Recent Developments in Ionization Methods for Mass Spectrometry: An Overview and Prospects

Presented at the 19th Meeting of the ANZSMS
February 2 – 6, 2003
Lorne, Victoria, Australia

Gary J. Van Berkel, Ph.D.

Organic and Biological Mass Spectrometry Group
Chemical Sciences Division, Oak Ridge National Laboratory
Oak Ridge, TN 37831-6131
phone: (865) 574-1922
website: http://www.ornl.gov/csd or email: vanberkelgj@ornl.gov

Ionization Methods Discussed

- Restricted to Organic and Biological Analysis
- Selected from publications over last 2 years
- Subjective choices made

Major Categories:

- Electron Ionization
- Chemical Ionization
- Atmospheric Pressure Plasma Ionization
- Laser Desorption Ionization
- Electrospray Ionization

Electron Ionization

- Field Emitters

- LC/MS Coupling

Electron Ionization - Field Emitters

Robust electron source in trend toward miniature mass spectrometers for field and space applications

- **Higher pressure operation**
- **Low power consumption**
- **Associated savings in weight and space**

Electron Ionization - Field Emitters

J. Vac. Sci. Technol. B 17(5), Sep/Oct 1999

Cold cathode field emitter array on a quadrupole mass spectrometer: Route to miniaturization

T. E. Felter[a)]
Sandia National Laboratories, Livermore, California 94550

J. Vac. Sci. Technol. B 19(3), May/Jun 2001

Field emitters for space application

S. E. Huq,[a)] B. J. Kent, R. Stevens, and R. A. Lawes
Rutherford Appleton Laboratory, Chilton, Didcot, Oxfordshire OX11 0QX, United Kingdom
N. S. Xu and J. C. She
Department of Physics, Zhongshan University, Guangzhou 510275, People's Republic of China

Anal. Chem. **2000**, *72*, 559 562

Field-Emission Cold-Cathode EI Source for a Microscale Ion Trap Mass Spectrometer

Oleg Kornienko, Peter T. A. Reilly, William B. Whitten,* and J. Michael Ramsey
Oak Ridge National Laboratory, P.O. Box 2008, Oak Ridge, Tennessee 37831-6142

Electron Ionization –Field Emitters

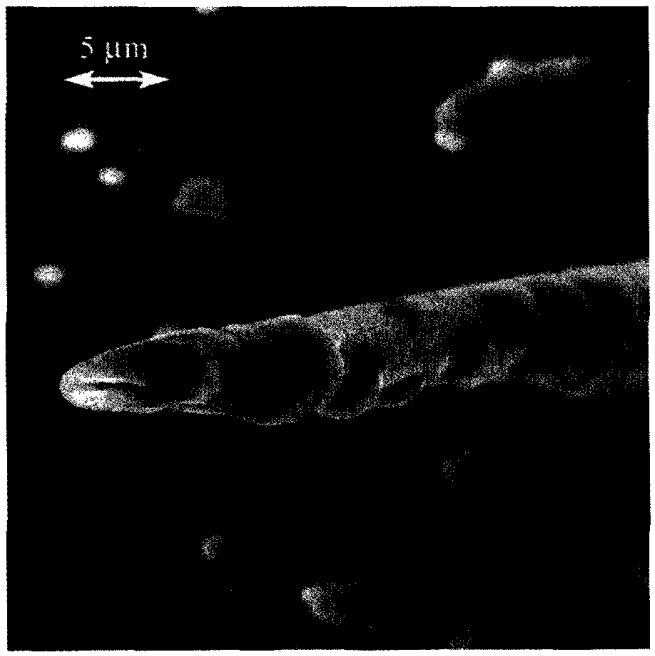


Figure 1. Scanning electron micrograph of a diamond-coated whisker.

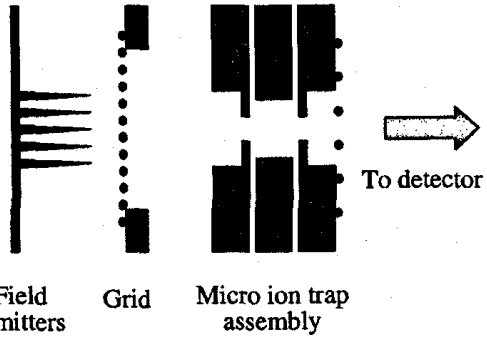


Figure 2. Microscale ion trap mass spectrometer assembly.

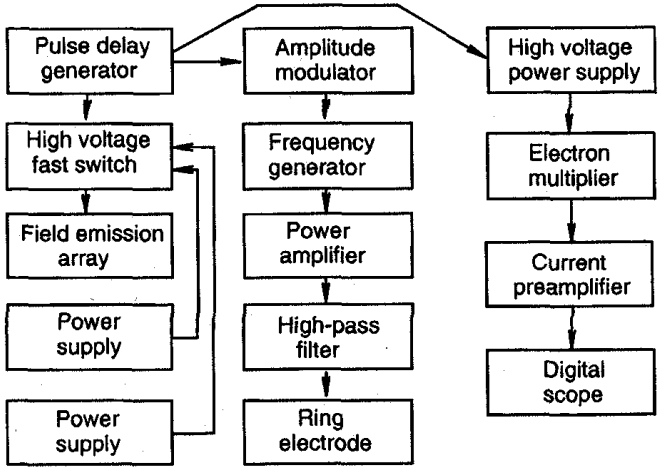


Figure 3. Block diagram of the electronics circuitry.

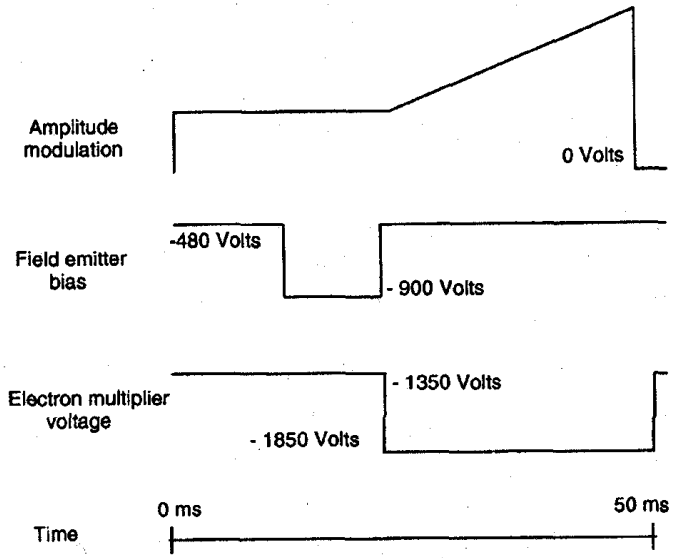


Figure 4. Timing diagram for a typical mass scan.

Electron Ionization –Field Emitters

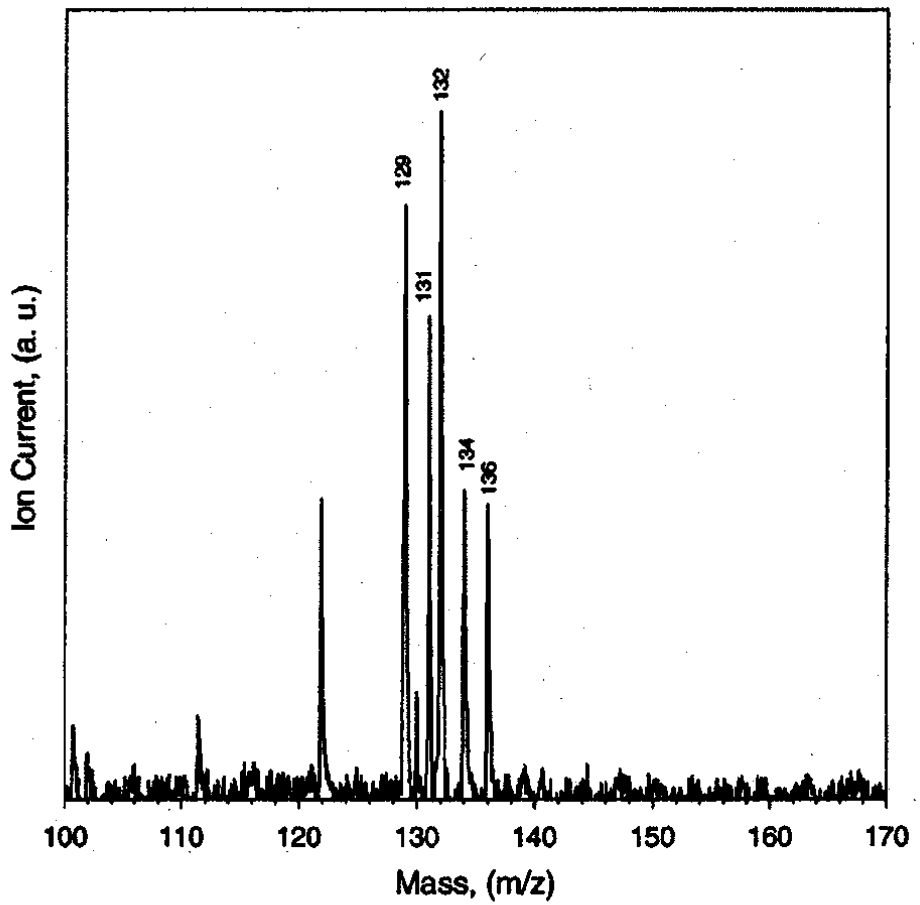


Figure 6. Single-scan mass spectrum of xenon.

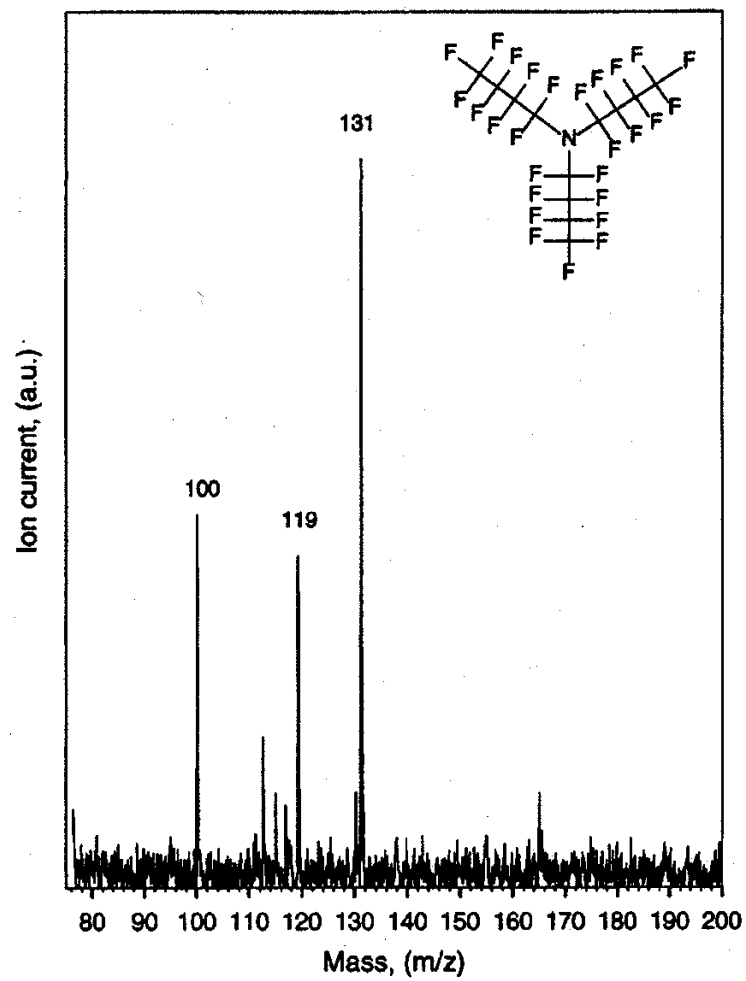


Figure 7. Single-scan, partial mass spectrum of PFTBA.

Electron Ionization - LC/MS Interfacing

Alternative ionization methodology to atmospheric pressure ionization (i.e., APCI or ES) for low to medium molecular weight compounds

- library searchable spectra -

Mass Spectrometry Reviews, **2001**, *20*, 88–104

NEW TRENDS IN THE APPLICATION OF ELECTRON IONIZATION TO LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY INTERFACING

Achille Cappiello, Giorgio Famiglini, Filippo Mangani, and Pierangela Palma
Istituto di Scienze Chimiche, Università di Urbino, Piazza Rinascimento 6, 61029 Urbino, Italy

Received 30 January 2001; received (revised) 16 April 2001; accepted 17 April 2001

I. Introduction . . . 88
II. Cap-EI Interface . . . 90
A. Interface Performance . . . 92
III. Direct-EI Interface . . . 93
A. Interface Performance . . . 97
IV. Conclusions . . . 103
Acknowledgments . . . 103
References . . . 103

Electron Ionization - LC/MS Interfacing

Cap-EI is a particle beam system optimized for flow rates in the 1.0 µL/min range

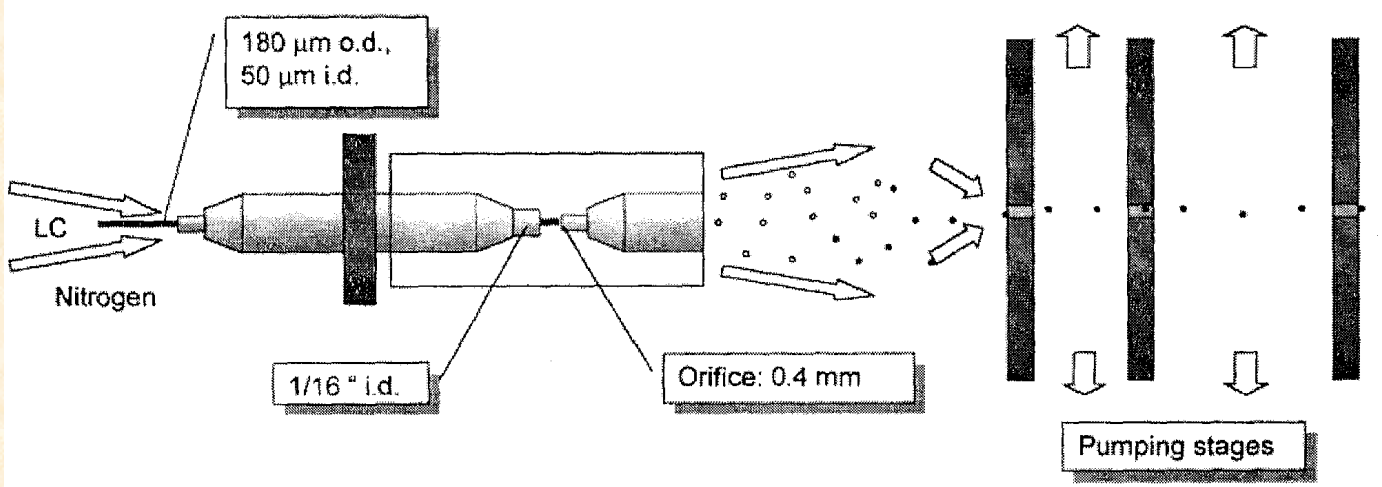


FIGURE 1. Scheme of the Cap-EI nebulizer.

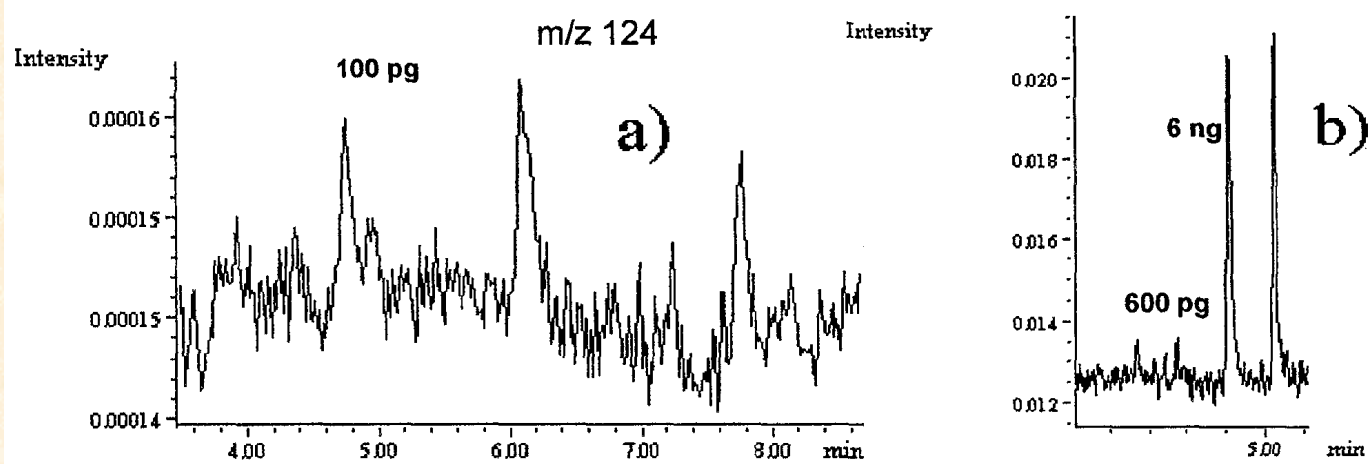


FIGURE 4. (a) *m/z* 124 ion profile relative to three injections of 100 pg of testosterone in the SIM mode. (b) Mass chromatogram relative to two injections of 600 pg and two injections of 6 ng of testosterone in the scan mode.

Electron Ionization - LC/MS Interfacing

- Direct-EI similar to older inlets of the same type
- Takes advantage of robustness of modern HPLC at or below flow rates of 1.0 µL/min
- More sensitive than particle beam approach

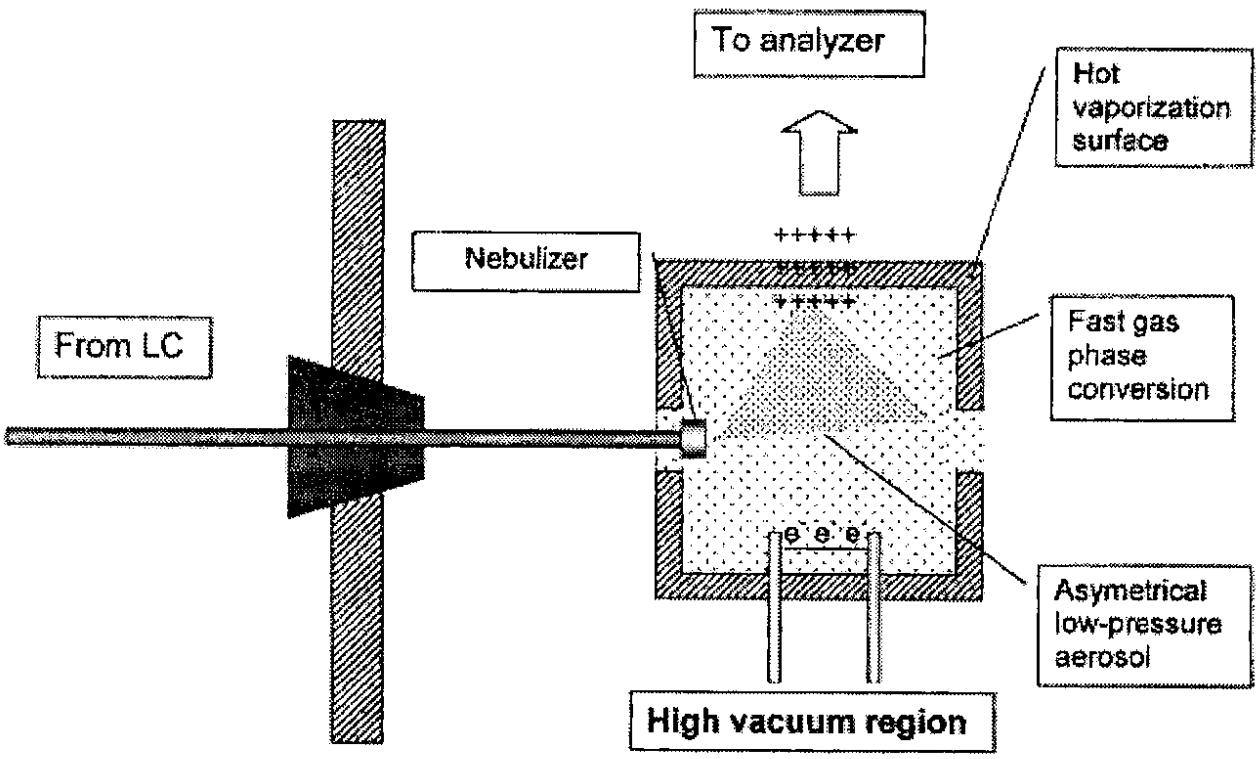


FIGURE 11. Scheme of the Direct-EI interface.

Electron Ionization - LC/MS Interfacing

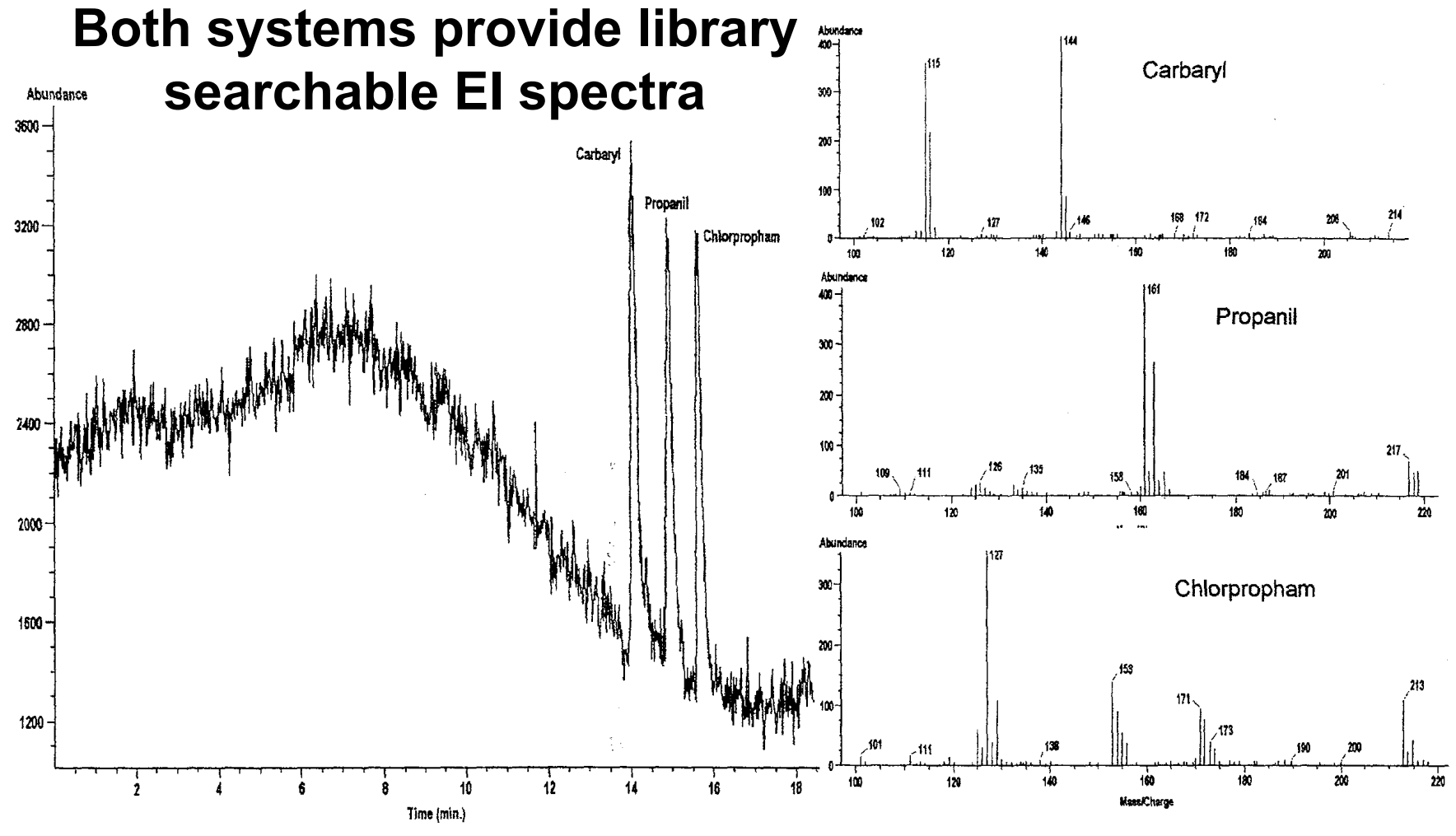


FIGURE 17. Full spectrum profile relative to the separation of three pesticides, carbaryl, propanil, and chlorpropham. The separation was obtained in gradient condition using a reversed phase, C18 15 cm × 75 μm i.d., 3 μm particle size nano-column. The flow rate was 300 nL/min, and the mobile phase composition was changed from 10 to 80% acetonitrile in water in 20 min.

Chemical Ionization

- Electron Capture
- Desorption Chemical Ionization
- Selected Reagent Ion Chemical Ionization

Chemical Ionization - Electron Capture

Journal of Chromatography A, 892 (2000) 329–346

Review

Electron–capture mass spectrometry: recent advances

Roger W. Giese*

Department of Pharmaceutical Sciences in the Bouvé College of Pharmacy and Health Professions, Barnett Institute and Chemistry Department, Northeastern University, 122 Mugar Building, Boston, MA 02115, USA

Tunable electron energy provides added selectivity of ionization by electron capture

Anal. Chem. **2000,** *72,* 2428–2432

Electron Monochromator Mass Spectrometry for the Analysis of Whole Bacteria and Bacterial Spores

Michael B. Beverly and Kent J. Voorhees*

Chemistry and Geochemistry Department, Colorado School of Mines, Golden, Colorado 80401

Ted L. Hadfield

Armed Forces Institute of Ptahology, Building 54, CPS-m, Washington D.C. 20306

Robert B. Cody

JEOL USA, Inc., 11 Dearborn Road, Peabody, Massachusetts 01960

Chemical Ionization - Electron Capture

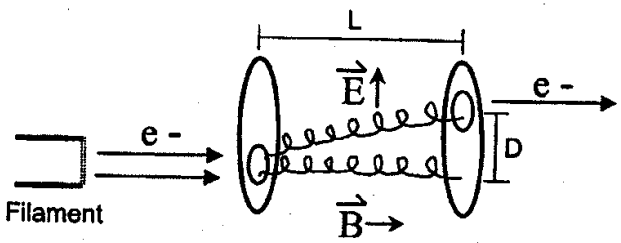


Figure 1. Electron energy selection principle.[21] Electrons (e^-) have different trochoidal paths in the plane parallel to the magnetic field (*B*). The drift velocity of the electron allows control of the electron's energy via the electron's time-of-flight in the electric field (*E*) and magnetic crossed field ($E \times B$) region of length (*L*). The electron drifts in a direction perpendicular to both fields to produce a drift distance of *D*. Electrons of a given energy are selected by having offset entrance and exit openings.

