https://doi.org/10.1016/j.aca.2021.339136

Electrospray ionization mass spectrometry with wooden tips: A review

Bin Hu[1*], Zhong-Ping Yao[2,3**]

[1]Institute of Mass Spectrometry and Atmospheric Environment, Guangdong Provincial Engineering Research Center for On-line Source Apportionment System of Air Pollution, Jinan University, Guangzhou 510632, China

[2]State Key Laboratory for Chemical Biology and Drug Discovery, Research Institute for Future Food and Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong SAR, China

[3]State Key Laboratory of Chinese Medicine and Molecular Pharmacology (Incubation) and Shenzhen Key Laboratory of Food Biological Safety Control, Shenzhen Research Institute of The Hong Kong Polytechnic University, Shenzhen, 518057, China

Authors to whom correspondence should be addressed:

*Bin Hu
Institute of Mass Spectrometry and Atmospheric Environment
Jinan University
Guangzhou 510632, China
Tel./Fax: +86-20-8522 5991
E-mail: bin.hu@jnu.edu.cn

**Zhong-Ping Yao
Department of Applied Biology and Chemical Technology
The Hong Kong Polytechnic University
Hung Hom, Kowloon
Hong Kong
Tel.: +852 34008792; Fax: +852 23649932
Email: zhongping.yao@polyu.edu.hk

Abstract

Electrospray ionization (ESI) is a powerful ionization technique in mass spectrometry (MS). There has been an increasing interest for the new development of ESI technique to extend its applications. ESI-MS with wooden tips (wooden-tip ESI-MS), an ESI technique invented in 2011, enabled not only new applications but also new insights into the ESI mechanism. In this review, the technical aspects of wooden-tip ESI-MS are described, the new features of wooden-tip ESI-MS for sampling and ionization of analytes are highlighted, and the important applications of wooden-tip ESI-MS in various fields in the past 10 years, including food safety, forensic investigation, environmental analysis, biomedical analysis and protein study, are summarized. The perspectives on the further development and applications of wooden-tip ESI-MS are also discussed.

1. Introduction

Electrospray ionization (ESI) is an important ionization technique used in mass spectrometry (MS) for analysis of various samples [1-5]. Conventional ESI typically uses a hollow capillary to deliver sample solutions for ionization under the application of gas and a high voltage. The capillary-based ESI can be traced back to the very first electrospray experiment by Abbé Jean-Antoine Nollet in the mid-18th century [6], and it has been further developed with the use of nano-capillary, which requires smaller sample volumes and allows softer ionization [7]. Various coupling methods, mainly based on the capillary-based technique, have been extensively explored for ESI [1, 8, 9]. ESI has become one of the most commonly used ionization techniques in MS, but conventional capillary-based ESI has shown a number of limitations. For example, the use of capillary for sample introduction is susceptible to clogging. Loading sample into the capillary is not convenient, especially for nanoESI capillary. Moreover, ESI-MS analysis of complex samples usually require time-consuming and labor-intensive sample pretreatments. ESI-MS is also difficult to directly analyze raw samples such as biological tissue and living organisms.

The ESI technique makes use of the electrospray phenomenon. When a high voltage is applied to a tip, a solution on the tip can protrude to form a ‘Taylor cone”, and when the Rayleigh limit is reached, the solution would spray out to form fine droplets with analytes in the solution ionized [10, 11]. The tips for ESI are thus not necessary to be hollow capillaries. In fact, the use of this technique on various solid substrates has already been developed [12-14], particularly with the emergence of ambient ionization techniques [15-18]. In the late 1990s, Shiea’s group employed copper wire to generate electrospray [19], and demonstrated that ESI on metal wire could avoid the aforementioned clogging problem and provided a promising

technique for the direct analysis of complex samples [20]. This group later developed a disposable plastic chip that contained eight open channels for sequential ESI analysis [21]. In 2001, Fenn *et al* [22] proposed the use of bundle fiber made of non-conductive materials such as glass, paper and cotton to introduce polar solutions for ESI-MS analysis with easy formation of the Taylor cone. In 2002, Kameoka *et al* [23] used triangular thin polymer tips for ESI-MS analysis of microfluidics. In 2003, glass rod was used as an ESI emitter after surface modification with a thin gold or Nafion film [24]. Pointed carbon fiber was developed as an ESI emitter for interfacing nano-liquid sampling technique with MS in 2004 [25]. In 2007, Hiraoka *et al* [26] developed probe ESI by using a sharp stainless steel needle for sampling soft solid or liquid samples at microliter or smaller volumes. In 2010, paper spray ionization was introduced for sampling and analysis of raw samples [27], and its development and applications have been reviewed [28-30]. In 2011, ESI on wooden tips (termed as wooden-tip ESI) was introduced [31]. Later on, other substrates such as aluminum foil [32], nylon brush [33], bamboo nib [34], fibers [35], polymer tip [36-38], medical swab [39-41], strips [42] and cotton thread [43, 44] have also been developed as ESI emitters. ESI techniques on other functionalized substrates such as thin-layer chromatography (TLC) plates [45, 46], coated blades [47, 48] and solid-phase microextraction (SPME) fibers [49, 50] have also been utilized to simplify analysis of complex samples. ESI on solid substrates has also been extended to the direct analysis of biological tissue [51-53], with applications in herbal medicine, food safety and clinical diagnosis [54-58], and *in vivo* and real time analysis of living organisms has been explored [59, 60].

This review aims at providing a comprehensive review on wooden-tip ESI-MS, a widely used

technique that has not yet been reviewed since its development 10 years ago. Wooden-tip ESI-MS makes use of ordinary wooden toothpicks for sampling/ionization of samples and it can be directly compatible with the nano-ESI ion source [31], making it readily adopted by various users. The different features of wooden-tip ESI compared with those of conventional ESI have given us new insights into the ESI process and new possibilities of the ESI technique. The development of wooden-tip ESI-MS in the last decade has seen the applications of the technique in various fields. These features and applications are discussed in details in this review, together with the technical aspects and prospects of the further development of the technique.

2. Technical aspects of wooden-tip ESI

2.1 Fabrication and configurations of wooden tips

A schematic diagram of wooden-tip ESI is shown in Fig. 1a. The wooden tips used are ordinary disposable wooden toothpicks (Fig. 1b), which are readily available from stores at significantly low prices [31] . The slim and hard properties of wooden tips allow sampling from specific locations (e.g., small openings), and the porous surfaces of the wooden tips enable the loading of appropriate samples and solvents. The commonly available toothpicks have a uniform size that is compatible with the capillary mount of typical nano-ESI ion sources and the elasticity of wood allows the tips to be steadily fixed without the use of screws, making the setup of wooden-tip ESI simple and easily achieved with different types of mass spectrometers without hardware modification or additional power supply. For laboratories without any nanoESI ion sources, the wooden tips can be mounted in front of MS inlets using clips with supports and power supplies from the ESI ion source or additional devices. The wooden tips can be oriented perpendicular to (Fig. 1c) or with an angle (Fig. 1d) to the MS inlet. It should be noted that the

tip ends of common toothpicks are usually required to be further cut into smaller sizes, typical at ~ 0.1~ 0.2 mm, to generate ESI with better signals and lower applied voltages according to the relationship between the tip size and threshold voltage for electrospray [61].

2.2 Conductivity and ESI process of wooden tips

How ESI can be generated from wooden tips, which are nonconductive, has been investigated [14]. It was found that the dry wooden tips of toothpicks had electric resistances larger than the measurement limit of the multimeter and they immediately became much more conductive when a solution was loaded on them. Further investigation revealed that upon loading onto the tip ends, the solution would diffuse along the longitudinal microchannels due to capillary action. Such diffusion was very rapid (about 0.24 s for a wooden tip of 20 mm), immediately allowing the wooden tips to become conductive and generate spray ionization. When a high voltage (e.g., 3.5 kV) was applied onto a wooden tip with a solution loaded onto the tip end, the Taylor cone and spray plume could be observed (Fig. 1e), showing a typical ESI process.

2.3 Potential background interferences and contamination to the MS inlet

Recently, there is concern about the potential background interferences of ESI-MS with subtracts such as wooden tips and paper [38]. The wooden toothpicks used in wooden-tip ESI-MS are typically purchased from stores or even collected from canteens, and directly used normally with low background signals [31, 62]. Soaking them in methanol before use could much reduce the possibility of background signal interferences caused by potential chemical residues in their manufacturing and storage [14].

Potential contamination of the MS inlet is another common problem of wooden-tip ESI-MS and other ambient MS techniques. To eliminate or reduce such contaminations, orthogonal

configuration (Fig. 1c) is preferred since its unionized species is not directed into the MS inlet, and if the parallel configuration (Fig. 1b) is used, the wooden tips should point at an angle rather than directly to the MS inlet. Controlling the sample volumes and concentrations as well as using optimized spray solvents and ionization voltages could also be helpful in reducing potential contamination to the MS inlet.

