

A Green Chemistry Analysis of Metal Complexes by MALDI-TOF

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Table of Contents

Title	p. 1
Abstract	p. 3
Introduction	p. 4
Methodology	p. 7
Results	p. 10
Discussion	p. 12
Conclusion	p. 18
Citations	p. 24
Appendix	
Appendix A	p. 25
Appendix B	p. 26

Abstract

Matrix-Assisted Laser Desorption/Ionization (MALDI) is a type of ionization that is commonly used for the analysis of high molecular weight biological compounds, but has also been used for metal complex analysis. By combining the work presented in previously published literature on low molecular weight techniques and metal complexes, an analysis of different methods was evaluated. Spectra of transition metals chelated by three different ligands using different chelating atoms were acquired. To analyze the complexes, four different matrices were used with three different plating methods. In the evaluation of the different methods, the amount of solvent used was recorded and compared to a similar ionization technique, electrospray ionization (ESI). The experiment demonstrated that MALDI had the capability to ionize more complexes while using less solvent than ESI.

Introduction

Matrix-Assisted Laser Desorption/Ionization (MALDI) is a type of ionization that is often paired with a Time-of-Flight (TOF) mass analyzer to determine the mass of a given compound. MALDI-TOF works by firing a laser at a mixture of the analyte and a matrix. The matrix is an added substance that can absorb the laser intensity. The matrix subsequently decomposes, transferring the energy to ionize any of the compounds in the mixture. While some of the laser intensity is absorbed by the matrix, the remainder of the laser intensity creates a small explosion on the plate creating a gaseous plume of matrix and analyte species. Upon ionization and vaporization of the mixture, the ionized gaseous analyte is held in a chamber by a voltage potential for a given amount of time, “delay time”, to allow the vacuum to remove neutral species. After this delay time, the ions are released into the TOF chamber to be analyzed. In the plume, the ionized compounds can interact with each other to create some novel and unwanted species.

MALDI-TOF analysis performs well for the analysis of high molecular weight compounds, like proteins and polymers, but has trouble accurately quantifying low mass molecules¹. The reason that MALDI-TOF analysis is unable to accurately detect low molecular weight (LMW) species is a result of the ionization. In matrix-assisted ionization, a relatively high concentration of excited matrix molecules autoionize and decompose to generate dimeric and trimeric matrix species, water adducts and carbon dioxide adducts². As most matrices have molecular weights below 300 Dalton, the system becomes saturated in the low mass range². Due to ion saturation and matrix interference, compounds with a mass-to-charge ratio (m/z) less than 500 (or 1500 m/z in some cases) are not usually evaluated using MALDI-TOF¹. Previous work has found that manipulations in the matrix-to-analyte ratios, isotopic labeling, and “background

subtraction” are able to improve low mass range detection¹⁻². By using methods published in the literature, MALDI’s mass detection range can be expanded and compared to Electrospray Ionization (ESI) analysis.

ESI is comparable to MALDI in that both are soft ionization techniques that often work in tandem with TOF mass analysis³. Soft ionization focuses on ionizing an intact analyte. In contrast, hard ionization such as electron ionization, uses a higher voltage to promote fragmentation of the analyte. ESI and MALDI are on the surface similar, but differ in the formation of the ionized analyte and transportation of the ions into the TOF chamber⁴. Due to the similarities and differences, both techniques were used and compared due to their quick analysis of high molecular weight species. However, the two major differences between the systems are the ability to detect LMW species and the amount of solvent used.

The sample preparation and ionization of analytes requires different amounts of solvent for the analyte to be detected. MALDI requires the sample to be in a crystalline state before ionization, while ESI requires the analyte to be in solution before and during ionization³⁻⁴. A focus on the use of solvent and finding alternative, less wasteful, methods is an application of green chemistry⁵. Green chemistry focuses on the dangers of solvent buildup and potential cost savings of solvent reduction⁵. When assessing mass spectral techniques using a green chemistry approach, a comparison of qualitative, quantitative, and environmental parameters should be investigated. A method that has comparable qualitative and quantitative performance, yet drastically less solvent use, should be the preferred technique.

In the analysis of certain metal-containing compounds, the use of isotopic abundancies and understanding of the ligand and counter ion structures can be used for mass spectrometric analysis and identification of analyte-specific peaks. Certain transition metals, such as copper

and palladium, have unique isotopic patterns that deviate greatly from the typical organic isotopic pattern. The uniqueness of certain metals' isotopic abundances can allow for accurate identification of the analyte of interest from the background spectra. On the other hand, the type, charge and size of ligand or counter-ion that is associated in the metal complex can play a role in the strength of metal-ligand bonds⁶. In this case, neutral ligands and chelators (NH_3) will have no effect on the charge, while charged species (COO^- and Cl^-) can affect the overall charge on the metal species⁶. This is important when dealing with mass spectrometry as the analyte must be charged to be detected. Based on the stability of the metal-ligand bonds, the metal compound could have different ionization proficiencies. For example, a weak bond, such as cobalt chloride, would be more likely to break and form during mass spectrometry ionization. The way to determine the strength of the metal ligand interaction is to understand the Hard and Soft Acids and Bases (HSAB) theory⁶.

