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High-Performance SPME/AP MALDI System for High-Throughput Sampling and Determination of Peptides

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This paper presents the performance characteristics for a new multiplexed solid-phase microextraction/atmospheric pressure matrix-assisted laser desorption/ionization (SPME/AP MALDI) source configuration for a hybrid quadrupole-linear ion trap instrument. The results demonstrate that thorough optimization of parameters such as SPME coating material, optics configurations, extraction solvents, and fiber capacity provides dramatic sensitivity improvements (>1000×) over previous reports in the literature. The multiplexed SPME plate is capable of simultaneous extraction from 16 different wells on a multiwell plate, eliminating the need for extensive sample preparation. Subfemtomole sensitivity is demonstrated for peptide standards and protein digests with run−run reproducibility ranging from approximately 13 to 31%. This high-performance SPME/AP MALDI system shows potential for high-throughput extraction from biological samples.

Matrix-assisted laser desorption/ionization (MALDI) has become a powerful technique for the analysis of proteins and peptides by mass spectrometry (MS) since its introduction in 1988.[1] Though MALDI has enabled the routine identification of biomolecules, the need to increase the throughput of the method has been recognized. Sample preparation for MALDI is still the time-limiting step, since it dictates the quality of the MS spectra. Efforts have focused on trying to produce more uniform cocrystals between the analytes and the matrix to improve the performance and reproducibility.[2,3] Others have attempted to combine the sample extraction onto the MALDI target.[4] Surface-enhanced laser desorption/ionization (SELDI) protein chip arrays are commonly used in proteomic research. These SELDI devices still require substantial sample preparation, and the automation is also expensive.

As a simple and efficient sample preparation technique, solid-phase microextraction (SPME) has been widely used with gas chromatography (GC)[5] and more recently with liquid chromatography.[6] Recently, several research groups have coupled SPME to other types of mass spectrometers. Meurer and co-workers demonstrated direct coupling of SPME with an electron ionization mass spectrometer.[7] Referred to as fiber introduction mass spectrometry, this method was used to analyze volatile and semivolatile compounds by direct insertion of a poly(dimethylsiloxane)-coated SPME fiber into the ion source after headspace extraction. Teng and Chen reported the combination of SPME with MALDI-MS.[8] A sol−gel-derived 2,5-dihydroxybenzoic acid film was employed as the SPME extraction coating and the substrate to help ionization without the addition of matrix. After extraction, the SPME fiber was attached on a MALDI plate with double-sided carbon tape. This procedure was not amenable to automation, and only the analytes on one side of the SPME fiber could be introduced to the MS. Direct coupling of an SPME fiber to a laser desorption mass spectrometer has also been described with ion mobility [9] and time-of-flight (QqTOF) instruments.[10] The SPME/MALDI fiber was employed as both the SPME extraction phase and MALDI substrate; however, the sensitivity was poor (pmol μL^{-1} detection limits).

This paper further investigates the coupling of SPME/MALDI to mass spectrometry. A multiplexed SPME plate was coupled to a high-performance hybrid quadrupole-linear ion trap (QqLIT) with a modified AP MALDI source. Since extraction time (2−10 min) is typically the rate-limiting step for SPME/AP MALDI analyses (MALDI MS times can be as low as a few seconds when complete sample depletion is not required, or high repetition rate lasers are used), the sample throughput can be improved by a factor approaching the number of fibers on the device. In this case, a 16-fiber embedded SPME/MALDI plate was constructed for demonstration. The multiplexed plate permits 16 simultaneous

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extractions from a 96-well plate, substantially improving throughput over previous configurations where successive sampling was achieved in a serial fashion. In addition, the use of a single plate with the fibers embedded allows for highly reproducible extraction times between replicates as opposed to immersion of separate fibers. In addition to parallel sampling, this system also improves throughput by simplifying the sample preparation for MALDI. In addition, a number of operational parameters were optimized to improve the system performance. Optimization of the laser illumination geometry provided more than a 100-fold improvement in the S/N ratio for peptides. The addition of α-cyano-4-hydrocinnamic acid (α-cyano) matrix to the extraction solvent gave improvements of approximately 100× and 32× for the absolute signal and S/N ratio for peptides, respectively. Analytical performance was also improved by using extraction fibers with increased surface areas (larger extraction capacity) and an improved atmosphere−vacuum interface. The combination of all these improvements gave detection limits of less than 500 amol μL^{-1} for protein digests with typical fiber−fiber reproducibilities on the order of 13−31%. For these studies, two different SPME coatings were evaluated (polypyrrole and polythiophene) and the extraction efficiency was determined. This system presents a low-cost, easy to use, high-throughput sample preparation tool for AP MALDI-MS analysis.

