การแยกไอโซเมอร์โครงสร้างพอลิไซคลิกแอโรมาติกไฮโดรคาร์บอนด้วยลิควิดโครมาโทกราฟีโดยใช้สารลดแรงตึงผิวเป็นสารเหนี่ยวนำสภาพแข็งเกร็งของเฟสคงที่

นางสาวอนงค์นาถ โรจนกร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2551

SEPARATION OF STRUCTURAL POLYCYCLIC AROMATIC HYDROCARBON ISOMERS BY LIQUID CHROMATOGRAPHY USING SURFACTANT AS STATIONARY PHASE RIGIDITY INDUCER

Miss Anongnart Rotjanakorn

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
Department of Chemistry
Faculty of Science
Chulalongkorn University

Thesis Title	SEPARATION OF STRUCTURAL POLYCYCLIC AROMATIC HYDROCARBON ISOMERS BY LIQUID CHROMATOGRAPHY USING SURFACTANT AS STATIONARY PHASE RIGIDITY INDUCER.
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อนงค์นาถ โรจนกร: การแยกไอโซเมอร์โครงสร้างพอลิไซคลิกแอโรมาติกไฮโดรคาร์บอนด้วยลิควิดโครมาโทกราฟีโดยใช้สารลดแรงตึงผิวเป็นสารเหนี่ยวนำสภาพแข็งเกร็งของเฟสคงที่ (SEPARATION OF STRUCTURAL POLYCYCLIC AROMATIC HYDROCARBON ISOMERS BY LIQUID CHROMATOGRAPHY USING SURFACTANT AS STATIONARY PHASE RIGIDITY INDUCER.) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: ดร. ลักษณา ดูบาส, 110 หน้า.

งานวิจัยนี้มุ่งพัฒนาประสิทธิภาพการแยกสารพอลิไซคลิกแอโรแมติกไฮโดรคาร์บอน (PAHs) โดยใช้มอนอเมอร์ริก ซี18 คอลัมน์ โดยการเติมสารลดแรงตึงผิวลงในเฟสเคลื่อนที่ในระดับความเข้มข้นต่ำกว่าความเข้มข้นต่ำสุดที่รวมตัวเป็นไมเซลล์ (CMC) เพื่อให้ถูกดูดซับบนเฟสคงที่ การดูดซับบนเฟสคงที่นี้คาดว่าสามารถเพิ่มความแข็งเกร็งให้แก่เฟสคงที่และเพิ่มค่ารีเทนชันแฟคเตอร์ (k') และค่าการเลือกจำเพาะ (α) ของสารตัวอย่างได้ ในงานศึกษานี้ใช้สารลดแรงตึงผิวทั้งหมด 5 ชนิดได้แก่ เอธิลแอมโมเนียมคลอไรด์, ไดเอธิลเอมินไฮโดรคลอไรด์, บิวธิลเอมิน, เฮกซิลเอมิน และ ออกทิลเอมิน เพื่อทำหน้าที่เป็นสารเพิ่มความแข็งเกร็งให้แก่เฟสคงที่ เฟสเคลื่อนที่ที่ใช้คือสารละลายคาร์บอเนตบัฟเฟอร์ที่พีเอช 6 และอะซิโตไนไตรล์ในอัตราส่วน 30:70 ปริมาณของสารลดแรงตึงผิวที่ดูดซับบนเฟสคงที่ คำนวณโดยใช้วิธีวิเคราะห์ด้วยฟรอนทอน อุณหภูมิที่ใช้ในการศึกษานี้มีค่าตั้งแต่ 0 ถึง 40 องศาเซลเซียส โดยเพิ่มขั้นละ 10 องศาเซลเซียส สารตัวอย่างที่เลือกมาศึกษาครั้งนี้ได้แก่ ไตรฟีนิลีน, เบนซ์[เอ]แอนทราซีน, เบนโซ[เค]โฟโอแลนทีน และไดเบนซ์[เอ,ซี]แอนทราซีน จากการทดลองพบว่าสารลดแรงตึงผิวถูกดูดซับบนเฟสคงที่ได้ ณ พีเอชที่ทำการศึกษา เมื่อใช้เอธิลแอมโมเนียมคลอไรด์เป็นสารเหนี่ยวนำให้เกิดความแข็งเกร็งให้ค่ารีเทนชันแฟคเตอร์ และค่าการเลือกจำเพาะสูงกว่าระบบที่ใช้สารเหนี่ยวนำความแข็งเกร็งเป็นสารลดแรงตึงผิวชนิดอื่นและเมื่อเทียบกับระบบที่ไม่มีการเติมสารลดแรงตึงผิว พบว่าค่ารีเทนชันแฟคเตอร์ และค่าการเลือกจำเพาะมีค่าสูงสุดที่อุณหภูมิ 0 องศาเซลเซียส และคอลัมน์ที่ถูกพัฒนาด้วยวิธีนี้จะให้ค่าความเที่ยงสูง (ค่าความคลาดเคลื่อนสัมพัทธ์ < 1.0เปอร์เซ็นต์) ในการแยกสารตัวอย่าง ได้นำวิธีนี้ไปใช้แยกสารตัวอย่าง 5-เมทอกซีฟลาโวล และ 6-เมทอกซีฟลาโวล พบว่าเมื่อใช้เอธิลแอมโมเนียมคลอไรด์เป็นสารเหนี่ยวนำให้เกิดความแข็งเกร็งที่อุณหภูมิ 0 องศาเซลเซียส ให้ค่าการแยกดีที่สุดเมื่อเปรียบกับสารลดแรงตึงผิวชนิดอื่น

ภาควิชา เคมี ลายมือชื่อนิสิต อนงค์นาถ โรจนกร

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KEY WORD : POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)/MONOMERIC COLUMN/SURFACTANT/FRONTAL ANALYSIS

ANONGNART ROTJANAKORN: SEPARATION OF STRUCTURAL POLYCYCLIC AROMATIC HYDROCARBON ISOMERS BY LIQUID CHROMATOGRAPHY USING SURFACTANT AS STATIONARY PHASE RIGIDITY INDUCER. THESIS PRINCIPAL ADVISOR: LUXSANA DUBAS, Ph.D., 110 pp.

This study was aimed to enhance the separation efficiency of a monomeric C18 column on the separation of isomeric polycyclic aromatic hydrocarbons (PAHs) by adding a surfactant into the mobile phase with a concentration lower than its critical micellar concentration (CMC). This surfactant will be adsorbed onto the stationary phase. The adsorbed surfactants lead to an increase in stationary phase rigidity which increase the selectivity (α) and retention factor (k') of the compound separation. In this study, 5 types of surfactants (ethylammoniumchloride, diethylamine hydrochloride, butylamine, hexylamine and octylamine) were employed as rigidity inducers. A pH6 carbonate buffer/acetonitrile (30/70) was used as mobile phase. The amounts of surfactant adsorbed on the column were determined by frontal analysis. The temperature was varied from 0 to 40 ^{0}C with a 10 degree increment. Triphenylene, benz[a]anthracene, benzo[k]fluoranthrene and dibenz[a,c]anthracene were used as the solute probes. Our results showed that surfactants were adsorbed onto the surface of a monomeric C_{18} stationary phase via electrostatic interactions. Selectivity and retention factors obtained from using ethylammoniumchloride as the rigidity inducer were higher than the other surfactants. In comparison with a system without addition of the surfactant, the selectivity and retention factors were most improved at 0 ºC. This technique gave the higher precision on the separation of PAHs (%RSD < 1.0%). This method was used for separation of 5- and 6-methoxyflavone. Selectivity and retention factors of 5- and 6-methoxyflavone obtained from using ethylammoniumchloride at 0 ºC as the rigidity inducers were higher than the other surfactants.

Department: Chemistry Student's signature Anongnart Rotjanakorn

Field of study: Chemistry Principal advisor's signature

Academic year: 2008

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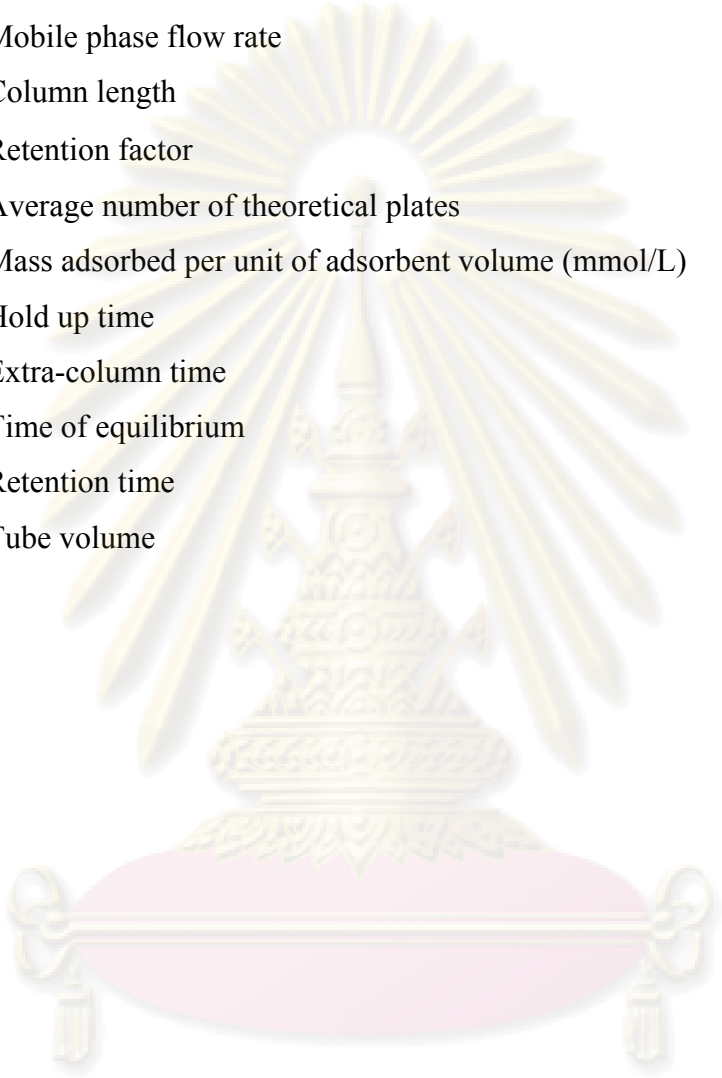
LIST OF ABBREVIATIONS AND SYMBOLS

CEP	The computation of elution profiles
CMC	Critical micellar concentration
DTAB	Dodecyltrimethylammonium bromide
ECP	Elution by characteristic points
FA	Frontal analysis
FACP	Frontal analysis by characteristic point
GC	Gas chromatography
HPLC	High Performance Liquid Chromatography
LS	Light scattering
L/B	Length-to-breadth
MEKC	Micellar electrokinetic chromatography
MS	Mass spectrometer
Near-IR	Near-infrared
NMR	Nuclear magnetic resonance
NPLC	Normal phase liquid chromatography
ODS	Octadecylated silica
PAHs	Polycyclic aromatic hydrocarbons
PBCs	Polychlorinated biphenyl congeners
PHAs	Polyaromatic hydrocarbons
RI	Refractive index
RPLC	Reversed-phase liquid chromatography
Rs	Resolution
SDBS	Sodium dodecylbenzenesulfonate
SDS	Sodium dodecyl sulfate
Sil-DSG	Dioctadecyl L-glutamide-derived lipid-grafted porous silica particles
UV	Ultra-violet
Wh	Bandwidth at half height
ΔG^0	Gibbs free energy
ΔH^0	Standard enthalpy
ΔS^0	The standard entropy

R	The gas constant
T	The temperature studies
α	The selectivity
d	Column diameter
Fv	Mobile phase flow rate
h	Column length
k′	Retention factor
N	Average number of theoretical plates
q*	Mass adsorbed per unit of adsorbent volume (mmol/L)
t0, tm	Hold up time
te	Extra-column time
teq	Time of equilibrium
tR	Retention time
Vc	Tube volume



CHAPTER I

INTRODUCTION

1.1 Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are the compounds that consist of two or more fused aromatic rings. These highly toxic compounds have been detected throughout the environment. The main sources of PAHs are incomplete combustion processes of organic matter, and to a smaller extent in forest fires and more scarcely microbiological syntheses or transformations [1, 2]. PAHs can have either mutagenic and carcinogenic properties or genotoxic potential. The complex mixtures of PAHs in environmental sample contain numerous isomeric structures. Since certain isomers are often more hazard than other isomers. The separations of individual PAHs are more important of these compounds [3, 4].

