

Max Karambelas, Yael Chalom and Alon Granek
Mentored by Dr. Shifi Kababya, and Prof. Asher Schmidt
Schulich Faculty of Chemistry, Technion, Haifa 32000, Israel

Introduction

Biomimetic mineralization is the process of elements in an environment being utilized as functional structures by biological organisms. The effects of biomimetic mineralization on the environment can be traced back to almost 600 million years ago through the fossil record. This stems from the cycling of elements such as calcium, carbon, and phosphorus over millions of years. These elements are recycled through biomimetic mineralization involving processes that allow organisms to create incredible inorganic structures. These ancient examples of inorganic materials can show the marine climate, chemical conditions, and water salinity of a different time.

More recently, however, biomimetic mineralization has been studied under a more chemical perspective. Researchers now seek to explain what biomimetic minerals are composed of and how to categorize them. And also to understand the functional properties of these biomimetic minerals. But the most important aspect of biomimetic mineralization studied today is understanding the process by which organisms can control the synthesis of inorganic compounds; and how they tune functional macroscopic properties. More specifically, how organisms are able to construct inorganic compounds in such an efficient manner.

Organisms

Coccolithophores [fig. 1], a species of Phytoplankton, are organisms who produce the most stable crystalline Calcium Carbonate, Calcite, for their shells.



Figure 1

The Blue Lobster [fig. 2] uses Calcium Carbonate to create an exoskeleton which serves as the organism's protective barrier and provides structural support. The exoskeleton is formed through the process of ecdysis, and through the intake of calcium from the lobsters environment. The exoskeleton consists of a chitin matrix hardened by a Calcium Carbonate deposit of calcite and stable amorphous calcium carbonate (ACC). Pre-molt, bio available Calcium reservoirs are prepared within special organs, Gastroliths [fig. 3]. Bioavailability amounts to the storage of highly soluble, stabilized ACC.



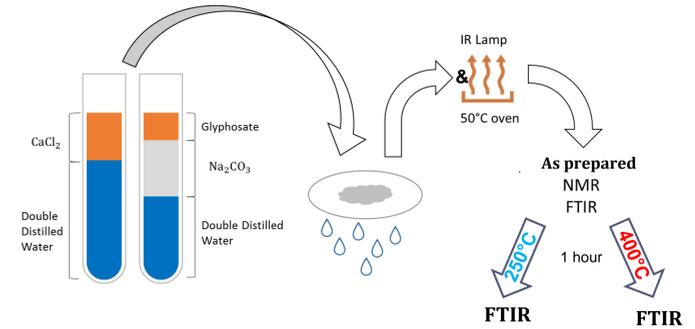
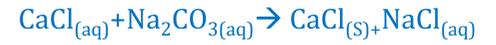
Figure 2



Figure 3

To view the biomimetic mineralization process and the link between conditions and results in it, a molecular level approach must be employed. A Simplistic model that accounts for factors implicated in biomimetic mineralization-incorporation of biomacromolecules (proteins, amino acids), and phosphorylation- we use the precipitation of CaCO₃ in the presence of the Glyphosate (Glp) molecule.

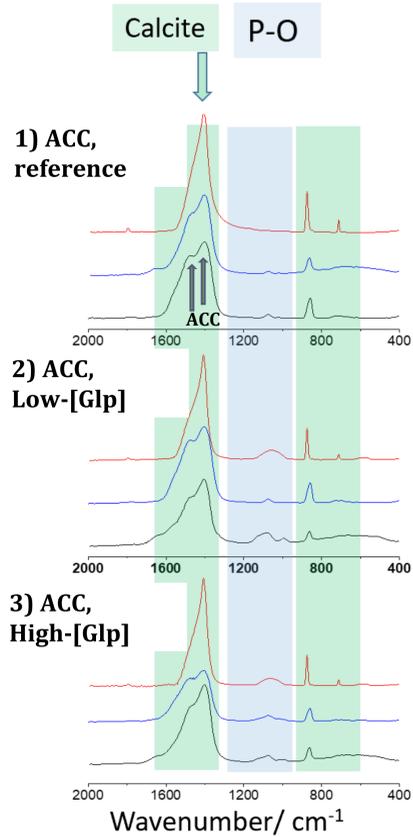
Biomimetic Model System Experimental Setup (Fig. 4)



