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Small Volume and Low Flow-rate Electrospray Ionization Mass Spectrometry of Aqueous Samples

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A new electrospray ionization source (emitter) has been developed which allows the effective utilization of very small sample volumes at much lower flow rates than previously demonstrated. A small diameter etched-tip capillary has been incorporated into a pressure-infusion electrospray ionization source. The ability to electrospray aqueous solutions without the use of an ancillary sheath flow is demonstrated with several biopolymers. High signal intensities and stable signals are observed for this source in a comparison with a standard methanol-sheath source.

Electrospray ionization mass spectrometry (ESI-MS) has become an important analytical technique for chemical, biochemical and biological materials.[1,2] The further application of ESI-MS to small-volume and difficult-to-analyze samples will require a source that utilizes sample efficiently, affords high sample sensitivity and provides a stable electrospray for extended periods of time. Several approaches have been reported for either the continuous delivery of sample or of a sample plug to an ESI source, primarily based upon pressure-driven flow. One of the most widely used approaches, initially developed at this laboratory,[3] involves the use of a coaxial sheath liquid. The coaxial-sheath-liquid ESI source design was originally developed to facilitate interfacing of capillary electrophoresis (CE) and mass spectrometry and provides considerable flexibility in solvent choice and flow rate.[3,4] The use of a sheath liquid, however, results in several disadvantages. These include the unavoidable addition of ionic and neutral species through the sheath liquid, which compete in the ESI process for available charge. The maximum sensitivity obtainable is lowered, to an extent which depends on the details of mixing of the analyte and sheath-liquid streams. Past experience in our laboratory has shown that stable signals from sheath-liquid type sources can, in certain applications, be difficult to obtain for extended periods of time. Obtaining a stable electrospray with a water sheath, when non-denaturing solvent conditions are desired and/or required, is often particularly difficult to achieve. Chowdhury and Chait have described a non-sheath ESI source constructed from a high-performance liquid chromatogaphy (HPLC) syringe that functions at conventional electrospray flow rates.[5] The syringe needle tip was electropolished to a tapered point which enabled the stable electrospraying of proteins from aqueous solutions. Wahl and coworkers have recently reported the use of a non-sheath source incorporating an etched tip at the terminus of small-i.d. fused silica capillaries for CE-MS applications, and have achieved subfemtomole detection of proteins.[6,7] The combination of low flow rates with small i.d. fused-silica capillaries resulted in high ESI-MS sensitivity due to an increase in sample ionization efficiency.

A non-sheath source for pressure-infusion ESI-MS, incorporating a small-diameter silica capillary with an etched terminus (i.e., 'tip'), is expected to demonstrate greatest sample sensitivity at low flow rates. By minimizing the dead volume of the source, high sample utilization efficiency and overall performance should be possible. We have used these concepts to guide the development of a new low-dead-volume non-sheath pressure infusion ESI source, incorporating an etched tip, small-diameter, fused-silica capillary (5–20 μm i.d.). This non-sheath source is capable of effective operation at low flow rates, which results in lower sample consumption and an improved ability to analyze small sample volumes. The ESI-MS of proteins, peptides and oligonucleotides in a variety of solution conditions can be obtained easily and at low flow rates without the addition of any non-aqueous solvent. In this paper, we describe this non-sheath source and its performance under positive and negative electrospray conditions. We also provide a comparison to a standard liquid-sheath source to quantify improvements in electrospray stability and sample sensitivity.

EXPERIMENTAL

Samples were pressure-infused at set flow rates with a Harvard (South Natick, MA, USA) syringe pump. The modified Sciex (Thornhill, ON, Canada) TAGA 6000E mass spectrometer, electrospray interface, and required power supplies used in these experiments have been described previously.[8,9] The sheath source design, consisting of a 50 μm i.d. analytical capillary (Polymicro Technologies, Phoenix, AZ, USA), a stainless steel capillary for coaxial methanol-sheath flow, and a stainless steel capillary for coaxial SF_6, has been described previously.[3,4] For the sheath source, methanol at a flow rate of 2.5 μL/min was used as the sheath liquid. The tip of the analytical capillary for the sheath source design extended approximately 0.5–1.0 mm beyond the co-axial stainless steel capillary for optimum performance. An outer coaxial flow of gaseous SF_6 was used to prevent electrical discharge for all positive and negative ESI-MS experiments with the sheath and non-sheath sources.[10,11]