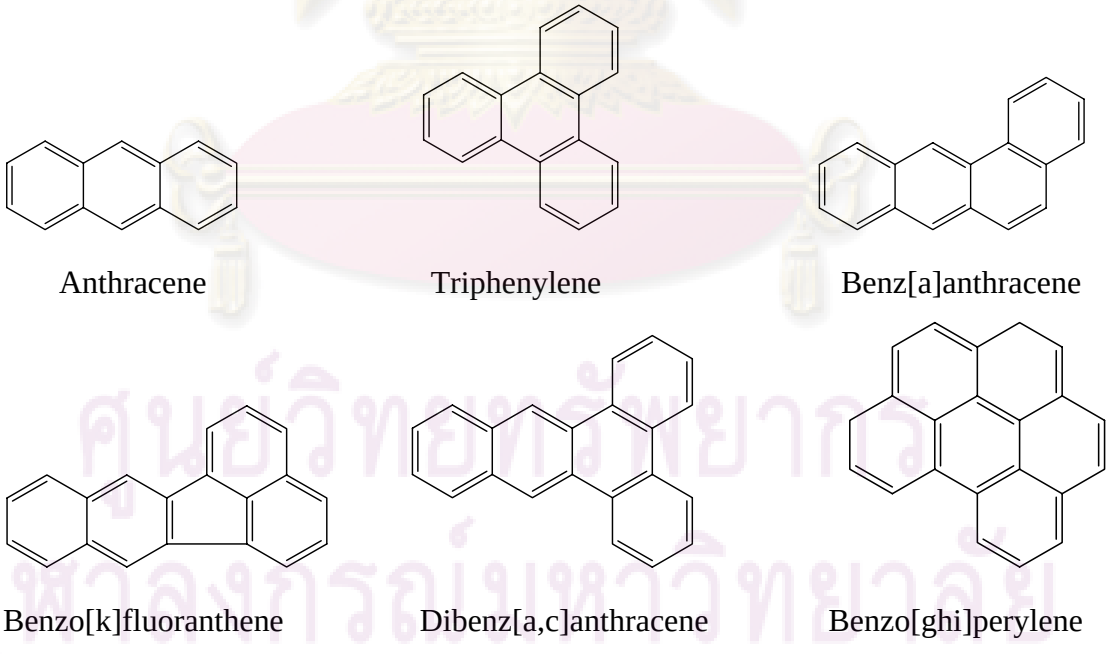


Figure 1.1 Chemical structures of some PAHs.

1.2 Analysis of PAHs

Gas chromatography (GC) is one of the most widely used in analysis of PAHs. The fundamental characteristic for a compound to be analyzed by GC is its volatility within the temperature range used. By this measure, PAHs containing up to 24 carbon atoms may be analyzed by GC. Structural differences may play a significant role in determining the volatilities of PAHs with the same carbon number; for example, the less condensed pyranthrene (which has thirty carbon atoms) can be easily analyzed by GC whereas its counterpart, naphtho[8,1,2-abc]coronene, requires special high-temperature GC [5]. However, the decomposition of analyte is the problem when analyzing PAHs using the GC.

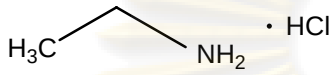
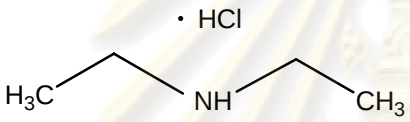
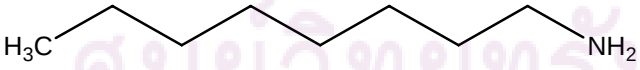
Reversed-phase liquid chromatography (RPLC) is the popular mode for separation and analysis of PAHs [6]. Particularly, octadecylated silica (ODS) has been most widely used as a stationary phase because of its wide applicability. Retention in RPLC is often described in terms of solute polarity and a like dissolve like process. However, it failed to explain the retention behavior of many classes of structural isomer such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyl congeners (PCBs) The retention mechanism of these groups are described by shape selectivity phenomenon. The shape selectivity is used to show a chromatographic quality exhibited by certain stationary phase for which separation of geometric isomers is based on their molecular structures [6]. Shape selectivity is enhanced by increased phase loading, longer chain length bonded phase ligands, reduced column temperature, increased organic modifier composition in the mobile phase, and the use of polymeric phases [4, 6]. Among these parameters, the phase density and polymeric phase play a major role in the solute retention. The commonly used high density monomeric and polymeric C18 stationary phases give better resolution (R_s) and longer retention factors (k') because these phases contain rigid structure [4, 6].

1.3 Purpose of the study

This study has for objective to enhance the separation efficiency of a monomeric C18 column on the separation of isomeric polycyclic aromatic hydrocarbons (PAHs) by adding a surfactant into the mobile phase with a concentration lower than its critical micellar concentration (CMC) to induce rigidity of stationary phase. Surfactants employed in the study are diethylamine hydrochloride, ethylamine hydrochloride, butylamine, hexylamine and octylamine as shown in Table 1.1. The positive part of the surfactants would interact with ionized silano groups on the silica supports via ionic interaction. The modified surface of stationary phase is covered with a layer of surfactant ions as shown schematically in Figure 1.2. The adsorbed surfactants lead to an increase in stationary phase rigidity which increases the resolution (R_s) and retention factor (k') of the PAHs. Solute probes are used in this study listed in Table 1.2 and 1.3. Column temperature will be varied from 0 to 40°C with a 10 degree increment.

First, we will study the adsorption isotherm of each surfactant as a function of concentration and column temperature. At each condition, the improvement of retention factor, resolution of PAHs solute probe will be evaluated. Finally, the separation efficiency of polar isomers will be studied under the best condition of each surfactant.

Table 1.1 Properties of studied surfactants

Surfactants	Carbon atoms	pK_a
Ethylammonuimchloride 	2	10.64
Diethylamine hydrochloride 	4	10.77
Butylamine 	4	10.70
Hexylamine 	6	10.56
Octylamine 	8	10.55

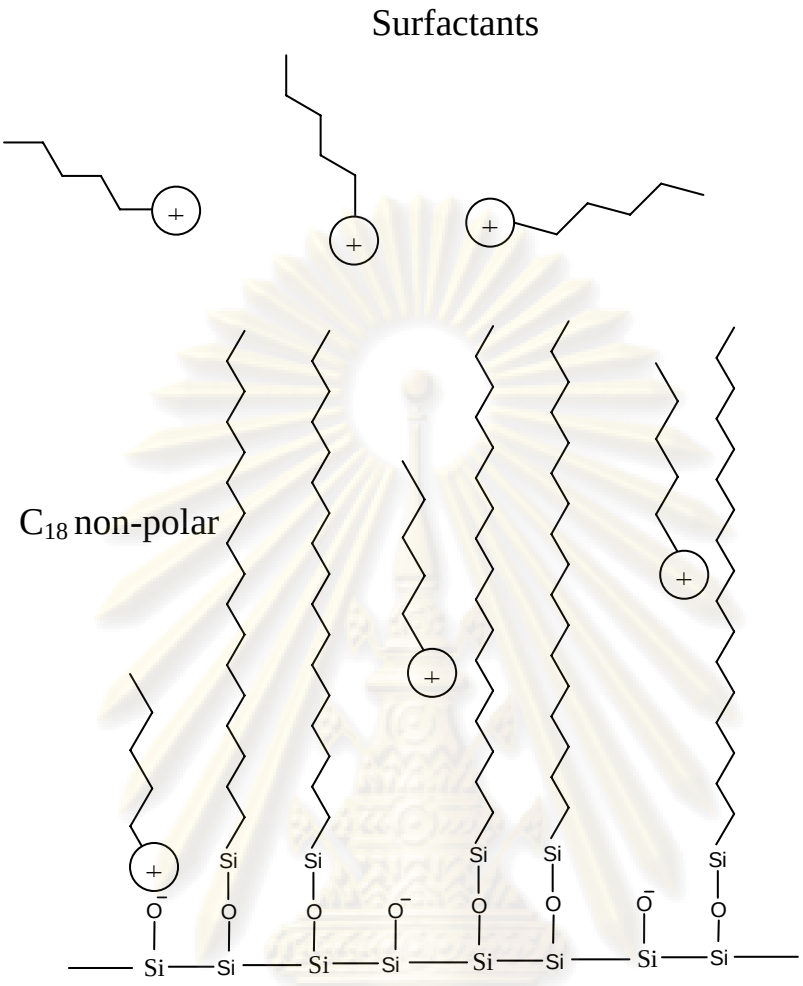
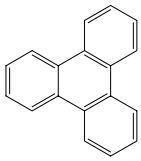
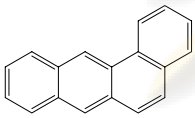
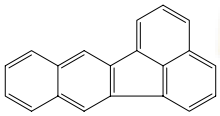
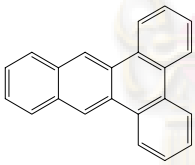


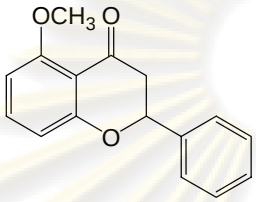
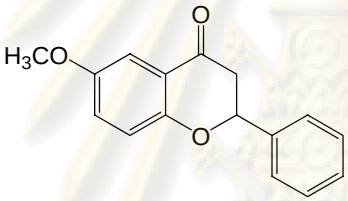
Figure 1.2 Stationary phase modified with surfactants.

Table 1.2 Properties of polycyclic aromatic hydrocarbons (PAHs, mixture I) used as solute probe: triphenylene, benz[a]anthracene, benzo[k]fluoranthene and dibenz[a,c]anthracene

Structure	Formula	Molecular Weight	L/B[a]	log P_{ow}[b]
Triphenylene 	$C_{18}H_{12}$	228	1.119	5.45
Benz[a]anthracene 	$C_{18}H_{12}$	228	1.599	5.91
Benzo[k]fluoranthene 	$C_{20}H_{12}$	252	1.474	6.84
Dibenz[a,c]anthracene 	$C_{22}H_{14}$	278	1.238	7.19

- [a] ref. [7]
- [b] ref. [8]

Table 1.3 Properties of isoflavonoids (mixture II): 5-methoxyflavone and 6- methoxyflavone were used for the performance study of the modified column

Structure	Formula	Molecular Weight
5-Methoxyflavone 	$C_6H_{12}O_3$	252.26
6-Methoxyflavone 	$C_6H_{12}O_3$	252.26

CHAPTER II

THEORY

2.1 High performance liquid chromatography (HPLC)

In the HPLC technique, the sample is forced through a column that is packed with irregularly or spherically shaped particles or a porous monolithic layer (stationary phase) by a liquid (mobile phase) at high pressure. HPLC is historically divided into two different sub-classes based on the polarity of the mobile and stationary phase. Technique in which the stationary phase is more polar than the mobile phase (e.g. toluene as the mobile phase, silica as the stationary phase) is called normal phase liquid chromatography (NPLC) and the opposite (e.g. water-methanol mixture as the mobile and C_{18} octadecylsilyl as the stationary phase) is call reversed phase liquid chromatography (RPLC). In general, HPLC system consists of four parts such as pump, sample introduction (injector), analysis column and detector [9], as shown in Figure 2.1.

