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Thermospray liquid chromatography–mass spectrometry with a quadrupole ion trap mass spectrometer

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ABSTRACT

A thermospray ion source using corona discharge ionization was interfaced to a quadrupole ion trap mass spectrometer via a multi-element lens system. Ions were injected into the trap periodically where they were stabilized by collisions with helium bath gas. Mass spectra were recorded on the trapped ions using the mass-selective instability scan mode. Data are shown for a peptide and a nucleoside and the effects of some experimental variables on the spectra are explored.

INTRODUCTION

As both a reactor for gas phase ion–molecule reactions and as a sensitive mass analyzer, the quadrupole ion trap mass spectrometer has proven to be a versatile tool for the qualitative and quantitative analyses of volatile organic compounds [1,2]. The sensitivity of ion traps [3] and the ability to efficiently dissociate ions in the course of tandem experiments [4–6] have increased the value of these instruments. Ions are typically generated within the cavity of the trap by electron-impact ionization [7], chemical ionization [8] or photoionization [9]. However, internal ionization is not the only means of ion trap operation; ionization outside of the trapping volume, followed by injection of the externally generated ions, has also been described. In particular, laser desorption [10] and Cs^+ desorption [11], followed by injection of the desorbed cations, have been reported. It is well known that the development of the family of techniques known generically as desorption ionization (DI) has had a major influence in expanding the scope of mass spectrometry (MS), particularly in facilitating the characterization of biological compounds.

The combination of high-performance liquid chromatography (HPLC) with MS has also been widely recognized as possessing enormous potential for the

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analyses of polar, non-volatile or thermally unstable compounds not amenable to gas chromatography–mass spectrometry. This has stimulated attempts to interface particle beam [12], electrospray [13] and thermospray (TSP) [14] sources to mass spectrometers [15]. Because TSP is one of the most widely used and successful interfaces for LC–MS [16] its utilization with an ion trap is detailed here. In parallel with this effort, an electrospray source has also been interfaced to the ion trap [17]. Because electrospray yields multiply charged ions of biological compounds, it is particularly valuable for molecular weight measurements, even of high-molecular-weight compounds. This capability plus a limitation to low flow-rates have led to a successful ion trap interface [17].

The TSP technique has been useful for the analysis of many types of compounds commonly encountered in biological systems including those that previously required extensive sample clean-up and chemical derivation prior to MS analysis. The success of TSP is attributed to the fact that it is a convenient LC–MS interface that is compatible with conventional HPLC systems. It permits the use of reversed-phase chromatography at flow-rates of 1–2 ml min^{-1}. Chromatographic integrity is maintained since the interface does not create significant peak broadening.

A Finnigan TSP source was coupled to the ion trap mass spectrometer, operated in the mass-selective instability mode [7], to study the feasibility of interfacing an LC to the trap [18]. The weakness of TSP, such as relatively poor sensitivity for some compounds, poor quantitative capabilities and a strong dependence on experimental parameters, should be alleviated by accumulating ions formed over a period of time. The capability of the ion trap for characterization of molecular ions by either ion–molecule reactions or by collision-activated dissociation is potentially a further advantage of this combination given the limited degree of fragmentation seen in TSP. Finally, TSP has often been interfaced to quadrupole mass spectrometers with mass ranges of less than 2000 dalton. As recently demonstrated, the ion trap offers a much higher usable mass range than these devices and therefore should allow the analysis of higher-molecular-weight compounds [11,19].

The original approach by Vestal *et al.* [14] for the development of a combined LC–MS system employed a 50 W CO_2 laser to rapidly vaporize both the sample and solvent. A redesigned, somewhat simpler version was later introduced, which used an oxy-hydrogen torch to vaporize the LC effluent [16]. This concept soon led to the development of an electrically heated vaporizer using cartridge heaters [20]. Today, direct electrical heating of the vaporizer by passing an electric current through the capillary vaporizer is used in most TSP ion sources. Passage of the column effluent through the vaporizer results in a supersonic jet of vapor composed of a mist of fine droplets. The droplets, which contain both positive and negative charges, continue to vaporize inside the hot TSP ion source. At a certain critical size, the net charge on a droplet is sufficient to produce an electric field at the surface (estimated to be on the order of 10^9 V/m) capable of inducing

ion evaporization [21]. An auxiliary pumping line located directly opposite the vaporizer provides additional pumping capacity. Ions are sampled with the aid of a repeller electrode through a conical orifice into the mass analyzer region. A source of this type, the Finnigan TSP system, which originally was designed for use with quadrupole mass filters, was utilized in these experiments.

