

A Mars-analog sulfate mineral, mirabilite, preserves biosignatures

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ABSTRACT

Various sulfate minerals exist on Mars; except for gypsum, they are understudied on Earth. Extremophiles have been documented in modern gypsum and halite and ancient halite, but other chemical sediments have not been evaluated for biosignatures. Here, we present the first observations and analysis of microorganisms and organic compounds in primary fluid inclusions in the Mars-analog mineral mirabilite, $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$, from Great Salt Lake, Utah, USA. Microscopy by transmitted light and ultraviolet-visible (UV-vis) light, and Raman spectroscopy, show abundant bacteria and/or Archaea, algae, fungi, diatoms, protozoa, and organic compounds such as beta-carotene. This discovery expands our current knowledge of biological materials trapped in salt and aids the search for life on Mars, both for sample selection by rover and for analyses of return samples on Earth.

INTRODUCTION

The Great Salt Lake in Utah (USA) hosts Na_2SO_4 -saturated springs with cryobrine that develop during the winter at air temperatures $<5^\circ\text{C}$ (Fig. 1; Jagniecki et al., 2021). Mirabilite ($\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$), a hydrous sodium-sulfate mineral, precipitates from these springs. Mirabilite forms as ~ 1 – 10 -cm-long, clear, bladed and tabular crystals. Continuous hydrologic discharge creates multiple layers of mirabilite, forming crystalline terraced mounds. The mirabilite precipitates in an aqueous environment with a diversity of species of halophilic microorganisms, many of which are tolerant of hypersalinity, high irradiation, and desiccation (i.e., Baxter et al., 2005; Weimer et al., 2009; Tazi et al., 2014; Jones and Baxter, 2017). The goal of this study was to determine whether mirabilite entraps primary fluid inclusions and whether those fluid inclusions contain microorganisms.

From studies of the chemical sediments halite and gypsum, we know that fluid inclusions form along growth bands as the host crystal grows (Goldstein and Reynolds, 1994). These primary fluid inclusions contain parent surface brine. Fluid inclusions and/or their different organic

phases, including solid, liquid, and gaseous organic compounds, can be observed and analyzed in situ and non-destructively (Norton and Grant, 1988; Lowenstein et al., 2011; Conner and Benison, 2013; Winters et al., 2013; Benison and Karmanocky, 2014; Benison, 2019).

There is a variety of hydrated and anhydrous sulfates on Mars, strongly suggesting past aqueous activity and possible habitable environments (e.g., Clark, 1978; Clark and Van Hart, 1981; Squyres et al., 2004; Clark et al., 2005; Murchie et al., 2009; Ehlmann and Edwards, 2014). It is likely that water on Mars was hypersaline. The presence of mirabilite on Mars is a possibility. Although mirabilite dehydrates to thenardite at some places on Earth when exposed, it can remain unaltered in cold environments and at shallow depths (Azzaro et al., 2022). It is possible that cold environments, shallow burial, and/or coating with Fe-oxides and/or dust may have protected any mirabilite on Mars from irradiation and/or dehydration. A discovery of biological materials in mirabilite would expand our knowledge about habitability and preservation style of life on Earth and for potential life on Mars.

Water enables the survival of microbial cells over vast time periods, whether in fresh water, ice, seawater, brines, or other contexts (Hallsworth, 2021). Several studies have shown that a range of extremophilic microorganisms survive

over geologic time scales within primary fluid inclusions in halite and gypsum (Vreeland et al., 2000; Lowenstein et al., 2011; Benison, 2019; Schreder-Gomes et al., 2022). However, other chloride and sulfate chemical sediments have not been investigated for their microbiology. It is important to show that other salt minerals can preserve biological products over geologic time and can provide micron-scale ecosystems for life to continue post-mineral crystallization. Because Great Salt Lake contains a diverse assemblage of halophilic microorganisms and organic compounds, we hypothesized that mirabilite precipitating there would trap and preserve representative microorganisms and organic compounds from that ecosystem.

