Anal. Chem. **2007,** *79,* 434−444

Small-Molecule Analysis with Silicon-Nanoparticle-Assisted Laser Desorption/ Ionization Mass Spectrometry

Xiujuan Wen, Shai Dagan,† and Vicki H. Wysocki*

Department of Chemistry, University of Arizona, Tucson, Arizona 85721

Silicon nanopowder (5−50 nm) was applied as a matrix for the analysis of small molecules in laser desorption/ ionization mass spectrometry. In contrast with conventional matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry, the matrix background interference in the low mass range was significantly reduced. Effects of the particle size and sample preparation procedures on the background mass spectra and the analyte signal intensity have been investigated, and an optimized powder and sample preparation protocol was established. Several surface characterization tools have been applied as well. Both positive mode and negative mode laser desorption/ionization have been applied to different analytes including drugs, peptides, pesticides, acids, and others. Detection limits down to the low femtomole per microliter levels were achieved for propafenone and verapamil drugs. The method developed was found relatively tolerant to salt contamination, which allowed the direct analysis of morphine and propaphenone in untreated urine and triazine herbicides in a soil extract. The new silicon-nanoparticle-assisted laser desorption ionization method was found to be highly selective, which may be due to analyte-dependent precharging in solution, prior to vacuum laser desorption. Some aspects of the charge-transfer mechanism have been studied and discussed. In comparison with standard MALDI matrixes, the silicon nanopowder requires much lower laser fluence (contributing to a reduced background) has much better surface homogeneity, and is more tolerant to salt interference, which makes it an easily applicable practical tool at a potentially low cost.

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) has been extensively used in the analysis of large molecules, such as synthetic polymers and biomolecules, since the late 1980s.[1−3] Small-molecule analysis has been limited in MALDI applications because of the background interference of small organic matrixes that may overlap with the analyte.[4,5] This low mass chemical background is typically up to about m/z 500 and depends mainly on the matrix used and the laser fluence of the instrument. To explore the full range of MALDI applications, several groups have investigated different types of matrixes to minimize the low mass background.[6] Early in 1988, Tanaka et al. first used 30 nm inorganic cobalt powder mixed with a glycerol solution to analyze the protein lysozyme.[7] They suggested that cobalt nanoparticles have a large surface area, high photoabsorption, and low heat capacity, which makes them successful for analyte desorption/ionization. Sunner and co-workers developed surface-assisted laser desorption/ionization (SALDI) using 2−150 μm graphite particles mixed with glycerol as the matrix.[8,9] They observed the surface desorption process and obtained spectra with reduced background in the low mass region. Active carbon powder,[10] carbon nanotubes,[11,12] gold nanoparticles,[13] and other inorganic nanoparticles such as Al, Mn, Mo, Sn, W, ZnO, TiO_2, TiN, Gu, and Fe_3O_4 have been studied as potential SALDI matrixes.[14−18]

Since 1999, Siuzdak and co-workers have developed and extensively explored a novel approach of desorption/ionization on porous silicon (DIOS) as a matrix-free ionization method for mass spectrometry.[19−22] Porous silicon is made by electrochemical

* To whom correspondence should be addressed.

† Israel Institute for Biological Research (IIBR), P.O. Box 19, Ness Ziona 74100, Israel.

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10.1021/ac061154l CCC: $37.00
Published on Web 12/15/2006

etching of crystalline silicon chips with hydrofluoric acid (HF) under white-light illumination. Instead of using chemical matrixes, DIOS-MS uses porous silicon surfaces to trap analyte molecules and efficiently absorb UV laser energy. Thus, the signal-to-noise ratio is enhanced, and background peaks are minimized. Siuzdak et al. further developed the DIOS chip by incorporating silylation,[23,24] which made the surface more stable and inert to chemical reactions. Small, as well as larger, molecules, from 150 to 12000 Da, including pharmaceuticals, peptides, carbohydrates, and natural products, have been detected by DIOS,[25−29] some with impressive detection limits. In 2005, Siuzdak and co-workers also developed silicon nanowire arrays grown on silicon substrates for desorption/ionization.[30] Compared with common MALDI and DIOS, these 10−40 nm diameter silicon nanowire arrays require much less laser fluence and thus further reduce the background ion interference. Although the efficient photon absorption of silicon and subsequent energy transfer to the analyte have been widely discussed,[31] the nature of proton transfer inducing the formation of ionized species in DIOS as well as in other chemical matrixless desorption/ionization methods remains unclear.

Si and SiO_2 micrometer-scale particles have also been studied as SALDI matrixes.[14] The results showed that Si (but not SiO_2) was useful as a matrix. Zou et al. used porous silicon powder scraped from the etched silicon wafer as a matrix. The scraped powder provided fewer background peaks compared with DIOS.[32] Recently, modification of a commercially available silicon powder as a matrix was first studied by Credo et al.[33] The best sensitivity for some drugs was obtained by the smaller size silicon particles around 50 nm compared with the larger, micrometer-scale particles. However, the background peaks could not be avoided.

Here we further explore the application of commercially available silicon nanoparticles for desorption/ionization mass spectrometry. The preparation procedure is optimized, the powder is characterized, and some mechanistic aspects are studied. The application of the new silicon-nanoparticle-assisted laser desorption/ionization (SPALDI) method to pharmaceuticals, peptides, pesticides, nucleic acids, and other acids is illustrated and discussed. Finally, the potential use of the silicon nanopowder is compared to that of standard MALDI as well as to that of matrix-free LDI methods.

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EXPERIMENTAL SECTION

Materials. Water was purified by a Millipore water purification system (Millipore, Bedford, MA) and had a resistivity of >18 MΩ cm. Organic solvents, including methanol, ethanol, acetone, and 2-propanol (HPLC grade), the peptide Arg-Gly, the compounds propafenone hydrochloride, verapamil hydrochloride, adenine, morphine, ametryn, altretamine, trioctylamine, chlorodimethylphenethylsilane (PHP), trimethylsilane (TMS), and the MALDI matrix α-cyano-4-hydroxycinnamic acid (CHCA) were all purchased from Sigma-Aldrich (St. Louis, MO). Dibutylphosphoric acid and other free acids tested in the negative ion mode were all purchased from Sigma-Aldrich as well. The peptide YGGFL was obtained from American Peptide Co. (Sunnyvale, CA). [(Pentafluorophenyl)propyl]dimethylchlorosilane (PFP) and other fluorinated derivatives were purchased from Gelest Inc. (Morrisville, PA). Urine samples were collected from a healthy woman on the same day of the experiment. Silicon nanopowders with a particle size of 50 nm were purchased from Sigma-Aldrich, and those with particle sizes of 30 and 5 nm were purchased from Meliorum Technologies Inc. (Rochester, NY).

Preparation of Si Powder. (1) Fifty Nanometer Si Powder. An 8 mg sample of 50 nm silicon powder was put in a 0.6 mL siliconized microcentrifuge tube and etched by 0.4 mL of 10% HF for 30 min with sonication. The powder was then washed with 1.5 mL of 2-propanol, dried in a vacuum concentrator for 30 min, and then oxidized by adding 0.4 mL of 10% HNO_3 for 30 min with sonication. Then the powder was washed with 2-propanol again and dried in a vacuum concentrator, SpeedVac SC110 (Savant Instruments Inc., Farmingoale, NY), with low heating (40 °C) for 1 h. The powder was derivatized with 90 μL of PFP (neat) for 10 h at 90 °C in an oven and washed with 2-propanol before use.

