ANALYSES OF MOLECULAR AND COMPOUND-SPECIFIC C, N, AND H ISOTOPIC RATIOS OF VOLATILE AMINES IN MURCHISON USING HEADSPACE SOLID-PHASE MICROEXTRACTION ON-FIBER DERIVATIZATION.

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Introduction: Volatile organic compounds, many of which have been spectroscopically observed in the interstellar medium (ISM), are particularly abundant in carbonaceous chondrites. For example, volatile monocarboxylic acids are the most abundant soluble organic compounds in Murchison (up to 100 times more abundant than amino acids). Low molecular weight (often volatile) organic compounds such as C_1 to C_6 amines are fundamental building blocks for more complex organics such as amino acids and are critical for elucidating pathways of prebiotic organic synthesis. A pristine mission-returned sample from a carbonaceous asteroid Bennu (the OSIRIS-Rex mission) offers a golden opportunity to examine these compounds in unprecedented detail with minimal contamination [1].

Currently published analytical methods to analyze volatile amines require "one-pot" derivatizations using 2,3,4,5,6-pentafluorobenzyl chloroformate (PFBCF) [2] or (S)-(–)-N-(trifluoroacetyl) pyrrolidine-2-carbonyl chloride (TPC) [3]. These derivatization methods also permit compound-specific carbon isotopic analyses of amines in Murchison. The one-pot derivatization methods for analyzing low molecular weight volatile amines in carbonaceous chondrites [3] consists of two main steps: **1)** addition of derivatization reagents to the aqueous extracts to convert the volatile compounds to low-volatility, less-polar derivatives; **2)** extraction of the derivatized compounds in the solution with organic solvents (e.g., dichloromethane) and then concentrate the solvent solution prior to analysis. The advantages of derivatization methods include, **1)** molecular weights of resulting derivatives are sufficiently high to avoid losses during the evaporative concentrating step; **2)** using an optically pure derivatization reagent allows quantification of chirality in compounds that possess chiral centers (Aponte et al., 2014b); and **3)** separation can be achieved using non-polar, durable GC columns.

However, there are a number of important caveats to using the one-pot derivatization methods: **1**) sample consumption is high: derivatization reagents are functional group–specific, with volatile compound classes not targeted by derivatization reagents being lost in the subsequent concentrating step. **2**) Derivatization reactions in solution may result in the formation of artifacts from reaction by-products or non-intended products (e.g., amine group in amino acids may be derivatized during amine derivatization). These artifacts will mix in the parent solution, hence affecting analysis of the other compound classed not targeted in the derivatization.

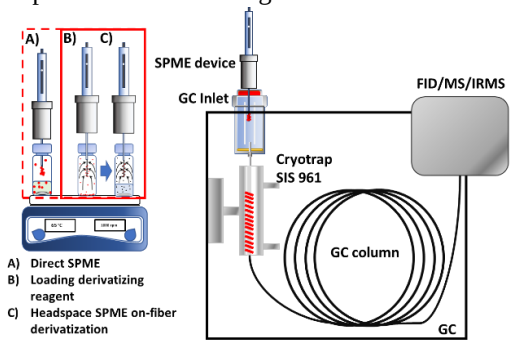

Fig. 1. Direct and headspace SPME on-fiber derivatization

An alternative approach to overcoming problems associated with one pot derivatization is to use headspace or immersion solid-phase microextraction (SPME) (Fig. 1). We have previously demonstrated successful applications of SPME to analyze volatile monocarboxylic acids in carbonaceous chondrites [5]. There are a number of important advantages of SPME over the traditional one-pot derivatization methods: **1**) SPME is non-destructive; i.e., aqueous extracts after analyses of one class of compounds can still be used for analysis of other compound classes such as amino acids. This greatly reduces sample consumption as it is not necessary to divide the bulk sample into small portions for different compound classes. For rare, high-value samples, such as the one from asteroid Bennu, the SPME approach is invaluable. **2**) The SPME extraction procedure is non-exhaustive, allowing repetition of analyses, and analyses of the sample compound sets using different instruments — e.g., gas chromatography–mass spectrometry (GC-MS) for identification, gas chromatography–isotope ratio mass spectrometry (GC-IRMS) for compound-specific isotopic analyses. By using fibers with different amount of absorbents (e.g., SPME fiber, SPME Arrow with 10 times higher capacity, SPME thin film with 100 times higher capacity), analysts can readily change the amounts of compounds de-

