

Utilizing the Electrospray Membrane Probe to Map Electrospray Signal Response in Gradient LC/ES/MS

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Overview

Purpose – Mapping Electrospray signal response in gradient LC/MS using an Electrospray Membrane Probe to scan Electrospray current at each solvent gradient step.

Methods – Dual Syringe pumps were used to simulate gradient LC/ES/MS separations to scan Electrospray current in order to map signal response for various analytes, solvents and acids.

Results – Results show the Electrospray signal response during LC gradients for different compound classes. The Electrospray Membrane Probe pH and ES current scanning shows the difference between Electrospray signal intensity in LC/MS and optimal ES performance for the same chemical system.

Introduction

The Electrospray MS signal response of a chemical species eluting from a gradient liquid chromatography run will vary depending on where in the organic solvent gradient the peak elutes. A study was conducted to map the Electrospray signal response for different compound classes during typical reverse phase LC gradient conditions. The Electrospray Membrane Probe was used to scan Electrospray current at different organic solvent ratios Electro sprayed in gradient LC/MS. Three dimensional maps of ES/MS signal versus organic solvent ratio and Electrospray total ion current were generated to determine the relative Electrospray ionization efficiencies occurring during an LC/ES/MS run. The ES Membrane Probe was used to rapidly assess relative Electrospray MS response in LC/ES/MS method development for different sample types.

Experimental

The experiments conducted map Electrospray signal response versus Electrospray total ion current over typical reverse phase LC solvent gradients. A standard Electrospray inlet probe and an Electrospray Membrane Probe interfaced to an Analytica of Branford Time-Of-Flight mass spectrometer were used with both Electrospray probes operating at ground potential. Electrospray TOF MS ion signal was measured using two different solvent gradients. 1 μM solutions of hexatryosine were prepared in 100% Water .01% TFA, 100% ACN .01% TFA and 100% MeOH .01% TFA. A dual syringe pump was then used to simulate a reverse phase LC/ES/MS gradient by mixing the water solution with either non polar or polar organic solvent solution in a mixing tee prior to ES/MS analysis. A linear gradient was run from 100% water to 100% organic in each experiment while holding the concentration of analyte and acid (electrolyte) constant. The observed ES TOF MS signal intensity and Electrospray total ion current at various organic percentage were recorded and plotted. In addition to these experiments, leucine enkephalin and caffeine were run in a similar manner but were also run with formic acid added to solution. Each study was complemented by running gradients of acid through the Electrospray Membrane Probe second solution flow channel at various solvent ratios in order to map the ES signal intensities at varying solvent composition and Electrospray currents. When the Electrospray Membrane Probe was interfaced to an Analytica of Branford Time-Of-Flight mass spectrometer a fused silica tube extended directly from the Electrospray Membrane Probe assembly through the pneumatic nebulization Electrospray probe where sample solution was Electro sprayed directly off the fused silica tube exit tip. The inlet of the mass spectrometer was then held at high potential to conduct Electrospray ionization. In order to connect the electrical circuit, the ground connection was made through the membrane electrode in contact with the second solution flow path of the Electrospray Membrane Probe.

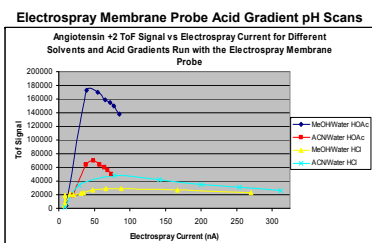


Figure 1A: This figure is a schematic of the ES Membrane Probe configured with no electrically conductive surface in the sample solution flow channel. The two flow channels are separated by a proton exchange membrane with an acid electrolyte run in the second solution flow channel. Protons pass through the membrane during operation allowing pH scanning in the sample solution flow channel.

Figure 1B: This figure shows ES TOF MS signals detected vs Electrospray Current for angiotensin +2 ToF Signal vs Electrospray Current for Different Solvents and Acid Gradients Run with the Electrospray Membrane Probe. The plot shows signal intensity vs current for MeOH/Water HOAc, ACN/Water HOAc, MeOH/Water H2, and ACN/Water H2.

