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luizno.bjp@gmail.com

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Mechanism of Insulin Emission in Matrix Assisted Laser Ionization

F. Fernández-Lima¹, V. M. Collado², C. R. Ponciano², L. S. Farenzena²,
E. Pedrero³, and E. F. da Silveira²

¹*Instituto Superior de Tecnologías y Ciencias Aplicadas,*

Ave Salvador Allende esq Luaces, s/n, CP 10600, Ciudad de la Habana, Cuba

²*Pontificia Universidade Católica, CP 38071, Rio de Janeiro, 22452-970, Brazil*

³*Physics Faculty-IMRE, University of Havana, Cuba*

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In the present paper the emission of intact insulin quasi-molecular ion $[M+H]^+$ during laser ablation (MALDI) is studied. It was observed in particular that the insulin TOF molecular peak increases as the laser power increases. The DE-Vestal method for the initial velocity measurements was improved theoretically including the distance (d_0) until the free expansion regime can be considered. According to the present analysis, the v_0 parameter given by the DE-Vestal method is interpreted as the initial velocity that the desorbed ion would have if no collision occurs in the plasma. The improved method interprets v_0 as the “final” initial velocity, i.e., as the velocity that the desorbed ions have when the plasma free expansion starts and, effectively, collisions no longer occur. The new method allows also the determination of d_0 , the distance to the solid when the free expansion starts. The data fitting shows that the distance (d_0) has a linear dependence on the laser's intensity. Extrapolation of these values gives $I = 0.69 \text{ G W cm}^{-2}$ as the minimum energy density necessary to produce high density plasma during the insulin ions desorption when using α CHCA matrix.

1 Introduction

Lasers have been used in the last three decades to produce high-temperature and high-density plasmas for fusion devices by vaporizing a small amount of material with high-powered nanosecond pulses. Great instrumental developments have been accomplished in the last 15 years but there is still a lacking of a satisfactory understanding of the chemical-physical phenomena involved in laser ablation. One of the major applications of laser ablation in modern biological analysis is the matrix-assisted laser desorption/ionisation mass spectrometry technique (MALDI-MS) [1,2]. As it is typical for the history of the desorption techniques, progress has been driven by the empirical experience, rather than by a more profound understanding of the underlying physical-chemical processes. In the early 1990's, in order to provide first-order correction for the initial distribution of emitted ions, the delayed extraction (DE) technique was introduced to improve mass resolution and sensitivity in time-of-flight (TOF) mass spectrometry (MS) coupled with MALDI.

In a first set of experiments, the initial velocity of the MALDI ions has been thoroughly investigated and it was concluded that it is the superior tool to characterize the laser ablation of the MALDI process [3,4]. The importance of the initial velocity analysis of desorbed ions is twofold: i) the absolute value of the average initial velocity, in respect to practical performance of MALDI/DE-TOF systems, is needed for accurate calibration procedures, and ii) the width

of the velocity distribution is an important factor for mass resolution.

Numerous experiments on the velocity measurements of laser desorbed ions and neutrals and theoretical calculations have been reported [3-9]. Average initial velocities of sample ions vary between 300 and 1000 m/s and between 300 and 1700 m/s for matrix ions. The effect of matrix substance and wavelength has been analyzed for several peptide, protein and DNA samples up to 25000 u [3]; however, laser irradiance influence has not been explored systematically. Most of these reports were carried out routinely at low irradiation regimes, and the initial velocity was considered as the first moment of the distribution of the velocity component in the TOF expansion. However, these considerations break down when the interaction of the desorbed ions in the plume is not negligible.

It is the aim of the current investigation to determine absolute and accurate values of the average axial initial velocity of insulin ions in α CHCA matrix (4-Hydroxy- α -cyanocinnamic acid). Substantial effort has been made in this study to consider all possible experimental factors involved in the ablation process. It is relevant to express the consideration of the leakage field influence, the laser intensity variations and the reproducibility of sample preparation.

