Chiral Determination of Amino Acids using X-Ray Diffraction of Thin Films D. Dragoi, J. Kulleck, I. Kanik and L. W. Beegle, Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Drive, Pasadena, Ca 91109, ddragoi@cco.caltech.edu, James.G.Kulleck@jpl.nasa.gov, ikanik@pop.jpl.nasa.gov, Luther.Beegle@jpl.nasa.gov

The astrobiological search for life, both extinct and extant, on other solar system bodies will take place via several planned lander missions to Mars Europa and Titan. The detection and identification of organic molecules that have been associated with life is a major technical challenge. Terrestrial life utilizes organic molecules, such as amino acids, as its basic building block. Amino acids can be synthesized by natural processes as is demonstrated by their detection in meteoritic material. In this process, the organic molecules are produced roughly in a even mixture of D and L forms. Biological process, however, can utilize almost uniquely one form or the other. In terrestrial biology, only the L-amino acids is common in biological processes. If signature of life existed elsewhere in the D form it then be concluded that life had evolutionary beginning on that body. Detection of an enantiomeric excess of L over D would also be a powerful sign that life had existed on that body at one time.

We have begun a series of experiments to extract organic molecules from rock and soil samples utilizing both direct heating and laser desorption. Our intended goal is to be able to separate organic molecules from the rock and soil samples and collect them on a collector plate without changing their structure. If this apparatus is operated over an extended period of time it will act as an organic molecule concentrator, which should aid in the identification of molecules which may exist in very small concentrations on the Martian surface and subsurface.

We have designed, constructed and characterized a new high-pressure (5 Torr, representing the Martian atmospheric pressure) hollow cathode ionization source (HCIS) that can be utilized as an ionizer in a wide variety of mass analyzers [1]. It is able to function under ambient Martian atmospheric conditions without modification. The ionizer produces a stream of H_3O^+ ions from pure water vapor (H_2O) introduced into the ionizer from a small liquid water container. These ions react with air-borne samples and ionize organic species present via proton-transfer-reaction (PTR) without ionizing the atmospheric components. The constituents of the Martian atmosphere and most non-organic species present on the surface will not react with H_3O^+ as they have lower proton affinities than H_2O [1], while organic species tend to have higher affinities than H_2O.

To facilitate the ionization of target molecules through the PTR process, a beam of (H_3O^+ ions, generated by the HCIS (see Fig. 2), are extracted into a reaction chamber. In our system the reaction chamber consists of 8 concentric rings having different electrical potential applied to them. This creates a near-uniform electric field in which the ions migrates toward the collection plate. At the 5 torr pressure, this allows for the breaking-up of H_2O clusters and provides a long path length for the PTR to take place.

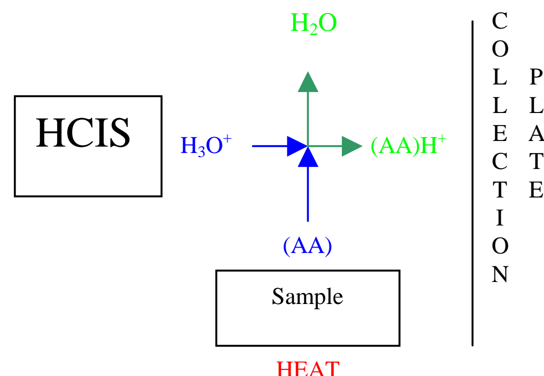

Fig.1 A schematic of the PTR ionization process. The HCIS creates H_3O^+ which reacts with amino acids (AA) released from a sample via heating. The ion and the molecule collide causing the H^+ to transfer from the AA which is then collected on a plate.

X-Ray Diffraction of Organics: Upon collection, the samples would be analyzed via HPLC or ESI/IMS to determine if structural changes have occurred on the sample organic. In order to do the analysis, the specimen holder needs to be removed, weighted to asses the efficiency of the collection process. The collected sample is then removed from the collection plate for analysis. It was realized that it would be more convenient if the sample could be analyzed with minimal sample preparation. Therefore, we explored X-Ray Diffraction (XRD) to determine the structure of the sample.

To test the feasibility of the method for determining structural changes using x-ray diffraction, we placed both L- and D-phenylalanine (min 98% and >99% purity, respectively; purchased from Sigma Aldrich Co. St. Louis Mo) directly on the x-ray sample holder, alumina single crystal (0001) face. The XRD pattern of both the L- or D-Phenylalanine were found to be identical. This was primarily due to the fact that the chirality of the original amino acids does not favor a specific orientation on the sample holder in the powder form.

