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International Journal of Mass Spectrometry 226 (2003) 107–116

International Journal of Mass Spectrometry

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Desorption/ionization on silicon (DIOS) mass spectrometry: background and applications

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Received 14 September 2001; accepted 7 July 2002

Abstract

Desorption/ionization on silicon mass spectrometry (DIOS-MS) is a matrix-less laser vaporization method for generating gas-phase ions. The physical properties of the silicon surfaces are crucial to DIOS-MS performance and are controlled by the selection of silicon type and the silicon etching conditions. DIOS-MS has been examined for its applicability to small-molecule analysis, quantitative studies, reaction monitoring, chromatography, protein identification, and protein functional characterization. In organic chemistry, DIOS has been applied to the analysis of reactions directed toward development of new catalysts and transformations. Because DIOS offers a chip-based format, it is capable of being used to raster the silicon surface for biological and chemical screening applications, such as enzymatic activity assays. DIOS-MS extends the mass analysis capabilities of laser desorption to small biomolecules and thus offers a platform on which multiple experiments can be performed on a wide variety of molecules.

Keywords: Desorption/ionization on silicon (DIOS); Silicon; Matrix-less; High-throughput

1. Introduction

Since the original desorption/ionization experiments performed in 1910 [1], desorption mass spectrometry (MS) has undergone significant improvements. In the early 1980s, the most dramatic change occurred with the introduction of an organic matrix which served as a medium for energy transfer to the analyte. Matrix-assisted desorption/ionization MS was originally applied with high-energy particle beams, such as those employed in secondary ion mass spectrometry (SIMS) [2] and fast atom/ion bombardment (FAB) [3]. The broad success of matrix-assisted desorption/ionization is related to the ability of the matrix to incorporate and transfer energy to the analyte [3–6]. As a result, mass measurements could be made to exceed 10,000 Da, but the sensitivity required for biomolecular analysis was not achieved until a matrix medium [5] was used in conjunction with laser desorption; the resulting MALDI technique has revolutionized biomolecular analysis [4].

Direct laser desorption/ionization (without a matrix) has been extensively studied on a variety of surfaces [7–12], yet has not been widely used due to the rapid molecular degradation usually observed upon direct exposure to laser radiation. However, just

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1387-3806/02/$ – see front matter
PII S1387-3806(02)00973-9

as SIMS has had a profound effect on surface science [13], the utility of direct laser desorption/ionization for biomolecular analysis could be highly beneficial due to the dramatically simplified sample preparation, the elimination of matrix background ions, and the potential for rapid analyses.

Since the matrix serves to trap analyte molecules as well as absorb UV radiation, nanoporous materials were investigated, such as porous polystyrene beads because they could potentially mimic the features of the MALDI matrix. While porous polystyrene was not found to be useful, porous silicon was found to be an effective medium for desorbing compounds and generating intact ions in the gas phase [14]. A nano-structured silicon thin film prepared by plasma-enhanced chemical vapor deposition also recently yielded very similar laser-desorption/ionization properties as porous silicon [15]. We speculate that this is, in part, due to the ability of its nanometer-sized pores to trap analyte molecules while the silicon surface, well known to be photoactive and even photoluminescent, effectively absorbs UV light (vide infra) [16]. Thus, desorption/ionization on porous silicon (DIOS) (Fig. 1) has demonstrated characteristics similar to MALDI in that intact molecules are observed at the femtomole and attomole level with little or no fragmentation [14]. This stands in contrast to what is typically observed with other direct desorption/ionization approaches [7–12]. Most importantly, the absence of matrix material [3,5,6,17] allows the technique to be applied to small molecules. With MALDI it is also possible to perform small-molecule analysis [18] and matrix suppression can be achieved under certain circumstances [19], but matrix interference presents limitations that a matrix-free technique such as DIOS does not encounter.