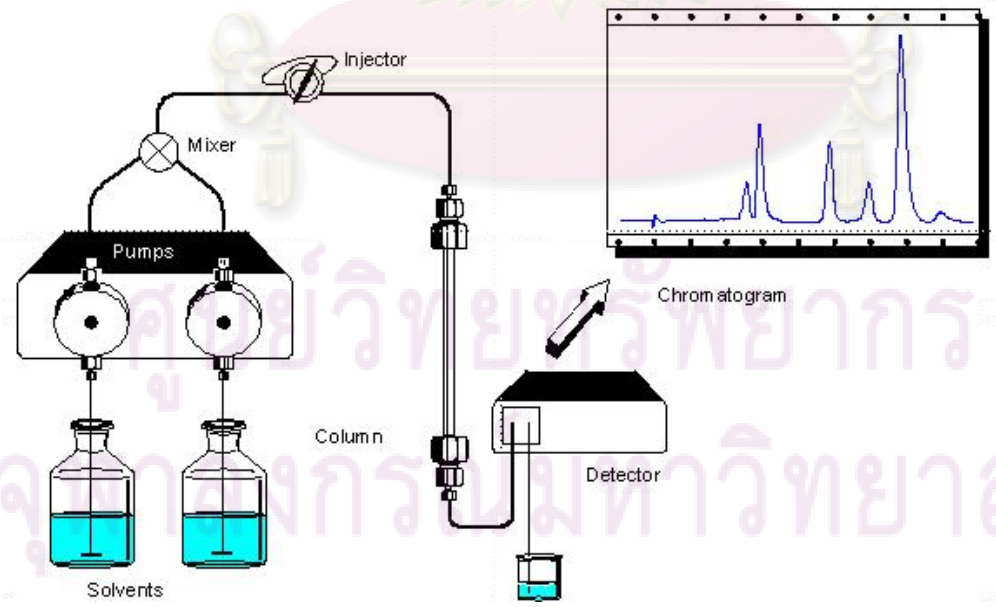


Figure 2.1 Schematic diagram of the HPLC system.

2.1.1 Pump

There are several types of pumps available for use with HPLC analysis. They are: reciprocating pumps, syringe type pumps, and constant pressure pumps.

The popular pumps used is reciprocating piston pump because this pump provides stable flow rate. However, it has to be pulse free and able to deliver constant flow rate [9].

2.1.2 Injector (sample introduction)

The injection port of HPLC consists of an injection valve and the sample loop. The sample is drawn into a syringe and injected into the loop via the injection valve. A rotation of the valve rotor closes the valve and opens the loop in order to inject the sample into the stream of the mobile phase. Loop volumes can range between 10 µl to over 500 µl. In modern HPLC systems, the sample injection is typically automated [9].

2.1.3 Stationary phase (column)

The commonly used stationary phase in RPLC is silica based modified surface with n-alkyl chains such as C-8 and C-18. The hydrophilic compounds elute earlier than hydrophobic compounds. The type of stationary phase in RPLC can be classified in two types: monomeric and polymeric.

2.1.3.1 Monomeric stationary phase

Monomeric phase is synthesized from the reaction of mono-functional silanes with silanols at the silica surface. Surface modification reactions are sterically limited and even under rigorous reaction conditions, only about half of the available silanol groups are covalently modified [6].

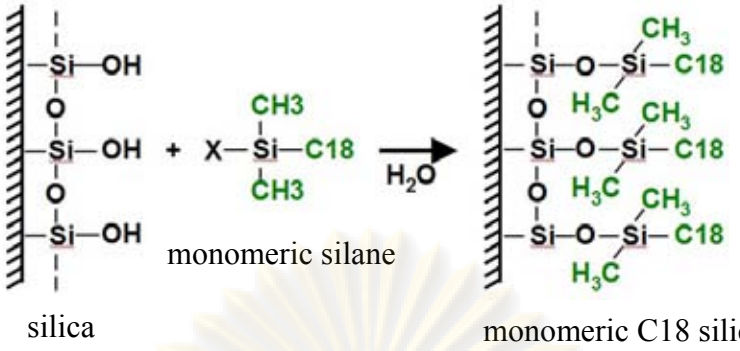


Figure 2.2 Synthesis schemes for monomeric C_{18} stationary phase [6].

2.1.3.2 Polymeric stationary phase

Polymeric stationary phase can be prepared by the reaction of di- or trifunctional silanes with silica, with the presence of water. Higher bonding densities can be achieved with polymeric synthesis. If water is added to the reaction slurry, the synthesis is referred to as solution polymerization since silane polymerization occurs in solution with subsequent deposition and links to the silica surface. Surface polymerization denotes a synthetic scheme in which the water is adsorbed onto dry silica prior to the silane introduction [6].

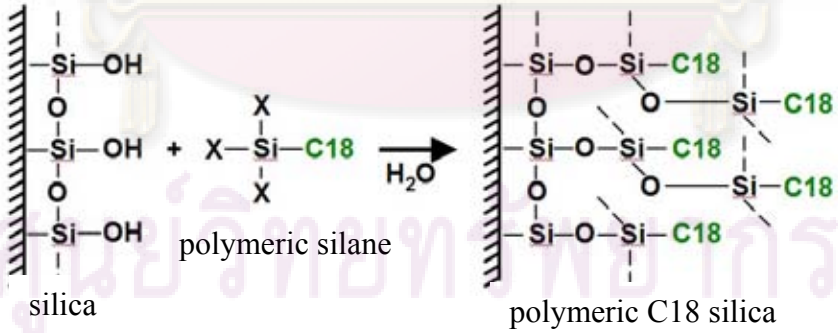


Figure 2.3 Synthesis schemes for polymeric C_{18} stationary phases [6].

2.1.4 Mobile phase

The mobile phase can be altered in order to manipulate the interactions of the sample and the stationary phase.

2.1.4.1 Isocratic elution: compounds are eluted using constant mobile phase composition.

2.1.4.2 Gradient elution: compounds are eluted by changing the mobile phase composition. In RPLC, percents of organic solvent in mobile phase increase with time [9]**.**

2.1.5 Detector

The common detectors used are ultraviolet (UV) detector, refractive index (RI) detector, fluorescence detector, radiochemical detector, electrochemical detector, near-infrared (Near-IR) detector, mass spectrometer (MS) detector, nuclear magnetic resonance (NMR) detector, and light scattering (LS) detector.

The ultraviolet spectrophotometer is the most popular and useful LC detector that is available to the analyst at this time. UV absorption detectors respond to those substances that absorb light in the range 180 to 350 nm. Many substances absorb light in this wavelength range. This can be detected at one (fixed wavelength detector) or several wavelengths (multi-wavelength detector) [9].

2.2 Parameter

Hold up time (t_M or t_0) is the time taken for the mobile phase to pass through the column. The unretained solute molecule is used to measure the hold up time because this compound is not retained by stationary phase and travels through column at the same rate as the mobile phase. The common unretained are potassium nitrate (KNO_3), uracil ($C_4H_4N_2O_2$) and deuterium oxide (D_2O) [9].

Retention time (t_R) is the time of analyte to travel through the column. Each analyte in a sample has a different retention time.

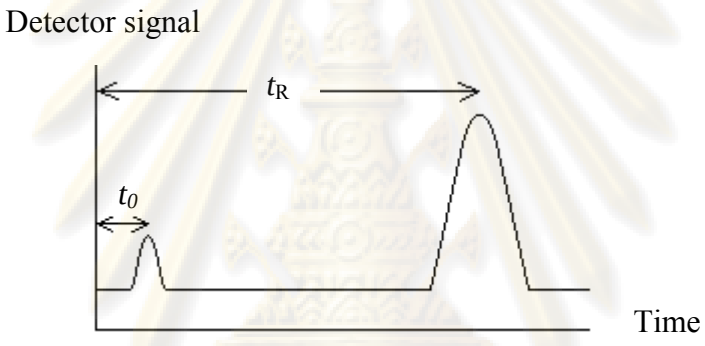


Figure 2.4 Shown the hold up time (t_0) and retention time (t_R).

The capacity or retention factor (k') is the ratio of amount of time a solute, spending in the stationary phase and mobile phase. It can be calculated using the following equation:

$$k' = \frac{t_R - t_0}{t_0} \qquad (2.1)$$

Where t_R is the retention time of the analyte (min) and t_0 is column hold-up time (min).

The selectivity (α) is a measure of the time or distance between the maxima of two peaks. If α = 1, then the peaks co-elute. It is calculated band with Equation (2.2).

$$\propto = \frac{k_2'}{k_1'} \tag{2.2}$$

Where k_1' and k_2' are retention factor adjacent bands 1 and 2.

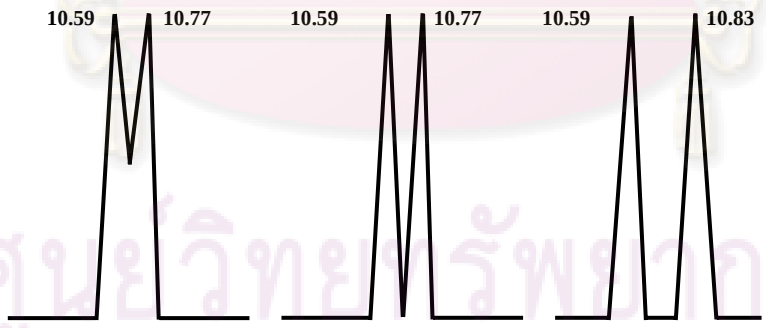
Although the selectivity factor describes the separation of band centers, it does not take into account peak widths. Another measure of how well species have been separated is provided by measurement of the resolution, R_S

$$R_S = 1.18\left(\frac{t_{R_2} - t_{R_1}}{W_{h_1} + W_{h_2}}\right) \tag{2.3}$$

Where W_{h_1} and W_{h_2} are their bandwidth at half-height (min) for adjacent bands 1 and 2.

t_{R_1} and t_{R_2} are retention time for adjacent bands 1 and 2 (min).

Baseline resolution is where resolution value is equal or above 1.5.



W_h = 0.126 min	W_h = 0.071 min	W_h = 0.071 min
R_s = 0.84	R_s = 1.50	R_s = 1.99
% = 50	% = 100	% = 100

Figure 2.5 Example of resolution.

2.3 Shape selectivity

Retention behavior in liquid chromatography is influenced by a wide variety of physical and chemical properties of both the chromatographic system and the solute. The development of retention models reflects an effort to describe the interaction processes between the solute and the stationary and mobile phases which are responsible for retention. The separation of isomeric compounds is described by "shape selectivity" mechanism, which is usually used to indicate a chromatographic quality exhibited by certain stationary phase for which separation of geometric isomers is based on their molecular structures, rather than other physical or chemical differences of the solutes [6].

2.3.1 Controlling factors of the shape selectivity

2.3.1.1 Bonding density

Stationary phase bonding densities control the shape discrimination of columns. Shape discrimination for geometric isomer increases with increasing bonding density because of the increase in phase rigidity [6, 10-12].

Table 2.1 The phenyl selectivity at 10 ºC as a function of octadecyl bonding density with acetonitrile/water (50/50) as mobile phase [12].

C_{18} bonding density ($\mu mol/m^2$)	Phenyl selectivity: mixture I[a]	Phenyl selectivity: mixture II[b]
1.44	2.48	3.94
2.74	2.67	4.31
3.43	2.82	4.65
4.77[c]	3.18	5.59

[a] Mixture of benzene, naphthalene and anthracene

[b] Mixture of benzene, biphenyl and p-terphenyl

[c] Polymeric C_{18} stationary phase

2.3.1.2 Bonded phase type

The polymeric C_{18} especially the highly loaded phase showed an excellence in the shape discrimination compared with monomeric phase.