Results

Total Electrospray Current vs. Acid Added to Different Ratios of Solvent to Water

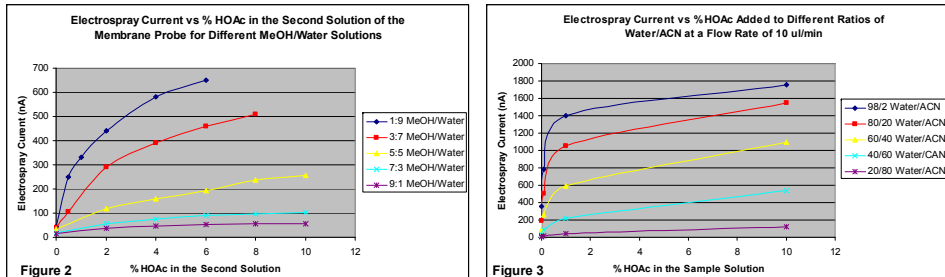


Figure 2 shows measured Electrospray current (nA) vs % HOAc flowing through the second solution of the Electrospray Membrane Probe for different ratios of Methanol and Water Electro sprayed at a flow rate of 10 μl/min. The range of Electrospray current measured for a constant % HOAc varies with the organic solvent ratio. For example, electrospray current can range from 52 nA to 650 nA when 6% HOAc is flowing through the ES Membrane Probe second solution and the ratio of MeOH/Water is changed from 9/1 to 1/9 MeOH/Water. This change in Electrospray current affects the observed Electrospray ion signal.

Figure 3 shows measured Electrospray current (nA) vs % HOAc added directly to different ratios of Water/Acetonitrile solutions. In direct correlation to ES Membrane Probe studies, different acetonitrile to water solvent ratios yield different Electrospray total current for constant HOAc electrolyte concentration. When the amount of acid added to the sample solution is held constant the Electrospray current can vary by over a factor of ten with changing organic solvent ratio typically encountered in a reverse phase LC gradient. The ES Membrane Probe provides a tool for rapidly scanning ES current with finer detail than can be achieved when adding electrolyte species directly into the sample solution.

Simulated LC/ES/MS MeOH/Water Gradient Running from 100% Water to 100% MeOH with Constant 0.01% TFA and 1 μM Hexatryosine

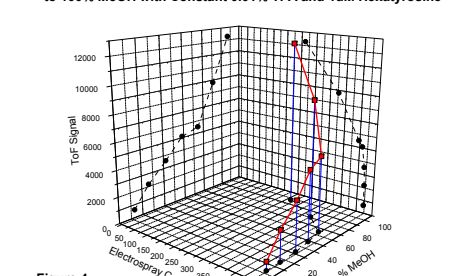


Figure 4: In this experiment 1 μM hexatryosine was dissolved in 100% water .01% TFA and in a second solution of 100% MeOH with .01% TFA. Each sample solution was loaded into separate syringes with outputs connected to a mixing tee. The mixing tee was then connected to an Electrospray source and the solution was analyzed with ES TOF MS. A gradient was run from 100% water to 100% MeOH while the acid and hexatryosine concentrations remained constant. During the experiment, the Electrospray current and ES TOF MS signal intensity of the singly charged species were measured and plotted. As shown above, Electrospray hexatryosine signal intensity increases with increasing Methanol percent and decreasing Electrospray current. This experiment indicates that when running an LC/ES/MS experiment the ES/MS signal of a specific analyte may not be optimized if the sample peak elutes early in the LC gradient. ES signal can vary if the sample peak LC retention time shifts, affecting quantitation performance.

