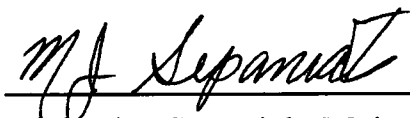


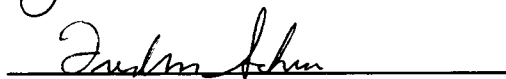
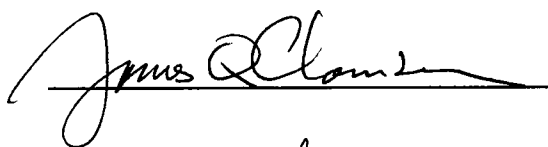
To the Graduate Council:

I am submitting herewith a thesis written by Victoria Tropina entitled "Evaluation of a Separation-Based Fiber-Optic Sensor in a Micellar Electrokinetic Capillary Chromatography Mode of Operation". I 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, with a major in Chemistry.



Michael J. Sepaniak, Major Professor

We have read this thesis and
recommend its acceptance



Accepted for the Council:



Associate Vice Chancellor and
Dean of The Graduate School

**Evaluation of a Separation-Based Fiber-Optic Sensor
in a Micellar Electrokinetic Capillary Chromatography
Mode of Operation**

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Victoria Tropina
August 1998

DEDICATION

This work is dedicated to my parents

Galina and Vasily Tropin

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ABSTRACT

The research presented in this thesis involves the evaluation of a separation-based fiber-optic sensor (SBFOS) for remote analyses, that combines the selectivity of capillary electrophoresis with the sensitivity provided by laser-induced fluorescence detection. The performance of the sensor is shown in a micellar electrokinetic capillary chromatography mode of separation which extends capillary electrophoresis to neutral analytes.

The design and construction of the sensor are demonstrated which is based on a single-reservoir, linear configuration. The gravity-driven hydrostatic flow resulting from nonlevel positioning of the separation capillary ends is reduced by incorporating a narrow-bore restrictor capillary. A single fiber optic is used in the optical detection arrangement of the SBFOS to transmit both the laser excitation and sample fluorescence signal to and from the sensor side of the separation capillary.

The evaluation experiments are performed in a frontal mode of operation which at some point results in the displacement of the micellar phase in the capillary by the sample (reagent depletion). Studies involve varying the composition of the sample matrix with regard to buffer and surfactant, measuring signal over applied voltages in 2.0-4.0 kV range, as well as constructing linear calibration curves for single- and multi-component mixtures.

The results of the SBFOS evaluation include observed separation efficiencies of 10^3 - 10^4 theoretical plates for an 8-cm separation capillary,

migration time and bandfront height reproducibilities of less than 10%, low-mid nanomolar limits of detection, and a linear response over two orders of magnitude in concentration. A preliminary SBFOS-MECC separation and detection of important pollutants, aflatoxins G_1 and G_2 , demonstrates the potential of the sensor for environmental monitoring.

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LIST OF ABBREVIATIONS

CD	cyclodextrin
CE	capillary electrophoresis
CMC	critical micelle concentration
EOF	electroosmotic flow
i.d.	inner diameter
k'	capacity factor
LIF	laser-induced fluorescence
MECC	micellar electrokinetic capillary chromatography
μ_{ep}	electrophoretic mobility
NA	numerical aperture
NBD-Cl	7-chloro-4-nitrobenzofurazan
o.d.	outer diameter
RSD	relative standard deviation
SBFOS	separation-based fiber-optic sensor
SDS	sodium dodecyl sulfate
t_m	retention time of the micellar phase
t_R	retention time of the solute
v_{hy}	hydrostatic flow velocity

CHAPTER I

INTRODUCTION

Fiber optics have provided a significant source of innovation and improvement in the field of analytical chemistry. This is the reason why the application of optical fibers to sensing and measurements has received great attention in recent years [1-4]. Most often, the fiber optic is used as a path to provide the spectroscopic information to the detector. This ability of efficient light transmission over long distances makes it possible to remotely monitor chemical substances in industrial process streams as well as in environmental and biological systems.

For these *in situ* measurements the question of selectivity and sensitivity becomes very important. As a result, fiber optic sensors have been designed to provide selective detection in infrared [5], Raman [6, 7] and surface-enhanced Raman spectroscopy [8], which is due to the unique nature of vibrational spectra. Unfortunately, these techniques show limited sensitivity, especially in comparison with fluorescence-based detection. Laser-induced fluorescence (LIF) is much preferred because of its great gain in sensitivity [9-13], however, the problem of spectral selectivity arises due to the broad-band nature of fluorescence spectra. The situation can be improved if the fluorescence signal results from a reaction between an analyte and some reagent phase immobilized at the fiber's sensing tip. The reagent phases can be chosen from a variety of

components and the reactions can be very selective, as, for example, in the case of chemically selective affinity reagents [9-14] or permeability-selective membranes [15-19]. The main drawbacks of such systems are that they are usually useful for only one particular analyte and can not be reused.

Another possibility to enhance selectivity in fiber optic-based sensing can be provided by chemical separations. For example, potential advantages of capillary electrophoresis (CE) in sensing applications include high separation efficiency, speed, small capillary size, low sample volume, versatility, reusability, remote control and automation.

Research described in this thesis concerns the development and evaluation of an integrated separation-based fiber optic sensor (SBFOS) for remote analyses that incorporates CE, more appropriately a variant of CE referred to as micellar electrokinetic capillary chromatography, directly at the fiber sensing terminus. Since the underlying principle of the sensor is to achieve high selectivity by CE separation and excellent sensitivity by LIF detection, the following sections will briefly discuss the relevant background and theory of these techniques.

1. Capillary electrophoresis (CE) – an overview

1.1. Separation principles and apparatus

Separation by electrophoresis is based on the migration of solutes in an applied potential field [20]. Positive ions under an electric field are attracted by the cathode, and negative ions by the anode. The migration velocity is given as the product of the electrophoretic mobility (μ_{ep}) and an electrical field (E) [21]:

$$V_{ep} = \mu_{ep} \cdot E \quad (1)$$

μ_{ep} is proportional to the size-to-charge ratio of the ions, which is described as:

$$\mu_{ep} = \frac{q}{6 \pi \eta r} \quad (2)$$

where q is the net charge of a solute, r is the ion radius, and η is the solution viscosity.

The net ionic charge can be influenced by the medium's pH as in the case of solutes with acidic or basic functional groups (e.g. proteins). As a result, the net charge on a solute will affect the magnitude and sign of μ_{ep} . Solvation of small ions by solvent molecules and viscosity of the buffer change the conductivity of the solution, and therefore the mobility.

The ionic strength of the buffer can also be an important factor affecting the mobility because it influences the amount of heat generated in an electrophoretic separation.

In capillary electrophoresis, separations are performed in narrow 25-75 μm i.d. fused silica capillaries [22]. Capillary electrophoretic techniques comprise a wide variety of separation methods, thus, extending the applications of CE toward the analysis of large range of compounds from small ions and neutral molecules to peptides, proteins and DNA materials [23].

The basic CE methods include capillary zone electrophoresis (CZE), micellar electrokinetic capillary chromatography (MECC), isotachophoresis (ITP), isoelectric focusing (IEF), and capillary gel electrophoresis (CGE). The separation mechanisms of these modes are based on differential mobility (CZE), hydrophobic/ionic interactions with micelles (MECC), size and charge (CGE), focusing in a pH gradient according to isoelectric point (IEF), or focusing in an ion mobility gradient between leading and terminating electrolytes (ITP) [23].

Figure 1 is a schematic diagram of the basic components of a CE instrument. A narrow-bore, fused silica capillary is filled with running buffer, and its ends are placed in two vials with buffer solution. Platinum electrodes, one of which is grounded and the other is charged, are immersed in the vials to make electrical contact between the high voltage power supply and capillary. Injection of sample in the capillary is accomplished by replacing one of the vials with a sample vial and applying either

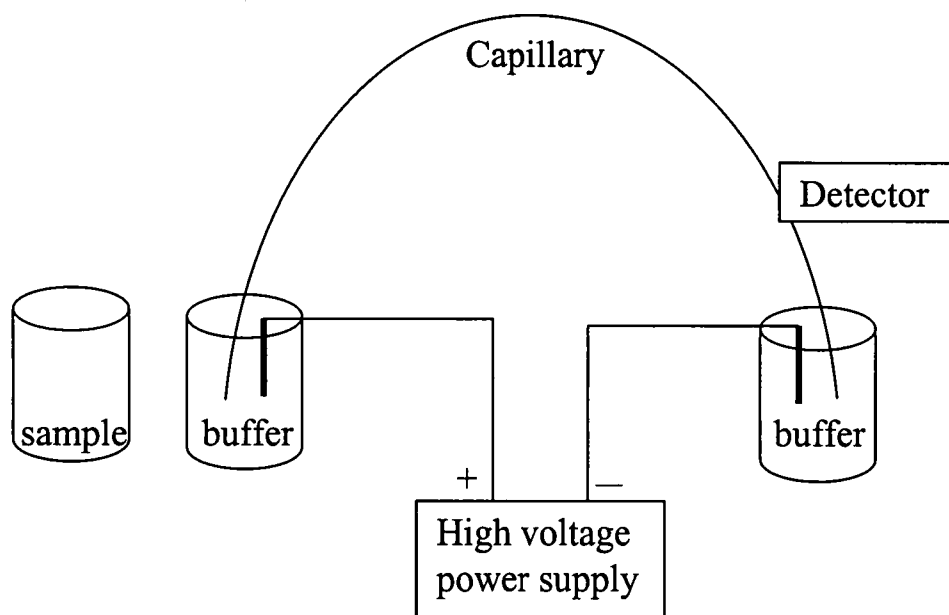


Figure 1. Diagram of a basic system for capillary electrophoresis

a potential field (electromigration injection) or an external pressure (hydrostatic injection). After replacing the sample vial, the potential field is applied across the capillary, and the separation is conducted. In general, because of the cathodic electroosmotic flow (see below), detection is made at the cathode end directly through the capillary wall or by coupling the capillary with other detectors through specially designed interfaces.

After contacting the buffer solution, the acidic silanol groups at the surface of the fused silica capillary undergo ionization, which results in a negatively charged capillary wall. The diffuse layer of hydrated cations is formed next to the wall, which balances the anionic charge. When voltage is applied across the capillary, the cations in the diffuse layer will migrate toward the cathode, causing the bulk solvent within the capillary to move at a constant velocity in the same direction. The process is called electroosmosis, and the electroosmotic mobility (μ_{EOF}) is:

$$\mu_{\text{EOF}} = \frac{\varepsilon \xi}{4 \pi \eta} \quad (3)$$

where ε is the dielectric constant of the medium, η is the viscosity, and ξ is the zeta potential across the solid-liquid interface.

Therefore, within the capillary, the observed migration velocity of ions results from the sum of the electrophoretic velocity and the velocity of electroosmotic flow (EOF):

$$V = (\mu_{\text{ep}} + \mu_{\text{EOF}}) \cdot E \quad (4)$$

As a result, anions will migrate in the same direction as cations if the magnitude of the EOF is greater than the mobilities of anions. Neutral compounds will be carried together by the EOF at its velocity.

1.2. Advantages of CE

The major disadvantage of normal electrophoresis is the generation of heat caused by the applied voltage (E) and the current (I) produced, i.e. power (heat) = $E \cdot I$. This so-called Joule heating becomes a problem because it can cause temperature gradients that increase zone broadening, influence electrophoretic mobilities, and can also result in solvent evaporation. In gel electrophoresis stabilizing media (gel) help to dissipate heat and reduce heat related convective flow. However, at the same time the gel increases the surface area available for solute adsorption and causes eddy diffusion [23]. The important advantage of narrow-bore capillaries is the high electrical resistance of the capillary and the efficient heat dissipation due to an increased surface area-to-volume ratio of these narrow-bore tubes. This allows higher applied voltages, which results in fast, efficient separations, and short analysis time.

In addition to the improved heat dissipation, another advantage of performing electrophoresis in small capillaries is the flat “plug-like” velocity profile of the electroosmotic flow. As discussed earlier, the flow originates at the diffuse region of the double layer. Because of the extremely small size of this layer and uniform distribution at the walls,

there is no pressure drop within the capillary. That is why the velocity profile is nearly constant across the entire diameter of the capillary (Figure 2). Consequently, band broadening due to resistance to mass transfer in the mobile phase is minimized, which contributes to highly efficient electrophoretic peaks. This is in contrast to a laminar or parabolic flow generated by hydrostatic pressure. In this case the velocity in the center of the capillary is greater than that at the edges, which leads to the dispersion of solute bands and lower efficiency.

Electroosmotic flow is also beneficial since cations, neutrals, and anions can be analyzed simultaneously (Figure 3). Cations migrate fastest because they move in the same direction as the EOF, towards the cathode. Neutral species elute together at the velocity of the electroosmotic flow. Anions migrate with the slowest velocity since they are attracted to the anode but can still be transported by strong EOF in the direction of the cathode.

