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The origin of electrons in MALDI and their use for sympathetic cooling of negative ions in FTICR

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Abstract

In MALDI, electrons were found to be formed by the photoelectric effect on the metal/organic matrix interface. The thin layer of organic matrix reduces the metal work function, resulting in a high electron yield in a field-free MALDI source. These electrons were detected in a FTICR mass spectrometer indirectly, by the observation of SF_6^- ions, produced by electron capture. The number and velocity distribution of these electrons were estimated. It is shown that the yield of electrons strongly depends on the thickness of the organic sample. In the case of non-metallic substrates or thick matrix layers, much fewer electrons were detected. Electrons confined in a FTICR trap can cool negative ions produced by MALDI by long range Coulomb interaction, thus increasing the resolving power and sensitivity. (Int J Mass Spectrom 220 (2002) 11–19)

Keywords: MALDI; Electrons; FTICR MS; Ion cooling

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1. Introduction

The yield of charged species in matrix-assisted laser desorption/ionization (MALDI), is rather low, on the order of 10^{-4} [1,2]. In negative mode, a signal for electrons is normally detected in addition to negative ions from the MALDI matrix and the analyte. The origin of these electrons has been unclear. It has, for example, been reasoned that they are produced by photoionization of matrix [3] but photoionization has been shown to be of minor importance in MALDI [4]. The fate of electrons in a MALDI plume is also of interest. For example, are they able to produce negative ions by electron capture? Will they neutralize positive ions thus decreasing the overall useful ions yield? Are they responsible, as it has been suggested [3], to reduce multiply charged analyte positive ions to charge state $z = 1$? The possibility that must be considered is that electrons are simply extracted from the plume by the high acceleration field usually present in MALDI ion sources. Some MALDI experiments, however, are carried out field-free, e.g., in delayed extraction time-of-flight MS, and, in particular, in internal source MALDI Fourier transform ion cyclotron resonance (FTICR) mass spectrometry [5,6]. Our internal source FTICR set-up permitted us to study the origin and effects of electrons in MALDI in more detail. One key idea of this work was to use these electrons for cooling of negative MALDI ions, thus improving sensitivity and resolution.

A variety of experimental approaches have been pursued for improving the sensitivity and efficiency of

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FTICR mass spectrometry [7–9]. Reduction of the ion translational energy leads to a decrease in ion axial oscillation amplitudes and their axial energy distribution, resulting in an increase in resolution and relative peak intensities. A number of experimental techniques have been developed for cooling of ions prior the excitation and detection events. The buffer gas cooling is a widely used method [10,11] but it is not always convenient because of the low pressure requirements of FTICR. Other techniques include adiabatic cooling [12,13], evaporative cooling [14], radiative cooling [15], resistive cooling [15], and laser cooling [16].

Electrons trapped in a magnetic field have a very high cyclotron frequency and are cooled down fast by electromagnetic emission. This radiative decay is the dominant energy relaxation mechanism for electrons in a strong magnetic field at low pressure. Electrons can be cooled to near room temperature in a few milliseconds. In a "sympathetic" cooling, cold electrons (or ions) cool translationally hot ions by electron–ion (or ion–ion) long range Coulomb interactions [17,18]. The exponential time constant for energy damping by sympathetic cooling was calculated [19]. This constant is inversely proportional to the number density of the cold ions (electrons) and proportional to the product of their masses. For example, it was shown that the time constant for sympathetic cooling of Au^- ions by electrons produced by an electron gun (number density $10^7\,cm^{-3}$) is close to 2 s [20]. The application of sympathetic cooling for FTICR mass spectrometry has been demonstrated recently [20,21]. Sympathetic cooling of trapped negative ions by self-cooled electrons in a MALDI FTICR has also been shown [22].

