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Single-Pulse Nanoelectrospray Ionization

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A new electrospray ionization (ESI) source that provides a means of generating single packets of ions for mass spectrometric analysis is presented. Sample solution held at a high potential is ejected from a glass capillary with a small dispensing aperture (20-μm i.d.) by constriction of a cylindrical piezoelectric element. Unlike conventional ESI sources that are continuous, this source dispenses fixed volumes of solution as small as 10 pL and provides detection sensitivity in the attomole range when coupled to an orthogonal time-of-flight mass spectrometer. In addition to picoliter-level control over the dispensed volume, the source permits control of the frequency with which ionization pulses are generated as well as the ability to start and stop the pulses without altering the applied solution potential. The source was characterized by analysis of both protein and DNA samples from a variety of different solution compositions. This source design should be compatible with virtually any ESI mass analyzer.

Piezoelectric dispensing of sample solutions has proven useful in many applications, including, but not limited to, ink-jet printing[1], studies of droplet evaporation and combustion,[2] droplet collision and coalescence,[3] automated titration,[4] automated reagent dispensing,[5] single molecule fluorescence analysis,[6] and as a source for inductively coupled plasma,[7] quadrupole[8] and MALDI-TOF[9] mass spectrometers. There are two piezoelectric dispensing methods that produce monodisperse droplets with directed momentum: continuous production by Rayleigh breakup of a liquid jet (vibrationally induced breakup of a continuous stream) and drop-on-demand production by rapid pressure pulsation.[10] In the latter method, a single droplet is released from the end of a capillary as a result of a rapid pressure pulsation generated by a radially contracting piezoelectric element. The size of the droplet produced depends on the solution conditions, the orifice diameter, and the amplitude and duration of the pressure pulse (which is controlled by the amplitude, duration, and shape of an electronic pulse applied to the piezoelectric element).[11]

Hager et al. successfully coupled a droplet source that utilized the Rayleigh breakup of a liquid jet with a Sciex TAGA 6000E mass spectrometer.[8] The droplets were charged by corona discharge from a platinum rod electrode placed near the droplet stream (which was running orthogonal to the mass spectrometer ion aperture). A mass spectrum of dodecyldiamine (M_w = 201 amu) in methanol was obtained, but no work was done with high-molecular-weight species. This work offered an alternative ionization method useful for evaluating the ESI process,[12] but it is not amenable to single or discretely controlled pulsed ionization.

Murray and He demonstrated the feasibility of performing mass spectrometry on discretely produced droplets using a MALDI process for generating ions.[9] In their work, a piezoelectric dispenser was used to create dried aerosol particles consisting of matrix and sample. This method relies on the transport of neutral particles into the mass spectrometer where they were ionized by laser irradiation in a MALDI instrument equipped for atmospheric sampling. This method was reported to consume ~4500 droplets (or 50 pm of analyte)/mass spectrum. It was speculated that the low sensitivity of the method was due to poor particle transmission efficiency.

We describe here the development of a new pulsed ionization source that employs piezoelectric solution dispensing to produce discrete packets of ESI-generated ions. Detection limits comparable to those obtained by nanoelectrospray mass spectrometry[13] are obtained when this source is employed in conjunction with orthogonal extraction TOF mass spectrometry. As with nanoelectrospray sources, a narrow capillary is used to dispense the sample solution; however, the exit aperture is 20 μm in diameter, as compared to the 1−10-μm diameter apertures used in conventional nanospray. This larger tip diameter has been found to be resistant to particle clogging, a persistent problem associated with traditional nanospray tips.[14] The ability to control the number and frequency of ionization pulses, as well the total volume dispensed per mass spectrum (down to 10 pL) distinguishes this new ionization technique from current electrospray techniques that continuously produce ions over longer periods of time.