The ability to work with very low sample volume (1 to 10 nL is typical) is another advantage of capillary electrophoretic techniques. This allows analyzing samples which are difficult to prepare in large volumes or studying microenvironments such as a single cell. Buffer is also consumed in very small amounts, and very little waste is generated. A few microliters is usually enough to flush the capillary several times. Thus, expensive buffer additives can be tried to improve selectivity with less cost.

One of the great advantages of CE is its wide application range due

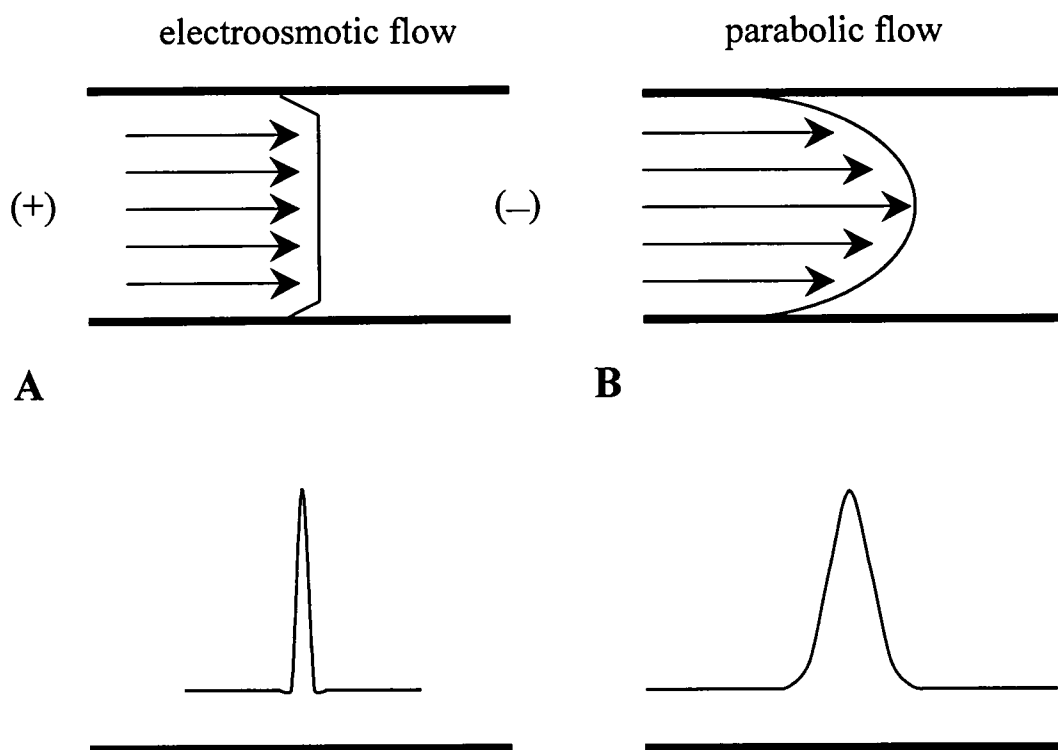


Figure 2. Depiction of (A) electroosmotic and (B) parabolic flow profiles and resulting peak shape

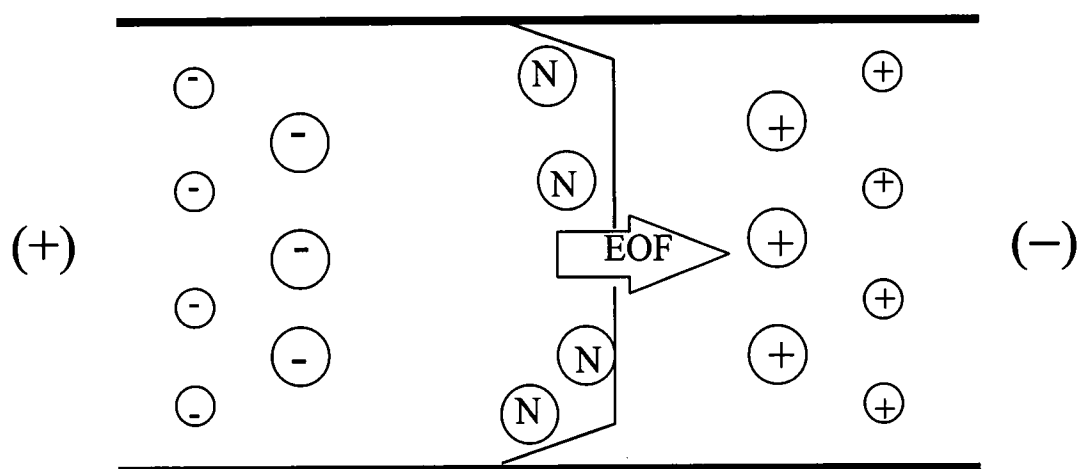


Figure 3. Representation of differential migration of cations, neutrals and anions in the presence of electroosmotic flow

to the numerous modes offering different separation mechanisms and selectivities. CE is effective for separations of inorganic ions, amino acids, pesticides, dyes, surfactants, chiral drugs, vitamins, organic acids, carbohydrates, peptides and proteins, oligonucleotides and DNA fragments, and even single cells and single-enzyme molecules.

All the advantages mentioned above plus simplicity of instrumentation and ease of automation make capillary electrophoresis an important rapidly growing separation technique.

2. Micellar electrokinetic capillary chromatography (MECC)

To extend CE to neutral analytes, surfactants can be added to the mobile phase. This separation technique was introduced by Terabe in 1984 [24] and is called micellar electrokinetic capillary chromatography (MECC). When the concentration of surfactant is above its critical micelle concentration (CMC), aggregates of individual surfactant monomers form the micellar phase. The most common surfactants used in MECC are anionic, e.g. sodium dodecyl sulfate (SDS) with the CMC of 8 mM and the aggregation number of 60. SDS forms negatively charged micelles with the hydrophobic tail groups oriented towards the center, and the charged head groups oriented toward the hydrophilic buffer. Depending on the charge, the micelles migrate with or against the EOF. Anionic SDS migrates against electroosmotic flow, toward the anode. However, if the EOF is of sufficient magnitude (e.g. at neutral or basic

pH), the net micelle movement is in the same direction as the electroosmotic flow, but at a reduced velocity (Figure 4). The slower moving micellar phase acts as a pseudo-stationary phase, and the running buffer represents the mobile phase. The solutes are separated based on their differential partitioning between these two phases [25]. MECC is also used for the separation of ions since it provides additional selectivity to electrophoresis, e.g. for compounds with very similar electrophoretic velocities (the same charges and similar structures).

The use of different surfactants gives significant changes in selectivity. Such important factors as the length of hydrophobic alkyl chain, the electric charges, and the structure of the hydrophilic group can affect the interaction of the analytes with the micellar phase.

Addition of surfactant above the CMC does not change its concentration; however, it leads to higher micelle content. It has been shown that for electrically neutral solutes the capacity factor and retention time increase with increased micelle concentration [23]. Nonionic and zwitterionic surfactants have low conductivities, which is advantageous in terms of low Joule heating and the possibility of using high concentrations of surfactants. However, nonionic surfactants, in contrast to ionic ones, do not separate electrically neutral compounds. The use of ionic surfactants at high concentrations can cause the increase in generated current, and band broadening due to Joule heating.

Interaction of the analytes with the micellar phase are hydrophobic and electrostatic. The more hydrophobic compounds interact stronger with

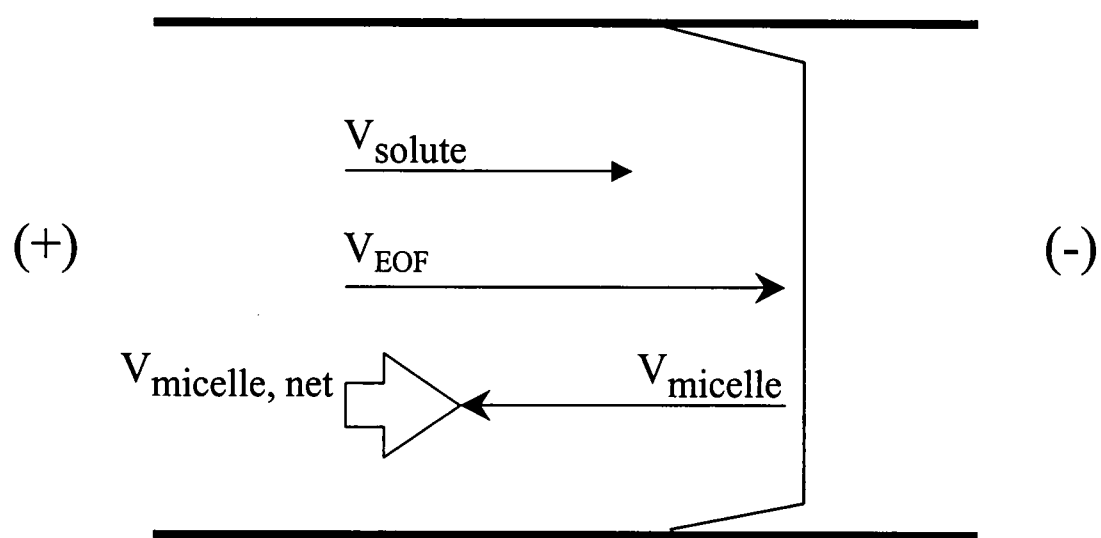


Figure 4. Depiction of migration velocities for electroosmotic flow, solute and anionic micelle in a MECC system

the micelles, and that increases their migration time (t_R). Highly hydrophilic solutes tend to elute with the electroosmotic flow (t_0), while solutes that are totally solubilized by the micelles will elute with the micellar phase (t_m). In contrast to conventional chromatography, the pseudo-stationary phase elutes from the column. This results in a limited elution window for MECC (Figure 5).

Retention times of solutes in MECC are given by equation 5 [24]:

$$t_R = \frac{(1 + k') t_0}{1 + (t_0/t_m) k'} \quad (5)$$

where t_0 and t_m are the retention times of an unretained solute and the micellar phase, respectively. k' is the capacity factor, the ratio of the amount of solute in the micellar phase to the amount of solute in the mobile phase, and is given by

$$k' = \frac{t_R - t_0}{t_0 (1 - t_R/t_m)} \quad (6)$$

If the micellar phase becomes truly stationary, t_m approaches infinity, and both equations 5 and 6 are reduced to the ones used in conventional chromatography.

In MECC, the limited elution window restricts peak capacity, n , of this technique:

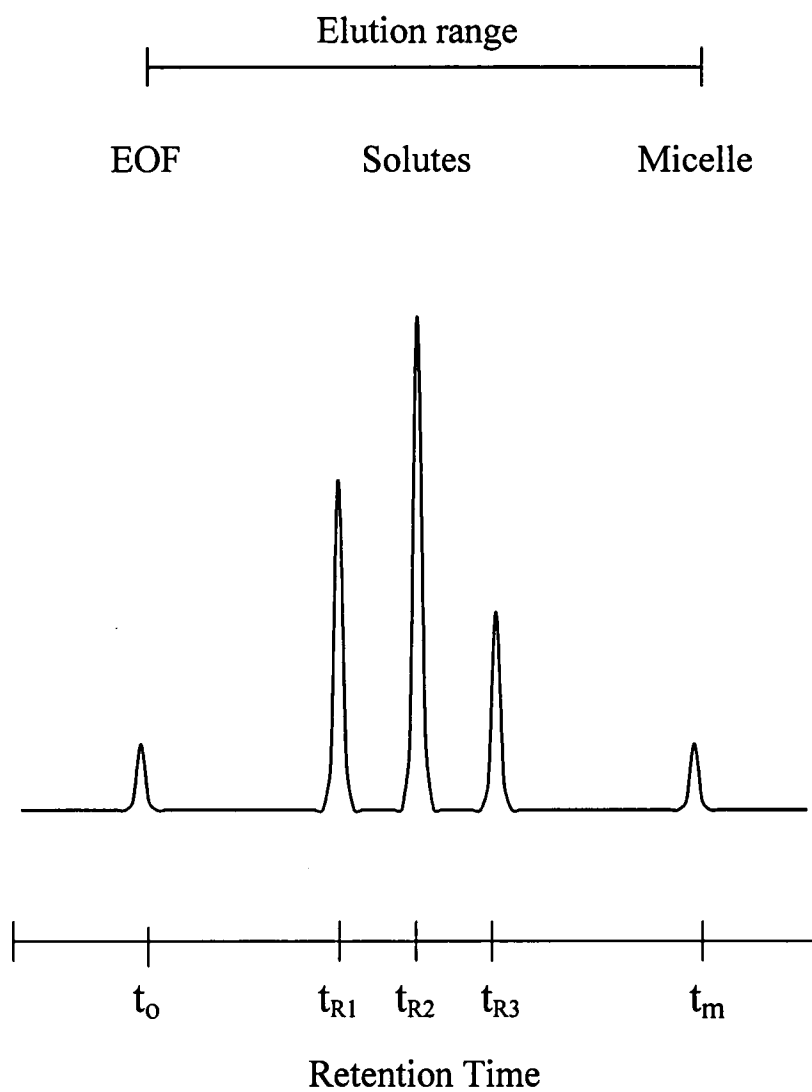


Figure 5. Depiction of a limited elution range in MECC

$$n = 1 + \frac{\sqrt{N}}{4} \ln \frac{t_o}{t_m} \quad (7)$$

where N is efficiency.

