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Development of a Multi-purpose Electrospray-Electron Impact Tandem Mass Spectrometer

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Abstract

Mass spectrometry (MS) is ubiquitous in nearly all facets of chemical research and industry. Modern MS techniques include coupling various ionization schemes and sample introduction systems for multiple applications. Commercial devices that combine different types of ionization schemes and sample introduction systems are routinely available; however, are difficult to obtain at smaller, primarily undergraduate universities such as Humboldt State University (HSU) due to the high cost of these instruments. To solve the dual problems of instrument cost and flexibility, this presentation describes the proof of concept development and construction of an Electrospray Ionization-Electron Impact Tandem Mass Spectrometer (E-EITMS) at a fraction of the cost of a commercial instrument. This instrument, compared to commercially available instruments, will be novel and innovative, as it will be tailored to fit the needs of many applications. The E-EITMS will be capable of direct gas-phase injection electron impact tandem mass spectrometry, electrospray tandem mass spectrometry, and vacuum *in-situ* molecular beam photo-chemistry. Lastly, eventual goals for the instrument is to stream line the concept model to be able to take it outside the laboratory and perform real-time atmospheric analysis. Thus far a detector quadrupole (UTI 100C) has been implemented into a working tandem vacuum chamber, where a custom LabView^{TM} program has been written to digitize the data. The vacuum chambers are capable of reaching base pressures of 10^{-8} torr when closed and 10^{-5} torr when open to the atmosphere through a 50-micron pinhole and differentially pumped through 3 stages. Current work includes designing a skimmer system to reduce the pressure in the chambers when open to the atmosphere and implementing the mass selector quadrupole to work in tandem with the detector quadrupole through a series of Einzel lenses.

Keywords: Mass Spectrometry, Electrospray, Vacuum

1. Introduction

Mass spectrometry (MS) is one of the most commonly used techniques in the chemical sciences and also has applications in nearly every discipline of the biological sciences.[1-5] The principle of the technique relies on producing gas–phase ions, separating the ions based on their mass-to charge ratio (*m*/*z*) using an electric or magnetic field under high vacuum conditions, and detecting the ions in various ways.[1] In electron impact (EI)–MS, molecules are bombarded with high-energy electrons, often shattering the molecules into charged fragments that can be separated by their *m/z*. Many molecules share similar or identical fragmentation patterns, often making the identification of individual constituents in a complex sample very difficult. Coupling the MS to various separation

techniques such as gas chromatography (GC), high-pressure liquid chromatography (HPLC), and even capillary electrophoresis (CE) can circumvent this problem.[1] These types of MS techniques are ubiquitous in undergraduate chemical and biological laboratories as well as in industrial and graduate laboratories. Even so, the separation techniques are often slow and are generally not uniform for all types of molecules and phases.

Electrospray ionization MS (E–MS) has been proven to be widely useful as a means of "soft ionization".[1, 6-8] This ionization scheme forces a solution of a desired analyte(s) through a narrow, highly charged capillary, where analytes *pick up* background ions and the resulting analyte-background ion cluster is ejected from the capillary tip in the gas phase. The advantage of this technique is that the molecular mass of the molecule in question is preserved, which is often not possible in EI-MS. The E-MS process does not generally provide a fragmentation pattern similar to that observed in EI-MS, which is often used as a means of *fingerprinting* a molecule. Thus, coupling EI-MS and E-MS is a powerful combination.[1] Various analytes can be electrosprayed into a MS to determine the molecular mass of the analytes and subsequently the ion can be bombarded with high-energy electrons to provide the fragmentation pattern traditionally used to identify the molecule. Coupling E-MS and EI-MS is routine with chromatographic separation techniques discussed above,[1, 9-10] thus making the technique sensitive for solutions containing multiple analytes that share similar fragmentation patterns.

