

Development of a Mass Spectrometry Sampling Probe for Chemical Analysis in Surgical and Endoscopic Procedures

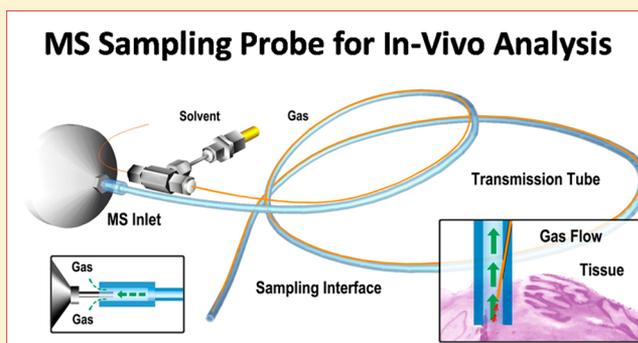
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ABSTRACT: A sampling probe based on ambient desorption ionization was designed for *in vivo* chemical analysis by mass spectrometry in surgical and endoscopic procedures. Sampling ionization of analytes directly from tissue was achieved by sealing the sampling tip against the tissue surface without allowing leakage of the auxiliary gas used for desorption ionization. The desorbed charged species were transferred over a long distance (up to 4 m) through a flexible tube of internal diameter as small as 1/16 in. to the inlet of the mass spectrometer used for analysis. The conditions used for desorption electrospray ionization (DESI) were optimized to achieve biocompatibility for clinical applications while obtaining adequate efficiency for the analysis. This optimization involved the removal of high voltage and use of pure water as a spray solvent instead of the organic solvents or aqueous mixtures normally used. Improved sensitivity was achieved under these conditions by increasing the gas flow rate in the transfer tube. The destructive effect on tissue surfaces associated with typical desorption ionization was avoided by altering the local gas dynamics in the sample area without compromising the overall analysis efficiency.



Mass spectrometry (MS) is a powerful tool for general purpose analysis of complex mixtures at high sensitivity and selectivity. Routine analytical procedures using mass spectrometry require sample preparation and chromatographic separation^{1,2} although direct analysis of complex mixtures is possible using tandem mass spectrometry provided that the ionization process is soft.^{3–5} The recently developed ambient ionization methods allow direct ionization of complex chemical and biological samples in their native state.^{6–14} Ambient ionization MS has been shown to provide adequate sensitivity and to be compatible with MS/MS for the identification of mixture components.^{7,15–20} High precision in quantitation has been shown recently using the ambient ionization method of paper spray.^{18,21}

For MS imaging of tissue samples, very limited sample preparation is possible both from a perspective of access to the tissue and also so as to preserve the original distribution of the analytes in the sample.^{22–25} In future planned applications to diagnostics during surgery, only a limited amount of time is available for sample manipulation.^{22–25} These considerations and the desire to obtain chemical information at localized positions on the surface of an organ mean that ambient ionization has particular advantages for imaging tissue, at atmospheric pressure without pretreatment and on a dimensional scale compatible with surgery.

Though MS imaging is being developed as an important tool for drug discovery,^{26,27} biomarker discovery,²⁸ and disease diagnosis,^{29–31} quick tissue analysis^{32–35} or profiling^{36,37} with the ambient ionization method can also play an important role in biomedicine to provide highly specific chemical information of the sample in a timely fashion.³⁸ As demonstrated by prior experiments using desorption electrospray ionization on excised tissue sections³⁹ and by results from the rapid evaporative ionization mass spectrometry (REI-MS) method,^{40,41} ambient ionization can be effectively used for real-time chemical analysis during surgery. In this study, we explored the development of a sampling/ionization probe using a modified form of desorption electrospray ionization (DESI) for surgical and endoscopic procedures. Previous studies on small sample sets of human liver,³⁶ bladder,⁴² kidney,⁴³ prostate,²⁸ testicular,⁴⁴ and brain^{29,45} cancers performed using DESI-MS imaging show correlations of lipid distributions with pathology. A mass spectrometry sampling probe could enable an *in vivo* tissue characterization to facilitate the diagnosis as well as the decision making during an open or laparoscopic surgery and the endoscopic procedures. Colon rectal (CR) cancer is the fourth leading cause of cancer morbidity in the U.S., and early

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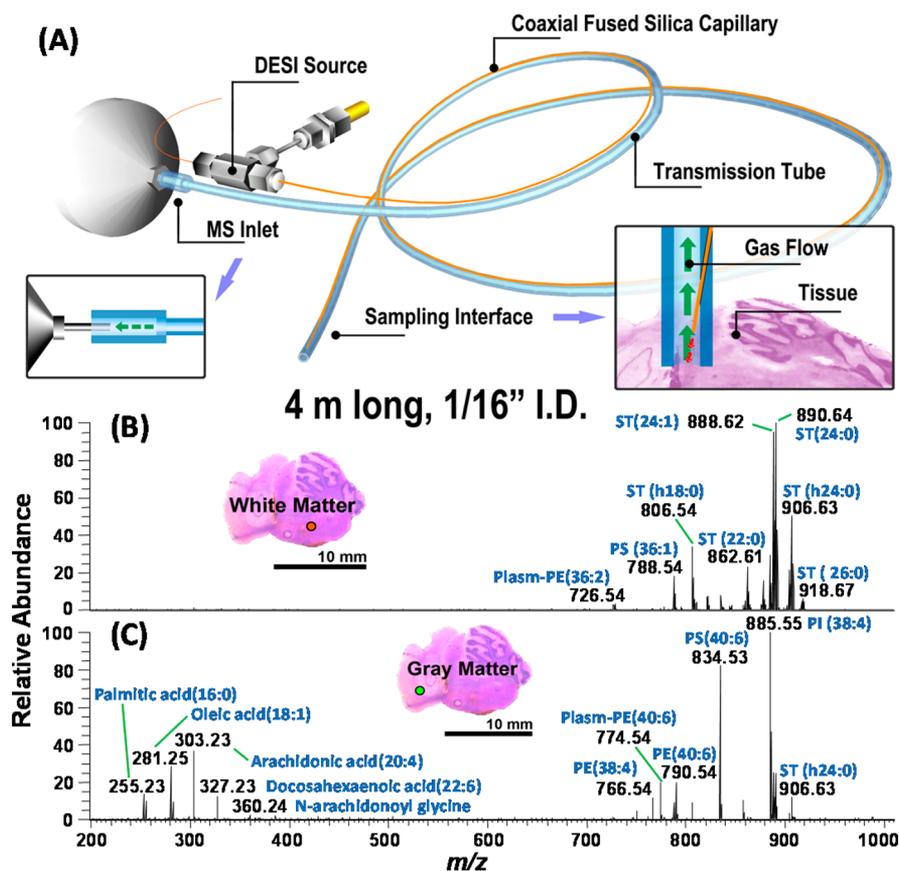