EXPERIMENTAL SECTION

Chemicals. Pyrrole, thiophene, anhydrous ferric chloride, tetraoctylammonium bromide, formic acid, angiotensin II, angiotensin I, bradykinin, glufibrinopeptide b, and bovine serum albumin (reduced and carboxymethylated BSA) were purchased from Sigma-Aldrich (St. Louis, MO). 2-Propanol and ammonium persulfate were purchased from VWR (Toronto, ON, Canada). The α-cyano-4-hydrocinnamic acid MALDI matrix was purchased from Agilent (Palo Alto, CA). Prior to digestion, protein samples were prepared at 5 mg/mL in 50 mM ammonium bicarbonate (BDH Chemicals, Toronto, ON, Canada) buffer adjusted to pH 8.5 with ammonium hydroxide (Fisher Scientific, Nepean, ON, Canada). Digestions were carried out with a ratio of 20:1 protein/trypsin. Proteins were digested for 4 h at 37 °C, and the digests were stored at −20 °C prior to use. Digests were reconstituted in water with 0.1% formic acid. Nanopure deionized water was exclusively used in these experiments. A four-peptide mixture containing angiotensin II, angiotensin I, bradykinin, and glufibrinopeptide b was prepared in water with 0.1% formic acid.

Preparation of the SPME Fibers. High OH silica optical fibers with core diameters of 600 μm were purchased from Polymicro Technologies Inc. (Phoenix, AZ) for the assessment of performance with different optical configurations. The connector ferrule, F-112 epoxy glue, polishing disk, and polishing films (5, 3, 1, and 0.3 μm) were purchased from Thorlabs Inc. (Newton, NJ). The silica optical fibers were cut into 1-m sections with a capillary cutter from Restek (Bellefonte, PA). One end of the optical fibers was glued to a connector ferrule with F-112 epoxy glue. After 24-h curing time, this fiber connector end was polished with polishing films to ensure the maximum light throughput. The other end of the optical fiber, hereafter called the sampling end, was coated with polymer coating and used for extraction. About 1 cm of the optical fiber was first cut from the sampling end to have a fresh clean surface to work with. Then the fiber tip was etched with 400-grit silicon carbide polishing paper. The etching step ensured that the polymer adhered to the fiber tip. The tip was then sonicated in methanol to remove the impurities on the fiber tip. After rinsing with water, the fiber tip was ready for the coating process.

Preparation of Multiplexed SPME/MALDI Plate. Glass rods with 2-mm diameter obtained from the University of Waterloo glass shop were used to prepare the SPME fiber tips for the multiplexed SPME/AP MALDI plate. The glass rods were cut into 3-cm sections, and then one tip and the sides of the rods were etched with 400-grit silicon carbide polishing paper. The tip was then cleaned with the same procedure described above.

A standard ABI stainless steel MALDI plate (AB/MDS SCIEX, Concord, ON, Canada) was used to prepare the multiplexed plate. A total of 16 holes were drilled on the plate, and 16 coated SPME tips were glued into place. The tips were cut so that the sampling ends protruded from the flat surface of the plate by ∼5 mm. The tips were positioned to provide alignment with the wells on a 96-well plate to permit simultaneous extraction from multiple wells.

Preparation of Polypyrrole (PPY)-Coated Fibers and Tips. Polypyrrole was prepared by chemical oxidation of pyrrole monomer with ammonium persufate. All the solutions were prepared fresh prior to the coating procedure. Up to 10 fibers or glass tips could be prepared simultaneously. First the fiber tips were immersed in 20 mL of 0.4 M ammonium persulfate aqueous solution. Then 20 mL of 0.4 M pyrrole solution in 2-propanol/water (50:50) was added dropwise. The mixture was stirred for 3 h. After stopping the reaction, a layer of black polymer coating could be observed on the fiber tips and the sides. The tips were then rinsed with deionized water and left to air-dry.

Preparation of Polythiophene (PTH)-Coated Fibers and Tips. Polythiophene-coated fibers and tips were prepared using the following chemical polymerization method.[11] A total of 2.4 g of $FeCl_3$ was first thoroughly dried at ∼100 °C in a reaction flask for 1 h under reduced pressure with the presence of dry nitrogen gas. This was followed by the addition of 50 mL of dry chloroform. Up to 10 optical fibers or glass tips were prepared in the same flask by dipping the tips in the $FeCl_3$ and $CHCl_3$ mixture. Subsequently 0.42 g of thiophene monomer was added dropwise into the mixture with stirring. The reaction mixture was then stirred for 48 h at room temperature under a continuous flow of nitrogen. The fibers were then removed and rinsed with methanol. A dark-red color could be observed after rinsing off ferric chloride from the fiber tips.

