Chemical Gardens Under Mars Conditions: Imaging Chemical Garden Growth In Situ in an Environmental Scanning Electron Microscope

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Abstract We have performed and visualized chemical garden growth experiments directly in situ in an environmental scanning electron microscope. The microscope chamber simulates the surface of Mars. We demonstrate that chemical gardens can form under the conditions of temperature and pressure existing on Mars in the presence of liquid water, silicates, and metal salts. Using chemical gardens from aluminum salts, tubes were formed where the external surface is formed principally by silicate and the inner surface is predominantly aluminum oxide-hydroxide. It should thus be expected that similar growths of metal salts may be found in geological explorations of water-rich environments during the search for life on Mars.

Plain Language Summary Mars today is a desert planet with scarcely any water, but we know that it used to have much more. So the search for life on Mars now is a search for water in the desert, and the search for fossil life is a search for places where there used to be water. We show with experiments in an environmental scanning electron microscope that chemical gardens should form on Mars under these conditions. It is important to be aware of this so as not to fall into the trap of thinking that these lifelike forms are fossils, but also important because on Earth, so perhaps on Mars too, chemical gardens may be where life was incubated.

1. Introduction

Although we now know that there are no canals on Mars, nonetheless the picture painted by that dramatic illusion of nineteenth-century astronomy and twentieth-century science fiction turns out to be broadly correct: Mars is a desert planet that has gradually lost its water (Kass & Yung, 1995). It is possible that life may no longer be extant on Mars but may have evolved there in earlier geological periods in which liquid water was common on the surface of the planet (Bibring et al., 2006), and it is also possible that there may still exist life on Mars in environments where liquid water persists (Davila & Schulze-Makuch, 2016). These hypotheses underlie the current search for signs of life, either extant or fossil, on Mars (Knoll & Grotzinger, 2006).

In the recent past, and even today, morphology has been and is taken as a good indicator for life (Jav-ux, 2019). It goes without question that if one digs up a dinosaur fossil, that is a reliable indicator that such an organism once lived. But for smaller, particularly for single-celled organisms, morphology is an increas-

ingly unreliable indicator of a biotic origin, because there exist abiotic processes that produce extremely lifelike forms (Livage, 2009). Chemical gardens, so called from their resemblance to biological structures, are one such system (McMahon, 2019) that has been known since the beginnings of chemistry (Glaub-

er, 1646). They are today of interest both because they demonstrate how one might make progress with the nascent technology of non-equilibrium self-assembled materials (Barge et al., 2015), and also because geological chemical gardens found in alkaline hydrothermal vents on Earth may have been the places where life on Earth got started (Barge et al., 2017; Cartwright & Russell, 2019; Cardoso et al., 2020; Martin & Rus-

sell, 2007; Martin et al., 2008; Russell et al., 1989; Sojo et al., 2016).
Primary minerals on Mars are similar to basaltic rocks on Earth (Taylor & McLennan, 2009). This implies a preponderance of silicate minerals containing iron, magnesium, and aluminum, in that order of abundance (McSween et al., 2009). Today, the surface of Mars is extremely dry (McKay et al., 1992), but there are indications that liquid water was once extensive over the surface (Di Achille & Hynek, 2010), as it is on Earth today. We know that water has been present on Mars from the presence of abundant fluviodeltaic and lacustrine geomorphological features. There may exist specific places on the planet’s surface or in the subsurface where liquid water may still be present (Fischer et al., 2014). We note the recent detection of subglacial water in southern polar layered deposits (Lauro et al., 2021). These liquid environments on modern Mars are most likely of salty water: brines (Rivera-Valentín et al., 2020; Stamenković et al., 2018).

The search for both fossil and extant life on Mars is thus particularly focused on investigating environments where water once existed, and environments where water may still exist near to the surface. Secondary minerals formed through hydrothermal alteration and weathering of primary basaltic minerals have also been found on Mars; some are minerals requiring liquid water for their formation (Ehlmann et al., 2009; Murchie et al., 2009). Carbonates, nitrates, perchlorates, aluminosilicates, sulfates are examples of such minerals. For instance, sulfates have been found in a number of locations containing fluvial landforms indicating that abundant water was once present, including near Juventae Chasma, Ius Chasma, Melas Chasma, Candor Chasma, and Ganges Chasma (Weitz et al., 2010), and at Cross Crater, Terra Sirenum (Ehlmann et al., 2016), and nitrates have been detected in mudstone deposits at Gale Crater (Stern et al., 2015). It is these brine environments in which the search for life on Mars is concentrated (Davila & Schulze-Makuch, 2016; Knoll & Grotzinger, 2006).

