HIGH-RESOLUTION ELECTROSPRAY IONIZATION/ION MOBILITY SPECTROMETER FOR DETECTION OF ABIOTIC AMINO ACIDS. L.W. Beegle, C.A. Terrell, H. Kim, and I. Kanik. Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Dr. Pasadena, Ca 91109, Luther.Beegle@jpl.nasa.gov, Charles.A.Terrell@jpl.nasa.gov, ikanik@mail1.jpl.nasa.gov

Introduction: One of the primary goals of the current NASA thrust in Astrobiology is the detection and identification of organic molecules as part of an in-situ lander platform on the surface of Mars or Europa. The identification of these molecules should help determine whether indigenous organisms exist on the surface of Mars or in an undersea environment on Europa. In addition, a detailed organic chemical inventory of surface and near surface molecules will help elucidate the possibilities of life elsewhere in the Universe.

Terrestrial life has, as its backbone, the family of molecules known as the amino acids (AA), and while AA can be found in the terrestrial environments as part of more complex molecules, such as peptides, and proteins. They also exist as individual molecules due to of the hydrolyses of biopolymers. In terrestrial biochemistry, there are 20 principal amino acids which are necessary for life. However, some forms of these molecules can be found in nature synthesized via abiotic process. For example, they are known to exist extraterrestrially as a component of carbonaceous meteorites. The idea that amino acids are readily created by abiotic means has been demonstrated by their positive identification in the Murchison CM2 meteorite, which fell in 1969. This meteorite was analyzed before contamination by terrestrial microbes could result. Three laboratories individually tested parts of the meteorite [1] and concluded that the amino acids present in them were indigenous to the meteorite because, among other reasons, they had equal L- and D- enantiomers. Final identification of the constituents of the Murchison included 33 amino acids which have no known biotic source, 11 amino acids which have limited distribution and 8 (Glycine, Alanine, Valine, Proline, Leucine, Isoleucine, Aspartic Acid, and Glutamic Acid) which readily occur in terrestrial proteins [2].

Ion Mobility Spectroscopy: Ion mobility spectrometry (IMS) has many features that make it attractive as an analytical separation device. IMS is simple, fast, rugged, highly selective and very sensitive to a wide range of compounds. Electrospray ionization is a soft-ionization method where molecules can be ionized at atmospheric pressures which make it a natural ionization method for ion mobility spectroscopy, which also operates at atmospheric pressures. The development of ESI/IMS greatly expanded the range of compounds to include non-volatile samples dissolved in a liquid sample that could be analyzed by IMS.

The fundamentals and applications of the IMS technique are reviewed in detail elsewhere [3]. In brief, the operation of an IMS analyzer is similar to that of a time-of-flight mass spectrometer except that it can function at atmospheric pressure. When an ion is placed in a constant electric field, it migrates along the direction of the field until it collides with a neutral molecule, it then accelerates again until it suffers another collision, and so forth. The energy gained from the electric field is randomized by these collisions, and the combination of acceleration and collision over macroscopic distances results in a constant average ion velocity ($\bar{v}_d$ in the equation below) which is directly proportional to the electric field (**E**). In order to remove the dependence on number density, ion mobilities are reported by normalizing to standard temperature (273 K) and Pressure (760 torr). The ratio of the ion velocity to the magnitude of the electric field at standard temperature and pressure is called the reduced ion mobility (K^m_0), which is usually specified in units of $cm^2\ V^{-1}\ s^{-1}$, and is given by

$$K^m_o = (273/T)(P/760)(L^2/Vt_d) \qquad (1)$$

Here, P is the pressure (in torr), T is the temperature (in K), L is the ion drift distance in cm, V is the voltage drop across L, E is the electric field and t_d is the time required for the ion to traverse L. The separation process and selectivity in IMS are a function of both ion size and mass rather than being dependent solely upon the mass. Since amino acids represent basic building blocks of life, we used common amino acids to conduct the first part of our investigation in the detection of organic compounds. [4]

We have determined the drift times and the reduced mobility constants for 11 of the AA found abiotically in nature using N_2, and CO_2 as drift gases. All were purchased from Sigma Aldrich (St. Louis Mo) and used without further purification. In addition, we compared the Ko values of these abiotic amino acids with the amino acids commonly found in terrestrial biology, to determine separability of the compounds.

