БИОРАСПРЕДЕЛЕНИЕ ВЫСОКОПОРИСТЫХ ЧАСТИЦ КАРБОНАТА КАЛЬЦИЯ С ПОЛИМЕРНЫМИ ОБОЛОЧКАМИ В ЖИВОТНЫХ-ОПУХОЛЕНОСИТЕЛЯХ

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300 лет Российская Академия Наук

18.10.2023

$CaCO_3$ applications

Metallurgy and building

Manufacture of paints, paper, rubber, plastics, glass…

Cosmetics, personal hygiene products

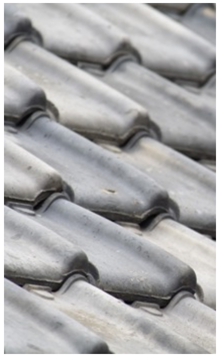
Food industry

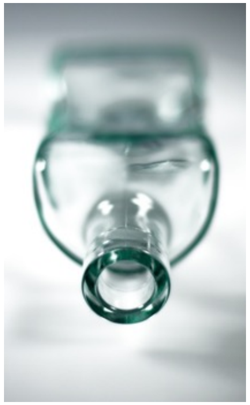
Water decontamination

Medicine

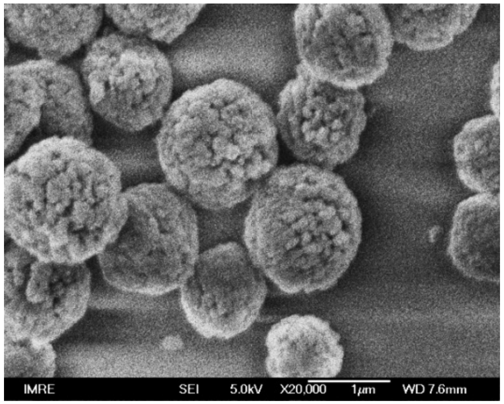
White color
Anti-caking property
Mechanical properties
Physical stability
Low cost

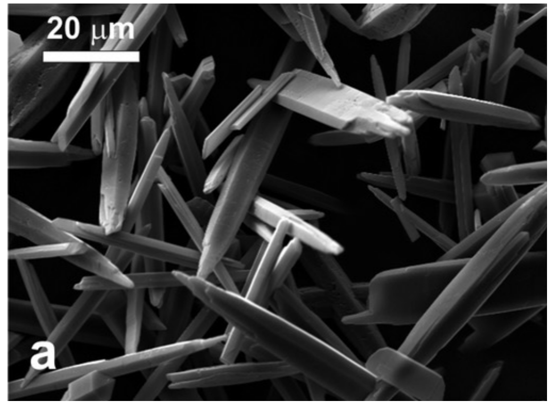
Biocompatibility
Low toxicity
Mild decomposition conditions
Biodegradability
Good absorption capacity
Ability to control particle parameters

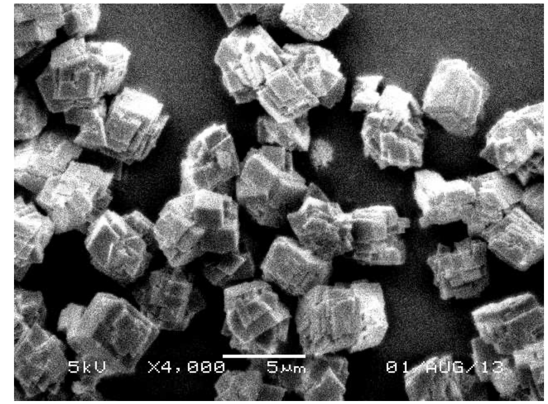




$CaCO_3$ Polymorphism

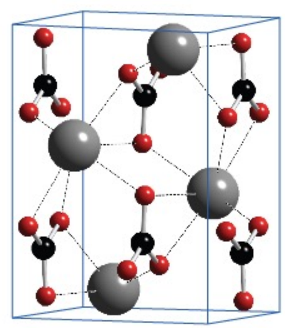




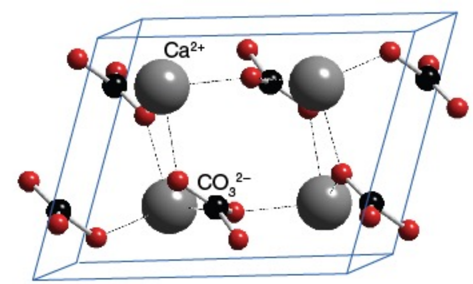


Metastable → Stable

Porous structure
Developed surface
↓
Efficient loading of active molecules



Aragonite



Calcite

Vaterite

Hexagonal crystal system

Orthorhombic crystal system

Trigonal crystal system

$CaCO_3$ particles: 3 polymorphs

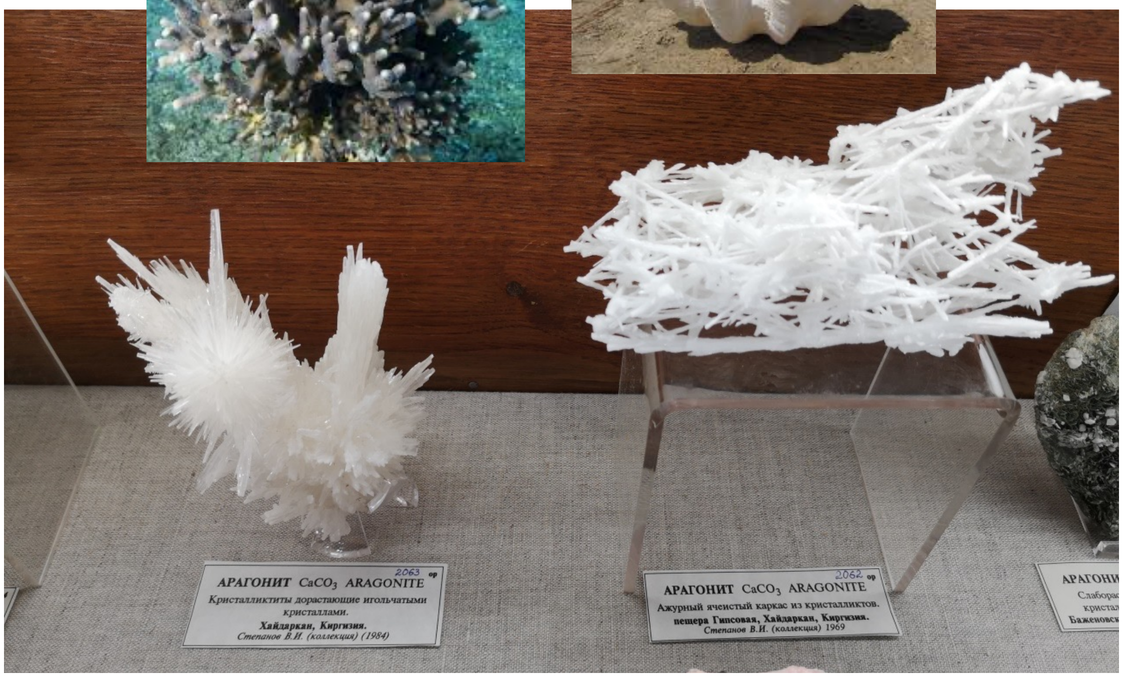
Calcite

Iceland spar, formerly known as Iceland Crystal, is a transparent variety of calcite (birefringence)

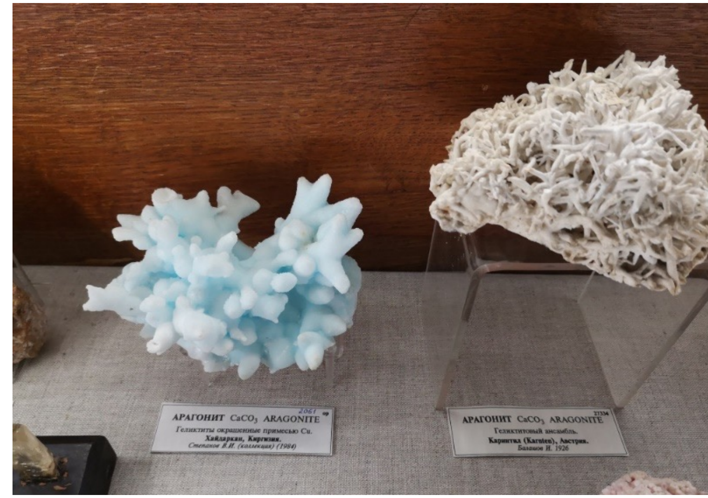
Fersman Mineralogical Museum RAS, Moscow

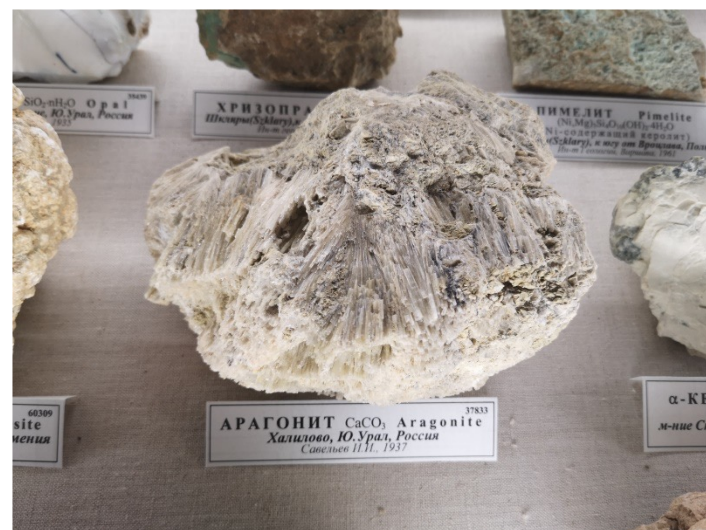
$CaCO_3$ particles: 3 polymorphs

Aragonite



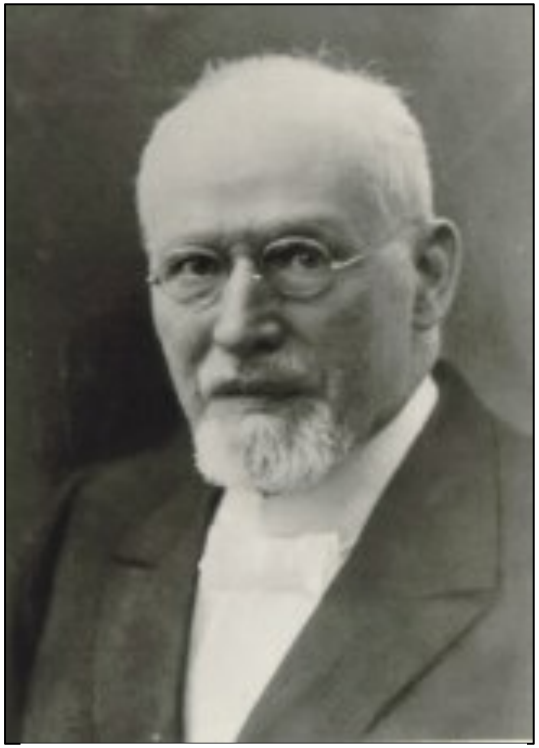
Fersman Mineralogical Museum RAS, Moscow





$CaCO_3$ particles: 3 polymorphs

Vaterite (1903) is less stable form of $CaCO_3$

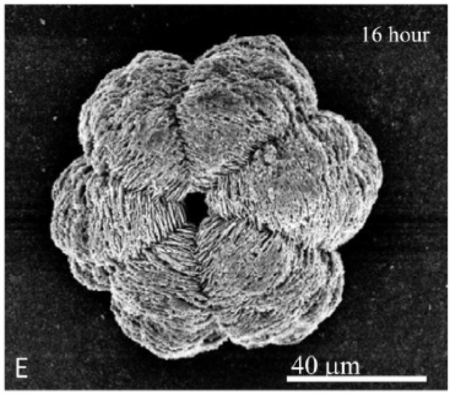


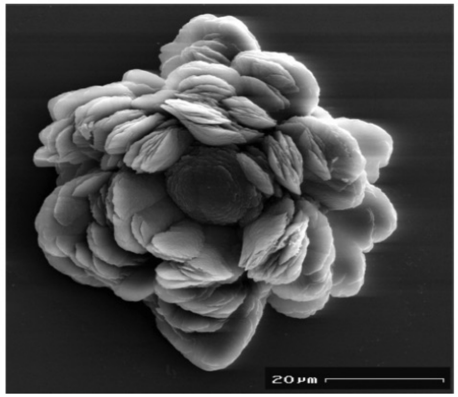
Heinrich Vater was a pioneer in the forest soil science, land evaluation, forest fertilization

