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Effect of Ultrasonic Microdroplet Generation in the Low-Temperature Plasma Ionization-Mass Spectrometry

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Abstract : Low-temperature plasma (LTP) ionization is one of the ambient ionization methods typically used in mass spectrometry (MS) for fast screening of chemicals with minimal or no sample preparation. In spite of various advantages of LTP ionization method, including simple instrumentation and *in-situ* analysis, more general applications of the method are limited due to poor desorption of analytes with low volatilities and low ionization efficiencies in the negative ion mode. In order to overcome these limitations, an ultrasonic vibrator of a commercial hand-held humidifier was interfaced with an LTP ionization source, which generated microdroplets from sample solutions and assisted with LTP ionization. Ionization behaviors of various chemicals in microdroplet-assisted LTP (MA LTP) were tested and compared with typical LTP ionization from dried samples applied on a surface. MA LTP efficiently ionized small organic, amino, and fatty acids with low volatilities and high polarities, which were hardly ionized using the standard LTP method. Facile interaction of LTP with ultrafine droplets generated by ultrasonic resonator allows efficient ionization of relatively non-volatile and polar analytes both in the positive and negative ion modes.

Keywords : ultrasonic microdroplet generation, low-temperature plasma ionization

Introduction

Low temperature plasma (LTP) ionization is one of the ambient ionization methods used in mass spectrometry (MS) for rapid and direct analysis of samples with minimal sample preparation.[1] A large variety of ambient ionization methods have been developed[2] since the initial desorption electrospray ionization (DESI) [3] and direct analysis in real-time (DART)[4] publications appeared. The success of ambient ionization of an analytes depends on desorption efficiencies, efficient interactions with ionizing species and final survival through the inlet to mass spectrometer.[5] LTP ionization is one of the discharge-based ambient ionization methods that have several advantages, including soft ionization, direct analysis from intact sample, minimum use of solvents, very simple and low cost instrumentation, for *in-situ* analyses. However, relatively polar molecules with low volatilities are not easily ionized by LTP ionization. Additional sample heating improved ionization efficiencies, but showed only limited success.[6,7]

Previously, LTP ionization has been successfully carried out from directly sprayed aerosol samples.[8] Direct spraying showed the potential to improve LTP ionization efficiencies for less volatile analytes. In this study, LTP ionization behavior was investigated using ultrafine microdroplets of sample solutions that were generated by a small ultrasonic resonator. For this purpose, a homemade LTP ionization source equipped with an ultrasonic microdroplet generator was developed. Ionization efficiencies of different classes of compounds were investigated using microdroplet-assisted (MA) LTP ionization and compared with those of typical LTP ionization from samples dried on glass slides.

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Experimental

Sample and reagents

Pyruvic acid, lactic acid, fumaric acid, oxaloacetic acid, malic acid, citric acid, glutamic acid, alanine, valine, tryptophan, glucose, fructose, glucose-6-phosphate, fructose-6-phosphate, adenosine monophosphate (AMP), adenosine tripphosphate (ATP), pentadecanoic acid,

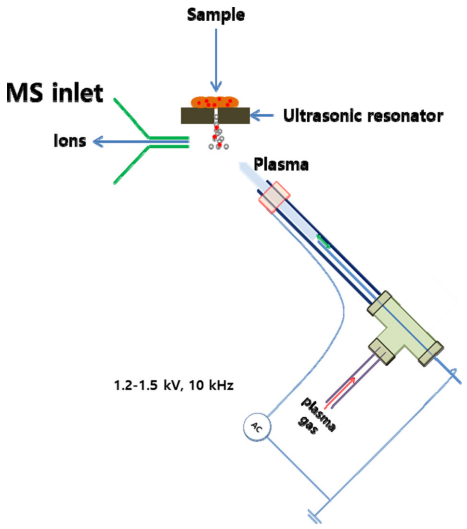

Figure 1. Schematic diagram of the homemade low-temperature plasma (LTP) ionization source for sample introduction by ultrasonic microdroplet generation. It is registered as Korean Patent No. 10-1768127.

