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Ambient ionization mass spectrometry

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Ambient ionization mass spectrometry emerged as a new scientific discipline only about ten years ago. A considerable body of information has been reported since that time. Keeping the sensitivity, performance and informativity of classical mass spectrometry methods, the new approach made it possible to eliminate laborious sample preparation procedures and triggered the development of miniaturized instruments to work directly in the field. The review concerns the theoretical foundations and design of ambient ionization methods. Their advantages and drawbacks, as well as prospects for application in chemistry, biology, medicine, environmetal analysis, *etc*., are discussed.
The bibliography includes 194 references.

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I. Introduction

The resolution and the range of detectable masses belong to the key parameters of mass spectrometers. Once these parameters were improved, mass spectrometry opened the world of complex natural biopolymers and hypercomplex mixtures (oils, humic substances). Yet another avenue of progress in mass spectrometry involves acceleration and simplification of analysis. Ambient ionization mass spectrometry methods implemented under normal conditions became a breakthrough in acceleration and simplification of analysis.

Until recently, numerous advantages of mass spectrometry were accompanied by a drawback, namely, fast, sensitive and informative analysis was often preceded by labour- and time-consuming sample preparation procedures. For instance, according to classical United States Environmental Protection Agency (US EPA) protocols, a laboratory analysis requires a 3 h sample preparation followed by 60-min gas chromatography/mass spectrometry (GC/MS) analysis. The sample preparation step may introduce severe analytical errors; therefore, simplification and even complete elimination of this procedure is topical.

A breakthrough was made in the early 21st century. In 2004, the first method for MS analysis without sample preparation called 'desorption electrospray ionization' (DESI) was proposed.[1] The year 2005 was marked by the discovery of the first plasma ionization method, *viz*., direct analysis in real time (DART).[2] Since then, one has dealt with a snowballing process of development of novel methods. Indeed, a table in a recent review [3] includes a total of 42 methods of ambient ionization mass spectrometry. Since there are numerous publications on ambient ionization mass spectrometry and their authors often present novel methods, there is urgent need to analyze and generalize the available information in the title field. Recently, a number of relevant reviews and tutorials have been reported.[3 – 18] Unfortunately, ambient ionization mass spectrometry methods are left almost out of consideration in the Russian Federation, while the only review in Russian [19] is devoted to a sole method, DART.

This review presents a general classification of ambient ionization mass spectrometry methods, describe the most efficient methods including details of experimental design and discuss the ionization mechanism and possible fields of application. Particular sections of the review are devoted to the new research avenues based on ambient ionization mass spectrometry, *viz*., surgical procedures guided by mass spectrometry, synthesis of organic compounds out of flask, design of miniaturized mass spectrometers and mass-spectrometric visualization of the distribution of components on the surface and in the bulk of samples (MS imaging). Listed

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below are the most valuable publications reported since 2004 to the early 2015. The review thus presents the state-of-the-art in ambient ionization mass spectrometry as applied to chemistry, biology, medicine, environmental and forensic analyses, *etc*.

Salient features of these methods consist in the possibility of using native samples, generation of ions outside the mass spectrometer and a rather short time taken to obtain results (from 1 s to 1 min). It was found that ions can be generated near a mass spectrometer at atmospheric pressure in open air under ambient conditions or directry in the field rather than using an ion source.[3–19] In some sense, ambient ionization mass spectrometry is a successor of atmospheric-pressure matrix assisted laser desorption/ionization (MALDI),[20] although the latter method involves a sample preparation step and samples are inaccessible in the course of analysis.

The ambient ionization mass spectrometry methods keep the advantages of classical MS methods, that is, selectivity, sensitivity, informativity, performance and the possibility to analyze mixtures in the tandem mass spectrometry (MS/MS) mode. Besides, they make it possible to carry out direct experiments without sample preparation, *i.e*., analyze native samples. Emphasize that most ambient ionization mass spectrometry methods involve the formation of analyte molecular ions and the degree of fragmentation is rather low, *i.e*., these are 'soft' ionization methods. A low excess energy of molecular ions is due to their efficient cooling as a result of numerous collisions with molecules of atmospheric gases. The potential of ambient ionization mass spectrometry methods is crucial to solving various present-day problems, *e.g*., detection of trace amounts of explosives in luggage, drug metabolites and narcotics in urea, lipids in biological tissues and active substances in pharmaceuticals.

Usually, the detection of an analyte takes a few seconds. For a simple matrix (small number of components in the sample), it is sufficient to detect any type of characterisitc ions, *viz*., MH^+, $[M+Cl]^-$, $[M+Na]^+$, *etc*. More reliable results, *i.e*., confirmation of the elemental composition of the analyte, can be obtained by high-resolution mass spectrometry. For a complex matrix, one can use tandem mass spectrometry if the conventional mass spectrum is doubtful. A good example is provided by the detection of L-aspartyl 4-phosphate in a dried 2 μl urine spot on paper without sample preparation.[4] The DESI mass spectrum of the spot and the collisionally activated dissociation (CAD) mass spectrum of a minor component (*m*/*z* 214) of the mixture is shown in Fig. 1. Based on the CAD mass spectrum, that component was identified as L-aspartyl 4-phosphate.

It is not necessary to hold the sample in close proximitiy to the inlet of the mass spectrometer. Ions can be transferred to long distances (more than 1 m) using silicone tubes.[21–23] A flexible ion transmission tube 4 m long for the sampling probe was developed and DESI conditions for continuous mass-spectrometric analysis of tissue markers in the course of surgical and endoscopic procedures were optimized.[24] A similar approach was used for online guidance of surgical operations by rapid evaporative ionization mass spectrometry.[25]

Summing up, ambient ionization mass spectrometry includes open-air methods for operation in ambient conditions or in the field using soft ionization outside the mass spectrometer. This can be done using any types of mass spectrometers without essential re-design and one can obtain results in the real time mode with minimum sample preparation or without it.[26]

II. Methods of ambient ionization mass spectrometry

Ambient ionization mass spectrometry methods can be divided into three large groups with respect to ionization mechanism. There are the spray, plasma and laser ionization methods. In all cases, desorption and ionization can occur either simultaneously or successively. Other classifications are also available. For instance, according to a

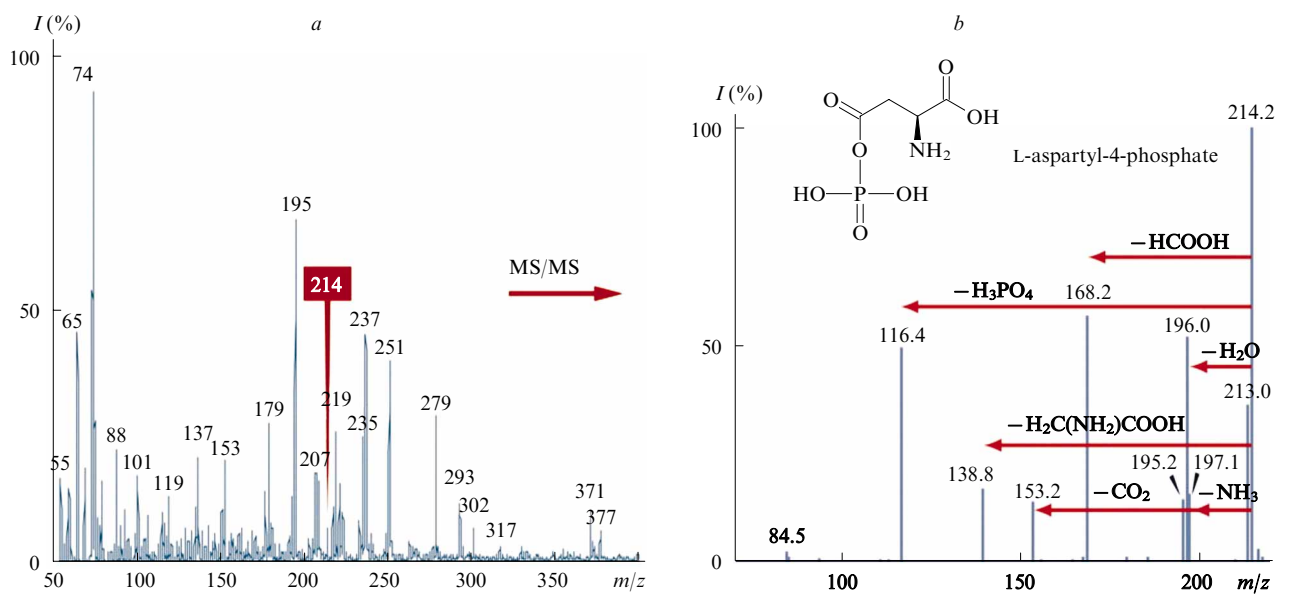


Figure 1. DESI mass spectrum of a dried urine spot on paper (*a*) and the tandem CAD mass spectrum of a minor component (*m*/*z* 214) of the mixture (*b*) (based on the CAD mass spectrum, the component was identified as L-aspartyl 4-phosphate).[4]

detailed study by Harris *et al.*,[15] the methods available by 2011 fall into seven categories.

II.1. Spray ionization methods

Important advantages of these methods include speed and high performance, the possibility to relate the intensity of a target signal to a particular point on the surface to be analyzed (mass-spectrometric visualization), as well as the possibility of carrying out chemical reactions immediately inside the spray droplets.

II.1.a. Desorption electrospray ionization

DESI can be called the 'most chemical' mass-spectrometric method. It involves extraction of analyte molecules with a solvent, derivatization of these molecules in solution and mass-spectrometric analysis of characteristic ions.[3] In DESI, the surface of a sample is bombarded with electrosprayed charged solvent microdroplets in order to extract analyte molecules, ionize them and transfer to the mass spectrometer inside secondary microdroplets (see below). In some sense the method is a relative of secondary-ion mass spectrometry (SIMS), electrospray ionization (ESI) and laser desorbtion/ionization (LDI) on porous silicon. Desorption electrospray ionization of analyte molecules is usually performed directly on their natural matrices or on auxiliary substrates.

Key advantages of the DESI technique include:

— high rate of analysis (1–5 s) and thus high performance of the method;

— low excess energy of ions (2 ± 1 eV) which is close to the case of electrospray ionization;

— possibility to obtain structural information (MS/MS);

— possibility to operate in both positive-ion and negative-ion modes;

— high sensitivity (at a level of a few picogrammes);

— weaker dependence on matrix effects;

— applicability to a wide range of compounds from hydrocarbons to biopolymers;

— possibility of quantification; and

— generation of multiply charged ions of large molecules.

Using the DESI method, one can vary the solvent composition, spray voltage, spray cone angle, as well as the distance from the sprayer tip to the sample and from the sample to the orifice of the atmospheric inlet of a mass spectrometer. The nature of the surface has almost no effect on the quality of mass spectra. Experiments with samples on paper, glass, plastic, *etc.* give identical results.

To obtain high-quality mass spectra, one should take into account the solvent nature, which becomes of great importance in this case.[27,28] Most often, a methanol + water mixture is used; acidification favours the formation of cations. The intensities of signals depend strongly on the solubility of analyte in the spray solvent. For instance, sprays based on water and mixtures with water enable ionizaion of various substrates from metal ions to complex biopolymer molecules irrespective of their molecular masses. Mixtures with chloroform, tetrahydrofuran and acetonitrile appeared to be much more efficient than aqueous solutions for analysis of hydrophobic compounds; the use of DMF–water or DMF–methanol mixtures leads to an increase in the signal intensities of ions of low-molecular-mass compounds.[29] DESI mass spectra of water-insoluble compounds were observed only in nonaqueous mixtures. Also, the octanol/water partition coefficient appeared to be useful for the determination of the most preferred solvent. The addition of solubilizers also causes a positive effect.[30] Salt additives also proved themselves; here, the analyte molecule acts as a Lewis base while the HOMO electrons interact with the LUMO of metal cation to give a corresponding complex.[31] In particular, olefins present in a sample can be detected with ease from the formation of adducts with Ag^+ upon the addition of $AgNO_3$ to the system under study.

Yet another important parameter is the solvent spray angle. At present, there are two types of DESI geometries. For DESI with independent geometry, the angle of incidence and the angle of reflection are fixed, being close to 90°. The other case, termed 'DESI with transmission geometry', is illustrated in Fig. 2. Here, the angle of incidence (α) and the angle of reflection (β) are variable; the maximum signal intensity is achieved at $\alpha = 52°$.

The DESI ionization mechanism involves the formation of a thin solvent film on the sample surface as the primary microdroplets of electrosprayed solvent arrive at the surface of a sample. The primary droplets 5 μm in diameter have a velocity of about 120 m s^{-1}.[32] Molecules of the desired components of the sample are extracted by the thin film, while new portions of charged solvent droplets collide with the film and trap the extracted analyte molecules. Splash (secondary droplets) thus produced contain the extracted analyte ions which then go to the gas phase by the electrospray mechanism through ion evaporation or residual charge.[33] Secondary droplets are 0.8–3.3 μm in size; their velocity depends on many parameters. It is worth noting that each primary droplet produces a few dozens of secondary droplets. The most characteristic ions generated in the DESI conditions include $[M+H]^+$ and/or $[M+Na(K)]^+$ for the positive-ion mode and $[M-H]^-$ for the negative-ion mode. The role of the proton transfer agent is played by the solvent. In the presence of inorganic anions, the formation of adduct ions (*e.g.*, $[M+Cl]^-$) is possible. To date, DESI proved itself in metabolomics, proteomics, forensic analysis, analysis of fuels, lipidomics, *etc.*[3–18]

DESI is an excellent method for imaging mass spectrometry. Unlike the most widely used vacuum MS methods

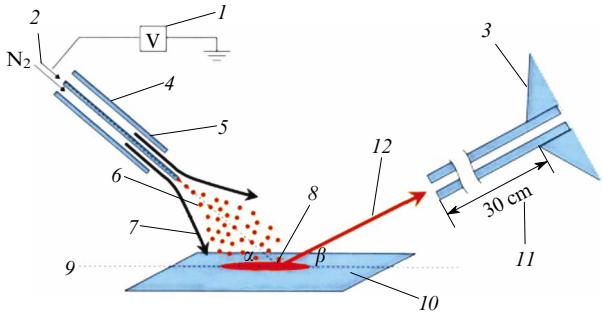


Figure 2. Schematic of a DESI experiment with transmission geometry.[1]
High voltage power supply (*1*), solvent (*2*), atmospheric inlet of mass spectrometer (*3*), spray capillary (*4*), nebulizer capillary (*5*), spray (*6*), gas jet (*7*), sample (*8*), freely moving sample stage in air (*9*), surface (*10*), ion transfer line (*11*) and desorbed ions (*12*).

(MALDI and SIMS), investigators have full access to the sample in the course of the DESI experiments; this enables all types of manipulations during the analysis. A rather high spatial resolution of DESI makes it possible to distinguish between tumour and normal biological tissues, as well as to locate the boundaries, type and grade of tumour.[34] Usually, the spatial resolution is 150–250 μm, although one can also achieve a resolution of 40 and even 35 μm.[35]

Having applied a high voltage to a syringe needle and directed a jet of charged droplets inside a cone (plastic pipette tip) containing a sample powder in the narrow end, one can obtain results similar to those obtained by tandem MS coupled with liquid chromatography and electrospray. The method was termed 'pipette-tip ESI'.[36] Coupling with corona discharge leads to additional ionization.[37]

Initially, ambient ionization mass spectrometry represented an alternative to gas chromatography–mass spectrometry. Recently, results of experiments on the use of ambient ionization mass spectrometry methods for chromatographic detection (*e.g.*, coupling of DESI with liquid chromatography) have been reported.[38,39] An interesting experimental design uses a micro-orifice ~100 μm in diameter in a polymeric plastic (PEEK) capillary tube connected to the outlet of a column of a preparative-scale liquid chromatograph.[40,41] The orifice is at a distance of nearly 1 cm from the capillary end and introduces a very small dead volume (~4 nl), *i.e.*, no broadening of peaks occurs. Continuous DESI with a time delay of about 0.01 s enables monitoring of the exit of components from the tube outlet and efficient fractionation of the starting mixture. The method allows one to work with compounds that are transparent to conventional UV detectors (*e.g.*, sugars). The reactive DESI technique (see below) can also be used when necessary. In particular, the addition of corresponding agents to the solvent causes the charge of protein ions to increase.[41] The method is also applicable under ultrafast separation conditions. Inorganic salts present in samples do not preclude analysis, as is the case for ESI. Since salts are of the highest polarity and thus leave the column first, subsequent fractions of organic components can be collected and used for all types of physicochemical measurements.

