MALDI Activity 2

MALDI-TOF Mass Spectrometry: Ionization

As discussed in MALDI Activity 1, MALDI-TOF MS is an acronym that stands for **M**atrix-**A**ssisted **L**aser **D**esorption **I**onization - **T**ime **o**f **F**light **M**ass **S**pectrometry. In figure 1 below, a schematic of a MALDI-TOF Mass Spectrometer and a MALDI mass spectrum are shown.

Model 1: Review of MALDI-TOF Mass Spectrometry

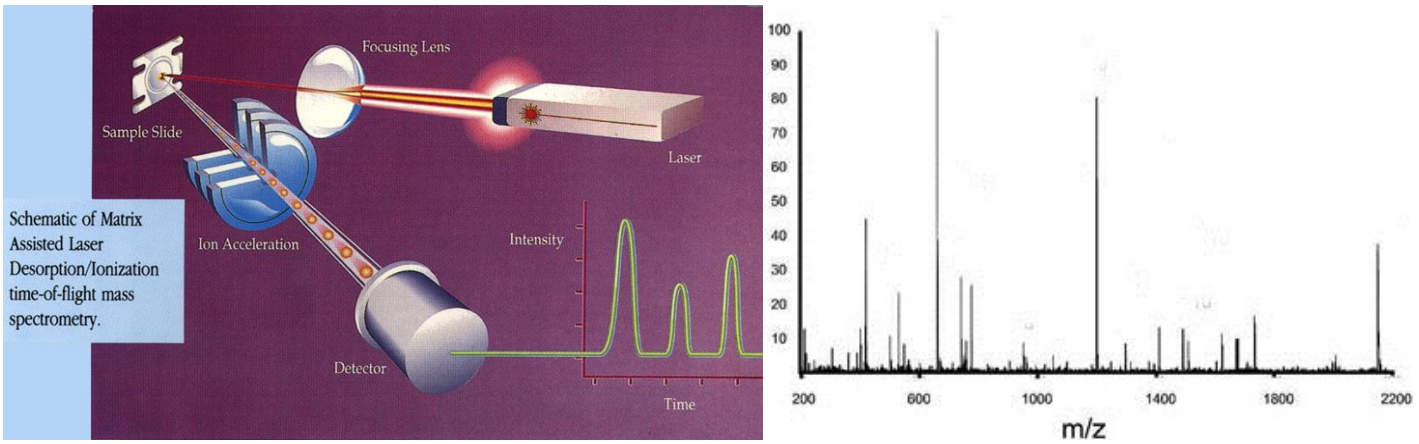


Figure 1: Schematic taken from http://www.protein.iastate.edu/maldi.html and data taken from reference 1.

Review of MALDI-TOF Sample Preparation and Data Collection:

A solution of the sample containing the molecule(s) of interest **(analytes)**, and a solution of the UV-absorbing matrix molecules are deposited at the same location on a stainless steel plate, yielding a solid sample mixture after evaporation of the solvent. Under vacuum in the instrument, a laser pulse is focused on the sample, causing the analyte and matrix molecules to vaporize (or **desorb**) and ionize. An Ion Accelerator accelerates the packet of ions down a flight tube toward the detector. The time of flight allows the determination of the mass-to-charge ratio (*m/z*) of each ion.

1. Choose the statement that **best describes** how a sample is introduced in MALDI-TOF mass spectrometry:

   A. A solution of the analyte is injected into a heated chamber in the spectrometer.
   B. The analyte molecule and the UV-absorbing matrix molecule are spotted at separated locations on a glass slide which is inserted into the spectrometer.
   C. The analyte molecule is spotted on a stainless steel plate which is inserted into the spectrometer.
   D. A pressurized solution of the analyte and UV-absorbing matrix molecule are atomized into an electron bombardment chamber in the spectrometer.
   E. Solutions of the analyte and UV-absorbing matrix molecules are spotted at the same location on a stainless steel plate and, after evaporation, the plate is inserted into the spectrometer.

