A METHOD DEVELOPMENT OF CAPILLARY ELECTROPHORESIS FOR THE DETERMINATION OF ORGANIC CONSTITUENTS IN GUN POWDERS

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บทคัดย่อ

ชื่อเรื่อง : การพัฒนาวิธีวิเคราะห์ด้วยเครื่องมือแคปิลลารีอิเล็กโทรโฟรีซีส เพื่อวัดปริมาณสารอินทรีย์ในดินปืน

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ศัพท์สำคัญ : แคปิลลารีอิเล็กโทรโฟรีซีส ไมเซลลาร์อิเล็กโทรไคเนติกโครมาโทกราฟี sweeping high salt stacking sweeping-high salt stacking

การศึกษาเทคนิค Nomal-MEKC ในการแยกสารอินทรีย์ในดินปืน 3 ชนิดได้แก่ 2,4 ไดไนโตรโทลูอีน (2,4 dinitrotrotoluene) 2,6 ไดไนโตรโทลูอีน (2,6 dinitrotrotoluene) และ 3,4 ไดไนโตรโทลูอีน (3,4 dinitrotrotoluene) โดยศึกษาปัจจัยที่มีผลต่อการแยกสาร ได้แก่ ชนิดของสารละลายอิเล็กโทรไลต์ ความเข้มข้นของสารละลายอิเล็กโทรไลต์ พีเอช สารลดแรงตึงผิว ศักย์ไฟฟ้าที่ใช้ในการแยก เวลาฉีดสาร เพื่อหาสภาวะที่เหมาะสมในการแยกสารโดยดูจากลักษณะของพีค และเวลาที่ใช้ในการแยกสาร พบว่าสภาวะที่ให้ผลดีที่สุดในการทดลองคือ การใช้ fused-silica capillary 75 μm i.d. x 64.5 cm (56 cm effective length) 25 mM phosphate buffer และ 75 mM sodium lauryl sulfate ที่ pH 7.5 ศักย์ไฟฟ้า +30 kV อุณหภูมิ 25 °C ตรวจวัดสัญญาณด้วย UV-Visible detector ที่ความยาวคลื่น 200 นาโนเมตร ซึ่งจะใช้เป็นสภาวะเริ่มต้นในการพัฒนาเทคนิคต่อไป

การพัฒนาเทคนิค on-line sample concentration เป็นการพัฒนาเทคนิคเพื่อเพิ่มประสิทธิภาพในการแยกสารที่มีความเข้มข้นในระดับต่ำๆ ได้ ซึ่งในงานวิจัยนี้ได้ศึกษาพัฒนา 3 เทคนิค คือ (1) sweeping (2) high salt stacking และ (3) sweeping-high salt stacking โดยเปรียบเทียบลักษณะของพีคและเวลาที่ใช้ในการวิเคราะห์ซึ่งให้ผลการทดลองดังนี้

(1) เทคนิค sweeping ศึกษาผลของความเข้มข้น phosphate buffer ที่ใช้ในการเจือจางสารตัวอย่างโดยไม่มีสารลดแรงตึงผิว (SDS) ควบคุมค่าการนำไฟฟ้า (Conductivity) ของสารตัวอย่างและสารละลายบัฟเฟอร์ให้มีค่าเท่ากัน พบว่าผลที่ดีที่สุดคือ การใช้ 25 mM phosphate buffer เจือจาง 25 เท่า

(2) เทคนิค high salt stacking ศึกษาผลของความเข้มข้นโซเดียมคลอไรด์ (Sodium chloride, NaCl) ที่ใช้ในการเจือจางสารตัวอย่างโดยไม่มีสารลดแรงตึงผิว (SDS) ควบคุมค่าการนำไฟฟ้าของสารตัวอย่างให้สูงกว่าค่าการนำไฟฟ้าของสารละลายบัฟเฟอร์ 2.5 เท่า พบว่าผลที่ดีที่สุด คือ การใช้ 150 mM NaCl

(3) เทคนิค sweeping-high salt stacking ศึกษาผลของความเข้มข้น phosphate buffer และ NaCl ที่ใช้ในการเจือจางสารตัวอย่างโดยไม่มีสารลดแรงตึงผิว (SDS) ควบคุมค่าการนำไฟฟ้าของสารตัวอย่างและสารละลายบัฟเฟอร์ให้มีค่าเท่ากัน พบว่าผลที่ดีที่สุด คือ การใช้ 25 mM phosphate buffer เจือจาง 25 เท่า ผสมกับ 50 mM NaCl

เมื่อทำการเปรียบเทียบผลการทดลองพบว่าเทคนิคที่ให้ผลการทดลองที่ดีที่สุด คือ high salt stacking ซึ่งสามารถนำไปวิเคราะห์ในตัวอย่างจริง (ดินปืน) ได้ภายใต้สภาวะที่เหมาะสม

ABSTRACT

TITLE : A METHOD DEVELOPMENT OF CAPILLARY ELECTROPHORESIS FOR THE DETERMINATION OF ORGANIC CONSTITUENTS IN GUN POWDERS

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This study was undertaken to separate three types of organic gun powder using Normal-MEKC. Factors influenced separation efficiency, including electrolyte concentration, pH, surfactant, applied voltage, and injection time, were investigated. The optimum condition, which was considered from peak shapes and separation time, was fused-silica capillary with 75 μm i.d. x 64.5 cm (56 cm effective length), 25 mM phosphate buffer and 75 mM sodium dodecyl sulfate at pH 7.5, +30 kV applied voltage, 25 °C, and on-column UV detection at 200 nm. This condition would be the initial condition to further develop this technique.

The development of on-line sample concentration technique is to increase separation efficiency in low concentration of solution. This study focused on the development of three separation techniques, (1) sweeping, (2) high salt stacking, and (3) sweeping-high salt stacking by comparing their peak shapes and migration times. The result is shown as follow.

(1) Sweeping is to study the effect of phosphate buffer used to dilute sample solution without injecting surfactant (SDS) to maintain equivalent conductivities of sample and running buffer. 25-time-diluted 25 mM phosphate buffer; consequently, was considered the optimum condition.

(2) High salt stacking is centered on effect of NaCl concentration used to dilute sample solution without injecting surfactant (SDS) to adjust conductivity of sample to 2.5 times of conductivity of running buffer. 150 mM NaCl, as a result, exhibited the optimum condition.

(3) Sweeping-high salt stacking is to investigate effect of phosphate buffer and NaCl solution used to dilute sample solution without injecting surfactant (SDS) to maintain equivalent conductivities of sample and running buffer. 25-time-diluted 25 mM phosphate buffer with 50 mM NaCl; as a consequence, demonstrated the optimum condition.

The comparison of the three techniques showed that high salt stacking was the most effective condition which can be practically used to analyze organic gun powder under suitable condition.

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LIST OF ABBREVIATIONS

ABBREVIATION	FULL WORD
CE	Capillary Electrophoresis
MEKC	Micellar Electrokinetic Chromatography
CZE	Capillary Zone Electrophoresis
CGE	Capillary Gel Electrophoresis
CIEF	Capillary Isoelectric Focusing
GSR	Gunshot Residue
GC	Gas Chromatography
HPLC	High-Performance Liquid Chromatography
EOF	Electroosmotic Flow
UV/VIS	Ultraviolet
DAD	Diode-Array Detection
PDA	Photo-diode Array
LIF	Laser Induced Fluorescence
CDs	Cyclodextrin
α-CD	α- Cyclodextrin
β-CD	β-Cyclodextrin
γ-CD	γ-Cyclodextrin
FASS	Field-Amplified Sample Stacking
BGE	Background Electrolyte
SM	Sample Matrix
i.d.	Internal Diameter
kV	Kilovolt
min	Minute
DNT	Dinitrotoluene
2,4 DNT	2,4 dinitrotoluene
2,6 DNT	2,6 dinitrotoluene

LIST OF ABBREVIATIONS (CONTINUED)

ABBREVIATION	FULL WORD
3,4 DNT	3,4 dinitrotoluene
SDS	Sodium Dodecyl Sulphate
CMC	Critical Micelle Concentration
L	Length of Capillary Column
LOD	Limit of Detection
N	Efficiency
R_s	Resolution
t_{mg}	Migration Time
t_r	Retention Time of the Solute
t_o	Retention Time of the Unretained Peak
t_{mc}	Retention Time of the Micelle
μ_{ep}	Electrophoretic Mobility
η	Viscosity
ε	Dielectric Constant
ζ	Zeta Potential

CHAPTER 1

LITERATURE REVIEW

1.1 Background

Forensic science plays a crucial role in criminal investigation. In cases which are related to gunshot, forensic investigation supplies useful information to identify whether suspects use guns as a weapon to commit the crime. It is currently admitted in judgements that Forensic Science, Royal Thai Police is responsible for examination of inorganic gunshot residue (GSR) by using atomic absorption spectrophotometry (AAS). AAS can detect only major elements including Sb, Ba, and Pb in primers. Organic GSR analysis; however, become essential to the characterization of gunshot residue when inorganic GSR examination provides unclear information due to analysis restriction.

An interest in the characterization of organic substances in gunshot residue and gun powders using capillary electrophoresis (CE) technique has been recently increased because CE is a novel separation technique which offers high efficiency and productivity. CE proves as effective as or sometimes prior to high performance liquid chromatography (HPLC). The fundamental concept of CE is difference between substances' moving capacity under applied voltage. CE can characterize not only substances with wide range of size, form ions to macropolymers, but also a small amount of samples. CE; in addition, can be operated in terms of quantitative and qualitative analysis. As CE requires only a small amount of samples, low content of chemical waste is released from the laboratory. CE; moreover, is a simple method which requires short operation time and low cost per unit.

The restriction of CE; however, is a high limit of detection (LOD). CE shows higher LOD than HPLC because it utilizes a capillary column with small diameter (50-75 μm) and small amount of injected sample (nL). To solve this problem, researchers attempt to develop several techniques, such as reducing content of injected sample. Another solution, called on-column concentration, is to stack up sample within narrow zone in a capillary column. On-column technique can be operated on CE without additional instrument. The major concept of this

technique is to maintain difference between electromigration velocity of each substance in a capillary column.

This research is a pioneer experiment in Thailand to analyze small amounts of organic substances in gunshot residue and gun powders using CE. The development of CE by using on-line sample concentration technique has not been widely studied in foreign. This research reveals various information which might be beneficial to criminal investigation of judgements in the further.

1.2 Gunshot Residues

1.2.1 General Considerations

When a firearm is discharged, an assortment of vapors and particulate material are expelled in the area around the firearm. These products of firearm discharge can be collectively referred to as gunshot residues (GSR) or sometimes cartridge discharge residues (CDR).

Gunshot residues have been used in criminalistics to estimate firing distances, identify bullet holes, and determine the discharge of a firearm. The ability to provide evidence is, based on analysis of gunshot residues, has been one of the most persistent goals of forensic scientists since the turn of the century. Several factors serve as motivation for this intensity of concern. Crimes associated with firearms are particularly serious and require perhaps the greatest investigative effort. It is often possible to recover spent bullets or cartridge casings and associate them with a particular gun. However, the remaining link in the chain of evidence is to associate a suspect with the firing of the gun in question. Gunshot residue evidence has been of substantial help in the investigation of many of these cases. Gunshot residue detection methods are based on analysis of the chemical residues produced by discharge of the firearm's cartridge. Normally, only trace amounts are deposited on the back of the hand, face, hair, and clothing of the firer, and this material can be used to determine if a person has discharged a weapon.

1.2.2 Source of Gunshot Residues

1.2.2.1 General

When a gun has been fired, gunshot residues can arise from the primer, propellant, lubricants and metals that are found in the bullet jacket, cartridge casing, and gun

barrel. The first step in the firing of a bullet is the detonation of the primer. The primer is detonated when it is crushed by the force of the firing pin of the firearm. This drives hot gases and hot particles into the propellant and ignites it. The ignition of the primer mixture and the propellant occurs in a matter of a few ten thousandths of a second [3]. The ignited propellant decomposes and forms gaseous products. Simultaneously, heat, in enormous quantity, is released by this reaction. The high pressure from the heated gases forces the projectile out of the cartridge casing into the barrel and out of the muzzle of the gun.

The heat generated on ignition of the primer causes the inorganic ingredients of the primer mixture to vaporize. Because of supersaturation, these vapors recondense into droplets [4], which are further subjected to high pressure and temperature arising from the burning propellant powder. Some of these droplets will grow by coalescence. With the expansion and cooling on leaving barrel many of these droplets freeze in their existing form. The gunshot residues originating from the primer contain elements of primer components mainly lead, antimony, and barium [4]. Elements, such as copper, iron, and some other nonspecific elements, such as aluminum, silicon, sulfur, potassium, and calcium are also often found associated with them. These latter elements can originate from other sources such as etchted bullets, cartridge casings, or barrels. In addition to the "primer particle", when abundant, they can arise from the lead bullets themselves [5].

Under ideal circumstances, it would be expected that all of the propellant powder would be expected that all of the propellant powder would be consumed in the burning process and would be converted into gases. However, in practice, this is not the case because the whole powder charge is never totally burnt. When a firearm is discharged, unburnt and partially burnt powder granules are propelled out of the barrel along with the projectile toward the target. The size of these particles ranges from large visible particles to a fine dust. Gunshot residues are mainly composed of these organic and inorganic particles. A simplified probability equation has been described by Halberstam [6]. The equation is a tool to assess the probability of finding gunshot primer residues when only a portion of a specimen is to be searched.

1.2.2.2 Primers

Ammunition primers consist of four basis chemical components: the initiating explosive, oxidizing agent, fuel, and sensitizer. Each component can be expected to

contribute gunshot residues after a gun has been fired. The standard explosive initiator in primers is lead stypnate. Formerly, lead azide and mercury fulminate had been used as initiator in primers, but are no longer commonly used because the intensity of flame produced is insufficient and a corrosive effect is imparted by mercury fulminate to gun barrels.

Oxidizing agents are used in primers to increase the heat of ignition. Barium nitrate is most commonly used in small-arms ammunition, but barium peroxide, lead nitrate, or lead peroxide may also be encountered. Antimony sulfide is commonly used as fuel in primers, as well as calcium silicide, lead thiocyanate, powdered aluminum, and powdered zirconium, magnesium, and titanium have also been used.

The standard sensitizing material used in small-arm primers is tetracene (1-(5-tetrazolyl)-4-guanyltetrazene hydrate). Pentaerythritol tetranitrate, trinitrotoluene, and tetryl are also used as sensitizers in primers.

1.2.2.3 Gunpowders

The use of black powder as a propellant for bullets has largely been discontinued. Black powder is composed of 75% potassium nitrate, 15% sulfur, and 10% charcoal. Smokeless powders, which are commonly used as propellants, are composed of nitrocellulose in single base powders and nitrocellulose together with nitroglycerine in double base powders. In some double base powders a portion of nitrocellulose and nitroglycerine has been replaced by nitroguanidine. These are sometimes referred to separately as triple base powders. Typical single based powders are found in rifle cartridges and some rim fire rifle and revolver cartridges, whereas double based powders are used in revolver and pistol as well as shotgun cartridges. Rim fire rifle and revolver cartridges may also be double based [7].

Nitrocellulose is used, not only in propellants and explosives, but also in lacquers, varnishes celluloid films and the printing and pharmaceutical industries [8]. Nitroglycerine occurs in both pharmaceutical preparations and explosives.

All smokeless powders, in addition to explosive ingredients, contain a number of additives. These additives fulfill the role as stabilizers, plasticizers, flash inhibitors, coolants, moderants, surface lubricants, and antiwear additives. A particular propellant powder will contain one or more of these additives depending on its use.

Common stabilizers used are diphenylamine (DPA), the centralites, and resorcinol. Diphenylamine is the most common stabilizer used in smokeless powders, especially in single base powders. In smokeless powders, the diphenylamine content is single base powders about 1% [7]. In addition, the main reaction products nitric oxide (NO_2) with DPA, 2-nitrodiphenylamine, 4-nitrodiphenylamine, and N-nitrosodiphenylamine have also been reported to be frequently encountered in gunpowder's [9-11]. Another group of stabilizers used in smokeless powders are the centralites. The most common is ethyl centralite, but sometimes methyl centralite may also be used. Ethyl centralite is usually found in double base powders. Another stabilizer found in some smokeless powders is resorcinol.

In the process of making powder grains, plasticizers are mixed with the powder components. These provide strengthened flexibility to the grains. Some of the plasticizers used are glyceryl triacetate (triacetin), dimethyl phthalate, diethyl phthalate, and dibutyl phthalate. Dinitrotoluene is used as a flash suppresser in some smokeless powders. Nitroguanidine fulfills the same roles. The role of a flash suppresser is to produce nitrogen gas to dilute the muzzle gases. The powder grains are also coated with graphite which reduces any hazards which could arise from static electricity and it also acts as a surface lubricant to improve the flow properties of powder during cartridge manufacture.

The bullet in an automatic pistol cartridge contains a lead core that is covered with a full metal jacket of copper alloyed with 5 to 10% zinc. Revolver bullets are generally composed of lead, or lead plated with a thin layer of copper, or the lead is alloyed with antimony or tin, or with both. In some bullets, such as hollow- point and soft- point bullets, a copper jacket covers the base and cylindrical portion, leaving a soft metal at the tip.

Cartridges cases are usually composed of brass and the brass case may sometimes be coated with nickel which leads to this element being present in some residues. Materials present on the interior surface of a gun barrel can give rise to gunshot residues. Iron and oil as well as rust and fouling from previous shots can be found inside a gun barrel. Bullet Lubricants are common in cartridges providing a smooth release of the bullet from the cartridge casing. The usual components found in gunshot residues are summarized in Table 1.1

Table 1.1 The usual components found in gunshot residues [4]

Gunshot Residues Components	Usage
Organic	
Nitroglycerine	Propellent
Resorcinol	Stabilizer
2,4 Dinitrotoluene	Flash inhibitor
2,6 Dinitrotoluene	Flash inhibitor
2,3 Dinitrotoluene	Flash inhibitor
Dimethyl phthalate	Plasticizer
Diethyl phthalate	Plasticizer
Dibuthyl phthalate	Plasticizer
Methyl centralite	Stabilizer
Diphenylamine	Stabilizer
Ethyl centralite	Stabilizer
Inorganic	
Antimony	Fuel
Iron	Bullet material
Barium	Oxidizing agent
Calcium	Fuel
Magnesium	Fuel
Aluminium	Fuel
Nickel	Bullet material
Zinc	Bullet material
Lead	Explosive (lead stypnate)
Copper	Bullet material

1.2.3 Charecteristics of Gunshot Residues

When a firearm is discharged, a residue is produced that can be deposited on the hands of the shooter. On targets and in weapons, the residue is made up of unburned powder, as well as metallic particles from the primer, the cartridge casing and the barrel of the gun [12].

There are three major reasons for analyzing explosive and gunshot residues: 1) to calculate firing distances; 2) identify bullet holes; and 3) to determine whether a person has fired a gun or participated in a terrorist blast. Due to these reasons, the analysis of explosives and gunshot residue has become one of the most crucial parts of forensic science. Since the work of Harrison and Gilroy [13], gunshot analysis has been based on the evaluation of the metals lead, barium, and antimony, the primary metals found in bullets. These evaluations require various techniques of metallurgical analysis, including neutron activation analysis (NAA) [14], atomic absorption spectroscopy (AAS) [15], and inductively coupled plasma mass spectrometry (ICP-MS) [16].

A review of all aspects of gunshot residue analysis included a summary of the specific nature of inorganic and organic residues, how they arise, and how they may be collected from a variety of matrices. Techniques discussed included scanning electron microscopy with energy dispersive X-ray analysis (SEM-EDX) [17], flameless AAS [18], NAA, energy dispersive X-ray fluorescence (EDXRF) [19] for inorganic residues and GC [20], HPLC [21] and supercritical fluid chromatography (SFC) [22] with special detectors for organic residues.

For now, the most accepted technique is SEM-EDX, which combines morphological (SEM) and elemental (EDX) identification of the metal residues. Although it is considered to be specific, heavy-metal analysis offers moderate diagnostic sensitivity, requires expensive instrumentation and is highly demanding in terms of professional skills, manpower, maintenance, and service. As a result, its application as a routine technique is difficult. Besides, it has been recently discovered that some particles found in gunshot residue can be misidentified, decreasing the specificity of SEM-EDX analysis [23]. The modern trend to produce primers free of heavy metals is now causing a new problem to arise, possibly leading to an increase of false negative results. For these reasons, there is a need for a fast, cheap and specific technique that can be routinely used in laboratories with a high workload.

Capillary electrophoresis (CE) is one of the most important analytical techniques that can provide rapid, high-resolution separations of complex mixtures. In CE, separation is carried out by the two related electrokinetic effects, electrophoresis, and electroosmosis. Although electrically neutral substances such as organic gunshot residues cannot be separated by conventional CE, Micellar Electrokinetic Capillary Electrophoresis (MEKC) permits the separation of these neutral substances.

In 1984, Terabe and co-workers [24] introduced an important development in the use of micelles to carry out separation of neutral species in CE. When a high voltage is applied to a capillary tube filled with a sodium dodecyl sulfate (SDS) micelle solution, the negatively charged SDS micelles can migrate at a velocity V_{ep} toward the positive electrode by electrophoresis and the aqueous solution can flow at a velocity V_{eo} toward the negative electrode by electroosmosis Because $IV_{eol} > IV_{epl}$, the micelles will move slowly toward the negative electrode. When a neutral analyte is added into the micellar solution, some portion of them may be solubilized in the micelles. When they are inside the micelles, the solubilizate will migrate with the micelles, whereas in free solution the solubilizate will migrate with the bulk flow. Thus selective partitioning of the analytes into the micellar phase causes them to migrate at different rates from that of the bulk electroosmotic flow rate. The micelles can be considered as the "stationary phase," and the free solution is the "mobile phase". MEKC may be classified as a type of liquid-liquid partition chromatography. In conventional elution chromatography, a totally retained compound is never eluted. Conversely a compound that is totally solubilized by the micellar phase in MEKC is eluted at a time that is equivalent to the effective retention time of the retarded micelles. Hence, MEKC is characterized by a limited elution range.

Munder et al. [25] also examined these same gunpowder and explosive components using SFC. Due to the lack of complete chromatographic resolution, unambiguous identification of all components by SFC required three different detectors (UV, FID and ECD). For instance, 2,4 dinitrotoluene, diphenylamine, and N-nitrosodiphenylamine all coeluted in the system described.

In 1991, Northrop et al. reported the use of MEKC in separation and identification of organic gunshot and explosive constituents [26]. The instrument used was a commercially available capillary electrophoresis system. Sodium dodecyl sulfate (SDS) was used to form micelles. The running voltage was constant at 20 kV. Electroinjection at 5 kV for 2 s was used to introduce samples. A multiple-wavelength UV absorbance detector was used to select an ideal monitoring wavelength for different compounds. Standard solutions of 11 gunpowder constituents and 15 high explosive constituents, reloading powders, plastic explosives, and gunshot residues swabbed from spent ammunition casings, were analyzed. Qualitative identification of the components in each of the samples was made by comparison to the capacity

factors of standard solutions, by sample spiking, and by monitoring at selected wavelengths. Eleven gunpowder constituents were completely separated. In the analysis of a mixture of 26 gunpowder and high-explosive components, coelutions were limited to two of the mononitrotoluene isomers and two of the dinitrotoluene isomers. In analysis of gunshot residues collected from two spent ammunition casings, both casing showed the presence of ethylcentralite and nitroglycerin. The plasticizer dibutyl phthalate (DBP) was also found in all gunshot residue samples and swab blanks. The swabbing solvent was obtained from a plastic storage container. The reloading powders and plastic explosives have also been successfully analyzed and identified.