HSAB explains that the strength of a bond is built upon the characteristic of the bond between the acid and the base interaction. In this case, hard acids make stronger bonds with hard bases, while the same is true for soft acid-base interactions. Based on HSAB theory, the ionization of compounds will result in the breaking of weaker bonds – i.e. bonds which don't include an acid and base of similar "hardness" – while stronger bonds will stay intact throughout the detection process. In addition to HSAB theory, metal complexes can make bonds through electrostatic and covalent interactions. The electrostatic bonds are built on the interaction of two charged species and, once broken, will generate ionic species. The potential for these bonds to be easily broken could indicate that certain metal complexes are easily ionized and detected by mass spectrometry. Synthetic and metallic chemistry researchers have taken advantage of the minimal

solvent usage and soft ionization of MALDI to capitalize on these aspects of metal chemistry to characterize metal complexes⁷.

In recent years, research has been reported on low molecular weight detection mass spectrometry, organometallic MALDI-TOF mass spectrometry (MALDI-TOFMS), and MALDI-TOF metal complex analysis^{1,8}. A variety of different techniques have been tested for the detection of organic compounds below 500 m/z. This area of research is growing with more researchers considering solvent free and matrix manipulation techniques to reduce the background of the MALDI matrix¹. Where solvent-free options provide a niche for green chemistry techniques and low-solubility molecules, the methodology requires substantial amounts of analyte. In addition, MALDI-TOF and MALDI-TOF MS has been previously used to verify organometallic and metal complexes⁷⁻⁸. Previous research has used a variety of different combinations of matrices, metals and ligands. Most of the current literature uses MALDI as a secondary detection method with other mass spectrometric and analytical (NMR and IR) techniques⁷. The current literature is lacking an investigation of MALDI as an analytical method that can detect LMW metal containing compounds with green chemistry practices. In this thesis, I will be looking at the green chemistry application of the MALDI system as it applies to the detection of LMW species.

Methodology

Sample Preparation

Four matrices, 2-(4'-Hydroxybenzeneazo)benzoic acid (HABA), 1,4 di[2-(5-phenyloxazolyl)]benzene (POPOP), α -Cyano-4-hydroxycinnamic acid (HCCA), and 2,5-Dihydroxybenzoic Acid (DHBA) were acquired from Sigma-Aldrich and used as arrived for

each of the following method developments. Matrices were prepared and diluted to 100 and 10 millimolar solutions using the matrix specific solvent.

Seven metal chloride salts (copper, iron, manganese, cobalt, zinc, chromium, and nickel) were acquired and used as arrived from Sigma-Aldrich and Fischer. Two 100 millimolar and two 10 millimolar solutions for each of the seven salts were prepared using acetone or deionized water.

Three different metal-chelating agents (Calcein Blue⁹, Xantphos, and (3aS,3'aS,8aR,8'aR)-2,2'-Methylenebis[3a,8a-dihydro-8H-indeno[1,2-d]oxazole]) were acquired and used as arrived from Sigma-Aldrich. A 100 and 10 millimolar solution were made for each of the three chelating agents. The final calcein Blue solution was made by adding 10 μ l of a 1 molar solution of sodium bicarbonate to 990 μ l deionized (DI) water to the solution. A 1:1 solution of methanol and dichloromethane was prepared and used as the solvent for the Xantphos solution. The (3aS,3'aS,8aR,8'aR)-2,2'-Methylenebis[3a,8a-dihydro-8H-indeno[1,2-d]oxazole] (BOX) solution was prepared by using methanol as the solvent.

The metal-complex solutions were made by mixing 10:1, 1:1 and 1:10 molar ratios of metal salt solution and chelating ligand solution. The solutions were stored at room temperature and mixed in open air conditions. The metal chelate mixtures were made to 100 μ l volumes and millimolar concentrations. The mixtures were left to react for both a 2 hour and a 2-day period.

Other metal containing compounds were synthesized by labs within the Duke Chemistry Department. These compounds included metal complexes containing cobalt, manganese, gold, rubidium, and palladium. All sets of compounds have had verification of structure either by nuclear magnetic resonance (NMR) or elemental analysis.

For the MALDI analysis of the metal-containing compounds, three different plating methods were used to analyze the compounds as seen in **Figure 3**. The first method, the *Sandwich* method, includes the separate deposition of matrix and analyte on the sample plate. The first step includes the deposition of 0.75 μl of matrix solvent in a sample well and allowing it to dry. Subsequently, 1.0 μl of analyte was deposited in the sample well containing the deposited matrix. The second method is referred to as the *Mix* method and results in the mixing of 10 μl of both the analyte and matrix solution first, and then subsequently an aliquot of 1.0 μl of the new mixture is plated in a sample well and allowed to dry. The final method is referred to as the *No-matrix* method, which plates 1 μl of analyte without any matrix added.

Instrumentation Setup

The instrument used was an Applied Biosystems, Inc. Voyager-DE™ PRO with a 337-nm nitrogen laser. The instrument was set to have a 20 kV potential, 90 nsec delay time and run on reflectron mode to increase precision with mass analysis from ~50 to 1000 Daltons.

ESI-TOF was also used to validate the mass of the analyte. A 4-minute isobaric method was used on an Agilent Technologies, Inc. Liquid Chromatography Mass Spectrometry (LC/MS) instrument to acquire the high accuracy masses of the analyte. The solvent was a 1:1:1 ratio of acetone, isopropyl alcohol and dichloromethane. One μl of the sample was injected and was run through a LC system, but did not pass through a column. On this instrument, ions are observed from 100-1100 daltons.