(2) Thirty Nanometer Si Powder (Optimized Procedure). Thirty nanometer Si powder was found to perform best with no HF etching. An 8 mg sample of this 30 nm powder was oxidized by 0.4 mL of 10% HNO_3 directly for 30 min with sonication. Then the powder was washed with 2-propanol and dried in a vacuum concentrator with low heating (40 °C) for 1 h. The powder was derivatized with 90 μL of neat PFP for 10 h at 90 °C in an oven and washed with 2-propanol before use.

Final powder solutions of 8 mg of powder/0.4 mL of storage solvent were prepared. The storage solvent was perfluorohexane. Before application of the powder on the MALDI plate, the perfluorohexane was washed with 2-propanol/H_2O (v/v, 50:50) and then mixed with the analyte solution.

Optimization of Silicon Powder Preparation Conditions. Most of the original optimization of the powder preparation conditions was carried out with the 50 nm particles and then extended to the 30 nm particles, with propafenone as a model compound, usually in concentrations of 1−10 pmol/μL. The signal intensity and the background intensity and identity were examined and compared. The powder preparation concept was based on prior knowledge (refs 19−22 and 33). In short, it included particle etching by HF (eventually not required with the 30 nm particles), followed by oxidation (presumably hydroxylation) with HNO_3 and finally modification of the Si−OH groups by derivatization.

(1) Concentration of the HF Solution. The function of HF etching for the silicon powder may be minimizing the particle size or making pores on its surface. Different concentrations of HF, 10%, 25%, and 48%, have been tested. They showed similar results. A 10% HF concentration was selected for safety and economy. Eventually, 30 nm particles were shown to perform well without HF etching.

(2) Etching Time. Periods of 15, 30, and 60 min were tested. Powder etched for 15 min led to poor analyte sensitivity. Powder etched for 30 min provided higher analyte sensitivities with a trace background. Powder etched for 60 min gave the best analyte sensitivity, but the background was also significant. An etching time of 30 min was selected for the 50 nm powder.

(3) Concentration of HNO_3. HNO_3 oxidation was performed to form Si−OH groups on the silicon particle surface to facilitate the derivatization step afterward. It may also help remove contaminants from the powder. HNO_3 of 10% concentration and concentrated (70%) HNO_3 were tested. Concentrated HNO_3 caused ignition and burning of the dried powder. A 10% HNO_3 concentration was selected.

(4) HNO_3 Oxidation Time. Periods of 15, 30, and 60 min were tested. Powder with 15 min of oxidation showed more background than powder with 30 min of oxidation. Powder with 60 min of oxidation showed no signal. A 30 min oxidation time was selected.

(5) Drying Time after HF Etching. Periods of 15, 30, and 60 min were tested. A time of 30 min was found to provide a stronger analyte signal than 15 min, and 60 min gave signals similar to those of 30 min, so 30 min was selected for efficiency.

(6) Drying Time before Derivatization. A period of at least 60 min of drying before derivatization provided the most stable performance. The drying step before derivatization was found to be very critical in the preparation procedure. If the powder is not dry enough before derivatization, the extra solvent (2-propanol) in the bulk powder may react with the derivatization reagent, thus affecting the modification efficiency and causing poor performance of the powder.

(7) Derivatization Time. Periods of 1, 4, and 10 h were tested for derivatization. A time of 10 h gives the best analyte signal intensity. Similar considerations and methods have been reported for HPLC column stationary phase particle derivatization.[34,35]

(8) Cleaning Solvent. Methanol, ethanol, acetone, and 2-propanol (analytical grade) were investigated as cleaning solvents during the preparation procedures. The best results were obtained with 2-propanol cleaning. It provided the lowest background and the highest analyte signal intensity among the four solvents examined. It could be that 2-propanol provides the best wetting of the silicon powder among the four organic solvents. This result is similar with DIOS analysis as reported by Waters Corp.[36]

Analyte Sample Preparation. Analytes were diluted in 2-propanol/H_2O (50:50) mixtures to obtain stock solutions (typically 1 mg/mL) and then further diluted in 2-propanol/H_2O (v/v, 50:50) to the final required concentration (usually in the picomole per microliter down to the femtomole per microliter range). The analyte solutions were mixed with the powder solutions in a ratio of 1:1 prior to spotting 1 μL droplets on standard MALDI plates.

Mass Spectrometry. All the mass spectra were obtained using a Reflex III (Bruker, Billerica, MA) time-of-flight mass spectrometer equipped with a reflectron and a 1.25 m flight tube. The accelerating voltage was set to 19 kV, and the reflectron voltage was set at 20 kV. The delay times were 200 ns. All the experiments were carried out in the reflectron mode.

The mass spectrometer was equipped with a 337 nm nitrogen laser (LSI-Laser Science Inc., Newton, MA). The laser has an average maximum fluence of 400 μJ/pulse and was focused to a spot diameter of ~100−150 μm. Typical laser fluences used: with powder, attenuation 99−80% (1−20% of the total laser intensity); with chemical matrixes, 60−70% (40−30% of the total laser intensity). The laser fluence, measured by an external sensor (Coherent-Fieldmate), responded linearly under different attenuation levels. A total of 200 single-shot signals at a rate of 3 shots/s were accumulated for each spectrum acquired. Random rastering of the sample spot was applied manually, moving typically every 5−10 laser shots, during the 200-shot acquisition.

Mass calibrations were performed externally using the mass peaks of the dimer of CHCA and bradykinin standards.

Surface Characterization. Transmission electron microscopy (TEM) was performed with a Hitachi 8100S model at a 200 kV acceleration voltage with a high-brightness LaB6 electron source and a CCD high-resolution camera. Scanning electron microscopy (SEM) images were obtained using a Hitachi S-4000 (Tokyo, Japan) field-emission scanning electron microscope with magnification to 500000×, accelerating voltages of 0.5−30 KV, and 4.0 nm resolution at 1 kV. Atomic force microscopy (AFM) was performed with a Digital Instruments Bioscope (Veeco Metrology Group, Santa Barbara, CA).

Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) analysis was performed using a Nicolet 4700 FT-IR spectrometer (Thermo Electron Corp.) with a diffuse reflectance measuring attachment. Samples were prepared by mixing 10 wt % sample with 90 wt % FT-IR-grade KBr (Sigma-Aldrich). FT-IR-grade KBr was used as the reference. Sample spectra were collected under a nitrogen environment, over the range of 4000−400 cm^{-1} at a resolution of 4 cm^{-1}, and the reported data represent the average of 256 scans. The data were recorded by applying Omnic E.S.P. software (version 5.2a, Nicolet).

RESULTS AND DISCUSSION

Optimizing Powder Preparation. To explore the function of each of the preparation steps mentioned in the Experimental Section (concentration of HF, etching time, concentration of HNO_3, HNO_3 oxidation time, drying time after HF etching, drying time before derivatization, derivatization with no etching, derivatization time), we performed analyses of silicon raw powder, silicon powder without HF etching, silicon powder without HNO_3 oxidation, and silicon powder without derivatization. The analyte was propafenone, an antiarrhythmic drug, at a concentration of 10 pmol/μL, in all the tests.

