

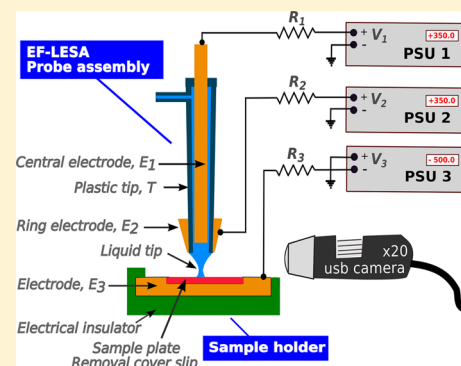
Electro-Focusing Liquid Extractive Surface Analysis (EF-LESA) Coupled to Mass Spectrometry

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Supporting Information

ABSTRACT: Analysis of the chemical composition of surfaces by liquid sampling devices interfaced to mass spectrometry is attractive as the sample stream can be continuously monitored at good sensitivity and selectivity. A sampling probe has been constructed that takes discrete liquid samples (typically <100 nL) of a surface. It incorporates an electrostatic lens system, comprising three electrodes, to which static and pulsed voltages are applied to form a conical “liquid tip”, employed to dissolve analytes at a surface. A prototype system demonstrates spatial resolution of 0.093 mm². Time of contact between the liquid tip and the surface is controlled to standardize extraction. Calibration graphs of different analyte concentrations on a stainless surface have been measured, together with the probe’s reproducibility, carryover, and recovery. A leucine enkephalin-coated surface demonstrated good linearity ($R^2 = 0.9936$), with a recovery of 90% and a limit of detection of 38 fmol per single spot sampled. The probe is compact and can be fitted into automated sample analysis equipment having potential for rapid analysis of surfaces at a good spatial resolution.



Analysis of surfaces has exploited mass spectrometry as a versatile detection system for many years. Extractive sampling of surfaces by direct contact of a liquid interfaced to electrospray ionization mass spectrometry (ESI-MS)^{1–3} is highly attractive as it can provide continuous monitoring and potentially high sensitivity for targeted compounds. Further downstream chromatographic separation could be applied to complex samples. A common hurdle for liquid sampling is the size of the contact face and lack of control of the liquid meniscus, this device has the potential to overcome these limitations and allow automated operation. This study examines an electro-focusing system to control droplet contact size and time of contact. The generic method demonstrated could be further improved and miniaturized through precision engineered electrode/probe assemblies.

The past decade has witnessed the invention of numerous methods to achieve ambient sampling/ionization,⁴ significantly Cooks et al. invented desorption electrospray ionization (DESI)⁵ for surface analysis. Neutral desorption sampling of surfaces, a variant of DESI, employing a hot gas stream was implemented by Chen and Zenobi in 2007.⁶ Laskin et al. devised a nano-DESI system offering high sensitivity, good spatial resolution and with imaging capability.⁷ On the other hand, microfluidics has been applied to the analysis of surfaces by the contact of a liquid stream or droplet, from an appropriate device, that is resampled and taken to a detector. Digital microfluidics (DMF) is an emerging sample preparation for mass spectrometry.⁸

In 2004, Luftmann described a simple device for sampling TLC spots directly to ESI-MS with an inlet and outlet solvent flow using a circular cutting edge to define a spot.⁹ Van Berkel’s group has published a number of strategies for direct liquid

sampling of surfaces^{10–17} and constructed liquid microjunction surface sampling probes, one having a contact face of $\approx 635 \mu\text{m}$ in diameter.¹¹ Operating at a flow rate of $15 \mu\text{L}/\text{min}$ with an aqueous/methanol mixture, it was assessed to have quantitative capability, a limit of detection (LOD) of 50 ng/mL by selected reaction monitoring (SRM), for drugs in dried blood spots and thin tissue sections.¹⁰ Chip-based infusion nanospray ionization system has been described as liquid extractive surface analysis (LESA).² Walworth et al. stated a lower limit of detection (LLOD) of 0.1 ng for propranolol using LESA with SRM detection, with extraction efficiencies of up to 77% reported for samples on a stainless steel plate.¹⁸

The above cited research represents a considerable body of work for the analysis of surfaces at atmospheric conditions. Compatibility of a sampling device to suit the characteristics of ESI-MS led us to the development of an EF-LESA probe to achieve enhanced spatial resolution of a contact liquid microjunction. The device incorporates an electrostatic lens system, shown schematically in Figure 1, that can shape the droplet at the end of the probe, sharpening it to a conical tip. The underlying concept for the EF-LESA probe was adapted from a micro droplet system of Yogi et al.^{19,20} They developed a trielectrode lens system for improving the positioning of a picoliters droplet dispensing system, with a $1 \mu\text{m}$ precision, using pulsed voltages applied to its electrodes. We also observed that a trielectrode system can shape sessile droplets