Figure 1. (a) Schematics of an endoscopic sampling ionization probe which was composed of a coaxial capillary sprayer and a transfer tube. A probe with 4-m-long, 1/16" i.d. tubing was used for the analysis of the rat brain tissue section, with the spectra recorded for (b) the white matter and (c) the gray matter. Gas flow rate of 4.3 L/min, high voltage at -4.5 kV, methanol/water 1:1 as spray solvent.

diagnosis is essential for successful treatment. *In vivo* chemical analysis could provide important information on the cause of the colon inflammation^{46,47} and subsequent colon cancer.^{48–51} To use such as sampling probe, the analytes on tissue surfaces need to be ionized and transferred over several meters in tubes thin enough to insert through probes for laparoscopic or endoscopic procedures. The main concerns relating to the design of an ambient ionization endoscopic probe are the sensitivity for the analysis and the safety of the operation. In the REIMS method, the analytes are evaporated by the surgical tools, transferred to the vicinity of the MS inlet using a gas flow, and ionized in the course of analysis. In our design, the desorption ionization event occurs at the sample surface. It has been shown previously that the species ionized by DESI can survive a long distance transfer with the gas flow from a DESI source.^{52,53} DESI has been shown to be effective in desorption ionization of nonvolatile organic compounds and biomolecules directly from tissue samples.³⁶ High voltage, high velocity gas flow, and organic solvents have been used to facilitate the desorption ionization at high efficiency;¹⁶ however, these conditions are not compatible with the safety requirement for clinical *in vivo* endoscopic operations. Recently, an *n,n*-dimethylformamide (DMF)/ethanol solvent system has been used for DESI to minimize the damage of the tissue,⁵⁴ which allows the same tissue sample to be used for other imaging procedures. Although these and other morphologically friendly solvents are still not biocompatible with *in vivo* applications,^{55,56} this approach is insightful to show that DESI

conditions could be varied while preserving the ionization efficiency.

In the course of this work, a probe with an inside diameter as small as 1/16 in. (outside diameter 1/8 in.) was developed in conjunction with a desorption ionization source. This probe easily fits into an endoscopic tube. The conditions for performing DESI were varied systematically and optimized to improve safety during *in vivo* operation while retaining the necessary sensitivity for chemical analysis.

EXPERIMENTAL SECTION

Rat brain tissue sections (thickness = 10 μm) were sliced by a microtome inside a freezer and put on glass slides. *E. coli* polar lipid extract was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Tygon tubings (R-3603) were purchased from the VWR Scientific (San Francisco, CA). Intact rat kidneys and intestine were taken after the sacrifice of rat. The intestine was cut open so the mucosal surface could be analyzed. Solvents used in the experiments include pure water (D.I. water from Milli-pore Milli Q system), methanol/water (1:1), and pure methanol (Mallinckrodt Baker, Inc., Phillipsburg, NJ).

Exacte Orbitrap and LTQ linear ion trap mass spectrometers (Thermo Scientific Inc., San Jose, CA) were used for mass analysis. The original heated capillary was replaced by an extended capillary. A diaphragm pump (four-stage diaphragm pump N813.4 from KNF Inc., free flow rate = 13 L/min) was connected to the back end of the Tygon tubing when operating in intact organ analysis. A vacuum gauge (series 925C micropirani transducer) which offers a measurement

