Innovative Technologies and Sustainable Use of Mediterranean Sea Fishery and Biological Resources
(FishMed-PhD)

Teaching week 2025

Crystallization in biomineralization and in the environment

3. Calcium carbonate - Model Systems in Biomineralization

4. Calcium carbonate - Model System in Environment

Damir Kralj
Ruđer Bošković Institute, Zagreb, Croatia

Biomineralization – study of processes that lead to the formation of hierarchically structured organic–inorganic materials, generated by living organisms

Interdisciplinary – chemistry, biology, **materials science**...

Importance – paleontology, geology, geochemistry, biomedicine, ..., **materials science (!!)**

$CaCO_3$ biomineralization - marine, freshwater and terrestrial organisms
- **Calcite** (e.g. foraminifera, coccolithophores)
- **Aragonite** (e.g. corals)
- **Aragonite and Calcite** (e.g. bivalve shells)
- **Vaterite and amorphous $CaCO_3$** (precursor phases, fish otoliths)

Relevance for materials science - production of advanced materials by simple process, at mild temperatures, pressure and chemical environment …

Shell formation in mollusks

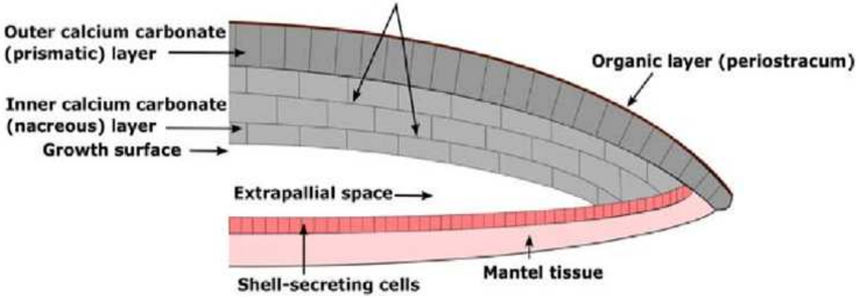
Molluscan shells → 95–99% calcium carbonate, 1–5 % organic component

Organic-inorganic composite → fracture toughness ≈ 3000 × greater than inorganic crystals (ADVANCED MATERIALS)

Soluble and insoluble (macro)molecules (proteins, sugars and lipids) → responsible for crystal nucleation and growth

Organic components → characteristic of specific mineral layer and of specific species

Different mineral layers → different polymorphs (calcite and aragonite)



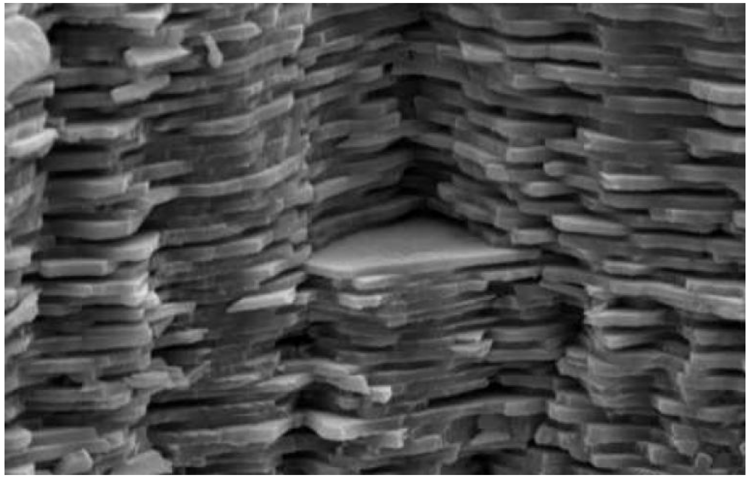
KELLY R. MARTIN et all. Teaching an Old Shell New Tricks: Extracting DNA from Current, Historical, and Ancient Mollusk Shells, BioScience 71: 235–248

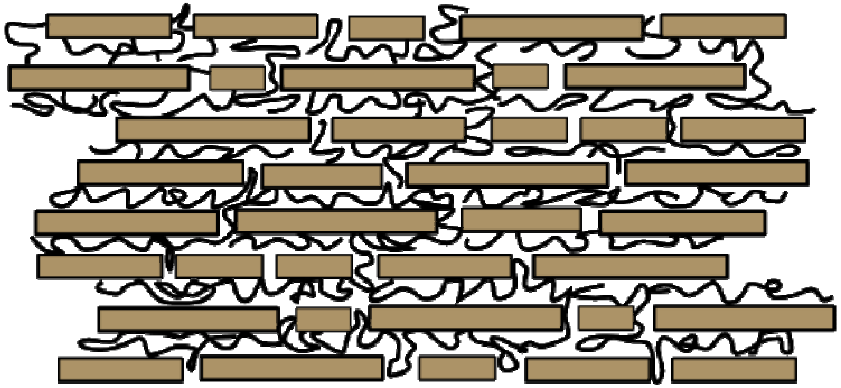
http://en.wikipedia.org/wiki/

Nacre

Inorganic component → aragonite platelets (10–20 μm wide, 0.5 μm thick) arranged in a continuous parallel lamina

Organic component → chitin, lustrin and silk-like proteins





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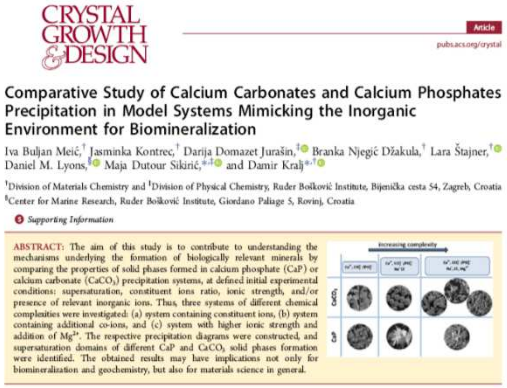
Model system 1

Influence of initial concentration (**supersaturation**), **ionic ratio** and **chemical complexity** of the system on $CaCO_3$ precipitation



CRYSTAL GROWTH & DESIGN

Article

pubs.acs.org/crystal

Comparative Study of Calcium Carbonates and Calcium Phosphates Precipitation in Model Systems Mimicking the Inorganic Environment for Biomineralization

Iva Buljan Meić,[†] Jasminka Kontrec,[†] Darija Domazet Jurašin,[‡] Branka Njegić Džakula,[†] Lara Štajner,[†] Daniel M. Lyons,[§] Maja Dutour Sikirić,[*,‡] and Damir Kralj[*,†]

[†]Division of Materials Chemistry and [‡]Division of Physical Chemistry, Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia
[§]Center for Marine Research, Ruđer Bošković Institute, Giordano Paliage 5, Rovinj, Croatia

Supporting Information

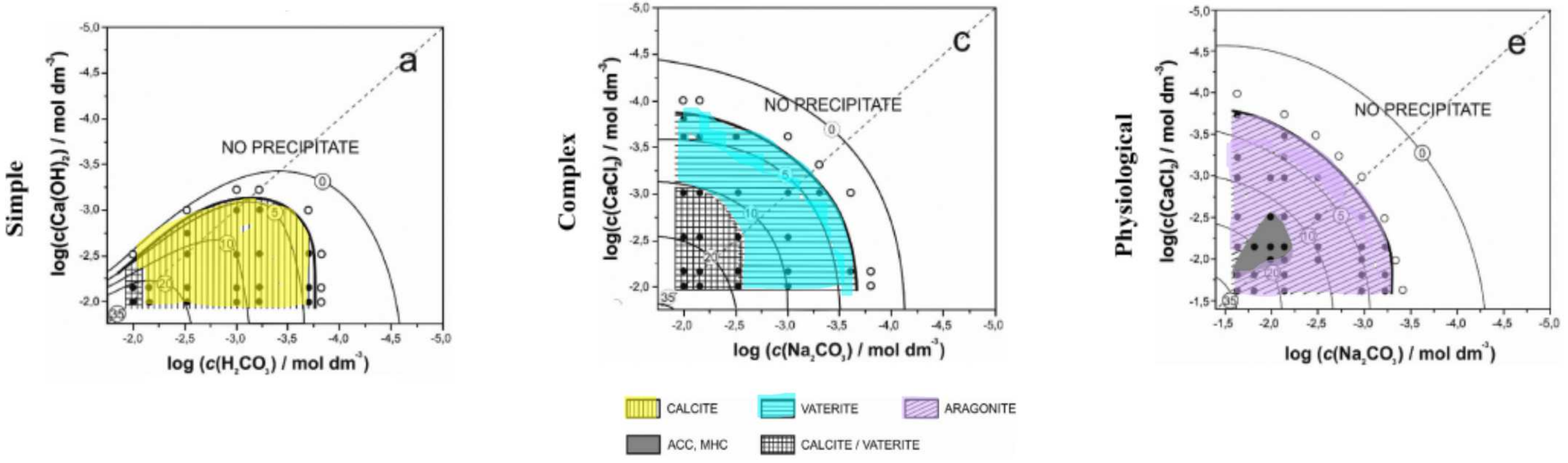
ABSTRACT: The aim of this study is to contribute to understanding the mechanisms underlying the formation of biologically relevant minerals by comparing the properties of solid phases formed in calcium phosphate (CaP) or calcium carbonate ($CaCO_3$) precipitation systems, at defined initial experimental conditions: supersaturation, constituent ions ratio, ionic strength, and/or presence of relevant inorganic ions. Thus, three systems of different chemical complexities were investigated: (a) system containing constituent ions, (b) system containing additional co-ions, and (c) system with higher ionic strength and addition of Mg^{2+}. The respective precipitation diagrams were constructed, and supersaturation domains of different CaP and $CaCO_3$ solid phases formation were identified. The obtained results may have implications not only for biomineralization and geochemistry, but also for materials science in general.

I. Buljan Meić, J. Kontrec, D. Domazet Jurašin, B. Njegić Džakula, L. Štajner, D. M. Lyons, M. Dutour Sikirić, D. Kralj: Comparative Study of Calcium Carbonates and Calcium Phosphates Precipitation in Model Systems Mimicking the Inorganic Environment for Biomineralization, Crystal Growth and Design 2017, 17, 1103−1117.

Model Systems Mimicking the Inorganic Environment for $CaCO_3$ Biomineralization

Biomineralization
Predominant role of small and large **organic molecules** (soluble and insoluble)
Role of **inorganic** ions typically **not considered**

Question:
How the **initial supersaturation** influence the polymorphic composition and **morphology** of $CaCO_3$?
How the **constituent ions ratio** influence the **morphology** of $CaCO_3$?
How the **ionic strength** and presence of relevant **inorganic components** influence the **polymorphism** and the **morphology** of $CaCO_3$?



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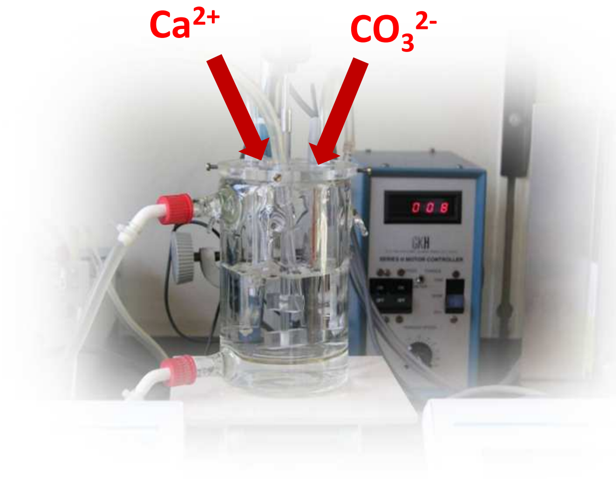
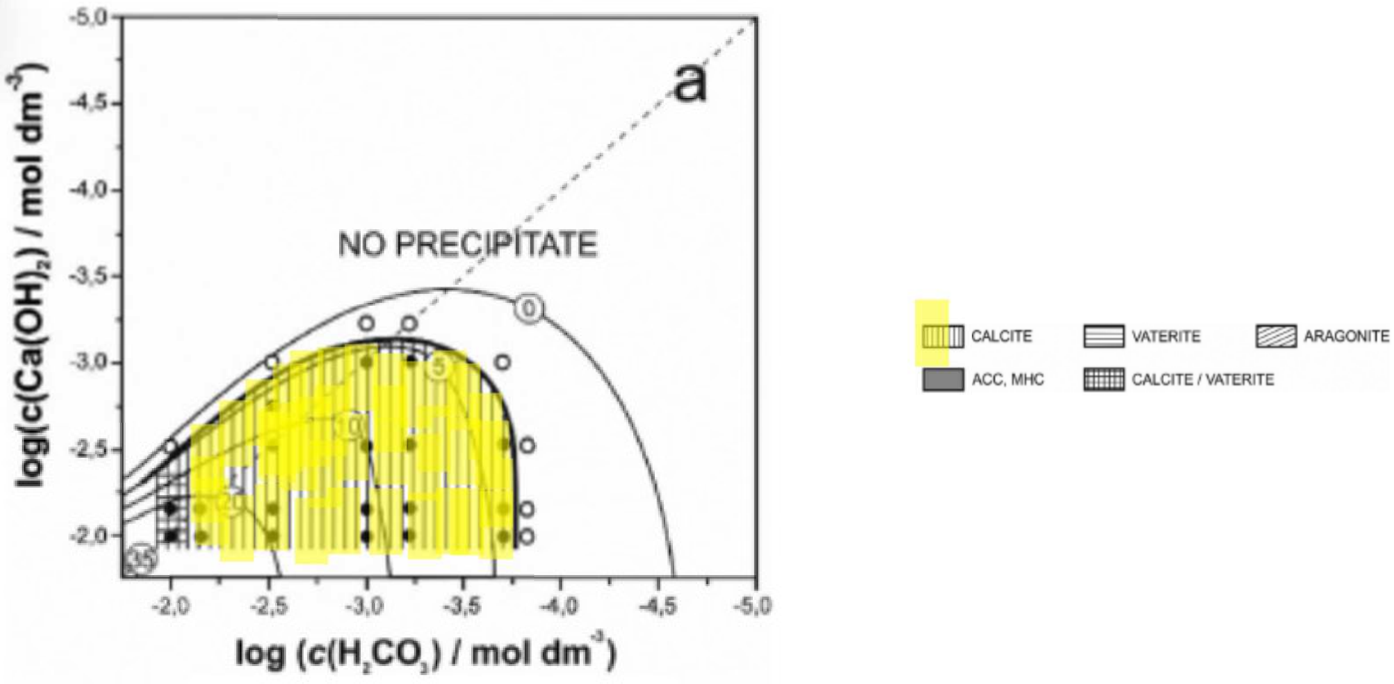


"Simple" $CaCO_3$ precipitation system
$Ca(OH)_2$ - H_2CO_3

(Constituent ions + H_2O: Ca^{2+}, CO_3^{2-}, H^+, OH^-)
$Ca(OH)_2(aq) + H_2CO_3(aq) \leftrightarrows CaCO_3\ (s) + H^+ + OH^-$

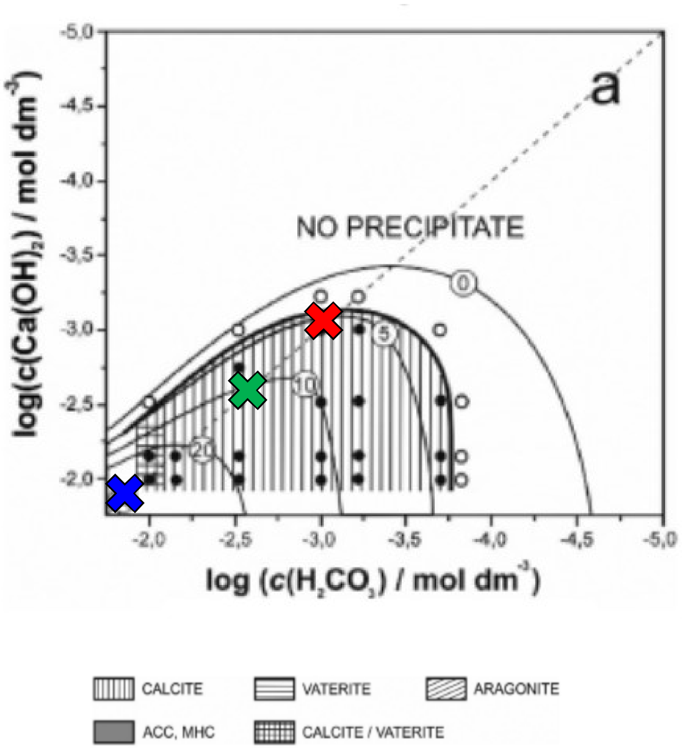
$0 < (S-1)_i < 35$
$\theta = 25$ °C, $t = 60$ min



Why Simple?
Isergones are strongly bending (ion pair formation)
Two domains (calcite, calcite + vaterite)

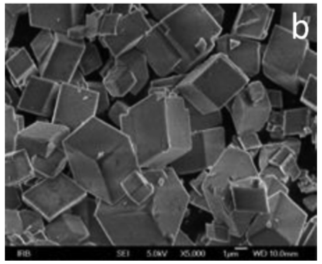
Simple system: role of **supersaturation** in equimolar systems of reactants

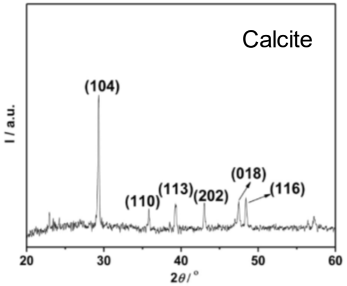
$Ca(OH)_2 : H_2CO_3 = 1 : 1$



$(S\text{-}1)_i = 5$ ✖

polydispersed, irregular rhombohedrons

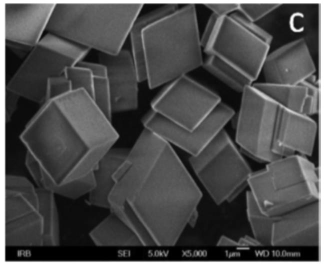


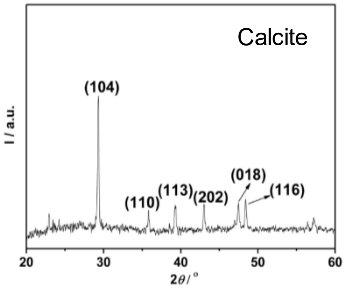


Low supersaturation domain, slow heterogeneous nucleation irregular particles

$(S\text{-}1)_i = 10$ ✖

monodispersed, regular rhombohedrons

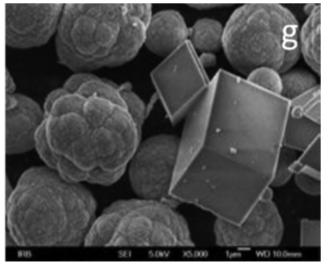


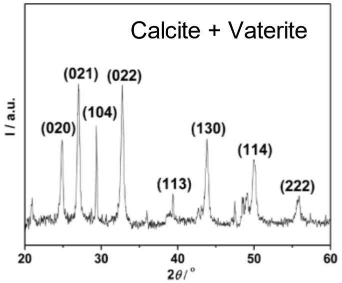


medium supersaturation domain, faster heterogeneous nucleation, regular size distribution and shape

$(S\text{-}1)_i = 30$ ✖

polydispersed calcite rhombohedrons and spherulitic vaterite

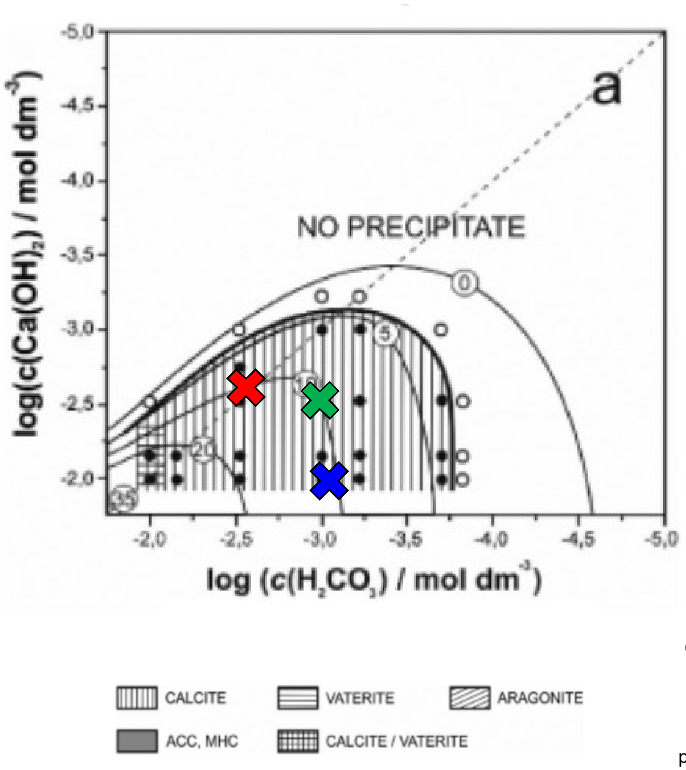




high supersaturation domain, fast homogeneous nucleation, unstable polymorphs, transformation into stable

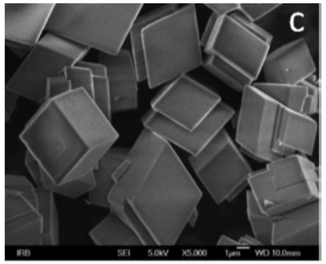
Simple system: role of **constituent ions ratio**

