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Review Article

Ambient Mass Spectrometry for Forensic Analysis

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Abstract

Ambient mass spectrometry has emerged as a significant tool to facilitate forensic investigation considering its well-known advantages of rapid, direct, nondestructive, high-throughput detection of a broad range of raw samples, as well as its potential capability of miniaturization and automation. New mass spectrometry technologies include more versatile ionization sources, allowing the next generation of instrumentation to be more multipurpose and adaptable to the needs of the discipline. This review assesses and highlights forensic applications of ambient mass spectrometry. In addition, the instrumentation and principles of some popular and promising ambient ionization techniques are summarized.

INTRODUCTION

Forensic science has been developed in the twentieth century and came to be recognized by the courts and law enforcement. Forensic analysis and investigation capabilities are vital in criminal and civil cases, national security, environmental protection, and public safety. The manufacture and trafficking of illicit drugs and counterfeiting of legitimate pharmaceuticals, new emerging challenges from organised crime and terrorist groups, also underline the vital need for sensitive and selective rapid identification of substances such as explosives and toxic spills in field, drug mixture. Presently, practical analytical technologies are needed to enable the "in field" screening and analysis of evidence to provide fast, accurate, scientific information to support the forensic investigation[1]. Many analytical techniques become developed for forensic application [2-8] and some are accepted by the court, including the use of infrared spectrum (IR) to test the driver's breath gas so as to assess blood alcohol content, or high performance capillary electrophoresis (HPCE) to detect the content of methamphetamine, morphine, cocaine and heroin in blood, urine and other biological samples, or liquid chromatography mass spectrometry (LC-MS) and gas chromatography mass spectrometry (GC-MS) to qualitative or quantitatively analyze illicit drugs and metabolites in biological matrices (e.g. blood, urine or hair).

Among the numerous modern analytical techniques, mass spectrometry (MS) is a widely used approach in forensic science [1] for its merits in identifying a large range of compounds in trace amounts with high sensitivity and short response time. However, the conventional MS experiments have proven huge inconvenient in terms of size, cost, complexity and time consuming due to laborious sample preparation or/and isolation procedures. These drawbacks make it difficult to analyze forensic evidence directly and effectively, leaving problems in maintaining sample integrity and traceability.

Recently, ambient mass spectrometry (ambient MS), which allows for direct and rapid analysis of raw sample in their native environment with high specificity and minimal or no effort in sample preparation, has aroused blooming interest. With ambient MS, analysis operation has been greatly simplified, and more types of samples could be interrogated. In this review, we will introduce the principles, instrumentation and forensic application of some popular ambient ionization techniques, and finally discuss the future prospects and challenges.

Ambient mass spectrometry

Since the introduction of desorption electrospray ionization (DESI) by Cooks and his co-workers in 2004[9], numerous ambient ionization techniques were developed [10-16]. Till now, several groups have reviewed the principle and applications of ambient mass spectrometry [15,17-21]. According to the traditional technique that plays the central role in the overall ionization process, they can be separated into two classes, *i.e.*, electrospray ionization (ESI)-related techniques and atmospheric pressure chemical ionization (APCI)-related techniques[18]. In comparison with desorption/ionization, processes such as DESI and DAPCI, occur on a two-dimensional (2-D) surface. While during the extractive electrospray ionization (EESI) process, analytics in the matrix are subjected to extractive ionization in a three-dimensional (3-D) space, in which the matrix disperses over a relatively large volumn. Chet *et al.* proposed the theoretical models based on the principles of energy conservation and charge immortalization, as well as energy spread and charge transfer occurring under ambient conditions (Figure 1)[20].

Over the past ten years, ambient MS using direct ionization techniques has been applied in food analysis [22], forensic and public safety monitoring, proteomics, metabolomics [23] and clinical diagnostics, in vivo analysis[24], direct mass spectrometry imaging[25], selectively chemical reaction methods study[26,27] and many other fields[28]. Especially in forensic analysis, ambient MS has been successfully used for detection of explosives at nano-gram levels; chemical composition of counterfeit pharmaceutical tablets, detection of drugs of abuse from biological liquids such as urine and plasma; breath analysis of metabolites; and imaging analysis for document verification and fingerprint identification.

Forensic applications of ambient mass spectrometry

Ambient mass spectrometry allows the in situ, real-time, online, high throughput, and low sample-consumption analysis of trace analytes in complex matrix to be performed with no/minimal sample pretreatment. In following sections, we will briefly introduce some ambient mass spectrometry and their forensic applications. A summary of the major ambient MS techniques emphasized in this review is presented, in chronological order, in Table 1.

