MALDI ToF ToF Instrumentation Optimised for Chemically Aided Fragmentation.

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Overview

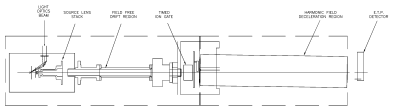
Use of a simple maldi ToF retarding field analyzer to study the physical properties of Chemical Assisted Fragment.

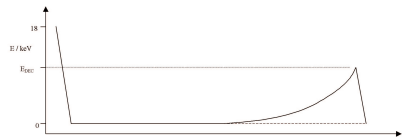
- Dissociation Rate Constant
- Internal Energy

These values form the basis for a new Maldi ToF ToF optimised for Chemical Assisted Fragment.

Method

The ToF retarding field analyzer consist of a pulsed maldi ion source, field free drift region, timed ion gate, deceleration region and detector as shown below.





The fragmentation which occurs in the field free drift region will produce three types of species at the entrance to the deceleration region according to the equation.

$$(P+H)^+ \rightarrow (F+H)^+ + F^n \qquad \textbf{Equation 1}$$

- Protonated Parent Ions $(P + H)^+$
- Protonated Fragment ions $(F + H)^+$
- Neutral Fragment ions F^n

The deceleration region effects each of the species differently.

- Neutral Fragment Ions — These are unaffected by the deceleration field and hit the detector first.
- Protonated Parent Ions — Are slowed by the decelerating field an so arrive after the neutrals.
- Protonated Fragment Ions — Those with energy greater than E_{DEC} will hit the detector later than the parent ions while those with energy less than E_{DEC} will not be detected.

The amount of fragmentation which occurs during the field free drift region is defined as the ratio of the number of unfragmented precurser ions to the total number of ions and is proportional to the exponential of the drift time. This can be seen from equation 2 to give the rate equation.

$$\frac{(P+H)^+}{(P+H)^+ + F^n} = e^{-kt} \qquad \textbf{Equation 2}$$

Since the retarding field analyser separates the neutral and precurser ions then this can be evaluated by comparing the relative peak areas for two different drift times. This gives an effective decay rate constant for the fragmentation for a fixed energy input.

The laser power was set at 10% above threshold to ensure that the samples received the same amount of energy and the vacuum in the source was keep at 5 x 10^{-7} mbar.

The internal energy given to the fragment ions can be estimated by the temporal spread of the neutral fragment ions. Assuming that the largest temporal spread is due to ions dissociating on axis then an upper limit for the internal energy given to the fragments in the centre of mass frame can be found.

It can be shown that the velocity given to the fragment ions in the centre of mass frame is given by the equation

$$\Delta V_{F^+} = \sqrt{\frac{2q\cdot\Delta E}{m_{(F+H)^+}\left(1+\frac{m_{(F+H)^+}}{m_{F^+}}\right)}} \qquad \textbf{Equation 3}$$

The time difference of the neutral fragments is given by

$$\Delta t = t_+ - t_- = L\left(\frac{2\cdot\Delta V_{F^+}}{V_{F^+}^{\ 2} - \Delta V_{F^+}^{\ 2}}\right) \qquad \textbf{Equation 4}$$

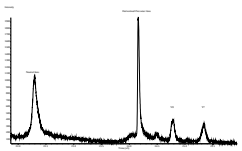
Substituting equations 3 and 4 gives the temporal spread of the neutral fragment ions in terms of the internal energy.

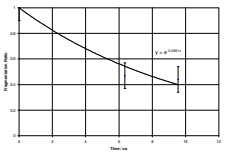
$$\Delta t = 2\cdot L\left(\frac{\sqrt{\frac{2q\cdot\Delta E}{m_{(F+H)^+}\left(1+\frac{m_{(F+H)^+}}{m_{F^+}}\right)}}}{\frac{2q\cdot E}{m_{(F+H)^+}} - \frac{2q\cdot\Delta E}{m_{(F+H)^+}\left(1+\frac{m_{(F+H)^+}}{m_{F^+}}\right)}}\right) \qquad \textbf{Equation 5}$$

Results

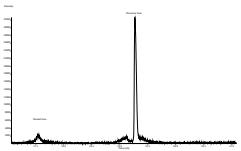
Two peptides where investigated both derivatized and native species in alpha cyano.

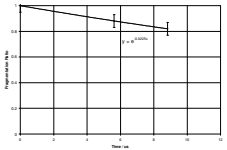
- Sulfonated ASHLGLAR.



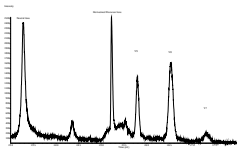


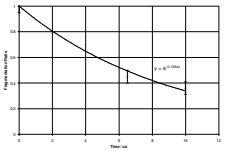
- Native ASHLGLAR.



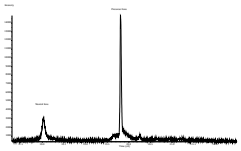


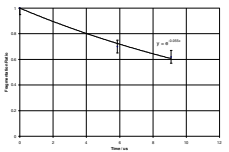
- Guanidinated & Sulfonated VGGYGYGAK.





- Native VGGYGYGAK.





Conclusion

- The effective dissociation rates for the derivatized peptides are several times that of the native peptides.
- The temporal spread of the neutral peaks are similar for the derivatized and native peptides and give an upper limit on the internal energy of the order of ~ 1eV.

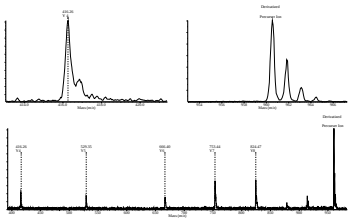
Chemically Aided Fragmentation Optimised Instrumentation

The physical properties of the derivatized peptides lead to a new design of Maldi ToF ToF instrument design for Chemical Aided Fragmentation.

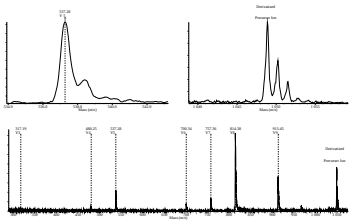
- ToF 1 is optimised to allow for the maximum amount of fragmentation while minimizing the temporal spread due to the internal energy of the fragments.
- ToF 2 is a large bandwidth harmonic mirror allowing for a complete fragment spectrum for each shot of the laser.

Preliminary data for this new instrument is shown below for the derivatized samples investigated previously.

- Sulfonated ASHLGLAR.



- Guanidinated & Sulfonated VGGYGYGAK.



Acknowledgements

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