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Do organic contaminants influence the formation of calcium carbonate structures in simulated biological environments?

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Background

Contaminants of emerging concern (CECs) are pollutants that may cause adverse environmental impacts but are typically not regulated under current environmental laws. Organic CECs are commonly used in food additives, personal care products and pharmaceuticals, have high chemical stability and are usually not removed during conventional wastewater treatment (Brumovsky et al., 2017). Recent studies report the identification of many such chemicals in estuarine and coastal marine waters (Mijangos et al., 2018) where they can occur at concentrations equivalent to or exceeding that of natural dissolved organic matter (Sommerville and Preston 2001).

Organic molecules can play significant roles in controlling the precipitation of calcium carbonates ($CaCO_3$) and regulate the formation of shells, tests and skeletons by marine calcareous organisms (Kellock et al., 2020). Amino acids can promote $CaCO_3$ precipitation rate by decreasing the energy barrier to the attachment of solutes (Elhadj et al., 2006) or may inhibit precipitation by blocking subsequent ion attachment (Sikirić and Füredi-Milhofer, 2006) or by binding the $Ca^{2+}_{(aq)}$ required for $CaCO_3$ precipitation on carboxylic acids groups (Tong et al., 2004). Organic molecules also affect $CaCO_3$ strength and hardness (Kim et al., 2016). Foraminifera and corals transport seawater (and any dissolved contaminants therein) to their calcification sites where they increase the seawater pH and alter its dissolved inorganic carbon (DIC) chemistry to promote $CaCO_3$ precipitation (Allison et al., 2021). In this research we investigated how CECs at the calcification site could affect the formation and structure of aragonite, the biomineral formed by reef building corals. We conducted aragonite precipitations *in vitro* in an apparatus simulating the conditions at the coral calcification site.

Study

Our group has recently built apparatus to precipitate $CaCO_3$ under conditions analogous to those of the calcification sites of marine organisms (Kellock et al., 2020, Figure 1). The coral biomineralisation process is not completely understood. Until recently, the skeleton was believed to form at an extracellular calcification site between the base of the coral tissue and the underlying skeleton and to proceed via the attachment of aqueous ions (CO_3^{2-} and Ca^{2+}) to the existing aragonite skeleton

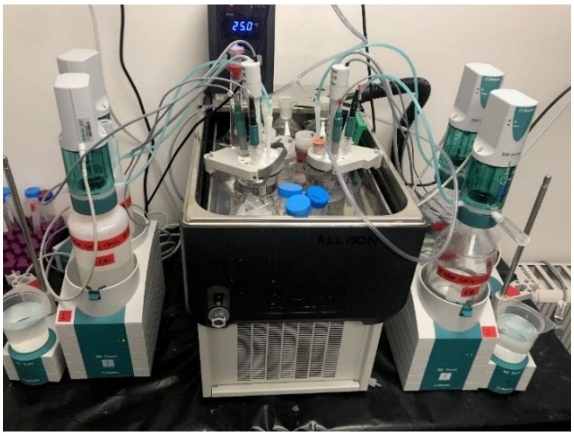


Figure 1. The precipitation apparatus. Synthetic aragonite was precipitated from seawater using a pH stat titrator (Metrohm Titrando 902). Precipitation of aragonite decreases the pH of the seawater solution and triggers the addition of titrants (Na_2CO_3 and $CaCl_2$ to replace the ions consumed in $CaCO_3$ formation. Experiments were conducted at 25°C.

(Allemand et al., 2011). Recent research suggests that coral aragonite also forms through a second crystallisation pathway, via intracellular formation of amorphous calcium carbonate (ACC) precursors in vesicles in the basal layer of coral cells, the calicoblastic epithelium (Mass et al 2017, Sun et al., 2020). The contents of the vesicles are released at the base of the cells and the ACC particles move through the extracellular calcification media and attach to the skeleton, converting to aragonite either during transit or after attachment (Sun et al., 2020). In either case the coral is believed to increase the pH of the calcification media.

