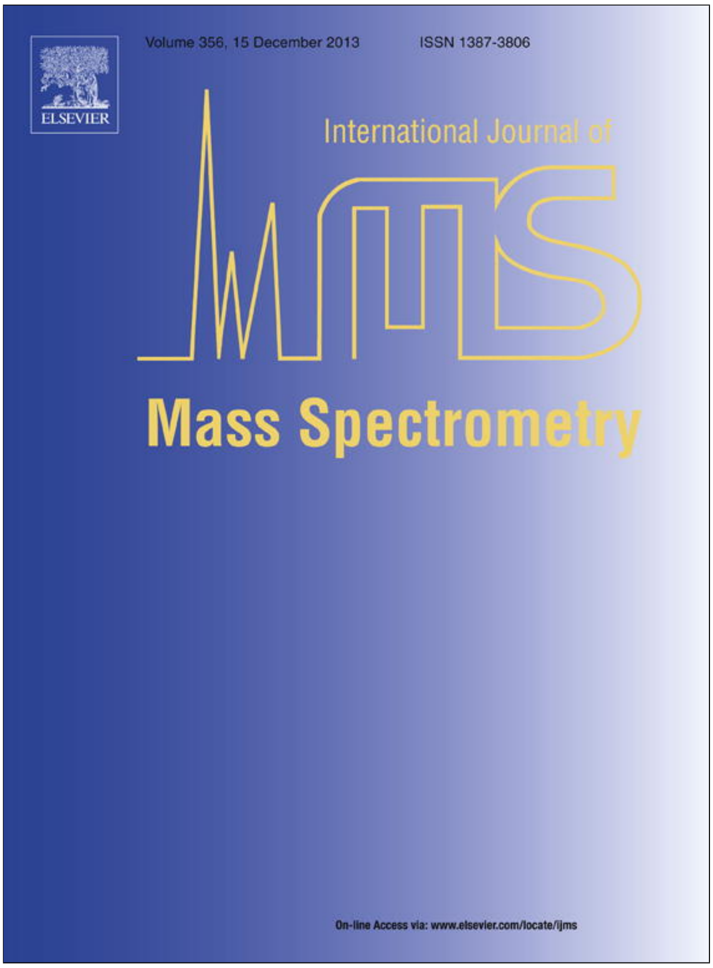
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Electrospray ionization using a bamboo pen nib

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ABSTRACT

A novel and highly sensitive electrospray ionization method, nib spray-mass spectrometry (Nibs-MS), is described. A bamboo pen nib was shaped and used for sample loading and ionization. The nib can be directly connected to the ESI ion sources of various mass spectrometers. The sample solution can be loaded onto the nib by normal pipetting, or simply dipping the nib into a sample solution. The sample solution enters the seam (width ~7.5 μm) of the nib by capillary attraction. Meanwhile, the seam functions as a micro reservoir and prevents the sample solution from spreading to the surface. When a high voltage is applied to the nib, the sample solution is rapidly ejected and ionized toward the mass inlet. Since ionization occurs within a very short period, an abundance of ions is formed, leading to a dramatic improvement in the limit of detection. Using 4-chloro-amphetamine as a model compound, the limit of detection was determined to be 10 ng/mL. In contrast to this, when the traditional methods, including paper-spray and wooden tip-spray were used, the limits of detection were 100 and 50 ng/mL, respectively. Detailed information on how to prepare a suitable bamboo pen nib, the optimized position for ionization and its application to the analysis of a saliva sample are also reported.