2. In MALDI-MS, desorption and ionization are accomplished using **a laser pulse** or **a temperature gradient**. *(circle one)*

3. *Check you work*: Is your answer to question 1 consistent with figure 2?

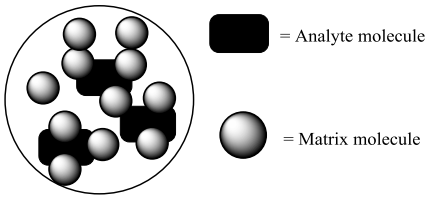
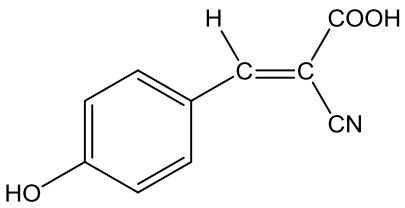


Figure 2: A properly prepared spot on a MALDI plate.

Model 2: Desorption and Ionization in MALDI-TOF using a Matrix

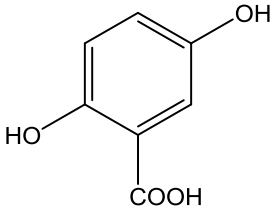
As shown in figure 2, the analyte molecule and **UV-absorbing matrix** molecule are mixed together at the same location on the stainless steel MALDI plate. Why is the matrix molecule necessary for MALDI-TOF mass spectrometry? The matrix is required to absorb the energy of a UV-laser pulse and enables **desorption** (release from the solid surface to the gas phase) and ionization of the analyte. The fact that the matrix must be able to absorb the laser energy, often at a **337 nm** wavelength, means not all molecules can function as matrix molecules, but it also means UV-Absorbing analytes can be analyzed without a matrix! Figure 3 shows three examples of compounds that can function as a matrix molecule. **Note the acronyms for each example**.

α-cyano-4,2-hydroxycinnamic acid (**CHCA**)



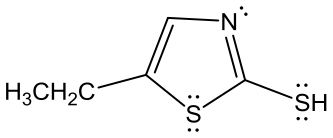
MW = 189.17

2,5-dihydroxybenzoic acid (**DHB**)



MW = 190.15

5-ethyl-2-mercaptothiazol (**EMT**)



MW = 145.00

Figure 3: Some commonly used matrix molecules.

4. Select the letter that best describes the hybridization state of the ring atoms for each molecule in figure 3.

CHCA: ____ **DHB**:____ **EMT**:____

A. Two sp^3 and the rest sp^2
B. All sp^3
C. All sp^2
D. One sp^3 and the rest sp^2

Check your work:

5. a) Is the answer you selected for each compound in question 4 the same? **Yes** or **No** (*Circle one*)

b) If you circled "Yes", move onto question 6. If you circled "No", consult further with your group or ask another group or your instructor for help.

Review of Aromaticity:

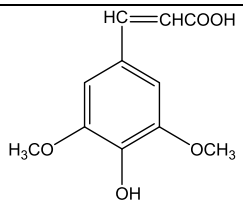
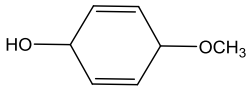
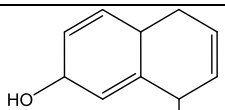
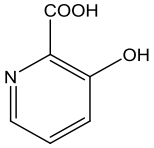
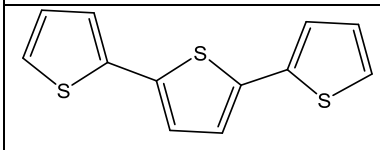
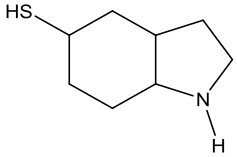
Recall that for a molecule to be aromatic it must have a π-system that:

1) is cyclic
2) has a continuous set of parallel p orbitals (no sp^3 hybridized atoms in the ring)
3) has 4n+2 π–electrons (where n = 0, 1, 2, 3, etc.; **Hückel's rule**)
4) has an overall planar geometry allowing the p-orbitals to be parallel.

