Anal. Chem. **2005,** *77,* 1641−1646

Desorption/Ionization on Silicon Nanowires

E. P. Go,[†,‡] J. V. Apon,[†,‡] G. Luo,[§] A. Saghatelian,[†] R. H. Daniels,[∥] V. Sahi,[∥] R. Dubrow,*[,∥] B. F. Cravatt,[†] A. Vertes,[§] and G. Siuzdak*[,†]

Department of Molecular Biology and The Center for Mass Spectrometry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, Mass Consortium Corporation, San Diego, California 92109, Department of Chemistry, George Washington University, Washington, DC 20052, and Nanosys, Inc., 2625 Hanover Street, Palo Alto, California 94304

Dense arrays of single-crystal silicon nanowires (SiNWs) have been used as a platform for laser desorption/ionization mass spectrometry of small molecules, peptides, protein digests, and endogenous and xenobiotic metabolites in biofluids. Sensitivity down to the attomole level has been achieved on the nanowire surfaces by optimizing laser energy, surface chemistry, nanowire diameter, length, and growth orientation. An interesting feature of the nanowire surface is that it requires lower laser energy as compared to porous silicon and MALDI to desorb/ionize small molecules, therefore reducing background ion interference. Taking advantage of their high surface area and fluid wicking capabilities, SiNWs were used to perform chromatographic separation followed by mass analysis of the separated molecules providing a unique platform that can integrate separation and mass spectrometric detection on a single surface.

Surface-based mass spectrometry approaches have been widely applied to problems in protein identification, small-molecule metabolite characterization, and synthetic organic chemistry. Among the most established techniques is matrix-assisted laser desorption/ionization (MALDI), which provides for soft ionization and high-sensitivity analysis.[1,2] However, MALDI is typically limited to the analysis of molecules above ~700 Da. The requirement of a matrix material imparts this limitation due to matrix background signals observed in the low-mass range. As an alternative, nano- to micrometer-size particles consisting of metals[1,3] and carbon[4,5] have been investigated to extend the observable mass below 500 Da. Good quality mass spectra were obtained from organic compounds, peptides, proteins, and polymers using a UV laser. More recently, laser desorption/ionization of analytes from a porous silicon (pSi) surface has been reported.[6] Porous silicon was developed as a matrix-free desorption/ionization approach,[6−8] where the absence of matrix-related ions extends the observable mass range to small molecules. It is believed that the high surface area, low thermal conductivity, and high UV absorptivity of pSi enabled its successful application to desorption/ionization on silicon mass spectrometry (DIOS-MS).[6,7,9]

Single-crystal silicon nanowires (SiNWs) have been the subject of extensive research in electronics, photonics, optoelectronics, sensing, and other novel device applications.[10−15] They share many of the same basic properties of pSi and appear to be a good platform for surface-based mass spectrometry. In contrast to pSi, SiNWs are catalyzed and grown on the surface of a substrate and their physical dimensions, composition, density, and position can be precisely controlled at the nanoscale level thus offering even greater potential for designing mass spectrometry active surfaces.

SiNWs can be prepared through chemical vapor deposition, laser ablation of Si targets, liquid crystal templating methods, laser-assisted catalytic growth, vapor−liquid−solid (VLS) growth mechanism, and supercritical fluid methods.[16−23] In this study, surface-deposited Au colloid with a defined diameter was used as a growth catalyst. This method is very flexible and allows the control of multiple growth parameters such as length, diameter, and density as well as being compatible with growing SiNWs on a variety of

* To whom all correspondence should be addressed, E-mail: rdubrow@nanosysinc.com; siuzdak@scripps.edu.

[†] The Scripps Research Institute.

[‡] Mass Consortium Corp.

[§] George Washington University.

[∥] Nanosys, Inc.

