**INTEGRATED PAPER**

**Development of New Ionization Methods for GC/MS and LC/MS Interfaces**

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New ionization methods for GC/MS and LC/MS interface which have been developed in our laboratory will be described. The atmospheric pressure Penning ionization was developed for GC/MS interface. Its sensitivity was found to be as good as that of the electron impact ionization method. The laser spray developed for LC/MS interface, which can be regarded as the electric-field assisted MALDI, gave about one order of magnitude higher ion detection sensitivity than the conventional electrospray. In addition, laser spray can detect ions with different surface activities more non-selectively than electrospray.

1. Introduction

For GC/MS interface, the electron ionization (EI) has been used as the most sensitive and standard ionization method. For EI, the analyte gas effusing out of the gas chromatograph (GC) is introduced into the vacuum chamber and ionized by an electron beam at the reduced pressure. In the process of the gas expansion from one atmospheric pressure (about 760 Torr) to about $10^{-4}$-10$^{-5}$ Torr, the number density of analyte gas molecules reduces by a factor of about $10^{-7}$-10$^{-8}$. This leads to the inherent decrease of the ionization efficiency of the analyte gases because the yield of ions is roughly proportional to the number density of the gas molecules. Despite of this disadvantage, the EI method is capable of detecting the analyte molecules with a high sensitivity. This is mainly due to the fact that the ions formed in the ion source can be transported to the ion detection system efficiently by the appropriate electric field. If the analyte gas samples effusing out of the GC capillary could be ionized at an atmospheric pressure, no reduction of the number density of the sample molecules would take place. This means that the disadvantage inherent to the EI method can be totally avoided and much higher ionization efficiency could be realized if the ions formed in the atmospheric pressure ion source can be transported to the analyzer system with high efficiency. In this respect, atmospheric pressure Penning ionization (APPeI) has been developed in our laboratory. In this method, the analyte gas molecules effusing out of the capillary outlet of GC are ionized by metastable rare gas atoms. The ions formed are sampled through the orifice into the vacuum chamber and analyzed by an orthogonal time-of-flight mass spectrometer (TOF-MS). Recently, it has been confirmed that ion detection sensitivity obtained by APPeI is of the same order with that by the EI method. The details for the APPeI will be described in this paper.

About 15 years has passed since the original works for electrospray (ES) and matrix-assisted laser desorption ionization (MALDI) had appeared. Since then, a lot of research has been performed to improve these techniques. In the study on the electrospray, nanoES has been developed which makes it possible to treat much less quantity of liquid samples leading to the higher throughput of the sample. In general, electrospray is rather inferior to the aqueous solution because of the high surface tension of water that makes the aqueous solution more difficult to electrospray than the mixed solution of water with some other organic solvents such as methanol. Recently, we have developed a new interface, laser spray. In this technique, the infrared laser with the wavelength of 10.6 $\mu$m was used. Because the liquid water has a rather high absorption coefficient for the 10.6 $\mu$m infrared light, this method is found to be most suitable for aqueous solution. Because water is ubiquitous in the universe and is a natural solvent for biological systems, the laser spray would become one of the most suitable methods for biological samples which easily suffer from the denaturation with the presence of other solvents such as alcohols other than water. In this article, the principle and application of laser spray will be described in detail.

2. Atmospheric Pressure Penning Ionization for GC/MS Interface

Basic research on the Penning ionization reaction (1) of long-lived metastable rare gas atoms (Rg*) and the following fragmentation of M+ has been studied over the two decades.\(^1\)\(^-\)\(^4\)

$$\text{Rg}^* + \text{M} \rightarrow \text{Rg} + \text{M}^+ + e$$  \hspace{1cm} (1)

Reaction (1) takes place only when the ionization energy (IE) of M is lower than the internal energy of Rg*. Since the work of Jones and Harrison in 1970,\(^1\) not much mass spectrometric work has been made to gain further information on Penning ionization reactions (1).\(^2\)

Ionization methods that operate under atmospheric pressure such as electrospray and APCI (atmospheric-pressure chemical ionization) are now indispensable for measuring nonvolatile organic compounds, and they provide useful interfaces for a liquid chromatograph/...
mass spectrometer (LC/MS). These methods, however, have several disadvantages; e.g., less sensitivity for less polar compounds (e.g., hydrocarbons and organometallic compounds) and a limitation in the use of solvent. In 1984, Tsuchiya and coworkers first applied the atmospheric pressure Penning ionization for the study of liquid ionization mass spectrometry.\(^5\), \(^6\) This method utilizes Ar\(^+\) to ionize liquid films on the tip of a metal needle at atmospheric pressure. It provides information about the hydrogen-bonded clusters that are present in the gas phase and at the liquid surface, and also about solvent effect. It is also applicable to the analysis of a mixture that contains nonvolatile, either polar or nonpolar, organic compounds.

In 1993, Bertrand \textit{et al.} applied the Penning ionization at reduced pressure for the use of an energy-tunable rare-gas metastable beam source generated by a corona discharge as an ionization source for mass spectrometry.\(^2\) The Rg\(^+\) beam was produced externally to the ion source by the dc discharge of tens Torr rare gas and its excitation energy is controlled by the respective internal energy of Rg\(^+\). This beam source was found to provide sensitivities of the same order as those obtained by electron impact.\(^2\) They further studied the mass isotopomer abundance mass spectrometry of methyl palmitate.\(^7\) While the ionization using the \(^3\)P\(_2\) and \(^3\)P\(_0\) states of Kr\(^+\) (9.92 and 10.56 eV, respectively) gave isotopomer abundances essentially independent of sample concentration, that of Ar\(^+\) (11.55 and 11.72 eV, respectively) showed molecular ion fragmentation intermediate between that of electron impact and the metastable atom bombardment (Kr\(^+\)) and showed some isotopomer concentration dependence.

Another unique application of the Penning ionization is the Penning ionization electron spectroscopy in glow discharge for gas chromatography detectors.\(^8\) This method is based on the Penning ionization reaction (1) for He\(^+\). The electron produced by reaction (1) has a kinetic energy \(E\) determined by

\[
E = E_m - IE
\]

Here, \(E_m\) is the energy of He\(^+\) (19.8 eV for the 2\(^2\)S\(_1\)) and \(IE\) is the ionization energy for the species. Both \(E_m\) and \(IE\) are atomic or molecular constants, and therefore, the Penning ionization electron energy, \(E\), is specific for each species and does not depend on the conditions of the experiment or on the presence of other species. The electron energy distribution in the helium discharge afterglow was measured using a Langmuir probe. The measured electron energy spectra displayed distinct characteristic peaks (fingerprints) for nitrogen and carbon monoxide gases. This method can be a good candidate for the analytical application, particularly, in GC instrumentation.

In the following section, the application of the atmospheric pressure Penning ionization to the high-sensitive detection of polar as well as nonpolar gaseous molecules will be described.

2.1 Experimental

Figure 1 displays the conceptual idea of the Penning ionization source coupled with an orthogonal time-of-flight mass spectrometer (Accu-TOF, JEOL, Musashino, Tokyo). The position of the stainless steel capillary (0.1 mm inner diameter and 0.3 mm outer diameter) was adjusted using 6 bolts in the acrylic resin needle holder. The strongest ion signals were obtained with the distance of about 2 mm between the tip of the stainless steel capillary and the ion sampling orifice. The Ar gas (reagent grade, Iwatani, Tokyo, 99.99\%\(^6\)) was used without further purification. The Ar gas introduced into the Penning ionization source was activated by the negative-mode corona discharge using 10 sharp tungsten needle electrodes which were spot-welded onto the stainless steel ring wire with 30 mm in diameter (see the inset in Fig. 1). The produced Ar\(^+\) atoms were entrained in the reagent Ar gas toward the tip of the

![Fig. 1. Conceptual idea of the Penning ionization source which is coupled with the orthogonal time-of-flight mass spectrometer (Accu-TOF, JEOL, Musashino, Tokyo). RP: rotary pump, TMP: turbo-molecular pump.](image-url)
stainless steel capillary and flowed out of needle holder. The sample gas was introduced into the ion source through a stainless steel capillary with a flow rate of about 0.1 cm$^3$ min$^{-1}$ ($\sim 10^{-7}$ mol min$^{-1}$ or $1 \times 10^{-7}$ mol s$^{-1}$) using Ar as a carrier gas. The produced ions were sampled through the ion sampling orifice (0.4 mm in diameter), passed through the ion guide and were mass-analyzed. The flow rate of the reagent Ar gas was 4 L min$^{-1}$. The temperature of the ion source was about 40°C. The voltages applied to the orifice 1, the ring lens, and the orifice 2 in Fig. 1 were 30, 40, and 2 V, respectively.

