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The Possibilities of the Mass-spectrometric Analysis of Involatile and Thermally Unstable Bio-organic Compounds

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The possibilities of the mass-spectrometry of thermally unstable highly polar and organic and bio-organic compounds, based on the direct (without preliminary evaporation) formation of ions of the test compound, are examined.
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I. INTRODUCTION

The development of modern organoelemental and bio-organic chemistry has led to the need to determine the compositions and structures of highly polar, involatile and thermally unstable compounds, which can be studied by traditional mass-spectrometric methods.

The development of "mild" methods for the ionisation of organic compounds, namely chemical ionisation (Field and Manson,[1] 1968), field ionisation, and field desorption (Beckey,[2] 1969) began in the 1960's and techniques on a wide scale in the 1970's. The mass spectra obtained by these procedures contain intense peaks due to molecular and quasi-molecular ions, but the transfer of the test molecules to the gas phase by heat treatment was not required only in the field desorption method. The field desorption method is therefore used successfully for the analysis of involatile compounds. The advances achieved with the aid of this method have been surveyed in several reviews.[2-5]

During the last decade communications have been published on new methods for the determination of the mass spectra of involatile and thermally unstable compounds (TUC), in which the vaporisation of the test substances before ionisation has been eliminated. These ionisation procedures can be divided into two groups: the methods for the generation of ions from the solid state without heating the specimen and the methods from the selective removal of ions from a solution containing the test compound at room temperature.

The first group comprises methods based on secondary ion emission (SIE) induced by bombarding the test specimen with a beam of primary ions (secondary ion–ion emission) or a beam of neutral atoms (atom–ion emission), the method based on plasma desorption by ^{252}Cf fission fragments, and ionisation by laser radiation (LD).

The second group of methods, which began to develop somewhat later, includes the electrohydrodynamic ionisation method,[6-8] the method based on the field of evaporation of ions from solutions,[9,10] including solutions in involatile solvents deposited on the surface of field desorption emitters,[11] the "electro-scattering" method,[12-16] the "mist formation" method,[17-19] and the method based on the extraction of ions from solutions at atmospheric pressure.[20] Heat treatment of the test substances has been eliminated from all these methods and the latter make it possible to determine the mass spectra of highly polar and involatile thermally unstable compounds without their preliminary chemical modification; the high content of quasi-molecular and in most cases of intense fragment ions ensures that the molecular weights and structures of the test molecules can be determined.

The volatility and in many cases also the stability of the compound diminish with increase of its molecular weight and the scope of a method can be characterised by the upper limit to the mass of the ions obtained with its aid. Towards the middle of 1982, major advances had been achieved by investigators using the methods based on the atom–ion emission in liquid matrices (see below) and plasma desorption by ^{252}Cf fission fragments. Quasi-molecular ions of the unprotected peptide glucagon (a pancreatic hormone), the molecular weight of one of the isotopes of which is 3481.6,[21] and a biological product (bovine insulin) with a molecular weight (MW) of 5729.6 [22] were obtained by the first procedure. A record success was achieved with the aid of the method based on the plasma desorption by ^{252}Cf fission fragments, which yielded the mass-spectrum of an oligonucleotide with a molecular weight of 6278.[23] The absence of the need for enzymic and chemical treatment made it possible to reduce the size of the sample required for the analysis by the new methods and at the same time gave rise to the possibility of identifying compounds present in unpurified biological preparations. Thus the determination of the molecular weight of a peptide by the atom–ion emission method now requires 0.1 nmol of the test substance, while the determination of the aminoacid sequence in it requires 2–50 nmol.[24]

The aim of the present review is to assess the possibilities of those of the above methods which have now found extensive practical applications in the mass-spectrometric analysis of thermally unstable compounds. These include secondary emission ionisation, plasma desorption by ^{252}Cf fission fragments, and laser desorption. The review does not deal with the studies in which the mass spectra of TUC are obtained by traditional ionisation methods—electron impact or chemical ionisation but with updated types of heating or procedures for the introduction of the specimen into the ion formation region, which reduce the degree of thermal decomposition of the product.[25-28] For the analysis of biomolecules, this procedure is not likely to be useful, since an increase in

the molecular weight and in the polarity of the test molecules leads to the requirement for stronger heating and the fraction of quasi-molecular ions in the mass spectrum sharply diminishes or the ions disappear altogether.[26]

II. SECONDARY EMISSION IONISATION

1. Secondary Ion Ionic Emission

When a primary ion beam impinges against a surface, positive and negative ions derived from the adsorbed layers and the material of the support are always present among the secondary particles leaving the surface. The yield of these ions depends on many factors.[29–33] The most important characteristics, which determine quantitatively the ion formation process, are two parameters: the secondary ion emission coefficient $S^{\pm}$ (the parameter $S^{\pm}$ has been called[34,35] the absolute yield of secondary ions), i.e. the number of ions $A^{\pm}$ leaving the surface following the impact by one primary particle, and the ionisation efficiency, i.e. the ratio of the number of dislodged ions to the number of neutral particles which have left the surface.

Impact by the primary ions alters the state of the surface layer. Calculation has shown that at a current of 1×10^{-8} A cm^{-2} the number of ions incident on the surface is 6×10^{-6} ions s^{-1} $Å^{-2}$. Assuming that the energy of a single ion is transmitted to a zone with a radius of 10 Å, we find that such a surface element is exposed to impact after every 8 min. This time is longer by several orders of magnitude than that required to dissipate the energy of the nucleus. For higher current densities of the primary beam, the layers adsorbed on the support are rapidly worn away, which is manifested by a change in the intensity and composition of the ions in the secondary beam.

Benninghoven[34] introduced the concept of the "dynamic" and "static" ion formation regimes under the conditions of secondary ion–ion emission (SIIE). Bombardment of the surface by the primary beam at a current density not exceeding 1×10^{-8} A cm^{-2} has been called the static model; in this case the disruption of the surface layer is insignificant. A method employing the static regime for the preparation of the ions of an organic substance deposited in the support has been called "organic"[36] or molecular[37] SIIE. It has been shown experimentally that irradiation of a thin layer of an organic product deposited on a metallic support by a beam of Ar^+ ions at a current density of 4×10^{-9} A cm^{-2} can give rise to a steady emission of quasi-molecular ions $(M-H)^-$ for several minutes.[38] The ionisation regimes achieved at primary beam current densities exceeding 10^{-8} A cm^{-2} have been called dynamic and are used mostly for the layer-by-layer analysis of metals and semiconductors. The first secondary-emission-mass spectra of organic substances were obtained in 1971 by Tantsyrev and Nikolaev.[39]

In 1976 Benninghoven and co-workers published the secondary-emission mass spectra of four aminoacids (alanine, phenylalanine, cystine, and arginine)[38] and a year later of a further 19 aminoacids and other biologically active compounds (short peptides, vitamins, sulphamide preparations, etc.).[40–42] The spectra of pyrimidine and purine bases, nucleotides and nucleosides,[43] and folid acid[44] were published by the same workers in subsequent years. Bombardment of a specimen in the form of a thin film on a silver support was carried out in the static regime by a primary beam of Ar^+ ions with an energy of 2.25–3.00 keV and at a current density of 4×10^{-9} A cm^{-2} and above. The spectra contained fairly intense peaks due to the ions $(M+H)^+$ and $(M-H)^-$ together with characteristic fragment ions. Stable emission persisted for several minutes but came to a rapid stop if the regime ceased to be static. Subsequently direct evidence was obtained by the method of electronic spectroscopy for chemical analysis (ESCA) that irradiation by a more powerful primary beam entails the disruption of the surface layer of the organic species.[45]