Green Analysis

A complete analysis of the amount of solvent was determined by calculating the average solvent used per sample for each of the methodologies. The amount of solvent use

includes the solvent volume for the analysis and cleanup for each sample. For the MALDI analysis, the amount used for the plating of the analytes and for the washing of the plate were calculated and divided by the number of samples analyzed in the experiment. For the ESI analysis, the volume of solvent used during the analysis and purge were calculated and recorded for each sample tested.

Results

The spectra for metal complexes were observed for single, and some double charged, metal species with varying ligands attached that could be present in the mixture, water and chlorine. The amount of background ions was a qualitative and subjective observation of the number of peaks that were present in and around the theoretical metal ligand ion range. Minimal background was defined as the spectra that contained 10 or less non-analyte ions in the range of the calculated analyte mass-to-charge ratios. Spectra with more than 15 background peaks were determined to have excessive background peaks. All the ions found for each of the metals are shown in Table 1.

The *Mix* method was only able to consistently ionize metal species with HCCA as a matrix. With the HCCA, multiple ions were detected by this method with little background, as determined by the qualitative observation of the number of non-identifiable ion peaks. The *Mix* method was not as effective in ionization with the other matrices, but the *Mix* method did consistently show a peak for the protonated free ligand.

The *No-matrix* method did not show consistent ionization for any of the three ligands or metals. In one specific case, a nickel-BOX complex with little background showed a protonated

ligand, charged metal-ligand complex and a hydrated charged metal-ligand complex ions, as seen in Figure 4. The spectrum was not reproducible in future experimentations.

The *Sandwich* method consistently could induce ionization across the spectrum of metals and matrices. The method in tandem with the DHBA matrix could ionize with little background the highest number of complexes when compared to other matrices. POPOP provided minimal background with this method, but was incapable of consistently ionizing a majority of the complexes.

The ESI spectra did not show ions for the Xantphos and Calcein Blue metal complexes. The BOX ligand showed the protonated free ligand form in most the runs. As seen in Figure 5, a sample containing an iron-BOX complex showed peaks corresponding to a cobalt-BOX complex. In addition, all BOX samples showed a peak following a copper-like isotope pattern, but the mass of the peaks did not match calculated masses of the complex. When the ligands were run without metal present, Xantphos and BOX showed a protonated species, while Calcein Blue did not show any peaks.

An average of 237, 207, and 213 μl of solvent per sample were used for the *Sandwich*, *Mix* and *No-matrix* method, respectively. The standard deviations of the three methods vary slightly and correspond to 85, 77, and 61 μl of solvent per sample, respectively. The average and standard deviation of the three methods and overall average for the MALDI solvent usage is 223 μl and 78 μl of solvent per sample, respectively. The amount of solvent used by the ESI system was constant because of the programmed method and sample injection. The calculated solvent usage per sample was 3,375 μl of solvent per sample. The ESI system uses approximately 15 times more solvent than the MALDI methods by sample.

Discussion

Matrix comparison

Each of the different matrix compounds were chosen based on the ability to absorb at the 337 nm wavelength of light and due to their capability to reduce the amount of background ions produced during ionization. Based on the structures, POPOP was proposed to be the best of the matrices for the reduction of background. All the structures except POPOP contain carboxylic functional groups capable of decarboxylation with proton transfer to the analytes under high heat conditions (due to the high laser intensity). This results in the increase in background as the carbon dioxide can aid in the vaporization and aerosolization of different neutral small molecules. Throughout the experiment, POPOP demonstrated consistent ionization of the Schiff base cobalt and manganese compounds, but HCCA and DHBA provided better and more consistent ionization for the bulk of the LMW metal compounds tested. The ranking of the different matrices was a result of the ability to detect the metal complex, inertness to the analyte and a reduction of background ions.

To be considered a reliable matrix, the different matrices had to induce ionization across the spectrum of metals. In this case, DHBA was the best matrix for ionization of the most metal complexes through the *Sandwich* method, while HCCA was favored using the *Mix* method. The ability to ionize the metal complex was determined by the consistent presence of a dominant metal ion species within one matrix when compared to the others. DHBA could consistently ionize a metal ion peak, while other matrices provided greater fluctuations in intensity or complete lack of ionization. As will be described later, MALDI has the undesired characteristic of variable sampling and inconsistency. In this regard, DHBA was the most successful of the

matrices to consistently provide an intense ion peak. In the analysis of metals by the *Mix* method, HCCA provided more consistent peaks than the other matrices.

In the comparison of the different matrices regarding generation of background ions, HCCA and DHBA produced the least background. HCCA clearly produced the least background in the *Mix* method, while DHBA and HCCA present relatively equal amounts of background ions for the *Sandwich* method. POPOP was the preferred matrix for background reduction as only two common peaks were present during analysis. Unfortunately, POPOP was unable to ionize most of the metal complexes. On the other hand, HABA was preferred for ionizing some of the larger mass metal complexes (gold, iron and rhodium species), but produced the most background of any of the species. During the analysis of the metal spectrum, HABA consistently provided a range of ions with a m/z from 200 to 500. As this is the prime region of the LMW detection, HABA background ions overlapped and reduced the chance of verification that a peak was background or analyte.

An advantage of the *Sandwich* method is that the solubility of the matrix and analyte did not play a role in the ability to inoculate the matrix with analyte. The only limiting factor to the plating and analysis of the analytes was the time needed for either the matrix and analyte solvent to individually evaporate. On the other hand, the *Mix* method relies heavily on the solubility of the two solvents. The Schiff base metal complexes required non-polar solvents which did not mix well with the matrix solutions. In this case, the *Mix* method was not a viable method for plating.

