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Analytica Chimica Acta 442 (2001) 183–190

ANALYTICA CHIMICA ACTA

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Desorption–ionization on silicon mass spectrometry: an application in forensics

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Received 12 January 2001; received in revised form 7 May 2001; accepted 7 May 2001

Abstract

Desorption–ionization on silicon (DIOS) is a new, matrix-free laser desorption mass spectrometry approach that allows for the direct identification of low molecular weight compounds in the presence of potentially interfering compounds. The porous silicon surfaces provide a scaffold for trapping analyte molecules, and are readily adaptable to commercial time-of-flight instruments. As an example of its utility in forensic cases, DIOS mass spectrometry was used to distinguish between similar synthetic polymers and identify specific polymers from complex biological media. Despite the absence of matrix, specific low molecular weight polymers were rapidly identified without any fragmentation. This method was applied to the rapid identification of ethoxylate polymers during a criminal investigation.

Keywords: Forensic science; Desorption–ionization; DIOS; Mass spectrometry; Polymers

1. Introduction

The versatility of modern mass spectrometry techniques such as matrix-assisted laser desorption–ionization (MALDI) and electrospray ionization (ESI) allows for molecular characterization with speed, accuracy, and sensitivity unequalled by many other analytical techniques [1]. While MALDI and ESI have been particularly useful for high-throughput genomic and protein characterization [2–4], they have also been widely used in other areas and recently have been recognized as tools in forensic analysis [5]. MALDI and ESI have enhanced mass spectrometric analysis by enabling the efficient generation of intact gas phase ions from nonvolatile, labile molecules [6,7]. ESI mass spectrometry, however, can be intolerant of contaminants and mixtures without prior separation such as with liquid chromatography (LC–ESI–MS). MALDI requires a matrix to assist in the laser desorption and ionization from the condensed phase to the gas phase by absorbing most of the laser energy and providing a means for vaporization and ionization. Tanaka et al., demonstrated the potential of MALDI time-of-flight mass spectrometry for the characterization of water soluble polymers [8]. More recently, Tabet and coworkers have described the significant effects of solvents, pH, and matrix composition on the MALDI analysis of large synthetic polymers [9]. Clearly, MALDI is a sensitive technique, yet the matrix can cause interference, particularly in the low-mass range (<700 Da). Here we describe the utility of a new, matrix-free laser

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0003-2670/01/$ – see front matter
PII: S0003-2670(01)01107-2

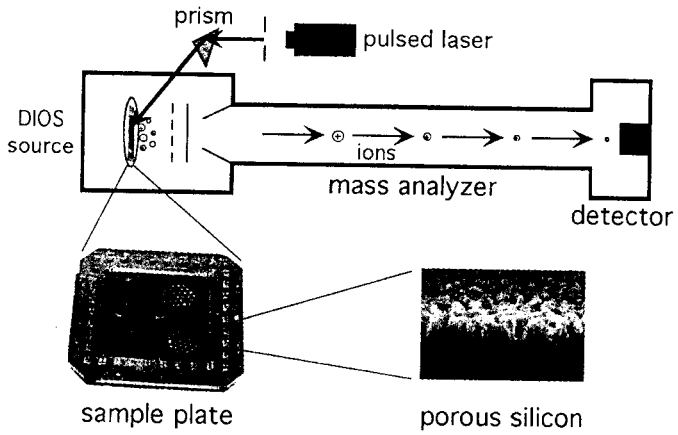

Fig. 1. DIOS surfaces are attached to the modified commercial MALDI sample plates. In the source, analyte ions are directly desorbed from the DIOS surface, and subsequently accelerated into the mass analyzer. The scanning electron microscopy figure (lower right) shows the typical morphology of the DIOS surfaces used in these analyses.

desorption–ionization approach on porous silicon [10,11] for a forensic case requiring chemical identification of synthetic polymers below 2000 Da. We have chosen to use desorption–ionization on silicon mass spectrometry (DIOS–MS) in this investigation because of the potential to identify small mass molecules without significant sample preparation.

