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I am submitting herewith a thesis written by Samuel Arthur Lewis, Sr. entitled "Design of a Low Sheath Flow Electrospray Tip for Improved Capillary Electrophoresis/Mass Spectrometry (CE/MS) Performance." We have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science degree, with a major in Chemistry.

Dr. M. J, Sepaniak

Dr. K. D. Cook

I have read this thesis and
recommend its acceptance:

Accepted for the Council:

Associate Vice Chancellor and
Dean of The Graduate School

Design of a Low Sheath Flow Electrospray Tip for Improved Capillary Electrophoresis/Mass Spectrometry (CE/MS) Performance

A Thesis Presented

for the Master of

Science Degree

The University of Tennessee, Knoxville

Samuel A. Lewis, Sr.

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DEDICATION

The author wishes to dedicate this work to his wife and family for their love and support.

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ABSTRACT

A low sheath flow Capillary Electrophoresis Mass Spectrometry (CE/MS) electrospray interface design is presented and proven to be a major improvement over conventional CE electrospray. A tapered-tip capillary is employed in the electrospray interface. The modified tip reduces the required sheath flow rate by 50% (to 2.5 μL/min), increases sensitivity for Ho and Pr, and reduces background signals compared to the conventional design. The feasibility of using the modified tip for CE/MS analysis is evaluated in the separation and detection of β-cyclodextrins and mono-substituted carboxymethyl cyclodextrins, and the separation and detection of a mixture of sulfonated β-cyclodextrins.

TABLE OF CONTENTS

CHAPTER PAGE

LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

FIGURE PAGE

CHAPTER I

INTRODUCTION

Since the introduction of modern electrospray mass spectrometry (ESMS) by Fenn, Yamashita, and Aleksandrov et al.,[1-3] the interfacing of mass spectrometry with liquid separation techniques, such as HPLC, micro HPLC, and CE, has become more feasible. Generally the electrospray interface transfers nonvolatile polar analytes from an ionized condensed phase to a gas phase as isolated entities.[4] The electrospray process is initiated when a capillary filled with ionic solution is held at a high potential. The high electric field draws ions to the surface as the solution emerges from the tip, generating a Taylor cone. By increasing the potential further a liquid filament is formed from the Taylor cone, producing charged droplets (Figure 1).[4] The charged droplets undergo desolvation and uneven droplet fission until individual molecular ions are produced. Several electrospray interface designs for capillary electrophoresis/mass spectrometry (CE/MS), including the widely used liquid sheath electrode, have been published.[5-8]

The liquid sheath interface consists of a triaxial probe in which the CE column (typically fused silica, 50 μm i.d., 365 μm o.d.) is placed in the center of an inner stainless steel sleeve (typically 400 μm i.d., 1400 μm o.d.) and a volatile make-up solution (e.g. methanol) is pumped between the column and inner sleeve

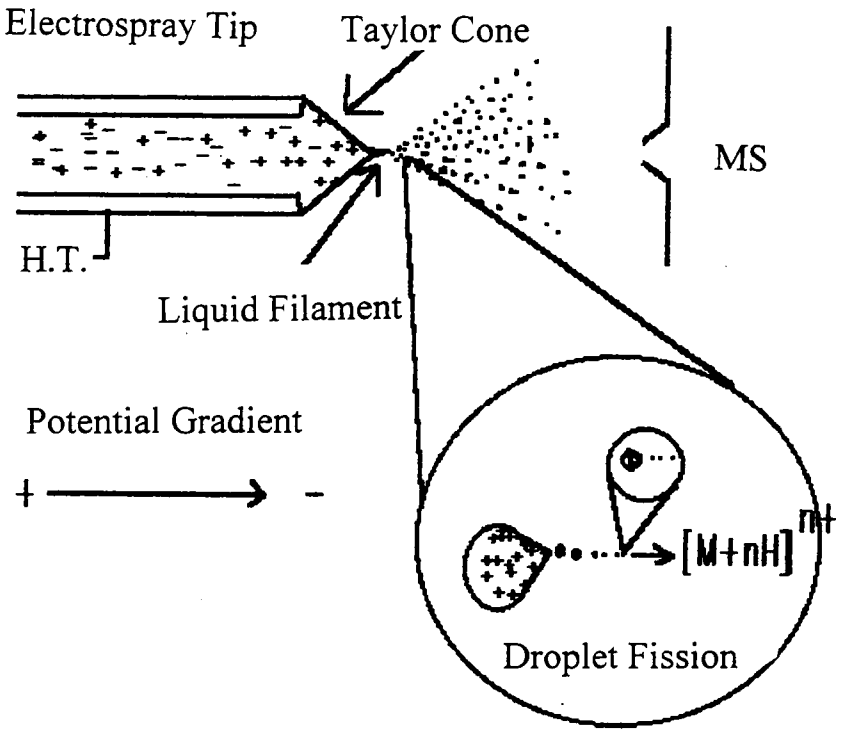

Figure 1. Electrospray Process

allowing the makeup solution to envelope the tip of the CE column. The inner metal sleeve is located within an outer metal sleeve (typically 1500 μm i.d., 2800 μm o.d.) forming a region for the introduction of a nebulizing gas. The liquid sheath configuration provides two important parameters necessary to interface a CE column with a mass spectrometer. (CE, ES, and MS fundamentals are addressed in Chapter II.) First, the make-up solution provides an electrical contact for both the CE column and the electrospray interface, and second, the design allows for the introduction of additional buffer into the make-up solution to ensure a constant pH to be maintained within the CE column. Ordinarily a CE instrument is configured with a buffer reservoir at both the cathode and anode to maintain constant pH within the column. Under an electrospray configuration, the buffer reservoir is not present at the spray tip. Thus, to maintain a constant pH, it is essential to add buffer through the make-up solution to the CE tip region. The relatively large flow rate of 5 to 10 μL/min. required to maintain the electrospray is a disadvantage because the high rate leads to the dilution of the solute band emerging from the CE column at a typical flow rate of 250 nL/min. In order to overcome the solute dilution problem, CE/MS interfaces have been designed that require either no make-up solution or a reduced amount of make-up solution. In 1995, Krieger, Cook, and Ramsey described a sheathless CE/MS interface design

incorporating a gold-coated, conically ground capillary column tip.[9] The article demonstrated promising results for the detection of the peptide leucine-enkephalin. The major limitation of a sheathless system is the inability to replenish the buffer solution at the CE tip when it is necessary to maintain a constant pH. Jorgenson et al. designed a low flow rate (nL/min.) biaxial sheath tip interface incorporating two concentric fused silica capillaries.[10] The outer capillary is drawn to a 20 μm tip, and the inner capillary, with a ground tip, is placed within a minimum 5 to 10 μm of the tip of the outer capillary. Buffer solution can be replenished at the tip using the biaxial sheath interface while minimizing the solute band dilution from the CE column. However, the construction of the interface is difficult since an inner thin-walled glass capillary must be placed within an outer thin-walled glass capillary. In addition, clogging within the interface easily occurs due to the small distance of less than 20 μm between the two tips, and the small inner diameter of the inner tip.

A. Research Goals

An alternative design for a low sheath flow CE/MS electrospray interface is presented in this thesis. In this system, a reduction of approximately 50% from conventional sheath requirements for the make-up flow is permitted. The reduced amount of make-up flow is made possible by modifying the tip of the CE column.

When the tip of the column is ground to a conical form, slightly larger than the inner diameter of the CE column, the base of the Taylor cone produced when spraying into the MS is reduced. Lowering the diameter of the electrospray tip leads to a lower flow rate requirement to sustain an electrospray.[11]

Wilm and Mann illustrate the relationship between the radius of curvature at the tip of the emitter cone (r_a) and the radius of the zone at the tip of the Taylor cone (r_e). They developed a theoretical equation to determine the size of the zone at the tip of the Taylor cone from which the droplets are ejected:[11]

$$r_e = \{\rho/ 4\pi^2\gamma\tan(\pi/2-\varphi)[(U_a/ U_T)^2-1]\}^{1/3} \times (dV/dt)^{2/3} \qquad (1)$$

where r_e is the radius of the emission region, ρ and γ are the density and surface tension of the liquid, respectively, φ is the liquid cone angle, U_a and U_T are the applied and threshold voltages respectively, and dV/dt is the solution flow rate. Evaluation of the above equation reveals that the emission radius, r_e, is proportional to the 2/3's power of the flow rate, dV/dt. By decreasing the flow rate, r_e is reduced leading to an increased desorption ionization efficiency.[11] The droplet size emerging from the tip of the CE column is larger than the emission diameter; however with a lower flow rate, a better correspondence is established between the droplet size and the emission diameter. Wilm and Mann conducted infusion experiments using peptides, and successfully verified their proposed

theories concerning Taylor cone zone size from which droplets are ejected. The experiments showed a significant increase in ions detected (total signal) with the micro-electrospray over conventional methods.

Upon evaluation of Wilm and Mann's proposed theory of Taylor cone droplet and emission zone size, the theoretical concepts were applied to the low sheath flow CE/MS electrospray interface design presented in this thesis. By grinding the electrospray end of the CE column, the tip shape approaches that of the micro electrospray tip of Wilm and Mann. An increase in analyte signal with a decrease in background are expected with the modified tip design over conventional sheath designs.

B. Experimental Overview

In order to demonstrate that the proposed design offers advantages over conventional sheath flow CE/MS designs, several experiments were conducted. Performance parameters such as stability, sensitivity, and ease of construction and maintenance must be considered.

1. Modified Tip Flow Rate Evaluation

To evaluate the stability of an electrospray emission from a modified tip at low flow rates, standard conditions should be set and the signal monitored for an extended period of time. The total ion current signal may be monitored and

reviewed for stability. In addition, the modified tip should be evaluated for potential problems such as salt formation after extended use.

2. Conventional Tip Performance at Low Flow Rates

In order to thoroughly compare the performance of a conventional tip to that of a modified tip, the ability of conventional tips to spray at low flow rates must be determined under the same conditions established in the previous experiment. The total ion current signal stability may be monitored to evaluate the electrospray stability at low flow rates.

3. Analyte Signal Comparison Between the Conventional and Modified Tips

A comparison of the analyte signal for the conventional and modified tips should be evaluated.

4. Applications

Finally CE/MS applications using the modified tip must be evaluated. Two applications have been chosen for this thesis. The first application is the separation and detection of β-cyclodextrins and mono-substituted carboxymethyl cyclodextrins, and the second application is the separation and detection of a mixture of sulfonated β-cyclodextrins. The chemical structure of a β-cyclodextrin is given in Appendix A.

CHAPTER II

ANALYTICAL INSTRUMENTATION

The low sheath flow electrospray for CE/MS was evaluated using a Fisons VG Quattro II tandem mass spectrometer in the single quad mode. Separations were carried out using capillary electrophoresis (CE). The low sheath flow electrospray source was used as an interface and ionization system for coupling the CE to the mass spectrometer. A general diagram of this CE/MS system is given in Figure 2.

A. Capillary Electrophoresis (CE)

Modern CE instruments offer an extremely efficient analytical separation technique for a wide range of components in small sample volumes. Separations have been conducted on analytes ranging from ionic inorganic species to high molecular weight proteins and peptides.[12] The use of narrow-bore fused-silica capillary columns (20 - 100 μm i.d.) for electrophoretic separations allows high-potential fields to be applied for extremely efficient separations within short periods of time, because efficiency is directly proportional to the applied voltage. The small capillary columns aid in heat dissipation allowing the essential high potentials to be applied.[12]

In a typical CE system, a buffer-filled capillary is placed between two reservoirs containing buffer solution, and a potential is applied across the length of

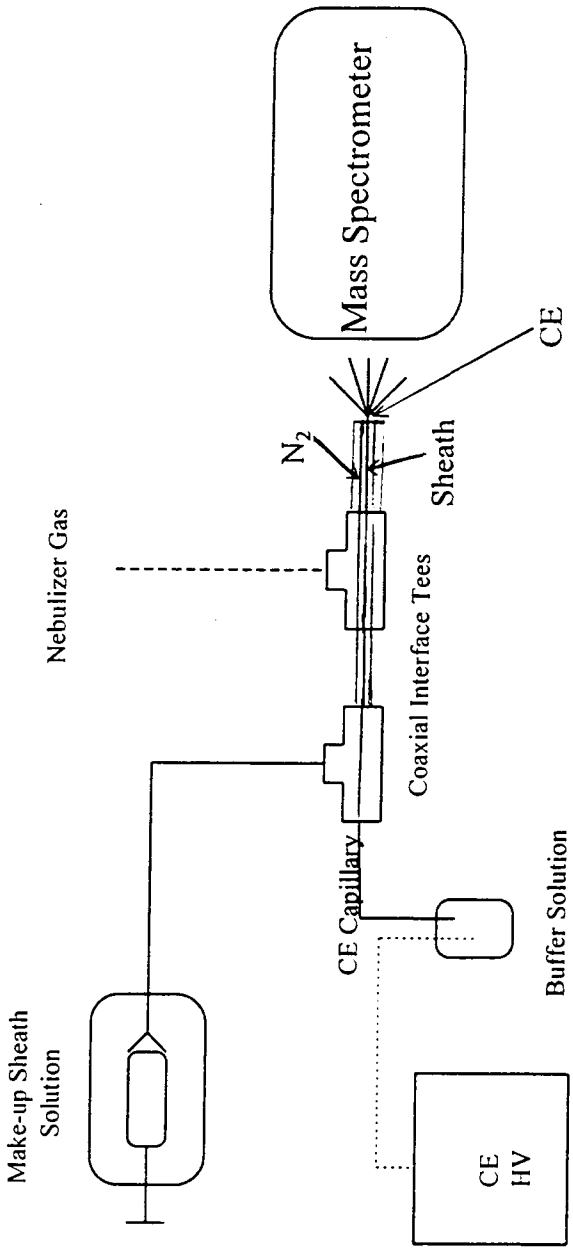

Figure 2. General CE/MS diagram.

the capillary. A negative anionic charge develops on the capillary surface due to the ionization of surface silanol functional groups. At the silica-solution interface, the anionic silica surface is surrounded with solvent and solute counterions forming a stagnant double layer (Helmholtz plane). Cations in the outer portion of the diffuse layer tend to migrate towards the cathode when electrophoretic fields are applied, dragging solvent with them.[12] This comprises the electroosmotic flow of the solvent. Factors such as buffer pH and ionic strength affect the magnitude of the electroosmotic flow. Motion of solute ions depends on the bulk flow and the electrophoretic mobility of the individual ions. Cations within the solution migrate toward the cathode with the electroosmotic flow, and anions within the solution migrate against the electroosmotic flow toward the anode. Neutral components migrate with the electroosmotic flow unless charged additives, such as micelles or cyclodextrins, are added to the buffer solution. By employing additives, neutral components may be separated based upon the differential electrophoretic mobilities of the neutral/additive adducts. Figure 3 illustrates the net flow within a CE column.