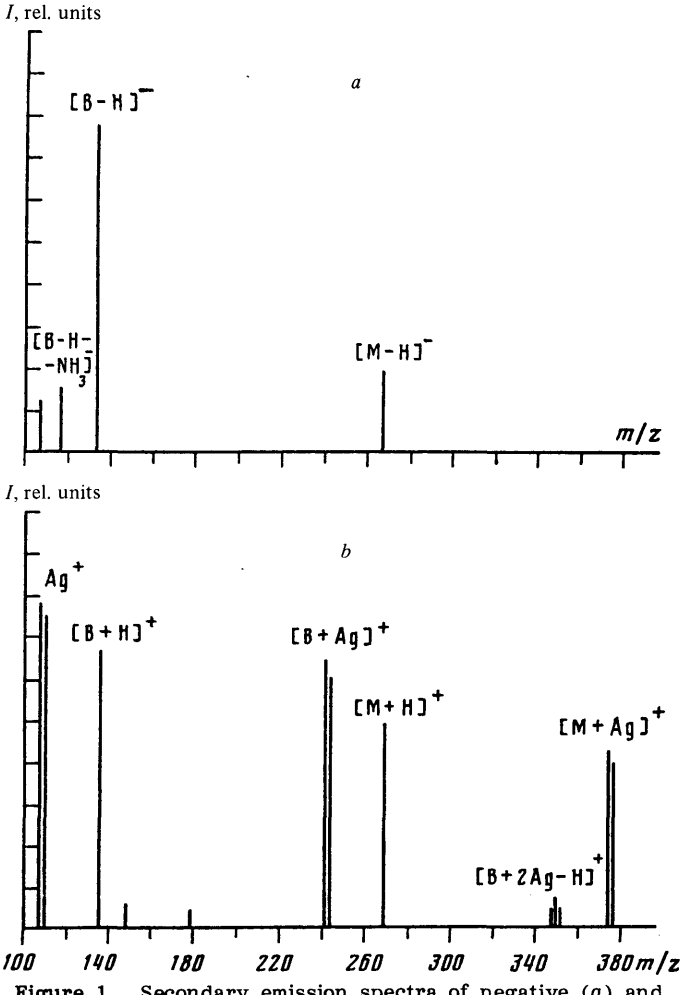


Figure 1. Secondary emission spectra of negative (*a*) and positive (*b*) adenosine ions.[43]

Apart from the protonated molecular and fragment ions of the test substance, the following can appear in secondary-emission mass spectra: (1) cationised molecular ions $(M+Ct)^+$, where Ct^+ is the cation of an alkaline metal or the metal of the support; (2) the intrinsic cations Ct^+ (or anions X^- in the spectra of the negative ions); (3) cluster ions of the type $(M_n + Ct)^+$, $(M_n + H)^+$, and $Ct.(Ct_n + X)^+$; (4) ions of the type $C_mH_n^+$ giving rise to a background spectrum in the region of low masses subsequently referred to as "chemical noise".[46] The spectra of certain compounds also contain clusters $(M - H + 2Ct)^+$ and molecular radical-ions $M^{\pm}$ and $M^{\mp}$. Clusters appear in the spectra of compounds having an

"acid" hydrogen atom,[43,47] while the source of the radical-ions has not been established. Presumably they arise at the phase interface by the interaction of neutral molecules of the test compound with the secondary electrons dislodged from the surface. Radical-ions and their fragmentation products have been observed in the spectra of atropine and nicotinamide.[41,42]

The fragmentation of the $(M + H)^+$ ions has been thoroughly investigated and takes place in accordance with the rules governing the decomposition of even-electron ions.[48,49] For example, the characteristic fragment for aminoacids is $(M - 45)^+$ [38,40-42,50] formed via the reaction

$$R{-}\underset{\overset{|}{\overset{+}{N}H_3}}{CH}{-}COOH \xrightarrow[-CO;\ -H_2O]{} R{-}\overset{+}{C}H{-}NH_2 .$$

The spectra of the purine and pyrimidine bases contain, apart from the intense peaks due to the ions $(M + H)^+$, $(M - H)^+$, or $(M + Ct)^+$, also the peaks due to the $(M + H - NH_3)^+$ and $(M + H - 43)^+$ fragments. In the spectra of the simplest nucleotides and nucleosides the peaks of the ions derived from the bases (B) and formed as a result of the decomposition of the $(M + H)^+$ ions are the most intense[43] (Fig. 1).

According to some data,[40-42] the average yield of the protonated ions from a film of an organic compound is 0.1; however, even for related compounds the values of $S^{\pm}$ differ markedly. In respect of aminoacids the yields of the ions $(M + H)^+$ from glycine and leucine differ by a factor of 150, while the ratio of the peak intensities of the fragment ions $(M - COOH)^+$ and $(M + H)^+$ varies from 10 in the alanine spectrum to 0.1 in the leucine spectrum.[42] A detailed study of the dependence of the yields of the ions $(M + H)^+$ and $(M - H)^-$ in the glycine spectrum on the experimental conditions showed that the ratio $(M + H)^+/(M - H)^-$ can be altered by a factor of 100 by changing the pH of the initial aqueous solution.[42] The yield of the ions $(M + H)^+$ increases with increasing acidity of the solution, while the addition of alkali promotes an increase of the yield of $(M - H)^-$. This is the first mention of the fact that the yield of ions under SIIE conditions can be altered and that for ionic compounds it is always higher than for neutral molecules. Using the zwitter-ionic character of the aminoacids, glycine has been converted[42] into one of the following ionic forms by the addition of acid or alkali:

$$CH_2{-}COOH \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} CH_2{-}C(=O)-O^- \underset{+H^+}{\overset{-H^+}{\rightleftharpoons}} CH_2{-}C(=O)-O^- \quad (NH_3^+ \text{ and } NH_2 \text{ respectively}).$$

Variants of this procedure for the conversion of the compound to be analysed into an ionic form was subsequently widely employed in studies by other investigators.

The possibility of changing $S^{\pm}$ makes it necessary to indicate the conditions used to prepare secondary ions in the determination of the sensitivity of the SIIE method. Benninghoven[51,52] estimated the limits within which substances of different types can be detected, assuming that not less than 10% of the number of molecules of the test substance on the target leave the surface in the form of quasi-molecular ions. If 10^{-10} of a mole of the product, i.e. 6×10^{13} molecules, have been deposited on the support, then in such a case $6 \times 10^5 - 3 \times 10^{12}$ ions arrive on the detector, depending on the type of mass-spectrometric analyser used (the relative aperture varies from 10^{-7} for static magnetic instruments with double focussing to 0.5 for time-of-flight instruments). Under ion counting conditions, it is possible to record reliably the signal from 1000 ions; consequently the limit for the detection of organic substances with the aid of SIIE is in the range 10^{-12}–10^{-19} of a mole. However, under real conditions, such high sensitivity cannot be achived owing to the presence of "chemical noise" and peaks due to admixtures.

Benninghoven[53] demonstrated the high sensitivity of the SIIE method in the solution of a practical clinical problem, namely the identification of medicinal drugs in urine. Narcotic drugs (morphine, codeine, meperidine, methadone) were introduced artificially into urine in an amount equivalent to 1 mg per 100 ml. A dilute (1 : 20) solution of 1 μl of urine (~0.5 g of each drug) was deposited on a support, which was placed in the focus of the primary ion beam. The $(M + H)^+$ ions from each preparation (Fig. 2) and certain fragments were observed in the mass spectra. Sodium cyclohexene-sulphonate introduced into the organism in an amount of 200 mg was not detected in the patient's urine after 12 h, but, when 18 h elapsed, its presence was demonstrated in an amount of 6.2 mg ml^{-1}.[53] The possibility of identifying 10^{-10} g of phenylalanine on a commercial double foccussing instrument

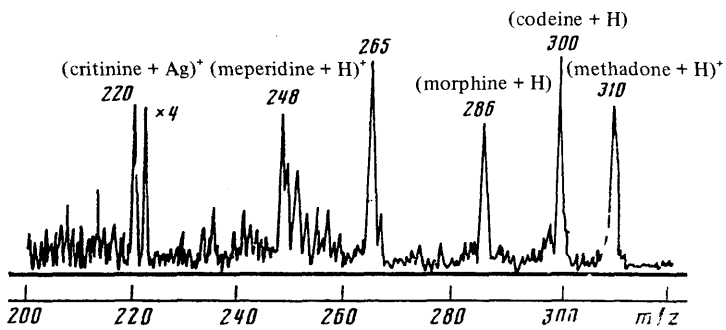


Figure 2. A fragment of the secondary emission spectrum of a mixture of narcotic substances.[53]