The work of MacCrehan et.at. [27] explained the sampling protocols for the recovery of the organic components under a variety of sampling conditions to improve the MEKC analysis. They described the collection of residue samples where external contaminations (e.g., grease or blood that were present on the residue substrate) were investigated using protocols for both tape lifts and solvent swabs. The same group analyzed organic additives in smokeless gunpowder by MEKC to evaluate residues contamination from previously fired ammunition.

A protocol was also presented recently for collecting and analyzing gunshot residues from hair using a fine-tooth comb [28]. Residues were collected from four weapons including a revolver, a semi-automatic pistol, a rifle and a shotgun. One characteristic additive, nitroglycerine, was detected by CE in the majority of the collection experiments.

In casework, MEKC has also been used to examine characteristic organic gunpowder components (i.e., nitroglycerin, diphenylamine, and ethylcentralite) [29-30]. The first study compared the results of MEKC analysis of the organic gunshot residues from firing-range samples with SEM results for inorganic residues, indicating that this methodology is a potentially valuable tool in the examination of gunshot-residue evidence for characteristic organic gunpowder compounds. Due to the numerous experimental and instrumental variables to be optimized in the development of a MEKC methodology, Casamento et.at. [31] used artificial neutral networks to optimize variables to separate 12 explosives. Also, a work based on comparing MEKC employing SDS and gradient reverse-phase HPLC, separated 14 organic explosive constituents of gun powders, concluding that, using diode-array UV detection, CE is more feasible than gradient HPLC.

In order to separate inorganic and organic gunshot residues, a CE method was developed [32]. Pre-capillary complexing agent diaminoethanetetraacetic acid (EDTA) as used, since it forms stable anionic complexes with 10 inorganic components of different gunshot residues. Thus, so as to separate 11 organic residues, it was necessary to add a micellar phase to the background electrolyte because almost all of the organic residues lacked any acid-base properties. To test the possibility of applying the developed method to real cases, residues from shot samples from different firearms were analyzed and their results compared with those obtained with Electrothermal Atomic Absorption Spectrometry (ET-AAS), the conclusion being that there was good agreement between both techniques. The method was tested on real samples collected from weapons and from hands after firing.

The gunshot residue samples analyzed by Northrop were swabbed from spent ammunition casing that were abundant in organic gunshot residues and free contamination. However, gunshot residue samples collected from a firer's hand were usually heavily contaminated and of low concentration. It becomes necessary therefore to carry out a further investigation to verify the feasibility of using MEKC for the analysis of gunshot residues sampled from a firer's hand. In 1992, these same authors reported their further work in gunshot residue detection by MEKC [33]. The samples were collected by adhesive tape lift from the firer's hands immediately after discharging a handgun three times. Suspected particles were either/directly removed from the adhesive tape or a section of the tape was extracted with ethanol. Ethylene glycol was added to the extracts to prevent the sample from going to dryness during the evaporative concentration which resulted in the loss of analytes. The extracts were then evaporated under a stream of nitrogen and reconstituted with the MEKC buffer. The reconstituted extracts were introduced to the MEKC by gravity injection to avoid the small bias when electrokinetic injection was used. The running voltage was constant at 25 kV. They found that the tape lift sampling technique was able to circumvent the analyte losses by adsorption during collection and handling, and minimized coextraction of sample matrix interferents associated with solvent swabbing collection methods.

1.2.4 On-Line Sample Preconcentration

Most reports have been published describing gunshot residues analysis by MEKC with a UV detector, however, detection sensitivity is a limitation. The sensitivity of CE

can be improved by applying sample concentration either prior to analysis or at the injection step. Of considerable interest to improve concentration sensitivity in CE is the development of on-line concentration techniques, which include sweeping and high salt stacking which may be performed individually or in combination. On-line techniques are easily implemented technologies because of their simplicity and economy.

A relatively new on-line sample preconcentration technique in MEKC called sweeping, is defined as the sorption and accumulation of analyte molecules by the pseudo-stationary phase (PS) that enters and fills the sample zone upon application of voltage. This phenomenon was initially observed by Gilges [34], however, it has not been well studied until recently.

Sweeping in CE involves the interaction of a pseudo-stationary phase (PS) in the separation solution and a sample in the matrix that is free of the PS used [35]. The PS includes not only the PSs employed in electrokinetic chromatography, but also complexation reagents such as borate. The sample matrix could have a lower, similar, or higher conductance than the separation solution. Thus, the basic condition for sweeping is a sample matrix free of the additive. The accumulation of analyte molecules during the interaction makes this interesting phenomenon very useful as on-line preconcentration method for CE. Preconcentration occurs due to chromatographic partitioning, complexation, or any interaction between analytes and PS. Contact between analyte and PS is facilitated by the action of electrophoresis and is independent of electroosmosis. The analyte, PS, or both should have electrophoretic velocities when an electric field is applied. The extent of preconcentration is dictated by the strength of the interaction involved. From tens to several thousand-fold improvements in detector response for many neutral and charged analytes have been achieved with this technique, suggesting sweeping as a general approach to on-line preconcentration in CE. The mechanism and applications of the sweeping phenomenon under different experimental conditions are discussed in the following review, with particular emphasis on a better understanding of the sweeping mechanism under reduced electric field (high conductivity) in the sample zone.

Gong et al. [36] reported a new sweeping carrier for the preconcentration of negatively charged solutes with a cationic surfactant, dodecyltrimethylammonium bromide (DTAB), at a concentration below the CMC. The sweeping power of DTAB below the CMC was

investigated. The optimal conditions were determined, and the concentrating capability was evaluated.

Palmer et al. [37] have developed a method of stacking based simply on manipulation of the sample matrix. Many solutions for sample stacking in MEKC are based on strict control of pH, micelle type, electroosmotic flow (EOF) rate, and separation-mode polarity. However, a universal solution to sample stacking in MEKC should allow for free manipulation of separation buffer parameters without substantially affecting separation of analytes. Analogous to sample stacking in capillary zone electrophoresis by invoking field amplification of charged analytes in a low conductivity sample matrix, the proposed method utilizes a high conductivity sample matrix to transfer field amplification from the sample zone to the separation buffer. This causes the micellar carrier in the separation buffer to stack before it enters the sample zone. Neutral analytes moving out of the sample zone with EOF are efficiently concentrated at the micelle front. Micelle stacking is induced by simply adding salt to the sample matrix to increase the conductivity 2-3 folds higher than the separation buffer. This solution allows free optimization of separation buffer parameters such as micelle concentration, organic modifiers, and pH, providing a method that may complement virtually any existing MCE protocol without restricting the separation method.

The separation and determination of four parabens (methyl, ethyl, propyl, and butyl p-hydroxybenzoate) which are commonly used as preservative in cosmetic products, by micellar electrokinetic capillary chromatography (MEKC) with and without large volume sample stacking (LVSS) technique were compared [38]. As an effective on-line concentration technique, LVSS was successfully combined with MEKC to determine neutral parabens in an acidic media. The effect of some typical parameters, such as sample volume, buffer pH, temperature, and concentration of surfactant were examined.

A selective on-line sample preconcentration technique of anion selective exhaustive injection (ASEI)-sweep-micellar electrokinetic chromatography (MEKC) was evaluated using a cationic surfactant [39]. To suppress the electroosmotic flow, a polyacrylamide-coated capillary was introduced. Some aromatic carboxylic acids, dansyl amino acids, and naphthalene-disulfonic acids were used as test analytes. About 1,000- to 6,000-fold increases in detection sensitivity were obtained in terms of peak heights by ASEI-sweep-MEKC.

Quirino et al. [40] have investigated sample concentration by sample stacking and sweeping utilizing microemulsion and a single isomer sulfated β-CD as pseudo-stationary phase in MEKC. Again, Quirino et al. [41] have explained the concentrating mechanism in MEKC when the sample matrix is a high resistivity non-micellar aqueous solution. Theoretical and experimental studies were undertaken. It was found that the total focusing effect is brought about by the cumulative effect of sweeping and sample stacking. For better analytical results, compounds showing low to moderate retention factor (k) and compounds showing high values of k must be dissolved in low and high conductivity matrices, respectively.

Quirino and Terabe [42] have devised a mixed mode for the further stacking of analytes in which the sample is concentrated first by field-amplified injection under non-micellar conditions. The buffers are changed, and the polarity is reversed to induce sweeping of the analytes into a micellar (SDS) solution giving about a million-fold increase in sensitivity for some cations.

The concept of sweeping neutral analytes applying a high salt containing matrix or under a reduced electric field in MEKC using anionic micelles, and in the presence of electroosmotic flow is presented by Quirino et al. [43]. Three important processes were identified. First, stacking of the micelles at the cathodic interface between the sample solution and background solution zone was identified, then followed by the sweeping of the analytes molecules by the stacked micelles that enter the sample zone. Finally, the destacking of the stacked micelles at the anodic interface between the sample and background electrolytes zones occurred.

High salt stacking in electrokinetic chromatography (MEKC) is defined and contrasted to the sweeping method [44]. A recent paper argued the two methods are identical, where high concentrations of micelle in the sample were intended to mimic the effect of high salt staking. However, high micelle concentration in the sample matrix in MEKC is analogous to using a high conductivity sample instead of a low conductivity sample in field amplified stacking. High salt staking does not require a sample free of pseudo-stationary phase, only a sample with a high mobility co-ion compared to the separation buffer electrokinetic vector. High salt stacking uses a discontinuous buffer system and should not be confused with continuous buffer stacking systems such as sweeping.

1.3 Research Objectives

The following objectives have been addressed with necessary requirements either in terms of testing the hypothesis or to explain against theoretical perspectives.

1.3.1 To develop the methods for the efficient separation of 2,4 DNT, 2,6 DNT and 3,4 DNT using optimized MEKC methods.

1.3.2 To investigate optimized condition by varying level of surfactants, types of buffer (acetate, phosphate and borate), buffer pH, applied voltage and detection wavelength.

1.3.3 To improve CE sensitivity by on-line sample concentration methods.

1.3.4 To analyze real sample under optimum condition by using the selected method.

CHAPTER 2

SEPARATION MECHANISM IN CAPILLARY ELECTROPHORESIS

2.1 Capillary Electrophoresis

Electrophoresis has been one of the most widely used techniques for the separation and analysis of ionic substances by attraction or repulsion in an electric field. It is stated that the cathode attracts positive ions under an electric field, and the anode attracts negative ions. In 1937, Tiselius was first introduced a separation technique based on this simple principle. He found that when a protein mixture was placed in a buffer solution, and an electric field was applied, the sample components migrated in a direction and at a rate determined by their charge and mobility [1]. This work of Tiselius on electrophoresis led him to win a Nobel Prize in 1948.

Hjerten described zone electrophoresis in free solution in 1967 [1]. He performed zone electrophoresis in tubes of quartz glass, having inner diameters of 1-3 mm and coating of methylcellulose to prevent electroosmosis. This free zone electrophoresis technique was applied to the separation of a great variety of samples including proteins and nucleic acids, as well as viruses. The most widely accepted initial demonstration of the potential of the use of CZE was performed by Jorgenson and Lukacs in fused silica capillaries [2]. This technique was first applied to the analysis of biological macromolecules, such as proteins, peptides, amino acids, nucleotides, oligonucleotides, etc. Terabe [45] developed the technique of MEKC, which further widened the applications of CE to include separations of neutral substances. In the following years many improvements with respect to detection systems as well as performance has been made. Additionally, many sub-techniques related to CZE have been developed to meet the need for powerful separation techniques.

The performance of electrophoresis in a narrow fused-silica tube, i.e. internal diameters less than 75 μm, has granted electrophoresis extremely high power in terms of high separation efficiency, fast response time, and very low sample volume requirement, i.e. less than 50 nl. The low required sample volume makes CE an ideal microscale analytical technique for probing microenvironmental and microbiological samples, e.g. single cells. It is also effective

for the analysis of a large range of compounds from small ions and neutral molecules to macromolecules. CE has been successfully used in a wide range of application areas. The applications cover food analysis [46], including the analysis of vitamins [47], food additives [48-49], environmental [50], biomedical [51] as well as pharmacological analysis [52]. There are thousands of CE instruments installed in laboratories worldwide, and applications of CE have been reported in every major industry. Currently, there are in excess of 7000 publications describing CE applications and developments, and this number is sharply increasing [53]. This is because one of the greatest advantages of CE is its diverse application range.

Figure 2.1 is a schematic diagram of the basic components of a CE instrument. The ends of a fused-silica capillary (narrow-bore) are placed in buffer reservoirs. The reservoirs are filled with the buffer electrolyte .The electrodes are immersed in the reservoirs to make electrical contact between the high-voltage power supply and the capillary. Sample is loaded into the capillary by replacing one of the reservoirs with a sample reservoir and applying either an electrical field (electromigration injection) or an external pressure or vacuum (hydrostatic injection). After replacing the sample reservoir, the electrical field is applied, and the separation is performed. Detection is made at the opposite end directly through the capillary wall for optical detection or by coupling the capillary with the other detection techniques, such as mass spectrometry or electrochemical detection through specially designed interfaces.

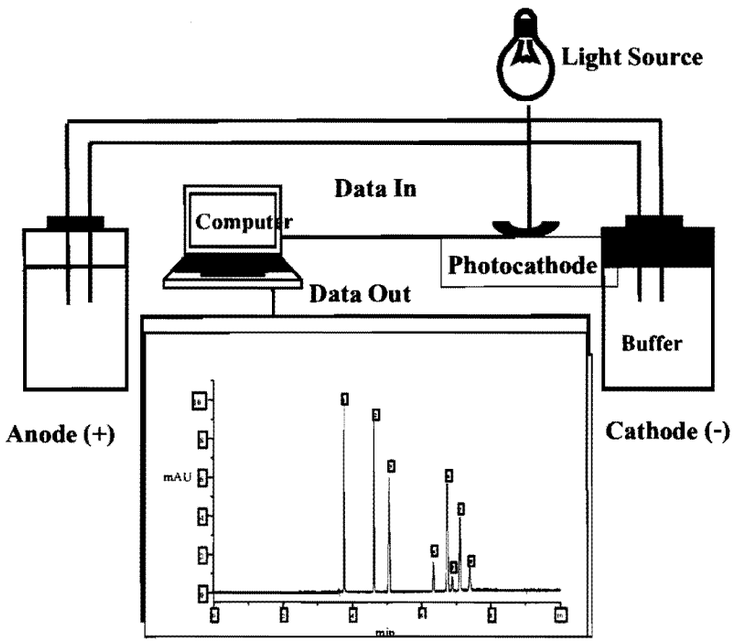


Figure 2.1 Schematic diagram of the basic components of a CE instrument [54]

2.1.1 Electrophoretic Mobility

The movement (migration) of charged species under the influence of an applied field is characterised by its elctrophoretic mobility (μ_{ep}). Mobility is dependent not only on the charge density of the solute but also on the dielectric constant and viscosity of the electrolyte. Mobility is also strongly dependent on temperature. In the presence of EOF, the apparent mobility (μ_a) is the sum of electrophoretic mobility of the analyte, μ_{ep}, and the mobility of the EOF, μ_{eof}.

$$\mu a = \mu ep + \mu eof \tag{2.1}$$

The apparent mobility may be determined using the following equation [1].

$$\mu_a = \frac{l}{tE} = \frac{lL}{tV} \tag{2.2}$$

Where l is the effective capillary length (cm). i.e. to the detector, L is the total capillary length (cm), t is the migration time (s), E is the electric field (V/cm) and V is the applied voltage (V). The apparent mobility of the solute is given as μa (cm^2/Vs), and it is measured mobility in the presence of EOF. The mobility of EOF (μeof) can be measured using a neutral marker that moves at a velocity equal to the EOF. Examples of neutral markers include methanol, acetone, benzene, and dimethyl sulfoxide [1].

2.1.2 Electroosmotic Flow (EOF)

EOF is the term used to describe the movement of a liquid in contact with a solid surface on the interior capillary wall when a tangential electric field is applied. This movement is also known as electroosmosis or electroendoosmosis. EOF occurs in fused silica capillaries because acidic silanol (Si-OH) groups at the surface of the capillary dissociate when in contact with an electrolyte solution (buffer), according to:

$$SiOH(s) \rightleftharpoons SiO^-(s) + H^+(aq) \qquad (2.3)$$

Hydrated cations in the electrolyte solution are attracted to the negatively charged silanol and became arranged into two layers. As illustrated in Fig 2.2, one layer is tightly bound by electrostatic forces (compact layer), and the other is more loosely bound (diffuse layer).

EOF occurs when an electric field is applied. Cations are attracted to the cathode and anions are attracted to the anode. Excess cations in the diffuse part of the double layer develop a net momentum toward the cathode. This brings the bulk solution towards the cathode, leading to electroosmotic solution flow.

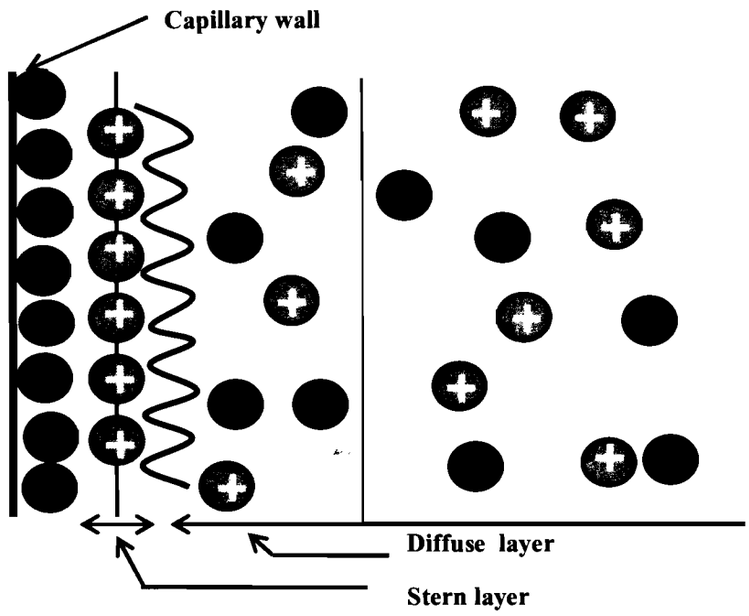


Figure 2.2 Representation of the double layers at the capillary wall [55]

The magnitude of the EOF can be expressed in terms of velocity or mobility by:

$$V_{EOF} = \mu_{eof}.E = (\varepsilon\zeta/\eta)E \qquad (2.4)$$

μ_{eof} = $\varepsilon\zeta/\eta$); EOF mobility

V_{EOF} = velocity (cm/s)

ζ = Zeta potential (V)

η = viscosity (Ns/m^2)

ε = dielectric constant (C^2/Jm)

E = electric field (V/m)

The speed of the EOF is directly related to the magnitude of the zeta potential (ζ). Factors affect ζ will affect the EOF. The zeta potential is the potential at the plane of shear and is dependent on both the nature of the solid surface and the ionic state of the liquid.

EOF is determined/controlled by pH. At low pH EOF is not very significant due to protonation of the silanol group at the capillary wall. High pH results in deprotonation of the silanol group making the EOF quite significant. Depending on the specific conditions, the EOF can vary by more than an order of magnitude between pH 2 and 12, Parameters, such as ionic strength can also have significant effect on the EOF. A unique feature of EOF in the capillary is the flat profile of the flow, as depicted in Figure 2.3.

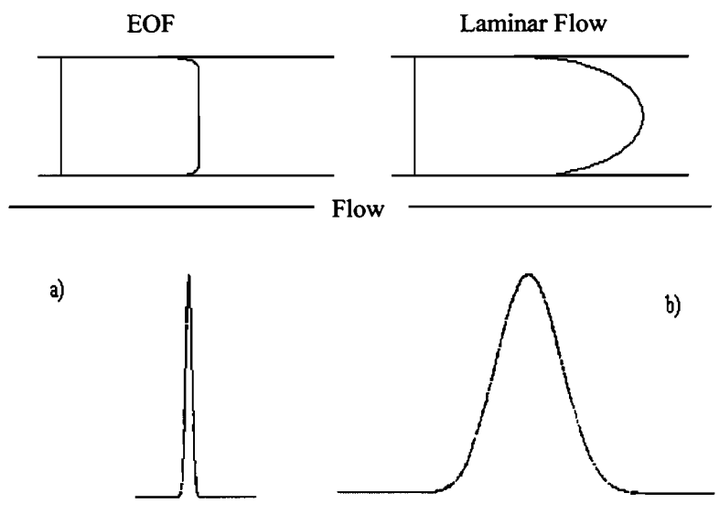


Figure 2.3 Flow profile and corresponding solute zone: a) EOF flow, and b) laminar flow [56]

There is no pressure drop within the capillary, and the flow is nearly uniform throughout as the driving force of the flow is uniformly distributed along the capillary. Figure 2.3a shows that a flat flow profile is beneficial since it does not directly contribute to the dispersion of solute zones. Note that the zone dispersion at the limit arises from molecular diffusion. This is in contrast to the flow that generated by an external pump, which yields a laminar or parabolic flow (Figure 2.3b), due to the shear force at the wall. Here, molecular diffusion is supplemented by multi-flow effects to give a broadened zone.

EOF causes movement of nearly all species, regardless of charge, in the same direction. Under normal conditions, giving a negatively charged capillary surface, the flow is from the anode to the cathode. Cations migrate fastest since the electrophoretic attraction is toward the cathode, and the EOF is in the same direction. Neutrals are all carried at the velocity of the EOF,

but are not separated from each other, and anions migrate slowest since they are attracted to the anode, but are still carried by the EOF toward the cathode.

2.1.3 Factors Affecting Efficiency

CE separation is based on differences in solute mobility. Different solutes have different mobilities, therefore, they migrate through the capillary at different speeds. Dispersion can cause the zone length to change, and as a result affects solute separation. The factors are Joule heating, injection plug length, electromigration dispersion and solute interactions with the capillary walls cause band spreading, and hence affect separation efficiency. These are described below:

2.1.3.1 Joule Heating

The conduction of electric current through an electrolytic solution results in the generation of heat arising from frictional collisions between mobile ions and buffer molecules. The degree of heating is determined by the capillary dimensions, i.e. a narrow bore capillary prevents joule heating due to ease of heat dissipation, conductivity of the buffer, and the applied voltage. Excessive heating may lead to thermal gradients across the column, which can affect solute mobilities and cause zone broadening.

2.1.3.2 Injection Plug Length

Sample plug length minimisation is important during injection. If the length is longer than the dispersion causes by diffusion, efficiency and resolution will be sacrificed. A practical limit of injection length is less than 1% to 2% of the total capillary length unless special injection methods, such as sweeping or high salt stacking are used, which cause injection zone compression.