Due to the extremely small size of the double layer, the flow of the solution within the CE column originates at the walls of the capillary causing a flat flow profile. The flat flow profile combined with the lack of a stationary phase lead to

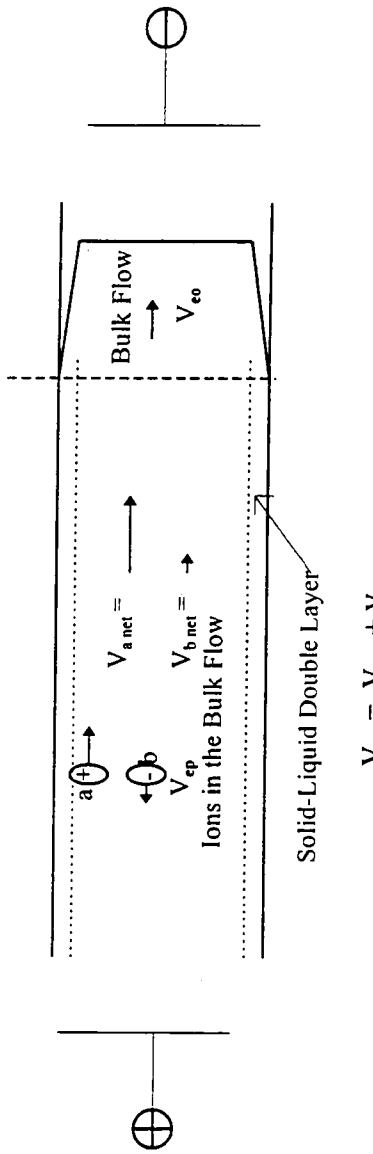

Figure 3. Illustration of the net flow (V_x) within the CE capillary.

very high efficiency. Factors in CE separations that lead to losses in efficiency include nonoptimized conditions for column temperature, separation time, column geometry, injection and detection volumes, solute adsorption, and sample concentration.[12]

Considering the electroosmotic flow, the solute velocity (v) and retention time (t) are expressed as follows:

$$v = (\mu_e + \mu_{eo})V/L \qquad (2)$$

$$t = L^2/(\mu_e + \mu_{eo})V \qquad (3)$$

where μ_e is the electrophoretic mobility, μ_{eo} is the electroosmotic mobility, V is the applied voltage, and L is the column length. Under conditions where efficiency (N) is limited solely by axial diffusion, the maximum separation efficiency (N) is calculated using the equation:

$$N = (\mu_e + \mu_{eo})V/2D \qquad (4)$$

where D is the diffusion coefficient. The resolution (R) is proportional to the time the solute spends in the capillary. The resolution of two solute zones may be expressed as:

$$R = \{(N)^{1/2}\Delta v\}/4v \qquad (5)$$

where N is the average number of theoretical plates, Δv is the difference in zone velocities, and v is the average zone velocity. Using Equations 2 and 4 to

substitute the terms for v and N into Equation 5, the resolution may be expressed as:

$$R = \{(\mu_{e,1} - \mu_{e,2})[V/D(\mu_e + \mu_{eo})]^{1/2}\}/5.6 \qquad (6)$$

where $\mu_{e,1}$ and $\mu_{e,2}$ are the electrophoretic mobilities of the two solutes, and μ_e is the average electrophoretic mobility.

B. Electrospray CE/MS Interface

A CE separated sample can be introduced into a mass spectrometer using an electrospray introduction system. The electrospray is electrostatically generated when a stream of fluid emerging from the CE column is placed in an electrostatic field. The electrospray technique was first demonstrated by Zeleny in 1917.[13]

When a drop of solution is placed on a metal tip and an electrostatic field is applied, two forces, surface tension and electrostatic force, interact with the drop. The surface tension causes the liquid to minimize the surface area of the solution, and the electrostatic force acts to pull the surface of the drop toward a target maintained at a lower potential. Once the field strength reaches a critical value, a Taylor cone is formed and an electrospray is temporally produced on the tip under this configuration. The Taylor cone formation theory was first published by Taylor in 1964.[14] Wilm and Mann developed a theoretical description of the electrospray process based on the original Taylor cone model.[11] They derived the

following equation describing the relationship between the threshold potential for spraying and the distance between electrodes:

$$U_T = 0.863(\gamma r_1 / \epsilon_0)^{0.5} \qquad (7)$$

where U_T is the threshold voltage, γ is the surface tension, ϵ_0 is permittivity, and r_1 is the distance between the liquid tip and the target surface (Figure 1).

Once an electrospray is initiated, charged particles are propelled toward a target surface. As the droplet travels toward the target, smaller charged droplets are formed as the result of solvent evaporation. As the solvent concentration decreases, the charge density increases until the resulting coulombic repulsion overcomes the surface tension force leading to droplet fission. To date, two major theories exist explaining the formation of gas phase ions from the smaller charged droplets. Fenn proposed that the charged analyte molecule penetrates the surface of the charged droplet, and the coulombic repulsion between the charged portion of the molecule and the droplet surface expels the entire molecular ion out of the droplet.[15-16] An alternative theory is based upon Dole's premise that nanometer sized droplets containing one macromolecule are produced by solvent evaporation and repetitive uneven droplet fission, and upon complete desolvation, ionized molecules are formed.[17] Organic solvents are typically added to the make-up solution in order to increase volatility and aid in desolvation. Resolution of the gas phase ion formation controversy lies outside the scope of this thesis.

A conventional electrospray ionization interface diagram is given in Figure 4. The liquid sheath interface consists of a triaxial probe in which the CE column (fused silica, 50 μm i.d., 365 μm o.d.) is placed in the center of an inner stainless steel sleeve (400 μm i.d., 1400 μm o.d.) and a volatile make-up solution (e.g. methanol) is pumped between the column and inner sleeve allowing the makeup solution to envelope the tip of the CE column. The inner metal sleeve is located within an outer metal sleeve (1500 μm i.d., 2800 μm o.d.). The tip of the CE column must extend at least 0.2 mm from the liquid sheath electrode stainless steel capillary in order to produce an analyte signal.[18] The flow of the sheath electrode make-up solution is typically maintained at a rate of 5-10 μL/min.

The modified low sheath flow electrospray ionization interface design presented in this thesis is shown in Figure 5. The same dimensions listed above are used with the low sheath flow CE/MS electrospray interface with the exception of the tip modifications and positioning in the metal sleeve. Inspection of the Taylor cones generated from the electrospray units illustrated in Figures 4 and 5 helps to give a visual image of the difference in amounts of sheath liquid make-up solution required to sustain a spray in each of the designs.

C. Mass Analysis

Electrospray sampling takes place within the inlet and source sections of the VG Quattro II tandem mass spectrometer (Figure 6). Dry nitrogen is

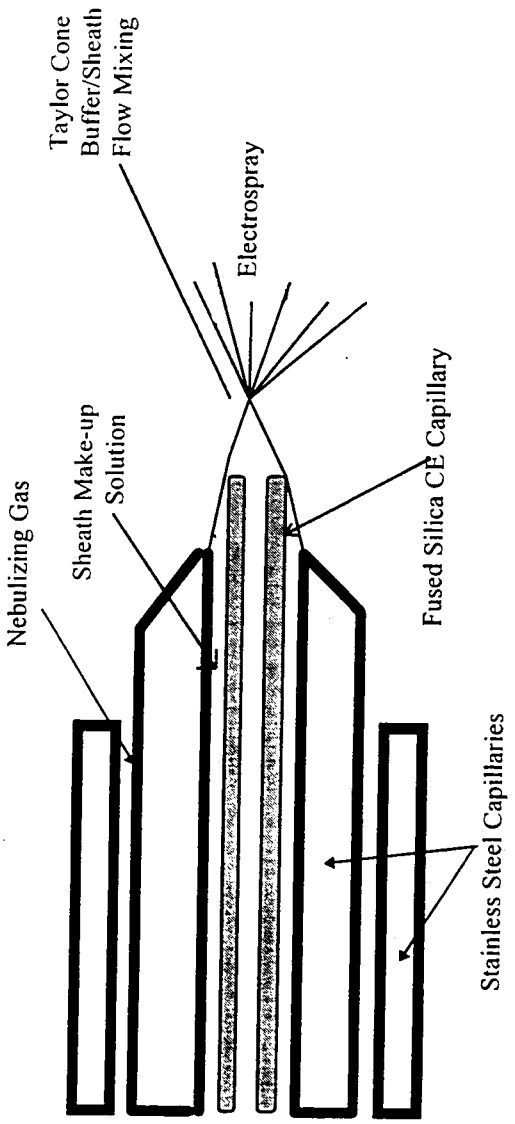

Figure 4. Conventional electrospray diagram.

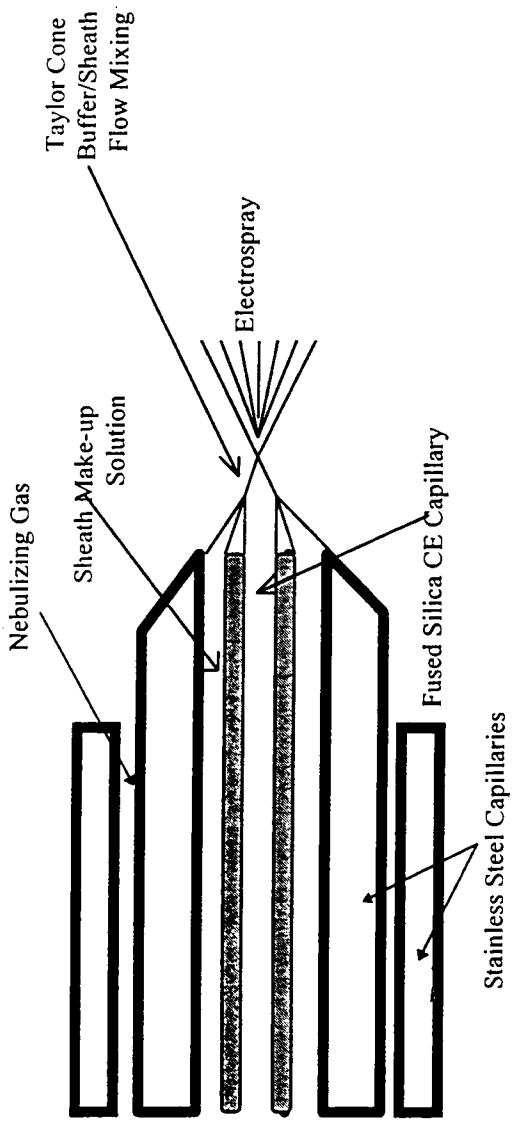

Figure 5. Modified low sheath flow electrospray diagram.

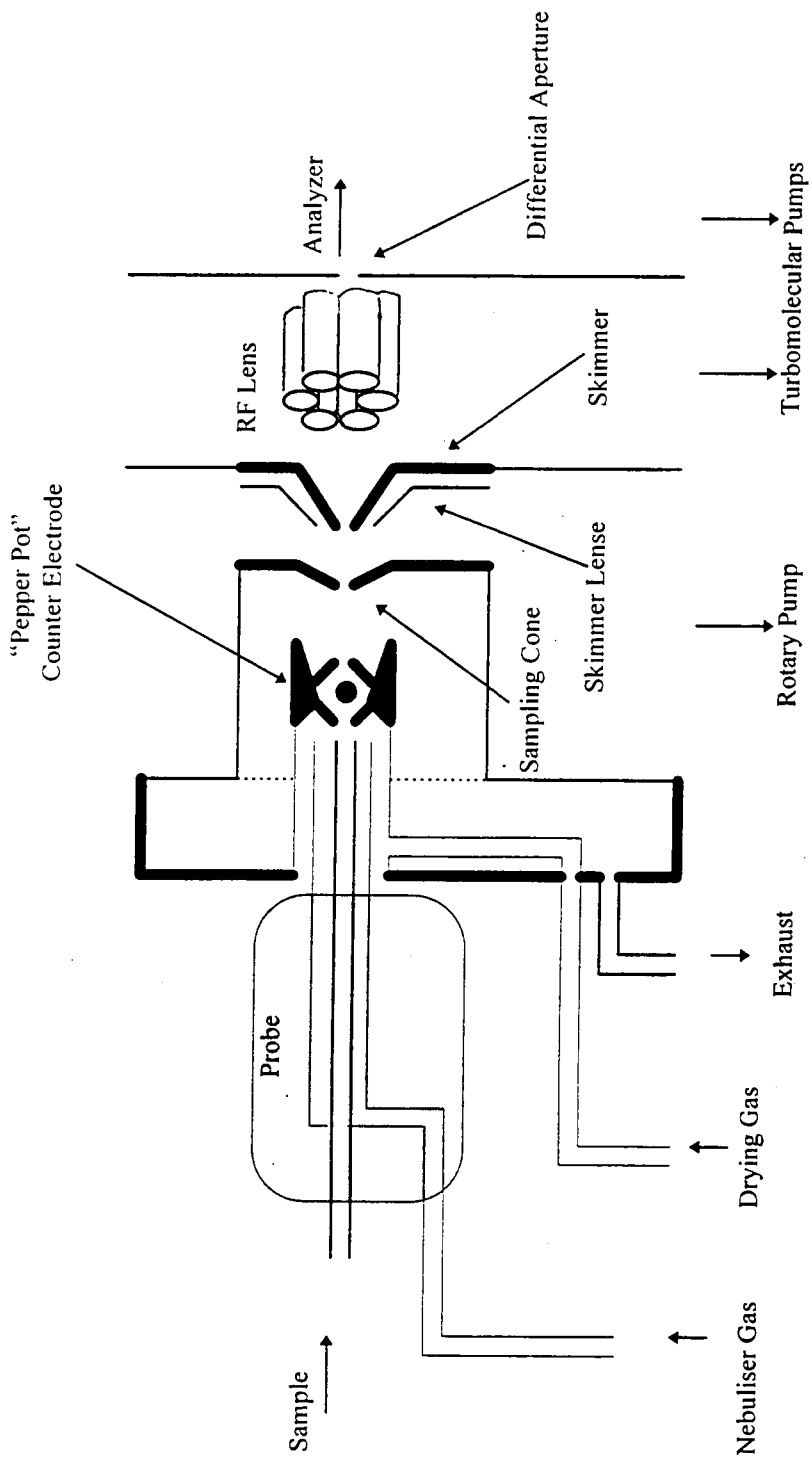

Figure 6. VG Quattro II electrospray/mass spectrometer interface.

introduced through a capillary to enhance nebulization conducted at atmospheric pressure. The charged droplets formed are accelerated in an electric field towards the "pepper pot" (counter electrode), then on to the analyzer section of the instrument held at high vacuum. The pepper pot aids in the desolvation of small liquid droplets, and prevents a straight line of sight between the electrospray tip and the sampling cone. This prevents large liquid droplet from entering the vacuum system. The desolvated ions pass through a sampling cone, skimmer lens, skimmer, RF lens, and differential aperture before entering the quadrupole mass analyzer. The ions are detected within the first quadrupole assembly (MS1) using an Altrincham lens and associated detector (Figure 7).

Ions of a given *m/z* ratio are transmitted through the quadrupole mass filter and are detected by the detector assembly system (see below). The mass resolving capability of the filter is based upon the intrinsic stability/instability characteristics of an ion trajectory in a superimposed D.C./R.F. electric field. In order to prevent residual gases from disrupting ion trajectories, it is essential to maintain a high vacuum environment.

