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Enhancement of Analyte Ionization in Desoprtion/Ionization on Porous Silicon (DIOS)-Mass Spectrometry (MS)

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Abstract Desorption/ionization on silicon mass spectrometry (DIOS-MS) is a relatively new laser desorption/ionization technique for mass spectrometry without employing an organic matrix. This present study was carried to survey the experimental factors to improve the efficiency of DIOS-MS through electrochemical etching condition in structure and morphological properties of the porous silicon. The porous structure of silicon structure and its properties are crucial for the better performance of DIOS-MS and they can be controlled by the suitable selection of electrochemical conditions. The fabrication of porous silicon and ion signals on DIOS-MS were examined as a function of silicon orientation, etching time, etchant, current flux, irradiation, pore size, and pore depth. We have also examined the effect of pre- and post-etching conditions for their effect on DIOS-MS. Finally, we could optimize the electrochemical conditions for the efficient performance of DIOS-MS in the analysis of small molecule such as amino acid, drug and peptides without any unknown noise or fragmentation.

Keywords: porous silicon, desorption/ionization on silicon (DIOS), matrix assisted laser desorption/ionization (MALDI), small molecule analysis

INTRODUCTION

In the past decade, porous silicon (PSi) has received a great deal of attention due to its application in silicon based optoelectronics [1,2], in chemical and biochemical sensing [3,4], and for its direct application in mass spectrometry [5]. In addition, porous silicon is well characterized, and versatile inorganic materials can be produced through galvanostatic, chemical, or photochemical etching procedure in the presence of hydrogen fluoride (HF) [1].

In general, matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) has found widespread use in the analysis of biological macromolecules. In MALDI-MS, the energy absorbing organic matrix transfers energy from a pulsed laser beam to the co-crystallized analyte molecule. This soft ionization technique affords little or no fragmentation of the analytes. However, the use of matrix has several disadvantages including an intolerance to salt, heterogeneous sample incorporation into matrix crystals (hot spots), and significant background ions often obscure and suppress signals from analyte ions in the lower mass range, especially below 500 m/z, which is limiting the use of MALDI-MS. Furthermore, the choice of matrix is often critical for optimal desorption/ionization of particular analytes, and the point to be emphasized is that matrixes are usually limited to UV-absorbing organic acids. To overcome these problems, direct laser desorption/ionization from a variety of surfaces has been studied extensively, but it is not widely used because a high degree of a molecular fragmentation occurs on direct exposure of analytes to laser radiation [8,9]. Nevertheless, direct laser desorption/ionization techniques remain attractive and plausible to small molecule analysis, because they provide simplified sample preparation and eliminate matrix background ions.

Based on the above scenario, Wei *et al.* recently described the use of porous silicon (PSi) as a platform for matrix free laser desorption/ionization (DIOS-MS) [5]. A utility of direct laser desorption/ionization for biomolecular analysis could be highly beneficial owing to dramatically simplified sample preparation, elimination of matrix background ions, and other advantages. PSi is typically derived from crystalline silicon by oxidatively degrading the surface, resulting in a material with significantly enhanced surface area and altered electronic and thermal properties. Despite continuing disagreement in the literature over certain details of the structure-property relationships in PSi, there is widespread agreement that the highly variegated morphology is a defining characteristic of the material. It has been suggested that the morphological features of the PSi in DIOS-MS provide framework in

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which solvent and analyte molecules are retained. The combination of large optical absorption cross section and high thermal conductivity promotes efficient energy transfer from the substrate to adsorbed analyte by which desorption/ionization of the intact analyte occurs upon irradiation with a pulsed laser [5,8].