The ability to detect multiple analytes simultaneously by combining E-MS and EI-MS is not limited to chromatographic separations. Coupling the electrospray and EI ionization schemes with a *tandem m/z* filter can actually circumvent the need for any chromatographic separation processes.[1, 11] Such a system is often referred to as Tandem MS (TMS) or sometimes MS–MS and allows the analysis of complex samples in a fraction of the time required for chromatography. The technique works by electrospraying ion(s) into the first MS and filtering for one *m/z*; the selected ion is then sent to the second MS, which contains the means for quantitative detection and further ionization capabilities to produce a fragmentation fingerprint pattern. The first MS will then select subsequent ions corresponding to different molecules within the same sample and the procedure is repeated. The electrospray portion of the MS technique requires some sample preparation and the analytes must generally be polar,[1] but the tandem EI technique can also be used to directly sample the gas phase and measure samples in real time without the need for chromatographic separation or sample preparation, minimizing this limitation of electrospray ionization.

While the combined E-EI-tandem MS techniques are commonly used in industry, the systems are often expensive (>$100,000), making them difficult to obtain at smaller, non-Ph.D. granting Universities. Even so, in a prior publication[12] it was demonstrated that exposing undergraduate students to these types of techniques (where available) leads to easily obtainable data that would otherwise require lengthy experiments — which few undergraduate students could accommodate into their heavy course loads — and also provided solid career preparation for advanced techniques used in the chemical industry. Therefore, this paper outlines the development and construction of an E–EITMS for future use by undergraduate students. This instrument will be novel in comparison to a commercial device, as this E–EITMS will be designed to have multiple applications, such as direct gas-phase injection for real time analysis, electrospray injection, and *in-situ* vacuum studies. As outlined herein, preliminary work has already begun on this project with great success in the use of LabView™ programming, vacuum technology, and experimental design. The majority of the technical equipment — high vacuum and instrumentation components — required for this project has already been obtained. Once completed, this instrument will be made available as a department resource, providing many other students with a unique experience in their undergraduate education and career preparation as well as enhancing collaboration amongst faculty members through a plethora of future projects and experiments.

2. HSU Physical and Analytical Chemistry Research Laboratory

The development of or the acquisition of an electrospray-electron impact tandem mass spectrometer (E-EITMS) can be quite expensive; however, this will be accomplished with a significant reduction in cost. The late William Golden (a former Professor of Chemistry at HSU) was a research chemist for IBM and over the course of his tenure at HSU was able to broker the donation of roughly one hundred thousand dollars' worth of ultra-high vacuum equipment. This equipment includes, among other items, ultra-high vacuum (UHV) chamber components, turbo molecular pumps, rough pumps, a variety of power supplies, several ion gauges, and even a handful of quadruple EI–MS detectors. This equipment is available for student research at the Humboldt State University Physical and Analytical Chemistry Research Laboratory, where many items from this donated equipment have been put into use for this specific project.

3. Progress to date

3.1 The Working Prototype

Initially, a working prototype was designed and constructed to simplify the daunting task of constructing the full E-EITMS. This consisted of a simple gas-phase injection EI–MS. The working prototype included one EI–MS detector (UTI 100C Quadrupole Analyzer), a small six-way six-inch conflat-type vacuum chamber, a 230 L/s turbomolecular pump (Pfeiffer-Balzers TCP 240) backed by a rough pump, and a calibrated leak valve for the introduction of gas-phase samples. Initially acquired mass spectra of ambient air were collected using an oscilloscope to digitize the data. Shortly thereafter a LabView™ program was written and coupled the detector to a National Instruments™ data acquisition card to acquire MS data that can be recorded on a computer. The data acquisition program allows the acquired mass spectra to be viewed over any pre-determined sensitivity range of the quadrupole detector and record the spectrum as a text file. Additionally, the program also allows the selection of any *m/z* or range of *m/z* and also allows monitoring of the integrated signal over time, thus allowing the researcher to view the change in concentration/abundance of any one (or many) analyte(s) in real-time. Figure 1(a,b) shows representative data acquired using the LabView™ program as proof of principle.