To explore whether chemical garden structures may be found in such environments, we reproduce Mars pressures and temperatures in an environmental scanning electron microscope, into which we place a small amount, a drop, of liquid water containing sodium silicate, together with which we put a grain of aluminum sulfate or nitrate as examples of possible Martian chemistries that may be encountered in these environments. Hydrated aluminum sulfate has been observed on Mars as alunite (Ehlmann et al., 2009) and a range of hydrated aluminum sulfates could potentially occur on Mars (Cao et al., 2019). Hydrated transition metal sulfates probably form as a consequence of the acidic weathering of basaltic minerals, something that happens today around fumaroles on Earth, and variously hydrated iron and magnesium sulfates have also been observed on Mars. Although aluminum nitrate has not yet been detected, nitrates are being sought, being an important part of biogeochemical cycling on Earth (Wang et al., 2018), and nitrates have been detected in the soil at Gale Crater (Stern et al., 2015). Silica-rich, alkaline fluids can be produced by serpentinization (oxidative hydration/hydrolysis of iron and magnesium silicates) (Sleep et al., 2004), and this could provide the other reactant for chemical garden growth (Sainz-Díaz et al., 2018). Serpentinization is known to have occurred on Mars (Ehlmann et al., 2010), and other workers have suggested that alkaline, silica-rich fluids are likely to have circulated in the subsurface (Michalski et al., 2013).

Chemical gardens are plant-like structures formed in a non-equilibrium self-organizing process that creates complex structures at the microscale (Barge et al., 2015; Cardoso et al., 2020). These biomimetic structures are chemobrionic systems that offer confined microspaces within which prebiotic reactions could occur in micro- or nano-reactors (Ding et al., 2019). These chemobrionic structures are formed when a seed crystal of a metallic salt is immersed into an anionic solution, often of silicate, but many other anions, phosphate, carbonate, etc. also. In this solution, the crystal of the salt starts to dissolve, forming a gel around it, which acts as a semipermeable membrane. The water from the silicate solution flows in through this membrane toward the salt crystal driven by osmotic pressure. Then, the crystal continues dissolving and the internal volume increases. When the pressure inside the membrane rises, the membrane can break, forming a hole. A jet of the internal fluid flows through the hole and flows up under buoyancy forces, and the contact of this fluid with the external solution produces the precipitation of silicate owing to the different pH of the internal and external solutions, forming the walls of tubes (Barge et al., 2015).

Previous planetary science work has examined the possibility that carbonate-hydroxide chemical gardens might be found in the soda ocean of Enceladus (Cardoso et al., 2019); that ice brinicles, oceanic tubular ice formations akin to submarine vents on Earth (Cartwright et al., 2013), might hang from the ice cover of subsurface oceans on Europa and other icy ocean worlds (Vance et al., 2019). These works have discussed how differences in gravity on other celestial bodies will alter chemical-garden tube growth rates and sizes.
compared to on Earth, as the fluid mechanics of buoyancy is affected by differences in $g$, but that the chemistry and the microstructure are most probably less affected. As in the case of Mars, all of these possible instances are of interest for the emergence of life, being far-from-equilibrium geochemical systems possessing thermodynamic and chemical phenomena that are driven into being by these disequilibria (Barge et al., 2017).

We have performed chemical garden growth experiments directly in situ in an environmental scanning electron microscope (ESEM) chamber. An ESEM allows the presence of water in the chamber during imaging. The pressures involved, in the range of hundreds of Pascals, and the temperatures, around 0°C (273 K), are not dissimilar to those that may be present, for example, on the surface of Mars today (Haberle, 2015) (~400–800 Pa; ~150–300 K). So it is of interest to try to form a chemical garden in situ within an ESEM both to observe the growth process directly, and also to simulate the formation of chemical gardens in extreme conditions of pressure and temperature, such as on Mars. The Mars atmosphere in bulk is 95% CO$_2$ and is tremendously dry, but very locally, near to sources of liquid water, the quantity of water vapor will be much greater than in the atmosphere in toto. Our simulated Mars conditions are of a locally wet environment in the surface or subsurface with a high partial pressure of water vapor.

