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2D Wax-printed paper substrates with extended solvent supply capabilities allow enhanced ion signal in paper spray ionization

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Paper-based microfluidic channels were created from solid wax printing, and the resultant 2D wax-printed paper substrates were used for paper spray (PS) mass spectrometry (MS) analysis of small organic compounds. Controlling fluid flow at the tip of the wax-printed paper triangles enabled the use of lower spray voltages (0.5-1 kV) and extended signal lifetime (10 minutes) in PS-MS. High sensitivity (sub *ng*/mL levels) and quantitation precision (<10% RSD) have been achieved in the analysis of illicit drugs in 4 µL of raw urine (fresh and dry), as well as corrosion inhibitors and pesticides in water samples. The reported study encourages the future development of disposable 3D microfluidic paper-based analytical devices, which function with simple operation but capable of on-chip analyte detection by MS; such a device can replace the traditional complex laboratory procedures for MS analysis to enable on-site in-situ sampling with portable mass spectrometers.

Introduction

Microfluidic paper-based analytical devices (μPADs) have emerged as a promising technology to develop simple, low-cost, portable, and disposable detection platforms for resource-limited settings.1-3 As a variant of conventional microfluidics typically constructed from glass or polymer substrates,4-6 μPADs are made from paper. The use of paper brings three mains benefits in microfluidic platforms: (i) it serves to pump fluids passively across the device by capillary wicking and eliminates external pumps that are typically not micro in size; (ii) like other porous substrates, paper allow the analysis of complex samples with little or no sample preparation; and (iii) using a paper substrate enables on-chip/on-surface detection. In effect, the ideal µPAD analytical systems are self-sustaining with all components necessary to perform an analytical assay (e.g. sample transport, sample pre-treatment, assay reagents, and signalling system) integrated into the device. Since their inception, µPADs have been utilised mainly for healthcare-related diagnostics;7-16 subsidiary applications have included environmental monitoring,17-21 explosive detection22-25 and detection of food contaminants.11,18,26-28 In order to maintain simplicity and portability, low power detection techniques, such as photometric, electrochemical, electrical conductivity, chemiluminescence, and electrochemiluminescence have been used for analyte signal transduction.29-35 Unfortunately, detection limits of µPADs based on these detection platforms are often inadequate and give only semi-quantitative results. The motivation behind this work is to generate next-generation μPADs that can enable the direct, rapid, sensitive, and on-surface detection of a variety of analytes – including small organics and large (kDa) biomolecules – through the use of handheld mass spectrometers.

Specifically, we are interested in the establishment of protocols that allow on-surface splitting of analyte solution for multiplexed detection, rapid immuno-extraction and concentration of analyte, and ambient paper spray (PS)36,37 ionization mass spectrometry (MS), all from a single paper device.38 In pursuit of this significant goal, the current study focuses on the optimization and characterization of small molecule ionization from 2D wax-printed paper substrates using low spray voltages under ambient conditions.

Solid wax printing represents an efficient approach to create microfluidic channels on paper in which the working hydrophilic regions are surrounded by hydrophobic wax barriers.39,40 We hypothesised that the well-defined hydrophilic channels generated from the wax-printing methodology can be utilised to confine DC potentials in narrow regions of the paper for efficient use of electrical power. Unlike other ambient ionization techniques,41-50 PS is well suited for on-site in-situ sample analysis, because no pneumatic assistance is needed to transport the analyte to the inlet of the mass spectrometer. Transfer of analytes occurs when the sample present on the paper substrate is solubilised by applying a spray solvent, which is typically a methanol/water mixture (1:1, v/v). Under this condition, charged micro-droplets are emitted from the tip of the wet paper triangle after applying 3-5 kV DC voltage to the paper triangle. Performance of PS-MS has been shown to depend on the geometry of the paper.51 Recently, Colletes *et. al.* described a novel PS-MS approach in which paper substrates with paraffin hydrophobic barriers (prepared by manual stamping of microfluidic shapes) allowed ~10x increase in PS signal from mono- and disaccharides.52We show in the present study that electronic solid wax-printing is an efficient way to optimize the generation of hydrophobic barriers for use in PS-MS. The resultant wax-printed paper substrates enables (i) the use of lower voltages (0.5 kV) for analyte ionization, and (ii) extended signal lifetime which permits both improved signal averaging and provides an increased timeframe to monitor multiple fragmentation/reaction pathways in tandem MS (MS/MS) experiments.