2.1.3.3 Solute-wall Interactions

Interaction between the solute and the capillary wall is detrimental to CE separation. The primary causes of adsorption to the fused silica walls are ionic interaction (i.e. between cationic solutes and the negatively charge wall) and hydrophobic interactions. Thus, coated capillaries may be employed in some applications to reduce such deleterious interaction. Note that generally sorptive partitioning will not be desirable at the coated surface.

2.1.3.4 Electrodispersion

Differences in sample zone and running buffer conductivity can have major effects resulting in skewed peak shapes, solute concentration or focusing (low conductivity sample) or solute defocusing (high conductivity sample) [57]. When the solute zone has a higher mobility than the running buffer, the leading edge of the solute zone will be diffused and the tailing edge will be sharp. An opposite effect is observed when the solute zone has a lower mobility than the running buffer, the leading edge will be sharp and the tailing edge will be diffused. When the conductivities are equivalent, no such peak distortions will occur, and symmetrical peaks are produced.

2.1.4 Sample Injection Methods

To obtain high resolution in CE, the volume of the injected sample must be very small compared with the volume of the capillary. As a rule of thumb, the sample plug length should be less than 1 to 2% of the total length of the capillary. The two most common injection techniques are hydrodynamic and electrokinetic [1, 58-59], which are further outlined as follow.

2.1.4.1 Hydrodynamic Injection

Hydrodynamic injection is the most widely used sample introduction in CE. Sample is introduced to the capillary by a pressure difference maintained between the ends of capillary. This is realised by imposing pressure at the injection end of the capillary, a vacuum at the exit end of the capillary, or the siphoning action obtained by elevating the injection reservoir relative to the exit reservoir. The volume of sample injected is a function of the pressure difference across the capillary, the injection time, the capillary internal radius [60], solute solution and buffer viscosities. The hydrodynamic injection volume, V_{inj}, is estimated by the equation [61].

$$V_{inj} = \pi r^2 \left(\frac{\Delta P r^2 \times t_{int}}{8\eta \times L} \right) \qquad (2.5)$$

ΔP = pressure difference across the capillary (N^2/m)

r = internal radius of the capillary (cm)

t_{int} = introduction time (s)

η = viscosity of the solution in a column (Ns/m^2)

L = the length of the column (cm)

2.1.4.2 Electrokinetic Injection

Electrokinetic injection may often be the method of choice because of its simplicity and easy control. Replacing the injection end reservoir with the sample vial and applying the low voltage for a short period, accomplishes electrokinetic injection. In electrokinetic injection, sample enters the capillary by electrophoretic mobility and pumping action of the EOF. The sample matrix greatly impacts the quantity of sample loaded as does charge on the ions. This produces a bias in the injection amounts, and this may not be representative of the relative amount of each analyte present in the original sample. The electrokinetic injection volume, V_{inj}, is estimated by the equation [61].

$$V_{inj} = \frac{\pi r^2 \times \mu_{app} \times V_{int} \times t_{int}}{L} \tag{2.6}$$

μ_{app} = apperent mobility (cm^2/s)

V_{int} = introduction voltage (V)

2.1.5 Resolution

The simplest way to characterise the separation of two components is to divide the difference in migration time by the average peak width [58].

$$R = \frac{2(t_{m2} - t_{m1})}{w_1 + w_2} \tag{2.7}$$

t_m = migration time (s)

w = baseline peak width (in time) (cm)

The time (t_m) taken by the solute to migrate the distance (l) from the injection end of the capillary to the detection point (capillary effective length) is given by the expression:

$$t_m = \frac{lL}{(\mu_{ep} + \mu_{eof})V} \tag{2.8}$$

μ_{eof} = electroosmotic mobility flow (cm^2/s)

μ_{ep} = electropheretic mobility (cm^2/s)

l = capillary effective length (cm)

2.1.6 Column Efficiency

The efficiency of a column is a number that describes that peak broadening as a function of retention, and it is described in terms of the number of theoretical plates *(N)*, where

$$N = \frac{(\mu_{ep} + \mu_{eof})V}{2D} \tag{2.9}$$

For the best separation results, the efficiency of the system must be optimized in order to minimize band broadening, and the column should have the capacity to retain the analytes and sufficient selectivity to resolve them.

2.2 Modes of Separation in CE

The basic electrophoretic separation modes encompassed by CE including CZE, Capillary Gel Electrophoresis (CGE), Capillary Isoelectric Focusing (CIEF), and Capillary Isotachophoresis (CITP) [58]. A few basic CE theoretical concepts will be introduced below.

2.2.1 Capillary Zone Electrophoresis

CZE is the basic operation mode of CE, and has been widely employed in CE. In CZE the composition of the electrolyte is constant in the capillary and the reservoirs surrounding the two electrodes. The electrolyte provides an electrically conducting and buffering medium (continuous system). On introduction of a sample, each species of analyte ions migrates in the buffer in a discrete zone and at a different velocity from the other species. Neutral molecules are carried along by the EOF as a single zone. Both anions and cations can be separated in a single run. Molecules elute from the capillary in order of decreasing positive charge, when

using "normal" potential electrolyte bias. In separations conducted at pH above 4, electroosmosis occurs and the EOF ensures that both migration of negatively charged ions and EOF are toward the cathode. The orders of elution are cations, then neutrals followed by anions; the species with smaller size and greater negative charge elutes slowest, with μ_{ep} towards the anode (injection end). The difference in migration results mainly from the different charge densities of the ionic species, which may be affected by the pH of the buffer electrolyte. In most cases, a separation optimisation can be achieved by adjusting the buffer pH. When buffer pH changes do not facilitate a satisfactory separation, selectivity can be obtained by the addition of additives into the buffer electrolyte. The additives include ion-pairing agents, complexing agents, i.e. polycarboxylic acids and chelating agents, organic solvents [62], polycyclic ethers and their derivatives, and various shape-selective additives. For example, cyclodextrin additives (CDs) are well known as chiral selectors, and they can contribute to shape selectivity for improving separation in certain conditions [59, 63]. CDs are widely used in the separation of chiral compounds [64] since the interaction energies of the two enantiomers of a chiral molecule with the CD often show different magnitudes, which provide a mechanism for differential mobility of the enantiomers.

CDs are also useful as shape-selective additives, applicable to all analytes whether or not they are optically active. As different types of association (host-guest interaction) are possible the differential apparent mobility (μ_a) leads to improvement in separation.

The schematic diagram for CZE separation is shown in Figure 2.4 and is adapted from [64].

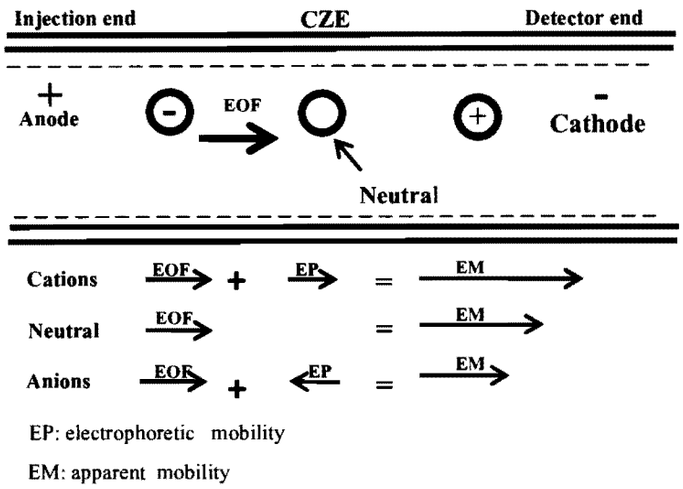


Figure 2.4 Schematic diagram showing the CZE separation of analytes with different charges (Normal potential) [65]

2.2.2 Micellar Electrokinetic Capillary Chromatography

Terabe introduced MEKC in 1984 [45]. It is distinguished for its capability to deal with the separation of electrically neutral molecules. The key for MEKC is the addition of a surfactant into the buffer electrolyte. When the concentration of surfactant is above its critical micelle concentration (CMC), aggregates of the surfactant molecules form the micellar phase, which acts as a pseudo-stationary phase. Micelles are amphiphilic (10-50 C units) with hydrophobic tail and hydrophilic head group [66].

In the absence of the micelles, all neutral molecules would reach the detector at the same time t_o, i.e. migration time of unretained solute moving at the EOF rate. Micelles injected with the sample reach the detector at time t_m (micelle migration time), which is longer that t_o as the anionic micelles migrate upstream. If a neutral molecule equilibrates or partitions between free solution and the inside of the micelles, its migration time is increased because it migrates at the slower rate of the micelle part of the time. In this case, the neutral molecule reaches the detector at time between t_o and t_m. The more time the neutral molecule spends inside the micelle, the longer is its migration time. Migration times of cations and anions are also affected by micelles because ions partition between the solution and the micelles and interact electrostatically with the micelles.

A representation of an ionic micelle is depicted in Figure 2.5 [45]. The differential interaction between the micelle and the neutral solutes causes the separation of analyte amongst the class of neutral solutes. A more complete illustration of the MEKC process for an ionic micelle is given schematically in Figure 2.6.

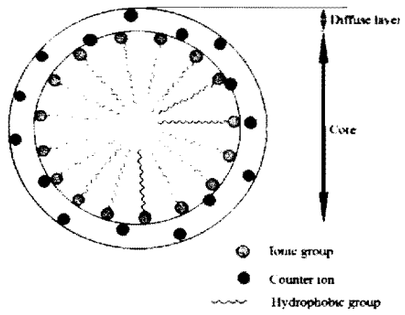


Figure 2.5 Schematic diagram of an ionic micelle [45]

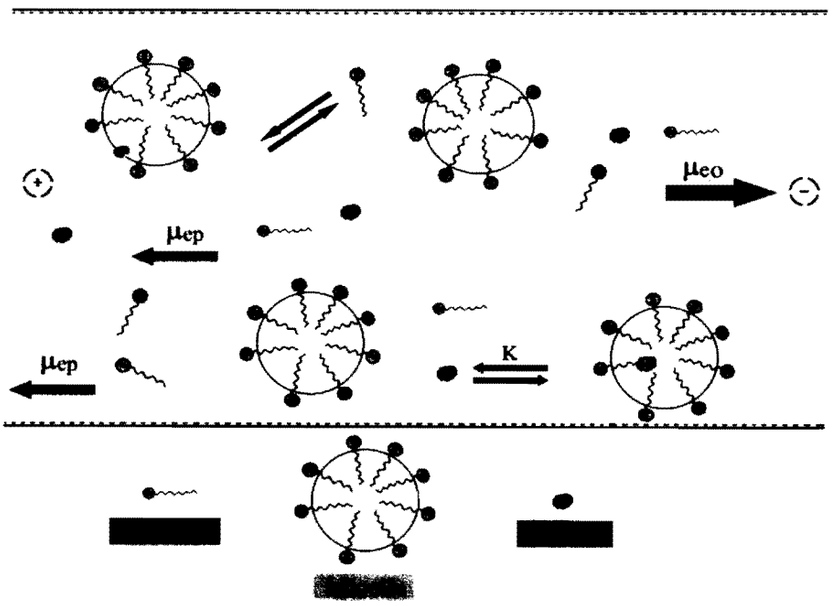


Figure 2.6 Schematic representation of the principle of MEKC. K = solute-micelle association constant [67]

By using anionic, cationic or neutral surfactants, the partition coefficients (K) of analytes can be changed. Cationic surfactants also change the charge on the wall, hence, the direction of EOF may alter.

MEKC is suitable for low to medium hydrophobicity compounds. Analyses have been performed with inorganic ions, phenols, organometallics, amino acids, carbohydrates, peptides, protein, nucleic acids, and most herbicides and pesticides, some of which may be difficult to handle with CZE. For some larger peptides and proteins, modifiers, such as organic solvents, cyclodextrins, and urea have to be added to the buffer electrolyte in order to increase the aqueous solubility of the analytes by reducing the interaction with the micellar phase, but the concentration of organic solvent is very limited due to the effect on critical micelle concentration [60].

The capacity factor (k') is a measure of the ratio of total moles of solute in the micellar phase versus those in the aqueous phase. It can be determined from the following equation:

$$k' = \frac{t_R - t_O}{t_O\left(1 - \frac{t_R}{t_M}\right)} \qquad (2.10)$$

t_R = migration time of the solute (s)

t_o = migration times of unretained solute moving at the EOF rate or "dead time" (s)

t_m = micelle migration time (s)

k' = capacity factor

Migration of solutes occurs within a time window as shown in Figure 2.7, where electrically neutral molecules elute between t_o and t_m. To improve resolution the time window needs to be increased. Hydrophilic solutes elute with EOF as they do not interact with the micelle. It is preferable to run under condition, where the time window is large that is slow EOF and mobility of micelle [68]. This effectively increases the capacity of the MEKC method, equivalent to fitting more peaks into the separation window.

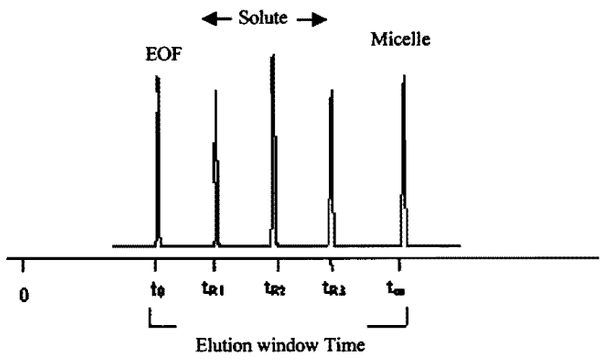


Figure 2.7 Typical elution window description for neutral species in MEKC [69]

2.3 Selectivity Control in MEKC

Selectivity is the relative order of solute migration and is determined by the mechanism that effects the separation. The control of selectivity can improve resolution and also yield complementary information that is useful for validation of a separation. The different modes of CE (e.g. CZE, MEKC), yield different selectivity due to different separation mechanisms while the focus in this section is on selectivity in MEKC only. In MEKC, selectivity of neutral analytes can be manipulated through various factors. The distribution coefficient can be modified by changing the distribution equilibrium through various experimental factors such as choices of surfactants [70], capillary surface characteristics [71], and choices of buffer additives, such as organic solvents [72] and cyclodextrin [73]. Choice of surfactants is the most effective among various factors that result in a successful separation in an acceptable migration window where the efficiency is not compromise and the outcome of the experiment is controlled by the selectivity optimization of additives and surrounding aqueous phase. It is to be noted that while these and other manipulations also result in changes in EOF, the EOF is itself not responsible for the changes in selectivity, only in migration time and resolution.

2.3.1 Surfactants

A surfactant consists of hydrophilic and hydrophobic portions. At a certain concentration, known as critical micelle concentration (CMC), surfactants are able to form

micelles which equilibrate with the monomer surfactant molecules in the solution. Micelles provide a different microenvironment (a pseudo-stationary phase) in the bulk solution [74]. Micelles contain various sites of interaction with solutes. For hydrophobic solutes, interaction between solutes and micelles based on hydrophobic force provides selectivity in differential partitioning.

After the first publication on micellar electrokinetic chromatography (MEKC) more than 10 years ago, many researchers have explored various types of monomeric surfactants above their critical micelle concentration (CMCs) as pseudo-stationary phases for the separation of both ionic and nonionic compounds. Among the pseudo-stationary phases investigated, sodium dodecylsulfate (SDS) has been successful in the MEKC separation of many water-soluble solutes [30]. However, in the case of highly hydrophobic analytes such as polycyclic aromatic hydrocarbons (PAHs), the binding with SDS micelle is often too strong to permit adequate resolution of these compounds.

The type of surfactant has a significant influence on solute migration/retention behaviour in MEKC. Ionic surfactants have an ionic group and a hydrophobic long alkyl chain in their molecule. The long alkyl-chain surfactants are believed to form the spherical micelle having ionic groups on the surface and the hydrophobic core. The analyte can be solubilised by the micelle in various ways [75]. Hydrophobic compound will be incorporated into the hydrophobic core. Therefore, it is generally predicted that the higher hydrophobicity, the stronger the interaction. High-molecular weight surfactant can form a micelle with one molecule, which cause some advantages over low-molecular weight surfactants; CMC is essentially zero; very low concentration of the micelle will be possible and, therefore, the concentration of the micelle is constant irrespective of temperature, buffer concentration, and additives; the size of the micelle is constant; a high content of organic solvent will not break down the micelle. Therefore, it has been reported that several copolymers (molecular weight ca. 40,000) offered remarkably different selectivity from SDS in MEKC.

2.3.2 Capillary Temperature and Voltage

CE separations normally performed around room temperature. Most commercial CE units are equipped with temperature control system that can keep capillary temperature in a range from 4 to 60 ^{0}C. The direct effect of the increase of temperature is an

increasing of analyte mobility and a decreasing of separation time. In some cases, increased temperatures are essential for good resolutions. Negative effects of temperature increasing include possible thermal denaturation of the sample and evaporation of some buffer components such as organic solvents. Increasing voltage may also have both positive and negative effects. The increased voltage can increase analyte electrophoretic mobility, and thus shorten analysis time. It also may increase the sharpness of the peaks. However separation at higher voltages may result in a lower efficiency and resolution [76].

2.3.3 Buffers

The running buffer selection is also important for a successful separation in any CE techniques. A buffer for use in common CZE should possess some qualities e.g. good buffering capacity in the pH range of choice, low absorbance at the wavelength of detection and low mobility to minimize current generation.

In most MEKC separations, neutral or alkaline buffers are employed to generate a strong EOF. Due to the high chemical stability of the fused silica capillary, the accessible pH range can vary from below 2 to more than 12, but may have limitation in relation to solute stability by extremely low or high pH. In addition to affecting solute charge, changing the pH will also cause a concomitant change in EOF. For instance, adequate resolution may be obtained at low pH, but when it is increased to faster EOF so that solutes may elute before the resolution is achieved.

2.3.4 Organic Modifiers

Buffer modifiers are added in the separation buffer for increase of sample solubility, adjustment of the migration, improvement of the selectivity, and extension of the elution window. The addition of an organic solvent in the micellar solutions of MEKC was first reported to improve the separation of highly hydrophobic compounds [78].

Organic solvents may influence the MEKC separation process in several ways: they can extend the solution migration time with an increase in peak capacity, increase sample solubility, provide a more equitable distribution of hydrophobic analytes between micellar and mobile phases, give an element of selectivity, and degrade separation performance at relatively high concentrations [79].

2.3.5 UV Detection

On-column UV absorption has been the most commonly used detection technique in MEKC as well as in other CE modes [80]. The main reasons for its popularity include its relatively universal nature and its widespread availability for former HPLC work. The small capillary dimensions and the small volumes employed in all CE modes present a challenge to achieve a sensitive detection without introducing zone dispersion. In on-column UV detection, a window has to be made on the polyimide coating of the fused silica capillary. The simplest way that can be used to form the window is by burning off a small section of the polyimide. Most micellar solutions are considerably transparent down to 200 nm and therefore a range of 200-220 nm is often used.

Many other detection techniques have been explored with varying degree of success. Few approaches have been made to improve the detection sensitivity by using more sensitive mode of detection, such as laser-induced fluorescence (LIF) detection [81]. But the application of CE-LIF is limited as it requires pre-derivatization of solutes with a suitable fluorescent tags priors to the analysis if the solutes does not pose any inherent fluorescence properties. Separation of neutral particles by MEKC, combined with sensitive mass spectrometry detection is rare [82] and the sensitivity is yet reach appreciably low levels.

2.4 On-line Sample Concentration

2.4.1 Sweeping

Sweeping was referred to "the picking and accumulation of analytes by pseudo-stationary phase that enter into the sample zone under an applied voltage." The principle of the sweeping technique is that the conductivity of a sample matrix is equivalent to that of the running buffer, but an analyzed solution contains no surfactant, sodium dodecyl sulfate [83-84]. The definition of "sweeping" was later expanded by Terabe and coworkers [85], "sweeping" comprises of any condition in which a running buffer contains a separation vector and the sample matrix does not. Figure 2.8 shows the mechanism of stacking for an equal conductivity sample matrix and a system with negatively charged micelles and normal EOF. In summary, under a homogeneous electric field, neutral analytes are carried toward the negative electrode only to be swept up in the micelle at the boundary of the sample region. The efficiency of the stacking relies

on an analytes' affinity for a micelle, an increase in the affinity of micelles results in the higher extent of stacking. Terabe derived the following equation representing an extent of stacking as a function of an analytes affinity for micelle.

$$l_{sweep} = l_{inj}(1/1+k) \tag{2.11}$$

where l_{sweep} is the length of the swept analyte (cm), l_{inj} is the length of the injected plug (cm), and k is the retention factor for a given analyte.

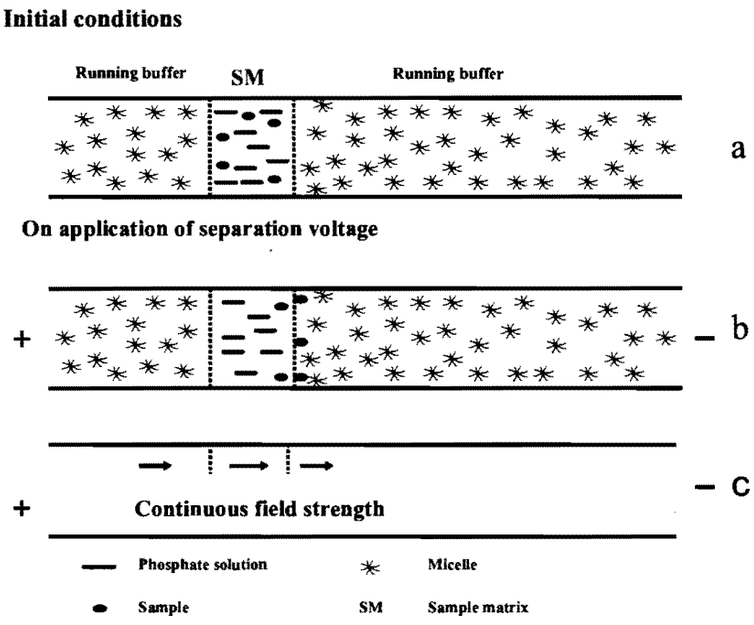


Figure 2.8 Schematic representations of sweeping (a) Starting situation: Sample solution prepared in sample matrix having conductivity similar to the running buffer. (b) Application of voltage at position polarity (c) Continuous buffer system (adapted from Landers [86].)

