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New approaches to the laser mass spectrometry of organic samples

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Contents

Abstract. Three new approaches to the laser mass spectrometry of organic samples are presented, which are based on the soft ionization of organic and bioorganic molecules and on the use of pulsed laser radiation.

Keywords: organic compounds, mass spectrometry, laser

1. Introduction

The effectiveness of research and development in medical diagnosis, molecular biology, and other fields is, to a large extent, determined by the reliability and speed of the data used, dramatically highlighting the need for high-sensitivity methods for determining and identifying organic and bioorganic compounds. The key current trend is the development and active use of new instrumental analysis methods.

Mass spectrometry methods are among the most informative analysis techniques, allowing hundreds of thousands of compounds with fundamentally different physical and chemical properties to be identified. Admittedly, however, standard and currently well-developed ionization methods are occasionally ineffective in producing ions and detecting different classes of chemical compounds. Currently, it is precisely the preparation of ions that emerges as one of the key problems in the further development of the mass spectrometry of organic compounds. Specifically at issue are the insufficient efficiency of ionization and the complex and labor-consuming procedure of preparing a test sample. These two factors are stimulating the development of new approaches to the mass spectrometry analysis of objects of complex compositions. In this context, laser methods seem to hold extreme promise due to their combining the necessary properties, such as a high ionization probability, a wide dynamic range, a high spatial resolution, and the potential to simplify the sample preparation procedure. In this paper, we consider new approaches to the use of laser technology in the mass spectrometry of organic compounds.

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2. Surface-assisted laser desorption/ionization (SALDI) method

First proposed back in the mid-1990s, SALDI used suspensions of the micro- and nanoparticles of graphite in glycerin or water as a means for laser desorption/ionization [1, 2]. By exposing such suspensions to laser radiation after first doping them with peptides or proteins, it is possible to measure the mass spectrum of these compounds. Of key importance to the ionization process is the well-developed surface of the microparticles involved — hence the name SALDI.

In its early days, SALDI was considered to be an alternative to the matrix-assisted laser desorption/ionization (MALDI) method, and its development was mainly stimulated by the problem of high background signals in the MALDI method. The means for the laser desorption/ionization of compounds in MALDI is a matrix usually consisting of organic acid crystals that are good absorbers of laser radiation [3]. The percentage concentration of the matrix molecules is several orders of magnitude in excess of the concentration of the analyte (the chemical compound being measured in the analysis), with the result that the ion signal from the matrix makes it difficult and often impossible to analyze compounds in the low-mass range (about 500 atomic mass units, amu).

In a later, advanced version of SALDI, the microparticle suspension was replaced by a specially prepared surface of an ion-emitting solid substrate made, with varying success, from graphite [4, 5], porous titanium and tungsten oxides [6], and zinc oxide [7]. It was silicon materials, however, that proved to be the most widely used due to their advantages, such as (most importantly) the high ionization efficiency, extremely high purity, and easy processing. The last property is particularly important, because the laser absorption-ioniza-

tion of molecules on the surface of single crystal silicon substrates is extremely inefficient [4], and hence, for the purpose of high-sensitivity analysis, an active surface is a prerequisite. As the method developed, a large number of SALDI-active layers of different morphologies and structures were proposed, including porous silicon [8], amorphous silicon [9], and silicon nanothreads [10].

In Ref. [9], a comparative analysis of various silicon materials under conditions of inserting a gas phase probe allowed concluding the conclusion that the efficiency of SALDI is determined by the chemical and electronic properties of the material, including the density of structural defects and their associated localized states and, accordingly, a model of laser desorption-ionization on silicon surfaces was proposed that involves the following key stages in the SALDI process:

— laser cleaning of the substrate to ensure the desorption of organic contaminants from its surface;

— the dissociative adsorption of water molecules to form proton-donating surface groups such as the silanol SiOH surface groups;

— molecules of the compounds being determined are adsorbed on proton-donating surface groups;

— photogeneration of nonequilibrium charge carriers; their separation under conditions of high density of structural defects; and hole localization near proton-donating surface groups;

— protonation of the absorbed molecules. From quantum mechanical calculations, the localization of a positive charge decreases the energy barrier for proton transfer from surface SiOH groups by about 4 eV, thus ensuring effective proton transfer to the molecules adsorbed on the groups;

— ion desorption. According to the results in [10], desorption occurs by a thermal mechanism and is due to the surface being rapidly heated locally by laser radiation.