Mobility of Ions: The amino acids were dissolved in a solvent at a concentration of 10 ppm in a solvent and introduced into the electrospray ionizer at a rate of 5 μL/min. The solvent consists of 47.5% Methanol, 47.5% H_2O, 5% acetic acid and was utilized for two important reasons: a) It helps for better dissolving of the amino acids for ESI process and b) it increases the

ionization efficiency and thus signal strength of the ions is increased. The ion mobilities were determined with N_2 and CO_2 as a drift gases. All spectra were obtained with a 0.2 msec gate pulse width while averaging 500 spectra. The temperature of the IMS cell was held at 500K at local atmospheric pressure (728 torr in our laboratory as measured with a heated Baratron pressure gauge.)

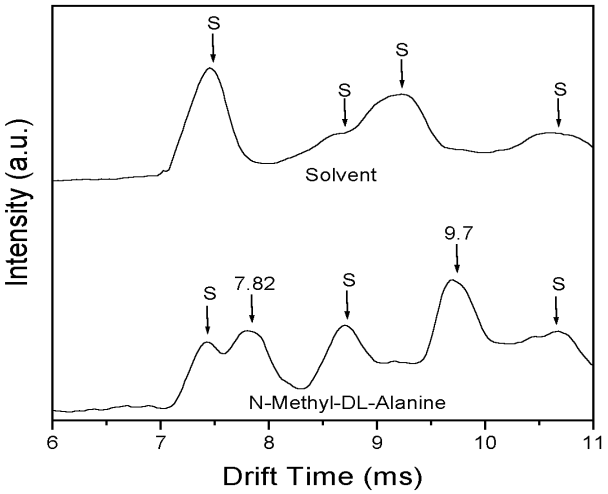
Table 1. Ion mobility constants for abiotic amino acids in N_2.

Amino Acid	Molecular Weight (a.m.u.)	K_o in N_2
Sarcosine	89	1.906
α-Aminoisobutyric Acid	103.1	2.219/ 1.761
N-Methyl-DL-Alanine	103.1	2.290/ 1.810
DL-β- Amino-n-Butyric Acid	103.1	1.809
DL-α- Amino-n-Butyric Acid	103.1	1.833
γ-Amino-n-Butyric Acid	103.1	1.833
L-Norvaline	117.1	
DL-Pipecolinic Acid	129.2	1.579
L-Norleucine	131.2	1.565
DL-Threo-β-Methylaspartic Acid	147.1	
N-Methyl-DL-Aspartic Acid	147.1	1.576
α -Methyl-DL-Aspartic Acid	147.1	1.614
L-α-Aminoadipic Acid	161.2	
DL-α-Aminopimetic Acid	175.2	1.499

The spectrum of amino acids were clearly visible in each run above residual solvent peaks in the spectra (See Fig 1). In that figure the residual solvent (*top*), N-Methyl-DL-Alanine (*middle*) and DL-α-Aminopimetic Acid (bottom). Table 1 gives the molecular weight, the drift times and ion mobility constants (K_0^m) for abiotic amino acids with N_2 as a drift gas. Three of those listed were not detected because they were not soluble in the solvent mixture we used, which was consistent with at least one biotic one (cysteine). Experiments to determine a better solvent mixture are in progress.

As shown in the Table 1, there were several abiotic AA which seemed to fragment in the ESI process. This included α-Aminoisobutyric Acid and N-Methyl-DL-Alanine which are two isomers that of methylated Alanine. Since ESI is a soft ionization technique, it is more likely that the elevated temperatures cause the methyl group to be stripped when the ion collides with the drift gas. Of note here that we utilized elevated temperatures so that cluster of H_2O molecules does not occur. We will show temperature dependent spectra of those two molecules showing the dissociated methyl group. The temperature where the dissociation occurs would be the temperature that an in situ instrument would operate at while minimal clustering of H_2O.

Figure 1. Top: IMS spectra of the solvent mixture (S). Bottom: the abiotic amino acid N-Methyl-DL-Alanine. which shows the fragmentation of the AA in the drift region.



It has been shown that changing the drift gas composition can improve the ion mobility separation in IMS [5,6]. This can be utilized to improve the selectivity. In present investigation, we demonstrated that changing the drift gases (i.e. N_2 and CO_2) can drastically improve the separability of ions.

Conclusions: We present spectra of abiotic AA and compare them to mobility spectra of biotic ones to demonstrate the utility of an ESI/IMS for use as an in situ instrument on the surface of Mars. In addition, we will present data on both temperature and solvent studies which will help define precise parameters which an *in situ* IMS, as part of a scientific package of instruments, would operate.

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