Automated Surface Sampling Electrospray Mass Spectrometry

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OVERVIEW

- HandsFree TLC/MS© software was developed to control the surface sampling probe-to-surface distance during operation of a surface sampling electrospray system using an image analysis approach.
- The software enables "hands-free":
  - Formation of the liquid microjunction used to sample material from the surface.
  - Re-optimization of the microjunction thickness during a surface scan to achieve a fully automated surface sampling system.
- The practical implementation of the monitoring and automated adjustment of the sampling probe-to-surface distance and liquid microjunction thickness are presented.
- The added capabilities of the surface sampling electrospray system enabled through this software are illustrated.

INTRODUCTION

- In several reports we have demonstrated the use of a combined surface sampling probe/electrospray (ES) emitter as the interface for coupling thin-layer chromatography (TLC) and mass spectrometry (MS)[1-4].
  - This device exploits a surface sampling probe-to-surface liquid microjunction and a self-aspirating ES emitter to sample material from the stationary phase of developed TLC plates for analysis by ES-MS.
- The analytical utility of this TLC/ES-MS coupling has been demonstrated by the qualitative[2,3] and quantitative[4] analysis of a variety of analytes separated on commercially available reversed-phase (RP) C8 and C18 TLC plates.
- Computer-controlled scanning of one development lane at a time on a TLC plate has been accomplished in a prior report[3], however, initial formation of the liquid microjunction and maintenance of the optimum junction thickness during the course of an experiment have required manual adjustments by a skilled operator.
- Analysis of additional development lanes or "spot" sampling required further manual positioning of the surface relative to the probe.
- We report the implementation of computer-controlled adjustment of the probe-to-surface distance to automate the surface sampling system.
- The HandsFree TLC/MS© software package discussed is also used for processing of the acquired data for display.
- The added capabilities of the surface sampling electrospray system afforded through this software are illustrated by
  - an example of automated scanning of multiple development lanes of rhodamine dyes separated on a reversed-phase C8 TLC plate
  - imaging inked lettering (containing a rhodamine dye) on paper surfaces

EXPERIMENTAL

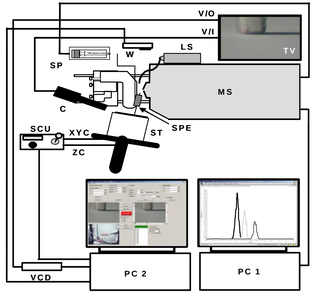
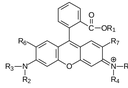


Figure 1. Schematic illustration of the automated TLC/ES-MS experimental setup (not to scale).

- *LS* = light source, *SP* = syringe pump, *W* = webcam, *TV* = TV monitor, *V/I* = video in, *V/O* = video out, *MS* = mass spectrometer, *C* = camera, *PH* = plate holder, *SPE* = sampling probe emitter, *ST* = XYZ stage, *SCU* = stage control unit, *XYC* = XY axis control, *ZC* = Z axis control, *VCD* = video capture device, *PC1* = computer running Analyst software for control of the mass spectrometer, *PC2* = computer running HandsFree TLC/MS© software for stage control, for data acquisition from the stage control unit, and for image acquisition from the webcam and the camera.
- An MS2000 XYZ stage (Applied Scientific Instrumentation Inc., Eugene, OR) was used to move the surface to be sampled relative to the stationary surface sampling probe/emitter during an experiment. The surface was held in the vertical XY plane, perpendicular to the Z axis of the sampling probe. The platform was operated under computer control using HandsFree TLC/MS© software version 1.2 developed in-house.



rhodamine 6G (cpd 1, *m/z* 443)
$R_1, R_3, R_4 = CH_2CH_3$;
$R_2, R_5 = H$; $R_6, R_7 = CH_3$