6. Based upon your answers to questions 4 and 5 and the information above, what do the compounds in figure 3, which are all good matrix molecules, have in common?

7. Use your answer to question 6 to help fill in the third column of table 1 using **G** (good matrix molecule) or **P** (poor matrix molecule) for each potential matrix molecule.

Table 1

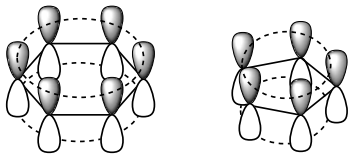
Structure	Name (abbreviation)	Good or Poor Matrix Molecule?
 MW = 224.07	3,5-Dimethoxy-4-hydroxy-cinnamic acid or Sinapinic Acid (SA)	
 MW = 126.15	3-hydroxy-6-methoxycyclohexa-1,4-diene (HMC)	
 MW = 173.21	7-hydroxy-1,4,4a,7-tetrahydronaphthalene-1-carbonitrile (HTC)	
 MW = 139.11	3-Hydroxypicolinic acid (3HPA)	
 MW = 248.39	2,2':5',2''-Terthiophene (2252T)	
	octahydro-1*H*-indole-5-thiol (OIT)	

Check your work:

8. a) Does your table have 3 "**G**" molecules and 3 "**P**" molecules? **Yes** or **No** (*Circle one*)

   b) If you circled "Yes", move on to question 9. If you circled "No", consult further with your group or ask another group or your instructor for help.

9. Based on your answer to question 7, which answer best describes how the diagram below relates to the good matrix molecules in table 3 (and also the compounds in figure 3)?



A. They are both black and white drawings.
B. They both represent rings made only of carbon atoms.
C. The diagram shows lowest energy molecular orbitals for aromatic rings and table 3 shows good matrix molecules that contain aromatic rings.
D. The diagram shows molecular orbitals with out-of-phase interactions, just like the good matrix molecules in table 3.

Molecular Orbital Theory and MALDI Matrix Molecules

Figure 4 illustrates molecular orbital diagrams of ethene, benzene and the matrix molecule **CHCA**. The Highest Occupied Molecular Orbital (**HOMO**) and the Lowest Unoccupied Molecular Orbital (**LUMO**) in each diagram are labeled. The double-headed arrows in figure 4 represent the energy gap (ΔE) between the **HOMO** and **LUMO** for each molecule. When energy of an appropriate wavelength is absorbed by a molecule it will often cause an electron to be promoted from the HOMO molecular orbital to the LUMO molecular orbital of the molecule. This usually corresponds to the wavelength of light that is most strongly absorbed, denoted lambda-max (λ_{max}).