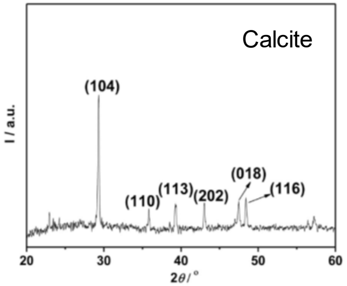
$(S-1)_i = 10$



$Ca(OH)_2 : H_2CO_3$
1 : 1

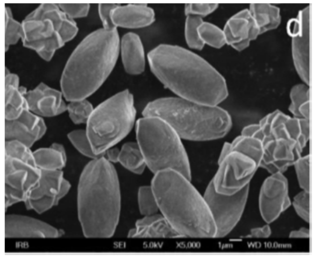
monodispersed, regular calcite rhombohedrons

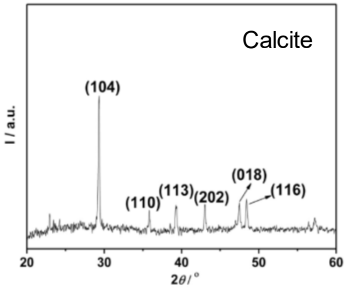




$Ca(OH)_2 : H_2CO_3$
8 : 1

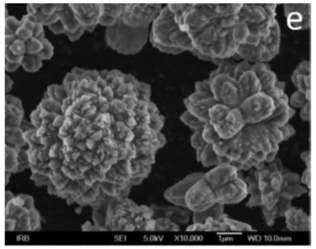
predominantly calcite scalenohedrons

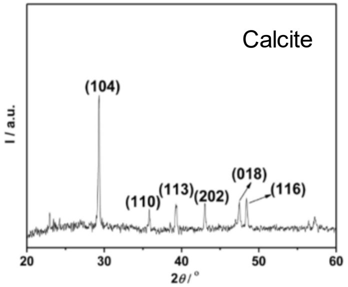




$Ca(OH)_2 : H_2CO_3$
10 : 1

predominantly spherulitic calcite aggregates





scalenohedral and spherulitic calcite particles formed at higher alkalinity (pH ≈ 12) – result of predominant adsorption of hydroxyl ions

"Complex" $CaCO_3$ precipitation system

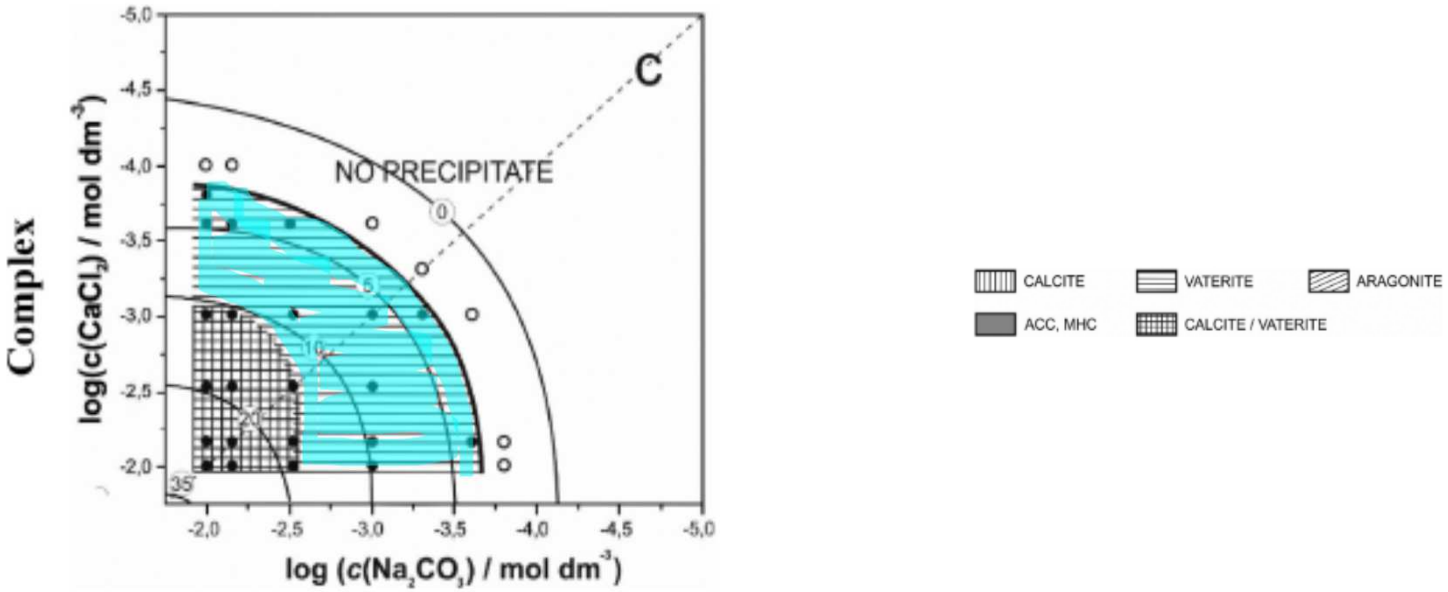
$CaCl_2$ - Na_2CO_3

(Constituent ions + co-ions + H_2O)

$$CaCl_2(aq) + Na_2CO_3(aq) \leftrightarrows CaCO_3\ (s) + 2Na^+ + 2Cl^- + H^+ + OH^-$$

$0 < (S-1)_i < 35$

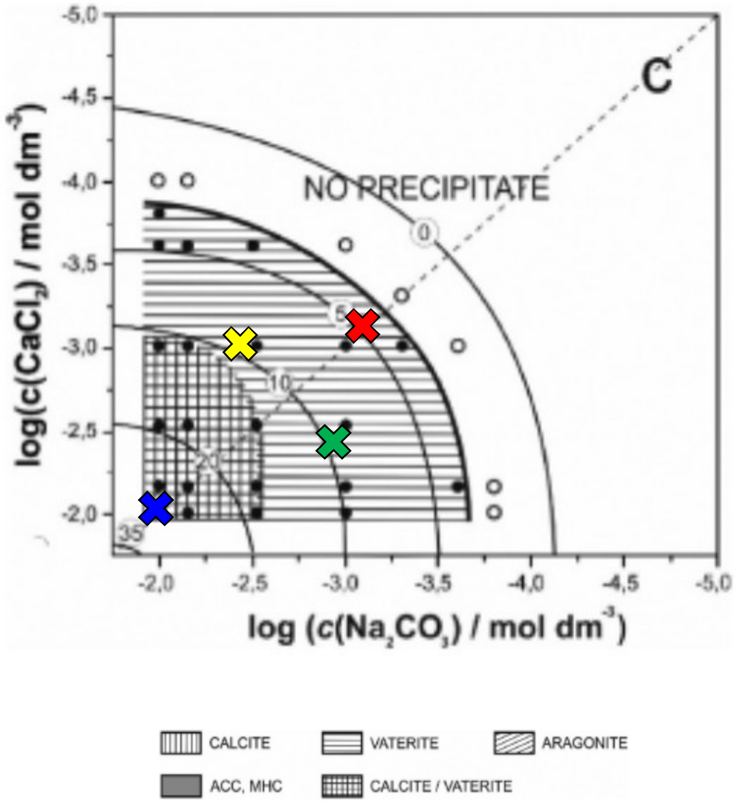
$\theta = 25\ °C$, $t = 60$ min



Why complex system?
Isergones are bended (ion pair formation)
Two domains (vaterite, calcite + vaterite)

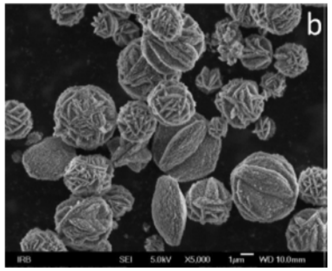
Complex system: role of supersaturation and ion ratio

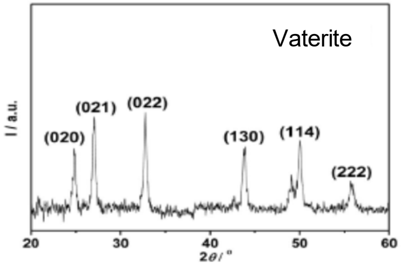
$CaCl_2 : Na_2CO_3 = 1 : 1$



$(S-1)_i = 5$ ✖

lens-like vaterite aggregates

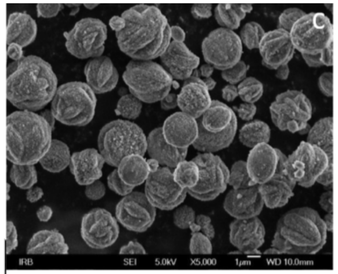


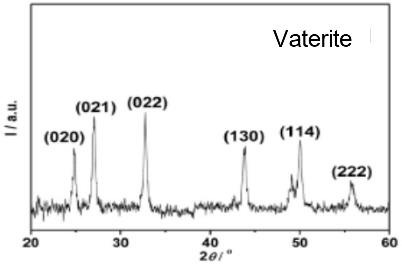


Low supersaturation domain, slow heterogeneous nucleation metastable polymorph

$(S-1)_i = 10$ ✖

Excess calcium
lens-like vaterite aggregates

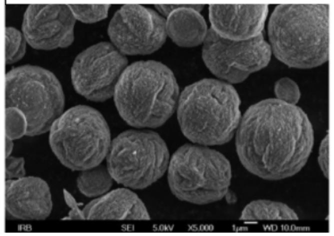


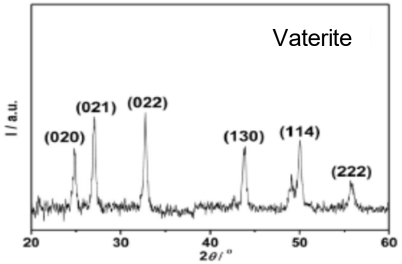


medium supersaturation domain, faster heterogeneous nucleation, **high calcium**

$(S-1)_i = 10$ ✖

Excess carbonate
spherical vaterite aggregates

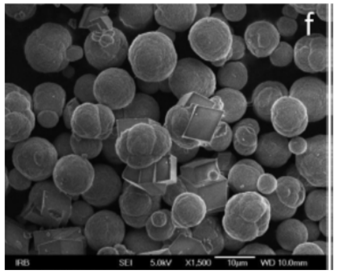


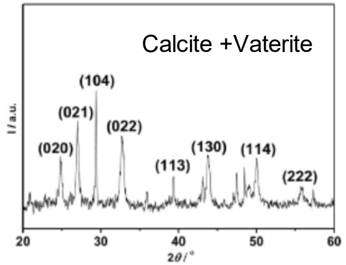


medium supersaturation domain, faster heterogeneous nucleation, **high carbonate**

$(S-1)_i = 30$ ✖

calcite rhombohedrons
and
spherulitic vaterite





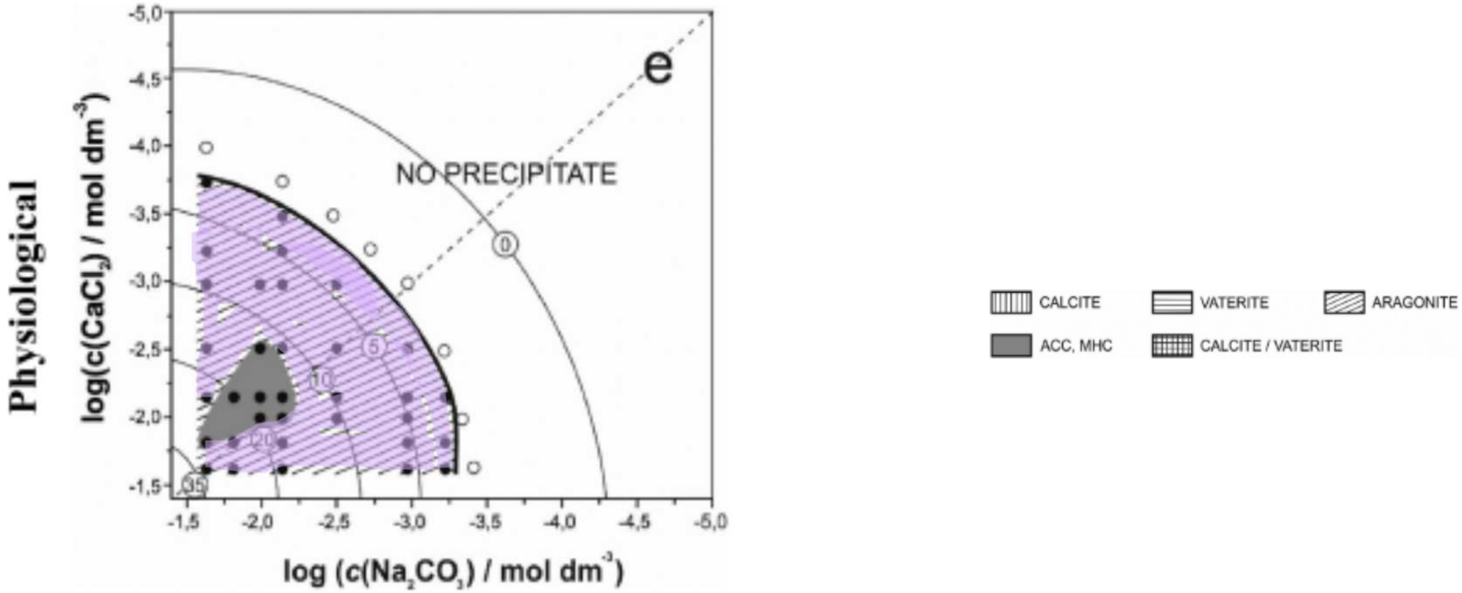
high supersaturation domain, fast homogeneous nucleation, unstable polymorphs, transformation into stable

"Physiological" $CaCO_3$ precipitation system

$CaCl_2$ - Na_2CO_3 – 0.15 M NaCl – $MgCl_2$
(Constituent ions + co-ions + magnesium ions + H_2O)

$CaCl_2(aq) + Na_2CO_3(aq) + NaCl(aq) + MgCl_2(aq) \leftrightarrows CaCO_3\ (s) + 2Na^+ + 2Cl^- + H^+ + OH^-$

$0 < (S-1)_i < 35$
$\theta = 25\ °C,\ t = 60\ min$

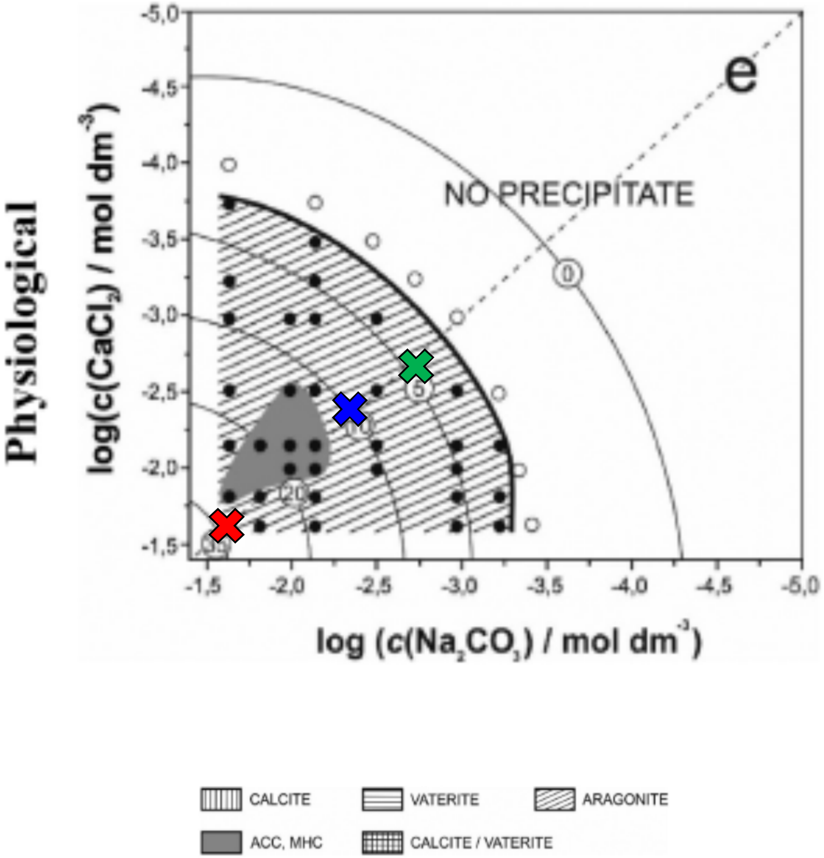


Why physiological system?
Lower supersaturation at comparable Na_2CO_3 *and* $CaCl_2$ *concentrations (increased ionic strength, Mg-ion pairs)*
Two domains (aragonite, aragonite + monohydrocalcite MHC)
Metastable zone broadening

Physiological system: role of supersaturation and Mg^{2+} ions addition (1)

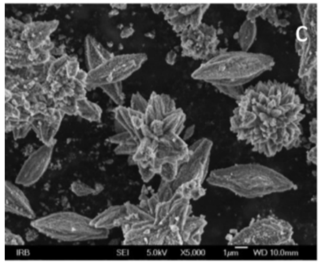
$CaCl_2 : Na_2CO_3 = 1 : 1$

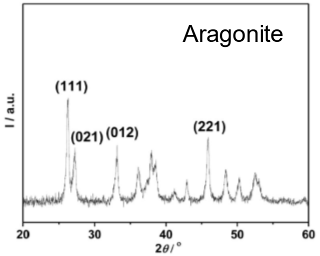
$CaCl_2 : MgCl_2 = 1 : 2$



$(S-1)_i = 5$ ✖

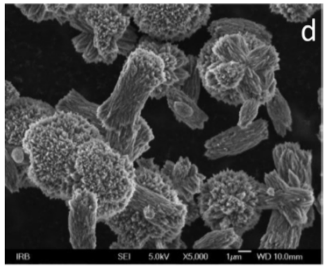
lens-like structures

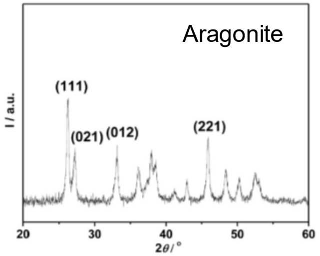




$(S-1)_i = 10$ ✖

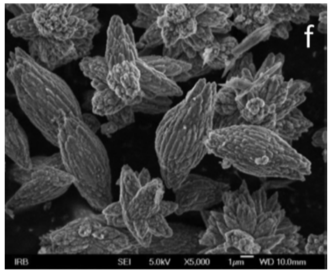
wheat sheaves forms

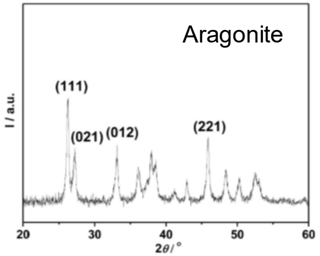




$(S-1)_i = 34$ ✖

lens-like structures

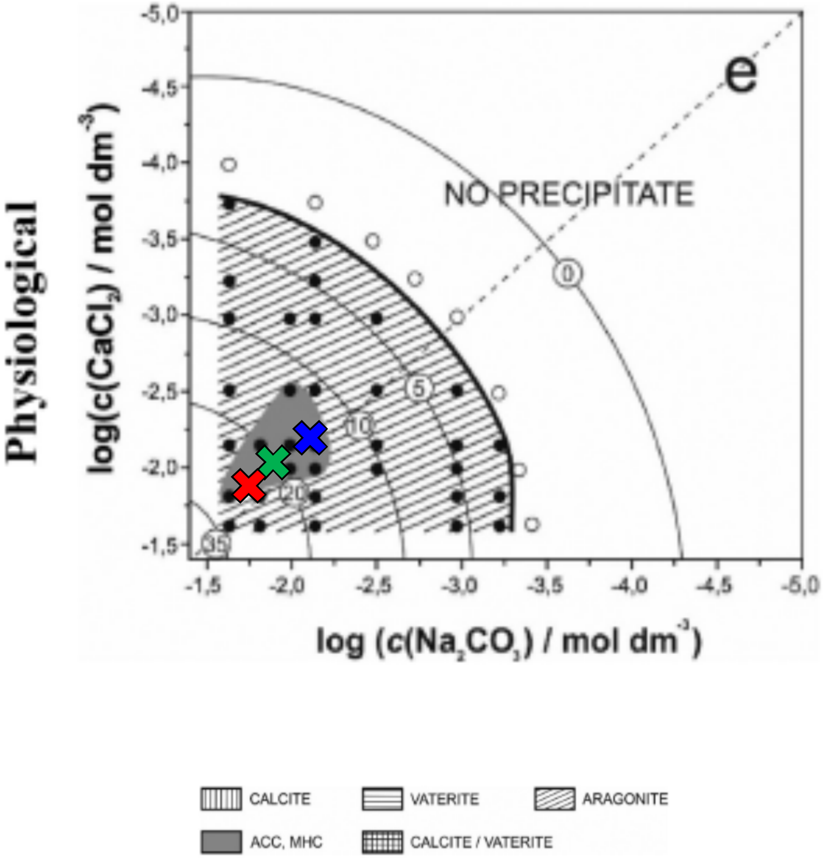




No difference on morphology with changing constituent ion ratio
Different mechanism of aragonite formation at low and high supersaturation

