

High Sensitivity and Analyte Capture with Desorption/Ionization Mass Spectrometry on Silylated Porous Silicon

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Silylation chemistry on porous silicon provides for ultra-high sensitivity and analyte specificity with desorption/ionization on silicon mass spectrometry (DIOS-MS) analysis. Here, we report that the silylation of oxidized porous silicon offers a DIOS platform that is resistant to air oxidation and acid/base hydrolysis. Furthermore, surface modification with appropriate hydrophobic silanes allows analytes to adsorb to the surface via hydrophobic interactions for direct analyte extraction from complex matrices containing salts and other nonvolatile interferences present in the sample matrix. This enables rapid cleanup by simply spotting the sample onto the modified DIOS target and removing the liquid phase containing the interferences. This approach is demonstrated in the analysis of protein digests and metabolites in biofluids, as well as for the characterizing of inhibitors from their enzyme complex. An unprecedented detection limit of 480 molecules (800 ymol) for des-Arg⁹-bradykinin is reported on a pentafluorophenyl-functionalized DIOS chip.

Desorption/ionization on silicon mass spectrometry (DIOS-MS) is a “soft” ionization method that uses a porous silicon (pSi) surface without a matrix to generate gas-phase ions.^{1,1–3} One challenge in the development of this technology is to produce a chemically modified pSi surface that retains DIOS-MS activity as this could potentially offer numerous advantages in surface stability, sample preparation, and selective analyte capture. Efforts with hydrosilylation and Lewis acid-mediated pSi functionalization with alkenes and alkynes^{4–9} have provided some success.

However, the silylation of oxidized pSi surfaces reported here produces far superior results. By using a variety of commercially available silylating agents, a diverse selection of functionalized pSi surfaces were synthesized and tested for DIOS performance.

The simplicity of the silylation chemistry, which has been used for decades in gas and liquid chromatography, allows for the easy modification of the DIOS surface properties by selecting appropriate silylating agents.¹⁰ Because of this flexibility, we have exploited the silylated surfaces for the development of sample cleanup techniques using the property of differential adsorption to selectively capture analytes from a solution containing contaminants that impede mass spectrometry analysis. DIOS with differential adsorption simply involves the deposition of a droplet containing analytes such as a protein digest and its subsequent removal with a pipettor. The van der Waals forces between the analyte and surface selectively extract the peptides or small molecules, while the hydrophilic contaminants such as salts are removed with the droplet. Similar sample preparation methods on various types of surfaces include preconcentration with a number of commercially available cleanup devices, such as the MALDI Anchor-Chip (Bruker Biosciences),¹¹ ProteinChip Array System from Ciphergen based on SELDI-MS,¹² the MALDI Mass-PREP PROtarget plates (Waters Corp.), or ZipTips (Millipore Corp.). This last product is a miniaturized solid-phase extraction (SPE) device that typically uses a C₁₈ sorbent to bind, wash, and elute a sample directly onto the plate. One limitation of silica-based sorbents is that significant losses can occur from strong irreversible adsorption onto the stationary-phase surfaces especially when subpicomole quantities are used.¹³ Advantages of modified DIOS surfaces include ultra-

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- (1) Wei, J.; Buriak, J. M.; Siuzdak, G. *Nature* **1999**, *399*, 243–6.
- (2) Shen, Z.; Thomas, J. J.; Averbuj, C.; Broo, K. M.; Engelhard, M.; Crowell, J. E.; Finn, M. G.; Siuzdak, G. *Anal. Chem.* **2001**, *73*, 612–9.
- (3) Thomas, J. J.; Shen, Z.; Crowell, J. E.; Finn, M. G.; Siuzdak, G. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 4932–7.
- (4) Dubin, V. M.; Vieillard, C.; Ozanam, F.; Chazalviel, J. N. *Phys. Status Solidi B* **1995**, *190*, 47–52.
- (5) Lees, I. N.; Lin, H. H.; Canaria, C. A.; Gurtner, C.; Sailor, M. J.; Miskelly, G. M. *Langmuir* **2003**, *19*, 9812–7.

