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Electrochromatographic studies of select pharmaceutical compounds

Elham Moslehi
San Jose State University

ELECTROCHROMATOGRAPHIC STUDIES OF SELECT PHARMACEUTICAL COMPOUNDS

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Elham Moslehi

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APPROVED FOR THE DEPARTMENT OF CHEMISTRY

Dr. Joseph J. Pesek

Dr. Bradley M. Stone

Dr. Roger Terrill

Dr. Brooke Lustig

APPROVED FOR THE UNIVERSITY

ABSTRACT

ELECTROCHROMATOGRAPHIC STUDIES OF SELECT PHARMACEUTICAL COMPOUNDS

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This study presents analyses of electrochromatographic migrations of select pharmaceutical compounds in two types of capillaries, prepared via etching and modification using 4-cyano-4′-pentoxy biphenyl and C-18 stationary phase, as well as a bare silica capillary. The liquid crystal stationary phase for open-tubular capillary electrochromatography (OTCEC) is prepared through silanization and hydrosilation processes. CEC in an open tubular format overcomes many problems associated with the use of packed column electrochromatography.

While OTCEC with liquid crystal and C-18 bonded stationary phases has a wide range of applications, this report presents focused CE & OTCEC studies of theophylline, dihydroxy-theophylline (dyphylline), aminophylline, and nortriptylline. The experimental conditions covered various buffer pH values (2.14 to 8.14), organic modifier volume ratios (0% to 50%), and applied voltages (±25 kV). The experimental results, including migration time and capillary efficiency, are analyzed for different conditions. The optimal migration efficiency conditions and capillary type are determined for each specific compound.

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Symbols and Abbreviations

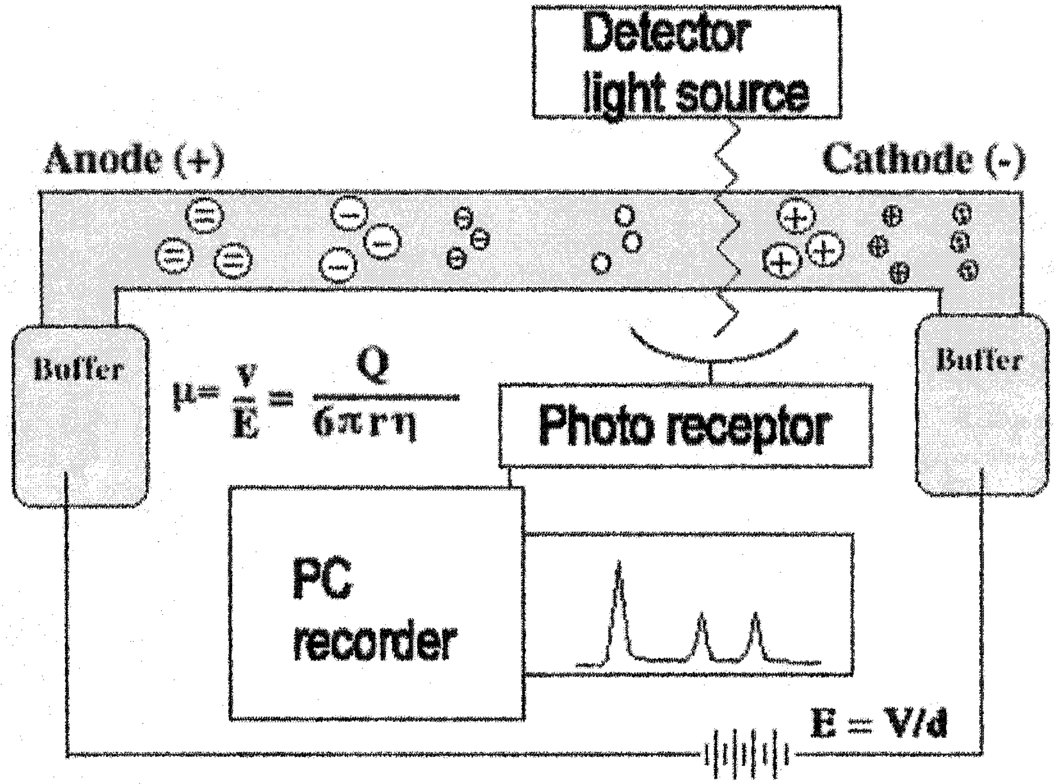
C-18	octadecyl
CAS	Chemical Abstract Service
CE	Capillary Electrophoresis
CEC	Capillary Electrochromatography
CGE	Capillary Gel Electrophoresis
CIFE	Capillary Isoelectric Focusing
CZE	Capillary Zone Electrophoresis
E	Electric Field
EOF	Electroosmotic Flow
FTIR	Fourier Transform Infrared Spectroscopy
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
l	Effective Capillary Length
i.d.	Internal Diameter
L	Total Capillary Length
LCD	Liquid Crystal Display
MEKC	Micellar Electrokinetic Chromatography
MS	Mass Spectroscopy
NMR	Nuclear Magnetic Resonance
OT-CEC	Open Tubular Capillary Electrochromatography
PC-CEC	Packed Column Capillary Electrochromatography

pH	(chemistry) p(otential of) H(ydrogen)
Q	Ion Charge
r	Ion Radius
SEM	Scanning Electron Microscopy
t	Migration Time
TES	Triethoxysilane
UV	Ultraviolet
v	Ion Migration Velocity
V	Applied Voltage
η	Solution Viscosity
μ_a	Sample Mobility
μ_e, μ_{ep}	Electrophoretic Mobility
μ_{EOF}	Electroosmotic Flow Mobility

1. Introduction

1.1. Capillary Electrophoresis (CE) Overview

Electrophoresis refers to the migration of electrically charged particles through an electrolyte (within a capillary) under the action of an electric field. These charged particles migrate through a capillary in a particular direction at specific rates depending on their charge-to-size ratio. More specifically, the electrophoresis process can be defined as *the differential movement or migration of ions by attraction or repulsion in an electric field.* Cations migrate toward the negatively charged electrode (cathode) and anions move toward the positively charged electrode (anode). Capillary Electrophoresis (CE) is an analytical method based on conducting electrophoresis in buffer-filled, narrow-bore capillaries. The practical implementation of CE is conceptually quite simple (see the schematic diagram in Figure 1). A positive electrode (anode) and a negative electrode (cathode) are placed in a solution containing the analyte (ions). When an electrical voltage is applied between the two electrodes, the negatively charged anions and the positively charged cations will drift through the solution toward the electrode of opposite charge polarity. Because of their high separation speeds and good analytical efficiencies, capillary column separation techniques are among the most vital tools for characterizations of organic and inorganic materials in many fields, such as chemistry, medicine, and pharmaceutical industries.



μ = Electrophoretic Mobility

v = Ion Migration Velocity

E = Electric Field

Q = Ion Charge

η = Solution Viscosity

r = Ion Radius

Figure 1. Schematic diagram of the capillary electrophoresis (CE) technique and its operating principle.

Electrophoretic analyses using free solutions were performed and reported, for the first time, by Hjerten *et al.* in 1958 [1]. Their analytical work involved the use of a quartz column with a 1 - 3 mm inner diameter (i.d.). It was reported that using columns with smaller i.d. values would diminish or reduce the undesirable Joule-heating effects, hence the advent of capillary electrophoresis (CE). The use of capillary analysis with different types of sample materials has since demonstrated enhanced analytical stability and reproducibility in the coming years.

Capillary electrophoresis has evolved towards a variety of separation techniques, all involving the application of a high electrical voltage across the buffer-filled capillaries in order to achieve separations. Electrophoretic techniques include: (1) The capillary separation phenomenon, based on ion size and charge differences among samples or analytes (i.e., capillary zone electrophoresis or CZE); (2) separation of neutral compounds using surfactant micelles, known as micellar electrokinetic capillary chromatography (MEKC); (3) moving of solutes through a gel (capillary gel electrophoresis or CGE); and (4) separation of zwitterionic solutes within a pH gradient (known as capillary isoelectric focusing or CIFE) [2]. MEKC is a commonly used separation technique in pharmaceutical applications. CGE and CIEF are used for separation of biomolecules such as DNA and proteins, and are increasing utilized in the development of biotechnology and drugs. Many of the CE separation techniques rely on the presence of an electrically induced flow of solution or electroosmotic flow [3].

1.1.1. Electrophoresis Theory

Electrophoresis theory is the foundation for the CE operating principle and can be described by a few simple equations. As discussed earlier, electrophoresis is the migration or movement of solutes or ions under the action of an applied electric field. Separation by electrophoresis (electrophoretic separation) is based on the differences in the migration speeds of ions (solutes) in an electric field. The ion migration velocity is proportional to the electric field, as shown in the following equation:

$$V = \mu_e E$$

Where: V = ion migration velocity ($m\ s^{-1}$)

μ_e = electrophoretic mobility ($m^2\ V^{-1}\ s^{-1}$)

E = applied electric field strength ($V\ m^{-1}$)

The electric field is simply the applied voltage divided by the total capillary length.

The electrophoretic mobility, μ_e, is a parameter that describes how fast a given ion or solute may move through a medium such as a buffer-filled solution. It is essentially a measure of the balance of the electrical force (acting in favor of motion) and the frictional force (acting against motion) affecting each ion. The electrophoretic mobility is a constant for a specific ion and under a given set of experimental conditions. When an ion is migrating, these opposing forces are equal and opposite. The electrophoretic mobility is a function of the ion charge (q), the solution viscosity (η), and the ion radius (r), as follows:

$$\mu_e = \frac{q}{6\pi\eta r}$$

Electrophoretic mobility is a characteristic constant for a given analyte or ion. The charge-to-size ratio of a given analyte ion determines its electrophoretic mobility. Higher ion charges and/or smaller ion sizes result in higher electrophoretic mobility values, whereas lower ion charges and/or larger ion sizes produce lower mobility values. Electrophoretic mobility in turn determines the migration velocities of the ions. Different solutes usually have different electrophoretic mobilities, thus, it is possible to separate mixtures of different ions and solutes using electrophoresis.

1.1.2. Electroosmotic Flow

Electroosmotic flow (EOF) is the bulk flow of liquid through the capillary that results from the longitudinal electric field acting on ions in the capillary diffuse layer (to be described by Stern's model below). EOF is an important parameter in CE and must be well controlled [4]. The capillary tube used in CE is usually made of fused silica. The inner surface of the capillary has silanol groups that are negatively charged when deprotonated. When the capillary is filled with the buffer solution, the negatively charged capillary wall attracts positively charged ions from the buffer solution. As shown in Figure 2 (i.e., Stern's model), this phenomenon produces an electrical double layer and a potential difference

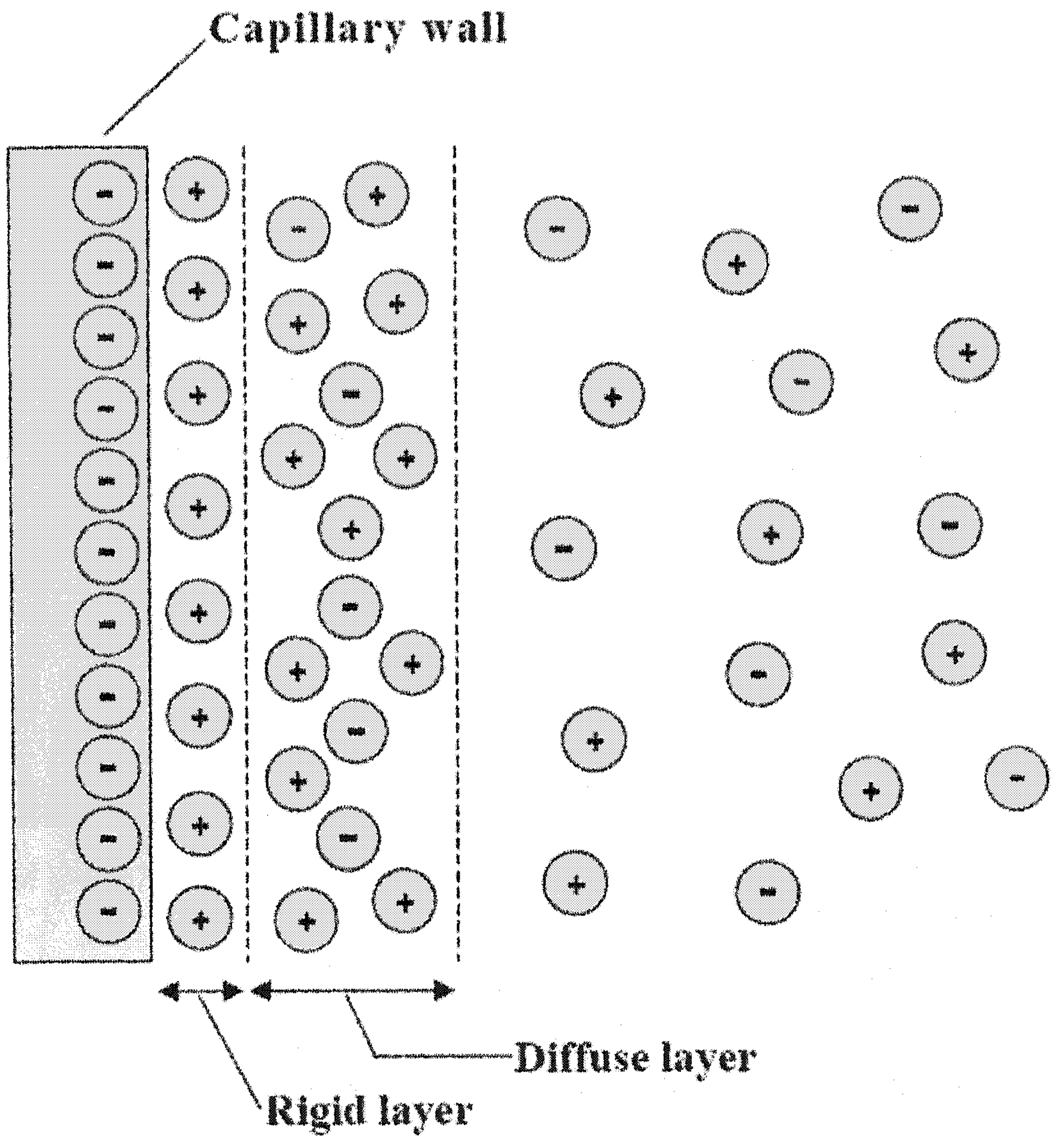


Figure 2. Stern's model for the double-layer charge distribution at a negatively-charged capillary wall, resulting in generation of zeta potential and EOF.

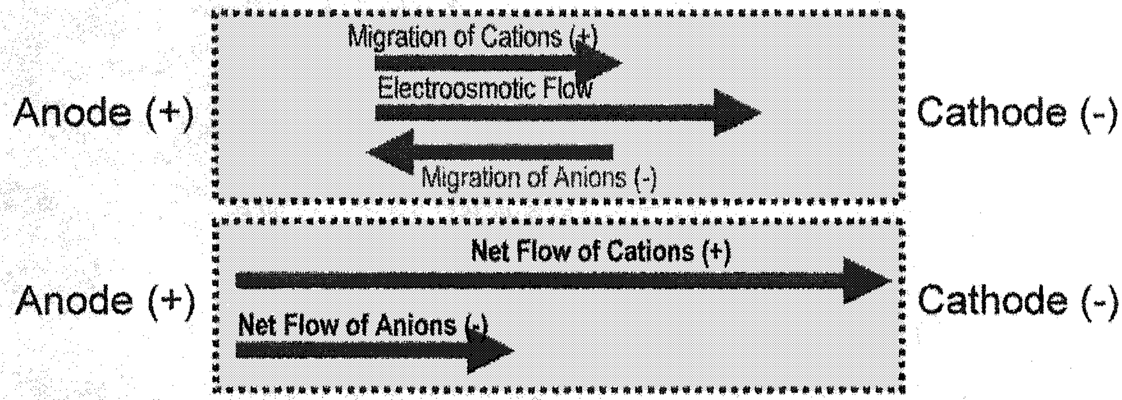
(called zeta potential) near the capillary wall. Stern's model for an electrical double layer comprises a rigid layer of adsorbed molecules on the capillary wall and a diffuse layer, where ion diffusion can take place. The closest layer to the surface is called "Inner Helmholtz" or "Stern Layer" and the second more diffuse layer is called "Outer Helmholtz Plane" or the diffuse layer. The zeta potential is the potential difference between the capillary and solution. The potential of the solution drops exponentially by moving away from the capillary surface. The zeta potential is a function of the nature and concentration of the ions in the diffuse layer. For example, a negatively charged surface and a positively charged solution result in a positive zeta potential. In this case, the solution bears a slightly positive net charge and, therefore, will tend to flow towards the cathode end of the capillary.

When a voltage is applied across the capillary, cations in the diffuse layer move toward the cathode, pulling the bulk solution along with them [see Figures 3(a) and 3(b)]. Neutral molecules also migrate along with the buffer as a result of EOF [4]. The result is a net bulk flow in the direction of cathode with an electroosmotic flow velocity described by the following equation:

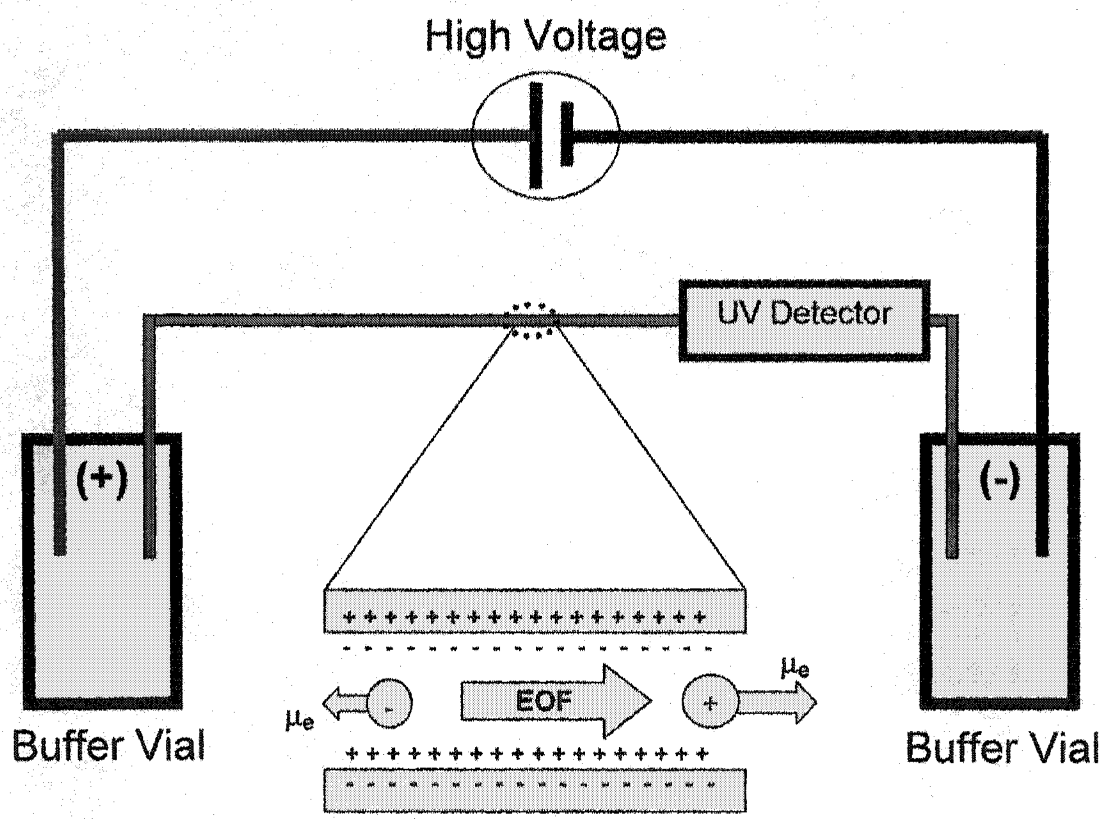
$$v_{\mathrm{EOF}} = \left(\frac{\varepsilon_0 \varepsilon \zeta}{4\pi\eta}\right) E$$

where: υ_{EOF} = electroosmotic flow velocity (bulk flow velocity)

ε_0 = dielectric constant of vacuum



(a)



(b)

Figure 3. Schematic diagrams of: (a) electroosmotic and electrophoretic migration components in a capillary, and (b) electroosmotic flow (EOF) in a capillary.

ε = dielectric constant of the buffer solution

ζ = zeta potential

E = electric field strength

The term inside parentheses corresponds to the electroosmotic flow mobility, μ_{EOF}. The relationship between electroosmotic flow velocity and electroosmotic flow mobility ($\upsilon_{EOF} = \mu_{EOF}$ E) is similar to the relationship between migration velocity and electrophoretic mobility (V= μ_e E). The primary parameters affecting EOF mobility are the zeta potential value, the dielectric constant, and the viscosity of the buffer solution. In summary, EOF is essentially the result of an electrical potential difference on the capillary surface where the ions in the diffuse layer experience a force parallel to the surface.

The zeta potential is proportional to the charge density on the capillary wall, which itself is highly pH dependent. So, EOF mobility changes with the buffer pH and is larger at higher pH. At low pH values the EOF decreases due to protonation of the silanol groups and reduced attraction between the positively charged solutes and the capillary wall. The EOF can even approach zero at very low pH values. At high pH (pH > 7), the EOF mobility is sufficiently large that even anions are swept towards the cathode. Thus, the measured migration velocity of a solute may not be directly related to its electrophoretic mobility but instead, depends on a combination of both its electrophoretic mobility and EOF mobility. The solute's apparent electrophoretic mobility (μ_a) derived from its measured migration velocity is the sum of its electrophoretic mobility (μ_e) and EOF mobility (μ_{EOF}), as indicated below:

$$\mu_a = \mu_e + \mu_{EOF}$$

The apparent electrophoretic mobility (μ_a) may also be described as follows:

$$\mu_a = (lL) / (tE) = l / (tV)$$

Where: V = applied voltage E = electric field

t = migration time L = total capillary length

l = effective capillary length

Since samples are usually introduced at the anode side of the capillary, and EOF moves from the anode to the cathode, cations have $\mu_e > 0$, neutrals have $\mu_e = 0$, and anions have $\mu_e < 0$. As a result, cations migrate faster than the EOF, anions migrate more slowly than the EOF, and neutrals migrate with the same velocity as the EOF [see the schematic diagrams of Figures 3(a) and 3(b)].

1.1.3. EOF Flow Profiles

As shown schematically in Figure 4, EOF produces a flat flow profile [see Figure 4(a)], versus a pressure-driven or hydrodynamic flow, which is a parabolic flow [see Figure 4(b)]. EOF has a flat, plug-type flow profile because its driving force (charge on the capillary wall) is uniformly distributed along the capillary. This feature implies that there are no pressure drops and the flow velocity is uniform across the capillary. This observation is different from pressure-driven flow

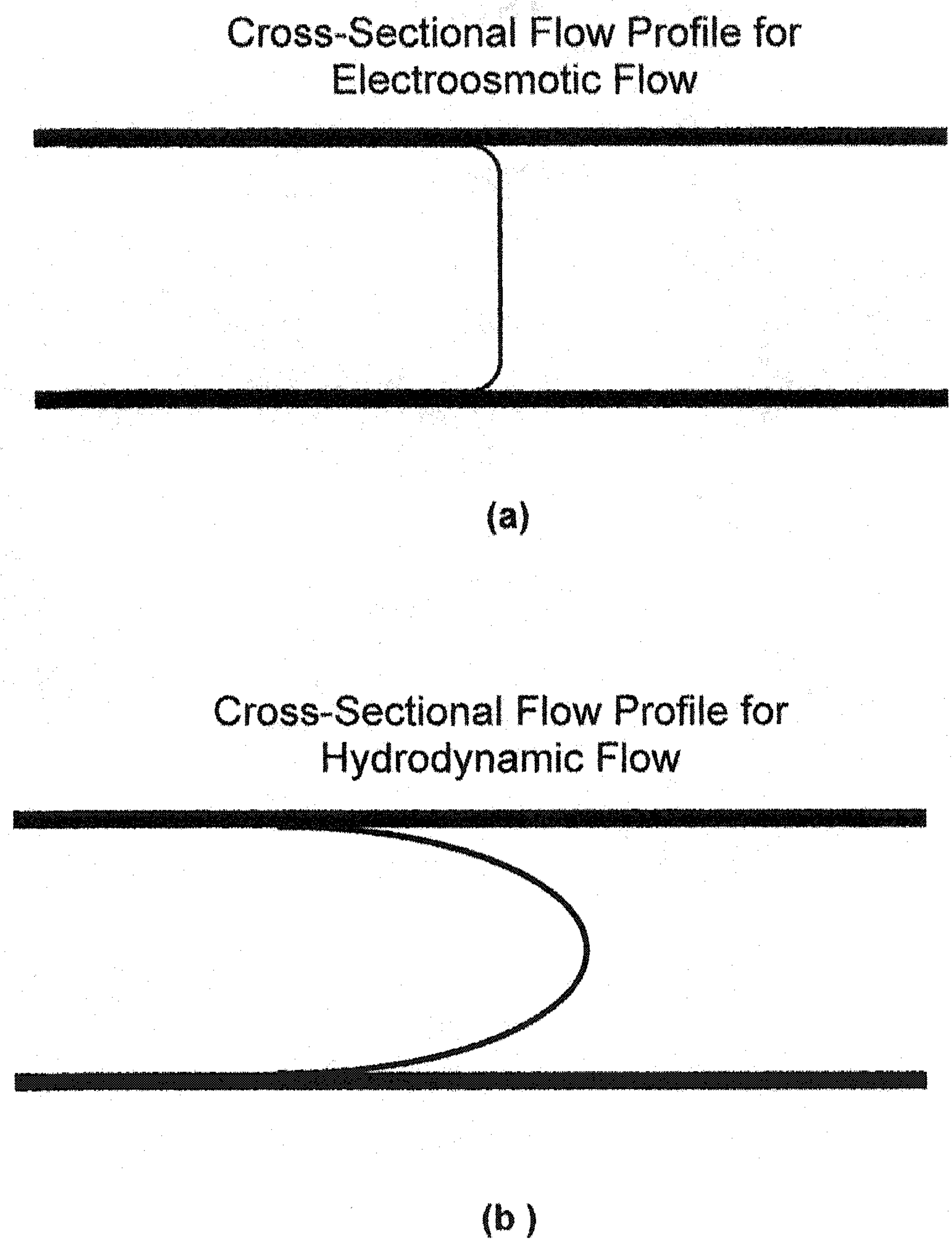


Figure 4. Schematic diagram of cross-sectional flow profiles as a result of: (a) electroosmotic flow, and (b) hydrodynamic flow.

(e.g., HPLC), in which pressure-driven forces at the column walls produce a pressure drop across the column, resulting in parabolic flow profile. The flat flow profile of EOF minimizes band broadening and narrows the detection zone, leading to high separation efficiencies that enable separations based on minute mobility differences.

1.1.4. CE Capillary Properties

The capillaries used in CE are normally made of fused silica covered with an external polyimide protective coating to give them bending flexibility and strength. Capillaries are usually 25-100 cm long and have volumes on the order of a few μl. A small portion of this coating is removed to form a window for detection. In commercial CE instruments, the capillary is held in a cartridge to protect the delicate detection window. The window is aligned at the optical center of the detector.

The inner surface of the capillary can be chemically etched and modified by covalently bonding certain molecules. When the modifier is adsorbed on the wall, the capillary is referred to as coated capillary, while a capillary with a covalently attached substance to the wall is called covalently modified. These coatings should be stable over a wide range of pH values. The coatings are used for a variety of purposes such as to reduce sample adsorption or to change the ionic charge of the capillary wall (i.e., change the zeta potential).

1.2. Capillary Electrochromatography (CEC)

Capillary electrochromatography (CEC) is a variant of CE where the capillary is covalently modified. CEC is useful for separation of a wide range of analytes, including small ions, basic compounds, proteins, and peptides. The CEC separation mechanism is due to the combined effects of: (i) chromatographic partitioning, and (ii) electrophoretic migration (or based on the combined features of both HPLC and CE [5]).

CEC offers the high efficiency of CE combined with the high selectivity of micro-HPLC. CE is only capable of separating charged species via their different electrophoretic mobilities and cannot resolve neutral species. Micro-HPLC, on the other hand, produces high selectivity in a wide range of applications, including analyses of mixtures containing neutral components, due to the variety of available stationary phases. Since the mobile phase in micro-HPLC is driven through the column by pressure, resulting in a parabolic flow profile, the column efficiency is typically lower than that of CEC. CEC typically employs columns similar to those used in micro-HPLC, but the mobile phase is driven by an applied electrical voltage, as in CE.

CEC can be performed in a standard CE instrument using a micro-HPLC column (employing an electrophoresis capillary packed with or attached to a chromatographic medium). As described before, CEC separation takes place because of both electrophoretic and chromatographic phenomena. CEC can be implemented in two different types of capillaries: (i) packed column (PC-CEC) that utilize stationary phases like HPLC, and (ii) open tubular (OTCEC) [6]. In OT-CEC, the stationary phase is attached to the capillary inner wall, while in PC-CEC the solid packed material is held in by frits that act as a stationary phase. In this study, the results of OT-CEC experiments are presented and discussed.

In CEC, packed columns offer faster separation times than HPLC. The CE capillaries used in CEC are packed with the HPLC packing material, and an electrical voltage is applied across the capillary in order to generate an electroosmotic flow (EOF). The EOF transports solutes through the column towards the ultra-violet (UV) light detector. Both differential partitioning and electrophoretic migration of the solutes occur during their movement towards the UV detector, leading to CEC separations of solutes.

Electrochromatography has a plug-like flat flow profile, similar to the flow profiles observed in CE. The plug-like flow in both open and packed capillaries is more uniform than the parabolic laminar flow of a pressure-driven system like HPLC. The beneficial plug flow profile of EOF reduces flow-related band broadening normally associated with pressure-driven parabolic flow. Since flows are electroosmotically and not pressure driven, small-diameter particles can be used in order to achieve very high separation efficiencies.

The primary analytical parameters in CE and CEC are very similar, including migration time, mobility, and dispersion. The migration time of the sample refers to the time required for the sample to migrate from the injection point to the point of detection (usually a UV absorbance detector). Migration time as well as other important CEC experimental parameters (such as peak width and area) must be known in order to determine the mobility of the sample in different capillaries [7].

1.2.1. CEC Apparatus

Operation of a CE system involves application of a high electrical voltage (5 kV - 30 kV) across a narrow-bore (25 μm – 100 μm) capillary, which results in electrical currents in the range of 10 – 100 μA [see the schematic diagrams in Figures 1 and 3(b)]. The capillary is filled with a suitable buffer, which conducts current through the inner bore. The capillary ends are placed into buffer reservoirs, and the capillary is filled with an identical buffer. Electrodes made of an inert material such as platinum are also inserted into the buffer reservoirs to complete the electrical circuit for current flow. A small volume of sample is injected into one end of the capillary. The sample may be injected into the capillary either electrokinetically or hydrodynamically. After the buffer reservoir is replaced, an electric field is applied and the separation process is performed. The capillary passes through a detector, usually a UV absorbance device, at the opposite end of the capillary (i.e., cathodic end of the capillary). Application of an electrical voltage causes movement of sample ions towards their appropriate electrode. The migration of cations results in an accompanying migration of buffer solution through the capillary, resulting in EOF.

As discussed earlier, the EOF is usually greater than the electrophoretic mobility of the individual ions of the sample in a bare capillary. As described in CE, both cations and anions can be separated in the same CEC run. Cations are attracted toward the cathode due to the electric field effects [Figure 3(a)]. While anions are electrophoretically attracted toward the anode, they are carried toward the cathode with the EOF of the buffer. Migration of cations is based on the charge-to-mass ratios, and the cations with the highest charge-to-mass ratios will migrate with the highest velocity, arriving first at the UV detector. Next, neutral components migrate with the same velocity as the EOF, and lastly, the anions migrate. The EOF is an essential feature of CEC and must be well controlled (e.g., at high pH values the EOF may be too great to allow sufficient separation of all components in a sample mixture).

1.2.2. Sample Injection

The sample solution is forced into the end of the capillary that is the farthest from the detector [see the schematic diagrams illustrated in Figures 1 and 3(b)]. Typical sample injection volumes are about 10-100 nl. There are two modes of sample injection; one is vacuum or pressurized injection and the other is injection of the sample by applying an electrical voltage, also known as electrokinetic sampling.

1.2.3. Detectors

The most commonly used detector is based on UV absorbance, which is standard on commercial CE instruments. Alternative detection modes available include fluorescence, laser induced fluorescence, and indirect detection methods. The coupling of CE to mass spectrometers is frequently used for detection and also obtaining structural information about the analytes.

1.2.4. Data Acquisition Device

The detectors can be interfaced to a data acquisition device (e.g., a computer) to calculate and store the results. Appropriate software (e.g., Chemstation) computes peak migration time, height, symmetry, peak width, and other useful information.

1.2.5. Separation Parameters

There are various experimental parameters that have to be considered and controlled in order to achieve optimum CEC separation results. The main parameters to be controlled are described below.

1.2.5.1. Applied Voltage

Separations are normally performed in the range of 10 - 30 kV [as indicated in Figure 3(b)]. Application of these voltages generates electrical currents in the range of 10 - 100 μA. Higher applied voltages lead to faster migration and higher EOF. Separation operations with currents beyond the above-mentioned range produce unstable and irreproducible conditions as well as analyte diffusion, resulting in band broadening. On the other hand, decreasing the applied voltage can lead to higher resolution due to reduced Joule heating, lower sample dispersion, slower migration of solutes, and more on–column interaction. However, the applied voltage cannot be too low and must be sufficiently high in order to establish reasonable ion velocities and stable (low noise) CEC currents. Constant-voltage and constant-current electrical sources are the most commonly used modes of operation used for CEC separation.