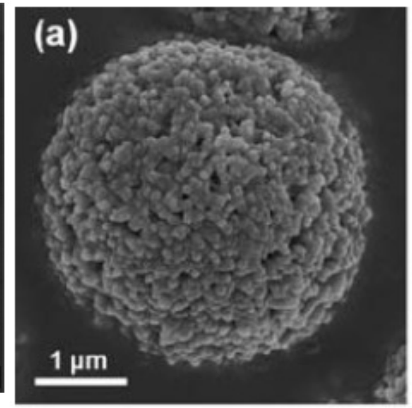
Saxifraga sempervivum, an alpine plant produces a greenish-white crust, which contains vaterite

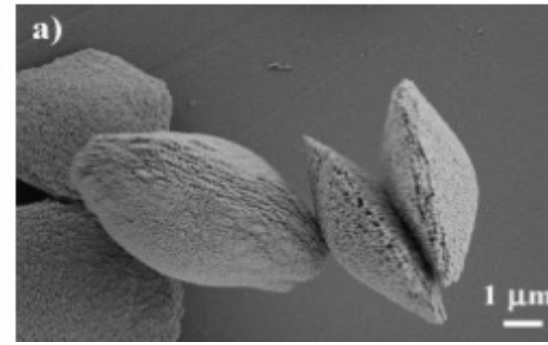
Spicules from the simple sea creature Herdmania momus contain large single crystals of vaterite of higher quality than those in the synthetic vaterite used in previous structure determinations (ESRF)

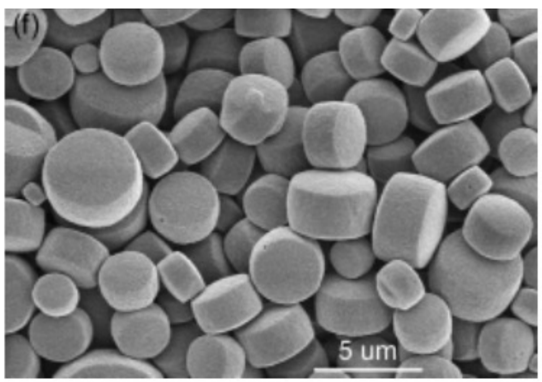
Variety of vaterite morphologies

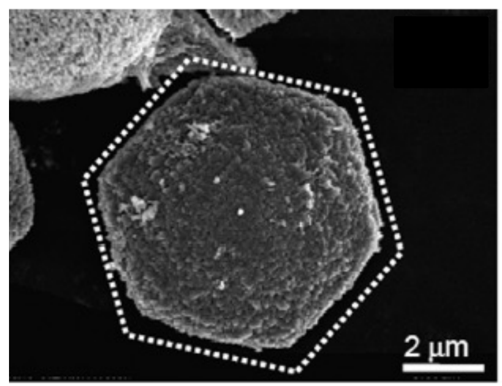


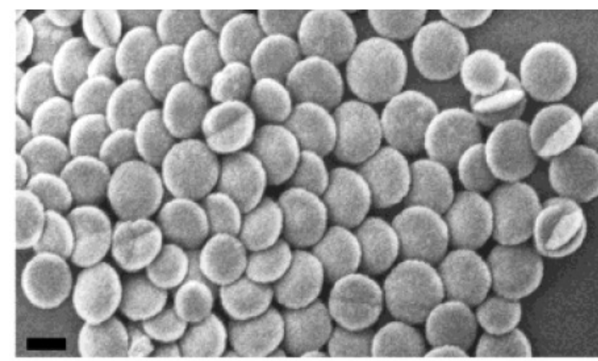












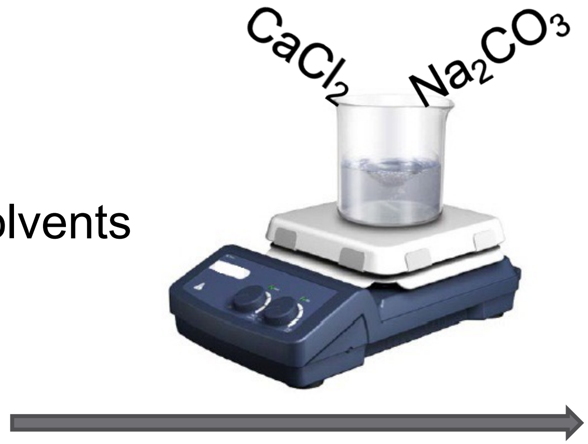
- Trushina D.B. et al. Calcium carbonate vaterite particles for drug delivery: Advances and challenges // *Mater. Today Adv.* **2022**. Vol. 14, P. 100214
- Trushina D.B., Bukreeva T. V., Antipina M.N. Size-Controlled Synthesis of Vaterite Calcium Carbonate by the Mixing Method: Aiming for Nanosized Particles // *Cryst. Growth Des.* **2016**. Vol. 16, № 3. P. 1311–1319
- Mikheev A. V et al. Hybrid Core–Shell Microparticles Based on Vaterite Polymorphs Assembled via Freezing-Induced Loading // *Cryst. Growth Des*. **2023**. Vol. 23, № 1. P. 96–103

Vaterite

Size-controlled crystallization

$$CaCl_2 + Na_2CO_3 = CaCO_3\downarrow + 2NaCl$$

Concentration of reagents
Temperature
pH
Presence of additives, co-solvents
Volume ratio of reagents
Mixing time and intensity
Influence of external fields



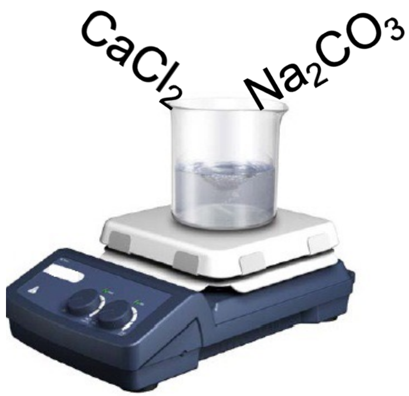
Polymorph composition
Size
Form
Porosity