The previous literature favored the use of DHBA and HCCA as matrices that can detect LMW species and metal containing compounds; however, the literature had little description of studies combining these two groups of chemicals. From the experiment done here, I have

concluded that HCCA and DHBA are indeed the preferred matrices for evaluation of LMW metal compounds using MALDI.

Ligand analysis

As seen in Figure 1, the three different ligands contain three different chelating atoms (nitrogen, oxygen and phosphorous). The different chelating atoms can change the strength of the bond of the chloride counter ion atoms¹⁰. Altering the strength of the chloride bond can potentially alter the ionization efficiency of the metal complex. By choosing a variety of different binding atoms, an understanding of the classes of metal complexes that can be ionized by the different methods could be better understood. The ionization of the metal complex is presumed to be done by the release of the chloride counterion to generate a metal ion species. The complexity of the ionization process and the validity of this hypothesis will be addressed later in the thesis, but for now the ionization process will be taken as done by the release of the chloride ion.

To test the range of chelators in the LMW range, the three ligands were chosen with relatively low molecular weights. Xantphos has a molecular mass above 500 Dalton, but it is a commonly used phosphorous chelator. As some biological and MALDI literature considers LMW to be less than 1000 Dalton, the 600 Dalton molecular weights of the Xantphos metal complexes were used for LMW analysis. Xantphos and the BOX ligands did not require the addition of a weak base to induce chelation, while the Calcein Blue ligand did require the deprotonation of the acidic protons. Due to the Lewis basicity of many nitrogen bases and hydroxide, bicarbonate was used to deprotonate Calcein Blue without introducing competing basic ligand. Each of the chelating ligands contain benzene backbone structures capable of absorbing light in the ultraviolet (UV) range. The wavelength of the laser is tuned to 337nm

corresponding to an absorbance for a ortho hydroxy group on a phenol which does not apply to any of the ligand backbone structures. This would imply that the possibility that the benzyl group of the chelating ligands absorbing a significant intensity of light is improbable.

Ionization mechanism

Two mechanisms for ionization by MALDI are firmly understood, the lucky survivor model and the gas protonation model. The lucky survivor model claims a pre-established state of the ions before the laser strikes, while the gas phase protonation model indicates that the ionization of the compound occurs in the gaseous state with a transfer of a proton from the excited matrix to the analyte¹¹. For the lucky survivor model the matrix aids in the plasma plume formation, while ionization occurs beforehand. In the ionization of the metal species a derivation of the lucky survivor model seems to be the observed form of ionization. For the case of metal complexes, the counter ion (chloride in most cases) seems to be dissociating, adding a positive charge to the metal complex and referred to as the dissociation mechanism. The evidence to support this ionization mechanism comes from the lack of a chloride isotope pattern and the molecular weight. Chlorine has a distinct 100:30 ratio between the chlorine-35 and chlorine-37 isotopes. Many of the peaks identified in the experimentation did not demonstrate this pattern, while also matching up with an unprotonated mass. In the dissociative mechanism, the matrix is not needed to protonate the complex and induce ionization. Where this is not the case in all circumstances, the chelating ligands still have the capability to readily pick up a proton (oxygen and nitrogen). In this case, the free chelating ligand and some metal complexes (Rh and Pd complexes) have demonstrated ionization through the two accepted models. MALDI has demonstrated the capability to ionize a variety of different compounds and depending on the structure of the complex one mechanism of ionization will be favored.

The dissociation method has a greater capability for reducing the amount of solvent required for the complete analysis. For dissociation, the matrix is not needed for the protonation and ionization of the analyte. This would imply that the *No-matrix* method would be the preferred method for the dissociation mechanism. This was not the case as only a few ions were detected by the *No-matrix* method. This is presumed to be the case due to the inability to absorb the 337nm wavelength of light. Without the significant energy to generate the plasma plume and gaseous species, minimal ions were detected. The *No-matrix* method had the greatest potential to reduce solvent use, but the method was more like a Laser Desorption Ionization technique without a tunable wavelength. In this case, the lower concentration matrix with respect to the analyte was accepted.

ESI analysis

The ESI analysis did not consistently present peaks for the different metal complexes. The only peaks that seemed to be consistently apparent were the free BOX ligand. When the ligands were run alone, the peak was seen clearly, but with the metal mixture the presence of the free ligand intensity decreased. This would imply that the ligand is being bound up with the metal complex, but none of the peaks from the Xantphos and Calcein Blue runs matched with possible metal complexes. Xantphos contains a free lone pair (on an ether group), to which an acidic proton could attach, but the instability of this bond would decrease the potential for ionization and detection. The Calcein Blue does have a free lone pair on the nitrogen atom, but unfortunately it is surrounded by two carboxylic acid groups. For the free Calcein Blue ligand, the possibility of deprotonation by the acid and self-protonation of the amide giving a net neutral complex. With the acidic oxygens bound to the metal, the nitrogen would be free to be protonated, and positively charged (without a competing negative charge), but this was not the

case. Xantphos and Calcein Blue complexes did not show promising peaks, but the BOX ligand did present possible metal complex ions. Most of the BOX ligand runs showed a few number of reoccurring peaks, corresponding to the free ligand, a potential copper complex (potential due to isotopic match, but single mass unit off) and a cobalt complex. From this observation, the aqueous metals from each of the previous runs, as the BOX ligand samples were the last to be tested by ESI, could have deposited along the tubing or junctions of the LC system. As a result, the free BOX ligands in solution were able to interact and form complexes with metals not present in the original sample. Though the ESI system had issues with depositing metals and ionization of complexes, it does give higher mass accuracy and less background.

