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Electrospray Ionization Using Wooden Tips

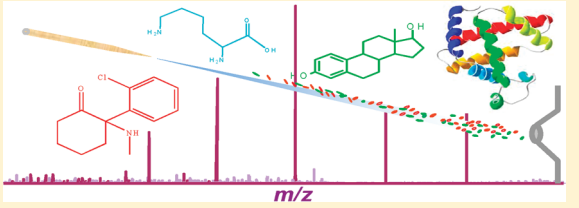
Bin Hu,[†,‡] Pui-Kin So,[†] Huanwen Chen,[*,‡] and Zhong-Ping Yao[*,†]

[†]State Key Laboratory for Chirosciences and Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong Special Administration Region, China

[‡]Jiangxi Key Lab for Mass Spectrometry and Instrumentation, East China Institute of Technology, Nanchang, 330013, China

Supporting Information

ABSTRACT: Electrospray ionization (ESI) is a mass spectrometric technique widely used in various fields including chemistry, biology, medicine, pharmaceutical industry, clinical assessment, and forensic science. In this study, we report a simple and economical ESI-mass spectrometry (MS) technique, which makes use of disposable wooden tips (wooden toothpicks) for loading and ionization of samples. Samples could be loaded by normal pipetting onto the tip or simply dipping the tip into sample solutions. The hydrophilic and porous nature of wood allows effective adhesion of the sample solution for durable ion signals. The tip can be directly connected to nano-ESI ion sources of various mass spectrometers. Upon application of high voltage to the tip, desirable mass spectra could be obtained. We demostrated that this new technique is applicable for analysis of various samples, including organic compounds, organometallic compounds, peptides, proteins, and samples that cannot be directly analyzed by conventional ESI techniques, e.g., slurry samples and powder samples. The slim and hard properties of the wooden tip enable sampling from specific locations such as corners and small openings, indicating potential applications of the new technique in forensic investigations. The observation of electrospray ionization from wooden materials also allows us to get new insights into the materials that can be directly ionized for mass spectrometric analysis.



Electrospray ionization mass spectrometry (ESI-MS) is a technique commonly used for analysis of various compounds. Since its introduction for analysis of biomolecules in the late 1980s,[1] considerable efforts have been made to further improve the sampling and ionization of this technique. Development of nanoelectrospray ionization (nano-ESI) allows softer ionization and consumption of smaller volume of samples.[2,3] The introduction of ambient ionization techniques, e.g., desorption electrospray ionization (DESI),[4] extractive electrspray ionization (EESI),[5] and other successively invented techniques[6−8] has enabled direct analysis of complex samples with no or little sample preparation. In the past 2 decades, noncapillary emitters have also been developed to avoid the clogging problem in conventional capillary-based ESI and for more convenient sample loading. Fenn attempted to generate ESI from a wicking element, e.g., cotton wire, and found the possibility of ionization from paper or a thin layer chromatography (TLC) plate.[9] Other materials, including a copper wire and a stainless steel needle,[10−14] optical fibers wired with a copper or platinum coil,[15,16] a surface-modified glass rod,[17] and nanostructured tungsten oxide,[18] have been successfully developed as emitters for ESI and applied for analysis of various samples.[10,11,13−22] More recently, paper spray, which used paper as a medium for sample loading and ionization, was introduced.[23−28]

We herein introduce an ESI method that uses disposable wooden tips for loading and ionization of samples. The wooden tips utilized here are wooden toothpicks readily available at a significantly low price worldwide (about 500 toothpicks/1 USD in supermarkets in Hong Kong). The sample preparation of the present method is very simple. Sample solutions can be applied to the sharp tip-end of the wooden tips by normal pipetting or simply dipping the sharp tip-end into sample solution, from which a layer of sample solution can be coated on the surface of the tip-end (Figure 1). The hydrophilic and porous nature of wood allows effective adhesion of sample solution on the tip surface without surface modification. The narrow-stick shape of wooden tips allows a lower extent of sample solution spreading compared to flattened paper used in paper spray,[28] reducing the surface area for evaporation and the tendency of drying of sample solutions and thus enables acquisition of ion signals of longer duration.

