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Analysis of deprotonated acids with silicon nanoparticle-assisted laser desorption/ionization mass spectrometry

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Chemically modified silicon nanoparticles were applied for the laser desorption/negative ionization of small acids. A series of substituted sulfonic acids and fatty acids was studied. Compared to desorption ionization on porous silicon (DIOS) and other matrix-less laser desorption/ionization techniques, silicon nanoparticle-assisted laser desorption/ionization (SPALDI) mass spectrometry allows for the analysis of acids in the negative ion mode without the observation of multimers or cation adducts. Using SPALDI, detection limits of many acids reached levels down to 50 pmol/μl. SPALDI of fatty acids with unmodified silicon nanoparticles was compared to SPALDI using the fluoroalkyl silylated silicon powder, with the unmodified particles showing better sensitivity for fatty acids, but with more low-mass background due to impurities and surfactants in the untreated silicon powder. The fatty acids exhibited a size-dependent response in both SPALDI and unmodified SPALDI, showing a signal intensity increase with the chain length of the fatty acids (C12-C18), leveling off at chain lengths of C18-C22. The size effect may be due to the crystallization of long chain fatty acids on the silicon. This hypothesis was further explored and supported by SPALDI of several, similar sized, unsaturated fatty acids with various crystallinities. Fatty acids in milk lipids and tick nymph samples were directly detected and their concentration ratios were determined by SPALDI mass spectrometry without complicated and time-consuming purification and esterification required in the traditional analysis of fatty acids by gas chromatography (GC). These results suggest that SPALDI mass spectrometry has the potential application in fast screening for small acids in crude samples with minimal sample preparation.

Keywords: silicon nanoparticles; laser desorption/ionization; matrix-less; small molecule acids; fatty acid analysis; chain length effect; MALDI

Introduction

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry is widely used in daily sample assay and features easy sample preparation and high sample throughput. The application of MALDI, however, is limited to macromolecules (typically larger than 1 kDa) such as biomolecules[1] and synthetic polymers[2] due to the low mass background peaks associated with organic matrices. In order to make MALDI applicable to small molecule analysis, several approaches have been explored and developed in order to reduce this limiting low-mass background. Many of these approaches incorporate the usage of inorganic materials as matrices, including graphite,[3–6] carbon nanotubes,[7,8] silicon,[9–11] gold nanoparticles[12] and many other inorganic particles such as Sn, SnO_2, Zn, ZnO, W, WO_3, TiO_2, Al, Mn and Mo.[13] Among these techniques, desorption/ionization on porous silicon (DIOS) has been extensively studied for its enhanced signal-to-noise ratio, minimized background and reported excellent limits of detection.[14] The success of DIOS led to several other types of silicon morphologies being explored including silicon nanowires[15] and silicon microparticles.[13] Nanostructured-initiator mass spectrometry (NIMS), a new matrix-free technique, involves surface modification on DIOS chips, provides soft ionization and high sensitivity.[16,17] Silicon nanoparticle-assisted laser desorption/ionization (SPALDI), an alternative approach to DIOS, is an ionization technique with high sensitivity, molecular selectivity and salt tolerance.[18] Compared to DIOS and standard MALDI with organic matrices, SPALDI requires lower laser fluence and induces lower internal energy of the desorbed ions.[19,20] The modification of the commercially available silicon nanoparticles does not involve dealing with toxic hydrofluoric acid or electrochemical etching which is required in the preparation of DIOS and NIMS chips. In addition, SPALDI does not need any special instrumental setup. The mixture of the silicon nanoparticles and the analyte solutions can be simply spotted on a standard MALDI plate and analyzed in a regular MALDI setup. These characteristics make SPALDI a practical and convenient tool for the analysis of small molecules. As shown in our previous work, SPALDI is especially efficient for compounds which can be easily pre-charged.[18,19] This makes SPALDI also applicable to the analysis of acids in the negative ion mode. We have already shown the efficient ionization of dibutylphosphoric acid by SPALDI.[18] In this paper, we further explore the application of SPALDI in the analysis of acids, especially fatty acids. The analysis of fatty acid composition in lipids is conventionally accomplished

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by gas chromatography (GC) after transforming the lipids to fatty acid methyl esters by transesterification.[21–23] Additionally, the GC peaks of some unsaturated fatty acid components are not completely resolved.[21] Analysis of fatty acids by MALDI does not require esterification or separation in advance.[21] However, the analysis of small molecules by MALDI often suffers from the background peaks resulting from the organic matrices. SPALDI with minimal background interference is therefore promising for the analysis of fatty acids. In addition to the study of a series of commercial sulfonic acid and fatty acid standards, the analysis of fatty acids in milk and in tick nymphs by SPALDI mass spectrometry is also demonstrated in this paper.