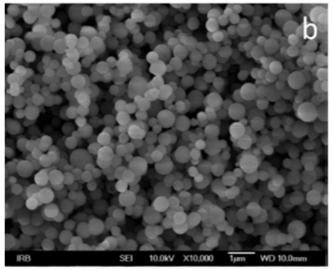
Physiological system: role of supersaturation and Mg^{2+} ions addition (2)

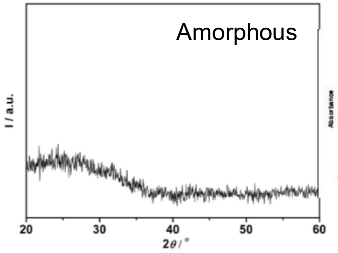
$CaCl_2 : MgCl_2 = 1 : 2$

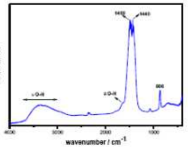


$(S-1)_i = 20$ ✖

ACC ($CaCO_3 \times n\ H_2O$)
smooth regular, monodispersed
spherical agglomerates

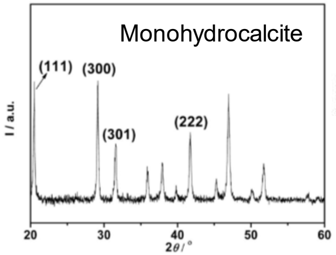


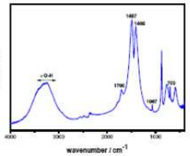




$(S-1)_i = 13$ ✖

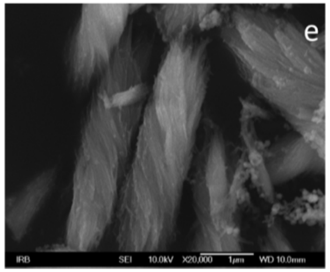
MHC ($CaCO_3$ x H_2O) (low S)
compact agglomerates of primary
particles

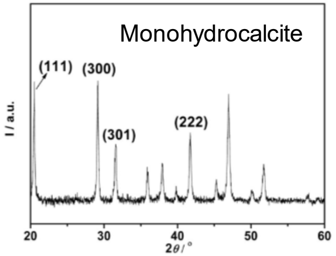


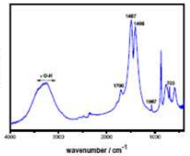


$(S-1)_i = 24$ ✖

MHC ($CaCO_3$ x H_2O) (high S)
elongated, intimately enfolded
particles





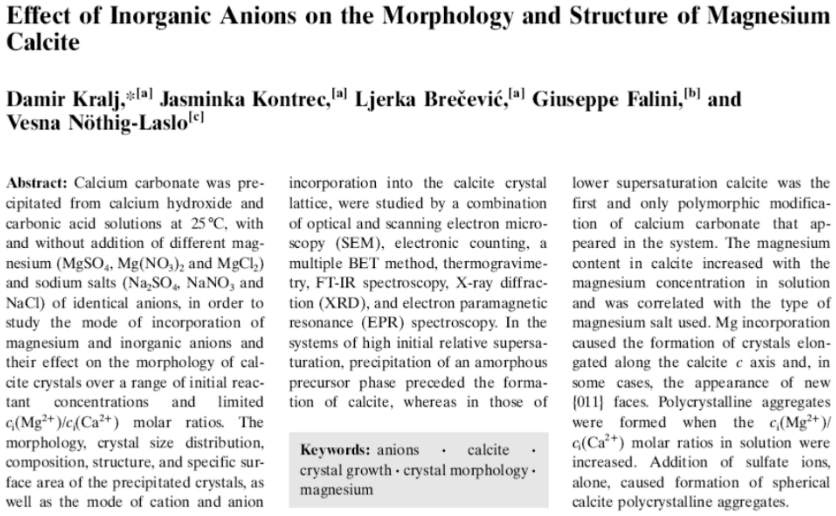


Stabilization of amorphous calcium carbonate after 1 hour of aging by Mg^{2+} presence!!
Formation of monohydrocalcite stabilized by Mg^{2+}. In literature at low temperature!!
Monohydrocalcite in literature: regular, smooth spherical particles

Model system 2

Influence of inorganic additives (Mg^{2+}, Cl^-, NO_3^-, SO_4^{2-}) on properties of calcite

(**Simple system**)



Effect of Inorganic Anions on the Morphology and Structure of Magnesium Calcite

Damir Kralj,*[a] Jasminka Kontrec,[a] Ljerka Brečević,[a] Giuseppe Falini,[b] and Vesna Nöthig-Laslo[c]

Abstract: Calcium carbonate was precipitated from calcium hydroxide and carbonic acid solutions at 25 °C, with and without addition of different magnesium ($MgSO_4$, $Mg(NO_3)_2$ and $MgCl_2$) and sodium salts (Na_2SO_4, $NaNO_3$ and NaCl) of identical anions, in order to study the mode of incorporation of magnesium and inorganic anions and their effect on the morphology of calcite crystals over a range of initial reactant concentrations and limited $c_i(Mg^{2+})/c_i(Ca^{2+})$ molar ratios. The morphology, crystal size distribution, composition, structure, and specific surface area of the precipitated crystals, as well as the mode of cation and anion incorporation into the calcite crystal lattice, were studied by a combination of optical and scanning electron microscopy (SEM), electronic counting, a multiple BET method, thermogravimetry, FT-IR spectroscopy, X-ray diffraction (XRD), and electron paramagnetic resonance (EPR) spectroscopy. In the systems of high initial relative supersaturation, precipitation of an amorphous precursor phase preceded the formation of calcite, whereas in those of lower supersaturation calcite was the first and only polymorphic modification of calcium carbonate that appeared in the system. The magnesium content in calcite increased with the magnesium concentration in solution and was correlated with the type of magnesium salt used. Mg incorporation caused the formation of crystals elongated along the calcite *c* axis and, in some cases, the appearance of new {011} faces. Polycrystalline aggregates were formed when the $c_i(Mg^{2+})/c_i(Ca^{2+})$ molar ratios in solution were increased. Addition of sulfate ions, alone, caused formation of spherical calcite polycrystalline aggregates.

Keywords: anions · calcite · crystal growth · crystal morphology · magnesium

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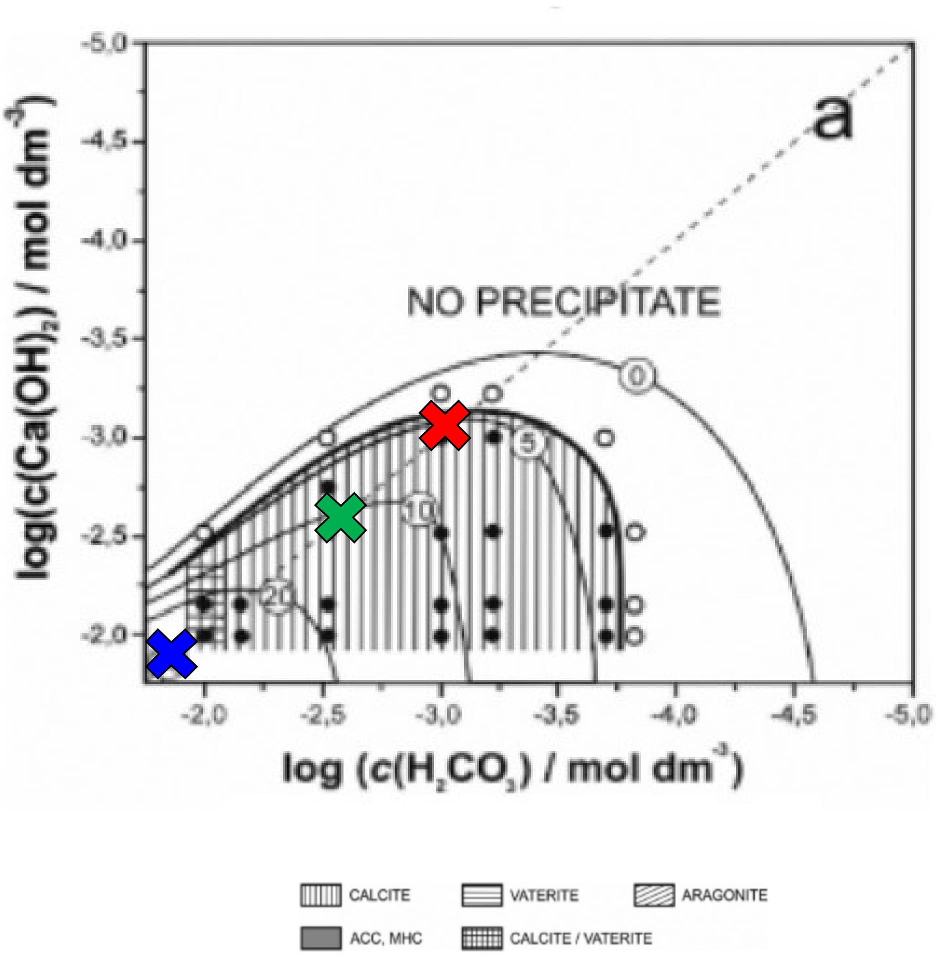
Facts about Mg^{2+} and $CaCO_3$ precipitation

- Mg^{2+} very often appears simultaneously with Ca^{2+} (seawater ≈ extrapallial fluids: Mg/Ca ≈ 5/1)
- Mg^{2+} effective inhibitor of $CaCO_3$ (calcite) nucleation and growth
- Mg^{2+} initiate precipitation of aragonite
- Mg^{2+} readily incorporate into calcite crystal lattice (Mg calcite!!)
- Dolomite, $CaMg(CO_3)_2$, almost impossible to produce at laboratory conditions

Simple system: role of **Mg^{2+}**

$Ca(OH)_2$ - H_2CO_3 – ($MgSO_4$, $MgCl_2$, $Mg(NO_3)_2$)



✖ (S-1)$_i$ = 5

Low supersaturation domain, slow heterogeneous nucleation
irregular **calcite rhombohedrons**

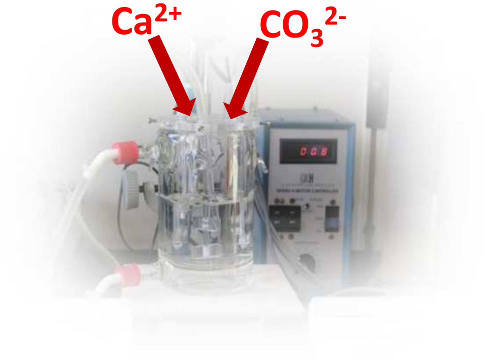
✖ (S-1)$_i$ = 10

medium supersaturation domain, faster heterogeneous nucleation, regular size distribution and **calcite rhombohedrons**

✖ (S-1)$_i$ = 30

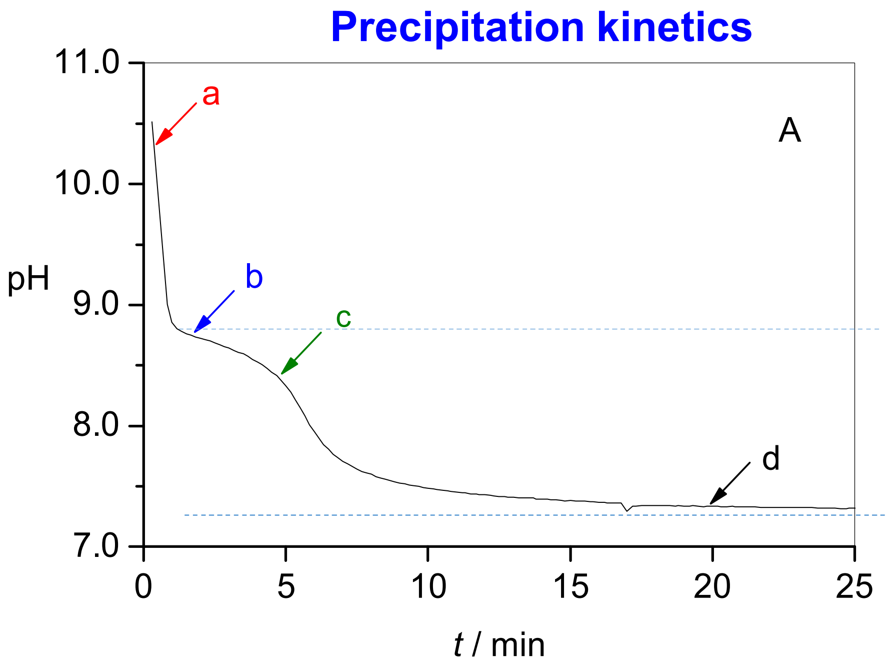
high supersaturation domain, instantaneous homogeneous nucleation, **precursor phases** formation, transformation into stable polymorphs

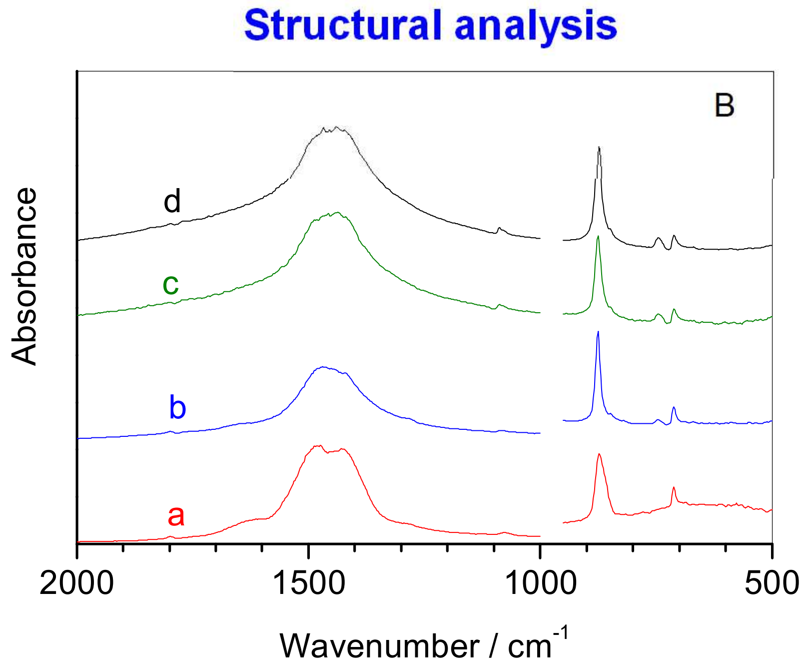
High initial supersaturation
Initial formation of precursors (metastable polymorphs or amorphous phases) → calcite



Solution mediated transformation
(a-b) Nucleation and growth of calcite, vaterite and amorphous $CaCO_3$
(b-c) Dissolution of amorphous $CaCO_3$ and vaterite and growth of calcite
(c-d) Growth of calcite

Amorphous $CaCO_3$ (a-b) → Vaterite (b-c) → Calcite (c-d)


Precipitation kinetics

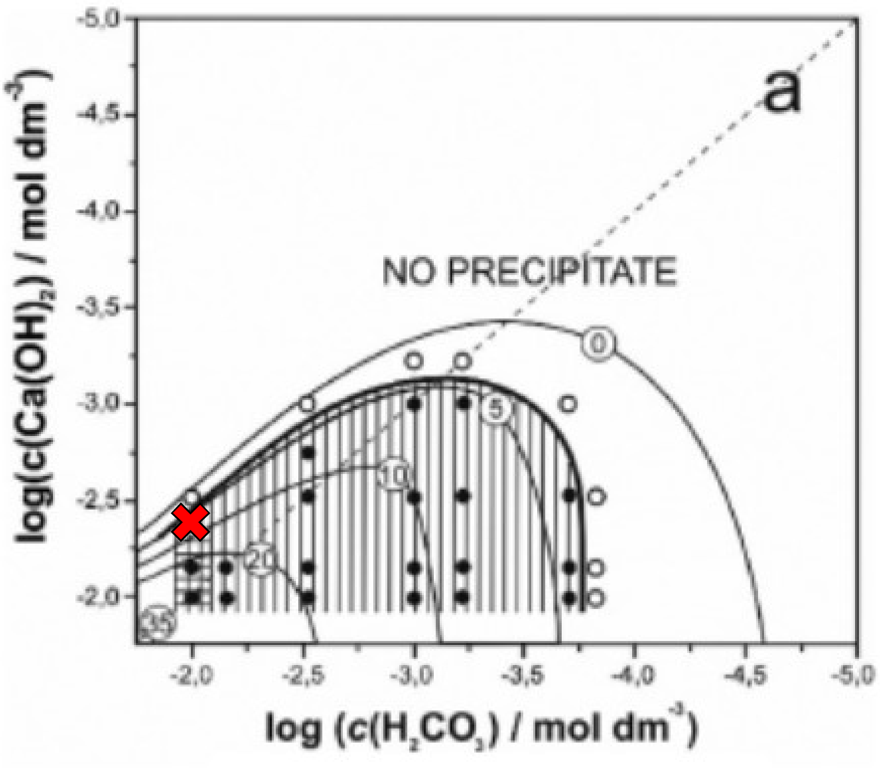

Structural analysis

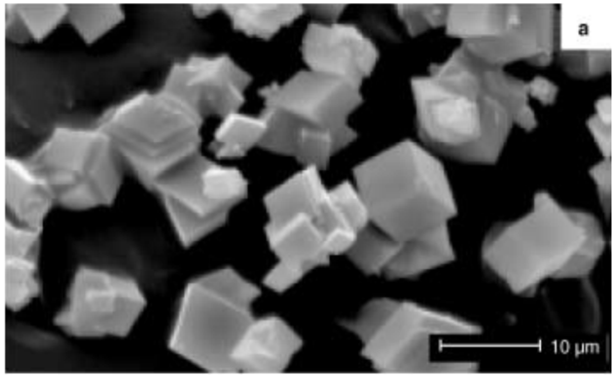
Model system for investigation of the role of magnesium salts

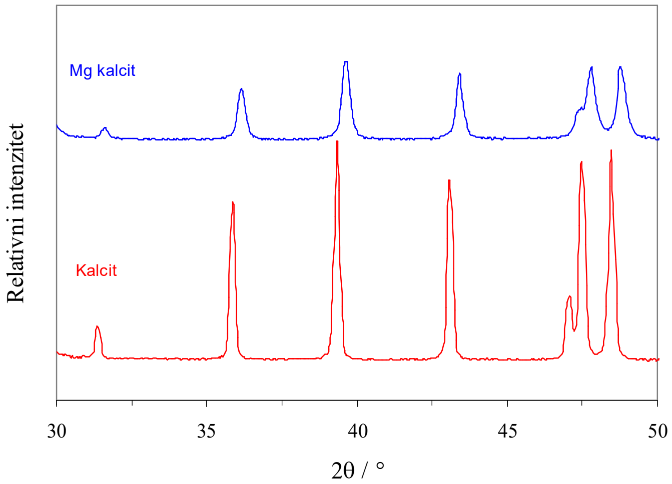
$c_i(Ca^{2+})$ = 0.005 mol dm^{-3}, $c_i(CO_3^{2-})$ = 0.01 mol dm^{-3}, t = 20 min

0.002 mol dm^{-3} < $c_i(MgCl_2, MgSO_4, Mg(NO_3)_2)$ < 0.010 mol dm^{-3}

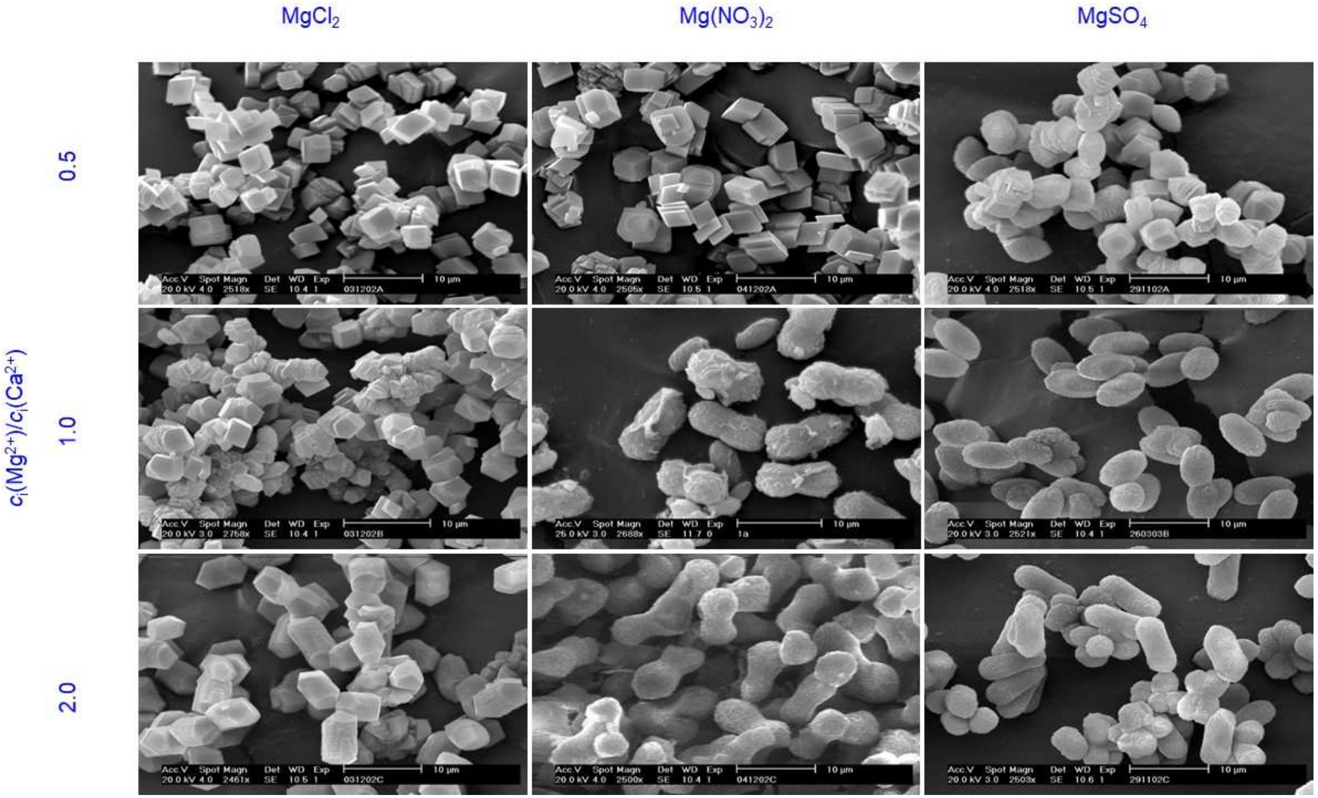
- Absence of Mg^{2+} - rhombohedral calcite, stable {104} faces
- No precursor - slow precipitation – crystal growth dominant over nucleation
- pH < 9.45 (no $Mg(OH)_2$ coprecipitation)







