MALDI Activity 1

Introduction to MALDI-TOF Mass Spectrometry

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. Molecular mass determinations of large synthetic or biological polymers by mass spectrometry (MS) use either Electrospray Ionization (ESI) Mass Spectrometry or MALDI-TOF Mass Spectrometry. These types of MS are typically used for molecules that are too polar and/or non-volatile to be vaporized in a heated chamber as is required in Electron Impact (EI)-MS. Although both ESI and MALDI are used in to analyze such macromolecules, our focus will be on MALDI-TOF Mass Spectrometry. Figure 1 shows a schematic of collecting MALDI MS data and a typical mass spectrum.

Model 1: Obtaining a MALDI-TOF Mass Spectrum

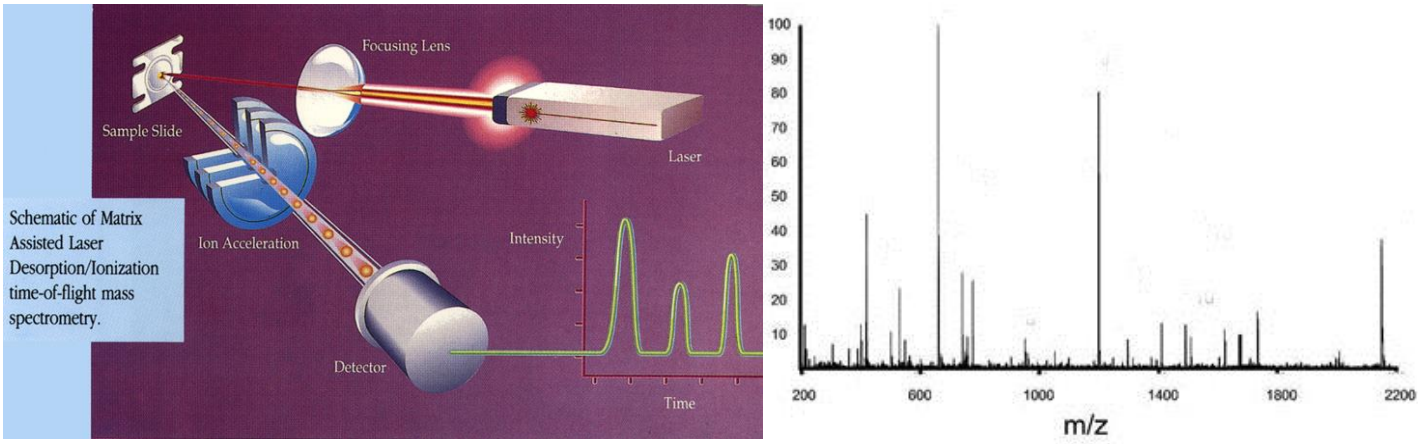


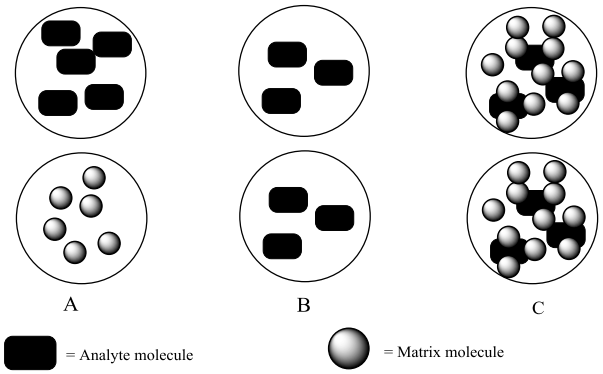
Figure 1: Schematic taken from http://www.protein.iastate.edu/maldi.html and data from reference 1.

The schematic above gives a general idea of how a MALDI MS spectrum is obtained.

The steps are:

1. A solution of the molecule of interest, called the **analyte**, is deposited on a stainless steel plate.
2. A large molar excess of a solution of a UV-absorbing "helper" molecule (i.e. more matrix molecules than molecules of analyte), called the **matrix**, is deposited **at the same location** on the plate.
3. The solvent is allowed to evaporate leaving a small solid spot of a mixture of analyte and matrix molecule on the steel plate.
4. The plate is inserted into the mass spectrometer and a vacuum is pulled on the sample chamber.
5. In the mass spectrometer, a UV laser pulse (typically 337 nm) is focused on the analyte and matrix mixture causing the mixture to vaporize (also called "**desorption**.")
6. The vaporized and charged analyte(s) are accelerated in the Ion Accelerator down the flight tube.
7. The detector uses the ion time-of-flight (TOF) to calculate and display the mass-to-charge (*m/z*) ratio of the analytes in the sample.

1. Which choice below shows properly prepared MALDI MS sample spots? (Circle one)



2. Which choice below describes the type of species that can be detected in MALDI MS? (Circle one)

A. Neutral molecules B. Ions C. Radicals

3. What phase is the sample in **before** the laser pulse? (Circle one)

A. A Bose-Einstein condensate B. Liquid C. Solid D. Gas

4. What does a mass spectrometer measure? (Circle 1)

A. The mass of a molecule B. The mass of an ion C. The mass-to-charge-ratio of an ion (*m/z)*

Model 2: EI or MALDI Mass Spectrometry?