This matrix-free approach simplifies sample preparation and has a higher salt tolerance than MALDI. While the detection limit of DIOS-MS rivals that of MALDI-MS (high attomole range), the reduction of background ions represents a significant advantage of DIOS-MS, making it a useful technique for the analysis of low molecular weight analytes [5-7]. The applicability of DIOS-MS for quantitative analysis, post source decay (PSD) sequencing, chromatographic separations, and organic reaction monitoring has recently been reported [12]. These studies suggest that analysis of small organic and biological molecules on DIOS-MS is compatible with silicon-based microfluidics and lab-on-a-chip technology [13-15], providing motivation for developing a sound scientific understanding of the principles that govern ion formation and extraction in DIOS. Thus, understanding the factors that affect vaporization and ionization of analytes on PSi is important for optimizing the performance of DIOS-MS.

Even if surface morphology affecting ionization efficiency is conceptually recognized as an important determinant of ionization yields [8,9], there are only fewer studies on the structure-property relationships in DIOS-MS. In the present study, the relationships between surface property of porous silicon in fabrication conditions and DIOS performance were examined as a function of etching solution, etching time, current flux, and post treatment. Therefore, optimization of surface of porous silicon that would avoid manufacturability issues and present a more controlled and homogeneous morphology without matrix promises to give reproducible ion yields, mass accuracy, high resolution, and a significant impact on efforts such as genomic, proteomic, and metabolomic screening.

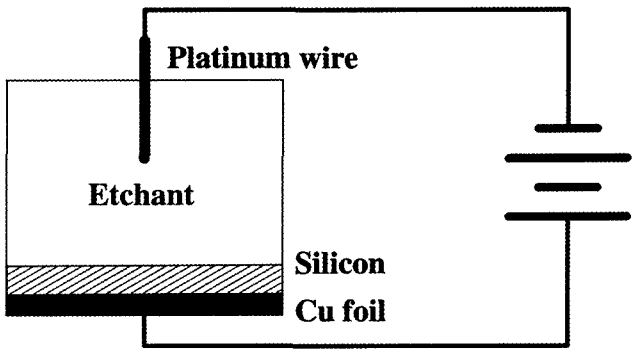

Fig. 1. The schematic diagram of experimental apparatus used to make porous silicon.

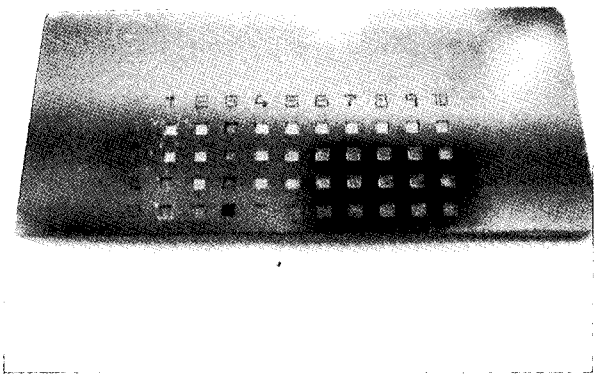

Fig. 2. The photograph of finally fabricated porous silicon substrate. The patterns were generated by illumination through a simple film mask. The translucent areas on the transparency film mask were transformed into active areas on the DIOS substrate.

MATERIALS AND METHODS

Materials

Alanine, Gly-Asp, arginine, atenolol and peptides (bradkynin, d-Arg-bradkynin, melanomacyte stimulating hormone) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Ethanol was obtained from Merck (Darmstadt, Germany). HF (48%) was obtained from J. T. Baker (Mallinckrodt Baker, Inc., NJ, USA). n-type silicon (P-dope,100) wafers (resistivity 0.001~0.02 Ω cm, thickness 500 ± 25 μm) were obtained from Semiconductor Materials & Services (AZ, USA).

Preparation of Porous Silicon

Porous silicon surfaces were prepared by electrochemical etching with an HF/ethanol solution under illumination and constant current flux (Fig. 1). Prior to the etching process, the wafers were sequentially cleaned with ethanol solution in sonic bath and 4:1 (v/v) of H_2SO_4/H_2O_2 solution for 5 min, and finally were rinsed with copious amount of Mili-Q-distilled water. These wafers were dried under nitrogen gas and mounted in a Teflon etching cell. After the electrochemical etching procedure, the etched wafer was rinsed with ethanol and nitrogen. To create the patterns on DIOS substrate during etching, the light was passed through the image mask. This simple procedure produces reproducible patterns of porous silicon on the bulk silicon wafer (Fig. 2).