II.1.b. The nano-DESI method

A drawback of DESI consists in analyte splash on the sample surface when the gas jet and solvent droplets collide with the surface. A possible method for eliminating this drawback is as follows.[42–46]

Under the nano-DESI conditions,[42] an analyte is desorbed from the surface at the point where two capillaries filled with a liquid contact the surface (Fig. 3). The first capillary is used to deliver the solvent to the sample surface, while the second capillary transfers the extract to the mass-spectrometer. The potential difference between the first capillary and the inlet of the mass spectrometer is 3–5 kV, while the distance from the nanospray capillary to the inlet of the mass spectrometer is about 0.5 mm. In this case, sampling in the 'liquid bridge' and ionization are separated in time. The size of the 'liquid bridge' can be controlled by varying the capillary diameters and the solvent flow rate. The highest efficiency was attained at a flow rate of 600 nl min^{-1}. The process does not require momentum transfer and, therefore, there is no need for nebulizing gas and splashing; also, no undesirable analyte transport across

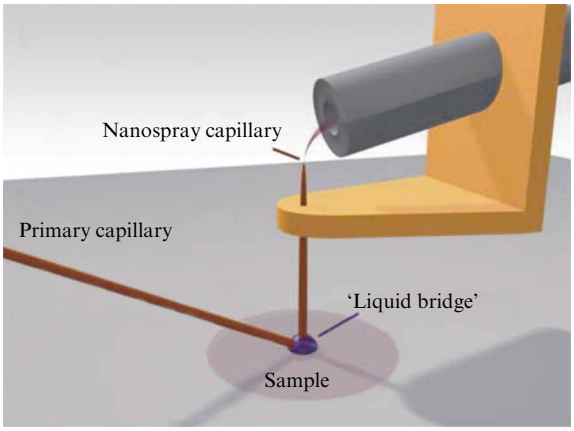


Figure 3. Experimental configuration of nano-DESI.[42]

the surface occurs. The ion current is stable and can be controlled with ease by varying a few parameters. The nanospray capillary should be filled with the liquid before application of the spray voltage. Otherwise, the process will not start. Air bubbles in the capillary have an adverse effect and should be removed by heating or by simply replacing the capillary. The method allows almost all types of surfaces (metals, paper, synthetic polymers, glasses, biological tissues) to be analyzed.

The nano-DESI technique makes it possible to deal with various analytes and their mixtures. Useful applications of nano-DESI include imaging of biological tissues (including tumours) with a spatial resolution of 12 μm without sample preparation[43] and quantification of compounds with the same functional groups (reactive nano-DESI,[44] see Section IV). Coupling electrochemistry with nano-DESI offers prospects for research on the oxidation, reduction and derivatization reactions on electrode surfaces.[45]

Nano-DESI appeared to be very useful for solving problems in petroleomics.[46] Crude oil samples analyzed using an acetonitrile + toluene mixture as solvent give mass spectra that are similar to those obtained under ESI or atmospheric pressure photochemical ionization (APPCI) conditions. If mixtures of polar solvents (acetonitrile + water or methanol + water) are used, ion peaks of the O_x and O_xS_y compounds dominate in the mass spectra. Using the positive-ion and negative-ion modes, one gets complementary mass spectra. Since the O_x and O_xS_y compounds are water-soluble, the possibility of detecting them in crude oil without sample preparation is an important advantage of the nano-DESI method, especially, taking into account problems in the ESI detection of these compounds.[46]

If a thin short capillary is used to drive liquid samples from a microreservoir filled with a sample solution, the liquid will move towards the tapered tip oriented vertically. Microdroplets leaving the capillary when moving towards the atmospheric inlet of the mass spectrometer lose the solvent molecules, so only desolvated analyte ions are transferred to the gas phase.[47] The method is referred to as 'atmospheric pressure contactless ionization'. It enables operation with varoius compounds and their mixtures, is characterized by high sensitivity and allows one to obtain high-quality mass spectra of complex molecules. For instance, the detection limit for bradykinin was ~5 aM (5×10^{-18} mol $litre^{-1}$).

II.1.c. Easy ambient sonic-spray ionization and Venturi easy ambient sonic-spray ionization

Easy ambient sonic-spray ionization (EASI) developed in the research group headed by Eberlin[48,49] is one of the simplest methods of mass-spectrometric analysis. EASI creates a bipolar (±) stream of very small charged droplets of solvent mixtures containing methanol as major component which cause efficient ionization and desorption of analytes from the surface in open air. Spray droplets deeply penetrate into matrices of solid samples, thus ensuring high homogeneity of sampling procedure and long-term detection of stable signals. Here, no application of electric voltage, use of a photon source or even heating is required.[50] The method can be treated as ultrasoft. It is suitable for analyzing labile molecules and complex mixtures by detecting a single ion determining the molecular mass; this is convenient for qualitative analysis and quantification. One can detect either positive ions {$[M+H]^+$ and/or $[M+Na(K)]^+$} or $[M-H]^-$ negative ions rather than $M^{+\bullet}$ or $M^{-\bullet}$ radical ions. A drawback of the method consists in the possibility of dusting small particles of sample powders off with the spray jet.

The EASI method is successfully used in analysis of various samples including fuels, pharmaceuticals, surfactants, wood, *etc*.[16] For instance, coupling of EASI with the simplest chromatography method, thin-layer chromatography, appeared to be a very effective tool for analyzing fuels, biofuels and their blends with vegetable oils. The method enables rapid and reliable typification of samples using biomarkers (fatty acids, triglycerides and alkylpyridines).[51]

Thermal imprinting EASI was used to analyze lipids in various samples of biological tissues with complex multicomponent matrices.[52,53] A slice of meat, fat or another food product is placed on a paper surface and 3 or 4 droplets of solvent (usually, a $MeOH+CHCl_3$ mixture) are dripped on the sample surface, the sample is illuminated for 1–2 min with a 150-W lamp and then the lipid spot on the paper is analyzed by the EASI method using methanol and nitrogen. The same thermal imprint is suitable for repeated analysis. No substantial changes were observed in the lipid distribution profile after a period of seven days. It follows that samples thus obtained can be mailed to other laboratories for comparative analysis.[52]

Utilization of the Venturi effect simplifies the EASI method, which now should be referred to as 'Venturi easy ambient sonic-spray ionization' (V-EASI) suitable for efficient analysis of solid and liquid samples. An example is provided by fast and reliable discrimination between different types of wood with similar appearance.[54] A modification of the V-EASI method using a 'Spartan' ion source[55] is indeed the simplest method for ionization of different types of molecules (Fig. 4).

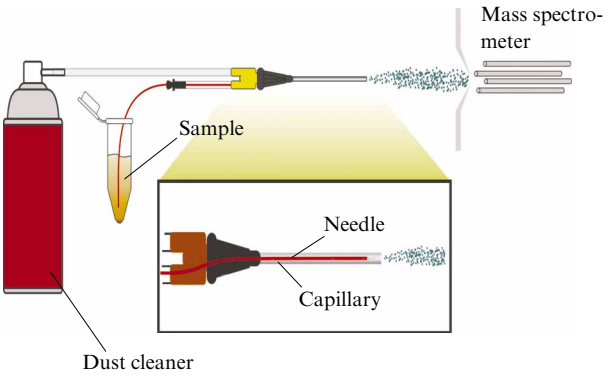


Figure 4. 'Spartan' V-EASI: a schematic.[26]

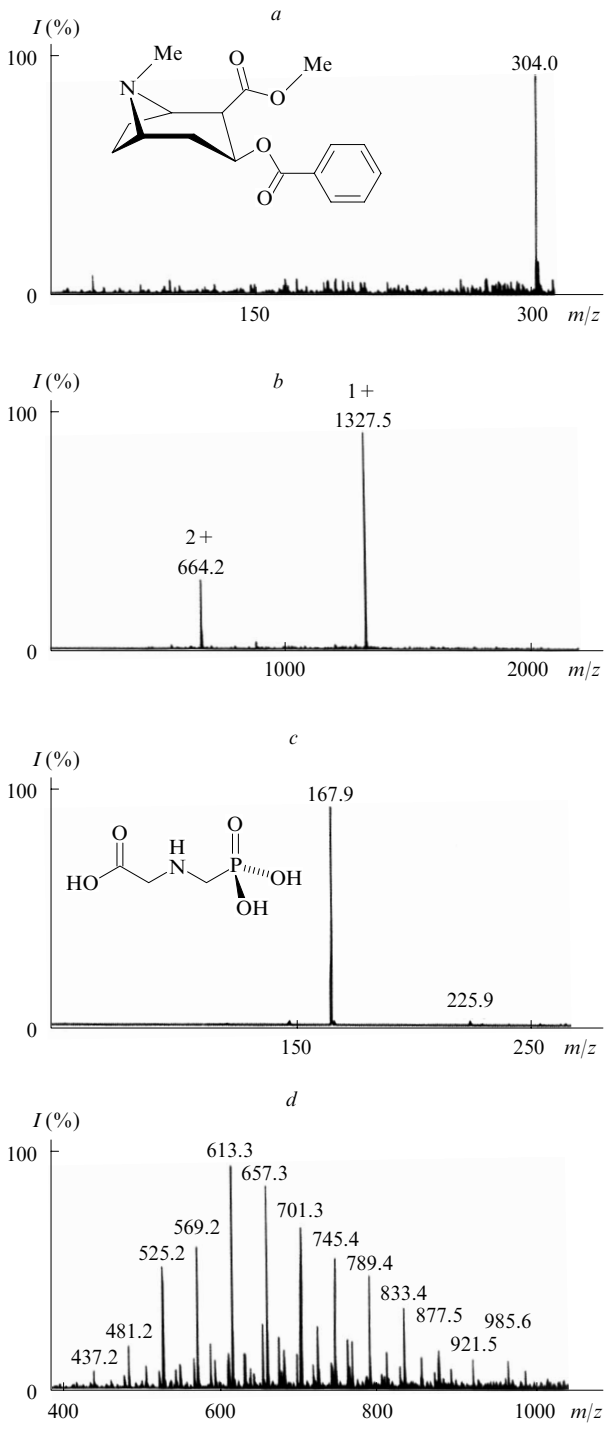


Figure 5. V-EASImass spectra of a 25 ng ml^{-1} cocaine solution (*a*); an acidified solution of the peptide SIS[pT]RK[pY]SPE (150 μg ml^{-1}) (*b*); glyphosate solution (50 μg ml^{-1}) (*c*); and poly(ethylene glycol) solution (50 μg ml^{-1}) (*d*).[55]

The 'Spartan' ion source is made of simple and readily available parts including a surgical catheter, an aerosol spray can with compressed gas (*e.g.*, dust cleaner), a small fused silica capillary and a syringe needle. The device can be assembled with ease even by unskilled persons. In spite of a very simple design of the ion source, ionization occurs with high efficiency. Using a commercially available can containing compressed air and chlorofluorocarbons, one gets no problems with the quality of mass spectra obtained in the positive-ion and negative-ion modes. Pressing the can valve for 2 min produces a constant gas flow of 4 litre min^{-1}; this is sufficient to carry out targeted analyses. When working with liquid samples, the end of the capillary is dipped into the analyte solution. In the case of solid samples, the gas flow from the syringe is directed towards the sample which is positioned close to the atmospheric inlet of the mass spectrometer. The signal-to-noise ratio in the mass spectra of various compounds including narcotics, pesticides, peptides and polymeric materials (Fig. 5) obtained with the 'Spartan' ion source was very high.[55] Undoubtedly, coupling the 'Spartan' ion source with an appropriate portable mass spectrometer seems to offer great prospects.

II.1.d. Extractive electrospray ionization

DESI loses its efficiency when applied to analysis of volatile compounds. In this case, the method of extractive electrospray ionization (EESI)[56] may appear to be useful. A schematic of the method is shown in Fig. 6.

The EESI method requires two kinds of spray, one for spraying the analyte solution and the other to produce charged microdroplets of solvent, like in DESI. To operate in the positive-ion and negative-ion modes, one should apply a voltage of 3 to 5 kV. Two spray flows intersect at a short distance from the atmospheric inlet of the mass spectrometer. Analyte molecules are extracted into electrosprayed solvent droplets and ionized by a mechanism similar to electrospray ionization. The angles between the spray flows and the atmospheric inlet can vary over a wide range. For instance, an increase in the angle α (see Fig. 6) favours a higher sensitivity, whereas long-term reproducible operation occurs at $\alpha = \beta = 90$. The method is efficient for direct analysis of complex liquid samples (for *e.g.*, urine) within a few hours [*cf.* a few minutes for ESI or atmospheric pressure chemical ionization (APCI)].

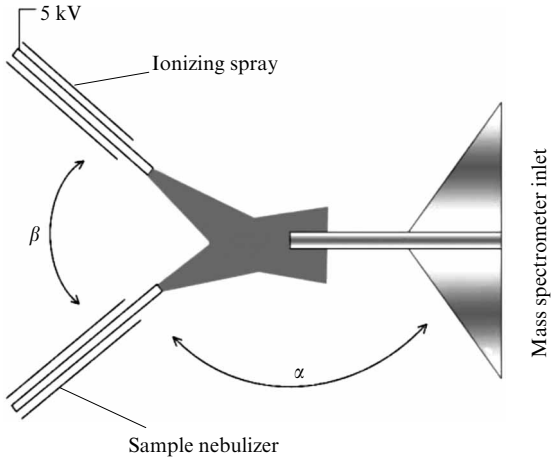


Figure 6. A schematic of EESI experiment.[56]

The EESI method is successfully used in online clinical diagnosis by analyzing exhaled air (various volatile and nonvolatile components of samples are detected).[57] To determine particular classes of compounds, one can modify the composition of the electrosprayed solvent. In particular, the addition of minor amounts of $AgNO_3$ to water leads to an abrupt increase in the intensities of signals of the molecules with high affinity to Ag^+ ion. For instance, an analysis of exhaled air leaves in the mass spectrum only peaks from solely sulfur-containing compounds.[57] Emphasize that the sensitivity of EESI (and other methods based on electrospray) is compound (analyte) dependent.

A version of the method in question, namely, EESI with desorption of neutral molecules (EESI-NM), involves initial desorption of sample molecules in any state of aggregation into a neutral gas flow which is mixed with the ESI spray. As a consequence, the EESI-NM mass spectra are similar to ESI mass spectra.[58] Usually, nitrogen is used as desorbing gas (in ESI, it is used for desolvation). The key advantage of EESI-NM consists in the possibility to analyze samples in complex matrices due to separation of sampling and ionization in space and time. Efficient ionization is achieved for almost all compounds with molecular masses below 1000 Da. The EESI-NM technique was used for, *e.g.*, rapid analysis of meat and fish, determination of bacterial contamination of spinach, *in vivo* identification of compounds on human skin,[58] rapid screening of drug components[59] and differentiation of maturity of fruits.[60]

The sensitivity of EESI-NM is rather high. In particular, the detection limits of the target components in the analysis of meat[58] were in the range from 10^{-14} to 10^{-12} g cm^{-2}. Taking into account a short time taken to collect a mass spectrum (1–2 s), the method seems to be suitable for automated high-performance analysis.[61] Besides, the EESI-NM mass spectra depend on the desorbing gas. For instance, the addition of acetone vapours to nitrogen leads to significant changes in the mass spectrum of compounds desorbed from human skin as compared to the mass spectrum obtained using pure nitrogen.

Organic chemists may take an interest in the possibility of studying mechanisms of reactions including the formation of intermediates. Ambient ionization mass spectrometry methods appeared to be highly efficient for continuous monitoring of chemical reactions. One can not only obtain quantitative information on the starting compounds and target products in the course of a reaction, but also monitor side products and elucidate the structures of intermediates including those formed in trace amounts and short-lived ones. In particular, EESI was used to monitor a one-step Michael addition reaction of phenylethylamine and acrylonitrile in ethanol, as well as a multistep acetylation reaction of benzyl alcohol with acetic anhydride catalyzed by 4-dimethylaminopyridine in dichloromethane.[61] A schematic view of the experimental setup is shown in Fig. 7 *a*. Reactions occurred in a three-necked flask. A nitrogen gas flow (50 litre h^{-1}) was supplied at regular intervals through one neck. Components of the reaction mixture transferred by the nitrogen flow were ionized with a flow of electrosprayed solvent and then transported to the atmospheric inlet of a mass spectrometer. The response time was shorter than 1 s. The study of the acetylation reaction revealed a number of side products and intermediates involving

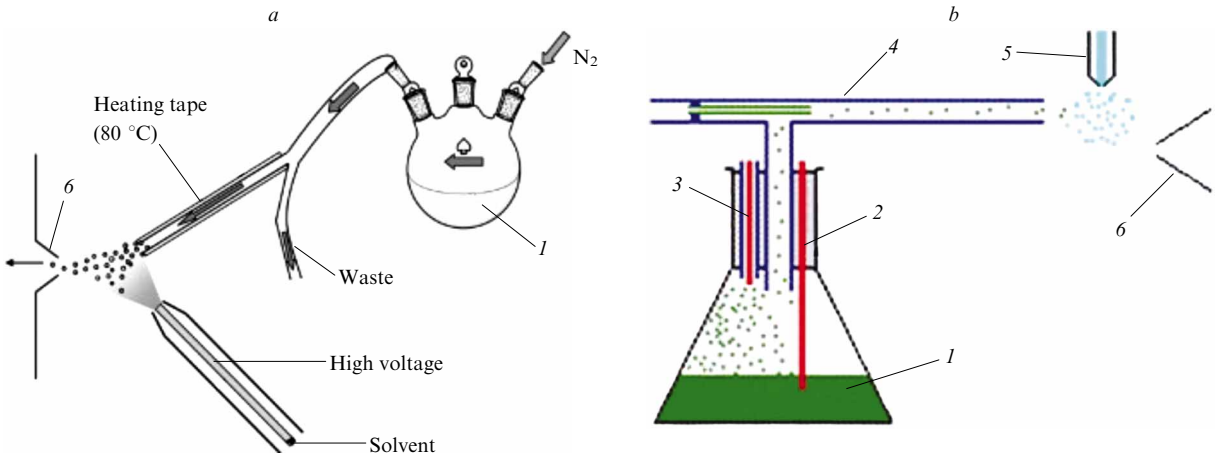


Figure 7. A schematic view of the EESI setups for online monitorng of chemical reactions reported in Refs 61 (*a*) and 62 (*b*). Reaction mixture (*1*), a capillary for drawing the reaction mixture with a peristaltic pump (*2*), a capillary for nebulizing the reaction mixture upon splitting the flow from the first capillary (*3*), aerosol sampling using the Venturi effect (*4*), ESI solvent nebulizer (*5*) and conical inlet of the mass spectrometer (*6*).