(1) Tanaka K.; Waki H.; Ido Y.; Waki H.; Yoshida Y.; Yoshida T. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 151.
(2) Karas, M.; Hillenkamp, F. *Anal. Chem.* **1988**, *60*, 2299−301.
(3) Schurenberg, M.; Dreisewerd, K.; Hillenkamp, F. *Anal. Chem.* **1999**, *71*, 221−29.
(4) Dale, M. J.; Knochenmuss, R.; Zenobi, R. *Anal. Chem.* **1996**, *68*, 3321−9.
(5) Sunner, J.; Dratz, E.; Chen, Y. C. *Anal. Chem.* **1995**, *67*, 4335−42.
(6) Wei, J.; Buriak, J. M.; Siuzdak, G. *Nature* **1999**, *399*, 243−6.
(7) Shen, Z. X.; Thomas, J. J.; Averbuj, C.; Broo, K. M.; Engelhard, M.; Crowell, J. E.; Finn, M. G.; Siuzdak, G. *Anal. Chem.* **2001**, *73*, 612−9.
(8) Cuiffi, J. D.; Hayes, D. J.; Fonash, S. J.; Brown, K. N.; Jones, A. D. *Anal. Chem.* **2001**, *73*, 1292−5.
(9) Kruse, R. A.; Li, X. L.; Bohn, P. W.; Sweedler, J. V. *Anal. Chem.* **2001**, *73*, 3639−45.
(10) Cui, Y.; Wei, Q. Q.; Park, H. K.; Lieber, C. M. *Science* **2001**, *293*, 1289−92.
(11) Cui, Y.; Lieber, C. M. *Science* **2001**, *291*, 851−3.
(12) Huang, Y.; Duan, X. F.; Lieber, C. M. *Abstracts of Papers of the American Chemical Society* **2002**, *224*, U308.
(13) Zhou, X. T.; Hu, J. Q.; Li, C. P.; Ma, D. D. D.; Lee, C. S.; Lee, S. T. *Chem. Phys. Lett.* **2003**, *369*, 220−4.
(14) Duan, X. F.; Huang, Y.; Agarwal, R.; Lieber, C. M. *Nature* **2003**, *421*, 241−5.
(15) Hahm, J.; Lieber, C. M. *Nano Lett.* **2004**, *4*, 51−4.
(16) Morales, A. M.; Lieber, C. M. *Science* **1998**, *279*, 208−11.
(17) Lieber, C. M. *Solid State Commun.* **1998**, *107*, 607−16.
(18) Cui, Y.; Lauhon, L. J.; Gudiksen, M. S.; Wang, J. F.; Lieber, C. M. *Appl. Phys. Lett.* **2001**, *78*, 2214−6.
(19) Gudiksen, M. S.; Lieber, C. M. *J. Am. Chem. Soc.* **2000**, *122*, 8801−2.
(20) Duan, X. F.; Lieber, C. M. *Abstr. Pap. Am. Chem. Soc.* **2000**, *219*, U874−5.
(21) Lyons, D. M.; Ryan, K. M.; Morris, M. A.; Holmes, J. D. *Nano Lett.* **2002**, *2*, 811−6.
(22) Wu, Y.; Yan, H.; Huang, M.; Messer, B.; Song, J. H.; Yang, P. *Chem.* **2002**, *8*, 1260−8.
(23) Ma, D. D. D.; Lee, C. S.; Au, F. C. K.; Tong, S. Y.; Lee, S. T. *Science* **2003**, *299*, 1874−7.

10.1021/ac048460o CCC: $30.25
Published on Web 02/17/2005

substrates including silicon, glass, ceramics, and metals. In addition, SiNWs can be grown in continuous fields or patterned using lithographic methods to provide precise positional control of the nanostructured surface at the micro- to millimeter scale. Typically, SiNWs are grown from 10 to 60 nm in diameter and up to 100 μm in length, and the nanowire density can also be controlled by varying the density of the catalyst deposited onto the growth surface (typical densities for this application are between 1 and 10 wires/μm^2).

Herein, we examine the effect of laser energy, nanowire density, nanowire size, and growth orientation on the SiNW performance as a platform for matrix-free mass spectrometry using peptides and small drug molecules as model compounds. Recently developed chemical modification on ozone-oxidized pSi, which has proven essential to achieve high sensitivities,[24] was employed on the SiNWs. In addition, we have observed that this surface has strong fluid wicking properties driven by capillary action generated in the interstitial spaces between SiNWs. We exploit this property in the chromatographic separation and subsequent MS analysis of endogenous and xenobiotic metabolites in biofluids and small drug molecules. The use of planar separation prior to MS analysis has been widely reported.[25−27] The combination of the specific detection of MS and the simplicity of planar chromatography allow for the direct analysis of complex mixtures.

EXPERIMENTAL SECTION

Materials. Aqueous stock solutions of verapamil (MW 454.6), propafenone (MW 341.4), midazolam (MW 325.8), and des-Arg^9-bradykinin (MW 904.0) were prepared by reconstituting lyophilized samples in deionized water at 1 mg/mL followed by subsequent serial dilution done as needed. Stock solutions of tenoxicam and piroxicam were prepared at 2 mg/mL in dichloromethane. The MALDI matrixes α-cyano-4-hydroxycinnamic acid (CHCA), sinapinic acid (SA), and 2,5-dihydrobenzoic acid (DHB) were obtained from Sigma. The thermometer molecule (TM) used for internal energy determination, 3-methoxybenzylpyridinium chloride, was custom synthesized and characterized by Celestial Specialty Chemicals (Nepean, ON, Canada). Bovine serum albumin (BSA) and flock house virus (FHV) proteolytic digests were prepared with trypsin (1:30 enzyme-to-protein ratio by mass). The proteins were denatured at 90 °C for 20 min. FHV was reduced and alkylated with dithiothreitol and iodoacetamide, respectively. The tryptic digests were incubated overnight at 37 °C in 5 mM ammonium citrate buffer (pH 7.5). The enzymatic reaction reached completion within 18 h, yielding a final BSA and FHV concentration of 1 μM, respectively. Samples (0.5 μL) were pipetted directly onto the chemically modified SiNW surfaces. High purity grade reagents were all obtained from Sigma except for PFPPDCS and trypsin, which were obtained from Gelest, Inc. and Promega, respectively.

