A SIMPLE MODEL FOR THE MALDI EFFECT DESCRIPTION

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RESUMO

A técnica MALDI atingiu grande popularidade desde a sua descoberta. Ela é uma técnica que proporciona resultados de forma rápida, acurada com grande sensibilidade. No entanto os processos Físicos que governam a dessorção de íons moleculares não estão totalmente entendidos. Neste trabalho, é apresentado um estudo realizado com um espectrômetro projetado e construído no Laboratório de Instrumentação e Partículas da USP utilizando a insulina humana. A intensidade dos picos no espectro de tempo de vôo foi estudada como função da concentração relativa entre a insulina e a matriz. Um modelo simples para dessorção foi desenvolvido para descrever aspectos gerais do processo MALDI e da emissão iônica.

ABSTRACT

Since its discovery the MALDI technique has achieved a great popularity. It is a fast, sensitive and accurate analytical tool. However, the Physical processes underlying the ion emission are still unclear. In this work, a characterization of a home-made MALDI instrument, using human insulin as a probe, is presented. The time of flight peaks intensities were studied as a function of the relative concentration of the analyte – matrix system. A simple model is developed to describe general features of the MALDI process and of the ion emission.

1. INTRODUCTION

Time of flight mass spectrometry has an increasingly importance in modern science. Its combination with novel ion production techniques, like Matrix Assisted Laser Desorption Ionization (MALDI) and Electro Spray Ionization (ESI), introduced new tools for many different research areas. The successful achievements on protein analysis, including identification and sequencing, by MALDI are one of the important applications of the technique for which it received the Chemistry Nobel prize in 2002 [1].

In MALDI, a pulsed laser is used to bombard a composite solid that contains analyte molecules (proteins, for instance) embedded in a light absorbing substance (matrix) [2]. In general, a high power UV-laser is used and the UV absorbing substance is an aromatic compound. The energy deposition process induced by the laser light leads to ion ejection (or desorption) from the solid. The time of flight (TOF)

spectrometry permits a compositional analysis of the bombarded solid through mass to time calibration.

From the basic Physics point of view, the description of the MALDI process is highly complex. Standard sample preparation protocols (see next session) produce inhomogeneous polycrystalline targets. Also the laser spot is non-uniform in space and time so the energy deposition varies from shot to shot. As a consequence, reproducibility is difficult and only the general behaviour of averaged physical quantities can be described [3].

In this work, the signal of human insulin ions was studied as a function of the relative analyte-matrix concentration. A first principles model, that includes the basics of the MALDI process, is developed and used to describe the data.

2. EXPERIMENTAL

Insulin has become a standard for MALDI-TOF analysis. It forms clusters with high masses (figure 1), permitting not only an accurate mass to time calibration, but also a way to study the efficiency and resolution of a given spectrometer for a wide range of mass. The matrices used for the present characterization were the 2,5-dihydroxy-benzoic acid (DHB) and the 3,5-dimetoxy-4-hydroxy-cinnamic acid (sinapinic acid) at different concentrations.
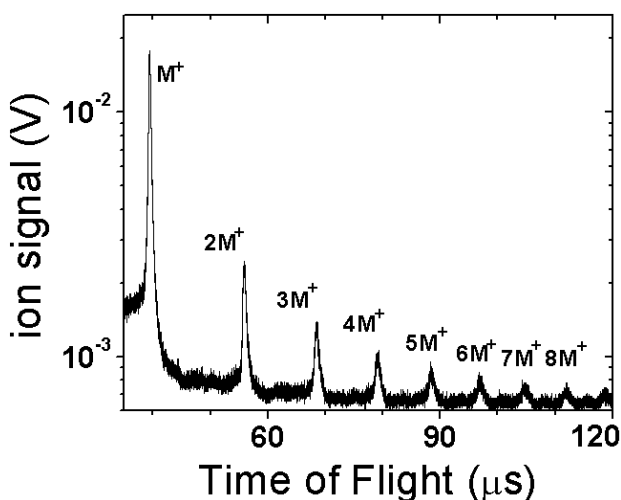

Figure 1 – Time of flight spectrum of a 100 pmol human insulin sample (insulin mass ~ 5806u).

