Understanding and Optimizing the MALDI Process using a Heated Sample Stage: a 2,5-Dihydroxybenzoic Acid Study[†]

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Since its demonstration in 1991 as a suitable matrix for matrix-assisted laser desorption/ionization (MALDI) mass spectrometry, 2,5-dihydroxybenzoic acid (2,5-DHB) has been the subject of many investigations of both its solid-state and gas-phase physical and chemical properties. In this regard 2,5-DHB may be the most thoroughly studied of all MALDI matrices. In the past, experiments were conducted on the laser fluence threshold from room temperature down to -100 °C. Using the typical nitrogen gas laser at 337 nm it was found that the laser fluence to directly desorb radical molecular ions increased as the temperature decreased and the fluence required to convert desorbed neutral molecules to radical molecular ions, in the gas-phase by photoionization with 248 nm laser, also increased as temperature decreased. These observations were interpreted using a quasi-thermal model based on the Arrhenius equation with the temperature augmented by the addition of the heat imparted to the sample by the laser. In this work temperature was raised from +20 °C to +160 °C and the laser-desorbed ion production was measured as a function of temperature by laser desorption/ionization (LDI) time-of-flight (TOF) mass spectrometry.

DHB was purified by re-crystallization (2X) from a solution of 90 % distilled water and 10 % methanol by volume fraction.   The solution was heated to 55 °C and placed in a freezer at –10 °C for 1.5 h followed by filtration and washing at room temperature. Single crystals were grown by dissolving 1 g of purified DHB in 20 mL of distilled water at 70 °C then placing the capped vial in a water bath at 36 °C overnight. The temperature of the water bath was then reduced 1.5 °C/d for 14 d. The largest crystal facet was always used in the ablation experiments. The LDI experiments were conducted on a reflectron TOF mass spectrometer. The sample is held at ground during ablation with a nitrogen laser at 337 nm. After the delay, a potential is applied to the sample plate which repels the ions into the flight tube. The mass spectrometer was modified allow resistive heating of the sample plate to 200 °C. The instrument is also equipped with a residual gas analyzer (RGA) quadrupole mass spectrometer that uses an electron impact ionization source.   Single-crystal samples were mounted on the target using conductive silver paste adhesive. A quartz crystal microbalance (QCM) was mounted in a rough-pumped vacuum bell jar and single crystals were heated using thin resistive heating elements embedded in a polyimide film. The thermal desorption under vacuum was measured as the temperature was raised from 20 °C to 200 °C.
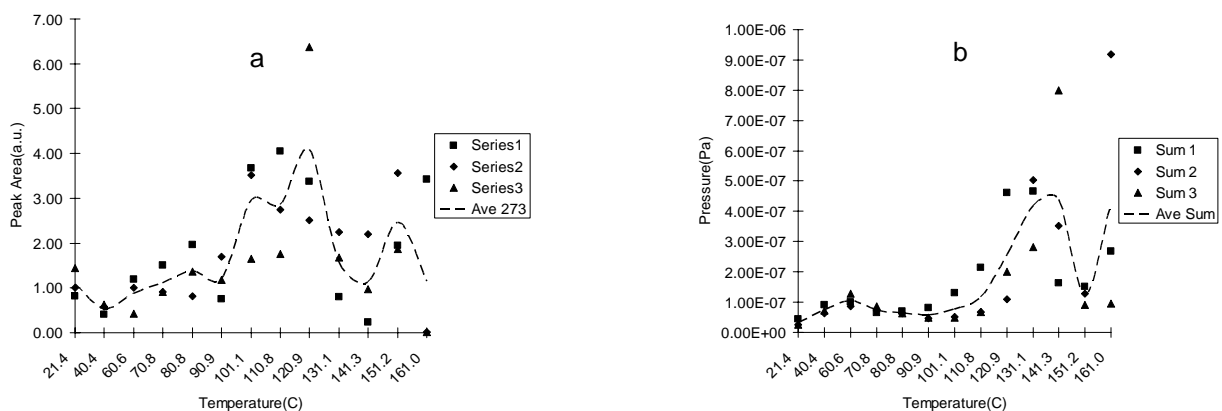

