

"This is the peer reviewed version of the following article: Picca, R.A., Calvano, C.D., Lo Faro, M.J., Fazio, B., Trusso, S., Ossi, P.M., Neri, F., D'Andrea, C., Irrera, A., Cioffi, N. (2016) Functionalization of silicon nanowire arrays by silver nanoparticles for the laser desorption ionization mass spectrometry analysis of vegetable oils. *J. Mass Spectrom.*, 51: 849– 856, which has been published in final form at <https://doi.org/10.1002/jms.3826>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions."

FUNCTIONALIZATION OF SILICON NANOWIRE ARRAYS BY SILVER NANOPARTICLES FOR THE LASER DESORPTION IONIZATION MASS SPECTROMETRY ANALYSIS OF VEGETABLE OILS

Rosaria Anna Picca^{1,*}, Cosima Damiana Calvano¹, Maria Josè Lo Faro^{2,3}, Barbara Fazio², Sebastiano Trusso², Paolo Maria Ossi⁴, Fortunato Neri⁵, Cristiano D'Andrea³, Alessia Irrera^{2,*}, Nicola Cioffi¹

¹*Dipartimento di Chimica, Università degli Studi Bari "Aldo Moro", Via E. Orabona 4, 70126 Bari, Italy*

²*CNR-IPCF, Istituto per i Processi Chimico-Fisici, V. le F. Stagno D'Alcontres 37, 98158 Messina, Italy*

³*MATIS CNR-IMM, Istituto per la Microelettronica e Microsistemi, Via Santa Sofia 64, 95123 Catania, Italy*

⁴*Dipartimento di Energia & Center for NanoEngineered Materials and Surfaces-NEMAS, Politecnico di Milano, Milano, Italy*

⁵*Dipartimento di Scienze matematiche e informatiche, scienze fisiche e scienze della terra, Università degli Studi di Messina, Messina, Italy*

*Corresponding Authors: rosaria.picca@uniba.it, irrer@its.me.cnr.it

ABSTRACT

In this work, novel hybrid nanostructured surfaces, consisting of dense arrays of silicon nanowires (SiNWs) functionalized by Ag nanoparticles (AgNP/SiNWs), were used for the laser desorption/ionization time-of-flight mass spectrometry (LDI-TOF MS) analysis of some typical unsaturated food components (e.g. squalene, oleic acid) to assess their MS performance. The synthesis of the novel platforms is an easy, cost-effective process based on the maskless wet-etching preparation at room temperature of SiNWs followed by their decoration with AgNPs, produced by pulsed laser deposition. No particular surface pretreatment or addition of organic matrixes/ionizers was necessary. Moreover, oil extracts (e.g. extra virgin olive oil, peanut oil) could be investigated on AgNP/SiNWs surfaces, revealing their different MS profiles. It was shown that such substrates operate at reduced laser energy, typically generating intense silver cluster ions and analyte adducts. A comparison with bare SiNWs was also performed, indicating the importance of AgNP density on NW surface. In this case, desorption/ionization on silicon was invoked as probable LDI mechanism. Finally, the influence of SiNW length and surface composition on MS results was assessed. The combination of typical properties of SiNWs (hydrophobicity, antireflectivity) with ionization ability of metal NPs can be a valid methodology for the further development of nanostructured surfaces in LDI-TOF MS applications.

KEYWORDS: silicon nanowire; silver; nanomaterial; SALDI-MS; unsaturated bonds; DIOS; food analysis

INTRODUCTION

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) can be applied to food components allowing rapid analyses with easy sample preparation^[1] or even directly on food extracts^[2]. Unfortunately, many organic matrixes generate intense ions in the low mass range (especially below 700 m/z) causing severe background noise and sweet-spot phenomena (inhomogeneous co-crystallization) with reduced analyte sensitivity^[3]. Alternatively, porphyrin-based matrixes^[4], that do not present interference in this m/z range, caused problems with calibration in low m/z regions^[5] and are less useful for the analysis of unsaturated fatty acids^[6], or other unsaturated compounds, which is the aim of this work. ~~As a result,~~ Nanostructured materials have emerged as alternative substrates for LDI-TOF MS introducing a different approach generally named as surface assisted LDI-MS (SALDI-MS)^[7]. In 1999, desorption/ionization based on (porous) silicon (DIOS) was proposed by Wei et al.^[8], who used porous silicon to test a broad range of analytes, such as carbohydrates, peptides, natural products, and small drug molecules. The efficacy of this approach was mainly related to the high UV absorptivity of the material. Afterwards, silicon nanowires were firstly reported as SALDI substrates in 2005^[9]. This pioneering work was soon followed by other examples, sometimes employing surface-modified NWs, for the MS characterization of low molecular weight analytes with sensitivities down to the picomole^[10-13]. Conventional MALDI is outperforming nanostructured surfaces in many applications, even achieving attomole sensitivity. On the other hand, “matrix-free” approaches based on nanostructures may allow the analysis in low molecular range, where it is very difficult to reach attomole sensitivity, especially with scarcely ionizable compounds.

Silicon-based substrates are considered highly tolerant to salts and lead to the generation of analyte molecular ions, with limited fragmentation, if any.

Metal nanoparticles (e.g. Au-, Ag-, Pd-, or Pt-NPs) have also been employed due to good thermal and electrical conductivity as well as light absorption ability on a wide wavelength range^[5,14-18]. Moreover, an increased detection capability towards high affinity compounds (e.g. thiols for AuNPs or olefins for AgNPs) was often reported. For example, a clear isotopic pattern, typical of silver, can be achieved thanks to the formation of Ag-analyte ion adducts when such NPs are used^[15]. This feature can be particularly appealing in several applications, ranging from biological^[19] to food analysis^[20]. Some authors have also highlighted that silver cationization may present some drawbacks^[21], however (nano)silver is especially useful when low polarity unsaturated compounds (such as squalene) are studied. Indeed, in this specific case, the ionization, which would be particularly difficult due to the absence of polar groups, is favored thanks to three-center two-electron bond with silver. Considering food matrixes ~~In the latter case,~~ main constituents of edible oils are

triacylglycerols (TAGs), mono- and diacylglycerols, free fatty acids, which can present one or more unsaturation sites. In particular, the use of supported AgNPs as well as Ag nanostructured layers, compared to colloidal AgNPs, may be more efficient for LDI and Imaging MS applications, as recently demonstrated^[22,23].

As a result, substrates based on hybrid semiconductor/metal nanostructures can combine physicochemical properties of both materials thus providing advanced platforms for LDI-TOF MS applications. Similar materials have been successfully applied in other fields (e.g. (photo)catalysis^[24,25] and sensing^[26]), but their use as SALDI substrates is not fully explored^[27-30]. Moreover, the mentioned studies are relevant to Pt^[27], Cu^[28], and Au^[29,30] nanostructures and no example on AgNP/SiNWs systems for LDI-MS is yet reported in literature.