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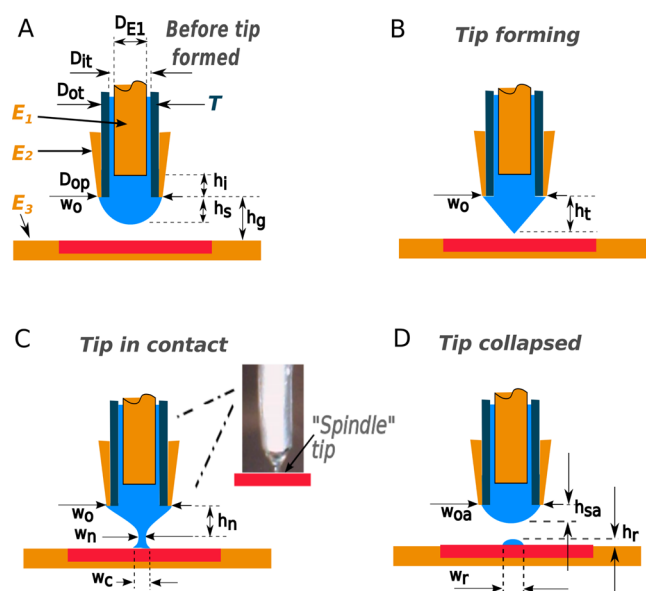


Figure 1. Schematic diagram of the stages of deformation of (A) a sessile droplet under the influence of an applied electrical field to form (B) a liquid tip, that contacts a (C) surface, and finally collapses to a (D) droplet that can be sampled by the mass spectrometer. Electrodes, E_1 , E_2 , and E_3 , shown in 1A, have voltages V_1 , V_2 , and V_3 applied. Plastic tip (T) and the removable stainless steel coverslip are shown in red. A photograph of a spindle tip is inset in (C). The dimensions of the probe assembly are D_{E1} , outer diameter of the electrode, E_1 ; D_{it} , inner diameter of the insulating plastic tip (T); D_{ot} , outer diameter of T; D_{op} , outer diameter at the end of T, coated in silver glue. Dimensions of the liquid are w_0 , diameter of the liquid wetting the probe plastic tip prior to liquid tip formation, usually $D_{op} = w_0$; h_i , distance between the end of the electrode E_1 and the end of the plastic tip; h_s , height of the sessile droplet; h_g , gap between the probe and the sample plate; h_n , height of the conical liquid tip during formation; w_n , diameter of the neck of the liquid tip and w_c , the diameter at contact; w_{oa} and h_{sa} are the diameter and height of the residual droplet on the probe, and w_r and h_r are the diameter and height of the residual drop on the sampling plate.

that can be resampled by discrete sampling or direct infusion ESI-MS. An important aspect of the design is the applied voltage to deform the liquid surface to a conical tip; it is significantly lower than the onset voltage for electrospray, V_{on}^{21} (see eq 1.1 below), allowing EF-LESA to form a stable liquid tip for enhanced liquid sampling.

The shape of a sessile droplet at the end of a capillary tip transforms when a voltage is applied; the liquid menisci formed with increasing applied voltage have been classified by Cloupeau and Prunet-Foch.²² Jaworck and Krupa have further detailed the classification of electrohydrodynamic (EHD) spraying modes on the size and charge of droplets generated.²³ In order of increasing voltage, the modes are described as dripping, spindle, oscillating jet, precession, and cone-jet mode. The spindle mode is the lowest voltage EHD mode that can form liquid tips and is employed in this study. The temporal progression that spindle-mode liquid tip shapes take on has been classified.²⁴ A near conical-shaped tip forms at the apex, with a proboscis-like spindle forming prior to its ultimate breakdown with the formation of droplets. Theoretical descriptions of the fluid dynamics of “Taylor cones” has recently been reviewed by de la Mora²⁵ and for electrospray by Kebarle and Verkerk.²¹ These theories have been applied to pure liquids, from low conductivities to charged liquids. Most

theories to our knowledge require a flow of liquid to describe dynamic behavior. Liquid flow through the EF-LESA probe into the tip is stopped, at the time of sampling, however, a transient local flow exists within the liquid tip as it reshapes from a sessile droplet to a conical liquid tip under the influence of an applied electric field, prior to contact with the surface. These conditions applied to EF-LESA prevent electrospray initiation and confine liquid contact to a small region on the surface.

While the present implementation is a single sampling device demonstrating proof of principle, we intend to further develop EF-LESA to allow continuous mobile phase flow through the liquid tip, for online MS operation. In this case, the EF-LESA liquid tip will be formed by automated control of a pulsed electric field. No studies are reported in the literature for a sampling system operating in the “spindle mode” domain. While there is significant literature for pulsed-mode droplet spraying^{25,26} and pulsed-mode electrospray,^{27–30} these have been demonstrated as spraying devices (not sampling) on a range of liquids and conductivities. Our approach could be adopted to operate as a pulsed sampling probe where the liquid tip contacts a surface at a point in a periodic fashion, providing higher spatial resolution than an equivalent liquid junction micro sampling system.

Preliminary research was conducted to find optimum voltages for the three electrodes of the EF-LESA system and the position of the probe tip relative to the surface. Early prototypes showed there was a need for a precision-engineered probe to achieve reproducible liquid tips; the prototype used in this investigation is based upon a plastic engineered dispensing tip (refer to Experimental Section for details). The system has been tested with a stainless steel surface and coated with analyte over a range of concentrations. Calibration graphs of analyte concentration were measured for leucine enkephalin (LE) and angiotensin II (AT), both using an internal standard of *N*-acetylarginine (NAA). The reproducibility, carryover, and recovery of the device were measured as part of the characterization study of EF-LESA.

