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Small- and Large-sized Iron (II, III) Oxide Nanoparticles for Surface-assisted Laser Desorption/Ionization Mass Spectrometry of Small Biomolecules

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Abstract

Rationale: Common surface-assisted laser desorption/ionization (SALDI) surfaces are functionalized to improve mass spectrometry detection. Such surfaces are selective to certain group(s) of compounds. The application of universal and sensitive SALDI surfaces with appropriate size/surface area is paramount. In this study, two different sizes/surface areas of Fe₃O₄ are compared as SALDI surfaces.

Methods: For accurate surface area comparisons, the physical properties of the Fe₃O₄ nanoparticles used as SALDI surfaces were determined using scanning electron microscopy, X-ray diffractometry, and N₂ Brunauer-Emmet-Teller adsorption techniques. SALDI mass spectrometry (MS) data were acquired using a time-of-flight mass spectrometer operated in the linear mode and equipped with a 50-Hz pulsed nitrogen laser (at 337 nm). Small biomolecules (adenosine, glucose, sucrose, tryptophan, and tripeptide) and a real sample (human serum) were analyzed.

Results: The average size/specific surface area of the small-and large-sized Fe₃O₄ nanoparticles' SALDI surfaces were ~21 nm/~82 m²/g and ~39 nm/~38 m²/g, respectively. An overall ~2.0-fold enhancement in *signal-to-noise* ratios was observed for the analyzed biomolecules' ionic species in SALDI-MS using small-sized Fe₃O₄ in comparison to large-sized Fe₃O₄ nanoparticles. MS sensitivity from adenosine calibration curves (concentration between 0.05 and 10 mM) was ~2.0-fold higher for small-sized than large-sized Fe₃O₄ nanoparticles as SALDI surfaces.

Conclusions: We have shown that transition metal oxides such as Fe₃O₄ nanoparticles are suitable and efficient surfaces for SALDI time-of-flight MS analysis of small biomolecules. We observed improvement in *signal-to-noise* ratios and detection sensitivity for the analyzed samples from SALDI surfaces using small-sized (possessing larger surface area) than large-sized Fe₃O₄ nanoparticles.

Keywords

Iron (ii, iii) oxide; mass spectrometry; nanoparticles; surface-assisted laser desorption/ionization; transition metal oxide

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1. INTRODUCTION

Mass spectrometry (MS) has become a major analytical tool for the analysis of biomolecules including gas-phase molecules and large groups of organic and inorganic compounds.^{1–3} Matrix-assisted laser desorption/ionization (MALDI) is one of such important MS techniques that involves the use of organic matrices such as 2,5-dihydroxybenzoic acid (DHB), 3,5-dimethoxy-4-hydrocinnamic acid (sinapinic acid), and α -cyano-4-hydroxycinnamic acid (α -cyano CHCA) to enhance desorption/ionization of analytes for MS detection.^{4–6} The matrix absorbs UV light from the pulsed laser and transfers the absorbed energy to the analytes to generate ions with minimal fragmentation.^{5–6} Some common challenges in MALDI include: (i) matrix interferences at low-mass region ($<m/z$ of 500) because of the presence of matrix ions and/or its degradation products in the mass spectrum and (ii) high MS signal irreproducibility partly due to the difficulty of obtaining uniform/consistent analyte-matrix surface.⁷ To circumvent the matrix interference at lower m/z regions and signal irreproducibility associated with MALDI, surface-assisted laser desorption/ionization (SALDI) MS was introduced which involves the use of inorganic materials to enhance and facilitate reproducible desorption/ionization of various compounds (50 Da – 12 kDa).^{8–11} Since the first use of an inorganic matrix by Tanaka et al where 30 nm cobalt powder was suspended in glycerol for the MS detection of proteins and peptides,¹² several materials have been employed as surfaces/substrates for SALDI-MS.^{13–17}

