Super-Atmospheric Pressure Ion Sources: Application and Coupling to API Mass Spectrometer

Lee Chuin Chen,*1 Md. Matiur Rahman,2 and Kenzo Hiraoka2

1Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, 4–3–11 Takeda, Kofu, Yamanashi 400–8511, Japan
2Clean Energy Research Center, University of Yamanashi, 4–3–11 Takeda, Kofu, Yamanashi 400–8511, Japan

Pressurizing the ionization source to gas pressure greater than atmospheric pressure is a new tactic aimed at further improving the performance of atmospheric pressure ionization (API) sources. In principle, all API sources, such as ESI, APCI and AP-MALDI, can be operated at pressure higher than 1 atm if suitable vacuum interface is available. The gas pressure in the ion source can have different role for different ionization. For example, in the case of ESI, stable electrospray could be sustained for high surface tension liquid (e.g., pure water) under super-atmospheric pressure, owing to the absence of electric discharge. Even for nanoESI, which is known to work well with aqueous solution, its stability and sensitivity were found to be enhanced, particularly in the negative mode when the ion source was pressurized. For the gas phase ionization like APCI, measurement of gaseous compound also showed an increase in ion intensity with the ion source pressure until an optimum pressure at around 4–5 atm. The enhancement was due to the increased collision frequency among reactant ion and analyte that promoted the ion/molecule reaction and a higher intake rate of gas to the mass spectrometer. Because the design of vacuum interface for API instrument is based on the upstream pressure of 1 atm, some coupling aspects need to be considered when connecting the high pressure ion source to the mass spectrometer. Several coupling strategies are discussed in this paper.

Please cite this article as: L. C. Chen, et al., Super-Atmospheric Pressure Ion Sources: Application and Coupling to API Mass Spectrometer, Mass Spectrom (Tokyo) 2014; 3(2): S0024; DOI: 10.5702/massspectrometry.S0024

Keywords: high pressure ion sources, gaseous breakdown, electrospray, probe electrospray, field desorption, corona discharge ionization

INtroduction

Ionization source is a key part in mass spectrometry, and the gas pressure around the ion source is an important parameter for ion formation and ion transmission to the mass analyzer. It is true for ionization methods like electrospray ionization (ESI), chemical ionization (CI), and perhaps, matrix-assisted laser desorption/ionization (MALDI) as well. The pressure of the ion source determines the gas phase density and the collision frequency of ion with the background gas molecule. In the early mass spectrometer that used electron ionization (EI) exclusively, the pressure in the ion source was in the order of 10−4 Pa, which was low enough to ensure no interaction between the EI generated ion and the background gas molecule. Increasing the pressure in the EI source gives rise to the gas phase ion–molecule reactions. When the reagent gas is intentionally introduced, the EI source becomes a chemical ionization (CI) source, an ionization method which is softer than EI. The early term of “high pressure ion source” refers to this type of ionization taking place under the gas pressure of 0.1 to 5 Torr, and it is still widely used for the study of ion–molecule reaction.

The atmospheric pressure chemical ionization (APCI), a popular ionization method used in liquid chromatography mass spectrometry along with ESI, is, as denoted by its name, a variant of CI done under atmospheric pressure (~105 Pa) condition. For gas phase ionization, one might expect that its performance can potentially be enhanced by further increasing the ion source pressure. Some theoretical treatment has been worked out by Fernandez de la Mora on the gaseous ionization efficiency of secondary electrospray, and it has proposed that “super-atmospheric operation would be more preferable in space-charge-limited situations.”

For spray related ionization methods, electrospray and its variants (nanoESI, ion spray, etc.) are so far the most efficient techniques for ion generation. Prior to the era of electrospray, the ionization methods that relied on high electric field, such as field desorption, and electrohydrodynamic spray, had been attempted under ultra high vacuum with certain degree of success. However, the problem of electrohydrodynamic spray under vacuum is that the choices of solvents are limited to those with low vapor pressure, and the desolvation process is not as efficient as that of atmospheric pressure electrospray. Fenn et al. revo-
lutionized the ESI method by bringing the ion source to the atmospheric pressure ambient, and the bath gas provided sufficient thermal energy to the charged droplets for the vaporization of solvent without freezing them. Generation of ions in the atmospheric pressure rather than in vacuum also helps to keep the internal and translational energies of the ions at a level corresponding to the bath gas temperature. As can be seen from the history of ESI development, the key issues for success are the operating pressure and the efficiency of transporting ions from the high pressure side to the vacuum of mass spectrometer. Surprisingly, there has been little effort to explore the workability of ESI in the pressure range other than atmospheric pressure. Investigation of the pressure and space charge effects on the electrospray current has been performed up to 2 atm, but the ESI process under such condition was not well studied. Recently, an attempt has been made to conduct the electrospray under sub-atmospheric pressure for improving the ion transmission. However, under the gas pressure of 1–10 Torr, it is rather easy to induce corona or glow discharge that can disrupt the stability of electrospray severely.

