ON-CHIP PURIFICATION OF AMINO ACIDS FOR OCEAN WORLD IN SITU BIOSIGNATURE ANALYSES K. L. Craft, T. B. Van Volkenburg, K. A. Ohiri, J. K. Skerritt, K. M. Irons, A. Y. Kilhefner, M. A. Hagedon, C. R. Glein, C. E. Bradburne, Johns Hopkins University Applied Physics Laboratory (JHU APL, Kate.Craft@jhuapl.edu), JHU APL (Tess.VanVolkenburg@jhuapl.edu), JHU APL (Korine.Ohiri@jhuapl.edu), JHU APL (Jen.Skerritt@jhuapl.edu), Univ. North Carolina, Chapel Hill (kmirons@live.unc.edu), JHU APL (Ashley.Kilhefner@jhuapl.edu), JHU APL (Matt.Hagedon@jhuapl.edu), Southwest Research Institute (cglein@swri.edu), JHU APL (Chris.Bradburne@jhuapl.edu).

Introduction: Whether exploring the icy ocean worlds Europa and Enceladus or subsurface aquifers on Mars, the search for life in these environments presents a number of challenges. One major challenge is that planetary samples are likely chemically complex, with high salinity, and are expected to have very low biomass, if present. These environmental factors can confound the instruments/techniques currently used to analyze in situ samples for biosignatures (e.g. amino acids and their chirality, or DNA/RNA). Instruments/techniques may include mass spectrometers, fluorescence-based optics, and nanopore sequencers [e.g.1-3]. One solution we are developing is an on-chip sample preparation technique capable of purifying and desalinating proteinogenic amino acids, independent of the pH, salinity, and type of salt in the sample. This device can be used in situ as a single purification tool for multiple downstream biosignature detection analyses. The technique employed follows similar methods developed for desalinating meteorite samples on Earth [4], however it has yet to be developed as an in situ flight instrument, robust to environmental factors and sample chemistries.

Our previous work proved the capability of the chip and beads (Figure 1) to separate samples containing amino acids of known concentrations from salty solutions [e.g. 5] and further work reported here details the improved automation work for the system runs and additional performance tests. In culmination, a geyser sample collected in Utah was run as a complex planetary geyser sample analog.

Figure 1. Microfluidic chip that purifies a fluid sample by separating out any salts and enables amino acids to be eluted for downstream analyses. (a) and (d) are the inlet and outlet, respectively, (b) is the bead bed, and (c) are the bead capture columns.

Methods: Salts and amino acids have been tested as planetary analogs for environments of icy ocean worlds such as Europa and Enceladus [6, 7] and the amino acids used by terrestrial biology [8]. Three salt types have been tested, NaCl, CaCl2, and MgSO4, with the amino acid, phenylalanine (Phe), in various concentrations, as well as variances on the sample pH to explore its effect on separation capability.

Work to automate the system has improved the capability to send fluids from filled reservoirs at controlled flow rates. Additionally, the automated setup has enabled improved accuracy in sample volume collection. Figure 2 shows the current automated setup.

Figure 2. Automated set-up of the breadboard instrument with pumps, reservoirs, control module, and chip.

A geyser sample, collected in UT, was run as a complex planetary analog sample, along with several control samples of known amino acid levels of glycine and alanine. The geyser sample was not of high salinity at ~11 ppt (~1/3 seawater), but had a basic pH= 9 that could challenge separations and was composed of other chemical compounds that provided a complex, planetary analog test of the on-chip purification for HPLC detection of amino acids. For our tests, samples were first purified on-chip, then the samples were dried and reconstituted for the derivatization, and then analyzed with High Performance Liquid Chromatography (HPLC).

Results: Sample purifications have been performed successfully for control samples containing phenylalanine and the three analog salt types, NaCl, CaCl2, and MgSO4 producing varying capture yields indicating that there is some competition of the Ca2+ cation competing with the amino acid for binding sites (see Figure 3). Increasing the bead bed size or running the sample...
through a second bead bed can be planned as a mitigation for environments found to be rich in \( \text{Ca}^{2+} \) composition to maximize amino acid capture.

Tests varying the pH of the sample show that with higher pH, there is lower salt-free yield of the amino acid due to proximity of the pH to the isoelectric point (for Phe isoelectric point ~ 5.5). As typical separations occur with the beads in a low pH solution, these tests indicate a high pH sample could cause lower amino acid salt-free capture yields and therefore strategies to lower pH of a sample before processing may be needed.

**Figure 3.** Two of the salt types tested with 50 \( \mu \text{L} \), 100 mM Phe, showing that the \( \text{Ca}^{2+} \) cation of \( \text{CaCl}_2 \) is likely competing for binding sites, more so than the \( \text{Na}^+ \) in \( \text{NaCl} \), and causes less amino acids to bind and elute earlier with the higher salt part of the sample (dark blue).

Before running the geyser sample through the purification process, control tests were performed to validate the preparation and analysis protocol for HPLC-UV. The sample was dried down, reconstituted, and a pre-column derivatization was performed. Figure 4 shows results for a pseudo-geyser sample consisting of Glycine (Gly) and Alanine (Ala) at targeted concentrations of 0.875 \( \mu \text{M} \) and 80 \( \mu \text{M} \), respectively, as reported by [9]. Differences between actual and target amino acid concentrations can be attributed to inherent error associated with the analytical methodology. Further tests on the actual UT geyser sample will be presented.

**Future Work:** Work is continuing to improve design and evaluate performance for the on-chip purification of complex samples. Further development will also work to construct a chip with flight materials and capability for robust connections to valves and tubing for connection to upstream sample delivery and downstream sample analyses devices including gas chromatographs and mass spectrometers. Complete system sample processing and analyses testing will then occur.

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**References:**