Samples:

- 1) ACC, reference ([0] mM Glp)
- 2) ACC, Low-[Glp] ([6] mM Glp)
- 3) ACC, High-[Glp] ([18] mM Glp)

Precipitates Characterization by FTIR

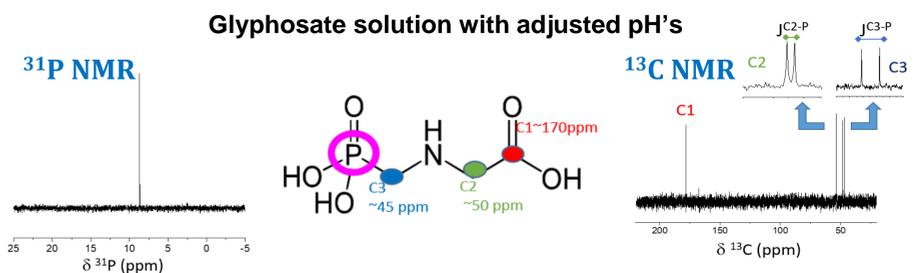


In Fourier Transform Infrared Radiation FTIR spectroscopy IR light is sent to the material and by absorption is detected. This makes it a sensitive analytical tool that distinguishes between the different polymorphs and amorphous forms of Calcium Carbonates.

FTIR [fig. 5] shows that all initial precipitates are ACC. Only upon hitting 400C ACC crystallizes to Calcite. FTIR can not tell apart ACC and ACC with Glyphosate.

Figure 5: FTIR spectra of ACC, low-Glp-ACC, and high-Glp-ACC samples. Data collected as prepared (black), data collected after 1h of 250°C heat treatment (blue), and 1h at 400°C (red). Assignment⁽¹⁾: ACC: split v3 peak at 1420 cm⁻¹ and 1485 cm⁻¹, v2 peak at 865 cm⁻¹. Calcite: v3 peak at 1420 cm⁻¹, v2 peak at 875 cm⁻¹, and v4 peak at 713 cm⁻¹

Titration curves of Glp by ³¹P & ¹³C High-Resolution Nuclear Magnetic Resonance (NMR)



The phosphonate and carboxylate are most sensitive for deprotonation of the -NH²⁺ group at pH~10.5.

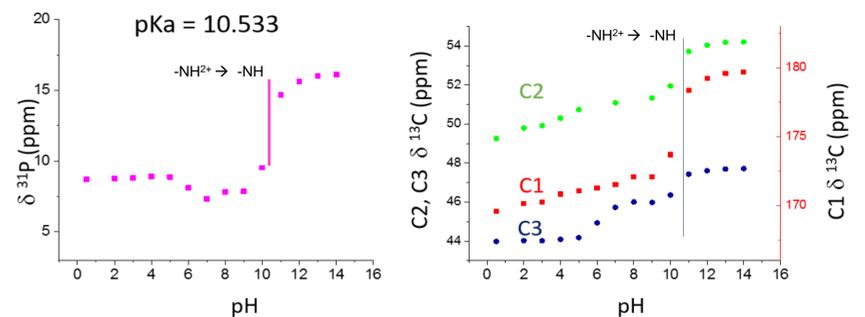
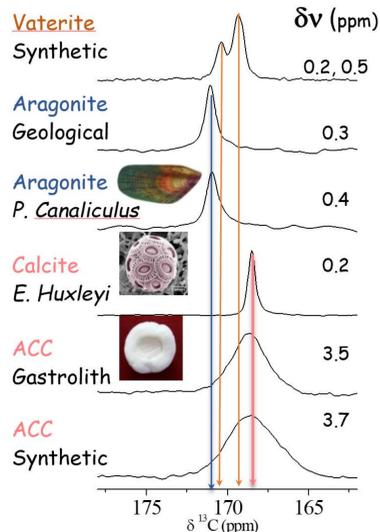


Figure 6: 162.08MHz, 100.68MHz ³¹P and ¹³C solution NMR spectra of the Glyphosate solution (upper part) titration curves from as function of ³¹P and ¹³C NMR chemical shift as function of pH. (lower part). Glyphosate molecule in the middle.

