ELECTROKINETIC CAPILLARY CHROMATOGRAPHY METHODS FOR SEPARATION AND TRACE ENRICHMENT OF DILUTE ANALYTES USING OPEN TUBULAR AND PACKED CAPILLARIES

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TABLE OF CONTENTS

Chapter Page

Chapter Page

LIST OF TABLES

Table Page

Chapter V

LIST OF FIGURES

Figure Page

Chapter I

Chapter II

Figure Page

Chapter III

Chapter IV

Figure Page

Chapter V

LIST OF SYMBOLS AND ABBREVIATIONS

α	Selectivity
δ	thickness of the double layer
ε	dielectric constant
ε'	porosity of the stationary phase
ε_0	permitivity of the vacuum
ζ	zeta potential
ζ_p	zeta potential on a stationary partical
η	viscosity of the electrolyte solution
κ	Debye-Hückel constant
μ_c	apparent mobility of a charged solute in CEC
μ_{eo}	electoosmotic mobility
μ_{ep}	electrophoretic mobility
μ_{app}	apparent analyte mobility
$\mu_{ep,mc}$	electrophoretic mobility of the micelle
$\mu_{ep,eff}$	effective electrophoretic mobilty
v_{ep}	electrophoretic velocity
v_{eo}	electroosmotic velocity
v_{app}	apparent velocity
Δp	pressure drop over the column

ρ	surface charge density of the capillary surface
σ_L	standard deviation of the peak in unit length
σ_l	standard deviation of the peak in unit length
σ_1, σ_2	standard deviations of the peaks 1 and 2, respectively
ψ_d	electric potential at the interface of the mobile and imobile region of the double layer
ψ_0	electric potential at the silica-solution interface
a	radius of the ionic species
c^*	conductivity of packed column and empty column
c_b	conductivity of empty column
D	diffusion coefficient of the solute
d_p	particle size of the stationary phase
E	electrical field strength
f	translational resistance
F_d	drag force
F_e	electrical field force
I	ionic strength of the medium
k'	chromatographic retention factor
$k_c^{'}$	retention factor for a charged analyte in CEC
$k_e^{'}$	retention factor in CE
L	total length of the separation capillary
l	length of the separation capillary from the inlet to the detection point

N	number of theoretical plates
q	net charge of a species
r	radius of the species
R_s	resolution
t_0	migration time of a neutral solute
t_M	migration time
t_{mc}	migration time of the micelle
t_r	retention time in CEC
$t_1^{'}$, $t_2^{'}$	adjusted retention times of components *1* and *2*, respectively
V	external applied voltage
w_b	peak width at the base
w_h	peak width at the half-height
w_i	peak width at the inflection point
ACN	acetonitrile
Ald	aldicarb
Ami	aminocarb
APK	alkyl phenyl ketones
Bay	baygon
Ben	bendiocarb
CE	capillary electrophoresis
Car	carbaryl
Carn	carbofuran
CEC	capillary electrochromatography

CGE	capillary gel electrophoresis
CMC	critical micellar concentration
CZE	capillary zone electrophoresis
CIEF	capillary isoelectric focusing
CITP	capillary isotachophoresis
DOSS	di-2-ethylhexyl sulfosuccinate
EC-ODS	Nucleosil octadecyl-silica
ECC	elution electrochromatography
EKC	electrokinetic capillary chromatography
EOF	electroosmotic flow
FEC	frontal electrochromatography
FASS	field amplified sample stacking
GC	gas chromatography
HC-ODS	J.T. Baker octadecyl-silica
HM-ODS	home made octadecyl-silica
HPCE	high performance capillary electrophoresis
HPLC	high performance liquid chromatography
LC	liquid chromatography
LIF	laser-induced fluorescence
LOD	limit of detection
MS	mass spectrometry
Met	methiocarb
Meth	methomyl

MECC	micellar electrokinetic capillary chromatography
MEGA 10	decanoyl-*N*-methylglucamide
MeOH	methanol
NMC	*N*-methyl carbamates
NMR	nuclear magnetic resonance spectroscopy
ODS	octadecyl-silica
Oxa	oxamyl
RPC	reversed-phase chromatography
SM-CEC	surfactant mediated electrochromatography
SFC	supercritical fluid chromatography
SDS	sodium dodecyl sulfate
THF	tetrahydrofuran

CHAPTER I

SOME BASIC PRINCIPLES OF ELECTROKINETIC CAPILLARY CHROMATOGRAPHY. SCOPE OF THE STUDY

Introduction and scope of the study

Capillary electrophoresis (CE) and capillary electrochromatography (CEC) are miniaturized analytical techniques that utilize direct electric current as the driving force for mass transport resulting in high resolution and high separation efficiency. CE and CEC consume small volumes of samples and reagents and employ automated instrumentation, which make them ideal tools for chemical and biochemical analyses. In CE, the separation of charged molecules comes about by electrostatic attraction or repulsion of the charged analytes toward the electrodes under the influence of an electric field. In CEC, charged and neutral molecules are separated on the bases of differences in partitioning between the mobile and stationary phase, and in addition charged molecules are also influenced by the electric field as in CE. Therefore, CEC is a hybrid of CE and high performance liquid chromatography (HPLC).

A modification to CE is to add selectors (also called pseudo-stationary phases) to the running electrolyte. Unlike CEC and HPLC where the stationary phase does not

move, the pseudo-stationary phase is moving in the same or opposite direction with respect to the running electrolyte and at a different velocity. In CE, the selectors or pseudo-stationary phases (e.g., micelles, cyclodextrins, crown ethers, charged or uncharged soluble polymers, etc.) interact with the molecules much the same way as the stationary phase in HPLC or in CEC interacts with the solute molecules. This interaction changes the mobility of the solute molecules, and thus resultes in the separation of neutral molecules and enhancing the separation of charged molecules. A CE separation system that consists of a pseudo-stationary phase and an aqueous or hydro-organic phase is referred to as electrokinetic capillary chromatography (EKC) system. In the special case where a surfactant is added to the running electrolyte above its critical micellar concentration (CMC), the technique is referred to as micellar electrokinetic capillary chromatography (MECC). CEC is also a member of EKC techniques where the only difference is that separation is performed in the presence of a true stationary phase (i.e., non moving phase). Therefore, the goal of this chapter is to enlighten the reader with the history of CE and CEC, to introduce the instrumentation used, to portray the basic separation principles, and to describe the similarities and differences in both techniques. Moreover, several equations and parameters pertinent to the CE and CEC studies conducted in this dissertation will be discussed.

The scope of this dissertation encompasses the development of novel trace enrichment and separation approaches in CE and CEC using pesticides as the model solutes. This was conducted with the intention that other classes of molecules could be potential candidates for the methods and concepts developed in this dissertation. Both CE and CEC are known for their high separation efficiencies. However, and as in any

given separation process, no separation can take place in the absence of adequate selectivity (i.e., discriminating power) even if millions of theoretical plates are easily attained. This dissertation recognizes the importance of selectivity by introducing and evaluating novel separation media for maximizing separation. The combination of high separation efficiency with adequate selectivity leads to high resolving power, which is essential for the separation of multicomponent mixtures usually encountered in chemical and biochemical analyses. Another important aspect of any separation technology is its ability to deal with trace analysis because high resolving power alone is not sufficient for achieving the analysis of dilute samples. The detection sensitivity in CE and CEC with photometric detectors is curtailed by the narrow pathlength offered by the inner diameter ($\leq$ 100 μm) of the capillary for on column detection. This drawback makes CE and CEC not directly amenable for the analysis of dilute samples. This dissertation addresses this need by introducing on column trace enrichment approaches.

Besides this introductory chapter, the dissertation contains 4 additional chapters which describe the trace enrichment and separation approaches developed. Chapter 2 deals with the investigation of MECC systems for the separation and trace enrichment of environmentally important solutes, the carbamate insecticides. Chapter 3 develops novel approaches for trace enrichment and separation by CEC of the carbamate insecticides using packed capillary columns. Chapter 4 further elaborates the trace enrichment methodologies introduced in Chapter 3 by using segmented capillaries for enhancing the detectability of ultra dilute samples of pyrethroid insecticides. Segmented capillaries are packed end to end with two types of silica-based stationary phases. The first stationary phase is a relatively high carbon load or high retention bonded silica used for the trace

enrichment, and the second stationary phase is a specially coated silica used to produce high flow and excellent separation. Chapter 4 also involves the use of a high sensitivity cell to further lower the limit of detection of the pyrethroid insecticides. Chapter 5 introduces the use of mobile phases rich in sodium di-2-ethylhexyl sulfosuccinate (DOSS) surfactant to modulate CEC selectivity, and the novel approach is referred to as surfactant mediated capillary electrochromatography (SM-CEC). In SM-CEC, as in EKC, the surfactant functions as a pseudo-stationary phase and is moving with or against the mobile phase. In SM-CEC, the solute molecules partition between the stationary phase, the pseudo-stationary phase, and the mobile phase to yield unique selectivity and separations. This novel separation method was demonstrated in the separation of the geometrical isomers and diastereomers found in the pyrethroid insecticides.

Historical Background and Development of Capillary Electrophoresis and Capillary Electrochromatography

Electrophoresis was introduced in 1937 by the Swedish scientist Tiselius and his co-workers as an important separation tool in the area of biomacromolecules, e.g., proteins, DNA and RNA.[1] However, traditional gel electrophoresis is not very quantitative and is labor intensive requiring casting the gel, staining, and destaining for the localization of the separated solute bands. These time consuming tasks involved in traditional electrophoresis have led to developing faster electrophoretic systems. Hjertèn[2] in 1967 was the first to use glass tubes of 3 mm internal diameters (i.d.) using high electric field strength. The solute molecules where detected on line as they passed in front of an UV detector. As time passed, and tubes with smaller i.d., such as Pyrex glass columns, became available, it was realized that they would allow for better

dissipation of heat and a more uniform thermal cross-section of the sample within the tube. Using potentiometric detection combined with the Pyrex glass tube, Virtanen[3] in 1974 was able to separate alkali cations. Later that same year and using 200 μm PTFE tubing, Mikkers et al.[4] were able to separate inorganic and organic compounds and identify them using UV and conductometric detection. In the first 50 years of its development, CE had come a long way but there were still major problems with low separation efficiency, large injection volumes, and poor detection sensitivity.

In 1981, these complex problems were confronted by Jorgenson and Lukacs.[5] By using a 75 μm fused-silica capillary and on column fluorescence detection the problem of injection and detection was solved in the separation of amino acids and peptides. This major breakthrough in capillary technology and on column fluorescence detection instrumentation led to achieving high separation efficiency and high sensitivity in CE, thus opening the door for further exploration and marking the era of modern CE.

With the modern era of CE in the early 1980s came a number of separation modes. A few methods were adapted from the traditional gel electrophoresis to the capillary format including isoelectric focusing,[6] and gel electrophoresis.[7] Terabe et al.[8] in 1984 introduced one of the most important modes of CE called micellar electrokinetic capillary chromatography (MECC). MECC uses a micelle as a pseudo-stationary phase where the solute molecules undergo differential partitioning between the micelle and the mobile phase, which leads to separation. MECC can enhance the separation of charged compounds and also allows the separation of neutral molecules by the introduction of sodium dodecyl sulfate (SDS),[9,10] a charged micelle that will migrate against the electroosmotic flow (EOF). The introduction of small capillaries, better detectors, and

other separation modes, such as ligand exchange,[11] ion exchange[12] and MECC, have greatly improved the separation value of CE. These major advances were capped in the early 1990's by a new and advanced platform of CE, the CE on a microchip.[13,14] Progress made in the microelectromechanical and microelectronic industries provided the needed technology for the construction of the first microchip channels that are etched on the surface of a glass wafer and produce fast multiplex assays. In addition, polymers are being used to produce microchips with high flow and high separation efficiencies. As CE separation modes become more sophisticated they will accompany gas chromatography (GC) and HPLC in meeting the needs of industrial companies. Indeed, CE has been used in DNA-sequencing, protein analysis, and carbohydrate analysis.[15-18]

Capillary electrochromatography (CEC) was first introduced in the late 1930's[19] when Strain was able to demonstrate the use of electroosmotic flow (EOF) in chromatography. The first true pumping action from EOF for analytical separation was not introduced until the mid 1970's by Pretorius et al..[20] In the 1980's and 90's CEC progressed on several fronts, including (i) the introduction of automated instrumentation that allow pressurizing both inlet and outlet vials resulting in reduced bubble formation, (ii) the development of specialized stationary phases to produce desired separation, and (iii) the advancement of ways to control the EOF.

As in HPLC, separation in CEC is based on differences in solutes partitioning between a stationary phase and a mobile phase. Although, CEC and HPLC are comparable in the separation mechanism, CEC has a number of advantages over HPLC. The first being that CEC is a microcolumn separation technique that uses microliters of solvents as compared to HPLC that uses liters of solvent. More important is the plug-like

flow profile in CEC due to EOF, which yields relatively high separation efficiencies as compared to HPLC, which has a parabolic flow resulting from a mechanical pump. When CE and CEC are compared, CEC has higher peak capacity and selectivity due to the stationary phase being packed in the column. In addition, CEC has the advantage of tailoring the ligand attached to the stationary phase to the molecules of interest. Another major advantage when developing a stationary phase is to consider the ability of the latter to produce a strong EOF resulting in fast separation times.

Since the 1980's, interest in separation sciences has focused mainly on the advancement of CE and CEC to produce powerful microseparation techniques for the analysis of neutral and ionic species of varying size and shape. Today the number of national and international meetings dealing with CE and CEC are countless, giving good indication to the value of these separation techniques.

Electrokinetic Capillary Chromatography Methods

General Aspects and Instrumentation

The instrument used in CE and/or CEC is the same in design and is shown in Fig. 1. The instrument is comprised of several basic parts, which are the same for manual or automated instrumentation. The first part is the high voltage power supply capable of delivering up to 30 kV. The second is a detector that is typically placed on-column with the ability to detect the analytes by UV or fluorescence. The third component of the instrument is some type of recording device, either as simple as a basic strip chart

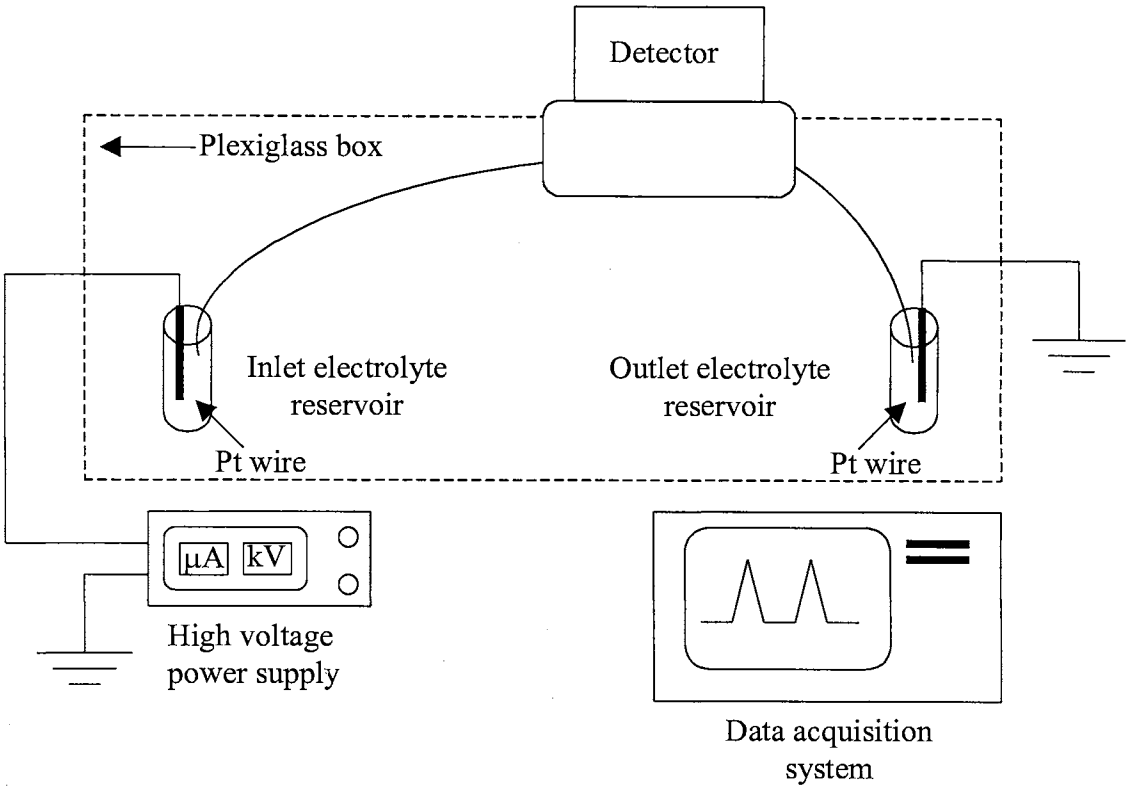

Figure 1. Schematic illustration of a manual instrument set-up used for CE/CEC

recorder or a sophisticated computer with integrated software. Other important parts of the CE/CEC system are the capillary, platinum electrodes, and a plexiglass box used to protect the analyst from the high voltage used. The plexiglass box is also equipped with a safety switch that shuts the power off when the lid is opened. The automated instrument offers several options that are not available on the manual instrument such as temperature control, automated sample injection, autosampler, and for CEC pressurization that offers the ability to reduce bubble formation.

The separation and usually the detection takes place in a fused-silica capillary. Fused-silica has a protective polyimide coating that enables it to be flexible and durable. At the detection point (~2 mm in length), the polyimide coating must be removed so that

on column detection can be performed. The polyimide coating can be removed by heating with a flame or an electrical wire stripper (~600 °C) resulting in a window that is UV transparent. An alternative to using excessively high heat that can stress or damage the fused-silica is to remove the coating by using concentrated sulfuric acid heated at 100 °C. Depending on the nature of the experiment the internal diameter of the capillary can range from 10 to 100 μm.

Sample Injection

Injection of the sample in CE and CEC is very important for obtaining reproducible results. There are basically two methods for sample injection, which are hydrodynamic and electrokinetic, depending on the automation of the instrument. Hydrodynamic injection can be broken down into three types called head-space pressurization, vacuum injection, or gravimetric (siphoning). When using an automated instrument, head-space pressurization or vacuum injection are used most frequently. A low pressure is applied to the inlet end of the capillary, resulting in the sample matrix being pushed into the capillary, or a vacuum is applied to the outlet end resulting in the sample being pulled onto the capillary. Automated instruments also have the ability to use electrokinetic injection. When using electrokinetic injection, the amount of sample injected is dependent upon the ionic strength of the sample matrix. Unfortunately, when injecting electrokinetically, the quantity injected is affected by variation in conductivity between the sample and running electrolyte. This difference in conductivity results in the concentration of the sample and sample plug injected being different. Although this is a

major shortcoming when dealing with electrokinetic injection, it is still very popular when dealing with viscous media or gels. In addition, when dealing with CEC where the flow is restricted by the packing, electrokinetic injection is typically the method chosen. When using a manual instrument, the method typically used is siphoning where the inlet end of the capillary is inserted in the sample matrix which is elevated above the outlet end of the capillary. This results in the sample matrix being loaded onto the capillary with the height and time of elevation determining the amount injected. As with automated instruments, manual instrumentation also utilizes electrokinetic injection.

Detection in CE and CEC

Detection is a major concern when dealing with CE or CEC due to the very small path length the capillary offers. UV absorbance is the most popular detection technique used due to the fact that the detectors are inexpensive and most analytes absorb in the UV part of the spectrum. A modification to the capillary itself to acquire higher sensitivity was introduced by Hewlett-Packard and called the bubble cell. The bubble cell is a portion of the capillary that is expanded directly within the capillary, resulting in a three fold expansion.[21] A second type of detection pathlength enlargement was devised from the introduction of the high sensitivity cell or the Z-shaped cell.[22] The path of light is aligned with the long axis of the capillary. The major problem with the Z-shaped cell is that the absorbance is measured along the long axis of the capillary, resulting in band broadening and lower efficiencies. Laser-induced fluorescence (LIF) [23-30] is a second type of detection that is becoming more popular and can be used with the bubble cell or

Z-shaped cell. Some other methods used are amperometry,[31-36] conductivity,[36-42] and indirect detection techniques.[42,43] CE and CEC have also been integrated with mass spectrometry (MS) [44-50] and nuclear magnetic resonance spectroscopy (NMR) [42,44,51-54] both of which are powerful detection tools.

Modes of separation

Since the introduction of CE, several separation modes have been developed to accomplish the separation of a wide range of analytes differing in their charge, size or shape, isoelectric point, polarity and hydrophobicity. The various modes are: capillary zone electrophoresis (CZE), micellar electrokinetic capillary chromatography (MECC), capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF), and capillary isotachophoresis (CITP).

The most common mode for separation is CZE, which is carried out in a uniform buffer at constant pH. The separation is based on differences in the analyte charge-to-mass ratios with size and bulkiness contributing to the selectivity. Both anionic and cationic analytes can be separated using CZE whereas neutral solutes will migrate with the EOF and elute in the dead volume. The number of separations that have been performed by CZE is countless ranging from small ions to viruses and bacteria.

In MECC, ionic, cationic, and neutral analytes can be separated through the hydrophobic interaction of solutes with charged micelles (e.g., sodium dodecyl sulfate). When an ionic surfactant is added above its critical micellar concentration (CMC), it serves as a pseudo-stationary phase which permits the solute to partition between the

micelle and the mobile phase. At the CMC or higher concentrations, the surfactant monomers are in equilibrium with micelles. As in reversed phase liquid chromatography, the more hydrophobic analytes interact with the micelle stronger than do the less hydrophobic analytes. When dealing with charged analytes, the separation results from both the interactions with the micelle and by CZE where the analytes are separated via differences in their charge-to-mass ratios.

In CGE, as in traditional gel electrophoresis there is no EOF generated. The analytes progress through the capillary by electrophoretic mobility and are separated based on their size as they progress through the pores in the gel-filled capillaries. This method has been very successful with large molecular weight compounds. Moreover, since the gel has anticonvective properties, the diffusion of the analytes is very small and theoretical plates as high as 30 million plates per meter have been reported[55] for this type of separation.

In CIEF, the capillary is filled with a pH gradient buffer formed by a polyampholyte system that is stationary or no flow. The analytes are separated on the basis of their isoelectric points or pI values.[2] To sum up the process, the analytes migrate through a stagnant medium by electrophoretic mobility as long as they are charged. As the analytes migrate in the capillary to a pH zone equaling its pI value, the molecule becomes neutral and in turn loses its electrophortic mobility or becomes stationary in the capillary. A second step is a mobilization step where an electrolyte is used to mobilize the focused molecules past the detector and out of the capillary.

Capillary isotachophoresis (CITP) uses a discontinuous buffer medium for the separation of analytes based on distribution of the solutes into continuous but discrete

sharp zones. The sample is injected into the capillary between a leading electrolyte with the highest mobility in the capillary and a terminating electrolyte with the lowest mobility in the capillary. The analytes condense between the leading and terminating electrolytes producing a configuration composed of consecutive sample zones moving at the same velocity. The analytes are detected with zone length proportional to the concentration.

Column Technologies for CE and CEC

Due to the fact that fused-silica is easy to fabricate into capillaries, is electrically resistant, is optically transparent, is mechanically strong, and is inexpensive, makes it the most widely used capillary material in CE and CEC. Fused-silica behaves as a weak acid ionizing within the pH range from 3.5 to 9 with an average surface coverage of 8 $\mu mol/m^2$ of silanol groups.[56] When the pH of the running electrolyte is between 3.5 to 9, the silanol groups become negatively charged and in turn allow for the production of EOF. When dealing with cationic compounds, the negative charge can be unfavorable due to the positively charged analytes sticking to the walls of the capillary causing peak tailing or extremely large or infinite retention times. When dealing with a positively charged analyte, several remedies have been successfully demonstrated such as manipulation of the buffer pH,[57] modification of the capillary surface by adding modifiers,[58-61] and chemically altering the capillary surface.[62] These issues were addressed very recently in two review articles.[63,64]

CEC Column Fabrication Typically, CE and CEC are performed with the same type of capillary with the inner diameter being 75 or 100 μm for CEC. Figure 2 shows the configuration of a capillary column used in CEC. There are mainly two configurations used when dealing with packed capillaries. The first is a partially packed capillary, see Fig 2a. A partially packed capillary has the detection point 1 to 2 cm past the separation bed where the sensitivity would be the same as using an open tube. For a fully packed capillary (see Fig. 2b), the sensitivity is lower due to light scattering form silica particles at the detection point. The fully packed column has the advantage over the partially packed column in the fact that bubble formation is usually minimized whereas in the partially packed column restriction in the flow imposed by the frit introduces bubbles in the empty section of the capillary. Another reason for bubble formation in the partially packed column is because of its segmented configuration in which flow velocity is not homogeneous over the entire capillary. The flow turbulence at the interface of the packed, and open capillary brings about bubble formation resulting in more difficult operating conditions.

In CEC, as in HPLC, the making of the column is the most important parameter affecting reproducibility, efficiency, peak shape, and resolution. The CEC column is made of several components including the capillary, stationary phase, and retaining frits. There are two main types of stationary phases used with the first being a silica-based stationary phase with the ligand covalently bonded to the silica.[65,66] The stationary phase is held in place by a frit sintered on each end of the capillary. The frit is a crucial part of the capillary column due to the fact it must be strong enough to hold the stationary phase

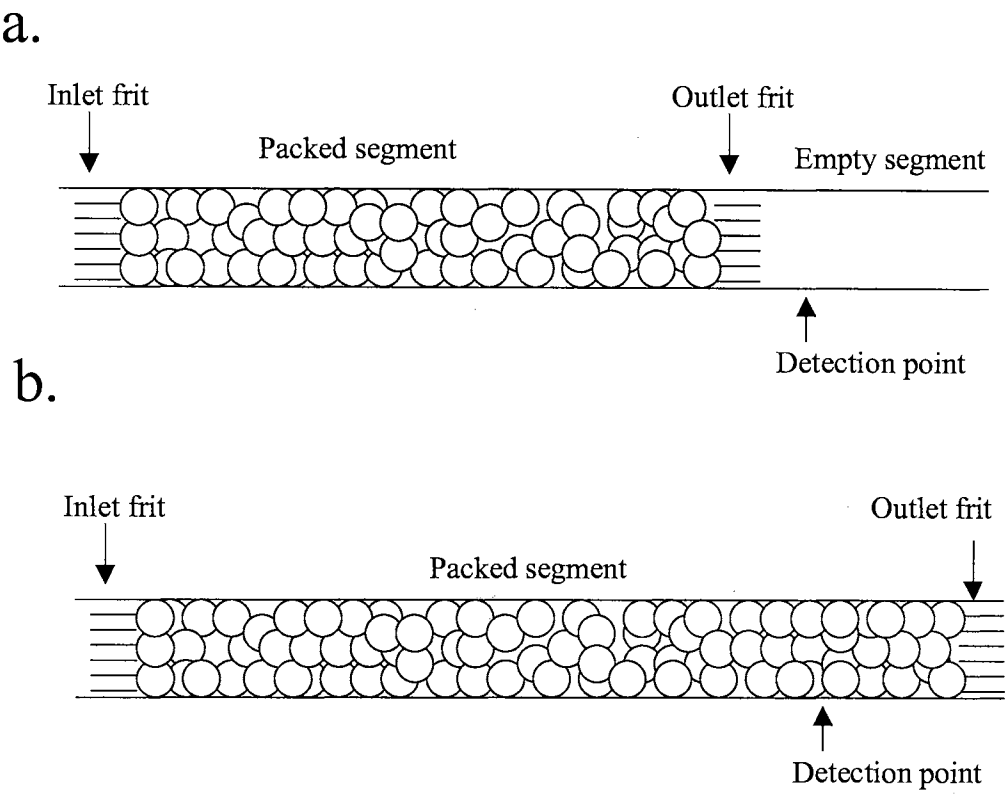

Figure 2. Configuration of packed CEC column. (a) Partially packed column with post-column detection window. (b) Fully packed column with on-column detection window.

in place but yet porous enough to allow fast flow with little to no band broadening. The second type of stationary phase is a monolithic sorbent made up of a porous polymer network with ligands extending from the surface.[65,67] Unlike silica-based stationary phases that use frits, the porous polymer monolithic column is fritless because the stationary phase is covalently bonded to the surface of the capillary. Another approach to fritless columns involves the entrapment of the silica stationary phase with silicate material.[68] Moreover, tapered ends on the capillary column can be used to retain the silica.[69] When using fritless columns, such as the ones just mentioned, the result seems to be higher separation efficiencies and significantly improved mechanical robustness.

Column Packing Material The first types of packing materials used in CEC were designed for use in HPLC. The particle size ranged in size from 3 to 5 μm with a pore size of 8 to 10 nm.[70] Due to the fact that reversed-phase packing seems to perform better when dealing with CEC, octadecyl-silica (ODS) has been the most commonly used stationary phase. However, ODS stationary phases used in HPLC are produced with the aim of having low silanol group content and sometimes are even endcapped. This lead to ODS columns with little or no EOF when used in CEC. Since the separation mechanisms of HPLC and CEC are similar, the modification of the stationary phase surface to improve selectivity is relatively well understood unlike the ability to improve the EOF in packed capillaries which has not been explored to a great extent.[71] With time being a major concern in all separation techniques, understanding how to produce a strong EOF with high separation efficiencies is critical for optimizing CEC. As CEC progresses, manufacturers of silica are starting to address the need to develop new stationary phases which are more suited for CEC.[72] A few articles in the literature have addressed this issue.[73-80] The current trend is to incorporate permanent charges (positive or negative) to control the magnitude and polarity of the EOF while keeping an adequate density of retentive ligands on the surface of the stationary phase to yield good chromatographic selectivity. In HPLC, mechanical pumps generate the flow whereas in CEC the flow is generated by electroosmotic mobility resulting in no back pressure and thus allowing for very small particle sizes of 1 μm or less. With the new stationary phases specially designed for CEC, it is common practice to acquire 100,000 plates per meter or more. In

another report, using a specially modified silica with permanently charged sulfonic acid groups allowed the pH to be used in the range of 2.5 to 10.[81]

Monolithic columns for CEC are receiving increasing attention as an alternative to capillary columns packed with microparticles and having retaining frits. With monolithic stationary phases there is no need for retaining frits, thus eliminating bubble formation and yielding stable columns. There are two categories of monolithic columns: rigid porous polymer-based monoliths[82-84] and porous silica-based monoliths.[85-87] In the category of polymer-based monoliths, acrylamide-,[83,88] methacrylate ester-[84,89,90] and polystyrene-based monoliths[91] have been described. Silica-based monoliths were prepared mostly by a sol-gel techniques.[85-87] *In situ* preparation of monolithic columns offers the advantage of the large number of readily available chemistries which can be performed to produce tailor-made columns for solving particular separation problems.

