J. Braz. Chem. Soc., Vol. 23, No. 9, 1762-1766, 2012.
Printed in Brazil - ©2012 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Short Report

Development of Nanoinjector Devices for Electrospray Ionization - Tandem Mass Spectrometry (ESI-MS^n)

Nilson A. Assunção,,[a] Ernesto S. Nakayasu,[#,b] Sirlei Daffre[b] and Emanuel Carrilho[c]*

[a]*Departamento de Ciências Exatas e da Terra, Universidade Federal de São Paulo, R. Prof. Artur Riedel, 275, Jd. Eldorado, 09972-270 Diadema-SP, Brazil*

[b]*Instituto de Ciências Biomédicas, Universidade de São Paulo, Av. Prof. Lineu Prestes, 1374, 05508-900 São Paulo-SP, Brazil*

[c]*Instituto de Química de São Carlos, Universidade de São Paulo, Av. Trabalhador São-carlense, 400, CP 780, 13560-970 São Carlos-SP, Brazil*

Em espectrometria de massas (MS) no modo de ionização eletrospray (ESI), as amostras podem ser analisadas com um método prévio de separação (cromatografia líquida ou eletroforese capilar) ou por infusão direta da amostra. A injeção direta apresenta um grande benefício que é a redução do volume de amostra consumido e a possibilidade de amostragem contínua por um período estendido. Este maior tempo de amostragem possibilita a análise completa através de fragmentações sucessivas por espectrometria de massas, crítico quando se busca elucidação estrutural de novos compostos, ou quando se dispõe de um analisador de massas do tipo captura de íons. Neste trabalho, descrevemos uma metodologia de deposição de filme estável na extremidade cônica dos dispositivos de boro-silicato, visando o desenvolvimento de nanoinjetores estáticos. A formação de uma camada dupla de titânio e ouro proporcionou uma excelente fixação do filme, resultando em um nanoinjetor para amostragem por ionização/formação do aerossol no sistema de espectrometria de massas. O objetivo deste filme é manter o contato elétrico no sistema. Os nanoinjetores apresentaram repetibilidade e estabilidade elevadas por mais de 100 min de amostragem contínua com apenas 10 μL de amostra.

In mass spectrometric (MS) systems with electrospray ionization (ESI), the sample can be analyzed coupled to separation systems (such as liquid chromatography or capillary electrophoresis) or simply by direct infusion. The greatest benefit of the type of injection is the possibility of continuous use of small amounts of samples over a long period of time. This extended analysis time allows a complete study of fragmentation by mass spectrometry, which is critical for structure elucidation of new compounds, or when using an ion trap mass analyzer. The injector filled with the sample is placed at the ESI source inlet creating an electric field suitable for the continuous formation of a spray (solvent and sample) and consequently, the gradual and even release of the sample. For the formation of the spray, is necessary that the injector end is metalized. The formation of a bilayer of titanium and gold provided an excellent attachment of the film, resulting in a nanoinjector for ionization/spray formation in the system for MS. The nanoinjectors showed high repeatability and stability over 100 min by continuous sampling with 10 μL of sample.

Keywords: mass spectrometry, nanoinjector, microsystems

Introduction

Mass spectrometry (MS) has become an essential laboratory technique in chemistry, biology, medicine and environmental sciences.[1-5] Currently, there are several interfaces for sample introduction in MS systems. There are methods that promote the separation, concentration, desorption, or simply the injection of the sample by infusion.

In systems of mass spectrometry with nanoelectrospray ionization (nanoESI-MS), the sample may be injected either using separation systems (when coupled to

*e-mail: nilson.assuncao@unifesp.br
#Present address: Biological Science Division, Pacific Northwest National Laboratory, Richland WA 99352, USA.