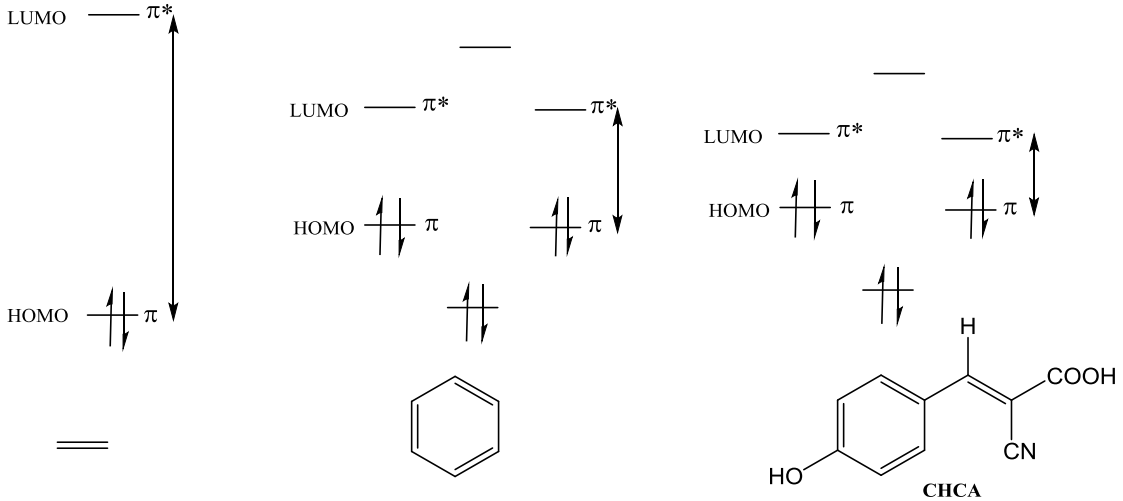


Figure 4: Molecular orbital energy diagrams that show the **HOMO**/**LUMO** gap and show the energy that corresponds to the wavelength of maximum absorbance (λ_{max}) using double-headed arrows (⟷).

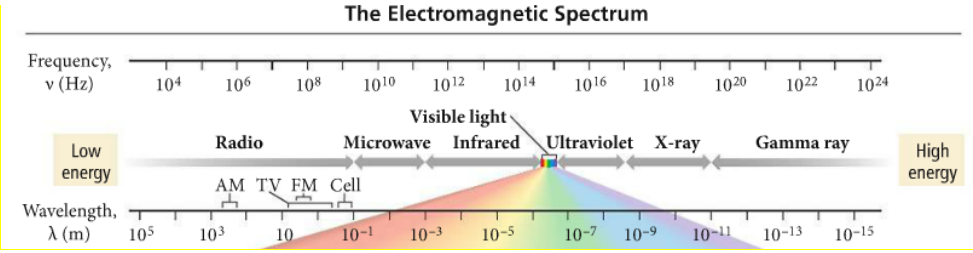


Figure 5: The electromagnetic spectrum. *(Taken from Tro's Principles of Chemistry: A Molecular Approach, 2nd Ed)*.

10. Recall that $\Delta E = \frac{hc}{\lambda}$ where λ = wavelength, **c** is the speed of light and **h** = Planck's constant. Using the molecular orbital diagrams in figure 4 above, match the wavelength of maximum absorption (λ_{max}) to the molecules from figure 4 below.

Possible wavelengths: 204 nm 337 nm 171 nm

CHCA

λ_{max} = _____ _____ _____

Check your work:

11. a) Do your values increase from left to right? **Yes** or **No** (*Circle one*)

b) If you circled "Yes", move on to question 12. If you circled "No", consult further with your group or ask another group or your instructor for help.

12. Based on question 10, why is CHCA a good matrix molecule while the aromatic benzene ring is not? *(You may want to review the introduction to Model 2 on page 2 to help answer this question).*

Additional Information for Matrix Molecules and Laser Desorption

The most important feature of a matrix molecule is that it should absorb light at or close to 337 nm. However, there are other properties that are important as well. Good matrix molecules should:

- have appropriate molecular weight to allow for easy vaporization with laser ablation.
- have high enough boiling points so that they don't evaporate before laser ablation.
- most often have acidic functional groups to provide H^+ ions to the analyte molecule.
- (often) have polar functional groups as solubility in aqueous solution is desirable.

Model 3: Ion Formation in MALDI-MS

When the analyte-matrix molecule mixture is irradiated with laser light of 337 nm, the matrix molecule absorbs the energy and is "desorbed" (vaporized) from the surface of the steel plate. Many of these matrix molecules become ionized during this desorption process. Along with the matrix, analyte molecules are also carried into the gas phase. **In positive ionization mode**, m/z signals come from the ionized acidic matrix molecules transferring a proton, or another available cation in the sample such as Na^+ or K^+, to the analyte molecules causing many of them to become positively charged.