EXPERIMENTAL SECTION

Chemical Reagents. Leucine enkephalin, angiotensin II, and *N*-acetylarginine were purchased from Fisher Scientific U.K. Ltd. (Loughborough, U.K.). Individual stock solutions (LE, 180 pmol/ μ L; AT, 956 pmol/ μ L; and NAA, 920 pmol/ μ L) were prepared in a solvent mixture A, consisting of pure water). Compounds were chosen because they were soluble in H₂O and provide a good ESI response. Solutions used in experiments and for construction of the calibration graphs were prepared at the desired concentration by serial dilution in water from the stock solution with further dilution, after surface sampling, with 50:50 water (with 0.1% formic acid):methanol solution (solvent mixture B). A solvent mixture of water with 0.1% formic acid was used as the EF-LESA probe mixture (solvent mixture C). HPLC-grade methanol and water were purchased from Fisher Scientific U.K. Ltd., and formic acid was supplied by Sigma Aldrich Ltd. (Gillingham, Dorset, U.K.).

Design of the EF-LESA Probe Apparatus. A generic design for an EF-LESA probe is shown schematically in Figure 1A, it comprises an insulated capillary tube (T, a commercially sourced plastic tip) and three metal electrodes E_1 , E_2 , and E_3 to which DC voltages V_1 , V_2 , and V_3 are applied, respectively. Electrode E_1 is contained within the insulating tube, T, and positioned at a height, h_i , from the tube end. There are many

possible designs for electrode E_1 , both a solid metal rod and metal capillary tube were tested allowing probe liquid to be dispensed either within electrode E_1 or around it. (Figure 1A shows an annular gap between E_1 and T, although this gap is closed in the prototype used.) An outer electrode, E_2 , is in contact and positioned flush with the end of the insulating tube, T. The surface to be studied is placed on the third electrode, E_3 ; it is a removable stainless steel coverslip ($10 \times 75 \times 1$ mm) retained in a guide channel milled into the base. Electrode E_3 is rotatable about a central axis, and the coverslip can be moved laterally. A USB camera (fixed magnification of $\times 20$; model Discovery VMS-001, Veho Europe, Southampton, U.K.) was mounted opposite the probe to capture frames or video. For the experiments reported below the probe mixture (solvent mixture C) was delivered by a $500 \mu\text{L}$ syringe (Unimetrics Corp, Illinois). The liquid entering the probe tip was contained within a sealed system so that probe liquid would not siphon during liquid tip formation. The surface to be sampled might be a thin nonconducting or a dielectric material, although we did not investigate this. The underlying surface, however, needs to be sufficiently conducted so as to support an electric field that drives tip information.

All results reported were obtained using a commercially available plastic tip (part no 1004763, Advion Inc., Itacha, NY), with a syringe needle acting as an inner electrode, E_1 . The outer electrode E_2 was made by carefully applying a thin coat of silver-loaded electrically conductive paint (part no. 186-3600, RS Components Ltd., Northants, U.K.) to the lower 15 mm portion of the plastic tip and the end face of the tip. A fine wire was employed during coating to clear and prevent silver glue entering the tip. Silvered tips were made in batches and dried overnight in a vacuum oven at room temperature under a vacuum of $<10^{-2}$ Torr. Due to the size of the syringe needle and the internal diameter (i.d.) of the plastic tip, the electrode E_1 had to be set at $h_i = 4$ mm (Figure 1A). Additionally, the device operated sufficiently well with $V_1 \approx V_2$ that E_1 was electrically connected to E_2 for all experiments. The gap between the probe was set at $h_g = 0.50$ mm and the initial droplet depth (h_s) set at 0.30 mm throughout. This allowed consistent liquid tips of width, $w_c \approx 0.34$ mm, to be formed in all experiments.

Procedure for Sampling. A manual process was employed for sampling the surface as follows. The stainless steel coverslip was cleaned with distilled water followed by a MeOH wash. The analyte was pipetted as a $1 \mu\text{L}$ volume of working solution. A grid had been marked on the coverslip and used to note the positions where $1 \mu\text{L}$ analyte solutions were pipetted and allowed to dry to solid residues typically covering a spot of ≈ 2 mm diameter. Photographic recording of the size of the region coated by the analyte and knowledge of the tip contact position and size, formed at sampling, allowed an estimate of the fractional area sampled. The probe tip was positioned so that the sample was taken within the region spotted by the analyte. Individual photographs were taken of liquid tips to estimate the contact area, the type of liquid tip formed, and the contact width (w_c) before and after a liquid probe tip broke leaving a residue droplet of width (w_r). The coverslip with analyte spotted on its surface was inserted into the base electrode, E_3 assembly. Each individual analyte spot was positioned directly under the EF-LESA probe and sampled as follows: (i) voltages V_1 , V_2 , and V_3 were initially set to zero. A sessile droplet was formed on the EF-LESA probe tip by syringing solution C, through the central electrode, to a depth of $h_s = 0.30$ mm. This