Extraction Process. Samples were diluted to various concentrations in water containing 0.1% formic acid. Various concentrations of α-cyano-4-hydrocinnamic acid MALDI matrix were mixed with a 1:1 volume ratio with the sample solutions prior to extraction. For some experiments, no matrix was added to the extraction samples.

The extraction process involved immersion of the SPME fiber tips in the sample solutions at 2−3 mm in depth. Typically extraction times were 2−10 min. The tips were air-dried for 2 min after extraction. Experiments showed that an aqueous rinse was insufficient to prevent carry-over, so after every run, the PPY fiber tips were cleaned by soaking in methanol for 1 min followed by

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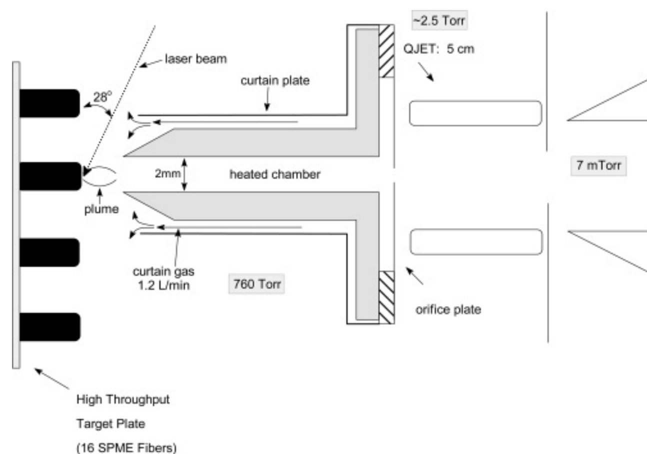

Figure 1. Schematic of the SPME/AP MALDI configuration used in these experiments. The target plate held an array of 16 SPME extraction fibers.

a rinse with water and methanol, respectively. The PTH tips were cleaned by soaking in acetonitrile/water (50:50) followed by rinsing with methanol/water (50:50) for 30 s each. Different rinse procedures were used for the two coatings to account for differences in stability with organic solvents.

The preliminary coating evaluation was carried out with a SRI 9300B GC system with a FID detector (SRI instruments, Torrance, CA). The column was a 1 m × 0.53 mm MXT-5 silicosteel GC column (Restek) with a 1.00-μm coating thickness. The temperature of the GC oven was initially held at 70 °C for 0.5 min and then ramped to 300 °C at 20 °C/min. The hydrogen carrier gas flow rate was set at 10 mL/min. Extracted analytes were then desorbed in methanol and injected into the GC for analysis. Both coatings were evaluated for comparison.

SPME/AP MALDI Coupled to a QqLIT. SPME devices were coupled to a modified 4000 QTRAP mass spectrometer (MDS SCIEX) with a modified AP MALDI ion source as reported previously.[12] The instrumental modifications involved increasing the gas throughput of the interface by a factor of 4 with a larger orifice plate aperture (0.6 mm). In addition, a QJET Ion Guide was incorporated to replace the standard skimmer as shown in Figure 1 to reduce the gas load on subsequent vacuum stages.[13] This configuration improved AP MALDI performance for peptides by a factor of ~2 over previous iterations (data not shown). The laminar flow chamber temperature was maintained at 200 °C for all experiments. A nitrogen laser from LSI (Frankin, MA) was used for all experiments with 10-Hz repetition rate. The AP MALDI source stage was repositioned by removal of shims so that the tips of the SPME rods could be placed ~2 mm in front of the laminar flow chamber entrance. Approximately 2000 V was applied to the stainless steel sample plate. For experiments with the optical fibers, the standard source flange was removed and the fibers were placed ~2 mm from the inlet of the laminar flow chamber. An alligator clip was fastened to the SPME fiber ~1 cm from the fiber tip to provide a potential onto the electroconductive polymer to improve the sampling efficiency for ions. The voltage used in these experiments was 2000 V.