Field Emission Scanning Electron Microscope (FE-SEM) Image Analysis

SEM images were obtained from field emission microscope using an accelerating voltage of 5.0 keV (XL 30FEG, Philips Electron Co., Netherlands). Top and side views were obtained at varying magnifications to examine the pore morphology. Average pore diameter and the size

distribution of pores on porous silicon surface were measured from enlarged photographs of SEM images using image analysis software (Scion image, Scion corp., ML, USA).

Laser Desorption/Ionization Mass Spectrometry

Laser desorption/ionization mass spectrometric analysis was performed with a Bruker Datonics Biflex IV time of flight mass spectrometry (Bruker, Germany) with delayed extraction condition, operating with a pulsed N_2 laser at 337 nm. The porous substrate with spots of analytes of interest was mounted onto a stainless steel MALDI target using double-side adhesive tape. Positive ion mass spectra were acquired using reflector mode with an accelerating voltage of 20.0 kV. Each spectrum was an average result of 100 laser pulses. The analytical laser intensity was adjusted to obtain good resolution and signal to noise ratio and mass calibration was achieved using external standards.

RESULTS AND DISCUSSION

The importance of preparation of porous silicon for DIOS-MS provides an insight into the strong correlation between surface morphology and performance of DIOS-MS governing desorption/ionization process. Preliminary our study showed that DIOS-MS performance and silicon type (p-type, n-type) were not correlated, suggesting that desorption/ionization process is not induced by only electronic perturbations. It is known that the strong correlation between the performance of DIOS-MS and both the pore size as well as overall porosity suggest that surface morphology plays an essential role in desorption/ionization process [5,11,12,16].

Initially, the relationship among etching time, surface morphology and performance of DIOS-MS under the condition of 24% HF in ethanol and 4 mA/cm^2 was investigated. In Table 1, the longer etching time produced the wider pore size and deeper pore depth rendering sample signals below 3 min etching time. While the smaller pore size (below 20 nm) and lower porosity were the ineffective parameters for DIOS-MS, it is clear that the 30 nm pores are enough for the efficient performance of desorption/ionization (Table 1).

Since the ionization efficiency of peptides is generally superior to that of small molecules, our results indicated that the small pores and shallow pores are efficient for ionization of peptide instead of small molecules (Table 1). It is reasonable to hypothesize that small and shallow pores have low energy trapping performance to necessitate ionization and desorption of small molecules. The relatively larger pore size (above 20 nm) produced reliable DIOS-MS signals for the intermediate-mass range (0~1,000 Da), but smaller pores did not produce efficient desorption/ionization of low mass analytes. Thus, it is evident that suitable pore size and porosity are the main determinants to provide an efficient platform for DIOS-MS. In addition, the increase of etching time resulted in the increase of pore depth. However, the deeper pore depth always do not produce reliable strong signal, which also indicates that even pore depth should be considered for an efficient ionization on DIOS-MS. The deeper pore depth may make the ionization of analytes difficult at certain level due to demand of high amount of desorption and ionization energy from the deeper site of pores.

Table 1. The effect of etching time and pore morphology on the effectiveness of DIOS-MS; Examined samples (each 100 nmol) are mixture of alanine (m/z 90.10), Gly-Asp (m/z 191.16), and bradkynin peptide (m/z 904.50); the calculated average values were obtained from three times experiment results using scion image analysis program

Etching time (min)	Pore diameter (nm)	Pore depth (nm)	Ala	GlyAsp	Bradk	Noise
0.5	17.4	180.2	×	×	Δ	medium
1	35.0	350.5	×	Δ	Δ	medium
2	32.5	740.1	Δ	Δ	○	low
3	39.5	870.2	○	○	○	low
5	N.D.	N.D.	×	×	×	high
10	N.D.	N.D.	×	×	×	high
30	N.D.	N.D.	×	×	×	high

