Optimization of the Electrospray Deposition Technique to Produce Better Quantitative MALDI Samples

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Introduction

Matrix-assisted laser desorption ionization (MALDI) mass spectrometry is now widely used for both the qualitative and *quantitative* analysis of a wide range of analytes, including small molecules, peptides, proteins and synthetic polymers. For quantitative analysis the choice of sample deposition technique largely affects the results that are obtained. The two methods that this work focuses on are the dry drop method and electrospray (ES) deposition. The dry drop method is known to generally produce heterogeneous samples than yield highly variable quantitative results, even with the use of internal standards[1]. Notwithstanding the development of the quite elegant isotopically labeled ICAT or iTraq tagging reagents (Applied Biosystems, Inc.) or isotopically labeled peptide standards (AQUA peptides, Aldrich, Inc.) now used in protein quantitation studies, the precision involved with the quantitation of these samples is still problematic. Previous work in our laboratory has shown that electrospray deposition produces a more homogenous sample surface; the ES surface yields more reproducible data, leading to improved quantitation of analytes via MALDI analysis[2]. While more laboratories are starting to use ES deposition to create MALDI samples, wide-spread efforts are generally hampered by the fact that an ES deposition apparatus is not yet commercially available. As a means of helping push towards wider use of the ES deposition technique, this poster will investigate details of the geometry of the ES deposition needle in order to identify the geometry that yields the best reproducibility.

Scope of this Work

Previous work in our laboratory clearly demonstrated that ES deposition produces more homogeneous MALDI samples which yield improved quantitative results. Samples included low to medium mass peptides (including analyte/internal standard pairs DDAVP/AVP, ~1080 Da and salmon/chicken calcitonin, ~3300 Da)[2,3] and protein (porcine/bovine insulin, ~5730 Da) mixtures[4]. In these early studies fairly large outer diameter (OD) SS HPLC tubing was used as the ES needle in a manual ES deposition device. In looking to develop an automated ES deposition device based on a commercially available HPLC-type autosampler system, we wished to investigate the use of commercially available syringe needles for use as the ES deposition needle. In this work several different ES needles were evaluated: 16 gauge rounded (KF hub type, point style 3, Hamilton, Inc., Reno NV), 22 gauge cut and 22 gauge rounded (RN type, point style 3, Hamilton, Inc.), and 26 gauge cut and 26 gauge rounded (RN type, point style 3, Hamilton, Inc.). Each of these syringe needles was compared to our "standard" 1/16" OD, 0.010" ID, 100mm length SS HPLC tubing (Alltech Associates, Deerfield, IL). An analysis of variance (ANOVA) approach was used to evaluate the experimental results, measured as the % coefficient of variation (%CV= 100*standard deviation/mean peak area) for the different needle types and spray distances for replicate mass spectra taken on a sample spot (the within spot variability). For each ES needle the spray height was adjusted between 15 and 25mm. As previous work also showed the precision was higher in the center of the sample spot[3], the precision of the edge versus the center of the ES spot was also evaluated.

Experimental

Materials

Experiments were performed using Angiotensin I (CAS: 484-42-4, Aldrich Chemical Company, Milwaukee, WI, Lot # 19H5800) and Angiotensin II (CAS: 68521-88-0, Aldrich, Lot # 68H58031) as 1 mg/ml solutions in DI water. The 0.10M α-cyano-4-hydroxycinnamic acid (CHCA, Aldrich, Lot # 12828JU) matrix solutions were prepared using a 90:10 (v/v) methanol:isopropanol solvent mixture. All MALDI samples were prepared by mixing 100 uL matrix to 5 uL analyte, yielding a M/A ratio of ~2000:1.

Experimental

Instrumentation-MALDI TOFMS

Mass spectra were collected on a Bruker (Bremen, Germany) Reflex III MALDI TOFMS running XACQ version 4.0 software on a Sun (Sunnyvale, CA) Sparcstation 5 workstation. The instrument was operated in reflectron mode with 20.0 KV on IS1, 16.3 KV on IS2 and 23.0 KV on the reflector. The Pulsed Ion Extraction (PIE) delay was set to medium. The voltage applied to the standard microchannel plate detector was 1.65 KV for all experiments. The laser intensity was adjusted to just above threshold for ion production. All sample spectra are digitized at 1 GSa/s. Each mass spectrum was the sum of 50 laser shots collected by continuously rastering the laser spot across the sample surface as the spectrum was accumulated. Five mass spectra were collected from the center and edge of each sample.