Basic Principles of Capillary Electrophoresis

Electrophoretic Migration

Separation in CE is accomplished through the movement of ions in the presence of an electric field. The anions migrate toward the anode or positive electrode while the cations migrate toward the cathode or negative electrode with electroneutrality of the solution being held constant through the dissociation of water. In solution, cations and anions carry the current. Therefore, the conductivity of the solution is dependent on the concentration and mobility of the electrolyte ions in the electric field. Also, the mobility

of the ionized solute is dependent on its charge-to-mass ratio with size and shape contributing to a lesser extent.

In the presence of an external electric field, a charged particle experiences a force F_e that is equal to the product of its net charge q and the electrical field strength E:

$$F_e = q \times E \tag{1}$$

E is given by:

$$E = \frac{V}{L} \tag{2}$$

where L is the total length of the separation capillary and V is the external applied voltage. F_e is positive for positively charged ions such that this force pushes them in the direction of the more negative electrode; the opposite applies to negative ions. The electrical force acting on the charged particle accelerates it, but there is an additional force acting on the particle called a drag force. The drag force results from the particle movement through the running electrolyte and is opposite to that of the electrical force experienced. This drag force F_d is directly proportional to the particle's electrophoretic velocity v_{ep}, and is given by:

$$F_d = -f \times v_{ep} \tag{3}$$

where f is the translational friction coefficient. For small spherical ions f can be expressed by Stoke's Law:

$$f = 6\pi\eta r \tag{4}$$

where η is the viscosity of the electrolyte through which the particles are migrating and r is the radius of the moving particle. The frictional drag is directly proportional to viscosity, size of the particle, and electrophoretic velocity. Due to the presence of the

frictional drag, a charged species is accelerated to a limiting velocity, which is dependent on both F_e and F_d. During electrophoresis the acceleration force is balanced by the retarding force and a steady state is attained. Under these conditions, the sum of the two opposite forces, F_e and F_d, is equal to zero, and a limiting velocity or electrophoretic velocity (v_{ep}) is acquired, which is given by equating equations 1 and 3 as follows:

$$v_{ep} = \frac{qE}{f} \tag{5}$$

Since the electrophoretic mobility, μ_{ep}, is defined as the electrophoretic velocity of the charged particle per unit field strength, a combination of equations (4) and (5) will give the following mathematical expression:

$$\mu_{ep} = \frac{v_{ep}}{E} = \frac{q}{6\pi\eta r} \tag{6}$$

By evaluating equation (6), it is evident that small particles with a large number of charges will have higher mobilities than large particles with a small number of charges. The magnitude of μ_{ep} depends on the net charge on a molecule and its frictional properties (size and shape) as well as the dielectric constant ε and the viscosity η of the running buffer.

Another relationship for electrophoretic mobility has been derived for colloidal particles, and is given by:[92]

$$\mu_{ep} = \frac{2\varepsilon\zeta}{3\eta} f(\kappa a) \tag{7}$$

where ζ is the zeta potential of the charged species, κ is the Debye-Hückel constant and a is the radius of the ionic species. The parameter $f(\kappa a)$ is a constant whose value depends on the shape of the migrating particle. The value of $f(\kappa a)$ varies between 1 and 1.5.

Electroosmotic Flow in Open Tubes

At the present time the most popular type of capillary used in CE is fabricated from fused-silica. The popularity of fused-silica is due to the fact that its chemistry is well known and understood. The surface of the fused-silica capillary under most conditions consists of ionized silanol groups (SiO^-). As a result, the walls of the capillary are lined with anions or negative charges. These negative charges will attract cations to the solid phase/liquid phase interface from the running electrolyte. Also, the negative ions (i.e., electrolyte anions) will be repelled from the capillary wall resulting in a distribution of charges and a potential gradient inside the capillary. Figure 3a is a representation of the charge distribution and potential gradient inside the capillary. The compact region is made of ions (mostly cations) closest to the capillary surface. These ions are tightly bound and form an immobile layer. Thermal motion in the compact region allows for a small number of cations to move out of the compact region. These cations form the diffuse region of the electric double layer, which is mobile. After the electric double layer and going towards the center of the capillary comes the bulk solution made up of equal concentrations of cations and anions. Figure 3b illustrates an electrical potential gradient as a result of the overall spatial distribution of ions within the electrical double-layer originating from the theory according to Stern-Gouy-Chapman.[92]

From examination of Fig. 3b, it is noticeable that the y axis is the electric potential (ψ) and ψ_0 which is located at the surface of the capillary wall has the largest potential. When moving across the x axis, which is the distance starting at the capillary

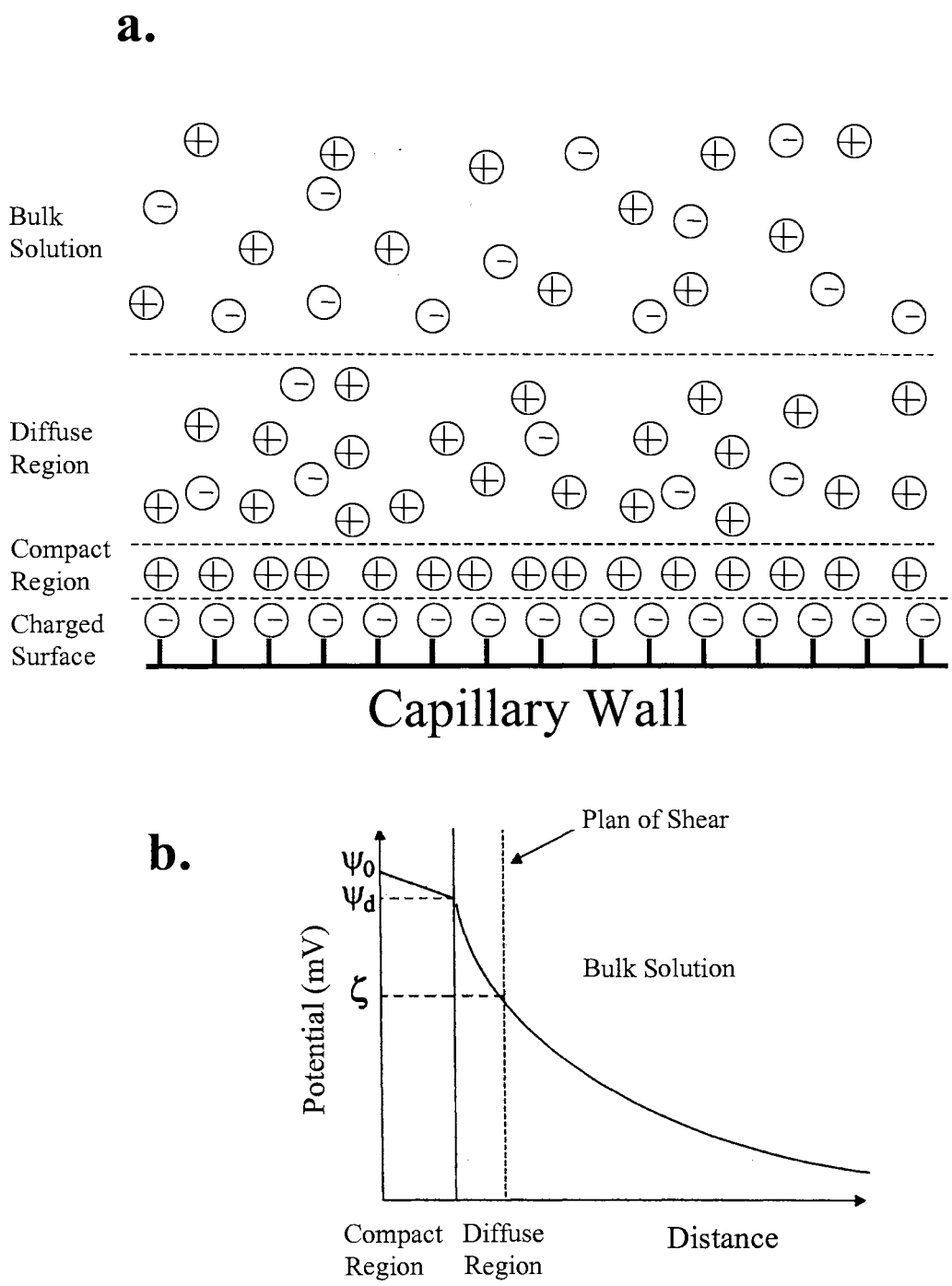

Figure 3. Schematic illustration of (a) the electric double-layer, (b) the electric double-layer potential gradient and vicinity region.

wall and moving inward, a linear decrease is observed. The linear decrease continues until ψ_d is reached which is the interface of the compact region and diffuse region. From ψ_d there is an exponential decay to ζ (the zeta potential), which is at the boundary of the diffuse region and bulk solution. Following the x axis through the bulk solution a drastic reduction in potential can be seen. The zeta potential is another characteristic used to define the potential at the plane of shear occurring when the liquid is forced to move. When an external voltage is applied, it causes the solvated cations forming the diffuse region of the electric double layer to migrate towards the cathode. As the cations move towards the cathode, they drag with them the bulk solution. Even though this diffuse layer is approximately 100 Å thick, the flow is transmitted throughout the bulk solution. This is the fundamental of electroosmotic flow (EOF) in CE. The magnitude of the EOF can be expressed in terms of velocity or mobility:[93]

$$v_{eo} = \mu_{eo} E = \frac{\varepsilon \zeta}{4\pi\eta} E \tag{8}$$

where μ_{eo} is the electroosmotic mobility. The zeta potential is governed by the surface charge on the capillary wall and is expressed as:[94]

$$\zeta = \frac{4\pi\delta\rho}{\varepsilon} \tag{9}$$

where ρ is the surface charge density of the capillary surface and δ is the thickness of the double layer. By implementing modern electrolyte theory which states that δ equals $1/\kappa$, equation (9) can be arranged to:

$$\zeta = \frac{4\pi\rho}{\kappa\varepsilon} \propto \frac{1}{\sqrt{I}} \tag{10}$$

where κ is the Debye-Hückel parameter and I is the ionic strength of the medium. Since ζ is directly proportional to the surface charge density on the capillary wall, and this surface charge is strongly dependent on pH, the magnitude of the EOF is also pH dependent. When the pH is high the silanol groups will be ionized and the EOF will be high, whereas when the pH is low the silanol groups are less ionized resulting in lower EOF. Furthermore, ζ is inversely proportional to $\sqrt{I}$ of the electrolyte as proposed in the electric double-layer theory. An increase in I will reduce EOF.

A unique feature of EOF is its flat profile due to electrical pumping action. In other chromatographic techniques, there is a laminar or parabolic flow profile due to mechanical pumping. Figure 4a depicts the EOF inside a capillary with electrical pumping action and Fig. 4b with mechanical pumping action.

Migration Time and Apparent Mobility

In CE, what actually is measured is the apparent mobility which contains both electrophoretic and eletroosmotic components. To obtain separation each of the solutes must have a unique apparent mobility (μ_{app}) which is given by:

$$\mu_{app} = \mu_{ep} + \mu_{eo} \tag{11}$$

where μ_{eo} and μ_{ep} are the electroosmotic and electrophoretic mobility, respectively. Likewise, the apparent velocity (v_{app}) of a solute is expressed by:

$$v_{app} = v_{ep} + v_{eo} = (\mu_{ep} + \mu_{eo})E = (\mu_{ep} + \mu_{eo})\frac{V}{L} \tag{12}$$

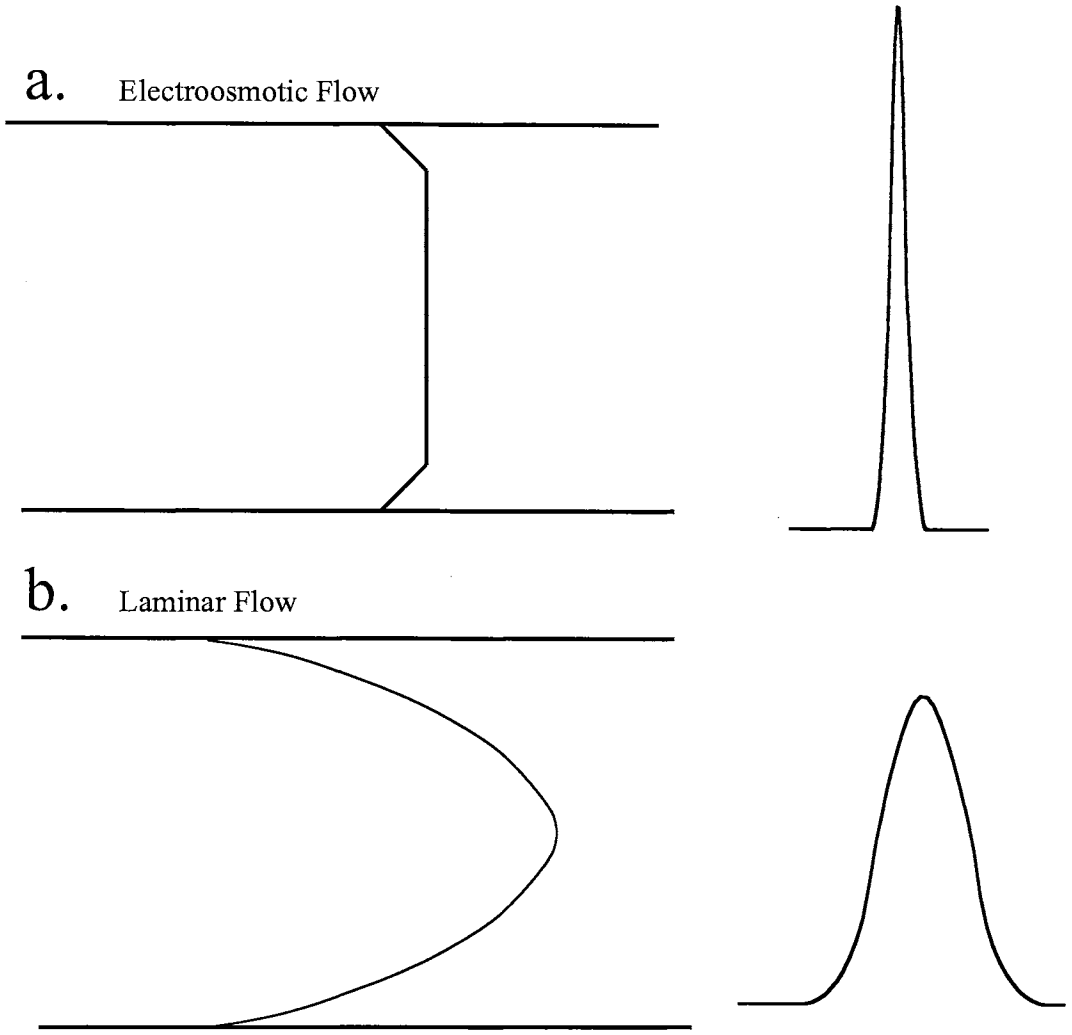

Figure 4. Schematic illustration of flow profiles and their effects on peak shape. (a) Plug profile when EOF is used as the driving force yielding sharper peaks as shown on the right hand side. (b) Laminar flow profile observed when a mechanical pump is used as the driving force yielding broad peaks as shown on the right hand side.

where V is the applied voltage, E is the applied electric field and L is the total length of the capillary. Migration time (t_M) is the actual time the analyte spends in the capillary from the moment of injection to detection, which is given by:

$$t_M = \frac{l}{v_{app}} = \frac{l}{(v_{ep} + v_{eo})} = \frac{lL}{(\mu_{ep} + \mu_{eo})V} \tag{13}$$

where l is the length of the capillary from the capillary inlet to the detection point. The migration time, t_o, of a neutral solute (i.e., EOF marker) can be expressed in terms of mobility through the following equations involving mobility and velocity:

$$\mu_{eo} = \frac{v_{eo}}{E} = \frac{lL}{t_o V} \tag{14}$$

Hence, the electrophoretic mobility, μ_{ep}, is given by:

$$\mu_{ep} = \mu_{app} - \mu_{eo} = \frac{v_{ep}}{E} = \frac{lL}{V}\left(\frac{1}{t_M} - \frac{1}{t_0}\right) \tag{15}$$

where μ_{app} is calculated using the observed migration time of the solute:

$$\mu_{app} = \frac{lL}{t_M V} \tag{16}$$

The direction (± sign) of μ_{ep} depends on the solute charge, and its sign is positive for cations and negative for anions.

Separation Efficiency

The separation efficiency in CE is typically very high compared to liquid chromatography (LC). There are several reasons for the higher separation efficiency. First and fare-most, there is no stationary phase in CE. Resistance to mass transfer between the stationary and mobile phases leads to band broadening. Another source of

band broadening in LC is dispersion (i.e., eddy diffusion and stagnant mobile phase) which are not important in CE. Since LC is a pressure driven process, frictional forces resulting from the mobile phase interacting with the walls of the capillary cause radial velocity gradients throughout the capillary. As a consequence of the wall drag, laminar or parabolic flow pattern are seen in Fig. 4b.

The separation efficiency in CE can be expressed by the number of theoretical plates, N, which is given by:

$$N = \left(\frac{l}{\sigma_l} \right)^2 \tag{17}$$

where σ_l is the standard deviation of the peak in unit of length. Under ideal conditions, longitudinal molecular diffusion (along the capillary) can be considered to be the only contributor to zone broadening. Thus, through the use of Einstein's law, the efficiency can be related to molecular diffusion:

$$\sigma_l^2 = 2Dt_M = \frac{2DlL}{\mu_{ep}V} \tag{18}$$

where D is the diffusion coefficient of the solute. By substituting equation (18) into equation (17), column efficiency can be expressed as:

$$N = \frac{\mu_{ep}Vl}{2DL} \tag{19}$$

When $l = L$, as in mass spectrometry or electrochemical detection, equation (19) becomes:

$$N = \frac{\mu_{ep}V}{2D} \tag{19a}$$

Equation (19a) shows that for large molecules (e.g., proteins, DNA, and RNA) N should reach millions of theoretical plates because of the small diffusion coefficient D. Usually, N can be calculated directly from the electropherogram using the same mathematical equation as that in liquid chromatography:[95]

$$N = 4\left(\frac{t_M}{w_i}\right)^2 = 5.54\left(\frac{t_M}{w_h}\right)^2 = 16\left(\frac{t_M}{w_b}\right)^2 \tag{20}$$

where w_i, w_h, and w_b are the peak widths at the inflection point, half-height and base, respectively.

Equations (17-20) predict several parameters that can be optimized for high separation efficiencies. Due to diffusion, the longer an analyte is in the column the lower the separation efficiencies. Thus, by using high running voltages the electrophoretic velocity increases and solutes tend to diffuse less. This is also seen in equation (19) where N is directly proportional to V.

Resolution and Selectivity

The selectivity, α, and resolution, R_s, for two adjacent zones is given by the following expressions:

$$\alpha = \frac{\Delta\mu_{ep}}{\overline{\mu}} = \frac{\Delta\mu_{ep}}{\overline{\mu}_{ep} + \mu_{eo}} \tag{21}$$

and

$$R_s = \frac{1}{4}\sqrt{N}\frac{\Delta\mu_{ep}}{\overline{\mu}} = \frac{1}{4\sqrt{2}}\Delta\mu_{ep}\sqrt{\frac{Vl}{DL\left(\overline{\mu}_{ep} - \mu_{eo}\right)}} \tag{22}$$

respectively. When $l = L$, equation (22) can be simplified to:

$$R_s = \frac{1}{4\sqrt{2}} \Delta\mu_{ep} \sqrt{\frac{V}{D(\bar{\mu}_{ep} - \mu_{eo})}} \tag{23}$$

In the last three equations, $\Delta\mu_{ep}$ and $\bar{\mu}$ are the difference in the electrophoretic mobility and average apparent mobilities of the two adjacent zones, respectively. The $\bar{\mu}_{ep}$ is the average electrophoretic mobility of the same adjacent zones. Equation (23) reveals that when the electrophoretic and electroosmotic mobility becomes equal, but opposite in sign, R_s approaches infinity. When these conditions are met, the analysis time will also approach infinity. It was shown that separation efficiencies are proportional to voltage, but resolution increases with the square root of voltage. Hence, voltage has less effect on resolution as compared to pH and composition of the running electrolyte, which largely influences $\Delta\mu_{ep}$ and in turn resolution.

Retention Factor and Resolution in MECC

An illustration of a neutral single-component separation in MECC is shown in Fig. 5. The fundamental equation for the retention factor (k') accounts for the presence of the mobile pseudo-stationary micellar phase. It is a modification of the equation for k' used in liquid chromatography:

$$k' = \frac{t_R - t_0}{t_0(1 - t_R / t_{mc})} \tag{24}$$

where t_{mc} is the time of the micelle. As the velocity of the micelle approaches zero, t_{mc} approaches infinity, thus this equation simplifies to the classical chromatographic expression for the retention factor, k'.

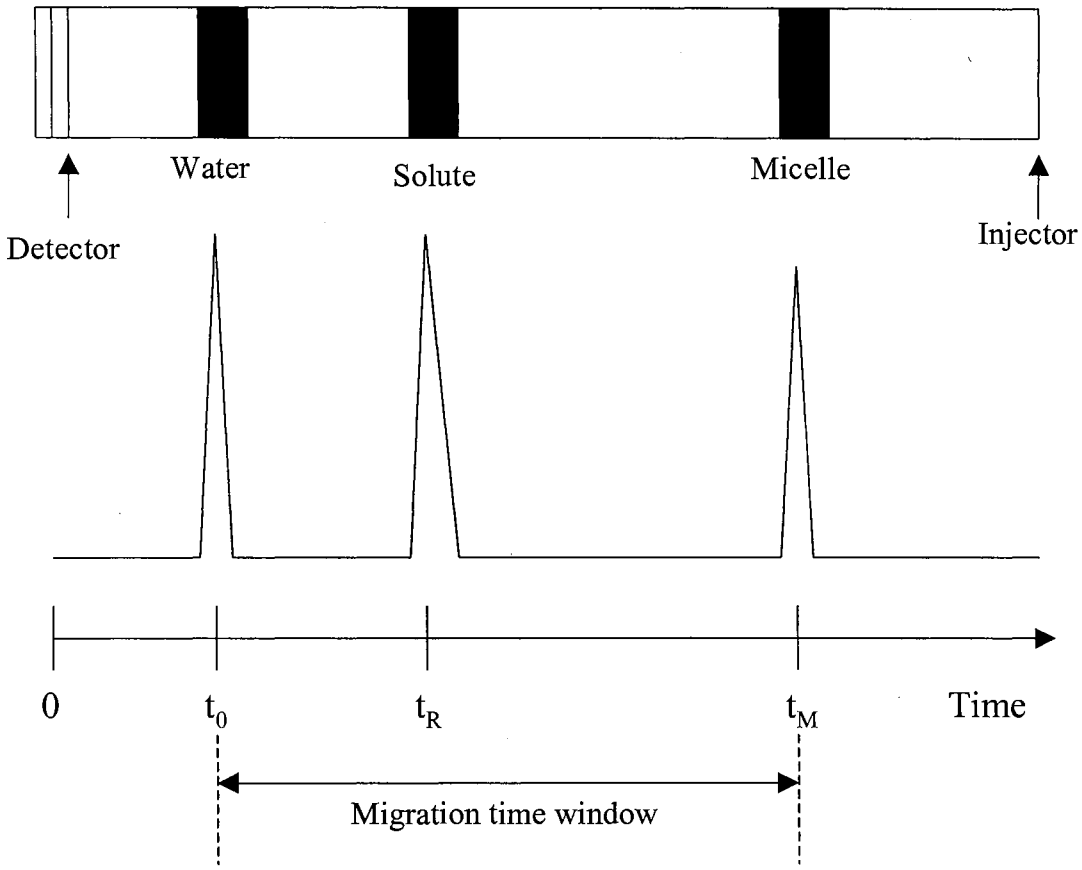

Figure 5. Schematic illustration of the separation principles in MECC (upper trace) and the detected electropherogram (lower trace) for a single component in MECC.

The resolution between two adjacent solutes in MECC is defined by:

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{k'_2}{1+k'_2}\right)\left(\frac{1-t_0/t_{mc}}{1+(t_0/t_{mc})k'_1}\right) \tag{25}$$

where α is the selectivity factor ($\alpha = k'_2/k'_1$) and t_0/t_{mc} is the elution range parameter. Since resolution is proportional to $\sqrt{N}$, the major players in optimizing resolution is α and t_0/t_{mc}. While α can be manipulated by varying the composition of the running electrolyte (e.g., pH, organic modifier, etc.), t_0/t_{mc}, which reflects the breadth of the

migration time window, can be adjusted by manipulating the surface charge density of the micelle and that of the capillary wall.

Like the MECC expression for k', the expression for resolution simplifies to the classical chromatographic equation when t_{mc} approaches infinity. The value of resolution between two adjacent peaks is directly calculated from the electropherogram by using the following expression:

$$R_s = \frac{2(t_{R2} - t_{R1})}{w_1 + w_2} \tag{26}$$

where w_1 and w_2 are the peak width at base for peaks 1 and 2, respectively. Equation 26 is valid for all electrophoretic and chromatographic methods.

Factors Affecting the Separation Efficiency in Open Tubes

Predicted through theory, equation (19) is the simplified calculation of N and does not account for all factors affecting band broadening. Since the magnitude of equation (20) is reflective of all band broadening processes that can occur simultaneously during solute migration, it will yield lower values than what is predict by equation (19). Thus, the total variance, σ_T^2, is predicted by the sum of all contributing variances:

$$\sigma^2_T = \sigma^2_D + \sigma^2_{\Delta T} + \sigma^2_i + \sigma^2_{\Delta ke} + \sigma^2_w \tag{27}$$

where the variances σ^2_D, $\sigma^2_{\Delta T}$, σ^2_i, $\sigma^2_{\Delta ke}$, σ^2_w are the contributions to the total variance from molecular diffusion, Joule heating, injection volume, conductivity differences, and solute-wall interaction, respectively. There is a large number of important contributors to solute zone broadening in CE shown in Table 1.

TABLE 1.

SOURCES OF ZONE BROADENING IN CE.

Source of zone band broadening	**Comments**
Longitudinal molecular diffusion	• Defines the fundamental limit of Efficiency • Solutes with lower diffusion coefficients form narrower zones
Joule heating	• Leads to temperature gradient and laminar flow
Injection plug length	• Injection length should be less than the diffusion controlled zone lengths • Dilute samples often necessitate longer than ideal injection lengths
Sample adsorption	• Interaction of solutes with capillary walls cause severe peak tailing
Conductivity differences	• Solutes with higher conductivities than the running buffer result in fronted peaks • Solutes with lower conductivities than the running buffer result in tailed peaks

Basic Principles of Capillary Electrochromatography

EOF in Packed Columns

Capillary electrochromatography (CEC) uses either fully or partially packed columns, typically with a silica-based material. Since silica and fused-silica (i.e., capillary wall) have the same properties, the flow will be generated not only from the capillary wall but also from the silica particles used to pack the column. In fact, the surface area of the silica particle is higher than that of the capillary wall. From this point of view, the packing having a much higher surface charged density would contribute to the EOF far more than that generated by the capillary wall. For illustration of overall EOF in packed capillaries, see Fig. 6. The overall EOF in packed capillary is not well understood. However, an expression for the average velocity of EOF, v_{eo}, generated in CEC by the stationary phase has been derived as:[96]

$$v_{eo} = -\frac{\varepsilon\varepsilon_0\zeta_p E}{\eta}\left(\frac{c^*}{c_b}\right) \tag{28}$$

where ζ_p is the zeta potential on the stationary particle, and c* and c_b are electrical conductivities of packed column and open column, respectively.

One limitation to equation (28) is that it does not account for the particle size of the packing. This seems to suggest that an infinite particle size could be used in CEC. It is well known that smaller particles in HPLC give better separation efficiencies. The disadvantage of using small particle in HPLC results in a large pressure drop, which is not seen in CEC due to EOF. Despite its being omitted in equation (28), particle size does

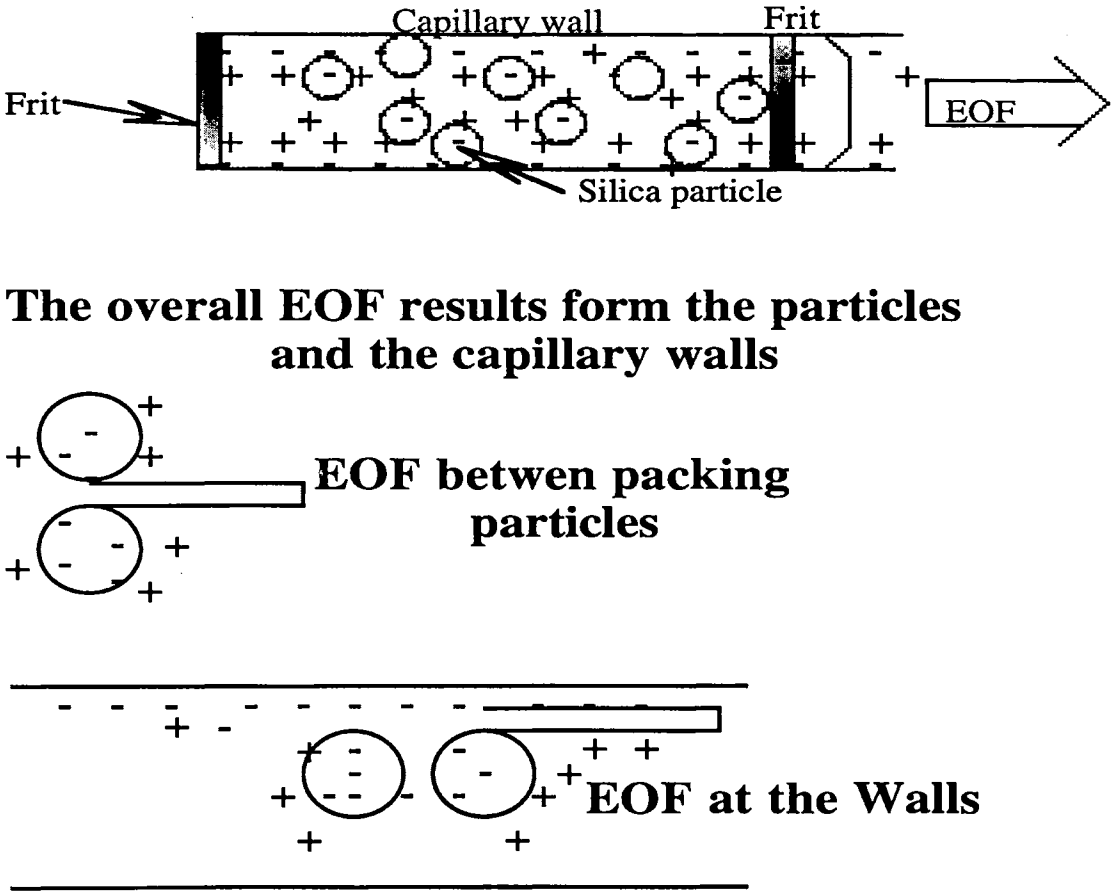

Figure 6. EOF in CEC.

have its limitation in CEC. When the particle size falls below 10 times that of the thickness of the electric double layer, a limit is reached. The theory is that double layers on the surface of the particles overlap and cancel each other out. This cancellation results in a dramatic decrease in the magnitude of EOF.