Simulated LC/ES/MS ACN/Water Gradient Running from 100% Water to 100% ACN with Constant 0.01% TFA and 1 μM Hexatryosine

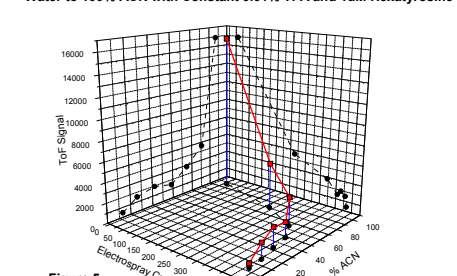


Figure 5: In this experiment 1 μM hexatryosine was dissolved in 100% water .01% TFA and in a second solution of 100% ACN with .01% TFA. Each sample solution was loaded into separate syringes with outputs connected to a mixing tee. The mixing tee was then connected to an Electrospray source and the solution was analyzed with ES TOF MS. A gradient was run from 100% water to 100% ACN while the acid and hexatryosine concentrations remained constant. During the experiment, the Electrospray current and the ES TOF MS signal intensity of the singly charged species were measured and plotted. The hexatryosine signal intensity increased with increasing acetonitrile percent and decreasing Electrospray current. As expected from the results in Figure 1B, the ES signal response for hexatryosine run in an acetonitrile/water gradient varies significantly from the ES signal response of hexatryosine run in a methanol/water gradient shown in Figure 4. ES signal can vary if the sample peak LC retention time shifts, affecting quantitation performance.

Simulated LC/ES/MS ACN/Water Gradient Running from 100% Water to 100% ACN with constant 0.1% Formic Acid for 1 μM Caffeine

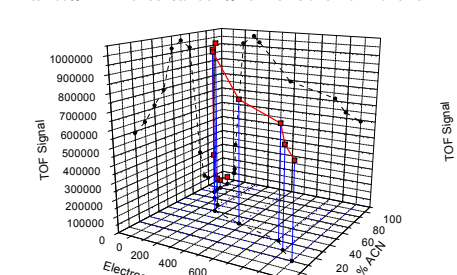


Figure 6: In this experiment 1 μM caffeine was dissolved in 100% water with 0.1% formic acid and in a second solution of 100% ACN with 0.1% formic acid. Each sample was loaded into a separate syringe with outputs connected to a mixing tee. The mixing tee was then connected to an Electrospray source and the solution was analyzed with ES TOF MS. A gradient was run from 100% water to 100% acetonitrile while the acid and caffeine concentrations remained constant. During the experiment, the Electrospray current and ES TOF MS signal intensity of singly charged caffeine ions was monitored and plotted. As shown above, with increasing acetonitrile percent, the ES current and caffeine signal intensity first increase and then rapidly decrease. The ES/MS caffeine signal maximized at 60% ACN while Electro spraying 176 nA of current. The above plot shows that caffeine signal in this solvent system can range by a factor of 20 times depending on LC retention time.

Simulated ACN/Water LC Gradient Running from 100% Water to 100% ACN with constant 0.01% TFA for 1 μM Caffeine

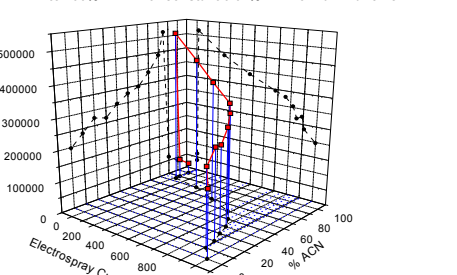


Figure 7: In this experiment 1 μM caffeine was dissolved in 100% water with 0.01% TFA and in a second solution of 100% ACN with 0.01% TFA. Each sample was loaded into a separate syringe with outputs connected to a mixing tee. The mixing tee was then connected to an Electrospray source and the solution was analyzed with ES TOF MS. A gradient was run from 100% water to 100% acetonitrile while the acid and caffeine concentrations remained constant. During the experiment, the Electrospray current and ES TOF MS signal intensity of singly charged caffeine ions was monitored and plotted. As shown above, with increasing acetonitrile percent, the ES current and caffeine signal intensity first increase and then rapidly decrease. The ES/MS caffeine signal maximized at 85% ACN while Electro spraying only 16 nA of current. Comparison of this plot with Figure 6, shows that the TFA electrolyte reduced the ES signal intensity of caffeine by a factor of two from that obtained with formic acid.