EXPERIMENTAL

A TSP source (Finnigan, San Jose, CA, U.S.A.) was interfaced to a prototype ion trap mass spectrometer (ITMS, Finnigan, San Jose, CA, U.S.A.) [4]. The rectangular ITMS manifold allows for easy adaptation of the TSP source for axial injection of ions into the ion trap. The effluent from the LC pump (typically at flow-rates between 0.2 and 0.5 ml/min) enters the vaporizer through a stainless-steel capillary tube (typically 1.5 mm I.D.). The jet of vapor could be ionized by either electron ionization or by discharge chemical ionization, though only discharge chemical ionization was used in these experiments. The vaporized effluent was differentially pumped with a belt-driven roughing pump and two turbomolecular pumps, 70 and 300 l/s. Typical operating pressures were maintained at approximately $1 \cdot 10^{-4}$ Torr (measured with an ionization gauge) at a flow-rate of 0.5 ml/min.

Upon ionization of the vaporized effluent, the ions are repelled from the ion volume into the ion injection system. Fig. 1 illustrates the lensing system for ion injection of the TSP-generated ions into the ion trap. Initial focussing of the ions is accomplished with three thin lenses on the TSP source. The ions are then focussed onto the aperture of the entrance endcap electrode of the ion trap using a three-element einzel injection lens assembly, previously described [10] and now commonly used in experiments which employ external ion sources [22]. A PTFE

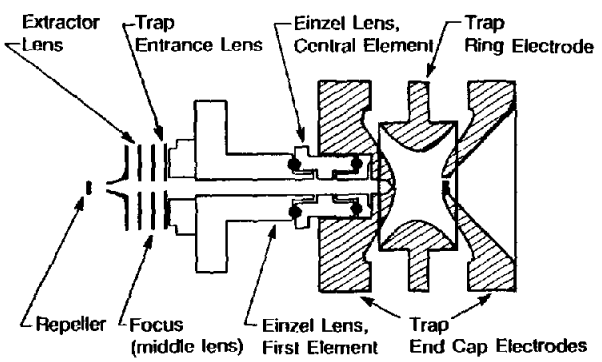

Fig. 1. Schematic diagram of the thermospray ion trap ion injection assembly. The central element of the injection einzel lens is gated to allow ions to enter the ion trap in pulses.

guide was machined and mounted to the first cylindrical einzel lens element to ensure that the TSP source was on-axis with the ion trap. The potentials applied to these lenses were controlled via independent power supplies. Because of the high pressure in the ion trap, the kinetic energy of the injected ions is collisionally damped to allow trapping. The presence of helium in normal electron-impact ionization experiments aids in trapping and in collisional damping of the trapped ions. Helium was used in these experiments at approximately 1 mTorr; however, it was observed that helium was not mandatory for successful trapping of ions generated by TSP because of the already high background pressure.

Fig. 2 illustrates typical scan functions for obtaining either mass spectra or MS–MS spectra on TSP generated ions. These scan functions were designed through modifications to the commercial ITMS software (Version 2.0). As shown in Fig. 2a, ions are introduced into the ion trap by gating the central einzel lens element from a positive potential to a negative potential. During the ion injection period, which typically lasts for 10–50 ms, the RF level on the ring electrode of the ion trap was held at a constant voltage chosen to provide optimum sensitivity for the injected species [22]. The voltage for the central lens element was supplied