Green Analysis

In the comparison between MALDI and ESI, MALDI methods used about 15 times less solvent. The dry and crystalline aspect of the MALDI method is what allows it to use so much less solvent. A caveat with this analysis is that the method used for the ESI analysis was a programmed method, where some ESI systems do have the ability to bypass column tubing and directly inject into the TOF system. The down side of direct injection of the analyte is that the solvent use per sample could fluctuate more than with the programmed system. Fluctuation in the solvent use was apparent in all the MALDI methods tested. Two major parameters dictated the amount of solvent use per sample, number of samples per plate and solubility of the analyte mixture. For all the MALDI experiments, 14 to 28 wells were used per plate. The number of wells used did play a key role in the amount of solvent that had to be used per plate. The solubility of the sample played a role in the amount of solvent required to remove and clean the plate after usage. Some analytes were more polar and were easy to remove with conventional solvents, methanol and acetone, while more non-polar samples required extra solvent to clean.

The solubility of the analyte created fluctuations between plates with a similar number of used wells.

Shortcomings of MALDI

MALDI-TOF in our previous unpublished experiments was shown to have performance issues with experimental error and uncertainty. Experimental error can be reduced using the reflectron mode; however, the reflectron mode increases the accuracy by increasing the flight distance¹². At the same time, increasing the distance is done by running ions into an ion mirror, which has the potential to cause decomposition of the ion species. Using reflectron mode has trade-offs by increasing selectivity, but decreasing sensitivity. For clarification, the accuracy of the linear mode is within plus or minus of one atomic mass unit, where the reflectron mode increased accuracy ten-fold. In addition, experiments done with MALDI-TOF have the potential for experimental variability as a result of non-uniform plating of the analyte on the Matrix sample plate¹³. The non-uniform distribution of the analyte is due to the crystallization of the sample on the MALDI plate; a homogenous solution in a test tube will not always produce evenly distributed crystals on the MALDI plate after plating.

Conclusion

In the comparison between the two soft ionization techniques, ESI and MALDI, each had their own advantages and disadvantages. The advantages observed by the MALDI system are the reduction of solvent usage, reduction of contamination and potential for alternative ionization. On the other hand, the advantages of ESI are the higher mass accuracy, lower mass detection and improved background reduction. The purpose of this analysis was to determine how effective and green MALDI was at the analysis of LMW metal complexes. In this case, MALDI is the

better technique for qualitative investigations into metal complexes. This was determined by the better reduction of solvent usage and increased number of ions detected when compared to ESI. MALDI consistently presents background ions below the 500 m/z region that has the possibility to overlap with analyte ions. MALDI is a useful method for the analysis of certain metal complexes in a qualitative sense. Our MALDI does not have the high mass accuracy and background removal that ESI does, but it is still capable of ionizing the analyte at hand. In this case MALDI can be used to verify that your analyte is present given that one knows the mass of the analyte. MALDI was not always able to accurately measure mass values with a comparable resolution to ESI. Though ESI could allow for increased accuracy, it was unable to ionize most of the complexes. It is important to point out that the MALDI-TOF system being used in this experimentation was manufactured in 2000. In the 17 years since the production of this system, the field of mass spectroscopy and MALDI systems have improved. MALDI has been set up in tandem with better TOF chambers and orbitrap systems, greatly improving the mass accuracy and precision of the instrument. The experimentation here demonstrates that the MALDI system has the potential to detect these complexes, where future research can demonstrate how accurate newer machines can be.

Given the complexes that were tested in this experiment, MALDI demonstrated an advantage in ionizing the metal complexes. The ESI system did not demonstrate any of the metal complexes for the Xantphos and Calcein Blue ligand in the metal spectrum tests. The BOX ligand was the only ligand to show ion peaks. In this case, MALDI, by ionizing most of the metal complexes for most of the metals, is shown to be a better technique for ionizing these complexes. The issues with MALDI will still inhibit the large-scale acceptance of the technique as a metal rather than biological analysis technique. As we have seen in the literature, MALDI

has been used for metal complexes and as the systems improve in accuracy so will the acceptance of the technique. The hope of this project is to demonstrate that MALDI can provide cheap, low solvent, reliable analytical verification for LMW metal complexes.

For Duke University and other research universities, this method can provide a quick analysis of metal complexes. As seen with the Xantphos and Calcein Blue ligands, some complexes do not ionize well by ESI, so MALDI is an alternative method to analyze complexes. As several Duke faculty do metallic chemistry, MALDI verification would make a viable alternative for the more expensive methods such as NMR, ESI and elemental analysis. This does not mean rely only on MALDI for verification, but it can act as a quick and cheap alternative to one of the other methods.