Experimental

Materials

p-Toluenesulfonic acid monohydrate and 1-dodecanesulfonic acid sodium salt were purchased from Acros (Pittsburgh, PA). Dodecanoic acid (C12), myristic acid (C14) and palmitic acid (C16) were purchased from Sigma (St. Louis, MO). (±)-Camphor-10-sulfonic acid, heptadecanoic acid (C17), stearic acid (C18), nonadecanoic acid (C19), arachidic acid (C20) and behenic acid (C22) were purchased from Fluka (St. Louis, MO). All these acids and the solvents including methanol, ethanol, chloroform and isopropanol (HPLC grade) were used without further purification. Silicon nanoparticles with a diameter of 30 nm were purchased from Meliorum Technologies, Inc. (Rochester, NY). (Heptadecafluoro-1,1,2,2-tetrahydrodecyl)dimethylchlorosilane (FC10) was purchased from Gelest, Inc. (Morrisville, PA) and used to silylate the silicon nanoparticles as described below. Whole milk (3–4% fat) was purchased from a local grocery store. Tick nymph samples were obtained from the tick rearing facility at Oklahoma State University. These tick nymphs were experimentally fed once in their life cycle with rabbit blood and collected after molting. They were kept at −80 °C until the experiment was carried out.

Preparation of silicon nanoparticles

The modification process of the silicon nanopowder was discussed in detail in our previous work[18] and is followed with some minor changes here. A set of optimized conditions to oxidize and silylate the silicon nanopowder for efficient laser desorption/ionization is as follows: 8 mg of silicon nanoparticles hydroxylated by 0.4 ml of 10% HNO_3 for 30 min with sonication in a heated water bath. Then the powder was centrifuged, underwent two cycles of washing by isopropanol and centrifugation to remove the HNO_3 residue. Finally, the powder was dried in a vacuum concentrator (SpeedVac® Plus SC110A, Thermo Savant, Holbrook, NY) under 'low' heating for 2 h. The dried powder was treated with 90 µl of pure (heptadecafluoro-1,1,2,2-tetrahydrodecyl)dimethylchlorosilane (linear 'FC10') for 30 min with sonication and then dried in an oven at 90 °C for about 24 h. An alternative simple solution preparation protocol for silicon nanoparticles was also developed and used for the preparation of some of the materials used in the experiment; this solution procedure does not require placement of the material in an oven at a temperature that exceeds the flash point (autoignition point not known). For the solution preparation, 16 mg of silicon nanoparticles hydroxylated by 0.8 ml of 10% HNO_3 for 30 min with sonication in a heated water bath. Then the powder was centrifuged and washed with isopropanol twice to remove the HNO_3 residue. Finally, the powder was dried in a vacuum concentrator under 'low' heating for 2 h. One hundred eighty microliters of (heptadecafluoro-1,1,2,2-tetrahydrodecyl)dimethylchlorosilane was dissolved in 10 ml of CCl_4. The dried powder was suspended in the silane solution with sonication for 30 min. Then the silane/silicon powder mixture was heated to reflux temperature for 24 h with stirring. Finally, the silylated silicon nanoparticles were stored in 0.4 ml of perfluorohexane for later use. Before application of the powder onto the MALDI plate, the perfluorohexane was replaced by isopropanol. Suspension of the silicon powder in isopropanol was achieved by sonication. A given amount of Milli-Q water was added to the powder suspension followed by sonication to obtain a final ratio of isopropanol and water of about 2 : 1 (v/v). Untreated silicon nanoparticles were washed three times with isopropanol and finally dispersed in isopropanol/water (2 : 1, v/v) before the application in SPALDI with unmodified particles.

Preparation of standard acid solutions

The sulfonic acids including *p*-toluenesulfonic acid monohydrate, 1-dodecanesulfonic acid sodium salt and (±)-camphor-10-sulfonic acid were dissolved in methanol/chloroform (1 : 1, v/v) to obtain stock solutions of 10 mM. Further dilution of the stock solutions was performed with methanol/chloroform (7 : 3, v/v) to achieve 50 pmol/µl *p*-toluenesulfonic acid and 100 pmol/µl for both (±)-camphor-10-sulfonic acid and 1-dodecanesulfonic acid sodium salt.

All the fatty acid standards (C12–C22) were dissolved in ethanol/chloroform (1 : 1, v/v) to obtain stock solutions of 5 mM. Desired final concentrations of the fatty acids (50–833 pmol/µl) were achieved by the dilution of the stock solutions with ethanol/chloroform (1 : 1, v/v) or by mixing several stock solutions of fatty acids.

Extraction of lipids and fatty acids from milk and tick nymph samples

The method to extract the lipids from milk samples was adopted from the literature[4] with slight modifications. Briefly, a mixture of 1 ml of milk and 3.75 ml of methanol/chloroform (2 : 1, v/v) was vortexed for approximately 30 s followed by centrifugation for 20 min at 3400 rpm. The supernatant was aspirated out and the residue was vortexed with 1.25 ml of chloroform. After centrifugation of the residue/chloroform mixture for another 20 min at 3400 rpm, the resulting supernatant was removed and combined with the primary supernatant set aside earlier. The combined supernatants were treated with 1.25 ml of 0.88% KCl solution to break micelles/bicelles formed by bipolar components. After brief agitation, the bottom organic layer was separated and treated with a small amount of anhydrous $MgSO_4$ to remove residual H_2O in the organic layer. After the organic layer was filtered, lipids in the organic phase were concentrated under reduced pressure rotavaporization (Rotavapor-R, Büchi Laboratoriums-Technik AG, Flawil, Switzerland) and stored in a freezer at −20 °C. The concentrated lipid solution was diluted 1 : 100 with methanol/chloroform (1 : 1, v/v) before analysis by SPALDI mass spectrometry. To extract the free fatty acids from the tick nymphs, the tick nymph samples were frozen in liquid nitrogen to facilitate efficient grinding. One tick nymph was ground using a mortar and pestle. Soluble fatty acids in the ground tick nymph samples were dissolved in 3–5 µl of methanol/chloroform (1 : 1,

