Mass Spectrometry

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Paper Spray for Direct Analysis of Complex Mixtures Using Mass Spectrometry**

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Mass spectrometry (MS) is a powerful tool for the analysis of complex mixtures, providing information on the molecular weights and chemical structures of the analytes. Solutions are normally ionized using electrospray ionization (ESI)[1] or atmospheric pressure chemical ionization (APCI),[2] while solids are often ionized using matrix-assisted laser desorption ionization (MALDI).[3] Complex mixtures are routinely separated using chromatography before the MS measurement in order to minimize suppression effects on analyte ionization and to preconcentrate the analytes. Ambient ionization methods,[4] including desorption electrospray ionization (DESI),[5] direct analysis in real time (DART),[6] and others,[7–12] have recently been developed to address the need for rapid methods of direct analysis of complex mixtures without sample preparation. Qualitative and semiquantitative analysis has been achieved using these experiments which typically take only a few seconds.[8–12]

The present study reports a paper spray (PS) method which has characteristics of both ESI and the ambient ionization methods and is useful for fast, qualitative, and quantitative analysis of complex mixtures. Analyte transport is achieved by wicking in a porous material with a macroscopically sharp point, and a high electric field is used to perform ionization. Pneumatic assistance is not required to transport the analyte: a voltage is simply applied to the wet paper, which is held in front of a mass spectrometer (Figure 1a).

These capabilities are demonstrated by the analysis of therapeutic drugs in whole blood. There is considerable interest in the pharmaceutical sciences in the storage and transportation of blood samples as dried blood spots on paper.[13] Dried blood spots or whole blood can be used in this experiment. For example, imatinib (Gleevec), a 2-phenylaminopyrimidine derivative, approved by the FDA for treatment of chronic myelogenous leukemia, is efficacious over a rather narrow range of concentrations. Whole bovine blood, spiked with imatinib at concentrations covering the therapeutic range, was deposited on a small paper triangle for analysis (Figure 1a). Protonated imatinib (m/z 494) can be identified among the many peaks in the mass spectrum (Figure S-1 in the Supporting Information). The tandem mass spectrum (MS/MS, Figure 1b) of imatinib is much simpler and shows a single characteristic fragment ion. Quantitation of

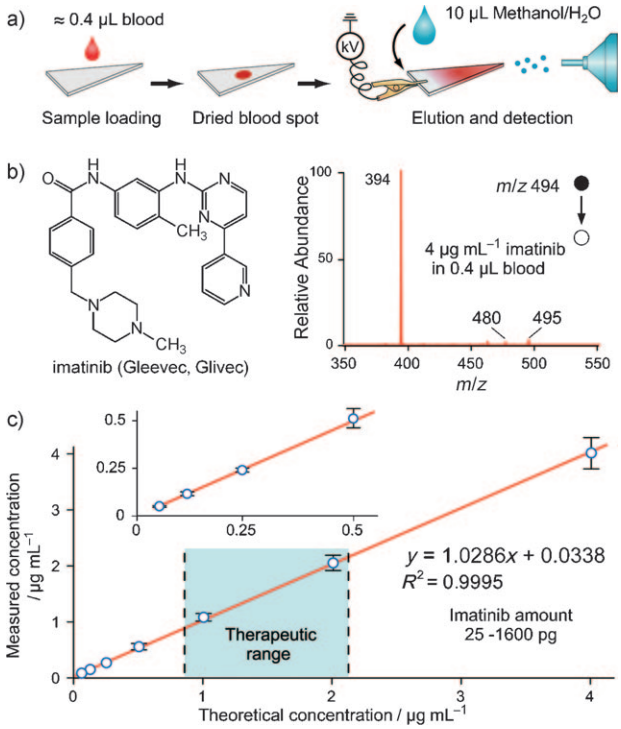

Figure 1. a) Analysis of a dried blood spot on paper. A drop of whole blood (0.4 μL) was applied directly to a triangular section of chromatography paper. A DC voltage (4.5 kV) is applied to the paper wetted with 10 μL methanol/water (1:1 v/v). b) Molecular structure of imatinib (Gleevec) and paper spray tandem mass spectrum of 0.4 μL of whole blood containing 4 μg mL^{-1} imatinib identified and quantified by the MS/MS transition m/z 494 → m/z 394. c) Quantitative analysis of whole blood spiked with imatinib (62.5 ng mL^{-1}–4 μg mL^{-1}) and its isotopomer [D_8]imatinib (1 μg mL^{-1}). Inset plot shows low-concentration range. Bars represent the standard deviation of analysis for three replicates. The intercept is 0.0338 μg mL^{-1} and the slope is 1.0286.

