Eur. Phys. J. D **38**, 225–229 (2006)
DOI: 10.1140/epjd/e2005-00282-6

THE EUROPEAN PHYSICAL JOURNAL D

Electrosprayed droplet impact/secondary ion mass spectrometry

K. Hiraoka[a], D. Asakawa, S. Fujimaki, A. Takamizawa, and K. Mori

Clean Energy Research Center, University of Yamanashi, Takeda-4, Kofu 400-8511, Japan

Received 20 July 2005
Published online 25 October 2005 –

Abstract. A new ionization method, electrosprayed droplet impact ionization (EDI), has been developed for mass spectrometry. The charged droplets formed by electrospraying 1 M acetic acid aqueous solution are sampled through an orifice with a diameter of 400 μm into the first vacuum chamber, transported into a quadrupole ion guide and accelerated by 10 kV after exiting the ion guide. The m/z of the primary droplet projectiles range from 10 000 to 50 000. The droplets impact on a dry solid sample deposited on a stainless steel substrate. No matrix was used for the sample preparation. The secondary ions formed by the impact are transported to a second quadrupole ion guide and mass-analyzed by an orthogonal TOF-MS. Intense molecular-related ions are detected for drugs, amino acids, peptides and proteins. EDI is found to be very sensitive to molecules present near the surface of the sample.

PACS. 34.50.Dy Interactions of atoms and molecules with surfaces; photon and electron emission; neutralization of ions – 36.40.Gk Plasma and collective effects in clusters – 39.10.+j Atomic and molecular beam sources and techniques

1 Introduction

Fundamentally different strategies, i.e., energy-sudden activation, nebulization, and the application of high electric fields, are employed in modern mass spectrometry for the formation of gaseous ions from condensed phase [1]. While electrospray makes use of nebulization and high electric fields, but not energy sudden activation, matrix-assisted laser desorption ionization (MALDI) is primarily based on pulsed laser-induced energy-sudden activation. Energy-sudden activation mass spectrometry methods are closely related to the concept of "limit of superheat".

The secondary ion mass spectrometry is one of the typical energy-sudden methods and has a long history. Notable among the various techniques are plasma desorption (PD) [2], and fast-atom bombardment (FAB) [3] or liquid secondary ion mass spectrometry (liquid SIMS) [4]. Various studies have related enhanced secondary ion yields with increasing mass of the incident projectile, i.e., "cluster ion sources" are more effective at desorbing molecules. Beuler and Friedman reported the use of heavy clusters $[H^+(H_2O)_n]$ with n up to 3 000 to study secondary ion emission by metal and carbon surfaces [5]. Brundle et al. [6] studied electron and ion emission from a CsI surface under the impact of molecular ions as heavy as bovine albumin (66 430 Da). The projectiles, SF_6, Cs_xI_y, gold clusters, C_{60}, and $(SO_2)_x$ enabled the acquisition of SIMS spectra with enhanced sensitivity for many polymer and organic thin films [7–11]. Massive-cluster impact (MCI), which utilizes a primary ion beam composed of large multiply charged glycerol clusters, satisfies the above features and has been shown to produce extremely soft desorption conditions for peptides and proteins [12–15].

In this article, we will report our preliminary results obtained by the newly developed electrosprayed droplet impact (EDI) ionization technique [16] which is a modified version of the MCI but much simpler than MCI in operation.

2 Experimental

The conceptual idea of the electrosprayed droplet impact (EDI) ion source coupled with an orthogonal time-of-flight mass spectrometer is displayed in Figure 1. The charged liquid droplets formed by electrospraying 1 M acetic acid aqueous solution are sampled through an orifice with 400 μm diameter into the first vacuum chamber, transported into a quadrupole ion guide and accelerated by 10 kV after exiting the ion guide. The electrosprayed droplets, i.e., the multiply-charged massive cluster ions, impact on a dry solid sample, deposited on a stainless steel substrate. The secondary ions formed by the droplet impact are transported into a second quadrupole ion guide and mass-analyzed by an orthogonal TOF-MS (JEOL, AccuTOF, Musashino, Japan). The m/z of the primary droplet projectiles was estimated by the RF voltage applied to the ion guide to be in the range of 1×10^4~5×10^4. The diameter of the beam of the electrosprayed droplets was estimated to be about 3 mm by observing the secondary ion intensities by moving the sample stage on which the sample was deposited.