Morphology



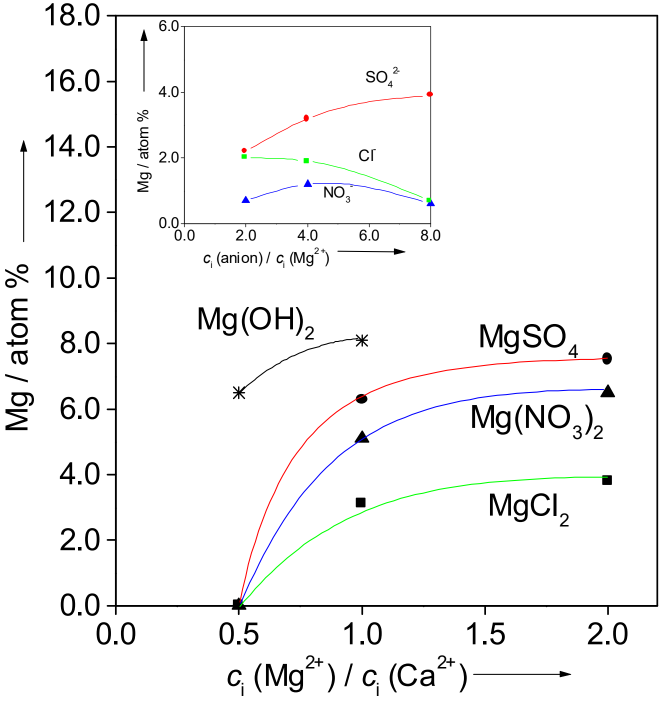
$MgCl_2$: rhombohedral {104} faces + {011} faces → {104} face-capped, elongated along c axis, no aggregation

$Mg(NO_3)_2$: rhombohedral calcite change to elongated dumbbell-shaped crystals, no {104} face-capping

$MgSO_4$: {104} face-capped at low concentration → at high concentration aggregates elongated along the c-axis

Chemical composition

Mg^{2+} incorporation into calcite crystal lattice



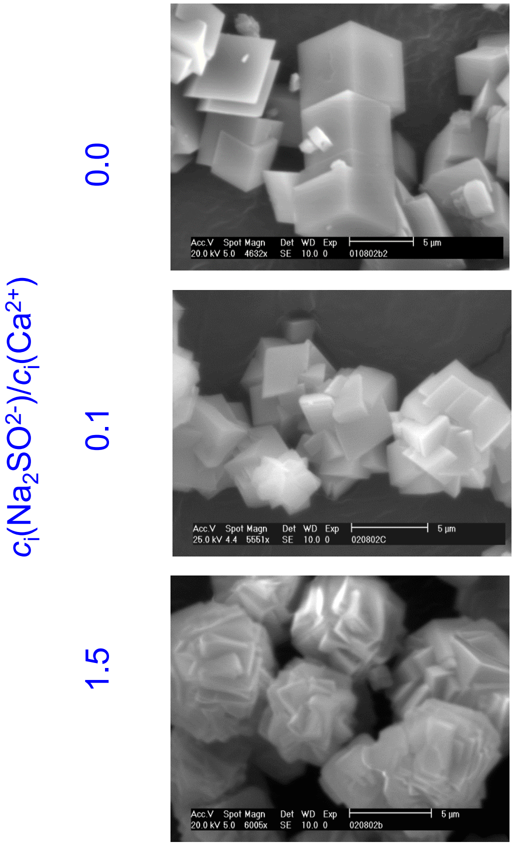
Use of $Mg(OH)_2$ limited with the solubility product, K_{sp} = 11.15, (not above pH = 9.3)

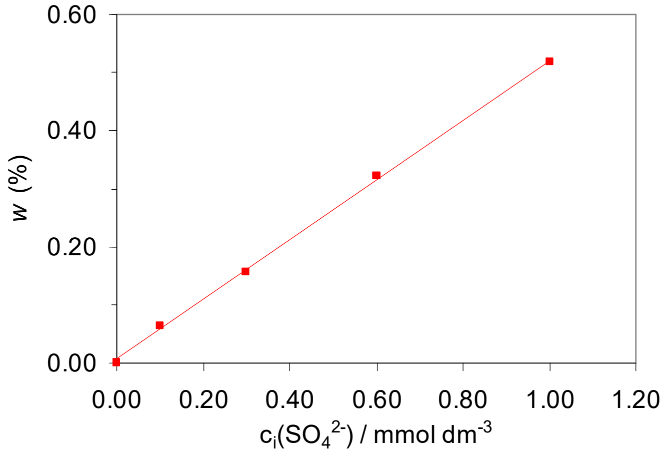
Extent of incorporation depends on Mg accompany anion

Isomorphic substitution consistent with observed morphological changes

Chemical composition and morphology

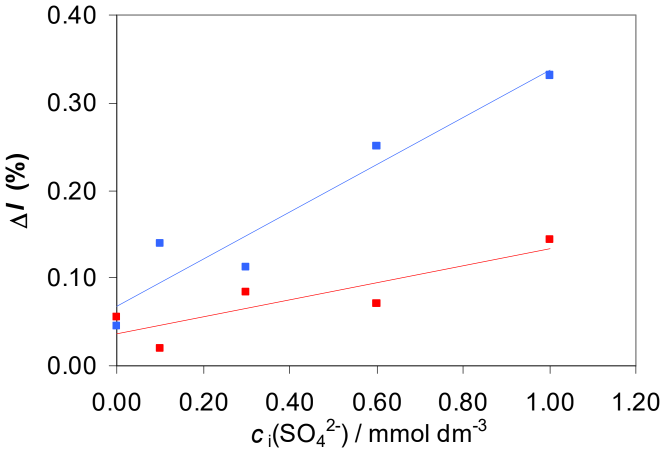
SO$_4^{2-}$ incorporation into calcite crystal lattice





Chemical analysis

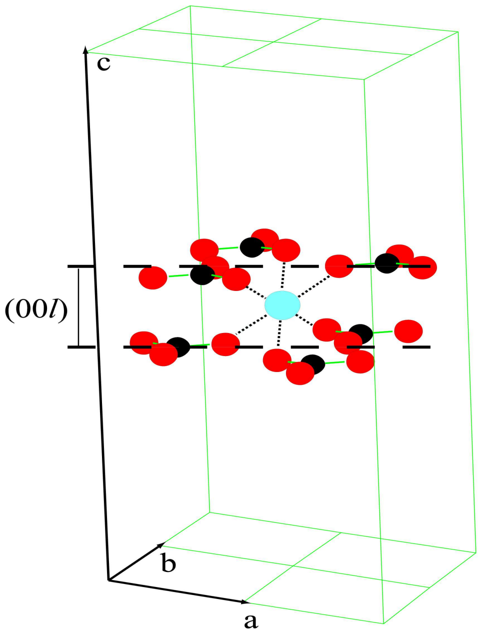
SO$_4^{2-}$ content in calcite

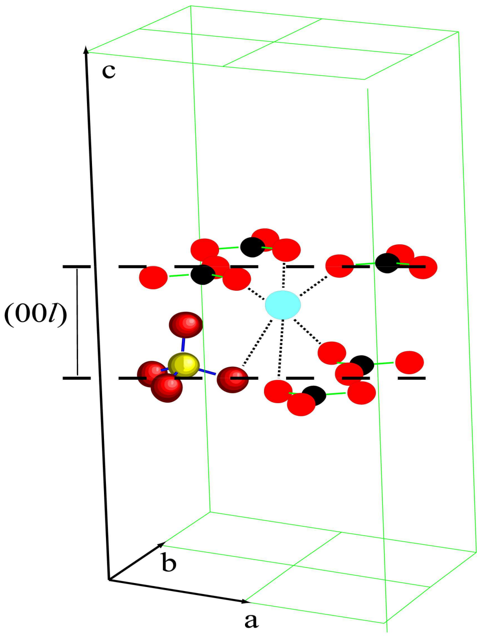


P-XRD structural analysis

Relative change of crystal lattice parameters (a, c)

Model of SO_4^{2-} substitution into calcite

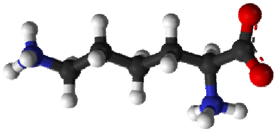


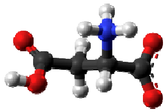


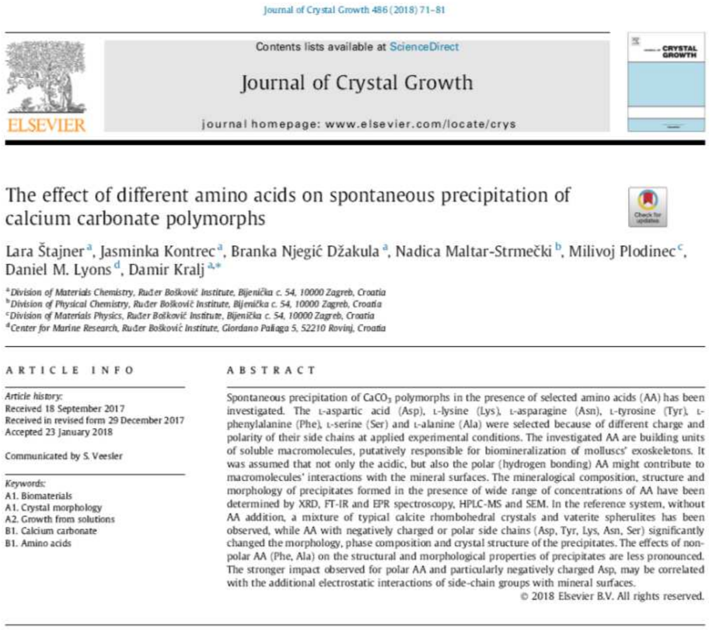
Model system 3

Influence of small organic molecules (amino acids) on properties of calcite

(Complex system)







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The effect of different amino acids on spontaneous precipitation of calcium carbonate polymorphs

Lara Štajner[a], Jasminka Kontrec[a], Branka Njegić Džakula[a], Nadica Maltar-Strmečki[b], Milivoj Plodinec[c], Daniel M. Lyons[d], Damir Kralj[a,*]

[a] *Division of Materials Chemistry, Ruđer Bošković Institute, Bijenička c. 54, 10000 Zagreb, Croatia*
[b] *Division of Physical Chemistry, Ruđer Bošković Institute, Bijenička c. 54, 10000 Zagreb, Croatia*
[c] *Division of Materials Physics, Ruđer Bošković Institute, Bijenička c. 54, 10000 Zagreb, Croatia*
[d] *Center for Marine Research, Ruđer Bošković Institute, Giordano Paliaga 5, 52210 Rovinj, Croatia*

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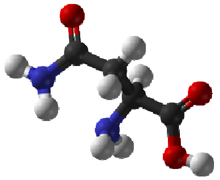
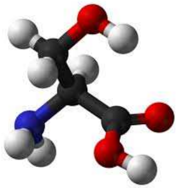
ABSTRACT

Spontaneous precipitation of $CaCO_3$ polymorphs in the presence of selected amino acids (AA) has been investigated. The L-aspartic acid (Asp), L-lysine (Lys), L-asparagine (Asn), L-tyrosine (Tyr), L-phenylalanine (Phe), L-serine (Ser) and L-alanine (Ala) were selected because of different charge and polarity of their side chains at applied experimental conditions. The investigated AA are building units of soluble macromolecules, putatively responsible for biomineralization of molluscs' exoskeletons. It was assumed that not only the acidic, but also the polar (hydrogen bonding) AA might contribute to macromolecules' interactions with the mineral surfaces. The mineralogical composition, structure and morphology of precipitates formed in the presence of wide range of concentrations of AA have been determined by XRD, FT-IR and EPR spectroscopy, HPLC-MS and SEM. In the reference system, without AA addition, a mixture of typical calcite rhombohedral crystals and vaterite spherulites has been observed, while AA with negatively charged or polar side chains (Asp, Tyr, Lys, Asn, Ser) significantly changed the morphology, phase composition and crystal structure of the precipitates. The effects of non-polar AA (Phe, Ala) on the structural and morphological properties of precipitates are less pronounced. The stronger impact observed for polar AA and particularly negatively charged Asp, may be correlated with the additional electrostatic interactions of side-chain groups with mineral surfaces.



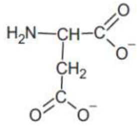


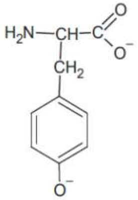
L. Štajner, J. Kontrec, B. Njegic Džakula, N. Maltar-Strmečki, M. Plodinec, D. M. Lyons, D. Kralj: The effect of different amino acids on spontaneous precipitation of calcium carbonate polymorphs, Journal of Crystal Growth 486 (2018) 71–81.

Selection of amino acids

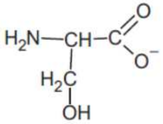
Charge, **polarity** and **chemical structure** of side chains groups

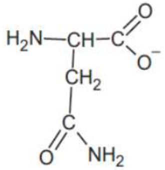
Asp, Tyr (pH = 10.7; negatively charged)

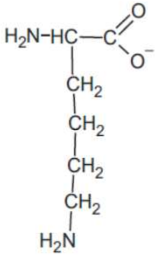




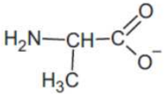
Ser, Asn, Lys, (pH = 10.7; polar, hydrogen bond donor)

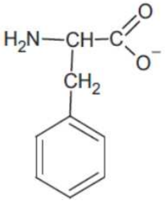


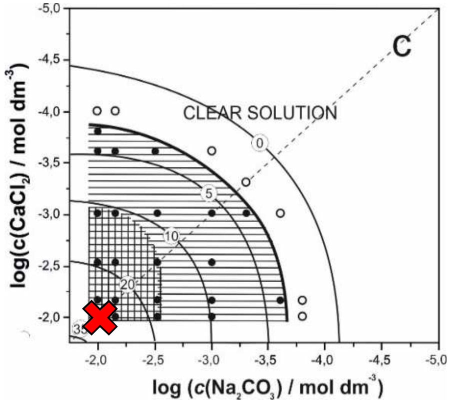




Ala, Phe (pH = 10.7; non-polar and hydrophobic)





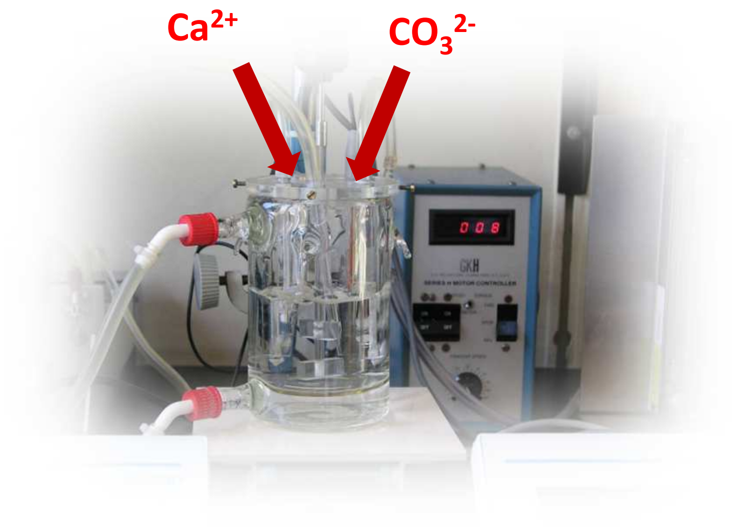


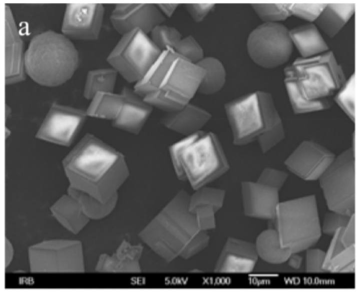
Spontaneous precipitation
(complex system)

$c_\mathrm{i}(\mathrm{Ca}^{2+}) = 0.01\ \mathrm{mol\ dm}^{-3}$
$c_\mathrm{i}(\mathrm{CO_3}^{2-}) = 0.01\ \mathrm{mol\ dm}^{-3}$

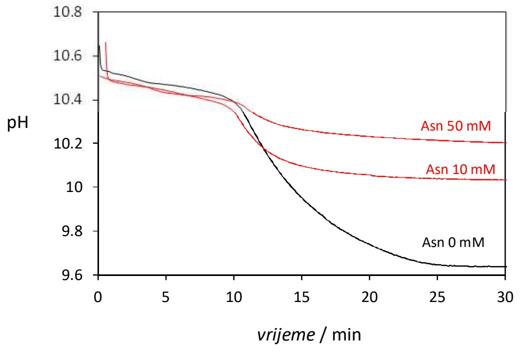
$10.0\ \mathrm{mmol\ dm}^{-3} < c_\mathrm{i}(\mathrm{AA}) < 75.0\ \mathrm{mmol\ dm}^{-3}$
Asp, Tyr, Ala, Phe, Ser, Asn, Lys

$\mathrm{pH_i} = 10.7$
$\theta = 25\ ^\circ\mathrm{C}$, $t = 30$ min

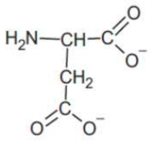




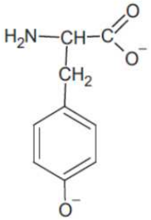
Absence of amino acids:
83% calcite + 17% vaterite



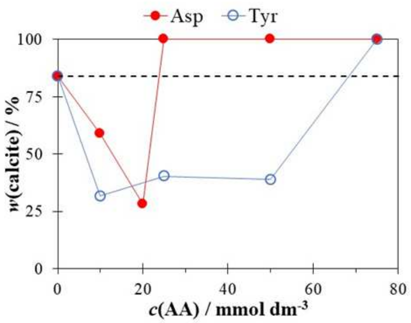
Polymorphic composition vs. AA concentration



