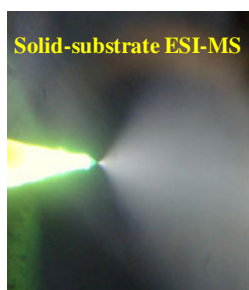


# Analytical Properties of Solid-substrate Electrospray Ionization Mass Spectrometry

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**Abstract.** Conventional electrospray ionization mass spectrometry (ESI-MS) uses a capillary for sample loading and ionization. Along with the development of ambient ionization techniques, ESI-MS using noncapillary emitters has attracted more interest in recent years. Following our recent report on ESI-MS using wooden tips (*Anal. Chem.* **83**, 8201–8207 (2011)), the technique was further investigated and extended in this study. Our results revealed that the wooden tips could serve as a chromatographic column for separation of sample components. Sequential and exhaustive ionization was observed for proteins and salts on wooden tips with salts ionized sooner and proteins later. Nonconductive materials that contain microchannels/pores could be used as tips for ESI-MS analysis with

sample solutions loaded to the sharp-ends only, since rapid diffusion of sample solutions by capillary action would enable the tips to become conductive. Tips of inert materials such as bamboo, fabrics, and sponge could be used for sample loading and ionization, while samples such as tissue, mushroom, and bone could form tips to induce ionization for direct analysis with application of a high voltage.

**Key words:** Electrospray ionization, Solid substrate, Separation column, Sampling techniques, Direct ionization

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## Introduction

Electrospray ionization (ESI) is a powerful mass spectrometric technique for analysis of various compounds. In conventional ESI [1] and nano-ESI [2], a sample solution is introduced into a capillary. Upon application of a high voltage to the capillary and usually with the assistance of gas, the sample solution would spray out of the capillary tip, leading to the formation of fine droplets and subsequently analyte ions. Noncapillary ESI techniques have also been developed. Materials such as wick element [3], copper wire [4], metal needle [5–7], optical fiber wired with a metal coil [8, 9], surface-modified glass rod [10], nanostructured tungsten oxide [11], and more recently paper [12, 13] and wood [14] have been used for sampling and ionization in ESI. Use of noncapillary emitters avoids the problem of clogging that could occur in capillary-based ESI and allows much easier sampling. Particularly, analysis of samples on

surfaces or in difficult-to-access corners could be greatly facilitated by collecting sample using paper [12, 13] or wooden tips [14] followed by paper spray or wooden tip ESI-MS. More recently, noncapillary ESI techniques have been further extended to direct mass spectrometric analysis of biological tissue [15–18].

Since the ionization conditions of noncapillary ESI such as the loading of sample solutions on surface and the absence of desolvation gas are different from those of capillary-based ESI, different features have been observed with noncapillary ESI. Some noncapillary tips such as stainless steel needle [6, 19], paper [13, 20–23], and wooden tips [14] enabled direct analysis of raw samples. Hiraoka and co-workers reported the sequential and exhaustive ionization of analytes on solid metal probe [24]. Separate ionization of proteins and peptides in a mixture sample was also observed with hydrophilic wooden tips [14]. The study on paper spray ionization of polar analytes using nonpolar solvents indicated that solvents could transport insoluble analytes to the paper tip, and that field desorption and surface tension of solvents might play important roles in the ionization of analytes [20]. Further investigation on characteristics and emitters of

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noncapillary ESI is necessary for development and applications of this kind of techniques.

We recently reported the ESI using wooden tips (toothpicks) [14]. In this technique, sample solution was loaded onto the sharp tip-end of a wooden tip. Upon application of a high voltage to the other end of the wooden tip, spray ionization was induced from the cusp and characteristic mass spectrum was observed. In the present study, the analytical properties of solid-substrate ESI were investigated and various tips were further developed for solid-substrate ESI-MS analysis.

## Experimental

### Materials

Wooden toothpicks used for wooden tips in this study were of the brand BESTbuy from PARKnSHOP (Hong Kong). Bamboo toothpicks used for bamboo tips were of the brand Zhouzi from Zhennan Bamboo and Wood Co. Ltd. (Hangzhou, China). The fabric material was of 35 % cotton and 65 % polyester and was cut from a lab coat from Guandong Tianyi Co. Ltd. (Zhongshan, China). Cellulose sponge was of the brand Scotch-Brite from 3 M (Hong Kong). PEEK tubing was from Waters (Milford, MA, USA). Stainless steel needles and silver needles were from Tongyong Office Co. Ltd. (Shanghai, China) and Chow Sang Sang (Hong Kong), respectively. Fresh coriander leaves, spinach leaves, mushroom, and ribs were purchased from supermarkets in Hong Kong. Reserpine, myoglobin (from horse heart), cytochrome *c* (from bovine heart), lysozyme (from chicken egg white), dimethoate, acetone, ligroin, ammonium acetate, sodium chloride, and formic acid were purchased from Sigma (St. Louis, MO, USA), and losartan from Gracure Pharmaceuticals Ltd. (New Delhi, India). Ketoconazole cream was purchased from Dihon Pharmaceutical Co. Ltd (Kunming, China). Green fluorescer was purchased from Zebra (Lincolnshire, IL, USA). Methanol was purchased from Tedia (Fairfield, OH, USA). Water used in this study was Milli-Q water generated from a Synthesis A10 Milli-Q water generator (Millipore, Billerica, MA, USA). Extract of spinach leaf was prepared as described in the literature [25]. Wooden tips were prepared as described previously [14]. Other tips were prepared by modifying the materials to form sharp ends pointing to the MS inlet. Washing of tips with methanol before use could reduce contamination and background if necessary.