METHODS

Modern mirabilite crystals were sampled from surface deposits during the winter of 2020 at Great Salt Lake State Park (40.736788°N , 112.209550°W ; Fig. 1). Samples were placed in a double-sealed zippered plastic bag with a cloth saturated with mirabilite spring brine and stored in a refrigerator at -1°C to prevent dehydration to its anhydrous counterpart, thenardite (Na_2SO_4). Small chips of mirabilite crystals (~ 1 – 3 cm wide) were cut with a razor blade and hand polished with sandpaper and/or a glass plate to ~ 0.5 – 1 mm thickness. To prevent dehydration, samples were placed in a customized sample chamber (see Fig. S1 in the Supplemental Material¹).

Crystals were microscopically examined with an Olympus microscope with plane-transmitted light with long-working-distance objectives and magnifications of as much as $480\times$ to verify the presence of primary fluid inclusions, suspect microorganisms, and organic compounds. Ultraviolet-visible (UV-vis) light (at combined 330 nm and 385 nm wavelengths) was used to test for fluorescence.

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¹Supplemental Material. Experimental setup showing sample chamber used to prevent dehydration of mirabilite crystals and Raman signatures of mirabilite crystal, Great Salt Lake brine, and inclusion liquid. Please visit <https://doi.org/10.1130/G51256.1> to access the supplemental material, and contact editing@geosociety.org with any questions.

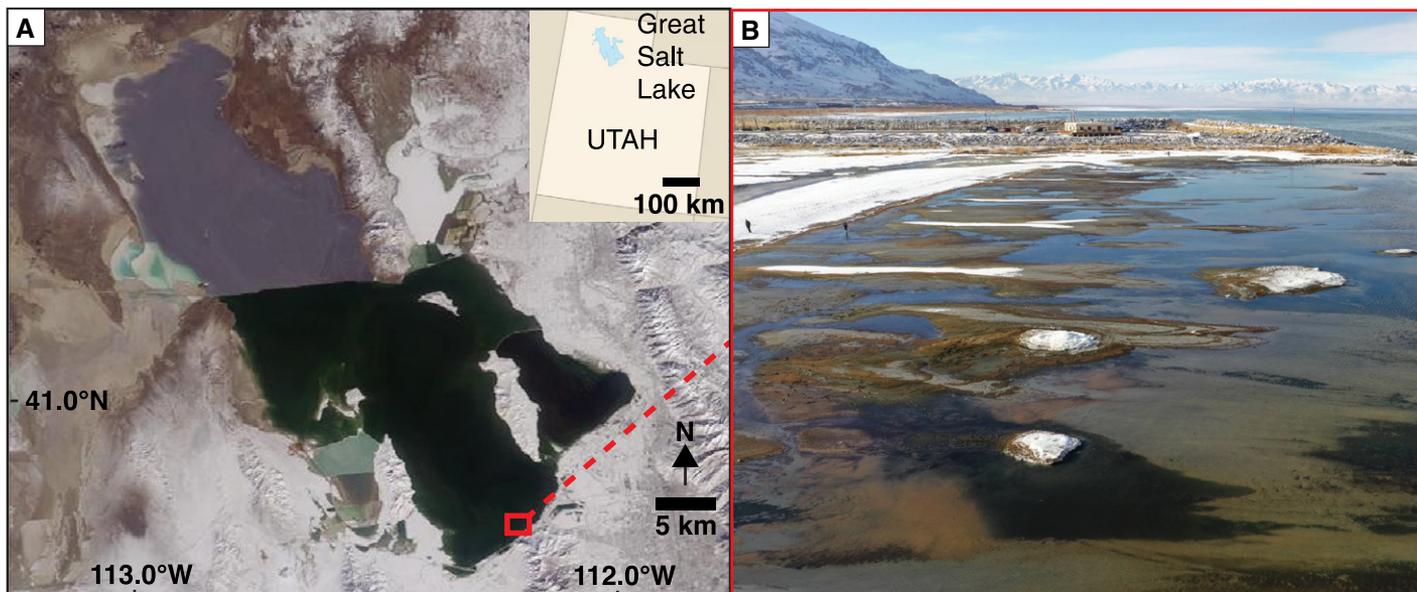


Figure 1. Great Salt Lake (Utah, USA). (A) U.S. Geological Survey Earthshot satellite image of Great Salt Lake; inset map shows location in Utah. (B) Unmanned aircraft system aerial photograph of spring pools with mirabilite mounds that formed during the winter of 2020.