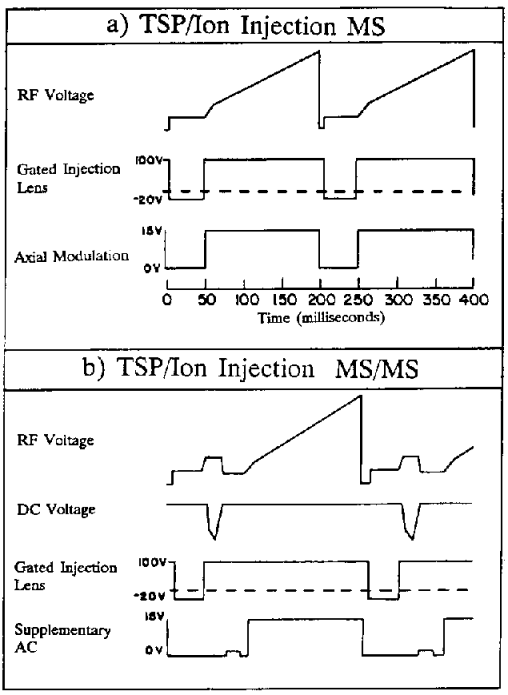

Fig. 2. Typical scan modes for thermospray-generated ion injection into the ion trap. (a) Scheme used to obtain mass spectra; (b) scheme to obtain MS–MS spectra.

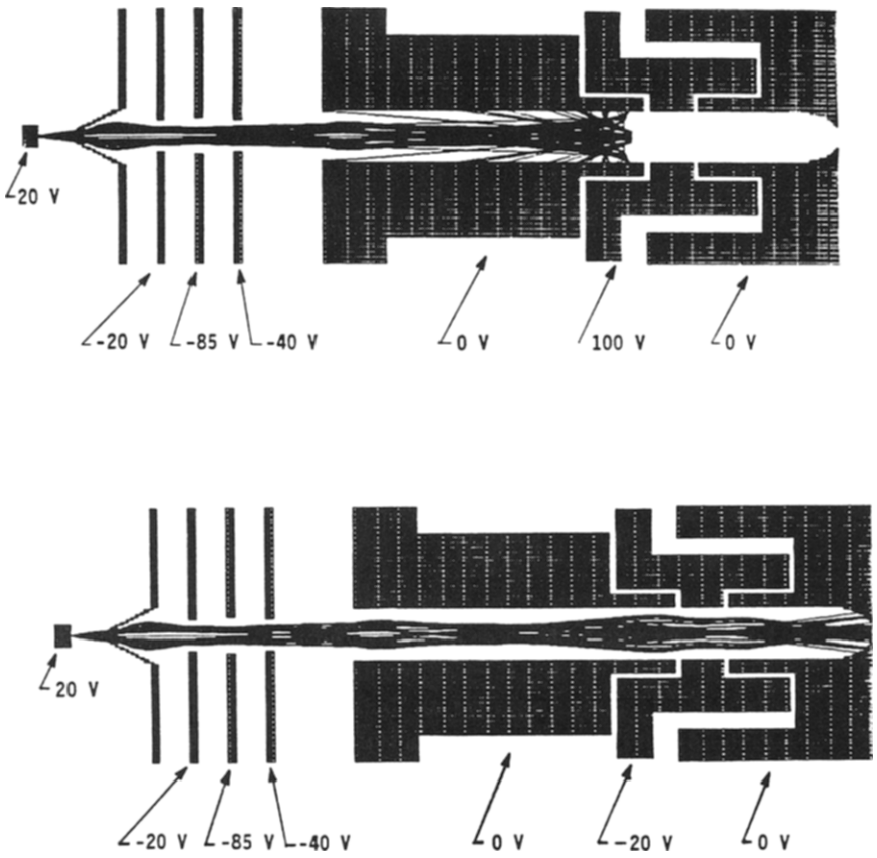

Fig. 3. SIMION modelling of the ion injection process when thermospray is used as the method of ionization. The central einzel injection lens is gated to either prevent ions from entering the ion trap or to focus the injected ions onto the aperture in the entrance endcap electrode.

by a programmable bipolar power supply consisting of a monopolar Kepco OPS-2000 programmable power supply and a floated Hewlett-Packard 6110A power supply. The control voltage was supplied by a 16-bit DAC, interfaced to the single board microcomputer which controls other timing sequences in the trap (such as scanning of the RF voltage and the application of the supplementary AC voltage for MS–MS experiments). Axial modulation, where a supplementary AC voltage is applied to the endcap electrodes, may be used for either sensitivity enhancement or for mass range extension [11]. Fig. 2b shows a scan function which allows MS–MS experiments to be performed on TSP-generated ions, though the results illustrated here are limited to the experiments shown in Fig. 2a.