Simulated LC/ES/MS MeOH/Water Gradient Running from 100% Water to 100% MeOH with constant 0.1% Formic Acid for 1 μM Caffeine

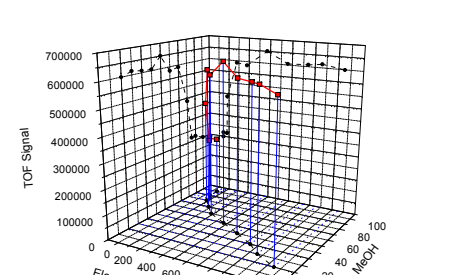


Figure 8

Simulated LC/ES/MS MeOH/Water Gradient Running from 100% Water to 100% MeOH with constant 0.01% TFA for 1 μM Caffeine

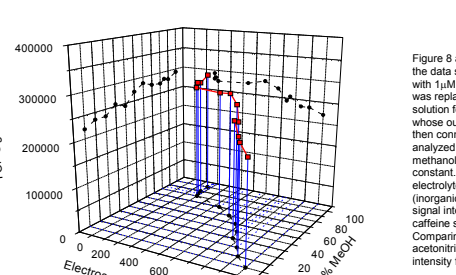


Figure 9

Figure 8 and 9: Extending the experiments that were performed for the data shown in Figures 6 and 7, the same study was performed with 1 μM caffeine, however, the acetonitrile non polar organic solvent was replaced by the methanol polar organic solvent. Each sample solution forming the gradient was loaded into separate syringes whose output was connected to a mixing tee. The mixing tee was then connected to an Electrospray source and the solution was analyzed with ES/MS. A gradient was run from 100% water to 100% methanol while the acid and caffeine concentrations remained constant. Figure 8 was run with 0.1% formic acid (organic acid electrolyte) in both solutions while Figure 9 was run with 0.01% TFA (inorganic acid electrolyte) in both solutions. Increased caffeine signal intensity was obtained with formic acid compared with the caffeine signal intensity measured with TFA as the acid electrolyte. Comparing Figures 8 and 9 with Figures 6 and 7, the combination of acetonitrile and formic acid yield the highest Electrospray signal intensity for caffeine at 60% ACN.

Simulated LC/ES/MS ACN/Water Gradient Running from 100% Water to 100% ACN with constant 0.1% Formic Acid for 1 μM Leucine Enkephalin

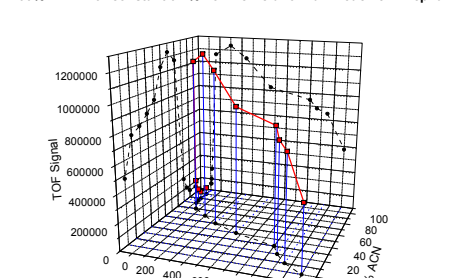


Figure 10

Simulated LC/ES/MS ACN/Water Gradient Running from 100% Water to 100% ACN with constant 0.01% TFA for 1 μM Leucine Enkephalin

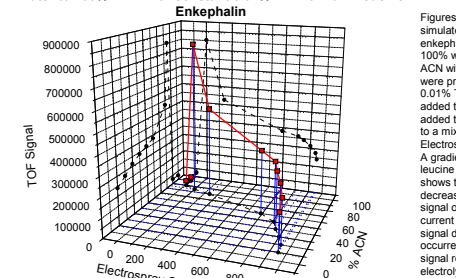


Figure 11

Figures 10 and 11 show the Electrospray signal measured during a simulated ACN/Water LC/ES/MS gradient for the peptide leucine enkephalin. In Figure 10, 1 μM leucine enkephalin was dissolved in 100% water with 0.1% formic acid and in a second solution of 100% ACN with 0.1% formic acid. In Figure 11, the same sample solutions were prepared except that the 0.1% formic acid was substituted with 0.01% TFA. In the two experiments, the water sample solution was added to one syringe while the organic solvent sample solution was added to a separate syringe. The two syringes were then connected to a mixing tee. The mixing tee outlet was connected to an Electrospray source and the solution was analyzed with ES TOF MS. A gradient was run from 100% water to 100% ACN while the acid and leucine enkephalin concentrations remained constant. Figure 10 shows that as the amount of ACN is increased the ES current decreased from 1200 nA to 6 nA. The maximum leucine enkephalin signal obtained during this run was at 60% ACN with the Electrospray current at 200 nA. As the ES current increased above 300 nA, the signal decreased. Figure 11 shows that a sharper maximum signal occurred at a higher acetonitrile percent when compared with the signal response with formic acid when it was used as the acid electrolyte. In Figure 11, the maximum leucine enkephalin signal occurred at 85% ACN and began to roll off for ES currents higher than 200 nA.