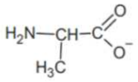
Asp



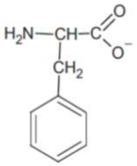
Tyr



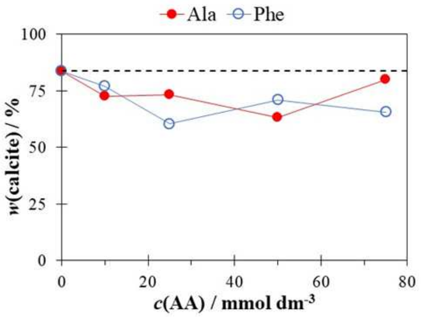
Negatively charged side chain



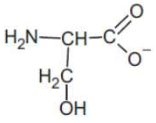
Ala



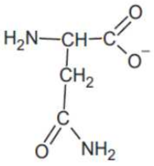
Phe



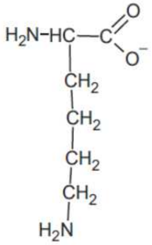
Hydrophobic side chain



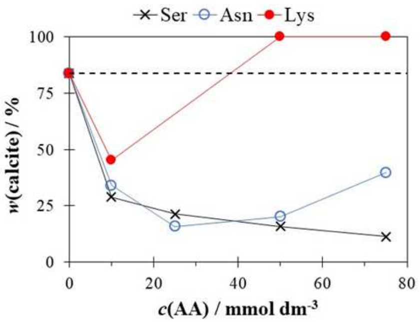
Ser



Asn

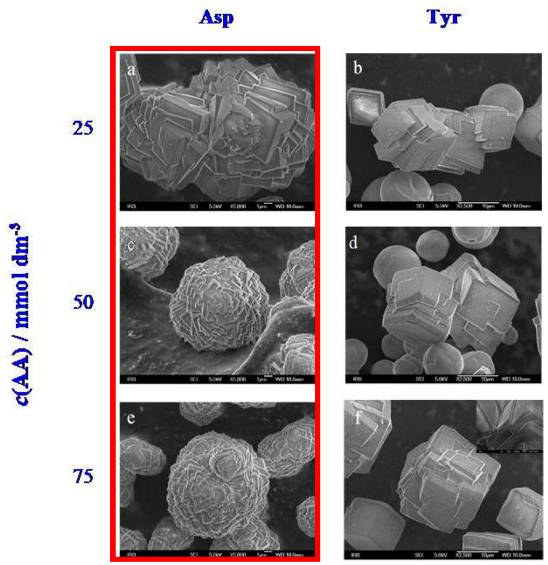


Lys

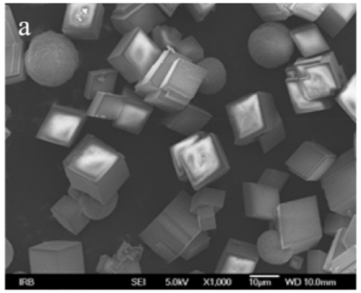


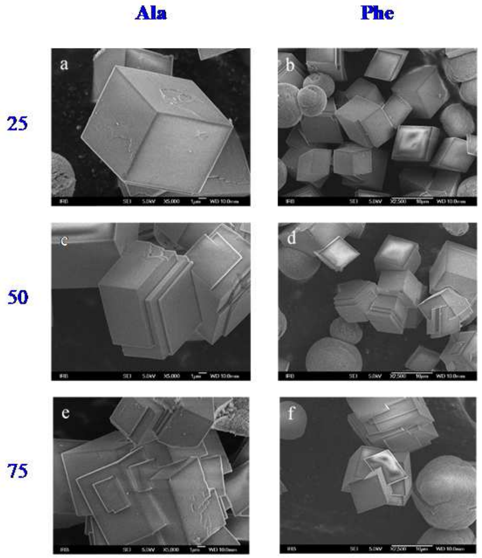
Polar side chain

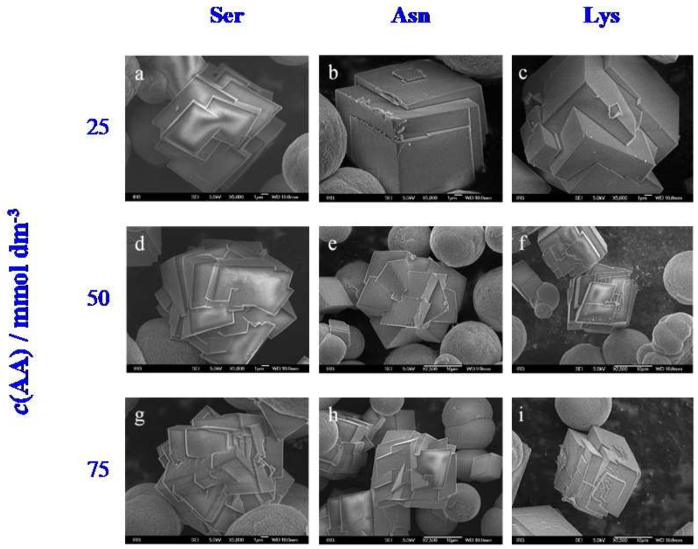
Morphology (SEM)



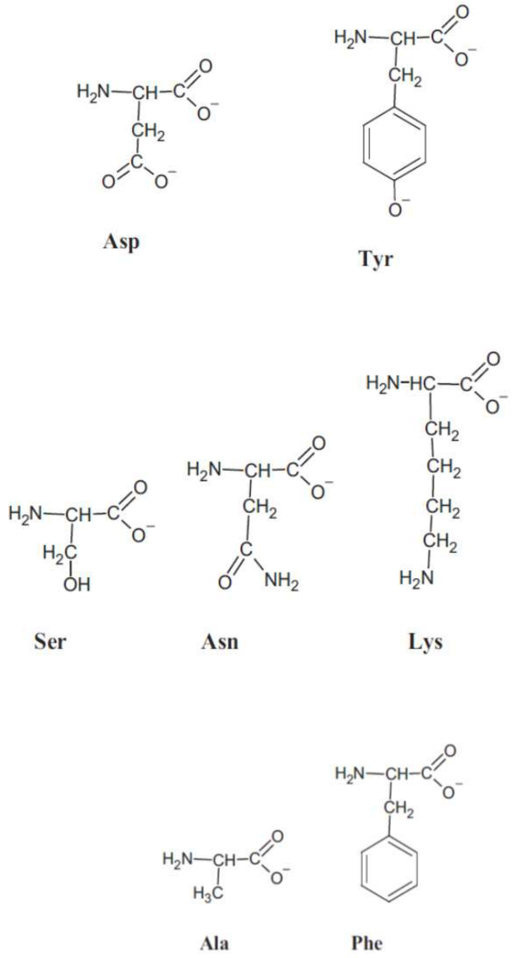
Referent

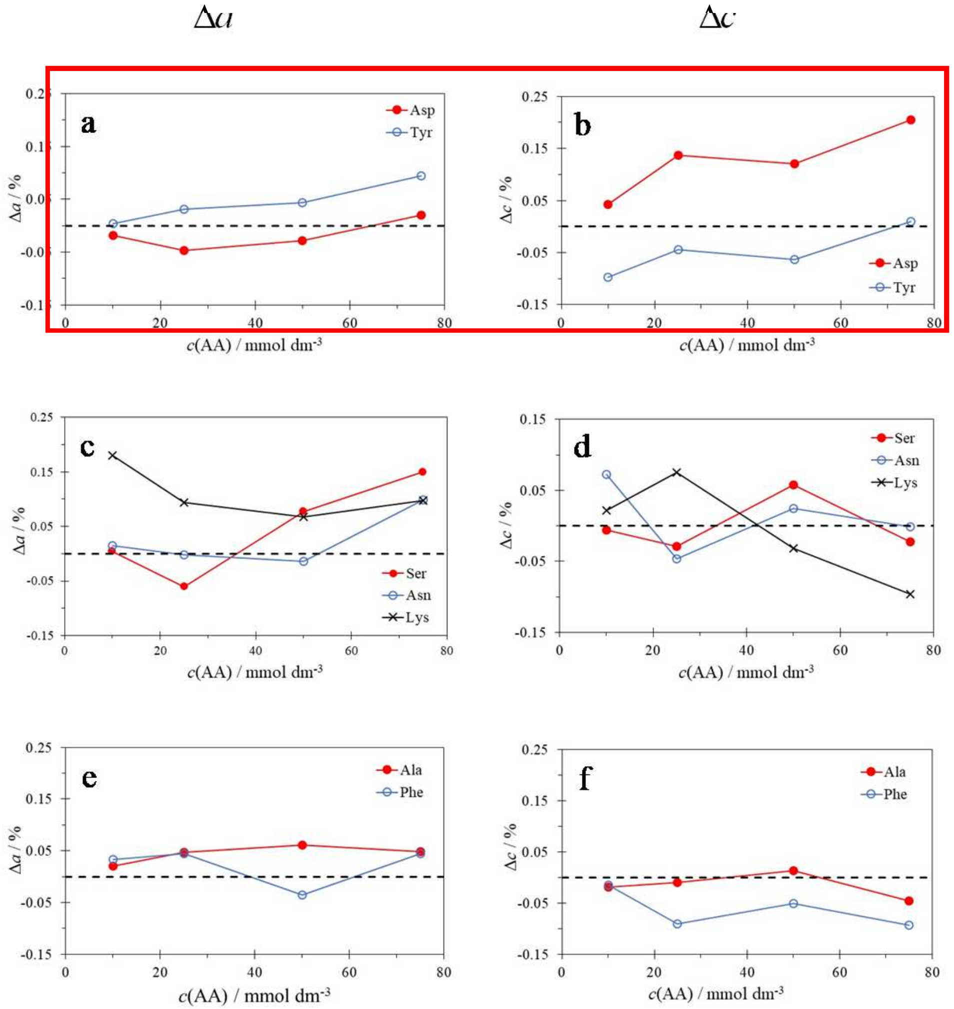




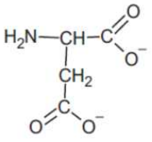


Calcite crystal lattice distortions (P-XRD)

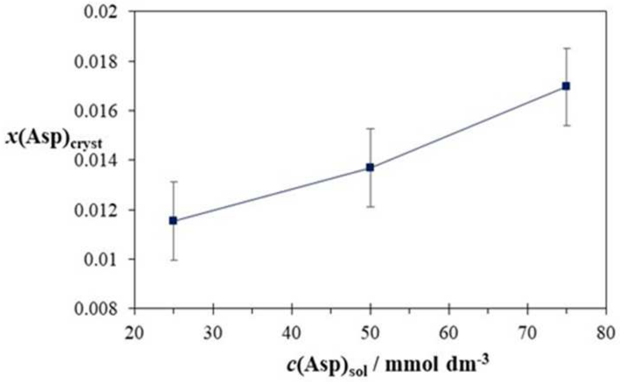


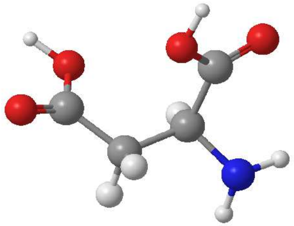


Asp incorporation into calcite lattice (chemical analysis)

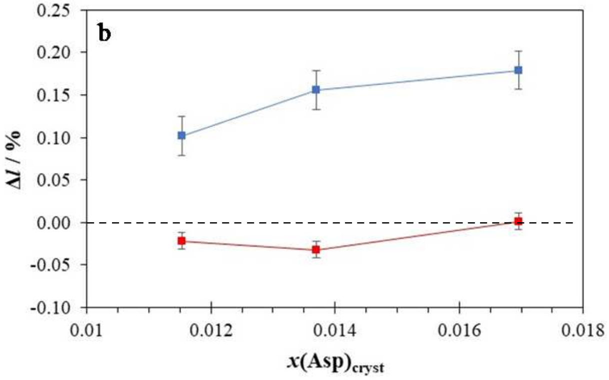


Asp



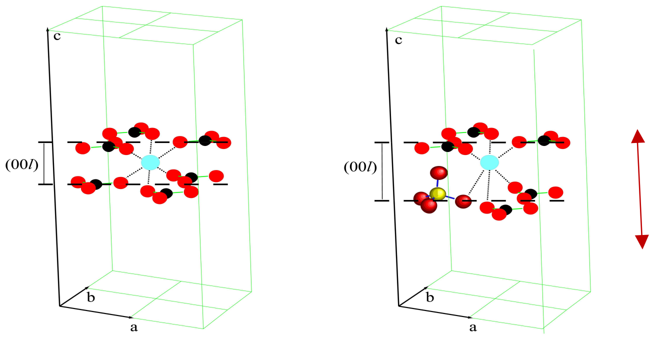


Relative changes of calcite crystal lattice (structural analysis)



Δc

Δa



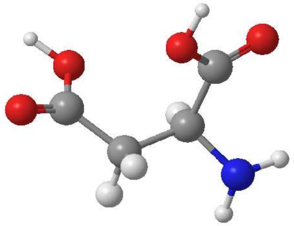
Conclusions

Non-polar amino acids (Phe, Ala) - insignificant effect on $CaCO_3$ precipitation

Charged and polar (hydrogen bonding) amino acids - (Asp, Tyr, Lys, Asn, Ser) changed the morphology, phase composition and crystal structure of the precipitates.

Asp had the **strongest effect** - significant change of calcite morphology and strong distortions of the crystal lattice.

Anisotropic distorsion of calcite ($\Delta c >> \Delta a$) - carboxylic groups on L-Asp substitute CO_3^{2-} and coordinate with Ca^{2+} ions from different layers in the crystal lattice.

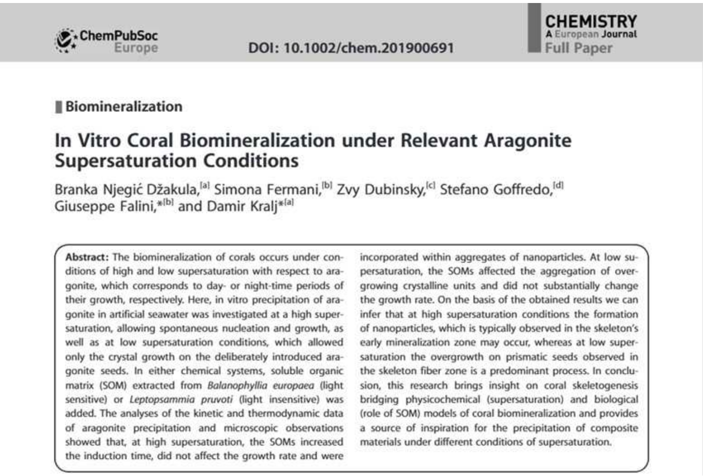


Relatively strong effect of polar AA may indicate that the **hydrogen bonding could influence** AA interactions with calcite surfaces during their diffusion on surface or along the step.

Model system 4

Role of macromolecules from soluble organic matrix extracted from corals, in $CaCO_3$ precipitation

(**Physiological system** – artificial seawater)



ChemPubSoc Europe
DOI: 10.1002/chem.201900691
CHEMISTRY
A European Journal
Full Paper

Biomineralization

In Vitro Coral Biomineralization under Relevant Aragonite Supersaturation Conditions

Branka Njegić Džakula,[a] Simona Fermani,[b] Zvy Dubinsky,[c] Stefano Goffredo,[d]
Giuseppe Falini,*[b] and Damir Kralj*[a]

Abstract: The biomineralization of corals occurs under conditions of high and low supersaturation with respect to aragonite, which corresponds to day- or night-time periods of their growth, respectively. Here, in vitro precipitation of aragonite in artificial seawater was investigated at a high supersaturation, allowing spontaneous nucleation and growth, as well as at low supersaturation conditions, which allowed only the crystal growth on the deliberately introduced aragonite seeds. In either chemical systems, soluble organic matrix (SOM) extracted from *Balanophyllia europaea* (light sensitive) or *Leptopsammia pruvoti* (light insensitive) was added. The analyses of the kinetic and thermodynamic data of aragonite precipitation and microscopic observations showed that, at high supersaturation, the SOMs increased the induction time, did not affect the growth rate and were incorporated within aggregates of nanoparticles. At low supersaturation, the SOMs affected the aggregation of overgrowing crystalline units and did not substantially change the growth rate. On the basis of the obtained results we can infer that at high supersaturation conditions the formation of nanoparticles, which is typically observed in the skeleton's early mineralization zone may occur, whereas at low supersaturation the overgrowth on prismatic seeds observed in the skeleton fiber zone is a predominant process. In conclusion, this research brings insight on coral skeletogenesis bridging physicochemical (supersaturation) and biological (role of SOM) models of coral biomineralization and provides a source of inspiration for the precipitation of composite materials under different conditions of supersaturation.

B. Njegić Džakula, S. Fermani, Z. Dubinsky, S. Goffredo, G. Falini and D. Kralj. In vitro coral biomineralization under relevant aragonite supersaturation conditions. Chemistry - A European Journal 2019, 25 (45) 10616-10624

Facts about corals

Biomineralization of aragonite in corals - hard skeleton
Marine invertebrates - class Anthozoa of the phylum Cnidaria
Form compact colonies of many identical individual polyps
Reef builders

Colony of corals - genetically identical polyps
Individual colonies - grow by **asexual** reproduction of polyps
Breed sexually - by spawning

http://en.wikipedia.org/wiki/

Aragonite precipitation in c**orals** at **high** and **low supersaturation** conditions (higher growth rate during day-time, slower during night-time periods)
Spontaneous precipitation – nucleation of aragonite
Crystal growth – on preformed aragonite crystals
Complex precipitation system / medium (extrapallial solution)

Specific goals of research

Extract soluble organic macromolecules (SOM) from two different species:
  Balanophyllia europaea (light sensitive)
  Leptopsammia pruvoti (light insensitive)

Apply "kinetic" approach to analyze biomineralization of aragonite

Discern a role of:
  Physicochemical parameters (supersaturation)
  Biological parameters (role of SOM)
    Spontaneous precipitation - nucleation (high supersaturation = **day-time growth period**, $S_a \approx 25$)
    Kinetics of aragonite seed - crystal growth (low supersaturation = **night-time growth period**, $S_a \approx 3.2$)

Hypothesis

Growth mechanisms - diffent during the night and day precipitation of aragonite in corals

A. Extraction of Soluble Organic Matrix (SOM) from model species

Balanophyllia europea (Scarlet coral)

Solitary stony coral

Only in the Mediterranean Sea

Photophilous species (needs sunlight to maintain alive symbiotic micro-algae)

Leptopsammia pruvoti (Sunset cup coral)

Solitary stony coral

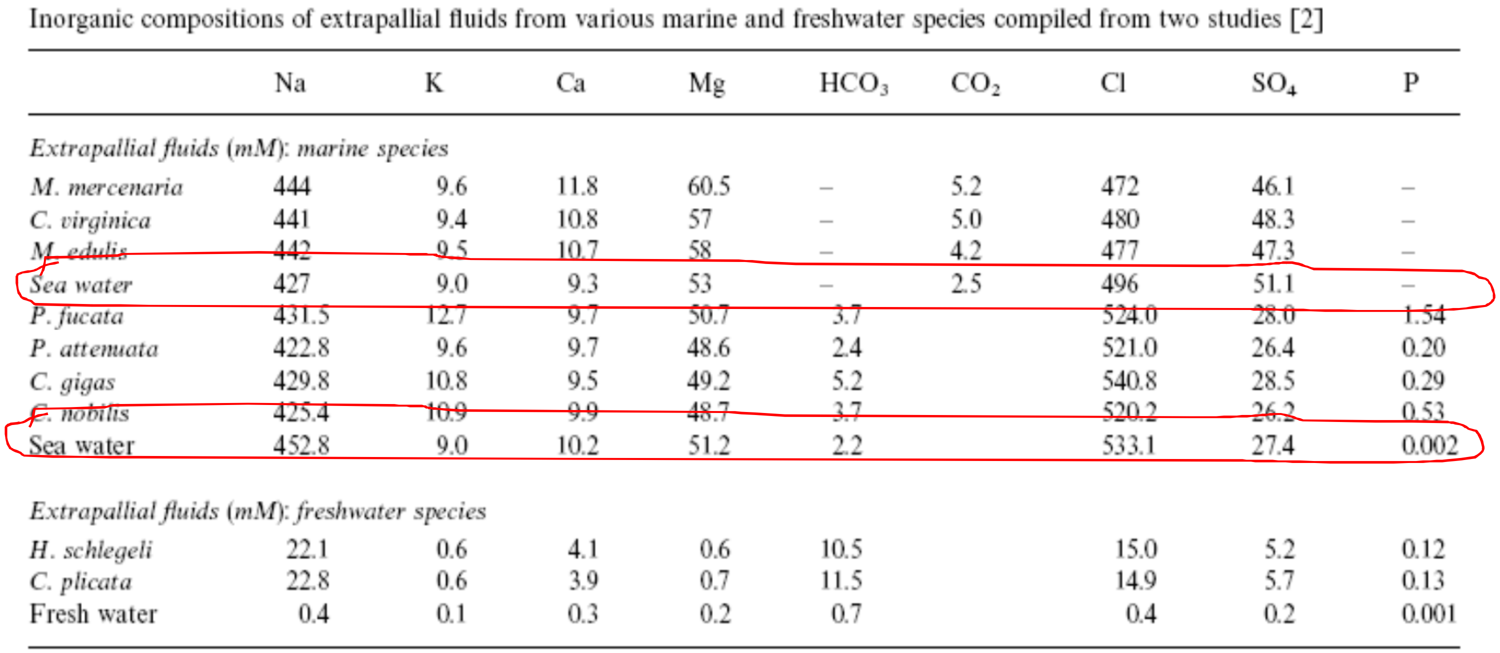
Azooxanthellate species (not contain the symbiotic unicellular algae)

Western Mediterranean Sea, Adriatic Sea,...

http://en.wikipedia.org/wiki/

B. Selection of model system !!

Artificial seawater ≈ extrapallial fluid



Inorganic compositions of extrapallial fluids from various marine and freshwater species compiled from two studies [2]

	Na	K	Ca	Mg	HCO_3	CO_2	Cl	SO_4	P
Extrapallial fluids (mM): marine species									
M. mercenaria	444	9.6	11.8	60.5	–	5.2	472	46.1	–
C. virginica	441	9.4	10.8	57	–	5.0	480	48.3	–
M. edulis	442	9.5	10.7	58	–	4.2	477	47.3	–
Sea water	427	9.0	9.3	53	–	2.5	496	51.1	–
P. fucata	431.5	12.7	9.7	50.7	3.7		524.0	28.0	1.54
P. attenuata	422.8	9.6	9.7	48.6	2.4		521.0	26.4	0.20
C. gigas	429.8	10.8	9.5	49.2	5.2		540.8	28.5	0.29
C. nobilis	425.4	10.9	9.9	48.7	3.7		520.2	26.2	0.53
Sea water	452.8	9.0	10.2	51.2	2.2		533.1	27.4	0.002
Extrapallial fluids (mM): freshwater species									
H. schlegeli	22.1	0.6	4.1	0.6	10.5		15.0	5.2	0.12
C. plicata	22.8	0.6	3.9	0.7	11.5		14.9	5.7	0.13
Fresh water	0.4	0.1	0.3	0.2	0.7		0.4	0.2	0.001

S. L. Tracy et al. The growth of calcite spherulites from solution I. Experimental design techniques Journal of Crystal Growth 193 (1998) 374-381

Model system 1

Spontaneous precipitation (ASW-1)

$c((Na_2CO_3) + (NaHCO_3)) = 5 \cdot 10^{-3}$ mol dm^3
+ SOM

$c(CaCl_2) = 0.01$ mol dm^3
$c(MgCl_2) = 0.05$ mol dm^3
$c(NaCl) = 0.3$ mol dm^3

$pH_i \approx 10.2$; $S_a \approx 11$

Model system 2

Kinetics - seeding precipitation (ASW-2)

