

Laser desorption ionization of small molecules assisted by tungsten oxide and rhenium oxide particles

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Inorganic metal oxides have shown potential as matrices for assisting in laser desorption ionization with advantages over the aromatic acids typically used. Rhenium and tungsten oxides are attractive options due to their high work functions and relative chemical inertness. In this work, it is shown that ReO_3 and WO_3 , in microparticle (μP) powder forms, can efficiently facilitate ionization of various types of small molecules and provide minimized background contamination at analyte concentrations below 1 ng/ μL . This study shows that untreated inorganic WO_3 and ReO_3 particles are valid matrix options for detection of protonatable, radical, and precharged species under laser desorption ionization. Qualitatively, the WO_3 μP showed improved detection of apigenin, sodiated glucose, and precharged analyte choline, while the ReO_3 μP allowed better detection of protonated cocaine, quinuclidine, ametryn, and radical ions of polyaromatic hydrocarbons at detection levels as low as 50 pg/ μL . For thermometer ion survival yield experiments, it was also shown that the ReO_3 powder was significantly softer than α -cyano-4-hydroxycinnaminic acid. Furthermore, it provided higher intensities of cocaine and polyaromatic hydrocarbons, at laser flux values equal to those used with α -cyano-4-hydroxycinnaminic acid. Copyright © 2015 John Wiley & Sons, Ltd.

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Introduction

One of the most robust and rapid mass spectrometry (MS) ionization techniques for large molecule analysis is matrix-assisted laser desorption ionization (MALDI).^[1–3] This technique, developed in the work of Karas and Hillenkamp^[4,5] and Tanaka *et al.*,^[6] relies on the properties of matrix molecules coprecipitated on a plate with a target analyte, to absorb laser irradiation and subsequently desorb protonated molecules. Typically, the matrix is a small (100–300 Da) organic aromatic molecule with either proton donating or abstracting features. The conjugated aromatic property allows for efficient absorption of an incident UV laser beam to excite and transfer energy to the analyte. The process by which this occurs, however, is still under debate.^[7–12] By having an acidic or basic character, the matrix is believed to assist in the protonation or deprotonation of analytes in either positive or negative mode mass spectrometry, respectively. When using this technique for smaller analytes, one major difficulty that arises is that, because the MALDI matrix is so abundant and a strong absorber of laser radiation, spectra can be plagued by matrix-derived interferences in the low mass range. This is true for many common matrices, such as 2,4-dihydroxybenzoic acid, α -cyano-4-hydroxycinnaminic acid (CCA), and sinapinic acid, which exhibit protonated molecules at m/z 154, 189, and 224, respectively. Furthermore, these matrices also produce proton-bound dimers, fragments, and other interfering matrix peaks in the laser desorption ionization (LDI) process.

Several techniques have been developed in recent years to eliminate complications in the low mass range and produce high ionization efficiency of small target analytes without background peaks resulting from the chemical matrix.^[13,14] In lieu of standard organic matrices, many of these methods involve the use of inorganic

substrates or particles so that there is no 'contamination' of spectra by either a small molecule matrix or the inorganic substrate itself. One of the well-known techniques based on the use of inorganic substrates is desorption/ionization on porous silicon (DIOS), developed by Siuzdak *et al.*^[15,16] In this process, the analyte is deposited onto a layer of porous silicon, which is generated via galvanostatic etching. The UV energy absorbed by the silicon wafer is transferred to desorb the analyte.^[16] With this method, small molecules such as drugs and short peptides, can be detected at a femto/attomole level with highly reduced matrix-derived background in the mass spectra. Earlier work in surface-assisted LDI utilized graphite as the surface and was applied to the analysis of small peptides, identifying several peptides from a tryptic digest, with no detrimental effects of matrix-derived interference in most cases. This technique was, however, limited by the production of carbon ion clusters at higher laser power irradiation.^[14,17] In recent years, silicon nanowire surfaces have been optimized as a commercial substrate under the name of nano-assisted laser desorption ionization (NALDI).^[18–24] Results have been promising for a wide array of analyte types, and it has been proposed that NALDI is a more sensitive technique than other current alternative matrix LDI methods.^[20] Additionally, Siuzdak *et al.* have recently developed a technique known as nano-structure initiator mass spectrometry (NIMS).^[25–28] This technique

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involved the use of etched silicon wafers with fluorinated and hydrocarbon polymeric 'initiator' molecules co-embedded in the Si nanostructures with the ions of interest.

