ON CHIP DESALINATION OF PROTEINOGENIC AMINO ACIDS FOR IN SITU EXTRATERRESTRIAL ANALYSES.

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Introduction: Many challenges exist for in-situ techniques searching for signatures of life in planetary environments. Whether exploring the ocean worlds Europa, Enceladus, or Titan or the dunes of Mars, collected samples are likely to contain high salinity and are expected to have very low biomass, if present. These environmental factors would act as significant challenges to the proven instruments/techniques currently used to analyze in-situ samples for biosignatures (e.g. amino acids and their chirality or DNA/RNA) including e.g. mass spectrometers, fluorescent tagging, and nanopore sequencers and would degrade their results. To surmount these challenges, we are developing a robust sample preparation method to desalinate proteinogenic amino acids independent of type and concentration of salt in the sample on a microfluidic chip. This technique can be used as a single purification tool for multiple downstream biosignature detection analyses. Though similar methods have been used to desalinate meteorite samples on Earth [e.g.1], this has yet to be developed as an in-situ space flight instrument.

Here we describe the separation achieved using a meso-scale ion exchange chromatography column that allows for non-destructive sample preparation to remove ionic interference, minimize loss of amino acids, and increase downstream detection sensitivity. We then discuss the current work to implement this technique into a modular microfluidic chip. Tests include those for two salts, NaCl and $CaCl_2$, and 3 amino acids: tryptophan, tyrosine, and phenylalanine. Salts and amino acids were chosen as planetary analogs for environments of icy ocean worlds such as Europa and Enceladus [e.g. 2, 3] and the amino acids used by terrestrial biology [4]. Further work will expand to test additional salts including $MgSO_4$ and additional amino acids.

Methods:

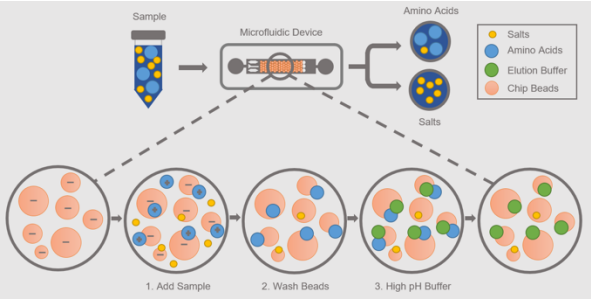

Figure 1. Ion exchange salt separation process

Chemistry (Figure 1) - The separation of salts and amino acids occurs through several steps. First, an ion exchange resin is buffered to a low pH to enable retention of the amino acids. Next, the sample is introduced and the amino acids bind to the beads within the solution. The next step is a water wash to remove salts and other contaminants, and then the exchange resin is buffered to a high pH to allow selective elution of the bound amino acids. Separation efficiency and yield for both salts and amino acids were measured using a conductivity probe and UV/Vis spectrometer, respectively.

Microfluidic chip (Figure 2) – A microfluidic prototype has been fabricated following a previous chromatography chip design [5] with a bed volume large enough to capture the needed amount of amino acids for downstream detection . Figure 2 shows the current chip design. Beads or ion exchange resin is loaded through port (a) at left and flow into the chip bed (b) until resting against the pillar array, (c), that prevents them from flowing further and allows packing. The chip design attempts to minimize bubbles during packing to minimize any reduction in binding sites available to the amino acids. Once the beads are packed in, the sample and subsequent reagents are all flowed in through port (a). Upon process completion the amino acids are collected from port (d).

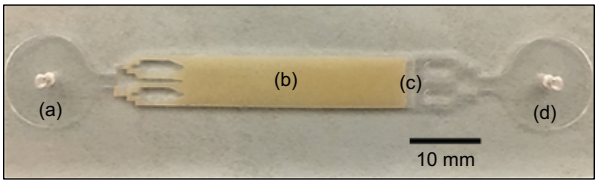

Figure 2. Microfluidic chip (beads loaded) that allows separation of salts and amino acids. (a) shows the port for insertion of beads, sample, and reagents. (b) is the bedding plane (c) denotes the location of the pillar array (d) is the collection port.

Results: Three aromatic amino acids, tryptophan, tyrosine, and phenylalanine, have been successfully purified from saturated salt solutions down to the millimolar level. To date, two types of salts, NaCl and $CaCl_2$, at multiple concentrations have been tested. Figure 3 shows results for mesoscale tests and Figures 4-5 show results for on chip tests. Mesoscale results were successful for separations of phenylalanine, tryptophan, and tyrosine from 5M NaCl with output yield percentages of 81% for phenylalanine, 46% for tryptophan, and 48% for tyrosine (Table 1). The lower output yields of tryptophan and tyrosine were expected as they are known

from literature to have the lowest yields of all amino acids for the type of bead used in this separation process.

Figure 4 shows results of on-chip separation with 50μL of 5M NaCl and 50 μL of 100mM phenylalanine. Higher concentrations NaCl were also tested and Table 2 gives the salt-free yields that were obtained. Salt-free yield is calculated as the % of phenylalanine (salt-free) collected during elution compared to the volume in the sample introduced. Yields are shown to decrease with higher salt concentration introduced as the salts begin to crowd out the amino acids.