As shown in Figure 1a, raw silicon powder without preparation gives spectra with a lot of background ion interferences. We suspect the main peaks (m/z 86, 102, 130) correspond to different kinds of easily ionized alkylammonium salts in the powder material. These salts can be attributed to surfactants present

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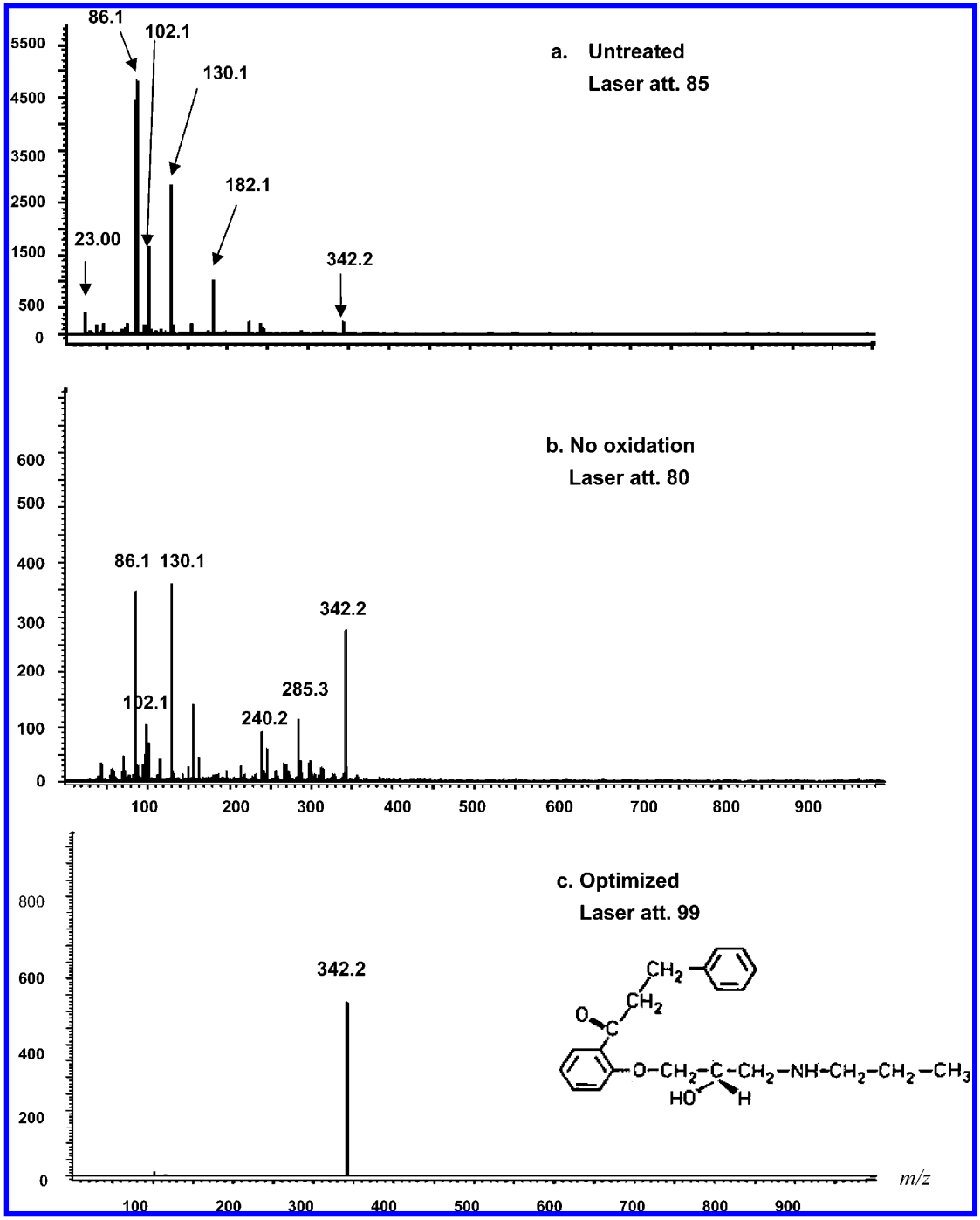

Figure 1. Effect of powder preparation: 1 μL drop of a 10 pm/μL propafenone solution (*m*/*z* 342). Analysis with (a) untreated 30 nm Si powder, (b) 30 nm Si powder prepared without the HNO_3 oxidation step but with 10 h of PFP derivatization, and (c) 30 nm Si powder with optimized preparation: 10% HNO_3 for 30 min, derivatized with PFP for 10 h. One percent of the laser intensity was used.

during the powder manufacturing process. Compared with the prepared silicon powder (as shown in Figure 1c), the raw material requires a relatively high laser fluence (attenuation 85%, 15% of laser intensity used) but still shows a relatively low analyte MH^+ signal at *m*/*z* 342.

As shown in Figure 1b, silicon powder without HNO_3 treatment, but including all the other preparation stages, exhibits more background ions and less signal intensity and requires higher laser fluence (attenuation 80%, 20% of laser intensity used) compared with the prepared silicon powder used to obtain the spectrum shown in Figure 1c. In comparison with untreated silicon (Figure 1a), the results imply that HNO_3 may have a cleaning effect, added to the hydroxylation of the silicon. Silicon powder treated with HNO_3 but without derivatization shows no signal at all for the analyte (not shown), probably due to active Si−OH groups on the surface that were formed by the HNO_3 treatment.

In Figure 1c, we demonstrate the utility of an optimized silicon powder with the absence of background and clear detection of propafenone. Interestingly, although the laser fluence used was extremely low at 99% attenuation (only 1% of the laser intensity was used), the signal was high and the signal/noise ratio was very good. For comparison, with standard chemical matrixes such

as CHCA on the same instrument, the typical laser attenuation used is 65−70% (35−30% of the laser intensity), with a threshold for obtaining any signal of about 30% of the laser intensity. The very low laser fluence required with the silicon powder may be the result of extremely efficient UV absorption and limited thermal transfer in the nanopowder, which may cause local overheating and thus induce efficient ablation/volatilization of the analytes. Similar behavior has been observed by Tanaka, who used 30 nm cobalt particles,[7] and Siuzdak et al. with nanowires assembled on silicon surfaces,[30] where the nanowire size was 40 nm, similar to the silicon nanoparticle size in the present work.

Derivatization Reagent. Derivatization is used to deactivate and stabilize the surface and results in reduced background and increased signal intensity. Several derivatization agents, among them PFP, PHP, and TMS, were tested in different combinations. PFP differs from PHP by containing five fluorine atoms on the aromatic ring, instead of hydrogens. Different procedures, including single PFP, single PHP, single TMS, double PFP (two consecutive cycles of derivatization with PFP), double TMS, PFP followed by TMS, and TMS followed by PFP, were tested. A single-step PFP derivatization provides the best signal intensity and the lowest background ion signal. PHP has a similar performance but shows a little more low m/z background such as the common background peaks at m/z 86, 102, and 130, and also some new peaks at m/z 72, 92, 98, 112, 116, 156, and 216. No MS peaks were observed with the TMS-derivatized powder (single or double derivatization). Preliminary experiments using a linear fluorocarbon chain (C10) derivative show promising results.

