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ABSTRACT

SEPARATION OF POLYCYCLIC AROMATIC HYDROCARBONS BY USING MICELLAR ELECTROKINETIC CHROMATOGRAPHY

by
Patricia Simmons

In this work, the use of micellar electrokinetic chromatography (MEKC) for the separation of five polycyclic aromatic hydrocarbon (PAH) compounds is investigated. All of the compounds studied are listed by the United States Environmental Protection Agency as priority pollutants.

In micellar electrokinetic chromatography, solutes partition between the aqueous phase and the micellar phase. This partition is based on the hydrophobicity of each compound. In some cases, organic modifiers are added to the electrophoretic solution to enhance the separation efficiency. The organic modifier used in this study is γ-cyclodextrin (γ-CD), and sodium dodecyl sulfate (SDS) is used as the micellar phase. When both γ-CD and SDS are in the electrophoretic solution, a water insoluble, hydrophobic solute is partitioned between the micelles and the γ-CD cavity. When the solute is included in the γ-CD cavity, which is neutral, it migrates toward the cathode with the electroosmotic velocity. When a solute is incorporated into the SDS micelle, it migrates with the micellar velocity. This differential partition of the solute between the γ-CD cavity and the SDS micelles enables the separation to be achieved.

The Hewlett Packard HP^{3D}CE system was used to perform the separation. Satisfactory separation was achieved by using an electrophoretic solution comprised of phosphate-borate buffer of pH 7.0 with 10 millimoles of SDS and 2 millimoles of γ-CD, in a 50 μm x 56 cm capillary at 15 kilovolts.

SEPARATION OF POLYCYCLIC AROMATIC HYDROCARBONS BY USING MICELLAR ELECTROKINETIC CHROMATOGRAPHY

by
Patricia Simmons

A Thesis
Submitted to the Faculty of
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in Partial Fulfillment of the Requirements for the Degree of
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APPROVAL PAGE

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This thesis is dedicated, with love, to
the memory of Miss Jamillah Monique Cobb

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CHAPTER 1

INTRODUCTION

Capillary electrophoresis (CE) has developed into an extremely powerful analytical technique in recent years (Heiger, 1992). Along with advances in instrumentation and separation methodologies, a wide range of applications has been developed in many areas including chemical, biotechnical, pharmaceutical, and environmental analysis (Li, 1992).

Electrophoresis is the differential movement of ions by attraction or repulsion in an electric field. According to Heiger, electrophoresis as a separation technique was introduced by Tiselius in 1937 (1992) . By placing protein mixtures between buffer solutions in a tube and applying an electric field, Tiselius found that the sample components migrated in a direction, and at a rate determined by their charge and mobility. This work won him a Nobel prize.

In high-performance capillary electrophoresis (HPCE), separation is performed in narrow-bore capillaries, typically of 25-75 micrometers (μm) inner diameter (id). In a CE system, the ends of the capillary are connected to electrodes, which are connected to a high voltage power supply. The capillary ends are placed into buffer reservoirs, and the capillary is filled with a buffer identical to that in the reservoirs. The sample is introduced into the capillary by replacing one of the buffer reservoirs with a sample reservoir (usually at the anode end); the sample may be injected either electrokinetically or hydraulically. After the buffer reservoir is replaced, the electric field is applied and the separation is performed. Either on-line or off-line optical detection can be made at the cathode end of the capillary.

A schematic diagram of a generic CE system is shown in Figure 1.1.

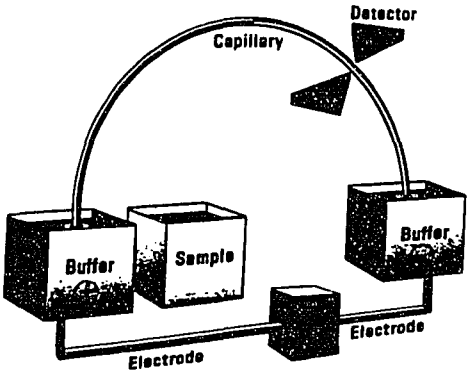


Figure 1.1 Schematic of CE System

Separation by electrophoresis is based on differences in solute velocities in an electric field. The velocity of an ion can be given by the following equation:

$$v = \mu_e E \tag{1.1}$$

where: v = ion velocity
μ_e = electrophoretic mobility
E = applied electric field

The electric field is a function of the applied voltage and the capillary length. The mobility for a given ion and medium is a constant which is characteristic of that ion, and may be defined as follows:

$$\mu_e = \frac{q}{6\pi\eta r} \tag{1.2}$$

where; q = ion charge
η = solution viscosity
r = ion radius

A fundamental part of HPCE operation is the electroosmotic flow (EOF). EOF is the bulk flow of liquid in the capillary. Most capillaries used for CE today are made of fused-silica, which contains surface silanol groups (Li, 1992; Heiger, 1992).

As shown in Figure 1.2, the interface between the fused-silica tube wall and the electrophoretic buffer consists of three layers; the negatively charged silica, the immobile layer, and the diffuse layer of cations adjacent to the surface of the silica, which tend to migrate toward the cathode. The migration of cations results in an accompanying migration of fluids through the capillary; this migration causes the EOF.

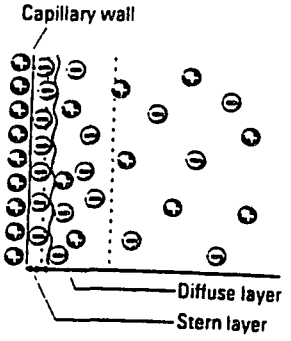


Figure 1.2 Representation of the Capillary Wall-Buffer Interface

The EOF is significantly greater than the electrophoretic mobility of the individual ions contained in the sample. Consequently, both anions and cations can be separated in the same run. Cations are attracted toward the cathode and their speed is augmented by the EOF. Although anions are electrophoretically attracted toward the anode, they are carried toward the cathode with the electroosmotic flow of the buffer. Cations with the highest charge/mass ratios will migrate first, followed by the cations with reduced ratios. Next, neutral components migrate with the same velocity as the EOF, and finally, the anions migrate. Those anions with lower charge/mass ratios migrate faster than those with greater ones (see Figure 1.3).

A unique feature of the EOF in the capillary is the flat flow profile (shown in Figure 1.4). Since the driving force of the flow is uniformly distributed along the capillary

facing 3

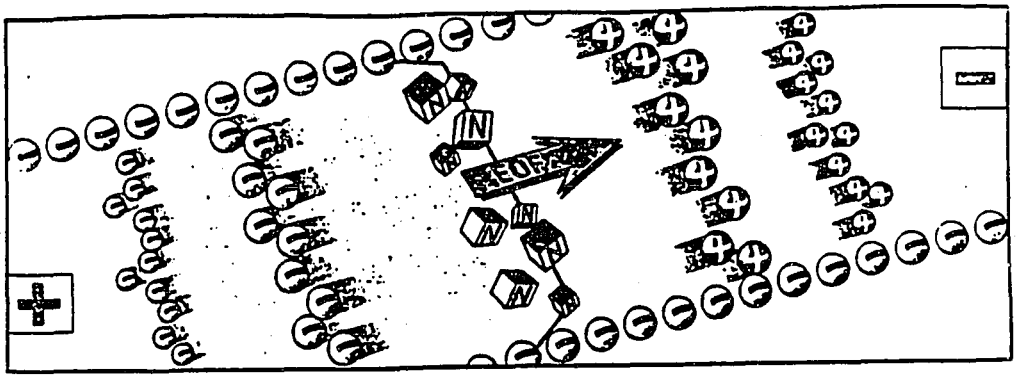


Figure 1.3 Differential Solute Migration Superimposed on EOF
Source: David N. Heiger, *High Performance Capillary Electrophoresis: An Introduction*, (France: Hewlett Packard Company, 1992) 19.