Resolution (R) of two analytes in MECC is described by

$$R = \frac{\sqrt{N}}{4} \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{k'_2}{k'_2 + 1} \right) \left(\frac{1 - (t_o / t_m)}{1 + k'_1 (t_o / t_m)} \right) \quad (8)$$

where k'_1 and k'_2 are capacity factors for two analytes, and α is the selectivity factor, the ratio k'_2 / k'_1 . A typical value of the elution range parameter, t_o / t_m , is ~ 0.3 [26], and a maximal resolution is achieved when k' is between one and five [27].

It can be seen from the equation 8 that the last two terms decrease for large k' values and, in this case, late eluting solutes are difficult to resolve. An increase in t_m relative to t_R would reduce k' values, and that should improve resolution of the late eluting components.

The limited elution window (and therefore t_m) can be extended by reducing electroosmotic flow. The EOF depends on the potential difference of the diffuse double layer along the capillary wall (equation 3). Thus, by reducing this zeta potential, the EOF will be decreased. For example, lowering the pH of the mobile phase causes the silanol groups on the capillary surface to be protonated, which decreases the zeta poten-

tial and therefore the electroosmotic flow [28, 29]. The result is the reduction in the net velocity of the micelles towards the detector, which extends the elution range and improves the separation of complex samples. However, there is a possible negative effect of decreasing pH for acid/base solutes. It can adversely change a solute's charge and therefore alter its solubilization and retention characteristics [30].

As an alternative, capillary wall treatment by a silanating agent such as trimethylchlorosilane (TMCS) has been demonstrated [26]. TMCS reacts with the silanol sites on the fused silica surface, and it reduces the electroosmotic flow and increases t_m .

The addition of organic solvents to the running buffer can also extend the elution window. Organic modifiers (e.g. methanol and isopropanol) interact with the negatively charged silanol groups on the capillary wall, reducing the EOF [31, 32]. In addition to the electroosmotic flow, they can also affect the viscosity and dielectric constant of the aqueous mobile phase, which decreases the net velocity of the micelles towards the detector [33]. The problem of using alcohol organic modifiers is the excessive increase in the elution window and therefore extremely long analysis times.

Another organic additive such as acetonitrile does not increase the elution range to the extent the alcohols do [34]. It decreases the polarity of the aqueous mobile phase and increases the time hydrophobic solutes spend in the mobile phase. This reduces the capacity factor and improves resolution. However, it can cause the early eluting peaks to co-elute. As a

solution to this problem and very large elution windows, solvent gradients can be employed [32, 35, 36]. The CMC of the micelles is found to increase with the addition of organic modifiers [25, 32] which concentration should be limited due to the damage of the micelles. They become unstable if the concentration of organic solvent is greater than about 20-30% (v/v).

To improve resolution of analytes eluting with the micelles or solutes that are very closely related, cyclodextrins (α -CD, β -CD, γ -CD) and their derivatives can be added to the MECC mobile phase [37]. These macrocyclic glucopyranose molecules are “tea-cup” shaped, with a void nonpolar cavity and an outer hydrophilic surface. Depending on the geometry, size, and chemical properties, certain solutes form inclusion complexes with the CDs. This selective interaction competes with the solute-micelle associations and improves the separation by changing mobility and retention time.

3. Laser-induced fluorescence detection

Due to the diminutive size of capillaries, the detection method in CE must be sensitive enough to detect the minute amounts of an analyte within the extremely small sample volumes. This must be done without introducing band dispersion. That is why on-column detection is more suitable for capillary electrophoresis and easily implemented. Absorbance and fluorescence detection modes are the most common methods used for

CE [23]. Various electrochemical detection methods and mass spectrometry detectors have also been studied extensively [38-41]. New detectors such as chemiluminescence, refractive index, thermooptical absorbance, and nuclear magnetic resonance detectors were also reported to be feasible for CE detection [42].

UV-visible detection can be applied to a wide spectrum of compounds, including proteins. However, detectibility is limited by short optical path length and poor light throughput in the capillary. That is why the usual concentration detection limit obtained with this method is 10^{-5} M, while fluorescence detection has attained LOD of 10^{-11} M or lower. This is the reason for wide acceptance of fluorescence detection scheme in biochemistry, pharmaceutical, and pharmacokinetics studies as well as biomedical research. Compounds that frequently require this detection mode include amines, amino acids, peptides, proteins, nucleic acids, and carbohydrates [43]. Since most of the compounds have no native fluorescence, various derivatization strategies have been developed [44].

Laser-induced fluorescence (LIF) is much preferred because of its great gain in sensitivity. Excitation radiation from a laser is focused onto a section of the capillary where the polymer coating has been removed. The resulting fluorescence is collected at an angle of 90° relative to the excitation beam. It is the unique properties of laser radiation that lead to improved performance for fluorescence detection [45].

Laser intensity gives higher sensitivity because in fluorescence, the greater the excitation source intensity (I_0), the higher the resulting emission signal (I_f) as seen below:

$$I_f = I_0 \cdot \phi_f \cdot k \cdot a \cdot b \cdot c \quad (9)$$

where ϕ_f is the fluorescence quantum efficiency of the solute, k is the fraction of emitted fluorescence that is detected, a is absorptivity, b is path length, and c is concentration. Thus, the high output powers of lasers produce larger signals. Equation 9 is only valid for small total absorbances.

Lasers emit radiation in a highly directional collimated beam with low divergence, which permits tight focusing into the small detection volumes that are typical for narrow-bore capillaries. This increases the incident intensity, I_0 , and therefore the fluorescence signal, and also gives a possibility to decrease specular scatter. Monochromaticity of laser beam helps to minimize background due to scattered light from Rayleigh and Raman processes by reducing the width of these bands.

Due to temporal coherence, pulsed lasers are able to deliver high photon fluxes in a short well defined period of time, and in pulses short compared to fluorescence lifetimes. It can be beneficial to fluorescence detection if the pulsed laser excitation is combined with time resolved detection. By observing emission at a set interval of time after excitation, fluorescence can be distinguished from Rayleigh and Raman scatter which are essentially coincident with the laser pulse and contribute to

background. Many lasers are inherently plane polarized because of the optical design. Since Rayleigh and Raman scattered light is generally polarized, it can be better rejected.

The overview presented in the above sections clearly demonstrates that the use of narrow-bore fused silica capillaries with laser-induced fluorescence detection combines high separation, selectivity and extreme sensitivity.

CHAPTER II

EXPERIMENTAL

1. Chemicals

Methylamine, n-propylamine, and n-hexylamine were obtained from Fisher Scientific (Fair Lawn, NJ). Ethylamine, n-butylamine, and SDS (at 99% purity) were purchased from Sigma Chemical (St. Louis, MO), as were aflatoxins G₁ and G₂. NBD-Cl (7-chloro-4-nitrobenzofuran) was acquired from Aldrich Chemical (Milwaukee, WI), and γ -cyclodextrin from Cyclodextrin Technologies Development, Inc. (Gainesville, FL). Methanol used was reagent grade (Burdick & Jackson, Muskegon, MI).

Buffer reagents (disodium hydrogen phosphate and sodium tetraborate) were purchased from Baker (Phillipsburg, NJ). Water used to prepare the buffer and all other aqueous solutions was purified with a Milli-Q+ filtration system (Millipore, Bedford, MA). The standard running buffer solution (pH 9) was composed of 10 mM Na₂HPO₄ and 6 mM Na₂B₄O₇. To perform MECC, it was made 50 mM in SDS. For some studies (e.g. in the derivatized amines sample preparation), the running buffer was varied in composition from 5/3 to 15/9 mM phosphate/borate.

Non-fluorescent alkylamines were derivatized with NBD-Cl which is not fluorescent of itself but yields highly fluorescent NBD-amines [46].

Derivatization was performed through mixing the amines with excess of NBD-Cl, followed by 10-fold dilution with the buffer solution. Stock solutions (1×10^{-3} M) were stored in glass vessels, wrapped in aluminum foil, and refrigerated. Assuming complete derivatization, NBD-amine solutions ranged from 5×10^{-8} to 5×10^{-4} M. Dilutions were performed with the running buffer solution, in some cases modified with the micelles.

Aflatoxin solutions (1 mg/ml) in acetonitrile were prepared by injecting the appropriate amount of the solvent into septum vials containing solid aflatoxin. Stock solutions (1×10^{-4} M) were made by dilution with the buffer, stored in the dark, and refrigerated. Samples of the aflatoxins G₁ and G₂ were diluted using the standard 10/6 mM phosphate/borate running buffer with 50 mM SDS and 15% methanol. Concentrations of the aflatoxins ranged from 2×10^{-5} to 5×10^{-5} M. When separating the aflatoxins, the aforementioned running buffer also contained 7 mM γ -CD.

Safety procedure

Taking into account the extremely toxic nature of aflatoxins, disposable latex gloves were worn at all times when preparing or handling solutions. Bleach (NaOCl, 5%) was used to clean all working areas and to dispose waste solutions.

2. The separation-based fiber-optic sensor (SBFOS)

Figure 6 depicts the SBFOS in a compact, linear configuration. A single optical fiber was employed for both excitation and emission transmission, and it probed the solution at the exit of a short capillary (Polymicro Technologies, Phoenix, AZ). To position the fiber optic on-axis with the separation capillary, they were placed in the two opposing ports of a non-metallic, low-volume PEEK tee (Upchurch, Model P712, Oak Harbor, WA). The third port was used to house the restriction capillary as well as flush the sensor. The side-arm restrictor capillary was employed to offset gravity-induced hydrostatic flow resulting from nonlevel positioning of the ends of the separation capillary.

All three ports of the PEEK tee were provided with finger-adjustable flangeless fittings. Each fitting had a flat-edged ferrule which fitted squarely against the bottom of the tee port well. The fiber optic and the separation and restriction capillaries extended from the respective fittings into the narrow-bore channels at the center of the tee which were of 0.02-in. diameter and 0.19-in. length.

Prior to assembling the sensor, the fiber optic and capillaries were polished to an even surface with lapping paper. They were also cemented (5-min epoxy, Devcon) within 1/16-in. o.d. PEEK tubing (Upchurch) to provide air-tight coupling to the flangeless ferrules.

For all studies, the length of the separation capillary was 8 cm (50 μm i.d., 365 μm o.d.), and that of the restrictor was 5 cm (25 μm i.d.,

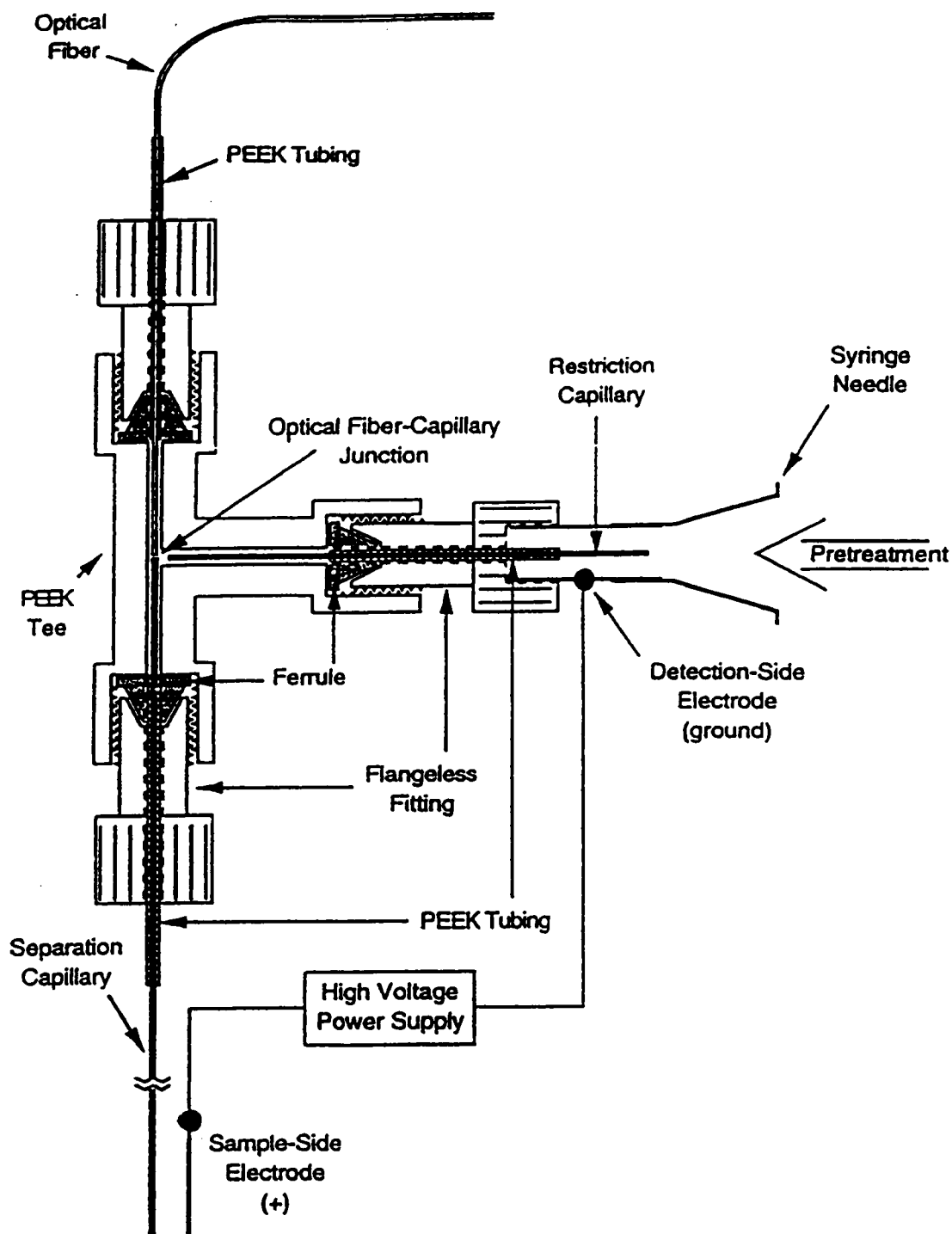


Figure 6. Depiction of the SBFOS

365 μm o.d.). The type of the fiber optic was varied between the studies of the derivatized amines (FSU100120) and aflatoxins (FVP100110125), with the dimensions being kept the same (1 m x 100 μm core diameter). The numerical apertures (NA) were 0.66 and 0.22 for the derivatized amine and aflatoxin studies, respectively. Since the fiber optic was smaller in outer diameter than 0.01-in. i.d., 1/16-in. o.d. PEEK tubing, the end to be inserted into the SBFOS was affixed within the bore of a 8 cm section of capillary tubing (180 μm i.d., 400 μm o.d.).

