

## OVERVIEW

Desorption Electro spray Ionization is demonstrated as a means to couple thin-layer chromatography with mass spectrometry

- The experimental setup and its optimization are described
- Fundamentals and practical application of the technique are demonstrated for a variety of TLC plate stationary phases and a variety of analytes

The instrumentation and ability to perform analyte electrochemistry at an electrode surface impinged upon by the Desorption Electro spray Ionization plume is demonstrated

## INTRODUCTION

Desorption electro spray ionization (DESI) is a new atmospheric pressure desorption ionization method introduced by Cooks and co-workers [1] for the analysis of analytes on surfaces

- This group described direct analysis of compounds on a number of different surface types using DESI-MS, including leather and nitrile gloves, a tomato skin, a medicine tablet and even a blood drop on a finger, for a variety of analytes, from small pharmaceutical molecules to large biopolymers

It appears that gas-phase ions can be generated from those compound types typically amenable to analysis by ES-MS (e.g., ionic and polar molecules and biopolymers)

More non-polar analytes, such as carotenoids, that are not particularly amenable to analysis by ES-MS may possibly be analyzed by DESI also

- These compounds appear to be ionized via electron-transfer processes during the desorption process or in the gas-phase following desorption

Another analytical surface that might be directly analyzed with DESI-MS is the chromatographic phase of a thin-layer chromatography (TLC) plate

DESI as a TLC/MS interface overcomes the limitations of other recently used interfaces

- Matrix-assisted laser desorption/ionization (MALDI) involves extensive post separation preparation of the plates prior to analysis, the need for specialized plates, low mass chemical noise from the MALDI matrix, and the requirement that the analysis be carried out in the vacuum chamber of the mass spectrometer have limited the technique [2]
- DESI circumvents surface wetting issues that have limited the surface sampling probe ES approach to TLC/MS [3]

We report the successful coupling of TLC and MS using DESI for a variety of hydrophobic and wettable TLC stationary phases [4]

We also show preliminary results using a planar electrode cell and DESI to perform electrochemistry of analytes impinged onto the electrode surfaces by DESI with subsequent mass spectrometric analysis of the reaction products

- Takáts, et al., *Science* 2004, 306, 471.
- Gusev, *Fresenius' J. Anal. Chem.* 2000, 366, 691.
- Van Berkel, et al., *Anal. Chem.* 2002, 74, 6216.
- Van Berkel, et al., *Anal. Chem.* 2005, 77, 1207.

# Desorption Electro spray Ionization for the Analysis of Analytes on Surfaces

Gary J. Van Berkel, Michael J. Ford, Michael A. Deibel

Organic and Biological Mass Spectrometry Group, Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6131

## EXPERIMENTAL

Figure 1. Structure and mass-to-charge ratio observed for the compounds investigated in the TLC/DESI-MS studies

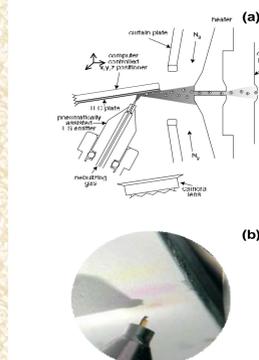
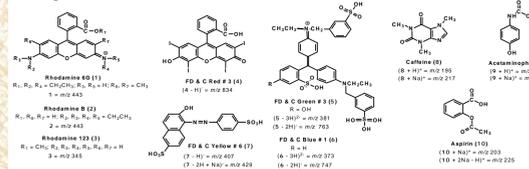
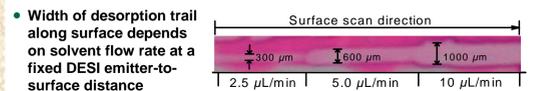


Figure 2. (a) Schematic illustration of the TLC/DESI-MS experimental setup and (b) a color photograph of the DESI emitter and the TLC plate as viewed through the camera monitor during a TLC/DESI-MS experiment. Separated bands of rhodamine 6G (1, orange band), rhodamine B (2, pink band), and rhodamine 123 (3, yellow band) are observed on the RP C8 TLC plate. The same basic setup was used to hold the electrode surface for the electrochemical studies.

