Desorption/ionization on silicon for small molecules: a promising alternative to MALDI TOF

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A method has been developed for laser desorption/ionization of catecholamines from porous silicon. This methodology is particularly attractive for analysis of small molecules. MALDI TOF mass spectrometry, although a very sensitive technique, utilizes matrices that need to be mixed with the sample prior to their analysis. Each matrix produces its own background, particularly in the low-molecular mass region. Therefore, detection and identification of molecules below 400 Da can be difficult. Desorption/ionization of samples deposited on porous silicon does not require addition of a matrix, thus, spectra in the low-molecular mass region can be clearly readable. Here, we describe a method for the analysis of catecholamines. While MALDI TOF is superior for proteomics/peptidomics, desorption/ionization from porous silicon can extend the operating range of a mass spectrometer for studies on metabolomics (small organic molecules and their metabolites, such as chemical neurotransmitters, prostaglandins, steroids, etc.).

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Abbreviations: DIOS, desorption/ionization on porous silicon; MALDI TOF, matrix-assisted laser desorption/ionization time of flight.
Catecholamines are important neurotransmitters. They act via dopaminergic and adrenergic receptors and affect regulatory systems. They participate in the regulation of learning and memory processes and response to stress. Catecholamines also play a significant role in neurodegenerative disorders, for example Parkinson’s disease, are involved in the control of regulatory functions of various systems, such as differentiation, proliferation, and induction of apoptosis in the lymphocytes. At least dopamine is involved in drug addiction through the reward pathway (Bergquist et al., 2002).

Due to their essential functions in the central nervous system and periphery, and our recent discovery of a separate pool of catecholamines in the immune system, it is important to develop sensitive and selective methods that enable identification and quantitation of these and similar compounds.

MALDI TOF (matrix-assisted laser desorption/ionization time-of-flight) is a powerful tool for identification of biomolecules (Mann et al., 2001; Kowalski & Stoerker, 2000; Andersen et al., 1996). This method has been extensively used for the detection and characterization of complex mixtures such as peptide fragments of an enzymatic digest for proteomics purposes, and can accurately analyze large biomolecules which can be detected as intact species with high accuracy. In this technique the analyte is typically co-crystallized with a solid ultraviolet-absorbing organic acid matrix, which vaporizes upon pulsed-laser radiation, carrying the analyte with it. The matrices are a limitation of MALDI TOF, because of the interference in the low-molecular mass region.

Desorption/ionization on porous silicon (DIOS) mass spectrometry is a cutting-edge method in proteomics but is also suitable for identification of low-molecular mass substances such as neurotransmitters, their metabolites, etc. This method is based on the MALDI TOF mass spectrometry but matrix is not required for ionization. Instead, ions are generated by employing direct laser vaporization. As a result, DIOS mass spectra are virtually free of the interfering matrix ions that are typical of MALDI mass spectra. DIOS is applicable to a wide range of compounds with molecular mass as low as 150 Da. In comparison with MALDI, DIOS simplifies sample preparation as well as enables direct laser desorption analysis (Wei et al., 1999; Thomas et al., 2001; Shen et al., 2001).

MATERIALS AND METHODS

Porous silicon wafers were prepared according to a procedure developed in our group. This new technology of silicon wafer fabrication differs from the method presented by Wei (Wei et al., 1999), although some of the start end procedures are similar. During the first phase, porous silicon is formed by electrochemical etching (Nieradko, 2001). Next, the porous silicon layer is transformed to porous silicon dioxide by thermal oxidation at high temperature in a water steam. In this process pores are obtained. According to thermal oxidation theory, a gain in wall thickness occurs, thus causing at this stage a reduction of pores diameter. By precise matching of the porous silicon fabrication procedure with thermal oxidation parameters, a proper geometry of the pores is obtained, with an increased surface of the porous silicon dioxide. A chemically-, and electrically passive and stable, porous dielectric layer is obtained in this manner. We designed DIOS wafers with 15 × 7 porous silicon dioxide spots (circles, squares and triangles). Silicon substrate type n, 380 μm thick, (111) oriented, with resistivity ρ = 1±5 Ω cm was used. The thickness of porous silicon produced in HF: ethyl alcohol (1:1 mixture in three electrode system under white light illumination) was about 30 μm. Silicon nitride CVD layers (0.1 μm) were used as a mask. Oxidation of porous silicon layer was performed at 1050°C. Wafers were washed in deionized water, dried in the infrared oven, and stored up to several months.
Synthetic catecholamines were purchased from Sigma-Aldrich (Poland) and dissolved to the desired concentrations in water. All other chemicals and solvents were of the highest purity grade (i.e. low-UV HPLC grade) and were purchased from various commercial sources (metanol: J.T. Baker (HPLC grade), HF: POCh Gliwice (Poland) (for microelectronics), H₂O: PROLAB s.c. PATEREK (aqua purificata, Pharmacopeia grade), ethanol: POCh Gliwice (Poland) (analytical grade)).