Desorption electrospray ionization (DESI) and its forensic applications

DESI [9] is an interesting ambient ionization technique combining both the ESI process and the surface desorption process. In a simplest form, the desorption electro spray experiment (Figure 2) uses an aqueous spray directing at the surface of analyte under ambient conditions. The spray compacts the surface, desorbs the analytes into the gas phase and subsequently ionises them. The desorbed ions are then transported to a commercial ion trap mass spectrometer through an atmospheric pressure interface. The experimental results show that DESI-MS spectra are very similar to those generated by ESI, and thus the ESI spectra can be used as the reference spectra for DESI analysis. DESI has been widely used for direct detection of various compounds. The principles, instrumental setup, and the applications of DESI have been reviewed previously [16-18,29-34]. Several tutorial papers have been reported about the forensic applications of DESI [35-37]. Morelato *et al.* assessed and highlighted the great advantages of DESI in detection of illicit drugs, explosives, chemical warfare agents, inks and documents, fingermarks, gunshots residues and drugs of abuse in urine or plasma specimens [37].

Surface desorption atmospheric pressure chemical ionization (DAPCI) and its forensic applications

DAPCI [38], combining the process of surface desorption and atmospheric pressure chemical ionization (APCI), has been considered as a variant of DESI. Instead of using liquid as solvent spray, gaseous solvent vapor is ionized by corona discharge in the alternative procedure, and the resulting plasma is used to create ions directly from the surface of interest. DAPCI is one type of plasma-based ambient MS techniques[39]. Quite different from spray techniques, plasma-based sources employ various

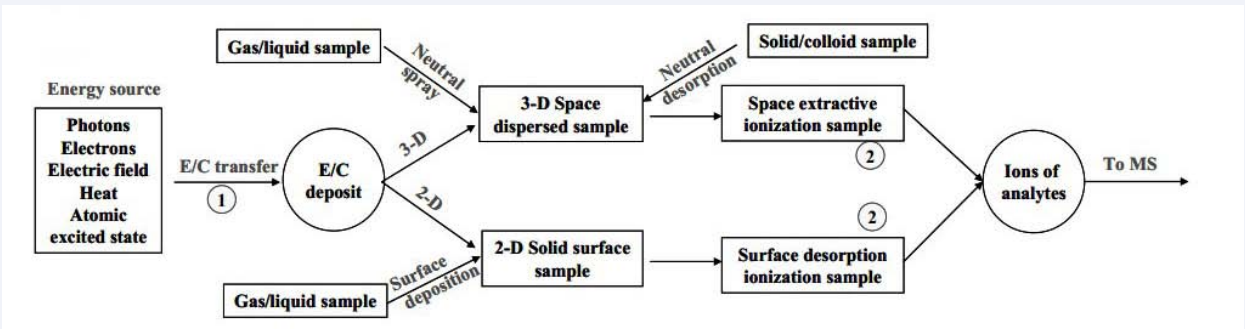

Figure 1 Conceptual illustration of 2-D and 3-D modes [20].

Table 1: Summary of major ambient MS techniques used in forensic application.

Ambient method	Basic technique	Year introduced	Desorption/ionization principles	Forensic application	Ref.
DESI	ESI	2004	Charged spray-jet	toxic industrial compounds, chemical warfare agents, illicit drugs and formulations, explosives, foodstuff, inks, fingerprint, skin	35-37
DAPCI	APCI	2005	Electric plasma species	explosives, chemical warfare, pharmaceutical, signatures	30,41,44
DART	GDI	2005	Metastable-thermal/indirect plasma	explosives	10
EESI	ESI	2006	Ambient gas/ extractive ESI	cocaine, explosives ,aerosol illegal drug, fingerprinting	74-81
DBDI	GDI [a]	2007	Electric plasma species	explosives	40
LTP	PI [b]	2008	Low-temperature plasma	drugs	14,88

[a] GDI, gas discharge ionization; [b] PI, photon ionization.

modes of electro-discharge instead of charged droplets to generate plasma gas that contains radicals, excited/metastable state atoms, and electrons.