The mass spectrometer was a 4000 QTrap (MDS SCIEX, Concord Ontario, Canada) hybrid triple quadrupole linear ion trap fitted with the nanospray interface orifice. The pneumatically assisted ES emitter was fashioned from a prototype "microionspray" head supplied by MDS SCIEX. The spray emitter was a 5.2 cm long taper tip fused silica capillary (100  $\mu\text{m}$ -o.d., 360  $\mu\text{m}$ -i.d., New Objective, Woburn, MA). The inner diameter of the nebulizing tube was 500  $\mu\text{m}$  providing a nebulizing gas (nitrogen) jet annulus area of about  $1.5 \times 10^{-7} \text{ m}^2$ . The ES emitter was mounted about 4 mm from the curtain plate of the mass spectrometer at an approximate 50 degree angle relative to the surface to be analyzed. Optimum spacing of the nebulizer tip was 0.5 to 1.0 mm from the surfaces investigated using a nebulizer gas flow rate of approximately 2.4 L/s (275 m/s nebulizing gas jet linear velocity). The surfaces were mounted so the edge nearest the mass spectrometer was in line with the far edge of the heater orifice and about 10 degrees off axis from the line of sight down the sampling orifice. TLC plates were cut or samples separated in development lanes near the plate edge to align the sample bands with the DESI plume. The ES high voltage ( $\pm 4.0$  - 4.5 kV) was applied to the stainless steel body of the microionspray head. The ES solvent was delivered to the emitter by a syringe driver, controlled by the mass spectrometer software, from a 500  $\mu\text{L}$  glass syringe connected to the emitter with about 30 cm of 254  $\mu\text{m}$ -i.d. (1/16 in. o.d.) Teflon tubing.

The MS2000 x, y, z robotic platform (Applied Scientific Instrumentation Inc., Eugene, OR) and control software used to manipulate the surfaces relative to the stationary DESI emitter was operated manually or by computer control in the x, y plane for development lane scanning. A side arm extension fabricated from plexiglass was attached to the platform to reach closer to the curtain plate of the mass spectrometer (Figure 2a). The surface was held on this extension in the vertical x, y plane using double sided tape, at approximately a 50 degree angle to the DESI emitter and about a 10 degree angle from the axis of the sampling orifice of the mass spectrometer. For initial positioning and adjustment the platform was controlled with the joystick (x, y) and jog wheel (z - surface - to - DESI emitter axis) of the manual control unit. The position of the surface relative to the stationary DESI emitter was monitored with a Panasonic GP-KR222 closed circuit camera (Panasonic Matsushita Electric Corporation of America, Secaucus, NJ) with an Optem 70 XL zoom lens (Thales Optem Inc., Fairport, NY). The camera image was output to the mass spectrometer PC and the image monitored and captured using VidCap32 software (Microsoft, Redmond, CA) (Figure 2b).

## TLC/DESI-MS



Width of desorption trail along surface flow rate at a fixed DESI emitter-to-surface distance

Read out Resolution,  $R = d/[(W_1 + W_2)/2]$  where  $d$  is the distance between the band centers and  $W_1$  and  $W_2$  are the widths of the two bands, respectively

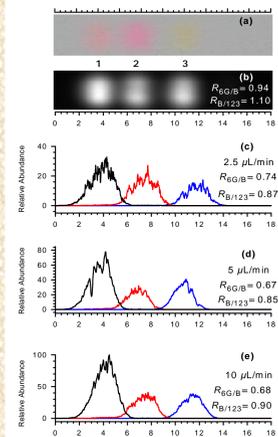


Figure 4. (a) Color photograph and (b) fluorescence image of RP C2 TLC separation of a mixture (50 ng each) of rhodamines 6G (1), B (2), and 123 (3) and the positive ion SRM ion current profiles for 1 ( $m/z$  443  $\rightarrow$   $m/z$  415, black trace), 2 ( $m/z$  443  $\rightarrow$   $m/z$  415, red trace), and 3 ( $m/z$  345  $\rightarrow$   $m/z$  285, blue trace) obtained during development lane scans (44  $\mu\text{m/s}$ ) of replicate development lanes using a DESI solvent (methanol) flow rate of (c) 2.5, (d) 5.0 and (e) 10  $\mu\text{L}/\text{min}$ . Dwell time was 100 ms dwell for each transition. Signal levels were normalized to maximum signal level in panel (e).