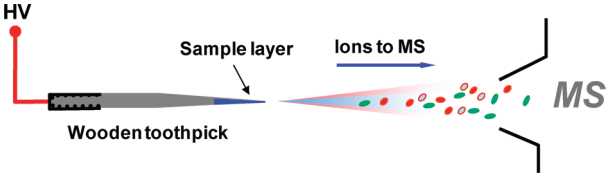


Figure 1. Experimental setup of ESI using a wooden tip.

The instrumental setup of using wooden tips for ESI analysis is simple and readily achieved in common laboratories. The o.d. of toothpicks and the elasticity of wood enable the tip to be steadily

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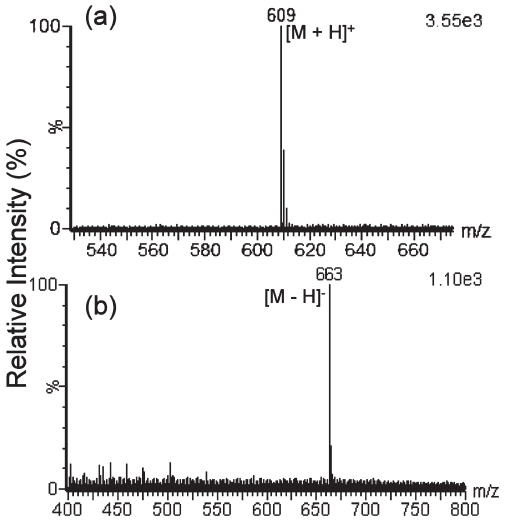

Figure 2. Mass spectra obtained by using wooden tips with the Q-TOF2 mass spectrometer: (a) positive ion mass spectrum of 1 μM reserpine in MeOH/H_2O (1/1) containing 0.1% formic acid and (b) negative ion mass spectrum of 10 μM angiotensin I/II in MeOH/H_2O (1/1).

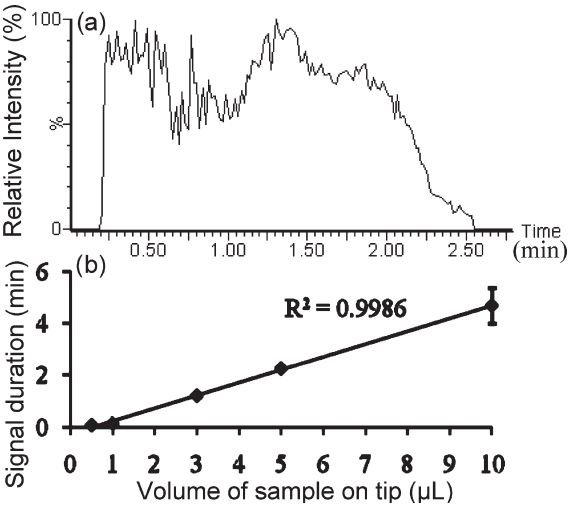

Figure 3. (a) Total ion chromatogram obtained by applying 5 μL of reserpine solution on wooden tip. (b) Duration of signals of the reserpine solution showed a linear relationship with the volume of solution applied on the tip. Each data point was the average obtained in three independent experiments, and the error bars represent the corresponding standard errors.

fixed to the capillary mount of typical nano-ESI sources for conducting electricity. Electric current can also be simply applied by electric cable with a clip. The tip can be oriented perpendicular or parallel to the aperture inlet. Such settings could be conveniently fitted with different types of mass spectrometers without major hardware modification (Figure S2 in the Supporting Information).

In this study, we demonstrated that this new technique could be used for analysis of a variety of compounds, including organic compounds, organometallic compounds, peptides, and proteins, and even samples that are less accessible to conventional capillary-based ESI, e.g., unfiltered slurry samples and powder samples, and has potential applications in forensic analysis.