Chemical analysis at zero volts from plain paper substrates was recently reported by the Cooks’ group53 in a solvent assisted inlet ionization54 experiment. PS-MS of various analytes was also achieved by spraying analyte solution from a carbon nanotube-impregnated paper substrate under the influence of only 3 V.55 In both cases, however, high quality mass spectra were obtained at ppm analyte concentrations. Attempts to increase analyte signal beyond the typical 1 minute lifetime55 have included the use of excess spray solvent,57 a hydrophilic wick,58 and by connecting the paper substrate to a solvent reservoir created in 3D printed cartridge59 or the nib of a fountain pen.60 In the present study, we created and optimised 2D solid wax patterns on paper that allowed sensitive (at sub-ppb levels) detection of illicit drugs (e.g., methamphetamine cocaine, amphetamine and benzoylecgonine), corrosion inhibitors (e.g., Duomeen), and pesticides (e.g., metaldehyde) in water samples using a spray voltage ≥0.5 kV. Up to 5x increase in sensitivity was achieved when analysing dried and wet urine samples at 3 kV from the 2D wax-printed paper substrate compared with analysis done on a plain/un-waxed paper surfaces. In this case, analyte signal lifetime lasted up to 6 times longer for wax-printed paper spray compared with the traditional paper spray experiments.

Experimental

Chemicals.

Standard solutions (1.0 *mg*/mL) of cocaine, methamphetamine and amphetamine were obtained from Cerilliant (Round Rock, TX). Methanol, metaldehyde, and atrazine-d5 were purchased from Sigma-Aldrich (St. Louis, MO). N-oleyl-1, 3-diaminopropane (Duomeen) was supplied by B&V Water Treatment, (Lamport Drive Heartlands Business Park Daventry Northamptonshire, NN11 8YH, UK) through the Department of Electrical Engineering and Electronics, Liverpool University.

Wax Printing.

A Xerox ColorQube 8870 wax printer (Norwalk, CT) was used to print patterns on Whatman (Maidstone, UK) grade 1 cellulose chromatography paper. The paper was then heated to 130 °C for 30 seconds to allow the wax to permeate the paper fibres. All wax printed and un-waxed triangles were cut into approximately 70 mm2 triangles (9 mm base and 16 mm height).

Mass Spectrometry and Current Measurement.

Samples were analysed by a Thermo Fisher Scientific Velos Pro LTQ linear ion trap mass spectrometer (San Jose, CA, USA). MS parameters used were as follows: 150 °C capillary temperature, 3 microscans, and 60% Slens voltage. Thermo Fisher Scientific Xcalibur 2.2 SP1 software was applied for MS data collecting and processing. Tandem MS with collision-induced dissociation (CID) was utilised for analyte identification. Current was measured in a separate experiment using a Keithley 485 autoranging picoammeter (Cleveland, OH).



**Fig. 1**. Procedure for preparing wax-printed paper. (**A**) Computer generated patterns are printed onto filter paper, followed by heating at 130 0C for 30 seconds to melt the wax; **insert (i)** shows the back of the filter paper after wax printing, but before heating and **insert (ii)** shows the back of the filter paper after heating the wax-printed paper. Finally, the wax-printed paper is cut into a triangle and used in paper spray ionization; MS inlet was grounded. (**B**) Cross section illustrations of paper before printing, after printing, and after melting, respectively. Printed wax rests on the surface of one face of the filter paper. Subjecting the paper to 130 °C causes the wax to melt into the paper pores, permeating the paper. (**C**) SEM images of the wax-paper barrier: **(C-i)** printed wax before melting, **(C-ii)** printed wax after melting. No morphological differences were observed in waxed areas after heating when compared to un-waxed areas.

Results and discussion

Optimisation of wax-printed channels

Since, for a given potential, electric fields are more intense for small objects (i.e., where there is a smaller distance between the applied potential and a charge), solid hydrophobic wax material was applied on chromatographic paper in order to control the area of paper substrate to be wetted by the spray solvent. In theory, manipulation of available area for wetting should be able to alter the electric field generated at the tip. Common aqueous-based electrospray solvent compositions containing up to 80% organic (e.g., methanol) and 100% acetonitrile could not penetrate the wax hydrophobic barrier, and are therefore suitable spray solvents for ionization of various organic analytes in the wax-printed paper spray methodology. The process for generating the 2D wax-printed paper triangles and subsequently using the resultant paper triangles in PS-MS is as illustrated in Fig. 1. First, five different designs of computer generated microfluidic channels were constructed using adobe illustrator (t1 to s3, with t0 representing a plain, un-waxed paper substrate; Fig. 2A). The shapes of the channels (blue regions in triangles) were made to (i) have different total wax coverage on the paper (ESI, Fig. S1), (ii) represent two main groups of geometries: tapered (t1 and t2) and straight (s1, s2, s3) channels, (iii) vary the channel width within each group (width of t2 > t1 at the paper tip; width of s3 > s2 > s1; see details in the ESI, Fig. S2), and (iv) allow comparison between tapered and straight channels with respects to channel width and percent area of paper covered by solid wax. After printing the desired channel onto the paper, the printed paper sheets were heated to melt the wax (Fig. 1A), which then spreads through the fibre core of the filter paper to produce hydrophobic barriers that extend through the thickness (180 µm) of the paper and effectively confines the spray solvent. After melting, no morphological difference is seen between wax and un-waxed regions (Fig. 1C), indicating a uniform spread of wax in the paper matrix.

