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Laser Microprobe Mass Spectrometry: Possibilities and Limitations

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Abstract. Laser microprobe mass spectrometry (LMMS) offers a great potential for inorganic analysis and speciation as well as for organic structural characterization. This remarkable flexibility makes the results critically dependent on the experimental conditions, however, so an exact description is hard to achieve, since inaccessible local parameters are involved. The paper deals in detail with this aspect, which is often neglected but remains essential for making LMMS acceptable as analytical tool. Our methods of procedure are presented in the context of instrumental aspects and experimental evidence. Tentative hypotheses for desorption and ionization in LMMS provide an adequate framework for interpreting results consistently. Selected examples illustrate the potential gained from our experimental procedure as well as the limitations imposed. For organic compounds, analysis of pure products and simple mixtures, and microprobe applications are highlighted. Improved differentiation of inorganic compounds is also shown.

Key words: laser microprobe mass spectrometry (LMMS), methodology, organic analysis, speciation.

The use of lasers in MS dates back about two decades in both inorganic and organic analysis. On the one hand, photon interaction is exploited for overall elemental determinations in non-conducting solids, for instance as an alternative to spark-source excitation [1]. On the other hand, laser desorption is widely appreciated as a so-called soft ionization method for thermolabile organics, yielding cationized molecules and the virtual absence of fragmentation [2]. These instruments have been used mainly in individually developed set-ups.

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The first laser microprobe mass spectrometry (LMMS) instrument was commercialized some 10 years ago [3, 4]. A highly focused UV-beam pulse is employed to interact with a microvolume of a solid at power densities between 10^6 and 10^{11} W/cm^2 to produce ions which are then mass analysed by time-of-flight (TOF) MS. A full mass spectrum is recorded for each laser shot, which typically evaporates about 1 μm^3 or 10^{-12} g of analyte. Depending on the mode of operation, the information obtained ranges from element location, through inorganic speciation, to structural characterization of organics. Prime assets such as full periodic table coverage, high sensitivity and spatial resolution, compatibility with both non-conductive and conducting samples, make LMMS complementary to the electron microprobe and secondary-ion MS in the microprobe version [5].

The current literature reflects a growing spectrum of application areas [6]. Quantification is difficult, but LMMS is excellent for qualitative characterization on a μm-scale, often with minimal sample preparation [7]. Use of the laser and MS in the reflection mode allows local analysis of bulk specimens without further treatment. Transmission-type instruments are suitable for analysing sections made by the normal electron microscopy (EM) procedures, as well as all kinds of μm particles, e.g. from powdered solids, aerosols, etc., held on an EM grid, coated with polymer film. In our experience, the equipment suffers from poor resolution during sample observation (less than that of a high-quality optical microscope), the extreme dependence of the results on the μm scale analysis conditions, and the limited mass resolution.

The ease of achieving different local regimes means that a wide range of analytical problems can be handled. Although the desorption and ionization (DI) mechanisms in LMMS are not yet fully understood, the potential of the technique can already be exploited. Experiments are conducted on the basis of know-how and experience in coping with the variety of μm-scale parameters. The procedure is often hard to describe, which means that results can sometimes not be reproduced. Moreover, the high versatility does not exclude incompatibilities. For instance, trace element determination requires conditions completely different from those for organic structural characterization. The same applies to detection of heavy ions and achievement of unit mass resolution. Use of films or sections provides another regime besides that for μm particles. In practice, an order of priority has to be assigned with regard to mass resolution versus m/z range, sensitivity versus material consumption, etc. It is often forgotten that there is no general methodology. A detailed description of the procedure is mandatory.

A systematic study of organic polyfunctionals has been started [8, 9], and guidelines have been developed for defining the experimental conditions precisely. Interpretation is attempted by using a model for DI in LMMS, initially based on tentative hypotheses [10], for which our steadily increasing data base now provides sustaining evidence [11]. It is shown that LMMS can cope with polar and ionic compounds that are intractable by conventional techniques, and hence deserves a distinct place within the range of organic MS instruments [12]. The potential for location of target elements in complex matrices by means of structurally relevant ions has also been explored, sometimes with success. Matrix simulation experiments have demonstrated the role of surface availability for organic molecules [13].

In this paper, our experimental procedure is described and discussed, then our tentative model for DI in LMMS is treated and selected examples are given to illustrate its merits for providing structural information on organics. The need for surface-availability of organic molecules in microprobe applications is stressed. Finally, examples of organic and inorganic microanalysis are given. LMMS is useful for qualitative characterization of simple mixtures. The very minute sample consumption is exploited in, for instance, the study of aerosols produced in graphite-furnace atomic-absorption spectrometry (AAS). This example also shows the improved speciation in the threshold regime.

Model calculations for semi-infinite targets in reflection geometry allow refinement of current concepts about the energy deposited in the region where ionization takes place. Insight is obtained into the relationship between laser output and local conditions, energy distributions of released ions, matrix-assisted desorption, shock-wave formation, etc. [14]. So far, the calculations provide guidelines but cannot yet substitute for the empirical approach to instrument optimization.

Experimental

Instrumentation

The LAMMA^R 500 (Leybold Heraeus, Köln, FRG) is described in detail elsewhere [15]. The original transient recorder (TR) is replaced by a 100 MHz LeCroy TR 8818-MM 8103 unit with 32 kbyte memory, interfaced to an IBM PC-AT by in-house developed software [16]. A Leitz projection screen is mounted on the microscope to monitor sample consumption.

Sample Preparation

Most work has been performed with pure organic products. Virtually all reference compounds were analytical grade and obtained from Janssen Chimica or Aldrich. Powders were ground between microscope slides and the fine particles collected by adhesion on a formvar-coated EM grid, brought into contact with the material.

For analysis of mixtures, equimolar solutions (about 5×10^{-4} M) of dyes in water were evaporated under nitrogen and the resulting crystals transferred to coated grids as usual. Alternatively, more concentrated solutions were applied directly to formvar-coated grids and dried.

Model samples for simulation of embedded specimens were made by cutting sections prepared on epon according to routine procedures. To coat one side of the carrier a water-bath was filled with aqueous target solution, and the sample section was dipped off carefully with a "sandwich" grid, and dried. The cover layer was collected on the other part of the grid, and after drying, both sections were pressed together firmly. The interlayer distance was measured by microscope after laser perforation. Vacuum sublimation was used as an alternative to wet coating.

The graphite-furnace aerosols were collected by exposing a formvar-coated grid to the emanating smoke. Details of the sampling periods during the heating procedure, which are less relevant to this paper, are available elsewhere [17].

Procedure for LMMS Analysis

For single-particle analysis with the LAMMA 500, settings for MS voltages, transient recorder and laser attenuation, lens selection, focus adjustment, etc. are optimized according to the following guidelines.

(1) *MS voltages.* Unit mass resolution is mandatory. The criterion is a 10% valley between two full-scale peaks at adjacent m/z, or complete baseline separation between a major signal at high m/z and its ^{13}C isotope contribution. The mass range is limited to the achievable resolution (i.e. $M/\Delta M$). Definition of peak shape by at least 10 points is required. The 100 MHz transient recorder (TR) is used at 10-nsec sampling intervals. To achieve a peak width of at least 100 nsec in the high m/z region, the accelerating voltage is decreased to the following typical values: 3.0 kV ($M/\Delta M = 150$), 2.2 kV ($M/\Delta M = 300$) and 1.4 kV ($M/\Delta M = 500$). The other potentials (lens, reflector) are decreased proportionally and tuned to maintain peak symmetry and sensitivity.