- (6) Buriak, J. M.; Stewart, M. P.; Geders, T. W.; Allen, M. J.; Choi, H. C.; Smith, J.; Raftery, D.; Canham, L. T. *J. Am. Chem. Soc.* **1999**, *121*, 11491–502.
- (7) Stewart, M. P.; Buriak, J. M. *J. Am. Chem. Soc.* **2001**, *123*, 7821–30.
- (8) Stewart, M. P.; Robins, E. G.; Geders, T. W.; Allen, M. J.; Choi, H. C.; Buriak, J. M. *Phys. Status Solidi A* **2000**, *182*, 109–15.
- (9) Saghatelian, A.; Buriak, J.; Lin, V. S. Y.; Ghadiri, M. R. *Tetrahedron* **2001**, *57*, 5131–6.
- (10) Moreno, J. D.; Guerrero-Lemus, R.; Martin-Palma, R. J.; Martinez-Duart, J. M.; Marcos, M. L.; Gonzalez-Velasco, J. J. *Mater. Sci.* **1999**, *34*, 3067–9.
- (11) Schuereberg, M.; Luebbert, C.; Eickhoff, H.; Kalkum, M.; Lehrach, H.; Nordhoff, E. *Anal. Chem.* **2000**, *72*, 3436–42.
- (12) Huthens, T. W.; Yip, T. T. *Rapid Commun. Mass Spectrom.* **1993**, *7*, 576–80.
- (13) Cuppett, C. M.; Doneski, L. J.; Wirth, M. J. *Langmuir* **2000**, *16*, 7279–84.

high sensitivity demonstrated for des-Arg⁹-bradykinin, lower background, and selective analyte absorption for on-surface sample preparation and the monitoring of protein–ligand interactions. By taking advantage of the differential adsorption property of the modified DIOS surface, one of the most sensitive mass spectrometry-based platforms can be achieved for the analysis of complex matrixes. Furthermore, this technique can be easily automated for high-throughput analysis.

EXPERIMENTAL SECTION

Materials. Optima grade acetonitrile, methanol, 2-propanol, ethanol, tribasic ammonium citrate, verapamil hydrochloride, midazolam, propafenone hydrochloride, maltotriose, sucrose, bovine serum albumin, hemoglobin *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) in 1% trimethylchlorosilane as catalyst, *N*-methyl-*N*-(trimethylsilyl)fluoroacetamide (MSTFA), hexamethyldisilazane (HMDS), octyldimethylchlorosilane (ODMCS), chloro(dimethyl)octadecylsilane (CDOS), and (3,3,4,4,5,5,6,6,6-nonafluorohexyl)chlorosilane (FHCS) were obtained in high purity from Sigma-Aldrich except when noted otherwise. (3-Aminopropyl)dimethylethoxysilane (APDMES), (pentafluorophenyl)propyldimethylchlorosilane (PFPPDCS), and trypsin were obtained from Gelest, Inc., Waters Corp., and Promega, respectively. Stock solutions for all small molecules were prepared by reconstituting the lyophilized sample in NANOpure water at 1 mg/mL and performing additional serial dilutions as needed. High-purity water from JT Baker (Baker Analyzed HPLC reagent) was obtained for the sensitivity measurements with des-Arg⁹-bradykinin.

DIOS Chip Preparation. The details of DIOS chip preparation have been described elsewhere.² Briefly, DIOS chips were prepared by etching low-resistivity (0.005–0.02 $\Omega\cdot\text{cm}$) n-type Si(100) wafers (Silicon Sense) in 25% v/v hydrofluoric acid (Fisher Scientific)/ethanol under white light illumination at a current density of 5 mA/cm² for 2 min. Typically, photopatterning was performed to create 100 sample spots on each chip. Immediately after etching, the DIOS chip was rinsed with ethanol and dried in a stream of N₂ to give an H-terminated surface, which was oxidized by exposure to ozone (flow rate of 0.5 g/h from an ozone generator directed at the surface for 30 s) and subsequently modified with the silylating reagent.

Chemical Modification of pSi Surfaces. Surface derivatization involved the modification of hydroxyl groups present on the ozone-oxidized porous silicon surface by silylation with BSTFA, MSTFA, HMDS, ODMCS, CDOS, APDMES, FHCS, or PFPPDCS. These modifications generated trimethylsilyl- (TMS), amine-, C₈-, C₁₈-, perfluoroalkyl-, and perfluorophenyl-derivatized surfaces. Except for C₁₈-derivatized surfaces, each silylation reaction was performed by adding 15 μL of the appropriate silylating reagent on the oxidized DIOS chip, which was placed in a glass Petri dish and incubated in an oven at 65 °C for 15 min. The modified DIOS chip was then rinsed thoroughly with methanol and was dried in a stream of N₂. For C₁₈ modification, the oxidized silicon surface is allowed to react with 5% chloro(dimethyl)octadecylsilane in toluene overnight at 80 °C. The surface was washed with excess toluene, followed by isopropyl alcohol and methanol. This simple derivatization procedure produces silylated porous silicon surfaces as verified by infrared (IR) spectroscopy.