[a] e-mail: hiraoka@yamanashi.ac.jp

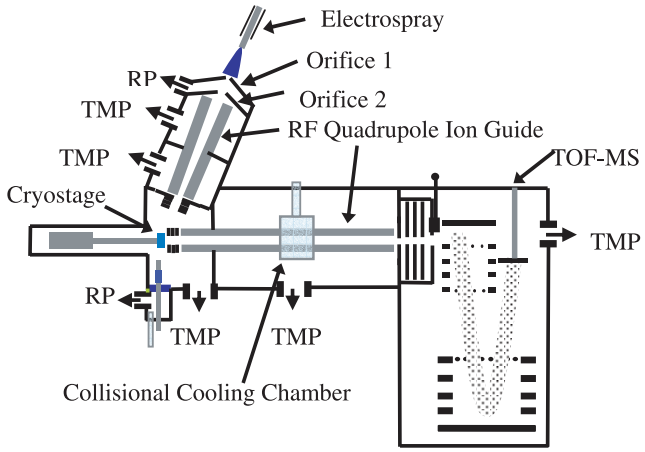

Fig. 1. Schematic diagram of the electrosprayed droplet impact (EDI)/secondary ion mass spectrometer. Electrosprayed droplets are sampled through an orifice (400 μm in diameter) and transported into the quadrupole ion guide. The droplets are accelerated by 10 kV after exiting the ion guide and impact the solid thin film deposited on the stainless steel substrate. Secondary ions formed are transported into the ion guide and mass-analyzed by an orthogonal time-of-flight mass spectrometer (JEOL, Accu-TOF, Musashino, Japan). Temperature of the sample stage: 300 K. RP: rotary pump, TMP: turbo-molecular pump.

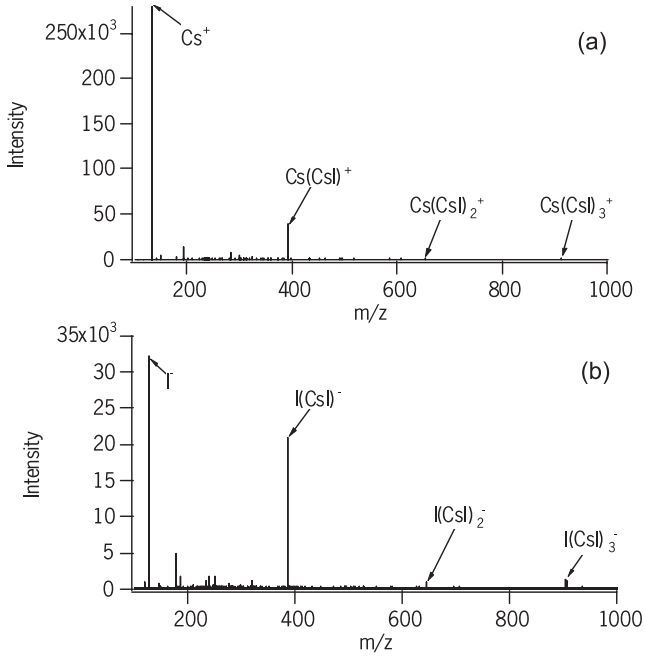

Fig. 2. EDI mass spectra from 1 nmol of dry CsI deposited on the stainless steel substrate with a diameter of 2 mm. (a) Positive-mode of operation, (b) negative-mode of operation. 1 M of acetic acid aqueous solution was electrosprayed and the charged droplets formed are sampled through a 400 μm ion sampling orifice. The electrosprayed droplets are accelerated by 10 kV and impact on the target. The m/z of the electrosprayed droplets are in the range from 10 000 to 50 000.

As a rule of thumb, about one extra charge can be carried per ∼1000 u in the globular cluster ions or peptide ions. For example, the maximum charge for lysozyme (molecular weight:14300) observed by electrospray is +8 or +9. By assuming the extra charges to be +10 in the electrosprayed charged water microdroplets with m/z 10^4, the mass of the cluster may be estimated to be $10\times10^4 = 10^5$ u. The kinetic energy of this projectile with 10 extra charges (e.g. $(10H)^{10+}(H_2O)_{6000}$) may be ∼100 keV by the acceleration with the potential of 10 kV. This corresponds to the energy per nucleon for the projectile to be ∼1 eV/u. This value is just the border line between the shock wave formation regime (≤1 eV/u) and the bond breaking and ionization regime (≥1 eV/u) [13]. The ion current of the electrosprayed droplets irradiated on the sample target was measured to be in the range of 10^{-10}∼10^{-11} A. That is, the flux of the charged droplets may be crudely estimated to be in the range of 10^7∼10^8 cm^{-2} s^{-1}.