In a previous study^[31], we showed the feasibility of this approach testing squalene as model analyte on AgNP/SiNWs hybrid platforms. Squalene, known as precursor for cholesterol biosynthesis, was selected as highly hydrophobic nonpolar compound with multiple double bonds. In this study, the proposed substrates are prepared by a metal-assisted wet etching method^[32], followed by an additional functionalization with AgNPs. The decoration step is achieved by a pulsed laser deposition method^[33-36]. In this work, we explored AgNP-functionalized SiNWs for the matrix-free analysis of low-molecular-weight unsaturated compounds and some vegetable oils. In particular, oils were selected as food samples to show the applicability of AgNP-decorated SiNWs in detecting unsaturated compounds (namely squalene and oleic acid) also in a complex matrix. A comparison with bare SiNWs was also carried out, evidencing the importance of NP decoration in terms of sensitivity and affinity towards ~~some~~ the aforementioned species.

EXPERIMENTAL

Synthesis of SiNWs and AgNP/SiNWs

Highly ordered and dense arrays of SiNWs ~~can be~~ were prepared starting from p-type (Boron concentration of $\sim 10^{16}$ cm⁻³) (100)-oriented single-crystal Si wafers^[32,37,38]. The wafers were UV oxidized and dipped in 5% HF to obtain clean and oxide-free Si surfaces. Afterwards, clean substrates were etched in a solution made of AgNO₃ 40%, H₂O 40% and HF 20% to form bare SiNWs with a length of 3.2 μ m. ~~Other experimental details can be found elsewhere~~^[26-28]. SiNWs were decorated by AgNPs, along their total extension, via a pulsed laser deposition method^[33-36] using 60000 laser shots. The process was realized in high vacuum chamber with a residual base pressure of 10⁻⁴ Pa. The laser beam from a KrF excimer laser (Lambda Physik CompEx 205, 25 ns pulse width, 248 nm wavelength, 10 Hz repetition rate) was focused onto the surface of a pure silver target using a quartz lens. The

silver target was mounted on a rotation holder in order to avoid excessive surface damaging. Si NWs samples were positioned 35 mm far from the target surface. The deposition process was performed in presence of 70 Pa of Ar. A scheme of the PLD apparatus is sketched in Fig. S1.

Surface characterization of AgNP/SiNWs

Morphological characterization of the as-prepared AgNP/SiNWs was carried out by Field Emission Scanning Electron Microscopy (FE-SEM) on a Zeiss Supra 25 Microscope (30 KV). The images were acquired in cross section by using 5 KV. Transmission electron microscopy (TEM) was performed with a FEI Tecnai 12 instrument (120 kV; filament: LaB₆) on AgNP/SiNWs, physically removed from the substrates and re-suspended in ultrapure Milli-Q (18.2 MΩ) water. 2-μL suspension was deposited on Formvar[®]-coated Cu grid (300 mesh, Agar Scientific).

The surface chemical composition of AgNP/SiNWs was investigated by means of X-ray Photoelectron Spectroscopy (XPS) using a Thermo Fisher Scientific Theta Probe spectrometer equipped with a monochromatized AlK α source and beam spot diameter of 400 μm. All spectra were registered in constant analyzer energy (CAE) mode at a pass energy of 150 and 100 eV, for survey and high-resolution (HR) spectra, respectively. Energy step size was 1.0 eV for survey spectra and 0.1 eV for the others. C1s, O1s, Si2p, Si2s, Ag3d, and AgM_{4,5}N₄₅N₄₅ regions were acquired in each experiment. No artifact due to sample irradiation with X-rays occurred. Spectra processing was performed by commercial Thermo Avantage software (v. 5.937, © 1999-2014 Thermo Fisher Scientific). Curve-fitting of HR spectra and elemental quantification (expressed as At%) were achieved applying a Shirley background removal and using *Scofield* sensitivity factors. Spectra were corrected for charge compensation effects by taking as reference the Binding Energy (BE) of adventitious carbon component of C1s spectrum (284.8 eV). Modified Auger parameter (α')^[39] was used to study silver chemical speciation^[15,40,41].

Mass spectrometry analysis

LDI-TOF MS experiments were carried out on a Micromass M@LDITM-LR TOF mass spectrometer (Waters MS Technologies, UK), equipped with a N₂ laser ($\lambda = 337$ nm, pulse duration 4 ns, laser firing rate 5 Hz), in reflectron positive ion mode between 100-1000 or 250-1200 *m/z*. Laser fluence was defined in terms of manufacturer's specification, using a Laser Energy% equal to 20%, which corresponds to about 10 mJ/cm². The following voltages were applied: pulse, 2610 V; source, 15000 V; reflectron, 2000 V; and MCP, 1900 V. A time lag focusing (TLF) delay of 500 ns was used between the laser pulse and the application of the accelerating voltage. For each spot, 60 spectra were averaged, background subtracted, and smoothed by a Savitzky-Golay algorithm. Raster mode was applied throughout the experiments as square substrates of mean area equal to 0.5 cm² were employed

by cutting bigger pieces with a diamond scribe. Substrates made of AgNP/SiNWs or SiNWs were directly attached on a 96-well stainless steel target by ultra-high-vacuum carbon tape (Agar Scientific). 1- μ L spots were deposited directly on the substrates, and allowed drying before loading in the spectrometer. Typically, reagents and solvents of HPLC or LC-MS grade Chloroform was HPLC-grade (Chromasolv® Plus), while the other solvents were of LC-MS grade (LC-MS Chromasolv®), all of them were purchased from Sigma-Aldrich, and used for mass spectrometry analysis. Oleic acid (OA, assay \geq 99%, Sigma) was diluted with methanol for quantitative analysis, and relative standard deviation (RSD) was calculated on three replicates, considering the ratio between the intensities of 389.2 m/z ($[\text{OA}+^{107}\text{Ag}]^+$) and 106.9 m/z ($^{107}\text{Ag}^+$). Squalene extraction from food samples was performed on extra virgin olive oil (EVOO) and peanut oil according to a previously reported procedure^[20]. Alternatively, 50 μ L of EVOO was diluted with 1 mL CHCl_3 to detect triacylglycerols (TAGs) on bare SiNWs. Vegetable oils were purchased from a local supplier, and stored in a dark dry place. 3.2 μm -long SiNWs (with or without AgNPs) were employed for MS experiments, unless otherwise stated.