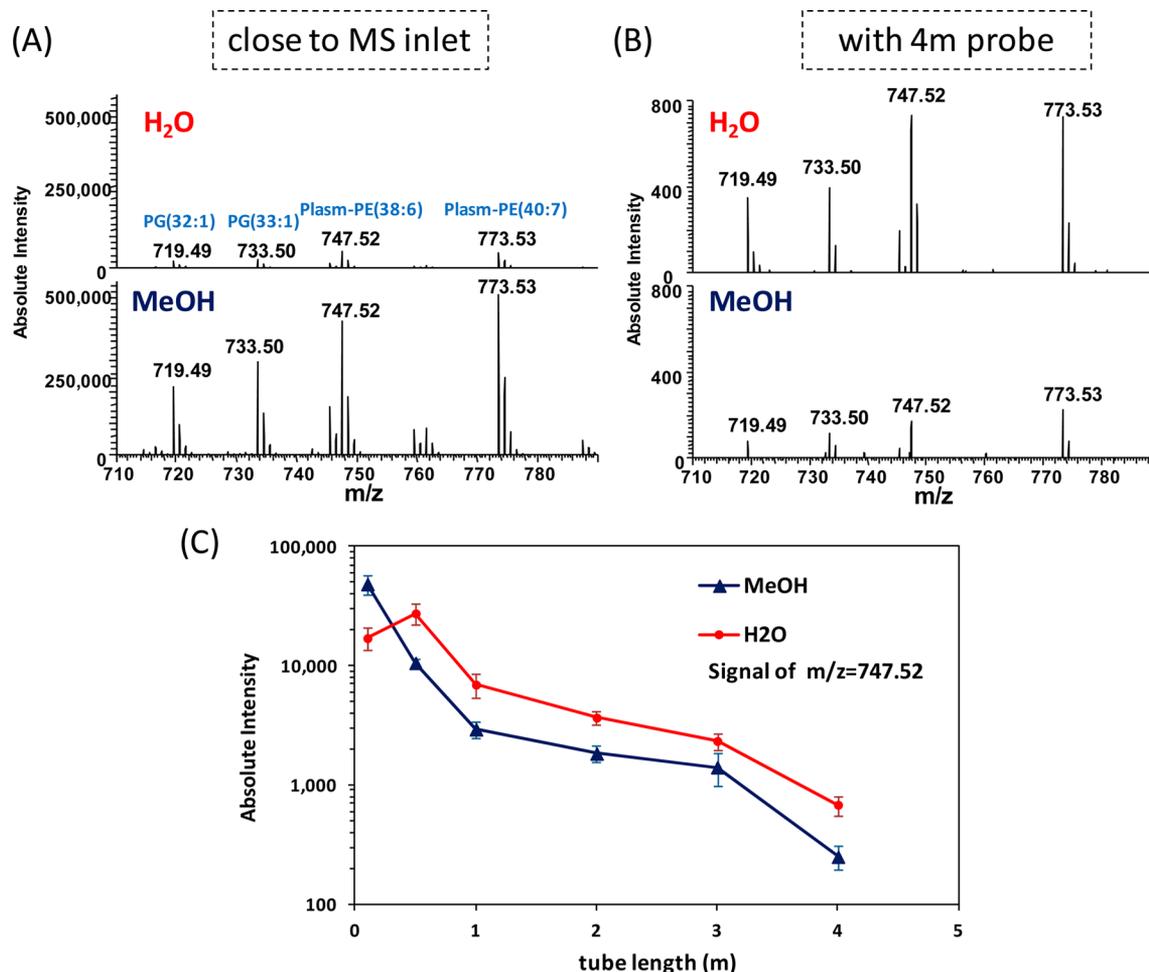


Figure 2. DESI analysis of 0.5 μg polar lipid extract deposited on the Teflon slides using pure water or methanol as the spray solvent. (a) Analysis with DESI performed close to the MS inlet, gas flow rate of 1.3 L/min, solvent flow rate of 3 $\mu\text{L}/\text{min}$. (b) Analysis with 4 m probe, gas flow rate at 4.3 L/min, solvent flow rate at 8 $\mu\text{L}/\text{min}$. (c) Signal intensity of plasma-PE (38:6; $m/z = 747.52$) recorded with probes of different tube lengths from 0.1 to 4.0 m.

range from 10^{-5} Torr to atmospheric was used here to measure the pressure at the end of the tubing.

RESULTS AND DISCUSSIONS

The design of the endoscopic probe is shown in Figure 1a. Tygon tubing of 1/8" (3.17 mm) o.d. and 1/16" (1.59 mm) i.d. and with a length of up to 4.0 m was used to transfer the ions from the sample to the mass spectrometer. The internal diameter of the working channel can be as large as 5 mm⁵⁷ for a laparoscope and 3.7 mm for a colonoscope.⁵⁸ For real clinical applications, custom-designed tubing with proper outside diameter needs to be made for specific applications. Tygon tubings used in this study for proof-of-concept demonstration were made from the nonconductive material Tygon-3603,⁵³ which was chosen for the design due to the good flexibility, softness, and chemical resistance of the material. A coaxial fused silica capillary sprayer was made by inserting a capillary of 50 μm i.d. and 150 μm o.d. into a capillary of 530 μm i.d. and 700 μm o.d. The inner capillary was used to deliver the solvent, while the outer capillary was used for the auxiliary nitrogen gas flow. The front end of the capillary sprayer was inserted through the wall of the Tygon tubing as shown in the inset of Figure 1A. During the sampling for analysis, the end of the Tygon tubing was pushed against the sample surface, with ~3

mm between the sprayer and the surface of the sample. When pushed against the sample surface, the soft edge of the Tygon tubing sealed the surface. The spray solvent and the auxiliary gas delivered at 4–8 $\mu\text{L}/\text{min}$ and 1.5–5.2 L/min, respectively, were contained inside the tubing without leaking. This is important for a real operation with an endoscopic probe.

It has been previously demonstrated that the analytes on the surface can be sampled and ionized by DESI and efficiently transferred by the gas flow from the DESI source through flexible bent tubing.^{52,53} The transfer efficiency of the ionic species is dependent on the speeds of transfer toward the MS inlet and the radial diffusion toward the inside wall of the tube.⁵³ With a much smaller 1/16" i.d. of the tubing used for the design of the endoscopic sampling probe, a low transfer efficiency potentially due to the diffusion of the ions to the tube wall was a significant concern. In an initial test, the typical conditions for DESI, viz. a high gas flow rate at 4.3 L/min, methanol/water (1:1) solvent as spray solution, and a high voltage of -4.5 kV, were applied to test the efficiency of the desorption and the transfer over a long distance using the Tygon tubing. The sampling probe was coupled with the MS inlet simply using short Tygon tubing of 1/8" i.d. and ~2 cm in length (Figure 1A). The MS capillary inlet was located at the center of the opening of the coupling Tygon tubing. The gas