Figure 1: The sum of all 4 ion peak areas at each temperature for three series of experiments from: a) LDI-TOF MS and b) Neutral production monitored by the RGA.
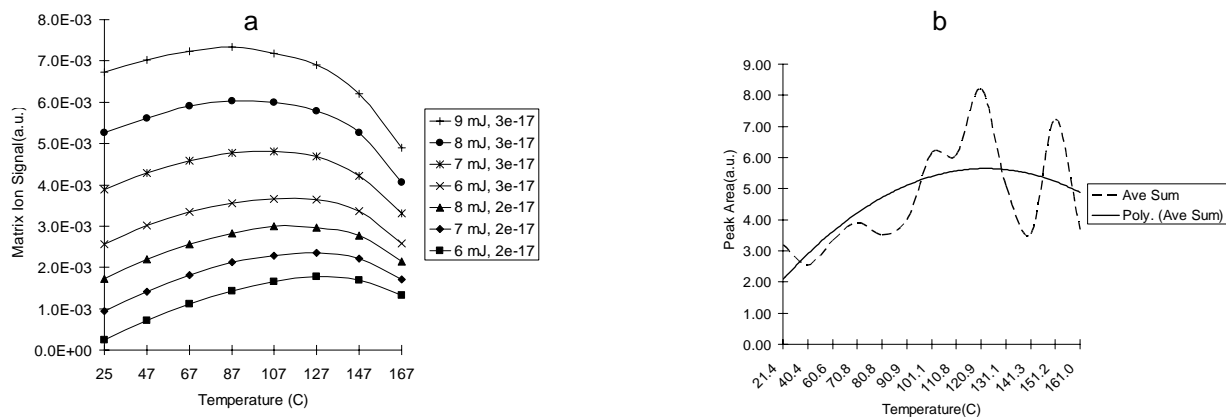
Figure 2: a) Calculation of the LDI process from the two step theory at constant fluences of 6 mJ to 9 mJ as a function of temperature at two different photon absorption cross-sections. b) The average of the summed peak areas for the three series of experiments with a second degree polynomial fitted to the line.

There are several peaks observed in the typical LDI-TOF mass spectrum taken from a 2,5-DHB single crystal and can be described as: a water-loss product ([DHB+H-H_2O]$^+$) from protonated DHB, the oxonium ion, at 137 u; the molecular ion of DHB ([DHB]$^{+\cdot}$) appearing at 154 u; protonated DHB ([DHB+H]$^+$) at 155 u, a dimer of DHB formed by condensation/dehydration (i.e. [2DHB – 2H_2O+H]$^+$) at 273 u, which can also be described as the protonated di-esterification product; and finally a trimer can also be formed from the tri-esterification of DHB ([3DHB – 3H_2O+H]$^+$) and can be seen at 409 u. The peaks produced by LDI at constant fluence were monitored using the TOF instrument and the total peak area summed for three series of experiments. The values were plotted and a graph of the average was overlaid to display the behavior of ion production as a function of temperature (Figure 1a). Figure 1 does not represent the quantitation of ion production at any given temperature and the line is to merely guide the eye. Each series of experiments was performed on a different crystal; therefore, it is difficult to quantitate the error in ion production. It can be seen that ion production remains relatively constant until a sharp increase at 80 °C until reaching a maximum at 120 °C. Ion production then decreases to a minimal value at 140 °C, approximately equal to that observed below 80 °C, then increases to a second maximum at 150 °C. Ion production beyond 150 °C falls to a minimum due to destruction of the crystal. Figure 2b displays neutral ion production as a function of temperature measured by the RGA from the 4 ions that are the known or accepted fragments of DHB at 70 eV and they are observed at 80 u, 108 u, 136 u and 154 u. The fragment ions have the structures [DHB – H_2O – 2CO]$^{+\cdot}$, [DHB – H_2O – CO]$^{+\cdot}$, [DHB – H_2O]$^{+\cdot}$, and [DHB]$^{+\cdot}$, respectively. The peak areas at each temperature can be summed to yield a plot of total neutral production as a function of temperature. Neutral production reaches its maximum at 140 °C, the same temperature at which LDI ion production is at its minimum. It is believed that this large increase in gaseous neutrals reduces ion production. Gas-phase ion neutralization (recombination) and charge stripping may cause ion production to decrease at elevated temperatures. Once the molecular ion is formed by multiphoton ionization, energy pooling or disproportionation, it is accelerated through the plume which contains positive and negative ions, free electrons and neutrals. It is possible at elevated temperatures, when the molecules are more thermally excited increasing their motion that the radical cations can recombine with free electrons or collide with radical anions, causing an electron to transfer neutralizing the radical. Ions that are protonated can go through multiple collisions in the source resulting in fragmentation or charge stripping. Both processes are possible, especially if increased sublimation at elevated temperatures creates a cloud of neutrals above the sample that the expanding plume and the ions must pass through upon desorption and acceleration. QCM experiments confirm the rapid thermal desorption at elevated temperature, which maximizes at 140 °C (not shown). Figure 2a shows the calculation of the LDI process from the two step theory at constant fluences of 6 mJ to 9 mJ as a function of temperature at two different photon absorption cross-sections. If the curve shown in Figure 1a is fitted with a second degree polynomial (Figure 2b), it can be seen that ion production from the LDI experiments resembles the predicted behavior at lower laser fluences (6mJ to 7 mJ) with a smaller cross section (2e-17). This model might be an accurate depiction of the general processes occurring during the LDI process because the experimental data shown was acquired just above threshold.