Interestingly, the performance of the wax-printed paper substrates did not follow any particular pattern when utilised in PS-MS analysis of amphetamine (*MW* 135, Log*P* 1.80), cocaine (*MW* 303, Log*P* 2.28), and methamphetamine (*MW* 149, Log*P* 2.24) using methanol/water (1:1, vol./vol.) spray solvent charged at 3 kV. The results of these experiments are summarised in Fig. 2B, which indicate that the wax-printed substrates generated relatively higher absolute ion signal compared with the plain paper substrate. When comparing the performance among the wax-printed substrates, s1 paper substrate (channel width 0.51 mm) was inferior in all cases compared with s2 (channel width 1.2 mm) and s3 (channel width 1.8). These results encouraged us to further optimise the two best wax-printed substrates s2 and s3 for PS-MS experiments using lower spray voltages. Here, the wax-printed paper substrate s3 was superior to s2 and un-waxed (t0) paper substrates at all potentials tested, including 0.5 and 1 kV spray voltages (ESI, Fig. S3). Based on these results, the s3 pattern was chosen to be used for all other experiments discussed in this study.

Characterisation of spray from wax-printed paper substrate

Before applying the selected wax-printed paper substrate (s3) in PS-MS for real sample analysis, differences in spray dynamics and loading capacity of wax paper were compared with the traditional paper spray experiment. For this, 4 µL of cocaine (100 *ng*/mL) prepared in methanol was deposited onto the wax-printed paper triangle, allowed to dry in ambient air after which the dried cocaine was sampled using methanol/water (1:1, vol./vol.) spray solvent. Typical selected ion (*m/z* 304) chromatograms generated in this experiment are shown in Fig. 3 for varying the volumes of the methanol/water spray solvent, applied in direct dumping mode.57 In each case, 3 kV of spray voltage was used. Using the typical 10 µL spray solvent, the signal from the wax-printed paper substrate lasted for more than 1.6 minutes. This spray lifetime was substantially extended to 10 minutes when 20 µL of solvent volume was used. A maximum solvent loading capacity of 25 L was determined for the paper size (9 mm × 16.5 mm) used in this experiment (insert (ii), Fig. 3). A similar experiment was performed on un-waxed paper; here, signal lasted for only 1.5 minutes when using 20 µL spray solvent (insert (i), Fig. 3).



**Fig. 2** (**A**) Wax-printed micro-fluid channels/patterns tested; t0 represents un-waxed paper whereas t1 and t2 have tapered channels (blue); s1-s3 represent straight channels with increasing width from s1 to s3. (**B**) Comparison of absolute intensity of major fragment ions from amphetamine (*m/z* 136 → 119), methamphetamine (*m/z* 150 → 119), and cocaine (*m/z* 304 → 182) sprayed from a normal un-waxed paper triangle with the corresponding wax patterns generated shown in (A). Wax pattern s3 was selected for further analysis. *I* = absolute fragment ion intensities.

Initial ion currents from the un-waxed paper triangle were two times higher than that from the wax-printed paper substrate. This observation suggests a higher flow rate, but the subtle differences in ion currents alone cannot explain the rapid depletion of solvent from the un-waxed paper. We believe solvent loss/depletion attributed to evaporation and electrospray are more effectively controlled when using the wax-printed paper triangle. The high surface area available in un-waxed paper enables easy spreading which enhances rate of solvent evaporation. Reducing the surface area of the paper triangle with solid wax printing allows a more stable electrospray for extended time by reducing solvent evaporation. The flow rate for the electrospray at the wax-printed paper tip varied as the solvent was consumed. Solvent flow was high at the onset of voltage application, which lasted for only a few seconds followed by a long stable spray period (insert (iii), Fig. 3).

A spike in ion current was consistently detected towards the end of the spray (ESI, Fig. S4); this observation is in accordance with a recently proposed paper spray mechanism61 in which corona discharge is thought to contribute to ion formation at lower solvent flow rates, typically occurring at that point where solvent is depleted. That no changes in ion type (protonated species versus radical cations) was detected in our wax-printed paper spray experiments when using 3 kV of spray voltage suggests minimal contribution from corona discharge (ESI, Fig. S4).



**Fig. 3**. Selected ion (*m/z* 304) chromatogram (XIC) of 100 *ng*/mL cocaine solution; 4 L sample was dried onto s3 wax-printed paper and sprayed with increasing volumes of MeOH/H2O (1:1, v/v) at 3 kV. Arrows show where each signal ceased. **Inset (i)**: 4 L of 100 *ng*/mL cocaine solution was dried onto an un-waxed paper triangle and sprayed with 20 L of MeOH/H2O (1:1, v/v). Spray time was approximately 1.5 minutes (0.2-1.7 minutes). **Inset (ii)**: signal lifetime varies with spray solvent volume applied to the paper triangle. Un-waxed paper signal lifetime does not increase after approximately 7 L of solvent, but wax-printed paper signal lifetime increases to ~10 minutes after 20 L is added to the triangle. **Inset (iii)**: zoomed-in XIC in 0.2 – 1.3 minute time range when using 10 µL spray solvent.