The non-sheath electrospray source, shown in Fig. 1, was constructed from a 25 μL gas-tight syringe (Hamilton, Reno, NV, USA) and a modified 1/16″ bored-through stainless steel reducer (Swagelock, Solon, OH,

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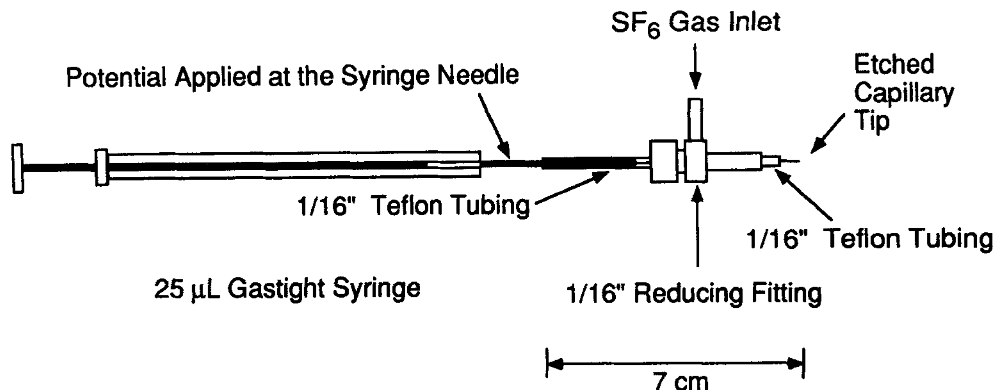

Figure 1. Non-sheath electrospray source, fabricated from a standard small-volume syringe, a combination low dead-volume needle-capillary union/coaxial SF_6 gas outlet, and an HF-etched small diameter fused-silica capillary (5, 10 or 20 μm i.d.).

USA). A piece of 1/16″ o.d. 0.007″ i.d. Teflon tubing (Alltech, Deerfield, IL, USA), is drilled out to permit the snug insertion of a blunt syringe needle. A 5, 10 or 20 μm i.d. capillary (5–6 cm long, Polymicro Technologies) was butted against the syringe needle and held in place by tightening the stainless steel Swagelock fitting. The result was a low dead-volume connection between the syringe needle and fused-silica capillary. (The capillaries used were etched in hydrofluoric acid for approximately 10 min. After etching, the capillaries were flushed with water, trimmed and visually inspected with a microscope.) The Swagelock fitting was modified to permit a coaxial flow of sheath gas. This was accomplished by drilling a 1/8″ hole and silver soldering a piece of 1/8″ stainless steel tubing (Alltech, Deerfield, IL, USA) to the Swagelock fitting. The electrical connection required for the electrospray source was made at the syringe needle. The electrospray currents for both the sheath and non-sheath sources were determined with an electrometer (Keithly, Cleveland, OH, USA) using a copper plate that served as the grounded counter electrode. The tip of the analytical capillary was placed 1.0 cm from the copper plate for both sources.

Horse-heart myoglobin ($M_r = 16\,951$) and ammonium acetate were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and used without additional purification. Acetic acid, hydrofluoric acid and HPLC grade methanol were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA) and used without additional purification. A 12-mer oligonucleotide (5′-dCCTCTCCTCCCT-3′, $M_r = 3468.3$), used for negative ion electrospray, was synthesized with an Applied Biosystems DNA synthesizer (Foster City, CA, USA) and purified by reversed-phase HPLC. Ultra-pure water was obtained from a Barnstead (Dubuque, IA, USA) distilled-deionized purification system. SF_6 was obtained from Matheson (Newark, CA, USA).