Nanoparticles and microparticles have also been reported as a stage for producing small molecule laser desorption/ionization spectra with reduced matrix interference.^[20–36] Previous work from our group in this area has focused on optimizing detection with 5–50-nm Si particles.^[29–31] Like the porous surface used in DIOS, the level of background from the nanoparticles of Si is low, and an enhanced ionization effect can be observed. Low-level detection of small drug molecules with almost no matrix peaks as interference has been readily achieved using this method. This technique, called silicon-nanoparticle-assisted LDI, is part of a continuing effort to use inorganic nanoparticles for matrix-free LDI of small molecule analytes. Silicon-nanoparticle-assisted LDI, along with the other alternative, successful LDI techniques (NALDI, DIOS, and NIMS), relies on the use of silicon as the main platform for ionization. In all of these techniques, however, chemical modification of the surface with a non-interacting species such as a perfluorinated compound is required to prevent oxidation and reduce the analyte–surface interactions that would suppress the analyte ion intensity in the mass spectra produced.

Other attempts have made use of metal oxides such as ZnO, MnO, CoO, WO₃, TiO₂, SnO₂, Fe₃O₄, CaO, and NiO; and bare metals such as Au, Ag, Sn, and Al.^[32–49] Clearly, the use of metal oxides as a base for LDI has been investigated for several compounds, and the Vorhees' research lab, responsible for studies using CaO and NiO, have coined this work as metal oxide laser ionization mass spectrometry (MOLI).^[47–49]

In addition to the ability to efficiently absorb UV incident laser radiation and then transfer that energy to desorb and ionize a target analyte without any unintended ions formed, there are other characteristics of inorganic particles to consider that make them successful in assisting LDI of a target analyte. One of these is the interaction of the inorganic surface with the analyte molecules themselves, i.e. determining whether the LDI environment induces unintended interactions between the analyte and the particle/surface. This would be an issue with any metal oxide that can covalently bind to functional groups of an analyte or that may induce strong analyte-particle electrostatic forces, reducing the intensity of signal during laser irradiation.^[50–52] Additionally, while the presence/transfer of protons is a necessity for many analyte molecules, the presence of strong electron donors or acceptors could also influence the ionization efficiency of small molecules, inducing electron transfers that could either enhance or decrease efficiency in the formation of cations and anions in an LDI environment.

In this work, we focused specifically on the performance of several high work function metal oxide particles, exploring their potential in MALDI of several small molecule analytes without any matrix signal interference. These included microparticle species of WO₃, WO₂, and ReO₃, in addition to nanoparticle size WO₃. In previous studies, the work functions of rhenium and tungsten metals in oxygen-rich environments were found to be very high, in the range of 6.4 and 6.0 eV, respectively,^[53,54] and surfaces of those metals provided promising platforms for ionization due to these work functions.^[55,56] These higher work functions are also conducive to photodetachment. Tungsten oxide microstructured surfaces have previously been explored for LDI and seem to be able to promote ionization and enhancement of both protonation and electron transfer events. Rhenium oxide has been studied for its charge transfer properties while doped in layers of organic light-emitting diodes.^[54,57,58] Using these metal oxide particles as bare metal surfaces for LDI,

we expect an enhancement in positive mode ionization without the need for any modification step to passivate the surface. The analytes chosen in this work encompass a range of compounds and include compounds with varying affinities for protonation, precharged bio-related species, and molecules that could form radical ions via photodetachment. They included the readily protonatable drugs cocaine and ametryn, quinuclidine, the natural product apigenin, the relatively difficult-to-protonate sugar glucose, the precharged species choline, and a standard mixture of polyaromatic hydrocarbons (PAHs) described in the succeeding texts, known to form radical ions upon laser desorption. Each metal oxide particle was compared with CCA as a standard MALDI matrix, whose wide use and performance with protonatable small-molecule species are well documented.^[59] Additionally, the fragmentation survival yields (SYs) of 3-methyl benzylpyridinium and cocaine were measured for all four matrices to assess the energy deposited upon LDI.

Experimental

WO₃ nanoparticles (nP) (<100 nm), WO₃ microparticles (μP) (<20 μm), WO₂ μP (<150 μm), and ReO₃ powders were purchased from Sigma-Aldrich (St. Louis, MO, USA). To make particle suspensions, 10 mg of each inorganic matrix was dispersed via sonication into 1 mL of nanopure H₂O purified in a Sartorius water purifier (18.3 Ω·cm). Standards of cocaine, ametryn, quinuclidine, apigenin, glucose, choline, and a PAH standard mix were purchased from Sigma-Aldrich, and each was serially diluted to final concentrations of 1 ng/μL in MeOH for cocaine, 1 ng/μL in H₂O for ametryn and quinuclidine, 50 ng/μL apigenin in H₂O, 100 ng/μL glucose in H₂O, and 500 pg/μL of PAH standard. Some of these solutions were further diluted in H₂O to lower concentrations as noted in the succeeding texts. To spot each sample, a 1 : 1 mixture of each inorganic particle solution and analyte solution was produced by mixing 4 μL of each and, after vortexing the suspension mixture, spotting 1 μL on a standard stainless steel MALDI plate.

