Ultrasonic-Nebulizer-Assisted Atmospheric-Pressure Chemical Ionization Mass Spectrometry

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ASMS 2019

Introduction

Antibiotics are widely used in dairy livestock management for the treatment of disease and as dietary supplements. The use of antibiotics may result in drug residues being present in food products. Monitoring and detecting the antibiotics are of routine analysis.

We have previously demonstrated surface acoustic wave nebulization (SAWN) with APCI as novel direct mass-spectrometry method.[1] The SAWN-APCI significant improved ion signals by up to 4 orders of magnitude obviating the need for modifications of mass spectrometers. This approach generated comparable ion signal to conventional ESI. Meanwhile, multiply charged ions of micro-molecules also enabled direct determinations of bio-analytes.

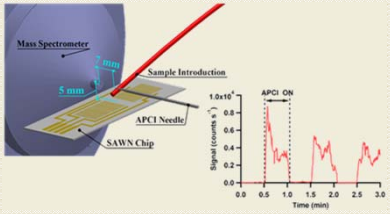


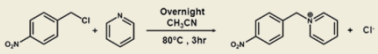
Figure 1. Schematic of SAWN-APCI coupling.

Unfortunately, the complexity of SAWN and its high cost ultimately limits its potential adoption in various applications. In the present work, an ultrasonic piezo that is originally designed as a room humidifier, termed ultrasonic nebulizer, is coupled with an APCI source. In order to obtain quantitative understanding of the SAWN and the ultrasonic nebulizer, a quantity termed survival yield (SY)[2] is used to gauge the "softness". Finally, raw milk is used as-is or with minimal pretreatment, to demonstrate the potential implementation of an ultrasonic nebulizer for rapid mass-spectrometric analyses.

Figure 2. Home-built ultrasonic nebulizer ionization source .

Method

- **Chemicals and sample preparation.** Optima LC/MS grade solvents, including methanol, water, acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ). Sodium ampicillin was obtained from GoldBio (St. Louis, MO). Ciprofloxacin was purchased from Fisher Scientific (Fair Lawn,NJ). The milk sample, sourced from a local super market, was spiked to 1000 μg/mL antibiotic drugs and used as stock solution. Subsequent dilutions were used directly or with minimal sample preparation.
- **Synthesis of benzylpyridium salts.** The 4-nitro-benzylpyridinium (BzPy) salts were synthesized following the reaction depicted in Scheme 1. All reagents and solvents used were obtained from Sigma-Aldrich Co (St. Louis, MO, USA). Briefly, 4-nitrobenzylchloride derivatives were refluxed in excess of pyridine (1.2 molar equivalents) in dry acetonitrile and stirred using a magnetic bar. Reactions were performed at room temperature (~25°C) overnight. After the reaction, diethyl ether was added to precipitate the BzPy salts. Solids of BzPy were collected by vacuum rotary evaporation at 35°C. The BzPy was further confirmed with ^{1}H nuclear magnetic resonance spectroscopy (NMR). The test solution of 4-nitro-BzPy was prepared by dissolving in a water methanol mixture (1:1 v:v) at 4 mg/L.



Scheme 1. Synthesis of a substituted benzypiridinium salt

- **Ionization Source.** The SAWN power supply (SAWN controller V2.0) and SAWN standing wave chips V2.0 were purchased from Deurion LLC (Seattle, WA). The ultrasonic piezo transducer was obtained by dissembling a commercial humidifier. A continuous-mode driver was built in-house. Liquid sample was introduced onto the surface of the SAWN chip continuously via LTQ syringe pump at a flow rate of 8 uL/min through a peek tubing.

  For ionization, an APCI needle from Thermo Fisher (Fair Lawn, NJ) was used to establish and sustain a corona discharge. The voltage and current of the needle was controlled and monitored with a high-voltage power supply (PS350, Stanford Research System, Sunnyvale, CA) at +3 kV and 3 μA, respectively. To obtain a stable corona discharge, a 6 kΩ current limiting resistor was connected between the power supply and the needle. Moreover, to mitigate potential arcing stimulated by periods of high droplet density, a toroid inductor of ~60 μH was connected in series with the resistor and needle.
- **Mass spectrometer**. All mass spectra were recorded in positive ion mode. In this study, we used a linear ion trap Orbitrap (LTQ orbitrap XL, Thermo Scietific, Germany). The temperature of the ion-transfer tube was maintained at 200°C unless otherwise specified. The capillary voltage was maintained at 100 V. The ion-injection time and acquisition parameters were set at 200 ms and 3 microscans, respectively. The mass ranges on the linear ion trap were set to *m/z* 75-400 for thermometer ions and *m/z* 250-400 for ampicillin and ciproflocaxin. The mass range on the Orbitrap was set to 200-750.