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imatinib in whole blood is achieved using the ratio of this fragment ion abundance to that of the corresponding fragment ion generated from [D_8]imatinib, which was also added to the blood as an internal standard. The relative response is linear across a wide range of concentrations, including the entire therapeutic range (Figure 1c).

A wide variety of molecules can be analyzed by PSMS, some of those examined include epinephrine, serine, atrazine, methadone, roxithromycin, cocaine, methyl violet, atenolol, phosphatidylcholine, angiotensin I, and cytochrome C. All display high-quality mass spectra and MS/MS product ion spectra from a variety of paper surfaces (see, for example, Figure S-2 in the Supporting Information). These experiments employ small volumes of solution, typically a few μL; analyte concentrations are 0.1 to 10 μg mL^{-1} (total analyte amount 50 pg to 5 ng); and signals last from one to several minutes.

Paper spray ionization can also be used for the analysis of surfaces by wiping the surface of interest with a paper triangle, adding solvent, and applying a high voltage (Figure S-3 in the Supporting Information). Small amounts (picogram range, in areas of 1 cm^2 to many cm^2) of cocaine and heroin can be detected by MS/MS.

Paper is an important medium in chemical separations, and this feature can be incorporated into a version of the PS experiment which utilizes separation by chromatography on paper. As shown in Figure 2, a mixture of two dyes, methylene blue (m/z 284) and methyl violet (m/z 358.5), was first separated on paper, and the partially separated spots were analyzed by MS after pieces had been cut from the paper medium. The ratios of signal intensities of the two dyes at the two positions indicated at the top of Figure 2 are 7.1 and 0.063, respectively. The results show that the dyes can be separated and detected by PSMS. Paper spray using chromatography paper is a new ambient ionization method which integrates three analytical procedures: sample collection, analyte separation, and analyte ionization. This represents a significant simplification in the coupling of chromatography with MS analysis. Because paper chromatography is an established technique for separating mixtures,[14] it could also be used to separate chemicals in biological fluids before in situ ionization. With the rapid development of paper-based microfluidic devices,[15,16] paper spray might play a role in the integration of future microfluidic MS systems. Experiments are underway to adapt other separation methods, including capillary electrophoresis to PSMS.

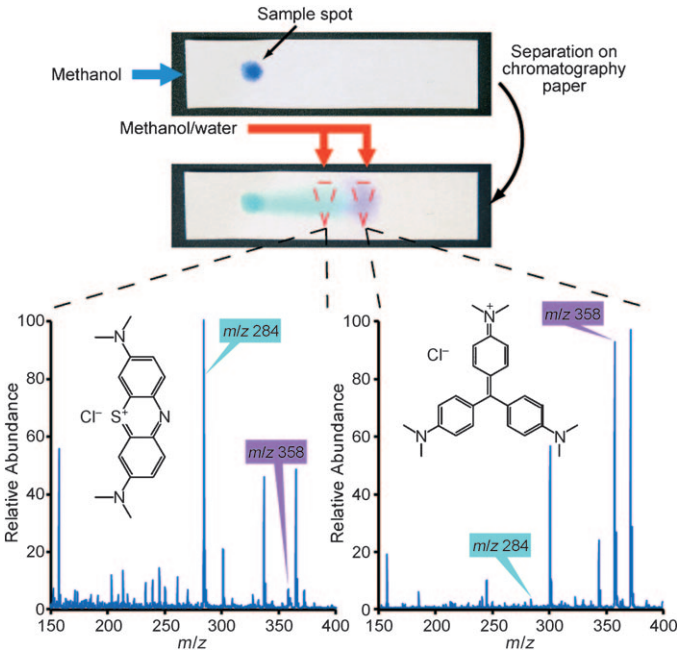

Figure 2. Paper spray MS analysis of two dyes, separated by paper chromatography. Methylene blue (m/z 284) and methyl violet (m/z 358.5) were dissolved and mixed in water, and the final concentration of each of these two dyes was 1 mg mL^{-1}. The mixture (0.1 μL) was applied to the chromatography paper (4 cm × 0.5 cm) forming a round spot 1 cm from the left edge. Then the dyes were separated by dipping the end of the paper into methanol. After elution for about 90 s, the two triangles indicated were cut from the paper for paper spray analysis.