### Solid-Substrate ESI Measurements

Mass spectra were acquired on a Q-TOF2 quadrupole time-of-flight mass spectrometer (Micromass, Manchester, UK). Various tips were connected to the high voltage supply of the mass spectrometers via a clip except for toothpick tips that were via a nano-ESI ion source. The tips were perpendicular to the MS inlet with the tip-ends ~10 mm away from the inlet. The capillary voltage and cone voltage were set at 3.5 kV and 30 V, respectively, unless otherwise specified. The source temperature

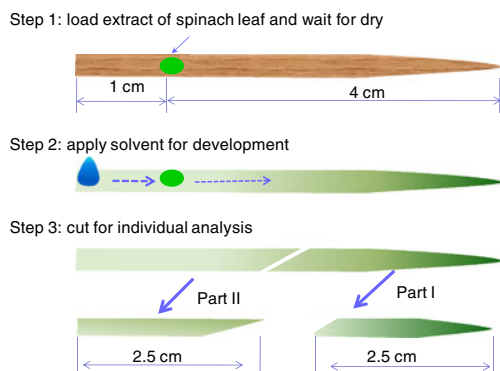
was set at 80°C. Unless specified, mass spectra were obtained by accumulating the data acquired in 1 min. Other settings were similar as those previously described for the wooden tips [14].

Electric resistances and voltages were measured using a multimeter from John Fluke Manufacturing Co. Inc. (Everett, WA, USA). Diffusion of the fluorescence solution on the wooden tips was visualized by an UV lamp from Macherey-Nagel (Düren, Germany) and recorded using a Sony T20 camera (Japan) with a speed of 28 frames/sec. Optical images of ESI plume were recorded using the same camera with the assistance of a KL 1500 LCD halogen cold light lamp (Schott, Mainz, Germany).

## Results and Discussion

### Separation Property of Wooden Tips

Wooden tips have hydrophilic surface of cellulose structures. To test whether wooden tips have separation effect like paper chromatography and thin-layer chromatography (TLC), a 5 cm wooden tip was used and 5  $\mu$ L extract of spinach leaf was loaded 4 cm away from the tip cusp (Figure 1). After the solution was dried, acetone/ligroin (1/9, vol/vol), a solvent system used for TLC separation of the extract [26], was added onto the coarse end of the tip to drive the components to the sharp end. The tip was cut into two halves after the tip became dry and each half was analyzed using the solid-substrate ESI technique with 5  $\mu$ L methanol added to the sharp end. Pheophytin-a and pheophytin-a O-allomer [27], compounds of lower polarity, were found at the front part (Figure 2a, compounds confirmed by MS/MS with data not shown), whereas polar compounds such as glucose were detected at the back part (Figure 2b). Some pigments such as chlorophylls were not observed probably because of their low signal responses under the ESI conditions. These results revealed that the wooden tip could serve as chromatographic column to allow separation of components based on their polarities. The wooden tip loaded with extract of spinach leaf at the 4-cm position was also analyzed online with a high voltage applied to the tip and solvents of different polarities added to the coarse end of the tip (Figure S1). Low-polar components pheophytin-a and pheophytin-a O-allomer (Figure S1a) and polar components such as glucose (Figure S1b) were predominantly detected when low-polar solvent acetone/ligroin (9/1, vol/vol) and polar solvent solvent/water (1/1, vol/vol) were used, respectively, as the mobile phase and ionizing solvents, confirming the separation effects of the wooden tips and the effects of running solvents. For comparison, the extract was directly analyzed with the extract solution loaded to the sharp tip-end of the wooden tips. Both polar and low-polar compounds and compounds such as monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG) were detected in this case (Figure 2c), covering most components observed by LC-MS [15]. These components, however, could not be

