CHEM 5181 Laboratory #1 – MALDI Time-of-Flight Mass Spectrometry (Pre-lab)

Introduction:

In this lab you will analyze a mixture of three peptides using a matrix-assisted laser desorption ionization (MALDI) time-of-flight mass spectrometer (TOFMS) from PerSeptive Biosystems (now Applied Biosystems). You will investigate how different matrices affect the outcome of a MALDI spectrum. Then you will manipulate various parameters of the instrument to see what effect they have on the resulting spectra (e.g., ion time of flight, resolution, signal to noise).

You will be provided:

- Instrument Data Sheet and description
- Simplified instructions for using the MALDI-TOFMS
- A MALDI sample plate with four sample spots on it

  Each spot contains a mixture of three peptides,
  - Angiotensin II mH^+ = 1046.62 Da
  - Angiotensin I mH^+ = 1296.69 Da
  - Glu1 fibrinopeptide B mH^+ = 1570.68 Da

  and a different matrix:
  - α-CHCA (α-Cyano-4-hydroxycinnamic acid)
  - SA (Sinapinic acid)
  - DHB (2,5-Dihydroxybenzoic acid)
  - (no matrix)

Description of Instrument Setup:

The instrument (Voyager-DE STR) consists of a typical MALDI-TOFMS setup with delayed extraction to compensate for the inherent distribution in the initial velocities of ions generated by MALDI (Figure 1).

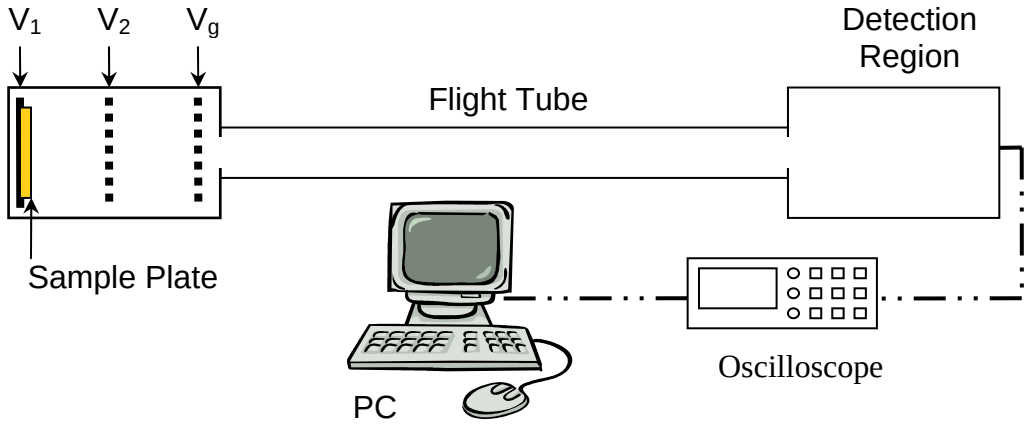

Figure 1. Block diagram of MALDI-TOFMS
(V_1; constant high voltage, V_2; delayed extraction voltage, V_g and Flight Tube; at ground)

A sample spot has been prepared by placing 0.3 µL of a matrix solution onto the plate immediately followed by 0.3 µL of the peptide solution, mixing them, and allowing it to dry.

The plate is inserted into the instrument via a sample arm and is placed at the back of the source chamber directly in front of the acceleration plate V_1. The acceleration plate and the delayed extraction grid voltage, V_2, are held at a voltage of +20 kV while the final grid voltage is held at ground potential (V_g = 0 V). The sample spot is then irradiated with a pulse from the N_2 laser emitting at 337 nm to create ions. Because both V_1 and V_2 are at the same potential, there is no effective electric field present and the ions are allowed to drift for a short amount of time (typically ~10^2 ns) without being accelerated.

After this initial delay time (user determined and set) the voltage of the grid, V_2, is quickly switched to a percentage (typically 80 – 95%, user determined and set) of the acceleration plate, V_1, so that the ions get accelerated past V_2 towards V_g and injected into the flight tube of the mass spectrometer. The ions are then separated by their time of flight (typically 10-100 µs) according to their mass-to-charge ratio and detected by the microchannel plate (MCP) detector.