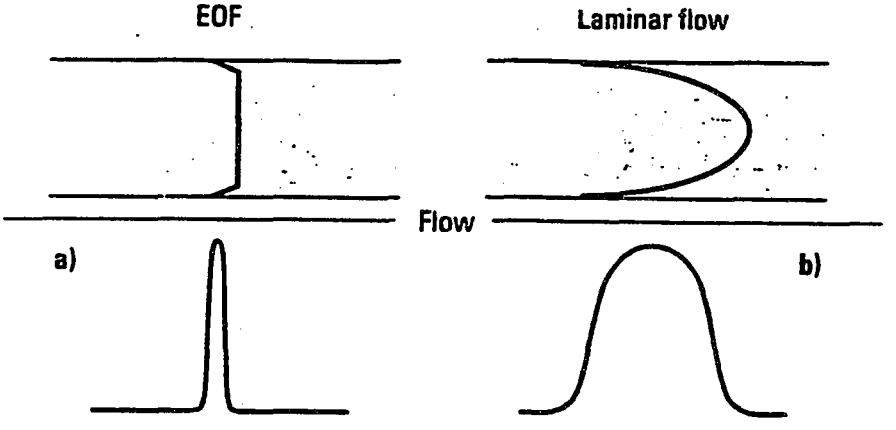


Figure 1.4 Flow Profile and Corresponding Solute Zone
Source: David N. Heiger, *High Performance Capillary Electrophoresis: An Introduction*, (France: Hewlett Packard Company, 1992) 18.

facing 4

Table 1.1 Methods to Control Electroosmotic Flow

Variable	Result	Comment
Electric field	Proportional change in EOF	• Efficiency and resolution may decrease when lowered • Joule heating may result when increased
Buffer pH	EOF decreased at low pH and increased at high pH	• Most convenient and useful method to change EOF • May change charge or structure of solute
Ionic strength of buffer concentration	Decreases zeta potential and EOF when increased	• High ionic strength generates high current and possible Joule heating • Low ionic strength problematic for sample adsorption • May distort peak shape if conductivity is different from sample conductivity
Temperature	Changes viscosity 2-3 % per °C	• Often useful since temperature is controlled instrumentally
Organic Modifier	Changes zeta potential and viscosity (usually decreases EOF)	• Complex changes, effect most easily determined experimentally • May alter selectivity
Surfactant	Adsorbs to capillary wall via hydrophobic and/or ionic interactions	• Anionic surfactants can increase EOF • Cationic surfactant can decrease or reverse EOF • Can significantly alter selectivity
Neutral hydrophillic polymer	Adsorbs to capillary wall via hydrophobic interactions	• Decreases EOF by shielding surface charge and increasing viscosity
Covalent coating	Chemical bonding to capillary wall	• Many modifications possible (hydrophillicity or charge) • Stability often problematic

Source: David N. Heiger, *High Performance Capillary Electrophoresis: An Introduction*, (France: Hewlett Packard Company, 1992) 21.

wall, there is no pressure drop within the capillary and flow is nearly uniform throughout. This increases the resolution in separations by reducing the band broadening of the analyte peak during its passage along the capillary (Heiger, 1992).

Electroosmotic flow is important in CE and must be controlled. For example, at a high pH the EOF may be too rapid. This may result in the elution of the solute before separation has taken place. Conversely, at a low pH the negatively charged wall can cause adsorption of cationic solutes through coulombic interactions (Heiger, 1992). Successful separations are usually obtained when the conditions optimize both EOF and solute mobility properties. Table 1.1 details several methods that may be used to establish optimal conditions.

The analytical parameters for capillary electrophoresis can be described in similar terms as those for column chromatography. These parameters include migration time, mobility, and dispersion. The migration time of a solute refers to the time required for it to migrate from the point of injection to the point of detection. Migration time, along with other experimental parameters may be used to calculate the apparent mobility, given by the following equation:

$$\mu_a = \frac{1}{tE} = \frac{1L}{tV} \qquad (1.3)$$

where; $\mu_a = \mu_e + \mu_{EOF}$
V = applied voltage
l = effective capillary length
L = total capillary length
t = migration time
E = electric field

In the presence of the EOF, the measured mobility is called the apparent mobility.

The effective mobility can be determined from the apparent mobility by independently measuring the EOF with a neutral marker that moves through the capillary at a velocity equal to the EOF (Terabe, 1990; Li, 1992).

Dispersion is the spreading of the solute zone, which results from differences in solute velocity within the zone, and can be defined as the baseline peak width, w_b;

$$w_b=4\sigma \tag{1.4}$$

where: s = standard deviation of peak width (in time length or volume)

and the separation efficiency, expressed in the number of theoretical plates, N, can be obtained by using the following equation:

$$N=(\frac{1}{\sigma})^2=\frac{\mu_e V l}{2DL} \tag{1.5}$$

where: D = diffusion coefficient (found in tables)

The theoretical plate number can also be determined from the electropherogram by using the following equation:

$$N=5.54(\frac{t}{w_{1/2}})^2 \tag{1.6}$$

where: t = migration time
$w_{1/2}$ = temporal peak width at ½ height

In practice, the measured efficiency is usually lower than the calculated efficiency because the theoretical calculation only accounts for zone broadening due to longitudinal diffusion (Heiger, 1992).

There are a number of other factors that contribute to zone broadening, including Joule heating and the adsorption of samples to the capillary wall. Joule heating is a result of the heat generated by the passage of an electrical current through the capillary.

The temperature increase depends on the power generated, and is determined by the capillary dimensions, the conductivity of the buffer, and the applied voltage. Joule heating causes a temperature gradient within the capillary; significantly elevated temperatures will result when the power generation exceeds the dissipation. Table 1.2 provides methods to control Joule heating and temperature gradients in the capillary.

Table 1.2 Methods to Control Joule Heating and Temperature Gradients

Variable	Effect
Decrease electric field	• Proportional decrease in heat generated • Reduces efficiency and resolution
Reduce capillary inner diameter	• Dramatic decrease in current • Decreases sensitivity • May cause increased sample adsorption
Decrease buffer ionic strength concentration	• Proportional decrease in current • May cause increased sample adsorption
Active temperature control	• Thermostats and removes heat from capillary

Source: David N. Heiger, *High Performance Capillary Electrophoresis: An Introduction,* (France: Hewlett Packard Company, 1992) 30.

Interaction between the solute and the capillary wall is detrimental in HPCE. The primary causes of adsorption to the fused-silica walls are ionic interactions between cationic solutes and the negatively charged wall, and hydrophobic interactions. The large surface area-to-volume ratio of the capillary, which is beneficial for heat transfer, actually increases the likelihood of adsorption. There are several strategies employed to reduce the solute-wall interaction. For example, an increase in the concentration of the buffer will result in a decrease in the solute interaction by reducing the effective surface charge. Another approach is coating the capillary wall. Coating the wall causes a decrease in the solute adsorption by

decreasing the free energy of interaction. Coatings can take various forms, including buffer additives and covalent modification of the capillary wall (Heiger, 1992).

There are several different modes of capillary electrophoresis. The following table presents each mode, and a brief description of its separation mechanism.

Table 1.3 Different Modes of High Performance Capillary Electrophoresis

CE Mode	Description
Capillary zone electrophoresis (CZE)	Separation is based on differences in the electrophoretic mobilities of the solutes, resulting in different velocities of migration of ionic species in the electrophoretic buffer contained in the capillary.
Capillary gel electrophoresis (CGE)	Separation is based on differences in solute size, as analytes migrate through the pores of the gel filled capillary.
Micellar electrokinetic chromatography (MEKC)	The main separation mechanism is based on solute partitioning between the micellar phase and the solution phase. This technique provides a way to resolve neutral molecules as well as charged molecules by CE.
Capillary electrochromatography (CEC)	The capillary is packed with a chromatographic packing which can retain solutes. The separation is based on the normal distribution equilibria upon which conventional chromatography depends.
Capillary isoelectric focusing (CIEF)	Substances are separated on the basis of their isoelectric points or pI values.
Capillary isotachorphoresis (CITP)	Separation is performed in a discontinuous buffer system. Sample components condense between leading and terminating constituents, producing a steady-state migrating configuration composed of consecutive sample zones.