When the analyte molecule adducts a hydrogen ion (H^+), it forms what is referred to as the $[M+H]^+$ ion, called the protonated molecular ion shown in equation 1. Equation 2 shows the $[M+2H]^{2+}$ ion that can also form.

$$M + H^+ \rightarrow [M + H]^+ \quad \text{(eqn. 1)}$$

$$M + 2H^+ \rightarrow [M + 2H]^{2+} \quad \text{(eqn. 2)}$$

Similarly, the result of sodium ion (Na^+) adduction is referred to as the $[M+Na]^+$, or sodiated molecular ion. Adduction of a potassium ion (K^+) is called the $[M + K]^+$, or potassiated molecular ion.

$$M + Na^+ \rightarrow [M + Na]^+ \quad \text{(eqn. 3)}$$

$$M + K^+ \rightarrow [M + K]^+ \quad \text{(eqn. 4)}$$

Theoretically, calcium and other common cation adducts could be formed too, but H^+, Na^+ and K^+ ions are the most abundant in a biological sample.

In **negative ionization mode,** instead of the hydrogen ion attaching to the analyte molecule as occurs in positive ion mode, *m/z* signals come from the basic matrix molecules accepting one or more hydrogen ions **from** the analyte molecules allowing the analyte molecules to take on a negative charge, as shown in equations 5 and 6.[2]

$$M + \text{matrix} \rightarrow [M - H]^- + \text{Hmatrix}^+ \text{ (eqn. 5)}$$

$$M + \text{matrix} \rightarrow [M - 2H]^{2-} + H_2\text{matrix}^{2+} \text{ (eqn. 6)}$$

Negative ionization mode requires that the polarity on the ion accelerator be inverted compared to **positive ionization mode**. **Note**: Although the charge is negative, the charge state, z, is given as an absolute value. Therefore *m/z* will **never** have a negative value.

1
H
1.008
3
Li
6.939
11
Na
22.99
19
K
39.10

13. Use the integer mass from table 2 below and the descriptions of common ions to fill in table 3 below for equations 2 - 6. Assume the molecular mass of the analyte molecule M is 125000 amu.

Table 2: Most abundant isotopes and masses for important elements in MALDI mass spectrometry.[3]

Element	Isotope	Mass (Da)	Integer Mass (Da)	Natural abundance (%)
Sodium (Na)	^{23}Na	22.9897677	23	100
Potassium (K)	^{39}K	38.9637074	39	93.2581
Hydrogen (H)	^{1}H	1.007825032	1	99.9885

Table 3

Equation	Ion symbol	Predicted ion mass-to-charge ratio
1	$[M+H]^+$	125001
2		
3		
4		
5		
6		

14. Label peaks in figure 6 (with ion labels from table 4) that would show up in **positive ionization mode**.

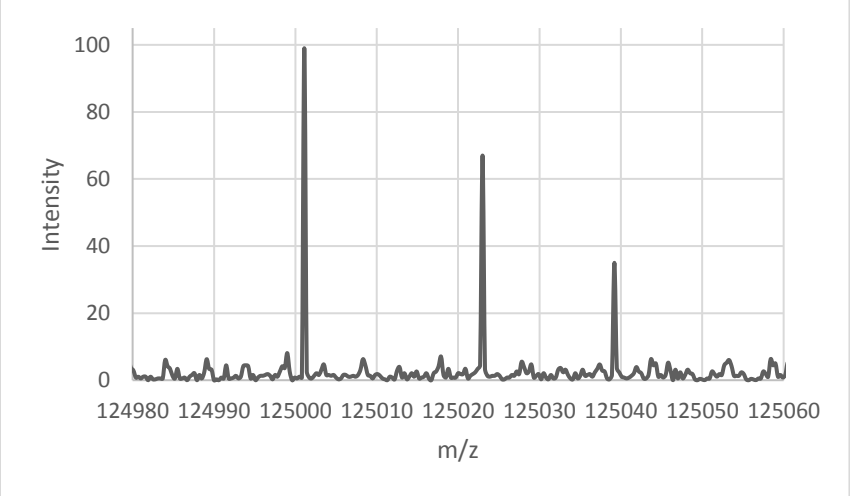


Figure 6: A portion taken from a MALDI mass spectrum in positive ion mode.