Recent developments in SALDI-MS technique involve the design of surfaces/substrates for efficient detection of organic and inorganic compounds for applications including, but not limited to pharmaceutical,¹⁴ proteomics (peptides and proteins),¹⁵ biological,¹⁶ and forensics.¹⁷ Materials such as silicon-based (*e.g.*, desorption/ionization on silicon; DIOS),^{18–20} organic-based (*e.g.*, immobilized carbon nanotubes,²¹ activated carbon surface,²² oxidized graphitized carbon black,²³ metal-based/metal-oxide (*e.g.*, Au,²⁴ Pd,²⁵ Pt,^{26–27} HgSe,²⁸ titania nanotube arrays,²⁹ TiO₂,^{30–31} MgO,³² lithium-rich composite metal oxide³³), metal-organic frameworks,^{34–36} quantum dots,³⁷ and multilayer-coated (*e.g.*, two-layered amorphous silicon³⁸, MoS₂/TiO₂/SiNW surface³⁹) have been utilized as surfaces for SALDI with advantages including high absorption coefficient for laser energy and low matrix interferences. Some of these SALDI surfaces are modified or functionalized to increase selectivity or to serve as affinity probes for enhanced MS detection.¹³ For example, Stark et al utilized functionalized graphene-coated cobalt nanoparticles for analysis of angiotensin II and small molecule drugs, including phenformin hydrochloride, ranitidine hydrochloride, verapamil hydrochloride and other environmentally important compounds such as perfluorochlorophenol and perfluorobutanesulfonate.⁴⁰ The application of surfaces that are readily available, sensitive, and require less sample preparation for SALDI-MS analysis of a wide variety of biomolecules are also essential. Herein, we show that transition metal oxide nanoparticles of iron (ii, iii) oxide (Fe₃O₄) are efficient and sensitive surfaces for SALDI-MS analysis of small biomolecules.

Knowledge of the SALDI surface properties is important for adequate MS analysis of biomolecules using the SALDI technique. It is known that the efficiency of desorption/ionization during SALDI partly depends on the size, surface area, and surface properties of the SALDI surface.^{8,13,41} In addition, the extent of UV absorption by the SALDI surface,

energy transfer to analyte, and analyte-SALDI surface interaction contribute to analyte's signal intensity.^{8,15–17} Transition metal oxide nanoparticles have properties such as high surface area-to-volume ratios, high photo-absorption, and low heat capacities that promote the desorption/ionization of analytes.^{42–43} A few SALDI-MS results have reported the utilization of surface-modified transition metal oxide nanoparticles as SALDI surfaces/affinity probes including albumin-modified Fe₃O₄ magnetic nanoparticles,⁴⁴ Fe₃O₄/TiO₂ core/shell nanoparticles,⁴⁵ and silanized Fe₃O₄ particles⁴⁶ for small molecule drugs, phosphoproteins, and peptides/protein analyses, respectively. Exploring MS sensitivity and selectivity using “as is” transition metal oxide nanoparticles (*e.g.*, Fe₃O₄ nanoparticles synthesized *via* co-precipitation method from ferrous and ferric solutions in basic condition and requiring no further surface modifications) as SALDI surfaces for biomolecule analysis is valuable. Our recent work shows that transition metal oxide nanoparticles such as Fe₃O₄, Co₃O₄, TiO₂, and NiO are useful surfaces for SALDI-MS analysis of complex mixtures such as asphaltenes.⁴⁶

In this report, we show that variable sized transition metal oxide nanoparticles of Fe₃O₄ (requiring no surface modifications) are efficient and sensitive surfaces for SALDI-MS analysis of small biomolecules. We present experimental data comparing the use of small- and large-sized Fe₃O₄ nanoparticles as surfaces for SALDI to analyze various classes of biomolecules (particularly those with hydroxyl (OH) and amine (NH) functional groups) including adenosine, glucose, sucrose, tryptophan, a tripeptide consisting of methionine, alanine, and serine (Met-Ala-Ser) amino acids, and adenosine-spiked supernatant human serum. To ensure accurate surface area comparisons, the nanoparticles' physical properties were characterized using scanning electron microscopy (SEM), X-Ray diffractometry (XRD), and nitrogen gas (N₂) Brunauer-Emmet-Teller (BET) adsorption techniques. We utilized Fe₃O₄ nanoparticles as SALDI surfaces due to their high UV absorption properties and simple sample-nanoparticle preparation. We also demonstrated that desorption/ionization efficiencies of these transition metal oxide nanoparticles for SALDI partly depends on surface area. Higher *signal-to-noise* ratios (S/Ns) are reported for MS data obtained using small-sized Fe₃O₄ nanoparticles as SALDI surfaces due to larger surface area of the particles than the large-sized Fe₃O₄ nanoparticles.