■ EXPERIMENTAL SECTION

Materials. Wooden toothpicks used in this study were BEST-buy brand toothpicks purchased from the PARKnSHOP supermarket in Hong Kong. Wooden toothpicks from other sources were also tested, but no significant difference was observed. The wooden toothpicks are made of birch wood without chemical modification on the surface during manufacturing. Reserpine, angiotensin I/II, myoglobin, cytochrome C, trypsin, melamine, cyanuric acid, amoxicillin, lysine, Gly-Ala-Phe, methyl yellow, dimethoate, formic acid, and iron(II) acetylaceonate were purchased from Sigma. Ketamine was purchased from Alfasan (Woerden, Holland). Methanol and acetonitrile were purchased from Tedia (Fairfield, OH). Papers used for paper spray experiments were grade 1 papers purchased from Whatman (Maidstone, U.K.), the same model used in the previous studies.[28] Melamine cyanurate (MC) was prepared in urine as previously described.[29] The completely digested cytochrome C sample was prepared by incubating 0.1 mg/mL of the protein with trypsin in a ratio of protein/enzyme (20/1, w/w) in 50 mM ammonium biocarbonate at 37 °C for 10 h. The corresponding incompletely digested sample was prepared by reducing the incubation period to 1 h.

Sample Preparation with Wooden Tip. The wooden toothpicks purchased from local stores typically have a length of ~5 cm, o.d. of ~2 mm, and one or two sharp-ends with an o.d. of ~0.5 mm (Figure S1 in the Supporting Information). The wooden toothpicks can be easily shortened or sharpened if necessary. Prior to sample loading, the toothpick was first cut into a length of ~2 cm, and the sharp tip-end was further striped to be sharper with a cutter. Note that a sharper tip-end is very important for good signals. The typical o.d. of the tip-ends used for measurements in this study was ~0.2 mm. After sharpening, the sharp tip-end may not be perfectly round, but this irregularity usually has no obvious effect on the ion signals. Sample solutions were loaded to the sharp tip-end by pipetting or dipping the sharp tip-end into sample solution for about 5 s. For application of powder samples, the sharp tip-end was first prewetted with MeOH/H_2O 1/1 containing 0.1% formic acid, then scraped with sample powder until a layer of solid powder was adhered on the tip surface. Subsequently, 5 μL of solvent (MeOH/H_2O 1/1 containing 0.1% formic acid) was pipetted onto the tip surface.

Mass Spectrometry. Mass spectra were acquired on quadrupole-time-of-flight (Q-TOF) mass spectrometers (Waters Q-TOF2 or ABI QStar Pulsar) or a triple-quadrupole mass spectrometer (Waters Quattro Ultima). For both Q-TOF instruments, the ionization source was set up using corresponding nano-ESI configurations and a wooden tip was mounted onto the capillary holder for analysis. The mounted wooden tip was perpendicular and parallel to the mass spectrometer inlet for the Q-TOF2 and API Qstar Pulsar mass spectrometers, respectively (Figure S2 in the Supporting Information). For the triple-quadrupole mass spectrometer, the wooden tip was held and positioned parallel to the mass spectrometer inlet via a clip, which was connected to the high voltage supply of the mass spectrometer (Figure S2 in Supporting Information). The tip-end was positioned ~0.5 mm away from the mass spectrometer inlet. For data acquisition of each instrument, the capillary voltage was adjusted in the range of 2.5−3.5 kV until optimal ion signals were obtained. The cone voltage of the Q-TOF2 and Quattro Ultima mass spectrometer was