Optimal performance of DIOS-MS is typically obtained for molecules less than 3000 Da (Fig. 2) and its application to whole proteins is currently being developed. The following factors help make DIOS a technique of practical interest. Since there is no matrix deposition, samples may be deposited in spot sizes of less than 1 mm in diameter, and, thus, detection limits are improved because it is easier to localize a sample into a very small region on the plate. Most existing MALDI mass spectrometers can be used to perform

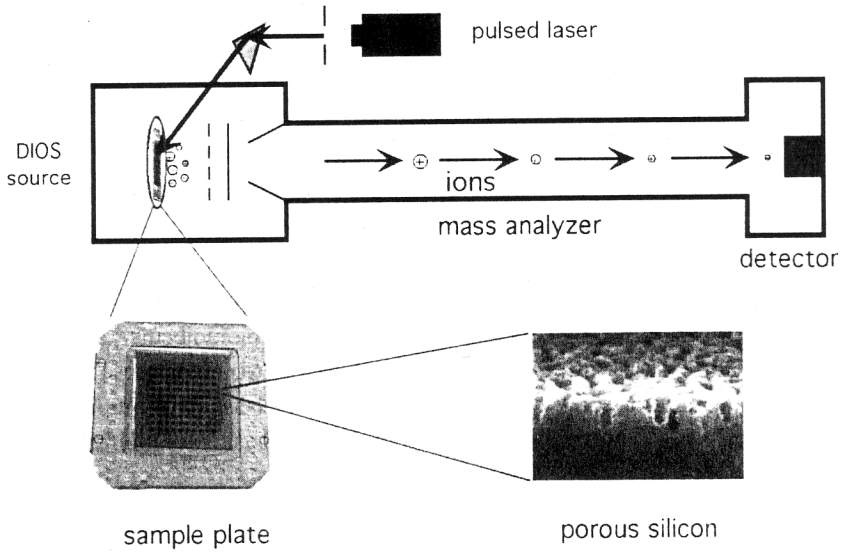


Fig. 1. DIOS surfaces are attached to the modified commercial MALDI sample plates. In the source, analyte ions are directly desorbed from the DIOS surface, and subsequently accelerated into the mass analyzer. The scanning electron microscopy figure (lower right) shows the typical morphology of the DIOS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

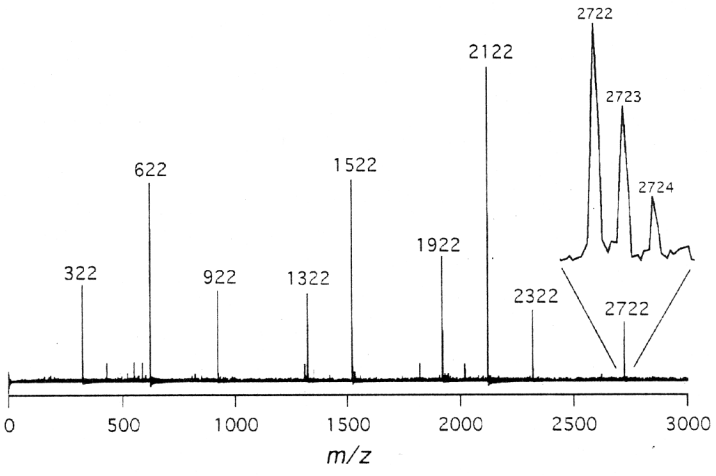


Fig. 2. Analysis of an Agilent ESI-MS tuning mixture demonstrating some of the analysis capabilities of DIOS-MS.

DIOS simply by changing the sample plate; no spectrometer modification is necessary. The rate of data acquisition with DIOS is fast. We normally average 128 spectra obtained over 50 s with a laser firing rate of 3 Hz, yet good spectral data may be obtained with as little as 20 pulses (7 s). Silicon wafers are inexpensive and their conversion to porous silicon is relatively simple. Signals from both porous silicon hydride surfaces and porous silicon surfaces capped with covalently bound organic monolayers, especially 2-phenethyl groups [14], are strong, suggesting that significant improvements can be made to DIOS-MS through further investigation of surface modifications (vide infra). Si–H termination can be selectively functionalized to make DIOS chips an integrated affinity capture and detection device. Because of its use in the semiconductor computer chip industry [20], a great deal is known about the properties [21–25], lithographic micropatterning, and micromachining [21,26,27] of silicon wafers; presently the interests of many chemists have been captured by the prospects of miniaturized microfluidic chemical reactors which are lithographically etched into crystalline silicon chips [22,28]. Therefore, significant potential does exist for the construction of chemical devices that employ direct desorption/ionization MS for rapid and informative readout.