2.2 Results and Discussion

2.2.1 Principles of the method

In the present experiment, ten sharp tungsten needles were used for generating corona discharge in the negative-mode of operation. The electric current of corona discharge between the corona discharge electrode (applied voltage of $-1 \sim -2$ kV) and the sampling orifice (ground potential) was measured to be in the range of $10^{-6} \sim 10^{-5}$ A. Figure 2 shows the dependence of voltage (negative) applied to the corona discharge electrode ($V_p$) on the signal intensity of the base peak C$_4$H$_9^+$ ($m/z$ 57) for n-butane. n-Butane was introduced through the stainless steel capillary into the Penning ionization source by the infusion method using Ar as a carrier gas. The voltage applied to the stainless steel capillary ($V_s$) was set at 800 V. With this $V_s$ value, no ion signal was detected with $V_p=0$ V indicating that no corona discharge was generated on the tip of the stainless steel capillary (no APCl took place). The ion signal of C$_4$H$_9^+$ appeared at $V_p=-330$ V in Fig. 2. This is due to the ignition of corona discharge at $V_p=-330$ V which can be recognized by the naked eye, i.e., the start of the Penning ionization. When only the Ar carrier gas without containing any sample gases (e.g., n-butane) was supplied through the stainless steel capillary, no Ar$^+$ was detected with $V_s=800$ V and $-V_p \geq 330$ V. This is reasonable because Ar$^+$ is formed by the negative-mode corona discharge and thus Ar$^+$ ions generated are well trapped by the strong electric field exerted near the tip of the needle electrodes. No Ar$^+$ ion signal also indicates that the electrons generated by the negative-mode corona discharge were thermalized by the multiple collisions with the ambient atmospheric pressure Ar gas and they do not have enough energy to ionize the sample gas molecules effusing out of the capillary. In Fig. 2, the ion current of C$_4$H$_9^+$ increases with decrease of $V_p$ and reaches the plateau above $-1$ kV. In our previous work,$^9$ we found that the atmospheric corona discharge current continued to increase up to about a few kV. The number density of Ar$^+$ produced by the corona discharge would increase with increase of the discharge current. The saturation of the ion signal for C$_4$H$_9^+$ in Fig. 2 suggests that the profile in Fig. 2 may reflect the relative concentration of Ar$^+$. Although the radiative lifetimes of the metastable $^1$P$_2$ and $^3$P$_0$ states are reported to be $\sim 60$ s and $\sim 50$ s, respectively,$^{10,11}$ the reaction lifetimes of Ar$^+$ are affected by several factors such as the occurrence of chemi-ionization reactions (3) and (4), and superelastic collision (5), the presence of impurities, etc.

$$\text{Ar}^+ + \text{Ar}^+ \rightarrow \text{Ar}^+ + \text{Ar}^+ + e^-$$

$$\text{Ar}^+ + \text{Ar}^+ \rightarrow \text{Ar}_2^+ + e^-$$

$$\text{Ar}^+ \rightarrow \text{e}^\text{(thermal)} \rightarrow \text{Ar}^+ + \text{e}^\text{(hot)}$$

With increase of the number density of Ar$^+$, the rate of annihilation of Ar$^+$ by chemi-ionization reactions (3) and (4) and superelastic collision (5) will concomitantly increase. Measurements of the rate constants for chemi-ionization reactions are rather scarce. Shevchenko et al.$^{12}$ measured the quenching rate constant $k_{q}$ for reaction $[\text{Ne}^+ + \text{Ar}^+ \rightarrow \text{products}]$ to be $(3.2 \pm 0.4) \times 10^{-12}$ cm$^3$ s$^{-1}$. The half-life $\tau$ of Ne$^+$ for reaction $[\text{Ne}^+ + \text{Ar}^+ \rightarrow \text{products}]$ is expressed as $1/2k_{q}[\text{Ne}^+]$, where $[\text{Ne}^+]$ is the number density of Ne$.^+$. In the case of $[\text{Ne}^+] = 10^{10}$ cm$^{-3}$, for example, $\tau$ is calculated to be only about 1 ms. Thus the number density of Rg$^+$ in the ion source may be self-determined by $[\text{Rg}^+]$ and the flow rate of reagent Rg gas.

In addition to reactions (3)–(5), the annihilation of Rg$^+$ due to the presence of impurities may also play a role. For example, the rate constant for reaction (6) was measured to be $8.1 \times 10^{-10}$ cm$^3$ s$^{-1}$ and only a 40 ppb of H$_2$O impurity in the atmospheric pressure Ar reagent gas leads to the decay lifetime of Ar$^+$ ($\tau = \ln 2/\kappa [\text{H}_2\text{O}])$ to be 1 ms.

$$\text{Ar}^+ ([\text{P}^2\text{H}] + \text{H}_2\text{O}) \rightarrow \text{OH} \text{(electronically excited A state)} + \text{H} + \text{Ar}$$

Thus it is highly desirable to use high purity reagent rare gas. Although the ionization energy of H$_2$O (12.6 eV) is higher than the internal energies of Ar$^+$ (11.55 eV for $^3$P$_2$ and 11.72 eV for $^3$P$_0$) and thus Ar$^+$ does not ionize the H$_2$O molecule, H$_2$O behaves as a very efficient quencher for Ar$^+$ by reaction (6). In the current experiment, it was found that the strong ion signal was obtained only when Ar flow rate was increased to 4 L min$^{-1}$. The Ar$^+$ residence time from the plasma source to the tip of the stainless steel capillary is about 0.1 s under the present experimental conditions. The high flow rate of the Ar reagent gas necessary for higher signal intensities may be due to the rapid annihilation of Ar$^+$ due to reactions (3)–(5) and also to the reaction of Ar$^+$ with impurity molecules such as H$_2$O (e.g., reac-
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Figure 3 shows the dependence of the voltage applied to the stainless steel capillary (\(V_s\)) on the signal intensity of C\(\text{H}_9\text{O}^+\) at \(m/z\) 57 for n-butane. The voltage applied to the corona discharge electrode (\(V_p\)) is \(-500\) V.

Figure 3. Dependence of the voltage applied to the stainless steel capillary for sample introduction (\(V_s\)) on the ion signal of C\(\text{H}_9\text{O}^+\) at \(m/z\) 57 for n-butane. The voltage applied to the corona discharge electrode (\(V_p\)) is \(-500\) V.

The Penning ionization leads to the formation of positive ions and also electrons. Thus, the recombination reaction between the positive ion and the electron will eventually take place sooner or later owing to the Coulomb attractive force between them. The absence of ion signals with \(V_s\leq500\) V is likely to be due to the annihilation of generated positive ions by the recombination reaction with electrons. That is, a greater part of charged particles generated by the Penning ionization may well be annihilated and only a small fraction of ions may survive. This is also the case for other atmospheric pressure ionization methods, e.g., atmospheric-pressure photoionization (APPI), inductively coupled plasma, MALDI, etc. The atmospheric pressure dc corona discharge is an exception. The dc discharge current corresponds to the net excess charges produced per unit time. This is the main reason why the APCI using a dc corona discharge can accomplish a high sensitivity. Because the Penning ionization produces the same number of positive ions and electrons, it is desirable to scavenge electrons selectively in order to suppress the recombination reaction. In the present experiment, a high positive voltage (\(V_s\)) was applied to the stainless steel capillary. Thus a strong electric field \(E\),

\[
E = 2V_s/[r \ln(4d/r)]
\]

was generated at the tip of the stainless capillary, where \(r\) is the radius of the capillary, and \(d\) is the distance between the tip of the capillary and the counter electrode. Under the present experimental conditions, \(E\) may be estimated to be \(~3\times10^4\) V cm\(^{-1}\) for \(V_s=800\) V, \(r=0.015\) cm, and \(d=0.2\) cm. Figure 3 clearly shows that this high electric field on the tip of the capillary is effective to scavenge electrons. In the present experiment, the electrons may be scavenged quite efficiently because Penning ionization of sample gas takes place only near the tip of the capillary where a strong electric field is generated. This high electric field will also make positive ions drift toward the ion sampling orifice. In Fig. 3, the ion signal appears at \(V_s=600\) V, increases steeply with increase of \(V_s\), reaches a maximum at about 900 V, decreases above 900 V and becomes voltage-independent above 1000 V. The sharp decrease in the ion current above 900 V is due to the start of corona discharge on the tip of the capillary. The ion signal with \(V_s\geq1000\) V is that for the APCI. Actually the signal intensities with \(V_s\geq1000\) V did not change by decreasing \(V_s\) to zero (i.e., no Ar\(^+\) present). Figure 3 indicates that the Penning ionization has even better sensitivity than APCI. In summary, the application of high positive voltage on the capillary for sample introduction is indispensable for the high-sensitive detection of ions produced by the Penning ionization.

2.2.2 Atmospheric pressure Penning ionization mass spectra for organic compounds

For all the organic compounds measured in this experiment, the base peaks were found to be molecular-related ions. Figure 4 summarizes the mass spectra obtained by APPeI for n-butane, n-hexane, cyclohexane, chlorobenzene, diethyl ether, and acetic acid. The flow rate of the sample gas supplied through the stainless steel capillary was about \(10^{-1}\) mol s\(^{-1}\). Cormack and Herman measured the mass spectra for the Penning ionization of Ar\(^+\) with n-butane at \(10^{-4}\) Torr.\(^{14}\) Obtained mass spectra were C\(\text{H}_\text{p}^+\) (18.3\%), C\(\text{H}_\text{p}^+\) (69.4\%), and C\(\text{H}_\text{p}^+\) (12.3\%). They did not detect the C\(\text{H}_\text{p}^+\) ion. In Fig. 4(a) for n-butane, however, the base peak is C\(\text{H}_\text{p}^+\) and no parent ion C\(\text{H}_\text{p}^+\) is detected. It seems likely that C\(\text{H}_\text{p}^+\) originates in a hydride-ion transfer reactions between the parent molecule and a variety of closed-shell fragment ions that are abundant in the reaction mixture, i.e.,

\[
\text{C}_\text{H}_\text{p}^+ + \text{C}_\text{H}_\text{p}^+ \rightarrow \text{C}_\text{H}_\text{p}^+ + \text{C}_\text{H}_\text{p}^+ + \text{C}_\text{H}_\text{p}^+ + \text{C}_\text{H}_\text{p}^+.
\]

(8)

If C\(\text{H}_\text{p}^+\) was formed, it should be detected in our experiment because reaction \([\text{C}_\text{H}_\text{p}^+ + \text{C}_\text{H}_\text{p}^+ \rightarrow \text{C}_\text{H}_\text{p}^+ + \text{C}_\text{H}_\text{p}^+ + \text{H}^+\text{e}^-]\) is endothermic by 17.2 kcal mol\(^{-1}\). At present we could not understand the absence of C\(\text{H}_\text{p}^+\).