1.2.5.2. Capillary Temperature

Most separation processes are performed at room temperature. Higher temperatures usually produce undesirable band broadening. It is very important to have the capillary temperature controlled in order to avoid band broadening. Depending on the sample compounds, the capillary temperature can be adjusted to optimize the separation process. Excessive temperatures can cause uncontrolled changes in modified capillaries, buffer viscosity, sample chemical structure, and degrade the separation efficiency [8].

1.2.5.3. Capillary Length & Inner Diameter (i.d.)

Increasing the capillary internal diameter leads to greater detection sensitivity due to an increase in the effective path length for the sample.

However, larger columns do not dissipate Joule heat as efficiently as smaller columns and can experience large radial temperature gradients that result in band broadening. Narrower capillaries have larger surface-to-volume ratios, so they dissipate Joule heat more efficiently [8]. On the other hand, the capillary i.d. cannot be made too small since it would result in substantial reductions in the electrical current, the capillary sample volume, and the resulting measurement sensitivity (thus, reduced signal-to-noise ratios).

1.2.5.4. Buffer Solutions

A separation process can be optimized by choosing an appropriate buffer. The buffer-detector compatibility must be taken into account in order to prevent UV absorbance near the sample detection wavelength. The buffer components should have low UV absorbance at the detection wavelength of the solute. The buffer pH value can also affect the electroosmotic flow. Buffer solutions with lower pH values result in decreased electroosmotic flow due to the protonation of silanol groups as shown in Figure 5. Higher buffer pH values lead to increased electroosmotic flow. Protonation of silanol groups can cause reduced attraction between the positively charged solutes and the capillary wall. The reagents used in the buffer preparation process should have high purity levels (to achieve controlled buffer pH and to prevent introduction of contaminants) and the buffer solution should be thoroughly degassed using an inert gas such as nitrogen or helium. The buffer solution must be stored in a refrigerator at approximately 4°C in order to prevent bacterial growth.

1.2.5.5. Organic Modifier

Organic solvents such as methanol can be used to achieve better resolution by decreasing the zeta potential and changing the buffer viscosity, resulting in reduced EOF and increased migration time. The most commonly used organic solvents are methanol, 2-propanol, and acetonitrile. By adding organic solvents, the sample will spend more time in the capillary because of the decreased EOF.

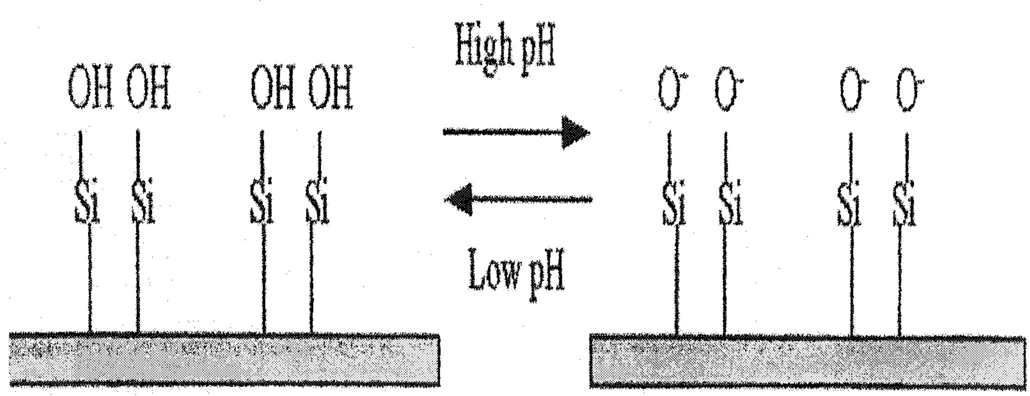


Figure 5. Illustration of the capillary inner surface termination at low and high pH values

1.2.6. Joule Heating

The capillary acts as an electrical resistor and generates heat by passage of an electrical current. The resulting temperature increase depends on the power dissipation (total power dissipation equals voltage squared divided by the capillary resistance: $P = V^2 / R$). Power dissipation is determined by the capillary dimensions, the buffer electrical conductivity, and the applied voltage. Joule heating increases with higher separation current. In general, an electrical current below 60 μA is preferred in order to limit the extent of Joule heating.

It is very important to utilize closed-loop capillary temperature control during the CEC separation process to maintain a controlled thermal environment and to reduce Joule heating. In order to achieve consistent and reproducible separations, the capillary temperature must be monitored and regulated in real time. The commercial CEC instruments are usually equipped with thermostats to achieve real-time capillary temperature monitoring and control.

1.3. CEC Columns

As mentioned earlier, there are two types of columns in CEC: (i) packed capillaries (PC-CEC), and (ii) open tubular (OT-CEC). The bare fused-silica capillary used in CE separation can cause irreversible adsorption of the sample to the wall, resulting in detrimental effects on analytical attributes, such as elution time and efficiency as well [5]. The main disadvantage of bare-silica columns in CE separation is the adsorption of positively charged particles to the SiO^-

groups. This can cause a high EOF in the capillary such that the samples move rapidly towards the cathode without allowing sufficient time for separation. In order to overcome this problem, capillaries are usually coated and chemically modified for CEC applications. It has been shown that the modification material can be covalently bonded to the etched capillary wall [5]. The organic moieties that are typically attached include: octadecyl (C-18), C-8, diol, and cholesterol derivatives.

These different modifications can provide a wide variety of chromatographic separations [9]. For instance, a mixture of tetracyclines has been successfully separated on a C-18 etched and modified column. Proteins and peptides have been separated with diol and octadecyl modified capillaries. Both coating and modification diminish the electroosmotic flow and analyte adsorption to the inner wall [10]. Surface coatings and bondings should be stable over a wide range of pH values and organic solvents for many injections.

The column in PC-CEC is similar to an HPLC column. In OT-CEC, the stationary phase is bonded to the capillary inner wall. Due to the presence of electrically driven flow in CEC in contrast to the pressure driven flow in HPLC, the use of small particle sizes in packed capillary CEC is possible. This is due to the fact that in the pressure-driven flow in an HPLC column can drive out the small particles from the column. Using smaller particle sizes leads to higher efficiency [11]. Column packing can be achieved by using a high-pressure packing technique through a pneumatic amplification pump, drawn packing, or electrokinetic packing.

As mentioned before, packed columns contain silica particles, which behave as a stationary phase. These particles can be much smaller than the particles in the HPLC columns due to the use of electrically induced flow [10]. The CEC columns are packed with particles as small as 1.5 μm. Packed columns have shown very good separation properties and are very versatile. However, there are some difficulties associated with the packing process. The most important issue is that the frit has to be sufficiently strong to hold the packing material in place, and porous enough to let the analyte and mobile phase pass through [12]. Another problem is bubble formation during operation, which leads to an unstable separation process.

Open tubular columns are made of fused silica in which the stationary phase is bonded to the inner walls of the capillary. OT-CEC capillaries overcome the problems of packed columns such as bubble formation [13]. In open tubular columns, the solutes have to travel across the capillary and interact with the stationary phase in order to achieve separation [6] (since the stationary phase is attached to the capillary wall). The main drawbacks of OT-CEC are its small capacity for sample analysis due to the low column surface area and the relatively long distance that the sample has to travel in order to interact with the bonded moiety. For samples with more chemical affinity to the stationary phase, this interaction leads to adsorption of the sample to the wall, resulting in band broadening. To prevent this effect, columns with smaller internal diameters (i.d.) are preferred [14]. In order to increase the effective inner surface area of column, the capillary can be chemically etched. The fused silica capillaries used in this study were etched using an ammonium hydrogen difluoride (NH_4HF_2) etchant.

The chemically etched surface can be modified with appropriate organic moieties. The inner wall bonded stationary phases can be hydrophobic or hydrophilic. For a specified set of experimental conditions, etched capillaries give higher resolution and a longer retention time compared to fused-silica capillaries due to enhanced interactions between the solute and the bonded phase [5,15]. Retention time in etched modified capillaries depends on the organic moiety bonded to the surface. The separation process is due to the differences in electrophoretic mobility and solute / bonded phase interactions (chromatography). Another possible analysis using the OT-CEC technique is separation of enantiomers by modifying the fused-silica capillary inner wall using chiral selector moieties such as cyclodextrin or cellulose derivatives [16].

1.3.1. Different Stationary Phases in CEC

There are several different types of bonded stationary phases in CEC, each providing a unique set of properties. A partial list includes:

Hydrophobic:

Octadecane (C-18)

Hydrophilic:

Diol

Liquid Crystals:

Cholesteryl undecanoate

4-cyano-4'- n-pentoxybiphenyl

1.4. Liquid Crystals

Liquid crystals are materials comprising a state of order between crystals and liquids, having imperfect molecular orientations and positions. Thus, they could behave like a liquid, and at the same time, have anisotropic properties like crystals (see Figure 6). Whereas plastic crystals have a predominantly positional order, the liquid crystal properties are more like a liquid than a solid. The physical properties of the system vary with the average alignment. If the liquid crystal alignment is significant, the material is very anisotropic. Otherwise, the material behaves very isotropically. These materials were first discovered by Freidrich Reinitzer in 1888. While working with an organic compound called cholesterol benzoate, he noticed two material phases. At the first temperature (145.5°C), the compound was cloudy like milk, and at a slightly elevated temperature, (178.5°C) the compound turned into a clear liquid [17]. Liquid crystal molecules are free to move for certain distance, so they have to align themselves along a particular axial orientation. The main reasons for formation of liquid crystals are as follows:

1. A simple geometrical form of molecules, which allows a closer fitting of the molecules in a mesophase (monophilic liquid crystal).

2. An intramolecular contrast, which allows microseparation of molecules (amphiphilic liquid crystals).

1.4.1. Various Types of Liquid Crystals

Different types of liquid crystal materials include nematic and smectic species, as described below.

Nematic liquid crystals: The simplest liquid crystalline phase is the nematic phase, in which there is no positional ordering between the molecules but the molecules tend to point in the same direction.

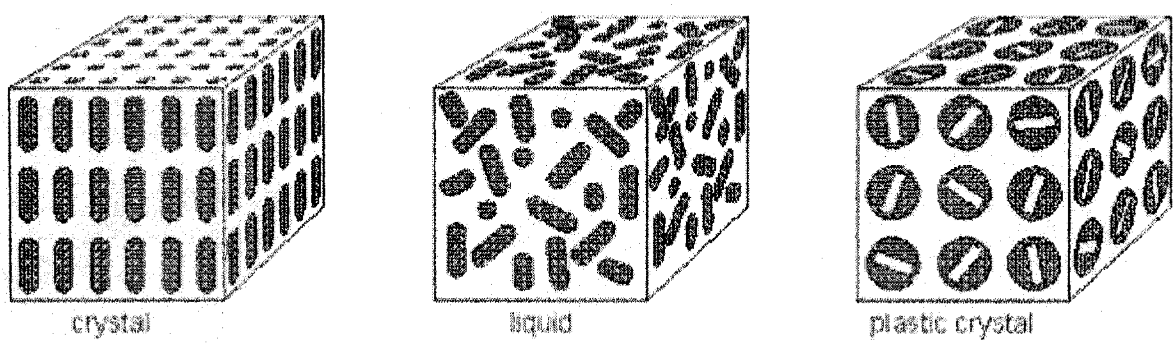


Figure 6. Orientation order in crystals, liquids, and plastic crystals.

Smectic liquid crystals: The word "smectic" is derived from the Greek word for soap. Smectic liquid crystals have layered structures and posses the highest axial alignment. In this state, the molecules maintain the general orientation order of nematics, but also have tendency to align themselves in layers or planes. The smectic phase is more "solid-like" than the nematic form. There are two different smectic phases:

- The "A" phase where molecules are aligned in a direction perpendicular to the plane.
- The "C" phase where the alignment is at a certain angle to the plane.

In both A and C phases, the molecules move randomly within each plane. Many other smectic phases have already been discovered. Figure 7 shows different phases of nematic and smectic structures.

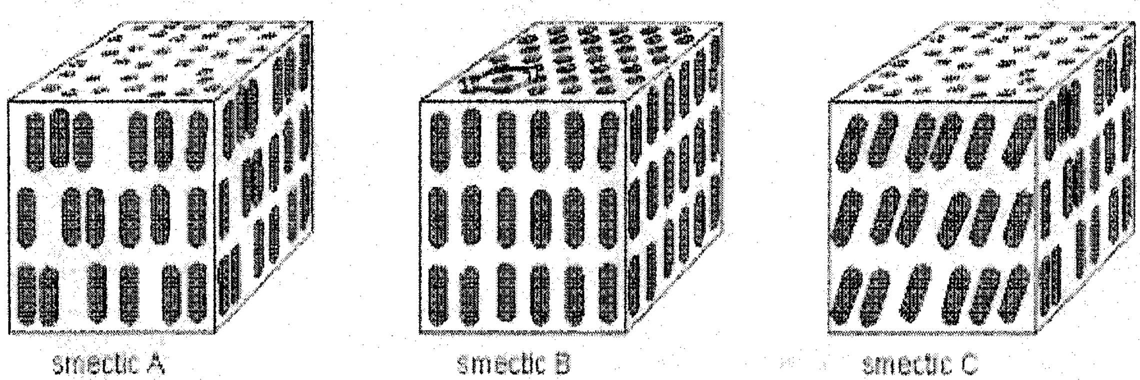


Figure 7. Structural picture of nematic and smectic liquid crystal.

1.4.2. Liquid Crystal Uses and Applications

Liquid crystal materials include a wide range of chemical structures and physical properties, with a variety of applications.

Typical chemical structures of liquid crystals are:

- Cholesterol ester
- Phenyl benzoates
- Surfactants
- Paraffines
- Glyco lipids
- Cellulose derivatives

Typical applications are:

- LCD displays
- Dyes (cholesterics)
- Advanced materials
- Membranes
- Temperature measurement by changing colors
- Solvents for GC, NMR

1.4.3. Liquid Crystals in Chromatography

Liquid crystals have been widely used in capillary chromatography (CEC) applications. The retention characteristic of these stationary phases are different from the widely used C-8 and C-18 bonded phases due to the more ordered nature of liquid crystal molecules. Liquid crystal materials used in chromatography separation have high thermal stability and low vapor pressure. Therefore, they are good candidates as stationary phase materials. Liquid crystal stationary phases were first utilized in GC applications. These materials can be deposited or directly coated onto the columns [18]. These materials have been used for separation of various isomers (cis and trans) in GC applications. In HPLC applications, the liquid crystal stationary phase has to be bonded since the coating can otherwise be removed under high-pressure conditions [19]. The development of successful covalently bonded liquid crystal stationary phases can have important implications in CEC. This research work extended the application of liquid crystals to the CEC area as a stationary phase due to the unique properties of liquid crystal as a bonded stationary phase material.

Liquid crystals demonstrate a different selectivity than the C-18 polymeric phase due to their structural order and microstructure (nematic and smectic). They have more shape selectivity than a polymeric material. These bonded liquid crystals on the inner surface of columns form 'slots'. Depending on their shape and size, solutes that diffuse into these slots are subsequently separated [20]. Separations of the solutes in these capillaries are shape and size dependent. Planar, flat, and thin solutes have shown longer separation times than the larger and bulkier solutes [21]. Cholesteryl 10-undecanoate has demonstrated very promising results as a bonded stationary phase in CEC.

1.4.3.1. Liquid Crystal Bonded Phase

The most commonly used material in chromatography columns is silica due to its favorable properties, such as its superior stability, low cost, and availability in different sizes. The bonding of an organic moiety as a stationary phase depends on the properties of the support material [22]. Various methods have been tried to attach liquid crystal molecules to the inner surface of the capillary. One of the more successful methods is based on organosilane chemistry. A silanization reagent is synthesized by reaction between a liquid crystal compound, which contains double bonds with a chloro-organosilane. The bonded phase is then obtained by reaction of the silanization reagent with the silica support. A liquid crystal compound, 4-(allyloxy)benzoyl-4methoxy phenyl, was employed to synthesize a bonded phase using this reaction [23]. Figure 8(a) shows the schematic diagrams of the bonding reaction of [4-(allyloxy) benzoyl]-4methoxy phenyl to silica. The stationary phase is attached to the silica surface by a siloxane linkage, i.e., Si-O-Si-C. However, a direct Si-C linkage is more desirable for a stable bonded phase.

Another method involves a silicon hydride intermediate formed by chlorination of the silica surface and immediate reduction with an inorganic hydride, mostly $LiAlH_4$. A bonded phase prepared by Sandoval et al. was found to be hydrolytically stable at low pH values [24]. In order to achieve optimum results, very dry conditions are required. Another method to prepare liquid crystal bonded stationary phases involves silanization followed by a hydrosilation reaction. In the silanization step, the silanols on the silica surface are converted to silicon hydrides through reaction with triethoxysilane (TES). A silicon hydride monolayer is formed. The hydrosilation step involves a reaction between silicon hydride and a terminal olefin in the presence of a Spiers catalyst, which is hexachloroplatinic acid. This reaction process produces a direct Si-C bond, which is highly stable. Figure 8(b) is a schematic diagram of the capillary wall surface reaction for Si-C bonded layer formation.

2. Experimental Procedures

2.1. Materials Used in This Study

The solutes used in this study were: theophylline and 7-(2,3-dihydroxypropyl)-theophylline (dyphylline) purchased from Aldrich (Milwaukee, WI), aminophylline and nortriptylline purchased from Sigma Chem. (St. Louis, MO). Figure 9 shows the molecular structures of the solutes. The liquid crystal used for the bonded stationary phase in the cyano pentoxy capillary was purchased from EM Industries (Hawthorne, NY). Table 1 provides a complete listing of the materials used in this research project.

2.1.1. Buffers and Related Materials Used in this Study

The buffers used in these experiments had pH values in the range of 2.14 to 8.14, as outlined below:

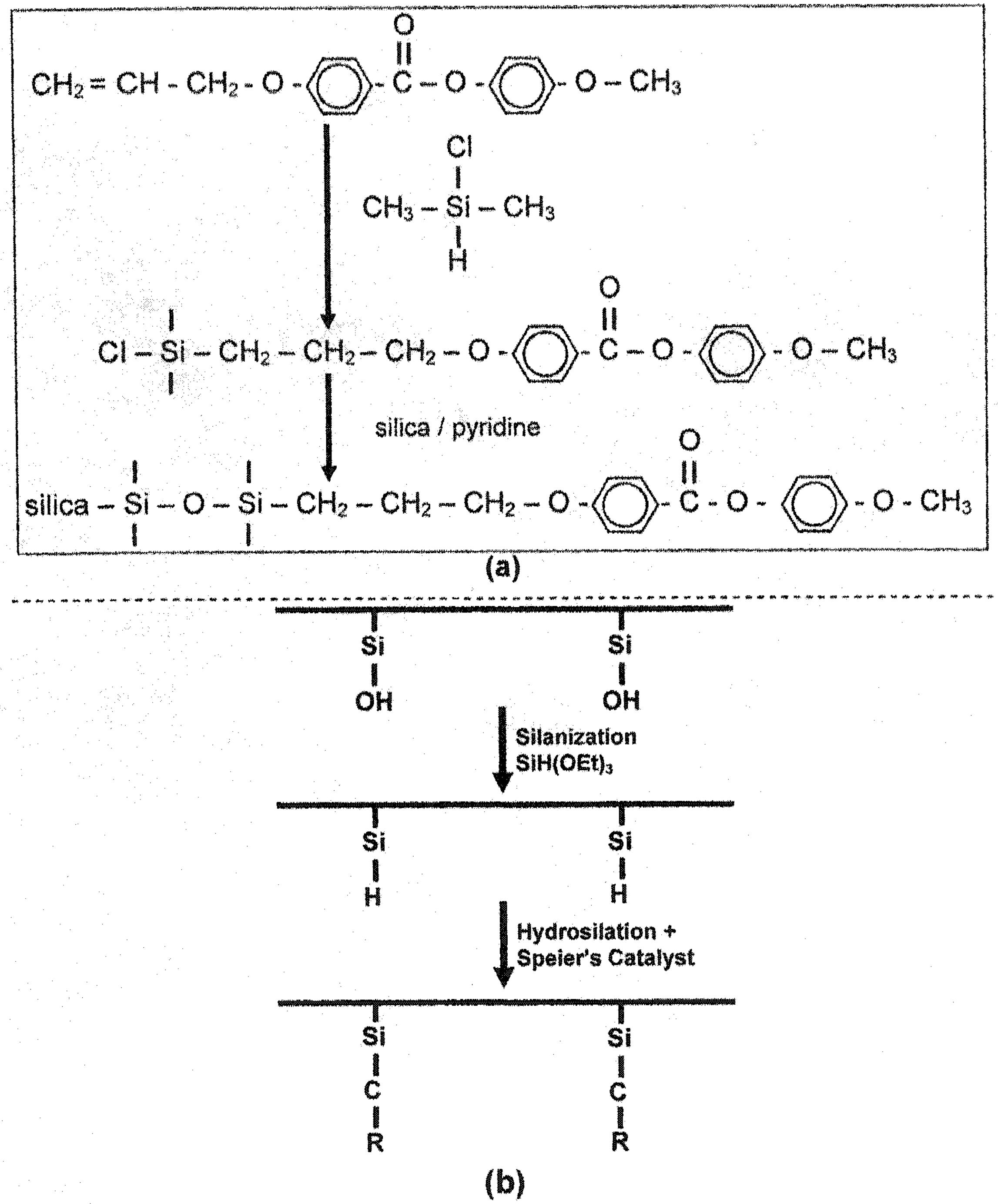
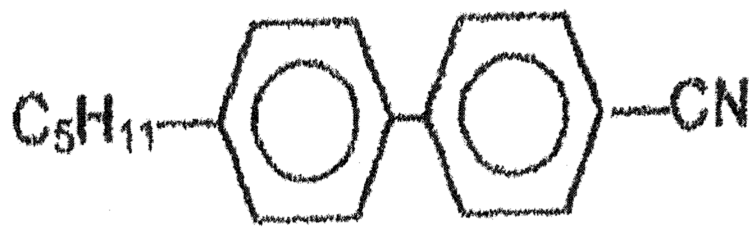


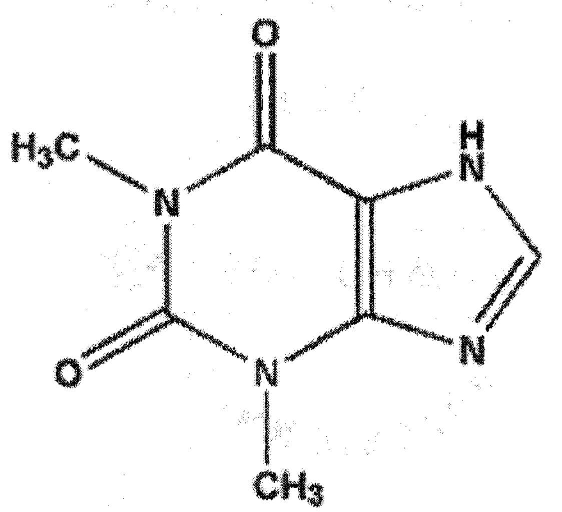
Figure 8. Schematic diagrams of: (a) the bonding reaction of *[4-(allyloxy) benzoyl]-4methoxy phenyl* to silica, and (b) the capillary wall surface reactions.

1. pH= **2.14**, 0.3 M phosphate; phosphate was from 85% H_3PO_4 (Fischer scientific, Pittsburg, PA) and 0.19 M Tris (Sigma, St Louis, MO, USA).
2. pH= **3.00**, 0.300 M citric acid and 0.25 M β-Alanine (Sigma, St. Louis, MO, USA).
3. pH= **4.41**, 300 mM acetic (Aldrich, Milwaukee, WI, USA) and 375 mM γ-amino butyric acid (Sigma, St. Louis, MO, USA).
4. pH= **7.06**, 300 mM Mops [3-(N-morpholinopropane sulfonic-acid)] and 215 mM Imidazole (1,3- Diaza-2,4- cyclopentadiene).
5. pH= **8.14**, 0.1 M Tris (Sigma, St. Louis, MO, USA) and 0.15 M Boric acid (Baker Analyzed reagent).

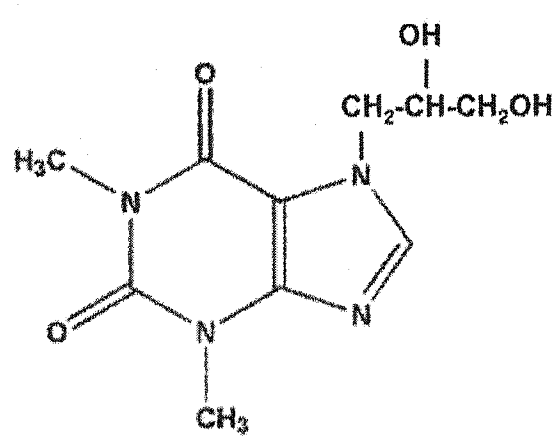
Deionized water (DI water) was obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA) and was filtered through a 0.20 μm Nylon 66 membrane (Altech Assoc., Deerfield, IL, USA). The buffers were filtered through a 0.22 μm nylon membrane, degassed with helium for 15 minutes before use, and diluted to a 1:10 volume ratio by deionized water.



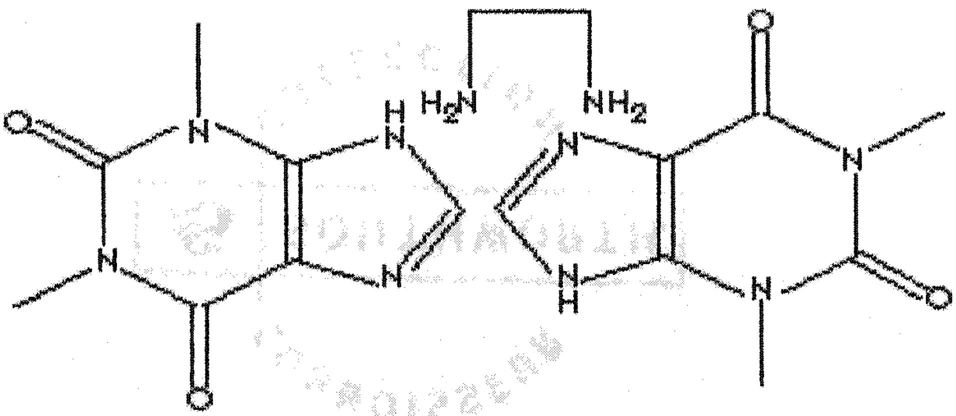
(a) 4-cyano-4′-n-pentoxybiphenyl



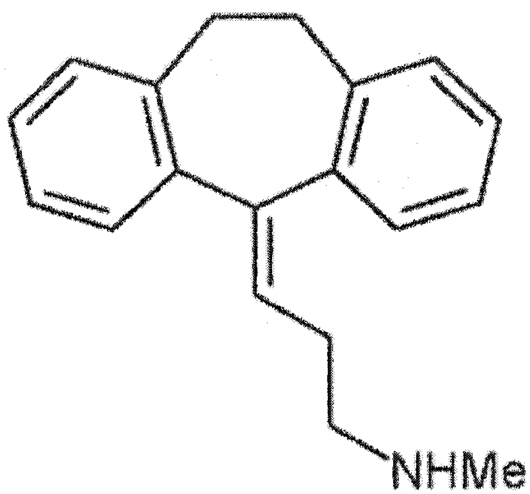
(b) Theophylline



(c) Dyphylline



(d) Aminophylline



(e) Nortriptylline

Figure 9. Structural drawings of the materials used in this study: (a) 4-cyano-4′-n-pentoxybiphenyl liquid crystal used for the bonded phase; (b) Theophylline; (c) Dyphylline; (d) Aminophylline; and (e) Nortriptylline.

Table 1. List of materials used in this study with their chemical abstract service (CAS) registry numbers.

Name	Source	CAS Registry Number
β-Alanine	Sigma	[107-95-9]
GABA	Sigma	[56-12-2]
Acetic acid	Aldrich Chemicals	[64-19-7]
Ammonium hydrogen difluoride	Aldrich Chemicals	[1341-49-7]
Citric acid	Sigma	[77-92-9]
Phosphoric acid	Fischer Scientific	[7664-38-2]
Tris	Sigma	[77-86-1]
GABA	Sigma	[56-122]
MOPS	-	-
Imidazol	-	[61H5008]
Methanol	General Chemicals	[67-56-1]
Theophylline	Aldrich Chemicals	[58-55-9]
Dihydroxy-theophylline	Aldrich Chemicals	[479-18-5]
Aminophylline	Sigma	[317-34-0]
Nortriptylline	Sigma	[894-71-3]

2.1.2. Organic Modifier

Various buffers (pre-mixed using a volume ratio of 1:10 buffer mixed with DI water) were prepared through additional mixing with methanol used as an organic modifier. The methanol volume ratios were in the range of 10% to 50% (10%, 20%, 30%, 40%, and 50%). The buffers without methanol as well as those with different methanol ratios were used in the migration experiments in order to study the impact of methanol as an organic modifier. The samples were labeled in glass vials and refrigerated at 4°C for subsequent use in the CE experiments. Before each run, the buffer-methanol mixtures were sonicated using an ultrasonic bath for 10 minutes at room temperature to remove any bubbles.

2.1.3. Etching Material

Ammonium hydrogen-difluoride, NH_4HF_2, used to etch the inner wall of the capillary, was purchased from Aldrich and triethoxysilane used to prepare the hydride intermediate was purchased from United Chemical Technologies (Bristol, PA, USA). Hexachloroplatinic acid and t-butyl peroxide were used as catalysts in the hydrosilation reaction and were purchased from Aldrich.

2.1.4. Capillaries

The capillary tubing (375 μm O.D. X 50 μm i.d.) for the preparation of etch-modified capillaries was purchased from Polymicro Technologies, (Phoenix, AZ, USA). Two etch-modified capillaries and an unetched bare silica capillary were used in this study; these capillaries are listed below:

- 4-cyano- 4 pentyloxy- biphenyl capillary (etch modified column)
- C-18 capillary (etch modified column)
- Bare fused silica capillary

2.2. Capillary Modification Processes

The etch-modified capillaries were prepared using a process sequence reported by Pesek and Matyska [13]. The specific etch process sequence used in this project is described below.

2.2.1. Etching Process

Two 50 μm bare (fused silica) capillaries were connected in the GC oven. The capillaries were filled with concentrated HCl from a plastic vial by applying 40 psi nitrogen pressure. The capillaries with HCl were heated at 80°C overnight. The capillaries were flushed with distilled water, acetone, and diethyl ether. The capillaries were subsequently dried with nitrogen gas for an hour. A 5% (w/v) saturated solution of ammonium hydrogen fluoride in methanol was used as an etching material. About 1 mL of this solution was used to fill the capillaries and treat them for an hour. Methanol was removed by using a uniform nitrogen flow through the capillaries for 5 minutes. The capillaries were then sealed at both ends by rubber corks and the rest of the capillaries were coiled and wrapped with aluminum foil and placed in a GC oven. The capillaries were heated at 300°C for 3 hours (without any gas flow) and then at 400°C for 1 hour in the presence of nitrogen flow. The capillaries were washed with methanol and dried with nitrogen flow for 4 hours. The etched capillaries were checked under a light microscope to detect any blockage.

2.2.2. Modification of Etched Surface

The etched capillaries were preconditioned by using 0.1 M NaOH solution at ambient conditions for 20 hours. They were rinsed with deionized water and flushed with 0.1 M HCl. Following the last step, they were rinsed again with deionized water and dried with nitrogen flow. After following all these steps, the capillaries are preconditioned and ready for modification. The capillaries were prepared to undergo silanization by washing with dioxine for 10 minutes and then treated with 1.2 mM TES in 8.4 mL dioxane at 90°C for 90 minutes. The formation of the hydride layer was done in this step. The capillaries were then washed with dioxane and THF, each for 2 hours at room temperature.

The monomeric stationary phase is bonded to the inner wall of the capillaries via two steps of silanization and hydrosilation, as shown below in Figure 10. Performing these two steps is necessary for attachment of organic moieties to the silica surface [25].

Silanization:

$$-Si-OH + (OEt)_3Si-H \xrightarrow{H^+} -Si-O-Si-H + 3EtOH$$

Hydrosilation:

$$-Si-H + R-CH=CH_2 \xrightarrow{cat.} -Si-CH_2-CH_2$$

cat. = free radical initiator (t-butyl peroxide)

or

cat. = Spiers catalyst

Figure 10. Silanization/ Hydrosilation reaction scheme.

2.2.3. Bonded Material to the Etched Capillary

The modification of the capillary inner wall by etching is performed in order to increase the surface area for better bonding of the stationary phase [13]. There are two types of capillary modifications: (i) neutral hydrophilic polymer modification comprising adsorption of molecules to a capillary wall via hydrophobic interactions; and (ii) covalently bonded coating with specific chemical bonds to the capillary wall. The neutral polymer coating decreases the EOF by shielding surface charge (SiO^-) and increasing viscosity. Liquid crystals such as cholesteryl moieties and cyano biphenyles show different degrees of association compared to C-18 capillaries due to the liquid crystal properties such as temperature sensitivity. Their structure can radically change by temperature elevation (the cyano molecules turn from the solid state to the nematic phase at 48°C).

2.2.3.1. Bonding of the 4-cyano-4′- n-pentoxybiphenyl

All of the glassware used in this bonding procedure was washed with deionized water and dried in an oven. About 7.5 mL of distilled toluene was placed in a 50 mL three-necked flask. One opening of three-necked flask was connected to a thermometer and sealed with parafilm. Another neck opening was closed with a stopper and sealed with parafilm as well. A magnetic stirrer bar was inside the flask. Then, 1.1g of 4-cyano-4′- n-pentoxybiphenyl was added to 7.5 mL of toluene while stirring. After it had dissolved, 40 μL t-butyl peroxide (catalyst) was added to the mixture and it was heated to 70°C for 1 hour. The liquid was passed through the capillary at 100°C, 70 psi for 5 days. After this step, toluene was run through the capillary overnight and dried by nitrogen flow. A 10 cm segment was cut off from both ends of the capillary. Figure 11 shows the cyano pentoxy bonding process.