RESULTS

The overall design of the non-sheath electrospray source is simple and compact, and can be readily adapted to various source configurations. In our experiments with an 'open' readily accessible mass spectrometer sampling aperture, the syringe and attached non-sheath source are placed on a syringe pump, and the high voltage lead and sheath gas connected. Due to the small overall dimensions and low dead-volume of the non-sheath source, samples can be quickly changed. The small i.d. capillaries used in this non-sheath source design require relatively low flow rates (typically < 0.25 μL/min) compared to conventional ESI sources (~1–10 μL/min), which is advantageous for the analysis of small sample volumes (< 2 μL). Instrumental parameters such as the mass spectrometer interface voltages (i.e., ΔNS) and gas flow rates for the non-sheath source are similar to those used with the methanol-sheath source. However, the non-sheath source can be located closer to the ion inlet (nozzle-skimmer or capillary interface) than the sheath source, because of a decreased susceptibility to electrical breakdown. This is due to the low flow rates and lower potentials required for electrospray with the non-sheath design. The low flow rate used with the non-sheath source makes visual inspection of the electrospray virtually impossible in normal laboratory lighting. This is due to the low volumetric flow rate, but may also be attributed to the formation of somewhat smaller droplets than produced with conventional source designs. The formation of a successful electrospray can be monitored, based upon the electrospray power-supply current. It has been observed that the electrospray power-supply current is significantly more stable with the non-sheath source than that observed for the methanol-sheath source.

The electrospray currents for the methanol-sheath and non-sheath sources, for identical samples, were compared. The i.d.s of the analytical capillaries used were 50 and 20 μm and the analyte flow rates were 0.50 μL/min and 0.20 μL/min for the methanol-sheath and non-sheath sources, respectively. The methanol-sheath liquid was infused at a rate of 2.5 μL/min. (The selected sheath capillary diameter and flow rates are the values most commonly used in our laboratory with this source for pressure infusion studies.) The non-sheath capillary diameter and flow rate represented a compromise between such factors as ease of use, sample consumption and sensitivity. For dilute, low-conductivity solutions (such as distilled water or desalted oligonucleotides in water), the measured electrospray current was approximately 10 times less for the non-sheath source than the current typically observed with the methanol-sheath source. For more-conductive solutions (horse-heart myoglobin in 5% acetic acid), the electrospray current observed for the non-sheath

source was approximately half that of the methanol-sheath source. These observations indicate that the electrospray current is effectively limited by the flow of charge-carrying species to the capillary tip by the low flow rates used with the non-sheath source, a situation where maximum achievable sensitivity is anticipated.

Shown in Fig. 2(a) is the ESI-mass spectrum of a 0.5 mg/mL horse-heart myoglobin/5% acetic acid solution, acquired with the non-sheath source. Shown in Fig. 2(b) is the ESI mass spectra for the same 0.5 mg/mL horse-heart myoglobin/5% acetic acid solution acquired with the methanol-sheath source. The experimental parameters for the two spectra were identical, with the exception of the source position, electrospray voltage and the flow rate of the sample, which were optimized for each source. The methanol-sheath source required an electrospray potential of +4.0 kV for the optimum combination of stability and signal intensity,