Particle Size. Si powders of 50, 30, and 5 nm size were selected and tested. The 50 nm particles gave the lowest analyte signal intensity. The 30 and 5 nm particles gave similar signal intensities, but 5 nm particles had more background peaks in the spectra, so 30 nm particles were selected. Micrometer size particles have already been investigated by other groups and have been reported to be less efficient than nanoparticles[14,15] where heat diffusion for nanoparticles and microparticles during the laser pulse was assumed to be different. Under the same laser fluence, the peak temperature increase for nanoparticles is considered to be an order of magnitude higher than for microparticles.[15] Interestingly, the optimal 30 nm nanoparticle size is much smaller than the N_2 laser wavelength (337 nm or, more importantly, ~168.5 nm for $\lambda/2$), suggesting that the efficient light absorption is obtained by a cluster of aggregated particles rather than by a single isolated one.

The HF etching time was investigated with the 50 nm silicon powder, and as described in the Experimental Section, 50 nm particles etched with 10% HF exhibited the best results. However, with the 30 nm powder, the best ion signal was obtained with no HF etching. HF etching is thought to increase the effective surface area of the silicon powder and may also affect the particle size. On the other hand, it seems to promote particle aggregation and particle cluster formation, as will be discussed later. The difference between the 50 nm particles (Sigma) (require HF etching) and the 30 nm particles (Meliorum) may be due to the different sizes or due to different surface properties.

Si Powder Characterization. The silicon powder was characterized by TEM, SEM, and AFM for surface morphology and by FT-IR and DRIFTS for identifying chemical bonds on the surface (results shown in Figure 2). Figure 2a shows TEM images of the 30 and 50 nm untreated powders. The 50 nm particles are larger than defined by the manufacturer (more than 100 nm) and much larger than the 30 nm particles. Both types of particles cluster in small groups. Similar findings were obtained by SEM analyses.

The HF etching effect was also explored using SEM as well as AFM. As shown in the AFM images in Figure 2b, it was found that etched, now hydrophobic particles tend to fuse together in large clusters and thus lose much of their nanostructure. It is assumed that the HF etching was required to prepare the larger "50 nm" particles to reduce their size or create some small pores on their surface, but was not beneficial for the small enough 30 nm ones.

FT-IR and DRIFTS measurements were carried out to uncover some of the chemical properties of the modified silicon powder. The current findings can confirm the presence of the derivatizing agent on the surface. As shown in Figure 2c, absorption lines of $Si-CH_3$ stretching, 2960 cm^{-1}, and bending, 1275−1250 cm^{-1}, $-CH_3$ stretching, 2880 cm^{-1}, Si−C stretching, 880−750 cm^{-1}, C_6F_5 C=C stretching, 1520−1500 cm^{-1} double peaks, and C−F stretching, 900−680 cm^{-1}, are related to the derivatizing agent PFP. No evidence for Si−H (2080−2280 cm^{-1}) is shown in any of the analyses. For aged powder, which became LDI inefficient after being stored dry or in water/alchohol solutions, we have found that the organic modifier absorption lines disappeared, suggesting back-exchange to Si−OH in the presence of water or alcohol (in the solvent or in the air) and implying that better storage may be obtained under an −OH-free environment.

Detection Limits, Dynamic Range, and Linearity. The minimum amount of sample needed for obtaining spectra of reasonable signal-to-noise ratios from a Si nanopowder was 33 fmol on spot for the basic, amine-containing drugs propafenone and verapamil, and this is demonstrated in Figure 3. The laser fluence needs to be increased to attenuation 85% (15% of total laser intensity) to collect these spectra. At a lower concentration of 10 fmol/μL (1 μL on spot) there was still signal, but it was marginal compared with the background neighboring peaks. The detection limits demonstrated here are comparable with the detection limits obtained with standard chemical matrixes on the same instrument.

The above drugs were analyzed in different concentrations from 10 fmol/μL up to >1000 pmol/μL. A signal increase was observed upon a concentration increase, from the detection limit up to more than 100 pmol/μL, although linearity was limited in that very broad dynamic range. Signal reproducibility was found to be relatively good compared with that of standard MALDI, presumably because the silicon powder matrix is much more homogeneous without the "sweet spots" typical of standard chemical matrixes.

Applicability to Different Analytes and Some Mechanistic Aspects. The technique was successfully applied to peptides, nucleobases, and even lipids in the positive ion mode. As shown in Figure 4a−c, for peptides and other compounds, higher laser fluence (typically attenuation of 90%, 10% of laser intensity used) is needed compared to that for drugs (attenuation 95−99%, 1−5% of laser intensity used). Peptides containing basic amino acids such as arginine are ionized more easily than others. With increasing laser fluence, some background ion interference