The fortuitous combination of properties of porous silicon allows a wide range of molecules to be directly desorbed and analyzed on commercially available mass spectrometers (Fig. 1). The porous silicon surface is believed to act as a scaffold for retaining analytes and as an energy receptacle for the UV-laser energy from lasers supplied by commercial time-of-flight mass spectrometers. Most samples are simply applied to the porous silicon surface, allowed to dry, and mass analyzed. Thus, DIOS provides a simplified means of directly analyzing molecules that would be obscured by matrix interference. Moreover, DIOS surfaces allow for the desorption–ionization of thermally labile molecules and have a high tolerance for common contaminants. DIOS surfaces are produced through a simple galvanostatic etching procedure as previously described [11], which yields a UV-absorbing porous silicon exterior with high surface area and thermal insulating properties. Unlike the conditions used for semiconductor and spectroscopic purposes [12–14], mild etching conditions (i.e. short etching times and low current densities) provide the best surfaces for DIOS–MS. DIOS surfaces comprised of a thin layer (~200 nm depth) of narrow (50–100 nm diameter) pores yield mass spectra for a wide range of analytes.

The utility of any new analytical technique is evaluated through its application to a variety of problems, such as those that require chemical identification. In criminal investigations, the recovery and characterization of physical evidence is important in reconstructing the crime, associating suspects with the crime, or in supporting or refuting claims of victims or suspects. In order to have value for forensic and biological applications, analytical techniques must have high selectivity, sensitivity and tolerance of contaminants. Numerous methods, including spectroscopic and separation techniques, have been used with limited success for the characterization of polymers in forensic applications. For example, Fourier transform infrared spectroscopy (FTIR) has been used to identify traces of large polymer lubricants obtained from sexual assault victims [15–17]. However, FTIR spectra of nonoxynol-9 (a nonionic surfactant) are not sufficiently distinct from detergent residues that might be extracted from clothing and bedding. Additionally, because these polymers do not contain chromophores, UV-visible spectroscopy is not a suitable technique for their detection. Silicon nuclear magnetic resonance (NMR) and proton-NMR were found to be too insensitive to provide reliable identification of polymer traces in forensic samples [15,18]. Thin-layer

chromatography (on silica gel plates) has also been used for detection of nonoxynol-9, however the bands could not be distinguished from possible detergent residues due to the poor resolution (i.e. band broadening) [19]. Other separation techniques such as high-performance liquid chromatography and gel-permeation chromatography require mass spectrometry detection to confirm the identity of trace quantities. Desorption chemical ionization mass spectrometry has also been used to characterize a silicone oil and nonoxynol-9 found in many contraceptive products [15].

Commercial contraceptive products contain specific compounds such as water soluble polymeric lubricants [15–18] and spermicides [15,16,20] that can be transferred to body fluids. These commercial products are polymeric, or at least contain polymers as part of their formulation. Polyethylene glycol (PEG) in the range of 200–1850 Da and spermicides are often found in contraceptive products. The two most common spermicides are the polyethoxylated phenol nonionic surfactants, nonoxynol-9 and octoxynol-9. The difference between these two polymers is one methylene unit located on the alkyl chain *para* to the ethoxylate group on the phenyl ring. Nonoxynol-9 has nine carbons located on this alkyl chain while octoxynol-9 has eight, therefore a mass difference of 14 Da is expected between the two polymer series. Nonoxynol-9 and octoxynol-9 are mixtures of ethoxylates with "9" being the average or most abundant ethoximer. The forensic case described in this work required the detection and characterization of low ethoxylate polymers such as PEG 1000 and nonoxynol-9. DIOS–MS affords the potential to detect and differentiate between similar low molecular weight polymers. Here we demonstrate that DIOS–MS is a powerful technique that provides unequivocal identification of trace amounts of commercial polymeric products from various media.

2. Experimental

2.1. Chemicals

PEG standards of 300, 1000, and 1500 average molecular weights and octoxynol-9 (IGEPAL CA-630) were purchased from Sigma–Aldrich (Milwaukee, WI), and used without further purification. The nonoxynol-9 standard (USP Reference) in the form of a 1 ml sealed glass ampoule was donated by Carter-Wallace, Inc., New York, NY). Encare (vaginal contraceptive inserts (Blairex Labs; Columbus, IN) and Ramses (spermicidally lubricated condoms (Schmid Laboratories; Sarasota, FL) were extracted into methanol/water solutions as described in the results. HPLC-grade solvents were purchased from EM Science (Gibbstown, NJ). Swabs (Puritan (cotton-tipped applicators, Hardwood Products Company, Guilford, ME) used in control experiments are similar to the cotton swabs included in most victim or suspect sexual assault investigation kits purchased by law enforcement agencies.