The main objective

Crystallization of stable porous vaterite particles in a size-controlled manner

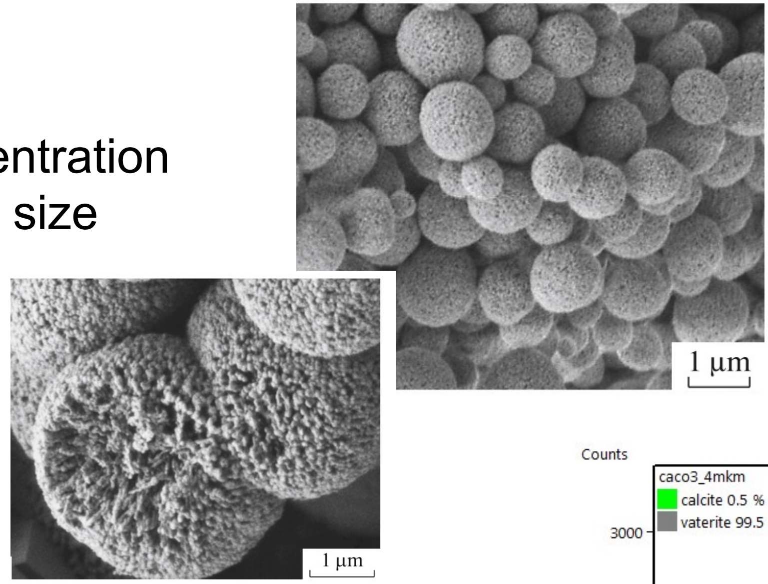
Focus on how to decrease the size

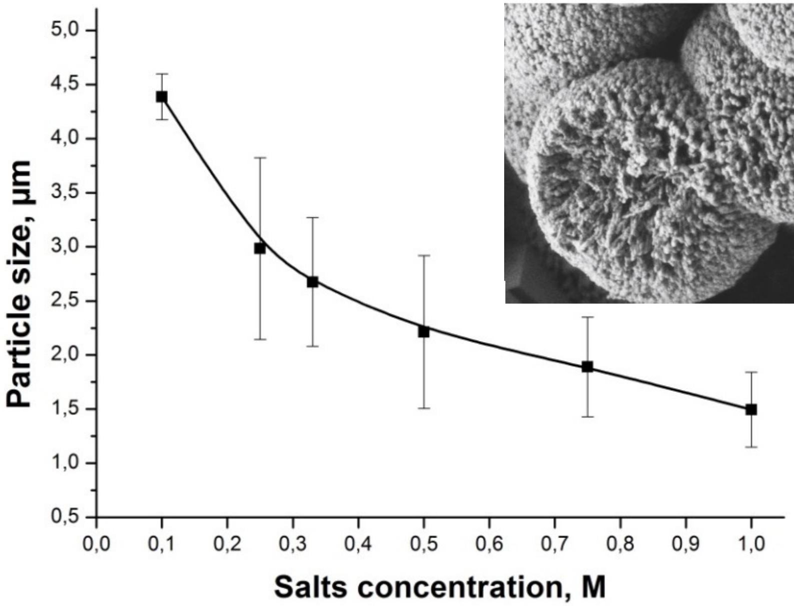
Micro-sized vaterite

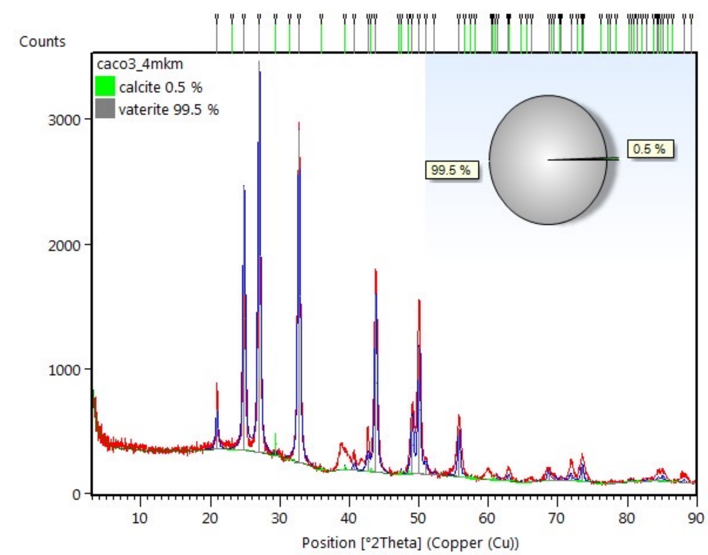


Standard protocol allows vaterite with the size ranging from 4.5 μm to 1.5 μm

Influence of the concentration of reagents on the size



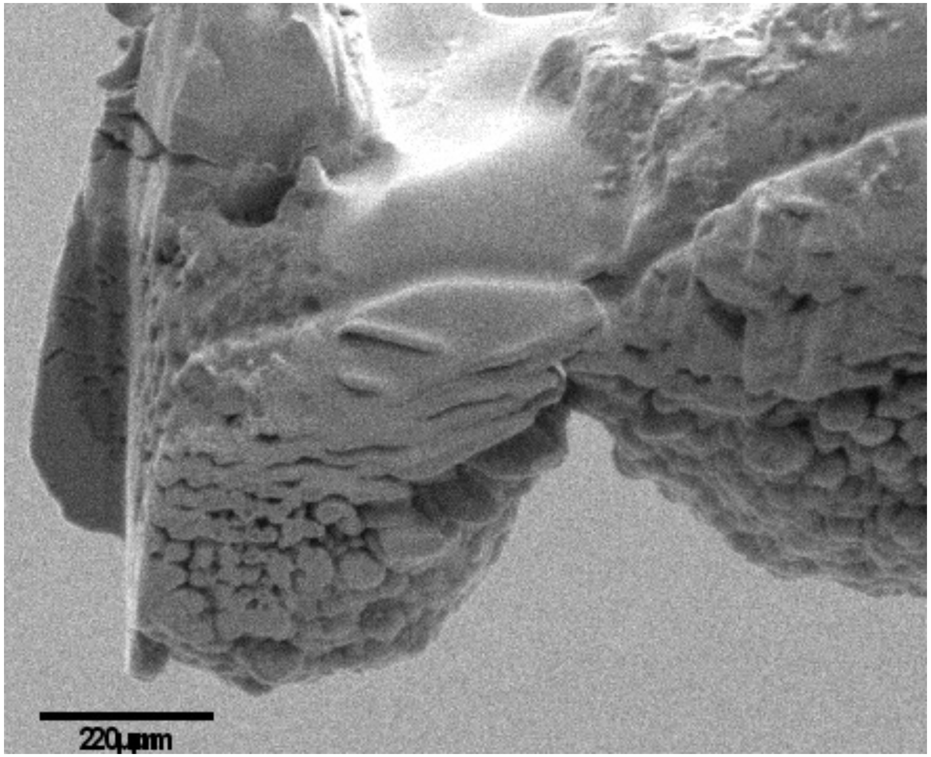


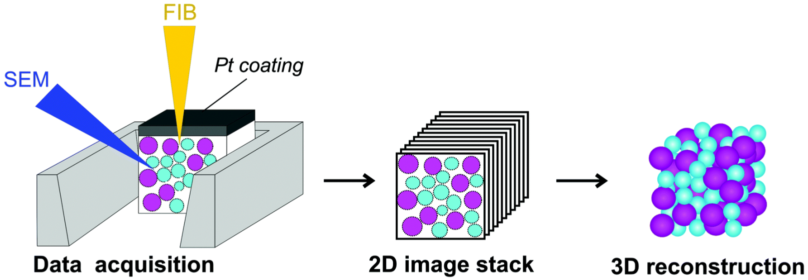


- Trushina, D. B.; Bukreeva, T. V.; Antipina, M. N. Size-Controlled Synthesis of Vaterite Calcium Carbonate by the Mixing Method: Aiming for Nanosized Particles. *Cryst. Growth Des.* **2016**, *16* (3), 1311–1319.

Micro-sized vaterite

FIB (Ga ions) milling of a Pt-coated $CaCO_3$ particle



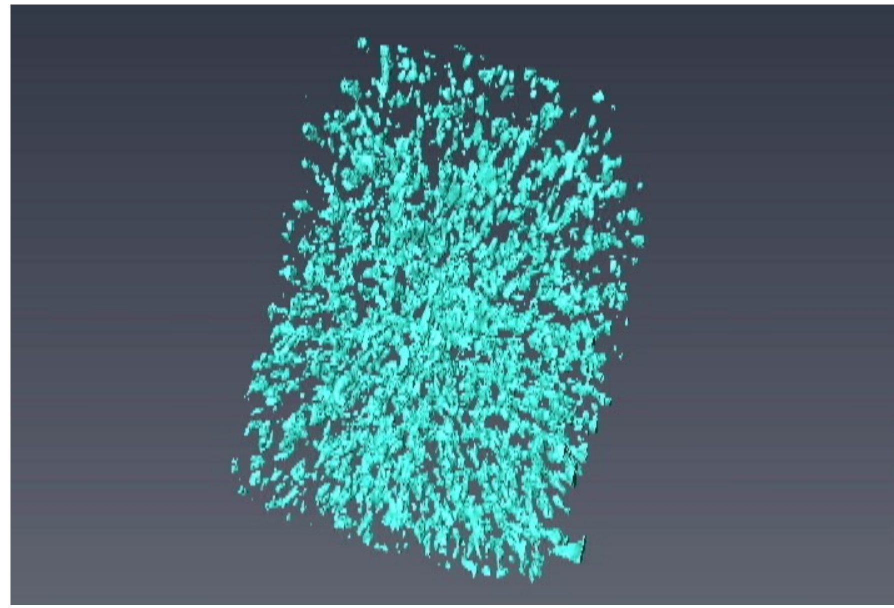


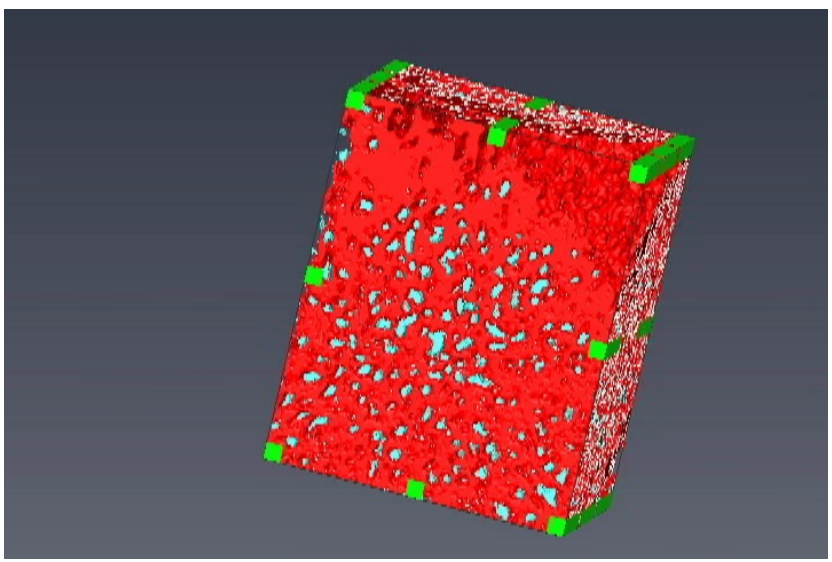
Micro-sized vaterite

3D reconstruction of vaterite particle structure according to SEM images

$CaCO_3$

Pores





Submicron-sized vaterite

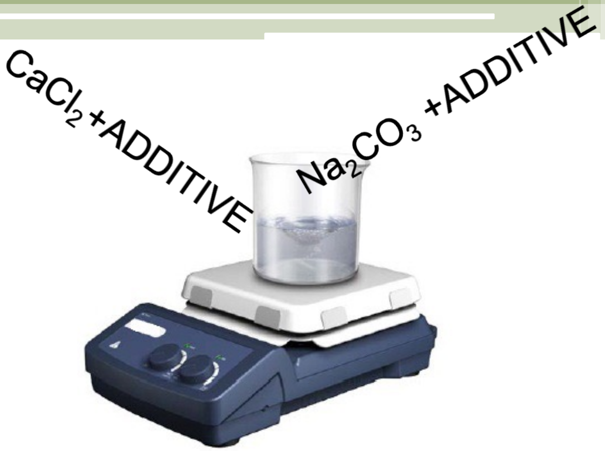
Modified protocol allows vaterite with the size ranging from 0.3 μm to 1.7 μm

Optimization of the reaction parameters:

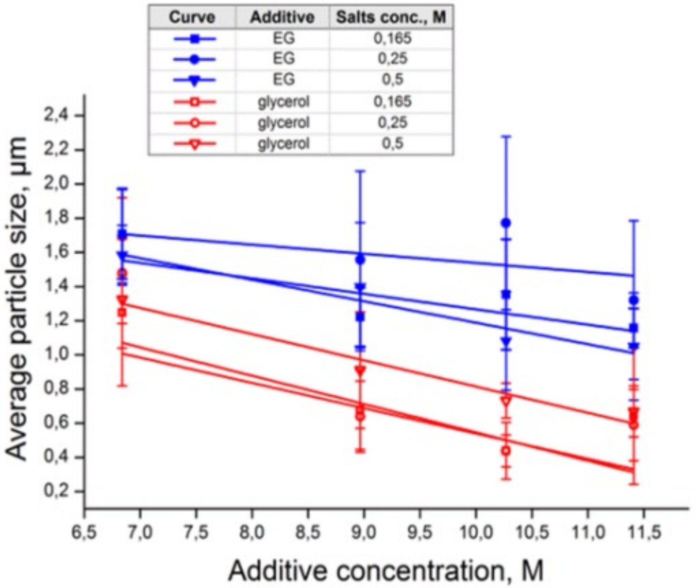
✓Varying salts:co-solvent volume ratio (1:1 – 1:5)

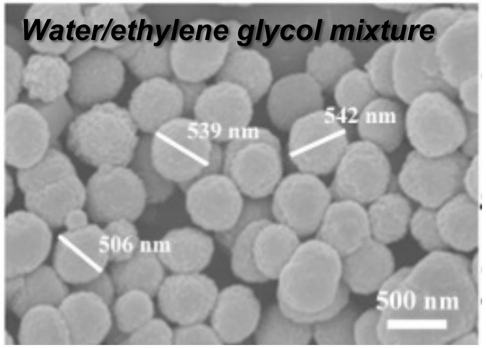
✓Different T (25°C – 40°C)

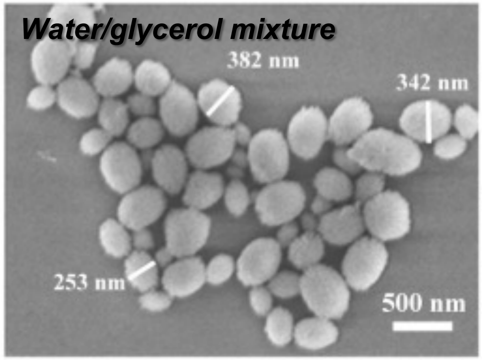
✓Mixing time from 30s to 3h

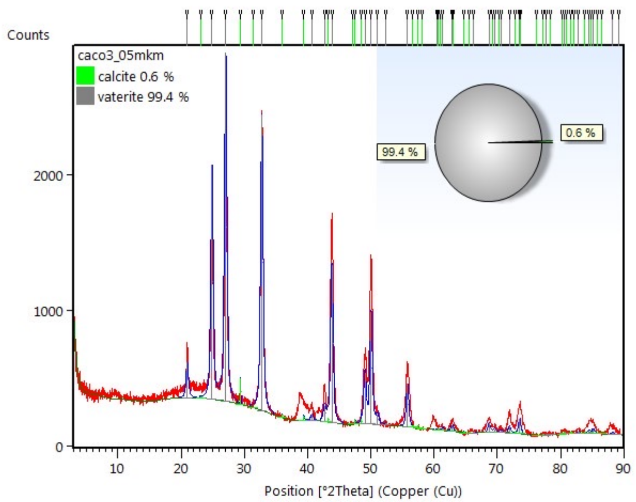


ADDITIVE (co-solvent): ethylene glycol/ glycerol









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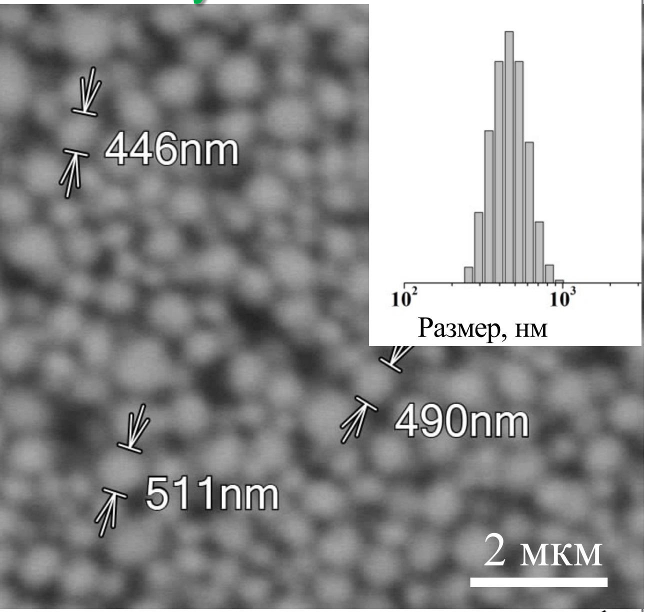
Mass crystallization