(2) *External calibration of m/z scale.* Carbon foil (thickness 25 nm) is perforated with about three times the laser energy used for organic analysis. Calibration constants are determined by linear regression from the values for positive clusters C_n^+ at m/z between 108 and 192, on an average of 20 spectra. The scale is maintained until the voltages are changed. Validity of the calibration means that ions are detected at a nominal mass correct within 0.1%. Individual spectra may show an offset over a constant number of channels, owing to fluctuations in the time delay between the laser trigger and TR stop pulse. These random shifts are levelled out during averaging.

(3) *Laser output attenuation and threshold energy.* The power density is kept as low as possible to obtain a fairly intense signal, cf. (4). Typically 1–10 nJ is applied in combination with the 32x lens, yielding a spot diameter of about 2 μm and a power density in the range 10^9–10^8 W/cm^2. Less than 10% of the available output (without laser amplifier) is used. An additional prefilter (27% transmission) is normally inserted before the sequential tandem attenuators.

(4) *Constant sensitivity.* A typical output is a full scale signal on the 100 mV input range, by use of a 10x preamplifier and 17 dynodes of the multiplier. When peaks become more abundant, it is better to lower the laser energy rather than to increase the intensity (which usually results in loss of resolution and calibration accuracy). Overload signals can be tolerated provided there is enough time for the detector to recover from saturation before the ions of interest arrive.

(5) *Microscope focusing.* The final adjustment is selected by monitoring the MS resolution and calibration, not by relying on the optical image. This is partly because of the difference between the red and UV laser focus in the sample plane, which is also affected by the filter setting. Hence, laser attenuation is kept constant and particles of a given size are selected. The supporting formvar film serves as a reference for focusing and a constant correction is then applied for analysis.

(6) *Particle size selection and material consumption.* Isolated material in the range 1–2 μm, i.e. about the diameter of the pilot laser spot, is preferentially analysed and normally allows a few consecutive shots. In practice, this requirement is difficult to meet for coarser particles. Use of very small particles, permitting only one shot, often provides a solution for organics that are difficult to desorb. The material around the focused particle is watched on the projection screen. Surrounding particles should not be removed or moved by laser impact. These "mechanical" effects are assessed on densely loaded grids.

(7) *Shot-to-shot variations.* The parameters above are optimized until a satisfactory reproducibility between consecutive spectra from different particles is obtained, cf. (9). In practice, fluctuations of up to a factor of 2 are tolerable for the total ion current on an absolute scale, provided the relative peak ratios of fragments agree within about 30% (average of 10 spectra). Larger variations of the ratios of molecular to adduct and/or daughter ions can be accepted in view of "stimulated fragmentation" but can often be minimized by using smaller particles.

(8) *Data acquisition frequency.* This is the ratio of number of shots to spectra saved. For single particle analysis, this depends on the operator's experience with particle-size selection and compensation for the offset between the pilot and UV laser in the plane of the sample. In practice, the sample position and focus can be readjusted, on condition that the next shot substantially improves the mass resolution, calibration and/or sensitivity. Whenever this is not feasible, the product is considered unsuitable for LMMS analysis.

(9) *Energy selection by resolving-power setting.* Operation of the instrument with maximal resolution (about 500) in the low m/z range carries the risk that baseline separation can be achieved without attenuation of the laser energy to threshold conditions. Therefore, in case of doubt, spectra are verified

with an accelerating voltage increased to allow no more than the required resolution. This sometimes permits elimination of peaks which are due to a local higher energy regime.

(10) *Long-term reproducibility.* Results from representative compounds are checked over a period of typically one year to evaluate the overall instrumental conditions.

Results and Discussion

Procedural Methods

The procedure was originally optimized for obtaining structural information with the LAMMA 500 by single-particle analysis of pure organic products or simple mixtures. However, the guidelines can be readily adapted for use in different situations, e.g. with thin films, sections, in inorganic samples, or with other LMMS instruments. Except for the configuration, i.e. transmission and/or reflection mode, and specific design features for the laser, viewing and ion optics, the main functional principles are the same [15, 18–21].

To some extent, the outstanding potential of LMMS arises almost coincidentally from the combination of pulsed ionization of solids, the TOF-type MS and the application of DC voltages for the transfer of charged species. A clear distinction is required between generated ions and detectable ions. The latter are those giving fully resolved and properly calibrated peaks. Hence, the implications of the instrument design deserve a short discussion to show the origin of our procedure.

The ions are formed by the interaction of photons with the solid sample during about 15 nsec, and are continuously extracted and focused into the TOF MS. An electrostatic mirror or reflector serves for energy-time focusing. Differences in flight time for ions with a given m/z may arise from different initial energies. As their initial momentum increases, the ions penetrate deeper in the rejecting field, have a longer trajectory and spend more time in the reflector in comparison to the low energy species. As a result, the peak width is reduced and hence, the mass resolution is improved. The arriving ions are sequentially detected and the signal is stored in a waveform digitizer or transient recorder (TR) after fast ADC conversion (100 MHz). These requirements imposed on the detector circuitry limit the dynamic range to about 10^2–10^3 [22].

As a result, in LMMS the m/z value and mass resolution, which are essential parameters for each MS measurement, depend inherently on the time-definition of the ionization process. The continuous extraction requires that charged species are released within a limited period, e.g. the laser pulse duration, to reduce the spread of the ion bunch at the entrance of the drift tube and to allow a reliable calibration. The mass is derived from the time measurement, which requires a suitable short event to start it. The initial uncertainty has to be negligible in comparison to the time-separation of the arriving ions. Fig. 1 shows that delayed ion formation leads to decreased mass resolution. At the same time, calibration can become problematic. Ions desorbed late on are detected at higher than their actual m/z. Finally, ions released continuously over several hundreds of nsec result only in a steady increase of the background. Therefore, the situation would be completely changed by insertion of a gate in front of the TOF MS or by use of a magnetic MS with photoplate detection (cf. Fig. 2).

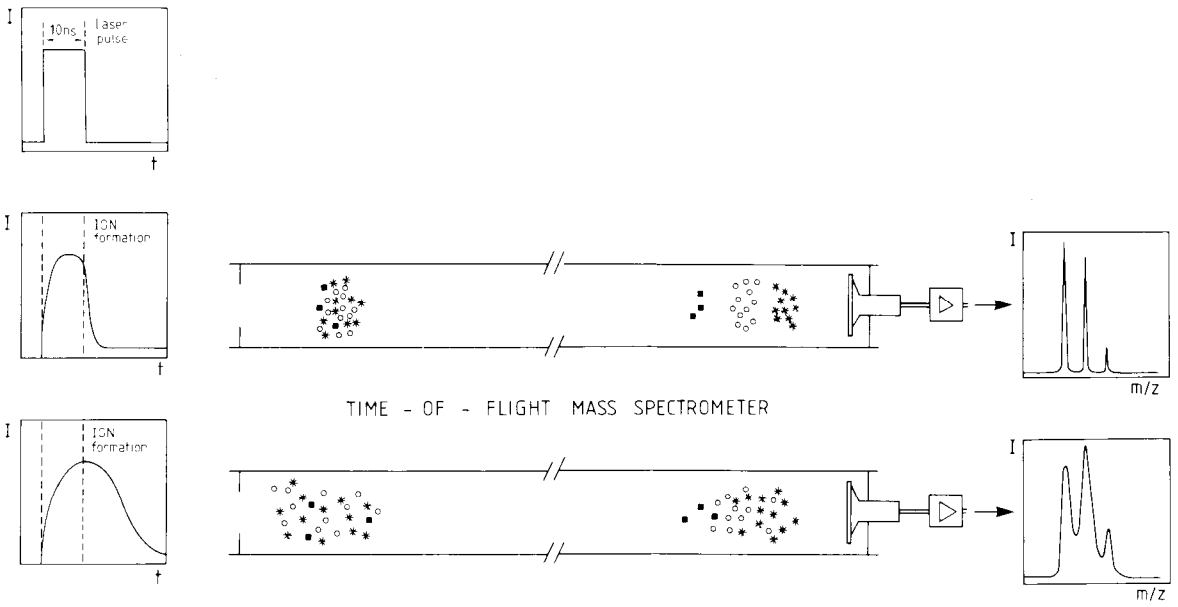

Fig. 1. Effect of the time-definition of the ion formation process on the mass resolution in LMMS