The 3-methyl benzylpyridinium (BP) thermometer ions were synthesized using methods described previously.^[30] 3-methyl benzylchloride and pyridine, purchased from Sigma-Aldrich, were mixed together at a 1–1.2 equivalent ratio for 12 h at room temperature in dry acetonitrile (ACN) (Fischer Scientific, Waltham, MA, USA). The resulting BP salt was crashed out of the ACN solution with the addition of cold diethyl ether, filtered, and then washed with hexane. Stock solutions of 1 mg/mL in nanopure H₂O were made from the solid salt and diluted to a concentration of 100 ng/μL in H₂O, for spotting onto the MALDI plate.

Spectra were collected on a Bruker 'ultrafleXtreme' MALDI-MS (Billerica, MA, USA) operated in reflectron mode with a mass range set from *m/z* 20 to 900. This system uses a Smartbeam™ laser (Bruker), which combines both nitrogen and Nd: Yttrium aluminum garnet (YAG) technology to produce focused beams at 355 nm. The laser settings were maintained at a frequency of 20 Hz, and in each run, 500 shots were collected. The laser attenuator was set to an offset, or lower limit of half the full laser power, of 66% with a range or upper limit of 10% this value. Within this subrange, relative percentages of 20–45% of the flux were selected to produce all spectra. The laser was manually rastered along the area of the spot, in each case, to sample for any potential hot spots and test whether the edge or the middle of the spot was a better source of analyte ions.

Results

Ionization of small molecular ions with ReO_3 and WO_3 microparticles

Figures 1 and 2 show collections of the low molecular weight analytes under LDI analysis with ReO_3 powder (Fig. 1) and WO_3 μP (Fig. 2) as the matrix. The four analytes of Fig. 1, cocaine (Fig. 1(a)), quinuclidine (Fig. 1(b)), ametryn (Fig. 1(c)), and a PAH standard mixture (Fig. 1(d)) exhibited the best LDI with ReO_3 μP compared with the other inorganic matrices (WO_3 nP, WO_3 μP , and WO_2 μP), as well as CCA and a 'blank' spot (no matrix and only the stainless steel plate as a platform for LDI). Likewise, Fig. 2 shows the three analytes that were ionized most efficiently by WO_3 μP : choline (Fig. 2(a)), apigenin (Fig. 2(b)), and glucose (Fig. 2(c)). The criteria for selection included the overall intensity of the precursor at equivalent laser shots and flux, the suppression of background, the survivability of the precursor ion with respect to possible fragment ions in the case of cocaine, and the ability to detect the widest range of analytes in the standard mixture of PAHs.

In the case of the drug cocaine co-spotted with ReO_3 μP (Fig. 1(a)), the dominant peak in the spectrum was the m/z 304.1 precursor, protonated via the single tertiary amine. At a concentration of 500 pg/ μL , an additional distinct peak is observed at m/z 182, identified to be a cocaine fragment ion formed from the loss of benzoic acid, which has been commonly observed in both MALDI and electrospray-MS investigations of cocaine.^[60,61] Both the precursor cocaine structure and the observed fragment are shown in the inset of the spectrum. The other two protonated analytes, quinuclidine and pesticide

ametryn, were also spotted with ReO_3 , and as with cocaine, no significant ReO_3 background was observed in these spectra (Fig. 1(b) and (c), respectively). Some background was, however, originating from impurities on the MALDI plate, which could be detected at lower concentrations of the analyte. These impurities became more dominant as the analytes approached their limits of detection, which for cocaine was 5 pg/ μL and which for quinuclidine and ametryn were slightly higher at 10 pg/ μL .

In addition to the protonated analytes cocaine and ametryn, ReO_3 proved useful in detecting a mixture of PAHs, as shown in Fig. 1(c). The mixture was spotted at a concentration of 250 pg/ μL . As each of the PAH analytes could directly absorb the laser and therefore did not necessarily require any assistance in ionization, the concentration was lowered to determine the presence of chemical background with the inorganic particles studied. The four observed peaks at m/z 228, 252, 276, and 279 correspond to chrysene, benzo [k] fluoranthene, benzo [ghi] perylene, and dibenz [a,h] anthracene, respectively.