**Analysis of illicit drugs**

Illicit drug quantitation using the wax-printed paper spray method was first optimised using pure methanolic solutions of methamphetamine. Standard solutions were prepared in 3 – 250 *ng*/mL range, and analysed both with un-waxed and wax-printed paper substrates using 0.5 and 1 kV spray voltages. All attempts to generate a linear calibration curve from un-waxed paper triangle at these low spray voltages failed.55 In contrast, good linearity and high precision were easily recorded for methamphetamine when analysed at the same voltages using wax-printed paper substrate (Fig. 4A and B). Detection limits (LODs) as low as 0.36 *ng*/mL and 2.53 *ng*/mL were recorded for 1 kV and 0.5 kV spray voltages, respectively. This performance was achieved in MS/MS mode using *m/z* 150 → 119 transition and with the use of internal standard (methamphetamine-d5, with MS/MS transition *m/z* 155 → 121), and monitoring analyte-to-internal standard ratios (A/IS) as a function of analyte concentration. Representative MS/MS product ion spectra for methamphetamine at 250 *ng*/mL concentration are shown in Figs. 4C and D for 0.5 kV and 1 kV spray voltages, respectively.

The method was then extended to detect other illicit drugs such as cocaine, amphetamine, and benzoylecgonine in raw urine (wet and dry). Direct analysis of small molecules in dried urine is possible with the traditional paper spray,56 but their quantification in fresh/wet samples is challenging due to ion suppression effects associated with sample extraction and ionization with aqueous-based solvents. Organic spray solvents have been used,62,63 but rapid evaporation makes it difficult to achieve good quantitation precision, especially in MS/MS experiments. We identified that pure acetonitrile does not penetrate the hydrophobic wax barrier and so permits extended analysis time.

**Fig. 4.** Calibration of methamphetamine standard solutions (3 – 250 *ng*/mL) analysed with MeOH/H2O (1:1, vol./vol.) solution using (**A**) 1 kV and (**B**) 0.5 kV spray voltages. Error bars show one standard deviation for three replicates. Representative spectra of methamphetamine fragmentation used for quantification are shown for (**C**) 1 kV and (**D**) 0.5 kV. RI = relative intensity; A/IS= ratio of analyte-to-internal standard signal. Internal standard used for methamphetamine was methamphetamine d5 with MS/MS transition m/z 155→123.

**Table 1.** Limit of detection (LOD) and limit of quantitation (LOQ) of selected illicit drugs spiked into urine, and analysed at 3 kV with 100% acetonitrile spray solvent.

|  |  |  |  |
| --- | --- | --- | --- |
| Analyte | LODs (LOQs)\*in urine matrix | | |
| Dry (*ng*/mL) | Fresh (*ng*/mL) | |
| Waxed paper | Waxed paper | Plain paper |
| Cocaine | 0.10 (0.87) | 0.62 (1.6) | 2.6 (13) |
| Benzoylecgonine | 0.21 (0.45) | 0.97 (2.5) | 5.9 (8.7) |
| Methamphetamine | 0.13 (0.60) | 0.51 (2.27) | 1.5 (8.7) |
| Amphetamine | 0.33 (0.38) | 0.76 (1.4) | - |

\*LODs and LOQs were calculated from respective calibration curves using signal corresponding to (Sblank) + 3×σblank, and (Sblank ) + 10×σblank, respectively where Sblank is the average blank signal and σblank is the standard deviation of the signal from 3 replicates

The results recorded for the detection of the selected illicit drugs from dry and fresh whole human urine samples using wax-printed paper and 100% acetonitrile spray solvent are shown in Table 1 (see ESI Fig. S5-S7 for calibration curves). LODs and LOQs ranged from 0.51 – 1.2 *ng*/mL and 1.4 – 3.2 *ng*/mL, respectively for fresh samples and 0.10 – 0.33 *ng*/mL and 0.38 – 0.87 *ng*/mL for dry samples. Relative standard deviations less than 10% were obtained for both wet and dry samples with concentrations higher than 3.0 *ng*/mL. Increased sensitivity (up to >5x) was observed when analysing dried urine samples compared with fresh urine (Table 1), and we attribute this effect to reduced ion suppression effects. Re-solubilisation of endogenous salts in dried urine is not efficient with acetonitrile and so their transfer to the mass spectrometer during PS ionization is limited. Identical conditions (10 µL and 3 kV) were used when comparing the performance of wax-printed paper detection of drugs in fresh urine to that of un-waxed paper (Table 1). When using 10 L pure acetonitrile as a spray solvent, the signal lasted for only 15 seconds on un-waxed paper (due to faster solvent evaporation); this time period was inadequate for signal acquisition of more than one ion (ESI Fig. S8). Increasing solvent volume to 20 L increased signal lifetime to ~1 minute and enabled linear regression analysis. Results are shown in Table 1 where LODs recorded from the un-waxed PS-MS analysis were at least 3x higher than LOD achieved on wax-printed paper substrates.