Laser Raman spectroscopy was conducted with a Renishaw InVia Raman spectrometer and microscope with 50 \times , 100 \times , and 400 \times magnification to identify covalently bonded materials, including gases, liquids, and solids. Calibration was done with a silicon reference sample. The spectrometer used a green laser with an excitation wavelength of 532 nm. Spectra were acquired over an extended wavelength of \sim 100–5000 cm^{-1} with an exposure time of 10 s. The spectra were processed with CrystalSleuth software (<https://rruff.info>) and mineral identification confirmed by the RRUFF database (<https://rruff.info>) and published literature (Hamilton and Menzies, 2010).

RESULTS

Mirabilite from Great Salt Lake consists of clear, vitreous, bladed and tabular crystals \sim 1–10 cm in length. Fluid inclusions are abundant within the mirabilite crystals, aligned along assemblages as growth bands parallel to crystal faces (Fig. 2A). Primary fluid inclusions range from \sim 20 to 400 μm in length and are sub-hexagonal in shape. Some fluid inclusions are all liquid water; others contain suspect microorganisms and solid and/or liquid organic compounds in addition to the liquid phase. Rare inclusions also contain a spherical, dark gas bubble. There is a range of abundance of microorganisms and organic compounds within and among individual growth bands in single crystals as well as among different mirabilite crystals. Approximately 40% of primary fluid inclusions contain microorganisms and organic compounds. Some crystals have abundant primary inclusions and growth bands that do not contain any obvious microorganisms.

Plane-transmitted-light views of fluid inclusions allowed the observation of a diversity

of organisms in the primary fluid inclusions, including prokaryotes (bacteria and/or Archaea), algae, fungi, diatoms, protozoa, and ostracods (Fig. 2). Prokaryotes appear as clear to pale yellow, high-relief, 1–2- μm -diameter spheres and clear to pale yellow, high relief, 3–5- μm -long rods (Figs. 2B–2C and 2F–2G). All prokaryotes fluoresce pale green and pale blue when exposed to UV-vis light. The cells tend to cluster in a corner of the fluid inclusion (Fig. 2B) or near other microorganisms (Fig. 2F) and appear to constantly move within the fluid inclusions, consistent with characteristics for bacteria and/or Archaea or due to Brownian motion.

Suspect green algae are relatively abundant in mirabilite from Great Salt Lake (Figs. 2D–K and 2R–2S). They appear as pale green and orange or red (if they have trapped carotenoids) spheres and ellipsoids ranging in size from 3 to 7 μm in diameter. They fluoresce pink, orange, green, and blue. Single inclusions, as large as \sim 400 μm , contain multiple cells that are typically found as clusters of helical and ribbon-like structures. Many appear similar in appearance to *Dunaliella* algae, which are well documented in Great Salt Lake waters and common in other evaporites (Schubert et al., 2010; Oren, 2014). Algal cells are commonly in proximity to suspect brown-black fungal spores in fluid inclusions (Figs. 2R and 2S). Suspect fungal spores are 5–20 μm in diameter, dark brown or orange, and are seen as tight clumps intertwined in loose contact with the algae. They fluoresce blue. Coccolid and pennate diatoms are trapped as clusters in primary fluid inclusions (Figs. 2L–2O). They are clear to pale yellow and pale green in transmitted light and are generally 10–20 μm in diameter. They fluoresce a splotchy blue or pink when exposed to UV-vis light. Suspect proto-

zoa with flagella are also present in this mirabilite (Figs. 2P–2Q). Although most protozoa (likely euglenid) appear green, some are clear or splotchy yellow or orange and green in transmitted light and fluoresce pale white, pale blue, and pale pink. The sizes and morphologies of these various objects within the primary fluid inclusions, as well as their colors of fluorescence, strongly suggest the presence of microorganisms and beta-carotene (Mormile and Storrie-Lombardi, 2005; Nadeau et al., 2008; Schubert et al., 2009; Lowenstein et al., 2011; Conner and Benison, 2013; Winters et al., 2013; Benison, 2019).