Preparation of SiNWs. SiNWs were synthesized using Au nanocluster catalyzed VLS growth mechanism.[18] Size-selected Au colloid particles were deposited on silicon wafers to produce high-quality SiNWs with a narrow diameter distribution. Briefly, this method employs Au nanoparticles with diameters of 10, 20, and 40 nm distributed on a silicon substrate by spin-coating. Colloids were deposited at densities of 1−10 wires/μm^2, which was verified by scanning electron microscopy (SEM). After removing solvents and organic residues, the substrates were placed in a 480 °C chemical vapor deposition furnace to grow SiNWs with silane (SiH_4) as the vapor-phase reactant.

Derivatization of SiNWs. SiNWs were etched in 5% HF solution to remove the native oxide layer and subsequently reoxidized with ozone. The surfaces were then modified with a silylating reagent. Surface derivatization involved the modification of OH groups present on the ozone-oxidized SiNWs by silylation with (pentafluorophenyl)propyldimethylchlorosilane (PFPPDCS). This modification generates a perfluorophenyl-derivatized SiNW surface. The silylation reaction was performed by adding 15 μL of the PFPPDCS on the oxidized SiNW, which was placed in a glass Petri dish and incubated in an oven at 90 °C for 15 min. The chemically modified SiNW surface was rinsed thoroughly with methanol and was dried in a stream of N_2.

Scanning Electron Microscopy. Nanowire diameter, length, and densities were measured using a JEOL 6460LV SEM. The samples were mounted on the stage with brass clips and analyzed in their native condition.

Mass Spectrometry. DIOS-MS measurements were performed with an Applied Biosystems (Framingham, MA) Voyager STR time-of-flight reflectron mass spectrometer. The SiNW surfaces were attached to a modified MALDI target plate using conductive carbon tape, and samples were irradiated with a nitrogen laser operated at 337 nm at 5 Hz (3-ns pulse duration) and attenuated with a neutral density filter. Ions produced by laser desorption were energetically stabilized during a delayed extraction period of 25−250 ns and then accelerated through the linear time-of-flight reflectron mass analyzer with a 2-kV pulse. The MS spectra were generated by averaging between 50 and 500 laser pulses. The laser intensity was set to optimize the signal-to-noise ratio and the resolution of the mass spectral data of the analyte.

Survival Yield Measurements and Calculations. Signal averaging of 300 TM spectra was used to determine the survival yield (SY) of TM molecular ions at various fluence levels. The survival yield method was used to determine the internal energy distribution of ions generated by electrospray ionization.[28−30] It is defined by the equation,

$$\mathrm{SY} = \frac{I_{\mathrm{M}}^{+}}{I_{\mathrm{M}}^{+} + I_{\mathrm{F}}^{+}} \qquad (1)$$

where I_M^+ is the intensity of the parent molecular ion and I_F^+ is the sum of the intensities of the fragment ions. The data acquisition protocol was designed to minimize the influence of spot-to-spot and shot-to-shot variations in the mass spectra. Identical procedures were followed for the three different SiNW

(24) Trauger, S. A.; Go, E. P.; Apon, J. V.; Shen, Z.; Finn, M. G.; Siuzdak, G. *Anal. Chem.* **2004**, *76*, 4484−9.
(25) Crecelius, A.; Clench, M. R.; Richards, D. S.; Parr, V. *J. Chromatogr., A* **2002**, *958*, 249−60.
(26) Guittard, J.; Hronowski, X. P. L.; Costello, C. E. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 1838−49.
(27) Gusev, A. I. *Fresenius J. Anal. Chem.* **2000**, *366*, 691−700.
(28) Drahos, L.; Heeren, R. M. A.; Collette, C.; De Pauw, E.; Vekey, K. *J. Mass Spectrom.* **1999**, *34*, 1373−9.
(29) Collette, C.; Drahos, L.; De Pauw, E.; Vekey, K. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 1673−8.
(30) Collette, C.; De Pauw, E. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 165−70.