EXPERIMENTAL SECTION

Chemicals. All protein samples were obtained from Sigma Chemical Co. (St. Louis, MO) and used without further purifica-

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Table 1

pulse parameters	pulse amplitude (V)	rise time (μs)	pulse width (μs)	fall time (μs)	no. pulses dispensed	pulse frequency (Hz)
typical range of value	10−80	0−40	0−30	0−40	1-continuous (single pulse increments)	1−1000
typical starting values	30	5	15	20	100	250

tion. For positive mode analysis, protein samples were diluted in a buffer of 1:1 H_2O/CH_3CN, 1% acetic acid or 1:1 H_2O/MeOH, 1% acetic acid to a concentration of 1 μM (unless noted otherwise). The 18-mer oligodeoxynucleotide (5′ TGT AAA ACG ACG GCC AAG 3′) was obtained reversed-phase HPLC purified from Integrated DNA Technologies, Inc. (Coralville, IA). For negative mode analysis, the DNA sample was diluted in a buffer of 1:1 H_2O/MeOH, 400 μM 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (adjusted to pH 7 with triethylamine) to a concentration of 5 μM.

Mass Spectrometer. All experiments were conducted on a Mariner Biospectrometry Workstation (PerSeptive Biosystems, Inc., Framingham, MA) orthogonal TOF mass spectrometer. The original pressure-assisted electrospray ion source employs a fused-silica polyimide-coated capillary (150-μm o.d., 25-μm i.d.). The inlet end of the capillary was immersed in the sample solution contained in a pressurized vessel. The positive pressure (10 psi) applied to the sample vessel resulted in a solution flow rate of 0.3 mL/min. The sample solution was maintained at a positive (or negative) potential up to 4900 V via contact with a platinum electrode. For the pulsed ionization analysis, the original pressure-assisted ESI source was replaced by the pulsed ionization source constructed in-house. The heated inlet nozzle of the spectrometer was lengthened by 2.5 cm through the addition of a 1-mm-i.d. stainless steel tube mechanically coupled to the inlet. Lengthening the nozzle facilitates alignment of the source with the sample inlet and provides additional desolvation time. No nebulizer, sheath flow, or curtain gases were employed. The Mariner's high voltage power supply was used to bias the pulsed ionization source. The mass range of the spectrometer was adjusted for each sample using the control software supplied with the instrument to maximize the duty cycle of the orthogonal time-of-flight mass analysis. Mass spectra presented were smoothed using a 98-point Gaussian smoothing algorithm provided with the PerSeptive Biosystems instrument (unless noted otherwise).

Source Construction. The pulsed ionization source is constructed from a glass capillary cemented into a cylindrical piezoelectric element. Prior to cementing, one end of the capillary is reduced to an inner diameter ~20 μm by flame polishing in a process similar to that described by Switzer.[11] The glass tubing (World Precision Instruments, Sarasota, FL), originally 1.5-mm o.d. × 0.8-mm i.d., is held vertically with one end over a Bunsen burner flame and rotated with the aid of an electric drill motor (100−200 rpm). This causes the end of the capillary to constrict and eventually close off. The flame-polished glass tubes are then ground and optically polished to produce a flat surface normal to the aperture opening. Grinding and polishing is accomplished through the use of a Buhler Ecomet 3 variable speed grinder−polisher (Lake Bluff, IL) that has been fitted with a custom holding fixture (designed and built in-house) that allows the capillary to be rotated around its central axis while being held normal to the polishing surface. Initial grinding is performed on a wetted 600 grit grinding disk (Buhler) and progressed with successively finer grit down to a 3 micron aluminum oxide abrasive film disk (South Bay Technology, San Clemente, CA). The flame polishing produces a tapered inside diameter; thus, the extent of grinding determines the size of the aperture, and it is necessary to microscopically monitor this process. A ground, polished, and cleaned glass tube of the desired aperture is then epoxied into a piezoelectric cylinder. The PZT-5A cylindrical piezoelectric element (Morgan Electro Ceramics, Bedford, OH) utilized has an outer diameter of 2.946 mm, an inner diameter of 1.778 mm, and is 12.7 mm in length. Electrical connections are made to the nickel-plated electrodes on the inner and outer surfaces of the piezoelectric cylinder via soldered 30-gauge wires.

Source Operation. The piezoelectric dispenser is driven with control electronics obtained from Engineering Arts (Mercer Island, WA). The drive electronics are controlled via RS232 communication with a personal computer (PC). To facilitate adjustments of the pulse parameters, we created a Windows-based user interface using LabWindows/CVI (National Instruments, Austin, TX). Table 1 lists the adjustable pulse parameters used for controlling the piezoelectric dispensing unit. It is possible to trigger the drive electronics externally with a TTL input pulse. This feature facilitates the timing of droplet illumination in our imaging system.