Recently, we have introduced a series of high pressure ion sources implemented with electrospray, nano and probe electrospray, and field desorption. These ion sources were pressurized with air or nitrogen to a pressure higher than atmospheric pressure (p>1 atm), and were connected to API mass spectrometer via various coupling strategies. These ion sources have some unique features that can supplement the existing ionization methods. For example, conducting the electrospray under super atmospheric pressure condition enhanced the dielectric strength of gas around the ESI emitter and the counter electrode, and that increased the onset potential of electric discharge (Paschen’s law). This feature is beneficial for ESI because the onset potential for electrospray, which is governed by the liquid surface tension is not affected by the gas pressure. If the ion source pressure is high enough, even pure water, which is notoriously difficult to handle with electrospray owing to its high surface tension, can be electrosprayed efficiently in both positive and negative ion modes. While organic solvent is typically used in ESI to reduce the liquid surface tension, for certain analysis like native protein detection, the use of aqueous solution buffer can disrupt the stability of electrospray severely.

RESULTS AND DISCUSSION

Except for miniaturized instrument, and some early version that use single stage vacuum system, most commercial API mass spectrometers adopt the differential pumping to sample the gas from the atmospheric pressure side, while keeping the high vacuum in the mass analyzer stage. The typical pressure in the first pumping stage is about 1 Torr, with an ion sampling orifice of ∼0.5 mm in diameter. Because the upstream pressure that can be tolerated by an API-MS is atmospheric pressure, the ion sources with operating pressure greater than 1 atm cannot be coupled directly without modification in the vacuum system. In our first high pressure ESI prototype, the source was not connected directly to the mass spectrometer but was separated with a distance of 2–3 mm from the sampling orifice. In the second prototype, improvement was made by coupling the ion source directly to the mass spectrometer using a modified ion transport tube with smaller inner diameter (0.25 mm). In this proceeding paper, we will discuss several coupling strategies and the latest prototypes that used an ion transport tube with larger i.d. (0.5 mm) incorporated with additional pumping system.

EXPERIMENTAL

Mass spectrometer

High pressure ion sources have been tested with time-of-flight (AccuTOF, JEOL, Tokyo, Japan), Orbitrap Fourier transform (Exactive, Thermo Fisher Scientific, Bremen, Germany), and linear ion trap (LTQ-Velos, Thermo Fisher Scientific, San Jose, CA) mass spectrometers. Most of the work in this paper was done with linear ion trap. The interface design of LTQ-Velos is somewhat different from the standard inlet-skimmer arrangement and a focusing stacked ring ion guide (S-lens) is used to focus the ion to the ion guide. The settings for the instrument were as follows: temperature for the ion transport tube: 150–300°C, magnitude for S-lens: 50–80%, and magnitude for the r.f. ion guide: 600–800 V.

High pressure ion source

The detailed design of the ion sources have been reported previously and their common features will be briefly described here. Basically, they consisted of a high pressure chamber made of aluminum alloy that could be attached/ detached easily to/from the ion sampling interface. The ionization took place inside the high pressure chamber that was pressurized with dry air from air compressor, or 99.995% nitrogen from high pressure tank. The ion sources for ESI and FD were equipped with transparent view ports to observe the effect of pressure on Taylor cone and ESI plume using microscope.

Figure 1 shows three coupling strategies for the high pressure ion source to the mass spectrometer. The ion source depicted in Fig. 1 is ESI, but it can also be implemented with other ionization methods like those shown in Fig. 2. In our first prototype, ions produced from the ESI were not fed directly to the vacuum stage of the mass spectrometer, but were transported out from the high pressure vessel to an atmospheric pressure side via a metallic capillary as shown in Fig. 1a). The ions entered the mass spectrometer via the...
sampling orifice. The arrangement might looked like a pneumatic-assisted ESI (such as ion spray or electrosonic spray), but the major difference was that the whole ESI emitter was placed inside the high pressure vessel. This strategy was the simplest one without any modification to the mass spectrometer. However, there was a shortcoming with this method. For example in our first prototype, the outlet diameter for the high pressure vessel was 0.8 mm and the gas flow rate was \( \sim 17 \) L/min. The typical gas sampling rate for API mass spectrometers with differential pumping and \( \sim 0.5 \) mm inlet orifice is about 1 L/min. This means that most of the ion rich gas from the ion source was not introduced to the mass spectrometer due to the limitation of gas load allowed for maintaining the operational vacuum level.

