

A New Liquid Chromatography/Mass Spectrometry Interface: Laser Spray

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Explosive vaporization and mist formation occurs when an aqueous solution effusing from the tip of the stainless steel capillary (inner diameter: 0.1 mm) was irradiated from the opposite side of the capillary by a 10.6 μm infrared laser focused to ≈ 0.1 mm. Application of a high voltage (3–4 kV) to the stainless steel capillary produced strong signals of singly and multiply charged ions. The ion intensities were more than one order of magnitude stronger than those obtained by conventional electrospray or ion spray for the same aqueous samples. For some amino acid samples (e.g. leucine), cluster ions $[\text{H}^+ + (\text{molecule})_n]$, $n=1-3$, were observed by the present method, laser spray. This suggests that the sample solution was concentrated during the laser spray due to the rapid vaporization of solvent. This enrichment of the sample concentration may explain the high sensitivity of the present method. The degradation of thermally labile compounds was not observed. This method was found to be very versatile for the analysis of aqueous solutions of peptides, surface-active agents, metal ions, halide ions, etc., which are rather difficult to handle by the electrospray and ion spray techniques. © 1998 John Wiley & Sons, Ltd.

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One of the ultimate goals for the analysis of ions in solution is to achieve the complete evaporation of the solvent molecules around the solvated ions, to isolate both positive and negative ions in solution as gas-phase ions and transport them efficiently to the mass spectrometer. In order to evaporate the solvent, the rotational, vibrational and/or electronic modes of the solvent molecules may be excited. The pioneering work on the vaporization of liquid using a 10.6 μm infrared laser was performed by Vestal *et al.*¹ This new approach for a liquid chromatography/mass spectrometry (LC/MS) interface used laser vaporization of the LC effluent at atmospheric pressure. The mist and vapor formed were sampled by the vacuum system as a molecular beam and were then ionized by chemical ionization or electron ionization.

In our recent work,² a similar study was made of the explosive vaporization of a liquid beam using a 10.6 μm infrared laser. When a liquid beam with a diameter smaller than 20 μm was irradiated by a 50 W infrared laser in a direction at right angles to the liquid beam, a non-directional plume of fine liquid droplets was generated. A minimum time required for the explosive vaporization of the liquid water beam was found to be ≈ 0.7 μs for a 50 W infrared laser with a spot of ≈ 0.1 mm diameter ($\sim 10^6$ W/cm²). Ions formed from a laser-irradiated aqueous solution were directly measured by a mass spectrometer. Ion signals were found to increase with an increase of voltage applied to the liquid. The sensitivity for the detection of ions in solution by this method was found to be about one order of magnitude lower than that by the conventional electrospray or ion spray. When the liquid is vaporized in the open atmosphere, the work ($P\Delta V$) made by the vaporization of the liquid is mainly wasted by expansion and it cannot be utilized efficiently for the atomization of liquid sample. This partly explains larger sizes of the liquid droplets and a lower

sensitivity for ion detection by this method. If vaporization of the liquid sample takes place inside the capillary tube, the jet vapor stream generated in the capillary would help to shatter liquid droplets into finer ones and carry the mist formed and gas-phase ions toward the ion sampling orifice of the mass spectrometer system. For this purpose, we prepared the ZnSe crystal block and drilled a fine channel through it with a diameter of 0.7 mm. When the aqueous solution in the channel was irradiated by the focused 50 W infrared laser at the right angle to the channel from outside of the crystal, the aqueous solution was found to be completely vaporized.³ This method seemed to be very useful for the instant vaporization of the liquid sample and for application to atmospheric pressure chemical ionization MS (APCI-MS). Unfortunately, however, fine cracks in the ZnSe crystal were easily formed along the path of the focused laser beam. Thus, we had to conclude that the ZnSe crystal is not a suitable material for the focused laser beam work.

Vaporization of the liquid sample in the metal capillary using the infrared laser without heating the capillary may be realized by the direct irradiation of the tip of the metal capillary coaxially from the opposite side of the capillary by a laser beam. By this means, it was found that explosive vaporization of the liquid sample took place and ions in the aqueous solution could be detected with a high sensitivity. The present work reports the new experimental results for aqueous solutions obtained by this method.