- Increasing the solvent flow rate up to 10  $\mu\text{L}/\text{min}$  increases the mass spectral signal during a surface scan along a development lane
- Read out resolution was largely unchanged with changes in solvent flow rate

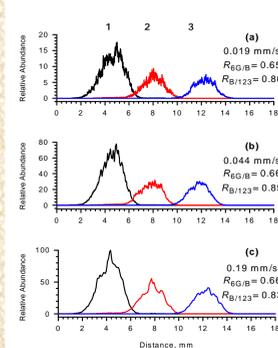


Figure 5. Positive ion SRM ion current profiles for 1 ( $m/z$  443  $\rightarrow$   $m/z$  415, black trace), 2 ( $m/z$  443  $\rightarrow$   $m/z$  415, red trace), and 3 ( $m/z$  345  $\rightarrow$   $m/z$  285, blue trace) obtained during development lane scans of replicate development lanes of the RP C2 TLC separation of a mixture (50 ng each) of rhodamines 6G (1), B (2), and 123 (3) at surface scan rates of (a) 19, (b) 44, and (c) 190  $\mu\text{m/s}$  using a DESI solvent (methanol) flow rate of 5.0  $\mu\text{L}/\text{min}$ . Dwell time was 100 ms for each transition. Signal levels were normalized to signal in panel (c).

- Read out resolution not affected by surface scan rate up to 0.190 mm/s
- Significantly faster surface scan rates resulted in diminished signal
- 0.19 mm/s translates to an analysis time of 4 min for a 5 cm long development lane

## RESULTS AND DISCUSSIONS

Figure 6. Plots of mass spectral peak areas versus amount spotted obtained from the positive ion SRM ion current profiles during a surface scan (44  $\mu\text{m/s}$ ) at a fixed RF value across a dilution series of (a) rhodamine 6G (1,  $m/z$  443 @  $m/z$  415) and (b) rhodamine 123 (3,  $m/z$  345 @  $m/z$  285) bands separated on a RP C8 plate. The DESI solvent (methanol) flow rate was 5.0  $\mu\text{L}/\text{min}$  and the dwell time was 100 ms for each transition. Signal levels are normalized with respect to the maximum signal for the respective dye.

- Lowest levels spotted and detected were 250 pg for each dye (0.56 pmol cpd 1, 0.72 pmol cpd 3)

Figure 7. (a) Picture of wettable RP C18 TLC plate development lane showing the separated bands of a four component spotted (1.0  $\mu\text{L}$ ) mixture of FD&C dyes containing approximately 320 ng Yellow # 6 (7), 250 ng of Green #3 (5), 260 ng of Blue #1 (6), and 240 ng Red #3 (4). (b) Base peak chromatogram from full scan negative ion EMS mode data ( $m/z$  300 - 1000) acquired scanning the development lane shown in panel (a) at 190  $\mu\text{m/s}$  from high to low RF. The background-subtracted, averaged mass spectra at the distance in the chromatogram corresponding to the respective band positions on the plate are (c) Yellow #6 (7,  $m/z$  407 and 429), (d) Green #3 (5,  $m/z$  381 and 763) and Blue #1 (6,  $m/z$  373 and 747), and (e) Red #3 (4,  $m/z$  834). The DESI solvent was methanol sprayed at 10  $\mu\text{L}/\text{min}$ .

In general, signal levels were superior for analytes desorbed from reversed-phase compared to normal phase surfaces

- Higher plate loading were required with the normal phase plates to acquire the quality mass spectra shown

Figure 8. (a) Picture of normal phase silica gel TLC plate development lane showing the separated components of a spotted Excedrin tablet extract (2.0  $\mu\text{L}$ ) containing approximately 2.5  $\mu\text{g}$  caffeine (8), 10  $\mu\text{g}$  of acetaminophen (9), and 10  $\mu\text{g}$  aspirin (10) (b) Base peak chromatogram from full scan positive ion EMS mode data ( $m/z$  60 - 300) acquired scanning the development lane shown in panel (a) at 190  $\mu\text{m/s}$  from low to high RF. The background-subtracted, averaged mass spectra at the distance in the chromatogram corresponding to the respective band positions on the plate correspond to (c) caffeine (8,  $m/z$  152 and 217), (d) acetaminophen (9,  $m/z$  152 and 174), and (e) aspirin (10,  $m/z$  203 and 225). The DESI solvent was methanol sprayed at 10  $\mu\text{L}/\text{min}$ .

## DESI/ELECTROCHEMISTRY(EC)-MS

Most of the current DESI fundamental studies and analytical applications have been limited to desorption from nonconducting surfaces. We found that DESI also works using conducting surfaces. We have attempted to exploit this fact to use DESI as a means to transport analytes to an electrode surface for electrochemical reaction with subsequent mass spectrometric analysis of the reaction products. In theory, DESI/EC-MS should provide an EC-MS platform with extremely fast response time for changes in the reactions on the surface with minimal chance for chemical follow-up reactions when compared to upstream or even emitter electrochemical cells on-line with ES-MS.