Sample solution is aspirated into the dispensing unit by immersing the dispensing end of the capillary in the sample solution and pulling a vacuum on a syringe connected to the back end. The dispenser tip is then wiped clean with a low lint lab wipe. The dispenser tip is mounted on an $x-y-z$ translational stage in front of the nozzle inlet of the mass spectrometer. A platinum electrode is inserted into the back end of the dispenser to hold the solution at high potential relative to the mass spectrometer inlet. Typically, the nozzle inlet is held at 100−200 V while the source voltage is adjusted for each sample, ranging from 2000 to 4900 V with a 1.5-mm separation between the dispenser tip and nozzle. The ion source region was imaged using a 6× microscope objective attached to a CCD camera system (JAI, CV-M50, Vision 1, Bozeman, MT) mounted directly above the dispenser tip on an $x-y-z$ stage. The droplets were illuminated at fixed positions via stroboscopic illumination from a light-emitting diode (LED) driven at the same frequency as the piezoelectric-voltage pulses. Two pulse generators (Philips, model PM 5715) were used for droplet imaging, one for setting the external trigger frequency on the dispenser drive electronics and the other for controlling the synchronized delay pulse on the LED strobe.

Dispensed Volume and Velocity Measurements. The volume of sample solution dispensed per pulse was determined using two techniques. The first technique involved calibrating the magnified CCD camera image using a microscope stage microme-

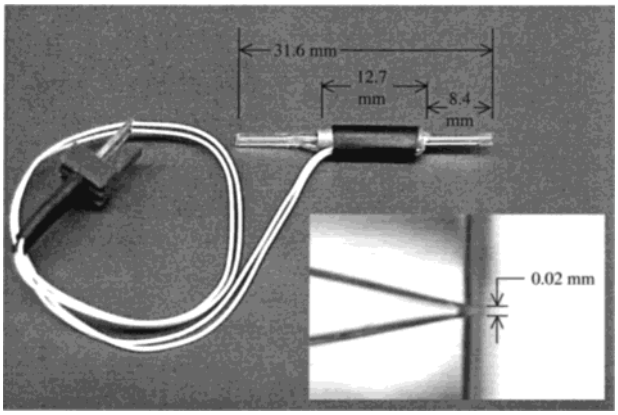

Figure 1. Photograph of the pulsed nanoelectrospray source. The glass tubing extends through the center of the piezoelectric cylinder (12.7 mm). The dispensing end of the source (8.4 mm) has the inner diameter reduced to a 20-μm exit aperture (magnified inset image).

ter. It was determined that a single CCD pixel corresponded to an area of 71 μm^2. From the expanded pixelized image, the droplet diameter can be measured and the volume calculated. The second technique involved dispensing a fixed number of droplets and weighing the dispenser before and after sample dispensing. The mass difference divided by the number of dispensing events and solution density gives volume per pulse. The two techniques produced values which were in agreement to within 20%.

The initial droplet velocity was calculated by measuring the distance the droplet traversed for a given time delay on the LED strobe. Droplet production was performed with the solution electrode held at 4900 V relative to the mass spectrometer. The distance the droplet traveled (~0.5 and 1 mm) was measured by reference to a stage micrometer, and the time delay on the LED strobe (~40 and 80 μs) from initial to final droplet position was measured using an oscilloscope. The nearly equivalent velocities calculated at 0.5 and 1 mm indicate the droplet has reached a steady-state velocity over the region examined.

RESULTS AND DISCUSSION

A commercially available source of piezoelectric dispensing devices compatible with the pulsed ionization source design was not available. Therefore, in-house construction of the dispensing units was performed as described in the materials and methods section. Figure 1 displays a photograph of a completed dispenser along with a magnified photograph of the dispenser tip. The flame-polishing technique employed to create the reduced dispensing aperture results in a narrowing of the inner diameter of the glass tubing while leaving the outer diameter nearly unchanged (see inset photograph of Figure 1). This produces a dispensing tip that is very robust, especially when compared to pulled capillaries. This robustness has been demonstrated by near daily use of a single dispenser over a three month period. The only maintenance required during this period was simple cleaning after each use by aspiration of purified water. This cleaning has been sufficient to eliminate any observable crossover contamination from one sample to the next. Additional dispensers have been constructed in which the distance the dispensing end of the glass tubing extends from the piezoelectric cylinder has been varied from 2.5 to 8.5 mm without noticeable effect on droplet formation.