For this project we precipitated $CaCO_3$ under the typical pH and DIC of marine organisms in the presence and absence of the CEC acetylsalicylic acid (commonly known as aspirin). Experiments were conducted at pH=8.445, [DIC]=4000, $\Omega_{aragonite}$=11 and pH=8.334, [DIC]=3000, $\Omega_{aragonite}$=7. The first scenario is representative of conditions at the coral calcification site (Sevilgen et al., 2019) while the second presents a lower saturation state, as likely occurs under ocean acidification. Aspirin has been detected in coastal seawater at concentrations up to 8.3 mg/l (Arpin-Pont et al., 2016) i.e. ~ 46 µM, and we conducted experiments at 0, 15 and 1500 µM aspirin.

Our preliminary data suggests that aspirin does not significantly affect aragonite precipitation rate at the typical concentrations occurring in coastal waters (Figure 2). However aragonites precipitated in the presence of aspirin have smaller and more spiky crystals than those precipitated without aspirin (Figure 3). Further work to use Raman spectroscopy to explore the influence of aspirin on the aragonite structure is ongoing.

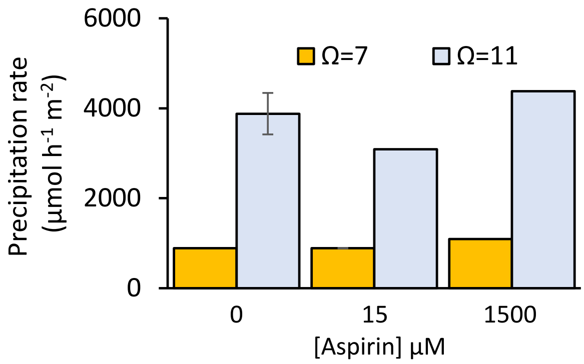
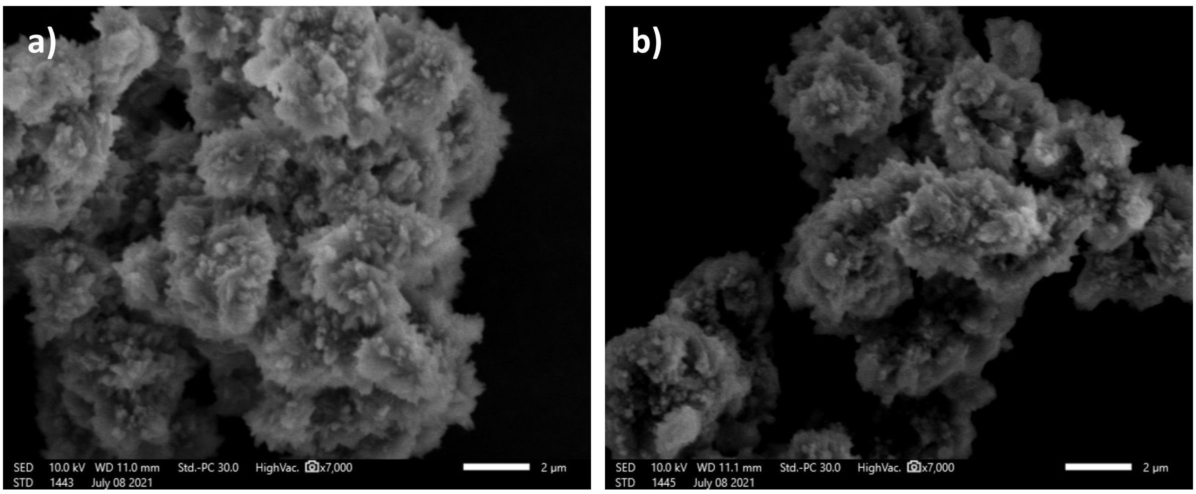


Figure 2. Aragonite precipitation rates from seawater *in vitro* as a function of [aspirin] at $\Omega_{aragonite}$ = 7 or 11. Error bars indicate standard deviations of replicate precipitations (n=2).

Figure 3. Scanning electron micrographs of aragonites precipitated a) with no aspirin and b) in the presence of 10 mM aspirin at Ω=11. Scale bars are 2µm.



Acknowledgements

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