livered to the instruments according to the specific instrument requirement (sensitivity). For example, compound-specific hydrogen isotopic analyses require larger sample masses. Analysts can use SPME Arrow or even thin film SPME to deliver appropriate amounts of compound masses to the GC-IRMS instrument. Careful selection of SPME phases allow minimal waste of target compounds. **3**) There are many different SPME phases commercially available covering a wide range of polarities, for targeting compounds of different polarity and bearing different functional groups. 4) The efficiency of SPME can be greatly enhanced by using on-fiber derivatization (Fig. 1B), especially for compound classes that are relatively difficult to analyze directly using gas chromatography. For example, Pan et al. [6] reported ~ 470 times higher detection sensitivity for C_1 to C_6 amines by using headspace on-fiber derivatization relative to direct headspace SPME with no derivatization, whereas Nair and Miskelly [7] reported an increase in detection sensitivity by 60 times for methamphetamine.

Our main objective in this study is to demonstrate the efficacy of using SPME on-fiber derivatization to analyze volatile amines in Murchison on GC-MS and GC-IRMS.

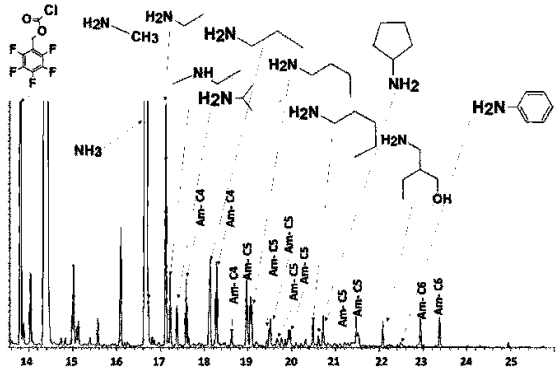

Fig. 2. Headspace SPME on-fiber derivatization of Murchison amines (and ammonia) as PFBCF derivatives allows direct GC-MS analyses of these volatile bases.

Methods and results: We have performed analyses of on-fiber derivatization for amines using 2.1 g of Murchison sample FMNH ME ME2644.26.92 (Fig.2). Our headspace SPME on-fiber derivatization methods for low-molecular-weight amines differ greatly from the conventional one-pot derivatization followed by solvent extraction previously used to characterize these compounds in carbonaceous chondrites [2] in the following important aspects: **1**) The derivatization reagent is preloaded by headspace SPME on to the fiber (Fig. 1B). Subsequently, the fiber is exposed to the air space equilibrated with aqueous extracts in a glass vial for a set period of time at a specific temperature (e.g., 5 minutes at 30°C). Derivatization takes place only on the fiber surface in the headspace of the aqueous extraction solution (pH is adjusted to 10). Thus, only the airborne amines in the headspace of the aqueous solutions are in contact with derivatization reagents on the fiber. The parent aqueous solution containing all other organic extracts is not in contact with the derivatization reagent on the SPME fiber (e.g., amino acids in solution will not be in direct contact with the derivatization reagent); **2)** Only the fraction of the target compounds that are airborne and to be analyzed by a specific instrument (GC-MS or GC-IRMS) are derivatized, whereas the remaining target compounds in solution stay in their original underivatized forms and do not contact the derivatization reagents. The derivatization reagent is functional group–specific (e.g., PFBCF only reacts with amines) and does not affect other compound classes in the vapor phase. Derivatized vapor phase target compounds on the fiber are transferred to GC-MS and GC-IRMS for analyses (hence there is no excess, or unnecessary derivatization). Our approach, therefore, minimizes any possible cross-contamination of the parent solution and minimizes sample consumption. In addition to various branched and cyclic C_1 to C_6 amines, we also found significant amounts of ammonia, consistent with findings in Murchison and many carbonaceous chondrites [7].

Conclusions: Our SPME on-fiber derivatization approach offers an efficient and non-invasive analysis of volatile amines in carbonaceous meteorites and Bennu. However, our current GC injector configuration only allows us to use common SPME fibers. The compounds delivered using common SPME fibers are sufficient for compound identifications using GC-MS, but they are insufficient for compound-specific isotopic analyses, unless repeated loadings are performed (which can contribute to lower chromatographic performance such as baseline fluctuations). We are in the process of setting up the adaption kits to allow us to use SPME Arrow, which will increase the compound delivery by ~ 10 times each loading. Once fully tested, SPME on-fiber derivatization approach can be directly applied for non-invasive analyses of amines in aqueous extracts of Bennu sample.

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