N.D.: Because the surface was highly interconnected pore structure and looks like stacked meshed network, the property of pore morphology cannot be determined.
○: reproducible and clear spectrum of $[M+H]^+$ ion
Δ : peaks detectable, but not adequate for reproducibility due to weak signal intensity
× : not detected

Furthermore, longer etching time (above 5 min) resulted in very fragile porous silicon surfaces and highly intense unknown low-mass background ion signals. Therefore, surface morphology appears to be interconnected and to propagate anisotropically (Fig. 3B). In contrast, the surface with shorter etching time (below 3 min) had much more compact and low porosity structure with small pore arrays as shown in Fig. 3A.

Subsequently, experiments were carried out by selecting optimized current density to investigate the variation of pore diameter at various current densities. By increasing the current flux from 1 mA/cm^2 to 10 mA/cm^2, the pore diameter was gradually increased. However, pore size did not enlarge from 3 mA/cm^2 within reliable measuring range (Fig. 4). According to the "current burst model" [17], the employed current density must be too high to allow selective pore growth at the formation of pores, because the average current density that can be carried by all current bursts fitting in the given area is smaller than the externally fixed current density. The current flowing through the liquid-solid interface was spatially and temporally inhomogeneous. Therefore, the pore formation was correlated with the amount of existing Si or oxidation component, *i.e.* electrolyte. Furthermore, the DIOS-MS gave almost the same resolution and signal to noise ratio of analyte under the conditions above 3 mA/cm^2,