dimension was monitored and recorded using the USB camera against a ruled scale to an estimated accuracy of ± 0.05 mm. The pipetted volume was estimated, from the on-screen display of the droplet dimensions and shape, to be ≈ 100 nL. The probe assembly was checked for leaks so that that liquid tip did not hydraulically draw liquid down through the tube, T. (ii) Voltage V_3 was switched from 0 to -500 V. (iii) Voltage $V_1 (=V_2)$ was slowly increased from 0 to $+350$ and then to $+700$ V (over 2 s). Typically $V_1 = +440$ V was required for the formation of the desired liquid tip (Figure 1B). (iv) The liquid tip was left in contact with the surface for 5 s prior to the probe assembly being retracted mechanically from the surface; that is, h_g was increased so that the liquid tip reproducibly retracted from the surface and broke in ≈ 2 s. (v) The probe was further retracted so that the residual sessile volume retained on the probe was extracted by manually syringing into a separate $50 \mu\text{L}$ syringe (Hamilton, Reno, NV) with $3 \mu\text{L}$ of solution B already in the syringe, to make up a volume of $\approx 3.1 \mu\text{L}$. This procedure was followed to clearly distinguish the plunger extracting the sample into the syringe. The fractional amount (F_s) injected into the MS is $2/3.1$ (i.e., 64.5% of that extracted by the probe). (vi) The top $2 \mu\text{L}$ of the volume was then directly injected using a $2 \mu\text{L}$ loop into the injector for SIM analysis. (vii) The probe was cleaned using washing method A outlined below. The probe tip could not be washed with a high content of organic solvent (e.g., methanol), as it was observed to erode and dissolve the silver coating on prolonged use.

Procedure for Washing the Probe and Coverslip.
Washing Method A. The probe was cleaned by eluting $15 \mu\text{L}$ of probe solvent C to a blank section of the coverslip and the probe tip was immersed in the resulting droplet for 1–2 s prior to removal. This was repeated three times.

Washing Method B. The coverslip was cleaned after removal by flooding the surface with water and then methanol. The plate was dried with a lint free wipe and allowed to evaporate to dryness. This was repeated three times.

Mass Spectrometry. Accurate mass data were obtained in selected ion mode (SIM) on a Thermo Scientific LTQ Orbitrap XL (Thermo Fisher Scientific GmbH, Bremen, Germany). A window of $m/z \pm 1$ was selected for SIM mode and centered on the target protonated molecule species, $[M + nH]^{n+}$. Samples were injected via a Rheodyne (Idex Health and Science GmbH, Wertheim-Modfeld, Germany) 6 port valve with a $2 \mu\text{L}$ loop and infused into the IonMax electrospray ionization (ESI) source operating with a $10 \mu\text{L}/\text{min}$ mobile phase flow of 50:50 water/methanol with 0.1% formic acid (solution B). The ESI source was operated in the positive ion mode with a spray voltage of $+3.5$ kV, nitrogen nebulizer gas flow of 10 (arbitrary units) indicated in Xcalibur (Thermo Fisher Scientific) at a capillary temperature of 275°C . The Orbitrap was operated at a mass resolution of 60000 (50% peak height definition at m/z 400) and mass measurement accuracy of ≈ 2 ppm (RMS). SIM spectra were recorded over approximately 2.4 to 3 s per scan. In our infusion setup, the SIM chromatograph had a peak width, at base, of approximately 30 to 40 s, corresponding to 10 to 15 scans per injection, sufficient for quantitation.

Safety Considerations. Exposed DC high voltages were present at the EF-LESA tip and on the surface sampling plate. The apparatus was insulated with appropriately machined PTFE parts to shroud high voltage components. Extreme care was employed to prevent electrical shock with the use of electrical isolation and earthing. Voltage sources were fitted

Table 1. Summary of Experimental Data^a

1. geometry of droplet	EF-LESA probe voltage	tip width measurement (mm)		cross-sectional area sampled per square millimeter (A_s)	ratio of cross-sectional areas for EF-LESA liquid tip (on and off) [$A_s(\text{on})/A_s(\text{off})$]	ratio of cross-sectional areas for the EF-LESA liquid tip (on) to the plastic tip area, based on $D_{\text{op}} = 0.85$ mm	
	on ^b	at neck, w_n	0.322 ± 0.080	0.080 ± 0.028	24.1%	14.1%	
	on ^b	at contact, w_c	0.344 ± 0.078	0.091 ± 0.028	26.6%	16.0%	
	off ^c	at neck, w_n	0.650 ± 0.035	0.332 ± 0.076			
	off ^c	at contact, w_c	0.660 ± 0.032	0.363 ± 0.069			
2. calibration	sample concentration (pmol/ μL)	internal standard concentration (pmol/ μL)	linearity R^2	standard error of blank (pmol/ μL)	standard error of intercept (pmol/ μL)	standard error of slope (response/unit concentration)	limit of detection (pmol/ μL)
leucine enkephalin	0	460	0.9936	0.48	0.27	0.0070	1.45
	0.9						
	1.8						
	3.6						
	7.2						
	14.4						
angiotensin II	0	460	0.9862	8.8	1.8	0.0014	26.4
	4.8						
	9.6						
	38.4						
	153.6						
3. recovery	% recovery from spot						
	sampling 1	sampling 2	sampling 3	sampling 4	sampling 5		
leucine enkephalin	89.5	21.4	7.8	5.5	6.3		
4. probe carry over	% carry over on probe						
	sampling 1 st blank spot	sampling 2 nd blank spot	sampling 3 rd blank spot	sampling 4 th blank spot	sampling 5 th blank spot		
leucine enkephalin	10.7	1.34	0.31	0.29	0.31		