4-dimethylaminopyridine. The sensitivity of the method can be improved by aerosolization of the reaction mixture.[61] The EESI approach also allows one to analyze less volatile compounds.[60]

A version of the EESI method for monitoring of chemical reactions with regular sampling using the Venturi effect (Fig. 7 *b*) was reported.[62] The efficiency of the experimental setup and the overall method was demonstrated in relation to monitoring of ethyl salicylate hydrolysis in methanol. A drawback of the approach consists in the dynamic range of 3.5 orders of magnitude, which precludes operation with trace amounts of intermediates and side products.

II.1.e. The 'paper spray' method

A very simple and versatile method of ambient ionization termed 'paper spray' was proposed by Cooks and co-workers in 2010.[63] A schematic of the technique is shown in Fig. 8. The role of a substrate is played by a triangular piece of paper or some other porous material. As a high voltage is applied to a wetted material, ions begin to move towards the tapered end of the paper triangle; gas-phase analyte ions are formed by the electrospray mechanism.

The method makes it possible to analyze both small organic molecules and large biopolymer molecules. Samples are prepared either by applying a few microlitres of solution to a triangular piece of paper or by wiping the surface under study with a triangular piece of paper. Microdroplets are sprayed through numerous microchannels in paper, *i.e.*, the 'paper spray' method is equivalent to the use of a group of nanosprayers. In this case, ion channels are not blocked and the ion currents achieved are rather high.

A version of the 'paper spray' method, namely, the 'dried blood spot' technique, appeared to be highly efficient. Paper samples thus obtained can be stored for long and mailed long distances for re-analysis. The linearity range of the method much exceeds the therapeutical concentration range of pharmaceuticals and their metabolites. Similarly to reactions in microdroplets (see below), analytes can be derivatized with ease immediately in the samples applied to paper. Main fields of application of this method include biomedical issues, *e.g.*, monitoring of pharmaceuticals and their metabolites in biological liquids.[64–66]

Since the 'paper spray' method requires no pumps, it can be used on-site. An important task is to correctly choose the solvent to dissolve the target compounds. Most often, a water–methanol (1 : 1) mixture is used. In this case, the optimum voltage is in the range from 2 to 4.5 kV and spraying at the sharp end of the paper triangle can be seen

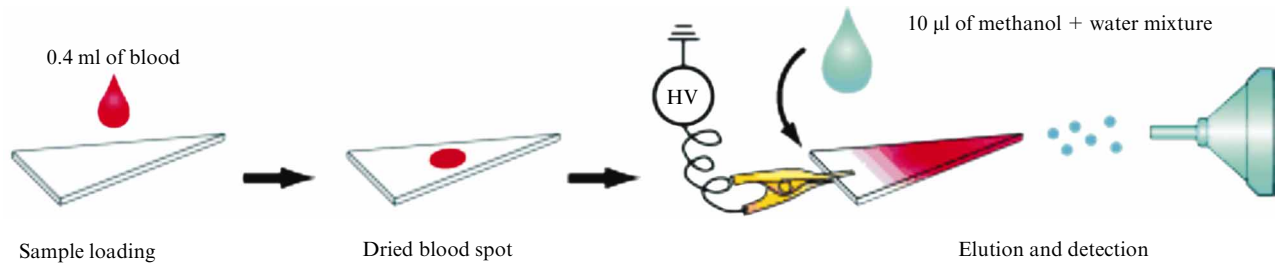


Figure 8. A schematic of the paper spray technique.[63] A chromatography paper triangle with an applied blood drop is hold with a clamp to which a high voltage is applied. The addition of a solvent droplet causes ions to move and go to the gas phase from the sharp paper tip adjoining the atmospheric inlet of the mass spectrometer.

by the naked eye provided good illumination. Other solvents are also used; furthermore, polar compounds such as phospholipids and peptides can be analyzed using polar solvents (*e.g.*, hexane).[67]

Desolvation in spray proceeds by conventional ESI mechanisms. Paper spray can occur in two modes.[68] In solvent-rich systems (mode 1), a number of spray jets emerge near the sharp end of the paper triangle. The droplet size varies from 0.5 to 1.9 μm; proton transfer reactions are the most competitive. In solvent-deficient systems (mode 2), only one Taylor cone is formed while the ion current increases and reaches a value of 0.8 μA. In this case, one deals with monodisperse droplets at most 1 μm in size. Proton transfer is accompanied by electron transfer resulting in ions with an odd number of electrons. This is termed 'field-enhanced electrospray'.[68] Here, the only electrospray cone is coupled to a corona discharge and ionization of molecules of lower polarity including aromatic hydrocarbon and fullerene molecules occurs more effectively. Mass spectra obtained in the negative-ion mode are characterized by domination of peaks of deprotonated molecules, although radical anions are also formed.

The spread mechanism of analyte solution on paper was studied.[69] Three possible processes causing motion of the mobile phase were considered:

— capillary forces (wetting),
— electrophoresis and
— bulk solution motion as a surface thin film.

It was found that the third mechanism dominates while electrophoresis is almost absent.

The efficiency of analysis depends on many parameters.[69] For each size of the paper triangle, there is particular, optimum amount of analyte. The spray tip angle is of great importance, namely, the smaller this angle the higher the electric field strength at the spray tip. The highest spray flow is achieved at a spray tip angle of 30°. As this angle further increases, the spray flow narrows and becomes less intense. At a spray tip angle of 150°, no spray is observed. Narrowing the spray flow leads to an increase in the proportion of ions transferred to the mass spectrometer; therefore, the optimum spray tip angle lies between 60 and 90°. Based on the optimized geometry and experimental conditions, a paper spray cartridge for clinical analyses was designed.[69]

If a dried analyte sample is placed in a ESI capillary, a solvent spiked into the sample will dissolve the sample components and generate a classical electrospray. This technique named 'extraction spray' can be more sensitive than the 'paper spray' method.[70]

At present, biomedical applications of the 'paper spray' approach dominate[69] because is it convenient for fast detection of pesticides, hormones, dyes, plasticizers in food samples, on various surfaces, on packages, *etc.*[71] With a portable mass spectrometer available, samples can be analyzed *in situ*.[72]

II.1.f. The 'leaf spray' method

The development of the 'paper spray' method was followed by elaboration of the 'leaf spray' (LS) technique (see Fig. 9).[73] If a leaf of a plant has a sharp tip, it can be used as a whole or a triangular piece can be cut. Not only leaves, but also any plant material including flowers, plant roots, squash, seeds, *etc.* can be used as samples. Optimum dimensions of a triangular sample ~3 mm thick are as follows: a ~5-mm base and a nearly 10-mm height. To

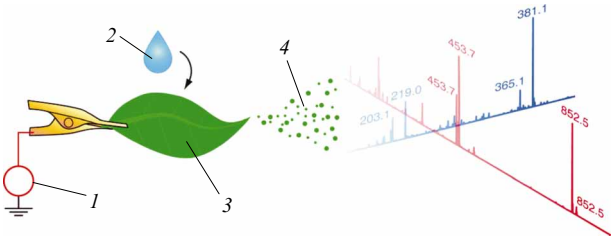


Figure 9. A schematic of the 'leaf spray' experiment.[73] High voltage power supply (*1*), methanol (optional) (*2*), plant tissue (*3*) and charged spray (*4*).

such a sample, a high voltage is applied. Experiments can be performed with any pointed goods including such unexpected items, as metallic needles in the probe electrospray ionization (PESI) method which penetrate the sample and then ionize adsorbed material upon application of high voltage and spraying the solvent,[74,75] or even wooden toothpicks.[76]

Application of a high voltage (~4.5 kV) of desired polarity to a triangular sample causes ions to move and go to the gas phase at the sharp tip (see Fig. 9). As a result, signals from various endogenous and exogenous compounds including amino acids, carbohydrates, fatty acids, alkaloids, lipids and pesticides present on the sample surface are detected. Usually, peaks from protonated and cationized (Na^+, K^+) molecules dominate in the mass spectra. In the negative-ion mode, deprotonated molecules are formed. In some cases, the results can be improved by introducing chloride ions into the sample (consequently, intense signals from $[M+Cl]^-$ appear).[77,78] No visual changes in the sample are observed in the course of experiments even if collection of the mass spectra takes a few minutes.

There is no need to add solvent droplets. For a fleshy fruit, its natural sap is sufficient for spraying to obtain high-quality mass spectra. Methanol appeared to be a versatile solvent. The shape of the mass spectrum did not change significantly as methanol volume was varied from 10 to 50 μl and the voltage duration was varied from 1 s to a few minutes. However, solvents may be useful to carry out targeted analysis. Fundamental changes in the mass spectra upon change in the solvent nature are illustrated in Fig. 10. Each solvent to a greater or lesser extent 'elutes' particular compounds.[73] Depending on the goal of the experiment, one can choose an optimum solvent to achieve a maximum selectivity and sensitivity of targeted analysis.

The LS method is convenient for direct detection of pesticides in vegetables and fruits, the detection limits being sufficient for operation at a level of maximum allowable concentrations.[79] The MS/MS analyses take at most 100 s and require a minimum amount of solvent. The detection limits for different matrices are 5–50 ng g^{-1}, while the linearity range is 5–15 000 ng g^{-1} (the last value is for potato peel).

When using a high-end mass spectrometer, the LS method can provide important structural information on complex organic compounds. Examples include identification of (i) glycosides in fresh leaves of *Stevia rebaudiana* or (ii) allergens from leaves of *Toxicodendron* genus plants.[77,78] A comparison of the results obtained by the DESI, 'paper spray', low-temperature plasma (see below)

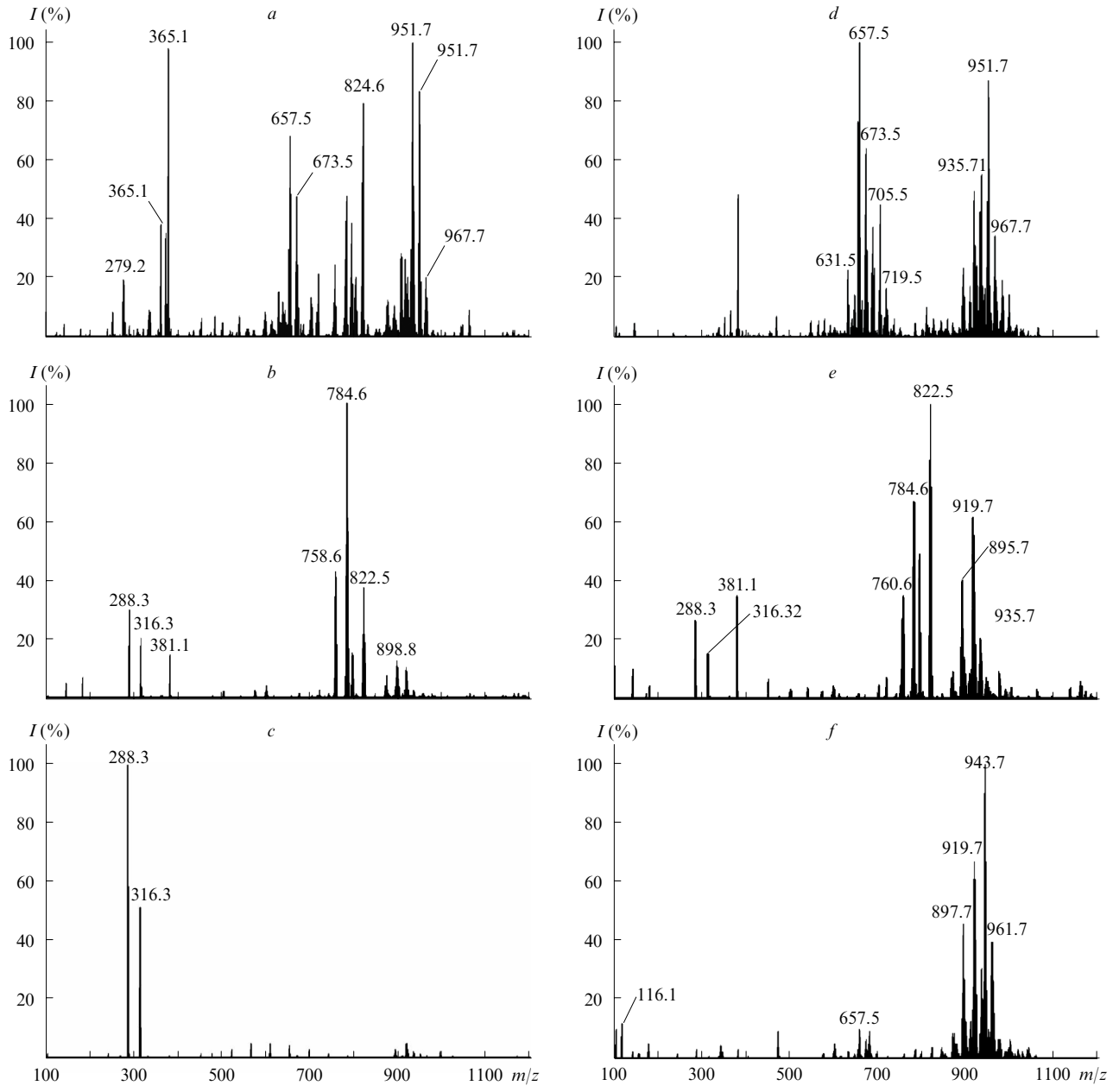


Figure 10. 'Leaf spray' positive-ion mode mass spectra of peanuts obtained with different solvents: methanol (*a*), dichloromethane (*b*), hexane (*c*), acetonitrile (*d*), chloroform (*e*) and acetone (*f*).[73]

and LS methods showed that the last-mentioned method gives the best results.

The LS approach can be practically implemented both in the laboratory and *in situ* using portable mass spectrometers (see Section VIII). The results obtained by this rapid method are of great importance for pharmaceutical industry, for research into primary and secondary metabolism, for controlling maturity of fruits and vegetables, for detection of pesticides, *etc*.

II.2. Plasma ionization methods

Equipment for plasma-based methods of ambient ionization mass spectrometry is even simpler than in the case of the spray ionization methods because no solvent is used and only gas (often, air) is needed as expendable material. The drawbacks of plasma-based methods include (i) low spatial resolution in the analysis of the distribution of substances on surfaces and (ii) impossibility to analyze high-molecular-mass compounds. All plasma desorption/ionization methods are based on thermally induced transition of analyte molecules from the surface to the gas phase followed by ionization. To date, a variety of experiments using different methods and discharge gases have been carried out and helium appeared to be the first in sensitivity and versatility of analytes; nitrogen, argon or air can compete with helium only rarely. However, one should keep in mind that in the experiments with, *e.g.*, low-temperature plasma, the use of helium instead of nitrogen or air often leads to production of high concentrations of background ions owing to higher ionizing ability of He.

It is commonly accepted that main ion/molecule reactions preceding ionization of analyte molecules are due to long-lived, high-energy, metastable helium atoms (He*) whose energies (19.8 or 20.9 eV depending on the type of the excited state) are high enough to ionize almost all kinds of molecules (M) by the Penning ionization mechanism

$$He^* + M \longrightarrow He + M^{+\bullet} + e \quad (1)$$

Atmospheric gases are also no exception while high concentrations of nitrogen and water vapours make generation of $N_2^{+\bullet}$ and $H_2O^{+\bullet}$ ions in the reactions similar to reaction (1) quite obvious. Plasma-based methods most often involve the formation of protonated molecules. The most efficient reagent ions in plasma are protonated water clusters, $(H_2O)_nH^+$, formed in the secondary ion/molecule reactions

$$H_2O^{+\bullet} + H_2O \longrightarrow H_3O^+ + OH^\bullet \quad (2)$$

$$H_3O^+ + n\,H_2O \xrightarrow{N_2} (H_2O)_{n+1}H^+ \quad (3)$$

Gas-phase organic molecules with proton affinities higher than that of water clusters can be ionized by the proton transfer mechanism [33]

$$(H_2O)_nH^+ + M \longrightarrow n\,H_2O + MH^+ \quad (4)$$

Nevertheless, it was shown [80] that $He_2^{+\bullet}$ radical ions produced in at least three possible processes

$$He^{+\bullet} + 2\,He \longrightarrow He_2^{+\bullet} + He \quad (5)$$

$$He^* + He \longrightarrow He_2^{+\bullet} + e \quad (6)$$

$$2\,He^* \longrightarrow He_2^{+\bullet} + e \quad (7)$$

play at least a comparable role to that of metastable helium atoms.