Analysis of corrosion inhibitors and pesticides in water

The wax-printed PS-MS method was also utilised to detect residual levels of Duomeen (a polyamine corrosion inhibitor) and metaldehyde (a tetracyclic acetaldehyde molluscicide) in water. Duomeen is a widely used chemical substance used to control corrosion in high pressure (HP) water-tube boilers.64 Direct detection and monitoring is necessary for boiler maintenance, and we have recently described a reactive paper spray method for on-site in-situ detection of Duomeen.65 We anticipated that the use of wax-printed paper substrates can improve the quantitative capabilities of the PS-MS method and that the use of low spray voltages will facilitate field analysis. These expectations have been met, and as shown in Fig. 5A and B Duomeen is sensitively detected with calculated LODs of 0.09 *pg*/mL and 0.68 *pg*/mL for 3 and 1 kV spray voltages, respectively. Excellent linearity (R2 = 0.9997) and precision (%RSD = 7.5%) were achieved without internal standards

**Fig. 5.** Calibration of (**A**) Duomeen sprayed at 3 kV, (**B**) Duomeen at 1 kV, (**C**) metaldehyde at 3 kV, and (**D**) metaldehyde at 1 kV in water samples. All samples were sprayed with 4:1 MeOH/H2O. AI = absolute fragment ion (*m/z* 308) intensity, A/IS= ratio of analyte-to-internal standard signal. Internal standard used for metaldehyde was atrazine with MS/MS transition *m/z* 221→179.

This performance is due in part to the high ionisation efficiency of the Duomeen and the occurrence of a long lasting stable spray from the wax-printed paper substrate, which enables ensemble averaging of many spectra. We applied the method to analyse two real water samples (pre- and post- treatment) collected from a large HP boiler system at Coventry waste treatment facility in the U.K. As expected Duomeen (*m/z* 325) was detected in the post treatment water sample (ESI Fig. S9).

Figs. 5C and D represent calibration curves recorded for the analysis of metaldehyde in water using methanol/water (4:1, vol./vol.) spray solvent charged at 3 and 1 kV, respectively. Analysis of metaldehyde is necessitated by recent reports of metaldehyde poisoning and death in both animals and humans.66,67 The molluscicide is used in agriculture to control slugs in order to protect crops. However, large residues of metaldehyde are mobilised during heavy rainfalls which end up in rivers and groundwater and finally in drinking water. In fact, the European Commission and U.S. Environmental Protection Agency have both issued instructions on permissible level for metaldehyde, restricting its use as a pesticide.68,69

In the current study, our interest was in the use of lower spray voltage for direct metaldehyde analysis. Metaldehyde formed sodiated ions in high abundance when analysed at 1 kV spray voltage, in which case calibration was obtained using *m/z* 199 [M+Na]+ → 111 MS/MS transition (through the elimination of neutral acetaldehyde dimer (*MW* 88); ESI Fig. S10). At 3 kV, however, the predominant peak was the protonated species [M+H]+; *m/z* 177, which fragmented to give product ion at *m/z* 149 via CH2=CH2 (*MW* 28) neutral loss. The formation of different ion types from metaldehyde when using different spray voltages is unique (Fig. 6), and ascribed to increased charged density in the microfluidic channel when using higher DC voltage (3 kV). Presumably, the increased charge causes increased proton abundance, which in turn suppresses sodium adduction. The different fragmentation pathways for [M+Na]+ (m/z 199 → 111) versus [M+H]+ (m/z 177 → 149) suggests different structures exist for both ions. We propose metaldehyde ring opening occurs in the presence of high proton abundance, at higher spray voltage, which subsequently fragments through eliminating ethylene in MS/MS. Similar effect is observed in acidic solution (ESI, Fig S11). LODs were determined to be 4.9 *ng*/mL and 5.2 *ng*/mL for 3 kV and 1 kV spray voltages using [M+H]+ and[M+Na]+ions, respectively. The ability to generate different ion types at different spray voltages will aid easy differentiation of metaldehyde from other interfering ions (having the same nominal mass), especially during field analysis.

**Fig. 6.** Depiction of the effect of spray voltage on metaldehyde ionization. Higher voltage (3 kV; green, right) generates high proton abundance at wax-printed paper tip producing protonated metaldehyde [M+H]+ ions with unique fragmentation pathway. Alternatively, at low voltage (1 kV; blue, left), the ionization process is dominated by sodium adduction forming [M+Na]+ ions at *m/z* 199. MS inlet was grounded