v/v). Three microliters of tick fatty acid extract was mixed with 3 µl of the SPALDI powder suspension and spotted onto a MALDI plate for analysis.

Mass spectrometry

Standard MALDI well plates were used for SPALDI experiments. For the commercial acid standards, 1 µl of silicon nanoparticle suspension was spotted on the MALDI plate immediately followed by 1 µl of the diluted acid solution placed on top of the silicon particles before the layer of the powder suspension dried. For the extracted lipid and fatty acid samples, the analyte solution was mixed with the silicon nanopowder suspension by a volume ratio of 1 : 1. Then, 0.5–1 µl of the analyte/powder mixture was placed on the MALDI plate. The spotted MALDI plates were always dried in air before introduction into a MALDI-time of flight mass spectrometer.

All the experiments were performed using a Reflex III (Bruker, Billerica, MA) time-of-flight mass spectrometer with a reflectron, operated in the negative ion mode. The accelerating voltage was set to 20 kV and the voltage on the reflectron was set to 23 kV. The mass spectrometer was equipped with a standard 337 nm nitrogen laser (LSI-Laser Science, Inc., Newton, MA). The laser beam has an average maximal fluence of 400 µJ/pulse and was focused to a spot in a diameter of 100–150 µm on the MALDI plate. Typically, 20–45% of the total laser fluence (attenuations of 80–55%) was applied in the experiments. Signals of 200 shots at a rate of 3 shots/s were accumulated for each spectrum acquired. Since the SPALDI samples do not have 'sweet spots',[18] random rastering of the sample spots was applied manually, typically moving every 5–10 laser shots.

Results and Discussion

SPALDI of substituted sulfonic acids

A series of substituted sulfonic acids including *p*-toluenesulfonic acid monohydrate, (±)-camphor-10-sulfonic acid and 1-dodecanesulfonic acid sodium salt was analyzed to evaluate the ionization of low-mass acidic compounds by SPALDI mass spectrometry (Fig. 1). As shown in Fig. 1(a) and (b), both 50 pmol/µl *p*-toluenesulfonic acid and 100 pmol/µl (±)-camphor-10-sulfonic acid can be efficiently ionized by negative ion SPALDI. For all the compounds, the negatively charged free acid is observed. With SPALDI, there were no multimers or adduct ions detected, whereas the desorption/ionization of *p*-toluenesulfonic acid and (±)-camphor-10-sulfonic acid on porous silicon is reported to generate Na^+ dimeric ($[Na^+ + 2(M - H)^-]$) and K^+ trimeric ($[K^+ + 3(M -$

