

THE EFFECT OF SALTS ON ELECTROSPRAY IONIZATION OF AMINO ACIDS IN THE NEGATIVE MODE. H. I. Kim, P. V. Johnson, L. W. Beegle, and I. Kanik, Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Drive, Pasadena, CA 91109 (Paul.V.Johnson@jpl.nasa.gov).

Introduction: The continued search for organics on Mars will require the development of simplified procedures for handling and processing of soil or rock core samples prior to analysis by onboard instrumentation. Extraction of organic molecules from rock and soil samples using a liquid solvent (H_2O) has been shown to be more efficient than heat extraction methods [1]. As such, liquid extraction (using H_2O) of organic molecules from rock cores or regolith material is a prime candidate for the required processing. In this scenario, electrospray ionization (ESI) of the liquid extract would be a natural choice for ionization of the analyte prior to interrogation by one of a variety of potential analytical techniques (mass spectroscopy, ion mobility spectroscopy, etc.). Aside from the obvious compatibility of ESI and liquid samples, ESI offers simplicity and a 'soft ionization' capability. However, a determination of the need for processing (i.e. desalting) of the liquid extract must be made.

Salts are expected to exist on the surface of Mars based on a number of observations. Indirect evidence for the presence of salts in Martian surface material at the Viking and Pathfinder landing sites has been presented in the literature [2-7] while direct measurement of extracted material from the Nakhla SNC meteorite has shown that presence of NaCl [8]. Recently formed gullies observed on Mars have been attributed to liquid water at or near the surface [9] despite the sub-freezing surface temperatures. It has been argued that the lower freezing point of brine mixtures could explain the presence of enough liquid to form these features [4, 10].

In terrestrial biological research, where many proteins, DNA, and other biology relevant substances, are often kept in a salt buffer solution, the presence of salts in electrospray solvents has proven to inhibit or prevent ionization of biomolecules [11]. As such, dramatic desalting is required before ESI can take place [12].

In order to show that liquid extraction and ESI can work as part of an *in situ* instrument on Mars, we must better understand the effect salts have on the ESI process. In the current work, we have endeavored to investigate the feasibility and limitations of negative mode ESI of Martian surface samples in the context of sample salt content using ion mobility spectroscopy (IMS).

Electrospray Ionization. Before continuing with the discussion of the current work, a short overview of the ESI process is given. In negative mode ESI, fluid emerges from a metal capillary held a few keV above a perpendicularly oriented planar electrode. Electrolytes

in solution undergo electrophoretic movement in response to the imposed electric field forming a net negative charge at the exit of the capillary. When the electrostatic forces are counterbalanced by the surface tension of the fluid, a 'Taylor cone' is formed. If the applied potential is such that the surface tension is insufficient to maintain a stable air/solvent interface, droplets with excess negative charge are expelled from the tip of the cone where the electric field is greatest. As solvent evaporates, the droplets become smaller until the Coulomb repulsion within the droplets exceeds the surface tension holding them together. At this point, the drops are 'blown apart' forming smaller charged drops. This process of evaporation and Coulombic explosion is repeated a number of times resulting in gas phase analyte ions. Further details on ESI are summarized by Cole (2000) [13].

Experiment: Previously, our group has investigated the use of electrospray ionization/ion mobility spectroscopy (ESI/IMS) for the detection and identification of amino acids [14, 15]. In the present study, IMS was used to investigate the effects of salt concentration on the ionization of amino acids by ESI in the negative mode. The fundamentals and applications of IMS are reviewed in detail elsewhere [16].

The ESI/IMS used in this study is based on previously described designs [17-19]. Briefly, the desolvation and drift regions consisted of concentric metal rings separated by macor rings. The drift tube was held at a constant temperature of 500 K at the local atmospheric pressure (~ 730 Torr at Pasadena, California). A counter flow of preheated N_2 drift gas was introduced at the end of the drift region at a flow rate of ~ 800 ml/min, while cooling gas (at ~ 400 ml/min) was introduced into a water cooled jacket surrounding the electrospray needle. This was done to prevent evaporation of the solvent inside the needle prior being sprayed.