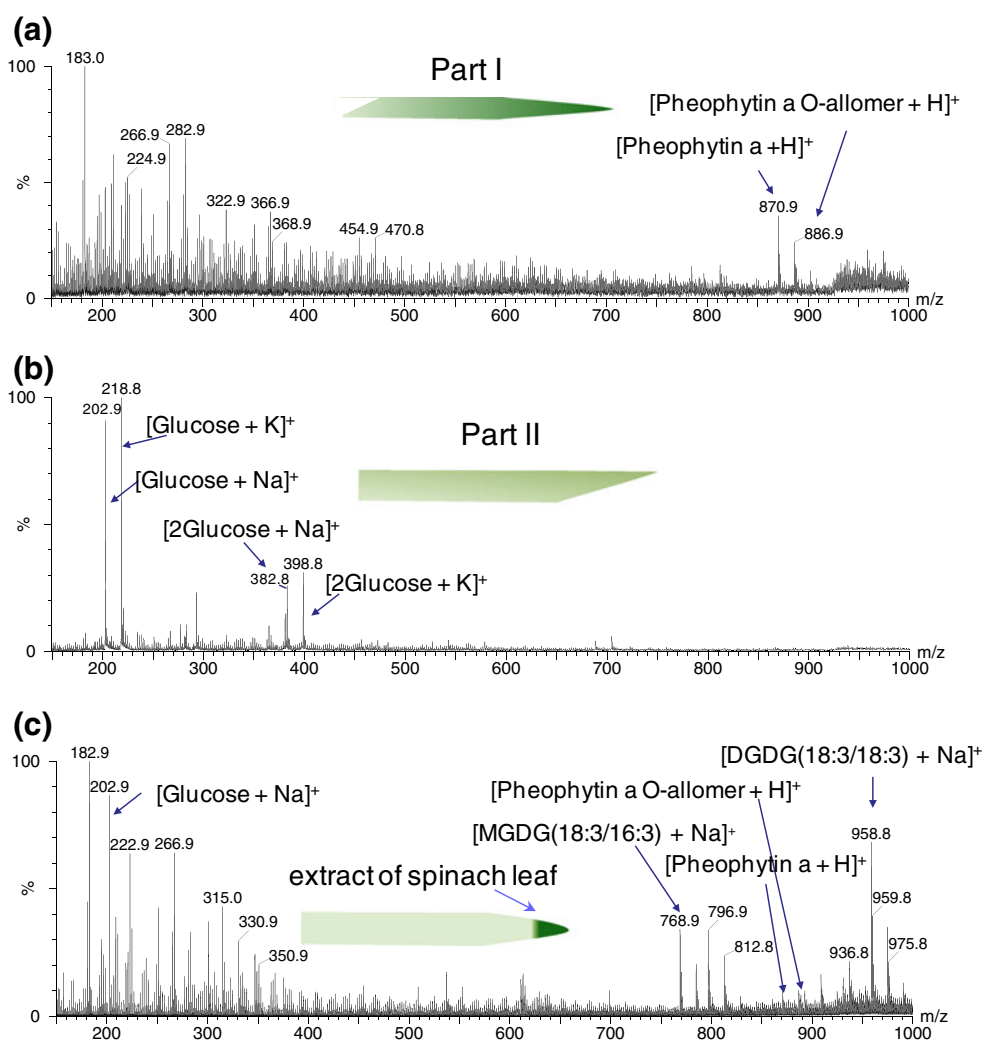


**Figure 1.** Procedures for testing the separation effect of the wooden tip; 5  $\mu\text{L}$  extract of spinach leaf was loaded and acetone/ligroin (1/9, vol/vol) was used as the mobile phase for development

detected by analysis of the extract using nano-ESI [15]. These results indicated that the solid-substrate ESI had lower

signal suppression and could provide a more complete profile of the sample.

The separation property of the wooden tips could be used for analysis of proteins containing high contents of salts. As shown in Figure S2a, 5  $\mu\text{L}$  solution containing 10  $\mu\text{M}$  lysozyme and 10 mM NaCl in 50 % methanol was loaded 0.5 cm away from the cusp of a 2 cm wooden tip and left for dry. The spot was subjected to solid-substrate ESI-MS analysis with driving and ionizing solvents added to the coarse end of the tip. When water was used as the solvent, only cluster ions of NaCl and almost no protein signals were observed (data not shown). When methanol, a solvent that favored elution of proteins, was used as the solvent, lysozyme signals were detected with low salt interferences (Figure S2b). However, such large molecules were found to have difficulty being carried through and detected if they were loaded too far away from the tip cusp, similar to the observation in TLC [28, 29].