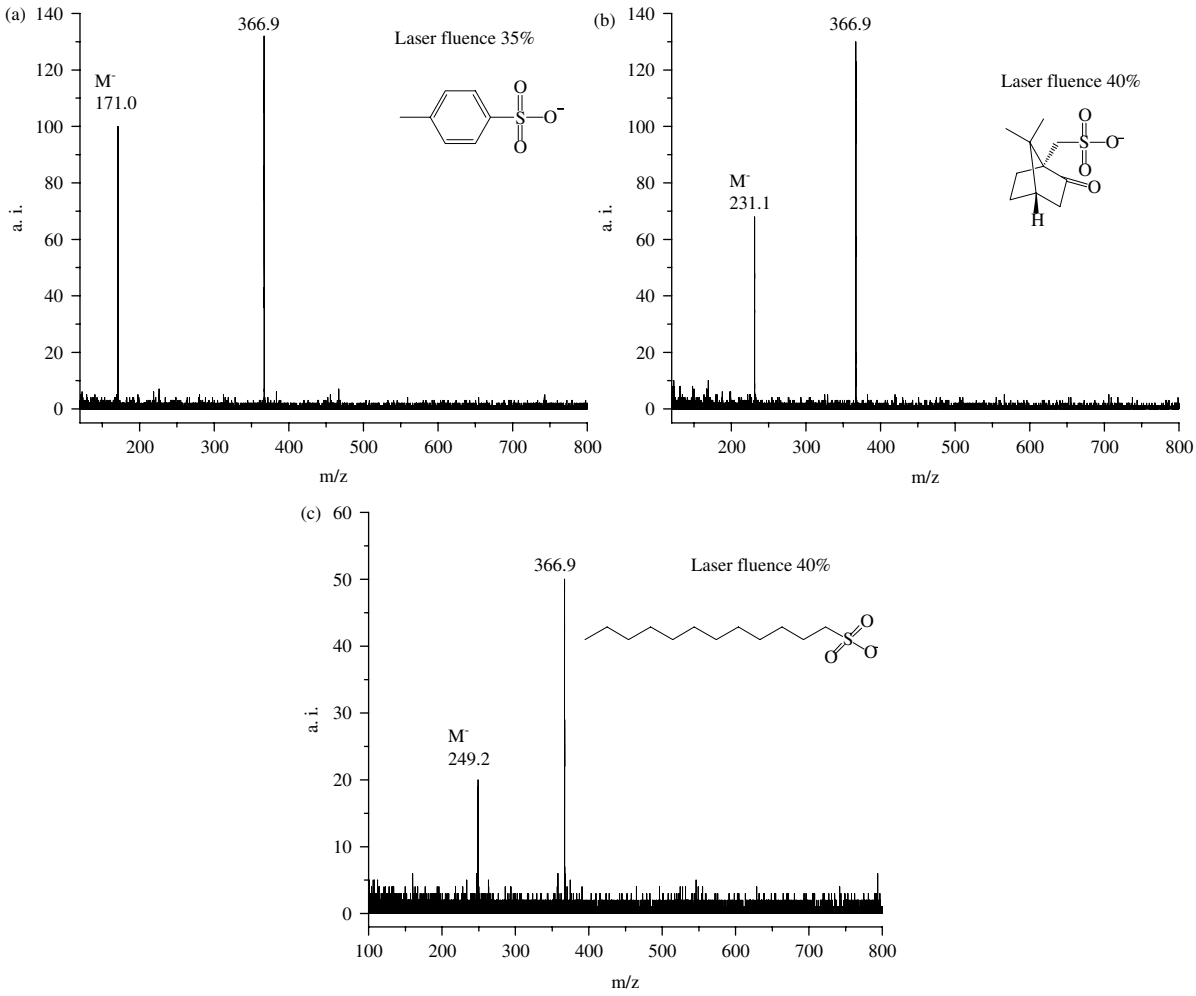

Figure 1. SPALDI-negative ion mass spectrometry of (a) *p*-toluenesulfonic acid at 50 pmol/µl, (b) (±)-camphor-10-sulfonic acid at 100 pmol/µl and (c) 1-dodecanesulfonic acid sodium salt at 100 pmol/µl.

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H)$^-$]) adducts[24] in the negative ion mode. In addition to these two substituted sulfonic acids, the sulfonic anion in 1-dodecanesulfonic acid sodium salt can also be detected by SPALDI mass spectrometry as shown for 100 pmol/μl concentration in Fig. 1(c). All mass spectra collected for these sulfonic compounds were clean with minimal background. The only interference was the peak at *m/z* 366.9. LDI on the FC10 modified silicon nanoparticles was performed and analyzed by a Fourier transform mass spectrometer (FT-MS). Exact mass measurement and MS/MS analyses reveal that the peak at *m/z* 366.9 may correspond to a fluorinated hydrocarbon, probably C10F13, which likely originates from the fluorinated modifier on the surface of the silicon nanoparticles. The sulfonic acid spectra suggest that SPALDI is capable of the relatively sensitive desorption/ionization of small acids. Similar to the SPALDI mass spectrometry of basic compounds in the positive ion mode,[18] multimeric species or cationized adducts of the acids were absent from the SPALDI mass spectra in the negative ion mode. It should be noted that the laser fluence required to induce efficient SPALDI in the negative ion mode was around 35–45% of the total laser fluence.

SPALDI of fatty acids

An equimolar mixture of fatty acids (C12, C14, C16, C18, C20 and C22) at a concentration of 833 pmol/μl each was successfully ionized by SPALDI and the mass spectrum is illustrated in Fig. 2. The concentration was set at a high level in order to observe the smaller C12 and C14 fatty acids which exhibited the poorest signals. It is noted that the laser fluence required for signal thresholds was about 30–35% with negative ion SPALDI, whereas a much higher threshold fluence of 60% was required for LDI of the fatty acids on the bare MALDI plate. The difference in the threshold laser fluence for efficient desorption/ionization of fatty acids in SPALDI and direct LDI is due to the efficient absorption of laser energy by nanoscale silicon particles and the local heating thereafter, as suggested in our previous work[18] as well as LDI on other silicon nanomaterials.[25] This could also be due to the relative inertness of the perfluorinated surface relative to the bare, contaminated and undefined metal.