2. EXPERIMENTAL

2.1. Materials.

Commercial Fe₃O₄ nanoparticles were obtained from US Research Nanomaterials, Inc., Houston, TX and they were used without any surface modifications. Standard acetonitrile (ACN, 99.9 %), and 0.1 % trifluoroacetic acid (TFA) solvents, adenosine, glucose, sucrose, tryptophan, Met-Ala-Ser, human serum, and ProteoMass™ Peptide MALDI-MS calibration kit were purchased from Sigma-Aldrich, St. Louis, MO. The solvents were used without further purification. Milli-Q (18 MΩ.m) water was prepared in-house using a Milli-Q water purification system, EMD Millipore, Billerica, MA.

2.2. Nanoparticle surface characterization.

Small- and large-sized Fe₃O₄ morphologies, average particle size distributions, and elemental compositions were obtained using a focused ion beam scanning electron microscope, Versa 3D (FIBSEM), FEI company, Hillsboro, OR operating under high vacuum at 30 kV and equipped with Everhart-Thornley detector. Data processing and particle size measurements (at 100000× and 120000× magnifications for small- and large-sized Fe₃O₄, respectively) were performed using AutoSlice and View G3 software. The energy-dispersive X-ray spectroscopy (EDS) spectra of the small- and large-sized Fe₃O₄ nanoparticles were obtained at 1000× and 1200× magnifications, respectively using the FIB-SEM instrument. XRD was performed on a Rigaku Miniflex II desktop X-Ray diffractometer (Rigaku, Woodlands, TX) equipped with a monochromator and Cu K α radiation ($\lambda = 1.540562 \text{ \AA}$, generated at 30 kV and 15 mA) source and operated in θ - 2θ mode. The specific surface area of the Fe₃O₄ nanoparticles were determined from an Autosorb iQ chemisorption gas analyzer (Quantachrome Instruments, Boynton Beach, FL) using N₂ and operating at an outgas duration, outgas temperature, and bath temperature of 16 h, 140 °C, and -196 °C, respectively under vacuum ($\sim 6.6 \times 10^{-5}$ Torr).

2.3. Sample, nanoparticle slurry, and MALDI matrix preparation.

Standard solutions consisting of adenosine, glucose, sucrose, tryptophan, and Met-Ala-Ser were prepared by dissolving appropriate amounts in a solvent mixture (50 % ACN, 25 % of 0.1 % TFA, and 25 % Milli-Q water) in separate sample vials. Adenosine concentrations of 0.05, 0.50, 1.00, 2.50, 5.00, and 10.00 mM were prepared for the adenosine calibration plots. An equal amount of human serum and ACN were placed in a glass vial for 15 min at 4 °C and subsequently centrifuged for 30 min at 5000 rpm using a Marathon 8K benchtop centrifuge (Fisher Scientific, now Thermo Fisher Scientific, Waltham, MA). The supernatant was collected and a portion of the supernatant was spiked with adenosine for SALDI-MS analysis based on the approach used by Hamdi et al where human serum was pretreated with acetonitrile, supernatant collected and diluted before spiking with glucose and glutathione (GSH) for SALDI-MS analysis.³⁸

Nanoparticle slurries were made by dissolving ~10 mg each of small- and large-sized Fe₃O₄ nanoparticles in 1 mL of a solvent mixture consisting of 50:50 ACN:Milli-Q water in separate sample vials. Nanoparticles-first, sample-first, dried droplet, and sandwich methods were employed for the sample-nanoparticle preparations (see Figure 2 for illustration). For the nanoparticles-first method, a 0.5- μ L nanoparticle slurry was deposited on the sample plate and vacuum dried for 5 min, then 0.5 μ L of analyte solution was layered on top of the vacuum dried nanoparticle slurry and vacuum dried again for 5 min. The sample-first method involves depositing a 0.5- μ L analyte solution on the sample plate, vacuum drying for 5 min, and subsequently layering 0.5 μ L nanoparticle slurry on top of the vacuum dried analyte solution and vacuum drying again for 5 min. For the dried droplet method, 1 μ L analyte-nanoparticle slurry mixture was deposited on the sample plate and vacuum dried for 5 min. The sandwich method was made by depositing a 0.5- μ L analyte solution between layers of 0.5 μ L nanoparticle slurries. The analyte solution and nanoparticle slurries layers were each separately vacuum dried for 5 min.

MALDI matrix was prepared by dissolving 10 mg of sinapinic acid in 1 mL of 50 % ACN (in 0.05 % TFA) solvent. To prepare the analyte for MALDI experiments, 10 μ L of Met-Ala-Ser was added to 10 μ L of the MALDI matrix (and vortexed), then 1 μ L of the analyte-MALDI matrix was deposited on the MALDI plate (and vacuum dried) for MS analysis.