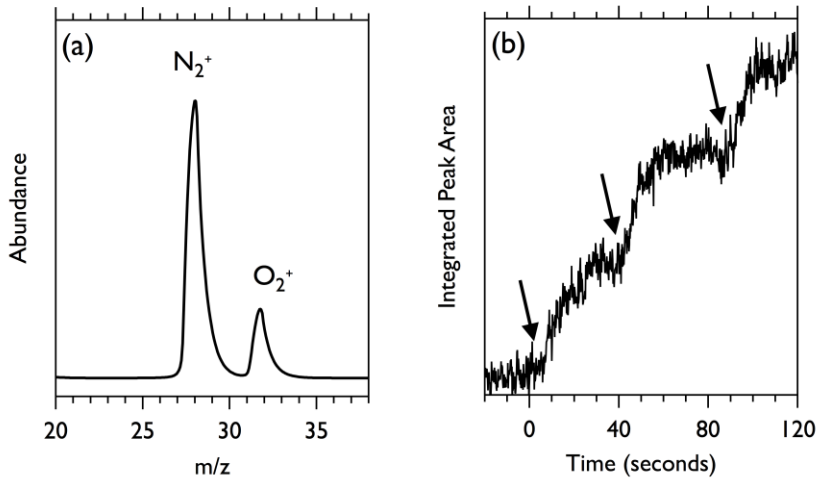

Figure 1: (a) A representative mass spectra of laboratory air, (b) real time analysis of laboratory air

In Figure 1(a) the calibrated leak valve was opened slightly to acquire a representative mass spectra of laboratory air. As can be seen, N_2 and O_2 are easily detectable and, while not shown in Figure 1(a), trace amounts of Ar, CO_2, and water vapor were also detected. Figure 1(b) demonstrates the capability to conduct real time analysis, where the arrows indicate points in time where the calibrated leak valve was opened in increasing amounts. The signal shown in Figure 1(b) is the real time integration of a user-selected area; in this case the region that was integrated corresponds to the area (20 – 38 *m/z*) shown in Figure 1(a).

3.2 Expansion For The Tandem MS Technique

Upon completion of the working proto-type and data acquisition program, work began to expand the vacuum chamber and support bench to the size required for the TMS technique. A large, mobile bench that houses the TMS and all support equipment (i.e. power supplies, electronics, etc.) was machined and this bench can be moved out of the laboratory and is quite robust. Upon completion of the TMS instrument, future projects can include monitoring samples outside of the laboratory in real-time or conducting experiments at a user facility such as The Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory (upon receipt of an accepted user proposal).[13] In addition to the construction of the mobile bench to support the TMS, students have also constructed the main vacuum chambers that house the TMS components.

Figure 2 is a photograph of the vacuum chambers and a rough schematic of how the TMS components have been integrated into the instrument.

