

Paper spray-MS for bioanalysis

This Review provides a general understanding of paper spray-MS, including the methodology and theory associated with a number of different related applications. This method has become a direct sampling/ionization method for mass spectrometric analysis at ambient conditions, and as a result, it has greatly simplified and increased the speed of mass-spectrum analysis. It has now become an increasingly popular and important method for MS. The first part of this review discusses the fundamentals of paper spray. Some modifications are also reviewed, including nib-assisted paper spray, droplet monitoring, high-throughput paper spray, leaf spray, tissue spray and wooden tip spray. The second part focuses on recent applications, including the analysis of DBS, foodstuffs, drugs and oil. These studies show that paper spray-MS has great potential for use as a fast sampling ionization method and for the direct analysis of biological and chemical samples at ambient conditions.

Within the last few decades, efforts have been made to increase the detection sensitivity for bio-samples. Soft ionization methods, MALDI-MS [1,2] and ESI-MS [3] have been greatly developed, evolved and refined. Apart from techniques that involve different mechanisms, such as electron ionization, gas discharge ionization, photoionization and supersonic spray ionization, MALDI and ESI are the major techniques used in MS analysis, especially for biosamples. Early studies on MALDI- and ESI-MS led to later success in various types of desorption and ionization methods. One of the earlier examples is atmospheric pressure(AP)-MALDI-MS [4–6]. Furthermore, in recent years, desorption electrospray ionization [7,8] and electrospray-assisted laser desorption/ionization [9,10] have developed. In the case of desorption electrospray ionization, a pneumatically assisted electrospray is employed to analyze surfaces in an ambient environment, whereas electrospray-assisted laser desorption/ionization involves the use of an electrospray plume to ionize neutral compounds that are thermally desorbed from a surface using laser ablation.

Once an ionization process can be achieved at ambient conditions, the most important benefits are that the experimental operation process can be simplified and the time required for mass spectrum analysis can be reduced. Shiea *et al.* recently authored a comprehensive review on the topic of ambient ionization MS [11]. Alberici *et al.* [12], Fernández *et al.* [13] and Ouyang *et al.* [14] have all authored comprehensive reviews on these topics. Undoubtedly, all of these techniques have expanded the range of analytes and samples. By the use of these methods, new fields have

opened up for investigating biomolecules and many of the drawbacks associated with vacuum system or ionization problems have now been overcome. Methods that combine sensitivity, speed and involve easy and direct sampling ionization at ambient conditions are current goals in mass spectrum analysis. **Paper spray** (PS)-MS has rapidly grown in popularity over the past few years, because it achieves these goals. Analogous to ESI, by applying a high electrical energy to a wet paper or other porous media, gas phase ions are produced from the surface. Either ionic species or neutral compounds in solution can thus be analyzed by a mass spectrometer.

Characteristic of both ESI and ambient ionization, PS-MS is currently a well-developed method and can be used for the fast, qualitative and quantitative analysis of complex mixtures, extracted from blood, urine, saliva or tissues, and so on. Since its debut in 2010, this method was developed as a direct sampling method as well as an ionization method for mass spectrometric analysis at ambient conditions, and as a result, it has greatly simplified and increased the speed of MS analyses [15]. Even a decade before, people would not have believed that MS analysis, including sample transformation, desorption and ionization, could be performed using just a piece of paper. Even though this technique is not ideal for measuring larger molecules (molecular weight >~500). It has now become increasingly popular and important. PS-MS is particularly efficient when used in conjunction with complex mixtures, either extracted from blood, urine, saliva or tissues, and so on. In this article, the mechanism, methodology and applications of PS-MS analysis, which are currently in use, are reviewed.

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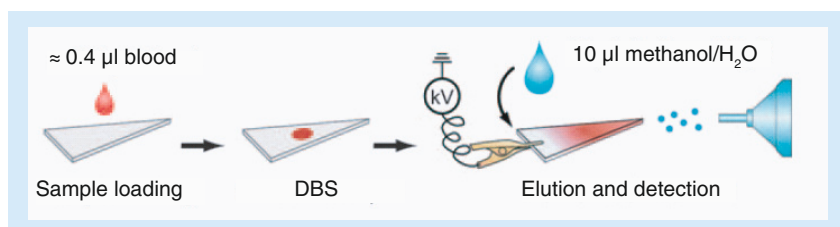


Figure 1. Analysis of a DBS on paper. A drop of whole blood (0.4 ml) was applied directly to a triangular section of chromatography paper. A DC voltage (4.5 kV) is applied to the paper wetted with 10 ml MeOH:H₂O (1:1 v/v). Reproduced with permission from [15] © 2010 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Key Term

Paper spray: A direct sampling ionization method for mass spectrometric analysis of complex mixtures using a piece of wetted triangular paper.

Fundamentals of paper spray

FIGURE 1 shows a schematic diagram of the PS method, when a DBS on paper and a drop of whole blood is applied directly to a triangular section of chromatography paper [15]. Following this, the paper is wetted with a MeOH:H₂O solution, and a high DC voltage is then simply applied to the wet paper, which is positioned in front of a mass spectrometer. In general, paper consists of porous fibers, in which the sample solution escapes from the surface or slowly migrates randomly through the fibers of the paper. **FIGURE 2** shows a scanning electron microscope photo of a triangular-shaped chromatography paper (Advantec, Japan); the inset shows the top view of the paper tip. Once a high voltage was applied, the sample solution is suddenly ejected and ionized toward the ground,