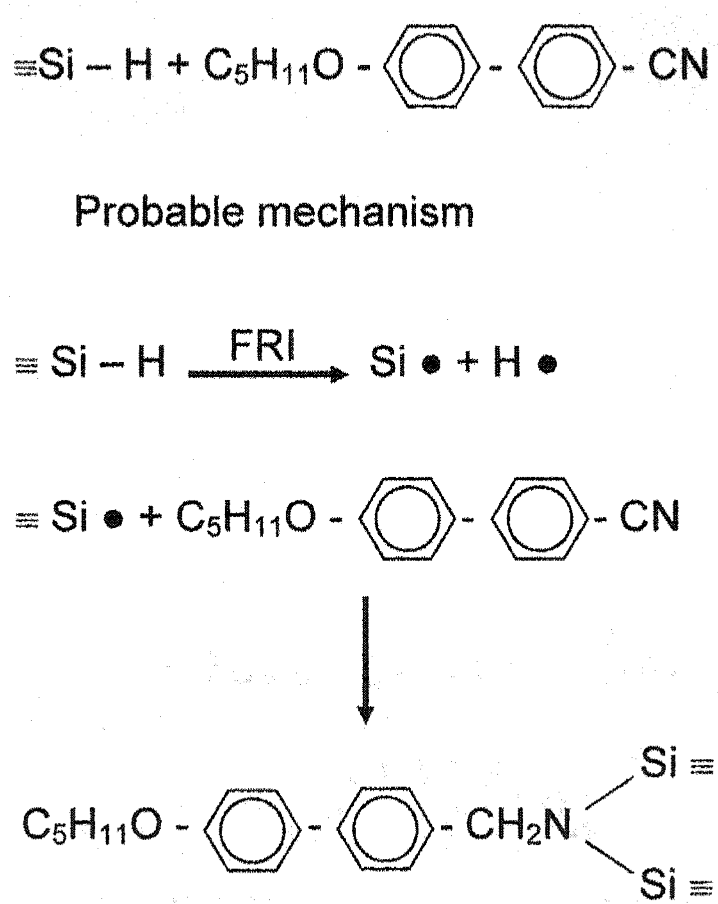


Figure 11. Reaction scheme for synthesis of cyano bonded phase using free radical initiator.

2.2.3.2. Bonding of C-18 (Octadecane) Stationary Phase

As discussed in the in the section above, the silanization / hydrosilation method was used to bond a C-18 moiety as a stationary phase to the capillary inner surface. Figure 12 shown below describes the reaction steps and final attachment of a C-18 moiety to the capillary [21]. The silanization process covalently attaches a silicon hydride layer to the reactive silanol groups [25]. In this reaction, HCl has been used as a catalyst. Finally, C-18 is bonded to the capillary surface through the hydrosilation process, as shown in Figure 12. The octadecane (C-18) bonded stationary phase is a hydrophobic material and shows different separation properties compared to the liquid crystal bonded phase which is more hydrophillic.

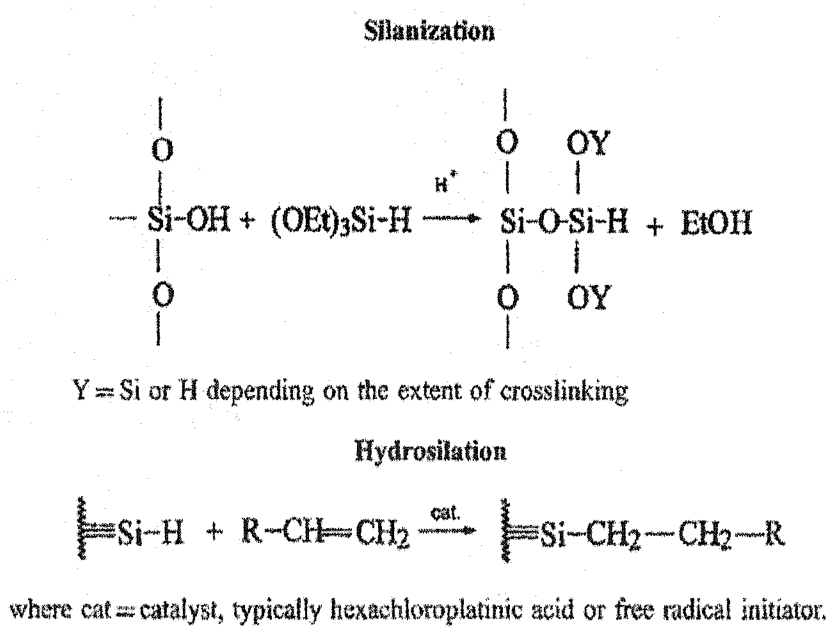


Figure 12. Schematic drawing of C-18 capillary bonding.

2.2.4. Preparing the Capillary Detection Window

The capillary was shortened to the appropriate length with a capillary cutter. The desired position of the detection window was measured and marked. A glass block with tiny grooves was heated by a flame. When the marked area on the capillary gets hot, the coating melts. The capillary was then wiped and cleaned gently with methanol and deionized water and was ready to be inserted in the capillary cassette. The capillaries were equilibrated by forcing buffer for 5 minutes and flushing with deionized water for 5 minutes as well.

2.2.5. Sample Preparation

All of the samples analyzed in this project are solid powders and water-soluble. About 1 mg of the sample was dissolved in 1mL of Tris buffer (pH = 2.14) using an ultrasonic bath. Samples were stored in labeled vials in a refrigerator as a stock solution. During the experimental work, the samples were diluted (1:5 volume ratio) with Tris buffer (pH=2.14) and run on each of the capillaries.

2.3. Experimental Overview

This research project focuses on capillary electrochromatography analyses of several xanthine-based pharmaceutical compounds such as theophylline, dihydro-theophylline, aminophylline, and a tricyclic antidepressant drug called nortriptylline. Moreover, comprehensive experimental work was performed in order to investigate the migration characteristics of three different type of capillaries. The CEC migration experiments were conducted in the C-18 and cyano pentoxy capillaries, which were chemically, etch and modified in order to perform in the OTCEC format. The same solutes were also studied using unmodified bare silica capillary. The matrix of the experimental conditions was the same for all of the capillaries in order to directly compare the resulting data.

Additional experiments were conducted in order to determine the effects of controlled variations in pH values and methanol as an organic modifier in different volume ratios, as well as different applied voltage polarities (+25 kV and –25 kV). Each experiment was performed twice for a any given set of experimental conditions in order to observe and ensure experimental reproducibility. Figure 9 shows the molecular structures of the samples. Buffers with five distinct pH values, ranging between 2.14 and 8.14 were used in these experiments. For various experiments, the buffer was mixed with 10% to 50% (volume ratio) methanol as an organic modifier.

The electrochromatographic peaks from various experiments were collected, tabulated, and analyzed in order to determine the effects of various experimental conditions, including the buffer pH values, methanol (organic compound) concentrations, and injection polarity on the peak signal profiles and amplitudes (including migration time and width) for various compounds in different types of capillaries.

The study involved using the open tubular approach to CEC (OTCEC), where the capillaries were chemically etched and modified by silanization with TES (tri-ethoxy silane), followed by hydrosilation where the organic moiety (liquid crystalline material and C-18) were attached to the capillaries inner surface. The liquid crystal used in this study was 4-cyano- 4′ n-pentoxybiphenyl. Figure 8 shows the structure of this liquid crystal.

2.3.1. Chromatographic Migration of Samples

Various solutes were tested using the cyano-pentoxy and C-18 modified capillaries in order to determine their separation capabilities. The same solutes were also tested in a bare silica capillary to compare the migration characteristics of these three capillaries (etch modified capillaries vs unetched bare silica capillary). The comparisons were performed for various sets of experiments, with each set conducted under identical experimental conditions. After the capillaries were installed in the CE instrument, data acquisition was performed. The retention times of the samples were measured individually. For each specific experiment, the sample was run twice through each capillary and the average of two data points was used to study the performance data and trends. The results were shown in the electrochromatogram form, which is a plot of the UV detector absorbance (mAU) versus time (minutes). Electrical currents passing through the capillary were measured in all experiments in order to confirm normal CE operating conditions. Therefore, we were assured that even the CE experiments without significant solute peaks had been performed with non-zero electrical current flows. See the experimental results tabulated in Tables 2 through 25 in Appendix I.

2.3.2. Experiment Conditions

Conditions: A voltage of ±25 kV was applied during all the migration experiments. Sample injections were performed for 3 seconds at 50 mbar. The capillaries were preconditioned with buffer and deionized water before each run. The multi-step capillary washes were the same throughout the entire series of experiments: water / methanol / water / buffer. Each of the four samples was run through the capillary for 40 minutes. The detection wavelength for theophylline, dihydroxy- theophylline, and aminophylline was at 270 nm. A different wavelength of 254 nm was used for nortriptylline detection. The geometrical dimensions of the capillaries used in this study are summarized below:

- Bare capillary: L= 58.2 cm l= 48.6 cm i.d.= 50 μm
- C-18 capillary: L= 50 cm l=41.5 cm i.d.= 50 μm
- Cyno pentoxy capillary: L= 33.4 cm l= 24.9 cm i.d.= 50 μm

As described below, four different materials belonging to two families of solutes were evaluated in this research study.

2.3.2.1. Xanthine Family of Drugs

The xanthines / methylxantines family of drugs are commonly used as heart and central nervous system stimulants. Xanthine is a purine base that can be found in most body tissue and fluid. Four important methylxanthine compounds are caffeine, theophylline, dyphylline and aminophylline, which are water-soluble. Major sources of these methylxanthine drugs are coffee, cocoa, cola, black teas, and chocolates.

Caffeine was first found to be helpful for asthmatic patients but had some unpleasant side effects. Other derivatives were quickly produced in hope of minimizing side effects and maximizing the airway relaxing effects. Theophylline and dyphylline are used as a pharmaceutical agent. The drug is used mainly as a bronchodilator in asthma related conditions. Aminophylline differs in its structure ($[\text{theophylline}]_2$ethylenediamine) from theophylline; it contains ethylenediamine and more water molecules so it works in the same way as theophylline and has most of its properties but tends to be less potent and shorter acting than theophylline. Aminophylline and dyphylline are essentially a theophylline derivative, as indicated in the structural drawing of Figure 8.

2.3.2.2. Tricyclic Antidepressants (Nortriptylline)

Nortriptylline is a tricyclic antidepressant used to treat depression, migraine, and panic disorder. This class of drugs has been developed by modification of the central ring in the phenothiazine molecule. Nortriptylline

($C_{19}H_{21}N \bullet HCl$) is one of the anti-depressant drugs and is a derivative of dibenzocycloheptene. The nortriptylline molecule has a –C-C- bridge and nitrogen atom of the phenothiazine ring replaced by a carbon double bond (=). For the structural diagram of the nortriptylline molecule, see Figure 9.

2.4. Instrumentation

2.4.1. Gas Chromatography Oven

Both the etching and modification processes of the capillaries were done using an HP-5890 (Hewlett-Packard, Model# 5890) GC oven. The GC was modified to allow placement of fused silica capillaries. Temperature was in the range of 300°C to 400°C in the GC oven, as outlined before in the etching section. A thick-walled glass tube was used as a reservoir to protect all of the reagent tubes for safety reasons. One end of the capillary was placed in the reagent tube and other end was placed in a small collection tube, also made of glass. Nitrogen gas was used to force reagent material from the capillary inlet that was in the reagent tube, to the outlet end of the capillary, which was in the collection vial. A nitrogen flow process was performed to dry the capillaries.

2.4.2. Capillary Electrophoresis

Capillary electrophoresis was performed using an HP^{3D} CE (Waldbronn, Germany) equipped with diode-array detection and the experimental data were acquired using Agilent Chemstation software (HP Chemstation), illustrated in

Figure 13. This CEC instrument offers all the benefits of HPLC tools, including reproducibility. The main features and benefits of this CEC instrument are:

- Easy to use: Single sample carousel, offers random vial access, and ideal for method development.
- Automated quantitative analysis.
- Superior diode-array on-capillary detection.
- High-pressure application: Only Agilent (HP) CE instruments can apply pressures up to 12 bar on both ends of the capillary, High pressure is also useful for rapidly flushing the column.

2.5. Data Analysis

After installing the capillaries in the capillary cassette of the CE instrument (HP 3D), various measurements were performed and the resulting data were collected. For each set of experiments, the retention or migration time of the solute was measured. As mentioned before, for each set of experimental conditions, each sample was injected and run through the capillaries twice to check the reproducibility (the average of two data points was used to study the performance trends). Each experiment was conducted at both +25 kV and –25 kV. Figures 15 through 37 show the plots of UV absorption vs migration time of the solutes in the electrochromatography format, as measured by the HP Chemstation software.

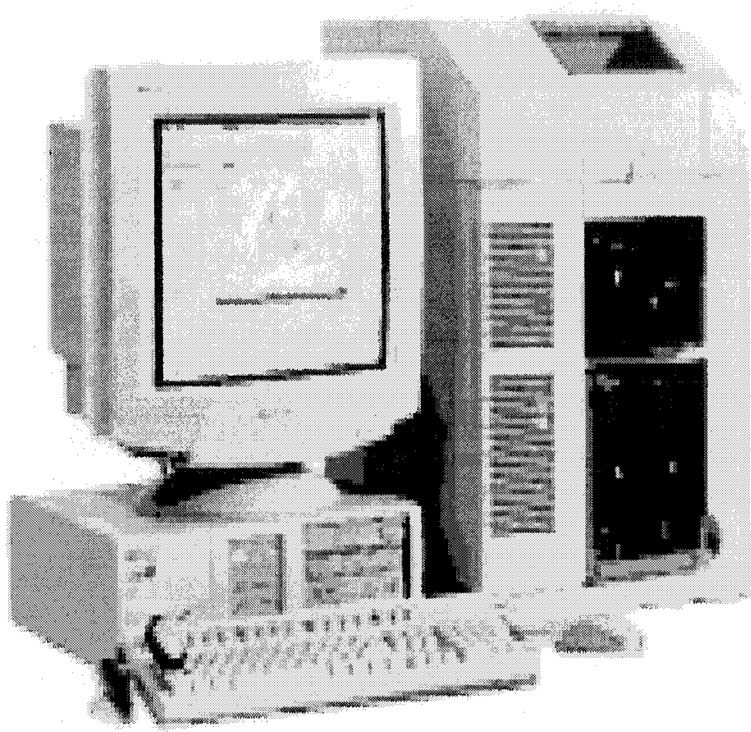


Figure 13. HP 3D CE system with HP Chemstation (See Figures 1 and 3 for related schematic diagrams).

2.6. Capillary Preconditioning

The bare silica capillary was preconditioned with 0.1 M sodium hydroxide (NaOH) for approximately 20 hours, and then manually washed via a syringe with Milli-Q water and, finally, treated with several buffer washes before each sample injection into the capillary. The two modified capillaries (cyano pentoxy and C-18) were also conditioned with buffer for 5 minutes followed by a Milli-Q water wash, before the start of each electrophoretic experiment.

2.7. Materials Characterization of Bonded Phase

2.7.1. SEM Analysis

Scanning electron microscopy (SEM) is a unique technique for characterization of the inner surface of a capillary to obtain surface roughness images and information. It has been used to study the surface microstructure of the etch-modified bonded phase. SEM is used in order to obtain both quantitative and qualitative data about the capillary surface morphology.

2.7.2. DRIFT Spectroscopy Analysis

DRIFT spectroscopy has been used in previous studies to confirm the presence of the bonded phase. The organic moiety attachment was investigated by a Mattson Instruments Infinity Series FTIR™ (Madison, WI, USA). The spectral range was between 4000 cm^{-1} to 450 cm^{-1} using 400 scans or 1000 scans with an MCT detector. For the detection of the organic moiety on the capillaries, the coatings were stripped, they were then crushed, and finally mixed with 5% spectral grade KBr. This mixture was processed into a fine powder. A pure sample of KBr was run to produce a background signal. The spectrum of the crushed sample was collected. The C-H bond stretching between 2800 and 3000 cm^{-1} reported in the literature proves that the cyano liquid crystal has been properly attached to the silica surface via silanization. However, DRIFT spectroscopy of the cyano pentoxy stationary phase was not done due to an insufficiency of this capillary. Instead, to confirm the cyano stationary phase bonding, the electroosmotic flow measurement with DMSO as a marker was performed.

2.7.3. Electroosmotic Flow Measurements in Modified Capillaries

The EOF of the cyano pentoxy capillary was measured in previous studies by using DMSO (dimethylsulfoxide) as a neutral marker. The EOF of the modified capillary was determined by meauring DMSO migrations at pH values of 2.14, 3.00, 3.70, 4.41, 6.00, and 8.19. As discussed earlier, the buffer pH value will affect the electroosmotic flow rate and direction. The higher pH buffers will increase the electroosmotic flow rate because there is more dissociation of SiOH groups on the inner surface of the capillary to SiO^-. The increase of negative charge leads to an increase of Zeta potential which itself increases the electroosmotic flow. In the cyano capillary at low buffer pH (between 2 and 4), there is anodic EOF. It changes to cathodic flow above pH = 4. These results indicate that there are some other species besides the silanol groups on the capillary wall that are affecting EOF. Photoelectron spectra of etched fused silica capillaries with organic moieties attach to the etched surface have been reported [26]. The spectra showed the presence of fluorine and nitrogen peaks besides the Si and O peaks from the silanol groups. The presence of F and N species is due to the residues left on the silica matrix by the etching agent ammonium hydrogen difluoride. Nitrogen species are responsible for the anodic electroosmotic flow at low pH [27].

3. Results and Discussions

3.1. Capillary Modification Process

3.1.1. Etching

The etching process is performed to increase the effective inner wall surface area. The freshly drawn fused silica capillary wall has a relatively smooth inner surface. Thus, the roughening of the inner wall is performed as a method to increase the surface area [28]. It has been reported that it is difficult to coat an unetched silica surface with a uniform layer of coating [29]. Various etching methods have been developed for glass capillaries such as etching with ammonium hydrogen difluoride. When the etching material passes through the bare capillary at an elevated temperature, ammonium hydrogen difluoride dissociates to gaseous hydrogen fluoride and ammonium species. The ammonium gas dissolves the silica surface, producing silicon tetrafluoride. Upon cooling, the silica is redeposited on the etched surface. This redeposition is not uniform and results in a needle-like rough surface with a much larger effective surface area. It has been reported that such as etching process can increase the surface area by a factor of about 1,000 [30].

The columns prepared with this etching process have many advantages. These capillaries resist the adsorption of basic molecules and have shown very good solute peaks along with reproducible retention times after multiple injections. The increased surface roughness in etched capillaries can be clearly seen in the scanning electron micrographs (SEM) as shown in Figure 14. The surface morphology and roughness pattern is both time and temperature dependent [31]. When the capillary is heated at 300°C for 3 hours (Figure 14.A), sharp needle-like extensions are formed. When the capillary is further heated to 400°C for 1 hour (Figure 14.B), a more uniform and wavy pattern is obtained, which is also more stable. Therefore, the capillary surface morphology could be tailored in a reproducible manner by controlling the etch time and temperature.

3.1.2. Bonded Layer

Figures 11 and 12 illustrate the silanization / hydrosilation reactions used for attachment of cyano pentoxy and C-18 stationary phases. The silanol groups on the silica surface are converted to a silicon hydride intermediate, upon reaction with TES. This hydride layer is modified with the specific organic moiety using a suitable catalyst, such as Spier's catalyst for the C-18 column and t-butyl peroxide for the cyano pentoxy column.

3.2. Chromatographic Studies of Solutes

Several solutes were tested on two different types of etch-modified capillaries: (i) C-18 capillary and, (ii) Cyano pentoxy capillary. The same samples were also tested on a bare silica capillary. The results of various types of capillaries were subsequently analyzed and compared. The comparisons were done under identical conditions over a wide range of experimental parameters.

3.2.1. Migration Study of Theophylline

The OTCEC and CE migration of theophylline in two different types of capillaries at different pH values and various methanol modifier volume ratios (v/v %) was studied. The experimental conditions used were as follows: Applied voltage= ±25 kV, sample injection for 3.0 s at 50 mbar, and UV detection wavelength of 270 nm. Tables 2 through 7 in Appendix I tabulate the measurement results (peak height in mAU, migration time in minutes, peak width (or capillary efficiency) in minutes, and peak area in mAU.sec). Each table lists the electrochromatographic results for a given applied voltage polarity (either +25 kV or −25 kV) in a specific capillary type (one of three types of capillaries), for pH values of 2.14, 3.0, 4.41, 7.06, and 8.14, as well as for

A. 300°C for 3 Hours

B. 300°C for 3 hours, 400°C for 1 hour

Figure 14. SEM micrographs of the etched capillary surface.

methanol organic modifier volume ratios in the range of 0% (no methanol) to 50%. The blank table positions without any data entries represent CEC measurements without any observable peaks (all of those conditions not yielding a peak did have stable and finite electrical current flows).

Figures 15 to 21 show some select representative CEC results for theophylline for various experimental conditions (see the figure captions for details of the specific experimental conditions). Moreover, Figures 38 through 43 illustrate various plots of migration time vs either methanol volume ratio (in volume ratio %) or vs pH value for various experimental conditions (most of these figures show families of plots). Moreover, Figure 44 shows the peak width (in minutes) for the pH value having yielded the greatest number of successful measurements (pH = 7.06). The capillary efficiency is determined based on the peak width in minutes. Thus, narrower widths with less tailing are preferred in terms of enhanced separation capabilities.

Based on the experimental data tables for theophylline in different capillaries, some specific behaviors and trends could be recognized. Good theophylline electrochromatographic migration was achieved in the cyano pentoxy modified capillary at a pH value of 7.06, +25 kV applied voltage, and with different methanol (v/v %) volume ratios. The migration studies in the cyano pentoxy capillary showed good peak shapes without much band broadening up to a 50% volume ratio of methanol. As expected, the migration time for theophylline was longer at maximum methanol volume ratio (50%); this behavior is due to the lower EOF rate with higher organic composition. The theophylline studies in the cyano pentoxy capillary at pH values of 2.14, 3.0, and 4.41, and for an applied voltage of –25 kV, showed some measurable CEC peaks, although not at all methanol composition ratios, but with good peak shape and no band-broadening. The best migration of the same solute in the C-18 modified capillary was obtained at pH values of 7.06 and 8.14 and with a positive applied voltage of +25 kV. The peak sharpness of the C-18 capillary was less than that of the cyano pentoxy capillary. At lower pH values (i.e., 2.14 and 3.0) there were no observable peaks in the C-18 capillary even with the negative injection (-25 kV), although finite and stable electrical currents were measured in all such experiments. In the bare silica capillary, the best theophylline migrations results (based on peak shape and width) were obtained at pH values of 4.41 and 7.06, although in most cases, elctrochromatogram peaks with different methanol volume ratios were observed at pH = 4.41 (+25 kV). Good peak shape was observed at pH= 4.41 and +25 kV.

The sample migration order in the two types of modified capillaries and the bare capillary at pH = 7.06, +25 kV, and no methanol were as follows. The theophylline elution time was shorter in the cyano pentoxy capillary (1.73 min) compared to the C-18 capillary (8.17 min) and the bare silica capillary at (9.81 min). Since the cyano pentoxy capillary with L=33.4 cm & l= 24.9 cm was shorter than the C-18 capillary with L=50 cm & l= 41.5 cm compared with the bare capillary with L= 50 cm, l= 41.5 cm, we could already expect a shorter migration time of the solute in the cyano modified capillary. Comparing the peak widths, the cyano modified capillary showed superior electromigration properties. The C-18 capillary showed some tailing for theophylline elution. This indicates that the solutes have more interactions with the C-18 bonded phase, leading to longer retention and, perhaps slower mass transfer. The peak sharpness degraded with higher methanol volume ratios, perhaps due to more interactions with the few remaining silanols. As shown in Figure 44, the bare silica capillary had superior peak sharpness compared to the cyano and C-18 etch-modified capillaries.

3.2.2. Migration Study of Dyphylline (Dihydroxy-Theophylline

The OTCEC and CE migration of dihydroxy-theophylline in two different types of capillaries at various pH values and for a range of methanol modifier volume ratios (v/v %) was studied and analyzed. The specific experimental conditions were: Applied voltage= ±25 kV, sample injection for 3.0 s at 50 mbar, and UV detection wavelength of 270 nm.

Tables 8 through 13 in Appendix I tabulate the measurement results (peak height in mAU, migration time in minutes, width or capillary efficiency in minutes, and peak area in mAU.sec) for dihydro-theophylline. Each table lists the electromigration results for a given applied voltage (either +25 kV or –25 kV) in a specific type of capillary, for pH values of 2.14, 3.0, 4.41, 7.06, and 8.14, as well as for methanol organic modifier volume ratios in the range of 0% (i.e., no methanol) up to 50%. The blank table positions without any data entries represent the CEC measurements without any observable peaks (all of those conditions not yielding a peak did have stable and finite electrical current flows; thus, the results are real and reproducible).

Figures 22 through 23 show some select representative CEC results for dihydroxy-theophylline under various experimental conditions (see the figure captions for details of the experimental conditions). Figures 45 through 50 illustrate various plots of migration time vs either methanol volume ratio or vs pH values for a wide range of experimental conditions. Moreover, Figure 51 shows the peak widths (in minutes) for the pH value having yielded the greatest number of successful measurements (i.e., pH = 7.06).

Based on the data tables for dihydroxy-theophylline in different capillaries, some specific behaviors and trends were recognized. Good dihydroxy-theophylline migration properties were achieved in the cyano pentoxy modified capillary at a pH value of 7.06 and a positive applied voltage of +25 kV, for various methanol (v/v %) compositions. The sample in the cyano pentoxy capillary gave a narrow peak width without band broadening, up to 50% methanol in the buffer. The sample migration time for dihydroxy-theophylline was longer for the highest methanol volume ratio (50%), due to a lower EOF. Migration studies of dihydroxy-theophylline in the cyano pentoxy capillary at pH values of 2.14, 3.0, and 4.41, and with an applied voltage of –25 kV showed some measured CEC peaks (although not at all methanol compositions); these peaks displayed good shape and no band broadening. The best electromigration results (based on peak width and shape) for this solute were obtained in the C-18 modified capillary at pH values of 7.06 and 8.14, and for an applied voltage of +25 kV.

The peak sharpness in the C-18 etch modified capillary was not as good as that of the cyano pentoxy capillary. At lower pH values of 2.14 and 3.0, there were no observable peaks in the C-18 capillary, even with the negative applied voltage (-25 kV). The best dihydroxy-theophylline migration result in the bare silica capillary were obtained at pH values of 4.41 and 7.06; however, the experiments performed at pH = 4.41 resulted in the greatest number of elctrochromatogram peaks at different methanol volume ratios. Good peak sharpness was achieved in the bare silica capillary at pH = 4.41 and with a +25 kV applied voltage.

The sample migration order in the etch-modified capillaries and bare silica capillary at pH = 7.06, +25 kV, and no methanol were as follows. The dihydroxy-theophylline elution time was shorter in the cyano pentoxy capillary (1.17 min) compared to the C-18 capillary (7.69 min) and unetched bare silica capillary (9.33 min). The shorter migration times of solute in the cyano modified capillary are partially due to the shorter capillary length. The cyano pentoxy capillary with L=33.4 cm & l= 24.9 cm was shorter than the C-18 capillary with L=50 cm & l= 41.5 cm, and the bare silica capillary with L= 50 cm, l= 41.5 cm.

A comparison of the peak widths measured in the three types of capillaries indicated superior behavior for the cyano modified capillary compared to the C-18 capillary (the dihydroxy-theophylline peaks showed some tailing in the C-18 modified capillary). This behavior is due to increased interaction of the solutes with the C-18 bonded phase, leading to longer retention; the peak sharpness is degraded by adding more methanol. The bare silica capillary yielded some solute peaks at pH = 7.06 for various methanol ratios (0% or no methanol, 20%, 40%, 50%). The best peaks for the bare silica capillary were obtained at pH = 4.41; these peaks also displayed good capillary efficiencies. A comparison of the peak widths of the three types of capillaries at the same pH value (Figure 51) indicates that the bare silica capillary provided the best peak sharpness, followed by the cyano pentoxy modified capillary, and lastly the C-18 modified capillary.

3.2.3. Migration Study of Aminophylline

The OTCEC and CE migrations of Aminophylline in the two different types of capillaries were studied at different pH values and for various methanol modifier volume ratios (v/v %). The experimental conditions were as follows: applied voltage = ±25 kV, sample injection for 3.0 s at 50 mbar, and UV detection wavelength of 270 nm.

Tables 14 through 19 in Appendix I tabulate the measurement results (peak height in mAU, migration time in minutes, width (in minutes), and peak area (in mAU.sec). Each table lists the migration results for a given applied voltage (either +25 kV or –25 kV) in a specific capillary type, for pH values of

2.14, 3.0, 4.41, 7.06, and 8.14, as well as methanol organic modifier volume ratios in the range of 0% (no methanol) to 50%. The blank table positions without any data entries represent measurements without any observable solute peaks (all of those conditions not yielding a peak did have stable and finite electrical current flows).

Figures 24 through 31 show some select representative CEC results for aminophylline for various experimental conditions (see the Figure Captions for details of the experimental conditions). Figures 52 through 56 illustrate various plots of migration time vs either methanol volume ratio or vs pH for various experimental conditions. Moreover, Figure 57 shows the capillary peak width (in minutes) for the pH value having yielded the greatest number of successful measurements (pH = 7.06).

Based on the experimental data (migration time, peak width, methanol%) tables for aminophylline in different type of capillaries, some specific behaviors and trends were recognized. Good aminophylline migration behavior was achieved in the cyano pentoxy modified capillary at pH = 7.06 and with an applied voltage of +25 kV, for different methanol (v/v %) compositions. The migration experiments in the cyano pentoxy capillary showed good peak symmetry and negligible band broadening, up to 50% in the buffer. As expected, the migration time for aminophylline was longer at maximum methanol volume ratio (50%) dilution due to lower EOF. Migration studies of aminophylline in the cyano pentoxy capillary at pH = 2.14, 3.0, and 4.41, and with an applied voltage of –25 kV showed some CEC peaks (although not at all methanol compositions); these peaks displayed good peak symmetry and no band broadening. The best migration results (based on peak width and shape) for the same solute in the C-18 modified capillary were obtained at pH values of 7.06 and 8.14, and with an applied voltage of +25 kV. The peak sharpness of the collected peaks from the C-18 modified capillary was less than that of the cyano pentoxy capillary. At lower pH values 2.14 and 3.0, there were no peaks in the C-18 modified capillary even with a negative injection voltage (-25 kV). The best migration results (based on peak width) of aminophylline bare silica capillary were obtained at pH values of 4.41 and 7.06; however, more successful measurements were made at pH = 7.06 for different methanol compositions. Good peak sharpness was achieved in the bare silica capillary at pH values of 4.41 and 7.06, and an applied voltage of +25 kV.

The aminophylline elution order in the two types of etched modified capillaries and the bare silica capillary at pH= 7.06 with 10% methanol modifier and +25 kV applied voltage (experimental condition) were as follows. The migration time in the cyano pentoxy capillary was shorter (3.39 min) compared to the C-18 capillary (8.81 min) and the bare capillary (11.04 min). The cyano pentoxy capillary with L = 33.4 cm & l = 24.9 cm was shorter than the C-18 capillary with L= 50 cm & l = 41.5 cm and the bare silica capillary with L = 50 cm & l = 41.5 cm. Therefore, the solute migration time in the cyano modified capillary was expected to be shorter compared to the C-18 modified capillary. The longer migration time of the bare silica capillary compared to the C-18 modified capillary is due to the higher solute and affinity in the bare silica capillary.

Figure 57 plots the peak width of the solute in the three different capillaries at pH= 7.06 and +25 kV. The cyano modified capillary showed higher efficiency compared to the C-18 modified capillary where the aminophylline peak showed some tailing in the C-18 modified capillary. This implies that that the solutes have increased interactions with the C-18 bonded phase, leading to longer retention time and reduced efficiency; the peak sharpness is degraded by adding more methanol due to poor mass transfer or interactions with silanols. The bare silica capillary showed some migration peaks at pH = 7.06 with various methanol compositions (no methanol, 20%, 30%, 40%, and 50% volume ratios of methanol). The best capillary efficiencies (based on peak width) were obtained in the bare silica capillary at pH= 7.06 and +25 kV. By comparing the efficiency results of all three capillaries at the same pH (7.06) value, the bare silica capillary yielded the best peak sharpness, followed by the cyano pentoxy modified capillary, and lastly the C-18 modified capillary.

3.2.4. Migration Study of Nortriptylline

OTCEC and CEC migrations of nortriptylline were studied in three different capillaries at various pH values and a wide range of methanol modifier volume ratios (v/v %) at a detection wavelength of 254 nm. The specific experimental conditions were: applied voltage= ±25 kV, sample injection time of 3.0 s at 50 mbar, and UV nm.

Tables 19 through 24 in Appendix I tabulate the migration measurement results (peak height in mAU, migration time in minutes, width (in minutes), and peak area in mAU.sec). Each table lists the migration results for a given applied voltage (either +25 kV or –25 kV) in a specific capillary type, for pH values of 2.14, 3.0, 4.41, 7.06, and 8.14, as well as for methanol organic modifier volume ratios in the range of 0% (no methanol) up to 50%. The blank table positions without any data entries represent CEC measurements without any observable solute peaks (all of those conditions not yielding a peak did have stable and finite electrical current flows).

Figures 32 through 37 show some select representative electromigration results for nortriptylline under various experimental conditions (see the Figure Captions for details of the experimental conditions). Figures 58 through 63 illustrate various plots of migration time vs either methanol volume ratio or vs pH for a wide range of experimental conditions. Figure 64 shows the peak width (in minutes) for the pH value having yielded the greatest number of successful measurements (i.e., pH = 7.06).