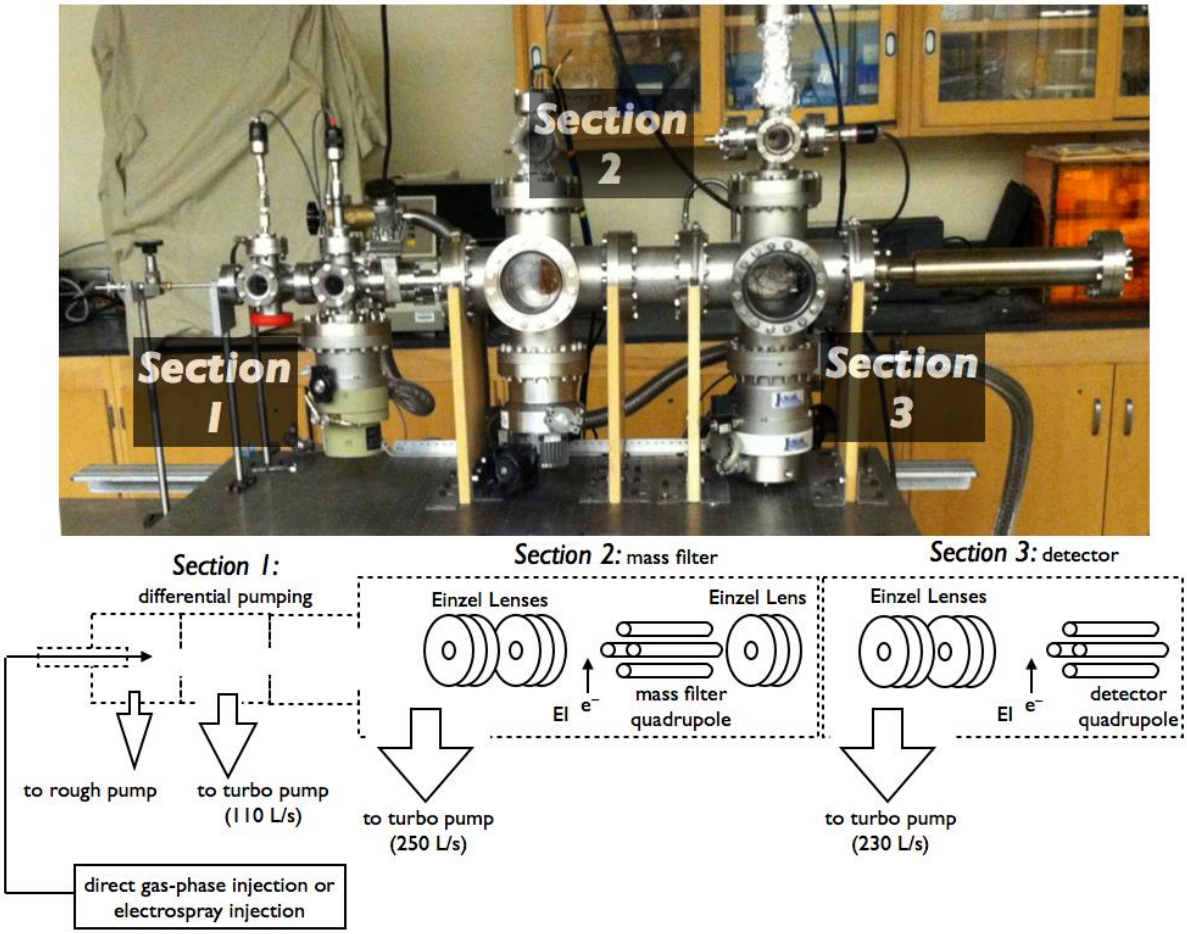

Figure 2: A schematic of the EITMS along with a recent photograph of the device

In Section 1, molecules are passed through a ¼ inch 316 stainless steel (SS) tube, where the molecules expand through a 50–µm pinhole into the first differentially-pumped region (rough pump). Molecules then travel into the second differentially pumped region (Pfeiffer TPU 110 turbomolecular pump operating at 110 L/s) isolated from the first region by a 1 mm pinhole. Finally, molecules travel into the third differentially pumped region separated from the second region by a 2 mm pinhole and enter Section 2. These stages of differential pumping bring the samples from atmospheric pressure to roughly 8×10^{-5} torr at the entrance to Section 2. In this mode of operation, the pressure in Section 3, where the MS detector is located, is roughly 4×10^{-5} torr. Section 3, as shown in Figure 2, is the same chamber, detector, and rough pump of the working prototype that generated the data shown in Figure 1(a,b). Generally, this is a reasonable pressure at which to conduct mass spectrometric analyses; however, the ultimate goal is to reduce the operating pressure in this chamber by at least magnitude factor of ten to limit the interference of background carrier gas molecules in the chamber and create molecular beam conditions[14] for future studies. The attainment of this goal has proven to be quite challenging! Currently a modified differential pumping section to that shown in Figure 2 is being explored and will be detailed in the next section.

3.3 Sample Introduction System

Designing and building a sample introduction system that can create molecular beam conditions from the introduction of direct gas phase injection or electrospray ionization is the current avenue of research on this project. Insofar, one design has been tested and will be outlined herein. It is expected several other iterations of this design will be tested in the immediately future.

Figure 3 shows the current sample introduction system, note that this schematic replaces Section 1 shown in Figure 2; however, many of the components are identical.