1.1. Preparing porous silicon

Microns-thick porous silicon is a photoluminescent semiconducting material with a high surface area (up to hundreds of square meters per cubic centimeter), produced from crystalline silicon by a straightforward electrochemical etching process (Fig. 3) [23,29,30]. Typically, n-type (phosphorus doped) silicon is etched under white-light illumination; p-type (boron doped) material is etched without special irradiation. For both, etching is accomplished by passing current in an electrochemical cell using an electrolyte with 20% aqueous HF in ethanol, in which the silicon wafer is the anode (anodization). This process produces a network of nanometer-scale Si structures, principally cylindrical pores [31,32], in which surface silicon groups are terminated by Si–H bonds. Although photoluminescence of porous silicon is usually attributed to quantum effects of nanometer-sized silicon domains [23,30], it has also been proposed that the etching process creates a layer of material at the surface resembling siloxene [$(Si_6H_6O_3)_n$], which is photoluminescent in bulk [33]. Siloxene prepared by the literature method does not give laser-desorption MS when tested on the standard gold MALDI plate. In our case, photoluminescence is not important; indeed, most of the photoluminescent materials that we prepared in our early

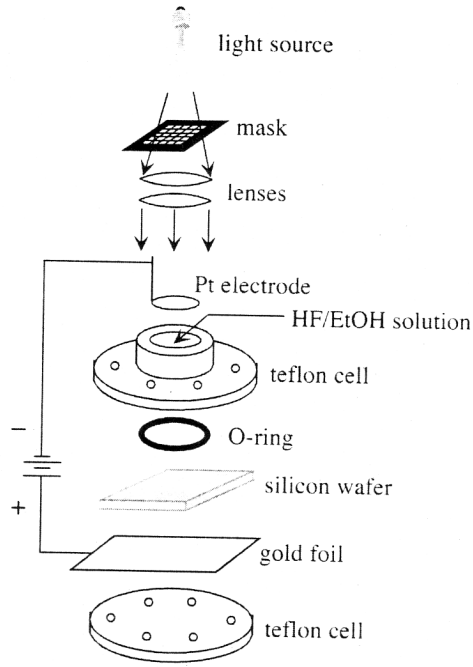


Fig. 3. The design of the apparatus that is used to make porous silicon.

investigations were not optimal supports for DIOS, and our now-standard DIOS surfaces (see the following description) are not photoluminescent. Similar conclusions were found independently by Sweedler and coworkers [34]. The structure, thickness, porosity, resistivity, and other characteristics of the material are sensitive to the choice of silicon wafer precursor and etching conditions (such as HF concentration, etching duration, current density, light exposure, and dopant type and concentration) [23,29,32,35–41]. Porous silicon materials are generally divided into microporous (pore diameters $\leq$ 2 nm), mesoporous (2–50 nm), and macroporous (>50 nm) categories [25,29,35]. We have investigated several of the variables in the etching procedure, with the following results:

(1) Porous silicon generated from heavily-doped n-type silicon wafers (resistivity 0.008–0.05 Ω cm) at low etching current densities (4 mA/cm^2) for short times (1–2 min) under moderate white-light intensity produce the best DIOS-MS results thus far. A material giving very similar DIOS results is also obtained with low light intensity, using less heavily doped silicon wafers (0.5–2 Ω cm) etched at larger current densities (20 mA/cm^2) for slightly longer times (5 min). The porous silicon samples appear to be fairly robust towards fracturing or peeling of the porous layer, perhaps because their porosities appear to be well below 50% [42].

