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JMS Letters

Dear Sir,

Applications of Multiple Channel Electrospray Ionization Sources for Biological Sample Analysis

One of the problems of connecting high-performance liquid chromatography (HPLC) to electrospray ionization mass spectrometry (ESMS) is that a good electrospray cannot be achieved owing to high flow-rate of the HPLC effluent. In the past few years, the development of methods to solve the problem has continued to attract considerable attention.[1–8] Methods include directly connecting the ES source to a small LC effluent by post-column splitting and the use of a packed capillary column[2] or an open-tubular HPLC column.[3] The source designs that have been reported include the ultrasonically assisted electrospray,[4,5] ionspray[6], turbo ionspray[7] and an ionspray source modified with a liquid shield placed between the electrosprayer and the ion sampling capillary.[8] Another possible approach to accommodate the high flow rate from the HPLC system involves a multiple channel electrospray ionization (MC-ES) source. However, ES sources with more than one sprayer are seldom found in the literature.[9–15] A Y-type dual-channel ES source was first reported by Smith and co-workers in 1991.[9–13] Experiments with arrays of electrospray capillaries have been performed by Rulison and Flagan[14] with the aim of scaling up the production of an aerosol. Two- and four-channel electrosprayers have been reported by Kostiainen and Bruins.[15] In this letter, we report the design and construction of two simple multiple-channel ES sources. Direct coupling of the HPLC effluent with the MC-ES source is demonstrated. Applications of MC-ESMS in biological sample analysis will also be discussed.

Figure 1(A) is a schematic diagram of a 16-channel ES source. Each electrosprayer was made by inserting a capillary column (170 μm o.d., 100 μm i.d.) through a PTFE tube (2 cm × 1.56 mm o.d. × 0.25 mm i.d.). The capillary column protruded 0.5 cm from the PTFE tube. The function of the PTFE tube was to prevent the capillary column from vibrating during electrospraying. All of the electrosprayers were then glued together with epoxy cement and inserted into a space drilled on an acrylic plate. The distance between two adjacent capillary columns was about 3 mm. Care was taken that the tips of all of the capillary columns were kept at the same distance from the interface plate, otherwise interference from a nearby electrosprayer might occur and an individual spray could become unstable. Although the flow-rate in each channel might differ slightly, it was found that the applied potential was high enough to generate a good electrospray from all the channels. The capillary columns were joined together at one arm of a three-way tee by a finger-tight HPLC connector. The joint was sealed with epoxy cement. The required high voltage for electrospray (6–10 kV, from a Glassman EH20R20 power supply) was introduced into the sample solution through a thin stainless-steel electrode which was inserted at another arm of the three-way tee. The source was mounted on a three-dimensional moving plate so that the position of the electrosprayers with respect to the sampling skimmer on the mass spectrometer could be adjusted. The best ion signal obtained by this MC-ES system was found to be at a distance about twice that of a single sprayer.

Figure 1(B) is a schematic diagram of a seven-channel ES source. The source consisted of a central electrosprayer surrounded by six electrosprayers. The analyte solution was pumped through the central electrosprayer by a syringe pump and the reagent was pumped through the outside of the six electrosprayers by another syringe pump. The construction of the seven-channel electrosprayer was similar to that of the above 16-channel electrosprayer except that each capillary column protruded 1 cm from the PTFE tube and the distance between two channels was about 1 cm. The positive ions generated by the MC-ES source were detected by a PE-Sciex API 1 mass spectrometer. The mass was scanned from m/z 10 to 2400 at a rate of about 2 s per scan. The temperature of the electrospray interface chamber was kept at 55 ± 1 °C.

All the biochemicals, polypropylene glycols and organic solvents were purchased from Sigma or Aldrich and used without further purification. The concentrations of the analytes in the sample solutions were between 2 and 10 pmol μl^{-1}.