(Varian 311 A) has been reported.[54] The combination of the SIIE method with a time-of-flight analyser is especially promising. This application has been developed in a number of studies.[55–57]

Cooks and co-workers[58,59] were the first to initiate the development of another application of the SIIE method based on the ability of the majority of organic molecules, particularly highly polar ones, to form complexes with alkali metal cations on bombardment with the primary beam. The authors published their communication one year after the publication of the first study by Benninghoven and co-workers,[42] where the intensities of the $(M + Ct)^+$ ion peaks in the mass spectra were smaller by an order of magnitude than those of the $(M + H)^+$ peaks and their appearance was attributed to incomplete coverage of the surface of the metal support by the layer of the specimen. This hypothesis was subsequently confirmed experimentally; the composition of a flux of secondary ions from a surface coated by an aminoacid (glycine, α- and β-alanine, and serine) has been investigated with the simultaneous observation of the surface layers with aid of X-ray[60,61] and Auger spectrometers.[61] On the grounds that the cationated complexes $(M + Ct)^+$ are more stable than the protonated ions $(M + H)^+$ [56,57,62,63] [the protonated adenosine 5-monophosphate protonated ions have a lifetime ≤10 μs, while that of $(M + Na)^+$ is greater at least by an order of magnitude[63]], Cooks and co-workers developed several procedures whereby it is possible to increase the content of the cationated ions in the spectra of test substances; the specimen was deposited on the support in a mixture with metal, sulphur, carbon, silicon,[64] and alkali metal salt powders[58,64–67] and was bombarded by ions of other elements (cobalt, copper and silver).[59,61,64] Complexes with 22 elements in the Periodic System have been obtained in this way.[37]

These methods of generation of TUC ions by the SIIE procedure rapidly found applications in studies by other investigators. A detailed investigation has been made[47,68] of the dependence of the yield of the ions $(M + H)^+$, (M + cation of added salt)$^+$, and (M + cation of the metal of the support)$^+$ for different aminoacids M deposited on the support in a mixture with LiCl. By adding sodium or lithium chloride, Japanese workers obtained secondary emission mass spectra of mono-, di-, tri-, and tetra-saccharides,[69–72] adenosine, and 13 aminoglycoside antibiotics.[70,72] The authors noted that the added salts increased the intensity of the peaks of the quasi-molecular ions and decreased the extent of their fragmentation. However, the excess salt reduces the $(M + Ct)^+$ peak intensity and in addition promotes an increase of the fraction of the $(M + 2Ct + H)^+$ ions, which complicates the interpretation of the mass spectra. Analogous results have been obtained in other investigations.[47,68] Cooks and co-workers, who detected the $(M + 2Ct + H)^+$ ions in the spectrum of aminobenzoic acid,[58] proposed a simple method whereby one can avoid such complications—the use of ammonium chloride as an additive.[66,73,74] In this case the $(M + H)^+$ ions appeared in the spectrum together with the intense peak of NH_4^+ ions.

The stabilities of the $(M + Ct)^+$ complexes differ for different cations. The alkali metal cations show a greater stabilising effect. In selecting the conditions for the solution of a specific problem, one must be guided by the following rules established by Grade and Cooks:[59] (a) the $(M + Ct)^+$ cluster is more stable the more polar the molecule [for example, the peak intensity ratio $(M + Ag)^+/M^+$ in the stilbene spectrum is 500 times greater, while that in the *p*-aminobenzoic spectrum is 1000 times greater than in the anthracene spectrum]; (b) the degree of fragmentation can be regulated by producing complexes with cations of different kinds; this can be done in the spectrum obtained under electron impact conditions by altering the energy of the ionising electrons. For example, in the ionisation of *p*-aminobenzoic acid on a support made of the Ag + Pd (60 : 40) alloy the peak intensities of the ions $(M + Ag)^+$ and $[(M - COOH) + Ag]^+$ were identical, while the ratio $[Pd + (M - COOH)]^+/(Pd + M)^+$ was 7. For molecules with a high electron affinity, an attempt was made to obtain anionated complexes; however, complexes with the Cl^- and CN^- ions were not detected among the negative ions in the mass spectra of o-, *m*-, and p-aminohydroxybenzoic and mercaptobenzoic acids, 1,5-dihydronaphthalene, pentachlorophenol, nicotinamide, and 1-hydroxyanthraquinone. The spectra contained intense peaks of the ions $(M - H)^-$, $M^{\cdot-}$, and $(M - H - CO_2)^-$. The ratio $(M - H)^-/M^{\cdot-}$ increases with increasing acidity of the product. It has been noted that the spectra of negative ions contain few lines and can be readily interpreted. The interpretation is especially facilitated by comparing the spectra of positive and negative ions derived from the same product.[75]

Thus, using the ability of organic molecules to give rise to complexes with cations, it is apparently possible to obtain the mass spectra of compounds with a high molecular weight and a high polarity. These studies have only just begun. Hitherto it has been possible to obtain the quasi-molecular ions of methylated nucleosides[67] and polynucleotides[76] and it has become possible to carry out the mass-spectrometric analysis of the compositions of certain DNA and RNA after their completely enzymic hydrolysis. The mass spectra of saccharides,[69–73,77] including pentasaccharides[71,77] and octasaccharides,[72] adenosine, certain antibiotics[72,78] and oligopeptides[79,80] have been published. The aminoacid sequence in peptides can be determined from such spectra using the method of activated collisions.[80]

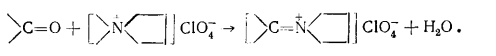
In estimating the sensitivity of the method employing cationated molecular ions, it is essential to take into account the differences between the capacities of the test substances for complex formation. The limit of the detection of sucrose in the mixture with LiCl proved to be 10 ng when the $(M + Ct)^+$ signal is 5 times more intense that the noise, while for adenosine the $(M + Ag)^+$ limit is 2 ng.[81] The sensitivity for glucose in a mixture of NaCl proved to be higher than 10^{-11} Cl g^{-1}.[69]

The mass spectra of organic ammonium salts and their analogues have been studied in detail under SIIE conditions.[81–87] Instead of complexes of the molecules with a proton or cation, the secondary ions are found to comprise undecomposed organic cations, some of which break down, giving rise to fragments which make it possible to infer the composition of the salt cation. Diquaternary salts do not give rise to dications in the vast majority of cases;[86] sometimes charge separation takes place, i.e. two singly-charged fragments of the dications are produced or a singly-charged cation is formed by loss of a proton.[87]

Comparison of the efficiencies of ion formation for ionic organic salts and compounds forming complexes with Ct has shown that the yield of ions derived from the quaternary ammonium salt $(C_2H_5)_4N^+I^-$ is 100 times greater than that of the ions derived from the mixture of dicyanobenzene and a copper(II) salt under identical conditions.[82] The absolute limit of the detection of the salt from the peak of the cation was 10 ng cm^{-2} (the signal exceeded the noise over 1 min).[81] A high sensitivity has been demonstrated in the identification of choline and choline esters[84] and also in the determination of muscarine and choline in thin-layer chromatographic spots.[85]

Thus the sensitivity of the method is highest for ionic compounds. Several procedures have been proposed whereby organic substances can be made to undergo a transition to a

formally ionic state; these transformations have been called a new type of "derivatisation".[87-89] For example, amphoteric compounds can be converted into an ionic form by adding inorganic acids or alkalis. As shown above, the addition of salts to basic products also increases the yield of quasi-molecular ions since metal ions are Lewis acids. By mixing amines with suitable alkyl iodides, it is possible to obtain the salts of quaternary nitrogen bases. On interaction with pyrrolidinium perchlorate, aldehydes and ketones are converted into immonium salts:



The proposed procedures extend the range of substances giving rise to intense quasi-molecular ions and characteristic fragments under SIIE conditions. Compounds not susceptible to analysis under field desorption conditions can nowadays be identified by the SIIE method. For example, after unsuccessful attempts to detect the ions of cymostatine and other thermally unstable antibiotics, the mass spectra of these molecules were obtained[78] by bombarding the specimen (in a mixture with NH_4Cl) by Xe^+ ions.