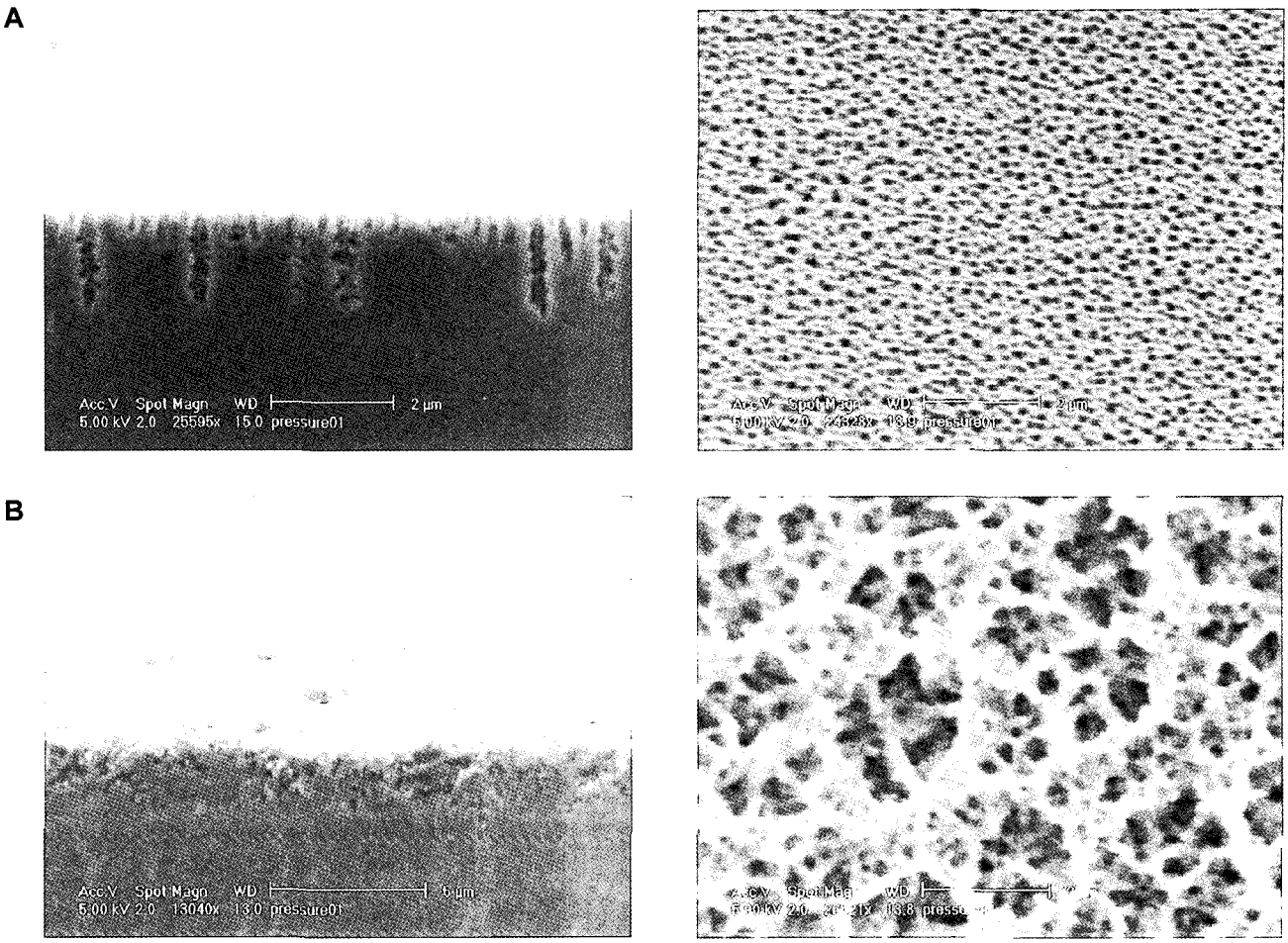

Fig. 3. The SEM images of pore morphology and overall porosity. A; Top and side view after 3 min etching time B; Top and side view after 10 min etching time.

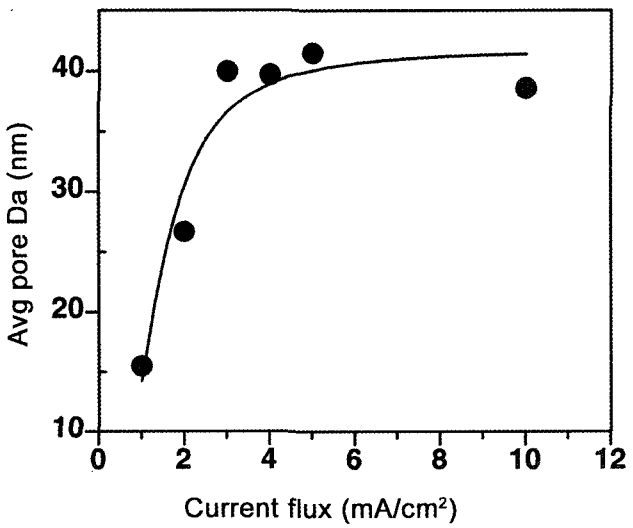

Fig. 4. The relationship between current density and pore size. Electrochemical etchings were performed in the presence of 24% HF for 3 min etching time with various current fluxes.

which indicated that the pertinent pore size for efficient desorption/ionization was approximately from 35 to 40 nm.

As it is well known that DIOS performance gradually degrades with increasing surface oxidation, previous study reported that by simply soaking the porous silicon surface in the HF solution ("double etching") removed the oxidized layer from the surface and regenerated the efficiency of DIOS-MS [12]. The double etched porous silicon surface showed wider openings at the surface, but the pore depths were similar to the single etched porous silicon surface (Fig. 5A). However, the double-etched surfaces have higher threshold energy for desorption and they require higher laser intensity in mass experiment and thus induced poor mass spectra at same concentration of analytes in our experiment conditions (Fig. 5B). It is reasonable to note that an increase of pore diameter and porosity is detrimental to the efficiency of DIOS-MS beyond optimized pore morphology.

