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Comparisons of ambient spray ionization imaging methods

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ABSTRACT

Ambient ionization methods allow for the examination of surfaces in their native conditions at atmospheric pressure with minimal or no preparation. Spray-based ambient ionization methods such as desorption electrospray ionization (DESI) and easy ambient sonic-spray ionization (EASI) have been successfully applied to imaging mass spectrometry. In 2012, a comparative study between DESI and EASI on spatial resolution and sensitivity was published by Janfelt and Nørgaard (J. Am. Soc. Mass Spectrom., 23 (2012) 1670–1678). We expand on that work by comparing DESI and EASI techniques for the assessment of the limit of detection (LOD) of several drugs on a PTFE surface and for the determination of the spray spot size varying flow rate and solvent composition for imaging purposes. MS/MS imaging was also done with both methods for performance comparison. The results showed that good ion images can be obtained by both techniques in the MS or MS/MS mode. No significant difference was observed in the spray spot size produced by DESI and EASI. DESI was found to have similar or higher sensitivity than EASI depending on the analyte interrogated.

1. Introduction

Imaging mass spectrometry (IMS) has established itself as an efficient tool that measures the analytes of interest and their spatial distribution by monitoring their mass to charge ratio (m/z) and spatial position [1]. IMS has been accepted worldwide as an effective system to detect and identify a broad range of molecules, due to high sensitivity, high speed of analysis and high chemical specificity [2,3]. Most mass spectrometry techniques require the introduction of the sample into vacuum; ambient ionization-based IMS methods are drawing popularity due to external ionization of the sample, at atmospheric pressure, outside of the vacuum system [4]. Ambient ionization methods allow introduction of ions, but not the entire sample, into mass spectrometer; in addition, ambient ionization methods require minimal or no sample pretreatment, facilitating rapid analysis of samples [5]. Among several developed ambient ionization methods, spray based techniques such as desorption electrospray ionization (DESI) and easy ambient sonic-spray ionization (EASI) have a wide range of applications. DESI has been successfully implemented in the field of forensics [6–8], imaging [9], metabolomics [10], pharmaceuticals [10], and polymers [11].

DESI adopts a soft ambient ionization technique leading to minimal fragmentation. Gas phase ions are produced from the condensed phase analytes by charged microdroplets. These gas phase ions are generated via a 'droplet pick up' mechanism where initial micron sized droplets wet the surface to be analyzed. Further collisions at the surface produce progeny droplets containing the analytes. Finally, gaseous ions are produced from charged progeny droplets which then undergo desolvation and proceed to the MS inlet [12]. Lateral spatial resolution, which is the capability to clearly distinguish between two adjacent spots on the surface, is typically 200 μm. However, the spatial resolution can be reduced to ~40 μm under specific conditions [13,14]. Typical limits of detection (LOD) have been reported in the range of picograms (pg) to femtograms (fg) making DESI-MS a sensitive method, useful for trace amount detection [15,16].

In 2006, Eberlin and co-workers introduced desorption sonic spray ionization (DeSSI) [17], later renamed in 2008 as EASI (easy ambient sonic spray ionization) [18]. EASI adopts a soft ionization method based on sonic spray that does not require high voltage or heating to produce gaseous ions at atmospheric pressure [19]. The mechanism of ionization involves production of gaseous ions due to unbalanced charge distribution in the resulting solvent daughter droplets induced from higher gas flow rates (>3.0 L/min). At sonic spray gas flow, droplets with less than 100 nm undergo fission and the resulting daughter droplets are charged [19]. These high gas flow rates are generated from the nebulizing gas pressure,

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generally 2–5 times higher in EASI (around 435 psi or 30 bar nebulizing gas backpressure) than in DESI standard conditions (around 120 psi or 8.2 bar) [17]. The intensity of the ions produced in a 'supersonic spray' strongly depends on gas velocity [19]. EASI has been successfully applied to quality control and forensics [7,17,20,21]. It also has been also applied to check for the purity of biodiesel [22,23].

For IMS purposes one has to take into account not only the ability to ionize a sample, but also the impact of the technique on the sample interrogated. EASI at standard conditions is not fully compatible with IMS because it requires high gas flow rates (>3.0 L/min) and high solvent flow rates (>20 μL/min) in order to promote the ionization. These conditions cannot be applied to all kinds of samples, especially biological tissues, because they damage the sample before it can be entirely mapped. Experiments can be done under specific conditions using low gas flow rates (<3.0 L/min) and low flow rates of volatile solvents (<10 μL/min). However, these conditions eliminate the "sonic spray" effect responsible for the high ionization efficiency. Indeed, ionization under these conditions was investigated in 2005 by the Cooks group [24].