In order to obtain more reliable data on the initial velocity distribution, the experimental setup was design to obtain the best performance avoiding the use of any ion focusing device [10].

2 Theory

In linear two-stage acceleration TOF system, the initial ion velocity (v_o) can be determined as an approximation from the slope of the curve of flight time vs extraction delayed. For this purpose, flight times and their dependence on the delay time are measured and an analytical solution/approximation of the flight time equation is used to determine v_o (DE-Vestal method [3,11,12]).

Briefly, the time-of-flight (TOF) equations can be written as functions of the two grid distances (d_1 and d_2), the field free drift length (L), the delay time (τ), the sample target potentials (U_0 and U_1 : initially U_0 switches into U_1 after the delay time τ), the first grade potential (U_0), the mass (m) and the charge (q) as follows:

$$TOF(\tau, v_o) = TOF(0, 0) + \tau + \left(\eta\tau - \frac{2d_1}{v_R} \right) \left(\frac{v_o}{v_R} \right) + \left(\tau - \frac{\eta d_1}{v_R} \right) \left(\frac{v_o}{v_R} \right)^2 \quad (1)$$

where $\eta = R\sqrt{R}\frac{L}{2d_1} + \frac{R}{1+\sqrt{R}}\frac{d_2}{d_1} - 1$, $v_R = \sqrt{\frac{2q}{m}(U_1 - U_0)}$ and $R = \frac{U_1 - U_0}{U_1}$.

$TOF(0,0)$ is the time of flight for an ion “emitted” with $v_o = 0$ and extracted promptly ($\tau = 0$):

$$TOF(0, 0) = \frac{d_1}{v_R/2} + \frac{d_2}{v_R/2} \left(\frac{\sqrt{R}}{1 + \sqrt{R}} \right) + \frac{L}{v_R} \sqrt{R}. \quad (2)$$

which represents the sum of time intervals that the ion takes to travel the distances d_1 , d_2 and L respectively.

Assuming $\tau \ll d_1/v_o$, $v_o \ll v_R$ and considering the plot $TOF(\tau, v_o) - \tau$ versus τ , the slope of the function ($d(TOF - \tau)/d\tau$) can be written as:

$$\frac{d(TOF - \tau)}{d\tau} = \eta \frac{v_o}{v_R} = \frac{v_o}{v_{ref}} \quad (3)$$

where $v_{ref} = \frac{v_R}{\eta} = \frac{1}{R^{3/2}\frac{L}{2d_1} + \frac{R}{1+\sqrt{R}}\frac{d_2}{d_1} - 1} \sqrt{\frac{2q}{m}(U_1 - U_0)}$

Although the width and overall shape of the velocity distribution are equally important parameters in any theoretical understanding of the plume expansion process, the above DE-Vestal method does not allow separation of these parameters [13].

One sees from equation (3), that the initial velocity v_o of the desorbed ions can be determined from the measurements of the slope $d(TOF - \tau)/d\tau$. In fact, the distributions of the slope of each part of the TOF peak allow, in principle, the determination of the initial velocity distribution.

Methods relying on the measurement of the drift velocity in a field-free region are very sensitive to residual electric fields. Penetration of the accelerating field into the field-free zone leads to an overestimate of the initial velocity. The effect of the residual field is function of $(m/q)^{-1}$ so it is less important at high mass-to-charge ratio, but it can cause substantial errors for low mass-to-charge ratio.

In a higher order approximation, the shape of the velocity distribution should also be taken into account. At short

extraction delays, the ions may have not reached their “final” initial velocity and collisions with neutrals can slow ions down. The time lag, until a free expansion regime can be considered, has been estimated from hydrodynamic calculations to be about 50-100 ns [14]. Therefore, the time lag also depends on the laser irradiance due to the substantial interaction between ions and neutrals.