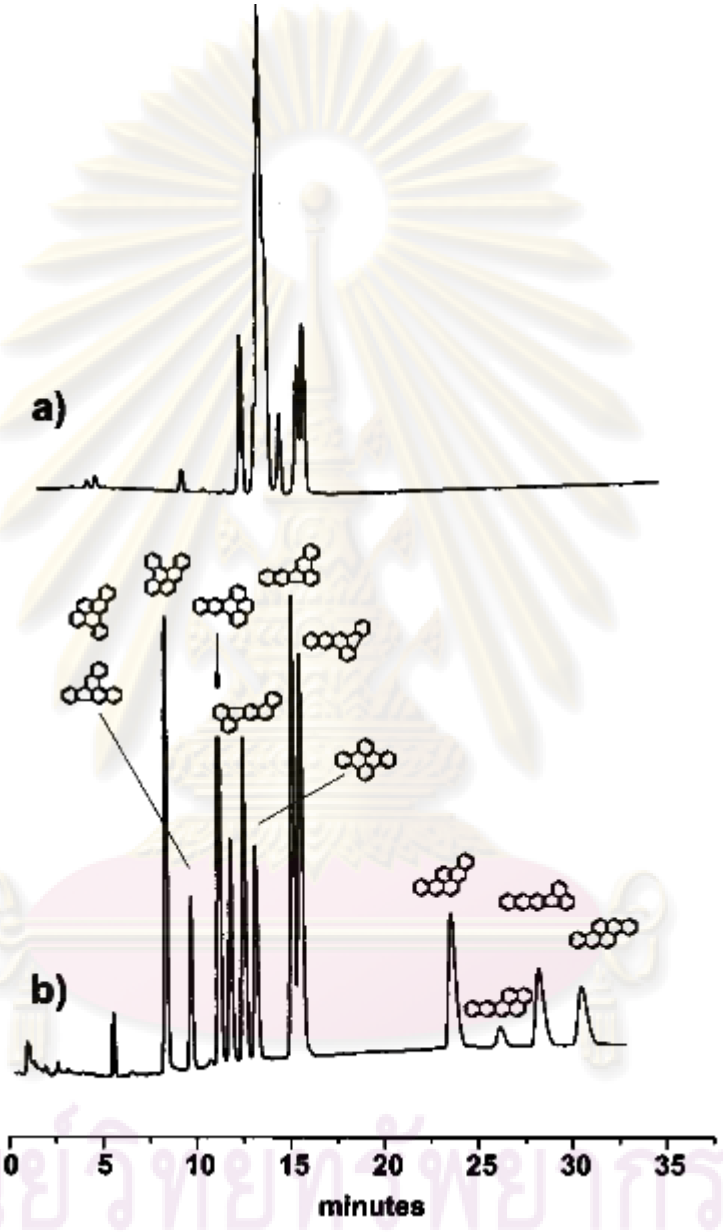


Figure 2.6 Separation of PAHs isomer of molecular mass 302 on (a) monomeric C_{18} column and (b) polymeric C_{18} column. Conditions: 90:10 acetonitrile/water (v/v) to acetonitrile over 10 min [6].

2.3.1.3 Alkyl chain length

The enhancement of the shape selectivity is proportionally related to the length of alkyl chain. Better separations of isomer sets are usually achieved with longer chain-length phases. This trend is observed for both monomeric and polymeric alkyl phases. For example, better shape discrimination of PAH isomers is possible with a monomeric C_{22} phase than with a monomeric C_8 phase. However, for any given alkyl length, polymeric phases still provide enhanced selectivity for structurally similar compounds [13].

2.3.1.4 Silica pore size

Shape selectivity depends on the pore size of the silica substrates. Little dependence of the column selectivity with silica pore size was observed in the C_{18} monomeric stationary phase. The polymeric stationary phase shape selectivity was improved with increasing pore diameter of the solid support [9].

2.3.1.5 Mobile phase composition

Mobile phase composition has been found to have a weak effect on shape selectivity. Shape selectivity increases slightly with the percent organic modifier. Mobile phase composition is probably of less importance than other variables for consideration during method development for isomers. However, mobile phase composition is often of importance for the separation of other compound [6].

2.3.1.6 Temperature

Column temperatures have been shown to have a strong effect on the shape selectivity in LC. The retention decreases with increasing temperature. Although temperature is sometime controlled to improve retention reproducibility or column efficiency [11, 14-15].

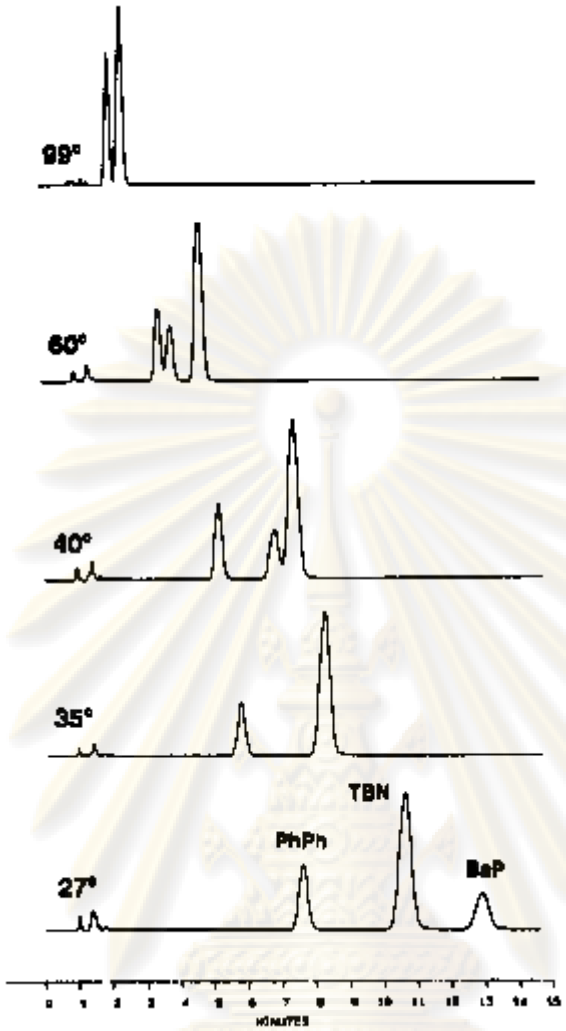


Figure 2.7 Separation of the phase selectivity test mixture on a Vydac 201TP C_{18} column at various temperatures [11].

2.3.2 Retention model of shape selectivity

The retention behavior of the PAHs can be explained in terms of schematic representation of the bonded phase, which we will refer to as the "slot model". Planar and linear molecules were preferentially retained over their nonplanar or nonlinear analogs. The planar or linear PAHs interact more strongly with the bonded phase than the nonplanar and nonlinear PAHs. A model can be envisaged in which the bonded phase consists of a number of narrow "slots" into which the solute molecules can penetrate (see Figure 2.8). Planar molecules would be able to fit more easily into these narrow slots and interact strongly with the C_{18} stationary phase,

whereas the nonplanar molecules would not penetrate as far into the slots, and so interact less strongly with the stationary phase [4].

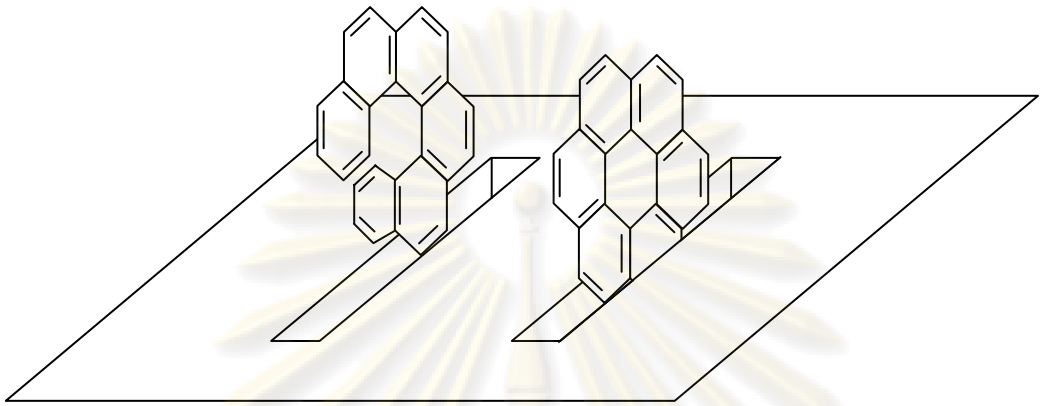


Figure 2.8 "Slot model" for retention of PAHs on C_{18} stationary phase [4].

2.3.3 Length-to-breadth (L/B) ratio

The L/B ratio was first used to correlate with the retention of PAHs in gas chromatography by Janini et al [15]. The molecules that have L/B ≈ 1 are nearly square and molecules that have L/B > 1 are linear or planar shape.

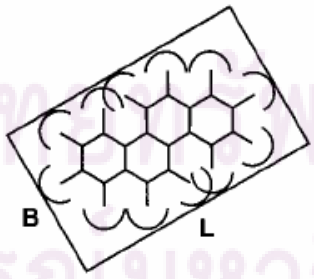


Figure 2.9 Molecular descriptors: length-to-breadth ratio [6].

2.3.3.1 Solute shape

In general, solute retention increases with increasing L/B ratio for PAHs isomer separation. The example of this trend illustrated in Figure 2.10 for the separation of molecular mass 278 PAH isomers on polymeric C_{18} stationary phase [6].

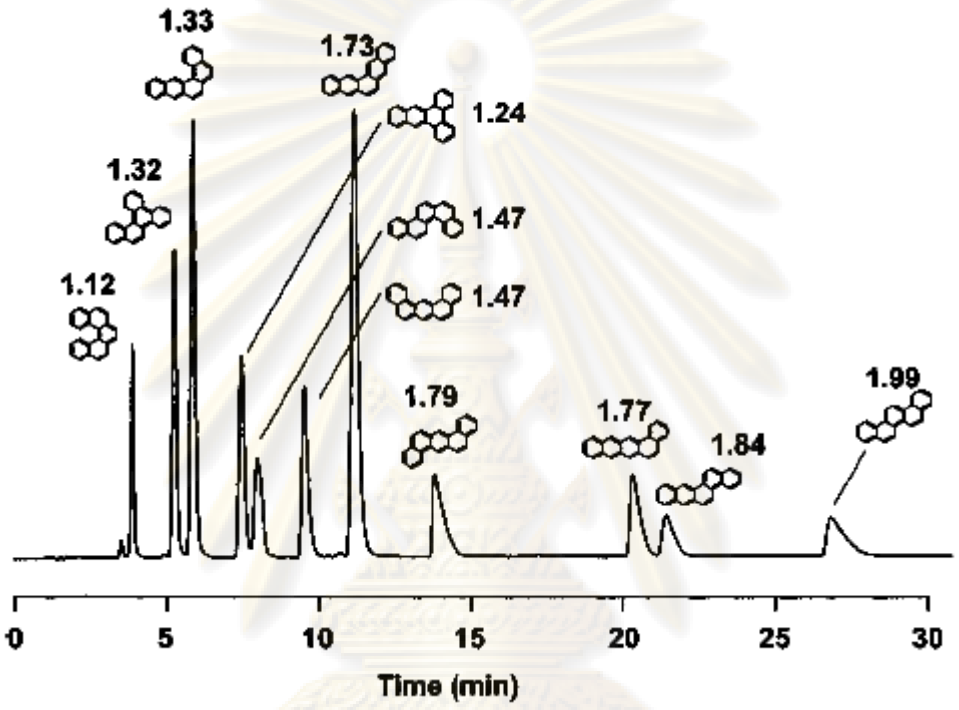


Figure 2.10 Separation of PAHs isomer of molecular mass 278 on polymeric C_{18} column. Values represent L/B ratios for the isomer. Conditions: 85:15 acetonitrile/water (v/v) to acetonitrile over 15 min [6].

2.4 Temperature dependence behavior of the bonded alkyl chain anchored on the stationary phase

The bonded phase association and the configuration change with changing temperature. The effect of column temperature on the alkyl chain configuration was also discussed by using NMR spectroscopic information.