Figure 2 shows the mass chromatograms of five biomolecules (myoglobin, cytochrome *c*, insulin, reserpine and renin) obtained with the 16-channel ESI/MS. The ES mass spectra of each analyte were recorded and are shown as insets in each mass chromatogram. The buffer solution (acetonitrile–water (90:10, v/v) containing 1% acetic acid) was delivered into the MC-ES source at a flow-rate of 0.3 ml min^{-1} by an HPLC pump (PE Series 200). The sample solutions (20 μl each) were introduced into the system by flow injection at 3 min intervals. No HPLC column was used in this analysis. Therefore, the analyte molecule would not be focused and the eluting peaks were wide and noisy. However, it could be seen that good ES mass spectra could still be obtained at such high flow-rates. The quality of the mass spectra degraded gradually as the LC flow-rate increased. At flow-rates higher than 0.6 ml min^{-1}, a good electrospray and satisfactory mass spectra

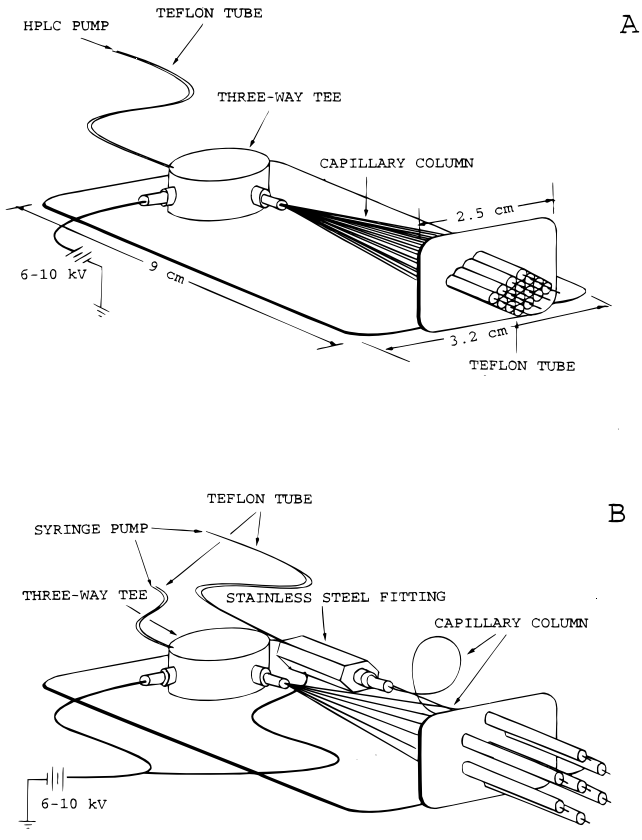

Figure 1. Schematic diagrams of (A) the 16-channel and (B) the seven-channel electrospray ionization source.

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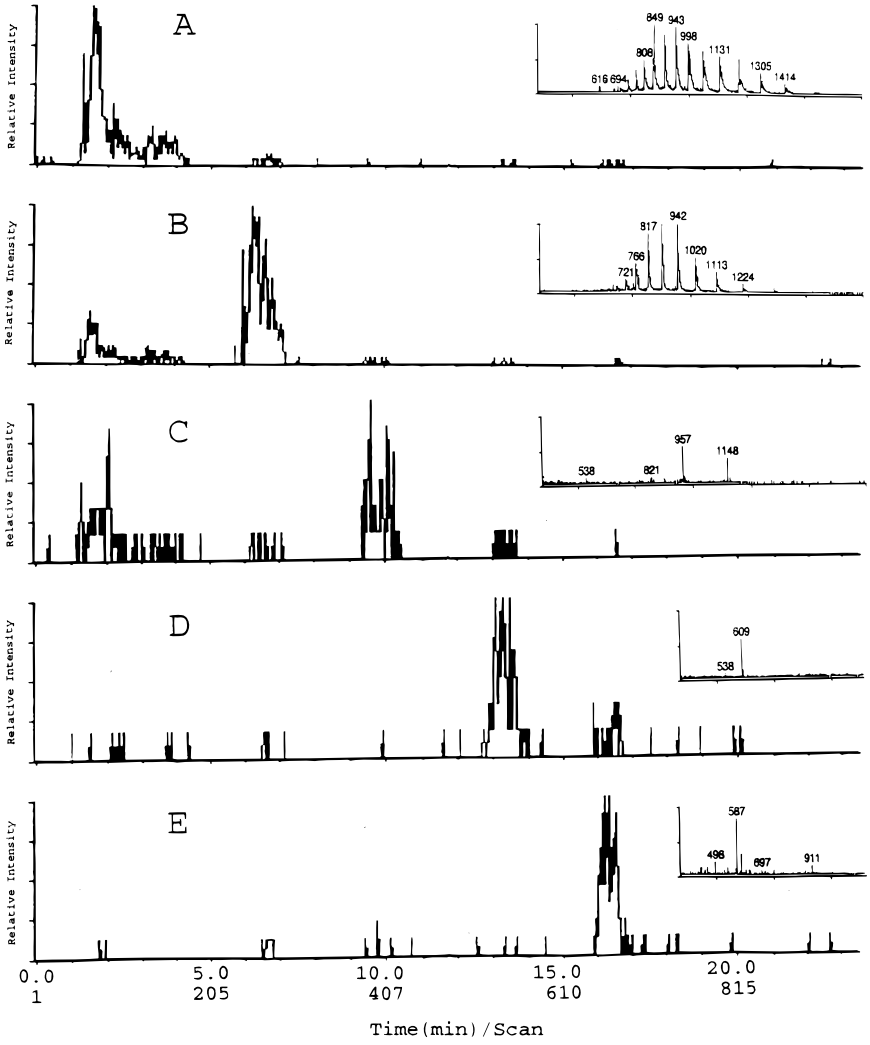