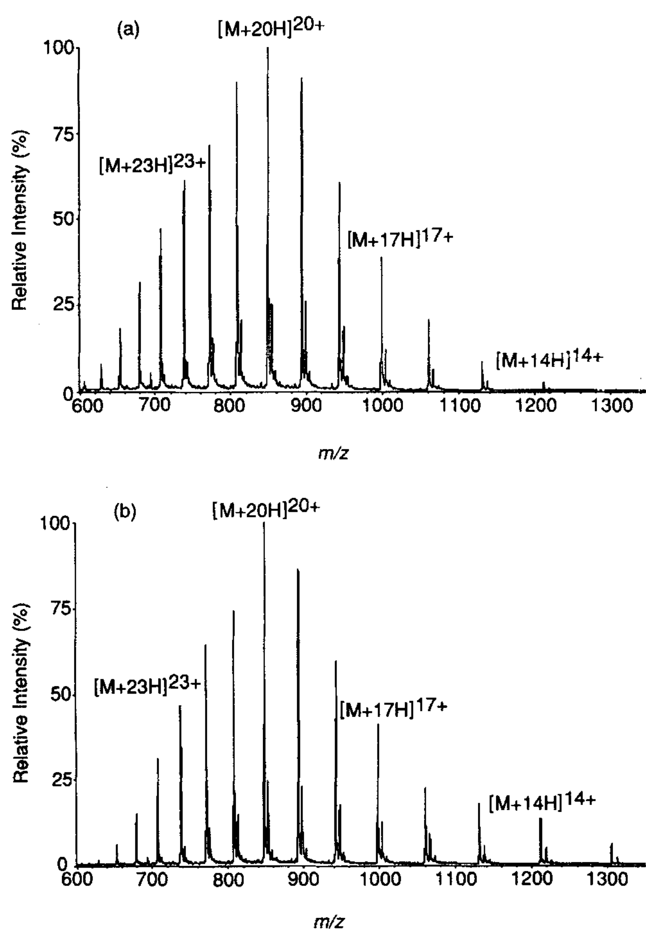

Figure 2. (a) Positive-ion electrospray mass spectrum of a 0.5 μg/mL sample of horse-heart myoglobin in 5% acetic acid, obtained with the non-sheath source. For the electrospray mass spectrum a total of 8 scans were summed with a step size of 0.2 *m/z* and a dwell time of 10 ms. The total amount of sample consumed was 30 pmol (1.02 μL). The i.d. of the etched capillary was 20 μm and the flow rate was 0.2 μL/min. The electrospray potential was +2.15 kV, lens potential was 700 V and ΔNS was 185 V. (b) Positive-ion electrospray mass spectrum of a 0.5 mg/mL sample of horse-heart myoglobin in 5% acetic acid, obtained with the methanol-sheath source. For the electrospray mass spectrum a total of 8 scans were summed with a step size of 0.2 *m/z* and a dwell time of 10 ms. The total concentration of sample consumed was 75 pmol (2.53 μL). The methanol-sheath flow rate was 2.5 mL/min. The i.d. of the analytical capillary was 50 μm and the flow rate of the horse-heart myoglobin was 0.5 μL/min. The electrospray potential was +4.0 kV, lens potential was 700 V and ΔNS was 185 V.

whereas the optimum potential for the non-sheath source was +2.15 kV. The lower electrospray potential for the non-sheath source can be attributed to the higher local electric field gradient at the tip of the non-sheath source due to the smaller diameter etched capillary terminus.[5] The non-sheath source was found to electrospray over a wide range of potential (typically up to 1000 V higher than the initial electrospray onset potential). However, the highest sensitivity and best stability were achieved at potentials slightly higher (~100–400 V) than the electrospray onset potential. Comparing the intensities for the base peak in the electrospray mass spectra from the two sources (20+ charge state of myoglobin for both) one finds that the signal for the non-sheath source is 8.4 times more intense than that of the methanol-sheath source, at a flow rate 2.5 times less, with all data acquisition parameters kept constant (number of scans, scan width, step size, multiplier voltage and dwell time), corresponding to an increase in sensitivity by a factor of 21. The overall charge-state distributions observed are similar; however, higher charge-state ions appear to be more intense for the non-sheath source and lower charge-states appear to be more intense with the methanol-sheath source. One explanation for the difference in the two spectra (Fig. 2(a) and (b)) may be related to the methanol solvent promoting greater gas-phase charge transfer due to the greater concentration of these solvent molecules, thus lowering the overall charge-state. A second possible explanation is that the electrospray process in the methanol sheath is saturated, due to the number of potentially charge-carrying species exceeding the available ESI current. The latter explanation seems more likely since high analyte concentrations are known to cause such shifts in charge-state distributions.

We have also observed from previous experiments in our laboratory that the sheath source can be relatively unstable over long periods of time. A primary goal of this work was to improve the stability of the electrospray signal. Shown in Fig. 3 are plots of the electrospray ion current measured for a 10 *m/z* range vs. time for both (a) the non-sheath and (b) the methanol-sheath sources, respectively. The range scanned, 843 to 853 *m/z*, corresponds to the most intense charge-state (20+) observed in the ESI mass spectra of a 0.5 mg/mL sample of horse-heart myoglobin/5% acetic acid for both the non-sheath and methanol-sheath sources. A total of 500 scans (total data acquisition time 17.2 min) were acquired. The average ion current of the (20+) charge-state is 7.4 times larger for the non-sheath source compared to that for the methanol-sheath source. The relative standard deviation (RSD) of the measured ion currents for the methanol-sheath source is 9.5% and for the non-sheath source 4.4%. These results indicate that the non-sheath source is more stable and sensitive compared to the methanol-sheath source, in the positive-ion mode, for this analyte.