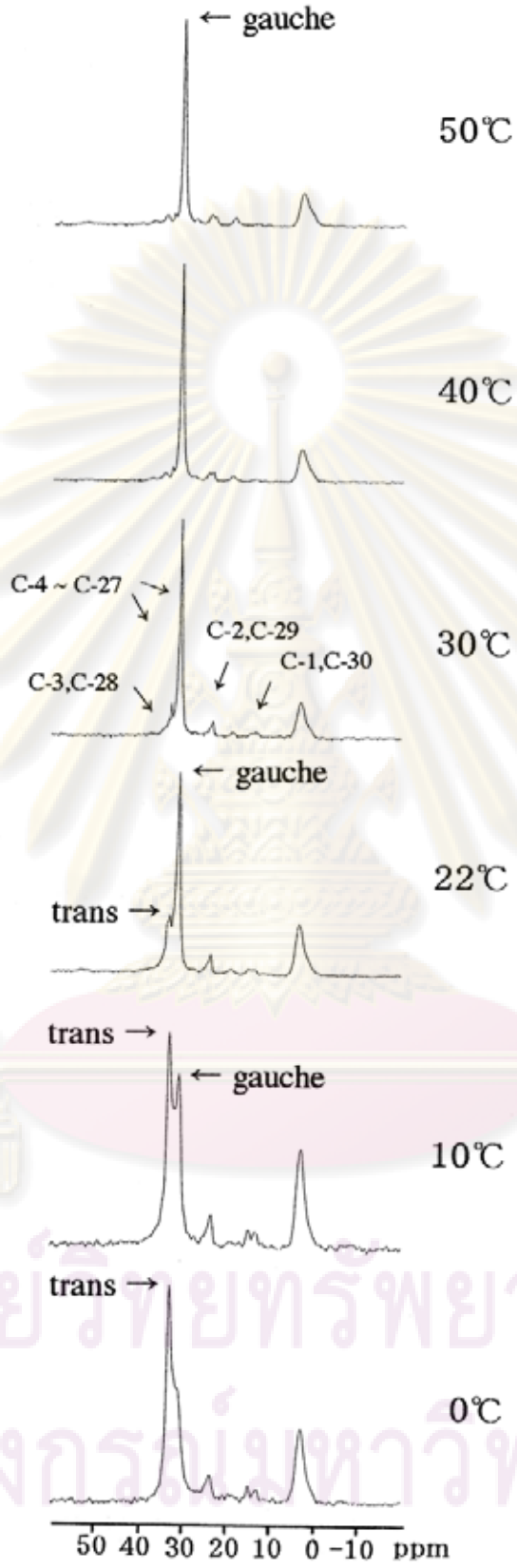


Figure 2.11 NMR spectra of the C_{30} bonded phase at various temperature [16].

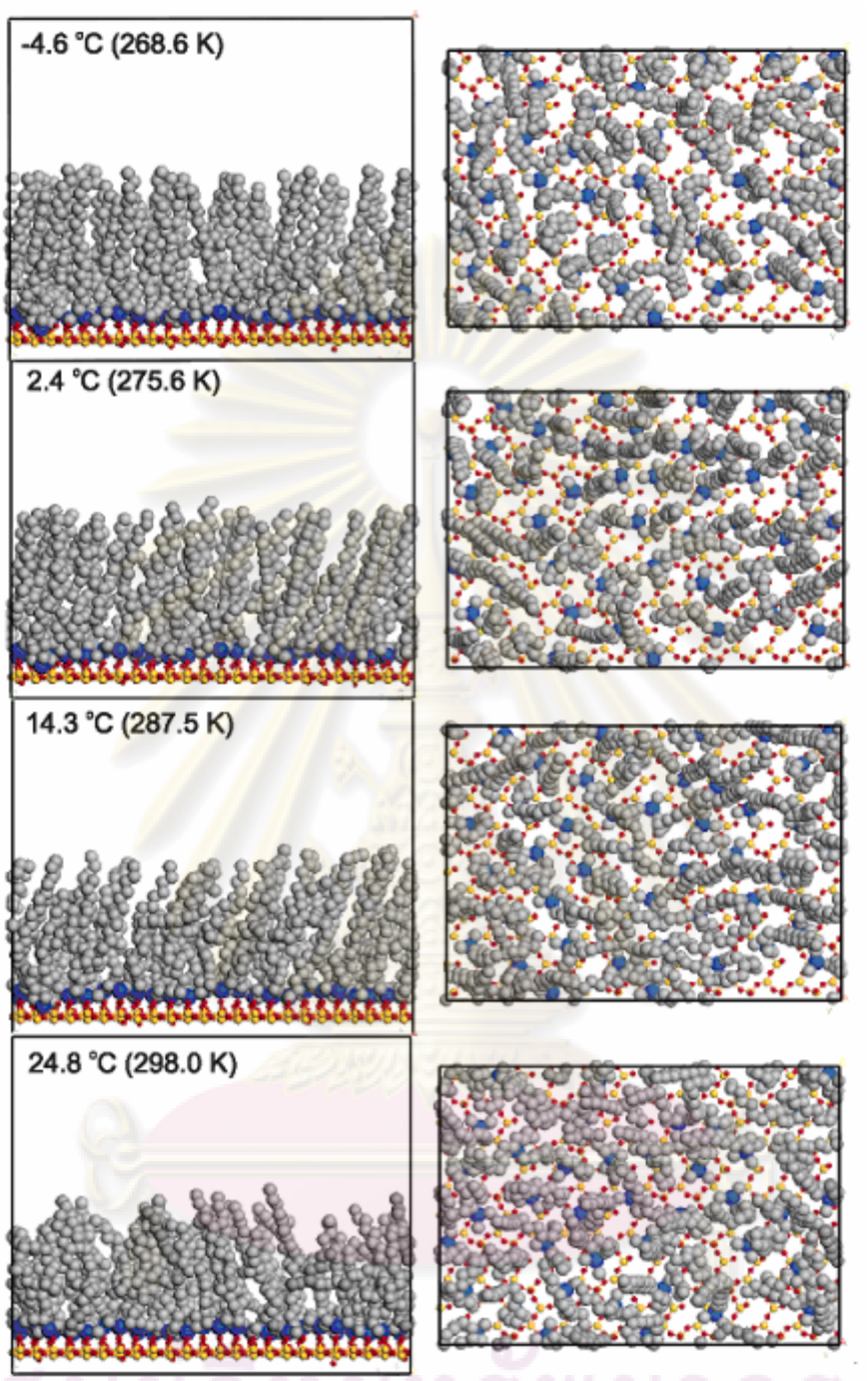


Figure 2.12 Side- and top-view snapshot of simulated monomeric C_{18} model at various temperatures [19].

Figure 2.11 and 2.12 show NMR spectral changes as a function of temperature for the monomeric type C_{30} phase. The proportion of the "gauche" configuration increases above 20 ºC and proportion of the "trans" configuration increases below 20 ºC [16-18].

The retention of planar molecule begins to increase at the same temperature that the proportion of the trans configuration just being to increase. At low temperature, more alkyl chains anchored on the stationary phase like slot structure of the bonded moiety so the planar PAHs can easily access to the space of the bonded moiety resulting in strong retention of planar PAHs. Figure 2.13 is a schematic for the proposed interaction between the monomer types C_{30} bonded phase and PAHs [16].

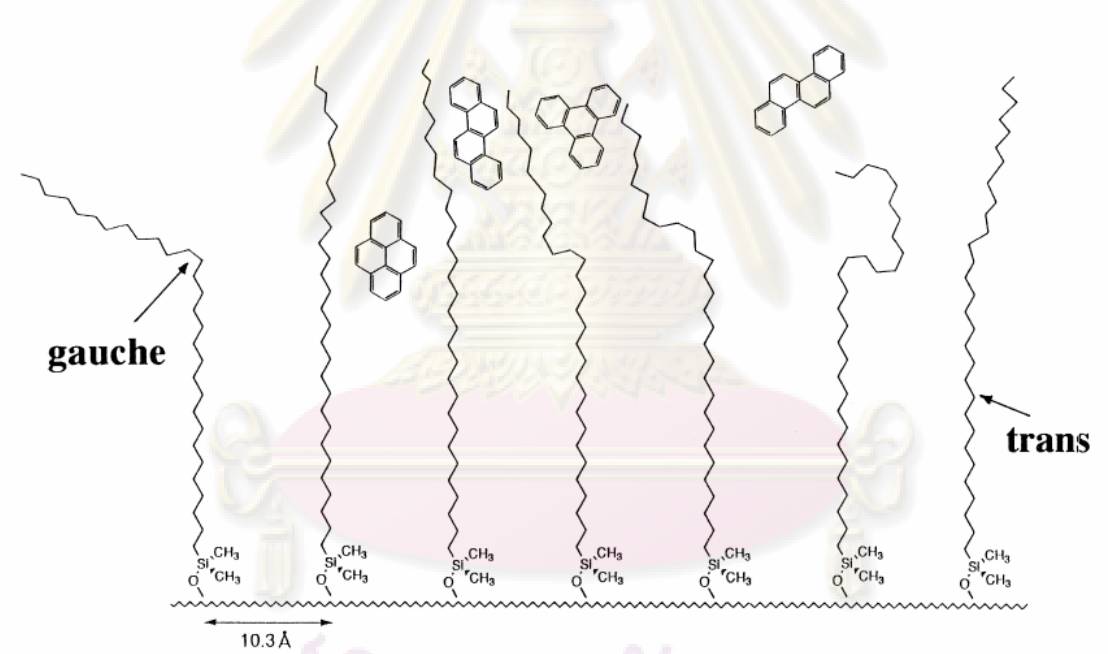


Figure 2.13 Schematic for the interaction between the C_{30} bonded phase and PAHs [16].

2.5 Surfactant

Surfactants are amphiphilic molecules that have both hydrophobic and hydrophilic properties. A surfactant molecule consists of a hydrophobic group, which has long chain hydrocarbon (tail) that dissolves in non-polar solvent and hydrophilic group (head) that dissolves in polar solvent [19].

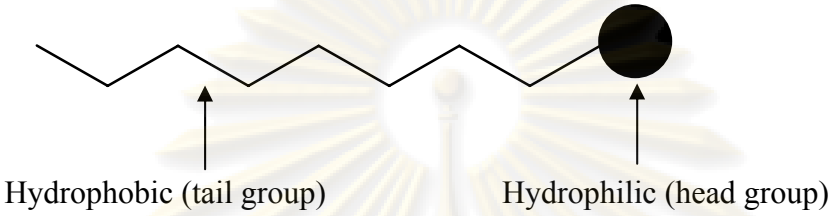


Figure 2.14 Structural of surfactants.

Critical micelle concentration (CMC) is the concentration of the surfactants in solution at which the formation of aggregates (micelles, round rods, lamellar structures etc.) in the solution is initiated.

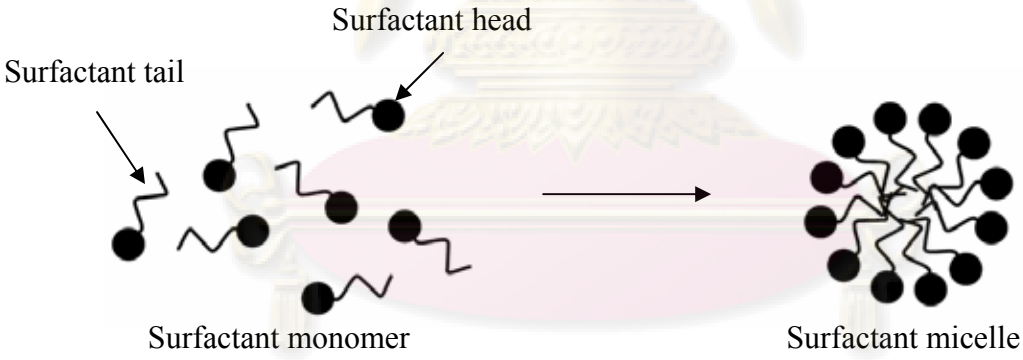


Figure 2.15 Structure of surfactant monomers and micelles.

2.5.1 Anionic surfactant

The head group of surfactant has negative charge in solution.