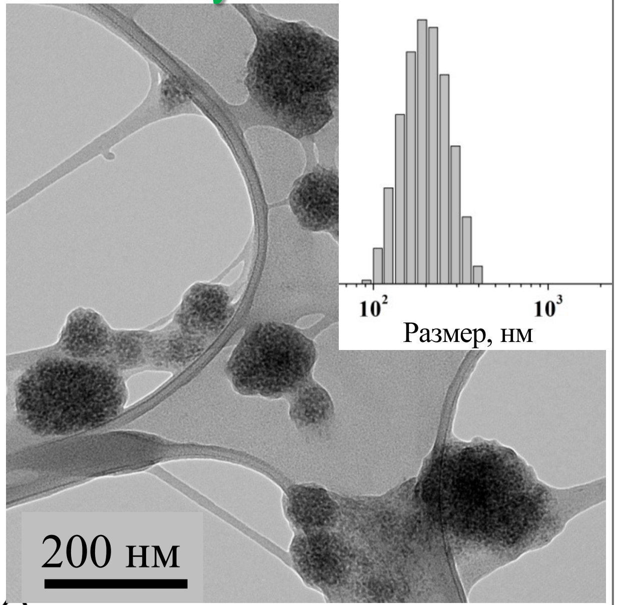


CaCl$_2$ +ADDITIVE

Na$_2$CO$_3$ +ADDITIVE

ADDITIVE (co-solvent): ethylene glycol/glycerol

Mass crystallization



CaCl$_2$ + ADDITIVE

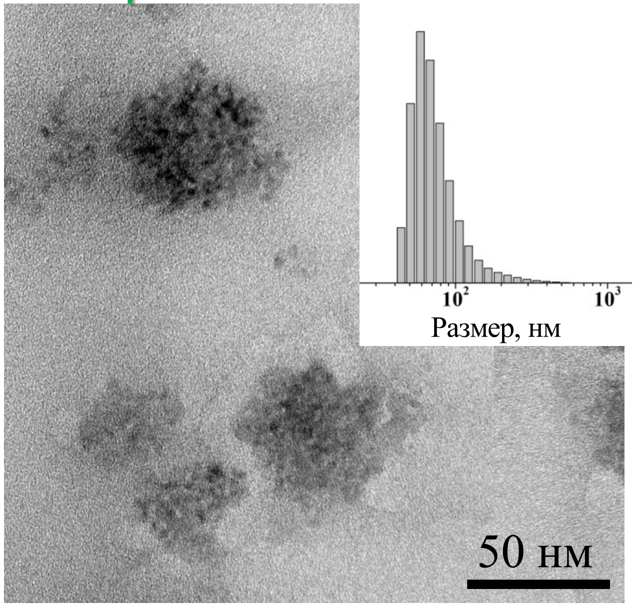
Na$_2$CO$_3$ + ADDITIVE

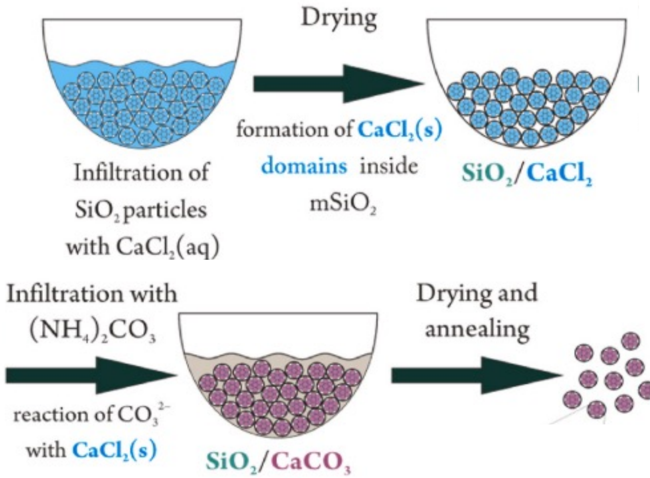
+US

- V. Popova et al. Nanomaterials (**2021**)

ADDITIVE: PEG+Tween-20+ DMEM + MgCl$_2$

Template method

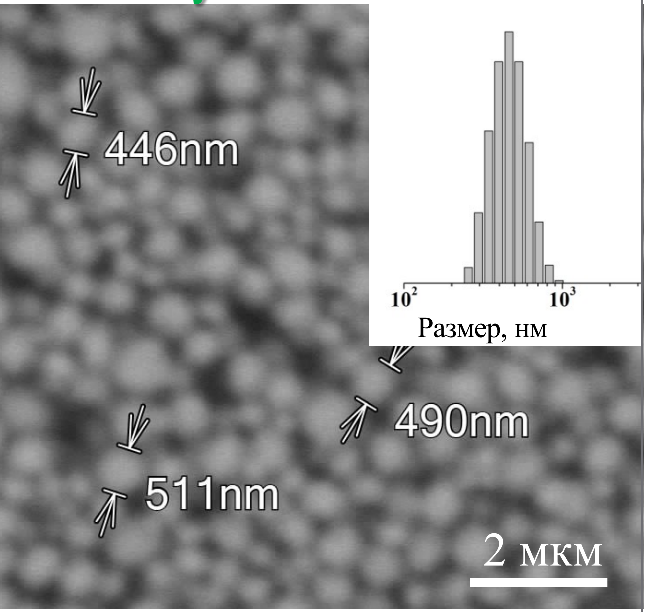


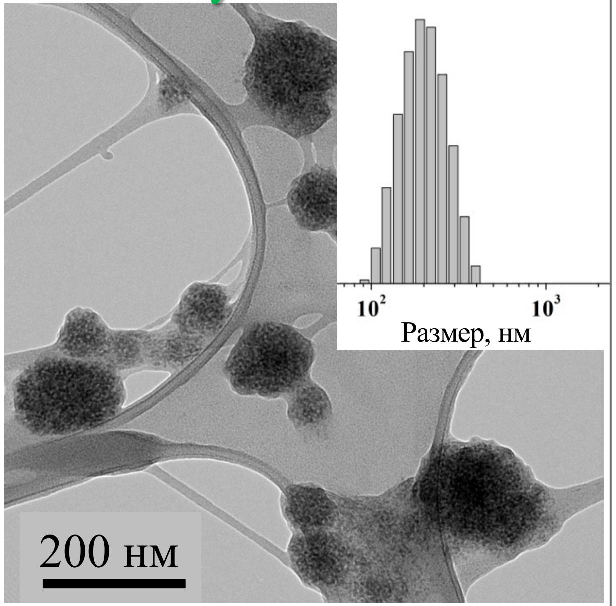


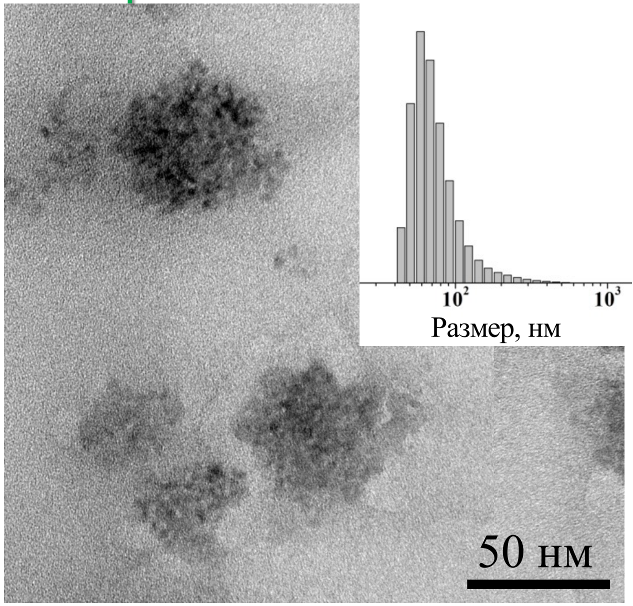
- D.A. Eurov et al. Microporous Mesoporous Mater. (**2022**)

- T. N. Pallaeva, A. V. Mikheev, D. N. Khmelenin, D. A. Eurov, D. A. Kurdyukov, V. K. Popova, E. V. Dmitrienko, D. B. Trushina. High-capacity calcium carbonate particles as ph-sensitive containers for doxorubicin, Crystallogr. Reports. 2 (**2023**) 309–315

Mass crystallization	Mass crystallization	Template method







500±90 nm ($CaCO_3$– 500)	**172±75 nm ($CaCO_3$–200)**	**65±15 nm ($CaCO_3$:Si:Fe–50)**

Polymorphic composition

99.4% vaterite	Amorphous	100%calcite

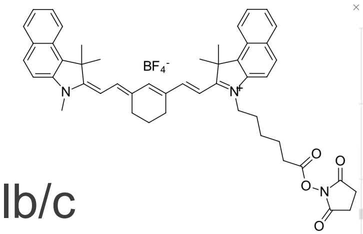
Loading with doxorubicin

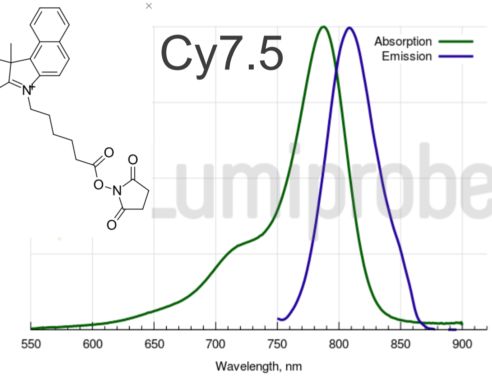
6.5 wt%	4.8 wt.%	4.0 wt.%

- T. N. Pallaeva, A. V. Mikheev, D. N. Khmelenin, D. A. Eurov, D. A. Kurdyukov, V. K. Popova, E. V. Dmitrienko, D. B. Trushina. High-capacity calcium carbonate particles as ph-sensitive containers for doxorubicin, Crystallogr. Reports. 2 (**2023**) 309–315

In vivo evaluation of 50/200/500nm $CaCO_3$ particles

Particle type	Blood half-life $t_{1/2}$, min
500 nm	1.6 ±0.7
500 nm/PEG	2.2±0.9
200 nm/PEG	9.2±4.7
50 nm/PEG	2.6±0.7