Based on the measured data for nortriptylline for migrations in different capillaries at the same pH, some specific trends and behaviors (refer to the above-mentioned tables and figures) can be identified. Good nortriptylline migration (based on peak shape and width) was achieved in the cyano pentoxy modified capillary at pH= 2.14, 3.0, and 4.14 at +25 kV, with different methanol (v/v %) ratios as an organic modifier. Migration in the cyano pentoxy capillary at pH = 3.0 and +25 kV showed better peak width and symmetry at 50% methanol volume ratio compared to the no methanol (0%) condition. As expected, the migration time for nortriptylline was longer at maximum methanol volume ratio (50%) due to lower EOF; the higher methanol ratios produced narrower peak width. The best migration results of the same solute in the C-18 modified capillary were obtained at pH values of 3.0 and 4.41, and an applied voltage of +25 kV, although the pH = 4.41 condition resulted in higher efficiency. However, the cyano pentoxy modified capillary can be considered as the preferred capillary for nortriptylline analysis, having produced better CEC Peaks. Measurement data were collected at pH values of 7.06 and 8.14, in the C-18 modified capillary and at an applied voltage of +25 kV. There were no observable peaks in the C-18 capillary with a negative applied voltage (-25 kV). The greatest numbers of successful measurements for nortriptylline analysis in the bare silica capillary were obtained at pH values of 2.14, 3.0, and 4.41. In the bare silica capillary and for different methanol composition, the pH = 4.41 and +25 kV condition produced the greatest number of successful elctrochromatograms and the highest efficiency, compared to the pH values of 2.14 and 3.0 and +25 kV. Good peak shapes were obtained in the bare silica capillary at pH values of 4.41 and 7.06, and for +25 kV applied voltage.

The following nortriptylline elution order was observed in the two types of modified capillaries and the bare silica capillary. The migration time in the cyano pentoxy capillary at pH = 4.41 and +25 kV with 10% methanol modifier was shorter (2.87 min) compared to the C-18 capillary (9.55 min) and bare silica capillary (5.72 min). The solute migration time was shorter in the cyano-modified capillary compared to the C-18 capillary. The cyano pentoxy capillary with L = 33.4 cm & l = 24.9 cm was shorter than the C-18 capillary with L = 50 cm & l = 41.5 cm and the bare silica capillary with L = 50 cm & l = 41.5 cm, Based on a comparison of the migration results obtained with the C-18 modified and bare silica capillaries, the bare silica capillary performed much better; the C-18 CEC peaks displayed broadening and tailing.

Figure 64 plots the peak widths of various measurements performed in the two different types of capillaries. As shown, the cyano modified capillary produced better efficiency compared to the C-18 capillary; the nortriptylline peaks displayed some tailing in the C-18 modified capillary. This result indicates that the solutes have more interaction with the C-18 bonded phase, leading to longer retention and reduced efficiency. The peak sharpness is degraded by adding more methanol (higher methanol volume ratios) due to additional interaction, possibly with the few remaining silanols. The bare silica capillary produced some observable peaks with good efficiency at the pH value of 4.41 with various methanol compositions (no methanol; 20%, 30%, 40%, 50% methanol). A comparison of the measured peak width results (at the same pH value of 4.41

and +25 kV) obtained in the three different capillaries indicates that the bare silica capillary offers the best peak sharpness (i.e., highest efficiency), followed by the cyano pentoxy modified capillary, and lastly C-18 modified capillary.

Figures 32 and 33 show impurities resolved by C-18 and cyano pentoxy etched modified capillaries that are not seen in a bare capillary. These results show importance and capabilities of etched modified capillaries.

3.3. Overview of Xanthine Family of Drugs Migration Studies

Theophylline, dihydroxy-theophylline (dyphylline), and aminophylline are in this group of pharmaceutical drugs and have similar molecular structures. Each of these compounds was tested both at +25 kV and -25 kV applied voltages in order to observe both anodic and cathodic migration patterns. The results of this work indicate that theophylline in the C-18 and cyano pentoxy etch-modified capillaries at buffer pH=7.06 and 8.14 with +25 kV applied voltage shows well-behaved and symmetrical electrochromatogram peaks. By adding methanol as an organic modifier, the electroosmotic flow was diminished in the C-18 and cyano pentoxy capillaries. Theophylline migration time in C-18 at +25 kV and pH=7.06 is around 8 minutes, whereas at the same pH and applied voltage in the presence of 50% methanol the peak migration time increases to 27 min. The same capillary (C-18) at pH=8.14 and +25 kV showed a theophylline elution time of approximately 6.6 minutes; adding 40% methanol resulted in a solute migration time of 10.30 min in the capillary. Similarly, for dihydroxy-theophylline at +25 kV and pH=7.06, the migration time was measured at 7.50 min (0% methanol), while it was shortened to 5.82 min at pH= 8.14 due to a higher EOF. Few migration peaks were observed with a negative applied voltage in the C-18 and cyano pentoxy capillaries at pH = 7.06 and 8.14 (although the electrical current flow was stable and finite).

3.4. Overview of Tricyclic Antidepressant (Nortriptylline) Migration Studies

Nortriptylline was successfully eluted in both the C-18 and cyano capillaries using a pH value of 4.41 and +25 kV applied voltage. The elution time at pH= 4.41 and +25 kV was the shortest in the cyano capillary, followed by the C-18 and bare silica capillaries. However, the cyano modified capillary produced higher efficiencies compared to the C-18 capillary, since the peaks in the C-18 capillary displayed some tailing. There are structural differences between the two etch modified capillaries; the cyano pentoxy stationary phase is a nematic liquid crystal whereas the C-18 stationary phase is simply a long-chain hydrocarbon molecule. Since these two capillaries are two different classes of materials with different structures, they offer different migration selectivities. Moreover, the molecular size and polarity of the solutes are considered important parameters affecting the migration process.

3.5. Effect of pH on Migration Behavior

In order to achieve optimum CE performance, various experimental conditions such as the applied voltage, capillary i.d., and pH must be optimized and controlled. It is necessary to control the buffer pH value in order to achieve good migration results with high resolution. In order to study the effects of pH on solute migration time and peak shape, various experiments were performed over a wide range of pH values. For instance, Figure 38 shows the migration results for theophylline with the bare silica capillary at buffer pH values in the range of 2.14 to 8.14. At low pH values of 2.14, 3.0, and +25 kV, the electroosmotic flow is very small due to protonation of the silanol group. At buffer pH values above 4.41, there is increased electroosmotic flow due to deprotonation of the silanols (negatively charged). Therefore, shorter migration times are expected at higher pH values, whereas longer migration times can be expected for pH values below 4.41. At a low pH value and +25 kV applied voltage, the resulting migration performance of the etch-modified capillaries was poor, with few or no resulting peaks. The optimum pH value for the migrations of theophylline, dihydroxy-theophylline, and aminophylline in the bare silica capillary was determined to be 4.41 and +25 kV. The pK_a values of these three samples (all from the xanthines family) are between 7.0 and 8.0. These compounds are positively charged at pH values of 2.14, 3.0, and 4.41. Since migration of the solutes in the bare silica capillary is based on the electroosmotic flow and electrophoretic migration, the pH value will have a direct effect on the migration time.

For the cyano pentoxy and C-18 etch-modified capillaries, the optimum pH value for the migrations of theophylline, dihydroxy-theophylline, and aminophylline was found to be at 7.06 and +25 kV applied voltage, based on the greatest number of successful measurements at pH= 7.06 with different methanol % as well as peak shape and width. Figure 42 shows the effect of pH on migration time for theophylline in the cyano pentoxy capillary at +25 kV with 10% methanol modifier. At low pH values of 2.14 and 3.0, no peaks were observed in the cyano pentoxy capillary with an applied voltage of +25 kV. With a –25 kV voltage, the observed peaks were due to a change in the direction of electroosmotic flow. As mentioned above, there is anodic flow at low pH values and the solutes move in the opposite direction of detector, which is the reason for lack of observed peaks. On the other hand, a reverse polarity of –25 kV resulted in measured peaks at the detector because of the strong anodic EOF, which was greater than the cathodic migration of the presented charged analytes.

4. Conclusions

The main objective of this research project was to determine the migration properties of selected basic pharmaceutical compounds in chemically etched and modified capillaries as well as a bare silica capillary. Moreover, this study assessed the ability of the CE and CEC techniques to analyze xanthine and tricyclic pharmaceutical compounds used in numerous medical applications. The effects of the specific capillary type, pH value, applied voltage (±25 kV), and methanol composition (% v/v) as an organic modifier on migration time and peak shape / width were studied through detailed experiments performed with each compound (see Tables 26 and 27 for the summary descriptions, ranges, and values of the experimental parameters in this study). The optimal conditions for migration of each sample were determined. The etch-modified capillaries were used in the open tubular electrochromatographic (OTCEC) analysis mode. Two different types of etch-modified capillaries were used in these studies: a cyano pentoxy modified capillary and a C-18 modified capillary. The bare silica capillary used in this work was etched and unmodified. The combination of etching and modification of the capillaries increased the effective surface area and facilitated more solute-bonded phase interactions. This observation was confirmed through comparative studies of the migration time as well as peak width and shape from cyano pentoxy and C-18 modified capillaries as well as bare silica capillary.

The synthesized liquid crystal capillary was previously characterized by FTIR studies. The C-H stretching bands observed in the FTIR spectra confirmed the liquid crystal was bonded as a stationary phase to the etched surface but the surface coverage of the bonded phase could not be ascertained. Since the monomeric C-18 (octadecane) moiety is much longer than the bulkier cyano pentoxy moiety, it is assumed that the two moieties would have different surface coverage values. It is also important to note that these two bonded moieties show different affinities with respect to molecular polarity. The octadecane (C-18) stationary phase is more hydrophobic compared to the cyano pentoxy (liquid crystal) moiety, which is a hydrophilic material. Thus, cyano pentoxy is expected to have more interaction with a protonated solute at low buffer pH values (below 4) than the C-18 modified capillary. This may be ascertained by capillary efficiency based on peak width and shape.

The electroosmotic flow for pH values below 4.0 is anodic and becomes cathodic at pH values above 4.0. Anodic flow has been previously studied and confirmed in bare etched and etch-modified capillaries. For etch-modified capillaries, the number of silanol groups greatly decreases after formation of the hydride layer on the capillary wall. The chemical etch process performed on the capillary inner wall with ammonium hydrogen difluoride (NH_4HF_2) chemical etchant leaves ionic NH_4^+ residues in the silica matrix of the capillary wall. These residual ions can cause anodic electroosmotic flow in the etch-modified capillary. The EOF depends on the buffer pH value, which can be adjusted to optimize the migration process.

Effects of the organic modifier in the mobile phase with various controlled volume ratios of methanol were studied. The higher methanol volume ratios resulted in longer migration times for the solutes. The use of methanol as an organic modifier in the bare silica capillary was also studied in this research work. As shown in Table 2, the greatest numbers of successful measurements were obtained at pH values of 4.41, 7.06, and 8.14. As the methanol volume ratios are raised, the elution times for all compounds were increased, and the peaks became broader due to the longer time for the solute to pass through the capillary.

The solute migration times in the cyano pentoxy modified capillary were the shortest, possibly due to the shorter column length (50 cm vs 33 cm). Both the C-18 and cyano pentoxy modified capillaries demonstrated well-behaved migration performance based on their peak shape and width at the same buffer pH (7.06) and +25 kV applied voltage. As outlined in Tables 2 through 18, the optimum migration conditions for theophylline, dihydroxy-theophylline, and aminophylline include the buffers with the pH values above 4.0 (pH = 4.41, 7.06, 8.14) and +25 kV and different methanol modifier (v/v %). These compounds have pk_a values in the range of 5,2 to 8.0. These results indicate better electrochromatographic performance for these materials at pH values near their pk_a value (see Tables 28 and 29 for the summary results).

The migration results for nortriptylline show that the best buffer pH values producing the greatest number of successful analyses are pH = 2.14, 3.0, and

4.41. Nortriptylline is compound with pK_a value of 9.7, indicating that the molecule is strongly basic and it is more likely to accept protons in a low pH value environment. By evaluating the CEC peak shapes and migration times of nortriptylline outlined in Tables 19 through 24, for pH = 4.41 and +25 kV, the migration time in the C-18 modified capillary was longer than in both bare silica and cyano pentoxy modified capillaries. As indicated in Table 21, there was slight peak tailing in the C-18 capillary, and such peak asymmetry could be improved by increasing the methanol volume ratio (see Tables 28 and 29 for the summary results).

This study demonstrates the valuable properties of chemically etched and modified capillaries in the open tubular format (OTCEC). These favorable characteristics are due to both the etching process used to increase the effective surface area and the modification process forming a hydride layer on the capillary inner surface and bonded with a specific moiety. The chemical modification of the capillary inner wall was done through silanization and hydrosilation. In this work, the unique characteristics of the liquid crystal material as a stationary phase in CEC were demonstrated for various pH values (2.14 through 8.14) and applied voltages (+25 kV and –25 kV) at room temperature. Compared to the bare silica capillary, the etch-modified capillaries are capable of resolving the sample and any residual impurities with excellent migration peak (time, width, and shape) measurement reproducibilities. The etch-modified capillaries (cyano pentoxy and C-18) are capable of providing enhanced analytical reproducibility (in terms of peak width, peak shape, and detection of sample impurities) compared to the bare silica capillary. Therefore, the etch-modified capillaries are the preferred candidates for analyzing the compounds evaluated in this study. Based on the capillary efficiency analysis data, the cyano pentoxy etch-modified capillary demonstrated superior performance (i.e., less tailing and more peak symmetry) compared to the C-18 etch-modified capillary.

Further studies would be useful to observe the effects of different applied voltages, electrokinetic injection (injection by voltage) instead of pressure injection, and temperature on migration and efficiency. In particular, the temperature studies should include the cyano pentoxy modified capillary due to the temperature sensitivity of liquid crystals.

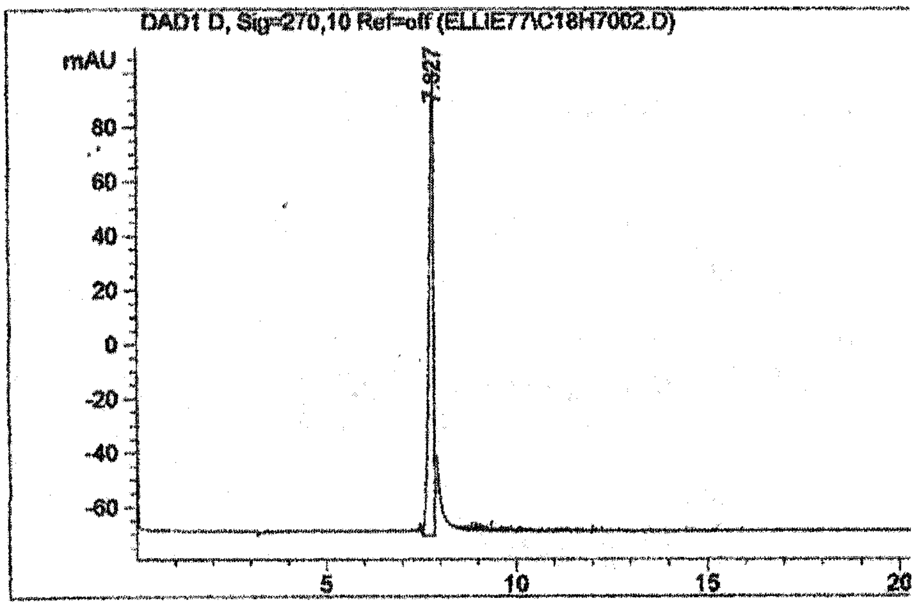


Figure 15. Migration of theophylline in C-18 modified capillary: pH=7.06, no methanol, +25 kV; migration time in min.

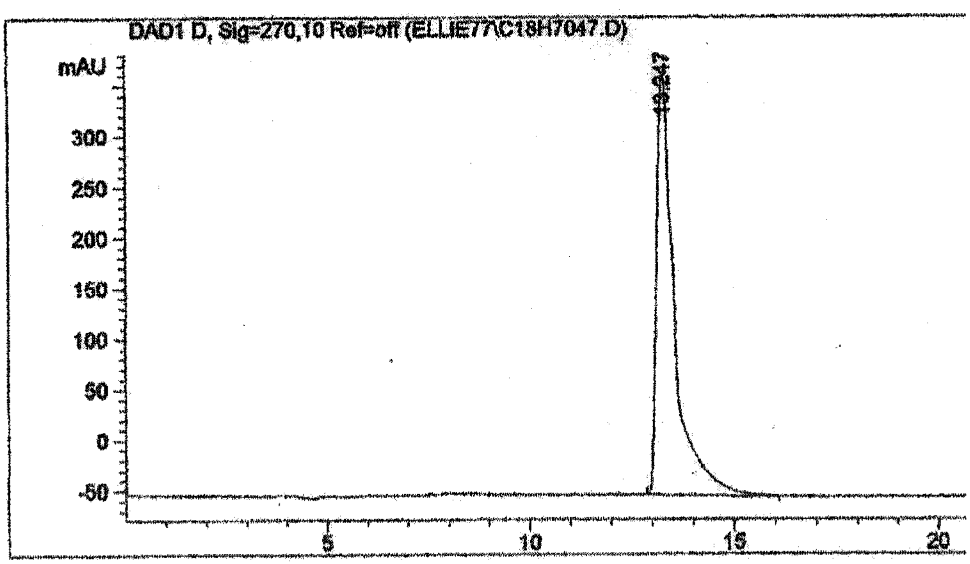


Figure 16. Migration of theophylline in C-18 modified capillary: pH=7.06, 30% methanol, +25 kV; migration time in min.

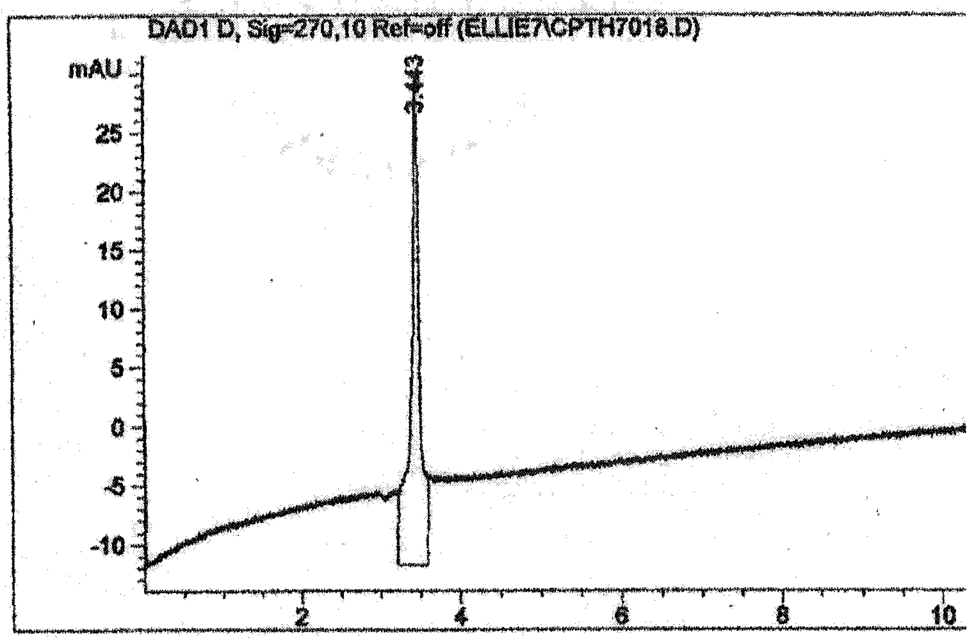


Figure 17. Migration of theophylline in cyano pentoxy modified capillary: pH=7.06, 10% methanol, +25 kV; migration time in min.

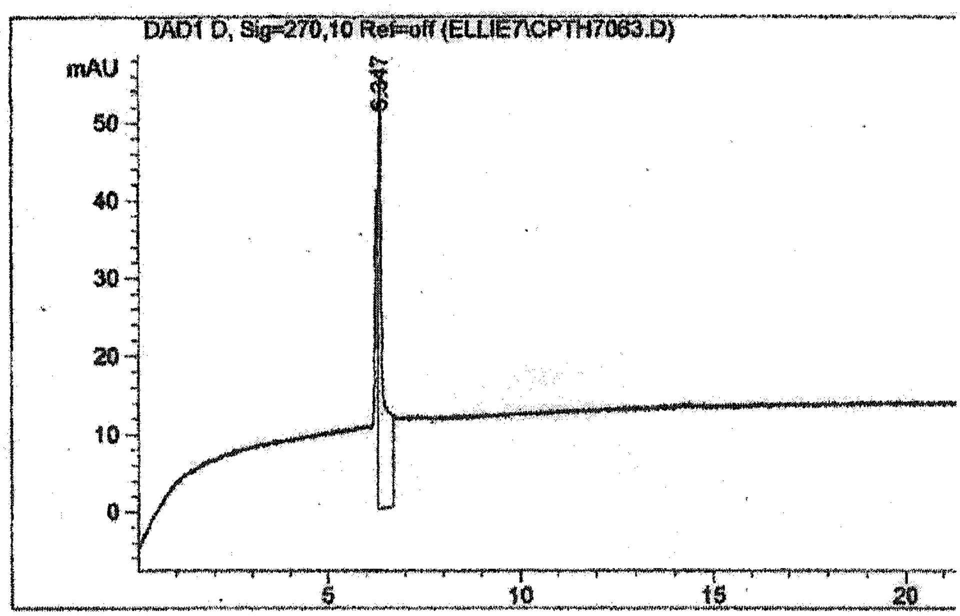


Figure 18. Migration of theophylline in cyano pentoxy modified capillary: pH=7.06, 40% methanol, +25 kV; migration time in min.

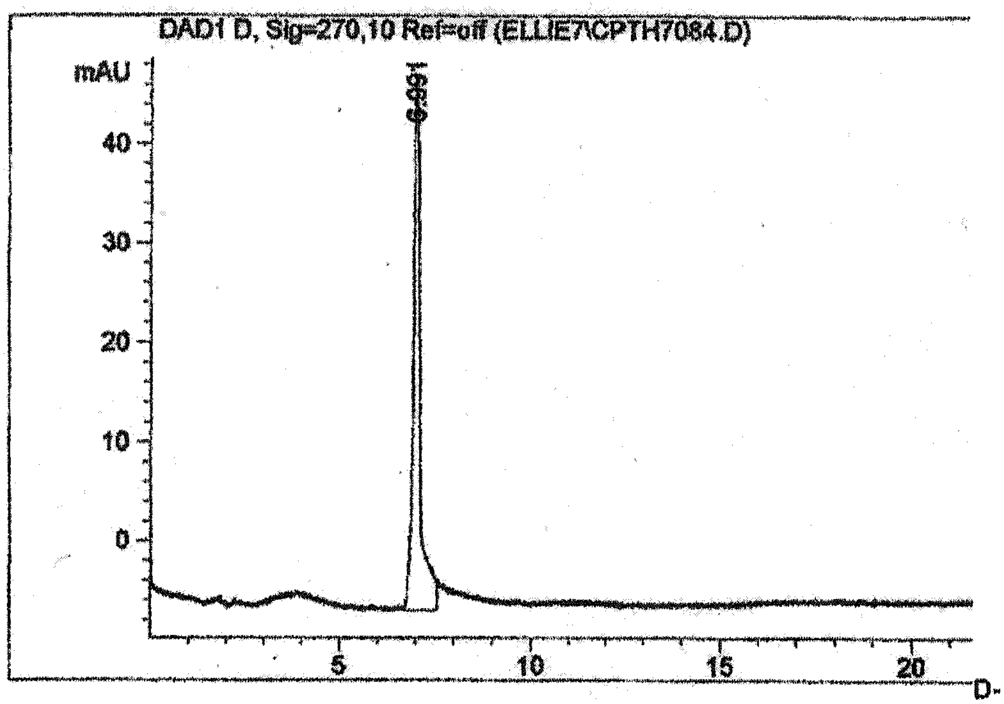


Figure 19. Migration of theophylline in cyano pentoxy modified capillary: pH=7.06, 50% methanol, +25 kV; migration time in min.

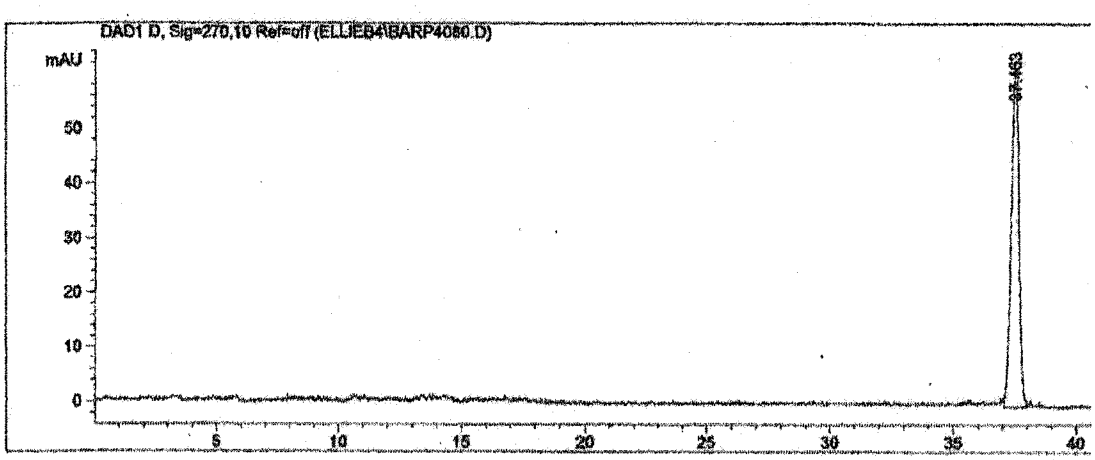


Figure 20. Migration of theophylline in bare silica capillary: pH=4.41, 50% methanol, +25 kV; migration time in min.

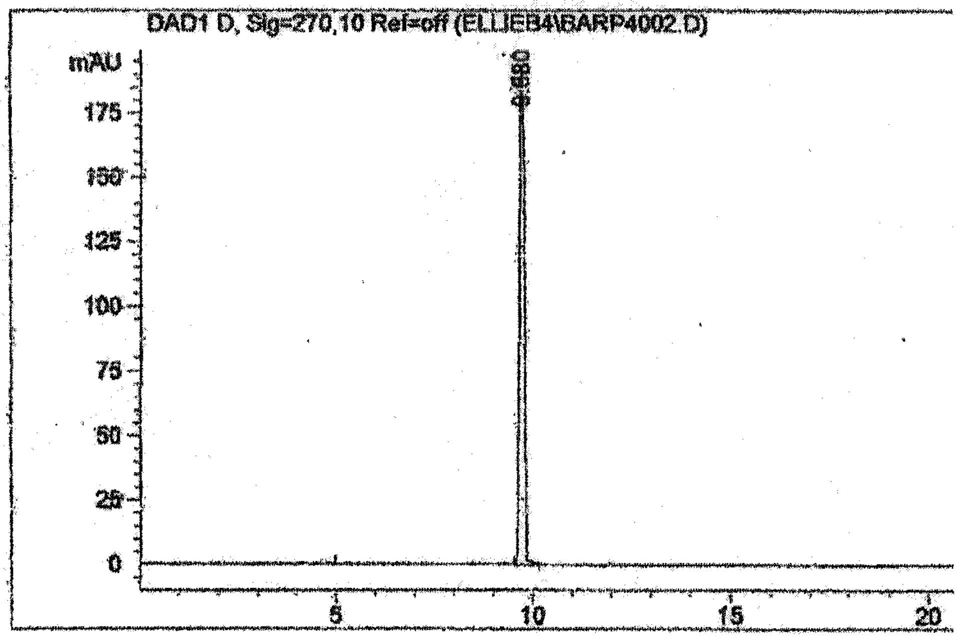


Figure 21. Migration of theophylline in bare silica capillary: pH=4.41, no methanol, +25 kV; migration time in min.

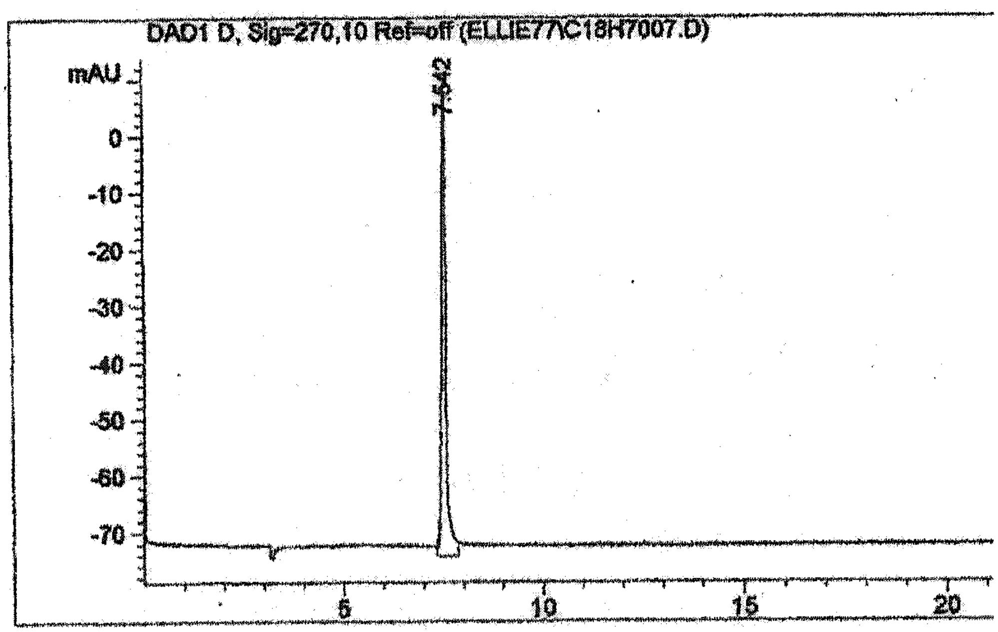


Figure 22. Migration of dihydroxy-theophylline in C-18 modified capillary: pH=7.06, no methanol, +25 kV; migration time in min.

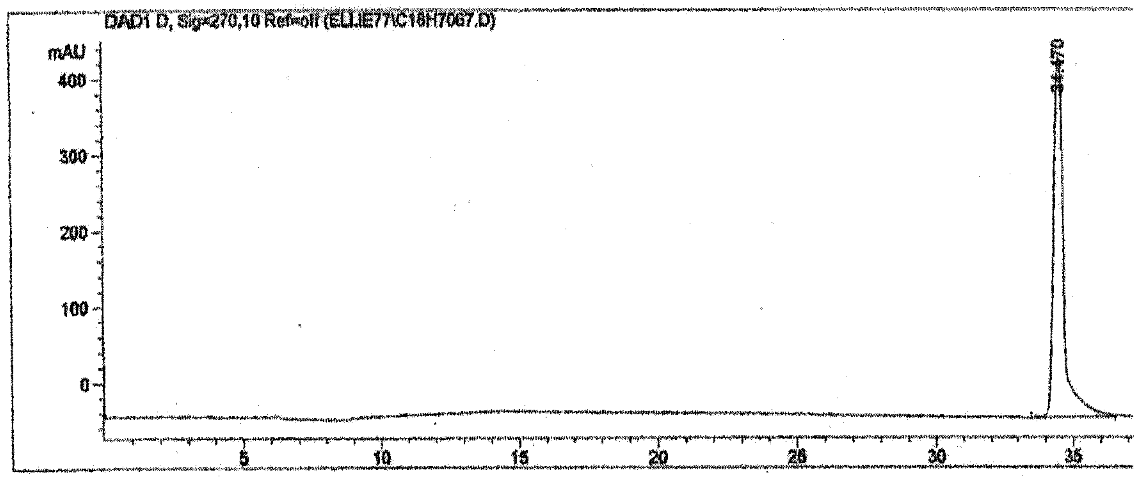


Figure 23. Migration of dihydroxy-theophylline in C-18 modified capillary: pH=7.06, 40% methanol, +25 kV; migration time in min.

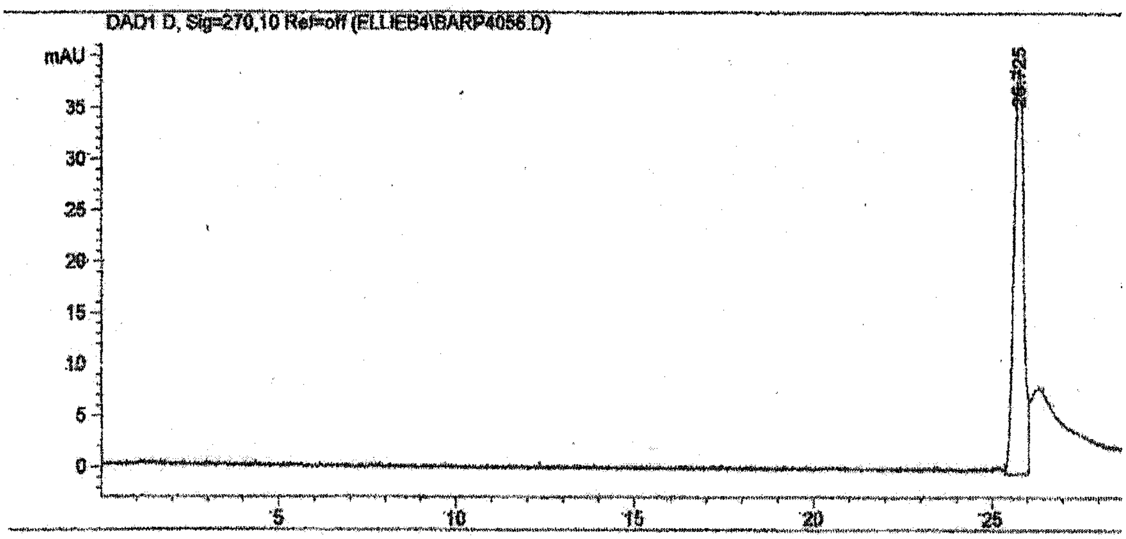


Figure 24. Migration of aminophylline in bare silica capillary: pH=4.41, 30% methanol, +25 kV; migration time in min.