The second strategy as shown in Fig. 1b) is to reduce the inner diameter of the ion transport tube from, for example, 0.5 mm to 0.25 mm. Some of the commercial instruments, such as LTQ and Exactive from Thermo Fisher Scientific, use an ion transport tube that can be easily detached from the interface for washing without breaking the vacuum of the instrument. It is achieved using a simple ball valve mechanism at the vacuum interface. The original ion transport tube has an outer diameter of approximately 1/16 inch, and it can be easily replaced by a modified capillary made from commercial 1/16 inch stainless steel capillaries that are commonly used in GC-MS. The coupling method depicted in Fig. 1b) was used in our second HP-ESI prototype, which featured a modified ion transport tube with inner diameter of 0.25 mm. When the ion source was pressurized to \( \sim 4 \) to 6 atm, the throughput of gas was about the same as that of the original API ion source condition, and the pressure in the first vacuum stage of the mass spectrometer would become the normal operating value, which was in the range of 1–2 Torr. This arrangement was simple, and the high pressure and the original AP ion sources could be switched in a plug and play basis without modification in the pumping system.

However, for the fundamental study of pressure effect on the ionization process, this experimental setup was not satisfactory as the pressure in the first pumping stage, which is known to be of paramount importance for the desolvation and fragmentation of ion,\(^{35}\) could not be kept constant when the pressure in the ion source was changed. The pressure in the first pumping stage also affected the ion transmission by the r.f. ion guide placed in the subsequent vacuum stage. Furthermore, since the inner diameter of the ion transport tube was smaller than the typical value, ion loss due to the collision with the inner metallic wall was expected to be greater.\(^{36}\) Despite these shortcomings, the performance of the second HP-ESI prototype (Fig. 1b)) was

---

**Fig. 1.** Strategies for coupling the high pressure ion source to API mass spectrometer. a) Indirect coupling, without any modification to the mass spectrometer. b) Direct coupling using a modified ion transport tube with smaller inner diameter (0.25 mm). c) Direct coupling using an ion transport tube of 0.5 mm inner diameter and an additional pump with variable pumping speed. The ion source depicted here is ESI.
better than the first one (Fig. 1a)). For example, as seen in Fig. 3, protein in the concentration of $10^{-7}$ M in water and ammonium acetate aqueous solution, which could not be easily handled using unassisted ESI, could be detected with good signal to noise ratio with HP-ESI. Besides positive mode, stable electrospray with good ionization efficiency could also be obtained in the negative ion mode.

To enhance the ion transmission efficiency, our latest ion source prototypes used an ion transport tube with i.d. of 0.5 mm as depicted in Fig. 1c). The original ion transport tube for the LTQ-Velos was substituted with a custom made ion transport tube made from commercial straight cut stainless steel tube (GL Sciences, Tokyo, Japan), and it was slightly longer (~7 cm) than the original one. To maintain the vacuum in the first pumping stage, a Roots booster pump (PMB 003C, ULVAC, Kanagawa, Japan) was added in between LTQ and the original rotary vane pumps. The maximum pumping speed for PMB 003C was 5,500 L/min. The pumping speed of the booster pump was controlled by a variable frequency controller (VF-AS1, Toshiba Schneider Inverter, Japan) to keep the pressure in the first pumping stage at 1.95 Torr. Although the local pressure around the ion transport tube could be slightly different from the value measured by the vacuum gauge, a same operating pressure could be maintained in the vacuum chamber that housed the r.f. ion guide.

A comparison between the ion transport tube with different i.d. (0.25 mm and 0.5 mm) using offline nanoESI is shown in Fig. 4. In both cases, the pressure in the ion source was separately optimized at around 2 bar. As expected, the tube with larger i.d. gave a better ion signal, and an enhancement of more than 5x had been observed for both positive and negative ion modes. In another measurement as shown in Fig. 5, the ion intensity was plotted against the ion source pressure using the ion transport tube with 0.5 mm i.d. The ion source was electrospray and the sample was $10^{-6}$ M cytochrome c. In order to have a fair comparison with the atmospheric pressure ESI, the solvent used here was a mixture of methanol/water so that the electrospray could be initiated without electric discharge under 1 atm. Enhancement in ion signal was observed when the ion source was pressurized to $\geq 2$ bar (1 bar–1 atm). Similar observation was also noticed with nanoESI. This result shows that the role of pressuring the ESI source is more than just preventing the corona discharge, but also improving the ionization efficiency and the ion transmission.