Source: S. F. Y. Li, *Capillary Electrophoresis: Principles, Practice and Applications*, (Amsterdam: Elseiver, 1992) 4-12.

The CE method used in this study is micellar electrokinetic chromatography. In MEKC, the main separation is based on solute partitioning between the micellar phase and the solution phase. Micelles form in solution when a surfactant is added to water in a concentration above its critical micelle concentration (cmc). Micelles consist of aggregates of surfactant molecules with typical lifetimes of less than 10 μs (Li, 1992). The most commonly used surfactant in MEKC is sodium dodecyl sulphate (SDS).

According to Li, SDS micelles can be considered as small droplets of oil with a highly polar surface which is negatively charged. Even though the micelles are negatively charged, they migrate toward the cathode end of the capillary because of the EOF. However, the micelles travel at a rate slower than that of the bulk aqueous flow because of their attraction toward the anode. Neutral molecules partition in and out of the micelles based on their hydrophobicity. For example, a very hydrophillic, neutral molecule like methanol will spend almost no time inside the micelle and will essentially migrate at the same rate as the bulk aqueous flow. Conversely, an extremely hydrophobic neutral molecule such as sudan III will spend nearly all the time inside the micelle and will be eluted with the micelle (Terabe, 1990). A number of other potentially applicable surfactant systems have been explored for use as the micellar phase in MEKC. Li provides a table listing typical surfactant systems used for MEKC (1992, 240).

In some cases, modifiers are added to the electrophoretic solution to enhance the separation efficiency. One of the commonly used modifiers is cyclodextrin. Cyclodextrin (CD) is an electrically neutral, organic polymer, and its outside surface is hydrophillic; it does not interact with the micelle. Therefore, CD in the micellar solution exists as another phase, which migrates with a velocity identical to that of the bulk aqueous solution and is capable

of selectively solubilizing certain solutes depending on their size, shape, and hydrophobicity. When a highly hydrophobic substance, which is insoluble in water, is injected into the CD-MEKC system, it will distribute itself between the micelle and the CD cavity (Li, 1992).

In this work, the author employed cyclodextrin-modified micellar electrokinetic chromatography to investigate the separation of five polycyclic aromatic hydrocarbons (PAH's). PAH compounds are of great environmental concern because of their carcinogenity. All five PAH compounds studied are listed by the United States Environmental Protection Agency (USEPA) as priority pollutants.

CHAPTER 2

LITERATURE REVIEW

Interest in high-performance capillary electrophoresis as a separation technique has increased steadily during recent years. This increasing interest could be attributed to the many advantages that HPCE provides over high-performance liquid chromatography, including higher efficiency and faster analysis times (Li, 1992). Several studies have been conducted to investigate the separations of compounds of environmental concern using micellar electrokinetic chromatography (MEKC). These compounds include phthalates, phenols, and polycyclic aromatic hydrocarbons (PAH's). While the theory and optimization may still be in the developmental stage, a considerable number of applications have already been documented (Chao *et al.*, 1995; Rasmussen *et al.*, 1990). Terabe, Otsuka, Ichikawa, Tsuchiya, and Ando were among the first to investigate electrokinetic separation with micellar solutions in open-tubular capillaries and they subsequently developed the method known as micellar electrokinetic chromatography (1984).

Terabe *et al.*'s studies were based on the theory of Nakagawa, who proposed that micelles of an ionic surfactant can migrate in an aqueous solution by electrophoresis. When a solute is added into a micellar solution, some portion of the solute may be solubilized into the micelle. Thus, the solubilization by micelles can constitute a mechanism of retention in chromatography (1981). During preliminary studies conducted by Terabe *et al.*, fourteen phenol derivatives were completely resolved by using a phosphate-borate buffer solution containing sodium dodecyl sulphate (SDS) as the electrophoretic medium.

Results from these studies indicated that the bulk SDS solution was carried from the anode to the cathode; negatively charged SDS micelles also migrated toward the cathode, against the electrophoretic attraction. The SDS micelles, however, migrated at a rate slower than that of the bulk aqueous phase because of their attraction toward the anode. Therefore, every sample that is injected at the positive end of the capillary tube can eventually be detected at the negative end of the tube.

Based on this information, the experimenters proposed that any electrically neutral sample should be eluted between the retention time of a completely insolubilized solute and a micelle itself. A solute which is not solubilized by micelles at all should migrate with the same velocity as the electroosmotic flow, v_{eo}, and be eluted first at the retention time t_0. On the other hand, a solute which is completely solubilized with the micelles should migrate with the same velocity as that of the micelle, v_{mc}, and be eluted last with the retention time t_{mc}. By using these assumptions, Terabe *et al.* developed an equation for the capacity factor, *k'*, which is defined as the ratio of the total moles of solute in the micelle to those in the aqueous phase;

$$k' = \frac{t_r - t_0}{t_0(1 - t_r/t_{mc})} \qquad (2.1)$$

where t_r, t_0 and, t_{mc} represent the retention times of the solute, the bulk aqueous phase and the micelle, respectively. Methanol was used to measure t_0 and sudan III was used to determine t_{mc}.

Based on Terabe *et al.*'s findings, Ong, Chong, Lee and Yi conducted a study of the separation of eleven priority phenols listed by the United States Environmental Protection Agency (USEPA) as priority pollutants (1990). Phenols are of great environmental concern because of their high toxicity. In this study the experimenters investigated the retention times

of the phenols by using MEKC with varying surfactants, applied voltages, pH values, and capillary diameters, in an attempt to optimize separation conditions.

Preliminary experiments were performed with two different types of electrophoretic solutions. One solution contained only SDS in a phosphate-borate buffer, pH 6.6, while the other solution consisted of potassium dodecyl sulfate (KDS), as well as, SDS in a phosphate-borate buffer. Capillaries with inner diameters (id.) of 180 μm and 150 μm were used with an applied voltage of either 10 kilovolts (kV) or 15 kV. Of the four sets of conditions investigated, the combination of the 180 μm id. capillary tubing with the KDS and SDS in the phosphate-borate buffer solution provided superior selectivity. All eleven phenols were satisfactorily separated using this set of conditions, and the capacity factors in this set were found to be higher than those for the other three sets.

Having established the optimum separation conditions, Ong *et al.* conducted subsequent experiments using this set of conditions at pH values of 7.0 and 7.5. Results showed that, in general, the capacity factors decreased with increasing pH values. The differences in the capacity factors among the eleven phenols were not very significant at pH 7.0 and 7.5, whereas larger differences were observed at pH 6.6. The reasoning behind this phenomenon, according to Ong *et al.*, is that as the pH value increases, more of the phenols ionize to anionic form; this ionization results in a higher electrostatic repulsion between the ionized solutes and the SDS micelles. Despite the increased interaction of the anions with the positive electrode, the effect of the stronger electrostatic repulsion with the micelles dominates, and tends to suppress micellar solubilization. Consequently, the net effect is that the capacity factors decrease at higher pH values.

Ong *et al.* also employed MEKC to separate a mixture of six phthalate esters, five of which are listed by the USEPA as priority pollutants (1991). Phthalates are widely used as

plasticizers in the formulation of polymers. These plasticizers are not chemically bonded to the polymer, and under suitable conditions, they can migrate into the environment. In this analysis, Ong *et al.* investigated the effects of the pH value of the buffer solution, the SDS concentration in the electrophoretic medium, and the voltage applied across the capillary, on the retention times of the phthalates. Initially, the tests were conducted by using only a phosphate-borate buffer solution as the electrophoretic medium. Results from these tests produced a chromatogram with a single broad peak for all of the phthalates, indicating that the phosphate-borate solution, in the absence of the SDS micelles, does not provide sufficient selectivity to separate the compounds.