Although the temperature of the sample stage which sits on the cold head of the cryocooler can be decreased down to 10 K, all of the present measurements were performed at ∼300 K.

3 Results and discussion

Figure 2 shows the EDI mass spectra for CsI. The $Cs^+(CsI)_n$ and $I^-(CsI)_n$ ions with $n = 0$ and 1 are observed as major ions for positive and negative mode of operation, respectively, but the cluster ions with $n \geq 2$ are almost negligible. This is a marked contrast to the fact that the cluster ions of $Cs^+(CsI)_n$ and $I^-(CsI)_n$ are observed up to m/z 10 000 and beyond when the same sample was analyzed by FAB (5 keV Xe) installed in the present apparatus. The appearance of much smaller-size cluster ions in Figure 2 clearly indicates that EDI is a much softer ionization method than FAB and only the top surface of the sample is stripped off into the vacuum by the colliding electrosprayed charged droplets. It seems likely that the impinging electrosprayed droplets pick up the top-surface fragments of solid CsI without causing the ablation of the solid.

Figure 3 displays the EDI mass spectrum for bovine insulin. The multiply charged ions with $(M+nH)^{n+}$ up to $n = 3$ are observed. The EDI mass spectrum in Figure 3 is quite similar to that obtained by MCI for dry bovine insulin (no matrix) except for a small appearance of a peak of $(M+4H)^{4+}$ in MCI spectrum [15]. The difference between MCI and the present EDI is the size and the components of the projectiles. In MCI, the projectile has an average of 10^6 glycerol molecules (mass of ∼10^8 u) with ∼+200 charges. In EDI, much smaller water cluster ions with masses of $10^5 \sim 5 \times 10^5$ u have been used. However, the energy per nucleon for MCI is of the same order as that for the present EDI, i.e., ∼1 eV/nucleon. This may be the main reason for the close resemblance of the mass spectra obtained by EDI and MCI. The operation of EDI is much simpler than MCI because the water cluster ions introduced into the vacuum are vaporized completely without leaving any residues in the vacuum system and thus there

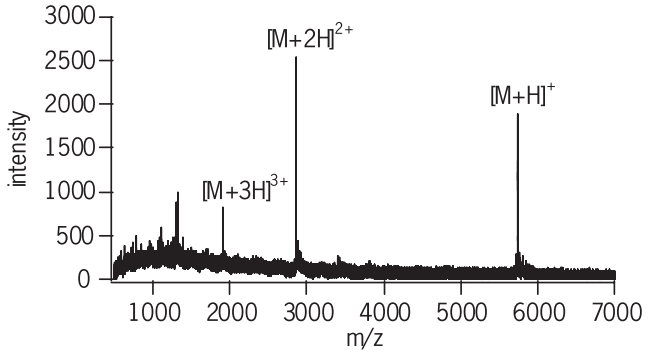

Fig. 3. EDI mass spectrum from 1 nmol of dry bovine insulin. No matrix is used.

is no worry about the contamination and charging of the electrodes by the sticky molecules like glycerol as in the case of MCI.

Figure 4 represents the EDI and FAB mass spectra for the drug, FK506. Figure 4a shows the EDI spectrum for the dried 1 nmol FK-506 deposited on the stainless steel substrate. Five molecular-related ions, $(M+H–2H_2O)^+$, $M+H\text{-}H_2O)^+$, $(M+H)^+$, $(M+Na^+)$, and $(M+K^+)$ are observed. The appearance of $(M+H)^+$, $(M+Na^+)$, and $(M+K^+)$ indicates that EDI is a soft ionization method, but the appearance of dehydrated ions, $(M+H\text{-}2H_2O)^+$ and $(M+H\text{-}H_2O)^+$, also indicate that degradation of the sample molecules takes place to some extent in EDI. Figure 4b shows the FAB mass spectrum just after FAB gun was turned on (the EDI gun was off). No molecular-related ions for FK-506 could be detected. However, strong chemical noise as well as weak signals of $(M + Na^+)$, and $(M + K^+)$ could be observed at 10 seconds after firing the FAB gun in Figure 4c. The reason for the time delay for the appearance of the molecular-related ions is not well understood. The molecular-related ions disappeared at 20 seconds after the start of FAB operation in Figure 4d (i.e., dose of $\sim 10^{13}$ of 5 keV Xe). The periodic peaks appearing in Figure 4d are spaced by 13 or 14 u, indicating that the sample surface of FK-506 was severely damaged and polymerized radiation products were formed on the top surface of the sample film. Figure 4e shows the EDI spectrum at 10 seconds after the damaged sample was bombarded by the electrosprayed droplets (the FAB gun was off). Figure 4e is almost the same as the original FK-506 in Figure 4a. This is somewhat surprising because the spot size of the Xe FAB beam covers about one third of the sample spot. The observed recovery of the mass spectrum for FK506 suggests that the electrosprayed droplet impact acts as a self-cleaning of the crust of the radiation products formed by the FAB treatment. Actually, the chemical noise level in Figure 4e becomes somewhat higher than that in Figure 4a. This kind of self-cleaning effect was also observed in MCI [17]. The cleaning effect may be extremely useful for the depth profiling of the labile biological samples.