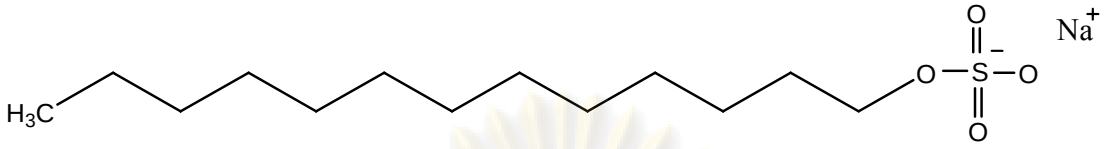


Figure 2.16 Structure of sodium dodecyl sulfate (SDS).

2.5.2 Cationic surfactant

The head group of surfactant has positive charge in solution.

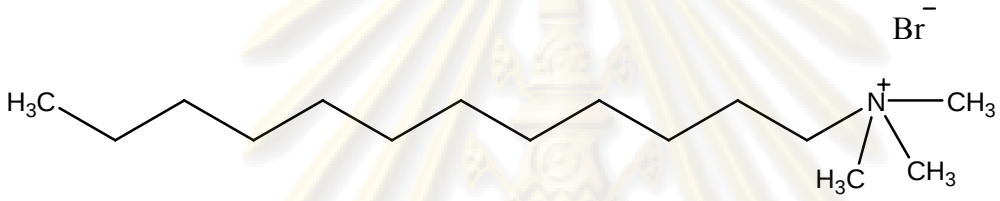


Figure 2.17 Structure of dodecyltrimethylammonium bromide (DTAB).

2.5.3 Non-ionic surfactant

The surfactant does not have an electrical charge.

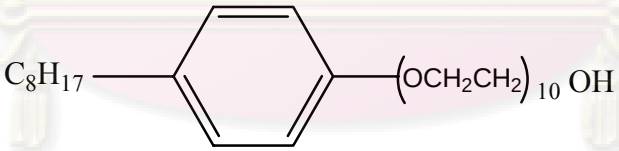


Figure 2.18 Structure of *t*-octylphenoxy-polyethoxyethanol (triton X-100).

2.5.4 Amphoteric surfactants

The surfactants can be either anionic (negatively charged), cationic (positively charged) or non-ionic (no charge) in solution, depending on the acidity or pH of the water.

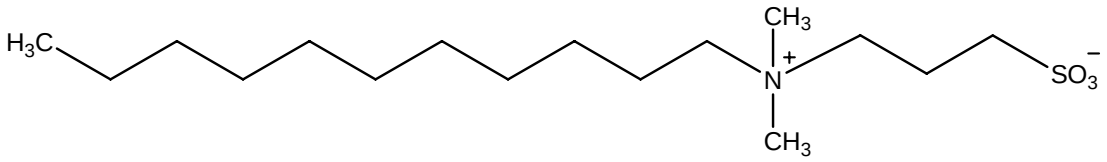


Figure 2.19 Structure of 3-(dodecyldimethylammonio) propanesulfonate.

2.6 Frontal analysis (FA)

An adsorption isotherm, which describes the relationship between solute concentration in the mobile and stationary phases under constant temperature at equilibrium, can be measured by many methods for example frontal analysis (FA), frontal analysis by characteristic point (FACP), elution by characteristic points (ECP), the pulse methods, and the computation of elution profiles (CEP) method or inverse method [20-21]. FA is the most convenient method because does not require detector calibration and does not depend on the column efficiency.

In FA, mobile phase is pumped into the column with surfactant solution at a known concentration in the mobile phase and recording the break-through curve of the column elute. From a breakthrough curve, the amount of the compound that is required to equilibrate the packing material in the column with the surfactant can be calculated as shown in Figure 2.20 [22-25].

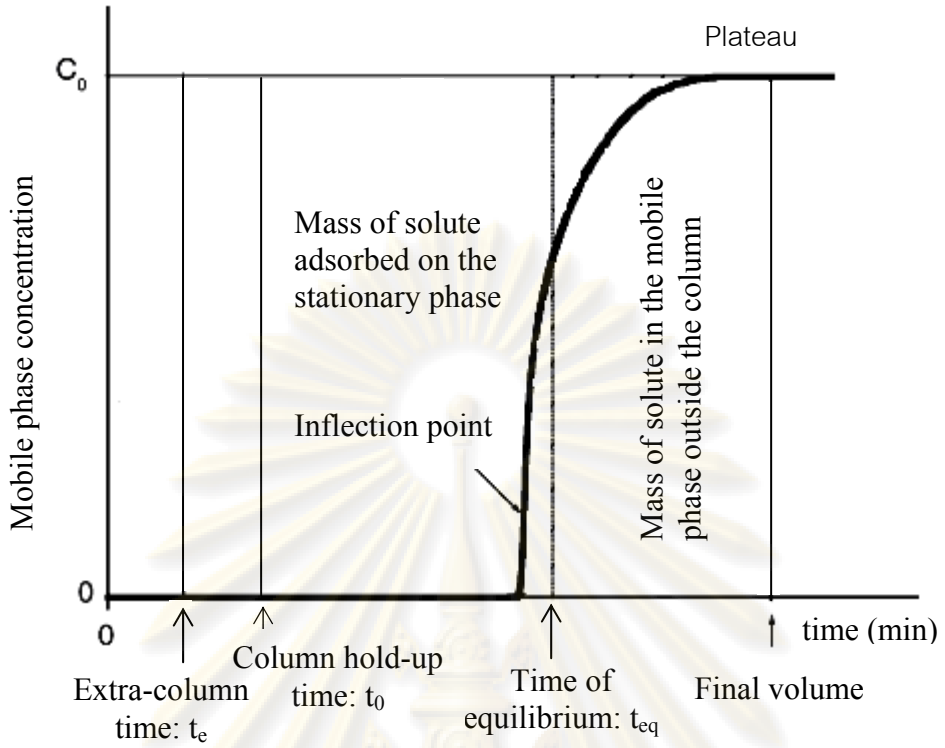


Figure 2.20 A frontal analysis curve and application of equal area method for the adsorption isotherm determination and description of the method used to derive the amount adsorbed from the frontal analysis curves [21].

The mass of compound adsorbed when plateau concentration is reached allows the calculation of the mass adsorbed per unit of adsorbent volume, q^* (mmol/L)

$$q^* = \frac{F_v\left(t_{eq} - t_0 - t_e\right)C}{V_c - F_v t_0} \tag{2.4}$$

F_v is a mobile phase flow rate, V_c is a column tube volume, t_0 is a column hold-up time, t_e is extra-column time, C is a concentration of mobile phase and t_{eq} is a time of equilibrium [26-27].

2.7 Literature reviews

2.7.1 Effect of stationary phase morphology for PAHs separation

Effect of bonded phase lengths was reported by Wise and Sander [28] by studying the retention of PAHs on monomeric and polymeric alkyl phases ranging in length in range from C_8 to C_{18}. The retention behavior of PAHs was observed to vary significantly with alkyl chain length where the C_{30} monomeric phase was observed to have selectivity toward PAH similar to C_{18} polymeric phase. Sander and Wise [10] studied molecular shape recognition of a series of C_{18} columns prepared using a variety of synthetic approaches. Mono-, di-, and trifunctional silanes were used to prepare stationary phases through monomeric and polymeric surface modification procedures. Shape selectivity was enhanced by increasing phase loading, longer chain length bonded phase ligands, reduced column temperature, increased organic modifier composition in the mobile phase, and the use of polymeric phases. Wise and Sander [4] separated PAHs by monomeric C_{18} and polymeric C_{18} stationary phase. Polymeric stationary phase has a high C18 surface coverage provided excellent selectivity for separation of PAHs than monomeric stationary phase. Sander *et al.* [6] presents an overview of column properties that influence shape selectivity for PAHs. Stationary phase contribution to shape recognition increase with polymeric surface modification chemistry, increasing bonding density, increasing alkyl chain length, increasing organic content of the mobile phase and decreasing temperature.

Kikta and Grushka [29] studied the retention behavior on alkyl bonded stationary phases for liquid chromatography. This work has been studied as a function of chain length, surface coverage, solute type, mobile phase composition and temperature. The small bonded nonpolar methyl groups do not interact strongly with the solutes.

Shundo *et al.* [30] newly synthesized and immoblilized poly(octadecylacrylate) having plural trimethoxysilyl groups in the side chain (co-ODA*n*) on to silica supported co-ODA*n* (Sil-co-ODA*n*) used as a stationary phase in HPLC. Sil-co-ODA_{16} showed higher selectivity for the separation of PAHs than Sil-ODA_{14} and octadecylated silica (ODS) due to the higher bonding density.

Kayillo *et al.* [31] studied the retention behavior of polycyclic aromatic hydrocarbons in reversed phase stationary phase on C_{18} and phenyl-type surface. The result revealed that phenyl-type column offered better separation performance for the linear PAHs. Because the propyl-phenyl column has the highest molecular-stationary phase interaction.

Takafuji *et al.* [32] newly synthesized a stationary phase in reversed phase liquid chromatography by dioctadecyl L-glutamide-derived lipid-grafted porous silica particles (Sil-DSG). Compared with conventional ODS (octadecylated silica), the Sil-DSG column showed remarkably higher selectivity for PAHs.

Rimmer *et al.* [33] synthesized from consecutive length alkylsilanes ranging from C_{13} through C_{18}, with three different bonding chemistries (monomeric, solution polymerized, and surface polymerized) at each phase length. The phases were characterized in terms of methylene selectivity, shape selectivity, and band broadening. As the shape selectivity is dependent on the bonding density of the phase, it is not surprising that surface polymerized phases exhibited the highest degree of shape recognition; monomeric phases showed the lowest degree of shape recognition, and solution polymerized phases are intermediate.

Núñez *et al.* [34] coated a monolithic silica capillary column with poly(octadecyl methacrylate) (ODM column) for the RPLC separation of some polar and non-polar compounds: PAHs, steroids, alkyl phthalates, and tocopherol homologues. The results were compared to those obtained by using a monolithic silica capillary column modified with octadecylsilyl-(*N*,*N*-diethylamino) silane (ODS column). The ODM column showed a better performance for polar and non-polar compounds compared to conventional ODS columns because the higher amount of C18 stationary phase bonded to the silica.

2.7.2 Surfactant application for PAHs and other compounds analysis

Hansen *et al.* [35] developed the reversed-phase HPLC base on dynamic modified of bare silica with long chain quaternary ammonium ions. The modified silica approach has been used in analysis drug metabolism and determination of the toluene metabolite. The main advantage is high reproducibility of the selectivity.

Delgado *et al.* [36] used micelles and alcohols as the modifiers of the aqueous mobile phase in RPLC in controlling retention and selectivity of polyaromatic hydrocarbons. The enhancement of separation selectivity with increasing of alcohol concentration was observed. Increasing micelle concentration showed an opposite effect on the separation selectivity, that the separation selectivity of PAHs was better with mobile phases with medium concentration of micelles and moderately high alcohol percentages. The selectivity enhancement can be explained in terms of the competing equilibria in micellar.

Kavran and Erim [37] used sodium dodecylbenzenesulfonate (SDBS) as an additive in separating a broad range of PAHs by micellar electrokinetic chromatography (MEKC) base on capillary electrophoresis. The separation mechanism was predicted as solvophobic association of the PAH molecules with hydrophobic chains of the SDBS surfactant and a possible ¶-¶ interaction between aromatic groups of PAHs and SDBS. A buffer of 20 mmol/l Tris, 40% MeCN and 50 mM SDBS at 25 kV was found to be optimal for complete separation.