At pressures >5 Torr, the concentration of $He_2^{+\bullet}$ radical ions in plasma is much higher than that of $He^{+\bullet}$ radical ions.[81] Under ambient ionization mass spectrometry conditions, reaction (5) is preferred to such an extent that the conversion time is in the range from 12 to 27 ns.[82] It is $He_2^{+\bullet}$ that makes the major contribution to the formation of $N_2^{+\bullet}$ and $N_4^{+\bullet}$ reagent ions:

$$He_2^{+\bullet} + N_2 \longrightarrow 2\,He + N_2^{+\bullet} \quad (8)$$

$$N_2^{+\bullet} + 2\,N_2 \longrightarrow N_2 + N_4^{+\bullet} \quad (9)$$

which then ionize water vapours

$$N_4^{+\bullet} + H_2O \longrightarrow 2\,N_2 + H_2O^{+\bullet} \quad (10)$$

Undoubtedly, depending on the experimental conditions, the contributions of ionization involving He* and $He_2^{+\bullet}$ species will be different. If the latter will be the preferred reagent ions in low-temperature plasma, the concentration of $He_2^{+\bullet}$ species in the region of interaction with the sample under, *e.g.*, DART conditions can be very low.[80]

II.2.a. Direct analysis in real time

A novel, second after DESI, method of analysis termed 'direct analysis in real time' (DART) was reported in 2005.[2] This approach allows one to analyze gaseous, liquid and solid samples almost without sample preparation. Good results were obtained in the analysis of components of chemical warfare, explosives, pharmaceuticals and their metabolites, ecotoxicants, as well as organic and organometallic compounds. Although significantly different, these compounds can be detected on any types of surfaces including concrete, asphalt, human skin, bank notes, glass, leaves, fruits, clothes, *etc*. Analysis of, *e.g.*, pharmaceuticals requires neither grinding nor extraction of analytes.

A schematic of a DART source is shown in Fig. 11. An inert gas (He or N_2) is passed through the source at a flow rate of 1–3 litre min^{-1}. A positive potential of 1–5 kV relative to the first perforated disk electrode is applied to the needle electrode and a glow discharge appears at a nearly atmospheric pressure. The discharge is accompanied by production of excited-state cations, electrons and neutral species (first of all, helium atoms or nitrogen molecules).[83] Plasma thus produced is transferred to the second compartment of the source where the second perforated disk electrode may be placed in order to remove ions. In the third compartment, the plasma temperature can be increased to 500 °C provided that the gas heater is in operation. At this temperature, less volatile compounds are desorbed from the surface. The terminal grid electrode reflects ions of the same polarity and eliminates ions of opposite polarity, which can cause weakening of the signal as a result of ion–ion interactions. The insulator cap protects the sample and operator from contact with electrodes.

Ionization occurs by interaction of gas plasma with atmospheric gases or with sample molecules on the surface at which the stream arrives. When excited plasma species interact with molecules of low polarity (and even nonpolar ones), ionization mainly proceeds by the Penning ionization mechanism [reaction (1)]. The interaction with analyte molecules of higher polarity mainly results in protonated molecules, MH^+ [reaction (4)[. Electrons can also be produced through Penning ionization; this enables an analysis in the negative-ion mode to detect interactions between analyte molecules and oxygen radical anions and water clusters.

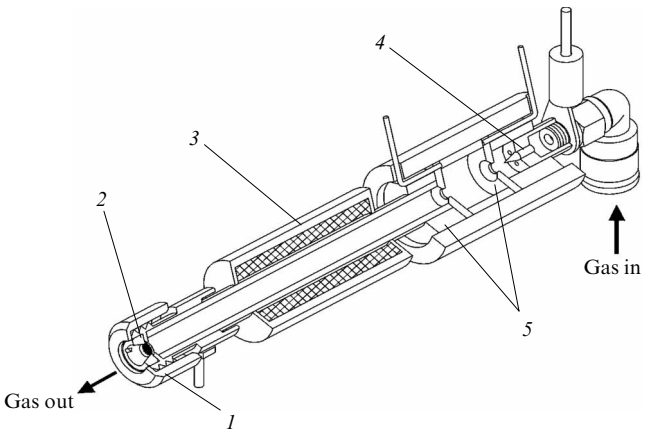


Figure 11. A schematic of the DART source.[2]
Insulator cap (*1*), grid electrode (*2*), gas heater (*3*), needle electrode (*4*) and perforated disk electrodes (*5*).

The DART gas stream can be directed immediately towards the atmospheric inlet of the mass spectrometer or reflected from the sample surface into the mass spectrometer. For each analyte, there is an optimum geometry of the DART experiment which includes the distances from the ion source to the sample and from the sample to the mass spectrometer, as well as the angle between the DART source and the mass spectrometer and the gas flow rate.[2]

An advantage of the DART method consists in insignificant suppression of ion production. Therefore, DART can be coupled with high-performance liquid chromatography (HPLC) with phosphate buffers. A comparison of the ion yields in the HPLC analysis of river and waste water samples followed by APPCI, ESI, APCI and DART analyses demonstrated a considerable advantage of DART.[84] Examples of DART application in analysis of pharmaceuticals, in forensic analysis, food industry and other fields are available.[15, 16, 19]

Online DART monitoring of chemical reactions was reported.[85] A glass capillary for melting point measurements used as sampler was periodically dipped in the reaction mixture and then transferred to the plasma region. Monitoring of the methylation and debenzylation reactions gave good results, although the sensitivity of DART was much lower than that of ESI.

DART mass spectrometry was used as detector for liquid chromatography.[86] The main advantage of this combination consists in the possibility to use phosphate buffers with concentrations up to 120 mmol litre^{-1} in the mobile phase at a conventional gas flow of 1 ml min^{-1}. No severe contamination of the ion source and ion suppression was observed. Also, operation in both the positive-ion and negative-ion modes with detection limits of the order of a few μg litre^{-1} is possible.

A device combining ion mobility mass spectrometry with DART was reported.[87] The first encouraging results were obtained in direct analysis of solid samples of pharmaceuticals.

II.2.b. The low-temperature plasma method

The low-temperature plasma (LTP) technique allows one to analyze the most 'sensitive' samples. For instance, an analysis of compounds present on human skin causes no skin damage or any discomfort. The method is based on dielectric barrier discharge at ambient temperature[88] using gas flows lower than 100 ml min^{-1} and a 1 W power supply. These parameters enable practical implementation of the LTP method in portable mass spectrometers that require small aerosol spray can filled with, *e.g.*, helium and electrical batteries instead of solvents and gas/liquid pumps. Analysis of solid, liquid or gaseous samples is possible. Often, ionization occurs using compressed air as the oxidizing agent,[89] with atmospheric nitrogen and oxygen as main reagent gases. Depending on the nature of analytes and experimental conditions, ionization can proceed by different mechanisms including protonation, deprotonation, electron capture, *etc.*, and produce positively or negatively charged molecular ions with odd number of electrons or protonated (deprotonated) molecules. When using helium as discharge gas, the most important ion generated in the discharge zone is $He_2^{+\bullet}$ radical cation, which has two main functions:

— energy transfer from the discharge zone to peripheral regions where analytical reactions occur; and

— ionization of atmospheric nitrogen by charge exchange to produce $N_2^{+\bullet}$ ions which in turn participate in the formation of secondary reagent ions, *e.g.*, protonated clusters of water molecules.

A comparison of helium and nitrogen (air) as discharge gases revealed[88] more intense peaks including background-ion peaks in the mass spectrum in the former case. This is because plasma contains excited helium states with energies >19 eV, which can ionize a larger number of compounds; this complicates the mass spectrum and can reduce the signal-to-noise ratio. However, the use of helium is more preferable if experiments are aimed at decreasing the detection limits.[23]

A schematic of the LTP method is shown in Fig. 12. There is a glass tube of an LTP source (dielectric barrier) with an internal grounded electrode arranged coaxially and a copper tape (outer electrode) surrounding the glass tube from the outside. An alternating voltage of 3–5 kV at a frequency of 2 to 5 kHz is applied to the outer electrode. Plasma in the tube is generated by gas discharge. The LTP probe is electrically insulated to protect against electric-shock hazard. Since plasma has a temperature of at most 30 °C, it causes no thermal damages of most surfaces including human skin.

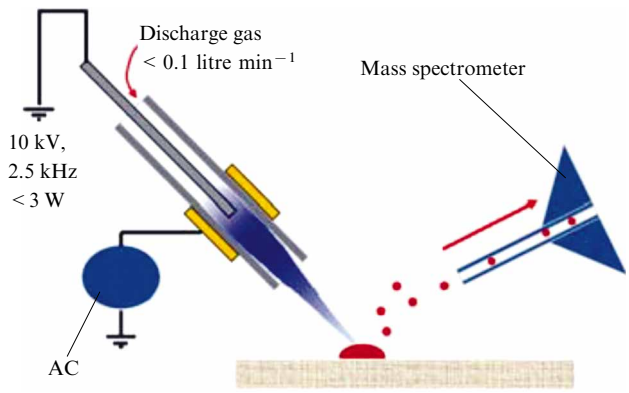


Figure 12. A schematic of the LTP method.[11]

The LTP approach is convenient when the desorption temperatures should be low, *e.g.*, in airport safety control[88, 90] or in portable mass spectrometers. In particular, in the negative-ion mode one can reliably detect a wide range of trace amounts of nitrogen-containing explosives.[91] For trotyl, the detection limit was only 0.6 pg (linearity range covered three orders of magnitude), with $[M-NO_2]^-$ and $[M + NO_2]^-$ being the characteristic ions. The sensitivity of detection can be improved by adding some agents to the discharge gas. In this case, one deals with reactive LTP. For instance, the addition of trifluoroacetic acid (TFA, 0.2%) causes a considerable enhancement of the signal from hexogen owing to $[M + TFA]^-$ ion.

The LTP method is highly efficient in the determination of double bond positions in the molecules of unsaturated fatty acids and their esters.[89] This is a topical problem in mass spectrometry because electron ionization and chemical ionization are accompanied by double bond migrations along the chain.[33] The LTP approach allows one to rapidly obtain reliable results. An example is provided by the LTP mass spectrum of pinolenic acid ethyl ester (see Fig. 13)

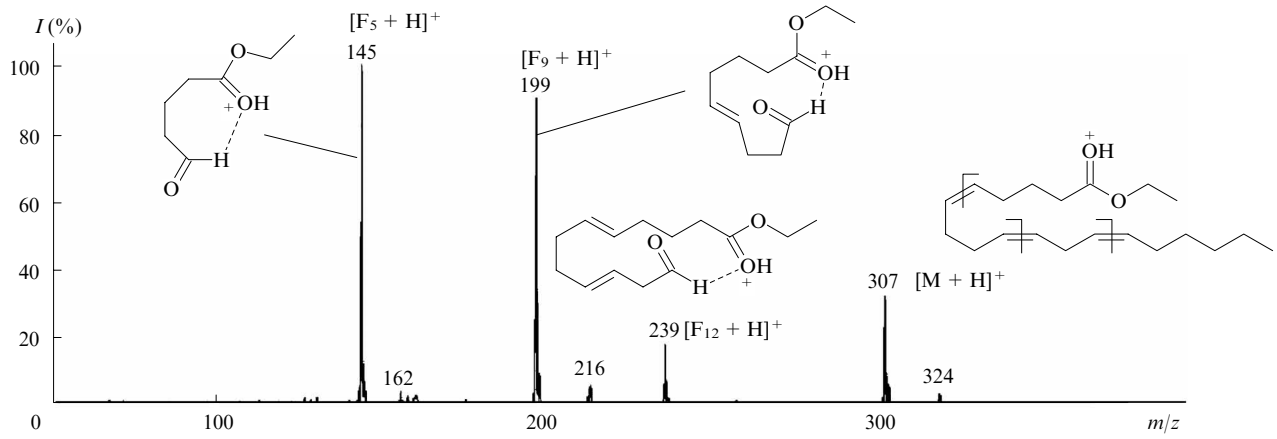


Figure 13. LTP mass spectrum of pinolenic acid ethyl ester.[89]

which exhibits peaks from highly characteristic species, *viz*., the protonated molecule (*m*/*z* 307) and $[F_n + H]^+$ fragment ions generated by ozonolysis of the initial molecule at double bonds. Ozone is generated under LTP conditions when using not only air, but also helium as discharge gas, because the process occurs in ambient conditions. Using, *e.g*., a protective helium stream to completely suppress oxidation, one can obtain a mass spectrum that exhibits only a peak from the protonated molecule.[89]

The LTP method was successfully used to detect explosives,[88] pesticides,[92] pharmaceuticals, narcotics[90] and viscous liquids (olive oil).[93]

II.2.c. Desorption atmospheric pressure chemical ionization

The desorption atmospheric pressure chemical ionization (DAPCI) technique has a a number of versions. One of them[37] can be treated as a method based on spray ionization because the classical DESI experiment with electrosprayed water–methanol mixture was coupled with corona discharge generated near the substrate whose surface was sprayed with the sample. This enhanced the intensities of signals in the mass spectra of some compounds under study. The term 'DAPCI' was for the first time proposed by Cooks and co-workers,[94] who used corona discharge near the substrate coated with the sample instead of electrospray of solvent. Molecules of solvent vapours supplied to the region of corona discharge were ionized using corona discharge ionization and became reagent ions for subsequent chemical ionization of the sample molecules. The solvents were toluene, methanol and some other compounds. Ionization proceeds by the proton or electron transfer mechanism (charge exchange).

There is yet another version of DAPCI, namely, atmospheric-pressure solid analysis probe (ASAP) proposed in 2005.[95] In this case, a solid sample in a melting point tube is placed into the APCI ion source while a stream of heated inert gas (conventional gas streams for spraying and desolvation in ESI/APCI) delivers analyte molecules to the region of corona discharge. The method seems to be competitive with electron ionization in the analysis of mixtures, because the mass spectrum usually exhibits only peaks from protonated (deprotonated) molecules. Since evaporation and ionization occurs at atmospheric pressure, the method is very fast and in some cases can indeed be used instead of the electron ionization technique.

A comparison of the efficiency of the DART, DESI and DAPCI methods in the analysis of pharmaceuticals and biologically active compounds showed[96] that DAPCI is more preferable in the case of compounds of low polarity. Two versions of corona discharge were used, a conventional one with water–methanol mixture and a solvent-free version similar to that described in Ref. 95. The DAPCI method with water–methanol mixtures demonstrated the highest efficiency.

Since it is desired to avoid the use of toxic chemicals (toluene, methanol), a solvent-free DAPCI with water vapour in atmospheric air as reagent gas was proposed.[97] A schematic of the method is shown in Fig. 14. Ionization requires neither solvent nor gas stream or dopants; the only necessary condition is to have an atmospheric air humidity of at least 20% (otherwise, water vapour should be delivered to the reaction zone). However, it should be noted that the signal intensity dramatically decreases at too high humidity (>60%). Since, as in most other plasma ionization methods, it is water that plays a key role, the most intense signals originate from protonated or deprotonated molecules. The sensitivity of the method is in the picogramme range. This type of ionization can be utilized to detect target analytes in food, on human skin and clothes.[97]

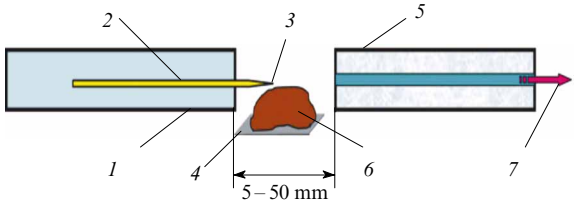


Figure 14. A schematic of the DAPCI mehod with no solvents and dopants.[97]
Insulated needle holder (*1*), high voltage (*2*), sharp needle (*3*), sample holder (*4*), capillary of mass spectrometer (*5*), sample surface (*6*) and ion inlet of the mass spectrometer (*7*).