2. Experimental

Experiments were performed on a Fourier transform ion cyclotron resonance mass spectrometer that consists of a home-built vacuum system with a 4.7 T superconducting magnet (Bruker, Fällanden, Switzerland) and an Odyssey data acquisition system (Finnigan FT/MS, Madison, WI). A home-built cylindrical open cell 6 cm in diameter was used for trapping of MALDI ions [6,23]. The instrument has an internal MALDI source located in the low-pressure region 5 cm from the ICR cell. For laser desorption, a Nd:YAG laser (Continuum, Minilite ML-10) operated at 355 nm was employed. Laser desorption/ionization was accomplished at a pulse energy of about 20 μJ. MALDI samples were prepared using the standard "dried droplet" method. 2,5-Dihydroxy benzoic acid (DHB) and trihydroxyacetophenone monohydrate (THAP) were obtained from Fluka (Buchs, Switzerland), and sulfur hexafluoride was purchased from Aldrich (Buchs, Switzerland).

The MALDI matrix layer was prepared by the "dried droplet" method, which resulted in many small crystallites on the sample surface [24]. Usually more than 100 shots were utilized in one experiment. We refer to "thin" matrix layers for cases where the substrate is partially exposed to the laser beam, and to "thick" matrix layers for cases where the target is completely covered for the duration of the experiment. We assume that even for "thin" samples, some monolayers of organic matter are present on the target surface, but the laser can penetrate through them. On the other hand, consider a situation where the laser beam is focused onto a single large crystallite: the crater depth (several micrometer per laser shot in our experiment, increasing with the number of laser shots [25]) would then be much less than the thickness of such a crystallite, and we can consider this to be a "thick" sample. Typically, we used depositions with a final thickness ≥1 mm for the "thick" samples to ensure that the target surface is not visible for thousands of laser shots. However, for both cases it is impossible to provide a number for the sample thickness, due to the erratic and heterogeneous nature of crystalline MALDI samples.

Upon laser desorption/ionization, the ions and electrons drifted in a field-free region into the open cell and became trapped. After an appropriate reaction delay period, analyte ions were excited by chirp excitation and detected. For each measurement 20 scans were co-added. Gated trapping was used for all experiments. The typical pressure in the cell region was 5×10^{-9} Torr. The SF_6 pressure was 1×10^{-6} Torr.

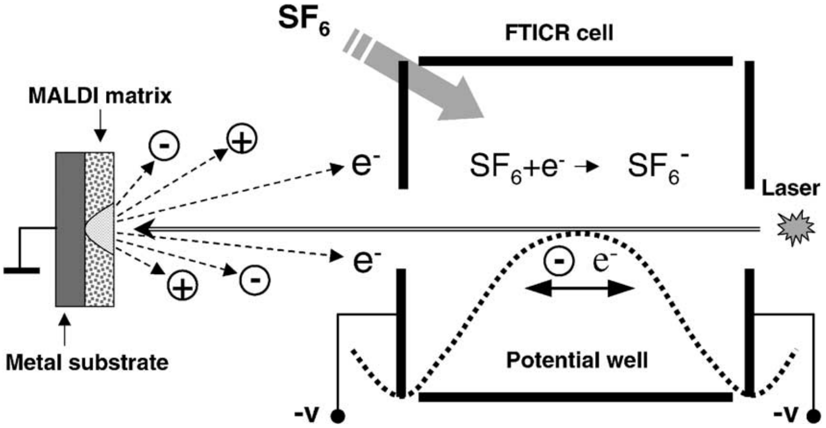

Fig. 1. Trapping of electrons and negative ions in a FTICR cell.