A MALDI TOF Reflex IV mass spectrometer (Bruker-Saxonia, Leipzig, Germany) was used for the experiments. The instrument was operated in a positive-ion mode and in the reflectron mode (3 m flight path). High voltage was set to 20 kV. Silicon surfaces were sonicated in methanol prior to sample application. Regeneration of the wafers was performed by immersion in a hot aqua regia, followed by extensive washing in water and methanol. Stainless-steel target plates were manufactured by the in-house workshop and their shape was adjusted to the dimensions of the silicon wafers, bearing in mind the overall thickness accepted by the ion-source. The wafers were attached to the target plates with a double sided conductive tape. Samples were irradiated with a nitrogen laser at 337 nm. Delayed extraction of ions was achieved using a 400 ns pulse. Resolution in this range was 3000 (10% valley).

RESULTS AND DISCUSSION

The manufacturing procedure reported here differs in several details from that described by Siuzdak’s group (Wei et al., 1999) and the technical details will be described in a separate paper. Silicon spots and thermal silicon dioxide spots fabricated on a wafer, ensure very low pore diameters and increased porous surface. A picture of a porous silicon wafer is shown in Fig. 1. The wafers can be produced in various shapes, with a desired number of positions.

To test the applicability of the new wafers for DIOS analysis, selected catecholamines were tested. An empty silicon wafer produces a very low background exclusively in the m/z range 20–40 which does not interfere with investigated samples. Figure 2 shows mass spectrum of a wafer irradiated with laser, where three signals are abundant at m/z of 22.68, 38.65, and 40.65. Analysis of dopamine (163 pmole/spot) produced an abundant spectrum (Fig. 3) containing molecular ion at m/z of 154.06 and its fragment at 137.04. The signals at m/z of 176.05 and 192.02 are sodium and potassium adducts of dopamine, respectively. Spectra of a similar quality were obtained for noradrenaline at a concentration of 59 pmole/spot, as shown in Fig. 4. Spontaneous fragmentation of catecholamines is a commonly occurring phenomenon, seen also in the MALDI TOF technique with α-cyano-4-hydroxycinnamic acid matrix. These data also prove that structural information can also be obtained using porous silicon.

In this paper we have presented our preliminary data with laser desorption/ionization from silicon wafers without addition of a matrix. This technique is simple, and production of the silicon target plates of various shapes and porosity can be arranged on a mass-scale. The lack of a matrix is advantageous for the low-molecular mass compounds, also of endogenous origin, and extends the working range of a mass spectrometer. Problems associated with high background produced by the
Figure 2. DIOS mass spectrum of empty spot irradiated with laser.

Figure 3. DIOS mass spectrum of dopamine (163 pmole/spot).

Figure 4. DIOS mass spectrum of norepinephrine (59 pmole/spot).
presence of matrices are eliminated. The differences in the manufacturing processes between Siuzdak’s group (Wei et al., 1999) and our laboratory indicates that there still might be a possibility to further improve the quality of porous silica surfaces, their handling and regeneration. MALDI TOF is a powerful tool for studies on proteomics and peptidomics where larger molecules such as proteins and peptides can be successfully analyzed with very high sensitivity. On the other hand, there are many low-molecular mass organic compounds such as chemical neurotransmitters, prostaglandins, steroids etc. that are objects of metabolomics. Such molecules can now be analyzed with the help of standard MALDI TOF equipment providing that some modifications of the target plate are performed.

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REFERENCES