II.2.d. Rapid evaporative ionization mass spectrometry

Production of ions of organic compounds upon heat treatment of solid samples has been known since the 1970s.[33] The phenomenon was used in the development of a direct (desorption) chemical ionization technique based on rapid heating of the sample to a temperature at which evaporation proceeds faster than thermal degradation. In open-air conditions, the thermal energy of a species efficiently decreases owing to collisions with gas molecules. This kind of cooling is characterized by additional suppression of thermal degradation (organic cations thermally desorbed in these conditions are characterized by minimum thermal degradation).[98] Formally, rapid evaporative ionization mass spectrometry (REIMS) is insensitive to the method of abrupt heating of the solid sample. One can use Joule heating, contact heating or radiative heating. For instance, mass spectra of biological tissues obtained using laser ablation (laser desorption ionization) and by the electrochemical Joule heating method are very similar.[99]

Although there are various applications of REIMS, the most in-demand application is still the analysis of biological tissues, including *in vivo* analysis which is of crucial importance for medicine. Experiments showed that REIMS of biological tissues provides information of the molecular masses of many biologically important groups of compounds (first of all, lipids);[25] possible heaters include commercially available surgical instruments, *viz*., surgical lasers and electrosurgical instruments.

An alternative mechanism involves desorption and chemical ionization of neutral organic molecules in the gas phase resulting in protonated (deprotonated) analyte molecules and primary products of their thermal decomposition as major charged species. It is due to the presence of ionized water molecules above the sample when tissues are dissected with an electrosurgical instrument. Since in these conditions, biological tissues are characterized by different sets of lipid markers, online determination of the nature of tissues is possible and one can discriminate with ease between not only fundamentally different, but also related tissues. This observation is basic to the design of a library of biomarkers for different tissues and for online guidance of tumour resection surgery.[25] Mass spectrometry enables fast and reliable identification of tens and even hundreds of biomarkers rather than a single, although very important, compound, thus providing reliable direct information on the nature of a biological tissue, cancer grading, necrosis, content of pharmaceuticals in the tissue, *etc*. A tissue identification system uses principal component analysis and linear discriminant analysis.[99]

An experimental setup for REIMS tissue analysis in surgery is schematically shown in Fig. 15. A tissue is evaporated along the contact surface of the electrosurgical instrument ('smoke' above the sample). Ions thus generated are transported to the mass spectrometer using a Venturi pump and a 4-atm nitrogen or air stream. To make ion transfer more efficient, ions are sucked from the flexible

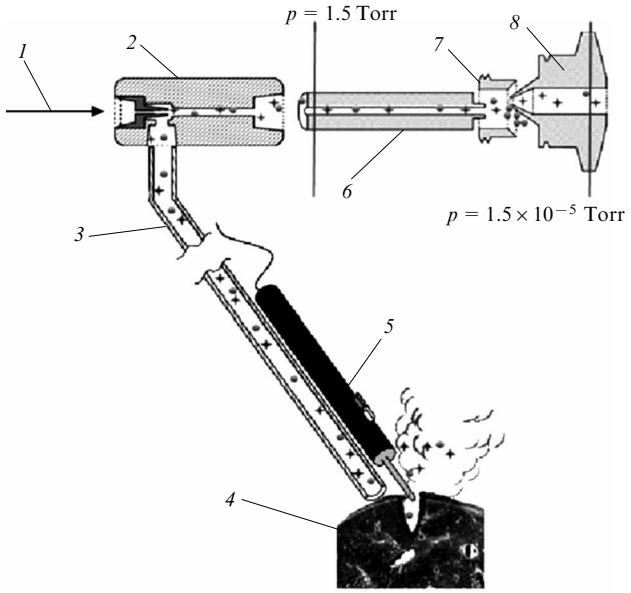


Figure 15. An experimental setup for REIMS tissue analysis.[25] Nitrogen flow (*1*), Venturi pump (*2*), Teflon tubing (*3*), tissue (*4*), needle cutting electrode (*5*), heated capillary (*6*), tube lens (*7*) and skimmer (*8*).

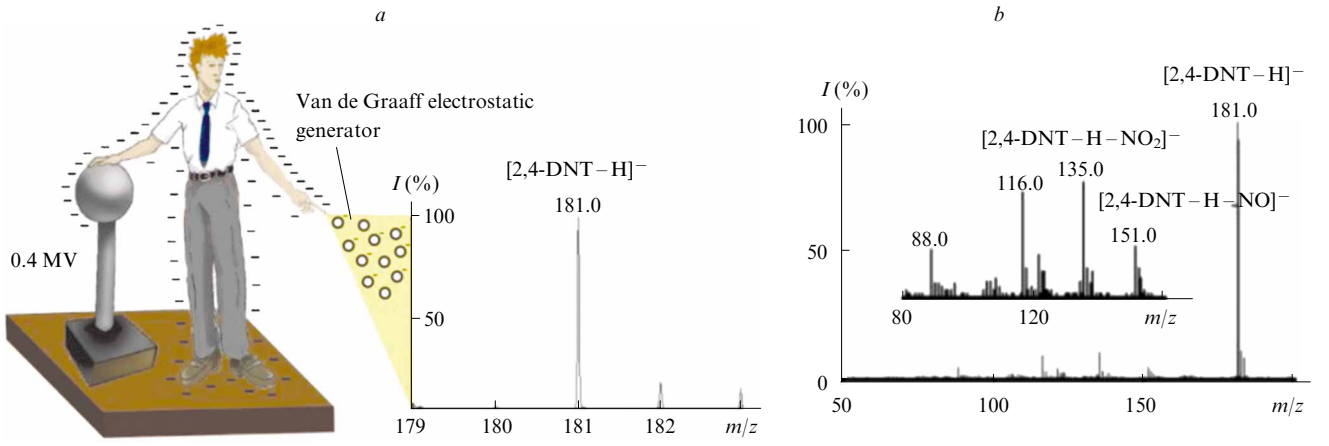


Figure 16. A schematic of the MV electrostatic ionization and the mass spectrum (*a*) and the corresponding tandem mass spectrum (*b*) of dinitrotoluene (DNT) deposited on the test subject's glove.[100]

transmission tube perpendicular to the heated capillary inlet of the mass spectrometer. Both positive and negative ions are generated; they can be separated with respect to their polarity in the skimmer region. Detection of both types of ions gives important complementary information on the composition of biomarkers. Owing to neutralization of simultaneously produced positive and negative ions no static charges appear on the walls in the course of ion transfer to the mass spectrometer *via* the flexible polymeric transmission tube.

As should be expected, the quality of the mass spectra depends strongly on the rate of heating. Experiments revealed an optimum heating regime.[99] Improvement of ionization efficiency with increasing the power of the heater is due to an increase in the rate of disintegration of biological tissues and to faster evaporation of analyte molecules. However, at very high power, high temperature causes thermolysis of the samples. For instance, an analysis of phosphoethanolamines is accompanied by visible carbonization of the sample; also, the analyte molecules lose ammonia.

There is an unusual version of ambient ionization, which seems to be treated as belonging to plasma ionization methods.[100] It involves application of a high electrostatic potential (up to 1 MV) to a human body by short-term contact ($\sim$2 s) with the van de Graaff electrostatic generator. All compounds exhaled or evaporated from the whole surface of human body are ionized (a kind of electric field ionization [33]) without electric shock in living individuals and then sucked into the atmospheric inlet of the mass spectrometer (Fig. 16). To prevent the mass spectrometer from electrostatic damage, the instrument is shielded by a number of electrically grounded metallic plates. The method was named 'MegaVolt electrostatic ionization mass spectrometry'. The possibility to detect explosives and flammable liquids on clothes, cocaine on human skin, acetaminophen in painkiller tablets, as well as numerous components in exhaled air was demonstrated.

II.3. Laser ionization methods

In the case of laser desorption, the concentration of neutral particles desorbed from the surface much exceeds the amount of ions;[101] therefore, subsequent ionization of the neutral particles is of interest from the standpoint of both enhancement of the range of analyzed compounds and reduction of detection limits of the target analytes. Yet another advantage of the laser methods of ambient ionization mass spectrometry consists in the possibility to analyze high-molecular-mass compounds by generating multiply charged ions. Owing to the smaller size of the laser spot, these methods are also characterized by higher spatial resolution compared to the spray methods. Usually, this concerns combined methods involving laser ablation/desorption of neutral particles from the sample surface followed by certain spray or plasma ionization. Harris *et al.*[7] emphasized that although experimental methods described in numerous studies are very similar, they are named in different way. Currently, this leads to confusion, so detailed examination of experimental conditions is necessary in each case.

Under resonant excitation, the absorption of radiation by an analyte, matrix or substrate can be increased by six orders of magnitude. This enables utilization of lasers with power densities of the order of 10^6 W cm^{-2}; here, molecules are desorbed by the phase explosion, thermal, impulsive or electronic-induced mechanisms.[102] Unfortunately, resonant excitation of most molecules is impossible when using lasers operating at particular frequencies falling in the optical region. Therefore, specific matrices are employed in order to absorb photons at particular frequencies. Non-resonant excitation is also possible; in this case, the analyte molecules are analyzed irrespective of their optical properties.

II.3.a. Electrospray laser desorption/ionization

Electrospray-assisted laser desorption/ionization (ELDI) was for the first time reported in 2005.[103] A conventional ELDI MS requires a nitrogen UV laser operating at 337 nm (pulses 4–5 ns long, pulse energies in the range from 150 to 300 μJ). If the angle of incidence of the laser beam is 45°, the spot on the surface has an area of 100 μm^2.[104] Desorbed species go to the stream of spray charged solvent droplets, undergo ionization and then are sucked into the atmospheric inlet of the mass spectrometer. Besides, Nd : YAG-lasers operating at 266, 532 and 1064 nm can be used; the most intense ion signal was detected at 1064 nm. This can be explained by the presence of water molecules in the samples analyzed (in particular, biological tissues). Laser irradiation of samples at λ = 1064 nm leads to excitation of O–H bending vibrations and causes an explosive transition of the material to the gas phase, the desorption/ionization intensity being almost independent of matrix effects.

Stainless steel appeared to be the best substrate material (probably, because of its high thermal conductivity), although experiments were carried out with a variety of materials. When analyzing particular groups of compounds, one should keep in mind that the target analytes should be well-soluble in the electrosprayed solvent. For instance, an analysis of cow's milk using water-rich water–methanol mixtures revealed predominance of peaks from water-soluble proteins and peptides (first of all, casein) in the mass spectra. Contrary to this, the most intense peaks in the mass spectra obtained using methanol-rich water–methanol mixtures originated from lipids characterized by high solubility in methanol.[92]

Nanosecond lasers are mainly used for resonant excitation and evaporation of analyte molecules. Femtosecond lasers also can operate in this mode, but the degree of fragmentation decreases because excitation proceeds faster than it is desired for start of atomic vibrations in the molecule; this leads to almost 100% suppression of fragmentation. If the dissociation time is shorter than the pulse duration, the primary fragments absorb an additional energy of the laser beam; as a result, secondary products are formed. For instance, a nanosecond pulse with a power density of 10^7 W cm^{-2} led to more intense fragmentation of gramicidin D than the femtosecond pulse (λ = 245–265 nm) with a power density of 10^9 W cm^{-2}.[105]

Atmospheric-pressure femtosecond laser desorption/ionization imaging mass spectrometry [106] was called laser electrospray ionization mass spectrometry (LEMS).[107] High-power-density ($\sim 10^{14}$ W cm^{-2}) non-resonant excitation in the near-IR region (λ = 800 nm) causes desorption and ionization of molecules. Here, no matrix is needed because the high-power radiation excites immediately the analyte molecules.

Pioneering experiments revealed a high efficiency of LEMS, especially, for the determination of low-molecular-

mass compounds; however, analyses of substances with higher molecular masses gave poorer results. An example is provided by the experiments[102] with a laser operating at 800 nm (70-fs pulses with a pulse energy of 1.5 mJ). As in other ELDI versions, the angle of incidence of the laser beam was 45° and a beam power density was about 10^{13} W cm^{-2}. The method was found to be admissible for the determination of peptides, porphyrins and vitamin B_{12}. It was emphasized that nonresonant excitation allows one to apply the method for analysis of many other compounds. It should be noted that the intensities of signals from protonated molecules in the matrix-assisted experiments (see below) were somewhat higher, but LEMS is almost free from sample preparation, which is of great importance for medical and forensic analyses of biological tissues.

The internal energies of ions produced in the analysis of dried and dissolved samples under femtosecond nonresonant LEMS conditions ($\lambda = 800$ nm) were compared.[108] The degree of fragmentation of benzylpyridinium salts and peptides was higher for solid samples, being similar to the ESI fragmentation for solutions owing to the protective action of solvent clusters. To obtain the 'softest' ionization, it was recommended to use solutions or to decrease the laser beam energy, especially if multi-photon resonance is possible for the analyte molecules.

II.3.b. Laser ablation electrospray ionization

Laser ablation electrospray ionization (LAESI)[109] is a relative of ELDI. The method involves no sample preparation, is applicable for analysis of a wide range of compounds with molecular masses up to 66 kDa, has a detection limit at a level of a few femtomoles and a dynamic range of four orders of magnitude for solving quantification problems. The penetration depth of the laser light was 5 μm. In the classical version of the method,[110] an IR laser operating at 2940 nm (pulse duration 5 s, pulse energy 100 μJ) excites O–H vibrations in water molecules present in samples (the method is thus particularly efficient in the analysis of samples with high water content). Usually, the laser beam is directed normal to the sample surface. As a result of a microexplosion at the point of ablation, analytes are ejected to the gas phase and appear in the stream of electrosprayed solvent. Analytes either coalesce with solvent droplets with subsequent ionization or react with gas-phase ions. The charged analyte species are sucked into the atmospheric inlet of the mass spectrometer.

The LAESI method proved itself in imaging under ambient ionization mass spectrometry conditions (see Section VI). For instance, an analysis of metabolites in plant leaves demonstrated a 2D spatial resolution of down to 350 μm and an in-depth resolution of 40 μm.[111] A LAESI MS imaging study of tissues in rat brain was characterized by improved 2D spatial resolution (the laser spot diameter was 200 μm).[110]

II.3.c. Other laser methods

Results of experiments with IR and UV lasers under ambient ionization conditions can be improved using matrix-assisted techniques, in particular, matrix-assisted laser desorption electrospray ionization (MALDESI).[112]

Using a CO_2 laser ($\lambda = 10.6$ μm) to analyze liquid samples with no matrix assistance, one can produce multiply charged ions of biological molecules with masses of up to 8–17 kDa.[113] For some proteins, signals were detected provided no ESI post-ionization.

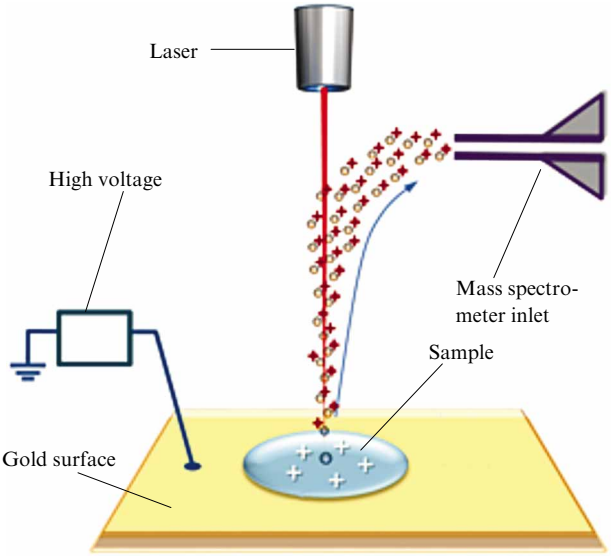


Figure 17. A schematic of the HALDI method.[114]

High-voltage-assisted laser desorption/ionization (HALDI) mass spectrometry was proposed.[114] Protonated molecules (including polyprotonated) of proteins, peptides, oligonucleotides, pharmaceuticals, *etc*. were produced using a Nd : YAG-laser ($\lambda = 1064$ nm) which desorbed charged species from the surface of liquid samples on a substrate to which a high voltage (up to 5.5 kV) was applied. The method does not require an organic matrix, the target surface can exhibit the properties of a conductor or a semiconductor, while small droplets desorbed from the surface under the action of laser radiation participate in processes that are similar to those observed under electrospray conditions. Ions generated are sucked to the atmospheric inlet of the mass spectrometer (Fig. 17). The method enables operation in both positive- and negative-ion modes. No ions are produced if no high voltage is applied or no laser irradiation occurs. Variable-voltage experiments showed that as the voltage increases from 0.5 to 5.5 kV the ion current intensity increases owing an increase in the charge density on the surface of liquid samples. Further increase in voltage is inappropriate because of possible electric breakdown.

II.4. Inlet ionization methods

When using atmospheric-pressure ion sources, most ions are lost during transfer from the atmospheric-pressure region to the vacuum region. The loss can be as high as > 99% of the total number of ions generated in the ion source (rim loss). However, this loss can be avoided by filling the interface between these regions with an analyte stream and subsequently ionizing the analyte molecules. These considerations gave rise to a new avenue of research termed 'inlet ionization'. Ionization occurs in the heated interface capillary and requires neither high voltage nor laser radiation (or ultrasonic gas jet). The intensities of analyte-ion peaks in the mass spectrum depend first of all on the capillary temperature.