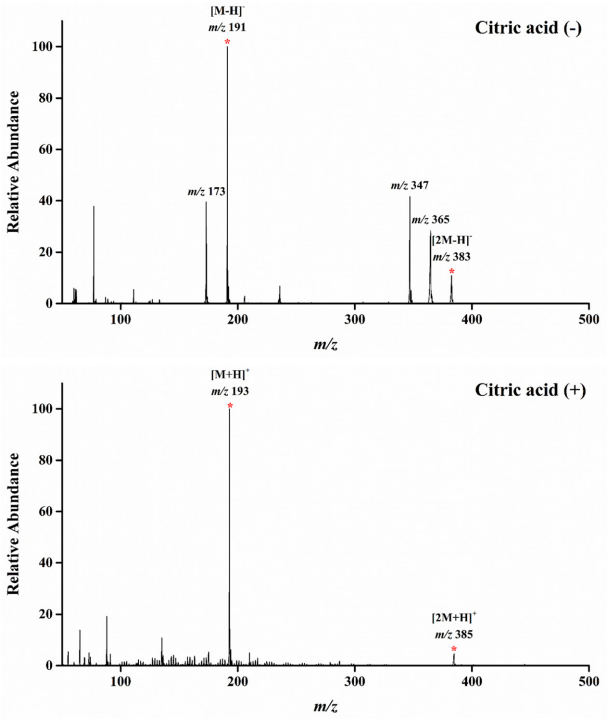

Figure 2. Microdroplet-assisted LTP ionization MS of citric acid in the positive and negative ion modes.

palmitic acid, linoleic acid, arachidic acid, melissic acid, and a standard fatty acid (FA) ethyl ester (FAEE) mixture (C4-C24 even carbon, saturated FAEEs, containing 1000 mg/mL each in hexane) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade water and methanol were also obtained from Sigma-Aldrich. For preparation of test solutions of about 0.5 mM each for LTP ionization MS, the pure chemicals were dissolved in water, methanol, methanol/water 1:1 mixture, ethanol, or hexane depending on their solubility. Sample solutions of ibuprofen and melatonin were obtained by methanol extraction of commercially available tablets.

Low-temperature plasma ionization source

An LTP probe described previously in the literature was used with minor modifications (Figure 1).[7,8] Briefly, the LTP probe consisted of a glass tube (outside and inside diameters 1/8 and 1/16 inches, respectively) with a central ground stainless steel electrode inserted inside the tube using a plastic tee connector, which was also used to allow helium discharge gas to flow through the glass tube. AC voltage of 1.2 to 1.5 kV and 10 kHz was supplied to an outer electrode surrounding the tube, which was made by wrapping the tube with Cu tape. The ultrasonic MA LTP ionization MS interface developed is this study is shown in Figure 1. An ultrasonic resonator from a commercial hand-held humidifier powered by a USB port was used to generate ultrafine microdroplets from liquid samples soaked in a piece of filter paper placed on the rear-side of the resonator. Sample solutions of about 0.1 mL were deposited and supplied through a pinhole to the front-side of the resonator, and ultrafine droplets were generated on the front-side. In the typical LTP setup, the glass slide sample stage is installed as previously described.[7]

Mass spectrometric analysis

LTP ionizations MS experiments were all performed using a linear ion trap mass spectrometer (Thermo LTQ; San Jose, CA, USA). An ESI source was removed, and the LTP ionization source was installed in its place. A full MS scan over the 50–1000 *m/z* range was done for the MS analysis. The capillary temperature was set at 270 °C. The MS analyses were conducted both in positive and negative ion modes.