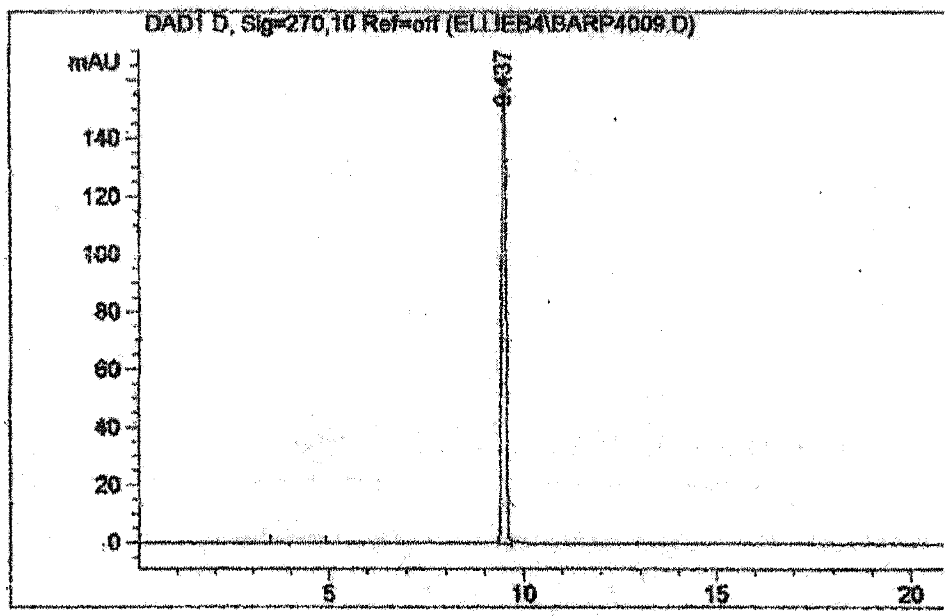


Figure 25. Migration of aminophylline in bare silica capillary: pH=4.41, no methanol, +25 kV; migration time in min.

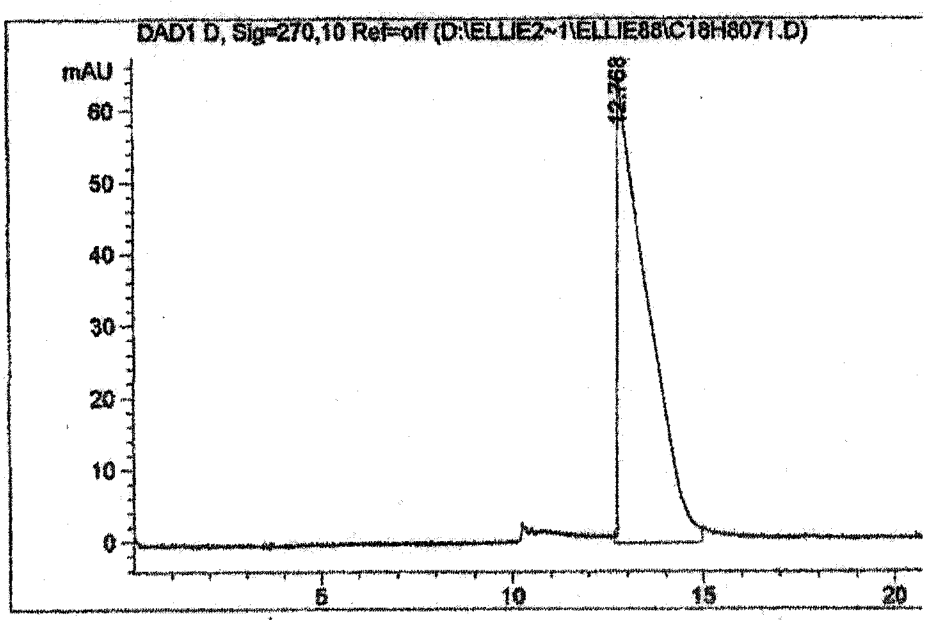


Figure 26. Migration of aminophylline in C-18 modified capillary: pH=8.14, 40% methanol, +25 kV; migration time in min.

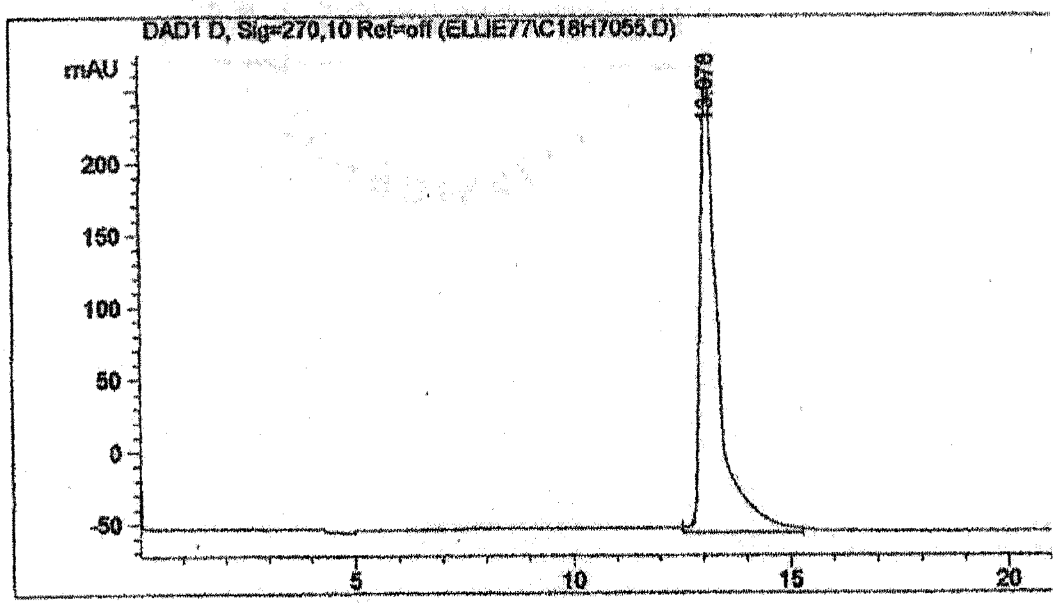


Figure 27. Migration of aminophylline in C-18 modified capillary: pH=7.06, 30% methanol, +25 kV; migration time in min.

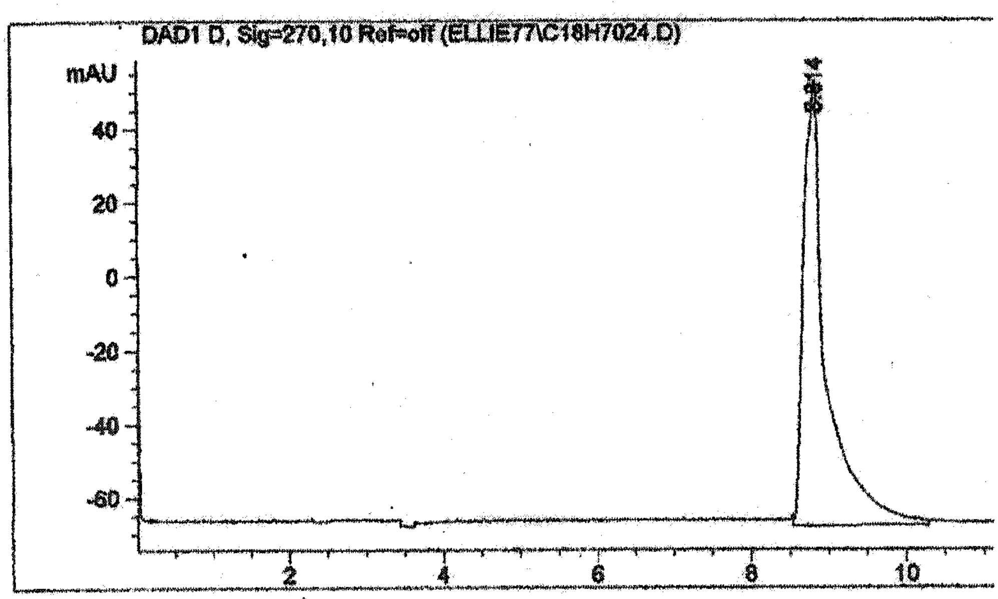


Figure 28. Migration of aminophylline in C-18 modified capillary: pH=7.06, 10% methanol, +25 kV; migration time in min.

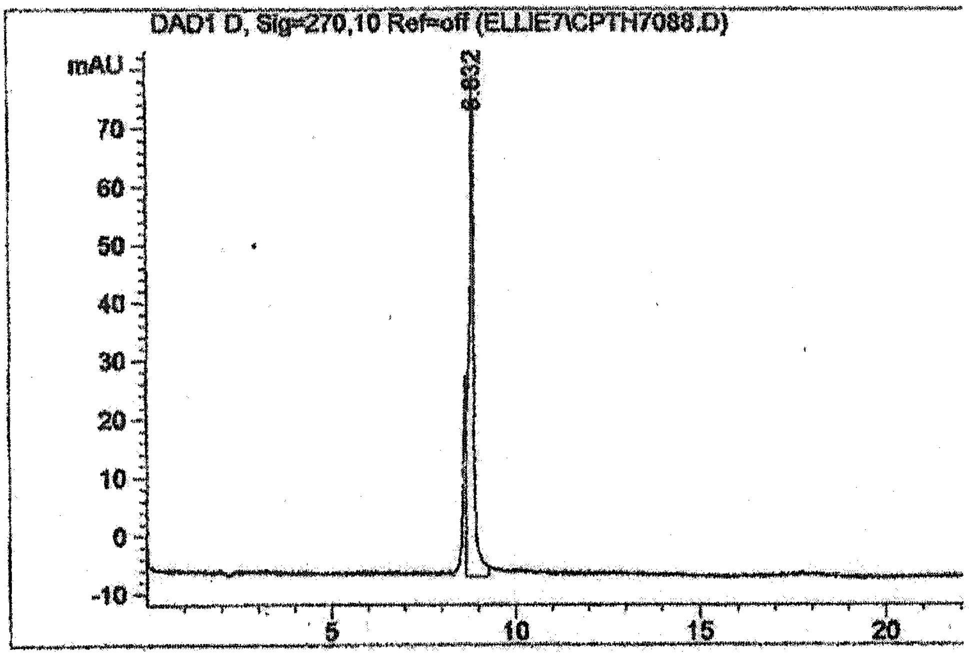


Figure 29. Migration of aminophylline in cyano pentoxy modified capillary: pH=7.06, 50% methanol, +25 kV; migration time in min.

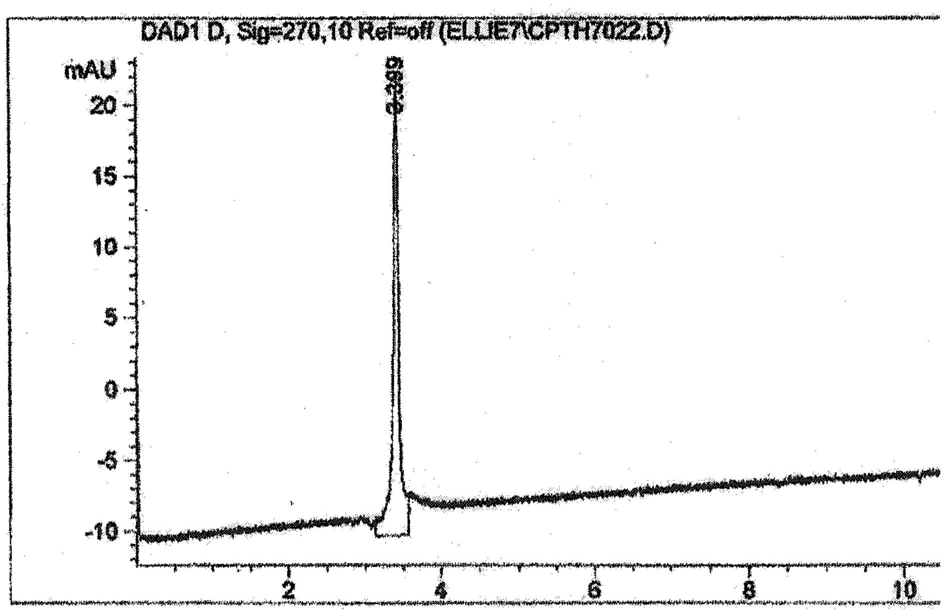


Figure 30. Migration of aminophylline in cyano pentoxy capillary: pH=7.06, 10% methanol, +25 kV; migration time in min.

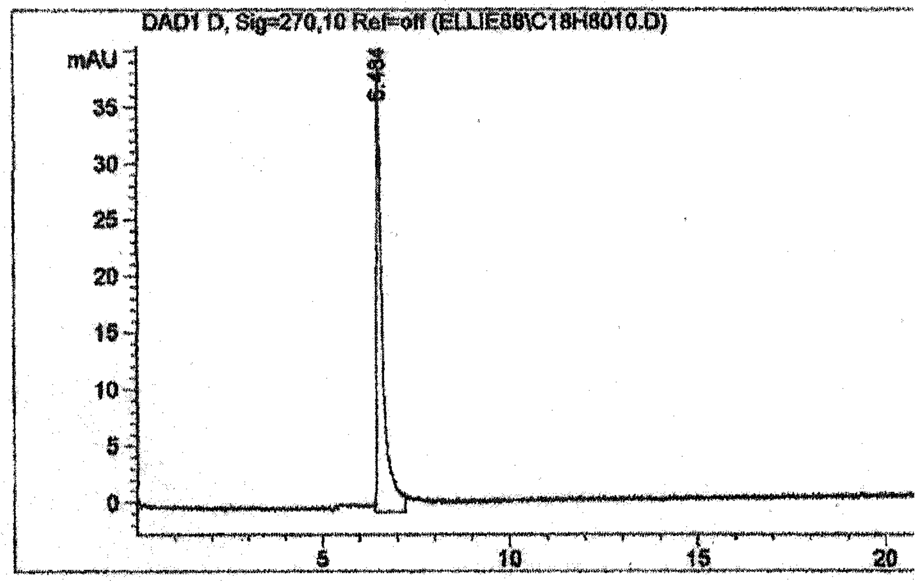


Figure 31. Migration of aminophylline in C-18 modified capillary: pH=8.14, no methanol, +25 kV; migration time in min.

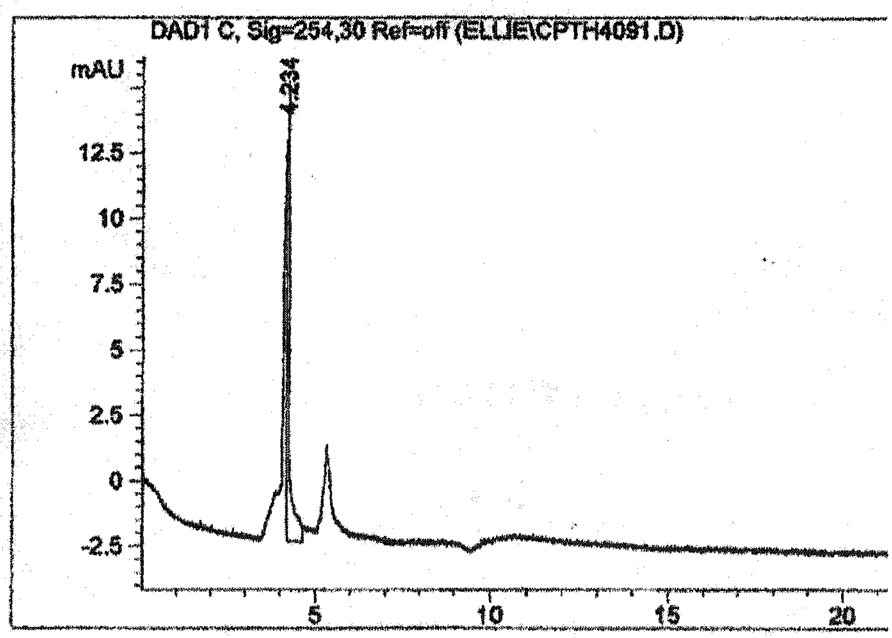


Figure 32. Migration of nortriptylline in cyano pentoxy modified capillary: pH=4.41, 50% methanol, +25 kV; migration time in min.

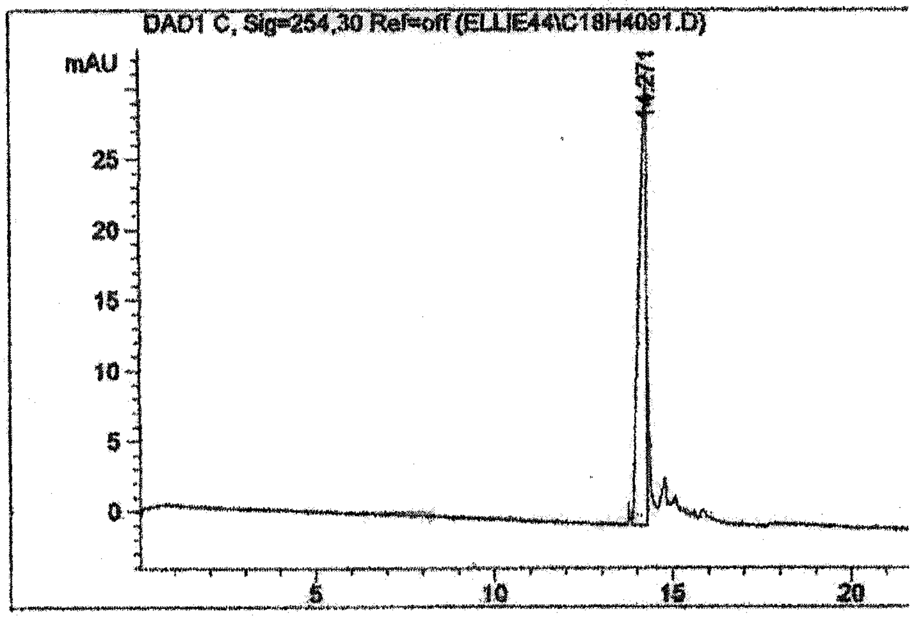


Figure 33. Migration of nortriptylline in C-18 modified capillary: pH=4.41, 50% methanol, +25 kV; migration time in min.

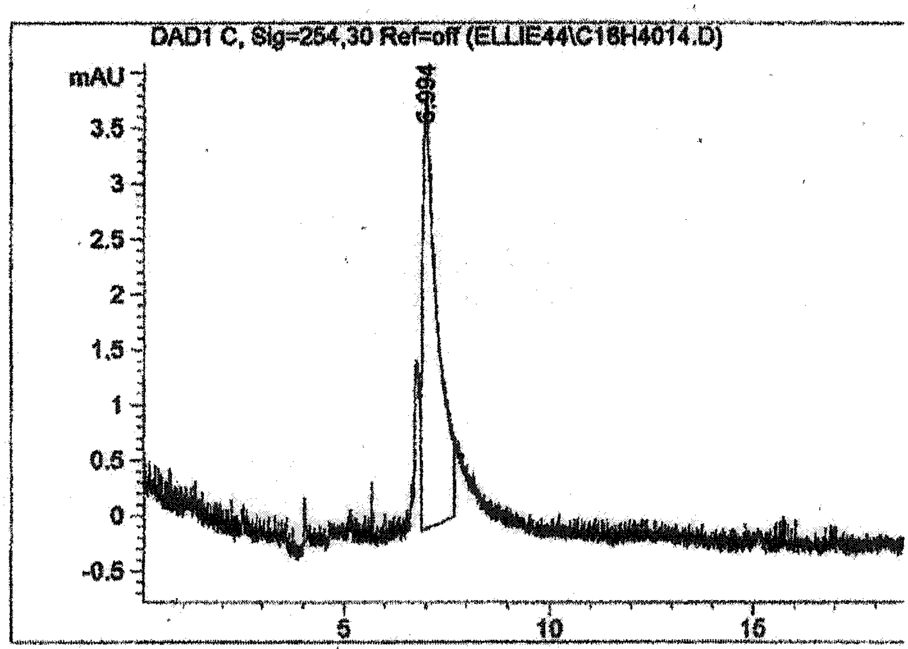


Figure 34. Migration of nortriptylline in C-18 modified capillary: pH=4.41, no methanol, +25 kV; migration time in min.

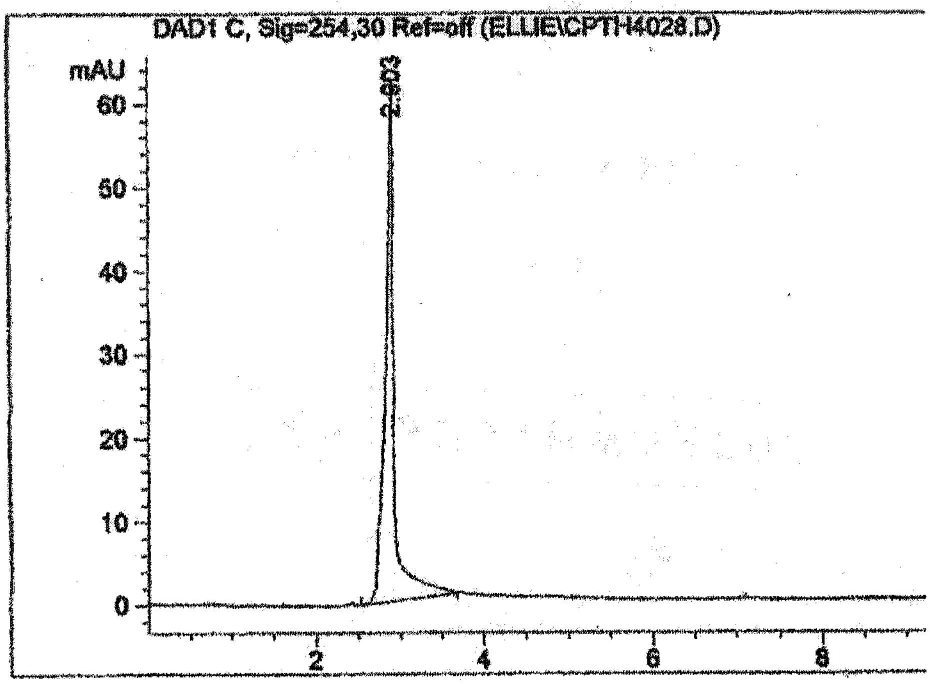


Figure 35. Migration of nortriptylline in cyano pentoxy modified capillary: pH=4.41, 10% methanol, +25 kV; migration time in min.

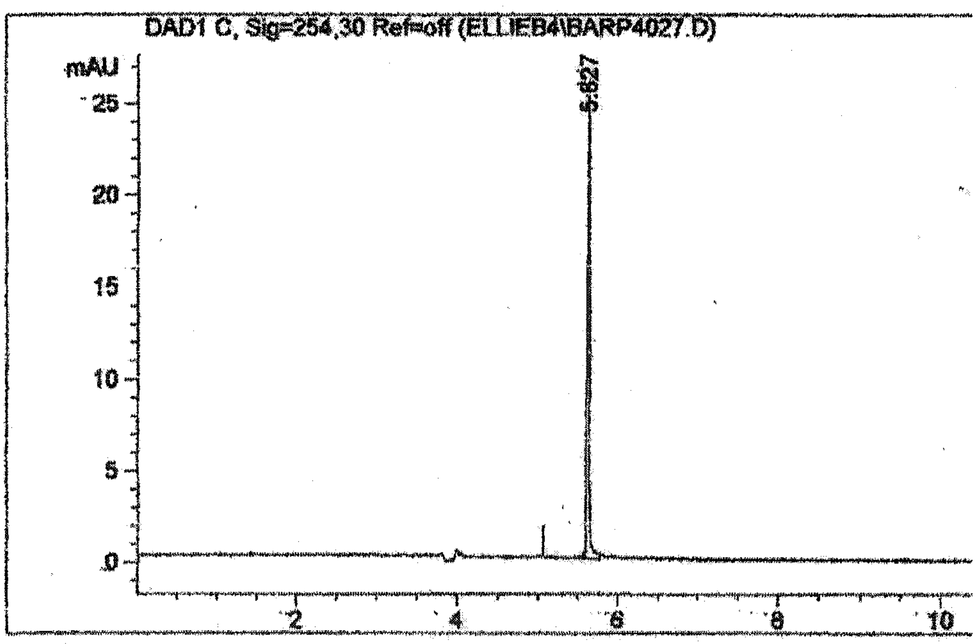


Figure 36. Migration of nortriptylline in bare silica capillary: pH=4.41, 10% methanol, +25 kV; migration time in min.

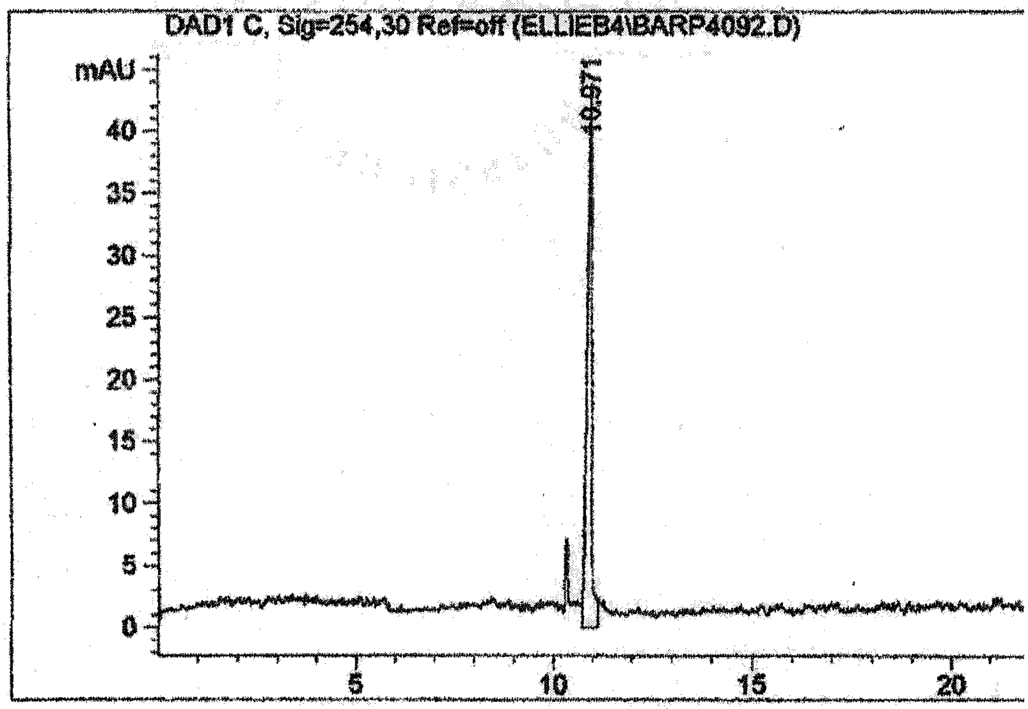


Figure 37. Migration of nortriptylline in bare silica capillary: pH=4.41, 50% methanol, +25 kV; migration time in min.

Note: In Figures 38 through 64 below, the trend lines are mostly obtained using the 2nd order through 5th order polynomial curve fits ('Poly'). Some other trend line curve fits are linear ('Linear') and exponential ('Expon.'), as indicated (all curve fits performed using Microsoft Excel).

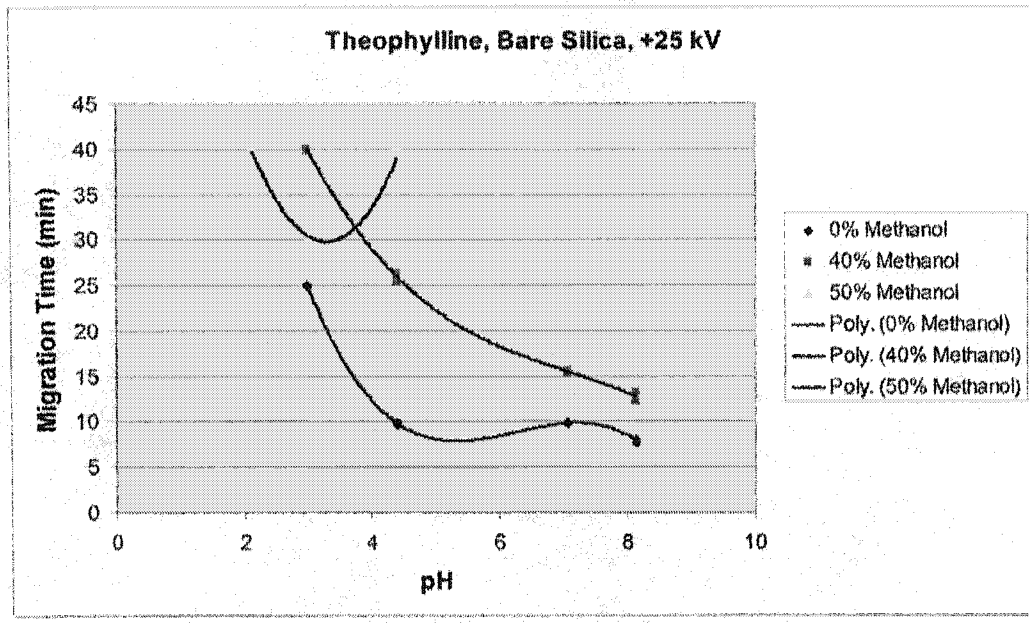


Figure 38. Theophylline migration time vs pH for methanol volume ratios of 0%, 40%, and 50% in bare silica capillary at +25 kV.

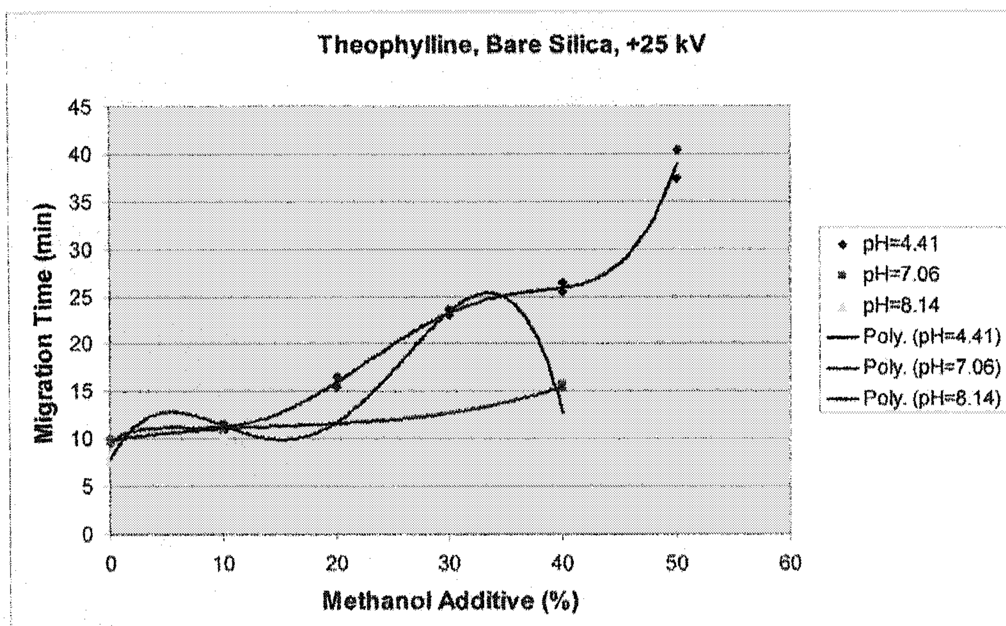


Figure 39. Theophylline migration time vs methanol volume ratio at pH = 4.41, 7.06, and 8.14 in bare silica capillary at +25 kV.

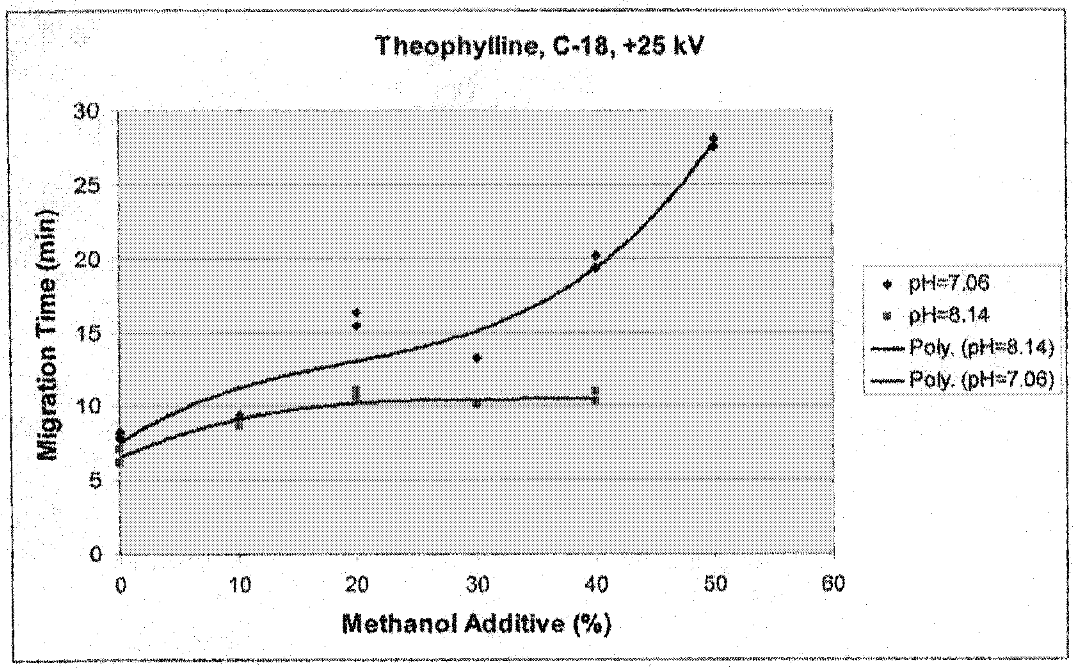


Figure 40. Theophylline migration time vs methanol volume ratio at pH = 7.06, and 8.14 in C-18 modified capillary at +25 kV.