Next, Ong *et al.* investigated the effects of the pH value on the retention of the phthalates by using SDS in addition to the phosphate-borate buffer solution, and pH values ranging from 6.0 to 7.5. No change in the migration order of the six phthalates was observed throughout the whole pH range examined, and the migration times corresponded with the hydrophobicity of each compound. Therefore, the experimenters deduced that the phthalates must be in neutral form within in the pH range investigated, and thus were not affected by the pH change.

Ong *et al.* also performed experiments using three different SDS concentrations. Results indicated that as the SDS concentration increased, the migration times for the phthalates also increased. In their discussion, the experimenters explained that with the higher SDS concentrations, the phase ratio of the micelles to the aqueous phase is larger; this larger ratio causes an increase in the retention times of the phthalates inside the micelles, leading to an increase in the migration times for these compounds.

Finally, Ong *et al.* conducted a third set of tests to examine the effect of the voltage applied across the capillary on the separation efficiency of the phthalates by applying a voltage

range of 15 kV to 30 kV. Results indicated that by increasing the voltage the separation efficiency can be increased. However, at 30 kV, even though all six phthalates were satisfactorily separated, an additional peak was observed in the chromatogram. The reasoning offered for this occurrence is that the excessive heat generated by such a high potential could have promoted the hydrolysis of the compounds to produce an alcohol and an acid. The additional peak was probably observed because of the presence of the acid. Thus, the optimum voltage for the separation of phthalates was determined to be 25 kV.

While it has been demonstrated that conventional MEKC can be employed to effectively separate hydrophillic and moderately hydrophobic neutral compounds, the process must be modified to achieve separation of highly hydrophobic compounds (Terabe *et al.*, 1990). Terabe *et al.* investigated the separation of twelve chlorinated benzene congeners, and eleven trichlorobiphenyl isomers by using cyclodextrin- modified MEKC.

Initially, Terabe *et al.* attempted to separate the chlorinated benzene congeners by using only SDS in a phosphate-borate buffer solution. The results indicated that the mono-, di-, and trichlorobenzenes are not very hydrophobic, and were not totally incorporated into the micelle. Three isomers of dichlorobenzenes were partially resolved, but those of the trichlorobenzenes were not, although they were separated from the other polychlorinated benzenes; tetra-, penta, and hexachlorobenzenes were eluted at migration times similar to that of the micelle. Addition of γ-CD to the separation solution that was used in the first set of experiments permitted the separation of all the chlorinated benzene congeners. The increased resolution was attributed to the differential partition of the isomers to the γ-CD cavity because these isomers were not resolved in the absence of γ-CD.

Similarly, Terabe *et al.* investigated the separation of eleven trichlorobiphenyl isomers by using a phosphate-borate buffer solution, both with and without the addition of γ-CD.

In the absence of the γ-CD, all of the isomers of the trichlorobiphenyls migrated with the same velocity as that of the micelle, indicating that the isomers were too hydrophobic to be separated without the addition of γ-CD. As seen with the chlorinated benzene congeners, addition of the γ-CD allowed the separation of all eleven isomers. Other polychlorinated biphenyls such as mono-, di-, and tetrachlorobiphenyls, and commercial PCB products were also subjected to the CD-modified MEKC separation under the same conditions and excellent resolution was achieved.

Yik, Ong, Khoo, Lee and, Li investigated the separation of PAH compounds by using CD-MEKC (1991). Since they are all neutral, non-ionizable, and of similar hydrophobicity, it was not expected that these compounds would be separated using conventional MEKC. The experimenters conducted tests using varying SDS and γ-CD concentrations in an attempt to optimize separation conditions for the PAH's.

As expected, the PAH compounds were not satisfactorily separated using conventional MEKC. Therefore, to further improve the separation, γ-CD was added to the background electrolyte which consisted of SDS in a phosphate-borate buffer solution. With the addition of the γ-CD, complete separation of the PAH's was achieved. The effects of using various concentrations of γ-CD and SDS in the electrophoretic solution were also investigated. In general, the observed trend was that as the concentration of γ-CD increased, the retention times decreased. Yik *et al.* contributed this to the fact that unlike SDS, which is negatively charged, γ-CD has a neutral cavity. Therefore, γ-CD migrates with the same velocity as the bulk aqueous phase, which is faster than the velocity of the SDS micelles; thus, the migration times of the solutes are reduced. It was also revealed that, in general, as the concentration of SDS was increased, the migration times of the solutes were also increased.

Yik *et al.* contributed this trend to the theory that as the SDS concentration increases, the micelle concentration also increases, thus the solutes are retained longer. Concentrations of 10 millimoles (mM) of SDS and 2 mM of γ-CD were found to provide the best selectivity for the separation of the PAH compounds.

CHAPTER 3

OBJECTIVE

The objective of this thesis was to explore the separation of five selected polycyclic aromatic hydrocarbon compounds by using the micellar electrokinetic chromatography process. The instrument used in this study is the Hewlett Packard $HP^{3D}CE$ system. This system was recently installed in the Geo-Environmental Laboratory at New Jersey Institute of Technology.

A mixture of five PAH compounds was analyzed, using the $HP^{3D}CE$ system, in an attempt to separate the compounds, and identify each by its migration time through the capillary.

CHAPTER 4

MATERIALS AND EXPERIMENTAL METHOD

4.1 Materials

4.1.1 Instrument

The experimenter used the Hewlett Packard HP ^{3D}CE system in this study. A fused-silica capillary tubing of inner diameter 50 μm and an effective length of 56 cm was used as a separation column. The temperature in the capillary was kept constant at 25°C by a built-in temperature control system, and a potential of 15 kV was applied across the capillary. Ion detection was carried out with a diode array detector at a sample wavelength of 254 nm, with an 80 mm band width, and a reference wavelength of 314 nm, with an 80 mm band width. Data were recorded and analyzed by Hewlett Packard analysis software.

4.1.2 Chemicals

The five PAH compounds studied were acenapthene, anthracene, benzo(a)pyrene, fluoranthene, and napthalene. All of these compounds were obtained from Ultra Scientific (North Kingstown, RI) and were 95-99% pure. The dimethyformamide (DMF), methanol, sodium hydroxide, and sudan III were all purchased from Fisher Scientific (Edison, NJ), the γ-cyclodextrin (γ-CD), sodium dodecyl sulphate (SDS), and the phosphate-borate buffer solution (pH 7.0) were purchased from Supelco (Belefonte, PA). Analytical grade reagents and HPLC grade solvents were used.

The electrophoretic solution was prepared by dissolving SDS, the micellar phase, and γ-CD, a modifier added to increase selectivity, in the phosphate-borate buffer solution. The SDS solutions were filtered through 0.45 μm pore size membranes prior to use. The PAH standard solutions were prepared by first dissolving the compounds in 1 to 3 milliliters of DMF, subsequently diluting, with methanol, to a concentration of 100 parts per million (ppm), and finally adding 250 ppm of sudan III, to mark the elution of the SDS micelles. Acenapthene and anthracene standards were prepared at a concentration of 250 ppm.

4.2 Experimental Method

4.2.1 Capillary Conditioning

Each day before testing was began, the capillary tubing was pre-conditioned. First, it was flushed for ten minutes with Milli-Q^{uv} filtered water. Next, it was flushed for thirty minutes with 0.1 normal sodium hydroxide (0.1N NaOH) to remove adsorbates and refresh the capillary surface. Finally, it was flushed for thirty minutes with the electrophoretic solution. After pre-conditioning, a blank was run, with only the electrophoretic solution as a sample, to establish a reference baseline.

The capillary was also conditioned before each analysis. This conditioning included flushing the capillary for one minute with Milli-Q^{uv} filtered water, for two minutes with 0.1N NaOH, and for three minutes with the electrophoretic solution. The electrophoretic solution in the inlet and outlet buffer vials was replaced after five runs.