Migration Time and Mobility in CEC

The migration time in CEC of a neutral solute is similar to that in HPLC and it is expressed as:

$$t_r = t_o(1 + k') \tag{29}$$

where t_r is the retention time of the solute, t_o is the dead time and k' is the conventional chromatographic retention factor, $k' = (t_r - t_o)/t_o$. For charged analytes not only is the electroosmotic mobility and retention from the stationary phase should be accounted for but the electrophoretic mobility must also be taken into consideration. The apparent mobility of a charged solute in CEC, μ_c, is given by:[97]

$$\mu_c = \left(\frac{1}{1+k'}\right)\left(\mu_{ep} + \mu_{eo}\right) = \frac{\mu_{ep} + \mu_{eo}}{1+k'} \tag{30}$$

From equations 28 and 29, the retention time of a charged solute in CEC is expressed as:

$$t_r = t_o\left(\frac{\mu_{ep} + \mu_{eo}}{\mu_c}\right) \tag{31}$$

It can be seen that when μ_{ep} is zero (neutral solute), equation (31) reverts back to (29) which is for conventional CEC.

Retention Factor

When dealing with charged analytes in CEC, the electrophoretic mobility and chromatographic retention must be taken into account. The retention factor for a charged analyte in CEC, k_c', is expressed as:

$$k_c' = k' + k'k_e' + k_e' \tag{32}$$

where $k_e^{'}$ is the velocity factor and is expressed by the following equation:

$$k_e^{'} = \frac{\mu_{ep}}{\mu_{eo}} \tag{33}$$

For neutral solutes $k_e^{'} = 0$, therefore equation (32) becomes:

$$k_c^{'} = k^{'} = (t_r - t_o)/t_o \tag{34}$$

which is the case as in chromatography.

Selectivity Factor

Selectivity in chromatography is defined as the segregation of components between two distinct zones (peaks), and is determined by the ratio of retention factors of the specified components:

$$\alpha = \frac{k_2^{'}}{k_1^{'}} = \frac{\left(\frac{t_{r2} - t_0}{t_0}\right)}{\left(\frac{t_{r1} - t_0}{t_0}\right)} = \frac{t_{r2} - t_0}{t_{r1} - t_0} = \frac{t_{r2}^{'}}{t_{r1}^{'}} \tag{35}$$

where $k_1^{'}$ and $k_2^{'}$ are chromatographic retention factors, t_{r1} and t_{r2} are the retention times, and $t_{r1}^{'}$ and $t_{r2}^{'}$ are the adjusted retention times of respective components.

For charged analytes the selectivity factor is expressed as:

$$\alpha = \frac{k_{c2}^{'}}{k_{c1}^{'}} = \frac{k_2^{'} + k_2^{'}k_{e2}^{'} + k_{e2}^{'}}{k_1^{'} + k_1^{'}k_{e1}^{'} + k_{e1}^{'}} \tag{36}$$

For neutral solutes $k_e^{'} = 0$, and therefore $k_{c2}^{'} = k_2^{'}$ and $k_{c1}^{'} = k_1^{'}$ which gives the same equation as in (35).

Resolution

Resolution, R_s, expresses the extent of the overlap of two adjacent specified component zones (peaks). For neutral solutes resolution can be expressed by the product of a selectivity factor α, efficiency N and retention factor k' terms as follows:

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2'}{1 + k_2'}\right) \tag{37}$$

Band Broadening Factors in CEC

There are six major factors that contribute to band broadening in column chromatography and these include (1) eddy diffusion ($h_{eddy,dif}$), (2) axial molecular diffusion ($h_{a,diff}$), (3) mass transfer resistance in the mobile phase ($h_{trans,m}$), (4) mass transfer resistance in the stationary phase ($h_{trans,s}$), (5) extra column effects (such as injector, connector, detector, etc) (h_{extra}), and (6) joule heating (h_{joule}). The six contributors are basically summed resulting in the observed band broadening expressed as plate height:

$$H_{obs} = h_{eddy,diff} + h_{a,diff} + h_{trans,m} + h_{trans,s} + h_{extra} + h_{joule} \tag{38}$$

Unlike in pressure-driven liquid chromatography, in CEC the two process (1) and (3) do not contribute to the overall band broadening, due to the presence of electroosmosis. The phenomenon of electroosmosis is believed to be the major reason for higher chromatographic efficiency in CEC, see Fig. 7. As can be seen in this figure, while solute molecules travel different pathways and reach the column end over a time interval (band broadening by eddy diffusion) in pumped flow, they travel the same pathways with

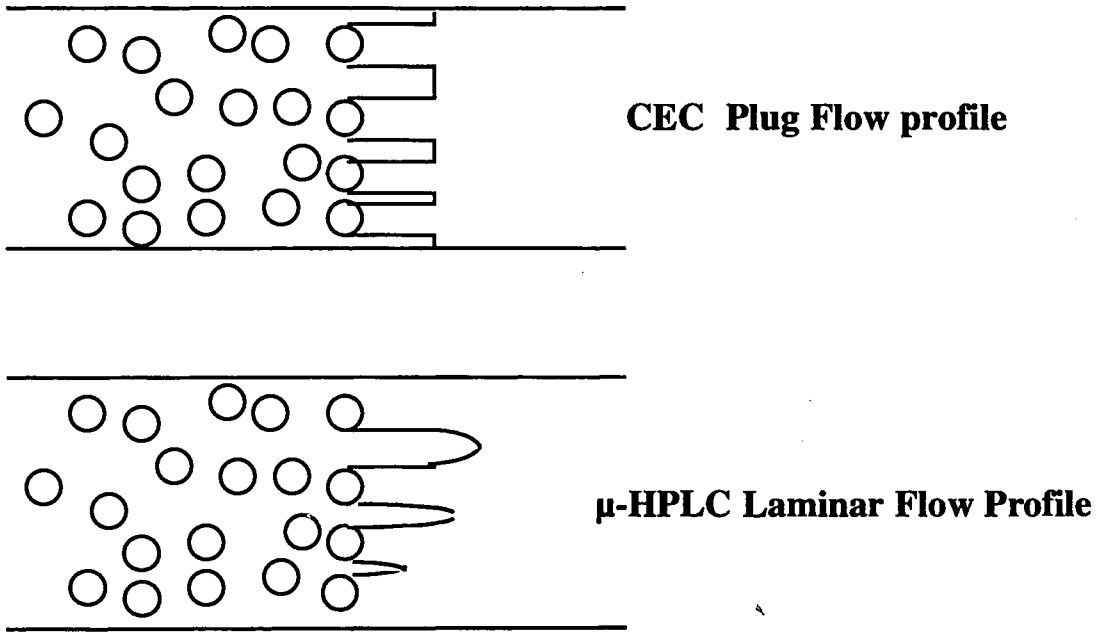

Figure 7. EOF versus pumped flow.

EOF. Also seen in Fig. 7 is the presence of inter particle mobile phase mass transfer resistance whereby solute molecules move faster in the middle of the channel than near the surface of the particles in pressure driven flow. Joule heating (6) is subdued by keeping the capillary diameter 100 μm or less, by using low buffer concentration, and by using applied voltages lower than 50 kV.

Conclusions

This chapter has outlined the scope of the dissertation and overviewed some of the basic principles of CE and CEC pertinent to the various interrelated studies conducted within the frame work of our investigation. Furthermore, this chapter shows that electrokinetic capillary chromatography (EKC), which consist of a host of capable separation methods, is well suited to solving analytical problems as the ones this dissertation has addressed.

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CHAPTER II

ELECTRICALLY DRIVEN MICROSEPARATION METHODS FOR PESTICIDES AND METABOLITES. I. MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY OF CARBAMATE INSECTICIDES WITH MEGA-BORATE AND SDS SURFACTANTS*

Introduction

Carbamate insecticides are the esters of carbamic acid (NH_2COOH) where one of the protons attached to the nitrogen atom is replaced by a methyl group. Thus, the name *N*-methyl carbamates (NMC) whose basic structure is CH_3NH-COO-R where R is either an aryl group or an aliphatic oxime. This leads to two major groups of NMC: aryl N-methyl carbamate and N-methylcarbamoyloximes.[1] In the present study, we investigated the electrophoretic behavior of both types of NMC, see Figure 1 for structures. Carbamates 1–3 belong to the latter, while structures 4–9 are representative of the former NMC.

N-Methyl carbamates were originally introduced to replace the organochlorine pesticides because of their effectiveness as insecticides and nematicides, their low

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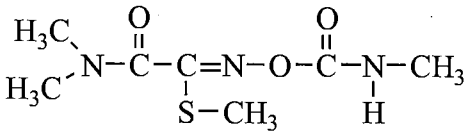
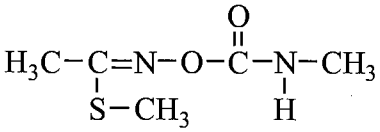
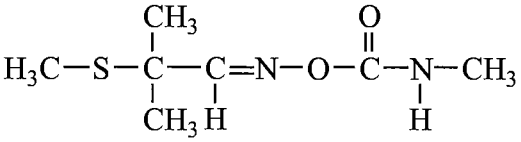


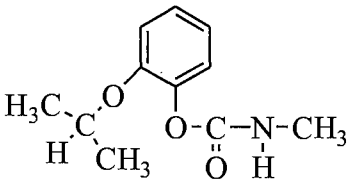
Oxamyl (Oxa) [1]



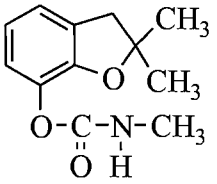
Methomyl (Meth) [2]



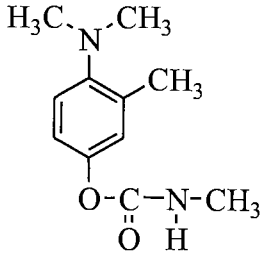
Aldicarb (Ald) [3]



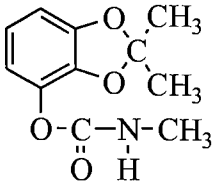
Baygon (Bay) [4]



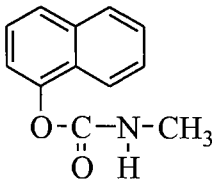
Carbofuran (Carn) [5]



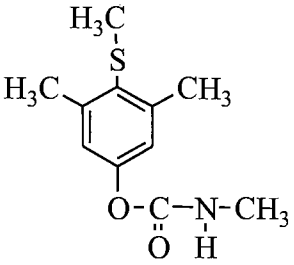
Aminocarb (Ami) [6]



Bendiocarb (Ben) [7]



Carbaryl (Car) [8]



Methiocarb (Met) [9]

Figure 1. Structures, abbreviations and MECC elution order of the carbamate insecticides used in this study.

mammalian toxicities and their low bioaccumulation potentials. NMC are widely used in agricultural set-ups to protect different kinds of crops from a variety of insects. Thus, there is a need for analytical methods for the determination of carbamate residues in fruits, vegetables and water to ensure food and drinking water safety. Also, the

availability of analytical methods for the control of the formulations of these insecticides is essential.

Thus far, high performance liquid chromatography (HPLC)[2,3] has been the most widely used technique for the determination of NMC, despite the recent significant progress made in electrically driven microseparation techniques [e.g., capillary electrophoresis (CE) and capillary electrochromatography (CEC)], and especially the proven potentials of CE in pesticide analysis.[4] There is a need for complementary techniques such as HPLC and CE on which control laboratories can rely to reduce false positives in the determination of pesticides. Despite the fact that CE already proved useful for the separation of several pesticides,[4] systematic studies involving MECC of NMC are scarce.[5,6]

This chapter is concerned with the study of the electrokinetic behavior of NMC in micellar electrokinetic capillary electrochromatography (MECC). MECC is the CE method of choice for the electrophoretic separation of neutral species such as NMC. To better understand the underlying phenomena, two different micellar systems were evaluated, namely the traditionally used sodium dodecyl sulfate (SDS) micellar phase [7] and the recently introduced in situ charged glycosidic micelles. In situ charged glycosidic micelles, which have been introduced by us in 1992,[8] have been shown very useful in CE of a wide variety of pesticides.[9-15] In situ charged glycosidic micelles are based on the complexation of the sugar head group of the glycosidic surfactant with borate. This allows the manipulation of the migration time window by conveniently varying the pH, surfactant concentration and borate concentration in the running electrolyte.[8-15]

Experimental

Instrument

Separations were performed on a home-built capillary electrophoresis instrument comprised of a positive and negative high-voltage power supply Model CZE1000/PN/R from Spellman (Plainview, NY, USA) and a UV-Vis variable wavelength detector Model SC100 from Thermo Separation Products (San Jose, CA, USA). Unless stated otherwise, both alkyl phenyl ketones (APK) and NMC were detected at 254 nm. Electropherograms were recorded with a computing integrator Model CR4A from Shimadzu (Columbia, MD, USA). All separations were carried out in fused-silica capillaries (Polymicro Technologies, Phoenix, AZ) of dimensions 50 μm i.d. x 365 μm o.d., 50 cm to the detector, 80 cm total length.

Reagents and Materials

The surfactant decanoyl-*N*-methylglucamide (MEGA 10), see Figure 2 for structure, was purchased from Anatrace (Maumee, OH, USA). Urea was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). Boric acid was from Fisher Scientific (Fair Lawn, NJ, USA). Sodium phosphate monobasic was from Mallinckrodt (Paris, KY). The carbamate insecticides (see Figure 1 for structures and abbreviations) were purchased from ChemService (West Chester, PA, USA). Sodium dodecyl sulfate (SDS) and alkyl phenyl ketones (APK), namely, acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone and heptanophenone, were from Sigma Chemical Co.

(St. Louis, MO, USA). All solutions were passed through a 0.2 μm Titan syringe filter obtained from Scientific Resources, Inc. (Eatontown, NJ, USA).

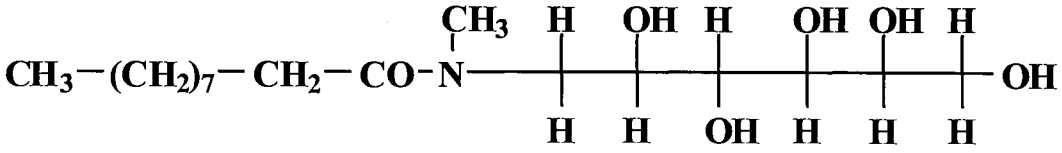


Figure 2. Structure of the MEGA 10 surfactant.

Field-Amplified Sample Stacking (FASS)

On-column concentration was performed according to the FASS procedure reported by Chien and Burgi.[16,17] Briefly, about 70% of the capillary length to the detection point was pressure filled with the dilute carbamate sample prepared in 25 mM borate, 25 mM MEGA 10, pH 9.0, and then a voltage of 24 kV was applied in the negative polarity mode (i.e., the cathode at the inlet end and the anode at the outlet end of the capillary). A capillary of 50 cm long and 50 μm I.D. offers a volume of ca. 982 nL. Thus, when this capillary is 70% filled with a dilute sample, the volume introduced into the capillary is about 687 nL. Upon the application of 24 kV, the current initially was 17μA due to the difference in conductivity between the running electrolyte and the large dilute sample plug. The running electrolyte was 1.0 *M* borate, 10 m*M* sodium phosphate, 125 m*M* MEGA 10, pH 7.0. As the aqueous sample plug was pumped out of the column by the cathodic EOF, the current kept increasing until it reached its normal value (85 μA) in the absence of the sample plug. At this time, the polarity of the system was switched back to the normal configuration (i.e., anode at the capillary inlet and cathode at capillary outlet) and the electrophoretic separation was performed.

Methods

In the case of MEGA 10 surfactant, the running electrolyte was prepared by dissolving proper amounts of boric acid, sodium phosphate, and MEGA 10 in water, and the pH was adjusted to 7.0 with sodium hydroxide. With SDS surfactant the running electrolyte was prepared by dissolving proper amounts (see figure captions) of boric acid and SDS in water, and adjusting the pH to 8.0 with sodium hydroxide. All samples were dissolved in HPLC grade acetonitrile form Burdick and Jackson (Muskegon, MI, USA) and injected neat. Sample injection was performed by hydrodynamic injection from a height of 10 cm above the outlet reservoir for a time of one second. Between runs the capillary was rinsed consecutively with 0.1 M NaOH, water and the running electrolyte.

In all calculations involving efficiency, the plate count was estimated from peak standard deviation taken as the half width at 0.607 of peak height and was reported as the average of at least two runs. The migration time of the unretained species, t_o, was determined by the deflection peak of acetonitrile. The migration time of the micelle, t_{mc}, was determined by the iterative method using alkyl phenyl ketones.[9]

Results and Discussion

Comparison of MEGA 10 and SDS

Figures 3 and 4 show the separation of 9 carbamate insecticides using MEGA 10 and SDS micellar systems, respectively, under optimum conditions. As expected, both systems yielded different selectivity. In fact, peaks of aminocarb (Ami) and bendiocarb

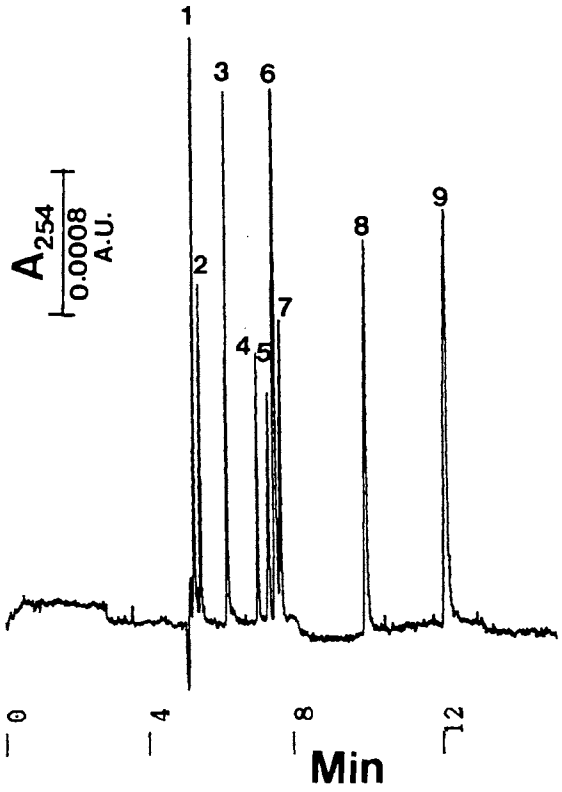


Figure 3. Electropherogram of the carbamate insecticides. Experimental conditions: running electrolyte, 1.0 *M* borate 10 m*M* sodium phosphate, 125 m*M* MEGA 10, pH 7.0; capillary, untreated fused silica, 80 cm (total length) x 50 μm I.D., 50 cm to detection point; voltage, 24 kV. Analytes: 1, Oxa; 2, Met; 3, Ald; 4, Bay; 5, Carn; 6, Ami; 7, Ben; 8, Car; 9, Met.

(Ben) switched order when going from MEGA-borate to SDS micellar systems. Furthermore, the separation of the 9 carbamate insecticides under investigation in the presence of SDS required the addition of 4.0 M urea to the running electrolyte. The SDS micellar system did not separate the carbofuran (Carn) and Ben insecticides without added urea. The resolution between Ben and Carn increased from 0 to 1.02 when going from 0 to 4.0 M urea. This contrasts the data obtained with MEGA 10 whereby the addition of urea to MEGA 10 surfactant resulted in no separation between the Carn and

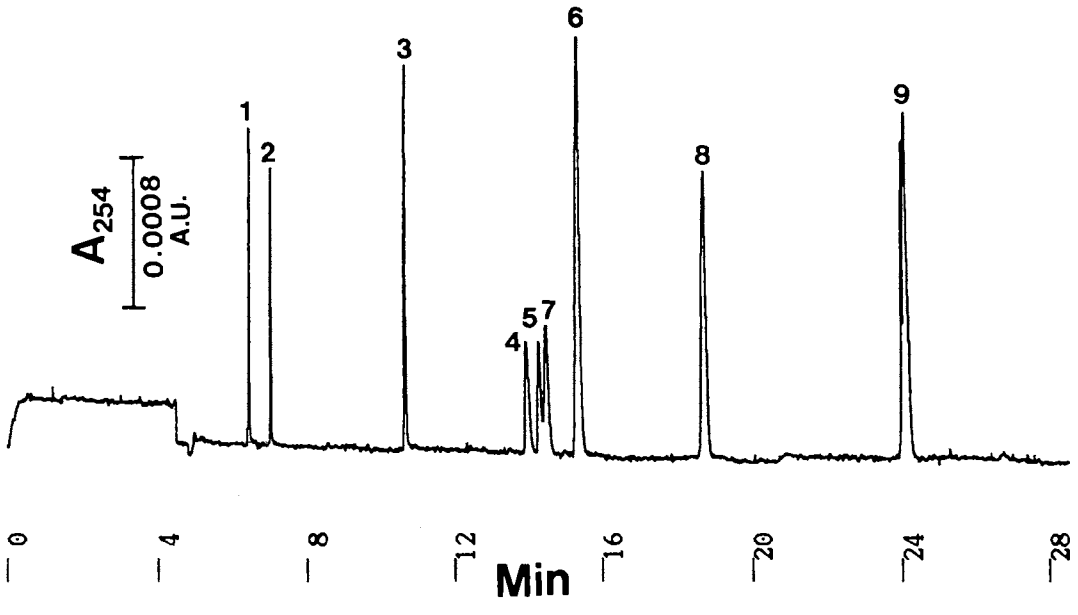


Figure 4. Electropherogram of the carbamate insecticides. Experimental conditions: running electrolyte, 100 m*M* borate, 100 m*M* SDS, 4.0 *M* urea, pH 8.0. All other conditions are the same in Figure 3. Analytes: 1, Oxa; 2, Met; 3, Ald; 4, Bay; 5, Carn; 6, Ami; 7, Ben; 8, Car; 9, Met.

Ben. In the absence of urea, the resolution for the two insecticides with MEGA 10 as the surfactant was 2.54 as opposed to no resolution in the presence of urea.

The analysis time with MEGA 10 is half that obtained with the SDS micellar system (compare Figures 3 and 4). This is in part due to the presence of 4.0 M urea in the SDS micellar system which increases the viscosity of the running electrolyte and in turn decreases the electroosmotic flow. The migration time window for the MEGA 10 extends between a t_o = 5.2 min and a t_{mc} = 14.3 min yielding a ratio t_{mc}/t_o = 2.75, while the migration time window for SDS spans between a t_o = 4.75 min and a t_{mc} = 32.7 min giving a ratio of t_{mc}/t_o = 6.88. In the absence of 4.0 M urea, the migration time window of the SDS micellar system extends between a t_o = 4.32 min and a t_{mc} = 14.78 min,

which corresponds to a t_{mc}/t_o ratio equals to 3.43, a slightly higher ratio than that obtained with the MEGA 10-borate system.

The average separation efficiencies for the SDS and MEGA systems were about the same 169,542 and 162,825, respectively. However, the MEGA system yielded 13,422 plates/min as opposed to 7044 plates/min with the SDS system. When comparing Figures 3 and 4, one can readily see the advantage of in situ charged micelles (i.e., MEGA 10-borate complex) over the traditionally used micellar systems (e.g., SDS) of predetermined migration time window and relatively strong hydrophobic character. With in situ charged micelles the migration time window is a freely adjustable parameter that can be manipulated by varying the pH and borate and surfactant concentration to suit a given separation problem in the shortest analysis time possible.[8-15]

In addition, due to its relatively weak hydrophobic character (i.e., possessing a large polyolic polar head group and an alkyl chain with 2 fewer carbon atoms), the MEGA 10-borate micellar system allowed more equitable distribution of the carbamate solutes between the mobile and stationary phases than did the SDS micellar system. The k' values of the various carbamates and the selectivity factor α are shown in Table 1. The k' values of the carbamates obtained with the MEGA 10 micellar system are much less than those obtained with the SDS-urea micellar system. Conversely, in most cases, α is higher with MEGA 10 than with SDS. In the absence of urea, the retention of the carbamates with SDS are substantially higher than those obtained in the presence of urea. Typically, the k' values for Bay, Carn/Ben, Ami, Car and Met are 3.38, 3.57/3.68, 4.2, 6.87 and 15.41, respectively, in the presence of urea as opposed to 6.17, 7.46/7.46, 10.45,

TABLE 1.

RETENTION FACTOR, k', AND SELECTIVITY, α, OF CARBAMATES INSECTICIDES OBTAINED WITH MEGA 10 AND SDS MICELLAR SYSTEMS. CONDITIONS AS IN FIGURES 3 AND 4.

Solutes	MEGA 10		Solutes	SDS	
	k'	α		k'	α
Oxa	0.03		Oxa	0.44	
		2.80			1.39
Met	0.084		Met	0.61	
		4.17			3.03
Ald	0.35		Ald	1.85	
		2.03			1.82
Bay	0.71		Bay	3.38	
		1.21			1.06
Carn	0.86		Carn	3.57	
		1.12			1.03
Ami	0.96		Ben	3.68	
		1.10			1.14
Ben	1.06		Ami	4.20	
		2.79			1.64
Car	2.96		Car	6.87	
		2.96			2.24
Met	8.75		Met	15.41	

18.67 and 74.29 in the absence of urea. Similarly, at constant surfactant and borate concentration and pH, the k' values for carbamate insecticides decreased monotonically as the amount of added urea increased (results not shown). At 100 mM MEGA 10 in 10 mM sodium phosphate containing 1.0 M borate, pH 7.0, the retention factor of Bay, Carn, Ben, Ami, Car and Met decreased (almost by 50%) from 0.62, 0.75, 0.80, 0.93, 2.58 and 6.88, respectively, in the absence of urea to 0.35, 0.39, 0.39, 0.52, 1.29 and 3.10 in the presence of 4.0 M urea.

Electrokinetic Behavior of MEGA 10-borate

It is well established that the magnitude of borate complexation is high in very alkaline solutions, and reaches a plateau at pH ≥ 12.[18] However, by using relatively high borate concentration (e.g., 1.0 M boric acid) at pH 7.0,[15] it is possible to yield a MEGA 10 micellar system that has a migration time window whose ratio t_{mc}/t_o is comparable to what can be achieved at pH 10 using 200 mM borate.[12] By changing the borate concentration over a wide range it was determined that at pH 7.0, 1.0 M boric acid was the optimum concentration for maximum resolution of the 9 carbamates.

To assess the approximate CMC value of the MEGA 10-borate system under investigation, a series of experiments involving various MEGA 10 concentrations at fixed pH and borate concentration were conducted using some carbamates and alkyl phenyl ketones (APK). In MECC, according to the following equation the retention factor k' is a linear function of the surfactant concentration:[19]

$$k' = \phi K \approx K v([S] - CMC) \qquad (1)$$

where K is the distribution coefficient of solute between micellar and aqueous phases, ν is the partial specific volume of the micelle, [S] is the concentration of the surfactant and CMC is the critical micellar concentration. The x-intercept of the line is [S] = CMC. Tables 2 and 3 summarize the data resulting from plotting k' of solutes versus MEGA concentration in the absence and presence of urea in the running electrolyte. The average CMC as determined from the plots of the carbamate insecticides was 10.5 mM and that as measured from plots of APK was 10.1 mM.

TABLE 2.

Y-INTERCEPTS, X-INTERCEPTS (CMC) AND SLOPES OF PLOTS OF log k' OF CARBAMATE INSECTICIDES VERSUS THE CONCENTRATION OF MEGA 10 IN THE ABSENCE (0 M) AND PRESENCE OF UREA (4 M). CONDITIONS: 10 mM SODIUM PHOSPHATE, 1 M BORIC ACID, pH 7.0. OTHER CONDITIONS ARE AS IN FIGURE 3.

Solute	y-intercept -Kν(CMC)		x-intercept CMC		Slope x 10^3 Kν		R	
	0 M	4.0 M	0 M	4.0M	0M	4.0 M	0M	4.0M
Bay	-0.078	0.024	12.6	-7.5	6.2	3.2	0.997	0.977
Carn	-0.086	0.006	11.3	-1.6	7.6	3.8	0.998	0.983
Ami	-0.10	0.006	12.0	-1.6	8.3	3.8	0.998	0.983
Ben	-0.11	0.008	11.8	-1.6	9.3	5.0	0.998	0.987
Car	-0.25	-0.056	10.0	4.2	25.1	13.5	0.997	0.991
Met	-0.34	-0.11	5.5	3.4	61.8	32.0	0.979	0.977

TABLE 3.

Y-INTERCEPTS, X-INTERCEPTS (CMC) AND SLOPES OF PLOTS OF log k' OF APK VERSUS THE CONCENTRATION OF MEGA 10 IN THE ABSENCE (0 M) AND PRESENCE OF UREA (4 M). CONDITIONS: 10 mM SODIUM PHOSPHATE, 1 M BORIC ACID, pH 7.0. OTHER CONDITIONS ARE AS IN FIGURE 3.

Solute	y-intercept -Kv(CMC)		x-intercept CMC (mM)		Slope x 10^3 Kv		R	
	0 M	4.0 M	0 M	4.0M	0M	4.0 M	0M	4.0M
Acetophenone	-0.068	-0.008	12.6	2.6	5.4	3.1	0.997	0.999
Propiophenone	-0.14	-0.052	11.5	7.3	12.2	7.1	0.996	0.999
Butyrophenone	-0.30	-0.15	11.0	9.5	27.2	15.8	0.997	0.999
Valerophenone	-0.66	-0.41	10.1	11.1	65.3	36.9	0.997	0.999
Hexanophenone	-1.29	-1.06	8.1	12.0	159.0	88.2	0.997	0.998
Heptanophenone	-2.58	-2.94	7.2	14.8	356.0	198.0	0.997	0.997

Performing the same experiments as in the preceding section but in the presence of 4.0 M urea yielded k' vs. [S] plots whose x-intercept values (i.e., CMC values) were scattered over a range of 2.6 to 14.8 mM for the APK, and averaging to a CMC of 7.6 M. k' vs. [S] plots for the carbamate insecticides gave negative values for the CMC. This would suggest that MEGA 10 is being affected in a negative way in the presence of urea. In other words, the addition of urea at the level of 4.0 M seems to disrupt the formation of the MEGA 10-borate micelle. Urea is a water structure breaker, a fact that leads to (i) an increase in the hydration of the hydrophilic group of the surfactant and (ii) disruption of the organization of the water produced by the dissolved hydrophobic group, thereby decreasing the entropy increase on micellization.[20] Since the hydration of the hydrophilic group and the decrease in entropy oppose micellization, the net result is an increase in

CMC.[20] This means a higher bulk concentration of surfactant is needed for micelle formation. The results shown in Tables 2 and 3 for carbamates and APK in the presence of urea shows either scattered values or negative values for the calculated CMC. This may indicate that at 4.0 M urea, the micellization of the MEGA 10 surfactant is partially disrupted so that Eqn 1 (page 54) may not describe any more the behavior of these systems. The linearity of k' vs. [S] plots may still be conserved because as the surfactant concentration is increased the phase ratio is increased and in turn k'.