RESULTS AND DISCUSSION

Morphological and spectroscopic analysis of AgNP/SiNWs

Typical SEM micrographs of 3.2- μm SiNWs, functionalized with AgNPs, are reported in Fig. 1. Cross-sectional view of the samples reveal the high density and order of SiNWs (Fig. 1a). Moreover, at higher magnification, the extremely homogeneous coverage with AgNPs is observable from the top to the bottom of elongated structures (Fig. 1b) demonstrating that the preparation of AgNP/SiNWs is well controlled and allows fabricating substrates of well-known nanostructured morphology. AgNPs present a spheroidal shape and diameters below 30 nm, as shown in the TEM image (Fig. 1c). XPS characterization was carried out to investigate the surface elemental composition of the proposed substrates with a particular focus on the chemical speciation of silver and silicon. Element abundances (At%) are presented in Table 1. Silver shows a higher abundance than silicon, as expected by the density of AgNPs onto wires. On the other hand, carbon contamination is particularly high, probably due to the remarkable reactivity of silver nanophases. In fact, similar results were also found on UV/ozone-cleaned surfaces, rapidly inserted into XP analysis chamber, thus indicating a fast adsorption of organic molecules onto samples. However, untreated AgNP/SiNWs could be employed as SALDI substrates even several months after their fabrication, provided they were stored under dry low rough vacuum (\sim 0.1 MPa) conditions. Regarding surface chemical environments, Si2p and AgM_{4,5}N₄₅N₄₅ regions are shown in Fig. 2, along with curve-fitting results. Si2p region (Fig. 2a)

presents two components centered at BE = 99.8 ± 0.2 eV and 103.6 ± 0.2 eV, ascribable to Si(0) and Si(IV), respectively^[42,43]. Both chemical states are in agreement with previous findings about the structure of pristine SiNWs, consisting of a crystalline Si core surrounded by an amorphous SiO₂ layer^[44]. Ag chemical speciation was achieved by fitting either Ag main photoelectronic and Auger signals^[15,41] (Fig. 2b). In fact, poor spectral resolution is associated to the sole Ag3d_{5/2} photoelectron peak^[40], falling at 368.4 ± 0.1 eV (not shown) for these samples. AgM_{4,5}N₄₅N₄₅ was fitted by six (a-f) peaks, following literature models^[15,40]. Position, intensity, and lineshape of the resulting components are compatible with nanostructured Ag(0)^[15]. The modified Auger parameter α , calculated as the sum of BE(Ag3d_{5/2}) and KE(Auger), is equal to 725.5 ± 0.2 eV, corroborating this assumption. Additionally, the so-called R ('d/e' area ratio) parameter was also calculated in this study obtaining 0.48 ± 0.02 , which is in agreement with the value reported for silver nanostructures at the elemental oxidation state^[15].

AgNP/SiNWs as SALDI-TOF MS platforms

In the past we have explored how different nanomaterials behave as MS platforms to detect highly unsaturated molecules (e.g. squalene)^[15,31]. In a previous work^[15], electrochemically synthesized Ag nanostructures were employed -exploiting the Ag⁺ affinity towards C=C moieties- as system assisting desorption/ionization of low-molecular-weight unsaturated compounds, thus detecting squalene down to 3 nmol/ μ L. However, the main criticism faced using such nanostructures was related to surface inhomogeneity with a consequent difficult search for sweet spots and a nonlinear response. Interestingly, squalene at the same concentration could be detected by bare SiNWs (1.6 μ m-long, without AgNPs) thanks to the presence of trace amounts of Ag, used as catalyst for the NWs synthesis. In this case, adduct of squalene with Ag traces was still formed, with a reduced number of background signals, as compared to the previous paper. When the NWs were modified with AgNPs, a more homogenous surface was assured with a resultant increase in sensitivity and the possibility to quantify squalene at a concentration as low as 0.1 nmol/ μ L^[31]. MS spectra relevant to squalene (SQ), acquired with SiNWs with Ag as a catalyst and AgNP/SiNWs, are compared in Fig. S2. In the hybrid system, a higher absolute intensity of [SQ+¹⁰⁷Ag]⁺ ion (517.3 m/z) and a higher S/N ratio were achieved with improved analytical performance of the surfaces proposed in this study.

~~Ag/SiNWs displayed good performance in the analysis of highly unsaturated molecules (e.g. squalene), exploiting the Ag⁺ affinity towards C=C moieties^[31]. In particular, we found that this triterpene could be detected at a concentration of 0.1 pmol/ μ L. Based on our previous results, Therefore, we considered to test other species bearing, for example, a single double bond. Oleic acid was investigated as model food component and a typical MS spectrum is reported in Fig. 3a. OA was identified as Ag-adduct with main components at 389.2 and 391.2 m/z , as expected from the isotopic~~

pattern of Ag (see the figure inset). Signal intensity was remarkably high, considering that desorption of OA from the Ag/SiNWs occurs at a relatively low laser fluence (i.e. ca 10 mJ/cm²). Several easily recognizable silver cluster ions can be observed (Ag⁺, Ag₂⁺, Ag₃⁺, labeled with asterisks); though the base peak is generally the ¹⁰⁷Ag⁺ (106.9 *m/z*). Other peaks (*m/z* = 142.2, 147.2, 242.3, 466.5) were typically detected with variable intensity also on the blank substrates and were ascribed to both silicon signals and/or organic contamination (Fig. 3b). Despite the presence of few interfering ions, a good signal-to-noise ratio (S/N ≈ 100) and a relative standard deviation below 20% were found for the analyte signal. Moreover, it was possible to quantify this fatty acid plotting a calibration curve in the range between 0.01 and 1 mg/mL with acceptable linearity (R² = 0.988) and a limit of detection of 40 ng with a S/N ratio = 3 (Fig. 3c). We also evaluated how SiNWs, without additional functionalization with AgNPs, behave as MS platform for OA. MS spectrum relevant to oleic acid (2 nmol/μL) spotted onto 3.2μm-long SiNWs (without AgNPs) is reported in Fig. 3d. “Bare” SiNWs showed a different ionization behavior for this analyte, resembling a typical DIOS mechanism. Indeed, oleic acid could be detected as radical cation [OA][•] at 282.3 *m/z*. Analysis of lower concentration of oleic acid onto such substrates did not provide reproducible results. Such finding suggests that the hybrid material is more reliable in terms of desorption/ionization efficiency of the analyte and of its quantification since it simultaneously exploits the features of silicon as efficient desorption surface and Ag as cation agent. It is also important to highlight that a direct comparison between AgNPs alone and AgNP/SiNWs platforms is in general very difficult and, in this case, not so straightforward. In fact, AgNPs of the present study are free of any capping agent; they are morphologically stable and easy-to-be-handled just because they are supported on SiNWs. On the contrary, when a colloidal suspension of AgNPs has to be compared to our nanocomposite system, several issues arise, which make intrinsically inhomogeneous data from the two LDI promoters, thus affecting the possible comparison.

Stabilized Ag nanocolloids show MS spectra with several peaks due to NP stabilizers, the latter giving rise to the most intense (interfering) signal in the spectrum. When prepared in the absence of stabilizers (for instance by Laser Ablation synthesis in solution), AgNPs tended to aggregate and precipitate and this affects the experiment reproducibility, making impossible any additional comparison. Moreover limited miscibility of analyte solvent and colloidal AgNPs is another source of problems, as recently reported^[45]. On the other hand, it has been already demonstrated that porous Si modified with AgNPs was a more efficient surface than AgNPs, also in consideration of temporal stability of the surfaces^[46]. As additional comparison to Ag salts, in the past we have already explored the use of silver trifluoroacetate as cationizer of squalene and other oil components^[20]. Comparable

results were obtained with the two methods; however, signal intensity was higher on AgNP/SiNWs since a homogenous surface was invariably obtained, contrary to salt.