In order to estimate the volume occupied by this initially expanding and interacting system, as function on the laser irradiance, a new parameter, d_0 , can be introduced into the equation (1): the length d_0 corresponds to the distance travelled by the desorbed ions until their velocity becomes constant (“final” initial velocity). Once the free expansion regime is reached, their “final” initial velocity is considered as the new “initial” velocity for the movement described by the equation (1). The “final” initial velocities of the plasma ions were calculated by the code TOF12 [10], developed by the authors, taking into account the leakage field effect influences on the ion dynamics inside the spectrometer.

3 Experimental

1) Instrumentation

Experiments were performed in a two-acceleration region linear mass spectrometer equipped with a delayed field extraction system. A pulsed-nitrogen laser radiation (337 nm) is brought into focus on the target with a 30° incidence angle.

The ions are detected with a microchannel plate (ion-electron conversion) and the delivered signals are digitalized at a rate of 500 MHz by a Tektronix TD520 digitizing oscilloscope (2 ns resolution). The ion extraction region is composed of two stages, each one ending by a 90 % transmission grid. The drift tube length L is 276.5 ± 0.1 mm and the distance d_2 from the first to the second grid is 20.0 ± 0.1 mm. (See Fig. 1).

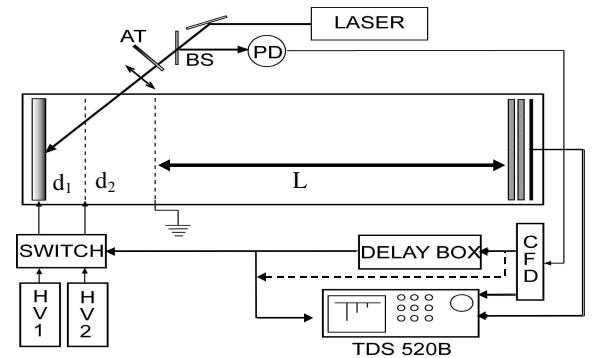


Figure 1. Schematic diagram of the mass spectrometer. AT – gradient neutral density attenuator, BS – beam splitter, PD – photodiode. The dashed line shows a second option for start signal generation also used.

TABLE 1. Comparison of DE-Vestal method with the improved new method results.

Laser intensity in [G W cm^{-2}]	DE-Vestal method initial velocity [m/s]	“Final” initial velocity [m/s]	Expansion distance d_0 [μm]
0.9	404 ± 122	362 ± 110	50.0 ± 15
1.1	643 ± 95	573 ± 85	75.0 ± 11
1.3	633 ± 35	576 ± 32	115.0 ± 6
1.5	442 ± 196	414 ± 184	180.0 ± 20

The distance d_1 from the target to the first grid is 2.43 ± 0.1 mm. The delayed extraction was set in such way that the target voltage is held to a potential of $U_{0i} = 10.99$ kV before the application of the ion extraction pulse and then switched to $U_1 = 12.50$ kV (U_{0i} was a correction of the leakage field influences). The first grid potential is permanently held at $U_0 = 11.00$ kV and the second grid is grounded. The extraction field was trigger using the laser pulse photo detector signal. Each spectrum represents ten-shot average acquisition. The delayed time was varied from 300 to 600 ns, and the laser pulse intensity was varied from 0.9 to 1.5 G W cm^{-2} .

II) Sample preparation

For the standard dried droplet method preparation, samples were prepared by applying $10\mu\text{L}$ of αCHCA matrix solution plus $1\mu\text{L}$ of insulin (Sigma) solution on a stainless-steel sample plate. Matrix solution was 10 mg/mL of 4-Hydroxy- α -cyanocinnamic acid in a mixture of acetonitrile / water (7:3 v/v). Analyte concentration was 0.1mg/mL in 0.3% Trifluoroacetic acid (TFA) aqueous solution.