There are two modes for the TOF:

Linear Mode:

In the linear mode, the ions exit the flight tube and are detected with an MCP located directly along the axis of flight. The flight length of ions is 1.3 m (quoted in the manual).

Reflector Mode:

In the reflector mode, the ions exit the flight tube and are turned around by a set of ion reflector mirrors and sent back towards a second MCP where they are detected. The flight length of ions is 2.0 m (quoted in the manual).

Acquisition of Data:

As the ions impinge upon the detector, the current pulse is sent to an oscilloscope that measures and displays the signal voltage as a function of time. After a pre-fixed number of laser shots, the oscilloscope signal is averaged over and sent to a computer, which converts the voltage peaks into ion intensities and the flight times into mass to charge (m/z) where mass is in Daltons. The computer is also used for processing of the acquired data.

During this experiment you will be using the computer to obtain and analyze mass spectra, compute the resolution and signal to noise of peaks, and save the data. You will

be using the oscilloscope to determine flight times of the various ions and to manually compute the resolution of the peaks from the voltage-time data.

Summary of Experiment:

I. Effect of Different Matrices:

The first step in obtaining a MALDI mass spectrum is to choose a proper matrix. In the first part of the experiment, you will attempt to obtain MALDI-TOF mass spectra for the peptide mixture using four different matrices. You will then compare the mass spectra to see what differences there may or may not be. The differences you should watch for include (but are not limited to) overall signal intensity and signal to noise. Based on these results, you will choose the best matrix to use for the rest of the lab.

II. Effect of Instrument Calibration:

In this part of the experiment, you will obtain a mass spectrum of the peptide mixture both before and after mass calibration of the instrument. You will determine how calibration affects your results.

III. Manual Calculation of Mass Resolution using Voltage/Time Data from the Oscilloscope:

In this part of the experiment, you will use the voltage/time data from the oscilloscope to manually calculate the resolution of peaks in the spectrum. To do this, we must first determine how the time resolution relates to the mass resolution.

The energy of an ion leaving the source of a mass spectrometer can be approximated by

$$E = qV$$

where q is the charge on the ion and V is the acceleration voltage in the source. This energy can also be written in terms of the ion's kinetic energy so that

$$qV = \frac{1}{2}mv^2$$

where m is the ion's mass and v is the velocity of the ion. Substituting in the relationship $v = \frac{d}{t}$ (d; flight length, t; time of flight) and rearranging to solve for the mass we obtain:

$$m = 2qV\frac{t^2}{d^2}$$

Taking the derivative of this equation while keeping in mind that q, V and d are all constants and making the approximation $dm \approx \Delta m$ and $dt \approx \Delta t$, we obtain

$$\Delta m = 2qV\frac{(2t\Delta t)}{d^2}$$

The resolution of a peak is therefore

$$\frac{m}{\Delta m} = \left(2qV\frac{t^2}{d^2}\right) \Big/ \left(2qV\frac{(2t\Delta t)}{d^2}\right) = \frac{t}{2\Delta t}$$

This equation tells us how the resolution of a peak on the oscilloscope in terms of the flight time can be converted to the resolution of a peak in the mass spectrum in terms of the ion's mass.

IV. Effect of Instrument Parameters:

In this part of the experiment, you will obtain MALDI-TOF mass spectra for the peptide mixture with various instrument settings. Settings that you will vary include the laser intensity and delay time/extraction voltage on grid V_2. You will determine how changing these settings affects the resolution, intensity, and ion time of flight.

V. Comparison of Linear and Reflector Modes:

In the final part of the experiment, you will obtain a mass spectrum of the peptide mixture using the reflector mode. You will then compare these results to the results already obtained using the linear mode and determine how using a reflector changes the quality of a mass spectrum. You will also be asked to explain what the reflector does that leads to these changes.

Prelaboratory Questions:

1. Draw the structures of the matrix molecules to be used for the lab.

2. In general, what role does the matrix play in MALDI?

3. What are the typical properties of a suitable matrix?

4. Make a more detailed sketch of the mass spectrometer. Be sure to label all important parts and provide a brief description for each.

5. How would you expect flight times to be affected by changes in the delayed extraction voltage (V_2) and delay time?