The determination of quantitative data concerning the mixture of organic products from the recorded secondary-emission mass-spectra is not always possible. In some investigations[67,76,90] the concentration of one of the components was estimated from secondary emission mass-spectrometric data using an isotopic isomer of the compound analysed as the internal standard. However, in many instances[81,91] it has been observed that formation of the ions of certain components is suppressed in the bombardment of a mixture of substances by the primary beam. For example, the spectrum of a mixture of five narcotic substances placed on a support was found to contain the $(M + H)^+$ ions derived from only three compounds.[91]

Summarising the data obtained in studied of thermally unstable compounds by the SIIE method, the following conclusions can be reached:

1. The spectra contain the intense peaks of the $(M + H)^+$, $(M - H)^-$, and $(M + Ct)^+$ ions, which make it possible to determine the molecular weight of the test substance.

2. Ionisation takes place in the solid state at room temperature or after slight heating; thermal degradation was not observed under these conditions.

3. The degree of fragmentation of the organic molecule can be regulated by altering the type of metal cation in the complex $(M + Ct)^+$ ion.

4. The method is highly sensitive, particularly for organic salts and polar compounds. Its sensitivity with respect to the three types of ion peaks decreases in the sequence organic cation > cationated molecules > protonated molecules. Under real conditions the determination of the mass spectrum under conditions where the support with an area of 0.1 cm^2 is scanned, it is necessary to deposit 10^{-10}–10^{-7} g of the test substance when the analyser employed is a commercial mass spectrometer with double focussing.[53,70]

5. The method can be used to identify products separated by thin-layer chromatography; in order to record the mass spectrum, the spot bombarded must contain at least 10 μg of the product. The first positive results using the SIIE method in a liquid chromatography–mass spectrometer system have been obtained.[46,92,93]

6. The method of preparation of the specimen for analysis is very simple and does not require a high experimental skill and lengthy preparations, as happens for example, in analysis by the FD method.

2. Secondary Atom–Ion Emission

The replacement of the primary ion beam by a beam of accelerated neutral particles has led to new possibilities for the study of involatile highly polar and thermally unstable compounds by the mass-spectrometric method. Data demonstrating that irradiation of the specimen by accelerated atoms makes it possible to ionise without decomposition heavier molecules than under SIIE conditions were surveyed towards the end of 1981.[94] For example, it has been possible to record the quasi-molecular ions of glucagon (a pancreatic hormone; MW = 3841) and the B-chain of insulin, separated from the rest of the molecule by oxidising disulphide bridges (MW = 3494).[95] The mass spectra of other biologically important products, obtained on bombardment with a beam of accelerated atoms have been published in reviews.[23,96-98] Compounds with MW > 4000 could be analysed at that time only by the plasma desorption method, with the aid of which the quasi-molecular ions of an oligonucleotide with MW = 6278 were recorded. However, by selecting the optimum balance of sensitivity and resolution, the spectra of protonated molecular ions derived from the hormone adrenocorticotropin (MW = 4538) and the undecomposed ox insulin molecule (MW = 5730) were obtained as early as 1982 on a magnetic mass spectrometer with suitable characteristics using the atom–ion emission (AIE) method[22] [FAB in Western literature (Ed.of Translation)].

The AIE method was developed and used for the first time by Tantsyrev and Kleimenov in 1973 in order to obtain the mass spectra of involatile organic molecules.[99] The object of the study consisted of thin films of polymeric substances deposited on a metallic support. The surface of the specimen was bombarded with a beam of Ar atoms having an energy of 300–3000 eV and a flux density of 10^9–10^{10} atom s^{-1} cm^{-2}. The AIE method proved to be convenient for the analysis of polymers, each of which has its characteristic spectrum suitable for its unambiguous identification; the spectra of copolymers represent a superposition of those of each polymer.[99-101]

The mutual orientation of the monomeric units in the macromolecules could be established from mass-spectrometric data[102-105] by determining the relative contents of hydrogen and deuterium when polymerisation took place with participation of a deuterium-containing monomer.[106] Apart from macromolecules, Tantsyrev and co-workers investigated the secondary-emission mass spectra of several TUC (aminoacids, secondary ammonium salts, and bases),[107] while in 1981 a systematic investigation of the mass spectra of biological substances was begun.[108-111]

In the non-Soviet literature Barber and co-workers or Surman and Vickerman (Great Britain), who published simultaneously in 1981 the secondary-electron mass spectra of several organic compounds,[112,113] are regarded as the discoverers of the AIE method as a new procedure for the ionisation of TUC. The rate of development of this new method, whereby it is possible to ionise without decomposition unstable organic molecules, is extremely high. Thus Barber and co-workers alone published in one year following the appearance of their first communication[112] not less than 17 papers devoted to the study of the mass spectra of bio-organic compounds of different classes.[22,114-129] At the present time the AIE method is widely used by many teams of investigators.[98,130-139]

The rapid spread of the new method for the ionisation of TUC is determined not only by the large amount of information derivable from the AIE spectra but also by the fact that their measurement does not require a major modification of commercial mass spectrometers.[140,141] On the basis of ion

gun designs proved a long time ago, the Kratos Company began the manufacture of AIE sources for the AEI MS902 apparatus,[117] while the Varian Mat company developed a design for a source with ionisation by an atomic beam for the MAT 731 mass spectrometer.[142]

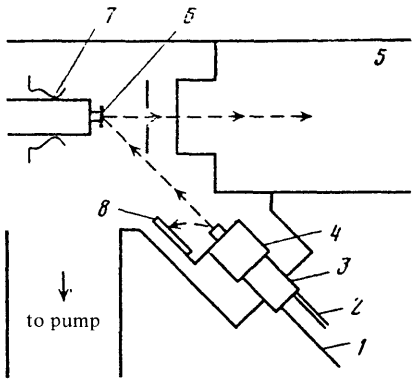


Figure 3. Schematic illustration of the ion source with the specimen bombarded by a beam of atoms[196] 1) high-voltage power supply; 2) injection of an inert gas; 3) region in which the primary ions are formed; 4) charge-transfer chamber; 5) mass analyser; 6) target with specimen; 7) vacuum lock; 8) deflecting electrode.

An apparatus has been described in which the secondary ions obtained as a result of ionic bombardment are separated by means of a static analyser with ordinary focussing;[143,144] a combination with quadrupole analysers is most frequently employed.[145] In order to obtain an accelerated atomic beam (usually a beam of Ar or Xe atoms), the ion beam initially generated is passed through a collision chamber. Fig.3 presents a systematic illustration of the AIE source. Ions undergo charge transfer on collision with the corresponding atoms in a collision chamber *4* filled with the gas from which the ions have been derived. The charge transfer cross-section is in this case large (10^{-16}–10^{-15} cm^2) [146] and approximately 50% of the ions undergo charge transfer without significant scattering of the beam in a chamber 100 mm long at a pressure of 10^{-4} mmHg.[104,146] An increase of pressure in the collision chamber by an order of magnitude decreases the degree of conversion of the ions into atoms to 10–30%.[143] The particles retaining the charge are removed from the beam by means of a special electrode *8*. The energy of the atom beam (of the order of magnitude of keV) differs somewhat from the nominal value of the applied potential difference accelerating the ions.[142] The beam of atoms bombarding the target *6* generates the maximum number of secondary ions at low angles of incidence relative to the target plane. Bombardment at an angle close to 90° leads to an increase of the fragmentation of the test molecules. The heavier the particles in the primary beam, the higher the yield of secondary, particularly quasi-molecular, ions.[94] For example, bombardment with mercury atoms increases the yield of the quasi-molecular sucrose, raffinose, and certain aminoacid ions by a factor of 10 relative to bombardment with Ar atoms.[147]

The principal distinctive feature of the preparation of secondary ions under AIE conditions involves the use of liquid targets made from volatile substances. A solution or a suspension of an organic compound in a liquid with a low vapour pressure is bombarded by accelerated atoms. This procedure has apparently led to the successful preparation of undecomposed quasi-molecular ions derived from extremely unstable and heavy biomolecules. Liquid targets were first used to obtain SE spectra by Tantsyrev, who showed that poly(propylene glycol) constitutes a convenient liquid target.[146] Glycerol is nowadays most often used as the involatile liquid, to which a small amount of an electrolyte, for example, oxalic acid, is added to ensure conductivity. The vapour pressure of glycerol at room temperature does not exceed 1×10^{-4} mmHg.[148] Apart from glycerol, epoxypropane,[139] organic and silicone oils,[95,128,149] α-monothioglycerol and its mixture with tetraethylene glycol,[95] polyglycol,[131] triglycerol, poly(ethylene glycol),[150] diethanolamine, tetraglyme, etc.[151] have been tested as solvents.

