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Analytical Methods

Dipping probe electrospray ionization/mass spectrometry for direct on-site and low-invasive food analysis

Dilshadbek T. Usmanov[a,b], Mridul K. Mandal[a], Kenzo Hiraoka[a,*], Satoshi Ninomiya[c], Hiroshi Wada[d], Masaya Matsumura[e], Sachiyo Sanada-Morimura[e], Hiroshi Nonami[f], Shinichi Yamabe[g,*]

[a] Clean Energy Research Center, University of Yamanashi, 4-3-11, Takeda, Kofu 400-8511, Japan
[b] Institute of Ion-Plasma and Laser Technologies, Durmon Yoli Street 33, 100125 Tashkent, Uzbekistan
[c] Graduate School, Department of Interdisciplinary Research, University of Yamanashi, 4-3-11, Takeda, Kofu 400-8511, Japan
[d] Kyushu Okinawa Agricultural Research Center, National Agriculture and Food Research Organization, 496 Izumi, Chikugo, Fukuoka 833-0041, Japan
[e] Kyushu Okinawa Agricultural Research Center, National Agriculture and Food Research Organization, Suya 2421, Goshi, Kumamoto 861-1192, Japan
[f] Plant Biophysics/Biochemistry Research Laboratory, Faculty of Agriculture, Ehime University, Matsuyama 790-8566, Japan
[g] Department of Chemistry, Nara University of Education, Takabatake-cho, Nara 630-8528, Japan

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ABSTRACT

Rapid, direct, on-site and noninvasive food analysis is strongly needed for quality control of food. To satisfy this demand, the technique of dipping probe electrospray ionization/mass spectrometry (dPESI/MS) was developed. The sample surface was pricked with a fine acupuncture needle and a sample of ~200 pL was captured at the needle tip. After drying the sample, the needle tip was dipped into the solvent for ~50 ms and was moved upward. A high-voltage was applied to the needle to generate electrospray when the needle reached the highest position, and mass spectra were measured with a time-of-flight mass spectrometer. For evaluation of the method, the technique was used to analyze foods such as vegetables, salmon flesh, cow's milk, yogurt, and soy-bean milk. The detected major ions for cow's milk and yogurt were $[(Lac)_n + Ca]^{2+}$ with n = 1–6 (where (Lac) is lactose), indicating that Ca^{2+} is tightly bound by Lac molecules.

1. Introduction

Electrospray ionization/mass spectrometry (ESI-MS) has become an indispensable tool for analysis in a variety of fields (Cole, 2010; Domin & Cody, 2015). To cope with the small amounts of analytes, miniaturized ESI ion sources using a narrow-bore capillary, such as nanoESI, have been developed (Emmett & Caprioli, 1994; Wahl, Goodlett, Udseth, & Smith, 1992; Wilm & Mann, 1994, 1996). In capillary-based electrospraying, direct analysis of samples with a high salt concentration is often problematic because the capillary tip can be easily clogged by the deposition of solid residues.

To avoid the clogging problem, several designs of electrospray ion sources that use noncapillary probes have been put forward. Hong et al. (1999) succeeded in electrospraying a sample solution deposited on a copper wire ring. The technique was further explored using optical fibers bundled with copper or platinum wires (Kuo, Yuan, & Shiea, 2000), a glass rod (Jeng & Shiea, 2003), and nanostructured tungsten oxide (Jeng, Lin, & Shiea, 2005). Electrospray from the solution deposited on micropillar chips has also been reported (Nissilä et al., 2007). In 2010, Ouyang and coworkers developed a paper spray for direct analysis of complex mixtures (Liu et al., 2010; Wang, Liu, Cooks, & Ouyang, 2010). Ions of analytes were generated by applying a high voltage to the paper with a small volume (≤10 μL) of solvent. Further, they developed a direct tissue-based spray using needle biopsy (Liu, Cooks, & Ouyang, 2011) and a plant leaf (Liu, Wang, Cooks, & Ouyang, 2011). Hu and coworkers (Hu, So, Chen, & Yao, 2011) developed a

* Corresponding authors at: Clean Energy Research Center, University of Yamanashi, 4-3-11, Takeda, Kofu, Yamanashi 400-8511, Japan (K. Hiraoka).
E-mail addresses: hiraoka@yamanashi.ac.jp (K. Hiraoka), yamabesh@nara-edu.ac.jp (S. Yamabe).