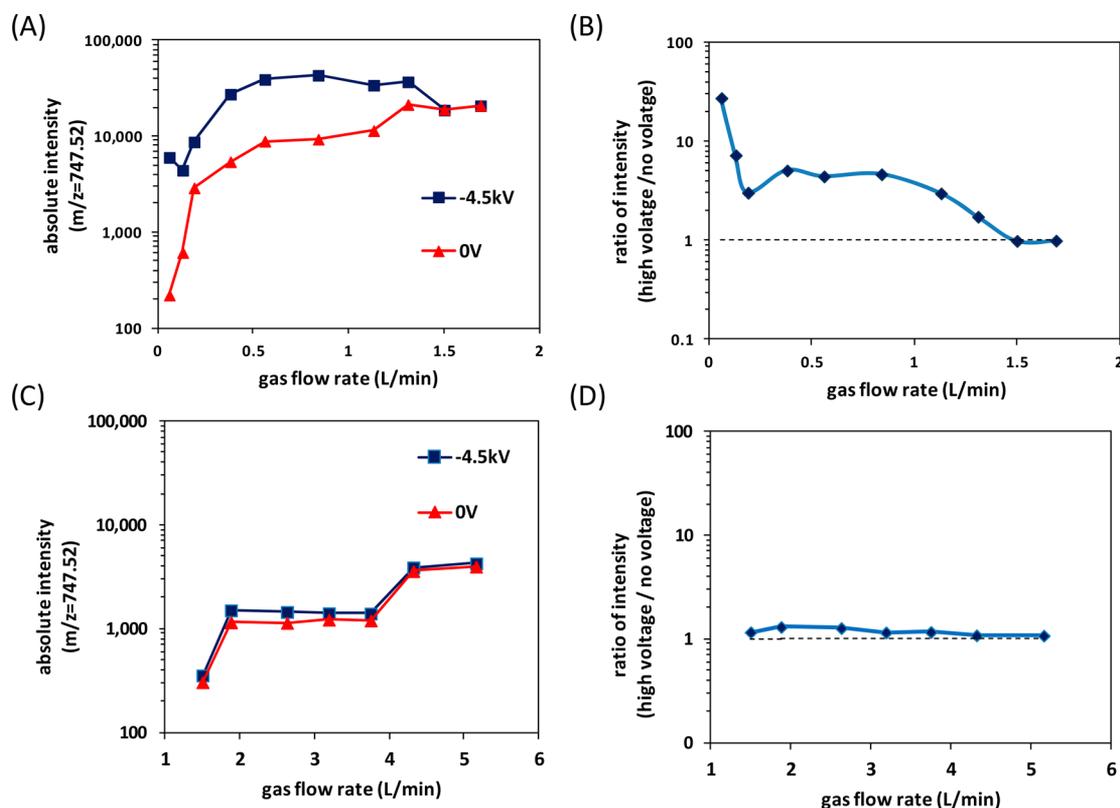


Figure 3. (a) Intensities and (b) ratio of the intensities of plasma-PE (38:6; $m/z = 747.52$) recorded as a function of gas flow rate with or without high voltage, desorption ionization performed close to the MS inlet. (c) Intensities and (d) ratio of the intensities of plasma-E (38:6; $m/z = 747.52$) recorded as a function of gas flow rate with or without high voltage, a sampling probe of 1 m used for the analysis.

flow from the sprayer was also used for ion transfer and allowed to exhaust at the end of the coupling Tygon tubing.

Probes of different lengths were made and tested for analyzing rat brain tissue sections. Surprisingly, excellent signals were obtained from lipids and fatty acids with a probe length of 4 m, which is sufficiently long for an endoscopic probe. The time delay between pushing the sampling probe against the tissue section surface and obtaining the signals was about 0.5 s. The mass spectra of white and gray matter are shown in Figure 1b and c. The profiles of lipids and fatty acids observed are pretty similar to those previously reported for DESI analysis.²² The fact that the ions survive long distance transfer through 1/16" i.d tubing supports the hypothesis previously revealed that the ions might be continuously generated from the charged droplets during the transfer.⁵³ This also indicates that ambient methods based on droplet extraction might be more suitable for the design of endoscopic probes for MS analysis. Although adequate transfer efficiency was obtained for the analyte ions from the sample, the conditions for desorption ionization must be altered to become compatible with the safety requirement for the endoscopic operation. A series of studies were done to characterize the roles of the organic solvent, high electric voltage, and the gas flow rate, on the basis of which the alternative conditions were suggested and tried experimentally.

Ideally, the methanol/water solvent should be replaced by pure water as the spray solvent. It is known from the studies of the spray-based ionization methods that the addition of methanol in the spray solvents helps the formation of smaller droplets during the spray and the subsequent desolvation of the analyte ions.⁵⁹ In previously studies, it has been shown that DESI analysis with methanol/water provides significantly

higher analyte signals than with pure water.⁵⁹ The results of a comparison study using the analysis of 0.5 μg of a lipid extract sample on a Teflon slide are shown in Figure 2. A high voltage of -4.5 kV was used, and the rates for the solvent and gas flow were optimized to get the maximum signals in each case. The signals of plasma-PE (38:6; $m/z = 747.52$) were three times higher with pure methanol than with pure water, when the DESI was performed at the MS inlet (Figure 2a); however, the relative intensities were reversed in the case with a 4-m-long probe (Figure 2b). The signals observed with pure water were three times higher than those with pure methanol.

To better understand this phenomenon, probes of different lengths were used for this comparison study. As shown in Figure 2c, the signal of plasma-PE (38:6) with pure methanol as a spray solvent has a monotonic decreasing trend as a function of the probe length. For pure water as the spray solvent, the signal increased when the desorption ionization occurred 50 cm away from the MS inlet. Although the signal intensity also decreased with longer probes, overall the DESI with pure water had better performance with probes of long lengths. This observation could be explained with the desolvation of the spray droplets and formation of the analyte ions with DESI. Relatively larger primary droplets with water as a DESI spray solvent might cause an inefficient desolvation in the formation of secondary dry ions for MS analysis, when DESI is performed close to the MS inlet. However, when the droplets containing the analyte ions are transferred over long distance, better desolvation could be achieved through collisions with gas molecules during the transfer, and the gradual desolvation might also help to protect the ions from losing charges through reactions. This leads to an overall

