SUPPORTING INFORMATION

Electro-focusing Liquid Extractive Surface Analysis (EF-LESA)

Coupled to Mass Spectrometry

by

A. Gareth Brenton* and A. Ruth Godfrey

Institute of Mass Spectrometry, College of Medicine
Swansea University, Singleton Park, Swansea SA2 8PP, U.K.

**Corresponding Author:** Prof. Gareth Brenton

Phone: +44 (0)1792 295300

Fax: +44 (0)1792 295554

E-mail: g.brenton@swansea.ac.uk
Measurement of Successive Liquid Tips And Residue Droplets To Assess Their Dimensions.
(Tables S1, S2 and S3)

The dimensions of the liquid tip were recorded from successive tip images taken on a x20 USB camera, calibrated against an engineer’s scaled ruler with 0.5 mm graduations. For simplicity it shall be considered that the tip has radial symmetry along its length so that it can be characterised by measurements of diameter and height indicated in Figure 1 and the text of the manuscript.

|   | (A)     |          |   | (B)     |          |
|---|---------|----------|---|---------|----------|
|   | n   | $w_n$/mm | $w_c$/mm |   | $w_n$/mm | $w_c$/mm |
|---|-----|---------|---------|---|---------|---------|
| 1 |  1  | 0.26    | 0.30    |   | 0.65    | 0.68    |
| 2 |  2  | 0.30    | 0.30    |   | 0.55    | 0.68    |
| 3 |  4  | 0.19    | 0.19    |   | 0.68    | 0.62    |
| 4 |  5  | 0.23    | 0.26    |   | 0.60    | 0.60    |
| 5 |  6  | 0.28    | 0.30    |   | 0.60    | 0.62    |
| 6 |  7  | 0.21    | 0.26    |   | 0.68    | 0.68    |
| 7 |  8  | 0.34    | 0.36    |   | 0.65    | 0.65    |
| 8 |  9  | 0.34    | 0.36    |   | 0.60    | 0.60    |
| 9 | 10  | 0.34    | 0.36    |   | 0.65    | 0.65    |
|10 | 11  | 0.34    | 0.34    |   | 0.70    | 0.72    |
|11 | 12  | 0.23    | 0.26    |   | 0.68    | 0.68    |
|12 | 13  | 0.38    | 0.40    |   | 0.68    | 0.68    |
|13 | 14  | 0.36    | 0.38    |   | 0.66    | 0.68    |
|14 | 15  | 0.36    | 0.42    |   | 0.60    | 0.60    |
|15 | 16  | 0.30    | 0.31    |   | 0.68    | 0.68    |
|16 | 17  | 0.28    | 0.30    |   | 0.67    | 0.67    |
|17 | 18  | 0.30    | 0.33    |   | 0.65    | 0.67    |
|18 | 19  | 0.43    | 0.45    |   | 0.62    | 0.62    |
|19 | 20  | 0.43    | 0.45    |   | 0.68    | 0.68    |
|20 | 21  | 0.17    | 0.19    |   | 0.65    | 0.67    |
|21 | 22  | 0.38    | 0.38    |   | 0.66    | 0.68    |
|22 | 23  | 0.43    | 0.42    |   | 0.62    | 0.64    |
|23 | 24  | 0.43    | 0.44    |   | 0.67    | 0.67    |
|24 | 25  | 0.34    | 0.36    |   | 0.69    | 0.70    |
|25 | 26  | 0.36    | 0.38    |   | 0.66    | 0.68    |
|26 | 27  | 0.42    | 0.45    |   | 0.67    | 0.68    |
|27 | 28  | 0.42    | 0.45    |   | 0.65    | 0.66    |
|28 |     | 0.20    | 0.23    |   | 0.66    | 0.67    |

|   | mean $w_n = 0.323$ | mean $w_c = 0.344$ |   | mean $w_n = 0.65$ | mean $w_c = 0.66$ |
|---|-------------------|-------------------|---|------------------|------------------|
| 1 std dev | $\pm 0.080$ | $\pm 0.078$ |   | $\pm 0.035$ | $\pm 0.032$ |
Table S1. Measurement of the liquid tip width, $w_n$ (at the neck) and $w_c$ (at contact) for 28 successive liquid tips formed on a stainless steel surface with $h_s = 0.30$ mm, $h_b = 0.50$ mm (refer to Figure 1). The average values of the widths $\bar{w}_n$ and $\bar{w}_c$ (errors to 1 s.d.) are tabulated for, (A) electro-focusing on, i.e. $V_1 (= V_2) = +440$V and $V_3 = -500$ V, and (B) electro-focusing off, $V_1 = V_2 = V_3 = 0$.

Table S2

| n  | $w_r$/mm | $h_r$/mm | Vol /nL |
|----|-----------|-----------|---------|
| 1  | 0.40      | 0.10      | 35      |
| 2  | 0.30      | 0.10      | 15      |
| 3  | 0.40      | 0.15      | 38      |
| 4  | 0.35      | 0.16      | 26      |
| 5  | 0.20      | 0.08      | 5       |
| 6  | 0.20      | 0.15      | 6       |
| 7  | 0.50      | 0.15      | 20      |
| 8  | 0.40      | 0.12      | 36      |
| 9  | 0.30      | 0.10      | 15      |
| 10 | 0.28      | 0.08      | 8       |
| 11 | 0.40      | 0.15      | 36      |

| mean | $\bar{w}_r = 0.339$ | $\bar{h}_r = 0.122$ | $V_g = 21.8$ |
| 1 std dev | $\pm 0.093$ | $\pm 0.031$ | $\pm 12.9$ |