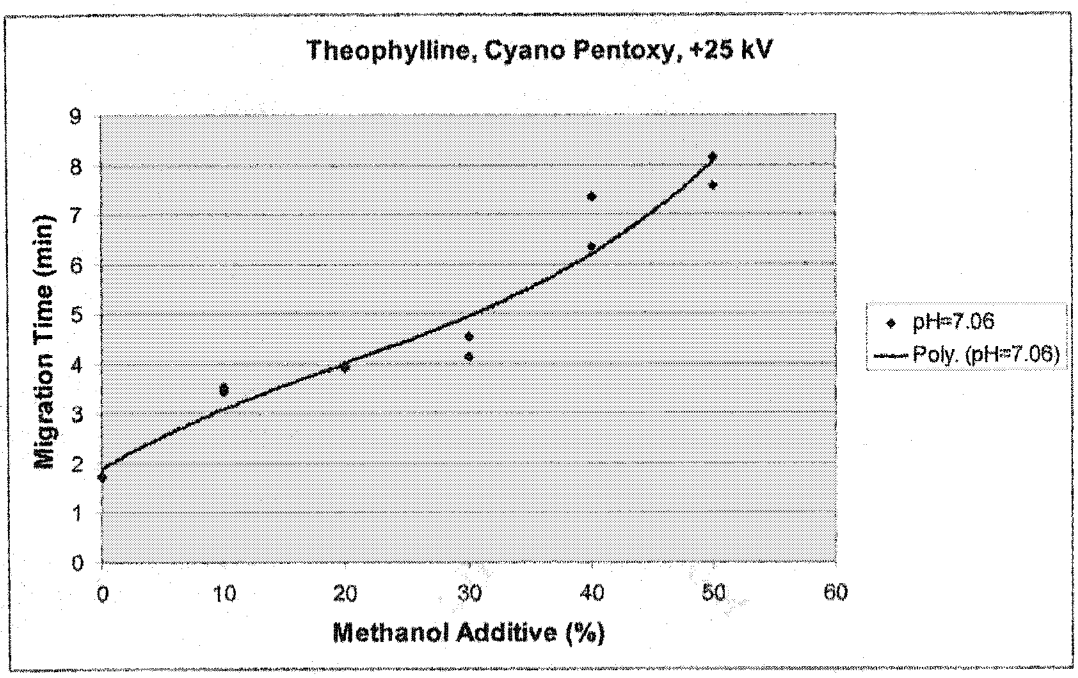


Figure 41. Theophylline migration time vs methanol volume ratio at pH = 7.06 in cyano pentoxy modified capillary at +25 kV.

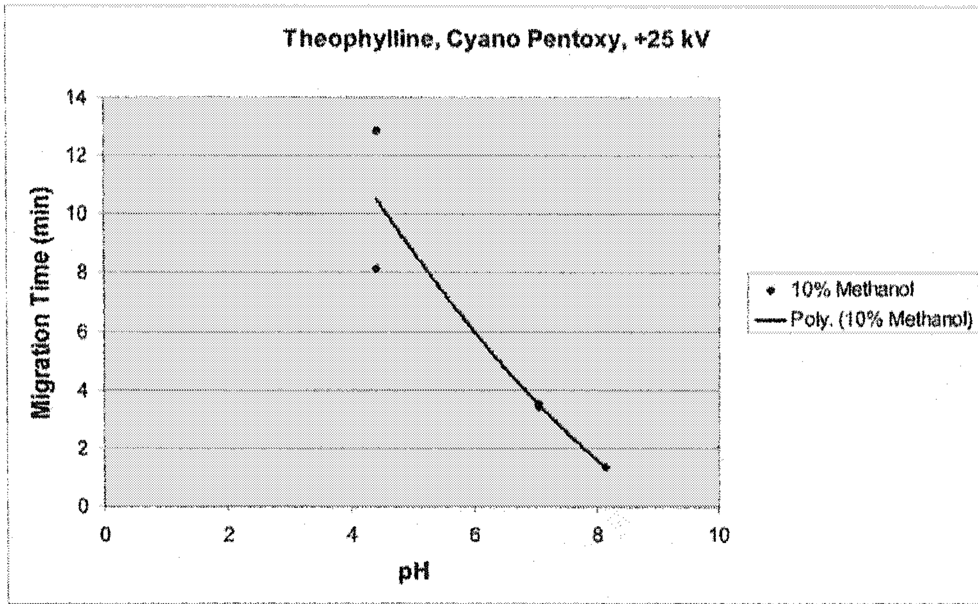


Figure 42. Theophylline migration time vs pH for methanol volume ratio of 10% in cyano pentoxy capillary at +25 kV.

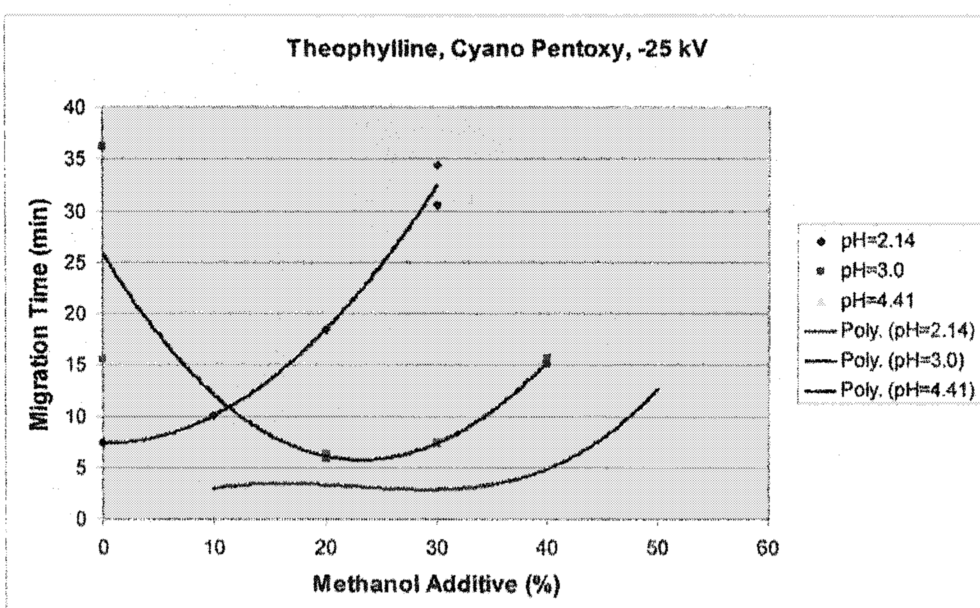


Figure 43. Theophylline migration time vs methanol volume ratio at pH=2.14, 3.0, & 4.41 in cyano pentoxy modified capillary at -25 kV.

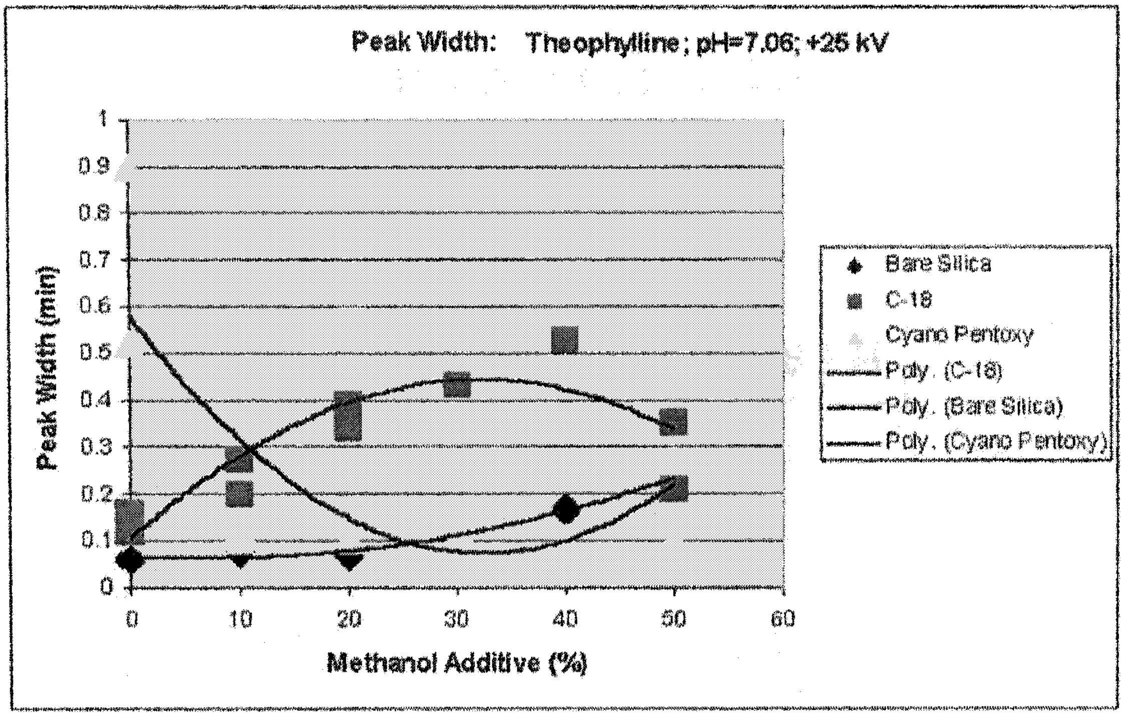


Figure 44. Capillary efficiency for theophylline vs methanol volume ratio for bare silica, C-18 modified, and cyano pentoxy modified capillaries at pH=7.06 and +25 kV.

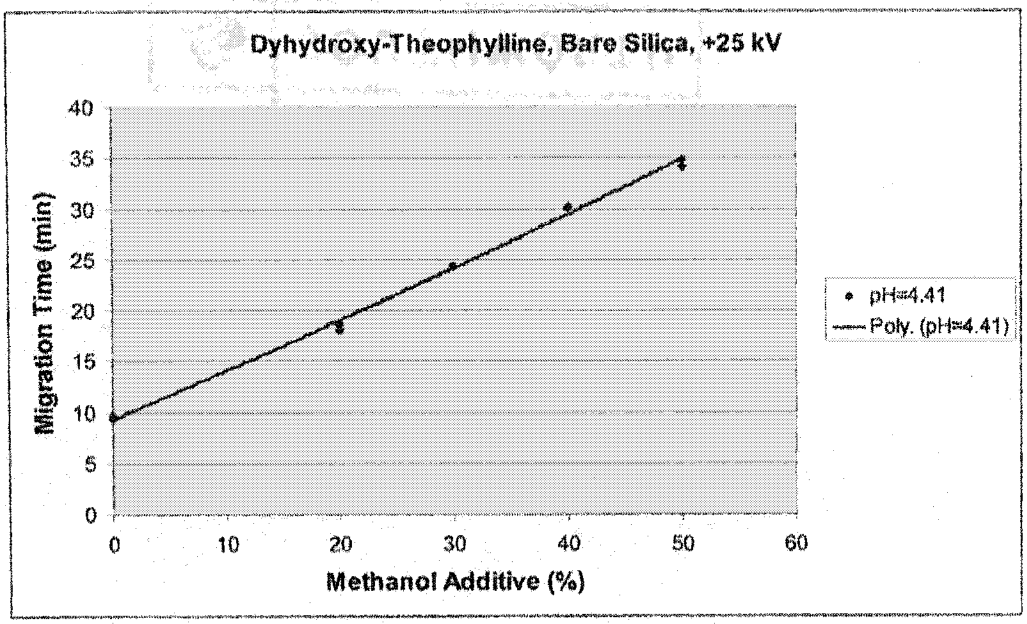


Figure 45. Dihydroxy-theophylline migration time vs methanol volume ratio at pH of 4.41, in bare silica capillary at +25 kV.

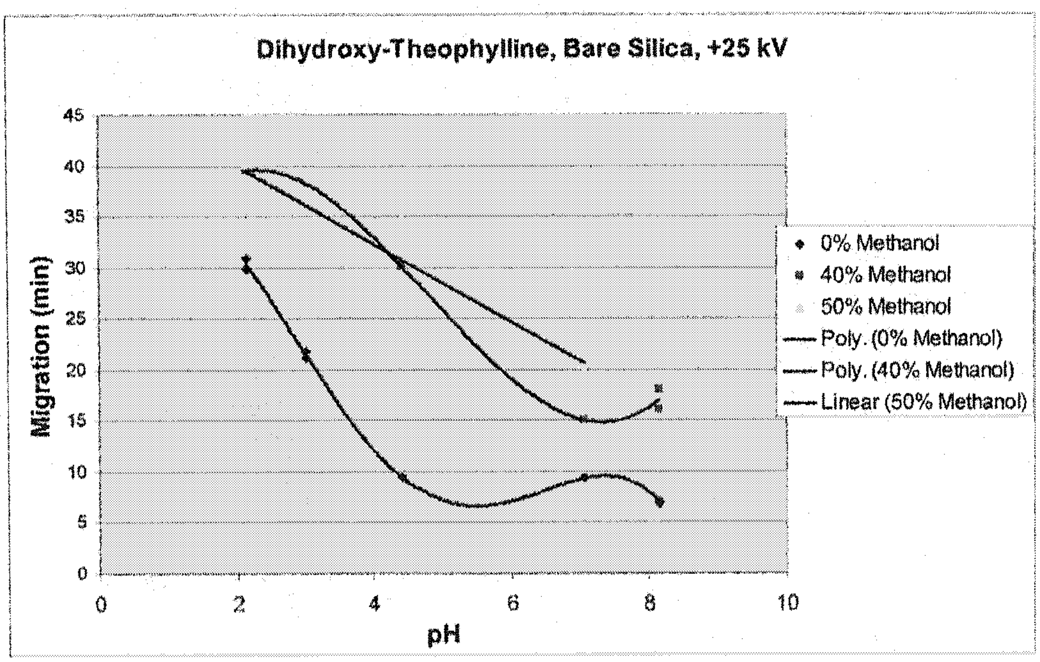


Figure 46. Dihydroxy-theophylline migration time vs pH for methanol volume ratios of 0%, 40%, and 50% in bare silica capillary at +25 kV.

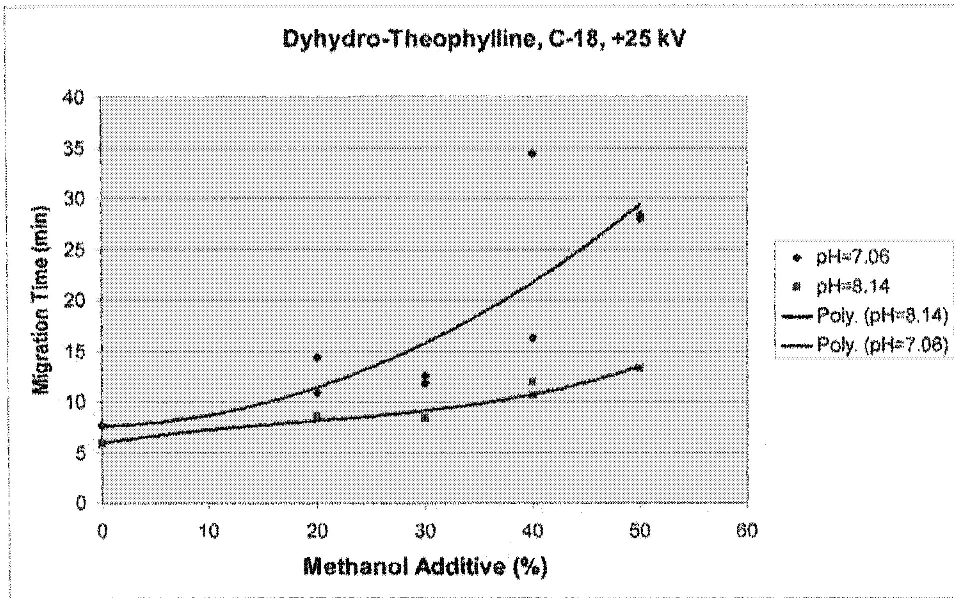


Figure 47. Dihydroxy-theophylline migration time vs methanol volume ratio at pH= 7.06, 8.14 in C-18 modified capillary at +25 kV.

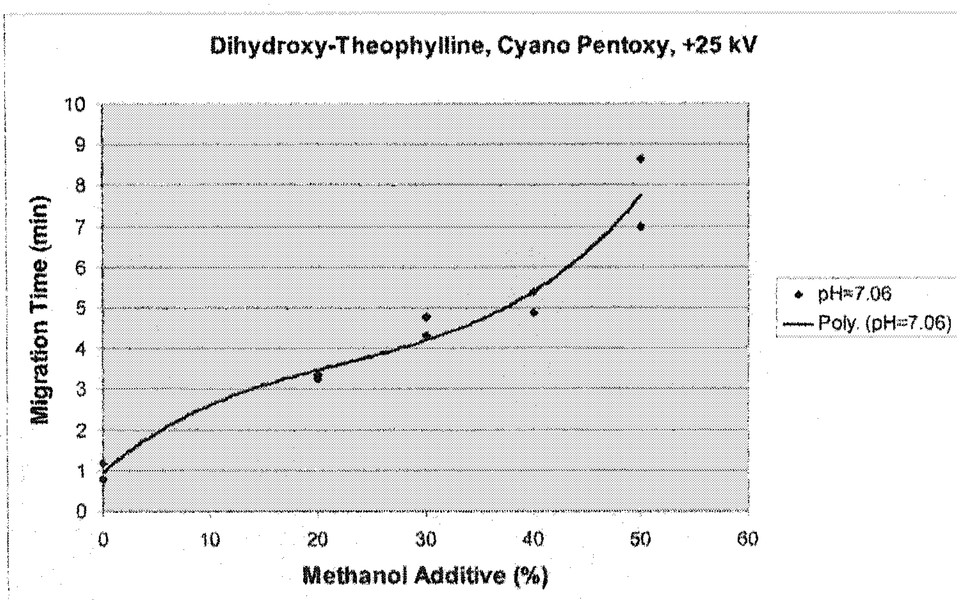


Figure 48. Dihydroxy-theophylline migration time vs methanol volume ratio at pH=7.06 in cyano pentoxy modified capillary at +25 kV.

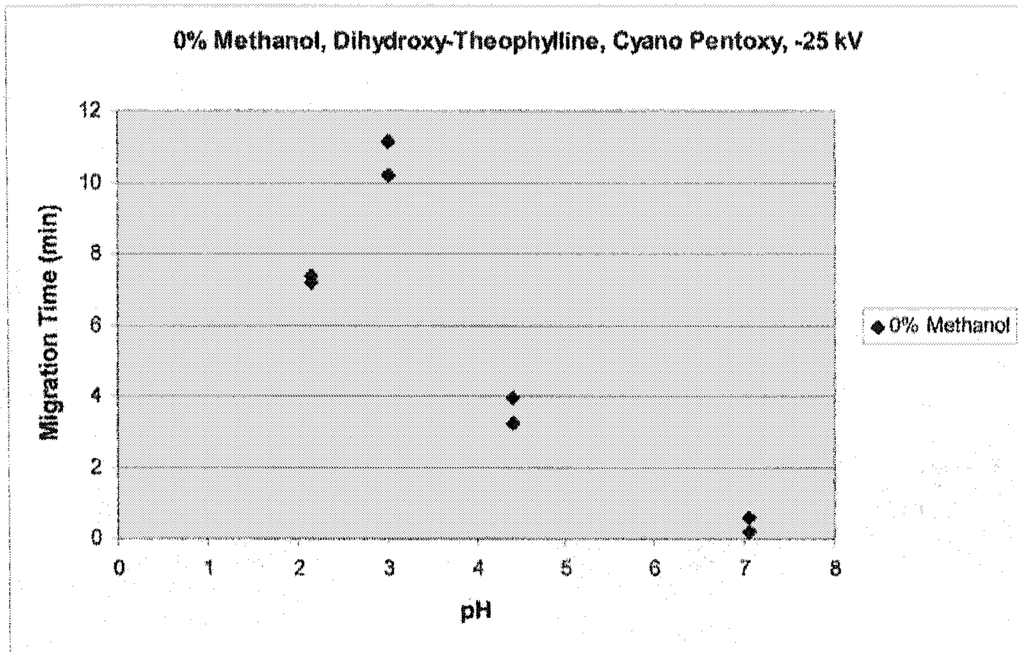


Figure 49. Dihydroxy-theophylline migration time vs pH for methanol volume ratio of 0% in cyano pentoxy modified capillary at -25 kV.

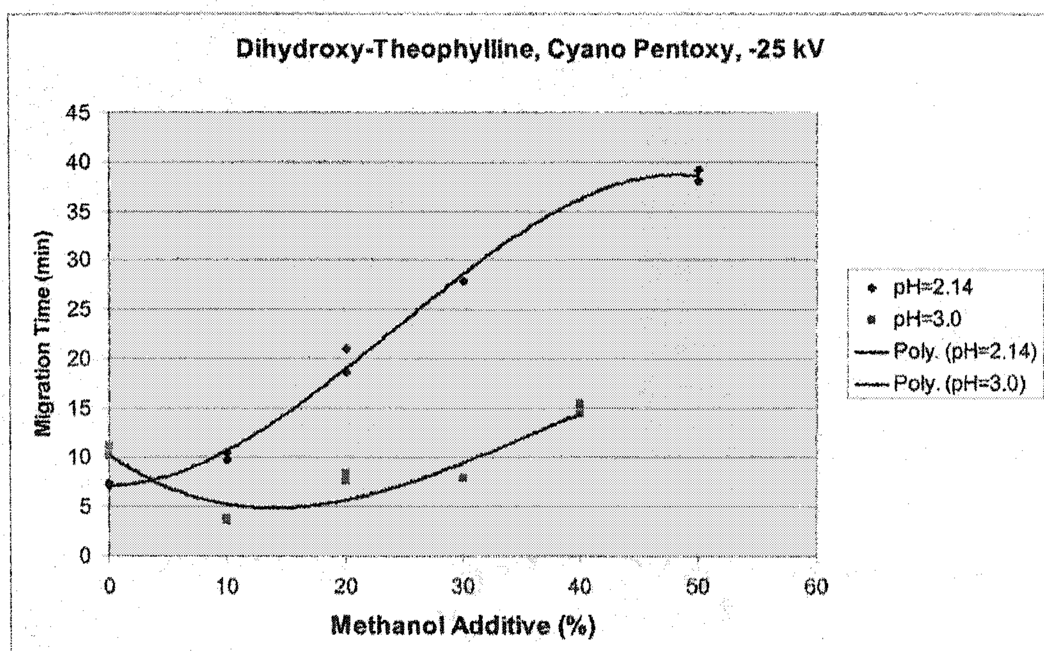


Figure 50. Dihydroxy-theophylline migration time vs methanol volume ratio at pH= 2.14 & 3.0 in cyano pentoxy modified capillary at +25 kV.

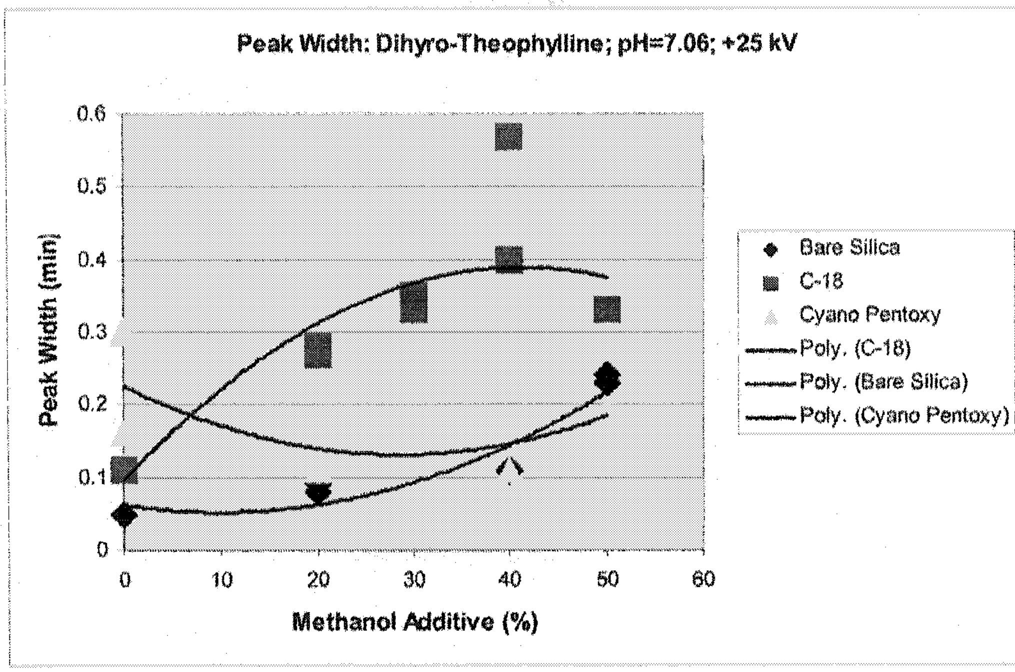


Figure 51. Peak width for dihydro-theophylline vs methanol volume ratio for bare silica, C-18 modified, and cyano pentoxy modified capillaries at pH=7.06 and +25 kV.

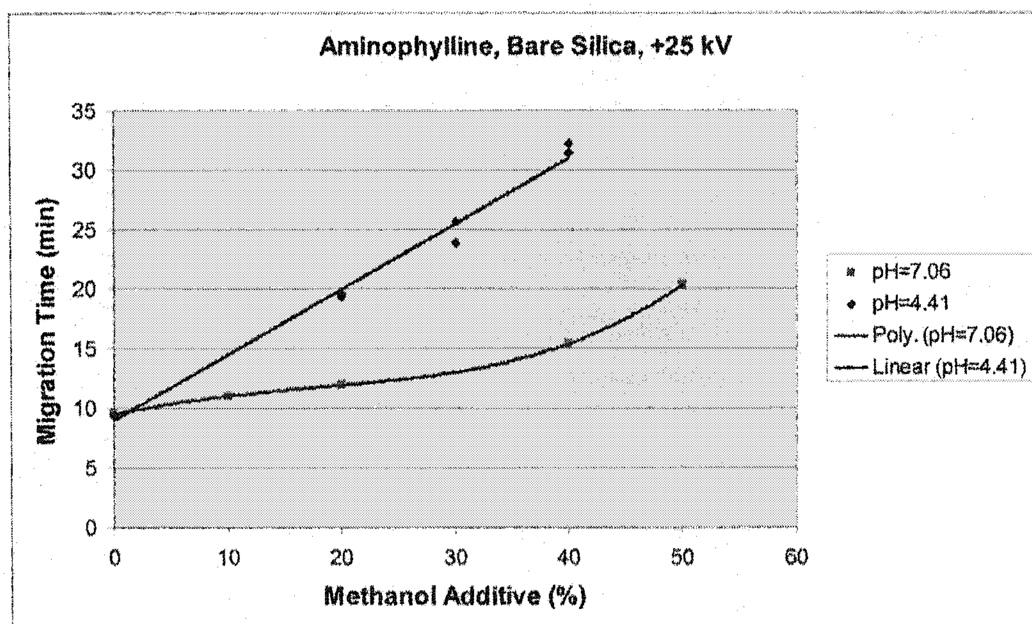


Figure 52. Aminophylline migration time vs methanol volume ratio at pH = 4.41 and 7.06, in bare silica capillary, at +25 kV.

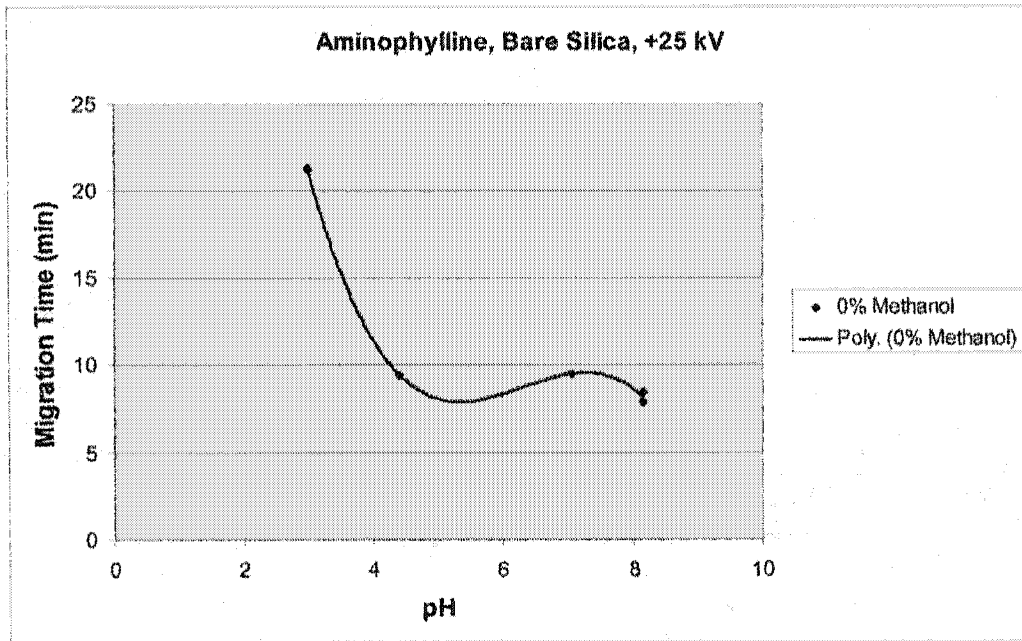


Figure 53. Aminophylline migration time vs pH in the range of 2.14 to 8.14 in bare silica capillary at +25 kV (no methanol modifier).

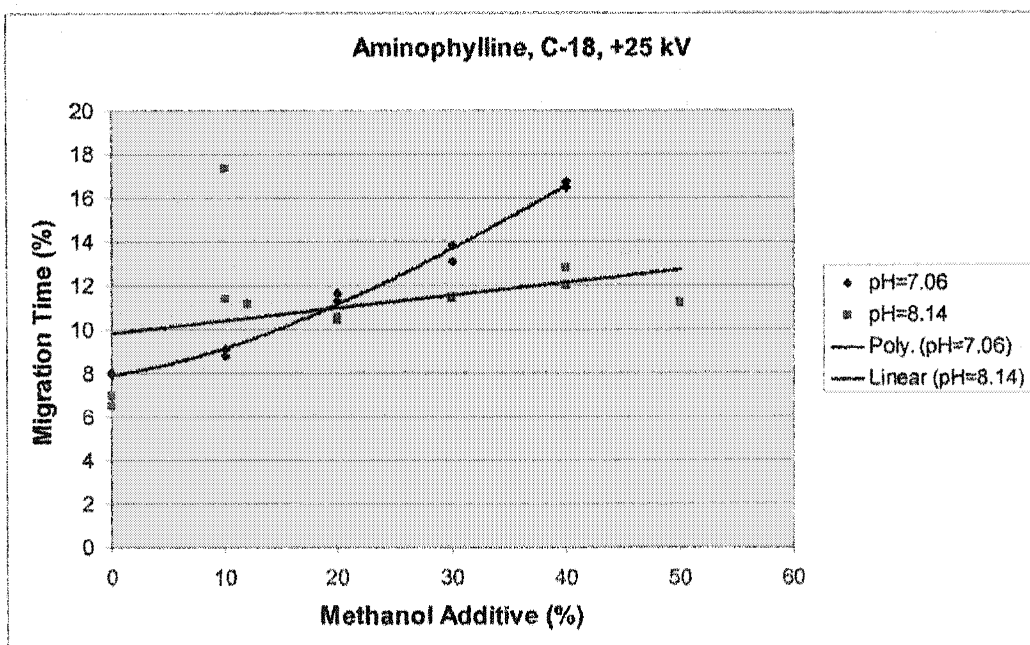


Figure 54. Aminophylline migration time vs methanol volume ratio at pH of 7.06 and 8.14, in C-18 capillary at +25 kV.

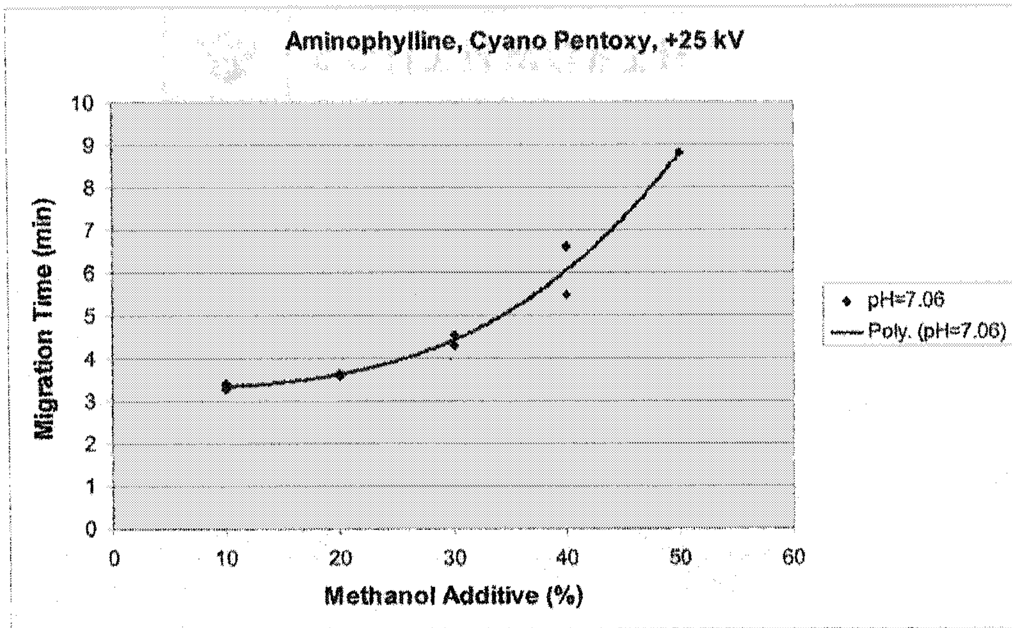


Figure 55. Aminophylline migration time vs methanol volume ratio at pH of 7.06, in cyano pentoxy capillary at +25 kV.

