JMS letters

Journal of MASS SPECTROMETRY

Received: 15 January 2014 Revised: 20 February 2014 Accepted: 3 March 2014 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/jms.3355

Droplet-based electrospray ionization mass spectrometry for qualitative and quantitative analysis

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Dear Sir,

Mass spectrometry (MS) is a rapid and sensitive analytical tool that can provide information such as molecular weight and structure.[1,2] One of the fast growing fields in MS in the beginning of this century is the development of ion sources at atmospheric pressure. Speed, simplicity and minimal sample preparation are the main characteristics of these recently developed ion sources at open air.[3–21] Most ion sources are mainly used in qualitative MS analysis. However, quantitative analysis can provide important information in different analyses, such as in the determination of the level of specific species in natural products[22,23] and the concentration of disease-related biomarkers in biological fluids.[24,25] Conventionally, chromatography combined with MS can provide reliable quantitative analysis. Nevertheless, newly developed ion sources could be potentially used in quantitative analysis,[15,26–31] such as the use of laser electrospray MS in the quantitative analysis of mixtures of small molecules.[26] Laser diode thermal desorption combined with atmospheric pressure chemical ionization was also used to quantify sulfonamide residues in dairy milk.[27] Paper spray has been demonstrated its potential in the quantitative analysis of abused drugs.[28] In addition, direct analysis in real-time ionization MS for the quantification of drugs in biological matrixes was also successfully demonstrated.[31]

We previously proposed the contactless atmospheric pressure ionization,[15–19] an ionization method that simply uses a tapered capillary as a sampling tube and a spray emitter. The capillary was placed approximately 1 mm from the front of the orifice of a mass spectrometer without electric contact. Electrospray is mainly induced by the high voltage applied on the mass spectrometer to generate gas phase ions. This approach suggests that ionization processes can be induced by the electric field provided by the mass spectrometer without making any direct electric contact with the emitter. Thus, we used an electronic pipette-operated tip as the emitter to generate the sample spray in the proximity of MS in this study. The electronic pipette can precisely dispense a given volume of droplets with a set interval for multiple ejections. No direct electric contact was made on the pipette tip. Thus, the setup can be easily established with the elimination of any extra accessories by simply placing the electronic pipette in the proximity (~1 mm) of a mass spectrometer. The mass spectra acquired from multiple droplets ejected from the pipette with a given dispensed volume and a fixed interval can be readily and quickly obtained. Multiple droplets from the same sample can be ejected in a fixed frequency by using one run of the electronic pipette. Thus, multiple analyses from the same sample can be repeatedly and quickly conducted. As a consequence, calibration curves based on the ion intensity of analytes *versus* concentration plots can be created for quantitative analysis. Initially, we demonstrated the feasibility of the use of an electronic pipette-operated tip as the emitter to obtain mass spectral results from the analytes with a wide mass range. The feasibility of the use of this setup for quantitative analysis was then studied.

All chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA) except acetonitrile (Merck, Darmstadt, Germany). Urine was collected from a healthy individual. Pipette tips (catalogue no. 15100; inner diameter: 390 μm, outer diameter: 570 μm) were purchased from Sorenson BioScience (Salt Lake City, Utah, USA). Scheme 1 shows the experimental setup used in this study. An Avegene electronic pipette (ePipette S20) obtained from the Pacific Image Electronics (New Taipei City, Taiwan) was adapted with a tip loaded with sample solution. This pipette was then placed vertically in front of a Bruker Daltonics Micro-Q-TOF II Focus (Bremen, Germany). The tip end was distanced from the orifice of the mass spectrometer for ~1 mm. The electronic pipette was powered by a lithium-ion chargeable battery (3.7 V). The voltage applied on the orifice of the mass spectrometer was −4000 V, whereas the temperature of the ion transfer capillary was set to 220 °C. The pipette can be set to dispense a series of programmed volumes repetitively with a set interval. Generally, 2 μl was set for multi-dispensing (×5 or ×10) every 10 s when conducting MS analysis in the study. The auto function was used, and the dwell time was set at 10 s, whereas the speed was set at 9 (full range pickup, 1 s; full range dispense, 1 s; hold at the end, 1 s). Once the trigger was pressed, 5 (or 10) droplets (2 μl) were repetitively dispensed every 10 s from the pipette with the set frequency. The generated gas phase ions were readily acquired by the mass spectrometer in real time. Unless specified, the samples were prepared in deionized water, and 1% formic acid was added to the deionized water when preparing the protein samples.