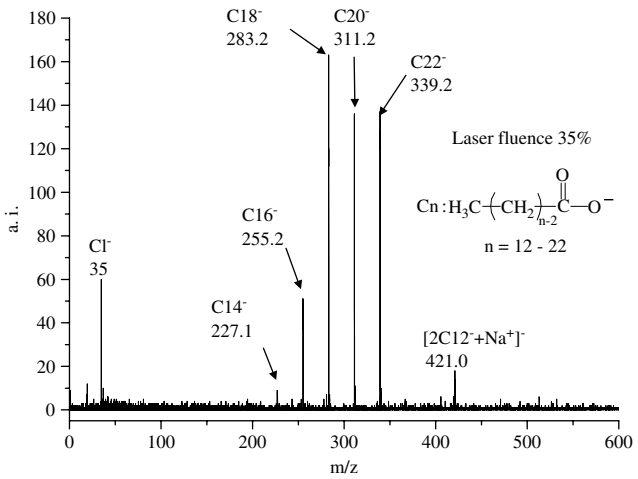

Figure 2. Fatty acid SPALDI – chain length dependence. Negative ion SPALDI mass spectrum of the equimolar mixture of dodecanoic acid (C12), myristic acid (C14), palmitic acid (C16), stearic acid (C18), arachidic acid (C20) and behenic acid (C22). The concentration of each fatty acid in the mixture was 833 pmol/μl.

An interesting phenomenon is the varying response of the SPALDI mass spectrometry to the different size of the fatty acids as illustrated in Fig. 2. The signal intensity of the fatty acids increases with the length of the carbon chain. While dodecanoic acid (C12) could not be observed and myristic acid (C14) and palmitic acid (C16) gave gradually increasing signals, the signal intensity almost leveled off for equimolar concentrations of fatty acids when the carbon chains were longer than 16 carbons. Chain-length-dependent signal intensity of fatty acid analysis in negative ion mode was recently reported for NIMS.[17] We suggest that the size-dependent response of the SPALDI mass spectrometry is due to the crystallization of the larger fatty acids on the silicon nanopowder. Dodecanoic acid (C12) and myristic acid (C14) seemed not to form aggregates or crystals from the observation by the camera in the ion source of the MALDI-TOF instrument. Some dispersed small crystals of the palmitic acid (C16) sample can be observed under the camera. When the alkyl chains of the fatty acids were 18 carbons and longer, white and sparkling crystals were seen under the camera.

In order to further study our hypothesis that crystallization of fatty acids on the surface of silicon nanoparticles contributes to the size-dependent signal response in SPALDI, three unsaturated fatty acids including oleic acid (*cis*-9-C18 : 1), elaidic acid (*trans*-9-C18 : 1) and linoleic acid (*cis*-9,12-C18 : 2) were ionized by SPALDI and compared with the saturated C18 acid. These unsaturated fatty acids have different numbers of double bonds and conformations in the long chains and thus have different crystallinities. Both oleic acid and elaidic acid have one double bond at the same position in the alkyl chain. However, oleic acid is a liquid and elaidic acid exists in small crystals at room temperature. Similar to oleic acid, linoleic acid is in liquid state because of its *cis*-conformation and the additional double bond in the alkyl chain. If our hypothesis is correct, both C18 and *trans*-9-C18 : 1 that are crystals are expected to show higher SPALDI signal intensity than *cis*-9-C18 : 1 and *cis*-9,12-C18 : 2 which are liquids. After mixing each fatty acid solution with silicon nanoparticles and spotting the mixture onto the MALDI plate, crystals of saturated C18 and smaller crystals of *trans*-9-C18 : 1 on the silicon nanopowder were observed under the camera. No crystals were seen for *cis*-9-C18 : 1 or *cis*-9,12-C18 : 2. The signal intensity of these unsaturated fatty acids in negative ion SPALDI mass spectrometry is shown in Fig. 3 and compared with C18. The results shown are an average of five experiments from five different spots for each fatty acid. As clearly illustrated in Fig. 3, the fatty acids which form crystals exhibited signal intensities two- to three-fold higher than those with liquid form, which is in accordance with our expectation. The correlation of the signal intensity of the fatty acids with crystallinity supports the hypothesis that crystallization on the modified silicon powder improves the signal intensity of the fatty acids in negative ion SPALDI mass spectrometry.

An equimolar mixture of C12–C18 was ionized by unmodified silicon nanoparticles as well. The silicon nanoparticles used were merely washed and sonicated with isopropanol and applied without further chemical modification. A size-dependent response of equimolar fatty acids was also observed for the unmodified particles showing signal intensity proportional to the chain length. This result indicates that the size-dependent response is not determined by the chemical modification of the silicon powder surface. Fatty acids with longer carbon backbones tend to crystallize on the unmodified silicon nanoparticles and strong signal intensities were also observed.

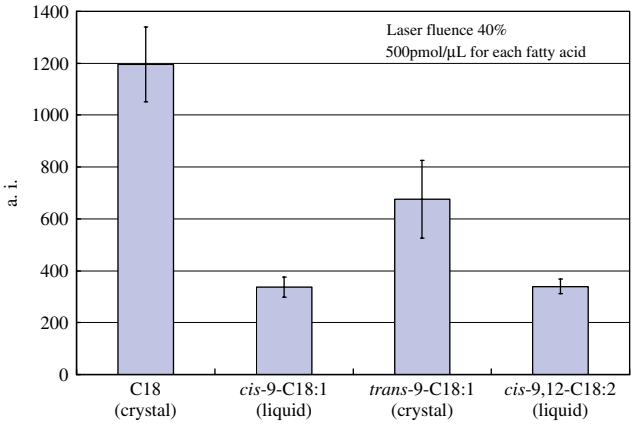

Figure 3. Signal intensity of stearic acid (C18), oleic acid (*cis*-9-C18 : 1), elaidic acid (*trans*-9-C18 : 1) and linoleic acid (*cis*-9,12-C18 : 2) in negative ion SPALDI mass spectrometry. The concentration of each fatty acid in the mixture was 500 pmol/μl. The intensity average and standard deviation for measurements of five sample spots per fatty acid are shown.

