Mass Spectrometry

DOI: 10.1002/anie.201007819

Synchronized Inductive Desorption Electrospray Ionization Mass Spectrometry**

*Guangming Huang, Guangtao Li, Jason Ducan, Zheng Ouyang, and R. Graham Cooks**

Mass spectrometry (MS) plays an important role in chemical analysis which is currently being enhanced by the increasing demand for rapid trace analysis in the areas of public safety, forensics, food safety, and pharmaceutical quality assurance, amongst others. These demands constitute an impetus to simplify MS instrumentation and methodologies. This in turn has resulted in the development of miniaturized instrumentation[1–3] and the invention of ambient ionization methods in which samples are examined without preparation in their native state.[4–12] The ambient ionization methods include spray-based,[13–20] plasma-based,[21,22] and laser-assisted methods.[23–27] Like other ambient methods, desorption electrospray ionization (DESI)[14] has the advantages of simple instrumentation, rapid and sensitive analysis, and broad applicability. In situ mass spectrometry requires both portable instruments and simple ionization/sampling methods. In our laboratory, this requirement has been met by fitting the small ion trap based Mini 10 and 11 instruments[1] with ambient ionization sources. The performance of the combined system is limited by the low pumping speed of small mass spectrometers and the large nebulizing gas and solvent volumes that must be handled. This problem was addressed by the development of the discontinuous atmospheric pressure interface (DAPI).[28,29] The DAPI interface is opened briefly to admit a bolus of ions, solvent vapor and gas, then closed while the neutrals are pumped away before the trapped ions are mass analyzed. The system operates well in spite of a duty cycle of just 1 %.[28,29]

Further improvement should be achievable by synchronization of the experiment (Figure 1) which requires the use of an inductive method to charge the primary microdroplets. This allows droplet creation to be synchronized with the opening of the sample introduction system (and also with the pulsing of the nebulizing gas). Synchronized inductive DESI shows good performance: 1) over 100-fold improvement in sensitivity (Figure 1c and 1d) while still using the 1:100 DAPI duty cycle, 2) reduced solvent spray flow rate from ca. 5 $\mu L\,min^{-1}$ to 0.5 $\mu L\,min^{-1}$, 3) reduced nebulizing gas usage from ca. 2 $L\,min^{-1}$ to 0.2 $L\,min^{-1}$, 4) improved sampling efficiency by a factor of 100, and 5) quasi-simultaneous recording of positive and negative ion spectra using a pulsed monopolar ion source.

These capabilities are based on accurate control of charged droplet creation by placing an electrode near a spray emitter (typically 2–5 mm distant) and pulsing it repetitively to high positive potentials (5–7 kV, 50–3000 Hz, pulse width 0.2–2 ms). The pulsed positive voltage was applied to a metal tube (inner diameter (i.d.) 250 μm), covering an inner silica capillary which served as the spray emitter tip (i.d. 50 μm). Electromagnetic induction produces high electrical fields in the DESI source that result in bursts of charged droplets. Precise synchronization with the DAPI interface is possible because the inductive pulsed DC high voltage has the necessary short on/off response times of ca. 1 ms (timing control data comparing inductive and conventional contact DC sprays are shown in Figure 2b and Supporting Information, Figure S1). The nebulizing gas flow was also synchronized to the MS scan function (Figure 1b). The DAPI pinch valve was opened for the first 10 ms while ions were being admitted into the MS then closed for the remainder of the scan period. Both the spray voltage and nebulizing gas were triggered on 20 ms before the pinch valve was opened, and remained open for the 10 ms ion introduction period. The spray solution flow rate was set at 0.5 $\mu L\,min^{-1}$. Other DESI conditions in the synchronized experiment remained the same as in the conventional DESI experiment (see Experimental Section and Table S1).