The adverse effects of urea on the MEGA 10 system have also manifested themselves by a lower reproducibility and separation efficiency. In fact, and on the average the %RSD for the migration times of Bay, Carn, Ben, Ami, Car and Met was found to be 3.4 in the absence of urea as opposed to 5.0 in the presence of urea. A similar trend was also found with the %RSD of the migration time of APK. For these solutes the average %RSD was found to be 1.7 in the absence of urea as compared to an average of 3.9 in the presence of urea. Also, the average separation efficiency calculated for 4 carbamates, namely Bay, Ami, Car and Met was 103,000 in the absence of urea as opposed to 61,000 in the presence of urea using 125 mM MEGA 10.

Limits of Detection and On-Column Sample Preconcentration

Table 4 lists the limits of detection (LOD) for six representative carbamate insecticides determined under the conditions used in Figure 3. The LOD were measured at the λ_{max} of the specific absorption band of each solute. As one would expect, the LOD is an inverse function of the molar absorptivity (ε) of the species. In fact, Car with

TABLE 4.

λ_{max}, MOLAR ABSORPTIVITY, ε, LOD AND MOLAR CONCENTRATION DETERMINED AFTER ON-COLUMN SAMPLE PRECONCENTRATION BY FASS. FOR FASS, SEE EXPERIMENTAL SECTION.

Solutes	λ (nm)	ε ($M^{-1}cm^{-1}$)	LOD (M)	FASS (M)
Oxa	248	6.1×10^3	1.7×10^{-3}	
Ald	248	2.0×10^3	1.6×10^{-3}	
Bay	270	1.8×10^3	2.9×10^{-3}	1.0×10^{-4}
Carn	282	3.5×10^3	8.3×10^{-4}	
Car	281	6.1×10^3	2.5×10^{-4}	9.9×10^{-6}
Met	266	2.6×10^3	8.0×10^{-4}	

the highest ε yielded the lowest LOD which is about one order of magnitude lower than the LOD exhibited by Ald whose ε is lower. These LOD are not sufficiently low to allow the determination of pesticides in environmental samples. However, environmental samples are usually subjected to various sample manipulations (e.g., sample clean-up, extraction, sample size reduction by solvent evaporation, etc.) before their analysis is carried out. These pre-analysis steps, and especially extraction (e.g., liquid-liquid extraction and more recently solid-phase extraction) and solvent evaporation bring about the enrichment of the analytes of interest. Following, the enriched and cleaned-up sample may be ready for analysis or may still require an on-column sample preconcentration to achieve an additional enrichment factor of 30 fold or less.

In CE, on-column trace enrichment methods for solutes including pesticides based on field amplified sample stacking (FASS),[21] on-column solid-phase preconcentration,[22]

and more recently sweeping by micelles[23] have been described. Among these three different approaches, FASS is the most useful method with the MEGA-borate micelle used in our studies. On-column solid phase extraction is excluded because in the presence of a surfactant such as the MEGA, the surfactant molecules will compete with the solute to the binding sites on the solid phase, thus lowering the effectiveness of the preconcentration. The sweeping approach, introduced very recently by Quirino and Terabe,[23] is only effective under conditions of negligible EOF, which is usually obtained at low pH. The low pH condition does not apply to the MEGA-borate system described in this report because the MEGA micelle is charged via complexation with borate ion at high pH.

FASS proved to be very effective in on-column preconcentration before CE separation. FASS is based on the fact that the electrophoretic velocity of an ion depends linearly on the field strength. In FASS, the region of the sample has a lower conductivity than the supporting electrolyte. Therefore, the analyte experiences locally an increased field strength and migrates at higher velocity. When reaching the background electrolyte, the ions experience a lower electric field in the supporting electrolyte than in the sample region, and consequently the velocity of the ions decreases as they cross the stationary boundary. The slower moving ions will stack up into a smaller volume, thereby increasing the analyte concentration in the sample zone.[16,17,24,25] Although FASS is powerful, only a few attempts have been made so far to introduce FASS for trace enrichment in MECC of neutral solutes such as the carbamate insecticides.[26,27]

In MECC, neutral solutes acquire an effective electrophoretic mobility, $\mu_{ep,eff}$, through their association with the micelle as follows:[19]

$$\mu_{ep,eff} = \left(\frac{k'}{1+k'} \right) \mu_{ep,mc} \qquad (2)$$

where $\mu_{ep,mc}$ is the electrophoretic mobility of the micelle. According to Eqn 2 the stronger the association of the solute with the micelle (i.e., large k') the higher the effective electrophoretic mobility of the solute and the better the stacking.

Two typical carbamate insecticides, namely Bay and Car, were selected to show the feasibility of sample stacking by FASS in MECC with neutral solutes. As can be seen in Figure 3, Bay is much less retained than Car under otherwise the same conditions. As indicated in the experimental section, the sample for stacking by FASS was prepared in 25 mM MEGA 10, 25 mM borate, pH 9.0. Using this low ionic strength electrolyte as the running electrolyte, the k' values for Bay and Car were determined to be 0.21 and 1.05, respectively. Based on these k' values, and from Eqn 2, the effective electrophoretic mobilities of Bay and Car are -7.3 x 10^{-5} $cm^2/V.s$ and -2.2 x 10^{-4} $cm^2/V.s$, respectively, which correspond to 17% and 51% of the electrophoretic mobility of the MEGA-borate micelle, which is -4.3 x 10^{-4} $cm^2/V.s$. Under these conditions, the effective electrophoretic mobility of Car is about 3 fold higher than that of Bay.

As shown in Table 4, FASS allowed the detection of 1.0 x 10^{-4} M and 9.9 x 10^{-6} M for Bay and Car, respectively, which are 25 and 29 fold lower than the LOD of Car and Bay, respectively. FASS resulted in similar enrichment for Bay and Car despite the fact that Car exhibited higher effective electrophoretic mobility than Bay. The effectiveness of sample enrichment is also determined by the solubility of the solute in the separation medium. The molar solubilities of Bay and Car in water are 9.6 x 10^{-3} M and 6.0 x 10^{-4} M, respectively.[28] For Car, the gain in preconcentration by FASS due to

its relatively higher k' value is offset by its lower molar solubility. In fact, if we assume (i) that the large sample plug of about 687 nL (see experimental section) shrinks down to 8 nL (i.e., 4 mm in length) when the stacking is finished, and (ii) that no sample loss during FASS occurs, a concentration factor of 86 will be readily realized. A concentration factor of 86 should have allowed the FASS determination of 2.9×10^{-6} M and 3.4×10^{-5} M for Car and Bay, respectively. These values are obtained by simply dividing the LOD values by 86. The fact that the observed FASS for Car is 9.9×10^{-6} M which is higher than the actual or expected FASS (2.9×10^{-6} M) may indicate that the amount of Car exceeding the molar solubility has precipitated out during sample stacking. In the case of Bay whose k' value is relatively low, the stacking was not very effective and yielded a relatively broad peak, a phenomenon that may explain the higher concentration of the observed FASS (1.0×10^{-4} M) than the actual or the expected FASS (3.4×10^{-5} M). In summary, these preliminary results indicate that more in-depth studies are needed to better understand the stacking of neutral solutes in MECC.

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CHAPTER III

ON-COLUMN TRACE ENRICHMENT BY SEQUENTIAL FRONTAL AND ELUTION ELECTROCHROMATOGRAPHY. I. APPLICATION TO CARBAMATE INSECTICIDES*

Introduction

In capillary electrochromatography (CEC), the mobile phase is transported through a capillary containing a stationary phase without a pressure drop by means of electroosmosis. The use of electroosmotic flow (EOF) as the driving force for differential migration yields a plug-like flow profile which results in a relatively high separation efficiency.[1-3] This high separation efficiency combined with the unique selectivity of the stationary phase have made CEC a microseparation technique of high resolving power very suitable to a wide variety of applications (for very recent reviews, see Refs [4,5]). However, CEC suffers from its rather poor concentration sensitivity with on-line photometric detection (e.g., UV-Vis detection), a fact that makes CEC not directly amenable to many real applications, especially those of biological and environmental significance where samples to be analyzed are usually very dilute. Therefore, there is a

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strong need for on-column trace enrichment (i.e., on-line preconcentration) schemes to enhance concentration detection limits.

The present investigation is concerned with (i) the introduction of an on-column trace enrichment method for dilute samples based on sequential frontal and elution electrochromatography and (ii) the application of the method to carbamate insecticides. This chapter is considered as a continuation to our very recent initial studies in the area of on-line preconcentration in CEC of pesticides [6,7] where the trace enrichment was based on introducing a relatively large plug of a dilute sample preceded by the introduction of a short plug of pure water. The plug of water produced zone narrowing and consequently concentration enhancement. The present investigation involved on-line preconcentration by frontal electrochromatography under conditions of strong solute binding to the stationary phase followed by step-gradient elution electrochromatography with a mobile phase of high eluting strength, see next section.

Thus far, only two articles have addressed the sensitivity enhancement in CEC but with limited success as far as the concentration detection limit is concerned.[8,9] As will be shown below, our studies involved systematic studies over a wide range of conditions in order to find the optimum conditions for on-column trace enrichment and to better understand the underlying phenomena.

Theoretical Treatment

The on-column trace enrichment method developed in this study comprised two distinct steps: a frontal electrochromatography step followed by an elution electrochromatography step, see Fig. 1. The dilute sample is first applied to the column

in a relatively large volume under conditions of strong solute binding to the stationary phase. In this frontal electrochromatography step, and in principle, solute molecules will accumulate at the column entrance in a relatively narrow band (i.e., zone compression or narrowing), see Fig. 1a. After this stationary phase mediated focusing step, the solutes molecules are swept with a mobile phase whose organic modifier content is relatively high to bring about the rapid elution and separation (i.e., elution electrochromatography by a step-gradient) of the enriched solute band. This step-gradient brings about a focusing effect on the enriched solute band, see Fig. 1b.

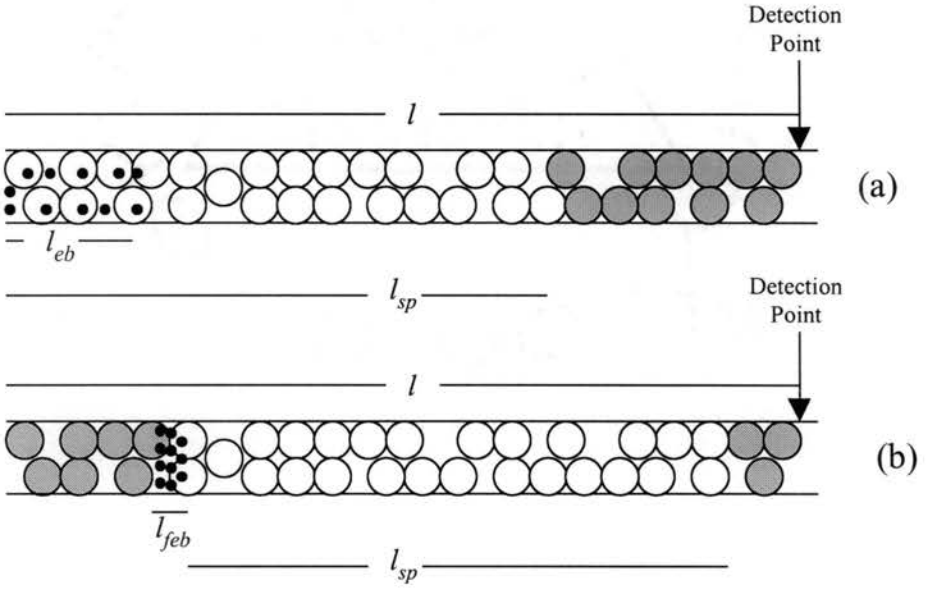

Figure 1. Schematic illustration of trace enrichment by frontal electrochromatography in (a) followed by zone focusing during step-gradient by elution electrochromatography. *l*, length of column to detection point; l_{eb}, length of enriched band; l_{sp}, length of sample plug; l_{feb}, length of focused enriched band.

The effectiveness of the on-column trace enrichment is largely influenced by the affinity of the individual solutes to the stationary phase during the frontal electrochromatography step. The stronger the retention of the solute the narrower the

concentrated band at the top of the column. In fact, and under a given mobile phase flow velocity u (i.e., electroosmotic flow velocity), the average solute velocity $\bar{\upsilon}$ is directly proportional to the fraction of time the solute spends in the mobile phase as follows:

$$\bar{\upsilon} = u\left(fraction\ of\ time\ the\ solute\ spends\ in\ the\ mobile\ phase\right) \quad (1)$$

Equation (1) is equivalent to writing

$$\bar{\upsilon} = u\left(\frac{moles\ of\ analyte\ in\ mobile\ phase}{total\ moles\ of\ analyte\ in\ mobile\ and\ stationary\ phases}\right) \quad (2)$$

thus, yielding the dependence of the average solute's velocity on its retention factor k' as follows:

$$\bar{\upsilon} = u\left(\frac{1}{1+k'}\right) \quad (3)$$

With ideal plug flow profile as in CEC, the distribution function for injection and detection can be considered as rectangular. Under this condition, and during sample introduction, equation (3) can be rearranged as follows:

$$\frac{l_{eb}}{t_i} = \left(\frac{l}{t_o}\right)\left(\frac{1}{1+k'}\right) \quad (4)$$

where l_{eb} is the length of the enriched solute band or compressed solute zone, l is the capillary length to the detection point, t_i is the injection time (i.e., time of the frontal electrochromatography step), t_o is the retention time of an unretained species (i.e., EOF marker), and k' is the retention factor of the solute being enriched in the injection solvent. Equation (4) can be further rearranged to yield:

$$l_{eb} = l_{sp}\left(\frac{1}{1+k'}\right) \quad (5)$$

where l_{sp} is the length of the sample plug, i.e., the distance traversed by the injection solvent, see Fig. 1. For a given sample plug length, eqn (5) shows that the effectiveness of zone compression (i.e., very small l_{eb}) during enrichment increases with increasing k'.

The peak width obtained during the elution step, and in turn the signal intensity, is influenced, among other things, by the length of the enriched band (l_{eb}) on the top of the column. The narrower the enriched band the stronger the signal intensity and the more effective the on-column trace enrichment. To ensure an optimal on-column trace enrichment, the high retention factor during sampling (i.e., during frontal electrochromatography) must be combined with negligible retention during desorption (i.e., during elution electrochromatography) in order to minimize band broadening during elution. This is usually accomplished by combining a strong eluent with a relatively strong EOF. In reversed-phase chromatography (RPC), an organic-rich mobile phase corresponds to a high eluent strength. A relatively strong EOF is ensured by using a relatively high running voltage, a column with high surface charge density, and a mobile phase of low viscosity.

During the elution electrochromatography step, a step-gradient elution occurs where the enriched sample band is further compressed or stacked. This stacking comes about from the fact that the solute molecules contacting the eluting mobile phase travel much faster than the solute molecules on the opposite side contacting the injection solvent which has a weaker eluent strength. The net result is an additional zone focusing until the eluting mobile phase reaches the slow migrating side of the injected enriched band. This zone focusing (i.e., zone narrowing) increases with (i) increasing the difference in the eluent strength between the injection solvent (weak eluent, high k') and

the mobile phase (strong eluent, low k') and (ii) decreasing the length of the sample plug (l_{sp}). Thus, the effectiveness of on-column trace enrichment is factored by all these variables. This report addresses the effects of the various parameters during trace enrichment in CEC.

Experimental

Instrumentation

The instrument used was an HP^{3D} CE system from Hewlett-Packard (Waldbronn, Germany) equipped with a photodiode array detector. Electrochromatograms were recorded with a computer running an HP 3D-CE Chemstation. A pressure of 10 bar was applied to both ends of the capillary during the experimental runs. The temperature was held constant at 25 °C. All samples were injected electrokinetically at various times and applied voltages which are stated in figure captions.

Reagents and Materials

Buffer solutions were prepared using either sodium phosphate monobasic from Mallinckrodt (Paris, KY, USA) or ammonium acetate from EM Science (Gibbstown, NJ, USA). The organic modifier used in the mobile phase was HPLC grade methanol, acetonitrile or tetrahydrofuran purchased from Fisher Scientific (Fair Lawn, NJ, USA). Benzene, alkyl benzenes, analytical grade acetone and HPLC grade toluene were from Aldrich (Milwaukee, WI, USA). All solutions were passed through a 0.2 μm Titan

syringe filter obtained from Scientific Resources, Inc. (Eatontown, NJ, USA). The Carbamate insecticides (see Figure 1 in Chapter II) were purchased from ChemService (West Chester, PA, USA). Dimethyloctadecylchlorosilane was purchased from Hüls Petrarch Systems (Bristol, PA, USA). Nucleosil silica was purchased from Macherey & Nagel (Düren, Germany). Fused silica capillaries with an internal diameter of 100 μm and an outer diameter of 360 μm were from Polymicro Technology (Phoenix, AZ, USA).

Stationary phase and column packing

Nucleosil 120-5 silica was converted in-house to an octadecyl-silica (ODS). The silica has a mean particle diameter of 5 μm, a mean pore diameter of 120 Å, and a specific surface area of 200 m^2/g. The following method was used to convert silica to ODS. To a 250 mL three neck round bottom flask, 30 mL of toluene were added. A condenser was connected to one neck, a stirring motor to the second and a glass stopper to the third. After the toluene was heated to 109 °C in an oil bath, 1.29 g of silica were added. The silica was heated for 15 min to purge all air from the pores. Subsequently, 0.5 g of dimethyloctadecylchlorosilane was added, and the reaction suspension was allowed to reflux for 24 hrs at 109 °C. Thereafter, the suspension was cooled to room temperature and then centrifuged for 10 min. The ODS stationary phase thus obtained was washed two more times with toluene and three more times with acetone. After the third acetone wash, the ODS was allowed to air dry for 24 hrs before use.

Untreated fused silica capillaries were packed using the slurry packing technique. The ODS stationary phase was suspended in acetone, and isopropanol was used as the packing solvent. The first step in the packing procedure was to sinter with a thermal wire

stripper a temporary porous frit on the outlet end of the capillary. A detection window approximately 10 cm from the temporary outlet frit was made using the same thermal wire stripper. The packing pressure was first ramped from 0 to 3000 psi within 3-5 min and then maintained constant at 3000 psi for 1 hr. The column was then rinsed with water for 30 min to 1 hr. Thereafter, the column was cut to the desired length and an inlet frit was sintered using the wire stripper. With the wire stripper a permanent outlet frit was sintered at 5 mm ahead from the detection window. The temporary outlet frit was then removed leaving a capillary segment of 8.5 cm from the detection window, which was then emptied from silica using an HPLC pump.

Procedures

Stock solutions of nine carbamate insecticides namely oxamyl, methomyl, aldicarb, baygon, carbofuran, aminocarb, bendiocarb, carbaryl, and methiocarb were prepared by dissolving 3 mg of each carbamate in individual 10 mL volumetric flasks filled with HPLC grade acetonitrile. This gave an approximate concentration of 1.5×10^{-3} M for each of the nine carbamates. A first dilution was performed by taking 1.0 mL of the stock solution and diluting it to 100 mL with HPLC grade acetonitrile in a volumetric flask to yield an approximate concentration of 1.5×10^{-5} M. The solvent used in the last dilution step consisted of pure buffer or various mixtures of acetonitrile/buffer (10/90 to 80/20 v/v). Since the carbamates were dissolved in pure acetonitrile, the stock solution pipetted was kept small (e.g., 10 µl or less) as not to affect the overall concentration of acetonitrile in the final dilution.

Results and discussion

Column evaluation

In order to ensure a relatively strong electroosmotic flow (EOF), the ODS stationary phase was intentionally prepared at a relatively low surface coverage in octadecyl ligands. Therefore, and as a first step in our studies, the ODS stationary phase was evaluated for its performance as a reversed-phase packing with benzene and alkylbenzene homologous series as the neutral model test solutes. Figure 2a shows a typical electropherogram of the test solutes under reversed-phase elution conditions. The average plate count is 25,200, which corresponds to 100,800 plates/m. The EOF velocity under the conditions of Fig. 2 is about 0.82 mm/s. This flow velocity is indicative of moderate surface coverage and matches well our previous surface modification[6,10,11] whereby only 25% of surface silanols (i.e., 2 $\mu mol/m^2$) are reacted leaving behind 75% of unreacted silanols (i.e., 6 $\mu mol/m^2$). Despite this relatively moderate surface coverage in octadecyl ligands (~ 2 $\mu mol/m^2$), the ODS stationary phase yielded the normal reversed-phase behavior as manifested form the quasi-linear plots of log k' versus the % acetonitrile in the mobile phase, see Fig. 2b.

Chromatographic behavior of carbamates

In order to introduce efficient ways for the on-line preconcentration of carbamate insecticides, the chromatographic behavior of these solutes (for structures see Fig. 1 in Chapter II) was examined over a wide range of mobile phase composition. This was performed by studying the effect of percent organic modifiers such as acetonitrile (ACN),

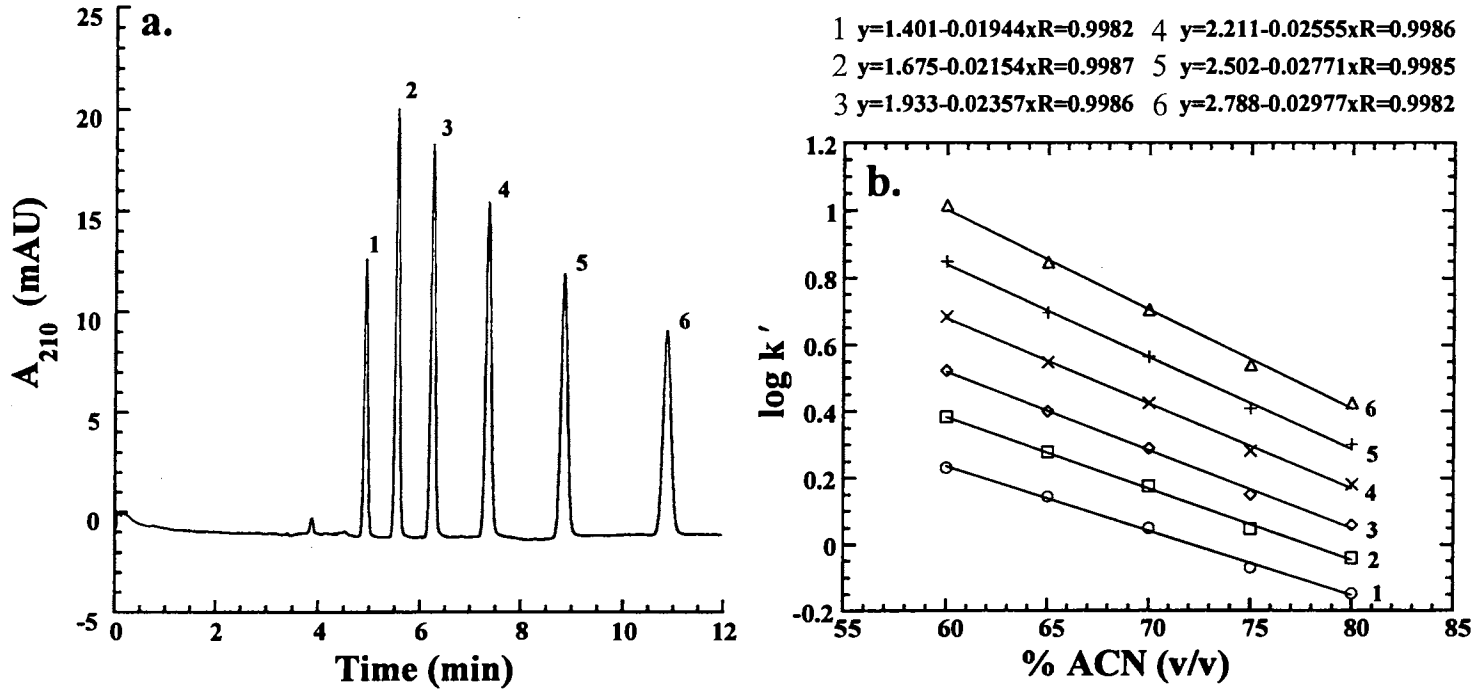

Figure 2. (a) Electrochromatogram of benzene and alkyl benzenes and (b) plots of logarithmic retention factor (log k') of benzene and alkyl benzenes vs. percent acetonitrile (v/v) in the mobile phase. Conditions: capillary column, 25 cm / 33.5 cm x 100 μm; mobile phases: (a), hydro-organic solution made up of 20% (v/v) of 10 mM NaH_2PO_4 (pH 6.0) and 80% acetonitrile (v/v); (b) various concentrations of NaH_2PO_4 to acquire an overall sodium phosphate concentration of 2 mM (pH 6.0) mixed with acetonitrile at different proportions; voltage, 20 kV; detection wavelength, 210 nm; column temperature, 25 °C. Solutes: 1, benzene; 2, toluene; 3, ethylbenzene; 4, propylbenzene; 5, butylbenzene; 6, amylbenzene.

methanol (MeOH), and tetrahydrofuran (THF) in mobile phases composed of sodium phosphate, pH 6.0, with an overall sodium phosphate concentration of 2 mM. As expected, the retention and selectivity (see Fig. 3) is largely affected by the % acetonitrile in the mobile phase with the best separation obtained at 55 % (v/v) ACN. Linear plots of log k' versus % acetonitrile were obtained in the percent range 50-80% (v/v) ACN with an R ranging from 0.981 to 0.999. Again, and as one would expect the stationary phase exhibited reversed-phase chromatography behavior for the nine carbamates studied.

The effect of mixed organic modifiers on retention and selectivity of the carbamates was also investigated. In this regards, MeOH or THF were mixed with ACN containing mobile phases and the results are shown in Fig. 3d, 3e and 3f. As shown in Fig. 3d and 3e, the addition of 5 and 10% THF led to completely destroying the selectivity between bendiocarb, baygon, carbofuran, and aminocarb. This indicated that a strong eluent such as THF is not suitable to bring about a baseline separation of the 9 carbamate insecticides. On the other hand, a weaker eluent such as MeOH in small amount improved the separation as shown in Fig. 3f. Maximum resolution was obtained when 5% methanol was added to a mobile phase containing 60% acetonitrile. With the mobile phase at 60% acetonitrile and 5% methanol, the selectivity between bendiocarb and baygon was 1.05 as opposed to 1.0 with the mobile phase at 55% acetonitrile. In summary, baseline resolution was not acquired with any of the mobile phases tested. This is in agreement with HPLC to the fact that no isocratic separation method was found in the literature. The method of choice for baseline resolution in HPLC is a methanol gradient[12] or an acetonitrile/methanol gradient.[13]

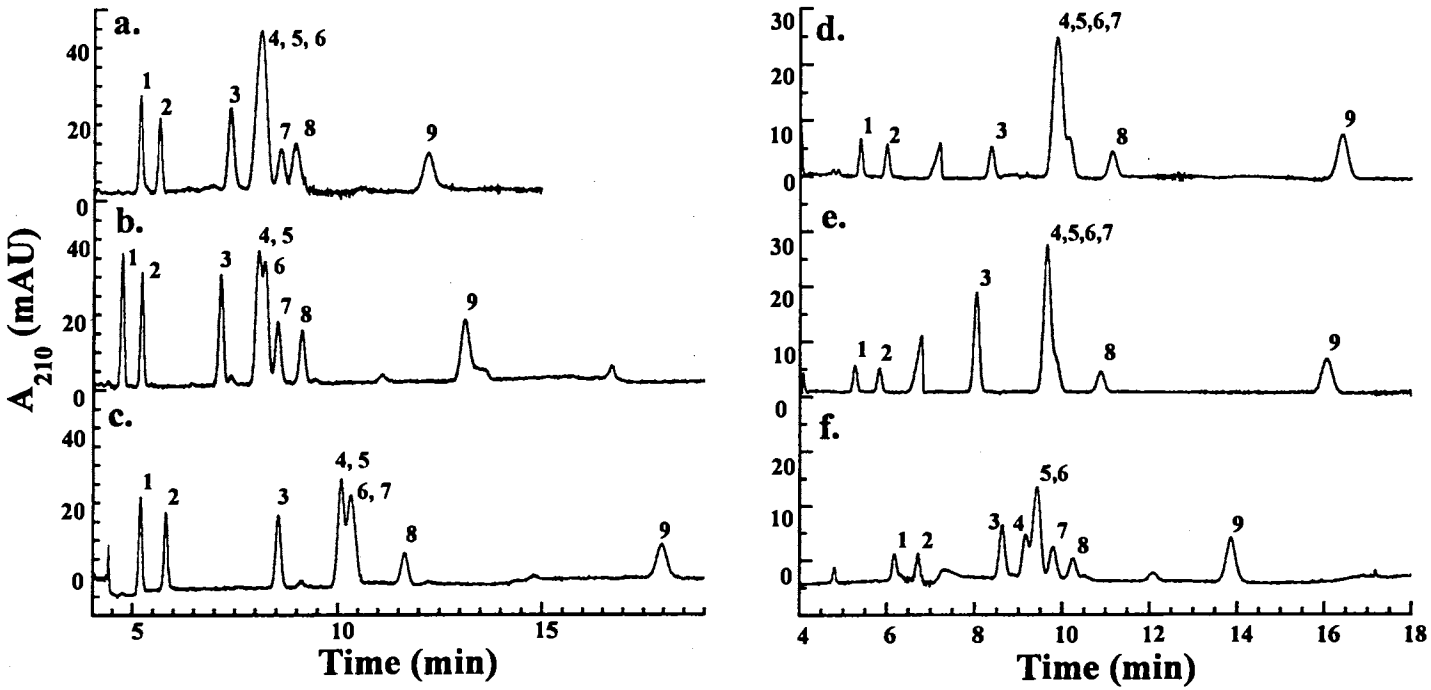

Figure 3. Electrochromatograms of *N*-methylcarbamates. Conditions: capillary column, 25 cm / 33.5 cm x 100 μm; voltage, 20 kV; detection wavelength 210 nm; column temperature, 25 °C. Mobile phases: (a) 40% (v/v) of 5 mM NaH_2PO_4 (pH 6.0) and 60% (v/v) acetonitrile; (b) 45% (v/v) of 4.5 mM NaH_2PO_4 (pH 6.0) and 55% (v/v) acetonitrile; (c) 50% (v/v) of 4 mM NaH_2PO_4 (pH 6.0) and 50% (v/v) acetonitrile; (d) 45% (v/v) of 4.5 mM NaH_2PO_4 (pH 6.0), 5% (v/v) tetrahydrofuran and 50% (v/v) acetonitrile; (e) 45% (v/v) of 4.5 mM NaH_2PO_4 (pH 6.0), 10% (v/v) tetrahydrofuran and 45% (v/v) acetonitrile; (f) 35% (v/v) of 5 mM NaH_2PO_4 (pH 6.0), 5% (v/v) methanol and 60% (v/v) acetonitrile. Solutes 1, oxamyl; 2, methomyl; 3, aldicarb; 4, bendiocarb; 5, baygon; 6, carbofuran; 7, aminocarb; 8, carbaryl; 9, methiocarb.