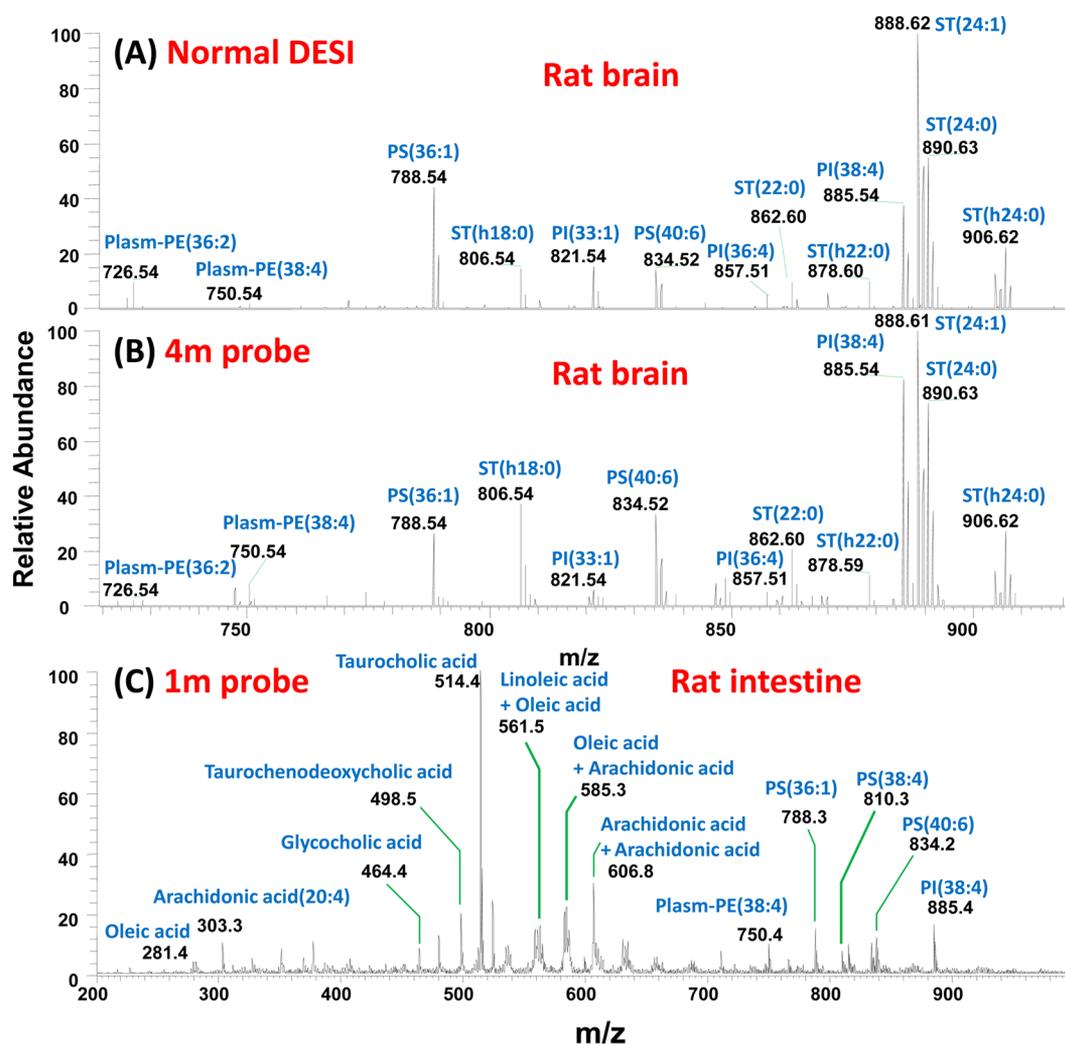


Figure 4. Analysis of rat brain tissue sections using (a) a DESI close to the MS inlet with MeOH/H₂O (1:1) as spray solvent and a high voltage of -4.5 kV and (b) a 4 m probe with water as spray solvent and no high voltage. (c) Analysis of rat intestine using a 1 m probe with water as spray solvent and no high voltage. Gas flow rate, 5.2 L/min for 4 m and 1 m probes and 1.5 L/min for DESI.

advantage of using water instead of methanol as spray solution for DESI in the design of an endoscopic probe.

The effectiveness of the high voltage for the spray in the DESI MS analysis has been characterized previously,¹⁶ while desorption ionization also using spray but without applying high voltage, such as easy ambient sonic-spray ionization (EASI), has also been shown to have high efficiency.^{60,61} The efficiency in generation of the secondary dry ions after desorption is dependent on the size and the charge density of the primary droplets, which are subjected to the spray conditions including the voltage and the gas flow speed. A comparison study was first done with the desorption spray source close to the MS inlet without transferring with Tygon tubing, where 0 V or -4.5 kV was used for spray while the gas flow rate was varied up to 1.75 L/min. The sample of 0.5 μ g of lipid extract on a Teflon slide was used for the analysis. Methanol/water (1:1) was used as the spray solvent. The intensity of plasma-PE (38:6; $m/z = 747.52$) was monitored as a function of the gas flow rate. As shown in Figure 3a and b, at a low gas flow rate, the desorption spray ionization benefits significantly (30 times higher) from the application of a high voltage. However, the impact by the gas flow is much more significant when there is no voltage applied for the spray. At a

flow rate of 1.5 L/min or higher, there is no significant difference in desorption ionization efficiency between applying a high voltage for spray or not. This is consistent with the findings in previous studies involving sonic gas flows⁶² where high velocity gas flows were found to be helpful to improving the ionization efficiency.

Ideally, the use of higher gas flow rates would lead to further increase the desorption ionization efficiency; however, practically this was difficult to achieve for desorption ionization performed close to the MS inlet, since the dispersion of secondary ionic species from the sample surface becomes severe and results in a poorer sampling by the MS inlet. Using a sample probe with its end sealed with the sample surface, this is not a concern since the gas containing the ions is forced toward the MS inlet. As shown in Figure 3c, the signal for the analysis with a 1 m probe could be further improved by 1 order of magnitude when the gas flow increased from 1.5 to 5.5 L/min. There is also no difference for applying a high voltage or not for the spray (Figure 3d).

With the understanding of each role played by the spray solvent, spray voltage, and the gas flow in the desorption spray ionization, a 4 m sampling probe operated with pure water, no spray voltage, and at a gas flow rate of 5.2 L/min was used in a

