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Solvent gradient electrospray for laser ablation electrospray ionization mass spectrometry†

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Most electrospray based ambient ionization techniques, *e.g.*, laser ablation electrospray ionization (LAESI), utilize a fixed spray solution composition. Complex samples often contain compounds of different polarity that exhibit a wide range of solubilities in the electrospray solvent. Thus, the fixed spray solution composition limits the molecular coverage of these approaches. Two-barrel theta glass capillaries have been used for the rapid mixing of two solutions for manipulating fast reactions including protein folding, unfolding, and charge state distributions. Here, we present a new variant of LAESI mass spectrometry (MS) by scanning the high voltages applied to the two barrels of a theta glass capillary containing two different solvents. In the resulting gradient LAESI (g-LAESI), the composition of the spray solution is ramped between the two solvents in the barrels to facilitate the detection of compounds of diverse polarity and solubility. Dynamic ranges and limits of detection achieved for g-LAESI-MS were comparable to conventional LAESI-MS. We have demonstrated simultaneous detection of different types of chemical standards, and polar and less polar compounds from *Escherichia coli* cell pellets using g-LAESI-MS. Varying the spray solution composition in a gradient electrospray can benefit from the enhanced solubilities of different analytes in polar and less polar solvents, ultimately improving the molecular coverage in the direct analysis of biological samples.

Introduction

Most conventional electrospray,[1,2] nanospray,[3,4] and electrospray-based ionization methods[5–7] utilize a spray solution of fixed composition from a single emitter. Typically, the composition of the spray solution is selected to match the polarity of the analytes and support spray stability. However, complex samples often contain compounds with diverse polarities and solubilities in the spray solvent. Combined with HPLC or other separation techniques, the molecular coverage of electrospray ionization (ESI) can be extended by implementing solvent composition changes through gradient elution. However, in direct ionization methods without sample preparation, *e.g.*, desorption electrospray ionization (DESI)[5,8] or probe electrospray ionization (PESI),[9,10] the solvent composition is usually fixed.

Recently, multiple channel electrospray based mass spectrometry (MS) techniques have been introduced to alleviate some of the limitations of a single electrospray. For example, multiple channel electrospray was developed to separate the nebulization and ionization processes. Varying the electrospray solvents selectively suppressed the signal from certain components in a sample mixture and facilitated the ionization of neutral molecules.[11,12] Two simultaneously operated ESI sources have been implemented to improve quantitation,[13] and rapidly alternating ESI sources in combination with laser desorption have been shown to enhance molecular coverage.[14] A dual-sprayer source was also used to introduce samples through one sprayer and to ionize the neutral molecules using the other sprayer,[15,16] and for enabling rapid gas phase ion/ion reactions.[17] In other applications, an internal calibrant is introduced by one of the sprayers to enhance the mass accuracy for the analytes ionized by the other sprayer.[18–20]

As an alternative approach to dual sprayers, a two-barrel theta glass capillary has recently been utilized as a nanospray emitter for the rapid mixing of two reactants in the liquid phase.[21–25] In a theta glass capillary, a septum in the middle creates two individual channels. These capillaries can be pulled to a sharp tip that retains the septum and the separate channels. Loading two different solutions in the channels and applying high voltages separately, rapid mixing of the solutions can be achieved in the Taylor cone at the tip. The utility of this rapid mixing has been demonstrated for manipulating protein folding, unfolding, altering charge state distributions,

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and following complex formation on timescales down to 1 μs.[22,23,25,26] To extend this technique to longer (milliseconds) timescales, mixing can be implemented in one of the channels through moving liquid from one barrel to the other by electroosmosis.[23]

Laser ablation electrospray ionization (LAESI), is an emerging direct ionization source that enables the analysis of biomedical samples at atmospheric pressure. In this technique, neutral particulates ejected from the sample by mid-IR laser ablation are ionized by charged droplets from an electrospray.[7,27] Without a need for a matrix, and with minimum sample preparation, LAESI-MS and the related imaging technique enable the direct detection of metabolites and lipids from biological tissues and cells.[28–30] However, analysis of complex samples with polar and less polar components, and imaging of heterogeneous tissues with local variations in the polarity of constituents result in limited molecular coverage due in part to the fixed composition of the electrospray solvent.

In this work, we introduce a new electrospray method with a rapidly changing solvent composition due to sweeping between solvents of different polarity. This gradient electrospray in combination with LAESI enables the detection of polar and less polar analytes simultaneously. The electrospray is produced by using a two-barrel theta glass capillary containing two different solvents, and by scanning the high voltages supplied to them. The resulting gradient (g) LAESI, extends the range of compounds that can be analyzed compared to the conventional fixed spray composition system. The capabilities of g-LAESI-MS have been demonstrated for the analysis of chemical standards and microbial cell pellets.

Experimental

Chemicals and samples

Solvents, water (W6-1), methanol (A452-4), and acetonitrile (A955-1) were obtained in HPLC grade purity from Fisher (Pittsburgh, PA), whereas HPLC grade toluene (34866) was procured from Sigma-Aldrich (St Louis, MO). The chemicals, D-serine (S4250) and verapamil (V4629) were purchased from Sigma-Aldrich (St Louis, MO), spermidine (85561) was obtained from Fluka (Munich, Germany), and phosphatidylcholine (PC(18:2/18:2)) (850385C) was bought from Avanti Polar Lipids, Inc. (Alabaster, AL). Glacial acetic acid (45727) and formic acid (06440) were obtained from Fluka (Munich, Germany). Aqueous stock solutions were made from arginine (1.3 mM), spermidine (7.9 mM), and D-serine (39.8 mM), whereas verapamil (1.0 mM) and PC(18:2/18:2) (1.3 mM) were dissolved in 50% (v/v) and 80% (v/v) methanol, respectively. For the experiments serial dilutions were prepared.