Paper spray ionization was compared with nanospray ionization to seek information on the ionization mechanism. A solution of cocaine (200 ng mL^{-1}, methanol/water, 1:1 v/v) was used to test these two ion sources in the positive mode. Droplets are visible (Figure 3a) under strong illumination but paper spray requires a much higher spray voltage (> 2.8 kV) than nanospray (> 0.8 kV), possibly because of differences in the effective size of the capillary channels. Signal intensities and the corresponding spray currents were also examined over a range of spray voltages. Paper spray shows an increase in both MS signal intensity and spray current with increasing spray voltage (Figure 3b). However, the nanospray signal intensity increased quickly (0.5–2 kV) and then decreased (2–4.5 kV) to zero while the nanospray current increased with increasing spray voltage. Collectively, these results suggest that 1) capillary action is responsible for fluid transport in the paper and 2) the experiment has similarities to ESI methods that use wicking.[17] The required macroscopically sharp tips may be needed for effective fluid transport or they might provide necessary high electric fields at the paper tip if it is these and not the fields in the individual fluid channels that are responsible for ionization. This point is still being investigated.

Despite the similarity between paper spray and nanospray in the positive-ion mode, significant differences were observed in the negative-ion mode. A stable spray could not be observed when a negative voltage was applied to paper wetted with methanol/water (1:1) solvent, even at voltages as high as 6 kV. However, electrons are emitted and can be captured using vapour-phase electron-capture agents like 1,4-benzoquinone at > 3.5 kV with the radical anion $[M]^{-\cdot}$ being generated (Figure S-4 in the Supporting Information). Although such an electron emission phenomenon also exists in nanospray experiments in the negative-ion mode (> 2 kV), it did not interfere with ion formation. The currents measured in paper spray are significantly higher than in nanospray at voltages above 2.5 kV (Figure S-6 in the Supporting Information). The reasons are not fully understood but could be related to the difference between the onset voltage of electrospray and that of electrical discharge. For paper spray, the onset voltage of electrospray appears to be lower in the positive mode; therefore stable electrospray ionization