The flangeless fitting of the side port was furnished with a 16-gauge syringe needle, to accommodate a syringe during flushing or filling the sensor. During separation this fitting was replaced by a modified one which, in addition to the syringe needle, also housed the restriction capillary. The reduced diameter of the restrictor (25 μm i.d.) served to reduce the gravity-driven hydrostatic flow. Otherwise, the applied electric fields needed to offset this flow would have resulted in excessive joule heating. The syringe needle acted as the detection-side electrode set at ground during sensor operation, and it was left open to the atmosphere. Residual liquid in the bore of the syringe needle fitting covered the outside end of the restrictor capillary, thereby completing the circuit between the detection-side and sample-side electrodes. A platinum wire, electrically isolated from the sensor and dipped in the sample, served as the sample-side electrode. It was set at positive bias voltages for all trials using a 20-kV high-voltage power supply (Hipotronics, Brewster, NY).

3. Apparatus (optics and instrumentation)

Figure 7 illustrates the single fiber-based optical detection system used for the SBFOS. A laser excitation beam was directed through the hole (2 mm diameter) of a plane mirror into a sensor-coupling lens which focused the laser beam on to the fiber optic of the SBFOS. The same fiber transmitted sample fluorescence signal from the sensor, which was then collimated by the lens. This collimated signal beam was reflected 90° off axis by the mirror (with the hole), oriented at 45° with respect to the excitation beam axis. An objective lens focused the signal beam onto an uncooled photomultiplier tube (PMT) detector (RCA, Model IP-28, Lancaster, PA). Right in front of the PMT, an analyte emission cut-on filter was inserted into the signal beam to reject Rayleigh scatter. Photocurrents from the PMT were amplified by a picoammeter (Keithley, Model 485, Cleveland, OH), and electrochromatograms were directly recorded with a strip chart recorder (Kipp and Zonen, Model BD 40, Delft, The Netherlands).

The laser was varied between the derivatized amine and aflatoxin studies, which required some changes in the general optical system. In the case of the derivatized amines, a 10-mW argon ion laser (Cyonics, Model 2201, San Jose, CA) was used with a 488-nm excitation bandpass filter and a 500-nm analyte emission cut-on filter. The visible excitation allowed the use of a 40 $\times$, 0.65 NA objective lens (Newport, M-40X, Irvine, CA) which coupled very efficiently with the 0.66 NA optical fiber of the sensor. Precise throughput optimization was enabled with an optical

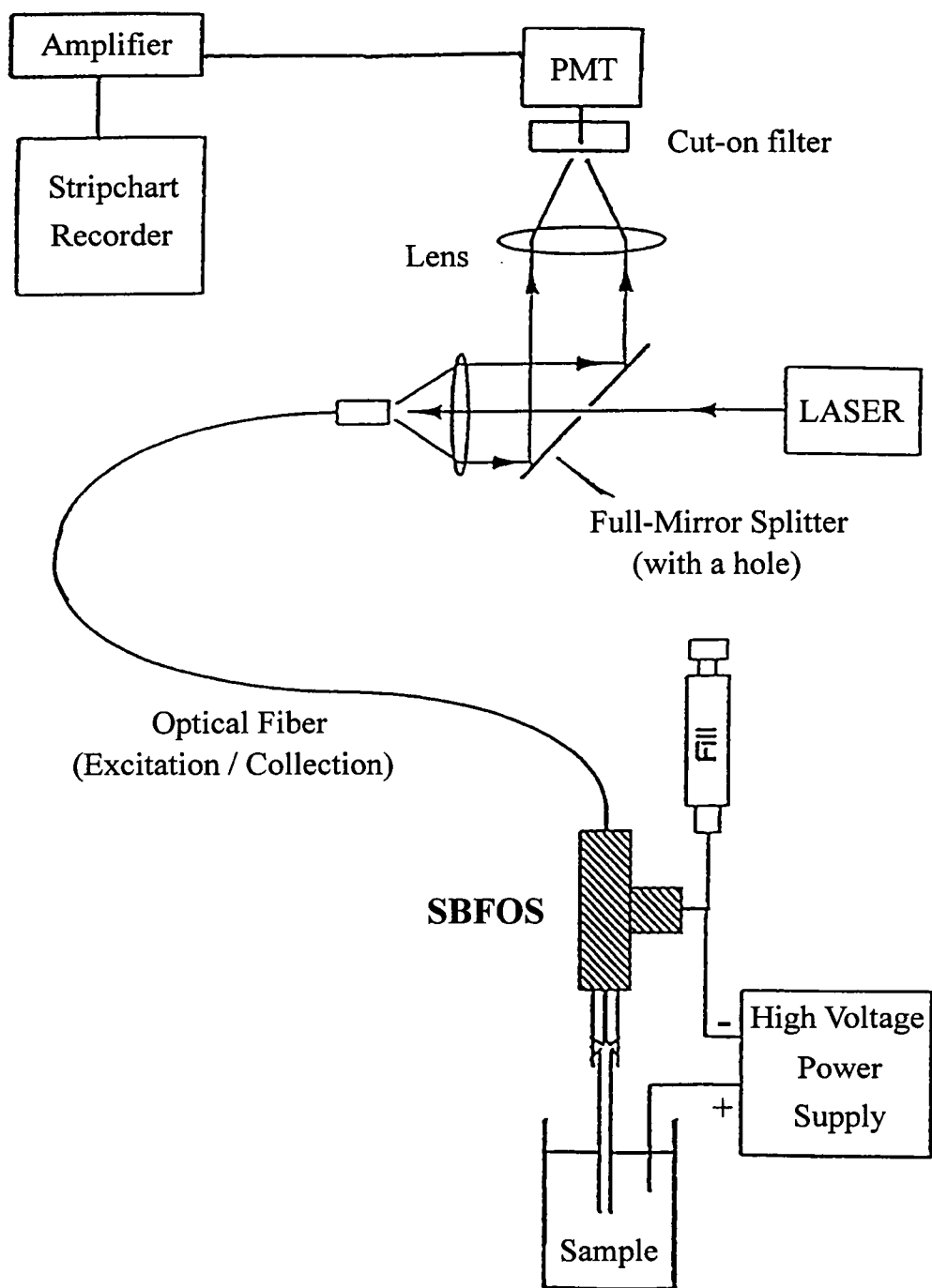


Figure 7. Diagram of the single fiber-based optical detection system

fiber coupler (Newport, Model F915-T).

For the aflatoxin experiments, a 20-mW He-Cd laser (Omnichrome, Series 74, Chino, CA) was used with a 325-nm excitation bandpass filter and a 400-nm analyte emission cut-on filter. Unfortunately, the ultraviolet source precluded the use of a tightly focusing, sensor-coupling objective lens. Instead, a plano-convex 1-in., f/1.5 quartz lens (Edmund Scientific, Barrington, NJ) was employed in conjunction with the 0.22 NA fiber. An x, y, z fiber-optic positioner (Newport, Model FP-1) facilitated throughput optimization.

4. Optical alignment and the sensor operation

Before conducting the experiments, the optical system was optimized to increase signal-to-noise ratio. First, the coupling of the laser excitation beam to the fiber was adjusted by visually observing the best light intensity at the distal end of the fiber (taken out of the sensor). Second, to mimic the fluorescence signal from the sensor, another laser was used to transmit radiation into the system from the distal end of the fiber. This helped to adjust the lenses and mirror, which provided the proper alignment with the detector. Third, the sensor was filled with a dye solution, and a signal level was optimized by further positioning of the optical components.

The pretreatment of the sensor was performed with the open-bore syringe needle housed in the side port fitting. Before each trial, the sensor was rinsed with 0.1 M NaOH and then with running buffer. Another

fitting modified with the needle and restriction capillary was carefully filled with the running buffer completely covering the restrictor end. This fitting was placed in the side port, replacing the one for flushing.

Samples were contained in plastic microcentrifuge vials. Applied voltages for the sensor separations varied from 2.0 to 4.0 kV. To initiate frontal mode operation, the sensor was immersed in the sample, along with the electrode which was set at positive bias. Upon applying the voltage, the sample was continuously injected in the separation capillary, where the sample components migrated toward the fiber optic at characteristic velocities.

Each component was detected as a bandfront, which produced a sharply increased signal, then remaining constant. Therefore, a mixture electrochromatogram appeared as a staircase pattern. The plate count (N) was determined from electrochromatograms using the equation $N = 16(t_R/W)^2$, where the bandwidth (W) corresponds to the rise time of the bandfront between the baseline and the plateau. The retention time (t_R) is measured from the moment of applying the voltage to the point of half-maximum height for the observed bandfront. To estimate resolution (R) between two solutes, the equation $R = 2\Delta t/(W_1 + W_2)$ was employed, where Δt is the difference in retention times, and W_1 and W_2 are the bandwidths of the two bandfronts.

CHAPTER III

RESULTS AND DISCUSSION

1. Preliminary studies and encountered problems

In order to choose optimal conditions for the work with the sensor, conventional MECC separations of neutral derivatized amines were performed using the CE-LIF detection system [25]. To mimic the sensor, the detection zone of the capillary (46.5 cm long) was positioned close to the inlet, at 8 cm. Figure 8 demonstrates frontal and zonal mode of separation of the derivatized NBD-amines which are highly fluorescent and known to be good model compounds.

In conventional MECC, the elimination of gravity-driven hydrostatic flow is accomplished by placing inlet and outlet of the capillary at equal heights. This is not possible in case of the SBFOS, where the sample is generally located 8 cm below the sensor-side reservoir. To observe the effect of the hydrostatic flow which would be expected in the SBFOS, again a short effective capillary length was used, with elevating the outlet end of the capillary. Figure 9 shows the increase in retention time and decrease in bandfront sharpness as the outlet of the capillary is raised 8 cm. With the SBFOS, hydrostatic flow should be reduced by using a narrow-bore restriction capillary.