We investigated if the sample solution dispensed from the pipette was affected by the high electric voltage (−4000 V) applied on the mass spectrometer (Scheme 1). The image of the droplet ejected from the tip was taken by a camera (Fig. 1). The sample droplet formed a Taylor cone (indicated by a red arrow), which was induced by the electric field of the mass spectrometer and was observed in front of the orifice of the mass spectrometer. Considering the limited magnification of the camera, we were unable to see the sprayed fine droplets in front of the orifice of the mass spectrometer. Nevertheless, the presence of the Taylor cone indicated that charge accumulation occurred at the edge of the sample droplet.

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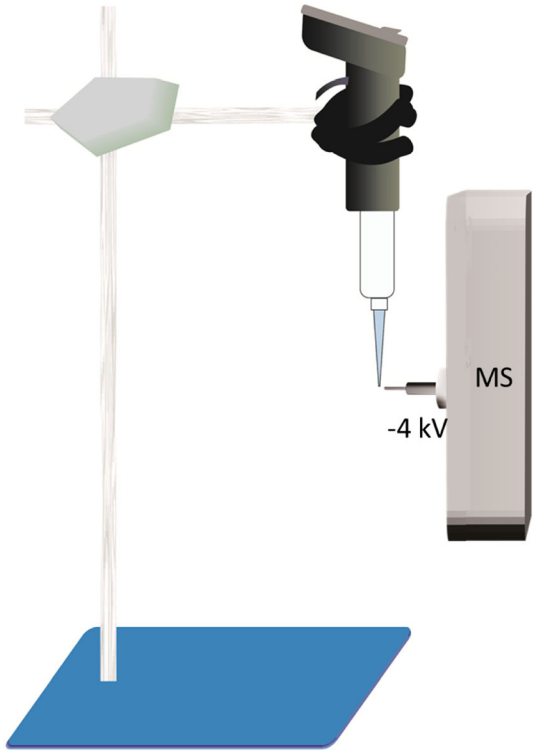

Scheme 1. Setup of the droplet-based electrospray ionization mass spectrometry. The distance between the tip and the orifice of the mass spectrometer was ~1 mm.

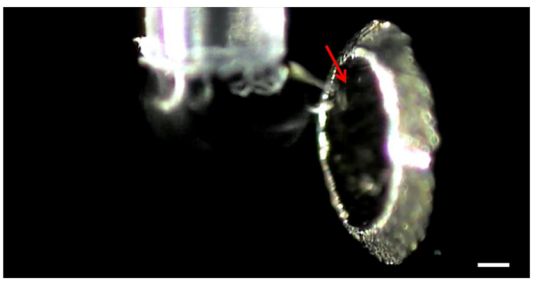

Figure 1. Photograph obtained when ejecting a liquid droplet from a pipette tip close to the orifice of a mass spectrometer applied with −4000 V. Scale bar, 150 μm. The red cursor indicates the formation of Taylor cone. This figure is available in colour online at wileyonlinelibrary.com/journal/jms.

We further investigated if any ions derived from the sample droplet were generated and were acquired by the mass spectrometer. Tryptophan ($(M+H)^+ = 205$) prepared in deionized water was first selected as the model sample. Multidroplets with a volume of 2 μl (×10) were repeatedly ejected from the tip every 10 s. Figure 2(A) shows the resulting plots of total ion current (TIC) chromatogram. A total of 10 peaks representing the 10 dispensed droplets from the electronic pipette were observed in the chromatogram, thereby indicating that the analyte ions were successfully generated from this approach. Furthermore, each peak was distanced from the adjacent peak every 10 s, which corresponds to the set interval between the dispensed droplets. The area of each peak is slightly different from one another, and Fig. 2 (B) shows the corresponding mass spectrum obtained from the plot. Protonated and sodiated tryptophan ions at *m/z* 205 and *m/z* 227, respectively, appear in the mass spectrum. The results indicate that the gas phase ions of the analytes can be successfully generated and detected by the mass spectrometer.