A quadrupole mass analyzer is made up of four electrically conducting metal rods arranged in a square array with an inscribed radius $\mathbf{r_o}$. The rods are biased to generate a time-dependent oscillating electric field that approximates an

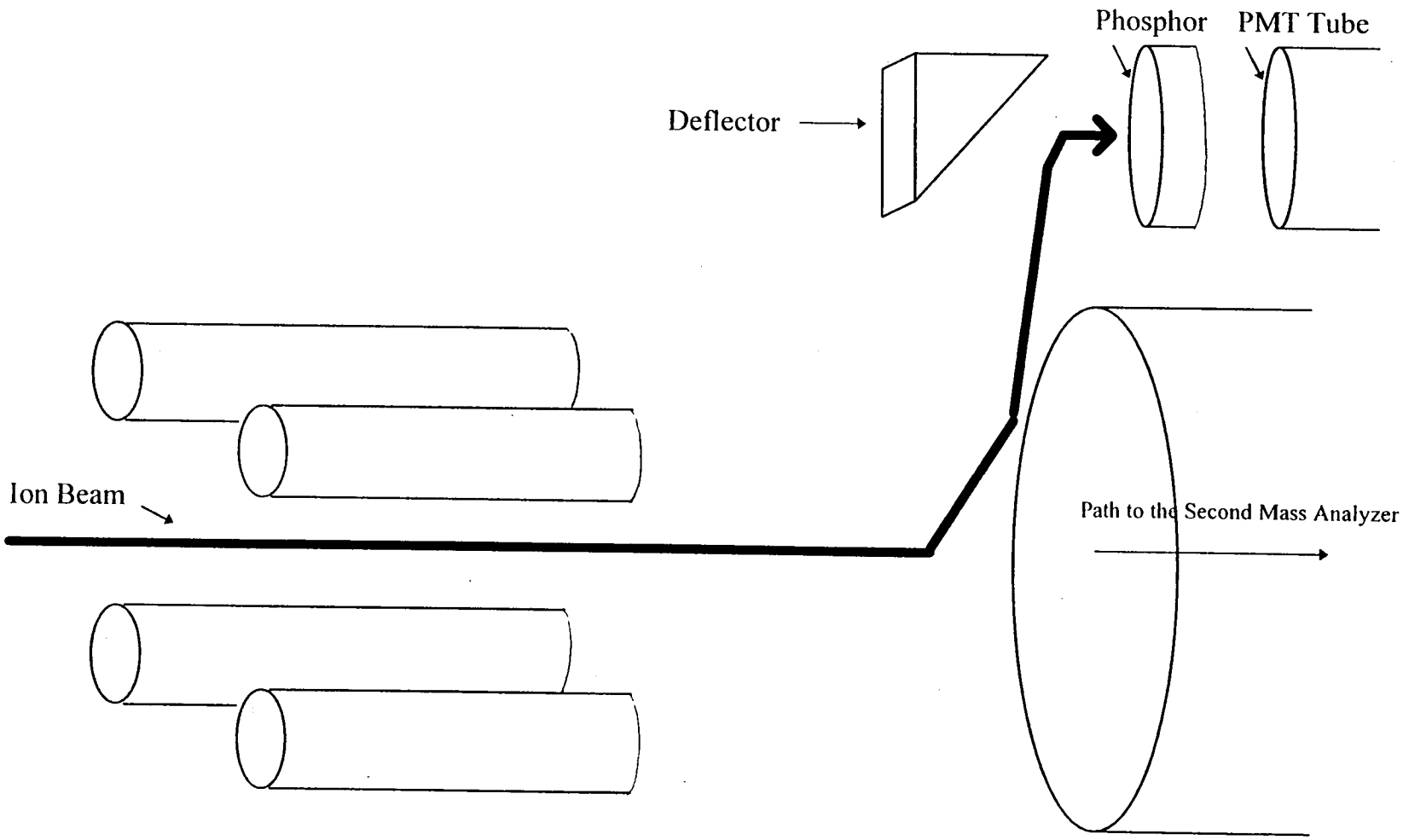

Figure 7. An Altrincham lens diverts ions at the first detector for MS analysis.

ideal hyperbolic field. There is no field component along the axis of the quadrupole (z) and equal and opposite potentials, **+Vo** and **-Vo**, are applied to the **x** and **y** rod pairs. When a varying RF potential (**V**) and a nonvarying potential (**U**) are applied, the overall sinusoidally varying field effect may be represented by the following equation:

$$\mathbf{Vo}(t) = \mathbf{U} + \mathbf{V_p}\cos(2\pi\mathbf{ft}) \qquad (8)$$

The overall product of the conditions given by Equation 8 is a band pass mass filter with a characteristic resolution component determined by the ratio of **U/Vp**.[19]

D. Ion Detection

The VG Quattro II tandem mass spectrometer allows ions to be detected in either MS or MS-MS mode. In the MS mode, ions exiting the first quadrupole mass filter are diverted at the Altrincham lens and enter the detector system (Figure 7). The ion detection system is made up of several components: a conversion dynode, a phosphor array, and a photomultiplier tube. Ions emerging from the quadrupole are deflected to the conversion dynode by the Altrincham lens, initiating the release of secondary electrons. The electrons from the conversion dynode in turn impinge upon a phosphor surface causing photons to be emitted. The photons are detected by a sealed low noise photomultiplier. The photomultiplier tube operates at a typical gain of 10^5, amplifying the ion current

collected. The VG Quattro II is controlled by the Fison's MassLynx software system to acquire and manipulate the data.[20]

CHAPTER III

EXPERIMENTAL

The low sheath flow electrospray design was evaluated using a CE/MS hyphenated separation/detection system. Refer to Figure 2 for a general diagram of the CE/MS configuration.

A. Apparatus

1. Capillary Electrophoresis

A 30 kV Spellman high voltage power supply (Plain View, NY) was used to apply the electrophoretic fields to the capillary column. Hydrostatic sample injections were made by placing the anodic end of the capillary into the sample at a height difference of +10 cm (relative to the cathode end) for 30 s. Once the injection was made, the capillary was wiped clean and placed into a buffer solution. Field strengths ranging from 220 to 280 V/cm were applied across the capillary column after placing the column into the buffer solution. The CE column used in all experiments was a 50 cm long, conventional fused silica capillary, 50 μm i.d. and 365 μm o.d.. The capillary [stock material] was obtained from Polymicro Technologies (Phoenix, AZ).

2. Capillary Column Tip Conditioning for the Electrospray Interface

A 60 to 70 cm piece of fused silica capillary was obtained. Using a column cutter, one end of the column was cut ensuring that the cut was smooth and square. Approximately 5 cm of the column was inserted into a #4 collet on a bench top minilathe (American Edestaal, NY). The square, precut end of the column was held like a pencil between the thumb and index finger. While the column was spinning at maximum rpm, the square tip was positioned at $\approx 40^{o}$ angle and pulled across the surface (Figure 8) of a very fine diamond dust sharpening stone (generic). A slight finger pressure was applied to the column while sharpening. Caution was used to ensure that the column was not pushed or left in one position on the sharpening stone while the column was spinning. Also, care was taken to ensure the tip was not ground too close to the inner wall column diameter. An over-ground tip tended to have an uneven tip surface leading to a poor electrospray. After moving the cut tip 10 to 15 times across one inch of the stone, the tip was observed under a 40x microscope. The column was inspected for the presence of defects such as uneven tip symmetry and divots. 2 to 3 cm of the polyamid coating was removed from the tip by heating in a butane flame to prevent possible coating degradation at the tip from interfering with the electrospray. Finally the tip was ultrasonically cleaned in methanol for

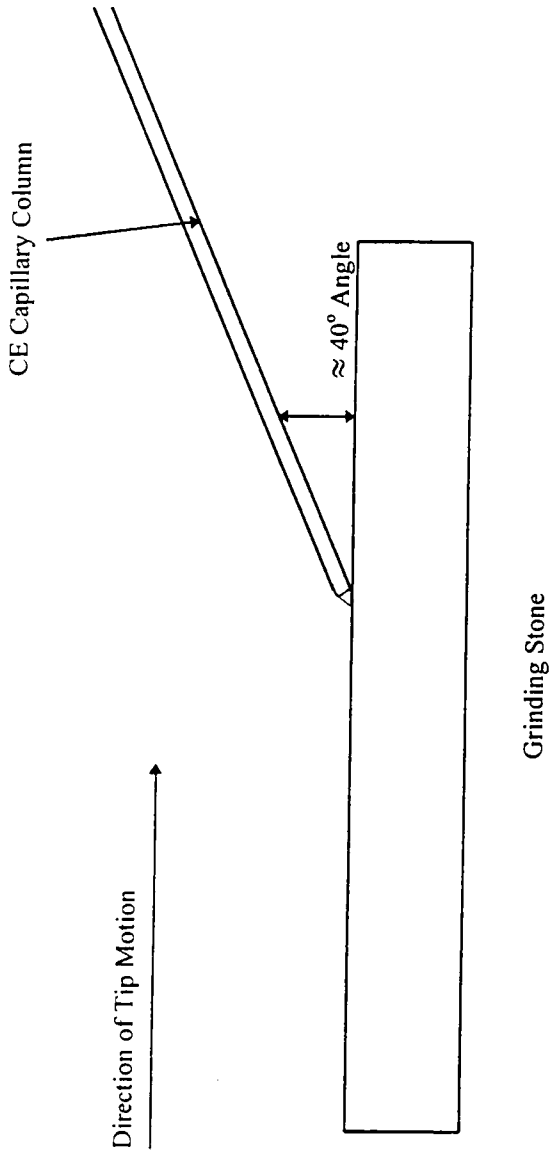

Figure 8. Construction of the modified tip.

approximately 2 min. The column was wiped to remove all dust particles from the column exterior. An electron micrograph of the modified tip is given in Figure 9.

3. ESMS

A Fisons VG Quattro II tandem mass spectrometer was used in this research project. The instrument was equipped with a standard Micromass triaxial electrospray sample introduction/ionization probe. The modified tip was placed in the probe and the tip position was adjusted (Refer to Figure 5) and secured by tightening a nut securing a graphite ferule at the sheath flow solution tee.

A Model 22 Harvard syringe pump (South Natick, MA) was connected to the make-up solution tee (flow rate range 2 to 5 μL/min.). Once in place, the electrospray capillary was cleaned with methanol at a flow rate of 20 μL/min. for 5 min. The nitrogen nebulizing gas metal sleeve was connected and visually centered around the stainless steel sheath capillary to ensure a uniform gas flow around the capillary was maintained.

The relative positions of the capillaries affect the intensity and stability of the ion beam. The performance was optimized by properly positioning the inner stainless steel capillary relative to the outer capillary. The inner capillary must extend forward, 0.5 mm past the outer capillary. Once adjusted, the tip lock nut was tightened onto the gas sealing O ring to prevent the nitrogen nebulizing gas

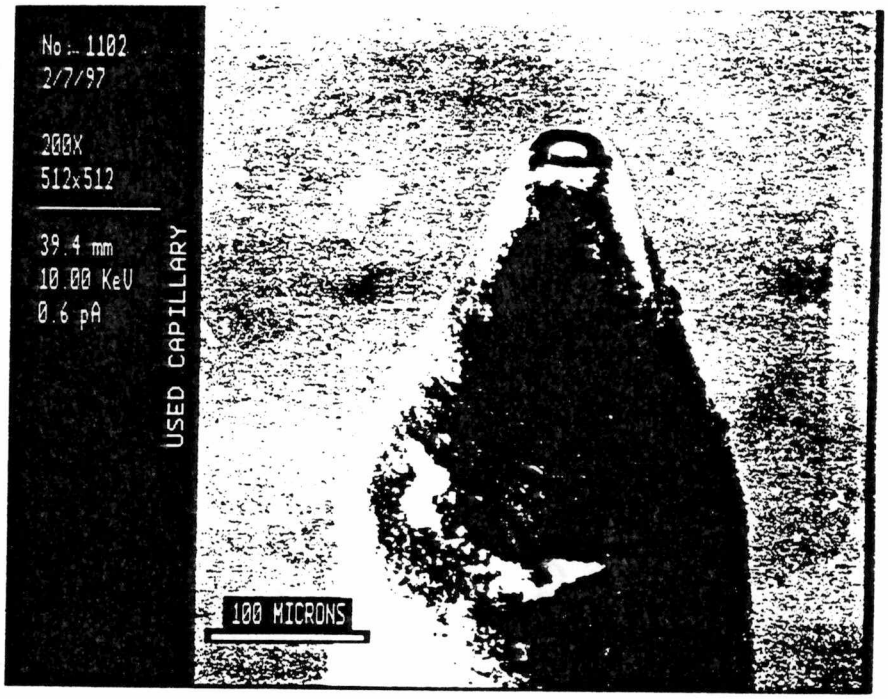

Figure 9. Electron micrograph of the modified tip.

from leaking. The triaxial configuration in the probe was visually inspected to ensure good seals by looking for leaks at all tees and unions. The electrical contact between the inner stainless steal capillary and the high voltage feed-through was checked by measuring the resistance between the two points.

Instrument tuning was initiated by setting the make-up flow to a given rate, then switching the bath and nebulizer gases (N_2) on at a flow rate of 50 and 25 L/hr respectively. The instrument interface temperature was set to 40 ^{o}C. The analyte was infused through the capillary using a syringe at a flow rate of 250 nL/min. This flow rate was chosen to simulate the osmotic flow through a CE column. The probe position and electrospray, counter electrode, sampling cone, skimmer lens, skimmer, RF lens, differential aperture, and prefilter voltages were optimized for a maximum signal at a selected *m/z* for each experiment.

B. Chemicals

18 MΩ purified water was used throughout the research project. The water was purified using a Millipore Milli-Q purification system (Marlborough, MA USA). The buffers α-hydroxy isobutric acid and ammonium acetate were purchased from Aldrich Chemical Co. (Milwaukee, WI USA). The holmium and praseodymium nitrate standards were obtained from Sigma Chemical Co. (St. Louis, MO USA). Sulfonated-β-cyclodextrins were acquired from American

Maize Products (Hammond, IN USA). Carboxymethyl-β-cyclodextrins were obtained from CTD (Gainesville, FL USA).

C. Spectra

The total ion current chromatogram, reconstructed ion chromatogram, and the mass spectra were generated from the Fisons VG MassLinks software. The total ion current chromatogram displayed the sum of all current intensities (both signal and noise) acquired in a scanning mode. Reconstructed ion chromatograms were generated using the software. A plot of the ion current from a requested *m/z* value was produced when the reconstruction option was requested. Mass spectra were generated for the mass region scanned. The percent intensity was graphed for each *m/z* monitored.

CHAPTER IV

RESULTS AND DISCUSSION

In order to test the proposed low sheath flow CE/MS electrospray interface design, several experiments were conducted. Performance parameters such as stability, durability, sensitivity, and efficiency were evaluated.