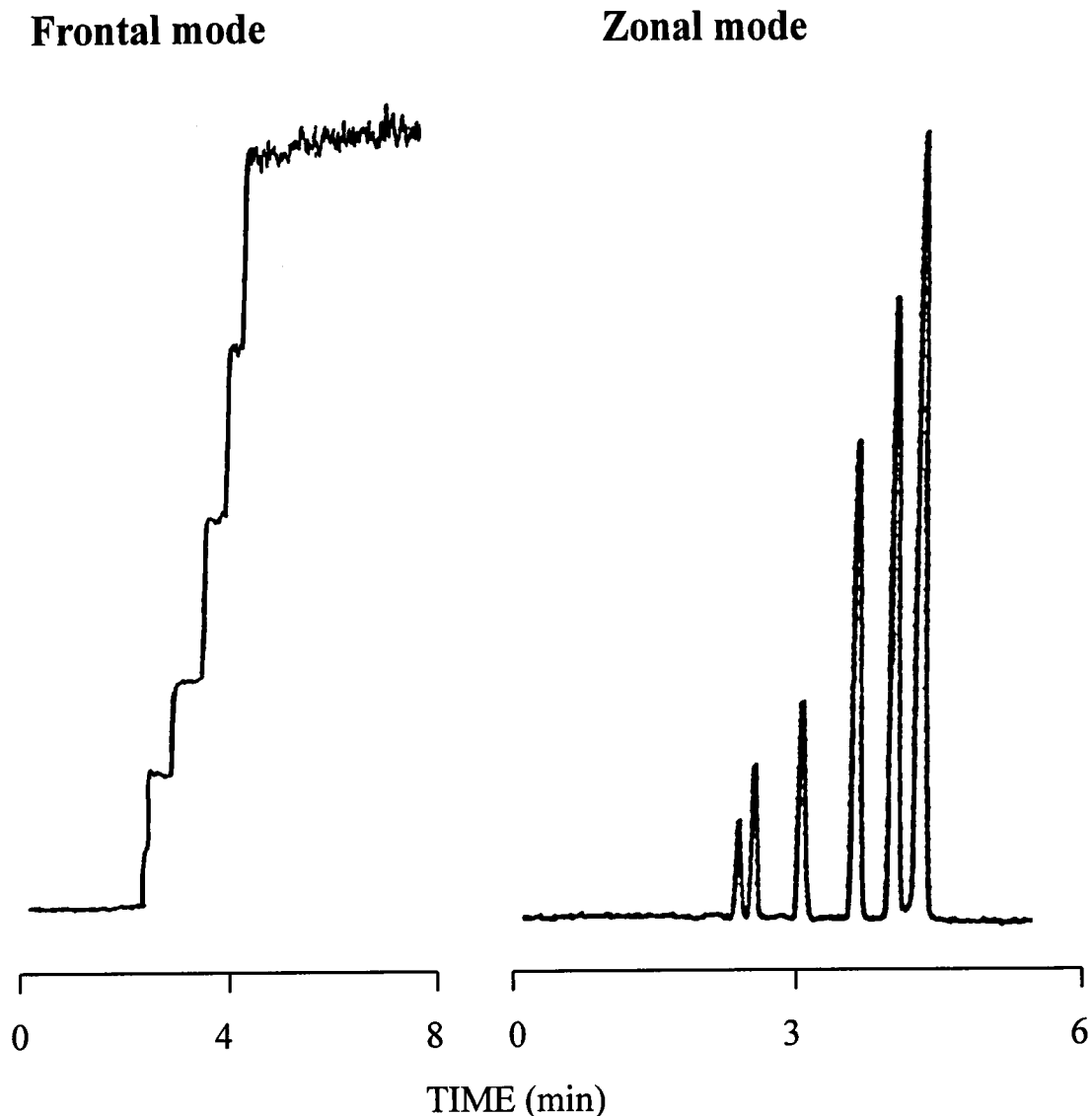


Figure 8. Frontal and zonal mode of conventional MECC separation of the five-amine mixture in the running buffer with SDS

Elution order: 1×10^{-5} M NBD-methylamine, -ethylamine, -n-propylamine, -n-butylamine, -n-hexylamine. Conditions: 50- μ m-i.d. $\times$ 46.5-cm capillary (effective length = 8 cm); applied voltage, 10 kV (negative polarity); mobile phase composition, phosphate/borate (pH 9) running buffer with 0.08 M SDS; detection, LIF using Ar^+ laser excitation.

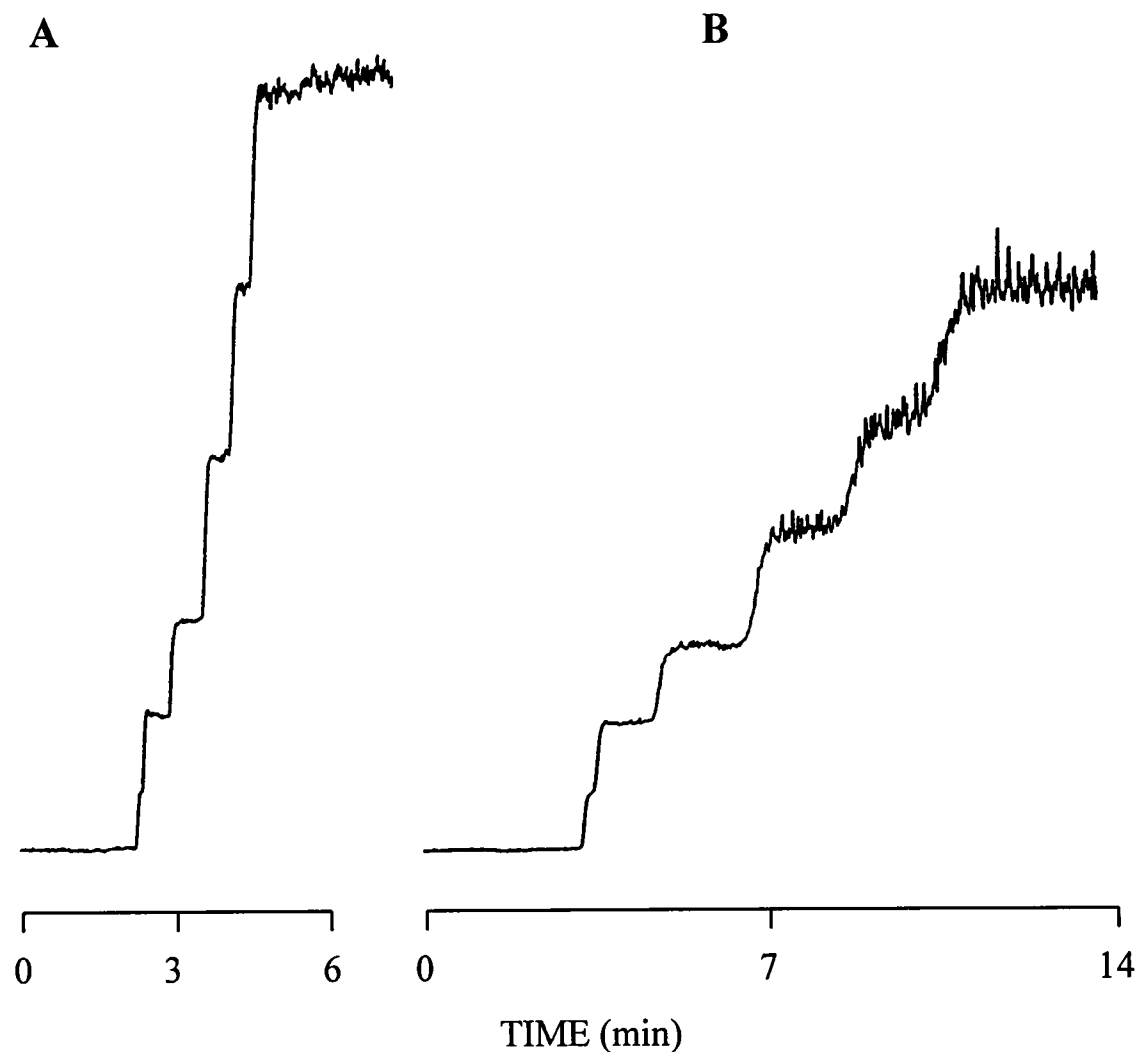


Figure 9. Hydrostatic effect on conventional MECC separation of the five-amine mixture in running buffer with SDS

(A) Equal heights and (B) 8-cm height difference between the inlet and outlet ends of the capillary. Elution order: 1×10^{-5} M NBD-methylamine, -ethylamine, -n-propylamine, -n-butylamine, -n-hexylamine. Conditions: 50- μ m-i.d. $\times$ 46.5-cm capillary (effective length = 8 cm); applied voltage, 10 kV (negative polarity); mobile phase composition, phosphate/borate (pH 9) running buffer with 0.08 M SDS; detection, LIF using Ar^+ laser excitation.

After preliminary studies had been done, the experiments with the sensor were performed. The purpose of the work presented in this thesis was to completely evaluate the SBFOS in the MECC mode of operation. However, at the beginning it was not easy to get the sensor to work properly, which mainly depended on how well it was fabricated and manipulated.

The problems seemed to arise from the hydrostatic flow in the SBFOS capillary. The use of a narrow-bore restrictor capillary in the side port of the sensor should reduce gravity-driven hydrostatic flow resulting from nonlevel positioning of the inlet and outlet ends of the separation capillary. In addition to gravity-driven hydrostatic flow, it is possible to build pressure in the sensor, due to the evolution of electrolysis gases or incomplete dissipation of pressure applied during rinsing steps. A larger volumetric flow rate in the separation capillary than in the restrictor may also contribute to a build-up of pressure in the PEEK tee, which can produce a detrimental hydrostatic flow.

During the construction of the sensor, the spacing between the capillary and the optical fiber must be made negligible to obtain relatively sharp bandfronts. If an extra-column dead volume is created, significantly broaden bandfronts will result, giving poor efficiency. On the other side, a tight optical fiber-separation capillary junction produces an extremely small dead volume, which may result in rapid build-up of pressure in the tee. Under such conditions, after applying the voltage, bandfronts were observed to migrate up the separation capillary, then turn around and

move downward under the influence of the opposing hydrostatic flow.

Since the volume in the center of the sensor tee is also occupied by the restrictor capillary assembly, the position of the restrictor in the side-port fitting is important. Experiments with varying this position were performed, which helped to find the optimal position. After the above-mentioned technical difficulties had been overcome, the work on the SBFOS evaluation was continued.

2. Effect of applied voltage on retention times

The SBFOS sensor should be operated at applied voltages that do not exceed the ability of the system to dissipate heat. Figure 10 demonstrates the effect of increasing voltage on retention times of the test solutes.

Normally in CE, band velocity is directly proportional to applied voltage (equation 4). Therefore, as the voltage increases, the solutes spend less time in the separation capillary. This relationship is observed until the voltage and current are too large, and the power generation exceeds heat dissipation. Under these conditions, the temperature of the running buffer increases, viscosity decreases, and migration rates increase (see equations 2 and 3).

Figure 10 illustrates the inverse relationship between applied voltage and retention time; however, the nonlinearity of the plots at higher

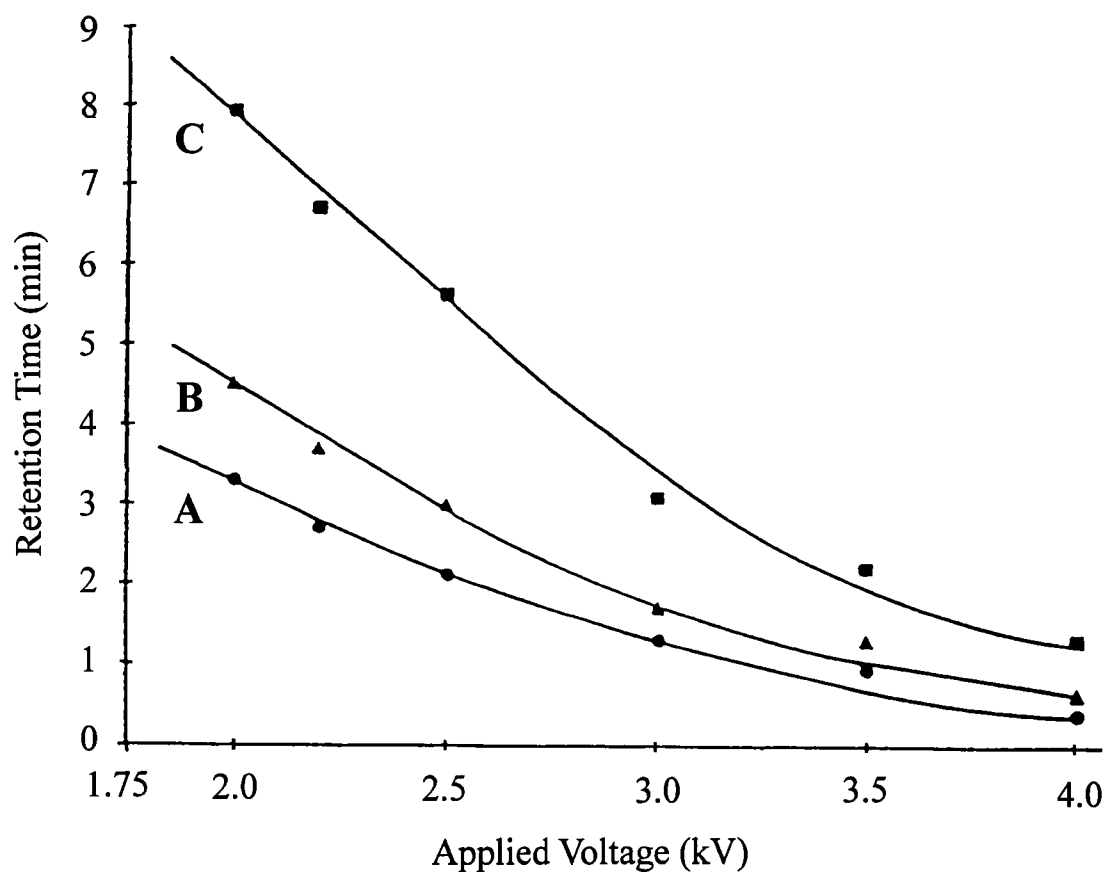


Figure 10. Plot of retention time versus applied voltage for the three-amine mixture in running buffer (without SDS)

(A) 4×10^{-4} M NBD-methylamine, (B) 3×10^{-4} M NBD-ethylamine and (C) 1×10^{-4} M NBD-n-propylamine. Conditions: 8.0-cm $\times$ 50- μ m-i.d. separation capillary and 5-cm $\times$ 25- μ m-i.d. restrictor capillary; mobile phase composition, phosphate/borate (pH 9) running buffer with 0.05 M SDS; detection, LIF using Ar^+ laser excitation.

voltages is probably due to hydrostatic flow (see below), rather than capillary heating. The actual field strength in the separation capillary was not possible to calculate accurately because the applied voltage was divided across the separation and restriction capillaries plus the optical fiber-separation capillary junction.