The ion optical program, SIMION [23], was used to optimize the voltages applied between the focussing lenses for optimum trapping efficiencies. Fig. 3

shows two simulations for ion injection of TSP-generated ions. The upper panel illustrates typical potentials used to prevent ions from entering the ion trap and the lower panel those used to focus the ion beam onto the aperture of the entrance endcap electrode. (See Fig. 2 for the sequences used to gate the central element). The aperture in the entrance endcap electrode is normally utilized in the unmodified ion trap system for injecting electrons into the ion trap for electron-impact ionization.

The mobile phase used in this study was methanol–water (50:50); typical flow-rates were between 0.5 and 1.0 ml/min. The vaporizer and jet temperatures were set to 120 and 200°C, respectively. Discharge ionization at potentials from 0.6 to 1 kV were used. Samples, dissolved in the mobile phase, were introduced into the TSP source via flow injection.

RESULTS AND DISCUSSION

The TSP source was first characterized by obtaining a mass spectrum of the methanol solvent. Fig. 4 illustrates that protonated methanol (m/z 33) occurs in relatively low abundance, the majority of ions in the mass spectrum being the result of ion–molecule reactions. The proton-bound dimer (m/z 65) loses H_2O to form the most abundant ion which is at m/z 47. The loss of H_2O from the proton-bound trimer generates the ion at m/z 79. The formation of these ion–molecule products is a result of both the high pressure in the source and the relatively long injection and trapping times. A notable attribute of this spectrum is the very broad ion responses. The peaks tail toward lower mass as a consequence of the high background pressure ($1 \cdot 10^{-4}$ Torr). In order to lower the operating pressure, it would be necessary to differentially pump the TSP source

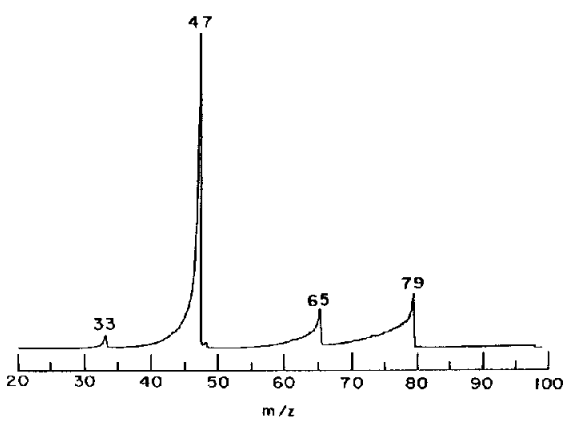

Fig. 4. Mass spectrum of TSP-generated methanol using discharge ionization. The flow-rate was 1.0 ml/min.

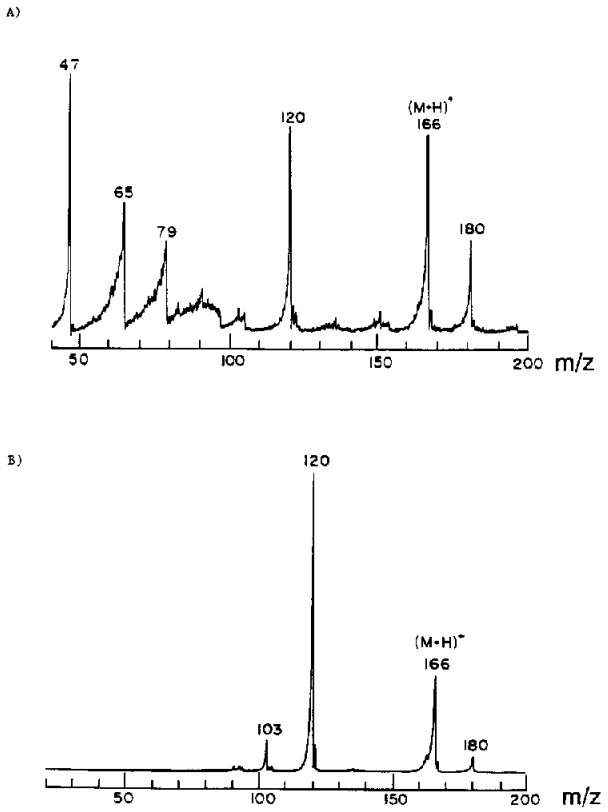

Fig. 5. (A) Mass spectrum of 100 ppm phenylalanine dissolved in methanol–water (50:50). The repeller potential was 20 V. The injection time was 100 ms. (B) Mass spectrum of 100 ppm phenylalanine dissolved in methanol–water (50:50). The repeller potential was 100 V with an injection time of 20 ms.