Results and Discussion

Small organic acids and amino acids

Organic acids found in the glycolysis and tricarboxylic acid (TCA) cycles are highly polar molecules, and many of them have limited volatilities. In a typical LTP ionization MS, they are not efficiently ionized. On the contrary, the MA LTP ionization generated strong molecular ions both in the positive and negative ion modes as shown in Table 1. This method produced more extensive adduct ions, especially in the case of smaller organic acids. For citric acid, the positive ion mode MA LTP generated $[M+H]^+$ (*m/z* 193) dominantly with a smaller $[2M+H]^+$ adduct ion (*m/*

Table 1. Comparison of ionization of various compounds by MA LTP versus standard LTP ionization mass spectrometry (MS).

compound	Formula	M.W.	Vapor Pressure[9] (mm Hg at 25°C)	MA LTP(+)	MA LTP(-)	Solvent for MA LTP	LTP(+)	LTP(-)
Pyruvic acid	$C_3H_4O_3$	88	1.29	$[2M+H]^+$, adducts	$[2M-H]^-$, $[3M-H]^-$, adducts	Water	X	X
Lactic acid	$C_3H_6O_3$	90	0.0813	$[M+H]^+$, adducts	$[M-H]^-$, $[2M-H]^-$, adducts	Water	X	X
Fumaric acid	$C_4H_4O_4$	116	1.54×10^{-4}	$[M+H]^+$, $[2M+H]^+$	$[M-H]^-$, $[2M-H]^-$	EtOH	X	X
Oxaloacetic acid	$C_4H_4O_5$	132	< 0.001 (20°C)	$[M+H]^+$,	$[M-H]^-$, $[2M-H]^-$	Water	X	X
Malic acid	$C_4H_5O_5$	134	3.28×10^{-8}	$[M+H]^+$, $[2M+H]^+$	$[M-H]^-$, $[2M-H]^-$	MeOH:water (1:1)	X	X
Citric acid	$C_5H_8O_7$	192	1.7×10^{-8}	$[M+H]^+$, $[2M+H]^+$	$[M-H]^-$, $[2M-H]^-$	Water	X	X
Alanine	$C_3H_7NO_2$	89	1.05×10^{-7}	$[M+H]^+$, $[2M+H]^+$	$[M-H]^-$	MeOH:water (1:1)	X	X
Valine	$C_5H_{11}NO_2$	117	5.55×10^{-9}	$[M+H]^+$, $[2M+H]^+$	$[M-H]^-$, $[2M-H]^-$	MeOH:water (1:1)	X	X
Glutamic acid	$C_5H_9NO_4$	147	1.7×10^{-8}	$[M+H]^+$	$[M-H]^-$	Water	X	X
Tryptophan	$C_{11}H_{12}N_2O_2$	204	2.1×10^{-9}	X	weak $[M-H]^-$	MeOH:water (1:1)	X	X
Glucose	$C_6H_{12}O_6$	180	No data	X	$[M-H]^-$, $[2M-H]^-$	MeOH:water (1:1)	X	X
Fructose	$C_6H_{12}O_6$	180	No data	$[M+H]^+$, loss of H_2O	$[M-H]^-$	Water	X	X
Glucose-6-phosphate	$C_6H_{13}O_9P$	260	No data	X	X	MeOH:water (1:1)	X	X
Fructose-6-phosphate	$C_6H_{13}O_9P$	260	No data	X	X	Water	X	X
AMP	$C_{10}H_{14}N_5O_7P$	347	No data	X	X	Water	X	X
ATP	$C_{10}H_{16}N_5O_{13}P_3$	507	No data	X	X	Water	X	X
Ibuprofen	$C_{13}H_{18}O_2$	206	4.74×10^{-5}	$[M+H]^+$	$[M-H]^-$	MeOH	$[H+H]^+$	X
Melatonin	$C_{13}H_{16}N_2O_2$	232	1.4×10^{-7}	$[M+H]^+$	$[M-H]^-$	MeOH	X	X
Pentadecanoic acid	$C_{15}H_{30}O_2$	242	4.35×10^{-7}	X	$[M-H]^-$, $[2M-H]^-$	Water	X	X
Palmitic acid	$C_{16}H_{32}O_2$	256	3.8×10^{-7}	X	$[M-H]^-$, $[2M-H]^-$	MeOH	X	X
Linoleic acid	$C_{18}H_{32}O_2$	256	8.68×10^{-7}	$[M+H]^+$	$[M-H]^-$, $[2M-H]^-$	EtOH	X	X
Arachidic acid	$C_{20}H_{40}O_2$	312	1.81×10^{-9}	X	weak $[M-H]^-$	EtOH	X	X
Melissic acid	$C_{30}H_{60}O_2$	452	No data	X	X	Chloroform	X	X
Ethyl caprylate	$C_{12}H_{24}O_2$	200	3.1×10^{-2}	$[M+H]^+$	X	Hexane	$[M+H]^+$	X
Ethyl laurate	$C_{14}H_{28}O_2$	228	7.44×10^{-3}	$[M+H]^+$	X	Hexane	$[M+H]^+$	X
Ethyl myristate	$C_{16}H_{32}O_2$	256	1.57×10^{-3}	$[M+H]^+$	X	Hexane	$[M+H]^+$	X
Ethyl palmitate	$C_{18}H_{36}O_2$	284	2.34×10^{-5}	$[M+H]^+$	X	Hexane	$[M+H]^+$	X
Ethyl stearate	$C_{20}H_{40}O_2$	312	2.59×10^{-6}	$[M+H]^+$	X	Hexane	$[M+H]^+$	X
Ethyl arachidate	$C_{22}H_{44}O_2$	340	1.92×10^{-8}	$[M+H]^+$	X	Hexane	$[M+H]^+$	X
Ethyl behenate	$C_{24}H_{48}O_2$	368	5.42×10^{-7}	$[M+H]^+$	X	Hexane	$[M+H]^+$	X
Ethyl tetracosanoate	$C_{26}H_{52}O_2$	396	No data	$[M+H]^+$	X	Hexane	$[M+H]^+$	X