3. Hydrostatic flow effect on retention times

When hydrostatic flow (ν_{hy}) is present, the retention time of a neutral analyte in a SBFOS-MECC system can be expressed by

$$t_R = \frac{L^2 (1 + k')}{V (\mu_m k' + \mu_{eof})} + \frac{L}{\nu_{hy}} \quad (10)$$

where L is the length of the separation capillary, k' is the MECC capacity factor, V is the voltage dropped across the separation capillary, and μ_m is the net mobility of a micelle.

With the sample-side electrode set at positive bias and the vertical position of the capillary in the sensor, μ_m and μ_{eof} are positive, producing upward flow, while negative ν_{hy} creates downward flow. The use of the 5 cm $\times$ 25 μ m i.d. restriction capillary should decrease ν_{hy} about 2.5-fold in the 8 cm $\times$ 50 μ m i.d. separation capillary. Close positioning of

the optical fiber against the capillary may further reduce the hydrostatic flow.

In spite of the decrease in v_{hy} , a constant gravity-driven hydrostatic flow will be generated due to the 8-cm height difference between the inlet and outlet of the capillary. It is evident from equation 10 that increases in voltage decrease the magnitude of the first term, thus v_{hy} becomes important at higher voltages and will largely determine t_R . This is seen in Figure 10.

Figure 11 demonstrates that with increasing voltage, differences in retention time between the NBD-amines are reduced, and retention times eventually level off. It was estimated that when going from 2 to 4 kV, the resolution between all test solutes diminishes almost threefold. Nevertheless, the bandfronts for each of the mixture components can be distinguished even in the 4-kV case.

Increasing the applied voltage changes efficiency as well. At 2 kV, the plate count for the NBD-ethylamine bandfront in Figure 11 is approximately 1.5×10^3 plates, which is roughly an order of magnitude less than would be expected in conventional frontal MECC. The efficiency at 4 kV degrades several-fold, probably due to heating effect and thermal-related dispersion. However, it is reasonable to expect the hydrostatic flow to be an important factor in producing the relatively poor efficiency, especially at the lower voltages.

Downward hydrostatic flow might distort the expected plug-like flow

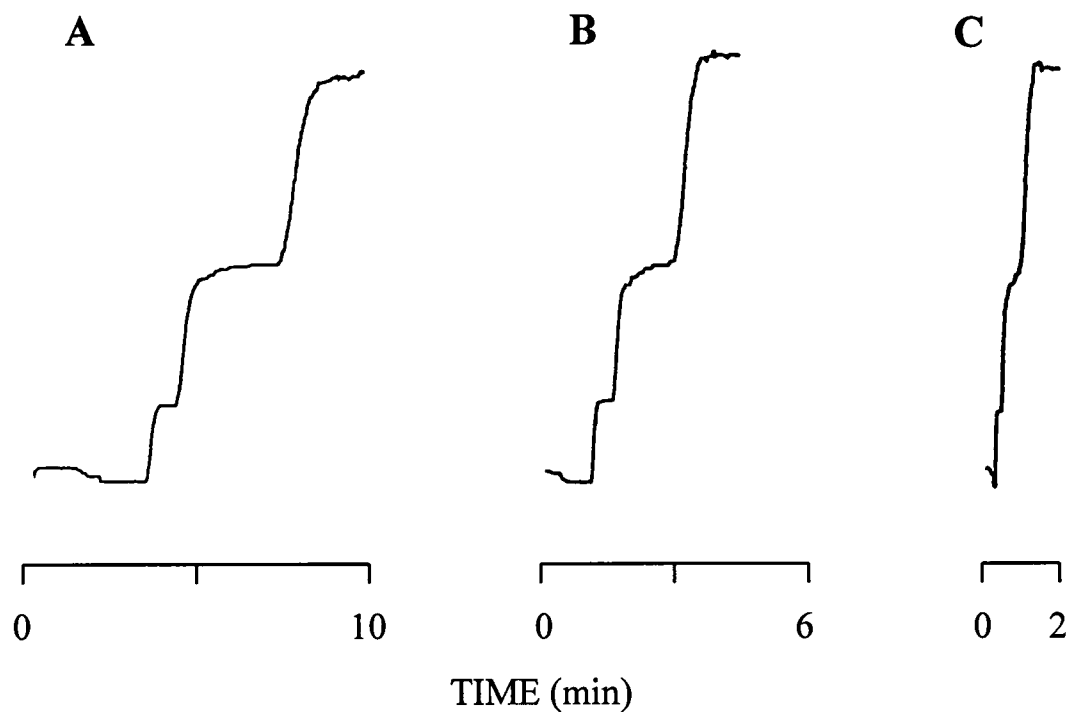


Figure 11. Frontal SBFOS separations of the three-amine mixture in running buffer at applied voltages of (A) 2.0, (B) 3.0, (C) 4.0 kV

Elution order: 4×10^{-4} M NBD-methylamine, 3×10^{-4} M NBD-ethylamine, and 1×10^{-4} M NBD-n-propylamine. Conditions: 8.0-cm $\times$ 50- μ m-i.d. separation capillary and 5-cm $\times$ 25- μ m-i.d. restrictor capillary; mobile phase composition, phosphate/borate (pH 9) running buffer with 0.05 M SDS; detection, LIF using Ar^+ laser excitation.

profile in the separation capillary as shown in Figure 12. The contribution of ν_{hy} to the plate height ($H_{\nu_{hy}}$) for an analyte may be approximated by

$$H_{\nu_{hy}} = \frac{(1 + 6k' + 11k'^2)}{(1 + k')^2} \frac{d_c^2 \nu_{hy}}{96 D_m} \quad (11)$$

Assuming a diffusion coefficient (D_m) of 1×10^{-5} cm²/s and a k' of 0.5 for NBD-ethylamine, and given that the capillary diameter (d_c) is 0.005 cm, $H_{\nu_{hy}}$ (in cm) is roughly given by $0.078 \nu_{hy}$.

A total observed plate height, H_{obs} , for the NBD-ethylamine band-front at 2 kV (Figure 11) is about 0.0053 cm. If the data for the NBD-ethylamine plot in Figure 10 are fitted to a linear form of equation 10, ν_{hy} is estimated to be approximately 0.042 cm/s. This gives a $H_{\nu_{hy}}$ of 0.0033 cm, which is more than 60% of H_{obs} . Although this rough estimation is based on many assumptions, it shows a significant contribution of the dispersion by ν_{hy} to the observed plate height.

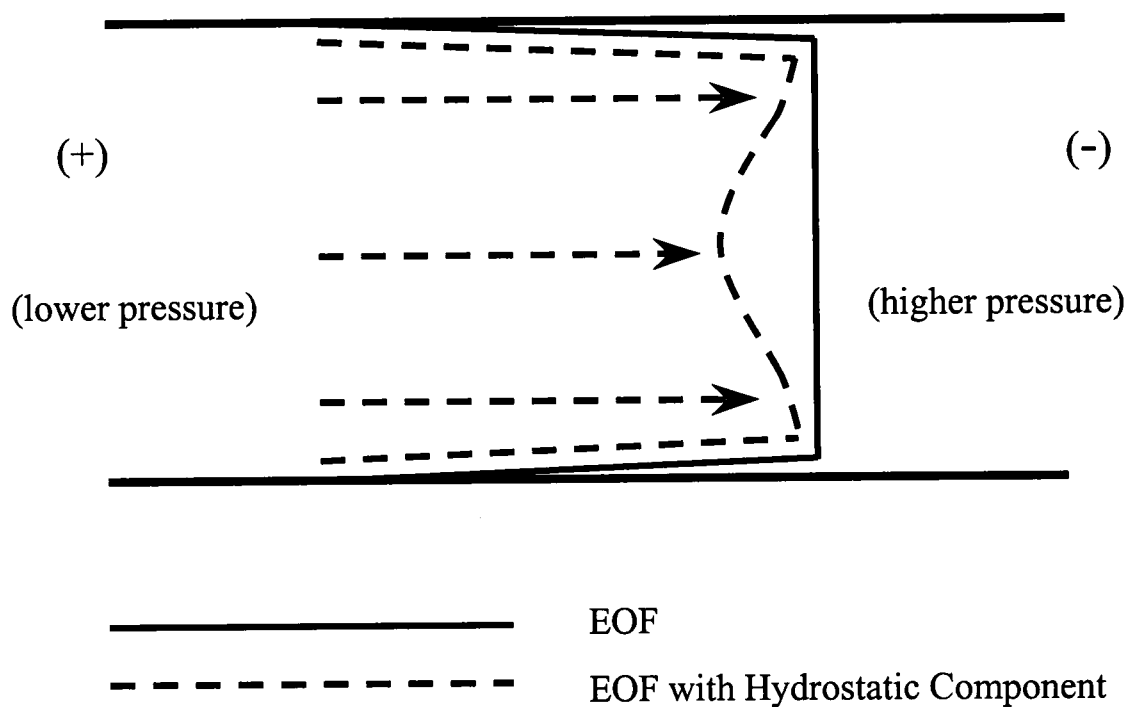


Figure 12. Depiction of flow profile in the SBFOS separation capillary based solely on EOF and in the presence of a hydrostatic counterflow

4. Influence of sample buffer concentration and surfactant in sample on SBFOS performance

The integration of capillary electrophoresis with fiber-optic sensing in the SBFOS offers the potential utility of the sensor for remote analysis. Since sensing applications involve in situ measurements, the ionic strength and composition of real sample solution will not be identical to that of the mobile phase in the sensor. This is an important factor in maintaining high separation efficiency. For example, in conventional zonal CE, differences in sample and running buffer conductivity can cause peak shape distortions which contribute to band dispersion. Samples with higher conductivities than the running buffer result in fronted peaks. Conversely, if the solute zone has a lower mobility than the running buffer, peak tailing occurs.

To observe the possible effects of different sample and running buffer compositions, two kinds of experiments were performed. The results of varying sample buffer concentration and the presence/absence of surfactant in the sample appear in Tables I and II, respectively.

First, three samples of NBD-amine mixture were prepared with 5/3, 10/6 and 15 mM Na_2HPO_4 /9 mM $\text{Na}_2\text{B}_4\text{O}_7$ buffer concentration. Since the standard running buffer solution filling the sensor was made of 10/6 mM phosphate/borate, sample buffer strength in these studies was lower, matched, and higher than that of the running buffer. The data in Table I show the trend for both retention time and bandfront height to increase

Table I

Effect of sample buffer concentration on SBFOS performance

NBD-amine	Sample buffer strength		
	lower^a	matched^b	higher^c
	Retention time, min / RSD (n = 5)		
methyl	1.6 / 7%	2.2 / 5%	3.0 / 9%
ethyl	1.9 / 9%	3.1 / 7%	4.3 / 9%
n-propyl	3.0 / 8%	6.1 / 10%	10.9 / 4%
	Bandfront height, mm / RSD (n = 5)		
methyl	18.1 / 6%	24.0 / 4%	31.5 / 9%
ethyl	19.8 / 5%	28.6 / 5%	30.7 / 5%
n-propyl	28.2 / 8%	36.4 / 10%	37.7 / 11%
	Efficiency, plates × 10³ / RSD (n = 5)		
methyl	~1.5 / 13%	~2 / 17%	~4 / 16%
ethyl	~1.5 / 11%	~1.5 / 13%	~2 / 14%
n-propyl	~0.9 / 8%	~1.3 / 16%	~1.0 / 15%

a: 'lower' - 5 mM Na₂HPO₄ / 3 mM Na₂B₄O₇ buffer concentration in the sample;**b:** 'matched' - 10 mM Na₂HPO₄ / 6 mM Na₂B₄O₇ buffer concentration in the sample;**c:** 'higher' - 5 mM Na₂HPO₄ / 9 mM Na₂B₄O₇ buffer concentration in the sample;

Table II

Effect of surfactant in sample on SBFOS performance

NBD-amine	Sample surfactant	
	without ^a	with ^b
	Retention time, min / RSD (n = 5)	
methyl	2.4 / 7%	2.9 / 6%
ethyl	3.2 / 6%	4.1 / 4%
n-propyl	5.4 / 9%	7.8 / 5%
	Bandfront height, mm / RSD (n = 5)	
methyl	9.8 / 9%	7.4 / 7%
ethyl	15.2 / 4%	10.1 / 10%
n-propyl	23.3 / 4%	10.9 / 8%
	Efficiency, plates × 10³ / RSD (n = 5)	
methyl	~6 / 19%	~10 / 12%
ethyl	~4 / 17%	~7 / 11%
n-propyl	~2 / 16%	~4 / 16%

a: 'without' - no SDS in the sample;**b:** 'with' - 0.05 M SDS in the sample;

when the sample buffer concentration is varied from lower to higher. The observed effects are probably due to changes in the rate of the electroosmotic flow. Neutral solutes, such as used in these studies, are swept in the separation capillary by the pumping action of the EOF. When the sample with higher buffer concentration enters the capillary, increased ionic strength of the buffer results in double-layer compression, decreased zeta potential, and reduced electroosmotic flow. As a result, solutes spend more time in the capillary, and the sample becomes concentrated, thereby increasing the response signals and therefore the bandfront heights. Although the varying of sample buffer concentration resulted in the changes in retention time and bandfront height, no distortion of bandfronts was observed as seen in Figure 13.