2.4. Surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS).

A home-built TOF mass spectrometer operated in the linear mode and equipped with a 50-Hz pulsed nitrogen laser; 337 nm (~ 3.68 eV) (model VSL-337ND, Laser Science Inc., Franklin, MA) was used to obtain the SALDI-TOF mass spectra presented herein.⁴⁷ All acquired mass spectra were a sum of 50 single laser shots. The voltages on the ion source acceleration lenses were set to 20 kV and 17 kV. The detection voltage was set to -1.7 kV. The TOF mass spectrometer was calibrated using ions from peptide calibration standards consisting of Bradykinin fragment 1–7, Angiotensin II, P₁₄R, and ACTH fragment 18–19. The vacuum chamber pressure measured using an Agilent Varian XGS-600 ion gauge controller (Agilent Technologies, Santa Clara, CA) and a T-075-P type ionization gauge tube (Duniway Stockroom Corp., Fremont, CA) during the experiment was $\sim 3.0 \times 10^{-6}$ Torr. All data acquisition was controlled with a home-written LabVIEW 2014 program (National Instruments Corp., Austin, TX) running on a Windows 7 (Microsoft Corp., Redmond, WA) Dell desktop computer. All SALDI-MS data were recorded using a Tektronix DPO3054 digital oscilloscope (Tektronix, Inc., Beaverton, OR) and mass spectra were processed using Igor Pro 6 technical graphing and data analysis software (WaveMetrics, Inc. Portland, OR). Multiple SALDI mass spectra were acquired to check reproducibility.

3. RESULTS AND DISCUSSION

3.1. Surface properties of small- and large-sized Fe₃O₄ nanoparticles.

In this report, we demonstrated the use of commercial small- and large-sized Fe₃O₄ nanoparticles (without any surface modifications) as SALDI surfaces for MS analysis of small biomolecules with average particle size/surface area of 21 nm/81.98 m²/g and 39 nm/38.32 m²/g, respectively (see Figures S1 (a) and (b)). Small-sized Fe₃O₄ nanoparticles have larger surface area than the large-sized Fe₃O₄, thus we expect higher SALDI-MS signal intensities with the small-sized Fe₃O₄ than the large-sized Fe₃O₄ nanoparticles as SALDI surfaces. Also, the utilized Fe₃O₄ nanoparticles possess only Fe and O atoms as expected (see the EDS spectra in Figures S2 (a) and (b)) and they show diffraction peaks that are characteristics of pure Fe₃O₄ particles⁴⁸ without any surface impurities (see the XRD spectra in Figures S3 (a) and (b)).

3.2. SALDI-MS of Small Biomolecules.

The goal of these experiments is to demonstrate that simple and variable sized transition metal oxide nanoparticles (requiring no surface modifications) can be used successfully for SALDI-MS of biomolecules. To verify the MS background signal from the Fe₃O₄ nanoparticles surfaces, we performed SALDI experiments of the small- and large-sized Fe₃O₄ nanoparticles in the presence of analyte solvent. Figure 1 shows the representative SALDI mass spectra of (a) small- and (b) large-sized Fe₃O₄ nanoparticles for m/z of 150 – 750 covering the mass range for the biomolecules analyzed in this report. Small-sized Fe₃O₄

nanoparticles showed no background peaks while the large-sized Fe₃O₄ nanoparticles showed a background peak at m/z 232 (corresponding to the protonated Fe₃O₄ ionic species (*i.e.*, [Fe₃O₄+H]⁺).

For adequate signal enhancement comparisons between the small- and large-sized Fe₃O₄ nanoparticles as SALDI surfaces, we utilized four known sample-nanoparticle preparation methods^{16, 49} including nanoparticles-first, sample-first, dried droplet, and sandwich to ascertain which sample-nanoparticle method to employ for our studies. Shown in Figure 2 are the average S/Ns obtained for sodiated (*i.e.*, [M+Na]⁺) species observed in the SALDI-MS of 2 mM Met-Ala-Ser. The calculated average S/Ns ($\pm$ relative standard deviations or RSDs) for the nanoparticles-first, sample-first, dried droplet, and sandwich preparation methods are 36 ($\pm$ 14 %), 58 ($\pm$ 16 %), 120 ($\pm$ 9 %), and 28 ($\pm$ 15 %), respectively. Among the four sample-nanoparticle preparation methods, the dried droplet method gave the highest S/Ns for the Fe₃O₄ nanoparticles as SALDI surfaces. Additionally, lower RSD was obtained in the dried droplet method indicating better signal reproducibility than the other three sample-nanoparticle preparation methods. As a result of the higher S/N and reproducibility, we used the dried droplet method for subsequent SALDI-MS analysis of the biomolecules presented herein. In the next section, we show SALDI-MS results for five simple biomolecules, selected to demonstrate the effectiveness of Fe₃O₄ transition metal oxide nanoparticles as SALDI surfaces, for improved MS detection of carbohydrate and protein moieties with OH/NH functional groups.