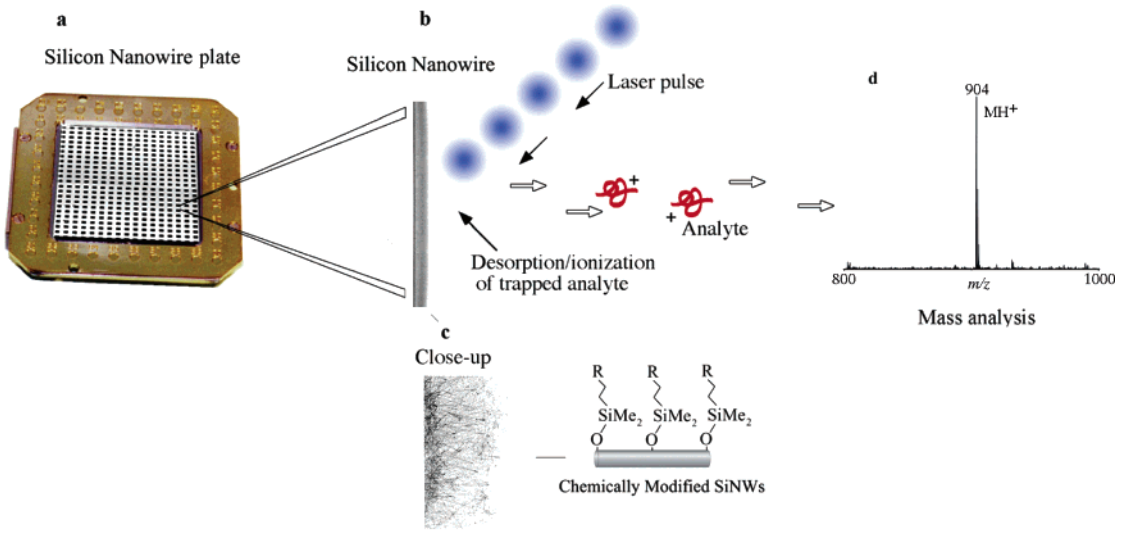

Figure 1. Configuration of laser desorption/ionization mass spectrometry experiment showing (a) patterned SiNWs grown on a Si substrate attached to a modified MALDI plate, (b) schematic of laser desorption/ionization of trapped analytes within the Si nanowire mesh, (c) closeup SEM image of SiNWs and an illustration of the functionalities by silylation, and (d) mass spectra of 500 amol of des-Arg^9-bradykinin using a 40-nm-diameter SiNW. The measured signal-to-noise ratio was 600 to 1.

surfaces, the pSi surface, and the CHCA, SA, and DHB MALDI matrixes. Details of the procedure are explained elsewhere.[31]

Preparation of Serum Samples. Human serum sample was spiked with cocaine (MW 303.1) at a concentration of 3 μM. The serum sample was deproteinized by adding 400 μL of cold methanol to 100 μL of spiked serum. The resulting mixture was mixed using a vortexer and centrifuged at 12 000 rpm for 10 min. The methanol extract was then transferred to an Eppendorf and evaporated to dryness in a stream of nitrogen. The dried sample was reconstituted in 50:50 methanol/water mixture, and a 5-μL aliquot of the sample was applied to the SiNW plate.

Extraction and Isolation of *N*-Acetylethanolamines (NAEs). A 2:1:1 extraction solution of $CHCl_3$/MeOH/Tris buffer (50 mM, pH8), 8 mL per brain and 4 mL per spinal cord, was placed into an 8-mL glass vial. The vials were shaken to mix the solutions and placed on ice along with the glass dounce tissue grinders. The isolated tissue was placed into the extraction solution and homogenized using dounce tissue grinders. The sample was poured back into the 8-mL vial and placed on ice while other samples were being prepared. The homogenized samples were centrifuged at 2500 rpm for 10 min at 4 °C by placing the 8-mL glass vials into 50-mL plastic conical tubes. After centrifugation, the organic layer (bottom) and the aqueous layer (top) were clearly distinguishable with a layer of insoluble material between them. A pipet was carefully inserted into the bottom layer and the organic layer was removed and transferred into a 4-mL vial and concentrated under a stream of nitrogen. After the sample was concentrated, methanol (50 μL) was added and gently swirled around the vial. This solution was used directly for the analysis.

Chromatographic Separation. Perfluorophenyl-derivatized SiNW surfaces were used as chromatographic separation plates. Prior to separation, the plates were heated at 90 °C for 15 min and were allowed to cool at room temperature. The SiNW plates were blotted with 5-μL aliquot of the sample. The separation of the small drug molecules and plasma sample was performed using methanol/water (8:2 v/v) mixture while a chloroform/methanol/ethyl acetate (58:24:8; v/v) mixture was used for the mouse spinal cord tissue. The separation was done in a beaker covered with transparent plastic film. The chromatogram was developed for 30 min. The SiNW surface was dried, and spots were visualized by illuminating the surface with 254-nm UV light. The retention factor (R_f) was calculated by taking the ratio of the distance of the sample spot from the origin to the distance of the solvent front from the origin.