3. Results and discussion

3.1. Origin of electrons in MALDI

Positive and negative ions, neutrals and electrons are emitted in a MALDI desorption process (Fig. 1). Negative ions and electrons can be trapped in a FTICR cell if a negative potential is applied to the trap electrodes. In MALDI, all ions regardless of their mass have roughly the same velocity and can be trapped in the cell after an appropriate drift delay period [26]. This drift delay time depends only on the ion velocity and distance from the target to the cell if the ion motion occurs in the absence of an electric field. The optimum drift delay time for our instrument is 80 μs, corresponding to an ion velocity of 800 m/s. Electrons cannot be detected directly in FTICR, due to their low mass and high cyclotron frequency. We expected that electrons would have much higher velocity and could be trapped at a shorter drift delay time. In order to check the presence of electrons in the cell, SF_6 gas was added to the vacuum system (Fig. 1). Sulfur hexafluoride has a high (1×10^{-15} cm^2) cross-section for low energy electron capture. We assume that the final number of generated negative SF_6^- ions is proportional to the number of trapped electrons.

SF_6 gas was added to vacuum system through a pulsed valve (general valve dual-solenoid pulsed valve) after the electrons had been trapped into the FTICR cell.

The spectra in Fig. 2 show the SF_6^- signal at different drift delay times. The sample was a DHB dried droplet prepared on a stainless steel target ("thin" matrix). At a drift delay time of 10 μs a strong signal of SF_6^- is observed. At longer drift delay times the SF_6^- signal becomes smaller, and signals from matrix ions ($[DHB]^-$, $[DHB–H]^-$, and $[DHB–2H]^-$) appear in the spectrum (Fig. 2B and C). The summary of this experiment is presented in Fig. 3. The maximum signal of SF_6^- ions, corresponding to the maximum number of trapped electrons was found at a short drift delay time of 10 μs. The most efficient trapping of MALDI ions was at 80 μs. This means the electron velocity distribution peaks at >8000 m/s, and that electrons have a rather broad velocity distribution. It was not possible to set a shorter drift time delay to measure the electron velocity distribution entirely because of limitations in the instrument control electronics. The energy of electrons was estimated to lie within the range from 0 to 2 eV from a measurement of the SF_6^- ion appearance curve [27]. Surprisingly, we found that the number of electrons in our experiment exceeds the number of negative and positive ions. This number can be estimated from the area under the curves in Fig. 3.

In order to find the source of electrons an experiment with a pure metal target was carried out. We

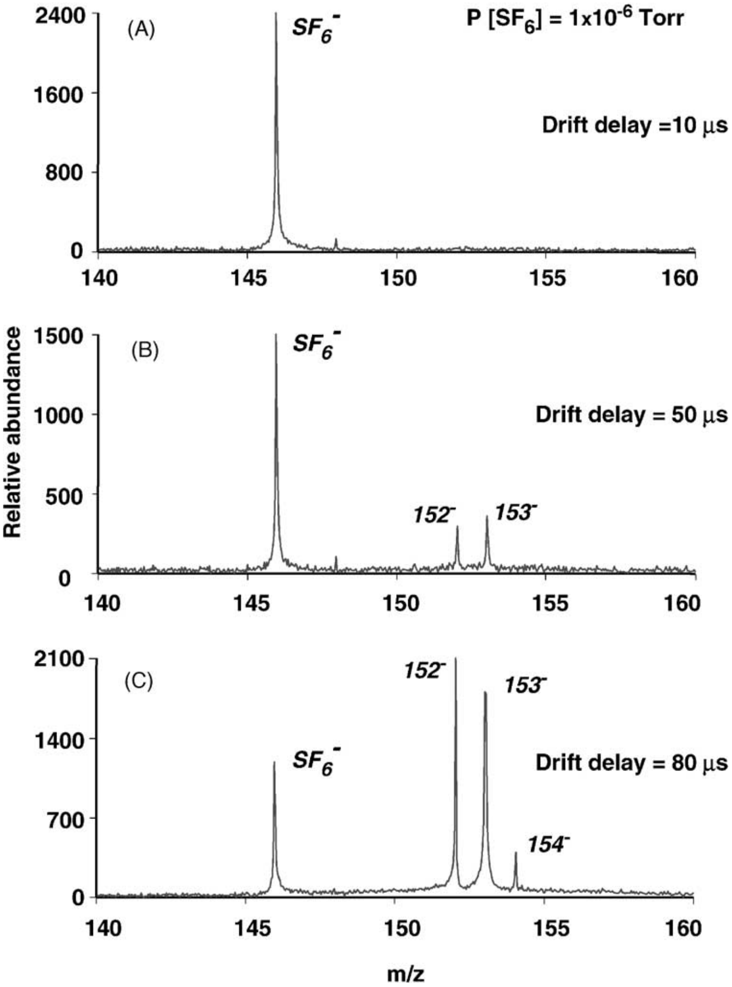

Fig. 2. Mass spectra of SF_6^- and negative DHB ions at different drift times.