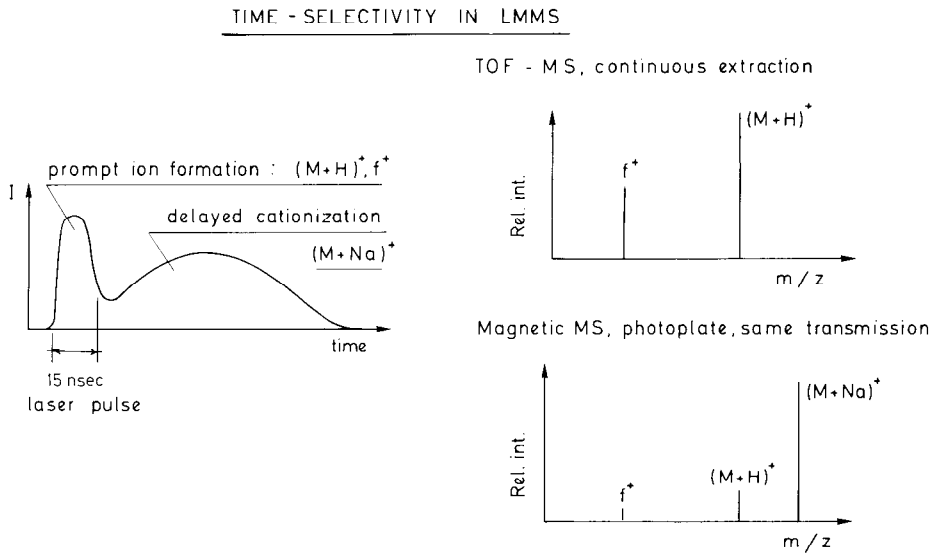

Fig. 2. Time selectivity of LMMS instrumentation compared with that of a magnetic instrument with photoplate detection for delayed ion formation

The laser pulse intensity profile is essentially the same from shot to shot, so the ionization kinetics should be similar. The relationship between the laser output applied and ion formation as a function of time depends on a combination of generally unknown μm-scale parameters. Monitoring of the laser energy per pulse does not give direct knowledge of the power density in the region where the ions are actually generated. Besides the UV absorption and dissipation properties of the

matrix [23], even such obvious aspects as particle size and laser focus position can be difficult to determine unambiguously. Indeed, at a magnification of 400x, imposed by the need to use the 32x lens, observation of sub-μm particles comes close to the limits of the human eye. The problem is aggravated by the chromatic aberration causing a difference in position between the visible and the UV beam in the sample plane. In practice, the inevitable slight misalignments must be overcome by trial and error.

The main idea behind our procedure is that the MS resolution, peak shape and calibration, which are strongly dependent on the correct optimization of μm-scale parameters, also serve as indices to evaluate the local conditions and the pulse profile of the ion formation. The principle is straightforwardly used to define the threshold regime. Voltages are selected to reduce the resolving power as far as possible, i.e. to not more than that strictly required to separate the highest m/z in the spectrum. The operator is thus forced to work at the threshold and to avoid as far as possible the initial time broadening during ion formation. The latter aspect also explains the difference between the practically achievable resolution, $M/\Delta M$ about 500 for organic compounds [24], and the maximum value of 850, calculated from lead isotopes [25]. Elemental ions permit the use of high power density to improve the pulse profile.

The procedure has been used for almost 5 years now, primarily for organic and recently for inorganic analysis, and is found to determine the experimental conditions quite strictly. Laser tuning remains a critical factor, partly monitored by the guidelines for long-term reproducibility and microscopic material consumption. Realignment or cleaning often cures the latter problem. In our experience, the long-term test is more helpful than use of energy meters for maintaining the equipment in a given state of operation.

The local conditions established by the procedure contribute not only to the reproducibility and reliability but also yield simple spectra. Obviously, threshold conditions lead inherently to selectivity and discriminate against plasma interactions, which are favoured in a high power-density regime. The latter is lethal for organic compounds. Inorganics may give a better elemental ion yield but the higher clusters are less reproducible and suffer from extensive recombination processes, which can be disturbing (cf. inorganic speciation, below). Moreover, in our experience the application of more energy consistently causes removal of several particles, and besides causing increased variability, is not compatible with high spatial resolution. These so-called "mechanical" effects are usually underestimated or not mentioned.

Use of threshold conditions may result in only the principal components in a given microvolume being characterized. Application of more energy to produce minor peaks can be tried as long as the resolution, calibration and material consumption remain reasonable.

The question of external or internal calibration has to be addressed. The former requires critical optimization of laser output and focus with respect to particle size. However, external calibration is sometimes no longer applicable and the mass peaks have a tendency to shift towards higher m/z. A typical example arises from the LMMS analysis of 1-*N*-benzyl-3-carbomethoxy-4-piperidone as a sodium salt. No problems occur with the positive and negative fragment ions but the cationized molecules or $(A^- + 2Na^+)^+$ species are detected at about 1 mass unit higher than

the nominal value, at least when external calibration with carbon foil is used. An internal calibration correction using the sodium peak to recalculate the time-mass conversion within each individual spectrum, permits the expected m/z value to be obtained. It should be noted that no correction is required for the positive or negative ions from the corresponding neutral analogue. The simple fact that the external calibration cannot be used for the $(A^- + 2Na^+)^+$ ions from the salt, in contrast to the fragments or intact cations, actually gives fundamental information about the moment and mechanism of ion formation. The release of the intact anions and all fragments occurs as a prompt process, of which the timing is adequately described by the clusters from carbon foil. Additionally, it is readily conceived that these ions share common formation processes and perhaps neutral precursors. In contrast, the behaviour of $(A^- + 2Na^+)^+$ ions points to a delayed formation in comparison with the carbon foil ions. The recombination species remain in phase with the majority of the co-desorbed Na^+ ions and both contributions remain well separated from the fragment ions and intact anion generation, as can be seen from the fluctuating relative shifts in mass. These data can be generalized within our data base.

With regard to acceptable reproducibility, a distinction is made between qualitative analysis and more quantitative work. So-called missing shots are virtually inevitable during particle analysis. This prevents quantification, but for diagnostic purposes it is enough that a given spectrum is reproduced from each particle, though not necessarily by the first shot. This substantially extends the application for organics. The procedure has played an essential role in the use of LMMS for diagnostic analysis. Indeed, external calibration has permitted key information to be obtained about generation of radical molecular and fragment ions [26–28], for instance.

The strict nature of the procedure partly results from the option to use the guidelines as a means to determine a given working range. Of course, there is a lot of interesting research outside this area, for instance on heavy ions with m.w. up to 10^5 amu or more [29]. The point is that each type of experiment requires full specification of criteria for resolution, calibration, etc. Our methods of procedure are certainly not generally applicable, and only try to define a way of operation which allows exploration of the potential of the technique.