15. Explain why the peaks associated with the ion labels you did **NOT** use in figure 6 would not be observed in the given mass spectrum.

Check your work:

16. a) Did your explanation include charge differences? **Yes** or **No** (*Circle one*)

b) Did the limited m/z scale factor into your explanation? **Yes** or **No** (*Circle one*)

If the answer to 16a and 16b were both "Yes", move on to question 17. If not, consult further with your group or ask another group or your instructor for help.

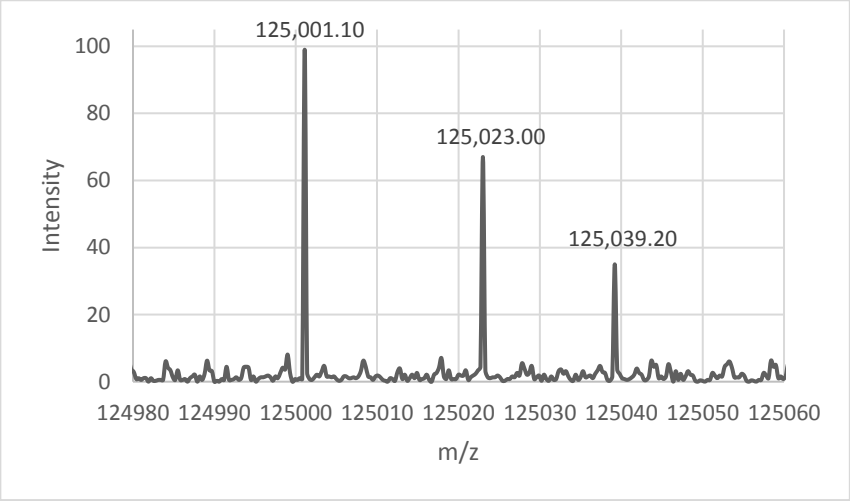


Figure 6: A portion taken from a MALDI mass spectrum in positive ion mode.

17. a) In the spectrum above in figure 6, identify any pairs of peaks that have the relationship X, X+22, and X, X+38?

List the masses of the <u>pairs</u> you identify on the lines below.

X and X+22 _____ and _____

X and X+38 _____ and _____

b) What is a likely relationship between these pairs of peaks?
(Use the terms $[M + H]^+$, $[M+ Na]^+$, $[M + K]^+$)

X, X+22 _____ _____

X, X+38 _____ _____

18. Why does X+22 demonstrate a sodium adduct in the MALDI mass spectrum example in figure 1 versus X+23?

a. Because sodium is ionized, and removing an electron caused a 1 amu decrease in mass.
b. Because X represents the $[M+H]^+$ ion and we need to subtract 1 amu (the mass of the most abundant isotope of H^+) from the mass of the $[M+H]^+$ ion signal before adding 23 amu (the mass of the only isotope of Na^+).
c. Because sodium atom has special isotopes that need to be considered in mass spectrometry.

19. *Check your work:* a) Does your answer to question 18 explain the difference in masses between the **first two labeled peaks** in figure 1? **Yes** or **No** (*Circle one*)

b) If you circled "Yes", you are done. If you circled "No", consult further with your group or ask another group or your instructor for help.

References:

1. Grant JE, Bradshaw AD, Schwacke JH, Baicu CF, Zile MR, Schey KL. Quantification of protein expression changes in the aging left ventricle of Rattus norvegicus. J Proteome Res. 2009 Sep;8(9):4252-63. doi: 10.1021/pr900297f.
2. Hillenkamp, F., Karas, M. The MALDI Process and Method in *MALDI MS. A Practical Guide to Instrumentatoin, Methods and Applications* Hillenkamp, F, Peter-Katalinić Ed. Wiley-VCH, Weinheim 2007, p. 11
3. http://www.webelements.com/ accessed 1/18/2016

Funding Provided by the National Science Foundation

"MALDI-based Research-like Experiences in a 2YC/4YC Collaboration with a Renewable Fuels Industry Partner" NSF ATE #1400885