(2) Storage in air for extended periods of time, or brief exposure to ozone or aqueous hydrogen peroxide, results in oxidation of surface groups to oxide (Si–O–Si) and hydroxide (Si–OH) moieties, which we characterize by IR spectroscopy and dramatic changes in wetting properties. In general, DIOS performance degrades with increasing surface oxidation, yet some hydrophilic compounds can be detected with better sensitivity on lightly oxidized surfaces or surfaces derivatized with polar functional groups.

(3) While pore depth generally increases with increasing HF concentration in the etching solution [43], we observed little effect on DIOS performance when the HF concentration was lowered (to 15%) or raised (to 35%) from the standard mixture (25%). In general, lowering the amount of ethanol below 30% leads to irreproducible results, probably because the formation of H_2 bubbles at the surface under such conditions is too vigorous.

(4) We have observed similar performance in DIOS-MS analysis of standard peptide samples (vide infra) on porous silicon prepared from both <1 0 0> and <1 1 1> silicon wafers using the same standard etching conditions (4 mA/cm^2, 1 min, with irradiation), suggesting that Si crystal orientation does not play an important role in DIOS performance.

(5) DIOS surfaces may be photopatterned by etching n^+-type silicon with illumination through a mask. Note that the low current densities employed here facilitate the creation of sharp boundaries since hydrogen bubble formation is minimized; such bubbles can stick to the surface and induce heterogeneity in both the lateral and vertical dimensions. A complementary type of photopatterning has

been demonstrated by Stewart and Buriak [44] in the covalent derivatization of porous silicon following the etching step.

2. Small-molecule characterization

Because of the hydrophobic nature of the surfaces, analytes are typically dissolved in water or water/methanol solutions for DIOS-MS analysis in order to localize (through beading) droplet formation on the surfaces. Freshly etched DIOS surfaces are hydrophobic; thus, aqueous samples bead up and stay localized to a relatively small area, whereas samples dissolved in nonpolar solvents spread over the hydrophobic porous silicon wafer. Aliquots of the sample are deposited directly onto the porous surfaces and are allowed to dry before DIOS-MS analysis.

A wide variety of compounds has been detected by DIOS-MS with little or no fragmentation, examples are shown in Fig. 4; note that DIOS-MS can be performed in both positive and negative modes on compounds that differ greatly in polarity and that range in mass from 100 to 3000 Da. In general, it appears that those molecules with ionizable functionalities are efficiently detected with both electrospray and DIOS. We are continuing to explore the relationship of surface structure and derivatization to DIOS sensitivity and selectivity.

Postsource decay (PSD) measurements can be used for structure analysis from the DIOS surfaces in a manner very similar to that observed with MALDI. However, the DIOS techniques can accommodate small molecules whereas MALDI often cannot, due to low mass interference of matrix-derived ions. Analysis of identical samples by PSD with MALDI and DIOS, and collision-induced dissociation with an electrospray triple quadrupole instrument reveal similar fragmentation patterns with the ESI-MS/MS technique showing more fragments, especially in the low *m/z* region [45].

A surface-based approach for performing mass measurements has the added benefit of allowing for rapidly scanning the surface on which compounds have been deposited and, thus, high throughput capability [presented at American Society for Mass Spectrometry 2002 in Orlando Florida] of DIOS-MS for the rapid analysis of analyte samples containing small molecules (less than 3000 Da). The analysis rate is currently limited by the data acquisition system and the instrument's translation platform rather than any inherit limitation in the DIOS-MS methodology.

3. Application in forensics

The utility of any new analytical technique is evaluated through its application to a variety of problems