2. Materials and Methods

Crystals of the aluminum salt hydrates, Al$_2$(SO$_4$)$_3$$\cdot$16H$_2$O and Al(NO$_3$)$_3$$\cdot$9H$_2$O at analytical purity were used. For the laboratory experiments at atmospheric (or room) conditions, these salts were homogenized with an agate mortar and pressed into 200 mg cylindrical pellets of 13 mm diameter and 1 mm height using a cell at 10 bar of pressure during 10 min to avoid initial conditions with different shapes and to obtain a systematically uniform composition. The sodium silicate solutions were prepared from a commercial concentrated solution composed of 27% SiO$_2$ and 15% NaOH. They were diluted with milli-Q water to several concentrations between 3 and 1 M.

The sample micrographs were obtained with a FEI Quanta 400 Environmental Scanning Electron Microscope (ESEM). Experiments were performed inside the ESEM chamber using water vapor as both imaging gas and hydrating agent. The temperature was controlled with a K-P Peltier stage. The silicate salt solution was equilibrated at 275 K. ESEM chamber evacuation was carried out through purge cycles keeping the range of pressures between 1,300 and 706 Pa at isothermal conditions (275 K). The reaction occurs inside the ESEM chamber, which is stabilized at around 700 Pa and constant temperature. In order to minimize scattering, we used a gaseous secondary electron detector (GSED) to visualize the samples at working distances of 6–8 mm and high vapor pressure. The samples were completely dried to observe the final result of the reaction. The microanalyses were performed at a pressure of approximately 67 Pa and working distance of 10 mm. To visualize compositional images at high vacuum conditions, the chemical gardens were taken out from the chamber and coated with carbon (EMITECH K975X). A back-scattered electron detector (solid state detector, SSD) set just above the specimen was used for obtaining images, while a Bruker detector XFlash 6/30 was used (range between Be-Am) for microanalysis and elemental mapping.

Chemical analysis of the solids was performed in situ in the microscope using Energy-dispersive X-ray spectroscopy (EDX) analysis. Subsequently the whole structure was ground in an agate mortar to be analyzed by Powder X-ray Diffraction (XRD) in a PANalytical X’Pert PRO diffractometer with a wavelength of 1.5418 Å. The identification of crystallographic phases in the diffractograms was performed with the Xpowder code (Martin, 2012).

The seed and solution were put into contact immediately before introducing them to the ESEM chamber. Reference experiments outside the ESEM chamber indicated that no reaction is produced before the first two minutes of mixing seed and solution at room temperature. Hence, at 275 K, we can consider that the reaction has not begun before entering the ESEM.

We chose aluminum salts, sulfate, and nitrate as reagents. A micrograin of an aluminum salt was placed on the sample support and a drop of approximately 0.05 ml of aqueous silicate solution was added. The sample support was previously cooled to 275 K with a K-T Peltier stage placed below it. The silicate salt solution was equilibrated at 275 K. ESEM chamber evacuation was carried out through purge cycles which kept relative
humidity (RH) at around 100%, that is, 5 purge cycles conducted at decreasing gas pressures, from 1,300 to 706 Pa, and constant temperature of 275 K. The vapor condenses when the partial water vapor pressure of the gas is higher than the saturated water vapor pressure; in the opposite case the liquid evaporates. Equilibrating the low vacuum and the internal vapor partial pressure of water it was possible to image the in situ formation of chemical garden inside the ESEM chamber, which is stabilized at around 700 Pa and constant temperature, and thereby to follow the growth in real time; see movie in supplementary material. A sample support with the ability to alter the tilt angle was used for a better 3D observation of the material formed.

3. Results

With 3 M silicate solution, growth was slow and the tips of the initial tubes were closed (Figure 1a). Using 1 M silicate solution a set of multiple tubes totaling 100 μm in diameter was formed (Figures 1b and 1c). This structure is wider at the top, having a planar upper surface and a total height close to 600–800 μm. This planar morphology of the upper part may be formed when this growth, like the trunk of a micro-tree, reached the upper surface of the liquid drop. The upper part is constructed of many linear fibers that can be observed in the inner part of this structure (Figures 1d and 1e).