**Conclusions**

In summary, a demonstration of 2D wax-printed substrates for expanding the applicability of paper spray ionization has been provided. By reducing the area of wetting on the paper triangle, spray solvent evaporation is minimized and stable electrospray can be generated that allows longer analysis times (~6x increase). Up to 80% methanol could be used for aqueous-based solvent systems and 100% acetonitrile spray solvents could be employed for the wax-printed paper spray experiment without penetrating the wax hydrophobic barrier. Microfluidic patterns suitable for low voltage paper spray ionisation were determined empirically using five different designs. The spray dynamics and loading capacity of the selected wax-printed paper substrate were characterized and compared with plain, un-waxed paper triangles. It is believed that the reduction in channel width improves the likelihood of analyte transport to the paper tip region where both the DC potential is more confined and proximity (i.e., distance) to the MS atmospheric interface is favourable for successful ion entry. Spray could be sustained for more than ten minutes, enabling tandem MS analysis of various analytes to be performed with high precision. The 2D wax-printed paper substrates do not require external solvent reservoirs or pumps to achieve continuous wetting.

The wax-printed PS-MS methodology was tested by analyzing water-based and whole human urine samples. Analytes detected include corrosion inhibitor Duomeen, pesticide metaldehyde, and illicit drugs such as cocaine and its metabolite benzoylecgonine, methamphetamine and amphetamine. Unlike un-waxed paper triangles which were unable to generate acceptable calibration curves at < 1 kV spray voltages, good linearity and precision were obtained for water samples analysed from wax-printed paper substrates. As low as 0.68 *pg*/mL sensitivity was recorded for Duomeen in water when using 1 kV spray voltage. A strong voltage dependence was observed for the analysis of the cyclic metaldehyde pesticide in water. Sodiated metaldehyde ions were detected from water at the reduced charged of 1 kV and fragmented differently upon collisional activation when compared with protonated species generated at high electrical charging of 3 kV. Such capability will aid field analysis with high selectivity. Higher voltage (>3 kV) was required for illicit drug analysis from raw urine and 5x improvement in signal sensitivity was recorded for dried urine samples compared with wet samples, which were in turn more sensitive for similar fresh urine samples analysed from un-waxed paper.

These results suggest that the wax-printing methodology can serve as an efficient approach to modify paper substrates for improved PS-MS analysis. The wax hydrophobic barrier may also serve to ease technical challenges (e.g., inaccuracies in quantitation) associated with sample collection in dried blood (biofluid) spot preparation by allowing a more uniform fluid distribution.70 Future work will focus on mathematical modeling and computer simulation to investigate the mechanism of fluid flow and how confined DC potentials influence analyte ionization and transport from the tip of the wax-printed paper substrates.