The application of liquid targets in AIE freed experimenters from many disadvantages characteristic of the SIIE method. Firstly, during bombardment by ions a positive charge is developed on the surface of the solid specimen, which must be compensated by a specially generated dark electron current. Otherwise, broad peaks, displaced towards higher mass numbers relative to integral mass peaks, appear among the recorded secondary ions peaks.[152] Charges do not accumulate on the surface of a liquid conducting target even in the presence of a small fraction of charged particles in the primary atom beam.[142] Secondly, bombardment with an atom beam makes it possible to employ lower potentials in the ion source in order to control the secondary ions; finally, under AIE conditions, the signal/noise ratio is higher by an order of magnitude than on irradiation with an ion beam.[80]

The role of the liquid phase in the mechanism of the transfer of the primary beam energy to organic molecules on the support has been investigated,[153–155] but is not as yet entirely clear. In a liquid the extent of the decomposition of the specimen molecules under the influence of high-energy primary particles apparently diminishes. On the other hand, the surface of the target is constantly renewed by undamaged portions of the specimen as a result of diffusion from internal layers. Furthermore, it has been shown[95] that certain test compounds shows an excess surface concentration, relative to the bulk-phase concentration, associated with the presence of hydrophobic groups. If these hypotheses are correct, liquid targets can be irradiated by a high-density primary beam without maintaining static bombardment conditions (for atom beams not exceeding 10^{10} atoms s^{-1} cm^{-2}), only making sure that the target does not become heated. An increase of the primary beam density entails an increase of the yield of secondary ions, i.e. ultimately leads to a diminution of the limiting concentrations of the products detected by the AIE method.

Serious experimental tests of the above postulates have not so far been published, but the advantage of liquid targets has been rapidly demonstrated and they have come to be used not only in AIE but also in the latest investigations by the SIIE method. This new procedure make it possible to obtain under SIIE conditions quasi-molecular ions derived from heavier and more unstable compounds.[77,80,156–158] It has been suggested that there are no radical differences between SIIE and AIE [89,115,139] and that the achievements by the AIE

method in the analysis of TUC are due mainly to the use of liquid targets. This has been confirmed by our study,[77] in which the use of a liquid target (diethanolamine) made it possible to obtain the quasi-molecular ions derived from an eight-membered cyclic oligosaccharide, namely γ-cyclodextrin (MW = 1679) on bombardment with Xe^+ ions. In the absence of a liquid matrix, it was possible to record only the ions of the tetrasaccharide stachyose (MW = 666).[71] Although quasi-molecular ions of heavier and more complex compounds have been obtained by the AIE method, the use of liquid targets in the SIIE method reduces the difference between the two procedures. In the future a liquid matrix will probably become an essential element of the study regardless of whether the specimen is ionised by a beam of atoms or ions. For the successful ionisation of different bio-organic substances, it is necessary in each case to select an appropriate liquid target matrix.[77,154] The elucidation of the role of the liquid phase in the ion formation mechanism has become an urgent taks in theoretical and experimental studies.

The mass spectra of organic compounds investigated under conditions involving irradiation with an atom beam do not show significant differences from the mass spectra recorded on bombardment with an ion beam.[94,147,157] A distinctive feature of the spectra obtained on a liquid target is the presence of the matrix ions. The molecules of the liquid appear in the spectrum in the form of cluster ions and fragments. For example, for glycerol (G) these are the positive ions $(G_n + H)^+$, $(G_n + Na)^+$, and to a lesser extent $(G_{n-1} + G - H + Na)Na^+$, where n reaches 15.[111] Clusters in which the molecules of the solvent and the test substance are combined sometimes appear. The spectra of negative ions contain significantly fewer clusters.[95] The presence of cluster ions has been used for the exact determination of the molecular weight of the test compound.[156] A method has been proposed for calibrating the mass scale under AIE conditions on the basis of the reference peaks of clusters comprising calcium toluenesulphonate and glycerol.[159]

Summarising the possibilities of the AIE method, we may note that the method increases the accuracy of the determination of the molecular weights of heavy molecules, since the $(M + H)^+$ and/or $(M + Ct)^+$ ions are always present in the spectra. The identification of the quasi-molecular ions among the peaks in the region of heavy masses is facilitated by comparing the mass spectra of positive and negative ions. The latter do not contain complex cluster ions and the heaviest peaks are due to the $(M - H)^-$ ions. The spectra of fragment ions make it possible to obtain information about the structure of the test compounds. The stability of the AIE spectra in time makes it possible to record the peaks of metastable ions, which are used to determine the mode of fragmentation of the test molecule.

The AIE method does not require the chemical modification of the test compounds, increases the reliability of the results, and diminishes by a factor of hundreds the amount of the test substance required for the analysis (for example, 1 μg instead of 1 mg is sufficient for the analysis of antibiotics[120]). These properties of the method altered radically the mass spectrometric procedure established previously for the determination of the aminoacid composition of peptides, in which chemical modification of the compounds was an essential stage.[3] The interpretation of the AIE mass spectrum yields nowadays information about the aminoacid sequence and about the composition of the terminal N- and C-acids.[114,115,118,119,121,130,132,161–163] The determination of the molecular weight of the peptide requires 0.1 nmol of the specimen and the determination of the aminoacid sequence requires 2–50 nmol.[24] However, one should note that sometimes not every aminoacid is represented in the spectrum by an individual peak.[132]

The high sensitivity of the AIE method is manifested in the analysis of bioproducts of different classes; data have been obtained for the molecular weights and structures of saccharides,[22,98,123,163–165] nucleotides and nucleosides,[24,117,137,166,168] lipids,[151,167,169] aminoglycoside and peptide antibiotics,[116,120,170,171] steroids,[23,134] sulphonated azo-dyes,[124–126] and a number of other compounds.[24,117,133,135,151,172] The identity of the AIE spectra of the synthesised and natural products has come to be regarded as confirmation of the structures of the natural products.[138]

Identical detection limits have been found in the mass spectra of positive and negative ions.[24] In order to increase the intensity of the quasi-molecular ions, the same procedures for the conversion of the test molecules to an ionic state are used in the AIE method as in the SIIE method.[88,95,117,124,132,173]

The quantitative aspects of the AIE method have not as yet been developed. However, the attempts to estimate the contents of the components in a mixture from the peak intensities of the quasi-molecular ions have already been made. Thus the determination of an admixture of a monomethyl-substituted quaternary amine in a mixture of dimethyl-substituted quaternary amines $(CH_2)_2N^+R^1R^2Cl^-$, entering into the composition of beef fat, has been reported.[23] The admixture concentration obtained (10%) agrees well with the result of chromatographic analysis (7.7%). At the same time it has not been possible by the AIE method to determine quantitatively nitrogen-containing cyclic compounds present in fuel shale,[131] although the qualitative identification of the individual components has been achieved successfully. The attempt to determine the content of bleomycins A_2 and B_2, differing only in the composition of the *N*-terminal aminoacids, also failed. The intensities of the formation of ions from these related compounds under AIE conditions differed appreciably.[116] A study has been made[174] of the causes of this phenomenon in order to obtain quantitative data for the dissociation constants of weak acids in solution.