Experimental

Instrumentation-Electrospray Deposition Apparatus

The electrosprayed samples were prepared using a custom-built ES deposition apparatus. The HV power supply (Bertan, Inc. PMT-75C-P-30) is a 250 μA precision PMT supply module giving an output of 0-7500V equipped with a digital readout (4-1/2 digit LCD display, Acculex, Inc.) and a 10-turn potentiometer for voltage adjustment. A three-pole LC filter was used to provide filtering of the HV output. A HV relay (Kilovac, Inc.) to the HV output line is activated by an operate/stand-by switch that grounds the programming input to the HV power supply, effectively driving the power supply to 0 volts. The pumping system consisted of a Harvard Apparatus, Inc. (South Natick, MA) model 22 infusion pump.

Experimental

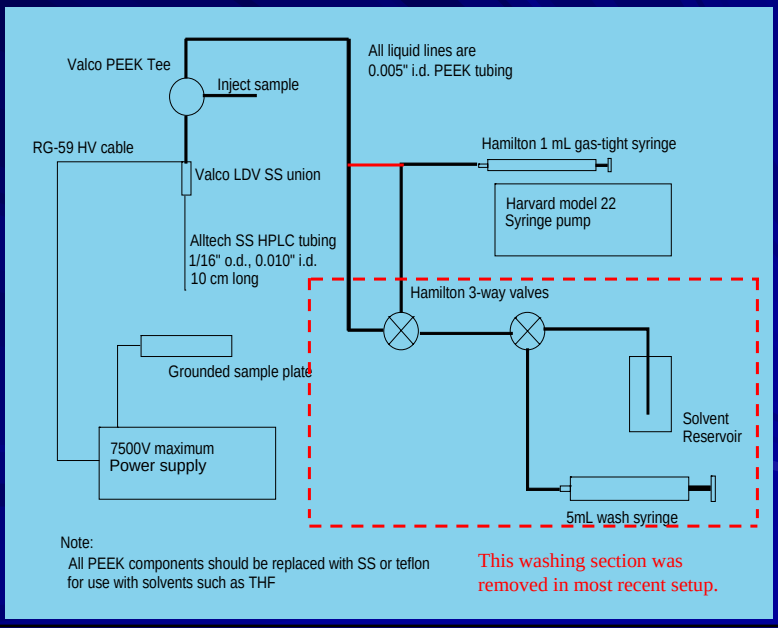
Methods- ES procedure

The sample and matrix were mixed in the selected solvent. Approximately 50 μL of this mixture is injected in one of the ports of a Valco tee (1/16“, 0.25mm ID stainless steel tee, Alltech Part # 30771) which is connected to the pumping system and the electrospray capillary with PEEK tubing (0.005" ID. x 1/16" OD). A solvent flow rate of 2 to 3 μL per minute pump the sample through the capillary. Electrospray is accomplished by applying approximately +5.5 KV to the capillary while the MS sample target is held at ground a distance of 15-25 mm away. Approximately 1-6 μL of a given sample is electrosprayed. With this set-up the circular spray pattern on the target was approximately 1.5-2.5 cm in diameter (depending on spray distance).

The distance between the capillary tip and grounded target, the flow rate, and the applied voltage were adjusted to obtain a stable, elongated Taylor cone. Too low of an applied potential resulted to round droplets at the end of the capillary while too high a potential resulted to formation of multiple small Taylor cones. The flow rate is adjusted according to the composition of the solvent and sample solutions. These three variables were optimized in order to obtain a stable Taylor cone.

After each sample, the sprayer is cleaned by switching off the solvent flow from the infusion pump and pushing at least 1 mL of solvent from a 10 mL-syringe. At least 1 mL of solvent is used to clean the tube and to get rid of air-bubbles. The injection port is also cleaned by injecting solvent (500 μL) at least twice or by manually pushing solvent from another syringe.