Because ionization results from proton transfer to the molecules of the compound being analysed, the only compounds that are effectively ionized in the SALDI process are those with high basicity, or proton affinity, in the gas phase [11]. This fact, on the one hand, imposes a bound on the number of the compounds to be identified, but on the other, provides a high selectivity of ionization and a low level of the background signal. The results obtained in Refs [10–13] indicate that with SALDI, high basicity compounds can in principle be detected at a level of one attomole (10^{-18} mole), which is several orders of magnitude better than with traditional methods — electron ionization, chemical ionization, electrospray, and MALDI.

An important structural-chemical factor indicating the possibility of effectively detecting compounds by SALDI is the presence in the molecule of reduced nitrogen, in particular, functional-structural groups such as piridine, piperidine, indole, diazepine, imidazole, and amino groups. These are constituents of many known medicinal products and are also widely used as structural units in developing new medicines, making SALDI extremely promising for solving important problems of modern medicine, such as the development of medical preparations, pharmacokinetics registration, and early disease diagnostics — the activities, parenthetically, where not only high sensitivity but also highly reproducible analysis results are needed. Because the ionization efficiency depends critically on the physical–chemical composition of the active surface, reproducibility becomes one of the greatest challenges for the method. To

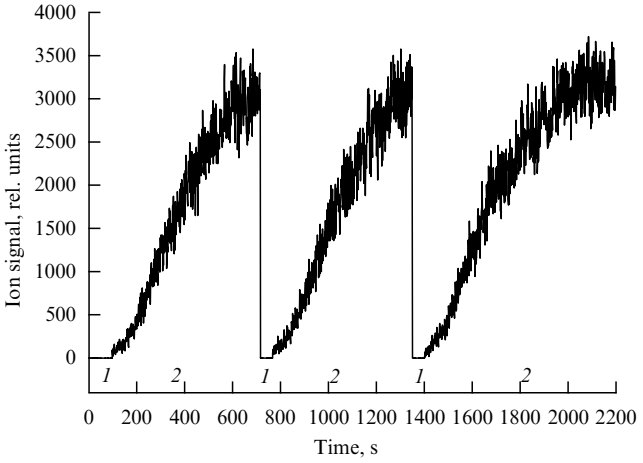

Figure 1. Time variation of the alanine ion signal in three successive procedures in the formation of an active layer on a silicon substrate. The initial *1* (subsequent *2*) portion of a plot corresponds to the first (second) stage of laser processing. The flow of beta-alanine molecules to the surface was created by using an effusive cell and remained constant over the entire time interval studied.

overcome it, an approach has been proposed that combines a new formation method of an active surface layer and a new way of inserting a probe into the mass spectrometer.

The layer formation method is based on the exposure of single-crystal silicon plates to laser radiation in the spectrometer vacuum chamber, first at an intensity above and then below the silicon melting threshold; in the latter case, the procedure is conducted in the presence of water vapor at a pressure of 10^{-6} mm Hg. As a result, a highly disordered layer with a high surface density of proton-donating groups forms. Importantly, what sets the proposed method apart from the known technique used for preparing a SALDI-active layer is high reproducibility and the possibility of using the same substrate repeatedly. This fact is illustrated in Fig. 1, which shows how the ion current of protonated beta-alanine molecules varies in the three successive procedures in the formation of the active layer.

Figure 2b is a schematic of how a sample is introduced into the mass spectrometer. The key element of the sample-introduction unit is a stainless steel ball rotating in vacuum seals, which has SALDI-active substrates placed symmetri-

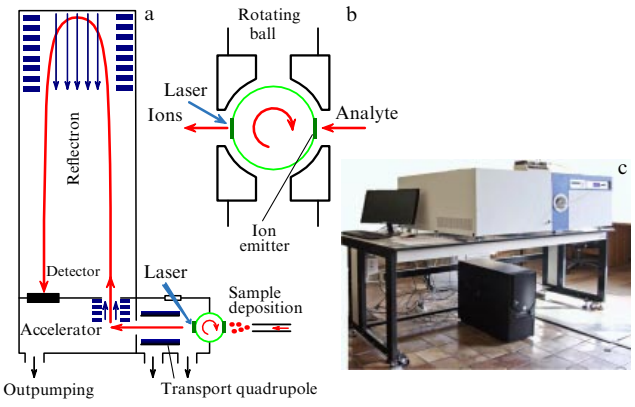

Figure 2. A schematic diagram of (a) the SALDIX mass spectrometer using SALDI ionization, (b) the sample introduction, (c) overall view of the experimental prototype.

cally in its indentations. One of the substrates is covered by deposition with analyte, whereas its counterpart located in the ion source is subject to laser radiation, and the ions that form are detected by the mass analyzer. Once all of the deposited material is desorbed and the surface activated, the ball (if necessary) is rotated and the process is repeated. The analyte can be deposited in a variety of ways, for example, by electrospraying solutions [14] or by thermodesorption [15].