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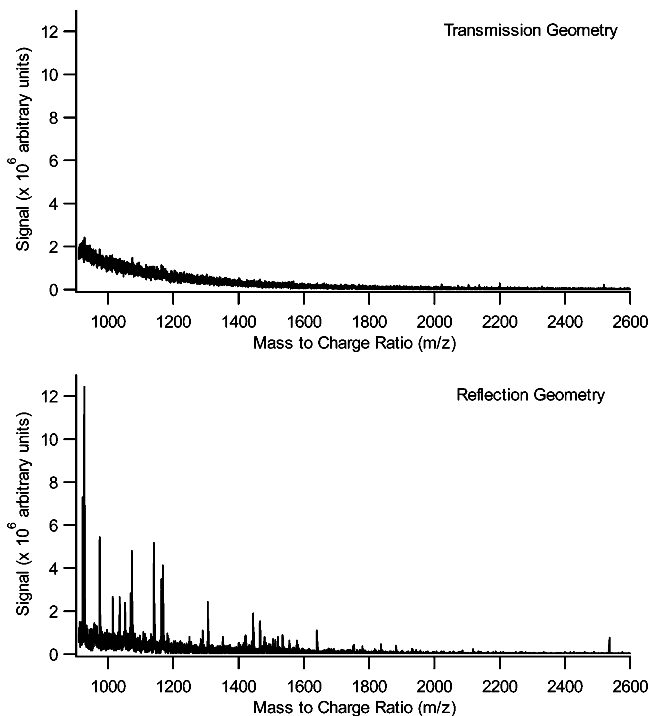

Figure 2. Comparison of performance for a 10 fmol μL^{-1} sample of BSA digest with transmission (upper pane) and reflection geometry (lower pane). The extraction time was 5 min and a PTH fiber was used for these experiments. Trap operational parameters: scan speed, 4000 Da/s; fill time, 150 ms.

Using the coated optical fibers, performance comparisons were made using two different laser illumination geometries. The first geometry involved attachment of the laser directly to the opposite end of the coated optical fiber in a fashion similar to experiments described in the literature.[10] With this configuration (hereafter referred to as transmission geometry), the laser light was transmitted through the optical fiber (600 μm), conductive polymer, and then sample extraction surface. This geometry essentially results in backside illumination of the polymer and sample. The second optical configuration (hereafter referred to as reflection geometry) involved attachment of the laser to the standard optics in the AP MALDI source (200-μm fiber) such that the light was directed at a ~28° angle to the front side of the fiber surface as described previously.[12]

RESULTS AND DISCUSSION

Optimization of Performance. I. Comparison of Transmission and Reflection Geometry. In previous direct couplings of SPME and AP MALDI[9,10] only transmission geometry was employed. Experiments were conducted with samples of BSA digest to compare the performance with the two illumination geometries. Similar laser fluence was used for both geometries. Figure 2 shows an example of the performance comparison for a 5-min extraction from a sample of 10 fmol μL^{-1} BSA digest using a single PTH fiber. The *Y* axes were scaled identically for the two sets of data so that they could be compared directly. Although these data were generated using a PTH fiber, similar trends were observed using PPY fibers as well. There was a substantial

background when using transmission geometry; however, peptide peaks could not be observed at the 10-fmol level. In contrast, a large number of peptide peaks could be observed with S/N ratios ranging up to 17 when using reflection geometry even though the optical fiber was positioned in the same location within the source region for both experiments. To achieve similar S/N ratios for this sample using transmission geometry, the BSA digest concentration had to be increased to greater than 1 pmol μL^{-1}. For these experiments, the sample surface was illuminated until no further ion current was generated with both optic configurations. Since the entire surface was illuminated simultaneously with transmission geometry, sample depletion from the surface required ~10 s. However, with reflection geometry, the laser was focused to a spot size of ~225 by 325 μm so that the ablation area was ~4 times smaller than the fiber surface area. Quantitative removal of analyte required rastering around the fiber surface. Under these conditions, analyte signals were observed for ~3 min. Even though no further ion current could be obtained from these fibers, some analytes were still present on the surface of the fibers. Additional analyte signal (much weaker) could be regenerated by respotting matrix onto the tips. Therefore, it was critical to use the aggressive wash procedures described in the Experimental Section between each sample to prevent carry-over. Reflection geometry was used for all other experiments described in this paper.