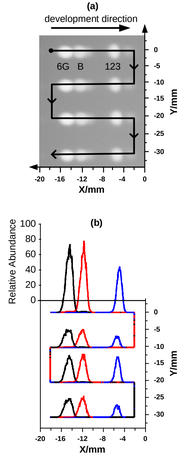
rhodamine B (cpd 2, *m/z* 443)
$R_1, R_6, R_7 = H$
$R_2, R_3, R_4, R_5 = CH_2CH_3$

rhodamine 123 (cpd 3, *m/z* 345)
$R_1 = CH_3$
$R_2, R_3, R_4, R_5, R_6, R_7 = H$

Figure 2. Structure and mass-to-charge ratio for the rhodamine dyes

- Chemicals. HPLC grade methanol and water were purchased from Burdick & Jackson (Muskegon, MI). Ammonium acetate (99.999%) and acetic acid (PPB/Teflon Grade) were obtained from Sigma Aldrich (Milwaukee, WI). Rhodamines 6G (cpd 1) and B (cpd 2) were purchased from Eastman Kodak Company (Rochester, NY) and rhodamine 123 (cpd 3) was obtained from Sigma Aldrich (Figure 2). Stock solutions were prepared by dissolving the dyes in methanol at a concentration of 200 ng/μL (0.45 nmol/μL for cpds 1 and 2; 0.57 nmol/μL for 3). Analytical standards were prepared from these stock solutions by dilution with methanol for TLC spotting and 60/40 (v/v) methanol/water with 0.1% acetic acid by volume for ES-MS optimization.
- Thin-layer chromatography. TLC was carried out using hydrophobic Merck RP C8 plates (P/N 13725/5, EM Science, Gibbstown, NJ). Dye standards were spotted on the plates in 0.5 μL aliquots. Plates were developed with 80/20 (v/v) methanol/water containing about 200 mM ammonium acetate. Developed plates were dried in an oven (110˚C) for 30 minutes just prior to mass spectrometric analysis. Photographs of the developed TLC plate were taken with a Coolpix 990 digital camera (Nikon, Tokyo, Japan) using long wavelength UV illumination.
- Inked Lettering on Paper. A red ink Stamp-Ever "COPY" logo stamp (U.S. Stamp and Sign, Cookeville, TN) and a red ink pen were used to impress the word "COPY" and write the word "OBMS", respectively, onto hp LaserJet Tough Paper (Hewlett-Packard Company, Palo Alto, CA). The red dye component of the stamp ink and the pen ink was compound 2 determined by ES-MS and MS/MS analysis (not shown). The Tough Paper (cut to 100 x 100 mm) was fixed to a glass backing plate (100 x 100 mm) with UHUstic glue adhesive (Manco Inc., Avon, OH). The tough paper was sufficiently hydrophobic to allow surface sampling using 60/40 (v/v) methanol/water (0.1 % acetic acid) as the eluting solvent system without wetting of the paper surface. Photographs of the impressed "COPY" logo were taken in white light.
- Surface Sampling Probe/ES-MS System. The mass spectrometer was a 4000 QTrap (MDS SCIEX, Concord Ontario, Canada) operated using Analyst software version 1.4 on computer PC 1. Data were collected in selected reaction monitoring (SRM) mode with the dissociation conditions optimized separately for each of the rhodamine dyes. The SRM transitions monitored were *m/z* 443 → *m/z* 399 (compound 1), *m/z* 443 → *m/z* 415 (compound 2), and *m/z* 345 → *m/z* 285 (compound 3) with a 500 ms dwell time for each transition. The eluting solvent used to sample material from the TLC plates was pumped (solvent pump, SP) from a 1.0 mL glass syringe with a syringe pump (Harvard Apparatus, Inc., Cambridge, MA). An Agilent 1100 LC pump (Agilent Technologies, Inc., Palo Alto, CA) was used to deliver the eluting solvent for the analysis of the ink stamp on the Tough Paper. The aspiration rate of the probe/emitter was matched to the pumped flow rate by adjustment of the nebulizing gas (nitrogen) flow rate. Analyte standard solutions used to optimize mass spectrometer detection conditions were infused through the probe/emitter via the eluting solvent line.
- SAFETY CONSIDERATIONS. The surface sampling probe/emitter floats at the high ES voltage and appropriate shields and interlocks should be used to avoid accidental contact with this device.