High-Sensitivity Measurements with Silylated DIOS-MS. Des-Arg⁹-bradykinin was obtained from Sigma-Aldrich in high

purity, and PFPPDCS was obtained from Gelest and was used to synthesize a modified silicon surface. To perform the dilutions, a 1.0 mg/mL stock solution was prepared in high-purity water (Baker Analyzed HPLC reagent), and a 1.0 μM solution was made from the initial 1 mg/mL solution. Serial dilutions were made at ratios of 1:100 in prewashed 15-mL Fisher brand glass vials. A final dilution of 1:2.5 was made to obtain the 4 fM concentration, and a 0.2- μL volume of this solution provided 800 ymol of the deposited sample. The vials were washed with methanol and water prior to use. A total of 990 μL of water was dispensed into the vials to which 10 μL of concentrated sample was added. Care was taken to minimize time between successive dilutions and therefore to minimize contact with the surface in the vial. A 10- μL concentrated sample was directly dispensed into each vial using a Rainin P-10 Pipetman containing 990 μL of water. The sample was gently vortexed for ~ 3 s before a fresh pipet tip was used to remove the diluted sample and dispense 10 μL into the next serial dilution vial containing 990 μL of water. A 0.2- μL sample was removed with a calibrated Rainin P-2 Pipetman with Rainin brand tips and applied to the DIOS surface. A blank DIOS analysis was performed from the water from the sample vial prior to making the serial dilution. The high-sensitivity DIOS-MS experiments were performed on the Applied Biosystems MALDI-TOF STR mass spectrometer. The mass spectrum generated on the 800 ymol was typically the result of a single shot for des-Arg⁹-bradykinin since subsequent laser shots produced no signal due presumably to complete sample consumption.

Preparation of Amino Acid Extract of Plasma Samples.

The preparation of the butyl ester derivatives of the amino acids in plasma involved deproteinization by simple cold methanol extraction followed by derivatization. A 400- μL methanol stock solution containing 8 nmol of stable deuterated analogue of phenylalanine was added to 100 μL of plasma, vortexed, and centrifuged. The methanol extract was then transferred to an Eppendorf vial and evaporated to dryness in a stream of nitrogen. The dried sample was dissolved in 100 μL of 3 N butanolic HCl and incubated at 65 °C for 30 min. Excess butanol was removed by evaporating the reaction mixture to dryness in a Speedvac. The derivatized plasma samples were reconstituted with water.

Preparation of Protein Digests. Bovine serum albumin (BSA) and hemoglobin proteolytic digests were prepared with trypsin (1:30 enzyme-to-protein ratio by mass). The BSA and hemoglobin 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. Samples (0.5 μL) were pipetted directly onto the chemically modified DIOS chip. A standard digest of BSA was also obtained from Agilent and further reconstituted as necessary.

Sample Cleanup on Silylated DIOS Targets. The alkylated DIOS surface was soaked in methanol prior to use in order to remove hydrophobic contaminants and to wet the surface. After drying the chip, a 0.5- μL aliquot of sample was applied using a pipet on the surface and the solution was aspirated back into the pipet tip after 3 s. Up to five repeat applications were performed with the sample to maximize loading. For certain complex samples, an on-target wash step with 0.5 μL of 0.1% formic acid was applied to remove proteins and small-molecule contaminants.

Surface Modification

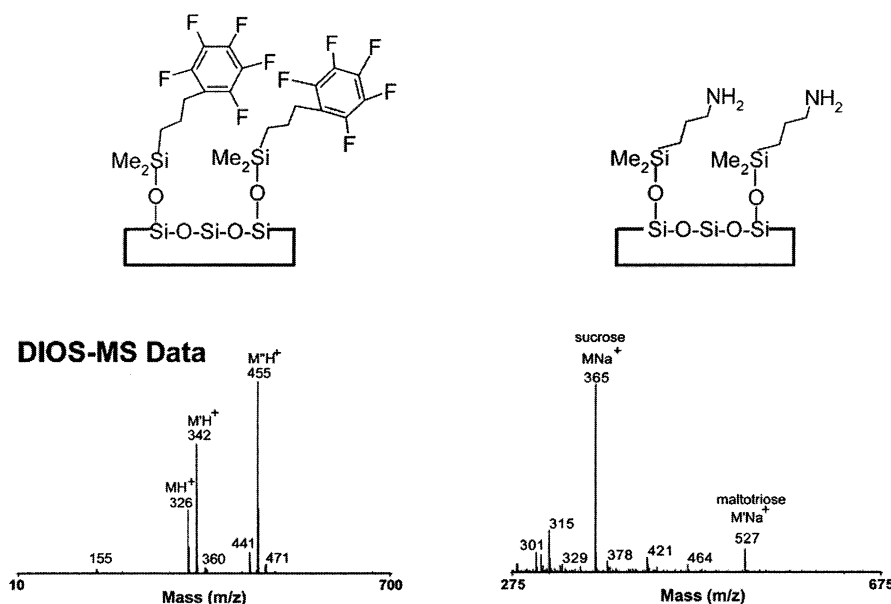


Figure 1. DIOS-MS spectra of small-molecule mix containing midazolam (MH^+ 326), propafenone ($M'H^+$ 342), and verapamil ($M''H^+$ 455) on perfluorophenyl silylated pSi surface (left) and carbohydrate mix containing sucrose (MNa^+ 365) and maltotriose ($M'Na^+$ 527) on amine silylated pSi surface (right). The hydrophobic perfluorophenyl-derivatized surface is amenable to hydrophobic molecules while the amine-derivatized surface is more amenable to hydrophilic molecules.