We further used larger biomolecules, such as peptides and proteins, as the samples to demonstrate the feasibility of using this approach for the analysis of large biomolecules. Figure 3(A) and (B) shows the plot of TIC obtained from the sample containing bradykinin (10^{-7} M) and its corresponding mass spectrum, respectively. Figure 3(C) and (D) shows the plot of TIC obtained from the sample containing insulin (10^{-6} M) and its corresponding mass spectrum, respectively. A total of 10 separated peaks were observed in these plots. The mass spectra were dominated by multiply charged ions as indicated in the mass spectra. The results indicate that the current approach is suitable for use in the analysis of peptides and small proteins. Moreover, like in electrospray ionization (ESI), multiple charged ions dominated the mass spectra. However, the intensity of the ion peaks derived from larger protein such as cytochrome c are much weaker than those observed in the mass spectra of bradykinin and insulin (results not shown). Usually, 1% formic acid was added to the protein solution to facilitate the ionization. The results demonstrate that this approach is also suitable for the analysis of large biomolecules although obtaining ion signals from analytes with lower molecular weights is easier. The choice of solvent is crucial in obtaining a reproducible peak area. Considering the surface tension and volatility, we selected water as the best solvent because of its desirable performance in the experiment. An additional organic solvent helps the ionization process; thus, better results for protein analysis can be obtained. However,

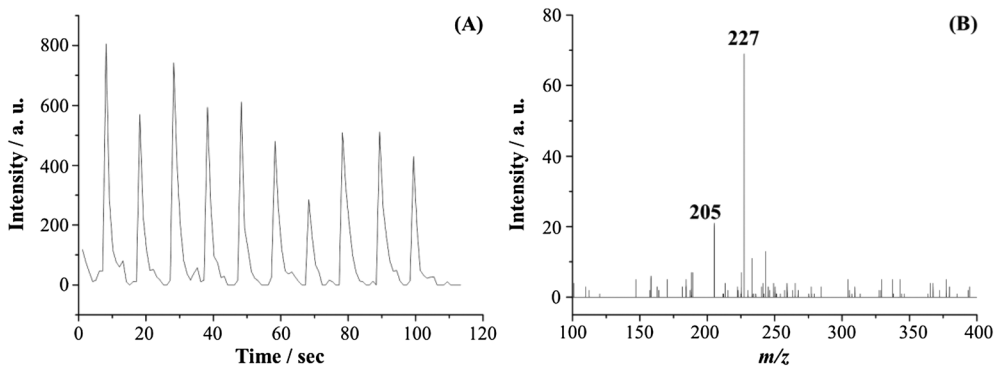

Figure 2. (A) Total ion current chromatogram of tryptophan (10^{-4} M) and (B) its corresponding mass spectrum acquired from the time points between 88.3 and 91.4 s. Ten droplets of the sample solution were dispensed from the pipette tip from every single run.