RESULTS AND DISCUSSIONS



- The plate was mounted in the XYZ stage and the length and distance of the lanes to be scanned set in the software. The surface was manually moved to position the probe above the starting point. The probe-to-surface distance was set at about ca. 300-400 μm. Following software-controlled formation of the liquid junction the actual surface scan began (44 μm/s) in manual synchronization with mass spectral data acquisition employing SRM detection. At the end of the multiple-lane scan the software moved the surface back from the probe 200 μm severing the liquid junction and the mass spectral data acquisition was stopped.
- The data files were recorded by the two computers into separate files, i.e. a file contained the time-*x*-*y*-*z* data sets (file A) stored in the computer that controlled the XYZ stage (PC 2 in Figure 1) and three files (files B-D) containing the time-SRM signals collected for compounds 1, 2, and 3 stored on the computer attached to the mass spectrometer (PC 1 in Figure 1), respectively. Files A and B were loaded into the software on PC 2 and by synchronizing the time axis in the files the program output one file containing the corresponding *x*-*y*-SRM ion current signal for compound 1. Similarly, files including *x*-*y*-SRM ion current signal data sets for compounds 2 and 3 were generated by loading files A and C and files A and D into the software, respectively.

Figure 3. (a) Photograph of a RP C8 TLC plate showing the separated components of a spotted mixture (0.5 μL, 65 ng each component) of rhodamine 6G (1), rhodamine B (2), and rhodamine 123 (3) showing the route of the probe during the automated surface sampling. (b) SRM ion current profiles for compound 1 (black line, *m/z* 443 → *m/z* 399), 2 (red line, *m/z* 443 → *m/z* 415) and 3 (blue line, *m/z* 345 → *m/z* 285) obtained during the automated multiple-lane scan. Development lanes were scanned at 44 μm/s using an eluting solvent (60/40 methanol/water, 0.1% by volume acetic acid) flow rate of 15 μL/min and a 500 ms dwell time for each transition.

- Figure 3b indicates that the peak heights (and areas) for the respective dyes were obviously not equivalent in the four lanes even though the same amount of material was spotted in each lane. The most likely explanation for this observation was off-center sampling of the bands. We have previously observed this effect when manually analyzing replicate development lanes on a plate. In this current case, lane 1 (Y = 0 mm) was most accurately lined up to start the experiment and the positions of the three other lanes were calculated and the scan parameters entered into the program.
- A possible but experimentally unexplored contributing factor to differences in intensity between lanes scanned in different directions may be the less than perfectly perpendicular position of the probe and the surface. This condition resulted in small differences in the average liquid junction thickness (ALJT, calculated by averaging the probe/surface distance during a lane scan) when scanning a flat surface in opposite directions as indicated in Figure 4. The software reoptimizes the liquid junction thickness when it reaches the lower or the upper limit of the required range by setting the optimal probe-to-surface distance (i.e. the center value of that range). For the experiments shown in Figure 3 the ALJT for both lanes 1 (Y = 0) and 3 (Y = -20) was 36 μm, while the ALJT for both lanes 2 (Y = -10) and 4 (Y = -30), which were scanned in the opposite direction was 40 μm. The actual effect of this small difference in ALJT on signal intensity will need further investigation. In any case, for qualitative work (i.e., compound identification) these changes in intensity would not compromise an analysis. Using isotopically labeled internal standards developed along with the analytes has been shown to eliminate this issue in quantitative analysis with our surface sampling system[4].

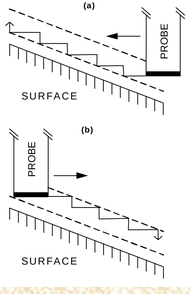


Figure 4. (a) Representation of the continuous reoptimization of the probe-to-surface distance illustrating the cause for different ALJT values observed in opposite surface scan directions (indicated by the arrows).

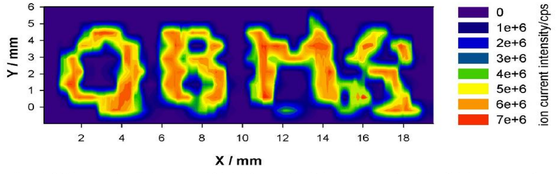


Figure 5. SRM ion current profile for compound 2 (*m/z* 443 → *m/z* 415) obtained during an automated multiple-lane scan over the "OBMS" word written on tough paper by hand. The lanes were scanned at 88 μm/s using an eluting solvent (60/40 methanol/water, 0.1% by volume acetic acid) flow rate of 15 μL/min.