Figure 4(b) shows the mass spectrum for cyclohexane. The base peak is found to be C\(\text{H}_\text{p}^+\) but with several weaker fragment ions. The ion at \(m/z\) 99 may be C\(\text{H}_\text{p}^+\) originating from impurities (probably heptanes). The APCI mass spectra for cyclohexane were also measured with \(V_s=1\) kV and \(V_p=0\) V. In the APCI mode, fragment ions of C\(\text{H}_\text{p}^+\) grew with the intensity of about 60% of the base peak C\(\text{H}_\text{p}^+\) and also other fragment ions such as C\(\text{H}_\text{p}^+\), C\(\text{H}_\text{p}^+\), C\(\text{H}_\text{p}^+\), and C\(\text{H}_\text{p}^+\) grew stronger. The intensity of the base peak C\(\text{H}_\text{p}^+\) by APCI decreased to about 75\% of that by APPeI. This indicates that APPeI is softer than APCI. For n-hexane in Fig. 4(c), the base peak is also the molecular-related ion C\(\text{H}_\text{p}^+\). The enthropy changes for reactions, Ar\(^+\) + M \(\rightarrow\) [M – H] + H\(^+\)e, for M = n-butane, cyclohexane, and n-hexane, are \(-1.0, -9.7\) and \(-6.3\) kcal mol\(^{-1}\), respectively. Thus the di-
rect formation of $[M-H]^+$ by the APPeI is energetically possible. However, the contribution from the hydride-ion transfer reactions for cyclohexane and $n$-hexane may also drive the formation of $[M-H]^+$ as in the case of $n$-butane. The experimental results obtained for aliphatic compounds clearly indicate that a variety of successive ion-molecule reactions take place after the primary ions are formed by APPeI. In another words, the primary ionic products formed by APPeI may well be modified by the following atmospheric-pressure chemical ionization reactions depending on the experimental conditions, e.g., the sample pressure, carrier gas flow rate, the presence of impurities, etc.

In contrast to aliphatic hydrocarbons, aromatic compounds such as benzene, toluene, xylene, and chlorobenzene (Fig. 4(d)) were found to give only the molecular ions $M^+$ as major ions and little or no fragment ions were formed. Figures 4(e) and (f) show the APPeI mass spectra for diethyl ether and acetic acid. For both cases, the protonated molecules were found to be the base peaks. Since oxygenated compounds (M gen-

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Fig. 4. Atmospheric-pressure Penning ionization (APPeI) mass spectra for $n$-butane, $n$-hexane, cyclohexane, chlorobenzene, diethyl ether, and acetic acid. The flow rate of sample gas introduced through the stainless steel capillary is $\sim 1 \times 10^{-7}$ mol s$^{-1}$. $V_p = -1.4$ kV, $V_s = 800$ V.
eraly have high proton affinities, the ion/molecule reactions take place to form the protonated molecules $H^+ M$.

$$M^+ + M \rightarrow H^+ M + \cdot (M - H) \quad (9)$$

The appearance of proton-bound dimer cations $H^+ (M)_2$ in Figs. 4(e) and (f) indicates that the concentrations of these samples in the ion source are high enough for the occurrence of the ion-molecule clustering reactions. Actually, the intensities of cluster ions decreased with decrease of sample concentration in the ion source (not shown).

Here it should be noted that no ions could be detected for chlorinated methanes (ionization energy in eV), $CH_3Cl (11.22 \text{ eV}), CH_2Cl_2 (11.32 \text{ eV}), CHCl_3 (11.37 \text{ eV})$, and CCl$_4$ (11.47 eV) under the experimental conditions. This is due to the fact that the positive ions and electrons recombine with each other efficiently in the atmospheric pressure Ar gas. When a high voltage was applied to the stainless steel capillary, strong positive ion signals appeared. This is due to the fact that electrons are scavenged by the stainless steel capillary resulting in the suppression of recombination reactions of positive ions and electrons.

The molecular-related ions are formed as major ions for all the gas samples measured, i.e., APPI is a soft ionization method. The observed major ions are classified into three groups, i.e., the radical cation $M^+$ (aromatic compounds such as benzene, toluene, xylene, chlorobenzene, etc.), the $[M - H]^+$ ion (aliphatic compounds such as n-butane, n-hexane, cyclohexane, etc.), and the protonated molecule $[M^+ H]^+$ (oxygenated compounds such as methanol, ethanol, acetone, etc.).

The methylated molecular ions such as $CH_3^+ (CH_3)_2CO$ for acetone are formed when the protonated methanol $H^+ CH_3OH$ coexists in the ion source as a reagent ion. APPI is a selective ionization method because only the molecules with ionization energies lower than the internal energy of Ar* (11.55 or 11.72 eV) can be ionized and thus it has inherently a high sensitivity. The sensitivity for APPI is found to be nearly equal to or even better than APCI. Although the radiative lifetimes of Ar* ($^3P_0$) and Ar* ($^3P_2$) are reported to be $\sim 50 \text{ s}$ and $\sim 60 \text{ s}$, respectively, the reagent Ar* is easily annihilated by chemi-ionization reactions and also by quenching reactions with impurities in the ion source and thus usage of high-purity reagent gas is recommended. The detection limit of the APPI under the present experimental conditions can be roughly estimated from the amounts of samples introduced and the noise level, i.e., $\sim 10^{-14} \text{ mol s}^{-1}$ which is comparable with the EI method. We believe that the sensitivity will be much improved by further optimization of the experimental parameters. For the quantitative analysis using APPI, the cross sections for the Penning ionization should be determined in advance. Such study is in progress in our laboratory. The APPI would be useful for the interface of GC/MS, especially for the analysis of non-polar compounds because of the soft ionization, selectivity and high sensitivity.

### 3. Development of the New Ionization Methods for LC/MS Interface

#### 3.1 Parallel electrospray interface for LC/MS

In recent years, electrospray has shown explosive development as a new means of handling intractable, higher molecular weight, polar compounds. In the early 1990’s, the capillary of the electrospray had been directed perpendicular to the interface plate for the ion sampling orifice. Under these experimental conditions, the electrospray system gave rather poor and unstable ion signals. This is due to the large liquid droplets formed by the electrospray and also due to the rapid contamination of the ion sampling orifice by the deposition of nonvolatile samples on its surface. In order to...
avoid the fouling of the orifice, we tried spraying the liquid with the capillary set parallel to the interface plate as shown in Fig. 5.15. To our surprise, it was found that ion intensities increased more than one order of magnitude and the contamination of the ion sampling orifice became practically negligible even after long-term use of the experimental system. In order to find the optimum position (direction) of the capillary, we changed the angle \( \theta \) of the capillary to the interface plate from 0° to 90°. It was found that the maximum ion intensity was always obtained with \( \theta = 0° \) and this was reproduced under almost all experimental conditions. Besides, the contamination of the ion sampling orifice was largely eliminated and the cleaning of the ion sampling orifice became unnecessary for long-term experiments with flow rates up to 2 mL min\(^{-1}\). This is a great advantage over the perpendicular ion spray method because the ion sampling efficiency became seriously deteriorated by the contamination of the ion sampling orifice.

Figure 6 represents the interdependence of the capillary position (i.e., values of \( r \) and \( d \)) and the intensity of \([M-2Cl]^2+\) ions for 10\(^{-3}\) M tubocurarine chloride (M) solution in isopropyl alcohol–water (4:1, v/v). With constant \( d \), the ion signal increases with increase of \( r \) between 7 and 30 mm, the maximum ion intensity being obtained with \( r = 30 \) mm. Because the N\(_2\) gas jet carries the charged droplets along the axis of the capillary, the charged droplets drift radially toward the interface plate, across the aerodynamic flow under the influence of the electric field.

In Fig. 6, the smaller the value of \( d \), the stronger the signal due to \([M-2Cl]^2+\) ions. This may be due to the increased value of the electric field between the capillary and the interface plate with smaller \( d \), leading to more efficient positive- and negative-ion separation on the tip of the capillary. In Fig. 6, the ion intensity is found to be rather insensitive to the change of \( r \), at low values of \( d \), with \( r \geq 10 \) mm. This indicates that the adjustment of the capillary positions is not very critical in the case of the parallel electrospray system.