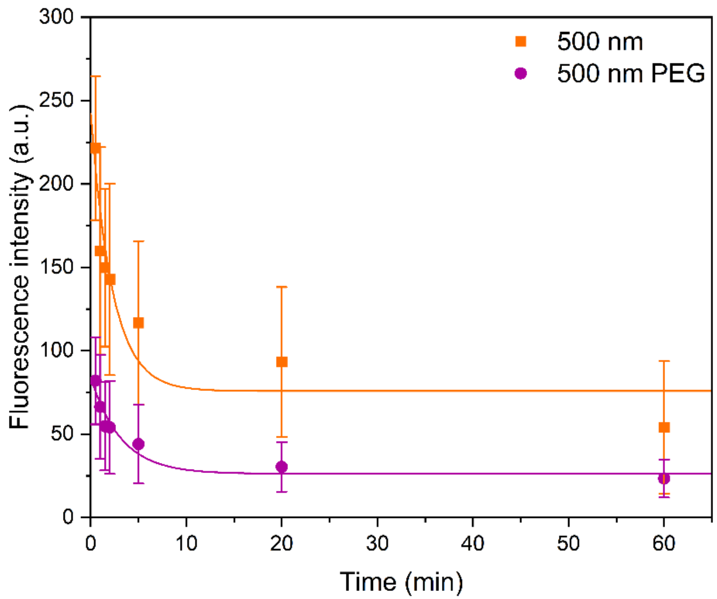


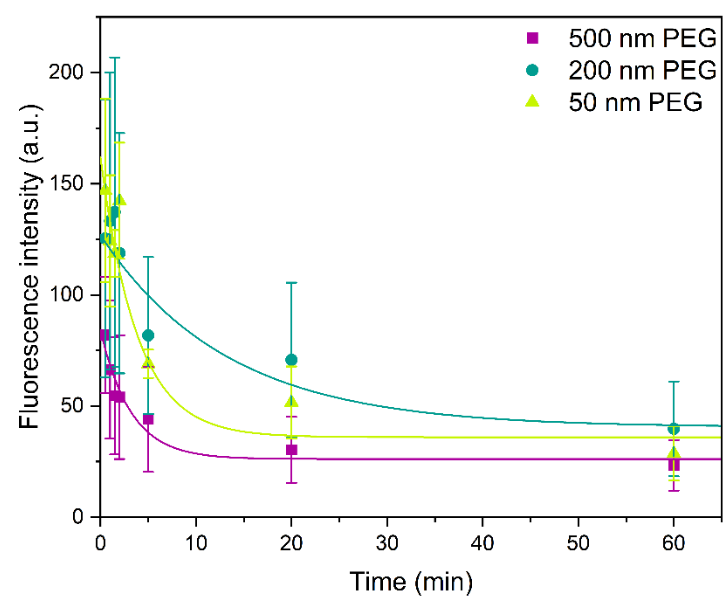


Balb/c

heart rates of 8 - 10 beats per sec

In vivo pharmacokinetic profiles of Cy7.5-labelled $CaCO_3$ in mice

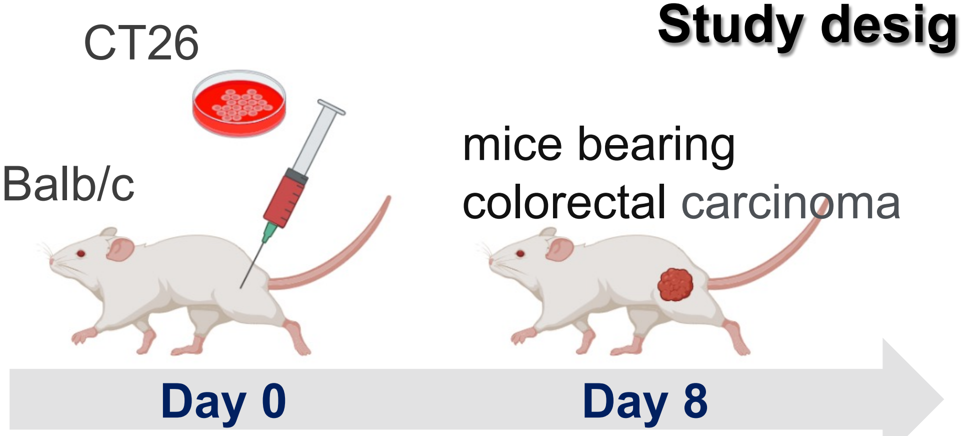




N=4

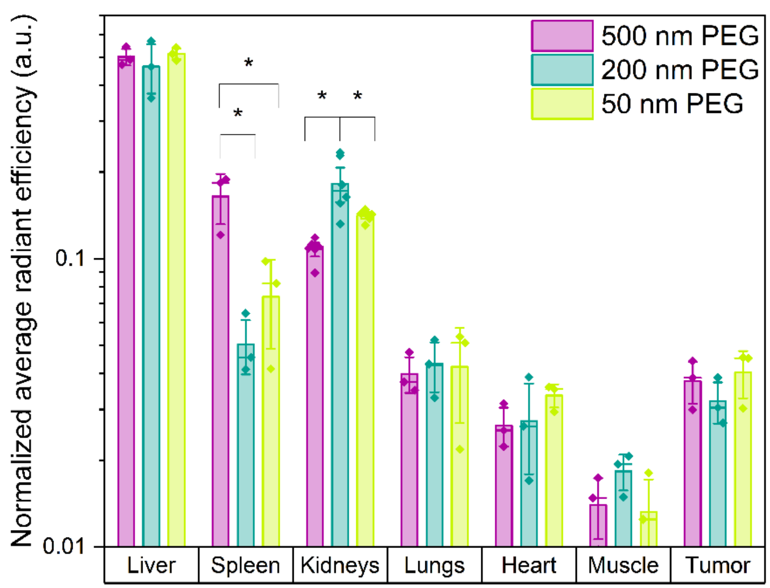
In vivo evaluation of 50/200/500nm $CaCO_3$ particles

Study design



Intravenous injection of Cy7.5-labelled $CaCO_3$

In vivo biodistribution of Cy7.5-labelled $CaCO_3$ in mice (IVIS Spectrum CT)



N=4

* p = 0.05

Результаты и Выводы

Для синтеза $CaCO_3$ использовали методику массовой кристаллизации в растворах, а также темплатный синтез. Оптимизация условий кристаллизации позволила получить частицы $CaCO_3$ с размерами в диапазоне от 500 до 50 нм.

Для частиц 500нм циркуляция увеличивается при пэгилировании: $t_{1/2}$ увеличивается с 1.6±0.7 до 2.2±0.9 сек.
Для частиц 200нм $t_{1/2}$ 9.2±4.7 сек.

При внутривенном введении большинство субмикронных и наночастиц $CaCO_3$– 500нм/PEG, $CaCO_3$– 200нм/PEG и $CaCO_3$–50/PEG аккумулируются в печени, селезенке, почках. Частицы одинаково накапливаются в опухоли в пределах погрешности. Т. о. применения наночастиц и полимерного покрытия недостаточно, чтобы значительно улучшить доставку препаратов к опухолевым клеткам.

Acknowledgment

Российский научный фонд

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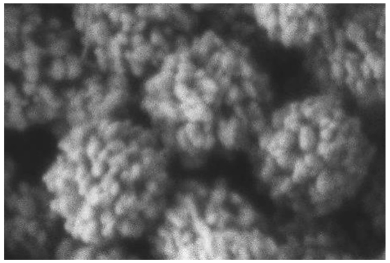

- ФНИЦ «Кристаллография и фотоника» РАН, Сеченовский Университет, Москва
- ИБХ РАН, Москва
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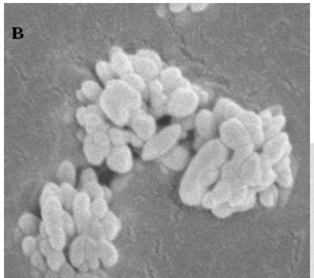
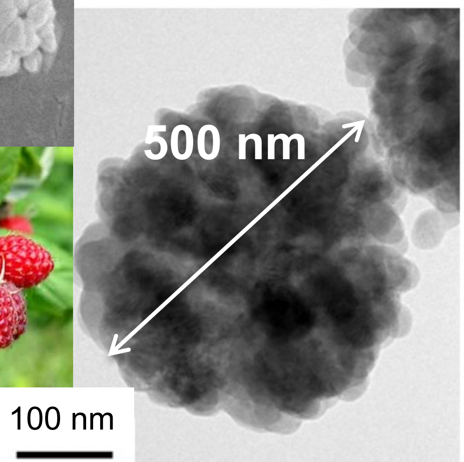
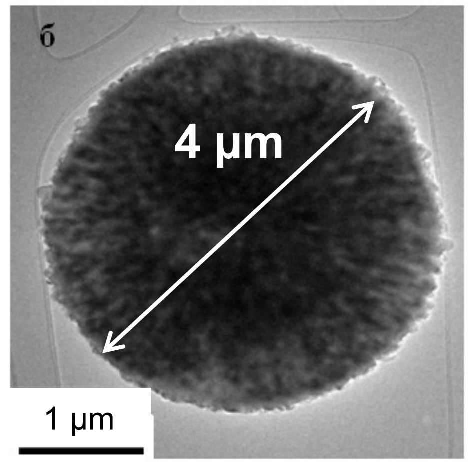
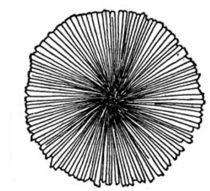
Two main types of vaterite achitecture



Framboids
(from fr. la framboise – raspberry)