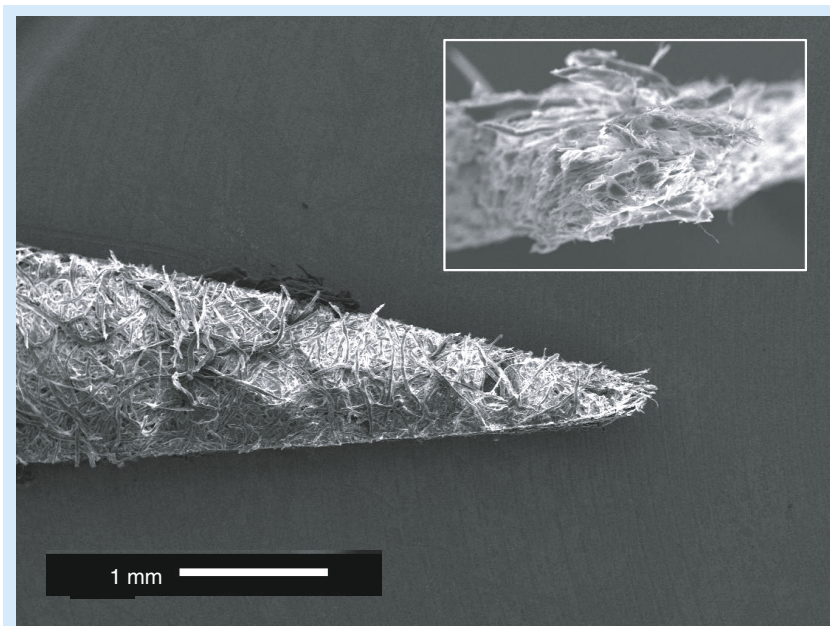


Figure 2. Scanning electron microscope photo of a triangle chromatography paper (Advantec, Japan). The inset shows the top view of the paper tip.

leading to the ESI process. The sharpness of the tip of the triangular paper has a substantial effect on ionization efficiency.

Cooks *et al.* recently proposed a mechanism that explains how the PS actually functions, even though the process is still not well-understood [16]. They found that two distinct spray modes operate during PS process. Mode one occurs in solvent-rich systems, in which multiple Taylor conejets are created, resulting in the production of a range of droplet sizes, whereas mode two occurs at low solvent flow rates and the higher currents that may be produced as the result of a corona discharge. As shown in **FIGURE 3**, conejets can be observed, of course, and this is dependent on the solvent composition (such as viscosity or the ratio of water to organic solvent), solvent flow rate and applied voltage [16]. The conejet diameters were approximately 1–10 µm in diameter, and it is possible that smaller jets are also produced but out of focus. The use of 100% water in conjunction with PS is difficult and, unless salt is added to the water to increase conductivity, the results can be erratic. The use of an additional organic solvent, usually a MeOH:H₂O mixture, is useful to produce spray jets.

The characteristic of the paper is also an important factor. Cooks *et al.* investigated the optimal type of papers by examining four types of filter papers with different pore sizes (pore sizes: 3, 4–7, 8 and 11 µm, respectively), glass fiber paper and chromatography paper (thickness: 0.18 mm), using cocaine in a MeOH:H₂O solution (10 µl, 200 ng/ml) as the model solution. The findings indicated that the optimized performance (highest quality spectrum with the highest S/N ratio), for cocaine analysis, was obtained when a chromatography paper was used [17]. The poorest performance for PS was observed for glass fiber paper. They noted that there was no observable deformation when the paper was wetted with a 10 µl volume of solution, and, after the spray solution had dried, the paper triangle could be re-used, the solution reloaded, and reproducible spectra were obtained. The tolerance to positioning was also examined, since this an important factor for judging the ease of use of an ionization source. As shown in **FIGURE 4A**, a paper triangle was mounted on a 2D moving stage. The translation distances were 8 and 3 cm, respectively, in the *y*-direction and the *x*-direction; a 2 mm increment for each step [17]. In this case, cocaine solution (1 µg/ml in MeOH:H₂O, 1:1 v/v) was

selected as the test sample. It was continuously fed to the paper from the bottom of the triangle to maintain the spray. In the meantime, when a high voltage was applied, the peak intensity of the protonated cocaine ion was appeared, and recorded as a function of the position of the triangle paper tip. As shown in **FIGURE 4B**, the peak intensities were normalized and then plotted as a contour map. It is clear that a stable high intensity can be obtained for PS with the paper tip being located anywhere in an area of about 5×10 mm (x - y plane). Therefore, it appears that accurate positioning may not be required for implementing PS.

An investigation of impacts by the substrates, solvents and elution methods were reported by Ren *et al.* [18]. PS ionization is a process that can be integrated with the fast extraction of the analyte from the raw sample by a solvent, the transport of the extracted analytes on the paper and spray ionization at the tip of the paper substrate with a high voltage applied [19]. As shown in **FIGURE 5**, it is clear that, if the angle of the paper tip was changed, the total spray current and the electric field intensity at the tip would all vary correspondingly, leading to differences in signal intensity. **FIGURE 5A–H** shows spray plumes on a rectangular paper substrate, paper substrates with different tip angles, MS and MS/MS spectra of a solution of $1 \mu\text{g/ml}$ of cocaine in MeOH:H₂O (1:1), the peak intensity of cocaine fragment ion m/z 182 and total spray current as a function of the spray voltage, and so on.