Figure 3 shows the representative SALDI-TOF mass spectra of small carbohydrates including 2 mM each of glucose (a monosaccharide) and sucrose (a disaccharide) using small- and large-sized Fe₃O₄ nanoparticles as SALDI surfaces. In Figures 3(a) and (b), the major ionic species observed for glucose is the sodiated adduct (*i.e.*, [Glucose+Na]⁺ at m/z 203). The ionic species observed in SALDI-MS of glucose is consistent with previous reports using metal-organic frameworks and activated carbon as SALDI substrates.^{22, 35–36} Most carbohydrates form metal adducts during MS ionization due partly to their low proton affinities and high cation affinities.^{21–22,36} Similarly, the major ionic species observed during the SALDI-MS of sucrose is [M+Na]⁺ at m/z 365 –Figures 3(c) and (d). In addition, the sodiated and potassiated forms of the fragment ion (due to the loss of glucose obtained from the breakage of the glycosidic bond in the sucrose moiety) were also observed. Fu, *et al.* has reported similar results for the SALDI analysis of sucrose using metal organic frameworks.³⁶ Upon comparisons of the S/Ns for [M+Na]⁺ in SALDI of glucose and sucrose, higher S/Ns were observed for small-sized Fe₃O₄ (*i.e.*, 226 ($\pm$ 3 %) and 198 ($\pm$ 9 %)) than large-sized Fe₃O₄ (*i.e.*, 110 ($\pm$ 7 %) and 131 ($\pm$ 9 %)) nanoparticles as SALDI surfaces, respectively. For example, a ~2.0- and ~1.5-fold S/N enhancements were obtained for [M+Na]⁺ of glucose and sucrose, respectively using small-sized Fe₃O₄ nanoparticles as SALDI surfaces.

Furthermore, Figure 4 shows the representative SALDI-TOF mass spectra of 2 mM each of tryptophan (an amino acid) and Met-Ala-Ser (a tripeptide) using small- and large-sized Fe₃O₄ nanoparticles as SALDI surfaces. In Figure 3, the SALDI-MS analyses of tryptophan and Met-Ala-Ser produced majorly [M+Na]⁺ at m/z 227 and m/z 330, respectively. Also, other ionic species including [M+H]⁺ (at m/z 205 and m/z 308) and [M+K]⁺ (at m/z 243 and

m/z 346) were observed in the SALDI mass spectra of tryptophan and Met-Ala-Ser, respectively using small- and large-sized Fe_3O_4 nanoparticles as SALDI surfaces. It is common for amino acids/peptides to produce protonated ($[\text{M}+\text{H}]^+$) and metal adducts of sodium ($[\text{M}+\text{Na}]^+$) and potassium ions ($[\text{M}+\text{K}]^+$) in MS analysis due partly to high proton affinities of the analytes, solvent composition and pH, and presence of metal-seeking functional groups (*e.g.*, NH and OH) on the analyte.^{22, 24, 27, 49} Upon comparisons of the S/Ns in SALDI of tryptophan and Met-Ala-Ser, higher S/Ns for $[\text{M}+\text{Na}]^+$ were observed for small-sized Fe_3O_4 (*i.e.*, 131 (± 7 %) and 120 (± 9 %)) than large-sized Fe_3O_4 (*i.e.*, 90 (± 8 %) and 74 (± 6 %)) nanoparticles as SALDI surfaces, respectively. Also, a ~ 1.5 - and ~ 1.6 -fold S/N enhancement was obtained for $[\text{M}+\text{Na}]^+$ of tryptophan and Met-Ala-Ser, respectively using Fe_3O_4 nanoparticles as SALDI surfaces.