1. Introduction

Liquid chromatography electrospray ionization-mass spectrometry (LC-ESI-MS) is currently one of the most popular, widely used and powerful techniques, not only for the analysis of illicit drugs [1] but also for biomolecules [2,3] and analogs thereof. A wide variety of ionization methods have been developed. Fernández et al., in a biennial review (2009–2011), reported on advances made in ambient sampling/ionization mass spectrometry (ambient MS), in which at 27 different techniques were mentioned [4]. The capabilities of ambient MS have become more common because they are very useful for rapid sampling and screening. Especially, the use of chromatography paper, so-called paper spray-mass spectrometry (PS-MS) [5–15], and even the use of a wooden tip spray-mass spectrometry (WTS-MS) [16,17] have opened new insights in the field of mass spectrometric analysis. In our previous study, we reported on a nib-assisted paper spray-mass spectrometry technique, in which designer drugs in saliva were rapidly screened and determined [18]. In this study, we report on the development of a novel and highly sensitive electrospray ionization method that uses a bamboo pen nib. By removing the paper spray and using the nib directly as the sample emitter, the sample leaves the sprayer in a short burst rather than in a more prolonged spay. Details of the procedures for preparing a bamboo pen nib, on mm scale, used for mass spectrometric analysis are also reported. 4-Chloro-amphetamine, an emerging drug of abuse, was selected as model compound, since it was used in our previous studies. The results obtained by nib-spray mass spectrometry (Nibs-MS), WTS-MS and PS-MS were compared and findings discussed.

2. Experimental

2.1. Materials

4-Chloro-amphetamine was generously donated by the Military Police Command, Forensic Science Center, Taiwan. The procedures for its synthesis have been described previously by Ann and Alexander Shulgin in their book entitled TiHKAL (Phenethylamines I Have Known And Loved) and are abbreviated herein. Chromatography paper was obtained from Advantec (Japan); bamboo tips (toothpicks) were purchased from a local supermarket. All the other chemicals were of analytical grade and were obtained from commercial sources.

2.2. Apparatus

A mass spectrometer (Finnigan LCQ Classic LC/MS/MS), which was identical to that used in our previous studies [18], was used in the nib-spray, wooden tip-spray and paper-spray mass spectrometry experiments, respectively. The mass signal was recorded under the full scan mode (m/z, 100–200), in which the data recording speed was ~0.63 dot/s. An Xcalibur data system was used for data

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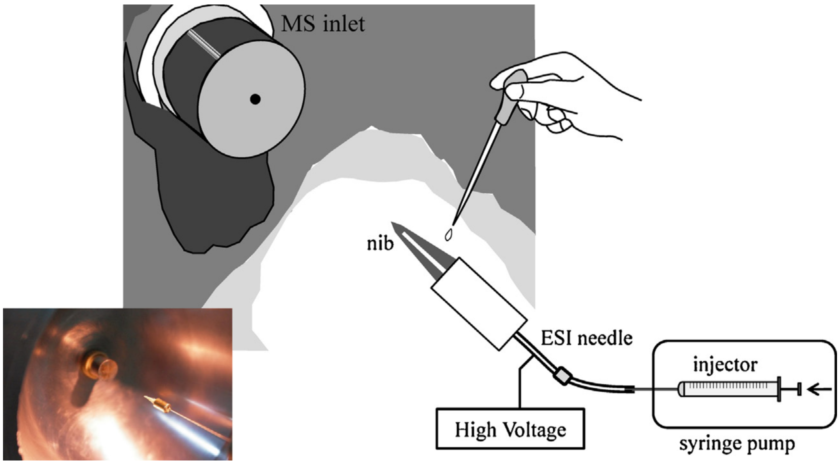

Fig. 1. Schematic diagram of the nib spray-mass spectrometry (Nibs-MS) method used in this study.

collecting, and the data were converted to an ASCII text file. The capillary temperature and spray voltage were set at 200 °C and 3.0 kV, respectively. The tube lens offset and capillary voltage were set at −36 V and 36 V, respectively.