As for the forensic applications, plasma-based ambient MS methods (e.g. DAPCI, DART[10], DBDI[40] and LTP[14]) have a clear advantage in trace explosive determination. In situ trace detection of peroxide explosives by DAPCI was first performed by Cooks and coworkers [30], then was used to detect nitro-aromatic explosives by monitoring their ion/molecule reaction products with reagent ions produced from acetonitrile and air [41]. DAPCI has also been used for detecting peroxide-based explosives (HMTD and TATP), where 15 ng of each explosive deposited on paper in a total area of 1 cm^2 was detected with or without ammonium acetate added as dopant to the carrier gas (N_2). With a relatively low proton affinity (PA), the TATP molecule cannot efficiently capture a proton from the ionized methanol/nitrogen plasma during DAPCI. While through doping the DAPCI gas with a reagent gas which can provide reagent ions such as the ammonium ion $NH4^+$, the sensitivity was greatly improved [42], so that DAPCI can directly detect chemical warfare agent

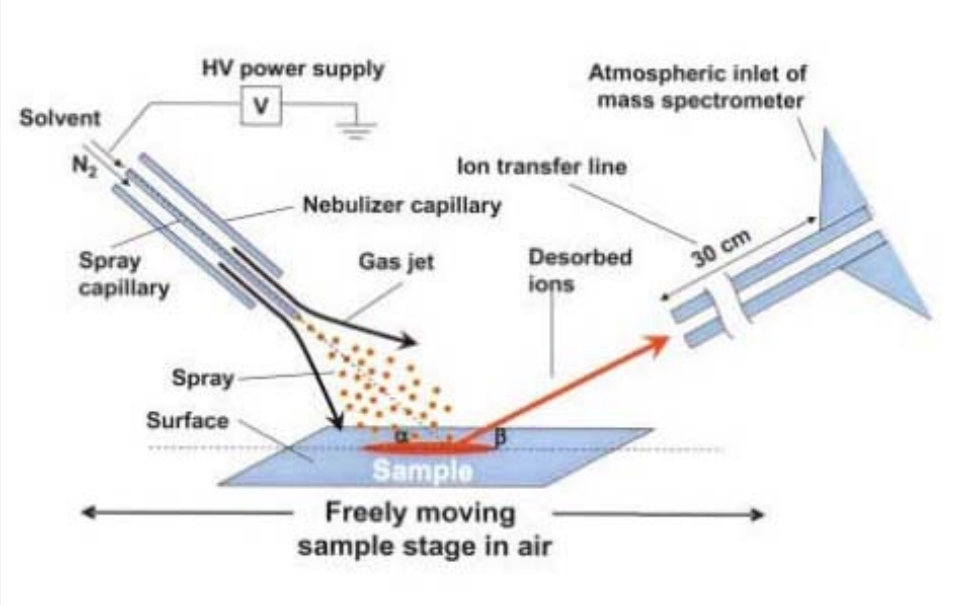

Figure 2 Schematic of typical DESI experiment [9].

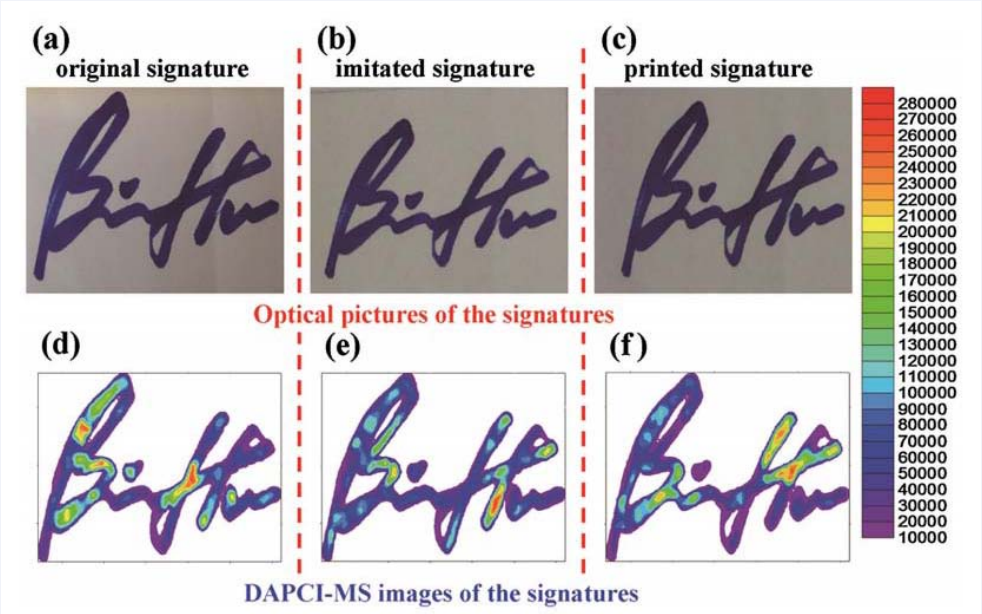

Figure 3 Detection of forged signatures by DAPCI-MS imaging. a, d: original signatures; b, e: imitated writings; c, f: facsimiled writings on a printed copy by using the same pen. (The peak at m/z 136 was monitored for analysis).