Enzyme Inhibition Studies. Staurosporine was obtained from Sigma-Aldrich, and Rho-Kinase (ROCKII) was obtained in active form (Upstate Biotechnology, Lake Placid, NY). Equimolar (5 μ M) solutions of ROCKII, staurosporine, verapamil, and heptylene bis-(tacrine) were prepared in 50 mM ammonium bicarbonate and incubated for 1 h at 37 $^{\circ}$ C. The enzyme was separated from the noninhibitors using a 10 000 molecular weight cutoff filter (Centricon, YM-10) by centrifuging the mixture at 13000g (Eppendorf model 5417 centrifuge) for 20 min. The enzyme-inhibitor complex was washed with a 500- μ L aliquot of 50 mM ammonium bicarbonate to further remove noninhibitors and centrifuged for an additional 10 min. DIOS-MS spectra were obtained of the mixture before and after passage through the MW cutoff filter to illustrate the selectivity obtained from this approach.

Instrumentation. DIOS-MS measurements were performed with an Applied Biosystems (Framingham, MA) Voyager STR or a Waters Micromass (Milford, MA) M@LDI-R time-of-flight reflectron mass spectrometer. The DIOS chips were directly attached to a modified MALDI target plate using Scotch Crystal Clear tape. The samples were irradiated with a 337-nm nitrogen laser operated at 5 Hz (3-ns pulse duration) and attenuated with a neutral density filter. A delayed extraction period of 10–250 ns was used to minimize the energy spread of the ions for optimum resolution, after which the ions were accelerated by a 20-kV pulse through a reflectron time-of-flight analyzer and detected using a multichannel plate detector. The DIOS-MS spectra were generated by averaging data obtained from 50–500 laser pulses. The laser intensity was adjusted to optimize the signal-to-noise ratio obtained for the mass spectral data. All data were externally calibrated except for the peptide mass fingerprint (PMF) obtained using DIOS capture of a BSA digest. The PMF was internally calibrated using trypsin autolysis peaks and searched using Mascot (Matrixscience Ltd.) using the Swissprot protein database. A score of

125 was obtained for the BSA digest, indicating a >95% confidence level in the match.

Safety Considerations. Extreme care should be taken in handling hydrofluoric acid solutions because of their toxicity and corrosiveness. All inhalation, ingestion, or skin and eye contact should be strictly avoided. Etching of silicon wafers should be conducted in a ventilated fume hood using proper double-layered nitrile gloves, lab coat, and goggles. Hydrofluoric acid solution spills and burns can be neutralized and treated with 2.5% calcium gluconate gel.

RESULTS AND DISCUSSION

Surface Modification. Silylation of protic heteroatom groups is a simple, versatile, and commonly used derivatization procedure for gas and liquid chromatography column preparation as well as molecule stabilization.^{14–17} This efficient reaction typically employs commercially available electrophilic silylating reagents containing a halide or stabilized heteroatom leaving group. In the initial experiments to modify pSi, trimethylsilylating reagents such as BSTFA and methoxysilane ($MeOSiMe_3$) were employed on oxidized pSi surfaces, resulting in the restoration of DIOS activity. These and subsequent experiments with different reagents (C_8 , C_{18} , dyes) led to two of the most effective modifications, a terminal amino group amenable for hydrophilic molecules and a pentafluorophenyl substituent for hydrophobic molecules shown in Figure

- (14) Evershed, R. P. In *Handbook of Derivatives for Chromatography*, 2nd ed.; Blau, K., Hackett, J. M., Eds.; John Wiley and Sons: New York, 1993; Chapter 4.
- (15) Plueddemann, E. P. *Silane Coupling Agents*; Plenum Press: New York, 1982.
- (16) Knapp, D. R. *Handbook of Analytical Derivatization Reactions*; John Wiley and Sons: New York, 1979.
- (17) Halket, J. M.; Zaikin, V. G. *Eur. J. Mass Spectrom. (Chichester, Eng.)* **2003**, *9*, 1–21.

1. It should be noted that di- and trialkoxysilanes polymerized¹⁸ to give a clearly visible coating and a concomitant suppression of DIOS activity.