The matrices solutions were prepared by diluting the DHB powder on trifluoroacetic acid (0.1%) + acetonitrile with a 2:1 proportion. The matrix concentrations was varied for

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5×10^{-2} mol/l up to saturation (3 mol/l). Similar procedure was adopted for the sinapinic acid from 5×10^{-5} mol/l up to 3×10^{-2} mol/l (saturated solution). For insulin, the concentration was varied from 1×10^{-6} mol/l up to 7×10^{-4} mol/l. The samples for MALDI-TOF analysis were produced dropping 1 μl of each solution on a metal sample holder and letting the mixture dry in air. For the least concentrated insulin solution there is 1 picomol of analyte in the sample. Figure 2 present the insulin samples picture, at different concentrations, after crystallization with sinapinic acid.

The sample holder was inserted in the vacuum chamber and the time of flight spectra are collected in a way described elsewhere [4]. The sample holder could be moved in the XY directions during the experiments so the laser spot (~ 0.2 mm^2) probes different areas of the sample. Everything was monitored by an optical system coupled to a TV camera. Higher ion signals are obtained when the laser spot hits the border of a crystal at the sample (white to black regions on figure 2b). For lower insulin concentrations the cluster ions presented lower signals. This suggests that the clusters are produced during the sample crystallization process.
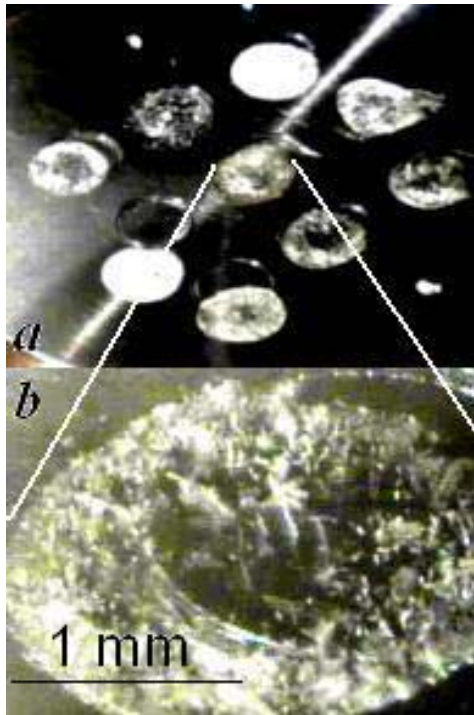

Figure 2 - *a***) Sample holder and** *b***) detailed view of the insulin sample mixed with sinapinic acid**

The peak amplitudes for the three first insulin clusters measured in the spectra were analysed as function of the relative concentration, x, of insulin (ρ_i) to matrix (ρ_M) and the data are presented in figures 3. One sees that there is an optimal value of $x = \rho_i / \rho_M$, which maximizes the ion signal, for different matrices. The presence of a maximum for all studied cases is a clear evidence of the MALDI effect. Small values of x represent a small number of analyte molecules in the sample and so the ion signal tends to zero since there are no analyte molecules to be desorbed. On the other hand, high values of x represent the situation where there is a

small number of matrix molecules in the sample. Correspondingly, the laser light is poorly absorbed and the ion signal tends to decrease as x becomes large.
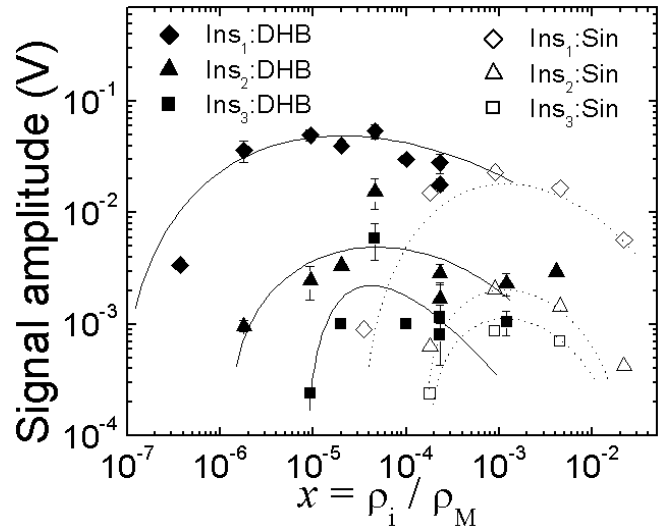

Figure 3 – Signal amplitudes of the three first insulin clusters as a function of the relative concentration of analyte to matrix $x = \rho_i / \rho_M$ for the two matrices. The lines are to guide the eye.