The dispenser dead volume, or minimum volume required for dispensing, is dictated by two parameters: (1) the inner diameter of the glass capillary and (2) the overall length of the glass capillary contained within the piezoelectric cylinder and extending out to the dispensing end of the device. The dispenser pictured in Figure 1 has a dead volume of 3.3 μL. The dead volume of a different dispenser which only has 2.5 mm of capillary extending beyond the piezoelectric cylinder is 2.4 μL. Further reductions in dead volume could be achieved by constructing the dispensers from piezoelectric cylinders and glass tubing with smaller inner diameters. We have not pursued further reductions in dead volume because the analysis of microliter to submicroliter samples volumes is easily accomplished with the current dispenser design by first back-filling the dispenser with a blank spray solution then drawing the desired volume of sample solution into the tip. We have not observed a significant dilution effect while analyzing submicroliter samples by this technique.

To generate a packet of ions, a voltage (up to 4900 V) is first applied to the platinum wire in contact with the sample solution holding it at a high potential relative to the mass spectrometer inlet. This establishes an electric field that results in migration of ions (same polarity as the voltage on the platinum wire) to the dispensing end of the piezoelectric tip. The electric field is maintained below the level that will spontaneously generate normal electrospray. A voltage pulse is then applied between the two contacts of the piezoelectric cylinder, causing it to constrict radially. This constriction is transmitted through the glass tube to create a shock wave in the sample solution. The resulting pressure fluctuation ejects solution from the small aperture opening of the dispensing end of the glass capillary. The ejected solution carries an excess charge due to the migration of the ions in the bulk sample solution. The volume of the ejected droplet is replaced by capillary action from the bulk solution, and the charge deficit is neutralized by the high-voltage power supply.

Figure 2 demonstrates the use of the pulsed ionization source to acquire mass spectra of a protein and a DNA sample. Figure 2a shows the mass spectrum of bovine ubiquitin (M_w = 8565.9) in positive ion mode and Figure 2b shows the mass spectrum of an 18-mer DNA oligonucleotide (M_w = 5465.6) in negative ion mode. The ubiquitin spectrum was generated from 100 individual pulses of the piezoelectric dispenser at a rate of 250 Hz. The analysis consumed 2.8 nL of sample at a concentration of 1 μM or a total of 2.8 fmol of sample with the solution held at a potential difference of +4500 V from the mass spectrometer. The DNA spectrum was generated from 1000 pulses generated at 250 Hz from a 5 μM sample solution held at −3000 V relative to the mass spectrometer. The analysis consumed 33 nL of sample at a concentration of 5 μM or a total of 165 fmol. Consistent with conventional ESI, the measured signal for DNA (in negative mode) is less than the signal obtained from proteins (in positive mode).[15]

The characteristics of the voltage pulse sent to the piezoelectric element influence the volume and form of the solution ejected from the dispenser. Table 1 lists the adjustable parameters for the voltage pulses, along with typical ranges employed. Tuning of these parameters is required for the stable dispensing of a fixed sample volume per voltage pulse applied to the dispenser tip. Each of the voltage pulse characteristics may be adjusted before or during dispensing operations. Tuning of the pulse parameters is monitored real-time via a CCD camera with a synchronized LED