chromatographic or electrophoretic systems) or simply by direct infusion of the sample. Despite essential for analysis of complex samples, a system for separation of components is not necessary when the goal is the generation of the ions for an extended time (long sampling time). The greatest benefit of this type of injection is the possibility of using small amounts of sample for continuous injection over a long period of time, what allows for multiple MS experiments. This resource is useful and applicable in tandem spectrometry equipments with a pure and isolated sample. The application of this type of device is necessary in experiments with the analysis of several generations of fragments (MS^n), such as in oligosaccharides or elucidation of natural products. Static nanoinjectors are available in the market as capillaries with total volume of a few μL for this kind of analysis. The nanoinjector is filled with the sample and placed at the nanoESI source inlet to create an electric field suitable for the continued formation of a spray (solvent and sample). For a stable formation of the spray, it is necessary a metallized injector end. Aiming to develop these injectors in our laboratory, it was used low cost boro-silicate tubes commonly used in the determination of hematocrits (Sigma-Aldrich, St. Louis, MO, USA).

In this work, it was developed a method for pulling the capillary tips and methodology for the deposition of metallic films onto glass-based surfaces (borosilicate and fused silica). This was applied to make nanoinjectors for direct injection of samples using MS without separation of the components of the sample. The static nanoinjectors are capillaries with total volume of approximately 10 μL. They are filled with the sample and placed at the ESI nanosource inlet. For the formation of the spray, it is necessary to place the metallized end of the injector close to the sampling cone of MS.[6-9] The objective of this work was to develop nanoinjectors and metallized silica capillaries for nanospray mass spectrometric systems.

Experimental

All reagents were of analytical grade and all solutions were prepared with deionized water (18.2 MΩ cm) obtained from a Milli-Q water purification system (Millipore, Milford, MA, USA). Methanol, acetonitrile (ACN), acetic acid (99%) and isopropyl alcohol were purchased from J. T. Baker (Mexico City, Mexico). The fused silica capillary was purchased from Polymicro Technologies (Phoenix, AZ, USA) and the borosilicate capillaries (Sigma-Aldrich, St. Louis, MO, USA) were cut to size after molding the end by heating and mechanical force applied to the ends. After reaching room temperature, the tubes were washed with an ethanol/methanol (1:1, v/v) solution and next dried in oven at 60 °C for 2 h. All MS experiments were performed using an LCQ DUO 120 ion trap mass spectrometer (Thermofinnigan, San Jose, CA, USA), equipped with a nanoLC electrospray source). The physical characterization of nanoinjetor was carried out by scanning electron microscopy (SEM) in a Leica-Zeiss LEO 440 model SEM coupled to an Oxford 7060 model analyzer (Waltham, MA, USA).

The film was deposited onto the borosilicate nanoinjector and silica capillary ends in a AJA (North Scituate, MA, USA) operating at 3×10^{-6} torr, room temperature (deposition at room temperature) and N_2 flow of 5 psi min^{-1}. The low-pressure metallization process occurs in cold plasma environment. The metallic targets were submitted to a potential of 500 V and bombarded with N_2 to create a vaporized ion environment inside the whole system. The material of interest (sample) was thus deposited.

The metallic targets used in our experiments were titanium and gold. Titanium was the first layer because of its good adherence to the silica-based material. Gold was used as the second layer because of its good conductivity, which facilitates the formation of the aerosol and good chemical resistance. A film with approximately 300 Å (total thickness) was deposited. Film thickness was indirectly measured. Glass plates were used as controls since the thickness measurement of a pointy material is an inaccurate process. Measurement of the metal layer deposited over the glass was carried out by the Rutherford backscattering (RBS) technique. This technique consists in measuring the energy of ions hit by a light beam. The difference of energy is proportional to the amount of material on the surface (film).

Results and Discussion

The deposition of the film was controlled by the thickness measurement of a film formed over a control glass plate, placed together with the capillaries and measured by RBS. Based on these experiments, the deposition times were set at 7 min for titanium and 13 min for gold, which were sufficient for the deposition of 15 nm thick films. Another parameter evaluated was the capillary end opening made by heating and the application of mechanical force, a process similar to that used to make fused silica capillaries. Figures 1A and 1B show SEM images of the nanoinjector tip produced by this process. The nanoinjectors are 5.0 cm long with 1.0 cm long tapered ends with a metallic film. The silica capillaries had different lengths, but all metallized ends were 1.0 cm long.