4 Results and Discussion

In the present section, mechanisms involved in the matrix-analyte ablation and emission yields as a function of the laser irradiance are discussed. An important point, in order to evaluate the initial velocity using the DE-Vestal method, is the criterion for choosing the reference point of the TOF peak ions signal in the case of MALDI-DE-TOF. In a previous work, the evolution of the peak shape for an initial Gaussian like velocity distribution was analyzed as function of the delayed time and the experimental spectrometer conditions [15]. In order to determine the initial velocity by the DE method, the peak centroide was considered the reference point for the TOF value, and FWHM the TOF peak centroide error. Fig. 2a shows the experimental spectra of the insulin ion $(\text{M}+\text{H})^+$ for the case of 1.3 G W cm^{-2} laser intensity and its correspondent maximum and centroide evolution. One sees that no dramatic changes occur in the peak shape as the extraction field is delayed. Most of the TOF peak shift is merely due to τ , the time interval between the laser shot and the application of the external electric field to extract the ions. The time difference between the TOF peak shift and τ , $\text{TOF}-\tau$, is the relevant quantity that allows the v_0 determination.

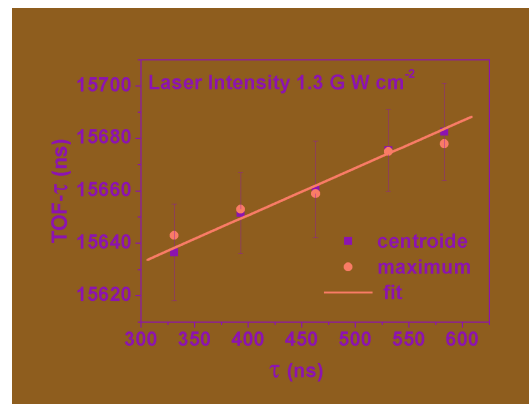
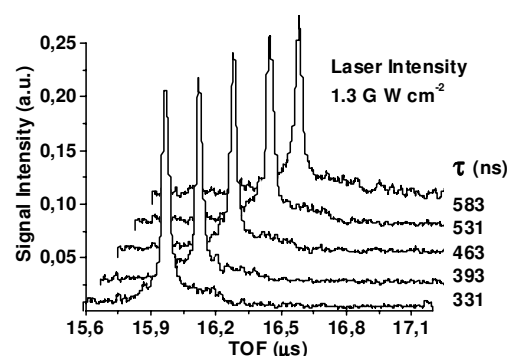


Figure 2. (a) The experimental spectra of the insulin ion $(\text{M}+\text{H})^+$ for the case of 1.3 G W cm^{-2} laser intensity. (b) The maximum and the centroide evolution of the TOF peak for different applied delayed extraction time, τ .

For this reason, Fig. 3 presents the analysis of the slope of the curve of flight time ($\text{TOF}-\tau$) vs extraction delayed time (τ) for the different applied irradiances. The initial velocities, calculated from these curves using the DE-Vestal method (equation (3)) for the different applied irradiances, are presented in the second column of table 1. Notice that the flight time evolution can not be explained by this method, because it is a consequence of the internal ion-neutral collision in the primary stages of the ions' evolution, a phenomenon not considered in the model. The same data of Fig. 3 is fitted with the new equation in which d_0 was introduced. The distance d_0 and the “final” initial velocity values as function of the applied laser intensities calculated using the program TOF12 are also shown in table 1.

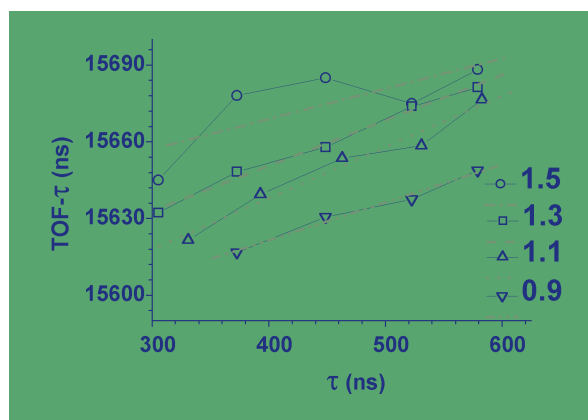


Figure 3. The flight time (TOF - τ) vs extraction delayed time (τ) for the different applied irradiances. The laser intensity values are given in [G W cm^{-2}].