ReO_3 provided a much cleaner signal than CCA with better detection of the lower mass peaks (m/z 228 and 252) and matched the signal of LDI with only water mixed 1:1 with the PAH standard solution (SI Fig. 1). This demonstrates the advantage of ReO_3 to detect both protonated and self-ionizing radicals simultaneously, in mixtures, without any interference or matrix-derived ion suppression observed for a standard matrix like CCA. As a matrix-free sample of only radical ions can be detected (SI Fig. 1a) without background, so too can ReO_3 . Additionally, ReO_3 can detect several other protonatable species with no background at low concentration, (Fig. 1) while a matrix-free spotting of these compounds would show no

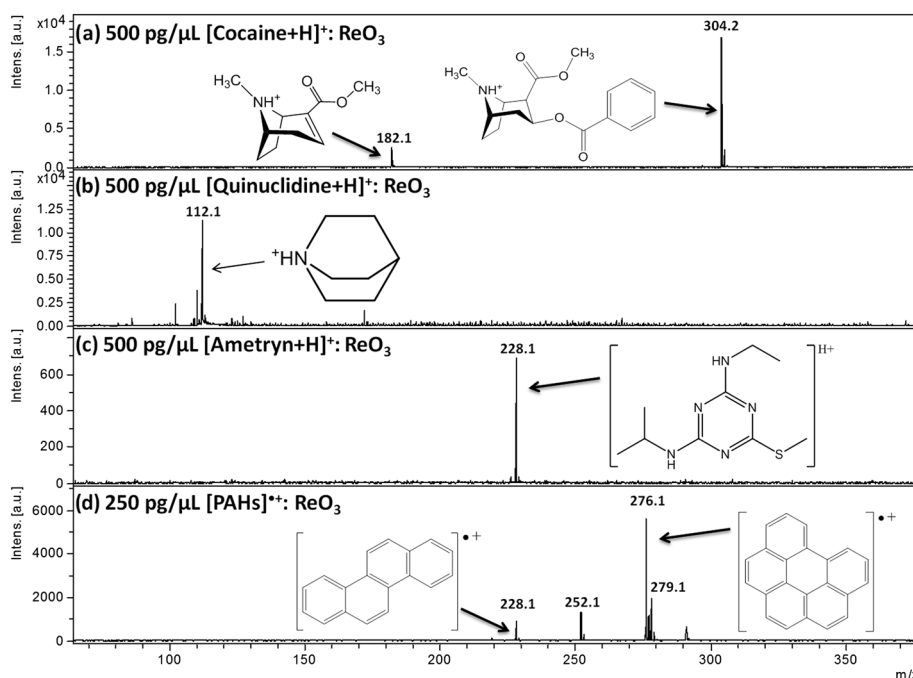


Figure 1. Performance of a rhenium oxide powder, ReO_3 , as an assisting ionization material for small molecule analytes in positive laser desorption ionization mode for (a) 500-pg/ μL protonated cocaine with its major fragment, (b) 500-pg/ μL protonated quinuclidine, (c) 500-pg/ μL protonated ametryn, and (d) radical ions of a polyaromatic hydrocarbon standard mixture with the four hydrocarbons: chrysene (m/z 228), benzo [k] fluoranthene (m/z 252), benzo [ghi] perylene (m/z 276), and dibenz [a,h] anthracene (m/z 279), at 250 pg/ μL each. Metal oxide particle suspensions were mixed 1:1 with each analyte (concentrations listed in the preceding texts after mixing) and 1 μL spotted on plate. Each case mentioned previously provides the highest and cleanest signal among all particles explored with each analyte.