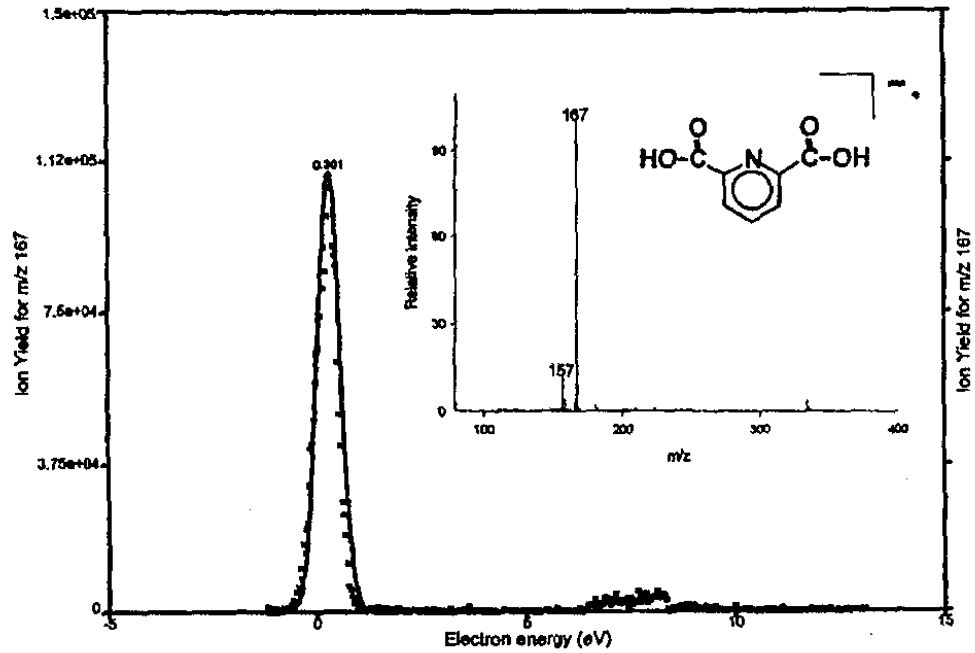


Figure 2. Electron energy scan of dipicolinic acid. The electron capture energy (eV) for dipicolinic acid that produces the greatest intensity of $M^{\bullet-}$ ions at *m/z* 167 occurs at 0.301 eV. The inset shows the ECNIMS spectra of a standard of dipicolinic acid with the $M^{\bullet-}$ ion at *m/z* 167.

Chemical Ionization - Electron Capture

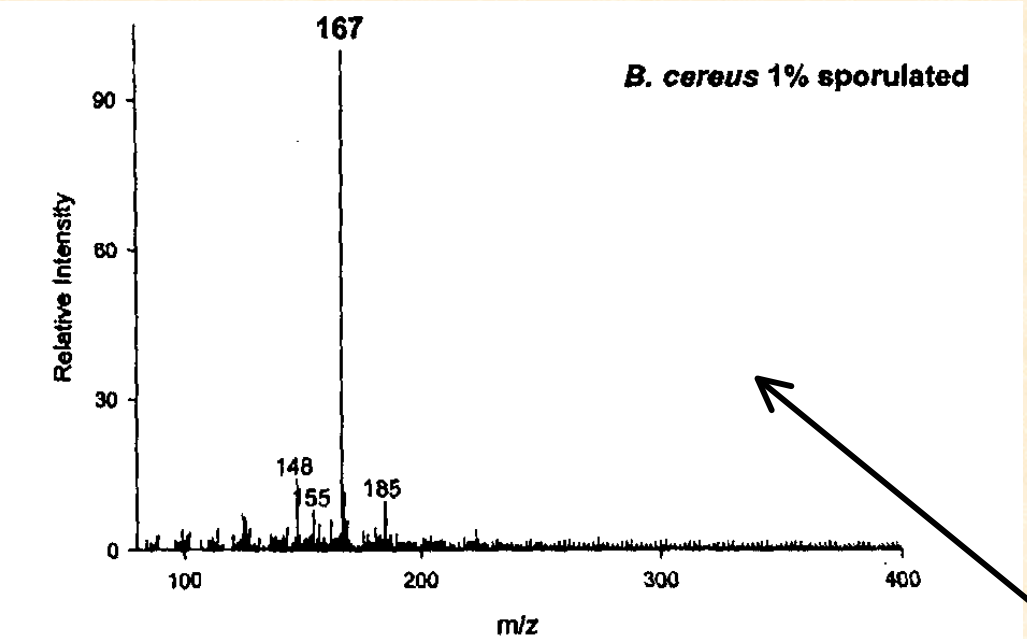


Figure 4. The 1% sporulated sample shown in Figure 3 analyzed with the monochromator tuned to the electron capture energy of dipicolinic acid, 0.301 eV.

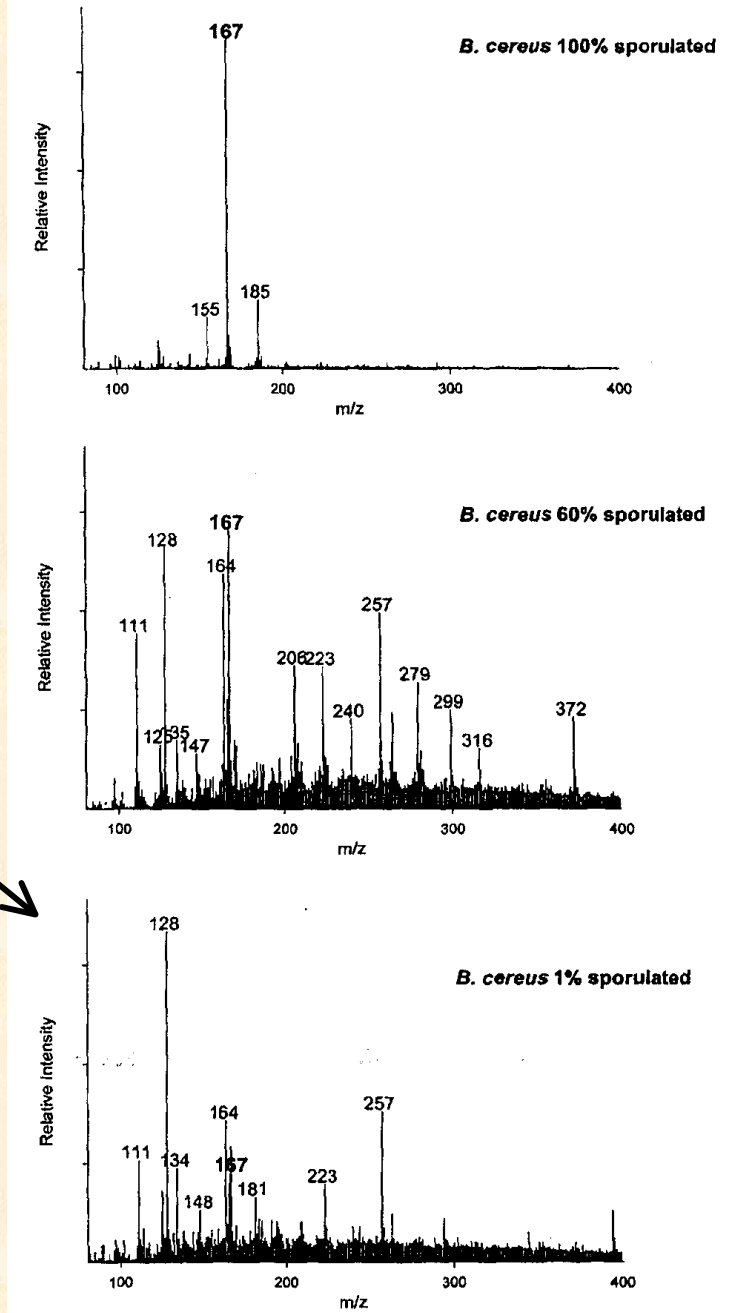


Figure 3. Standard ECNIMS spectra of samples of *B. cereus* at various degrees of sporulation.

Tuned electron energy improves S/N at low analyte levels because of selective ionization

Desorption Chemical Ionization

International Journal of Mass Spectrometry 212 (2001) 505–518

The renaissance of desorption chemical ionization mass spectrometry: characterization of large involatile molecules and nonpolar polymers

Marco Vincenti

Dipartimento di Chimica Analitica, Università di Torino, Via Pietro Giuria, 5 - 10125 Torino, Italy

- **Otherwise known as direct-CI, direct exposure CI, in-beam CI**
- **No real renaissance**
- **Substantial capabilities forgotten**

Among the positive aspects of the technique, the following should be mentioned. DCI-MS is: (1) fast, as it allows to carry out as much as 20–30 analysis per hour; (2) sensitive, as one nanogram of material is generally sufficient to record a good spectrum; (3) clean, since minimal sample loading prevents the contamination of the mass spectrometer; (4) cheap, since it requires minimal investment and inexpensive consumables; (5) noise-free; (6) easy-to-use, and (7) widely applicable.

Desorption Chemical Ionization

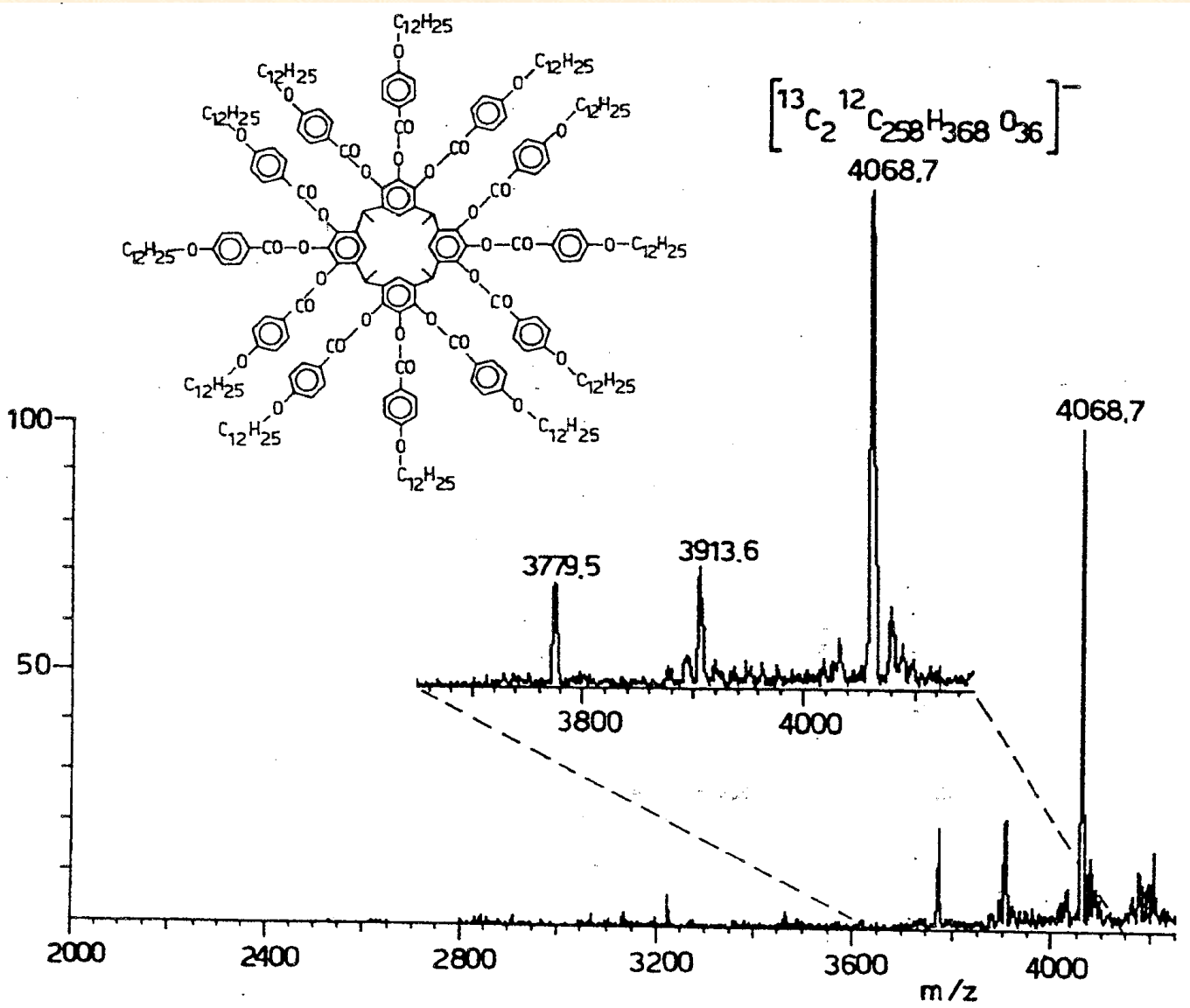


Fig. 1. Electron-capture (negative-ion) DCI mass spectrum of a macrocycle having the chemical formula $C^{260}H^{368}O^{36}$, recorded at 90 °C ion-source temprature. Reprinted with permission of Heyden & Son Limited from Rapid Communications in Mass Spectrometry 3 (1989) 106.

Selected Reagent Ion CI

- CI mass spectrum is dependent on the reagent ion distribution
- Alteration of reagent ion distribution or selection of one particular reagent allows precise control of reaction type and reaction energetics

Anal. Chem. **2000**, *72*, 5055–5062

High-Pressure Ion Source Combined with an In-Axis Ion Trap Mass Spectrometer. 1. Instrumentation and Applications

J. C. Mathurin, T. Faye, A. Brunot, and J. C. Tabet*

Laboratoire de Chimie Structurale Organique et Biologique, CNRS UMR 7613, Université Pierre et Marie Curie, 4 place Jussieu Boite 45, 75252 Paris Cedex 05, France

G. Wells

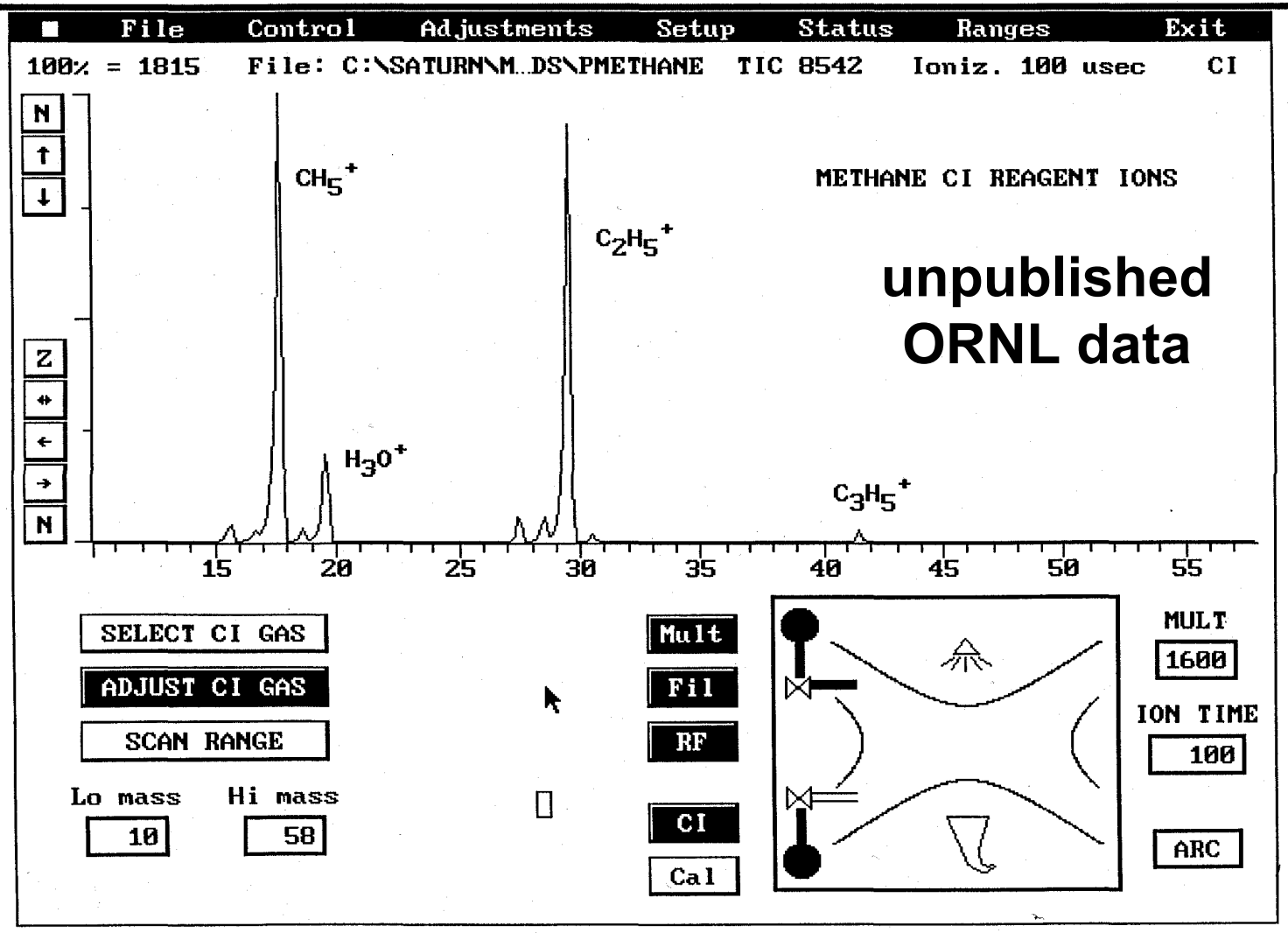
Varian Chromatography Systems, 2700 Mitchell Drive, Walnut Creek, California 94598

C. Fuché

CREL, Ministère de l'Intérieur, 168 route de Versailles, 78150 Le Chesnay, France

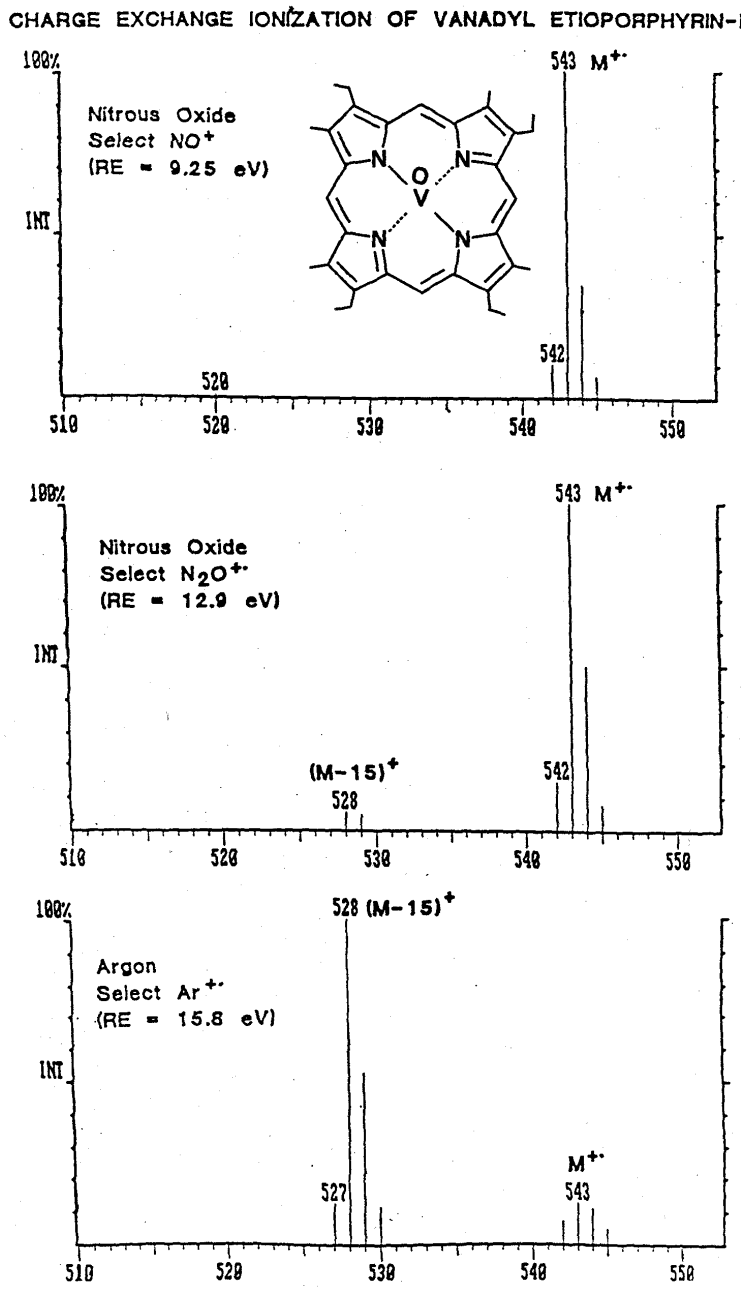
Selected Reagent Ion CI

Methane CI Reagent Ions



unpublished ORNL data

Selected Reagent Ion CI



$$M + X^{+\cdot} \rightarrow M^{+\cdot} + X$$
$$\Delta H = IE(M) - RE(X^{+\cdot})$$

reaction exothermicity increases

unpublished ORNL data

Atmospheric Pressure Plasma Ionization

- Microwave Induced Plasma Ionization (AP-MIPI)
- Dual Atmospheric Pressure Chemical Ionization (APCI)/Electrospray (ES) Ionization
- Atmospheric Pressure Photoionization (APPI)

Microwave Induced Plasma Ionization

- Alternative to APCI or ES for low to medium molecular weight compounds
- Good buffer tolerance

Atmospheric Pressure Microwave Induced Plasma Ionization Source for Molecular Mass Spectrometry

(J Am Soc Mass Spectrom 1998, 9, 42–49)

Mehdi Moini, Ming Xia, James B. Stewart, and Bernhard Hofmann
Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, Texas

Analysis of Underivatized Amino Acid Mixtures using High Performance Liquid Chromatography/Dual Oscillating Nebulizer Atmospheric Pressure Microwave Induced Plasma Ionization-Mass Spectrometry

(J Am Soc Mass Spectrom 2001, 12, 117–122)

Jun-Young Kwon and Mehdi Moini
Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, Texas, USA

Microwave Induced Plasma Ionization

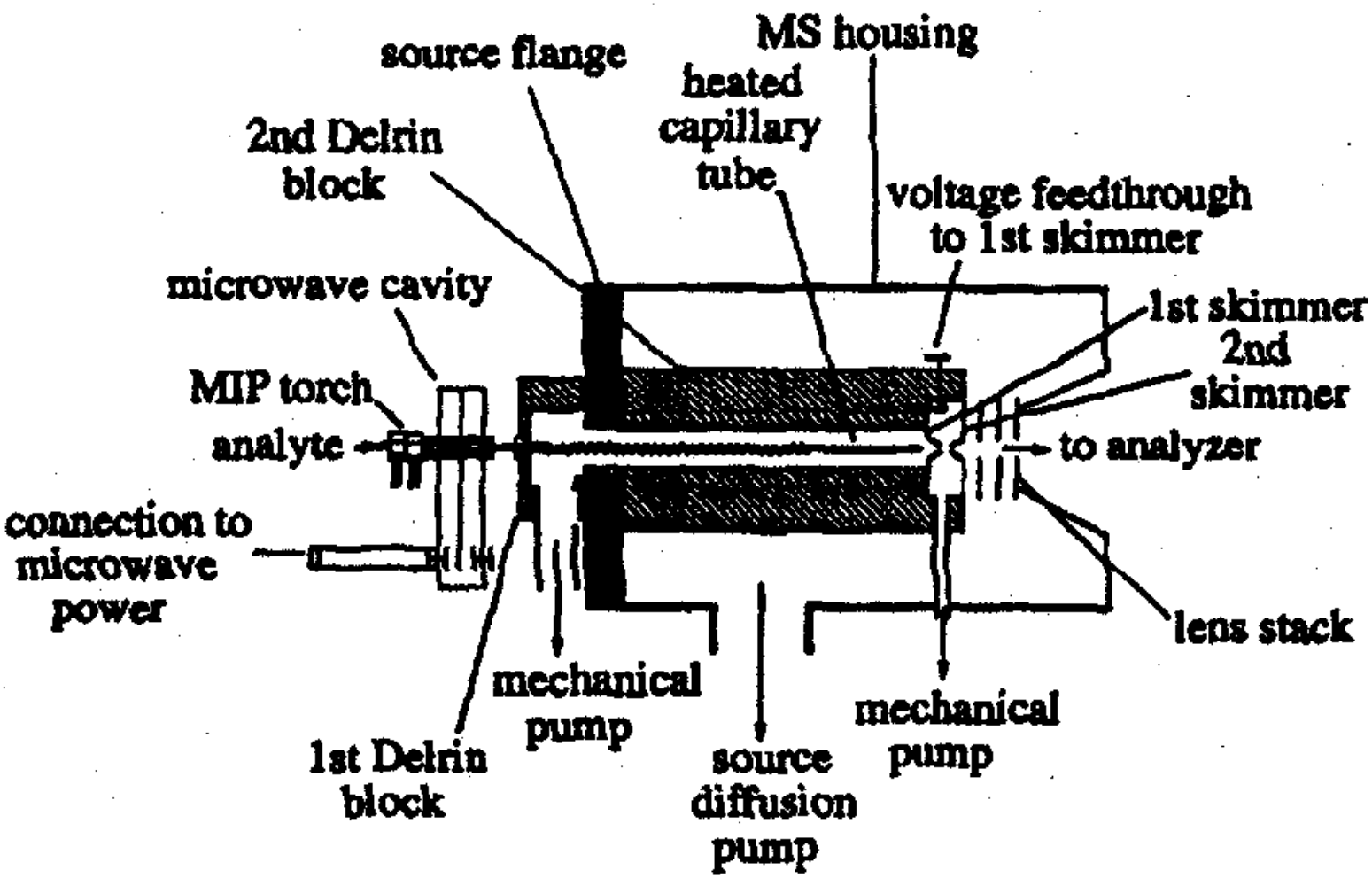


Figure 1. Schematic representation of the MIP atmospheric pressure ionization source and vacuum interface.

Microwave Induced Plasma Ionization

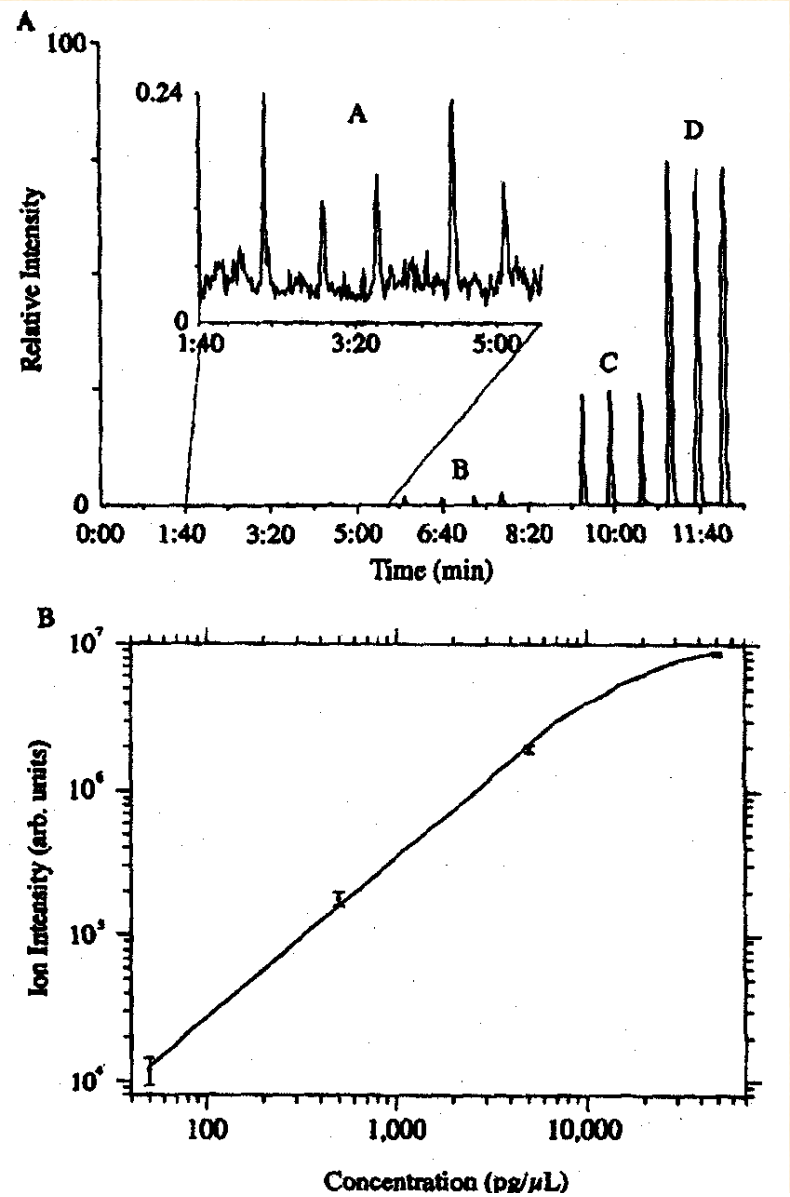


Figure 8. (a) Ion chromatogram (m/z 90) for a quantitative analysis of alanine at various concentrations: A: 10 pg/μL, B:100 pg/μL, C:1000 pg/μL, and D:10,000 pg/μL. The mass spectrometer was operated using the single ion monitoring mode with a dwell time of 1 s. (b) Log–log plot of data presented in Figure 8a.

