Application of Laser Spray for Liquid Chromatography/Mass Spectrometry Interface

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New liquid chromatography/mass spectrometry (LC/MS) coupled with a new interface, LC/laser spray MS was developed. The laser spray was found to be particularly suitable for the low-concentration aqueous sample solutions because the enrichment of the sample concentration takes place near the meniscus of the liquid sample protruded from the stainless steel capillary. The extracts from the young leaves were measured using the present method coupled with the dual spray technique. Owing to much better sensitivities for laser spray than for electrospray, molecular formulas for some components in the sample could be postulated.

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1. Introduction

Mass spectrometry for biological analysis has been recently demonstrating remarkable progress with development of new analytical tools and methods. In particular, development of soft ionization techniques, electrospray (ES) and matrix assisted laser desorption/ionization provides huge benefits to this field. For these researches, many biologists, pharmacists and physicians, however, expect an ionization technique with much higher sensitivity and throughput. Laser spray, developed by Hiraoka *et al.*^{1)–6)} is more suitable for the analysis of aqueous solution than ES. Because a 10.6 μm infrared laser excites vibrational modes of hydrogen bonds in water, the irradiation of the laser at the Taylor cone results in the rapid liquid vaporization. Therefore, laser spray is expected to be much more suitable for the detection of less surface-active ions than ES. We have reported the beneficial potential for ionization of small and biological macromolecules.^{1)–7)} Furthermore, another merit with laser spray was found that 10.6 μm laser irradiation dissociates non-covalent complexes only selectively without a fragmentation of the dissociated subunits.⁷⁾ In contrast, collision induced dissociation (CID) of non-covalent complex causes considerable cleavages of covalent bonds as well as the dissociation of non-covalent bonds.⁸⁾ Selective cleavage of the non-covalent bonds established by laser spray is highly preferable for the study of the stability and structure of non-covalent complexes.

Liquid chromatography/mass spectrometry (LC/MS) would be useful for identification of these unknown mixtures, such as drugs, urines and biopolymers.^{9)–16)} ES and atmospheric pressure chemical

ionization are mainly used for the interface of mass spectrometer. In particular, ES is used for characterizing small to medium sized proteins (<100 kDa) and thermally labile compounds. Reversed-phase liquid chromatography is an important component of the intact protein analysis because it has the ability to separate species based on minor structural heterogeneity and coupled to standard high performance liquid chromatography (HPLC) detectors, provides quantitative information about the main component, variants, and cleavage products. Conventional LC/MS techniques, however, can hardly deal with hydrophilic samples such as sugars and glycoproteins. The new LC/MS interface should be developed so that it can be applied to wider biological samples that cannot be dealt with by the conventional methods. To address this problem, we are applying laser spray to LC/MS interface. This article presents initial results of a novel approach, in which laser spray is first used for the interface of LC/MS, and measured unknown mixtures. We also evaluated this new technique, LC/laser spray MS.

2. Experimental

Aero-Smell (Chikyugi, Meguro, Tokyo, Japan) that is the aqueous solution of extracts from natural plants, such as pine, white birch, *etc.*, was used for the sample. It is claimed that negative ions are contained in the sample, but the components of the sample have not been analyzed yet. Preparation for the sample for the analysis by laser spray is made as follows. The sample of Aero-Smell, as an aqueous solution, was mixed with an almost equal amount of diethylether and the oil soluble components were extracted to the diethylether. The fractions extracted from the sample with diethylether were evaporated to a few μL , and 50 μL of methanol is added to the evaporated solution. This methanol solution was used for our measurements. Sodium trifluoroacetate (CF_3COONa) aqueous solution

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Fig. 1. Experimental setup of laser spray coupled with an orthogonal time-of-flight mass spectrometer. Here RP is rotary pump, and TOF is time-of-flight mass analyzer. Charge coupled device camera is used for monitoring Taylor cone. Laser beam alignment is accomplished by adjusting two Au mirrors (not shown). Another probe for reference solution is not irradiated by CO_2 laser.

was used for a reference in the negative ion mode.

Schematic diagram is shown in Fig. 1. The HPLC system consisted of Agilent 1100 series (HP-1100, Palo Alto, CA, USA) equipped with C-18 analytical column (XDB-C18, Agilent, $\phi 2.1 \times 50$ mm) and operated at a flow rate of $50 \mu\text{L}/\text{min}$. The sample was eluted by LC/MS utilizing an elution composition of distilled water including 0.1% acetic acid (Solvent A) with a gradient of HPLC grade methanol (Solvent B). After a 5 min isocratic elution at 95% A, a gradient started and increased to 15% B in 15 min, followed by 30% B in 20 min (0.75% B/min), 70% B in 20 min (2.0% B/min), and finally reached to 100% B at 100 min (0.75% B/min). The HPLC system was connected on-line to time-of-flight mass spectrometer (Accu-TOF, JEOL, Akishima, Tokyo, Japan) equipped with orthogonal ES ion source.