In the separate experiment, the direct ion currents were measured as functions of \( r \) and \( d \) using an electrometer. The results obtained are shown in Fig. 7. It can be seen that the radial distribution of ions has two components, viz., a sharp peak in the center of the electrospray and a concentric broader peak surrounding the central one. It was found that only the central part of the spray made the ion collector wet and the peripheral region was kept dry. This result suggests that the large liquid droplets are distributed mainly in the center of the electrospray and the finer charged liquid droplets are dispersed away from the center of the spray owing to the strong electric field around the capillary tip. In the present electrospray, the liquid sample was nebulized by the N\(_2\) gas jet under the influence of the electric field on the tip of the elec-
trospray capillary. The success of the present parallel electrospray may be due to the fact that finest charged droplets were selectively sampled and larger undried liquid droplets in the center of the spray cone were carried away from the ion sampling system in the parallel electrospray.

About one year after the appearance of this paper, the merit of the parallel electrospray was introduced in the journal of Analytical Chemistry ("News & Features"). After 1997, many mass spectrometer manufacturers recognized the advantage of the parallel electrospray and they started to install the parallel electrospray system. The parallel electrospray is generally known as "orthogonal" electrospray named by Hewlett Packard. The "orthogonal" electrospray means that the ions sprayed are sampled through the ion sampling orifice orthogonally to the axis of the electrospray cone. The history of the development of the LC/MS about parallel electrospray etc. has been nicely documented by Niessen.

3.2 Laser spray: electric-field assisted MALDI

The ultimate goal for the analysis of ions in liquid or solid samples is to achieve complete evaporation of the liquid samples or solid matrices, to isolate positive and negative ions generated in the gas phase and to transport all of them to the mass spectrometer. In electrospray, the electrosprayed charged liquid droplets suffer from the Rayleigh fission accompanied by the evaporation of the solvent resulting in the charge enrichment in the off-spring droplets. Some part of the excess charges will ultimately become gaseous ions by the ion evaporation and/or charged residue mechanisms.

It is known that the efficiency of the positive and negative ion separation in the Taylor cone becomes less than 1 for the ion concentrations of \( >10^{-5} \) M. In order to increase the sensitivity for electrospray, nanoES was developed. In fact, finer charged liquid droplets were formed by nanoES. Because the size of the electrosprayed charged droplets is controlled by the balance between the surface tension and the Coulomb repulsion, it would be difficult to scale down the size of the charged droplets to the atomic level by the spontaneous charged liquid generation which takes place on the tip of the sharp capillary needle, i.e., the size formed by the nanoES may be closer to the minimum attainable by the electrostatic liquid atomization method. In my opinion, the electrospray (or nanoES) has already reached the ultimate goal for this kind of technique and there may be not much more room to improve it further. For example, the aqueous electrolyte solution (water has a high surface tension) with a concentration of \( >10^{-5} \) M is intrinsically difficult to handle. It would be necessary to hybridize another techniques such as nebulizer, laser irradiation, etc. in order to improve the sensitivity of the electrospray.

MALDI is a possible alternative for the biological mass spectrometry. It relies on the ultraviolet or infrared laser irradiation on the liquid (e.g., glycerol) or solid matrices and there is a time delay of tens to hundreds of nanoseconds for the start of the ion desorption after the laser irradiation. In the process of ablation (or spallation) of the condensed matrix sample, various phenomena take place in the plume such as electronic excitation of matrix molecules followed by the exciton formation, collision of excitons leading to the formation of electrons and ions, secondary electron emission from the substrate, ion-molecule reactions (proton transfer reactions, ion-molecule clustering reactions, charge exchange reactions, etc.), recombination reactions of positive and negative ions, unimolecular dissociations of the sample and matrix molecules, etc. Therefore, there is a possibility for further modifications of the intact samples. In addition, enormous ejections of tiny droplets occur owing to the phase explosion followed by recoil-induced expulsion of a large mass of matrix. This leads to a serious loss of the sample prepared, i.e., the sacrifice of the sensitivity for the sample detection. If suppression of the liquid droplet formation and complete vaporization of the matrix were realized, tremendous improvement of the detection sensitivity could be achieved. Recently, Siuzdak and coworkers have developed a new type of MALDI, called DIOS (desorption ionization on porous silicon). DIOS doesn't require the matrix because adsorbed molecules in porous silicon, such as solvents used for sample preparation, moisture, N\(_2\) and O\(_2\), etc. tend to act as matrices desorbing suddenly when the 337 nm nitrogen laser was irradiated. Silicon is opaque at the 337 nm region and the surface temperature of the porous silicon will increase suddenly by the irradiation of 337 nm UV laser which results in the desorption of the adsorbed molecules. This method does not create droplets from the matrix and thus in principle it has a high sensitivity. However, DIOS is applicable for the molecules with molecular weights less than a few thousands because the larger molecules are not desorbed efficiently enough owing to the lack of cooperative movement of the matrix molecules to carry on the large biomolecules into the vacuum.

The pioneering work on the vaporization of liquid using a 10.6 mm infrared laser was done by Vestal et al. This new approach for LC/MS interface used laser vaporization of the LC eluent at atmospheric pressure. The mist and vapor formed were sampled into the vacuum as a molecular beam and were ionized by chemical ionization (CI) or EI. The experiments succeeded in measuring EI mass spectra of a number of compounds injected in liquid solution at flow-rates up to 1 mL min\(^{-1}\) for a number of common solvents such as methanol, acetonitrile, hexane, and chloroform. However, the maximum flow-rate of aqueous solution that was able to vaporize in a stable manner was about 0.5 mL min\(^{-1}\). They encountered problems with mist formation due to incomplete vaporization.

Li et al. reported results on continuous-flow MALDI. They succeeded in demonstrating that a solution of peptides and proteins can be continuously introduced into a TOF-MS via the flow probe and ionized by MALDI. By examining the results from flow injection analysis with continuous-flow MALDI, they showed that the flow probe interface does not introduce significant peak tailing and memory effect.

Murray and Russell developed a new method of liquid sample introduction for a TOF-MS by applying the method of MALDI to aerosols. Analyte bio-
molecules were dissolved in a methanol solvent along
with a UV-absorbing matrix and formed into an aero-
sol with a pneumatic nebulizer. The aerosol particles
are dried in a heated skimmer tube before ionization by
pulsed 355-nm UV laser radiation. Results for the
ionization of bovine insulin (5733.5 $\text{Mr}$) were re-
ported.

Brutschy et al. studied the laser induced liquid beam
ionization/desorption mass spectrometry.\textsuperscript{26} This
method allows ions to be desorbed directly from the
liquid beam injected into the vacuum chamber through
a 10 $\mu$m nozzle and can be analyzed using a TOF-MS.
They detected the intact hemoglobin using this
method for the first time. In this method, a greater part
of liquid sample was carried away to the cold trap at
liquid nitrogen temperature.

Recently, Capiello et al. applied the EI method for
LC/MS.\textsuperscript{27} A stream at a nanoscale flow rate is nebul-
ized inside the ion source and proceeds toward a vapor-
ization surface. During a very short journey, the dro-
plets are converted into solute particles that are vapor-
ized against the hot surface. Solvent vapors are re-
moved from the source by an additional opening, thus
reducing the risk of CI processes. The originality of
their approach is to squeeze all the interfacing process
into the small volume of the EI ion source of the mass
spectrometer.

In this section, the principles of the laser spray will
be described. As will be mentioned later, this method is
the hybrid of three basic techniques for the formation of
gaseous ions from the condensed phase, \textit{i.e.}, energy
sudden, nebulizer, and electric field.\textsuperscript{28} Owing to
the cooperative effect of these techniques, laser spray has
a better sensitivity than electrospray. Since the solvent
in the LC effluent acts as a matrix, the laser spray may
be regarded as the electric-field assisted MALDI.

\subsection*{3.2.1 Experimental}
A schematic diagram of the apparatus is shown in Fig. 8. A stainless steel capillary
(inner diameter: 0.13 mm, outer diameter: 0.2 mm) par-
allel to the interface plate is supplied with a sample
solution \textit{via} a computer regulated pump (Tosoh CDPD,
Akasaka, Tokyo, Japan) or \textit{via} a syringe pump (Har-
vard Apparatus, type 11 plus). Nebulizer gas, $N_2$, effus-
ing from the concentric stainless steel tube (inner diam-
eter: 0.7 mm) reduces the angular divergence of the
plume and entrains the mist and gas in a confined gas
stream going towards the sampling orifice. Distances
of 3–7 mm between the capillary and the interface
plate and 10–15 mm between the capillary tip and the
ion sampling orifice give the highest ion signals.

A SYNRAD (Bothel, WA, USA), model FSV20SFB, max.
20 W infrared laser (10.6 $\mu$m) is used. The tip of
the stainless steel capillary is irradiated axially from
the opposite side of the capillary by a laser beam
focused to $\approx 0.2$ mm. Although it is not very critical,
the finest liquid droplets are generally formed when
the outer diameter of the stainless steel capillary is
about the same as the diameter of the laser spot ($\approx 0.2$
m). When the diameter of the stainless steel capillary
becomes larger than the laser spot ($\approx 0.2$ mm), the size
of the liquid droplets becomes larger. When it is
smaller, the tip of the capillary melts easily owing to
overheating.

