

Review

Electrospray Ionization on Solid Substrates

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Development of electrospray ionization on solid substrates (solid-substrate ESI) avoids the clogging problem encountered in conventional capillary-based ESI, allows more convenient sampling and permits new applications. So far, solid-substrate ESI with various materials, *e.g.*, metals, paper, wood, fibers and biological tissue, has been developed, and applications ranging from analysis of pure compounds to complex mixtures as well as *in vivo* study were demonstrated. Particularly, the capability of solid-substrate ESI in direct analysis of complex samples, *e.g.*, biological fluids and foods, has significantly facilitated mass spectrometric analysis in real-life applications and led to increasingly important roles of these techniques nowadays. In this review, various solid-substrate ESI techniques and their applications are summarized and the prospects in this field are discussed.

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INTRODUCTION

Electrospray ionization mass spectrometry (ESI-MS) is an important technique for analysis of chemical and biological molecules.^{1,2)} In conventional ESI, sample solution is delivered to a capillary. Upon application of a high voltage to the capillary, electrospray is induced from the capillary, leading to formation of analyte ions. The development of nano-electrospray ionization (nano-ESI), which typically makes use of a tiny metal-coated capillary for sample introduction, allows consumption of smaller amount of sample and softer ionization.^{3,4)} However, these capillary-based prototypes of ESI have a number of limitations. First, the use of capillary for sample introduction is easily susceptible to clogging,⁵⁻¹²⁾ and sample loading into capillaries, particularly nano-ESI capillaries, is not very convenient. In addition, the capillaries used in conventional ESI, typically made of stainless steel and fused silica,⁵⁾ only serve for sample loading and ionization, and complex samples usually require time-consuming sample preparation and chromatographic separation prior to ESI-MS analysis. In the past two decades, extensive research efforts have been made to improve the sampling and ionization for ESI-MS analysis. Along with the emergence of other ionization techniques, *e.g.*, ambient ionization techniques,¹³⁾ the development of electrospray ionization on solid substrates (solid-substrate ESI) has significantly facilitated the analysis and extended the applications of ESI-MS.

In solid-substrate ESI, sample solution is typically applied on the surface of a solid, non-capillary substrate,

which could be various materials, *e.g.*, metals, cellulose-based materials, and fibers. Upon applying a high voltage to the solid-substrate, the sample solution is sprayed out of the solid-substrate and ions of analytes are generated for mass spectrometric detection. Ions of analytes might also be directly generated from solid biological tissues by applying ionizing voltage and, if necessary, addition of spraying solvents. As the use of capillary is not involved, sampling with these techniques is free from clogging.⁵⁻¹²⁾ Moreover, the feature of open-surface sampling allows high flexibility in the sampling materials. Separation of components in the sample is possible when materials with separating properties, *e.g.*, cellulose-based materials and chromatographic media, are applied as the substrates. These desirable features allow analysis of raw complex samples, *e.g.*, biological fluids, with no or only little sample preparation. Furthermore, solid-substrate ESI techniques typically involve simple and rapid sampling procedure, simple instrumental setup, and the use of low-cost materials for sampling, enabling mass spectrometric analysis easily accessible and effective. In this paper, various solid-substrate ESI techniques and their applications are reviewed and the prospects of this area are discussed.

SOLID-SUBSTRATE ESI WITH METALS

Copper and platinum probe

In the late 1990s, Shiea *et al.* first introduced the use of a copper wire, which was bended to form a ring, as a sampling solid substrate for ESI⁵⁾ (Fig. 1(a)). Upon loading of 1 μ L

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Abbreviations: electrospray ionization mass spectrometry (ESI-MS); nano-electrospray ionization (nano-ESI); probe electrospray ionization (PESI); thin layer chromatography (TLC); direct ionization (DI).