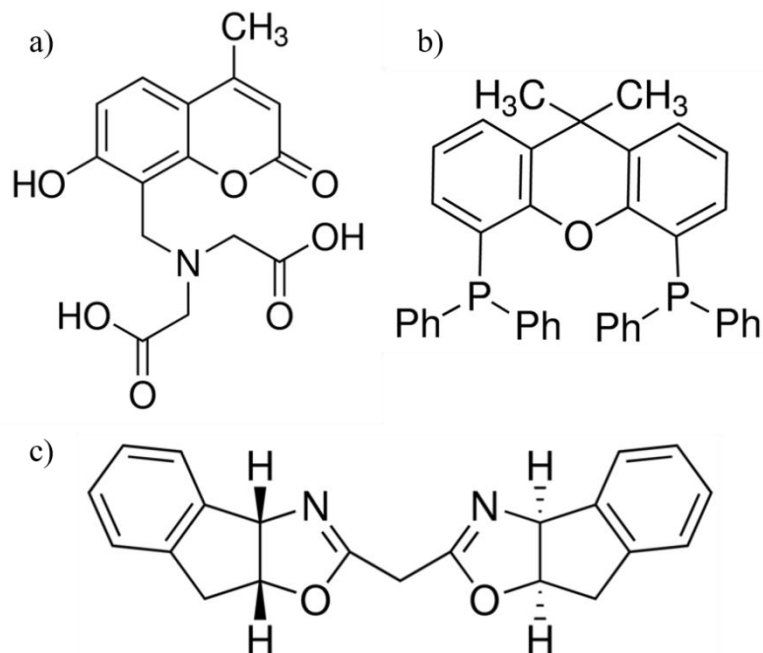


Figure 1. Each of the four ligands used for the metal spectrum analysis, a) Calcein Blue, b) Xantphos, c) (3a*S*,3'*a**S*,8a*R*,8'*a**R*)-2,2'-Methylenebis[3a,8a-dihydro-8H-indeno[1,2-d]oxazole] (BOX).

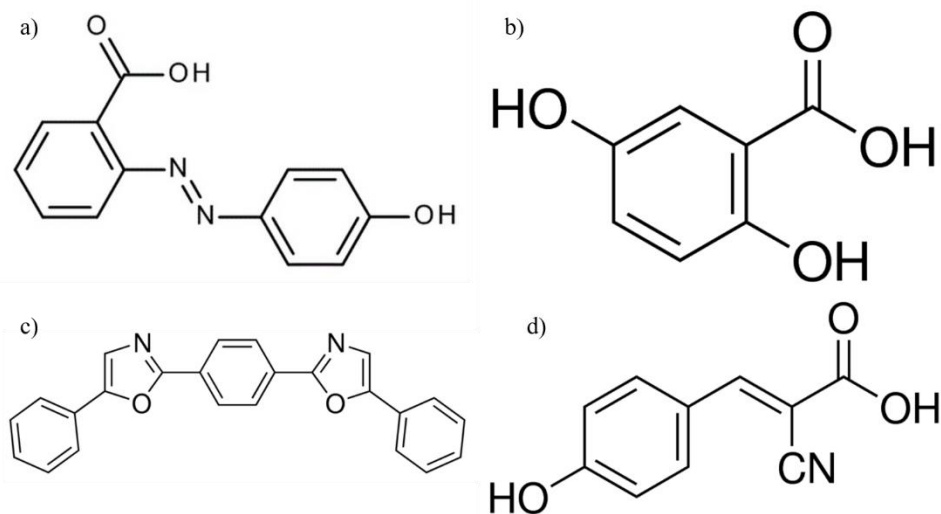


Figure 2. Each of the four matrices used for the MALDI analysis, a) 2-(4'-Hydroxybenzeneazo)benzoic acid (HABA), b) 2,5-Dihydroxybenzoic Acid (DHBA) c) 1,4 di[2-(5-phenyloxazolyl)]benzene (POPOP), and d) α -Cyano-4-hydroxycinnamic acid (HCCA).