**Figure 2.** (a) and (b) Mass spectra obtained by analyzing Part I and Part II in Figure 1, respectively; (c) mass spectrum obtained by analysis of the extract loaded to the sharp end of the tip

### *Sequential and Exhaustive Ionization of Wooden Tips*

When the solution containing lysozyme and NaCl was loaded to the sharp end of a wooden tip and directly analyzed, the protein and salt were found to be ionized separately. As shown in Figure 3, NaCl was ionized at the first stage whereas lysozyme was detected at the late stage, showing an ionization order different from that obtained by Mandal et al. [24] in the analysis of insulin and NaCl on metal needle. Mandal et al. [24] recently reported the sequential and exhaustive ionization of analytes on metal needles. They concluded that analytes were ionized and detected in the order of their surface activities (i.e., compounds of higher surface activity were exhausted sooner than those of lower surface activity). However, there were also controversial cases in their studies [24]. In the analysis of protein and peptide mixtures using wooden tips, we observed that protein signals were exhausted first while peptide signals lasted much longer [14]. In the present study, lysozyme and NaCl were found to be ionized in the order of increasing surface activity. This ionization sequence was further confirmed by analysis of a mixture of myoglobin and NaCl using wooden tips (Figure S3). Once again the salt was exhausted first and the protein signals were clearly observed at the late stage. Our results indicated that wooden tips could be used to directly analyze protein solutions with high salt concentrations. More investigations will be needed for a better understanding of the mechanism and order of the sequential and exhaustive ionization in solid-substrate ESI.

### *Quantitation Using Wooden Tips*

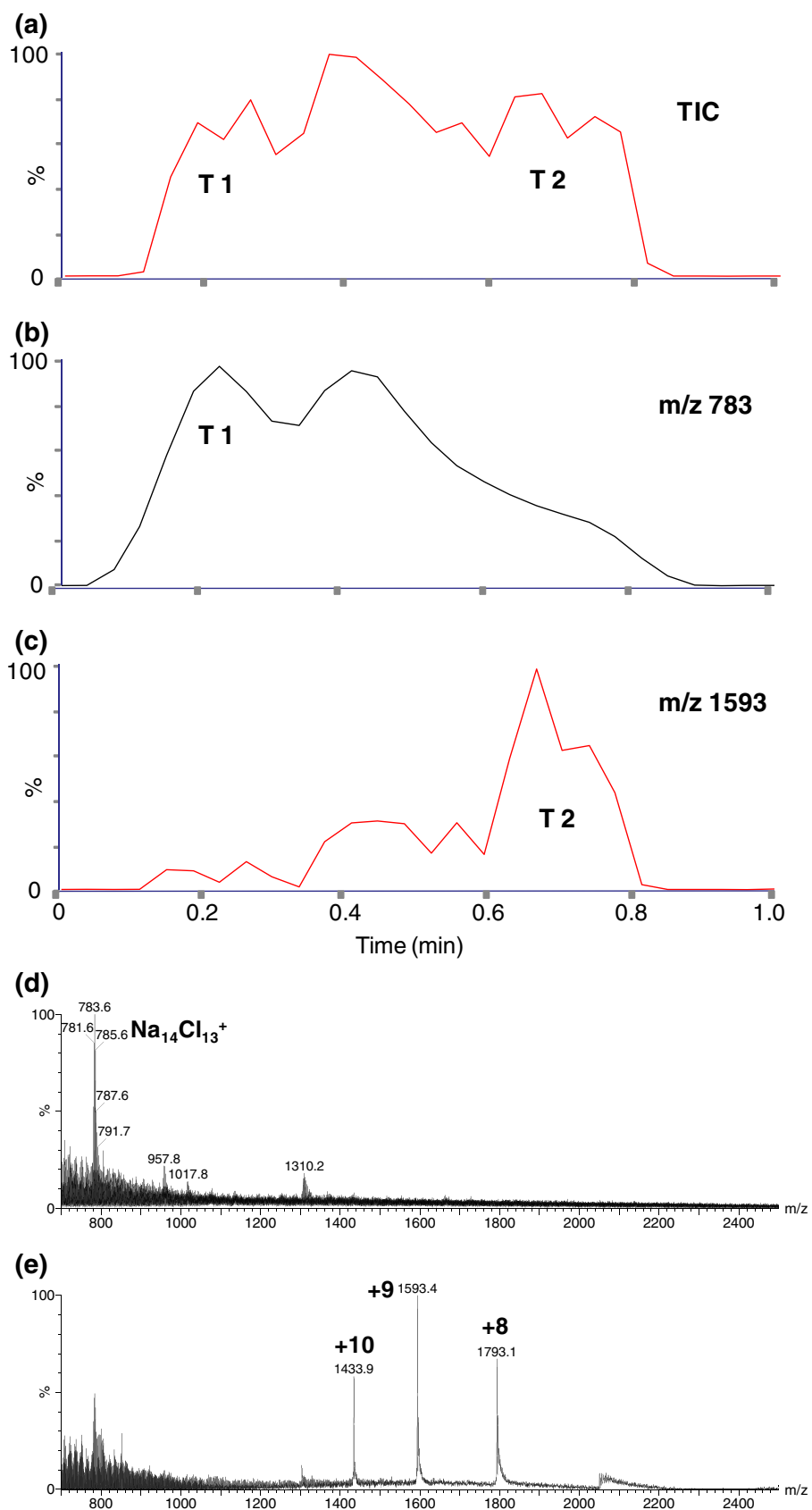
Wooden tips can be used not only for qualitative analysis but also for quantitative analysis of raw samples. In this study, a wooden tip was used to directly analyze losartan, a medicine for hypertension, in raw urine (see Figure S4a for the spectrum obtained). As shown in Figure S4b, selected reaction monitoring (SRM) intensity shows a good linear relationship against the concentration of the compound in urine, suggesting good reproducibility of the measurements and potential application of solid-substrate ESI in quantitation.