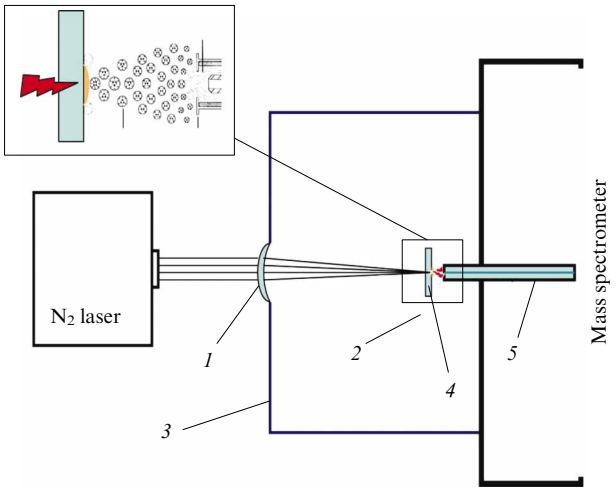


Figure 18. A scheme of the method of laser desorption with inlet ionization.[115]
Focusing lens (*1*), movable glass slide (*2*), atmospheric-pressure ion source (*3*), sample/matrix (*4*) and heated inlet capillary (*5*).

In 2010, a number of laserspray ionization studies have been reported.[115–118] Lasers were solely used to produce ions by laser ablation of matrices and analytes at atmospheric pressure. Samples were placed at a distance of 1–2 mm from the atmospheric inlet of the mass spectrometer (Fig. 18). The key condition was high temperature (300–400 °C) of the atmospheric inlet capillary where ionization of desorbed matrix–analyte complexes occurs. It is assumed that in this case the ionization mechanism is similar to the mechanism proposed for ESI, the matrix molecules playing the role of a solvent. A cold capillary is unsuitable to obtain signals from analyte ions.

By varying the procedure for sample application one can produce different types of ions. The classical MALDI 'dried droplet' method leads to production of mono-protonated molecules of low-molecular-mass compounds and poly-protonated molecules of high-molecular-mass compounds, *e.g.*, peptides. In particular, multiply charged cytochrome C ions with charges from +8 to +15 were detected.[115] These are typical ESI spectra which differ appreciably from conventional MALDI spectra. Nevertheless, peaks of just singly charged ions dominate in the mass spectra of samples obtained using solvent-free mixtures of analyte and matrix powders. Thus, one can obtain the desired type of mass spectra depending on the aim of the experiment and corresponding equipment. As applied to peptides, the method has a sensitivity of the order of a few attomoles. More recently, it was shown that multiply charged ions of macromolecules can be produced in a solvent-free manner and that a combination of ion mobility spectrometry followed by mass-spectrometric analysis makes it possible to analyze complex mixtures.[119]

There is no need to excite samples by high-power laser radiation.[118] Very similar results were obtained using a shock wave from a gas gun or even by touching the sample with a hot glass capillary. The key parameter was the temperature of the interface capillary. No high voltage, laser radiation or ultrasonic gas jets were needed for ionization. The method was named matrix assisted inlet ionization (MAII). Of course, laserspray ionization[115] is only a particular case for MAII.

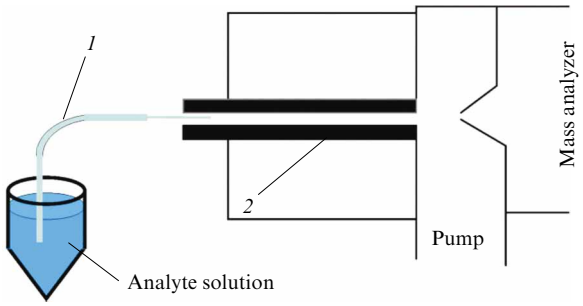


Figure 19. A schematic of the solvent-assisted inlet ionization method.[120]
A fused silica capillary with polyimide coating removed (*1*) and a heated ion inlet tube (*2*).

The inlet ionization methods can also be applied to liquid samples.[120] A method presented in Fig. 19 was called solvent assisted inlet ionization (SAII). As in the case of MAII, ionization occurs in the capillary interface. Consequently, there is no ion loss and the sensitivity of the method is higher than that of the classical versions of ESI. In particular, high-quality signals from peptide molecules can be obtained at attomolar and even zeptomolar concentrations of the samples; for low-molecular-mass compounds (*e.g.*, ciprofloxacin, acetaminophen), a detection limit below one ppt was demonstrated. The sensitivity is comparable with that of nanoelectrospray, but SAII is free from issues related to ultrasmall amounts of samples and solvent streams.

In fact, the inlet ionization methods make the ion source of the mass spectrometer unnecessary.

III. Quantitative issues of ambient ionization mass spectrometry

Numerous versions of ambient ionization mass spectrometry proved themselves in the detection of various compounds in different samples. However, the development of an actual tool in analytical chemistry requires elaboration of quantification procedures. The quantification accuracy achieved by methods of ambient ionization mass spectrometry is limited to matrix effects. Undoubtedly, the accuracy and reliability of results obtained is improved when using internal references; however, the addition of internal references to solid samples faces severe difficulties. Besides, in spite of impressive detection limits, the stability of ion signals is low; therefore, quantification requires a spectra averaging procedure characterized by a relative standard deviation of the order of a few per cent (usually, within 20%).[4]

The possibility to use methods of ambient ionization mass spectrometry (*e.g.*, 'paper spray') for quantification has been demonstrated.[63,66,121] However, it is desired that these methods be free from complex sample preparation procedures and take a rather short time. If the introduction of an internal reference takes a long time and requires skilled staff, the advantages of ambient ionization mass spectrometry vanish. Therefore, elaboration of simple and fast procedures is topical. Cooks and co-workers[122] proposed to use glass capillaries with inner surface coated with internal references. A liquid sample moves upward to the

upper end of the capillary by virtue of capillary forces and dissolves the specified amount of the internal reference. Subsequent application of the capillary content to the corresponding substrate surface leads to formation of a droplet that is ready to analysis. This sample preparation procedure demonstrated excellent results in the 'paper spray', LTP and DESI mass spectrometry studies, namely, reliable quantification of pharmaceuticals in blood, narcotics in urine and pesticides in natural water was made.

Nevertheless, it should be noted that the vast majority of studies on ambient ionization mass spectrometry are qualitative in character or include very rough estimates.

IV. Chemical reactions accompanying ambient ionization mass spectrometry studies

IV.1. Chemical reactions in air

Research into organic reactions belongs to the most chemically interesting avenues of progress in ambient ionization mass spectrometry. Classical mass spectrometry allows one to study gas-phase unimolecular reactions including thermolysis, photolysis or acid- and base-catalyzed processes and even predict the structures and yields of products of various reactions in solutions.[123, 124] Reactions of biomolecules within the electrospray source using a two-channel nanospray emitter have been reported.[125] Nevertheless, mechanisms of reactions in the ion source of a mass spectrometer and in solution are significantly different because in the former case there are no solvent and counter-ion effects and the energy of species is not averaged by collisions.

Numerous methods of ambient ionization mass spectrometry make it possible to study both unimolecular processes and bi- and trimolecular reactions or even to synthesize various compounds out of flask. Corresponding reaction products can be either directly transferred to the atmospheric inlet of the mass spectrometer or accumulated for further analysis or other purposes. For instance, a Claisen-Schmidt condensation of indanone-1 and 4-chlorobenzaldehyde was performed with a 92% yield within a minute using the electrosonic spray ionization method.[126] In fact, one deals with a chemical microreactor capable of generating reaction product at a rate of 0.6 mg min^{-1}. Taking into account that the electrospray methods allow one not only to rapidly analyze, but also to collect reaction products, they can be used for both analytical and preparative purposes. The available equipment enables application of the spray methods of ambient ionization mass spectrometry in combinatorial chemistry. Accelerated Michael, Claisen and other reactions involving formation of a new C–C bond in microdroplets, in thin films, as well as a result of ambient soft landing of ions have been reported.[126, 127] Would the yields of these processes be extended to a gramme or, better, kilogramme scale, a simple and cheap version of *out of flask* synthetic chemistry could emerge. In fact, this is a kind of reactions in a confined volume, among which reactions in micellar systems have been best studied.[128]

Such reactions in ambient conditions can be conducted in different manner, *e.g.*, by:

— intersection of two spray jets bearing charged droplets that contain all necessary reactants for the chemical reaction to occur (EESI);

— direction of the spray jet bearing charged species of a reactant towards the surface coated with the other solid or liquid reactant (reactive DESI);

— simply spraying the reaction mixture containing both reactants and any necessary additives (acids, alkali); and

— simultaneous or successive application of reactants followed by the determination by the 'paper spray' method.

An essential feature of all these methods consists in that the secondary spray microdroplets of radius ~ 1 μm reflected from the sample surface towards the atmospheric inlet of the mass spectrometer represent microreactors. As the volume of the microreactor decreases, the reactant concentrations significantly increase and the pH value of the medium changes; this causes additional acceleration of acid- and base-catalyzed processes.[129] As a result, even slow reactions carried out in a flask proceed at very high rates, which leads to quantitative formation of products within a few minutes and even seconds.[130] A similar effect of abrupt acceleration of chemical reactions is also observed in the thin films formed as the reaction mixture droplets spread across the surface.[127] If a solution is dried in ambient conditions, the corresponding thin film will also represent a microreactor with a confined volume. Acceleration of the reaction is due to a sharp decrease in the number of solvent molecules. For instance, an S_N2 ion/molecule reaction requires almost no activation energy to begin; this is similar to a gas-phase ion/molecule reaction where any collision between reactants results in a product.[131]

Large surface areas characteristic of microdroplets or thin films facilitate the occurrence of physical processes including evaporation and preferred distribution of solute species on the surface rather than in the bulk.[132] Therefore, electrospray is indeed suitable for easy and efficient generation of weakly solvated, highly reactive ions in ambient conditions. This observation gave rise to a new investigation line [133] known as ambient soft landing of ions (see below).

Analytically, an important advantage of the spray methods is the possibility to abruptly enhance signals from target compounds upon their derivatization with the formation of readily ionizable products. For instance, spraying of a 5% hydroxylamine solution on the surface of a multicomponent sample (reactive nanospray DESI) led to a sharp increase in the intensities of signals from steroids upon formation of corresponding oximes.[44] Contrary to this, ionization of other components of mixtures containing no keto groups was suppressed (Fig. 20). The 'paper spray' method was successfully used for the determination of natural aldehydes with a linearity range of three orders of magnitude. In the course of analysis, aldehydes react with 4-aminophenol to give charged iminium ions.[134]

A rapid test of the quality of 'Tamiflu' pharmaceutical was carried out using crown ethers as reactive additives to electrosprayed solvent (acetonitrile).[135] Molecular recognition with the formation of a host–guest complex under DESI conditions enabled reliable detection of the substance (oseltamivir) without significant fragmentation; the detection limit was ten times lower than in the case of neat acetonitrile as solvent. Since the mass spectrum exhibited only signals from the protonated complex ions, tandem mass spectrometry (MS^3) allowed one to develop approaches to quantification of oseltamivir by the reactive DESI method.

Since for DESI the time scale of reaction durations spans the range of micro- and milliseconds and the sensi-

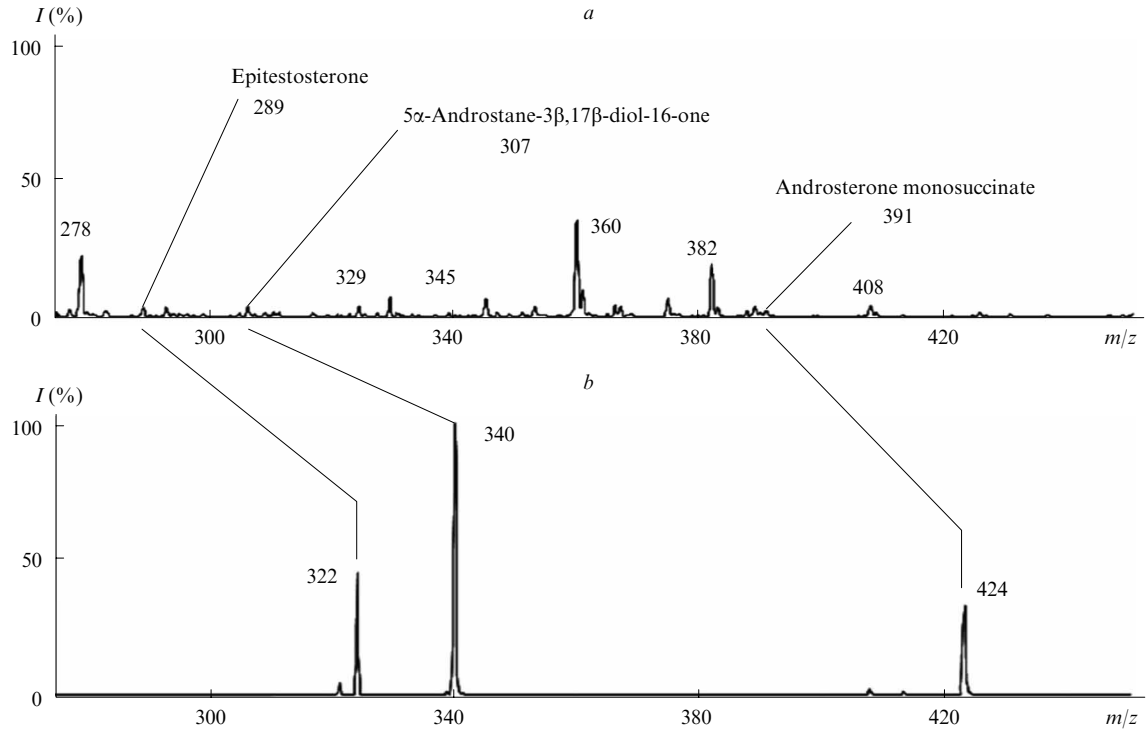


Figure 20. DESI (*a*) and reactive DESI (*b*) mass spectra of untreated urine obtained with hydroxylamine as reactant.[44]

tivity of the method is high, one can detect short-lived intermediates that cannot be detected by other methods (NMR and IR spectroscopies, ESI, *etc.*). Undoubtedly, this is of great value for research into reaction mechanisms. In particular, a number of intermediates of the hydrogenation of ketones using organometallic Ru-catalysts involving catalyst, substrate and solvent molecules were determined.[136] Some other examples are provided by the detection of (i) Meisenheimer complexes in the nucleophilic aromatic substitution reactions by the reactive DESI method[137] and (ii) intermediates of the Katritzky reaction (Scheme 1) between a triphenylpyrylium salt and amines.[138]

Reactive intermediates can also be studied by thermal activation of charged droplets in ESI. This kind of activation favours the occurrence of ion reactions out of flask at atmospheric pressure, in particular, the Fischer indole synthesis and the pinacol rearrangement.[139]

IV.2. Ambient soft landing of ions

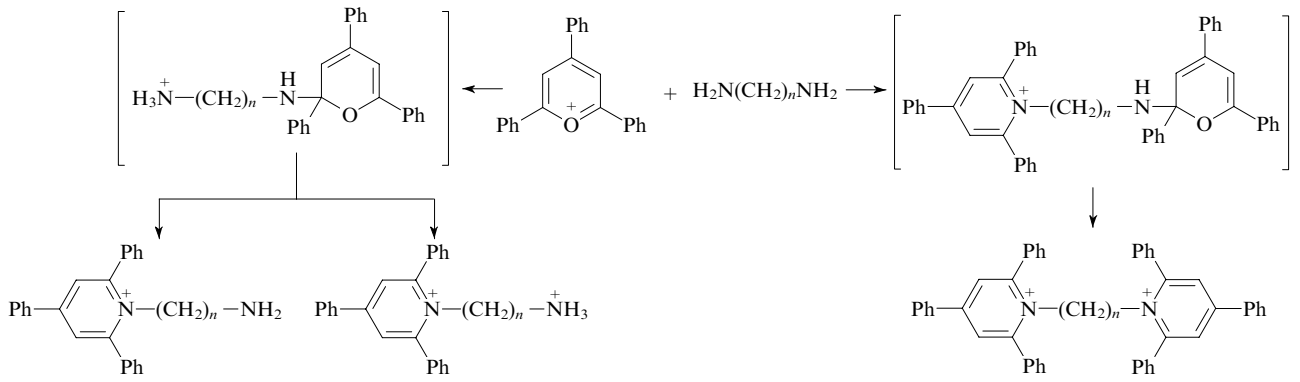
The term 'ambient soft landing' of ions denotes the *in vacuo* collisions between hyperthermal polyatomic ions with kinetic energies $\leqslant 100$ eV and the surface; this results in bonding of intact ions or corresponding molecules to the surface.[140] Ambient soft landing of ions always competes with other processes, *e.g.*, elastic collisions, dissociation induced by collisions with the surface[141] as well as numerous ion reactions on the surface. There are three main mechanisms of ambient soft landing of ions:

— trapping of an intact ion,[142]

— neutralization of an ion resulting in a corresponding molecule which retains the structure of the parent ion,[143] and

— reaction of the trapped ion with molecules on the surface (reactive deposition).[144]

Scheme 1



Competitiveness of these processes depends on the nature of ions and surface. The possibility of mass spectrometric detection of a desired ion with specified mass, kinetic energy and charge makes ambient soft landing of ions a promising method for production of materials with modified surface with specified properties. The method was tested taking separation of pure compounds from mixtures, deposition of clusters of preset mass on different substrates and immobilization of biomolecules on metallized surfaces in the design of novel catalysts as examples.[133]

In ambient soft landing of ions, charged electrosprayed droplets are directed towards the specially prepared surface using ion deflectors. The droplets should travel a distance of 10–12 cm at atmospheric pressure;[133] this is necessary to dry them through rapid evaporation of the solvent. Microsolvated ions thus formed easily enter into reactions in air. Reactive soft landing of ions implies the occurrence of heterogeneous reactions between ions and the solid surface in contrast to homogeneous reactions within droplets under the DESI conditions. Nevertheless, both types of reactions occur rapidly and efficiently.[127, 129] Application of the nanoelectrospray ionization method to the delivery of reactant and instantaneous quantitative derivatization of peptides on the surface was demonstrated.[145] Also, it was shown that reactions of 'dried' gas-phase ions with reactants on the surface may proceed in somewhat different manner as compared to the corresponding reactions in solution and that not only the rate, but also the selectivity of the reaction increases. For instance, the reaction of triphenylpyrylium ions with ethanolamine under conditions of reactive ambient soft landing of ions results in triphenylpyridinium derivatives only (*cf.* possible formation of undesired side products if the reaction in solution proceeds at the ethanolamine oxygen atom).[146] Besides, the reaction mechanism can vary.