A 12-base oligonucleotide, 5′-dCCTCTCCTCCCT-3′ in distilled deionized water, was used to evaluate the non-sheath source in the negative electrospray mode. Shown in Fig. 4 are the ESI mass spectra of a 8.6 μg/mL aqueous solution of 5′-dCCTCTCCTCCCT-3′ acquired with (a) the non-sheath and (b) the methanol-sheath sources respectively. The experimental parameters for Fig. 4 (a) and (b) were identical, with the exception of

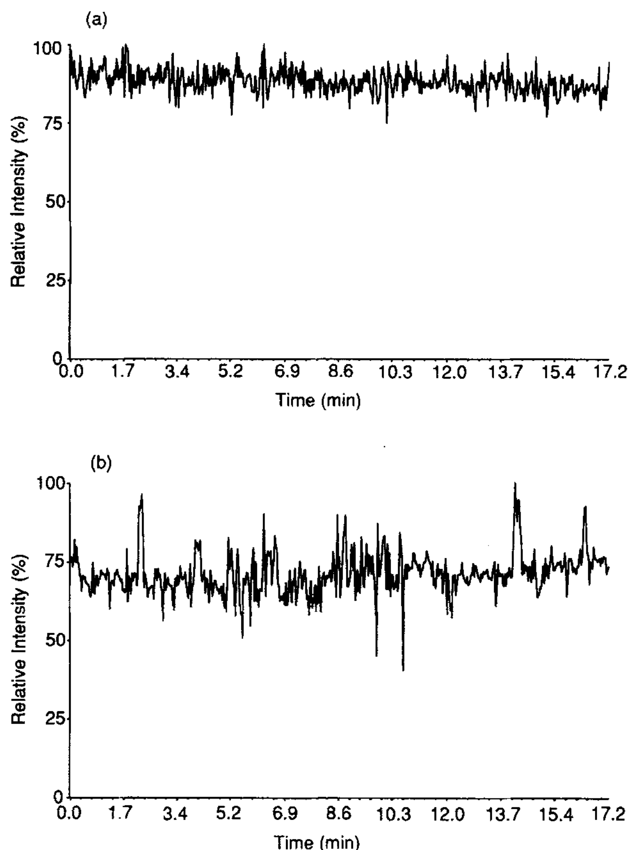

Figure 3. Plot of signal electrospray ion current for a 10 *m/z* range vs. time for (a) the non-sheath source and (b) the methanol-sheath source. The range scanned, *m/z* 843–853, corresponds to the most intense charge-state observed in the ESI mass spectra of horse-heart myoglobin (shown in Fig. 2). Other acquisition parameters: 500 scans acquired (17.2 min), step size 0.1 *m/z*, dwell time 20 ms. The average intensity for (a) was 8 650 000 with an RSD of 4.4% and for (b) was 1 167 000 with an RSD of 9.5%.

the electrospray voltage and the sample flow rates, which were optimized for each source. The methanol-sheath source required an electrospray potential of −4.0 kV, whereas the non-sheath source required a potential of −3.0 kV for the optimum combination of stability and signal intensity. The most abundant peak, corresponding to the (6−) charge-state of the oligonucleotide, was 4.6 times greater for the non-sheath source in comparison to that obtained using the methanol-sheath source. The observed charge-state distribution for the 12-mer oligonucleotide (Fig. 4 (a) and (b)) was similar for both sources. The non-sheath source mass spectrum exhibits enhanced signal-to-noise and was significantly easier to obtain than the methanol-sheath source mass spectrum.

The stabilities of the two sources were also compared in negative-ion mode with the 8.6 μg/mL aqueous solution of 5′-dCCTCTCCTCCCT-3′. Shown in Fig. 5 are plots of the electrospray ion current measured for a 10 *m/z* range vs. time for both (a) the non-sheath and (b) the methanol-sheath sources respectively. The *m/z* range scanned corresponds to the most intense charge state, (6−), observed in the ESI mass spectra of 5′-dCCTCTCCTCCCT-3′. A total of 500 scans (total data acquisition time 17.2 min) were acquired over the 572–582 *m/z* region. The average ion current of the (6−) charge-state was 5.1 times larger for the non-sheath source compared to that for the methanol-sheath source design. The RSD of the measured ion currents for the methanol-sheath source was 33.1% compared to 3.8% for the non-sheath source. Clearly, these results indicate that the non-sheath source is more stable and sensitive in the negative-ion mode compared to the methanol-sheath source design.