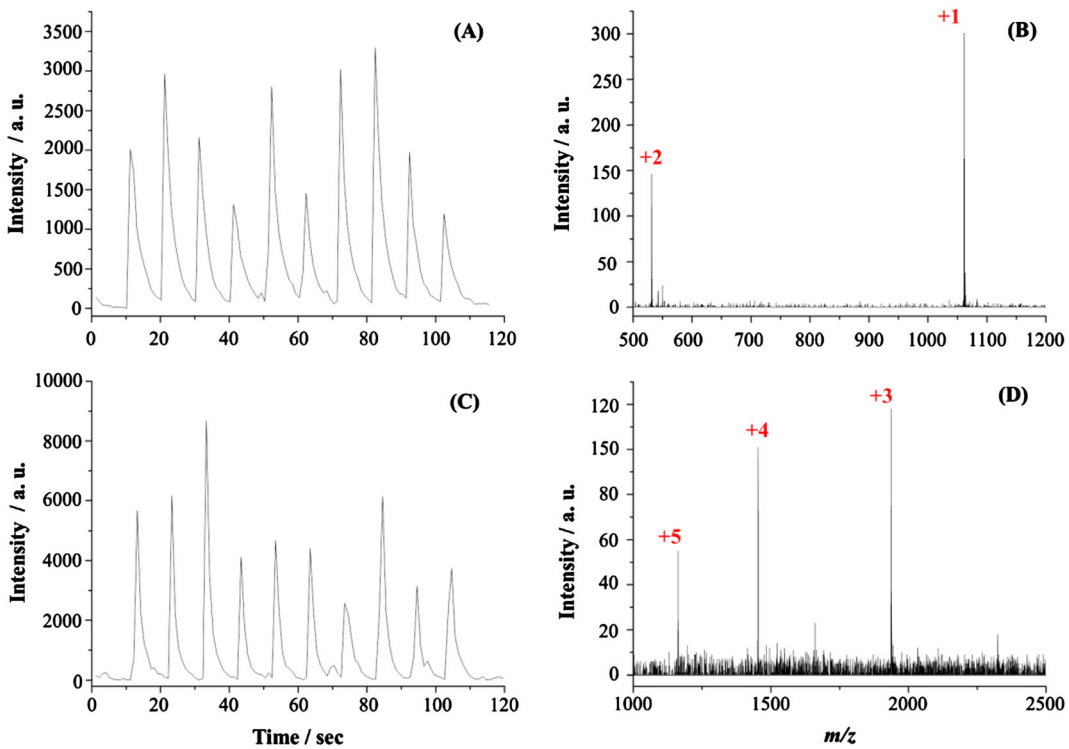

Figure 3. (A) Total ion current chromatogram of bradykinin (10^{-7} M) and (B) its corresponding mass spectrum. (C) Total ion current chromatogram of insulin (10^{-6} M) and (D) its corresponding mass spectrum acquired from the time periods of 81.4–88.4 s and 33.3–37.3 s, respectively. Ten droplets of the sample solution were dispensed from the pipette tip from every single run.

the reproducibility of the integrated area from peak to peak is not good. When an organic solvent was used, the ion intensity derived from the proteins can be improved, but the peak area from multiple analyses from one run greatly varied. Thus, adding organic solvent for protein analysis to obtain a higher intensity of ion peaks for qualitative analysis is effective. Nevertheless, the results indicate that the detectable upper molecular weight of the analytes when this approach was used is smaller than 20 kDa.

To demonstrate the reproducibility of this approach, replicate experiments using the same tip and different tips for droplet-based ESI-MS analysis were conducted. Bradykinin (10^{-6} M) prepared in deionized water was initially used as the model sample. Multidroplets with a volume of 2 μl (×5) were repeatedly ejected from the tip every 10 s. Figure S1A, S1B and S1C in the Supporting Information showed the resultant plots of the extracted ion chromatograms (EICs) at *m/z* 531, standing for the doubly charged ion of bradykinin, using the same tip for three replicates of droplet-based ESI-MS analyses. A total of five peaks representing the five dispensed droplets from the electronic pipette was observed in these chromatograms. The relative standard deviation (RSD) values derived from the peak area of the five peaks observed in Fig. S1A, S1B and S1C were estimated to be ~7%, ~11% and ~13%, respectively. Figure S2 showed the resultant EICs at *m/z* 531 using three different tips for the droplet-based ESI-MS analysis. The corresponding RSD values derived from the peak area of the five peaks observed in Fig. S2A, S2B and S2C were ~7%, ~5% and ~12%, respectively. The RSD values derived from Figs. S1 and S2 were $< 15\%$. The results indicate that the reproducibility for the analysis of aqueous samples using the same tip and different tips for the droplet-based ESI-MS analysis seems fine.

To show the reproducibility of this approach in complex samples, bradykinin (10^{-6} M) prepared in 50-fold-diluted urine was used as the model sample. Multidroplets with a volume of 2 μl (×5) were repeatedly ejected from the tip every 10 s. Figure S3A, S3B and S3C showed the resultant EICs at *m/z* 531 using the same tip for three replicates of droplet-based ESI-MS analyses. The corresponding RSD values derived from the peak area of the five peaks observed in Fig. S3A, S3B and S3C were 14%, 13% and 10%, respectively. The RSD values are slightly higher than those obtained from aqueous samples. It is understandable because urine is much more complex than aqueous solution. Thus, the ion intensity of the analyte ions obtained from droplet to droplet was varied slightly. Three different tips were also used to examine the reproducibility of the analysis of the urine sample by this current approach (Fig. S4). The RSD values of the peak area from the five peaks observed in Fig. S4A, S4B and S4C were 14%, 15% and 18%, respectively. The results show that the RSD values from different sets were $< 20\%$, indicating that the reproducibility of this approach for the analysis of complex samples seems still all right.