4.2.2 Sample Analysis

Samples were injected hydraulically with a built-in, automatic sample injector. Injections lasted for three seconds at a pressure of 30 millibars.

First, a sample containing only methanol was analyzed to establish its migration time. Next, a sample containing methanol and sudan III was analyzed to determine the migration time of the SDS micelles. Once this information was established, samples of each PAH standard were analyzed individually to determine the migration times of the compounds.

A second series of tests was conducted to verify the migration times established in the first set of tests. First, a sample of the benzo(a)pyrene standard was analyzed and its migration time was established. Next, a sample containing benzo(a)pyrene and fluoranthene was analyzed to determine the migration time for fluoranthene. The experimenter continued with this procedure, adding one PAH compound at a time to the mixture for each subsequent analysis, until ultimately a mixture containing all five PAH compounds was analyzed.

facing 21

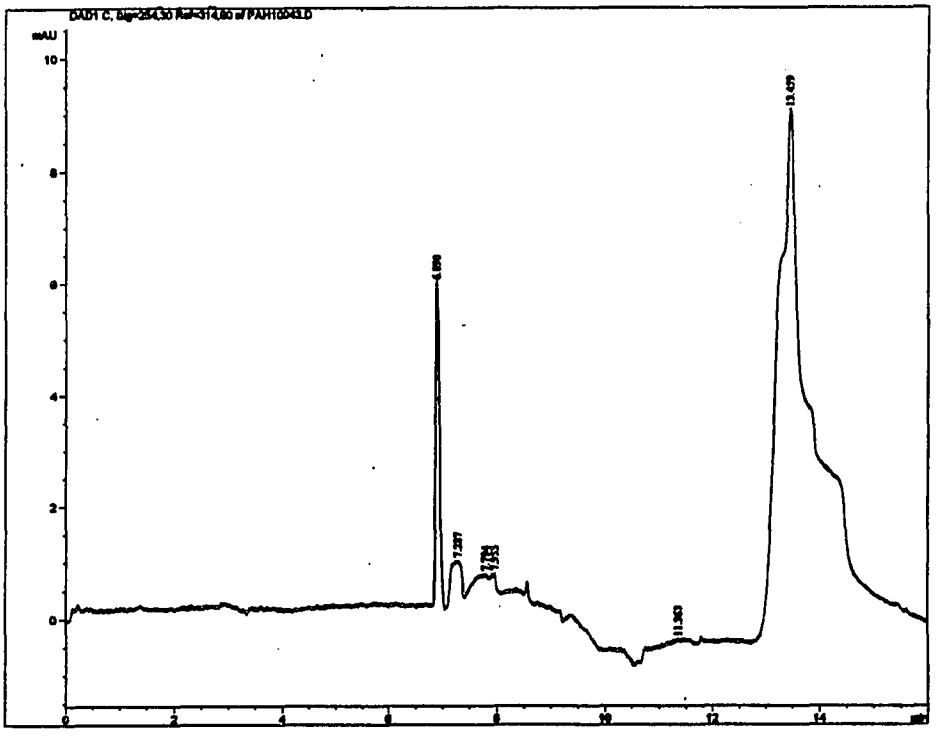


Figure 5.1 Electropherogram Output of Mixture Analysis

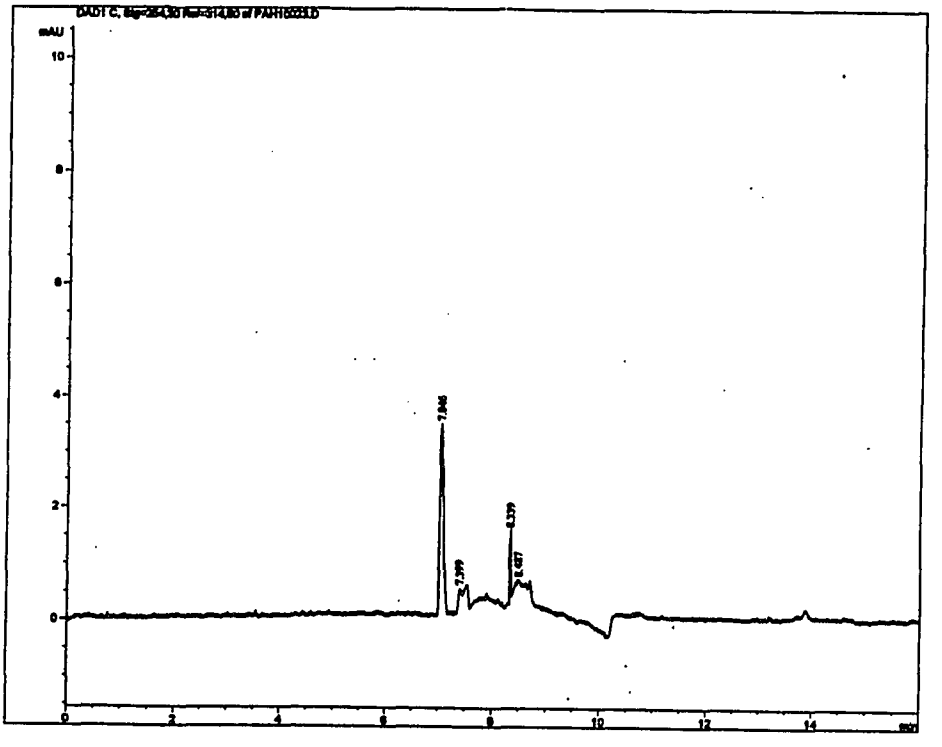


Figure 5.2 Electropherogram Output of Napthalene Analysis

CHAPTER 5

RESULTS AND DISCUSSION

Initially, a mixture containing all five of the PAH compounds was analyzed. However, the results from that analysis, shown in Figure 5.1, were inconclusive. The baseline is disturbed and not all of the peaks are distinguishable. When each PAH standard solution was analyzed individually, it was found that the electropherogram of the napthalene standard, shown in Figure 5.2, also contained baseline disturbance, as well as too many peaks. This leadto the suspicion that the napthalene may have been the cause of the inconclusive results.

In order to confirm this suspicion, several different mixtures of the compounds were analyzed in sequence. First, only one compound was analyzed. Then, for the next analysis, a mixture of two compounds was analyzed. This procedure was continued by adding the rest of the compounds one at a time, until a mixture of all five PAH compounds was analyzed. Since napthalene was suspected to be the cause of the problem, it was the last compound to be added to the mixture. The results from this series of tests are shown in Figures 5.3 through 5.7.

These results confirmed the suspicion that the napthalene was indeed causing the disturbance in the electropherogram. For all of the samples analyzed prior to the addition of the napthalene, electropherograms with clear identifiable peaks and minimal baseline disturbance were produced.