The simplicity of the procedure used to prepare the specimens to carry out the analysis have led to an increasing use of the AIE technique for the measurement of the spectra. It is displacing the FD method, which has been for a long time the principal procedure for the analysis of TUC. Extensive factual data concerning the determination of the compositions and structures of biomolecules by the AIE spectroscopic method can be found in reviews.[89,98,145,151,175]

The principal difficulty, involving the preparation of ions from thermally unstable highly polar heavy molecules, has been overcome by the AIE method. Whereas several years ago the low volatility and stability of the test products constituted the main obstacle in mass-spectrometric analysis, at present the limiting factor has been the maximum capacity of mass analysers as regards the range of masses and their resolving capacity in the recording of heavy quasi-molecular ions generated under AIE conditions.[22]

3. Bombardment with High Energy Ions

A new method called desorption by high energy ions (DHEI),[176] which makes it possible to record the mass spectra of TUC by irradiating the latter with like multicharged ions speeded up in accelerators to energies ranging from several units to several tens of MeV, has been proposed recently. The general form of the mass spectra obtained is characteristic of secondary ion emission and differs little from the SIIE and AIE spectra. A distinctive feature of the

new method is the possibility of altering within wide limits the yield of the secondary ions as a function of the mass, charge, and energy of the primary beam ions (in contrast to the bombardment of the specimen by the ^{252}Cf fission fragments, whose composition is inhomogeneous and whose charge and energy vary independently of the experimental conditions).

The mass spectra of valine, obtained by bombarding a solid film of the specimen deposited on a nickel support by ^{16}O, ^{18}O, and ^{32}S ions in different charge states with energies ranging from 0.1 to 50 MeV, have been investigated.[176-180] The primary beam density was 10^3–10^4 ions s^{-1} cm^{-2} and the angle of incidence was 30° (relative to the plane of the target). The peaks of the ions $(M + H)^+$, $(M + 2Na - H)^+$, and the highly intense peak of the $(M - COOH)^+$ fragment were detected in the mass spectra. The curve for the efficiency of the generation of the $(M + H)^+$ and $(M - COOH)^+$ ions contains two maxima, corresponding to the energies of the bombarding ^{16}O and ^{32}S ions of 10 keV and 8 MeV, regardless of the energy of the particles from the primary beam.[178]

In order to explain the observed phenomenon, two mechanisms of the interaction of the primary beam with the substance of the target have been proposed. On impact by relatively slow primary particles (up to 0.1 MeV), their energy is scattered in elastic collisions of the atomic nuclei, while the energy of fast particles (tens of MeV) is consumed mainly in the electronic excitation of the molecules.[94] The irradiation of the surface valine film by ^{18}O ions with energies of 20 and 25 MeV in different charge states has shown that, with increase of the positive charge of the bombarding ions, the yield of the secondary ions also rises.[179] It has been shown[91] that the relative yield of the ions $(M + H)^+$ derived from Gly – Gly increased by a factor of 20 following the replacement of the primary beam of Cs^+ ions with an energy of 3 keV by Ge^{4+} ions with an energy of 54 MeV. The effect of raising the charge state is greater when compounds with a molecular weight in excess of 1000 are bombarded. The yield of secondary ions increases sharply with decrease of the angle of incidence of the primary beam relative to the plane of the target.[181]

The mass spectrum of a peptide with a molecular weight of 6000 has been obtained by the DHEI method;[91] following the bombardment of a thin film of ox insulin by ^{127}I ions with a 20-fold charge and an energy of 90 MeV, the mass spectra of positive and negative ions, containing peaks in the range of the molecular weights of the A- and B-chains of insulin, were recorded.[182]

The advances made have shown that, despite the complexity of the apparatus, the DHEI method can be used successfully to obtain the mass spectra of TUC, although so far only the first steps have been taken in this direction.

III. PLASMA DESORPTION BY ^{252}Cf FISSION FRAGMENTS

A new method for the analysis of involatile and relatively involatile TUC, called the method of plasma desorption by ^{252}Cf fission fragments (PlD ^{252}Cf), was developed in 1974.[183-187] The ions of the test substance are formed in the following manner: the test specimen is deposited on a thin foil behind which a ^{252}Cf source is placed. Quasi-molecular and other ions arise as a result of the penetration into the specimen of the ^{252}Cf fission products. The energy liberated under these conditions (~1000 eV $Å^{-1}$) causes the formation of a heat pulse in the specimen up to 20 000 K lasting 10^{-12} s. A cylindrical microvolume of the specimen-support with a radius of 25 Å is converted into a hot gas in which molecules of the specimen and of the support react. The ionic products of these reactions are "withdrawn" by an external electric field and are analysed in the mass spectrometer.

The spontaneous fission of the ^{252}Cf nucleus results in the formation of two fragments with unequal masses. There is a possibility of the formation of up to 40 pairs of different particles, but the formation of $^{142}Ba^{18+}$ and $^{106}Tc^{22+}$ with kinetic energies of 79 and 104 MeV respectively is most probable. The pair of fission fragments arises simultaneously (within 10^{-9} s), which leads to exceptional possibilities for the employment of the time-of-flight technieque for the recording of the mass spectra.[184,185]

The main difficulties in the employment of this method consist in the high radioactivity of the source, which usually amounts to 10–100 microcuries and in the high toxicity of ^{252}Cf. The preparation of the source is therefore a complex task.[184,187] The source is obtained by evaporating a drop of a ^{252}Cf solution deposited on a nickel or aluminium foil 1–5 μm thick; it is then covered by a polychloroacetate film, which prevents the migration of ^{252}Cf on the surface of the foil. In order to attenuate the intensity of the source, the latter can also be covered by additional layers, for example, gold.[186,187]

The specimen in the form of a thin homogeneous film ~100 mg cm^{-2} thick is usually obtained by electrosputtering.[188-190] When the film is obtained by other procedures—by depositing a drop from the solution, by cataphoresis, etc., poorly reproducible results are obtained.[188-191] The limit of sensitivity depend on the type of molecules investigated; for aminoacids, it is of the order of 10^{-9} g cm^{-2}. The recording of the spectrum for the very lowest yield of ions takes ~12 h.[185,189,191]

In virtually all cases different modifications of the time-of-flight mass spectrometer are used to obtain the mass spectrum.[191-194] Its advantages are a theoretically unlimited mass range and the convenience of combining the method for the recording of ions with a pulsed ionisation method. The modifications of the mass analyser were made largely to increase the sensivity and the resolving power. In the range of heavy masses the accuracy of the mass number determined does not as a rule exceed hundredths per cent.[193-195] The inadequate resolving power of the time-of-flight mass spectrometers is a significant obstacle in certain investigations, for example, in the determination of the aminoacid sequence in peptides.[196-198]

The mechanism of the formation of ions in the PlD ^{252}Cf method has not been fully investigated, but the majority of investigators describe it qualitatively in the following manner. At the sites of incidence of the ^{252}Cf fission fragments local plasma zones arise in a specimen where the desorbed molecules are ionised by one of two pathways: (1) via the formation of ion pairs: $2MH \rightarrow MH_2^+ + (M - H)^-$. This pathway, which is most typical for aminoacids, peptides, nucleotides, etc., involves the transfer of a proton from the acid end of one molecule to the basic end of another. (2) Via ion–molecule reactions with H^+, Na^+, K^+, or H^- ions. This results in the appearance in the spectrum of quasi-molecular ions containing an atom of the corresponding alkali metal, while the spectrum of negative ions contains protonated quasi-molecular ions.[199,200]

Aminoacids,[183,201] peptides,[184,195,201] nucleotides,[194,196-198,202,203] saccharides,[164,188,201] chlorophyll a,[204,205] antibiotics,[192,206] polytoxins,[207,208] vitamins,[188,191,193] and steroids[188,209] have been investigated by the PlD ^{252}Cf method. The results of such studies are satisfactorily reproducible from experiment to experiment and from one group of investigators to another.[204,205]

At the present time the PlD ^{252}Cf method makes it possible to obtain the heaviest ions. The ions $(2M + Na)^+$ with a mass of 12 637,[210] derived from fully protected oligonucleotides (dodecanucleotide with MW = 6278), have been recorded in the analysis of oligonucleotides.