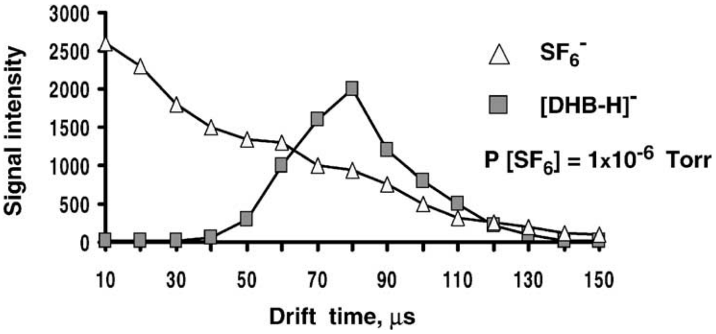

Fig. 3. Relative signal intensity as a function of the drift delay time for SF_6^- and negative matrix ions ($[DHB–H]^-$) for a thin DHB sample on a metal substrate.

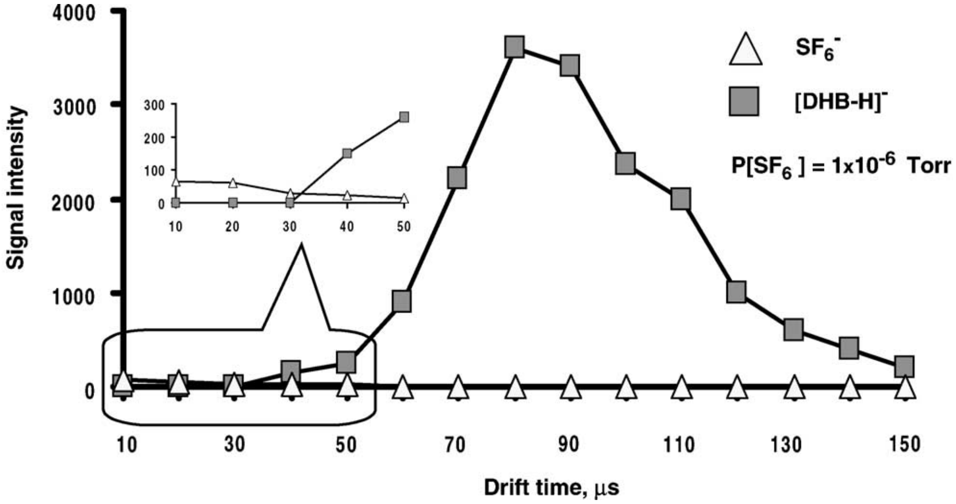

Fig. 4. Relative signal intensity as a function of the drift delay time for SF_6^- and negative matrix ions ($[DHB–H]^-$) for a thick (>1 mm) DHB sample on a metal substrate.

found that there were no photoelectrons emitted from the metal. The reason for this is the work function of the metal (stainless steel), which is greater than the photon energy. To estimate the number of photoelectrons from the matrix, experiments with thick (>1 mm) matrix and with matrix on a Teflon substrate were done. Fig. 4 shows the result obtained from a thick DHB sample on a metal substrate. There are very few photoelectrons produced from the matrix, which can be detected via SF_6^- signal at a short drift delay time. An identical result was obtained when a thin layer of DHB matrix was placed on Teflon substrate. The total number of SF_6^- ions was less than 1% of the number of negative ions.