RESULTS AND DISCUSSION

The application of silylated SiNWs to laser desorption/ionization mass spectrometry (Figure 1) was examined as a function of nanowire diameter, length, density, and growth orientation. BSA and FHV tryptic digests, small drug molecules (midazolam, MH^+ 325, propafenone, MH^+ 342; verapamil, MH^+ 455), and a standard peptide (des-Arg^9-bradykinin, MH^+ 904) were used as model compounds to examine desorption/ionization properties of SiNWs. In the initial set of experiments, we determined the material characteristics in terms of SiNW length and density to generate ion signals. Using the growth methods described here, the NW growth orientation randomly varies from horizontal to vertical and is independent of wire density and diameter. Our initial observation reveals that the performance was dependent on both the layer thickness (wire length) and wire density. Mass spectral data obtained with 10-, 20-, and 40-nm diameter silylated SiNW surfaces gave signal-to-noise levels and mass ranges very similar to the silylated pSi surface, and the data were obtained reproducibly with NW densities of less than 10 wires/μm^2 and wire length of less than 5 μm. The detection sensitivity of the silylated SiNWs used in this study was 500 amol for des-Arg^9-bradykinin which is 6 orders lower in magnitude compared to the sensitivity obtained from the silylated pSi reported recently.[24] As a control, silylated planar silicon substrate (with no SiNWs) was tested, and our results show no detectable signal for peptides at 5 μM. Figure 1 shows a scanning electron micrograph of the structured surface we investigated, the length of the wires

(31) Luo, G.; Marginean, I.; Vertes, A. *Anal. Chem.* **2002**, *74*, 6185−90.

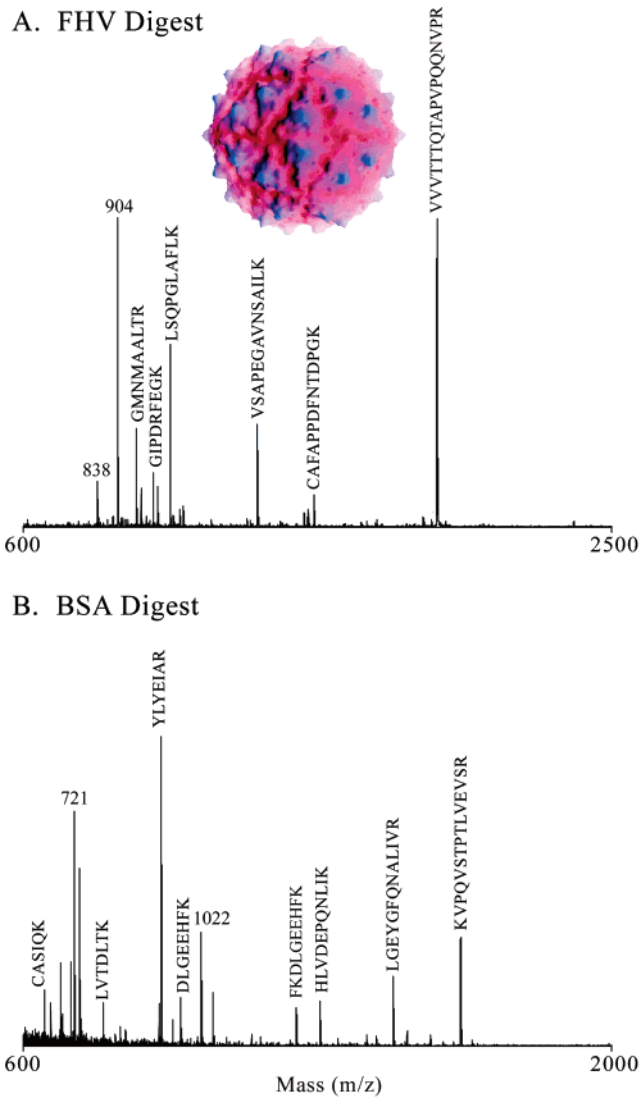

Figure 2. Laser desorption/ionization from a 40-nm-diameter silylated silicon nanowires of (A) 50 fmol of FHV) and (B) 5 fmol of BSA digests showing the cleavage peptides that have been identified. The data were searched with Mascot to identify the proteins with a confidence level of greater than 99%.

were varied from 0.5 to 10 μm, and the wires were deposited at various densities from 1 to 50 wires/μm^2.

Because the electrical and optical properties of the SiNWs are strongly dependent on the nanowire length and density, the effect of the diameter on the laser desorption/ionization performance was also examined. SiNWs with ~1 μm in length and diameters of 10, 20, and 40 nm were tested for the analysis of small molecules and peptides. In contrast to the strong dependence on wire length and density, we did not observe a clear difference in the performance of the nanowire surfaces with varying diameter. MS analysis of small molecules and protein digests were obtained reproducibly on SiNWs with diameters between 10 and 40 nm and 1 μm in length with sensitivity at the picomole to the attomole level. The data obtained from the digest were searched against SwissProt database using Mascot (MatrixScience) to identify BSA and FHV with a score greater than 99% confidence level (Figure 2). Typically, MS analysis on the 10−40-nm-diameter SiNWs provided a detection limit of 50 fmol for small molecules while 40-nm-diameter SiNWs provided a detection limit of 500 amol for peptides (Figure 1). With further optimization of SiNW fabrication and surface treatment, the detection limit should easily be improved.

Laser Energy Requirement for Desorption/Ionization. The minimum laser energy required to desorb/ionize analytes from SiNWs was also examined. Interestingly, SiNWs required significantly lower energy than pSi or MALDI (Figure 3A), and as a result, very little surface-related background ions were observed from the SiNWs. This characteristic is especially useful in the analysis of small molecules wherein desorption/ionization can be performed with laser energy as low as 0.3 μJ (Figure 3B).