To evaluate the stability and sensitivity of the derivatized chips, DIOS-MS experiments were performed on protein model systems (BSA and hemoglobin tryptic digests, 5 fmol), three small drug molecules (propafenone, MH^+ 342; verapamil, MH^+ 455; midazolam, MH^+ 326; 200 fmol each), and simple neutral carbohydrates (maltotriose, MNa^+ 527; sucrose, MNa^+ 365; 25 pmol each). A comparison of the DIOS performance of TMS-, C_8 -, NH_2 -, and perfluorophenyl-derivatized pSi with freshly etched pSi revealed that all of the derivatized surfaces gave signal-to-noise values and mass ranges very similar to Si-H surface. Our results show that the pentafluorophenyl-derivatized DIOS chips exhibited the highest sensitivity, lowest level of background ions, and lowest laser energies. The modified chips were also resistant to air and ozone oxidation (oxidation of Si-H surface completely eliminates DIOS activity), and ongoing longevity experiments with the silylated surfaces have thus far demonstrated a lifetime of greater than 9 months for TMS-derivatized pSi, with no noticeable loss of sensitivity. The stability of derivatized chips was also tested against acid/base hydrolysis over a wide pH range by immersing the derivatized DIOS wafers in aqueous methanol at the desired pH for 24 h at room temperature. No change in DIOS activity was observed within the pH range of 2–9, a result consistent with studies on luminescent pSi surfaces terminated with long-chain and aromatic alkynes.^{8,19,20}

An additional important observation is that hydrophobic-derivatized surfaces were shown to be suited to the detection of both hydrophilic and hydrophobic compounds, while the NH_2 -derivatized chips selectively ionized for very hydrophilic analytes. This led to the construction of a patterned chip designed to perform on-chip concentration to enhance sensitivity. To accomplish this, an oxidized pSi chip was spotted in an array format with 0.5 μ L of aminopropylsilyl reagent, incubated at 65 °C, washed, and then immersed in a solution of BSTFA. This resulted in a chip that contained ~1-mm-diameter hydrophilic spots within a surrounding hydrophobic region. Both hydrophobic and hydrophilic regions of the plate are DIOS active, yet the deposition of aqueous-phase samples on the amine-containing hydrophilic spots resulted in containment and preconcentration of analyte on each spot as each droplet dried. Using this type of wafer, it was possible to improve sensitivity on a carbohydrate mixture (sucrose and maltotriose) by a factor of 10 compared with underivatized target plates.

High Sensitivity with Silylated Silicon Surface. A set of experiments with an aqueous solution of des-Arg⁹-bradykinin was used to examine the sensitivity of the pentafluorophenyl silylated silicon surface. Des-Arg⁹-bradykinin was chosen because it is commonly used as a test of sensitivity by instrument manufacturers. A series of dilution experiments ultimately demonstrated a lower limit of detection for the peptide at 480 molecules (800 ymol) (Figure 2). This result was reproduced in 10 different sets of experiments by two different individuals (S.A.T. and J.V.A.).

Sample Cleanup on Silylated Silicon. Hydrophobically modified DIOS surfaces could be readily used to selectively

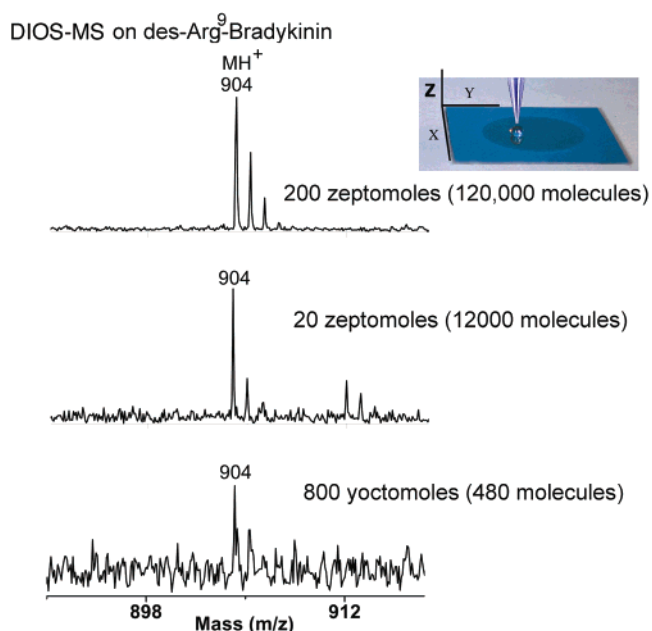


Figure 2. DIOS mass spectra obtained for 200 zmol, 20 zmol, and 800 ymol for des-Arg⁹-bradykinin using the perfluorophenyl silylated modified chip.