$c((Na_2CO_3) + (NaHCO_3)) = 5 \cdot 10^{-3}$ mol dm^3
+ SOM

$c(CaCl_2)=0.01$ mol dm^3
$c(MgCl_2)=0.05$ mol dm^3
$c(NaCl)= 0.3$ mol dm^3

$pH_i \approx 8.9$; $S_a \approx 5.8$

0.5 ppm < c_i(SOM-Beu) < 8 ppm
1 ppm < c_i(SOM-Lpr) < 8 ppm

$\theta = 21$ ° C, t = 30 min

Ionic equilibrium of relevant ionic species:

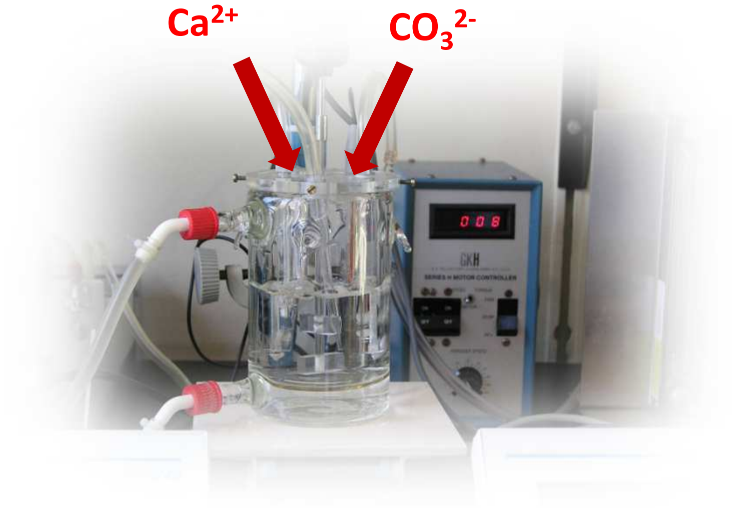
H^+, OH^-, Ca^{2+}, $CaCO_3^0$, $CaHCO_3^+$, $H_2CO_3^*$, HCO_3^-, CO_3^{2-},
Mg^{2+}, $MgCO_3^0$, $MgHCO_3^+$, Cl^-, Na^+

Initial conditions:

$[Ca]_{tot}$, $[Mg]_{tot}$, $[Na]_{tot}$, $[Cl]_{tot}$

Measurements:

pH



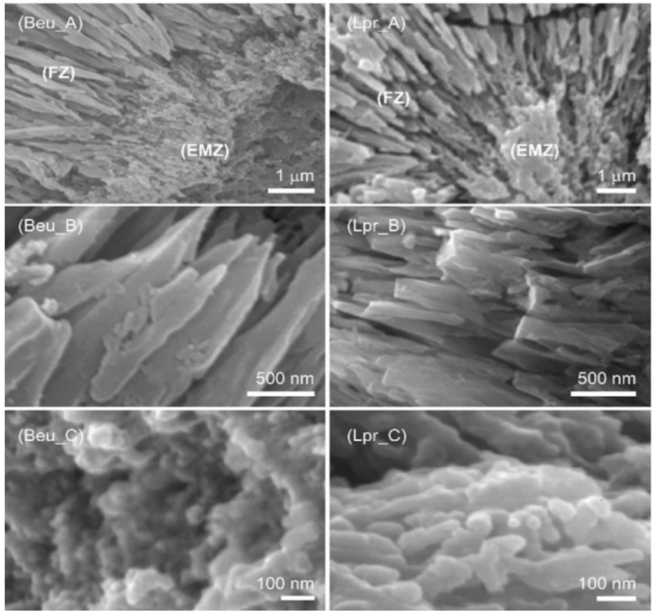
Spontaneous precipitation – nucleation of aragonite (ASW-1)

Morphology and polymorphism

Cross sections of septum skeletons (**aragonite**)

B. europaea

L. pruvoti



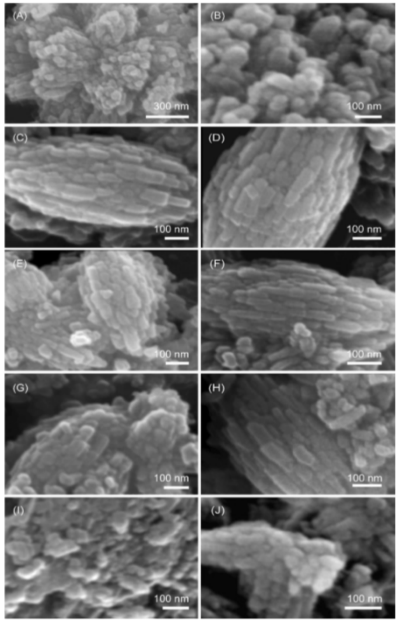
Fiber zone
(FZ)

Early mineralization
zone
(EMZ)

In vitro spontaneous **aragonite** precipitation

SOM-Beu

SOM-Lpr



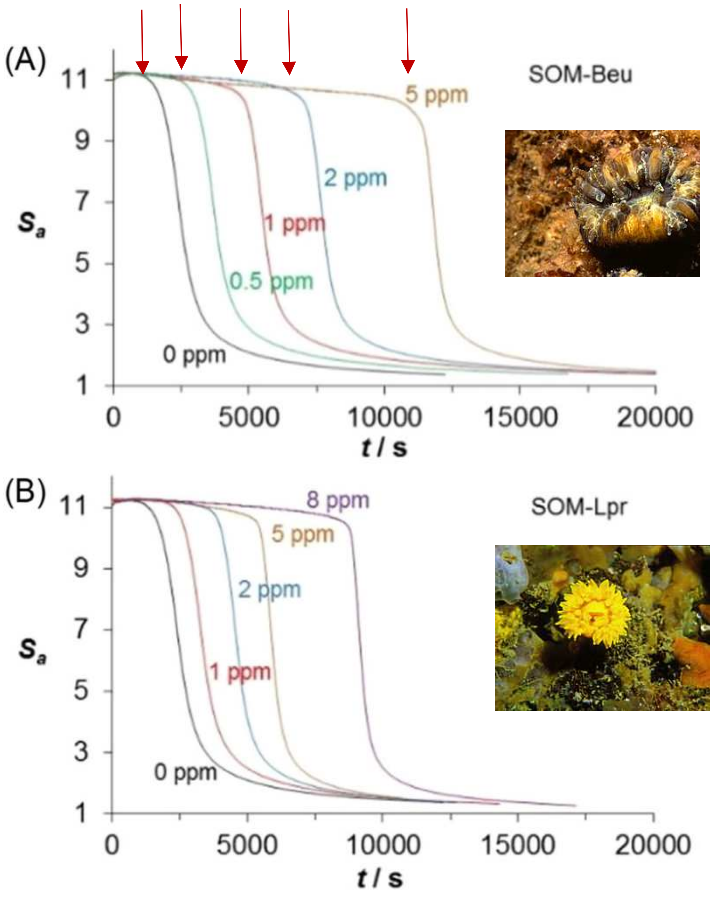
0.0 ppm
0.5 ppm
1.0 ppm
2.0 ppm
5.0 ppm

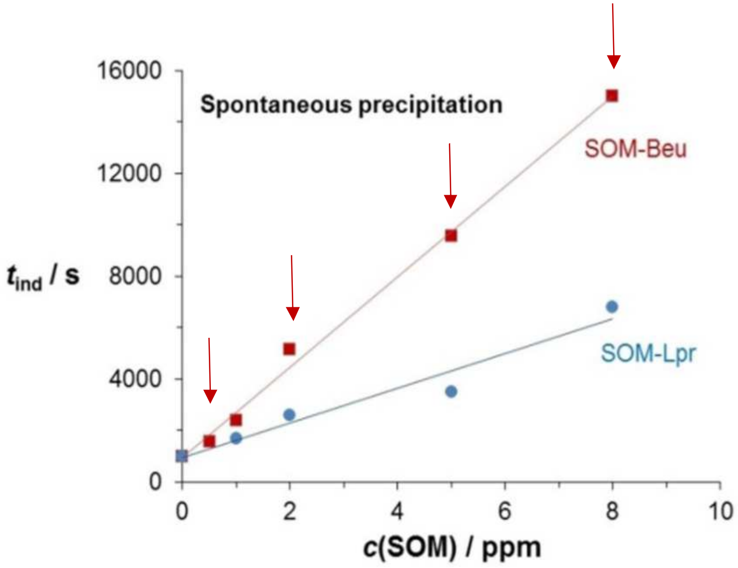
0.0 ppm
1.0 ppm
2.0 ppm
5.0 ppm
8.0 ppm

Highest concentrations of SOM → similarity with corals EMZ ?!

Spontaneous precipitation – nucleation of aragonite (ASW-1)

Induction time



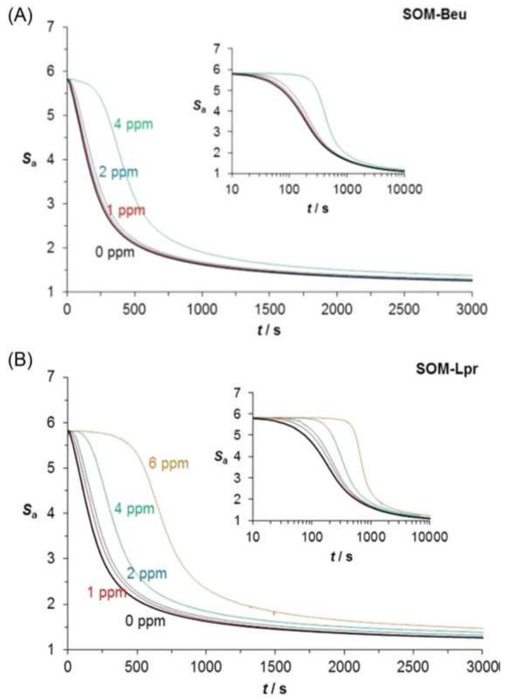


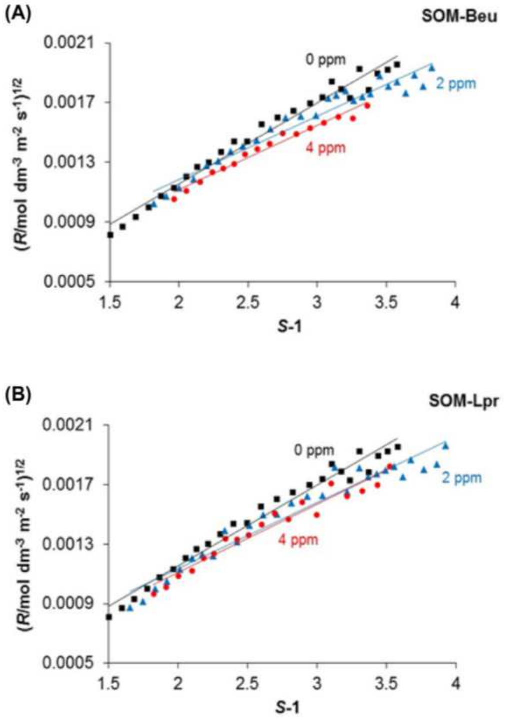
- Induction time - adsorption of SOM on nuclei and crystals!
- Increased induction time with increasing concentration!
- No change of mineralogical composition (aragonite)!

Seeded precipitation – crystal growth of aragonite (ASW-2)

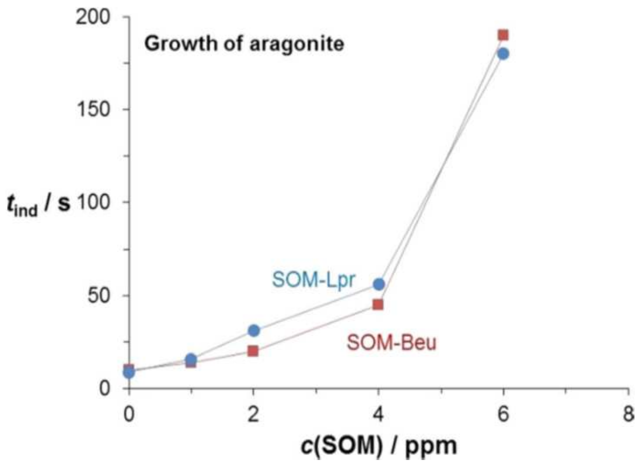
Kinetics

Growth kinetics → Growth mechanisms





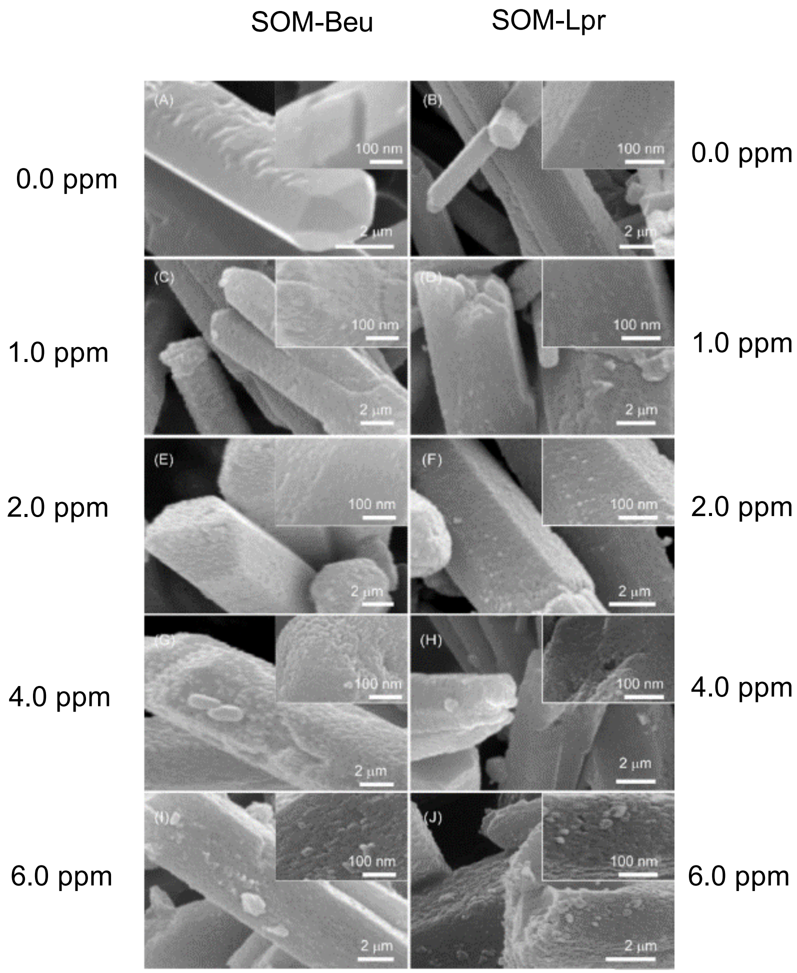
Induction time



- Adsorption of SOM on nuclei and crystals!
- No change of mineralogical composition (aragonite seed)!
- No change of growth mechanisms
- Inhibition increase with SOM concentration
- Increased induction time with increasing concentration!

Seeded precipitation – crystal growth of aragonite (ASW-2)

Morphology, size and polymorphism



- Seed – regular prismatic aragonite
- No change of mineralogical composition after overgrowth in presence of SOM
- No change of morphology at low SOM concentrations
- Increased roughness at higher SOM concentrations
- Highest Beu concentration - textural reorganization of particles

Conclusions about *in vitro* coral mineralization

- Under **high supersaturation** conditions - **significant incorporation** of macromolecules into **mineral phase** during the nucleation and growth of crystals observed.

- **SOMs incorporate in aragonite**, but **not in calcite** crystals under similar conditions.

- Precipitated aragonite appears as aggregates of nanoparticles resembling those observed in the EMZs.

- Inhibition of precipitation - observed as a systematic increase of induction period with increasing SOM addition

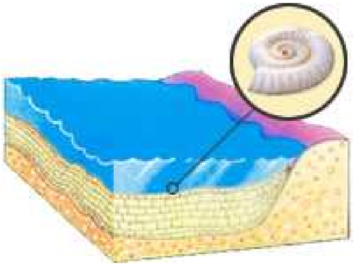
- The growth mechanism of aragonite obtained by seeding experiments (growth on the spiral step), did not change after the addition of SOM. However, the presence of high concentration of SOMs induces a change in the morphology and shape of the growing crystalline units.

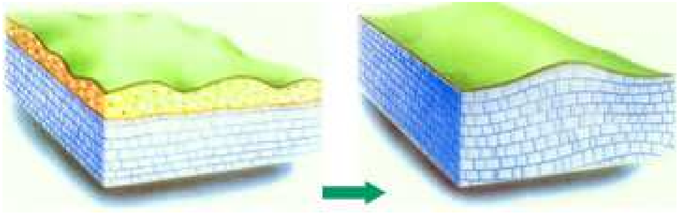
- **Inhibition activity of SOM-Beu is stronger than that of SOM-Lpr**

- **Calcification of corals is controlled by both, pure physical-chemical mechanism (supersaturation) and biological mechanism (SOM plays an active role in the process).**

Precipitation of calcium carbonates on mainland

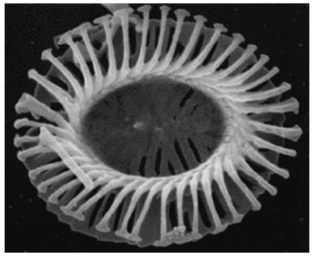
Formed by diagenesis of seawater sediments

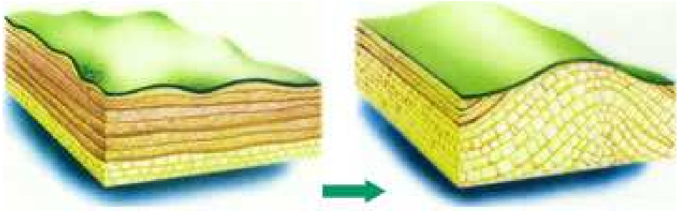




Chalk, 70 – 120 M years

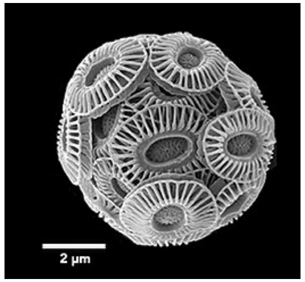
Planktonic or benthic protista (Foraminifera)

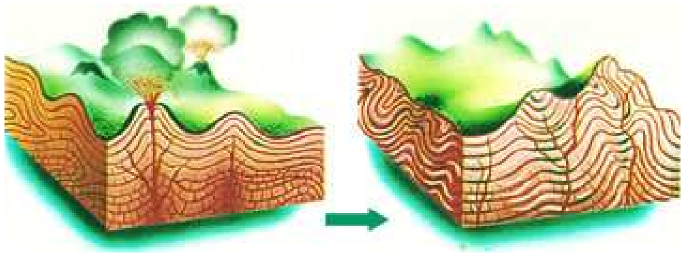




Limestone, 340 M years

Phytoplankton (coccolithophores (algae))



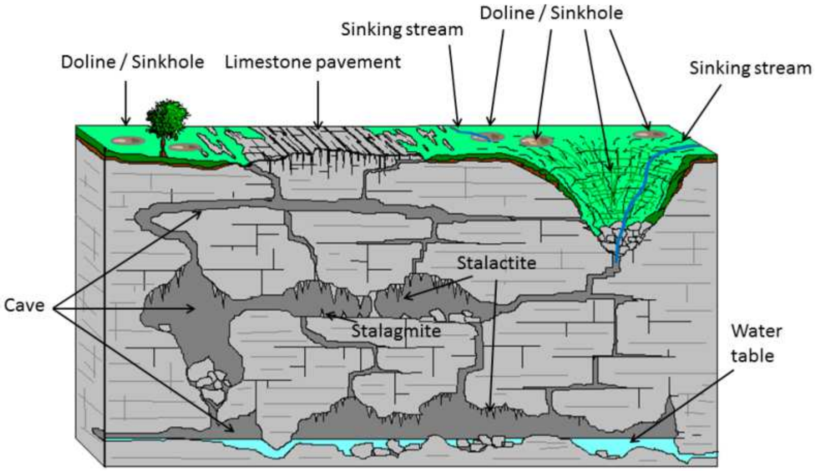


Marble = metamorphic limestone or chalk

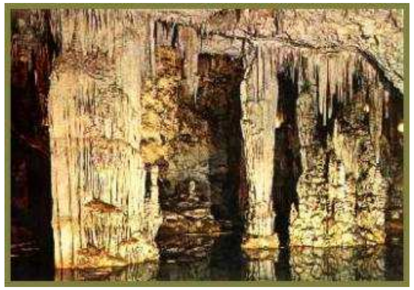
http://en.wikipedia.org/wiki/

Calcium carbonate on mainland and karst topography

Karst landscape → Earth's surface erosion of dense carbonate rock (limestone (calcite, aragonite) or dolomite)

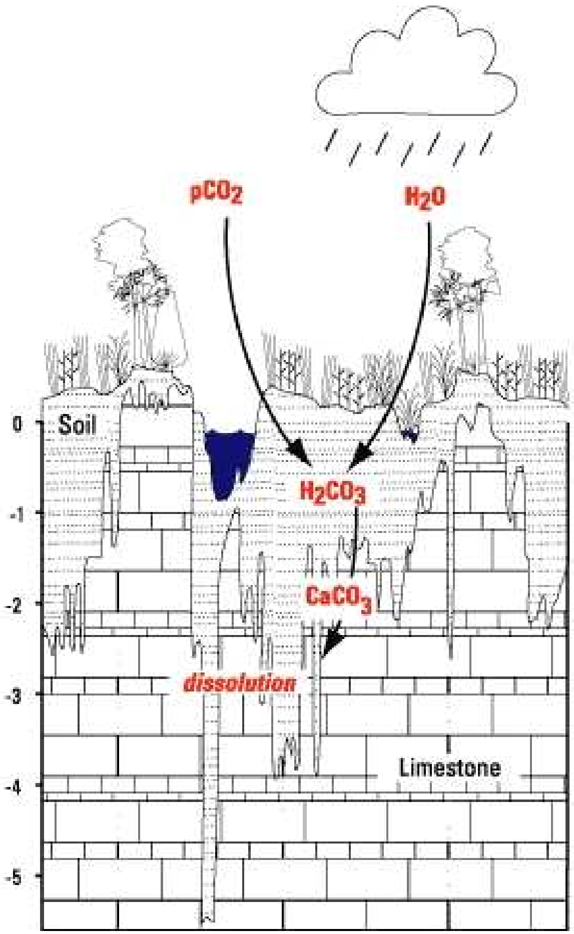


Typical karst landforms



http://en.wikipedia.org/wiki/

Mechanism of limestone dissolution and precipitation in karst



CO_2 dissolution

atmosphere → **$p_{CO2} \approx 10^{-3.4}$ atm**

$H_2O + CO_2\ (g) \leftrightarrows H_2CO_3\ (aq) \leftrightarrows H^+ + HCO_3^-$

underground → **$p_{CO2} >> 10^{-3.4}$ atm**

$CaCO_3$ dissolution

$H_2O + CO_2\ (g) \leftrightarrows H_2CO_3\ (aq) \leftrightarrows H^+ + HCO_3^-$

(CO_2 dissolution, pH decrease)

$CaCO_3(s) + H^+ \leftrightarrows Ca^{2+} + HCO_3^-$

($CaCO_3$ dissolution)

(atmosphere → **$p_{CO2} \approx 10^{-3.4}$ atm**)

$CaCO_3$ precipitation

$H_2CO_3\ (aq) \leftrightarrows H_2O + CO_2\ (g)$

(CO_2 degasification, pH increase)

$Ca^{2+} + CO_3^{2-} \leftrightarrows CaCO_3(s)$

($CaCO_3$ precipitation)

stalactite, stalagmite, tufa, lake sediment...