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solid-base electrospray ionization using wooden tips. The design of this work is similar to that of paper spray, but a longer spray time than for the paper spray can be attained because of the slower vaporization of the solvent from the substrate.

In 2007, probe electrospray ionization (PESI) using a sharp solid needle as an electrospray emitter was developed in our laboratory (Hiraoka, Nishidate, Mori, Asakawa, & Suzuki, 2007). In PESI, discontinuous sampling and single-shot electrospray cause a sequential and exhaustive electrospray (Mandal, Chen, & Hiraoka, 2011; Usmanov et al., 2015). PESI is robust, largely free from the suppression effect (Mandal et al., 2011) and is readily applicable to liquid and wet samples with little or no sample preparation (Chen et al., 2008a; Chen, Yu, Nonami, Hashimoto, & Hiraoka, 2009; Chen, Yoshimura, et al., 2009).

Because the electrospray is based on electrochemical reactions taking place at the interface between the metal electrode and the liquid, PESI is not directly applicable to dry samples. To circumvent this disadvantage, several methods have been proposed for the liquid solvent to wet the dry samples captured on the solid probe. Chen, Yoshimura, et al. (2009) made the acupuncture needle wet by condensing solvent vapor. The mapping of phosphatidylcholines and galactosylceramides was made with a spatial resolution of about 60 μm. Further, PESI was applied to single-cell analysis by several groups. Yu et al. (2014) inserted a surface-activated sharp stainless-steel probe into a plant cell of a rhododendron petal, soybean sprout and geranium leaf. After drying the sample, metabolites loaded on the tip surface were extracted using the auxiliary solvent generated by a piezoelectric microdroplet generator and were electrosprayed under a high electric field. Gong et al. (2014) detected metabolites at cellular and subcellular levels by PESI-MS. A tungsten probe with ~1 μm tip diameter was inserted into a live *A. cepa* (onion) cell. By condensing the solvent vapor on the needle tip, metabolites were directly desorbed/ionized from the tip of the probe. Recently, Chen et al. (2016) developed a method for single-cell analysis and lipid profiling by combining drop-on-demand inkjet cell printing and PESI-MS. Through the inkjet sampling of a cell suspension, droplets including single cells with a volume of ~500 pL were generated. They were precisely dripped onto a tungsten-made electrospray ionization needle and immediately sprayed under a high electric field.

The use of vapor condensation (Chen, Yu, et al., 2009; Chen, Yoshimura, et al., 2009) or liquid microdroplet generation (Yu et al., 2014) for supplying solvent to the needle requires sophisticated skills for reproducible results. In the current work, a much simpler method for supplying solvent to the needle, dipping PESI (dPESI), will be presented. Sampling was performed by pushing an acupuncture needle into the sample to a depth of ~0.5 mm. After drying the sample, the needle was positioned in front of the inlet of the mass spectrometer (MS). The needle was moved down and wetted by just dipping it for ~50 ms into the pure liquid solvent, and then lifted. At the highest position, the liquid sample was electrosprayed by applying a high voltage (HV) to the metal needle. This operation is exactly the same as in conventional PESI, except that the sample is *preloaded* at the needle tip and that the needle is dipped into *pure* solvent. Because the sampling by a needle and the MS measurement are performed independently, dPESI is applicable to any bulky samples for a low-invasive direct analysis of fruits, vegetables, meats and dairy foods.

2. Materials and methods

2.1. dPESI measurements

The mass spectrometer (MS) measurements were performed using orthogonal-acceleration time-of-flight mass spectrometry (oa-TOFMS) (JEOL, Akishima, Tokyo, Japan). The temperature of the ion sampling orifice was kept at 100 °C. The ions generated by ambient electrospray were sampled through a 0.4-mm-diameter ion sampling orifice into the vacuum and were collimated using a lens voltage of 17 V, an orifice(1) voltage of 50 V and an orifice(2) voltage of 5 V. The mass spectra were acquired using an ADC/continuous averager ion detection system. Mass Center V1.1.5 software (JEOL) was used for the data processing and the signal integration.

