POSITIVE GLYCINE INCORPORATION IN CERES ANALOGUE MINERALS. L. R. Reynoso[1*], K. J. Robinson[2], R. A. Root[3], L. B. Williams[4], J. Castillo-Rogez[5], M. Bose[4*], [1]School for Engineering of Matter, Transport, & Energy, Arizona State University, Tempe, AZ, 85287. [2]Beyond Center, Arizona State University, Tempe, AZ, 85287. [3]Department of Environment Science, University of Arizona, Tucson, AZ, 85721. [4]School of Earth and Space Exploration, Arizona State University, Tempe, AZ, 85287. [5]Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA. *Center for Isotope Analysis. (lrreynos@asu.edu)

Introduction: As the scientific world shifts its focus outward to the investigation of solar system bodies possessing internal oceans beneath their icy surfaces (e.g., Europa, Enceladus, and Titan) and related objects that were possibly ocean worlds in the past (e.g., Ceres), there is an increasing need for laboratory studies to better constrain the compositions and organic contents of these oceans. Do habitable environments with the key ingredients for life exist in these subsurface oceans? The recently released *Origins, Worlds, Life - Decadal Strategy for Planetary Science and Astrobiology 2023-2032* features several near-term robotic exploration mission concepts, such as the Enceladus flybys and Ceres sample return, aimed at investigating the physical and chemical processes prevalent in these environments in order to assess their habitability and identify signs of past or extant life. Solid ocean materials are expressed on these bodies in the form of surficial evaporites (Ceres) or ejected salts (Enceladus). Hence, there is an urgent need for laboratory investigations to (a) understand how organic molecules can be encapsulated and preserved during salt formation and, in turn, how organics influence the physical and chemical properties of the salts, and (b) to guide sample collection sites and methodology during *in situ* analysis, and guide experimental protocols for returned samples. Here we report on results from an ongoing project to investigate the likelihood of amino acid (specifically glycine, $C_2H_5NO_2$) incorporation into halite (NaCl) crystals growing in a saturated brine solution.

Project Results so far: We have conducted several experiments to grow NaCl crystals under equilibrium conditions in a supersaturated solution of 0.7 mM, 1.3 mM, 6.7 mM, 0.06 M, 0.13 M, and 1.3 M glycine. Reflected light optical microscope images with a Nikon Eclipse LV100ND revealed two prominent features within the glycine-bearing crystals: (a) blebs of dark material (ranging from 5 to 25 µm) scattered heterogeneously in the NaCl matrix and (b) a dark hue over the entire crystal [1]. Next, we used the highly-sensitive IMS 6F SIMS instrument at Arizona State University (ASU) to confirm that the blebs are indeed carbon-bearing. The blebs in the NaCl crystal show a $^{12}C/^{23}Na$ ratio >50 times higher than the glycine-free crystals [1]. Finally, we performed Fourier-transform infrared spectroscopy (FTIR) on the glycine-bearing crystals at the University of Arizona. The FTIR results confirmed that the glycine was intact within the crystals as several peaks were observed between 2000-1000 cm^{-1} due to C-C & NH_2 bending, C-C and NH_2 twisting, and CH_2 scissoring. Here, we report the results of gas chromatography and Brunauer-Emmett-Teller (BET) measurements on the NaCl crystals with variable amounts of glycine.

Methodology: *(1) Gas Chromatography.* A portion of evaporite crystals were analyzed to quantify bulk glycine concentrations at ASU. This was done by dissolving NaCl crystals in RO (reverse osmosis) water at a crystal:water mass ratio of ~1:20. Aliquots of the resulting aqueous solutions were then subjected to derivatization via ethyl chloroformate using methods similar to [2–3] for gas chromatography analysis. This derivatization procedure replaces the protic functional groups of amino acids, i.e., the carboxyl and amino groups, with ester and amide-ester groups, respectively, which contribute to increased volatility in the derivatized analyte product. Derivatized aqueous samples were then liquid/liquid extracted with dichloromethane containing millimolar dodecane as an internal standard. The resulting organic extraction was analyzed via gas chromatography with flame ionization detection. Glycine was ultimately quantified by comparing experimental sample intensities to 4-point standard calibration curves produced from aqueous glycine standards of varying concentrations that underwent identical derivatization and extraction procedures.

(2) Brunauer-Emmett-Teller (BET) Analysis. The TriStar II Plus instrument at ASU was used to probe the pore size distribution of a NaCl crystal with 0.13 M glycine and one without glycine. Nitrogen gas was used as the adsorbent, and the gas was held at 77 K using liquid nitrogen in a 4L dewar throughout the measurements. The NaCl crystallites were ground in a mill for two minutes to a fine powder. The powders were then placed into sample tubes and weighed using a Mettler Toledo balance. Afterward, the samples were placed into a Micrometrics FlowPrep 060 sample preparation system and were heated to 100°C under an N_2(g) stream for 24 hours. Afterward, sample tubes were cooled and reweighed to obtain their dry weight before analysis.

Results and Discussion: *(1) Gas Chromatography.* The measurements suggest glycine is incorporated intact in the NaCl crystal structure and does not decompose under the current experimental conditions. We also find that glycine abundance in the NaCl crystals increases with increasing glycine concentrations in pre-evaporate solutions (Fig. 1). However, this relationship is disproportionate and nonlinear. As examples, a solution prepared with 29.4% glycine relative to the total mass of glycine and sodium chloride incorporated only 1.7%

glycine in the resulting evaporite crystal. Separately, a solution prepared with 1.5% glycine produced 0.25% glycine in the resulting evaporite crystal (i.e., a greater fraction of glycine was incorporated at the lower concentration by a factor of ~3).