2.4.2 High salt stacking

High-salt stacking is a mechanism of analyte preconcentration provided by the stacking of micelles at the negative electrode side of the sample plug [87]. Analyte enters into the

stacked micelle region experiencing a local high retention factor caused by the high micelle concentration. High salt stacking is achieved under the following conditions:

$$\mu_{sample} E_{sample} < \mu_{ev} E_{ev} \quad (2.12)$$

$$\mu_{sample} > \mu_{ev} \quad (2.13)$$

Where μ_{sample} is the mobility of the sample stacking co-ion (cm^2/Vs), E_{sample} is the electric field in the sample zone (V/cm), μ_{ev} is the mobility of electrokinetic vector (micelle) (cm^2/Vs), and E_{ev} is the field in a running buffer (V/cm). To achieve the condition that the mobility of the sample stacking co-ion is higher than the mobility of the micelles, the field in the running buffer must be higher than the field in the sample zone; this is imperative to significantly increase conductivity of the sample zone associated with the running buffer. The stacking by high salt mechanism is presented in Figure 2.9

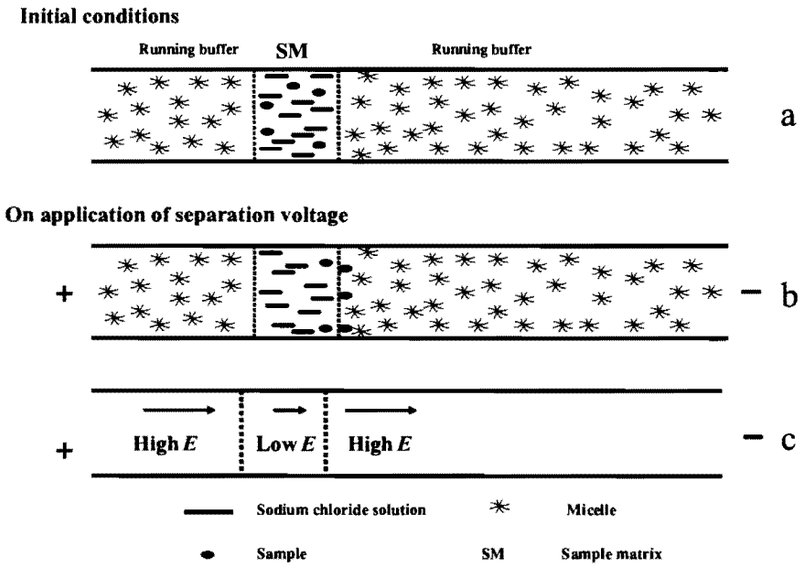


Figure 2.9 Schematic representations of high salt stacking (a) Starting situation: The conductivity of the sample zone was greater than that of the running buffer. (b) Application of voltage at position polarity (c) Discontinuous buffer system (adapted from Landers [86].)

2.4.3 Sweeping –High salt stacking

A combination of the different on-line sample concentration techniques increases concentration efficiency or simultaneous concentration of different types of analysis. The aim of this thesis is an attempt to develop two analyzing techniques, sweeping and high salt stacking by controlling conductivity of sample matrix and running buffer as can be seen in Figure 2.10

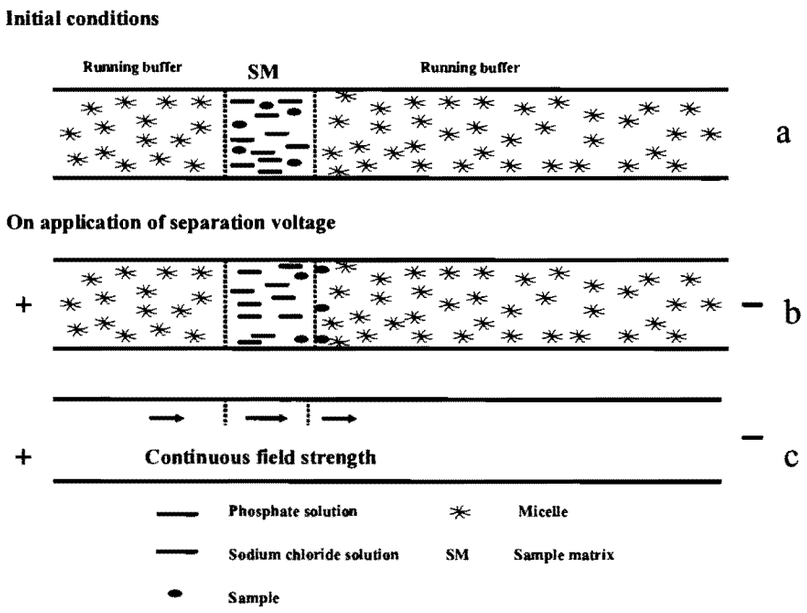


Figure 2.10 Schematic representations of sweeping-high salt stacking (a) Starting situation: The mixture was adjusted for the same conductivity as a running buffer. (b) Application of voltage at position polarity (c) Continuous buffer system (adapted from Landers [86].)

CHAPTER 3

EXPERIMENTAL PROCEDURES AND METHODS

3.1 Instrumentation

3.1.1. Capillary Electrophoresis

All experiments were carried out using a Hewlett-Packard^{3D} capillary electrophoresis system (Agilent Technologies, Germany) equipped with diode-array detection (DAD) in Figure 3.1. The HP-3D instrument fitted with UV diode-array detector was used to select the detection signal wavelength and record absorbance. Pressure and electrokinetic injection can be performed using the HP-3D. Electropherograms were generated at 25 °C.

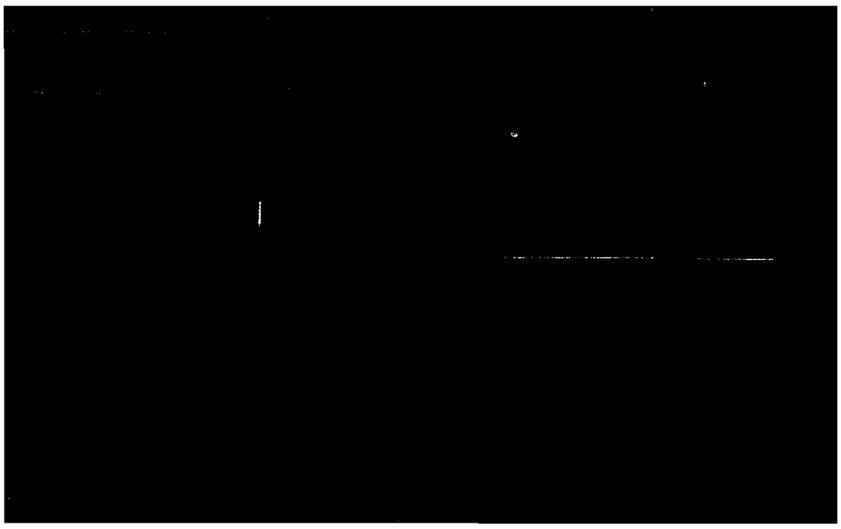


Figure 3.1 Hewlett Packard 3D Capillary Electrophoresis System

3.1.2 pH Meter

pH of solutions were measured by a Sartorius pH meter (Germany). The pH meter was calibrated with buffer pH 7 first, followed by pH 4 or pH 10. A glass electrode was rinsed with distilled water between each measurement.

3.1.3 Conductivity Meter

Conductivities were measured using an Eutech Instruments conductivity meter (Netherlands).

3.1.4 Ultrasonic

Ultrasonic cleaning uses high frequency (usually from 20–400 kHz) sound waves to agitate in a liquid using Crest Model 575HT Tru-Sweep.

3.2. Preparation of Separation Columns [29]

3.2.1 Capillary Column

Capillary columns used for analysis were prepared by cutting the appropriate length of uncoated fused silica capillary. Detection windows was created by either removing the polyimide coating using hot 97% sulfuric acid or burning it off using a gas flame at an appropriate distance from the outlet end of the capillary. A capillary window of about 0.5 cm was created to accommodate the light path slit on the detector of the CE instruments used. Care must be exercised when handling a capillary with a window since it was very brittle after the polyimide was removed.

All the separations were performed on a fused-silica capillary of 64.5cm (56 cm effective length) x 75 μm I.D. (Agilent, USA). This tubing was uncoated fused silica capillary column material.

3.2.2 Conditioning of Capillary Columns [32]

The new capillary columns were pressure-rinsed with 1 M NaOH for 15 minutes and then water for 30 minutes in order to activate the silica on the wall. The columns were treated with 1 M NaOH, then Deionized water, followed by the carrier electrolyte at the start of each working day. The following washing steps were used prior to each electrophoretic run: (1) water for 3 minutes; (2) running buffer for 3 minutes. The capillary column was cleaned daily by washing it with 1 M NaOH for 3 minutes and then water for 5 minutes.

The column was flushed with Deionized water and equilibrated with running buffer for 3 minutes between analyses. The capillary column was cleaned daily by washing it with 1 M NaOH for 3 minutes and then water for 5 minutes.

3.3 Chemicals

HPLC-grade solvents (methanol and ethanol), monosodium phosphate, disodium phosphate and sodium dodecyl sulfate from Carlo Erba were used for preparation of the running buffer. 2,4 dinitrotoluene (2,4 DNT) was obtained from Acros Organics. 2,6 dinitrotoluene (2,6 DNT), 3,4 dinitrotoluene (3,4 DNT), sodium fluoride (NaF), sodium chloride (NaCl), sodium bromide (NaBr), sodium iodide (NaI), lithium chlorides (LiCl), potassium chlorides (KCl), potassium sulfate (K_2SO_4), sodium sulfate (Na_2SO_4) and magnesium sulfate ($MgSO_4$) were obtained from Sigma-Aldrich.

3.4 Methods

3.4.1 Preparation of MEKC Buffer and Standard Solutions

The running buffer was composed of a combination of monosodium and disodium phosphate, adjusted with sodium hydroxide to maintain a pH of 7.5 at 25 mM phosphate concentration [90]. Sodium dodecyl sulfate, used for micellar separation, was added to the buffer at a concentration of 75 mM, and then the final volume was adjusted with deionized water. After a thorough mixing in a sonicator for 10 minutes, the final running buffers were filtered through a 0.45μm Nylon membrane filter. Organic standards were dissolved in methanol to provide 1000 ppm stock solutions.

3.4.2 Preparation of Sample Solutions

3.4.2.1 Normal-MEKC: standard solutions were prepared using running buffer (25 mM phosphate at pH 7.5 [90], 75 mM sodium dodecyl sulfate, 30 kV applied voltage, and on-column UV detection at 200 nm.)

3.4.2.2 Sweeping: standard solutions were prepared in diluted phosphate solution (no SDS micelles). The mixture was adjusted to the same conductivity as a running buffer. [42]

3.4.2.3 High salt stacking: standard solutions were prepared with sodium chloride or other salts as indicated by using deionized water. The conductivity of the sample zone was greater than that of the running buffer. [44]

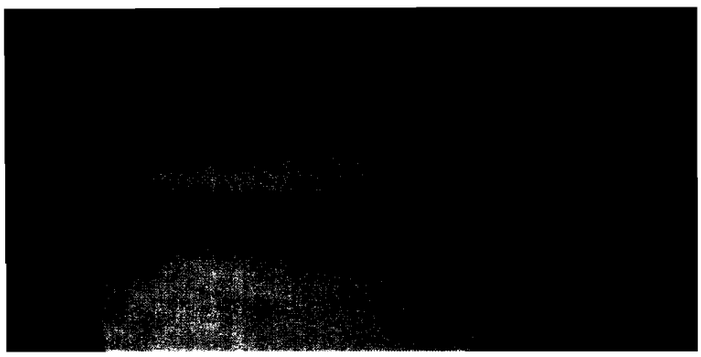
3.4.2.4 Sweeping-high salt stacking: standard solutions were prepared in diluted phosphate solution (no SDS micelles) and sodium chloride solution. The mixture was adjusted for the same conductivity as a running buffer.

3.5 The Steps of Deformed Cartridge Case Head and Mouth

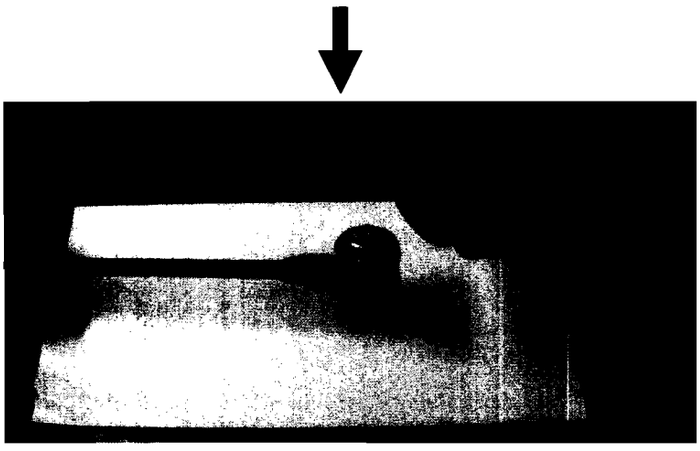
The deformation of cartridge case head and mouth is shown in the following Figure 3.2.

(a) Tool to deform cartridge case head and mouth.

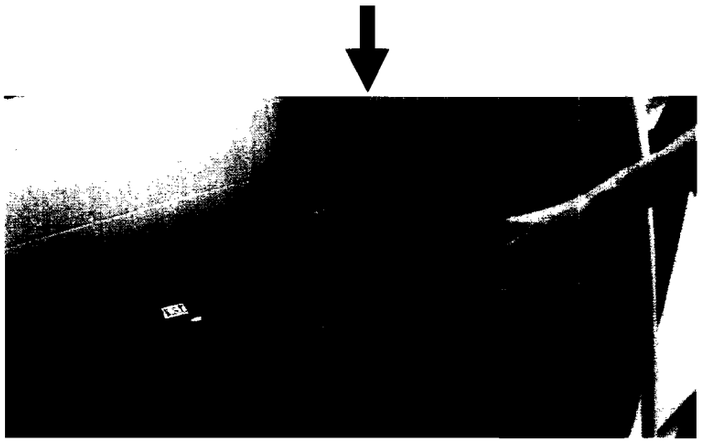
(b) Fix the bullet to the tool number 3.



(c) Put the tool from 3.2 b on the tool number 1.



(d) Put the tool number 2 on the tool from 3.2c.



(e) Hit with hardwood to depart the head from the mouth.

Figure 3.2 Flows diagram for deformed cartridge case head and mouth

3.6 Sample preparation with the Ultrasonic Extraction (USE) procedure and analysis by the High Salt Stacking

A simple ultrasonic extraction procedure was developed to expanded the range of applications. This method consisted on USE and it is indicated for the extraction of non-volatile and semi-volatile organic compounds from solids, such as unburned gun powder. Extractions were performed in triplicates (n=3) and included a method blank, whereas USE efficiencies of the experiments were checked by calculating of recoveries. In particular, the spiked unburned gun powder (0.05 g) was accurately weighed out in a centrifuge tubes and 5 mL of methanol was added. The sample was extracted a time by ultrasonication for 15 min in an ultrasonic bath [29]. After each extraction period, centrifugation followed at 1000 rpm for 10 min in order to obtain clear organic supernatants. The extracts were evaporated by a gentle stream of nitrogen gas at room temperature to near dryness and the residues were re-dissolved in 1 mL of 150 mM NaCl solution. After that, the solution was filtered by a 0.45μm Nylon membrane filtered. The amount of extracted DNT was determined by capillary electrophoresis system.

A blank unburned gun powder sample was extracted with 5 mL methanol by ultrasonic extraction, followed by the high salt stacking coupled with capillary electrophoresis analysis. The electropherogram of the blank sample was clean, no interference compounds were present in the extract, therefore an clean up step was not necessary.

3.7 Validation of the High Salt Stacking

3.7.1 Calibration curve (Operating range)

The calibration graph of each DNT was constructed using the standard solutions which a known amount of a mixture of the analytes with five different concentrations (1.0 to 10.0 μg/mL). The calibration standard mixture solutions over the concentration range of interest were prepared by serial dilution of the mixed standard solution with methanol and 150 mM NaCl solution. The detector response linearity was examined over five concentration ranges, the analyte peaks obtained were integrated and plotted as functions of concentration. The standard mixture solutions were analyzed in triplicates by the high salt stacking at each concentration level.

3.7.2 Precision

The precision of an analytical method is the agreement within the individual measurements of an analyte when the analytical procedure is applied repeatedly to the multiple aliquots of sample matrix. The accuracy of an analytical method is the degree of agreement between the true value of the analyte in the sample and the experimentally determined value. Both precision and accuracy can be calculated from the same analytical experiments. A spiked concentration of the unburned gun powder samples and five replicates for 5 μg/g level of DNT concentration was analyzed and the intra-day precision was calculated. The accuracy was determined as the mean of the measured value relative to the theoretical spiked values and is reported as a percentage (%). The precision is denoted by the intra-day relative standard deviation (RSD).

3.7.3 Limit of Detection (LOD)

The LOD is the lowest concentration of analyte that can be detected but not necessarily quantified, under the stated conditions of the test. The LOD known also as the lowest concentration of an analyte that can be determined with an acceptable precision and accuracy under the stated conditions of test. The LOD was evaluated as the signal-to-noise ratios of 3:1. The LOD was evaluated for each DNT as follows:

(1) Migration times were determined by running the electropherogram of a standard solutions.

(2) The lower standard solutions of DNT (<1.0 μg/mL) were analyzed by the High-salt stacking MEKC technique. From this electropherogram, the average noise levels were measured.

(3) The concentration that led to a signal three times the noise level was evaluated using the response obtained from three injections of the DNT standard solutions and taking into account the values of the noise level.

To determine the LOD in the DNT standard solutions with different concentration levels that led to a signal three times of the noise level.

3.7.4 Recovery

Recovery tests were carried out based on the addition of known amounts of DNT to the unburned gun powder samples. Since the ultrasonic extraction and analysis by the high salt stacking are the non-exhaustive extraction and analysis procedure, for this reason the relative recovery, defined as the ratio of the concentration found in samples and working solution, spiked with the same amount of analytes, instead of the absolute recovery (used in exhaustive extraction and analysis procedure) were employed. The recoveries of the methods were examined on the DNT-free unburned gun powder samples. The percentage recoveries were determined for five replicates samples at 5 μg/g of each DNT.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Optimization of Normal-MEKC

The main disadvantage of capillary electrophoresis as a general method of analysis is its low sensitivity in comparison with liquid chromatography. This is a consequence of the small sample volume which can be applied without incurring band broadening. The small optical path available for the generally used ultravilolet detection also limits the concentration sensitivity. Several methods have been developed to improve sensitivity. Some of these involve alternation of detection geometry [86]. Others depend upon preconcentration techniques either as part of the electrophoretic separation or by incorporating an additional on-line selective procedure. Such preconcentration methods have recently been reviewed [87] with particular reference to immunoaffinity chromatography linked to capillary electrophoresis. Methods such as stacking and field-amplified sample injection (FASI) [88] have been used to improve sensitivity and detection limits but require careful control of the relative ionic strengths of running buffer and sample solution. The nature of the original sample matrix can make this difficult and the concentration sensitivity obtained from purely aqueous matrices cannot always be realised when compounds are present in biological fluids. In addition, such stacking techniques are applicable only to charged compounds.

A method of using MEKC has been described which results in considerable preconcentration of a wide range of compounds by using surfactant. A surfactant is a molecule possessing two zones of different polarity, therefore, resulting in solutions of special characteristics. There is a non-polar zone, of a hydrophobic nature, constituted by a hydrocarbon chain. The other zone can be polar or even ionic and permits classification of the surfactants into three principle classes: ionic (cationic and anionic), non-ionic, and zwitterionic. If the surfactant is in sufficient concentration (above the surfactant's critical micelle concentration (CMC)), it form a micelle in aqueous solution, which has a desired pseudo-stationary phase property. An additional separation mechanism occurs via the partitioning of the solutes in and out of the micelles.

This constitutes the method of MEKC. Association with the micelle results in the retardation of analyte velocity (the micelle migrates slowly). MEKC may now enable the separation of neutral, ionic or non-ionic compounds. Note that in the absence of the partitioning process, neutral compounds will all co-migrate and so MEKC provides an ability to differentiate neutral substances. Surfactants suitable for MEKC should: a) have enough solubility in the buffer to form micelles: b) form a low-viscosity solution; and c) form a solution that is homogeneous and UV transparent. Thus, most MEKC applications have utilized aqueous sodium dodecyl sulfate (SDS) as the micelle phase, but cationic surfactants are also very popular. In addition, chiral surfactant, nonionic surfactants, zwitterionics, biological surfactants, or mixtures of each are finding increasing use.

In the study, MEKC use types of buffer, anionic surfactant, buffer concentration, effect of potential were developed for the separation of three types of dinitrotoluene (i.e. 2,4 DNT, 2,6 DNT and 3,4 DNT)

4.1.1 The Effect of Buffer

In the analysis of three types of dinitrotoluene using normal-MEKC, buffers which are widely used in the experiment includes acetate, phosphate, and borate, with pH ranges 3.76-5.76, 6.20-8.20, and 8.14-10.14, respectively. Dinitrotoluene is a weak base with pK_b approximately 12.2 [89]. In this experiment, phosphate buffer was used as a running buffer because its pH range covered pH of analyte, corresponding by Smith et.al (1999) and Oehrle (1996) that determined the DNT in gun powder by using phosphate buffer as running buffer [90-91]. Thus, phosphate buffer was selected for running buffer of CE system in the further experiments.

4.1.2 The Effect of Concentration of Anionic Surfactant

The separation of three types of dinitrotoluene is accomplished in MEKC by addition of surfactant into the running buffer. An ionic surfactant is placed in the buffer at a concentration exceeding the CMC (8-9 mM) and the surfactant monomers aggregate to form micelle. SDS is the most commonly cited surfactant for use in MEKC, owing to its high water solubility [89]. Figure 4.1 shows the separation of three types of dinitrotoluene obtained at different SDS concentration (25-100 mM). It was certain that SDS with this concentration could separate the sample because the concentration of SDS was higher than the CMC. The separations

of three types of dinitrotoluene were achieved at every concentration of surfactant; however, increasing the concentration of SDS resulted in longer migration times. Because the anionic surfactant concentration is further increased in the aqueous phase, the number of micelle as well as the micelle shape, size and conformation may change significantly. Figure 4.1 (a-b) showed low response and incompletely-separated peaks of the DNT while Figure 4.1 (c-d) showed high response and completely-separated peaks of the analytes. The 75 mM SDS was used in this technique because it provided the higher response and brought about shorter migration time. Migration order of the substances (2,4 DNT, 2,6 DNT and 3,4 DNT) depended upon the water solubility of target analytes. Water solubility of 2,4 DNT, 2,6 DNT, 3,4 DNT were 0.027 g/100 ml, 0.0182 g/100 ml, 0.010g/100 ml, respectively. Normally, substances with higher water solubility were better dissolved in water and aqueous solution than substances with lower solubility, then they were also able to move better along with the running buffer. The DNT with higher water solubility migrated first due to the higher the water solubility tendency. Whereas those with lower water solubility migrated later because they partition into micelle. Thus, the orders of migration were as follow; 2,4 DNT, 2,6 DNT and 3,4 DNT.

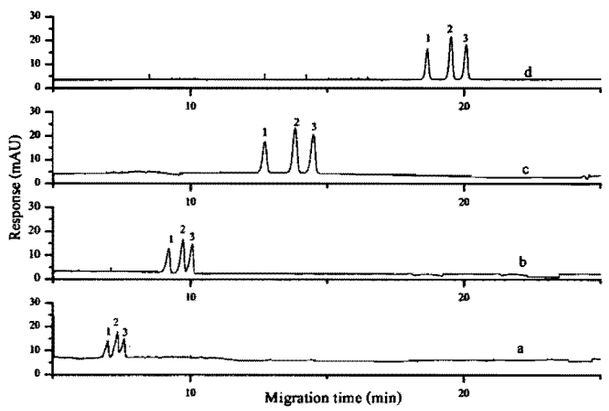


Figure 4.1 Effect of SDS concentration on separation of three types of dinitrotoluene in MEKC. Conditions used: sample concentration 50 ppm, buffer composition; 50 mM phosphate buffer, pH 7.0 with; a) 25 mM, b) 50 mM, c) 75 mM and d) 100 mM SDS, separation voltage +30 kV, hydrodynamic injection (50 mbar, 5 sec), UV detection at 200 nm, L=64.5/56 cm effective length, Peaks identification; 1: 2,4 DNT, 2: 2,6 DNT, 3:3,4 DNT.