Based on the proposed approach, a prototype mass spectrometer was developed in cooperation with Advanced Energy Technologies Ltd. and the Institute of Analytical Instrumentation, RAS, enabling rapid and highly sensitive quantitative analysis of liquid biological samples for medicine and pharmacokinetics (Fig. 2a, c). The instrument was granted a registration certificate by Russia's Federal Service for the Oversight of Healthcare and Social Development under the name SALDIX.

3. Laser-induced electron transfer desorption/ionization

An important limitation of SALDI is that the degree of ionization depends on the basicity (or proton affinity) of the analyte, making the method ineffective for analyzing samples containing nonvolatile low-basicity compounds. There is a wide range of such compounds, including, in particular, metal coordination complexes, hydocarbons, sugar, and some other classes of chemical compounds. The traditional mass spectrometry methods for nonvolatile compounds — MALDI and electrospray ionization — which also primarily use ionization by protonation, are only successful when used for the mass spectrometry detection of ion, polar, and high-basicity compounds. Therefore, one of the current challenges in the field of organic mass spectrometry is to develop new methods allowing the 'soft' noninvasive ionization of nonvolatile low-basicity nonpolar analytes.

To overcome this challenge, a new approach to ionizations was proposed (Fig. 3a) that combines the advantages of SALDI with those of the method of multiphoton laser ionization [16] (Fig. 3b). As in SALDI, the analyte is deposited on the surface of a solid substrate, followed by the exposure of the surface to pulsed laser radiation, but this time the parameters of laser radiation are chosen such that it be absorbed not only by the substrate material but also by the analyte molecules. When absorbing a laser photon, an analyte molecule adsorbed on the surface makes the transition from the ground to an excited state. If the energy level of the excited state lies above the bottom of the semiconductor conduction band, the effective transfer of an electron from the absorbed molecule to the semiconductor material is possible. This effect is widely used, for example, for the sensibilization of broad-band semiconductors [17]. As a result of the electron transfer, molecular ions form on the surface. If, further, the condition for the laser radiation being absorbed by the substrate material is fulfilled, then laser irradiation leads to a rapid heating of the substrate, followed by the desorption of the ions that form. Unlike methods using proton transfer ionization, the new approach uses laser-induced ionization via electron transfer; hence its name laser-induced electron transfer desorption/ionization (LETDI). The efficiency of ionization in the LETDI method is independent of the analyte basicity, offering the possibility of greatly expanding the range of analytes for laser mass spectroscopy.

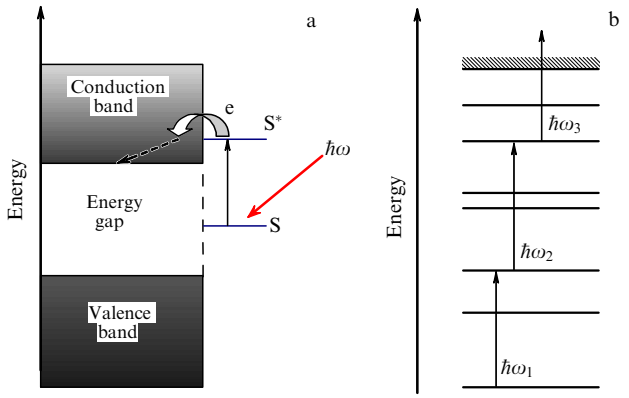

Figure 3. Basic schematics of the ionization of chemical compounds: LETDI (a) and laser-induced resonant multiphoton scheme [6] (b).

The LETDI method has been tested for the high-sensitivity determination and identification of metal coordination complexes used as chemical models of antitumor drugs for the chemotherapeutic treatment of cancer [18, 19].

As an example, Fig. 4 shows the mass spectrum obtained from Os complexes with 8-mercaptoquinoline using TiO_2 as an ion emitter. The measured mass-to-charge values m/z and the observed characteristic ratio of the isotope peaks readily identify the peaks in Fig. 4a as corresponding to the molecular ions of the complex $(Os_2L_5)^+$, where L is 8-mercaptoquinoline. For comparison, Fig. 4b shows the calculated isotope distribution for Os_2L_5 ($Os_2C_{45}N_5S_5H_{30}$). Very close agreement is seen between the observed and predicted isotope distributions. A close match between the calculated and experimental spectra indicates that the flow of desorbed ions does not contain protonated molecules, and therefore the main and only ionization channel for the osmium complex in LETDI is due to the electron transfer from the molecule to the substrate. A comparison using the same mass spectrometer indicates [19] that LETDI can be two orders of magnitude more effective than MALDI in identifying metal coordination complexes.