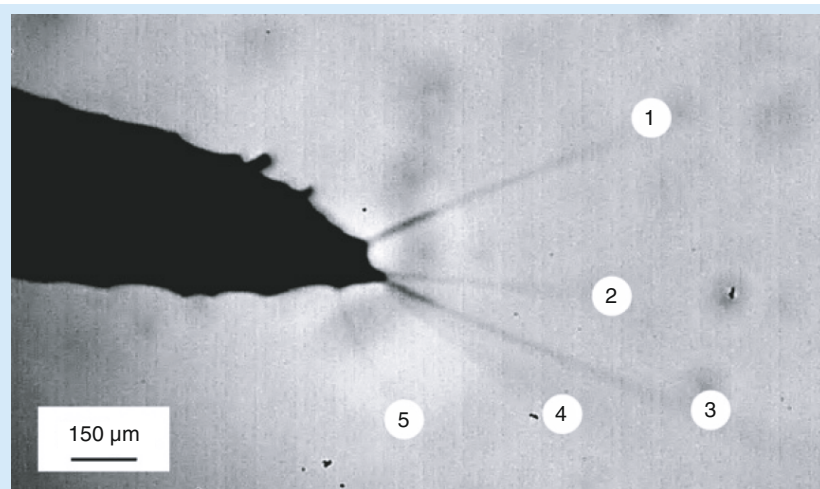


Figure 3. Image of a paper spray taken using a FASTCAM 1024 PCI with back-lit fiberoptic illumination. The substrate is Whatman 1 filter paper, the solvent is 80/20 MeOH:H₂O, the spray voltage is +4500 V. The spray is operating in Mode one. Reproduced with permission from [16] © 2012 Elsevier.

Modifications

■ Nib-assisted PS

When the PS-MS is applied, MeOH:H₂O solution should be added from time to time to maintain the paper in a wet condition. In contrast to this, a nib-assisted device can be used to slowly and continually wet the paper, as shown in **FIGURE 6** [20]. Herein, a piece of paper was cut into a triangular shape (5 mm in length and 3 mm wide at the base). The sample solution was dropped on the triangular spray-paper, and then directly placed on the nib. The nib was designed so as to easily connect with a capillary

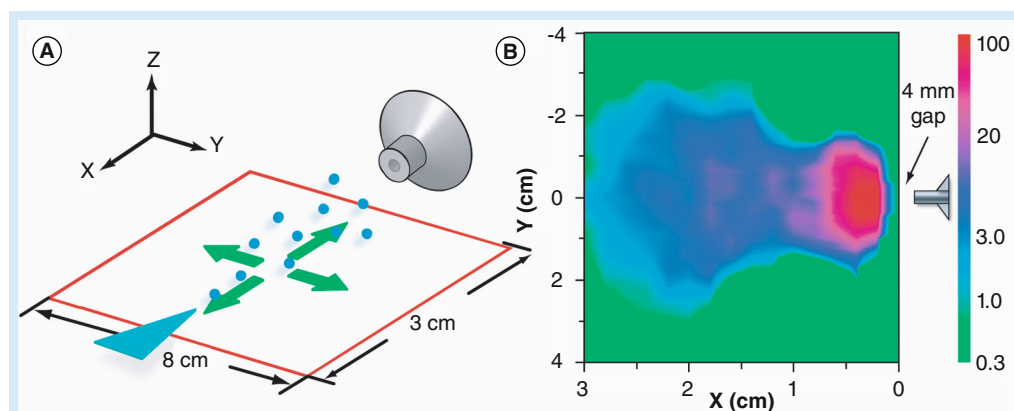


Figure 4. Characterization of the tolerance of paper spray ionization to the position of the paper tip, using a cocaine solution ($10 \mu\text{l}$ MeOH:H₂O, 200 ng/ml) as a standard and chromatography paper. (A) Illustration of the spatial movement of the paper triangle. (B) 2D contour plot showing the relative intensity of m/z 304 as a function of the paper tip location on the x - y plane.

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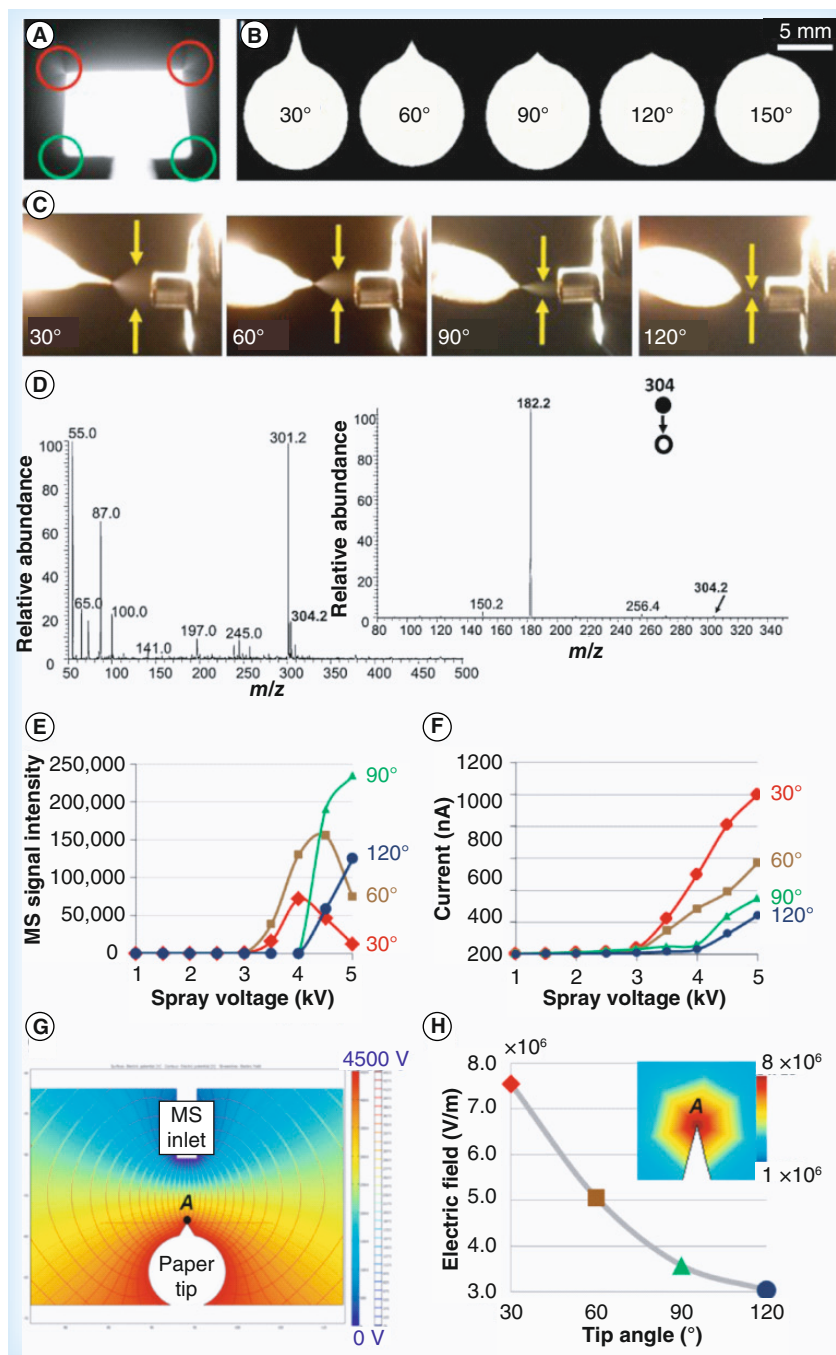