4.1.3 The Effect of pH

The effect of pH of the running buffer was examined from 6.0 to 8.0. Figure 4.2 showed the effect of buffer pH on separation of the DNT. The results showed that pH 6.0 to 7.0 gave the tailing peaks of all analytes but pH 7.5 gave the Guassian and non-broadening peak and shorter migration time but pH 8 gave the lower response of all analytes. Silanol groups on capillary wall underwent ionization because they became weak acid in buffer solution. More silanol groups ionized in solution with higher pH (basicity increased). When base pulled protons from silanol groups, the number of negative charges on capillary wall increased. Positive charges in buffer interacted with negative charges on capillary wall to maintain charge balance. When voltage was applied, positive charges in diffuse layer moved along with bulk solution to cathode. This phenomena leads to an increase in ζ and EOF which results in short migration time, but at pH = 8 the reaction did not take place according to this tendency because ionized silanol groups reached saturating point.

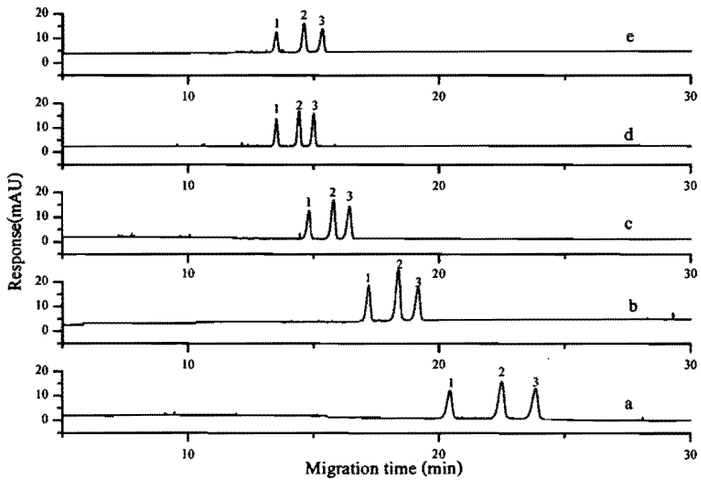


Figure 4.2 Effect of buffer pH on separation of three types of dinitrotoluene in MEKC. Conditions used: sample concentration 50 ppm, 25 mM phosphate buffer containing 75 mM SDS with; a) pH 6.0, b) pH 6.5 c) pH 7.0, d) pH 7.5 and e) pH 8.0, separation voltage +30 kV, hydrodynamic injection (50 mbar, 5 sec), UV detection at 200 nm, L=64.5/56 cm effective length, Peaks identification; 1: 2,4 DNT, 2: 2,6 DNT, 3:3,4 DNT.

4.1.4 The Effect of Buffer Concentration

The phosphate buffer composed of a combination of monosodium and disodium phosphate. The effect of the buffer concentration, range from 25 to 100 mM in the presence of 75 mM SDS, on the migration times and the resolution are shown in Figure 4.3. There is an obvious improvement in resolution when increasing the phosphate concentration at 25 mM. However, an optimum is reached and further increase (from 50-100 mM) results in increased migration times. Because increasing the ionic strength of the running buffer increase the thickness of the ionic double layer, and has the effect of decreasing EOF, hence, increasing the analysis time. Increasing ionic strength in addition to the obvious improvement in buffer capacity, will decrease analyte-wall interaction. The net effect on the separation, therefore, will increase resolution, provided that capillary thermosetting capability is not overcome and that unwanted analyte dissociation processes do not occur. On the other hand, an increase in ionic strength might improve resolution in mixtures by decreasing nonspecific analyte-analyte interaction.

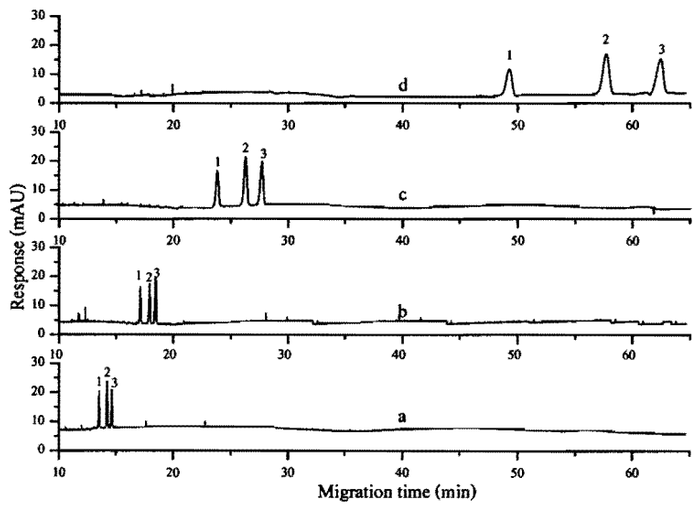


Figure 4.3 Effect of phosphate buffer on separation of three types dinitrotoluene in MEKC. Conditions used: sample concentration 50 ppm, 75 mM SDS, pH 7.5 with; a) 25 mM, b) 50 mM, c) 75 mM and d) 100 mM phosphate buffer, separation voltage +30 kV, hydrodynamic injection (50 mbar, 5 sec), UV detection at 200 nm, L=64.5/56 cm effective length, Peaks identification; 1: 2,4 DNT, 2: 2,6 DNT, 3:3,4 DNT.

4.1.5 The Effect of Applied Potential

The voltage applied during the run time of separation was varied by applying a series of voltages to the running buffer and monitoring the resulting current. The EOF as well as the migration velocity of ion are proportional to the applied voltage used. At a higher voltage, the analysis time was reduced. Note that even though solutes migrated faster at 30 kV, their resolution is not deteriorated because efficiency is improved at higher voltage in CE. The effect of varying the applied voltage (10 kV to 30 kV) on the separation of three types of dinitrotoluene was studied, as can be seen in Figure 4.4. An operating potential of 30 kV gave the minimal analysis time with baseline separation. Therefore, the potential of 30 kV was selected for further work, although greater selectivity is still required. An increase in voltage (> 30kV) resulted in joule heating. When current was applied, crashing between substance charges and buffer charges led to an increase in temperature within capillary at different area and time as well as changing in viscosity of the buffer. This might bring about peak dispersion.

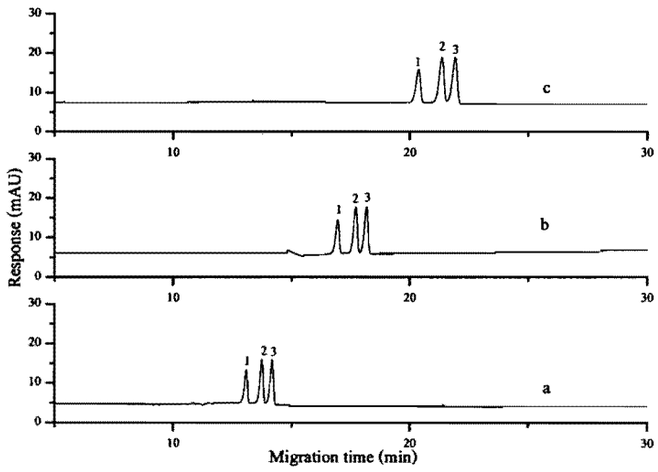


Figure 4.4 Effect of applied potential on separation of three types of dinitrotoluene in MEKC. Conditions used: sample concentration 50 ppm, buffer composition; 25 mM phosphate buffer pH 7.5 containing 75 mM SDS, hydrodynamic injection (50 mbar, 5 sec) L=64.5/56 cm effective length, UV detection 200 nm. Separation voltage; a) 30 kV, b) 20 kV and c) 10 kV, Peaks identification; 1: 2,4 DNT, 2: 2,6 DNT, 3:3,4 DNT.

4.2 Selection of Sample Injection Modes

Electropherogram of the three types of dinitrotoluene in sample matrix showing the effect of hydrodynamic injection (HDI) and electrokinetic injections (EKI) at various times on the peaks is depicted in Figure 4.5 and Figure 4.6, respectively. The HDI of up to 10 s produced a baseline separation of the dinitrotoluene isomers but at 15 s injection the peak start to be unresolved. Injection time of 10 s was taken as the optimum. The EKI of up to 50 s injection time produced peaks, which are well separated. Injection of 60 s produced peaks which is biforked and humped. The 50 s injection time with EKI was taken as the optimum. Hydrodynamic injection at 50 mbar 10 sec correlated with 0.2 nL/s injection volume, while the electrokinetic injection at 10 kV 50 sec correlated with 4.5 nL/s injection volume. Subsequently, the comparison between the optimal injection techniques of HDI and EKI exhibited that the efficiency of EKI was higher than HDI.

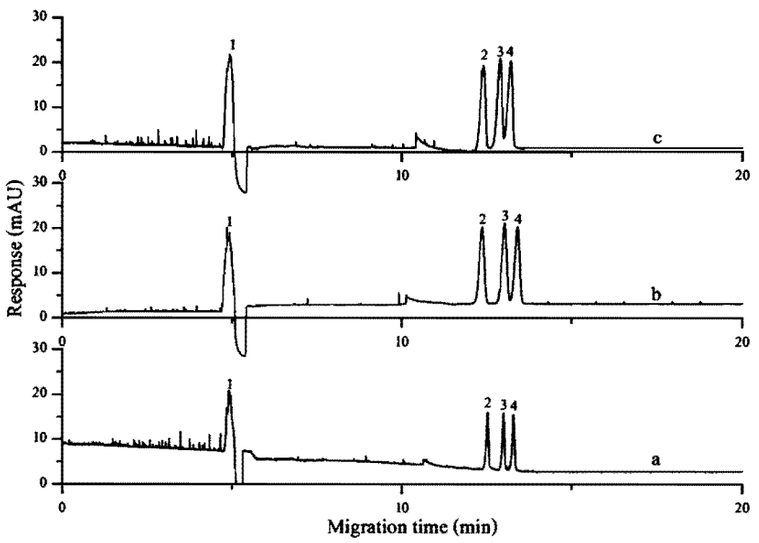


Figure 4.5 Effect of hydrodynamic injection on separation of three types of dinitrotoluene in MEKC. Conditions used: sample concentration 10 ppm, 75 mM SDS, pH 7.5 with; a) 5 s, b) 10 s, c) 15 s injection times at 50 mbar pressure, separation voltage +30 kV, at 200 nm, L=64.5/56 cm effective length, Peaks identification; 1: EOF, 2: 2,4 DNT, 3: 2,6 DNT, 4: 3,4 DNT.

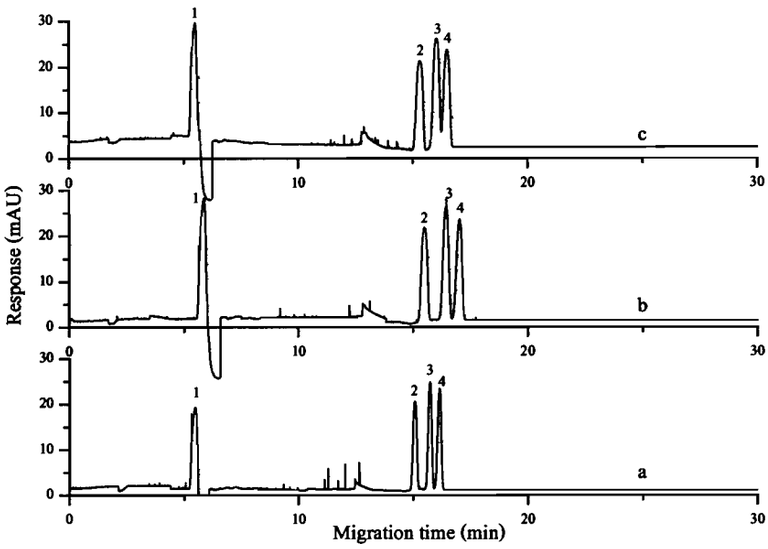


Figure 4.6 Effect of electrokinetic injections on separation of three types of dinitrotoluene in MEKC. Conditions used: sample concentration 10 ppm, 75 mM SDS, pH 7.5 with; a) 40 s, b) 50 s, c) 60 s injection times at 10 kV, separation voltage +30 kV at 200 nm, L=64.5/56 cm effective length, Peaks identification 1: EOF, 2: 2,4 DNT, 3: 2,6 DNT, 4: 3,4 DNT.

It can be concluded that the following experiment will use electrokinetic injection to separate the three types of dinitrotoluene. The optimum condition, which was considered from peak shapes and separation time, was fused-silica capillary with 75 μm i.d. x 64.5 cm (56 cm effective length), 25 mM phosphate buffer and 75 mM sodium dodecyl sulfate at pH 7.5, +30 kV applied voltage, 25 °C, and on-column UV detection at 200 nm. This condition would be the initial condition to develop this technique in the further.

4.3 Optimization of Sweeping

Sweeping, a new on-line sample concentration technique in EKC, is defined as the sorption and accumulating of analyte molecules by the pseudo-stationary phase (PS) that enters and fills the sample zone upon application of voltage. This phenomenon, initially observed by

Gilges [34], however, was not well studied until more recently. It occurs whenever the sample matrix is void of a charged carrier phase and it does not matter whether the sample matrix has a higher, similar, or lower conductivity compared to the running buffer. In sweeping, the analyte zones are narrowed due to the chromatographic or partitioning mechanism as the sample molecules experience and are sorbed into the pseudo-stationary phase zone (PS). Several papers have been published on the use of this concentration technique in MEKC. Quirino et al. [81] reported exceeding 5000-fold concentration of dilute analytes in MEKC using sweeping as on-line concentration technique without off-line treatment. Quirino et al. [31] developed the sample concentration technique by sample stacking and sweeping using microemulsion and a single isomer sulfated β-CD as pseudo-stationary phase in MEKC. Moreover, Monton et al. [39] used sweeping technique as an on-line preconcentration of charged analytes in MEKC with nonionic micelles to yield peak height enhancements up to 100-fold. Nunez et al. [92] reported analysis of the herbicides paraquat, diquat and difenzoquat in drinking water by MEKC using sweeping and cation selective exhaustive injection (CSEI-sweeping-MEKC). More recently, the sweeping principle has been extended to CZE separations of neutral solutes involving complexation reaction of *cis*-diols with borate demonstrating the versatility and wide applicability of the sweeping, referred to as CSEI-sweep has achieved almost a million-fold enhancement in detector response for cationic hydrophobic analytes [42]. In particular, on-line concentration by sweeping was successfully applied to microchip MEKC by Sera et al. [93].

In this study, sweeping approaches are investigated in order to improve concentration detection limits for a mixture of the target analytes. Sample was prepared in buffer of equivalent ionic strength to the running buffer but without any micelles, and a longer plug is injected. For simplicity, cations are generally continuous in either system, with hydrogen or sodium cations in both the sample matrix and running buffer. This approach is designed to focus the analyte into narrow bands within the capillary, thereby increasing the sample volume that can be injected. It utilizes the interactions between micelles in the running buffer and the sample in a matrix that is devoid of the micelles used. The accumulation occurs due to chromatographic partitioning, complexation or any interaction between analytes and micelles through electrophoresis. The extent of the preconcentration is dependent on the strength of interaction involved. Figure 4.7 showed the effectiveness of sweeping in the sample matrix. Samples were prepared in various concentrations

of phosphate solutions (25 mM phosphate, 25-time-diluted 25 mM phosphate and 50-time-diluted 25 mM phosphate). Sample was injected for 40 s at 10 kV; therefore 25-time-diluted 25 mM phosphate was selected to use as a sample matrix. From Figure 4.7 (a-c), electropherogram (a) showed the lowest response while responses of electropherogram (b) and (c) were not different. However, resolution of electropherogram (b) was higher than resolution of electropherogram (c) and peaks 2 and 3 of electropherogram (c) were overlapped; therefore 25-time-diluted 25 mM phosphate was selected to use as a sample matrix.

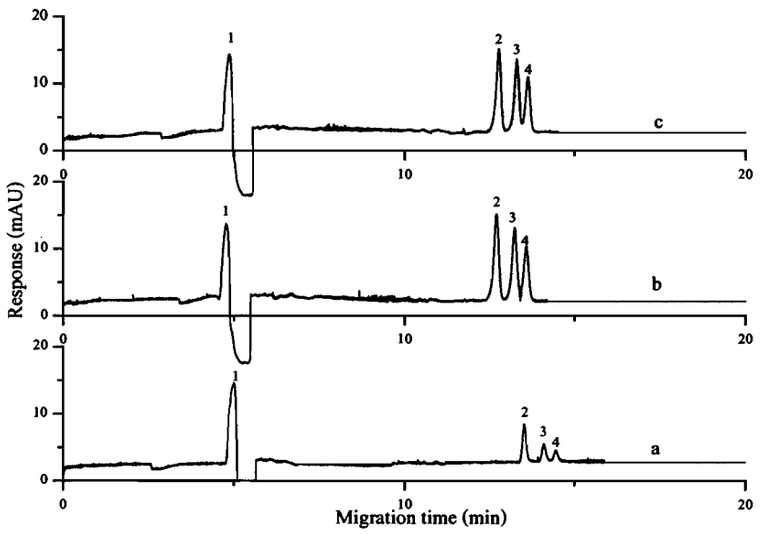


Figure 4.7 Electrophoregram obtained from sweeping a) 25 mM phosphate b) 25-time- diluted 25 mM phosphate and c) 50-time- diluted 25 mM phosphate. Conditions used: sample concentration 10 ppm, buffer composition; 25 mM phosphate buffer pH 7.5 containing 75 mM SDS, separation voltage +30 kV, electrokinetic injection 10 kV, 40 sec at 200 nm, L=64.5/56 cm effective length, Peaks identification; 1: EOF, 2: 2,4 DNT, 3: 2,6 DNT, 4:3,4 DNT

4.4 Optimization of High Salt Stacking

4.4.1 Types of Salt

The addition of salt to sample matrix is indicated for stacking analytes in MEKC as well as other modes of electrokinetic chromatography. Figure 4.8, 4.9, and 4.10 showed different salts added to sample matrix. The chlorides of lithium, sodium as well as the sodium salts of fluorine, chlorine, bromine and iodine were utilized at a concentration of 150 mM NaCl to sample matrix (10 ppm). There were no substantial or trending differences between the salt used and the effectiveness of the sample stacking. However, when a higher mobility salt was used, such as sodium chloride [44], it is also logical that the anion mobility of the sample matrix (e.g., chloride) be greater than that of the anionic micellar carrier (e.g., micelle) to avoid intrusion of the micelles into the sample zone during stacking. The sample stacking method efficiency is high as a result of the micelles being stacked before they enter the zone. While it is critical to maintain the highest possible surface tension in the sample matrix to maximize hydrophobic interaction with analytes.

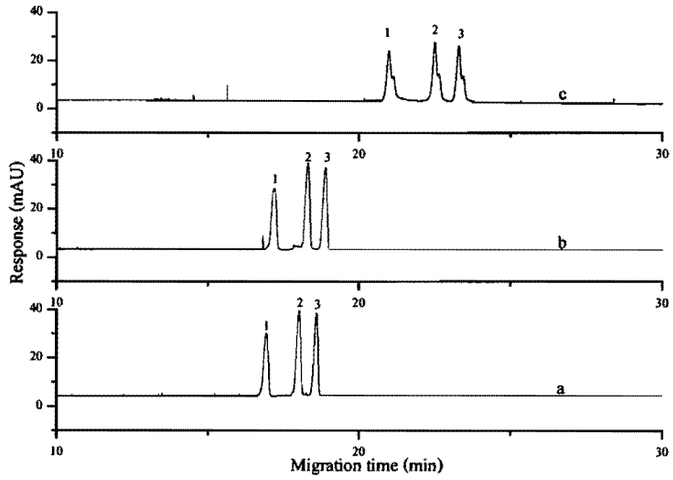


Figure 4.8 Effect of varying the types of salt a) LiCl, b) NaCl and c) KCl. Conditions used: sample concentration 10 ppm, buffer composition; 25 mM phosphate buffer pH 7.5 containing 75 mM SDS, separation voltage +30 kV, electrokinetic injection 10 kV, 40 sec, at 200 nm, L=64.5/56 cm effective length, Peaks identification 1: 2,4 DNT, 2: 2,6 DNT, 3:3,4 DNT

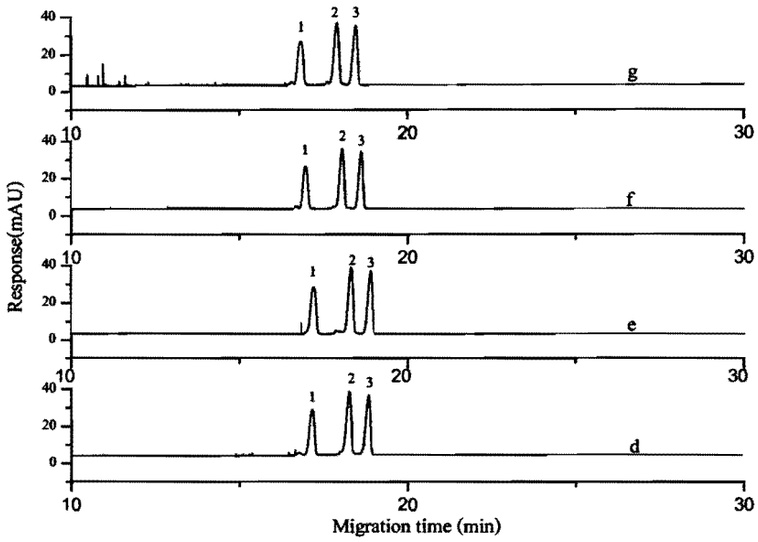


Figure 4.9 Effect of varying the types of salt d) NaF, e) NaCl, f) NaBr and g) NaI. Conditions used: sample concentration 10 ppm, buffer composition; 25 mM phosphate buffer pH 7.5 containing 75 mM SDS, separation voltage +30 kV, electrokinetic injection 10 kV, 40 sec, at 200 nm, L=64.5/56 cm effective length, Peaks identification; 1: 2,4 DNT, 2: 2,6 DNT, 3:3,4 DNT.