Empirical Model for DI of Organic Compounds in LMMS

LMMS results look totally different from those for conventional laser desorption (LD) MS [30, 31]. The latter method typically yields cationized molecules, even from thermolabiles, virtually without fragmentation and decomposition. An intermediate form between LDMS and LMMS, namely so-called bulk-analysis laser MS (LMS), has also been developed [32]. There are fundamental differences between these techniques with respect to power density and ion formation, for instance. Much has to be learned about the desorption and ionization (DI) mechanisms in LMMS. The current literature describes a wide variety of possibilities, e.g. direct ionization from the solid state, bond-dissociation in excited molecules on the surface, plasma formation, shock-wave induced processes, gas-phase mechanisms, resonance absorption in the condensed phase [33–38]. To assess these, we needed a simple and general framework to start tentative interpretation of the signals.

Organic LMMS data have a certain ambiguity. Even quite stable molecules are readily pyrolysed or completely disintegrated into carbon clusters, whereas thermolabile compounds can be desorbed intact, and soft adduct ionization processes seem to prevail, though in most cases fragmentation remains abundant. Some hypotheses from this behaviour have been merged into a tentative model for DI in LMMS. The final result of laser-induced processes is rationalized in terms of energy, pressure and time. Our approach is empirical in nature, based on internal consistency for the molecules studied so far. The term "electron ionization" will denote here the formation of radical molecular ions, positive and/or negative. This does not exclude possible photon ionization.

It is readily conceived that laser impact creates an energy gradient along the sample surface and starts an entire series of processes, ranging from complete disintegration in the central area, through pyrolysis and thermal decomposition, to gradually softer conditions in the periphery. A further simplification is the assumption that organics are initially desorbed as neutrals. Thus the internal energy of the released species ranges from high to very low, consistent with the intact escape of thermolabiles. Subsequently, electron ionization takes place, or adduct formation by attachment of H^+ or Na^+. Precursors with high internal energy are compatible with radical parents, and yield fragments, whereas soft ionization behaviour is assigned to adducts. For salts with preformed ions, the situation does not drastically change. Most signals are interpreted by assuming that thermal decomposition occurs and applying previous concepts to the resulting neutrals. Obviously, a given fraction of the preformed ions will be desorbed intact. In comparison to conventional direct-insertion probe MS, the presence of sample inside the source gives LMMS a distinct advantage when only a small number of preformed ions are available.

Pressure-related effects are considered next. The laser-generated cloud includes local density regimes, from relatively high pressure, which favours intermolecular interactions, to typical high vacuum, where no second-order processes take place. These regimes are denoted by classical terms, as the "chemical ionization" and "electron ionization" regions. The link between pressure, internal energy and volatility is important. The last term refers to the ease of desorption from the solid state by laser impact, and this can be affected by both the chemical structure and the physical properties. Low molecular-weight compounds show a pronounced tendency to form dimeric clusters for instance, even under threshold conditions [39], in the absence of fragments. Less volatile compounds produce fewer adducts but increased electron ionization and prominent daughter ions. Substances that are difficult to desorb yield no adducts and only fragments. This situation is completely different from conventional MS, where each compound receives a given constant amount of internal energy. In LMMS there is a gradual transition from chemical ionization to electron ionization conditions, depending on the physicochemical properties.

As pointed out before, LMMS exploits only the promptly generated ions. This time-selectivity may explain why only a fraction of the total yield for some processes is monitored in LMMS, especially cation attachment, which prevails in LDMS. In fact, we usually find that cationization leads to a clearly distinct peak shape, in contrast to protonated molecules or fragments. This observation is related to the delayed thermionic flux of alkali-metal ions [40–42].

Experimental evidence sustaining our DI model, is summarized elsewhere [11]. The next section deals with relevant examples, namely the comparison of methoxytryptamine hydrochloride with the free base, and the LMMS analysis of triphenylphosphonium salts and nucleotides. The data allow us to address the role of electron and adduct ionization and of thermal degradation for salts.

Structural Characterization of Organic Compounds by LMMS

A substantial fraction of the organic information obtainable from LMMS is carried by negative ions. Hence, there is little supporting background information from conventional MS to ease the problem of spectrum interpretation. Moreover, the method is preferentially applied to compounds which are intractable by other techniques. Finally, the instrument offers only low mass resolution, which does not allow unambiguous determination of the exact elemental composition. Hence, interpretation has to be based on comparison of related analogues having different substituents.

In most cases it is impossible to verify the precursors for a given fragment. Hence, we simply use the general notion that the energetically unfavourable nature of the radical state acts as the driving force to trigger fragmentation and that transition from an even-electron to an odd-electron species is unlikely. The assignment of soft ionization behaviour to parents with even-electron systems, for instance preformed ions from salts of $(M + H)^+$ from neutrals, can be argued. Indeed, all even-electron fragments can be formally explained as due to adducts. In contrast, our approach consistently uses molecular ions, in spite of the fact that $M^{+\cdot}$ signals are frequently absent in LMMS.

There are several reasons for our choice. First, the absence of the $M^{+\cdot}$ peak holds true for a substantial number of EI–MS results, where the formation of radical molecular ions is not questioned. Each daughter is detected at the expense of the corresponding parent peak intensity and this principle reasonably applies to LMMS as well. Secondly, the use of $M^{+\cdot}$ as initial precursor permits extrapolation of conventional background knowledge for initial charge localization and triggering fragmentation in the positive mode. The latter is less obvious when for instance $(M + H)^+$ is considered. Finally, the detection of odd-electron fragments ($f^{+\cdot}$), often encountered within our data base, is not compatible with exclusively adduct ion-formation.

The LMMS results for 9-anthracenylmethyltriphenylphosphonium chloride, shown in Fig. 3, are relevant to this discussion. The molecular region contains three major peaks, of which the one at *m/z* 452 obviously refers to the radical molecular ions ($M^{+\cdot}$) of the most likely thermal decomposition product, corresponding to $(C^+ - H^+)$ where C^+ denotes the intact preformed ion. The same signal is found in direct-insertion probe EI–MS, but at low intensity. The base peak there, for triphenylphosphine, lies at *m/z* 262 and is of less diagnostic interest. The peak at *m/z* 453 in LMMS is higher than the ^{13}C satellite of the signal at *m/z* 452 and hence includes an additional contribution from intact preformed cations and/or protonated adducts of the thermal degradation product $(M_d + H)^+$. In contrast to EI–MS, the LMMS base peak at *m/z* 191 gives specific information characterizing the anthracenylmethyl group. The peaks at *m/z* 297 and 299 obviously refer to