3. Results and discussion

Fig. 1 shows a schematic diagram of the nib spray-mass spectrometry (Nibs-MS) setup used in this study. The sample solution (typically 1–2 μL) can be loaded onto the nib, either by normal pipetting, or by simply dipping the nib into a solution. Because of the effect of capillary attraction, part of the sample solution diffuses into the seam of the nib. The seam is created by holding the two halves of the cut tooth pick close together by a brass ring. It functions as a micro reservoir and prevents the sample solution from spreading on the surface of the paper or bamboo tip. A syringe pump and an injector are used to supply ESI buffer and for wetting nib for use. Methanol was used for this purpose and it was possible to continuously elute the nib at a rate of 6 μL/min. The applied voltage used was +3 kV and the optimized distance from the nib to the MS inlet was ∼17 mm (as described below). Since bamboo is a natural material, it should be ultrasonically cleaned with methanol for 30 min prior to use. In Fig. 2, steps (a)–(c) show the details of the procedures for preparing a bamboo pen nib. In the beginning, as shown in step (a), the end of the bamboo toothpick (sharp side) is split into a Y-shaped tip using a regular cutter. Due to the nature of bamboo, a cross section contains vascular bundles, which allows half to be easily cut and removed from the middle. Following this, a section of a brass hollow tube (length 10 mm; outside/inside diameter 2/1 mm) was used as the nib holder, as shown in step (b). The portion of the toothpick containing the notch was threaded through the hollow tube and the remaining portion should be cut off (as shown in the gray line). The other side of the notched toothpick was shaped into the form of a nib. Although the nib is not exactly a "pen shape", the shaping needs to be done carefully, using a razor blade. The optimized length of the nib outside the brass holder was 5 mm. Finally, in order to insert an ESI-needle, a 0.6 mm drill was used to produce a hole 5 mm in depth, as shown in step (c). A hole is drilled into the bamboo nib and the ESI-needle rests on the bamboo. For comparison of the shape of the nib, the photos in Fig. 3 show a successful nib (photo A) and a failed nib (photo B), respectively, scale of 500 μm. As seen in the insets, the small photos show the phenomenon of Taylor cones, which are the characteristics of ESI. In Frame A (below photo), a clear seam with a width of about 7.5 μm can also be observed. In the remaining portion, the tip of the nib, is still connected but it is difficult to accurately measure its real scale.

In a previous study, we reported that the sharpness of the portion of the tip of a triangular paper has a substantial effect on ionization efficiency; S/N ratios are improved dramatically when the degree is sharper than 30° [18,19]. The angle of the tip of the nib is sharper than 12° and the bamboo nib is porous, factors that are very helpful for the ESI process. Once a high voltage was applied to the nib, the sample solution is suddenly ejected and ionized, leading to the ESI processes. The presence of a "micro reservoir" is a very important issue in terms of improving sensitivity. This is different from the case of a wooden tip-spray or a chromatography paper-spray, in which the sample solution escapes from the wood pores or slowly migrates through the paper fibers. We conclude that this is the major reason for the dramatic improvement in the limit of detection, when this method is used. Frame B (in Fig. 3) shows a failed case; the tip is notched. Two Taylor cones can be observed, indicating that the ESI processes are still underway. Despite the tip not being perfect, ESI can still be generated, once this occurs, it would be difficult to decide which cone to utilize for the measurement. In this case, detecting either cone, the sensitivity would drop to 1/3–1/4. Fig. 4 shows the relationship between the total ion intensity for a paper-spray (total ion current; m/z: 100–300) and the period

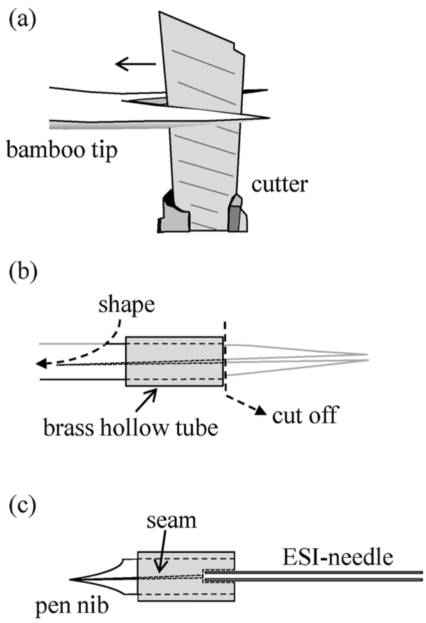

Fig. 2. Steps (a)–(c) show detailed procedures used for preparing a bamboo pen nib.