Summing up, heterogeneous reactions between ions and solid reactants on the surface are of interest not only as an individual avenue of chemical research, but also as a potential synthetic route to high-purity compounds. Using reactive ambient soft landing of ions, one can prepare modified surfaces of different compositions with confined size. Numerous examples of out-of-flask chemical reactions in air have been reported.[130, 147]

V. Miniaturized mass spectrometers

Miniaturization is one of the most interesting avenues of progress in modern mass spectrometry. For the first time, miniaturization has attracted the attention of researchers 25 years ago.[148] The development of ambient ionization methods made it possible to carry out on-site analyses using portable mass spectrometers.[149] Every year, a number of models of miniature mass spectrometers are announced. In particular, a 4-kg hand-held multisource mass spectrometer was designed in 2008.[150] To become in-demand and efficient, mass spectrometers should be sensitive, maintenance-friendly, fast and cheap. Since a recent review reported by researchers from the laboratory headed by Professor R G Cooks is available,[151] in this Section we will consider only the most interesting last-minute advantages in ambient ionization mass spectrometry achieved using portable instruments.

The inlet systems of portable mass spectrometers may be of the following types:

— membrane inlet,
— capillary inlet or a solid sorbent, and
— discontinuous atmospheric pressure interface (DAPI).

Membrane inlet mass spectrometry is one of the most convenient methods for online direct analysis of air and water samples using portable mass spectrometers. The method is reliable, simple, rather sensitive and provides continuous monitoring of various environmentally important compounds.[152] The solid sorbent is used in the classical analysis of vapours and gases in air by the thermal desorption method. The system can be easily automated and used for monitoring target analytes in the field. For instance, portable instruments based on a solid sorbent for monitoring toxic industrial compounds in air[153] and chemical warfare agent simulants in air[154] are available. The inlet through a capillary coated with polydimethylsiloxane[155] occurs by contact between the capillary and the sample followed by desorption of analytes in the mass spectrometer with electron ionization.

The development of ambient ionization mass spectrometry methods led to significant shortening of the time of analysis and to extension of the range of analytes. Classical mass spectrometers require powerful pumps to maintain high vacuum. Portable instruments use tailor-made units. A highly efficient discontinuous atmospheric pressure interface inlet[156] makes it possible to use not only ambient ionization methods, but also atmospheric-pressure ionization methods (ESI, APCI). The ion transfer channel is open for maximum ion introduction only for a short time ($\sim$15 ms). Air, analyte vapours and gas-phase ions arrive at the vacuum chamber where ions are trapped by the low-voltage radiofrequency field, while neutral species are evacuated until a necessary pressure of about 1 mTorr (usually, this takes 200–800 ms). Then, a high-voltage field is applied to acquire the mass spectrum (or MS/MS spectrum). This approach is efficient when analyzing samples in all states of aggregation and requires no sample preparation.

Depending on the ionization conditions (vacuum or ambient conditions), methods of ionization are classified into internal and external ones. Miniaturized mass spectrometers require external methods for most analytes (only volatile components can be introduced using a membrane or sorbent). The internal methods include electron ionization, ESI and APCI. By default, methods of ambient ionization mass spectrometry belong to external ones. For portable mass spectrometers, DESI, LTP, 'paper spray' and DAPCI are the most efficient methods.[157]

Portable mass spectrometers were designed on the basis of different-type analyzers; the best results in miniaturization are associated with ion traps that are characterized by not too strict vacuum requirements (at a level of a few mTorr) and can operate in the tandem MS mode. To date, instruments based on different types of traps are available.[151] In most cases, they operate in the positive-ion modes, although the negative-ion mode is more efficient for particular classes of compounds (*e.g.*, nitro compounds).[158]

A Mini 12 miniaturized mass spectrometer with ambient ionization was developed in 2013.[159] The maximum mass detectable is 900 Da and tandem MS experiments up to MS^5 are possible. The instrument weighs 25 kg, consumes a power of 100 W and can operate with 110 V a.c. or from a

battery. It is suitable for analysis of compounds of different polarity. The sample cartridges and software enable automated operation without sample preparation.[159] The instrument can be used for environmental analysis, analysis of pharmaceuticals and food quality control. Operation in the tandem MS/MS mode allows one to quantify target analytes using the internal reference method with a detection limit of 7.5 ng m^{-1} for a number of pharmaceuticals in blood.

A hand-held wireless LTP probe for 'point-and-shot' mass spectrometry using laboratory-scale and portable mass spectrometers was proposed.[23] A miniature spray can serve as a source of discharge gas (helium). The design is convenient to analyze samples in all states of aggregation without sample preparation. A small Li cell (7.4 V) is suitable for 2 h of continuous operation. A comparison with conventional LTP sources demonstrated the advantage of the new probe. Cooks and co-workers[160] developed a novel probe whose combination with a Mini 11.5 miniaturized mass spectrometer made it possible to detect signals from nanogrammes of explosives on a glass surface. The key advantage of the novel design consists in replacement of the compressed gas vessel by a small diaphragm pump, which delivers atmospheric air to the plasma probe. A portable mass spectrometer[161] can be used in the search for extraterrestrial features because it can analyze samples in the form of liquid and solid mixtures, perform targeted analysis and establish the structure of the novel compounds in the tandem mass spectrometry mode (up to MS^5).

Yet another portable mass spectrometer with modular design was proposed.[162] The inlet, ionization, ion separation and detection are carried out in one module while the power source, pumps and control electronics are in the other module. The modules are connected with a flexible stainless steel tube (length 1.2 m and inner diameter 25 mm, see Fig. 21 *a*). The inlet capillary was extended to 10 cm and its inner diameter was increased to 500 μm. The vacuum volume of the instrument thus increased, which is treated as a drawback for classical mass spectrometers. However, this appeared to be an advantage in the case of the portable instrument with DAPI because in this case the amount of ions is proportional to the vacuum volume. The system with large vacuum volume allows a larger volume of gas to pass through the DAPI until the necessary pressure will be achieved; therefore, a larger number of ions can be trapped. The system is three times more sensitive than the previously designed instrument without the ion transfer tube. This can be explained not only by the enlarged vacuum volume, but also by higher efficiency of ion transfer through the capillary of larger diameter. Disadvantages include the increase in time necessary for the pressure to decrease to a desired value (2 mTorr) to record mass spectra (1.25 s *vs.* 0.4 s) and the increase in the time for each scan from 0.7 to 1.5 s.[162]

This design became a prototype of a portable, dust cleaner-type mass spectrometer (Fig. 21 *b*).[163] A 2-kg sampling wand contacting the surface under study contains the ion trap with DAPI and an LTP source. This module is connected by a flexible tube to a 10-kg backpack containing a pump, a power supply and a control unit. The instrument is first of all designed to detect trace amounts of nonvolatile compounds on various surfaces. The ionization method, LTP, is efficient in both positive-ion and negative-ion modes. Plasma is formed upon application of a low voltage, the discharge gas flow rates are not too high, production of the source is not problematic and there is no need for solvents or other reactants. The instrument proved itself in real-time detection of nanogramme amounts of explosives and chemical agents, narcotics and other compounds immediately on the sample surfaces without any sample preparation. The surfaces under study could be of complex geometry, thermally sensitive and could contain complex matrices. The mass spectrometer consumes a power of 65 W when operates autonomously from a battery for 1.5 h. The maximum detectable mass is 925 Da at a mass resolution of 1 a.m.u. Emphasize that the device can be operated by an unskilled person.

Online MS monitoring of the course of chemical reactions in laboratories and at prodution sites provides information on the reaction mechanisms. This can be done by the EESI method (see above).[61, 62] Portable instruments can be arranged in a chemical laboratory or at the factory near the reaction setup. In particular, a 4000MiD mass spectrometer[165] with ESI was used for continuous monitoring of amide transformation to amines (Hofmann rearrangement).[164] Monitoring of the starting compound, end product, intermediate (isocyanate) and some impurities allowed the reaction conditions to be optimized. An offline analysis revealed a large number of impurities, but the portable mass spectrometer is suitable for rapid response to changes in reaction conditions.

Even more complex reaction chain including the synthesis of dehydrobenzene by diazotization and subsequent reaction of dehydrobenzene with furan (Scheme 2) was

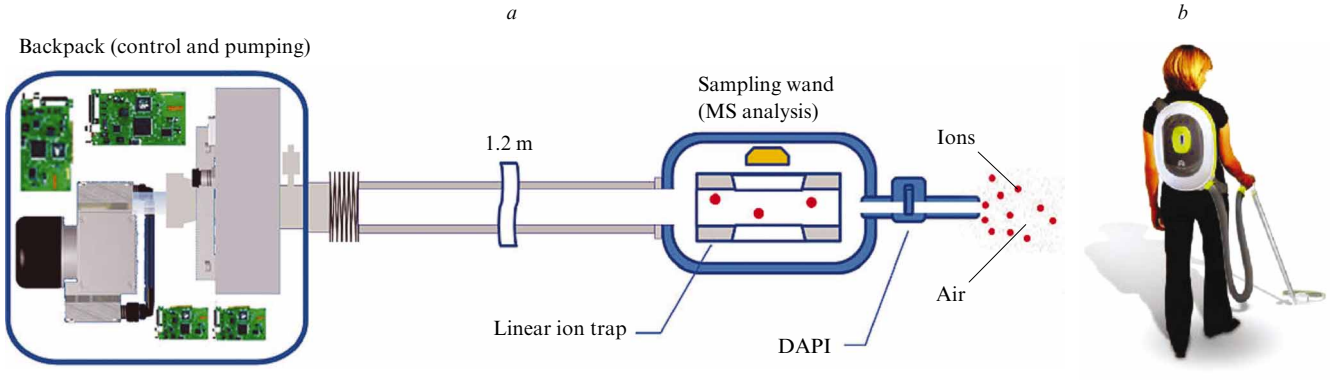
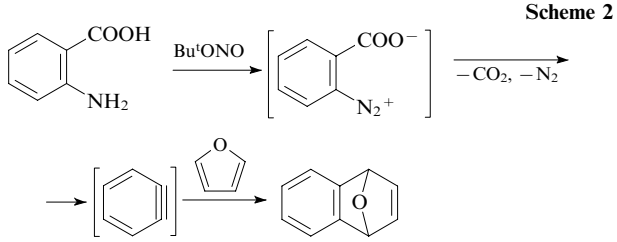


Figure 21. A miniaturized mass spectrometer wih a flexible bellow tube (*a*)[162] and a dust cleaner-type design (*b*).[163]

Scheme 2



monitored online using a similar instrument, a 3500MiD miniature mass spectrometer.[166] The optimum temperature and duration of the Diels–Alder reaction resulting in a desired product as well as pathways of side reactions resulting in acridone and other compounds were established.

VI. Ambient ionization mass spectrometry for molecular imaging

Molecular imaging became a very popular field of mass spectrometry owing to a huge volume of detailed chemical information obtained by this method. Classical mass spectrometry allows one to identify and quantify various compounds in a particular sample. Imaging mass spectrometry makes it possible to determine the distribution of such compounds or their groups over the surface or in the bulk of the sample (Fig. 22). This is of importance for analysis of biological samples; one can study the distribution of biopolymer molecules, components of pharmaceuticals and their metabolites and even particular chemical elements.[168]

The method involves registration of mass spectra at particular points of the sample surface with reference to the coordinates of the points. Data processing affords the distribution of various compounds over the surface under study (Fig. 23).[169]

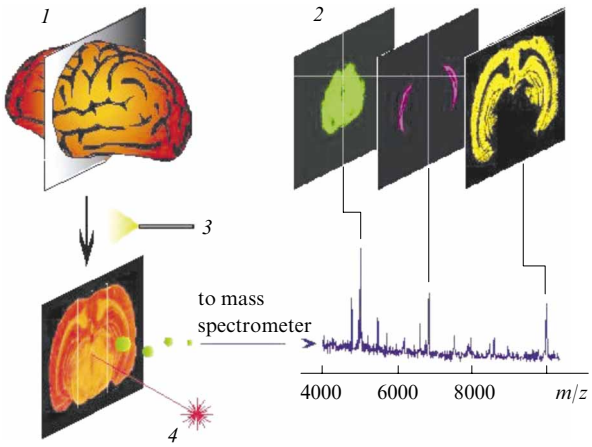


Figure 23. MALDI-MS imaging: a schematic.[169]
Frozen section (*1*), MS images (*2*), matrix application (*3*) and laser (*4*).

The spatial resolution is a crucially important parameter. At present, the most widely used microprobe approach is characterized by a pixel size of less than 10 μm. A record high resolution achieved [170] using C_{60} fullerene ion beams is 100 nm. An alternative is provided by microscope mode imaging mass spectrometry. This method is much faster, but practical implementation requires high-quality optics and vacuum.[171] Specific design enables an astigmatic projection of ions from the surface on the position-sensitive 2D detector and simultaneous acquisition of a larger field of view.