z 385). The negative ion mode LTP ionization MS also produced strong $[M-H]^-$ (*m/z* 191) and $[2M-H]^-$ (*m/z* 383) signals, but it also generated several fragment ions due to water loss (*m/z* 365, 347, 173) as shown in Figure 2. Ultrasonically-generated microdroplets from the sample solution enhanced the ionization of small organic acids with high polarity and low volatility. Considering the relatively high vapor pressures of pyruvic acid, lactic acid,

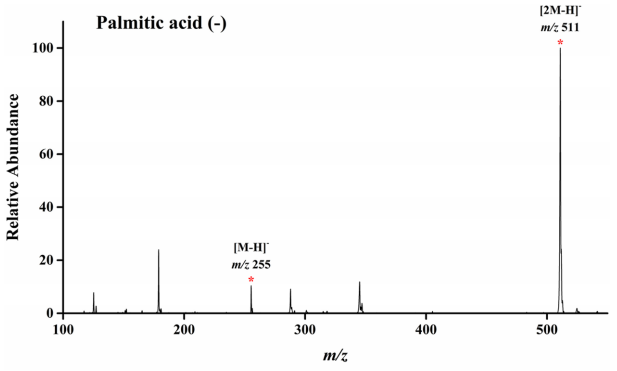

Figure 3. MA LTP ionization MS of palmitic acid in the negative ion mode.

and fumaric acid, volatility alone cannot explain the low ionization efficiency of a typical LTP ionization method, and further investigations are required.

Amino acids are non-volatile zwitter-ionic molecules that are hardly ionized in a standard LTP ionization MS. The MA LTP also boosts facile ionization of amino acids both in the positive and negative ion modes (Table 1).