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Notes and references

1. A. W. Martinez, S. T. Phillips, M. J. Butte and G. M. Whitesides, *Angew. Chem. Int. Ed.*, 2007, **46**, 1318–1320.
2. A. W. Martinez, S. T. Phillips and G. M. Whitesides, *Proc. Natl. Acad. Sci.,* 2008, **105**, 19606–19611.
3. A. W. Martinez, S. T. Phillips, G. M. Whitesides and E. Carrilho, *Anal. Chem.*, 2010, **82**, 3–10.
4. G. M. Whitesides, *Nature*, 2006, **442**, 368–373.
5. P. Yager, T. Edwards, E. Fu, K. Helton, K. Nelson, M. R. Tam and B. H. Weigl, *Nature*, 2006, **442**, 412–418.
6. E. K. Sackmann, A. L. Fulton and D. J. Beebe, *Nature*, 2014, **507**, 181–189.
7. A. K. Badu-Tawiah, S. Lathwal, K. Kaastrup, M. Al-Sayah, D. C. Christodouleas, B. S. Smith, G. M. Whitesides and H. D. Sikes, *Lab Chip*, 2015, **15**, 655–659.
8. N. R. Pollock, J. P. Rolland, S. Kumar, P. D. Beattie, S. Jain, F. Noubary, V. L. Wong, R. A. Pohlmann, U. S. Ryan and G. M. Whitesides, *Sci. Transl. Med*., 2012, **4**, 152ra129–152ra129.
9. S. A. Klasner, A. K. Price, K. W. Hoeman, R. S. Wilson, K. J. Bell and C. T. Culbertson, *Anal. Bioanal. Chem*., 2010, **397**, 1821–1829.
10. K. Abe, K. Suzuki and D. Citterio, *Anal. Chem*., 2008, **80**, 6928–6934.
11. Z. Nie, F. Deiss, X. Liu, O. Akbulut and G. M. Whitesides, *Lab Chip*, 2010, **10**, 3163–3169.
12. Y. Lu, W. Shi, L. Jiang, J. Qin and B. Lin, *Electrophoresis*, 2009, **30**, 1497–1500.
13. B. A. Rohrman and R. R. Richards-Kortum, *Lab Chip*, 2012, **12**, 3082–3088.
14. C. Li, K. Vandenberg, S. Prabhulkar, X. Zhu, L. Schneper, K. Methee, C. J. Rosser and E. Almeide, *Biosens. Bioelectron*., 2011, **26**, 4342–4348.
15. M. Li, J. Tian, M. Al-Tamimi and W. Shen*, Angew. Chem. Int. Ed*., 2012, **51**, 5497–5501.
16. E. Fu, T. Liang, J. Houghtaling, S. Ramachandran, S. A. Ramsey, B. Lutz and P. Yager, *Anal. Chem*., 2011, **83**, 7941–7946.
17. L. Wang, W. Chen, D. Xu, B. S. Shim, Y. Zhu, F. Sun, L. Liu, C. Peng, Z. Jin, C. Xu and N. A. Kotov, *Nano Lett*., 2009, **9**, 4147–4152.
18. S. M. Z. Hossain, R. E. Luckham, M. J. McFadden and J. D. Brennan, *Anal. Chem*., 2009, **81**, 9055–9064.
19. D. M. Cate, P. Nanthasurasak, P. Riwkulkajorn, C. L’Orange, C. S. Henry and J. Volckens, *Ann. Occup. Hyg*., 2014, **58**, 413–423.
20. A. Apilux, W. Dungchai, W. Siangproh, N. Praphairaksit, C. S. Henry and O. Chailapakul, *Anal. Chem*., 2010, **82**, 1727–1732.
21. G. G. Lewis, J. S. Robbins and S. T. Phillips, Chem. Commun., 2014, **50**, 5352–5354.
22. K. L. Peters, I. Corbin, L. M. Kaufman, K. Zreibe, L. Blanes and B. R. McCord, *Anal. Methods*, 2015, **7**, 63–70.
23. R. V. Taudte, A. Beavis, L. Wilson-Wilde, C. Roux, P. Doble and L. Blanes, *Lab Chip*, 2013, **13**, 4164–4172.
24. M. O. Salles, G. N. Meloni, W. R. de Araujo and T. R. L. C. Paixao, *Anal. Methods*, 2014, **6**, 2047–2052.
25. A. Pesenti, R. V. Taudte, B. McCord, P. Doble, C. Roux and L. Blanes, *Anal. Chem*., 2014, **86**, 4707–4714.
26. J. Lankelma, Z. Nie, E. Carrilho and G. M. Whitesides, *Anal. Chem*., 2012, **84**, 4147–4152.
27. Y. Zhang, P. Zuo and B.-C. Ye*, Biosens. Bioelectron*., 2015, **68**, 14–19.
28. S.-Q. Jin, S.-M. Guo, P. Zuo and B.-C. Ye*, Biosens. Bioelectron*., 2015, **63**, 379—383.
29. A. K. Ellerbee, S. T. Phillips, A. C. Siegel, K. A. Mirica, A. W. Martinez, P. Striehl, N. Jain, M. Prentiss and G. M. Whitesides, *Anal. Chem*., 2009, **81**, 8447–8452.
30. W.-J. Lan, E. J. Maxwell, C. Parolo, D. K. Bwambok, A. B. Subramaniam and G. M. Whitesides, *Lab Chip*, 2013, **13**, 4103–4108.
31. A. Arena, N. Donato, G. Saitta, A. Bonavita, G. Rizzo and G. Neri, *Sens. Actuators B: Chem*., 2010, **145**, 488–494.
32. C. Steffens, A. Manzoli, E. Francheschi, M. L. Corazza, F. C. Corazza, J. V. Oliveira and P. S. P. Herrmann, *Synth. Met*., 2009, **159**, 2329–2332.
33. J. L. Delaney, C. F. Hogan, J. Tian and W. Shen, *Anal. Chem.,* 2011, **83**, 1300–1306.
34. L. Ge, S. Wang, X. Song, S. Ge and J. Yu, *Lab Chip*, 2012, **12**, 3150–3158.
35. N. K. Thom, G. G. Lewis, K. Yeung and S. T. Phillips, RSC Adv., 2014, **4**, 1334–1340.