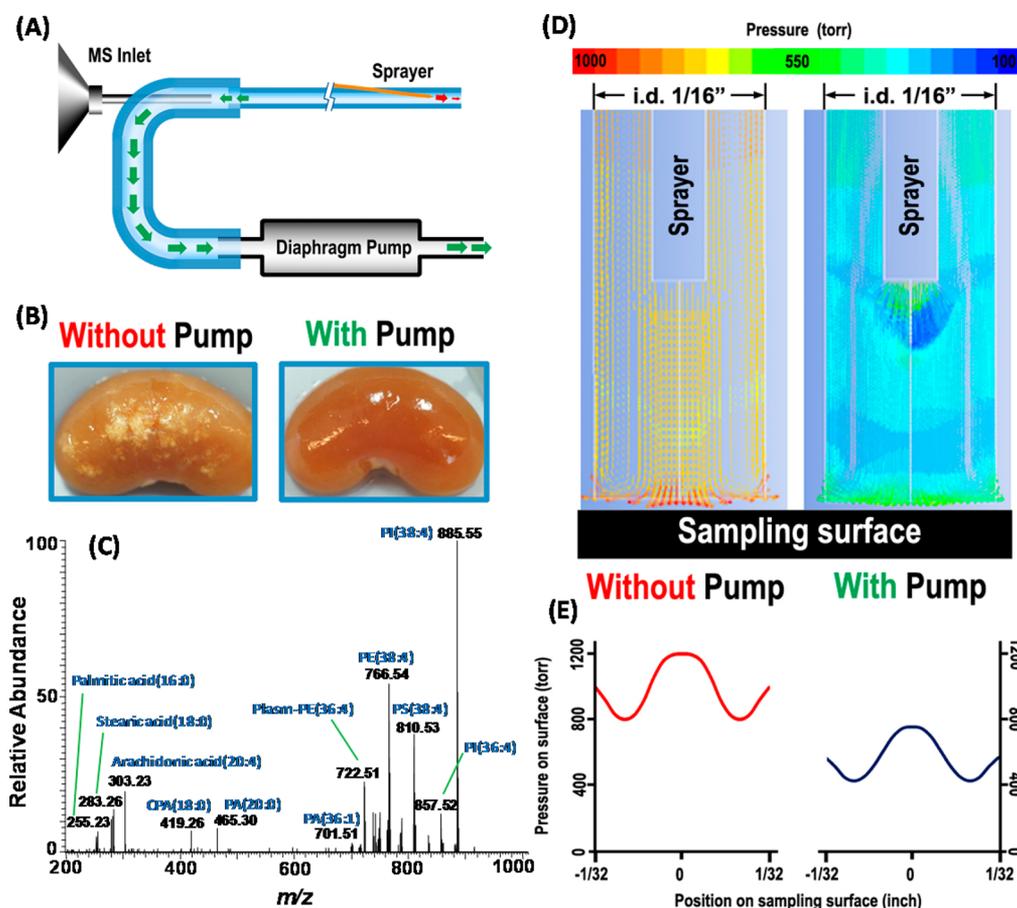


Figure 5. (a) Noninvasive sampling probe with the gas flow pulled by a diaphragm pump, 1-m-long 1/16" i.d. Tygon tubing, pure water as spray solvent, spray voltage of 0 V, gas flow rate from the sprayer at 3.8 L/min. (b) Comparison of the surfaces of rat kidneys after sampling without (left) and with (right) the diaphragm pump. (d) Contour maps with streamlines simulated for sampling without (left) and with (right) the diaphragm pump. (e) Pressure distribution along the radius on the sampled surface for sampling without (left) and with (right) the diaphragm pump.

performance comparison with DESI close to the MS inlet under a typical optimized condition (Figure 4a and b). They were both tested for analysis of rat brain tissue sections. The quality of the spectrum compares well for these two methods in terms of the signal intensity and the species identifiable with the spectrum. As a test for potential applications with endoscopic diagnosis, analysis of the mucosal surface inside the fresh rat intestine was performed using a 1-m-long probe under the biocompatible conditions for desorption ionization. Besides the fatty acids and the lipids, peaks at strong intensities were observed for the bile acids and dimers of the fatty acids (Figure 4c). The potential carryover between analyses was also characterized by moving the sampling probe between the surfaces of the intestine mucosa and a latex glove. It was found that the signals due to the chemicals on the previous sample disappeared completely 4.5 s after the probe was moved to a new surface.

Though efficient analysis using the sampling probe could now be performed without organic solvents or high voltage, which is harmful for in vivo endoscopic analysis, the damage to the tissues by the gas flow still needs to be addressed. The previously described method of using special organic solvents for DESI to minimize the tissue damage is not a solution for the design of this endoscopic sampling probe. Organic solvents generally are not biologically friendly, and the high gas flow is recognized to be important for eliminating the high spray voltage. In addition to its role in the droplet generation and ion

desolvation, the gas flow at a higher rate also helps to improve the efficiency in transfer of the ions, either as dry ions, partially solvated, or contained in the charged droplets. The gas flow is aiming at the sample surface and pushes back toward the MS inlet, which inevitably results in a worse impact by the gas molecules and the droplets to the tissue at a higher gas flow rate. To make the in vivo analysis minimally invasive with the sampling probe, this issue has to be addressed while retaining good sensitivity for the analysis.

A modification to the coupling of the sampling probe to the MS inlet was developed as shown in Figure 5a. A diaphragm pump was used to add a pulling force to drag the gas toward the MS inlet. This revision in the coupling has been found to be significant in terms of preventing damage to the sample surface. In a test with analysis of fresh rat kidneys, no visible damage was observed using the probe with the pump (Figure 5b), while spectra with good signals of the analytes were recorded with water and no high voltage applied for spray (Figure 5c). In comparison, marks were made on the kidney surface due to the damage during the sampling ionization when the probe was used without the pump (Figure 5b). The pressure inside the probe was found to be significantly changed with the pump. A vacuum gauge connected to the adapter between the end of the tubing and the MS inlet and the local pressure was measured to be 315 Torr with the diaphragm pump on. Although a 3.8 L/min gas flow rate was used, the pressure inside the probe was lower than the atmospheric pressure. This resulted in a gentle

suction that helped to form a sealing of the probe end to the sample surface. Although the Tygon material is not as soft as others such as silicone, the kidney tissue is soft, and a good sealing was easily achieved without carefully positioning the probe end. Simulations of the front end of the probe were done by ANSYS software as shown in Figure 5d to help better understand the experimental observations. The local gas dynamics in the sampling region are changed by the pulling force added with the diaphragm pump. As shown in Figure 5e, the average pressure at the sample surface is about 1000 Torr without the pump but is reduced to about 600 Torr with the pump, which results in a significantly reduced impact by the gas molecules and the droplets onto the sample surface.

CONCLUSIONS

An attempt has been made to design a sampling probe based on ambient ionization that potentially can be used for endoscopic analysis. Derived from the DESI and the long distance ion transfer method previously studied, the individual roles and overall impacts by applying the electric voltage, spray solvent, and gas flow were investigated, which led to the development of performing the sampling analysis in a biocompatible fashion. This work along with other efforts in this field shows the potential in the combination of the direct sampling analysis using ambient ionization and the gas flow assisted ion transfer. To be used in clinical diagnosis, much further development is needed to convert this probe to actual devices, and operation procedures, including cleaning the sample surfaces, need to be developed.

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Chace, D. H. *Chem. Rev.* **2001**, *101*, 445–477.
- (2) Domon, B.; Aebersold, R. *Science* **2006**, *312*, 212–217.
- (3) Kondrat, R. W.; McClusky, G. A.; Cooks, R. G. *Anal. Chem.* **1978**, *50*, 2017–2021.
- (4) Cooks, R. G.; Busch, K. L.; Glish, G. L. *Science* **1983**, *222*, 273–291.
- (5) Busch, K. L.; Glish, G. L.; McLuckey, S. A. *Mass Spectrometry: Techniques and Applications of Tandem Mass Spectrometry*; Wiley-VCH Verlag GmbH: Weinheim, Germany, 1989.
- (6) Cooks, R. G.; Ouyang, Z.; Takats, Z.; Wiseman, J. M. *Science* **2006**, *311*, 1566–1570.
- (7) Huang, M. Z.; Cheng, S. C.; Cho, Y. T.; Shiea, J. *Anal. Chim. Acta* **2011**, *702*, 1–15.
- (8) Weston, D. J. *Analyst* **2010**, *135*, 661–668.
- (9) Chen, H. W.; Zenobi, R. *Nat. Protoc.* **2008**, *3*, 1467–1475.
- (10) Miao, Z. X.; Chen, H. *J. Am. Soc. Mass Spectrom.* **2009**, *20*, 10–19.