Fatty Acids and Their Esters

Long chain fatty acids are non-volatile and frequently analyzed in the discharge-based ambient ionization MS after esterification to enhance volatility.[7] Both typical LTP and MA LTP ionizations efficiently generate strong molecular ions of FAEEs in the positive ion mode (Table 1). In a typical LTP ionization, additional heating is still required in order to achieve ionization of long-chain FAEEs.[7] Non-derivatized fatty acids cannot be analyzed by standard LTP ionization. In contrast, in the negative ion mode, MA LTP ionization MS showed dominant molecular ions of intact fatty acids (Table 1). Figure 3 shows MA LTP ionization mass spectrum of palmitic acid as an example. $[M-H]^-$, and a much stronger $[2M-H]^-$ can be found at *m/z* 255 and 511, respectively. Not all free FAs were efficiently ionized by MA LTP. Very long chain FA, such as arachidic acid and melissic acid failed to produce noticeable ions.

Other Molecules and Samples

MA LTP could not ionize many metabolites in the glycolysis and energy metabolism, such as glucose-6-phosphate, fructose-6-phosphate, AMP, and ATP. MA LTP ionization of fructose was successful, however, in generating $[M-H]^-$ at *m/z* 179 in the negative ion mode. In the positive ion mode, $[M+NH_4]^+$, and ions generated by sequential water loss from $[M+H]^+$ were identified at *m/z* 198, 163, and 145, respectively (Figure 4). Ionization of small carbohydrates was successful using MA LTP ionization MS, but further studies are required to extend

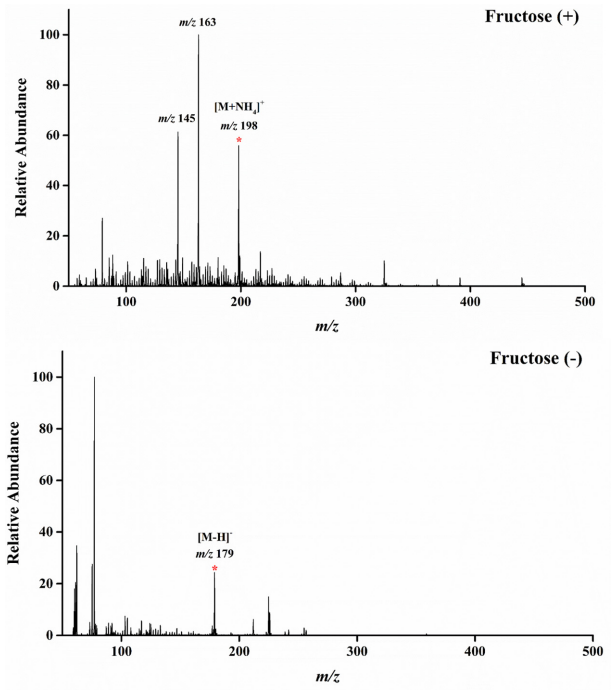

Figure 4. MA LTP ionization MS of fructose in the positive and negative ion modes.

this method to larger carbohydrate molecules or metabolites. Ionization of glucose-6-phosphate fructose-6-phosphate, AMP, and ATP were not successful. Pharmaceutical drug molecules, ibuprofen and melatonin, that were simply extracted from tablets were easily detected using MA LTP ionization MS both in positive and negative ion modes.

Conclusions

A low-cost home-built nebulization system was developed using an ultrasonic resonator obtained from a commercially available hand-held humidifier. It was interfaced with an LTP ionization source to enhance ionization by generating ultrafine microdroplets from a sample solution. Even without sufficient optimization of the interface geometry, MA LTP has made it possible to ionize wider variety of molecules, including polar and non-volatile molecules, which has been the limitation of standard LTP ionization MS. Lack of success in negative ion generation using standard LTP ionization was also overcome by the present method. It is believed that interactions of ultrafine sample droplets with plasma-generated reagent ions provides an additional electrospray-like ionization mechanism to the plasma-based ionization, which allows ionization of more diverse classes of molecules. The microdroplet generator using ultrasonic resonator has advantages including the small size, low cost,

and USB-powered operation. Taken together with the simple and easy instrumentation of LTP, the present method is highly amenable for portable MS in the future.

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