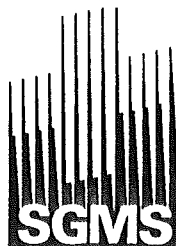


Swiss group for mass spectrometry
Schweizerische Gruppe für Massenspektrometrie



Groupe suisse de spectrométrie de masse
Gruppo svizzero di spettrometria di massa

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1991 Meeting and General Assembly

Hostellerie Rigi , November 7-8

PROGRAMME AND ABSTRACTS

This program has to be presented at the station in order to benefit of the special fare for the Rigi train or for the cable car (price : Frs 18.80)

Thursday, November 7

Chairman : W. Vetter

14.15 A.P. Bruins, University of Groningen
 "Instrumentation for Electrospray and Ion-Spray in Mass Spectrometry"

15.15 L.A. Savoy, M-Scan Ltd, Ascot
 "Electrospray Mass Spectrometry: a Users Perspective"

15.40 D. Waidelich, Uni. Tübingen
 "The Coupling of CZE to Ionspray Mass Spectrometry"

16.00-16.30 PAUSE

Chairman : U. Schlunegger

16.30 K. Jennings, University of Warwick
 "Tandem Mass Spectrometry: Fact, Fiction and Prejudice"

17.30 D. Evard, Spectrospin AG, Fällanden
 "Matrix Assisted Laser Desorption in Combination with FTMS and TOF"

17.50 PAUSE

18.05 GENERAL ASSEMBLY

19.00 Apertif , Dinner

Friday, November 8

Chairman : W. Richter

9.15 K. Rose, Biochimie Médicale, CMU, Genève

" Pharmacokinetics of Polypeptides and Proteins Using Mass Spectrometry: Dream or Reality?"

10.15 H. Reinhard, Inst. für Org. Chemie, Bern
 " The Quistor as a Reaction Chamber for Gas Phase Ion/Molecule Reactions"

10.35-11.00 PAUSE

Chairman : L. Rivier

11.00 W. Vetter, Hoffmann La Roche, Basel

"Mechanistic Studies on the Fragmentation of the Aliphatic Chain in Derivatized Fatty Acids and Macrocyclic Ketones"

11.20 G. Zhao, ICP-I , E.P.F.-Lausanne

" Structure of $C_7H_8^+$ and $C_7H_7^+$ Ions"

11.40 S. Schürch, Inst. für Org. Chemie , Bern

" Halocarbenes as Diagnostic Tools for Mass Spectrometrical Localisation of Double-Bonds "

12.00 F.Friedli, MSP Friedli & Co.,Köniz

" Networking Computers Used in Mass Spectrometry "

INSTRUMENTATION FOR ELECTROSPRAY AND IONSpray MASS SPECTROMETRY

A.P. Bruins, University Centre For Pharmacy
A. Deusinglaan 2, 9713 AW Groningen, The Netherlands

The exciting possibility of recording mass spectra of biopolymers such as proteins, glycoproteins, nucleic acids, etc. by electrospray in combination with a simple quadrupole mass analyzer, has spurred the development of new instruments by manufacturers and research groups. The prospective user is now confused by seemingly different solutions for the same problems.

Electrospray (ionspray) mass spectrometry is technically divided into 4 steps :

1. The generation of a charged aerosol from a sample solution either by electrospray in its simplest form, or with the aid of a coaxial sheath flow of a solvent with good spray characteristics such as methanol or with the aid of pneumatic assistance (ionspray). Electrospray and ionspray take place at atmospheric pressure. The dispersion of a liquid by electrospray requires a strong electric field at the tip of the spray capillary. Care must be taken however to avoid a corona discharge from the capillary by too strong an electric field.
2. Liberation of desolvated sample ions from solution into the gas phase, which is promoted or suppressed by a particular composition of the sample solution. A warm ion source promotes the evaporation of solvents, and is advantageous for the handling of aqueous solutions.
3. Introduction of sample ions from the atmospheric pressure region through an orifice into the vacuum system. A gas expanding into vacuum undergoes strong cooling with formation of big cluster ions by the condensation of solvent vapor (notably water) on sample ions. The introduction of solvent vapor into the vacuum is prevented by a counter current flow of dry nitrogen through the entire source or by a gas curtain just in front of the ion sampling orifice. Alternatively, the supply of heat may be used to prevent or cure the problem of cluster ion formation.
4. Adequate removal of gas, but efficient transportation of sample ions from the expanding beam into the mass analyzer, by the use of sampling nozzle,