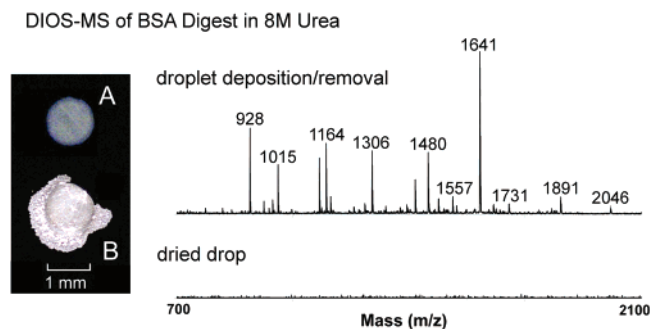


Figure 3. Comparison of mass spectra obtained from 500 amol of a BSA tryptic digest out of 8 M urea using (A) differential adsorption sample cleanup on the DIOS surface and (B) dried drop deposition. In the first example, the mass spectrum containing peaks from multiple peptides that readily allowed for protein identification, while the second approach, conventional dried drop deposition, generated virtually no signal as urea crystallized out and covered the chip.

remove interferences prior to analysis. The method involves depositing the sample on a modified pSi DIOS surface and then, ~3 s later, aspirating the sample with the same pipet. This short-term deposition allows for any molecule with a propensity for adsorption onto the surface to attach itself, yet any potential hydrophilic contaminants such as salts and buffers remain in solution, leaving the surface free of such contaminants. The DIOS-MS analysis for peptides and small molecules from complex matrixes was generally enhanced with this differential adsorption technique on the silylated silicon surface. For example, a peptide mass map obtained on 500 amol of a BSA digest in 8M urea with and without aspiration demonstrates the utility and sensitivity of this sample cleanup technique even in the presence of a large quantity of contaminant (Figure 3). The data could be searched against a database using Mascot to identify the model protein with a very high confidence score. No signal was obtained on these samples using the conventional dried drop deposition with DIOS (Figure 3) and MALDI (identical to flat mass spectral data in

(18) Kruse, R. A.; Rubakhin, S. S.; Romanova, E. V.; Bohn, P. W.; Sweedler, J. V. *J. Mass Spectrom.* **2001**, *36*, 1317–22.

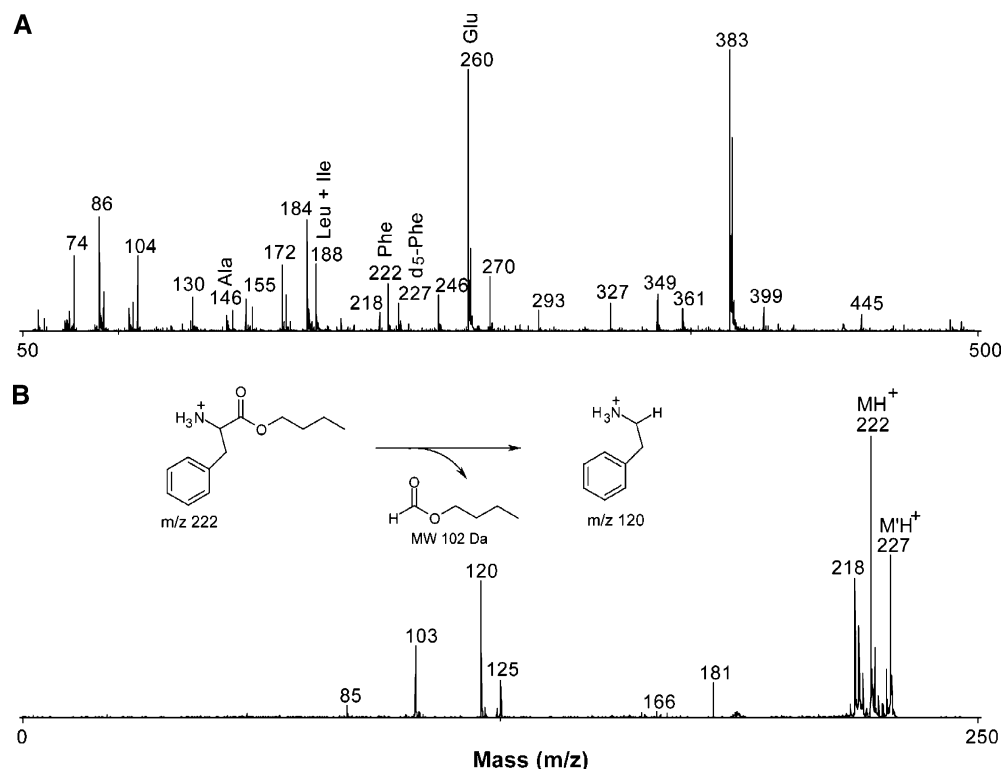


Figure 4. (A) On-target sample cleanup followed by DIOS mass analysis of amino acids in human plasma after esterification. (B) DIOS PSD profile of phenylalanine (MH^+ 222) and phenylalanine- d_5 (MH^+ 227) showing a characteristic neutral loss of 102 Da corresponding to butyl formate resulting in the product ions of the butyl ester derivatives of phenylalanine (MH^+ 120) and its corresponding deuterated internal standard (MH^+ 125).