Bacterial cultures

A lambda derivative of *Escherichia coli* (ATCC 12435, ATCC, Manassas, VA) bacteria was cultured for 24 hours in lysogeny broth (LB) medium (10855, Life Technologies, Frederick, MD) at 37 °C using an orbital shaker (MaxQ 4000, Thermal Scientific Inc., Waltham, MA). The *E. coli* cell pellets were prepared by centrifuging 1.0 mL of cell suspension at 5000 rpm for 2 min, aspirating the medium, and washing the pellet with 1.0 mL of water. After washing, the suspended cells were centrifuged again (5000 rpm, 2 min), the supernatant was removed, and the pellet was directly used for MS analysis.

Gradient electrospray

A theta-glass capillary (1.5 mm OD, 1.0 mm ID, with a 0.2 mm wide septum, Warner Instruments, Hamden, CT) was pulled into a nanospray emitter with a tip diameter of 3 ± 0.2 μm (see Fig. S1 in the ESI†) using a micropipette puller (P-1000, Sutter Instrument, Novato, CA). A theta glass holder (THS-F15PH, Warner instruments, Hamden, CT) was used to secure the pulled capillary. Two perfluoroalkoxy-coated platinum wires (bare OD 127 μm, coated OD 203 μm, A-M Systems, Sequim, WA) were inserted into the two barrels of the theta glass capillary and attached to the two jack type connectors in the holder. Two high voltage power supplies (PS350, Stanford Research Systems, Inc.) provided time dependent voltages to the two platinum wire electrodes in the emitter. The power supplies were controlled through their external voltage set input by a dual-channel function generator (Tektronix, Inc., Beaverton, OR) that scanned the high voltages according to the selected programs. In all experiments, triangular waveforms produced by the high voltage power supplies exhibited 700 to 1000 V peak-to-peak amplitudes and 0.1 to 0.2 Hz frequencies, and were superimposed on an 850–1000 V DC bias. For different solvents, waveforms with different voltage ranges were chosen to achieve ion signal with acceptable stability for the generated nanosprays. The liquid flow for the nanospray was induced by the high voltages, *i.e.*, there was no forced flow (syringe pump) involved in these experiments. Thus, the flow rate was determined by the properties of the spray solution, the capillary, and the applied voltages. The produced waveforms were monitored by a 500 MHz digital oscilloscope (Wavesurfer 452, LeCroy, Chestnut Ridge, NY) through a high voltage probe (P6015A, Tektronix, Inc., Beaverton, OR).

Gradient-LAESI-MS

The gradient electrospray setup was incorporated into a LAESI system similar to the configurations that had been described before.[7,31] In the schematic of the resulting g-LAESI (see Fig. 1) we indicate the time dependent voltages on the electrodes driving the gradient spray and the corresponding ion currents registered by the mass spectrometer. In the testing experiments, mid-IR laser pulses at 2940 nm with a repetition rate of 20 Hz were focused through a plano-convex lens onto the sample surface to generate an ablation plume. The particulates in the plume were merged with the gradient electrospray of ramped composition for ionization. Spray solutions of different polarities were loaded into the two barrels of the capillary. The resulting ions from g-LAESI were collected by a quadrupole time-of-flight mass spectrometer with ion mobility separation (Synapt G2 S, Waters, Co., Milford, MA). The limit

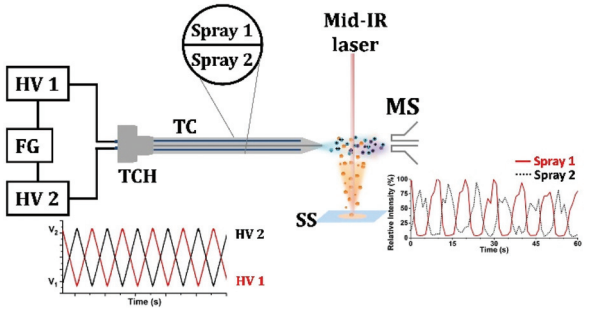

Fig. 1 Schematic of gradient LAESI (g-LAESI). FG: dual-channel function generator, HV 1 and HV 2: high voltage power supplies, TC: theta glass capillary, TCH: theta glass capillary holder, SS: sample stage, MS: mass spectrometer.

of detection and sensitivity for g-LAESI-MS was established based on experimental triplicates.

Data processing

The mass spectra were collected and processed (averaged, smoothed, and centered) in MassLynx V4.1 (SCN851, Waters, Co., Milford, MA). Chromatograms for selected ions were exported to track the signal intensities as a function of time. Both the mass spectra and ion chromatograms were normalized to the highest ion counts. The experimental results were plotted in a scientific visualization software (Origin 9.1.0, OriginLab Corporation, Northampton, MA). The detected ions for the cell pellets were tentatively identified by searching the *E. coli* Metabolome Database (ECMDB) V2.0 (http://www.ecmdb.ca, last accessed on May 16, 2017).

Results and discussion

Gradient electrospray

To assess the effective voltage range for inducing a stable nanospray, voltages 800 V, 1800 V, and 2800 V were supplied to the spray barrels. As shown in Fig. S2 of the ESI,† the verapamil ion intensity from the nanospray exhibited a transition from stable signal at 800 V through slight fluctuations at 1800 V to a highly unstable spray at 2800 V. The voltage range applied for the gradient nanospray was selected to enable a performance with reasonably stable signal. Compared to typical nano-ESI experiments, lower potential was needed to produce a stable spray. A possible reason might be the presence of the septum in the theta-glass capillary tip, effectively reducing the individual barrel inner diameters below half of the overall values.