The main advantage of this approach is multiple analyses that can be conducted within a very short period from one run, suggesting the feasibility of using this approach for quantitative analysis. The results obtained from multiple analyses of the same sample from the same run are good for constructing the data points in a quantitative calibration curve. The level of amino acids such as arginine in biological fluids in newborns[32,33] can be used as an indication of abnormal metabolism. Thus, we selected arginine as the model sample. Because creatinine is a basic metabolite excreted in urine, it was selected as the internal standard. Figure 4(A)–(E) shows the EIC plots obtained from different

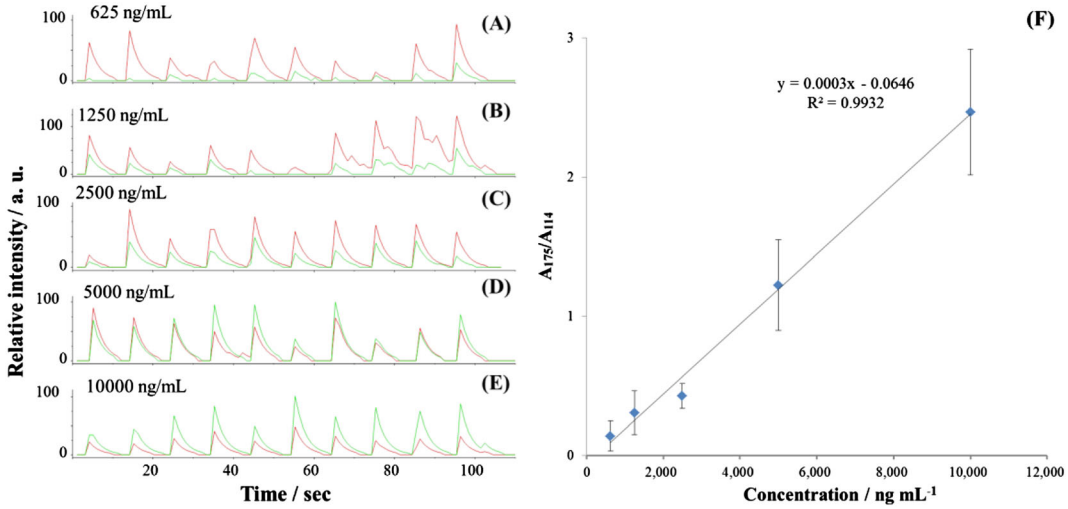

Figure 4. Extracted ion chromatograms at *m/z* 175 (arginine, green) and *m/z* 114 (creatinine, red) obtained from the samples containing arginine with the concentrations of (A) 625 ng/ml, (B) 1250 ng/ml, (C) 2500 ng/ml, (D) 5000 ng/ml and (E) 10 000 ng/ml. Each arginine sample contained creatinine (2 μg/ml), which was used as the internal standard. (F) Corresponding calibration curve obtained from the results of Panels (A–E). Ten droplets of the sample solution were dispensed from the pipette tip from every single run.

concentrations of arginine (*m/z* 175, green) spiked with a given concentration of creatinine (*m/z* 114, red). The intensity of the green peaks over that of the red peaks increased with increasing arginine concentration in the sample. Although the peak area slightly varied, the presence of an internal standard in the sample compensated for the variance. Figure 4(F) shows the resultant calibration curve based on the results obtained from Fig. 4(A)–(E). The linear regression ($R^2 \approx 0.993$) indicates a desirable linearity. The results suggest that this current approach may be potentially used in quantitative analysis.

In conclusion, we demonstrated a quite straightforward approach involving the use of a tip operated by an electronic pipette as a spray emitter without any extra accessories or electric contact. By placing the tip in front of a mass spectrometer, gas phase ions generated from the droplet that is ejected by the tip can be readily acquired by the mass spectrometer. The results showed that this approach can be used in the analysis of organic molecules, peptides and small proteins. Moreover, we demonstrated the possibility to conduct quantitative analysis by this simple setup. Desalting and concentration are possible with the use of a tip packed with stationary phase. We believe that our approach is potentially suitable in directly characterizing the eluent after treated by stationary phase-packed tips. The time spent in sample transfer and treatment can be reduced. This approach is potentially useful for quantitative analysis and quick characterization of enriched species that are eluted from stationary phase-packed tips in real time.

Acknowledgement

We thank the National Science Council of Taiwan (NSC 99-2113-M-009-003-MY3) for financial support of this research.

Yours,

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