2.2. Biological sample collection, storage, and preparation

Biological samples were a part of the sample evidence included in the victim/suspect investigation kit provided to hospitals and law enforcement agencies in California by the California Department of Justice. These samples were stored at 4°C in a refrigerator in a restricted access area. The cotton from air-dried swabs taken from a volunteer couple immediate post-coitus and 8 h post-coitus were removed from the swabs with different disposable scalpels (Feather sterile single-use disposable scalpels; Fisher Scientific, Pittsburgh, PA). The removed cotton was then placed in glass test tubes, covered with a minimum amount of methanol and vortexed for a few seconds.

2.3. Preparation of DIOS surfaces

DIOS chips were made from ⟨1 0 0⟩ low resistivity (0.005–0.02/cm) n-type silicon wafers (Silicon Quest, Inc.). As shown in Fig. 2c, the silicon chips were sandwiched by a Teflon (base and an upper portion containing the etching cell. Electrodes were placed in the etching solution (cathode) and on the other side of the silicon chip (anode). DIOS chips were constructed by etching the silicon surfaces in a 25% HF/ethanol solution under white light illumination for 1 min at a current density of 5 mA/cm^2. The product of the above procedure was further processed by rapid oxidation with ozone followed by chemical etching in 5% HF/ethanol for 1 min. The surfaces were ready for use after washing with ethanol.

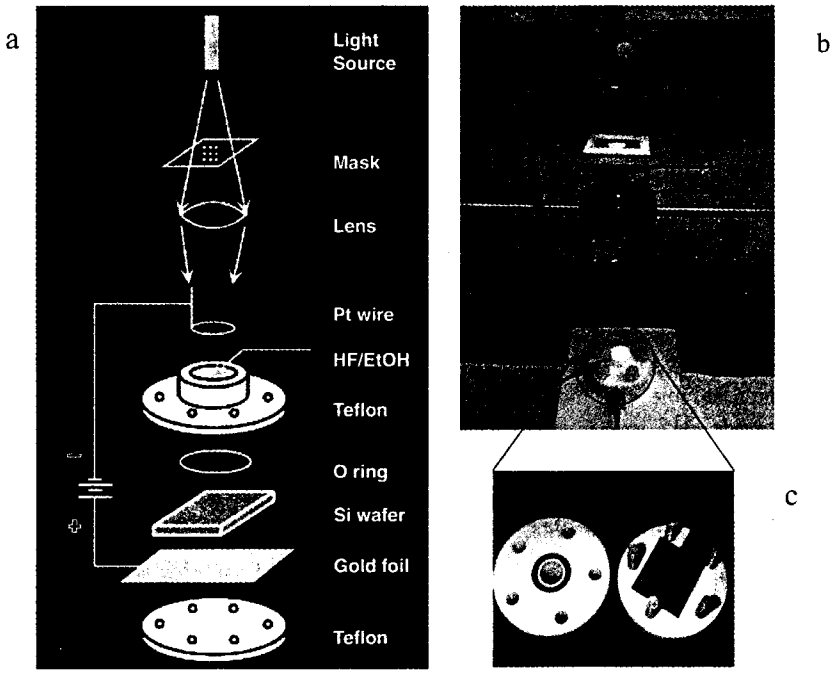

Fig. 2. (a) Schematic of the equipment design used to construct the DIOS surfaces; (b) the equipment scheme used to construct the surfaces includes a 50 mW white light source, two bifocal lenses, and the Teflon® etching cell; (c) the etching cell consists of a solution cavity that holds the etching solution and the base where the silicon chip rests upon one of the electrodes.

2.4. Mass spectrometry analysis

All DIOS experiments were performed on a Voyager DE-STR, time-of-flight mass spectrometer (PerSeptive Biosystems; Framingham, MA) equipped with a pulsed nitrogen laser operated at 337 nm. Desorbed ions were extracted into the mass analyzers with 20 kV after a 200 ns delay. Mass spectra were an average of 128 laser shots per sample.