Earth's oceans show dissolved free amino acid concentrations ranging from 0.05 to 1.78 µmol/L [4] and much lower particulate amino acid contents ranging from 90 to 260 nmol/L [5]. Amino acid concentration decreases with depth in the oceans and increasing distance from the continental shelf [4]. In the case of Ceres, we expect to have a high concentration of organics in the salts because the deep brine reservoir is likely saturated with organics accumulated as a consequence of freezing. Our current analytical detection limit is 0.0008% (8 ppm) glycine as a mass percentage of the crystal, similar to what is seen for the glycine-free experiment. We plan future experiments to reduce this detection limit by evolving protocols and synthesizing crystals in an anaerobic chamber to curb contamination.

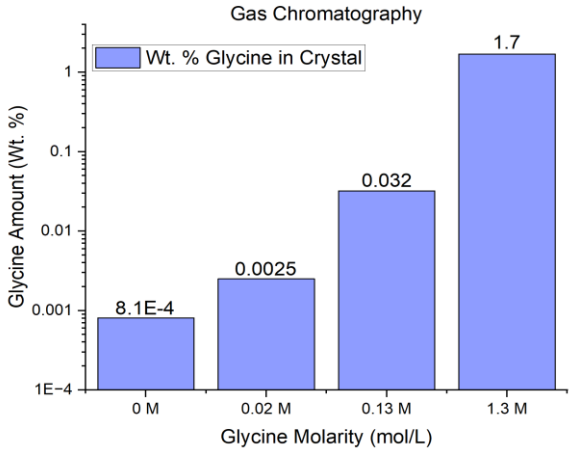

Figure 1. *Glycine amount (in Wt. %) observed in the glycine-free, 0.02 M, 0.13 M, and 1.3 M solutions.*

(2) Brunauer-Emmett-Teller (BET) Analysis. Based on the adsorption and desorption isotherms (Fig. 2), several deductions can be made about the crystal porosity. For the 0.13 M glycine crystal, the adsorption average pore diameter was found to be 16.7 nm. On the other hand, the average adsorption pore diameter for glycine-free salt was lower (12.4 nm). These pore sizes are classified as mesoporous (2 nm < widths < 50 nm) [6]. Both adsorption trends exhibit Type II isotherm characteristics where the monolayer coverage is complete at less than 0.1 relative pressure (Fig. 2). The initial steep slope of the adsorption curve indicates the crystal contains relatively few micropores as the monolayer forms quickly. The 0.13 M glycine crystal (Fig. 2b) has a steeper slope, indicating a more mesoporous surface as it takes longer to fill the individual pores with nitrogen molecules once multilayer adsorption is initiated.

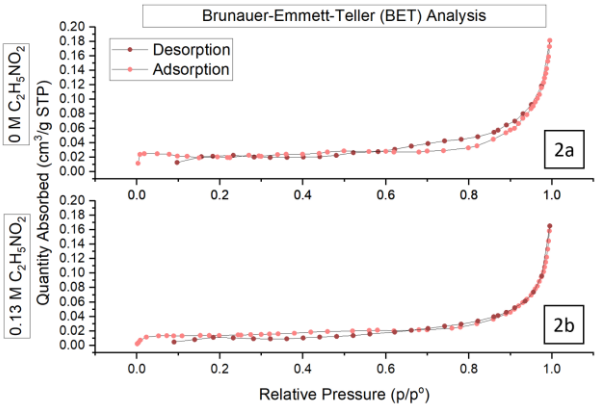

Figure 2. *Gas adsorption isotherms using N_2 gas at 77K for glycine-free and 0.13 M glycine crystals.*

Using the Barrett-Joyner-Halenda (BJH) adsorption cumulative pore volume curve, the relative crystal porosity can be calculated using the ratio between the volume of the pores and the crystal volume. Using the measured mass and known density, the relative crystal porosity was calculated to be 43.5% and 39.3% for the 0.13 M and glycine-free crystals, respectively. Results show that although the pore size distribution changed as a result of adding glycine, the relative porosity did not change significantly. Highly-porous silicate grains have stronger emissivity features than those with fewer pore spaces [e.g., 7]. Additional experiments to understand at what glycine concentrations the emissivity features of silicates mixed with salty/icy materials can be influenced will be done.

Conclusions: We show that glycine survives fragmentation or decomposition during its incorporation into NaCl crystals and can be detected as individual molecules with a current detection limit of 0.0008% (8 ppm) weight percent glycine. Additionally, inclusions of glycine can decrease the crystal porosity, especially if the crystallites form under non-equilibrium conditions. Sample porosity can ultimately influence the design of a sample acquisition system to be utilized on ocean world missions. Future experiments will allow an accurate assessment of the amount of glycine integrated into the NaCl crystals, which will aid in understanding the mass requirements for sample collection from ocean worlds.

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References: [1] Reynoso, L. R. et al. (2022) 54th Annual Meeting: AAS DPS, Abstract#505 [2] Cao, P., and Moini, M., (1997) *Rap. Comm. in Mass Spec., 11*, 349–352, [3] Hušek, P., (1991) *Journal of Chromatography A, 552,* 289–299. [4] Niu, J.et al. (2022)J. Ocean. Limnol., 41 [5] Siezen, R.J. et al. (1978) *Mar. Chm., 6,* 3¸ 215-231. [6] Sing, K.S.W et al. (1985) *Pure Appl. Chem., 57,* 603–619. [7] Young, C. L., (2019) *Icarus, 321,* 71–81.