Because SiNW required much lower laser energy to desorb molecules, we further examined the energy transferred from the SiNWs during ionization and compared this to pSi and commonly used matrixes (CHCA, SA, DHB) in MALDI. To quantitatively compare the amount of internal energy in the generated ions, the SY of the analyte ions generated on different types of SiNW surfaces was obtained using 3-methoxybenzylpyridinium chloride, as model TM. The SY method is an approach used to determine the internal energy of analyte ions produced after desorption. The internal energy, which is exclusively a combination of rotational and vibrational energies, is calculated based on the fragmentation pattern of the TMs and the intensity ratio between the molecular and fragment ions. [31,32] Our results show that, except for the perfluorinated, silylated abraded SiNW surface, 3-methoxybenzylpyridinium ions have the highest SY, i.e., the lowest internal energy originating from SiNW surfaces compared to CHCA, SA, and DHB MALDI matrixes (Figure 3C). However, it is also apparent from the data that abraded SiNW surfaces require the lowest laser energy to induce fragmentation of the TM molecule. This result suggests that not only SiNWs surfaces can efficiently absorb energy during desorption, but depending on its morphology, they can also efficiently transfer this energy to analyte molecules.

A possible explanation of the remarkable (7-fold) reduction in desorption/ionization threshold compared to DIOS and MALDI can be derived from the optical near-field effect. Upon laser irradiation, the SiNWs act as tiny antennas producing significant field enhancement in the vicinity of the nanometer sharp tip.[33,34] Thus, the laser energy is efficiently focused to a small area (commensurate with the cross section of the NWs). The resulting focusing effect [35,36] could generate a field desorption effect resulting in the gas-phase generation of the deposited analyte molecules. The field enhancement phenomenon resembles the light-focusing effect observed in apertureless scanning near-field optical microscopy with the benefits of multiple tips and the proximity of the target.

Applications to Chromatographic Separation of Molecules. One of the potentially useful applications of SiNWs is in the area of chromatography. Like thin-layer chromatographic plates, SiNWs can be employed as a platform for the separation

(32) Collette, C.; Drahos, L.; De Pauw, E.; Vekey, K. *Rapid Commun. Mass Spectrom.* **1998**, *12*, 1673−8.
(33) Royer, P.; Barchiesi, D.; Lerondel, G.; Bachelot, R. *Philos. Trans. Ser. A-Math. Phys. Eng. Sci.* **2004**, *362*, 821−42.
(34) Wang, K. L.; Barkan, A.; Mittleman, D. M. *Appl. Phys. Lett.* **2004**, *84*, 305−7.
(35) Bouhelier, A.; Beversluis, M. R.; Novotny, L. *Ultramicroscopy* **2004**, *100*, 413−9.
(36) Hartschuh, A.; Beversluis, M. R.; Bouhelier, A.; Novotny, L. *Philos. Transac. Ser. A-Math. Phys. Eng. Sci.* **2004**, *362*, 807−19.

