

AstroBionibbler: In Situ Microfluidic Subcritical Water Extraction of Amino Acids

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Introduction: Searching for trace levels of organic molecules on Mars or other rocky bodies presents a formidable challenge for robotic instrumentation. One particularly high value target, amino acids, are ideally suited to wet chemical analysis methods such as liquid chromatography (LC) and capillary electrophoresis (CE); because of this, automated microfluidic versions of LC and CE capable of separation and sensitive detection of amino acids and other organic molecules are already demonstrated or under development [1, 2].

However, less work has been done to develop suitable instrumentation for analyte extraction and liquid extract delivery to downstream microfluidic instruments. The AstroBionibbler instrument (ABN) [3, 4], focuses on this problem, with the primary aim of developing a chip based fluidic device for subcritical water extraction (SCWE) of amino acids from powder samples.

Background: The simplest analogy for SCWE and the ABN instrument is that of a French press coffee maker. In a French press a slurry of near boiling water and coffee grinds are allowed to mix for a period of time before a filter helps separate the desired coffee extract from the undesired residual particulates. The main difference in the ABN instrument (and SCWE in general) is the need to allow the system to pressurize so that temperatures greater than the boiling point of water can be reached (but less than the critical point).

The SCWE technique has three features that make it desirable for a planetary *in situ* extraction technique. The first is that it only requires water to perform the extractions, and not any other more reactive or difficult to contain solvents. Second, SCWE allows water to behave like other less polar solvents when desirable because the dielectric constant of water changes dramatically with temperature, especially above 100°C [5]. Third, the high temperatures reached in SCWE can be used to hydrolyze biopolymers such as proteins into their constituent amino acids, increasing our ability to separate and conclusively detect potentially very small amounts of material.

A number of proof-of-principle studies have shown that it is possible to extract both amino acids and polycyclic aromatic hydrocarbons from low organic content Atacama soils using SCWE [5, 6]. Commercially SCWE is a potentially environmentally friendly manner of converting food wastes into useful chemical precursors such as amino acids [7].

Instrument Design and Preliminary Results: The ABN instrument can be broken down into three components:

1. *Sample acceptance and slurry preparation:* Sample cups of 5 mL volume have been designed to accept solid powder samples and water to create homogeneously mixed slurries via sonication with piezoelectric actuators. Using a peristaltic pump it was possible to reproducibly move slurries up to 20% weight from the sample cups to another device. This cup also functions as a “gentle” extractor if desired, allowing comparison of the free soluble species, to those bound in larger polymers. Furthermore, this sort of sonic extractor may be all that is required for icy samples that simply need melting and handling.

2. *Microfluidic SCWE extraction chip:* A significant challenge in creating a SCWE instrument is withstanding the pressures and temperatures required during extraction. A bonded glass chip has the inherent strength, but proper fabrication/bonding and creating pressurized seals at the interfaces that are easily automatable is non-trivial. Work is ongoing to determine the most robust and reproducible extraction chip fabrication procedure. The problem of fluidic seals has been overcome by using ice plugs in the microfluidic channels as a sealing mechanism. Thermoelectric coolers have been demonstrated to hold ice plug seals against extraction area temperatures >200°C, with the heating generated by both ultrasonication and resistive methods [4].

A series of samples including pure solutions of a test protein (bovine serum albumin), as well as JSC Mars 1A, Atacama, and Antarctic soils have been extracted under a variety of temperatures and durations using a benchtop SCWE. Comparisons between the benchtop SCWE and the ABN system are underway.

3. *Particle removal and instrument reuse*: After extraction it is necessary to remove the residual solids and deliver only the extract to downstream instruments. ABN achieves this through a combination of a settling cup and an inline filter. By allowing the particles to gravitationally settle out, only those $< \sim 25 \mu\text{m}$ remain dispersed in the extract after a time period of ~ 1 hr. A 2 micron inline filter can then provide the purity of extract typically required for a microfluidic device without rapidly clogging due to the gravitational removal of the largest (and largest fraction) of particles.

After the extract has been delivered, piezo agitation can be applied to remix the slurry and then transfer the whole aliquot to waste for reuse of the system.

References:

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