EXPERIMENTAL

The instrumentation currently used is largely the same as that described previously.^{4,5} A schematic diagram of the apparatus is shown in Fig. 1. A stainless steel capillary (inner diameter: 0.13 mm, outer diameter: 0.3 mm) parallel to the interface plate was supplied with sample solution by means of a computer regulated pump (Tosoh CCPD, Akasaka, Tokyo, Japan). Nebulizer gas, N₂, effusing from

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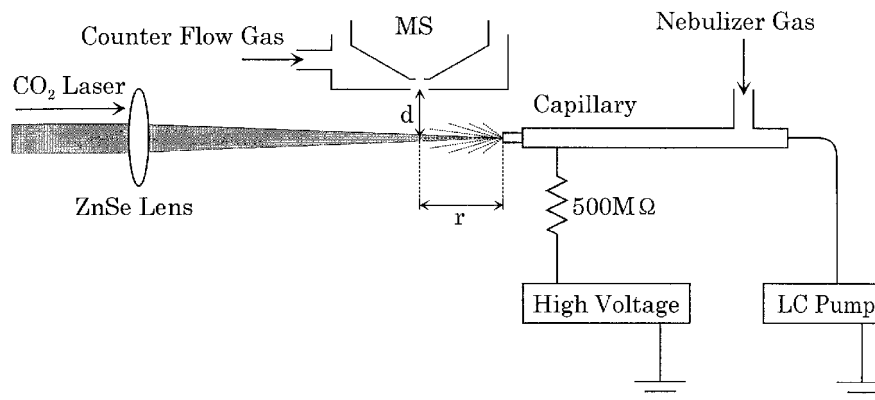


Figure 1. Spray and ionization region of the apparatus, drawing not to scale.

the concentric stainless steel tube (inner diameter: 0.7 mm) reduces the angular divergence of the plume and entrains the mist and gas towards the sampling orifice. A distance of 3–7 mm between the capillary and the interface plate and 10–15 mm between the capillary tip and the ion sampling orifice gave the highest ion signals.

A SYNRAD (Bothel, WA, USA), model 48-5, Duo-Lase 50 W infrared laser (10.6 μm) was used. The tip of the stainless steel capillary was irradiated from the opposite side of the capillary by a laser beam focused to ≈ 0.1 mm. We found that the finest liquid droplets were generated by laser irradiation when the inner diameter of the stainless steel capillary was about the same as the diameter of the laser spot (≈ 0.1 mm). When the diameter of the stainless steel capillary became larger than the laser spot (≈ 0.1 mm), the size of the liquid droplets became apparently larger. When it was smaller, the tip of the capillary melted easily due to overheating.

The ions formed at atmospheric pressure were sampled into the vacuum system through a 100 μm diameter orifice and mass analyzed by a quadrupole mass spectrometer, model MSQ-400 (ULVAC, Chigasaki, Japan) over the range m/z 1–1000.

The flow rate of the aqueous solution was 100 $\mu\text{L}/\text{min}$ through the measurements.

RESULTS AND DISCUSSION

Experimental Results

The present method, referred to as 'laser spray', is found to

be applicable to a wide variety of samples such as amino acids, peptides, surface active agents, metal ions, halide ions, etc., for both positive and negative ion modes. It needs no special skill for successful operation. As far as the laser spot is properly directed on the center of the capillary tip, stable ion signals could be obtained over the period of the measurements.

Figure 2 shows the ion intensity of $[\text{M}-2\text{Cl}]^{2+}$ vs. voltage applied to the stainless steel capillary for aqueous 10^{-5} M tubocurarine chloride solution with and without using nebulizer gas, N_2 . Laser power of 5 W was used to atomize the sample solution. When the applied voltage was low, no ion signals were detected although explosive vaporization of the liquid sample took place and the plume of fine mist and vapor emerged from the tip of the capillary. The absence of an ion signal may be due either to the less efficient positive and negative ion separation in liquid by the method, or to the recombination of generated positive and negative ions in ambient atmosphere. Increasing the voltage applied to the capillary, made the sprayed liquid droplets become apparently finer. The fine laser spray had a similar appearance as the normal electrospray. When the applied voltage was higher than +5 kV in the positive-ion mode, signal intensities decreased due to the occurrence of electrical discharge at the tip of the capillary. The gas-phase discharge breakdown took place at a lower voltage of about 3 kV in the case of the negative-ion mode operation.