Figure 5. Angle of the paper tips plays a key role in generation of the spray. (A) Spray plumes on a rectangular paper substrate. (B) Paper substrates with different tip angles of 30, 60, 90, 120 or 150°. (C) Spray plumes recorded for paper substrates with tip angles of 30, 60, 90 and 120°. (D) MS and MS/MS spectra of 1 g/ml cocaine in MeOH:H₂O (1:1) as spray solvent, paper substrate with a tip angle 90°, spray voltage 4.5 kV. (E) Peak intensity of cocaine fragment ion *m/z* 182 and (F) total spray current as a function of the spray voltage. (G) Simulated potential distribution (tip angle = 60°). (H) Electric field strength at the paper substrate tip as a function of tip angle. Inset: zoomed-in view of electric field distribution at a paper substrate tip (tip angle = 30°). Reproduced with permission from [19] © 2012 Elsevier.

that provided solution. As a result, it was possible to continuously elute the paper with methanol.

■ Droplet monitoring

Lin *et al.* reported on the development of a novel method for measuring submicroliter droplets [21]. As shown in **FIGURE 7**, droplets were generated by gravity and electrostatic attraction using a capillary tube. The parameters affecting the sizes and frequency of the droplets were investigated. The findings showed that the volume of droplets could be controlled in the range from 0.7 to 2.4 ml and the time interval from 15 to 60 s with appropriate parameters. By combining the droplets with online MS via paper-based ESI, it was possible to deliver a steady flow of solvent via the capillary tube to the base-side of the paper, therefore allowing the electrospray to be maintained in a consistent state. With this approach, each droplet can produce a peak in the ion chromatogram.

■ High-throughput PS

A high-throughput device was developed by Ouyang *et al.*, based on the recently developed PS technology [22]. **FIGURE 8A** shows a diagram of this system; a side view of the device is shown in the inset. The paper substrate with multiple triangles for loading samples is shown in **FIGURE 8B**. This system is a rapid method for the analysis of chemicals in complex mixtures, including therapeutic drug monitoring and food safety screening. As a result, this method was successfully applied to bovine blood samples spiked with sunitinib and nicotine. At optimized conditions, a relatively fast analysis speed of 7 s/sample was achieved with relatively good LOQs (1 ng/ml) and quantitation.

■ Leaf spray

Cooks' group [23,24] examined a simple spray method that provides real-time information regarding sugars, amino acids, fatty acids, lipids and alkaloids. Instead of paper, they used a piece of a leaf that permits the chemical constituents of intact plant material, including living plants, to be directly analyzed. **FIGURE 9** shows a diagram of a **leaf spray**. The leaf spray spectrum can be acquired directly from a green onion leaf in the positive ion mode, and sucrose and glucose ions were identified. The method can be applied to various plant parts and has been demonstrated to be useful for a wide variety of species. Furthermore, in their study, differences in spatial distributions and the possibility of studying plant metabolism were also demonstrated. As can be seen, leaf spray MS is now a fast and simple way for the

direct analysis of components in fresh leaves, without the need for any sample pretreatment, such as sweet glycosides in an untreated Stevia leaf [25]. Tulsi or Holy Basil (*Ocimum sanctum* Linn) is an important plant for medicinal source. Major constituents contain ursolic acid and oleanolic acid, which account for many medicinal activities of the plant [26]. The authors investigated changes in the relative amounts of the parent compounds (ursolic acid and oleanolic acid), and the findings show that their oxidized products show an increasing trend upon aging. Therefore, leaf spray is also extremely easy to implement with no need for nebulizing gas or sample preparation, and is suited to semi-quantitative determinations of complex materials.