Figure 1c and d shows that 1 μg of cocaine is needed to record a DESI spectrum in the conventional continuous mode comparable to that given by 10 ng cocaine in the synchronized mode. This and similar results for other compounds (atenolol, methamphetamine, and morphine) indicate an approximately two orders of magnitude increase in sensitivity for synchronized DESI over conventional DESI using a miniature MS (Figure S2). In addition to the decreased detection limits, synchronized DESI also provides higher sampling efficiency. For conventional DESI, 1 μg cocaine signal lasted for ca. 15 s, while with synchronization just 10 ng of sample provides signal for the same period (Figure S3). The improvement of two orders of magnitude in sensitivity should be particularly important for samples of small size, where ionization efficiency is most important. Other improvements due to synchronization include the decreased nebulizing gas flow rate from 2.1 $L\,min^{-1}$ to 0.15 $L\,min^{-1}$ and the spray solution flow rate decrease from 5 $\mu L\,min^{-1}$ to 0.5 $\mu L\,min^{-1}$. The decreased solution flow rate is not responsible for the increased efficiency. This is shown by the fact that for conventional contact DC DESI, no signal could be seen with pulsed DC high voltages and 0.5 $\mu L\,min^{-1}$ spray flow

[*] G. Huang, G. Li, J. Ducan, Z. Ouyang, Prof. R. G. Cooks
Department of Chemistry and Center for Analytical Instrumentation Development, Purdue University
West Lafayette, IN 47907 (USA)
Fax: (+1) 765-494-9421
E-mail: cooks@purdue.edu

[**] This research was supported by Office of Naval Research, Research Tools Program (N00014-05-1-0454).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201007819.

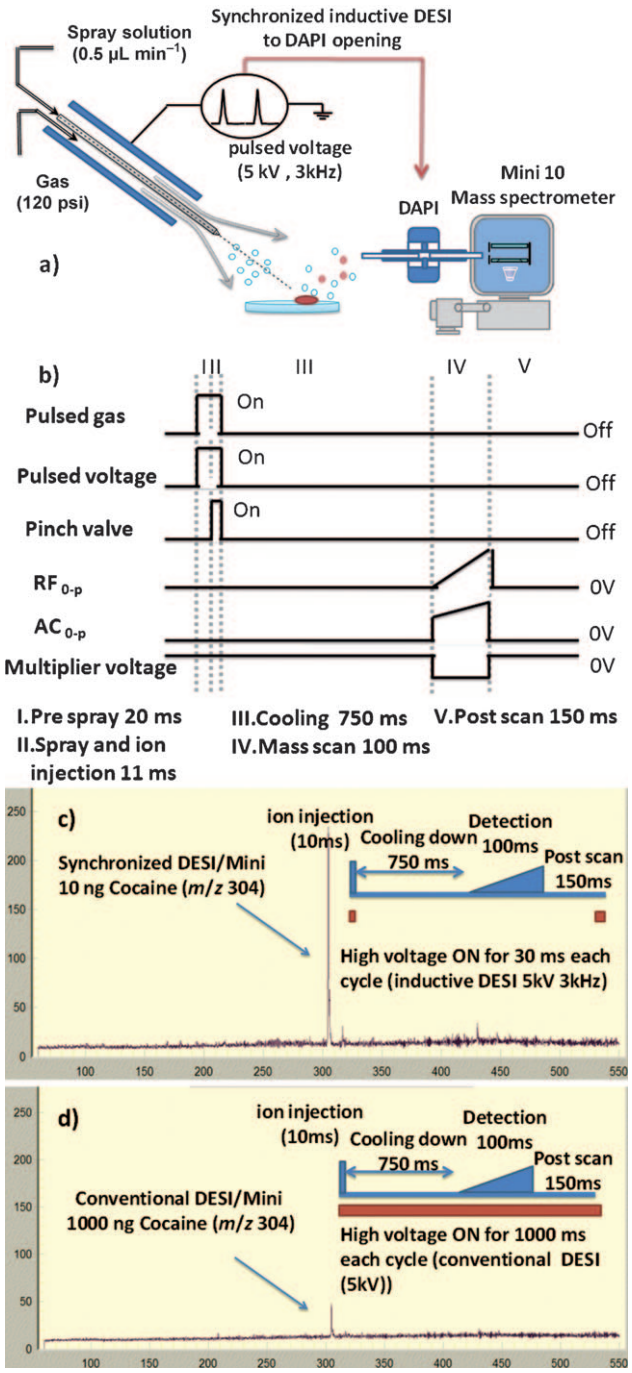

Figure 1. a) DESI experiment using a miniature MS in which charged droplet creation, nebulizing gas pulsing, and sample introduction into the MS are all synchronized. b) Pulse sequence used in synchronized experiment. c, d) Average of five DESI mass spectra recorded for cocaine on a glass substrate (spray solvent MeOH/water; 0.5 µL min^{-1}) using a mini MS interfaced to a DAPI operated at a duty cycle of 1:100. c) Synchronized DESI/DAPI-Mini experiment using 10 ng cocaine; d) conventional experiment using 1000 ng cocaine.