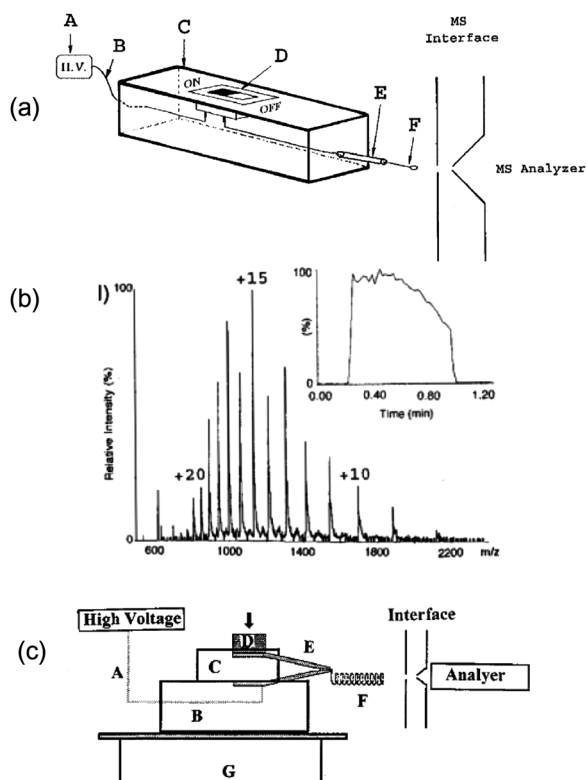


Fig. 1. (a) Schematic diagram of instrumental setup using a copper ring as sampling substrate for ESI (A: high voltage power supply; B: electric cable; C: acrylic box; D: switch; E: connector; F: copper ring). (b) Mass spectrum of myoglobin obtained by the instrumental setup shown in (a). The inset shows the total ion chromatogram obtained for the analysis. (Figs. 1(a) & (b) were reproduced with permission from ref. 5.) (c) Schematic diagram of instrumental setup using a copper coil as sampling substrate for ESI (A: electric cable; B, C, and D: acrylic boxes; E: alligator clip; F: copper coil). (Reproduced with permission from ref. 6.)

of sample solution onto the copper ring and application of a high voltage, quality mass spectra could be obtained for various protein samples and the sensitivity achieved was comparable to that of conventional ESI⁵⁾ (Fig. 1(b)). This study demonstrated that ESI with copper probe could be a fast, simple, and promising technique that avoided the clogging problem commonly encountered in conventional ESI. A similar experimental setup was further demonstrated to be applicable in rapid determination of drugs in beverage samples with only little sample preparation.¹⁴⁾

The instrumental setup with copper probe was further developed into a refined version, in which a copper coil was used for sampling⁶⁾ (Fig. 1(c)). The copper coil rendered a higher surface area for accommodation of larger volume of sample solution and generation of more durable ion signals. This refined configuration could be coupled with optical fibers for generation of more stable ion signals and with solid phase microextraction fibers for enrichment of trace analytes for mass spectrometric detection.⁶⁾ A similar prototype using a coil of platinum instead of copper for sampling was developed subsequently.¹²⁾ The merit of using platinum lies on its chemical inertness, thus the problem of corrosion commonly encountered in copper could be avoided.¹²⁾ Another study demonstrated the application of solid-substrate ESI with a stainless steel wire in analysis of neat ionic liq-

uids.¹⁵⁾

Probe electrospray (PESI)

In 2007, Hiraoka *et al.* introduced the technique of probe electrospray ionization (PESI),⁹⁾ which was further developed and applied by the same group in later years. In PESI, sampling is performed by using a tiny stainless steel needle with a tip diameter in micro-meter range to pick up small amount of liquid sample (Fig. 2(a)). Upon application of a high voltage to the needle, the adhered liquid sample is sprayed out and ionized. More recently, high-pressure PESI and sheath-flow PESI, two further advanced configurations of PESI, were developed to improve the stability and reproducibility of ion signals and enable analysis of dry samples, respectively.^{16,17)} Various studies indicated that PESI was applicable in analysis of a wide range of compounds, and importantly, has a higher tolerance to salts and other contaminants, *e.g.*, urea and detergents, than conventional ESI.^{9,18–21)} In addition, PESI was found to exhibit the phenomenon of sequential and exhaustive ionization of compounds, in which compounds could be separately ionized and detected in the order of their surface activity.²²⁾ These features led to the applicability of PESI in direct analysis of complex biological samples. A very recent study by the same group applied PESI in direct detection of various illicit drugs, *e.g.*, methamphetamine, cocaine, and morphine, in raw urine, oral fluid, and plasma.²³⁾ PESI was also applied in ambient imaging of mouse brain tissue, in which phospholipids and galactosylceramides contents of the tissue were mapped with a high resolution, *i.e.*, 60 μm lateral resolution (Fig. 2(b)), achieved due to the tiny size of the sampling needle.²⁴⁾ PESI was applicable in *in vivo* analysis of plants and animals, as sampling with the tiny needle is considered to be of low invasiveness.^{25,26)} Yu *et al.* demonstrated that by piercing a living tulip bulb with the solid sampling needle to pick up a small amount of tissue fluid, carbohydrate content during the growth of the bulb could be monitored *in vivo*.²⁵⁾ PESI was also applied for direct detection of lipid content in livers of normal and steatotic living mice^{26,27)} (Fig. 2(c)). Higher ion abundance of triacylglycerols was found for the steatotic mice, allowing differentiation of normal and cancerous cells^{26,27)} (Fig. 2(d)).