A. Stability of Spray with the Modified Tip

A mock CE/MS experiment using a modified tip with a 2.0 mM ammonium acetate in methanol sheath make-up solution at a flow of 2.5 μL/min. was initiated to evaluate electrospray stability. Tuning was accomplished by maximizing a ammonium acetate solvent peak (*m/z* 43) intensity. Refer to Table 1 for the modified tip instrument parameters used. A 15.0 mM ammonium acetate/5.0 mM α-hydroxy isobutric acid buffer solution was electroosmotically pumped through a 50 cm long, 50 μm i.d. fused silica capillary column with an applied voltage of 17 kV. After tuning the ESMS using a modified tip, a buffer solution was electroosmotically pumped through the CE column, and the total ion current (TIC) was monitored for a period of 20 min. An example of a total ion current chromatogram from the modified tip stability study is given in Figure 10 (TIC ≈ 2.5E5 +/- 8%). The stability test for the modified tip was repeated four times. The

TABLE 1

TUNE PARAMETERS FOR THE CE/MS MODIFIED TIP

Parameter	Setting
Capillary	3.60 kV
HV Lens	0.50 kV
Cone	164 V
Skimmer Lens Offset	5 V
Skimmer	1.5 V
RF Lens	0.2 V
Source Temperature	40 °C
API Current	0
LM Resolution	14
HM Resolution	14
Ion Energy	1.5 V
Lens 6	6.0 V
Analyzer Vacuum	1.2 E-5 mbar
Inlet	2.8 E-3 mbar

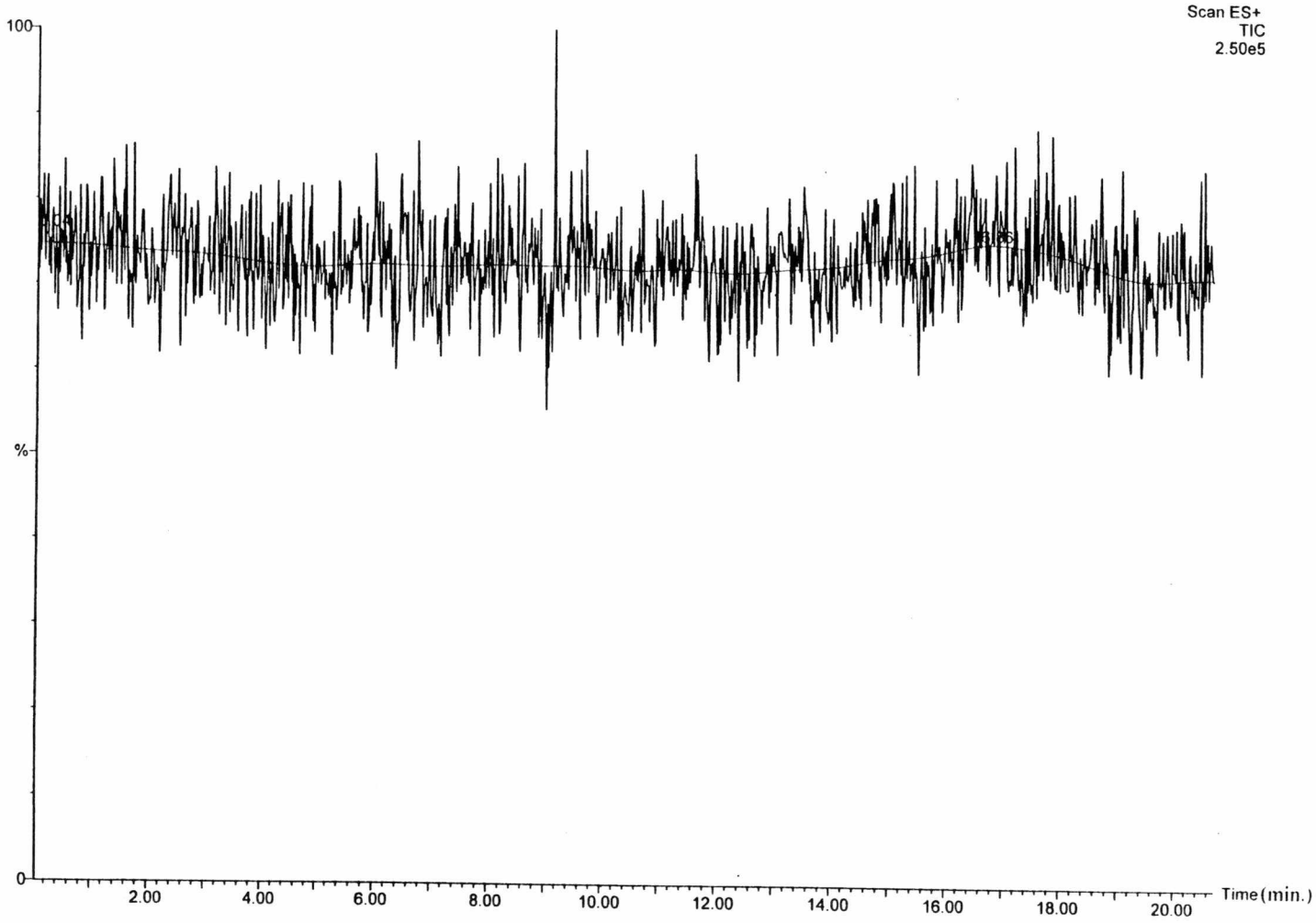

Figure 10. Total ion current chromatogram from the modified tip stability study.

main purpose of this experiment was to ensure that a stable electrospray was maintained for the 20 min. period. If the spray had not been stable, the total ion current would have dropped several orders of magnitude. The electrospray was stable for each of the four runs. After the evaluation, the tip was removed and examined for salt deposits and deterioration. The tip was found to be clean and in good condition.

B. Performance Evaluation of the Conventional Tip at a 2.5 μL/min. Sheath Flow Rate

In order to thoroughly evaluate the performance of a conventional tip in comparison with the modified tip, the ability of conventional tips to spray at low flow rates was determined. The conventional tip was observed under the same tune conditions established in Section A, using the make-up flow rate of 2.5 μL/min. Refer to Table 1 for the instrument parameters. The total ion current signal was monitored to evaluate conventional tip stability at this flow rate. The tip performance illustrated in Figure 11 was poor (TIC ≈ 3.0E5 +/- 30%), showing several periods of electrospray interruptions.

C. A Signal Comparison for both the Conventional and Modified Tips

A comparison of an analyte signal for both the conventional and modified tips was made under optimum conditions for each of the two tip designs. Refer to Table 1 and 2 for the modified tip and conventional tip instrument parameters,

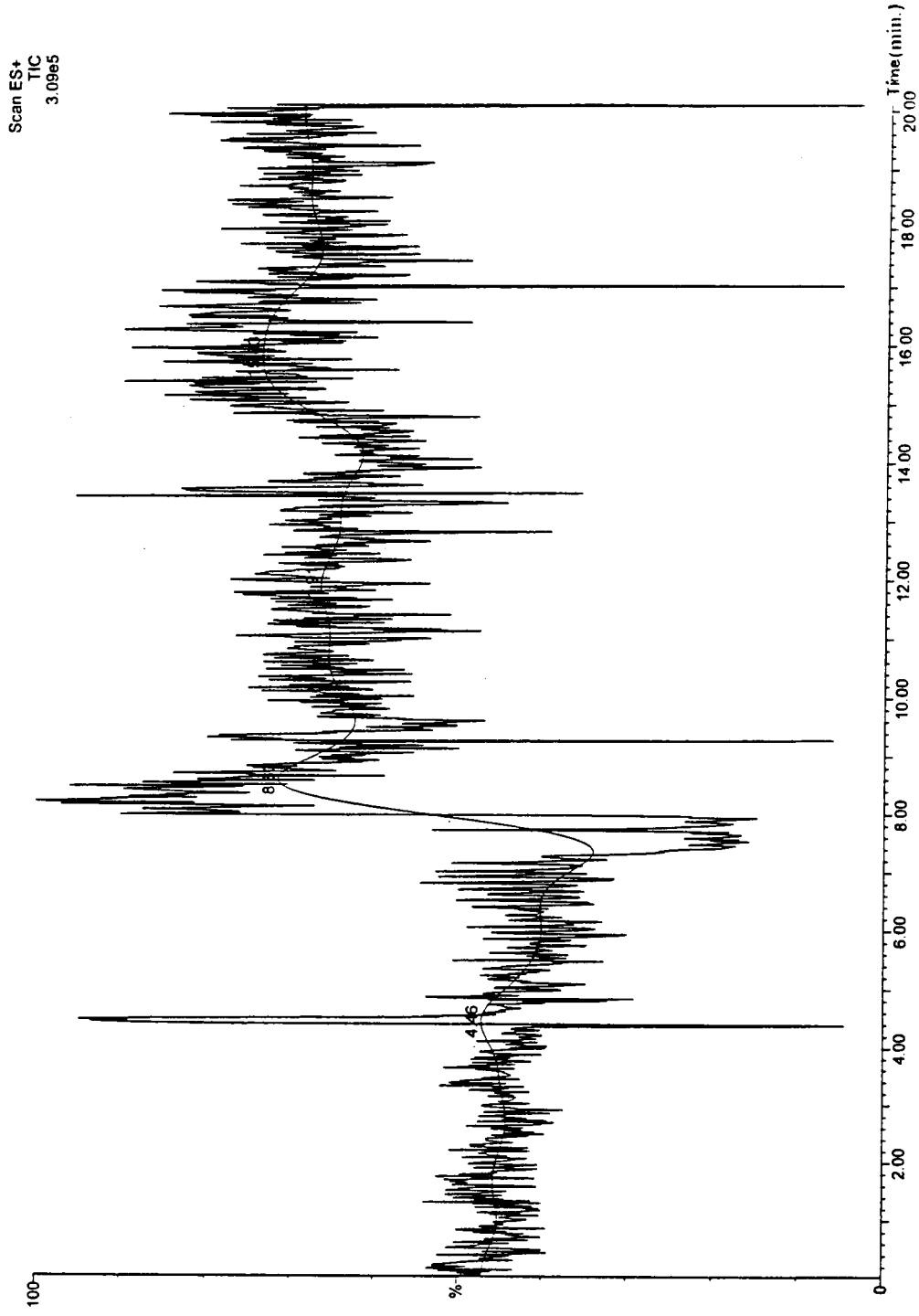

Figure 11. Conventional tip total ion current chromatogram at a 2.5 µL/min. make-up flow rate.

TABLE 2

TUNE PARAMETERS FOR THE CE/MS CONVENTIONAL TIP

Parameter	Setting
Capillary	4.0 kV
HV Lens	0.50 kV
Cone	164 V
Skimmer Lens Offset	5 V
Skimmer	1.5 V
RF Lens	0.2 V
Source Temperature	40 °C
API Current	0
LM Resolution	14
HM Resolution	14
Ion Energy	1.5 V
Lens 6	6.0 V
Analyzer Vacuum	1.2 E-5 mbar
Inlet	2.8 E-3 mbar

respectively. The sheath make-up flow rates were 5.0 μL/min. for the conventional tip and 2.5 μL/min. for the modified tip.

The mass spectrometer was tuned, and the settings were held during the evaluation of both tips. The quadrupole was scanned from *m/z* 40 to 250 at a rate of 1 s per scan. The make-up sheath flow solution was 2.0 mM ammonium acetate in methanol. A 100 ppm solution of Pr was pumped at a flow rate of 250 nL/min. for each setup. The nebulizing gas and drying gas were set at 25 and 50 L/min., respectively. Centering the nebulizing tube relative to the sheath and CE column was found to be essential. The centering was visually conducted using a 10x magnifying glass. The source temperature was set at 40 °C.

Figures 12 A and 12 B illustrate the total ion current chromatogram and the reconstructed ion chromatogram for PrO^+ (*m/z* 156) respectively using the conventional electrospray tip. Figures 13A and 13 B illustrate the corresponding data for the modified electrospray tip. This experiment was conducted twice, on different days. The ESMS results and precisions from these experiments, obtained by first smoothing the data using a six point mean function, graphically overlaying the raw data, and visually estimating precision, are given in Table 3. Using the stated conditions and make-up sheath flow rates, a PrO^+ signal obtained using the modified tip was 2 to 3 times larger and the total ion current was significantly

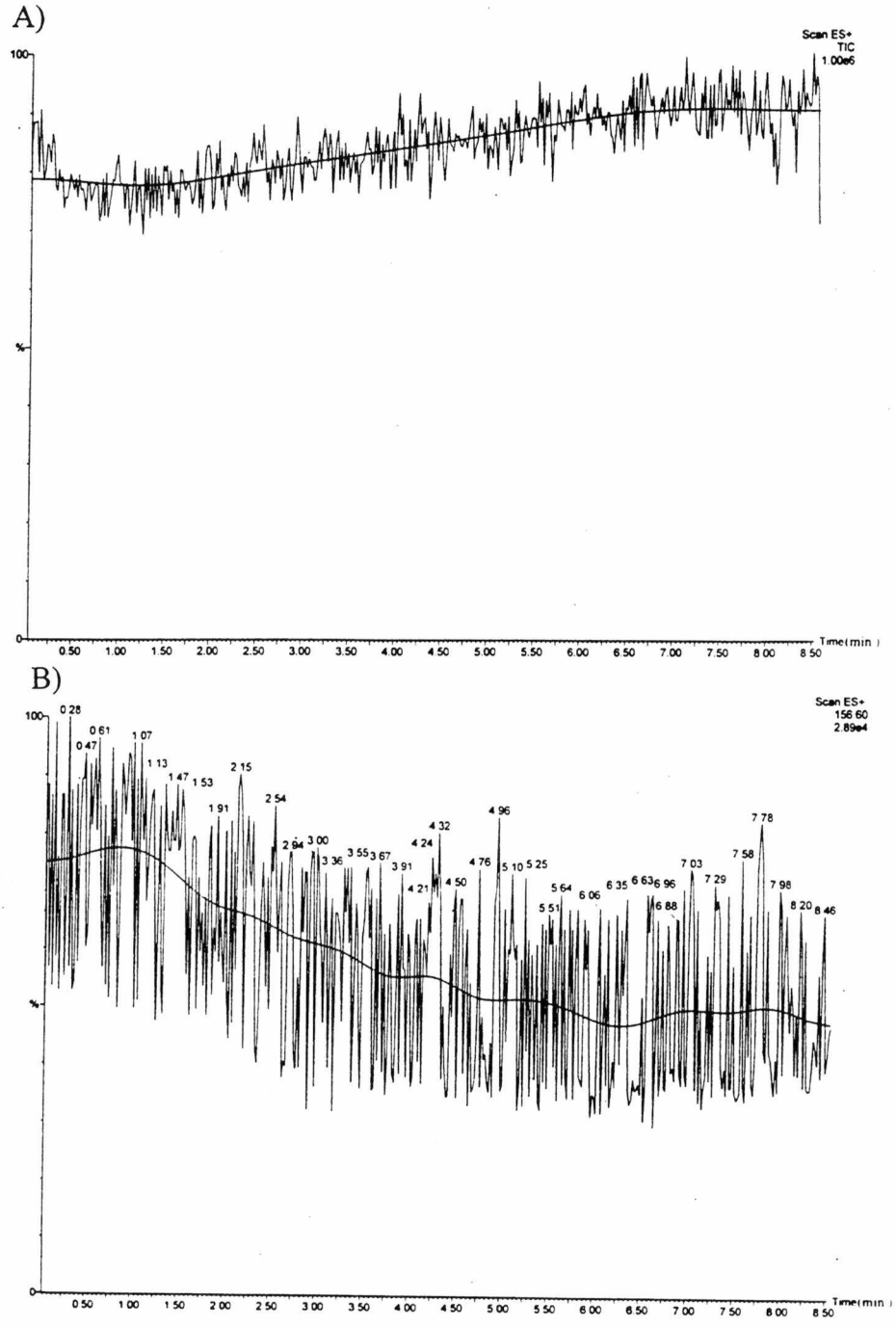

Figure 12 [A and B]. Total (A) and reconstructed (B; *m/z* 156.6) ion chromatogram for PrO^+ using the conventional electrospray tip.