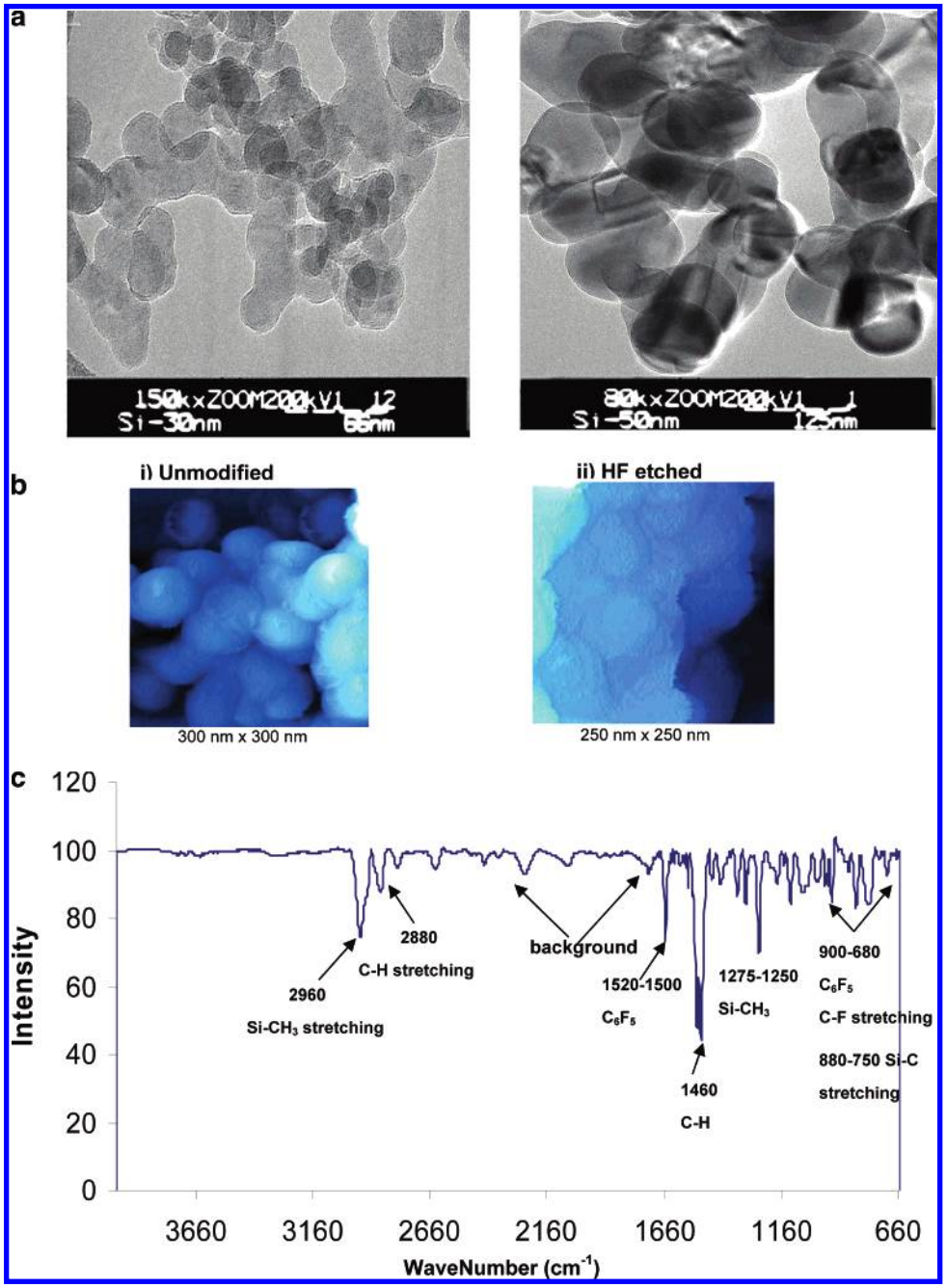

Figure 2. Characterization: (a) TEM scan for untreated 30 nm silicon particles (left) and 50 nm silicon particles (right); (b) AFM scan of (i) untreated raw 30 nm particles, compared to (ii) HF-etched and PFP-derivatized 30 nm particles; (c) FT-IR-DRIFTS spectrum of PFP-derivatized 30 nm particles. The optimized Si preparation was used (see the caption for Figure 1c).

appears around m/z 100 (mainly m/z 86, 102, 130). These peaks are similar to those appearing with the raw, untreated powder, but with the prepared powder their intensity is significantly lower. As mentioned before, we suspect those background ions represent alkylammonium salts originating from surfactants used during the manufacture of the silicon powder raw material. Those background peaks are repeatable from batch to batch and with different preparation methods using different particle sizes and brands.

Several acids, among them dibutylphosphoric acid, have been successfully tested as analytes in the negative ion mode, as shown in Figure 4d. The method is also selective in the negative ion mode for low pK_a acids, which may also support the hypothesis that silicon-powder-assisted laser desorption/ionization is functional for precharged compounds. Background ions in the negative ion mode were different from those in the positive one. An abundant peak at m/z 181 could not be identified and may correspond to the m/z 182 unidentified background peak in the positive ion mode. Another dominant peak was the chlorine peak pair at m/z 35 and 37, likely the counterion of the tetraalkylammonium ions detected in positive ion mode.

This technique is compound-selective and shows different sensitivities for different types of analytes such as peptides compared to drugs. We propose it may be highly selective for precharged compounds. It is suggested that the silicon powder functions only as a thermotransfer matrix which strongly absorbs UV laser energy and transfers it to the precharged analytes to

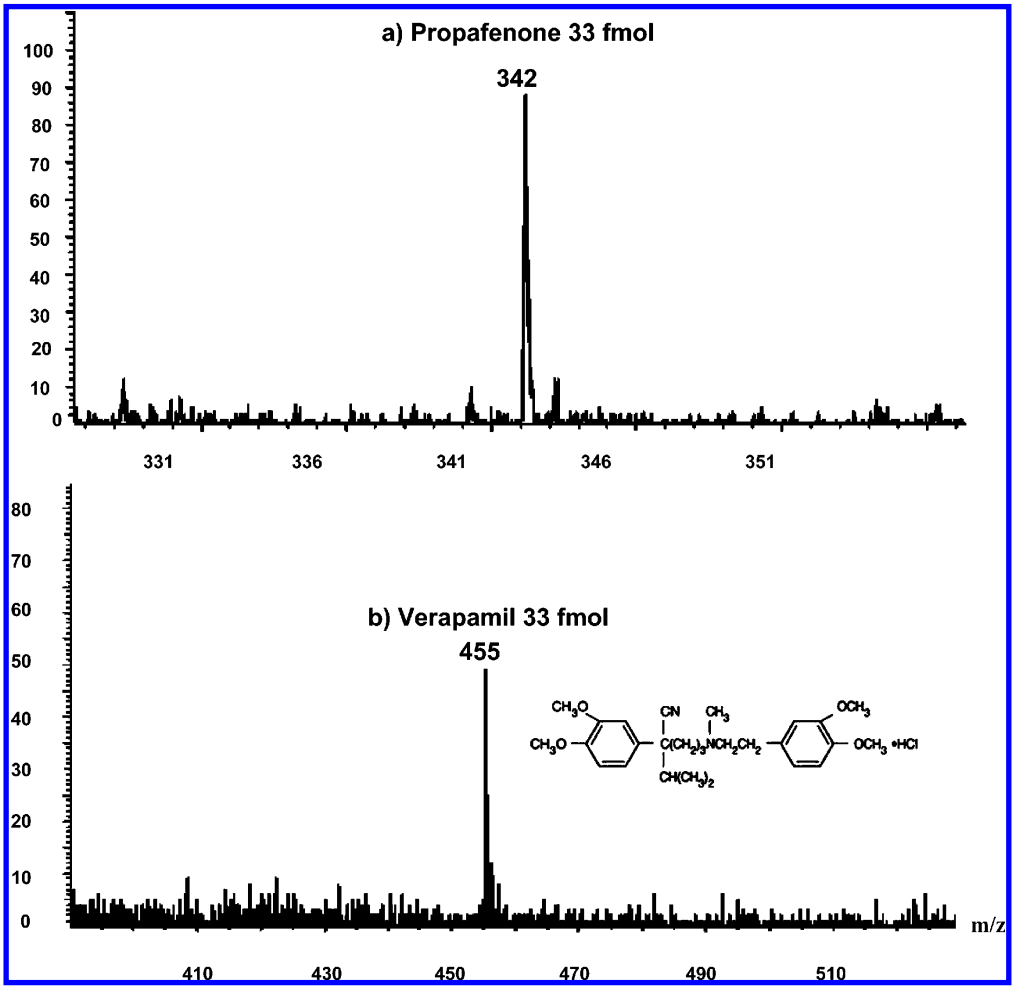

Figure 3. Detection limits for propafenone and verapamil drugs. The particle size was 30 nm and the optimized Si preparation was used (see Figure 1c). The laser fluence was 15%, the analyte concentration 33 fmol/μL, and the spotting volume 1 μL.

cause desorption. The level of precharging may strongly depend on the basicity (or acidity) of the analytes.

To partially explore this hypothesis, we used deuterated solvents (D_2O and C_3D_7OD) for the powder preparation as well as for analyte dissolution and compared the results to the regular procedure where H_2O and C_3H_7OH are used. The analytes chosen for that comparison were verapamil (structure shown in Figure 3) and trioctylamine, both having basic nitrogen atoms and no labile hydrogens. As shown in Figure 5, it is clear that, with the deuterated solvents, the MS peaks are shifted, indicating that the proton originates from the solvents (the results show only partial deuteration, perhaps because of some back-exchange with H_2O in the air). We have also confirmed the origin of the protons by using MTBE (methyl *tert*-butyl ether) as a powder and analyte solvent; with no proton-donating groups present on MTBE, no propafenone ion peaks appeared.