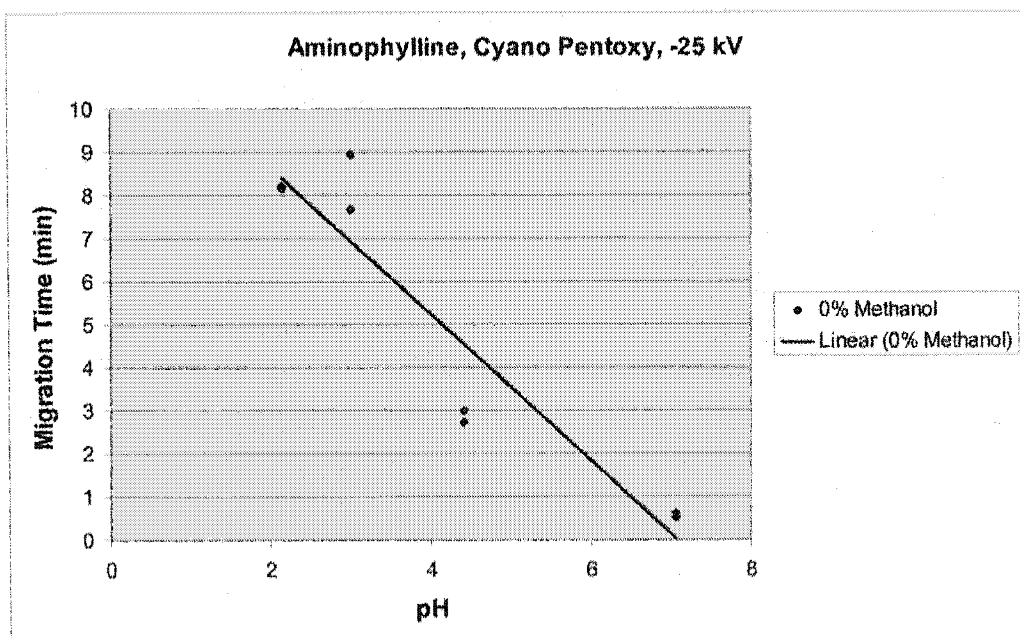


Figure 56. Aminophylline migration time vs pH in the range of 2.14 to 8.14 in cyano pentoxy capillary at -25 kV (no methanol modifier).

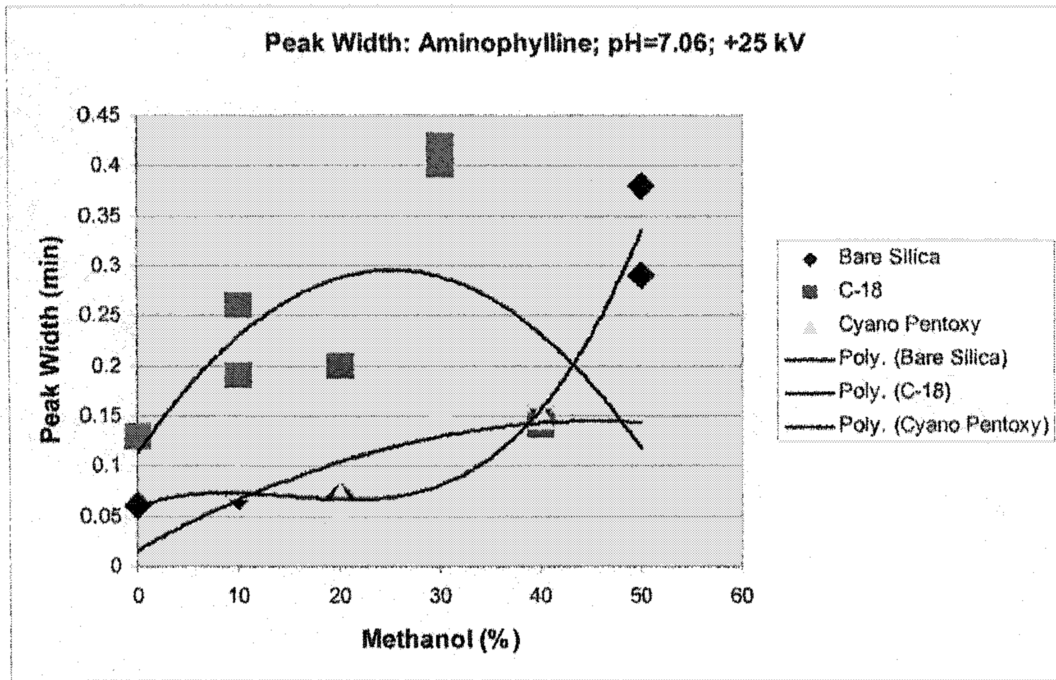


Figure 57. Peak width for aminophylline vs methanol volume ratio for bare silica, C-18 modified, and cyano pentoxy modified capillaries at pH=7.06 and +25 kV.

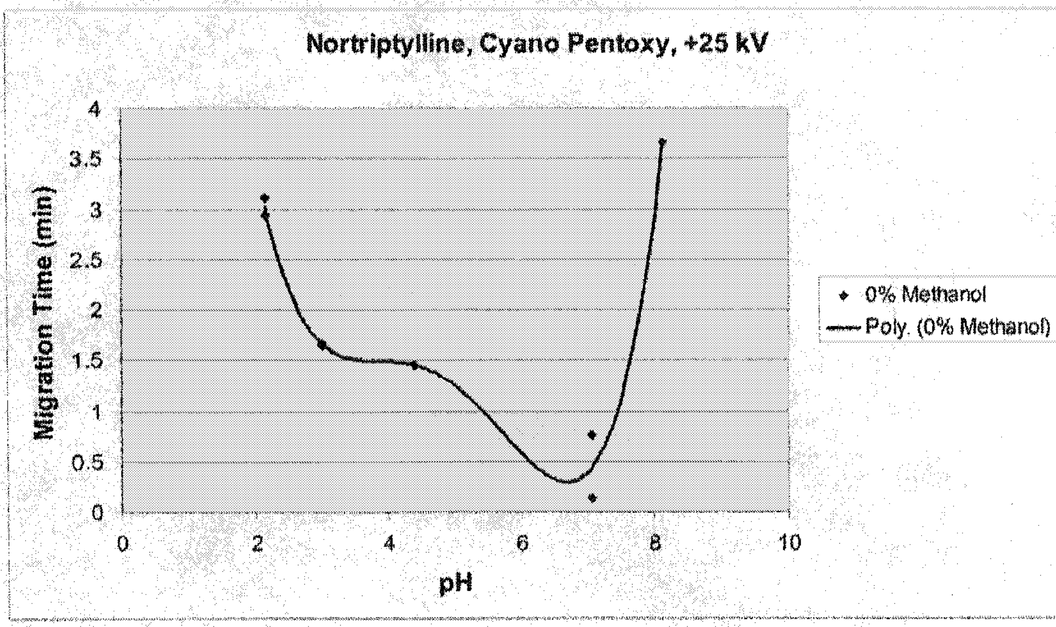


Figure 58. Migration time vs pH for nortriptylline in cyano pentoxy capillary at +25 kV (no methanol modifier).

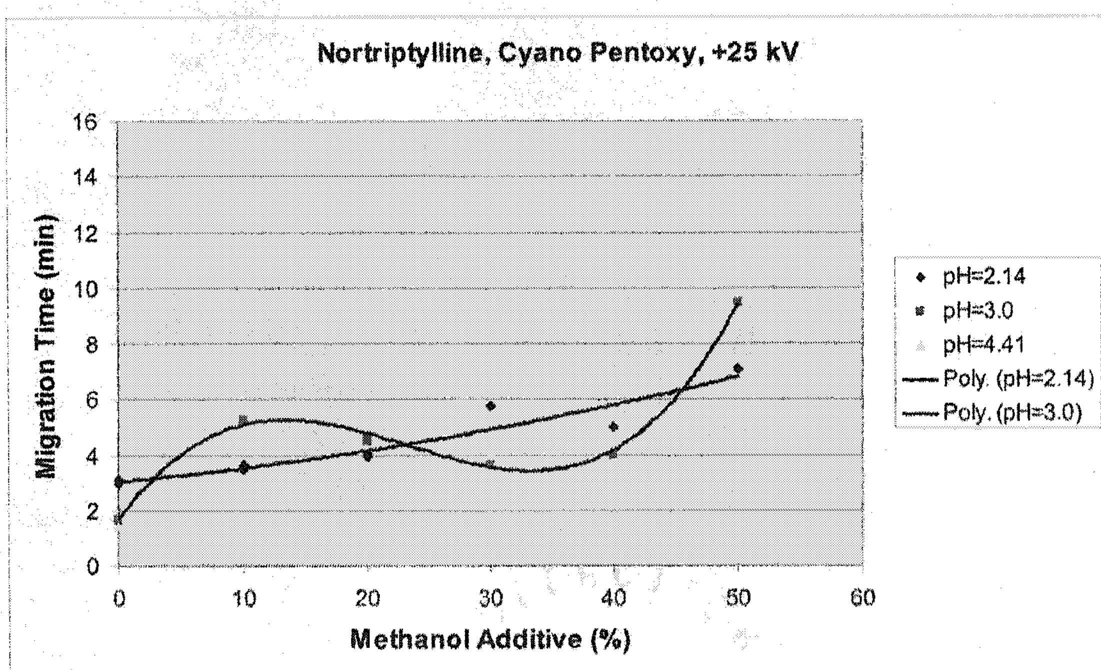


Figure 59. Nortriptylline migration time vs methanol volume ratio for pH = 2.14, 3.0, and 4.41, in cyano pentoxy capillary at +25 kV.

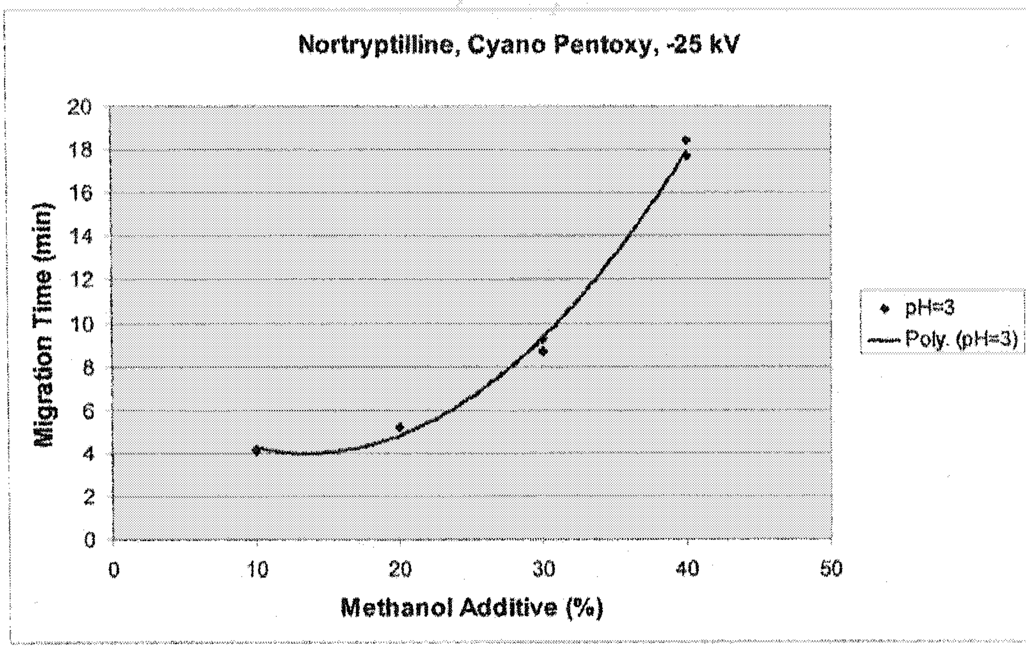


Figure 60. Nortriptylline migration time vs methanol volume ratio for pH = 3.0, in cyano pentoxy capillary at -25 kV.

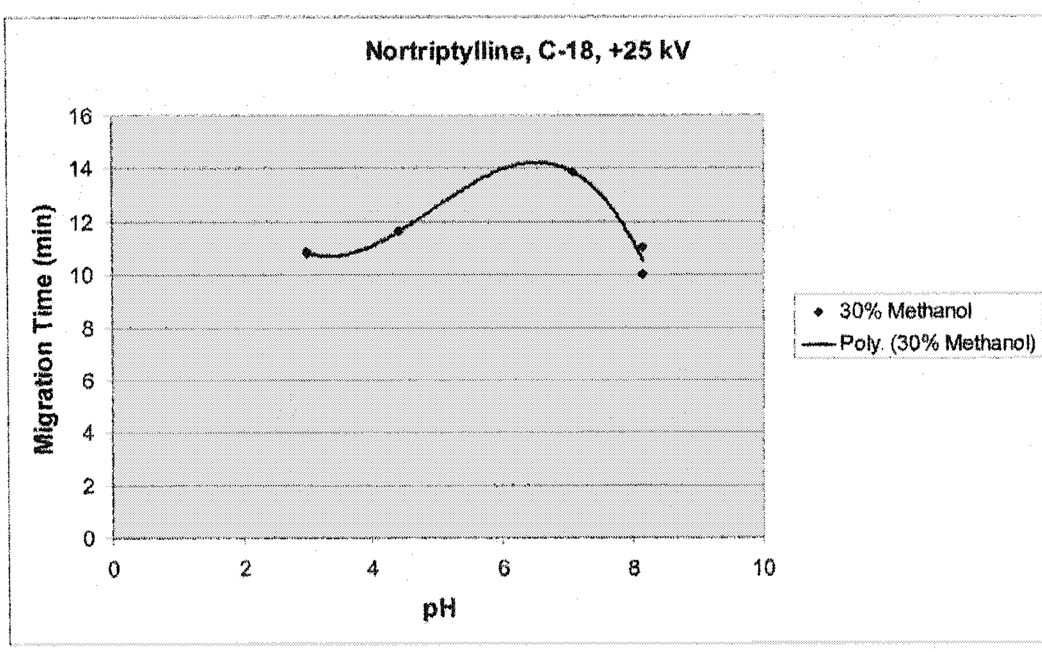


Figure 61. Nortriptylline migration time vs pH in the range of 2.14 to 8.14 with 30% methanol volume ratio in C-18 capillary at +25 kV.

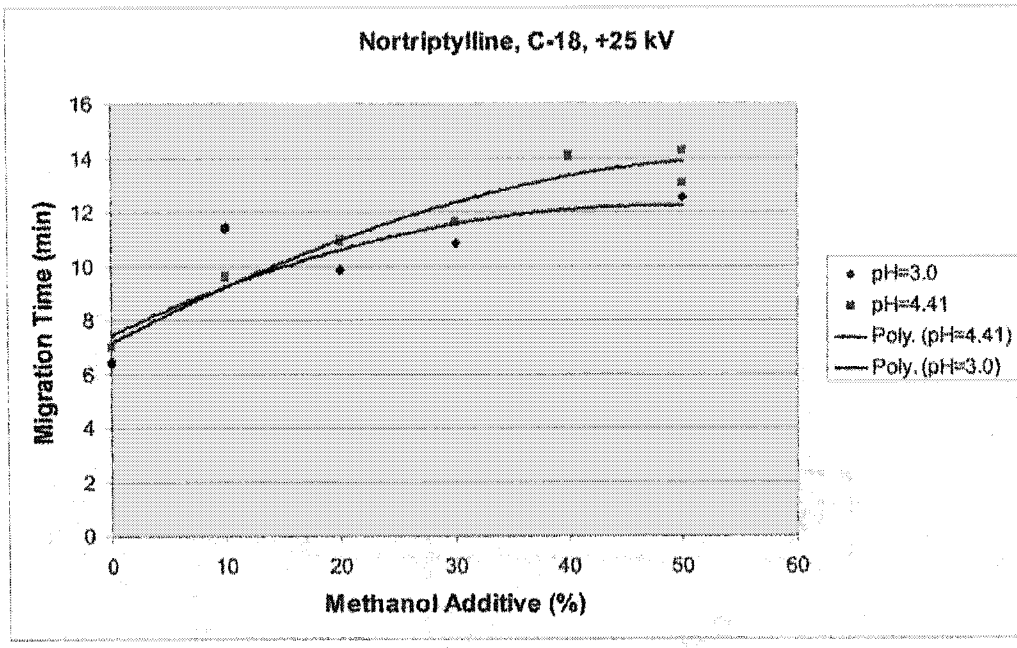


Figure 62. Nortriptylline migration time vs methanol volume ratio at pH of 3.0 and 4.41, in C-18 capillary at +25 kV.

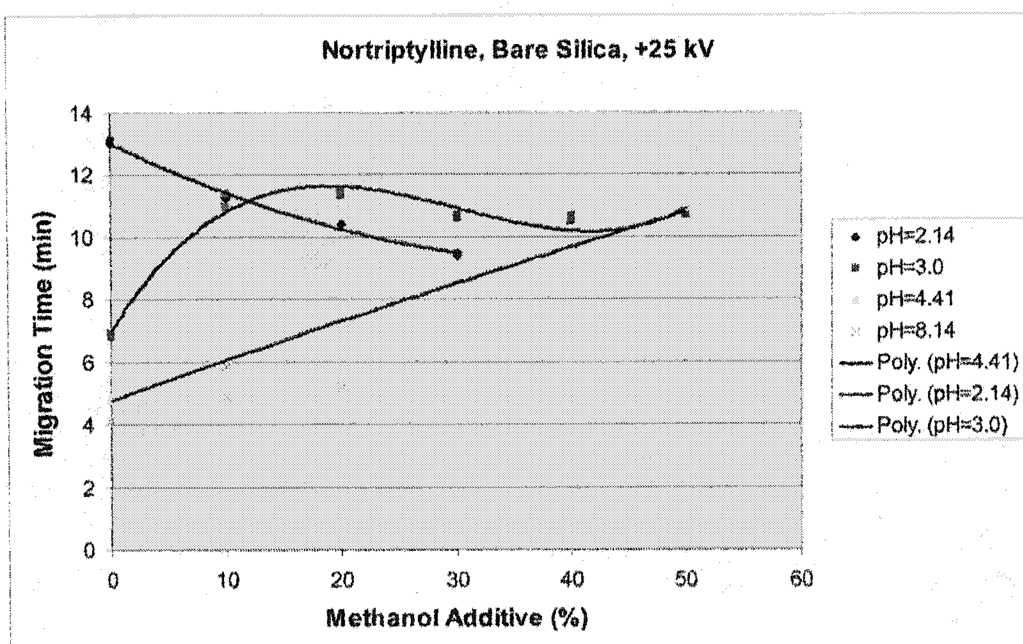


Figure 63. Nortriptylline migration time vs methanol volume ratio at pH = 2.14, 3.0, and 4.41, in Bare Silica capillary at +25 kV.

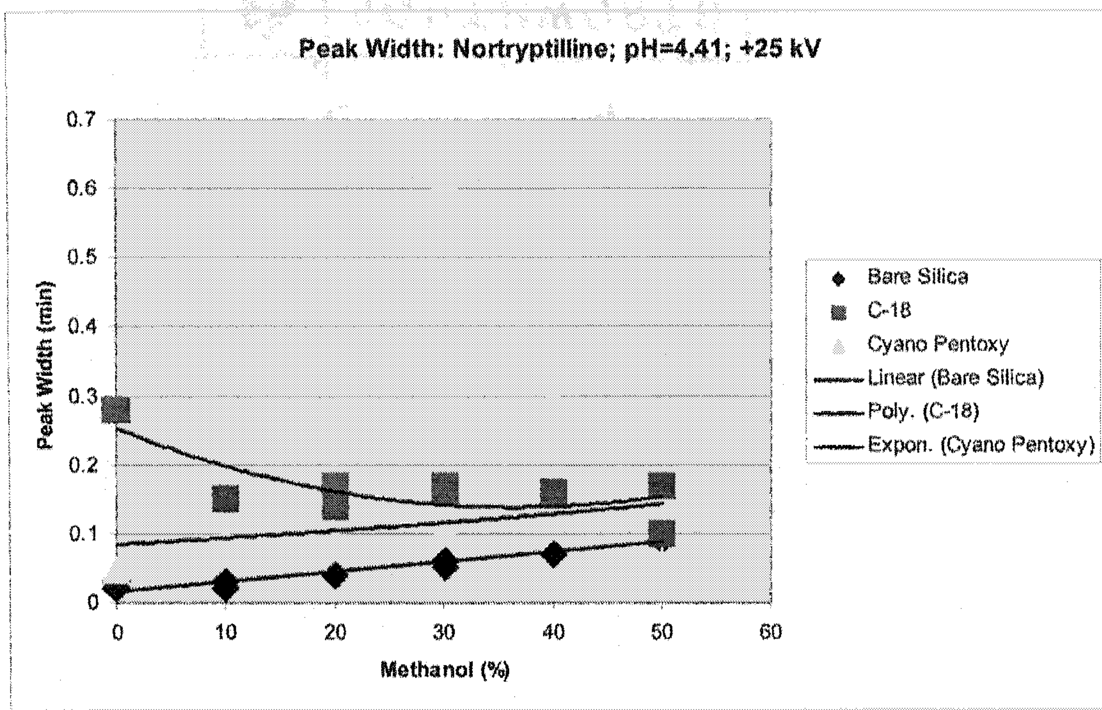


Figure 64. Capillary efficiency for nortriptylline vs methanol volume ratio for bare silica, C-18 modified, and cyano pentoxy modified capillaries at pH=7.06 and +25 kV.

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Appendix

Table 2. Theophylline Migration in Bare Silica Capillary (+25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0				
2.14	0				
2.14	10				
2.14	10				
2.14	20				
2.14	20				
2.14	30				
2.14	30				
2.14	40				
2.14	40				
2.14	50	183.6	39.23	0.4	5453.04
2.14	50	183.8	40.11	0.41	5568.18

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
3	0	4.32	24.98	0.18	67.25
3	0	4.35	24.9	0.19	67.32
3	10				
3	10				
3	20				
3	20				
3	30				
3	30				
3	40	4.11	40.03	0.13	33.85
3	40	4.07	39.87	0.1	33.62
3	50	61.48	30.7	0.22	1018.24
3	50	59.78	30.18	0.23	1015.27

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
4.41	0	248.61	9.9	0.16	2540.38
4.41	0	190.17	9.68	0.14	1655.25
4.41	10	111.21	11.66	0.12	934.29
4.41	10	84.86	10.91	0.11	599.09
4.41	20	37.17	16.48	0.16	377.16
4.41	20	32.9	15.39	0.13	282.8
4.41	30	37.6	23.55	0.2	640.52
4.41	30	38.25	23.01	0.19	618.75
4.41	40	47.31	26.38	0.24	860.97
4.41	40	45.5	25.44	0.24	821.14
4.41	50	61.82	37.46	0.27	1362.57
4.41	50	59.47	40.4	0.31	1403.62

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0	47.4	9.81	0.06	188.45
7.06	0	41.1	9.85	0.058	160.82
7.06	10	38.92	11.14	0.076	193.96
7.06	10	38.45	11.02	0.072	182.58
7.06	20	70.91	11.61	0.067	325.37
7.06	20	70.84	11.66	0.068	332.26
7.06	30				
7.06	30				
7.06	40	98.09	15.68	0.17	1266.78
7.06	40	95.36	15.38	0.16	1171.8
7.06	50				
7.06	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
8.14	0	75.25	8.1	0.14	846.48
8.14	0	85.1	7.6	0.14	2048.66
8.14	10	42.9	10.34	0.16	561.98
8.14	10	32.69	12.47	0.21	543.44
8.14	20	44.67	12.19	0.27	987.46
8.14	20	48.1	11.3	0.25	984.14
8.14	30	28.7	27.3	0.52	1292
8.14	30	41.69	19.45	0.98	3507
8.14	40	80.62	13.19	0.51	3354
8.14	40	80.15	12.28	0.43	2799
8.14	50				
8.14	50				

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Theophylline migration in Bare Silica capillary: L = 58.2 cm; l = 48.6 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 270 nm.

Table 3. Theophylline Migration in Bare Silica Capillary (-25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0	372.72	27.37	0.25	6777.24
2.14	0	334.18	29.02	0.23	6440.69
2.14	10				
2.14	10				
2.14	20				
2.14	20				
2.14	30				
2.14	30				
2.14	40				
2.14	40				
2.14	50				
2.14	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
3	0				
3	0				
3	10				
3	10				
3	20				
3	20				
3	30				
3	30				
3	40				
3	40				
3	50				
3	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
4.41	0				
4.41	0				
4.41	10				
4.41	10				
4.41	20				
4.41	20				
4.41	30				
4.41	30				
4.41	40				
4.41	40				
4.41	50				
4.41	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0				
7.06	0				
7.06	10				
7.06	10				
7.06	20				
7.06	20				
7.06	30				
7.06	30				
7.06	40				
7.06	40				
7.06	50				
7.06	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
8.14	0				
8.14	0				
8.14	10				
8.14	10				
8.14	20				
8.14	20				
8.14	30				
8.14	30				
8.14	40				
8.14	40				
8.14	50				
8.14	50				

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Theophylline migration in Bare Silica capillary: L = 58.2 cm; l = 48.6 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 270 nm.

Table 4. Theophylline Migration in C-18 Capillary (+25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0				
2.14	0				
2.14	10				
2.14	10				
2.14	20				
2.14	20				
2.14	30				
2.14	30				
2.14	40				
2.14	40				
2.14	50				
2.14	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
3	0				
3	0				
3	10				
3	10				
3	20				
3	20				
3	30				
3	30				
3	40				
3	40				
3	50				
3	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
4.41	0		NO peaks		
4.41	0				
4.41	10				
4.41	10				
4.41	20				
4.41	20				
4.41	30				
4.41	30				
4.41	40				
4.41	40				
4.41	50				
4.41	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0	291	8.17	0.16	3988
7.06	0	171	7.82	0.12	1345
7.06	10	171	9.09	0.27	3685
7.06	10	204	9.41	0.2	3176
7.06	20	208	16.36	0.39	6635
7.06	20	229	15.38	0.34	6345
7.06	30	415	13.24	0.43	12677
7.06	30	496	13.21	0.43	14915
7.06	40	7.1	19.3	0.53	323
7.06	40	4.2	20.16	0.53	190
7.06	50	1.94	27.57	0.21	35.77
7.06	50	2.19	28.08	0.35	66.6
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
8.14	0	48.23	7.09	0.22	779
8.14	0	52.04	6.16	0.14	599
8.14	10	41.36	8.67	0.22	747
8.14	10	43.3	8.74	0.24	861
8.14	20	41.1	10.54	0.31	1097
8.14	20	40.41	10.99	0.33	1141
8.14	30	65.98	10.034	0.38	2132
8.14	30	35	10.032	0.39	1148
8.14	40	3.05	10.26	0.08	19.74
8.14	40	2.66	10.96	0.1	22.5
8.14	50				
8.14	50				

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Theophylline migration in C-18 etch modified capillary: L = 50 cm; l = 41.5 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 270 nm.

Table 5. Theophylline Migration in C-18 Capillary (-25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0				
2.14	0				
2.14	10				
2.14	10				
2.14	20				
2.14	20				
2.14	30				
2.14	30				
2.14	40				
2.14	40				
2.14	50				
2.14	50				
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
3	0				
3	0				
3	10				
3	10				
3	20				
3	20				
3	30				
3	30				
3	40				
3	40				
3	50				
3	50				
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
4.41	0				
4.41	0				
4.41	10				
4.41	10				
4.41	20				
4.41	20				
4.41	30				
4.41	30				
4.41	40				
4.41	40				
4.41	50				
4.41	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0		No peak		
7.06	0				
7.06	10				
7.06	10				
7.06	20				
7.06	20				
7.06	30				
7.06	30				
7.06	40				
7.06	40				
7.06	50				
7.06	50				
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
8.14	0				
8.14	0				
8.14	10				
8.14	10				
8.14	20				
8.14	20				
8.14	30	8.06	4.69	0.05	32
8.14	30	7.84	6.19	0.11	67.25
8.14	40				
8.14	40				
8.14	50		2.36		
8.14	50		2.32		

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Theophylline migration in C-18 etch modified capillary: L = 50 cm; l = 41.5 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 270 nm.

Table 6. Theophylline Migration in Cyano Pentoxy Capillary (+25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0				
2.14	0				
2.14	10				
2.14	10				
2.14	20				
2.14	20				
2.14	30				
2.14	30				
2.14	40				
2.14	40				
2.14	50				
2.14	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
3	0	2.7	o.o59	0.032	7.02
3	0				
3	10				
3	10				
3	20				
3	20				
3	30				
3	30				
3	40				
3	40				
3	50				
3	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
4.41	0	367	13.48	0.52	15973
4.41	0	454	5.58	0.18	5456
4.41	10	345	8.13	0.15	3941
4.41	10	268	12.87	0.31	6077
4.41	20				
4.41	20				
4.41	30				
4.41	30				
4.41	40				
4.41	40				
4.41	50				
4.41	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0	83.51	1.73	0.52	3729
7.06	0	83.63	1.76	0.9	4551
7.06	10	35.23	3.52	0.13	321
7.06	10	41.02	3.44	0.1	319
7.06	20	15.16	3.9	0.1	157
7.06	20	18.16	3.94	0.11	163
7.06	30	65.98	4.12	0.12	678
7.06	30	60.99	4.55	0.13	653
7.06	40	25.11	7.36	0.47	1027
7.06	40	55.1	6.34	0.11	481
7.06	50	60	7.57	0.09	431
7.06	50	62.74	8.16	0.1	489
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
8.14	0				
8.14	0				
8.14	10	805.52	1.34	0.17	1.2
8.14	10				
8.14	20	11.99	2.78	0.22	230
8.14	20				
8.14	30				
8.14	30	3.13	34.93	0.028	5.37
8.14	40				
8.14	40				
8.14	50				
8.14	50				

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Theophylline migration in Cyano Pentoxy etch modified capillary: L = 33.4 cm; l = 24.9 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 270 nm.

Table 7. Theophylline Migration in Cyano Pentoxy Capillary (-25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0	11.96	7.36	0.11	86.15
2.14	0				
2.14	10	139	10.04	0.19	1984
2.14	10	130	10.06	0.19	1986
2.14	20	86	18.47	0.43	2896
2.14	20	48.56	18.46	0.39	1653
2.14	30	40.51	30.53	0.53	1859
2.14	30	55.94	34.48	0.86	4141
2.14	40				
2.14	40				
2.14	50				
2.14	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
3	0	201	36.27	0.36	6050
3	0	302	15.46	0.33	6882
3	10				
3	10				
3	20	375	5.82	0.17	4239
3	20	383	6.33	0.17	4555
3	30	368	7.38	0.2	4903
3	30	366	7.32	0.19	4556
3	40	326	15.62	0.42	9783
3	40	375	14.98	0.41	10130
3	50				
3	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
4.41	0				
4.41	0				
4.41	10	3.28	2.81	0.018	2.94
4.41	10	3.63	3.05	0.02	3.82
4.41	20	3.03	3.46	0.013	2.94
4.41	20	3.36	3.13	0.018	2.79
4.41	30				
4.41	30				
4.41	40	322	4.84	0.16	3332
4.41	40	327	4.89	0.16	3571
4.41	50	465	12.75	0.21	6237
4.41	50	469	12.52	0.21	6567

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0				
7.06	0				
7.06	10				
7.06	10				
7.06	20				
7.06	20				
7.06	30				
7.06	30				
7.06	40				
7.06	40				
7.06	50				
7.06	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
8.14	0				
8.14	0				
8.14	10	7.58	1.59	0.032	19.33
8.14	10				
8.14	20				
8.14	20				
8.14	30				
8.14	30				
8.14	40				
8.14	40				
8.14	50				
8.14	50				

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Theophylline migration in Cyano Pentoxy etch modified capillary: L = 33.4 cm; l = 24.9 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 270 nm.

Table 8. Dihyroxy-theophylline Migration in Bare Silica Capillary (+25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0	174.88	30.88	0.31	3751
2.14	0	192.7	29.77	0.307	4029
2.14	10				
2.14	10				
2.14	20				
2.14	20				
2.14	30				
2.14	30				
2.14	40	194.66	39.56	0.37	6013.55
2.14	40	192.46	39.54	0.36	6012.77
2.14	50	491.33	42.62	0.55	2.32
2.14	50	461.61	40.15	0.54	1.91
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
3	0	38.74	21.81	0.16	430.98
3	0	29.88	21.16	0.16	321.53
3	10				
3	10				
3	20				
3	20				
3	30				
3	30				
3	40				
3	40				
3	50	58.02	31.75	0.24	1019.9
3	50	55.42	30.95	0.23	907.3
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
4.41	0	103	9.5	0.13	867
4.41	0	97.841	9.46	0.13	739
4.41	10				
4.41	10				
4.41	20	16.78	18.61	0.15	186
4.41	20	20.95	18.14	0.17	234
4.41	30	24.69	24.38	0.21	439
4.41	30	24.75	24.27	0.2	409
4.41	40	34.71	30.08	0.27	801
4.41	40	33.38	30.2	0.27	772
4.41	50	52.25	34.13	0.26	1098
4.41	50	49.97	34.84	0.21	889

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0	16.49	9.33	0.048	52.03
7.06	0				
7.06	10				
7.06	10				
7.06	20	29.76	11.49	0.09	203
7.06	20	29.35	11.5	0.08	202
7.06	30				
7.06	30				
7.06	40	62.37	15.06	0.11	484
7.06	40	61.62	14.99	0.109	474
7.06	50	92.19	19.8	0.24	1677
7.06	50	92.19	19.81	0.23	1676
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
8.14	0	46.57	6.9	0.08	245
8.14	0	46.64	7	0.074	239
8.14	10				
8.14	10				
8.14	20	52.43	7.73	0.07	259
8.14	20	61.38	7.99	0.05	266
8.14	30	60.83	8.99	0.07	321
8.14	30	59.62	8.81	0.08	343
8.14	40	52.49	16.02	0.25	922
8.14	40	41.34	18.02	0.24	820
8.14	50				
8.14	50				

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Dihydroxy-Theophylline migration in Bare Silica capillary: L = 58.2 cm; l = 48.6 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 270 nm.