The performance of optimized porous silicon was examined with a mixture containing small amino acid like alanine, and various molecular weight of peptides below 2,000 Da range (Fig. 6). Fig. 6 shows the mass spectrum

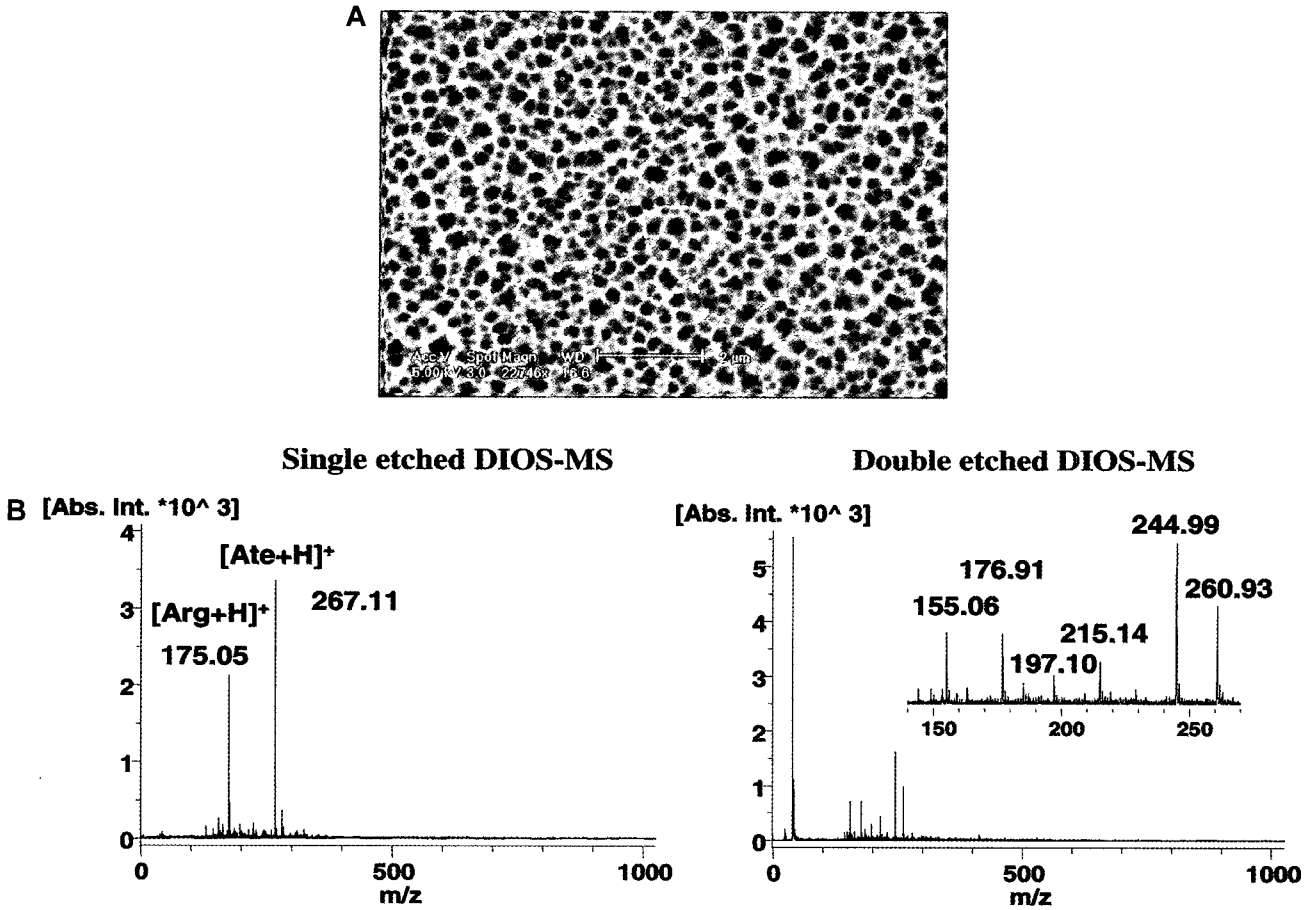

Fig. 5. SEM image of the "double etching porous silicon surface" prepared by dipping the silicon substrate in the 5% HF solution for 1 min and comparison of DIOS performance of single and double etched porous silicon surface. A; SEM image of the double etched surface: the average pore diameter was increased from 39.5 to 74.5 nm owing to post-treatment of HF solution for 1 min. B; Mass spectra difference between single and double etched surface; In the case of the double etched surface, the unknown noise ions (notably m/z 155, 177, 197, 215, 245, and 261) were detected without the signals of analytes. The examined samples are arginine (m/z 175.05) and atenolol (m/z 267.11) at a concentration of 100 nmol each.