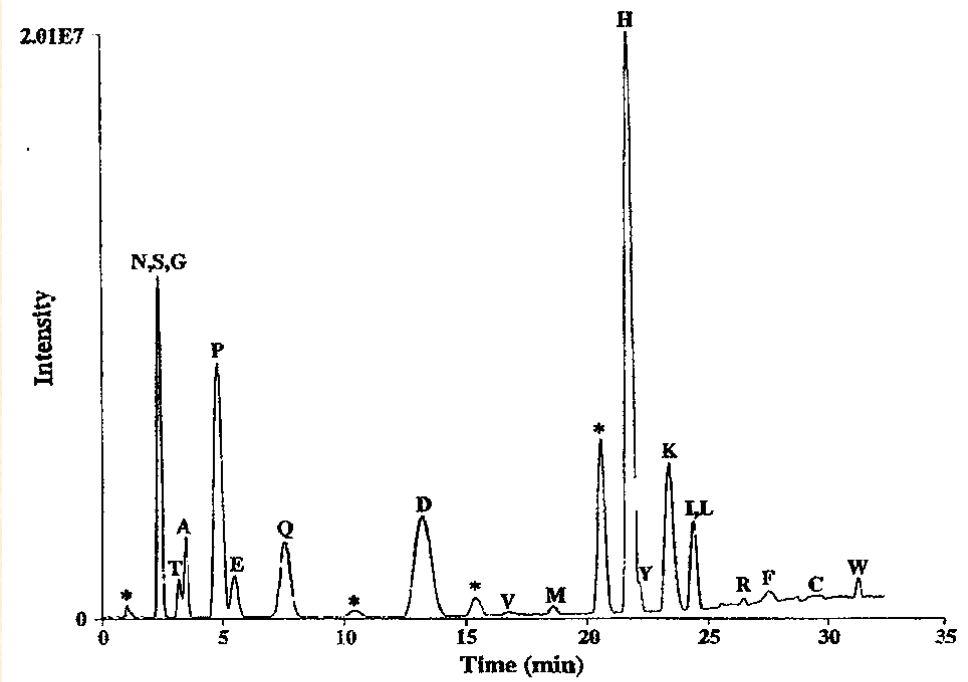


Figure 2. Total ion chromatograms for a mixture of 20 underivatized amino acids using HPLC/AP-MIPI-MS. Peaks marked with an asterisk were not identified.

Microwave Induced Plasma Ionization

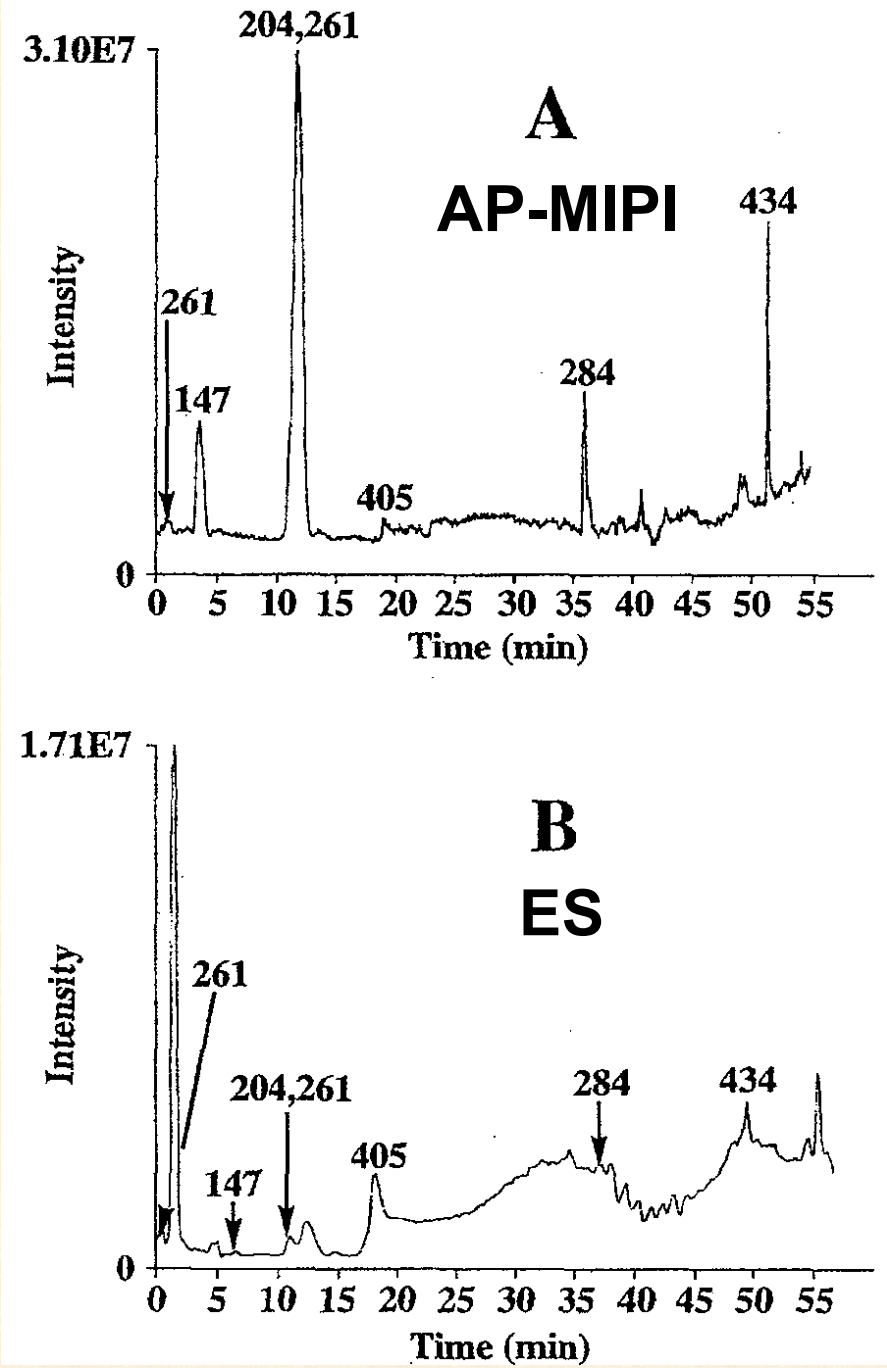


Figure 3. Total ion chromatograms of the six smallest components of tryptic digest of cytochrome *c* using HPLC/AP-MIPI-MS (**A**) and HPLC/ESI-MS (**B**). The marked peaks represent the protonated molecules: 147 (Lys), 204 (Gly-Lys), 261 (Asn-Lys), 261 (Gly-Gly-Lys), 284 (His-Lys), 405 (Thr-Glu-Arg), and 434 (Ala-Thr-Asn-Glu).

Dual APCI/ES

- Multiple ionization modes in a single chromatographic run
- Extends range of compounds detected
- Increases analysis throughput

RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2002; **16**: 300–319

Dual parallel electrospray ionization and atmospheric pressure chemical ionization mass spectrometry (MS), MS/MS and MS/MS/MS for the analysis of triacylglycerols and triacylglycerol oxidation products

Wm. Craig Byrdwell[1]* and William E. Neff[2]
[1]Department of Chemistry and Biochemistry, Florida Atlantic University, 777 Glades Road, Boca Raton, FL 33431, USA
[2]National Center for Agricultural Utilization Research, ARS, USDA, 1815 N. University St., Peoria, IL 61604, USA

Evaluation of a Dual Electrospray Ionization/ Atmospheric Pressure Chemical Ionization Source at Low Flow Rates (~50 μL/min) for the Analysis of Both Highly and Weakly Polar Compounds

(J Am Soc Mass Spectrom 1998, 9, 1196–1203)

Marshall M. Siegel, Keiko Tabei, Frank Lambert, Leonard Candela, and Bart Zoltan
Wyeth-Ayerst Research, Lederle Laboratories, Pearl River, New York, USA

Anal. Chem., in press

Combined Electrospray Ionization–Atmospheric Pressure Chemical Ionization Source for Use in High-Throughput LC–MS Applications

Richard T. Gallagher,* Michael P. Balogh,† Paul Davey, Mike R. Jackson,† Ian Sinclair, and Lisa J. Southern‡

AstraZeneca, Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TG, U.K.

Dual APCI/ES

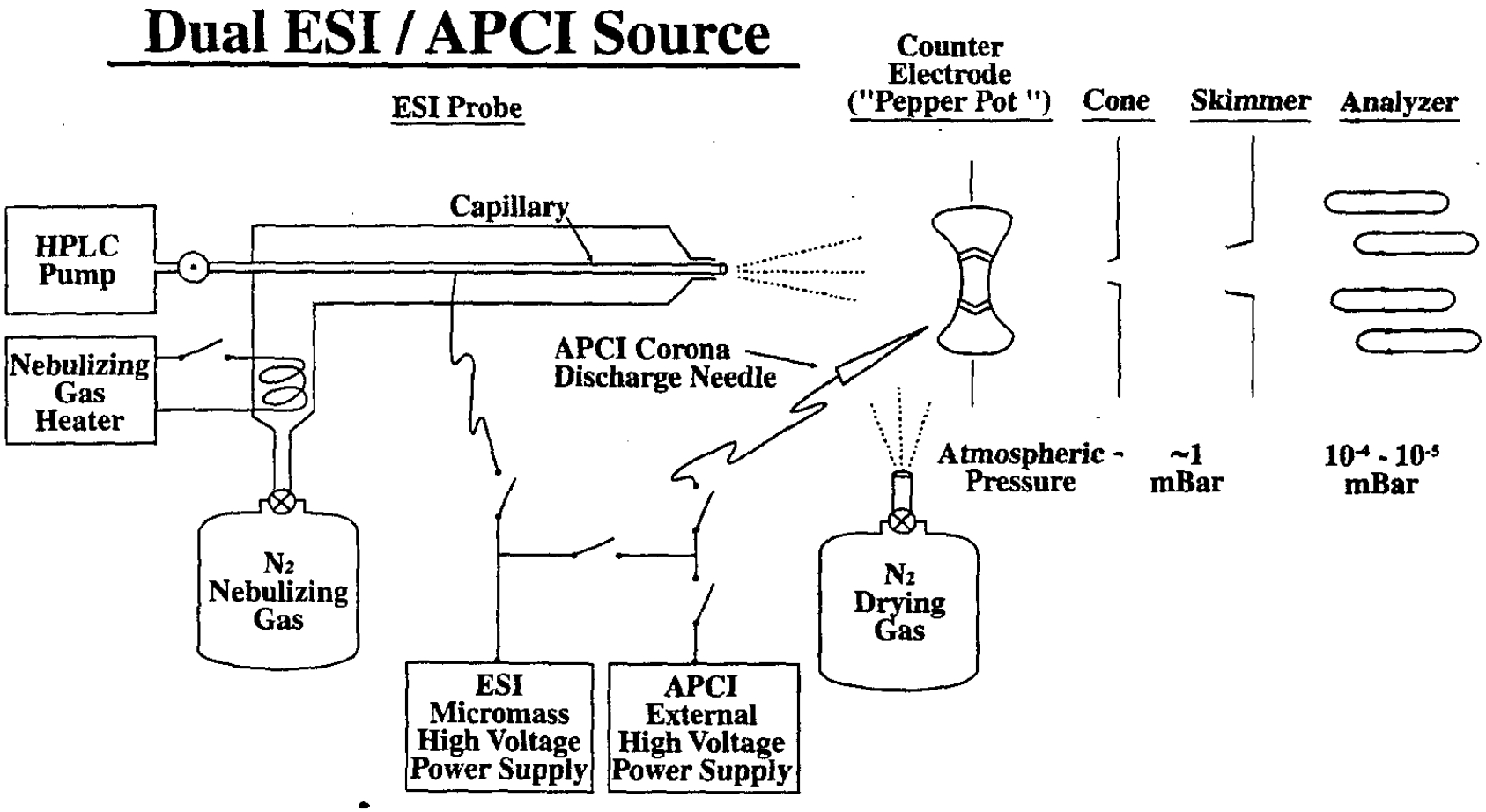


Figure 1. Schematic diagram of the dual ESI/APCI source with ESI probe and APCI corona discharge needle and block diagrams illustrating the high-voltage power supplies, the nitrogen tanks for the drying and nebulizing gases, the heater controller for the nebulizing gas, and the HPLC pump. All the switches illustrated are individually programmable from the mass spectrometer data system.

Dual APCI/ES

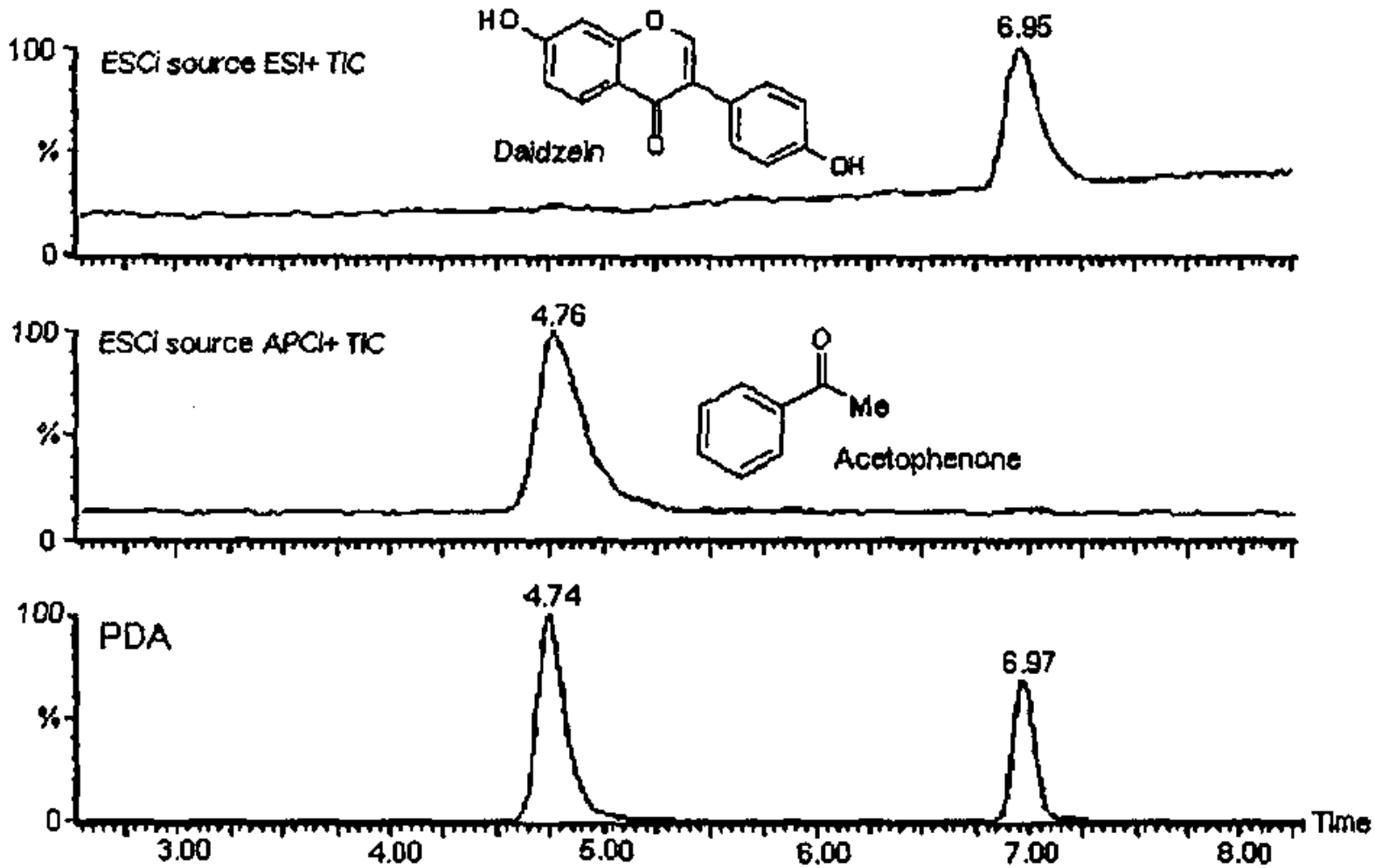


Figure 2. LC–ESCi-MS analysis on a mixture of daidzein and acetophenone. Total ion chromatogram (TIC) traces for ESI and APCI positive ion modes.

Atmospheric Pressure Photoionization

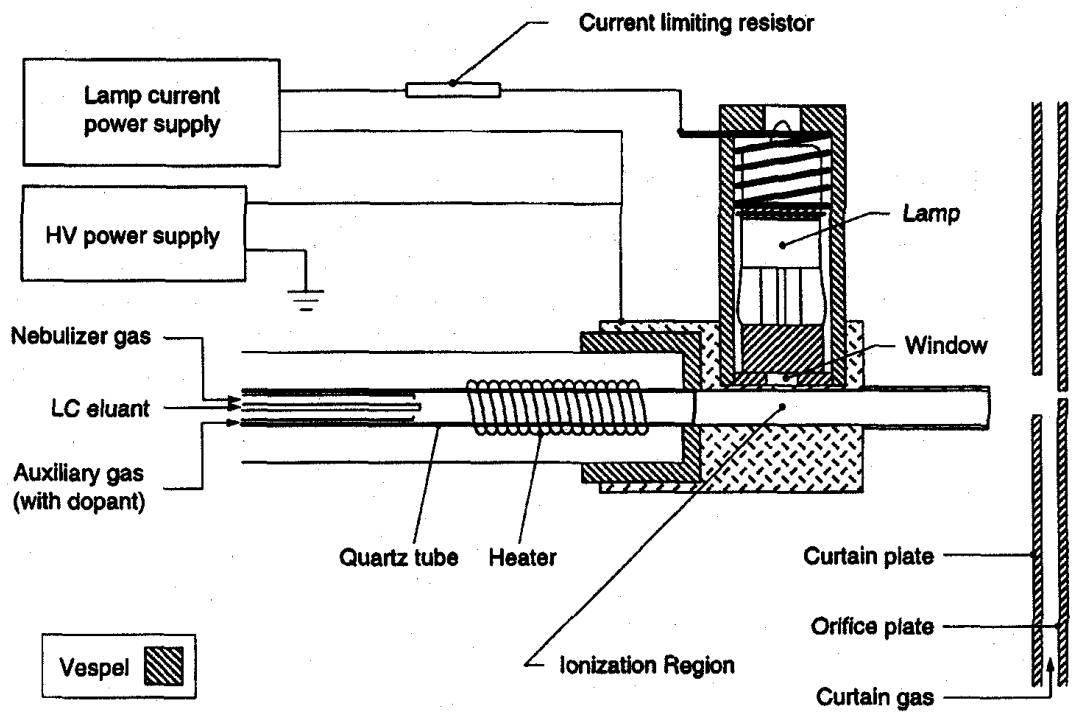
- Alternative to APCI
- Equal or better detection levels than APCI
- Extends efficient ionization to include less polar compounds than APCI

Anal. Chem. **2000**, *72*, 3653–3659

Atmospheric Pressure Photoionization: An Ionization Method for Liquid Chromatography–Mass Spectrometry

Damon B. Robb,[†,‡] Thomas R. Covey,[§,∥] and Andries P. Bruins[*,†]

University Center for Pharmacy, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands, and MDS SCIEX, 71 Four Valley Drive, Concord, Ontario, Canada, L4K 4V8



Schematic of the APPI ion source, including the heated nebulizer probe, photoionization lamp, and lamp mounting bracket.

Atmospheric Pressure Photoionization

- Direct photoionization statistically unfavorable
- Photoionize dopant
- Dopant ions react to ionize analyte
  - Proton transfer
  - Electron transfer

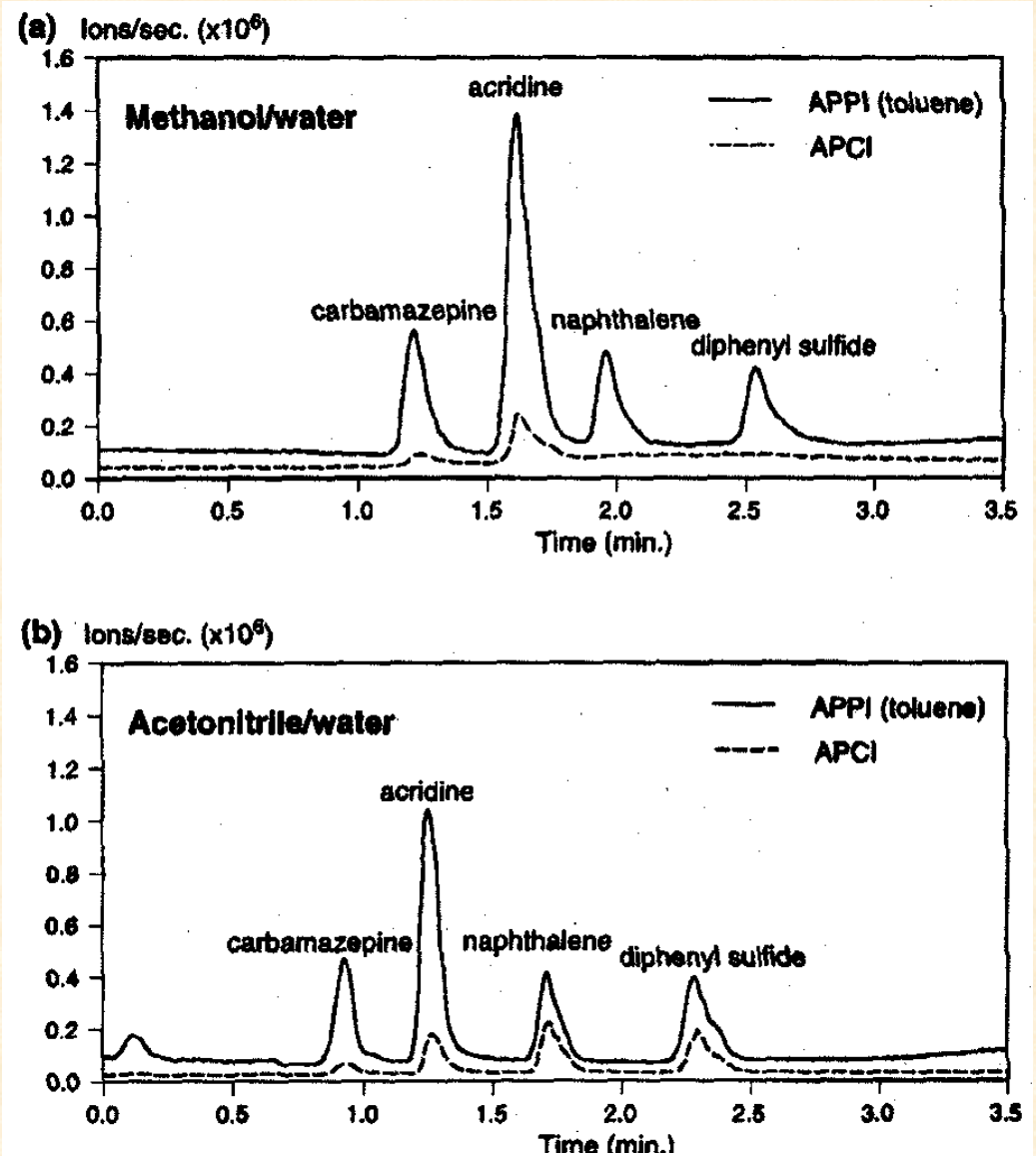


Figure 6. Comparison of APPI and corona discharge-APCI. (a) The LC eluant is methanol–water. APPI is more sensitive than APCI, especially toward naphthalene and diphenyl sulfide. (b) For acetonitrile–water, APPI is still more sensitive, but APCI shows improved sensitivity toward the low proton affinity compounds.

Laser Desorption Ionization

- Atmospheric Pressure Laser Desorption
  - Atmospheric Pressure Matrix Assisted Laser Desorption/Ionization (AP-MALDI)
  - Laser Desorption (LD) – APCI
- "Matrix-free" MALDI
- Coupling MALDI with Separations
- Imaging with MALDI
  - Tissues Sections
  - Virtual 2-D Gels
- Affinity Surfaces for MALDI
- Automated Sample Preparation/Analysis for MALDI

Atmospheric Pressure MALDI

- Interface MALDI with atmospheric sampling TOF and trapping instruments (ES,APCI, MALDI)
- Simplification and versatility of sample analysis provided by atmospheric pressure operation
- "Softer" ionization

Anal. Chem. **2000**, *72*, 652–657

Atmospheric Pressure Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry

Victor V. Laiko,[†] Michael A. Baldwin,[†,‡] and Alma L. Burlingame*[,†,§]

Mass Spectrometry Facility, Department of Pharmaceutical Chemistry, Department of Neurology and Institute for Neurological Diseases, and The Liver Center, University of California, San Francisco, California 94143-0446

Anal. Chem. **2002**, *74*, 1891–1895

Atmospheric Pressure Matrix-Assisted Laser Desorption/Ionization in Transmission Geometry

Marsha C. Galicia,[†] Akos Vertes,[†] and John H. Callahan*[,‡]

Department of Chemistry, The George Washington University, Washington, D.C. 20052, and Chemical Dynamics and Diagnostics Branch, Naval Research Laboratory, Code 6115, Washington, D.C. 20375

RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2002; **16**: 1737–1742
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/rcm.781

Atmospheric pressure laser desorption/ionization on porous silicon

Victor V. Laiko[1], Nelli I. Taranenko[1], Vadym D. Berkout[1], Brian D. Musselman[2] and Vladimir M. Doroshenko[1]*

[1]MassTech Inc., Subsidiary of SESI, 4032-A Blackburn Lane, Burtonsville, MD 20866, USA
[2]SciMarket Strategies Inc., 318 West Emerson Street, Melrose, MA 02176, USA

Atmospheric Pressure MALDI

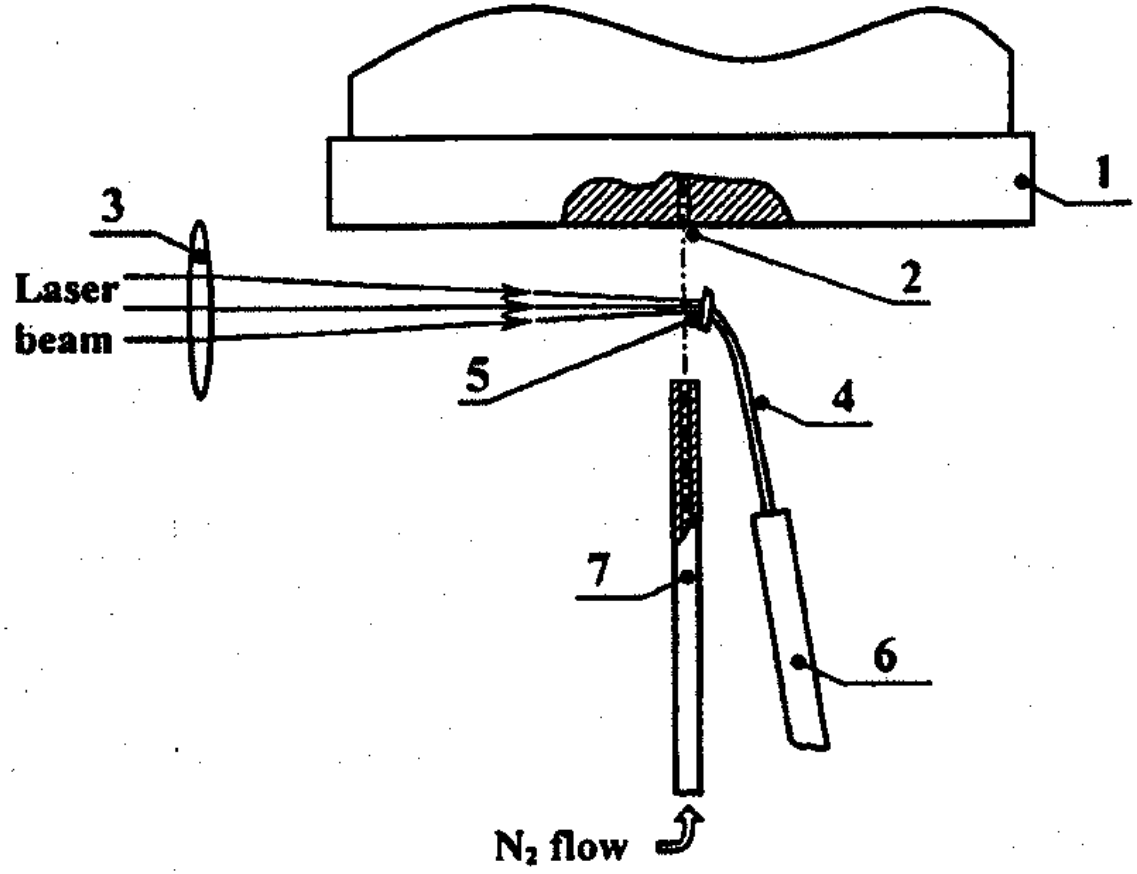


Figure 1. Schematic view of the PA-AP MALDI source. 1, Atmospheric pressure interface of Mariner oaTOF mass spectrometer; 2, inlet nozzle to MS instrument; 3, quartz lens; 4, replaceable target tip; 5, stainless steel MALDI target plate; 6, target holder; 7, stainless steel capillary gas nozzle.