Model system 5

Precipitation of $CaCO_3$ at condition which mimic the conditions in karst water and tufa formation

How to set-up a representative model system?

(**Complex system =** artificial karst water)

Nives Matijaković Mlinarić: THE EFFECT OF SELECTED ENVIRONMENTAL AND ANTHROPOGENIC MOLECULES ON CALCIUM CARBONATE PRECIPITATION IN ARTIFICIAL KARST WATER. PhD thesis 2021, Faculty of Science, University of Zagreb

moss on tufa

Facts about Tufa

Tufa – porous limestone formed by precipitation in karst water, at ambient temperature
(Travertine – precipitation at elevated temperatures)
Tufa formation – either in fluvial channels or in lakes

Fluvial tufa appearance

- **Spring** – deposits form on emergence from a spring/seep
- **Braided channel** – deposits dominated by oncoids (layered structures by cyanobacterial growth)
- **Cascade** – deposits form at waterfalls (accelerated flow)
- **Barrage** – deposits formed as phytoherm barrages ("freshwater reef"), contain detritus.

Lacustrine tufa

Formed at lakes' periphery and built-up phytoherms, stromatolites and oncoids (created by cyanobacteria, sulfate-reducing bacteria or proteobacteria)

Tufa formation endangered by human impact (temperature, acidification, phosphorous, organic matter...)

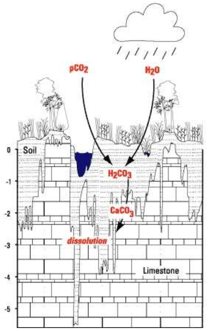
Collapse of tufa barrier

Mechanism of tufa formation

1. Dissolution of limestone and formation of soluble calcium bicarbonate

$H_2O + CO_2\ (g) \leftrightarrows H_2CO_3\ (aq) \leftrightarrows H^+ + HCO_3^-$

$CaCO_3(s) + H^+ \leftrightarrows Ca^{2+} + HCO_3^-$



2. Precipitation of tufa ($CaCO_3$)

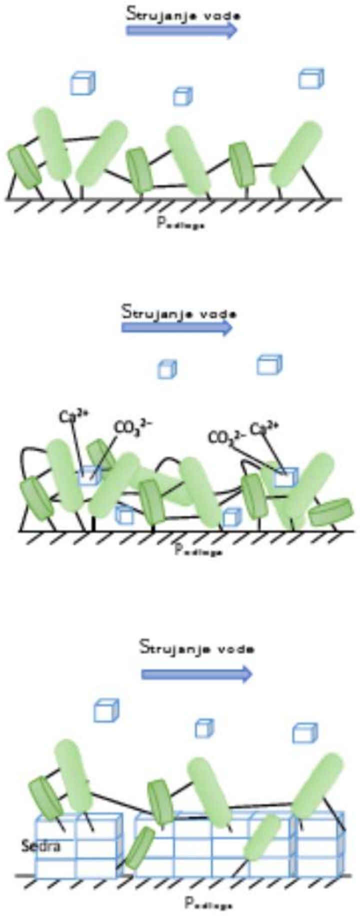
Increase of pH after CO_2 removal, either by

- Intensive water splashing at waterfalls,
- Photosynthesis of periphyton substrate (algae, cyanobacteria, diatoms...)

$Ca^{2+}\ (aq) + CO_3^{2-}\ (aq) \leftrightarrows CaCO_3\ (s)$

Sequence of events

a. Diatoms cover the substrate and extcrete the mucos
b. Microcrystalline $CaCO_3$ detritus trapped in mucus
c. Tufa growth at low supersaturation ($CaCO_3$ detritus seeding)



Nives Matijaković Mlinarić: THE EFFECT OF SELECTED ENVIRONMENTAL AND ANTHROPOGENIC MOLECULES ON CALCIUM CARBONATE PRECIPITATION IN ARTIFICIAL KARST WATER. PhD thesis 2021, Faculty of Science, University of Zagreb

Plitvice Lakes study area

https://np-plitvicka-jezera.hr/en/

Oldest and largest **national park** in Croatia, situated in the mountainous region of Croatia (**Dinarides karst region**)

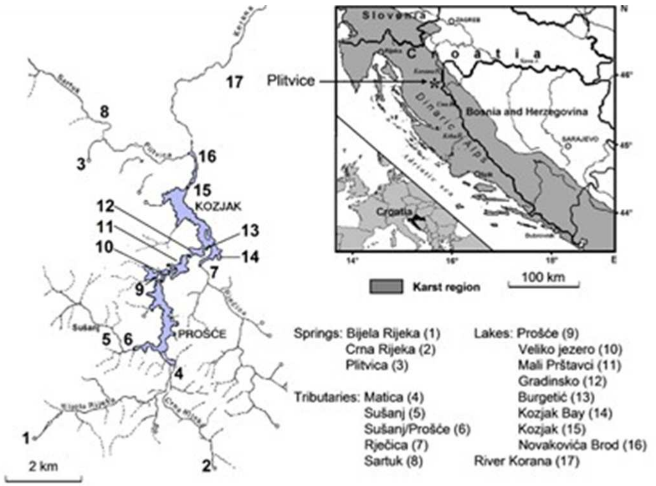
On UNESCO World Heritage List since 1979.

Lake system comprised of **16 cascading lakes**, divided into the Upper and Lower lakes

Upper Lakes (12: Prošćansko jezero, Ciginovac, Okrugljak, Batinovac, Veliko jezero, Malo jezero, Vir, Galovac, Milino jezero, Gradinsko jezero, Burgeti and Kozjak) formed on impermeable dolomite rock

Lower Lakes (4: Milanovac, Gavanovac, Kaluđerovac and Novakovića Brod) formed in permeable limestone substrate, deep canyon

Lakes system ended in waterfalls Sastavci - **outflow of the Korana River**



J. Dautovic,· Z. Fiket, J. Baresic, M. Ahel, N. Mikac. Sources, Distribution and Behavior of Major and Trace Elements in a Complex Karst Lake System. Aquat Geochem (2014) 20:19–38

Specific goals of research:

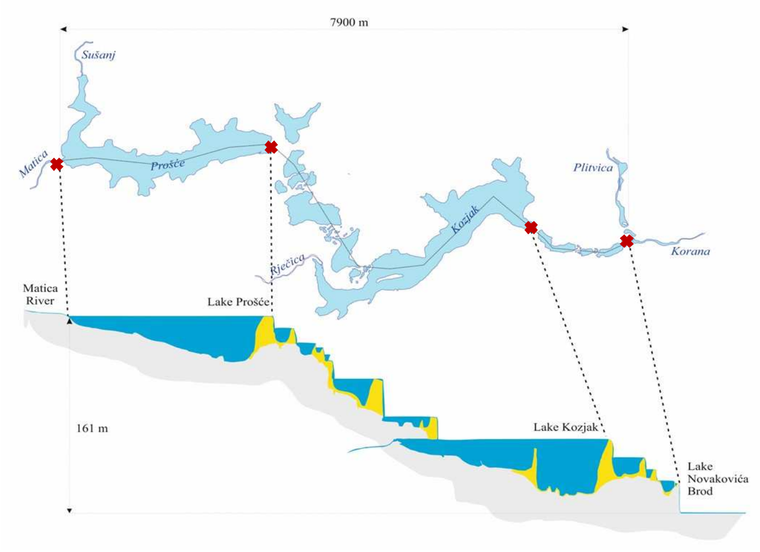
Determination of the **rate of tufa mineralization** at **Plitvice lakes (Croatia)**
Description of the **mechanism of tufa formation**, in a context of $CaCO_3$ growth mechanisms
  Role of **supersaturation** and **chemical composition** of lake water?
  Role of **substrate**?
  Role of **temperature**
  Role of dissolved **organic matter**

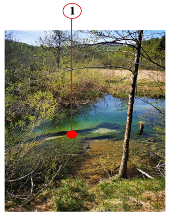
Research strategy:

1. **Physical-chemical characterization of lake water** (seasonal and spatial distribution of major parameters):
   (chemical composition (inorganic and organic matter), pH, temperature, ..., hydrodynamics, supersaturation
2. *In situ* determination of recent **tufa mineralization rate**
3. **Characterization** of recent tufa

4. *Off site* (laboratory) precipitation experiments
   Selection of representative precipitation model system (complex system)
5. ....
6. ....
7. ....

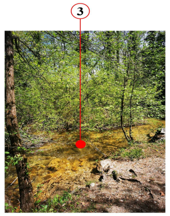
Vertical cross-section of Plitvice Lakes and sampling locations



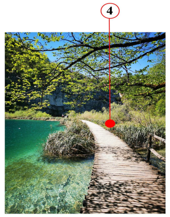


Matica

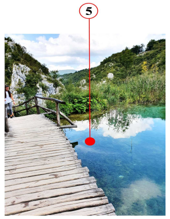
Prošće



Kozjak



Kaluđerovac

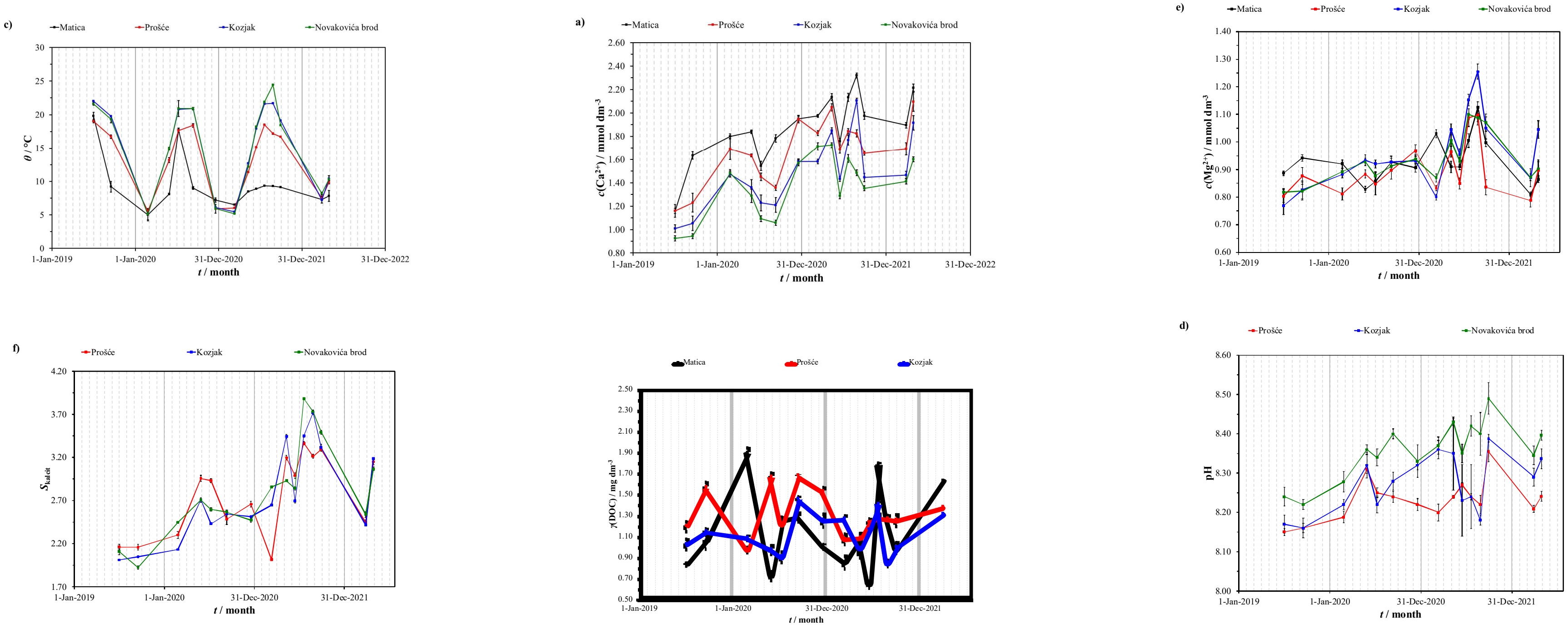


Novakovića brod

Physical-chemical characterization of lake water

Seasonal distribution of key parameters

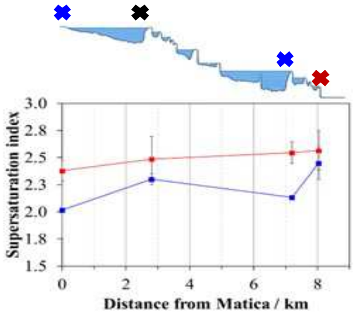
Novakovića brod	$c(Ca^{2+})$ /mM	$c(Mg^{2+})$ /mM	$c(Na^{+})$ /mM	$c(Cl^{-})$ /mM	$c(SO_4^{2-})$ /mM	$c(NO_3^{-})$ /mM	pH	t/C	c(CO3)/mM	c(K+) / mM	TOC	Sc
02-Jul-19	0.925	0.818	0.031	0.016	0.009	0.014	8.240	21.620	3.443	0.000	0.980	2.107
16-Sep-19	0.944	0.822	0.035	0.038	0.033	0.031	8.220	19.340	3.415	0.000	1.150	1.923
25-Feb-20	1.482	0.892	0.042	0.046	0.036	0.047	8.278	5.100	4.626	0.000	0.910	2.449
28-May-20	1.288	0.928	0.045	0.035	0.026	0.036	8.360	14.970	4.297	0.010	0.990	2.706
08-Jul-20	1.093	0.878	0.049	0.056	0.038	0.036	8.340	20.930	3.763	0.000	0.930	2.597
10-Sep-20	1.058	0.913	0.040	0.043	0.035	0.025	8.400	20.910	3.775	0.006	1.210	2.566
17-Dec-20	1.570	0.938	0.040	0.037	0.026	0.033	8.330	5.970	4.927	0.008	1.260	2.474
11-Mar-21	1.713	0.870	0.044	0.046	0.031	0.044	8.370	5.190	5.029	0.008	1.130	2.857
10-May-21	1.725	1.005	0.041	0.045	0.032	0.039	8.430	12.190	5.323	0.006	0.640	2.929
14-Jun-21	1.288	0.930	0.044	0.046	0.031	0.037	8.350	18.240	4.276	0.008	0.900	2.842
20-Jul-21	1.608	1.100	0.043	0.041	0.033	0.035	8.420	21.890	5.179	0.009	1.430	3.882
26-Aug-21	1.485	1.088	0.040	0.037	0.030	0.019	8.400	24.450	4.936	0.006	1.270	3.730
28-Sep-21	1.353	1.070	0.040	0.045	0.037	0.023	8.490	18.410	4.616	0.040	1.070	3.493
28-Mar-22	1.413	0.873	0.041	0.049	0.036	0.035	8.345	8.200	4.433	0.007	0.520	2.538
28-Apr-22	1.601	0.903	0.043	0.044	0.034	0.036	8.397	10.330	4.841	0.005	0.800	3.069
26-May-22	1.663	0.983	0.048	0.049	0.035	0.033	8.320	19.140	5.111	0.010	1.200	3.383
21-Jul-22	1.450	1.060	0.050	0.045	0.038	0.027	8.310	22.360	3.221	0.012	0.875	4.484
30-Aug-22	1.465	1.090	0.040	0.043	0.035	0.019	8.223	21.627	4.975	0.007		2.940

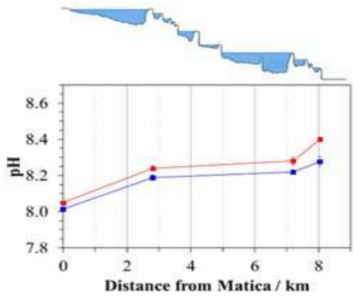


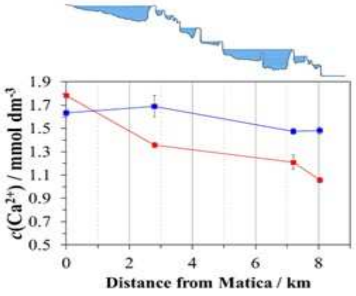
Results of periodical sampling and lake water analyses of key parameters on different locations

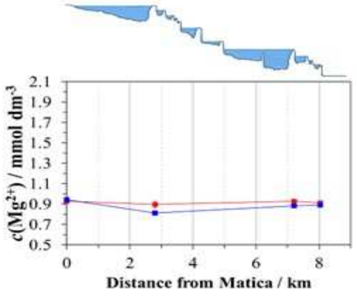
Physical-chemical characterization of lake water

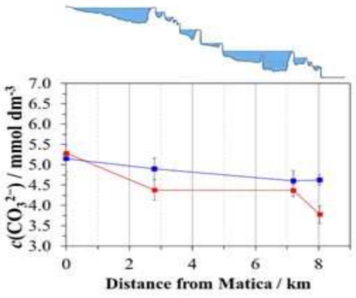
Spatial distribution of key parameters
(cold and warm season)