II. Ionization Efficiency for Conductive Polymers. Matrix addition to the extraction sample was also evaluated to try to improve the analytical performance of the SPME/AP MALDI system. Extractions were carried out using a sample of 100 fmol μL^{-1} angiotensin II. Data were collected sampling directly from the acidified aqueous sample solvent, sampling from solvent prepared by mixing the sample solvent directly with undiluted α-cyano matrix (1:1 ratio), and mixing the sample solvent directly with α-cyano matrix (1:1 ratio) that was diluted by a factor of 10 with water containing 0.1% formic acid. For each of the three extraction conditions, four separate runs were conducted with different tips. Average data obtained using PPY fibers are presented in Figure 3 with the Y axes scaled identically for comparison purposes. The addition of matrix to the extraction solution provided a dramatic improvement in SPME/AP MALDI performance. The addition of undiluted matrix provided increases of 57× and 31× for the absolute signal and S/N for protonated angiotensin II. Dilution of the matrix provided an additional signal improvement (a factor of ~2), but the S/N ratio was essentially unchanged. The increased ion intensity with matrix dilution was likely due to the resulting increase in aqueous content of the extraction solution (50% aqueous → 95% aqueous) since the matrix was diluted with acidified water. This may increase the distribution constant for the analyte in the extraction phase. In addition, the decrease of the total amount of matrix in the extraction solution may decrease the competition for the surface, allowing more peptides to be adsorbed. Therefore, for all further experiments in this paper, aqueous samples were mixed with a 1:1 ratio with α-cyano matrix diluted 10×. Similar behavior was observed with SPME devices coated with PTH.

III. Comparison of PPY and PTH Coatings. Conductive coatings of PPY and PTH were evaluated. GC experiments were initially conducted to compare the extraction ability and reproducibility for the two polymer coatings. GC experiments showed signal improvements of 1.9× for the PPY fibers as opposed to the PTH fibers. In addition, the run–run reproducibility (as measured by the relative standard deviation) was 9% for the PPY fibers and 21% for the PTH fibers. Experiments with the QqLIT also showed poorer performance and reproducibility with the PTH fibers (data not shown). Differences in performance and reproducibility may be related to differences in the fiber surface morphologies. Scanning electron microscopy (SEM) was used to evaluate the homogeneity of the extraction surfaces with the PPY- (Figure 4) and PTH-coated devices (Figure S-1, Supporting Information). Similar conditions were used for the acquisition of the images with the two coatings, and the magnification was 10000×. The top image depicts the surface of the coated fiber prior to extraction, and the bottom image depicts the surface of the coated fiber after extraction from a mixture containing 10 fmol μL^{-1} BSA digest. The surface of the PTH-coated fiber (Figure S-1, Supporting Information) showed a lack of surface uniformity with areas of

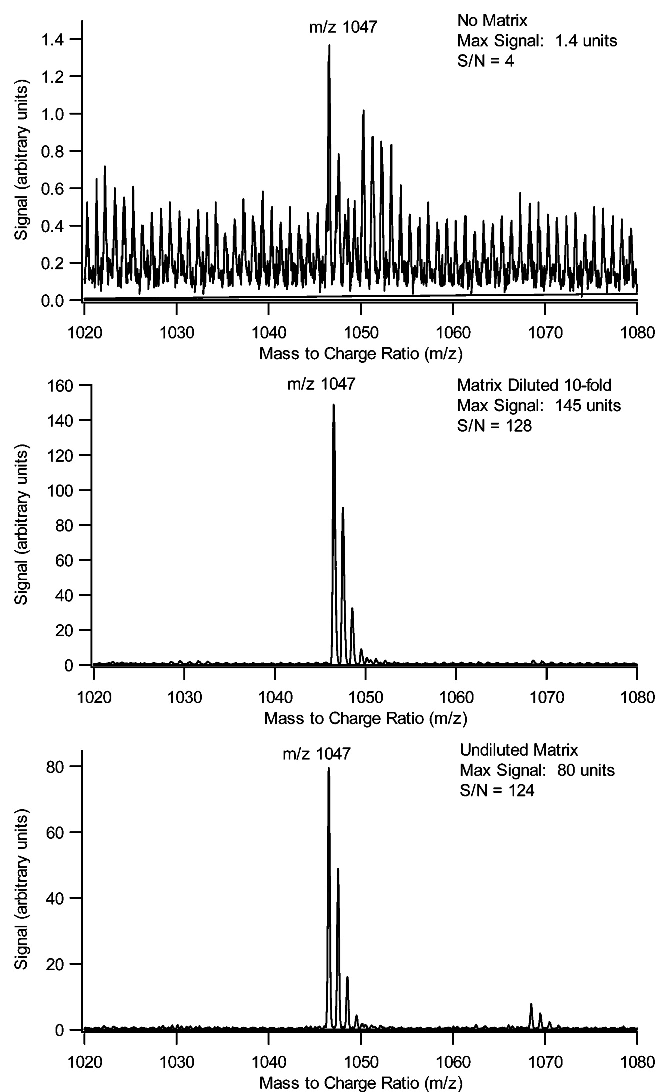

Figure 3. Comparison of performance for 100 fmol μL^{-1} angiotensin II using direct ionization from the surface of the PPY fiber (no matrix addition) and incorporating various amounts of matrix to the extraction solvent. Top pane, no matrix addition; middle pane, 10-fold diluted matrix added; bottom pane, undiluted matrix added. Trap operational parameters: scan speed, 250 Da/s; fill time, 150 ms.