Simulated LC/ES/MS ACN/Water Gradient Running from 100% Water to 100% MeOH with constant 0.1% Formic Acid for 1 μM Leucine Enkephalin

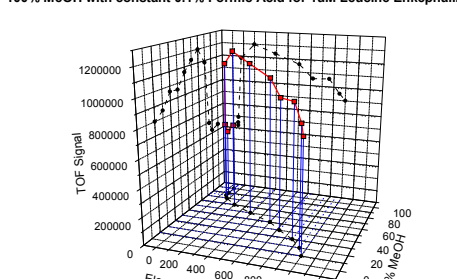


Figure 12

Simulated LC/ES/MS ACN/Water Gradient Running from 100% Water to 100% MeOH with constant 0.01% TFA for 1 μM Leucine Enkephalin

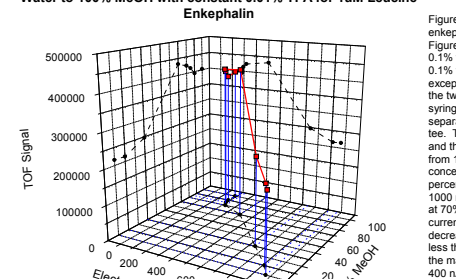


Figure 13

Figures 12 and 13 show the ES/MS signal response of leucine enkephalin over a simulated methanol/water LC/ES/MS gradient. In Figure 12, 1 μM leucine enkephalin was dissolved in 100% water with 0.1% formic acid and in a second solution of 100% methanol with 0.1% formic acid. In Figure 13, the same solutions were prepared except that the 0.1% formic acid was substituted with 0.01% TFA. In the two experiments, the water sample solution was added to one syringe while the organic solvent sample solution was added to a separate syringe. The two syringes were then connected to a mixing tee. The mixing tee outlet was connected to an Electrospray source and the solution was analyzed with ES TOF MS. A gradient was run from 100% water to 100% ACN while the acid and leucine enkephalin concentrations remained constant. Figure 12 shows that as the percent methanol is increased, the Electrospray current drops from 1000 nA to 44 nA. The maximum signal obtained during this run was at 70% MeOH while Electro spraying at 200 nA or current. As the ES current increased above 200 nA, the leucine enkephalin signal decreased. Figure 13 shows that the maximum signal obtained was less than half for TFA substituted for formic acid. In this experiment the maximum signal occurred at 70% ACN while Electro spraying at 400 nA of current.

1 μM Caffeine Dissolved in Different Ratios of ACN/Water Run on the Membrane Probe with 5% Formic Acid Gradients

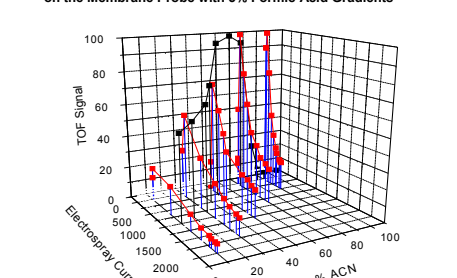


Figure 14

1 μM Caffeine Dissolved in Different Ratios of ACN/Water Run on the Membrane Probe with 5% TFA Gradients