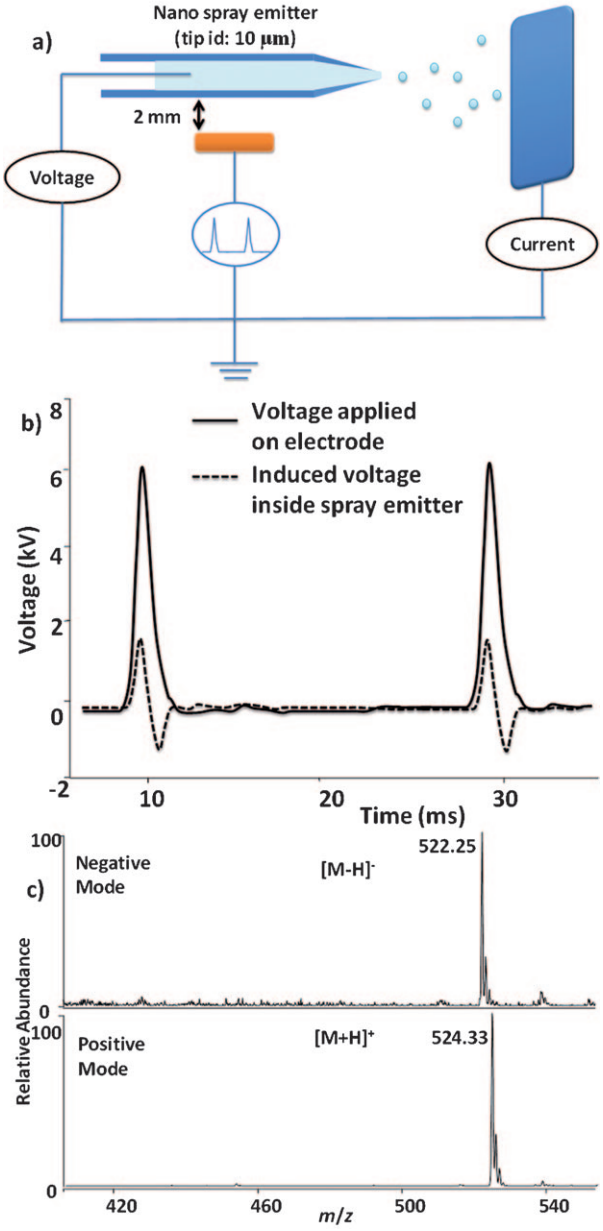

Figure 2. a) Measurement of nano sprayer voltage and current. b) Induced voltage recorded inside the DESI source when a nearby electrode voltage is pulsed. c) Synchronized DESI mass spectra of MRFA (Met-Arg-Phe-Ala) (20 ng on glass) showing both polarities recorded in successive scans made at 5 Hz without changing ion source potentials.

rates even for 50 ng cocaine using a benchtop MS instrument (i.e. without using a DAPI interface), while for inductive DESI 10 ng cocaine could be observed under the same experimental conditions (Figure S4).

As important as is the improved analytical performance, more striking are the new capabilities achieved in terms of virtually simultaneous production of ions of both positive and negative polarity from a single spray emitter without changing the polarity of the applied potential. This capability is illustrated by the spectrum obtained for the tetrapeptide MRFA (Figure 2c). The protonated molecule appears in the positive mode and the deprotonated form when the polarity of the detector is switched to negative. Detector switching can be done at 1 Hz, fast enough to record spectra of alternating polarities in successive scans. By contrast, conventional pulsed DC electrospray[30,31] provided ions with either positive or negative, but not both polarities (Figure S5). This bipolar