The ions formed at atmospheric pressure are sam-
ples into the vacuum system through an orifice and
mass analyzed by a quadrupole mass spectrometer,
model MSQ-400 (ULVAC, Chigasaki, Japan) over the
range of $m/z$ 1–1000 or an orthogonal TOF-MS (Accu-
TOF, JEOL, Musashino, Japan) over the range of $m/z$
100–10,000. The flow rate of the sample solution is 5–
100 $\mu$L min$^{-1}$

\subsection*{3.2.2 Results and Discussion}

\textit{Ion desorption by laser spray}

Without applying high voltage to the metal capil-
ary, a rather directional plume was generated when
the nebulizer gas was not used. However, with the
application of high voltage to the capillary, the plume
enlarged resembling a trumpet shape (inset of Fig. 8)
and at the same time the size of the liquid droplets
became apparently finer than that without applying
the high voltage. The angle of the spray cone was
found to be much wider than that of the electrospray.
This clearly indicates that the separation of positive
and negative ions in laser spray is more ef}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig8.png}
\caption{Laser spray interface, drawing not to scale. The inset represents laser spray with and without application of a high voltage on the stainless steel capillary. With $V$ on, a spreading of the spray appears around the central high-velocity jet.}
\end{figure}
for aqueous solution is defined by the ratio of laser penetration depth and studied nanosecond and femtosecond laser photochemistry.

They found that the start of the formation of shock wave takes about 22 ns for liquid toluene after the irradiation of the femtosecond laser (Ti:sapphire, 248 nm, 300–500 fs) at a laser fluence of 90 mJ cm$^{-2}$. This value roughly corresponds to $\tau_{sc} = \sim 10^{-8}$ s for the aqueous solution. We measured the minimum time required for the explosive vaporization of the liquid water to be $\sim 0.7 \mu$s for 50 W infrared laser with a laser spot of 0.1 mm diameter (irradiance: $\sim 10^6$ W/cm$^2$). This is also compatible with the start of spallation of the liquid toluene at about 580 ns measured by Hatanaka et al.$^{20}$ They found that the liquid spallation lasts as long as a few ms or even longer after the femtosecond laser pulse. This is one of the reasons why the resolution of the TOF mass spectra for MALDI is poor when the delayed extraction of desorbed ions is not adopted. Another finding made by Hatanaka et al.$^{20}$ is that a greater part of the liquid toluene was ablated as tiny liquid droplets, i.e., the efficient vaporization of the liquid sample by the laser irradiation is inherently difficult. This is related to the fact that only a small fraction of sample molecules mixed in the matrices is detected as gaseous ions in MALDI. In this respect, Karas et al. proposed the “lucky-survivor model”$^{33}$ Overall, these results demonstrate that the desorption of sample ions mixed in matrices from the condensed phase to the gas phase and their isolation for the mass spectrometric detection are very inefficient processes.

Figure 9 shows the ion intensity of [M–2Cl]$^{2+}$ vs. voltage applied to the stainless steel capillary for the aqueous solution of 10$^{-3}$ M tubocurarine chloride with and without using nebulizer gas N$_2$. Laser power: 5 W, flow rate of the sample solution: 100 $\mu$L min$^{-1}$.

through an orifice for the mass spectrometric analysis (see Fig. 8). Interestingly, little ions could be detected without applying high voltage to the stainless steel capillary although an explosive vaporization of the liquid sample took place. This result is similar to that obtained by the atmospheric pressure MALDI, in which no ions could be detected when the water (or ice) sample on the substrate was irradiated by a UV or an IR laser.$^{29,30}$

The time constant for acoustic energy dissipation, $\tau_{ac}$, is defined by the ratio of laser penetration depth and velocity of sound in the matrix. For aqueous solution $\tau_{ac}$ may be estimated to be $\sim 10^{-8}$ s. Hatanaka et al. studied nanosecond and femtosecond laser photochemistry and ablation dynamics of neat liquid toluene.$^{20}$ They found that the start of the formation of shock wave takes about 22 ns for liquid toluene after the irradiation of the femtosecond laser (Ti:sapphire: Al$_2$O$_3$/KrF hybrid laser, 248 nm, 300–500 fs) at a laser fluence of 90 mJ cm$^{-2}$. This value roughly corresponds to $\tau_{ac} = \sim 10^{-8}$ s for the aqueous solution. We measured the minimum time required for the explosive vaporization of the liquid water to be $\sim 0.7 \mu$s for 50 W infrared laser with a laser spot of 0.1 mm diameter (irradiance: $\sim 10^6$ W/cm$^2$). This is also compatible with the start of spallation of the liquid toluene at about 580 ns measured by Hatanaka et al.$^{20}$ They found that the liquid spallation lasts as long as a few ms or even longer after the femtosecond laser pulse. This is one of the reasons why the resolution of the TOF mass spectra for MALDI is poor when the delayed extraction of desorbed ions is not adopted. Another finding made by Hatanaka et al.$^{20}$ is that a greater part of the liquid toluene was ablated as tiny liquid droplets, i.e., the efficient vaporization of the liquid sample by the laser irradiation is inherently difficult. This is related to the fact that only a small fraction of sample molecules mixed in the matrices is detected as gaseous ions in MALDI. In this respect, Karas et al. proposed the “lucky-survivor model”$^{33}$ Overall, these results demonstrate that the desorption of sample ions mixed in matrices from the condensed phase to the gas phase and their isolation for the mass spectrometric detection are very inefficient processes.

Figure 9 shows the ion intensity of [M–2Cl]$^{2+}$ vs. voltage applied to the stainless steel capillary for the aqueous solution of 10$^{-3}$ M tubocurarine chloride with and without using nebulizer gas N$_2$. The ion intensity of [M–2Cl]$^{2+}$ increased at a greater rate with voltage applied to the stainless steel capillary. By the application of voltage (V) on the metal capillary, the strong electric field ($E$) is exerted on the tip of the capillary (see Eq. (7)). The value of $E$ was about 10$^5$ V cm$^{-1}$ under the present experimental conditions. The liquid effusing out of the stainless steel capillary experiences this high electric field and the positive ions (in the positive ion mode) are likely concentrated near the meniscus of the liquid and negative ions diffuse to the opposite direction and are neutralized on the metal surface (vice versa in the negative ion mode). The positive ions enriched near the surface of the liquid meniscus will be selectively desorbed by the laser irradiation resulting in high efficiency for the ion extraction. Hale and Querry$^{35}$ measured the absorption coefficient of water for 10.6 $\mu$m infrared light to be $\alpha = 832$ cm$^{-1}$. This value leads to the absorption thickness in bulk water ($1/\alpha$) to be $\sim 12 \mu$m. That is, the penetration depth of the laser light in the aqueous solution is at most $\sim 12 \mu$m thick. The thickness of the electric double layer depends on the electric field strength and the electrolyte concentration. If the electric double layer is thicker than $\sim 12 \mu$m, an efficient desorption of enriched ions near the liquid surface would be realized. For electrospray, the Taylor cone is formed spontaneously on the tip of the metal capillary owing to the high electric field exerted on the tip of the metal capillary. Since the average size of the electrosprayed charged droplets is about a few $\mu$m, the curvature diameter of the tip of the Taylor cone may have about the same size. From Gauss’ law and the number of excess charges in the electrosprayed charged droplets,$^{18}$ the electric field on the tip of the Taylor cone can be estimated to be more than one order of magnitude higher than that generated on the tip of the metal capillary ($\sim 10^5$ V cm$^{-1}$). This high electric field on the tip of the Taylor cone (i.e., thick electric double layer) is related to the formation of highly charged droplets. For laser spray, however, the stable formation of Taylor cone may be disturbed by the rapid vaporization of the liquid by the laser irradiation. In other words, the electric field strength generated on the liquid surface for laser spray may be weaker than that on the tip of the spontaneously formed Taylor cone (i.e., the electric double layer for laser spray may be thinner than that for electrospray). The fact that the laser spray has the better sensitivity than the electrospray suggests that the laser spray can form finer liquid droplets than the electrospray, in accordance with the experimental observation.

As shown in Fig. 9, the ion intensity increased to about a factor of 2 when the nebulizer gas N$_2$ was used (flow rate: 3 L min$^{-1}$). This may be due to the confinement of the widely diverged plume (Fig. 8) to the narrower gas stream resulting in the higher space charge density in the plume.

Figure 10 shows the dependence of the laser power.
on the ion signals [M – 2Cl]^{2+} for the aqueous solution of 10^{-8} M tubocurarine chloride with and without using the nebulizer gas. The flow rate of the sample solution: 100 μL min^{-1}, voltage applied to the capillary: + 3 kV.

Figure 10. Ion intensity of [M – 2Cl]^{2+} vs. laser power for aqueous solution of 10^{-8} M tubocurarine chloride with (flow rate: 3 L/min) and without using the nebulizer gas. The flow rate of the sample solution: 100 μL min^{-1}, voltage applied to the capillary: + 3 kV.

Observation of triply charged metal ions

Multiply charged metal ions have not received much attention in electrospray experiments owing in part to the belief that charge transfer to the ligands occurs exclusively. Kebarle and co-workers found that M^{2+} (H_{2}O)_{n} were formed as major gas phase ions when suitable liquid solutions containing the M^{2+} ions (M = Mg, Ca, Sr, Ba, Mn, and Co) were electrosprayed. They extended the study of the gas phase ion chemistry to the observation of triply charged ion ligand complexes, M^{3+}(ligand)_{n}, produced in the gas phase by electrospray of a solution of the M^{3+} salts (M = Y, La, Ce, Nd, and Sm) and with the ligand DMSO. We made a comparative study of electrospray and laser spray for the formation of gaseous cluster ions of triply charged metal ions M^{3+}(ligand)_{n} with M = Sc, Y, and La with the ligand DMSO in order to obtain information on the solvation of these ions in solution and in the gas phase. Figure 11 shows the intensities for all ions observed by laser spray and electrospray for a H_{2}O–CH_{3}OH (1:1, v/v) solution of mixed samples of 5 × 10^{-8} M ScCl_{3}, YCl_{3}, LaCl_{3}, and 2 × 10^{-2} M DMSO. The intensity of ion(DMSO)_{n} corresponds to the value summed up for all n observed. The transmission efficiencies of the quadrupole mass spectrometer were taken into consideration.