Generation of an electrospray with solvent gradient was initially demonstrated using the following two solutions: 0.6 μM verapamil in 2 : 1 (v/v) methanol/chloroform (spray 1), and 27.0 μM arginine in 1 : 1 (v/v) acetonitrile/water acidified to achieve 0.1% acetic acid concentration (spray 2). These solutions were filled into the two barrels of a theta capillary. A fixed high voltage at 1100 V was applied for spray 1, and high voltage with a triangular waveform (0.1 Hz frequency, 1000 V amplitude, and 1000 V offset) was supplied for spray 2 (see the top panel in Fig. 2a). The chromatograms for the two sample related molecular ions, shown in the bottom panel of Fig. 2a, varied with the frequency of the triangular waveform, and their intensities alternated in time. This indicated that the spray solvent composition also alternated between the two solutions from the capillary barrels, effectively producing 5 s concentration gradients.

To achieve greater control over the gradients, two triangular high voltage waveforms with a phase difference were applied to the two barrels of the theta capillary. This enabled us to adjust the solvent gradient by changing the phase difference between the two waveforms. For example, for the solutions of 0.8 μM verapamil in 1 : 1 (v/v) acidified methanol/water and 27.0 μM arginine in 1 : 1 (v/v) acidified acetonitrile/water at a voltage phase difference of 180°, the corresponding ion chromatograms indicated a phase shift of 108° (see Fig. 2b). This meant that the solvent composition for optimum ionization of one of the components fell between the solvent compositions found in the two barrels.

The influence of phase differences between the voltage waveforms (both with 0.1 Hz frequency, 800 V amplitude, and 850 V offset) on the produced solvent gradient was studied in the phase range between 0° and 300° in 60° increments, using spray solutions of 0.7 μM arginine in 1 : 1 (v/v) acidified aceto-

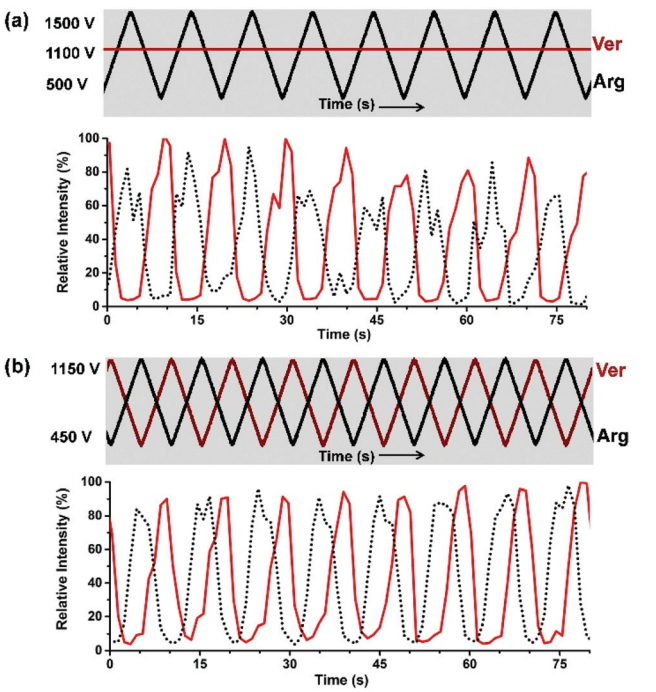

Fig. 2 Ion chromatograms for two sprays and corresponding high voltage waveforms with (a) one constant and one triangular pattern, and (b) two triangular patterns. Time dependence of relative ion intensities for arginine (black dotted line), and verapamil (red solid line) exhibited periodic behavior that followed the periodicity of the high voltage.

nitrile/water, and 0.1 μM verapamil in 2 : 1 (v/v) acidified methanol/chloroform in the two barrels.

The ion chromatograms of the two samples (see Fig. 3) illustrated that at the voltage phase differences of 180° and 240°, a sudden dip in the verapamil signal was observed at the time when the highest voltage was supplied to the corresponding solution. This suggested that due to the large potential difference between the two spray channels ($\Delta U \approx 800\ V = U_{Ver} - U_{Arg}$ = 1250 V–450 V), the current between the two barrels shunted the spray current for verapamil. The phase differences of 120° and 300° produced a relatively stable transition between the two solvents, whereas a partial overlap of the two sprays was observed for the phase difference of 0° and 60°.

To investigate the Taylor cone geometry generated at the tip of the theta glass capillary, a homebuilt long-distance microscope[32] was used for visualization of the emanating spray (see Fig. 4 in the ESI†). The two barrels of the theta capillary were filled with 50% acetonitrile and 50% methanol, respectively, both acidified by 0.1% acetic acid. Static voltages of 1800 V and 2200 V were applied to the two barrels. As shown in Fig. 4, two separate liquid filaments were formed at the tip of the capillary indicative of two Taylor cones. The phase difference between the two high voltage waveforms resulting in a potential difference between the two barrels, ΔU, might generate electroosmotic flow[24] leading to fluid exchange between the barrels and impede the formation of an electrospray. To avoid this electroosmotic flow, the tip diameter of the emitter required optimization. Typically, with a tip diameter below 4 μm, electrospray was formed (see Fig. 4), *i.e.*, electroosmotic flow could be avoided for most of the used solvents and voltages, whereas with a diameter larger than 5 μm spraying was not observed but electroosmosis occurred between the two barrels (see Fig. S3 of the ESI, and the ESI Video S1†).[24]