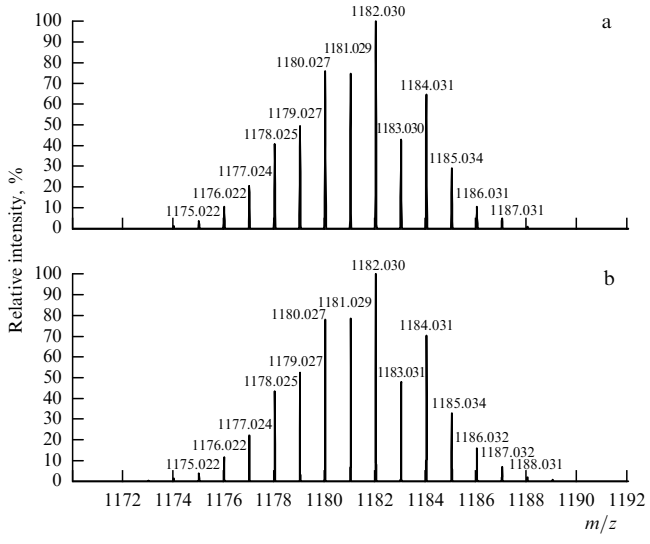

Figure 4. Mass spectra of the osmium coordination complex $OsC_{27}N_3S_3H_{18}$: (a) measured by LETDI and registered by the mass analyzed Orbitrap; (b) calculated theoretically.

4. Ionization by laser plasma radiation at atmospheric pressure

The laser methods discussed in Sections 2 and 3 for producing ions of complex organic compounds mainly apply to the analysis of volatile compounds or solutions. The explosive recent trend in the analysis of biological samples (tissue cuts and biological liquids) is toward ambient pressure, in situ setups, without pretreating the sample, and introducing it into the high-vacuum chamber of the mass spectrometer. This technology is currently of extreme topical interest for biology, pharmacology, and medicine because, when not pretreated, a biological object retains and provides the maximum information possible on its initial composition and structure.

Analyzing solid and liquid samples under ambient conditions requires that the sample be first converted into the gas phase and then ionized. The first task is most commonly accomplished by laser ablation, a process which, for an appropriately chosen laser source, ensures high spatial resolution both in the plane and along the depth of the sample.

For the subsequent ionization of the ablation products, a range of methods has been developed. The first deserving mention are those using the collision of the neutral flow of ablation products with the flow of charged particles produced in a special external source. All of these methods share the feature that the ionization of chemical compounds results from a sequence of chemical reactions that leads ultimately to the formation of protonated analyte molecules. This puts limitations on the ionization efficiency, which is determined by the proton affinity of the compound being studied. A second group of methods use photoionization by hard ultraviolet (UV) radiation, commonly from krypton lamps; we note, however, that the energy of the emitted photons does not exceed 10.6 eV in this case.

To extend the range of identifiable compounds and to enhance the analysis sensitivity, a new method is proposed in [20], with the laser ablation of the sample combined with the ionization of the ablation products by radiation from laser plasma created near the surface of a metal target. This approach came to be known as atmospheric pressure laser plasma ionization (APLPI). The spectrum of laser plasma radiation extends to soft X-ray wavelengths, allowing the use of an ionized water molecule (ionization potential 12.6 eV) as a proton source in proton exchange reactions, a molecule which is always present in atmospheric air and in the ablation products of biological tissues. Using hard UV radiation, it is also possible to achieve direct photoionization of analytes, including those with a low proton affinity value.