- The lettering measured approximately 0.6 cm x 1.8 cm as illustrated in Figure 5. In this experiment, 20 lanes were scanned with 1 mm distance parallel with the Y-axis. The "OBMS" word clearly recognizable in the ion current signal intensity vs. surface location filled contour plot.

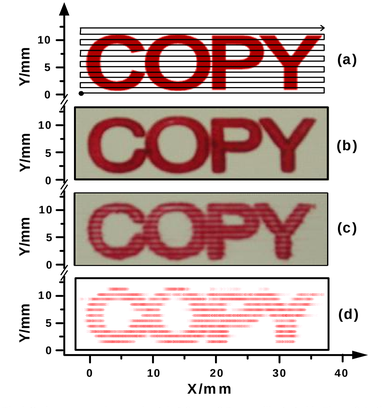


Figure 6. (a) Schematic representation of the impressed "COPY" logo showing the route of the surface sampler during the lane scanning. (b) Picture before lane scanning of the impressed "COPY" logo on tough paper applied by a stamp containing rhodamine B (cpd 2). (c) Picture of the impressed "COPY" logo on tough paper after the lane scanning. (d) Normalized SRM ion current profile for compound 2 (*m/z* 443 → *m/z* 415) obtained during the automated multiple-lane scan. Darker red color represents higher SRM ion signal at that surface location. The lanes were scanned at 88 μm/s using an eluting solvent (60/40 methanol/water, 0.1% by volume acetic acid) flow rate of 15 μL/min and a 500 ms dwell time.

- The lettering measured approximately 1.0 cm x 3.7 cm as illustrated in Figure 6. In this experiment, 13 lanes were scanned parallel with the X axis and the distance between the lanes was selected as 1.0 mm.
- The data files containing time, *x*, *y*, *z* position and time, SRM signal for compound 2 were processed by the software in a similar fashion to the TLC lane scans discussed in Figure 3. There was a direct correlation between the photograph of the scanned lettering (Figure 6c) and the scanned image (Figure 6d).
- The data in Figure 6 took 94 mins to acquire. During this entire time, the surface sampling system was under complete computer control; no operator intervention was required. The data in Figure 6d also show that the read-out resolution in these experiments was sufficient to create a readable image of the inked letters. This resolution might not be suitable for other imaging applications (e.g. smaller font lettering). With the current sampling probe (635 μm outer diameter), read-out resolution might be improved from 1.0 mm separated lane scan to about 650 μm separated scans. A smaller diameter probe could be used to further improve resolution by decreasing the necessary distance between lane scans. However, as the probe diameter shrinks less material will be sampled from the surface and signal levels would be reduced.

CONCLUSIONS

- HandsFree TLC/MS© software automates formation and real-time re-optimization of the sampling probe-to-surface distance using image analysis.
- We demonstrated automated multiple-lane scans along a surface of a TLC plate.
- Imaging of inked lettering on paper was also presented.
- The software synchronized the mass spectrometric data collected during the surface scan with the surface location where those data were measured.
- From these data it was possible to produce 2D contour plots, where the X and Y axis of the plot corresponded to the horizontal and vertical range of the scanned surface, and the Z axis or the pixel color represented the corresponding mass spectral signal intensity, respectively.

NEAR FUTURE

- Fabrication of an emitter with a smaller (inner and outer) diameter should increase the resolution of the surface imaging.
- With the ability to now precisely control the liquid microjunction thickness, we will more fully explore sampling efficiency and mass spectral signal intensity dependence on liquid junction thickness to better understand the sampling process.
- Read out of affinity arrays (e.g. protein chips): expanding the degree of automation to allow "spot-sampling" rather than just lane scanning.
- In general: imaging different surfaces using mass spectrometric detection, e.g. read out tissue samples.

REFERENCES

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