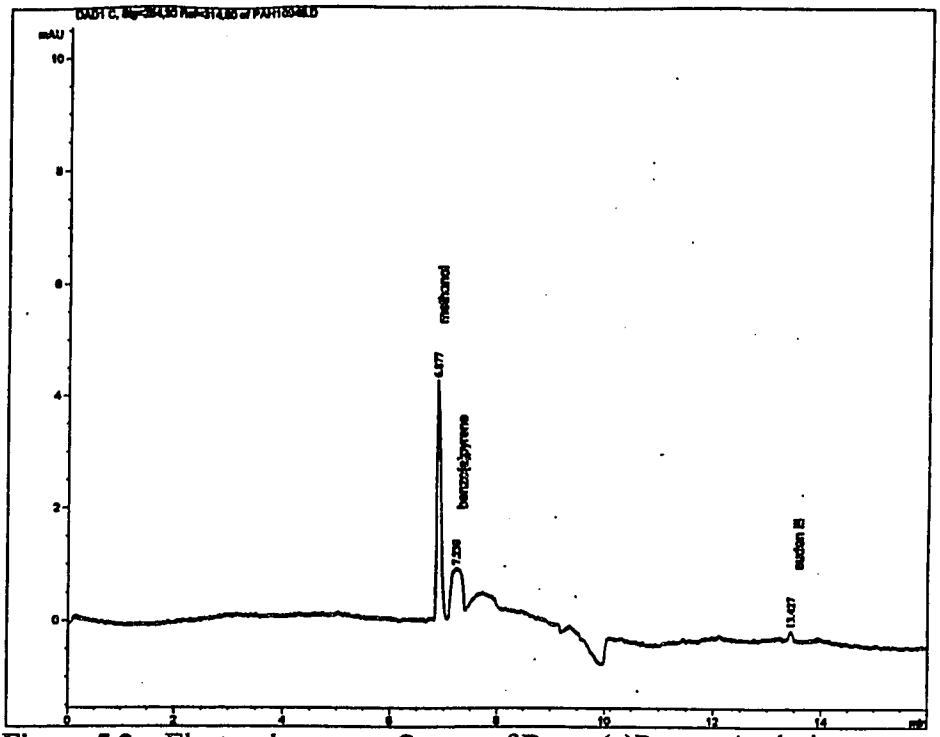


Figure 5.3 Electropherogram Output of Benzo(a)Pyrene Analysis

Figure 5.4 Electropherogram Output of Benzo(a)Pyrene and Fluoranthene Analysis

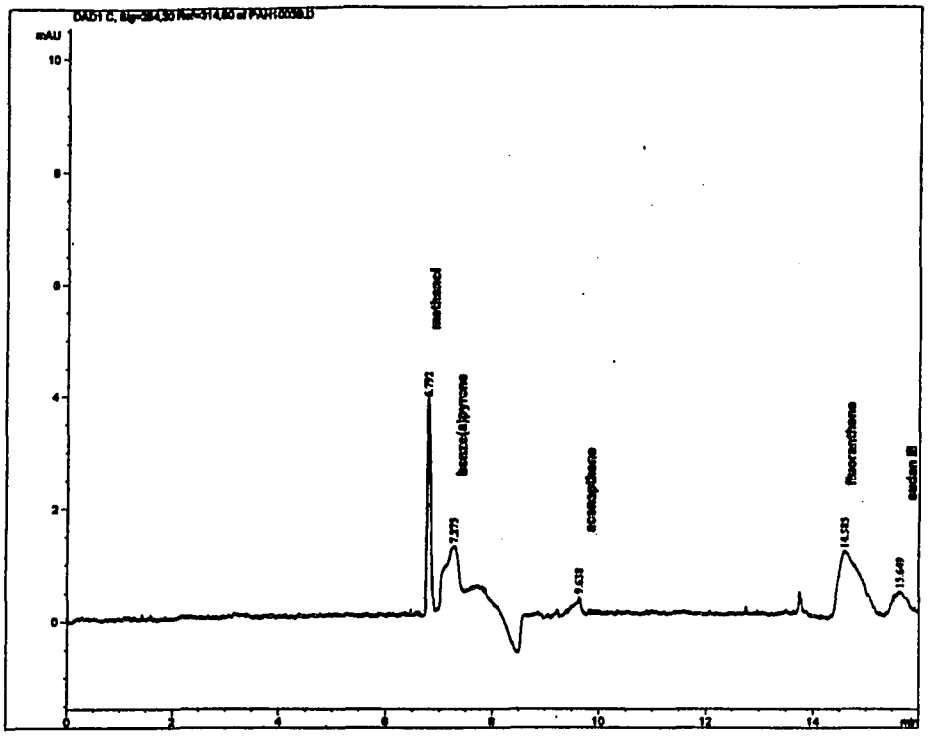


Figure 5.5 Electropherogram Output of Benzo(a)Pyrene, Fluoranthene, and Acenapthene Analysis

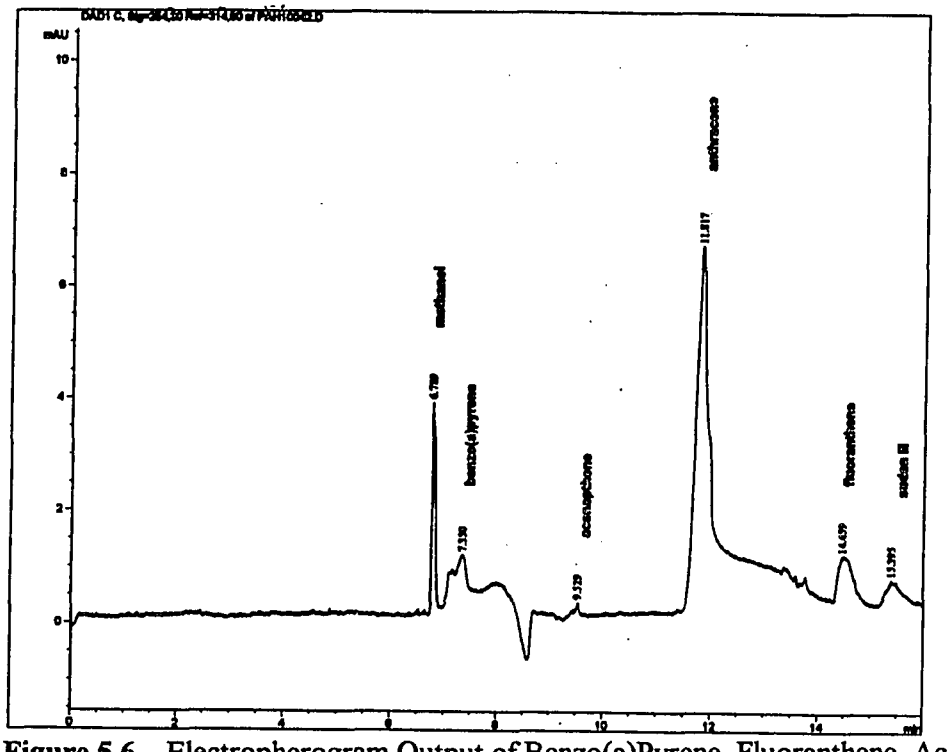
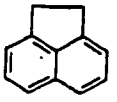
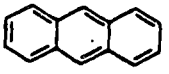


Figure 5.6 Electropherogram Output of Benzo(a)Pyrene, Fluoranthene, Acenapthene, and Anthracene Analysis

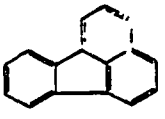
facing 24



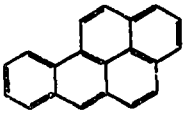
Acenapthene (Molecular Weight 154.21)



Anthracene (Molecular Weight 178.24)



Fluoranthene (Molecular Weight 202.26)



Benzo(a)Pyrene (Molecular Weight 252.32)

Figure 5.7 Molecular Structures of PAH'S

A possible explanation for this occurrence is that the napthalene obtained from the chemical company was either contaminated or expired. Since the napthalene was found to be unusable, the remainder of this discussion will focus on the separation of the remaining four PAH compounds.

When each PAH standard was analyzed individually, it was found that all of the compounds eluted between 7.90 and 8.40 minutes. However, when a mixture of the compounds was analyzed, the migration times for the compounds ranged from 7.35 to 14.46 minutes (see Figures A.1 through A.4 in Appendix A). A possible explanation for this occurrence may be that the compounds favor the γ-CD micelles over the SDS micelles. Therefore, when the compounds are analyzed individually, they tend to be totally incorporated into the γ-CDcavity and are eluted around the same time. However, when more than one PAH compound is contained in the sample, the ratio of γ-CD micelles to compound is lower, and the compounds are forced to partition between the γ-CD micelles and the SDS micelles because of their hydrophobicity. Thus, the compounds are eluted at varying times.