Figure 11. Intensities for all ions observed by laser spray and electrospray for a H_{2}O–CH_{3}OH (1:1, v/v) solution of mixed samples of 5 × 10^{-8} M ScCl_{3}, YCl_{3}, LaCl_{3}, and 2 × 10^{-2} M DMSO. The intensity of ion(DMSO)_{n} corresponds to the value summed up for all n observed. The significant difference between electrospray and laser spray mass spectra is that the cluster ions Sc^{3+}(DMSO)_{n} could not be detected by electrospray while strong signals of these cluster ions were obtained by laser spray. In contrast, the intensities of H^{+}(DMSO)_{n} measured by electrospray are stronger than those by laser spray. Because the size of Sc^{3+} is the smallest of the three metal ions studied (Sc^{3+}, Y^{3+}, and La^{3+}), Sc^{3+} may have the least efficiency for the formation of gas phase ions from the liquid phase due to its largest solvation energy. On the other hand, the H^{+}(DMSO)_{n} must be the easiest to transfer from the liquid to the gas phase because the proton H atom is sandwiched by two hydrophobic DMSO molecules, (CH_{3})_{2}OS···H^{+}···OS(CH_{3}). Figure 11 clearly indicates that laser spray is more suitable for the detection of ions with less surface-active ions.

In our previous work, we made an effort to detect halide ions (F^{−}, Cl^{−}, Br^{−}, and I^{−}) in mixed solutions containing four halide ions by electrospray without any success. In contrast, we found it straightforward to observe all four halide ions in aqueous or water–methanol solutions by laser spray. The strong appearance of all halide ions clearly confirms the laser spray to be a technique that can form ions less selectively.

Does the thermal decomposition occur for thermally
labile compounds?

In matrix-assisted laser desorption ionization (MALDI), fragment ions of thermally labile compounds can be readily formed by increasing the laser power. This makes the interpretation of mass spectra more complex but it is quite useful for the structural elucidation of sample molecules. Because the laser spray can be regarded as a kind of MALDI using the liquid solvent as a matrix, the occurrence of sample degradation by laser irradiation is possible. A detailed study was done to determine whether thermal degradation takes place in laser spray, using ribostamycin, acetylcholine chloride, and cholesteryl sulfate sodium salt as test samples (all of them are known to be thermally labile). It was confirmed that no fragment ions were formed when the laser beam was focused at the center of the stainless steel capillary, i.e., no wall heating. When the laser beam was slightly off-center, the sample ions suffered from thermal degradation by the heated wall of the stainless steel capillary to give fragment ions, which would be useful for the structural elucidation of the sample molecules.

The non-occurrence of thermal decomposition for thermally labile compounds indicates that the temperature rise of the sample in the process of desorption is suppressed under the critical temperature for thermal decomposition. The violent explosion and mist formation are due to the absorption of 10.6 μm infrared laser by the liquid water (absorption coefficient: 832 cm⁻¹). This absorption is mainly due to intermolecular interactions and decreases with the number density of H₂O molecules. This reduction in optical absorption occurs due to the weakening of the hydrogen bonds between adjacent water molecules at higher temperature that results in a change in the length and strength of the OH bond. The continuum absorption coefficient of saturated water vapor (∼300 K) is of the order of 10⁻⁶ cm⁻¹ which is about 9 orders of magnitude smaller than that of liquid water. That is, the absorption of 10.6 μm infrared laser decreases drastically by the phase transition from liquid to vapor and it becomes negligible for water vapor. Thus, the overheating of the aqueous solution by the 10.6 μm infrared laser is self-suppressed with decrease of the number density of the sample. This may partly explain why the degradation of the thermally labile compounds did not occur. We think that laser spray will be a versatile LC/MS interface for the analysis of high-molecular weight bio-molecules because it can handle the aqueous solution without worrying much about the sample degradation. The chemical noise may also be greatly suppressed compared to the conventional MALDI because the matrix used is water and it does not give any fragments or polymerized products.

The effect of the presence of foreign salts on the formation of gaseous ions for electrospray and laser spray

In electrospray and laser spray, the effect of the electric field on the tip leads to the destabilization of the meniscus and emission of charged droplets starts. Two mechanisms have been proposed for the formation of gaseous ions from the charged liquid droplets. The first is called the charged residue mechanism. In this mechanism, the charge density eventually gets large enough to overcome the surface tension of the droplet and cause it to divide and smaller droplets are formed by the successive droplet fission. The residual ions in the ultimate tiny liquid droplets are finally detected as gaseous ions after the solvent has evaporated from the droplets. The second mechanism, proposed by Iribarne and Thomson, is called the ion evaporation mechanism. In this model, too, the charge density increases while the solvent evaporates. However, instead of forming smaller droplets, the Coulomb repulsion overcomes the charged species adhesion to the surface and some ions are expelled directly from the surface to the gas phase. It is generally accepted that both mechanisms must be invoked for the formation of gaseous ions from the charged liquid droplets formed by electrospray.

In general, laser spray gives much stronger ion signals than electrospray, for both positive- and negative-mode of operation. Especially the signal enhancement in the negative-mode of operation is prominent. This is mainly due to the tolerance of laser spray to the occurrence of breakdown (formation of corona discharge) on the tip of the capillary. This is especially beneficial for aqueous sample solution because an application of a higher negative voltage is necessary to spray aqueous solution owing to the high surface tension of water.

In order to obtain further information on the mechanism for the formation of gaseous ions by laser spray, the comparative study on the effect of the presence of foreign salts for the formation of gaseous ions by electrospray and laser spray was performed.

Tang and Kebabie used Iribarne and Thomson’s ion evaporation mechanism to propose a model wherein the ion evaporation rate is proportional to the concentration of the ion in the droplet. They proposed the following equation:

\[ I(A^+, ms) = PF \cdot \frac{k_a[A^+] + k_e[E^+]}{k_a[A^+] + k_e[E^+]} I \]  (15)

to predict the mass spectrometrically detected anolyte ion intensity, \( I(A^+, ms) \), for a solution containing a single anolyte and electrolyte where \([A^+]\) and \([E^+]\) are anolyte and electrolyte concentrations, \(k_a\) and \(k_e\) are rate constants expressing the rates of transfer of anolyte and electrolyte ions to the gas phase, \(I\) is the electrospray current, \(f\) is the fraction of ions converted into the gas phase, and \(P\) is a proportionality constant expressing the “sampling efficiency” of the system. The ions in the gas phase are due to the excess charges on the droplets. Therefore, the current \(I\) leaving the electrospray or laser spray capillary is a measure of the rate at which excess positive electrolyte ions leave the capillary. The current \(I\) is easily measured and is found to be dependent on the conductivity \(\sigma\) of the solution. There is a minimum (threshold) conductivity below which there is no electrospray. The threshold occurs \(\sigma \approx 10^{-7} \text{ W}^{-1}\text{ cm}^{-1}\) which corresponds to a concentration of \(\approx 10^{-6}\text{ M}\) of the electrolytes. \(I\) is only a very weak function of the conductivity \(\sigma\) (or electrolyte concentration). It is generally accepted that Eq. (15) explains the relationship between \(I(A^+, ms)\) and \([A^+]\). In the following section, the relation-
ship between the ion intensity $I(\text{A}^+, \text{ms})$ and the concentration of foreign salt for electrospray and laser spray will be discussed from the viewpoint of the ion formation mechanism.

Figure 12 shows the relationship between the ion signal intensities of (Glu$^-$H)$^-$ and Br$^-$ and [tetra-$\eta$-hexylammonium bromide] for $10^{-4}$ M glutamic acid H$_2$O/CH$_3$OH (1:1, v/v) solution for electrospray. The rather high concentration of $10^{-4}$ M glutamic acid solution must be used because the ion signal intensity of (Glu$^-$H)$^-$ could not be detected for lower concentrations for electrospray. The tetraalkylammonium ions N$	ext{R}_4^+$ is known to be highly surface-active because it has four hydrophobic alkyl groups (R’s) bonded to the N atom. In Fig. 12, the (Glu$^-$H)$^-$ ion shows a steady decrease with increase of [tetra-$\eta$-hexylammonium bromide]. The ratio of the signal intensities $I[(\text{Glu}^-\text{H})^-]/I(\text{Br}^-)$ = 370/9600 = 1/26 with [Glu] = [N(C$_6$H$_{13}$)$_4$Br] = 10$^{-4}$ M indicates that (Glu$^-$H)$^-$ is much less surface-active than Br$^-$. This is because the (Glu$^-$H)$^-$ ion has three ionic sites, two negative and one positive.