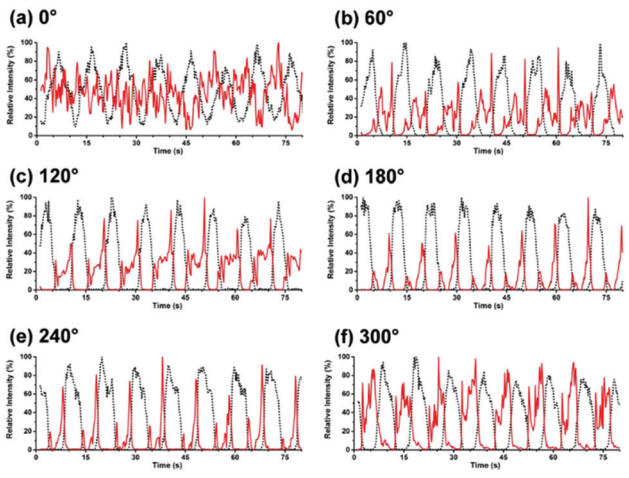

Fig. 3 Ion chromatograms for two spray solutions, 0.1 μM verapamil in 2 : 1 (v/v) acidified methanol/chloroform (red solid line), and 0.7 μM arginine in 1 : 1 (v/v) acidified acetonitrile/water (black dotted line), with two triangular high voltage waveforms (0.1 Hz frequency, 800 V amplitude, and 850 V offset) supplied at voltage phase differences of (a) 0°; (b) 60°; (c) 120°; (d) 180°; (e) 240°; and (f) 300°.

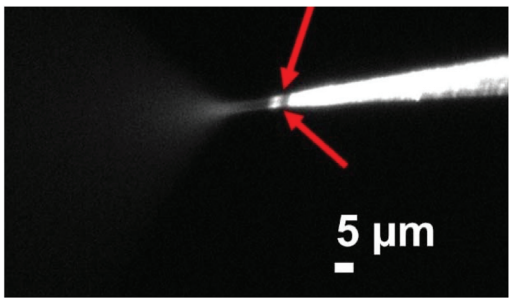

Fig. 4 Microscope image of spray geometry formed at two barrels of a theta capillary with static voltages of 1800 V and 2200 V applied. Two separate liquid filaments were formed, indicative of two Taylor cones.

To verify that the spray gradients were not the result of spray instability at different voltages, additional experiments were performed by operating the two spray channels with static voltages. The two barrels of the theta capillary were filled with 0.76 μM arginine and 0.20 μM verapamil in 50% methanol with 0.1% acetic acid, and static voltages of 1500 V and 1000 V were applied on the two channels, respectively. As shown in Fig. S4 of the ESI,† the time dependence of ion intensities from the two barrels showed good stability ($I_{arginine}$ = 77.2 ± 7.6 and $I_{verapamil}$ = 79.6 ± 9.1), and the ratio of arginine to verapamil intensities stayed at 2.07 ± 0.24 over time.

g-LAESI geometry optimization for nanospray

The frequency of the triangular high voltage waveforms was 0.1 Hz, corresponding to a period of 10 s. The repetition rate of laser sampling in LAESI was 20 Hz with a 0.05 s period that was much shorter than that of the high voltage waveforms. Therefore, the LAESI signal followed the changes in the solvent gradient in the electrospray.

To achieve a strong analyte signal from the ~3 μm spray capillary tip, the g-LAESI geometry had to be optimized. This was probably related to the smaller size of the droplets (<200 nm) emitted from the nanospray[4] in g-LAESI compared to the 5–10 μm droplets produced by the conventional electrospray[33] used in LAESI. Earlier studies indicate that for efficient coalescence of the ablated particles and the droplets in the spray, their diameters need to be comparable.[27,33,34] In atmospheric pressure laser ablation, the produced particles naturally segregate during the plume expansion process because a difference in their stopping distances.[27] The stopping distance, x_{stop}, of the ejected particles can be expressed as

$$x_{stop} = 2\rho R^2 v_0 / 9\mu, \quad (1)$$

where R and v_0 are the radius and initial velocity of the particle, respectively, ρ is the density of the ablated material, and μ is the dynamic viscosity of air. Thus, under the same conditions, smaller particles stop after traveling a shorter distance. This means that the nanospray axis has to be closer to the ablated surface for the smaller nanospray droplets to capture these smaller particles. Indeed, optimum signal in g-LAESI was achieved when the sample was placed ~7 mm

below the axis of the nanospray. Further optimization of the signal required reducing the distance between the mass spectrometer orifice and the emitter tip to 4 mm (from the 10 mm value in conventional LAESI) and positioning the laser beam 1 mm in front of the emitter tip.

g-LAESI-MS analytical figures of merit

To test the combination of the gradient nanospray with LAESI-MS, two spray solutions, 2 : 1 (v/v) methanol/chloroform (spray 1), and 1 : 1 (v/v) acidified acetonitrile/water (spray 2), were loaded into the two barrels with verapamil and arginine reference solutes to monitor the spray composition, respectively. A fixed high voltage (1100 V) was supplied for spray 1, and a triangular voltage waveform (0.1 Hz frequency, 700 V amplitude, and 1000 V offset) was applied for spray 2 to generate the gradient electrospray. To establish the figures of merit for g-LAESI, 5 μL of aqueous spermidine and PC(18:2/18:2) (in 80% methanol) sample solutions were deposited for ablation in the 8 nM to 8 mM and 13 nM to 1.3 mM concentration ranges, respectively. For comparison, conventional LAESI-MS experiments were conducted with a fixed voltage of 3300 V, and an electrospray solution of 1 : 1 (v/v) acidified acetonitrile/water.