Besides standard electrospray and nanoESI, the high pressure ion source had also been implemented with probe electrospray, field desorption and corona discharge chemical ionization as shown in Fig. 2. Probe electrospray ionization (PESI) is a variant of ESI, in which, instead of using capillary, a sharp solid needle (e.g. acupuncture needle) is used as the sampling probe as well as the ESI emitter. As shown in Fig. 2d), the PESI probe is actuated to dip into the liquid
surface for sampling, and moves up along the vertical axis to an ionization position to initiate the electrospray. Upon the sampling, the liquid adhered to the needle may not be on the end of the tip, but rather slightly away from it due to the liquid surface tension. The liquid has to be driven to the tip by the high voltage to form electrospray. Because the liquid amount is in the order of pico to nano liter, there are cases where the electrospray cannot be initiated due to large liquid surface tension before the drying out of solution. Pressuring the PESI source have been proven to overcome this problem and had improved the sensitivity and stability of PESI.

With sufficient dielectric strength for the gas under high pressure, field desorption (FD), which previously could only be done under high vacuum, had also be achieved under non-vacuum condition. FD can be seen as an electrospray or electrohydrodynamic spray of molten, or mobilized sample without solvent. The high potential applied to the FD is typically higher than that of ESI. For example, the FD emitter in Fig. 2c) was a tungsten wire (20 µm in diameter), positioned at a distance of ~2 mm from the counter electrode, and the applied voltage was 8 kV. Pictures in Fig. 2c) show the formation of conical protrusion from the molten sample without and with the application of high voltage. The mechanism of vacuum and non-vacuum FD are likely the same and they share a similarity with electrohydrodynamic (EHD) spray and ESI that, the sample molecules on the emitter have to be in a mobile state for the electrochemical reaction to take place for charge transfer. However, in the field desorption, the samples are not evaporative under atmospheric and super-atmospheric pressures, and the repeated jet fission process (owing to the shrinking of desorbed droplets) in ESI is unlikely to take place. Thus, the detected gaseous ions in our experiment should be generated directly from the apex of the protrusion of the liquefied sample layer where the strong electric field was concentrated.
For gas phase ionization like that of APCI, it makes sense that the ionization process, such as protonation can be further promoted under higher gas pressure owing to the increased frequency of collision between reactant ion and analyte molecule. Figure 6 shows the plot of ion intensities for the protonated methamphetamine and a fragment at m/z 91 (open circle) versus pressures, obtained by atmospheric pressure and super-atmospheric pressure chemical ionization under different gas pressures. The ionization method was similar to APCI where corona discharge was used as the plasma source to generate reactant ions, such as protonated water clusters. Higher needle potential was necessary under higher gas pressure to initiate the corona discharge as compared to APCI, but the discharge current and the measured ion signal were stable for all tested pressures. The ion intensities for protonated methamphetamine and its fragment first increased steadily with the pressure until an optimum value at 4 atm, and after that, it dropped with the further increase of pressure. Here, increasing the ion source did not particularly reduce the fragmentation of methamphetamine, but overall, the enhancement of analyte originated ion signal was more than five times when the ion source was optimized at 4 atm. Figure 6 shows a similar trend like that of ESI in Fig. 5. This trend is conceivable because as the gas throughput was excessively increased by the raise of pressure, the gas flow would turned from laminar to turbulent flow, and that would definitely contribute to the loss of ion current.

CONCLUSION

Gas pressure is an interesting parameter to play with, either to boost the performance of the ion source, or to study the ionization mechanism. But the acceptance of this approach depends on the availability of affordable ion transport technology that can efficiently transport the ions generated in the high pressure environment, to a high vacuum that houses the mass analyzer. Further development and innovation on this aspect is still in need. Besides the coupling techniques discussed in Fig. 1, another alternative is the discontinuous sample introduction such as that used in the pulsed molecular beam and miniature mass spectrometer. The repetition rate and the opening time of the sample inlet valve can, in principle, be adjusted to maintain a good vacuum for the mass spectrometer while keeping the ion source under high pressure. Such technique will be explored in the future.

Acknowledgements

This work was supported by the Grants-in-Aid for Scientific Research (Kakenhi, Grant No. 12015369) from JSPS, and the Program to Disseminate Tenure Tracking System from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES