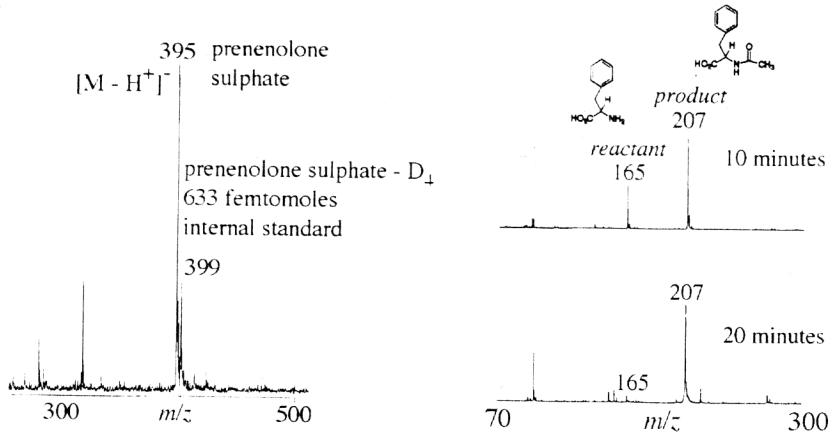


Fig. 4. DIOS mass spectra of two steroids and an amino acid as well as an acetylated amino acid.

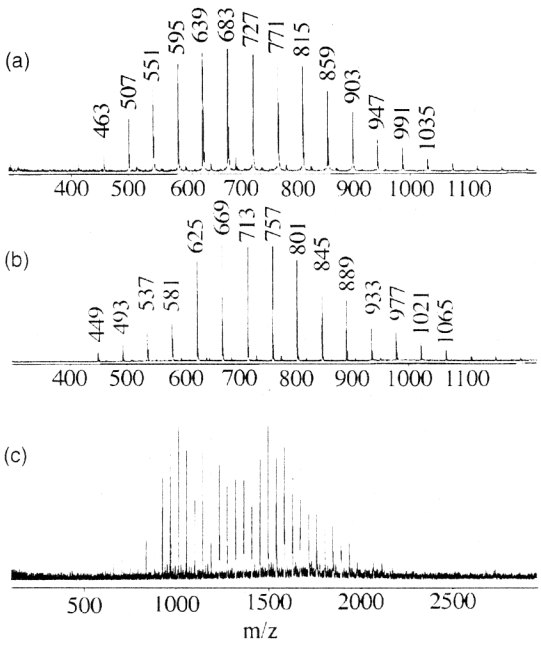


Fig. 5. DIOS mass spectra of (a) nonoxynol-9 (nonylphenylpolyethylene glycol), (b) octoxynol-9 (octylphenylpolyethylene glycol) standards, and (c) a mixture of PEG 1000 and PEG 1500.

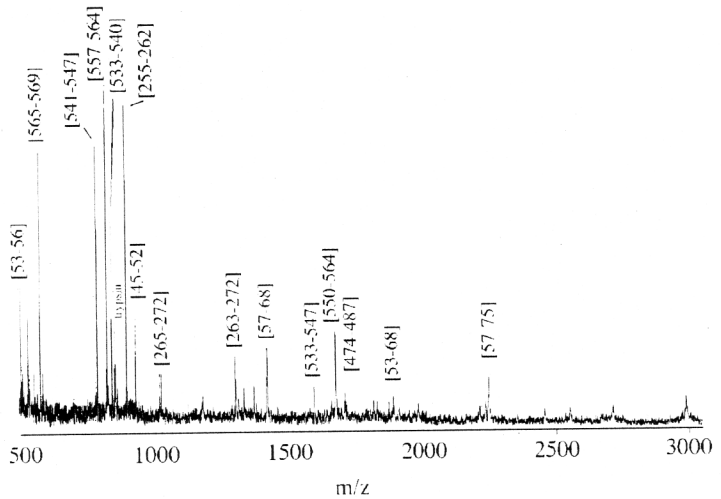
that require chemical identification. In criminal investigations, the recovery and characterization of physical evidence is important in reconstructing the crime, associating suspects with the crime, or in supporting or refuting claims of victims or suspects. In order to have value for forensics and 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. Commercial contraceptive products contain specific compounds such as water soluble polymeric lubricants and spermicides [46–49] that can be transferred to body fluids. Mass spectra of the polymers used in commercial contraceptive products contain distinct series of peaks separated by ethoxy moieties of 44 Da. Fig. 5 shows DIOS mass spectral data of (a) nonoxynol-9, (b) octoxynol-9, and (c) a mixture of PEG 1000 and PEG 1500.