from the mass analyzer region, though this was not done for these experiments. The observed peak broadening cannot be attributed to space charging [2] which is a common cause of reduced resolution in ion traps if too many ions ($> 1 \cdot 10^6$) are trapped. Under a space charged condition, tailing of the ion peaks is observed toward higher masses, and not toward lower masses as observed in Fig. 4.

The amino acid phenylalanine was dissolved in methanol–water (50:50) at a concentration of 100 ppm to obtain the mass spectrum shown in Fig. 5A. The ionization method was discharge ionization and the repeller voltage was set to +20 V. The sample and solvent ions were accumulated in the ion trap for 100 ms prior to obtaining the mass spectrum. The spectrum shows the same solvent peaks as seen in Fig. 4. In addition, the $[M+H]^+$ ion is observed at m/z 166 as is its fragmentation product m/z 120, which is due to loss of H_2CO_2. The ion–molecule product at m/z 180 results from loss of water from the phenylalanine–methanol adduct. At a higher repeller potential of +100 V and an ion injection time of only 20 ms, more fragmentation of the methanol cluster ions and of the

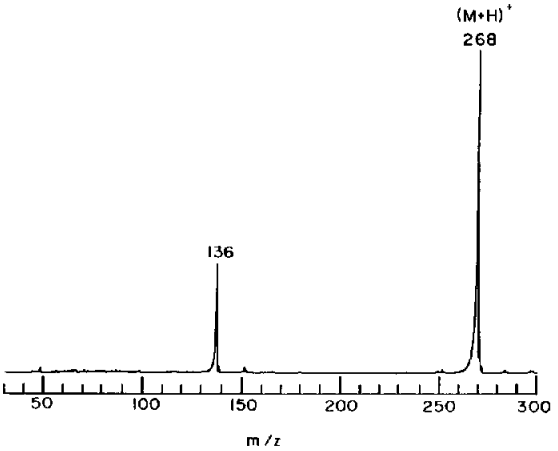

Fig. 6. Mass spectrum of adenosine dissolved in methanol–water (50:50). In this experiment, the repeller potential was 20 V with an ion injection time of 20 ms.

phenylalanine ion is observed and fewer ion–molecule side-reactions occur as illustrated in Fig. 5B. The formation of more intense fragment ions inside the TSP source through the use of high repeller voltages has been well documented by other research groups [24], and this effect is more pronounced under discharge ionization conditions. However, the fragments observed in this study could also arise in the ion trap itself.

The further example of an ion trap mass spectrum from TSP-generated ions is illustrated in Fig. 6 for a 100-ppm aqueous solution of the nucleoside adenosine. A single fragment ion at m/z 136 is observed from the $[M+H]^+$ ion at m/z 268. The injection time for this experiment was 20 ms at a repeller potential of 20 V.

CONCLUSIONS

A TSP source can readily be interfaced to an ion trap mass spectrometer. The combination displays the expected high sensitivity but in order to fully utilize this TSP interface, it will be necessary to differentially pump the source region. As illustrated in the mass spectra, the high operating pressures caused peak broadening at flow-rates less than 1 ml/min. To be used as a general LC interface, the flow-rates must be increased to 1.5 or 2.0 ml/min, again requiring that the source region be differentially pumped from the analyzer region. In spite of these limitations, the results reported here confirm that the ion trap is a useful mass analyzer for LC using TSP ionization. Recent data [25] which demonstrate that multiply charged ions can be generated by thermospray ionization add further interest to the experiments reported here. The high mass/charge range of the ion trap together with its capabilities for ion–molecule reactions should facilitate study of large biomolecules as the singly or multiply charged species.

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