SOLID-SUBSTRATE ESI WITH CELLULOSE-BASED MATERIALS

Although common cellulose-based materials are intrinsically not good conductors, their electrical conductivities could be significantly enhanced by addition of conducting medium, *e.g.*, solvents, enabling these materials to be used as ESI emitters.¹⁰⁾ The use of cellulose-based materials, *e.g.*, cotton wick and paper, for ESI was first reported by Fenn in 2001.²⁸⁾ In recent years, solid-substrate ESI using chromatography paper, termed as paper spray,^{29,30)} and wooden tip, termed as wooden-tip ESI,³¹⁾ were developed. The major merit of paper spray and wooden-tip ESI lies on their capability in direct analysis of complex mixtures, *e.g.*, biological fluids, mainly due to the ability of paper and wood in separating molecules and, at least to some extents, that the hydrophilic nature of these materials tends to retain polar interfering impurities, *e.g.*, salts. These advantageous characteristics contribute to the wide applications of these

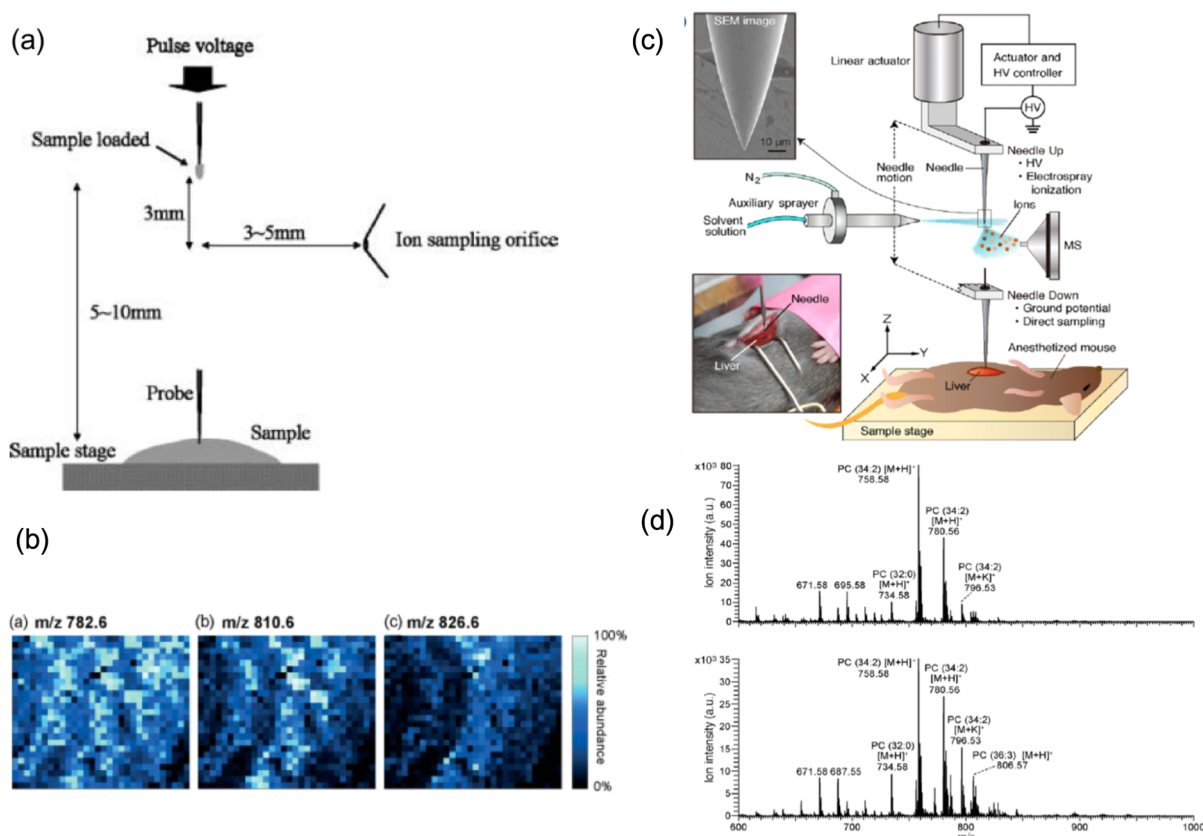


Fig. 2. (a) Schematic diagram of PESI setup. (Reproduced with permission from ref. 9.) (b) PESI-MS images of mouse brain section for different phospholipids: m/z 782.6 ([PC 34:1+Na]⁺), m/z 810.6 ([PC 36:1+Na]⁺) and m/z 826.6 ([PC 36:1+K]⁺). (Reproduced with permission from ref. 24.) (c) PESI setup for *in vivo* analysis of living mice. (d) PESI mass spectra acquired for normal (upper) and steatotic (lower) mice. (Figs. 2(c) & (d) were reproduced with permission from ref. 26.)

techniques in chemical and biological fields.

Paper spray

Paper spray was developed by Liu *et al.* in 2010.^{29,30} In this technique, a piece of typical chromatography paper was first cut into a small triangle (Fig. 3(a)). Sampling can be made by preloading the sample, which could be in solution or solid form, onto the paper or simply wiping the sample with the paper (Fig. 3(a)). Upon addition of wetting solvents and application of a high voltage, the sample is extracted by the added solvents and transferred to the tip end for ionization. This technique was demonstrated to be able to not only analyze complex samples, but also perform quantitative analysis.^{29,30,32-35} Paper spray was successfully applied in direct detection and quantitation of therapeutic drugs in blood and tissue samples, providing a rapid and reliable method in therapeutic monitoring.