Figure 5 shows on-chip separation results for varying volumes of 5M $CaCl_2$ and 50μL of 100mM phenylalanine. As with the NaCl results in Figure 4, the chip is being overloaded with salts and therefore allows some phenylalanine to exit during the water wash, and lower than introduced volumes are captured during the elution step. Table 3 shows the salt-free phenylalanine yield for the three $CaCl_2$ concentrations tested. These results show that the Ca^{2+} ion in $CaCl_2$ has a higher binding affinity and results in lower yields when compared to the NaCl salt tests.

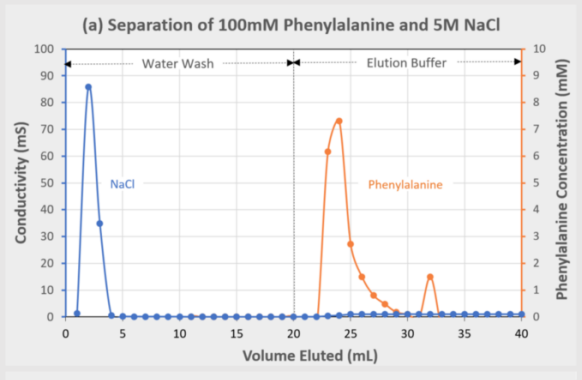

Figure 3. Mesoscale separation for NaCl and phenylalanine. Separations for tryptophan and tyrosine were also successful and Yield % are shown in Table 1.

Table 1. Mesoscale separation yields with 5M NaCl

Amino Acid	Yield %
Phenylalanine	81%
Tryptophan	46%
Tyrosine	48%

Table 2. On-chip separation salt-free yields with 50 to 250μL of 5M NaCl and 50μL of 100mM phenylalanine

Volume of NaCl	Salt-Free Yield %
50 μL	79%
150 μL	35%
250 μL	27%

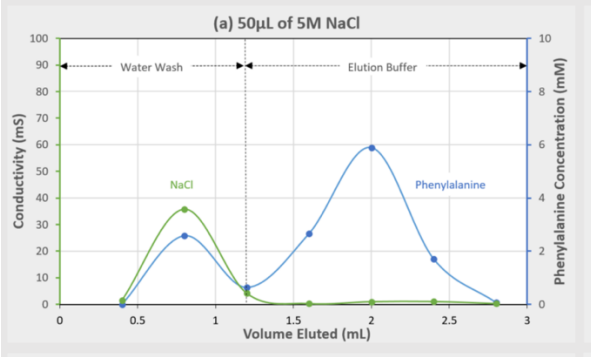

Figure 4. On-chip separation results for 50μL of 5M NaCl and 50 μL of 100mM phenylalanine

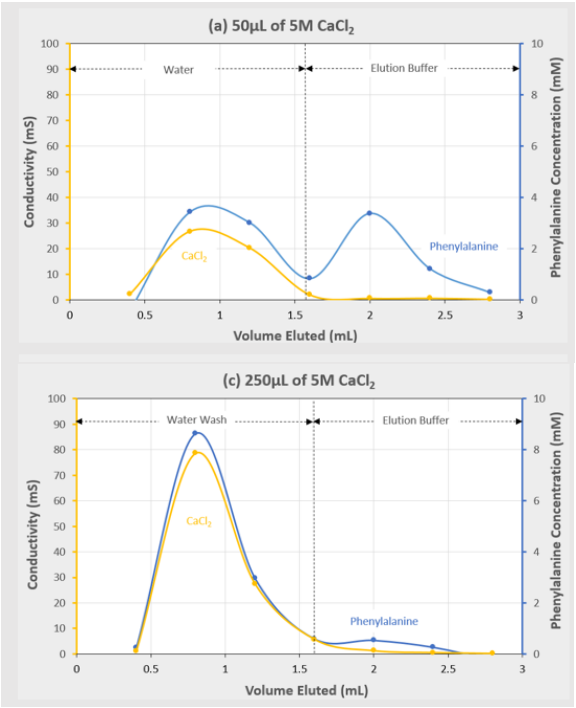

Figure 5. On-chip separation results for 50 to 250μL of 5M $CaCl_2$ and 50 μL of 100mM phenylalanine

Table 3. On-chip separation salt-free yields for 50 to 250μL of 5M $CaCl_2$ and 50μL of 100mM phenylalanine

Volume of $CaCl_2$	Salt-Free Yield %
50 μL	41%
150 μL	21%
250 μL	8%

References:

[1] Glavin et al. 2010, *Meteor. & Planetary Sci.*, *45*; [2] Brown and Hand 2013, *The Astronomical Journal*, *145*(4), 110; [3] Postberg et al. 2009, *Nature*, *459*. [4] McKay 2004, *PLoS Biol. 2*, e302. [5] Millet et al. 2015, *Lab Chip*, *15*, 1799.