In most cases, no multimers or cation adducts were observed for fatty acids with chain length greater than 12 carbons in SPALDI which is similar to the SPALDI of substituted sulfonic acids as mentioned before. However, the deprotonated molecule of C12 fatty acid was missing from the SPALDI mass spectrum, while a sodium adduct of a C12 dimer was detected. No multimeric species or cation adducts were detected for fatty acids on unmodified particles. In contrast, cationized multimeric adducts were reported for several fatty acids from heptadecanoic acid (C17) to lignoceric acid (C24)[24,26] in the negative ion DIOS mass spectrometry. In order to further confirm the minimal formation of cation adducts or multimeric ions in negative ion SPALDI mass spectrometry, $ZnCl_2$ and $CuCl_2$ were intentionally spiked into the nonadecanoic acid (C19) solution, based on their reported contribution to the formation of adduct ions.[26] Neither cation adducts nor multimers were detected at a concentration of C19 up to 3000 pmol/μl. Abundant Zn^{2+} and Cu^{+} adducts of multimeric species and monomeric $ZnCl_2$ or CuCl complexes ($[M + ZnCl_2 - H]^-$, $[M + CuCl - H]^-$ and $[M + (CuCl)_2 - H]^-$) were observed for C19 at the same concentration in negative ion DIOS mass spectrometry.[26]

The absence of cationized multimeric ions of fatty acids in SPALDI may be due to the surface morphology of the SPALDI sample spots. The long fatty acids tend to aggregate/crystallize on the surface of the silicon powder spots. The analyte ions are laser desorbed from the crystals on the silicon particles and then immediately released to the vacuum. Since dodecanoic acid (C12) does not form crystals on the silicon nanopowder, C12 molecules may be trapped in the interstitial space between silicon particles and have more opportunities to interact with each other or alkali cations on the nanoparticle surface to form cationized dimers. In the case of DIOS, analytes are trapped in the porous silicon nanopores with a depth of ~250 nm on the DIOS chips.[27,28] The ions that are laser desorbed were initially confined in the pores and have a higher probability of generating cation adducts or multimeric species.

Although the desorption/ionization behavior of fatty acids was similar for SPALDI and unmodified particles, the detection limit of fatty acids in SPALDI mass spectrometry was not as low as unsilylated SPALDI. C18, C19, C20 and C22 at various concentrations were analyzed with both SPALDI and with untreated silicon powder. SPALDI had a typical limit of detection of 200 pmol/μl for fatty acids with a backbone longer than 16 carbons, while SPALDI with unmodified silicon nanoparticles exhibited a limit of detection below 50 pmol/μl under the same experimental conditions as shown in Fig. 4. While no signal could be obtained at concentrations below the detection limits, monotonic rise of signal with concentration could be observed above the detection limits.

The inferior sensitivity of silylated particle SPALDI to fatty acids may result from the better electron donating property of the unsilylated silicon powder compared to the fluorinated silane chains tethered on the surface of silicon particles. In order to support this hypothesis, $CuCl_2$, which was reported to be reduced by the photoelectrons generated in MALDI,[29] was mixed with unsilylated and fluorinated FC10 silylated silicon powder, respectively. Reduction of Cu(II) to Cu(I) in unsilylated SPALDI generated much more intense reduction products than in SPALDI, suggesting that unmodified silicon particles donate more electrons to the plume than fluorinated FC10 modified silicon powder, perhaps decreasing the likelihood of anion neutralization. The negatively charged surface of the bare silicon particles, which is due to the deprotonation of the surface silanol groups, might also have an electrical repulsion to the deprotonated acids. In addition, the layer of fluorinated alkyl modifier on the silicon nanoparticles dissipates part of the laser energy as shown in our previous work.[19] Therefore, the bare silicon powder is leading to higher sensitivity than SPALDI in the negative ion mode.

However, unsilylated particles resulted in intense background peaks in the low mass region which are attributed to impurities and/or surfactants present from production of the silicon nanoparticles,[18] as illustrated in Fig. 4(b). As Fig. 4(a) shows, most of the background peaks can be eliminated or suppressed by the procedure of cleaning and silylation of the silicon powder, leaving a relatively clean low-mass region and minimizing the interference to analyte signals. The background peaks remaining in the negative ion SPALDI mass spectra resulted from salt in the analyte solutions and phosphate which is a common anionic salt surfactant counterion.[30] In any case, the spectra above *m/z* 100 are usually almost background free in negative ion SPALDI.