With small diameter capillaries, low flow-rate ESI-MS of proteins, peptides and oligonucleotides is possible. Shown in Fig. 6 is an electrospray mass spectrum of the 8.6 μg/mL aqueous solution of 5′-dCCTCTCCTCCCT-3′ acquired with a 5 μm etched tip capillary. The flow rate used for this analysis was 0.050 μL/min. A total of 20 nL of sample, which represents 50 fmol of the oligonucleotide, was consumed over the course of this analysis (less than 2 μL of sample required). Several other proteins have been analyzed and accurate mass measurements have been obtained with sample consumption with the 5 μm non-sheath source in the high-attomole to low-femtomole range. The stability of the electrospray from the 5 μm capillary non-sheath source was also investigated. A total of 500 scans of the most intense ion (6−), 10 *m/z* range, in the 5′-dCCTCTCCTCCCT-3′ mass spectrum were acquired. An RSD of 5.1% was obtained, indicating that the 5 μm i.d. non-sheath source is stable over long data acquisition times at low flow rates. The amount of sample consumed for the 5 μm i.d. capillary stability study (total data acquisition time 17.2 min) was less than 1 μL.

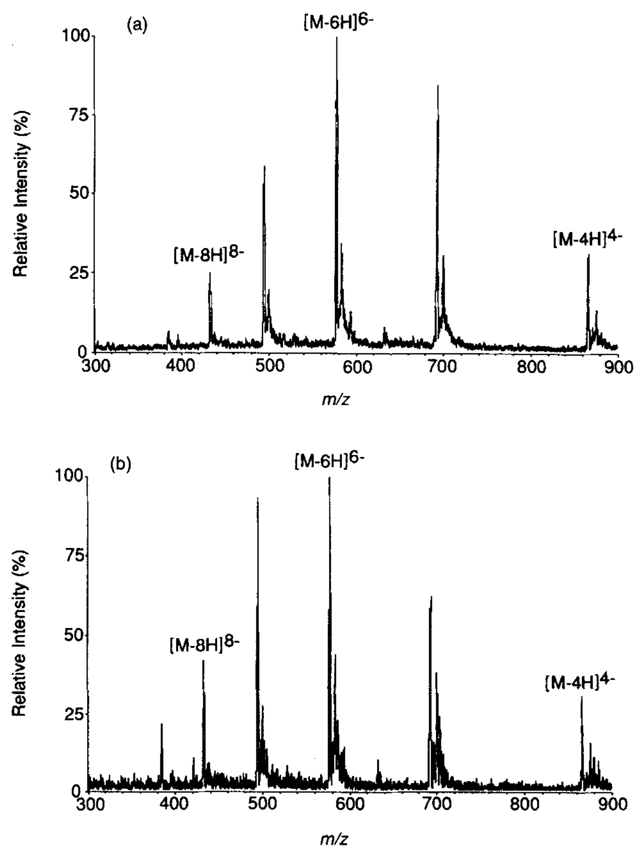

Figure 4. Negative-ion electrospray mass spectrum of a 8.6 μg/mL 5′-dCCTCTCCTCCCT-3′ sample obtained with (a) the non-sheath source and (b) the methanol-sheath source. For the electrospray mass spectra a total of 8 scans were summed with a step size of 0.2 *m/z* and a dwell time of 10 ms. The total amount of sample consumed for (a) was 1 pmol (1.02 μL). The total amount of sample consumed for (b) was 2.5 pmol (2.53 μL). The lens potential was −700 V and ΔNS was −120 V for both spectra.