^a(1) Geometry of the droplet (refer to Figure 1) both with electro-focusing on and off. (2) Analyte calibration figures of merit. (3) Recovery data from successive sampling of a single 1 μL spot of LE (14.4 pmol pipetted), and 1 μL of AT (153.6 pmol pipetted). (4) Analyte carry over on the probe from successive sampling of a single spot of 1 μL of LE (14.4 pmol pipetted) and 1 μL of AT (153.6 pmol pipetted). ^b $V_1 = V_2 = +440$ V, $V_3 = -500$ V. ^c $V_1 = V_2 = V_3 = 0$.

with current limiting 10^8 ohm blocking resistors to reduce risk and potential of electrical discharge. Full risk assessment was undertaken for the project prior to commencement. Material safety sheets for all chemicals employed were consulted in advance of performing experiments.

RESULTS AND DISCUSSION

Early studies involved evaluating prototype assemblies made in our workshop. Glass capillary tubing was initially used for the inner insulated tube, T, and various solid metal rods and tubes tested as a central electrode, E_1 (refer to Figure 1A). Liquid was fed by syringe pump operated manually to the probe tip with liquid passing around electrode E_1 or through the electrode, when a hollow tube was used. The tubing diameters of the inner electrode and insulated tube were chosen in the earliest prototypes to ensure liquid flow to the tip (i.e., $D_{E1} < D_{it}$) (Figure 1). These preliminary studies showed that electro-focusing of the sessile droplet meniscus was feasible with a trielectrode lens, and different dimensions for the electrode configuration were examined. Due to the home-built nature of the probe, we experienced difficulty with reproducibility and control of the liquid tip and opted to use an engineered plastic tip that gave good performance and stability of operation. Probe liquid was dispensed through the central electrode (syringe needle), which was a push fit sealed into the plastic tip. While this method employs off-line sampling of the liquid retained on the probe, it is expected that similar results could be obtained with a probe engineered to return

the sample directly into the mass spectrometer, although carry over is likely to be more important. In this study, we mechanically rupture the liquid bridge supporting the liquid tip in contact with the surface by moving the probe a fraction of a millimeter, prior to sampling the collapsed liquid tip, and transferring the residual liquid to the MS.

Experimental results including analytical figures of merit have been established for the EF-LESA probe's quantitative capability, limit of detection, recovery, and carry over (Table 1).

Liquid Tip Characterization. The liquid tip formed prior to the initiation of EHD spraying is a dynamic structure that can take on a number of shapes.^{22–25} Theoretical studies typically describe the characteristics of liquid droplet formation^{22,23,25} or electrospray.^{21,31} de la Mora's review²⁵ lays out the theory of "Taylor cone" formation, describing the balance between capillary and electrostatic forces, which are satisfied exactly on the liquid surface. The pressure differential, Δp , at the surface drives cone tip formation. In the special case of the Taylor cone the cone angle is (49.3°), for $\Delta p = 0$, and separates a family of menisci shapes both at $\Delta p < 0$ and $\Delta p > 0$. de la Mora states that "Rather than describing one Taylor cone structure, one should strive to describe a family of them", and describes regions of applied voltage where stable shapes exist, "one rounded" and the other "conical".²⁵

Figure 1 shows a schematic diagram of a sessile droplet forming a conical liquid tip, followed by contact with a surface, and finally detachment from the surface. These are four distinct phases of a single EF-LESA sampling event. The geometry of the EF-LESA device (Figure 1) employed was tip diameter,

$D_{\text{op}} = 0.85$ mm, electrode gap, $h_{\text{g}} = 0.50$ mm, and sessile droplet height (depth) of $h_{\text{s}} = 0.30$ mm. Experimentally liquid tip formation was initiated at a voltage $V_{\text{tf}} = +850$ V to $+1200$ V, $V_{\text{tf}} = (V_2 - V_3)$, comprising $V_2 \approx +350$ to $+700$ V (variable) and $V_3 = -500$ V (fixed). The average value for V_{tf} was (940 ± 52) V (refer to the Supporting Information).