The spectra of the test substances contain in all cases the peaks of fragment ions together with quasi-molecular peaks. The number of fragments arising in the PlD ^{252}Cf method is large even for relatively small molecules. Many fragmentation processes can be satisfactorily explained in terms of the concepts adopted in the chemistry of gas-phase ions. This enabled Field and co-workers[201] to assume, in contrast to Danigel and MacFarlane,[194] that the PlD ^{252}Cf method cannot be classified as a mild ionisation method. The method should rather be treated as one possessing dual properties: it makes it possible to obtain the intense peaks of quasi-molecular ions like the classical mild methods, and at the same time it yields an extensive fragment spectrum as in severe methods, for example, under electron impact. Such duality is not observed for other high-energy ionisation processes.

Investigators have noted that the plasma desorption method is promising and has considerable possibilities for the study of various classes of high-molecular-weight organic substances. The advantages of the method include, firstly, the possibility of the simultaneous study of the spectra of positive and negative ions, which permits a reliable determination of the molecular weight, structure, and the concomitant impurities in substances such as fully protected ribo-oligonucleotides up to heptamers inclusive.[174–176] Secondly, it became possible to establish the sequence of groups in complex organic compounds without a preliminary chemical modification in the intermediate phases of the synthesis (see, for example, Yoshihara[187]). Thirdly, with the aid of this method one can investigate unstable dissociation products of the ionised molecules and their fragmentation in the drift tube of the time-of-flight mass spectrometer.[173,184] Their presence broadens the packet of ions and impairs the resolving capacity of the spectrometer in the same way as the change in the real diameter of the ionisation spot on the target, which is related to the parameters of the ion source.[172] Finally, the method is distinguished by the simplicity of the introduction of the specimen and the lack of necessity for a special preparation of the sample, which makes it possible, in particular, to set up on the basis of this method an LC–MS (liquid chromatograph–mass spectrometer) system. In the description of the LC–MS apparatus it is stated that the fractions separated in the liquid chromatograph were deposited by electrosputtering through a capillary onto supports placed on sectors of a disc rotating at discrete time intervals (12 positions at a pressure of 10^{-3} mmHg); the sectors with the sample were inserted in succession through a vacuum lock into the chamber of the time-of-flight mass spectrometer and were exposed to the ^{252}Cf source. The LC–MS combination has been used for the quantitative analysis of biochemical compounds, in particular, of antitumour preparations: etoposide, teniposide, and alkaloids vindesine, vinblastine, and vincristine.[189]

IV. LASER DESORPTION

The first information about the generation of positive ions by means of laser radiation dates back to the beginning of the 1960's[212] and at the beginning of the 1970's laser desorption was used for the analysis of organic substances,[213,214] including thermally unstable compounds with formation of a molecular ion.[215] However, up to 1978,[216,217] laser desorption and ionisation had not yet been regarded as methods for the investigation of organic substances; mainly the use of laser in the mass spectrometry of solids was reported in literature up to 1978 and the effects of the interaction of laser pulses of different power with the test substance were investigated and classified.[218,219]

After the laser irradiation of involatile substances (salts, nucleotide–Na_2, sucrose, peptides), the cationated molecular ions and certain fragments, yielding valuable structural information, are abundant in the mass spectrum.[220] Hence it follows that at certain intensities laser radiation is capable of causing the desorption of the ionised molecules and that laser desorption (a) constitutes a mild ionisation method and (b) is promising for the analysis of thermally unstable and involatile compounds by mass-spectrometric methods. Experiments designed to investigate the mechanism of the formation of the $(M + Ct)^+$ ions have also been carried out and studies have been made of the rapid heating processes and of the role of multiphoton processes which yield a fragment spectrum.[220–222] So far the laser desorption method has been used to investigate a large series of TUC and relatively involatile compounds: oligosaccharides,[216,223–228] nucleotides and nucleosides,[224,227,229,230] glucosides,[224,225,230] aminoacids and oligopeptides,[224,226–228,230,231] vitamins and antibiotics,[232] and other bioorganic compounds.[232,233] Mass-spectrometric data obtained with laser desorption have been surveyed in two reviews.[234,235]

Parameters of the lasers used in the study of organic specimens by the laser desorption method.

Test substance	λ, nm	τ, s	W, W cm^{-2}	E, J cm^{-2}	Refs.
Ruby	694	10^{-3}	$5\cdot10^{4}$	50	[237]
Neodymium	1 064	10^{-4}	$2.5\cdot10^{5}$	25	[237]
Neodymium	1 064	10^{-8}	$2.5\cdot10^{9}$	25	[237]
Neodymium	265	$1.5\cdot10^{-8}$	10^{7}	0.15	[241]
Ruby	347	$3\cdot10^{-8}$	10^{8}	3	[238]
Ruby	347	$3\cdot10^{-8}$	10^{8}	3	[230]
Neodymium	265	$8\cdot10^{-9}$	10^{8}	0.8	[230]
Neodymium	1 064	10^{-8}	10^{8}	1	[242]
Neodymium	1 064	$8\cdot10^{-5}$	$2.5\cdot10^{3}$	0.2	[226]
CO_2	10 600	$4\cdot10^{-8}$	$3\cdot10^{6}$	0.12	[243]
Ruby	694	$3\cdot10^{-4}$	10^{6}	300	[213]
Ruby	694	$3\cdot10^{-4}$	10^{6}	300	[214]
CO_2*	10 600	5	20	100	[244]
Neodymium	1 064	10^{-4}	$5\cdot10^{6}$	500	[224]
CO_2*	10 600	10^{-1}	10^{3}	100	[244]
CO_2	10 600	$1.5\cdot10^{-7}$	$8.7\cdot10^{6}$	1.3	[222]

Notation: λ is the wavelength, τ the duration of the pulse, W the power density, and E the energy density.

*Under continuous irradiation conditions; in the remaining cases pulse lasers were used.

Several methods, similar as regards experimental technique, have been proposed for laser desorption. The specimen is normally deposited on a copper grid from solution in ethanol or water and is irradiated by a pulse of focussed laser radiation. The laser desorption products are analysed in time-of-flight,[213–215,217,228,229,238] quadrupole,[227,236] static,[220–222,224,226] or other types of mass spectrometer.[231] A wide variety of lasers, both pulse-type and continuous emission, are used for irradiation in the range from infrared to the ultraviolet region of the spectrum (see Table).[234]

Laser desorption usually involves the formation of cluster ions of the type $(M + Ct)^+$, and H, Li, Na, K, Cs, Cu, Ag, and Pt can function as the Ct^+. In certain cases the negative $(M - H)^-$ ions are formed.[224,231,234] The mechanism of the formation of cluster and quasi-molecular ions in laser desorption is insufficiently clear and requires further theoretical and experimental studies. Cluster formation has been regarded[94,199] as the manifestation of various mild ionisation mechanisms. Numerous investigations of relatively involatile organic compounds by laser desorption methods indicate the possible occurrence of the following processes: vaporisation of molecules from the surface of the support under conditions of very rapid rise in surface temperature;[237,238] surface thermoionisation;[239] decomposition of molecules and ion–molecule reactions;[37] multiphoton ionisation processes.[223,224]

The Table presents the conditions in the formation of various groups of ions with the aid of lasers having different parameters.[224] Evidently the wavelength of the laser radiation does not have a significant influence on the character of ionisation. Quasi-molecular ions are formed when the power of the radiation is varied in the range from 10 to 10^8 W cm^{-2}, which casts doubt on the claim that multiphoton processes are essential for the formation of ions.[223,224] In the cases presented in the Table, the duration of the radiation pulses varied from 10^{-8} to 1 s, but in all cases ions were formed. Evidently, when low power pulse lasers are used, quasi-molecular ions are formed after a comparativley prolonged irradiation.[216,223] On the other hand, in the studies with powerful lasers the resolving capacity deteriorated with time and quasi-molecular ions could not be detected.[240] Analysis of the tabulated data also shows that the relation between the peak intensities of the quasi-molecular ions and the irradiation energies is determined by the material of the support.[222] If the material of the support is characterised by ready desorption (for example, in the case of quartz), then quasi-molecular ions are formed even for a low irradiation energy density; in the case of metallic supports, it is necessary to employ higher energy densities.