The observed higher S/Ns for the ionic species of glucose, sucrose, tryptophan, and Met-Ala-Ser using small-sized Fe_3O_4 than large-sized Fe_3O_4 nanoparticles as SALDI surfaces is potentially due to small-sized Fe_3O_4 nanoparticles' larger surface area than large-sized Fe_3O_4 . Large surface area increases the loading capacity for analytes and also promotes increased heat transfer (during SALDI) for analyte desorption.^{13–17} The SALDI-MS data presented in Figures 3 and 4 suggest that transition metal oxide nanoparticles with larger surface areas are suitable SALDI surfaces for MS analysis of small biomolecules. The calculated RSDs for the SALDI-MS of glucose, sucrose, tryptophan, and Met-Ala-Ser are <10 % (for an average of three experimental trials), indicating acceptable signal reproducibility for the SALDI-MS experiments.^{15–16, 17, 36}

3.3. SALDI-MS of a mixture of standard samples.

In addition to the SALDI-MS analysis of individual small biomolecules, we carried out MS analysis on a mixture of four standard samples to examine analytes' sensitivity and desorption/ionization efficiencies on the small- and large-sized Fe_3O_4 nanoparticles' surfaces. High desorption/ionization efficiencies of the analyte from the surfaces of the nanoparticles will invariably lead to improved MS detection.^{8, 14, 17} In the next section, we discuss SALDI-MS analysis of a mixture of glucose, sucrose, tryptophan, and Met-Ala-Ser using small- and large-sized Fe_3O_4 nanoparticles as SALDI surfaces.

In Figure 5, a mixture of ~ 5 mM each of glucose, sucrose, tryptophan, and Met-Ala-Ser were mixed with small- and large-sized Fe_3O_4 nanoparticles and analyzed using the SALDI-MS technique. Sodiated ionic species of the standard samples were the major species observed in the SALDI mass spectra presented in Figures 5(a) and (b). The sodiated species of sucrose (*i.e.*, $[\text{Sucrose}+\text{Na}]^+$) and Met-Ala-Ser (*i.e.*, $[\text{Met-Ala-Ser}+\text{Na}]^+$) showed comparable and higher signal intensities than the sodiated species of neat glucose (*i.e.*, $[\text{Glucose}+\text{Na}]^+$) and tryptophan (*i.e.*, $[\text{Tryptophan}+\text{Na}]^+$) in both mass spectra obtained using small- and large-sized Fe_3O_4 nanoparticles as SALDI surfaces. It is important to note that the intensity of $[\text{Glucose}+\text{Na}]^+$ in Figure 5 is a combination of the total ion intensities from the neat glucose molecule and the fragmented sodiated glucose ion from sucrose (both appearing at m/z 203). This was verified by performing SALDI-MS of a mixture of sucrose, tryptophan, and Met-Ala-Ser (with no addition of glucose), and we also observed the $[\text{Glucose}+\text{Na}]^+$ species (data not shown) under similar experimental conditions with the MS

data presented in Figure 5. As shown in previous work, the observed higher ion intensities for [Sucrose+Na]⁺ and [Met-Ala-Ser+Na]⁺ than [Tryptophan+Na]⁺ and [Glucose+Na]⁺ could be due to charge competition, differences in cation affinity, available ionizable sites, and desorption efficiencies of the analytes.^{5–6} Comparing the observed MS signal intensities of the mixed analytes in Figures 5(a) and (b) indicate an overall 1.5-fold signal enhancement for the SALDI signal obtained using small-sized Fe₃O₄ over large-sized Fe₃O₄ nanoparticles as SALDI surfaces. The signal enhancement corroborates the observed S/N improvements discussed in the previous section.