The sample solution was delivered via an Eldex Micropro liquid chromatography pump into a metal spray needle at a flow rate of $5 \mu\text{l}/\text{min}$. A Bradbury-Neilson type ion gate was used to gate the ions into the drift region while the ion mobility signals were collected on a Faraday cup at the end of the IMS cell. The ion current was then fed into a Stanford Research Systems model SR570 low-noise current amplifier where the signal was amplified by a factor of 10^9 before being introduced into the data acquisition system.

The salts and amino acids which were studied in this investigation were purchased from the Sigma Chemical Company (St. Louis, MO) and used without further purification. All solvents (water, acetic acid and methanol) were HPLC grade and were purchased from J. T. Baker (Phillipsburg, NJ). Samples were prepared by weighing out known quantities of amino acids and then dissolving them in a solvent solution consisting of 50% water and 50% methanol.

Results and Discussion: Ion mobility spectra of aspartic acid (10 ppm and 30 ppm), glutamic acid (36 ppm) and serine (33 ppm) were collected with concentrations of NaCl ranging from 0-65 ppm. Figure 1 shows a waterfall plot of the aspartic acid (10 ppm) data set. As seen in the plot, the amino acid anion peak is the dominant feature of the IMS spectra (over the solvent feature) at salt concentrations between 0 and 1.3 ppm. However, at a salt concentration of 13 ppm, a dramatic change occurs in the character of the IMS spectra. At this point, there is a perceptible diminishing of the amino acid anion intensity while there is a concurrent and drastic increase in the solvent peak. This spectral character continues as the salt concentration is increased above 13 ppm with the amino acid anion peak slowly decaying until it is virtually indistinguishable above the baseline at 65 ppm salt concentration. This behavior was seen in all four amino acid samples tested, and Figure 1 can be regarded as representative of all four data sets. This indicates that the effect of salt on the electrospray process is independent of the amino acid species tested.

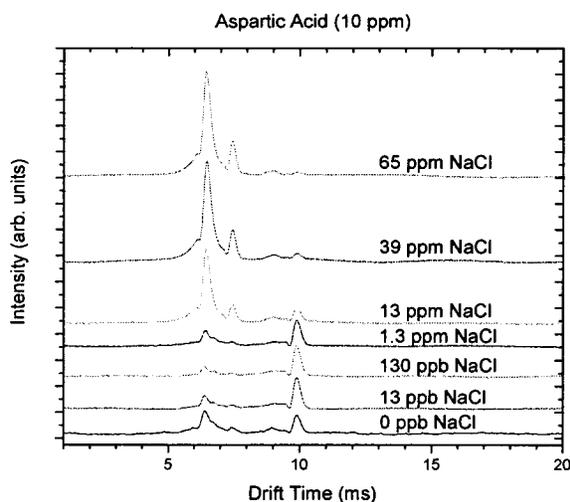


Figure 1. ESI/IMS of 10 ppm aspartic acid in a 50% water-50% methanol solvent mixture as a function of NaCl concentration. Aspartic acid peak is seen at ~9.9ms

In all four cases, the amino acids could be clearly detected at NaCl concentrations below 65 ppm. This therefore can infer an upper limit on the amount of NaCl that can be tolerated in a liquid extract to be analyzed with ESI. However, the reason for the sudden change in the ion mobility spectra between 1.3 and 13 ppm salt concentrations is not clear. It is possible that the decrease in the amino acid anion peak is due to the attachment of a Na^+ cation at the site of deprotonization. One might further speculate that the increased solvent intensity is due to an unresolved chloride peak.

IMS Spectra were also taken with other alkali metal chloride salts (LiCl, KCl, RbCl and CsCl). These spectra showed similar results as the NaCl data sets indicating that the above discussion is independent of metal cation species.

Conclusions: It is clear from the current data that the presence of salt in the sample solution has a profound affect on the ESI process. Clearly, if ESI is to be considered for inclusion in a future *in situ* Mars experiment, it is important to understand the degree to which salts are present on the surface and/or to develop desalting protocols that are amenable to robotic execution.

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