The potential 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 assault investigation kits provided by Naval Criminal Investigative Service Regional Forensics Laboratory, San Diego, involved the 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. Even from the most contaminated samples, DIOS-MS allowed for the identification of these polymers directly from biological sources.

4. Protein identification

Site-specific proteolytic digestion with enzymes, combined with mass analysis and database searching allows for protein identification as well as characterization of posttranslational modifications. For example an on-plate digestion combined with DIOS-MS analysis was used to identify the acetylcholine esterase (AChE) enzyme in straightforward fashion. An in situ tryptic digest of the enzyme was sustained on the porous silicon plate for 4 h at 37 °C, followed by drying and direct MS analysis of the residue by DIOS. The mass information was obtained primarily from peptides near the C- and N-termini, presumably because access is limited by disulfide bonds and glycosylation. The observed peptides were nonetheless sufficient to identify the 72 kD protein. DIOS-MS analyses of proteolytic digests of β-lactoglobulin, bovine serum albumin, and flock house virus capsid proteins were also performed and allowed for their identification.

The utility of DIOS analysis for protein identification is illustrated by the analysis of a digest and the resultant identification of an adenovirus penton protein (Fig. 6). After purification with a 10 kDa filter, the protein was incubated with trypsin on the DIOS chip at 37 °C at a reaction volume of 1.0 μL. The



Rank	Probability	Protein Description
1	0.64	PEN3_ADE02 PENTON PROTEIN (VIRON COMPONENT III BASE PROTEIN)
2	0.36	PEN3_ADE05 PENTON PROTEIN (VIRON COMPONENT III BASE PROTEIN)
3	2.1e-17	(M55059) HEMAGGLUTININ [influenza virus type A]

Fig. 6. Mass spectral data obtained from a digest of adenovirus penton protein as well as the subsequent identification of the correct serotype.

mass information obtained from the DIOS spectrum was sufficient to identify the type of protein and the correct serotype (type II). Although DIOS can have a high tolerance for contaminants, spectral quality does diminish in the presence of large quantities of interfering compounds. However, the addition of an ion-sequestering reagent, such as ammonium citrate, directly to the sample can significantly enhance DIOS peak intensities. It should be noted that such additives do not absorb light at the frequency of the UV laser, and therefore do not act as a matrix. In the case of adenovirus, the sample obtained from ion-exchange chromatography containing a high concentration of salts (40 mM Tris, 1 M NaCl) was simply purified with a molecular weight cutoff filter before analysis.

4.1. Protein functional characterization

DIOS is particularly suited to investigations of enzyme-catalyzed chemical events in metabolism and signaling. Biochemical methods for determining protein activity often require fluorogenic substrates or coupling to auxiliary biochemical reactions, which add to the complexity of assays and reduce the biological relevance of the analysis. Substrates containing radioactive isotopes allow tracking of molecules in biological systems, yet require careful handling and disposal. DIOS-MS facilitates analyses of natural substrates and products, and circumvents the use of potentially hazardous reagents or reagents with modified activity.

Some of the most compelling advantages of DIOS-MS over other methods emerge in qualitative and quantitative studies of the activity of proteins. The action of several enzymes, including phospholipase A2, mannosidase II, and AChE, were monitored through the simultaneous analysis of the substrate and the product. As each reaction progresses, a single spectrum from one acquisition provides an overview of the chemical process. An example is provided by