3. Results and discussion

Polymers typically desorb well in mass spectrometry, however spectral quality determines the amount of information that is obtained. In forensic cases, the ability to unequivocally identify trace amounts of molecular species may furnish critical evidence. While DIOS–MS has been effective for a range of compounds, spectral quality is still dependent upon construction of the DIOS surfaces, and sample preparation as previously discussed [11]. Generally, DIOS is less amenable than MALDI to high concentrations of analytes (i.e. millimolar concentrations), therefore high sample concentrations on DIOS surfaces require significantly higher laser fluence (i.e. higher desorption threshold) than lower concentrations of the same analytes. High laser intensities lead to greater background noise and less resolution, where both contribute to poor sensitivity and lowered accuracy. Sample spreading on DIOS surfaces can result in a concentration gradient from the center of the applied sample drop to the edge of the dried drop. The polymer samples spread less when the samples were dissolved in water versus samples dissolved in organic solvents. The spectra of the polymer samples were obtained by simply diluting (~1:2000) the sample into deionized water and mass analyzing the sample.

Mass spectra of the polymers used in commercial contraceptive products contain distinct series of natriated peaks separated by ethoxy moieties of 44 Da. Fig. 3 shows DIOS mass spectra of (a) nonoxynol-9,

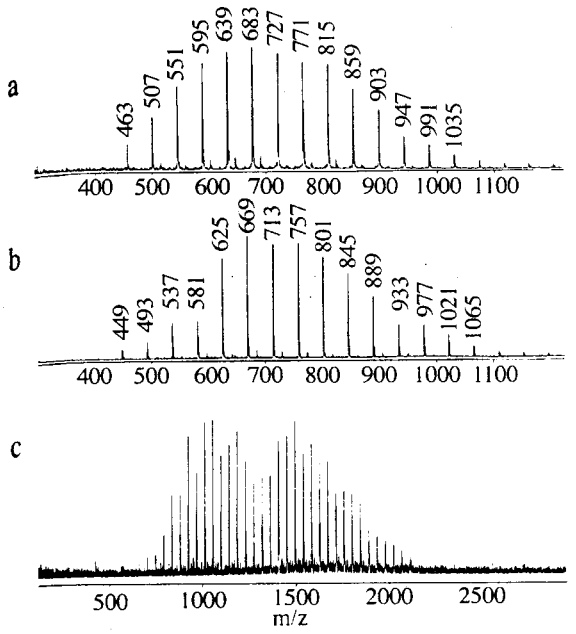

Fig. 3. DIOS–MS spectra of (a) nonoxynol-9 (nonylphenylpolyethylene glycol); (b) octoxynol-9 (octylphenylpolyethylene glycol) standards, and (c) a mixture of PEG 1000 and PEG 1500.

(b) octoxynol-9, and (c) a mixture of PEG 1000 and PEG 1500. As expected, the mass difference between the nonoxynol and the octoxynol series is 14 Da, corresponding to one methylene group. A relatively low intensity series of peaks in the nonoxynol and octoxynol standard spectra (16 Da higher than the primary series) is due to the potassium ion adducts. These polymers show preferential chelation of sodium as opposed to potassium or ammonia ions. Unlike previous MALDI analysis of ethoxylate polymers where the relative abundance of $[M + Na]^+$ and $[M + K]^+$ depends upon sample preparation [9], DIOS–MS analysis of these polymers yielded $[M + Na]^+/[M + K]^+$ ratios that were primarily dependent upon the amount of each alkali cation in the sample. MALDI analysis of nonoxynol-9 yielded significantly poorer quality spectra with several different matrices compared to DIOS analysis of the same amount of this low molecular weight compound. Additionally, the 'pyrolysis' fragmentation of ethoxylate polymers previously observed by MALDI [9,20] was not observed in the matrix-free DIOS–MS analysis of the polymers of interest despite the higher laser intensity used for DIOS analysis.