■ Tissue spray

The **tissue spray** ionization method was developed by Ng *et al.* and Hu *et al.* [27,28]. This method is simple, fast and very useful for the *in situ* analysis of solid samples for chemical profiling, as shown in **FIGURE 10**. Different from the optimized distance in the case of PS, a closer distance is needed. The herbal tissue is placed in front of the ion inlet of a mass spectrometer. With the application of a high electrical voltage, analyte molecules can be directly sprayed and ionized from the solvent-wetted tissues. It should be performed carefully, since a short distance (<1 mm) between the ion inlet and the tissue tip, or too high an electrical voltage (>3.2 kV), could easily induce an electrical discharge at the sample tip. As a result, the ion signals would be suppressed and the ion signal becomes unstable. The successful spraying of ions from the ginseng tissues was described in **FIGURE 10**, where the sharpness of the tissue tip is pointing toward the ion inlet. In order to detect ion profiles in tissue samples and to avoid electrical discharge, these parameters should be optimized to allow the formation of a stable spray of charged droplets. By this approach, the sample extraction process can be simplified and the sample transfer step can be reduced, leading to an increase in the throughput of analysis. In their study, raw herbs were directly analyzed, including analytes that were embedded in herbal samples [27]. Differences were found between wild-type and cultivated-type American ginseng. A needle biopsy (or fine-needle aspiration biopsy) is a diagnostic procedure used to investigate superficial lumps or masses. A major surgical (excisional

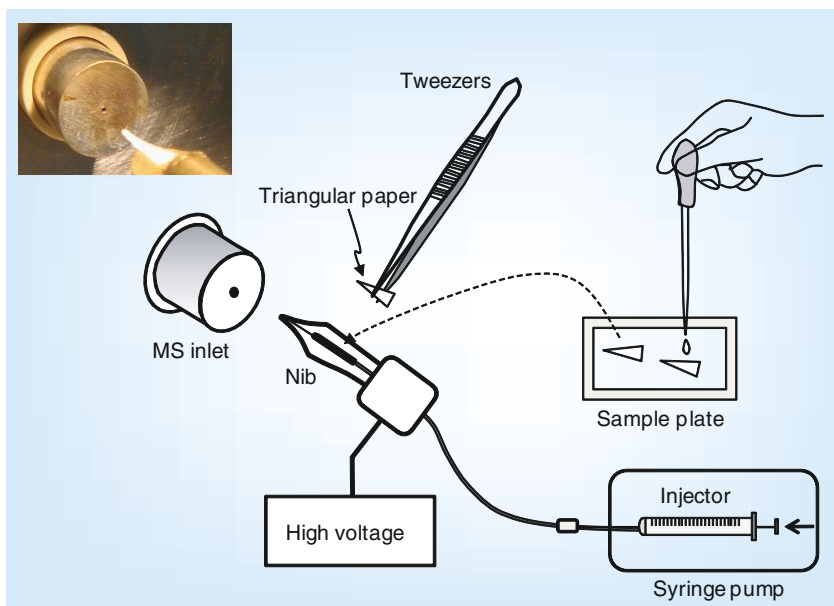


Figure 6. Nib-assisted paper spray-MS method.

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or open) biopsy can be avoided by performing a needle aspiration biopsy. Spray ionization directly from tissue in the biopsy needle is a new approach that may provide highly specific molecular information through MS analysis. For biological tissue analysis, the scheme used

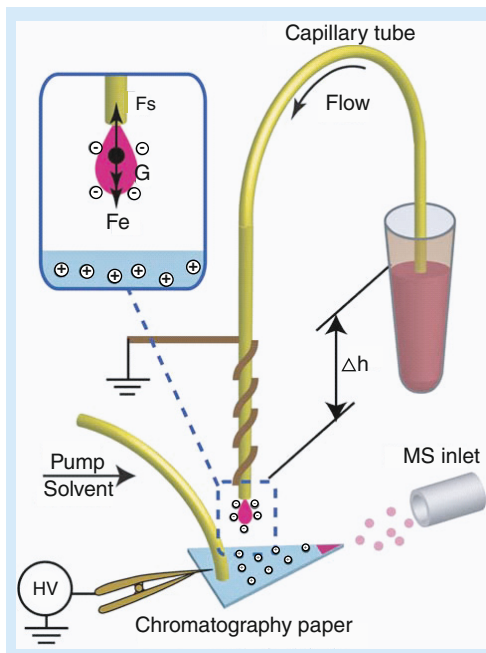


Figure 7. Generation of the submicrolitre droplets and direct MS analysis via paper-based ESI.

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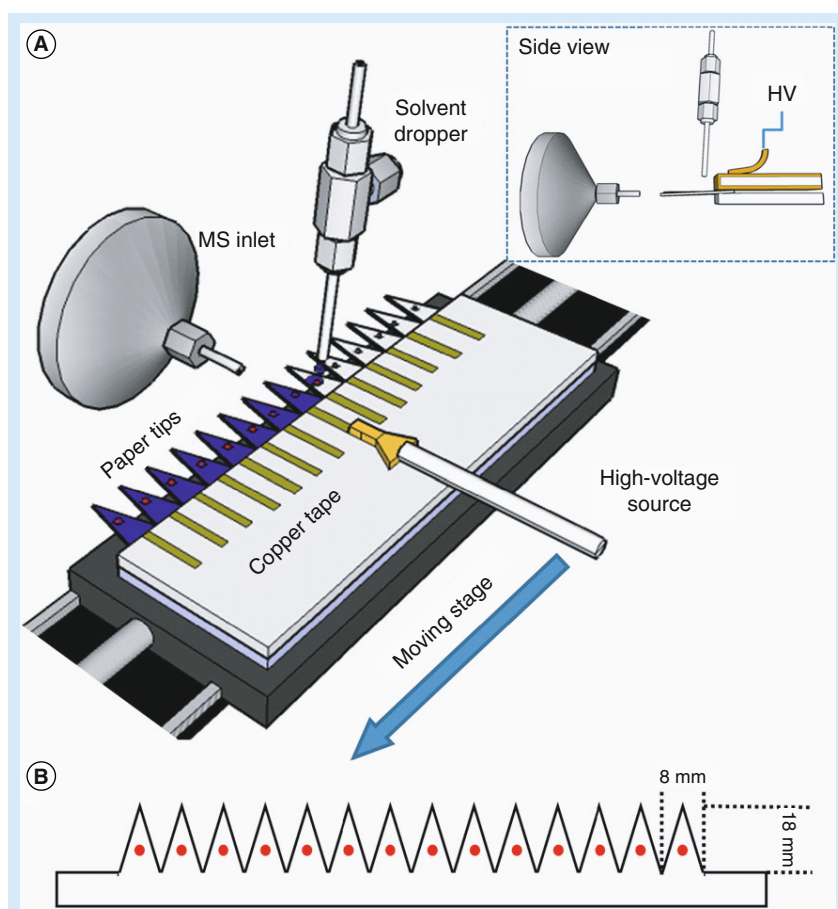