On this basis, we considered testing AgNP/SiNWs for the analysis of vegetable oils. In particular, we focused on squalene detection from some edible oils as a marker of good quality olive oil^[20]. In fact, this triterpene represents a 0.2–0.7% presence in extra virgin olive oil (EVOO), but only 0.002–0.03% in other common oils^[47]. To this aim, we applied a particular procedure suitable for selective extraction of squalene from such oils^[20].

Two typical MS spectra registered respectively for EVOO and peanut oil extracts deposited onto AgNP/SiNWs substrates are reported in Fig. 4. Noticeable differences emerge from the analysis of these spectra. Despite the usual presence of ions originating from the substrate, e.g. Ag-based cluster ions and other signals reported above, many peaks could be attributed to different components of the investigated oil samples. In Fig. 4a, starting from low m/z range and reported as inset in the same panel, the adduct between silver and free oleic acid ($[OA+Ag]^+$) can be found at m/z 389.2 along with free linoleic acid (LA) at m/z 387.2, with much lower intensity as expected from the natural free fatty acid composition of EVOO. Interestingly, the former being more concentrated was desorbed also as $[OA+Ag_2]^+$, with a characteristic isotopic pattern whose main peak falls at 497.1 m/z , as also shown in Fig. S3. Such intriguing behavior has also been found in previous works involving Ag commercial nanocolloids in LDI-MS experiments on p-aminothiophenol (ATP), where this molecule formed adducts with Ag_n clusters^[48]. High intense peaks are associated to squalene (SQ) in combination with silver ion (main ion at 517.3 m/z), as anticipated, due to considerable content of this molecule in EVOO. As previously reported^[47], other minor peaks signals could be identified thanks to silver ability as ion promoter, such as 533.3 and 549.3 m/z , which may be tentatively assigned probably associated to sterols^[20] as citrostadienol/cicloartenol ($C_{30}H_{50}O$) and uvaol/erythrodiol ($C_{30}H_{50}O_2$), respectively. This result is of particular interest since these compounds are not easily ionized using conventional matrices; the presence of the double bond in the ring allowed the formation of Ag adducts which is distinguishable thanks to the peculiar isotopic pattern. A non-sodiated diacylglycerol (DAG)-like fragment ($[OO]^+$, quite likely originating from hydrolyzed triolein TAG ($[OOO]^+$, not observed) due to the loss of oleic acid residue^[36], was detected at 603.6 m/z indicating that also SiNWs contribute to the detection capability of the proposed platforms. Such ion may be originated by two different processes, i.e. hydrolysis of triolein TAG forming a true molecule, or nearly complete fragmentation of unseen protonated triolein TAG ($[OOO]^+$)^[49] as related to gas-phase reactions, both being plausible hypotheses. Moreover, AgNPs allow detecting different DAGs as silver adducts such as diolein (DAG(OO)), dilinolein (DAG(LL)) and olein/linolein (DAG(OL)) in the region above 700

m/z . The complete probable attributions of the main ions are reported in Table 2 (of course, tandem mass analyses will be performed in the future to confirm these assignments).

On the other hand, the analysis of peanut oil (Fig. 4b) revealed the presence of SQ, again as adduct with silver, but with reduced abundance if compared to EVOO extract. Such result is in agreement with the lower content of squalene in peanuts. A part from a small contribution of linoleic acid detected as Ag-adduct [LA+Ag]⁺ at m/z 387.2, other components could not be discriminated.

We performed a comparison of the analytical performance of these platforms with that of similar SiNWs of different length, produced with silver as metal catalyst, without any additional surface functionalization with AgNPs. In particular, we analyzed EVOO extract on 275 nm-, 1.6 μ m-, and 3.2 μ m-long SiNWs to evaluate their response to squalene (Fig. 5a-c). In all those cases, 2- μ L of extracted sample was spotted to get comparable signals. It is clearly visible that the [SQ+Ag]⁺ intensity decreased considerably with NW length increase. Such result is easily explained by silver availability as catalyst dendrites at the bottom of SiNWs. We also plan to reduce the analytical background signal, a typical problem related to the use of highly reactive nanomaterials, by developing ultracleaning procedures allowing complete removal of possible contaminants of the Ag/SiNWs system, as recently demonstrated by our group on gold nanowires^[50].

Moreover, investigation of TAGs was also carried out on 3.2 μ m-long SiNWs (without AgNPs) by simply diluting a proper amount of EVOO in chloroform (see above) in order to evaluate their role as SALDI substrates. In this case, it was observed that they behave as the “active” component nanomaterial. In this sense, such nanosystems show a sort of DIOS-like mechanism favoring both desorption and ~~detection~~ ionization of these biomolecules as cation adducts. In fact, no adduct with silver was revealed, while triolein was detected as adduct either with Na⁺ and K⁺ (region above 900 m/z), and signals relevant to DAG-like fragments are again visible in the 600 m/z range (Fig. 7).

In the region above 850 m/z , sodiated and potassiated TAGs could be detected. For example, peaks at 881.8 and 907.8 m/z are related to sodiated dioleoyl-palmitoyl-glycerol and triolein, as for the characteristic difference of 26 due to replacement of oleic with palmitic acid found in olive oil^[6]. The other abundant peak groups at higher m/z (923.8) can be associated to the replacement of sodium with potassium as cationizer of triolein TAG as a difference of 16 is observed, in agreement with other works^[6]. Ion species in combination with silver were not observed. These results open the way to further application of the described substrates, especially in consideration of the low laser fluence required for successful desorption of different oil components as compared also to recent works with nanostructured materials^[16]. Obviously, the application of AgNP/SiNWs in quantitative analysis of real samples still needs strong optimization, as a full analytical protocol has not yet been developed. In particular, some critical issues can be represented by surface cleaning and spot size control. Further

investigations about preferential sensitivity to a particular class of molecules (e.g. saturated vs. unsaturated components) might push the reliability of these platforms. As example, however, it has already been reported that free fatty acids (FFAs) bearing unsaturated bonds (either one or more), have higher response factors than their saturated counterparts^[2,51]. In terms of reusability and storage stability, we already proved their reusability, even after storage for months, without losing their analytical performance as demonstrated by the similar results found on OA (Fig. S4). Additionally, it was also verified that, after a mechanical gentle removal of AgNP-layer, the described substrates still act as DIOS-like surfaces. On the other hand, sometimes we experienced a sort of memory effect when highly concentrated samples were tested. It should be mentioned, however, that the cost of preparation of such substrates are reduced suggesting that they can be regarded as disposable targets.