36. H. Wang, J. Liu, R. G. Cooks and Z. Ouyang, *Angew. Chem. Int. Ed.*, 2010, **49**, 877–880.
37. S. Maher, F. P. M. Jjunju and S. Taylor, *Rev. Mod. Phys*., 2015, **87**, 113–135.
38. S. Chen, Q. Wan, A.K. Badu-Tawiah, Touch Paper Spray Ambient Mass Spectrometry for Paper-based Immunoassays: Towards On-demand Diagnosis, 2016, submitted
39. E. Carrilho, A. W. Martinez and G. M. Whitesides, *Anal. Chem*., 2009, **81**, 7091–7095.
40. Y. Lu, W. Shi, L. Jiang, J. Qin and B. Lin, *Electrophoresis*, 2009, **30**, 1497–1500.
41. Z. Takáts, J. M. Wiseman, B. Gologan and R. G. Cooks, *Science*, 2004, **306**, 471–473.
42. R. G. Cooks, Z. Ouyang, Z. Takats and J. M. Wiseman, *Science*, 2006, **311**, 1566–1570.
43. R. B. Cody, J. A. Laramée and H. D. Durst, *Anal. Chem*., 2005, **77**, 2297–2302.
44. M. J. Ford and G. J. Van Berkel, *Rapid Commun. Mass Spectrom.*, 2004, **18**, 1303–1309.
45. J. Shiea, M.-Z. Huang, H.-J. HSu, C.-Y. Lee, C.-H. Yuan, I. Beech and J. Sunner, *Rapid Commun. Mass Spectrom*., 2005, **19**, 3701–3704.
46. P. Nemes and A. Vertes, Anal. Chem., 2007, 79, 8098–8106.
47. P. J. Roach, J. Laskin and A. Laskin, *Analyst*, 2010, **135**, 2233–2236.
48. M. E. Monge, G. A. Harris, P. Dwivedi and F. M. Fernández, *Chem. Rev*., 2013, **113**, 2269–2308.
49. A. K. Badu-Tawiah, L. S. Eberlin, Z. Ouyang and R. G. Cooks, *Annu. Rev. Phys. Chem*., 2013, **64**, 481–505.
50. D. J. Weston, *Analyst*, 2010, **135**, 661–668.
51. Q. Yang, H. Wang, J. D. Maas, W. J. Chappell, N. E. Manicke, R. G. Cooks and Z. Ouyang, *Int. J. Mass Spectrom*., 2012, **312**, 201–207.
52. T. C. Colletes**,** P. T. Garcia**,** R. B. Campanha**,** P. V. Abdelnur**,** W. Romão**,** W. K. T. Coltro and B. G. Vaz Analyst, 2016, **141**, 1707-1713.
53. M. Wleklinski, Y. Li, S. Bag, D. Sarkar, R. Narayanan, T. Pradeep and R. G. Cooks, *Anal. Chem*., 2015, **87**, 6786–6793.
54. V. S. Pagnotti, N. D. Chubatyi and C. N. McEwen*, Anal. Chem*., 2011, **83**, 3981–3985.
55. R. Narayanan, D. Sarkar, R. G. Cooks and T. Pradeep, *Angew. Chem. Int. Ed*., 2014, **53**, 5936–5940.
56. J. Liu, H. Wang, N. E. Manicke, J.-M. Lin, R. G. Cooks and Z. Ouyang, *Anal. Chem*., 2010, **82**, 2463–2471.
57. Y. Ren, H. Wang, J. Liu, Z. Zhang, M. N. McLuckey and Z. Ouyang, *Chromatographia*, 2013, **76**, 1339–1346.
58. N. E. Manicke, Q. Yang, H. Wang, S. Oradu, Z. Ouyang and R. G. Cooks, *Int. J. Mass Spectrom*., 2011, **300**, 123–129.
59. G. I. Salentijn, H. P. Permentier and E. Verpoorte, *Anal. Chem*., 2014, **86**, 11657–11665.
60. H. Lee, C.-S. Jhang, J.-T. Liu and C.-H. Lin, *J. Sep. Sci*., 2012, **35**, 2822–2825.
61. R. D. Espy, A. R. Muliadi, Z. Ouyang and R. G. Cooks, *Int. J. Mass Spectrom*., 2012, **325–327**, 167–171.
62. H. Wang, Y. Ren, M. N. McLuckey, N. E. Manicke, J. Park, L. Zheng, R. Shi, R. G. Cooks and Z. Ouyang, *Anal. Chem*., 2013, **85**, 11540–11544.
63. D. E. Damon, K. M. Davis, C. R. Moreira, P. Capone, R. Cruttenden and A. K. Badu-Tawiah, *Anal. Chem*., 2016. DOI: 10.1021/acs.analchem.5b03992
64. M. Behpour, S. M. Ghoreishi, M. Khayatkashani and N. Soltani, Mater. Chem. Phys., 2012, **131**, 621–633.
65. F. P. M. Jjunju, S. Maher, D. E. Damon, R. M. Barrett, S. U. Syed, R. M. A. Heeren, S. Taylor and A. K. Badu-Tawiah, *Anal. Chem*., 2016, **88**, 1391–1400.
66. K. C. Hallett, A. Atfield, S. Comber and T. H. Hutchinson, *Sci. Total Environ.,* 2016, **543, Part A**, 37–43.
67. N. Ruiz-Suárez, L. D. Boada, L. A. Henríquez-Hernández, F. González-Moreo, A. Suárez-Pérez, M. Camacho, M. Zumbado, M. Almeida-González, M. del Mar Travieso-Aja and O. P. Luzardo, *Sci. Total Environ*., 2015, **505**, 1093–1099.
68. Reregistration Eligibility Decision for Metaldehyde. U.S. Environmental Protection Agency Web Archive [Online], July 27, 2006. http://archive.epa.gov/pesticides/reregistration/web/pdf/metaldehyde\_red.pdf (accessed January 12, 2016).
69. T. Dolan, P. Howsam, D. J. Parsons and M. J. Whelan, *Environ. Sci. Technol*., 2013, **47**, 4999–5006.
70. P. A. Demirev, *Anal. Chem*., 2013, **85**, 779–789.