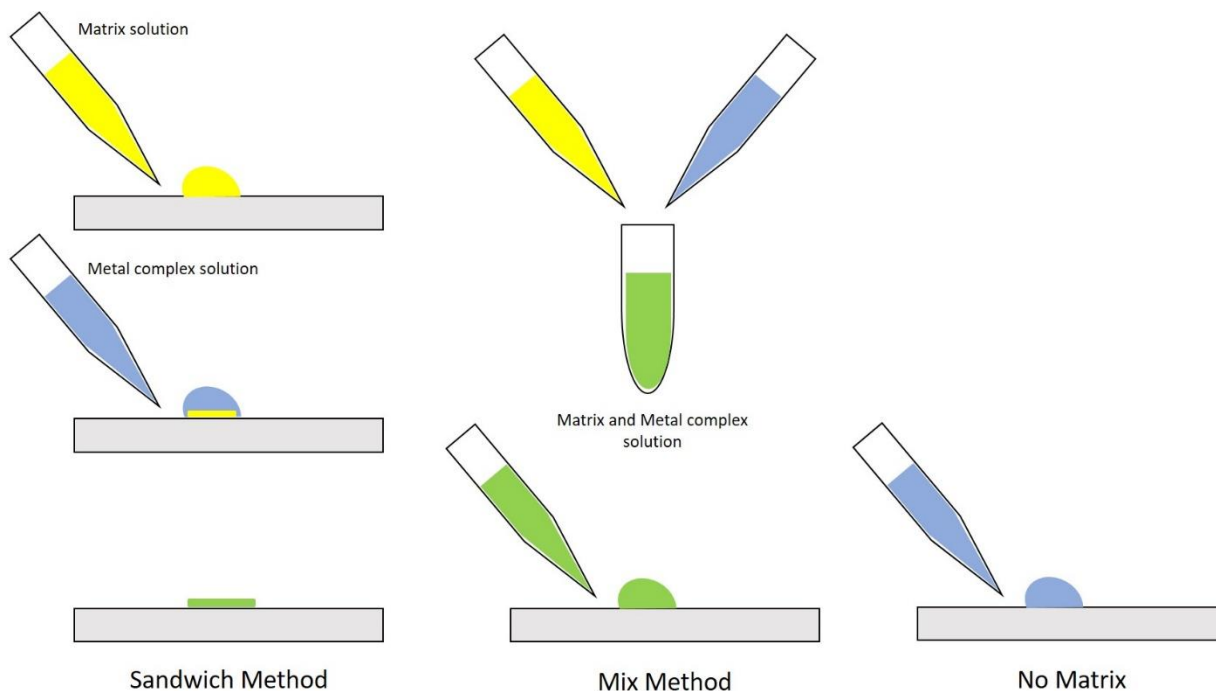


Figure 3. Each of the three MALDI plating methods.

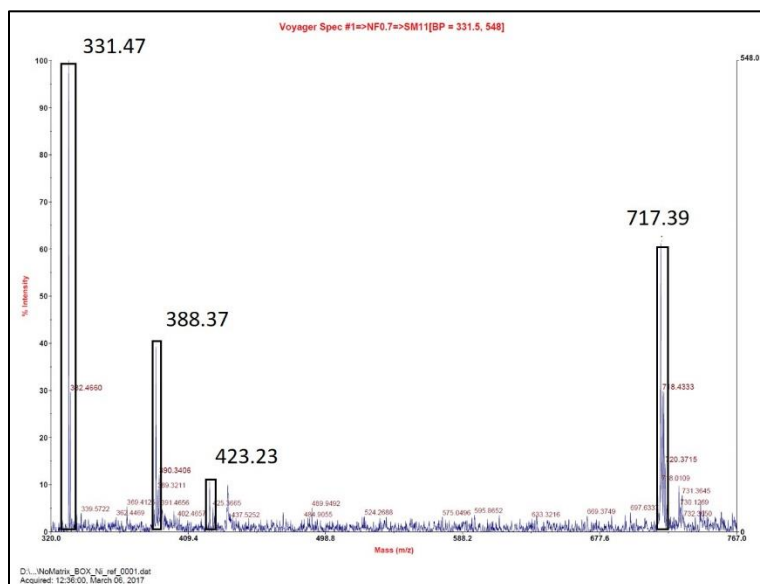


Figure 4. MALDI-TOF spectra of a nickel-BOX complex using the *No-matrix* method. The 331, 388, 423 and 717 peaks correspond to a protonated ligand, a nickel ligand complex, a hydrated nickel ligand, and a bi-ligand nickel complex respectively.

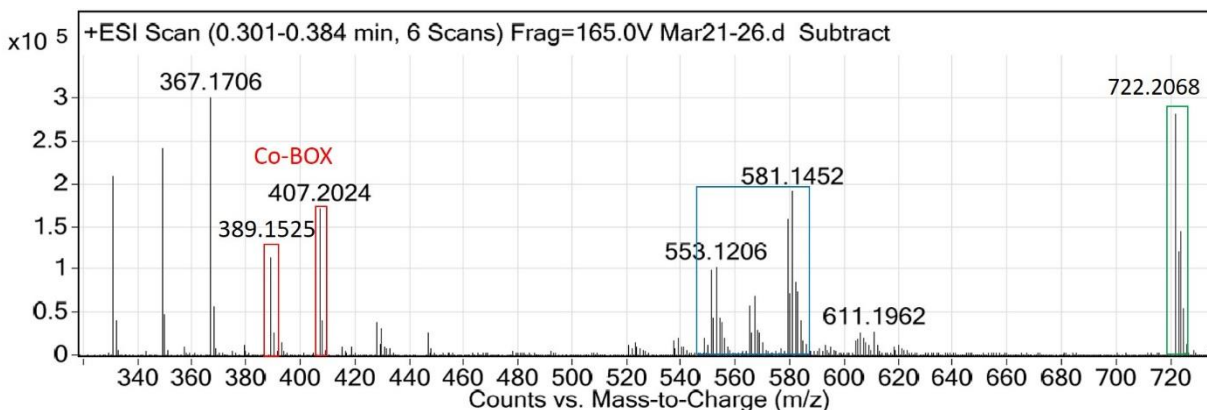


Figure 5. ESI spectra of an Iron-BOX complex. The red, blue and green box all highlight peaks that correspond to potential cobalt, iron and copper complexes.

Table 1. The types and prevalence of each ion were determined from all the MALDI-TOF spectra and tests. The table is separated by the ligand, metal and method used. The letters M, X, H, Cl, H₂O and O stand for the metal, ligand, chloride, water and oxygen components of the ionized complex, respectively. The number within the parentheses represents the reproducibility of the presence of the ion in repeated experimentation.