To perform MECC, a surfactant should be added to the mobile phase, making sample and buffer composition be different. That is why studies were carried out using the matched sample and running buffer concentration, with the addition of SDS not only to the mobile phase but to one of the samples as well. The results shown in Table II indicate that removing surfactant (hence micelles) from the sample decreases retention time and increases bandfront height, with the effects being greater for the more retained components. This may be attributed to sample stacking.

When the SBFOS is operated in the frontal mode, the sample solution tends to replace the running buffer in the separation capillary. The SDS-micelles are negatively charged and have a mobility that is opposite in sign but smaller in magnitude than electroosmotic flow. As a

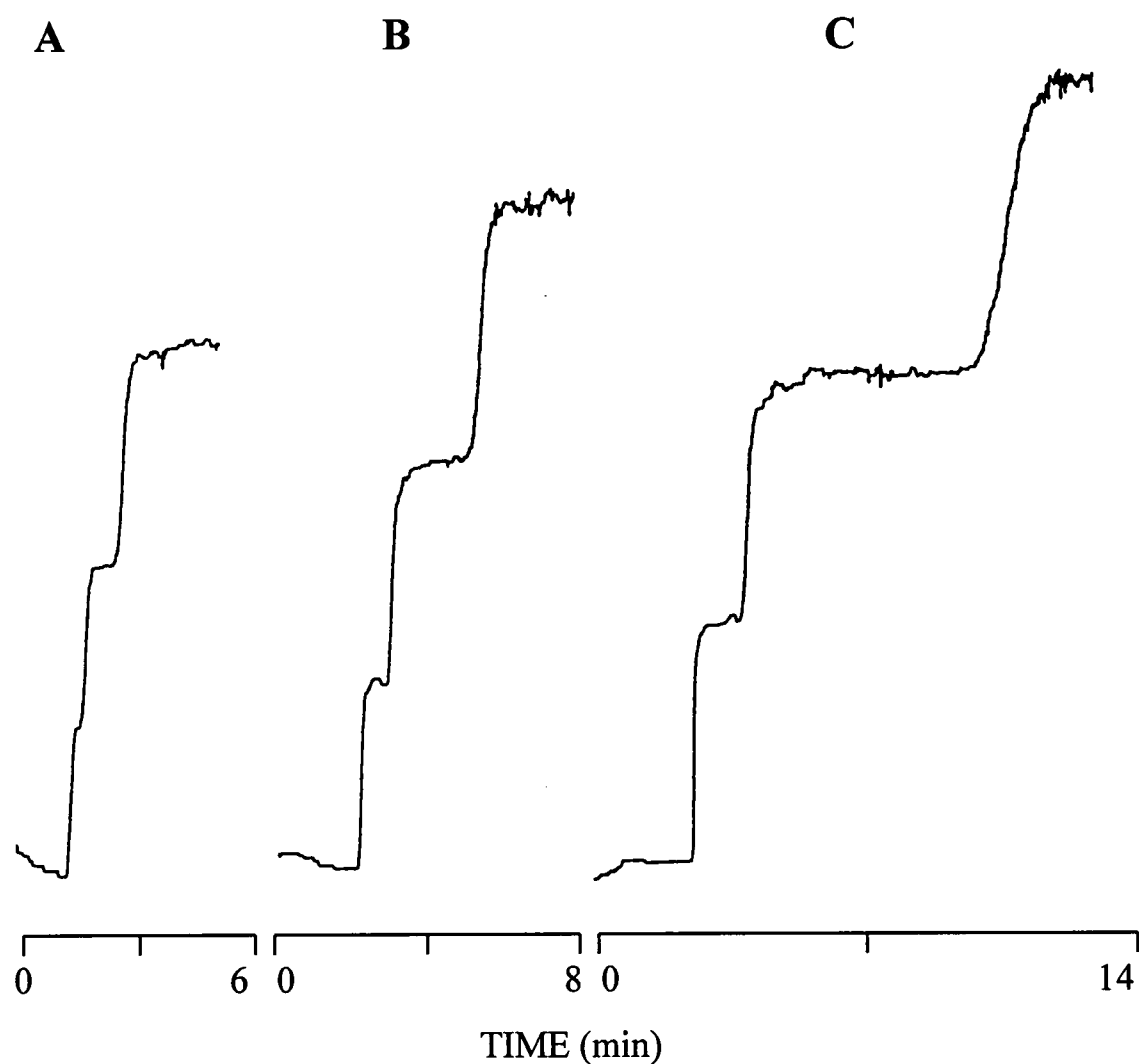


Figure 13. Electropherograms showing effects of varying sample buffer concentration on SBFOS performance

(A) 5/3, (B) 10/6, and (C) 15/9 mM phosphate/borate buffer concentration in the sample. Elution order: 5×10^{-4} M NBD-methylamine, 2×10^{-4} M NBD-ethylamine, and 1×10^{-4} M NBD-n-propylamine. Conditions: 8.0-cm $\times$ 50- μ m-i.d. separation capillary and 5-cm $\times$ 25- μ m-i.d. restrictor capillary; applied voltage, 2.5 kV; mobile phase composition, 10/6 mM phosphate/borate running buffer with 0.05 M SDS; detection, LIF using Ar^+ laser excitation.

result, the micellar phase migrates slowly toward the fiber optic. The first sample in Table II did not contain micelles. Because of this the fluorescence signal level resulting from the three analytes began to decrease at about 12 min and then dropped rapidly at about 20 min to the point where the signal was not enhanced by micelles. Before this complete micelle depletion occurs, it is possible to obtain considerable analytical information such as bandfront position and height. The second sample in Table II was made to contain micelles, and hence reagent depletion did not occur.

Bandfront efficiencies in Tables I and II were obtained directly from strip chart recordings and are only approximate values. However, it is generally best when the sample and buffer are matched. Since the measurements are made manually, the width of the sharper bandfronts is difficult to accurately determine. Therefore, values of 2×10^3 plates or greater are listed to one significant figure.

It has been shown that reduced volume of buffer reservoirs can cause significant changes in pH and column conductivity [47]. This reflects nonuniform conditions created in the capillary. A similar situation can occur in the SBFOS. After applying the voltage, the minute volume of the tee and the presence of the hydrostatic flow may result in spatial and temporal variations in running buffer composition within the separation capillary. When the sample and running buffers are not matched, nonuniform conditions are likely to develop.

The data for Tables I and II were collected at different days with

slightly changed concentrations of the NBD-amines. Nevertheless, it can be seen from RSD values that the analytical information present in retention times and bandfront heights is reasonably reproducible.

5. The reproducibility of the SBFOS in the MECC mode of operation

The reproducibility of the SBFOS in the MECC mode of operation was evaluated by performing five determinations on the samples used to collect the data for Tables I and II. The relative standard deviations in retention time for the bandfronts were $\leq 10\%$. Figure 14 illustrates the SBFOS reproducibility with the overlaid electrochromatograms for the sample with no SDS (from Table II). Obvious variations in t_R are probably due to rinsing procedure.

The side port with the restriction capillary was taken out, and the sensor was rinsed before each separation. Rinsing conducted in this manner was meant to save time over the numerous trials performed with the SBFOS. As discussed earlier, the position of the restrictor capillary assembly is important for satisfactory performance because it occupies some volume inside the tee. Since it is not possible to identically reseat the restrictor capillary fitting within the tee every time, it is likely that this inconsistency contributed to the observed t_R variations.

RSD values may improve if the sensor is rinsed with the restrictor capillary in place, for example, electrokinetically. However, it would be at the expense of time.

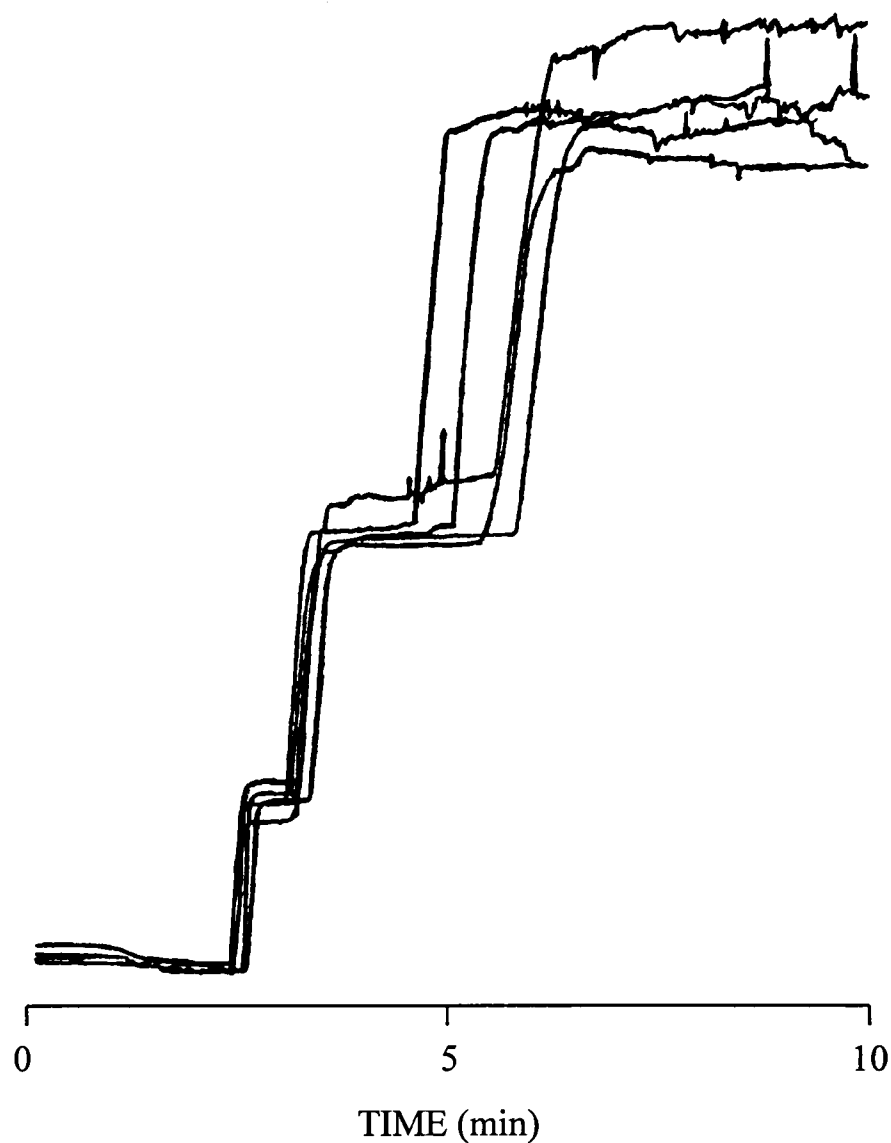


Figure 14. Five consecutive SBFOS-MECC electropherograms of the three-amine mixture in running buffer

Elution order: 4×10^{-4} M NBD-methylamine, 3×10^{-4} M NBD-ethylamine, and 1×10^{-4} M NBD-n-propylamine. Conditions: 8.0-cm $\times$ 50- μ m-i.d. separation capillary and 5-cm $\times$ 25- μ m-i.d. restrictor capillary; applied voltage, 2.5 kV; mobile phase composition, phosphate/borate (pH 9) running buffer with 0.05 M SDS; detection, LIF using Ar^+ laser excitation.

6. Calibration curves for one- and three-component mixture

The response characteristics of the SBFOS were evaluated by generating NBD-n-propylamine calibration curves over the concentration range of 5.0×10^{-8} - 1.0×10^{-5} M (Figure 15). They were constructed by obtaining electrochromatograms of one- and three-component mixtures. Each signal response plotted on the calibration graph is the average of three replicate separations. The limit of detection for the NBD-n-propylamine was found to be less than 5.0×10^{-8} M, and the linear dynamic range extended over more than two orders of magnitude in concentration. It is certain that the detection aspects of the SBFOS-MECC system can be improved by further optimization of the optics and instrumentation.