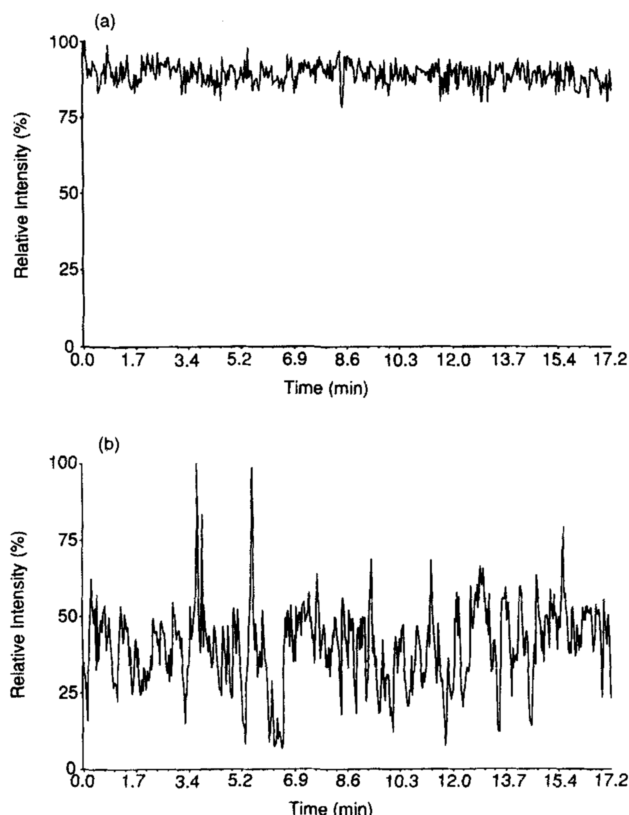

Figure 5. Plot of signal electrospray ion current for a 10 *m/z* range vs. time for (a) the non-sheath source and (b) the methanol-sheath source. The range scanned, *m/z* 572–582, corresponds to the most intense charge-state observed in the ESI mass spectra of 5'-dCCTCTCCTCCCT-3'. Other acquisition parameters: 500 scans acquired (17.2 min), step size 0.1 *m/z*, dwell time = 20 ms. The average intensity for (a) was 393 039 with an RSD of 3·8% and for (b) was 75 814 with an RSD of 33·1%.

CONCLUSION

A new electrospray ionization source for pressure-infusion studies has been developed. The non-sheath source has the attributes of high sensitivity, low required flow rates and long-term stability, which enable long acquisition times to be used for small volumes, dilute solutions and/or difficult-to-analyze samples. This is particularly useful in the study of limited biological samples, as demonstrated with oligonucleotides. The non-sheath source can more effectively electrospray a variety of proteins, peptides and oligonucleotides under a variety of aqueous conditions without the presence of non-aqueous solvents or sheath flows. The non-sheath source also generates stable electrosprays from aqueous solutions at substantially lower applied voltages than are required by the methanol-sheath source. The ability to electrospray from lower potentials minimizes possible contributions and experimental difficulties arising from electrical breakdown phenomena (corona). Stable electrosprays can also be generated without the need to vary the analyte flow rate to optimize stability, which enables the swift analysis of samples in a variety of solvent conditions. Space-charge limiting effects may also be reduced due to the lower electrospray currents, allowing a larger fraction of the ions formed to enter the mass spectrometer, resulting in greater sensitivity (i.e., lower absolute detection limits).[12] The non-sheath design eliminates the contribution of partially ionized species originating from the sheath, resulting in more effective ionization of the analyte and more effective utilization of the reduced electrospray current. The non-sheath source provides higher analyte signal intensities, typically 4–10 times greater, when compared to the standard methanol-sheath design for identical samples under nearly identical experimental conditions. This may be due to the production of smaller droplets from the small i.d. capillaries. Finally, the non-sheath source can operate at flow rates of magnitude similar to those of CE, which may be useful in the optimization of instrumentation parameters (interface type, heating, position, gas flow rates, etc.).

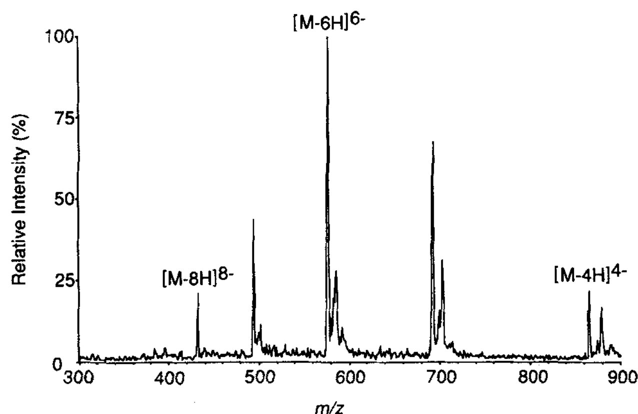

Figure 6. Negative-ion electrospray mass spectrum of an 8.6 μg/mL 5'-dCCTCTCCTCCCT-3' sample obtained with the non-sheath source. A total of 4 scans were summed with a step size of 1.0 *m/z* and a dwell of 10 ms. The total amount of sample consumed was 50 fmol (20 nL). The i.d. of the etched capillary was 5 μm and the flow rate of the 5'-dCCTCTCCTCCCT-3' sample was 0.050 μL/min. The electrospray potential was −3.0 kV, lens potential was −700 V and ΔNS was −120 V.

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