2.4. Ion suppression

Unlike the conventional approach that involves solvent extraction followed by chromatographic separation and MS detection, matrix effects and competitive ionization of co-existing compounds that occur during the direct analysis of raw samples by wooden-tip ESI-MS and other similar techniques, can cause significant ion suppression. The mass spectra obtained by wooden-tip ESI-MS might not give complete profiles of the compounds in the samples, and sometimes the target compounds might not be detected in the spectra. Changing spray solvents might allow detection of different compounds [14]. Surface modification of wooden tips with special functional groups is another strategy to improve the detection of target compounds. As discussed below, the modified wooden tips could serve not only as ESI emitters for sample loading and ionization, but also as extraction probes to enrich trace analytes in complex samples and diminish matrix effects, allowing sensitive detection of analytes in various raw samples [63-68]. Selected reaction monitoring (SRM) could be used to enhance the wooden-tip ESI-MS detection of target compounds [69], while coupling wooden-tip ESI with ion mobility mass spectrometry (IM-MS) could resolve isobaric ions that could not be separated without the help of chromatography [70]. Interestingly, a recent study [71] showed that compared to metal capillary, wooden capillary could allow much stronger ESI signals, particularly for negative ion mode, and reduced electrolytic interferences.

2.5. Quantitation

It has been demonstrated that wooden-tip ESI-MS can be used for quantitative analysis of target analytes in raw samples with good analytical performance. Using SRM with triple quadrupole mass spectrometers can significantly improve the specificities and sensitivities of the measurements [69]. To compensate for the signal fluctuations caused by the irregular tip ends as well as the inconsistent instrumental and operational conditions, it is important to add an internal standard (IS) to ensure good analytical results [69, 70, 72, 73]. IS is typically mixed with the raw sample prior to loading the sample onto the wooden tip, and could possibly be pre-loaded on the wooden tip or mixed into the spray solvent for fast and simple operation [39, 65, 74].

2.6. Tips made of other materials analogous to wood

Tips made of other materials analogous to wood have been attempted for ESI sampling and ionization with the sample solutions loaded onto the sharp ends [14]. No signals were observed with the nonporous nonconductive tips due to the intrinsic insulating property of the materials and there was no efficient diffusion of solutions along the tips to improve conductivity. Spray ionization could be observed with the porous tips that were made of bamboo, fabrics and cellulose sponges, respectively. Compared with wooden tips, bamboo tips were more rigid and held solutions less efficiently, and thus typically offered shorter signal durations. Shorter signal durations were also observed for paper tips and fabrics tips due to the easy spread and evaporation of sample solutions on these planar tips with larger surface areas.

3. Features that are different from those of conventional ESI

The use of wooden tips for sampling and ionization of analytes enables new features of ESI that are different from those of conventional capillary-based ESI. These new features include:

1) The replacement of capillary with wooden tips avoids the clogging problem and allows direct analysis of raw samples in various forms, including liquid, powder, semiliquid, bulk tissue. 2) Unlike capillary with inert surfaces, the relatively active surfaces of wooden tips can interact with samples to affect the detection of analytes, and can even be modified to separate or extract target analytes from complex samples. 3) Unlike hollow capillary that has a relatively closed environment to contain the samples prior to spraying, wooden tips expose samples to an open environment in the air, which can cause evaporation of solvents and condensation of moisture or other components in the air.

3.1. Direct analysis of raw samples in different forms

Direct analysis of raw samples is of significant importance in understanding the molecular compositions of raw samples in their native environments. With little or no sample pretreatment required, such a direct and rapid approach is beneficial to many fields. Wooden-tip ESI for sampling and ionization in an open environment allows direct analysis of raw samples in different forms. For example, raw liquid samples such as body fluids (e.g., urine, oral fluid) [69, 73, 75, 76], beverages [32, 77], medicines [63, 75], and environmental water samples [63, 66] can be directly loaded on wooden tips for ionization and detection. Furthermore, viscous samples (*e.g.*, ointment and cream) [14] and solid powder samples (*e.g.*, herbal medicines, capsules, pills, tablets, and food materials) [14, 63, 78-80] can also be loaded on wooden tips for direct analysis by adding optional extraction/spray solvents. The slim, hard, and porous properties of wooden tips also allow direct insertion into or touching of living organisms for *in vivo* sampling of bulk solid samples [67, 81]. Moreover, wooden-tip ESI-MS can be used for direct analysis of bulk biological tissue [51-53] and living organisms [81], and high throughput [82] and vibrating voltage-free [83] methods have been developed to facilitate the wooden-tip ESI-MS analysis. These desirable features of wooden-tip ESI allow direct analysis of various

raw samples with the advantages such as easy setup, short analysis time, and low cost.

3.2. Effects of substrate-analyte interactions

Unlike conventional ESI that delivers samples through a capillary with inert and smooth surfaces, in wooden-tip ESI samples are loaded and ionized on the surfaces of wooden tips which can have relatively active surfaces and porous structures, and thus the analytical performance of wooden-tip ESI might be influenced by the interactions between analytes and the tip surfaces. Wong *et al* [62] found that hydrophobic tips (*e*.g., polyester and polyethylene tips) allowed better detection sensitivity for polar compounds but not for non-polar compounds, while hydrophilic tips (*e.g*., wooden tips) showed the opposite effect. Studies also indicated that wooden tips could serve as a chromatographic column for separating small analytes with different polarities [14] and combination of the chromatographic and electric field effects was involved in the substrate-analyte interaction [68, 84]. To further investigate the substrate-analyte interactions in wooden-tip ESI-MS and to design new substrates with improved sensitivity and selectivity to enhance the detection of target analytes from complex samples, a series of wooden tips with surfaces modified with hydrophobic, basic, and acidic functional groups (Fig. 2a) were designed to compare two sampling methods, *i.e.*, extractive sampling and direct loading (Fig. 2b), for wooden-tip ESI-MS [67]. The results showed that in the direct sample loading method, analytes with weak interactions with the wooden surfaces could be readily sprayed out for detection; while in the extractive sampling method, analytes strongly retained on the wooden surfaces could be selectively enriched and detected, and a washing step after sample loading could effectively remove unbound components to reduce interferences. Modifying wooden surfaces with target functional groups by chemical reactions and molecular imprinting techniques was further developed for selective detection of target analytes [63-66, 85]. The surface modification strategy has also been employed for various substrates, including

paper [86], stainless steel needle [87], tungsten probe [88], aluminum foil [89] and stainless steel blade [90], to enhance the detection of target analytes in various samples. Surface modification is expected to provide more possibilities for the designing of surfaces that can provide higher extraction and ionization efficiency of target analytes.

In addition to small analytes, wooden-tip ESI could separate intact proteins and peptides [31]. Further mechanistic investigation revealed that there were two stages in the ionization process of protein samples on porous substrates [91]. As shown in Fig. 3, in the first stage (Fig. 3a and Fig. 3c, T1), liquid flow and resulting spectra (Fig. 3d) of bulk solutions on substrate surfaces were similar to those obtained with conventional capillary-based ESI-MS (Fig. 3f). In the second stage (Fig. 3b and Fig. 3c, T2), however, analyte-substrate interactions played a dominated role in the mobility and detectability of analyte ions (Fig. 3e). In this stage, the separation of analytes depended on hydrophobic interactions between analytes and substrate surfaces, and proteins could be separated based on their sizes and shapes, thus allowing the separation of different conformations and charge states of the same proteins and measurements of their cross sections [91]. Such two-stage ionization and elution order could be observed with wooden and other porous tips, and could be used for preparative separation of proteins. The separation effects of porous surfaces on wooden tips were similar to those of ESI on chromatographic papers [27], TLC plates [46] and other chromatographic materials [92]. Compared with conventional LC-ESI-MS, these separations and detections were more convenient and could be completed within a short time; however their separation resolution and efficiencies were typically still poor.

3.3 Ionization in open environment

Unlike convention ESI that keeps samples inside the capillary before spraying them out for

ionization, in wooden-tip ESI, sample solutions are exposed to an open environment in the air during the whole analysis process, leading to evaporation of solvents and potential interactions of the loaded samples with components in the environment. It was reported that under such conditions, non-polar solvents such as hexane that are of low electrical conductivity and that are usually considered as non-ESI-friendly solvents could be used for paper spray ionization of polar analytes [93]. A later study also showed that wooden-tip ESI-MS with non-polar solvents could allow direct detection of native proteins or protein complexes from raw biological samples such as egg white and bone marrow [94]. The mechanistic study revealed that during the process, the added nonpolar solvent was rapidly vaporized to cause a drop in temperature and consequent condensation of water from the ambient air that was subsequently deposited on the substrate surface, causing the dissolution and spraying out of analytes from substrate tip under the electric field (Fig. 4a) [94]. When non-polar solvents such as n-pentane were loaded onto the wooden tips, solvent evaporation was extremely rapid, causing a drop in the temperature to below -10 ºC and formation of ice dendrites on the wooden tips (Fig. 4b). By addition of non-polar solvents, low-polar compounds in the samples loaded on substrate surfaces could also be extracted and water-soluble gas (*e.g.*, ammonia) in the air could be condensed onto the substrate surfaces to be detected [95].