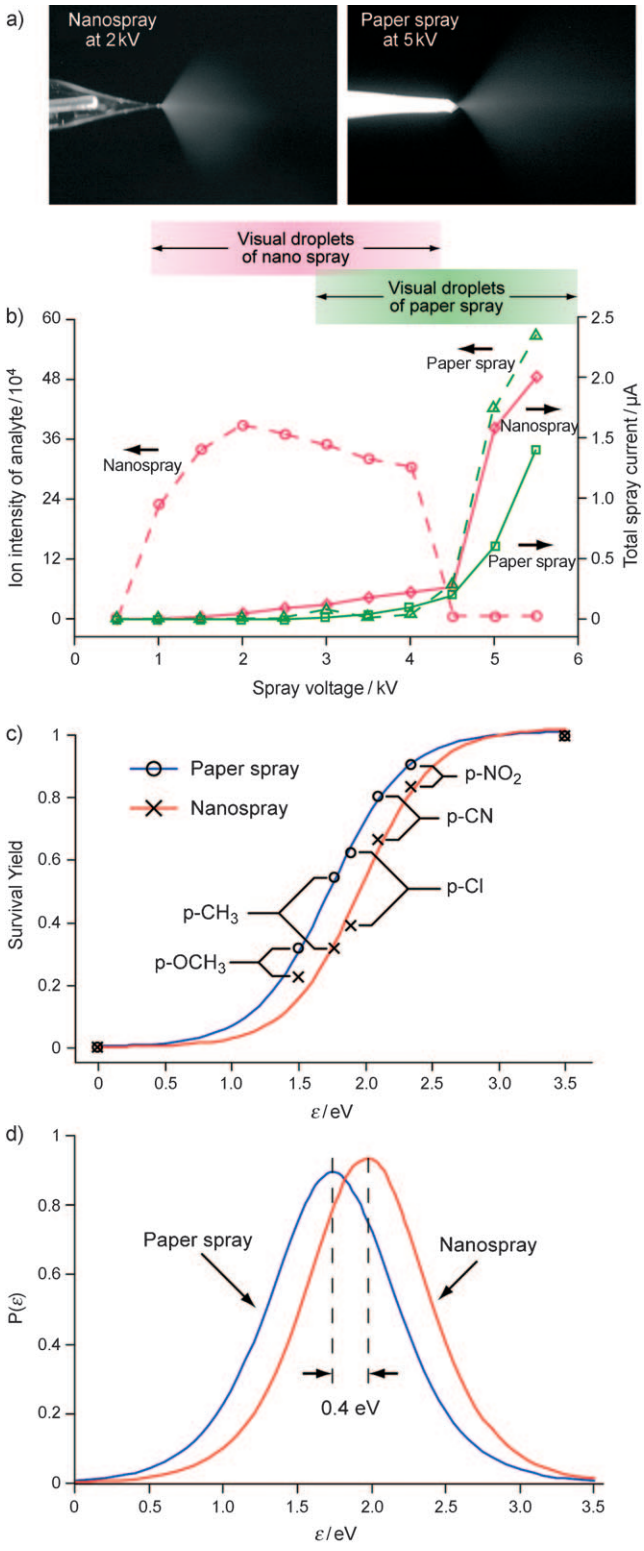

Figure 3. Comparison of nanospray ionization with paper spray ionization in the positive-ion mode. a) Optical images of the spray in nanospray and paper spray, showing the generation of droplets at the tips of the glass capillary and paper triangle, respectively. Spray voltage: 2 kV for nanospray and 5 kV for paper spray. b) Paper spray and nanospray show different molecular-ion abundances (cocaine, 200 ng mL^{-1}, m/z 304) and different spray currents when the spray voltage is increased from 0.5 to 5.5 kV. c) Calculated survival ion yields for paper spray and nanospray ionization. d) Internal energy distributions for paper and nanospray ionization.

can occur. However, the electrical discharge onset is always lower than the onset voltage of electrospray ionization in the negative-ion mode; and the performance of electrospray is therefore seriously degraded.[1] When the solvent was changed to pure methanol, a stable electrospray could be observed for paper spray in the negative-ion mode and the negative ions from analytes such as stearic acid could be detected readily. The fact that it is difficult to induce a discharge with pure methanol supports the above explanation.

The internal energy distributions of typical ions generated by paper spray were measured using the "survival yield" method, which is used to judge the internal energy deposition associated with soft ionization techniques.[18,19] We again compared the result of paper spray with corresponding data of nanospray to investigate the relationship between these two ionization methods using spray voltages of 4.5 kV for paper spray and 1.5 kV for nanospray, respectively, corresponding to optimum performance. Five thermometer ions, *p*-chlorobenzylpyridinium chloride (p-Cl), *p*-methylbenzylpyridinium bromide (p-CH_3), *p*-methoxybenzylpyridinium tetrafluoroborate (p-OCH_3), *p*-nitrobenzylpyridinium bromide (p-NO_2) and *p*-cyanobenzylpyridinium chloride (p-CN) were used as a mixture at 10^{-5} M in methanol/water (1:1, v/v). Figure 3c shows the breakdown curve, calculated as described in a previous study.[19] The corresponding internal energy distribution P(ε) (Figure 3d) was calculated by taking the derivative of the breakdown curve in Figure 3c. The internal energy distributions are similar in shape and slightly different in mean value ($\approx$0.4 eV) for paper spray and nanospray. This result of internal energy distributions suggests these two ionization methods might follow the same mechanism.

In summary, paper spray mass spectrometry (PSMS) has desirable features for clinical applications, including neonatal screening, therapeutic drug monitoring, and, possibly in the future, personalized medicine.[20] Two additional features indicate that it has the potential to increase the use of mass spectrometry in primary care facilities: 1) blood samples needed for analysis can be drawn by means of a pinprick rather than a canula; 2) the experiment is readily performed using a handheld mass spectrometer (Figure S-5 in the Supporting Information).[21] The mechanisms involved in sample transport and ionization in paper spray have not been fully elucidated, but the procedures for complex mixture analysis are simple and rapid; throughput is limited only by the rate of presentation of samples to the MS. The paper medium serves a secondary role as a filter, retaining blood cells; it can also be used for prior chromatographic separation. Significantly, the sample is analyzed directly on the storage medium. Both stored dried blood spots and whole blood can be analyzed. Owing to the porous characteristics of paper, there are no clogging problems. All experiments are done in the open lab environment.

Experimental Section

All experiments were carried out with a Finnigan LTQ mass spectrometer (Thermo Electron, San Jose, CA). Instrumental conditions used for positive-mode mass spectrometry unless specified

otherwise: paper spray voltage: 4.5 kV; nanospray voltage: 1.5 kV; capillary temperature: 150 °C; heated-capillary voltage: 15 V; tube-lens voltage: 240 V for the survival yield experiment and 65 V for all other experiments. Paper was cut into a triangle with typical dimensions height 10 mm and base 5 mm. A copper clip was used to hold the paper section and to apply the high voltage. The distance between the tip of the paper triangle and the inlet to the mass spectrometer was 5 mm. The paper used was Grade 1 chromatography paper purchased from Whatman (Maidstone, England). The blood used was bovine whole blood purchased from Innovative Research (Novi, MI).

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