3.4. Adenosine calibration plots.

To compare the MS sensitivities of the SALDI technique using the small- and large-sized Fe₃O₄ nanoparticles as SALDI surfaces, we performed MS analysis using adenosine at concentrations ranging between 0.05 – 10.0 mM, corresponding to 50 pmol – 10 nmol per μ L of adenosine on the SALDI plate. Figure 6 shows the calibration plots for adenosine based on the total ion intensities for the [M+H]⁺ and [M+Na]⁺ adducts of adenosine observed at m/z 268 and m/z 290, respectively (see Figure 6 inset). In Figure 6, the calibration curves with a solid line (solid square symbol) and a dashed line (empty circle symbol) represent the calibration plots obtained using small- and large-sized Fe₃O₄ nanoparticles as SALDI surfaces, respectively. Each of the calibration point in Figure 6 is an average of three experimental trials (RSD <15 %). R² values of 0.999 and 0.998 were obtained for the linear plots and error bars are reported at the 95 % confidence level (CL) for $n = 3$ experimental trials using small- and large-sized Fe₃O₄ nanoparticles as SALDI surfaces, respectively. The ratio of the slopes (related to sensitivities of the transition metal oxide-nanoparticle SALDI technique) showed a ~2.0-fold enhancement using the small-sized Fe₃O₄ nanoparticles as SALDI surfaces over the large-sized Fe₃O₄ nanoparticles. This improved MS sensitivity observed with the small-sized Fe₃O₄ nanoparticles as SALDI surfaces corroborates the MS data in Figures 3 to 5, which further demonstrates the large sample loading capacity of the small-sized Fe₃O₄ nanoparticles (related to its large surface area). This sensitivity improvement advantage of the small-sized Fe₃O₄ metal oxide nanoparticles could be useful for characterizing analytes and small biomolecules in a complex sample matrix such as in biological samples. The sensitivity and selectivity of the SALDI analysis depend on several factors such as analyte physical properties, analyte-nanoparticle affinity or interaction, and the nanoparticle energy absorption coefficient for efficient desorption/ionization.^{8–10, 13}

A summary of the limit of detections (LODs) reported by several researchers for various analytes (such as glutathione, angiotensin I, insulin, cytochrome C, chymotrypsin, amino acids, peptides, fatty acids, synthetic polymers, small biomolecules) using different functionalized and un-functionalized nanomaterials including Au, carbon nanorods, CdTe, diamond, HgTe, lithium-rich composite metal oxide, MgO, Pt, quantum dots, Se, TiO₂, and ZnO are between 1 pM – 500 μ M.^{15, 24, 32, 37} In this work, the estimated LODs at S/N = 3 for adenosine using small- and large-sized Fe₃O₄ nanoparticles as SALDI surfaces are 10 μ M and 30 μ M, respectively. The estimated LOD obtained using the small-sized Fe₃O₄ nanoparticles suggest that lower LOD could be achieved with SALDI surface possessing lower particle size or higher surface area.

3.5. SALDI-MS of adenosine-spiked supernatant human serum.

The SALDI results discussed thus far demonstrates the proof of concept for the application of Fe_3O_4 transition metal oxide nanoparticles as SALDI surfaces. To further show the application of the transition metal oxide-nanoparticle SALDI technique, we extended the experiments to include a real biological matrix using human serum. Figure 7 shows the SALDI-TOF mass spectra of unspiked supernatant human serum and supernatant human serum spiked with ~2 mM adenosine. The ionic species obtained in the analyzed unspiked supernatant human serum (treated with ACN to remove abundant serum proteins) are seen in Figures 7(a) and (b). The supernatant human serum was spiked with adenosine to verify if the transition metal oxide-nanoparticle SALDI technique using small- and large-sized Fe_3O_4 nanoparticles as SALDI surfaces would be suitable for detecting biomolecules in the presence of a complex matrix as serum. In Figures 7(c) and (d), the sodiated adduct of adenosine (*i.e.*, $[\text{M}+\text{Na}]^+$) at m/z 290 was observed in the SALDI mass spectra of the adenosine-spiked supernatant human serum. A ~2.0-fold MS signal enhancement was obtained for the $[\text{M}+\text{Na}]^+$ species of adenosine using small-sized Fe_3O_4 than with large-sized Fe_3O_4 nanoparticles as SALDI surfaces; results which are consistent with MS data presented in Figures 3 to 5.