It is probable that this micro chemical garden formed within a large drop of sodium silicate solution growing from the micro-seed in the bottom of the drop to the top surface inside the aqueous solution. This drop thus acts as a 3D nanoreactor (Ding et al., 2020). This structure was visualized after evaporating the water by lowering the chamber pressure. In general, the external surface is smooth and the internal surface is more complex. In the internal surface of the osmotic balloon, we observe bell-shaped forms, reminiscent of biomimetic forms of jellyfish, that have grown from the surface inwards to the internal solution (Figure 1f).

For the chemical analysis of these forms we combine EDX for elemental compositions on different surfaces (Figure 2) and XRD for crystalline phases (Figure 3). This combined analysis has been successfully applied to identify chemical composition in chemical gardens (Cartwright, Escribano, & Sainz-Díaz, 2011).
chemical analysis of these forms shows the presence of sulfur along with silicon oxides, together with a small amount of aluminum silicate (Figure 2a). This sulfur content is due to the presence of sodium sulfate found by XRD; see below. The amount of sodium found inside the osmotic balloon is not uncommon, it has been observed before in similar systems (Cartwright, Escribano, Khokhlov, et al., 2011; Cartwright, Escribano, & Sainz-Díaz, 2011), indicating that sodium cations have crossed the osmotic membrane, probably on account of their small size. When the sodium ions reach the internal solution, they encounter a high concentration of sulfate anions in a medium with different pH conditions, as we know from previous work (Barge et al., 2015), and hence the sodium sulfate precipitates. The highest concentration of sodium sulfate is seen in the medusa-like forms observed in Figure 1f. The external surfaces of the tubes are mainly silicon oxide with sodium silicate (Figure 2b). The relative amount of aluminum is larger in the internal surface of tubes (Figure 2c). However, no sharp boundary can be observed between silicon and aluminum oxides, and aluminosilicates with a gradient of aluminum content are probably formed.

Figure 2. Energy-dispersive X-ray spectroscopy microanalysis spectra of the main components of the formations observed in an environmental scanning electron microscope with 1 M silicate solution. (a) Internal surface of the lower osmotic balloon (Figure 1f), (b) external surface of tubes, and (c) internal surface of tubes.

Figure 3. Powder X-ray diffractogram of the solid formed with aluminum sulfate and an aqueous solution of (a) 1 M and (b) 3 M sodium silicate. a: aluminum hydroxide, Si: aluminum silicates, s: sodium sulfate.
The XRD patterns of these materials formed from 1 M sodium silicate showed a highly disordered amorphous solid phase (Figure 3a). In the sample formed with 3 M silicate, a certain proportion of amorphous phase is also observed, but additional reflections are detected (Figure 3b). Reflections corresponding to sodium sulfate can be seen, marked s in Figure 3b, along with other reflections that may tentatively be assigned to aluminum oxide/hydroxides or aluminum silicates (marked with a and Si, respectively). Nevertheless, a definitive assignment is not possible with this resolution.

The general behavioral difference between the processes using 1 and 3 M sodium silicate solution concentrations is also observed outside of an ESEM chamber. There, 1 M sodium silicate solution yields larger tubular forms than does 3 M solution (Figure 4). In these macroscopic systems, a planar morphology is also formed on the top surface of the liquid, similar to that observed in the nanoreactor of a drop in the ESEM chamber.

Using aluminum nitrate as the seed, unlike with aluminum sulfate, no micro-tree was obtained inside the ESEM chamber. A smooth surface was formed on one side; the other side is seen to be a foam with great porosity (Figure 5a). In some cases microspheres connect a fibrous network (Figures 5b–5d). Only occasionally were microtubes noted (Figure 5e). The initial concentration of sodium silicate solution was 0.5 M in these experiments. However, as in all these in situ experiments, the concentration increases over time owing to the constant evaporation of water during the experiment. A fast evaporation of water in the chamber was found to yield these foams.

EDX analysis and a mapping of element distribution was performed on the materials formed inside the ESEM chamber with aluminum nitrate (Figure 6). In the smooth surface and in the highly porous zone, the main component is sodium silicate. The aluminum and nitrate are in the same phase indicating that the reaction forming aluminum silicate was not produced.