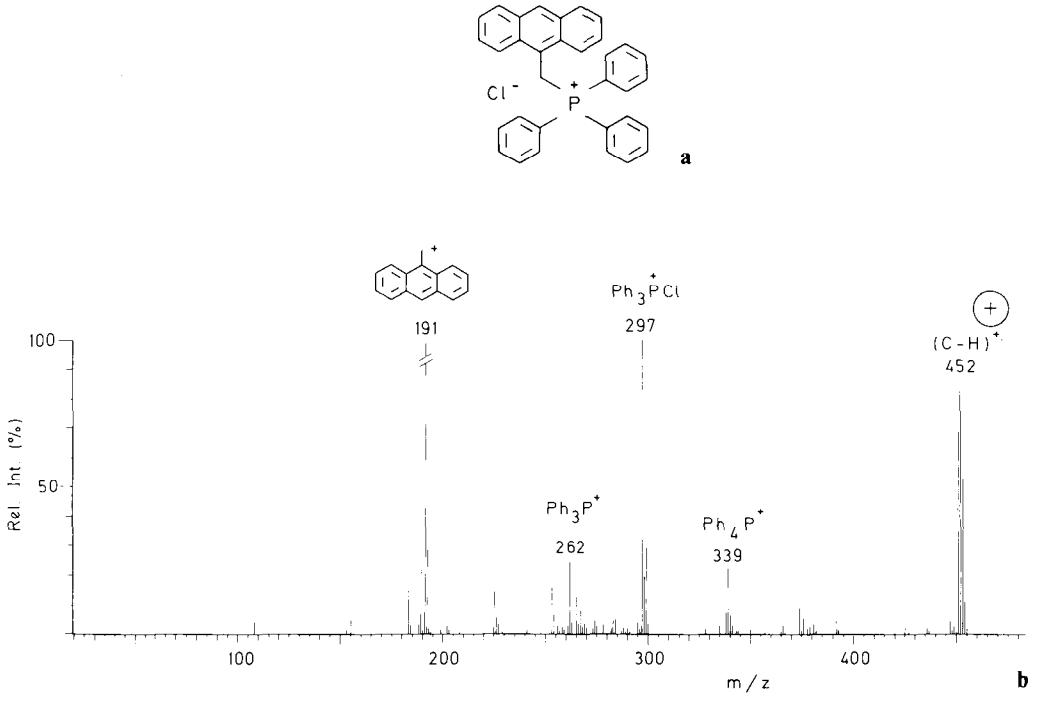

Fig. 3. LMMS results (positive-ion detection mode) for methoxytryptamine, analysed as the neutral form (**a**) and the hydrochloride (**b**). The molecular weight (m.w.) of the free base is 190

triphenylphosphine attached to one chlorine atom. These ions, absent in EI–MS, point to recombination in the laser-generated plume. Such interactions are rarely encountered under threshold conditions. A weak signal at *m/z* 262, detected for most triphenylphosphonium salts, is assigned to the $M^{+\cdot}$ of triphenylphosphine. These ions can result from thermal decomposition or, alternatively, from fragmentation of the $M^{+\cdot}$ at *m/z* 452. Anyway, their radical nature again makes preformed cations unlikely parents.

In conclusion, this molecule evidences thermal degradation as well as electron ionization. These features become less prominent when a simple instead of a polycyclic aromatic substituent is involved. This example is not unique within our data base [43, 44].

We have compared several free bases with the corresponding hydrochlorides. The most striking results are found for tryptamine-based analogues (cf. Fig. 4), but the main trends are generally followed. The results for the methoxy-derivative, analysed as the neutral form, show that the major signals point to odd-electron ions, namely $M^{+\cdot}$ at *m/z* 190 and $(M-HCN-H_2)^{+\cdot}$ at *m/z* 161. As a result, a predominant fraction of the total ion current in the positive mode is carried by radical species, which undoubtedly indicate electron ionization. Surprisingly, the same holds true for the hydrochloride. The major difference lies in the relative abundance of the molecular and fragment ions, which can be partly related to the fact that the degree of fragmentation depends on the ease of desorption. It has to be realized that the fragments, being of the $f^{+\cdot}$ type, can only come from $M^{+\cdot}$ precursors. To evaluate the contribution from adduct formation and/or release of preformed cations from the

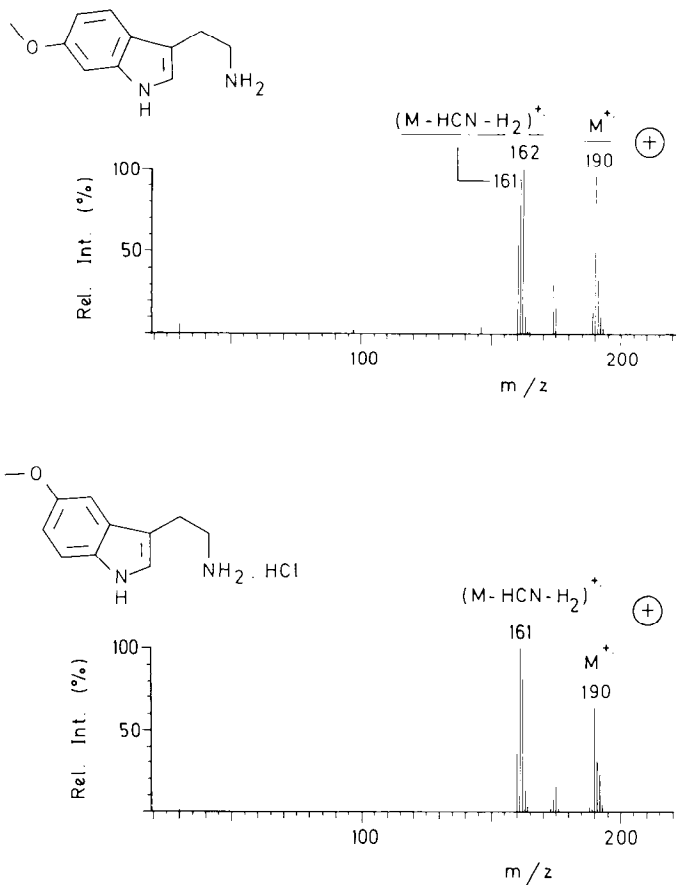

Fig. 4. LMMS results (positive-ion detection mode) for 9-anthracenylmethyltriphenyl-phosphonium chloride. The m.w. of the preformed cations is 453

hydrochloride, relative to that from electron ionization, the peak intensity at m/z 191 has to be corrected first for the ^{13}C satellite of the $M^{+\cdot}$ at m/z 190 (about 12%) and then compared to the sum of the peaks at m/z 190 and 161 ($M^{+\cdot} + f^{+\cdot}$). There is no real difference between the free base and the hydrochloride: even-electron precursors account only for 10 and 13% respectively, of the total ion current. These figures are unexpected for a soft ionization technique. Of course, hydrochlorides do not allow distinction between the ions, for instance at m/z 191 in this example, resulting from desorption of preformed cations, and those from protonation of a likely thermal degradation product, namely the free base. However, this does not affect the prominence of the role of electron ionization relative to adduct ionization.

Our approach remains consistent with the assignment of odd-electron daughters, which arise for a lot of polyfunctionals and are more frequently encountered, at least within our data base, than $M^{+\cdot}$ signals. Their relevance for electron ionization is similar. Formation of radical molecular ions $M^{+\cdot}$ is detected in the negative mode as well. Electron-capture ionization (ECI) even yields the base peak for a molecule such as Methyl Red, in spite of the carboxylic acid group present [26]. Evidence for radical molecular ions from fragments is also found for N-oxides in the positive and negative ion detection mode [28].