Using optimized parameters for g-LAESI-MS, in separate experiments a limit of detection of 79 and 13 fmol were achieved for spermidine and PC(18:2/18:2), respectively. These values were comparable to those obtained by conventional LAESI-MS (see Fig. 5). Similar to conventional LAESI-MS, g-LAESI-MS exhibited over four orders of magnitude dynamic range for the quantification of spermidine and PC(18:2/18:2). Correlation coefficients for the datasets in the fitted region, $r > 0.99$, indicated linear relationships for both analytes. The impurities in the spray solution generated a chemical background. The non-linear regions in the plot are the result of the sample related signal falling below the background level. We excluded the corresponding non-linear regions from the fitting process. The signal for g-LAESI was generally lower than that for conventional LAESI, due to the reduced sample flow rate of 40 nL min^{-1} for the former compared to 300 nL min^{-1} of the latter. The flow rate of 40 nL min^{-1} was measured by loading 50% methanol with 0.1% acetic acid into one barrel of the theta capillary with 1100 V voltage applied. We also measured the flow rates in the two channels for 50% methanol (spray 1) and 50% acetonitrile (spray 2), both acidified with 0.1% acetic acid. With 1500 V static voltage applied for spray 1, the measured flow rate was 80 nL min^{-1}, whereas with 1000 V static voltage applied for spray 2, the measured flow rate was 53 nL min^{-1}. These voltage-induced flow rates offered strong signal when combined with LAESI-MS. Increasing the voltages led to higher flow rates with no significant drop in the LAESI-MS performance as long as the spray stability was not compromised.

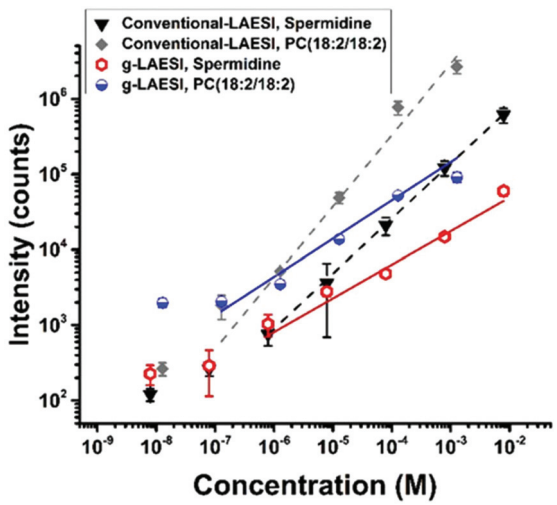

Fig. 5 Comparison of analytical figures for gradient and conventional LAESI-MS. Using g-LAESI, a limit of detection (LOD) of 79 and 13 fmol were obtained for spermidine (red hexagon) and PC(18:2/18:2) (blue circle), respectively. Same LODs were achieved for these two analytes using conventional LAESI (spermidine: black triangle, PC(18:2/18:2): gray diamond). Over four orders of magnitude dynamic ranges were observed for both gradient and conventional LAESI-MS.

Analyte signal as a function of electrospray solvent polarity in g-LAESI-MS

To evaluate the performance of g-LAESI for analyzing compounds with different polarities and solubilities in water, D-serine, spermidine, and PC(18:2/18:2) were tested with spray solutions of different polarity. Three samples, 1 mM D-serine, 8 μM spermidine, and 30 μM PC(18:2/18:2) in 80% methanol were deposited for ablation. The two barrels of the theta glass capillary were filled with 2 : 1 (v/v) methanol/chloroform and 1 : 1 (v/v) acidified acetonitrile/water with 0.6 μM verapamil (spray 1), and 27.0 μM arginine (spray 2) reference solutes, respectively. Applying a triangular high voltage waveform (0.1 Hz frequency, 1000 V amplitude, and 1000 V offset) and a fixed high voltage (1100 V) on spray 1 and 2, respectively, a gradient electrospray was generated corresponding to a transition from a less to a more polar solvent. The ion chromatograms for the reference solutes (bottom traces in the three panels of Fig. 6) reflected a periodic change in spray composition as a function of time, indicating alternating solvent polarity.

The time-dependent intensity profiles of the detected analyte signal from the three ablated samples showed distinct features (top traces in the three panels of Fig. 6). Spermidine exhibited persistent signal over the solvent gradients (see top trace in Fig. 6a). This can be attributed to its polar character and good solubility in both solvent mixtures. The lipid sample, PC(18:2/18:2), as a less polar compound, displayed a significantly stronger signal in spray 1 (see top trace in Fig. 6b), composed of the less polar solvent. D-Serine was only detected during the transition periods between the two sprays corresponding to quaternary mixtures of water, acetonitrile, methanol, and chloroform (see top trace in Fig. 6c). This might be explained by the relatively higher solubility of this water-soluble compound in methanol compared to acetonitrile. These results demonstrated the utility of g-LAESI-MS for the detection of compounds with different polarities and solubilities.

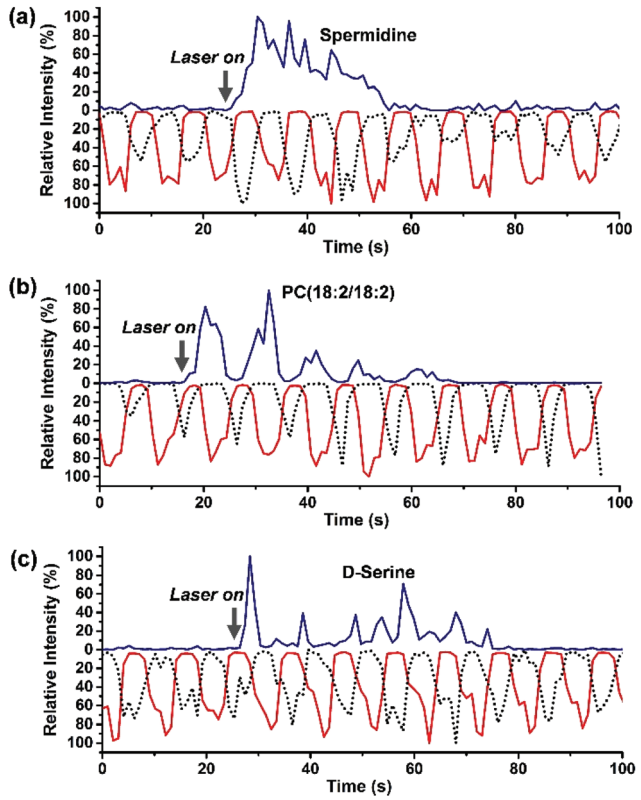

Fig. 6 Correlation between spray composition in g-LAESI and polarity/solubility of detected analyte. The bottom traces in all three panels show the ion chromatograms for the reference solutes: 0.6 μM verapamil in 2 : 1 (v/v) methanol/chloroform (spray 1, red solid line), and 27.0 μM arginine in 1 : 1 (v/v) acidified acetonitrile/water (spray 2, black dotted line). (a) Spermidine showed persistent signal irrespective of changes in spray composition. (b) The PC(18:2/18:2) analyte exhibited significantly stronger signal during spray 1. (c) D-Serine displayed high intensity at the overlap of sprays 1 and 2.