Table 9. Dihyroxy-theophylline Migration in Bare Silica Capillary (-25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0				
2.14	0				
2.14	10				
2.14	10				
2.14	20				
2.14	20				
2.14	30				
2.14	30				
2.14	40				
2.14	40				
2.14	50				
2.14	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
3	0				
3	0				
3	10				
3	10				
3	20				
3	20				
3	30				
3	30				
3	40				
3	40				
3	50				
3	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
4.41	0				
4.41	0				
4.41	10				
4.41	10				
4.41	20				
4.41	20				
4.41	30				
4.41	30				
4.41	40				
4.41	40				
4.41	50				
4.41	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0				
7.06	0				
7.06	10				
7.06	10				
7.06	20				
7.06	20				
7.06	30				
7.06	30				
7.06	40				
7.06	40				
7.06	50				
7.06	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
8.14	0				
8.14	0				
8.14	10				
8.14	10				
8.14	20				
8.14	20				
8.14	30				
8.14	30				
8.14	40				
8.14	40				
8.14	50				
8.14	50				

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Dihydroxy-Theophylline migration in Bare Silica capillary: L = 58.2 cm; l = 48.6 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 270 nm.

Table 10. Dihyroxy-theophylline Migration in C-18 Capillary (+25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0				
2.14	0				
2.14	10				
2.14	10				
2.14	20				
2.14	20				
2.14	30				
2.14	30				
2.14	40				
2.14	40				
2.14	50				
2.14	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
3	0	19.57	33.56	0.27	446
3	0	21.17	38.31	0.36	662
3	10				
3	10				
3	20				
3	20				
3	30				
3	30				
3	40				
3	40				
3	50				
3	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
4.41	0				
4.41	0				
4.41	10				
4.41	10				
4.41	20				
4.41	20				
4.41	30		8.65 @ 254nm		
4.41	30		8.92 @ 254nm		
4.41	40				
4.41	40				
4.41	50				
4.41	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0	83.95	7.54	0.11	628
7.06	0	91.06	7.69	0.11	709
7.06	10				
7.06	10				
7.06	20	102	14.35	0.27	2082
7.06	20	107	10.86	0.28	2099
7.06	30	140	11.84	0.33	3473
7.06	30	151	12.47	0.35	3891
7.06	40	474	34.47	0.4	12154
7.06	40	25.44	16.24	0.57	1247
7.06	50	2.2	27.93	0.33	65.07
7.06	50	1.89	28.35	0.33	53.67
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
8.14	0	60.13	5.89	0.14	615
8.14	0	80.28	5.84	0.14	799
8.14	10				
8.14	10				
8.14	20	89.27	8.46	0.17	1125
8.14	20	89.78	8.59	0.17	1165
8.14	30	96.89	8.27	0.22	1527
8.14	30	97.97	8.4	0.23	1602
8.14	40	230	11.97	0.19	2827
8.14	40	287.2	10.61	0.17	4087
8.14	50	22.09	13.26	0.46	832
8.14	50	11.14	13.27	0.41	391

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Dihydroxy-Theophylline migration in C-18 etch modified capillary: L = 50 cm; l = 41.5 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 270 nm.

Table 11. Dihyroxy-theophylline Migration in C-18 Capillary (-25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0				
2.14	0				
2.14	10				
2.14	10				
2.14	20				
2.14	20				
2.14	30				
2.14	30				
2.14	40				
2.14	40				
2.14	50				
2.14	50				
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
3	0				
3	0				
3	10				
3	10				
3	20				
3	20				
3	30				
3	30				
3	40				
3	40				
3	50				
3	50				
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
4.41	0				
4.41	0				
4.41	10				
4.41	10				
4.41	20				
4.41	20				
4.41	30				
4.41	30				
4.41	40				
4.41	40				
4.41	50				
4.41	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0				
7.06	0				
7.06	10				
7.06	10				
7.06	20				
7.06	20				
7.06	30				
7.06	30				
7.06	40				
7.06	40				
7.06	50				
7.06	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
8.14	0				
8.14	0				
8.14	10				
8.14	10				
8.14	20				
8.14	20				
8.14	30	2.2	8.65	0.15	28.17
8.14	30				
8.14	40				
8.14	40				
8.14	50	2.44	2.37	0.086	15.61
8.14	50	2.01	2.25	0.075	10.96

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Dihydroxy-Theophylline migration in C-18 etch modified capillary: L = 50 cm; l = 41.5 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 270 nm.

Table 12. Dihyroxy-theophylline Migration in Cyano Pentoxy Capillary (+25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0				
2.14	0				
2.14	10				
2.14	10				
2.14	20				
2.14	20				
2.14	30				
2.14	30				
2.14	40				
2.14	40				
2.14	50				
2.14	50				1
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
3	0	46.73	12.46	0.67	2711
3	0	26.19	12.51	0.35	781
3	10				
3	10				
3	20				
3	20				
3	30				
3	30				
3	40				
3	40				
3	50				
3	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
4.41	0				
4.41	0				
4.41	10				
4.41	10				
4.41	20				
4.41	20				
4.41	30				
4.41	30				
4.41	40				
4.41	40				
4.41	50				
4.41	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0	57.46	1.17	0.3	1495
7.06	0	196	0.78	0.16	2684
7.06	10				
7.06	10				
7.06	20	24.22	3.25	0.12	221
7.06	20	21.09	3.34	0.11	170
7.06	30		4.32		
7.06	30	37.15	4.76	0.18	551
7.06	40	54.16	4.89	0.18	772
7.06	40	34.83	5.39	0.11	287
7.06	50	52.96	6.99	0.19	681
7.06	50	17.16	8.63	0.17	226
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
8.14	0	7.3	1.86	0.18	112
8.14	0				
8.14	10				
8.14	10				
8.14	20	369	15.42	0.18	5706
8.14	20	369	15.46	0.11	3461
8.14	30				
8.14	30				
8.14	40				
8.14	40	168	37.01	0.18	2391
8.14	50				
8.14	50				

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Dihyroxy-Theophylline migration in Cyano Pentoxy etch modified capillary: L = 33.4 cm; l = 24.9 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 270 nm.

Table 13. Dihyroxy-theophylline Migration in Cyano Pentoxy Capillary (-25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0	169	7.19	0.14	1603
2.14	0	11.96	7.36	0.11	86.15
2.14	10	116	9.74	0.24	1711
2.14	10	112	10.47	0.26	1828
2.14	20	84.34	18.69	0.47	3349
2.14	20	76.36	20.9	0.58	3811
2.14	30	35.16	27.81	0.61	18.52
2.14	30				
2.14	40				
2.14	40				
2.14	50	18.58	38.12	0.25	69.84
2.14	50	5.7	39.1	0.8	10.26
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
3	0	102.97	11.16	0.25	2038
3	0	111	10.19	0.24	2025
3	10	114	3.58	0.09	660
3	10		3.76		
3	20	108	7.63	0.18	1372
3	20	107	8.45	0.19	1480
3	30	85.5	7.94	0.18	1063
3	30	105	7.82	0.17	1267
3	40	88.66	14.39	0.33	2526
3	40	86.11	15.3	0.38	2686
3	50				
3	50				
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
4.41	0	111	3.24	0.12	875
4.41	0	107	3.95	0.14	1015
4.41	10				
4.41	10				
4.41	20				
4.41	20				
4.41	30				
4.41	30				
4.41	40	106	4.3	0.12	820
4.41	40	105	4.07	0.13	925
4.41	50	106	18.84	0.27	2082
4.41	50	100	16.97	0.24	1756

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0	283	0.19	o.23	5484
7.06	0	124	0.58	0.04	428
7.06	10	5.39	0.059	0.02	6.11
7.06	10				
7.06	20				
7.06	20				
7.06	30				
7.06	30				
7.06	40				
7.06	40				
7.06	50				
7.06	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
8.14	0				
8.14	0				
8.14	10				
8.14	10				
8.14	20				
8.14	20				
8.14	30				
8.14	30				
8.14	40				
8.14	40				
8.14	50				
8.14	50				

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Dihyroxy-Theophylline migration in Cyano Pentoxy etch modified capillary: L = 33.4 cm; l = 24.9 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 270 nm.

Table 14. Aminophylline Migration in Bare Silica Capillary (+25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0				
2.14	0				
2.14	10				
2.14	10				
2.14	20				
2.14	20				
2.14	30				
2.14	30				
2.14	40	312	37.69	0.5	1.25
2.14	40	289	36.98	0.5	1.07
2.14	50	567	44.95	0.41	1.98
2.14	50	565	45	0.42	1.98
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
3	0	50.05	21.24	0.16	574
3	0	51.62	21.3	0.17	635
3	10				
3	10				
3	20				
3	20				
3	30				
3	30				
3	40				
3	40				
3	50	112	32.4	0.28	2213
3	50	124	31.57	0.28	2483
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
4.41	0	162.65	9.43	0.12	1197
4.41	0	162.65	9.43	0.12	1197
4.41	10		check		
4.41	10		check		
4.41	20	31.22	19.58	0.17	391
4.41	20	30.31	19.3	0.18	358
4.41	30	39.54	25.72	0.23	789
4.41	30	37.71	23.9	0.21	611
4.41	40	94.21	32.19	0.31	2301
4.41	40	89.9	31.46	0.31	2128
4.41	50				
4.41	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0	50.83	9.5	0.06	203
7.06	0	60.09	9.51	0.06	247
7.06	10	42.14	11.04	0.07	210
7.06	10	46.28	11	0.07	230
7.06	20	67.74	11.96	0.07	333
7.06	20	67.87	11.87	0.07	327
7.06	30				
7.06	30				
7.06	40	69.27	15.43	0.16	802
7.06	40	69.08	15.34	0.15	787
7.06	50	79.6	20.43	0.38	2267
7.06	50	76.25	20.25	0.29	1907
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
8.14	0	84.61	8.44	0.18	1186
8.14	0	48.88	7.93	0.16	606
8.14	10	42.3	13.86	0.3	1024
8.14	10	54.05	9.91	0.18	778
8.14	20	58.82	10.8	0.29	1405
8.14	20	59.99	10.59	0.28	1349
8.14	30				
8.14	30				
8.14	40				
8.14	40				
8.14	50				
8.14	50				

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Aminophylline migration in Bare Silica capillary: L = 58.2 cm; l = 48.6 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 270 nm.

Table 15. Aminophylline Migration in Bare Silica Capillary (-25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0	189	35.5	0.35	4842
2.14	0	182	37.08	0.35	4883
2.14	10				
2.14	10				
2.14	20				
2.14	20				
2.14	30				
2.14	30				
2.14	40				
2.14	40				
2.14	50				
2.14	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
3	0				
3	0				
3	10				
3	10				
3	20				
3	20				
3	30				
3	30				
3	40				
3	40				
3	50				
3	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
4.41	0				
4.41	0				
4.41	10				
4.41	10				
4.41	20				
4.41	20				
4.41	30				
4.41	30				
4.41	40				
4.41	40				
4.41	50				
4.41	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0				
7.06	0				
7.06	10				
7.06	10				
7.06	20				
7.06	20				
7.06	30				
7.06	30				
7.06	40				
7.06	40				
7.06	50				
7.06	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
8.14	0	5.06	16.37	0.18	77.72
8.14	0	2.44	12.6	0.1	20.6
8.14	10				
8.14	10				
8.14	20				
8.14	20				
8.14	30	3.6	8.14	0.18	54.48
8.14	30	2.2	9.05	0.13	24.55
8.14	40				
8.14	40				
8.14	50				
8.14	50				

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Aminophylline migration in Bare Silica capillary: L = 58.2 cm; l = 48.6 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 270 nm.

Table 16. Aminophylline Migration in C-18 Capillary (+25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0				
2.14	0				
2.14	10				
2.14	10				
2.14	20				
2.14	20				
2.14	30				
2.14	30				
2.14	40				
2.14	40				
2.14	50				
2.14	50				
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
3	0	35.44	39.73	0.33	1003
3	0				
3	10				
3	10				
3	20				
3	20				
3	30				
3	30				
3	40				
3	40				
3	50				
3	50				
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
4.41	0				
4.41	0				
4.41	10				
4.41	10				
4.41	20				
4.41	20				
4.41	30	2.99	8.97	0.09	22.03
4.41	30	2.73	9.02	0.09	20.48
4.41	40				
4.41	40				
4.41	50		band broadening		
4.41	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0	114	7.91	0.13	1129
7.06	0	118	7.97	0.13	1187
7.06	10	132	9.09	0.19	1932
7.06	10	120	8.81	0.26	2498
7.06	20	145	11.28	0.2	2189
7.06	20	78.71	11.63	0.2	1179
7.06	30	315	13.07	0.4	9062
7.06	30	322	13.84	0.42	9810
7.06	40	3.18	16.48	0.14	38.12
7.06	40		16.72		
7.06	50				
7.06	50				
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
8.14	0	31.96	6.99	0.15	388
8.14	0	39.17	6.48	0.13	420
8.14	10	22.24	11.39	0.27	490
8.14	10	18.82	17.36	0.52	835
8.14	20	32.15	10.56	0.27	736
8.14	20	32.49	10.44	0.26	717
8.14	30	40.16	11.46	0.39	1316
8.14	30	41.68	11.42	0.39	1365
8.14	40	64.39	12.76	0.64	3488
8.14	40	72.13	12.03	0.6	3671
8.14	50	2.43	11.2	0.08	15.2
8.14	50		11.14		

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Aminophylline migration in C-18 etch modified capillary: L = 50 cm; l = 41.5 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 270 nm.

Table 17. Aminophylline Migration in C-18 Capillary (-25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0				
2.14	0				
2.14	10				
2.14	10				
2.14	20				
2.14	20				
2.14	30				
2.14	30				
2.14	40				
2.14	40				
2.14	50				
2.14	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
3	0				
3	0				
3	10				
3	10				
3	20				
3	20				
3	30				
3	30				
3	40				
3	40				
3	50				
3	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
4.41	0				
4.41	0				
4.41	10				
4.41	10				
4.41	20				
4.41	20				
4.41	30				
4.41	30				
4.41	40	2.43	13.88	0.013	2.12
4.41	40	3.35	14.36	0.007	1.57
4.41	50				
4.41	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0				
7.06	0				
7.06	10				
7.06	10				
7.06	20				
7.06	20				
7.06	30				
7.06	30				
7.06	40				
7.06	40				
7.06	50				
7.06	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
8.14	0		4.45		
8.14	0		4.13		
8.14	10				
8.14	10				
8.14	20				
8.14	20				
8.14	30				
8.14	30				
8.14	40				
8.14	40				
8.14	50		2.48		
8.14	50		2.49		

Notes:

3. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
4. Experimental conditions for Aminophylline migration in C-18 etch modified capillary: L = 50 cm; l = 41.5 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 270 nm.

Table 18. Aminophylline Migration in Cyano Pentoxy Capillary (+25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0	s			
2.14	0				
2.14	10				
2.14	10				
2.14	20				
2.14	20				
2.14	30				
2.14	30				
2.14	40				
2.14	40				
2.14	50	4.97	3.42	0.044	18.11
2.14	50	4.5	3.78	0.036	11.94
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
3	0	153	14.46	0.81	1.06
3	0	135	17.73	1.23	1.42
3	10				
3	10				
3	20				
3	20				
3	30				
3	30				
3	40				
3	40				
3	50	15.84	33.02	0.13	166
3	50	9.59	18.64	0.14	113
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
4.41	0	226	3.98	0.14	2103
4.41	0	205	4.03	0.15	1972
4.41	10				
4.41	10				
4.41	20				
4.41	20				
4.41	30				
4.41	30				
4.41	40				
4.41	40				
4.41	50				
4.41	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0				
7.06	0				
7.06	10	32.08	3.39	0.08	179
7.06	10	46.02	3.28	0.09	332
7.06	20	21.2	3.59	0.07	115
7.06	20	45.81	3.65	0.05	182
7.06	30	44.6	4.3	0.14	473
7.06	30	45.21	4.55	0.16	597
7.06	40	98.82	6.61	0.17	1314
7.06	40	83.02	5.48	0.15	1044
7.06	50	85.83	8.83	0.12	755
7.06	50				
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
8.14	0	30.15	0.08	0.09	237
8.14	0				
8.14	10	839	6.29	0.04	2930
8.14	10	5.61	7.44	0.18	85.04
8.14	20				
8.14	20				
8.14	30				
8.14	30				
8.14	40				
8.14	40				
8.14	50				
8.14	50				

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Aminophylline migration in Cyano Pentoxy etch modified capillary: L = 33.4 cm; l = 24.9 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 270 nm.

Table 19. Aminophylline Migration in Cyano Pentoxy Capillary (-25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0	128	8.22	0.17	1374
2.14	0	121	8.15	0.17	1323
2.14	10	90.1	11.5	0.21	1457
2.14	10	89.52	11.42	0.21	1469
2.14	20	59	17.95	0.41	1299
2.14	20	64	17.94	0.41	2228
2.14	30	2.06	34.91	0.2	36
2.14	30				
2.14	40				
2.14	40				
2.14	50				
2.14	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
3	0	362	7.66	0.25	5897
3	0	345	8.93	0.27	6255
3	10	437	3.78	0.1	2753
3	10	445	3.85	0.1	2804
3	20	455	5.93	0.18	5380
3	20	451	5.57	0.17	5145
3	30				
3	30				
3	40				
3	40				
3	50				
3	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
4.41	0	1.99	2.72	0.03	4.09
4.41	0	1.9	2.99	0.01	1.64
4.41	10				
4.41	10				
4.41	20				
4.41	20				
4.41	30				
4.41	30	3.19	5.85	0.02	6.46
4.41	40	366	4.17	0.12	2955
4.41	40	417	4.14	0.15	4082
4.41	50	179	27.09	0.31	4089
4.41	50	89	29.05	0.29	34093

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0	148	0.52	0.16	1969
7.06	0	184	0.6	0.13	2058
7.06	10	4.55	0.09	0.03	11.7
7.06	10	4.8	0.092	0.03	11.2
7.06	20				
7.06	20				
7.06	30				
7.06	30				
7.06	40				
7.06	40				
7.06	50				
7.06	50				
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
8.14	0				
8.14	0				
8.14	10				
8.14	10				
8.14	20				
8.14	20				
8.14	30				
8.14	30				
8.14	40				
8.14	40				
8.14	50				
8.14	50				

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Aminophylline migration in Cyano Pentoxy etch modified capillary: L = 33.4 cm; l = 24.9 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 270 nm.

Table 20. Nortriptylline Migration in Bare Silica Capillary (+25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0	30.52	13.02	0.24	622
2.14	0	28.45	13.14	0.23	554
2.14	10	32.44	11.28	0.21	580
2.14	10	28.62	11.25	0.18	450
2.14	20	21.27	10.4	0.12	211
2.14	20	21.72	10.43	0.11	211
2.14	30	26.99	9.48	0.15	321
2.14	30	27.99	9.41	0.14	318
2.14	40				
2.14	40				
2.14	50				
2.14	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
3	0	21.91	6.91	0.07	141
3	0	23.44	6.8	0,08	165
3	10	19	11.37	0.14	209
3	10	19.32	10.96	0.12	196
3	20	15.74	11.49	0.17	223
3	20	15.93	11.36	0.16	215
3	30	18.8	10.57	0.15	237
3	30	18.8	10.71	0.16	251
3	40	22.2	10.67	0.18	348
3	40	22.16	10.52	0.18	329
3	50	22	10.73	0.26	482
3	50	22.76	10.83	0.25	486
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
4.41	0	4.98	4.79	0.02	12.9
4.41	0	5.07	4.88	0.03	12.94
4.41	10	24.97	5.72	0.03	47.01
4.41	10	25.5	5.62	0.02	46.31
4.41	20	25.93	7.8	0.04	76.86
4.41	20	27.15	7.76	0.04	76.8
4.41	30	30.33	8.54	0.05	112
4.41	30	30.42	8.47	0.06	108
4.41	40	38.71	9.36	0.07	175
4.41	40	38.48	9.38	0.07	170
4.41	50	43.03	10.95	0.09	343
4.41	50	44.02	10.97	0.1	366

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0				
7.06	0				
7.06	10				
7.06	10				
7.06	20				
7.06	20				
7.06	30				
7.06	30				
7.06	40				
7.06	40				
7.06	50				
7.06	50				
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
8.14	0	6.4	4.13	0.17	94
8.14	0	6.2	4.15	0.17	84
8.14	10	6.5	4.83	0.21	117
8.14	10	5.6	5.85	0.2	91.51
8.14	20	5.7	5.13	0.34	170
8.14	20	5.7	5.16	0.33	115
8.14	30				
8.14	30				
8.14	40				
8.14	40				
8.14	50				
8.14	50				

Notes:

3. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
4. Experimental conditions for Nortriptylline migration in Bare Silica capillary: L = 58.2 cm; l = 48.6 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 254 nm.

Table 21. Nortriptylline Migration in Bare Silica Capillary (-25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0				
2.14	0				
2.14	10				
2.14	10				
2.14	20				
2.14	20				
2.14	30				
2.14	30				
2.14	40				
2.14	40				
2.14	50				
2.14	50				
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
3	0				
3	0				
3	10				
3	10				
3	20				
3	20				
3	30				
3	30				
3	40				
3	40				
3	50				
3	50				
pH	**Methanol (%)**	**Peak Height (mAU)**	**Migration Time (min)**	**Width (min)**	**Area (mAU.sec)**
4.41	0				
4.41	0				
4.41	10				
4.41	10				
4.41	20				
4.41	20				
4.41	30				
4.41	30				
4.41	40				
4.41	40				
4.41	50				
4.41	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0				
7.06	0				
7.06	10				
7.06	10				
7.06	20				
7.06	20				
7.06	30				
7.06	30				
7.06	40				
7.06	40				
7.06	50				
7.06	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
8.14	0	3.29	13.54	0.12	32.88
8.14	0	4.45	13.82	0.05	19.49
8.14	10	1.87	13.79	0.03	24.92
8.14	10	3.38	11.76	0.1	29.47
8.14	20				
8.14	20				
8.14	30	3.43	7.74	0.16	46.59
8.14	30	3.57	7.59	0.12	35.67
8.14	40				
8.14	40				
8.14	50				
8.14	50				

Notes:

5. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
6. Experimental conditions for Nortriptylline migration in Bare Silica capillary: L = 58.2 cm; l = 48.6 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 254 nm.

Table 22. Nortriptylline Migration in C-18 Capillary (+25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0				
2.14	0				
2.14	10				
2.14	10				
2.14	20				
2.14	20				
2.14	30				
2.14	30				
2.14	40				
2.14	40				
2.14	50				
2.14	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
3	0	5.09	6.36	0.13	56.24
3	0	5.2	6.47	0.18	77.61
3	10	4.82	11.38	0.21	83.92
3	10	4.5	11.46	0.26	130
3	20	6.85	9.85	0.19	106
3	20	7.12	9.86	0.18	109
3	30	7.5	10.83	0.22	139
3	30	7.44	10.85	0.21	129
3	40				
3	40				
3	50	13.77	12.52	0.18	210
3	50	14.06	12.51	0.19	220
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
4.41	0	3.92	6.97	0.28	94.58
4.41	0	3.96	6.99	0.28	96.45
4.41	10	6.28	9.66	0.15	77.81
4.41	10	7.61	9.55	0.15	92.45
4.41	20	2.58	10.97	0.14	32.03
4.41	20	10.05	10.89	0.17	135
4.41	30	14.98	11.65	0.17	193
4.41	30	15.55	11.61	0.16	190
4.41	40	17.79	14.11	0.16	242
4.41	40	17.0-8	14.03	0.16	227
4.41	50	32.06	14.27	0.17	454
4.41	50	5.59	13.07	0.1	43.25

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0	193	8.02	0.14	1867
7.06	0	291	7.95	0.16	3385
7.06	10				
7.06	10				
7.06	20				
7.06	20				
7.06	30	2.87	13.88	0.33	82.87
7.06	30				
7.06	40				
7.06	40				
7.06	50	7.27	30.54	0.55	342
7.06	50	5.01	29.53	0.32	138
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
8.14	0				
8.14	0				
8.14	10				
8.14	10				
8.14	20		6.99		
8.14	20	3.94	7.06	0.035	11.37
8.14	30	2.74	10.03	0.03	4.95
8.14	30	1.71	11	0.06	8.24
8.14	40	22.09	10.79	0.46	832
8.14	40	2.2	14.57	0.13	24
8.14	50				
8.14	50				

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Nortriptylline migration in C-18 etch modified capillary: L = 50 cm; l = 41.5 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 254 nm.

Table 23. Nortriptylline Migration in C-18 Capillary (-25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0				
2.14	0				
2.14	10				
2.14	10				
2.14	20				
2.14	20				
2.14	30				
2.14	30				
2.14	40				
2.14	40				
2.14	50				
2.14	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
3	0				
3	0				
3	10				
3	10				
3	20				
3	20				
3	30				
3	30				
3	40				
3	40				
3	50				
3	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
4.41	0				
4.41	0				
4.41	10				
4.41	10				
4.41	20				
4.41	20				
4.41	30				
4.41	30				
4.41	40				
4.41	40				
4.41	50				
4.41	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0				
7.06	0				
7.06	10				
7.06	10				
7.06	20				
7.06	20				
7.06	30				
7.06	30				
7.06	40				
7.06	40				
7.06	50				
7.06	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
8.14	0				
8.14	0				
8.14	10				
8.14	10				
8.14	20	2.58	9.32	0.09	19.81
8.14	20	2.56	10.34	0.085	17.22
8.14	30	2.13	25.67	0.47	83.74
8.14	30		26.09		
8.14	40				
8.14	40				
8.14	50				
8.14	50				

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Nortriptylline migration in C-18 etch modified capillary: L = 50 cm; l = 41.5 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 254 nm.

Table 24. Nortriptylline Migration in Cyano Pentoxy Capillary (+25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0	3.08	3.11	0.05	11.93
2.14	0	2.06	2.94	0.04	5.86
2.14	10	32.05	3.62	0.08	214
2.14	10	33.82	3.48	0.08	213
2.14	20	3.95	4.03	0.05	15.06
2.14	20	3.61	3.92	0.015	13.61
2.14	30	7.63	5.74	0.11	65.84
2.14	30				
2.14	40	2.28	5.01	0.092	16.22
2.14	40				
2.14	50	11.85	7.08	0.24	235
2.14	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
3	0	59.9	1.65	0.08	370
3	0	57.02	1.67	0.08	355
3	10	29.7	5.23	0.38	920
3	10	29.24	5.18	0.37	858
3	20	29.88	4.47	0.15	362
3	20	28.81	4.63	0.25	585
3	30	22.68	3.8	0.1	169
3	30	25.26	3.77	0.2	410
3	40	65.21	3.97	0.09	490
3	40				
3	50	5.06	9.49	0.06	21.61
3	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
4.41	0	87.67	1.45	0.046	268
4.41	0				
4.41	10	41.58	2.87	0.08	231
4.41	10	61.97	2.9	0.09	455
4.41	20	29.47	3.1	0.09	225
4.41	20	47.07	2.85	0.08	314
4.41	30	41.31	4	0.62	2222
4.41	30	44.05	4.02	0.11	408
4.41	40	1.88	14.42	0.3	49.05
4.41	40	3.98	13.98	0.3	67
4.41	50	17.61	4.23	0.06	88.07
4.41	50	35.42	4.22	0.04	116

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0	169	0.76	0.1	1442
7.06	0	129	0.13	0.071	705
7.06	10	3.44	2.52	0.074	20.09
7.06	10	2.76	2.61	0.08	18.31
7.06	20				
7.06	20				
7.06	30				
7.06	30				
7.06	40				
7.06	40				
7.06	50				
7.06	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
8.14	0	549	3.67	0.056	2434
8.14	0				
8.14	10				
8.14	10				
8.14	20				
8.14	20				
8.14	30	10.68	10.57	0.16	137
8.14	30				
8.14	40				
8.14	40				
8.14	50				
8.14	50				

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Nortriptylline migration in Cyano Pentoxy etch modified capillary: L = 33.4 cm; l = 24.9 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 254 nm.

Table 25. Nortriptylline Migration in Cyano Pentoxy Capillary (-25 kV)

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
2.14	0				
2.14	0				
2.14	10	4.46	12.78	0.28	106
2.14	10	2.53	14.21	0.18	39.04
2.14	20				
2.14	20				
2.14	30				
2.14	30				
2.14	40				
2.14	40				
2.14	50	1.83	22.5	0.17	25.78
2.14	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
3	0				
3	0				
3	10	5.56	4.11	0.1	45.33
3	10	5.52	4.14	0.1	44.98
3	20	6.84	5.19	0.14	72.45
3	20	6.94	5.21	0.14	79.01
3	30	6.57	9.25	0.11	62.83
3	30	8.61	8.7	0.21	152
3	40	6.4	18.43	0.4	231
3	40	9.53	17.72	0.38	310
3	50				
3	50				
pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
4.41	0	138	23.03	0.2	2266
4.41	0				
4.41	10				
4.41	10				
4.41	20				
4.41	20				
4.41	30				
4.41	30				
4.41	40				
4.41	40				
4.41	50	7.23	39	0.21	188
4.41	50	8.33	38.23	0.29	204

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
7.06	0	20.55	1.093	0.3	530
7.06	0				
7.06	10				
7.06	10				
7.06	20				
7.06	20				
7.06	30				
7.06	30				
7.06	40				
7.06	40				
7.06	50		3.89		
7.06	50				

pH	Methanol (%)	Peak Height (mAU)	Migration Time (min)	Width (min)	Area (mAU.sec)
8.14	0	19.4	6.89	0.03	52.03
8.14	0	392	38.61	0.06	19.47
8.14	10	1.76	1.86	0.06	9.66
8.14	10				
8.14	20				
8.14	20				
8.14	30				
8.14	30				
8.14	40				
8.14	40				
8.14	50				
8.14	50				

Notes:

1. The blank table positions without any data represent CEC measurements without any observable migration peaks (all of those conditions had stable and finite electrical current flows).
2. Experimental conditions for Nortriptylline migration in Cyano Pentoxy etch modified capillary: L = 33.4 cm; l = 24.9 cm; injection 3 sec at 50 mbar; i.d. = 50 μm; detection wavelength = 254 nm.

Table 26. Summary descriptions of the effects and ranges of the experimental parameters in this study.

Parameters	Description	This Study
Applied Voltage	10-30 kV; higher V's → higher EOF and better resolution, but more Joule heating	**±25 kV**
Capillary Temperature	Typically room T; higher T's → Joule heating & band broadening	**Controlled Room T (20°C)**
Capillary Length and Inner Diameter	Larger i.d. → higher detection sensitivity, but higher Joule heating & band broadening	**i.d. = 50 μm** **L = 33.4 – 58.2 cm**
Buffer pH	Lower pH → decreased EOF Higher pH → increased EOF	**pH = 2.14 – 8.14**
Organic Modifier	Organic modifier to change zeta potential and EOF (methanol modifier decreases EOF)	**Methanol** **0% to 50% v/v**

Table 27. The primary experimental variables and their values.

Parameter	Attributes / Values
Capillary Type	Bare Silica, C-18 Modified, Cyano-Pentoxy Modified
Applied Voltage	+25 kV and -25 kV
Methanol Modifier	0%, 10%, 20%, 30%, 40%, 50%
Buffer pH	2.14, 3.00, 4.41, 7.06, 8.14

Table 28. The overall preferred experimental conditions for various compounds evaluated in this study (the pH values for obtaining the greatest numbers of successful measurements are shown).

Capillaries → Compounds ▼	Bare Silica	C-18	Cyano Pentoxy
Theophylline (greatest # of peaks for pH=7.06)	pH = 4.41, 7.06, 8.14, V=+25 kV; superior peak w<C-18 & cyano	pH = 7.06, 8.14, V=+25 kV; peak w > cyano; some tailing	pH = 7.06, V=+25 kV; peak w < C-18; better electromigration
Dyphylline (greatest # of peaks for pH=7.06)	pH = 4.41, 7.06, 8.14, V=+25 kV; superior peak w<C-18 & cyano	pH = 7.06, 8.14, V=+25 kV; peak w > cyano; some tailing	pH = 7.06, V=+25 kV; peak w < C-18; better electromigration
Aminophylline (greatest # of peaks for pH=7.06)	pH = 4.41, 7.06, V=+25 kV; superior peak w<C-18 & cyano	pH = 7.06, 8.14, V=+25 kV; peak w > cyano; some tailing	pH = 7.06, V=+25 kV; peak w < C-18; better electromigration
Nortriptylline (greatest # of peaks for pH=4.41)	pH = 2.14, 3.00, 4.41, V=+25 kV; superior peak w<C-18 & cyano	pH = 3.00, 4.41, V=+25 kV; peak w > cyano; some tailing	pH = 2.14, 3.00, 4.41, V=+25 kV; better peak w & electromigration

Table 29. The optimal capillary type, applied voltage polarity, pH value, and Me volume ratio for various compounds evaluated in this study (for obtaining the greatest number of successful measurements and good capillary efficiency).

Compound	**Optimal Conditions (greatest # of successful measurements; capillary efficiency)**
Theophylline	Cyano Pentoxy; +25 kV; pH = 7.06; 10-50% Me
Dyphylline	Cyano Pentoxy; +25 kV; pH = 7.06; 20-50% Me
Aminophylline	Cyano Pentoxy; +25 kV; pH = 7.06; 10-50% Me
Nortriptylline	Cyano Pentoxy; +25 KV; pH = 4.41; 0-20% & 50% Me