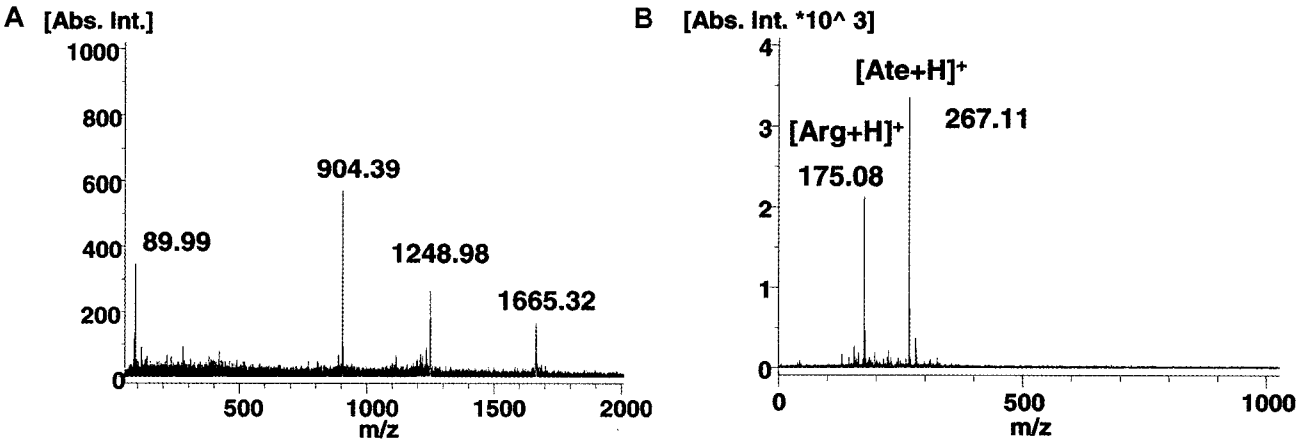

Fig. 6. Representative examples of mass spectrum obtained with optimized porous silicon surface. A; The DIOS mass spectrum of a mixture of small aminoacid (alanine m/z 89.99) and three peptides (10 nmol each) including bradkynin (m/z 904.39), d-arg-bradkynin (m/z 1248.98), and α-melanocyte stimulating hormone (m/z 1665.32), B; The DIOS mass spectrum of a mixture of 10 nmol of arginine (m/z 175.08) and atenolol (m/z 267.11).

on DIOS-MS for the sample mixture. All components in the mixture were simultaneously ionized and desorbed on the optimized porous silicon. Clearly, analysis of a mixture of small molecules using MALDI-MS with organic matrix could be achieved under suitable condition, but the matrix interference is still a real problem. However, this problem can be solved by using porous silicon as described in our experiments. Even if unknown noise ions appear below 300 Da as reported on the previous work by Shen *et al.* [12], our optimized porous silicon surface did not produced any significant noise signals from 0 to 2,000 Da. It is also noteworthy that none of the silicon containing adduct, fragment or unknown compounds have been observed in our optimized porous silicon, indicating the inert nature of the porous silicon material. Hence, the optimized porous silicon surface was able to detect a variety of small compounds including amino acid, drug, and peptides with no fragmentation.

CONCLUSION

The present study describes that the DIOS-MS techniques strictly depend on the formation and treatment of the porous silicon surfaces, which could determine the quality of the DIOS-MS spectra. The characterization of surface morphology indicated the correlations between porous silicon structure and the performance of DIOS-MS. The optimized experimental condition (current flux; 3 mA/cm^2, etching time; 3 min, etching solution; 24% HF) produced efficient signals of small molecules without any background ions. Even though the investigations of desorption/ionization mechanisms of DIOS are going in our laboratory, the DIOS may provide an important detection system of lab on a chip and can be a promising method for high throughput screening of small molecules.