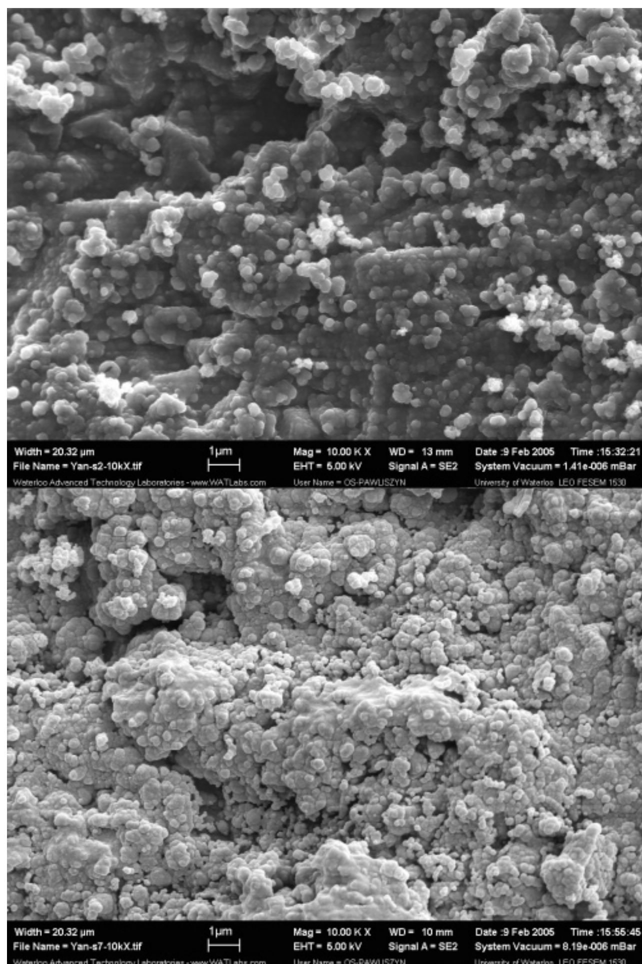

Figure 4. Scanning electron microscopy images of the PPY coating (upper pane) and the PPY coating after extraction from a mixture containing 10 fmol μL^{-1} BSA digest and matrix (lower pane). Operational parameters were 5000 V and 10000× for the accelerate voltage and magnification.

filament-shaped particles and areas with alternate morphologies. The picture taken after extraction showed the presence of a number of areas where there appeared to be gaps in the extracted material. The surface of the PPY-coated fiber (Figure 4) shows a more homogeneous morphology (ball-shaped structures). In addition, there appeared to be a more uniform and continuous layer of material on the surface after the extraction procedure. This difference in surface structure may be the reason for the improved performance with the PPY-coated fibers. PPY-coated fibers were used for all of the rest of the experiments described in this paper.

A number of PPY-coated tips were also examined with blank extraction from the 10× diluted matrix solution to look for the presence of extra peaks resulting from ionization of various subunits of the PPY polymer. It was not possible to observe any peaks that corresponded to polymer ions. These results suggest that the polymer coatings might not be able to be ionized with the current laser fluence (380 J/m^2). Another possible explanation for the lack of PPY-related ions in the mass spectra was that the proton affinity of the matrix may be substantially higher than the proton affinity of PPY-related molecules.[14]

Evaluation of Analytical Performance. I. Extraction Efficiency. The capacity of the SPME coating limits the amount of analyte that can be extracted from a particular sample. Typically this means that the actual amount of sample adsorbed on the fiber surface is very much lower than the amount of sample initially present in the extraction solution. Therefore, experiments were conducted with a four-peptide mixture to evaluate the extraction efficiency using the large-diameter (2 mm) fibers mounted on the surface of the multiplexed SPME plate. Extraction efficiencies depend on a number of parameters including solvent composition, temperature, analyte affinity for the solid phase, extraction time, and analyte concentration. Prior to these experiments, the solvent composition was optimized to water with 0.1% formic acid as described above for Figure 3. The optimum temperature was ~25 °C with reduced ion count rates at both higher and lower extraction temperatures (data not shown). To evaluate the extraction efficiencies, 1 μL of a sample containing 20 fmol μL^{-1} of four different peptides was spotted onto the tip of four separate SPME fibers. Data were accumulated from each fiber until the sample was depleted from the surface and the four runs were averaged. After cleaning, the same four fibers were used to extract from wells containing 40 μL of the same mixture (20 fmol μL^{-1}). Data were accumulated from each fiber until the samples were depleted from the surface and the four runs were averaged. The peak intensities were compared for the four peptides, and the extraction efficiencies were calculated using eq 1.