The bottom spectrum (Fig. 3c) shows the bimodal distribution from approximately a 2:1 (v/v) mixture of PEG 1500:PEG 1000. Strong signals from one polymer solution (i.e. PEG 1000 alone) results in a Gaussian distribution of single charged monomeric peaks. The distribution of overlapping series from this mixture demonstrates the ability to distinguish between mixtures of similar polymers from DIOS surfaces. A disproportionate amount of any polymer in these solutions would show a distorted distribution, which would also indicate the presence of a mixture. Identification of the polymers of interest therefore can be determined by qualitative appearance in addition to accurate mass measurements. For example, peaks from nonoxynol-9 (*m/z* 507, 551 595, 639, 683, ...) and PEG 300 (*m/z* 349, 393, 437, 481, 525, ...) were observed from condom extracts (Fig. 4). According to manufacturers' specifications, this product contains 15% (w/w) nonoxynol-9 in addition to PEG 300. Again, the distribution clearly suggests the presence of two polymers, and is confirmed by the masses of the peaks in the two series.

The tolerance of DIOS toward moderately high amounts of contaminants such as salts and the absence of low-mass background ions are major advantages for unpurified samples from biological sources. The high tolerance of DIOS–MS for contaminants was demonstrated with the contraceptive products as control experiments and forensic samples in which these products had been used. Fig. 5 shows a DIOS–MS spectrum of an extract from immediate post-coitus swabs when the contraceptive inserts had been used. The analysis of this extract produced a nonoxynol and PEG ion series corresponding to an expected polymer mix of PEG 1000 and PEG 1450 in addition to nonoxynol-9. To a greater extent than the octoxynol and nonoxynol standard spectra, the spectrum of the swab extract shows a small series of peaks 16 Da higher than the natriated polyethylene glycol species that corresponds to potassium adducts. The results are also consistent with mass spectra from unadulterated vaginal contraceptive control extract, which predominantly showed natriated peaks of nonoxynol-9 and the PEG polymers. The analyses of these samples were obtained from post-coitus swabs on individuals who did and did not use contraceptive inserts. Additional controls of extracts from the fresh cotton swabs showed a very weak polymeric series (ethoxylate

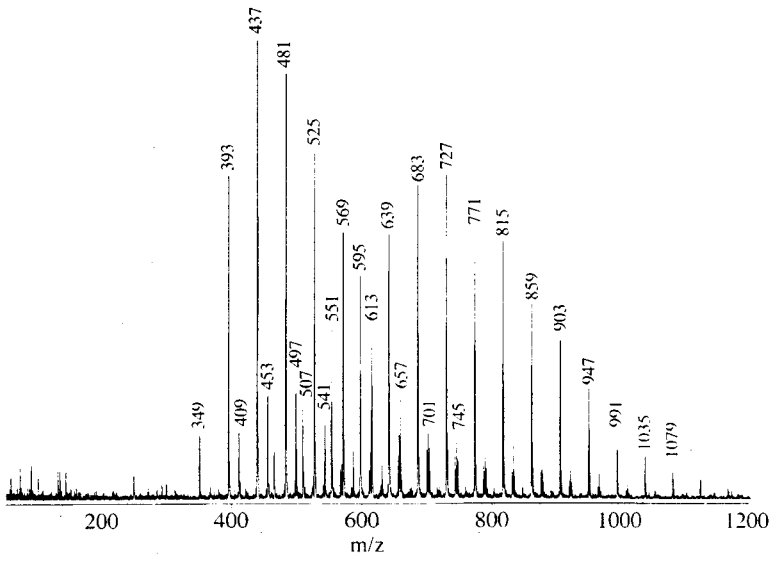

Fig. 4. DIOS mass spectrum of polymers extracted from a condom into 50 ml of 50% (v/v) methanol/water.

series, 44 Da apart) whose molecular weights did not correspond to either PEG or nonoxynol-9. This series was not identified, but could originate from the glue used to hold the cotton to the wood shaft. Except for this series of peaks, no other polymer traces were detected on extracts of control swabs.

The ability to use DIOS–MS to directly identify specific commercial polymers also allowed us to use this technique for a biological forensic sample. The victim and suspect sexual assault investigation kits provided by Naval Criminal Investigative Service Regional Forensics Laboratory in San Diego involved