Organic salts are another field where LMMS may provide an alternative to conventional MS. Structural information is readily available from nucleotides,

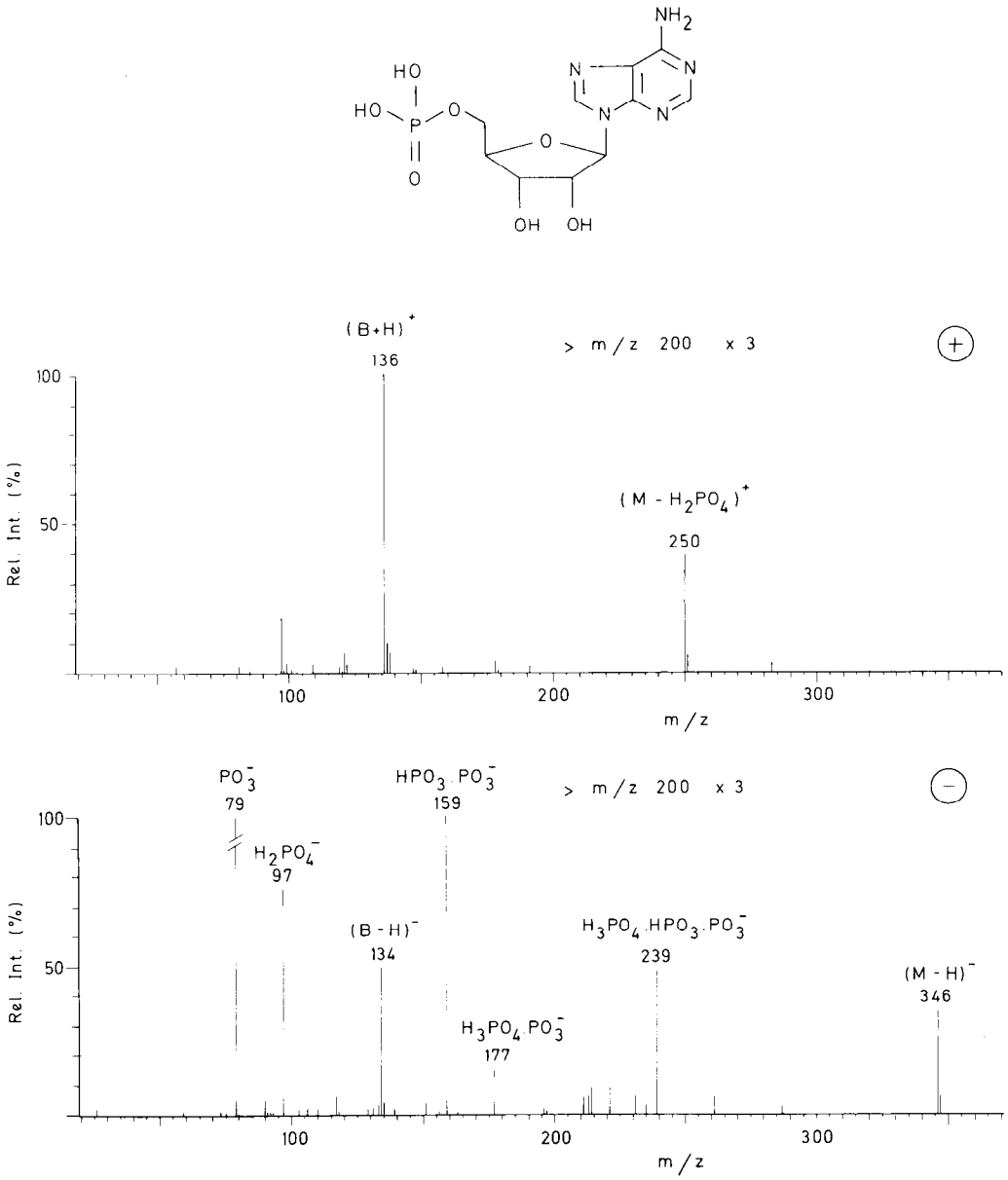

Fig. 5. LMMS results (positive- and negative-ion detection mode) for adenosine mononucleotide, analysed in the acidic form (m.w. 347). B refers to neutral adenine

analysed in the acidic form. The mass spectra are given in Fig. 5 for the adenosine analogue. The molecular weight is indicated by the $(M-H)^-$ ions in the negative mode, where the purine moiety and also the presence of a phosphate group is indicated. In the high mass-range of the positive LMMS spectra, the major peaks point to the typical loss of the phosphate group and the aromatic part is again confirmed. In contrast, when the salts are analysed, all organic information is removed from the spectra and only inorganic clusters are detected. This trend is shared by several organics containing preformed ions, and contrasts with the widely

accepted notion that the polar or even ionic nature of a compound makes it more suitable for methods employing direct DI of solids [35].

However, we have recently found that sulphonate diazo-dyes yield a lot of organic information by LMMS in the negative mode [27]. In view of the data discussed later, it should be mentioned that the positive ion mass spectra typically contain prominent signals from cationized molecules as well as from clusters at m/z 149 and 165, assigned to $Na_2SO_3 \cdot Na^+$ and $Na_2SO_4 \cdot Na^+$. The sensitivity of these peaks when obtained from organic sulphonates contrasts with that for inorganic sulphates, where these ions are hardly detected under threshold conditions.

Characterization of Organic Mixtures

Our experimental procedure accepts that the absolute intensity of consecutively taken spectra can vary by a factor of up to 2 because in practice it is not possible to assess exact particle size and laser focus position. Some of the problems involved are directly related to single-particle analysis in the sub-μm range, which yields the best structural information. Use of samples in the form of homogeneous thin films or coatings eases the operator's task, but their preparation is not always obvious, particularly for analysis in the transmission mode, where a thick supporting layer (metal) cannot be used. Powder samples are easily obtained from practically all kinds of solids but this procedure yields heterogeneous size distributions, which do not ease the operator's task. As long as the relative peak ratios agree reasonably, we find these fluctuations of absolute intensities acceptable for qualitative characterization. Obviously, the situation becomes different for quantification. So-called "missing" shots are then not tolerable. A suitable sample preparation procedure is required to obtain either films or a uniform size distribution.

We have analysed mixtures of organic dyes which are ionic and hence water-soluble. By trial and error it has been found that the best specimens are obtained from aqueous solutions, which can contain up to five components. All the particles then have virtually the same size. The shot-to-shot variability is substantially improved and the total ion current can be controlled to keep the peaks of interest in scale.

The results from individual shots are interpreted as a yes-or-no answer about the presence of the corresponding dye in the microvolume analysed. Quantification is not yet feasible, partly as a result of the lack of appropriate standards with composition checked by an independent method on the μm scale. The DI and fragmentation behaviour seem to be unaffected by the presence of other constituents. Otherwise stated, LMMS analysis of a mixture simply gives a superposition of the mass spectra for the corresponding pure products. Except for the trivial observation that the relative contributions of cationization and protonation depend on the overall alkali-metal concentration in the sample, no indications of additional interactions of molecules or fragments are found. An example is illustrated in Fig. 6.

A direct comparison between LMMS and PDMS has been made for these samples. For the pure components, the main characteristics are the same for both techniques, including the occurrence of electron-capture ionization. For mixtures, PDMS circumvents some of the inherent problems encountered in LMMS. In the former technique, the interaction of a single fission fragment with the solid remains