Atmospheric Pressure MALDI

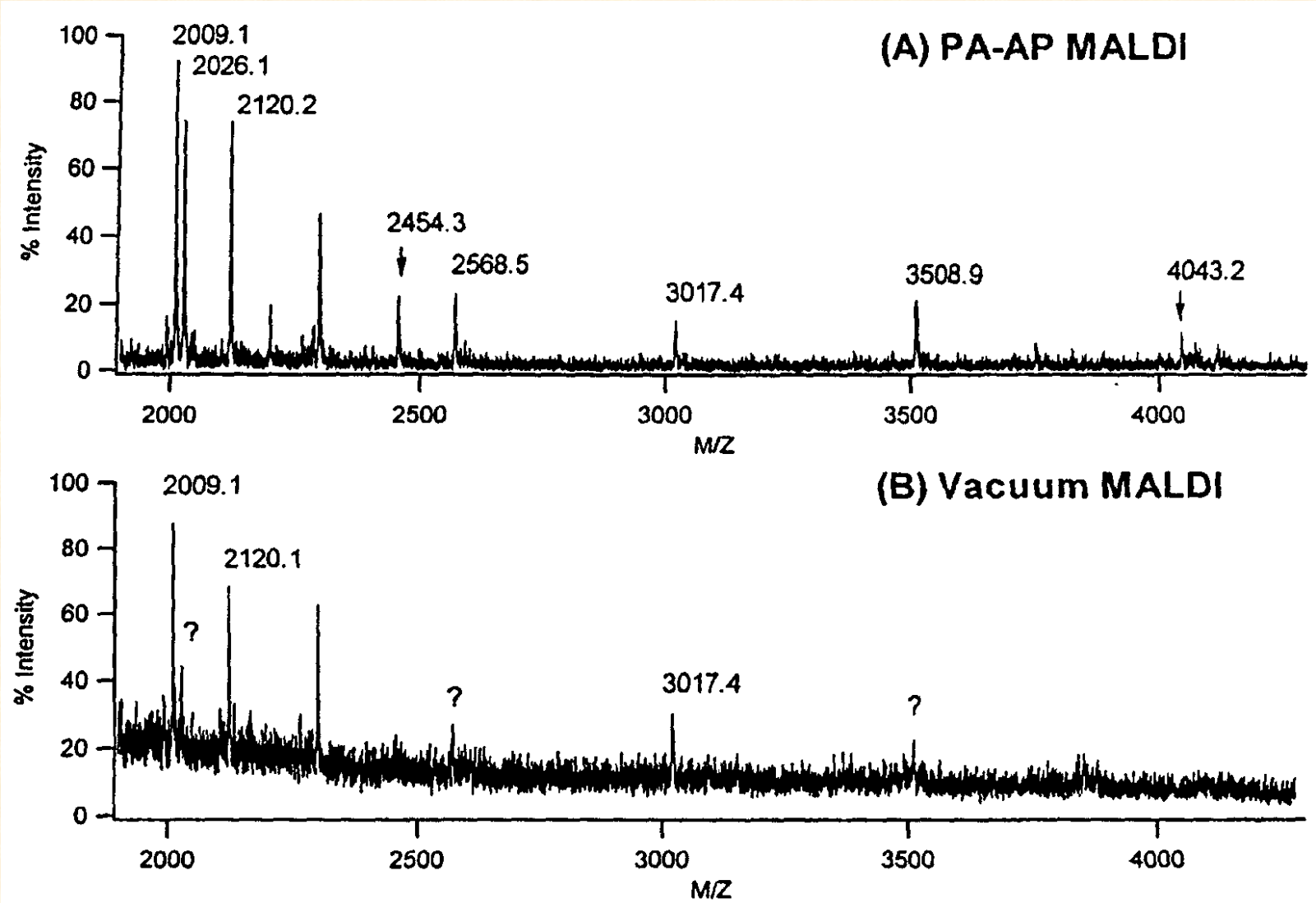


Figure 5. Partial mass spectra for tryptic digest of bovine fetuin. (A) PA-AP MALDI spectrum from 1.5 pmol deposition, showing eight peaks previously identified from LC/MS data.[6] (B) Vacuum MALDI spectrum from Voyager Elite instrument in delayed extraction reflectron mode for 1 pmol deposition, showing three peaks previously identified from LC/MS data.[6]

LD-APCI

- Decouples desorption and ionization
- Eliminates requirement that matrix facilitate both desorption and ionization
- Simplifies sample preparation and analysis
- Improves ionization efficiency

RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2002; **16**: 681–685
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/rcm.626

Atmospheric pressure laser desorption/chemical ionization mass spectrometry: a new ionization method based on existing themes

Joshua J. Coon, Kevin J. McHale and W. W. Harrison*
Department of Chemistry, University of Florida, Gainesville, FL 32611, USA

JOURNAL OF MASS SPECTROMETRY
J. Mass Spectrom. 2002; **37**: 1163–1167
Published online 6 November 2002 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jms.385

Laser desorption-atmospheric pressure chemical ionization: a novel ion source for the direct coupling of polyacrylamide gel electrophoresis to mass spectrometry

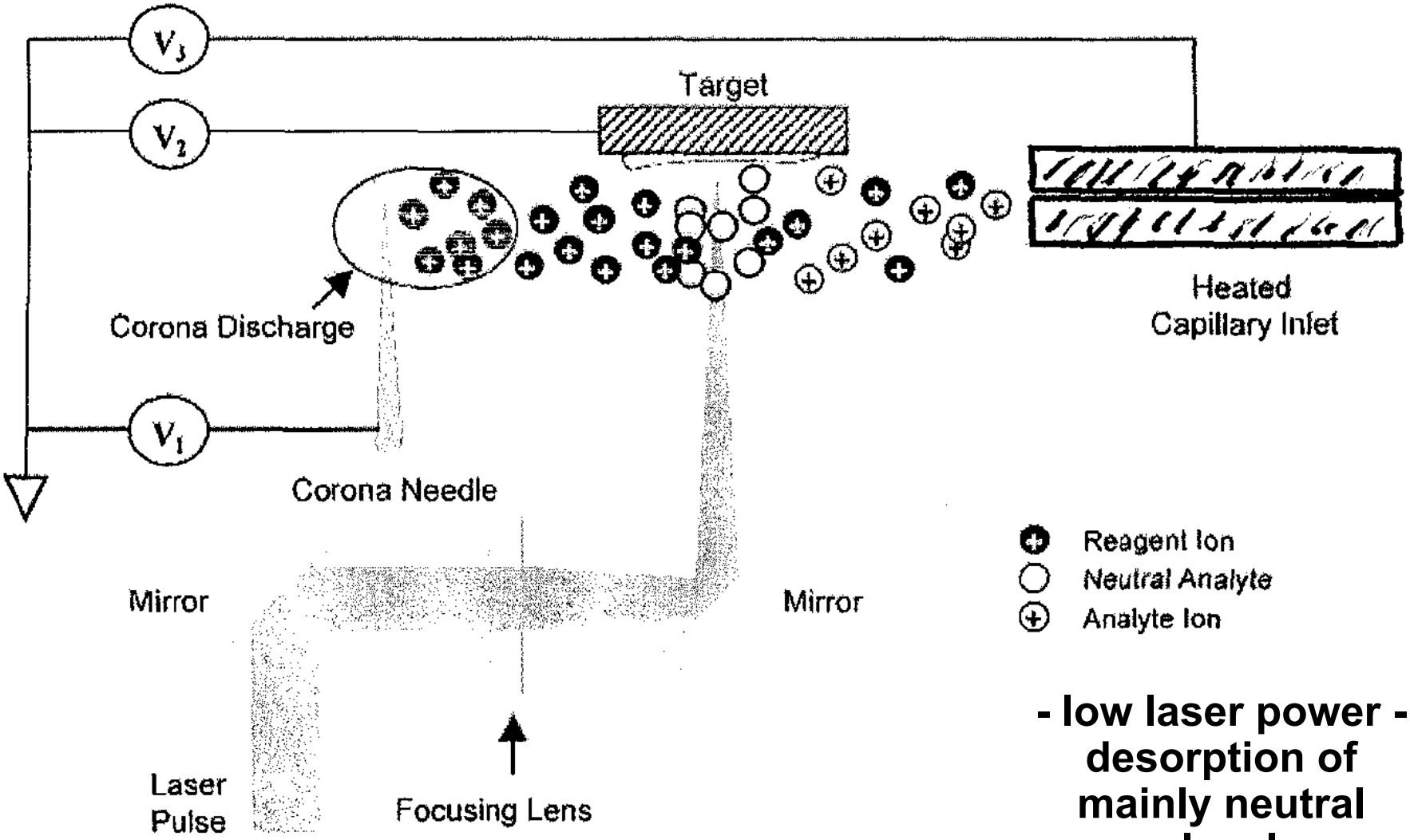
Joshua J. Coon,[1] Heather A. Steele,[2] Philip J. Laipis[2] and W. W. Harrison[1]*
[1] Department of Chemistry, University of Florida, Gainesville, FL 32606, USA
[2] Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, Florida 32606, USA

Anal. Chem. **2002,** *74,* 5600−5605

Laser Desorption-Atmospheric Pressure Chemical Ionization Mass Spectrometry for the Analysis of Peptides from Aqueous Solutions

Joshua J. Coon and W. W. Harrison*
Department of Chemistry, University of Florida, Gainesville, Florida 32611

LD-APCI



- low laser power - desorption of mainly neutral molecules

Figure 1. Schematic representation of LD-APCI source.

LD-APCI

Table 1. Expected peptides resulting from a tryptic digest of horse cytochrome c[a]

Theoretical m/z	Position	Peptide sequence
1495.69	61–72	EELMEYLENPK
1470.68	40–53	TGQAPGFTYDANK
1168.62	28–38	TGPNLHGLFGR
1018.44	14–22	CAQCHTVEK
964.53	**92–99**	**EDLIAYLK**
779.44	**80–86**	**MIFAGIK**
678.38	**74–79**	**YIPGTK**
634.39	**9–13**	**IFVQK**
604.34	**56–60**	**GITWK**
547.27	1–5	GDVEK
434.18	101–104	ATNE
405.20	89–91	TER
332.23	**6–8**	**GKK**[b]
284.17	26–27	HK
275.21	**87–88**	**KK**[b]
261.15	**54–55, 23–25**	**NK, GGK**
204.13	6–7	GK
147.11	8, 39, 73, 87, 88, 100	K

[a] Bold entries represent peptides detected during PAGE–LD-APCI-MS analysis.
[b] Detected missed cleavages.

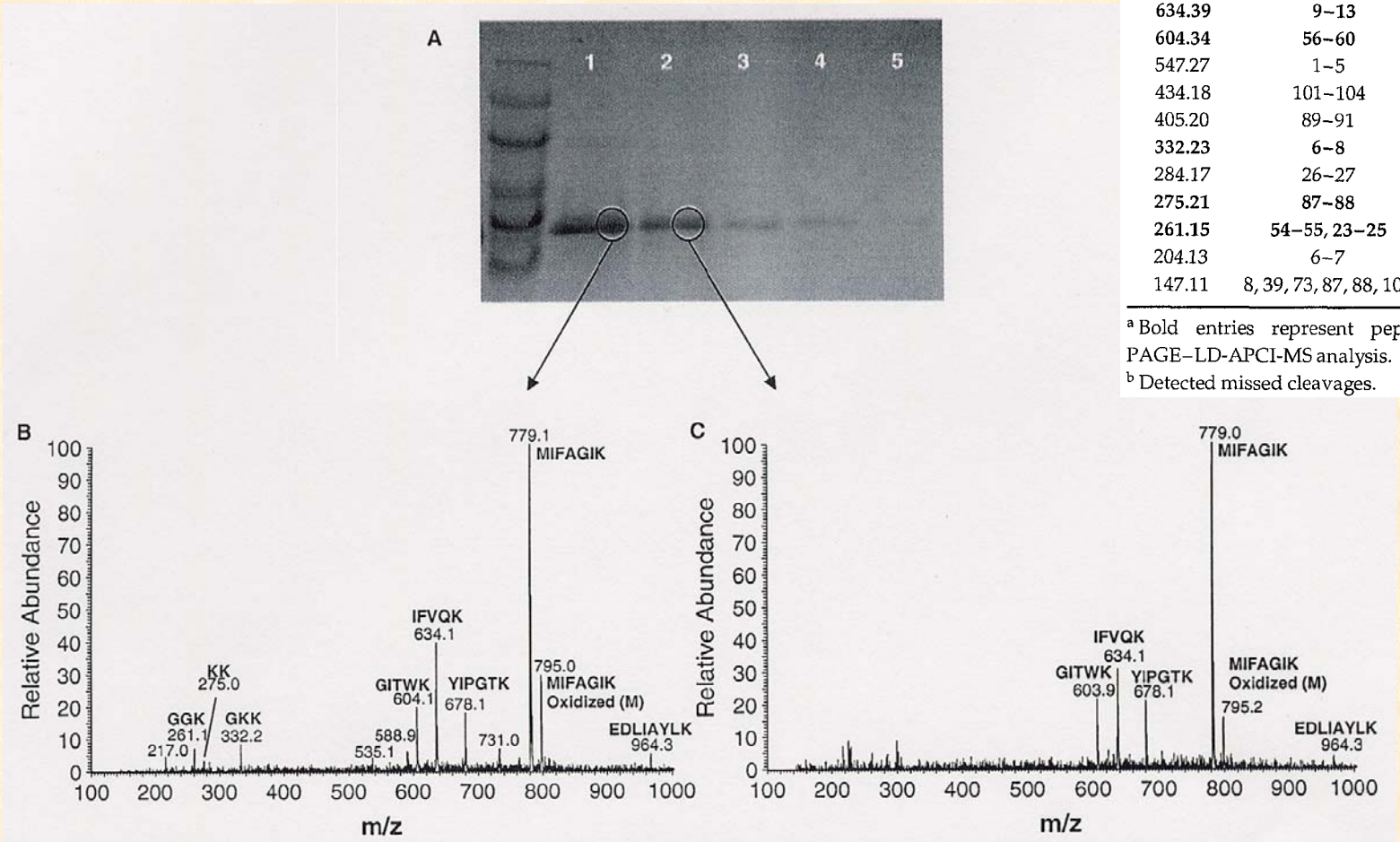


Figure 2. (A) Photograph of Coomassie blue stained polyacrylamide gel following electrophoresis. (B) LD-APCI-MS analysis of stained spot from lane 1. (C) LD-APCI-MS analysis of stained spot from lane 2.

"Matrix-Free" MALDI

- Simplifies sample preparation
- Minimizes low mass chemical noise
- Enhances analysis of low mass analytes

Anal. Chem. **2001**, *73*, 612–619

Porous Silicon as a Versatile Platform for Laser Desorption/Ionization Mass Spectrometry

Zhouxin Shen,[†] John J. Thomas,[‡] Claudia Averbuj,[§] Klas M. Broo,[‡] Mark Engelhard,[||] John E. Crowell,[†] M. G. Finn,[*,‡] and Gary Siuzdak[*,‡]

Departments of Chemistry and Molecular Biology and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, Mass Consortium Corporation, San Diego, California 92121, Department of Chemistry & Biochemistry, University of California, San Diego, La Jolla, California 92093-0314, and Environmental Molecular Sciences Laboratory, Department of Energy's Office of Biological and Environmental Research, Pacific Northwest National Laboratory, Richland, Washington 99352

Anal. Chem. **2001**, *73*, 1292–1295

Desorption–Ionization Mass Spectrometry Using Deposited Nanostructured Silicon Films

Joseph D. Cuiffi*

Nanofabrication Facility, The Pennsylvania State University, University Park, Pennsylvania 16802

Daniel J. Hayes, Stephen J. Fonash, Kwanza N. Brown, and Arthur D. Jones[†]

Mass Spectrometry Center, The Pennsylvania State University, Whitmore Laboratory, University Park, Pennsylvania 16802.

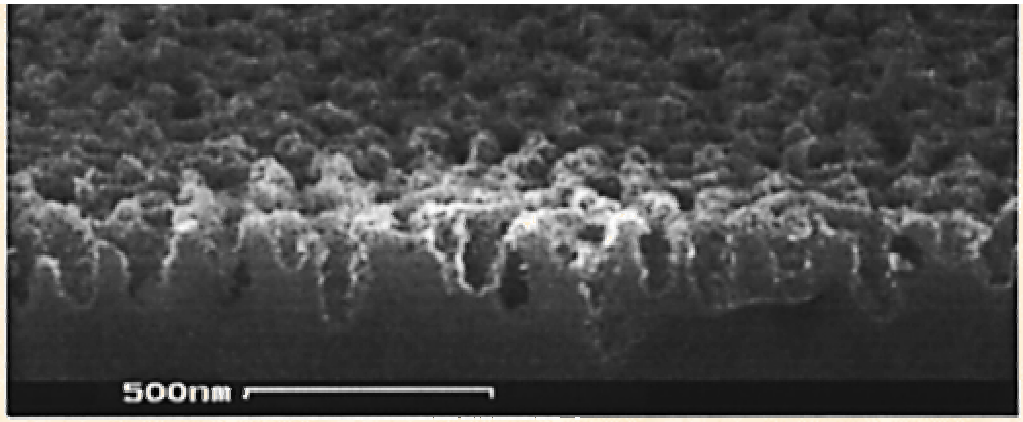
Anal. Chem. **2001**, *73*, 3639–3645

Experimental Factors Controlling Analyte Ion Generation in Laser Desorption/Ionization Mass Spectrometry on Porous Silicon

Rebecca A. Kruse, Xiuling Li, Paul W. Bohn,* and Jonathan V. Sweedler*

Department of Chemistry, Beckman Institute for Advanced Science and Technology, and Fredrick Seitz Materials Research Laboratory, University of Illinois at Urbana–Champaign, 600 South Mathews Avenue, Urbana, Illinois 61801

Figure 3. SEM analysis of the "double-etched" porous silicon surface prepared from low-resistivity n^+-Si material (0.001–0.005 Ω·cm).



"Matrix-Free" MALDI

- **Porous silicon surface takes on role of matrix**
- **Optical absorption, thermal conductivity, pore size and overall porosity critical parameters**

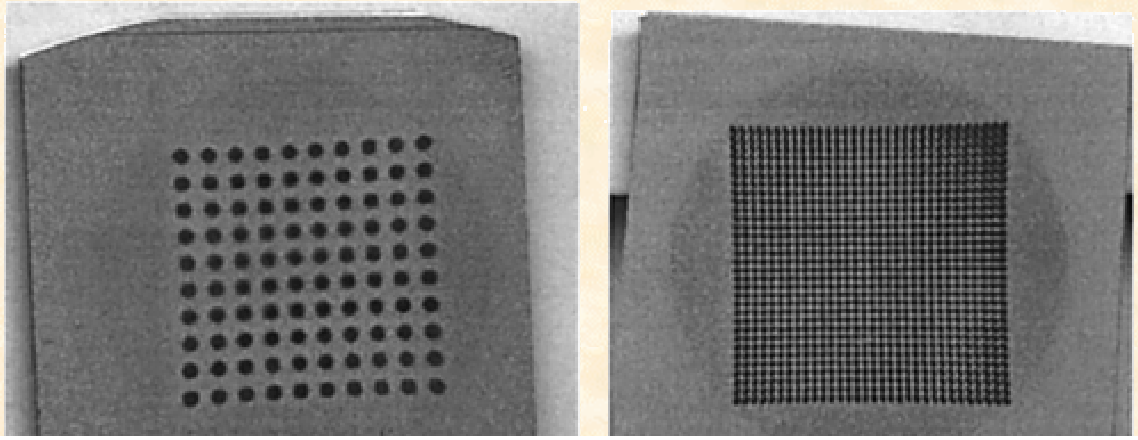


Figure 4. Porous silicon wafers suitable for DIOS having 100 positions (left) and 1000 positions (right) etched on a 3 cm × 3 cm surface. The patterns are generated by illumination through a simple transparency mask. Translucent areas on the transparency translate to dark active areas on the DIOS surfaces. The silicon wafers used in these studies were typically 0.5 mm thick.

“Matrix-Free” MALDI

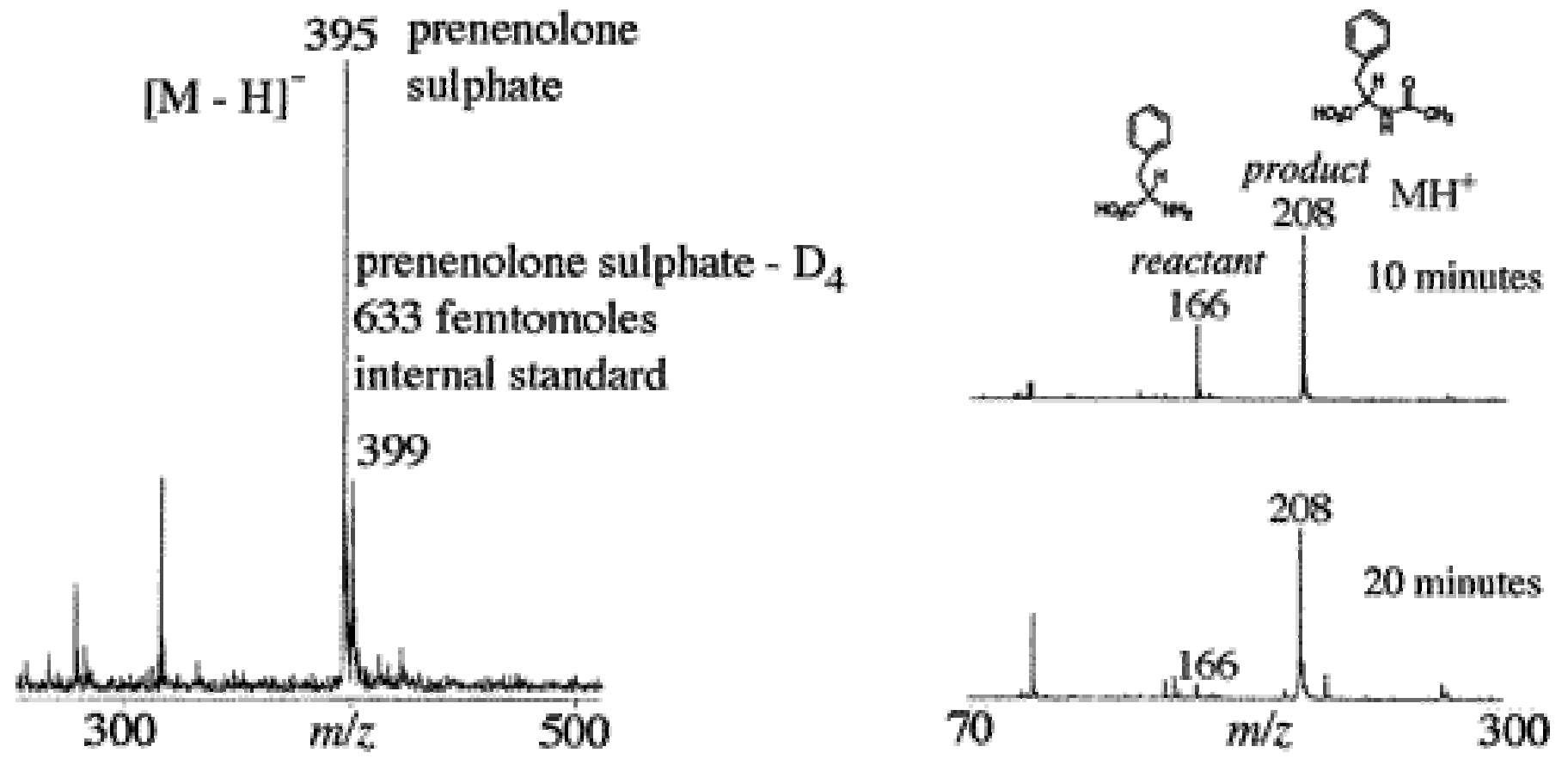


Figure 5. Examples of DIOS-MS spectra including a sulfated steroid (negative ionization mode (1 pmol), on-plate monitoring of the acetylation of an amino acid (positive ionization mode, 0.5-μL reaction volume, 50 pmol), and the identification of the components of a poly-(ethylene glycol) sample of high polydispersity (positive ionization,