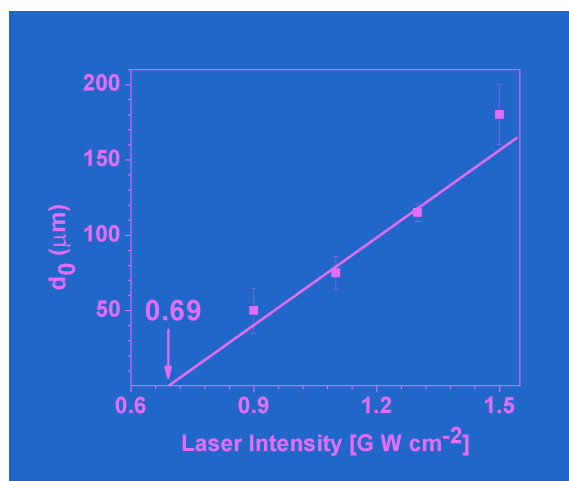


Figure 4. Starting point (d_0 distance) for the consideration of the “final” initial velocity as function of the applied laser intensity.

The DE-Vestal method for initial velocity lead to values which are larger than the “final” initial ones, because equation (1) does not take into account that ions cannot be accelerated by the external field while the plasma density is too high. The two values reported in the literature [3,4] (there is no reference to the applied irradiance) are lower than the one presented here (both are ~ 311 m/s, very close to that corresponding to our lowest applied irradiance). The d_0 values grow linearly as function of the laser applied intensity (at a rate of 194) as shown in Fig. 4. Extrapolation of these values gives $I = 0.69 \text{ G W cm}^{-2}$ as the minimum energy density necessary to produce high density plasma during the insulin ion desorption with αCHCA as matrix. Note that, when using lesser than $I = 0.69 \text{ G W cm}^{-2}$ (which implies $d_0 = 0$), the DE-Vestal method and the new method presented here are equivalent, i.e. predict the same “final” initial velocity value. There are no significant differences in the “final”

initial velocities values ($I = 1.1\text{--}1.3 \text{ G W cm}^{-2}$) up to the highest applied laser irradiance ($I = 1.5 \text{ G W cm}^{-2}$) when the effect of kinetic energy loss is clearly seen. We have also observed that, for laser irradiances higher than 1.5 G W cm^{-2} , the collision effects are responsible for the reduction of the insulin ($\text{M}+\text{H}$)⁺ ions signal, till the production of the TOF signal is completely extinguished for laser intensities larger than 1.6 G W cm^{-2} . The energy incorporated with the increase in laser irradiance reverts in collisions, de-excitations and fragmentations of the insulin ($\text{M}+\text{H}$)⁺ ions. Further studies of insulin ($\text{M}+\text{H}$)⁺ ions stability or neutralization as function of temperature could reveal more information of the ablation of this kind of systems.

5 Conclusions

The emission of intact insulin ion molecules during laser ablation (MALDI) is studied. It was observed in particular that the insulin TOF of the molecular peak increases as the laser power increases. The DE-Vestal method for the initial velocity measurements was improved theoretically with the inclusion of the distance (d_0) in the TOF equation, treating the free expansion regime correctly. According to the present analysis, the v_0 parameter given by the DE-Vestal method is interpreted as the initial velocity that the desorbed ion would have if no collision occurs in the plasma. The improved method interprets v_0 as the “final” initial velocity, i.e., as the velocity that the desorbed ions have when the plasma free expansion starts and, effectively, collisions no longer occur. The new method allows also the determination of d_0 , the distance to the solid when the free expansion starts. The data fitting shows that the distance (d_0) has a linear dependence on the laser’s intensity. Extrapolation of these values gives $I = 0.69 \text{ G W cm}^{-2}$ as the minimum energy density necessary to produce high density plasma during the insulin ions desorption when using αCHCA matrix.

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