3. MODEL FOR MALDI ION DESORPTION

A simple model was developed to describe the MALDI effect appearing in figure 3. It is explained with the help of figure 4. The laser pulse intensity is not uniform and a Gaussian intensity profile $\sim \exp(-r^2 / r_0^2)$ was assumed [5]. The laser energy absorption by matrix molecules was described using the Beer's law : $I = I_0 \exp(-\mu \rho_M z)$ where z is the penetration depth and μ is the molar absorption coefficient of the matrix. It is possible to define a fluence density, $i(r,z)$, combining these two expressions:

$$i(r,z) = i_o \exp(-r^2 / r_0^2 - \mu \rho_M z) \qquad (1)$$

The i_0 value can be found integrating $i(r,z)$ over the whole space and equating the result to the incident fluence I_0.

$$I_0 = \int_0^\infty \int_0^\infty 2\pi r\, i(r,z)\, dr\, dz \qquad (2)$$

The assumption that the fluence density has a minimum value, i_c, for desorption to take place leads to the definition of a critical volume, V_c, characterized by a critical radius r_c and a critical penetration depth z_c. The number of insulin molecules embedded in the critical volume, where $i(r,z) > i_c$, is given by $\rho_i V_c$. An effective laser fluence for desorption, I_{eff}, can be defined by integrating $i(r,z)$ inside V_c.

An equation relating r_c and z can be found making $i(r_c,z) = i_c$ in eq (1) resulting in $r_c = r_0\sqrt{A - Bz}$ with $A = \ln(i_0/i_c)$ and $B = \mu\,\rho_M$. For I_{eff} one has:

$$I_{eff} = \int_o^{z_c} \int_0^{r_c(z)} 2\pi r\, i(r,z)\, dr\, dz \qquad (3)$$
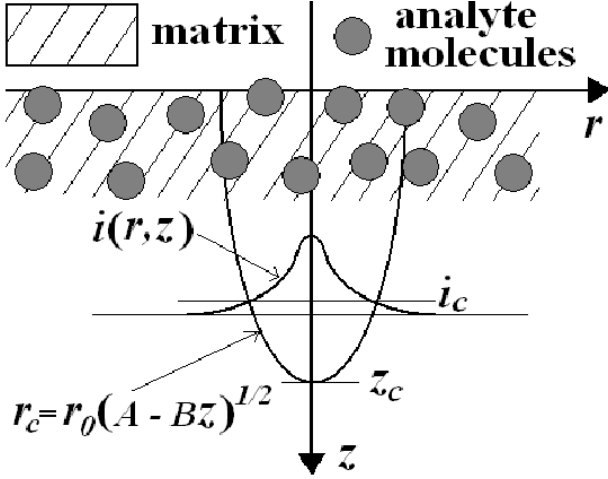

Figure 4 - Schematic view of the laser fluence density in a MALDI sample. The A and B values are $A = \ln(i_0/i_c)$ and $B = \mu\,\rho_M$

The ion signal, Y, should be proportional to the product $\rho_i V_c I_{eff}$. Writing in terms of the relative insulin-matrix concentration one has:

$$Y = \Lambda \frac{I_0^2}{2 i_c} \rho_i \left(\frac{ln\,\xi}{\xi}\right)^2 (\xi - ln\,\xi - 1) \qquad (4)$$

with $\xi = \rho_i x/\rho_c$, $\rho_c = \pi r_0^2 i_c / \mu I_0$ and $x = \rho_i / \rho_M$.

4. MODEL PREDICTIONS

The results of the calculation using eq (4) for the insulin ions are shown in figure 5a, for the DHB matrix, and in figure 5b, for sinnapinic acid, considering different ρ_c values. The best fit values are $\rho_c = 5 \times 10^{-4}$ mol / l for DHB and for sinapinic acid one has $\rho_c = 0.06$ mol / l. The ρ_c values are related to the other parameters of the model according to