capability is based on the characteristics of the voltages involved in inductive DESI. The induced potential measured inside the DESI spray emitter during the synchronized experiment was found to have the same frequency as the pulsed voltage applied to the outer electrode of the source and an amplitude of 1.2–2 kV, similar to that used in the normal contact experiments (Figure 2a and b). However the induced voltage inside the emitter shows ringing with both positive and negative components and a peak-to-peak voltage of ca. 3 kV. The short pulse width of the repetitively pulsed (5–2000 Hz) positive potential applied to the outer electrode caused the induced potential to swing from high positive to high negative values in 1 ms. An apparently stable electrospray plume could be observed, indicating that the induced potential is high enough to generate an electrospray, similar to that achieved in a direct contact AC electrospray experiment.[30,31] The result is that both positive and negative ions can be observed simply by switching the polarity of the mass spectrometer, without making any ion source changes by rapidly polarizing the spray solution in opposite polarities. These new capabilities should facilitate rapid chemical identification and minimize prior sample manipulation.

Figure 3 compares the performance of conventional and synchronized nanoelectrospray coupled to a Mini 10. Similar ion intensities and signal-to-noise ratios are achieved even though synchronized spray rates are ca. 80 times lower than conventional contact DC nanoelectrospray, corresponding to an 80-fold improvement in sampling efficiency. This highlights an advantage of synchronized ESI or DESI in applications when the sample amount is limited, as in single-cell mass spectrometry. The ability to detect ions of both polarities extends to electrospray ionization. Using Ultra mark 1621 (Figure 3c) as an example, both positive and negative ions can be detected when the synchronized ESI experiment is performed on a commercial benchtop instrument. Similar results were observed for *p*-toluenesulfonic acid, propranolol, and atenolol (Figure S6). Another advantage of the fast switching of the polarity of the induced potential inside the spray emitter was the elimination of unwanted electrochemical reactions during DESI/ESI (Figure S7).

In summary, both DESI and ESI benefit in terms of improved sensitivity from controlled droplet generation which is available through the use of induced rather than directly applied potentials.[32] These advantages should also extend to other ambient ionization methods including plasma-based methods. Synchronization of droplet creation with ion transfer into a miniature mass spectrometer reduces nebulizing gas and solution flow rates by an order of magnitude, with obvious implications to improved in situ operation. Synchronized DESI also offers significant new capabilities in temporal control of ion polarity on a scan-to-scan basis with millisecond inversion of solution polarity. Recent interest in DESI measurements on the ms time scale[33] and in the study of intermediates in solution-phase reactions while sampling on the ms time scale[34] might benefit from the bipolarity and enhanced sensitivity of the present methodology.