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REFERENCES

[1] Cullis, A. G., L. T. Canham, and P. D. J. Calcott (1997) The structural and luminescence properties of porous silicon. *J. Appl. Physics* 82: 909-965.

[2] Brus, L. (1994) Luminescence of silicon materials-chains, sheets, nanocrystals, nanowires, microcrystals, and porous silicon. *J. Phys. Chem.* 98: 3575-3581.

[3] Sailor, M. J. and E. J. Lee (1997) Surface chemistry of luminescent silicon nanocrystallites. *Adv. Materials* 9: 783-&.

[4] Lin, V. S. Y., K. Motesharei, K. P. S. Dancil, M. J. Sailor, and M. R. Ghadiri (1997) A porous silicon-based optical interferometric biosensor. *Science* 278: 840-843.

[5] Wei, J., J. M. Buriak, and G. Siuzdak (1999) Desorption-ionization mass spectrometry on porous silicon. *Nature* 399: 243-246.

[6] Tuomikoski, S., K. Huikko, K. Grigoras, P. Ostman, R. Kostiainen, M. Baumann, J. Abian, T. Kotiaho, and S. Franssila (2002) Preparation of porous n-type silicon sample plates for desorption/ionization on silicon mass spectrometry (DIOS-MS). *Lab on a Chip* 2: 247-253.

[7] Go, E. P., J. E. Prenni, J. Wei, A. Jones, S. C. Hall, H. E. Witkowska, Z. X. Shen, and G. Siuzdak (2003) Desorption/ionization on silicon time-of-flight/time-of-flight mass spectrometry. *Anal. Chem.* 75: 2504-2506.

[8] Cohen, S. L. and B. T. Chait (1996) Influence of matrix solution conditions on the MALDI-MS analysis of peptides and proteins. *Anal. Chem.* 68: 31-37.

[9] Westman, A., T. Huthfehre, P. Demirev, and B. U. R. Sundqvist (1995) Sample morphology effects in matrix-assisted laser-desorption ionization mass-spectrometry of proteins. *J. Mass Spectrometry* 30: 206-211.

[10] Garden, R. W. and J. V. Sweedler (2000) Heterogeneity within MALDI samples as revealed by mass spectrometric imaging. *Anal. Chem.* 72: 30-36.

[11] Stewart, M. P. and J. M. Buriak (2000) Chemical and biological applications of porous silicon technology. *Adv. Materials* 12: 859-869.

[12] Shen, Z. X., J. J. Thomas, C. Averbuj, K. M. Broo, M. Engelhard, J. E. Crowell, M. G. Finn, and G. Siuzdak (2001) Porous silicon as a versatile platform for laser desorption/ionization mass spectrometry. *Anal. Chem.* 73: 612-619.

[13] Lee, J. Y., J. J. Kim, and T. H. Park (2003) Miniaturization of polymerase chain reaction. *Biotechnol. Bioprocess Eng.* 8: 213-220.

[14] Li, C., W. C. Lee, and K. H. Lee (2003) Affinity separations using microfabricated microfluidic devices: *In situ* photopolymerization and use in protein separations. *Biotechnol. Bioprocess Eng.* 8: 240-245.

[15] Park, S. S., H. S. Joo, S. I. Cho, M. S. Kim, Y. K. Kim, and B. G. Kim (2003) Multi-step reactions on microchip platform using nitrocellulose membrane reactor. *Biotechnol. Bioprocess Eng.* 8: 257-262.

[16] Cuiffi, J. D., D. J. Hayes, S. J. Fonash, K. N. Brown, and A. D. Jones (2001) Desorption-ionization mass spectrometry using deposited nanostructured silicon films. *Anal. Chem.* 73: 1292-1295.

[17] Christophersen, M., J. Cartensen, and H. Foll (2000) Macropore formation on highly doped n-type silicon. *Physica Status Solidia Applied Research* 182: 45-50.

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