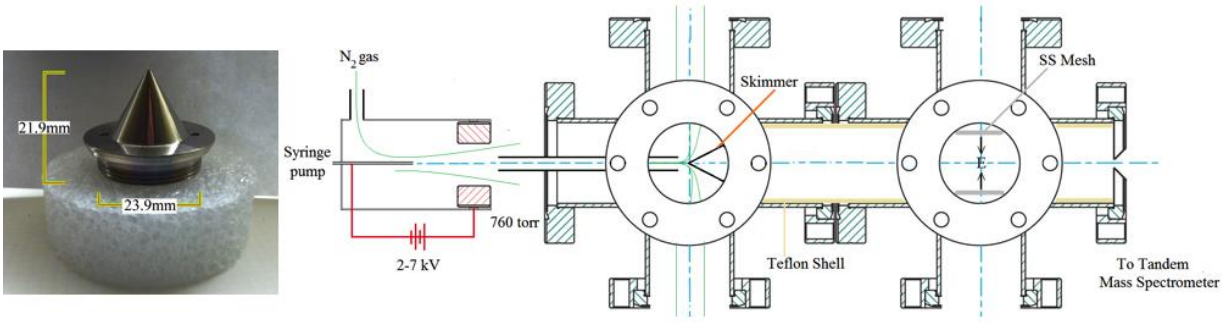

Figure 3: A schematic of the current sample introduction system.

Molecules are still passed through the same ¼ inch 316 SS tube with the 50–µm pinhole; however, ~1 cm after the tube opening in the first vacuum chamber stage they encounter a nickel skimmer cone with a 0.9 mm orifice (PerkinElmer WE021137) that is attached to a custom machined Teflon shell that acts as an insolating sheath for the metal chamber walls. This will allow future researchers to apply a focusing voltage on the skimmer without electrifying the metal chamber. In this schematic it can be seen a syringe pump is depicted upstream from the sample introduction tube. This is a proposed design; insofar this new sample introduction system has only been tested with direct gas phase injection. A SS mesh that can have a voltage applied to it is also proposed to follow immediately after the Teflon shell ends to further focus molecules through the 2 mm orifice that separates Section 1 from Section 2 (Figure 2).

4. Future Work And Conclusions

Many aspects of the development of the E-EITMS still remain; however, each aspect represents the possibility for future generations of students. For example, currently the mass filter quadrupole shown in Section 2 of Figure 2 has been installed but not tested. This was accomplished with a commercially available quadrupole mass filter (UTI 100C Quadrupole Analyzer) equipped with an EI source that was customized with Teflon rings, which allow the quadrupole component to fit snuggly against the metal walls of the bridge between Section 2 and Section 3. The Teflon rings allowed the quadruple to be electronically isolated from the rest of the chamber. Another example of future work is the Einzel lenses shown in Sections 2 and 3 of Figure 2. Einzel lenses are routinely employed as a means of focusing a beam of ions onto a specific target, such as the detector quadruple. The lenses have been custom machined and installed in Section 3 shown in Figure 2; however, they have not been tested yet either. Lastly, the electrospray portion is being constructed and tested currently and offers a plethora of research that can be completed by future undergraduate students.

The development of an E-EITMS by undergraduate students has been outlined. This project will provide undergraduate students the unique opportunity to conduct research and be involved in experimental design at a higher level than normally available to undergraduate students. Completion of this device will also facilitate future collaborations among faculty members, where many different types of projects will be investigated. For example, synthetic chemists can use the device to identify and quantify reaction products and/or intermediates. Kinetic studies can be conducted by taking advantage of the real-time, direct gas-phase injection scheme. Additionally, *in-situ* molecular beam photo-chemistry studies can be investigated by irradiating various mass selected electrosprayed ions and using the MS detector to investigate photo-products. These experiments will be complementary to those[13] that can be conducted at the ALS upon receiving an accepted user proposal. Development of this device is innovative in that it will have many applications hitherto unknown at an undergraduate institution.

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