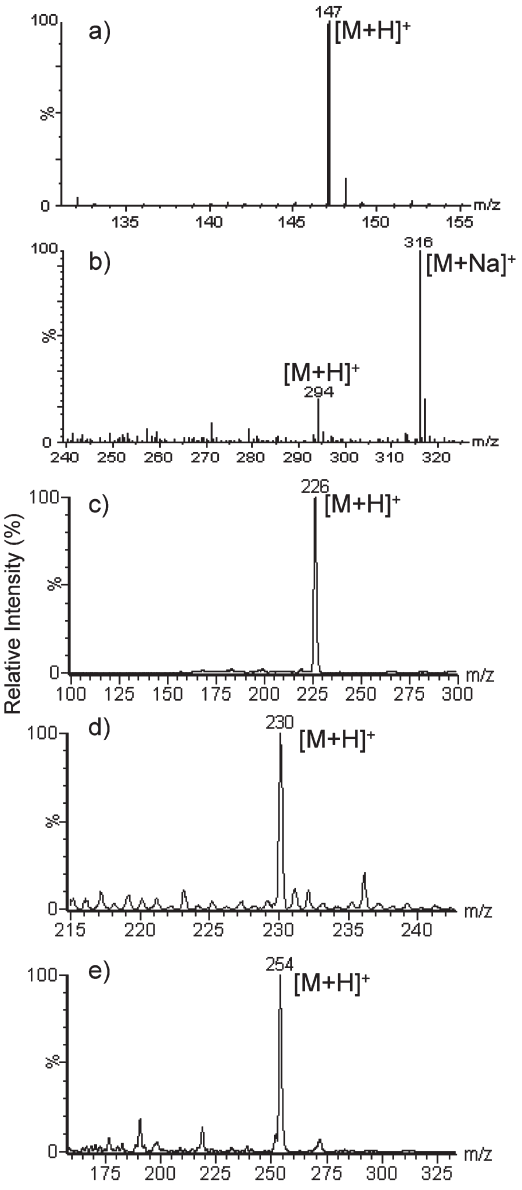

Figure 4. Mass spectrum of (a) lysine, (b) Gly-Ala-Phe, (c) methyl yellow, (d) dimethoate, and (e) iron(II) acetylaceonate obtained by using a wooden tip. Sample solutions were typically 1 μM in MeOH/H_2O (1/1) containing 0.1% formic acid. Spectra a and b were acquired using the Qstar mass spectrometer and spectra c−e were acquired using the Quattra Ultima mass spectrometer.

set at 30 V, and declustering potential of the API Qstar Pulser mass spectrometer was set at 50 V. All desolvation gases were turned off, and the ion source temperature was set at 40 °C. Other settings were similar to those in nano-ESI operation.[30−32] Paper spray experiments were performed as in the literature.[28] Unless specified, mass spectra were obtained by averaging the data acquired in a time window of at least 20 s (one mass spectrum acquired per second).

RESULTS AND DISCUSSION

Analysis of Small Molecules. The mass spectrum obtained for 1 μM of reserpine, a pharmaceutical small molecule, is shown in Figure 2a. The protonated molecules ($[M + H]^+$) (m/z 609) were clearly observed with no significant interfering background

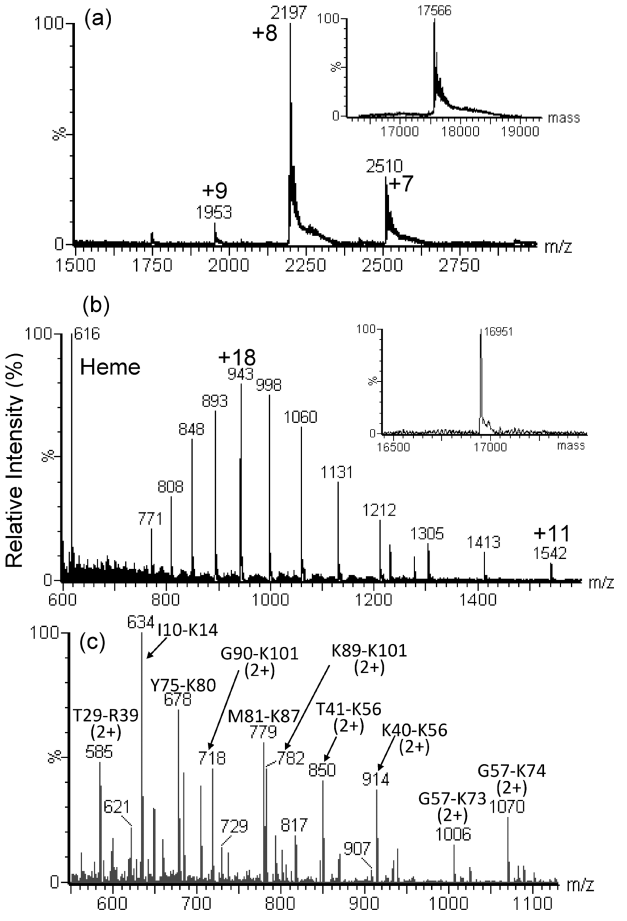

Figure 5. Positive ion mass spectrum obtained by using a wooden tip for 20 μM myoglobin in (a) 20 mM ammonium acetate and (b) ACN/20 mM CH_3COONH_4 (1/1) containing 0.1% formic acid. (c) Mass spectrum of the completely digested cytochrome C obtained by using a wooden tip. All mass spectra were acquired on the Q-TOF2 mass spectrometer.