Polymorph ¹³C SSNMR Fingerprint [Figure 7] (2)



Chemical shift: polymorph "id": local structure

Peak width: spread of structural environments uniform vs. heterogeneous crystalline vs. disorder

Precipitates Characterization by Solid State NMR

In solution NMR peaks are narrow because of the rapid tumbling of molecules. In solid state NMR limited mobility results in spectra with broad peaks. To make peaks narrower again: sample is spun at the "magic angle" [Fig. 8].



¹³C NMR [Fig. 9]:

ACC is stable upon heating 250C

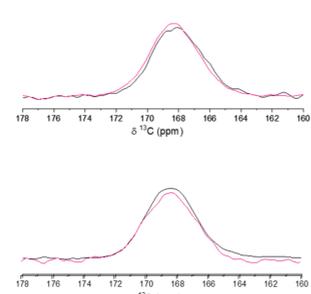


Figure 9

³¹P NMR [Fig. 10]:

Glp in two forms: protonated and deprotonated Amine. Deprotonated form is dominant in Low Glp ACC; they are about the same in High Glp ACC: These populations become equal in low Glp ACC upon heating.

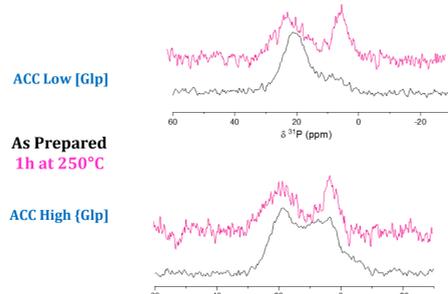
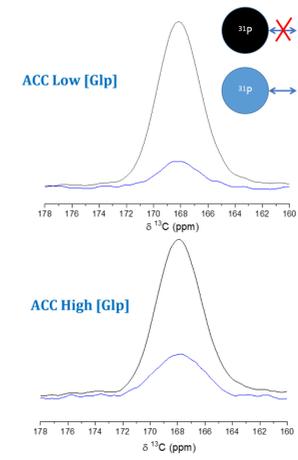


Figure 10

REDOR ¹³C-³¹P [Fig. 11]



ACC Low [Glp]: Glp is within 10Å distance of 17% of the Carbonates.

ACC High [Glp]: Glp is within 10Å distance of 27% of the Carbonates.

Glyphosate forms a solid solution within the ACC.

Figure 11: ¹³C(³¹P) CP-REDOR MAS NMR spectra of the as prepared ACC Low [Glp] and ACC High [Glp] S0, black and difference spectrum, ΔS, blue, are overlaid. While the S0 spectrum accounts for all carbon species, the difference spectrum ΔS (blue) shows peaks only for species that are nearby a P atom. The recoupling period is 64T_R (6.4 ms).

❖ After heating at 250 C: water goes out (1H NMR not shown), Glyphosate remains dispersed, and the amorphous "lattice" contracts.

Summary & Conclusions:

- ❖ Precipitation of Calcium Carbonate in the presence of High and Low [Glp] produce ACC (FTIR and NMR Figs. 5 & 9).
- ❖ REDOR NMR shows Glp is homogeneously and randomly dispersed throughout ACC (Fig. 11).
- ❖ Embedded Glp exhibits deprotonated and protonated states (titration NMR Figs. 5 & 10).
- ❖ ACC Low & High Glp are thermally stable to 250 C; both partially transform to Calcite at 400 C (Figs. 5, 9 and 10).
- ❖ Along with the information presented above, more work needs to be done to fully understand the stabilization process of ACC presented in our system.

References:

- (1) S. Kababya, A Gal, K Kahil, S Weiner, L Addadi, A Schmidt Journal of the American Chemical Society 137, 990-998
- (2) Ira Ben Shir, Shifi Kababya, and Asher Schmidt Isr. J. Chem. 2014, 54, 74 - 85

Acknowledgements:

We would like to thank Dr. Shifi Kababya and Prof. Asher Schmidt for hosting and guiding us through our research in their laboratory. We would also like to thank the foundations and donors for their generous support of the SciTech Program.