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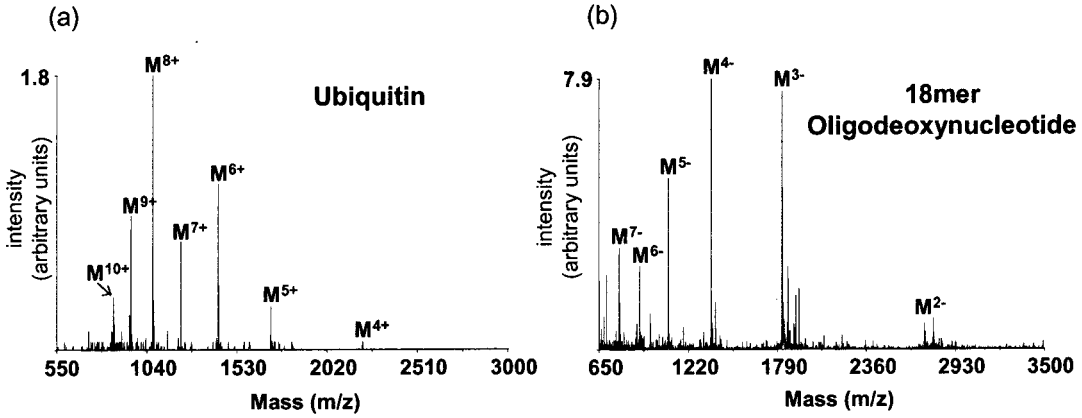

Figure 2. Mass spectra acquired using the pulsed nanoelectrospray source: (a) positive mode mass spectrum of a protein sample and (b) negative mode mass spectrum of a DNA sample. Part a is from a solution of ubiquitin (M_w = 8565.9) at 1 μM in 1:1 acetonitrile/water with 1% acetic acid. Pulses (100) were dispensed at 250 Hz, with each pulse volume ~28 pL. The solution was held at +4500 V relative to the mass spectrometer inlet. Part b is from a DNA oligonucleotide, mixed-base 18-mer (M_w = 5465.6) at 5 μM in 1:1 water/acetonitrile with 400 μM HFIP. Pulses (1000) were dispensed at 250 Hz, with each pulse volume ~33 pL. The sample solution was held at −3000 V relative to the mass spectrometer inlet.

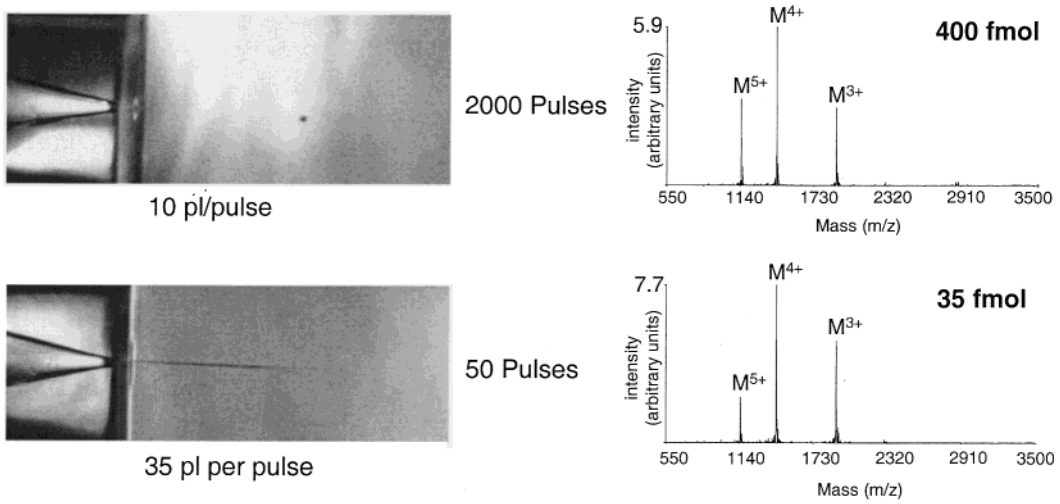

Figure 3. The piezoelectric pulsed nanoelectrospray source can be tuned to dispense either single droplets per pulse (top left) or an elongated pulse stream (bottom left). The corresponding mass spectra for the given pulse numbers are shown on the right. The spectra are of roughly equivalent signal intensity, but the spectrum from the elongated pulse required ~12 times less sample than the single drop spectrum. The same sample solution, 20 μM insulin in 1:1 methanol/water, 1% acetic acid, was used for both pulsing conditions. The sample solution was held at a potential difference of +4500 V relative to the mass spectrometer inlet, with source operation at 250 Hz. The following are piezoelectric voltage pulse parameters for each mode. Single droplet mode: pulse amplitude, 20 V; rise time, 13 μs; pulse width, 5 μs; and fall time, 3 μs. Elongated pulse stream: pulse amplitude, 35 V; rise time, 13 μs; pulse width, 5 μs; and fall time, 3 μs.

strobe. User interface software developed in-house allows rapid, mouse-click control of all parameters listed in Table 1. Once the pulse parameters have been tuned for a particular piezoelectric dispensing tip, those parameters can generally be used to reproduce the desired pulse characteristics for a given sample solution and applied solution potential.