4. Applications

The applications of wooden-tip ESI-MS for identification and quantification of analytes in various fields have been extensively explored in the past decade (see Table 1 for a summary of the analytical performances of wooden-tip ESI-MS for detection of target analytes from various raw samples). Some featured applications are shown below.

4.1. Food safety

ESI-MS with disposable, low-cost, efficient wooden tips is a desirable technique for rapid sampling and analysis of food samples as it enables quick responses to food safety issues. For example, Yang *et al* [77] applied wooden-tip ESI-MS for rapid qualitative and quantitative detection of trace toxic and hazardous compounds in food samples, with detection limits as low as ~10 pg and analysis of single sample within 2 min. Zhang *et al* [96] applied wooden-tip ESI-MS to directly analyze chemical components of navel orange juice under positive and negative ion modes and semi-quantitatively determine ascorbic acid in navel orange juice from different origins. Hu *et al* further developed an automated high-throughput wooden-tip ESI-MS for rapid screening of illegal additives in dietary supplements with an analysis speed of ~15 seconds per sample [78]. The screening results of totally 33 adulterated drugs and 144 dietary supplements in this work were in good agreement with those obtained by using conventional LC-MS. Liu *et al* [65] developed molecularly imprinted polymer (MIP)-coated wooden-tip ESI-MS for enhanced analysis of trace macrolide antibiotics in food samples. This work showed that the MIP-coated wooden-tip probe could be applied to offer enrichment factors of 244–1604, 72–370 and 12–82 folds as well as limits of detection in the range of 0.003–0.05, 1.1–5.1 and 1.9–15.8 ng/g for detecting five macrolide antibiotics in drinking water, honey and milk samples, respectively. These results also indicated that MIPs, which contain molecular recognition sites to accommodate and retain specific molecules [97], could be integrated with wooden-tip ESI-MS for selective and sensitive detection of target analytes in complex samples.

4.2 Forensic investigation

The sampling trace ketamine, a commonly abused drug, from a tiny crack on a concrete floor using a pre-wetted wooden tip is one of the examples of the forensic application of wooden-tip

ESI-MS [31]. So *et al.* [69] further demonstrated rapid quantitation of ketamine and its metabolite norketamine in urine and oral fluids by wooden-tip ESI-MS with acceptable analytical performance in analytical speed, linear range, accuracy, precision, and sensitivity. Following this work, Ng *et al.* [73] extended the application of wooden-tip ESI-MS for detection and quantitation of various commonly abused drugs, including methamphetamine, methylenedioxymethamphetamine (MDMA), cocaine, heroin and tetrahydrocannabinol (THC), in urine and oral fluids. It was found that the limit-of-detection for methamphetamine could fully fulfill the cut-off values of the international standards and those of MDMA and cocaine could fulfill some of the requirements, and analysis of one sample could typically be completed within minutes. The sensitivity for detecting abused drugs in human body fluids could also be further improved by surface-modified wooden-tip ESI-MS, *e.g.*, sulfoacid-modified wooden-tip ESI, for enhanced detection of cocaine in oral fluids [67]. Millán-Santiago *et al.* developed a polyamide-coated wooden-tip ESI-MS to improve the determination of methadone, cocaine and methamphetamine in oral fluids, with the limits of detection as low as in the μg/L level obtained [85]. The performance of ESI-MS tips made of wood, polyester and polyethylene were compared for the detection of two common nitroaromatic-based explosives, 2,4-dinitrotoluene and 2,4,6-trinitrophenol (picric acid), in soil samples [98], and the result suggested that the detection sensitivity was dependent on electrostatic charging capability of the porous materials.

4.3 Biomedical analysis

Yang *et al.* [75] established a wooden-tip ESI-MS method for the rapid screening of classic phenylketonuria (PKU) and atypical PKU diseases by quantitative detection of neopterin and biopterin in clinical urine samples. The limits of detection for the analyses of neopterin and biopterin were determined to be 30 ng/mL and 50 ng/mL, respectively. Compared with wooden

tips, hydrophobic polymer substrates enhanced detection of biopterin and neopterin in clinical urine samples [76], with improved limits of quantification for the detection at 1.0 ng/mL. Wooden-tip ESI coupled with IM-MS was used for rapid screening of drug-resistant bacteria from various clinical samples including sputum, blood and ascites by using β-lactam antibiotics as the indicator [70]. Hydrophilic wooden tips, polyester tips with medium hydrophobicity and hydrophobic polyethylene tips were compared, and the results showed that more hydrophobic tips offered stronger signals for detection of the indicator because of their weak interactions with the hydrophilic β-lactam antibiotics.

Yang *et al.* [63] investigated a wide variety of active components present in pharmaceuticals in the forms of tablets, capsules, granules, dry suspensions, suspensions, drops and oral liquids using wooden-tip ESI-MS, and showed that active components and trace degradation products could be sensitively detected. Lu *et al.* [99] compared the use of ESI-MS with wooden tips and herbal materials for directly analyzing aconite (*Aconitum carmichaeli* Debx), showing that alkaloids in aconite tuber could be detected by both methods, but the direct herbal ionization method was better than the wooden-tip ESI method except when methylene chloride was used as the extract/spray solvent. Xin *et al.* [79] demonstrated rapid identification of Fritillaria species in powder using wooden-tip ESI-MS. Du *et al.* [75] applied wooden-tip ESI-MS for rapid quality assessment of herbal formula. Yang *et al* [82] designed a field-induced wooden-tip ESI-MS device for high-throughput analysis of herbal medicines, demonstrating that by combining wooden-tip ESI-MS with multivariate statistical analysis, rapid tracing and assessing of the quality consistency and stability of herbal medicines could be achieved.

4.4 Environmental analysis

The presence of contaminants and their potential harmful environmental effects have attracted

global attention and applications of wooden-tip ESI-MS. Deng *et al.* [63, 66] applied surface-modified wooden-tip ESI-MS in the analysis of ultra-trace perfluorinated compounds (PFCs), fluoroquinolone and macrolide antibiotics in different water samples including river water, lake water and tap water. The LOD and LOQ of PFCs and antibiotics were in the ranges of 0.06–0.59 and 0.21–1.98 ng/L, and 1.8–4.5 and 5.9–15.1 ng/L, respectively, showing excellent performance for the ultra-trace analysis. Huang *et al.* [64] applied surface-modified wooden-tip ESI-MS for rapid and sensitive detection of trace malachite green and its metabolite in water and fish samples, exhibiting high enriching capacity and good analytical performances. These studies demonstrated the enrichment capacity and enhanced sensitivity of surface-modified wooden-tip ESI-MS for detecting target analytes in aqueous samples.

Wooden-tip ESI is an attractive tool for direct sampling and analysis of living organisms [69]. Wu *et al.* [81] performed *in vivo* sampling and rapid analysis of alkaloids in flowers, leaves, stems, veins and roots of living *C. roseus* under low-phosphorus stress using wooden-tip ESI-MS, with the detection results in good agreement with those obtained by conventional LC-MS. These studies demonstrated that wooden-tip ESI-MS could be used to provide endogenous metabolic information and information on chemical residuals in plants and animals for monitoring changes in ecological environments.

4.5 Protein study

Wooden-tip ESI is a kind of ESI technique and thus shares the features of soft ionization of protein samples. Intact and digested proteins can be directly characterized using wooden-tip ESI by loading protein solutions on the wooden tips [31]. Separation and isolation of proteins from mixtures and biological fluids according to their sizes and shapes were reported with wooden tips and other porous substrates under ESI conditions [91]. Wooden-tip ESI-MS,

especially hydrophobic wooden tips after surface modification, was used for enhanced detection of proteins from solutions containing salts and detergents [14, 67] due to the hydrophobic interactions between substrates and proteins [89, 92].