stimulant, explosives and human-related samples with a detection limit of sub-pg level. Besides, many pharmaceutical powder compounds are desirable for analysis in gasless DAPCI without toxic chemical contamination [43]. Recently, Li *et al.* for the first time reported the use of DAPCI imaging in document authentication at the molecular level [44]. Among many signals detected, the peak at m/z 136, which is absent from the blank spectrum, is of the highest intensity (4.89 × 106 cps), indicating that this signal might be ascribed to the major component of the blue ink. Thus, the peak at m/z 136 was selected as target ion for handwriting analysis. Non-destructive forensic analysis of forged signatures either handwritten or computer-assisted was achieved according to the difference of the contour in DAPCI images, which was attributed to the strength personalized by different writers (Figure 3). Many experimental results show that DAPCI-MS imaging provides rich information at the molecular level and thus can be used for the reliable document analysis in forensic applications [45].

Extractive electrospray ionization (EESI) and its forensic applications

As schematically shown in Figure 4, extractive electrospray ionization (EESI) [12] has a unique design utilizing two sprays and aligning them along a certain angle with respect to the mass spectrometer. A number of studies have investigated the ionization mechanism during EESI process in detail. Liquid samples, such as raw urine, milk and waste water, can be directly monitored by EESI mass spectrometry without sample contamination by toxic chemicals. However, it was difficult for EESI to analyze solid surfaces until recently Chet *et al* have further developed the EESI ion source to allow analysis of many kinds of samples (solid, cream, gel, liquid, gas or aerosols) with a neutral desorption (ND) sampling gas beam (Figure 5) [46]. The ND-EESI-MS analysis process requires no sample pretreatment because it can tolerate an extremely complex matrix due to the separation of sampling and ionization processes in both space and time, thus allowing real-time, online chemical profiling of highly viscous samples under ambient conditions. Thereby, EESI

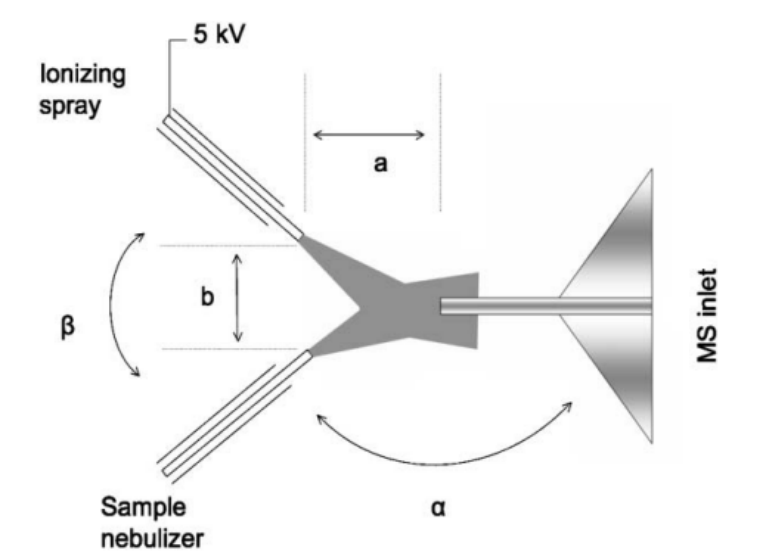

Figure 4 Schematic diagram of a traditional EESI ion source for direct liquid sample analysis[12].