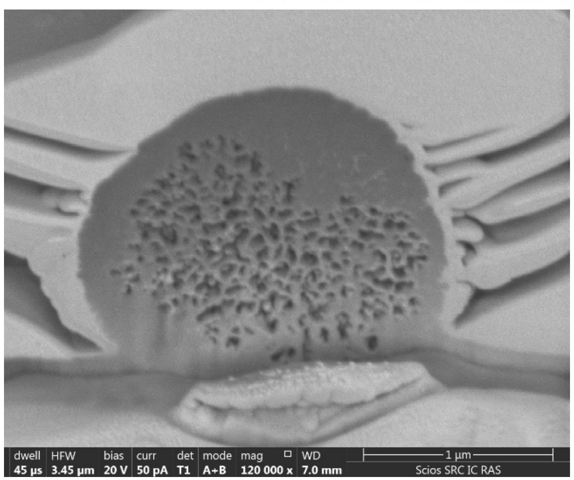


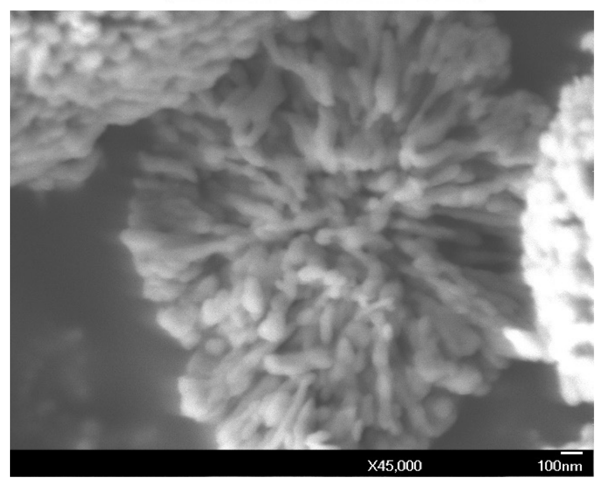




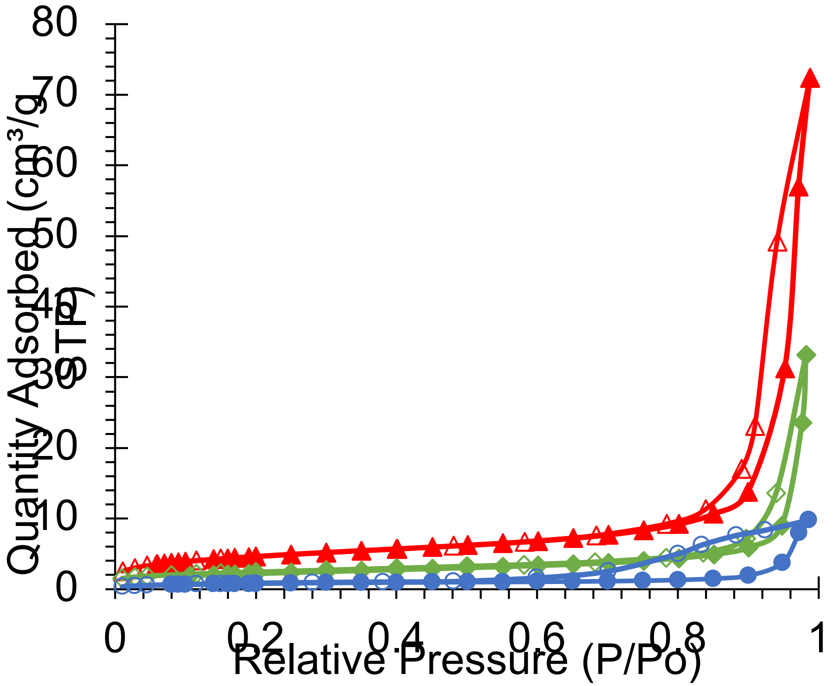
Spherulites

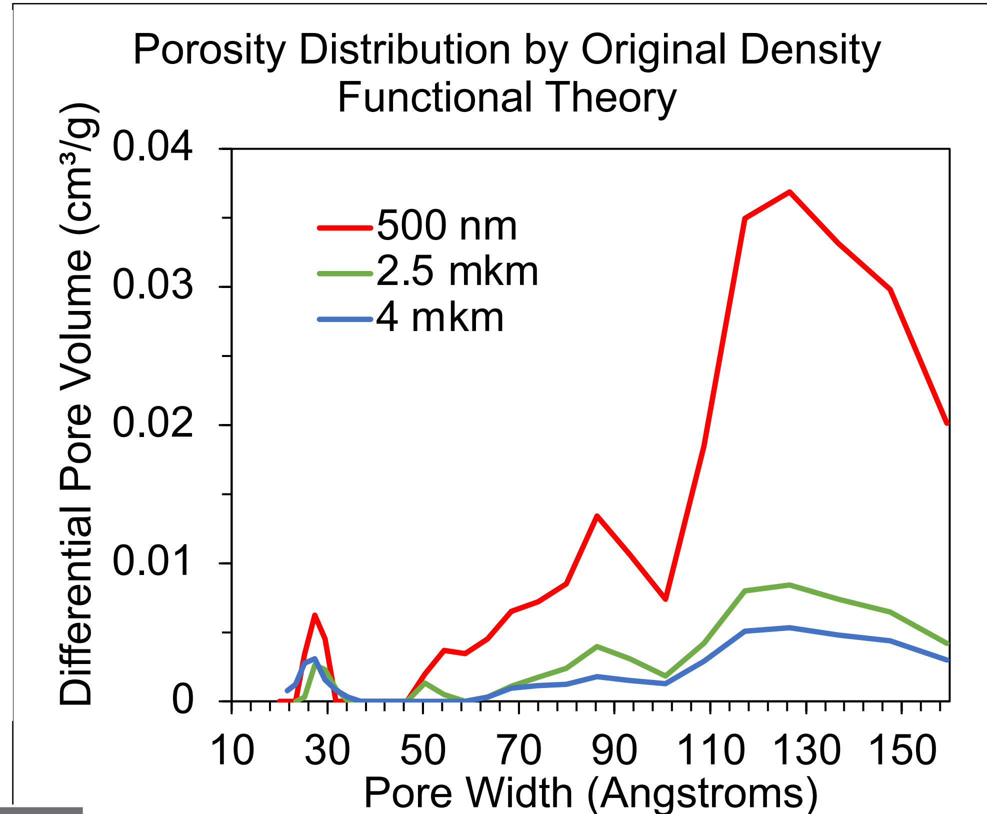
All directions are equal, framboids are the result crystal growth *via* oriented attachment of primary grains (non-classical crystal growth)





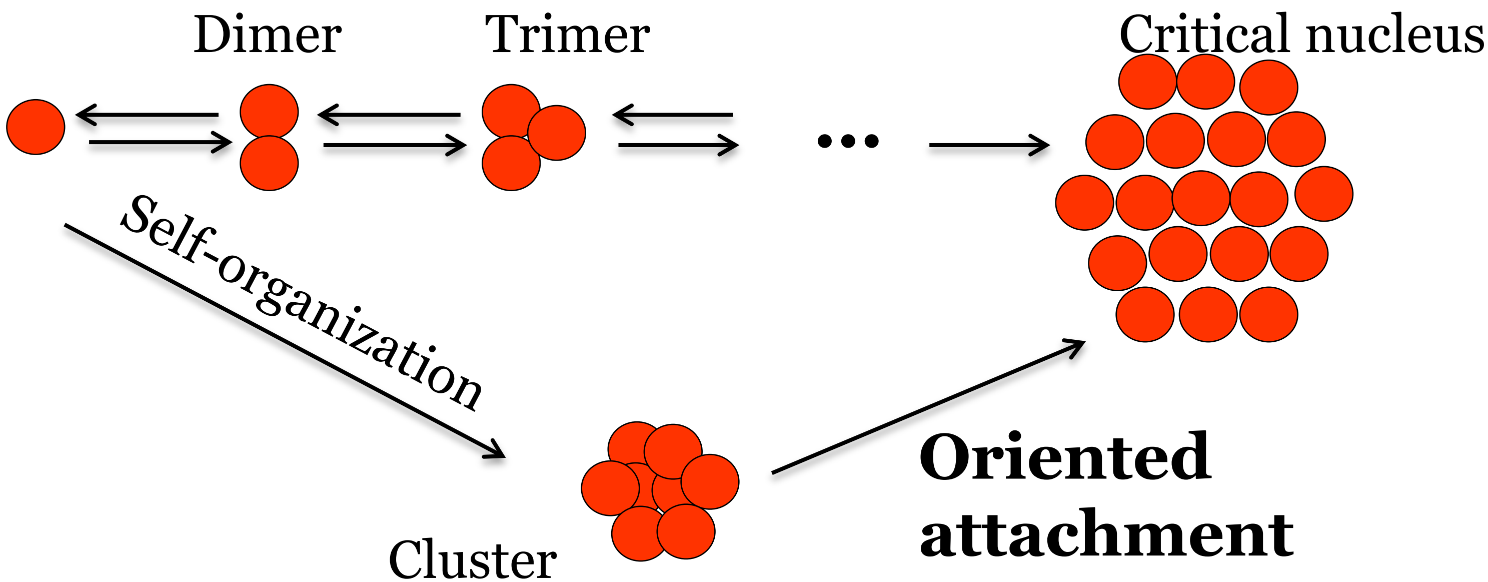
Spherulites are characterized by a radial-radiant internal structure due to the peculiarities of crystal growth



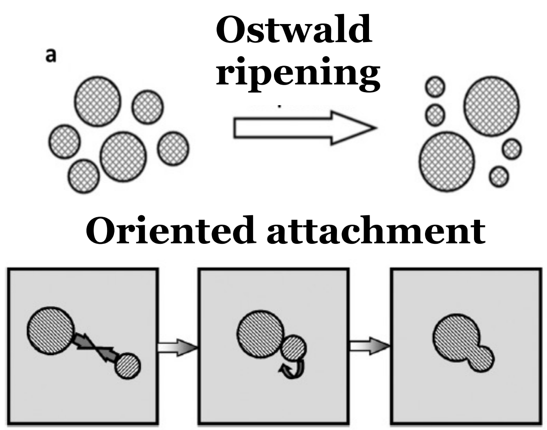


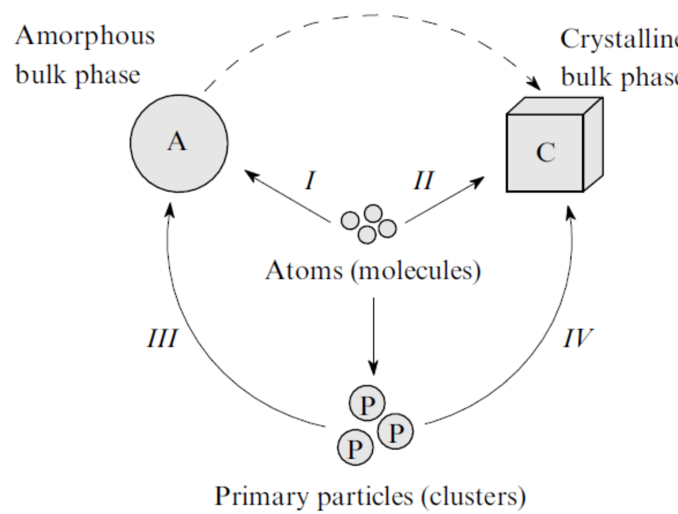
Diameter	BET Surface Area, m^2/g
500 nm	16.4
2.5 μm	7.8
4 μm	2.8

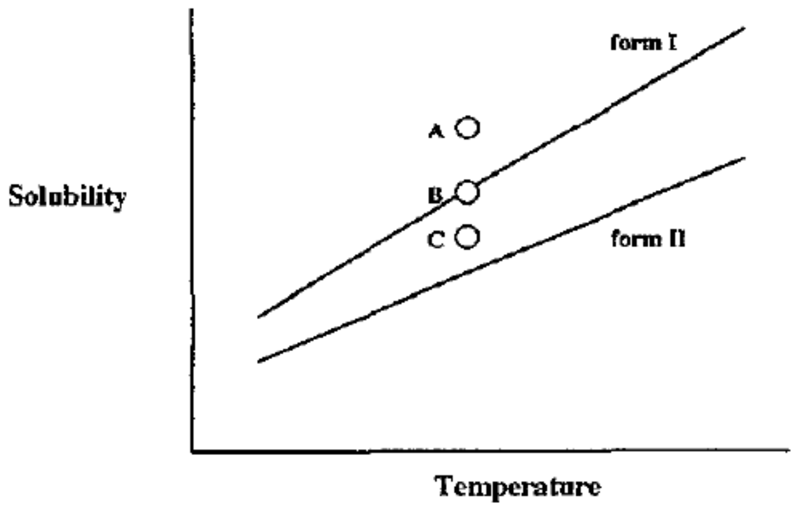
Classical theory (Ostwald ripening)

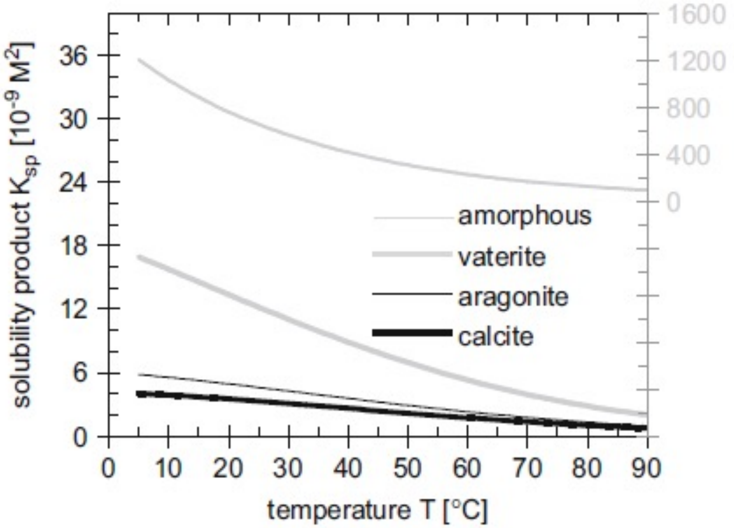


Building element:atom
VS nano cluster









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Nuclei formation*

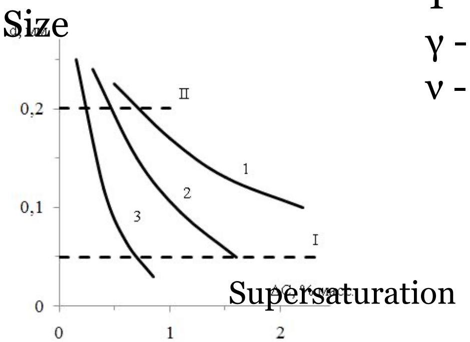
Radius of spherical critical nucleus at given supersaturation

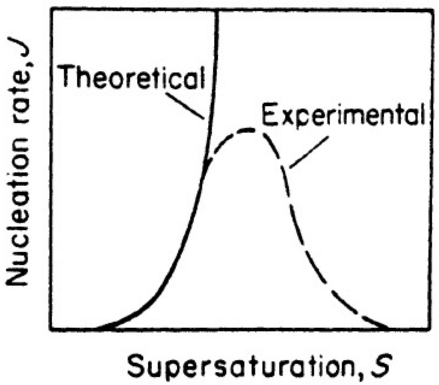
$$r_c = 2\gamma v / \mathbf{k} T \ln S$$

Rate of nucleation – the number of nuclei formed per unit time per unit volume

$$J = A \exp\left[-\frac{16\pi\gamma^3 v^2}{3\mathbf{k}^3 T^3 (\ln S)^2}\right]$$

S – supersaturation,
K – Boltzmann constant,
T – temperature,
γ - interfacial tension,
ν - molecular volume