^{29,30,32-34} Very recently, this technique was utilized in determination of drugs-of-abuse in biological fluids³⁵ and trace amount of illicit drugs on a solid surface (desktop).³⁰ Furthermore, the use of paper spray in food analysis, *e.g.*, determination of Sudan azo-dyes in chilli pepper,³⁶ detection of agrochemicals on fruit peels,³⁰ chemical fingerprint analysis of Bansha herbal tea³⁷ and determination of nonsteroidal anti-inflammatory drugs in olive oil,³⁸ were demonstrated.

The paper spray technology is still being further developed. For example, a high throughput experimental setup³⁹ (Fig. 3(b)) and paper spray cartridge devices for biomedical analysis⁴⁰ (Fig. 3(c)) were developed recently, further ad-

vancing the applications of paper spray. This technique was also found to be able to analyze compounds soluble only in non-polar solvents using non-polar solvents as the wetting solvent, which is less accessible in conventional ESI.⁴¹ This feature widens the range of compounds that could be detected by ESI-MS.

Wooden-tip ESI

In 2011, our research group developed the technique of wooden-tip ESI, which makes use of disposable wooden tips (wooden toothpicks) for sampling.³¹ By applying sample, which could be in form of solution, semi-solid or solid, to the sharp tip end and application of a high voltage to the wooden tip (Fig. 4(a)), ESI could be induced and high quality mass spectra could be obtained. Commercially available wooden toothpicks are directly compatible with nano-ESI ion sources, thus requiring no hardware modification or additional setup to common mass spectrometers for the technique. This technique is applicable for analysis of various samples, including organic compounds, organometallic compounds, peptides and proteins, as well as direct analysis of complex samples, *e.g.*, detection of melamine cyanurate, major cause of fatal kidney stones in the melamine incident in 2008, in urine.³¹ The slim and hard properties of the wooden tip allow easy sampling, including sampling from corners and small openings, *i.e.*, sampling of ketamine powder in a small hole on floor, indicating the potential applications of this technique in forensic investigations.³¹ Our further studies demonstrated the ability of wooden tip