On-line trace enrichment

Various parameters were investigated in order to find the conditions under which optimum trace enrichment can be performed. This involved the study of the effects of the organic modifier concentration in the sample solution, the sample injection voltage, the length of the sample plug and the composition of the mobile phase used in the elution step.

Effect of Acetonitrile Content in the Sample Plug. Figure 4 shows the effect of the ACN content in the sample plug on the effectiveness of the on-line preconcentration by means of frontal electrochromatography. The results are expressed in terms of peak height and peak width. Methiocarb was used as the model solute. The sample plug was introduced electrokinetically at 3 kV from a sample solution at 4.0 x 10^{-6} M in methiocarb for an injection time of 180 s. The content of the sample in ACN was varied between 0 and 80% (v/v). On the average, the length of the sample plug (l_{sp}) was around 4.0 cm, which is about 16% of the effective length of the column. In all cases, the elution step was carried out with a mobile phase composed of 80% (v/v) acetonitrile and 20% (v/v) of 10 mM sodium phosphate, pH 6.0, using 20 kV as the elution voltage. As can be seen in Fig. 4, there is virtually no change in either peak height or peak width as a function of % ACN in the sample in the % range from 0 to 30%. The near plateau obtained for peak height and peak width of the eluted band which was originally enriched from a sample containing from 0 to 30% ACN (see Fig. 4) can be attributed to two opposing effects. Increasing the ACN content improves the surface contact with the

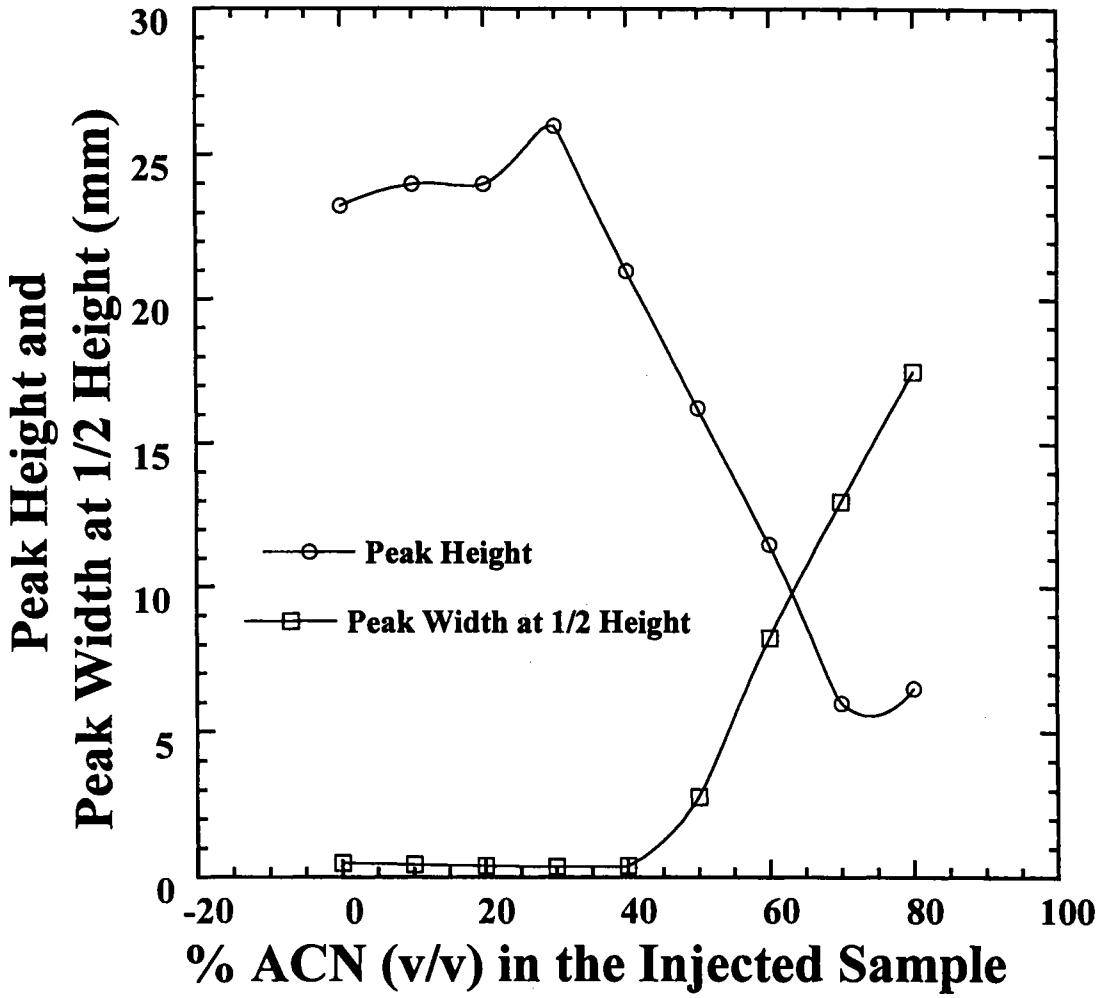

Figure 4. Plots of peak height and peak width at half height vs. percent acetonitrile in the injection sample. Conditions of frontal electrochromatography: organic content in the injected sample, 0, 10, 20, 30, 40, 50, 60, 70 and 80% (v/v) acetonitrile; sample plug was introduced electrokinetically at 3 kV from a sample solution at 4.0 x 10^{-6} M in methiocarb for an injection time of 180 s. Conditions of elution electrochromatography: mobile phase, 20% (v/v) of 10 mM NaH_2PO_4 (pH 6.0) and 80% (v/v) acetonitrile; voltage, 20 kV; detection wavelength, 266 nm. All other conditions as in Fig. 3.

hydro-organic solution being enriched by opening the hydrophobic chains (i.e., the octadecyl ligands), thus increasing the effective hydrophobic surface area. This is in agreement with the findings that in solid-phase extraction (SPE) with ODS cartridges, the SPE materials are usually pretreated or pre-wetted with an activating solvent such as ACN, MeOH or acetone to improve surface contact.[14] On the other hand, as the % ACN

is increased, the solute affinity to the stationary phase is decreased. In the 0 to 30% ACN range, the solute accumulates at the entrance of the column occupying a distance l_{eb} of 0.7, 1.2, 2.1 and 3.4 mm at 0, 10, 20 and 30% ACN in the sample plug, respectively. These values were estimated through eqn (5) by using the extrapolated k' values of methiocarb obtained from plots of log k' vs. % ACN in the mobile phase and by considering that the distance traversed by the solvent front of the sample plug is l_{sp} = 4.0 cm. Although the length of the enriched solute band increases from l_{eb} = 0.7 to l_{eb} = 3.4 mm as the amount of ACN is increased from 0 to 30% (v/v), the elution step is able to compress the band and to decrease the initial band spreading during sample introduction. However, after 40% ACN content, the peak height and peak width decreased and increased rapidly, respectively, indicating a significant decrease in the effectiveness of the on-line preconcentration. At 40% ACN and higher, not only the initial solute band becomes wider and wider (increasing form l_{eb} = 5.6 to l_{eb} = 23 mm when going from 40 to 80% v/v ACN) but also the elution step is unable to compress the band as the solute molecules move faster and faster in the head sample solvent in which the solute was originally dissolved because its ACN content is increased (see theoretical treatment). In other words, as the amount of ACN in the sample solvent is increased, the difference in the eluent strength between that of the sample solvent and the eluting mobile phase decreases, thus diminishing the focusing effect of the mobile phase during the elution electrochromatography step. As can be seen in Fig. 4, the peak width at half height is in very close agreement with the peak height. As one would expect as peak height increases peak width will decrease. Therefore, 30% ACN in the sample solvent seems to be optimal for trace enrichment.

Applied Voltage During Sample Enrichment. Figure 5 shows the effect of varying the injection voltage during on-line sample enrichment, i.e., during frontal electrochromatography. All conditions are the same as in the preceding section with sample plug composed of 30:70 acetonitrile/buffer (v/v). The applied voltage during sample enrichment was varied from 3 to 20 kV. In all cases, the sample plug length l_{sp} was kept constant at approximately 4.4 cm which represents 18% of the effective column length, l (i.e., length to the detection window). Typically, this sample plug is introduced within 27 s at 20 kV as opposed to 180 s at 3 kV. Increasing the voltage increases the magnitude of the intraparticle or perfusive EOF (i.e., the flow within the pores). This means that the relative stagnant volume decreases and the apparent mobile phase volume increases, thus leading to an increased mass transport within the pores (i.e., reduction in the mobile-phase mass-transfer resistance). This translates into higher capacity and better focusing of the sample. Since the mass transfer within the pore is directly proportional to the magnitude of the perfusive EOF, a linear relationship is obtained between peak height and the applied voltage during sample enrichment, see Fig. 5. Under CEC conditions, a significant intraparticle or perfusive flow was reported and this flow was shown to increase with pore size.[15,16]

Injection Time or Length of Sample Plug. It is apparent from the above results that an applied voltage of 20 kV for sample introduction and a relatively low acetonitrile content in the sample are the best choice for on-line trace enrichment. Next, it was necessary to examine the maximum length of sample plug l_{sp} that needs to be introduced for enhanced trace enrichment. In this regards, the length of the sample plug was varied

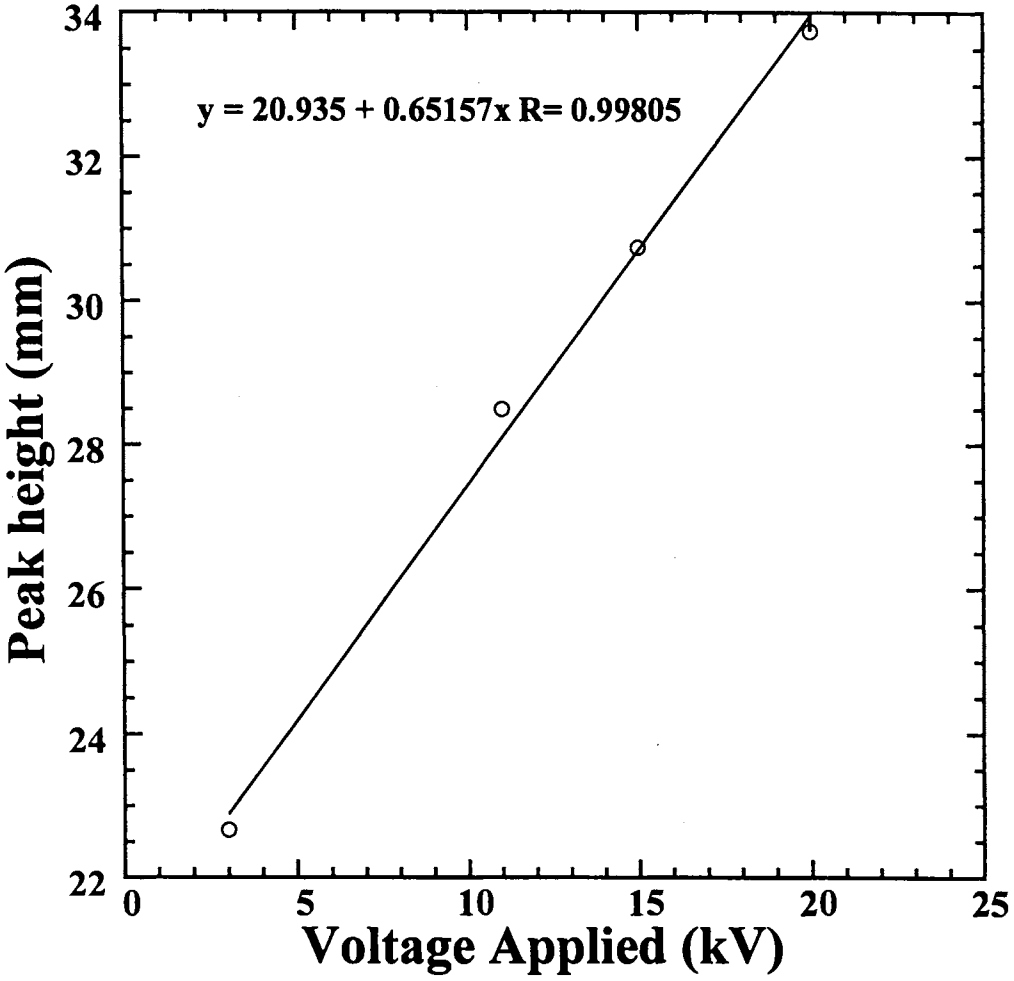

Figure 5. Plots of peak height vs. voltage applied during injection. Conditions of frontal electrochromatography: injection voltage, 3, 11, 15 and 20 kV for a duration of 180, 49, 36 and 27 sec, respectively; solute, methiocarb at 3.7 x 10^{-6} M dissolved in 30/70 acetonitrile/buffer (v/v). Conditions of elution electrochromatography: mobile phase, 20% (v/v) of 10 mM NaH_2PO_4 (pH 6.0) and 80% (v/v) acetonitrile; voltage, 20 kV; detection wavelength, 266 nm. All other conditions as in Fig. 3.

by changing the injection time at a constant applied voltage (i.e., 20 kV). These studies were performed with carbofuran as the test solute at 3.0 x 10^{-7} M. As can be seen in Fig. 6a, the peak height increases sharply as the injection time is increased from 1 to 3 min under four different elution conditions (see next section). Above 3.5 min, only a moderate increase in peak height has been observed. For a 1 min injection, the sample

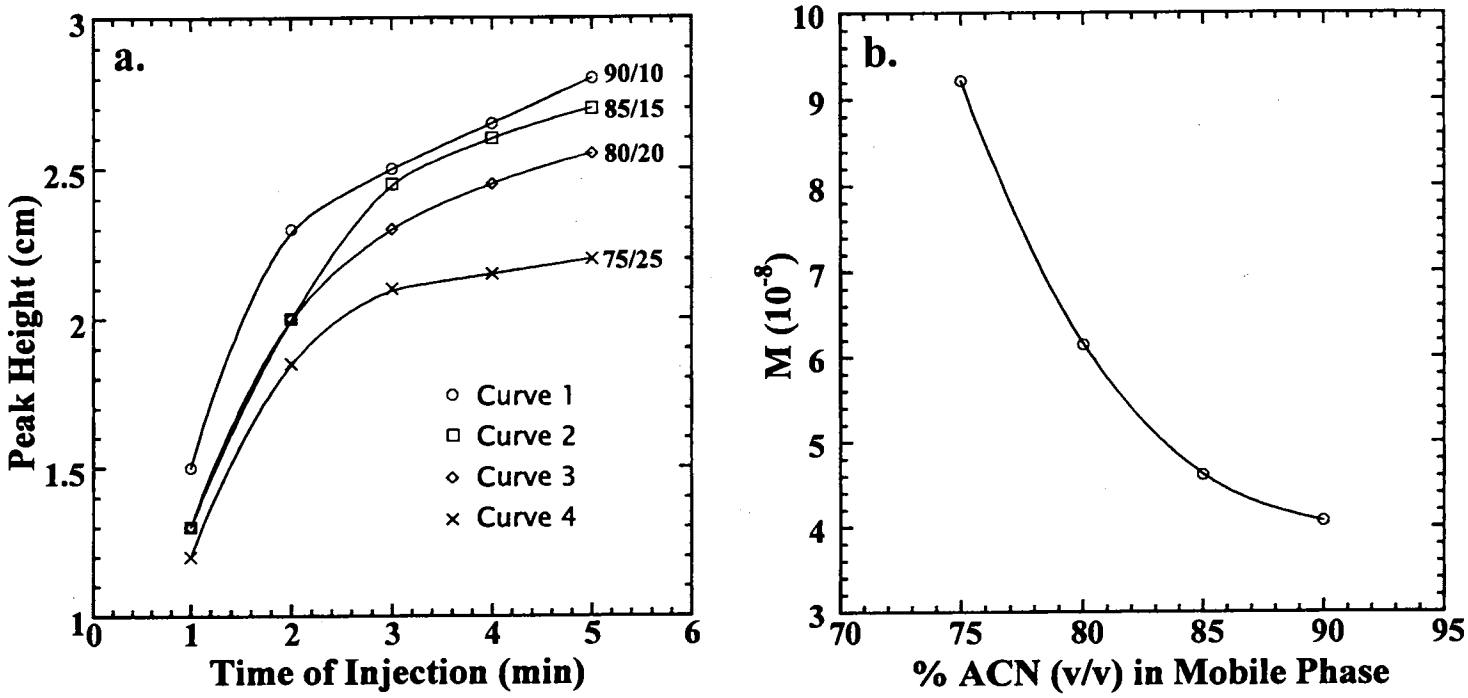

Figure 6. (a) Plots of peak height vs. time of injection and (b) trace enrichment as a function of % ACN in the mobile phase. (a) Conditions of frontal electrochromatography: injection voltage, 20 kV; injection time, 1, 2, 3, 4 and 5 min; solute, carbofuran at 3.1 x 10^{-7} M dissolved in 30/70 acetonitrile/buffer (v/v). Conditions of elution electrochromatography: curve 1, 10% (v/v) of 5 mM CH_3COONH_4 (pH 6.0) and 90% acetonitrile (v/v); curve 2, 15% (v/v) of 7.5 mM CH_3COONH_4 (pH 6.0) and 85% acetonitrile (v/v); curve 3, 20% (v/v) of 10 mM CH_3COONH_4 (pH 6.0) and 80% acetonitrile; curve 4, 25% (v/v) of 12.5 mM CH_3COONH_4 (pH 6.0) and 75% acetonitrile; detection wavelength, 281 nm. All other conditions the same as Fig. 3.
(b) Conditions of frontal electrochromatography: solute, carbofuran at various molarity in 30/70 acetonitrile/buffer (v/v). Conditions of elution electrochromatography: mobile phase, 2 mM CH_3COONH_4 (pH 6.0) at various % ACN. All other conditions as in (a).

plug is about l_{sp} = 6.31 cm long (l_{eb} = 1.1 cm) while for a 5 min injection the length of the sample plug l_{sp} is about 28.7 cm (i.e., 3.7 cm longer than the column length to the detection window and l_{eb}= 5 cm). This corresponds to increasing the sample size from 0.25 to 1.15 column length when going from a 1 min to 5 min injection. The flow velocity at 3.5 min was used to calculate the sample plug length at 5 min assuming a negligible change in flow velocity. For a 3.5 min injection time, the sample size l_{sp} reaches 20.1 cm (l_{eb} = 3.5 cm) or 0.80 column length. Thus, for carbofuran (relatively low k') the maximum allowable sample plug length seems to be around 20 cm. Beyond this length, the eluted band becomes excessively broad. Based on the theoretical treatment discussed in section 2, and under a given set of preconcentration conditions, one can state that the higher the solute retention in the frontal electrochromatography step, the longer the permissible sample plug length and consequently the higher the analyte signal in the subsequent elution electrochromatography step.

Percent Acetonitrile in the Mobile Phase Used in the Elution Step. Figure 6a also shows a large dependence of trace enrichment on the composition of the mobile phase used in the elution step following the on-line sample accumulation (i.e., frontal electrochromatography) from dilute solutions. With all other conditions held constant, the percent acetonitrile in the running mobile phase was varied from 75 to 90% in the acetonitrile/buffer mobile phase. As can be seen in Fig. 6a, the higher the organic content in the mobile phase the greater the peak height was. This is in agreement with the discussion in section 2 in the sense that the larger the difference between the eluent strength of the mobile phase and the injection solvent the better the effectiveness of the

zone compression during the step-gradient elution. In addition, stronger eluent leads to decreasing longitudinal molecular diffusion as the solute residence time in the column is decreased with increasing mobile phase eluent strength. Figure 6b shows the dependence of the trace enrichment on the acetonitrile content of the mobile phase used in the elution step. In other words, Fig. 6b shows the smallest detectable concentration by the on-column trace enrichment scheme as a function of the % ACN in the mobile phase. At 90% ACN in the eluent, very dilute sample of concentration as low as 4.0×10^{-8} M of carbofuran could be detected.

Sensitivity Enhancement Under Optimal Conditions. To determine the sensitivity increase (i.e., enhancement factor) by the on-line trace enrichment, the limit of detection (LOD) was determined under analytical injection conditions. Using a mobile phase at 90% ACN and an applied voltage of 20 kV, and injecting carbofuran for 2 sec, the LOD was found to be 2×10^{-5} M. As shown above, trace enrichment under optimal conditions including 30% ACN in the sample plug, 20 kV as the injection voltage, 3.5 min as the injection time and elution with a mobile phase at 90% ACN, a 4.0×10^{-8} M solution of carbofuran could be readily enriched and detected. This trace enrichment allows a sensitivity higher by a factor of 500 compared to straight CEC (i.e., without preconcentration) with UV detection.

Simultaneous On-Line Preconcentration and Separation of Carbamates Spiked in Deionized and Tap Waters. Figure 7a and 7b show typical electropherograms for the simultaneous on-line preconcentration and separation of carbofuran, carbaryl and

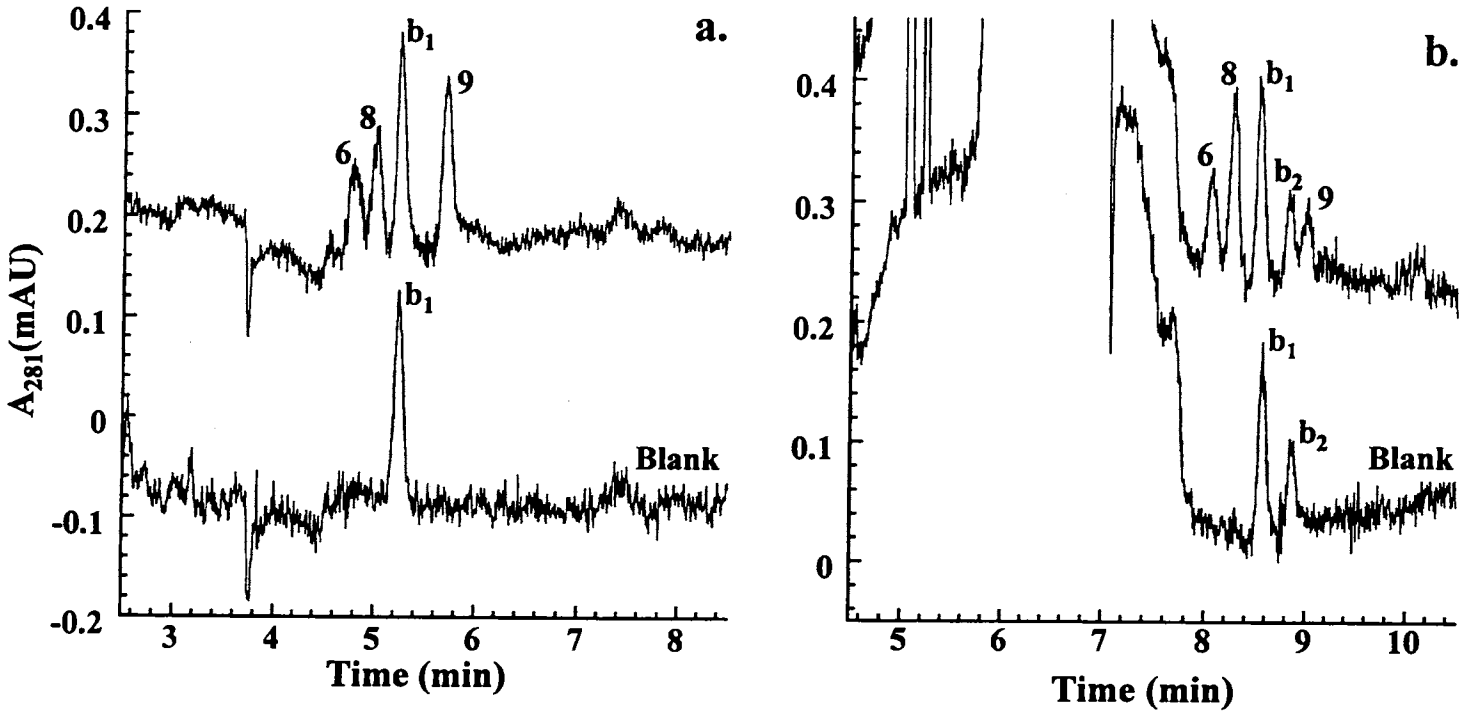

Figure 7. Simultaneous on-line preconcentration and separation of carbamates spiked in deionized (a) and tap (b) waters. Conditions of frontal electrochromatography: injection time, 3.5 min in (a) and 4.39 min in (b); injection voltage, 20 kV; solutes: 6, carbofuran; 8, carbaryl; 9, methiocarb at 10^{-7} M spiked in deionized water or tap water at 30% (v/v) ACN. Conditions of elution electrochromatography: mobile phase, 20% v/v of 10 mM CH_3COONH_4 (pH 6.0) and 80% (v/v) acetonitrile. All other conditions the same as in Fig. 3.

methiocarb spiked in deionized and tap waters, respectively. The deionized water sample spiked with the 3 carbamates was injected for 3.5 min at a concentration of 10^{-7} M for each solute in a solvent composed of 30/70 acetonitrile/buffer (v/v). The elution and separation were then achieved with a mobile phase at 80% ACN. Figure 7a shows the baseline resolution of the 3 carbamates under investigation.

Figure 7b illustrates the electropherograms obtained for a blank injection and an injection of 3 carbamates spiked in tap water at 10^{-7} M. Both the blank and the carbamate sample were injected for 4.39 min. The EOF in the presence of tap water in the blank and sample decreased by a factor of 1.25 with respect to that with deionized water under otherwise the same running conditions. This is why the injection time with tap water was prolonged to 4.39 min in order to introduce the same sample plug length as in the case of deionized water. The lower EOF in the case of tap water is due primarily to the presence of various inorganic ions, specially the divalent calcium ion, and humic and fulvic substances which collectively confer to the tap water a higher ionic strength and higher viscosity than the deionized water. In fact, comparing Fig. 7b to Fig 7a reveals a large breakthrough in the tap water injection which is absent in the deionized water injection, which is most often attributed to the presence of fulvic and humic substances.[17,18] The %RSD (n = 3) for retention times for trace enrichment and separation of spiked tap water were 1.57, 1.60 and again 1.60 for carbofuran, carbaryl and methiocarb, respectively. Also, the %RSD (n = 3) for peak areas for the 3 carbamates were 1.51, 6.81 and 2.89, respectively, while for peak heights the %RSD (n = 3) were 2.39, 1.46 and 6.46, respectively.

The retention time of the peak b_1 observed in the blank injection for both deionized and tap water corresponds to the time at which the injection solvent (i.e., 30:70 % ACN: water) plug has emerged out entirely from the column end, thus reflecting a sudden change in conductivity as well as in the average EOF across the column. The peak b_2 observed in the blank injection of tap water may be attributed to a retained interference from the tap water.

Conclusions

Capillary electrochromatography with ODS packed capillary columns allows the rapid and efficient separation of closely related pesticides and the introduction of a relatively large sample plug equivalent to almost one column dead volume thus permitting the determination of dilute samples of carbamate insecticides at concentration of ~ 10^{-7} to 10^{-8} M with a UV detector. The on-column trace enrichment by sequential frontal and elution electrochromatography has the potential of allowing multiple column dead volumes of sample to be injected because the analytes are adsorbed onto a stationary phase, which will not only result in the preconcentration of analytes but also in the removal of interferences.

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CHAPTER IV

ON-COLUMN TRACE ENRICHMENT BY SEQUENTIAL FRONTAL AND ELUTION ELECTROCHROMATOGRAPHY. II. ENHANCEMENT OF SENSITIVITY BY SEGMENTED CAPILLARIES WITH Z-CELL CONFIGURATION — APPLICATION TO THE DETECTION OF DILUTE SAMPLES OF MODERATELY POLAR AND NONPOLAR PESTICIDES*

Introduction

As with other microcolumn separation techniques, capillary electrochromatography (CEC) suffers from its rather poor concentration sensitivity due to the small sample volume and the short path length for on-column photometric detection. This fact is the major drive for introducing new ways for enhancing CEC concentration sensitivity and enabling the technique to analyze dilute samples. The alleviation of this shortcoming is essential for the exploitation of the high resolving power of CEC in the analysis of dilute samples of biological and environmental provenance in order to meet

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current challenges such as those imposed by the era of proteomic and safe drinking water whereby low abundance proteins must be isolated and characterized and trace amounts of toxic pollutants must be determined, respectively. Although extensive research efforts have been dedicated to improving detection sensitivity in capillary electrophoresis by developing various approaches for on-line preconcentration (for recent reviews, see Refs [1-4]), only a few reports have appeared so far concerning on-column trace enrichment for enhancing detection sensitivity in CEC.[5-9] The on-line preconcentration approaches used in CE are several including field amplified sample stacking, large volume sample stacking, pH-mediated focusing, isotachophoretic stacking, chromatographic extraction and sweeping.[1-4] The approach allowed a maximum sensitivity enhancement of 100 fold. Very recently, our approach to on-column trace enrichment involved sequential frontal and elution electrochromatography whereby relatively long sample plug (21 cm) dissolved in a solvent of weaker eluting strength than the mobile phase is first introduced followed by elution with a mobile phase of stronger eluent strength thus effecting a step-gradient elution and zone narrowing in the separation step.[9] This approach allowed a 500 fold sensitivity enhancement. To further boost the concentration sensitivity of CEC, we are describing here the sequential frontal and elution electrochromatography with a segmented capillary and a z-cell configuration for detection. The present approach allowed a sensitivity enhancement of 817 and 1100 fold for permethrin (a pyrethroid insecticide) and methiocarb (a carbamate insecticide), respectively. In the segmented capillary configuration, the capillary column consists of two contiguous segments each packed with a different stationary phase: the inlet segment is a short segment for preconcentration and the second segment is a longer segment and functions as the

separation segment. While the preconcentration segment should provide the highest affinity towards the enriched analytes, the separation segment approaches used in CEC are based on the capability of the chromatographic column packed with microparticles to play the simultaneous role of preconcentrator and separator.[5-9]

This chapter is a continuation to our earlier contribution to on-column trace enrichment of dilute samples in CEC (see also chapter III).[6,7,9] In our initial studies,[6,7] a water plug of a few millimeters long (3 to 5 mm) was injected first followed by prolonged injection of the dilute sample (ca. 12 mm sample plug) dissolved in a solvent of the same composition as the mobile phase used in the separation step. The water plug (the more retentive mobile phase) brought about an enhanced accumulation of the dilute samples into a narrow band at the inlet of the CEC column. This should exhibit the highest selectivity and separation efficiency.