Application of negative ion SPALDI mass spectrometry

SPALDI mass spectrometry was used to analyze milk and tick nymph samples in order to illustrate applications of negative ion mode SPALDI in real-world samples. Through simple extraction and mixing the lipid extract with silylated silicon nanoparticles, five fatty acids were successfully detected in a milk sample including C14, C16, linoleic acid (C18 : 2), oleic acid (C18 : 1) and C18, which are the most abundant fatty acid components in the milk lipids determined by GC[22] and HPLC,[31] as illustrated in Fig. 5. Compared to fatty acid analysis by GC, the analysis of fatty acid components in lipid mixtures by SPALDI did not require esterification or saponification,[21] making the sample preparation easier and faster. In addition, the fatty acids detected were free of cation adducts while fatty acids were observed as cation adducts in the analysis of lipids using other matrix-less LDI techniques.[4] Comparison of the milk lipid extract SPALDI mass spectrum with the spectrum of the equimolar mixture of fatty acids (Fig. 2) reveals that the shorter C14 and C16 are more abundant in the extract. The ratio between C18 : 2, C18 : 1 and C18 in the milk revealed by SPALDI is 0.2 : 1 : 0.5, similar to the ratio of 0.2 : 1 : 0.4 for full fat milk determined by HPLC.[31] C18 showed a higher abundance in SPALDI compared to the reported value which should be due

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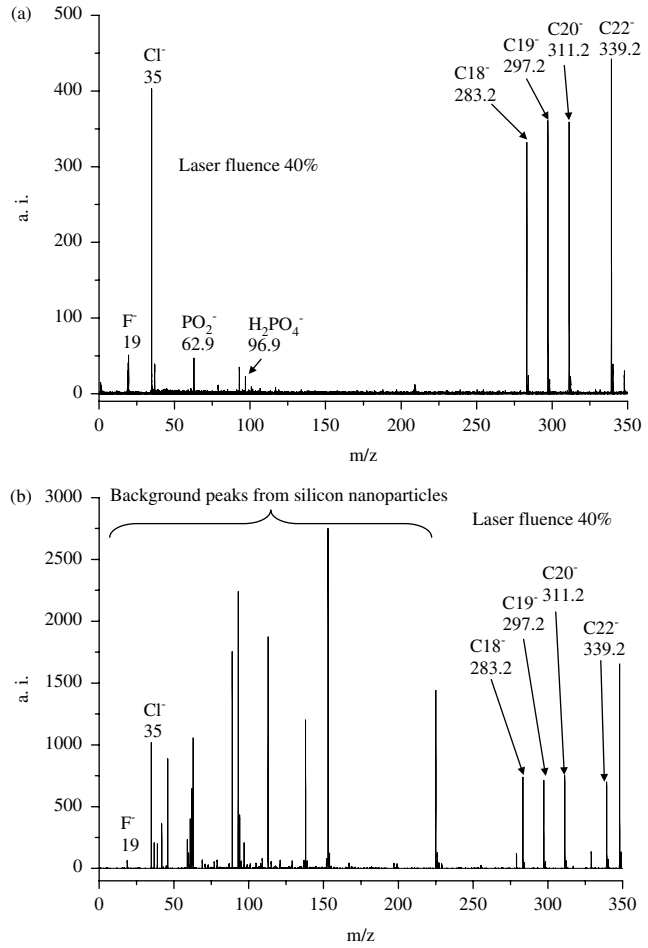

Figure 4. Negative ion mass spectra of an equimolar mixture of stearic acid (C18), nonadecanoic acid (C19), arachidic acid (C20) and behenic acid (C22) with (a) SPALDI and (b) unsilylated SPALDI. The concentration of each fatty acid in the mixture was (a) 313 pmol/μl and (b) 50 pmol/μl.

to the higher SPALDI response of C18 than that of C18 : 2 and C18 : 1 as illustrated in Fig. 3. Taking advantage of the fact that no adducts or multimers form, fatty acids can be semi-quantified by negative ion SPALDI mass spectrometry after size-dependent signal response is calibrated by fatty acid standards.

Free fatty acids in tick nymph samples were also analyzed by SPALDI mass spectrometry. Profiling of fatty acid composition in ticks that have acquired a vertebrate blood meal is a potential way of identifying mammals on which nymphs have fed, assisting disease prevention efforts. A single tick nymph was simply ground and mixed with methanol/chloroform mixture. As exhibited in Fig. 6, palmitic acid (C16), linoleic acid (C18 : 2), oleic acid (C18 : 1) (the dominant peak, *m/z* 282 is the isotopic peak of C18 : 1) and stearic acid (C18) were successfully detected in the ground tick nymph by negative ion SPALDI mass spectrometry, indicating that fatty acids in the tick nymph samples can be directly analyzed by SPALDI with minimal sample preparation. In addition, the analysis of fatty acids in tick nymphs by SPALDI can be obtained with one nymph only, whereas GC usually requires a significantly larger number ranging from 20 to 200 nymphs.[32,33] In our lab we pushed the number of tick nymphs used below 15 to analyze fatty acid profiles by GC. Desorption electrospray ionization (DESI) and direct analysis in real-time (DART), which are relatively new ionization techniques that require 'no sample preparation', have