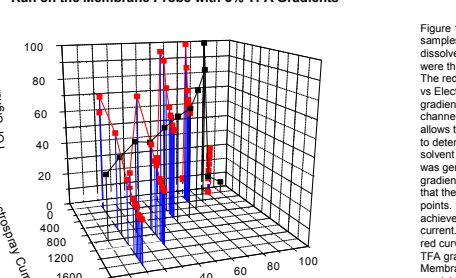


Figure 15

Figure 14 and 15: In each of these experiments five different samples were prepared. The samples included 1 μM caffeine dissolved in 10, 30, 50, 70 and 90% ACN. Each of these samples were then Electro sprayed with the Electrospray Membrane Probe. The red curves in Figure 14 show the measured ES TOF MS signal of caffeine vs Electrospray Current vs % ACN while running acid electrolytes gradients from 0% to 5% formic acid in the second solution flow channel of the ES Membrane Probe. The ES Membrane Probe allows the generation of more comprehensive signal intensity maps to determine where signal maximums occur for each percent organic solvent to water ratio. The black curve corresponds to the curve that was generated in Figure 6, generated using a simulated LC/ES/MS gradient without the use of the Membrane Probe. Figure 14 shows that the two sets of data track each other well at their intersection points. A significant increase in caffeine signal intensity can be achieved for higher acetonitrile percent solutions by decreasing ES current. Figure 15 shows the results from a parallel study where the red curves plot caffeine ES TOF MS signal intensity while running TFA gradients from 0% to 5% through the second solution of the ES Membrane Probe. The black curve is the data shown in Figure 7 overlaid onto the ES Membrane Probe data.

1 μM Caffeine Dissolved in Different Ratios of MeOH/Water Run on the Membrane Probe with 5% Formic Acid Gradients

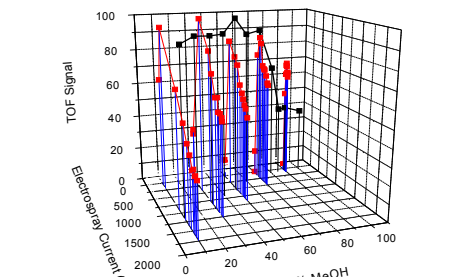


Figure 16

1 μM Caffeine Dissolved in Different Ratios of MeOH/Water Run on the Membrane Probe with 5% TFA Gradients

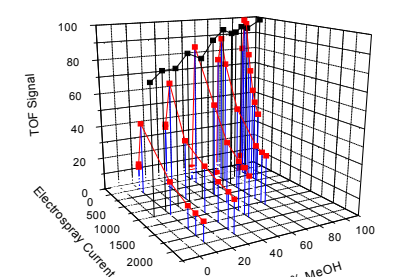


Figure 17

Figure 16 and 17: In these experiments five different samples were prepared. The samples included 1 μM caffeine dissolved in 10, 30, 50, 70 and 90% MeOH. Each of these samples were then Electro sprayed using the Electrospray Membrane Probe. The red curves in Figure 16 show the ES TOF MS signal of caffeine vs Electrospray Current vs % MeOH while running acid electrolyte gradients from 0% to 5% formic acid in the ES Membrane Probe second solution flow channel. Caffeine signal intensity maps were generated to determine where signal maximums occur for each methanol/water ratio. The black curve shown in Figure 16 corresponds to the curve that was generated in Figure 8, which was generated without the use of the ES Membrane probe. Figure 17 shows the results of a parallel study where the red curves are the caffeine signal measured while running TFA gradients from 0% to 5% through the ES Membrane Probe second solution flow channel. The black curve in Figure 17 is the data shown in Figure 9 overlaid onto the ES Membrane Probe data.

1 μM Leucine Enkephalin Dissolved in Various Ratios of ACN/Water Run on the Membrane Probe with 5% Formic Acid Gradients

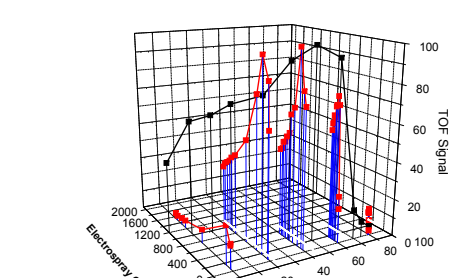


Figure 18

1 μM Leucine Enkephalin Dissolved in Various Ratios of ACN/Water Run on the Membrane Probe with 5% TFA Gradients