Basic Principles

The basic principles of the on-column trace enrichment method used in this study was previously described in detail (see chapter III).[9] Briefly, it comprised two distinct steps: a frontal electrochromatography (FEC) step followed by an elution electrochromatography (EEC) step.

As shown in Fig. 1, we have devised a segmented capillary column configuration with a z-cell in order to accomplish the highest performance in terms of trace enrichment and detection sensitivity. In this configuration, while the concentrating segment provides strong solute binding during FEC step (i.e., the thinnest l_{eb}), the separation segment provides the selectivity and plate counts during EEC step. The length of the concentrating

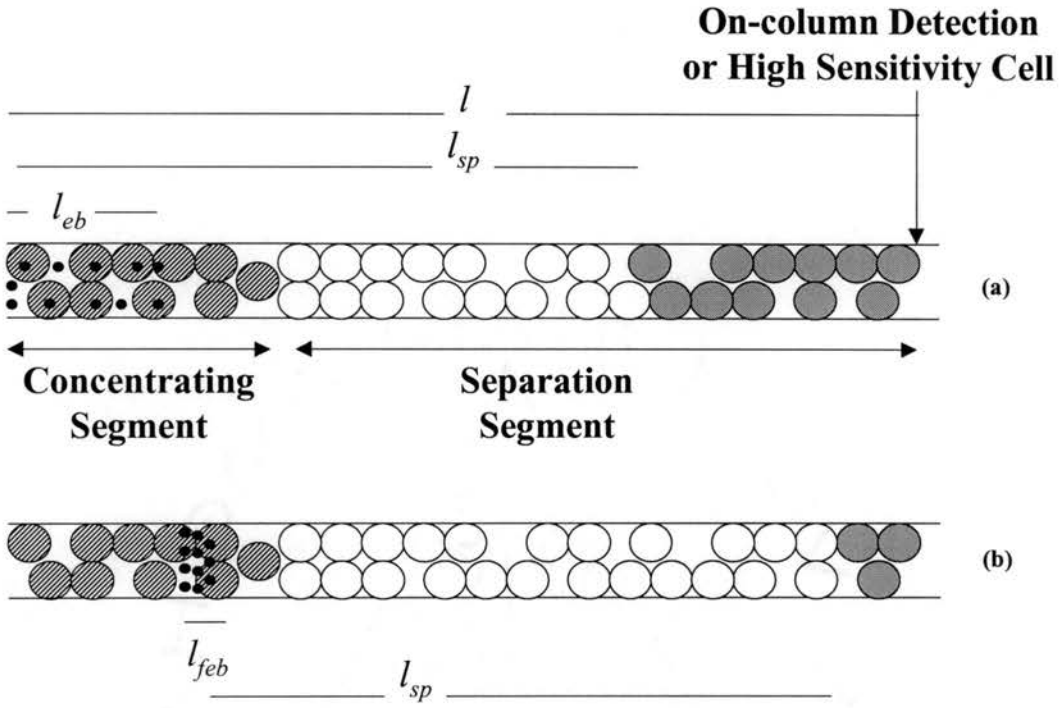

Figure 1. Schematic illustration of trace enrichment by frontal electrochromatography in (a) followed by zone focusing during step-gradient by elution electrochromatography in (b). This illustration also shows the segmented capillary design consisting of two contiguous segments packed with different stationary phases: a concentrating segment preceding a separation segment. *l,* length of column to detection point; l_{eb}, length of enriched band; l_{sp}, length of sample plug; l_{feb}, length of focused enriched band.

segment need not to exceed the length of the enriched solute band l_{eb} (i.e., 3 to 5 mm). But for convenience and ease of packing the capillary column, we have set this length in our work to 2.5 cm which is about 10% of the effective length of capillaries used in our studies. For details of packing segmented capillaries, see Experimental section and also Ref..[10] Previously, we have introduced the concept of segmented capillaries for the control of EOF in CE[11,12] and CEC[10] separations. In these work, we have shown that the overall EOF in segmented capillaries is a linear function of the fractional length of a given segment as follows:

$$\mu_{eo,av} = (\mu_{eo,1} - \mu_{eo,2}) \bullet \frac{l_1}{l_t} + \mu_{eo,2} \qquad (1)$$

where $\mu_{eo,1}$ and $\mu_{eo,2}$ are the electroosmotic mobilities in segment 1 (e.g., concentrating segment) and segment 2 (e.g., separation segment), respectively, when operated individually. l_t, l_1 and l_2 are the total column length, the length of segment 1 and segment 2, respectively. The total column length is $l_t = l_1 + l_2$. Equation 1 shows that knowing the individual EOF in each segment, the overall EOF in segmented capillaries can be readily predicted.

During the EEC step, a step-gradient elution occurs where the enriched sample band is further compressed or stacked yielding a band of length l_{feb}, see Fig. 1. This stacking comes about from the fact that the solute molecules contacting the eluting mobile phase travel much faster than the solute molecules on the opposite side contacting the injection solvent which has a weaker eluent strength. The net result is an additional zone focusing until the eluting mobile phase reaches the slow migrating side of the injected enriched band. This zone focusing (i.e., zone narrowing) increases with (i) increasing the difference in the eluent strength between the injection solvent (weak eluent, high k') and the mobile phase (strong eluent, low k') and (ii) decreasing the length of the sample plug (l_{sp}).

Experimental

Instrumentation

For instrumentation see Chapter III.

Reagents and Materials

Buffer solutions were prepared using either tris from Fisher Scientific (Fair Lawn, NJ, USA), sodium phosphate monobasic from Mallinckrodt (Paris, KY, USA) or ammonium acetate from EM Science (Gibbstown, NJ, USA). The organic modifier used in the mobile phase was HPLC grade acetonitrile or tetrahydrofuran purchased from Fisher Scientific (Fair Lawn, NJ, USA). All solutions were passed through a 0.2 μm Titan syringe filter obtained from Scientific Resources, Inc. (Eatontown, NJ, USA). The insecticides (see Fig. 2 and Chapter II) were purchased from ChemService (West Chester, PA, USA). Nucleosil silica was purchased from Macherey-Nagel (Düren, Germany). Fused silica capillaries with an internal diameter of 100 μm and an outer diameter of 360 μm were from Polymicro Technology (Phoenix, AZ, USA).

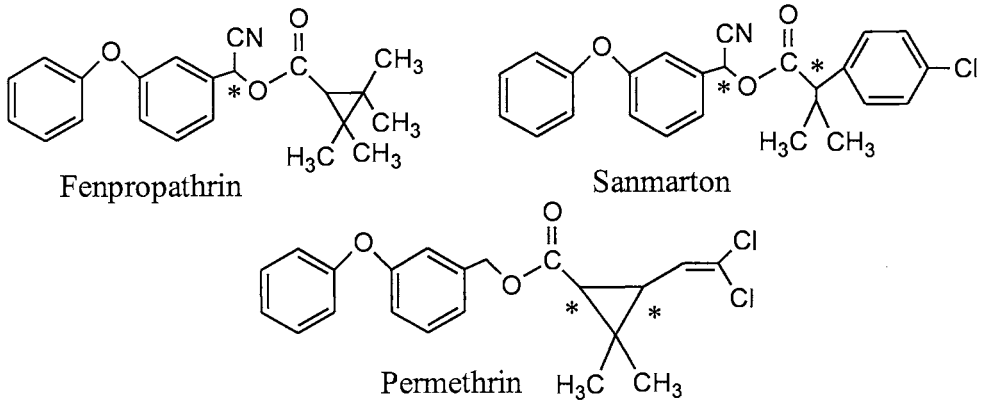

Figure 2. Structures of the pyrethroid insecticides (fenpropathrin, sanmarton and permethrin) used in this study. For structures of the *N*-methylcarbamates insecticides (carbofuran, carbaryl and methiocarb) see Chapter II.

Stationary Phases and Column Packing

Four types of nonpolar silica-based stationary phases were examined in this study. The first stationary phase was based on a 5 μm Nucleosil 120-5 silica with an average pore diameter of 120 Å and a specific surface area of 200 m^2/g. This silica was converted in-house to an octadecyl-silica (denoted HM-ODS) according to our previously described procedures.[9,13] The second stationary phase investigated was 5μm Nucleosil C_{18}-silica from Macherey-Nagel (Düren, Germany) having an average pore diameter of 100 Å and a specific surface area of 250 m^2/g. This stationary phase (denoted EC-ODS) is endcapped with a carbon coverage of 15% w/w as stated by the manufacturer. The third sorbent was a 5 μm ODS (denoted HC-ODS) with an average pore diameter of 120 Å and came from J.T. Baker (Phillipsburg, NJ, USA). The fourth silica was a 5 μm C_8-Zorbax from DuPont (Wilmington, DE, USA).

Untreated fused silica capillaries were packed using the slurry packing technique.[9,13] All capillaries used in this study were 25 cm packed / 33.5 cm in total length. See Fig. 1 for the configuration of segmented capillaries. When packing capillaries with two segments (each segment with a different stationary phase), the concentrating segment was first packed to an approximate length of 8 cm. A pressure of 200 bars was applied and the silica length was measured to the nearest millimeter. Following, the separation segment was packed at the same pressure as the concentrating segment. Thereafter, the concentrating segment was cut to the desired length followed by sintering the inlet frit. Throughout the length of the concentrating segment was 2.5 cm. The outlet frit was sintered to form an overall effective length (i.e., to the detection point) of 25 cm. When using the high sensitivity cell, the capillary was cut at 5 mm after the

outlet frit. When using on-column detection, the outlet end of the capillary was cut at 8.5 cm after the outlet frit. The remaining silica in this 8.5 cm segment was flushed out using an HPLC pump, and the detection window was formed 5 mm from the outlet frit.

To acquire a greater sensitivity an $HP^{3D}CE$ high-sensitivity detection cell was employed. The z-shape of the cell allows for a 1200 μm path length as compared to the 100 μm path length of a regular capillary column. The increased path length can lead to a seven-fold increase in sensitivity when compared to the 100 μm capillary (see Agilent Technologies on line, magazine Peak 1-98).

Procedures

Stock solutions of three carbamate (i.e., carbofuran, carbaryl, and methiocarb) and three pyrethroid insecticides (i.e., fenpropathrin, sanmarton and permethrin) were prepared by dissolving 10 mg of each insecticide in individual 100 mL volumetric flasks filled with HPLC grade acetonitrile. This gave a concentration of 2×10^{-4} to 5×10^{-4} M depending on the insecticides. A first dilution was performed by taking 1.0 mL of the stock solution and diluting it to 100 mL with HPLC grade acetonitrile in a volumetric flask to yield an approximate concentration of 4×10^{-6} M. The solvent used in the last dilution step consisted of pure buffer or various mixtures of acetonitrile/buffer (10/90 to 80/20 v/v). Since the insecticides were dissolved in pure acetonitrile, the stock solution pipetted was kept small (e.g., 10 μl or less) as not to affect the overall concentration of acetonitrile in the final dilution.

Results and Discussion

Capillary Configuration—Selection of Stationary Phases Best Suited For Preconcentration and Separation

Four different non-polar stationary phases were first examined for their plate counts with alkyl benzenes as the test solutes as well as for their EOF using a running voltage of 20 kV and a mobile phase of ACN:water (4:1 v/v) whose aqueous component (10 mM ammonium acetate) had a pH of 6.0. The home made ODS Nucleosil (HM-ODS) stationary phase provided the highest separation efficiency (123,400 plates/m) as well as a relatively fast EOF (1.21 mm/s). This ODS stationary phase was intentionally prepared at a relatively low surface coverage in octadecyl ligands[9] to ensure a relatively strong EOF. Under the same running conditions and for the same test solutes, the capillary packed with C_8-Zorbax provided a slightly higher EOF velocity of 1.35 mm/s but a significantly lower separation efficiency of 67,000 plates/m. The capillaries packed with the ODS from J.T.Baker (HC-ODS) or the endcapped ODS Nucleosil (EC-ODS) from Machery Nagel provided the slowest EOF velocities of 1.02 and 0.91 mm/s, respectively, with an average plate count of 78,000 and 67,000 plates/m, respectively. Besides the HM-ODS, which was specially designed for use in CEC, the other 3 non-polar silica-based stationary phases were manufactured for use in HPLC. From the above experiments, it is obvious that the stationary phase best suited for separation is the HM-ODS because it exhibited the highest separation efficiency with a relatively strong EOF.

In order to select the stationary phase best suited for preconcentration, the various stationary phases were evaluated with the pesticides under investigation. Three carbamate insecticides (namely carbofuran, carbaryl and methiocarb) and three

pyrethroid insecticides (namely fenpropathrin, sanmarton and permethrin) were analyzed at various acetonitrile content of the mobile phase. The results are shown in Figs. 3a and 4a in terms of log k' vs %ACN in the eluent. The plots shown in Figs 3a and 4a are for carbofuran and permethrin, respectively, as representative solutes of *N*-methylcarbamate and pyrethroid insecticides, respectively. In all cases, linear plots were obtained. Since the carbamates insecticides are relatively polar solutes, it is not surprising to see that the HM-ODS (of low surface coverage in octadecyl ligands) and the C_8-Zorbax yielded the highest retention toward the carbamate insecticides. In both cases, the unreacted surface silanols are most likely contributing to enhanced solute retention *via* silanophilic interactions. Using these data, the lengths of the compressed solute bands l_{eb} during the FCE step were calculated from Eqn 5 (see Chapter III) at the various %ACN in the injection solvent. Plots of l_{eb} versus % ACN for the various stationary phases are shown in Fig. 3b for the representative solute carbofuran. As can be seen in this figure either C_8-Zorbax or the home-made ODS Nucleosil are ideal for use in the preconcentration segment in segmented capillaries depending on the %ACN used in the injection solvent. At relatively low %ACN (below 40%) C_8-Zorbax provides thinner l_{eb} while the home made ODS Nucleosil is the opposite and yields thinner l_{eb} at high % ACN.

On the other hand, since the pyrethroids are relatively more hydrophobic than the carbamates, the ODS with the highest surface coverage (i.e., the EC-ODS from Machery Nagel) provided higher retention than the HM-ODS Nucleosil (see Fig. 4a) and in turn the thinnest compressed enriched band l_{eb} at all %ACN in the sample solvent (see Fig. 4b). Unexpectedly, the C_8-Zorbax exhibited the

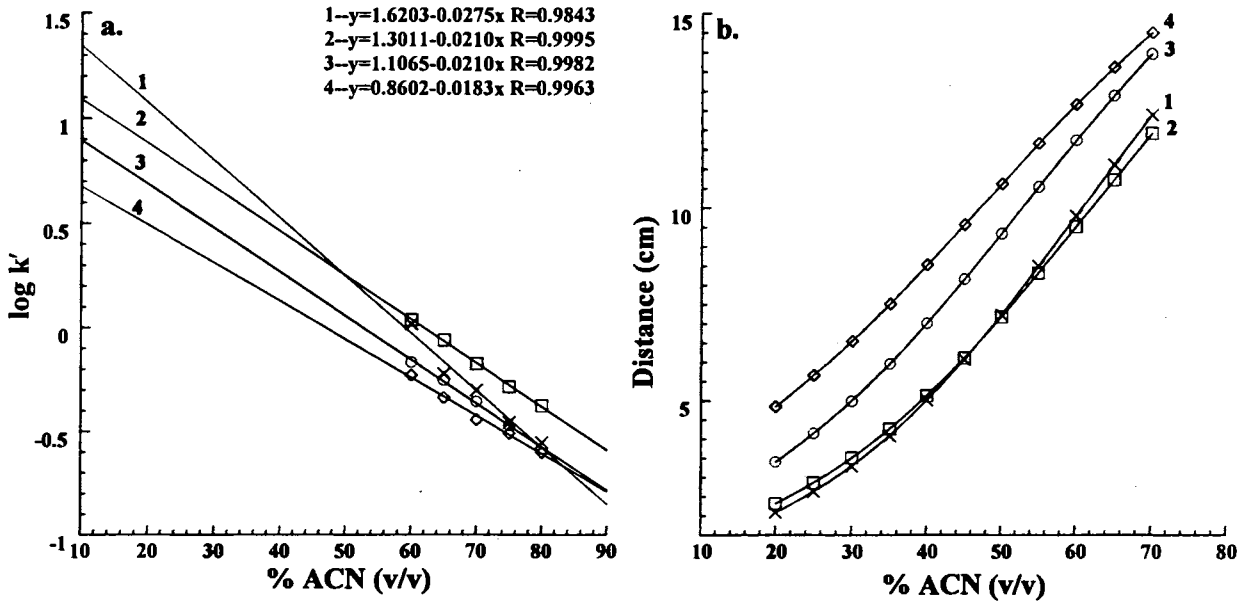

Figure 3. (a) Plots of logarithmic retention factor (log k') for carbofuran vs. percent acetonitrile (v/v) in the mobile phase and (b) plots of the compressed band of carbofuran l_{eb} vs. % acetonitrile (v/v) in the injected sample. Conditions: capillary column, 25 cm / 33.5 cm x 100 μm; mobile phase hydro-organic solution made up of various concentrations of CH_3COONH_4 to acquire an overall ammonium acetate concentration of 2 mM (pH 6.0) mixed with acetonitrile at different proportions; voltage, 20 kV; column temperature 25 °C. (b) Data points calculated from equation 5 chapter III using l_{sp} = 21 cm and k' values at different % ACN. 1, C_8-Zorbax; 2, HM-ODS Nucleosil; 3, HC-ODS; 4, EC-ODS Nucleosil.

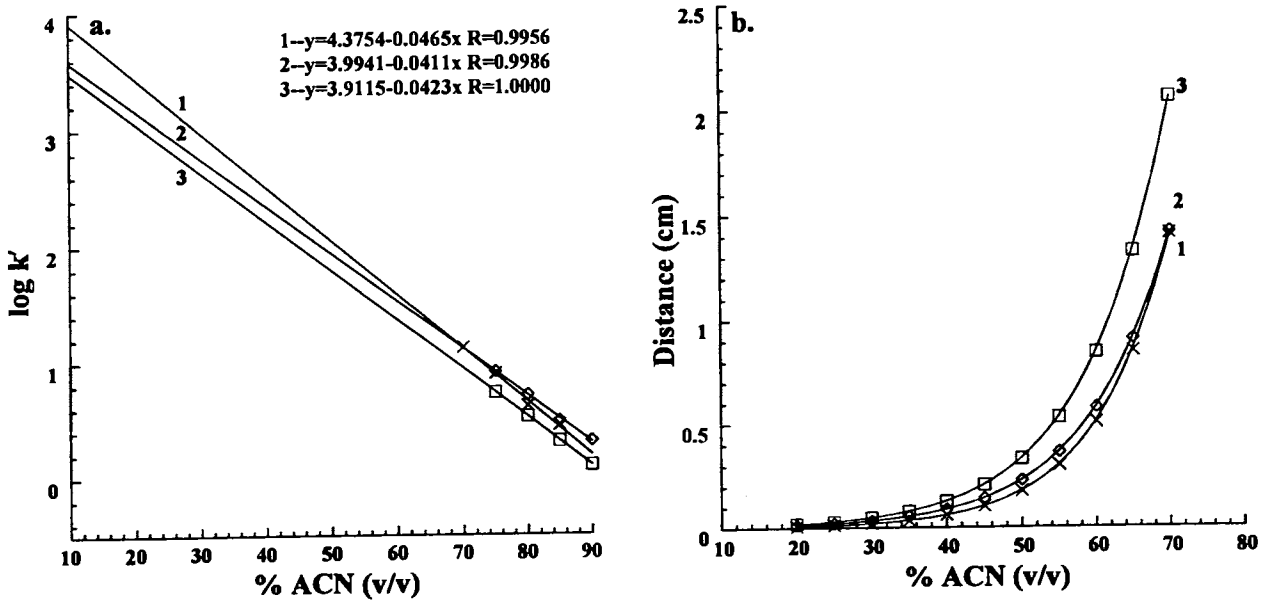

Figure 4. (a) Plots of logarithmic retention factor (log k') for permethrin vs. percent acetonitrile (v/v) in the mobile phase and (b) plots of the compressed band of permethrin l_{eb} vs. % acetonitrile (v/v) in the injected sample. Conditions: capillary column, 25 cm / 33.5 cm x 100 μm; mobile phase hydro-organic solution made up of various concentrations of CH_3COONH_4 to acquire an overall ammonium acetate concentration of 2 mM (pH 6.0) mixed with acetonitrile at different proportions; voltage, 20 kV; column temperature 25 °C. (b) Data points calculated from equation 5 chapter III using l_{sp} = 21 cm and k' values at different % ACN. 1, C_8-Zorbax; 2, EC-ODS Nucleosil; 3, HM-ODS Nucleosil.

same or slightly higher retention toward the pyrethroids than the ODS with the high %C (i.e., HC-ODS) (Fig. 4a). This can be explained by the fact that the relatively short C8 chains of the C_8-Zorbax do not "stack" as extensively as the longer octadecyl ligands on the ODS column (see Fig. 5 for illustration of the stack configuration). In other words, the effective nonpolar phase ratio available for solute interaction is about the same or higher on the C_8-Zorbax column than on the EC-ODS column from Machery Nagel with high carbon load. Since the EC-ODS with high surface coverage yielded the lowest EOF, C_8-Zorbax was elected as the stationary phase for preconcentration segment.

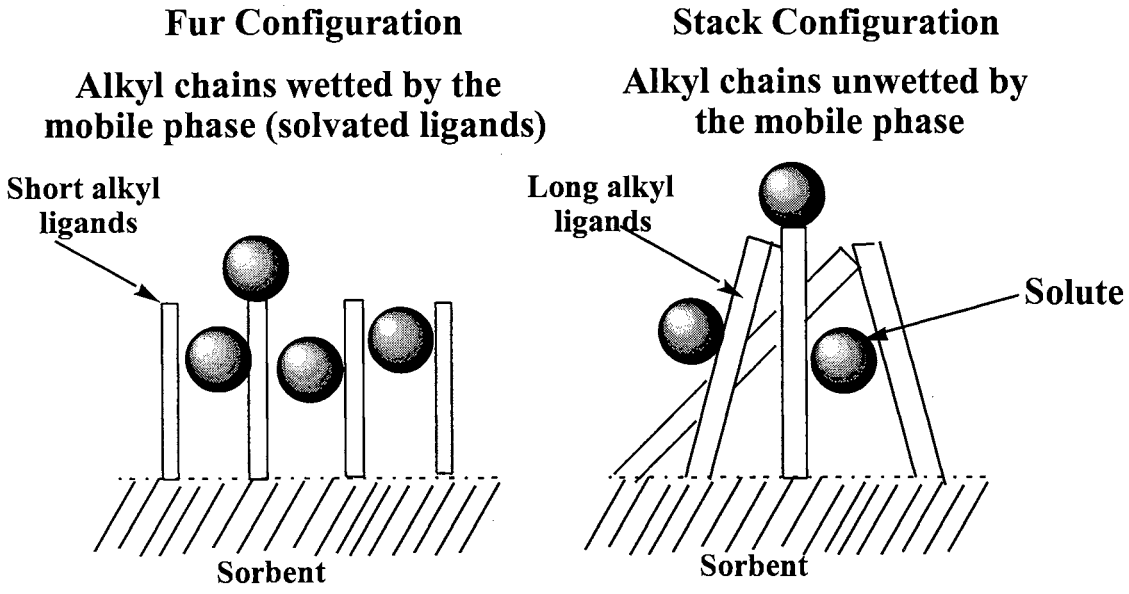

Figure 5. "Stack" configuration versus "fur" configuration for alkylated stationary phases.

Previously, we have investigated the CEC behavior of carbamate insecticides as a function of the organic content of the mobile phase,[9] and found that an eluent at 80 to 90% (v/v) ACN was very suitable for the rapid separation of the carbamate insecticides.

Therefore, and for completeness, it was necessary to evaluate in this study the retention of pyrethroid insecticides in reversed-phase chromatography (RPC) with the HM-ODS Nucleosil so that the amount of organic modifier in mobile phase needed for separation could be determined. Figure 6a illustrates the separation of the three pyrethroids using a mobile phase of 15% (v/v) of 14 mM ammonium acetate , 5% THF (v/v) and 80% acetonitrile (v/v). As shown in Fig. 6b, linear plots of log k' versus percent acetonitrile in the mobile phase were obtained demonstrating that the stationary phase exhibited RPC behavior toward the three pyrethroids studied. It was found that by adding 5% THF (v/v)

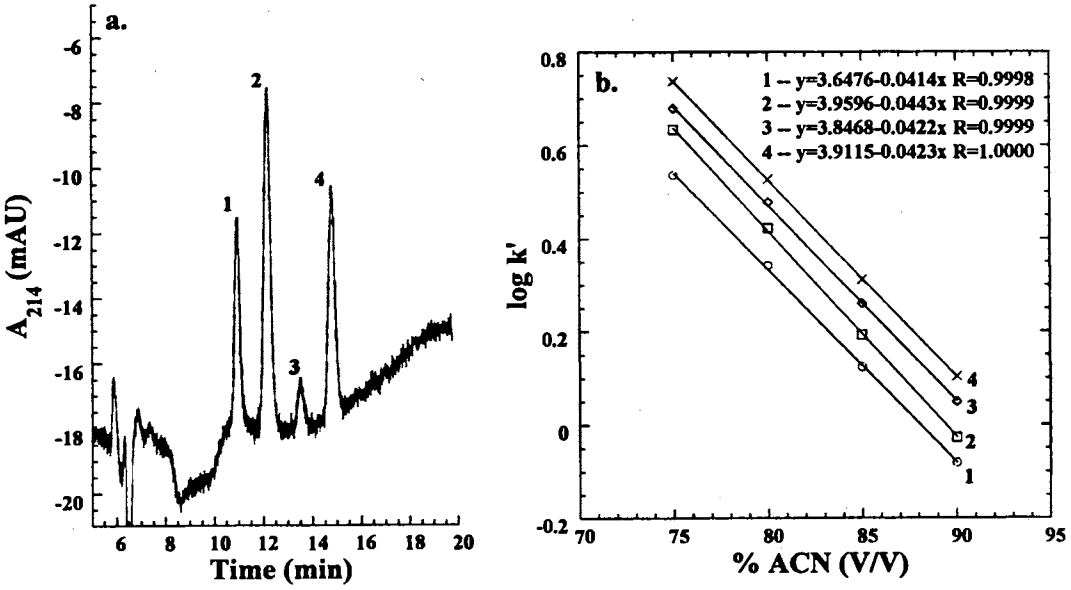

Figure 6. (a) Electrochromatogram of pyrethroids used in this study and (b) plots of logarithmic retention factor (log k') of the three pyrethroids vs. percent acetonitrile (v/v) in the mobile phase. Conditions: capillary column packed with HM-ODS, 25 cm / 33.5 cm x 100 μm; mobile phase: (a), hydro-organic solution made up of 15% (v/v) of 14 mM CH_3COONH_4 (pH 6.0), 80% acetonitrile (v/v) and 5% THF (v/v). (b) various concentration of CH_3COONH_4 to acquire an overall ammonium acetate concentration of 2 mM (pH 6.0) mixed with acetonitrile at different proportions; voltage, 20 kV; detection wavelength, 214 nm; column temperature, 25 °C. Solutes: 1, fenpropathrin; 2, sanmarton; 3, *trans*-permethrin; 4, *cis*-permethrin.

to the mobile phase as compared to using acetonitrile alone decreased the k' for fenpropathrin from 1.5 to 0.9 and for permethrin from 2.3 to 1.6. This indicates that THF has a strong eluting power towards the pyrethroids. This strong eluting power is necessary in on-line trace enrichment for the sweeping, which occurs in EEC step after the injection or stacking during the FEC step so that the enriched band is reduced in size (i.e., zone compression) before the final separation step.

On-Line Trace Enrichment

In a very recent contribution from our laboratory,[9] the effects of the sample injection voltage during trace enrichment by FEC, the length of the sample plug and the composition of the mobile phase used in the elution step were investigated in order to find the conditions under which optimum trace enrichment can be performed.[9] We have shown that increasing the applied voltage during sample enrichment by FEC led to increasing signal intensity of the enriched band upon elution. This was attributed to increasing the magnitude of perfusive EOF (i.e., the flow within the pores) which lead to increased mass transport within the pores and consequently higher capacity and better focusing of the sample. An applied voltage of 20 kV for sample introduction was a good compromise. Also, the study of the effect of sample plug length revealed that the higher the solute retention in the FEC step, the longer the permissible sample plug length and consequently the higher the analyte signal in the subsequent elution electrochromatography step. As stated in section 2, for achieving the most effective on-column trace enrichment, the FEC step should be combined with the fastest EEC step. This means that the larger the difference between the eluent strength of the mobile phase

and the injection solvent the better the effectiveness of the zone compression during the step-gradient elution.