The sensitivity for the NBD-n-propylamine in the presence of the other two NBD-amines was only slightly less than that of the single component case. However, the SBFOS frontal mode of operation influences the detection since each bandfront reaching the fiber optic contributes the baseline noise to subsequent bandfronts. This effect is clearly seen in Figure 14. If signal-to-noise ratio is plotted versus analyte concentration, the difference in sensitivity between three- and one-component curves would be larger because of increasing fluorescence background.

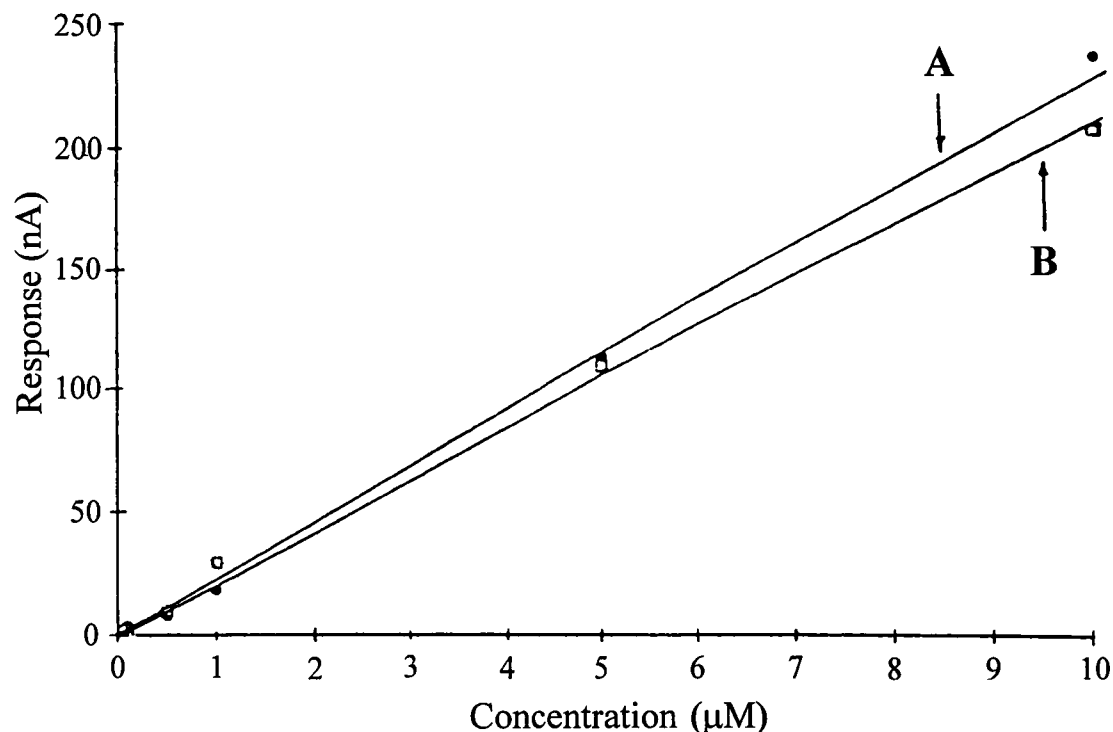


Figure 15. NBD-n-propylamine calibration curve for (A) one-component and (B) three-component mixture (NBD-methylamine, -ethylamine, -n-propylamine) in running buffer

Conditions: 8.0-cm $\times$ 50- μ m-i.d. separation capillary and 5-cm $\times$ 25- μ m-i.d. restrictor capillary; applied voltage, 2.5 kV; mobile phase composition, phosphate/borate (pH 9) running buffer with 0.05 M SDS; detection, LIF using Ar^+ laser excitation.

7. The use of the SBFOS for environmentally significant aflatoxin separations

The demonstrated results on selectivity, sensitivity, dynamic range, speed and reproducibility, obtained for the model compounds, show the feasibility of using the SBFOS-MECC system in real-world remote sensing applications. One of such analyses can be the determination of aflatoxins, naturally occurring toxic compounds.

These substances are produced by *Aspergillus* fungi, which are common contaminants of feeds and foods [48]. The International Agency for Research on Cancer has placed aflatoxins on its list of human carcinogens [49]. They are known to cause liver cancer and weaken the immune system, making a person abnormally susceptible to the effects of HIV and the hepatitis B virus. Since the Gulf War, aflatoxins have been included in the arsenal of potential biological weapon. According to the United Nations, Iraq has admitted to making bioweapons in the form of producing not only large amounts of anthrax, along with botulinum and carcinogenic aflatoxin, but also missile warheads and bombs filled with toxins [50].

The capability of the SBFOS to perform aflatoxin analysis was investigated by taking advantage of their native fluorescence, and using a helium-cadmium laser to provide the excitation light. It can be seen from Figure 16 that aflatoxins G₁ and G₂ used in this study have similar structures and are fairly hydrophobic.

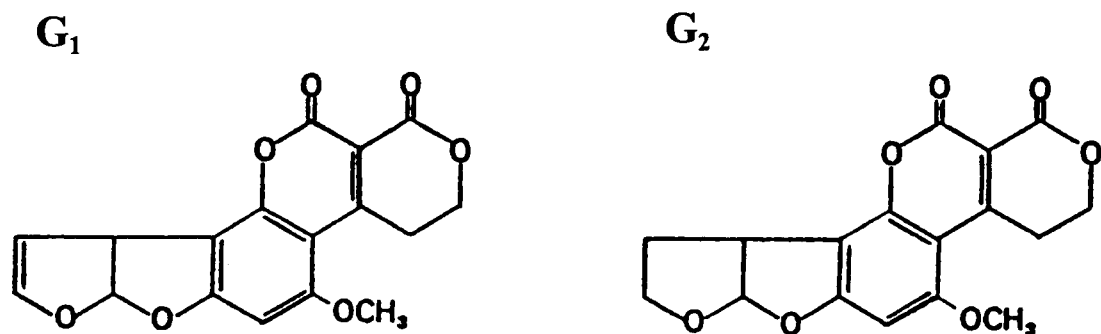


Figure 16. Structures of aflatoxins G₁ and G₂

Differing selectivity for these toxins can be accomplished by adjustment of running buffer composition. For example, adding organic solvent increases the mobile phase solubility of hydrophobic compounds, and hence reduces their capacity factors, improving resolution. The use of additives such as cyclodextrins leads to specific interactions with solutes and affects selectivity.

The organic solvent studies were carried out by varying ethanol, acetonitrile, methanol and their concentration in the running buffer which was modified with different amounts of γ -CD. The best selectivity toward the aflatoxins was achieved using phosphate/borate running buffer with 50 mM SDS, 15% v/v methanol and 7 mM γ -CD. To match the running buffer, the sample was modified with 50 mM SDS and 15% MeOH. The SBFOS separation and detection of aflatoxins G₁ and G₂ is shown in Figure 17. The order of elution was confirmed by spiking procedure.

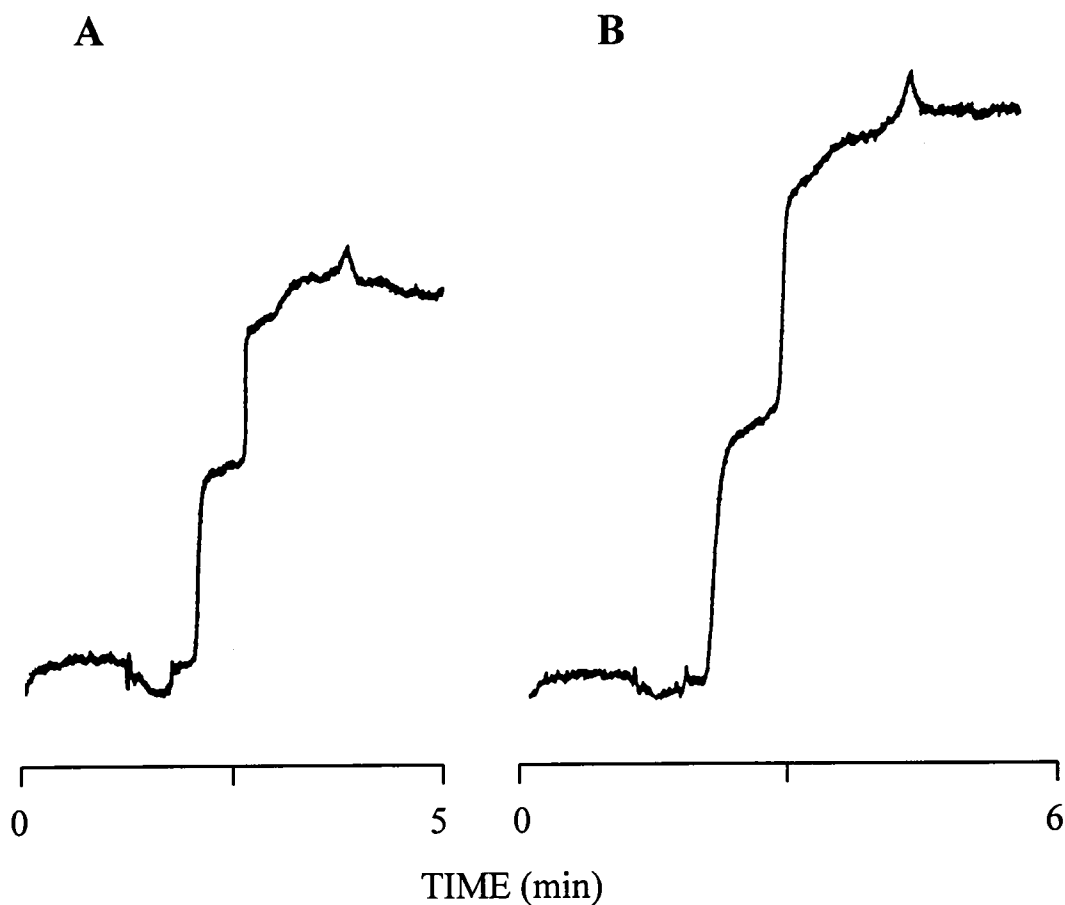


Figure 17. SBFOS-MECC electropherograms of aflatoxins G_1 and G_2
 (A) 20 μM G_1 , G_2 and (B) 25 μM G_1 , 30 μM G_2 . Conditions: 8.0-cm $\times$ 50- μm -i.d. separation capillary and 5-cm $\times$ 25- μm -i.d. restrictor capillary; applied voltage, 2.5 kV; mobile phase composition, phosphate/borate (pH 9) running buffer with 50 mM SDS, 7 mM γ -CD; LIF detection using He-Cd laser.

Since the attention in this study was focused on testing the feasibility of performing aflatoxin analysis with the SBFOS, an optical system used (lens, fiber, filters) was not optimized for high sensitivity. Nevertheless, the potential for the simultaneous, remote and repetitive analysis of the toxic compounds in ground and subsurface waters is clearly evident.

CHAPTER IV

CONCLUSIONS

The work presented in this thesis has shown that the separation-based fiber-optic sensor is a viable chemical analysis system for multicomponent remote determinations of neutral analytes separated by MECC.

The performance of the SBFOS was fully characterized using the mixture of the NBD-derivatized amines as the test solutes. The effects of the applied voltage and the sample matrix on retention time and signal were investigated. The observed behavior is not fully understood. Nevertheless, through refinements in the design and operation of the sensor, encouraging results in terms of retention time reproducibility and efficiency were obtained. Signal calibration plots were presented, which provided the information about the limit of detection and the linear dynamic range.

The frontal mode of the SBFOS operation makes it well suitable for remote, in situ monitoring of chemicals because the sensor resides in the sample. To examine the applicability of the SBFOS-MECC system for "real" analyses, the preliminary separation of environmentally significant aflatoxins was carried out.

Unfortunately, in situ measurements are more problematic and can affect the selectivity, sensitivity and reliability demonstrated by the system within the lab environment. In addition, the influence of sample

matrix on both retention time and bandfront height will clearly complicate calibration. One solution to decrease the potential for error between 'real' samples and standards is to obtain some of the sample to be measured, e.g. from a waste storage tank or ground water, and to use this as the solvent when preparing standard solutions for calibration.

If the sample is not easily accessible, another approach might be to receive information about the major components in a sample and prepare a similar matrix for calibration. The analytes of interest are usually present as minor components in samples for which the matrices do not change significantly with respect to major components. Therefore, it should be possible to collect fairly reliable calibration data before placing the sensor in the sample for periodic remote analyses.

As already mentioned, there are some problems in the current design and operation of the SBFOS that should be addressed in future work. In an effort to improve reproducibility, the rinsing of the sensor electrokinetically might be involved, without removing the side port with the restrictor capillary. Further optimization of the optical system should reduce the limit of detection as well as improve the dynamic range and sensitivity.

To alleviate some of the problems associated with calibration and reagent depletion, a modification may be possible by incorporating a gated injection procedure such as has been developed for CE separations performed on a chip [51]. Since the SBFOS approach in this case would involve zonal operation and the active delivery of running buffer to the sample side of the capillary, this could help to overcome the effect of

reagent depletion occurring in the frontal mode of operation. To sum up, the further development of the separation-based fiber-optic sensor should be continued, because it shows great promise for future applications in the biomedical, environmental and industrial fields.

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