- (11) Chen, H.; Yang, S.; Wortmann, A.; Zenobi, R. *Angew. Chem., Int. Ed.* **2007**, *46*, 7591–7594.
- (12) Shiea, J.; Huang, M. Z.; Hsu, H. J.; Lee, C. Y.; Yuan, C. H.; Beech, I.; Sunner, J. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 3701–3704.
- (13) Ifa, D. R.; Wu, C. P.; Ouyang, Z.; Cooks, R. G. *Analyst* **2010**, *135*, 669–681.
- (14) Monge, E. M.; Harris, G. A.; Dwivedi, P.; Fernandez, F. M. *Chem. Rev.* **2013**, *113*, 2269–2308.
- (15) Liu, J. J.; Wang, H.; Manicke, N. E.; Lin, J. M.; Cooks, R. G.; Ouyang, Z. *Anal. Chem.* **2010**, *82*, 2463–2471.
- (16) Takats, Z.; Wiseman, J. M.; Cooks, R. G. *J. Mass Spectrom.* **2005**, *40*, 1261–1275.
- (17) Garcia-Reyes, J. F.; Harper, J. D.; Salazar, G. A.; Charipar, N. A.; Ouyang, Z.; Cooks, R. G. *Anal. Chem.* **2011**, *83*, 1084–1092.
- (18) Wang, H.; Liu, J. J.; Cooks, R. G.; Ouyang, Z. *Angew. Chem., Int. Ed.* **2010**, *49*, 877–880.
- (19) Huang, G. M.; Xu, W.; Visbal-Onufrak, M. A.; Ouyang, Z.; Cooks, R. G. *Analyst* **2010**, *135*, 705–711.
- (20) Garcia-Reyes, J. F.; Mazzoti, F.; Harper, J. D.; Charipar, N. A.; Oradu, S.; Ouyang, Z.; Sindona, G.; Cooks, R. G. *Rapid Commun. Mass Spectrom.* **2009**, *23*, 3057–3062.
- (21) Manicke, N. E.; Abu-Rabie, P.; Spooner, N.; Ouyang, Z.; Cooks, R. G. *J. Am. Soc. Mass Spectrom.* **2011**, *22*, 1501–1507.
- (22) Wiseman, J. M.; Ifa, D. R.; Song, Q. Y.; Cooks, R. G. *Angew. Chem., Int. Ed.* **2006**, *45*, 7188–7192.
- (23) Laskin, J.; Heath, B. S.; Roach, P. J.; Cazares, L.; Semmes, O. J. *Anal. Chem.* **2012**, *84*, 141–148.
- (24) Nemes, P.; Woods, A. S.; Vertes, A. *Anal. Chem.* **2010**, *82*, 982–988.
- (25) Huang, M. Z.; Cheng, S. C.; Jhang, S. S.; Chou, C. C.; Cheng, C. N.; Shiea, J.; Popov, I. A.; Nikolaev, E. N. *Int. J. Mass Spectrom.* **2012**, *325*, 172–182.
- (26) Wiseman, J. M.; Ifa, D. R.; Zhu, Y. X.; Kissinger, C. B.; Manicke, N. E.; Kissinger, P. T.; Cooks, R. G. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 18120–18125.
- (27) Lanekoff, I.; Thomas, M.; Carson, J. P.; Smith, J. N.; Timchalk, C.; Laskin, J. *Anal. Chem.* **2013**, *85*, 882–889.
- (28) Eberlin, L. S.; Dill, A. L.; Costa, A. B.; Ifa, D. R.; Cheng, L.; Masterson, T.; Koch, M.; Ratliff, T. L.; Cooks, R. G. *Anal. Chem.* **2010**, *82*, 3430–3434.
- (29) Eberlin, L. S.; Dill, A. L.; Golby, A. J.; Ligon, K. L.; Wiseman, J. M.; Cooks, R. G.; Agar, N. Y. R. *Angew. Chem., Int. Ed.* **2010**, *49*, 5953–5956.
- (30) Gerbig, S.; Golf, O.; Balog, J.; Denes, J.; Baranyai, Z.; Zarand, A.; Raso, E.; Timar, J.; Takats, Z. *Anal. Bioanal. Chem.* **2012**, *403*, 2315–2325.
- (31) Schafer, K. C.; Balog, J.; Szaniszló, T.; Szalay, D.; Mezey, G.; Denes, J.; Bogner, L.; Oertel, M.; Takats, Z. *Anal. Chem.* **2011**, *83*, 7729–7735.
- (32) Wang, H.; Manicke, N. E.; Yang, Q. A.; Zheng, L. X.; Shi, R. Y.; Cooks, R. G.; Zheng, O. Y. *Anal. Chem.* **2011**, *83*, 1197–1201.
- (33) Liu, J. J.; Cooks, R. G.; Ouyang, Z. *Anal. Chem.* **2011**, *83*, 9221–9225.
- (34) Luo, Z. G.; He, J. M.; Chen, Y.; He, J. J.; Gong, T.; Tang, F.; Wang, X. H.; Zhang, R. P.; Huang, L.; Zhang, L. F.; Lv, H. N.; Ma, S. G.; Fu, Z. D.; Chen, X. G.; Yu, S. S.; Abliz, Z. *Anal. Chem.* **2013**, *85*, 2977–2982.
- (35) Schafer, K. C.; Szaniszló, T.; Gunther, S.; Balog, J.; Denes, J.; Keseru, M.; Dezso, B.; Toth, M.; Spengler, B.; Takats, Z. *Anal. Chem.* **2011**, *83*, 1632–1640.
- (36) Wiseman, J. M.; Puolitaival, S. M.; Takats, Z.; Cooks, R. G.; Caprioli, R. M. *Angew. Chem., Int. Ed.* **2005**, *44*, 7094–7097.
- (37) Agar, N. Y. R.; Golby, A. J.; Ligon, K. L.; Norton, I.; Mohan, V.; Wiseman, J. M.; Tannenbaum, A.; Jolesz, F. A. *Neurosurgery* **2011**, *68*, 280–290.
- (38) Liu, J. J.; Ouyang, Z. *Anal. Bioanal. Chem.* **2013**, *405*, 5645–5653.