Effect of Acetonitrile Content in The Sample Plug. Figure 7a and b shows the effect of %ACN in the sample plug on the effectiveness of the on-line preconcentration by means of FEC on a single sorbent capillary and a segmented capillary, respectively, using permethrin as the model solute. The single sorbent capillary consisted of the home made ODS Nucleosil while the segmented capillary column was made of a C_8-Zorbax segment (2.5 cm) and HM-ODS Nucleosil segment (22.5 cm). The results shown in Fig. 7a and b are expressed in terms of peak height in mAU versus %ACN in the sample plug. The sample plug was introduced electrokinetically at 20 kV from a sample solution at 1.3 x 10^{-7} M in permethrin for an injection time of 3.5 min. The content of the sample in ACN was varied between 0 and 80% (v/v) for the segmented column (C_8-Zorbax→HM–ODS Nucleosil) while for the single phase capillary column (packed with HM-ODS Nucleosil) was varied between 30 and 60% (v/v). On the average, the length of the sample plug (l_{sp}) was around 21 cm, which is about 84% of the effective length of the column. In all cases, the elution step was carried out with a mobile phase composed of 80% (v/v) acetonitrile and 20% (v/v) of 10 mM ammonium acetate, pH 6.0, using 20 kV as the elution voltage. As can be seen in Fig. 7b, the peak height increases slowly first in the 0 to 20% ACN range and then rapidly in the 20 to 40% ACN range. Increasing the ACN content improves the surface contact with the hydro-organic solution being enriched by opening the hydrophobic chains (i.e., the octadecyl ligands), thus increasing the effective hydrophobic surface area, see Fig. 5. This is in agreement with

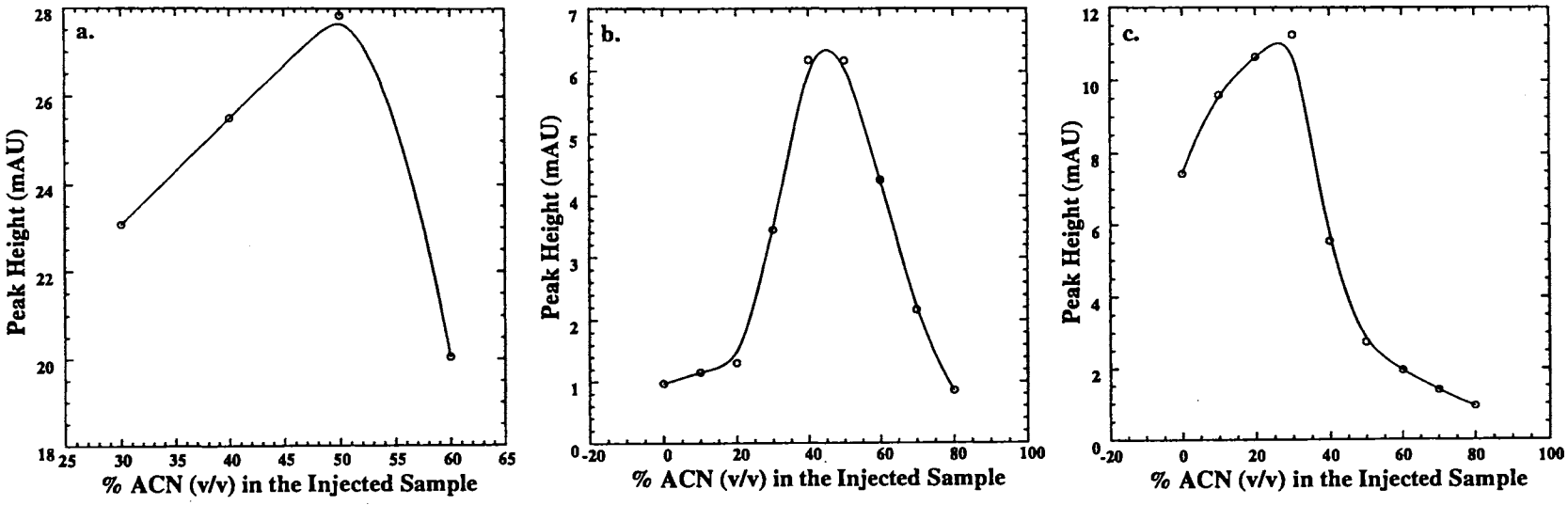

Figure 7. (a) Plot of peak height of permethrin vs. percent acetonitrile (v/v) in the injection sample for single sorbent capillary packed with HM-ODS Nucleosil silica. (b) Plot of peak height of permethrin vs. percent acetonitrile (v/v) in the injected sample for a segmented capillary column consisting of 2.5 cm C_8-Zorbax segment followed by 22.5 cm of HM-ODS Nucleosil silica segment. (c) Plot of peak height of carbaryl vs. percent acetonitrile (v/v) in the injected sample for a segmented capillary column consisting of 2.5 cm C_8-Zorbax followed by 22.5 cm of HM-ODS Nucleosil silica segment. Conditions of frontal electrochromatography: organic in the injected sample, 30, 40, 50 and 60% ACN for (a) and 0, 10, 20, 30, 40, 50, 60, 70 and 80% (v/v) ACN for (b) and (c); sample plug was introduced electrokinetically at 20 kV from sample solution at 1.28×10^{-7} M in permethrin in (a) and (b) or 1.28×10^{-6} M in carbaryl in (c) for an injection time of 3.5 minutes. Conditions of elution electrochromatography: mobile phase, 20% (v/v) of 10 mM CH_3COONH_4 (pH 6.0), and 80% (v/v) acetonitrile; Voltage, 20 kV; detection wavelength, 214 nm for permethrin and 281 nm for carbaryl.

the findings that in solid-phase extraction (SPE) with ODS cartridges, the SPE materials are usually pretreated or pre-wetted with an activating solvent such as ACN, MeOH or acetone to improve surface contact.[14] In the 0 to 50% ACN range, the solute accumulates at the entrance of the column occupying a distance l_{eb} of 0.0009, 0.003, 0.008, 0.02, 0.06 and 0.2 cm at 0, 10, 20, 30, 40 and 50% ACN in the sample plug, respectively. These values were estimated through eqn 5 chapter III by using the extrapolated k' values of permethrin obtained from plots of log k' vs. % ACN in the mobile phase on the C_8-Zorbax capillary and by considering that the distance traversed by the solvent front of the sample plug is l_{sp} = 21 cm. Although the length of the enriched solute band increases from l_{eb} = 0.0009 to l_{eb} = 0.2 cm as the amount of ACN is increased from 0 to 50% (v/v), the elution step is able to compress the band and to decrease the initial band spreading produced during sample introduction. However, after 50% ACN in the sample plug, the peak height decreased indicating a significant decrease in the effectiveness of the on-line preconcentration. At 50% ACN and higher, not only the initial solute band becomes wider and wider (increasing form l_{eb} = 0.2 to l_{eb} = 3.8 cm when going from 50 to 80% v/v ACN) but also the elution step is unable to compress the band as the solute molecules move faster and faster in the head sample solvent in which the solute was originally dissolved because its ACN content is increased (see section 2). In other words, as the amount of ACN in the sample solvent is increased, the difference in the eluent strength between that of the sample solvent and the eluting mobile phase decreases, thus diminishing the focusing effect of the mobile phase during the elution electrochromatography step.

Similar behavior was obtained on the single phase capillary column, see Fig. 7a. As can be seen in Fig. 7a, the maximum is approximately at 50% ACN compared to Fig. 7b where the peak height is at a maximum around 45% ACN. The slightly higher ACN content required for maximum peak height on the single phase ODS capillary than on the segmented C_8-Zorbax→HM–ODS Nucleosil capillary is due to the longer octadecyl chains which necessitate more organic solvent to open the hydrophobic chains. The C8 chains on the C_8-Zorbax being shorter than the C18 ligands can withstand higher aqueous concentration without undergoing significant stack configuration (see Fig. 5 for illustration). As shown in Fig. 7, 50% ACN in the sample solvent seems to be optimal for trace enrichment of the pyrethroids.

In our previous contribution to trace enrichment in CEC,[9] the optimum organic modifier concentration in the sample solution was determined to be 30% for the home made ODS Nucleosil using methiocarb as the model solute for carbamate insecticides. In the present study, the effect of ACN in the sample plug was studied on a segmented column (C_8-Zorbax→HM–ODS Nucleosil) using carbaryl as the model solute. The results are shown in Fig. 7c by a plot of peak height vs. % ACN in the injection sample. The sample plug was introduced electrokinetically at 20 kV from a sample concentration of 1.3×10^{-6} M in carbaryl for an injection time of 3.5 min. When comparing Fig. 7c to 7b, one can see the same trends. Due to the fact that the carbamates are less hydrophobic than the pyrethroids, the percent organic in the sample injection is less for the carbamates (30% ACN) than for the pyrethroids (45% ACN) for maximum peak height obtained in the subsequent EEC step.

Sensitivity Enhancement Under Optimal Conditions. Tables 1 and 2 list the LOD's with and without on-column trace enrichment for carbamates and pyrethroid insecticides, respectively, under various column and detection configuration. First, without preconcentration and as expected, the LOD decreased in the presence of the z-cell configuration by a 2.5 and 3.7 fold for carbaryl and methiocarb, respectively, at 282 nm. Similarly, for fenpropathrin, sanmarton and permethrin, the LOD decreased by 2.1, 2.2 and 2.7 fold, respectively, at 214 nm. As can be seen in Tables 1 and 2, significant sensitivity enhancement was achieved by on-column trace enrichment. In the case of carbamate insecticides, and under optimum conditions, 282 and 620 fold sensitivity increase were obtained with carbaryl and methiocarb, respectively, with on capillary detection at 282 nm. This sensitivity enhancement grew to 500 and 917 fold for carbaryl and methiocarb, respectively, when the capillary was connected to the z-cell. The segmented capillary with a z-cell at 282 nm boosted the sensitivity to 752- and 1100-fold for carbaryl and methiocarb, respectively. At 208 nm, although the sensitivity enhancement was smaller, ca. 112 fold for both carbaryl and methiocarb with on-capillary detection and 346 fold for combined segmented capillary and z-cell, more dilute samples were determined at 208 nm, see Table 1. In the case of pyrethroid insecticides, 125, 133 and 167 fold increase in sensitivity were obtained by the trace enrichment of fenpropathrin, sanmarton and permethrin, respectively, under optimal conditions using on-capillary detection. This sensitivity enhancement became 286, 250 and 292 fold for fenpropathrin, sanmarton and permethrin, respectively, when using the z-cell for detection. This sensitivity increase was further enhanced by combining segmented

TABLE 1.

LIMIT OF DETECTION WITH OR WITHOUT PRECONCENTRATION FOR TWO CARBAMATE INSECTICIDES. ALL SAMPLES WERE MADE IN DEIONIZED WATER. CONDITIONS FOR ELUTION: MOBILE PHASE, 20% v/v OF 10 mM AMMONIUM ACETATE (PH 6.0), 80% v/v ACN; VOLTAGE, 20 kV; OTHER CONDITIONS AS IN FIG. 8.

	No Preconcentration (M) 2 sec injection at 20 kV		Preconcentration (M) 3.5 min injection at 20 kV		
Solute/ Wavelength	On-Capillary Detection	High Sensitivity Cell	On-Capillary Detection	High Sensitivity Cell	Segmented Capillary With High Sensitivity Cell
Carbaryl					
@ 282 nm	2.24×10^{-5}	8.95×10^{-6}	7.95×10^{-8}	4.48×10^{-8}	2.98×10^{-8}
@ 208 nm	6.34×10^{-6}		5.63×10^{-8}		1.83×10^{-8}
Methiocarb					
@ 282 nm	5.54×10^{-6}	1.51×10^{-5}	8.93×10^{-8}	6.04×10^{-8}	5.03×10^{-8}
@ 208 nm	4.33×10^{-6}		3.85×10^{-8}		1.25×10^{-8}

TABLE 2.

LIMIT OF DETECTION WITH AND WITHOUT PRECONCENTRATION FOR SOME PYRETHROIDS. ALL SAMPLES WERE MADE IN DEIONIZED WATER. CONDITIONS FOR "NO PRECONCENTRATION" AS IN FIG. 6A AND FOR "PRECONCENTRATION" AS IN FIG. 8.

	No Preconcentration (M) 2 sec injection at 20 kV		Preconcentration (M) 3.5 min injection at 20 kV		
Solute	On-Capillary Detection	High Sensitivity Cell	On-Capillary Detection	High Sensitivity Cell	Segmented Capillary With High Sensitivity Cell
Fenpropathrin	6.20×10^{-6}	2.98×10^{-6}	4.96×10^{-8}	2.17×10^{-8}	1.15×10^{-8}
Sanmarton	8.73×10^{-6}	3.93×10^{-6}	6.55×10^{-8}	3.49×10^{-8}	1.42×10^{-8}
Permethrin	5.72×10^{-6}	2.06×10^{-6}	3.43×10^{-8}	1.96×10^{-8}	7.00×10^{-9}

capillary with the z-cell and grew to 539, 615 and 817 fold for fenpropathrin, sanmarton and permethrin, respectively.

As expected, the magnitude of sensitivity enhancement paralleled the order of solute retention. In other words, the more retained the solute the more effective the trace enrichment.

Simultaneous On-Line Preconcentration and Separation of Pyrethroids and Carbamates Spiked in Deionized, Tap and Lake Waters. Figure 8a, b and c show typical electrochromatograms for the simultaneous on-line preconcentration and separation of fenpropathrin, sanmarton and permethrin spiked in deionized, tap and lake waters, respectively. In all cases, the water sample was spiked with the 3 pyrethroids at a concentration of 10^{-8} M for each solute in a solvent composed of 50/50 acetonitrile/buffer (v/v). While the deionized water sample was injected for 3.5 min at 20 kV that of tap and lake waters was introduced at the same voltage but for 4.1 min and 5.0 min, respectively. The elution and separation were then achieved at 20 kV with a mobile phase at 5% (v/v) THF, 80% (v/v) ACN and 15% (v/v) of 14 mM ammonium acetate, pH 6.0. The injection time in the case of tap water was prolonged to 4.1 min due to the fact that the EOF in the presence of tap water in the blank and sample decreased by a factor of 1.17 with respect to that with deionized water under otherwise the same running conditions. Under these circumstances, prolonging the injection time with tap water to 4.1 min insured the introduction of the same sample plug length as in the case of deionized water (i.e., 21 cm sample plug). Also, the EOF in the presence of lake water was almost identical to that of tap water. The injection for lake water was prolonged for an extra 0.9 min with respect to tap water due to an interfering peak in the electrochromatogram when

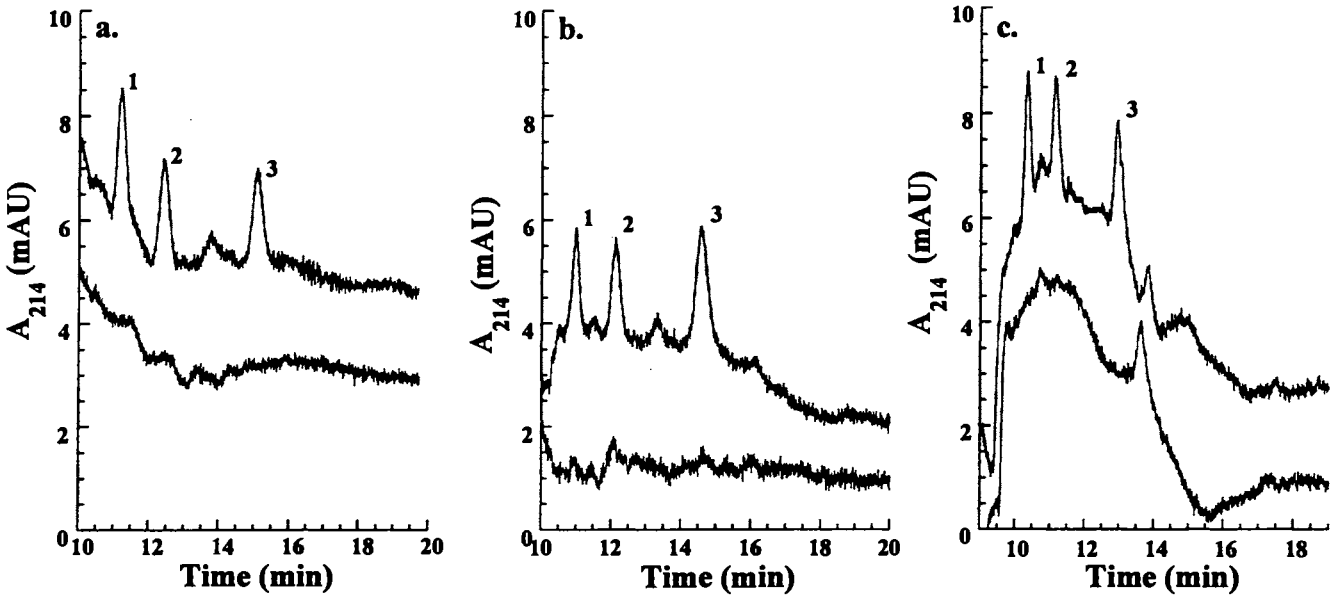

Figure 8. Simultaneous on-line preconcentration and separation of pyrethroids spiked in deionized (a), tap (b) and lake (c) waters. Conditions of frontal electrochromatography: injection time, 3.5 min in (a) 4.1 min in (b) and 5.0 min in (c); injection voltage, 20 kV; solutes: 1, fenpropathrin; 2, sanmarton; 3, permethrin at ca. 10^{-8} M spiked in deionized, tap or lake water at 50% (v/v) ACN. Conditions of elution electrochromatography: Voltage, 20 kV; mobile phase, 15% v/v of 14 mM CH_3COONH_4 (pH 6.0), 80% (v/v) acetonitrile and 5% (v/v) THF. High sensitivity cell used for detection; 33.5 cm capillary with concentrating segment of 2.5 cm packed with C_8-Zorbax and a separation segment of 22.5 cm packed with HM-ODS Nucleosil.

the sample is introduced for 4.1 min as in the case of tap water. As shown in the blank and sample electrochromatograms in Fig. 8c, prolonging the injection time by an extra 0.9 min moved the interferring peak away from the analyte peak #3. According to our previous findings,[9] a longer injection than 4.1 min or 21 cm would not yield a significant increase in peak height. The lower EOF in the case of natural waters (e.g., tap water and lake water) may be attributed to the presence of various inorganic ions, specially the divalent calcium ion, and humic and fulvic substances [15,16] which collectively confer to natural waters a higher ionic strength and higher viscosity than deionized water.

Under optimal conditions using segmented capillary with high sensitivity cell, the trace enrichment by sequential frontal and elution electrochromatography allowed the detection of very dilute samples of pesticides prepared in deionized, tap and lake waters. At 208 nm, the LOD's for carbaryl in deionized, tap and lake waters were 1.83×10^{-8}, 1.97×10^{-8} and 2.53×10^{-8} M, respectively. Under the same trace enrichment conditions, the LOD's for methiocarb in deionized, tap and lake waters were 1.2×10^{-8}, 1.35×10^{-8} and 1.73×10^{-8} M, respectively. These figures which are very close in magnitude demonstrate that the trace enrichment is not influenced by the nature of the water matrix. Also the LOD's for the three pyrethroids were largely independent of the nature of the water matrix. In fact, the LOD's for fenpropathrin in deionized, tap and lake waters were found to be 1.15×10^{-8}, 1.14×10^{-8} and 1.14×10^{-8} M, respectively. The LOD's for sanmarton were 1.42×10^{-8}, 9.76×10^{-9} and 9.76×10^{-9} M while the LOD's for permethrin were found to be 7.0×10^{-9}, 1.21×10^{-8} and 1.21×10^{-8} M in deionized, tap and lake water, respectively.

Conclusions

Capillary electrochromatography with segmented packed capillary columns using a high sensitivity cell (i.e., z-cell) allows the rapid and efficient separation of closely related pesticides and the introduction of a relatively large sample plug equivalent to almost one column dead volume. This permitted the determination of dilute samples of carbamate and pyrethroid insecticides consisting of tap and lake water spiked at concentration of ~ 10^{-8} to 10^{-9} M with a UV detector. These LOD's were largely independent of the nature of the water matrix.

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CHAPTER V

EFFECT OF THE NATURE OF ALKYL BONDED SILICA-BASED STATIONARY PHASES IN CAPILLARY ELECTROCHROMATOGRAPHY. SURFACTANT MEDIATED CAPILLARY ELECTROCHROMATOGRAPHY FOR THE SEPARATION OF NONPOLAR PYRETHROID INSECTICIDES*

Introduction

Capillary electrochromatography (CEC) with silica bonded stationary phases has already demonstrated its high resolving power in various applications including carbohydrate species,[1-3] pesticides,[4-6] small and large nucleic acid fragments,[7,8] and various chiral substances [9-13] just to name a few (for recent reviews on CEC applications, see Refs. [14-17]). Despite these advances, the exploitation of the full potentials of CEC is yet to come. This will largely depend on the introduction of more specially designed stationary phases for CEC and other components of the electrochromatographic system.

** The content of this Chapter has been submitted to Electrophoresis for publication.*

In this chapter, we focused our investigation on enhancing the resolving power of CEC by introducing a novel concept for separation by CEC based on surfactant-rich mobile phases. For this purpose, we selected pyrethroid insecticides because of their importance as agrochemicals and environmental species and also because of their various isomeric forms to challenge the resolving power of CEC. In order to achieve this goal, we first compared various alkyl bonded silica-based stationary phases of different levels of surface coating with alkyl ligands in the separation of isomeric pyrethroids. Second, mobile phases containing a charged surfactant, namely sodium di-2-ethylhexyl sulfosuccinate (DOSS), were introduced to enhance the resolving power of CEC. The new CEC system is referred to as surfactant mediated capillary electrochromatography (SM-CEC). In this SM-CEC system, DOSS plays the role of a slowly moving pseudo-stationary phase so that the solutes are partitioned between a mobile phase, a fixed stationary phase and a slowly moving pseudo-stationary phase. As will be shown in this chapter, the SM-CEC provided an enhanced resolution for all the pyrethroids, thus permitting the resolution of isomeric pyrethroids.

Principles of Surfactant-Mediated Capillary Electrochromatography

Surfactant-mediated capillary electrochromatography (SM-CEC) refers to a CEC system consisting of a fixed stationary phase packed in the capillary column (e.g., octadecyl-silica (ODS)) and a slowly moving pseudo-stationary phase dissolved in the mobile phase. The pseudo-stationary phase described here consists of sodium dioctyl sulfosuccinate (DOSS) surfactant (see Fig. 1 for structure of DOSS) dissolved in an

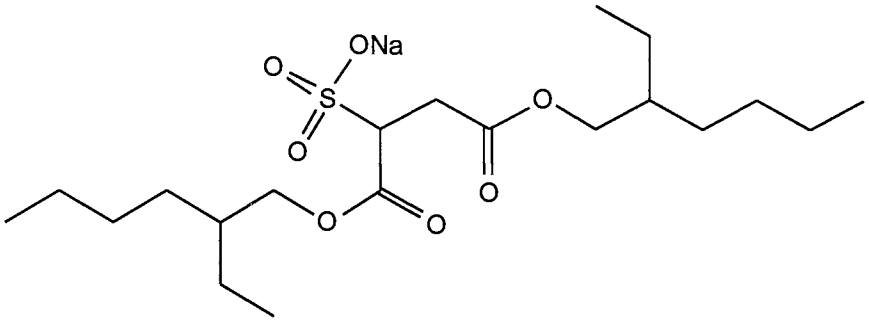


Figure 1. Structure of sodium di-2-ethylhexyl sulfosuccinate surfactant (DOSS).

organic-rich mobile phase. At the ACN concentration customarily used in CEC (20% or greater), it is well established that micelle formation is inhibited, and consequently the DOSS surfactant dissolves in a large fraction as monomers.[18] The inhibitory effect of acetonitrile on micellization is based on the reduction of the dielectric constant of the aqueous phase by the organic solvent which would cause increased mutual repulsion of the ionic heads in the micelle, thus opposing micellization. Recently, DOSS has been used in capillary electrophoresis with hydroorganic electrolyte systems for the separation of neutral, hydrophobic compounds.[19,20]

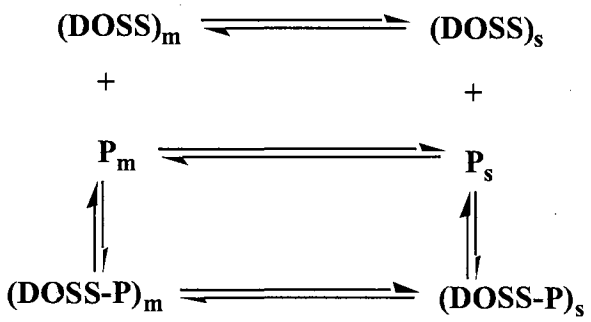
As can be seen in Fig. 1, DOSS is a branched surfactant where the polar head group occupies a central position in the hydrophobic chain. The branched nature of the DOSS surfactant may explain its relatively high critical micellar concentration (CMC) in pure water,[18] which is 2.5 mM. At concentration higher than the CMC, DOSS forms a cloudy solution in aqueous buffer solutions, but dissolves freely in hydro-organic solutions such as those used for mobile phase in reversed-phase CEC. Under this mobile phase condition, the surfactant DOSS consists of monomers moving electrophoretically against the electroosmotic flow (EOF). However, the relatively strong EOF sweeps the

DOSS towards the detection point at an apparent velocity slower than the mobile phase velocity. A neutral solute (e.g., pyrethroids) that associates with DOSS will acquire an electrophoretic mobility as shown in the following equation:

$$\mu_{eff,ep} = f_c \mu_{ep,c} \quad (1)$$

where $\mu_{eff,ep}$ is the effective electrophoretic mobility of the solute and f_c is the mole fraction of DOSS-solute complex whose electrophoretic mobility is $\mu_{ep,c}$. A neutral solute will acquire the electrophoretic mobility of the complex when f_c approaches 1, i.e., when the solute associate with the DOSS surfactant avidly. Thus, the stronger the association of the solute with the DOSS surfactant the higher the effective electrophoretic mobility of the solute and vice versa.

In SM-CEC, the solute partitions between 3 phases (a mobile phase, a fixed stationary phase and a slow moving pseudo-stationary phase), thus leading to an enhanced separation. The following equations describe the various equilibria that control the partitioning of the solute between the mobile phase and the stationary phases (i.e., DOSS and ODS):



where m and s stand for mobile and stationary phase, respectively, and P stands for pyrethroid or any other neutral solute. When P is in the mobile phase, it is either free or

interacting with the DOSS pseudo-stationary phase. Whether free or complexed with DOSS, the solute is also interacting with the ODS stationary phase. Moreover, the DOSS is distributed between mobile and stationary phases.

Experimental

Reagents and Materials

Buffer solutions were prepared using either Tris from Fisher Scientific (Fair Lawn, NJ, USA), sodium phosphate monobasic from Mallinckrodt (Paris, KY, USA) or ammonium acetate from EM Science (Gibbstown, NJ, USA). The organic modifier used in the mobile phase was HPLC grade acetonitrile purchased from Fisher Scientific. Analytical grade acetone as well as the surfactant sodium di-2-ethylhexyl sulfosuccinate (DOSS) were purchased from Aldrich (Milwaukee, WI, USA); for structure of DOSS see Fig. 1. All solutions were passed through a 0.2 μm Titan syringe filter obtained from Scientific Resources, Inc. (Eatontown, NJ, USA). The pyrethroid insecticides (see Fig. 2 and also Fig. 2 in chapter IV) were purchased from ChemService (West Chester, PA, USA). Dimethyloctadecylchlorosilane was purchased from Hüls Petrarch Systems (Bristol, PA, USA). Fused-silica capillaries with an internal diameter of 100 μm and an outer diameter of 360 μm were from Polymicro Technology (Phoenix, AZ, USA).

Instrumentation

For instrumentation see Chapter III.

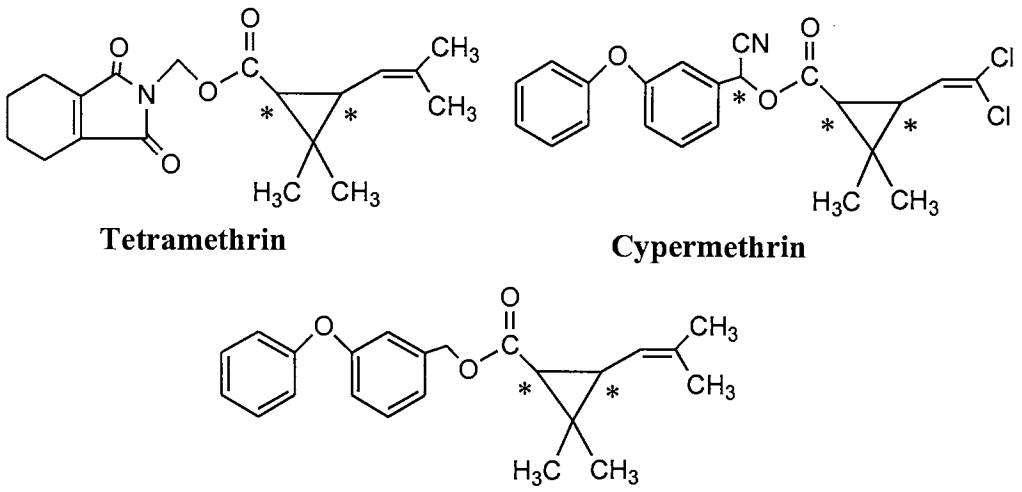


Figure 2. Structures of three pyrethroid insecticides used in this study, see Fig. 2 in Chapter IV for others.

Stationary Phase and Column Packing

For stationary phase and column packing used see Chapter IV.