Figure 3 represents the ion intensity of $[\text{M}-2\text{Cl}]^{2+}$ vs. laser power for aqueous 10^{-5} M tubocurarine chloride solution with and without using the nebulizer gas. The ion intensities with laser power set at zero just correspond to

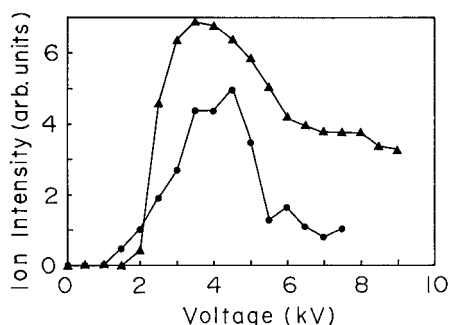


Figure 2. Ion intensity of $[\text{M}-2\text{Cl}]^{2+}$ vs. voltage applied to the stainless steel capillary for aqueous 10^{-5} M tubocurarine chloride solution with (flow rate: 3 L/min) and without using nebulizer gas N_2 . Laser power: 5 W, flow rate of the sample solution: 100 $\mu\text{L}/\text{min}$.

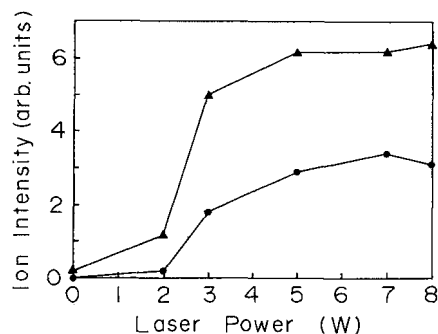


Figure 3. Ion intensity of $[\text{M}-2\text{Cl}]^{2+}$ vs. laser power for aqueous 10^{-5} M tubocurarine chloride solution with (flow rate: 3 L/min) and without using the nebulizer gas. The flow rate of the sample solution: 100 $\mu\text{L}/\text{min}$, voltage applied to the capillary: +3 kV.

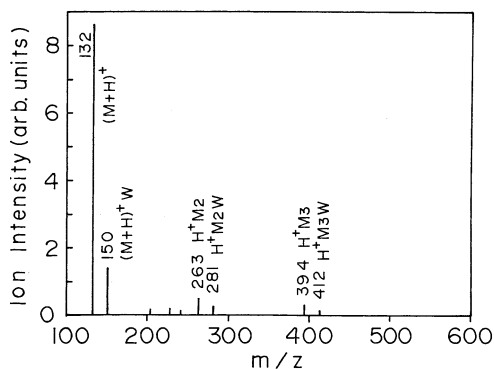


Figure 4. Mass spectrum for aqueous 10^{-5} M D-leucine solution. Flow rate of the sample solution: 100 μ L/min, voltage applied to the capillary: +3 kV, laser power: 6 W, flow rate of nebulizer N_2 gas: 3 L/min.

those obtained by the conventional ion spray (nebulizer gas used) and electrospray (nebulizer gas not used). The ion intensity increased by more than 30 times under laser irradiation with 5–8 W laser power, compared to the normal ion spray operation. When nebulizer gas was not used, no ion signals could be detected with the laser power set at zero, i.e. electrospray failed to spray the aqueous solution used. With an increase of laser power to about 10 W, the stainless steel tip glowed red and the signal intensities decreased to about 60% of the maximum value. Laser powers higher than 10 W caused the tip of the stainless steel capillary to melt and the laser spray to cease. Under the present experimental conditions, a laser power of 5–7 W gave the best results. This laser power of 5 W nearly corresponds to the heat necessary for the aqueous solution with a flow rate of ≈ 100 μ L/min to raise its temperature from 300 K to boiling point and to vaporize it completely. Since liquid mists were visible to the naked eye by illuminating the spray with a diode laser, it is apparent that not all but only some fraction of the laser power was used for the vaporization of aqueous solution.

In the present experiments, a laser power density of about

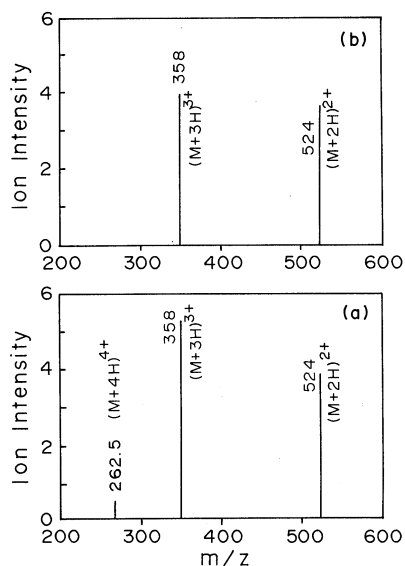


Figure 5. Laser spray mass spectra for aqueous 10^{-5} M angiotensin II solution (a) with and (b) without addition of 10^{-2} M acetic acid. Flow rate of sample solution: 100 μ L/min, voltage applied to the capillary: 3 kV, flow rate of nebulizer N_2 gas: 3 L/min.