The orange or pink fluorescent response is primarily due to carotenoid pigments in the membranes of microorganisms and is commonly associated with *Dunaliella*-like algal cells (Teller, 1987). Carotenoids are not only important for protection from irradiation and oxidative damage in photosynthetic organisms but can be potential extraterrestrial biomarkers in ancient sediment due to their long-term stability (Sies and Stahl, 2004; Simoneit, 2004; Vítek et al., 2009).

In order to recognize any organic materials with laser Raman spectroscopy, we first analyzed the host mirabilite crystal, spring brine, and inclusion liquids to confirm the mirabilite identification and to establish the background peaks from the host mineral and inclusion liquid (Fig. S2). The Raman spectra confirm that these crystals are mirabilite (Hamilton and Menzies, 2010). Brine from mirabilite mound springs and inclusion liquids have a similar Raman signature to mirabilite crystals. Raman spectra of organic material in primary fluid inclusions in mirabilite crystals have peaks consistent with beta-carotene standards at 1010, 1158, and 1518 cm^{-1}

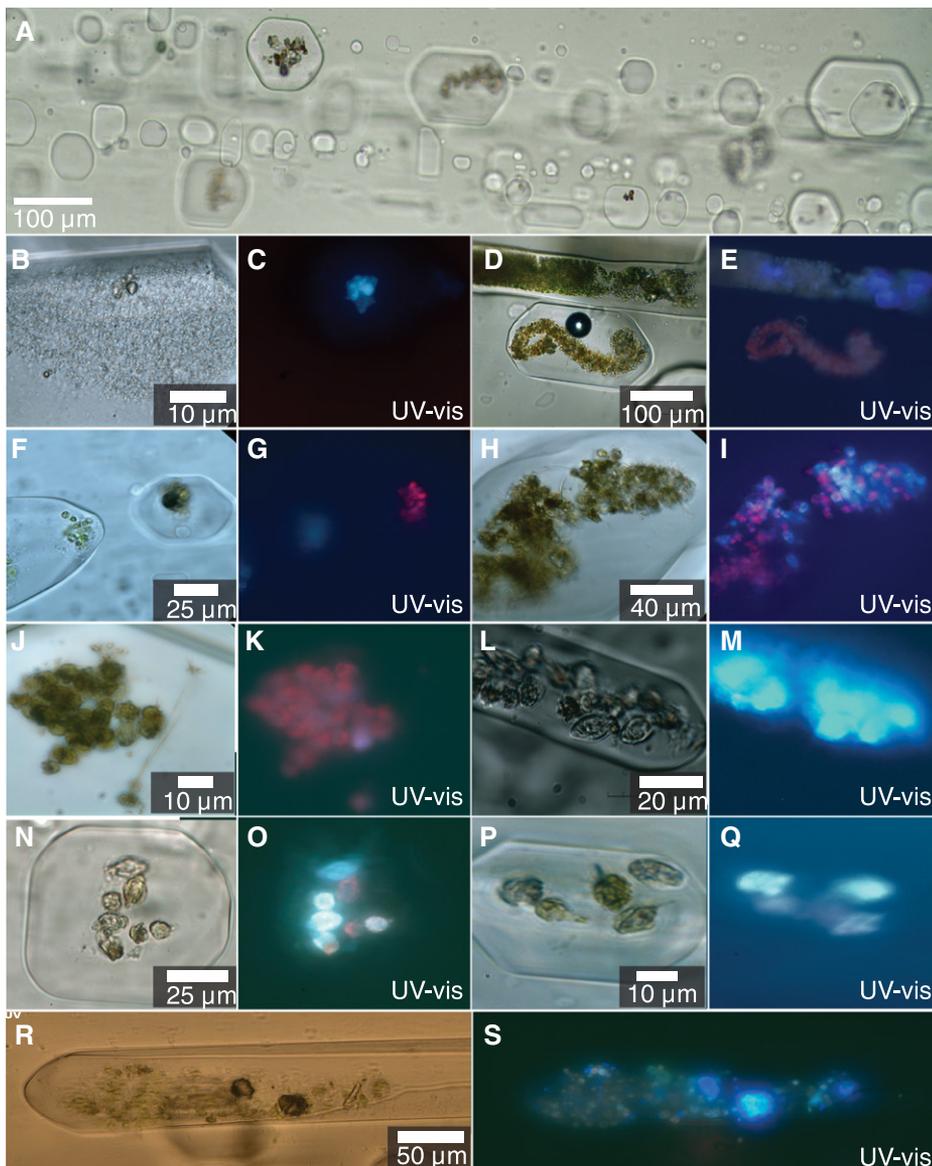


Figure 2. Microorganisms and organic compounds in primary fluid inclusions in mirabilite. (A) Primary fluid inclusions along growth bands with abundant microorganisms. (B–S) Paired photomicrographs taken with plane-transmitted light (left) and UV-vis light (right) to show fluorescence. (B–C) Prokaryotes. (D–E) Suspect green algae with carotenoid in helical and ribbon-like structure. (F–G) Green algal cells with prokaryotes. (H–K) Different forms of suspect green algal cells with carotenoid. (L–O) Coccoid and pennate diatoms. (P–Q) Protozoa with flagella. (R–S) Algal cells with suspect brown-black fungal spores.

(Fig. 3; Vítek et al., 2009; Osterrothová and Jehlička, 2011; Conner and Benison, 2013; Winters et al., 2013).

To test whether organics could be detected in the dehydrated form of mirabilite (thenardite, Na_2SO_4), we conducted an experiment in which we documented changes to a mirabilite crystal as it dehydrated. The crystal used was one in which abundant microorganisms had been documented. As the crystal was exposed to air ($\sim 25^\circ\text{C}$) on the microscope stage, we observed and documented changes at 5, 15, 30, and 45 min and 1, 3, 24, and 96 h with plane-transmitted light and UV-vis light. During desiccation, the crystal became a white powder that obscured the view of the organic material in plane-transmitted

light. However, UV-vis light produced fluorescent responses to localized spots in the white powder for at least several days after dehydration (Fig. 4).