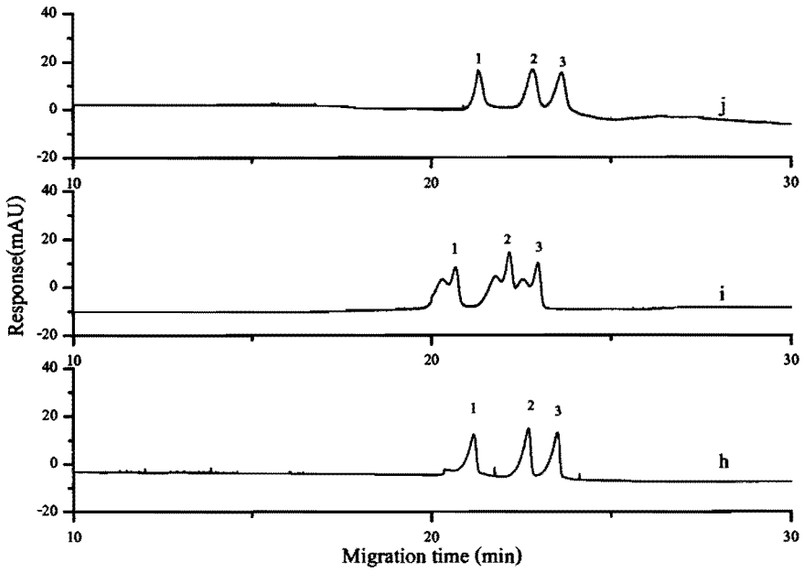


Figure 4.10 Effect of varying the types of salt h) Na_2SO_4, i) K_2SO_4 and j) $MgSO_4$. Conditions used: sample concentration 10 ppm, buffer composition; 25 mM phosphate buffer pH 7.5 containing 75 mM SDS, separation voltage +30 kV, electrokinetic injection 10 kV, 40 sec, at 200 nm, L=64.5/56 cm effective length, Peaks identification; 1: 2,4 DNT, 2: 2,6 DNT, 3:3,4 DNT.

4.4.2 The Effect of Conductivity

The effect of varying the conductivity of the sample zone was typical 1.5x3.0 that of the running buffer in Figure 4.11. The reason for failure of stacking with less, equal conductivities in the sample matrix and running buffer when the sample matrix has a lower conductivity than the running buffer, it is well-known that the sample matrix constituents experience and enhanced field upon initiation of separation voltage, and the micelle on the detector side of the sample plug would be expected to electrophoresis into the sample zone. However, because of the amplified field in the sample matrix, the micelle enters and accelerates through the sample matrix. It will accumulate at the opposite interface with the running buffer, never reaching a higher concentration than the running buffer. The zonal micelle concentration

entering the sample matrix is decreased by the low-ionic strength sample matrix, and therefore, micelle complexation of analytes is decreased and no stacking.

At equivalent sample matrix and running buffer conductivities, micelle enters the zone without any component of field amplification, yet does not exhibit a sufficient concentration for stacking of the analytes.

With conductivity of the sample matrix from 1.5-2.5 folds that of the running buffer (as seen in figure 4.11 (a-c)), field amplification is transferred from the sample zone to the running buffer. The micelles within the running buffer experiencing the enhanced filed migrated rapidly to the detector side of the sample matrix interface. When the high conductivity sample matrix induces a stacking of the micelles at the interface, analytes experience a reduction in velocity upon encountering the stack micelle/sample zone interface. This is due to the different velocities of analytes in the sample zone (pure EOF velocity) versus the velocities of analytes in the stacked micelle zone (greatly enhanced counter-EOF mobility and reduced velocity due to a high local micelle concentration). Separation mode commences when the micelle front has exited the high salt matrix and the chloride component has diffused to separation buffer. Thus, increasing the field in the running buffer by increasing conductivity in the sample zone will cause an increase in the mobility of micelle in the running buffer and a decrease in mobility of micelle at the sample matrix/running buffer interface. This, in combination with the maximum velocity of analytes in the sample matrix versus decreased velocity in the concentrated micelles at the sample matrix/running buffer interface, cause analyte stacking. However, when the conductivity of the sample matrix exceeds that of the running buffer (as seen in Figure 4.11 (d). It appears that overlapping peak. It was postulated that maintaining sample matrix conductivity 2.5 folds above that of the running buffer was fundamental aspect of maximizing peak efficiency.

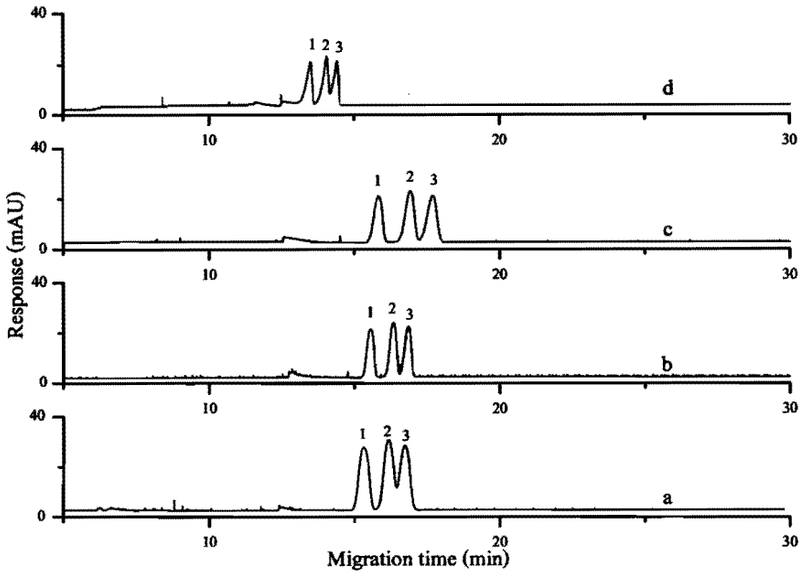


Figure 4.11 The effect of varying the conductivity a) 1.5x, b) 2.0x, c) 2.5x and d) 3.0x. Conditions used: sample concentration 10 ppm, buffer composition; 25 mM phosphate buffer pH 7.5 containing 75 mM SDS, separation voltage +30 kV, electrokinetic injection 10 kV, 40 sec, at 200 nm, L=64.5/56 cm effective length, Peaks identification; 1: 2,4 DNT, 2: 2,6 DNT, 3:3,4 DNT.

4.4.3 Concentration of NaCl

Since our earlier works [94 -95] showed that high ionic strength sample matrices play a role in MEKC, varied salt concentration sample matrices were examined in conjunction with the 25 mM phosphate buffer/75 mM SDS/pH 7.5 running buffer. Figure 4.12 shows the electrophoretic results when the three type of dinitrotoluene (10 ppm each) were injected in sample matrices with sodium chloride at concentration from 50 to 200 mM. As shown in Figure 4.12 (a), poorly shaped peak expected with such large injection (40 s) were observed with sample matrixes at low salt concentration ($\leq$ 50 mM), however, there was a dramatic improvement in peak shape and detectability with increasing of sodium chloride concentration ($>$ 50-150 mM, Figure 4.12 (b-c). An overlapping peak of 200 mM NaCl in sample matrix was found in Figure 4.12 (d). However, stacking is starkly apparent when the conductivity of the

sample matrix is raised substantially above (100-150 mM NaCl) that of the running buffer. Thus, it appears that the sharp increase in efficiency with all analytes that is obvious in Figure 4.12 (c) is associated with a sample matrix that has a higher conductivity than the running buffer. Since zones with different conductivities influence the relative fields experienced by chloride components, a mechanism of stacking is described with explains the necessity of a high-conductivity sample matrix in this mode. It was found that 150 mM NaCl in the sample matrix gave the best result with baseline separation.

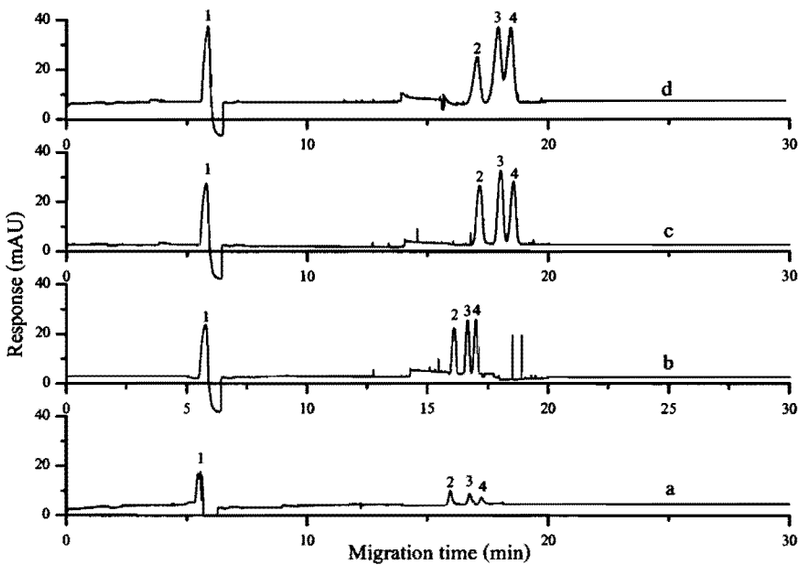


Figure 4.12 The effect of varying the concentration a) 50 mM b) 100 mM c) 150 mM and d) 200 mM NaCl. Conditions used: sample concentration 10 ppm, buffer composition; 25 mM phosphate buffer pH 7.5 containing 75 mM SDS, separation voltage +30 kV, electrokinetic injection 10 kV, 40 sec, at 200 nm, L=64.5/56 cm effective length, Peaks identification; 1: EOF, 2: 2,4 DNT, 3: 2,6 DNT, 4:3,4 DNT.

4.4.4 Factors Affecting High Salt Stacking

4.4.4.1 The Effect of Organic Modifiers

In MEKC, organic solvent can be added to the running buffer to afford separation of analytes by altering the affinity of the analyte for the micelle phase. Organic

additives alter hydrophobic analyte/micelle interactions by displacing the analytes from the micelle, by offering alternative hydrophobic binding sites, or by decreasing the surface tension of the running buffer [96]. Thus, the addition of organic modifiers to the running buffer provides a means of interrogating the dependence of the stacking mechanism on analyte/micelle complexation in the two zones. Electrophoresis of the three type of dinitrotoluene in a 150 mM NaCl sample matrix was carried out with running buffer including methanol and ethanol at a concentration of 10% in Figure 4.13. While increasing ethanol concentration in the running buffer diminished stacking of the three type of dinitrotoluene, its presence in the running buffer affords an increase in the peak efficiency relative to the unmodified running buffer. Alcohols contribute to repression of EOF [97] to varying degree as illustrated in the Figure 4.13 (a-d). This can contribute to an initial increase in analysis times for analytes. However, as the hydrophobic character of the alcohol is increased (longer carbon chain), the effect of decreasing the surface tension of the running buffer and likewise decreasing the affinity of the analyte for the micellar phase occurs.

The effect of organic modifier in the running buffer was examined by adding ethanol from 5% to 20%. From electropherograms, 10% ethanol presented the highest response in Figure 4.14 (b). The result is a reasonable analysis time, even for very hydrophobic compounds. We may expect a similar effect with the use of organic solvent in high salt stacking. However, it should be noted that a high concentration of the organic solvent may break down the micellar structure. Generally, concentrations of up to 20% organic solvent can be used without difficultly in high salt stacking.

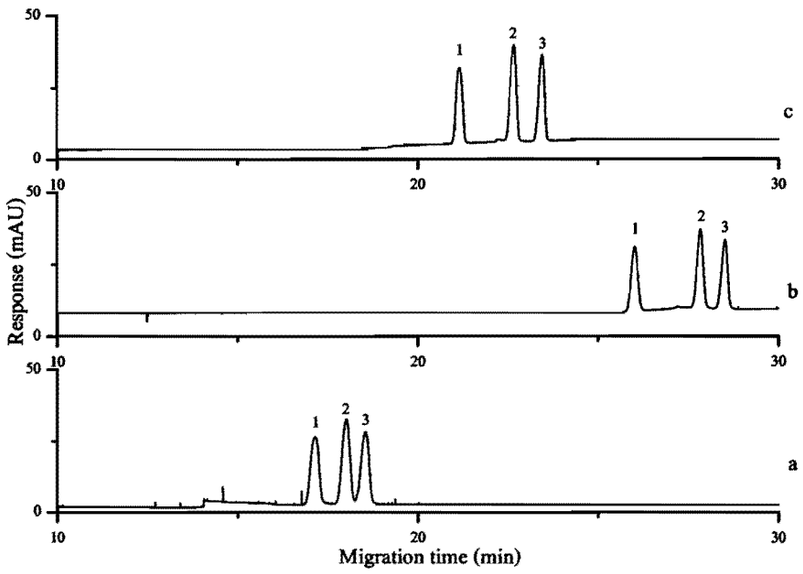


Figure 4.13 Effect of organic solvents on separations of three types of dinitrotoluene in high salt stacking. Conditions used: sample concentration 10 ppm, buffer composition; 25 mM phosphate buffer pH 7.5 containing 75 mM SDS with; a) no solvent, b) 10% methanol and c) 10% ethanol, separation voltage +30 kV, electrokinetic injection 10 kV,40 sec at 200 nm, L=64.5/56 cm effective length, Peaks identification; 1: 2,4 DNT, 2: 2,6 DNT, 3:3,4 DNT.

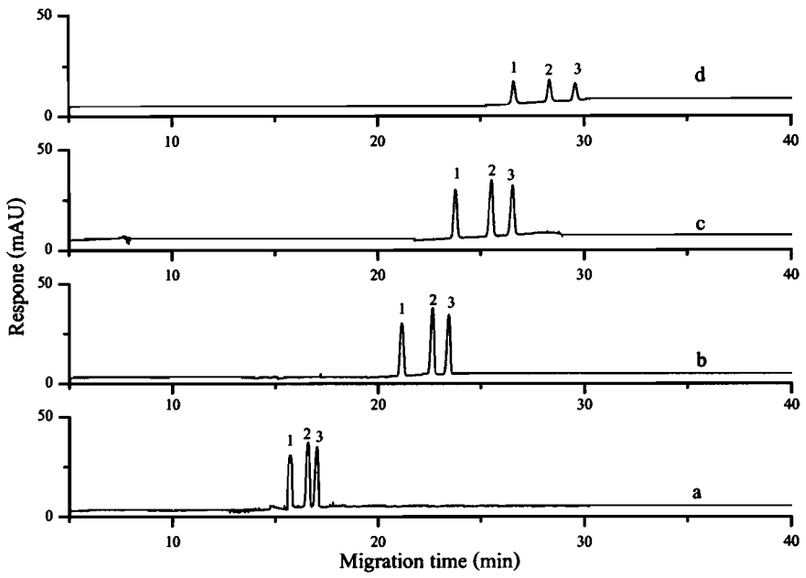


Figure 4.14 Effect of ethanol on separations of three types of dinitrotoluene in high salt stacking. Conditions used: sample concentration 10 ppm, buffer composition; 25 mM phosphate buffer pH 7.5 containing 75 mM SDS with; a) 5%, b) 10%, c) 15% and d) 20% of ethanol, separation voltage +30 kV at 200 nm, L=64.5/56 cm effective length, Peaks identification; 1: 2,4 DNT, 2: 2,6 DNT, 3:3,4 DNT.

4.4.4.2 The Effect of Capillary Tube Temperature

The electropheretic mobility and the electroosmotic expression both contain a viscosity term in the denominator. Viscosity is a function of temperature; therefore, precise temperature control is important. Increasing the temperature of capillary tube reduced the viscosity of the buffer and therefore increased the EOF, resulting in overall faster migration times of the analytes. By increasing the temperature from 20-45 ^{0}C (Figure 4.15 (a-f)) shows that increasing the temperature of the capillary gave sharper peaks, but resolution was not improved due to a closer migration time. At 25 % (Figure 4.15 (b)) decrease in migration time was observed in the separation of three types of dinitrotoluene because effected of temperature increasing include possible thermal denaturation of the sample and evaporation of some buffer components such as organic solvents.

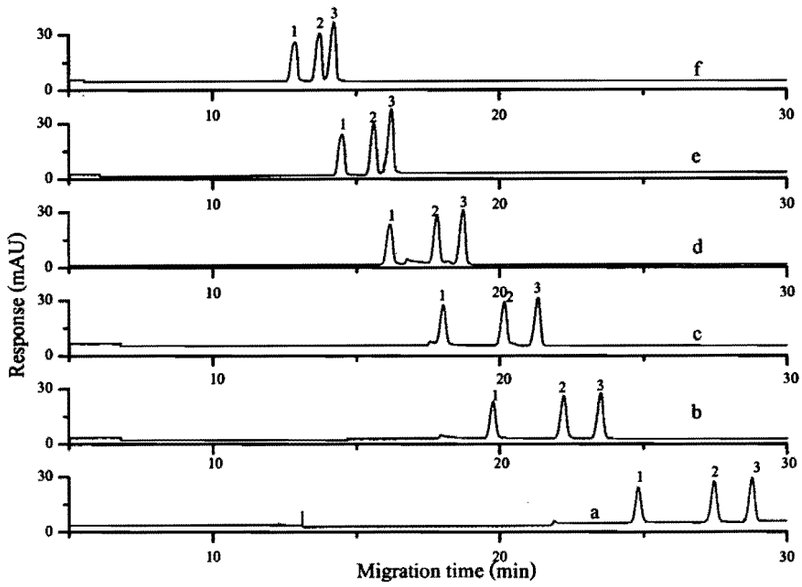


Figure 4.15 Effect of various temperatures on separation of three types of dinitrotoluene in high salt stacking. Conditions used: sample concentration 10 ppm, buffer composition; 25 mM phosphate, 75 mM SDS, pH 7.5 with; a) 20 °C, b) 25 °C, c) 30 °C, d) 35 °C, e) 40 °C and f) 45 °C, separation voltage +30 kV, electrokinetic injection 10 kV, 40 sec at 200 nm, L=64.5/56 cm effective length, Peaks identification 1: 2,4 DNT, 2: 2,6 DNT, 3:3,4 DNT

4.5 Optimization of Sweeping –High Salt Stacking

This technique shares the same principle as sweeping in terms of an equivalence of conductivity between sample matrix and running buffer. However, in this technique NaCl solution or co-ion was added into sample matrix whereas sweeping contains no co-ion in the system. For this technique, sample matrix with analytes and NaCl solution tended to migrate or diffuse to anode because electropheretic mobility of chloride ion was higher than those of micelles. When the voltage was applied to the system, micelles migrated to analyte. This mechanism was similar to the mechanism of sweeping and resulted in new length of the bands of analytes as shown in Figure 4.16. When NaCl solutions (25, 50 and 75 mM) were added into 25-time-diluted

25 mM phosphate solutions (from sweeping), the result showed electropherogram (a) with the lowest response and electropherogram (c) with overlaping peaks between peak 2 and 3. Therefore, electropherogram (b) was considered to be the best result because it indicated the highest response and resolution.

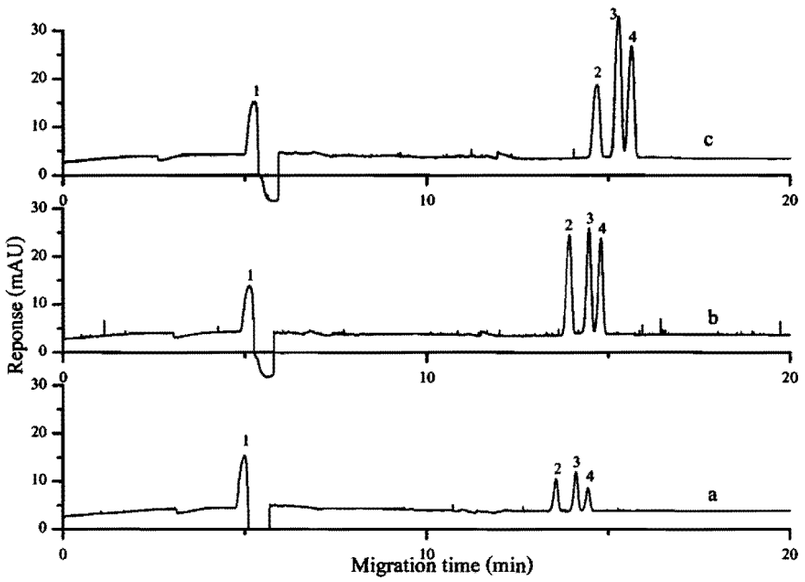


Figure 4.16 Electropherogram obtained from sweeping-high salt stacking a) 25-time- diluted 25 mM phosphate+ 25 mM NaCl. b) 25-time- diluted 25 mM phosphate +50 mM NaCl and c) 25-time- diluted 25 mM phosphate + 75 mM NaCl. Conditions used: sample concentration 10 ppm, buffer composition; 25 mM phosphate buffer pH 7.5 containing 75 mM SDS, separation voltage +30 kV, electrokinetic injection 10 kV, 40 sec at 200 nm, L=64.5/56 cm effective length, Peaks identification; 1: EOF, 2: 2,4 DNT, 3: 2,6 DNT, 4:3,4 DNT.

4.6 Comparison of Normal MEKC, Sweeping, High Salt Stacking and Sweeping-High Salt Stacking

Normal MEKC was used to investigate three types of dinitrotoluene in this experiment. Sample matrix was dissolved in running buffer (25 mM phosphate/ 75 mM SDS/ pH 7.5). The result showed that the three peaks of dinitrotoluene were completely separated and resolution was lower than 5, but response was still low (Figure 4.17 (a) and Table 4.1). This condition was later used to develop on-line concentration in this experiment.

In this study, three techniques of on-line concentration were compared to investigate the technique with the highest efficiency to separate three types of dinitrotoluene. The first technique was sweeping (Figure 4.17 (b) and Table 4.1); sample matrix was designed to be continuous with the running buffer and equivalent in conductivity. There was no micellar stacking observed, and the velocity of analytes in the sample zone in the presence of micelles was the same as the velocity of the analytes in the running buffer. From this experiment, Sweeping showed higher response with apparent signals, but resolution was 1.5-2, and 2,6 DNT coeluted with 3,4 DNT.

Another technique is high salt stacking (Figure 4.17 (c) and Table 4.1); the system was termed "discontinuous" since the mobility of sample matrix ions differed from the mobility of running buffer. High salt sample matrix brought about the stacking of micelles at the sample/running buffer interface. From the result, high salt stacking demonstrated the highest peak height and peak areas among the three techniques. However, the sample was able to be separated only when the conductivity of sample matrix was higher than the conductivity of running buffer. From the experiment, response and resolution indicated that the three peaks were completely separated within 20 minutes of analysis time.

The last technique, sweeping-high salt stacking (Figure 4.17 (d) and Table 4.1); was initially developed in this study. For this technique, the condition used in this experiment was a combination between the ultimate condition of sweeping (25-time-diluted 25 mM phosphate, and ultimate condition of high salt stacking (NaCl solution). For sweeping-high salt stacking, when the same electric field was applied throughout the capillary, chloride ions migrated from sample zone to micelle zone. This phenomena generated a narrow zone in which the number of analyzes was higher than the number of analyzes in a narrow zone of sweeping technique.

The result exhibited that the three substances required approximately 15 minutes of analysis time, but resolution and response decreased. Therefore, high salt stacking was considered the best technique to separate the three types of nitrotoluenes due to its highest response and complete resolution of all analytes.