The mode in which the sample solutions were ejected from the capillary could be changed by adjusting the shape or amplitude of the voltage pulse applied to the piezoelectric element. Two stable sample ejection modes are shown in Figure 3. In one mode, single droplets are formed, whereas in the other, a small stream is formed that quickly breaks apart into a series of smaller droplets. The two different dispensing modes were obtained by changing the amplitude of the applied pulse to the dispenser (in the example shown, increasing the pulse amplitude from 20 to 35 V changes the form of the dispensed solution from a single droplet to a stream). The amount of sample dispensed per pulse was 10 pL for the droplet and 35 pL for the stream. Even though the dispensed volume increased by a factor of only 3.5 in the stream mode, the observed signal increased by a factor of nearly 12. This observation is consistent with the current understanding of ESI mechanisms. The smaller droplets, generated by breakup of the pulsed stream, have a higher surface-to-volume ratio, which makes a larger proportion of the analyte molecules available for desorption.[13] All data shown, with the exception of the discrete droplet data in Figure 3, were generated using the stream mode.

The pulsed ionization source was compared to a continuous microspray-ESI source over a range of solution concentrations for the protein sample insulin (5735 Da). Figure 4 gives the mass spectra acquired using the pulsed ionization source (left column) and the continuous ESI source (right column) for three sample solution concentrations (1 μM, 50 nM, and 2.5 nM). For each

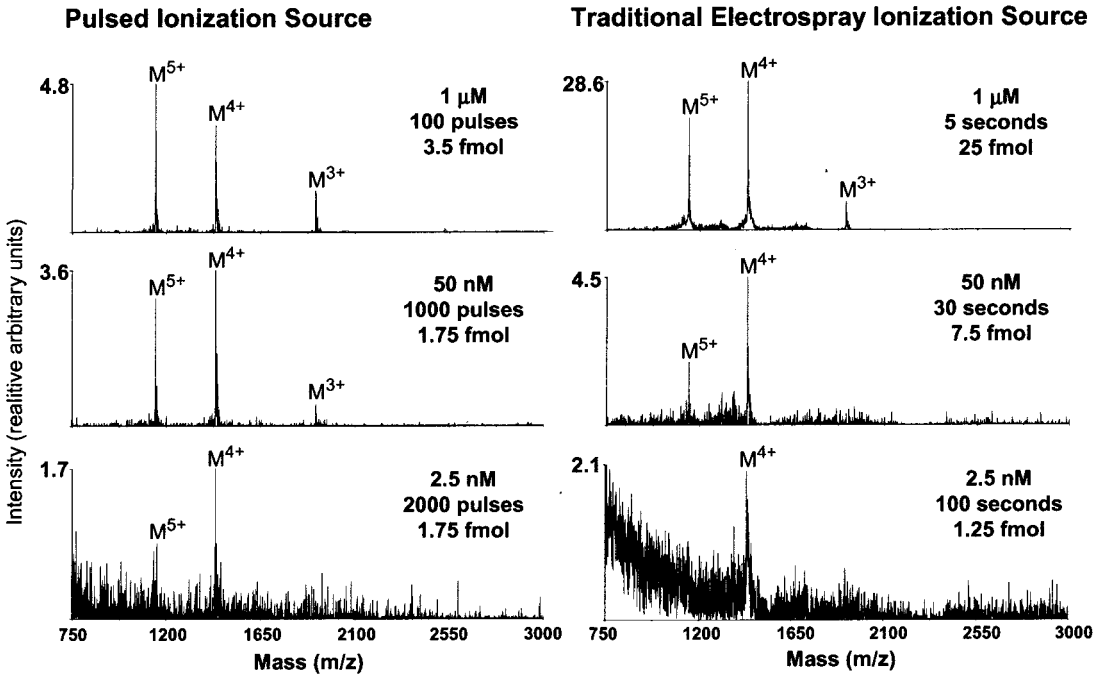

Figure 4. The mass spectra obtained from the pulsed ionization source are compared to those obtained from a continuous ESI source (pneumatically assisted forced flow microspray) over a concentration range of insulin (1 μM, 50 nM, and 2.5 nM). The insulin sample solutions contained 1:1 methanol/water with 1% acetic acid.