Table S2. Exemplar data illustrating the residue droplet dimensions, on a stainless steel surface immediately after liquid tip contact is broken, and estimation of the mean width $\bar{w}_r$, height $\bar{h}_r$, and volume, $V_g$ calculated from the width $w_r$ and height, $h_r$ of 11 successive liquid tips formed with $h_s = 0.30$ mm, $h_b = 0.50$ mm (refer to Figure 1) and with electro-focusing, i.e. $V_1 (= V_2) = +440$V and $V_3 = -500$ V.
Table S3. Measurement of the mean surface area of 20 successive pipetted drops of 1 µL of Leucine Enkephalin (LE) at a concentration of 14.4 pmol/µL dissolved in 100% water and 1µL of Angiotensin (AT) at a concentration of 153.6 pmol/µL dissolved in 100% water, on the surface of a stainless steel plate. This data is used to estimate the mean area, $A_d$, of each analyte spotted so that the analytical figures of merit can be calculated from EF-LESA sampling experiments. Errors are to 1 s.d.
Recovery Characteristics Of The EF-LESA Probe.

Recovery was measured for an initial sampling of 14.4 pmol/µL of a LE spot (1µL), followed by four successive samplings of the same spot for three replicate experiments, without washing between sampling events. The signal fall off from 100% (initial analyte sample) was: 89.5%, 21.4%, 7.8%, 5.4% and 6.3%. The data is plotted below in Figure S1, error bars are calculated to 2 s.d. (95% confidence). A nominal recovery of 90% is achieved. Further work with different analytes, substrates and larger data size n is required to further accurately characterise recovery.

100% recovery is an analyte signal = (Amount of analyte on a spot) × F_s × F_v pmol directly infused in the MS. This is equivalent to 100% recovery from sampling a 1µL pipetted spot, where F_s is the fractional area sampled (see main text, F_s = A_s/A_d, 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% recovery is the mass spectrometer signal equivalent to 14.4 × 0.042 × 0.645 = 0.37 pmol directly infused.

Figure S1. Recovery characteristics of the EF-LESA probe for an initial sampling of a 14.4 pmol/µL LE pipetted spot (1 µL) followed by 4 successive samplings of the same spot without washing between sampling events.
Carry over Characteristics Of The EF-LESA Probe.

Carry over was measured for an initial sampling of 14.4 pmol/µL LE spot (1µL deposited), followed by five successive samplings of neighbouring blank spots, i.e. spots of blank solvent, 100% water. The probe was not washed between successive samplings. The signal fall off from 100% (initial analyte signal equivalent to 0.37 pmol) was: 10.7%, 1.3%, 0.3%, 0.3% and 0.3%. The data is shown below in Figure S2, errors are calculated to 2 s.d. (95% confidence). It can be seen that the LE signal falls off exponentially and follows

\[ c = c_0 e^{-kn} \]

where c is the amount (or concentration) remaining after n samplings, \( c_0 \) is the initial amount (or concentration) and k is a constant \( \approx 2 \).

Figure S2. Carry over characteristics of the EF-LESA probe for sampling 14.4 pmol/µL of a LE pipetted spot followed by 5 successive samples of blank solvent spots without washing between sampling events.
Calculation of the conical tip height, $h_t$

The volume of liquid in the sessile droplet, $V_g$, will be equal to the volume in the conical tip, $V_c$ (refer to Figures 1A and 1B), where

$$V_g = \pi h_s \left( \frac{D_{op}}{8} + \frac{h_s^2}{6} \right)$$

and

$$V_c = \frac{1}{3} \pi \left( \frac{D_{op}}{2} \right)^2$$

solving gives the height of the conical tip, $h_t$ (refer to Figure 1B and equation 1.2 in the main manuscript)

$$h_t = 12 h_g \left( \frac{1}{8} + \left( \frac{h_s}{6D_{op}} \right)^2 \right)$$

$h_t = 0.518$ mm, for the experimental settings used throughout all experiments of $h_s = 0.30$ mm and $D_{op} = 0.85$ mm.
Higher Spatial Resolution Operation

It was found after a few days the EF-LESA probe became more stable and reproducible in operation; some probes gave higher performance routinely. It is not clear what process conditioning takes, whether it relates to improved wettability to the circumference of the plastic tip or reducing imperfections of the silver coating to produce more reproducible and finer liquid tips. Two cases of finer liquid tip formation were observed (see Figures S3A and S3B). Both cases were initiated when a higher voltage than normal $V_1$ voltage is applied i.e. $\approx +500$ to $+1200$ V, inducing other tip modes. The voltage is controlled to be below ESI onset and immediately followed by reducing $V_1$ quickly to $\approx +450$ V. The first case (Figure S3A), a tip is formed that is $<100$ µm. A second mode (Figure S3B) is observed to show a glow discharge established and a liquid tip size $\approx 100 – 200$ µm. Without adaptation of the apparatus this cannot unambiguously be verified and requires further investigation. These modes were observed to be reproducible.

Figure S3. Pictures of higher spatial resolution operation taken of liquid tips under higher initial voltage conditions for $V_1 (=V_2)$ where the probe surface has conditioned to produce fine liquid tips, (A) a stable fine tip is formed by reducing the voltage immediately on liquid tip contact (a reflection of the tip can be seen in this image); (B) the case where a glow discharge appears to be formed with a fine but ill defined liquid tip (the photograph was taken at an angle, the tip was mounted vertically).
Photograph of the EF-LESA assembly

The apparatus consists of a central electrode, $E_1$ a syringe within a plastic tip, $T$ coated in a silver conducting glue to form the outer electrode, $E_2$. A third electrode, $E_3$ is positioned below and acts as the sample stage. A cover slip made of stainless steel can slide in a groove in $E_3$ and be readily removed to change samples.