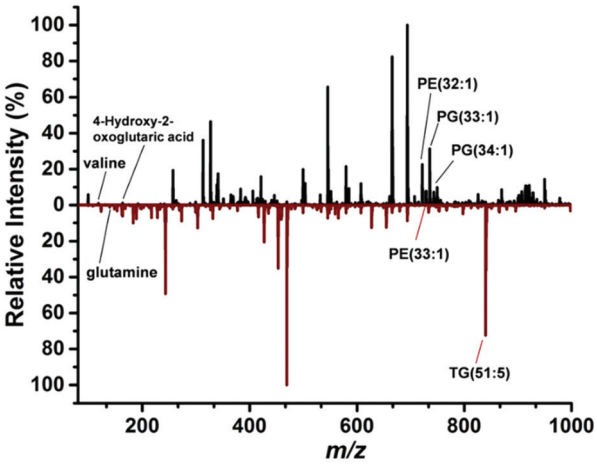

Fig. 7 g-LAESI mass spectra of *E. coli* cell pellets acquired in two different phases of the gradient electrospray (10 to 15 scans each) produced from 1 : 1 (v/v) acidified methanol/water (top trace), and 10 : 7 : 3 (v/v/v) acetonitrile/methanol/toluene (bottom trace).

Analysis of biological samples by g-LAESI-MS

To evaluate the efficacy of g-LAESI-MS for the detection of compounds with various polarities in biological samples, we applied this approach to study *Escherichia coli* cell pellets. Using 1 : 1 (v/v) acidified methanol/water (spray 1) and 10 : 7 : 3 (v/v/v) acetonitrile/methanol/toluene (spray 2) as spray solutions, alternating solvent gradients were established from polar to semi-nonpolar and back by supplying a triangular high voltage waveform (0.1 Hz frequency, 600 V amplitude, and 1000 V offset) and a fixed high voltage at 1200 V to spray 1 and 2, respectively.

Mass spectra from the *E. coli* cell pellets produced by g-LAESI at different times during the gradient electrospray displayed profoundly altered patterns (see Fig. 7). Polar compounds, *e.g.*, acetylspermidine at *m*/*z* 188.177 exhibited strong signal and were mainly detected in the more polar spray. Less polar compounds, *e.g.*, a triglyceride, TG(51:5), at *m*/*z* 839.651, were found during the less polar phase of the gradient electrospray.

The *E. coli* spectrum acquired at the more polar spray phase (top trace in Fig. 7) revealed 683 peak features, which were reduced to 191 chemical species after deisotoping and peak deconvolution. During the less polar spray phase (bottom trace in Fig. 7), 693 features were detected, which were reduced to 212 chemical species after deisotoping and peak deconvolution. Comparing the obtained peak lists from the two spectra, approximately 60 chemical species were present in both, and over 130 different chemical species were unique to one or the other deisotoped spectrum. These results indicated that g-LAESI-MS substantially extended the molecular coverage for metabolites and lipids in a biological sample.

Conclusions

In this report, we described the combination of a solvent gradient electrospray with LAESI-MS for the detection of analytes of diverse polarity and solubility in the spray solvent. The gradient electrospray was established by applying triangular high voltage waveforms to at least one of two different solutions in the barrels of a theta capillary. Dynamic ranges and limits of detection attained using g-LAESI-MS were similar to those of conventional LAESI-MS.

We have demonstrated the simultaneous detection of different types of chemical standards, and polar and less polar compounds from biological samples. Varying the spray solution composition in a gradient electrospray can potentially take advantage of the enhanced solubilities of different analytes in polar and less polar solvents, and improve molecular coverage. The properties of the gradient electrospray can be adjusted by changing the phase between the two waveforms. Further enhancements can be expected from combining the ability of g-LAESI to produce ions from a wider array of components with ion mobility separation followed by MS. Direct analysis by g-LAESI can also enhance throughput in applications that conventionally call for extractions by aqueous and organic solvents

because of the alternating solvent composition of the gradient spray. Applications with other electrospray based direct ionization methods, for example, desorption electrospray ionization can also expand the molecular coverage for samples with compounds differing in polarity and/or solubility.

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Electronic Supplementary Material (ESI) for Analyst.

Supporting information for

Solvent Gradient Electrospray for Laser Ablation Electrospray Ionization Mass Spectrometry