Still, it is not clear whether the protonation process is precharging in solution or a laser-induced proton transfer on/near the surface. However, for a laser-induced proton-transfer mechanism, it would be difficult to explain the proton origin on the derivatized and dried powder in vacuum. We also altered the pH of the powder and analyte mixture solution to better control the ionization, following Kruse et al.,[31] but failed to observe any effect both in the positive and in the negative ion modes. Since the samples have to be dried on the plate to be introduced into the vacuum, it is assumed that no solution-phase equilibrium exists anymore, and thus, the charge condition of the spotted analytes becomes unclear. Wetting experiments with glycerol added to the powder in various concentrations to have an effect on charging of the analytes and/or the heat-transfer mechanism were not successful. The above observations have to be further explored to facilitate better understanding of the selective protonation mechanism of different analytes.

We have also mixed low concentrations of a standard CHCA matrix with the silicon nanopowder in an attempt to obtain better ionization with improved laser absorption, but no effect was observed, perhaps because no CHCA crystallization could be accomplished.

Salt Effect. Contamination of MALDI samples with alkali-metal salts is well-known to reduce MALDI sensitivity by ion suppression and matrix cluster formation.[37] Similarly to DIOS,[19] Si-powder-assisted LDI has higher salt tolerance. With salt concentrations up to 0.5 mmol/μL the signal exhibits only a slight decrease in comparison to signals from samples with a low salt concentration. Different salts have been tested. LiCl, NaCl, KCl, NaAc, and NH_4-Ac show similar performance. Surprisingly, none of those produced any detectable metal adducts of the molecular ion in the LDI-TOF spectra of various analytes. This also supports the

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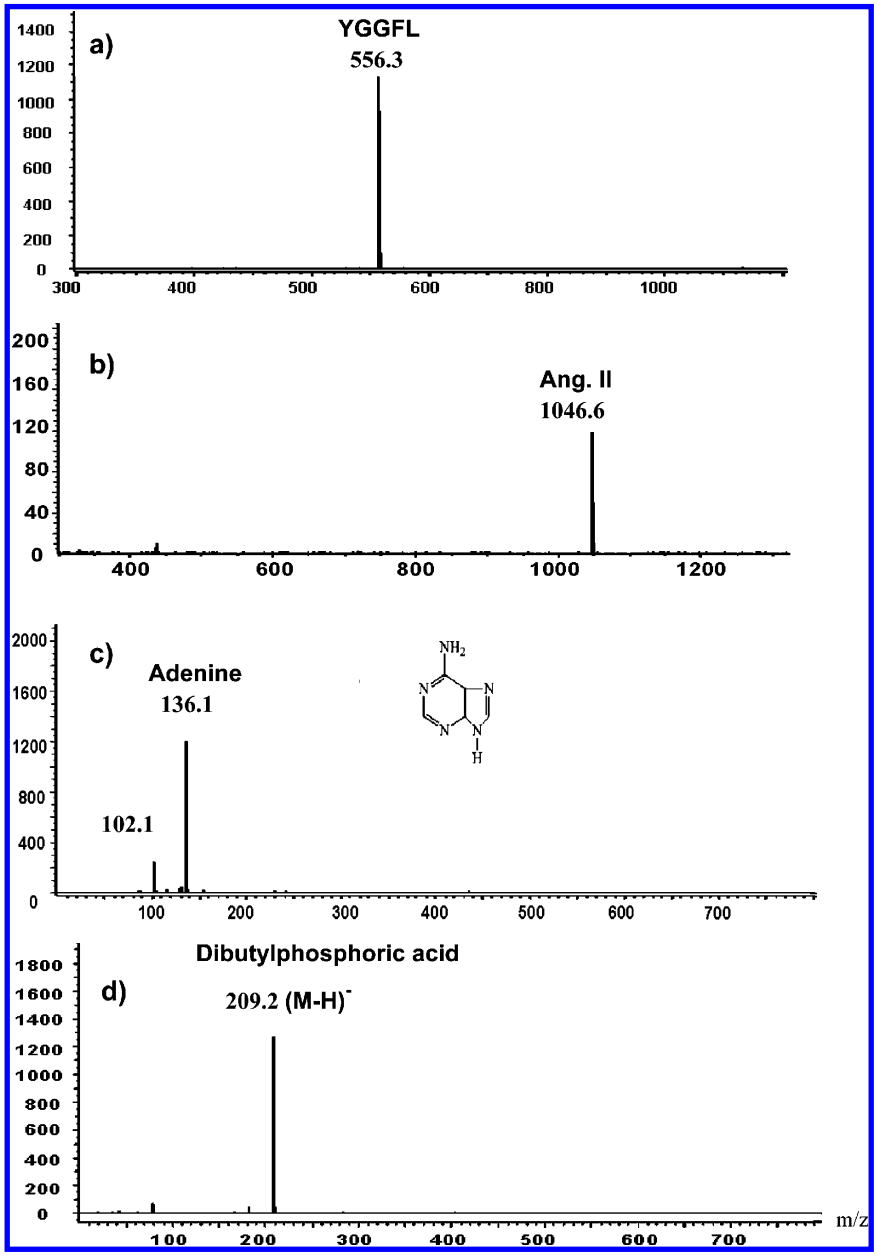

Figure 4. Mass spectra of (a) peptides leucine enkephalin (YGGFL) (200 μg/mL (360 pmol/μL)), (b) angiotensin II (DRVYIHPF) (100 μg/mL (~100 pmol/μL)), (c) nucleobase adenine (400 μg/mL ~2900 pmol/μL), and (d) in negative ion mode for dibutylphosphoric acid, $(M - H)^-$ at *m*/*z* 209. The particle size was 30 nm, and the optimized Si preparation was used (see the caption for Figure 1c). The laser fluence was 10% for (a)−(c) and 20% for (d).

hypothesis that no charge transfer occurs in vacuum during the laser desorption/ablation process. To demonstrate the salt tolerance in practical analyses, two examples of drugs in urine and pesticides in soil are shown.

Applications. (1) Urine Direct Analysis. Urine samples were spiked with morphine or propafenone and analyzed without any pretreatment (Figure 6). Both compounds show only a slight decrease of signal intensity in urine compared to a standard solution with the silicon particles. However, with the standard CHCA, neither compound was detected when it was in the urine environment as shown and compared in Figure 6. The salt tolerance of the silicon particle method is a potential advantage in analyzing biological samples.

(2) Soil Analysis. A demonstration of the compatibility of the silicon powder method with an environmental matrix is the analysis of pesticides in soil. Two triazine pesticides, ametryn and altretamine, were spiked in 5 g of soil (taken from the University of Arizona campus). After 5 min, 5 mL of tap water for extraction was added to each centrifuge tube, and the tubes were sonicated for 20 min. The samples were centrifuged, and the extract was directly applied with the modified Si nanopowder for laser desorption/ionization mass spectrometry. As shown in Figure 7, the analytes are detected with the silicon powder without any suppression from the environmental sample. Concentrations as low as 1 μg of pesticide/g of soil could be easily detected for ametryn.