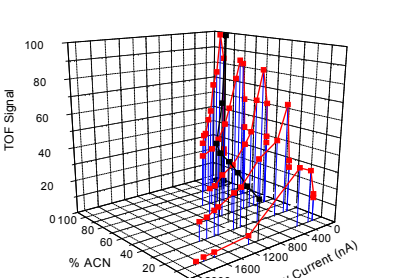


Figure 19

Figure 18 and 19: In these experiments five different samples were prepared. The samples included 1 μM leucine enkephalin dissolved in 10, 30, 50, 70 and 90% ACN. Each of these samples were then Electro sprayed using the Electrospray Membrane Probe. The red curves in Figure 18 show the measured ES TOF MS signal of leucine enkephalin vs Electrospray Current vs % ACN while running acid electrolytes gradients from 0% to 5% formic acid through the ES Membrane Probe second solution flow channel. Signal intensity maps were generated to determine where the signal maximums occur for each acetonitrile/water ratio. The black curve in Figure 18 corresponds to the curve that was generated in Figure 10 without the use of the ES Membrane Probe. Figure 19 shows the results from a parallel study where the red curves here show the data acquired while running TFA gradients from 0% to 5% using the ES Membrane Probe. The black curve in Figure 19 is the data shown in Figure 11 overlaid onto the ES Membrane Probe data.

1 μM Leucine Enkephalin Dissolved in Various Ratios of MeOH/Water Run on the Membrane Probe with 5% Formic Acid Gradients

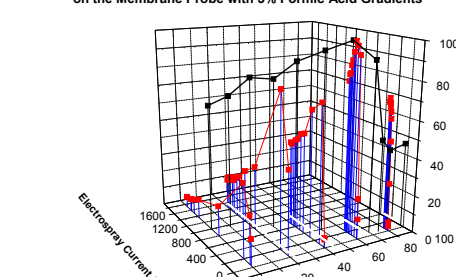


Figure 20

1 μM Leucine Enkephalin Dissolved in Various Ratios of MeOH/Water Run on the Membrane Probe with 5% TFA Gradients

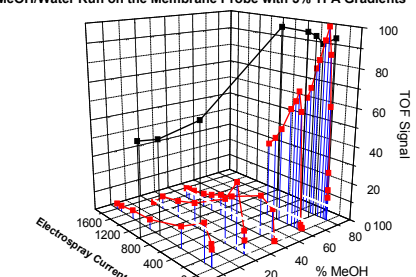


Figure 21

Figure 20 and 21: In these experiments five different samples were prepared. The samples included 1 μM leucine enkephalin dissolved in 10, 30, 50, 70 and 90% Methanol. Each of these samples were then Electro sprayed using the Electrospray Membrane Probe. The red curves in Figure 20 show the measured ES TOF MS signal of leucine enkephalin vs Electrospray Current vs % Methanol while running acid electrolytes gradients from 0% to 5% formic acid through the ES Membrane Probe second solution flow channel. Signal intensity maps were generated to determine where the signal maximums occur for each methanol/water ratio. The black curve in Figure 20 corresponds to the curve that was generated in Figure 12 without the use of the ES Membrane Probe. Figure 21 shows the results from a parallel study where the red curves here show the data acquired while running TFA gradients from 0% to 5% using the ES Membrane Probe. The black curve in Figure 21 is the data shown in Figure 13 overlaid onto the ES Membrane Probe data.

Conclusions

The experimental results presented provide a detailed view of Electrospray ionization efficiency for different analyte species versus varying organic solvent ratios and types and different electrolyte species typically run in LC gradients. Depending on the percent organic, with a constant amount of acid, the electro spray current and ultimately the detected signal intensities of analyte ions can vary significantly for different LC retention times. The highest Electrospray signals were achieved at lower Electrospray currents. The Electrospray Membrane Probe provides a tool to expand the ES ionization response field to determine if maximum ES ionization efficiency is achieved for a given LC method. In this study the ES Membrane probe was used as a tool to rapidly assess the relative performance of LC/ES MS methods for a range of solvent and analyte combinations and to shed light on the fundamental mechanisms underlying Electrospray ionization processes.