Figure 3), because of the very high urea concentration that forms crystals on the silicon surface. Similar performance can be achieved for 500 amol of the BSA digest, which contains other buffers that cause extreme signal degradation for both MALDI and DIOS due to the presence of soluble nonvolatile species using conventional "dried drop" deposition. Other problematic solution systems that have been successfully tested include 6 M guanidine hydrochloride, 50% glycerol, TBS buffer (500 mM sodium chloride and 20 mM Tris-HCl), PBS (10 mM sodium phosphate, 150 mM sodium chloride), and neat dimethyl sulfoxide.

Sample Cleanup and DIOS-MS for Plasma Metabolite Analysis. While the availability of a versatile array of MS instrumentation has provided sensitivity, speed, and ease of use in the detection and identification of metabolites in biofluids, sample preparation prior to MS analysis remains a time-consuming process. We extend the utility of sample cleanup using selective adsorption with DIOS to the analysis of amino acids in plasma. Using a simple cold methanol extraction to remove the proteins followed by a butyl ester derivatization.^{21–23} Phenylalanine (MH^+ 222), alanine (MH^+ 146), isoleucine/leucine (MH^+ 188), glutamic

acid (MH^+ 260), and the doped deuterated internal standard of phenylalanine (MH^+ 227), used to quantify the amino acid, were detected with good sensitivity after performing a sample cleanup step (Figure 4). The specificity and sensitivity of this technique using derivatized pSi surfaces was further examined by employing MS/MS analysis. The selective MS/MS analysis of phenylalanine is demonstrated using the postsource decay (PSD) method to confirm the detection of the amino acid in plasma. PSD analysis of the butyl ester derivative of phenylalanine and its deuterated analogue shows a characteristic neutral loss of 102 Da associated with butyl formate resulting in the product ions at 120 and 125 Da from the collision-induced dissociation of butylated phenylalanine and its deuterated internal standard, respectively. A rapid desorption ionization technique like DIOS-MS for the direct analysis of amino acids in plasma could potentially be powerful since many inherited metabolic disorders such as phenylketonuria can be routinely and accurately diagnosed by monitoring the concentration ratio of certain amino acids in extracts of neonatal blood specimens.^{18–20}

Drug Molecule Analysis from Drug–Protein Complexes with DIOS-MS. An important application of mass spectrometry is in monitoring enzyme reactions where it is capable of detecting most natural substrates or products, and therefore, assay development is rapid (typically less than a day), and false positives and false negatives are rare (m/z overlap with a contaminant). The absence of low-MW matrix-related ions in DIOS-MS, the ability to analyze samples rapidly, and the ease of automation make it a potentially useful tool for the direct identification of inhibitors. However, sample preparation is often necessary due to the

- (19) Janshoff, A.; Steinem, C.; Lin, V. S. Y.; Ghadiri, M. R.; Sailor, M. J. *Abstr. Pap. Am. Chem. Soc.* **1998**, 216, U89.
- (20) Janshoff, A.; Dancil, K. P. S.; Steinem, C.; Greiner, D. P.; Lin, V. S. Y.; Gurtner, C.; Motesharei, K.; Sailor, M. J.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1998**, 120, 12108–16.
- (21) Chace, D. H.; Millington, D. S.; Terada, N.; Kahler, S. G.; Roe, C. R.; Hofman, L. F. *Clin. Chem.* **1993**, 39, 66–71.
- (22) Chace, D. H.; Millington, D. S.; Terada, N.; Kahler, S. G.; Roe, C. R.; Hofman, L. F. *Clin. Chem.* **1993**, 39, 66–71.
- (23) Millington, D. S.; Kodo, N.; Terada, N.; Roe, D.; Chace, D. H. *Int. J. Mass Spectrom. Ion Processes* **1991**, 111, 211–28.