"Matrix-Free" MALDI

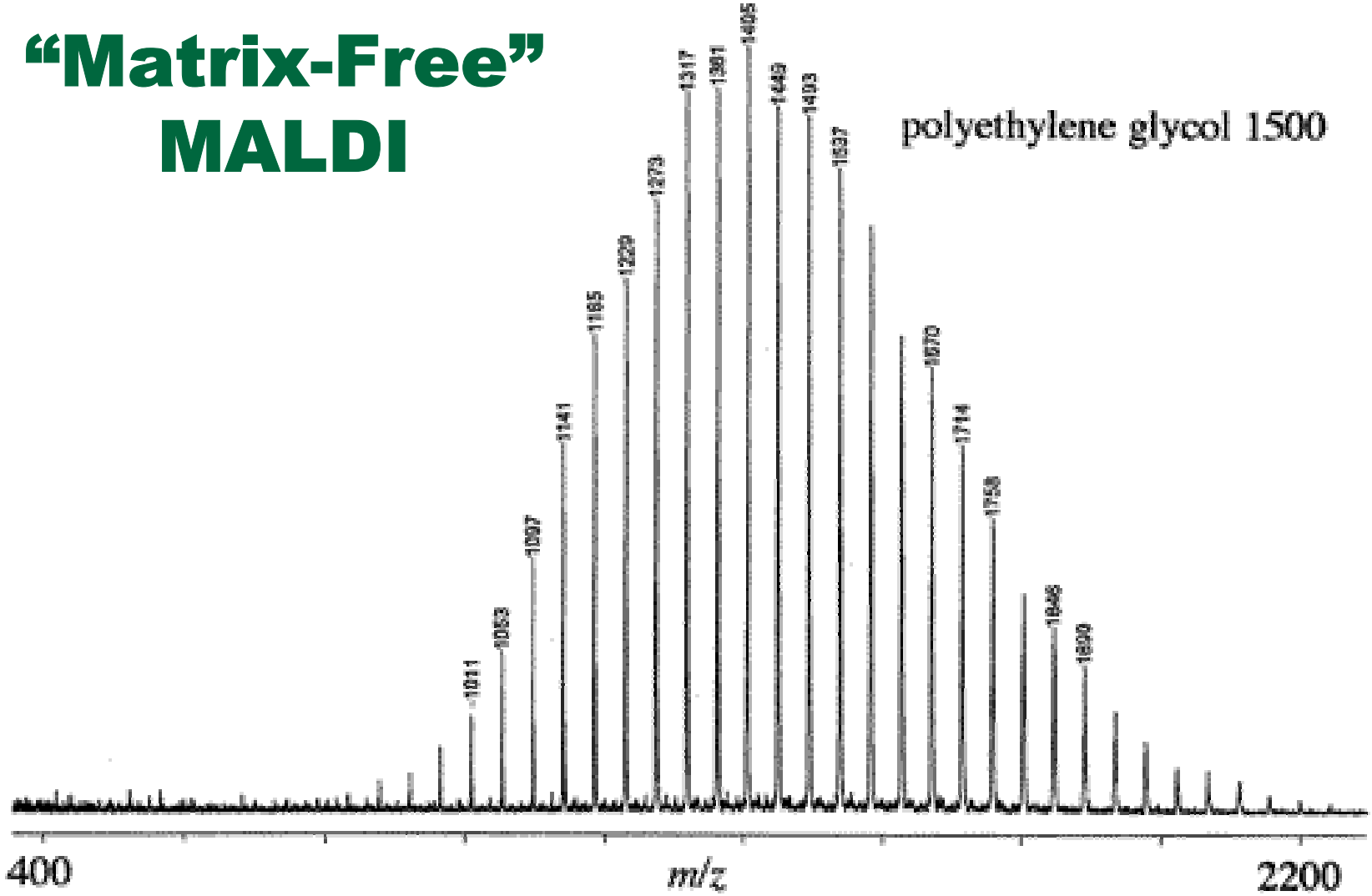


Figure 5. Examples of DIOS-MS spectra including a sulfated steroid (negative ionization mode (1 pmol), on-plate monitoring of the acetylation of an amino acid (positive ionization mode, 0.5-μL reaction volume, 50 pmol), and the identification of the components of a poly-(ethylene glycol) sample of high polydispersity (positive ionization,

Coupling MALDI with Separations

- Obtain advantages of other LC-MS couplings
- Numerous coupling approaches
  - fraction collection,
  - automated spotting
  - continuous deposition
  - continuous desorption
  - and others

Arkady I. Gusev
Fresenius J Anal Chem (2000) 366 : 691–700

Interfacing matrix-assisted laser desorption/ionization mass spectrometry with column and planar separations

Proteomics 2002, 2, 360–372

Liquid phase interfacing and miniaturization in matrix-assisted laser desorption/ionization mass spectrometry

František Foret[1]
Jan Preisler[2]
[1]Institute of Analytical Chemistry, Czech Academy of Sciences, Brno, Czech Republic
[2]Department of Analytical Chemistry, Masaryk University, Brno, Czech Republic

Interfaces for on-line liquid sample delivery for matrix-assisted laser desorption ionisation mass spectrometry

Chem. Soc. Rev., 2001, **30**, 104–112

Henrik Orsnes† and Renato Zenobi*

Department of Chemistry, Swiss Federal Institute of Technology (ETH) CH-8092. Zürich, Switzerland. E-mail: ZENOBI@org.chem.ethz.ch; Fax: 0041-1-632-1292; Tel: 0041-1-632-4376

Coupling MALDI with Separations

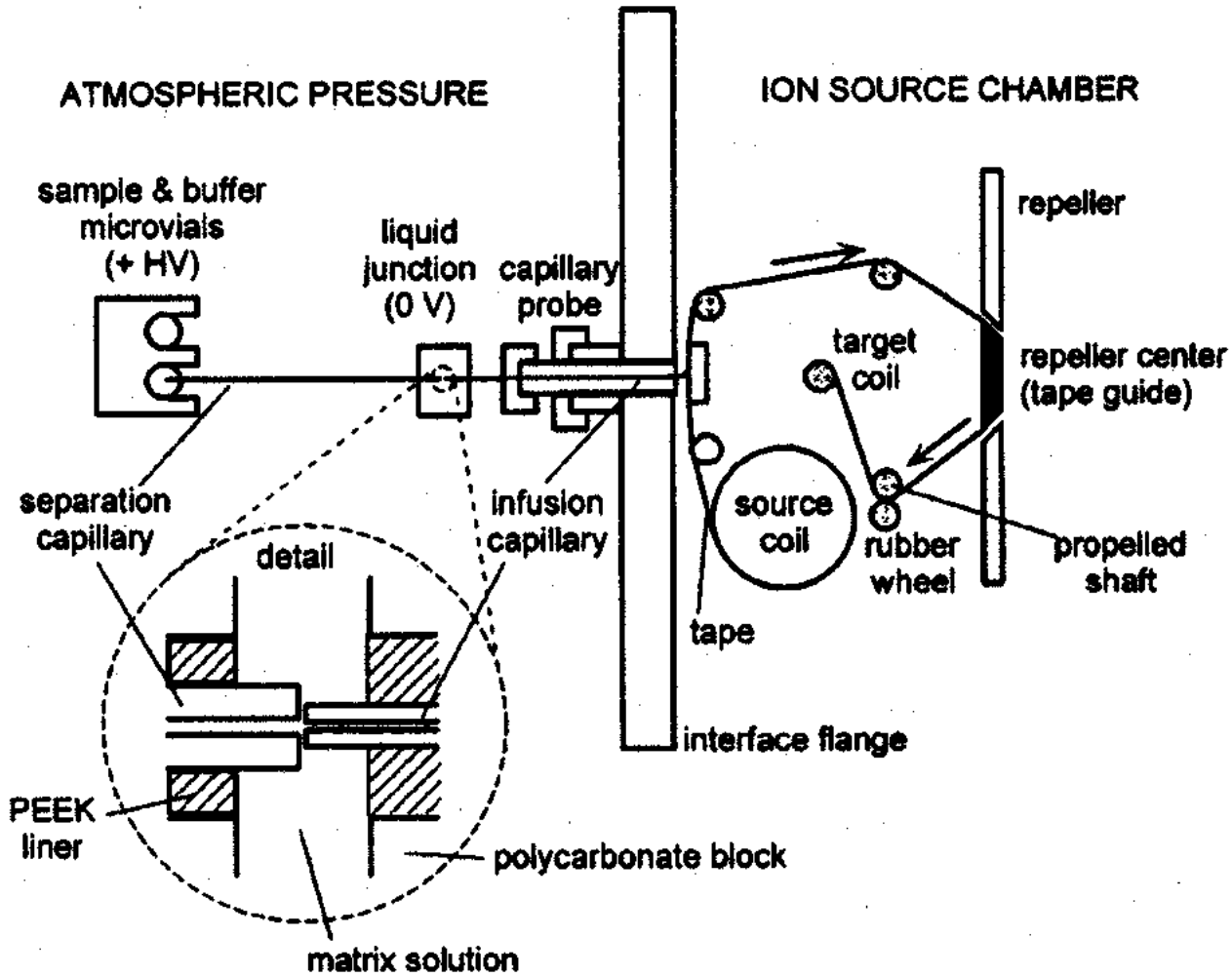


Figure 2. Top view of the vacuum deposition interface with the tape cartridge and the liquid junction. The design of the repeller region is also shown. Details can be found in the Experimental Section.

Coupling MALDI with Separations

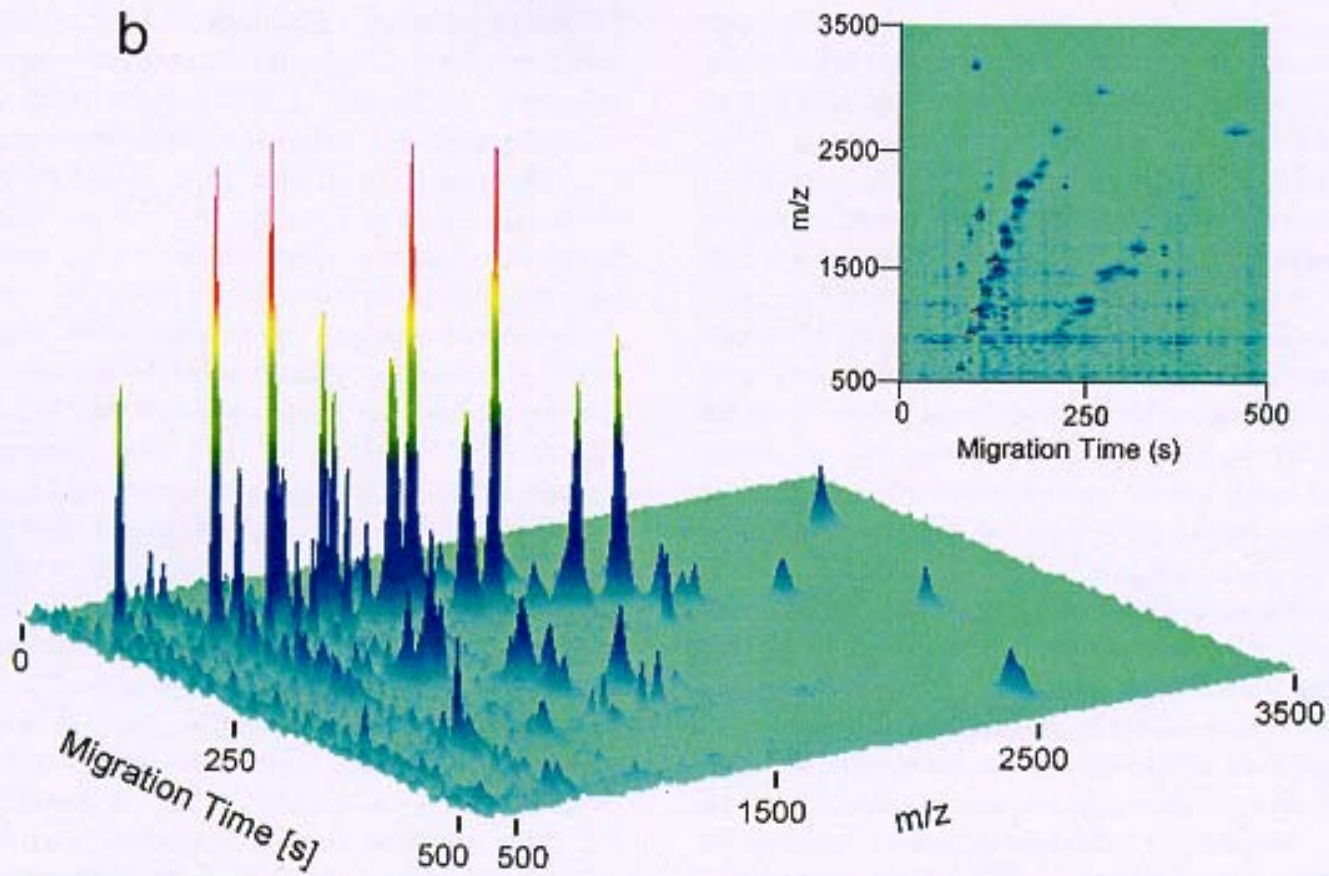


Figure 6. MALDI MS spectra of enolase digest. (a) MALDI MS spectra of enolase digest mixture after SPE sample preparation. Upper trace: sample desalted with ZipTip$_{C18}$, 50% ACN elution. Lower traces: sample fractionated by SPE using 15, 20, and 50% ACN. Both experiments used 200 μM enolase digest samples. After SPE elution, samples were diluted in MALDI matrix (50 mM αCHCA in ACN/0.1% TFA 50/50) before dried-droplet deposition. Final enolase digest concentration, 10 μM. MS: Bruker Proflex MALDI/DE-mass spectrometry, 50-shot average. (b) 3D spectrum of enolase digest obtained by CE−MALDI MS. Sample: 200 μM enolase digest desalted with ZipTip$_{C18}$, 50% ACN elution, sample diluted in water before CE injection. Same experimental conditions as indicated in Figure 4, except that 20 μM sample was hydrodynamically injected at 5 cm for 10 s (330 fmol). CE separation was carried out in 20-cm-long PVA-coated capillary (75-μm i.d., 375-μm o.d.) at 1000 V/cm.

Imaging with MALDI Tissue Sections

- Map distribution of targeted compounds in tissue
- Altered molecular marker distributions are diagnostic for various diseases

JOURNAL OF MASS SPECTROMETRY
J. Mass Spectrom. 2001; **36**: 355–369

Organic ion imaging of biological tissue with secondary ion mass spectrometry and matrix-assisted laser desorption/ionization

Peter J. Todd,[1] T. Gregory Schaaff,[1] Pierre Chaurand[2] and Richard M. Caprioli[2]*

[1] Chemical and Analytical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, USA
[2] Mass Spectrometry Research Center and Department of Biochemistry, Vanderbilt University, Nashville, Tennessee 37232, USA

Imaging mass spectrometry: a new tool to investigate the spatial organization of peptides and proteins in mammalian tissue sections

Pierre Chaurand, Sarah A Schwartz and Richard M Caprioli*

Mass Spectrometry Research Center and Department of Biochemistry,
Vanderbilt University, Nashville, TN 37232-6400, USA
*e-mail: r.caprioli@vanderbilt.edu

Current Opinion in Chemical Biology 2002, **6**:676–681

Stoeckli M, Chaurand P, Hallahan DE, Caprioli RM: **Imaging mass spectrometry: a new technology for the analysis of protein expression in mammalian tissues.** *Nat Med* 2001, **7**:493-496.

Imaging with MALDI Tissue Sections

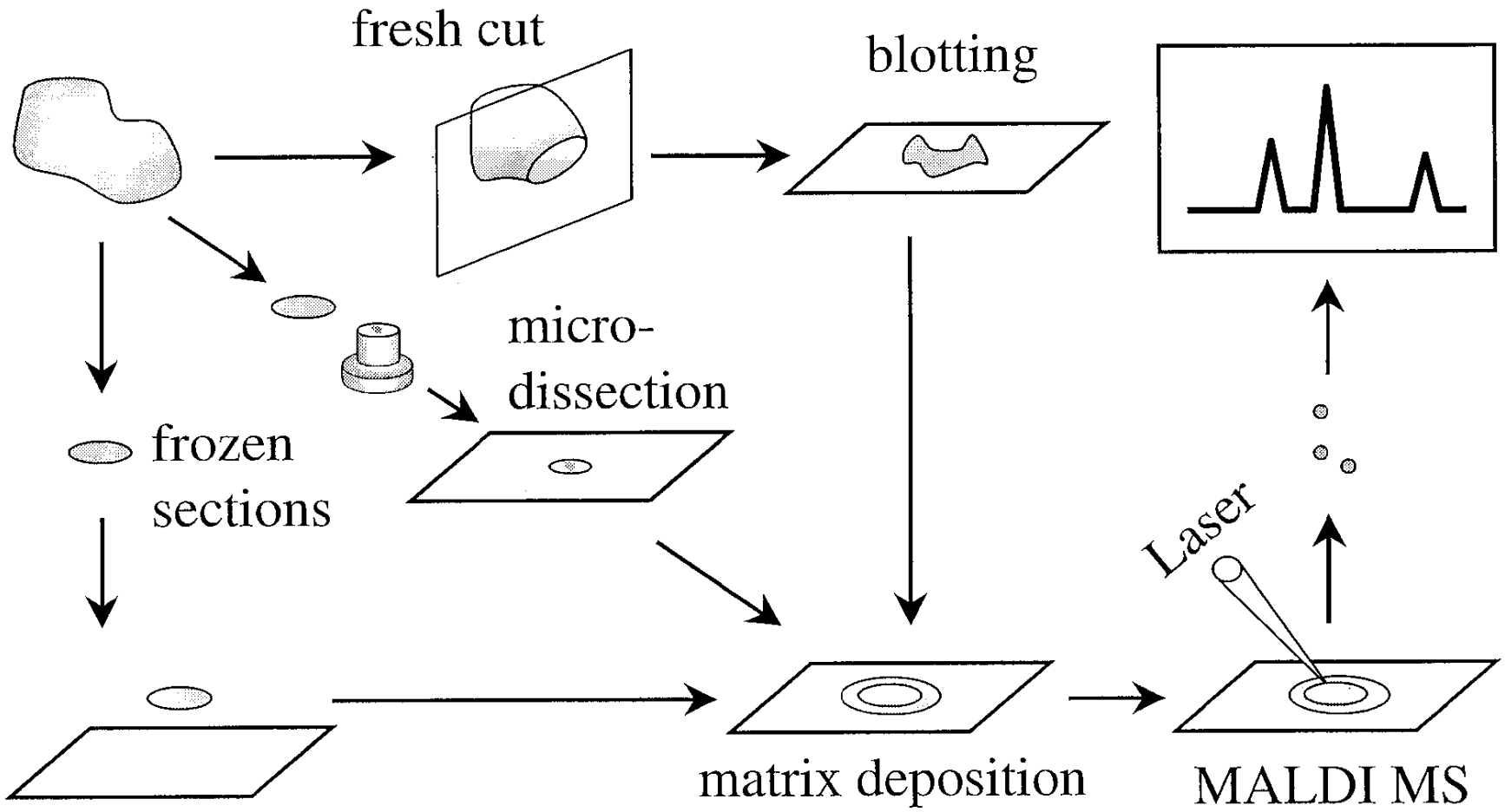
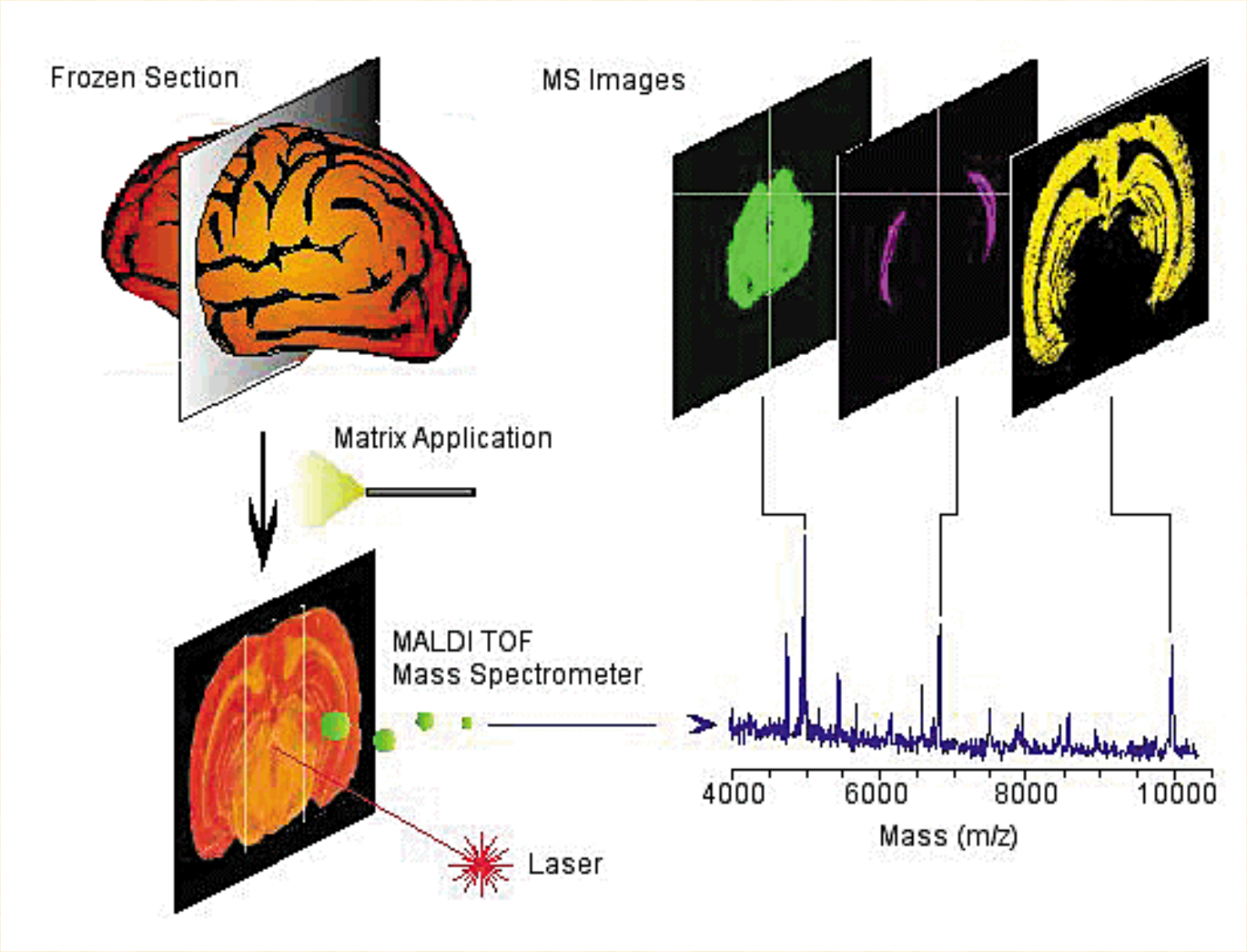


Figure 6. Schematics of the different strategies and sample preparation methods employed to investigate protein populations in tissue samples.

Imaging with MALDI Tissue Sections



Imaging with MALDI Virtual 2-D Gels

- MALDI analysis of separated components on 1-D isoelectric focusing or pH gradient gels
- Mass analysis substituted for size separation of SDS - PAGE

Electrophoresis 2001, *22*, 933–945

Mass spectrometric imaging of immobilized pH gradient gels and creation of "virtual" two-dimensional gels

Angela K. Walker
Gary Rymar
Philip C. Andrews

Department of Biological Chemistry, University of Michigan, Ann Arbor, MI, USA

Anal. Chem. **2001**, *73*, 4063–4070

Virtual 2-D Gel Electrophoresis: Visualization and Analysis of the *E. coli* Proteome by Mass Spectrometry

Rachel R. Ogorzalek Loo,[*,†,‡] James D. Cavalcoli,[§] Ruth A. VanBogelen,[§] Charles Mitchell,[†,∥] Joseph A. Loo,[§] Brian Moldover,[§,⊥] and Philip C. Andrews[*,†]

Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan 48109-0606, and Pfizer Global Research and Development, 2800 Plymouth Road, Ann Arbor, Michigan 48105

Imaging with MALDI Virtual 2-D Gels

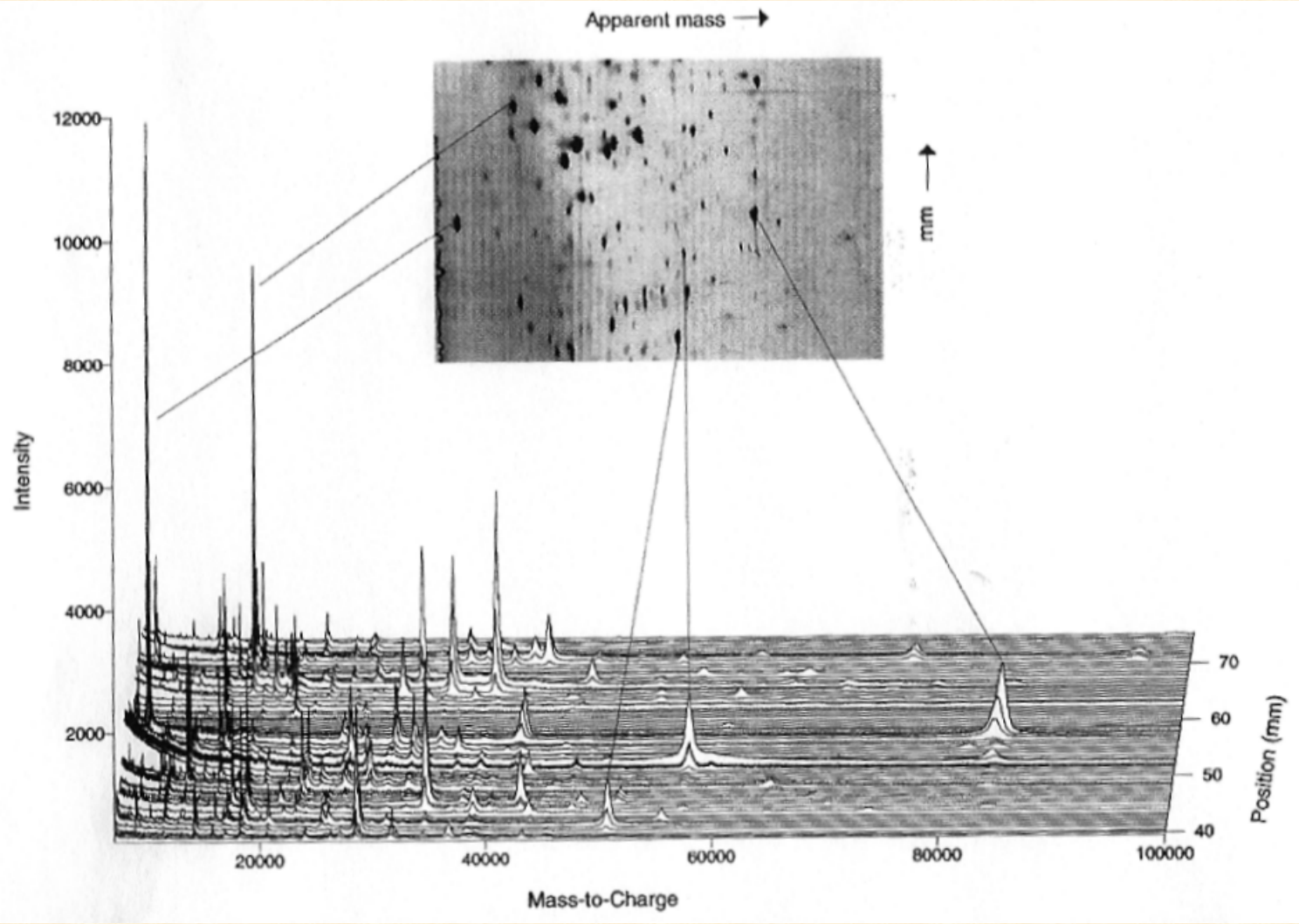


Figure 2. Mass spectra assembled from discrete scans across the pH 6.0–5.5 range (40–75 mm from basic edge) of an IPG gel (10-μg load) and correspondence to a silver-stained 2-D gel (10-μg load) across the same pH gradient.