From the measurement described above, it is evident that EDI can sputter only the top-surface molecules of the sample film without damaging the sample underneath

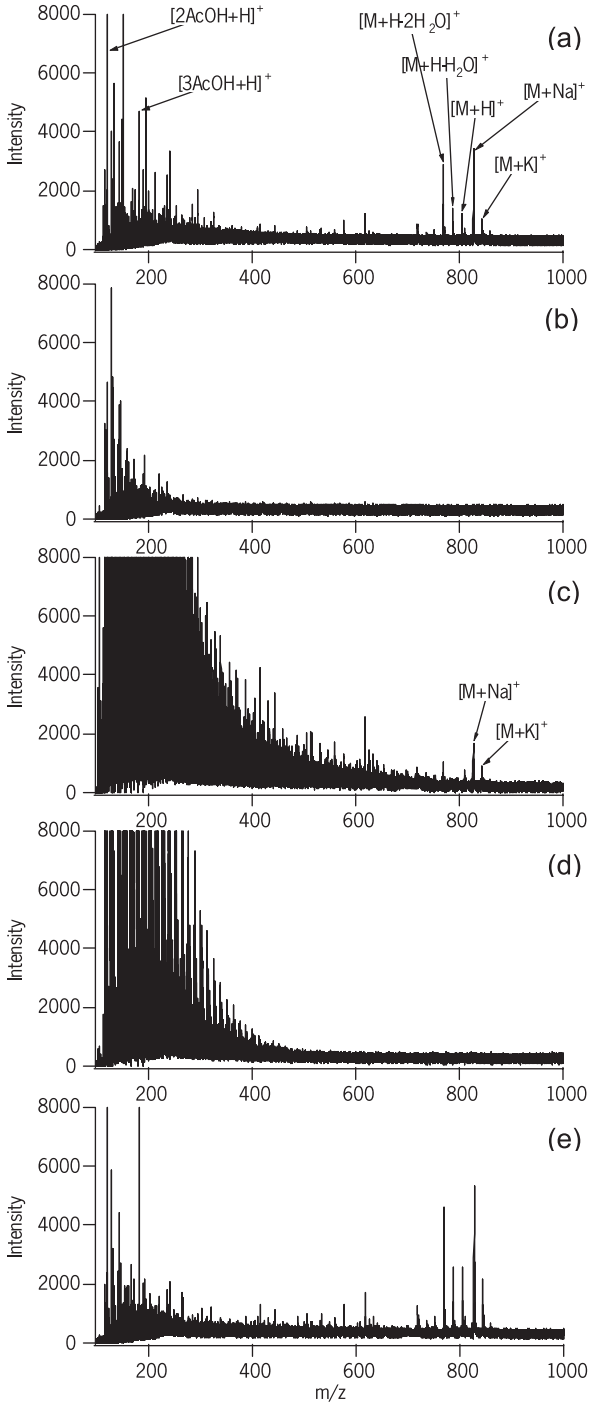

Fig. 4. EDI and FAB mass spectra from 1 nmol of dry FK506. (a) EDI mass spectrum. (b) FAB mass spectrum right after the FAB gun ignition (5 keV Xe FAB with a beam flux of about 0.1 μA). No molecular-related ions for FK-506 could be detected. (c) FAB mass spectrum at 10 s after the FAB gun ignition. Strong growth of chemical noise as well as the appearance of molecular related ions, $(M+Na)^+$ and $(M+K)^+$ were observed. (d) FAB mass spectrum at 20 s after the FAB gun ignition. Intensities of chemical noises decreased and the molecular-related ions of FK-506 disappeared. (e) EDI mass spectrum after the sample was irradiated by Xe FAB for 20 seconds. The molecular-related ions of FK-506 are restored but some increase in the chemical noise is noticeable.