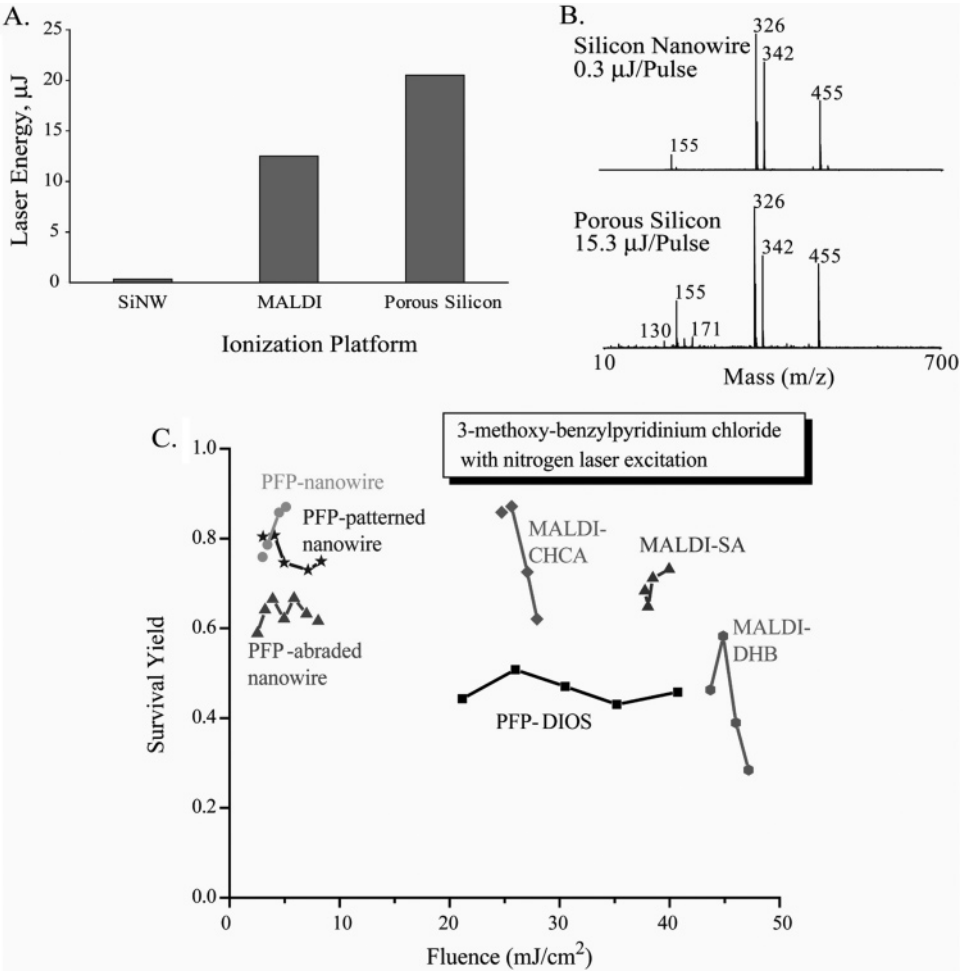

Figure 3. (A) Laser energy per pulse vs MS platforms used for laser desorption/ionization analysis. (B) A comparison of the laser energy required to desorb/ionize small molecules (midazolam, *m*/*z* 326; propafenone, *m*/*z* 342; verapamil, *m*/*z* 455, 500 fmol) on the two MS platforms. (C) Survival yield results for 3-methoxybenzylpiridinium ion on silylated SiNWs, silylated pSi, and the commonly used matrixes (CHCA, SA, DHB) in MALDI.

of molecules. As in thin-layer chromatography, capillary forces are employed to transport the analytes in the mobile phase, allowing analytes applied on the stationary phase to move at different rates ultimately allowing separation.[37] The capability of SiNWs to separate a simple sample mixture lies in its high surface-to-volume ratio and in the differences in analyte−surface interactions. When combined with its ability to support laser desorption/ionization mass spectrometry, chromatographic separation followed by MS analysis with SiNWs provides a simple, inexpensive, rapid, and qualitative means to separate and analyze sample mixtures. We demonstrate this in the analysis of a mixture of two small drug molecules (tenoxicam *m*/*z* 338; piroxicam *m*/*z* 332). The silylated SiNW surface allowed for the migration and separation of the sample mixture (R_f = 0.69, 0.56 for tenoxicam and piroxicam, respectively) as demonstrated by the fluorescence of the drug molecules when irradiated with 254-nm UV light. The resolution of the two drug molecules was evident from the two characteristic fluorescent spots observed from the SiNW plate. At least three different SiNW plates were developed under the same condition. MS scanning of the SiNW plate along the sample track revealed two strong signals at *m*/*z* 332 and 338 corresponding to piroxicam and tenoxicam, respectively. It should be noted that mass spectra were only observed from the two adjacent spots (Figure 4a), and analysis above and below those spots generated no signal.

We further employed the SiNWs in the analysis of xenobiotic and endogenous metabolites in biofluids on the basis of the aforementioned success of the technique in the separation of the two drug molecules. Its utility as an assay in the detection of drugs of abuse in human serum and endogenous metabolites present in mouse spinal cord tissue is illustrated. Separation experiments followed by MS analysis of human serum spiked with cocaine shows the separation cocaine (MH^+ = 304) and its known metabolite (MH^+ = 200) (Figure 4b). This result illustrates the viability of this technique in the analysis of biological samples for forensic applications. In a similar experiment, we extended the utility of the SiNW assay in the detection of NAEs in mouse spinal cord tissue. NAEs are biologically active lipids that participate in numerous functions in cellular processes including membrane stability, antiinflammatory and antiviral properties, drug distribution and metabolism, and regulation of feeding behavior.[38,39] Initial

(37) Sherma, J. *Anal. Chem.* **1988**, *60*, R74−86.

(38) Schmid, H. H.; Schmid, P. C.; Natarajan, V. *Prog. Lipid Res.* **1990**, *29*, 1−43.

(39) Fu, J.; Gaetani, S.; Oveisi, F.; Lo Verme, J.; Serrano, A.; de Fonseca, F. R.; Rosengarth, A.; Luecke, H.; Di Giacomo, B.; Tarzia, G.; Piomelli, D. *Nature* **2003**, *425*, 90−3.