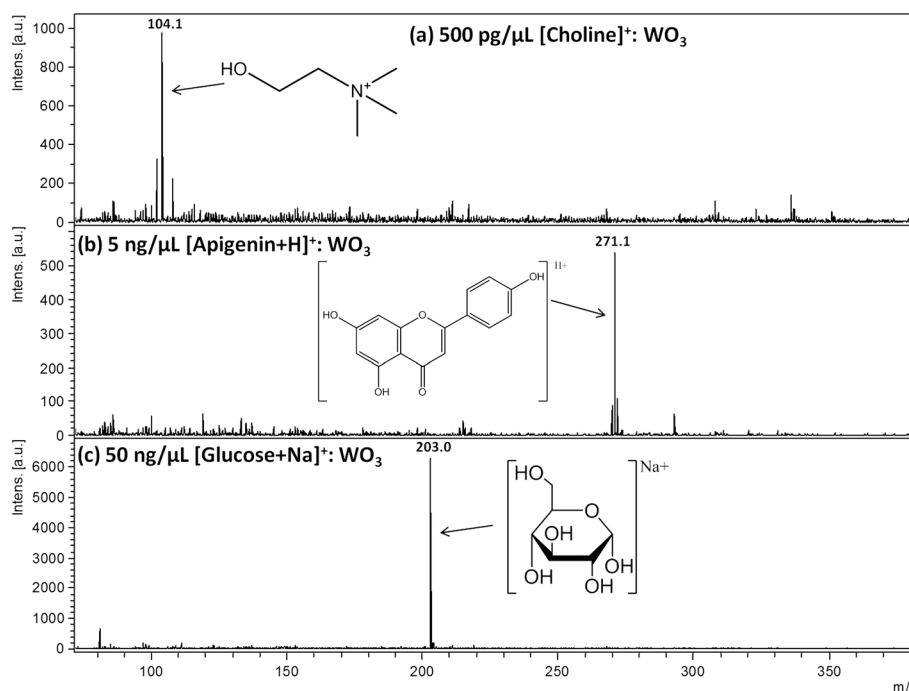


Figure 2. Performance of a tungsten oxide micropowder, WO_3 , as an assisting ionization material for small molecule analytes in positive laser desorption ionization mode for (a) 500 pg/ μL of the precharged choline analyte, (b) 5-ng/ μL protonated apigenin, and (c) 50-ng/ μL glucose with no additional source of sodium used.

detection. Therefore, mixtures that contain both radical ion species along with protonatable compounds should show simultaneous detection by using ReO_3 as the matrix. On the whole, ReO_3 μP provided the best LDI of the two protonated drugs as well as the radical ions of the PAH mixture. This may be due to the higher work function of rhenium trioxide compared with other particles, where both radical and protonated ions would survive the laser desorption and energy transfer processes with little chance for neutralization on the particle surface via an electron transfer from, or proton transfer to, the surface.

For the precharged quaternary amine choline, an essential nutrient, at m/z 104, WO_3 μP provided a strong and relatively isolated signal. Because choline does not require protonation, this analyte provided an interesting test for the nanoparticle and microparticle matrices. Despite not outperforming CCA in the intensity of analyte (SI Fig. 2), it did provide limited background interference, much lower than with the matrix derived peaks of CCA as well as a limit of detection (LOD) of 100 pg/ μL . Additionally, it may be argued that a matrix-free analysis of choline would result in strong LDI signal, because no protonation is required. However, SI Fig. 2(a) shows the analyte spotted with water alone on the stainless steel plate, and while this is a non-assisted LDI process, there is no m/z 104 signal observed in this spectrum, suggesting that analyte/surface interactions may hamper LDI of choline on the stainless steel surface.

The natural product apigenin, shown in Fig. 2(b), is an analyte that, unlike those analytes observed in Fig. 1, does not contain an amine group. For this reason, apigenin was more difficult to ionize than cocaine, ametryn, or quinuclidine and therefore required a higher concentration of 5 ng/ μL for adequate signal-to-noise. At this concentration, a limited amount of background is observed at a relatively low intensity and does not appear to

be matrix-derived. The LOD of apigenin was 250 pg/ μL for WO_3 μP and also showed a similar LOD of 300 pg/ μL with higher and well-isolated signal for ReO_3 , as seen in SI Fig. 3. In SI Fig. 3(a), ReO_3 μP provides higher signal than WO_3 μP , yet WO_3 μP shows fewer impurities. In SI Fig. 3(c), CCA showed a signal several times higher than both ReO_3 and WO_3 , but at this laser flux, an overwhelming amount of matrix background interferences was present.

The analyte requiring the highest concentration on spot (50 ng/ μL) was glucose, as shown in Fig. 2(c). Glucose is a very difficult analyte to ionize and was detected as the sodiated m/z 203 ion with minimal background with WO_3 μP as matrix. The signal at this concentration provided the cleanest spectrum, but this analyte could even be detected as low as 1 ng/ μL . Glucose is a difficult molecule to detect regardless of the ionization method chosen, and its detection by WO_3 μP is likely due, in part, to the 'edge' effect present for this matrix particle when spotted at a suspension concentration of 10 mg/mL. The 'edge' effect (enhancement of analyte signal at the edge) is believed to be a spatial concentration of the analyte on the boundary of the spot as it moves with the solvent upon spotting, which results in an increased intensity of analyte at this area. This effect, however, did not appear to be a factor for the other three inorganic oxide particles. At the same analyte concentration, the other oxides tested produced a substantially weaker signal in comparison with that obtained with WO_3 microparticles, either on the edge or within the boundaries of the spot. Furthermore, sodium iodide was added to all the matrices to determine if a possible impurity of Na^+ was contributing to the elevated intensity of $[\text{glucose} + \text{Na}]^+$ for WO_3 μP . Interestingly, with the addition of this salt at 100 ng/ μL , no improvement to the $[\text{glucose} + \text{Na}]^+$ signal was observed in any other matrix, while WO_3 μP still provided a clear m/z 203 peak.