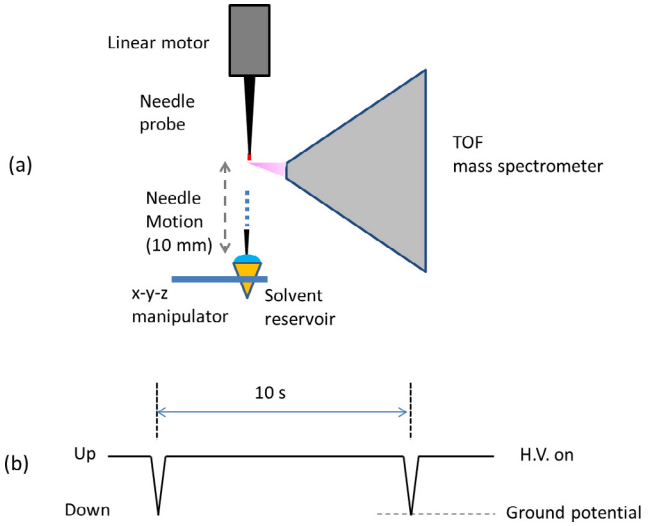

Fig. 1. (a) Schematic diagram of the dPESI system. The needle tip bearing sample was dipped in the solvent to a depth of ≤1 mm. (b) The timing of the probe operation and application of a high voltage to the needle is synchronized. The high voltage is applied to the needle at its highest position.

The default desolvation chamber was removed and the dipping PESI (dPESI) system was installed (see Fig. 1(a)). Sampling was performed using an acupuncture needle (J type No. 02, body diameter 0.12 mm, tip diameter 700 nm, Seirin, Shizuoka, Japan) by manually pushing the needle tip into the sample to a depth of ≤0.5 mm. The sample amount captured at the tip was estimated to be ~200 pL for nonviscous wet samples such as fruits and vegetables (Yoshimura, Chen, Asakawa, & Hiraoka, 2009). For viscous samples such as salmon flesh, a shallower invasion depth (< 0.5 mm) is recommended to avoid unnecessary contamination of the ion source. After drying the captured sample, the needle was positioned vertically in front of the inlet of the mass spectrometer. The optimum position of the needle tip was 3 mm to the side and 2 mm above the apex of the ion-sampling cone of the mass spectrometer. The needle was driven down along the vertical axis by a linear motor-actuated system and was dipped into the pure methanol/water (1/1) solvent in the reservoir to a depth of ~1 mm for 50 ms (Usmanov, Ninomiya, & Hiraoka, 2013). The level of the solvent meniscus in the reservoir was adjusted manually by using an *x-y-z* manipulator. After dipping the needle tip into the solvent, it was lifted up at the highest position to generate electrospray by applying a high voltage of +2.5 to +3 kV to the needle. The solvent volume of ~600 pL (Yoshimura et al., 2009) that was attached to the needle tip was totally depleted by the electrospray in a period of less than 1 s (Chen et al., 2008b). The timing of the application of the high voltage and the needle motion was synchronized using a homemade control system (Fig. 1(b)). The needle was operated with a frequency of 0.1 Hz.

2.2. Chemicals and samples

Water was purified and deionized using Simplicity UV (Millipore, Bedford, MA, USA). High-performance liquid chromatography grade methanol was purchased from Kanto Chemicals (Tokyo, Japan). Tomato, spinach, onion, salmon flesh, cow's milk (Meiji Co. Japan, sodium chloride (salt): 220 mg, proteins: 6.8 g, carbohydrates: 9.9 g, calcium: 227 mg, lipids: 7.8 g in 200 mL), yogurt (fermented milk, Meiji Probio, 1073R-1, sodium: 48 mg, proteins: 3.6 g, calcium: 129 mg, lipids: 0.67 g, sugars: 13.3 g, carbohydrates: 13.9 g in 112 mL), and soybean milk (Kikkoman Co. Japan, proteins: 7.7 g, lipids: 7.7 g,