Initially, only gold was deposited on the tip of the silica capillary without the protection of polyimide.

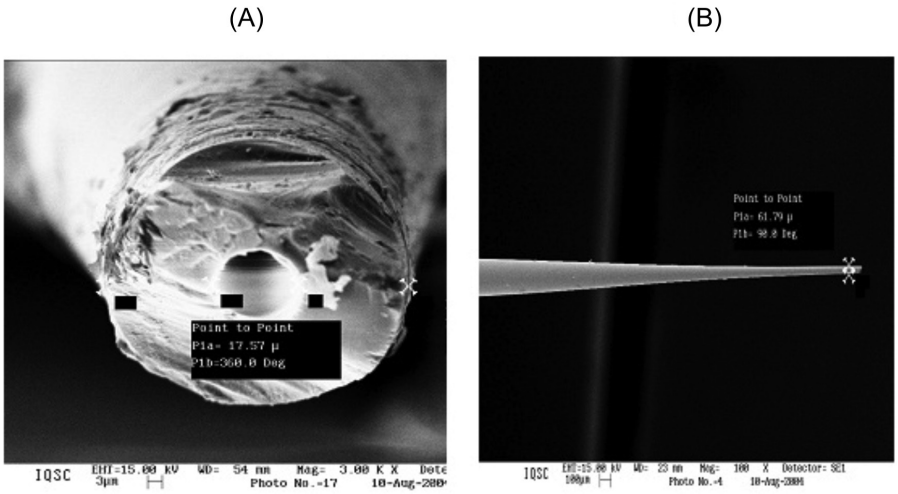

Figure 1. Scanning electron microscopies of nanoinjectors. (A) Frontal and (B) lateral view of the metallized tip. Both SEM images of the nanoinjector tip were obtained after double deposition of titanium and gold layers. Enlargement: 8000X.

However, the experiments with MS showed a continuous degradation of the gold film due to loss of adhesion to the silica capillary. The gold layer degradationresulted in a lower lifetime of metal layer and instability in the signal generated. Based on these results, it was verified the need to use materials with good adhesion to glass (silica), and then the deposition of a second layer of gold. This approach facilitated the formation of a stable two-layer system. The choice of titanium was due to its high adhesion to glass, and in turn, titanium has good adhesion to gold. This composition was ideal for the double layer to generate a conductive material, yet stable when submitted to an electric potential, high temperature, and organic vapors, which is the environment of an ESI interface. Other chemical elements were assessed in the composition of the double layer, including cobalt and nickel, but the formation of the film was not homogeneous as the system titanium/gold.

Figure 2 displays the analysis results of a phospholipid by static injection. The sample was dissolved in 50% ACN,