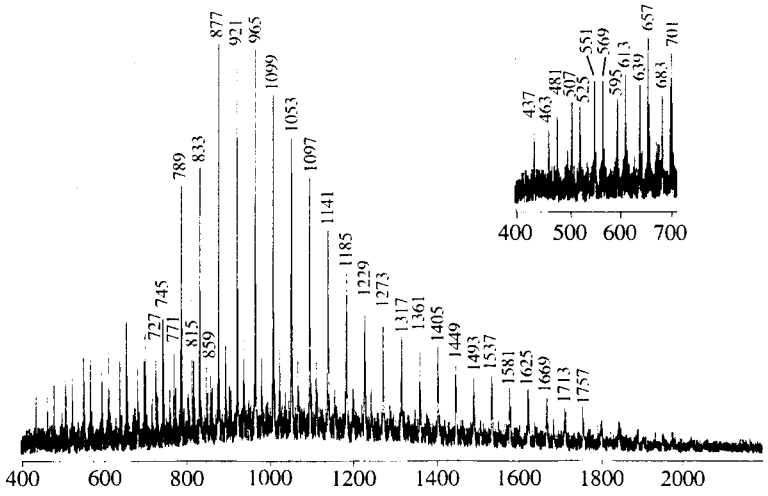

Fig. 5. DIOS mass spectrum from a swab extract from a volunteer immediately following the use of the commercial contraceptive product. The methanol extract was diluted (4×) into water.

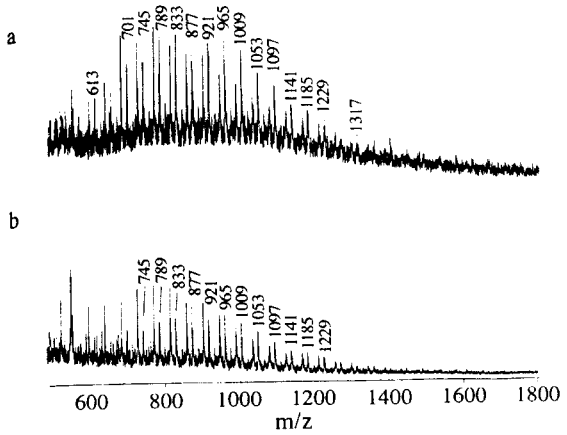

Fig. 6. Evidence of the commercial product taken from swabs of (a) female, and (b) the male believed to have used this product. The DIOS mass spectra show the presence of the low molecular weight polymer distribution despite delayed collection of the samples and no purification prior to analysis.

the sexual assault of a juvenile female. To avoid any suggestion of possible cross-contamination, separate porous silicon chips were used for DIOS–MS of control samples, victim evidence samples, and suspect evidence samples. Due to the hydrophobic nature of the porous silicon chips, samples with high organic content (>25% v/v) can spread on DIOS surfaces. All of the standard polymeric samples used in these studies spread to some extent even in predominantly aqueous solution. However, diluting the extracts of the post-coitus samples into water contained the amount of sample spreading. Fig. 6 shows the DIOS–MS spectra from two forensic evidence samples. Spectrum (a) was obtained from an extract of a vaginal swab from the female victim, while (b) is from an extract of a swab of the male suspect. A PEG distribution (*m/z* 701, 745, 789, 833) similar to the commercial product controls is observed in these spectra, thereby confirming the presence of the contraceptive products on the victim and assailant.

Due to the nature of these samples and the time following sample collection, the spectra from the crime samples had fewer peaks than controls, including the immediate post-coitus sample. Despite the limited amount of information obtained from the most contaminated samples, DIOS–MS allowed for the identification of these polymers directly from biological sources.

4. Conclusion

DIOS mass spectrometry has been shown to be a simple, but highly effective analytical technique for the analysis of forensic samples. The specificity, accuracy, and tolerance of DIOS–MS allows for the identification of small molecular weight polymers from biological sources. Here we have demonstrated the potential to identify spermicides (nonoxynol-9 and octoxynol-9) and polyethylene glycol polymers from commercial products and in bodily fluids when these products were used. Unlike commonly used mass spectrometry ionization techniques such as MALDI and LC–ESI, DIOS–MS enables these analyses to be performed in a short time without additives (i.e. matrices), which can obscure analyte ions, cause analyte suppression and add to sample preparation time.

The previous success of DIOS–MS for a variety of applications including proteomics [21] and small organic molecule analysis [10,11,21], along with polymer analysis in this study have demonstrated the potential of this technique for future forensic work. It can easily be envisioned that DIOS–MS analysis of illicit drugs, inks, explosives, hair, and hypervariable regions of DNA would enable scientists to perform accurate and rapid identification of analytes.

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