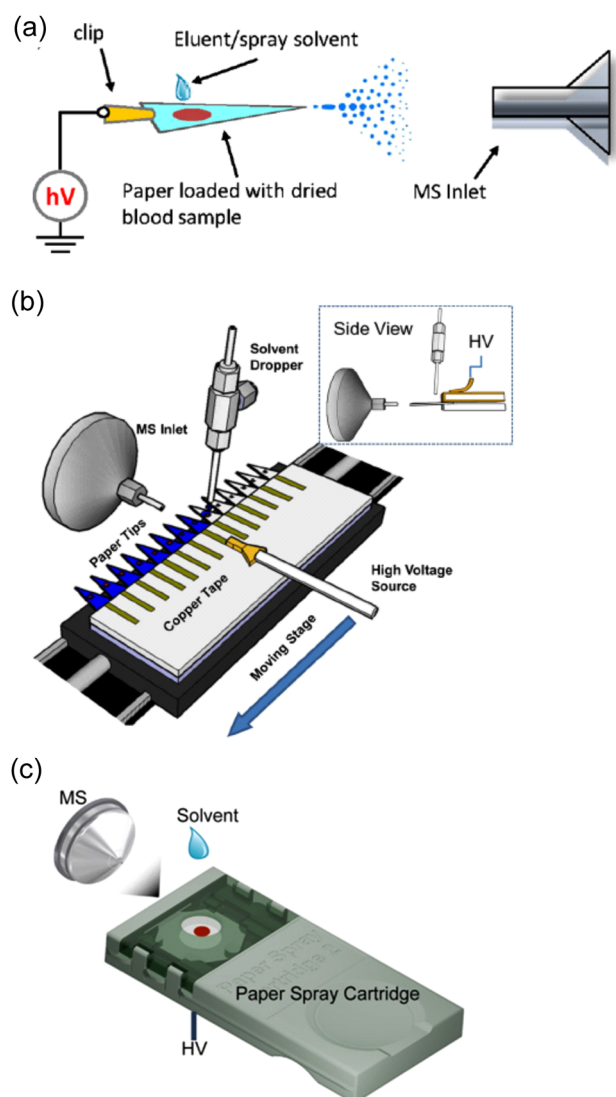


Fig. 3. (a) Schematic diagram of paper spray for mass spectrometric analysis. (Reproduced with permission from ref. 29.) (b) & (c) Schematic diagram of a high-throughput paper spray setup and a paper spray cartridge device for biomedical analysis, respectively. (Figs. 3(b) & (c) were reproduced with permission from refs. 39 & 40, respectively.)

for chromatographic separation of compounds in mixtures such as extract of spinach leaves¹⁰ (Fig. 4(b)). Sequential and exhaustive ionization was also observed in wooden-tip ESI with an order different from that in PESI,²² enabling separated ionization of salts and proteins thus direct analysis of protein samples with high salt content by the technique.¹⁰ More recently, we demonstrated the application of wooden-tip ESI in direct detection and quantitation of drugs-of-abuse in raw oral fluid and urine with acceptable sensitivity, linear range, accuracy and precision⁴² (Figs. 4(c) & (d)). This technique is expected to be further extended to other applications, e.g., food analysis, environmental analysis and therapeutic drug monitoring, in the future.

SOLID-SUBSTRATE ESI WITH OTHER MATERIALS

Fiber materials

Jeng and Shiea demonstrated the use of a surface-modi-

fied optical fiber for ESI.⁷ By coating a thin film of gold or Nafion on the surface of an optical fiber to increase its hydrophilicity thus wettability, aqueous sample solution could be strongly adhered on the surface, which could prevent the loss of sample solution caused by repelling of the sample solution droplet from the probe surface before the onset of electrospray.⁷ However, modification of the optical fiber surface with Nafion might result in formation of protein-Nafion adducts during the analysis.⁸ The same research group subsequently developed another solid-substrate ESI technique using a tungsten oxide nanowire (TON) fiber, which could be produced by heating a tungsten wire under an argon atmosphere, for sampling.⁸ TON was highly inert and did not lead to the formation of adducts with proteins.⁸ In addition, the surface of TON fiber was highly hydrophilic, allowing strong adhesion of and generation of electrospray from an ultra-low volume, i.e., 50 nL, of sample solution.⁸ Other studies introduced the use of polymer microchips for ESI in order to analyze trace amount of analytes.^{43,44} The application of other materials, such as polyester, polyethylene, bamboo, fabrics and sponge, as sampling solid substrates for ESI was also demonstrated in recent studies by Wong *et al.*⁴⁵ and by our group.¹⁰

Thin layer chromatography (TLC) plate

Thin layer chromatography (TLC) is a classical technique for separation of mixtures, yet identification of an unknown on a TLC plate is not an easy task.⁴⁶ Shiea *et al.* developed two interfaces to connect TLC plate to ESI source, allowing online TLC-MS analysis for compound identification.⁴⁶ In one interface, two bound optical fibers were inserted into C-18-packed channels, and the other end of the two optical fibers were pointed towards the mass spectrometer inlet to induce ESI (Fig. 5(a)). In the other interface, a commercial TLC strip was cut to produce a sharp tip, which was connected to a high voltage and oriented towards the mass spectrometer inlet (Fig. 5(b)). Mobile phase solvent from a reservoir delivered the compounds along the TLC plate to the tip end for ionization. These setups were successfully applied to analyze organic mixture samples with significantly small quantity, i.e., 0.2 μ L.