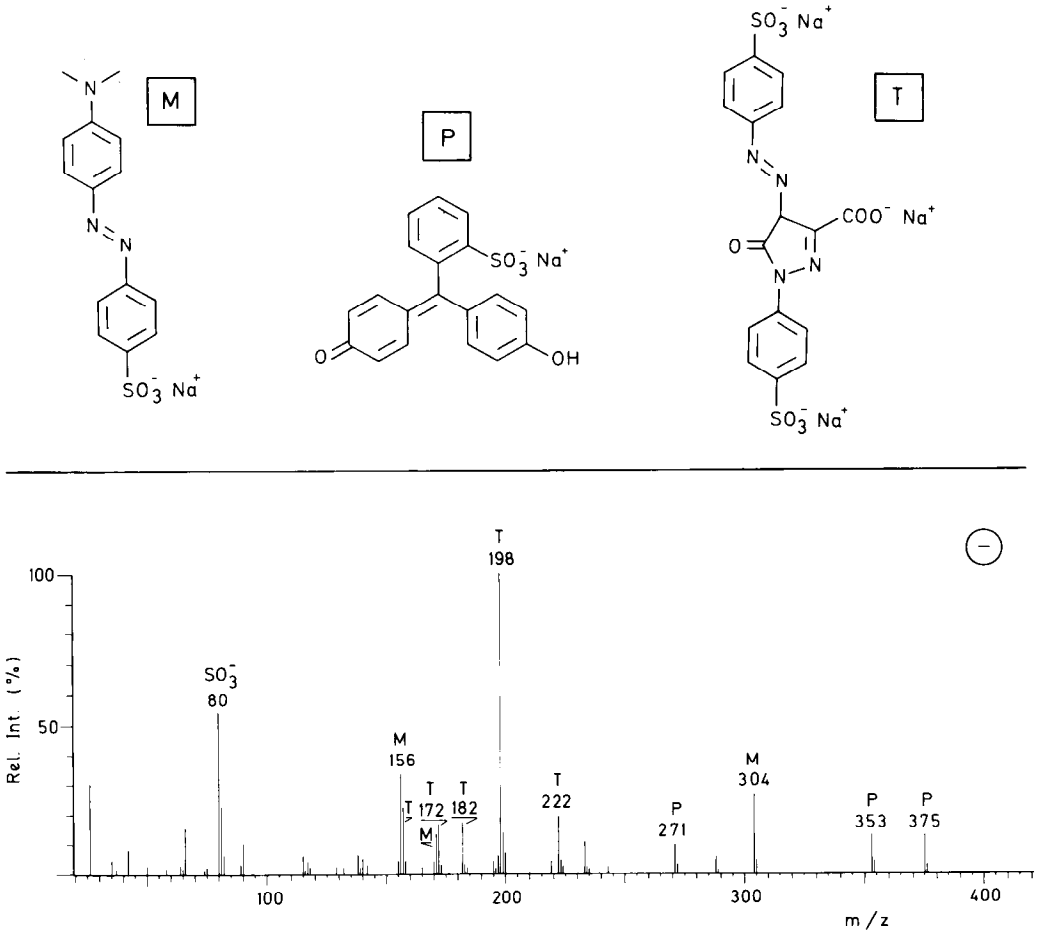

Fig. 6. LMMS for the characterization of simple organic mixtures. Typical negative ion detection mode spectrum of a sample containing Methyl Orange (*M*), Phenol Red (*P*) and Tartrazine (*T*)

highly localized in nature, but a total area of about 2–3 mm^2 is sampled randomly. The specimen, therefore, does not have to be homogeneous on a microscopic scale, because the results are averaged over a large area. Hence, PDMS allows a better assessment of the ion yields. The data are reported in detail elsewhere [45].

In conclusion, LMMS allows detection of the presence of a given compound by means of structurally relevant ions, down to the 20% level in organic mixtures. This seems disappointing but it has to be realized that no preseparation is involved, as opposed to current practice in organic MS. In gas chromatography-MS, detection of a compound in the presence of 5 and 10 co-eluting constituents is not trivial either. Obviously, the detection limit of 20% in LMMS is not a strict limit, and depends on the ion yield and interferences from other constituents in the mixture. Analysis of commercial tablets of aspirin, vitamin C, etc., has revealed that the diluting agents such as talc or amylose scarcely hinder characterization of the active product. Major components with a relatively low ionization efficiency reduce the number of shots needed to yield information on the substance of interest. This again illustrates how the range of application of LMMS depends on experimental options.

Depth Limitation for Analysis of Organic Compounds in Matrices

The reason for failure of initial attempts to locate drugs or metabolites in embedded tissues by means of structurally relevant ions has been associated with the complications resulting from interactions between the released targets and the co-desorbed constituents of the heterogeneous biological environment. It is readily conceivable that, in addition to the straightforward concentration problem, modification of the ion yield and formation or fragmentation mechanism by the other components leads to a very complicated situation, requiring a stepwise approach.

Model systems have been designed to study the relative importance of the different interactions separately. Embedding polymers as well as homogenized tissues are used for matrix simulation. The results pointed to the predominant role of surface-availability, whereas the expected interactions with co-desorbed constituents, e.g. cationization and recombination of fragments, proved negligible.

To define the thickness of the surface layer from which structurally relevant ions are generated, sandwich samples have been analysed, consisting of microcrystalline targets between two sections of matrix-simulating material. The total thickness is kept constant at 1 μm, and non-porous polymer films, namely 50 nm epon, 25 nm carbon foil and 5 nm formvar, are used as cover. Laser perforation is achieved with about 10 times more energy than normally used for particle analysis.

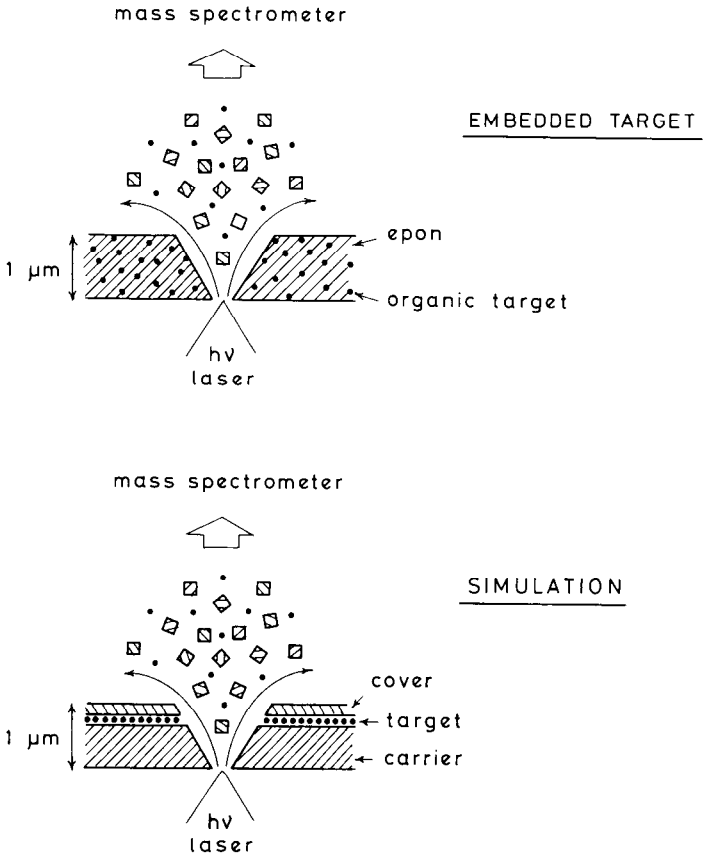

Fig. 7. Model sample simulation for the analysis of embedded targets by LMMS, and definition of carrier and barrier layers

The presence of even a very thin covering section drastically decreases the sensitivity of target detection, and the mass resolution, peak shape and calibration are severely degraded. This peak distortion is associated with time and/or space broadening of the ion-release through the leak orifice created in the upper layer. Targets present in the lower part of the section do not contribute to the organic ion current. In fact, as shown in Fig. 7, a homogeneously doped resin section can be considered as analytically equivalent to a carrier, which should not contain targets and serves only to absorb and dissipate energy, combined with a surface layer in which all the effectively measured targets are accumulated. In order to reach the detection limit, a concentration of 10% or more in the upper region is required, which is hardly realistic for most biological applications. More details are available [46].

It should be emphasized that these results concern a given test case. Successful microprobe applications in different matrices have been achieved. The porosity of the matrix plays an essential role, as shown by the analysis of microlichen cryosections [47]. The detection of crown ether complexes between silicate layers is favoured by the mineral nature of the matrix, which has a poor ionization yield under threshold conditions [48]. Hence, material consumption can be increased without overloading the detector. Intercalation is not a real problem since several layers are evaporated at once, permitting release of the cationized ligands.