(3) Storage and Other Practical Use Aspects. Derivatized Si powder was first stored in 2-propanol or stored dry in the oven. Under those storage conditions, the powder deteriorated and kept its original performance for only 4−5 days. The analyte signal

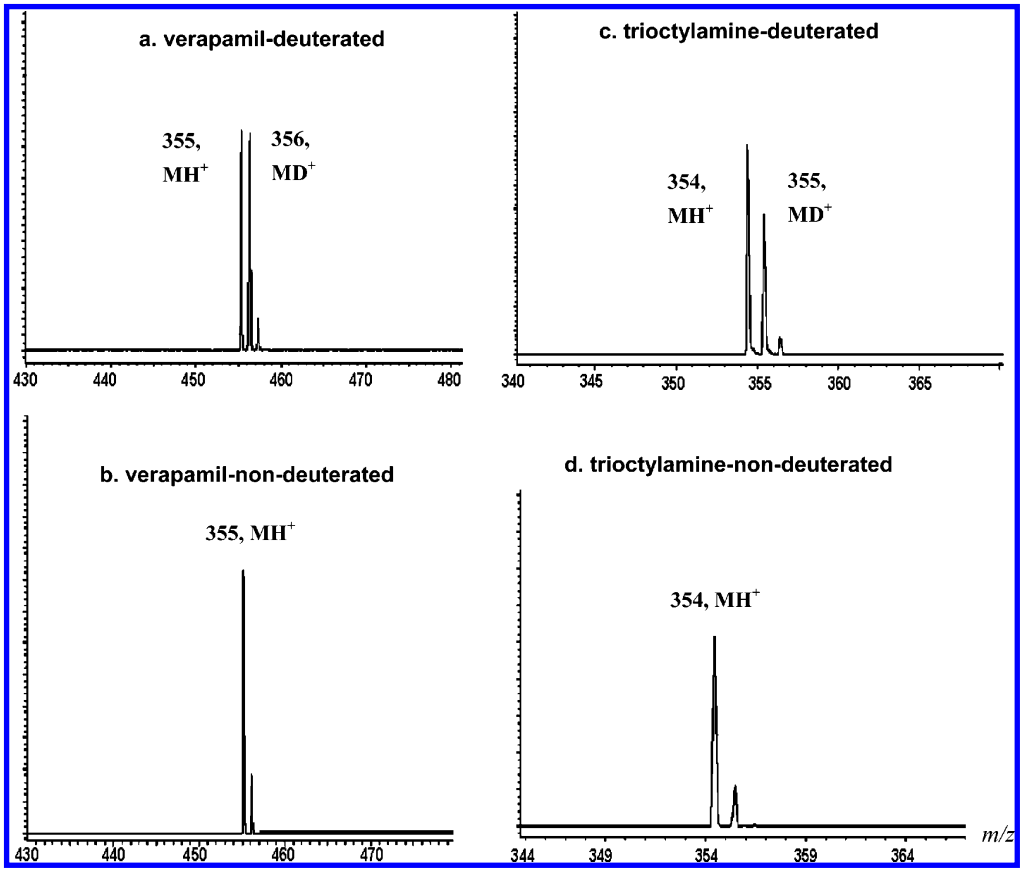

Figure 5. Proton origin with silicon-assisted laser desorption/ionization mass spectrometry analysis. Analysis of veparamil (10 pmol/μL) and trioctylamine (~80 μg/mL) using (a, c) deuterated solvents, laser fluence 5%, and (b, d) standard nondeuterated solvents, laser fluence 1%.

would then decrease, and the background ion peaks would increase. IR measurements we have performed indicate complete loss of the derivatizing group on the silicon particles after 2 weeks. It is likely that the −OH group from 2-propanol reacted with the modified particle surface or that humidity in the air reacted with the modified powder and caused the powder to lose the derivative functionality. Perfluorohexane ($CF_3(CF_2)_4CF_3$) is now used to store the modified Si nanopowder. As this solvent does not contain any −OH groups, the silicon particles are isolated from attack by alcohols or water in the solution or in the air. So far, with perfluorohexane storage, only minor deterioration in performance is observed after 30 days, and the powder is still functional even after more than 3 months.

Given the potential for long-term storage and the very modest consumption of powder per MALDI spot (less than 1 mg/spot), the silicon nanopowder can become a useful low-cost matrix, as conveniently used as any other MALDI chemical matrix.

CONCLUSIONS

This work has demonstrated the feasibility of using Si nanoparticles for desorption/ionization of small molecules in TOF mass spectrometry. The new technique has proved to be selective with high efficiency and good detection limits for basic analytes in the positive ion mode and acidic ones in the negative ion mode.

In comparison with standard MALDI ionization, the silicon nanopowder technique has several advantages, including some that are similar to the advantages of DIOS or other matrix-free techniques. First, the low mass ion background is much less abundant. Second, the analyte selectivity as well as the better salt tolerance can eliminate many of the problems encountered with MALDI analysis of samples in complex media. These may enable direct drug and metabolite analyses in urine, in other biological fluids, and in tissues and environmental analysis of salt-containing samples. Third, sample spots with the silicon nanopowder are homogeneous and do not form any "sweet spots". This results in better reproducibility and makes quantification more feasible (also true with DIOS[38,39]), although many of the quantification problems encountered with laser desorption TOF instruments would still remain.

The silicon nanopowder method seems to have characteristics similar to those of DIOS and other silicon-based methods with presumably similar mechanisms for energy transfer and ion formation involved. The molecular selectivity, solvent-dependent ionization efficiency, salt tolerance, and minimal formation of salt or other adduct ions may all be evidence for analyte precharging in solution.

Although it is similar in many respects to DIOS, the new silicon nanoparticle method is distinguished in some aspects that may affect its usefulness.

(1) The laser fluence required is typically much lower than that required for both standard MALDI and DIOS and is similar to that used for the nanowire DIOS or other nanosized particle desorption methods. This is assumed to be a result of efficient