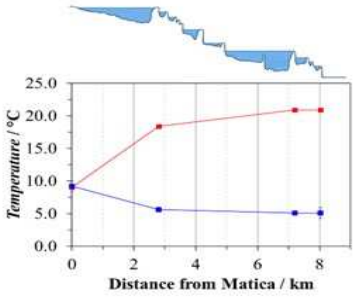






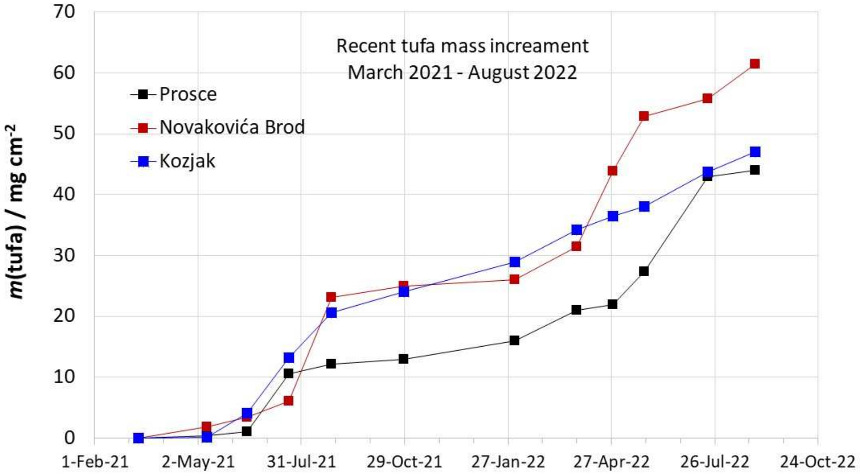


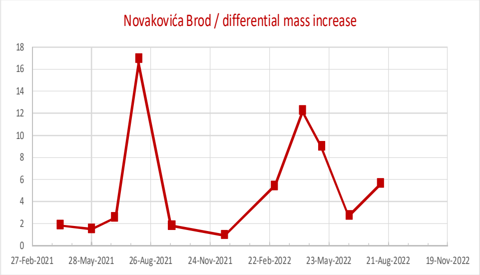




Recent tufa mineralization rate
In situ measurements

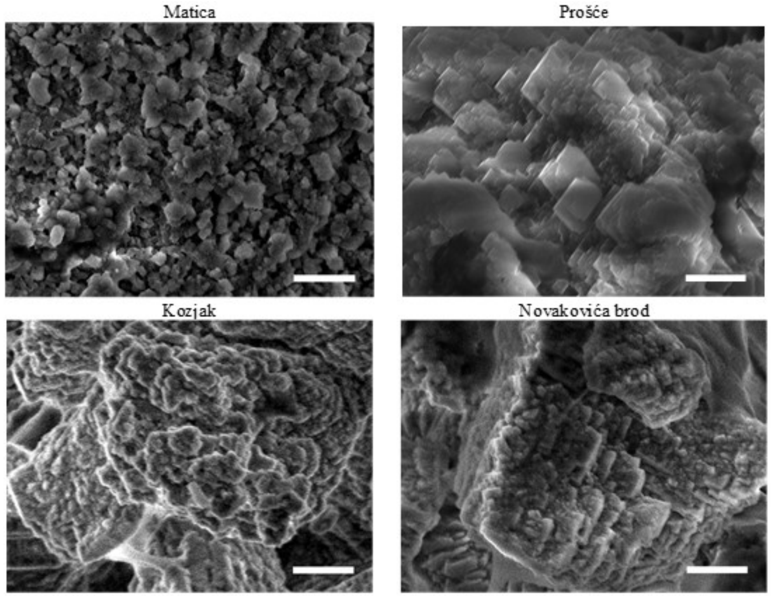
Substrate: microscope glass slides



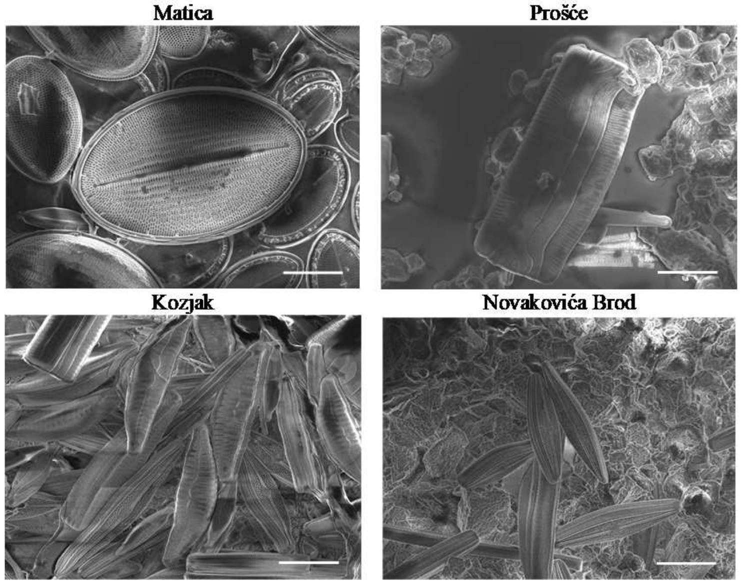


Recent tufa characterization

Morphology



Morphology of tufa after 12 months of mineralization

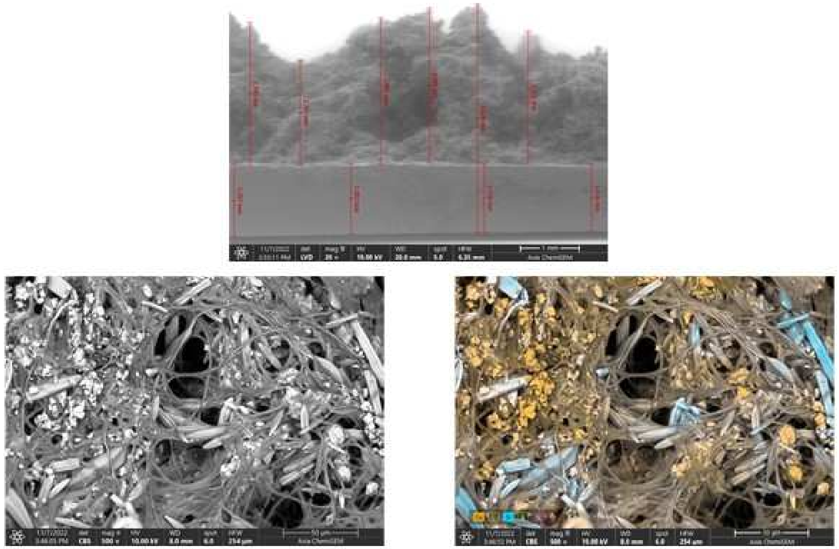


Diatoms on substrate after 2 months of mineralization

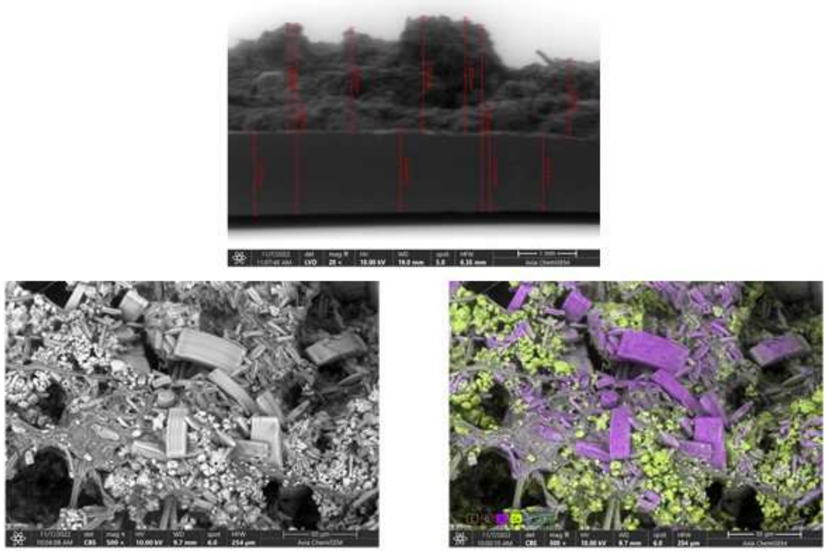
Recent tufa

Chemical analysis

(SEM and Energy Dispersive X-ray Spectroscopy)



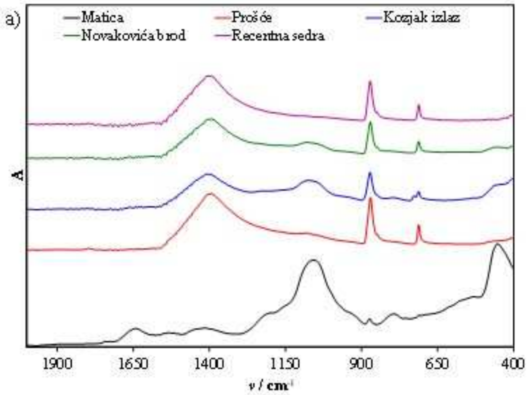
Novakovica brod

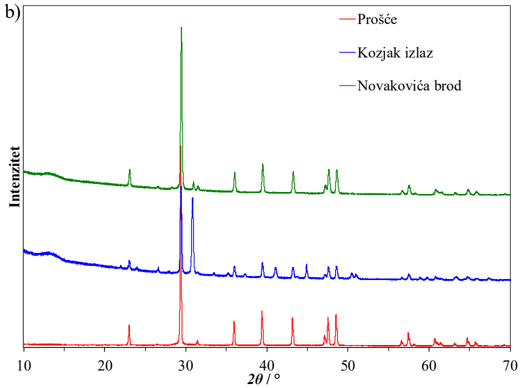


Prosce

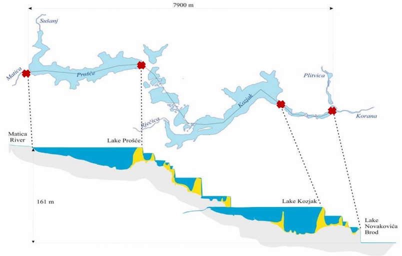
Recent tufa characterization

Mineralogical composition





	Calcite %	Mg-calcite %	Dolomite %	SiO_2%
Prosce	95	-	4	1
Novakovica brod	95	-	4	1
Kozjak	54	12	32	2



Laboratory experiments

Selection of precipitation model system
High mineralization rate, high supersaturation, high pH

Inorganic components
CO_2, $[Ca]_{tot}$, $[Mg]_{tot}$, $[Na]_{tot}$, $[K]_{tot}$, $[SO_4]_{tot}$, $[Cl]_{tot}$, $[NO_3]_{tot}$

Initial concentrations – average of composition at location of maximum mineralization
Novakovica brod

Protocol for preparation of reactant solutions (simple and reliable)

Comparison of the karst water and artificial karst water composition

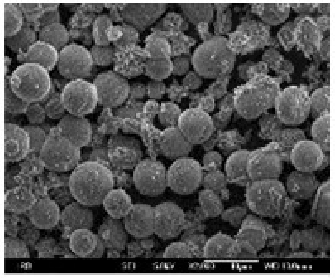
Ions	c_i(Novakovica brod) mmol dm^{-3}	c_i(ASW) mmol dm^{-3}
Na^+	0,043	0,043
Ca^{2+}	1,520	1,520
Mg^{2+}	0,910	0,910
K^+	0,015	0,015
Cl^-	0,034	0,034
NO_3^-	0,00	0,00
SO_4^{2-}	0,020	0,010
HCO_3^-	4,770	4,484
pH	8,24	8,80

Smith, E. J., Davison, W., Hamilton-Taylor, J., 2002. Methods for preparing synthetic freshwaters. Water Research 36 ,1286–1296.

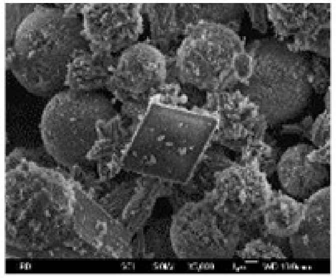
Spontaneous precipitation in artificial karst water (Novakovica brod)

Morphological and mineralogical composition

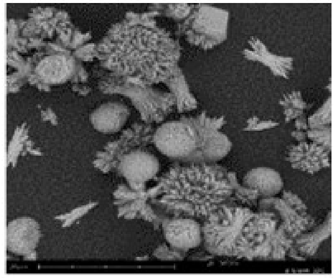
10 °C



20 °C

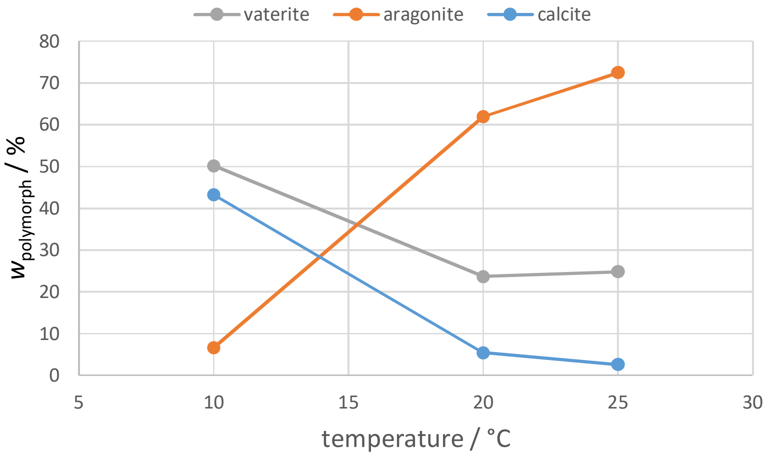


25 °C



95 % calcite + 4 % dolomite

t / °C	calcite	aragonite	vaterite
10	43.2	6.6	50.2
20	5.4	61.9	23.7
25	2.6	72.5	24.8



Spontaneous precipitation in artificial karst water

Organic matter addition (humic and fulvic acid)

Humic compounds

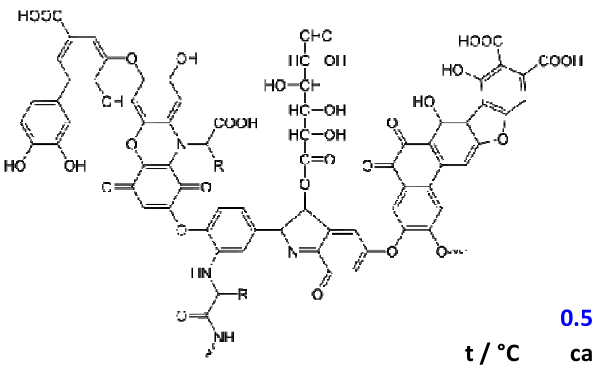
Organic compounds formed by decomposition of biomass

Major components of dissolved organic matter (DOM) in lakes

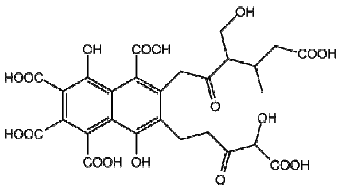
Humic acid (HA) - soluble in water at neutral and alkaline pH, insoluble at pH < 2

Fulvic acid (FA) - soluble in water at any pH.

Humic acid



Fulvic acid



0.5 ppm fulvic acid

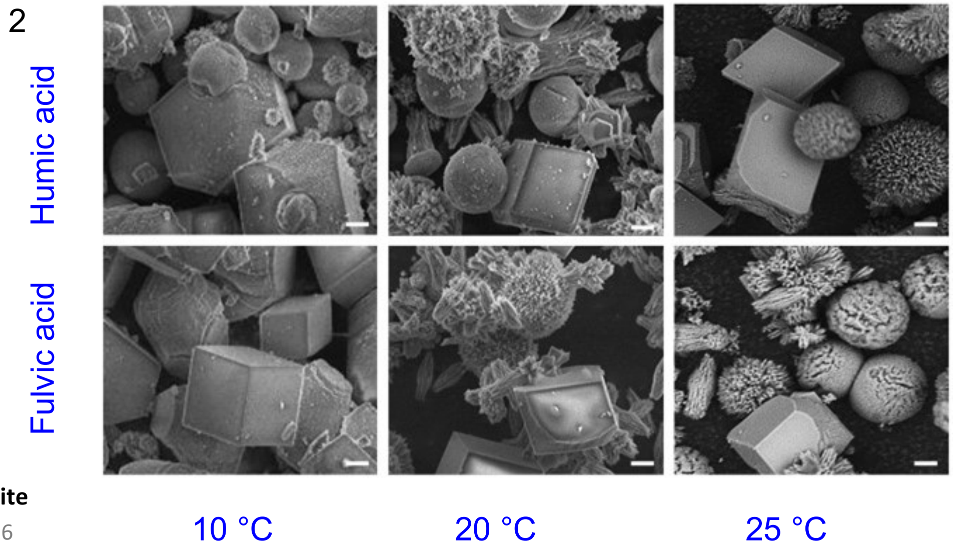
t / °C	calcite	aragonite	vaterite
10	79.24	2.04	18.26
20	22.05	77.95	0
25	17.07	73.2	9.73

0.5 ppm humic acid

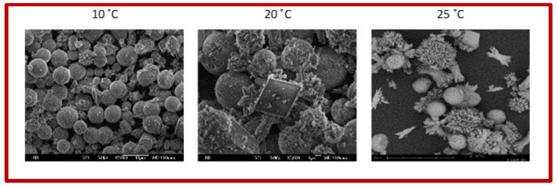
t / °C	calcite	aragonite	vaterite
10	0.9	0.9	99.8
20	42.83	34.88	22.81
25	73.92	6.84	19.23

Spontaneously precipitated $CaCO_3$

c(additive) ≈ 0,5 ppm

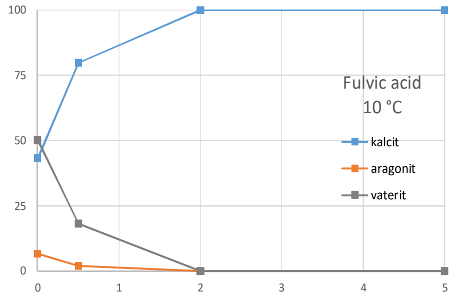


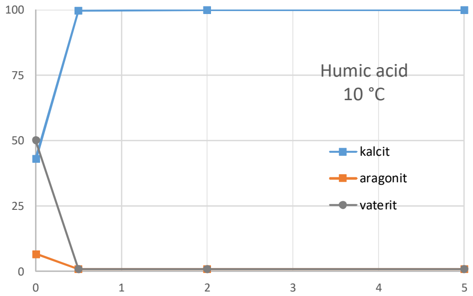
no additive

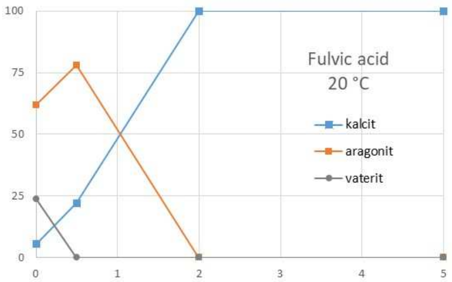


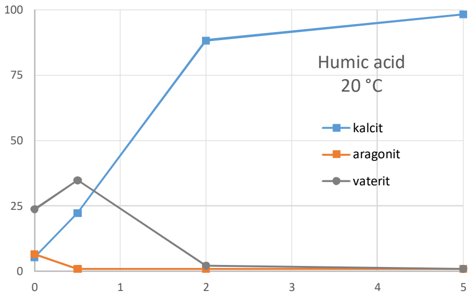
Spontaneous precipitation in artificial karst water

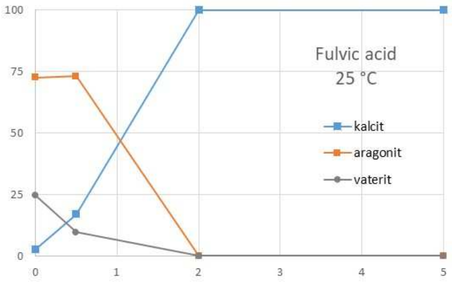
Organic matter addition (humic and fulvic acid)

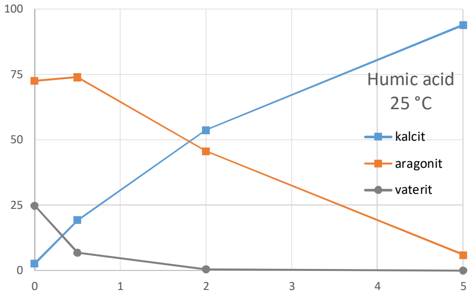






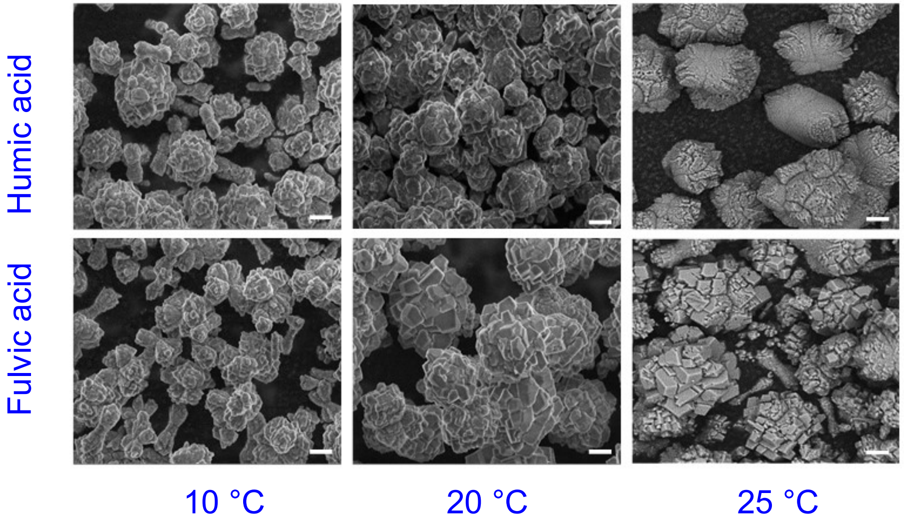






Spontaneously precipitated $CaCO_3$

c(additive) ≈ 2.0 ppm



95 % calcite + 4 % dolomite

$CaCO_3$ precipitation system (artificial karst water)

$CaCl_2$ - Na_2CO_3 - NaCl - KCl - $MgCl_2$ – Na_2SO_4 – $NaNO_3$
(Constituent ions + co-ions + H_2O)
0 < DOM < 5 ppm

$3.7 < (S-1)_i < 12$

10 °C < θ < 25 °C
t = 60 min

+
ADDITIVES??? (phosphates, polyphosphates, ... specific organic molecules...)

Conclusions

- **Investigation** of tufa mineralization at locations **on watercourse is difficult** due to interplay of several factor which could influence the mineralogical and chemical composition and mineralization rate
- **Concentration** of major inorganic components and other physical-chemical properties of karst water, **varied along the watercourse**
- **Concentration** of major inorganic components and other physical-chemical properties of karst water varied **during the season**
- **Recent tufa** obtained after 3 seasons of continuous mineralization is **predominantly calcite** and magnesium calcite, while diatoms could be also found
- **Laboratory model** assumes **reproducible preparation** of artificial karst water which closely mimic the chemical composition of natural waters
- Mixture of **3 polymorphs (calcite, aragonite and vaterite**) precipitate when **only inorganic components** are present in the system, while **magnesium calcite** is the only component when at least **2 ppm of fulvic acid** is introduced as a model organic molecules

Recommended literature

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4. Yu-Qin Niu, Jia-Hui Liu, C. Aymonier, S. Fermani, D. Kralj, G. Falini and Chun-Hui Zhou. **Calcium carbonate: controlled synthesis**, surface functionalization, and nanostructured materials. Chemical Society Reviews, 2022, 51, 7883 – 7943.

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