For sinapinic acid one has $\mu = 4\times 10^3$ l / mol.cm and the laser parameters were estimated to be $\pi r_0^2 = 10^{-3}$ mm^2 and $I_0 = 0.12$ J/cm^2.
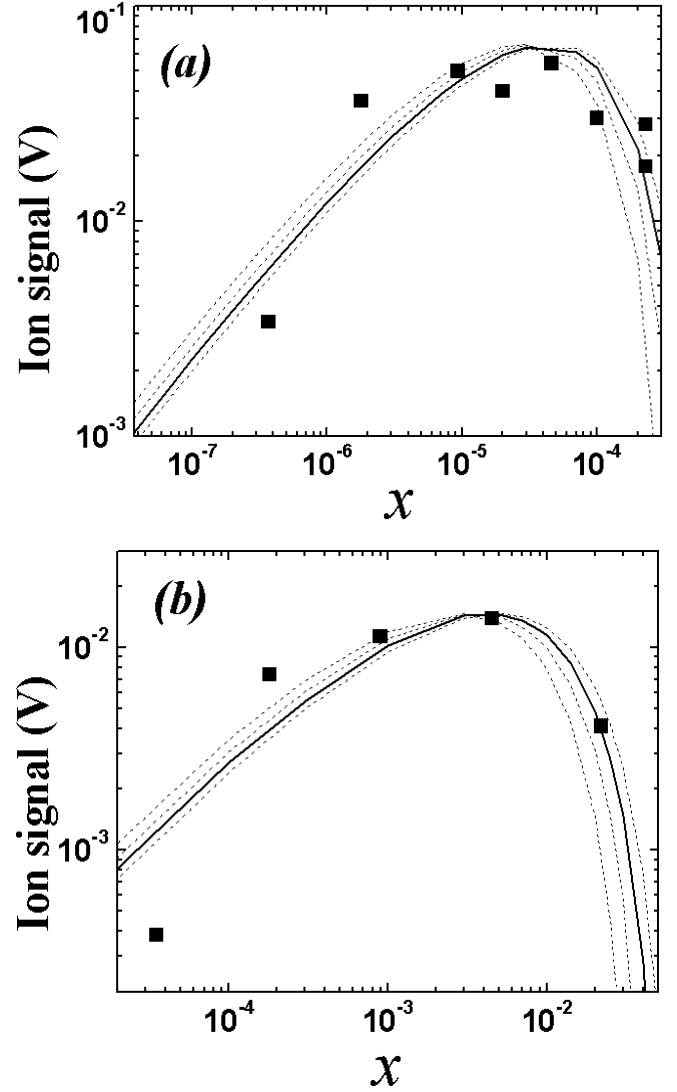

Figure 5 - Data for insulin ions, appearing in figure 3 (ins$_1$), together with the curves described by eq (4). *(a)* for the DHB matrix and *(b)* for sinnapinic acid.

Considering these estimations and $\rho_M = 3$ mol / l one can extract $i_c = 2.8 \times 10^6$ J/cm^5. Other important output values are the critical volume

$$V_c = \pi r_0^2 \frac{ln^2(i_0/i_c)}{2\mu\rho_M} \qquad (6)$$

and the effective intensity

$$I_{eff} = I_0\left[1 - \frac{i_c}{i_0}\left(1 + ln\frac{i_0}{i_c}\right)\right] \qquad (7)$$

The model predicts an intensity threshold, I_0^{th}, which is a well known feature of MALDI analysis [6]. This is found for $i_0 = i_c$ corresponding to $I_{eff} = 0$. One has

$$I_0^{th} = \frac{\pi r_0^2}{\mu\rho_M} i_c = I_0\frac{\rho_c}{\rho_M} \qquad (8)$$

According to the above formulae the values of the critical volume and of the intensity threshold are $V_c = 6400$ μm^3 and $I_0^{th} = 25 \times 10^{-4}$ J/cm^2 respectively. These values are in the same order of magnitude to those observed in other experiments. Measurements made with scanning electron microscopy showed that the diameter of the craters left by laser pulse impact to be ~ 14000 μm^3 [7]. The ion signal measurement for varying incident intensity, I_0, showed an intensity threshold $I_0^{th} \sim 20 \times 10^{-4}$ J/cm^2 [6]. The value of V_c is proportional to the laser parameter πr_0^2 which was actually not measured in this work and depends on focusing conditions. A better estimation of this parameter could improve the agreement between scanning electron microscopy results and the model prediction. On the other hand, the value of I_0^{th} is independent of πr_0^2 and there is a quite good agreement with the calculation made with the fitting parameter ρ_c.

5. CONCLUSIONS

The insulin ion signal was measured as a function of the relative concentration of analyte − matrix in a home made MALDI-TOF instrument. A first principles model was able to describe the MALDI effect, *i.e.* the ion signal is maximum for a certain value of the relative concentration *x*, and outputs reasonable values for the crater dimension and fluence threshold. The total energy deposited by the laser pulse is not completely used to produce ion emission and not distributed uniformly inside the solid. That is the reason this model uses the energy density, which is proportional to the fluence density $i(r,z)$, for the ion signal description. According to this assumption, the ion emission process can only occur when the energy deposited locally in a small volume is higher than a certain critical value. The model presented here is in fact an adaptation of a former description to the ion emission induced by MeV particles [8].

6. REFERENCES

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