skimmer, ion optics and vacuum pumping in one or more stages. In an instrument having one pumping stage, ions and gas expand directly into the analyzer section of the instrument. The pressure in the analyzer must be kept low enough to ensure proper operation of the mass analyzer. A high sensitivity is possible at a high throughput of gas and ions, which is achieved by high speed cryogenic pumping (SCIEX). All other manufacturers make use of multiple stage vacuum pumping systems, equipped with a rotary pump and turbomolecular or diffusion pumps. Without going into details and using a simple approximation, one can show that a well pumped multiple stage instrument can be equally sensitive as a single stage instrument.

The ion currents measured for the base peak in an electrospray mass spectrum are of the order of a million ions per second or less. Fundamental limitations in ion statistics preclude the acquisition of good quality mass spectra at 0.1 a.m.u. intervals over a wide mass range at a high scan speed. Mass measurement to the nearest 0.1 m/z value takes time, either via a slow scan, or via the accumulation of multiple scans.

ELECTROSPRAY MASS SPECTROMETRY: A USERS PERSPECTIVE

L.A. Savoy, M-Scan Ltd, Ascot, England

Some practical aspects of electrospray mass spectrometry (ESMS) will be presented including :

- The use of ESMS to aid the characterization of high molecular weight recombinant proteins (purity, S-S bridges, etc...)
- The use of ESMS as a tool for quantitative analysis of a toxin in water.
- The use of ESMS for the analysis of low molecular weight compounds.

THE COUPLING OF CZE TO IONSPRAY MASS SPECTROMETRY

Dietmar Waidelich, Ernst Bayer - Universität Tübingen

Capillary zone electrophoresis (CZE) offers some advantages in separation technique: high separation potentials, low sample amounts, and short running times. Its coupling to mass spectrometry offers the possibility to analyze the eluents with a highly specific detection method. Pneumatically assisted electrospray, also called ionspray, enables an easy way of coupling.

Experiments of CZE/MS coupling must regard to two basic conditions of CZE technique which are independent on what kind of mass spectrometry and interface system is used: the small injection volume of the samples in the low nl range according to a total sample amount in the low picomole or femtomole range and the CZE buffers which are transported by the electroosmotical flow to the ionization place and may influence the obtained MS spectrum of the investigated sample. This influence of CZE buffer may be (1) the presence of sometimes strong background MS signals, (2) a change or further presence of quasi-molecular ions (i.e. sodiated instead of protonated), and (3) an effect on signal intensity of the analyte.

Representative examples of CZE/ionspray MS are shown demonstrating the potentials and limitations of this new analytical method.

TANDEM MASS SPECTROMETRY- FACT, FICTION AND PREJUDICE

Keith R. Jennings, Department of Chemistry,

University of Warwick, Coventry, CV4 7AL, UK

A tandem mass spectrometer consists essentially of two mass spectrometers in series separated by a collision cell. In the most frequently-used mode of operation, the first mass spectrometer (MS1) is used to select an ion of chosen mass-to-charge ratio, m_1^+ , which then undergoes collision-induced dissociation within the collision cell which contains a low pressure of collision gas such as helium or argon. The second mass spectrometer, MS2, is scanned to give a mass spectrum of the fragment ions arising from the chosen precursor ions. Other modes of operation allow one to obtain a mass spectrum of all product ions formed by the loss of a neutral species of chosen mass.

Applications of tandem mass spectrometry fall under two general headings.

- (1) Structure elucidation, eg. the obtaining of structural information from the fragment ions formed in the collision-induced decomposition of MH^+ ions of a compound for which a soft ionisation technique gives very few fragment ions directly.
- (2) The identification of a component in a mixture without prior separation of the components by, eg. chromatographic methods. The fragment ion spectrum of the chosen precursor ion is compared with that of an authentic sample in order to demonstrate the presence or absence of the component of interest in the mixture.

Examples of each type of application based both on work at Warwick and from the literature will be presented.

Several types of instrumentation are now available for tandem mass spectrometry, the most commonly-used being the following.