peaks. Upon application of 5 μL of reserpine sample solution on the tip, ion signals could last for ~2.5 min (Figure 3a). This time scale is generally sufficient for obtaining desirable mass spectrometric data. The duration of ion signals showed a linear relationship with the volume of sample solution applied (Figure 3b). Useful ion signals with a duration of ~5 s could be obtained by applying as few as 0.5 μL of sample on the tip. Longer duration of ion signals could be obtained by introducing a larger volume of sample solution on the tip, but extra precaution should be taken against gravity-driven falling of the liquid droplet and contamination of the voltage connector by diffusion of liquid sample on the tip surface. After the sample solution was used up or dried out, ion signals could be conveniently regenerated by pipetting more sample solution onto the mounted tip. It was noted that ion signals could be obtained by loading sample solution only to the tip-end, revealing that wetting of the entire tip body was unnecessary for conducting voltage. It was assumed that although not apparently visible, the sample solution loaded on the tip-end might diffuse along channels and pores of the wooden tip by capillary action, making the entire wooden tip become conductive.

Comparison of the current technique with nano-ESI and paper spray was investigated with reserpine. For 1 μM of the sample solution, the spectra obtained with the three techniques are comparable (Figure S3 in the Supporting Information).

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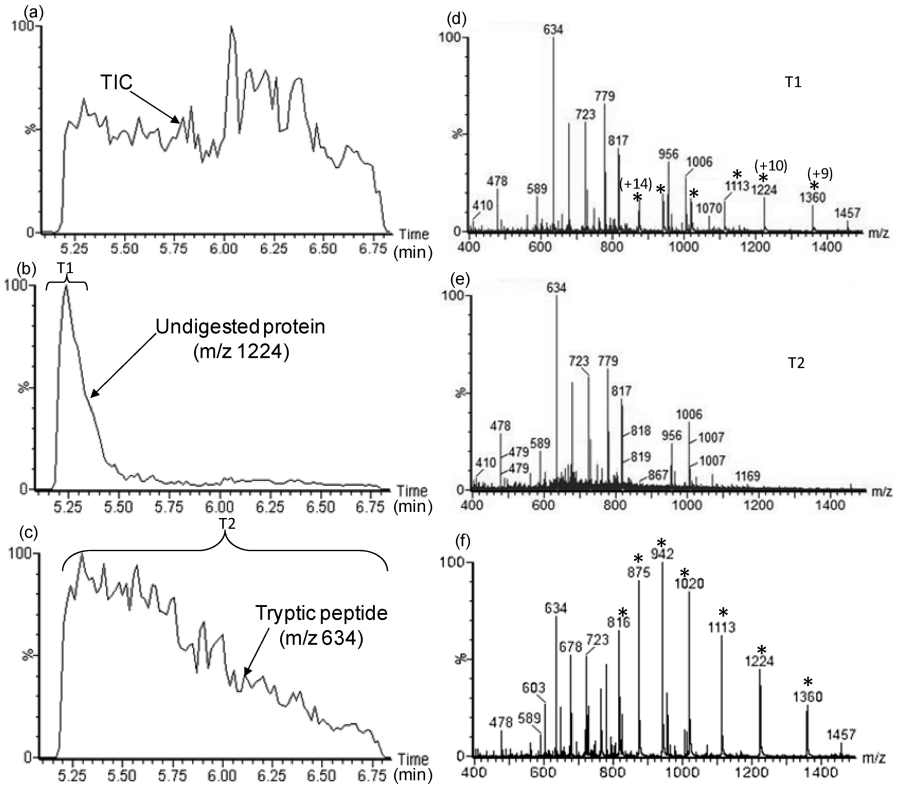