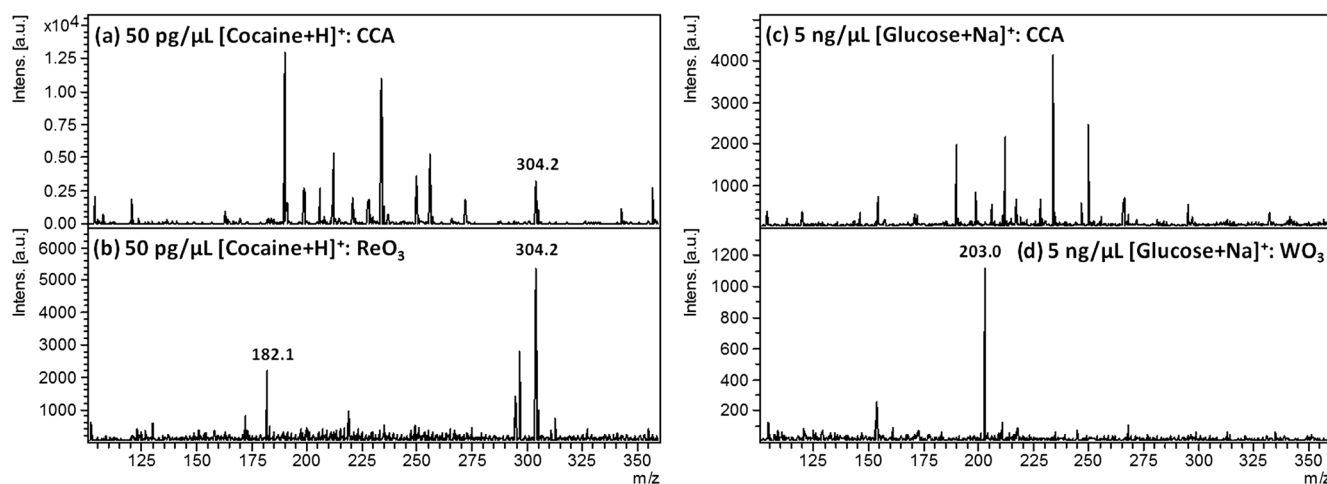


Figure 3. Sensitivity of cocaine and glucose assisted by the ReO_3 and WO_3 μP laser desorption ionizations versus matrix-assisted laser desorption ionization using α -cyano-4-hydroxycinnamic acid (CCA): (a) 50- $\mu\text{g}/\mu\text{L}$ cocaine with CCA as matrix, (b) 50- $\mu\text{g}/\mu\text{L}$ cocaine with ReO_3 as matrix, (c) 5- $\text{ng}/\mu\text{L}$ glucose with CCA as matrix, and (d) 5- $\text{ng}/\mu\text{L}$ glucose with WO_3 μP as matrix.

Figure 3 presents lowered concentrations of cocaine (Fig. 3(a) and (b)) and glucose (Fig. 3(c) and (d)), at 50 $\mu\text{g}/\mu\text{L}$ and 5 $\text{ng}/\mu\text{L}$, respectively. The performances of ReO_3 μP for cocaine and WO_3 μP for glucose are compared with CCA at low concentrations where analyte detection is crucially dependent on minimization of background. This set of spectra highlights the promising ability of these metal oxides as matrices for providing small molecule signal with limited background interference derived from the matrix. In the case of cocaine, Fig. 3(a) CCA, shows

the m/z 304 precursor peak at less than 20% the relative base peak intensity, while five CCA matrix-derived peaks dominate the spectrum. The sensitivity distinctions between CCA and WO_3 μP for sodiated glucose in m/z 203 are even more pronounced, where, for a concentration of 5 $\text{ng}/\mu\text{L}$, the sodiated glucose peak is not even detectable for CCA, but for WO_3 μP , it is the most intense peak with very few background peaks and only one peak at m/z 158 reaching about 25% relative intensity of the dominant sodiated glucose peak.