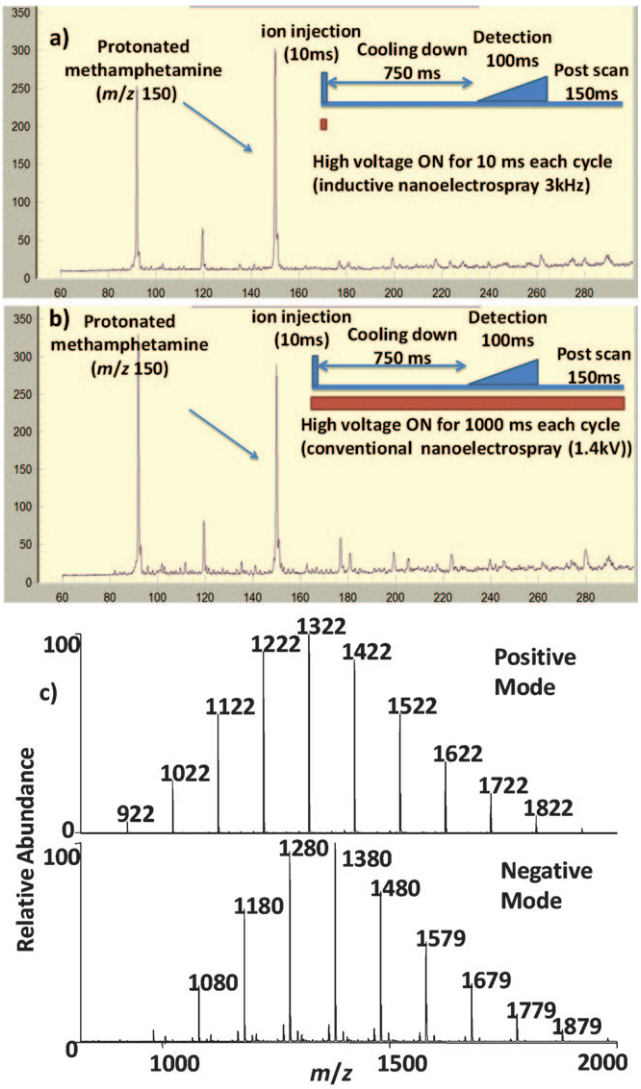

Figure 3. Nanoelectrospray using 1 μg mL^{-1} methamphetamine in MeOH/water with DAPI interface (duty cycle 1:100) on a Mini 10, averaging signal for 5 min: a) synchronized experiment (80 pL per scan, flow rate 5 nL min^{-1}) and b) conventional experiment (6.5 nL per scan, flow rate 400 nL min^{-1}). c) Synchronized electrospray MS of 100 ng mL^{-1} Ultra mark 1621 recording both polarities in successive scans without any changes in ion source potentials using benchtop MS.

Experimental Section

Experiments were carried out using a custom-built miniature mass spectrometer (Mini 10)[1] or a Thermo LTQ mass spectrometer (Thermo Scientific, San Jose, CA). Capillary temperature: 150 °C; capillary voltage: 15 V; tube lens voltage: 240 V. A custom power supply provided a pulsed output of 50–5000 Hz and 0–8 kV. DESI[14] conditions were: nitrogen gas 150 psi, a metal tube (i.d. 250 μm, 5 cm long) serves as outer electrode, an inner silica capillary serves as the spray emitter (i.d. 50 μm), angle of DESI sprayer to substrate set at 40°, distance between spray tip and sample set at 2 mm, distance between sample and MS inlet, 3 mm. The spray solution was MeOH/water (1:1 v/v). Commercial silica nanoelectrospray tips of 20 μm were obtained from New Objective (Woburn, MA, USA).

Received: December 12, 2010
Published online: February 18, 2011

Keywords: desorption electrospray ionization · in situ analysis · mass spectrometry · surface analysis · trace analysis