Diagram of Electrospray Deposition Apparatus



Owens Lab Electrospray Deposition Apparatus

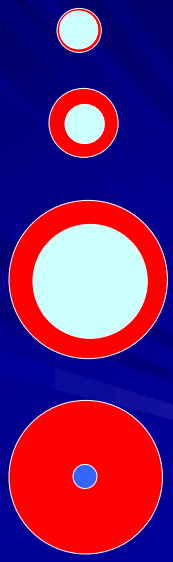
Needle Sizes

26 gauge 0.46mm OD, 0.26mm ID

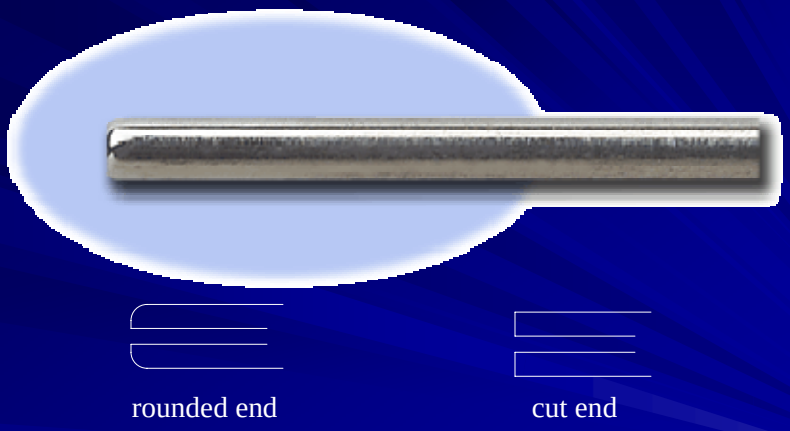
22 gauge 0.72mm OD, 0.41mm ID

16 gauge 1.65mm OD, 1.19mm ID

HPLC tubing 1.6mm OD, 0.25mm ID



Cut versus Rounded End



rounded end

cut end

The commercially obtained Kel F hub type and RN-type (point style 3) syringe needles (Hamilton, Inc., Reno, NV) came with a rounded end; they were cut flat using a wire EDM (Drexel University Machine Shops).

Example Mass Spectrum of Angiotensin II

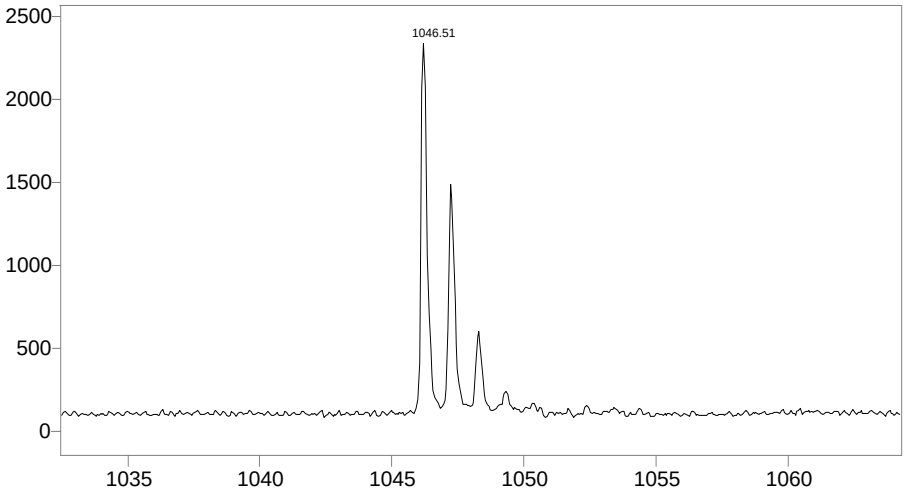


Figure 1: MALDI mass spectrum of Angiotensin II obtained by ES deposition of a sample in 0.1M CHCA in 90:10 (v/v) MeOH:H_2O, 2 minute spray, 5670V.

Figure 2: ES spot 0.1M CHCA in 90:10 (v/v) MeOH:H_2O, 2 minute spray, 5670V, 60X magnification.

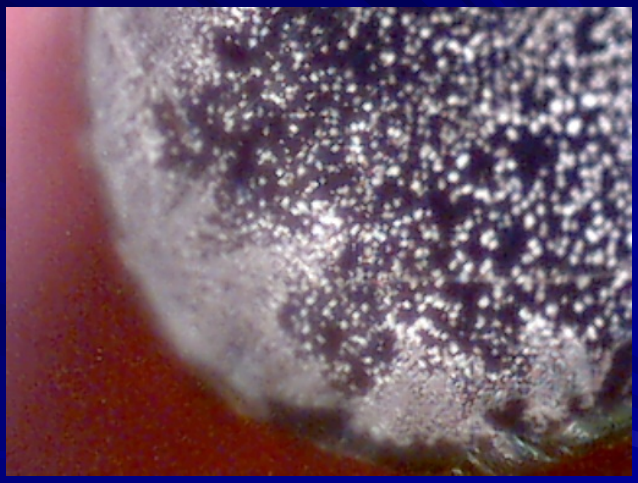


Figure 3: Dry Drop spot 0.1M CHCA in 90:10 (v/v) MeOH:H2O 60X magnification.