Direct detection of native proteins and protein complexes could also be achieved by wooden-tip ESI-MS. With the use of non-polar solvents, condensed cold water could facilitate the direct detection of native proteins and protein complexes from raw biological samples (*e.g.*, egg white, fresh bone marrow) [94]. Conformational changes in protein-ligand complexes, *e.g.*, protein-anion complexes, protein-metal complexes, were also investigated using wooden-tip ESI-MS and non-polar solvents [95]. Furthermore, formation and characterization of binding of native proteins with ligands in raw biological samples could be monitored by reactive wooden-tip ESI-MS [100]. When sodium dodecyl sulfonate (SDS) solution was applied to the electrospray plume of raw viscous hen egg white generated from wooden-tip ESI, lysozyme-DS complexes were formed and subsequently detected by MS. In this work, the reaction between the viscous egg and SDS occurred in the microdroplets, rather than in the bulk solution where the raw hen egg white could be aggregated, flocculated and denatured by the SDS solution, making the native protein complexes detectable. These results showed that wooden-tip ESI is a potential approach for analysis of proteins and investigation of protein structures and conformations.

5. Conclusions and prospects

Invented in 2011, wooden-tip ESI-MS, a technique that makes use of readily available, cheap and disposable wooden tips, has attracted much research attention in the past 10 years. The replacement of capillary with wooden tips extends the scope and applications of ESI-MS and allows us to gain new insights into the ESI process and mechanism. Wooden-tip ESI provides new features, *e.g.*, direct ionization of raw samples in different forms, interaction and

separation effect, and operation in open environment, which are unavailable to conventional capillary-based ESI. These new analytical features have turned the wooden-tip ESI techniques into a robust, simple, convenient, and rapid means for analysis of a large variety of raw samples in various applications. More effective and profound developments and wider applications of wooden-tip ESI-MS are expected, *e.g.*, the developing of automatically sampling for high-throughput analysis, the functionalizing of wooden tips to improve the selectivity and sensitivity for detecting target analytes, the coupling with miniatured MS for onsite application, and the performing of *in-vivo* analysis of intermediates of important biological processes in living organisms. Further mechanistic study of the interactions among substrates, analytes and solvents is still needed for a better understanding of ESI-MS with wooden tips and other solid substrates.

Acknowledgments

We would like to thank the National Natural Science Foundation of China (Grant Nos. 21804053 and 81874306), Research Grants Council (Grant Nos. 15304020, 15304117, R5013-19, R4005-18 and C4002-17G), the University Research Facility in Chemical and Environmental Analysis and the University Research Facility in Life Sciences of The Hong Kong Polytechnic University for their supports to this work.

References

[1] J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong, C.M. Whitehouse, Electrospray ionization–principles and practice, Mass Spectrom Rev, 9 (1990) 37-70.

[2] H. Awad, M.M. Khamis, A. El-Aneed, Mass Spectrometry, Review of the Basics: Ionization, Appl Spectrosc Rev, 50 (2015) 158-175.

[3] P.M. Peacock, W.-J. Zhang, S. Trimpin, Advances in Ionization for Mass Spectrometry, Anal Chem, 89 (2017) 372-388.

[4] F. Rigano, P.Q. Tranchida, P. Dugo, L. Mondello, High-performance liquid chromatography

combined with electron ionization mass spectrometry: A review, TrAC-Trend Anal Chem, 118 (2019) 112-122.

[5] R.D. Smith, J.A. Loo, R.R.O. Loo, M. Busman, H.R. Udseth, Principles and practice of electrospray ionization—mass spectrometry for large polypeptides and proteins, Mass Spectrom Rev, 10 (1991) 359-452.

[6] Q. Dumont, R.B. Cole, Jean-Antoine Nollet: The father of experimental electrospray, Mass Spectrom Rev, 33 (2014) 418-423.

[7] M. Wilm, M. Mann, Analytical properties of the nanoelectrospray ion source, Anal Chem, 68 (1996) 1-8.

[8] L. Konermann, E. Ahadi, A.D. Rodriguez, S. Vahidi, Unraveling the Mechanism of Electrospray Ionization, Anal Chem, 85 (2013) 2-9.

[9] P. Kebarle, U.H. Verkerk, Electrospray: From ions in solution to ions in the gas phase, what we know now, Mass Spectrom Rev, 28 (2009) 898-917.

[10] P. Kebarle, U.H. Verkerk, Electrospray: From Ions in Solution to Ions in the Gas Phase, What We Know Now, Mass Spectrometry Reviews, 28 (2009) 898-917.

[11] N.B. Cech, C.G. Enke, Practical implications of some recent studies in electrospray ionization fundamentals, Mass Spectrometry Reviews, 20 (2001) 362-387.

[12] P.-K. So, B. Hu, Z.-P. Yao, Electrospray Ionization on Solid Substrates, Mass Spectrom, 3 (2014) S0028.

[13] B. Hu, Z.-P. Yao, Principles and applications of solid-substrate electrospray ionization mass spectrometry, Sci Sin Chim, 47 (2017) 1365-1378.

[14] B. Hu, P.-K. So, Z.-P. Yao, Analytical properties of solid-substrate electrospray ionization mass spectrometry, J Am Soc Mass Spectrom, 24 (2013) 57-65.

[15] C.L. Feider, A. Krieger, R.J. DeHoog, L.S. Eberlin, Ambient ionization mass spectrometry: Recent developments and applications, Anal Chem, 91 (2019) 4266-4290.

[16] Z. Takáts, J.M. Wiseman, B. Gologan, R.G. Cooks, Mass spectrometry sampling under ambient conditions with desorption electrospray ionization, Science, 306 (2004) 471-473.

[17] M.Z. Huang, C.H. Yuan, S.C. Cheng, Y.T. Cho, J. Shiea, Ambient Ionization Mass Spectrometry, Annu Rev Anal Chem, 3 (2010) 43-65.

[18] C.W. Klampfl, M. Himmelsbach, Direct ionization methods in mass spectrometry: An overview, Analytica chimica acta, 890 (2015) 44-59.

[19] C.-M. Hong, C.-T. Lee, Y.-M. Lee, C.-P. Kuo, C.-H. Yuan, J. Shiea, Generating electrospray from solutions predeposited on a copper wire, Rapid Commun Mass Spectrom, 13

(1999) 21-25.

[20] Y.-C. Chen, A. Hu, Simultaneous determination of trace benzodiazepines from drinks by using direct electrospray probe/mass spectrometry (DEP/MS), Forensic Sci Int, 103 (1999) 79-88.

[21] C.H. Yuan, J. Shiea, Sequential electrospray analysis using sharp-tip channels fabricated on a plastic chip, Anal Chem, 73 (2001) 1080-1083.

[22] J.B. Fenn, Method and apparatus for electrospray ionization, US Patent 6,297,499, 2001.

[23] J. Kameoka, R. Orth, B. Ilic, D. Czaplewski, T. Wachs, H.G. Craighead, An electrospray ionization source for integration with microfluidics, Anal Chem, 74 (2002) 5897-5901.

[24] J. Jeng, J. Shiea, Electrospray ionization from a droplet deposited on a surface-modified glass rod, Rapid Commun Mass Spectrom, 17 (2003) 1709-1713.

[25] J. Liu, K.W. Ro, M. Busman, D.R. Knapp, Electrospray ionization with a pointed carbon fiber emitter, Anal Chem, 76 (2004) 3599-3606.

[26] K. Hiraoka, K. Nishidate, K. Mori, D. Asakawa, S. Suzuki, Development of probe electrospray using a solid needle, Rapid Commun Mass Spectrom, 21 (2007) 3139-3144.

[27] H. Wang, J. Liu, R.G. Cooks, Z. Ouyang, Paper Spray for Direct Analysis of Complex Mixtures Using Mass Spectrometry, Angew Chem Int Edit, 49 (2010) 877-880.

[28] E.M. McBride, P.M. Mach, E.S. Dhummakupt, S. Dowling, D.O. Carmany, P.S. Demond, G. Rizzo, N.E. Manicke, T. Glaros, Paper spray ionization: Applications and perspectives, TrAC-Trend Anal Chem, 118 (2019) 722-730.

[29] B.S. Frey, D.E. Damon, A.K. Badu-Tawiah, Emerging trends in paper spray mass spectrometry: Microsampling, storage, direct analysis, and applications, Mass Spectrom Rev, 39 (2020) 336-370.

[30] S. Chiang, W. Zhang, Z. Ouyang, Paper spray ionization mass spectrometry: recent advances and clinical applications, Expert Rev Proteomics, 15 (2018) 781-789.

[31] B. Hu, P.-K. So, H. Chen, Z.-P. Yao, Electrospray ionization using wooden tips, Anal Chem, 83 (2011) 8201-8207.

[32] B. Hu, P.K. So, Z.P. Yao, Electrospray ionization with aluminum foil: A versatile mass spectrometric technique, Analytica chimica acta, 817 (2014) 1-8.

[33] J.-Y. Liu, P.-C. Chen, Y.-W. Liou, K.-Y. Chang, C.-H. Lin, Development and application of a brush-Spray derived from a calligraphy-brush-style synthetic hair pen for use in ESI/MS, Mass Spectrom, 6 (2017) S0058-S0058.