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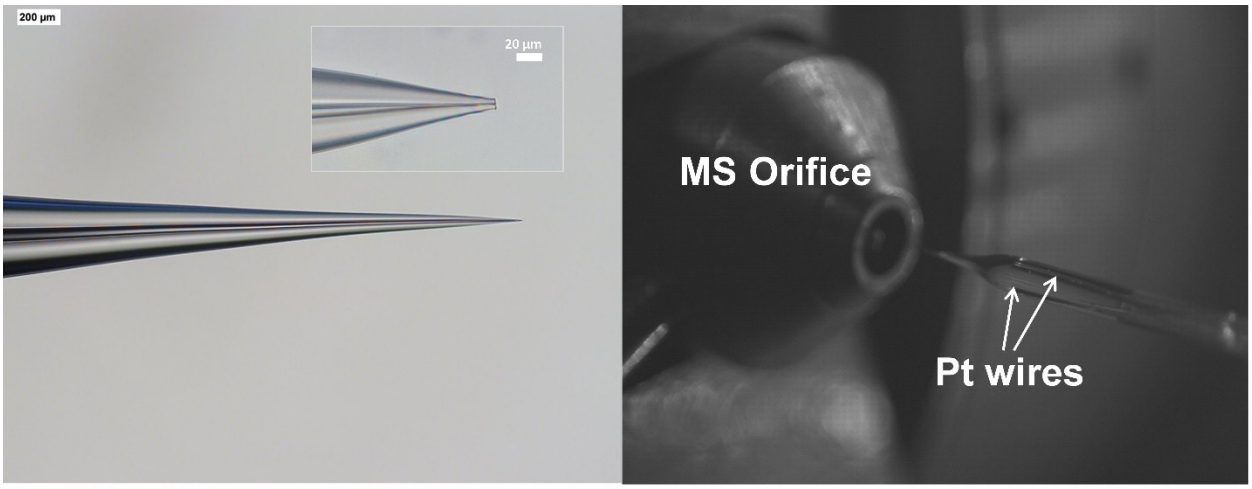

Figure S1. (a) Pulled theta glass capillary with a typical tip diameter of ~3 µm. Image in the inset at higher magnification shows a larger tip diameter of ~8 µm with a ~1 µm wide septum that reaches the tip of the capillary. (b) Pulled theta glass capillary in front of the mass spectrometer inlet orifice with two platinum wire electrodes inserted for generating gradient electrospray.

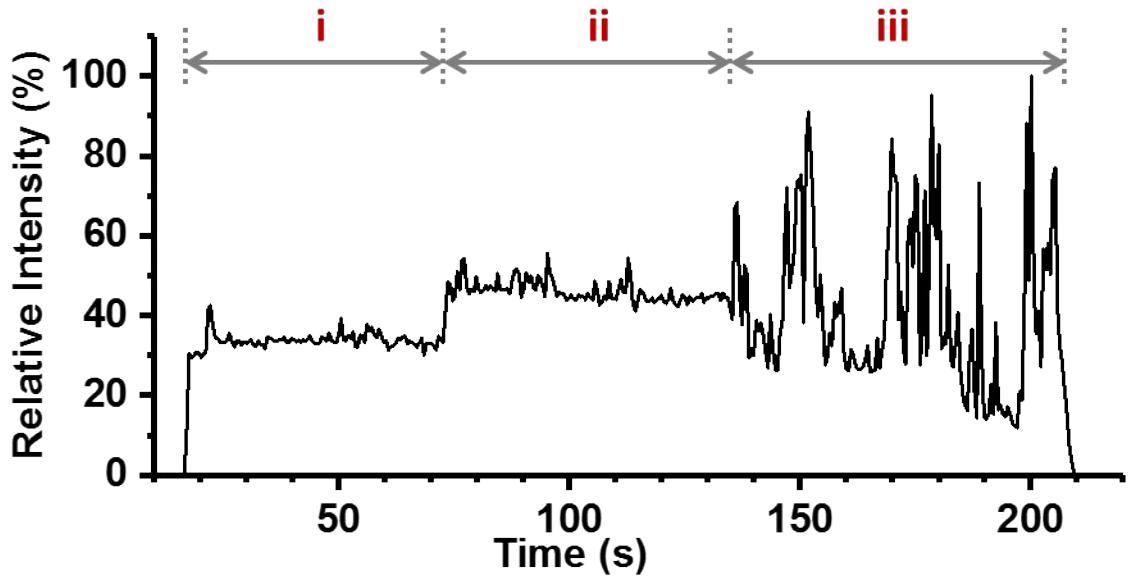

Figure S2. Time dependence of verapamil ion intensity showed a transition from (i) stable signal at 800 V spray voltage, (ii) through slight fluctuations at 1800 V, (iii) to a highly unstable spray at 2800 V.