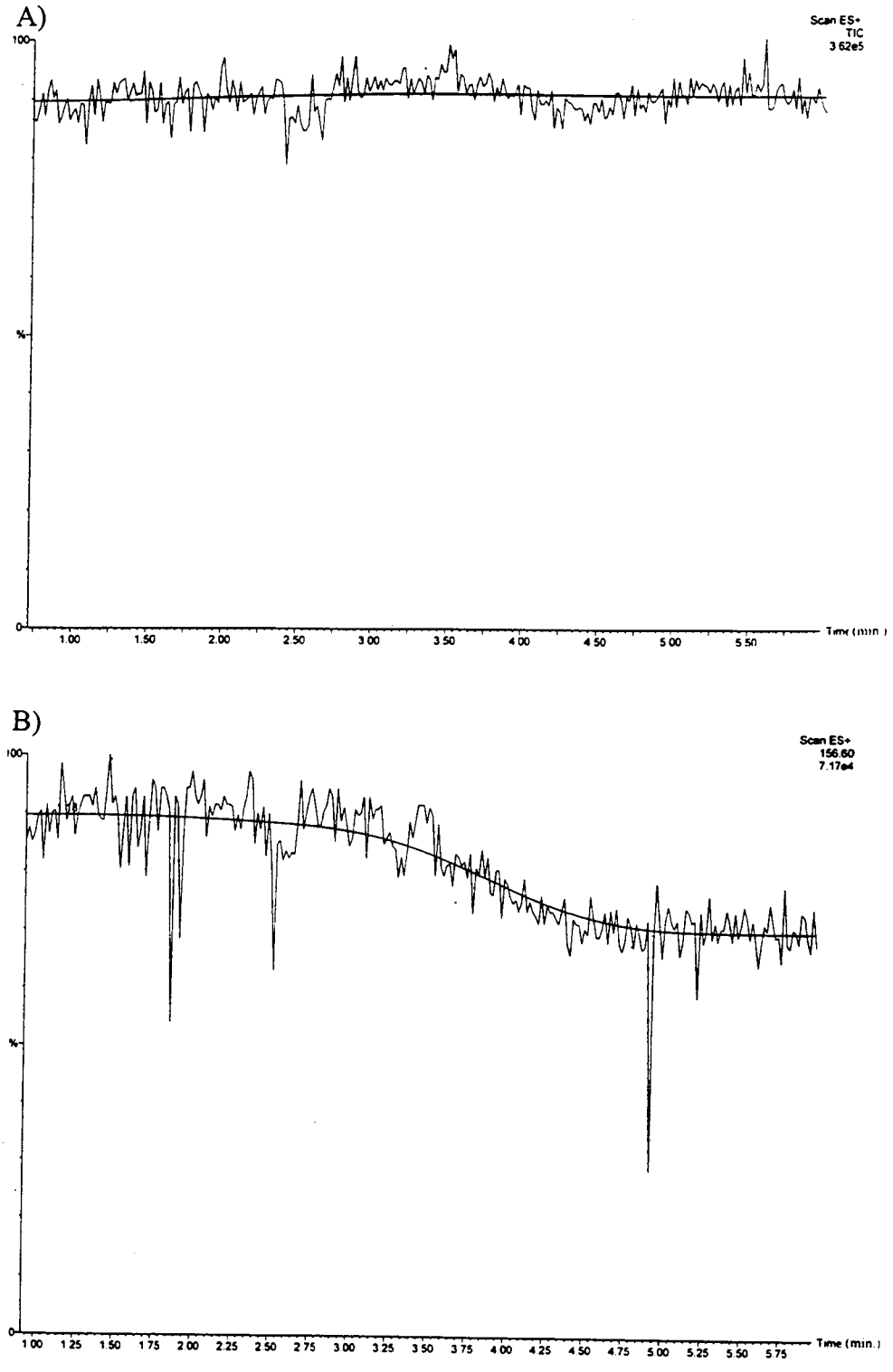

Figure 13 [A and B]. Total (A) and reconstructed (B; *m/z* 156.6) ion chromatogram for PrO^+ using the modified electrospray tip.

TABLE 3

Pr INFUSION RESULTS FOR THE CONVENTIONAL AND MODIFIED TIPS[1]

Tip Type	Make-up Flow Rate	Day 1 Reconstructed Maximum Ion Current At *m/z* 156.6	Day 1 TIC	Day 2 Reconstructed Maximum Ion Current At *m/z* 156.6	Day 2 TIC
Conventional	5.0 μL/min.	2.89E4 +/- 25%	1.00E6 +/- 5%	2.59E4 +/- 22%	1.12E6 +/- 6%
Modified	2.5 μL/min.	7.17E4 +/- 5%	3.62E5 +/- 5%	6.55E4 +/- 5%	2.55E5 +/- 6%

[1]Uncertainties represent estimated signal fluctuation throughout the analysis period.

reduced compared to the conventional tip. The reduced total ion current was indicative of achieving a lower background with the modified tip.

An additional comparison of the two tips was made using CE/MS runs. The buffer solution contained 5.0 mM α-hydroxy-isobutric acid and 12.0 mM ammonium acetate. The buffer solution was chosen based upon preliminary capillary electrophoresis/ultraviolet experiments. The make-up sheath flow solution was 2.0 mM ammonium acetate in methanol. A 190 ppm (each) Pr and Ho mixed standard solution was separated and detected in this experiment. A 30 s hydrostatic injection was made at a height of 10 cm. A voltage of +17 kV was applied to the buffer solution, and the electrospray tip was held at +3.5 kV yielding a potential drop of 270 V/cm. The tuning parameters were identical to those stated in Table 1. The selected ion monitoring detection mode was used for data acquisition with a dwell time of 0.2 s per ion and a 0.01 s inter-scan delay. The CE/MS electropherograms for the separation of Ho and Pr using the conventional and modified tips are given in Figures 14 and 15 respectively. The results of this experiment were comparable with the previous Pr infusion experiment, in that the ion sensitivity for the modified tip was 2-3 times greater and the background current was lower than that obtained using the conventional tip. The small drops

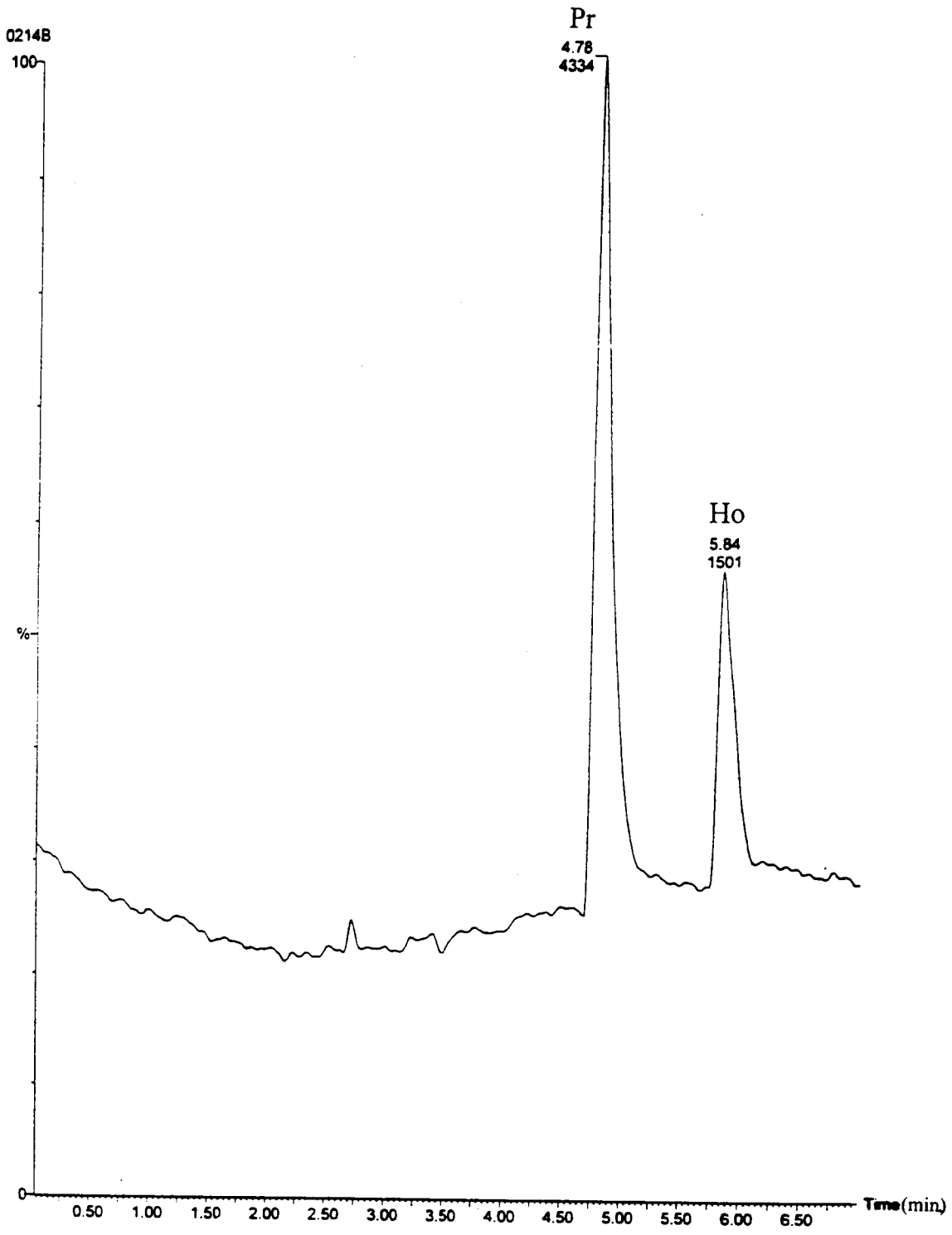

Figure 14. Electropherogram for the separation of Ho and Pr using the conventional electrospray tip.

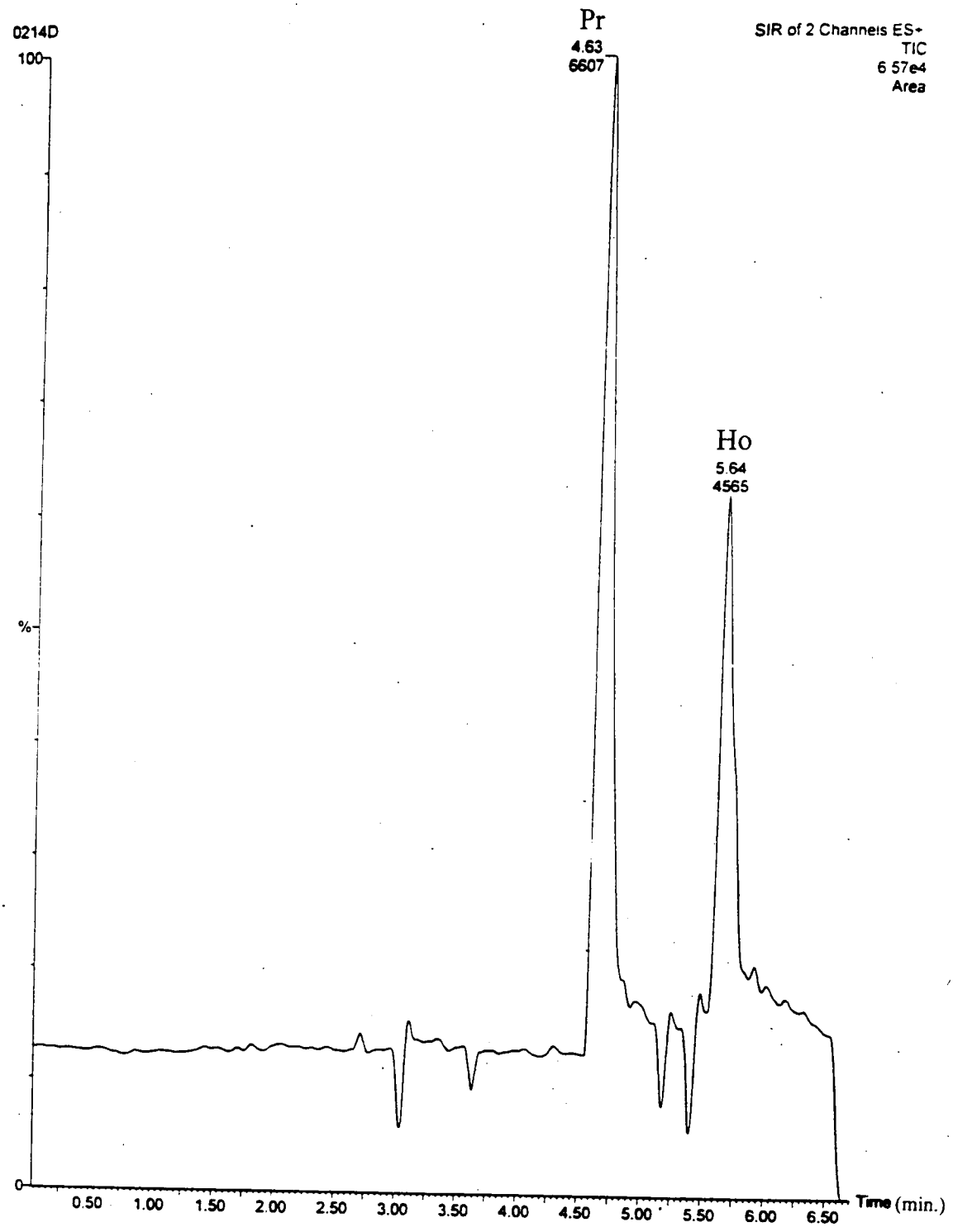

Figure 15. Electropherogram for the separation of Ho and Pr using the modified electrospray tip.

in the baseline of the modified tip electropherogram were due to air bubbles in the makeup solution leading to the momentary loss of electrospray.

D. Applications Using the Modified Tip Electrospray in CE/MS

Two applications were chosen for assessment in this thesis. The first application was the separation and detection of β-cyclodextrins and mono-substituted carboxymethyl cyclodextrins, and the second application was the separation and detection of a mixture of sulfonated β-cyclodextrins.

1. Separation and detection of β-cyclodextrins and mono-substituted carboxymethyl cyclodextrins

The separation and detection of β-cyclodextrins (β-CD) and of mono-substituted carboxymethyl cyclodextrins [CM-β-CD, Degree of Substitution = 1 (DS-1)] was evaluated for this CE/MS application. This application is a useful method for determining purity and degree of substitution for the synthesis of anionic substituted β-CDs. A 25.0 mM aqueous ammonium acetate buffer solution was used in the CE column with a -8.0 kV potential applied to the buffer reservoir. The makeup sheath solution was a mixture of methanol and 2% formic acid. Refer to Table 4 for the CE/MS instrumental parameters for this application. A *m/z* range of 300 to 1500 was monitored in multichannel analyzer (MCA) mode. A 50 cm capillary column with a modified tip was used. A make-up flow rate of 2.0 μL/min. was set.