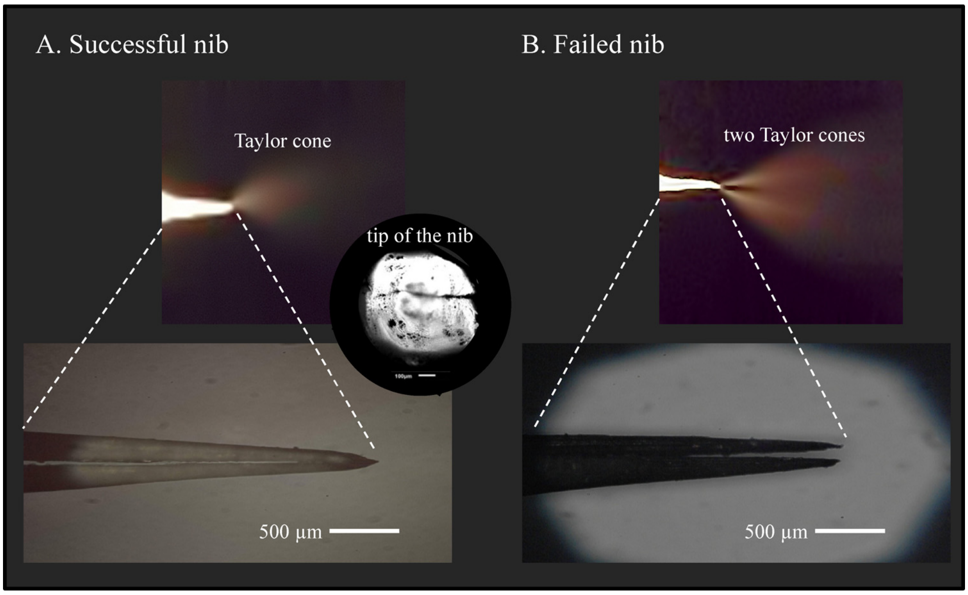

Fig. 3. Photos showing a successful nib (photo A) and a failed nib (photo B), respectively, scale in 500 µm. The insets, small photos, show the Taylor cones and a SEM image of the tip of the nib.

of ion production (time) using the nib-spray, wooden tip-spray and paper-spray, respectively. Herein, 4-chloro-amphetamine was selected as a model sample at a 0.5 µg/mL concentration level. As can be seen, the use of the nib-spray provides the most intense peak (black line). When a 1 µL sample solution was loaded onto the nib by normal pipetting, a major peak appears at ~1 s, while a high voltage was applied. Under optimized conditions, the LODs can be dramatically improved to 0.01 µg/mL. It should be noted that the width at half maximum of the peak is ~3.1 s. Compared to this peak area and the area of the remaining portion (5 s–2 min), we found that 1/3 of the sample solution was ionized and continuously ejected from the "micro reservoir" within the initial ~3 s of the procedure; 2/3 of that was released slowly for the other within ~2 m, as shown in the decay curve. This is because bamboo is hydrophilic and porous, which allows effective adhesion of the sample solution, thus resulting in the production of durable ion signals. A similar phenomenon was observed when a wooden tip was used (blue line). In this case, however, no sharp peak was observed. The initial ion signal appears at ~5 s and remains for a further ~120 s. In contrast to this, when a chromatography paper was used, (red line), the ion intensity was weak and decreased very slowly. The inset in Fig. 4 shows the relationship between the ion intensity (Y-axis) and the distance (X-axis) from the nib-tip to the MS inlet. It is clear that, in the case of Nibs-MS, a specific position exists that provides maximum ion intensity. The optimized distance was ~17 mm and the degree between the nib-tip and