CONCLUSIONS

SiNWs functionalized with highly dense spheroidal AgNPs were used for the first time as SALDI-MS substrates for the analysis of vegetable oil extracts. Morphological characterization showed that dense arrays of micrometer-long SiNWs were formed by the proposed maskless wet-etching approach. Moreover, a further functionalization step with AgNPs allowed their homogeneous decoration. Surface chemical characterization carried out by XPS demonstrated that SiNWs consist of Si(0) and Si(IV) in agreement with previous findings, while silver surface chemical state is certainly attributed to nanostructured Ag(0). The hybrid nature of this nanomaterial was important for exploiting both DIOS and metal-like LDI mechanisms. AgNP/SiNWs were first tested for the SALDI-MS detection of oleic acid, chosen for its high abundance in vegetable oils and the presence of a single unsaturation bond. Good linearity and LOD were assessed in this case detecting this food component as adduct with Ag. Extra-virgin olive and peanut oils were selected and suitably treated by an extraction procedure for squalene determination. Several oil components could be determined, other than squalene as silver adducts with high S/N ratio and very few interfering peaks. Moreover, the density and availability of AgNPs revealed fundamental for proper SQ detection, as assessed by a comparative study performed on bare SiNWs. Interestingly, the use of a different protocol for EVOO treatment allowed enhancing a DIOS-like effect associated to the nanostructured surfaces as triolein TAG could be detected as sodiated and potassiated ions. We have shown that the AgNP/SiNWs system is very sensitive towards double bonds, though in principle also saturated TAGs could be detected through the observed DIOS mechanism on the same platforms.

The proposed study represents a proof-of-concept of the feasibility of the proposed nanomaterials as SALDI-MS platforms, though analytical protocol optimization needs to be performed. However, AgNP/SiNWs (as well as SiNWs modified with other metal nanophases) could be a promising

technology for further studies on other (food) matrixes without employing time-consuming complex sample treatment.

ACKNOWLEDGEMENTS

R.A.P. and N.C. acknowledge the Italian Project “Nanomaterials & laser ionization mass spectrometry: a new bio-analytical approach” FIRB Futuro in Ricerca 2008 (grant n. RBF088SW7), funded by the Ministero dell’Istruzione, dell’Università e della Ricerca, for financial support.

References

- [1] C. D. Calvano, C. De Ceglie, C. G. Zambonin, *Journal of Mass Spectrometry* **2014**, *49*, 831.
- [2] A. Pirkl, M. Meier, Y. Popkova, M. Letzel, A. Schnapp, J. Schiller, K. Dreisewerd, *Anal. Chem.* **2014**, *86*, 10763.
- [3] Y. E. Silina, D. A. Volmer, *Analyst* **2013**, *138*, 7053.
- [4] F. O. Ayorinde, P. Hambright, T. N. Porter, Q. L. Keith, *Rapid Communications in Mass Spectrometry* **1999**, *13*, 2474.
- [5] J. Nizioł, W. Rode, Z. Zieliński, T. Ruman, *International Journal of Mass Spectrometry* **2013**, *335*, 22.
- [6] J. Schiller, R. Süß, M. Petkovi, K. Arnold, *Journal of Food Lipids* **2002**, *9*, 185.
- [7] T. Guinan, P. Kirkbride, P. E. Pigou, M. Ronci, H. Kobus, N. H. Voelcker, *Mass Spectrometry Reviews* **2015**, *34*, 627.
- [8] J. Wei, J. M. Buriak, G. Siuzdak, *Nature* **1999**, *399*, 243.
- [9] E. P. Go, J. V. Apon, G. Luo, A. Saghatelian, R. H. Daniels, V. Sahi, R. Dubrow, B. F. Cravatt, A. Vertes, G. Siuzdak, *Anal. Chem.* **2005**, *77*, 1641.
- [10] G. Piret, H. Drobecq, Y. Coffinier, O. Melnyk, R. Boukherroub, *Langmuir* **2010**, *26*, 1354.
- [11] M. F. Wyatt, S. Ding, B. K. Stein, A. G. Brenton, R. H. Daniels, *J. Am. Soc. Mass Spectrom.* **2010**, *21*, 1256.
- [12] F. Lapierre, G. Piret, H. Drobecq, O. Melnyk, Y. Coffinier, V. Thomy, R. Boukherroub, *Lab Chip* **2011**, *11*, 1620.
- [13] C.-W. Tsao, P. Kumar, J. Liu, D. L. DeVoe, *Anal. Chem.* **2008**, *80*, 2973.
- [14] J. A. McLean, K. A. Stumpo, D. H. Russell, *J. Am. Chem. Soc.* **2005**, *127*, 5304.
- [15] N. Cioffi, L. Colaianni, R. Pilolli, C. Calvano, F. Palmisano, P. Zambonin, *Analytical and Bioanalytical Chemistry* **2009**, *394*, 1375.
- [16] Y. E. Silina, C. Fink-Straube, H. Hayen, D. A. Volmer, *Anal. Methods* **2015**, *7*, 3701.
- [17] H. Kawasaki, T. Yonezawa, T. Watanabe, R. Arakawa, *J. Phys. Chem. C* **2007**, *111*, 16278.
- [18] R. Pilolli, F. Palmisano, N. Cioffi, *Anal Bioanal Chem* **2012**, *402*, 601.
- [19] A. U. Jackson, T. Shum, E. Sokol, A. Dill, R. G. Cooks, *Analytical and Bioanalytical Chemistry* **2010**, *399*, 367.
- [20] C. G. Zambonin, C. D. Calvano, L. D'Accolti, F. Palmisano, *Rapid Communications in Mass Spectrometry* **2006**, *20*, 325.
- [21] Y. E. Silina, M. Koch, D. A. Volmer, *Journal of Mass Spectrometry* **2015**, *50*, 578.
- [22] T. M. Guinan, O. J. R. Gustafsson, G. McPhee, H. Kobus, N. H. Voelcker, *Anal. Chem.* **2015**, *87*, 11195.
- [23] J. Sekula, J. Nizioł, W. Rode, T. Ruman, *Analyst* **2015**, *140*, 6195.
- [24] S. Naama, T. Hadjersi, H. Menari, G. Nezzal, L. B. Ahmed, S. Lamrani, *Materials Research Bulletin* **2016**, *76*, 317.
- [25] Y. M. A. Yamada, Y. Yuyama, T. Sato, S. Fujikawa, Y. Uozumi, *Angewandte Chemie* **2014**, *126*, 131.
- [26] X. Han, H. Wang, X. Ou, X. Zhang, *J. Mater. Chem.* **2012**, *22*, 14127.
- [27] H. Kawasaki, T. Yao, T. Suganuma, K. Okumura, Y. Iwaki, T. Yonezawa, T. Kikuchi, R. Arakawa, *Chemistry – A European Journal* **2010**, *16*, 10832.
- [28] Y. Coffinier, I. Kurylo, H. Drobecq, S. Szunerits, O. Melnyk, V. N. Zaitsev, R. Boukherroub, *Analyst* **2014**, *139*, 5155.
- [29] X. Li, J. Tan, J. Yu, J. Feng, A. Pan, S. Zheng, J. Wu, *Analytica Chimica Acta* **2014**, *849*, 27.
- [30] C.-W. Tsao, Z.-J. Yang, *ACS Appl. Mater. Interfaces* **2015**, *7*, 22630.
- [31] N. Cioffi, R. A. Picca, M. J. Lo Faro, C. D. Calvano, B. Fazio, M. C. Sportelli, S. Trusso, P. M. Ossi, F. Neri, C. D'Andrea, A. Irrera, *SPIE Newsroom* **2015**, DOI 10.1117/2.1201509.006086.