$$\text{extraction efficiency} = \frac{\text{signal intensity}_{\text{extracted sample}}}{\text{signal intensity}_{\text{depostied sample}}} \times \frac{20\ \text{fmol}\cdot\mu\text{L}^{-1} \times 1\ \mu\text{L}}{20\ \text{fmol}\cdot\mu\text{L}^{-1} \times 40\ \mu\text{L}} \times 100\% \quad (1)$$

Table 1. Quantitative Comparison of Fiber−Fiber Reproducibility for Extractions from Four Different Sample Wells[a]

fiber no.	angiotensin II signal ($\times 10^7$ au[b])	angiotensin II S/N ratio	bradykinin signal ($\times 10^7$ au[b])	bradykinin S/N ratio	angiotensin I signal ($\times 10^7$ au[b])	angiotensin I S/N ratio	glufibrinopeptide b signal ($\times 10^7$ au[b])	glufibrinopeptide b S/N ratio
1	8.9	153	6.2	108	0.81	26	5.2	224
2	7.6	201	4.1	109	0.83	31	4.9	257
3	12.0	180	7.1	106	1.0	25	5.7	199
4	10.0	129	5.2	64	1.5	30	4.1	130
average	9.6	166	5.7	97	1.0	28	5.0	203
RSD (%)	19	19	23	23	31	11	13	27

[a] Sample contains 20 fmol μL^{-1} angiotensin II, bradykinin, angiotensin I, and glufibrinopeptide b with 10-fold diluted matrix. The extraction time was 5 min. Trap operational parameters: scan speed, 4000 Da/s; fill time, 150 ms. [b] au, arbitrary units.

The extraction efficiencies were 0.3, 0.2, 0.1, and 0.4% for angiotensin II, bradykinin, angiotensin I, and glufibrinopeptide b, respectively. It is important to note that the calculated extraction efficiencies only accounted for peptides adsorbed on the coated end of the fiber tips, as it was not possible to sample from the sides of the fiber tips with this configuration. The differences in peptide structure likely account for differences in the extraction efficiencies. In addition, since extraction efficiencies depend on sampling time and concentration, it is important to note that the calculated efficiencies provide an efficiency estimate that is only valid for the conditions described above.

II. Reproducibility and Sensitivity. Tip−tip reproducibility was evaluated with four PPY-coated tips using samples of the four peptide mixture containing 20 fmol μL^{-1} concentrations of each peptide. In each case, data were acquired until the sample was completely depleted from the tip of the fiber. Separate SPME tips (labeled fibers 1−4) were used for simultaneous extraction from four different sample wells. The four spectra were qualitatively similar, showing the presence of the protonated peptides as well as a small peak corresponding to the dehydration of glufibrinopeptide b. A quantitative comparison of the signal and S/N ratio for the four peptides is presented in Table 1. Typical RSDs ($N = 4$) for the signal intensity and S/N ratio ranged from 13 to 31% and 11 to 27%, respectively. This was a substantial improvement over previous iterations and was likely due to a number of factors such as the improved control with simultaneous sampling, improved SPME technique, improved laser optics, and the more stable atmosphere to vacuum interface.[12] In addition, the multiplexed plate allowed all extractions to be conducted simultaneously, reducing the total analysis time. The S/N ratios from these experiments can be used to estimate detection limits of 362 amol μL^{-1}, 619 amol μL^{-1}, 2.2 fmol μL^{-1}, and 295 amol μL^{-1} for angiotensin II, bradykinin, angiotensin I, and glufibrinopeptide b, respectively. These detection limits represent improvements on the order of 1000−7500× over previously published data.[10]