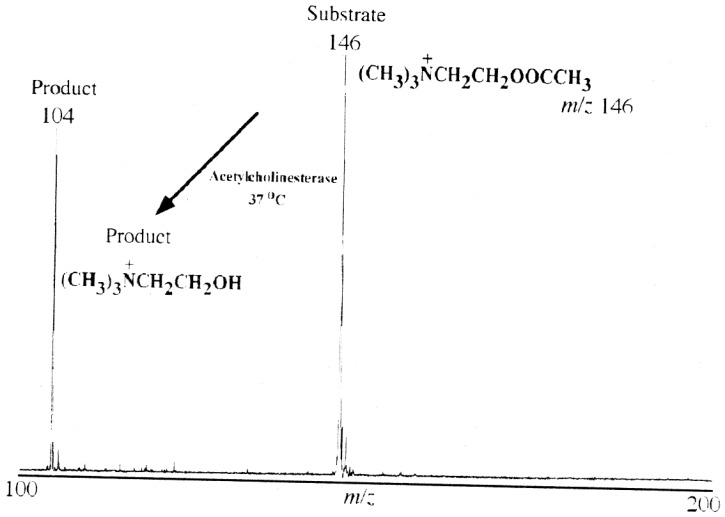


Fig. 7. DIOS mass spectral data of the AChE enzyme-catalyzed reaction of the substrate (ACh) to product (choline).

DIOS-MS monitoring of AChE which catalyzes the conversion of the neurotransmitter acetylcholine to choline and acetate (Fig. 7). AChE is the enzyme primarily responsible for the termination of signaling events at cholinergic synapses. Quantitation of choline levels during the enzymatic reaction was achieved by adding an internal standard (d_9-choline). A standard curve showed that d_9-choline allows accurate choline quantitation (Fig. 8). Inhibition of the enzymatic activity can also be assayed by following product formation. Both huperzine and tacrine, potent inhibitors of AChE, strongly inhibited

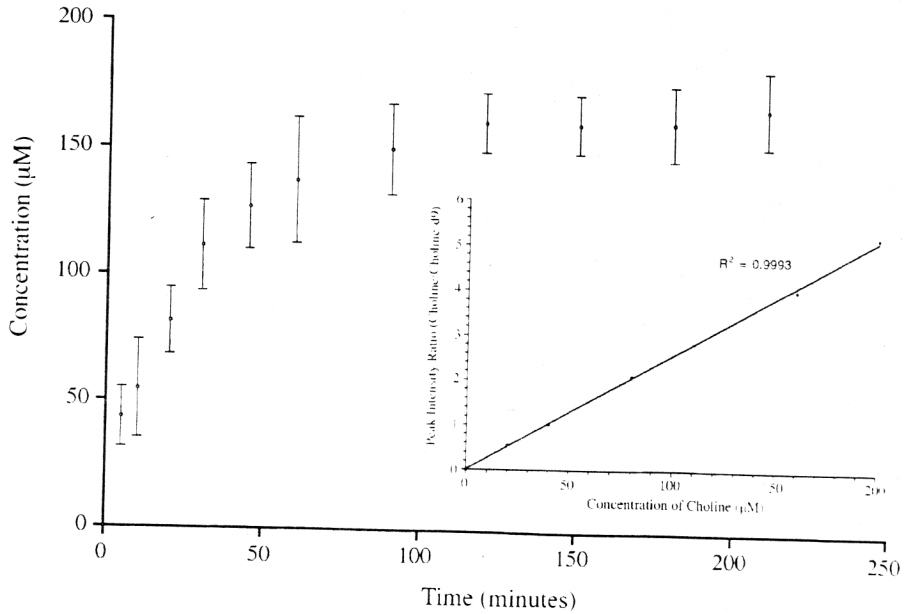


Fig. 8. Quantitative DIOS-MS analysis of the conversion of ACh to choline at 25 °C catalyzed with AChE. d_9-Choline was added as an internal standard. The data follows the increase in the product (choline) as a function of time. Inset: linear calibration of choline vs. internal standard used to relate observed choline peak intensities to concentration.

AChE, and little conversion of ACh to choline was observed.

5. Conclusions

DIOS is an effective MS tool with unique capabilities for chemical, biochemical, and protein characterization [14,15,34,45,50–53]. Because DIOS offers a chip-based format, it is capable of rastering the surface for biological and chemical screening applications, such as assays for enzymatic activity. In organic chemistry, DIOS is being applied to the analysis of reactions directed toward development of new catalysts and transformations [51,52]. Because DIOS is a matrix-free laser-desorption technique, DIOS-MS extends the observable mass range to small biomolecules and it has the ability to rapidly scan/analyze across the surface. Thus, DIOS offers a platform on which multiple experiments can be performed on a wide variety of molecules.

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