Figure 6. (a) Total ion chromatogram (TIC) obtained for the incompletely digested cytochrome C, (b and c) extracted ion chromatograms of representative mass peaks of undigested protein (m/z 1224) and the tryptic peptide (m/z 634), respectively. (d and e) Mass spectra obtained for the period of T1 in part b and T2 in part c, respectively. (f) Mass spectra obtained for the incompletely digested cytochrome C using nano-ESI. Mass peaks of undigested cytochrome C are labeled with an ∗. All mass spectra were acquired on the Q-TOF2 mass spectrometer.

Informative mass spectra could be obtained with 0.1 μM of the sample solution for the wooden tip and paper spray method, but a higher level of background noises or more background mass peaks were observed compared to nano-ESI (Figure S3 in the Supporting Information). With further dilution of the sample to 0.01 μM, chemical noises and background mass peaks were dominant and ion signals of interest could not be observed with the wooden tip and paper spray method, but an informative mass spectrum could still be obtained for nano-ESI using our instrument (data not shown). These data indicated that the current wooden tip method is of similar sensitivity compared to paper spray but less sensitive than nano-ESI. As for signal duration for the same volume of sample solution, obviously nano-ESI normally can last a much longer time than the wooden tip method. For the paper spray method, the duration of ion signals obtained by directly loading 5 μL of the sample solution onto the flat paper was measured to be ~30 s in our study, similar to the value in the literature.[28] The signal duration of the paper spray method is much shorter than that obtained by the wooden tip method, most likely due to the higher tendency of spreading and thus drying of sample solution on the paper surface.

In general, no significant background noises and interfering mass peaks were observed during the detection of ion signals of interest when samples with sufficient concentration were analyzed by the wooden tip method. The chemical noises might become more significant when the sample solution is approaching completely consumed, i.e., in approximately the last five spectra obtained before complete depletion of ion signals (Figure S4a in the Supporting Information), but this usually did not remarkably affect the spectral quality after averaging of the experimental data. Similar phenomenon was observed with the paper spray method in our study (Figure S4b in the Supporting Information).

Negative mode ionization can also be performed using the wooden tips. As shown in Figure 2b, the negative ion mass spectrum of 10 μM angiotensin I/II displays a predominant peak of deprotonated molecules (m/z 663) and minimal background peaks. Upon introduction of 5 μL of the sample solution on the tip, negative ion signals could last for ~1.2 min, a duration slightly shorter than that from positive ionization. In addition, in the wooden tip sampling and ionization method, the sensitivity of the negative ionization mode tends to be lower than that of the positive mode.

The wooden tip sampling and ionization method has been applied for analysis of various compounds using different instruments. Spectra obtained from some of the compounds, such as lysine, Gly-Ala-Phe, methyl yellow, dimethoate, and iron(II) acetylaceonate, are shown in Figure 4. These results demonstrated the applicability of the new method for routine analysis of chemical compounds.

Analysis of Proteins. Applications of the wooden tip method for protein studies were investigated. The mass spectrum obtained for 20 μM myoglobin in 20 mM ammonium acetate (CH_3COONH_4) buffer is shown in Figure 5a. A narrow charge state distribution ranging from 7+ to 9+, similar to that obtained by EESI,[33] was observed, indicating maintenance of the native structure of the protein during the ionization process. The deconvoluted mass 17 566 Da was in good agreement with the

noncovalent complex between apo-myoglobin (16 951 Da) and the heme moiety (616 Da). When the solvent of the myoglobin solution was changed to ACN/20 mM CH_3COONH_4 (1/1) containing 0.1% formic acid, the charge state distribution shifted to the lower m/z region and became broader, and the ion corresponding to the heme moiety was also observed (Figure 5b), indicating that the protein was denatured and the noncovalent complex was dissociated into the apo-myoglobin and heme moiety. These results revealed the softness of the wooden tip ionization and the applicability of the technique for analysis of protein samples.