In general, we found that electrons can only be produced from the metal substrate when it is covered with a thin layer of matrix. The electron yield strongly depends on the thickness of the matrix. It has a maximum at very low matrix thickness (several nanometer) and decreases when the matrix thickness is increased. A possible explanation of this effect is that the metal/organic interface has lower work function as compared to a pure metal [28,29]. A thin layer of organic material shifts the Fermi level of the metal and decreases the work function.

Photoelectrons ejected from the metal/matrix interface may play role in ion formation. In an internal MALDI FTICR experiment all particles move into the cell in the absence of an electric field. Positive ions, negative ions, and some of the electrons can interact with each other during the drift time. Neutralization is probably the dominant process such that mostly negatively charged ions are survived. This explains some puzzling observations in internal MALDI FTICR experiments, i.e., that the total signal of negative ions usually exceeds that of positive ions.

Fig. 5 shows a comparison of positive and negative mode spectra for thin and thick matrixes. The presence of electrons apparently results in a reduction of the positive ion signal in the experiment with a thin matrix. Thus, the positive mode spectrum shows only very low signals. In the negative mode, on the other hand, there is an additional peak of $[DHB]^-$ due to electron attachment. When a thick matrix layer was used, there are only few photoelectrons generated from the matrix, which does not change the charge balance; in this case, the number of positive ions was found to be similar to the number of negative ions.

It should be noted that the situation is different in a standard MALDI TOF experiment. There is strong

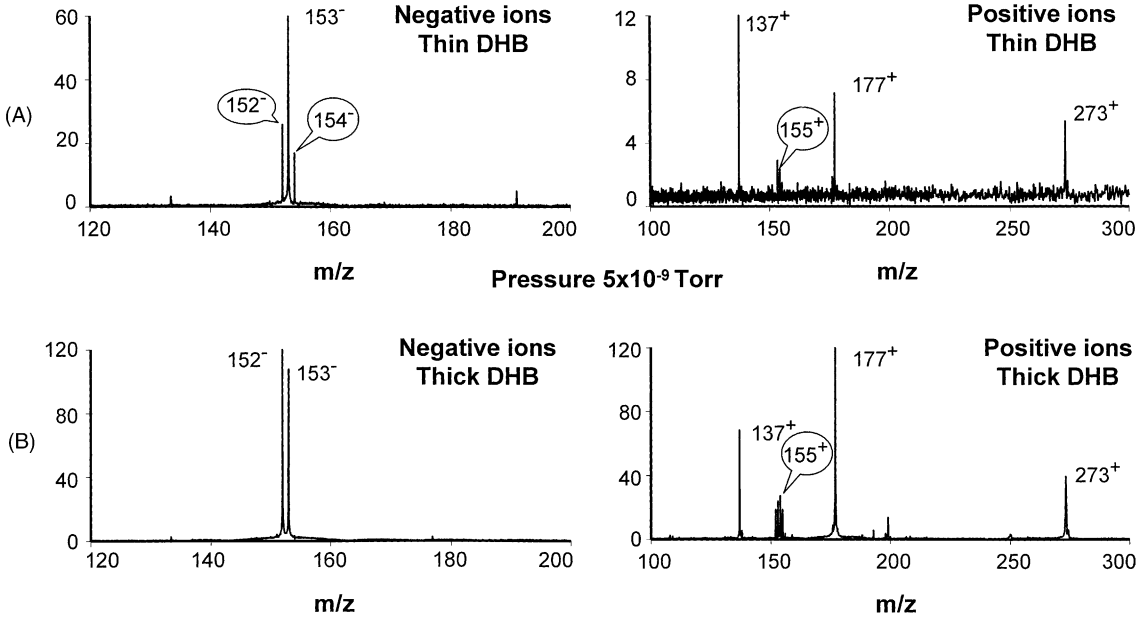

Fig. 5. Comparison of positive and negative mode mass spectra for thin and thick matrixes layers. (A) Thin DHB matrix; (B) thick DHB matrix.

extraction field (~10^6 V/m) in a TOF mass spectrometer ion source. This field will extract electrons from the plume in negative mode, while it accelerates them onto the target in positive mode. The Coulomb force for positive ions and electrons formed at a distance of 1 μm is weaker than the force from the extraction field. It is possible that for delayed extraction experiments some neutralization of positively charged ions will take place.