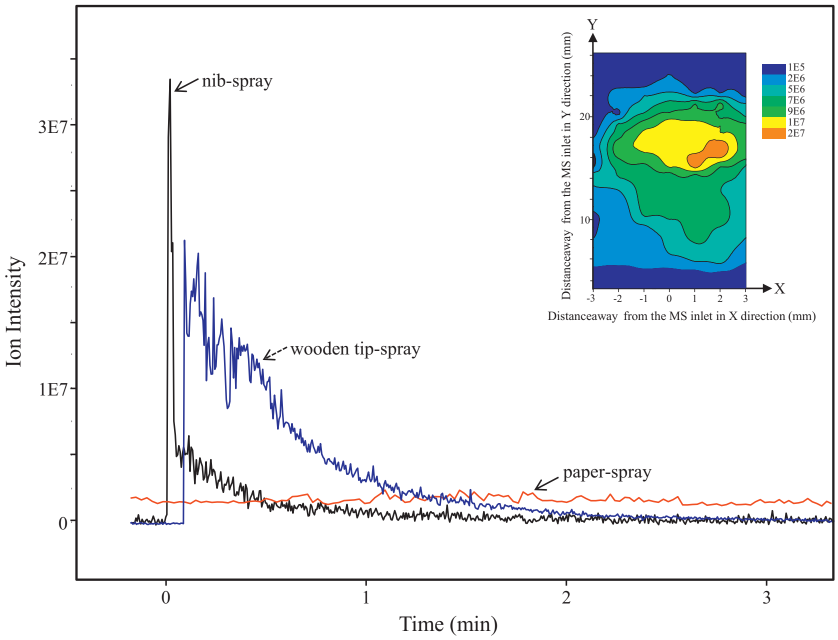

Fig. 4. The relationship between the nib-spray ion intensity (total ion current; m/z: 100–300) and the period of ion occurrence (time) when nib-spray (solid line), wooden tip-spray (broken line) and paper-spray are used (dotted line) applied, respectively; the model sample, 4-chloro-amphetamine (0.5 µg/mL). The inset shows the relationship between the ion intensity (Y-axis) and the distance (X-axis) from the nib-tip to the MS inlet.

Table 1
Comparison of limit of detection (μg/mL) values for a 4-chloroamphetamine standard solution and saliva extracts by Nibs-MS, WTS-MS, PS-MS, NAPS-MS, AP-MALDI-MS and ELDI-MS methods, respectively.

Methods	Sample (4-chlror-amphetamine)
Nibs-MS	0.01
WTS-MS	0.05
PS-MS	0.1
NAPS-MS	0.1
AP-MALDI-MS	8
ELDI-MS	3

Nibs-MS, nib-spray mass spectrometry; WTS-MS, wooden tip spray mass spectrometry; PS-MS, paper spray mass spectrometry; NAPS-MS, nib assisted paper spray mass spectrometry; AP-MALDI-MS, atmospheric pressure-matrix assisted laser desorption ionization-mass spectrometry; ELDI-MS, electrospray-assisted laser desorption ionization mass spectrometry.

the MS inlet was determined to be ~10°. In a previous study, we reported on the LODs for amphetamines [18], based on different methods, including AP-MALDI-MS (atmospheric pressure-matrix assisted laser desorption ionization-mass spectrometry) [20–25], ELDI-MS (electrospray-assisted laser desorption ionization mass spectrometry) [26–31], and NAPS-MS (nib assisted paper spray-mass spectrometry). For comparison, the LODs (at $S/N = 3$) for the 4-chloro-amphetamines obtained by these various method are summarized in Table 1. Hence, it can be concluded that nib-spray mass spectrometry (Nibs-MS) is, under most circumstances, the most favorable method for rapid "drug-screening" under ambient conditions.

4. Conclusions

The development of a novel ESI technique based on a bamboo pen nib, nib-spray mass spectrometry, is described. This method, which is simple and economical, makes use of disposable bamboo pen nibs for the loading and ionization of samples. It is suitable for use in the rapid screening of drugs, since it has a high degree of sensitivity, the operating procedure is simple and an ion signal can be observed immediately. We believe this method has the potential for use in practical analyses and can also be regarded as a helpful tool for use, not only in forensic and clinical analysis, but also biomolecules.

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