3.6. Comparison of the Fe_3O_4 Nanoparticle-SALDI Technique to MALDI.

We compared MS S/Ns and signal reproducibility observed with the SALDI technique using small-sized Fe_3O_4 nanoparticles as SALDI surfaces with MALDI. Figure 8 shows the representative MALDI mass spectrum of 2mM Met-Ala-Ser obtained using sinapinic acid as MALDI matrix. The major ionic species obtained in MALDI of Met-Ala-Ser is $[\text{M}+\text{H}]^+$ as opposed to $[\text{M}+\text{Na}]^+$ in SALDI mainly because of the abundant proton source in the MALDI matrix than in the SALDI slurry. A comparison of the MS intensity scale obtained for MALDI (Figure 8) and SALDI (Figure 4(c)) showed a ~2-fold increase in ion intensity for Met-Ala-Ser ionic species. The sum of the ion intensities ($\pm$ RSDs) for the $[\text{M}+\text{H}]^+$, $[\text{M}+\text{Na}]^+$, and $[\text{M}+\text{K}]^+$ of the Met-Ala-Ser peaks observed using the nanoparticles-first, sample-first, dried droplet, sandwich (on small-sized Fe_3O_4 nanoparticles), and MALDI (using sinapinic acid) are 5200 ($\pm 19\%$), 12000 ($\pm 25\%$), 25000 ($\pm 9\%$), 4300 ($\pm 22\%$), and 18000 ($\pm 15\%$), respectively. The total ion intensity and RSD results suggest that the dried droplet method of the Fe_3O_4 nanoparticles-SALDI technique is more sensitive and reproducible than MALDI. However, MALDI generated a better MS signal and reproducibility than the other three sample-nanoparticle methods using the small-sized Fe_3O_4 nanoparticles as SALDI surfaces for MS analysis of the tripeptide.

4. CONCLUSION

We reported the use of a simple, sensitive, and efficient small- and large-sized Fe_3O_4 nanoparticles as surfaces for SALDI-TOFMS of adenosine, glucose, sucrose, tryptophan, tripeptide (Met-Ala-Ser), and human serum. The surface characteristics of the transition metal oxide nanoparticles including morphology, average particle size, and specific surface area were obtained using SEM, XRD, and N_2 BET adsorption techniques. An overall 2.0-fold enhancement in the MS signal of the analyzed samples was observed using small-sized (larger surface area) Fe_3O_4 nanoparticles than large-sized

Fe₃O₄ nanoparticles as SALDI surfaces. These transition metal oxide-nanoparticle SALDI-MS experiments will be extended to explore detection capabilities for large biological molecules such as lipids, nucleic acids, and phosphoproteins. We will also correlate other transition metal oxide nanoparticle type and surface area differences to energy transfer to analytes for soft desorption/ionization processes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Representative background SALDI-TOF mass spectra of (a) small- and (b) large-sized Fe_3O_4 nanoparticles as SALDI surfaces.

Figure 2.

Average S/N s for $[M+Na]^+$ species of Met-Ala-Ser obtained using nanoparticles-first, sample-first, dried-droplet, and sandwich sample-nanoparticle preparation methods on Fe_3O_4 nanoparticles as SALDI surfaces. Error bars are relative standard deviation (%) for $n = 3$. Inset on each bar plot is the pictorial representation of each sample-nanoparticle preparation. ● Nanoparticle surface ● Sample

Figure 3.

SALDI-TOF mass spectra of 2 mM each of glucose and sucrose obtained using (a, c) small-sized and (b, d) large-sized Fe_3O_4 nanoparticles as SALDI surfaces. S/Ns are average of 3 experimental trials with relative standard deviation in parenthesis. *: $[\text{Fe}_3\text{O}_4+\text{H}]^+$

Figure 4.

SALDI-TOF mass spectra of 2 mM each of tryptophan and Met-Ala-Ser obtained using (a, c) small-sized and (b, d) large-sized Fe_3O_4 nanoparticles as SALDI surfaces. S/Ns are average of 3 experimental trials with relative standard deviation in parenthesis.. *:

Figure 5. SALDI-TOF mass spectra of a mixture of ~5 mM each of glucose, sucrose, tryptophan, and Met-Ala-Ser obtained using (a) small-sized and (b) large-sized Fe₃O₄ nanoparticles as SALDI surfaces. *: [Fe₃O₄+H]⁺

Figure 6.

Calibration plots of total ion intensities for $[M+H]^+$ (at m/z 268) and $[M+Na]^+$ (at m/z 290) as a function of adenosine concentration (0.05 – 10.0 mM) using small-sized Fe_3O_4 (solid square symbol) and large-sized Fe_3O_4 (empty circle symbol) nanoparticles as SALDI surfaces. Error bars are reported at the 95 % CL for average of 3 experimental trials. Inset is the representative SALDI mass spectrum of adenosine at 2.5 mM.

Figure 7.
SALDI-TOF mass spectra of unspiked and adenosine-spiked supernatant human serum obtained using (a, c) small- and (b, d) large-sized Fe_3O_4 nanoparticles as SALDI surfaces.

Figure 8.

MALDI-TOF mass spectrum of 2 mM Met-Ala-Ser obtained using sinapinic acid as MALDI matrix. *: sinapinic acid matrix.