DISCUSSION

The sulfate mineral mirabilite traps and preserves microbial life and environmental conditions in primary fluid inclusions. Extremophilic organisms, including bacteria and/or Archaea, algae, fungi, diatoms, protozoa, and ostracods, along with organic compounds such as beta-carotene, were documented within mirabilite from Great Salt Lake. Here, transmitted light and UV-vis petrography, paired with Raman spectroscopy, allowed us to both visualize and chemically

analyze organic materials. Furthermore, this approach studies the microorganisms as well as inclusion liquids and host minerals, both in situ and non-destructively. These methods should be conducted prior to destructive techniques, such as crushing bulk mineral samples or extracting fluid from individual inclusions, to understand the context and target types of microorganisms to be identified by more advanced biological methods (i.e., Vreeland et al., 2000; Mormile et al., 2003). Fluid inclusions in sulfate minerals are an important repository of organic materials that should be carefully examined in the search for ancient microorganisms on Earth, on Mars, and possibly elsewhere in the solar system.

Pockets of brine isolated and trapped as primary fluid inclusions in mirabilite crystals directly reflect the chemical environment of the parent liquid along with the microorganisms and molecules that were in the surface brine at the time of mineral precipitation (Goldstein and Reynolds, 1994; Lowenstein et al., 2011). Fluid inclusions are microenvironments within crystals that may serve as an oasis for microorganisms and organic compounds (Lowenstein et al., 2011). Although our study did not definitively identify the genus and species of these microorganisms, it showed: (1) that there is diversity and abundance of microorganisms in the mirabilite; and (2) the context of the microorganisms within primary fluid inclusions, showing that they were trapped during primary crystal precipitation in the depositional environment and not later.

Mirabilite is stable in saturated solution but unstable in much of the Earth's ambient surface atmosphere where it dehydrates to the mineral thenardite (Na_2SO_4). This decomposition is very rapid and can occur within minutes. However, there are some terrestrial environments, such as in glacier salt cones in Antarctica, where mirabilite is a stable mineral (Azzaro et al., 2022). Mirabilite is also potentially stable on Mars. Regardless, biosignatures may be preserved and detected by some methods, including UV-vis petrography, in the dehydrated daughter mineral.