Terabe *et al.* (1990) suggested that smaller PAH compounds are more easily included into the γ-CD cavity. Based on this assumption, the expected elution order is as follows: acenapthene, anthracene, fluoranthene, benzo(a)pyrene (molecular structures are shown in Figure 5.7). The actual elution order for the PAH compounds in this experiment was found to be: benzo(a)pyrene, acenapthene, anthracene, flouranthene. While benzo(a)pyrene was expected to elute last, it was actually the first PAH compound to be eluted.

The number of theoretical plates obtained in this work is not as high as was expected. This could be because the instrument settings may need to be adjusted to obtain optimum operating conditions.

Another possible explanation is that the equation used to calculate the number of theoretical plates, Eq. 1.6, does not account for the asymmetric shape of the peaks observed in the electropherogram output. Table B.1 in Appendix B contains the capacity factors (calculated by using Eq. 2.1) and the number of theoretical plates for the four PAH compounds.

CHAPTER 6

CONCLUSIONS

Results have shown that micellar electrokinetic chromatography is an effective technique for the separation of PAH compounds. While four of the five compounds studied were satisfactorily separated, further investigation is warranted to establish optimum separation and instrument conditions, which will allow for increased separation efficiency.

APPENDIX A

ELECTROPHEROGRAM OUTPUT OF ANALYSES

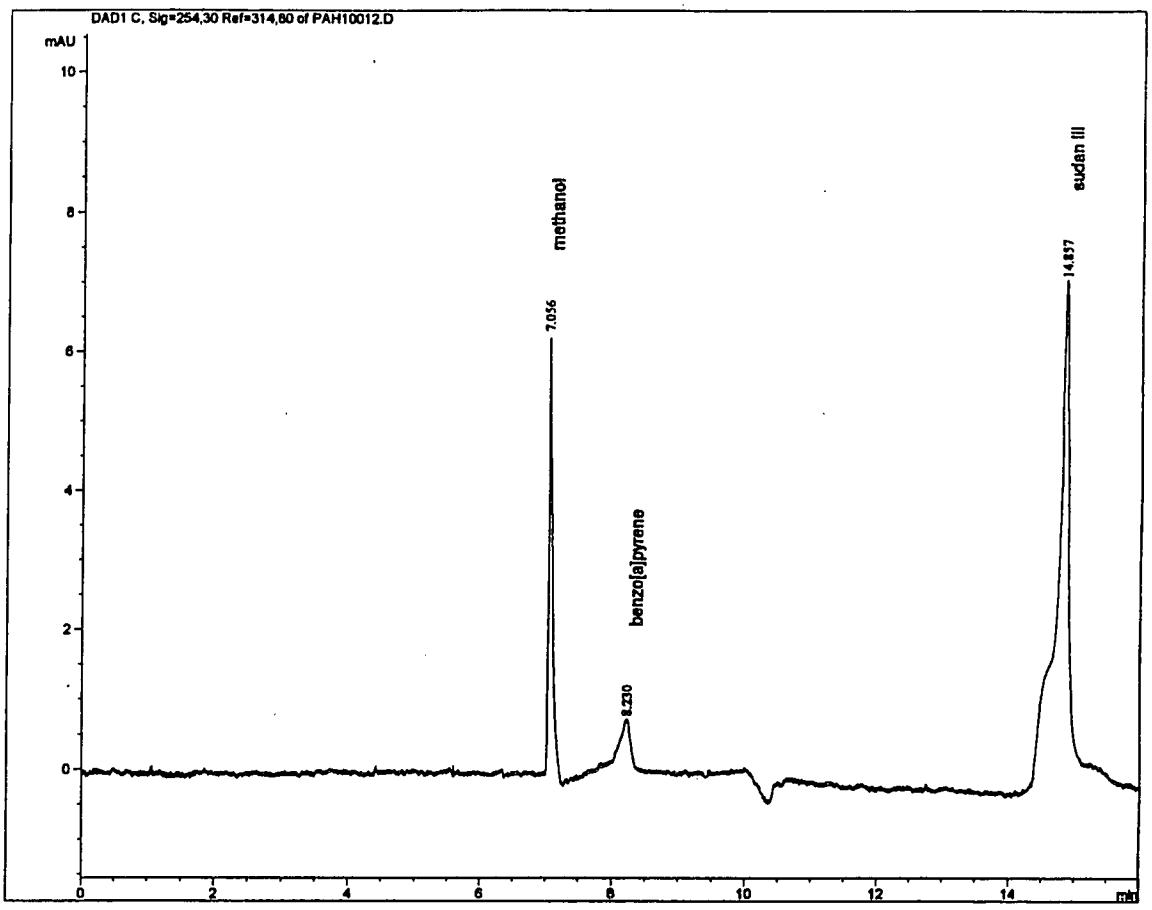


Figure A.1 Electropherogram Output of Benzo(a)Pyrene Analysis

APPENDIX A
(Continued)

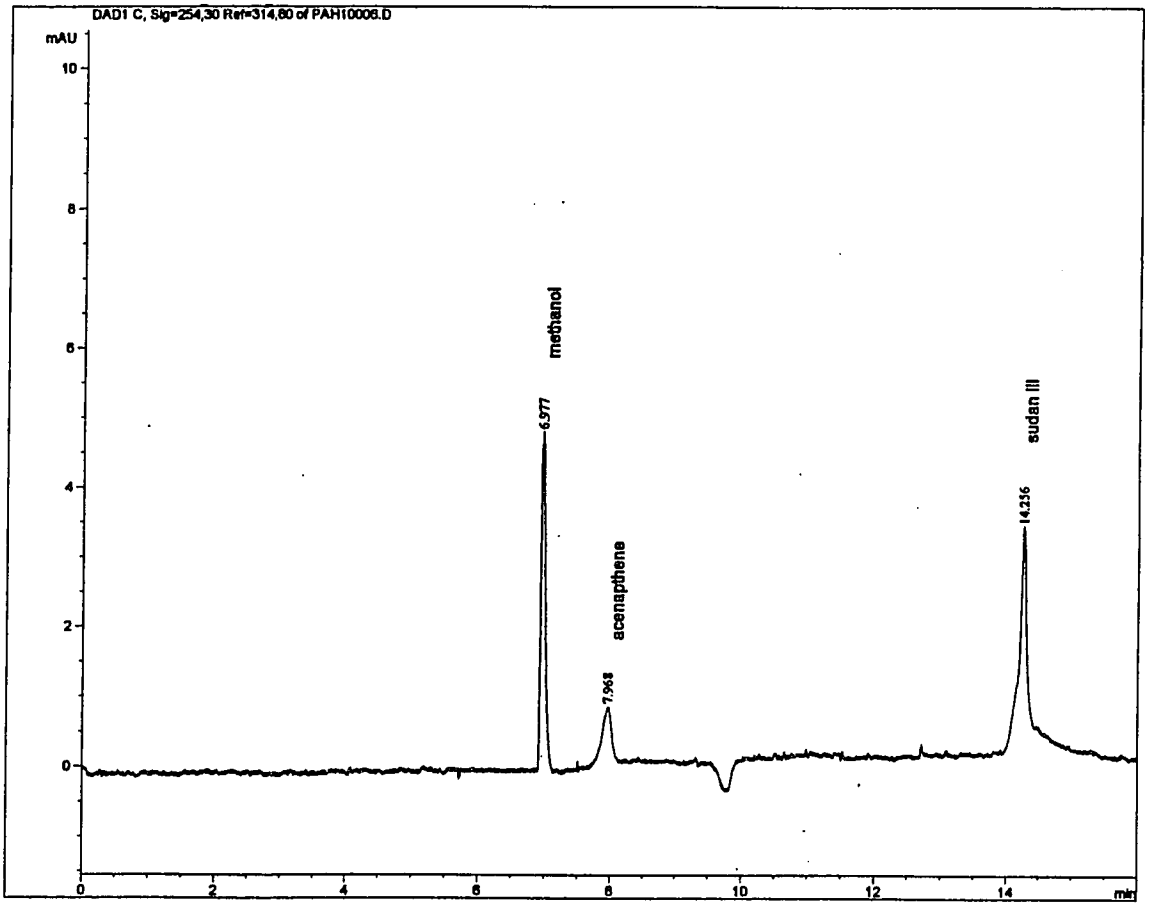


Figure A.2 Electropherogram Output of Acenapthene Analysis

APPENDIX A
(Continued)