### *Conductivity of Wooden Tips*

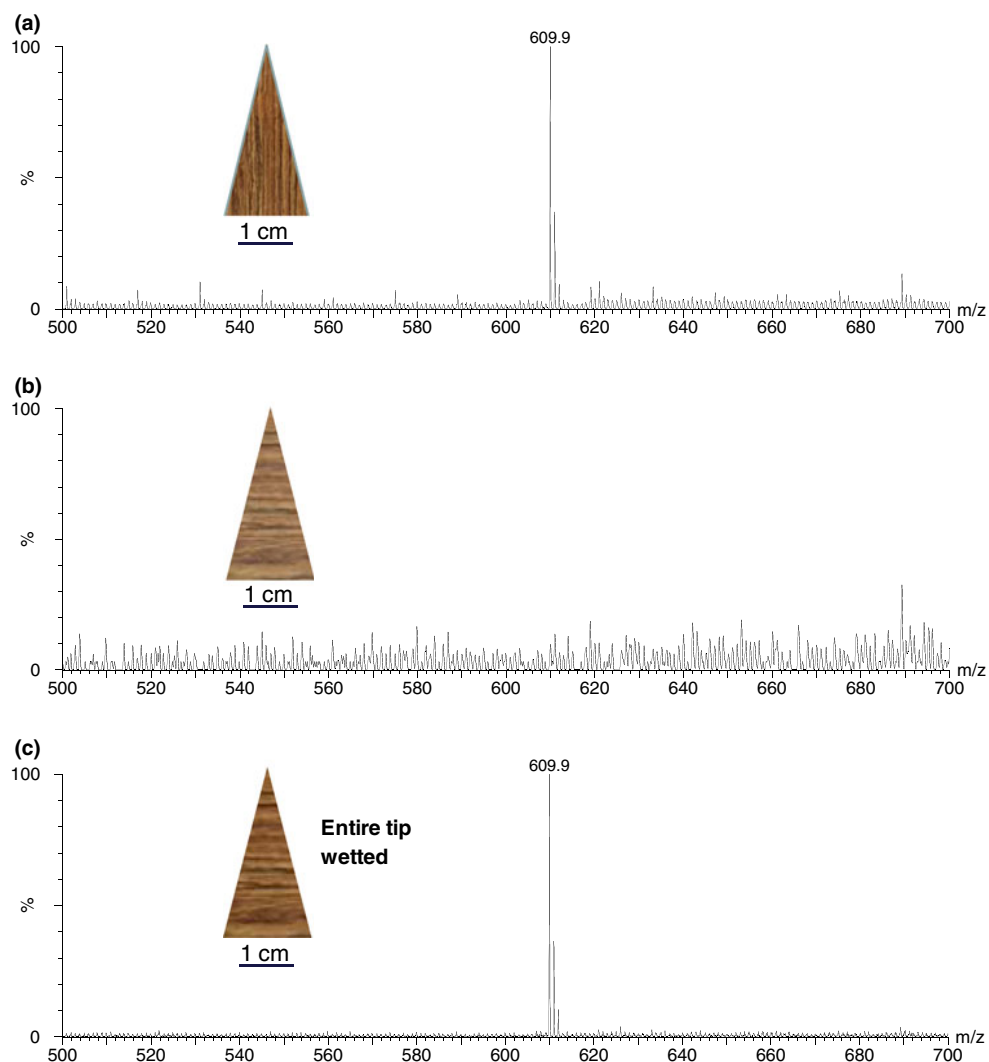
To investigate the conductivity of wooden tips used in the study, the electric resistance of a typical wooden toothpick (length: 20 mm, root diameter: 2 mm) was measured. An electric resistance larger than the measurement limit of the multimeter was obtained when the wooden tip was dry. When 5  $\mu\text{L}$  methanol was loaded onto the sharp end of the tip, immediate measurement revealed that the electric resistance of the tip became  $5.0 \pm 0.5 \text{ M}\Omega$  (three independent measurements), indicating that the wooden tip became much more conductive even though the solvent was not applied to