The voltage for the onset of electrospray, V_{on} , is defined as²¹

$$V_{\text{on}} = \left(\frac{D_{\text{op}} \gamma \cos \theta}{4\epsilon_0} \right)^{1/2} \ln \left(\frac{8h_{\text{g}}}{D_{\text{op}}} \right) \quad (1.1)$$

where γ = surface tension, ϵ_0 = permittivity of vacuum, and $\theta = 49.3^\circ$, h_{g} and D_{op} are given above. Water with 0.1% formic acid was used in the EF-LESA probe as the mobile phase, assuming $\gamma = 0.73$ N m⁻¹ (i.e., water) and then $V_{\text{on}} = +1725$ V. The experimental value of V_{tf} for liquid tip formation was consistently measured to be in the range from $(+850$ to $+1200$ V), deviations from approximately $+940$ V were due to variation in setting, h_{s} . This establishes $V_{\text{tf}} \ll V_{\text{on}}$, demonstrating that a lower voltage mode, most likely to be spindle mode, is formed. The outer diameter, D_{E1} of the central electrode E_1 was chosen to be slightly greater than the inner diameter of the plastic tip D_{it} (i.e., $D_{\text{E1}} \leq D_{\text{it}}$), thus sealing the flow of liquid through the probe. In this case, a sessile droplet is preloaded by dispensing the liquid through the central electrode, E_1 . The volume of liquid in the sessile droplet (V_{g}) will be equal to the volume in the conical tip (V_{c}) (refer to Figure 1, panels A and B), and it is shown (in Supporting Information) that the height of the conical tip, h_{t} is

$$h_{\text{t}} = 12h_{\text{g}} \left(\frac{1}{8} + \left(\frac{h_{\text{s}}}{6D_{\text{op}}} \right)^2 \right) \quad (1.2)$$

where $h_{\text{t}} = 0.518$ mm, $h_{\text{s}} = 0.30$ mm, and $D_{\text{op}} = 0.85$ mm. Thus the liquid tip will just touch the surface prior to the tip reaching its full conical extent. Once the liquid tip touches the surface, it spreads to a contact face of diameter, w_{c} , and subsequently collapses (retracts) to a sessile droplet on the tip of diameter w_{oa} and height h_{sa} , with a residue droplet on the surface of diameter w_{r} and height h_{r} (refer to Figure 1C).

Feasibility of routine operation depends upon the ability to reproducibly form liquid tips of suitable shape, stability of position, transfer of surface analytes to the probe liquid, and consistency of time-in-contact. Van Berkel et al. studies report transfer efficiencies to a liquid microjunction probe in the range from 77% to 1%¹⁸, indicating that surface sampling efficiency varies considerably dependent on many parameters, including the type of sample, its solubility, and contact time.

Repeated measurements of liquid tip formation with a EF-LESA probe (with $h_{\text{s}} = 0.30$ mm, $h_{\text{g}} = 0.50$ mm, and solvent C as probe liquid) demonstrated that consistent liquid tips can be formed. The measured average size of the liquid tip was $\bar{w}_{\text{n}} = (0.322 \pm 0.080)$ mm, at the neck, and $\bar{w}_{\text{c}} = (0.344 \pm 0.078)$ mm at contact (from 28 successive measurements, refer to Table S1A in the Supporting Information). Figure 1C (inset) shows a well-formed liquid tip in contact with a surface, with a spindle tip diameter of ≈ 200 μm . The liquid tip size when no electro-focusing is applied (i.e., $V_1 = V_2 = V_3 = 0$) was measured to be $\bar{w}_{\text{n}} = (0.650 \pm 0.035)$ mm and $\bar{w}_{\text{c}} = (0.660 \pm 0.032)$ mm (refer to Table S1B of the Supporting Information). In this case, the probe was lowered to a point where the sessile droplet just touched and wetted the surface. The area of the liquid tip, at contact, is approximately circular and with quoted width, w_{c}

(at contact) and of the residue droplet, w_{r} are estimated diameters of equivalent circular spots. Characterization results, summarized in Table 1, indicate that the EF-LESA probe enhances the spatial resolution of surface sampling by a factor of four or a factor of eight based on the plastic tip diameter ($w_{\text{c}} \approx D_{\text{op}}$).

The size of the residue droplet on the coverslip, immediately after sampling, was measured for successive sampling events. The mean values of the residue droplet width were $\bar{w}_{\text{r}} = 0.34$ mm and height, $\bar{h}_{\text{r}} = 0.12$ mm corresponding to an average surface area, $A_{\text{r}} = 0.096 \pm 0.049$ mm² (1 s.d.) and an average residual volume, $V_{\text{r}} = 22 \pm 13$ nL (1 s.d.) (refer to Table S2 of the Supporting Information). In order to assess the limit of detection (LOD) per sampling event and recovery from the surface, the concentration of the coatings per unit area were estimated from the average area of coating, A_{c} , and the standard line concentration used (Table 1). Small but significant differences in the measured coating area were found, and dependent on the analyte used and its concentration, different analytes were observed to wet slightly differently. Nevertheless, these coatings were tested to assess quantitation. The area sampled (A_{s}) and the area analyte coated (A_{d}) and the recovery (R) were statistically measured, allowing the amount of analyte sampled to be calculated. Additionally, the fact that only 64.5% of analyte collected by syringe from the tip was injected into the MS had to be included in the calculations.