Figure 8. Paper spray-MS platform. (A) High-throughput paper spray-MS platform. The inset shows a side view of the device. (B) The paper substrate with multiple triangles for loading samples. Reproduced with permission from [22], © 2013 Elsevier.

for direct spray ionization from a biopsy needle is shown in **FIGURE 11** [29]. Herein, by inserting the needle, tissue is extracted from the organ (**FIGURE 11A**). By pushing the plunger along the barrel, the extracted tissue is partially released (1–3 mm) from the needle, and it is retained on the needle tip. Following this, a high voltage

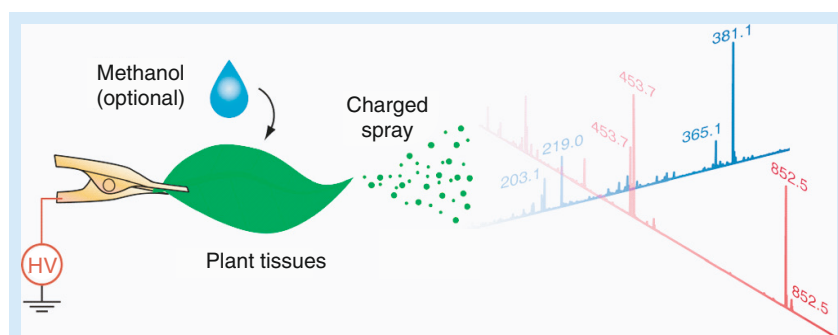


Figure 9. Leaf spray-MS. Reproduced with permission from [23] © 2011 American Chemical Society.

(4–4.5 kV) and spray solvent (2–5 μ l, methanol) are applied to the needle and the exposed tissue, respectively. Charged droplets would be generated, carrying chemicals from the tissue into the MS inlet. A schematic drawing (as shown in **FIGURE 11B**) and a photo of direct-spray ionization in conjunction with a needle biopsy on tissue is shown as **FIGURE 11C**.

■ Wooden tip spray

FIGURE 12 shows a schematic diagram of a **wooden tip spray** (toothpick). It uses disposable wooden tips for the loading and ionization of samples. The largest benefit is that samples could be loaded by normal pipetting onto the tip. It can also be done by simply dipping the tip into the sample solution [30]. The porous nature and hydrophilic property of wood allows the sample solution to be adhered effectively, therefore resulting in the production of durable ion signals. On the other hand, the tip can be directly connected to the nano-ESI ion source of various mass spectrometers. When a high voltage was applied to the tip, desirable mass spectra was obtained. This technique is new and it is applicable for the analysis of various samples. It is particularly useful for samples that are hard to directly analyze by regular ESI techniques, for example, slurry samples and powdered samples. Another advantage is that the hard portions, including the slim and hard properties of the wooden tip, enable sampling from specific locations. It is useful for sampling from corners and small openings. It is clear that potential applications of the new technique in forensic investigations can be expected.

Applications

■ Blood

The storage and transportation of blood samples as DBS on paper is convenient; the analysis of DBS or whole blood is easily accomplished by PS-MS. The first application of PS was reported for the analysis of therapeutic drugs in whole blood by Cooks and Ouyang *et al.* [15]. As shown in **FIGURE 1**, the authors successfully analyzed a blood spot, in which 0.4 μ l of whole blood containing 4 μ g/ml of imatinib was identified and quantified by the MS/MS transition m/z 494 and m/z 394. Quantitative analysis was performed by spiking with imatinib (62.5 ng/ml \sim 4 μ g/ml) and its isotopomer [D_8] imatinib (1 μ g/ml). It should be noted that pharmaceuticals from DBS can be easily investigated. During clinical trials or for therapeutic drug monitoring

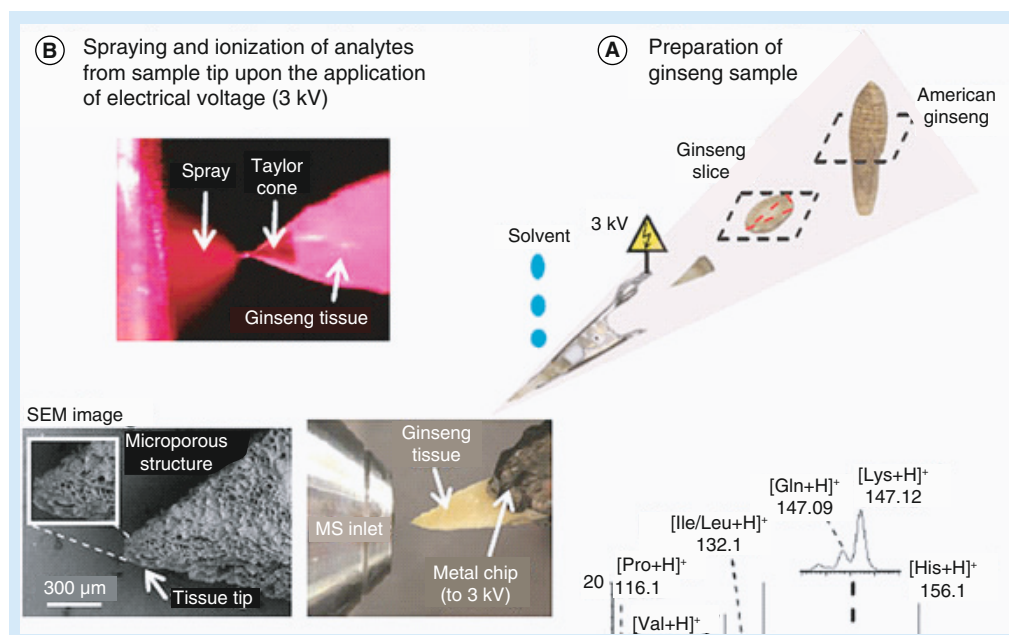


Figure 10. Tissue-spray ionization MS for *in situ* chemical analysis of American ginseng.