- (1) Various linked-scans of two-sector magnetic sector instruments.
- (2) Triple quadrupole instruments.
- (3) Four sector magnetic sector instruments

- (4) Hybrid instruments, eg. a two-sector magnetic sector instrument coupled to two quadrupoles.
- (5) FT ICR instruments
- (6) Ion Trap instruments

Each type of instrumentation has its own characteristics: (1) and (3) employ high collision energies (eg. several keV) whereas the remainder employ low collision energy (< 100 eV). The precursor ion can be selected at high mass resolution in (3) and (4), unit mass resolution in (2), (5) and (6) and relatively poor mass resolution in (1). Only (2) allows better than unit mass resolution for the fragment ion spectrum. Helium has normally been used as collision gas for high energy collisions (and more recently in (6)) whereas argon is normally used for low energy collisions. The useful upper mass range depends to some extent on the type of problem but currently, the upper mass limit of m/z 2 500-3 000 reflects the inefficiency of the collisional activation process rather than of the initial ionisation process.

The spectra produced by the various instrumental techniques differ considerably and even different experimental conditions give different spectra from the same instrumentation. One therefore has to ask questions such as the following.

- (1) Is there a "best" type of instrumentation ?
- (2) Are there readily identifiable "best" conditions to work with for an unknown sample ?
- (3) What mass resolution is required in solving most problems ?
- (4) How reproducible are the spectra ?
- (5) Are the sensitivities of the different methods similar ?

In formulating the answers, it is important to distinguish between fact, fiction and prejudice - not always straightforward from reading the literature !

MATRIX ASSISTED LASER DESORPTION IN COMBINATION WITH FTMS AND TOF

D. Evard, Spectrospin AG, Fällanden

Matrix Assisted Laser Desorption (MALD) is a technique which is applicable for the desorption and ionization of biological molecules. The choice of detection method depends upon the specific molecules under investigation and the desired information which is to be obtained. Fourier Transform Mass Spectrometry (FTMS) provides high resolution and mass accuracy for the detection of ions up to 15,000 amu. Time of Flight (TOF) mass spectrometry has proven to be an excellent method for the detection of high mass biological compounds, up to $m/z = 300,000$ but is limited in resolution.

The Bruker APEX 47e FTMS spectrometer with an external ion source can be used to study biological molecules using MALD desorption and ionization. The advantages of FTMS, namely high resolution and mass accuracy, as well as MS/MS capabilities make this a very powerful technique for the characterization of biological systems such as proteins and peptides. The standard external ion source was modified slightly to allow the use of MALD ionization technique. Results are shown which demonstrate the high resolution capability of FTMS instrument on such samples as gramicidin S and D, and angiotensin.

The technique of MALD-TOF is also very important in the study of high molecular weight biological compounds. Experiments on the Bruker MALD-TOF apparatus have focussed on effects which limit the mass resolution and accuracy. Results will be presented which illustrate the effects of ion energy, the use of the reflectron and postacceleration in the ion detector. Cytochrome C, m/z 12,360 has been observed with a resolution of 1,200. Clusters of bovine albumin have been measured up to m/z 266,000 which represents $[4M+H]^+$. Further work is in progress both in the areas of MALD-TOF and MALD-FTMS and it is clear that exciting developments in both fields will result from this work.

PHARMACOKINETICS OF POLYPEPTIDES AND PROTEINS USING MASS SPECTROMETRY: DREAM OR REALITY?

Keith Rose, Département de Biochimie Médicale, Centre Médical Universitaire, 11 rue Michel Servet, 1211 Geneva 4, Switzerland

Over one hundred polypeptides and proteins are either already in use in the clinic or are being intensively studied with a view to clinical application, and this number is likely to increase substantially over the next few years. However, although the pharmacokinetics of protein drugs in animals has made progress, mainly through the use of radioactively labelled molecules, there is understandable concern regarding the use of radioisotopes in Man for purely investigational purposes. If stable isotopes are to be used successfully in this field, then means must be found (i) to synthesise polypeptides and proteins specifically labelled with an appropriate isotope, (ii) to extract in good yield tiny amounts of polypeptide from complex biological matrices such as blood plasma and (iii) to achieve qualitative and quantitative analysis by mass spectrometry with adequate sensitivity. The challenge is not trivial, but the prize (a better understanding of protein drugs, and perhaps discovery of fragments having biological activity) is great. The field will be reviewed, and our attempts to solve problems (i)-(iii) presented.