Figure 13 shows the relationship between the ion signal intensities for (Glu$^-$H)$^-$, (Glu$^-$H)$^-$/(Glu$^-$H) and Br$^-$ and [N(C$_6$H$_{13}$)$_4$Br] for $10^{-4}$ M glutamic acid H$_2$O/CH$_3$OH solution for laser spray. About 25 times stronger ion signal intensity for (Glu$^-$H)$^-$ is obtained by laser spray (Fig. 13) than by electrospray (Fig. 12). This is one example that laser spray is more suitable for the detection of negative ions than electrospray. The ratio of the signal intensities $I[(\text{Glu}^-\text{H})^-]/I(\text{Br}^-)$ = 2800/12,000 = 1/4 with [Glu] = [N(C$_6$H$_{13}$)$_4$Br] = 10$^{-4}$ M is much larger than that (1/26) for electrospray in Fig. 12. This is another example that laser spray is more suitable for the detection of ions rather non-selectively than electrospray.

The weak appearance of the dimer anion (Glu$^-$H)$^-$ (Glu) in Fig. 13 should be noted. As was already pointed out in our previous paper, selective vaporization of solvent takes place by the laser irradiation resulting in the increase of the sample concentration near the meniscus of the sample solution effusing out from the capillary. The increase of the sample concentration leads to the formation of cluster ions. The rather slow decrease of (Glu$^-$H)$^-$ with increase of [N(C$_6$H$_{13}$)$_4$Br] in the range of $10^{-4}$ to $10^{-5}$ M indicates that the presence of surface-active ions N(C$_6$H$_{13}$)$_4$ is minor for laser spray in this concentration range. Apparently, the presence of foreign salts for laser spray is less effective than that for electrospray. The rapid decrease of (Glu$^-$H)$^-$ and increase of Br$^-$ with [N(C$_6$H$_{13}$)$_4$Br] > $10^{-5}$ M can be qualitatively interpreted by Eq. (15).

In the previous sections, the effect of the presence of tetra-$\eta$-hexylammonium bromide in solutions of glutamic acid was investigated. For the analysis of biopolymers such as polypeptides, DNA, and their complexes, etc., ammonium acetate (CH$_3$COONH$_4$) is often added in aqueous solution as a buffer. It would be interesting to investigate the effect of the addition of CH$_3$COONH$_4$ on the signal intensities of biopolymers. Here, lysozyme was adopted as a test polypeptide. In this experiment, the mass spectra for electrospray were measured first and then the infrared laser was irradiated on the tip of the stainless steel capillary for laser spray with the sample flow rates of 5 µL min$^{-1}$. The experimental conditions are the same for electrospray and laser spray except for the laser on (laser spray) and off (electrospray).

Figure 14 displays the relationship between the signal intensity of ions (lysozyme$^+\text{H}^+$) summed up for all n and [CH$_3$COONH$_4$] for the $10^{-6}$ M aqueous solution of lysozyme chloride for electrospray and laser spray. In Fig. 14 for electrospray, no lysozyme ions are detected with [CH$_3$COONH$_4$] ≤ $10^{-4}$ M. The ions (lysozyme$^+\text{H}^+$) start to be observed with [CH$_3$COONH$_4$] = 5×$10^{-4}$ M, reach the maximum with $10^{-3}$×$10^{-2}$ M, and decrease with >$10^{-2}$ M. This trend was reproduced for repeated experiments. For laser
spray, the (lysozyme+nH)^n+ ions could be detected without the addition of CH3COONH4. In Fig. 14, the signal intensities of (lysozyme+nH)^n+ show an increase with $5 \times 10^{-3}$, reach the maximum at $10^{-3}$ M and decrease with $>10^{-3}$ M of [CH3COONH4]. The decrease of the (lysozyme+nH)^n+ ion signal intensities with increase of [CH3COONH4] with $>10^{-3}$ M for laser spray can be interpreted by Eq. (15), but the increase of ion signals with increase of [CH3COONH4] from $10^{-5}$ to $10^{-3}$ M observed for both electrospray and laser spray cannot be explicable by Eq. (15). There must be some mechanism for the enhancement of the evaporation of analyte lysozyme ions with increase of [CH3COONH4].

Enke et al. investigated the effects of salt concentration on analyte response using electrospray ionization mass spectrometry. They observed an increase in the analyte response factor with increasing electrolyte concentration for electrolyte concentrations below $10^{-3}$ M. This result argues against the prediction of Eq. (15). They proposed the electrical double layer model suggested by Enke et al. could account for the increase in the ion signals for (lysozyme+nH)^n+ for electrospray and laser spray in Fig. 14.

Figure 15 displays some examples of electrospray and laser spray mass spectra for $10^{-6}$ M lysozyme aqueous solution with several concentrations of CH3COONH4 buffer taken from the data in Fig. 14. For both electrospray and laser spray, the average charge $n$ of (lysozyme+nH)^n+ decreases as [CH3COONH4] increases. This is likely to the salt formation of (lysozyme+nH)^n+ with the negative acetate ion CH3COO− leading to the reduction of the positive charges.

\[
\text{(lysozyme+nH)^n+ + CH}_3\text{COO}^- \\
\rightarrow \text{(lysozyme+nH)^n+} \cdots \text{CH}_3\text{COO}^- \\
\rightarrow \text{(lysozyme+(n-1)H)^n-1/}+\text{CH}_3\text{COOH} \quad (16)
\]

Mirza and Chait investigated the effects of anions on the positive ion electrospray mass spectra of peptides and proteins. They found that certain anionic species in the spray solutions were observed to cause a marked decrease in the net average charge of peptide and protein ions in the mass spectra compared to the average charge measured in the absence of these anions, with the propensity for neutralization following the order: CCl3COO− > CF3COO− > CH3COO− = Cl−.

It should be noted that the peaks for (lysozyme+nH)^n+ in Fig. 15 are not contaminated by the adductions of the elements of buffer salts of CH3COONH4. It is evident that the CH3COONH4 is a versatile buffer because the cleanup of CH3COONH4 during the desolvation stage is quite efficient owing to its relatively high volatility. This is not the case for alkali halides and (NH4)2SO4.

In Fig. 15, the average charge of (lysozyme+nH)^n+ for laser spray is smaller than that for electrospray with the same CH3COONH4 concentrations. This is the...
general trend observed for the samples of oligopeptides and proteins. The formation of less highly charged ions for laser spray may be due to the fact that the process of charge enrichment by successive uneven droplet fission is less prominent by laser spray than by electrospray. That is, the average size of the liquid droplets formed by laser spray is smaller than that by electrospray and the charged residue mechanism is more important in laser spray than in electrospray.

Selective dissociation of noncovalent bonds in the biological molecules by laser spray

The advent of electrospray has enabled analyses of various biomolecules, including noncovalent complexes, such as protein–protein, protein–DNA, protein–ligands, and DNA–drug etc. It is desirable to maintain the sample in native conformation to observe such noncovalent interactions by electrospray, thus sample solution should be prepared in neutral condition without the presence of organic solvents. Ionization efficiency for aqueous sample solution containing no organic solvents for electrospray is not so good as that for electrospray with organic solvents, but it can give accurate molecular mass of the intact noncovalent complexes. From the information of molecular mass of a complex, the binding stoichiometry can easily be determined, which is a great help for better understanding of biologically significant reactions and characters of native proteins. In addition, with changing mass spectrometric parameters, dissociation of these complexes can also be achieved. In collision-induced dissociation (CID) experiments, for example, either in the 1st vacuum chamber source or in the collision cell, of multiply protonated molecules generated by electrospray, stability of the noncovalent complex can be analyzed. In some cases, however, CID of noncovalent complex causes considerable cleavages of covalent bonds rather than selective dissociation of noncovalent interactions. Specific cleavage of only the noncovalent bonds is highly preferable for the study of the stability and structure of the noncovalent complexes. Laser spray can be a promising tool for characterization of non-covalent bond complexes because the laser irradiation induces the local heating of the meniscus of the aqueous solution on the tip of the stainless steel capillary. In this section, an application of laser spray to the characterization of biomolecules, such as a protein, double-strand DNA (dsDNA), and a protein–DNA complex is described.

A protein c-Myb is a transcription factor, and its DNA binding domain (DBD), 13 kDa, specifically recognizes dsDNA with the consensus sequence of AACNG (N denotes A or T or G or C). Figure 16 shows electrospray (laser on) and laser spray mass spectra of a complex of c-Myb DBD and 22mer dsDNA, whose solution had been determined to be $2.2 \times 10^{-9} \text{ M}$. In electrospray mass spectrum the complex of c-Myb DBD and dsDNA gave three peaks, with 8, 9, and 10 positive charges, while peaks of free c-Myb DBD were observed with 7–18 positive charges. No free dsDNA ions were observed with laser off. The charge distribution of the free c-Myb DBD was wider than that of the complex in Fig. 16. This indicates that some population of c-Myb DBD should have been denatured and cannot recognize
the dsDNA, resulting in the appearance as molecular ions with wide charge distribution. Since the sensitivity of a DNA molecule was much lower than that of a protein molecule in the positive electrospray mode, no protonated dsDNA was observed although the equal amount of 22mer dsDNA and c-Myb DBD were present in the sample solution.