Analysis of Tryptic Digests. The mass spectrum obtained for tryptic digest of cytochrome C was shown in Figure 5c. A total of 11 peptides were identified, covering 73% of the amino acid sequence of the protein. The duration of the ion signals allows performing tandem mass spectrometry (MS/MS) analysis of peptide ions (Figure S5 in the Supporting Information). The peptide masses and the sequence information obtained by MS/MS allowed the protein to be identified in high score (320 A.U.) through a database search using the Mascot program (data not shown). These results demonstrated the wooden tip sampling and ionization method could be used as an alternative method to nano-ESI for protein identification in proteomics.

An interesting finding was that when an incompletely digested cytochrome C was analyzed using the wooden tip method, the undigested protein and the tryptic peptides were detected in a separate way. As shown in Figure 6a−e, the ion signals of the undigested protein completely depleted within ~10 s, while those of the tryptic peptides could be detected for a sustained period. When the same sample was analyzed by nano-ESI, the signals of the undigested protein significantly dominated over those of tryptic peptides (Figure 6f). These results demonstrated that the wooden tip method could be advantageous in analysis of incomplete digestion products, which is common in proteomics studies.

The separation effect between the protein and peptides in the wooden tip method was proposed to be brought out by two scenarios. First, the hydrophilic nature of wood might retain the protein less readily than its digested peptides, as the former is more hydrophobic than the later. Second, the protein and peptides might be sequentially ionized due to their differences in surface activity.[34−37] It has been reported that in ESI, more hydrophobic components exhibit higher surface activity and are preferentially ionized, while the components with lower surface activity tend to leave in the primary ESI droplets and are less readily ionized.[34−37] Such sequential ionization has recently been observed in ionization using a metallic solid tip.[38] We believe that the sequential ionization phenomenon might also occur in ionization with a wooden tip, allowing ionization of protein and peptides separately according to their differences in hydrophobicity and thus surface activity.

Analysis of Raw Biological Liquid Samples. The characteristic of ionizing samples in an open environment in the present wooden tip method allows analysis of samples that are less accessible to conventional capillary-based ESI, such as unfiltered slurry samples. Melamine cyanurate (MC) is an insoluble complex between melamine and cyanuric acid and is the cause of fatal kidney stones in the melamine incident in 2008.[29] Direct detection of MC in urine samples by mass spectrometry has been a challenging task.[29] In this study, a slurry of MC in urine (5 μL) was directly analyzed using the wooden tip sampling and ionization method without filtering. The $[M + H]^+$ of the melamine moiety (m/z 127) was clearly observed in the mass spectrum (Figure 7a), similar to the previous results obtained using matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS) after sample cleanup.[29] Impurity mass peaks due to the chemical complexity of urine were also present in the spectrum, but they did not interfere with the observation of the peak of interest, revealing that the present method exhibits high tolerance to impurities. This result demonstrated that wooden tip sampling and ionization could be a rapid and a simple screening method for MC in urea samples for clinical assessment.