the entire tip. To further investigate how the conductivity was improved in this case, 5  $\mu\text{L}$  of green fluorescer solution (1 mg/mL in methanol) was loaded onto the sharp end of a wooden tip, and a UV lamp and a camera were used to trace the movement of the solution. It was found that the solution diffused rapidly and longitudinally along the microchannels (the vascular system) on the surface of the wooden tip by capillary action (Figure S5a), and it took about 0.36 s (10 recorded frames) for the solution to travel 30 mm. This result suggested that for a wooden tip of 20 mm, a common length used in our studies, it took about 0.24 s for the solution to reach the other end to make the tip become conductive. It was noted that only a trace amount of solution had diffused along the tip, and only the sharp end of the tip onto where the sample solution had been loaded was apparently wet, while other parts of the tip still looked or felt dry after the tip had become conductive.

It was noted that the fluorescer solution only diffused longitudinally along the microchannels and almost no transverse diffusion was observed (Figure S4a). To further investigate the effects of microchannel direction on diffusion and spray ionization of sample solutions, wooden tips with longitudinal and transverse microchannels were made, respectively (Figure 4). Investigation using the green fluorescer solution confirmed that when the solution was applied to the sharp tip-end, rapid solution diffusion to the other end occurred only on the tips with longitudinal microchannels but not on those with transverse microchannels. The electric resistance of the wooden tips with transverse microchannels remained larger than the measurement limit of the multimeter after sample loading to the sharp end, in contrast to the much improved conductivity of the wooden tips with longitudinal microchannels. Wooden tips with longitudinal microchannels and those with transverse microchannels were connected to a high voltage (3.5 kV), and they were compared for ESI-MS analysis. When 5  $\mu\text{L}$  reserpine solution (1 ppm in methanol) was applied to the sharp end of each tip, reserpine signals were immediately observed for the tips with longitudinal microchannels (Figure 4a), while no spray and no reserpine signals were observed for the tips with transverse microchannels (Figure 4b). When the tips with transverse microchannels got entirely wet with 20  $\mu\text{L}$  methanol, reserpine signals were detected (Figure 4c, also see Figure S5b for an image of the plume of spray from a wooden tip). These results further confirmed that it was the diffusion of solution along the wooden tips that caused conductivity and, subsequently, spray ionization of the wooden tips. Commercially available wooden toothpicks usually have longitudinal microchannels and, thus, can be conveniently used for ESI-MS analysis with sample solution loaded onto the sharp tip-end. Not requiring to get the entire tip wet allows less sample consumption and more convenient sample loading.



**Figure 3.** Sequential and exhaustive ionization on a wooden tip for 5  $\mu\text{L}$  solution containing 10  $\mu\text{M}$  lysozyme and 10 mM NaCl in 50 % methanol. (a) Total ion chromatogram (TIC); (b) extracted ion chromatogram for m/z 783 ( $\text{Na}_{14}\text{Cl}_{13}^+$ ); (c) extracted ion chromatogram for m/z 1593 ([lysozyme + 9H] $^{9+}$ ); (d) spectrum acquired at T1; (e) spectrum acquired at T2



**Figure 4.** Mass spectrum obtained after loading 5  $\mu\text{L}$  reserpine (1 ppm in methanol) onto the sharp end of a wooden tip. (a) Reserpine signals were immediately obtained from the tip with longitudinal microchannels; (b) no reserpine signals were obtained from the tip with transversal microchannels; (c) reserpine signals were obtained from the tip with transversal microchannels after the entire tip was wetted by methanol

### Sampling with Various Tips

Conductivity is an important factor for fabrication of ESI emitters, since the applied high voltage needs to reach the emitter cusp in order to induce spray ionization. Conventionally, emitters coated with or made of conductive materials or with a wire inserted have been widely used [30]. The high voltage can also be conducted via sample solutions. In this way, the emitters are not necessary to be conductive [30]. For example, spray ionization has been induced from materials such as glass [31], PMMA [32], carbon fiber [33], and silica fiber [34], with high voltages applied through solution channels. Capillary-channeled polymer fiber tips were also used for desalting to improve ESI-MS analysis of protein samples [35].