spectrum, the solution concentration, the number of pulses or time of acquisition, and the number of moles of sample consumed are given. The number of moles consumed was calculated by the product of sample concentration with total volume consumed during spectrum acquisition. For the pulsed source, an average pulse volume of 35 pL multiplied times the number of pulses dispensed gives total volume. For the continuous ESI source, the experimentally determined flow rate of 0.3 μL/min was multiplied times the spectrum acquisition time to give total volume. Comparing the pulsed and continuous sources for the lowest solution concentration (2.5 nM, Figure 4, bottom row) it can be seen that roughly the same number of moles are consumed to yield spectra of comparable signal intensities and signal-to-noise ratios. For the 50 nM solution concentration, the pulsed source consumes roughly 4 times less sample to achieve a mass spectrum approximately equivalent to that from the continuous source. For the 1 μM sample, the two sources can be compared in two ways. First, in terms of number of moles consumed for a given signal intensity, the two sources are fairly equivalent, with the pulsed source yielding a factor of 6 less in signal intensity for a factor of 7 less in moles consumed. Second, in terms of signal-to-noise ratio, the two spectra are roughly equivalent, and the pulsed source has consumed 7 times less sample. The larger sample consumption for the continuous source is due to a reasonable minimum time (5 s) required for spectrum acquisition. This last comparison illustrates one of the significant differences between the new pulsed ionization source and a continuous ESI source, namely that the pulsed source offers a higher degree of control over the amount of sample consumed in the ionization process. The ability to precisely dispense the required amount of sample solution during the ionization process helps to conserve analyte.

The data shown in Figures 3 and 4 were obtained using multiple sequential dispensing pulses (as noted) and summing

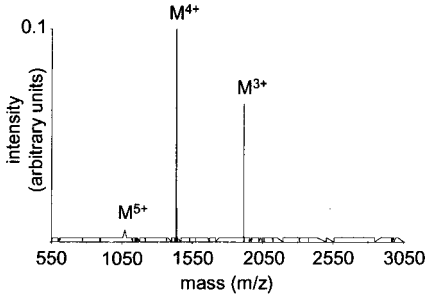

Figure 5. Mass spectrum acquired from a single 80-amol pulse of insulin solution from the pulsed nanoelectrospray source. The sample solution is 1 μM in 50% methanol with 1% aqueous acetic acid. The pulse volume was measured at 80 pL. The sample solution was held at +4700 V.

the signal over the total dispensing period. It is also possible to obtain high quality mass spectra from a single pulse. An example is shown in Figure 5, which was obtained from a single 80-pL pulse of 1 μM insulin, corresponding to 80 amol of analyte. This level of sensitivity is comparable to that attained with nanoelectrospray mass spectrometry.[5] Signal intensity is the principle factor determining the need for single or multiple sequential pulses. The intensity of the single pulse spectrum (Figure 5) is adequate for chemical identification, but certain applications, such as multiple MS/MS experiments, may require a more substantial signal, such as the 100-pulse spectrum demonstrated in Figure 4 (1 μM).

An advantage of traditional electrospray is the ability to employ a variety of different sample solutions, ranging from aqueous to organic, to enhance the solubility of various analyte species and maximize the rate of desolvation of electrosprayed droplets. The pulsed ionization source was evaluated for a range of solution compositions. Figure 6 displays the mass spectra obtained from 100 pulses of a 5 μM insulin sample from each of four different solution compositions: (1) 75% methanol in water, (2) 50%