[34] H.-K. Chen, C.-H. Lin, J.-T. Liu, C.-H. Lin, Electrospray ionization using a bamboo pen

nib, Int J Mass Spectrom, 356 (2013) 37-40.

[35] Y.-W. Liou, J.-S. Wang, C.-C. Chen, C.-H. Lin, Development of an on-line microextraction method for use in fiber-spray/mass spectrometry, Int J Mass Spectrom, 421 (2017) 178-183.

[36] X. Song, H. Chen, R.N. Zare, Conductive polymer spray ionization mass spectrometry for biofluid analysis, Anal Chem, 90 (2018) 12878-12885.

[37] M.T. Dulay, R.N. Zare, Polymer‐spray mass spectrometric detection and quantitation of hydrophilic compounds and some narcotics, Rapid Commun Mass Spectrom, 31 (2017) 1651-1658.

[38] R. Narayanan, X. Song, H. Chen, R.N. Zare, Teflon Spray Ionization Mass Spectrometry, J Am Soc Mass Spectrom, 31 (2020) 234-239.

[39] L. Wu, Z.C. Yuan, Z.M. Li, Z.X. Huang, B. Hu, In vivo solid-phase microextraction swab sampling of environmental pollutants and drugs in human body for nano-electrospray ionization mass spectrometry analysis, Analytica chimica acta, 1124 (2020) 71-77.

[40] N.s.M. Morato, V. Pirro, P.W. Fedick, R.G. Cooks, Quantitative swab touch spray mass spectrometry for oral fluid drug testing, Anal Chem, 91 (2019) 7450-7457.

[41] V. Pirro, A.K. Jarmusch, M. Vincenti, R.G. Cooks, Direct drug analysis from oral fluid using medical swab touch spray mass spectrometry, Analytica chimica acta, 861 (2015) 47-54.

[42] Y.-N. Yao, D. Di, Z.-C. Yuan, L. Wu, B. Hu, Schirmer Paper Noninvasive Microsampling for Direct Mass Spectrometry Analysis of Human Tears, Anal Chem, 92 (2020) 6207-6212.

[43] S. Jackson, D.J. Swiner, P.C. Capone, A.K. Badu-Tawiah, Thread spray mass spectrometry for direct analysis of capsaicinoids in pepper products, Analytica chimica acta, 1023 (2018) 81-88.

[44] D.J. Swiner, S. Jackson, G.R. Durisek, B.K. Walsh, Y. Kouatli, A.K. Badu-Tawiah, Microsampling with cotton thread: Storage and ultra-sensitive analysis by thread spray mass Spectrometry, Analytica chimica acta, 1082 (2019) 98-105.

[45] F.L. Hsu, C.H. Chen, C.H. Yuan, J. Shiea, Interfaces to connect thin-layer chromatography with electrospray ionization mass spectrometry, Anal Chem, 75 (2003) 2493-2498.

[46] B. Hu, G.-z. Xin, P.-K. So, Z.-P. Yao, Thin layer chromatography coupled with electrospray ionization mass spectrometry for direct analysis of raw samples, J Chromatogr A, 1415 (2015) 155-160.

[47] G.A. Gómez-Ríos, J. Pawliszyn, Development of Coated Blade Spray Ionization Mass Spectrometry for the Quantitation of Target Analytes Present in Complex Matrices, Angew Chem Int Edit, 53 (2014) 14503-14507.

[48] A. Khaled, G.A. Gomez-Rios, J. Pawliszyn, Optimization of Coated Blade Spray for Rapid

Screening and Quantitation of 105 Veterinary Drugs in Biological Tissue Samples, Analytical Chemistry, 92 (2020) 5937-5943.

[49] S. Ahmad, M. Tucker, N. Spooner, D. Murnane, U. Gerhard, Direct Ionization of Solid-Phase Microextraction Fibers for Quantitative Drug Bioanalysis: From Peripheral Circulation to Mass Spectrometry Detection, Anal Chem, 87 (2015) 754-759.

[50] B. Hu, G. Ouyang, In situ solid phase microextraction sampling of analytes from living human objects for mass spectrometry analysis, TrAC-Trend Anal Chem, 143 (2021) 116368.

[51] S.L.F. Chan, M.Y.M. Wong, H.W. Tang, C.M. Che, K.M. Ng, Tissue‐spray ionization mass spectrometry for raw herb analysis, Rapid Commun Mass Spectrom, 25 (2011) 2837-2843.

[52] B. Hu, Y.-H. Lai, P.-K. So, H. Chen, Z.-P. Yao, Direct ionization of biological tissue for mass spectrometric analysis, Analyst, 137 (2012) 3613-3619.

[53] J. Liu, H. Wang, R.G. Cooks, Z. Ouyang, Leaf spray: direct chemical analysis of plant material and living plants by mass spectrometry, Anal Chem, 83 (2011) 7608-7613.

[54] N. Malaj, Z. Ouyang, G. Sindona, R.G. Cooks, Analysis of pesticide residues by leaf spray mass spectrometry, Anal Methods, 4 (2012) 1913-1919.

[55] H.-Y. Wong, B. Hu, P.-K. So, C.-O. Chan, D.K.-W. Mok, G.-Z. Xin, P. Li, Z.-P. Yao, Rapid authentication of Gastrodiae rhizoma by direct ionization mass spectrometry, Analytica chimica acta, 938 (2016) 90-97.

[56] H.-Y. Wong, M.Y.-M. Wong, B. Hu, P.-K. So, C.-O. Chan, D.K.-W. Mok, Z.-P. Yao, Rapid differentiation of Ganoderma species by direct ionization mass spectrometry, Analytica chimica acta, 999 (2018) 99-106.

[57] G.-Z. Xin, B. Hu, Z.-Q. Shi, J.-Y. Zheng, L. Wang, W.-Q. Chang, P. Li, Z. Yao, L.-F. Liu, A direct ionization mass spectrometry-based approach for differentiation of medicinal Ephedra species, J Pharm Biomed Anal, 117 (2016) 492-498.

[58] Y.P. Wei, L.R. Chen, W. Zhou, K. Chingin, Y.Z. Ouyang, T.G. Zhu, H. Wen, J.H. Ding, J.J. Xu, H.W. Chen, Tissue spray ionization mass spectrometry for rapid recognition of human lung squamous cell carcinoma, Sci Rep-Uk, 5 (2015).

[59] B. Hu, L. Wang, W.-C. Ye, Z.-P. Yao, In vivo and real-time monitoring of secondary metabolites of living organisms by mass spectrometry, Sci Rep-Uk, 3 (2013) 2104.

[60] W. Li, Y.-N. Yao, L. Wu, L. Wang, B. Hu, Contactless electrospray ionization mass spectrometry for direct detection of analytes in living organisms, J Mass Spectrom, 56 (2021) e4539.

[61] D.P.H. Smith, The Electrohydrodynamic Atomization of Liquids, IEEE Trans Ind Appl,

IA-22 (1986) 527-535.

[62] M.Y.M. Wong, H.W. Tang, S.H. Man, C.W. Lam, C.M. Che, K.M. Ng, Electrospray ionization on porous spraying tips for direct sample analysis by mass spectrometry: enhanced detection sensitivity and selectivity using hydrophobic/hydrophilic materials as spraying tips, Rapid Commun Mass Spectrom, 27 (2013) 713-721.

[63] Y. Yang, J. Deng, Z.-P. Yao, Pharmaceutical Analysis by Solid-Substrate Electrospray Ionization Mass Spectrometry with Wooden Tips, J Am Soc Mass Spectrom, 25 (2014) 37-47.

[64] Y. Huang, Y. Ma, H. Hu, P. Guo, L. Miao, Y. Yang, M. Zhang, Rapid and sensitive detection of trace malachite green and its metabolite in aquatic products using molecularly imprinted polymer-coated wooden-tip electrospray ionization mass spectrometry, RSC Adv, 7 (2017) 52091-52100.

[65] Y. Liu, Q. Yang, X. Chen, Y. Song, Q. Wu, Y. Yang, L. He, Sensitive analysis of trace macrolide antibiotics in complex food samples by ambient mass spectrometry with molecularly imprinted polymer-coated wooden tips, Talanta, 204 (2019) 238-247.

[66] J. Deng, T. Yu, Y. Yao, Q. Peng, L. Luo, B. Chen, X. Wang, Y. Yang, T. Luan, Surface-coated wooden-tip electrospray ionization mass spectrometry for determination of trace fluoroquinolone and macrolide antibiotics in water, Analytica chimica acta, 954 (2017) 52-59.