Affinity Surfaces for MALDI

- Molecular selective or specific concentration and cleanup of analytes from complex mixtures
- Simplifies sample handling and analysis

Advances in surface plasmon resonance biomolecular interaction analysis mass spectrometry (BIA/MS)

JOURNAL OF MOLECULAR RECOGNITION
J. Mol. Recognit. 1999;**12**:77–93

Randall W. Nelson[1]* and Jennifer R. Krone[2]

[1]Intrinsic Bioprobes Inc., 2009 E. 5th Street, Ste. 11, Tempe, AZ 85281, USA
[2]Lovelace Respiratory Research Institute, Inhalation Toxicology Laboratory, P.O. Box 5890, Albuquerque, NM 87185, USA

Electrophoresis 2000, *21*, 1164–1167

Recent advancements in surface-enhanced laser desorption/ionization-time of flight-mass spectrometry

Maggie Merchant
Scot R. Weinberger
Ciphergen Biosystems, Inc.,
Palo Alto, CA, USA

Affinity Surfaces for MALDI

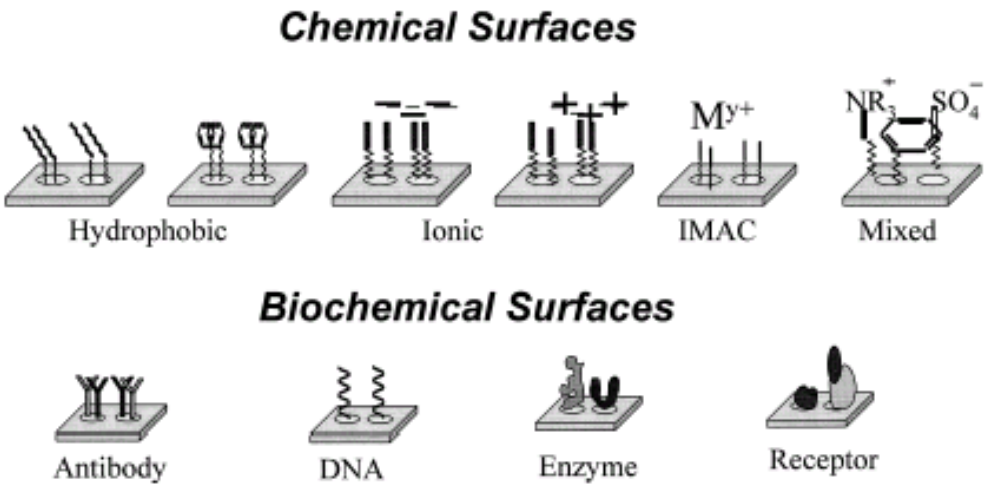


Figure 1. SELDI ProteinChip surfaces. See Section 2 for details.

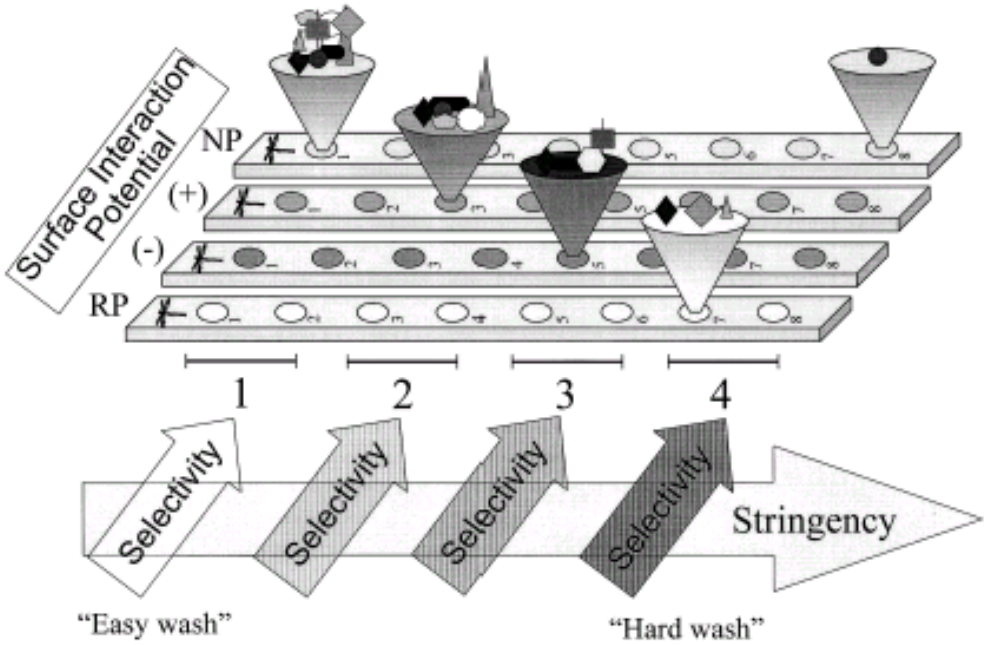
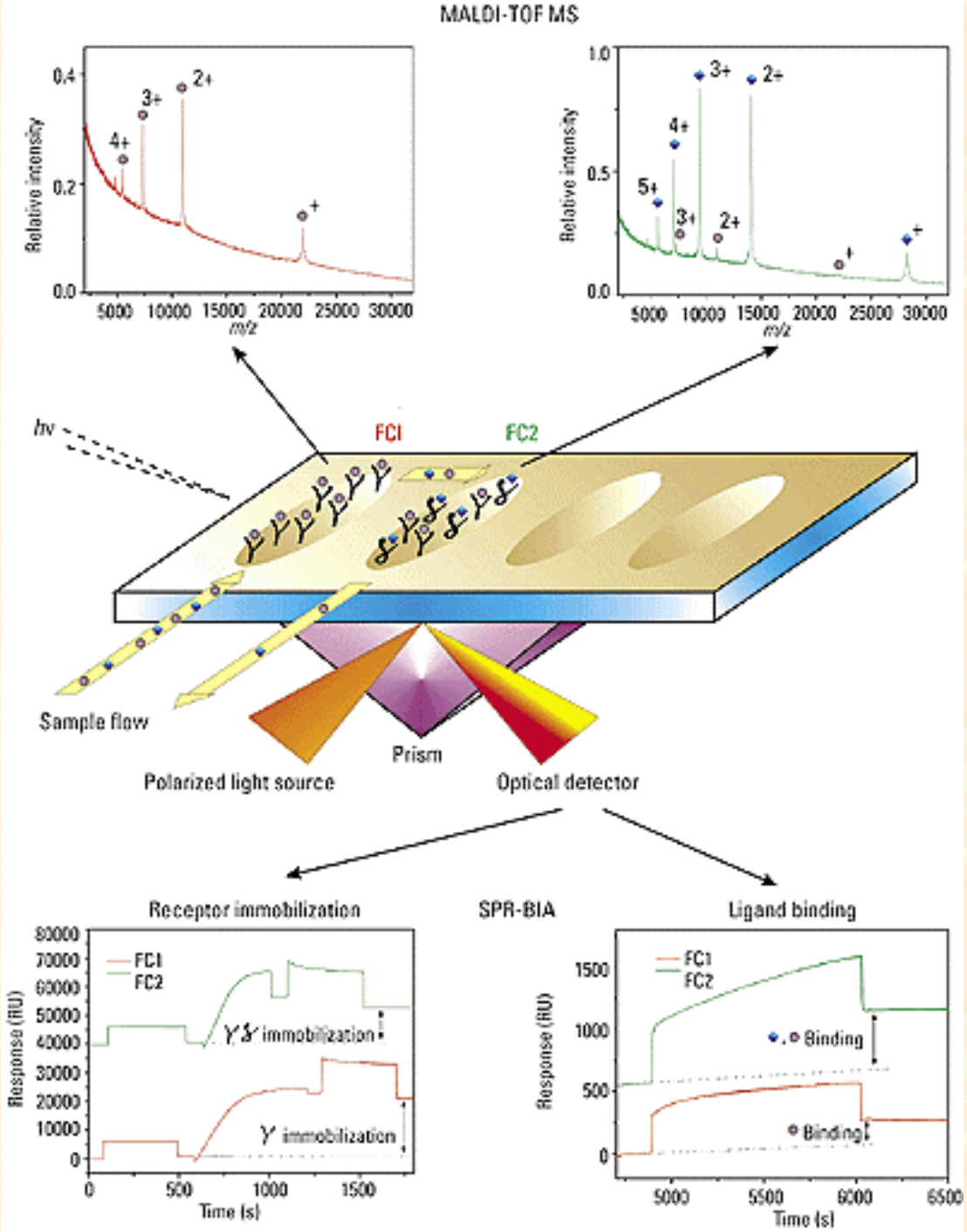


Figure 2. SELDI on-chip sample fractionation. Four ProteinChip Arrays, each with distinct affinity capture properties, are aligned side by side. Each array contains eight specific regions or "spots" with defined surface chemistry. The large circles show schematically that, due to the specific surface chemistries, different proteins are retained on the different arrays. Differential washing influences selectivity on a given array. See Section 2 for further details.

Affinity Surfaces for MALDI

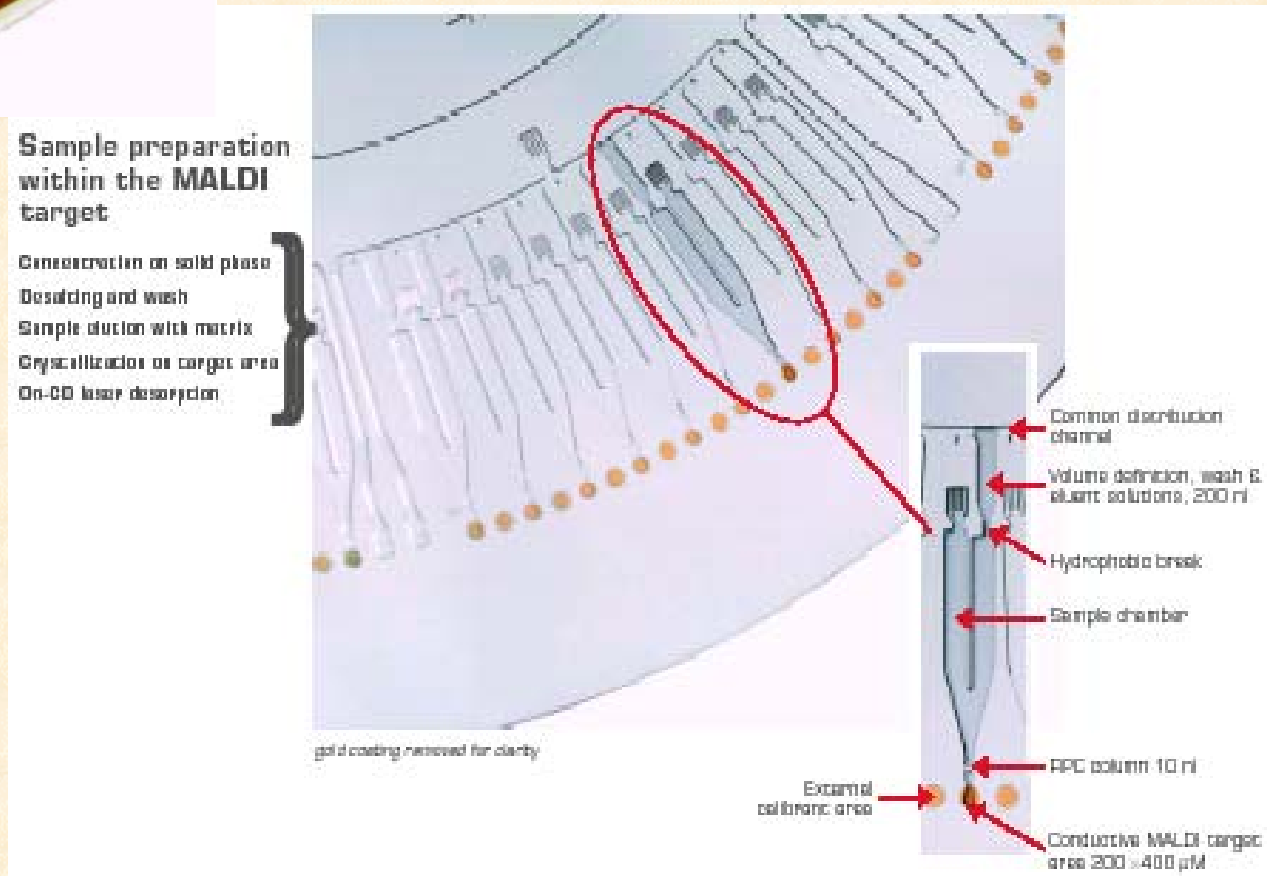


Automated Sample Preparation for MALDI

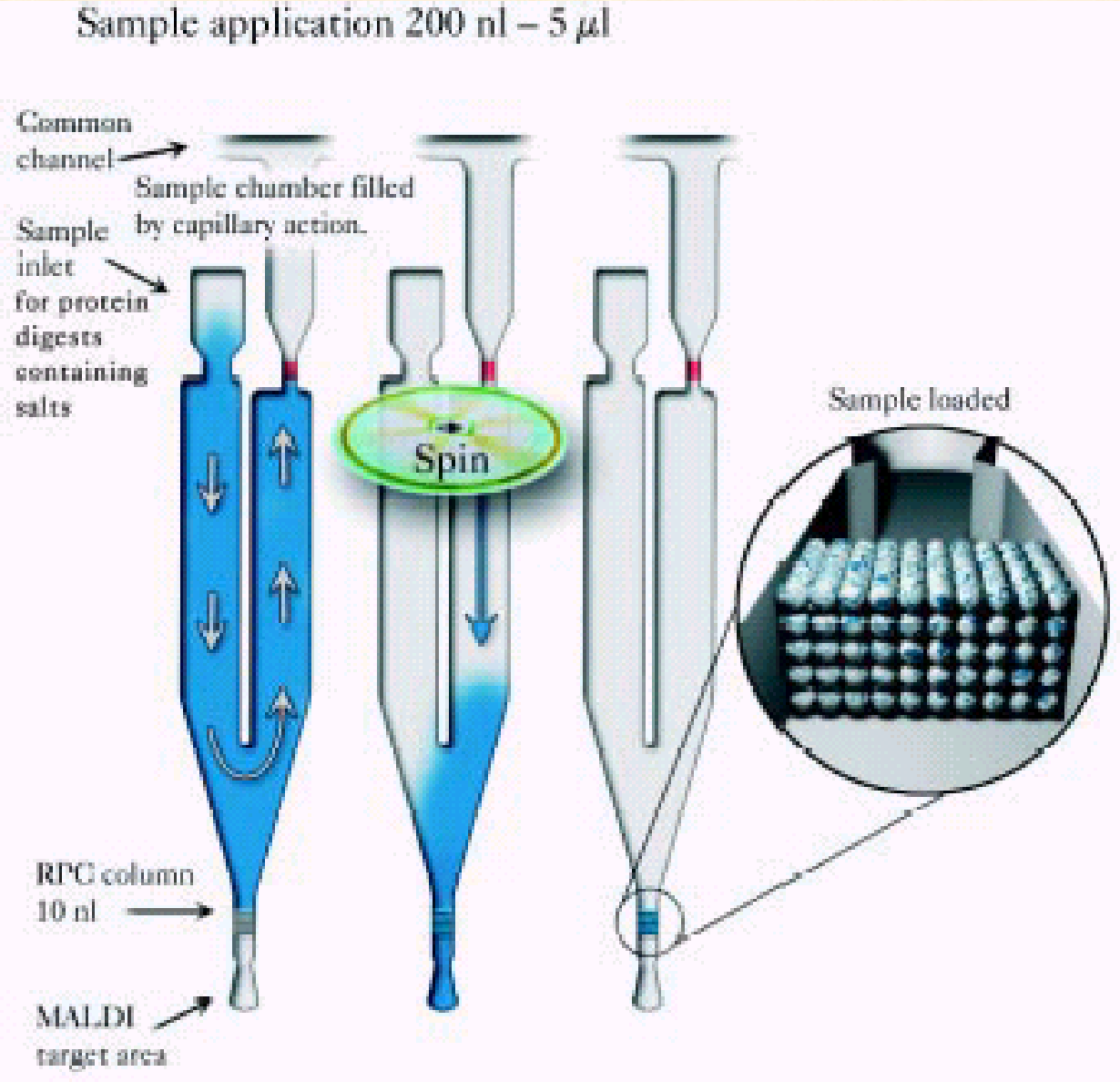
Gyros AB

Uppsala Science Park

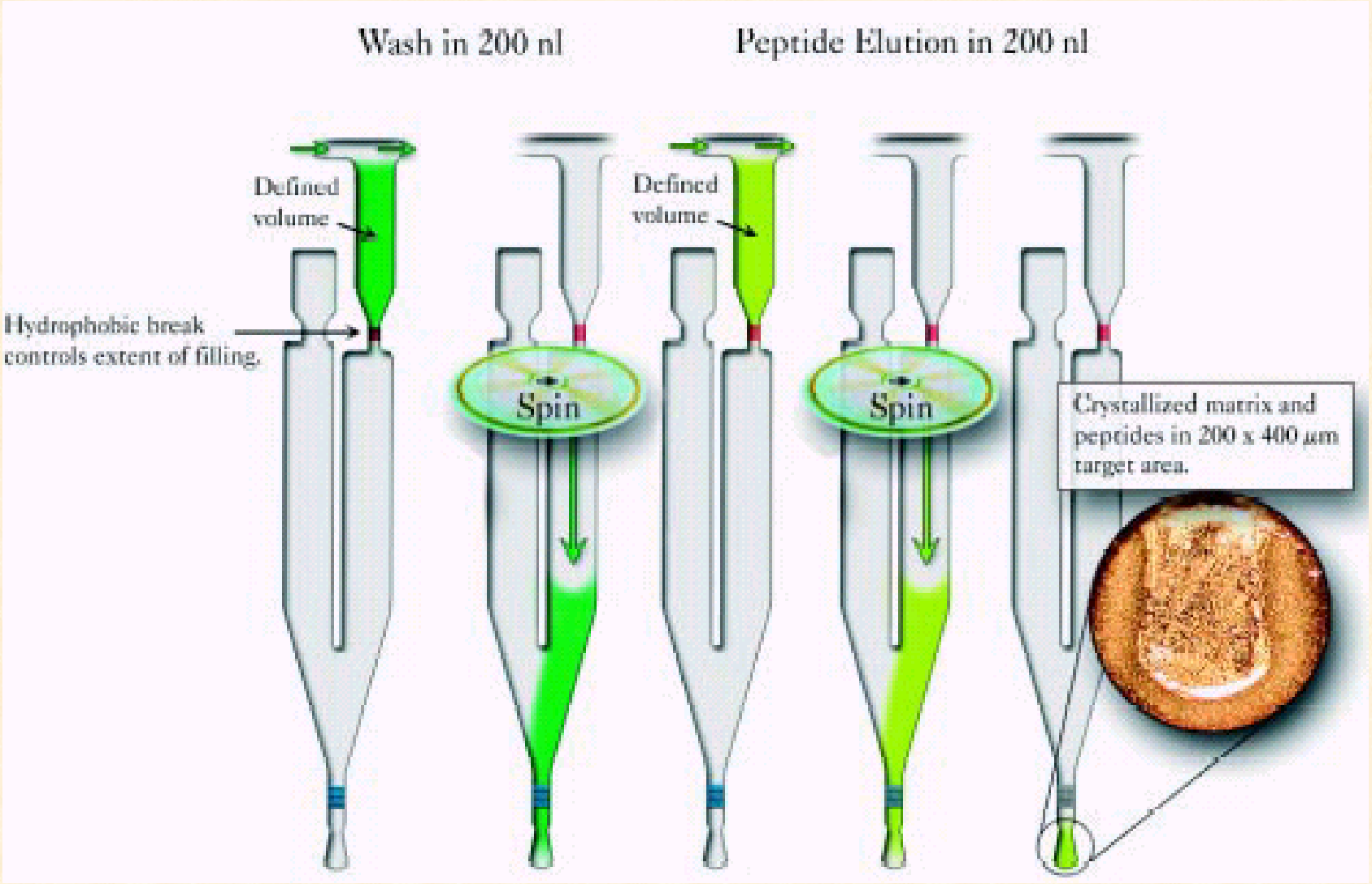
SE-751 B3 Uppsala, Sweden



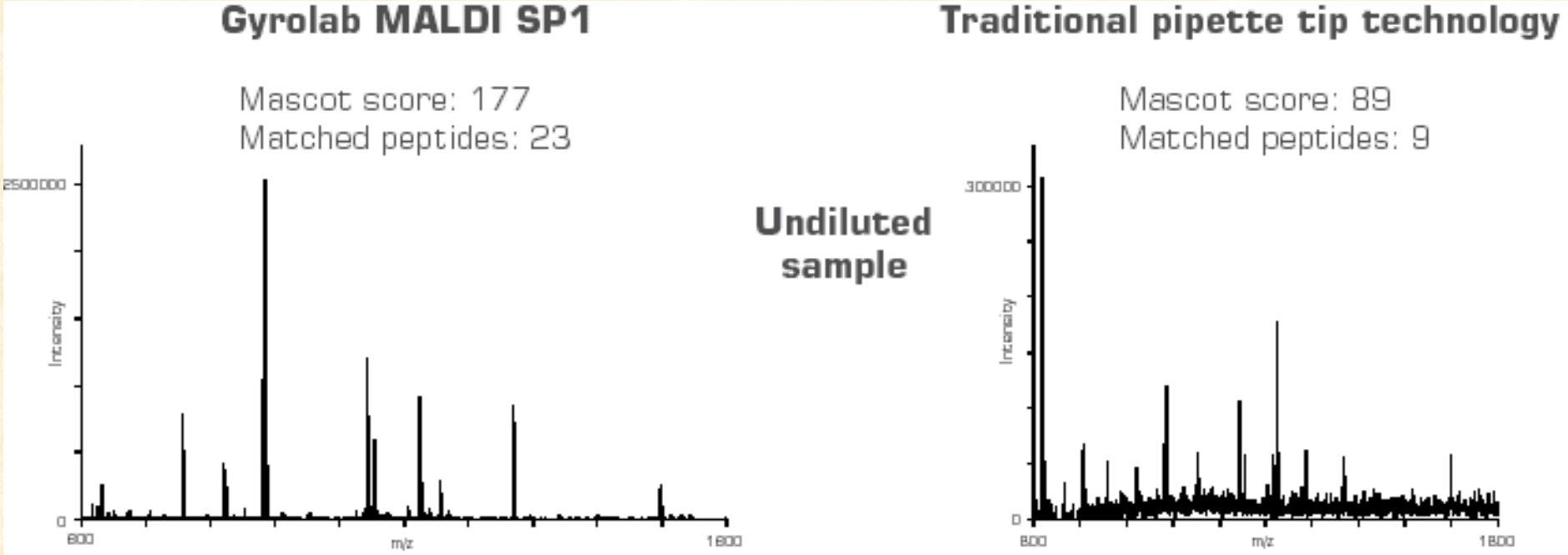
Automated Sample Preparation for MALDI



Automated Sample Preparation for MALDI



Automated Sample Preparation for MALDI



By miniaturizing sample preparation steps into an integrated and automated process within Gyrolab MALDI SP1, we were able to routinely achieve results close to the detection limit for MALDI mass spectrometry instruments. Improved sensitivity compared to traditional techniques, high reproducibility and the ability to process up to 96 samples in parallel offer a significant step towards greater productivity and a higher success rate for protein identification.

Electrospray Ionization

- Electrochemistry of Electrospray
- Surface Sampling
- Dueling Ion Sources
  - Alternate Ionization Modes (ES/APCI)
  - Ion/Ion Reactions
- Emitter Arrays

Electrospray Electrochemistry

- EC is inherent to operation of ES ion source
- Devise means to avoid or exploit EC of ES

JOURNAL OF MASS SPECTROMETRY
J. Mass Spectrom. 2001; **36**: 204–210

Minimizing analyte electrolysis in an electrospray emitter

Vilmos Kertesz and Gary J. Van Berkel*

Chemical and Analytical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37381-6365, USA

JOURNAL OF MASS SPECTROMETRY
J. Mass Spectrom. 2001; **36**: 1125–1132

Redox buffering in an electrospray ion source using a copper capillary emitter†

Gary J. Van Berkel[1]* and Vilmos Kertesz[2]

[1] Organic and Biological Mass Spectrometry Group, Chemical and Analytical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6365, USA
[2] Győr, Móra Ferenc tér 14.I/3, H-9028, Hungary

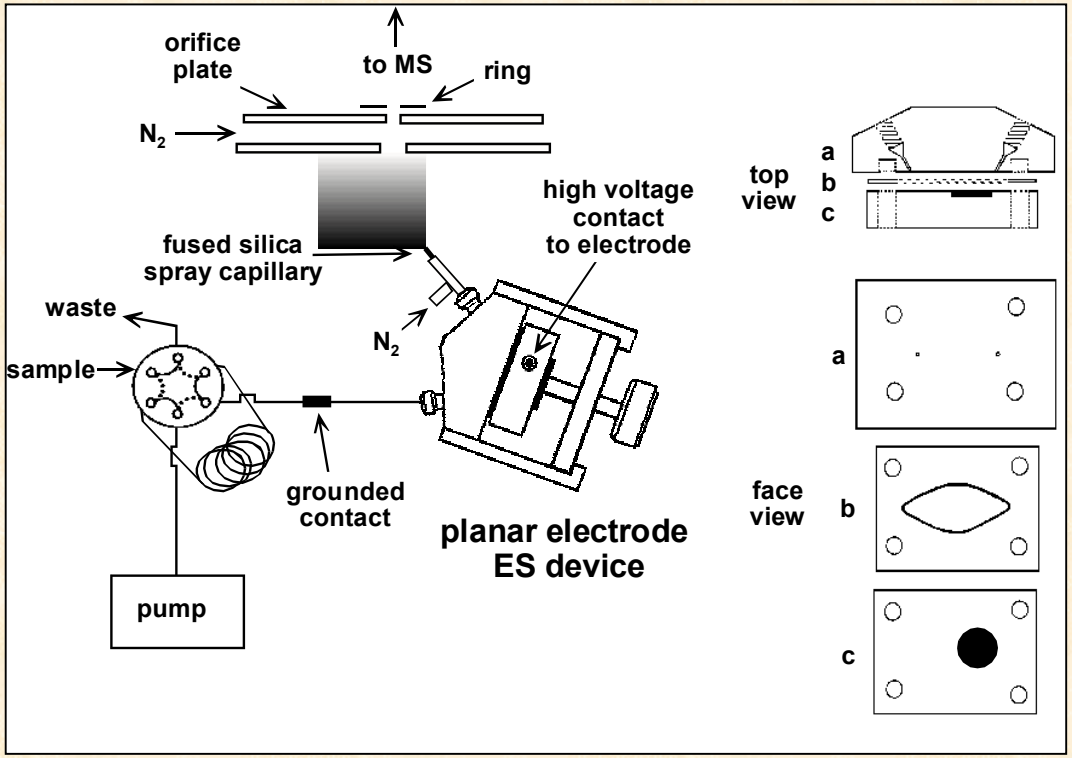
Anal. Chem. **2002**, *74*, 5047–5056

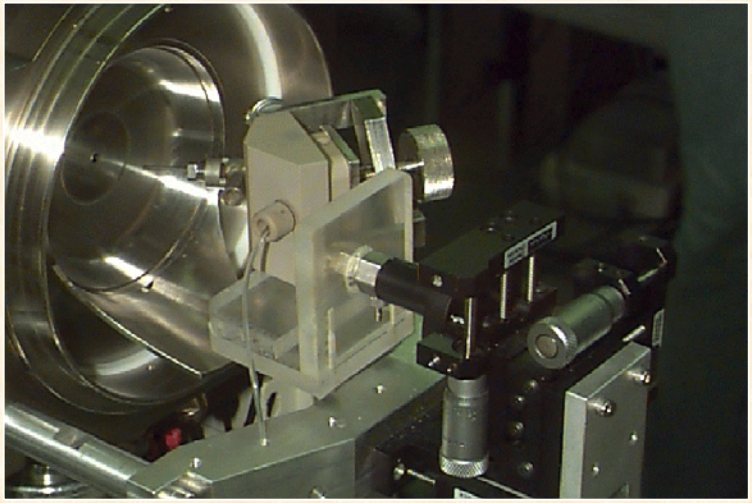
Enhanced Study and Control of Analyte Oxidation in Electrospray Using a Thin-Channel, Planar Electrode Emitter