Zhao *et al.* [38] used cationic surfactants attached to cation-exchange silica and unmodified silica to create hydrophobic solid-phase extraction sorbents. Various chain lengths and numbers of amine, ammonium and pyridinium based cationic surfactants were investigated to reach sufficient sorbent hydrophobicity to capture PAHs. The result showed that the hexadecylamine gave a greater density of alkyl chains than the trimethylhexadecylammonium bromide and N-cetyloxycarbonylmethyl pyridinium bromide, resulting in a more hydrophobic sorbent. Steric hindrance of the more bulky trimethyl ammonium and pyridinum groups prevented a smaller amount of surface alkyl groups than the least bulky amine group. The trimethyloctadecylammonium bromide chain attacheed as efficiently as

the trimethylhexadecylammonium bromidechain, therefore the greater carbon content from the C18 chain resulted in greater %C and greater hydrophobicity. The C16 trimethylammonium bromide was less steric hindrance so it better attached to surface than di-C16 dimethylammonium bromide.

From our knowledge, there are no studies on the effects of surfactant: chain length, concentration and column temperatures on the separation efficiency of PAHs and polar rigid compounds on the monomeric C18 stationary phase.

CHAPTER III

EXPERIMENTAL

3.1 Instrument and Apparatus

3.1.1 High Performance Liquid Chromatography (HPLC): Varian, Prostar.

3.1.2 Milli-Q, Ultrapure water systems, with Millipak® 40 Filter unit 0.22 µm, model Millipore ZMQS5VOOY, Millipore, Billerica, MA, U.S.A.

3.1.3 HPLC column: HyperClone 5 µm ODS (C18) 4.6 x 150 mm, Phenomenex, USA.

3.1.4 A glass filter holder set (300 mL funnel, 1 L flask, glass base with tube cap, and 47 mm spring clamp) for HPLC mobile phase filtration, Millipore, Billerica, MA, U.S.A.

3.1.5 Vacuum pump with pressure regulator, Model DOA-P504-BN, Gast®, Michigan, U.S.A.

3.1.6 pH meter, Model 744, Metrohm, Herisau, Switzerland.

3.1.7 Micropipettes 10-100, 100-1000 µm and tips, Eppendorf, Hamburg, Germany.

3.1.8 Nylon filter membrane 47 mm, 0.45 µm, Sartorius, Goettingen, Germanny.

3.1.9 Round bottle flask 250, 500 and 1000 mL.

3.1.10 Volumetric flask 10.00, 25.00, 250.00 and 1000.00 mL.

3.1.11 Beakers 10, 100, 250, 500 and 1000 mL.

3.1.12 Graduated cylinders 10.0 and 25.0 mL.

All glassware was washed sequentially with detergent and follow by rinsed with deionizer water.

3.2 Chemicals

3.2.1 Standard compounds

Triphenylene, benz[a]anthracene, benzo[k]fluoranthene and dibenz[a,c]anthracene was purchased from Fluka, Sigma-Aldrich, USA. D_2O and acetylsalicylic acid were purchased from Aldrich, Sigma-Aldrich.

3.2.2 Organic solvents

Acetonitrile (HPLC grade) was purchased from Fisher Scientific, Leicestershire, U.K. Methanol (HPLC grade) was purchased from E. Merck, Darmstadt, Germany.

3.2.3 Other chemicals

Hydrochloric acid (HCl) was purchased from E. Merck, Darmstadt, Germany. Sodium hydrogen carbonates ($NaHCO_3$) and sodium hydroxides (NaOH) were purchased from Carlo Erba Reagenti. Butylamine, hexylamine and octylamine were purchased from Aldrich, Sigma-Aldrich, USA. Ethylammoniumchloride was purchased from E. Merck, Darmstadt, Germany. Diethylamine hydrochloride was purchased from Fluka, Fluka chemical AG, Switzerland. Glycerol was purchased from Fisher Scientific, Leicestershire, U.K.

3.3 Preparation of standard solutions

3.3.1 Stock of standard solution

A 200 ppm single solution of each standard triphenylene, benz[a]anthracene, benzo[k]fluoranthene and dibenz[a,c]anthracene was prepared by dissolving 2.0 mg in 10 mL volumetric flask with 1 mL acetonitrile and making up to scale with 70% acetonitrile: 30% water. All single stock standard solutions were kept in amber bottle glasses.

A 20 ppm of each solute probe solution was prepared by pipetting 1.00 mL of each standard stock solution into a 10.00 mL volumetric flask and making up to scale with 70% acetonitrile : 30% water. These solutions were kept in amber bottle glasses.

A 20 ppm of acetylsalicylic acid was prepared by dissolving 2.0 mg in 10 mL volumetric flask and making up to scale with 70% acetonitrile: 30% water. D_2O was used as t_0 marker.

3.3.2 Preparation of carbonate buffer

A 500 mL of 40 mM carbonate buffer pH6 was prepared by dissolving sodium hydrogen carbonates ($NaHCO_3$) 1.6801 g with water and adjusting pH of solution to the required pH with hydrochloric acid (HCl).

3.3.3 Preparation of surfactant solution

A 10 mM surfactant solution pH6 was prepared by dissolving surfactant with sodium hydrogen carbonates ($NaHCO_3$) in 250.00 mL volumetric flask. The pH6 was adjusted with hydrochloric acid (HCl), the solution was made up to scale with sodium hydrogen carbonates solution.

3.4 High performance liquid chromatography analysis

3.4.1 Parameter of chromatographic system

Table 3.1 Physico-chemical properties of the C_{18}-bonded packed (4.6 x 150 mm) and other parameters

Particle shape	Spherical
Particle size (μm)	5
Pore size (°A)	130
Pore volume (mL/g)	0.6
Surface area (m^2/g)	155
Total carbon (%)	10
Column length (mm)	150
Column diameter (mm)	4.6

Information is provided by the manufacturers (phenomenex®)

3.4.2 High performance liquid chromatography instrument

Varian Prostar model 230 HPLC was used in this study. This instrument includes a multi-solvent delivery system, a manual injector with a 20 μL sample loop, a diode-array UV-detector (PAD). Frontal analysis was monitored at 210 nm. Detection wavelength of solute probes was set at 254 nm. Data processing was performed by Varian workstation software. Column was water-jacketed with a circulating water-glycerol mixture using a Lauda Brinkmann model RC6 refrigerated circulator. The isocratic program of appropriated mobile phase composition obtained from optimize condition study was used in the study. The flow rate was set at 1 mL/min for the whole study. The 40 mM carbonate buffer and acetonitrile were used as mobile phase.

3.4.3 Optimization composition of mobile phase

The appropriated mobile phase composition was developed by varying percentage of acetonitrile. The composition of acetonitrile was varied from 60 to 100 percentages with a 10 percentage increment. Column temperature: 25 ^{0}C, flow rate: 1 mL/min and detection wavelength: 254 nm. PAHs were used as the solute probe.

3.5 Chromatographic measurements

The column hold-up time (t_0) was derived from the retention time of injections of D_2O as the unretained marker. The extra-column volume (t_e) is the transit time between the pump mixer and the column inlet, and between the column outlet and the detector cell, that can be calculate using the elution time of D_2O. The column tube volume (V_c) was calculated by the column diameter and its length. These parameters were used to calculate the amounts of surfactant adsorbed on the stationary phase. t_0 was used to calculate the retention factor.

3.6 Study the relationship between the adsorbed amounts of surfactant on the stationary phase with the number of carbon atoms, concentrations and column temperatures

Frontal analysis is used as the method to quantify the adsorbed amount of surfactant on the stationary phase surface as the function of surfactant types, concentration and column temperatures by monitoring the breakthrough curve. The investigated parameters are the carbon numbers, surfactant concentrations (1, 2, 3, 4 and 5mM), and column temperatures. The temperature was varied from 0 to 40 ^{0}C with a 10 degree increment and at room temperature.

The measurement of breakthrough curve of a surfactant was made by the follow procedure:

Firstly, the column is equilibrated with an aqueous carbonate buffer (40 mM, pH 6.0) and acetonitrile (70/30) for 1 hr.

Then, the carbonate buffer solution (40 mM, pH 6.0) containing a fixed amount of a surfactant was continuously loaded at the same flow rate, and the frontal elution of a surfactant was monitored by measurement of absorbance at 210 nm. The breakthrough curves were recorded only one time at each concentration.

The column was equilibrated back with water/acetonitrile (70/30) for 1 hr before performing the next measurement.

3.7 Study of an adsorbed amount of surfactant on the separation efficiency

After equilibrating the column with each mobile phase condition, a 20 μL of a 20 ppm mixture of triphenylene, benz[a]anthracene, benzo[k]fluoranthene dibenz[a,c]anthracene and acetylsalicylic acid was injected into the column. HPLC-UV detector operated at range 254 nm. All experiments in this section were performed in triplicate runs.

The improvement of separation efficiency between the systems with and without surfactants will be evaulated by the determination of changes on the retention factors (k'), resolution (R_s) and selecitivty (α).

CHAPTER IV

RESULTS & DISCUSSION

4.1 Optimization of the chromatographic system

As shown in Table 4.1, the increase in the percentage of acetonitrile in the mobile phase resulted in the decrease of resolution. At 100% acetonitrile, which one compounds could not be separated. The best mobile phase composition for the separation of PAHs solute probes is 30% carbonate buffer and 70% acetonitrile.

Table 4.1 Separations of PAHs on the monomeric C_{18} phase with various percentage of pH6 carbonate buffer and acetonitrile (v/v) as the mobile phase

Conditions	Resolution (R_s)	
	T^A/B[a]A^B	B[k]F^C/D[a,c]A^D
Carbonate buffer/acetonitrile: 0/ 100(v/v)	0.0	0.0
Carbonate buffer/acetonitrile: 10/ 90(v/v)	0.2	2.0
Carbonate buffer/acetonitrile: 20/ 80(v/v)	2.1	3.5
Carbonate buffer/acetonitrile: 30/ 70(v/v)	2.6	4.9
Carbonate buffer/acetonitrile: 40/ 60(v/v)	4.2	6.1

[A]: triphenylene [B]: benz[a]anthracene, [C]: benzo[k]fluoranthene,
[D]: dibenz[a,c]anthracene.

4.2 Amount of surfactants adsorbed on the stationary phase (q*)

In order to allow the surfactant adsorption onto the stationary phase via electrostatic interactions, the pH of the mobile phase needed to be kept at a value 6, at which the silanol groups (Si-OH) on the stationary phase are ionized to $Si\text{-}O^-$.

This part of our study was to investigate the relationship between the amount of adsorbed surfactants on the stationary phase (q^* values) and temperatures. The temperatures were varied from 0 to 40 ^{0}C (0, 10, 20, 25, 30, 40 ºC).

The mass of surfactants adsorbed on the stationary phase was calculated by the following equation:

$$q^* = \frac{F_v(t_{eq} - t_0 - t_e)C}{V_c - F_v t_0} \qquad (2.4)$$

where: F_v is the mobile phase flow rate

V_c is the column tube volume

t_{eq} represents the time of the surfactants that is adsorbed on the stationary phase inside the column at equilibrium.

t_0 represents the time during which the compound is contained in the volume of the mobile phase inside the chromatographic column or hold-up volume.

t_e represents the time during which the compound is present in the volume of the mobile phase outside the column, between the mixing point and the detector.