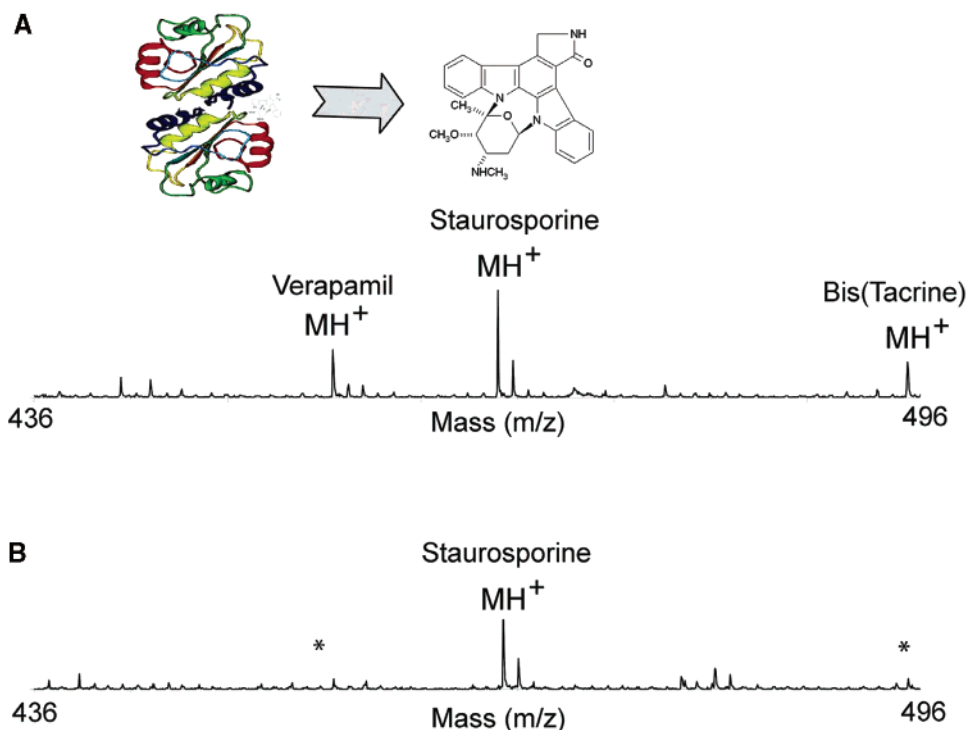


Figure 5. Monitoring of enzyme inhibitors with DIOS-MS following sample cleanup: Rho-kinase II, staurosporine (a potent kinase inhibitor), verapamil, and bis(tacrine) before (A) and after (B) passage through a 10 000 MW cutoff filter in the presence of a stabilizing buffer allowing for the identification of a known inhibitor.

presence of salts and proteins in biological samples. The presence of high quantities of salt and protein often results in the formation of a crust on the target inhibiting both MALDI and DIOS-MS activity. However, most of these contaminants can be removed by differentially adsorbing the analytes relative to polar salts onto a surface-modified DIOS target. An experiment was designed to demonstrate that DIOS-MS with sample cleanup can separate inhibitors from nonbinding molecules for a model enzyme. Figure 5 shows the selective extraction of the potent kinase inhibitor, staurosporine in the presence of two other nonbound molecules by using a 10 000 MW cutoff filter to isolate the Rho-kinase II–staurosporine complex, spotting the sample onto the DIOS target, and then removing polar interferences prior to MS analysis. The hydrophobic interaction of the drug with the fluoroalkyl-modified DIOS surface was able to extract the drug, even in the presence of protein, salts, and stabilizing agents for the active enzyme (25% glycerol, 75 mM NaCl, 0.5 mM benzamidine, 0.1 mM PMSF, 0.05 mM EGTA, 0.015% Brij 35, and 0.05% 1-mercaptoethanol in the original mixture). Therefore, this cleanup technique, which was performed directly on the DIOS surface, could serve as a useful tool for monitoring drugs in biological fluids in the presence of high amounts of proteins and soluble nonvolatile contaminants.

CONCLUSIONS

We have demonstrated that modification of the oxidized pSi surface by the formation of O–Si bonds to functionalized silanes alters surface properties, improves stability against oxidation and hydrolysis, and promotes DIOS-MS activity over an extended period of time. The ease of incorporating functional groups to

modify the pSi surface for mass spectrometric applications demonstrates that silyl derivatization is a flexible approach for preparing functionalized DIOS chips for analyte-specific applications and, for the selective adsorption of analytes, enables on-target sample cleanup and analysis of analytes from complex matrixes. Differential adsorption sample cleanup directly on the DIOS target provides a sensitive and rapid method for the selective extraction of peptides and small molecules out of complex matrixes, including nonvolatile buffers and stabilizing agents frequently present in mass spectrometry samples and biofluids. If these nonvolatile and hydrophilic substances are not removed prior to analysis, they can cause severe signal suppression. Due to its simplicity, this cleanup technique can be easily automated for high-throughput applications with commercially available robots designed for sample processing and spotting on MALDI targets, therefore eliminating the necessity for additional SPE components such as ZipTips or matrix deposition. Most importantly, we are only in the initial stages of exploring the chemical possibilities for modifying these surfaces. And with these early successes in sensitivity and improved specificity, the next generation of modifications hold even greater promise.

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