3.2. Electron cooling of negative MALDI ions

As follows from the previous discussion, electron cooling in FTICR will occur when normal, thin layers of matrix are employed. The electron yield is negligible from thick (>1 mm) organic samples. The maximum of the electron velocity distribution was found to be in the >8000 m/s range and the optimum drift delay time that could be set for trapping electrons was less than 10 μs. However, as follows from the Fig. 3, there are still a lot of electrons with a velocity similar to the velocity of the ions. These low energy electrons are always trapped together with negative ions and can influence the spectrum.

In the electron cooling experiment shown here, some of the faster electrons were trapped in the cell first, and the "gate" was opened again to admit slowly moving negative ions into the same trap. Electrons were trapped in a −1 V potential, and after the ion introduction the "gate" was closed and trap potentials were set to −3 V.

Electrons can always be trapped regardless the drift time delay, as already discussed. In order to compare the spectra obtained with and without electrons in the cell the electrons were ejected from the trap by a quench pulse. The duration of this pulse was rather short (10 μs, ±9 V) in order to eject electrons only. Because of the difference in the axial velocity, electrons will be ejected much faster than ions.

Fig. 6 presents the signal intensity as a function of the cooling time for ions trapped together with

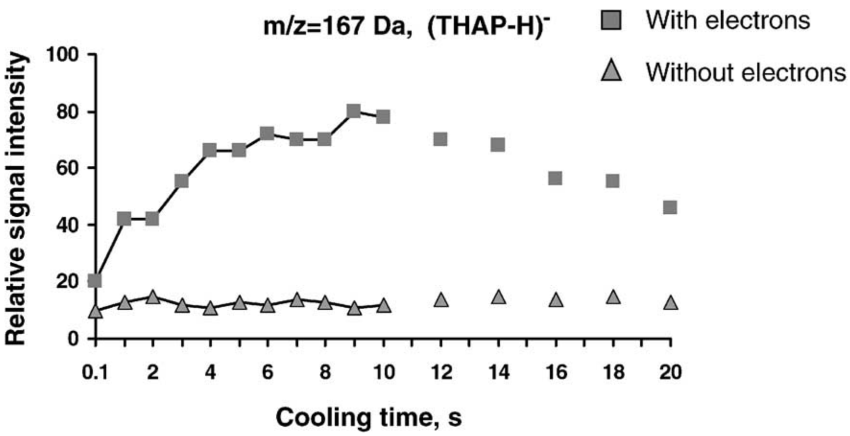

Fig. 6. Sympathetic cooling of negative THAP ions. The signal intensity as a function of the cooling time is shown.