[67] B. Hu, P.-K. So, Y. Yang, J. Deng, Y.-C. Choi, T. Luan, Z.-P. Yao, Surface-Modified Wooden-Tip Electrospray Ionization Mass Spectrometry for Enhanced Detection of Analytes in Complex Samples, Anal Chem, 90 (2018) 1759-1766.

[68] Y.-N. Yao, B. Hu, Analyte-substrate interactions at functionalized tip electrospray ionization mass spectrometry: Molecular mechanisms and applications, J Mass Spectrom, 53 (2018) 1222-1229.

[69] P.-K. So, T.-T. Ng, H. Wang, B. Hu, Z.-P. Yao, Rapid detection and quantitation of ketamine and norketamine in urine and oral fluid by wooden-tip electrospray ionization mass spectrometry, Analyst, 138 (2013) 2239-2243.

[70] P.-K. So, B.-C. Yang, W. Li, J. Zheng, B. Hu, Development of tip-desorption electrospray ionization coupled with ion mobility-mass spectrometry for fast screening of carbapenemase-producing bacteria, Talanta, 201 (2019) 237-244.

[71] J. Lin, J. Yan, Q. Xu, X. Wang, Study on properties of wooden capillary electrospray ionization mass spectrometry, Rapid Commun Mass Spectrom, 34 (2020) e8600.

[72] Y. Yang, J. Deng, Internal standard mass spectrum fingerprint: A novel strategy for rapid assessing the quality of Shuang-Huang-Lian oral liquid using wooden-tip electrospray ionization mass spectrometry, Analytica chimica acta, 837 (2014) 83-92.

[73] T.-T. Ng, P.-K. So, B. Hu, Z.-P. Yao, Rapid detection and quantitation of drugs-of-abuse

by wooden-tip electrospray ionization mass spectrometry, J Food Drug Anal, 27 (2019) 428-438.

[74] N.E. Manicke, Q. Yang, H. Wang, S. Oradu, Z. Ouyang, R.G. Cooks, Assessment of paper spray ionization for quantitation of pharmaceuticals in blood spots, Int J Mass Spectrom, 300 (2011) 123-129.

[75] Q. Du, J. Deng, Y. Liu, X. Zhang, Y. Yang, J. Chen, Rapid assessment of the quality of Qingkailing products using wooden-tip electrospray ionization mass spectrometry combined with multivariate statistical analysis, Anal Methods, 7 (2015) 4803-4810.

[76] X. Yang, H. Huang, Q. Lu, S.-h. Chen, F. Wang, O.-p. Huang, B. Hu, B.-c. Yang, High-throughput polymer tip-electrospray ionization mass spectrometry for enhanced detection of neopterin and biopterin in clinical urine samples, J Mass Spectrom, 54 (2019) 189-194.

[77] B.-c. Yang, F. Wang, W. Deng, Y. Zou, F.-y. Liu, X.-d. Wan, X. Yang, H. Liu, O.-p. Huang, Wooden-tip electrospray ionization mass spectrometry for trace analysis of toxic and hazardous compounds in food samples, Anal Methods, 7 (2015) 5886-5890.

[78] B. Hu, Y. Huang, G. Yin, G. Zhang, L. Zhang, T. Wang, Z.-P. Yao, Rapid detection of adulterated drugs in herbal dietary supplements by wooden-tip electrospray ionization mass spectrometry, Anal Methods, 8 (2016) 6840-6846.

[79] G.-Z. Xin, B. Hu, Z.-Q. Shi, Y.C. Lam, T.T.-X. Dong, P. Li, Z.-P. Yao, K.W.K. Tsim, Rapid identification of plant materials by wooden-tip electrospray ionization mass spectrometry and a strategy to differentiate the bulbs of Fritillaria, Analytica chimica acta, 820 (2014) 84-91.

[80] Z. Liu, Y.-L. Guo, Q.-X. Xu, S.-Y. Liu, Study on Analytical Properties of Sunflower Seed Husk as a New Wooden Electrospray Ionization Material, Chin J Anal Chem, 47 (2019) 1411-1418.

[81] L. Wu, Y.-N. Yao, B. Hu, Investigating distributions and changes of alkaloids in living Catharanthus roseus under low-phosphorus stress using wooden-tip electrospray ionisation mass spectrometry, Phytochem Anal, 31 (2020) 739-746.

[82] Y. Yang, J. Deng, Z.P. Yao, Field-induced wooden-tip electrospray ionization mass spectrometry for high-throughput analysis of herbal medicines, Analytica chimica acta, 887 (2015) 127-137.

[83] Y.-N. Yao, L. Wu, D. Di, Z.-C. Yuan, B. Hu, Vibrating tip spray ionization mass spectrometry for direct sample analysis, J Mass Spectrom, 54 (2019) 772-779.

[84] P. Shi, M. Li, X. Fu, B. Xia, Y. Zhou, Study on the separation mechanism of solid-substrate electrospray ionization mass spectrometry, J Sep Sci, 44 (2021) 1026-1035.

[85] J. Millán-Santiago, M. Teresa García-Valverde, R. Lucena, S. Cárdenas, Polyamide-coated wooden tips coupled to direct infusion mass spectrometry, a high throughput alternative for the

determination of methadone, cocaine and methamphetamine in oral fluid, Microchem J, 162 (2021) 105843.

[86] W. Han, Y. Zheng, T. Muyizere, Z. Zhang, Development of paper substrate for paper spray MS in high-sensitivity analysis of biological samples, Bioanalysis, 10 (2018) 2047-2067.

[87] G.L. de Araújo, D.V.A. de Aguiar, I. Pereira, L.C. da Silva, A.R. Chaves, B.G. Vaz, Polypyrrole-coated needle as an electrospray emitter for ambient mass spectrometry, Anal Methods, 12 (2020) 3235-3241.

[88] J. Deng, W. Li, Q. Yang, Y. Liu, L. Fang, Y. Guo, P. Guo, L. Lin, Y. Yang, T. Luan, Biocompatible Surface-Coated Probe for in Vivo, in Situ, and Microscale Lipidomics of Small Biological Organisms and Cells Using Mass Spectrometry, Anal Chem, 90 (2018) 6936-6944.

[89] P.-K. So, B.-C. Yang, W. Li, L. Wu, B. Hu, Simple Fabrication of Solid-Phase Microextraction with Surface-Coated Aluminum Foil for Enhanced Detection of Analytes in Biological and Clinical Samples by Mass Spectrometry, Anal Chem, 91 (2019) 9430-9434.

[90] G.A. Gómez-Ríos, M. Tascon, J. Pawliszyn, Coated blade spray: shifting the paradigm of direct sample introduction to MS, Bioanalysis, 10 (2018) 257-271.

[91] B. Hu, Z.-P. Yao, Mobility of proteins in porous substrates under electrospray ionization conditions, Anal Chem, 88 (2016) 5585-5589.

[92] H. Wang, P.-K. So, T.-T. Ng, Z.-P. Yao, Rapid analysis of raw solution samples by C18 pipette-tip electrospray ionization mass spectrometry, Analytica chimica acta, 844 (2014) 1-7.

[93] A. Li, H. Wang, Z. Ouyang, R.G. Cooks, Paper spray ionization of polar analytes using non-polar solvents, Chem Commun, 47 (2011) 2811-2813.

[94] B. Hu, Z.-P. Yao, Detection of native proteins using solid-substrate electrospray ionization mass spectrometry with nonpolar solvents, Analytica chimica acta, 1004 (2018) 51-57.

[95] Z. Huang, Y.-N. Yao, W. Li, B. Hu, Analytical properties of electrospray ionization mass spectrometry with solid substrates and nonpolar solvents, Analytica chimica acta, 1050 (2019) 105-112.

[96] T. Zhong, X.L. Zhang, H. Zhang, M.L. Yang, P. Zhou, Z.G. Le, Analysis of navel orange juice components by wooden-tip electrospray mass spectrometry, J Chin Mass Spectrom Soc, 37 (2016) 75-81.

[97] F. Canfarotta, A. Poma, A. Guerreiro, S. Piletsky, Solid-phase synthesis of molecularly imprinted nanoparticles, Nature Protocols, 11 (2016) 443-455.

[98] M.Y.-M. Wong, S.-H. Man, C.-M. Che, K.-C. Lau, K.-M. Ng, Negative electrospray ionization on porous supporting tips for mass spectrometric analysis: electrostatic charging effect on detection sensitivity and its application to explosive detection, Analyst, 139 (2014)

1482-1491.

[99] J. Lu, J. Yan, Q. Xu, Comparative analysis of aconitine alkaloids in aconite tube using direct ionization and wooden tip spray mass spectrometry, Acta Pharm Sin, 54 (2019) 1271-1276.