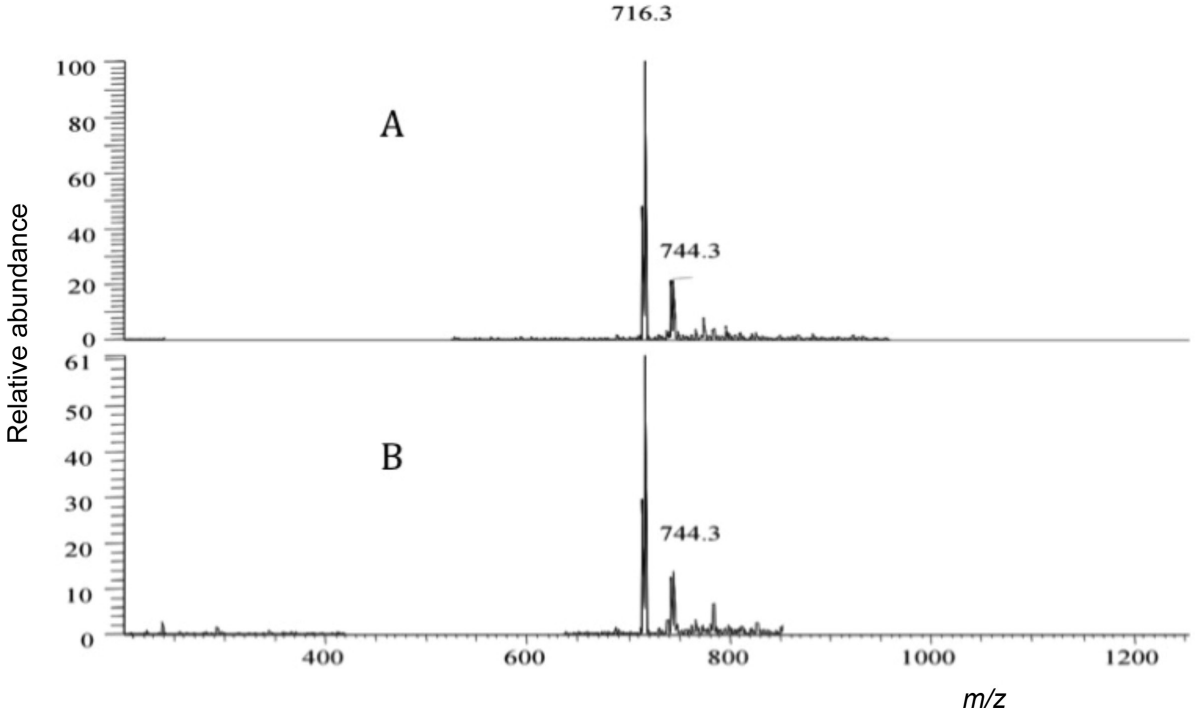

Figure 2. Comparison of static nanoinjectors. (A) Commercial tip injector, i.d = 1 mm, length = 100 mm (Sigma-Aldrich, St. Louis, MO, USA). (B) Homemade tip injector (i.d = 1 mm, length = 100 mm, Sigma-Aldrich, St. Louis, MO, USA). Sample was 1.4 nmol μL^{-1} phosphatidylethanolamine. Temperature ESI 180 °C, voltage 2.5 kV.

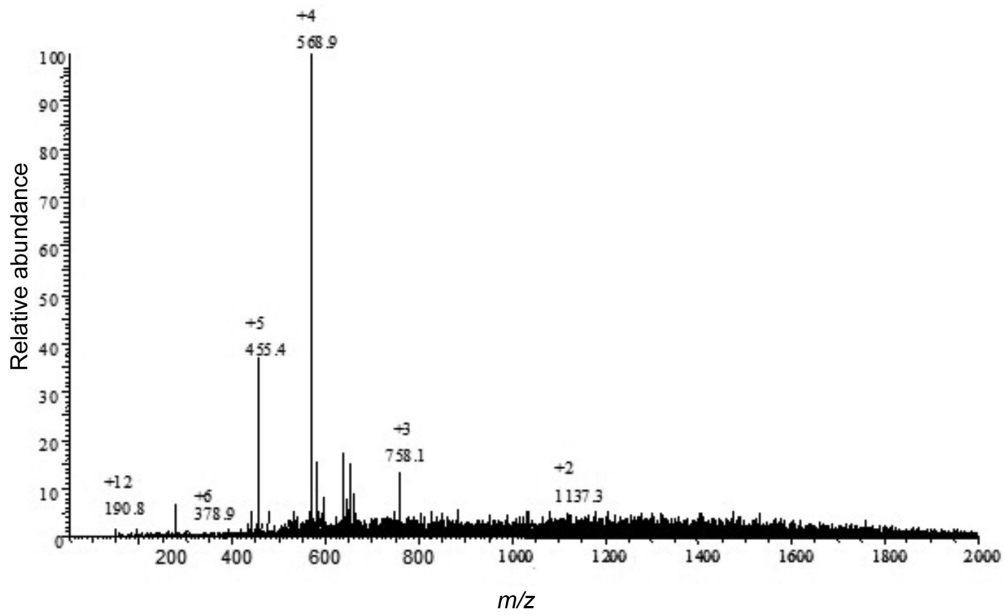

Figure 3. Spectrum of peptide gomesin after 80 min of continuous infusion. The nanotip prepared was the same shown in Figure 1 and was filled with 10 μL gomesin at 10 pmol μL^{-1}. Temperature ESI 180 °C, voltage 2.5 kV.