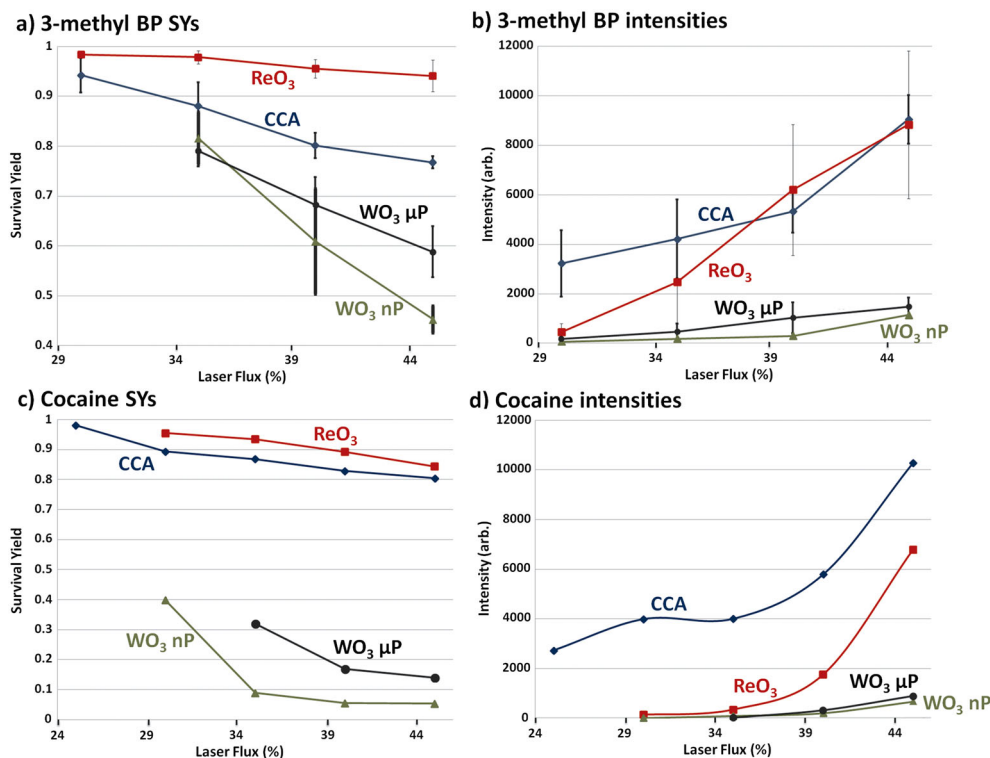


Figure 4. Plots of survival yields versus laser flux percentage of (a) 3-methyl benzylpyridinium (BP) ion at 50 $\text{ng}/\mu\text{L}$ and for (c) cocaine at 1 $\text{ng}/\mu\text{L}$, for α -cyano-4-hydroxycinnamic acid matrix (blue diamonds), ReO_3 μP (red squares), WO_3 μP (black circles), and WO_3 nP (green triangles). The overall intensity for 500 laser shots for (b) 3-methyl BP and (d) cocaine was shown (arbitrary units). In plots (a) and (b), error bars represent the standard deviation for an average of at least three runs taken at different days.

Survival yields of thermometer ions with each metal oxide matrices

In addition to the signal intensity and background, another interesting aspect of LDI is the amount of internal energy deposited into the analyte, reflecting on the 'softness' of the LDI method. Results of the nano-size *versus* micro-size particles are discussed in more detail in the succeeding texts with consideration of each particle's 'softness' on cocaine and the thermometer ion, 3-methyl BP.

Apart from the ReO_3 μP and WO_3 μP results shown in Figs 1–3, WO_3 nP and WO_2 μP were also studied, and Fig. 4 summarizes the LDI survivability of the thermometer ions 3-methyl BP (Fig. 4(a) and (b)) and cocaine (Fig. 4(c) and (d)) for three particle types. The results with the WO_2 microparticles exhibited relatively high background and some instability upon the increase of laser flux (not shown). This μP did not give improved results over standard CCA matrix and therefore was not included in the thermometer ion comparison. Both cocaine and 3-methyl BP have an easily formed fragment ion, and the relative ratio of the major fragment peak to the precursor was shown to vary significantly with the laser flux when either CCA or the three trioxide particles (ReO_3 , WO_3 μP , and WO_3 nP) were co-spotted. The SY is calculated as the peak intensity ratio between the precursor and the (precursor + fragments) and can be used to determine the overall energy deposition for the ionization method being used. In the case of 3-methyl BP, the precursor at m/z 184 is fragmented by the loss of neutral pyridine to form the m/z 105 fragment, where $\text{SY} = I [184] / (I [184] + I [105])$, so that the higher the SY, the less fragmentation and the 'softer' the resulting ionization of the sample being laser desorbed with the matrix. The same process was carried out for cocaine with the precursor at m/z 304 and the major fragment at m/z 182, as shown in Fig. 1(a).