[1] L. Gao, Q. Y. Song, G. E. Patterson, R. G. Cooks, Z. Ouyang, *Anal. Chem.* **2006**, *78*, 5994 – 6002.
[2] I. Cotte-Rodriguez, D. R. Justes, S. C. Nanita, R. J. Noll, C. C. Mulligan, N. L. Sanders, R. G. Cooks, *Analyst* **2006**, *131*, 579 – 589.
[3] I. Cotte-Rodriguez, H. Hernandez-Soto, H. Chen, R. G. Cooks, *Anal. Chem.* **2008**, *80*, 1512 – 1519.
[4] A. Venter, M. Nefliu, R. G. Cooks, *TrAC Trends Anal. Chem.* **2008**, *27*, 284 – 290.
[5] R. G. Cooks, Z. Ouyang, R. Noll, N. Manicke, A. Costa, C. Wu, Y. Xia, A. Dill, D. Ifa, *Biopolymers* **2009**, *92*, 297 – 297.
[6] D. R. Ifa, C. P. Wu, Z. Ouyang, R. G. Cooks, *Analyst* **2010**, *135*, 669 – 681.
[7] J. Shiea, M. Z. Huang, H. J. Hsu, C. Y. Lee, C. H. Yuan, I. Beech, J. Sunner, *Rapid Commun. Mass Spectrom.* **2005**, *19*, 3701 – 3704.
[8] M. Z. Huang, C. H. Yuan, S. C. Cheng, Y. T. Cho, J. Shiea, *Annu. Rev. Anal. Chem.* **2010**, *3*, 43 – 65.
[9] H. Chen, G. Gamez, R. Zenobi, *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 1947 – 1963.
[10] W. S. Law, H. W. Chen, J. H. Ding, S. P. Yang, L. Zhu, G. Gamez, K. Chingin, Y. L. Ren, R. Zenobi, *Angew. Chem.* **2009**, *121*, 8427 – 8430; *Angew. Chem. Int. Ed.* **2009**, *48*, 8277 – 8280.
[11] K. Chingin, V. Frankevich, R. M. Balabin, K. Barylyuk, H. W. Chen, R. Wang, R. Zenobi, *Angew. Chem.* **2010**, *122*, 2408 – 2411; *Angew. Chem. Int. Ed.* **2010**, *49*, 2358 – 2361.
[12] D. J. Weston, *Analyst* **2010**, *135*, 661 – 668.
[13] R. G. Cooks, Z. Ouyang, Z. Takats, J. M. Wiseman, *Science* **2006**, *311*, 1566 – 1570.
[14] Z. Takats, J. M. Wiseman, B. Gologan, R. G. Cooks, *Science* **2004**, *306*, 471 – 473.
[15] N. Talaty, Z. Takats, R. G. Cooks, *Analyst* **2005**, *130*, 1624 – 1633.
[16] J. J. Liu, H. Wang, N. E. Manicke, J. M. Lin, R. G. Cooks, Z. Ouyang, *Anal. Chem.* **2010**, *82*, 2463 – 2471.
[17] H. Wang, J. J. Liu, R. G. Cooks, Z. Ouyang, *Angew. Chem.* **2010**, *122*, 889 – 892; *Angew. Chem. Int. Ed.* **2010**, *49*, 877 – 880.
[18] V. Kertesz, G. J. van Berkel, *Anal. Chem.* **2008**, *80*, 1027 – 1032.
[19] V. Kertesz, G. J. Van Berkel, M. Vavrek, K. A. Koeplinger, B. B. Schneider, T. R. Covey, *Anal. Chem.* **2008**, *80*, 5168 – 5177.
[20] M. S. Bereman, D. C. Muddiman, *J. Am. Soc. Mass Spectrom.* **2007**, *18*, 1093 – 1096.
[21] R. B. Cody, J. A. Laramee, H. D. Durst, *Anal. Chem.* **2005**, *77*, 2297 – 2302.
[22] E. Block, A. J. Dane, S. Thomas, R. B. Cody, *J. Agric. Food Chem.* **2010**, *58*, 4617 – 4625.
[23] J. J. Brady, E. J. Judge, R. J. Levis, *Rapid Commun. Mass Spectrom.* **2010**, *24*, 1659 – 1664.
[24] E. J. Judge, J. J. Brady, D. Dalton, R. J. Levis, *Anal. Chem.* **2010**, *82*, 3231 – 3238.
[25] P. Nemes, A. Vertes, *Anal. Chem.* **2007**, *79*, 8098 – 8106.
[26] P. Nemes, A. A. Barton, Y. Li, A. Vertes, *Anal. Chem.* **2008**, *80*, 4575 – 4582.
[27] P. Nemes, A. A. Barton, A. Vertes, *Anal. Chem.* **2009**, *81*, 6668 – 6675.
[28] L. Gao, R. G. Cooks, Z. Ouyang, *Anal. Chem.* **2008**, *80*, 4026 – 4032.
[29] L. Gao, G. T. Li, Z. X. Nie, J. Duncan, Z. Ouyang, R. G. Cooks, *Int. J. Mass Spectrom.* **2009**, *283*, 30 – 34.
[30] S. Maheshwari, H. C. Chang, *Appl. Phys. Lett.* **2006**, *89*, 234103.
[31] N. Chetwani, C. A. Cassou, D. B. Go, H. C. Chang, *J. Am. Soc. Mass Spectrom.* **2010**, *21*, 1852 – 1856.
[32] T. Tu, A. D. Sauter, M. L. Gross, *J. Am. Soc. Mass Spectrom.* **2008**, *19*, 1086 – 1090.
[33] G. K. Barbula, M. D. Robbins, O. K. Yoon, I. Zuleta, R. N. Zare, *Anal. Chem.* **2009**, *81*, 9035 – 9040.
[34] R. H. Perry, M. Splendore, A. Chien, N. K. Davis, R. N. Zare, *Angew. Chem.* **2010**, *123*, 264– 268; *Angew. Chem. Int. Ed.* **2010**, *50*, 250 – 254.

