

C₆₀ ASSISTED LASER DESORPTION-IONIZATION MASS
SPECTROMETRY

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An application of C₆₀-assisted laser desorption/ionization mass spectrometry of two organic samples including insulin (5734 Da), bovine albumin (66 000 Da) and a heteropoly inorganic system: phosphotungstic acid H₃PO₄(WO₃)₁₂×H₂O, is presented. A nitrogen laser (337 nm) on a linear tandem time-of-flight mass spectrometer is used for the observation of the laser-power dependence of mass spectra.

1. Introduction

Application of mass spectrometry in chemical, biomedical materials and earth science has recently grown very much. In many cases, samples for a mass analysis need to be vaporized to allow ionization to take place [1-4]. If the sample is thermally stable then it can be heated and evaporated in the source of the mass spectrometer. For thermally unstable compounds, slow heating often leads to fragmentation. Alternatively, it is possible to heat the sample with lasers of nanosecond pulse duration so rapidly that the molecules desorb before they reach an equilibrium with the heated surface. In this process the desorption and ionization are performed as the one process. Kistemaker and co-workers were the first to demonstrate this

so-called "laser desorption" (LD) process in the source of a double-focusing mass spectrometer [5].

The development and evolution of LD-mass spectrometry has recently been reviewed by Miller [6] and in general this technique is usually combined with the time-of-flight (TOF) analysis and pulsed lasers. The laser desorption has been differentiated into separate ionization techniques usually referred to as matrix-assisted laser desorption and ionization (MALDI) [7], laser desorption with post ionization [8], substrate-assisted laser desorption [9], surface-enhanced neat desorption (SEND) [10] and ultrafine metal powder laser desorption [11]. In MALDI [7,12] the sample is diluted in matrix, often an aromatic acid, and the mixture is irradiated with a nanosecond laser pulse which vaporizes intact sample ions from the matrix. To be effective, matrix materials must possess several characteristics which allow:

- absorption of the laser radiation;
- evaporation of the matrix during the laser irradiation to transfer analyte molecules into the gas phase;
- protection of the analyte from thermal decomposition during the laser irradiation;
- proton or cation attachment to the sample of interest.

Many compounds possess the characteristics described above and they have been successfully used in MALDI experiments [12,13].

Since the discovery of buckminsterfullerene there has been much interest toward fullerene research. In this work, we present the performance of the fullerene, C₆₀, in assisting laser desorption/ionization mass spectrometry of two organic samples including insulin (5734 Da), bovine albumin (66000 Da) and one inorganic sample, phosphotungstic acid, H₃PO₄(WO₃)₁₂×H₂O [14,15].

2. *Experimental*

The linear tandem TOF mass spectrometer, used for the investigations presented here, was built at the University of New South Wales, Australia. The nitrogen laser (VSL-337, LSI Laser Science Inc.) of a wavelength of 337 nm, an output pulse length of 3 ns, has a maximum pulse energy specified as 120 μJ and a rectangular output shape of approximately 3 mm×8 mm. The angle of incidence upon the sample was 30° (see Fig. 1). The maximum power density of the focused laser used in this work was ≈ 70 MWcm⁻². This power was varied by defocusing the beam. An ion acceleration voltage of 9 kV was applied to the sample holder. The ions were detected using a three multichannel plate (Galileo Electro-Optics Corp.) ion detector operated at 2.8 kV. The spectra were acquired as an average of 10 (organic samples) or 1000 (phosphotungstic acid) laser shots. Acquisition and averaging was performed using a LeCroy 9310M, 300 MHz oscilloscope. The spectra were then transferred to a Macintosh IIvx computer for processing.

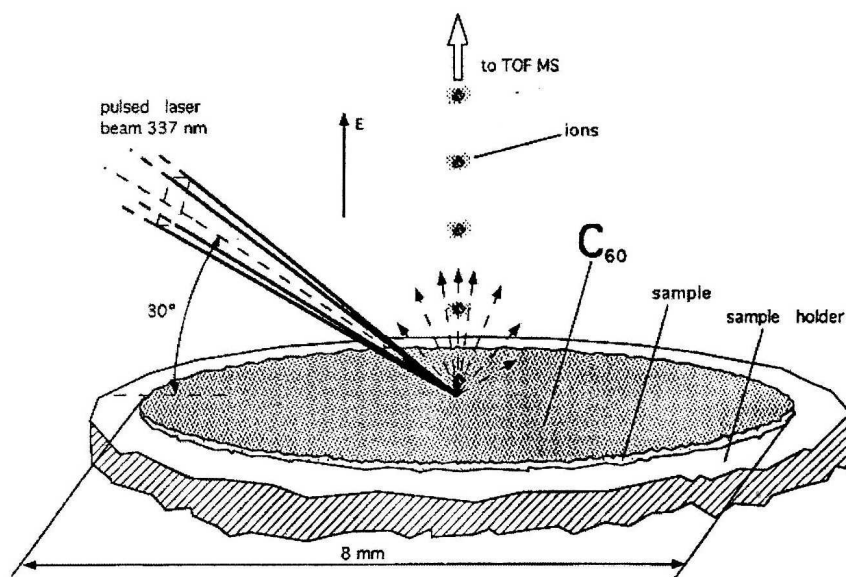


Fig. 1. Sample deposition for C₆₀ assisted laser desorption/ionization mass spectrometry.