THE QUISTOR AS A REACTION CHAMBER FOR GAS PHASE ION/MOLECULE REACTIONS

H.Reinhard, P.Kofel, S.Konig, M.Sägesser and U.P.Schlunegger

Institut für organische Chemie, Universität Bern, Freiestrasse 3, 3012 Bern

Our recently built Quadrupole, Quistor (quadrupole ion store), Quadrupole tandem mass spectrometer allows for mass selected ion injection into the Quistor. Ions are trapped by the combined action of damping collisions with helium gas and the Quistor RF field applied to the ring electrode. The reaction gas is introduced by means of a capillary into the Quistor acting as a reaction chamber. A series of experiments can then be performed over a wide range of pressure and storage times. After the reaction time all products are extracted and mass analyzed. Mass scanning and data acquisition are performed by a TMS320C25 board in a personal computer.

The reaction between selectively injected protonated acetaldehyde and neutral methanol was studied in the Quistor to investigate the pressure dependence of the products built.

MECHANISTIC STUDIES ON THE FRAGMENTATION OF THE ALIPHATIC CHAIN IN DERIVATIZED FATTY ACIDS AND MACROCYCLIC KETONES.

W. Vetter, Hoffmann La Roche, Basel

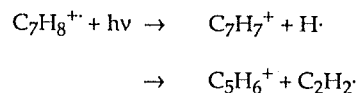
The EI-mass spectra of the methyl esters of fatty acids are remarkably similar to the spectra of the ethylene ketals of macrocyclic ketones. Even more similarity is observed between the spectra of the reaction products of 2-hydrazinopyridine with fatty acids and with macrocyclic ketones.

Based on these observations and in view of the fact that some derivatives of fatty acids are well suited for the deduction of structural features of the chains, the mechanism of the fragmentation was studied.

STRUCTURES OF $C_7H_8^+$ AND $C_7H_7^+$ IONSGuohong Zhao and Tino Gäumann

Institut de Chimie Physique, Ecole Polytechnique Fédérale de Lausanne
CH-1015 Lausanne

Photodissociation is a powerful tool for the determination of the structure and a possible isomerization of trapped ions in FT-ICR. The photodecomposition of toluene, cycloheptatriene, and norbornadiene ions is measured at wavelengths in the visible. The structures of $C_7H_8^+$ and $C_7H_7^+$ ions generated from the three parent neutrals are identified by photodissociation kinetics. In the case of the toluene and the cycloheptatriene, two isomer $C_7H_8^+$ cations that fragment in the visible range by loss of a hydrogen atom are assigned to a toluene and a cycloheptatriene structure respectively. Two competitive fragmentation pathways are observed for the norbornadiene molecular cation:



The main photofragmentation reaction of these three excited molecular cations leads to at least two distinct product $C_7H_7^+$ ions that are identified with the help of ion-molecule reactions: one reacting with the parent or other neutrals corresponds to a species assigned to have the benzyl structure; the other is totally unreactive and corresponds to the tropylium ion. The reaction mechanisms for the benzyl ion are explained. The mechanisms are dependent on the structure of the neutral, but independent on the benzyl ions produced from the different precursors.

HALOCARBENES AS DIAGNOSTIC TOOLS
FOR MASS SPECTROMETRICAL
LOCALISATION OF DOUBLE-BONDS.S. Schürch, M. Howald, H. Gfeller and U.P. Schlunegger

Institute of Organic Chemistry, University of Berne,
Freiestr. 3, CH-3012 Berne, Switzerland

Different halogen cyclopropanes have been prepared by addition of halocarbenes to olefins by phase transfer reactions and analyzed by GC-MS. In order to determine the position of the double bond in the molecules, the following topics have to be fulfilled: simple fragmentation and clearly recognizable isotopic patterns in the mass spectrum.

Several halocarbene olefin adducts have been checked. Fragmentation pathways, reactions yields and diagnostic potentials have been evaluated.

The bromo fluoro cyclopropanes were considered to be the most promising derivatives. The results and the spectra of a series of these adducts will be discussed.

NETWORKING COMPUTERS USED IN MASS SPECTROMETRY

E. Friedli, MSP Friedli & Co., Köniz

Various computers running different mass spectrometry data systems require quite different network solutions. Unexpected pitfalls arise out of the fact that the evolution of computer software is uncoordinated and unpredictable.