TABLE 4

INSTRUMENT PARAMETERS FOR THE β-CD AND CM-β-CD

SEPARATION

Parameter	Setting
Capillary	3.6 kV
HV Lens	0.50 kV
Cone	25 V
Skimmer Lens Offset	5 V
Skimmer	1.5 V
RF Lens	0.2 V
Source Temperature	40 °C
API Current	0
LM Resolution	11
HM Resolution	11
Ion Energy	1.5 V
Lens 6	6.0 V
Analyzer Vacuum	1.2 E-5 mbar
Inlet	2.8 E-3 mbar

In preliminary studies each separate component was infused with a syringe pump at a rate of 250 nL/min., and optimized tuning conditions were determined. At a cone voltage of 25 V, both CD's had a large ammonium adduct peak in the spectrum. Figure 16 gives an example of the β-CD ammonium observed at *m/z* 1152 using 25 V. Figure 17 shows the CM-β-CD ammonium adduct spectrum obtained at 50 V. The spectrum clearly shows the protonated CM-β-CD at *m/z* 1193, the CM-β-CD ammonium adduct at *m/z* 1210, and the CM-β-CD sodium adduct at *m/z* 1215. After reviewing the infusion data, it was determined that the CE/MS analysis should be performed in selected ion monitoring mode to obtain maximum sensitivity. First a 2.0 mM β-CD solution was analyzed by CE/MS using the tune parameters listed in Table 4. The β-CD was found to have a retention time of approximately 19 min. (Figure 18). Next, a 2.0 mM CM-β-CD solution was analyzed and found to have a retention time of approximately 14 min.(Figure 19). Finally a 1:1 mixture of the two CDs was analyzed. Figure 20 is the electropherogram obtained from the CD mixture. Excellent separation and peak shape were obtained with a calculated resolution of 2.5. The calculated efficiencies for β-CD and CM-β-CD were 2443 and 2990 respectively. Normally, diffusion coefficients are approximately 1E-6 cm^2/s; however, experimental diffusion coefficients for the β-CD and CM-β-CD were calculated to

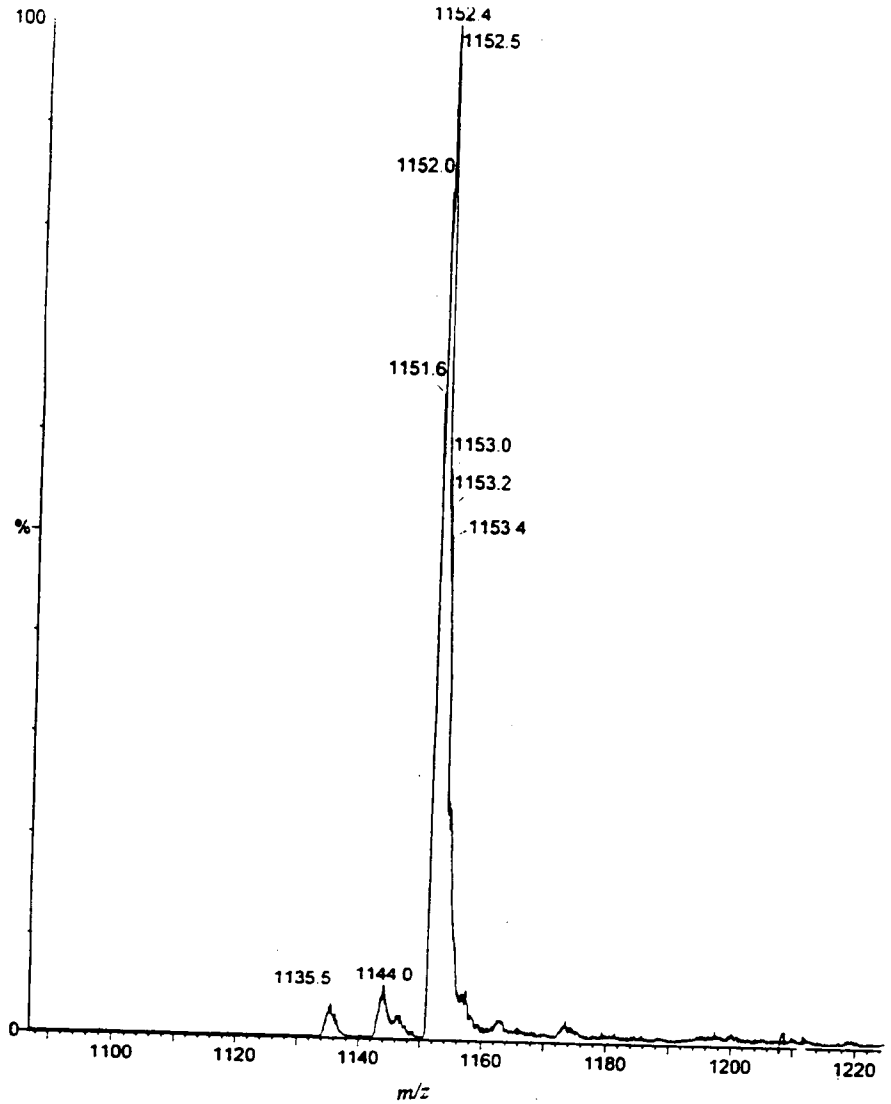

Figure 16. Example of the β-CD ammonium adduct obtained at *m/z* 1152 using 25 V.

Figure 17. The CM-β-CD ammonium adduct obtained at 50 V.

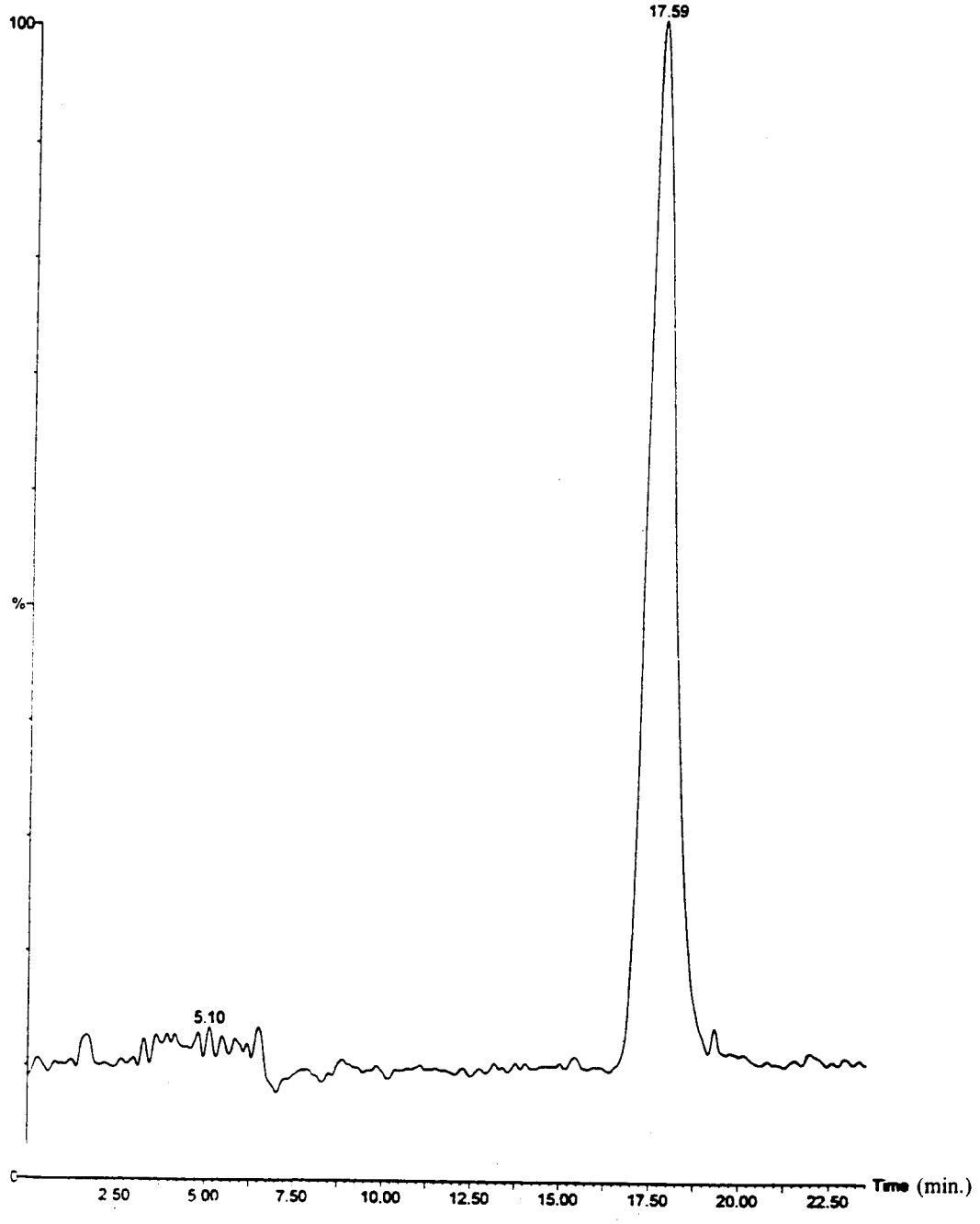

Figure 18. Electropherogram of a 2.0 mM β-CD solution.

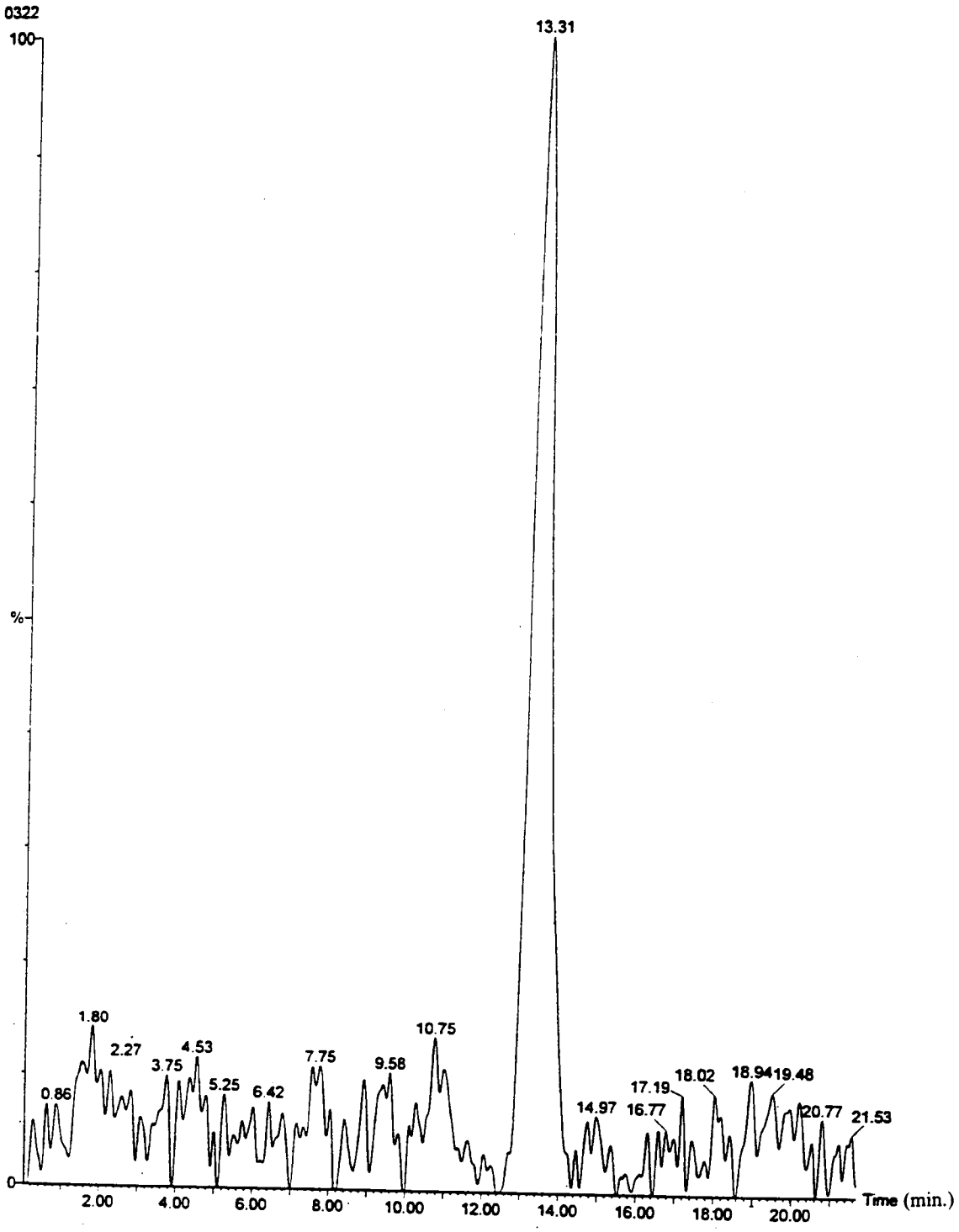

Figure19. Electropherogram of a 2.0 mM CM-β-CD solution.

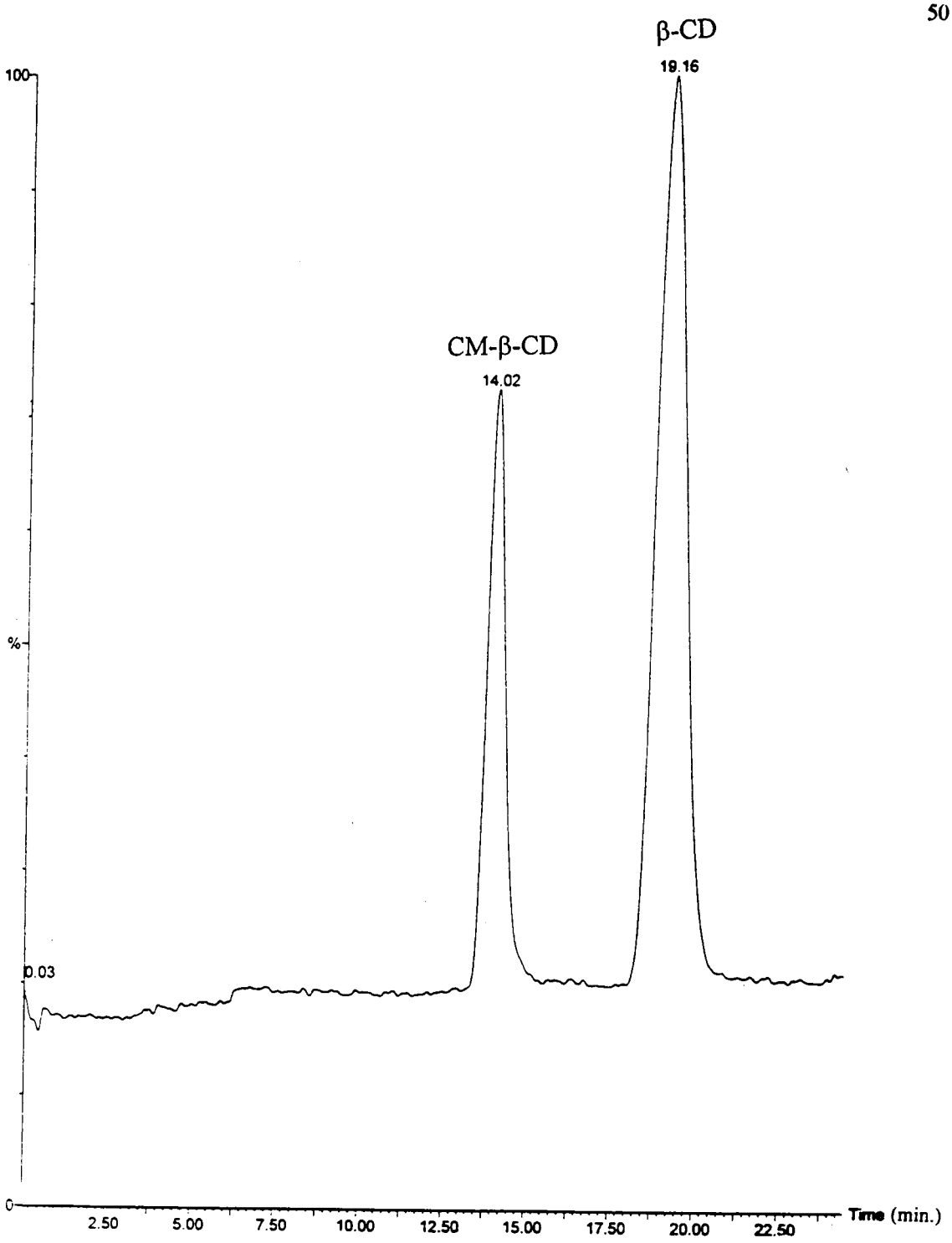

Figure 20. Electropherogram of a 2.0 mM β-CD and CM-β-CD mixture.

be 5.6E-4 and 6.0E-4 cm^2/s respectively. The diffusion of the two compounds was greater than expected due to two factors, large injection plug and possible hydrodynamic flow. Actual formulas used to determine these values are stated in Appendix B.