Figure 9. Electrical schematic and picture of two-electrode cell used as surface in DESI experiments

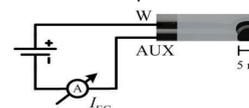


Figure 10. Picture of two-electrode cell during DESI experiments



Figure 11. Reserpine structure (11), proposed oxidation pathways and ions observed

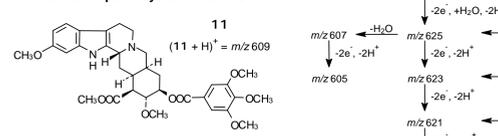
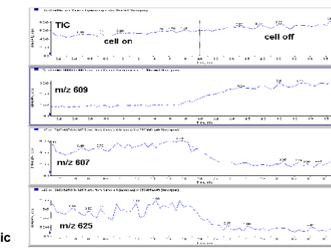
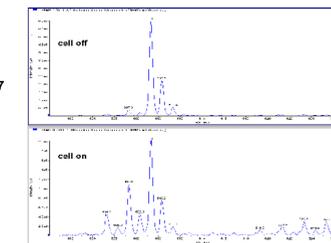


Figure 12. Extracted ion current profiles for the protonated molecule of reserpine ( $m/z$  609) and the major reserpine oxidation products observed ( $m/z$  607 and 625) with the cell off and cell on



2  $\mu\text{M}$  reserpine, 50 mM ammonium acetate in water/acetonitrile (1/1 v/v), 1% by volume acetic acid, 5  $\mu\text{L}/\text{min}$  flow rate

Figure 13. Reserpine mass spectra recorded with cell off and cell on (27 V, 1.0  $\mu\text{A}$ )



With cell on reserpine oxidation products (\*) are observed at  $m/z$  607, 605, 625, 623, 621 and 619

## CONCLUSIONS

The use of DESI for coupling TLC and MS was demonstrated

- Analytes separated on commercially available hydrophobic RP C8 and C2 plates, wettable RP C18 and normal phase plates were amenable to analysis in positive or negative ion mode
- Positioning of the DESI emitter, TLC plate surface, and the atmospheric sampling orifice of the mass spectrometer were found to be crucial for obtaining maximum analyte signal levels
- Close positioning of the desorption region to the sampling orifice was somewhat constrained by the configuration of our particular mass spectrometer
- This meant that TLC development lanes needed to be run near the plate edge or the plates cut following development to provide the proper positioning for DESI-MS
- With a change in the sampling orifice similar to that shown by Cooks and co-workers [1], this constraint on the TLC would be eliminated
- These same changes might be expected to improve the sampling efficiency and thus absolute detection level

The results presented point to other further TLC/DESI-MS studies

- Desorption ionization from all TLC phases was not equivalent
- The normal phase surface did not provide the lower detection levels garnered when using the reversed-phase plates
- The mechanistic aspects and practical implications of this observation will need to be addressed through analysis of a wide range of analytes on these and other TLC phases using a variety of DESI solvents and conditions
- Means to enhance desorption ionization efficiency while minimizing damage to the TLC plate surface will also need to be addressed
- Physical damage to the TLC plates occurred because of the use of aqueous solvents or less frequently because of the mechanical forces of the pneumatic DESI gas jet
- Operation under conditions that damaged the chromatographic phase hindered the generation of analyte gas-phase ions and necessitated relatively frequent cleaning of the mass spectrometer interface to remove the sputtered stationary phase particles and restore optimum instrument performance

The instrumentation and ability to perform analyte electrochemistry at an electrode surface impinged upon by the Desorption Electro spray Ionization plume was demonstrated

- Utility of DESI-EC-MS is currently limited by poor electrochemical efficiency
- Means to enhance the electrochemical conversion efficiency and to probe other processes at electrode surfaces with DESI are under investigation

## ACKNOWLEDGMENTS

- Vilmos Kertesz (ORNL) is thanked for assistance with the DESI/EC-MS experiments
- MDS Sciex is thanked for the microionspray and ESA Biosciences for the electrodes
- M.J.F. acknowledges an ORNL appointment through the ORNL Postdoctoral Research Associates Program
- M.A.D. acknowledges an appointment to the U. S. DOE Higher Education Research Experiences (HERE) Program for Faculty at ORNL
- The DESI-MS research was sponsored by the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences, U. S. Department of Energy
- The TLC/MS plate scanning platform was developed with funding from the Laboratory Directed Research and Development Program of ORNL and support from ORNL Technology Transfer and Economic Development (TTED) Royalty Funds
- ORNL is managed by UT-Battelle, LLC for the U.S. Department of Energy under contract DE-AC05-00OR22725