3. Matrix and sample preparation

The desorption matrices that were used for this work were C₆₀. The preparation and purification of C₆₀ has been described previously [16].

3.1. Matrix and organic sample preparation

The insulin and bovine albumin were purchased from Sigma Chemical Ltd., and were used without further purification.

For the C₆₀-assisted LD mass spectra reported in this chapter, 0.5 μ l of 1.0 mg/ml C₆₀ solution in benzene was placed on a flat stainless steel sample holder and dried for 20 s at room temperature to remove the volatile compounds. Evaporation of the solvent produced an optically (visible) homogeneous spot of C₆₀, calculated to be \approx 10 nm thick. This matrix layer was then coated with a solution containing the sample molecules.

We found that the yield of high mass ions largely depended on the method of sample preparation. In our experiments best results were obtained for samples of insulin (0.2×10^{-7} M) and bovine albumin (2×10^{-7} M) made by dissolution in 30% acetonitrile (v/v) distilled water. Then, 10 ml of the solution to be investigated was placed on the layer of C₆₀ and dried (about 10 minutes) at room temperature.

The TOF mass spectra with C₆₀ assisted Laser Desorption–Ionization are presented in Fig. 2 (insulin) and Fig. 3 (bovine albumin).

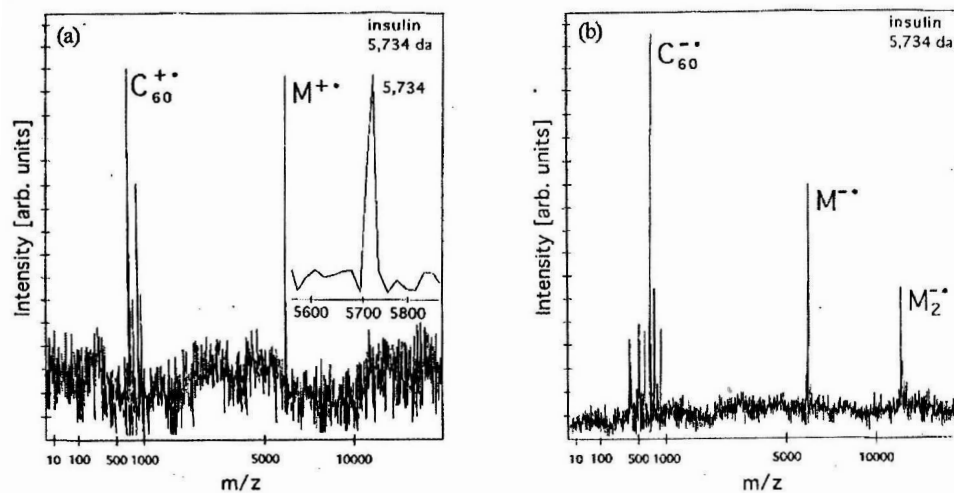


Fig. 2. (a) Positive and (b) negative ion LD-mass spectra of insulin (5,734 Da) with C₆₀. Laser power density was $\approx 35 \text{ MWcm}^{-2}$ at a wavelength of 337 nm.

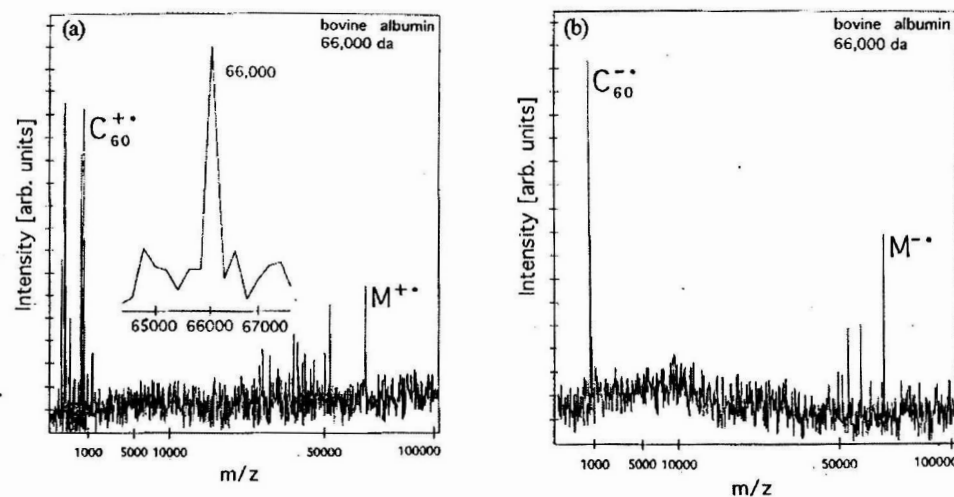


Fig. 3. (a) Positive and (b) negative ion LD-mass spectra of bovine albumin (66,000 Da) with C₆₀. Laser power density was $\approx 35 \text{ MWcm}^{-2}$ at a wavelength of 337 nm.