the surface. In order to obtain further information on the sputtering phenomena in EDI, the signal intensities of the gramicidin S were measured as a function of irradiation time. The 1 nmol, 100 pmol, and 10 pmol of gramicidin S were deposited on the stainless steel substrate with a spot diameter of 2 mm. The roughly estimated film thicknesses for 1 nmol, 100 pmol, and 10 pmol are 100, 10, and 1 monolayers, respectively, assuming that the sample molecules are dispersed uniformly on the surface of the substrate. Figure 5a shows the intensities of $(M+H)^+$, $(M+2H)^{2+}$, and the fragment ion $(M/2 + H)^+$ for 1 nmol of gramicidin S as a function of exposure time. The $(M+H)^+$ ion gradually increases after the ignition of EDI up to the initial 1 min and reaches a plateau after 1 min. The initial signal increases in $(M + H)^+$ may be due to the surface contamination of the sample prepared on the stainless steel substrate. It was made sure that the signal intensity stays constant as long as 30 min. The measurements for longer time exposure were not performed. The observed exposure-independent signal abundance clearly indicates that the damage accumulation is negligible and EDI is effective at removing any accumulating chemical damage. This is a marked contrast to FAB which uses the atomic projectile. Figure 5b shows the results for 100 pmol of gramicidin S. The signal intensities for $(M + H)^+$, $(M+2H)^{2+}$, and the fragment ion $(M/2 + H)^+$ are basically independent on the exposure time. It should be noted that the signal intensities for 1 nmol and 100 pmol samples are about the same. This is reasonable because only the molecules near the top surface was desorbed and thus signal intensities are independent on the film thickness as far as the film is thicker than a few monolayers.

The 10 pmol sample is about one monolayer thick on average. The signal intensities were found to decrease with exposure time as shown in Figure 5c. This is reasonable because the surface of the substrate is covered by only one monolayer average of the sample molecules. In Figure 5c, the "half-life" of the decay curve is about 5 min. This suggests that it takes about 5 min to desorb (or sputter) the 50% of the sample deposited on the surface under the present experimental conditions.

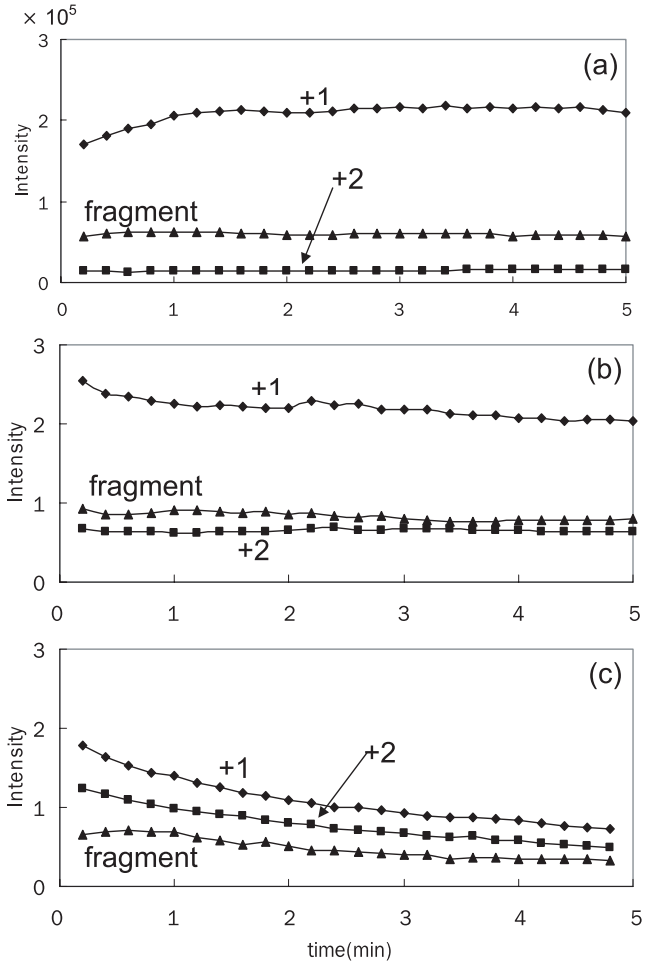

Fig. 5. Plots of molecular-related ions, $(M+H)^+$ and $(M+2H)^{2+}$ and the fragment ion $(M/2+H)^+$ vs. exposure time for a dry sample of (a) 1 nmol, (b) 100 pmol, and (c) 10 pmol gramicidin S, obtained by using 10 kV EDI beam.