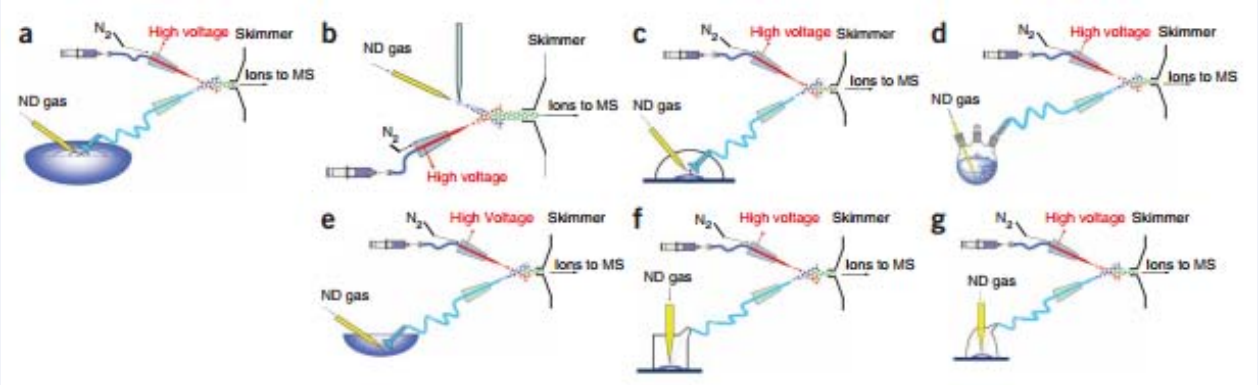

Figure 5 Schematic diagrams of seven types of ND devices.

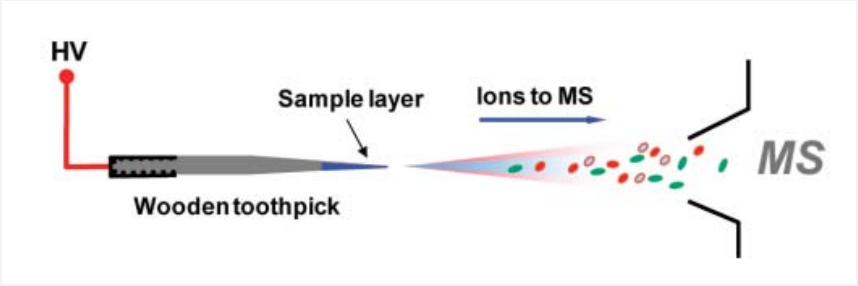

Figure 6 Experimental setup of ESI using a wooden tip [83].

mass spectrometry provides a platform for multiple disciplines including food analysis [47-52], consumer product safety [53-57], environmental investigation [58-61], proteins [62-64], metabolomics [65-70], chemical reaction monitoring [71-73], forensic analysis and public safety [12,74-81].

Without sample pretreatment, a novel air-tight neutral desorption enclosure has been fabricated to noninvasively sample picograms of explosives 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetraazocine (HMX), triacetone triperoxide (TATP), and nitroglycerin (NG) from human skin using a nitrogen carrier gas over a 4m distance. Selective ion/molecule reactions can be easily implemented in the EESI process, resulting in enhanced specificity for the detection of trace amounts of explosives present in complex matrices such as biological surfaces [74]. Nano-EESI, evolving from EESI, developed also by Chen's group, requires nebulizing gas to produce the reagent ion plume and the neutral sample aerosols, allows proper analytical performance using manual sample introduction with no gas assistance cite the ref only. Intrinsically, nanoEESI has advantages, including high sensitivity, good tolerance of matrices, readiness for miniaturization and integration, simple maintenance, easy operation, and low cost. The LOD for cocaine was found to be 7-15 fg for various effervescent drink samples [76].

Other newly emerging ambient MS

In the past two decades, noncapillary emitters have been developed to avoid the clogging problem in conventional capillary based ESI and for more convenient sample loading. Some materials have been successfully developed as emitters for sample loading and ionization [82-87], such as paper spray [82] and disposable wooden tips [83]. The wooden 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 (Figure 6). This new technique is applicable for analysis of various samples, which brings a new vision to ambient MS technique. Moreover, the slim and hard properties of the wooden tip enable sampling from specific location such as corners and small opening, as indicates potential application in forensic investigation.

SUMMARY

Ambient mass spectrometry techniques, including DESI-MS, DAPCI-MS and EESI-MS, have emerged as important tools in different areas of forensic analysis. Features such as in situ, real time, online, high throughput and nondestructive analysis of raw samples, enable ambient MS techniques meet the requirement for short timer, sample integrity and tracebility, having a huge development potention for forensic analysis. The new emerging ambient MS technology is still in the theoretical and laboratory research stage and most evidence analysis are simulation using standard materials (such as explosive, illegal drugs). With the further development of novel ionization technique, it is expected to have universal application of mass spectrometer to the actual field and provide credible evidence for forensic investigation.

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