- [32] C. D'Andrea, M. José Lo Faro, P. Musumeci, B. Fazio, F. Iacona, G. Franzò, P. Gucciardi, C. Vasi, F. Priolo, A. Irrera, *physica status solidi (c)* **2014**, *11*, 1622.
- [33] P. M. Ossi, F. Neri, N. Santo, S. Trusso, *Applied Physics A* **2011**, *104*, 829.
- [34] N. R. Agarwal, F. Neri, S. Trusso, A. Lucotti, P. M. Ossi, *Applied Surface Science* **2012**, *258*, 9148.
- [35] E. Fazio, F. Neri, P. M. Ossi, N. Santo, S. Trusso, *Applied Surface Science* **2009**, *255*, 9676.
- [36] C. D'Andrea, F. Neri, P.M. Ossi, N. Santo, S. Trusso, *Nanotechnology* **2009**, *20*, 245606.
- [37] A. Irrera, P. Artoni, R. Saija, P. G. Gucciardi, M. A. Iatì, F. Borghese, P. Denti, F. Iacona, F. Priolo, O. M. Maragò, *Nano Lett.* **2011**, *11*, 4879.
- [38] M. J. Lo Faro, C. D'Andrea, E. Messina, B. Fazio, P. Musumeci, R. Reitano, G. Franzò, P. G. Gucciardi, C. Vasi, F. Priolo, F. Iacona, A. Irrera, *Scientific Reports* **2015**, *5*, 16753.
- [39] G. Moretti, *Journal of Electron Spectroscopy and Related Phenomena* **1998**, *95*, 95.
- [40] S. Bera, P. Gangopadhyay, K. G. M. Nair, B. K. Panigrahi, S. V. Narasimhan, *Journal of Electron Spectroscopy and Related Phenomena* **2006**, *152*, 91.
- [41] F. Paladini, R. A. Picca, M. C. Sportelli, N. Cioffi, A. Sannino, M. Pollini, *Materials Science and Engineering: C* **2015**, *52*, 1.
- [42] F. J. Himpsel, F. R. McFeely, A. Taleb-Ibrahimi, J. A. Yarmoff, G. Hollinger, *Phys. Rev. B* **1988**, *38*, 6084.
- [43] National Institute of Standards and Technology, "XPS Database," can be found under <http://srdata.nist.gov/xps>, **2012**.
- [44] A. Irrera, P. Artoni, F. Iacona, E. F. Pecora, G. Franzò, M. Galli, B. Fazio, S. Boninelli, F. Priolo, *Nanotechnology* **2012**, *23*, 75204.
- [45] A. Schnapp, A.-C. Niehoff, A. Koch, K. Dreisewerd, *Methods* **2016**, *104*, 194.
- [46] H. Yan, N. Xu, W.-Y. Huang, H.-M. Han, S.-J. Xiao, *International Journal of Mass Spectrometry* **2009**, *281*, 1.
- [47] C. V. Rao, H. L. Newmark, B. S. Reddy, *Carcinogenesis* **1998**, *19*, 287.
- [48] B. Nie, R. N. Masyuko, P. W. Bohn, *Analyst* **2012**, *137*, 1421.
- [49] J. Gidden, R. Liyanage, B. Durham, J. O. Lay, *Rapid Communications in Mass Spectrometry* **2007**, *21*, 1951.
- [50] L. Colaianni, S. C. Kung, D. K. Taggart, R. A. Picca, J. Greaves, R. M. Penner, N. Cioffi, *Analytical and Bioanalytical Chemistry* **2014**, *406*, 4571.
- [51] H. Yu, E. Lopez, S. W. Young, J. Luo, H. Tian, P. Cao, *Analytical Biochemistry* **2006**, *354*, 182.

Captions to Figures

Figure 1. Microscopy images of AgNP/SiNWs: a) SEM cross-sectional view of a dense array, b) detailed view of AgNP decoration along the wires, c) TEM observation of AgNPs attached to SiNWs. SiNWs are 3.2 μm in length.

Figure 2. Typical Si2p (a) and AgM_{4,5}N₄₅N₄₅ (b) fitted regions relevant to AgNP/SiNWs, as acquired by XPS measurements.

Figure 3. a) MS spectra of oleic acid ~~0.5 mg/mL~~ (2 nmol/ μL) on AgNP/SiNWs. Ag_n⁺ clusters are labeled with an asterisk. In the inset the mass profile of OA adduct with Ag is reported; b) MS profile of AgNP/SiNWs surface. Labels as in (a); c) Calibration curve for OA obtained on AgNP/SiNWs; d) MS spectra of oleic acid (2 nmol/ μL) on bare SiNWs. In the inset the mass profile of [OA]⁺ is reported. SiNWs are 3.2 μm in length.

Figure 4. MS profiles acquired using AgNP/SiNWs of a) EVOO extract, b) peanut oil. Asterisks denote Ag_n⁺ cluster ions. Circles denote peaks attributed to sample. A zoom on the m/z region relevant to adducts of OA and LA with silver for EVOO extract is presented in the inset.

Figure 5. MS profiles of EVOO extract for SQ determination registered using unmodified a) 275 nm-, b) 1.6 μm -, and c) 3.2 μm -long SiNWs. Ag was only used as metal-catalyst for growing NWs. Dashed lines indicate the cluster ions relevant to [SQ+Ag]⁺.

Figure 6. MS profile in the 500-1100 m/z region of EVOO diluted with chloroform (5% v/v), acquired using SiNWs.

Tables

Table 1. Typical surface chemical composition of AgNP-functionalized 3.2- μm long SiNWs. Data acquired in triplicate.

Element	At%
Ag	30 ± 2
Si	12 ± 3
O	19 ± 4
C	39 ± 5

Table 2. Typical ions detected in the investigated oils samples. For silver adducts, the monoisotopic ions of ^{107}Ag is reported. L = linoleic acid, O = oleic acid, SQ = squalene, DAG = diacylglycerol.