Figure 2. Mass chromatograms of five biomolecules, (A) myoglobin, (B) cytochrome *c*, (C) insulin, (D) reserpine and (E) renin, obtained by coupling the HPLC effluent to the 16-channel ESMS system. Each sample solution (20 μl) was flow injected at 3 min intervals. The solution was delivered to the ES source by an HPLC pump at a flow-rate of 0.3 ml min^{-1}. The inset in each mass chromatogram shows the ES mass spectrum of the analyte recorded.

could no longer be obtained. In this case, an ES source with more sprayers or with a nebulizing gas is required.

Another advantage of using an MC-ES source in sample analysis is the increase in electrospraying area and angle. The electric field distribution and charge density distribution in an ES source were simulated by Sunner and co-workers.[16,17] The results showed that the optimum location of a sampling orifice was 2–4 cm from the center line of the spraying needle. Hence, the position of the electrospray needle has to be well adjusted in order to obtain a good ES mass spectrum. Since the spraying area by the MC-ES source is much larger than that of a single sprayer, the position of the electrospray needles relative to the aperture of the sampling skimmer is not so critical. The ESI mass spectra of a mixture of polypropylene glycols obtain by placing the 16-channel ES source at different locations (2 cm apart from the center of the sampling skimmer) were nearly identical (data not shown). However, it is worth noting that the intensity of the ion signals obtained with the MC-ES source did not increase. This is consistent with results reported previously.[15]

MC–ES can also be used to study the protonation and deprotonation of large biomolecules in the gas phase. Previously, three different types of ES sources have been reported.[9–13,18,19] The sheath flow sprayer was actually an interface which was designed for connecting capillary electrophoresis (CE) with ESMS. For the Y-type ES source, different ions or molecules could be introduced into the two arms of the Y-tube. Several ion–ion or ion–molecule reaction categories have been studied by using this dual-channel flow reactor. For example, the reaction of multiply charged peptide ions with gaseous amines gave a simple ES mass spectrum which was predominant with singly charged peptide ions.[20] The advantage of deprotonating the analyte ions (to form singly charged ions) is that the interpretation of the mass spectra for samples which contain a mixture of peptides becomes much easier.[20] The third design is the so-called bubble-gas assisted electrospray source described by Lin and Sunner.[21] The changes of the charge states and conformations of protein ions by acid gases were studied.

The ES source with seven electrosprayers can also be used for similar studies. It is known that native myoglobin ion can be generated by a single electrosprayer from solutions between pH 4 and 9. However, the intensity of the peak of such an ion in a positive-ion ES mass spectrum is usually low. By using the seven-channel ES source, the signal of native myoglobin ion was enhanced about threefold. To obtain the ES mass spectra, an aqueous solution containing native myoglobin was pumped through the central channel. The solution flowing through the other six channels was methanol–water (1:1, v/v) containing 1% acetic acid. The two solutions were