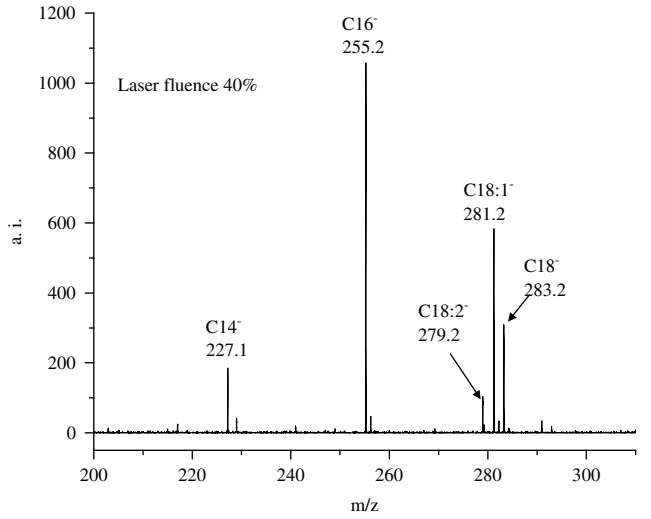

Figure 5. Myristic acid (C14), palmitic acid (C16), linoleic acid (C18 : 2), oleic acid (C18 : 1) and stearic acid (C18) detected in milk extract by SPALDI mass spectrometry in the negative ion mode.

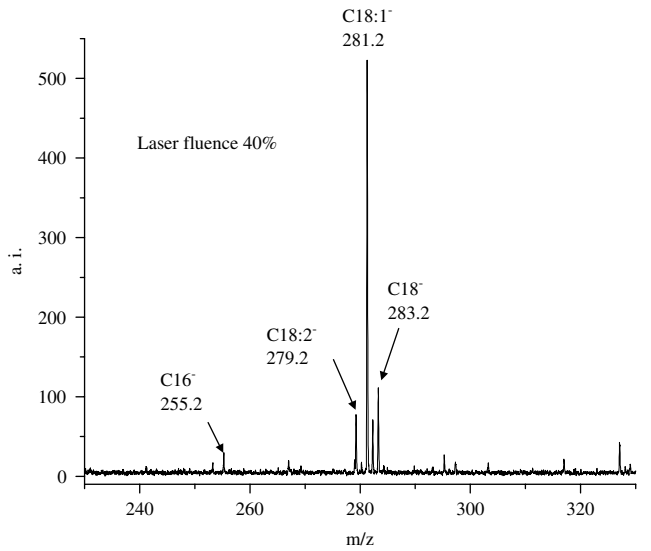

Figure 6. Palmitic acid (C16), linoleic acid (C18 : 2), oleic acid (C18 : 1) and stearic acid (C18) detected in a ground tick nymph by SPALDI mass spectrometry in the negative ion mode.

been reported to provide more sensitive detection[34] or complete profiling of fatty acids[35] in intact bacteria. However, the mass spectra of fatty acids acquired were interfered with abundant or intense unidentified peaks. Recently, strong base was reported as a novel matrix of MALDI for small anions, and fatty acids analysis in crude samples was demonstrated.[36] While we were in the process of submitting the present manuscript, NIMS was reported for rapid screening of fatty acids in plant oil and cell extract, with the detection limit of 100 fmol.[17] SPALDI provides an alternative method for fatty acids screening with minimal sample preparation, especially when analytes are required to be detected in positive ion mode as well.

These results suggest that SPALDI can be used to directly analyze fatty acids and lipids with high sensitivity and simple

extraction and has potential applications in fast screening of those compounds in various crude samples.

Summary

It is demonstrated here that SPALDI mass spectrometry is capable of analyzing small acids in the negative ion mode. The detection limit of SPALDI was 50 pmol/μl for substituted sulfonic acids and 200 pmol/μl for the less acidic fatty acids. Compared to DIOS, SPALDI was slightly more sensitive to acids and almost no multimers or cation adducts were observed in the SPALDI mass spectra although they are commonly seen with DIOS. Fatty acids showed a size effect in their SPALDI response – saturated or *trans*-unsaturated fatty acids with longer carbon backbones exhibited higher signal intensity in the mass spectra. This phenomenon might be related to the aggregation/crystallization of fatty acids on the silicon particles. This hypothesis was also supported by the SPALDI of unsaturated fatty acids with different crystallinities (but similar molecular weight), showing that better crystallinity facilitates higher signal intensity of fatty acids in negative ion SPALDI mass spectrometry. SPALDI was less sensitive than unsilylated SPALDI for fatty acids. However, SPALDI suppressed most of the low-mass background peaks resulting from impurities and surfactants in the untreated silicon nanopowder. SPALDI was also applied to the analysis of fatty acids in milk and tick nymph samples. Both saturated and unsaturated fatty acids were successfully detected and semi-quantified by SPALDI mass spectrometry in the negative ion mode with minimal sample preparation. SPALDI mass spectrometry can potentially become a routine and relatively sensitive analytical technique to perform fast sample screening in the negative ion mode.

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