Results and Discussion

Comparison of the Stationary Phase

The overall performance of four alkyl-bonded stationary phases was evaluated using six pyrethroids as the model solutes. Figure 3 illustrates the electrochromatograms of the six pyrethroids obtained on the four stationary phases used in this study. The separations were performed using a hydroorganic mobile phase which consisted of 75% (v/v) acetonitrile and 25% (v/v) of 20 mM ammonium acetate, pH 6.0. As can be seen in

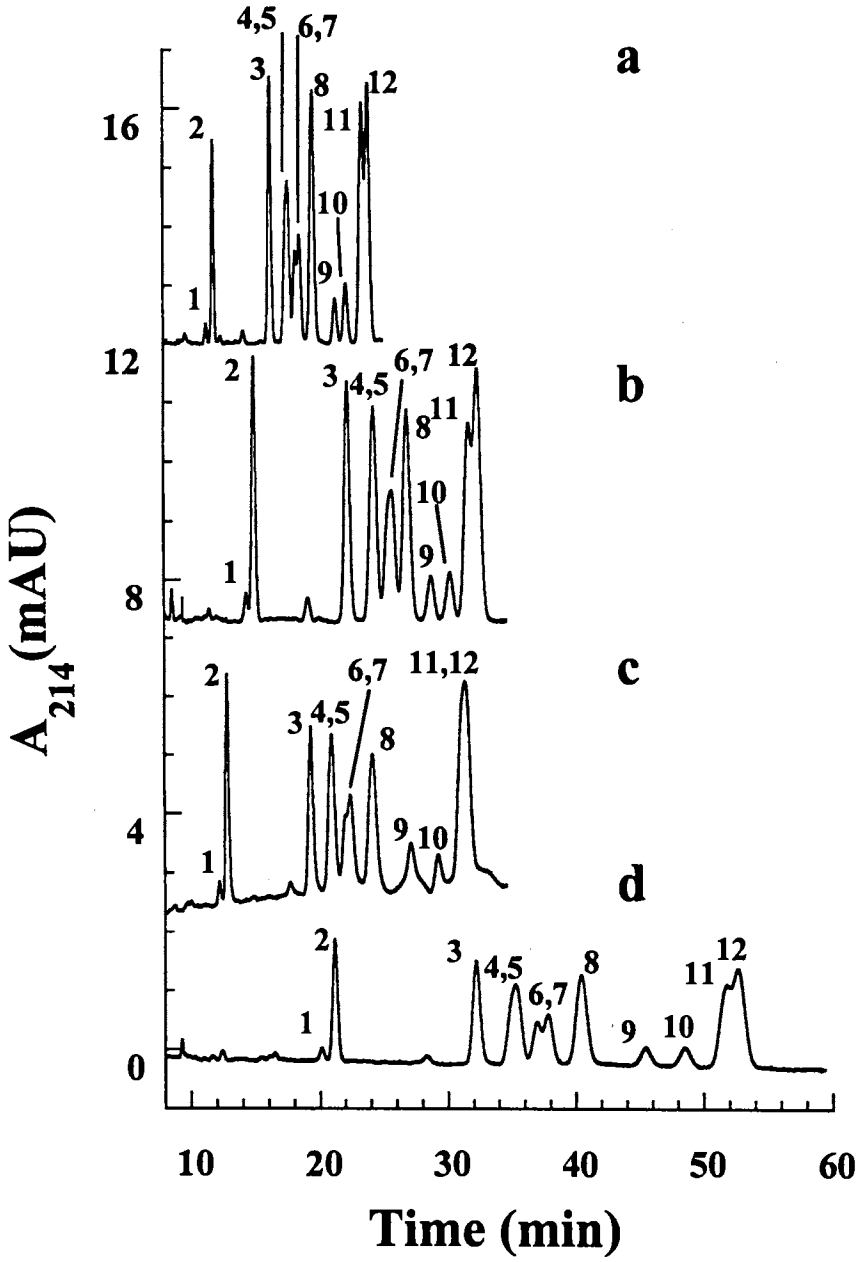


Figure 3. Electrochromatograms of some pyrethroids using various stationary phases. (a), HM-ODS; (b), C_8-Zorbax; (c), HC-ODS; (d), EC-ODS. Conditions: mobile phase, 25% (v/v) of 20 mM CH_3COONH_4, pH 6.0, and 75% (v/v) acetonitrile; Voltage, 20 kV; detection 214 nm. Solutes; 1 and 2, tetramethrin; 3, fenpropathrin; 4-7. cypermethrin; 8, sanmarton; 9 and 12 permethrin; 10 and 11, phenothrin.

Fig. 3, the home-made ODS Nucleosil 120-5 (i.e., HM-ODS) gives the best separation for the different analytes and their geometrical isomers (i.e., *cis* and *trans*) and diastereomers in the least amount of time. Overall, the HM-ODS and C_8-Zorbax material provided the highest EOF velocities over a wide range of mobile phase composition, see Table 1. The HM-ODS was intentionally prepared in house with a low surface coverage in octadecyl ligands (ca. 2 μmole octadecyl ligands/m^2) in order to yield an ODS stationary phase with a higher number of silanol groups on the silica surface, thus causing higher EOF velocities, and in turn faster separation times. The EC-ODS had the slowest EOF velocity due to endcapping. Endcapped stationary phases have a limited number of silanol groups available on the silica surface to produce EOF.

TABLE 1

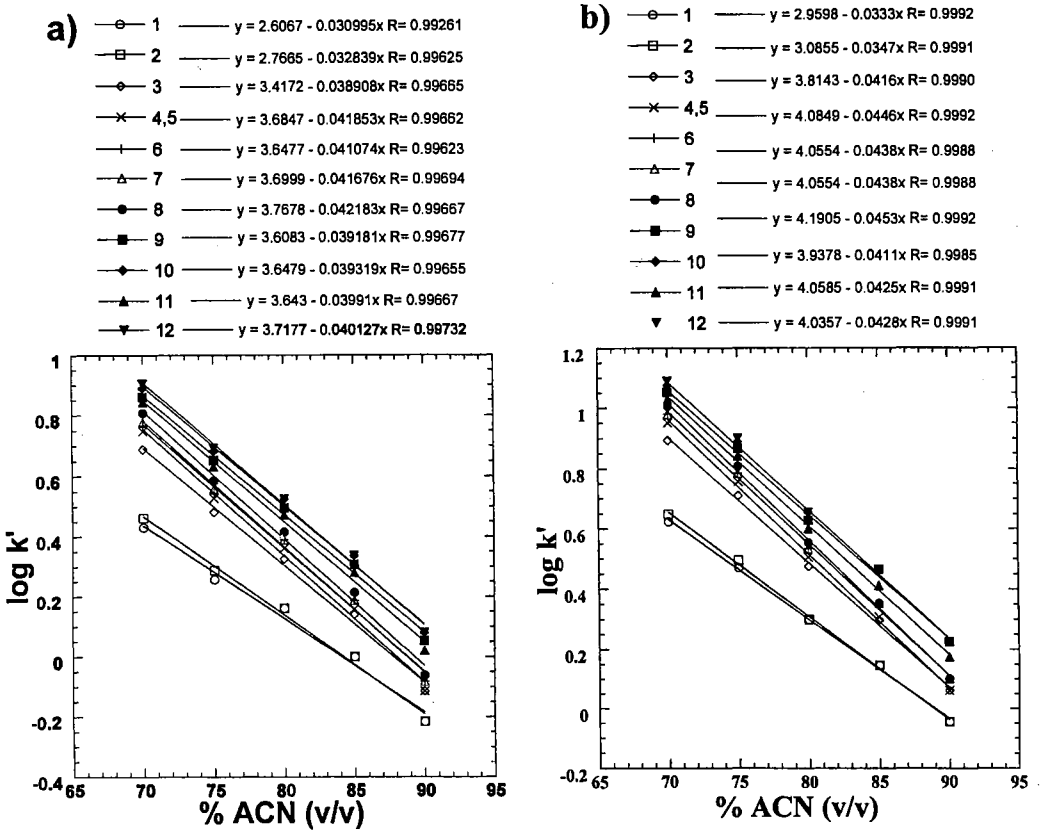
ELECTROOSMOTIC FLOW VELOCITY FOR THE DIFFERENT STATIONARY PHASES AT DIFFERENT ACN CONTENT OF THE MOBILE PHASE. OTHER CONDITIONS AS IN FIG, 3

% ACN in mobile phase	EOF velocity, mm/s			
	HM-ODS	C_8-Zorbax	HC-ODS	EC-ODS
70.00	0.91	1.08	0.92	0.64
75.00	1.04	1.13	0.97	0.71
80.00	1.02	1.20	1.03	1.01
85.00	1.17	1.26	1.08	0.97
90.00	1.23	1.32	1.16	0.98

Returning to Fig. 3, some of the pyrethroids may yield more than one peak because these solutes are geometrical isomers as well as diastereomers which in principle can be separated using an ODS or C_8 stationary phase. The various stereoisomers of the pyrethroids under investigation are not mentioned in this study because they can not be separated by reversed-phase CEC. While fenpropathrin which is not diastereomeric or geometric isomer is expected to give only one peak, tetramethrin, phenothrin and permethrin which exist as geometric isomers (i.e., *cis* and *trans*) could yield two peaks each. Aslo, sanmarton, which exist as two diastereomers may yield two separate peaks. Furthermore, cypermethrin has 4 diastereomers of which could result in a possible four peaks. Figure 3a-d shows that the two isomers of sanmarton could not be separated with the four stationary phases investigated. For cypermethrin, three of the four possible diastereomeric peaks were separated (see Fig. 3a, b, d). The *cis* and *trans* isomers of phenothrin and permethrin were separated with three of the four stationary phases, see Fig. 3a, b, and d. The four stationary phases investigated were able to separate the *cis* and *trans* isomers of tetramethrin, see Fig. 3. These results show the effects of the surface coverage in alkyl ligands and in turn the interplay of hydrophobic/silanophilic interactions on the selectivity of the stationary phases towards the solutes under investigation. Also, the results show the importance of the stationary phase morphology or texture (e.g., porosity) which affects the solute accessibility to the interactive ligands.

Linear regression analysis for the relationship of log k′ versus percent acetonitrile in the mobile phase was carried out, and the results are shown in Fig. 4 for the four stationary phases. In all cases, the plots of log k' vs. %ACN are linear, and the absolute values of the slopes of the lines obtained on the four stationary phases increased with the

hydrophobicity (i.e., solute size) of the solute as usually encountered in reversed-phase chromatography (RPC). This trend strongly supports the notion that the four stationary phases exhibit RPC behavior toward the investigated analytes. The retention of the various solutes increased with carbon load of the stationary phase; that is at any percent ACN in the mobile phase solute retention increased in the order the EC-ODS > HC-ODS > HM-ODS. Unexpectedly, the C_8-Zorbax exhibited the same or slightly higher retention towards the pyrethroids than the EC-ODS which is endcapped and has a %C (15% C) as was reported earlier (see Chapter IV).[21] This was explained by the fact that the short C8 chains of the C_8-Zorbax do not "stack" as extensively as the longer octadecyl chains of the ODS stationary phase. This means that the effective nonpolar phase ratio available for solute interaction is about the same or higher on the C_8-Zorbax than on the EC-ODS.



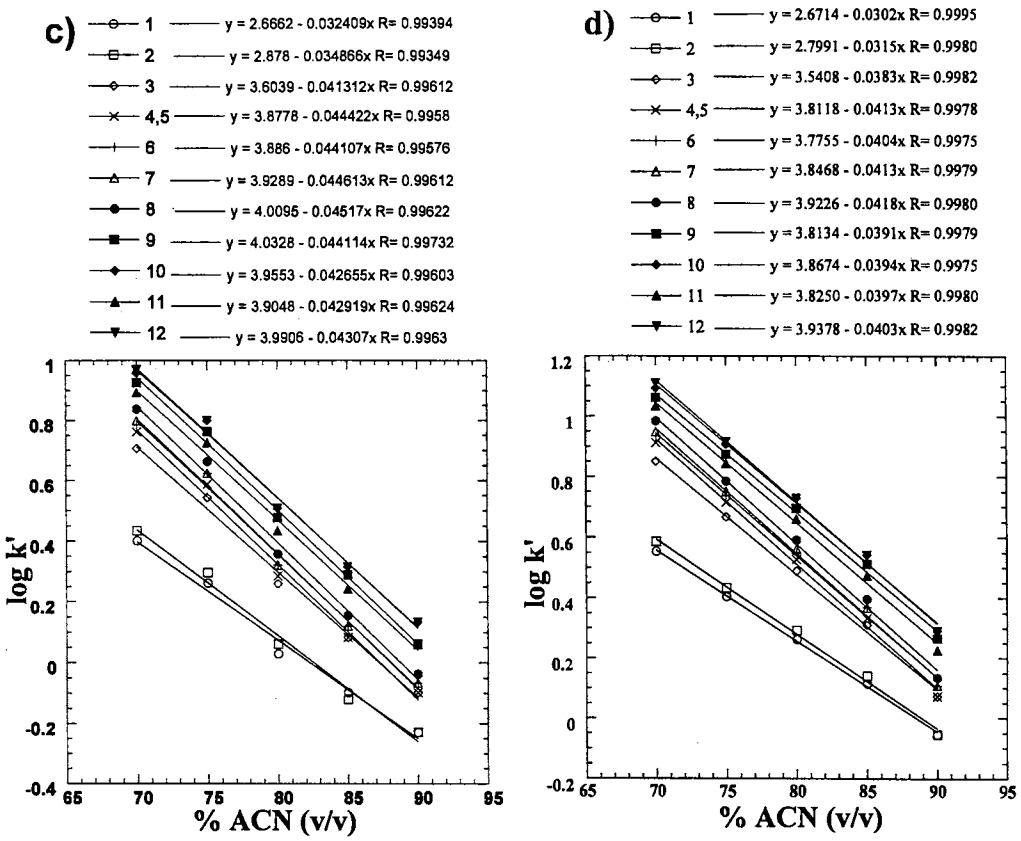


Figure 4. Plots of logarithmic retention factor (log k') for pyrethroids vs. percent acetonitrile (v/v) in the mobile phase. (a), HM-ODS; (b), C_8-Zorbax; (c), HC-ODS; (d), EC-ODS. Conditions: mobile phase, hydro-organic solution made up of various concentrations of CH_3OONH_4 to acquire an overall ammonium acetate concentration of 5 mM (pH 6.0) mixed with acetonitrile at different proportions; voltage 20 kV. Solutes; 1 and 2, tetramethrin; 3, fenpropathrin; 4-7. cypermethrin; 8, sanmarton; 9 and 12 permethrin; 10 and 11, phenothrin.

Table 2 lists the separation efficiencies for fenpropathrin and sanmarton obtained on the four stationary phases tested. These two solutes were selected because they each exhibited a single peak. The highest separation efficiencies are obtained when using home-made ODS (i.e., HM-ODS). The lower separation efficiencies obtained with the EC-ODS and HC-ODS capillary columns both having high carbon load on their surfaces may be attributed to an increased mass transfer resistance into the relatively thick

TABLE 2

SEPARATION EFFICIENCY, *N*, OBTAINED ON THE VARIOUS STATIONARY PHASES AT DIFFERENT ACN CONTENT OF THE MOBILE PHASE. OTHER CONDITIONS AS IN FIG. 3.

	Separation efficiency, N							
%ACN in	HM-ODS		C_8-Zorbax		HC-ODS		EC-ODS	
mobile phase	Fen*	San*	Fen*	San*	Fen*	San*	Fen*	San*
70.00	18800	15500	13800	12500	13500	7900	16200	10950
75.00	20900	16600	12850	10300	12400	6800	13750	9900
80.00	13800	11150	11000	5500	11300	7100	14700	9900
85.00	16400	10400	--	--	--	7700	12500	9900

* Fen and San stand for fenpropathrin and sanmarton, respectively
-- Peaks were not sufficiently resolved to determine *N*

retentive layer on the surface of the stationary phase of higher carbon coverage. On the other hand, the lower separation efficiency obtained on the C_8-Zorbax may be attributed to an increased silanophilic interaction of the solutes with the surface silanols than on the home made ODS-Nucleosil (HM-ODS) because of the shorter alkyl chain on the former than on the latter.

Surfactant Mediated Capillary Electrochromatography

Based on the above investigation, HM-ODS provided the best performance as far as the overall separation and speed of analysis of the pyrethroid insecticides are concerned. Therefore, the HM-ODS was selected to demonstrate the usefulness of the new SM-CEC concept in enhancing the resolving power of CEC. In this regards, the

SM-CEC based on the DOSS surfactant (see section 2) was investigated in the separation of the pyrethroids and their geometric isomers and diastereomers under different mobile phase conditions including the concentration of DOSS surfactant and organic modifier (e.g., acetonitrile) in order to determine the optimal conditions.

Figure 5a and b shows the dependence of the adjusted retention time (t_r-t_o) on DOSS concentrations in the mobile phase at 60 and 70% (v/v) ACN, respectively. At 60% ACN, the adjusted retention time goes through a maximum at 20 mM DOSS (Fig. 5a) while at 70% ACN, the adjusted retention time increases steadily with increasing the DOSS concentration (Fig. 5b) in the range studied. Changing the DOSS concentration and the ACN content of the mobile phase is expected to influence various parameters of the SM-CEC system, e.g., ionic strength, viscosity and dielectric constant of the mobile

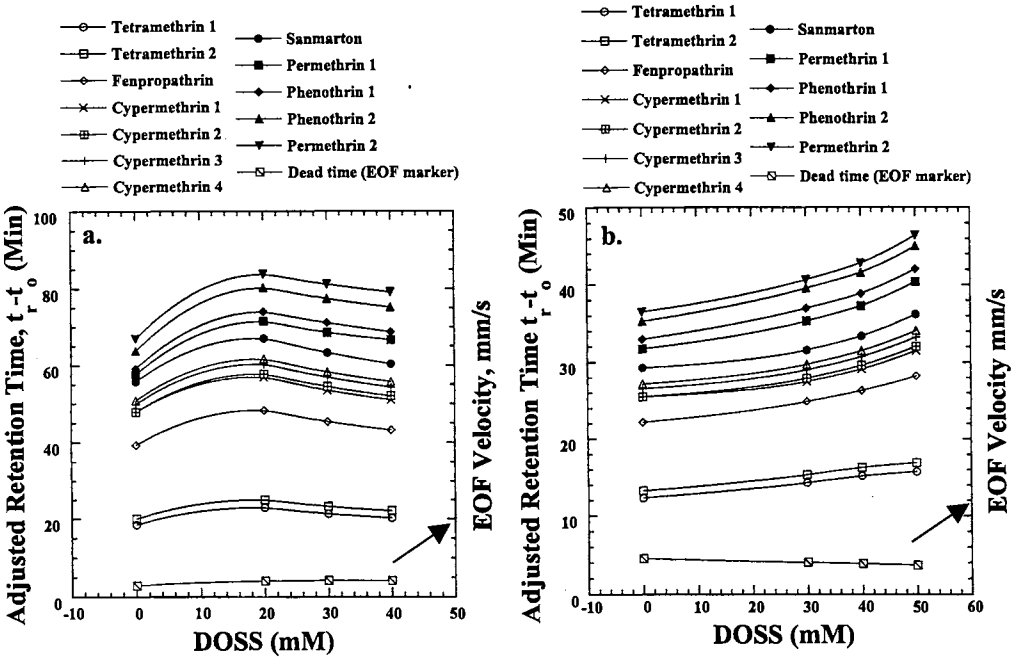


Figure 5. Plots of adjusted retention time (t_r-t_o) for pyrethroids and EOF velocity vs. DOSS concentration in the mobile phase: (a), 60% (v/v) acetonitrile, 40% (v/v) 12 mM Tris, pH 8.00; (b), 70% (v/v) acetonitrile, 30% (v/v) 17 mM Tris, pH 8.00. Other conditions: voltage, 20 kV; detection, 214 nm.

phase as well as the amount of DOSS adsorbed onto the stationary phase and the extent of solute-DOSS association. At 60% ACN, the EOF velocity decreased from 1.43 to 1.00 mm/sec when going from 0 to 40 mM DOSS in the mobile phase, see Fig. 5a. On the other hand, at 70% ACN, the EOF velocity increased from 0.91 to 1.12 mm/sec when the DOSS concentration in the mobile phase increased form 0 to 50 mM, Fig. 5b. The increase in the EOF velocity at 70% ACN parallels the expected increase of DOSS adsorbed onto the stationary phase surface with increasing surfactant concentration. This should result in increased surface charge density and in turn the EOF velocity. This effect seems to be higher in magnitude than the opposing effect resulting from increasing the ionic strength of the mobile phase with increasing DOSS concentration which should decrease the EOF velocity. At 60% ACN the two opposing effects seem to reverse in magnitude such that the second effect (i.e., ionic strength effect) is the predominant one, and the net result is a decrease in the EOF velocity as the DOSS concentration in the mobile phase at 60% ACN is increased.

Increasing the DOSS concentration in the mobile phase results in increasing the solute-DOSS association in both the mobile and the stationary phase and in turn increasing the adjusted retention time. This expected trend is seen in Fig. 5b (i.e., at 70% ACN) for the entire surfactant concentration range studied despite the fact that the EOF has increased with increasing the DOSS concentration, thus indicating that the magnitude of the EOF increase is well below the magnitude of other retention promoting effects. This expected trend is also seen in Fig. 5a (i.e., at 60% ACN) but with a maximum in the adjusted retention time at 20 mM DOSS. The shallow decrease in the adjusted retention time at DOSS concentration higher than 20 mM may be explained by the viscosity

increase of the mobile phase that is richer in the aqueous component, which then slow the electrophoretic mobility of the DOSS-pyrethroid complex in the opposite direction to the EOF.

As can be seen in Fig. 5a and b, the selectivity factor for two of the 4 diastereomers of cypermethrin increased from ~ 1.00 (no separation) to 1.02 (partial separation) at both 60 and 70% ACN when going from 0 to 40 mM DOSS in the mobile phase. For the other two diastereomers of cypermethrin, the selectivity factor increased from 1.02 to 1.03 at both 60 and 70% ACN when increasing the DOSS concentration from 0 to 40 mM. Another noticeable increase in selectivity was observed for the solute pair consisting of sanmarton and the first eluting permethrin isomer. In this case, the selectivity factor increased substantially from 1.03 to 1.10 when going from 0 to 40 mM DOSS at 60 % ACN.

The separation efficiencies for six representative isomeric pyrethroids were chosen and plotted, see Fig. 6. As these plots suggest, the efficiencies tend to rise as the concentration of DOSS goes up. These results indicate that the DOSS pseudo-stationary phase works as an anti-convective medium reducing solute longitudinal diffusion in the mobile phase. In fact, upon solute binding to the DOSS, the resulting complex has a higher molecular size than the free solute, thus exhibiting smaller diffusion coefficient.

The combined enhancing effects of DOSS on selectivity and separation efficiency resulted in improving the resolution of the pyrethroids and their geometric isomers and diastereomers in SM-CEC. This is shown in Table 3 which lists the resolution between neighboring peaks in the absence and presence of DOSS in the mobile phase. With a few

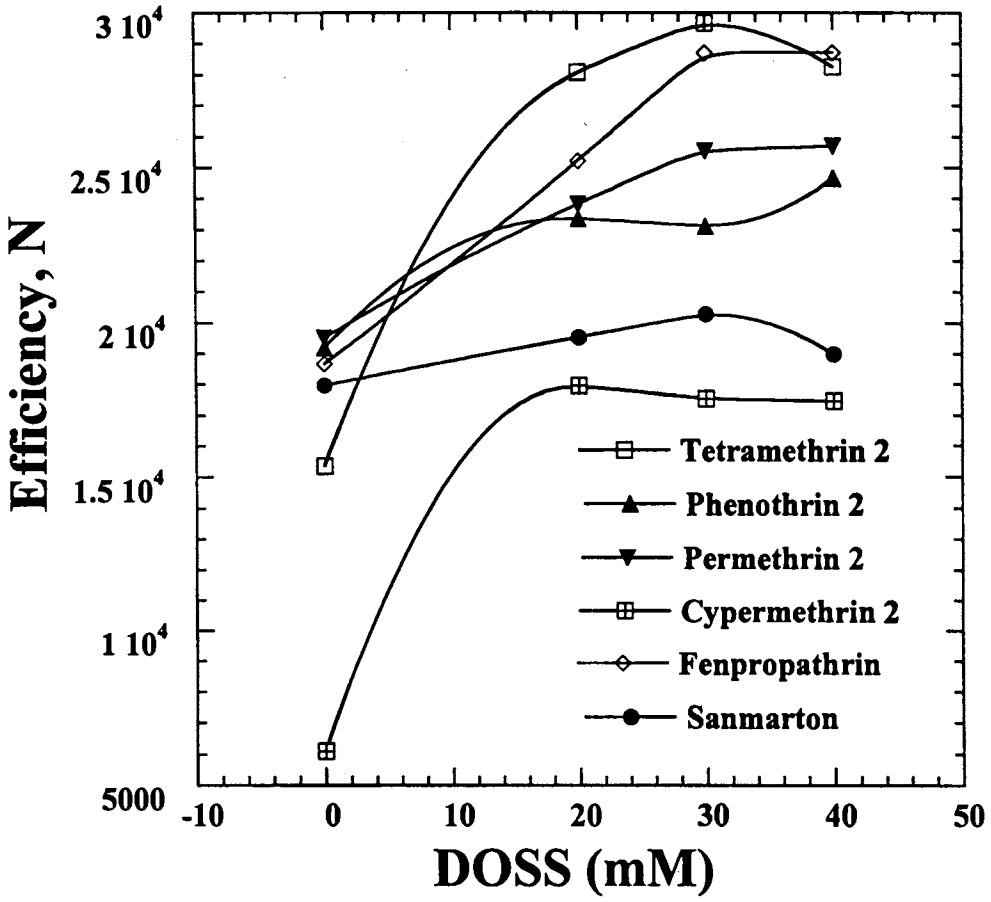


Figure 6. Plot of separation efficiency *N* vs. DOSS concentration for the six pyrethroids investigated. Conditions: mobile phase, 40% (v/v) of 12 mM Tris, pH 8.0, and 60% (v/v) acetonitrile; Voltage, 20 kV.

exceptions, resolution increased substantially with increasing DOSS concentration in the mobile phase in the concentration range studied. A noticeable case is the diastereomers of cypermethrin.

Based on the above evaluation of the various conditions, it was concluded that 60% (v/v) acetonitrile, 40% (v/v) of 12 mM Tris, pH 8.0, containing 30 mM DOSS gave the best separation in the least amount of time. Fig. 7a and b shows the six pyrethroids without DOSS and with 30 mM DOSS, respectively. The resolution of the cypermethrin diastereomers was increased from 0.0 with no DOSS to 0.60 with 30 mM DOSS, see Fig.

TABLE 3

RESOLUTION FOR THE SIX PYRETHROIDS AND THEIR DIASTEREOMERS UNDER INVESTIGATION AT VARIOUS DOSS CONCENTRATION IN THE MOBILE PHASE. OTHER CONDITIONS AS IN FIG. 7.

	Resolution, Rs			
Solute	0 mM DOSS	20 mM DOSS	30 mM DOSS	40 mM DOSS
Tetramethrin	1.98	2.88	3.15	2.50
Tetramethrin	19.56	23.13	24.61	23.81
Fenpropathrin	4.45	6.38	6.14	5.97
Cypermethrin	0.00	0.49	0.60	0.61
Cypermethrin	0.95	1.38	1.34	1.26
Cypermethrin	0.59	0.70	0.81	0.81
Cypermethrin	2.81	2.66	2.94	2.66
Sanmarton	1.01	1.97	2.38	3.13
Permethrin	0.73	0.99	0.97	1.07
Phenothrin	2.18	2.66	2.53	3.12
phenothrin	1.65	1.59	1.74	1.91
Permethrin				

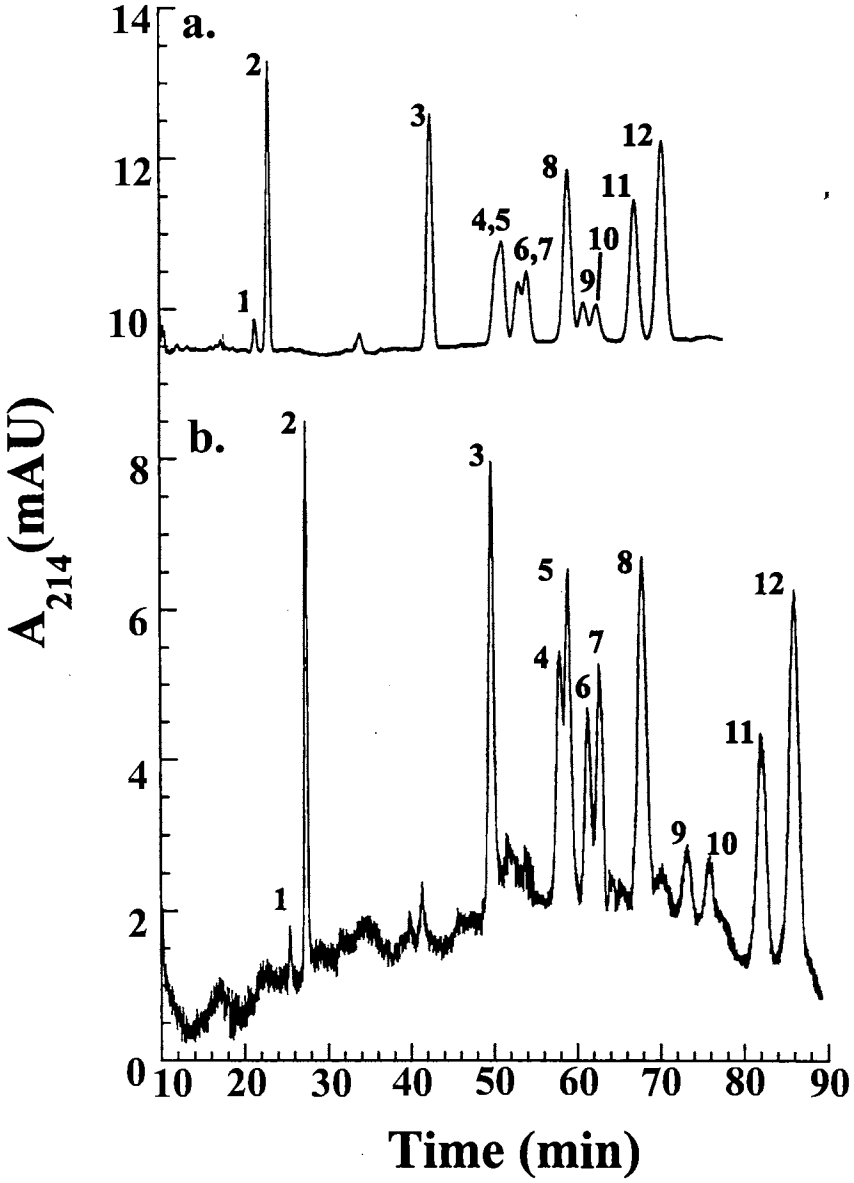


Figure 7. Electrochromatograms of the pyrethroids used in this study: (a) without DOSS and (b) with 30 mM DOSS. Conditions: mobile phase, 40% (v/v) of 12 mM Tris, pH 8.0, and 60% (v/v) acetonitrile; Voltage, 20 kV; detection 214 nm. Solutes; 1 and 2, tetramethrin; 3, fenpropathrin; 4-7, cypermethrin; 8, sanmarton; 9 and 12 permethrin; 10 and 11, phenothrin.

7. Unfortunately, the time also increased for cypermethrin by 8 min and for the last peak by 15 min. The increase in time is acceptable considering the improvement in resolution obtained when using DOSS in the mobile phase.

Conclusion

We have shown that an ODS stationary phase with moderate surface coverage in octadecyl ligands is useful in the separation of pyrethroid insecticides. Furthermore, SM-CEC is an effective separation system for the separation of isomeric forms of pyrethroid insecticides. The presence of a charged surfactant (e.g., DOSS) in the mobile phase introduces another factor for manipulating retention, selectivity and separation efficiency. The net result of optimizing these three parameters is a significant enhancement in the resolving power of the CEC system.

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VITA

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Doctor of Philosophy

Thesis: ELECTROKINETIC CAPILLARY CHROMATOGRAPHY METHODS FOR SEPARATION AND TRACE ENRICHMENT OF DILUTE ANALYTES USING OPEN TUBULAR AND PACKED CAPILLARIES

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