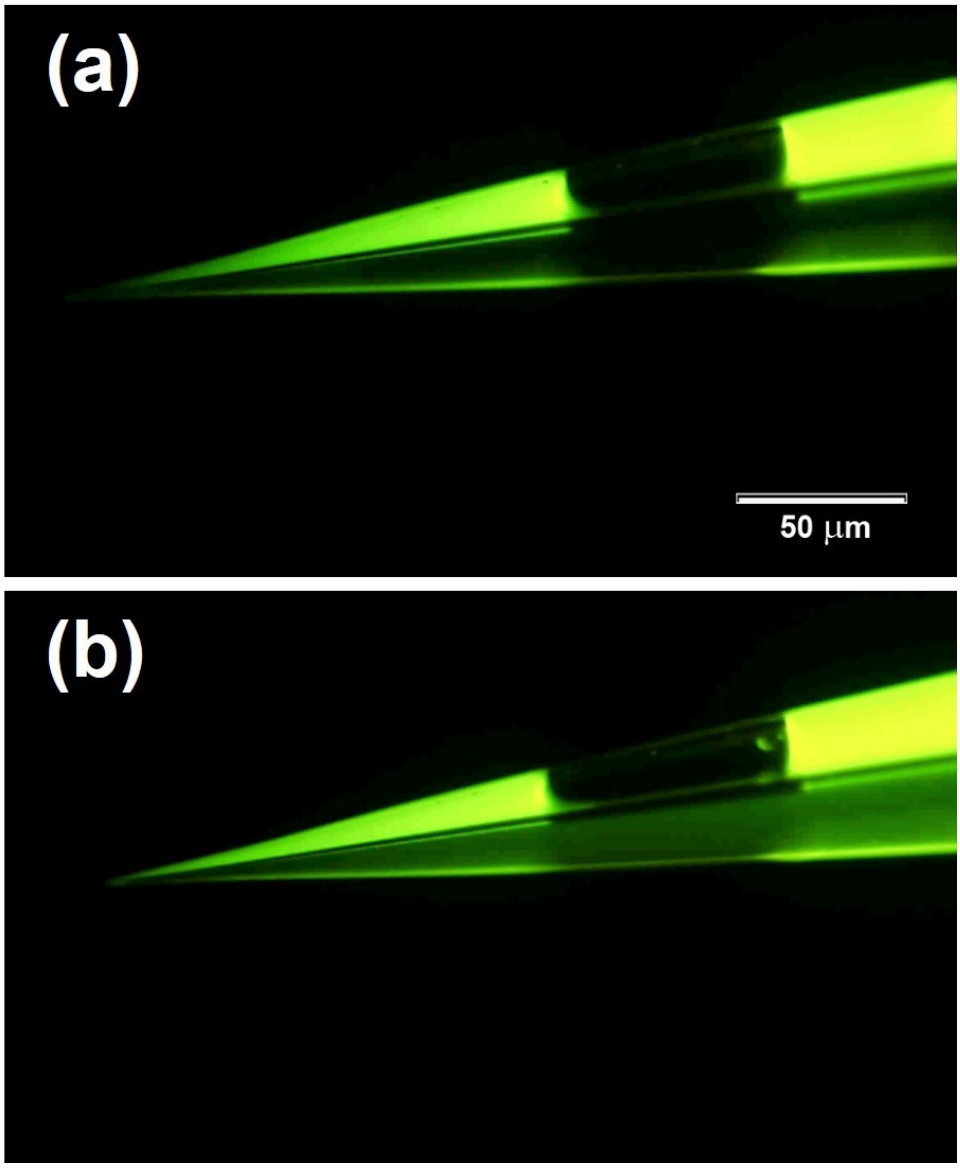

Figure S3. (a) Fluorescence microscope image (excitation at 470-490 nm and emission at 520 nm) of theta capillary of >5 μm tip diameter with barrels loaded with 6 mM rhodamine 6G and water before voltages are applied. (b) Applying 500 V and 300 V to the barrels containing rhodamine 6G solution and water, respectively, results in electroosmosis.

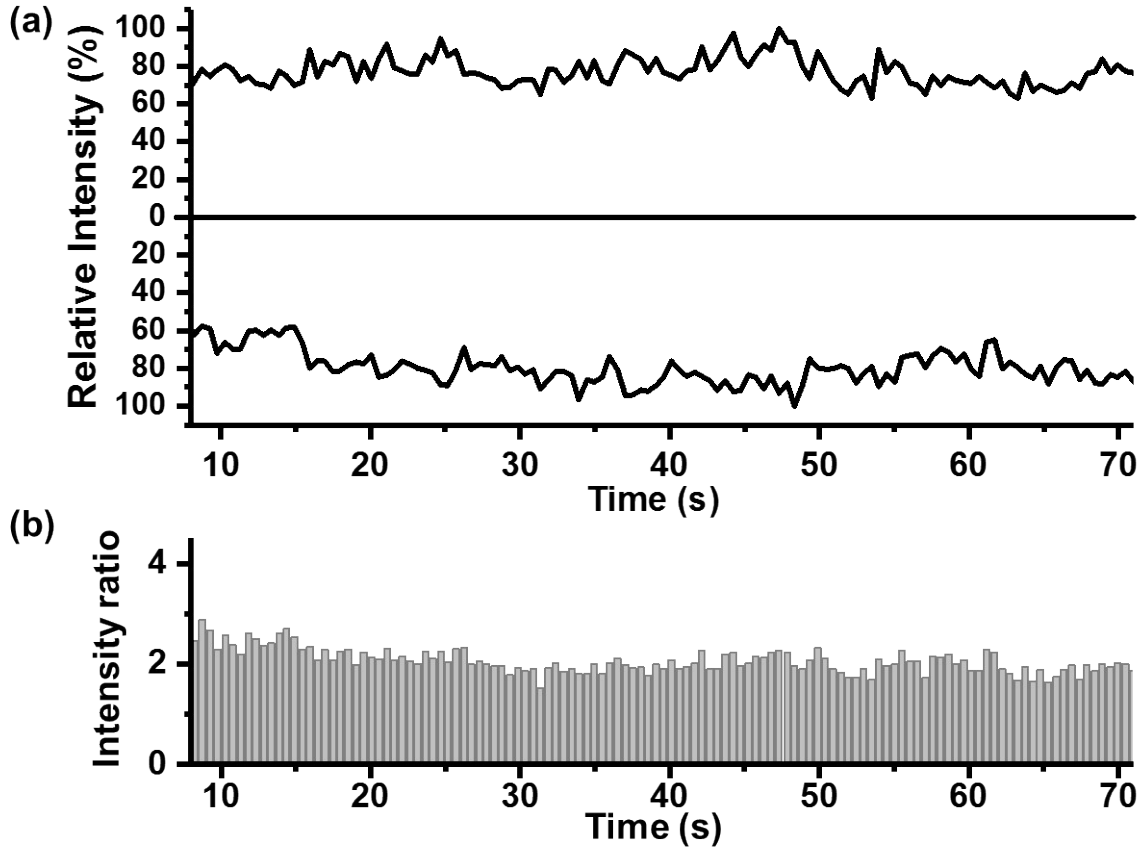

Figure S4. (a) Time dependence of ion intensities from two barrels of a theta capillary. Barrels are loaded with 0.76 μM arginine at 1500 V (spray 1, top trace), and 0.20 μM verapamil at 1000 V (spray 2, bottom trace). (b) Signal intensity ratio of arginine to verapamil (spray 1 to spray 2) as a function of time.