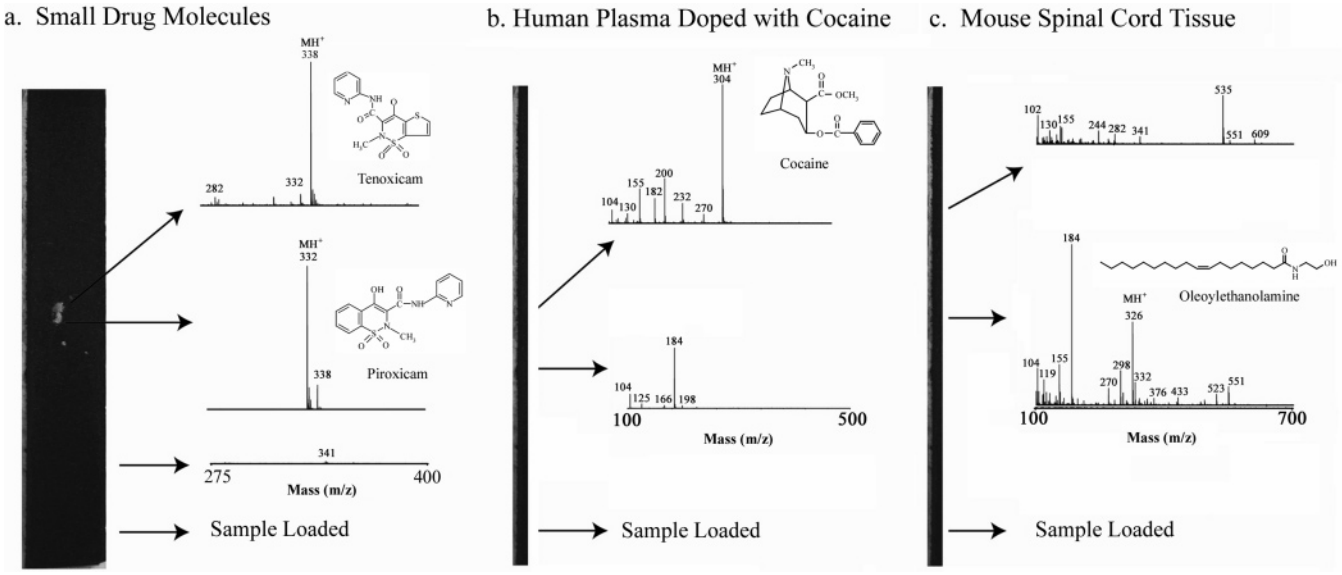

Figure 4. Silicon nanowires (40 nm in diameter) as a platform for chromatographic separation of (a) a mixture of 1 mg/mL each of the small drug molecules (tenoxicam, *m*/*z* 338; piroxicam *m*/*z* 332), (b) human serum spiked with 5 μM cocaine (*m*/*z* 304), and (c) oleoylethanolamine (*m*/*z* 326) and other unknown endogenous metabolites in mouse spinal cord tissues. The samples were deposited 0.5 cm from the edge of the plate and allowed to separate using methanol/water mixture as mobile phase for (a) and (b) and chloroform/methanol/ethyl acetate mixture for (c).

attempts to separate and identify oleoylethanolamine, an NAE that regulates feeding, in mouse spinal cord tissue is demonstrated in the data shown in Figure 4c. Peaks corresponding to oleoylethanolamine ($MH^+ = 326$), doped deuterated internal standard of a long-chain NAE, and other unknown endogenous metabolite with $m/z > 400$ Da in mouse spinal cord tissue were observed. The separation of the NAEs was performed in less polar solvent containing chloroform/methanol/ethyl acetate. These preliminary data demonstrate the capability of the SiNW assay in the detection of these metabolites in biofluids. It should be noted that the metabolite spots were not visible when the SiNW plates were held under the UV lamp. Due to the absence of fluorescence, the detection of metabolites was done by MS analysis of the SiNW plates (Figure 4b,c). Presently, experiments are being performed to improve its zone detection and further explore the effect of the nanowire size on separation and extraction efficiency, its application in column chromatography, and the effect of different silylating reagents. With these improvements, chromatographic separation with SiNWs followed by subsequent MS analysis is attractive compared with existing planar chromatography coupled with MALDI-MS[25,27] because it offers a one-step procedure that does not require matrix deposition.

CONCLUSIONS

The results described here demonstrate the promising potential of silylated SiNWs for direct biomolecule analysis. It was shown that the size, high surface area, and fluid wicking properties play an important role for its application in mass spectrometry and chromatographic separation. This study also found that SiNWs require lower laser energy for analyte desorption/ionization compared to MALDI or pSi-DIOS. Furthermore, it provided for the analysis of a wide range of molecules, and the material could serve as a platform that can integrate chromatographic separation followed by MS analysis. Significant improvement in sensitivity and chromatographic properties is expected by tailoring the surface properties through additional chemical and structural modifications.

ACKNOWLEDGMENT

The authors are grateful for the interesting insight of R. Graham Cooks concerning the mechanism of DIOS. This work was supported by NIH grant GM05775 and Nanosys, Inc. Financial support to A.V. from the Department of Energy (DE-FG02-01ER15129) is appreciated. DOE's support does not constitute an endorsement by DOE of the views expressed in the article.

Received for review October 18, 2004. Accepted December 30, 2004.

AC048460O