DIRECT IONIZATION (DI) OF SOLID SAMPLES

The successful development of wooden-tip ESI drove us to further investigate the possibility of direct ionization on similar materials such as plant tissue. We subsequently developed a simple and convenient ionization method, direct ionization (DI), for direct analysis of plant and animal tissues.⁴⁷ In DI, a small piece of plant or animal tissue is fixed ~0.5–1 cm away from the mass spectrometer inlet with a simple tool, such as a clip or needle (Fig. 6(a)). By applying a high voltage to the tissue sample and adding some solvents if necessary, spray ionization is directly induced from the bulky tissue sample (Fig. 6(a)). Different kinds of plant tissues, including leaf, root, stem, fruit and rhizome, and animal tissues, including heart, lung, liver, kidney, spleen and medulla, have been analyzed and various compounds such as lipids, alkaloids, glucosides, lignans, pharmaceuticals and proteins could be detected.⁴⁷ Similar techniques, termed as tissue-spray⁴⁸ and leaf spray,⁴⁹ were

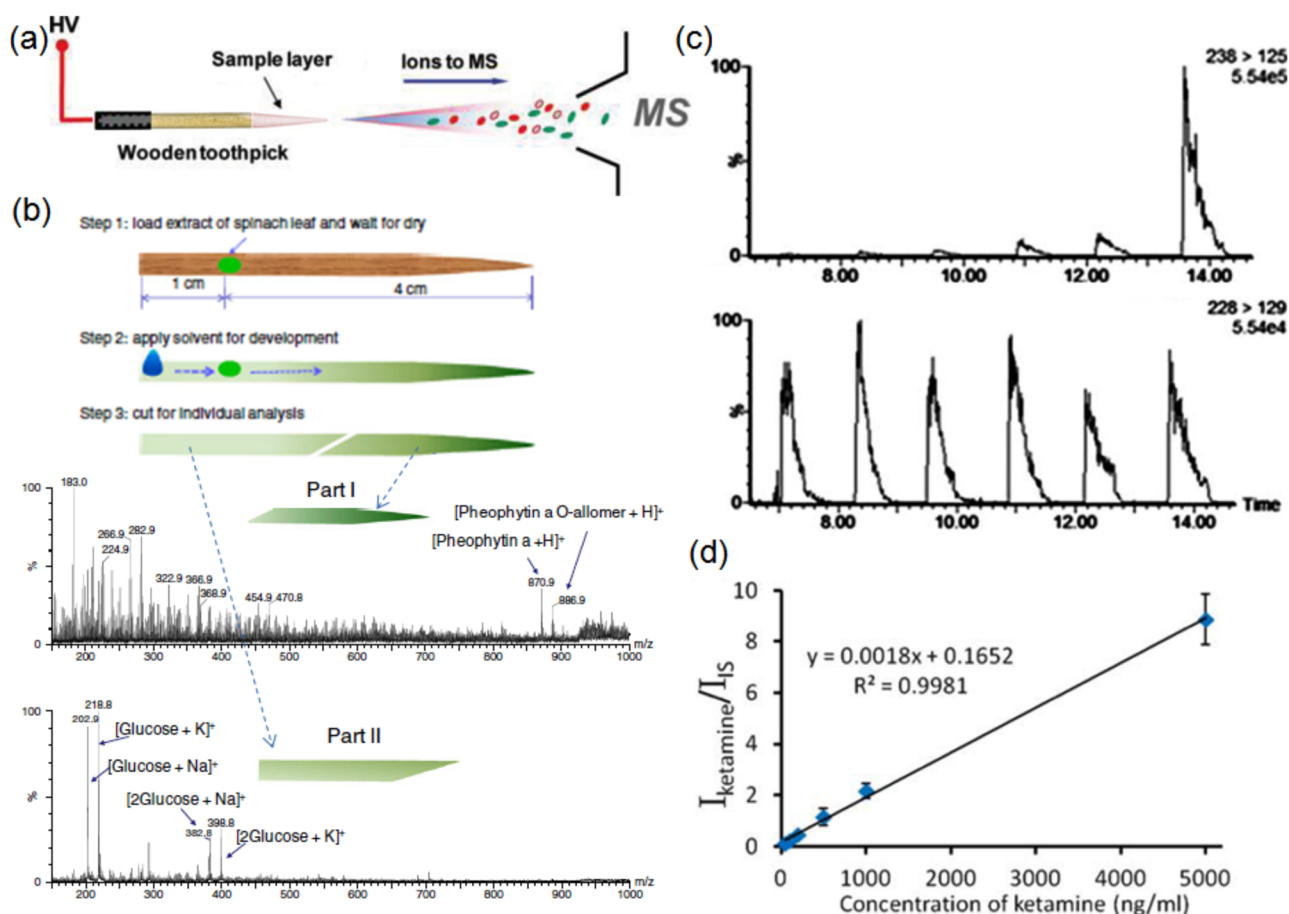


Fig. 4. (a) Experimental setup of wooden-tip ESI. (b) Procedures for testing the separation effect of wooden tip and the mass spectral results obtained. (Reproduced with permission from ref. 10.) (c) Selected-reaction-monitoring (SRM) chromatograms obtained for 50–5,000 ng/mL of ketamine (upper) and 333 ng/mL of d_4 -norketamine internal standard (lower) in oral fluid. (d) A calibration curve obtained for direct quantitation of ketamine in oral fluid with d_4 -norketamine as internal standard. (Figs. 4(c) & (d) were reproduced with permission from ref. 42.)

also coherently developed for direct analysis of plant tissues by two other research groups. These techniques have recently been successfully applied for detection of allergenic urushoils directly from poisonous ivy leaves,⁵⁰⁾ determination of pesticides in peel and pulp of different fruits and vegetables,⁵¹⁾ and differentiation of Chinese and Japanese star anises based on their differences in anisatin content.⁵²⁾ An ionization technique using biopsy needle, which is commonly used in medical examination, for sampling was also developed for direct analysis of animal tissue.