[100] L. Wu, Y.-N. Yao, Z.-C. Yuan, D. Di, L. Li, B. Hu, Direct detection of lysozyme in viscous raw hen egg white binding to sodium dodecyl sulfonate by reactive wooden-tip electrospray ionization mass spectrometry, Anal Sci, 36 (2020) 341-346.

Figure captions

Figure 1. Setup of wooden-tip ESI-MS. a) Schematic diagram of wooden-tip ESI-MS; b) photos of nano-ESI capillary, wooden toothpick and wooden tip; c) wooden tip connected to a nano-ESI ion source with an orthogonal configuration; d) parallel configuration of wooden-tip ESI; e) optical image of the plume of methanol spray from a wooden tip, and an inset to show the view of the Taylor cone. a-d) Reproduced from Ref. [31] with permission; e) reproduced from Ref. [14] with permission.

Figure 2. Surface-modified wooden-tip ESI-MS. a) Wooden surfaces modified with hydrophobic, basic and acidic functional groups; b) extractive sampling and direct loading. Reproduced from Ref. [67] with permission.

Figure 3. **Solution movements on porous substrates under ESI conditions.** Reproduced from Ref. [91] with permission.

Figure 4. Wooden-tip ESI-MS with nonpolar solvents. (a) Illustration of the process of non-polar solvents on wooden tips under ambient conditions, G: gas, L: liquid, S: solid. (b) Ice dendrites formed on the wooden tip surface and spray plume from ice dendrites with non-polar n-pentane as the added solvent. Reproduced from Ref. [94] with permission.

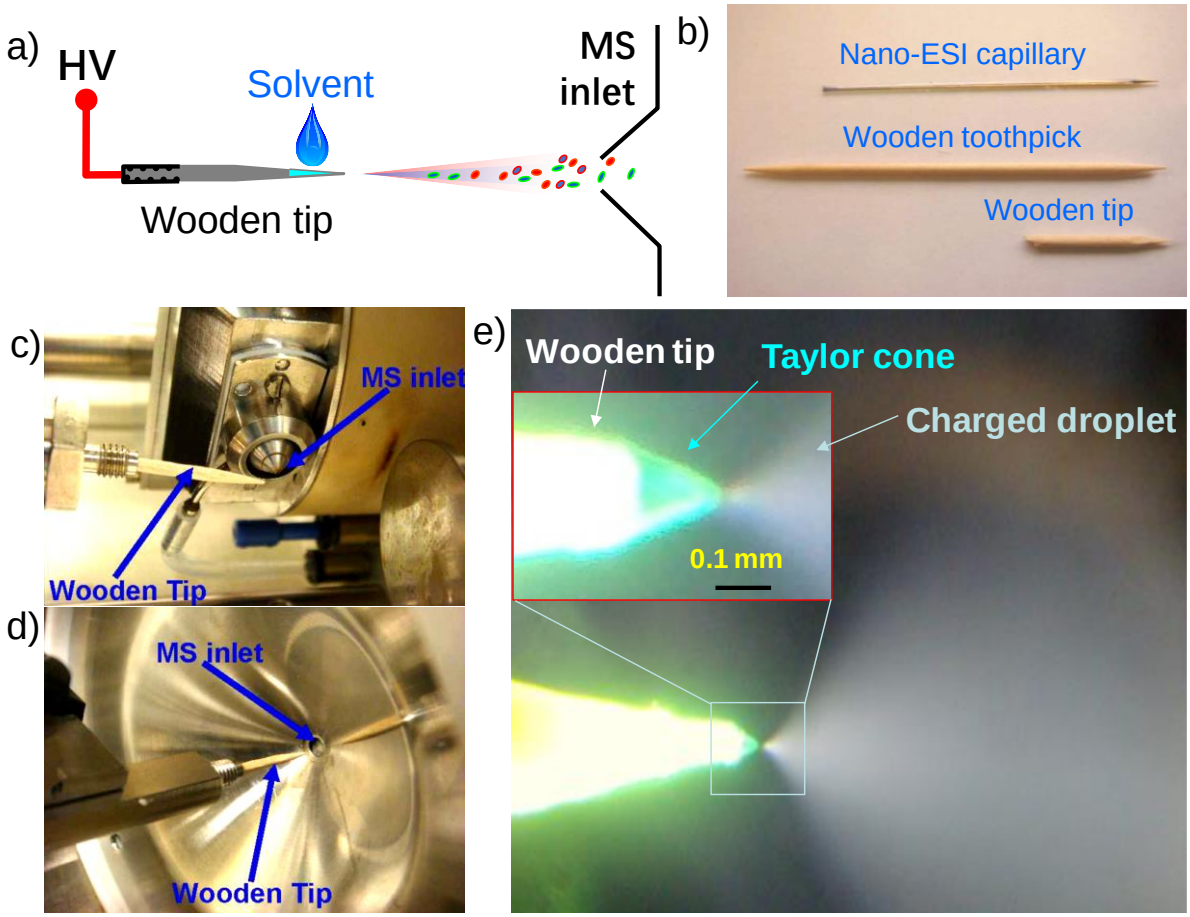
a)
HV
Solvent
MS
inlet
Wooden tip
b)
Nano-ESI capillary
Wooden toothpick
Wooden tip
c)
MS inlet
Wooden Tip
d)
MS inlet
Wooden Tip
e)
Wooden tip
Taylor cone
Charged droplet
0.1 mm

Figure 1

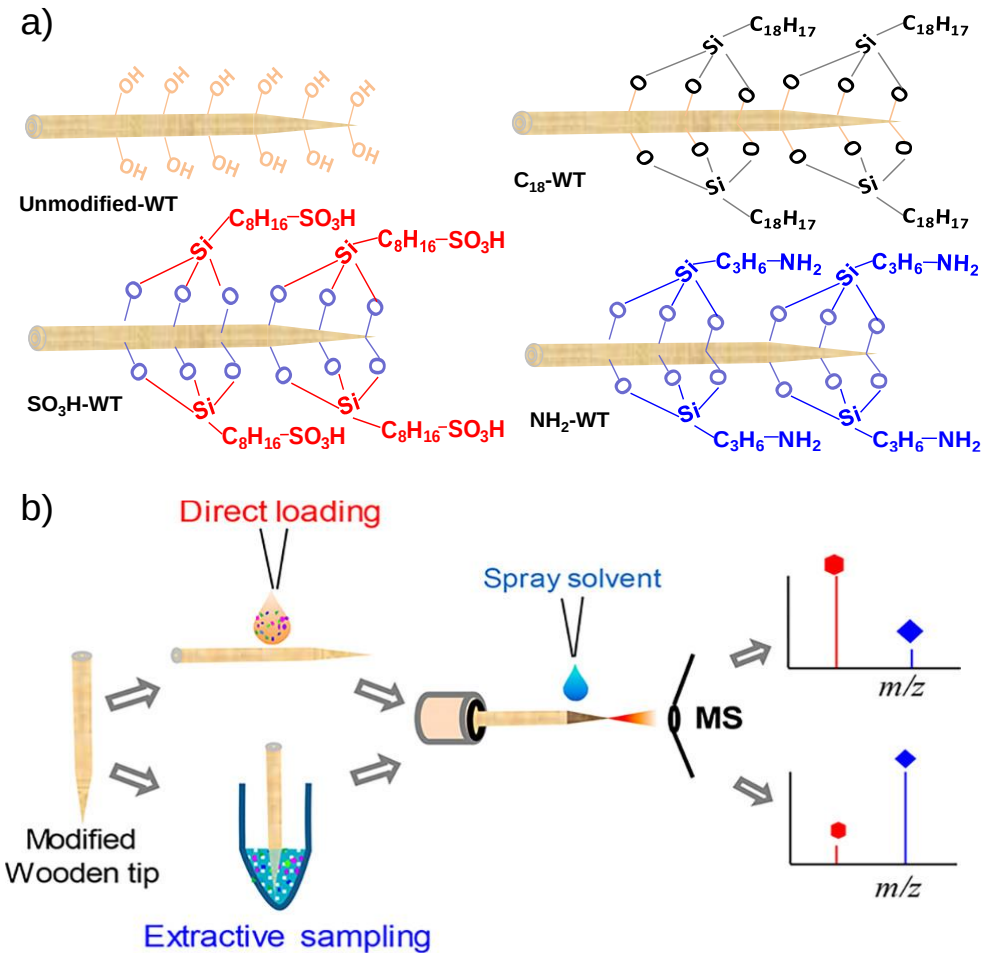
a)
Unmodified-WT
C_{18}-WT
SO_3H-WT
NH_2-WT
b)
Direct loading
Spray solvent
MS
m/z
m/z
Modified
Wooden tip
Extractive sampling