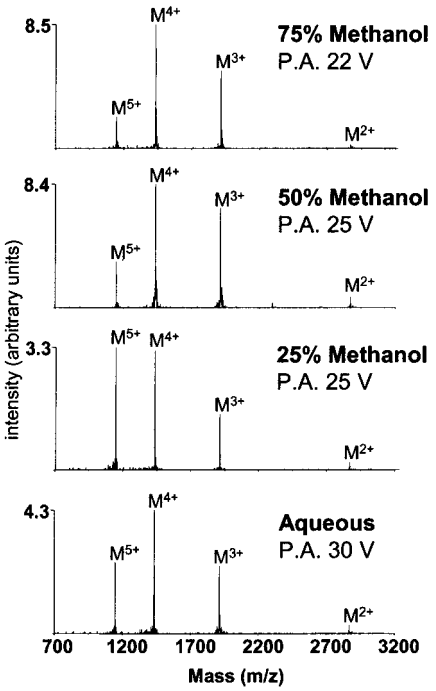

Figure 6. Demonstration of the compatibility of the pulsed ionization source with various methanol:water mixtures, each containing 1% acetic acid and 10 μM insulin. The piezoelectric voltage pulse amplitude (P.A.) used to achieve optimal signal is given for each solution composition.

methanol in water, (3) 25% methanol in water, and (4) a straight aqueous solution; all sample solutions contained 1% acetic acid. The piezoelectric pulse parameters were optimized for the 50% methanol solution to the following: pulse amplitude 25 V; rise time, 20 μs; pulse width, 7 μs; fall time, 12 μs; with the solution potential held at +4900 V. For the remaining solutions, the only pulse parameter adjusted was the pulse amplitude (P.A.), and the values for optimal signal are listed in Figure 6 under the percent methanol values for each spectrum. In general, as the percentage of methanol was decreased, an increase in the pulse amplitude was required to maintain maximum signal. This is consistent with the general observation that increasing the percentage of organic solvent facilitates the formation of the pulsed stream mode, which operates at higher efficiency as discussed previously. The measured signal varied by <3-fold over this range.

A possible means of increasing detection sensitivity using the pulsed ionization source with orthogonal-TOF analysis would be to synchronize the droplet generation events with the orthogonal extraction pulse events. Current orthogonal-TOF ESI mass analyzers transmit and collimate gas phase ions into the high-vacuum region along an initial axis. These ions are then periodically extracted by a pulsed electric field perpendicular to their initial trajectory, which initiates the TOF analysis. It is essential that the generated packets of ions be temporally separated by adequate spacing to avoid overlap of consecutive mass spectra. Thus, a new packet of ions cannot be extracted into the flight tube until the slowest ion from the previous packet reaches the detector. In the case of orthogonal extraction, in which the beam of ions entering the extraction region is monoenergetic, the duty cycle is proportional to $(m/z)^{1/2}$ with a systematic discrimination against low-m/z ions.[16] Most ESI-TOF instruments have duty cycles between 5 and 50%, depending on the m/z range of the ions being analyzed.[16]

If the spatial extent of the ion packet produced from an individual pulsed droplet was comparable to the size of the orthogonal extraction region, synchronization of the orthogonal extraction pulse with the droplet generation event could in principle allow a 100% duty cycle to be obtained. Experiments were performed to evaluate this possibility. Briefly, an oscilloscope was set to trigger from the droplet generation pulse, and the subsequent ion signal from the instrument microchannel plate detector was monitored over time. A 5 μM solution of insulin in 1:1 H_2O/MeOH, 1% HOAC was employed as a sample solution. The first ion signal was observed ~3 ms after the droplet generation pulse and persisted subsequently for another 190 ms (or >2000 orthogonal extraction events). The measured droplet velocities were ~14 m/sec (see Experimental Section), and the gap between the end of the dispenser and the mass spectrometer inlet nozzle was 1.5 mm. This leads to a time of 0.1 ms for the droplet to cross the gap, indicating that the substantial spread in ion arrival times observed is due to processes occurring within the mass spectrometer rather than stemming from properties of the pulsed ionization source. This observation is consistent with previous work in which it has been shown that the differentially pumped regions used to bring the analyte ions into the high-vacuum region, in combination with the collisional cooling that most orthogonal ESI instruments employ, have the effect of turning a discrete source into a quasicontinuous source.[17] The substantial temporal extent of the ions produced from a single pulsing event makes unfeasible the strategy of increasing ion extraction efficiency by synchronization of the source and orthogonal extraction pulses. Future work will explore instrument designs to permit such a synchronization of droplet generation and mass analysis, to obtain the desired increases in ion extraction efficiency.

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