Estimation of EF-LESA Cross-Sectional Area Sampled and Recovery. To determine the recovery and LOD from a surface, the average surface area coated (A_{d}) had to be determined from depositing individual 1 μL pipet droplets for both analyte solutions and was estimated from images of the initial spot size. This estimation process assumes the analyte is evenly coated, which is unlikely; replicate and random sampling over the analyte spot will help to average out such effects. For 1 μL of pipet drops of (LE + IS) and (AT + IS) analyte solutions on the stainless steel coverslip, it was found that $A_{\text{d}} = 2.30 \pm 0.20$ mm² and $A_{\text{d}} = 3.16 \pm 0.50$ mm² (1 s.d.), respectively (see Table S3 of the Supporting Information). These results were reproducible with AT coverage being 39% greater than LE due to changed wetting properties of the solution.

The fractional amount (F_{s}) of the coated area (A_{d}) sampled is

$$F_{\text{s}} = \frac{A_{\text{s}}}{A_{\text{d}}} \quad (1.3)$$

and is 4.0% for LE + IS and 2.9% for AT.

As a separate test of recovery, a 14.4 pmol/ μL sample of LE was spotted (1 μL) in triplicate. Each spot was sampled five times without washing the probe tip and without a change in its physical position or movement of the coverslip. These results shown in Table 1 were compared to a loop injection of 0.37 pmol LE solution (i.e., amount of analyte deposited per spot) $\times F_{\text{s}} \times F_{\text{v}}$ pmol, which is a 100% recovery (see Supporting Information for full details). The % recovery was measured from the average of the three replicates, and for the first sampling was calculated to be 89.5% (Figure S1 of the Supporting Information). After five successive "samplings" of the same spot, the analyte signal reduced by a factor of 14 for LE.

100% recovery is an analyte signal = (amount of analyte on a spot) $\times F_{\text{s}} \times F_{\text{v}}$ pmol directly infused in the MS. This is equivalent to 100% recovery from sampling a 1 μL pipetted spot, where F_{s} is the fractional area sampled (4% for LE) and F_{v} is the fractional volume (=0.645) injected into the MS from the sample collected from the EF-LESA probe tip. For LE, 100%

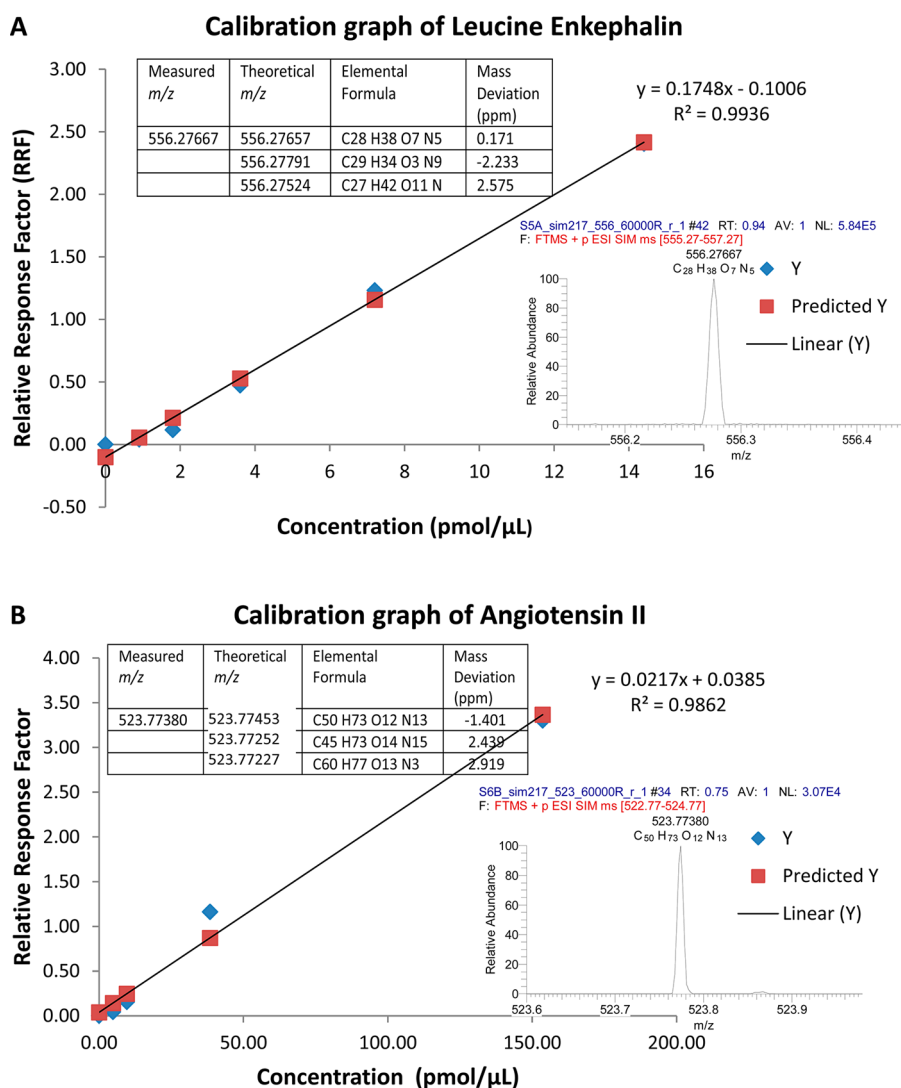


Figure 2. Calibration graphs for Leucine Enkephalin (LE) and Angiotensin II (AT). Data to construct the graphs are given in Table 1. The line fit for both graphs are shown in the figure and have corresponding R^2 values of 0.9936 and 0.9862 for LE and AT, respectively. SIM mass spectra and elemental composition assignments are inserted. Elemental formula search criteria are as follows: (A) leucine enkephalin: C₃₀, H₆₀, O₁₅, N₁₀ and (B) angiotensin II: C₆₀, H₁₀₀, O₁₅, N₁₅. Note: the results reported are those < 3 ppm and for protonated species only. Concentrations quoted are not accounting for partial injection volume (64.5%) and the surface area sampled.