As you have learned in previous chemistry classes, as the molecular weight of a molecule increases, its boiling point increases as well. In many instances, when attempts are made to vaporize a large molecule with a high MW using heat, the molecule will often decompose into a complex mixture of smaller molecules at a temperature below the boiling point. Some molecules do not have a boiling point as they decompose before they can boil.

Table 1

Compound	**Molecular Weight (amu)**	**Boiling Point (°C)**	**Decomposition Point (°C)**
Trypsin (a polypeptide)	~23,300	---	130
Cotton (a polysaccharide)	~81,000	---	106
Polyethylene (Low Density)	~20,000	---	217
3,5-dichlorobiphenyl	223	324	1000
Stearic acid (a fatty acid)	284	361	232
Limonene (a terpene)	136	176	~400
Triglyceride composed of palmitic, oleic and α-linoleic acid side chains	855	300	~400

Table 1 shows a number of compounds of varying molecular weight, along with their corresponding boiling points and/or decomposition points. The decomposition point is the temperature at which a compound breaks down into smaller molecules.

When a molecule decomposes in electron-impact mass spectrometry (EI-MS), it generates a complex mass spectrum that can be difficult to interpret. MALDI-TOF Mass Spectrometry overcomes this major barrier of EI-MS. In MALDI-TOF Mass Spectrometry the analyte molecule is vaporized using a "soft" ionization technique that involves the use of a large excess of matrix molecules that absorb laser light of a specific wavelength. When the laser light is absorbed by the matrix, it induces a co-vaporization of the analyte molecule that occurs without decomposition. Formation of molecular ions occurs during this step as well.

Using the data in Table 1, answer the following questions.

(E) 4. Which molecules:

a) have a boiling point that is BELOW its decomposition temperature (T_d)?

b) have a boiling point that is ABOVE its T_d?

c) have no boiling point listed?

(CTQ) 5. Identify which mass spectrometry technique, **Electron Impact**, **MALDI** or **BOTH**, can be used to analyze each compound. *(Place an X in the appropriate box).*

Table 2

Compound	**Molecular Weight (amu)**	**Electron Impact (EI)**	**MALDI**	**BOTH EI and MALDI**
Trypsin (a polypeptide)	~23,300			
Cotton (a polysaccharide)	~81,000			
Polyethylene (Low Density)	~20,000			
3,5-dichlorobiphenyl	223			
Stearic acid (a fatty acid)	284			
Limonene (a terpene)	136			
Triglyceride composed of palmitic, oleic and α-linoleic acid side chains	855			

Check your work

6. a) Does your **Table 2** have 4 "X" marks in the MALDI column and 3 "X" marks in the BOTH EI and MALDI column? **YES** or **NO** *(Circle one)*

b) If you circled "YES", move on to question 7. If you circled "NO", consult further with your group or ask another group or your instructor for help.

7. From your answers in table 2, what is the general relationship between molar mass and choice of mass spectrometry technique?

From your answers in **Table 2**, it may seem that MALDI would work equally well for both large and small molecules. In practice, however, the ions that form from the matrix molecules can interfere with the data from low mass analytes due to their similar molecular weights. This leads to less precision and more difficult data interpretation for the analysis of smaller molecules using MALDI mass spectrometry. For these reasons MALDI is used most often, though not exclusively, for analyte molecules **significantly larger** than the matrix molecules.

Model 3: Time of Flight ion separation

Once the analyte and matrix molecules have been vaporized and ionized by the laser pulse, an Ion Accelerator brings all ions in the sample to the **same kinetic energy** (KE) using an electric field. Kinetic energy can be described using the equation $KE = ½ mv^2$ where m is mass (in kg) and v is velocity in m/s. In this example we will only consider a **singly-charged** protonated molecular ion.

Table 3

Compound	Protonated Mass (amu)	Equivalent Mass (kg)	Kinetic Energy (J)	Velocity (m/s)
A	50.	8.3×10^{-26}	4.2×10^{-20}	
B	500	8.3×10^{-25}	4.2×10^{-20}	
C	5000	8.3×10^{-24}	4.2×10^{-20}	

8. Fill in the velocity column for table 3 (use $KE = ½ mv^2$ and a +1 charge).

9. If the compounds in table 3 are accelerated to 4.2×10^{-20} J of kinetic energy down a 1 meter flight tube, which arrives at the TOF detector first and which arrives last?

First _____ Last _____

10. The compound that arrives:
    a) **first** at the detector is the ion with the (***highest, middle, lowest***) mass-to-charge ratio. (Circle one)
    b) **last** at the detector is the ion with the (***highest, middle, lowest***) mass-to-charge ratio. (Circle one)

11. Assuming all ions are singly charged, when would a 2000 amu species arrive at the detector in a TOF instrument?

A. Before compound A
B. Between compound A and B
C. Between compounds B and C
D. After compound C

12. After consultation with your group, summarize the two most important concepts learned from this activity. (Use the next page if needed.)

References:

1. Grant JE[1], 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.

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