Gary J. Van Berkel,* Keiji G. Asano, and Vilmos Kertesz†

Organic and Biological Mass Spectrometry Group, Chemical Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831-6365

Thin-Channel Electrode Electrospray Emitter



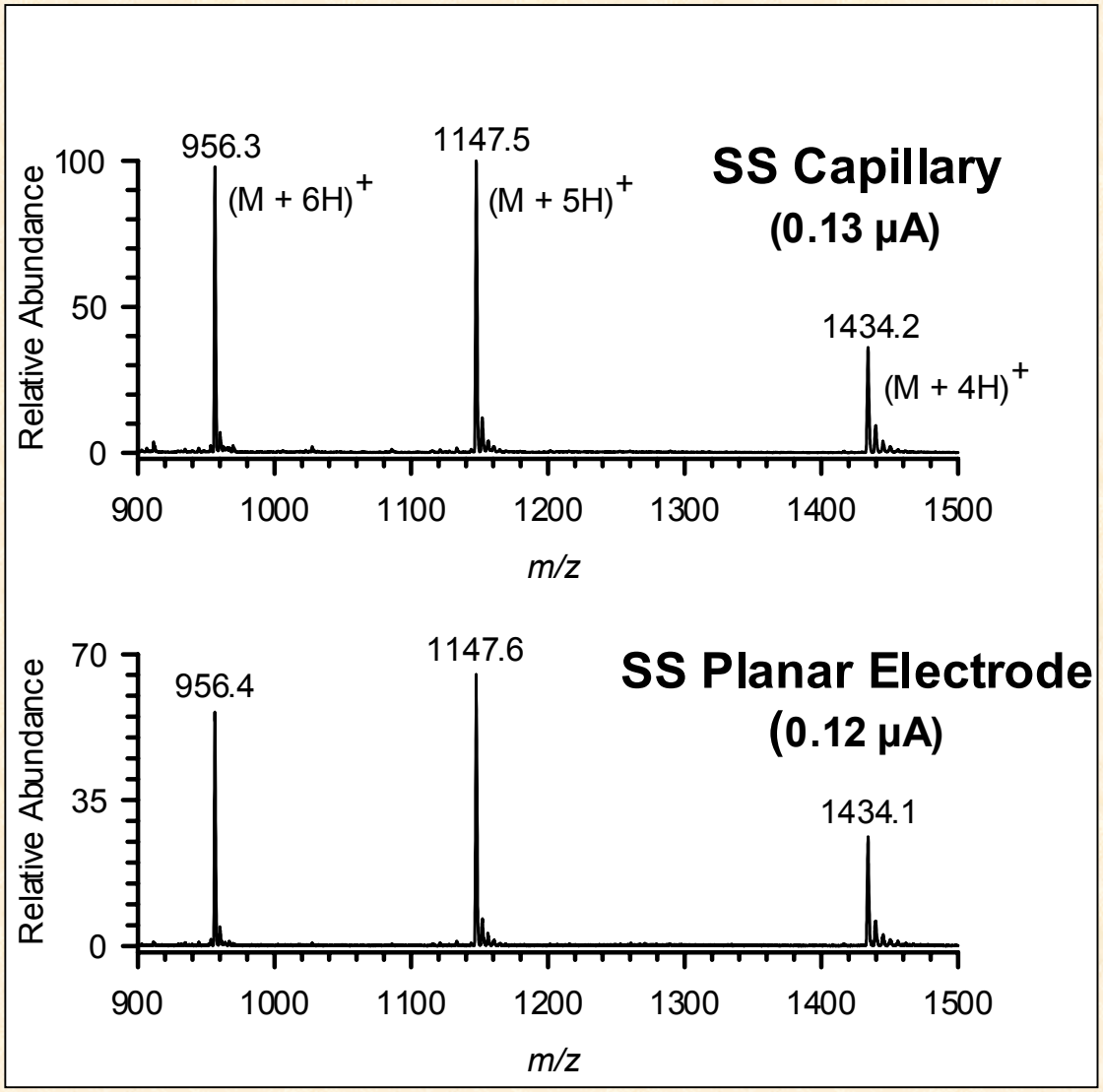


Facilitates the study and utilization of key parameters that influence the electrochemical processes:

Easily and quickly -

- **change electrode material (SS, Pt, Au, Ag, Zn, Cu, Ni, etc.) and area,**
- **alter flow dynamics through change in electrode area, thin-layer channel thickness, or flow rate**
- **use as electrodes materials not available in tubular form (glassy carbon)**
- **make and use modified electrode surfaces (conductive polymers, catalytic electrodes)**

Thin-Channel Electrode Electrospray Emitter

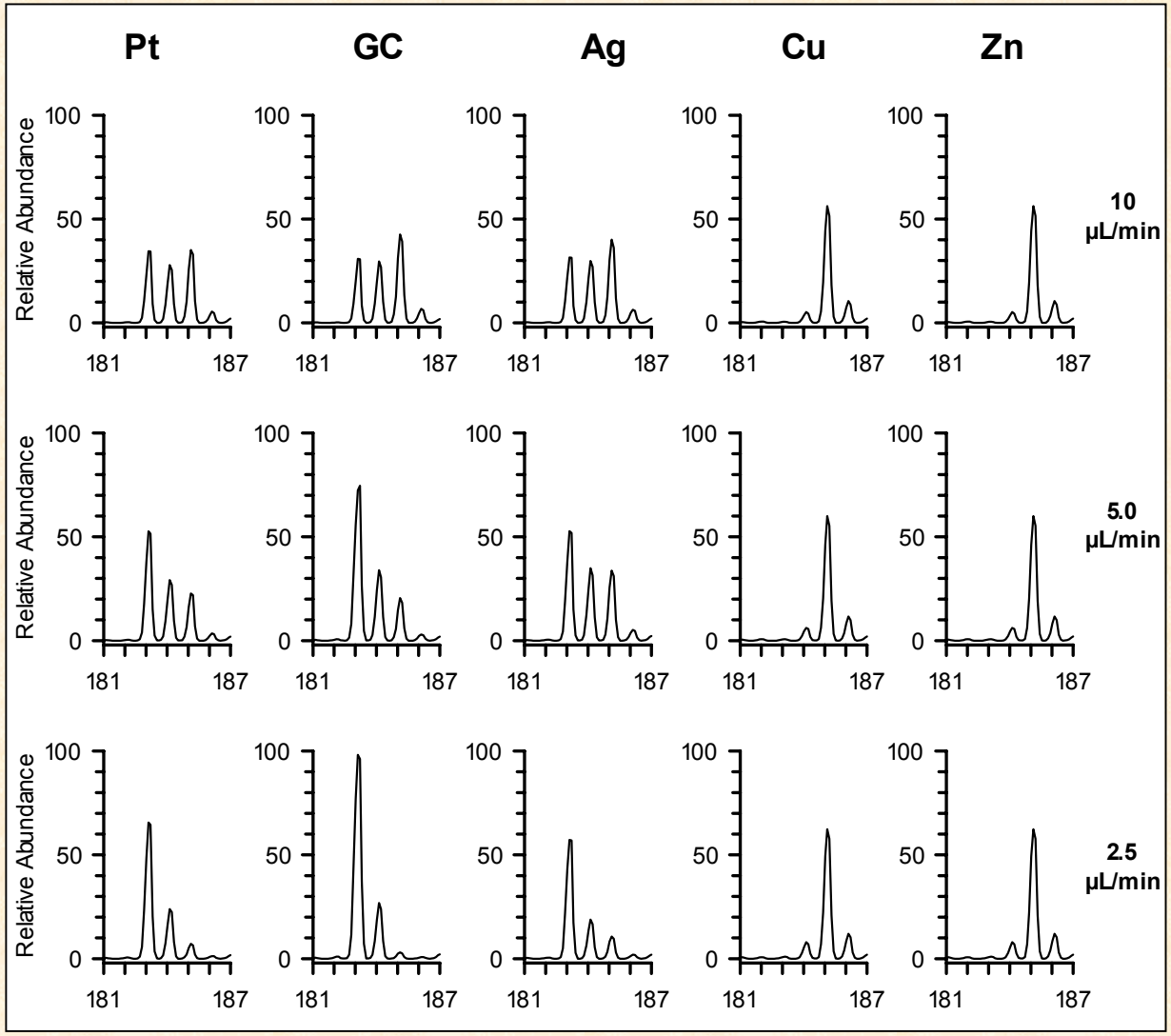


1.7 µM bovine insulin
1/1 v/v water/acetonitrile
0.1% by vol HOAc
10 µL/min, 4.5 kV

TurboIonSpray™ source with a 304 stainless steel capillary emitter electrode

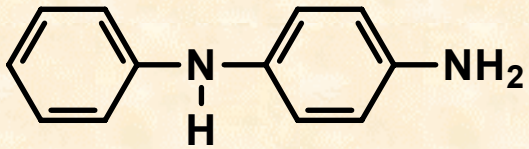
Thin-channel electrode emitter device with a 6.0 mm dia., 316L stainless steel disk electrode

Thin-Channel Electrode Electrospray Emitter

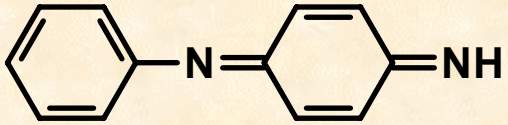


Effect of emitter electrode material on the oxidation of and signal from 20 µM *N*-phenyl-1,4-phenylenediamine

Red = Ox + $2H^+$ + $2e^-$



$(Red + H)^+$ = *m/z* 185



$(Ox + H)^+$ = *m/z* 183

Electrospray Ionization Surface Sampling

- Sampling of material at, in, or just above a surface
- Monitor electrode reaction products, components on TLC plates, in gels, etc.

Anal. Chem. **2001**, *73*, 4229–4240

Scanning Capillary Microscopy/Mass Spectrometry for Mapping Spatial Electrochemical Activity of Electrodes

Alexander D. Modestov, Simcha Srebnik, Ovadia Lev,* and Jenny Gun

Division of Environmental Sciences, Fredy and Nadine Herrmann School of Applied Science, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Anal. Chem. **2001**, *73*, 632–638

Electrospray Device for Coupling Microscale Separations and Other Miniaturized Devices with Electrospray Mass Spectrometry

Timothy Wachs and Jack Henion*

Analytical Toxicology, Department of Population Medicine and Diagnostic Sciences, New York State College of Veterinary Medicine, Cornell University, 927 Warren Drive, Ithaca, New York 14850

Anal. Chem. **2002**, *74*, 6216–6223

Thin-Layer Chromatography and Electrospray Mass Spectrometry Coupled Using a Surface Sampling Probe

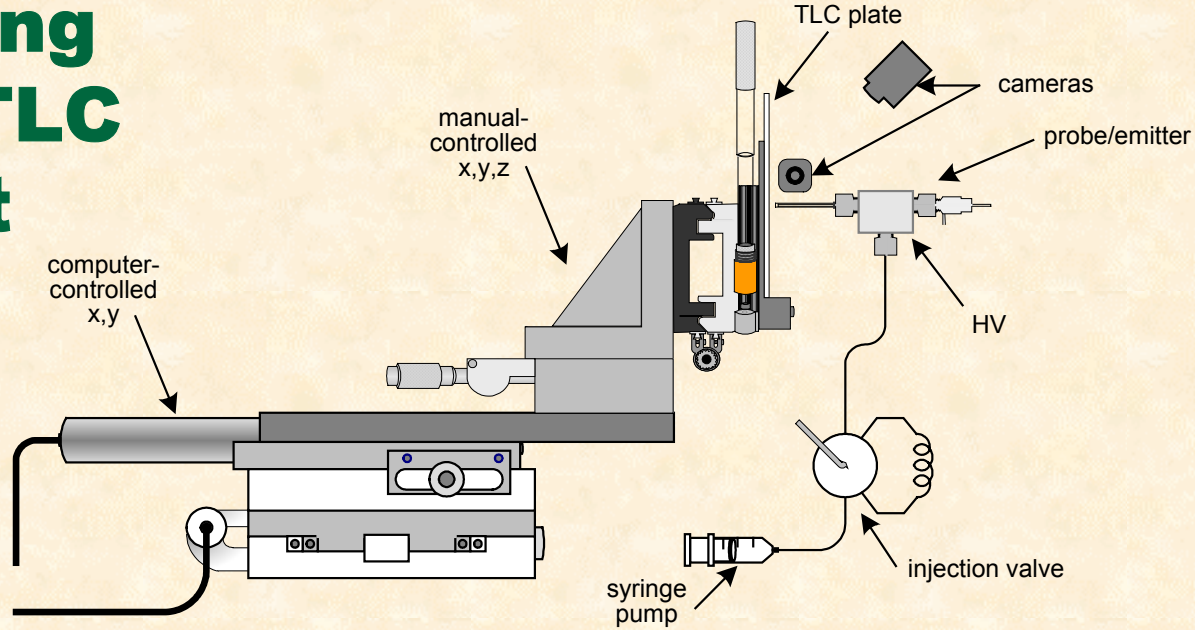
Gary J. Van Berkel*

Organic and Biological Mass Spectrometry Group, Chemical Sciences Division Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831-6131

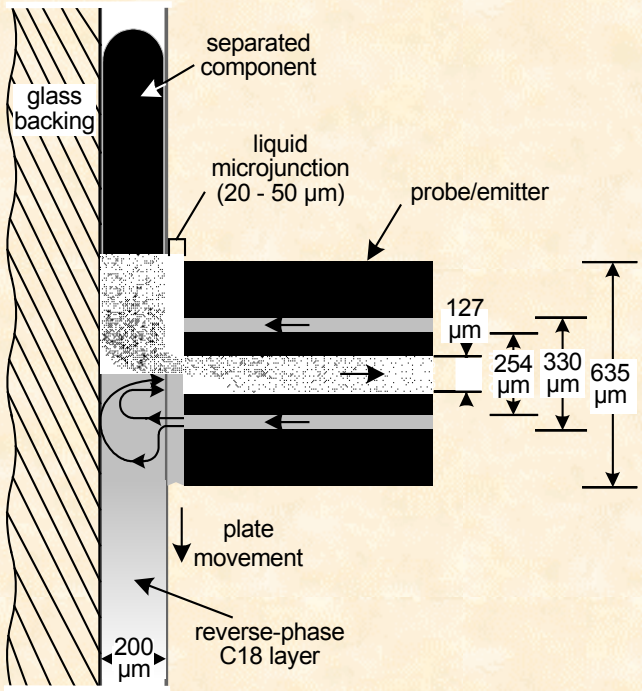
Amaury D. Sanchez and J. Martin E. Quirke

Department of Chemistry, Florida International University, Miami, Florida 33199

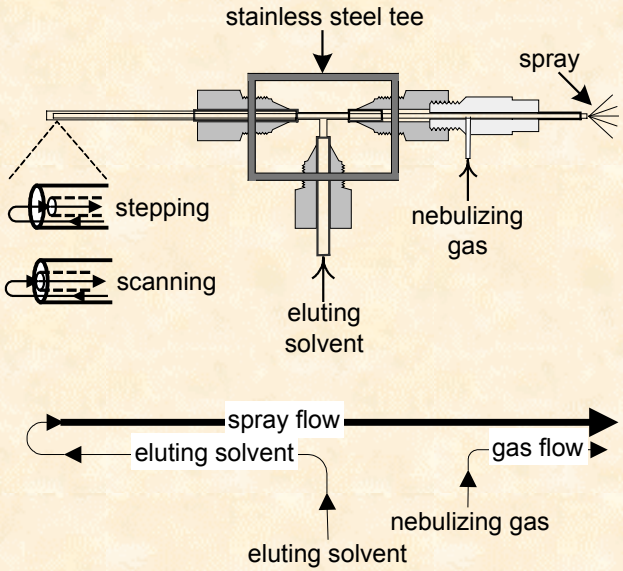
ES Sampling Probe for TLC Readout



(b) Sampling Detail

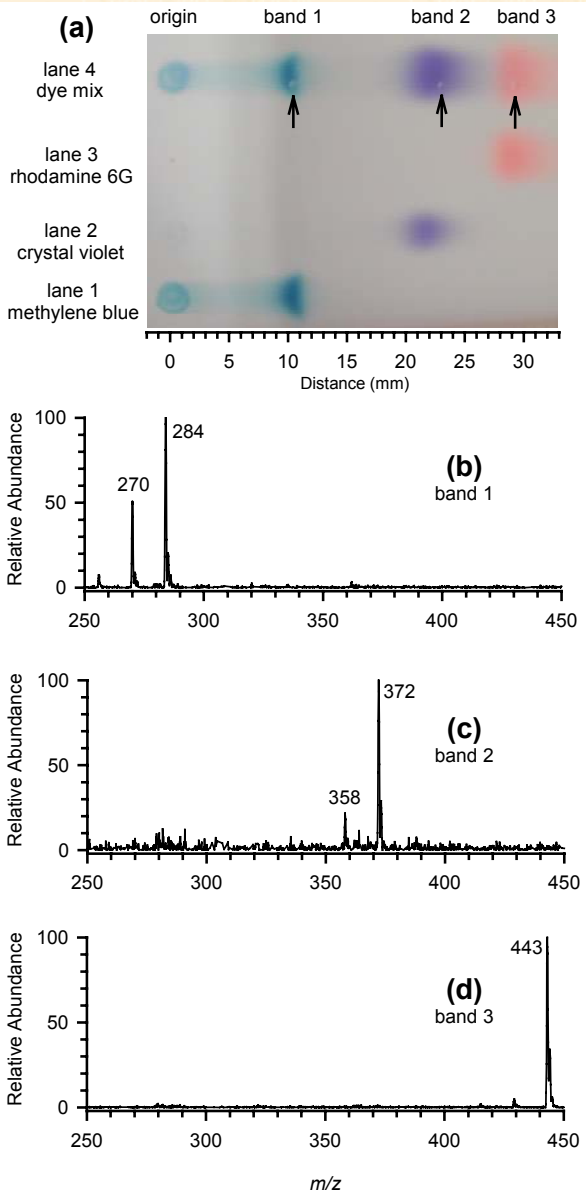


(a) Probe Detail



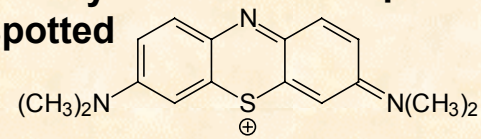
TLC/ES-MS

stepping sampling



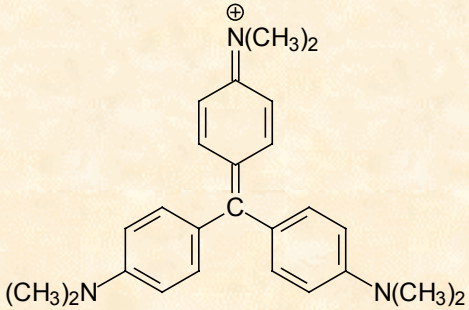
ca. 1 µg each dye spotted

ca. 250 ng each dye spotted



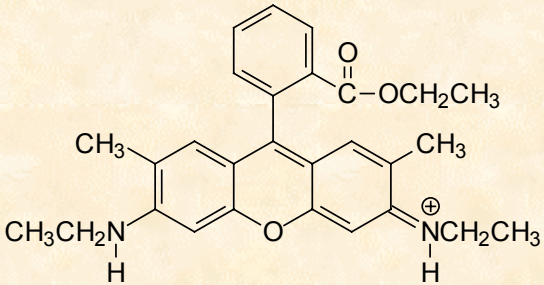
methylene blue

$(M)^+$ = *m/z* 284



crystal violet

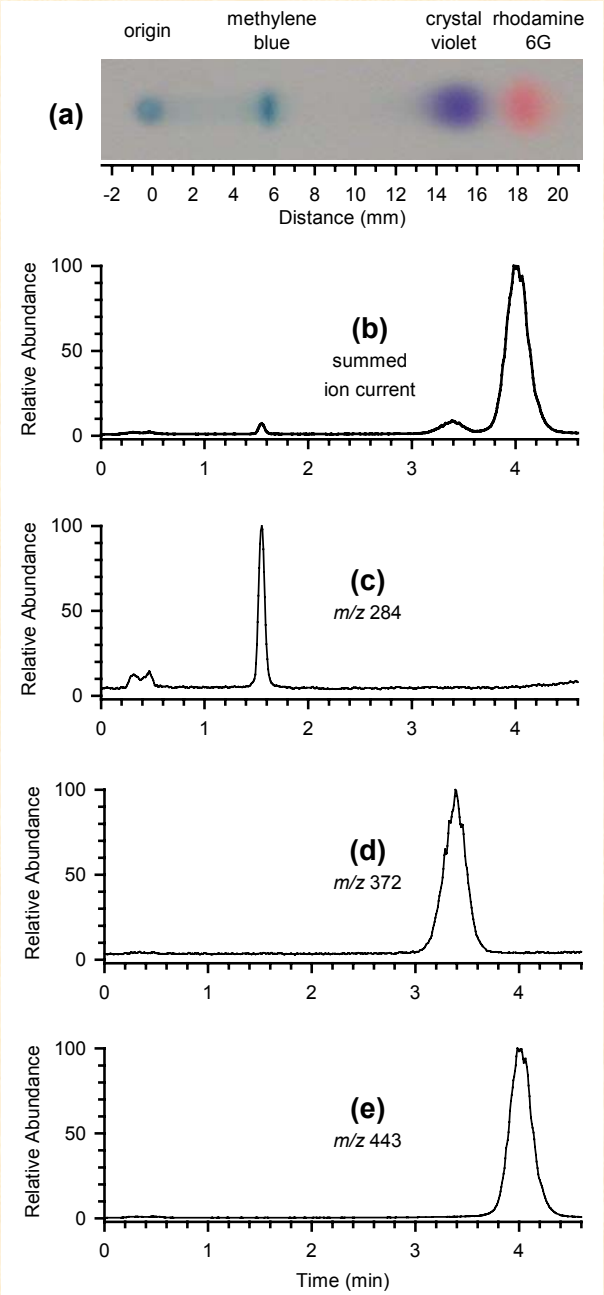
$(M)^+$ = *m/z* 372



rhodamine 6G

$(M)^+$ = *m/z* 443

scanning sampling



Dueling Ion Sources
Ion/Ion reactions

- Expanded analytical capabilities via charge state manipulation of gaseous analyte ions
  - simplification of ES spectra
  - study of reaction mechanisms
  - creation of novel ions

Anal. Chem. **2000**, *72*, 5158–5161

Corona Discharge in Charge Reduction Electrospray Mass Spectrometry

Daniel D. Ebeling, Michael S. Westphall, Mark Scalf, and Lloyd M. Smith*

Department of Chemistry, University of Wisconsin–Madison, 1101 University Avenue, Madison, Wisconsin 53706

"Dueling" ESI: Instrumentation to Study Ion/Ion Reactions of Electrospray-generated Cations and Anions

(J Am Soc Mass Spectrom 2002, 13, 614–622)

J. Mitchell Wells, Paul A. Chrisman, and Scott A. McLuckey

Department of Chemistry, Purdue University, West Lafayette, Indiana, USA

Ion/Ion reactions

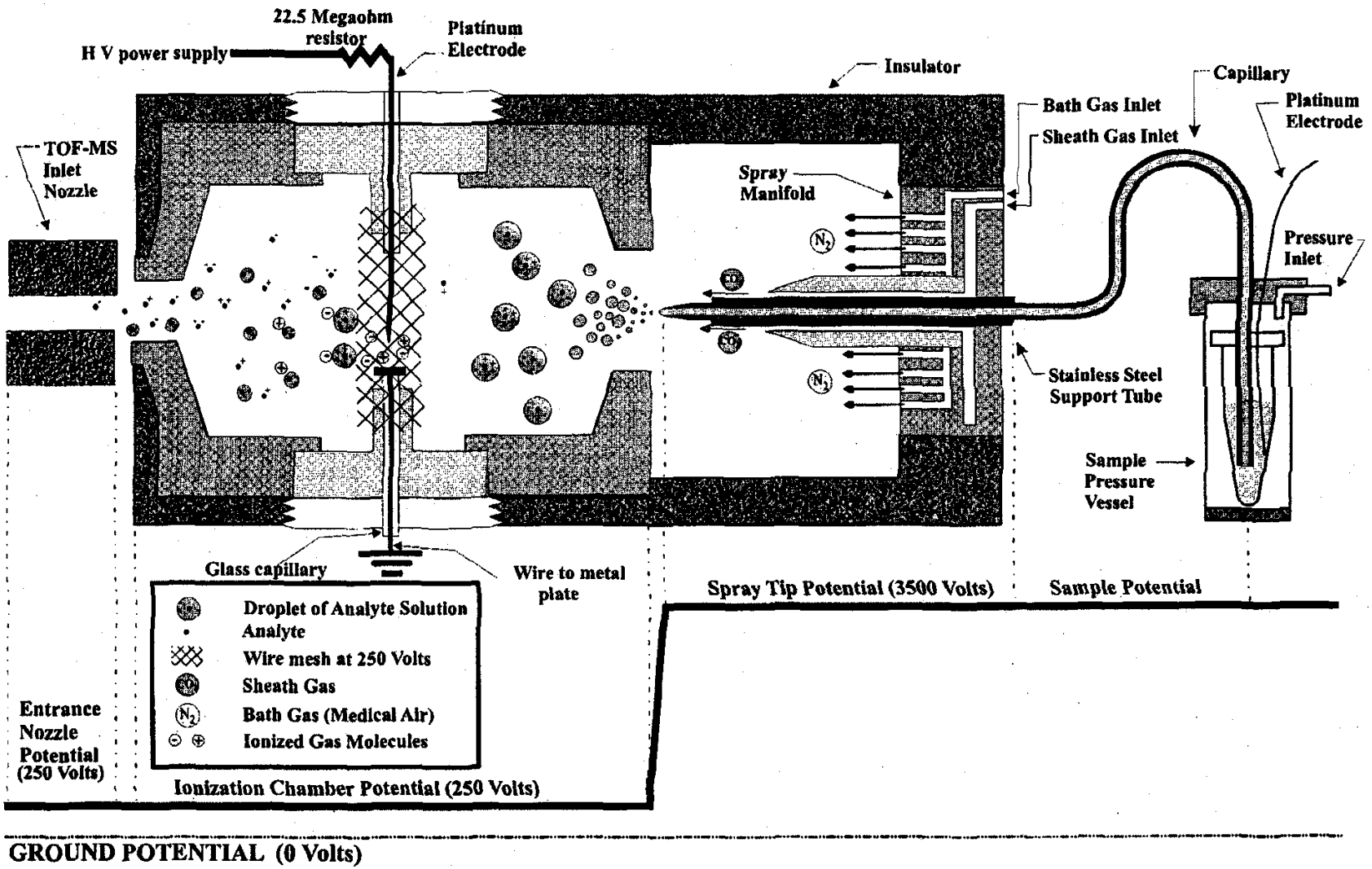


Figure 1. Schematic diagram of ESI source and corona discharge in the neutralization chamber.