A stainless steel capillary (inner diameter: 0.1 mm, outer diameter: 0.2 mm) parallel to the interface plate is supplied with a sample solution from HPLC. Nebulizer gas, N_2 , effusing from the concentric stainless steel tube (inner diameter: 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. A SYNRAD (FSV20SFB, Bothel, WA, USA), max. 20 W infrared laser ($10.6 \mu\text{m}$) was used for that. The tip of the stainless steel capillary is irradiated axially from the opposite side of the capillary by a laser beam focused to ≈ 0.3 mm. The power was adjusted to avoid having the capillary burned. The voltage applied to the capillary was -2800 V. Reference sample was flowed into another stainless steel capillary with an off-axis by using a syringe pump (type 11 Plus,

Harvard Apparatus, Holliston, MA, USA) and the flow rate was $20 \mu\text{L}/\text{min}$. The applied voltage was the same as the capillary directly connected to HPLC.

3. Results and Discussion

Since the present mass spectrometer system is originally designed for electrospray, it is possible to compare the sensitivities of electrospray with that of laser spray operated under the similar experimental conditions. Figure 2 shows ES and laser spray total ion current (TIC) chromatogram for sample solution. The laser power is 3.6 W, and it seems to be higher than our conventional measurements.^{1), 6), 7)} The flow rate of sample solution, however, is much higher than that of previous reports. Because the sample contained quite a lot of elements, the separation was impossible for a short time and this LC/MS measurement needed higher flow rates than normal flow injection measurements. Therefore, higher laser power must be selected. From 0 to 5 min, sharp peak was observed with both laser off (a) and laser on (b). These peaks identified halogen compounds from isotopic abundances, but the detail could not become clear. In (a), broad peaks were observed from 5 to 20 min and were judged to ions created by discharge of acetate ion and these peaks

Fig. 2. TIC chromatogram for oil soluble components of Aero-Smell with (a) ES (laser off) and (b) laser spray. Insets on the right side of each chromatogram show enlarged illustration in 70 to 110 min. Full time scale of both measurements are 130 min. The expression of a.u. means arbitrary units.

were often observed as polar solvents were used. A gas phase breakdown takes place by an application for high voltage in negative ion modes and destroys analyte ions. In this experiment, the voltage of the needle was set as high as possible for detecting the analytes effectively in methanol, therefore, electrical discharge happened in early time when water was included a lot. In (b), these peaks were not observed, and this trend often happens in laser spray measurements. Because a gas phase pressure in laser spray is lower than that of ES due to effective vaporization of liquid by the irradiation of CO₂ laser, the firing potential must increase from Paschen's low. For other possibility, higher temperature liquid by laser irradiation may eject molecules in liquids, such as N₂ and O₂, which may decrease the efficiency of discharge.

The intensity for TIC with laser spray was much higher than conventional ES except when discharge compounds were detected and all of peak intensities for mass spectra in the peaks of TIC chromatogram were higher than the peak intensities with ESI. In particular, the sensitivity became more than one order of magnitude higher in 70 min to 110 min (see inset of Fig. 2). These results show that the ionization with laser spray is more effective to detection of species at low concentration. The irradiation of CO₂ laser induces concentration of ions including first formed droplet while liquid vaporization is accelerated. Oppositely it is apparent that the enrichment of the sample concentration for laser spray by the laser heating led to the less efficient ion desorption due to the formation of thinner electric double layer formed on the meniscus of the liquid sample. The number of detected peaks with ES was 10 while the number with laser spray was 23. This means that 13 species cannot be detectable with ES, despite the fact that the solution included a large amount of methanol in this timescale. And it is clear that the laser spray is applicable to aqueous solutions as well as methanol solutions. Without the laser (a), two small peaks were observed about from 78 min, and these peaks gave similar signal intensities. With the laser on (b), however, the first detected peak had twice as large intensity than the other peak. This result implies that the first species may be surface-active ion, such as terpene. In positive ion modes, similar results were obtained, but the increasing sensitivity was not as large as that of negative ion modes. It is considered that the laser spray in negative ion modes is more useful for the detection of multi elements than conventional ES as the generation of gas phase breakdown is controlled with decreasing pressure.

Figure 3 is mass spectrum of chromatographic peak detected from 112 to 113 min with laser spray. The assignment is executed using the following manner. Theoretical mass of reference was decided by the calculation. In this case, the mass of trifluoroacetate dimer (CF₃COO⁻(CF₃COONa)) was 248.960 u. Obtained mass of unknown peak was calibrated with difference between obtained and calculated mass of reference peak. From this analysis, unknown peak in Fig. 3 was assigned at C₁₆H₃₁O₂. It is considered that this formula expresses saturated fatty acid. However, it could not be judged whether this compound was included in an

Fig. 3. Laser spray. ESI mass spectrum of the chromatographic peak detected from 112 to 113 min.

original sample or not. The compound may be impurity originated from C-18 column for the separation of sample. Some other peaks were also assigned, but the information for the structure could not be obtained. Tandem mass spectrometry, CID, or some other techniques have to be performed to obtain the detail for the structure.

4. Conclusion

In conclusion, we developed the new LC/MS technique, LC/laser spray MS. The use of laser spray for the interface gave better results than conventional ES. In particular, the sensitivity became more than one order of magnitude higher in negative ion modes. It was also found that this technique has a potential benefit for the low concentration samples due to condensation effect of the formed droplet by the irradiation of laser. In future, we will apply this method to protein, sugar and urine. Assignments of inclusion in Aero-Smell were incomplete in the detail of the structural information. The present system was insufficient as yet for deciding molecular formulas. A more systematic study is under way in our laboratory. Ultimately this method has to be combined with tandem mass spectrometer or other techniques providing structural information.

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