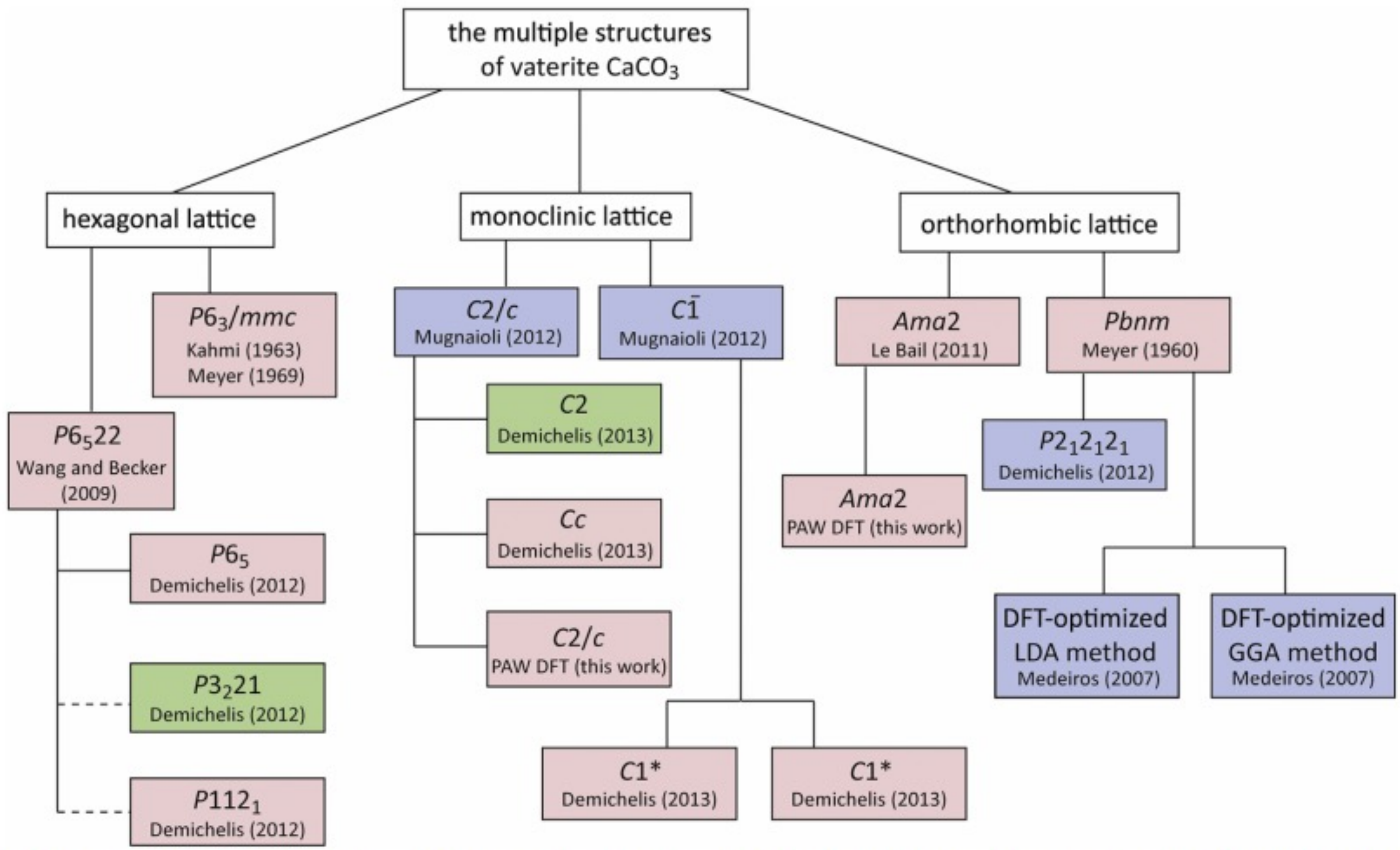


* Girshick, Steven L.; Chiu, Chia-Pin //The Journal of Chemical Physics, 1990, 93, 2, 1273

Разнообразие предложенных структур ватерита

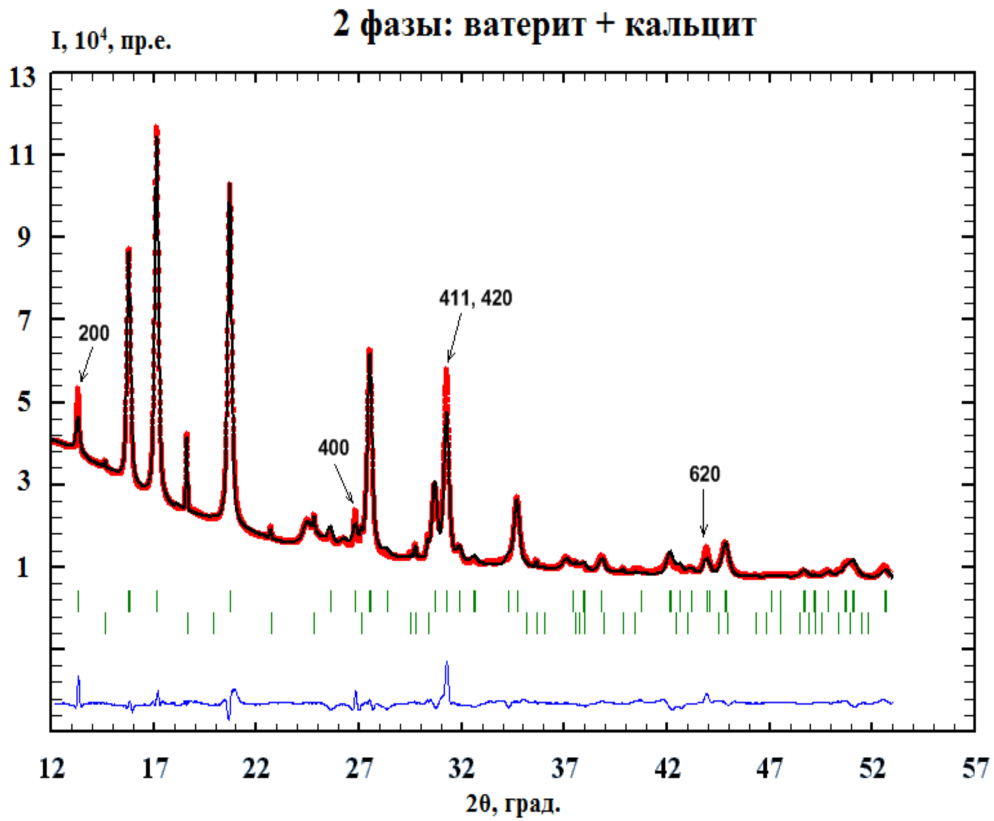
K.M.N. Burgess, D.L. Bryce / Solid State Nuclear Magnetic Resonance ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Accepted 29 August 2014



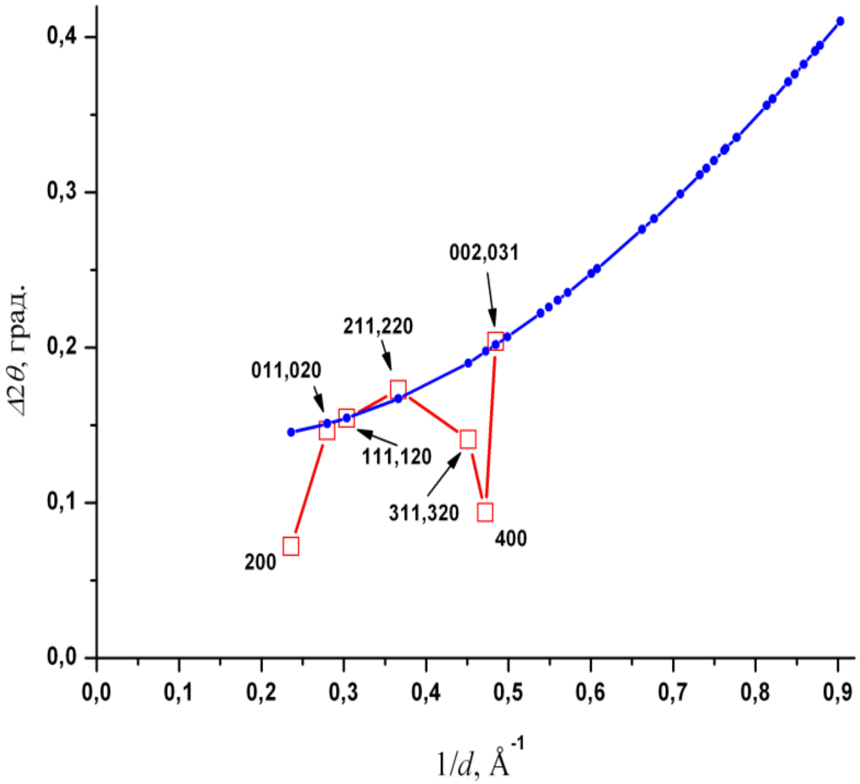
Science AAAS

Vaterite Crystals Contain Two Interspersed Crystal Structures
Lee Kabalah-Amitai *et al.*
Science **340**, 454 (2013);
DOI: 10.1126/science.1232139



Исходная рентгенограмма и результат уточнения методом Ритфельда для одного из образцов карбоната кальция

Чем больше первый индекс *h* рефлекса, тем уже пик, т.е. тем больше размер наночастиц в направлении параметра решётки *a*.



Зависимость ПШПВ дифракционных максимумов от обратного межплоскостного расстояния (построение по Williamson-Hall)

Каждый символ на гладкой линии (рассчитанной методом Ритфельда) означает ФУ (**ПШПВ**) для конкретного пика в модели Le Bail. Величины, обозначенные полыми квадратами, получены отдельно интерполяцией функцией pseudo-Voigt на линейном фоне.

Используя методику Вильямсона-Холла и соотношение Шеррера получены размеры нанокристаллитов (области когерентного рассеяния)- значения длин главных осей эллипсоида $2r_a$ = 1000(90) Å и $2r_b \approx 2r_c$ = 500(50) Å.

Cite this: *CrystEngComm*, 2012, **14**, 44

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COMMUNICATION

A new structural model for disorder in vaterite from first-principles calculations†

Raffaella Demichelis,*[a] Paolo Raiteri,[a] Julian D. Gale[a] and Roberto Dovesi[b]

Received 31st July 2011, Accepted 13th October 2011
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Table 1 Structures proposed for vaterite: space group (*SG*) and lattice parameters [Å]

Reference	*SG*	*a*	*b*	*c*
Meyer[11]	*Pbnm*	4.13	7.15	8.48
McConnell[12]	$P6_322$	7.135	7.135	8.524
Kahmi,[13] Sato and Matsuda[14]	$P6_3/mmc$	4.13	4.13	8.49
Bradley *et al.*[15]	$P6_322$	7.135	7.135	8.524
Meyer,[7] Gabrielli *et al.*[6]	$P6_3/mmc^a$	7.15	7.15	16.96
Dupont *et al.*[16]	$P6_3/mmc^a$	7.169	7.169	16.98
Le Bail *et al.*[9]	*Ama2*	8.7422	7.1576	4.1265
Medeiros *et al.*[17]	$Pbnm^b$	4.531	6.640	8.480
Wang and Becker[18]	$P6_522^b$	7.290	7.290	25.302

J. Wang et al. / Journal of Crystal Growth 407 (2014) 78–86

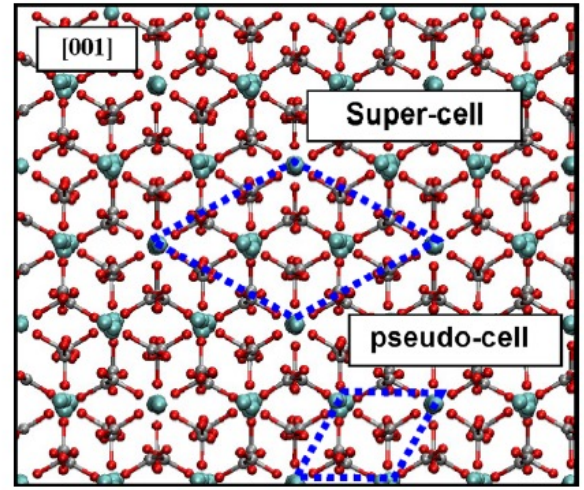


Fig. 3. Structure of a snapshot from molecular dynamics simulation with the dashed lines highlighting the basic pseudocell and supercell. The structure is projected on (001) plane. The large balls are calcium atoms. The balls and sticks are carbonate groups.