Result

Direct Source Comparison

- The dissociation of 4-nitro-BzPy was used to gauge the "softness" of an ionization method.
- During mass-spectrometric analysis, the ion signal ratio of the parent ion and fragments can be used as a benchmark to access energy deposition. Quantitatively, the softness (or hardness) of the ionization process is accessed via survival yield (SY). The SY is calculated by:

$$SY = \frac{S_{parent}}{S_{parent} + \sum S_{fragment}} \times 100\%$$

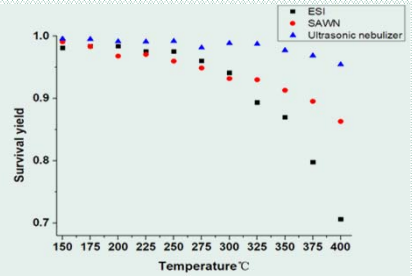


Figure 3. SY as a function of temperature for 4-nitro-BzPy with various ionization methods at the same sample feeding rate.

- Experimentally, the SY of the ultrasonic nebulizer was found to be more resistive to temperature changes on the inlet capillary.

Direct Milk Analysis

- To show the potential application and performance, two different antibiotics drugs (ampicillin and ciprofloxacin) were tested separately and compared with SAWN-APCI.

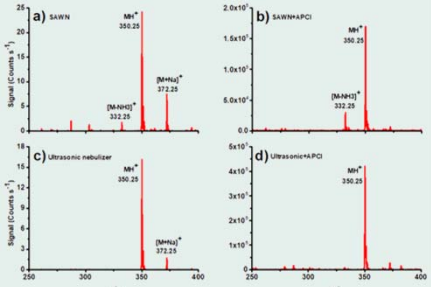


Figure 4. Mass spectra of 10ppm ampicillin with LTQ (a) SAWN (b) SAWN-APCI (c) Ultrasonic nebulizer (d) Ultrasonic nebulizer-APCI

Result

- To access the rapid detection performance of the ultrasonic nebulizer-APCI combination with complex samples, samples of unfiltered raw milk were spiked with two antibiotic drugs. The total sample volume in these measurements, consumed over a time interval of 5 s, was ~ 3 μL per trial.

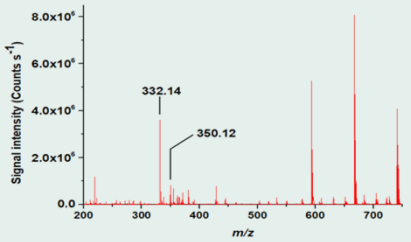


Figure 4. Mass spectrum of milk spiked with 100 ppm drugs with LTQ Orbitrap

- Both protonated ampicillin and ciprofloxacin were observed.
- Limits of detection (LoD) of 4.0 ppb for ciprofloxacin and 5.6 ppb for ampicillin were obtained.

Conclusion

- Ion signal could be improved by coupling with APCI.
- SAWN and ultrasonic nebulizer could have similar performance as ionization source.
- Ultrasonic nebulizer-APCI is an inexpensive and efficient ionization method for rapid detection.

Future Work

- The relationship between internal energy and the particle size distributions of the droplets need to be explored.
- Diagnostic applications towards dry blood spot analysis will be explored.

Acknowledgement

We thank Dr. Erik Nilsson for guidance in the use of the Deurion SAWN device.

References

(1) Song, L.; You, Y.; Evans-Nguyen, T. *Anal. Chem*. **2019**, 91(1),912-918

(2) Huang,Y.; Yoon, S.H.; Heron, S.R.; Masselon, C.D.; Edgar, J.S.; Tureček, F.; Goodlett, D. R. *J. Am. Soc. Mass Spectrom*. **2012**, *23* (6), 1062–1070.