2. Separation and detection of a mixture of sulfonated β-cyclodextrins

A 15.0 mM solution of sulfonated-β-cyclodextrins (su-β-CD, Average DS = 3.5) was infused at a rate of 250 nL/min. through the CE column. The 15.0 mM concentration was experimentally found to provide an adequate signal-to-noise ratio for each of the su-β-CDs. The make-up solution was a mixture of methanol and 2.0 % formic acid, and the make-up flow rate was 2.0 μL/min. All experimental conditions were identical to those in the previous application, with the exception of cone voltage discussed later in this section. The mass spectra were obtained from cone voltage settings of 40, 90, 160 and 190 V given in Figures 21 - 24 respectively. The cone voltage of 40 V produced a poor spectrum containing doubly charged sodium adducts of the su-β-CDs. The cone voltage of 90 V produced both singly and doubly charged sodium adducts of the su-β-CDs. The cone voltages of 160 and 190 V gave a very clean spectrum of only singly charged sodium adduct peaks; however, the 190 V cone voltage lead to a weaker signal. In the 160 V spectra, unreacted β-CDs appear to be present along with a

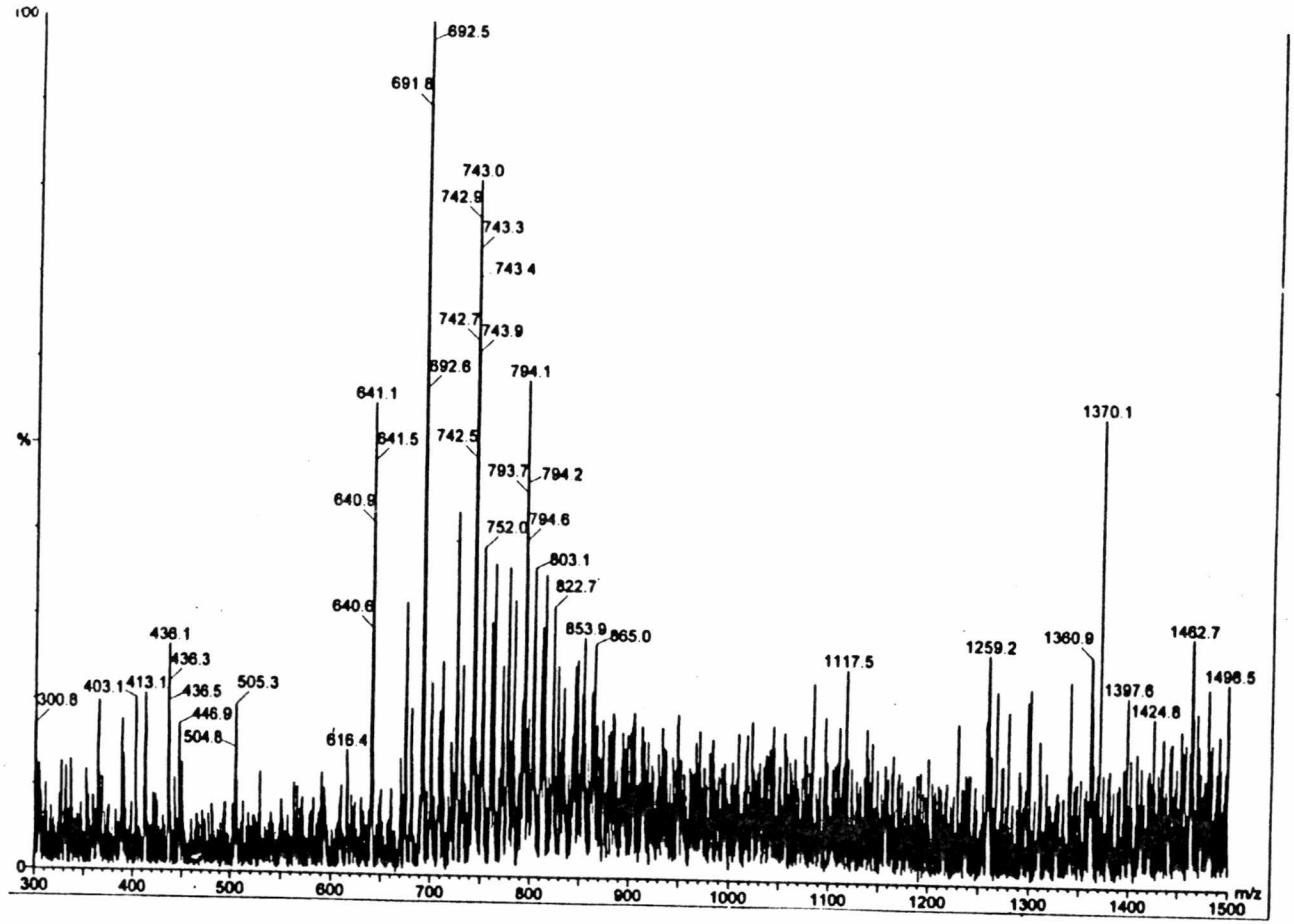

Figure 21. Mass spectra of sulfonated-β-cyclodextrins at a cone voltage of 40V.

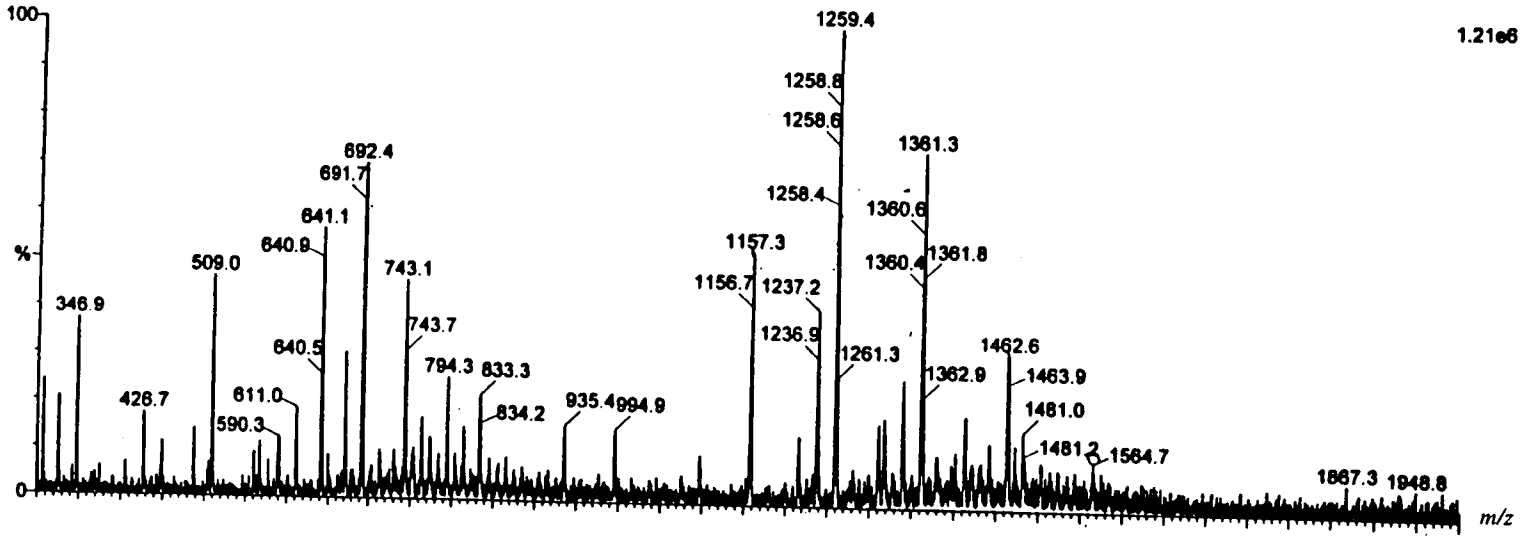

Figure 22. Mass spectra of sulfonated-β-cyclodextrins at a cone voltage of 90V.

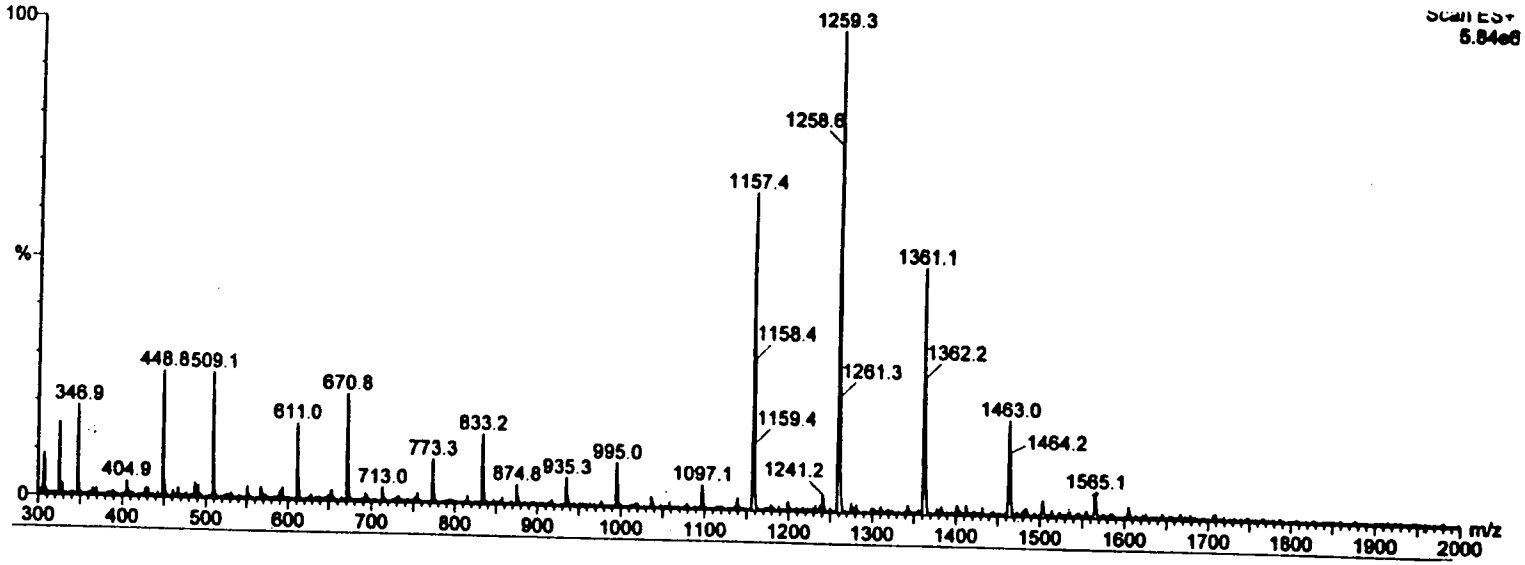

Figure 23. Mass spectra of sulfonated-β-cyclodextrins at a cone voltage of 160V.

Figure 24. Mass spectra of sulfonated-β-cyclodextrins at a cone voltage of 190V.

mixture of su-β-CDs ranging from 4 to 1 in degrees of substitution. If present, higher degrees of substitution were not at levels that could be detected.

A CE/MS analysis was conducted on the 15.0 mM solution of su-β-CDs using the 160 V cone voltage. A 25.0 mM ammonium acetate buffer solution was found to be acceptable for this experiment. At the buffer solution, -14 kV was applied, and +3.1 kV was applied at the make-up solution. A 30 s hydrostatic injection was made. The CE separation was performed while monitoring each singly charged molecular ion (Figure 25). Close inspection of Figure 25 revealed that the SO_3 groups were being removed, and that no original unsubstituted β-CDs were present . The unsubstituted sodium adduct β-CDs $[M + Na]^+$ (m/z = 1157) plot contained 4 peaks through the electropherogram; the singly substituted sodium adduct β-CD (m/z = 1259) contained 3 peaks; the doubly substituted sodium adduct β-CD (m/z = 1361) contained very small peaks; and the three and four substituted sodium adduct β-CD (m/z = 1463 and 1565 respectively) contained no measurable peaks. The SO_3 group removal was initiated at 60 V. At the higher cone voltages, sufficient energy was supplied to permit a simple hydrogen rearrangement to form a hydroxyl site on the CD and a neutral SO_3 compound.

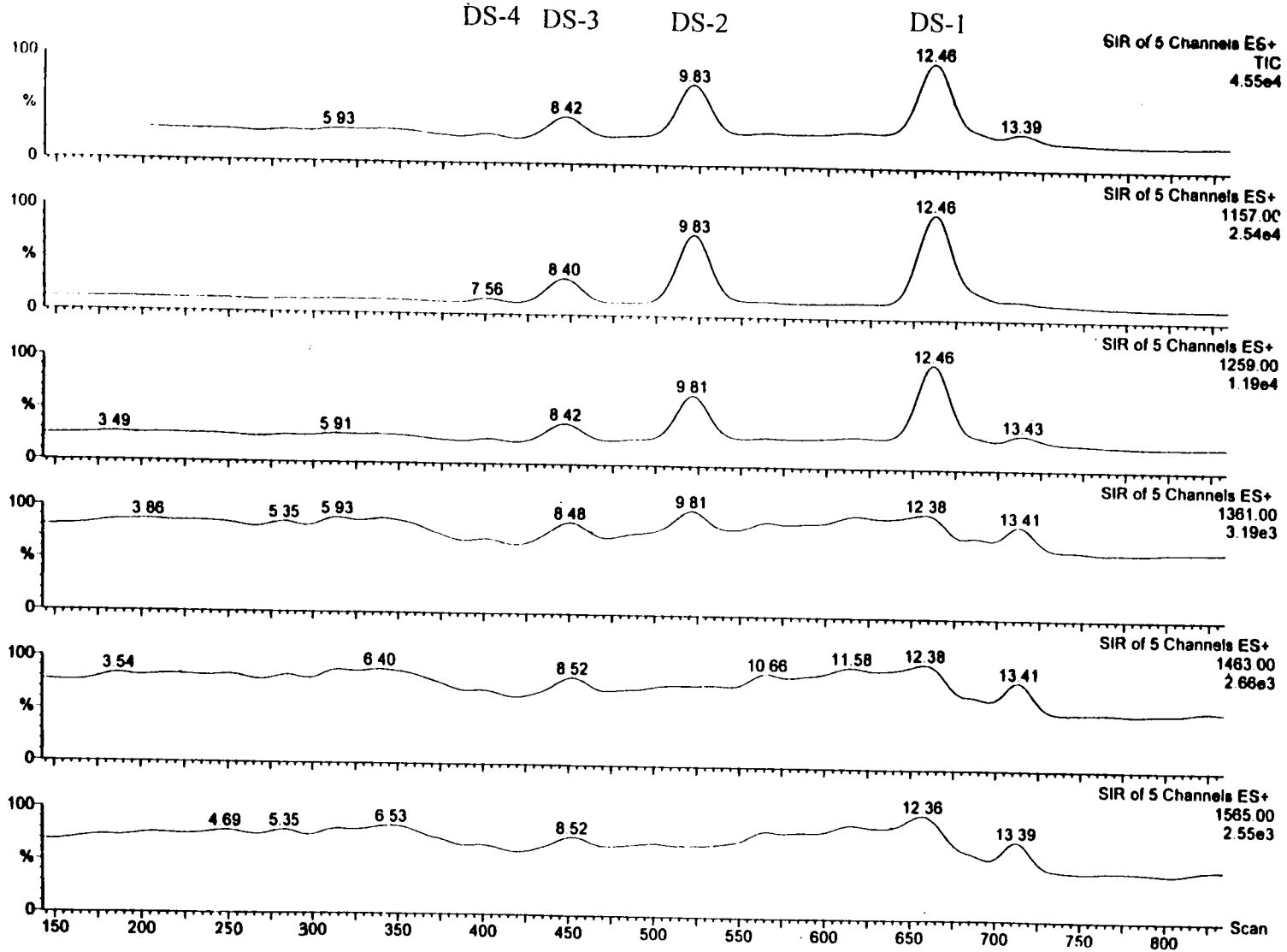

Figure 25. Electropherogram sulfonated-β-cyclodextrins obtained at a cone voltage of 160 V.