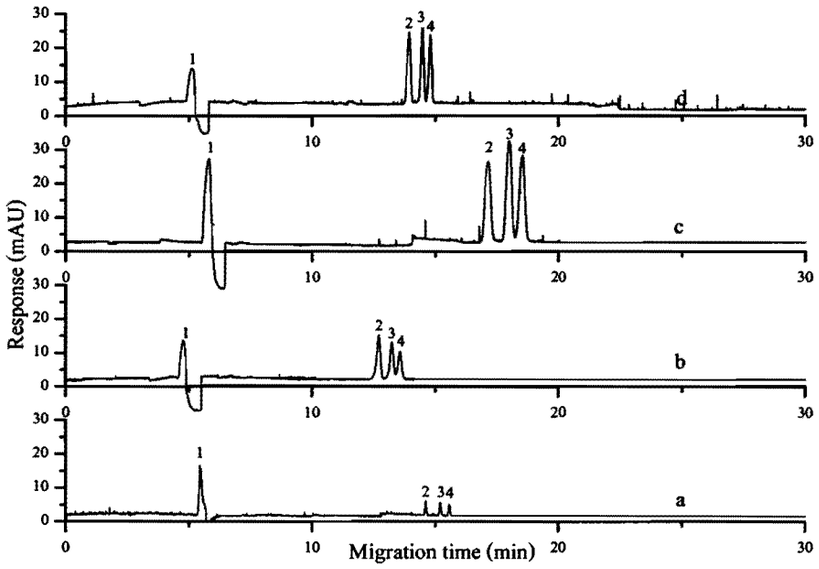


Figure 4.17 Comparison of concentration techniques in three types of dinitrotoluene a) normal MEKC b) sweeping c) high salt stacking and d) sweeping-high salt stacking. Conditions used: sample concentration 10 ppm, buffer composition; 25 mM phosphate buffer pH 7.5 containing 75 mM SDS, separation voltage +30 kV, electrokinetic injection 10 kV, 40 sec at 200 nm, L=64.5/56 cm effective length, Peaks identification; 1: EOF, 2: 2,4 DNT, 3: 2,6 DNT, 4:3,4 DNT.

Table 4.1 Resolution and Peak area of techniques in MEKC

Methods	Compounds	Peak area (n=4)	Resolution (R_s)
Normal-MEKC	1) 2,4 DNT	14.81	$R_{1,2}$ = 8.39
	2) 2,6 DNT	16.49	$R_{2,3}$ = 5.31
	3) 3,4 DNT	13.79	
Sweeping	1) 2,4 DNT	85.01	$R_{1,2}$ = 2.14
	2) 2,6 DNT	71.38	$R_{2,3}$ = 1.60
	3) 3,4 DNT	53.97	
High salt stacking	1) 2,4 DNT	314.15	$R_{1,2}$ = 3.51
	2) 2,6 DNT	373.55	$R_{2,3}$ = 2.18
	3) 3,4 DNT	376.50	
Sweeping-High salt stacking	1) 2,4 DNT	178.14	$R_{1,2}$ = 3.18
	2) 2,6 DNT	182.59	$R_{2,3}$ = 2.12
	3) 3,4 DNT	178.18	

4.7 Validation of Quantitative High Salt Stacking

When the high salt stacking has been developed, it is important to validate it to confirm that it is suitable for its intended purpose. The validation shows how reliable the methods are, specifically for the DNT analyzed application. In this study, the analytical performance characteristics of the optimized high salt stacking were validated.

4.7.1 Calibration curve (Operating range)

The linearity of an analytical method is its ability to produce test results that are directly proportional to the concentration of the analyte within a given ranges. For the establishment of linearity, a minimum of five different concentrations should be used. Usually, the spiked solutions are made with a known amount of a mixture of the analytes and calibration curves are drawn by relating the peak areas. Series of five concentration levels were obtained by analysis of all DNT in a concentration ranging from 1.0 to 10.0 µg/mL. Each solution was analyzed in triplicates. The linearity of the method has been investigated over the range of

1.0 to 10.0 μg/mL and the three types of DNT had correlation coefficients of the calibration graphs greater than 0.995. The results showed in Table 4.2. Calibration curves of each DNT were shown in Appendix E.

Table 4.2 Operating Ranges, linearity and linear equation of DNT analysis by the high salt stacking

DNT	Operating Range (μg/ml)	Linearity (5 levels)	Linear equation
2,4 DNT	1.0-10.0	0.9959	y = 25.817x - 16.988
2,6 DNT	1.0-10.0	0.9954	y = 43.855x - 21.976
3,4 DNT	1.0-10.0	0.9987	y = 39.180x - 23.159

4.7.2 Precision

The precision of an analytical method is the closeness of the individual measurement of an analyte when the analytical procedure is applied repeatedly to multiple samplings of the spiked sample. The precision is usually expressed as the relative standard deviation (RSD). The measured RSD can be subdivided into three categories: repeatability (intra-day precision), intermediate precision (inter-day precision) and reproducibility (inter-laboratory precision). In this study, repeatability precision of the developed high salt stacking was investigated.

The repeatability of an analytical method refers to the use of the procedure within a laboratory over a short period of time, and carried out by the same analyst with the same equipment. Table 4.3 showed the repeatability of the high salt stacking, was obtained by analyzing five replicates spiked samples consecutively at a concentration level (5 μg/g). For spiked the sample at 5 μg/g, the obtained %RSD was ranging from 0.99 to 3.16%, and the precision of this method was good.

4.7.3 Limit of Detection (LOD)

The LOD is defined as the concentration of analyte that results in a peak height three times the noise level when injected into the electrophoresis system. The LOD is the lowest

concentration of the analyte in a sample that can be detected but not necessarily quantifiable. The LOD was evaluated by comparing the signal to noise ratio (S/N) of the lowest concentration to a S/N = 3. The results showed that the method allowed detection of DNT in the solution at concentration lower than 1.0 μg/mL. The LODs were between 0.25 and 0.50 μg/mL. The results were shown in Table 4.3 and electropherogram in Appendix F.

4.7.4 Recovery

Accuracy can be measured by analyzing samples with known concentrations and comparing the measured values with the true values. Accuracy can be illustrated with mean recovery value. Recovery of the analytes from the matrix is a desirable outcome of the sample preparation, and is therefore an important characteristic of the extraction procedure. The relative recovery was applied to instead of absolute recovery as used in exhaustive extraction procedures. According to the expected levels of real concentrations, the spiking was performed at three fortification levels; high, middle and low regions of the linear ranges. The recovery of the DNT at each fortification level was evaluated. The DNT-free samples were spiked with target DNT at a fortification level (5 μg/g). The peak areas obtained on these samples were analyzed and compared with the peak areas obtained when analyzing standard solutions with the same concentration by the same procedure. The mean % recoveries obtained for the DNT spiked samples. The recoveries of all analytes ranged between 68.03 and 90.78%, as shown in Table 4.3

Table 4.3 Analytical performance for the determination of DNT in unburned gun powder with the ultrasonic extraction and analysis by the high salt stacking

DNT	Repeatability (%RSD)	Mean % Recovery (n=5)	LOD (μg/ml)
2,4 DNT	3.16	90.78	0.50
2,6 DNT	0.99	68.03	0.25
3,4 DNT	1.42	70.11	0.25

4.8 Application of High Salt Stacking in the Analysis of Bullets

Applicability of the high salt stacking to real sample was evaluated by testing in eight different commercial bullets (38 Special w-w, 38 Special ww, 38 Special s&w, 38 Special Winchester, 38 Special Speer, .357 Magnum (R-P), 380 Auto Winchester and 9 mm Luger Winchester). Table 4.3 showed that only 2,4 DNT was found in 38 Special w-w, 38 Special ww (for example in Figure 4.18), 38 Special s & w, 380 Auto win, and Win 9 mm Luger. 2,6 DNT and 3,4 DNT were not found in the eight commercial bullets.

Table 4.4 Organic compounds that found in commercial bullets

Type/sizes	**Composition**		
	2,4 DNT	**2,6 DNT**	**3,4 DNT**
38 Special w-w	22.00	nd	nd
38 Special ww	25.80	nd	nd
38 Special s&w	38.40	nd	nd
38 Special Winchester	nd	nd	nd
38 Special Speer	nd	nd	nd
.357 Magnum (R-P)	nd	nd	nd
380 Auto Winchester	24.80	nd	nd
9 mm Luger Winchester	26.80	nd	nd

nd = not detect

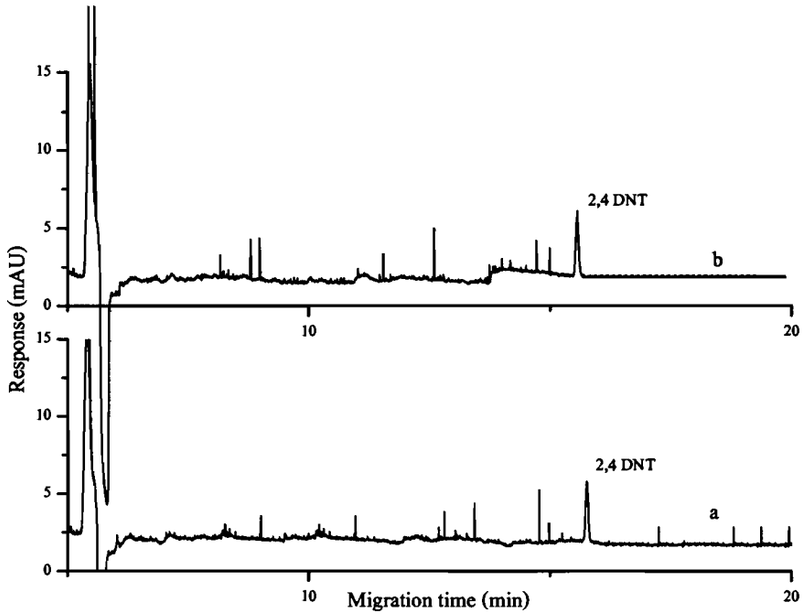


Figure 4.18 Separation of 2,4 DNT by high salt stacking in bullets a) 380 Auto Winchester and b) 9 mm Luger Winchester. Conditions used: buffer composition; 25 mM phosphate, 75 mM SDS, pH 7.5; electrokinetic injection 10 kV, 40 s, separation voltage +30 kV, UV detection at 200 nm, L=64.5/56 cm effective length.

CHAPTER 5

CONCLUSIONS

In this research normal-MEKC technique was used to separate three types of organic gunshot residues. Influence of factors, such as electrolyte concentration, pH, surfactant, applied voltage, and injection time, on separation efficiency were studied. The result showed that optimum condition, which was evaluated by using peak shapes and separation time, was fused-silica capillary wtih 75 μm i.d. x 64.5 cm (56 cm effective length), 25 mM phosphate buffer and 75 mM sodium dodecyl sulfate at pH 7.5, +30 kV applied voltage, 25 ^{0}C, and on-column UV detection at 200 nm. In further researches, this condition would be an advantage to develop this technique.

In this study, separation efficiency of on-line sample concentration technique in low concentration solution was developed by emphasizing on three separation techniques, (1) sweeping, (2) high salt stacking, and (3) sweeping-high salt stacking. Comparison of peak shapes and migration time among the three techniques brought about the result in the following.

(1) Sweeping, the effect of phosphate buffer used to dilute sample solution without injecting surfactant (SDS) to maintain equivalent conductivities of sample and running buffer: The optimum condition is 25 mM 25-times-diluted phosphate buffer.

(2) High salt stacking, the effect of NaCl concentration used to dilute sample solution without injecting surfactant (SDS) to adjust conductivity of sample to 2.5 times of conductivity of running buffer: The optimum condition is 150 mM NaCl.

(3) Sweeping-high salt stacking, the effect of phosphate buffer and NaCl solution used to dilute sample solution without injecting surfactant (SDS) to maintain equivalent conductivities of sample and running buffer: the optimum condition is 25 mM 25-times-diluted phosphate buffer with 50 mM NaCl.

Effectiveness of sweeping, high salt stacking and sweeping-high salt stacking techniques were compared. High salt stacking was selected because this gave successful separation of three types of dinitrotoluene, with good peak shape and completely separation. The chlorides of lithium and sodium as well as the sodium salts of fluorine, chlorine, bromine and

iodine were effective to separate in the sample matrix. Organic solvent such as methanol and ethanol in the running buffer had strong effect on the resolution of three types of dinitrotoluene. Capillary temperature was found to have a strong influence on the migration of these components. The applicability of the estabilished high salt stacking method was demonstrated by analyzing organic gun powder under suitable condition.

REFERENCES

REFERENCES

[1] D. N. Heiger. High performance capillary electrophoresis. 2nd ed. Germany: Hewlett-Packard, 1992.

[2] J. W. Jorgenson and K. D. Lukacs. "Zone electrophoresis in open tubular glass capillaries", J. High Resol. Chromatogr. 4: 230-231, 1981.

[3] C. G. Wilber. Ballistic science for the law enforencement office. Springfield: Charles C Thomas, 1997.

[4] S. Basu. "Formation of gunshot residues", J. Forensic Sci. 27: 72-91, 1982.

[5] G. M. Wolten and R. S. Nesbitt. "On the mechanism of gunshot residues particles formation", J Forensic Sci. 25: 533-545, 1980.

[6] R. C. Halberstam. "A simplified probability equation for gunshot primer residue (GSR) detection", J. Forensic Sci. 36: 894-897, 1991.

[7] R. A. D. Kumarrawickrama. The characterization and identification of organic gunshot residue. Master's Thesis. UK: University of Strathclyde, 1985.

[8] S. A. Peak. "A thin-layer chromatographic procedure for confirming the presence and identity of smokeless powder flakes", J. Forensic Sci. 25: 679-681, 1980.

[9] A. W. Archer. "Separation and identification of minor components in smokeless powders by thin-layer chromatography", J. Chromatog. 108: 401-404, 1975.

[10] J. C. Via and L. T. Taylar. "Chromatographic analysis of nonpolymeric single base propellant components", J. Chromatog Sci. 30: 106-110, 1992.

[11] E.O'N. Espinoza and J.I. Thornton. "Characterization of smokless powder by means of diphenylamine stabilizer and its nitrated derivatives", Anal Chim Act. 288: 57-69, 1994.

[12] F. S. Romolo and P. Marget. "Identification of gunshot residue: a critical review", Forensic Sci. Int. 119: 195-211, 2001.

[13] H. C. Harrison and R. Gilroy. "Firearms discharge residues", J. Forensic Sci. 4: 184-199, 1959.

[14] P. M. Halina and et al. "Instrumental neutron activation analysis for steel analsis and certification", Nukleonika. 56(4): 311-315, 2011.

REFERENCES (CONTINUED)

[15] S. Walter and S. Sibina. "Recent Trends in analytical Atomic Absorption Spectroscopy", Applied Spectroscopy. 23: 421-553, 1969.

[16] R. R. Nageswara and M. V. Talluri. "An overview of recent application of inductively coupled plasma-mass spectrometry (ICP-MS) in determination of inorganic impurties maceuticels", J Pharm Biomed Anal. 43: 1-13, 2007.

[17] E. Copur and et al. "Determination of inorganic elements in poppy straw by scanning electron microscopy with energy dispersive spectrometry as a means of ascertaining origin", Bull Narc. 57(1-2): 183-202, 2005.

[18] J. L. Kacprzak and et al. "Determination of methyl mercury in fish by flameless atomic absorption spectroscopy and comparison with an acid digestion method for total mercury", J Assoc off Anal Chem. 59: 153-157, 1976.

[19] L. L. Jackson and et al. "Geological and inorganic Materials", Anal Chem. 65: 12R-28R, 1993.

[20] R. W. Taylor and et al. "Simultaneuos identification of amphetamine and Methaphetamine using solid-phase extraction and gas chromatography/nitrogen phosphorus detection or gas chromatography/mass spectrometry", J Assoc off Anal Chem. 59: 153-157, 1976.

[21] L. Rottmann and et al. "Determination of heavy metal interaction with dissolved organic materials in Natural Aquatic systems by Coupling a High-Performance Liquid Chromatography System with an inductively Couple Plama Mass Spectrometer", Anal Chem. 66(21): 3709-3715, 1994.

[22] L. X. Qian and et al. "Supercritical Fluid Extraction-Supercritical Fluid Chromatography with Fraction Collection for sensitive Analytes", J Chromatogr Sci. 27(7): 365-370, 1989.

[23] L. Garofano and et al. "Gunshot residue: Further studies on particles of environmental and occupational", Forensic Sci. Int. 103: 1-21, 1999.

[24] S. Terabe and et al. "Electrokinetic separations with solutions and open-tubular capillaries", Anal chem. 56: 111-113, 1984.

REFERENCES (CONTINUED)

[25] A. Munder and et al. "Microanalysis of explosives and propellants by on-line supercritical fluid extraction/chromatography with triple detection", J Microcolumn Sep. 3: 127-140, 1991.

[26] D. M. Northrop and et al. "Separation and identification of organic gunshot and explosive constituents by micellar electrokinetic capillary electrophoresis", Anal Chem. 63: 1038-1042, 1991.

[27] W. A. MacCrehan and W. F. Rowe. "Sampling protocols for the detection of smokless powder residues using capillary electrophoresis", J. Forensic Sci. 43: 119-124, 1998.

[28] W. A. MacCrehan and et al. "Hair combing to collect organic gunshot residues (OGSR)", Forensic Sci. Int. 103: 167-173, 2003.

[29] D. M. Northrop. "Gunshot residue analysis by micellar electrokinetic capillary electrophoresis: assessment for application to casework. Part I", J Forensic Sci. 46(3): 549–559, 2001.

[30] D. M. Northrop. "Gunshot residue analysis by micellar electrokinetic capillary electrophoresis: assessment for application to casework. Part II", J Forensic Sci. 46(3): 560–572, 2001.

[31] S. Casamento and et al. "Optimization of the separation of organic explosives by capillary electrophoresis with artificial neutral", J. Forensic Sci. 48: 1075-1083, 2003.

[32] E. B. Morales and A. L R.Vazquez. "Simultaneous determination of inorganic and organic gunshot residues by capillary electrophoresis", J Chromatogr.A. 1061(2): 225–233, 2004.

[33] D. M. Northrop and W. A. MacCrehan. "Sample collection, preparation and quantitation in the micellar electrokinetic capillary electrophoresis of gunshot residues", J. Liquid Chromatog. 15: 1041–1063, 1992.

[34] M. Gilges. "Determination of impurities in an acidic drug substance by micellar electrokinetic chromatography", Chromatographia. 44: 191-196, 1997.

REFERENCES (CONTINUED)

[35] J. P. Quirini and et al. "Sweeping: Concentration mechanism and application to high-sensitivity analysis in capillary electrophoresis", J. Chromatogr. A. 965: 357-373, 2002.

[36] M. Gong and et al. "On-line sample preconcentration by sweeping with dedecyltrimethylammonium bromide in capillary zone electropheresis", J Chromatogr. A. 1125: 263-269, 2006.

[37] J. Palmer and et al. "A universal Concept for Stacking Neutral Analytes in Micellar Capillary Electrophoresis", Anal. Chem. 71: 1679-1687, 1999.

[38] S. He and et al. "Comparative study for the analysis of parabens by micellar electrokinetic capillary chromatography with and without large-volume sample stacking technique", Talanta. 69: 166-171, 2006.

[39] M. R. N Monton and et al. "Separation and on-line preconcentration by sweeping of charged analytes in electrokinetic chromatograph with nonionic micelles", J. Chromatogr. A. 939(1-2): 99-108, 2001.

[40] J. P. Quirino and et al. "Sample concentration by sample stacking and sweeping using a microemulsion and a single-isomer sulfated *[beta]* –cyclodextrin as pseudostationary phase in electrokinetic chromatography", J. Chromatogr. A. 838(1-2): 3-10, 1999.

[41] J. P. Quirino and S. Terabe. "Sweeping with an enhanced electric field of neutral analyte zones in electrokinetic chromatography", J.High Resol. Chromatogr. 22(7): 367-372, 1999.

[42] J. P. Quirino and S. Terabe. "Approaching a million-fold sensitivity increase in capillary electrophoresis with direct ultraviolet detection: Cation-selective exhaustive injection and sweeping", Anal Chem. 72(5): 1023-1030, 2000.

[43] J. P. Quirino and et al. "Sweeping of neutral analytes in electrikinetic chromatography with high-salt-containing matrixes", Anal Chem. 72: 1934-1040, 2000.

[44] J. Palmer. "High salt stacking principles and sweeping: comments and contrast on mechanisms for high-sensitivity analysis in capillary electrophoresis", J. Chromatogr. A. 1036: 95-100, 2001.

REFERENCES (CONTINUED)

[45] S. Terabe and et al. "Electrokinetic separations with micellar solutions and open-tubular capillaries", Anal Chem. 56: 111-113, 1984.

[46] M. Zeece. "Capillary electrophoresis: a new analytical tool for food science", Trends in Food Science and Technology. 3: 6-10, 1992.

[47] M. Chiari and et al. "Determination of total vitamin C in fruits by capillary zone electrophoresis", J. Chromatogr. A. 761: 297-305, 1993.

[48] Mg. C. L. and et al. "Analytical of food additives by ion-pairing electrokinetic chromatography", J. Chromatogr. Sci. 30: 167-170, 1992.

[49] M. Jimidar and et al. "Comparison of capillary zone electrophoresis with high-performance liquid chromatography for the determination of additives on food stuffs", J. Chromatogr. 636: 179-186, 1993.

[50] B. Paterson and et al. "Determination of phenols in environmental water using liquid chromatography with electrochemical detection", J. Chromatogr. A. 731: 95-102, 1996.

[51] R. Lehmann and et al. "Capillary electrophoresis in clinical chemistry", J. Chromatogr. B. 697: 3-35, 1997.

[52] W. Steuer and et al. "Comparison of high-performance liquid chromatography, supercritical fluid chromatography and capillary zone electrophoresis in drug analysis", J. Chromatogr. 507: 125-140, 1990.

[53] K. D. Altria. "Overview of capillary electrophoresis and capillary electrochromatography", J. Chromatogr. A. 856: 443-463, 1999.

[54] J. P. Lander. Introduction to Capillary Electrophoresis. In J. P. Lander, editor. Handbook of capillary and microchip electrophoresis and associated microtechniques. New York: CRC, 2000.

[55] J. P. Lander and R. P. Oda. Introduction to Capillary Electrophoresis In J. P. Lander, editor. Handbook of capillary electrophoresis. New York: CRC, 1997.

[56] S. F. Y. Li. Capillary Electrophoresis" Principle, Practice and Applications. Amsterdam: Elsevier, 1994

REFERENCES (CONTINUED)

[57] A. Foret. Electrophoresis library. New York: Radola B. J., 1993.

[58] A. Weston and P. R. Brown. HPLC and Principles and Practice. san Diego: Academic Press, 1997

[59] D. C. Harris. Quantitative chemical analysis. 5th ed. New York: W. H. Freeman, 1999.

[60] Q. Yang and et al. "Trends in capillary electrophoresis: 1997", J. Chromatographic Science. 35: 358-373, 1997.

[61] J. P. Lander. Introduction to Capillary Electrophoresis. In J. P. Lander, editor. Handbook of capillary and microchip electrophoresis and associated microtechniques. New York: CRC, 2000.

[62] S. H. Hansen and et al. "Selectivity enhancement in capillary electrophoresis using non-aqueous media", Trends in Anal Chem. 15: 175-180, 1996.