In 2012, Janfelt and Nørgaard published a comparative study between DESI and EASI by producing ion images of tissue sections [25]. In this study, a pixel to pixel comparison was performed as it can distinguish between signal and noise. It was concluded that EASI can be as efficient as DESI for imaging and direct analysis of tissue sections as long as a higher solvent flow rate (10 μL/min) is maintained. Improved EASI signals were observed as long as the pressure was kept at 10 bar which is approximately 145 psi. It was found that DESI is more sensitive than EASI toward analytes that are present at low abundance for both rat brain and plant imprints deriving the fact that there must be a difference in dynamic range for both DESI and EASI.

With these previous reports in mind, the experiments reported here were not performed under the standard conditions for either DESI [24] or EASI [17,21], but rather a single set of conditions which allow a comparison of the results (Table 1). Note that the classification of the techniques (DESI and EASI) based on the gas pressure component is not well established. For instance, EASI was reported using as low as 100 psi for nebulizing gas backpressure [20] and DESI was reported using as high as 170 psi gas backpressure [26]. We chose to compare both techniques at 145 psi or ~10 bar, a pressure that can achieve effective ionization in EASI, but still allows imaging experiments without damaging the sample as reported by Janfelt and Nørgaard [25]. However, we found that an even lower flow rate (5 μL/min) could be used to avoid smearing effects. Both techniques were further compared here in terms of the sensitivity (limits of detection), spray spot size and lateral spatial resolution in order to gauge the capabilities in terms of imaging performance. Spray spot size is one of the main components for creating an image with good resolution. Spot analysis was done by measuring spot size on a water sensitive paper under various conditions. Limits of detection, on a porous PTFE surface, of various compounds were recorded to investigate the ionization and transfer efficiency with both methods. MS/MS imaging experiments were performed in order to illustrate IMS applications for forensic analysis. Finally, the coronal sections of rat brain were analyzed to create ion images allowing us to compare both the ionization profiles and the image quality. All these results obtained from new experiments taken together help us further understand the capabilities of DESI and EASI and to assess the viability of these techniques for IMS.

2. Experimental

2.1. Materials and reagents

The solvents (HPLC grade) and the compounds used in the limit of detection experiments: propranolol, testosterone, dobutamine, verapamil, chloramphenicol, ibuprofen, diazepam, roxithromycin, and angiotensin, were obtained from Sigma–Aldrich Canada. Porous PTFE sheets 1.5 mm thick with a medium porous size of 7 μm were purchased from Berghof (Eningen, Germany). Microscope slides 26 mm × 77 mm thickness 1 mm were purchased from Bionuclear diagnostics Inc. (Toronto, ON, Canada). Rat brains were purchased from (Rockland Immunochemicals Inc., Gilbertsville, PA, USA) and the water sensitive paper, paper that changes its color when exposed to water, was obtained from TeeJet Technologies (Harrisburg, Dillsburg, PA). Red pens containing Rhodamine B and Rhodamine 6G, BIC Company, used in MS/MS experiments were purchased from a bookstore at York University.

2.2. Sample preparation

2.2.1. Water sensitive paper

Water sensitive paper was cut to working size and was secured on the moving stage with tape on all sides.

2.2.2. Rat brain

Frozen rat brains were sectioned into 15 μm thick coronal section (12 mm × 15 mm) using a Shandon Cryotome FE (Thermo Fischer Scientific, Nepean, ON, Canada). These tissue sections were thaw mounted onto glass slides, stored at −40 °C and brought to room temperature before analysis.

2.2.3. Limit of detection

Standards of 1 mg/mL were prepared in methanol solvent. The spotting solutions were created from the 1 mg/mL standards using serial dilution to 100, 10, 1, 0.1, and 0.01 ng/μL prepared in a 1:1 ratio of methanol to water solution. The solvent used to spray was also prepared with methanol to water ratio of 1 to 1.

2.2.4. MS/MS imaging

Two different red pens were used attempt forgery on a piece of paper. The paper was secured to the running stage with tape and MS imaging was performed.

Table 1
Standard DESI and EASI conditions versus experimental conditions.

	Standard conditions		Experimental conditions chosen	
	DESI [24]	EASI [17,18]	DESI	EASI
Nebulzing gas back pressure (psi)	50–120	400	140	140
Solvent flow rate (μL/min)	0.5–5	20–25	1.5/5.0[a]	1.5/5.0[a]
Spray voltage (kV)	2–5	0	5	0

[a] Solvent flow rates used in the limits of detection/rat brain experiments.