Results & Discussion

Table 1: Example results using the 1/16" OD HPLC tubing.

spray height (mm)	applied voltage (V)	spray time (s)	spot size (mm)	%CV
25	5980	12	20	15.7
20	5590	4	16	2.6
15	5310	5	10	7.3

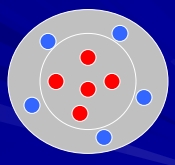
The results shown in table 1 were typical for the various ES needles tested. As the spray height increased, the required voltage for obtaining a stable spray also increased. Note that the applied electric field does not increase linearly with distance. Also note that the spot size on the sample plate increases with spray distance; the time required to produce the spot also increases, but at a slower rate than the spot area. As the ES flow rate is constant for these experiments the thickness of the final ES spot also varies with spot size and spray time. The final column shows the obtained reproducibility of the angiotensin peak area (from N=5 mass spectra). While obtaining a smaller spot may be desired (to fit more samples on a plate), the reproducibility is minimized at a fixed distance. Presumably there is an optimum distance corresponding to the drying time of the ES droplets, which will depend upon the sample composition.

Results & Discussion

Table 2: Example results using the 22 gauge rounded needle.

spray height (mm)	applied voltage (V)	spray time (s)	spot size (mm)	%CV	
				Edge	Center
25	4610	20	22	25.0	8.2
20	4480	20	17.5	5.9	11.2
15	4180	20	14	14.3	29.8

The results shown in table 2 show similar trends in applied voltage and spot size to those shown in table 1 for the HPLC tubing. As the spray time remained constant the thickness of the electrospray spot did decrease with spray height. The final two columns in table 2 contain data showing another general trend; the improved reproducibility observed when analyzing mass spectra from the center of the spot. Here data from N=5 mass spectra collected at the center of the spot are compared to N=5 spectra collected from the edge. The reproducibility is generally better in the center of the electrospray samples.



Sample locations for collection of center and edge mass spectra

Results & Discussion

Table 3: Example results (for N=5 mass spectra) using the 22 gauge cut needle.

spray height (mm)	%CV Center	%CV Edge
25	15.97	6.72
20	10.45	8.63
15	11.67	10.20

Table 4: Two-way ANOVA table (sample= spray height, column= center vs edge) for the 22 gauge cut needle.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	795656.7	2	397828.3	59.23	0.000	3.40
Columns	46207.1	1	46207.1	6.88	0.015	4.26
Interaction	48348.9	2	24174.5	3.60	0.043	3.40
Within	161189.9	24	6716.2			
Total	1051402.6	29				

The ANOVA results in table 4 indicate there is a statistically significant difference in the reproducibility obtained as a function of both spray height and center versus edge. There is no significant interaction between the two variables.

Results & Discussion

Table 5 shows provides information of the reproducibility obtained for each of the ES needles used in this study. For comparison the results of dried drop samples are provided in table 6. It must be noted that the values given in these tables are for the absolute area of the angiotensin peak- not the analyte to internal standard peak area ratio.

	22 gauge cut	22 gauge round	26 gauge cut	26 gauge round	HPLC Tubing
Height, mm	25	20	15	20	20
Spot	edge	center	center	center	center
%CV	6.7	5.9	16.1	12.8	2.6

Table 5: Results obtained under optimized ES sample deposition parameters.

	%CV Edge	%CV Center
Dried Drop	45.0	86.8

Table 6: Results obtained for a dried drop sample preparation.

Conclusions

- Overall the best result (i.e., highest precision) was obtained with the 1/16” OD SS HPLC tubing at a spray distance of 20mm. The %CV for the *absolute peak area* (not analyte to internal standard peak ratio) was 2.6%.
- There was no significant difference in precision obtained using the cut or rounded edges on the commercially available syringe needles.
- Better stability of the spray was obtained from the HPLC tubing in comparison to the 16 gauge needle, even though it had a comparable OD. The 16 gauge needle had a very large ID, however, and it was difficult to obtain a stable spray for any length of time.

Future Work

- We want to investigate 1/16” OD SS HPLC tubing with larger IDs to further understand how the ID affects the stability of the Taylor cone.
- We want to investigate the commercially available 26s and 22s gauge needles. As the ID of these needles is smaller than that of their standard counterparts, there is a larger metal surface to stabilize the Taylor cone. The overall smaller diameter of these needles (compared the HPLC tubing) may enable the production of smaller electrospray droplets, which would allow the needle to be moved closer to the sample surface, producing a smaller ES spot.
- To enable larger numbers of samples to be analyzed, we want to continue the transfer from our original manual to an automated ES system based on an HPLC autosampler. The ability to use commercially available syringe needles for the ES will assist in the conversion.

Acknowledgements

The authors would like to thank Dr. Russel Hensel and Dr. Cynthia Chavez-Eng for initial construction and characterization of the manual electrospray deposition apparatus.

References

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