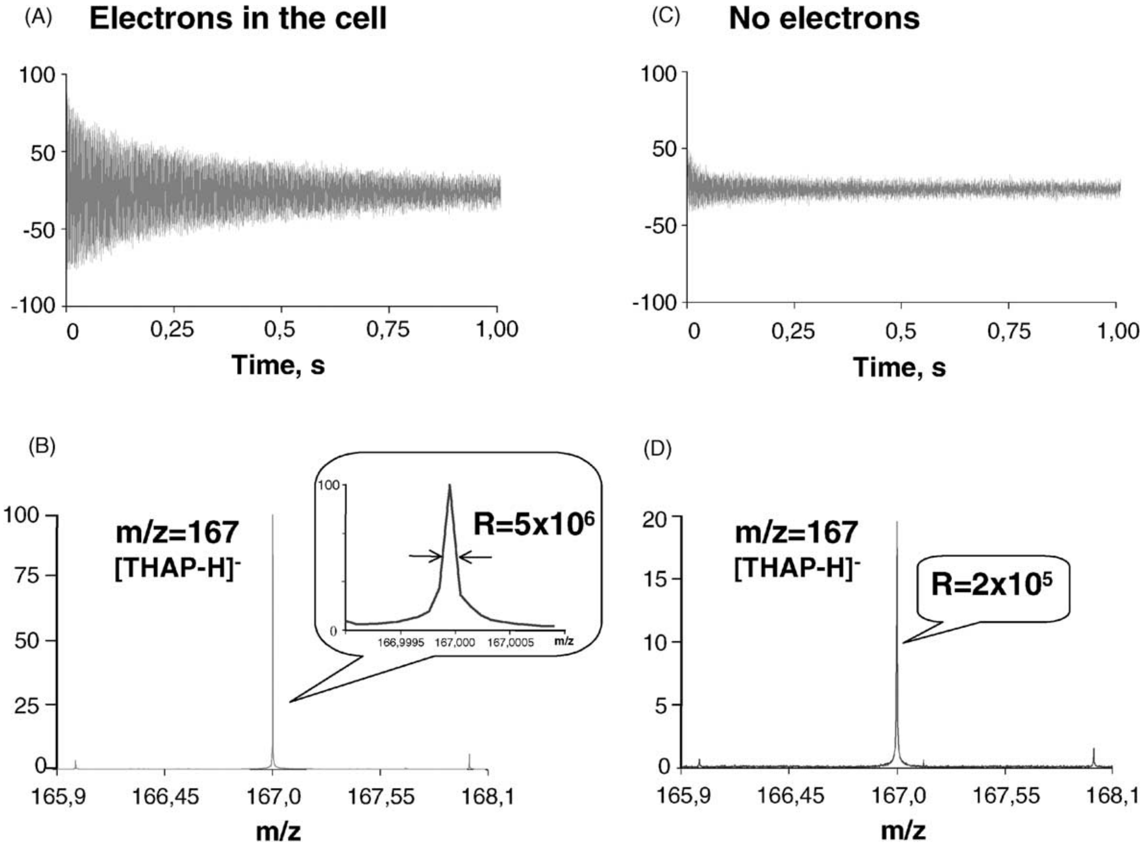

Fig. 7. Signals and mass spectra of negative THAP ions trapped with electrons (A and B) and without electrons (C and D).

electrons and ions only. For short cooling times there is no large difference in the signals. Sympathetic electron cooling becomes effective after several seconds of simultaneous trapping of both THAP negative ions and electrons. In contrast, buffer gas cooling is not effective at this low (5×10^{-9} Torr) pressure. Fig. 7 shows the signal and mass spectra of negative THAP ions trapped with and without electrons. The cooling period was 10 s. There is a better resolving power and higher signal-to-noise ratio when electrons were trapped together with ions. A very respectable resolving power, greater than 10^6, was obtained without any special efforts. The space charge can increase in an ICR cell due to the presence of electrons. However, no frequency shift due to space charge effects was observed in our experiment [30–32], indicating that the cell geometry is well suited to handle the higher number of negative charges present. The cooling period is inversely proportional to the electron number density, so it strongly depends on the number of trapped electrons [20]. Sympathetic cooling could be observed even in a standard negative mode MALDI FTICR experiment at long drift delay time where some electrons were still present, although the cooling time required will be much longer.

4. Conclusions and outlook

Metal substrates covered with a thin layer of organic material emit a photoelectrons under UV laser radiation. The electron yield depends on the thickness of the organic material and becomes negligible for thick (>1 mm) samples. These electrons play an important role in internal source MALDI FTICR experiments because they reduce the positive charge and disturb the charge balance. Free photoelectrons from the metal/organic interface can be used for sympathetic cooling of negative MALDI ions or for electron attachment ionization of neutrals with high electron affinity. Sympathetic cooling of negative ions results in an increase of resolution and sensitivity.

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