Calcein Blue									
Method	HCCA-Mix	HCCA-SW	DHBA-Mix	DHBA-SW	POPOP-Mix	POPOP-SW	HABA-Mix	HABA-SW	No-matrix
Zn	XH+, MX+	-	-	-	-	-	-	MXH+	-
Cr	XH+	-	-	MXH+*Cl	-	-	-	MX+	-
Co	XH+, MXH+	-	-	MXH+, XH+	-	-	-	XH+	-
Mn	XH+, MXH+	-	-	-	-	-	-	MXH+	-
Fe	XH+, MXH+	-	-	-	-	-	-	-	-
Ni	XH+, MXH+	-	-	-	-	-	-	-	-
Cu	XH+, MXH+	MXH+*H ₂ O	-	MXH*H ₂ O+(2), MXH*(H ₂ O) ₂ +	-	-	-	-	-
Xantphos									
Method	HCCA-Mix	HCCA-SW	DHBA-Mix	DHBA-SW	POPOP-Mix	POPOP-SW	HABA-Mix	HABA-SW	No-matrix
Zn	-	-	-	XH+	-	XH+	-	MX+	-
Cr	-	-	-	XH+	-	MXCl+	-	-	-
Co	-	MX*Cl+, MX*H ₂ O+	-	XH+, MX*H ₂ O+	-	MXCl ₂ +	-	-	-
Mn	-	-	-	XH+	-	-	-	-	-
Fe	-	-	-	XH+	-	MXCl*H ₂ O+	-	Oxides	Oxides
Ni	-	MX*H ₂ O+	-	-	-	-	-	XH+	-
Cu	-	MX+(2), XH+, Oxides	-	MX+, Oxides	-	-	-	XH+, MXO ₂ +	-
BOX									
Method	HCCA-Mix	HCCA-SW	DHBA-Mix	DHBA-SW	POPOP-Mix	POPOP-SW	HABA-Mix	HABA-SW	No-matrix
Zn	XH+	MX+	-	XH+	-	-	-	XH+	-
Cr	XH+	-	-	MXH+*H ₂ O	-	-	-	XH+	-
Co	XH+	MX+(2)	-	-	-	-	-	XH+, MX+	-
Mn	XH+	MXH+	-	-	-	-	-	XH+	-
Fe	XH+	MXH+	-	XH+(2), MX+	-	-	-	XH+, MX ₂ +	-
Ni	XH+	MX+, MXH+	-	XH+	-	-	-	XH+, MXCl+	XH+, MX+, MX*H ₂ O+
Cu	XH+, MX ₂ +	MX+(2), XH+	-	XH+, MX+	-	-	-	-	-

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Appendix A - Safety Considerations

Chemical	CAS	Health	Fire	Reactivity	Contact	Standard Procedure
Dichloromethane	75-09-2	3	1	1	2	RD
Acetone	67-64-1	1	4	2	1	RD
Methanol	67-56-1	3	3	1	3	RD
Nickel	7791-20-0	3	0	0	2	RD
Cobalt	7791-13-1	3	0	0	1	RD
Iron	13478-10-9	2	0	3	3	RD
Manganese	13446-34-9	3	0	3	4	RD
Chromium	10060-12-5	2	0	3	2	RD
Zinc	7646-85-7	1	0	3	2	RD
Copper	10125-13-0	2	0	2	1	RD
(3aS,3'aS,8aR,8'aR)-2,2'-Methylenebis[3a,8a-dihydro-8H-indeno[1,2-d]oxazole])	175166-49-1	2	0	0	1	RD
Xantphos	161265-03-8	0	0	0	0	RD
Calcein Blue	54375-47-2	2	0	0	1	RD

Appendix B - Acronyms

Term	Explanation
HABA	2-(4'-Hydroxybenzeneazo)benzoic acid - A type of MALDI matrix used for the ionization of the analyte from the sample plate to the mass analyzer
POPOP	1,4 di[2-(5-phenyloxazolyl)]benzene - A type of MALDI matrix used for the ionization of the analyte from the sample plate to the mass analyzer
HCCA	α-Cyano-4-hydroxycinnamic acid - A type of MALDI matrix used for the ionization of the analyte from the sample plate to the mass analyzer
DHBA	2,5-Dihydroxybenzoic Acid - A type of MALDI matrix used for the ionization of the analyte from the sample plate to the mass analyzer
LMW	Low Molecular Weight - In this experimentation this is taken as a molecule or complex that has an actual mass or m/z of less than 500 m/z. In some cases, this term can be extended to molecules below 1000 m/z.
MALDI	Matrix Assisted Laser Desorption/ Ionization - A soft ionization technique that works by firing a laser at a mixture of the analyte and a matrix. The matrix then allows for the ionization of the analyte to allow for mass analysis.
TOFMS	Time of Flight Mass Spectrometry - A tandem mass spectrometer that relies on the TOF to shuttle ions while a tandem mass analyzer is used to induce collisions and dissociations of the analyte. The dissociated and fractured molecules are later analyzed for their fragment ions.
ESI	Electrospray Ionization - A soft ionization technique that relies on the solvent to protonate the analyte before the spray eject microscopic droplets into the mass analyzer.
TOF	Time of Flight - A technique that relies on the size and of an ion to dictate the speed of flight after a potential is applied to give an initial force. The technique is used to separated and analyze the mass of a mixture of ions.
BOX	(3aS,3'aS,8aR,8'aR)-2,2'-Methylenebis[3a,8a-dihydro-8H-indeno[1,2-d]oxazole] - A chelating agent that primarily uses imine nitrogen groups to bind the metals. Classified as a low molecular weight ligand in this experiment due to its mass below 500 dalton.
LC/MS	Liquid Chromatography/ Mass Spectrometry - A analytical technique that uses a column to separate the sample mixture before being directly injected into a mass analyzer. Allows for a tandem molecular separation and mass analyzation.