⁵³⁾ By picking up small amount of animal tissue using the biopsy needle and upon application of a high voltage, different physiologically relevant compounds could be directly detected from a wide range of tissue samples. Our recent study showed that other solid samples such as mushroom and bones could also be readily analyzed using DI-MS and compounds such as amino acids, saccharides, lipids and proteins could be directly detected from these samples.¹⁰⁾

More recently, we developed an alternative prototype of DI, field-induced DI, in which the high voltage for ionization is applied to the mass spectrometer inlet, while the sample is maintained at electric ground^{54,55)} (Fig. 6(b)). Such prototype avoids connection of the sample to a high voltage and significantly facilitates the sampling and analysis, particularly for *in vivo* study since it also prevents the prior perturbation caused by the high voltage to the analyzed liv-

ing organisms when doing a stimulation *in vivo* study. Our results showed that secretion of venoms by a living scorpion and toad upon attack (Fig. 6(c)) and variation in alkaloids in a living plant, *Catharanthus roseus*, upon stimulation could be readily monitored in real-time using this technique.⁵⁴⁾ These studies revealed the applicability of field-induced DI in *in vivo* and real-time monitoring of secondary metabolites of living organisms.

CONCLUSION AND PROSPECTS

This review summarizes ESI techniques using various solid substrates. Compared to conventional ESI, solid-substrate ESI has the general superiority that it is not susceptible to clogging and allows more convenient sampling. More importantly, a range of solid-substrate ESI techniques possess significant advantages such as high tolerance to impurities and the ability to separate compounds, which allow direct analysis of complex samples with no or only little sample preparation. These features significantly facilitate mass spectrometric analysis in various applications, *e.g.*, clinical diagnosis, therapeutic monitoring, food analysis, and *in vivo* studies.