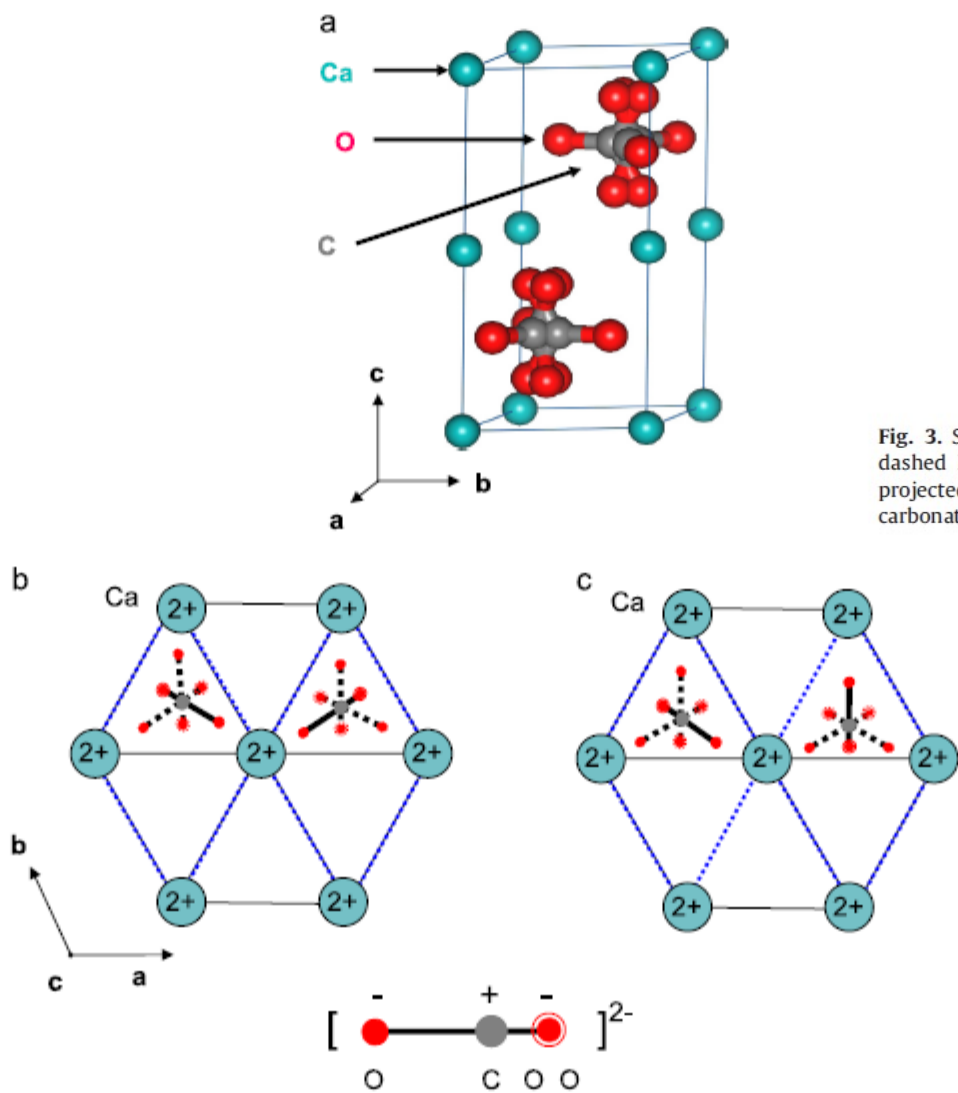


Fig. 2. (a) Crystal structure of vaterite with disordered CO_3^{2-} group [16]. Each CO_3 has a one-third occupancy. Ca^{2+} ions form a hexagonal lattice. (b) and (c) Schematic view of Ca^{2+} lattice (shown as 2+ charge inside a circle) and CO_3^{2-} orientations. Thin dashed lines highlight the pseudo unit cells. Three possible orientations of CO_3 ions are shown in dashed and solid symbols. The configuration in (b) where the two neighboring CO_3 ions are pointing to the same edge of the prisms is less stable than the one in (c) where the two neighboring CO_3 ions are pointing to different edges because of electrostatic repulsion between the oxygen atoms of CO_3 in the former configuration.

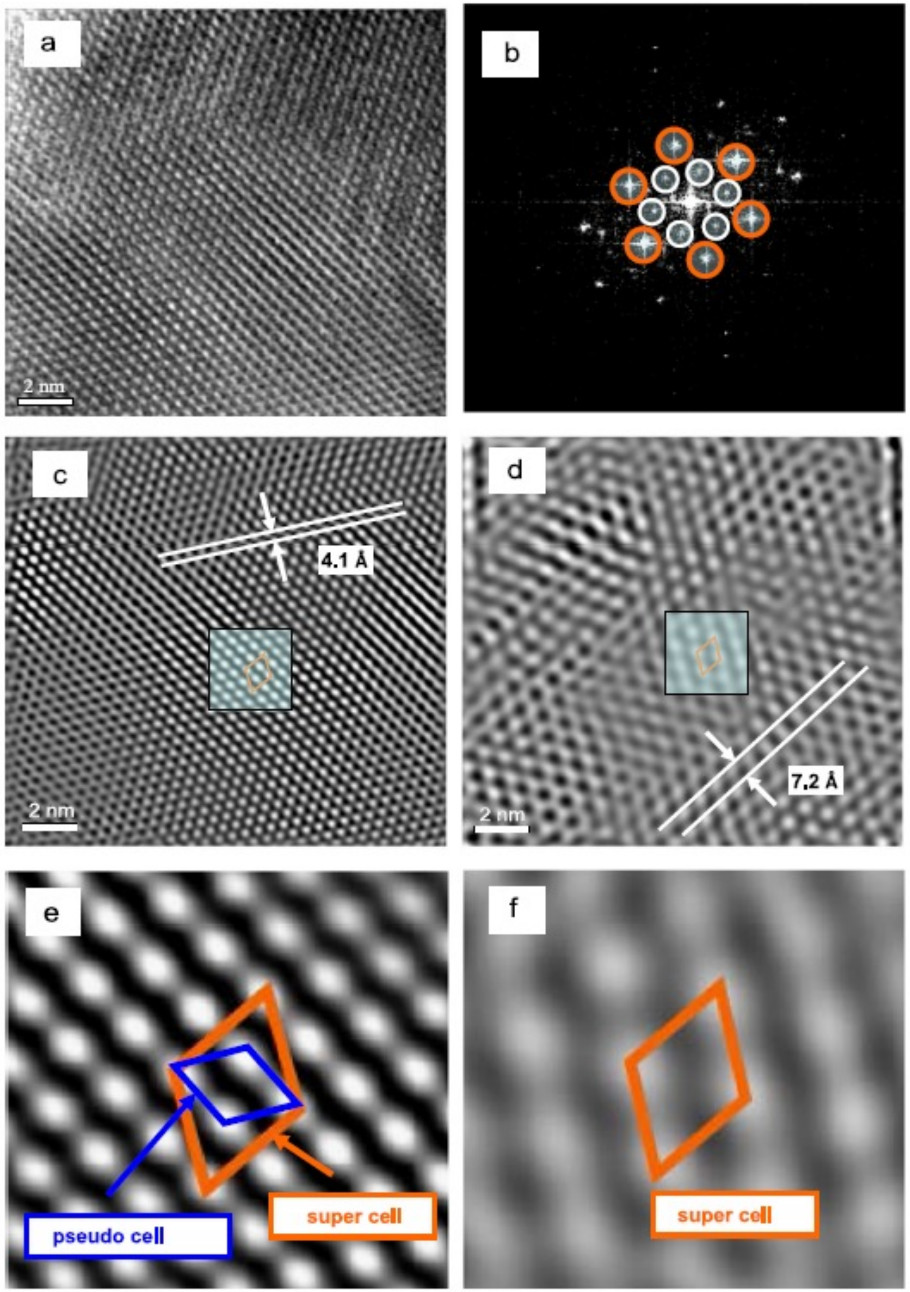
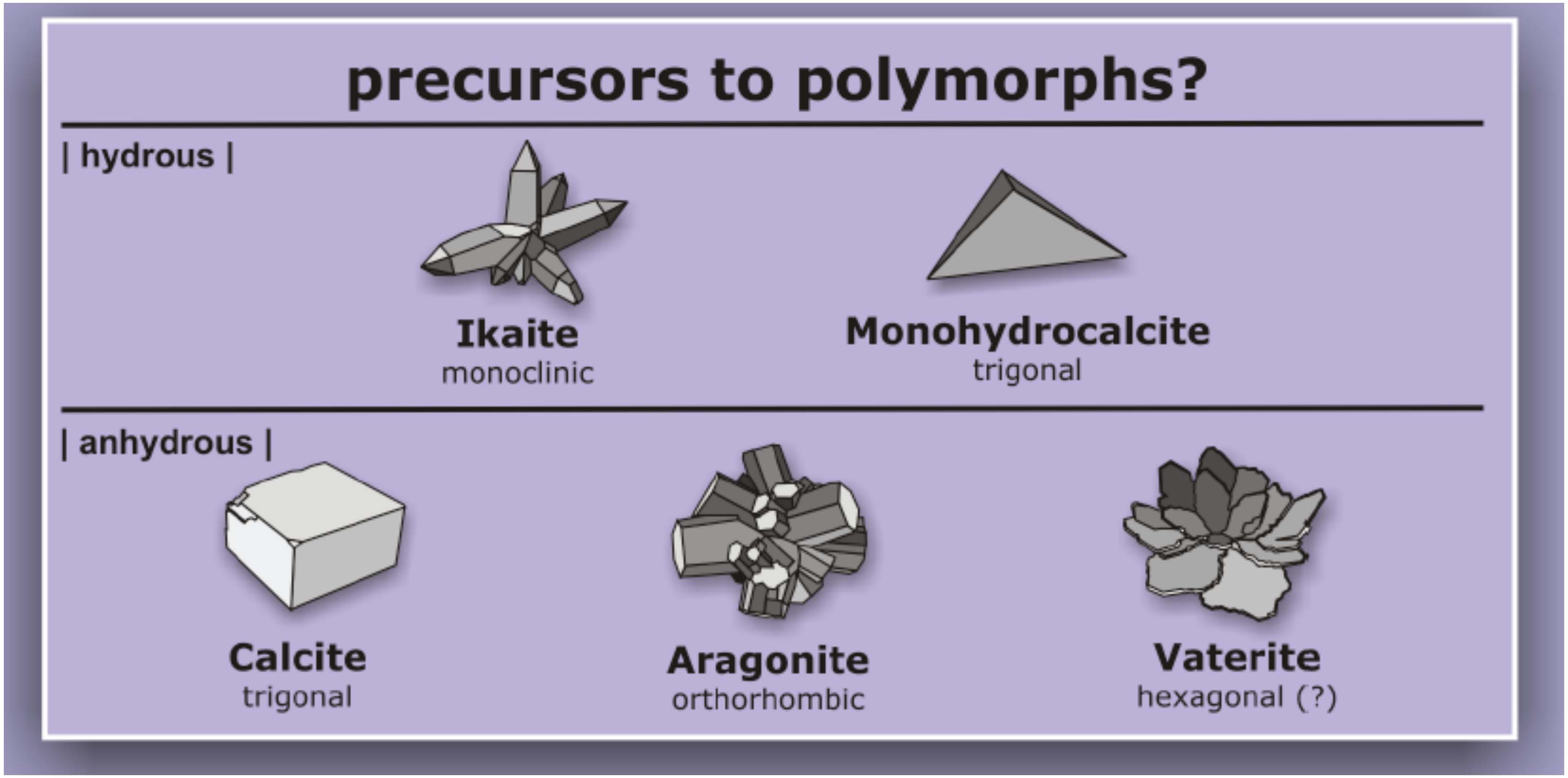


Fig. 6. (a) An HRTEM image shows the threefold symmetry along [001]. (b) A fast Fourier transform corresponding to the image (a). Two sets of diffraction peaks present and were highlighted with small and larger circles, respectively. (c) The HRTEM image generated from the primary diffraction peaks in Fig. 4b. (d) The HRTEM image generated from the satellite diffraction peaks in Fig. 4b. (e) The HRTEM image of the highlighted region in Fig. 4c. (f) The HRTEM image of the highlighted region in Fig. 4d. The basic lattice and superlattice are highlighted in Figs. 4e, f.


precursors to polymorphs?
| hydrous |
Ikaite
monoclinic
Monohydrocalcite
trigonal
| anhydrous |
Calcite
trigonal
Aragonite
orthorhombic
Vaterite
hexagonal (?)