However, when collecting molecules using PTR, substantial amount of water would be present which should change the diffraction pattern of the sample. Therefore we experimented on L- and D- amino acids in liquid water solution, by slowly evaporating the water and examine the resulting powder with XRD. A thin D- and L-Phenylalanine powder layer was crystallized in a slow-evaporation process using ultra pure, spectrophotometric grade water and was deposited on a low x-ray scatter membrane in order to increase x-ray S/N ratio. A standard laboratory x-ray analysis was conducted utilizing a monochromatic x-ray beam aimed to the surface of the specimen at an angle θ, the Bragg angle. The scattered x-rays were then collected at an angle 2θ by an x-ray detector. Both detector and specimen are coupled while the detector is moving around the sample and collecting the diffracted x-rays that contain information about type of atoms and their periodic distribution in space which is usually limited to the crystal unit cell. In order to obtain a complete x-ray diffraction pattern, one must obtain a plot of diffracted x-ray (in units of counts s^{-1}), versus angle of collection (2θ in units of degrees) over a large range of angles θ. The rotation of the arm detector coupled with the sample, called θ/2θ scan, is controlled by a computer, so the entire spectrum can be stored and used later for structural refinements.

The present investigation was conducted by utilizing an updated version of Siemens D500 with Cu-K_α radiation with a Kevex semiconductor x-ray detector, a rotating holder for specimen to remove the preferred orientation effect. The instrument is automated using Materials Data, Inc. (MDI) software.

Fig. 2 shows an example of the diffraction pattern for two specimens of D- and L- phenylalanine, fresh crystallized in pure water under the same conditions. For raw phenylalanine D and L enantiomers the laboratory x-ray diffraction pattern matches, despite the fact that Powder Diffraction File (PDF) [2] that correspond to PDF# 11-0827 for D form and PDF# 37-1771 indicates detectable differences. This is due to a structure factor, which is invariant for both D and L forms. For fresh crystallized specimens of D and L forms, a difference can be shown (see Fig 2.). We are currently studying this splitting effect further using different solutions of varying Ph levels for crystallization. Comparing diffraction pattern for the entire range produced by L and D crystallized Phenylalanine in pure water, shifts occur in peak position and intensity. These differences are possibly due to stereo-specific interactions of D- and L- phenylalanine molecules with water molecules.

The x-ray diffraction pattern for D and L form, a small part shown in Fig. 2, reflects new structural configurations such as rotation, slight deformation and/or translation of the phenylalanine molecule inside the unit cell. It is possible that D form crystallizes with more molecules of water than L form. Structural solution for freshly crystallized phenylalanine can be found using Rietveld refinement technique [4] which is a fairly mature method for determining crystal structure from powder diffraction. This method has been demonstrated to work for some protein structures by Von Dreele [3].

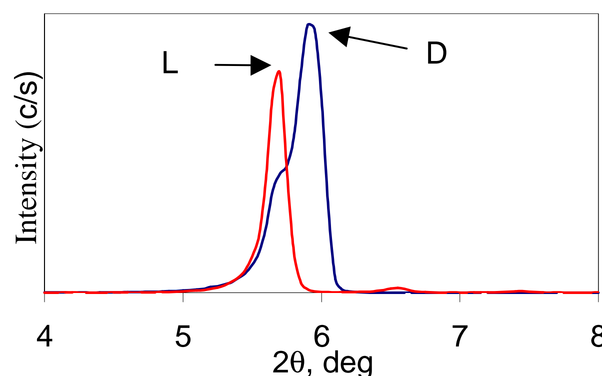

Fig. 2 Two x-ray diffraction patterns for phenylalanine using fresh crystallized specimens of D and L enantiomers.

Conclusions: The ability of X-Ray diffraction to not only identify molecules but to determine the chirality of them will be further investigated. This will include differences in diffraction patterns dependent upon the Ph levels of solution when crystallization takes place. This might lead to a determination of those conditions such as salinity, present at the time of crystallization. The efficiency of the collection process as mentioned above is also currently being investigated. The major technical obstacles to overcome is increasing the time the volatile spend in the gas phase by either slowing the evaporation process preventing freezing of the molecules on the wall of the chamber.

Acknowledgment:
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References: [1] Beegle L.W. et al. (2001) *LPSC 32nd Abstract #2139*., [2] International Centre for Diffraction Data, Newtown Square, PA 19073-3273 U.S.A., [3] Von Dreele R.B. (2000) 49th Denver X-ray Conference, Colorado, [4] The Rietveld Method, Ed. R.A. Young (1993) International Union of Crystallography, Oxford University Press)