Analytical performance was also evaluated for protein digests. Figure 5 shows an example of this for samples of BSA digest in which the *Y* axes have been scaled identically for comparison purposes (the *m/z* values of the peptide peaks are shown in Table S-1, Supporting Information). Figure 5 shows data acquired (average of 8 runs each) for samples of 5 fmol μL^{-1} BSA digest, 500 amol μL^{-1} BSA digest, and a blank sample containing only matrix mixed with acidified water. The extraction times were 5, 10, and 10 min, respectively. Blank runs were taken before and after each of the measurements with the fibers to ensure that carry-over was not an issue. On average the peak heights and S/N ratios were approximately 6× and 5× lower for the 500 amol μL^{-1} extractions than the 5 fmol μL^{-1} extractions, respectively. These data suggest that the data generated with the lower concentration may have benefited from the extended extraction time. This is expected since the extraction process relies upon diffusion of analyte molecules to the fiber surface. Therefore, longer extraction times result in more analyte adsorbed on the fiber surface before the extraction equilibrium is reached. Future research will be focused on improving performance further with extended extraction times as well as improving the homogeneity of the extraction material on fiber surfaces with the goal of achieving quantitative analysis. New extraction materials such as antibody coating are also under investigation for this purpose.

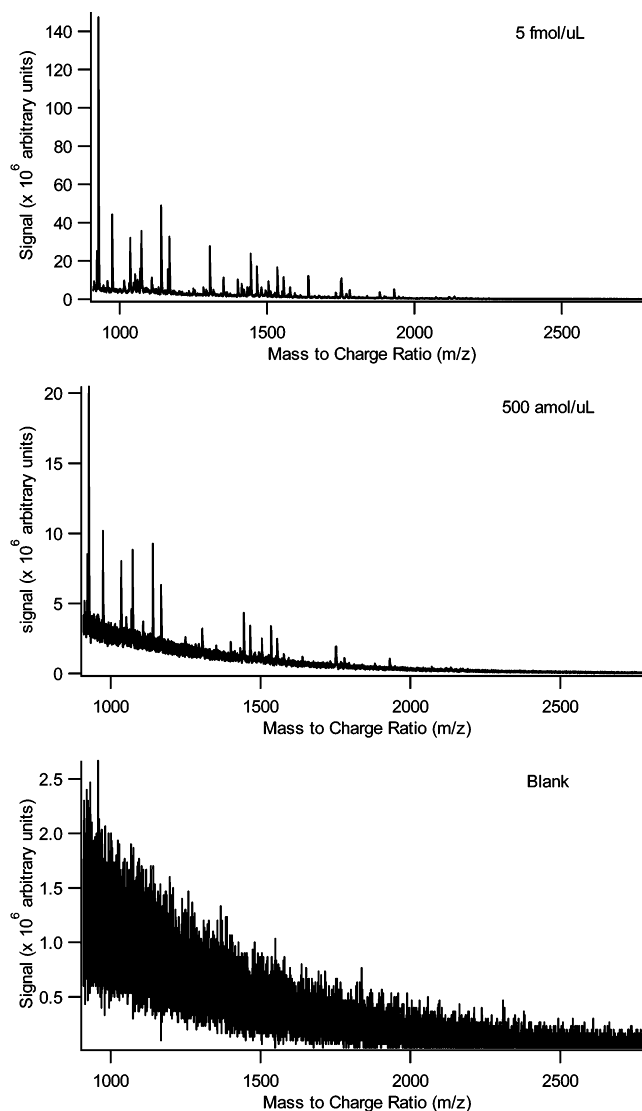

Figure 5. Analytical performance for samples of 5 fmol μL^{-1} BSA digest (top pane), 500 amol μL^{-1} BSA digest (middle pane), and a blank (bottom pane). Samples were extracted using a PPY-coated fiber for 5, 10, and 10 min, respectively. Trap operational parameters: scan speed, 4000 Da/s; fill time, 20 ms; and Q0 trapping enabled.

CONCLUSION

A new multiplexed SPME/AP MALDI plate was designed and evaluated on a QqLIT with a modified AP MALDI source. The experimental parameters were optimized to obtain a significant improvement in performance. The incorporation of diluted matrix to the extraction solution improved the absolute signal and S/N by 104× and 32×, respectively. The incorporation of reflection geometry for the laser illumination improved the S/N ratio by more than 2 orders of magnitude. Reproducibility was also improved as a result of these changes and the improved atmosphere−vacuum interface used for these experiments. The fully optimized high-throughput SPME/AP MALDI configuration described in this paper generated detection limit improvements on

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the order of 1000−7500× those achieved prior to these modifications. This system presents a possible alternative for qualitative proteomics and drug screening.

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SUPPORTING INFORMATION AVAILABLE

The SEM images of PTH coating and the table of the protein digest peptide peaks in Figure 5. This material is available free of charge via the Internet at http://pubs.acs.org.

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