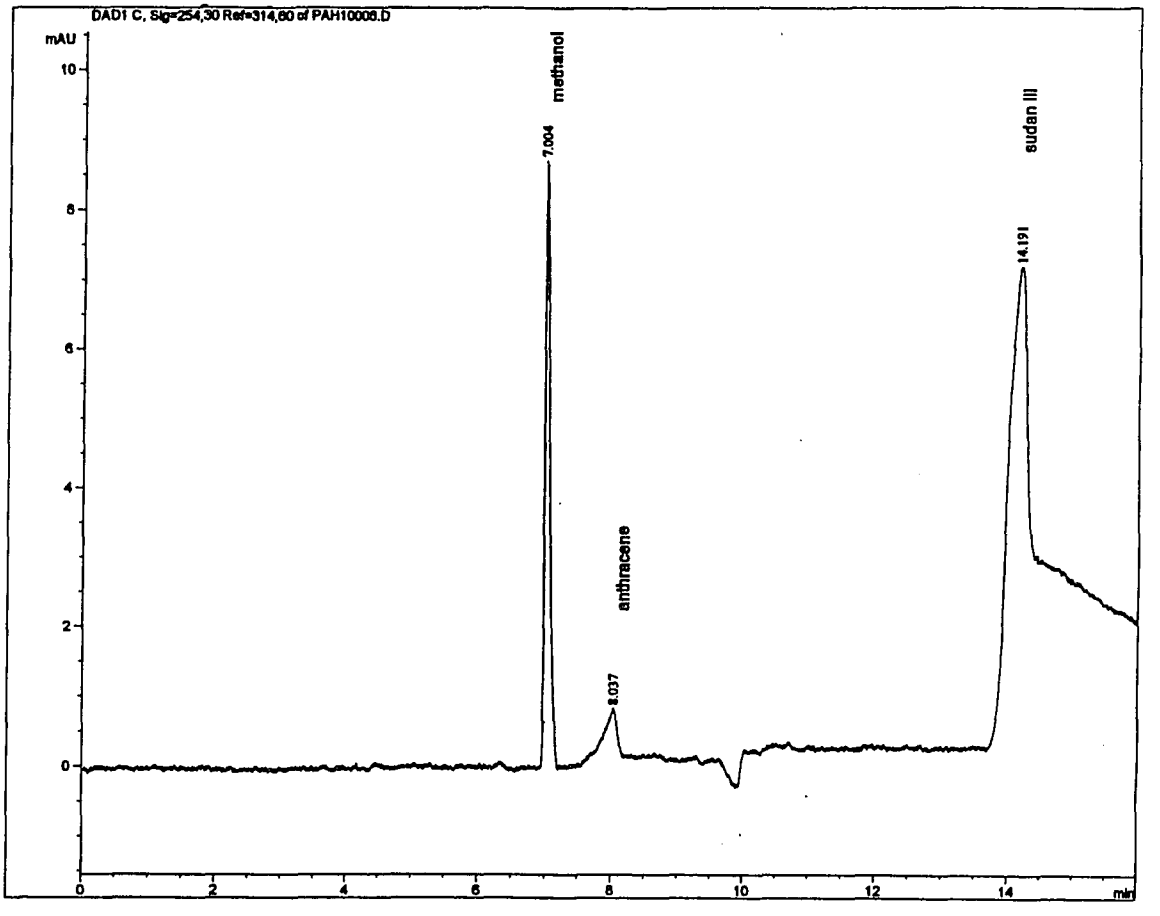


Figure A.3 Electropherogram Output of Anthracene Analysis

APPENDIX A
(Continued)

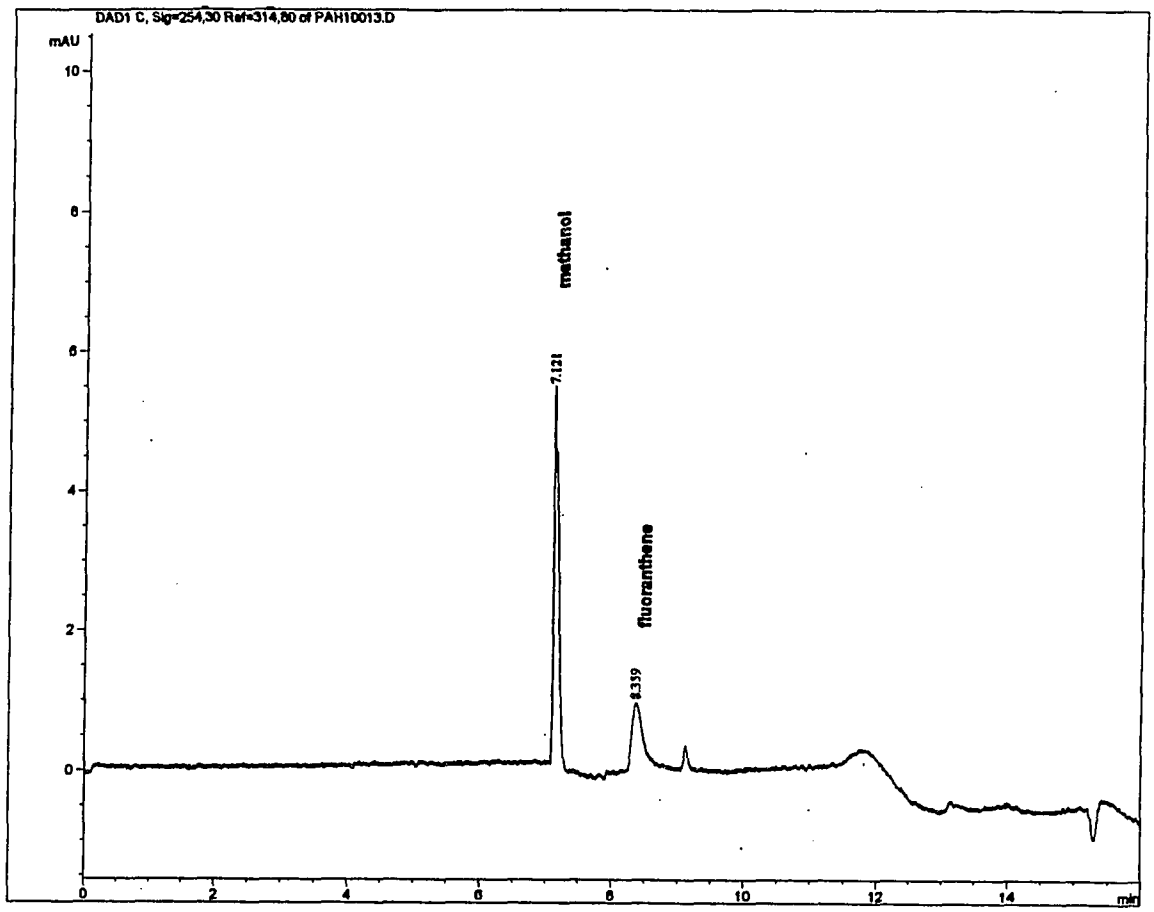


Figure A.4 Electropherogram Output of Fluoranthene Analysis

APPENDIX B

CAPACITY FACTORS AND THEORETICAL PLATE NUMBERS

Table B.1 Capacity Factors and Number of Theoretical Plates

Compound	Capacity Factor	# of Theoretical Plates
Benzo(a)Pyrene	0.158	7,000
Acenapthene	1.030	18,000
Anthracene	3.190	20,000
Fluoranthene	18.580	21,000

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