One may conclude that a definite minimum temperature, characteristic of the given specimen–support system and ensuring an energy balance of the system, is essential for the formation of quasi-molecular ions. The properties of the lasers (energy density, duration of pulses, the wavelength of the radiation) and those of the specimen–support system (energy absorption coefficient, thermal conductivity, heat of evaporation of the test substance, etc.) are taken into account in the balance. Lasers with a lower power density have been used in the study of the formation of quasi-molecular ions as a function of temperature.[94,200,220,245,246] The studies shows that it is possible to distinguish two temperature regimes: the lowest temperatures, at which neutral molecules are desorbed and higher temperatures at which surface ionisation and desorption of complexes with alkali metals are the most characteristic processes.[222] In all cases ion-molecule reactions in the gas phase, to which relatively involatile organic molecules are transferred without decomposition by the radiation, play a considerable role in all cases in the establishment of the final mass spectrum.

V. THE MECHANISMS OF ION FORMATION

It has been frequency noted that the mass spectra of organic and bio-organic molecules, obtained from the solid phase by different ionisation methods, are very similar. The similarity of the mass spectrum has been noted by many investigators.[51,54,66,67,94,139] Thus alanine and arginine, quaternary nitrogen-containing salts, sucrose, vitamin B_{12}, and adenosine 5-monophosphate give rise to identical mass-spectra under conditions where the specimens are bombarded by both potassium and caesium ions with energies ranging from 3.5 to 10 keV and ^{252}Cf fission fragments.[55] Under laser desorption conditions, ions can be formed with participation of thermal processes; nevertheless similar mass spectra of $(C_4H_9)_4N^+I^-$,[239] nucleotide bases,[239,240] and sucrose[239] have been obtained on irradiation of the solid products both by a low-power laser beam and by ^{252}Cf fission fragments. The spectra of sucrose recorded by the SIIE and LD methods are almost identical.[242]

The similarity of the mass spectra is determined by the following characteristics:

1. The principal ions in the spectra are even-electron particles. They include the quasi-molecular ions $(M + H)^+$ and/or $(M + Ct)^+$ and the cluster ions $(M + H)^+$, $(M + Ct)^+$, and $(M - H + 2Ct)^+$; in addition, odd-electron fragments (with odd mass numbers) appear in the spectra of compounds having the composition $C_xH_yO_z$. Tantsyrev and Saraev[248] compared the spectra of benzene obtained by the ionisation of the vapour and of a solid film (frozen on a support) by exposure to an atomic beam. The spectra had different intensities of the peaks, those due to odd mass ions proved to be significantly higher under conditions involving ionisation from the solid state.

2. The ions of organic substances leaving the surface are energetically stable. The internal energy of the secondary particles is low. The ionisation is accompanied by ion-molecule interactions. As a result, cluster ions and their fragmentation products appear in the spectra obtained by many of the methods discussed above.

3. Multistage decompositions are not characteristic of the spectra recorded. Only in the ionisation the atom-ion emission method (using a "light matrix") is it sometimes possible to record peaks of metastable ions.[249] Under the conditions of ionisation from the solid state, the recorded ions are formed during a very short time interval (several nanoseconds), which makes it convenient to combine these ionisation methods with time-of-flight analysers.

The similarity of the mass spectra discussed above permits the conclusion that the main processes involving the formation of the ions detected in the methods considered differ little from one another. Nevertheless, it was initially assumed that each ionisation method is based on an energy transfer principle characteristic only of that particular method. For example, it has been suggested that a beam of heavy multicharged ions with an energy amounting to tens of MeV transmits its momentum to the material of the support mainly as a result of electron retardation,[247] while the primary beam with an energy amounting to several keV is retarded as a result of interaction with nuclei.

We shall consider the question why the energy of the primary beam and the mass and charge state of the primary particles do not influence significantly the composition and ratios of the secondary ions in the spectrum of the given products. A common feature of all the methods involving secondary ion emission from the solid state is ultrafast transfer of momentum. When the specimen is bombarded by fast ions or atoms, the time interval during which energy is transferred amounts to 10^{-12} s, while on irradiation with a laser beam it is 10^{-8} s.[37,51] The short interaction time of the primary beam of particles or quanta with a layer of test molecules is the physical basis which justifies the combination in one group of all the ionisation methods considered. As a consequence of the ultrafast transfer of momentum, the organic molecules present on the surface at the site of impact receive, according to the majority of investigators, a small

amount of excitation energy. A rough estimate of its value under the conditions of ionisation by the $^{252}Cf^{+}$ fission fragments yield on average 1 eV.[199] This value has been confirmed by measurements in another study.[250] It becomes clear why quasi-molecular ions can be obtained in an undamaged form on exposure to a beam of ions with an energy exceeding by a factor of thousands or hundreds of thousands the bond energy in organic molecules. Theoretical calculations relating to the dynamics of the dislodgement of the ions of organic multiatomic molecules have shown that the probability of the expulsion of an undamaged molecular ion is fairly high.[251–255]

In order to account for the processes involving the formation of secondary ions on bombardment of a solid film of the test compound by a beam of fast atoms, Tantsyrev and co-workers were the first to put forward the idea of a "hot spot",[105,107,146,256,257] which reduces to the following statement: most of the energy of the primary beam is dissipated in a localised region—the "hot spot". As a result of a cascade of collisions, the set of molecules in the activated volume can be regarded as a highly heated dense gas. The size and depth of the "hot spot" depend on the character of the primary irradiation, but on the whole this mechanism explains the ion formation process in any of the methods examined, for example, that of Barber et al.[123] and Van der Peyl et al.[221] The transfer of momentum from the bombarding particles to the atoms or molecules of the target takes place isotropically, but in macromolecules with an ordered structure (polymers) the process has its characteristic features,[105,146] which will not be discussed here.

In the activated volume a fraction of the molecules are in a vibronically excited state and some are ionised. The positive and negative ions formed can leave the surface without undergoing any change. According to Benninghoven,[51] this is precisely the origin of the ions recorded in the SIE spectra. Cooks and co-workers[37,74,75,83] suggest that this mechanism is valid only for compounds with an ionic structure (for example, organic salts). When such compounds are irradiated, the "completed" ions are scattered" into a vacuum and no vibrational excitation of the ions takes place, since the process involving their formation resembles adiabatic expansion into a vacuum accompanied by cooling. Another portion of the ions formed within the hot spot are able to interact during their lifetime with the surrounding molecules on the surface. According to Tantsyrev, the cluster ions $(M_n + H)^+$ and $(M_n + Ct)^+$ arise in this way. This is not the only view in existence. Cooks and co-workers[39,73] suggest that the cluster ions appear as a result of ion–molecule interactions not on the surface but in the dense gas cloud above the spot. In one way or another, ion–molecule reactions make a significant contribution to the formation of secondary ions.

Finally a fraction of the secondary ions which have appeared are capable of undergoing unimolecular decompositions in the gas phase. The fragment ions produced make it possible to derive structure information from the SIE spectra. The three types of ions enumerated apparently determine the ionic composition of the mass spectra.

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The development of new methods for the ionisation of thermally unstable compounds over a short period has extended the scope of mass-spectrometric studies of the composition and structure of bio-organic compounds. This required in the first place a further improvement of the analytical components of the apparatus and of the methods for the determination and treatment of mass-spectrometric data and also the solution of many theoretical problems. It became necessary to increase the range of the mass numbers with the simultaneous retention of a high resolving power and accuracy of the mass determinations. The establishment of the overall formula of the test compounds (for MW in excess of 3000), taking into account the distribution of the intensities of the isotopic peaks of the quasimolecular ions, was found to be difficult.[258] The determination of the ion formation mechanisms and also the elucidation of the role of the liquid matrix in the formation of undecomposed ions of TUC with high molcular weights is important for the correct interpretation of the results of investigations.

Studies of the characteristic features of the unimolecular decomposition reactions of heavy organic ions in activated collisions under the conditions of tandem mass spectrometry have been initiated.[259] The possibility of combining the new ionisation methods with ion-cyclotron resonance spectroscopy is being considered.[260] Further development of these trends will undoubtedly extend the applications of mass spectrometry in studies on bio-organic compounds.

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