The main conclusion is that LMMS essentially remains a surface-sensitive method, especially for organic ions and the threshold regime. In this context, results reported for matrix-assisted desorption deserve attention [49]. The term refers to the use of selected compounds which strongly absorb at the laser wavelength used, in order to increase the ion yield from an analyte with unfavourable desorption characteristics.

Inorganic Speciation: A Test Case

The experimental procedure for operation under threshold conditions serves not only for organic measurements but can also be profitable for inorganic analysis. It is found that single-particle analysis at low irradiation density improves speciation.

An on-going project on the volatilization losses and chemical transformations of $SnCl_2$ and Na_2SO_4 during heating in electrothermal graphite-furnace AAS, has produced the following problem. So far, explanations for the suppression and enhancement effects on the atomic Sn signal by the sulphate matrix are based on analysis of furnace residues and molecular absorption in the gas phase, complemented by thermodynamic data. However, molecular absorption bands are hard to assign, because of the virtual impossibility of obtaining adequate reference samples in the gas phase. Heating a given substance in the furnace always involves the risk of chemical transformation. Hence, there is a distinct need for positive identification of the gas phase constituents arising from volatilization or decomposition. We have exploited the merits of LMMS for dealing with microscopic sample amounts and simply collected aerosol samples by exposing a formvar-coated EM grid to the smoke emanating from the furnace [17]. It should be mentioned that the amount of product which can be obtained without risk of change in the graphite furnace surface properties excludes application of classical techniques.

Speciation of tin compounds in the presence of a 1000-fold ratio of sulphates is not feasible with LMMS. In practice, the tin salts and matrices are studied separately. The need to use high analyte concentrations limits the comparability of the atomization process with the normal analytical situation. Nevertheless, these criticisms do not affect the relevance of the laser microprobe data to assignment of molecular absorption bands.

LMMS analysis of aerosol samples at high-power laser density has yielded complex spectra, suffering from the variety of recombination clusters from Sn, N, O, C and S, and no conclusions have been possible. A solution has been found by application of the "organic procedure". The use of threshold conditions gives reproducible and simple spectra, related directly to the chemical nature of the major component. $SnCl_2$ and SnO or SnO_2 are measured in the negative-ion detection mode by means of the sensitive $SnCl_2 \cdot Cl^-$ adducts or the set of SnO_2^-, SnO^- and Sn^- ions. No diagnostic distinction is made between SnO and SnO_2. The interfering information from recombination with contaminants during DI in LMMS is eliminated. At the same time, the reproducibility is improved by limiting the material consumption. The removal of surrounding particles seems a major, but often underestimated, source of variability in LMMS.

It is observed that $SnCl_2$ volatilizes during heating, with less than 10–20% conversion into oxides. This holds true even in the presence of ammonia solutions, in which case most particles consist of ammonium chloride. SnO or SnO_2 is detected in the aerosols collected when a suspension of SnO_2 in water is heated in the furnace.

The positive LMMS results for aerosols from the Na_2SO_4 matrix are characterized by ions at *m/z* 149 and 165. With the sulphide, a prominent signal at *m/z* 101 is obtained, due to $Na_2S \cdot Na^+$, as verified by analysis of pure Na_2S. The clusters at *m/z* 149 and 165 are assigned to $Na_2SO_3 \cdot Na^+$ and $Na_2SO_4 \cdot Na^+$ respectively. The link to Na_2SO_4 seems obvious. However, analysis of reference compounds has revealed that these ions are almost negligible in comparison to Na^+ and Na_2O^+ for sodium sulphate or pyrosulphate. These peaks become prominent in the mass spectra of sodium pyrosulphite whereas sulphite leads to an intermediate situation. An aerosol of primarily $Na_2S_2O_5$ is logical in the context of the oxidation and reduction processes taking place in graphite furnace during heating. Nevertheless, the disagreement between the data obtained for $Na_2S_2O_5$ under threshold conditions and those reported for Na_2SO_4 and Na_2SO_3 [50] illustrates again the importance of careful evaluation of the practical methods of procedure in LMMS.

Conclusion

Owing to the remarkable flexibility for variation of local operating conditions, laser microprobe mass spectrometry is a valuable tool for inorganic and organic analysis. Element location and quantification on the μm scale can be achieved, and extensive speciation possibilities are available. LMMS is also suitable for structural characterization of organics by the combination of so-called soft ionization with extensive fragmentation.

The strength of the method arises from the ease of adapting the experimental conditions, e.g. power density for a given problem. In practice, however, this asset tends to become a weakness, since the general appearance and specific features of

LMMS results critically depend on the procedural details. This holds true for the basic parameters, e.g. resolution and calibration, and the fundamental aspects, e.g. major ion-formation mechanism, degree of fragmentation, etc. It is difficult to describe the local situation in terms of accessible parameters. This may ultimately lead to results which depend very much on the operator, and are hard to reproduce.

The proposed procedure defines the experimental conditions by directly available and generally applicable criteria. In practice, the reproducibility has been greatly improved. The guidelines permit unit mass resolution to be achieved, and external calibration up to 500 and 600 amu.

We have demonstrated that LMMS is promising for structural characterization of pure organic compounds. The results look unfamiliar in comparison to conventional MS, and interpretation requires introduction of new fragmentation routes. As a purely empirical approach, we have developed a tentative model for desorption and ionization to rationalize the final effect of the actual mechanisms, in terms of simple concepts.

Results for tryptamine-based analogues show the prominent role of radical molecular cations ($M^{+}\cdot$). The situation hardly changes when the corresponding hydrochlorides are analysed, reflecting a limited contribution from the intact release of preformed cations. The same trend is followed by triphenylphosphonium salts with polycyclic groups. Thermal degradation followed by formation of $M^{+}\cdot$ yields the predominant peak in the parent region. If LMMS is not "soft" for ionization, it certainly is for desorption. Molecular weight information is normally obtained, even for thermolabiles. Comparison of fragmentation with that of conventional direct-introduction probe electron-impact MS also points to intact release in LMMS. Structural data are complementarily distributed between positive and negative ions. Nucleotides can be analysed easily as the acids, not as the salts. The possible assets arising from the presence of preformed ions are not obvious.

Characterization of mixtures requires suitable powder samples with uniform particle-size distributions. Thin films are better, but their preparation is not always possible. LMMS results are interpreted as a yes-or-no answer about the presence of an individual target. The depth limitation is critical in organic microprobe experiments. The presence of a barrier layer covering the targets dramatically reduces the detection possibilities. LMMS is a surface-sensitive method. The thickness of the useful region is estimated as about 5 nm in a total of 1 μm. Considering the number of ions required to reach the detection limit, local concentrations of at least about 10% are mandatory. The *in situ* location of pigments in plant material and of crown ether complexes in silicates shows that the situation is improved by use of cryo-sections or mineral matrices.

The experimental procedure offers definitive advantages for inorganic studies. Analysis of graphite-furnace produced aerosols by use of the high power-density regime leads to complex spectra and confusing information, but under threshold conditions specificity is gained and the support needed to assign molecular absorption bands is obtained.

Model calculations may provide useful hints for solving the problems faced by the analytical chemist in using LMMS. The results allow refinement of concepts about the energy actually deposited in the ionization region, with respect to amount and redistribution mechanisms. Recent calculations have confirmed that a separate

description of the energy deposition and ionization processes is required for the high power-density regime and the typical conditions for laser desorption and organic analysis. However, a lot of further research will be necessary to fully elucidate the relationship between the macroscopically accessible parameters and the local ionization conditions on a microscopic scale.

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