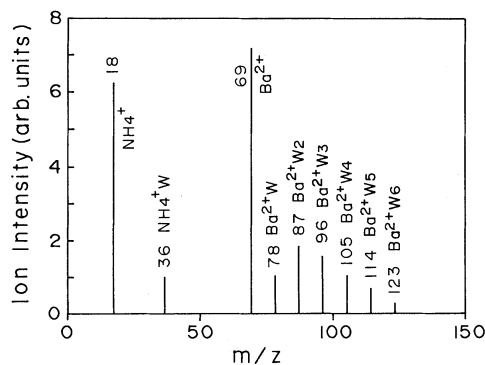


Figure 6. Mass spectrum for aqueous 10^{-5} M $Ba(NO_3)_2$ solution. Flow rate of the sample solution: 100 μ L/min, voltage applied to the capillary: +3 kV, flow rate of the nebulizer gas: 3 L/min.

10^6 W/cm² was used. It is necessary to check the thermal degradation of the labile compounds. We did not observe any fragment ions formed from amino acids and peptides. As a test compound to check the thermal degradation, mass spectra of cholesterol 3-sulfate were measured. This compound is known to be rather labile and decompose to form HSO_4^- (m/z 97) and SO_3^- (m/z 80) ions as fragment ions.⁶ In the laser spray mass spectra of this compound (not shown), a strong molecular ion $[M-H]^-$ was observed but neither HSO_4^- nor SO_3^- was detected. These results indicate that thermal degradation is negligible in the laser spray. It seems likely that the laser power is efficiently converted to the heat for vaporization of aqueous solution and the local overheating leading to the thermal degradation of sample molecules does not take place to any serious degree.

Figure 4 shows the positive-ion mode mass spectra for aqueous 10^{-5} M D-leucine solution. It should be noted that a strong molecular ion $[M+H]^+$ was detected even though acid was not added to the solution. This is also the case for the strong appearance of the $[M-H]^-$ ion signal for the same sample in the negative-ion mode operation, without the addition of base in solution. In the figure, it is worthwhile noting that the cluster ions of $H^+(M)_n$ with $n = 2$ or 3 are observed in addition to the major monomer molecular ion, $[M+H]^+$. The appearance of such cluster ions was also observed for other amino acid samples in the laser spray mass spectra. We confirmed that such cluster ions were not observed by conventional electrospray. The appearance of the cluster ions formed by laser spray may be due to the efficient vaporization of water solvent during the laser spray which resulted in the enrichment of the sample. Such a process of sample enrichment may partly explain the high sensitivity established in the present method.

Figure 5 shows the laser spray mass spectra for 10^{-5} M angiotensin II with and without addition of 10^{-2} M acetic acid in aqueous solution. Without addition of acetic acid, only the $[M+2H]^{2+}$ and $[M+3H]^{3+}$ ions are observed as major ions. When acetic acid was added, a new peak of $[M+4H]^{4+}$ with m/z 262.5 appeared. We made considerable effort to observe this multiply charged ion by electrospray and ion spray by optimizing the experimental conditions (e.g. solvents, sample concentration, addition of acids, applied voltage, etc.), but we failed to detect this ion. This suggests that laser spray could be superior to electrospray and ion spray for the observation of higher, multiply

charged ions. This may be related to the fact that the aqueous solution was used in the present work. It is well known that a more polar solvent is better for the observation of higher, multiply charged ions.⁷

Figure 6 shows the mass spectrum for aqueous 10^{-5} M $\text{Ba}(\text{NO}_3)_2$ solution. The Ba^{2+} ion appears as the base peak. In the current work, it was also found that other alkaline earth and transition metal cluster ions $\text{M}^{2+}(\text{H}_2\text{O})_n$ could be easily detected as major ions by the laser spray.

In our previous work, we failed to observe the halide ions from a mixed solution of 10^{-5} or 10^{-6} M NH_4X (X=F, Cl, Br and I) by means of electrospray or ion spray.⁵ In contrast, halide ions of F^- to I^- could be easily observed with nearly equal intensities for mixed samples of aqueous 10^{-5} M NH_4X (X=F, Cl, Br and I) solution by the laser spray. This also indicates the versatility of the laser spray technique.

Mechanism for ion formation in laser spray

As described above, the present laser spray was found to be a very versatile method for observing a wide variety of analyte ions, either positive or negative ions, and especially suitable for the observation of multiply charged ions. In some cases, higher multiply charged ions could be observed than by electrospray or ion spray.