Our above results indicated that nonconductive materials containing microchannels or pores to allow longitudinal

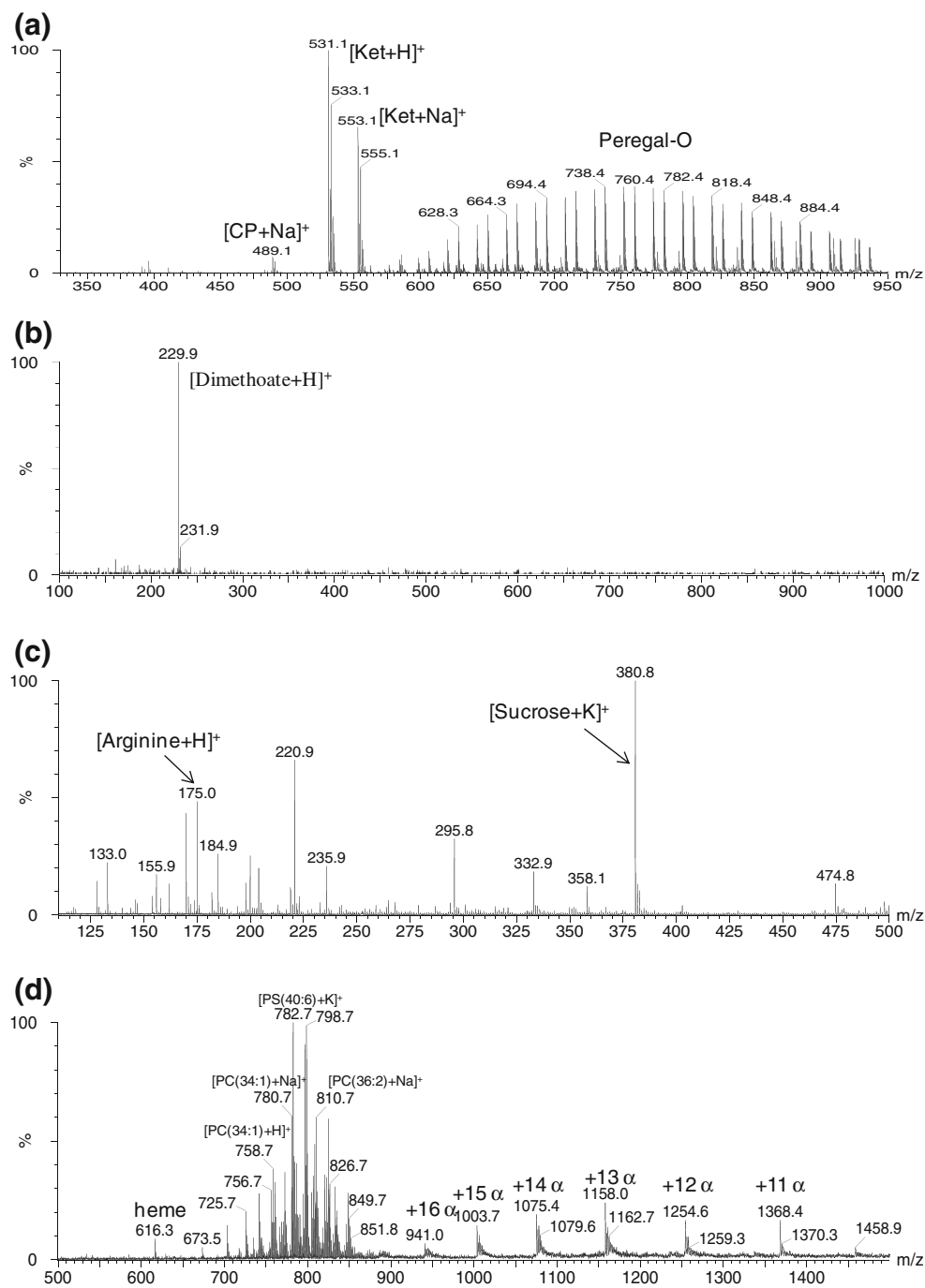
diffusion of solution could be used as ESI emitters even when the sample solution was loaded to the tip ends only. Rapid diffusion of the solution by capillary action would enable the materials to become conductive and spray ionization could thus be induced. Various other materials were tested as tips for ESI analysis in this study.

Common nonconductive materials, including porous materials such as bamboo, fabrics, and sponge, and nonporous materials such as poly(ether ether ketone) (PEEK) were attempted for ESI sampling and ionization with the sample solutions loaded to the sharp-ends. No signals were observed with the nonporous tips because of the intrinsic insulating property of the materials, and the solution could not be efficiently diffused along the tips to improve conductivity. Spray ionization could be observed with the porous tips made of bamboo, fabrics (35 % cotton and 65 % polyester) or cellulose sponge. With these tips, the

diffusion of solutions along microchannels/pores enabled the applied voltage to reach the tip end to induce ionization. Some of the spectra obtained using these porous tips are shown in Figure S6. Bamboo is more rigid than wood and holds solution less efficiently. The signal span of bamboo tips was thus shorter than that of wooden tips for the same volume of the solutions. Shorter signal durations were also observed for the tips of paper and fabrics, indicating that

solutions on these planar materials of larger pores spread out and evaporated more easily. Sponge, including cellulose sponge and other sponge, could also be used as the ESI tips. Compact sponge with smaller pores tended to offer better signals and longer signal durations.