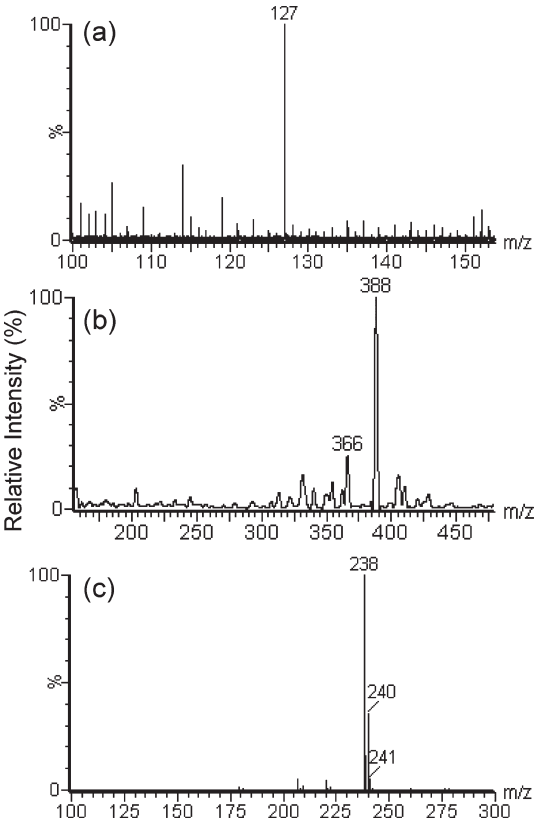

Figure 7. Positive ion mass spectrum of (a) MC, (b) amoxicillin powder, and (c) ketamine powder from a tiny crack on the concrete floor obtained by using the wooden-tip sampling method. Spectra a and c were acquired using the Q-TOF2 mass spectrometer, and spectrum b was obtained using the Quattra Ultima mass spectrometer.

Analysis of Powder Samples. Ionization of samples in an open environment also allows direct analysis of powder samples. To investigate this application, a wooden tip, prewetted with MeOH/H_2O (1/1) containing 0.1% formic acid on the tip-end, was scraped with a powder of amoxicillin, a commonly used β-lactam antibiotic,[39] to form a layer of sample powder at the tip-end. The tip was then mounted into the ion source, and subsequently 5 μL of driving solvent (MeOH/H_2O (1/1) containing 0.1% formic acid) was loaded onto the tip deposited with sample powder. The role of the driving solvent is to dissolve and deliver the sample to the tip-end for electrospray ionization, similar to that in the liquid extraction surface sampling probe/ionization methods.[40] As shown in Figure 7b, a quality spectrum with predominant $[M + Na]^+$ (m/z 388) and minor $[M + H]^+$ (m/z 366) of the target compound could be observed, indicating the applicability of the powder-sampling method.

Applications in Forensic Analysis. The slim and hard properties of the wooden tip allow collection of samples from

corners or small openings, which could be useful for some applications, such as forensic analysis. In our study, an attempt was made to collect the powder of ketamine, a commonly abused recreational drug,[41] from a tiny crack on a concrete floor for MS analysis. Ketamine was previously spiked on the floor. A pre-wetted wooden tip was inserted into the crack and scraped in a circular motion until a layer of powder was adhered to the tip-end. The tip was then mounted onto the ion source, and driving solvent was added for mass spectrometric analysis. Although other particulates might also be collected along with the sample powder, the mass spectrum obtained (Figure 7c) showed insignificant background peaks and a distinct mass peak corresponding to the $[M + H]^+$ of ketamine (m/z 238), which could be further confirmed by MS/MS analysis (data not shown). These observations again indicated the high impurity tolerance of the current method and demonstrated its potential application in forensic analysis.

CONCLUSIONS

Electrospray ionization using a wooden tip has been demonstrated in this study. The wooden tips used in the new method are cheap, are readily available, and can be directly connected to nano-ESI ion sources of various mass spectrometers. The sampling of the new method is relatively simple. The hydrophilic and porous nature of wood allows effective adhesion of sample solution and thus acquisition of durable ion signals. The slim and hard properties of wooden tips also allow sampling from specific locations, e.g., corners and small openings. The mechanism of the ionization process on wooden tips is still unclear, but our results indicated that this new method can be used for routine analysis of various compounds and for analysis of tryptic digests for peptide sequencing and protein identification. The ionization of the new method is very gentle and enables observation of native proteins, noncovalent complexes (e.g., myoglobin), and organometallic complexes (e.g., iron(II) acetylaceonate). Furthermore, analytes in some mixture samples, e.g., a mixture of a protein and some peptides, could be separately detected with this new method. This method was applicable for direct analysis of raw biological samples and powder samples, indicating the potential applications in clinical and forensic analysis. The successful use of wood as sampling and ionization media in ESI not only simplifies ESI analysis but also brings us a new vision to the ESI technique. Direct ionization of similar materials, e.g., plant tissue, for mass spectrometric analysis, is being investigated.

ASSOCIATED CONTENT

Supporting Information. Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Zhong-Ping Yao: phone, (852)34008792; fax, (852)2364-9932; e-mail, bczpyao@inet.polyu.edu.hk. Huanwen Chen: fax, (+86) 791-3896370; e-mail, chw8868@gmail.com.

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