0.1% formic acid. In Figure 2(A), it is shown the mass spectrum using a commercial injector and in Figure 2(B) using the injector developed here. The intensity of the signal in each case is within a normal difference between injectors, being considered acceptable for mass spectrometry with ESI source. The *m/z* values and the relative intensities of all peaks of the two spectra are coincident.

Figure 3 presents the spectrum of gomesin, a peptide with antimicrobial activity isolated from hemocite of spider *Acanthoscurria gomesiana* with molecular mass of 2270.4 Da. This peptide has 18 amino acids, including pyroglutamic acid, a C-terminal arginine, and four cysteine residues forming two sulfide bridges. The sample was purified and isolated at the Laboratório de Bioquímica e Imunologia de Artrópodes (ICB-USP).[10] Gomesin samples were dissolved in 50% ACN, 0.1% formic acid. The greatest benefit of this type of injection is the possibility of using minimal amounts of sample for injection over a long time. The results in Figure 3 also show that continuous injection of minimal amounts of sample over a long time was successfully attained. The intensity of the signal obtained was in the order of 10^7. The peptide was ionized with +2, +3, +4 and +5 charges, corresponding to ion *m/z* 1136.5, 758.1, 568.9 and 455.3. A total of 30 nanoinjectors was made and tested with 100% of repeatability. These results allowed us to make reproducible static nanoinjectors. The experiments presented in Figure 3 show the stability of the spectrum after over 80 min of continuous sampling. The intensity of the signal remained constant until the end of the sample, which occurred after 120 min.

Conclusions

The metallization of nanoinjectors and fused silica capillaries by the titanium-gold double layer process is already a laboratory routine in our group. This methodology reduces significantly the cost of analysis by eliminating the need of importing expensive nanoinjectors and may be extended to capillary electrophoresis couple to mass spectrometry detector (CE-ESI-MS). Metallized capillary is necessary in sheathless mode CE-ESI-MS systems and this fabrication process allows further development of this technique in Brazil.[11]

Acknowledgments

We gratefully acknowledge financial support from FAPESP, CNPq and FAP-Unifesp.

References

1. Aebersold, R.; Mann, M.; *Nature* **2003**, *422*, 198.
2. Geoghegan, K. F.; Kelly, M. A.; *Mass Spectrom. Rev.* **2005**, *24*, 347.
3. Mann, M.; *Org. Mass Spectrom.* **1990**, *25*, 575.
4. Pramanik, B. N.; Bartner, P. L.; Mirza, U. A.; Liu, Y. -H.; Ganguly, A. K.; *J. Mass Spectrom*. **1998**, *33*, 911.
5. Zahn, J. A.; Higgs, R. E.; Hilton, M. D.; *Appl. Environ. Microbiol*. 2001, *67*, 377.
6. Wahl, J. H.; Gale, D. C.; Smith, R. D.; *J. Chromatogr.* **1994**, *659*, 217.

7. Moini, M.; *Anal. Bioanal. Chem*. **2002**, *373*, 466.
8. Janini, G. M.; Conrads, T. P.; Wilkens, K. L.; Issaq, H. J.; Veenstra, T. D.; *Anal. Chem*. **2003**, *75*, 1615.
9. Issaq, H. J.; Janini, G. M.; Chan, K. C.; Veenstra, T. D.; *J. Chromatogr*. **2004**, *1053*, 37.
10. Silva, P. I.; Daffre, S.; Bulet, P.; *J. Biol. Chem.* **2000**, *275*, 33464.
11. Assunção, N. A.; Bechara, E. J. H.; Simionato, A. V. C.; Tavares, M. F. M.; Carrilho, E.; *Quim. Nova* **2008**, *31*, 2124.

Submitted: February 27, 2012
Published online: September 13, 2012

FAPESP has sponsored the publication of this article.