Unlike capillary-based ESI, in solid-substrate ESI, sample is ionized on the surface of a solid substrate, therefore the analytical performance might be influenced by the interac-

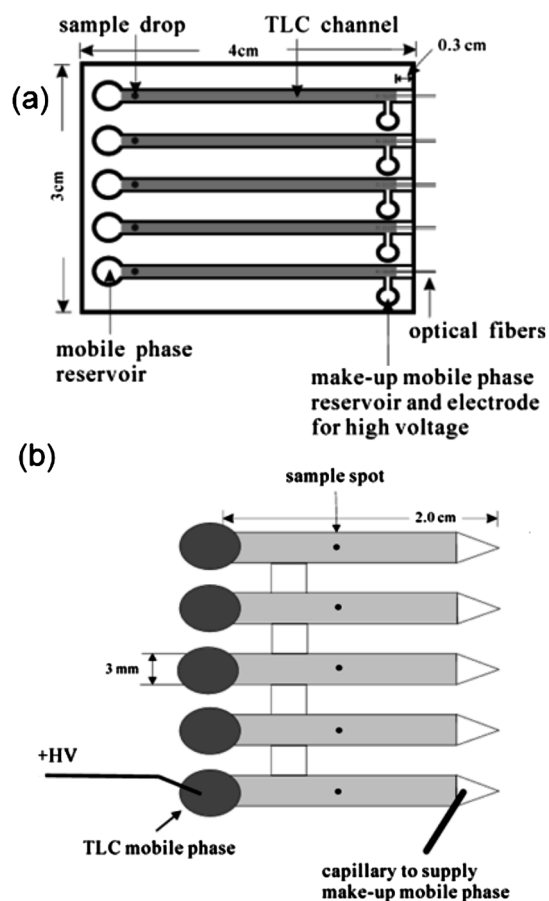


Fig. 5. Schematic diagram of TLC-ESI interface with (a) C18-packed channels inserted with optical fibers and (b) commercial TLC strips. (Figs. 5(a) & (b) were reproduced with permission from ref. 46.)

tions between analytes and the solid-substrate surface. It is thus important to understand such interactions and their effects to the signals and to further improve solid-substrate ESI for various applications. A recent study by Wong *et al.* revealed that solid-substrate ESI with hydrophobic materials showed higher detection sensitivity for polar compounds than for non-polar compounds, while the opposite effect was observed for hydrophilic materials.⁴⁵⁾ Our recent results showed⁵⁶⁾ that solid-substrate ESI with inert, hydrophobic and heat-tolerant aluminum foil enabled on-target sample extraction, cleaning and heating, extending ESI device from usually only for sample loading and ionization to including sample treatment. Solid-substrate ESI replaces capillaries in conventional ESI with solid substrates, allows selection of a wide range of materials, and thus opens many new possibilities for further developments and applications.

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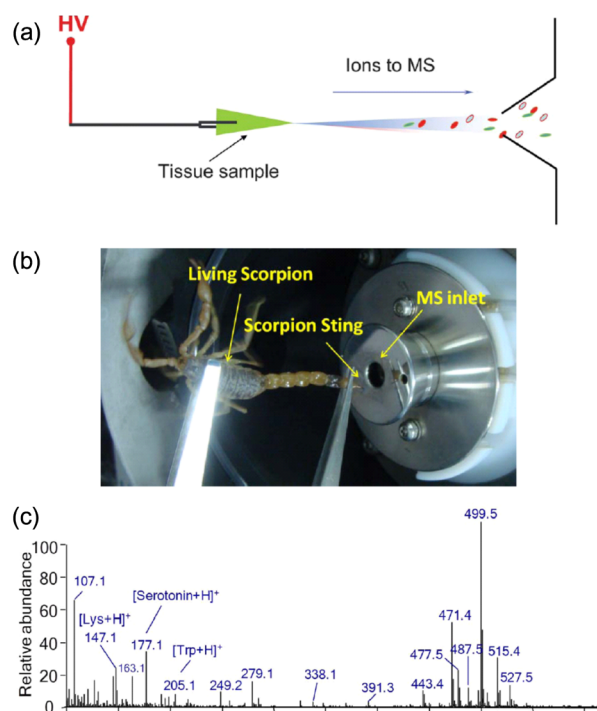


Fig. 6. (a) Experimental setup for direct ionization of biological tissues for mass spectrometric analysis. (Reproduced with permission from ref. 47.) (b) A photo of *in vivo* analysis of a living scorpion by field-induced direct ionization mass spectrometry. (c) Mass spectrum obtained for monitoring of secretion released from a living scorpion upon stimulation. (Figs. 6(b) & (c) were reproduced with permission from ref. 54.)

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