Using a cone voltage of 60 V, predominately doubly charged sodium adduct molecular ions were observed. At this cone voltage, singly charged adducts of β-CDs should also be present, but were not observed. A CE analysis was also conducted using the 60 V cone voltage setting. At the buffer solution, -8 kV was applied, and +3.1 kV was applied at the make-up solution. After examining the previous 160 V electropherogram, it was determined that a lower applied voltage could be used due to the efficient separation of the of 1-4 su-β-CDs. A lower applied voltage was desirable due to safety concerns. The sodium adduct β-CD (*m/z* = 1157) and the doubly charged ions sodium adduct ions of 1-4 su-β-CD (*m/z* = 641, 692, 743, and 794 respectively) were detected in the selected ion monitoring mode. The resulting electropherogram is given in Figure 26. The doubly charged ions of the sodium adducts of 1-4 su-β-CDs are present, as well as fragments of the substituted ions. For example, the su-β-CD, DS-4 was observed in the *m/z* = 794 electropherogram with a retention time of 10.59 min., and a fragment was obtained in the *m/z* = 743 electropherogram with a retention time of 10.57 min. The presence of original unsubstituted β-CDs was not observed as a single peak in the electropherogram at *m/z* = 1157. From the sulfonated-β-cyclodextrin experiment, the usefulness of CE separation prior to mass detection was demonstrated. Looking at the mass spectra alone, one would not necessarily

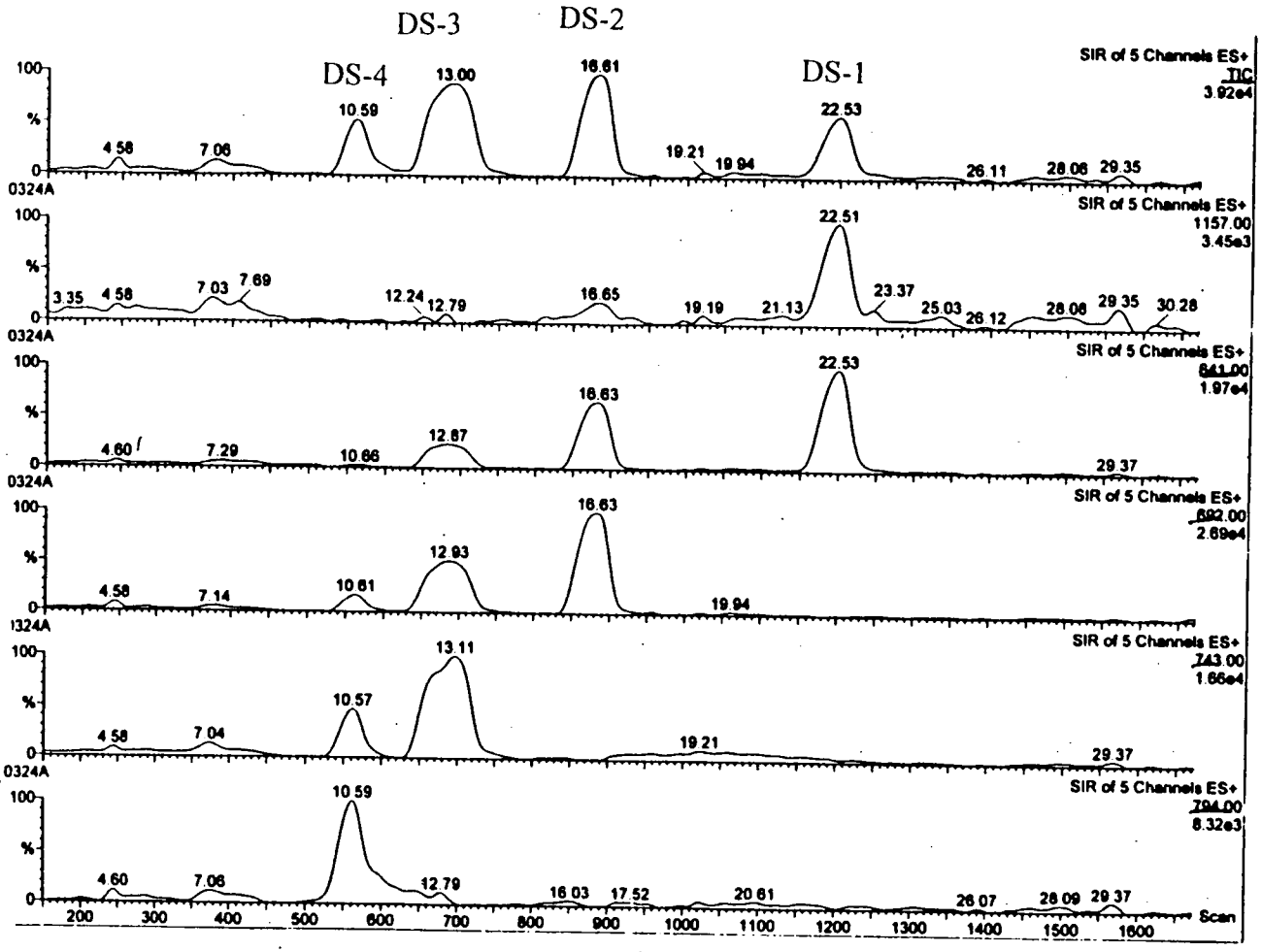

Figure 26. Electropherogram of sulfonated-β-CD doubly charged ions obtained in selective ion monitoring mode at 60 V.

conclude that the 1-4 su-β-CDs had undergone fragmentation at the different cone voltages.

From the data obtained in this study, the theoretical efficiency and resolution values were calculated. The calculated values are given in Table 5. Evaluation of the calculated results and electropherograms revealed good resolution, but poor efficiency and peak shape. Some band broadening was probably due to the long (30 s) injection time and differences in mobilities of the positional isomers. Hydrodynamic flow could also contribute to the poor efficiency and peak shape. The inability to accurately maintain a level position between the column tip at the buffer reservoir and the spray tip could result in gravitational effects on the solution flow within the column. It was doubtful that dispersion due to wall interactions was present since the compounds were large in size and anionic in solution. Diffusion in the mass spectrometer was minimal due to the extremely short time that the ion traveled before detection.

Good correlation was found between migration times and ionic charges of the species. DS-4 was the first to migrate from the CE column followed by DS-3, DS-2, and DS-1. If the electroosmotic velocity is constant, a plot of electrophoretic velocities (v_b) vs. degree of substitution yields a linear relationship in which the y-intercept equals the electroosmotic flow (v_{eo}) and the slope equals

TABLE 5

CE/MS PERFORMANCE VALUES FOR THE SU-β-CD'S SEPARATION

Substitution Term	Resolution	Experimental Efficiency
DS-4		1396
	1.7	
DS-3		1074
	2.3	
DS-2		1728
	4.5	
DS-1		3171

electrophoretic velocity of an unsubstituted compound. Plotting data from Figures 25 and 26 yield two reasonably linear lines (Figure 27), demonstrating that a constant electroosmotic velocity was maintained throughout the analysis.

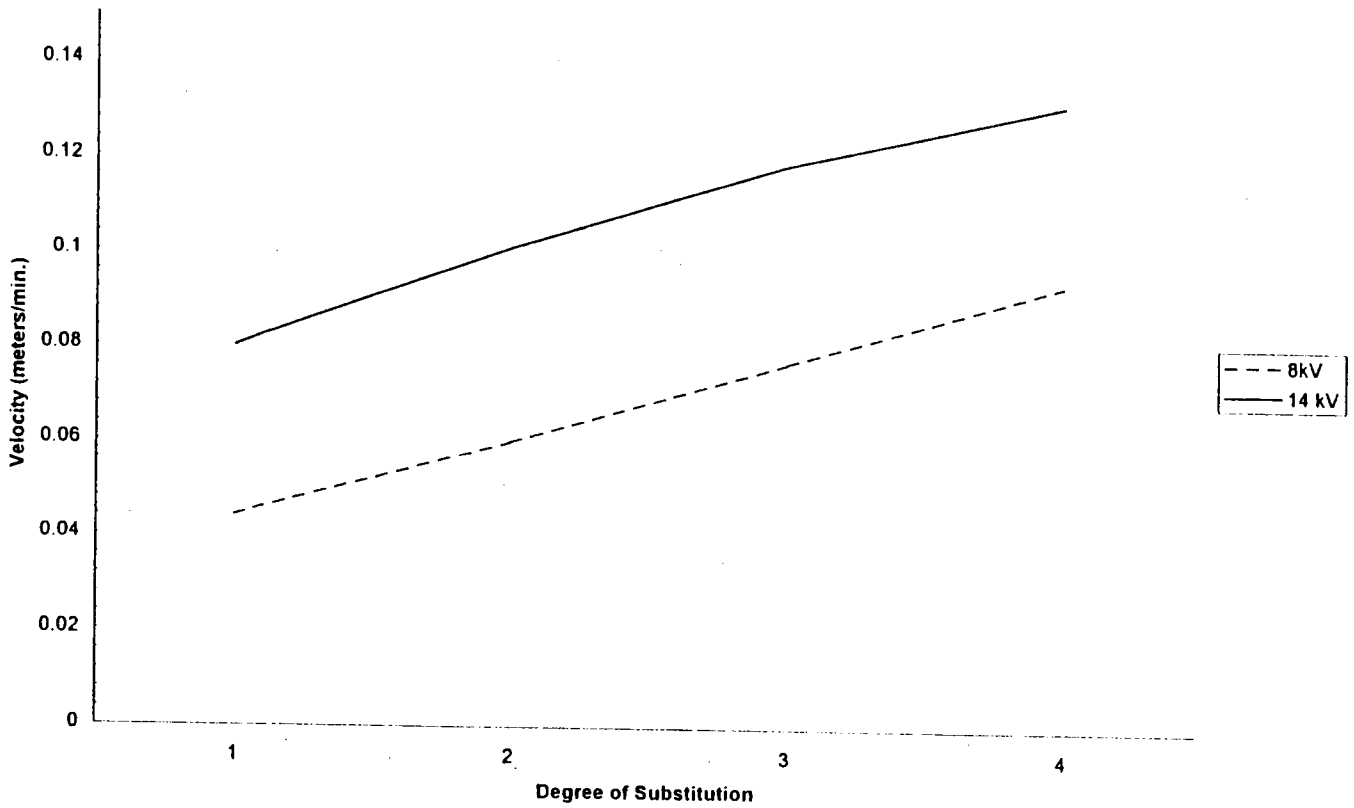

Figure 27. Calculated velocities for the 1- 4 su-β-CDs at -8 kV and -14 kV.

CHAPTER V

CONCLUSION AND SUGGESTIONS FOR FUTURE WORK

The low sheath flow CE/MS electrospray interface design was proven to be a major improvement over conventional electrospray. The dilution of the solute band and introduction of interferences through the sheath flow solution were reduced using the low sheath flow electrospray tip. The tapered tip capillary employed in the electrospray interface was easy to construct and assemble. The modified tip reduced the required sheath flow rate by 50% (2.5 μL/min.), increased the sensitivity for Ho and Pr, and reduced background signals compared to the conventional design. The feasibility of using the modified tip for CE/MS analysis was successfully demonstrated by the separation and detection of β-cyclodextrins and mono-substituted carboxymethyl cyclodextrins, and the separation and detection of a mixture of sulfonated β-cyclodextrins.

Future studies concerning the make-up solution composition would be beneficial. Even lower make-up flow rates may be obtained with different mixtures. Evaluation of capillary coatings to improve conductivity between the applied electrospray voltage and the actual voltage at the electrospray tip would be useful.

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LIST OF REFERENCES

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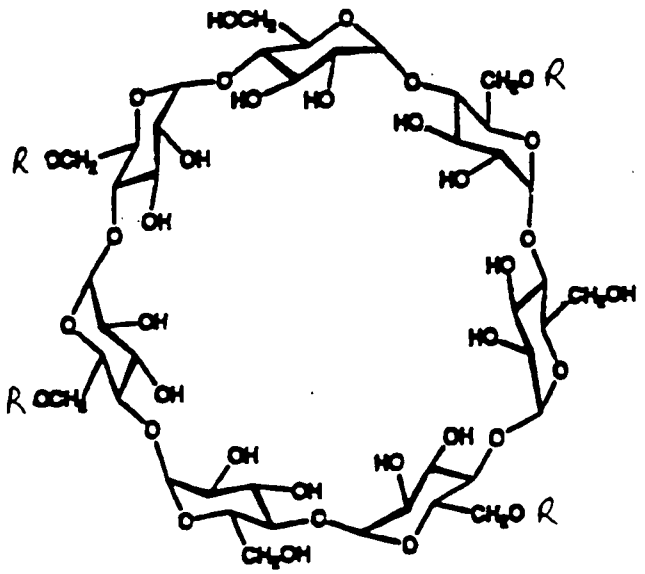
APPENDIX

APPENDIX A

β-CYCLODEXTRINS CHEMICAL STRUCTURE



R = H, - CH_2COOH, - $SO_3^-Na^+$

APPENDIX B

FORMULAS

Theoretical Plate Number (N):

$$N = 5.54\ (t_R/w_{1/2})$$

where: t_R = migration time
$w_{1/2}$ = temporal peak width at half height.

Diffusion Coefficient (D)

$$D = \sigma^2/2\ t_R$$

where: t_R = migration time
σ^2 = standard deviation of the peak in length.

Standard Deviation of the Peak In Length (σ)

$$\sigma = L/(N)^{1/2}$$

where: L = capillary effective length
N = theoretical plate number.

Resolution (R)

$$R = (t_2 - t_1)/\ 0.5(w_2 + w_1)$$

where: t_1 = retention time of component 1
t_2 = retention time of component 2
w_1 = base width of component 1
w_2 = base width of component 2.

VITA

Samuel A. Lewis, Sr. son of John and Loretta Lewis, was born in Grundy, VA on September 7, 1960. He graduated from Abingdon High School in Abingdon, VA in June of 1978. In June of 1988, he graduated from Georgia Southern University with a Bachelor of Science degree in Chemistry. He began graduate school on a part-time basis in August 1993 while employed by Lockheed Martin Energy Systems, Inc.

He married Linda A. Lewis, daughter of Rev. Leonard and Mary Arnold, in March of 1982. He has three children, Mary Beth, fifteen years of age, Samuel Jr. eleven years of age, and Katherine, nine years of age.