recovery is the mass spectrometer signal equivalent to $14.4 \times 0.042 \times 0.645 = 0.37$ pmol directly infused.

Quantitation Using the EF-LESA Probe. An evaluation of the quantitative behavior of the EF-LESA probe was assessed for two biochemical compounds; leucine enkephalin (LE) and angiotensin II (AT) with *N*-acetylarginine (NAA) as an internal standard (IS). The linearity and LOD of the method was measured with calibration graphs (Figure 2, panels A and B), also showing SIM spectra and mass measurement data of the protonated species. LODs were 1.45 and 26.4 pmol/ μ L spotted for LE and AT, calculated from $3 \times$ standard error of the blank. Calibration graphs were measured at five nonzero concentrations with a matrix blank, S_o , and solvent blank, S_b , to ensure the reagent used for the calibration solvents was analyte free and to test for potential carry over and/or contamination of the coverslip sample plate prior to sampling standards. Three repeat measurements were made at each standard concentration, and the probe was washed after each sampling by cleaning method A to limit carry over. Each concentration level (including S_b and S_o) was pipetted onto the stainless steel

coverslip. Between concentration levels, the coverslip was removed and washed using cleaning method B; calibration was built from low to high concentration. Between each individual SIM scan, the injector was washed with 20 μ L of solvent B, with 2 μ L of blank (solvent B) injected and monitored by a SIM scan for the protonated analyte and IS as a check that no analyte and IS remained. The results show pleasing limits of quantitation demonstrating promise of the EF-LESA technique for quantitative surface sampling. Calibration graph linearity, R^2 for LE + IS and AT + IS were 0.9936 and 0.9862, respectively.

Use of the internal standard is important for quantitation as the area sampled during each liquid tip contact depends on the area wetted during that sampling event, the transfer of analyte to the probe, and residue on the probe tip. Using the fractional area sampled (F_s) (eq 1.3), the LOD can be estimated from

$$\begin{aligned} & \text{limit of detection (LOD) per sampling event} \\ & = \text{LOD} \times F_s \times F_v \end{aligned} \quad (1.4)$$

where LOD is derived for each calibration graph (Figure 2), F_s is the fractional surface area sampled, and F_v ($= 0.645$) is the partial injection volume into the MS of the collected sample. Thus the effective LOD per sampling event is estimated to be 38 fmol for LE and 494 fmol for AT.

Carry Over. Carry over was examined by spotting a cover-slip with three replicate spots of LE (concentration = 0.9 pmol/ μ L) containing IS and five S_b spots in triplicate. An LE spot was sampled first, then n individual S_b spots until extinction of the LE (and IS). This was repeated on two remaining replicate (and n S_b) spots, with a method A wash of the probe tip between each batch of LE spots to thoroughly clean the tip (the procedure is given in Supporting Information). Carry over for both LE and NAA is given in Table 1, for LE the signal fall off from 100% (initial analyte sample) is to 10.7%, 1.3%, 0.3%, 0.3%, and 0.3% and is an exponential decay (refer to Figure S2 of the Supporting Information).

CONCLUSIONS

A liquid sampling device has been designed and tested as a method for analytically sampling a surface at higher spatial resolution than is currently offered by a similar diameter liquid micro junction device. It has been demonstrated to operate as a single event liquid sampling device. This trielectrode system can shape the liquid meniscus, and its geometry has been optimized. For leucine enkephalin, an effective LOD per spot sampled is 38 fmol. A surface recovery of 90% and a carryover of 10.7% both for a 14.4 pmol/ μ L solution was demonstrated for a coated stainless steel surface. Calibration graphs for two analytes ($R^2 = 0.9936$ and 0.9862) showed good promise for quantitative operation. Operation of an EF-LESA probe over a few days showed improving performance with more consistent and reproducible liquid tips (30–200 μ m) being formed (refer to the Supporting Information). Further work will require new designs of engineered plastic tips to enable continuous flow operation and control of liquid tip formation by a voltage pulse sequence to the electro-focusing electrodes. These modifications could lead to enhancements and an automated sampling–analysis system coupled to MS, using aqueous media.

ASSOCIATED CONTENT

Supporting Information

Tables S1, S2, and S3: measurement of liquid tip and residue droplet dimensions. Figure S1: recovery characteristics of the EF-LESA probe. Figure S2: carry over characteristics of the EF-LESA probe. Figure S3: pictures of higher spatial resolution operation and the EF-LESA assembly. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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