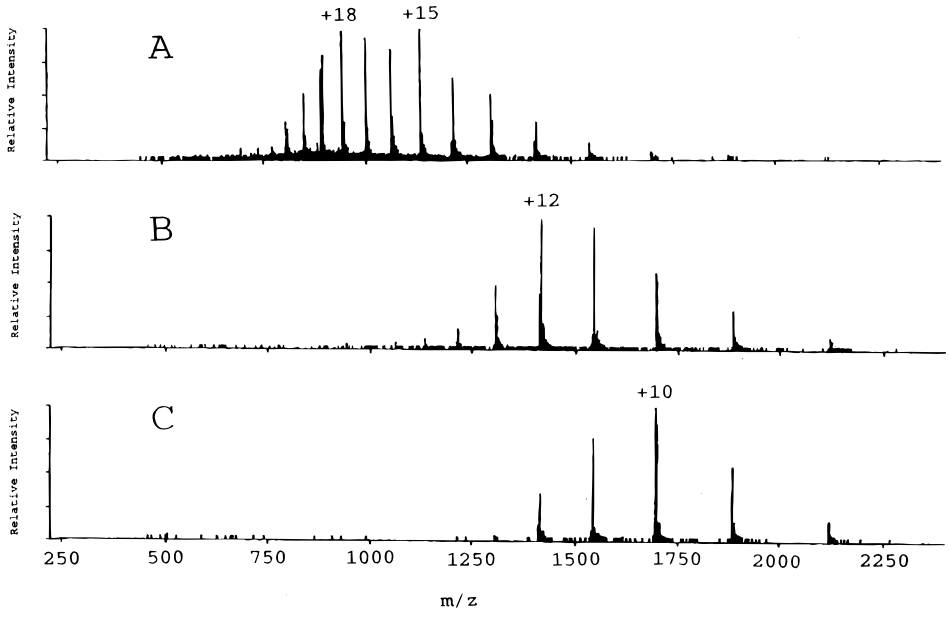

Figure 3. Positive ES mass spectra of myoglobin obtained by simultaneously electrospraying solutions containing denatured myoglobin molecule and diethanolamine (DEA) by the seven-channel ES source. Concentration of DEA (in methanol) in the solution: (A) 0, (B) 0.5 and (C) 1 mM.

electrosprayed at the same time. It was found that the ES mass spectrum was dominated by the native myoglobin ion. It seemed that the efficiency of protonation of the native myoglobin molecule was better than that in the bulk solution. Similar results were also reported by Lin and Sunner[21] using their bubble-gas electrospray source.

It seemed very possible that the acid molecules in gas phase could be formed by rapid evaporation from the fine droplets. The molecules might then be absorbed into the liquid surface of the charged droplets and subsequently interact with myoglobin inside the droplets. The time for this interaction should be very short, since it would take less than 10^{-3} s before the ions or charged droplets arrived at the mass analyzer. This explained why myoglobin ion with the native conformation was produced. In contrast, if the myoglobin was dissolved directly in the bulk solution, the acid molecules had a much longer time to interact with myoglobin. This would complete the denaturation process and accomplish heme group dissociation. However, the explanations described above still need to be substantiated with further studies.

For the study of the deprotonation of denatured myoglobin ions, a common fast atom bombardment matrix with a high gas-phase basicity, diethanolamine (DEA, in methanol),[22] was used as the reagent. The reagent flowed through the outside six electrosprayers. At the same time, a solution containing denatured myoglobin (in methanol–water (1:1, v/v) containing 1% acetic acid) was flowing and electrospraying at the central electrosprayer. It was found that increasing the concentration of DEA in the reagent solution caused more significant deprotonation and the number of charges on the myoglobin ion decreased (Fig. 3). According to the explanation given previously, the gaseous DEA molecules could be produced by rapid evaporation from the fine droplets that were produced from electrospray. These molecules then reacted with the myoglobin ions within the spraying droplets, resulting in a decrease in the charge number of the protein ions.

In conclusion, we have reported the design and construction of two multiple-channel ES sources. The two sources are both time saving and much simpler to construct. This should lead to easy implementation in other laboratories. Directly coupling the HPLC effluent to MC-ESMS for biological sample analyses has been demonstrated. Other applications such as increasing the electrospraying area and angle and in studies of protonating and deprotonating reactions of protein ions within droplets can also be performed using MC-ESMS.

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Yours,

JENTAIE SHIA[1]* and CHIN-HSIUNG WANG[1]
[1] Department of Chemistry,
Natinal Sun Yat-Sen University,
Kaohsiung,
Taiwan 804

* Correspondence to: J. Shia.

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