(A) Preparation of a herbal tissue slice from the raw herb of American ginseng and (B) spraying and ionization of analyte molecules from the solvent-wetted ginseng tissue upon the application of high electrical voltage. The spraying was observed by light scattering upon irradiation by a laser beam (650 nm, 1 mW), and the microporous structure of the tissue tip was observed under scanning electron microscope examination. (C) Chemical profiles of the wild-type and cultivated-type American ginseng tissues.

Arg: Arginine; Di: Disaccharide; Gln: Glutamine; His: Histidine; Ile: Isoleucine; Leu: Leucine; Lys: Lysine; Pro: Proline; Tri: Trisaccharide; Val: Valine.

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in a hospital or clinic, this method would be potentially useful for quantitation of drugs. Some papers have reported on topics related to the analytical characteristics of PS for analyzing drugs in DBS [31–33]. One such example is the investigation of protein–drug interactions, by comparing the ratio of propranolol and atenolol [31]. Traditionally, HPLC–MS is used in fields such as proteomics and pharmaceutical research. However, by taking a punch from a blood spot and adding an IS solution prior to ionization, quantitation can be performed by PS more easily. From DBS, the weaknesses and strengths of several procedures for implementing PS ionization for the quantitation of small molecule pharmaceuticals were examined by their studies [31]. The use of PS permits total drug concentration in blood to be determined, especially, protein binding does not affect analyte signals. In addition, when IS was added to the paper, either before adding the blood or when it was added afterwards to the dried blood punch, the variance of this method was less than 8%. Another study was reported regarding the rapid quantitation of acylcarnitines in serum and whole blood

[32], where dried serum and whole blood containing a mixture of ten acylcarnitines at various concentrations were analyzed as spots from paper directly without any sample pretreatment, separation or derivatization. The findings showed that the composition of the spray solvent used was a critical factor: for serum samples, a spray solvent of MeOH:H₂O:formic acid (80:20:0.1) was used because that gave the best signal intensity, while for blood samples, which contain more matrix components, acetonitrile/water (90:10) was a much more suitable spray solvent. Studies of eight oncology drugs, including pazopanib, tamoxifen, imatinib, cyclophosphamide, paclitaxel, irinotecan, docetaxel and topotecan, were also reported [33], in which the LODs ranged from 0.5 to 17 ng/ml. All of these findings indicate the applicability to point-of-care therapeutic drug monitoring in a clinical setting [33]. Demirev recently authored a comprehensive review on recent progress in the analysis of DBS and related applications [31]. The review reported on the number of publications that contained the term dried blood spot (DBS) during the last 20 years. More than 2300 papers

Key Terms

Leaf spray: Direct chemical analysis of plant material, including sugars, amino acids, fatty acids, lipids, and alkaloids.

Tissue spray: Direct *in situ* chemical analysis of tissues under ambient conditions.

Wooden tip spray: Using disposable wooden tips for the loading and ionization of samples.

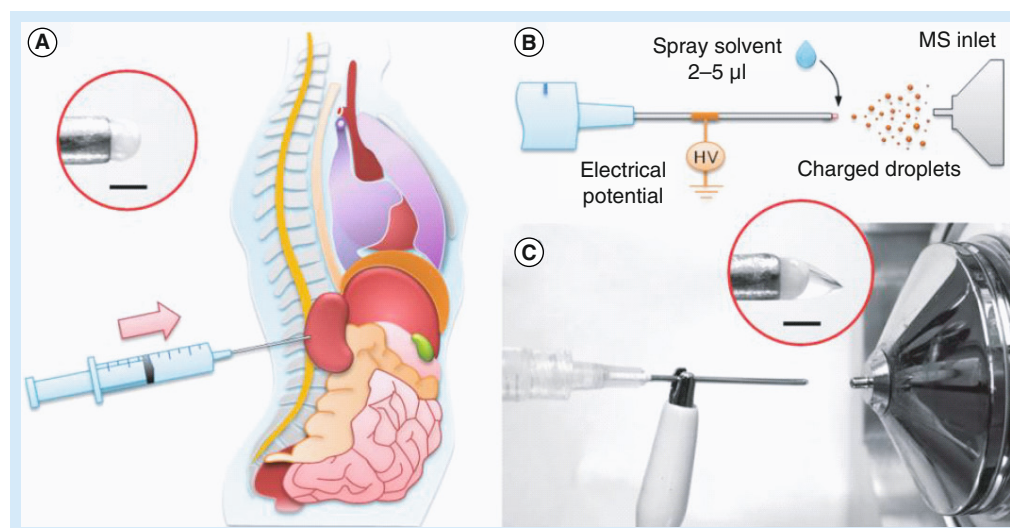


Figure 11. Direct spray ionization from a biopsy needle for the analysis of biological tissue. (A) Tissue is extracted from the organ by inserting the needle into the organ. The extracted tissue is partially released (1–3 mm) from the needle by pushing the plunger along the barrel, and it is retained on the needle tip. High voltage (4–4.5 kV) and spray solvent (2–5 μl , methanol) are applied to the needle and the exposed tissue, respectively, to generate charged droplets carrying chemicals from the tissue into the MS inlet. (B) Schematic and (C) photo of direct spray ionization with needle biopsy on tissue. Inset in part a, biological tissue held by the needle without added spray solvent. Inset in part c, Taylor cone formed at the tissue after applying high voltage and spray solvent. Scale bar is 1 mm. Reproduced with permission from [29] © 2011 American Chemical Society.