Ion / m/z	Attribution
387.2	$[\text{L}+\text{Ag}]^+$
389.2	$[\text{O}+\text{Ag}]^+$
411.2	$[\text{A}+\text{Ag}]^+$
497.1	$[\text{O}+\text{Ag}_2]^+$
517.3	$[\text{SQ}+\text{Ag}]^+$
533.3	citrostadienol/cicloartenol ($\text{C}_{30}\text{H}_{50}\text{O}$)
549.3	uvaol/erythrodiol ($\text{C}_{30}\text{H}_{50}\text{O}_2$)
601.6	DAG-like fragment $[\text{OL}]^+$
603.6	DAG-like fragment $[\text{OO}]^+$
725.5	$[\text{DAG}(\text{OL})+\text{Ag}]^+$
727.4	$[\text{DAG}(\text{OO})+\text{Ag}]^+$

Figure 1

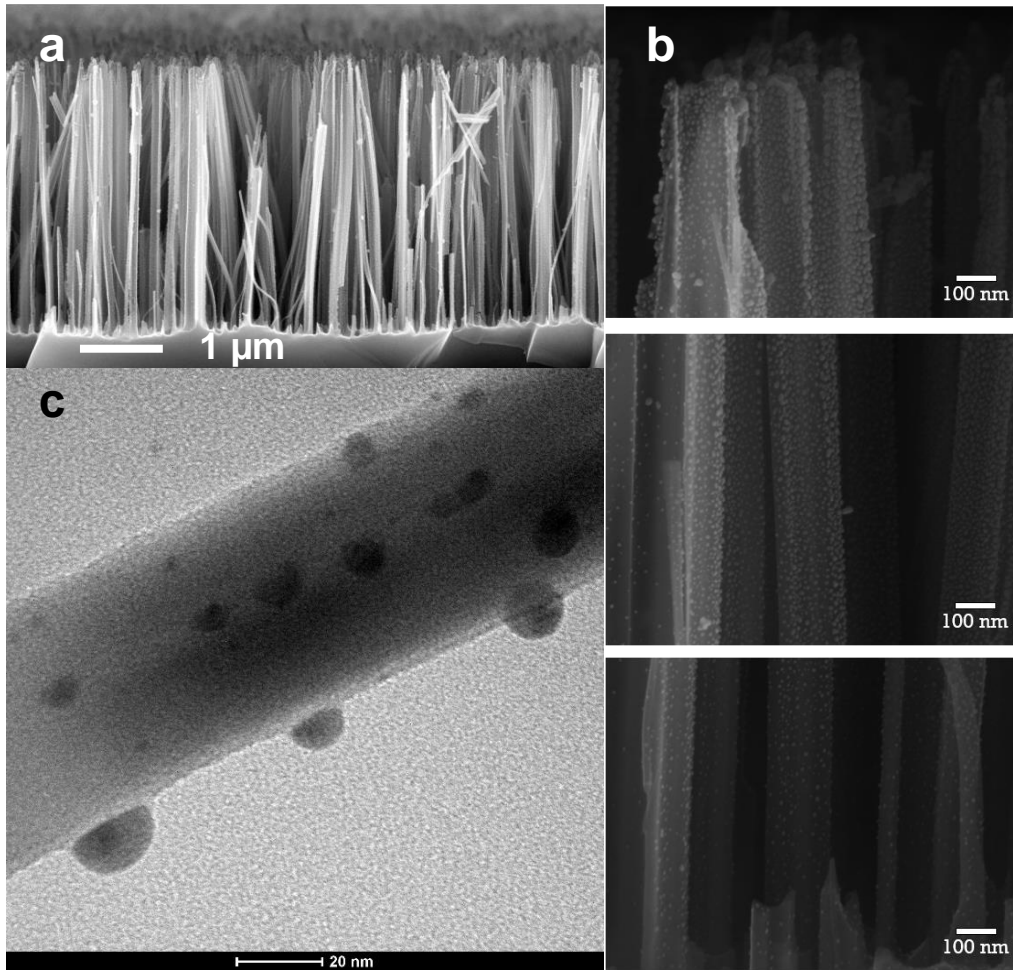


Figure 2

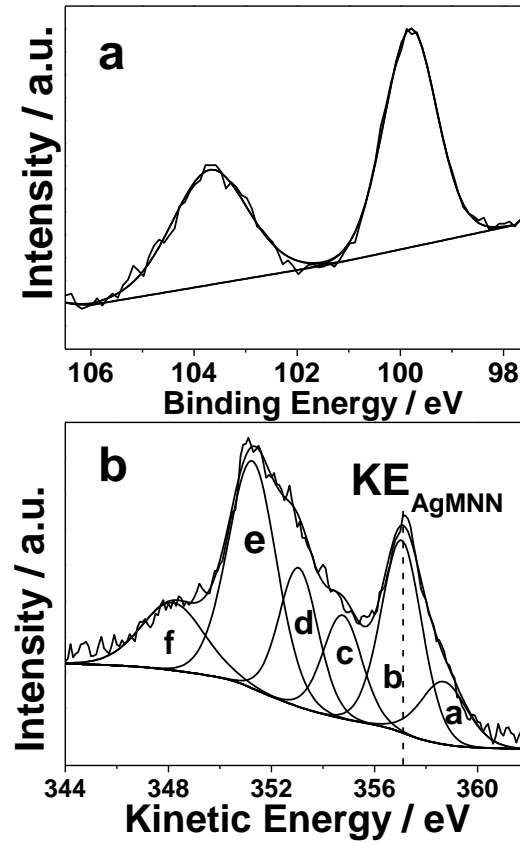


Figure 3

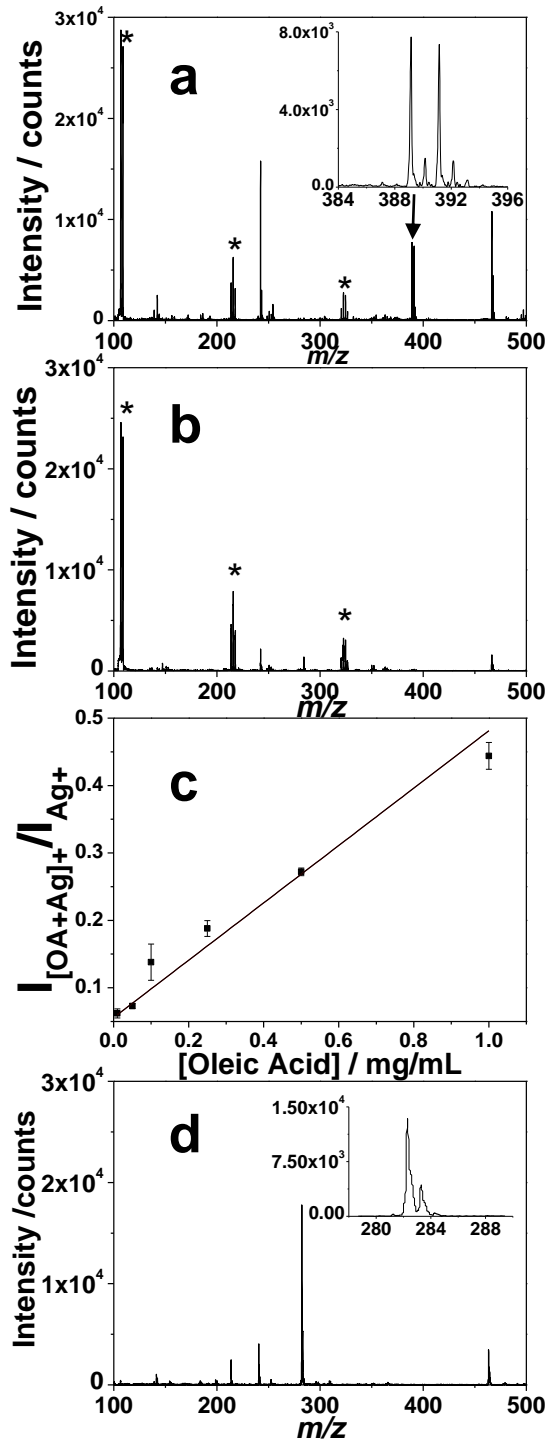


Figure 4

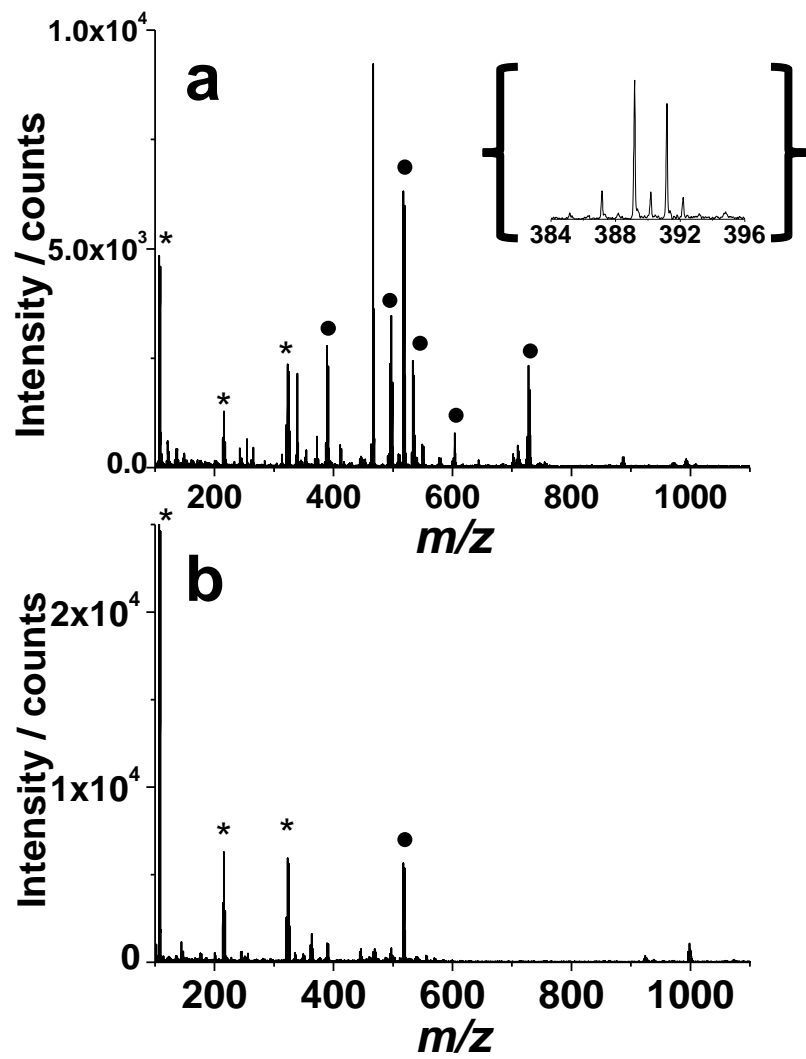


Figure 5

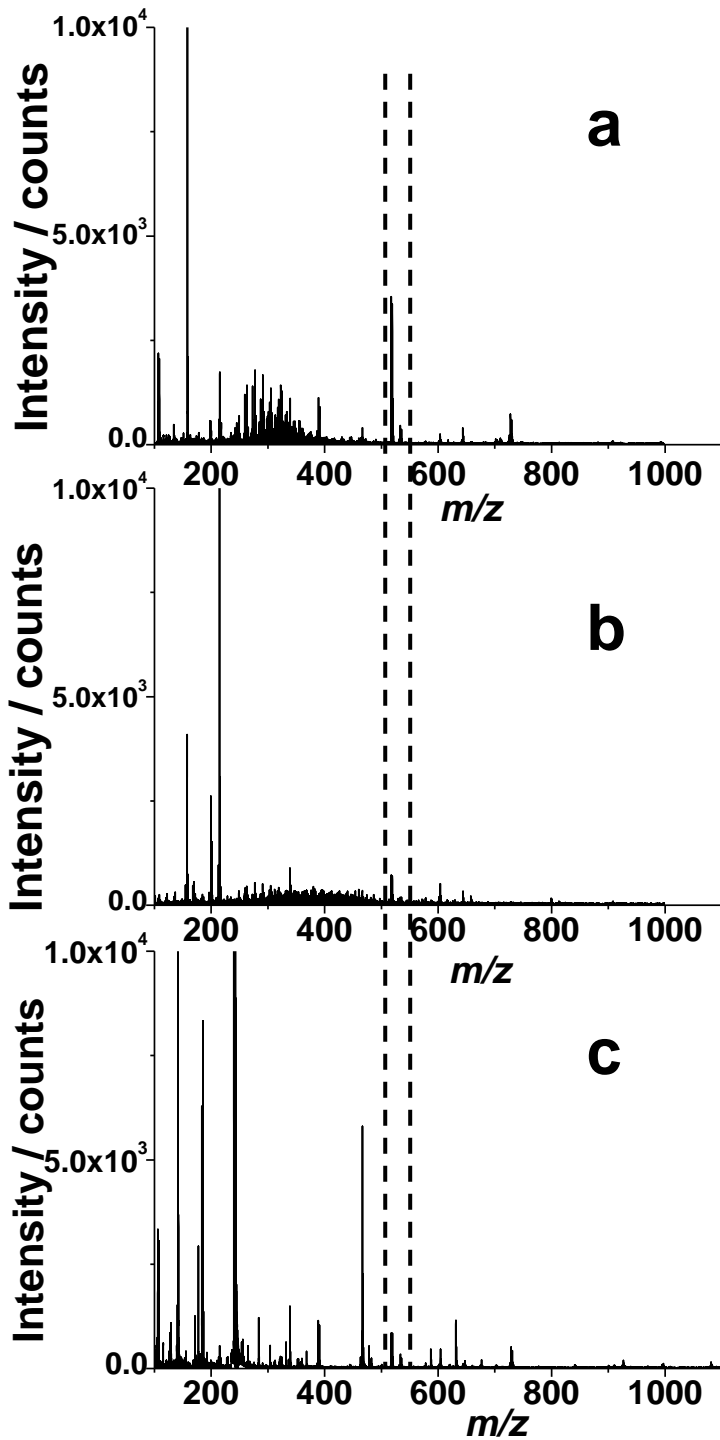


Figure 6