Figure 2

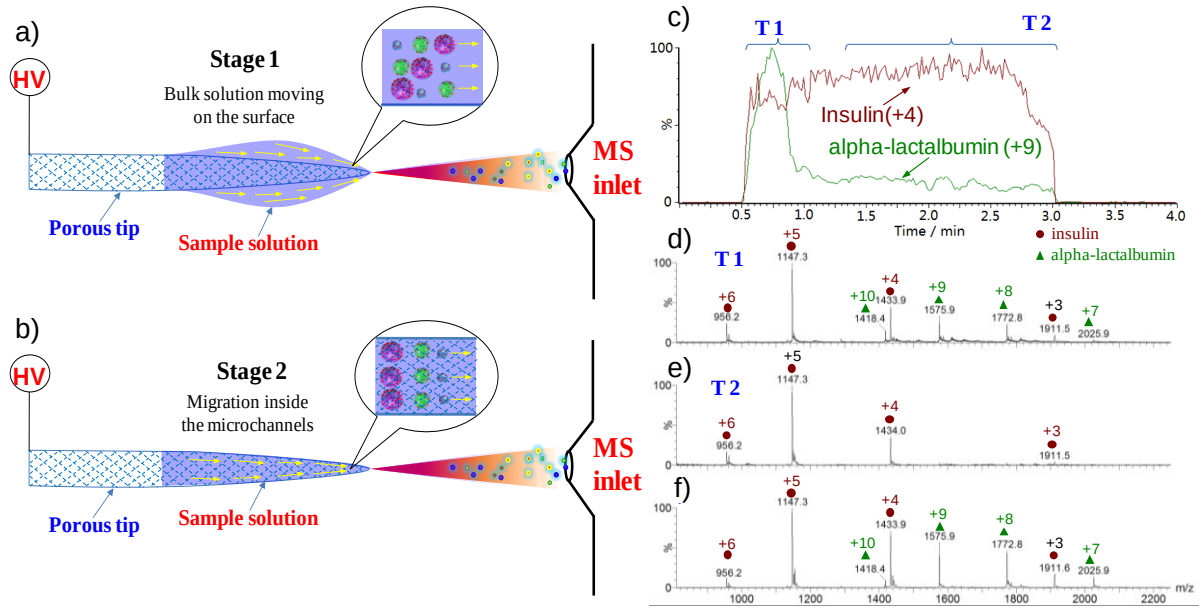
a)
HV
Stage 1
Bulk solution moving
on the surface
Porous tip
Sample solution
MS
inlet
b)
HV
Stage 2
Migration inside
the microchannels
Porous tip
Sample solution
MS
inlet
c)
T 1
T 2
Insulin(+4)
alpha-lactalbumin (+9)
Time / min
d)
T 1
e)
T 2
f)
insulin
alpha-lactalbumin
m/z

Figure 3

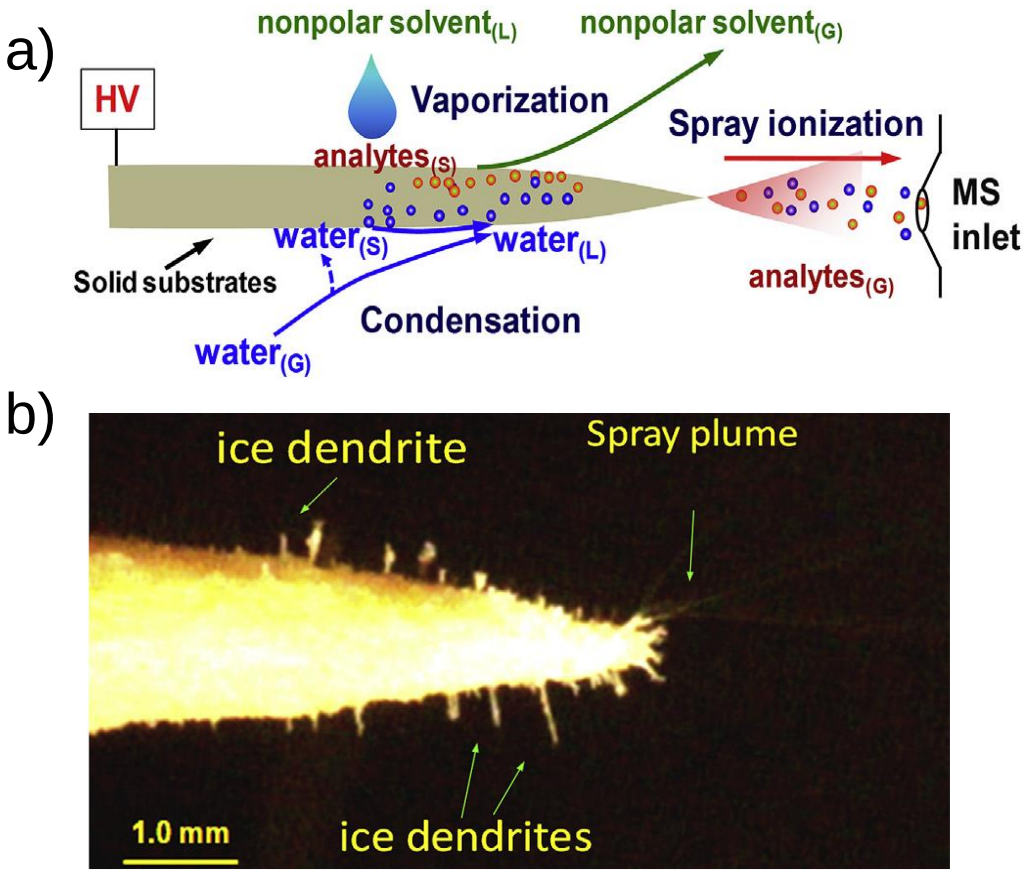
a)
nonpolar solvent(L)
nonpolar solvent(G)
HV
Vaporization
Spray ionization
analytes(S)
MS
inlet
water(S)
water(L)
Solid substrates
analytes(G)
Condensation
water(G)
b)
ice dendrite
Spray plume
1.0 mm
ice dendrites

Figure 4

Table 1. Analytical performances of wooden-tip ESI-MS for detecting various analytes from raw samples.

Analytes	Samples	LOD	LOQ	Linear Range	R^2	RSD (%)	Refs
3 hazardous compounds	Beverage and food	30.0 ng/mL, 0.1 μg/kg	NA	0.1-20 μg/mL, 0.5-20 μg/kg	0.97-0.98	8.7-23.2	[77]
Ascorbic acid	Navel orange juice	3.7 μg/L	NA	0.01-100 μg/L	0.992	7.9-9.7	[96]
33 adulterated drugs	Herbal dietary supplements	0.1 μg/g	NA	NA	NA	19.6	[78]
5 macrolide antibiotics	Beverage and food	0.003-15.8 ng/g	0.01-52.8 ng/g	0.1-100 ng/g, 100-5000 ng/g	0.9911-0.9986	4.3-10.7	[65]
Ketamine and norketamine	Oral fluid and urine	20 ng/mL	50 ng/mL	50-5000 ng/mL	0.9981-0.9999	1.8-15.2	[69]
9 drugs of abuse	Oral fluid and urine	20-40000 ng/mL	50-10000 ng/mL	50-5,000 ng/mL, 250-10,000 ng/mL	0.9903-0.9998	3.2-25.5	[73]
Cocaine	Oral fluids	0.01 ng/mL	0.1 ng/mL	1–200 ng/mL	0.9978	5.3-16.5	[67]
3 drugs of abuse	Oral fluid	1.5 μg/L	5 μg/L	5-250 μg/L	0.9876-0.9963	3.4-18.2	[85]
Explosives	Soil	0.4-20 ppm	NA	0.03 mM-0.9 mM	0.9945	NA	[98]
Neopterin, biopterin	Human urine	30-50 ng/mL	NA	50–5000 ng mL	0.9963-0.996	6.3-9.5	[75]
Neopterin, biopterin	Human urine	1.0 ng/mL	5.0 ng/mL	5-1000 ng/mL	0.9983-0.9986	12.2-13.4	[76]
Losartan	Human urine	NA	10 ng/mL	10-1000 ng/mL	0.9910	6.60-22.05	[14]
Perfluorinated compounds	Pure water	0.06-0.59 ng/L	0.21–1.98 ng/L	0.5-100 ng/L	0.9931-0.9992	2.9-16.4	[63]
6 antibiotics	Pure water	1.8 - 4.5 ng/L	5.9 - 15.1 ng/L	10-5000 ng/L, 20-5000 ng/L	0.9940-0.9998	1.5-14.3	[66]
Malachite green	Aquatic products	0.01 ng/mL	NA	0.1-100 ng/mL	0.9988-0.9990	4.6-10.6	[64]
Alkaloids	Catharanthus roseus	NA	NA	0.1-10 μg/mL	0.9796-0.9989	NA	[81]