The matrices shown in Fig. 4 include CCA in blue (diamonds), ReO_3 in red (squares), WO_3 μP in black (circles), and WO_3 nP in green (triangles). In Fig. 4(a), the SY of 3-methyl BP clearly demonstrates the 'softest' ionization coming from ReO_3 , with a small standard deviation between trials run on separate days. It is, however, evident that when the laser flux percentage was below 35%, SY with CCA improved dramatically and that below the lowest attenuation shown for both the 3-methyl BP (Fig. 4(a)) and cocaine (Fig. 4(c)), the CCA SY exceeds that of ReO_3 . As laser flux increases, the SY with ReO_3 μP is retained and is higher than CCA, for both the precharged 3-methyl BP and the protonated cocaine analytes, even though in the case of cocaine, the signal intensity is lower than with CCA.

Micro-sized *versus* nano-sized particle comparison

It is important to comment on the advantage of the WO_3 μP over the WO_3 nP. It might have been presumed that because the nano-particle is <100 nm, it would absorb better in the region of the 355-nm wavelength and impart a greater amount of energy into the analytes, increasing the amount of LDI relative to the micro-sized particle. This is not the case, however, and throughout the 3-methyl BP and cocaine intensity plots (Fig. 4(b) and (d), respectively), the WO_3 μP provides higher signal and better SY (i.e. 'softer') for all cases, except for the 35% laser flux of 3-methyl BP, where the two are equivalent within error. Particle analysis with scanning electron microscopy or transmission electron microscopy was not performed to determine whether aggregation was a factor, resulting in the differences observed between the two particles. Whether it is the case that the 355-nm laser flux absorbance did not impart enough energy to overcome the advantage in crystallization and

uniformity of the larger size particles or that the 'edge' effect, where more analyte concentrated in the edge of the spot, could have been better facilitated by the greater space between particles of microparticle size, is unclear. For the WO_3 μP and nP, the edge was a considerable factor in concentrating the analytes. For ReO_3 , there was a crystallization observed on the stainless steel plate but little effect in signal from focusing the laser on the edge of the spot. While WO_3 formed tight, well-defined circular spots with edges containing more of each compound, ReO_3 spots were more diffuse, as if the crystallization followed the path of the solution as it traveled left and right along the imperfections of the machined plate. More investigation is required to adequately determine the main cause for these observations and the differences in edge effects. It is, however, clear that there is a pronounced difference in 'edge' effect between the smaller nano-sized and larger micro-sized particles of WO_3 with a greater enhancement of signal for the micro-size and little advantage for nano-size.

Conclusions

The data reported here show that two commercially available and stable metal oxides, ReO_3 and WO_3 μP , without any treatment but rather procured and spotted as is, can be used as LDI matrices for various applications, providing clean signal for trace amounts of analytes in the low mass range for specific classes of compounds as summarized in SI Table 1. Not only did the ReO_3 and WO_3 microparticles show clean and significant ionization for the analytes chosen, rhenium oxide either outperformed or matched CCA in intensity and SY of cocaine and 3-methyl BP at comparable laser flux, while tungsten oxide was able to detect sodiated glucose where no such peaks could be seen for CCA. The ReO_3 microparticles had a particular advantage over CCA at higher laser fluxes because even as laser flux increased, the inorganic particle was not observed to produce any matrix-derived peaks cluttering the low mass regions, unlike CCA. It should be noted however, that in the case of lower laser fluence, CCA did provide higher analyte signal than ReO_3 , but always with more chemical noise.

In many cases in previous literature, particles used for assisting in LDI have required pretreatment with chemical modification, etching, or with the addition of proton sources to achieve the desired signal.^[29,30,48] From the experiments performed in direct comparison with the high work function particles in our lab, the lower work function metals stainless steel (blank matrix) and untreated Fe_3O_4 (SI Fig. 4) performed poorly. Only upon chemical modification, as has also been observed elsewhere in the literature,^[36] does iron oxide show any reasonable signal from spotted analytes. The clear advantage of these particles over other untreated compounds could be the result of a number of reasons, and further mechanistic study is required to better understand this behavior. Additionally, having shown that a promising set of untreated particles can be utilized for a variety of analytes, future study could focus on the use of these compounds for biologically relevant compounds and metabolites, as per their ability to produce limited matrix derived background and the advantage of the apparent concentrating 'edge' effect using WO_3 .

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