[63] R. Weinberger. Practical Capillary Electrophoresis. San Diego: Academic Press Inc, 1993.

[64] Backman. Introduction to Capillary Electrophoresis. Californai: Beckman Instruments Inc, 1994.

[65] D. Heiger. High performance capillary electrophoresis-An introduction. Waldbronn: Agilent Technologies, 2000.

[66] S. Terabe. Micellar Electrokinetic Chromatography. Californai: Beckman Instruments Inc, 1992.

[67] P. Camilleri. Capillary Electrophoresis: Theory and Practice. Florida: CRC Press, 1991

[68] S. Yang and et al. "Chemical selectivity in micellar electrokinetic chromatography II. Rationalzation of elution patterns in different surfactant systems", J. Chromatogr. A. 738: 265-274, 1996.

[69] S. Terabe and et al. "Electrokinetic chromatography with micellar solution and open-tubular capillary", Anal Chem. 57: 834, 1985.

[70] M. Sepaniak and R. O. Cole. "Collumn Efficiency in Micellar Electrokinetic Chromatrography", Anal Chem. 59: 472-476, 1987.

REFERENCES (CONTINUED)

[71] S. Katsuta and K. Saitoh. "Control of separation selectivity in micellar electrokinetic chromatography by modification of the micellar phase with solubilized organic compounds", J. Chromatogr. A. 780: 165-178, 1997.

[72] Y. S. Wu and et al. "High-performance chiral separation of fourteen triazole fungicides by sulfated β-cyclodextrin-mediated capillary electrophoresis", J. Chromatogr. A. 912: 171-179, 2001.

[73] M. G. Khaledi. Miceilar electrokinetic capillary chromatography. Florida: CRC Press, 1994.

[74] C. F. Poole and S. K. Poole. "Interphase model for retention and selectivity in micellar electrokinetic chromatography", J. Chromatogr. A. 792: 89-104, 1997.

[75] G. M. McLaughlinand et al. "Pharmaceutical drug separation by HPCE: practical guidelines", J. Liq. Chromatogr. 15: 961, 1992.

[76] A. T. Balchunas and M. T. Sepaniak. "Extension of elution range in micellar electrokinetic capillary chromatography", Anal. Chem. 59: 1466, 1987.

[77] R. O. Cole and et al. "Bile salt surfactants in micellar electrokinetic capillary chromatography: application to hydrophobic molecule separations", J. Chromatogr., 557: 113-1 23, 1991.

[78] M. J. Sepaniak and et al. Fundamentals of Micellar Electrokinetic Capillary Chromatography. Capillary Electrophoresis Theory&Practice. UK: Academic Press Inc, 1995

[79] K. V. Penmetsa and et al. "Herbicide analysis by micellar electrokinetic chromatography", J. Chromatogr. A. 745: 201-208, 1996.

[80] M. Molina and et al. "Use of partial filling technique and reverse migrating micelles in the study of N-methylcarbamate pesticides by micellar electrokinetic chromatography-electrospray ionization mass spectrometry", J. Chromatogr. A. 927: 191-202, 2001.

[81] J. P. Quirini and S. Terabe. "Exceeding 5000-fold concentration of dilute analytes in micellar electrokinetic chromatography", Science. 282: 465-468, 1998.

REFERENCES (CONTINUED)

[82] J. P. Quirini and S. Terabe. "Sweeping of analyte zones in electrokinetic chromatography", Anal. Chem. 71: 1638-1644, 1999.

[83] J. P. Quirini and et al. "Sweeping: Concentration mechanism and application to high-sensitivity analysis in capillary electrophoresis", J. Chromatogr. A. 965: 357-373, 2002.

[84] J. P. Landers. Online Sample Preconcentration for Capillary Electrophoresis. NewYork: CRC Press, 2008.

[85] J. Palmer and et al. "A universal concept for stacking neutral analytes in micellar electrokinetic chromatography", Anal. Chem. 71: 1679-1687, 1999.

[86] N. H. H. Heegard and et al. "Principles, high resolution methods, and application", J. Chromatogr B. 715: 29-54, 1998.

[87] N. A. Guzman. "New directions for concentration sensitivity Enhancement in CE and microchip technology", LC-GC. 17(I): 14-31, 1999.

[88] R. L. Chien and et al. "Analysis of basic antimalarial drugs by CZE and MEKC. Part 1-2 Critical factors affecting separation", J. Chromatogr. 559: 141-152, 1991.

[89] A.M. Kaplan. "Dinitrotoluene", http:// www.chemicalbook.com. March, 2012

[90] K. D. Smith et al. "Detection of Smokless Powder Residue on Pipe Bombs by Micellar Electrokinetic Capillary Electrophoresis", J. Forensic Sci. 44(4): 789-794, 1999.

[91] S. A. Oehrle. "Analysis of Nitramine and nitroaromatic explosives by capillary electrophoresis ", J. Chromatogr A . 745: 233-237, 1996.

[92] O. Nunez and et al. "Analysis of the herbicides paraquat, diquat and difenzoquat in drinking water by micellar electrokinetic chromatography using sweeping and cation selective exhaustive injection", J. Chromatogr. A. 961: 65-75, 2002.

[93] Y. Sera and et al. "Sweeping on microchip: Concentration profiles of the focused zone in micellar electrokinetic chromatography", Electrophoresis. 22: 3509-3513, 2001.

[94] J. Palmer and et. al. "Proceeding, Symposium on High Performance Capillary Electrophoresis", in HPCE'97. CA: Anaheim, 1997.

REFERENCES (CONTINUED)

[95] N. J. Munro and et. al. "Proceeding, Symposium on High Performance Capillary Electrophoresis", in HPCE'98. Orlando: FL, 1998.

[96] J. Gorse and et. al. "Effects of organic mobile phase modifiers in micellar electrokinetic Capillary Chromatography", J High Resolut. Chromatogr. 11: 554-559, 1988

[97] N. Chen and et. al. "Effects of organic modifier concentrations on electrokinetic migrations in micellar electrokinetic chromatography", Electrophoresis. 16(8): 1457-1462, 1995.

APPENDICES

APPENDIX A

SELECTION OF CAPILLARY INNER DIAMETER

APPENDIX A

Selection of capillary inner diameter (μm)

The narrow separation window (path length) of a capillary is a definate limitation in CE, especially in UV detection. Capillaries must remain narrow (20-100 μm) in order to control and minimize the amount of heat generated by high running voltages. To compensate for this, specialized extended light path capillaries exist which have a standard internal diameter but which have an extended light path at the capillary window (360 μm), allowing for increased sensitivities due to the longer pathlength. These capillaries were tested during the optimization process, and they were found to result in twice the sensitivity achieved with a regular capillary of 75 μm i.d. in Figure A.1

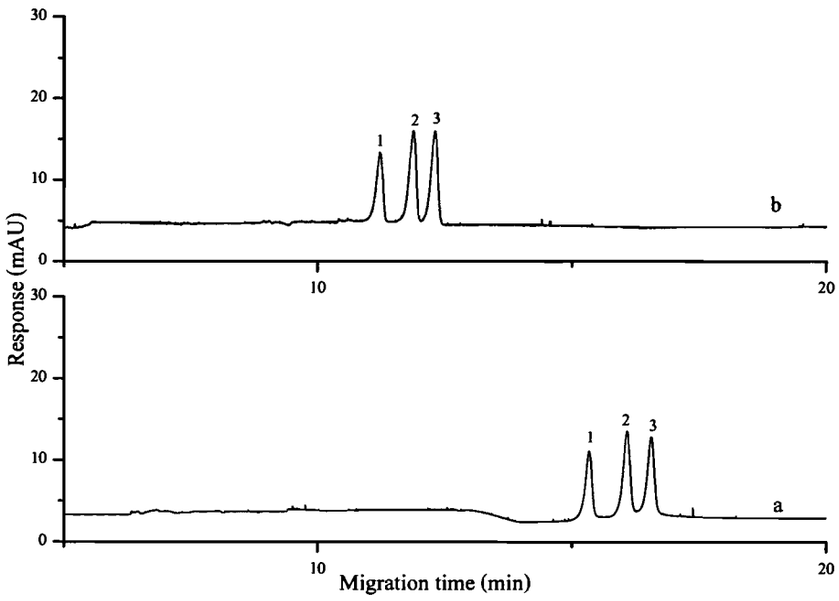


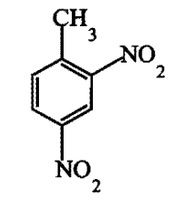
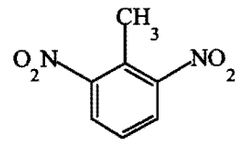
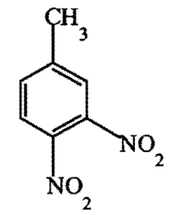
Figure A.1 Selection of capillary inner diameter on separation of three types of dinitrotoluene in MEKC. Conditions used: 75 mM SDS, pH 7.5 with; a) 50 μm and b) 75 μm, separation voltage +30 kV at 200 nm, L=64.5/56 cm effective length.
Peaks identification; 1: 2,4 DNT, 2: 2,6 DNT, 3: 3,4 DNT.

APPENDIX B

PROPERTIES OF COMPOUNDS AND SPIKING OF STANDARDS

APPENDIX B

Table B.1 Properties of compounds

2,4 DNT  2,4 DNT	Molecular weight : 182.14 g/mole Boiling point : 300 ^{0}C Melting point : 71^{0}C Solubility in water: 0.027 g/100ml Easily soluble in acetone. Solubility in diethyl ether and ethanol.
2,6 DNT  2,6 DNT	Molecular weight : 182.14 g/mole Boiling point : 300 ^{0}C Melting point : 56-61^{0}C Solubility in water: 0.0182 g/100ml Solubility in organic solvent
3,4 DNT  3,4 DNT	Molecular weight : 182.14 g/mole Boiling point : 250 ^{0}C Melting point : 54-57^{0}C Solubility in water: 0.010 g/100ml Solubility in organic solvent

Spiking

Neutral analytes a high affinity for the micellar carrier (i.e., very hydrophobic) spend a greater amount of time complexed with the micelles and hence have a velocity lower than EOF, while those with less affinity (i.e., less hydrophobic) have velocity dependent more on EOF. Peaks in the electropherograms were identified by spiking the standards mixture solutions with the standard of interest in Figure B. Peak identification: 1, 2,4 DNT; 2, 2,6 DNT; 3, 3,4 DNT.

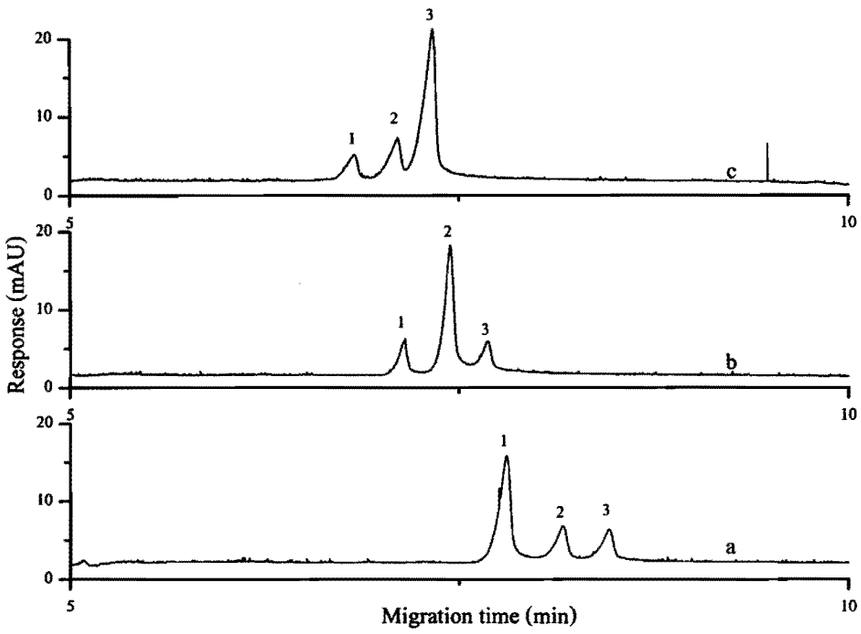


Figure B.1 Effect of spiking on separation of three types of dinitrotoluene in MEKC. Conditions used: 75 mM SDS, pH 7.5 with; a) spiking 2,4 DNT, b) spiking 2,6 DNT, and c) spiking 3,4 DNT, separation voltage +30 kV at 200 nm, L=64.5/56 cm effective length, Peaks identification; 1: 2,4 DNT, 2: 2,6 DNT, 3:3,4 DNT.

APPENDIX C

MONOMERIC SURFACTANT

APPENDIX C

Monomeric Surfactant

Sodium Dodecyl Sulfate (SDS) is the most widely used surfactant in MEKC. It is available in highly purified forms and is inexpensive. Its molecular weight is 288 and the CMC is 8 mM. The aggregation number (number of molecules/micelle) is 62. Molecular structure of SDS is shown in Figure C.

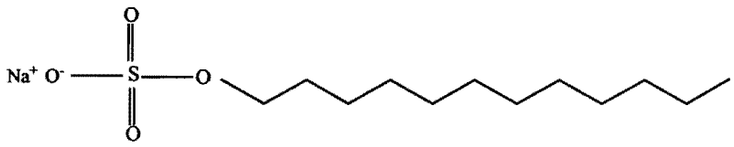


Figure C.1 Structure of the surfactant SDS

APPENDIX D

NUMBER OF THEORETICAL PLATES (N)

ELECTROOSMOTIC FLOW (μ_{eof})

ELECTROPHERETIC MOBILITY (μ_{ep})

PEAK AREA AND PERCENTAGE RSD

APPENDIX D

Table D.1 Number of Theoretical Plates (N), electroosmotic flow (μ_{eof}), electropheretic mobility (μ_{ep}), peak area and percentage RSD

a) Normal-MEKC

compounds	μ_{eof}	μ_{ep}	t_m(min)	N	**Peak area (n=4)**	**%RSD (n=4)**
2,4DNT	4.12×10^{-4}	-2.23×10^{-4}	14.87	3.08×10^{5}	14.81	4.69
2,6DNT	4.12×10^{-4}	-2.28×10^{-4}	15.48	2.94×10^{5}	16.49	4.38
3,4DNT	4.12×10^{-4}	-2.31×10^{-4}	15.86	2.86×10^{5}	13.79	3.79

b) Sweeping

compounds	μ_{eof}	μ_{ep}	t_m(min)	N	**Peak area (n=4)**	**%RSD (n=4)**
2,4 DNT	4.08×10^{-4}	-2.61×10^{-4}	13.64	3.15×10^{5}	85.01	1.64
2,6 DNT	4.08×10^{-4}	-2.67×10^{-4}	14.23	3.01×10^{5}	71.38	1.68
3,4 DNT	4.08×10^{-4}	-2.71×10^{-4}	14.61	2.94×10^{5}	53.97	1.25

c) High salt stacking

compounds	μ_{eof}	μ_{ep}	t_m(min)	N	**Peak area (n=4)**	**%RSD (n=4)**
2,4DNT	3.77×10^{-4}	-2.50×10^{-4}	15.85	2.71×10^{5}	314.15	4.36
2,6DNT	3.77×10^{-4}	-2.58×10^{-4}	16.93	2.53×10^{5}	373.55	2.87
3,4DNT	3.77×10^{-4}	-2.63×10^{-4}	17.70	2.43×10^{5}	376.50	3.19

d) Sweeping - High salt stacking

compounds	μ_{eof}	μ_{ep}	t_m(min)	**N**	**Peak area (n=4)**	**%RSD (n=4)**
2,4DNT	4.12×10^{-4}	-2.69×10^{-4}	13.95	3.08×10^{5}	178.14	3.04
2,6DNT	4.12×10^{-4}	-2.75×10^{-4}	14.59	2.94×10^{5}	182.59	3.76
3,4DNT	4.12×10^{-4}	-2.79×10^{-4}	15.00	2.86×10^{5}	178.18	3.15

APPENDIX E

CALIBRATION CURVE FOR THE SEPARATION OF DNT

APPENDIX E

Calibration curve for the separation of DNT obtained by using high salt stacking

a) 2,4 DNT

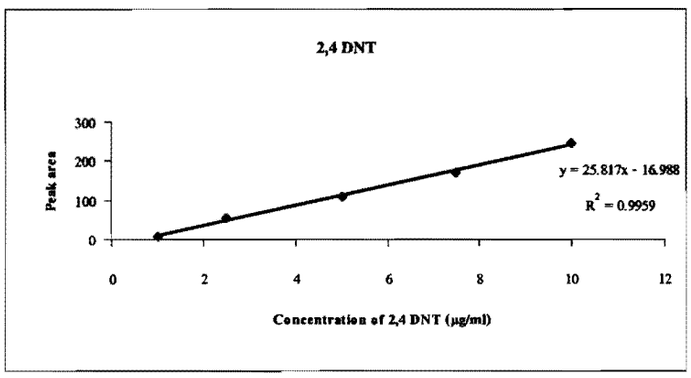


Figure E.1 Calibration curve of 2,4 DNT

b) 2,6 DNT

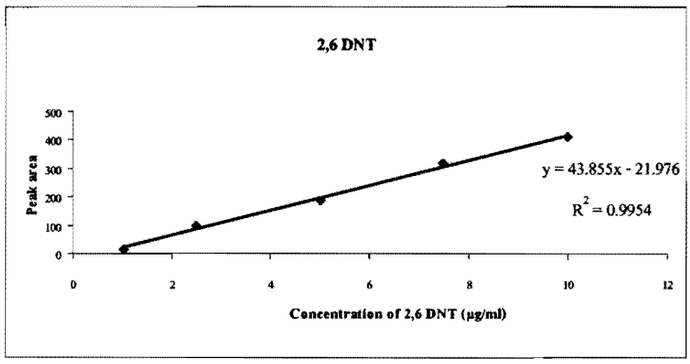


Figure E.2 Calibration curve of 2,6 DNT

c) 3,4 DNT

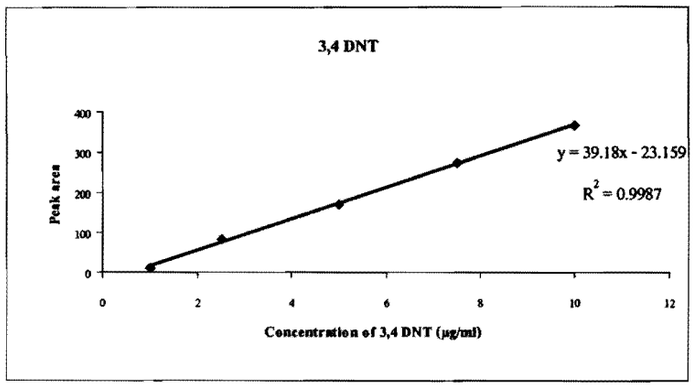


Figure E.3 Calibration curve of 3,4 DNT

APPENDIX F

LIMIT OF DETECTION OF THREE TYPES OF DINITROTOLUENE

APPENDIX F

Limit of Detection of three types of dinitrotoluene in high salt stacking

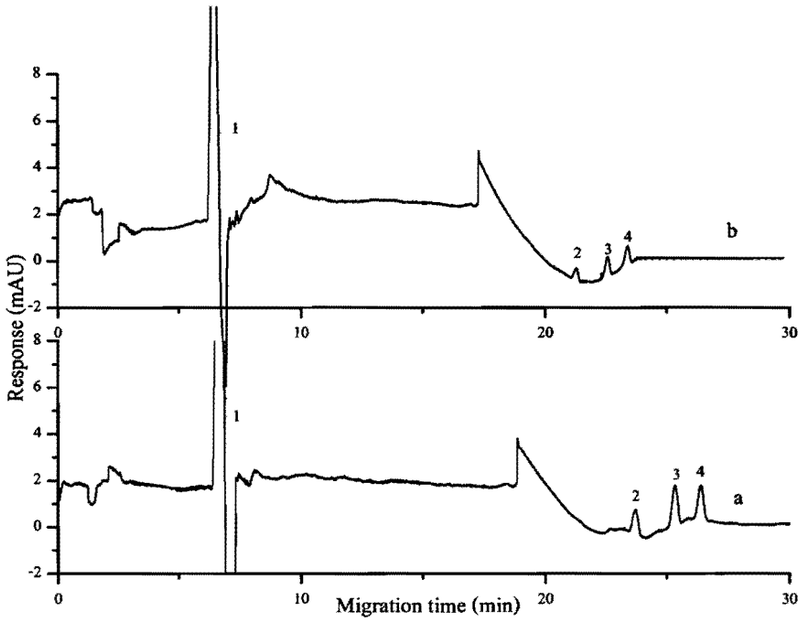


Figure F.1 Electropherogram of three types of dinitrotoluene for limit of detection a) 0.5μg/ml and b) 0.25μg/ml Conditions used: buffer composition; 25 mM phosphate, 75 mM SDS, pH 7.5; electrokinetic injection 10 kV, 40 sec, separation voltage +30 kV at 200 nm, L=64.5/56 cm effective length, Peaks identification; 1: EOF, 2: 2,4 DNT, 3: 2,6 DNT, 4:3,4 DNT.

APPENDIX G

SPIKED STANDARDS SOLUTION IN UNBURNED GUN POWDER

APPENDIX G

Spiked standards solution in unburned gun powder by high salt stacking

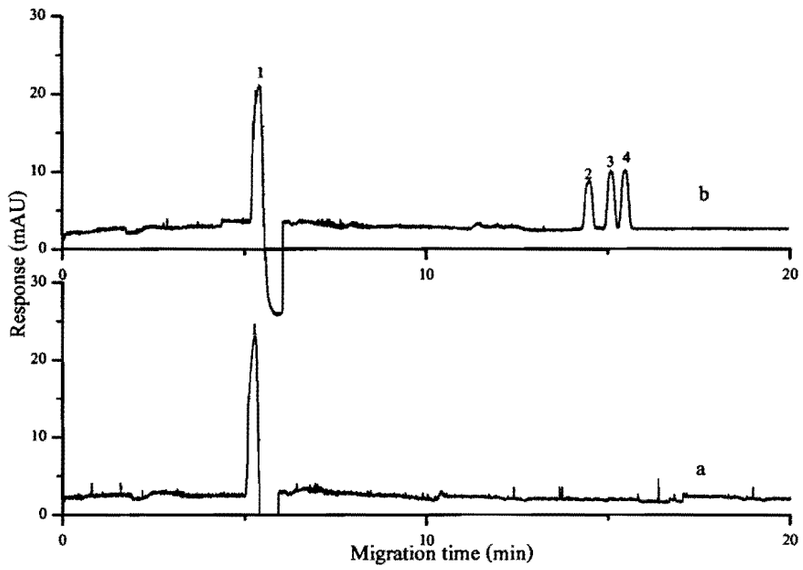


Figure G.1 Electropherogram of unburned gun powder by high salt stacking a) unburned powder of 38 special speer and b) spiked sample Conditions used: buffer composition; 25 mM phosphate, 75 mM SDS, pH 7.5; electrokinetic injection 10 kV, 40 sec, separation voltage +30 kV at 200 nm, L=64.5/56 cm effective length. Peaks identification; 1: EOF, 2: 2,4 DNT, 3: 2,6 DNT, 4:3,4 DNT

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