Metal tips such as silver and stainless steel needles were also examined as the solid-substrate ESI tips in this study. These materials are intrinsically conductive and do not



**Figure 5.** Mass spectrum obtained by analysis of (a) ~2  $\mu$ L ketoconazole cream loaded on a stainless steel needle without addition of solvents, (b) intact fresh coriander leaf with 5 ng dimethoate spiked on the surface, (c) a piece of mushroom with 5  $\mu$ L methanol as the added solvent, and (d) a piece of porcine rib with 5  $\mu$ L methanol containing 0.1 % formic acid as the added solvent

require solution diffusion to improve conductivity and induce spray ionization. Sample signals could be observed with these metal needles, but the signal durations were very short because the surface of these needles was smooth, hydrophobic and, thus, less capable of holding solutions. This result was consistent with the previous observation with metal needles [5, 24]. Although metal needles have short signal duration for analysis of solution samples, their good conductivity and relative inertness are good for direct analysis of some special samples. Analysis of viscous samples is a challenging task in mass spectrometry [36]. Metal needles could hold viscous samples well and, thus, could be used in their direct analysis. Figure 5a is a spectrum for ketoconazole cream, a common medicine for fungal infections on skin [37], obtained by direct analysis of the cream on a stainless steel needle without addition of solvents. Ketoconazole (Ket) and clobetasol propionate (CP), two major ingredients of the medicine, and the cream Peregol O were detected with excellent signal-to-noise ratios in the spectrum. With ~2  $\mu\text{L}$  of the cream, the signals lasted for about 15 min. Metal needles can also be used to hold soft animal tissue for direct ionization analysis [15].

Direct ionization could also be achieved with samples that could form tips to allow for solid substrate ESI-MS analysis. These samples could be intrinsically conductive or become conductive with addition of solvents. Added solvents are also usually required to dissolve and facilitate ionization of chemicals from the samples. Figure 5b is a spectrum obtained by solid substrate ESI-MS analysis of an intact fresh coriander (*Coriandrum sativum*) leaf previously spiked with 5 ng of dimethoate, a pesticide. After adding 5  $\mu\text{L}$  methanol and applying a high voltage, a plume of spray could be observed from the leaf tip and protonated dimethoate ( $m/z$  230) was predominantly detected in the spectrum. As no damage was done to the leaf and the waxy layer on the leaf surface prevented extraction of internal compounds by the solvent, only chemicals on the leaf surface were detected. This provides a simple and rapid method for analysis of substances on leaf surface. Leaves could also be cut into V-shape [15, 16] to allow their endogenous compounds to be analyzed using the solid-substrate ESI method.

Mushroom is a fruiting body of fungi. Figure 5c is a spectrum obtained by solid substrate ESI-MS analysis of a piece of mushroom (~20 mg) with 5  $\mu\text{L}$  methanol as the added solvent. Compounds such as arginine and sucrose could be observed. Animal bones could also be directly analyzed using the solid substrate ESI-MS method. Figure 5d is a spectrum obtained for a small piece of fresh porcine rib (~5 mg). One side of this triangular rib was connected to a high voltage of 3.5 kV via a clip. Upon addition of 3  $\mu\text{L}$  methanol containing 0.1 % formic acid to the rib, a spectrum was obtained with ingredients of marrow such as lipids and hemoglobin detected.

## Conclusions

The hydrophilic surface of wooden tips could serve as a chromatographic column for separation of sample components. Sequential and exhaustive ionization was observed for proteins and salts on wooden tips with salts ionized sooner and proteins later, allowing direct analysis of protein samples with high salt contents. Further investigation is required to understand the mechanism and order of sequential and exhaustive ionization on wooden and other tips. Our results also revealed that rapid longitudinal diffusion of solutions/solvents by capillary action enabled the wooden tips to become conductive and the high voltage applied to the tip could thus reach the tip cusp to induce spray ionization. Nonconductive porous materials could thus be used as tips for ESI-MS analysis and ionization could be induced with sample solutions loaded to the sharp ends of the tips only. We demonstrated that in addition to wood, paper, and metals, materials such as bamboo, fabrics, and sponge could also be used as tips for sample loading and ionization of ESI-MS analysis, and ionization could be induced from tips of plant and animal tissues, mushroom, and animal bones to allow them to be directly analyzed. All these can be considered as solid-substrate ESI-MS since their ionization processes have a common feature: spray ionization is induced from solid substrates with application of an electric field. In solid-substrate ESI-MS, tips of inert materials can be used for sample loading and ionization, and bulky solid samples can form tips to be directly ionized and analyzed. Searching for tips of higher efficiency for sample loading and ionization and extending solid-substrate ESI-MS for direct ionization analysis of a broader range of samples are under investigation.

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