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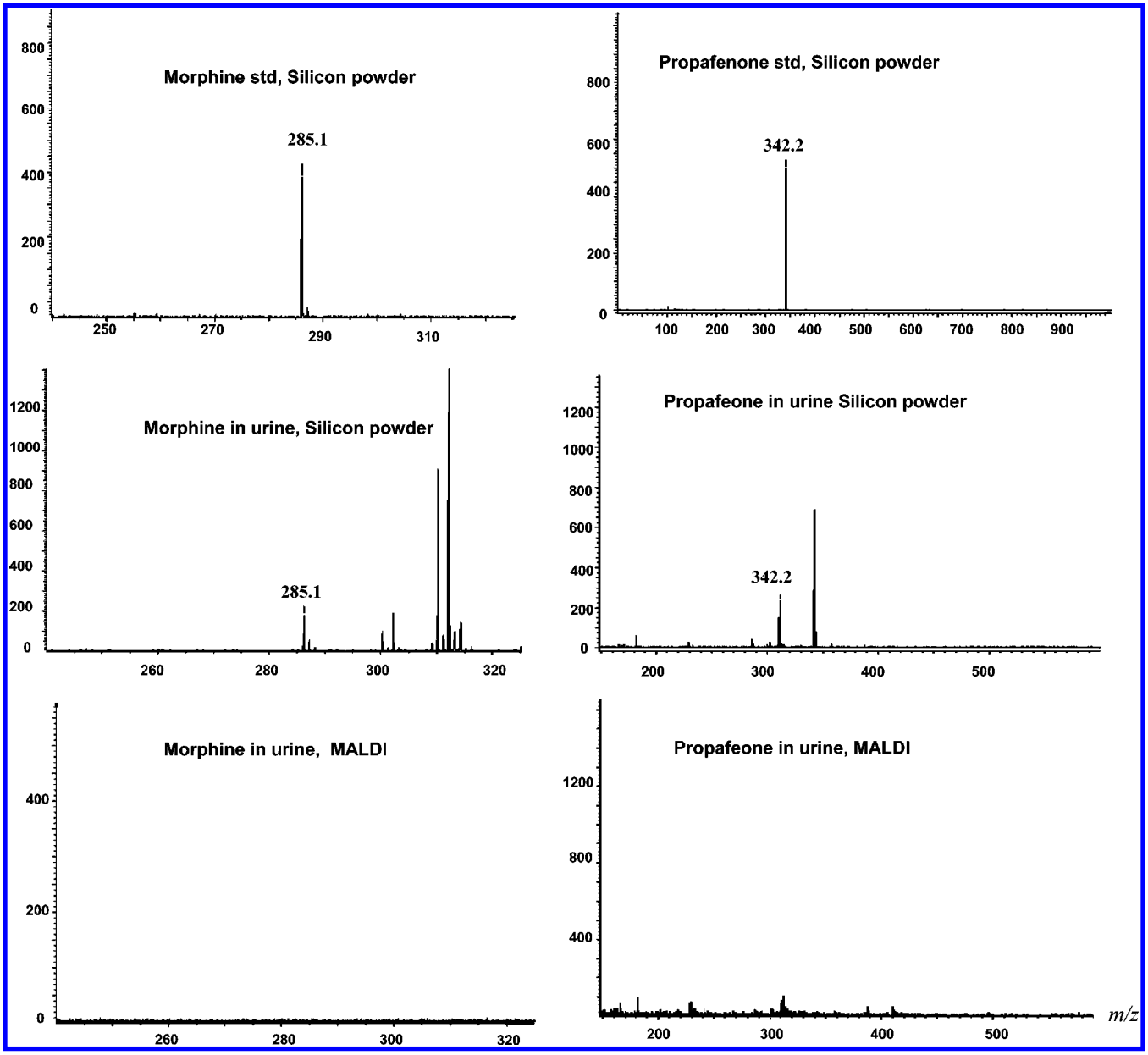

Figure 6. Analysis of morphine (left) and propafenone (right) spiked into untreated urine (final concentration of 10 pmol/μL each) with silicon powder LDI (middle) (laser fluence 15%) and with CHCA MALDI (bottom) (laser fluence 35%). For comparison, standard solutions with the same concentrations were also analyzed with the silicon powder (top). The particle size was 30 nm, and the optimized Si powder preparation was used.

UV absorption followed by high local overheating of the small particles. This feature may allow good analyte signals to be obtained with even lower background levels and may also be beneficial in imaging experiments, where a well-focused low-fluence laser beam can be used. The low laser fluence used may also be beneficial in the ionization of thermally labile compounds, where it may reduce the internal energy deposition.

(2) The use of a powder may enable better mixing and interaction of the analyte and the light-absorbing element compared with DIOS, which may also contribute to the lower laser fluence required and may induce better sample homogeneity. This may lead to less dependence on instrumental features such as laser alignment and incidence angle that has been observed for DIOS.[33]

(3) Powder preparation is simpler and safer than DIOS chip preparation. It does not require the use of UV light, electrochemical etching, or HF etching.

(4) The nanopowder instrumental application is simple, similar to standard MALDI, on a regular MALDI plate, without the need for any special setups. The silicon powder can be applied on a routine basis and even together with standard MALDI samples on the same plate.

The commercial availability of the 30 nm silicon nanopowder, the relatively simple preparation procedure, and its potential for long-term storage, together with the small amounts of powder required per analysis, can make this method a simple, practical, and low-cost ionization technique.

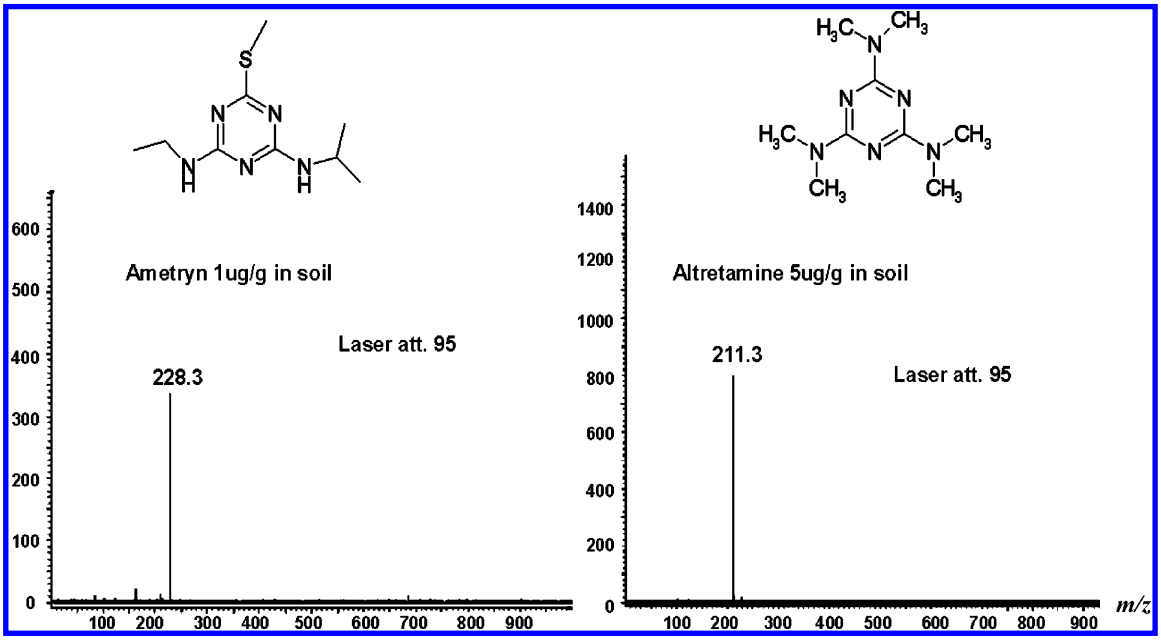

Figure 7. Tap water extraction of triazine pesticides ametryn and altretamine from soil (taken from the University of Arizona). The particle size was 30 nm, and the optimized Si powder preparation was used. The laser fluence was 5%.

ACKNOWLEDGMENT

We thank Dr. Arpad Somogyi and the Mass Spectrometry Facility at the University of Arizona for continuous support and guidance in the operation and maintenance of the MALDI instrument as well as fruitful discussions. We thank the surface facility laboratory in the Material Science and Engineering Department of the University of Arizona for the TEM and SEM tests. We also thank Mr. Michael Legg for the AFM experiments. We are grateful to the Siuzdak group for their guidance and assistance in assembling a DIOS setup in our laboratory. This work was supported by NSF-CHE Grant No. 0416338.

Received for review June 26, 2006. Accepted October 4, 2006.

AC061154L