As already described, strong ion signals could be observed only when a high voltage was applied to the stainless steel capillary. This indicates that the high electric field on the tip of the stainless steel capillary plays an important role for the separation of positive and negative ions. The electric field E_c on the tip of the capillary of radius r_c which is at a distance d from a planar electrode can be evaluated with the approximate relationship given in Eqn. (1),^{8,9,10} where V_c is the potential applied to the capillary.

$$E_c = 2V_c/[r_c \ln(4d/r_c)] \quad (1)$$

The value E_c , found under the present experimental conditions, is estimated to be $\approx 6 \times 10^4$ V/cm. The electric field penetrated in the liquid may be estimated to be about 10^3 V/cm by dividing E by the dielectric constant of the liquid. In such a field in liquid, the drift velocity of H_3O^+ , for example, with the mobility of 4×10^{-3} cm²/V s is estimated to be about 4 cm/s. In the present experiment, the linear velocity of the liquid in the capillary (diameter: 0.13 mm) is ≈ 3 cm/s. That is, the ion drift velocity in liquid and the linear velocity of liquid in the capillary are of the same order. Thus, the positive and negative ions have enough time to be pulled apart in liquid by the high electric field and the positive ions are enriched over the surface of the liquid in the positive ion mode, for example. The negative ions drift against the direction of the liquid flow toward the capillary and discharge there. The typical thickness of the drift region is estimated to be $\approx 10^{-2}$ cm.¹¹ In this drift region, the positive ions are being enriched. Hale and Querry¹² measured the absorption coefficient of water for 10.6 μm infrared light to be $= 832$ cm⁻¹. This value leads to the absorption length in bulk water ($1/\alpha$) to be 12 μm . That is, the penetration depth of the laser light in aqueous solution is only about 12 μm thick. Thus, the explosive vaporization of aqueous solution may take place within the top surface layer of about this thickness. This guarantees the formation of a fine mist by the laser spray. The penetration depth of 12 μm is about one order of magnitude thinner than the thickness of the drift region (10^{-2} cm). This suggests that the top surface liquid

layer enriched by the positive ions are selectively vaporized by the laser irradiation. This may explain the high sensitivity of the present method.

CONCLUDING REMARKS

When an aqueous solution (flow rate: 100 $\mu\text{L}/\text{min}$) effusing from the tip of the stainless steel capillary was irradiated by a focused infrared laser, positive and negative ion signals of amino acids, peptides, surface active agents, metal ions (alkali metal, alkaline-earth metal and transition-metal ions), halide ions, etc. were obtained at high sensitivity. Laser spray is particularly suitable for the analysis of aqueous solution samples which are rather difficult to handle by electrospray and ion spray.

The laser beam has the Gaussian-type intensity distribution with ≈ 0.1 mm half-width at half-maximum. Some fractions of laser power may be absorbed by the charged liquid droplets present in the laser beam path and by the stainless steel capillary. Thus only a fraction of laser power may be used for liquid heating. If vaporization of the liquid takes place on the tip of the capillary but not inside, the liquid surface being vaporized on the tip experiences a high electric field and the charge separation must take place there. The enriched ions (positive or negative) on the liquid surface are selectively vaporized by the laser irradiation. The use of a capillary with an inner diameter about the same as that of the laser spot is necessary because all the surface of the sample liquid must be illuminated by the laser in order to establish the efficient atomization. The high electric field on the tip of the capillary must also function to separate the gas-phase positively and negatively charged droplets and also vaporized ions.

A decrease in ion signals was found with increasing electrolyte concentrations, but it was less pronounced when compared with electrospray or ion spray. The decrease observed may partly be due to the decrease in the thickness of the ion drift region formed near the liquid surface since the thickness of the drift region is dependent on the ion concentration.

The present method was found to be applicable not only to aqueous solution but also to other organic solvents such as alcohols and acids (e.g. acetic acid). Further investigation on this aspect is in progress in our laboratory.

Ikonomou and Kebarle found that an electrospray interface provides high sensitivity and signal stability for mass spectrometric detection of analytes in solvents with high water content including 100% water when the electrospray capillary tip section is heated close to the boiling point of the solvent.¹³ In this method, the dispersion of the liquid sample into droplets could be driven partially by the electric field and partially by the temperature. That is, their method could be a form of electro-thermospray, where droplet charging is provided by the field whereas droplet dispersion is a product of the field and the near boiling temperature. In the present laser spray, the droplet dispersion is mainly driven by the laser heating because the explosive mist formation takes place without the electric field, although the sprayed liquid droplets become finer by the application of the high electric field. The high sensitivity of the present method may partly be due to the efficient vaporization of water solvent by the laser irradiation which results in the enrichment of the sample concentration.

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