- (39) Eberlin, L. S.; Norton, I.; Orringer, D.; Dunn, I. F.; Liu, X. H.; Ide, J. L.; Jarmusch, A. K.; Ligon, K. L.; Jolesz, F. A.; Golby, A. J.; Santagata, S.; Agar, N. Y. R.; Cooks, R. G. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 1611–1616.
- (40) Schafer, K. C.; Denes, J.; Albrecht, K.; Szanislo, T.; Balog, J.; Skoumal, R.; Katona, M.; Toth, M.; Balogh, L.; Takats, Z. *Angew. Chem., Int. Ed.* **2009**, *48*, 8240–8242.
- (41) Balog, J.; Szanislo, T.; Schaefer, K. C.; Denes, J.; Lopata, A.; Godorhazy, L.; Szalay, D.; Balogh, L.; Sasi-Szabo, L.; Toth, M.; Takats, Z. *Anal. Chem.* **2010**, *82*, 7343–7350.
- (42) Dill, A. L.; Eberlin, L. S.; Costa, A. B.; Zheng, C.; Ifa, D. R.; Cheng, L.; Masterson, T. A.; Koch, M. O.; Vitek, O.; Cooks, R. G. *Chem.—Eur. J.* **2011**, *17*, 2897–2902.
- (43) Dill, A. L.; Eberlin, L. S.; Zheng, C.; Costa, A. B.; Ifa, D. R.; Cheng, L.; Masterson, T. A.; Koch, M. O.; Vitek, O.; Cooks, R. G. *Anal. Bioanal. Chem.* **2010**, *398*, 2969–2978.
- (44) Masterson, T. A.; Dill, A. L.; Eberlin, L. S.; Mattarozzi, M.; Cheng, L.; Beck, S. D. W.; Bianchi, F.; Cooks, R. G. *J. Am. Soc. Mass Spectrom.* **2011**, *22*, 1326–1333.
- (45) Eberlin, L. S.; Norton, I.; Dill, A. L.; Golby, A. J.; Ligon, K. L.; Santagata, S.; Cooks, R. G.; Agar, N. Y. R. *Cancer Res.* **2012**, *72*, 645–654.
- (46) Rhee, K. J.; Wu, S. G.; Wu, X. Q.; Huso, D. L.; Karim, B.; Franco, A. A.; Rabizadeh, S.; Golub, J. E.; Mathews, L. E.; Shin, J.; Sartor, R. B.; Golenbock, D.; Hamad, A. R.; Gan, C. M.; Housseau, F.; Sears, C. L. *Infect. Immun.* **2009**, *77*, 1708–1718.
- (47) Wu, S. G.; Powell, J.; Mathioudakis, N.; Kane, S.; Fernandez, E.; Sears, C. L. *Infect. Immun.* **2004**, *72*, 5832–5839.
- (48) Dejea, C.; Wick, E.; Sears, C. L. *Future Microbiol.* **2013**, *8*, 445–460.
- (49) Goodwin, A. C.; Shields, C. E. D.; Wu, S. G.; Huso, D. L.; Wu, X. Q.; Murray-Stewart, T. R.; Hacker-Prietz, A.; Rabizadeh, S.; Woster, P. M.; Sears, C. L.; Casero, R. A. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 15354–15359.
- (50) O'Hagan, H. M.; Wang, W.; Sen, S.; Shields, C. D.; Lee, S. S.; Zhang, Y. W.; Clements, E. G.; Cai, Y.; Van Neste, L.; Easwaran, H.; Casero, R. A.; Sears, C. L.; Baylin, S. B. *Cancer Cell* **2011**, *20*, 606–619.
- (51) Wu, S. G.; Rhee, K. J.; Albesiano, E.; Rabizadeh, S.; Wu, X. Q.; Yen, H. R.; Huso, D. L.; Brancati, F. L.; Wick, E.; McAllister, F.; Housseau, F.; Pardoll, D. M.; Sears, C. L. *Nat. Med.* **2009**, *15*, 1016–U1064.
- (52) Cotte-Rodriguez, I.; Mulligan, C. C.; Cooks, G. *Anal. Chem.* **2007**, *79*, 7069–7077.
- (53) Garimella, S.; Xu, W.; Huang, G.; Harper, J. D.; Cooks, R. G.; Ouyang, Z. *J. Mass Spectrom.* **2012**, *47*, 201–207.
- (54) Eberlin, L. S.; Ferreira, C. R.; Dill, A. L.; Ifa, D. R.; Cheng, L.; Cooks, R. G. *Chembiochem* **2011**, *12*, 2129–2132.
- (55) Nemes, P.; Vertes, A. *TrAC, Trends Anal. Chem.* **2012**, *34*, 22–34.
- (56) So, P. K.; Hu, B.; Yao, Z. P. *Mol. Biosyst.* **2013**, *9*, 915–929.
- (57) Khosla, A.; Ponsky, T. A. *J. Minimum Access Surg.* **2011**, *7*, 116–120.
- (58) Varadarajulu, S.; Banerjee, S.; Barth, B. A.; Desilets, D. J.; Kaul, V.; Kethu, S. R.; Pedrosa, M. C.; Pfau, P. R.; Tokar, J. L.; Wang, A.; Wong, L.-M.; Rodriguez, S. A. *Gastrointest. Endosc.* **2011**, *74*, 1–6.
- (59) Green, F. M.; Salter, T. L.; Gilmore, I. S.; Stokes, P.; O'Connor, G. *Analyst* **2010**, *135*, 731–737.
- (60) Haddad, R.; Catharino, R. R.; Marques, L. A.; Eberlin, M. N. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 3662–3666.
- (61) Janfelt, C.; Norgaard, A. W. *J. Am. Soc. Mass Spectrom.* **2012**, *23*, 1670–1678.
- (62) Yang, P. X.; Cooks, R. G.; Ouyang, Z.; Hawkridge, A. M.; Muddiman, D. C. *Anal. Chem.* **2005**, *77*, 6174–6183.