By 1.2 W of laser firing, intensity of c-Myb DBD peaks derived from the dissociation of the complex increased dramatically, and multiply protonated molecules of dissociated ssDNA and complexes of c-Myb DBD and ssDNA appeared in the mass spectrum. By increasing the laser power up to 1.4 W, peaks of protonated molecules of the c-Myb DBD/c8141 dsDNA complex were barely recognized while intensities of multiply protonated subunits of c-Myb DBD, ssDNA, and c-Myb DBD-ssDNA increased by a factor of about 2. Since the charge distribution of c-Myb DBD was very broad (7/c8141 to c8140 21i nl) in laser spray mass spectra (middle and upper panels in Fig. 16), the dissociated protein seems to have been denatured. No dissociated ions generated by the loss of neutral bases from the intact duplex DNA ions or fragments from ssDNA were observed in laser spray mass spectra. This indicates that laser heating cleaves hydrogen bonds only selectively, with no cleavage of covalent bonds.

In Fig. 16, not only the protonated molecules of the dissociated protein but also the ssDNA and protein–ssDNA complexes appeared in the laser spray mass spectrum (middle and upper panel). The protein–ssDNA complexes might have been generated by the reconstruction of the protonated molecules of dissociated protein and each ssDNA. However, protonated molecules of the protein–ssDNA complexes carried only eight or nine positive charges while dissociated c-Myb DBD had 7–21 positive charges in laser spray mass spectra in Fig. 16. Since wide charge distribution of the protonated protein molecules designates denaturation of the protein, it is more likely that the protein–ssDNA complex is generated by the dissociation of the original protein–dsDNA complex to a protein–ssDNA complex and a ssDNA, i.e., a protein–ssDNA complex is a transient intermediate for the complete dissociation of protein–dsDNA to three subunits.

CID experiments in the conventional ion source have been carried out to investigate the binding interactions of protein–DNA complex.61), 65), 66) An increase in cone voltage in the 1st vacuum chamber led only to partial dissociation of the complex with the appearance of ions derived from loss of neutral base for protein–dsDNA complex prepared in 20 mM CH3COONH4 buffer. In order to dissociate the protein–DNA complex selectively without any degradation of DNA, high salt
(CH₃COONH₄) concentration (~1 M) is required for sample preparation to weaken the electrostatic interaction between protein and dsDNA. Thus, many sets of samples containing different salt concentrations should be prepared for the minute investigation of the binding affinity of protein and dsDNA. Figure 16 shows that laser spray enables prompt heating of the sample resulting in the rapid dissociation of hydrogen bonds and electrostatic interactions that are thought to be strengthened in vacuo because of the paucity of solvent water molecules. Therefore, laser spray will be a new methodology for the investigation of non-covalent bond complexes such as enzyme–substrate, receptor–ligand, host–guest, protein–DNA, transporter–biopolymer, drug–protein, drug–DNA, etc.

4. Summary and Future Prospect

A new ionization method, the APPeI for GC/MS interface has been described. In this method, the sample gas is ionized by the metastable rare gas atoms just after it flows out from the capillary of GC. On the view point of sample number density of the sample molecules to be ionized, APPeI is inherently superior to EI because it can largely suppress the sample diffusion that can only be established by the atmospheric pressure ionization methods. It was found that the detection sensitivity of APPeI is as good as that of EI under the present experimental conditions. The author believes that the ultimate sensitivity of APPeI would be much more improved by the modification of the Penning ionization source, the hardware of the mass spectrometer, etc. In addition to its high sensitivity, APPeI was also found to be a much softer ionization method than EI. For example, the mass spectral pattern of EI (m/z (relative intensity)) for n-octane is 43(100), 57(30), 85(25), 14(12), 71(19), 29(17), 56(14), 42(10), 27(10), 70(10), 39(6), 84(5), 114(3). That is, the molecular ion C₈H₁₈⁺ at m/z 114 for n-octane is only 3% of the base peak of the C₈H₁₄⁺ ion at m/z 43 for EI. In contrast, the mass spectral pattern of He⁺ APPeI was measured to be 114(100), 113(85), 71(60), 85(46), 57(44), 43(9). That is, the base peak is the molecular ion (C₈H₁₈⁺) and the relative intensity of the fragment ion C₈H₁₄⁺ which is the base peak for EI is only 9% of that of the base peak, C₈H₁₈⁺, for APPeI. These results strongly suggest that APPeI could be one of the most suitable methods for the high-sensitive and soft ionization method for various gaseous molecules including non-polar hydrocarbons. The study on the ionization mechanisms for APPeI is now under progress in our laboratory.

In MALDI, it takes about sub ms or longer to initiate the sample desorption after the laser irradiation. This time scale is much longer than the half-life of the recombination reactions of positive and negative ions. For example, the half-life in the spinodal state (high-pressure dense gas) for 1 M analyte solution is about 10⁻¹² s. In order to suppress the positive and negative ion recombination reactions, there may be two solutions, first is to vaporize the liquid or solid sample within ~1 ps and separate the positive and negative ions formed in the gas phase by a strong external electric field, and second is to generate charged solution and extract excess ions by electrostatic repulsive force (electrospray or cylindrical capacitor interface) or by laser irradiation (present method).

The sample desorption/vaporization within ~1 ps is realized by FAB/SIMS and massive-cluster impact ionization (MCI). FAB/SIMS is a rather hard ionization method and not very suitable for biological samples. In contrast, MCI is a soft ionization method and is well suited for biopolymers. Recently, Aksyonov and Williams studied the impact desolvation of electrosprayed microdroplets (IDEM) for mass spectrometry of large biomolecules. Analytes are dissolved in an electrolyte solution which is then electrosprayed in vacuum, producing highly charged micron and sub-micron sized droplets. These microdroplets are accelerated through potential differences ~5–10 kV to velocities of several km s⁻¹ and allowed to impact a target surface. Energetic impacts vaporize the droplets and release desolvated gas-phase ions of analyte molecules with high efficiencies of ion extraction. The authors suggested that the rapidity of the droplet disruption does not allow efficient re-association of all the liberated ions. I think this kind of method will be a very powerful technique for the biological mass spectrometry in the near future.

Many useful LC/MS interface and soft ionization methods have been developed so far. They rely on nebulizer, electric field, and/or energy sudden techniques. The laser spray developed in our laboratory is a hybrid of all these. The high electric field exerted on the liquid effusing out of the metal capillary enriches the positive (or negative) ions on the surface of the liquid. The thin layer of liquid which is enriched by the positive (or negative) ions is selectively desorbed by the laser irradiation. The evaporated solvent acts as a nebulizer because the jet flow would lead to further atomization/vaporization of the liquid droplets entrained in the jet stream, e.g., destruction and evaporation of liquid droplets at the boundary of the Mach disc. The angle of the laser spray cone is found to be much wider than that of the electrospray, indicating higher efficiency for the positive (or negative) ion extraction from the liquid solution. Consequently, these effects result in better sensitivity than electrospray for the majority of compounds studied in this work. As described above, the laser spray may be regarded as the electric-field assisted MALDI and it is well suited for the analysis of biological samples because it can readily be applied to aqueous solutions.

Laser spray is appropriate for the observation of ions with less- or non-surface active ions. This may be mainly due to the formation of fine charged liquid droplets by laser spray. In other words, the charged residue mechanism may be more important for the formation of gaseous ions for laser spray than for electrospray. This is the reason why laser spray gives multiply charged ions with fewer charges than for electrospray.

The surface energy per unit mass (ε_surface), i.e., the energy stored in the surface of liquid per unit mass, is the specific surface area (S) multiplied by the surface tension (σ), ε_surface=σ(S/cm²)g⁻¹. When a 1 cm³ water droplet (surface area: 4.8 cm²) is atomized to droplets with a diameter of 1 mm, the surface area of

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the liquid is increased to 59,000 cm². However, this increase of surface area corresponds to an increase of surface energy of 1.7 × 10⁻⁴ × 59,000 = 0.1 cal which is only 0.02% of the heat necessary to vaporize the 1 g water droplet. Energetically liquid plume formation plays a negligible role in vaporizing the liquid. Thus, the vaporization of the liquid sample must rely solely on the successive heat supply to the liquid droplets. Unfortunately, the complete vaporization of liquid or solid by laser irradiation is inherently difficult because the formation of liquid droplets in the ablated plume prevails.\(^{20, 21}\)

The Gibbs free energy, \(\Delta G\), that describes the thermodynamics of bubble formation is given by

\[
\Delta G = \left(\frac{4}{3}\pi r^3 \mu_i - \mu_l\right) + 4\pi r^2 \sigma
\]

(18)

where \(\mu_i\) and \(\mu_l\) are chemical potentials of the vapor and liquid states, respectively, \(r\) is the size of the vapor nuclei, and \(\sigma\) is the surface tension of the surrounding liquid. Nuclei grow only if they are larger than a critical radius, \(r_{cr}\), such that the chemical potential difference exceeds the barrier posed by the surface tension. An increase in the superheat temperature increases the chemical potential difference between the superheated liquid and the vapor inside the bubble and results in a reduction in the critical embryo size that can spontaneously grow. When the temperature of the liquid reaches the spinodal temperature, the occurrence of liquid bumping can be avoided and a smooth vaporization will take place.\(^{21}\) For heating conditions that the laser power was kept low enough not to cause the liquid bumping. The study on the laser spray using a diamond tip coupled with the cylindrical capacitor interface is now in progress in our laboratory. This method will be a versatile interface for coupling of mass spectrometer and the on-line liquid separation technique.

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**References**


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