Ion/Ion reactions

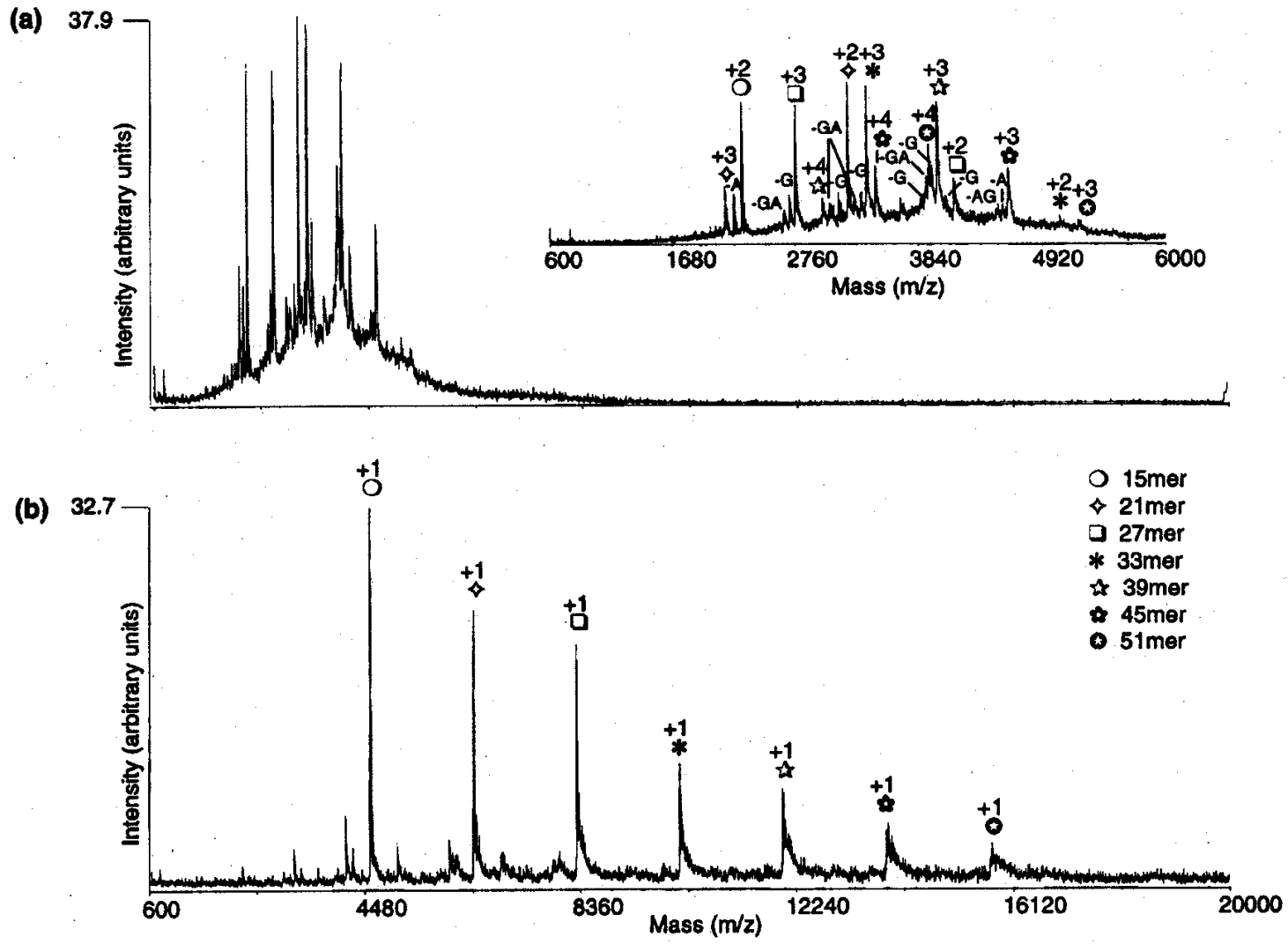


Figure 5. Positive mode analysis of an equimolar mixture of seven oligonucleotides (5 μM in 1:1 H_2O/CH_3OH, 400 mM HFIP (adjusted to pH 7 with triethylamine)[49] (a) without charge reduction and (b) with charge reduction. Both spectra are plotted on the same *m/z* scale. The inset shows an expanded view of (a) without charge reduction. The spectra were smoothed by convolution with a Gaussian function using software supplied with the spectrometer.

Ion/Ion reactions

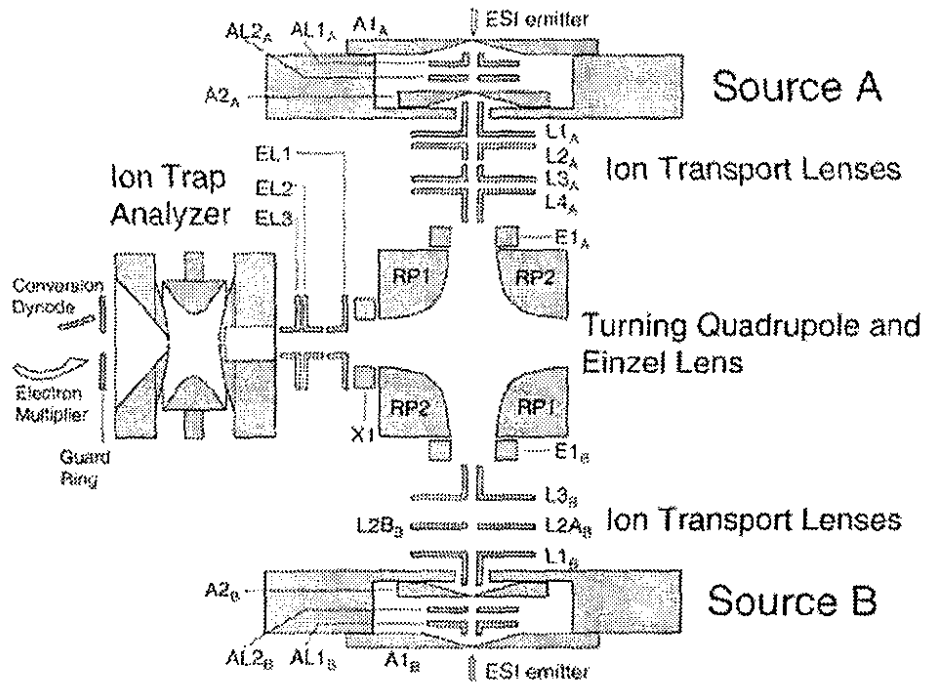


Figure 1. Schematic diagram (not to scale) of the dueling ESI ion trap mass spectrometer.

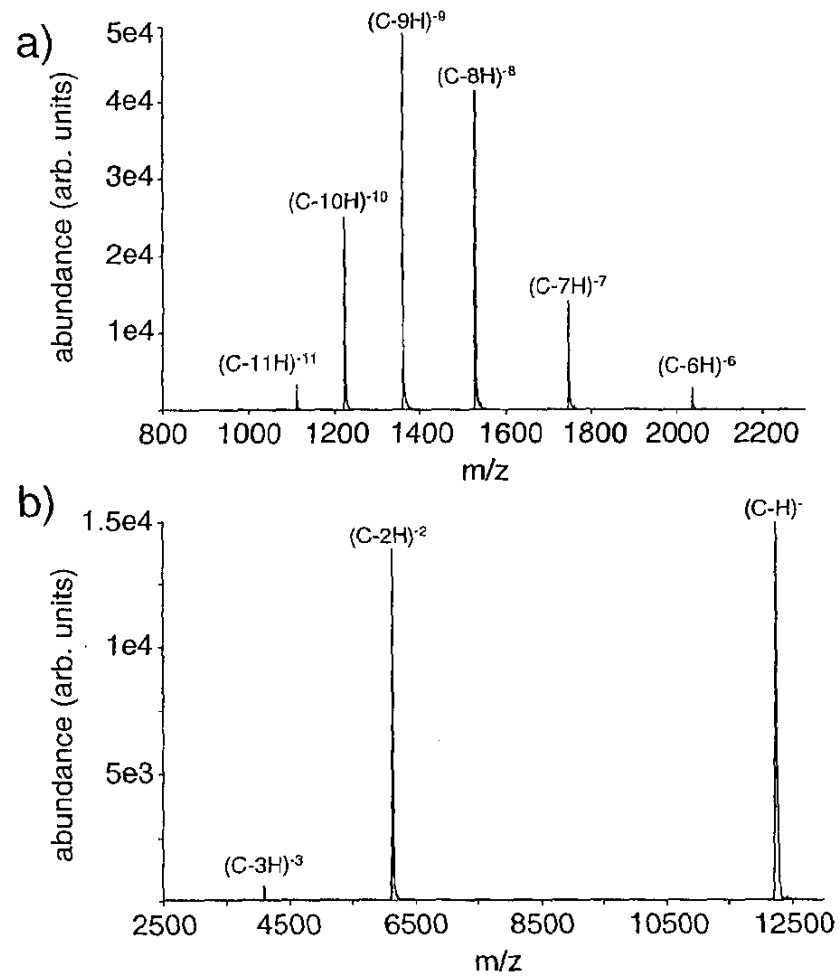


Figure 6. (a) Pre-ion/ion reaction mass spectrum of cytochrome *c* anions formed via nano-ESI from 2 % NH_4OH and injected into the ion trap for 30 ms. (b) Mass spectrum resulting from reaction of the ion population in (a) for 500 ms with protonated benzo-(f)quinoline injected for 270 ms.

Manipulation of reactions within ion trap provides:

- **Larger number of ion/ion reaction types**
- **Better defined reactions**

Electrospray Emitter Arrays

- High-throughput analysis
  - nanospray-MS
  - no carryover
  - limited sample
- Tend towards total microanalysis system using ES-MS detection
- On-chip sample
  - preparation
  - concentration
  - separation

Anal. Chem. **2001**, *73*, 1658–1663

Generation of Multiple Electrosprays Using Microfabricated Emitter Arrays for Improved Mass Spectrometric Sensitivity

Keqi Tang, Yuehe Lin, Dean W. Matson, Taeman Kim, and Richard D. Smith*

Environmental Molecular Science Laboratory, Pacific Northwest National Laboratory, P.O. Box 999, Richland, Washington 99352

Anal. Chem. 2000, *72*, 3303–3310

Development of Multichannel Devices with an Array of Electrospray Tips for High-Throughput Mass Spectrometry

Hanghui Liu,† Chantal Felten, Quifeng Xue,‡ Bailin Zhang, Paul Jedrzejewski, Barry L. Karger,* and Frantisek Foret*

Barnett Institute and Department of Chemistry, Northeastern University, Boston, Massachusetts 02115

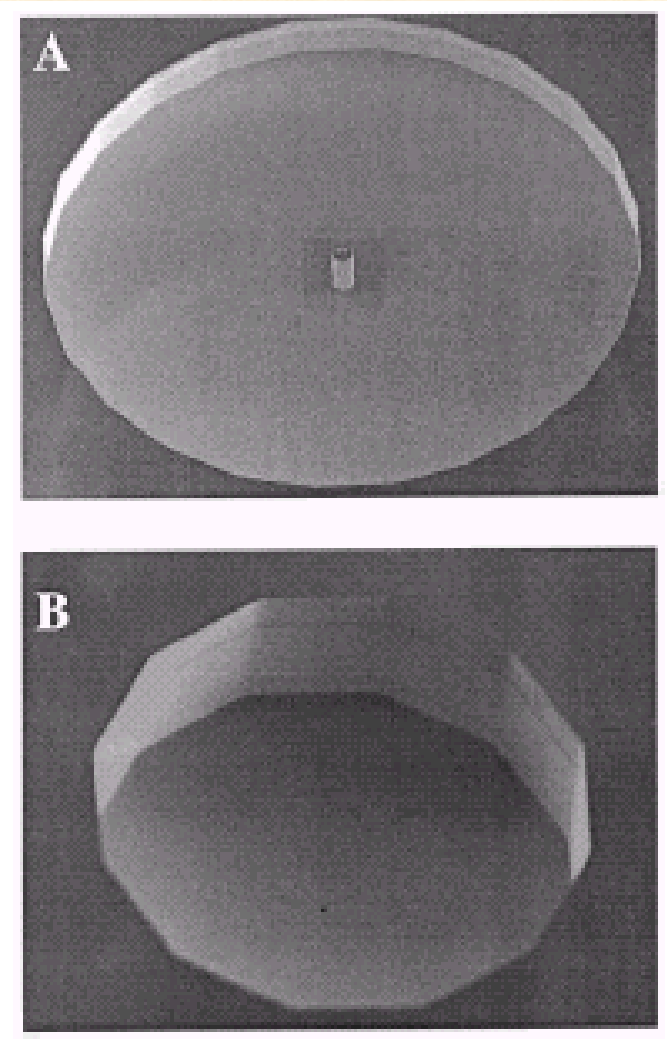
Anal. Chem. 2000, *72*, 4058–4063

A Fully Integrated Monolithic Microchip Electrospray Device for Mass Spectrometry

Gary A. Schultz,* Thomas N. Corso, Simon J. Prosser, and Sheng Zhang

Advanced BioAnalytical Services, Inc., 30 Brown Road, Ithaca, New York 14850

Electrospray Emitter Arrays



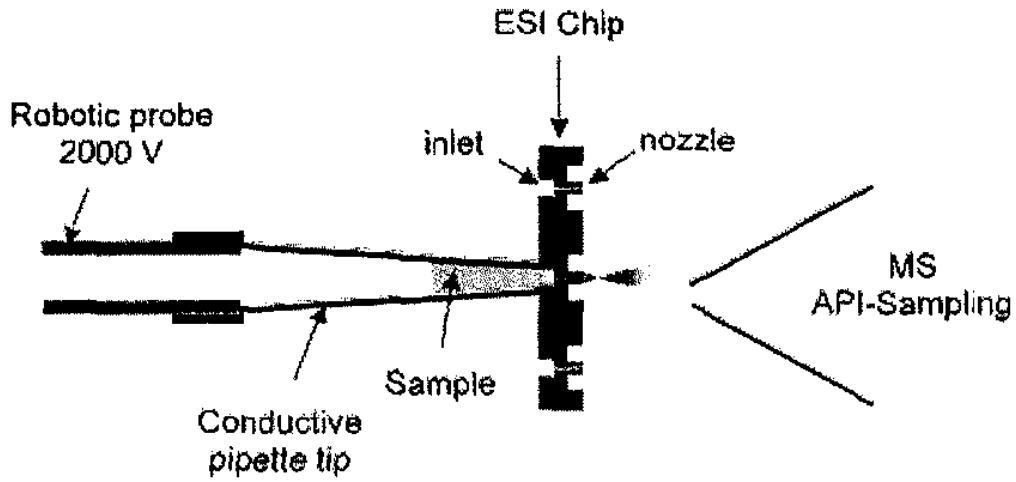
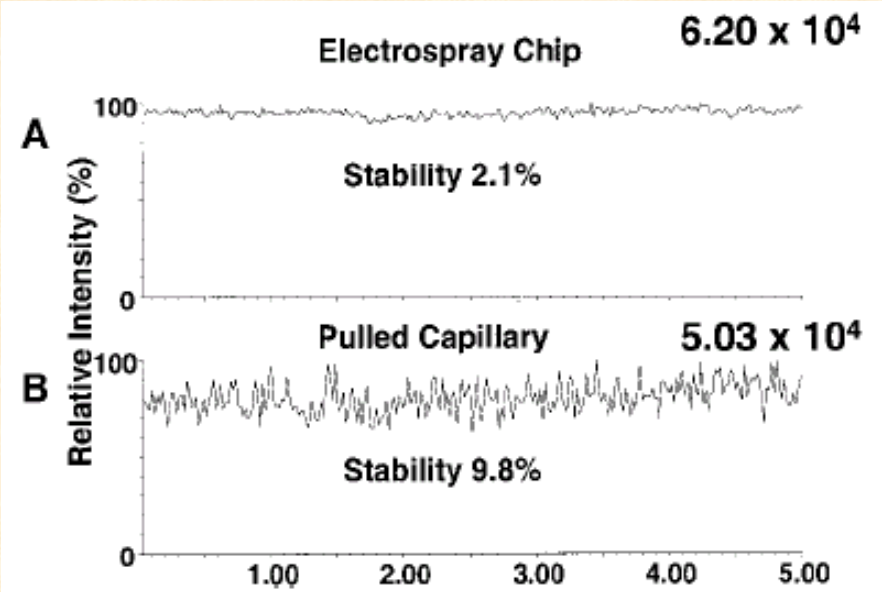


Figure 2. Illustration showing the interface between the pipet tip sample delivery system and the ESI Chip. A robotic probe delivers sample (up to 10 μL) through a conductive pipet tip, which interfaces directly to the back plane of the ESI Chip. Voltage required for nanoelectrospray along with a slight positive pressure (N_2) is delivered to the sample through the robotic probe. The ESI Chip was positioned near the atmospheric pressure ionization (API) sampling orifice of a triple quadrupole mass spectrometer.

- **One well/emitter per sample**
- **Samples brought to chip/analyzed serially**

Electrospray Emitter Arrays



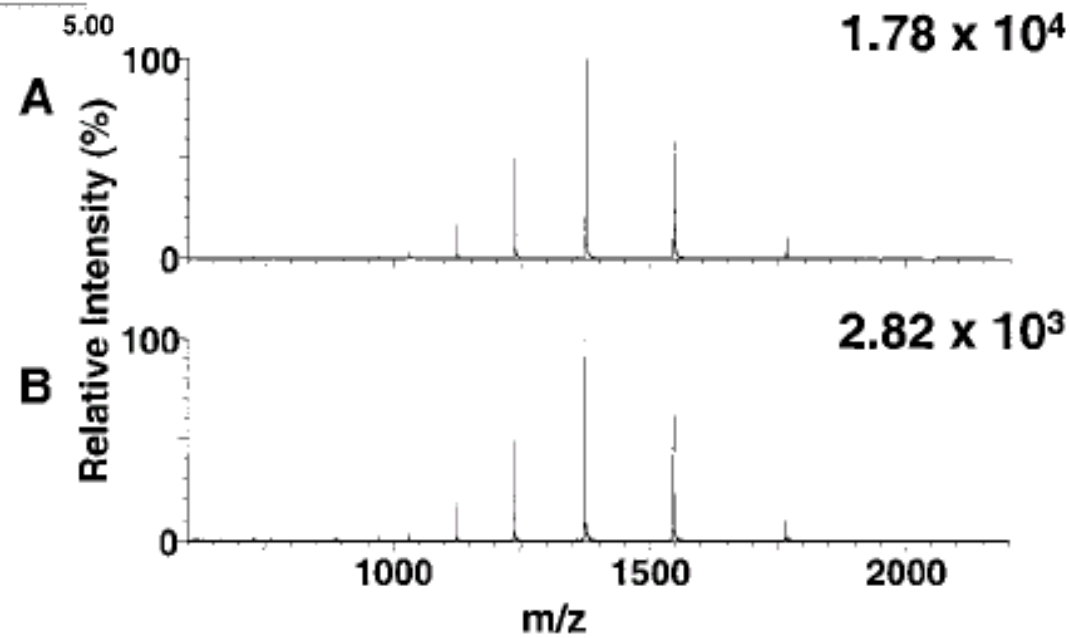


Figure 8. (A) Mass spectrum of 100 nM cytochrome *c* in water from a microchip electrospray device resulting from summing one minute of data. (B) 10 nM cytochrome *c* in water from a microchip electrospray device resulting from summing of one minute of data.

Electrospray Emitter Arrays

- One well/ emitter per sample
- All samples loaded on chip
- Samples analyzed serially

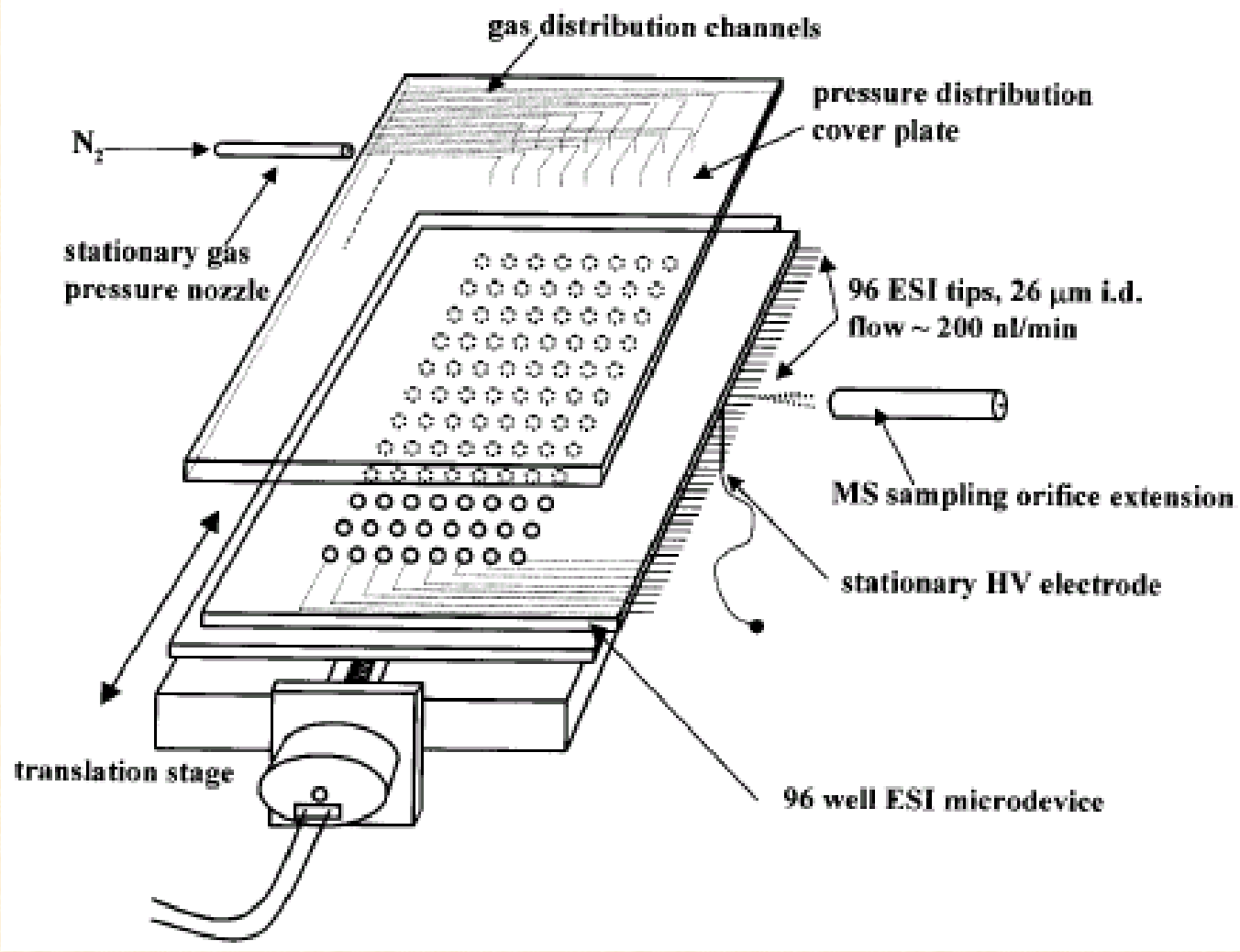


Figure 3. Exploded view of the total system design. The 96-well plate with individual channels and electrospray tips was positioned on a translation stage in front of the extension of the MS sampling orifice. The electrospray analysis of individual samples was activated by sequential pressurization of the sample wells through the pressure distribution cover plate and connection of the ESI high voltage (HV) through the stationary HV electrode positioned under the ESI device. The silicone rubber sealing gasket placed between the ESI device and the pressure distribution cover plate as well as the aluminum clamping plate was omitted for simplicity. See Experimental Section for details.

Electrospray Emitter Arrays

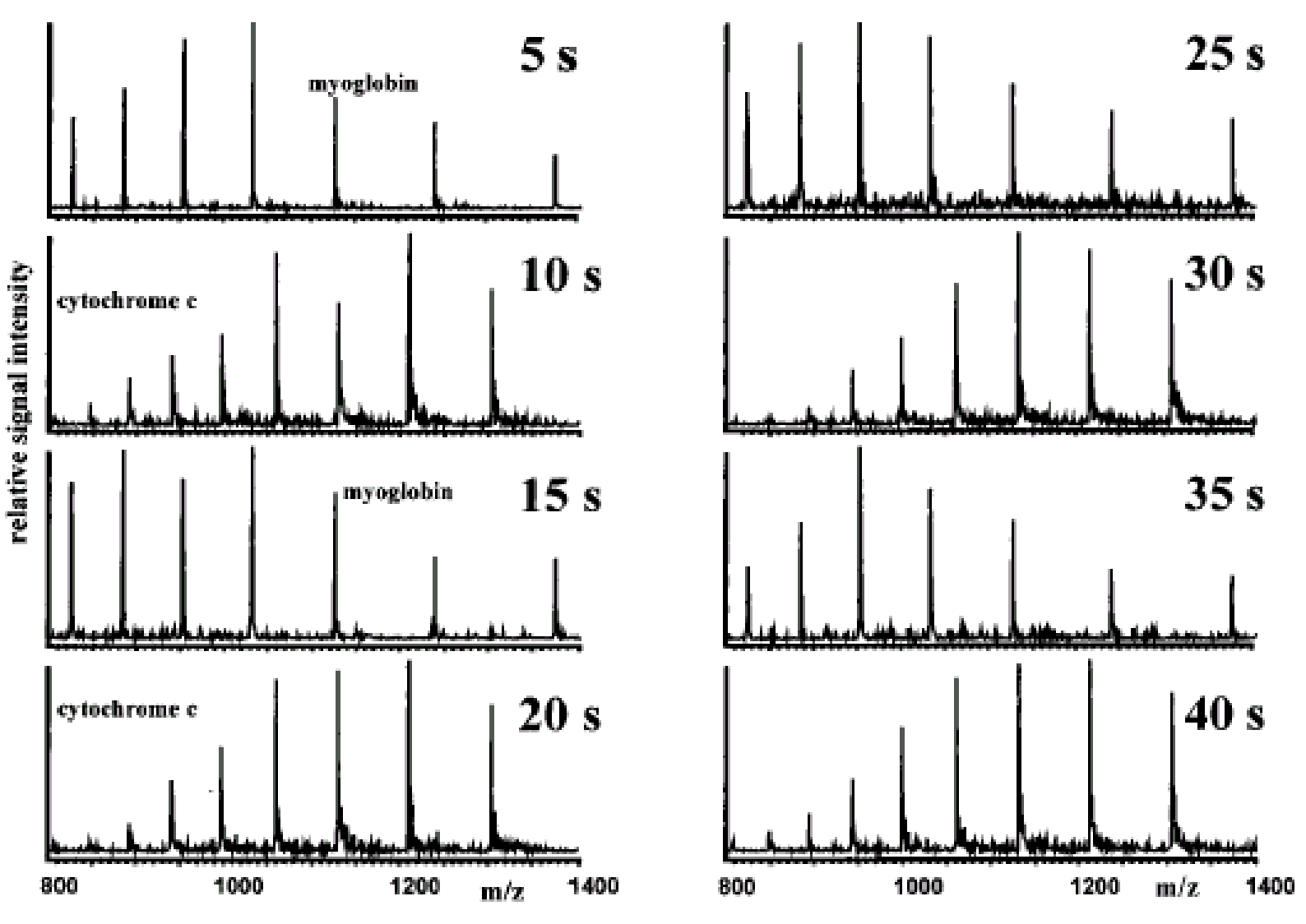


Figure 4. High-throughput ESI-MS analysis using the plastic microwell plate with 96 electrospray tips. (A) cytochrome *c* and myoglobin solutions (5 μL) were alternately loaded into consecutive sample wells, and each well was analyzed every 5 s over a 40-s time period. The concentrations for both proteins were 0.1 mg/mL.

Recent Developments in Ionization Methods for Mass Spectrometry: An Overview and Prospects

What are the prospects?

- **Totally automated sample preparation and analysis**
- **Total microanalysis systems that use mass spectrometric detection**
  - **engineering of current ion sources to small size**
- **What advances in ion sources are required to:**
  - **solve problems currently intractable using mass spectrometry?**
  - **continue the advance (versus plateau) of the field of mass spectrometry?**