have been published since 1936. The number in the last 3 years alone (2010–2012) exceeded 1200. The data was obtained from Thomson-Reuters Web of Science bibliographic database. The same descriptors returned more than 1.5 million hits by a Google search. Methods for the analysis of DBS including chromatography/MS and direct MS [34,35], in which prior treatment to MS analysis is necessary, are not needed. In this work, the time taken for sample preparation to analysis is less than 30 s; LODs for small molecules in blood, for example a chemically diverse set of therapeutics, including hydrophobic and weakly basic drugs (sunitinib, citalopram and verapamil), are routinely less than 1 ng/ml [36].

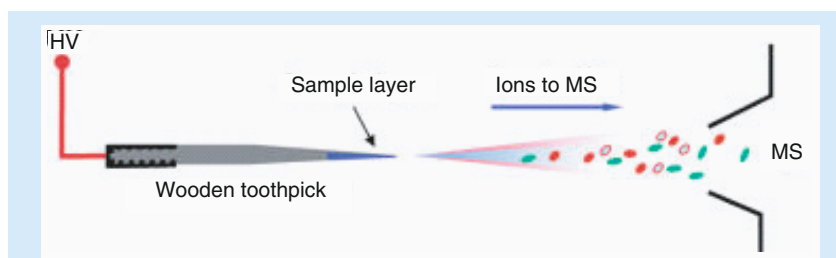


Figure 12. Experimental setup of ESI using a wooden tip. Reproduced with permission from [30] © 2011 American Chemical Society.

■ Foodstuffs & food safety

Food safety has gained increasing attention due to the number of incidents involving food contamination, including cases involving additives such as Sudan red, melamine, clenbuterol, plasticizers and chemical dyes. A high-throughput mass spectrometric method for the simultaneous detection of Sudan I, II, III, IV and Para-Red azo-dyes in foodstuffs was reported [37]. Based on the use of PS–MS and deuterium-labeled IS, Sudan dyes can be determined above the threshold of 1 ppm. Various contaminants, including clenbuterol, melamine, plasticizers and sudan red in various foodstuffs (e.g., meat, milk, sports drinks and chili powder) were also identified by means of PS–MS/MS [38]. Furthermore, a chemical fingerprinting methodology for tracing the origins of such contaminants yielded the best chemical profiling information [39]. For pharmaceutical analysis, these studies show that the PS–MS is a simple, rapid, and robust methodology.

■ Drugs

Quantitative analyses of therapeutic drugs in DBS samples are very important. The entire time for preparation and analysis of blood samples is around 30 s. It has reported on the LODs of 15 therapeutic drugs [36]. Hydrophobic and weakly

basic drugs, such as sunitinib, citalopram, and verapamil, were found to be routinely detectable at approximately 1 ng/ml. Using PS-MS, drug concentrations can be measured quantitatively over several orders of magnitude, with accuracies within 10% of the expected value. By prespotting an IS solution onto the paper prior to the application of the blood sample, a RSD of around 10% can be obtained. On the other hand, a drug-screening system, consisting of PS-MS and a CE-ESI-MS method was recently developed [40]. This system can be easily switched either to PS-MS (for rapidly screening samples) or to the traditional CE-ESI-MS method (for separation and to obtain detailed mass spectral information). The S/N ratios are dramatically improved when the degree of the nib is sharper than 30° and the optimized degree is about 15°. When 4-chloroamphetamine was selected as a model compound, the LOD for PS-MS and CE-ESI-MS were found to ~0.1 and 0.25 ppm, respectively. More recently, as active components in the formulation of corrosion inhibitors, alkyl quaternary ammonium salts in a complex oil matrix was investigated by PS using a portable mass spectrometer [41]. These salts were identified in oil and confirmed by their fragmentation patterns that were obtained by MS/MS. As a result, individual quaternary ammonium compounds were detected at low concentrations (<1 ng/ml) with a dynamic range from 5 to 500 pg/ml. Furthermore, cations of

alkyl- and benzyl-substituted quaternary ammonium salts demonstrated characteristic neutral losses of C_nH_{2n} and C_7H_8 , respectively.

Future perspective

PS is now recognized as a fast sampling ionization method for the direct analysis of raw biological and chemical samples using MS. It is noteworthy that the method is operated at ambient conditions, this is very useful in terms of sample preparation prior to MS analysis. By applying a high voltage, analyte ions can be generated from a wet paper or porous substrate and only a small volume (~10 µl) of solvent is needed. Matrix effects are minimal, samples obtained by chromatographic separation, extraction or other techniques used to purify samples can all be easily applied. Furthermore, besides chromatograph paper, leaf or tissue can also be used for spray ionization and further applications might emerge.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Executive summary

- Paper spray-MS is a fast sampling ionization method for the direct analysis of raw biological and chemical samples.
- It can be operated at ambient conditions, this is very useful in terms of sample preparation prior to MS analysis.
- Only a small volume of solvent is needed.
- Matrix effects are minimal, samples obtained by chromatographic separation, extraction or other techniques used to purify samples can all be easily applied.
- Besides chromatograph paper, leaf or tissue can also be used for spray ionization.

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