CONCLUSIONS

As a result of this study, we propose that all sulfate minerals on Mars are potential hosts of biosignatures or even viable cells (Vreeland et al., 2000; Mormile et al., 2003; Lowenstein et al., 2011). The optical methods and Raman analysis we employ should be considered as a first step to guide further studies that may lead to chemical and DNA identification of microorganisms. Even if cells are no longer present, the chemistry of past fluids and potential biological molecules can provide clues about past life on Earth, Mars, and potentially elsewhere. In addition, the in situ and non-destructive nature of the observations and analysis eliminates concerns about possible contamination and may protect

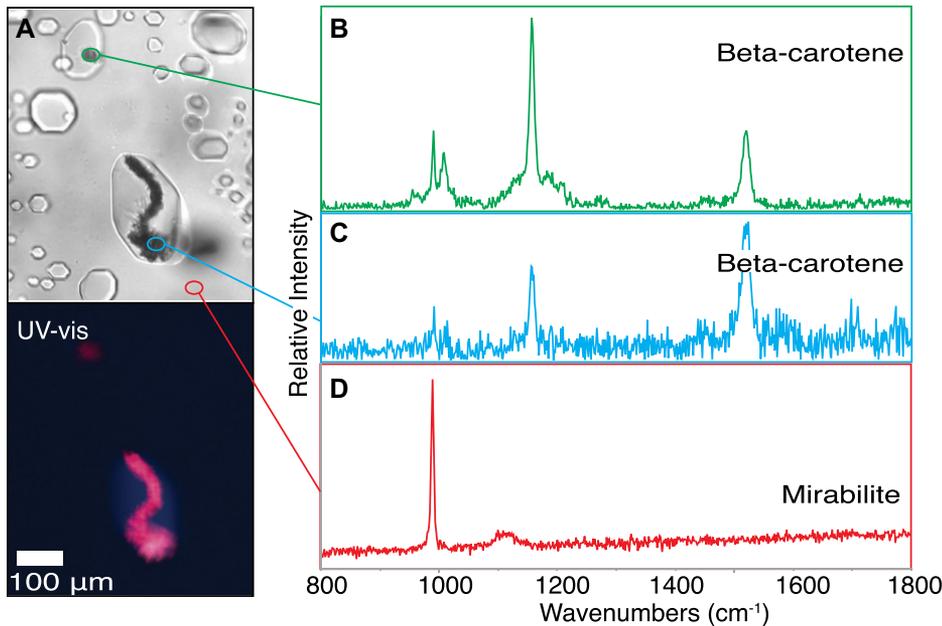


Figure 3. Microorganisms and organic compounds in a mirabilite crystal with corresponding Raman spectra. (A) Primary fluid inclusion growth band containing organic materials in plane-transmitted light (top) and UV-vis light (bottom). (B, C) Raman spectra of organic compounds showing three peaks at 1010, 1158, and 1518 cm^{-1} , consistent with beta-carotene standards. (D) Raman spectra for mirabilite crystal with a strong band at $\sim 989 \text{ cm}^{-1}$.

against degradation by irradiation and/or oxidation. Optical observations of microorganisms and organic compounds are easily achieved in a laboratory with microscopes and confirmed with Raman spectroscopy using a focused laser. The use of high-resolution transmitted light and UV-vis light petrography and Raman spectroscopy is a highly effective combination for determinations of the mineralogy, brine composition, and organic materials of sulfate minerals. On Mars, microorganisms and organic compounds would possibly not be detected in situ with current technology but may be found in sulfate minerals in return samples. This study has expanded the current knowledge on the potential of fluid inclusions in salt minerals to trap and preserve microbial communities. Cryogenic saline ecosystems, those with extremophiles preserved within mineral precipitates, could be long-lasting microhabitats of life. Our documentation of cells and associated beta-carotene in individual primary fluid inclusions in mirabilite suggests that it, along with other salt minerals, may be amongst the best places to search for life in Mars return samples.