Application software makes it possible to construct 2D distribution maps using the mass spectra corresponding to each pixel. Same-colour intensities denote point-to-point

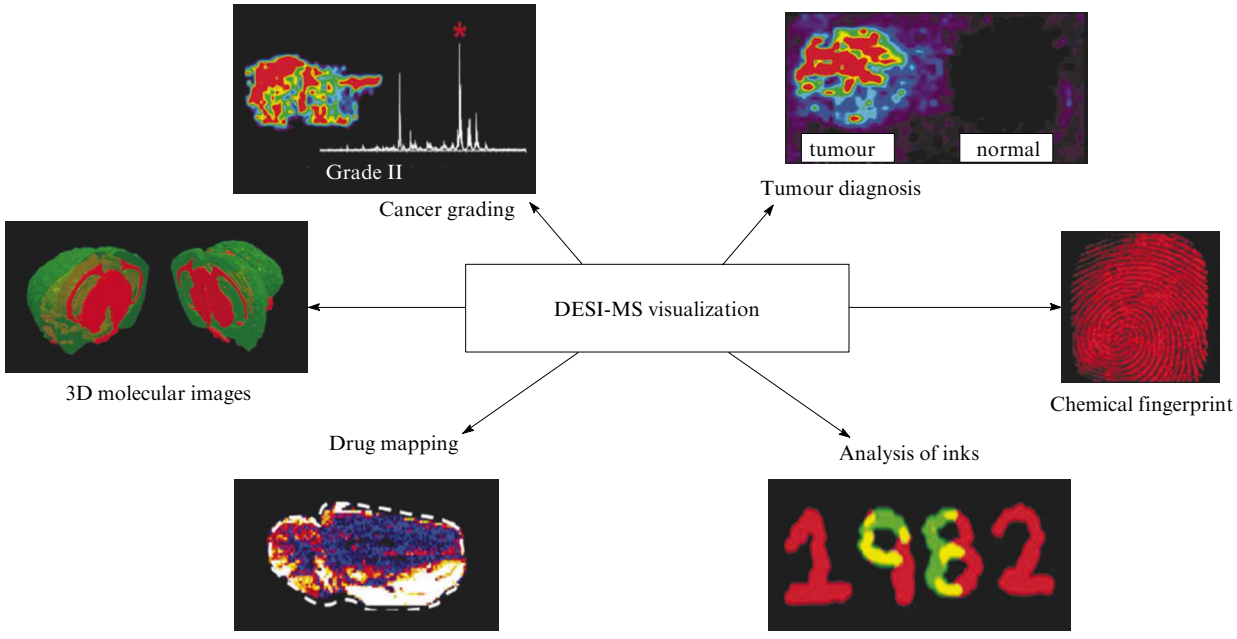


Figure 22. Selected DESI-MS imaging applications.[167]

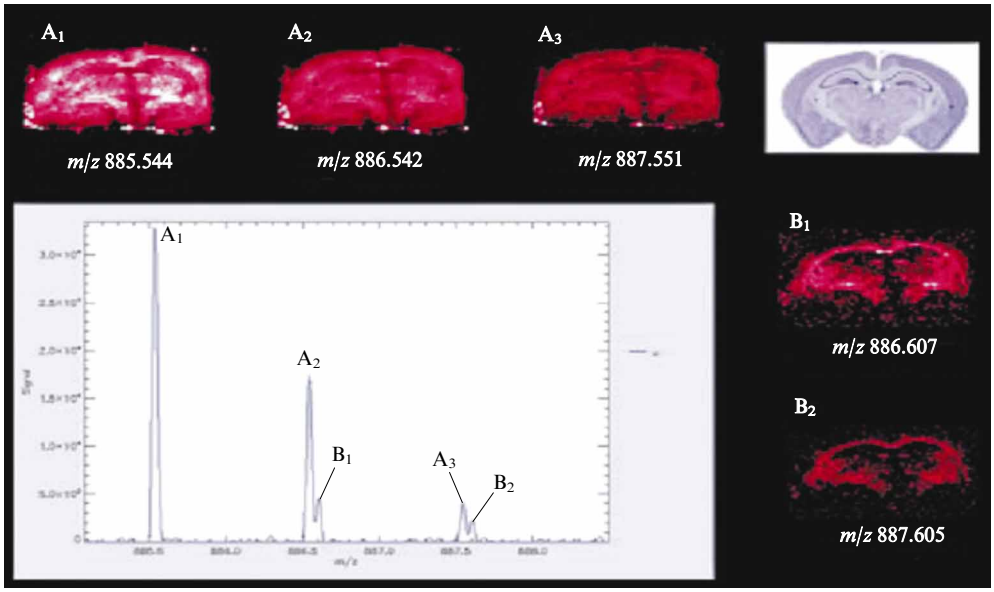


Figure 24. High-resolution DESI-MS imaging of mouse brain (mapping of phospholipids) and a DESI-MS image of mouse brain (upper right corner).[11]

differences between same-type ion intensities. An MS image of an ion can also be superimposed onto the image of another ion.[172] For high-resolution mass spectrometry, the image file size may appear to be very large (to a few Gbytes). Too large files are hard to process and therefore one should search for a balance between unveiling the potential of the instrument and convenience of interpretation of the available information.

Three-dimensional images can be constructed by combining information obtained by scanning individual sections. Alternatively, successive depth profiles of samples can be constructed. If the primary beam scanning the surface completely removes the upper layer, multiple scans can provide information on the depth profile of analytes. The possibility of such experiments depends on the ionization method and on many parameters including the pixel size, increment value, the nature of the solvent, *etc*. For instance, since DESI involves selective interaction of charged droplets with the surface (elution of particular groups of compounds), experiments on direct 3D imaging are impossible. On the contrary, the SIMS [173] and LAESI [111] methods allow one to carry out 3D imaging studies with a depth resolution of <100 nm and 30 μm, respectively. Also, 3D images enable a direct correlation between the distribution of particular molecules in the bulk of a sample and the tissue structure.[174] More detailed information on experimental techniques and applications of the method is available in the literature.[172, 175–179]

Mass spectrometric imaging began with experiments by Hillenkamp *et al*.,[180] who developed the first instrument for mass spectrometric analysis of microsamples using laser desorption and demonstrated its efficiency in the determination of the elemental and molecular compositions of biological samples with admissible spatial resolution. By the end of the 20th century, MALDI and SIMS became the main methods of molecular imaging. The emergence of ambient ionization mass spectrometry re-vitalized the field. The most efficient methods are DESI, nano-DESI and

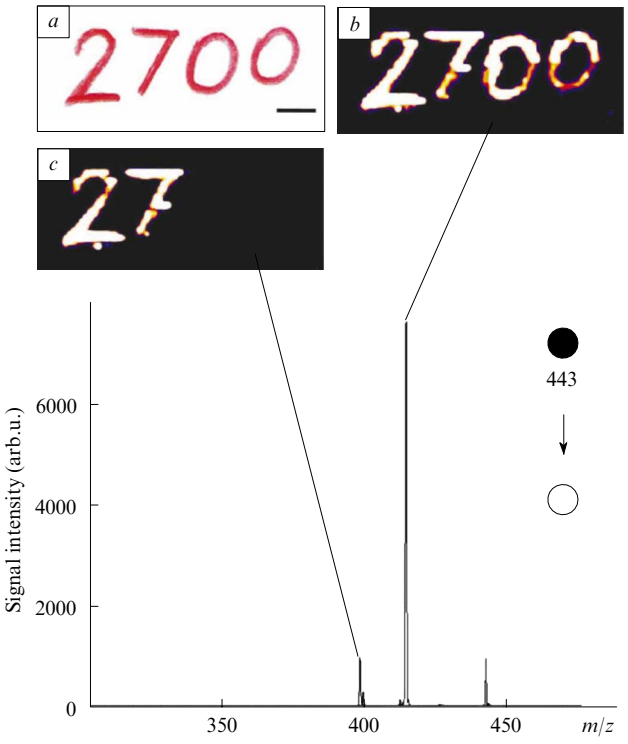


Figure 25. Application of tandem DESI-MS/MS to molecular imaging.[10]
For *a*–*c*, see text.

LAESI. Their advantages include the lack of sample preparation, the possibility of analyzing complex surfaces including rough and large-area ones, online derivatization of analytes by adding reactants to the DESI spray solvent and even mass spectrometric guidance of surgical operations (see below). These methods are particularly conven-

ient for imaging of the distribution of small molecules, because their signals do not overlap with signals of matrix ions (as in MALDI) and the degree of fragmentation is very low (in contrast to SIMS). Two processes, desorption and ionization, which underlie these methods, can occur either simultaneously (DESI) or successively (LAESI).

When necessary, modern equipment enables efficient imaging experiments in high-resolution mass spectrometry and tandem mass spectrometry modes. In particular, if one deals with complex biological molecules, the probability to detect isobaric ions having the same integer mass in the mass spectrum is very high. The results of molecular imaging of phospholipids in a mouse brain sample are shown in Fig. 24. The mass difference between the A_2 and B_1 ions is merely 0.065 Da and mass spectrometric imaging of A_2 and B_1 requires a resolving power of 15000.

Whereas high-resolution mass spectrometry has been successfully used to distinguish between isobaric molecules, tandem mass spectrometry can be useful for differentiation of isomers. For instance, two red pens were used to create the characters '2700'. The pen used to write '27' contained a mixture of two pigments, rhodamine B and rhodamine 6G, while the other pen (for '00') contained only rhodamine B. The DESI method with detection of protonated molecules makes it possible to read the all characters usng the ion current of ions with m/z 443 (Fig. 25 *a*). Only the areas containing rhodamine 6G (Fig. 25 *b*,*c*) are visible for DESI MS/MS with detection of the m/z 443 $\rightarrow$ m/z 415 transition (loss of ethylene molecule).

Most studies on molecular imaging of biological tissues by methods of ambient ionization mass spectrometry were carried out using lipids.[179] These compounds readily undergo ionization and give intense signals; however, they can mask the presence of other compounds in the sample. A MALDI detection of, *e.g.*, proteins requires that lipids be preliminarily removed.[181] This is not necessary for DESI studies, although signals from dyes, peptides and alkaloids are to some extent suppressed, especially if the masses of the compounds of interest fall in the mass range of lipids. The problem can be solved by detecting ions of other polarity[8] or by using reactive DESI.

Mass-spectrometric chemical fingerprints (see Fig. 22) allow one to unambiguously correlate a particular compound and a particular person. Stable chemical fingerprints on glass, paper and plastic were studied by the DESI method. It was shown that positive results can be obtained even if a number of fingerptints overlap. Moreover, the fingerprints make it possible to determine compounds from spots on the surface touched by a person and even to identify medicines taken and food consumed.[182]

Among numerous studies on molecular imaging using ambient ionization mass spectrometry, mention may be made of construction of 3D maps showing the distribution of particular molecules in internal organs[111,183] and determination of cholesterol distribution in biological tissues by the reactive DESI method.[184]

Conventional solvents (water + methanol or acetonitrile + water mixtures) for DESI studies can damage biological tissues.[29] In this connection, as well as to avoid splash of materials, samples should be exactly positioned below the spray jet. The problem can be solved with recently developed solvent systems based on *N*,*N*-dimethylformamide (with acetonitrile and ethanol). These mixtures allow one to obtain intense signals from lipids by the DESI method, to leave the morphology of the tissue analyzed and the protein distribution intact; therefore, samples can be re-used for MALDI imaging.[185]

Although most published molecular imaging mass spectrometry studies were carried out using DESI and LAESI, other methods of ambient ionization mass spectrometry are also tested for molecular imaging applications. The PESI method[74] uses metallic needles as samplers and electrospray emitters. A needle is dipped into the sample and some amount of material is adsorbed. Then, the needle is positioned near the atmospheric inlet of the mass spectrometer and a high voltage is applied; a solvent stream desorbs and ionizes components by the electrospray mechanism. The spatial resolution of the imaging experiments first of all depends on the diameter of the needle. Usually, needles 100 μm in diameter are used. The needle diameter can be decreased to, *e.g.*, 50 μm,[186] although subsequent analysis was based on thermal desorption rather than electrospray. An interesting feature of PESI is successive desorption of different classes of compounds depending on their surfactant properties. In particular, an analysis of samples of malignant tumours revealed[187] that at first intense signals from proteins were observed in the mass spectrum, followed by signals from lipids in later stages of the experiment. Drawbacks include the possibility of contamination of new samples with incompletely desorbed material from the preceding samples and ambiguity of quantification when compounds of different nature are sorbed on the metallic needle.

A robotic version of molecular imaging using DART and metallic needles was proposed.[188] The method allows one to analyze nonplanar surfaces while the limiting stage consists in the need to anneal the needle prior to re-sampling.

Very high spatial resolution can be attained using the nano-DESI approach.[42] This is related to the diameter of capillaries and to the lack of splash (*cf.* DESI where transfer of the sample substance to the surrounding areas is possible). An imaging study of samples of malignant tumours and rat brain demonstrated a spatial resolution of 12 μm achieved without any sample preparation.[43]

Mass-spectrometric imaging with ambient ionization can also be exemplified by other methods.[168,179] Their efficiency in solving problems in biology, medicine, as well as forensic and environmental analyses is obvious. The challenges precluding widespread application of mass-spectrometric imaging include suppression of signals from certain analytes by other analytes, insufficient reproducibility of experimental results because of the need to control a number of parameters and to maintain them at a specified level, as well as the problem of reliable quantification (although positive results can be obtained by homogeneously sputtering an internal reference on a surface).[189]

VII. Online control of surgical procedures by methods of ambient ionization mass spectrometry

Intraoperative use of mass spectrometers began in the 1980s taking online monitoring of exhaled air during anesthesia as an example.[190] Surgical operations are the main method of treatment of malignant neoplasms, in particular, brain tumours. The aim of an operation is to remove what should be removed and to leave what should be left. Undoubtedly, one can draw up a detailed plan of an operation using the

results of a preliminarily conducted MRI procedure. However, issues related to the nature of particular tissue areas may appear intraoperatively. At present, intraoperative ultrasonography[191] or magnetic-resonance imaging[192] of tissues are used in well-equipped medical centres; however, the former method is of limited use with respect to tissue differentiation, while the latter approach has a limited spatial resolution. Besides, both methods give no idea of actual molecular characteristics of tissues, being used only for indirect proof of the presence of tumour.[193]

Histopathological examination of frozen section biopsy is the most informative and reliable method of evaluating cancer grading and tumour diagnosis. In modern medical centres, this takes nearly 20 min and the surgical procedures should be suspended. Also, a limited number of samples are analyzed and the results thus obtained usually provide no information on the molecular composition of the sample. That is why methods of ambient ionization mass spectrometry (ambient conditions, no sample preparation, time scale is comparable with the duration of a surgical operation) are considered as the most promising for the determination of the tissue type.[24] They are suitable to solve a variety of issues. Continuous sampling of tissues at any point of interest provides valuable information about tumour type and grade as well as specific information including level of biomarkers for rapid decision-making. Besides, DESI and magnetic resonance imaging can provide complementary information.[193]

Main problems in using ambient ionization mass spectrometry during endoscopic procedures and surgical interventions are associated with safety of patients and medical staff. Organic solvents, high voltage, as well as liquid or gas streams that damage tissues are undesired. In particular, in REIMS[99] ionization is achieved using conventional electrosurgical instruments (Fig. 26 *a*). Ions are transferred to the mass spectrometer *via* a flexible tube. The actual mass spectrum on the operator's monitor (see inset) is processed using application software within a few seconds to reveal tumour markers. During the operation, the surgeon can identify the type of the tissue (tumour or intact) contacting the electrosurgical instrument at a given instant (Fig. 26 *b*).

In the case of DESI, desorption/ionization occurs on the surface of tissues. Conditions for ionization and efficient transfer of ions to the mass spectrometer using a 4-m-long flexible ion transmission tube with an outer diameter of 3 mm were optimized.[24] The tube, which can be arranged with ease within an endoscope tube, contains coaxially arranged capillaries of smaller diameters necessary to deliver electrosprayed solvent and auxiliary gas. A diaphragm pump prevents the loss of gas, solvent and droplets containing analytes (they are formed upon desorption/ionization) through contact of the sheath of the transmission tube with biological tissue. The time delay in obtaining signals is only 0.5 s.

Usually, DESI gives better results (smaller droplets are formed) with a methanol + water mixture as solvent.[27] However, experiments with the 4-m transmission tube revealed more efficient ion transfer using water. (This is of importance because the use of methanol to analyze tissues from living organisms is impossible.) A possible reason consists in just larger size of water droplets. Complete desolvation occurs near the inlet of the mass spectrometer and gas-phase ions produced do not lose on the walls of the interface. As the auxiliary gas flow rate increased to 5.2 litre min^{-1}, the ion signal increased irrespective of the application of spray voltage. Summing up, DESI mass spectrometry can be used in medicine because no high voltage is needed, only water and air are used as reactants and the sensitivity is high enough to obtain necessary results.

* * *

Studies on ambient ionization mass spectrometry, which emerged as a new scientific discipline only about ten years ago,[1] are rapidly progressing. Short response time, informativity and simplicity of the methods attract the attention of not only analysts, but also medical professionals, forensic analysts, environmentalists and synthetic chemists. Novel methods emerge every year and the field of application of ambient ionization mass spectrometry extends correspondingly. Organic synthesis out of flask, mass-spectrometric analysis in the field, online guidance of surgical operations and mass-spectrometric imaging of biological and environmental samples become individual avenues of chemical research.

In the near future, a breakthrough in miniaturization of mass spectrometers may occur so they may become household devices. All necessary prerequisites have been created to date and key problems to be solved are solely related to reduction of the high cost of instruments.

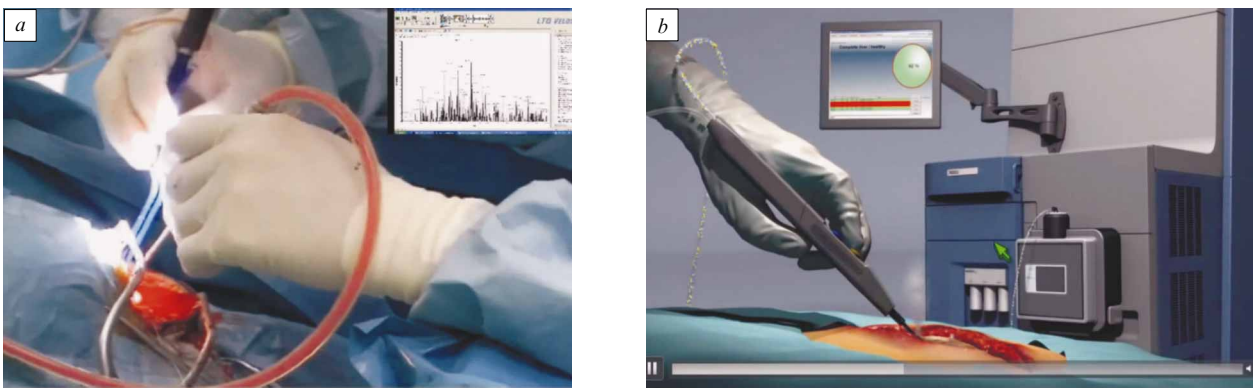


Figure 26. Rapid evaporative ionization mass spectrometry guidance of a surgical intervention on removal of tumour.[194] For *a* and *b*, see text.

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