Our present results indicate that it is possible to form chemical gardens in extreme conditions of pressure, temperature, and strong electron radiation and electrical voltage. The porous microstructures formed are nanoreactors that are ideal confined environments within which aqueous chemistry can take place (Ding et al., 2019). The ESEM chamber has conditions not found in our terrestrial environment. But the extreme conditions of an ESEM chamber used in this work could occur on other planets and on other Solar System bodies where salts are exposed to conditions of low atmospheric pressure, low temperature, and under
strong radiation. Mars is one such celestial body, so that we can reproduce in the ESEM Mars surface brine conditions.

4. Discussion

We have in past research compared cations from group II of the periodical table (Cartwright, Escribano, Khokhlov, & Sainz-Díaz, 2011), and transition metal cations from period 4 (Cartwright, Escribano, & Sainz-Díaz, 2011), as seeds for chemical garden formation. We found that there are differences in reactivity, tube morphology and mechanical properties. These differences are based on experimental conditions, concentrations, and different solubilities of the silicate and oxide-hydroxides of these cations, and the permeability of the membranes. As reported in previous works of ours and of others, the anionic nature of the seed salt has little effect on the chemical gardens growth. Collins et al. (1999) found catalytic properties

Figure 5. Micrographs of porous microstructures formed inside the environmental scanning electron microscope chamber with an aluminum nitrate seed and 0.5 M sodium silicate solution.

Figure 6. (a) Back-scattered electron image, (b) map of element distribution, and (c) chemical analysis of external surface of a microstructure formed inside the environmental scanning electron microscope chamber with an aluminum nitrate seed and 0.5 M sodium silicate solution.
in the powder derived from aluminum silicate chemical gardens. This group also explored the formation of chemical gardens with aluminum nitrate in silicate solution, observing a hierarchical microstructure of microtubules (Collins et al., 1998). Chemical gardens can be also formed under microgravity conditions in space, as was done previously in the Space Shuttle (Jones & Walter, 1998) and the International Space Station (Cartwright, Escribano, Sainz-Díaz, & Stodieck, 2011).

In previous work, we simulated the conditions of interplanetary space in a cryo-ESEM chamber in which we grew amorphous ices (Cartwright et al., 2008). In this work, we have produced chemical gardens in situ within the chamber of an environmental scanning electron microscope. An ESEM is a laboratory that allows us to observe and simulate different abiotic and biotic processes of extreme environments; some of these are possible analogs to the conditions on Mars.

Despite some limitations, the ESEM can be considered as a tool able to provide valuable information on the genesis of the chemical gardens at particular pressure and temperature conditions. Furthermore, an ESEM can be used to estimate an approximate elementary chemical composition of the product which is forming during the process. However, it is difficult to obtain precise element information, since the work conditions at a certain gas pressure within the ESEM chamber implies some degree of dispersion of the primary electrons (skirt effect; see Stokes (2008) for a detailed description). With these caveats in mind, we nevertheless consider that the information obtained during in situ growth can serve as a complement to the results yielded by other experiments and analyses.

Chemical gardens are more likely to have formed on early Mars than today since water was much more abundant 3 or 4 billion years ago, and early Mars was a more dynamic world geochemically than it is today. The point for astrobiology is not just that we find chemical gardens on Mars, but that we find them preserved in martian rocks that formed in habitable environments, perhaps hydrothermal systems, 4 billion years ago. Nevertheless, even today, with Mars a frozen desert, there is a possibility for chemical gardens to form should silica-rich, alkaline fluids ever make contact with suitable salts. Chemical gardens could potentially have formed on Mars at any point in the past 4 billion years. The possible presence of these chemical gardens on Mars should be taken into account when planning the search for life on Mars, so as not to fall into the morphology trap, as has too often happened in the past. On one hand we should not be surprised to find chemical gardens on Mars. On the other hand, on Mars as on Earth, it is possible that compartments within such chemical gardens are nanoreactors that may have acted as incubators for the first life, these have been termed warm little pores (Ding et al., 2019), and so their presence is compatible with the emergence of life.

**Data Availability Statement**

The data obtained from the analyses of the samples produced in the laboratory experiments are given in the figures. Data sets for this research are available in http://hdl.handle.net/10261/237126.

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