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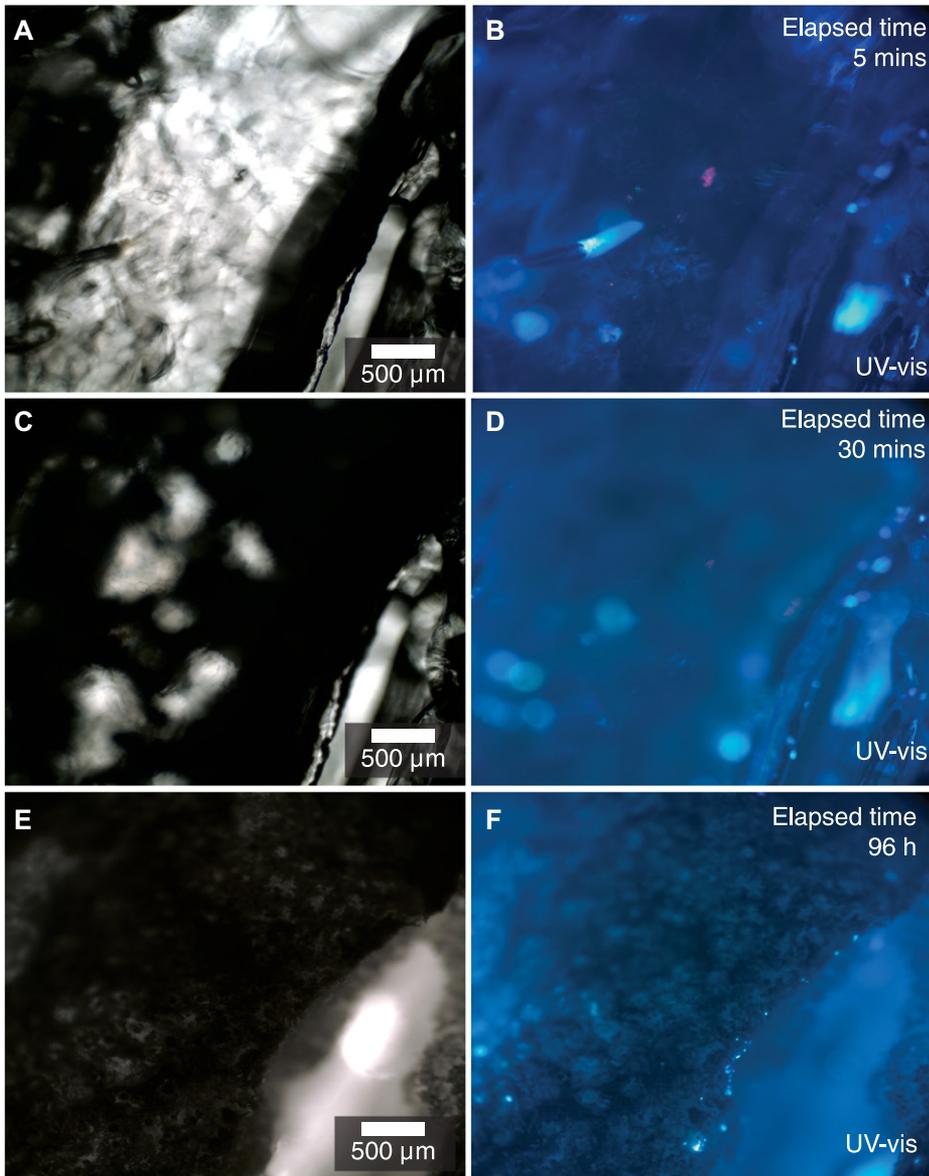


Figure 4. Paired transmitted light (left) and UV-vis light (right) photomicrographs of crystals of mirabilite dehydrating to thenardite over time in ambient air (~40%–50% relative humidity) and temperature (~25 °C). (A, B) Mirabilite crystal after 5 min of exposure. Suspect organic materials in primary fluid inclusions are visible with transmitted light and fluoresce bright pink and blue. (C, D) Mirabilite crystal after 30 min of exposure showing partial desiccation. Some suspect organic materials in primary fluid inclusions are still visible with transmitted light and have bright fluorescence responses. (E, F) Completely desiccated mirabilite (now thenardite) crystal after 96 h of exposure, with fluorescent responses still visible in localized spots of the white thenardite powder.

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