3.2. Matrix and inorganic sample preparation

The phosphotungstic acid, $\text{H}_3\text{PO}_4(\text{WO}_3)_{12}\times\text{H}_2\text{O}$, was purchased from British Drug Houses and used without further purification.

For the C₆₀-assisted LD mass spectra reported in this chapter, 10 ml of phosphotungstic acid dissolved in methanol (0.03 M) was placed on a flat stainless steel sample holder and dried for 10 s at room temperature. Evaporation of the solvent produced a visible and apparently homogeneous spot of phosphotungstic acid of ≈ 8 mm diameter. A C₆₀ solution in benzene was then deposited on top of the phosphotungstic acid layer. We found that the yield of the sample ions depended on the ratio of C₆₀ solution to the phosphotungstic acid solution used. In our experiments, the best results were obtained from a fullerene layer produced from 5 ml of 1.0 mg/ml C₆₀ solution in benzene. The C₆₀ layer was calculated to be ≈ 25 nm thick on top of the ≈ 8 mm diameter spot of the phosphotungstic acid.

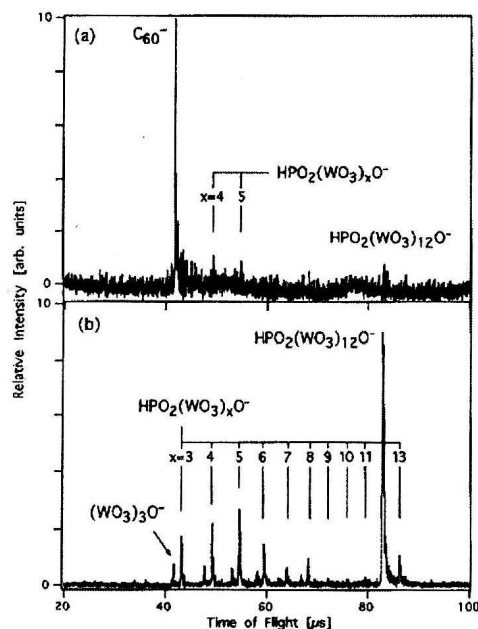


Fig. 4. Negative-ion LD mass spectra of phosphotungstic acid with C₆₀, at a wavelength of 337 nm and (a) laser power density was $\approx 40\text{MWcm}^{-2}$ and (b) a laser power density was $\approx 65\text{MWcm}^{-2}$.

The negative-ion LD mass spectra of phosphotungstic acid with C₆₀, are shown in Fig. 4. It is important to note that the mass distribution of the LD negative-ion mass spectra was strongly dependent on laser power density. For low laser power densities (see Fig. 4(a)), at $\approx 40\text{MWcm}^{-2}$ for example, we observed a large abundance of C₆₀⁻ and a small abundance of ions which are assigned as $\text{HPO}_2(\text{WO}_3)_{12}\text{O}^-$ and two associated ions $\text{HPO}_2(\text{WO}_3)_x\text{O}^-$, where $x = 4$ and 5. At increased laser

powers (see Fig. 4(b)), at $\approx 65 \text{ MWcm}^{-2}$ for example, the intensity of C₆₀⁻ ions decreases along with a corresponding increase of the intensity of series of negative ions which are assigned as HPO₂(WO₃)_xO⁻ ($x = 3\text{--}13$). The most intense ion of this sequence, HPO₂(WO₃)₁₂O⁻ with measured $m/z = 2861 \pm 3$, is closest in mass to the molecular phosphotungstic acid and possibly arises from dehydration of the H₃PO₄ component of acid.

4. Conclusion

Most significant from this study is that we have provided evidence that a layering technique with C₆₀ as the substrate for laser desorption of organic or inorganic molecules can be used as a useful alternative to the well-known MALDI method.

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LASERSKA DESORPCIJSKO-IONIZACIJSKA MASENA SPEKTROSKOPIJA
POTPOMOGNUTA SA C₆₀

Opisuje se primjena laserske desorpcijsko-ionizacijske masene spektroskopije sa dva organska pripravka, insulin (5734 Da) sa crijevnim albuminom (66 000 Da), te heteropolni anorganski sistem od fosforovolframske kiseline, H₃PO₄(WO₃)₁₂×H₂O. Primijenjen je dušikov laser pri linearnom tandem masenom spektrometru s mjerenjem vremena proleta i proučavana je ovisnost masenih spektara o snazi laserskog snopa. Pokazano je da se tehnika slojeva sa C₆₀ kao substratom za desorpciju organskih ili neorganskih molekula, može primijeniti kao alternativna dobro poznatoj MALDI metodi.