4 Conclusion

This is the first report on the newly developed desorption ionization method, the electrosprayed droplet impact ionization (EDI). The mass spectra obtained by EDI are found to be quite similar to those obtained by MCI. In MCI, the massive cluster ions composed of glycerol were used for the projectile. The ion signal becomes unstable after some time of operation because of the charging of the lens electrodes in the vacuum chamber due to the contamination by less-volatile glycerol [18]. In contrast, the present method, EDI, utilizes the volatile solvents such as 1 M acetic acid aqueous solution and thus EDI is totally contamination-free. Actually, the ion signals stay constant for a long period of operations for the samples with a few monolayers or thicker. EDI does not need any matrices and thus can be readily applied to a wide variety of the samples, e.g., drugs, amino acids, peptides, proteins, alkaloids, etc. Although the broad distributions in kinetic energy, charge distribution, and mass of the electrosprayed droplets used in these experiments do not permit detailed analysis of the parameters such as penetration depth, range and stopping power, only a few monolayers on the top surface of the sample film can be stripped and detected by the mass spectrometer. That is, EDI would be a versatile and high-sensitive technique for trace analyses in many applied fields. Since EDI is very sensitive to molecules present near the sample surface and no matrix is needed, it could be a versatile sniffing technique for drugs and explosives, and for the imaging of biological samples by scanning the projectile beam across the sample surface. The combination of the high layer-by-layer desorption/ionization efficiency and the imaging technique would revolutionize the biological nanotechnology.

References

1. A. Takamizawa, S. Fujimaki, J. Sunner, K. Hiraoka, J. Am. Soc. Mass Spectrom. **16**, 860 (2005)
2. B. Sundqvist, R.D. Macfarlane, Mass Spectrom. Rev. **4**, 421 (1985)
3. M. Barber, R.S. Bordoli, R.D. Sedwick, A.N. Tyler, J. Chem. Soc. Chem. Commun. 325 (1981)
4. W. Aberth, K.M. Straub, A.L. Burlingame, Anal. Chem. **54**, 2029 (1982)
5. R.J. Beuhler, L. Friedman, Int. J. Mass Spectrom. Ion Process. **94**, 25 (1989)
6. A. Brundle, P. Chaurand, S. Delola-Negra, G.B. Baptista, Int. J. Mass Spectrom. Ion Process. **126**, 65 (1993)
7. Z. Postawa, J. Phys. Chem. B **108**, 7831 (2004)
8. M.G. Blain, Phys. Rev. Lett. **63**, 1625 (1989)
9. A. Novikov, M. Caroff, S. Della-Negra, J. Depauw, M. Fallavier, Y.L. Beyec, M. Pautrat, J.A. Schultz, A. Tempez, A.S. Woods, (Rapid Commun.) Mass spectrom. **19**, 1851 (2005)
10. N. Winograd, Anal. Chem. April 1, 143A (2005)
11. F. Eusepi, A. Tomsic, C.R. Gebhardt, Anal. Chem. **75**, 5124 (2003)
12. J.F. Mahoney, J. Perel, S.A. Ruatta, P.A. Martino, S. Husain, T.D. Lee, (Rapid Commun.) Mass Spectrom. **5**, 441 (1991)
13. J.F. Mahoney, J. Perel, T.D. Lee, P.A. Martino, P. Williams, J. Am. Soc. Mass Spectrom. **3**, 311 (1992)
14. J.F. Mahoney, D.S. Cornett, T.D. Lee, (Rapid Commun.) Mass Spectrom. **8**, 403 (1994)
15. D.S. Cornett, T.D. Lee, J.F. Mahoney, (Rapid Commun.) Mass Spectrom. **8**, 996 (1994)
16. 53rd ASMS confererence, ThP 229, San Antonio, Texas, 2005
17. J.M. McMahon, N.N. Dookeran, P.J. Todd, J. Am. Soc. Mass Spectrom. **6**, 1047 (1995)
18. S.A. Aksyonov, P. Williams, (Rapid Commun.) Mass Spectrom. **15**, 2001 (2001)