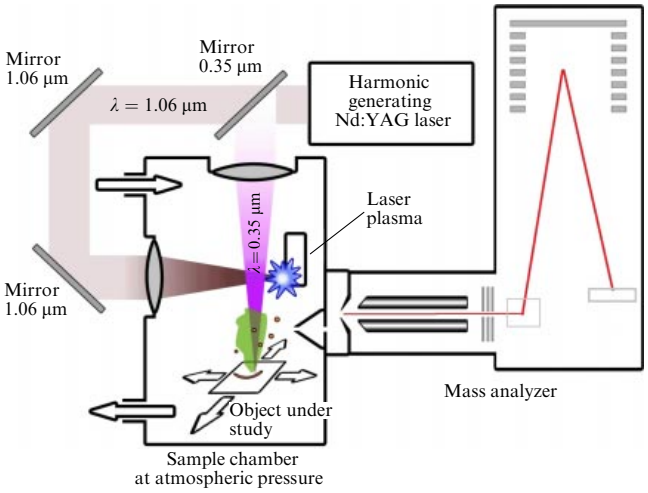

Figure 5. Schematic layout of an APLPI spectrometer for the analysis of solid samples.

The energy conversion of laser radiation to plasma radiation can occur with an efficiency of tens of percent, making it possible to place the radiation source directly within the ionization chamber using compact diode pumped pulsed Nd:YAG lasers as in the first two approaches.

Figure 5 presents a schematic of the instrumental realization of the APLPI method for the analysis of solid samples [20]. The sample to be studied is mounted on a two-axis 2 μm pitch stage placed in a hermetic chamber at atmospheric pressure. The chamber can be blown through by pure gases, which in some cases is necessary in order to reduce the background signal arising from the ionization of trace amounts of organic compounds in the atmospheric air (nicotine, amines, alcohols, etc.). The relative concentration detection threshold for these impurities in atmospheric air is at the level of 10^{-10} (100 ppt), allowing APLPI also to be used for the high-sensitivity analysis of the atmosphere. The method uses the third harmonic of the Nd:YAG laser (355 nm) to vaporize the sample and its fundamental frequency (1064 nm) to produce plasma on a special-purpose

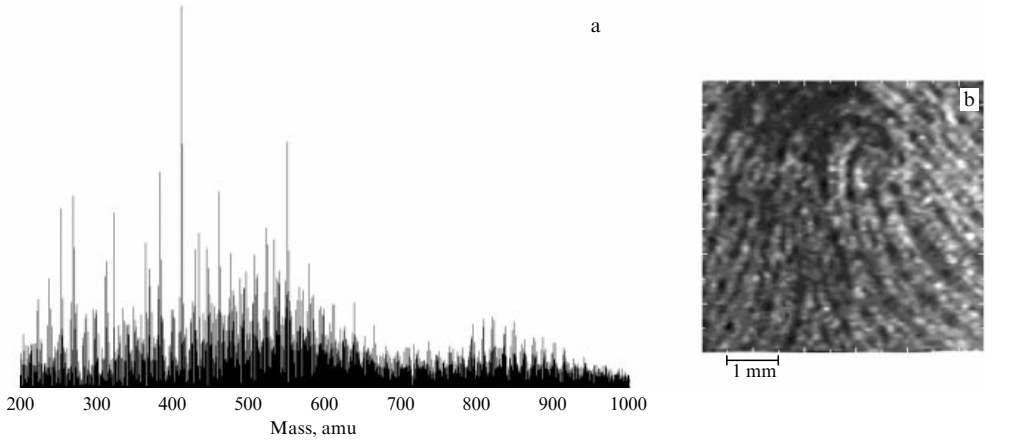

Figure 6. (a) APLPI mass spectrum from a laser ablated fingerprint on stainless steel; (b) Mass spectrometry image of a papillary pattern for the mass range 500–600 amu.

metal target placed near the ionization zone. To introduce the produced ions into the mass analyzer, a gas-dynamic interface is used.

One of the most promising applications of APLPI is the mass spectrometry imaging of the distribution of chemical compounds in biological materials. As an example, Fig. 6 shows the mass spectrum of a fingerprint on a metal substrate and a mass spectrometry image of a fingertip for the mass range 500–600 amu (which mainly correspond to lipid group compounds). The image can be constructed based on any selected portion of the spectrum or an individual characteristic peak in the mass spectrum of the sample composition with a spatial resolution at the level of 100 μm or better.

The APLPI method has also been successfully applied to the composition and spatial distribution analysis of various components of biological tissue cuts. Moreover, APLPI is very promising for developing a 'smart scalpel' that uses laser radiation as a surgical instrument and employs mass spectrometry to analyze the vaporization products.

5. Conclusion

The three organic mass spectrometry approaches proposed in this paper combine pulsed laser radiation with soft ionization technology that does not destroy complex organic and bioorganic molecules. They markedly improve the potential of mass spectrometry as a tool for the analysis of solid, liquid, and gaseous samples and can be used for solving a variety of problems in present-day medicine, biology, and analytical chemistry.

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