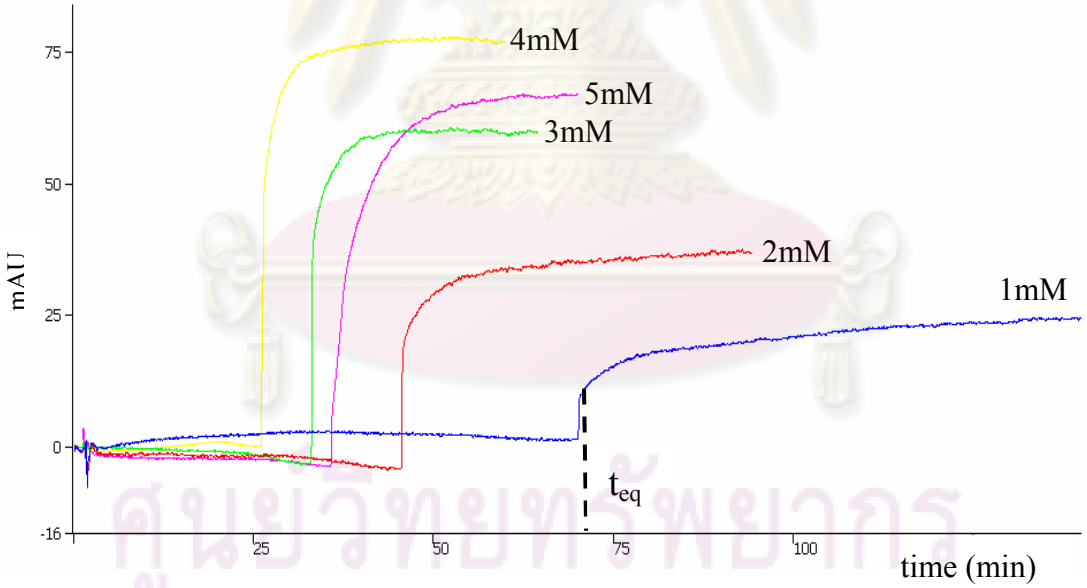
Table 4.2 Parameter used for calculating q* value

Flow rate (mL/min), F_v	1.00
Extra-column time: t_e	3.00
Column tube volume: V_c (mL)*	2.49

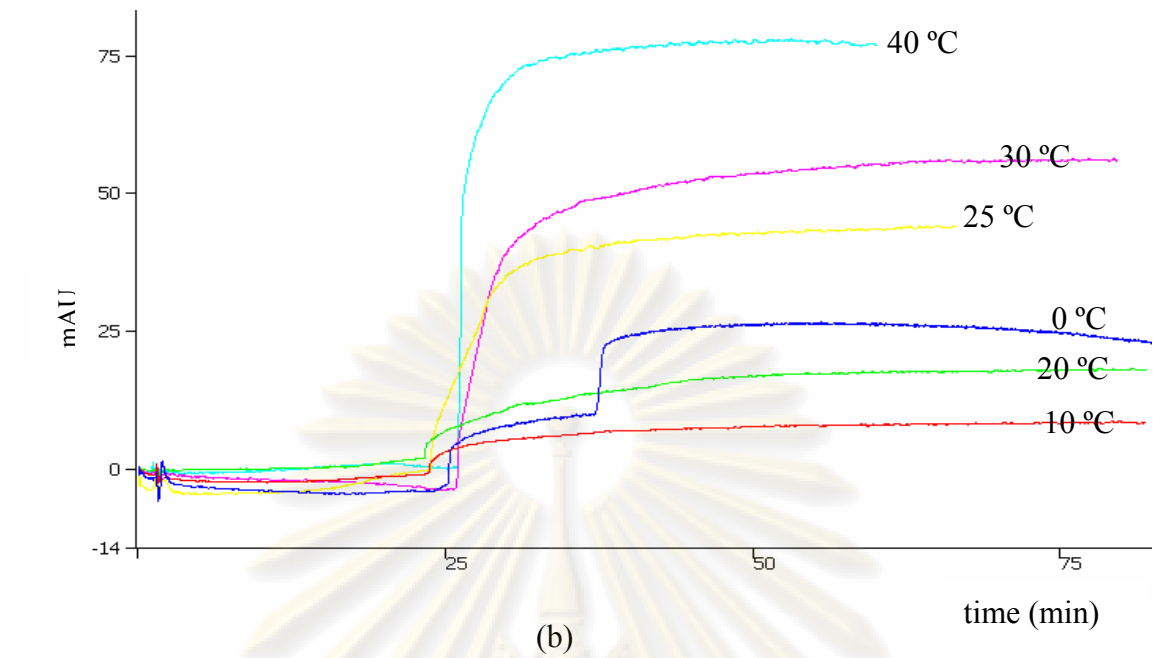
*: $Vc = \frac{\pi d^2 h}{4}$ Where; d= column diameter

h= column length

4.2.1 Frontal analysis



(a)



(b)

Figure 4.1 (a) Frontal analysis curves of the octylamine at 40 ºC as a function of concentration and (b) frontal analysis curves of the 4mM octylamine at 0, 10, 20, 25, 30 and 40 ºC.

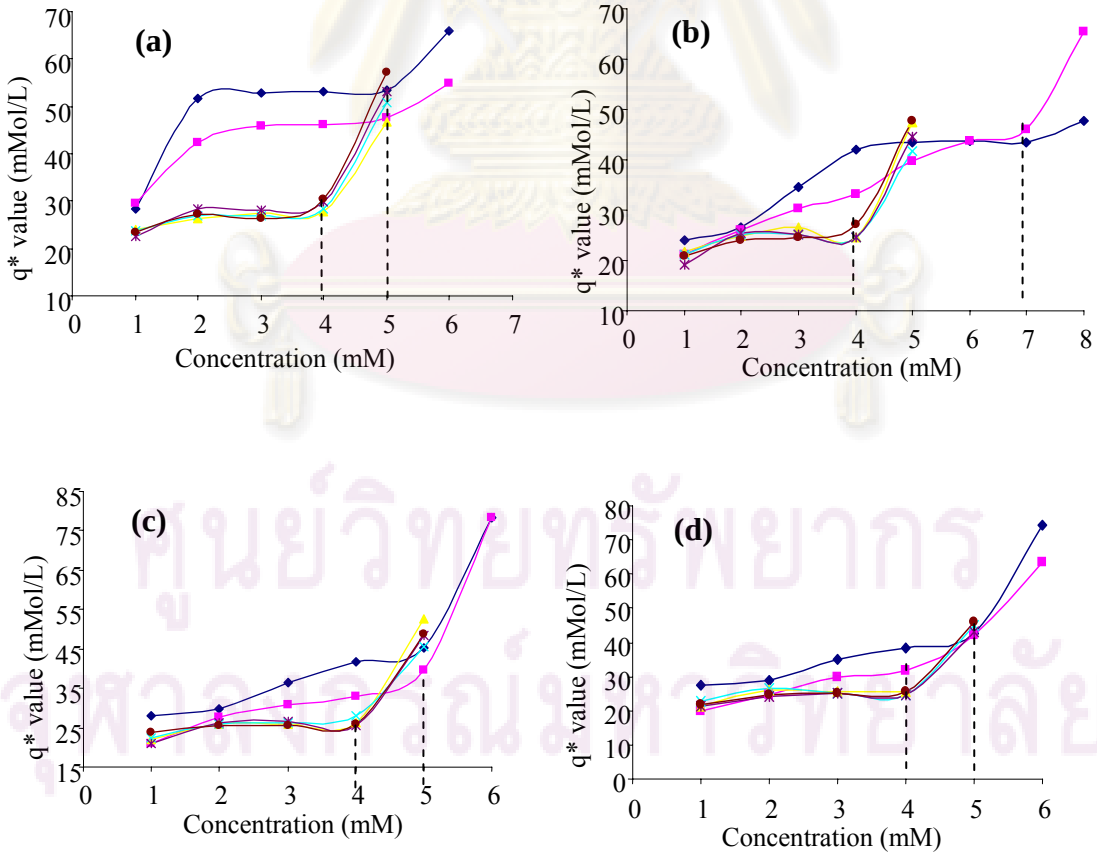
As example of the frontal analysis for octylamine adsorption obtained in our study shown in Figure 4.1. The equilibrium time (t_{eq}) decreased with increasing in surfactant concentration in the mobile phase. There results demonstrated that the change of surfactant concentration affected the equilibrium time (t_{eq}). This could be explained by the fact that adsorption sites are being covered quicker with the increment of surfactant concentration. This phenomenon shown that the adsorption is diffusion control because of the dependence of concentration with q* value [23].

However, at concentrations above the CMC, the equilibrium time was found to increased because the physical properties of the surfactants changed when the concentration reached the CMC point leading to a change in absorbance of the surfactant [38-39]. At CMC point, the self-diffusion of the surfactant was lower than that below the CMC. Resulting the in a increased in equilibrium time [39].

When focusing on the shape of the front of the breakthrough curves at low temperatures (0 and 10ºC) in Figure 4.1 (b), the curves display two plateau steps, which indicated adsorption of the different solute species [24]. In our system, we may have the adsorption of the surfactant-carbonate and the surfactant-ion pairs carbonate (CO_3^{2-}). In contrast, at high temperatures, the single-component adsorption front was observed. This can be explained by a decrease in kinetic energy with decreasing temperature. When the temperature is decreased, the random motion of individual molecules is slowed down. The surfactant and ion pair carbonate can be form to surfactant-ion pairs carbonate easier than at high temperature, thus the curves have two plateau steps in low temperature.

4.2.2 Factors affecting the q* value

4.2.2.1 Concentrations and temperatures



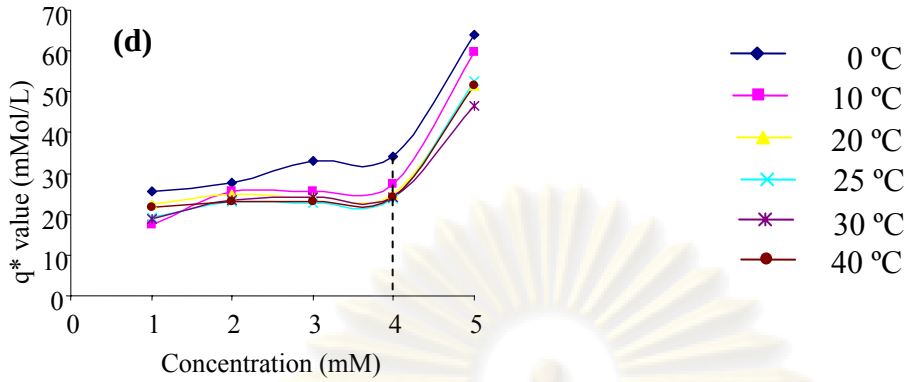


Figure 4.2 Plot of the q* values versus surfactant concentrations at each temperature; (a)ethylammoniumchloride, (b)diethylamine hydrochloride, (c)butylamine, (d)hexylamine and (e)octylamine. (·····) Dash line shows the highest concentration of surfactant before CMC level.

Firstly, the effect of the concentration on the calculated q* values was studied. The q* values can be calculated from Equation 2.4. As seen in Figure 4.2, the calculated q* values slightly changed with increasing concentration of surfactant. Similar results were found in previous works [21, 35]. At concentration of surfactant above CMC, the q* value was found to increased dramatically. This result can be explained by the fact that when the surfactant concentration is below the CMC the self-diffusion of surfactants is higher than that above CMC so the increased equilibrium time (t_{eq}) resulting in higher q* value (from Equation 2.4).

Considering the CMC values of each surfactant between low and high temperatures observed in Figure 4.2. The CMC values of all surfactants increased with decreasing column temperatures. This is due to the fact that at low temperature, the hydrocarbon chains adopt to trans conformations. This made the packing of the surfactant tail into micelle more difficult and energetically unfavorable. Therefore, it required a higher surfactant concentration to reach the CMC. This phenomenon is consistent with previously published [17, 39].

However, the amount of adsorbed surfactants increased with decreasing column temperatures. When focusing on the low temperature (0 and 10 ºC), the amount of surfactant adsorbed on the stationary phase was larger than at high temperatures (20-40 ºC) for all surfactants at all concentrations. This is due to the

decrease of the column temperatures resulting in the decrease of gauche conformations of C_{18} alkyl chains anchored on the stationary phase. Therefore, the space between alkyl chains should present less steric hindrance as shown in Figure 4.3 (a). Surfactant molecules are able to penetrate between C_{18} alkyl chains easier compared to the higher temperature range which resulted in higher q* values at low temperatures [6, 13, 16-17]. Surprisingly, at 0 and 10 ºC a linearly increase in q* value with increasing surfactant concentration was observed.

At high temperature (20-40 ºC), all surfactant gave similar the q* value for all temperature because of the space between alkyl chain should present more steric hindrance as shown in Figure 4.3 (b). The lower amount of surfactant that can be adsorbed on the stationary phase results in q* value for all surfactant significant change with increasing temperature.

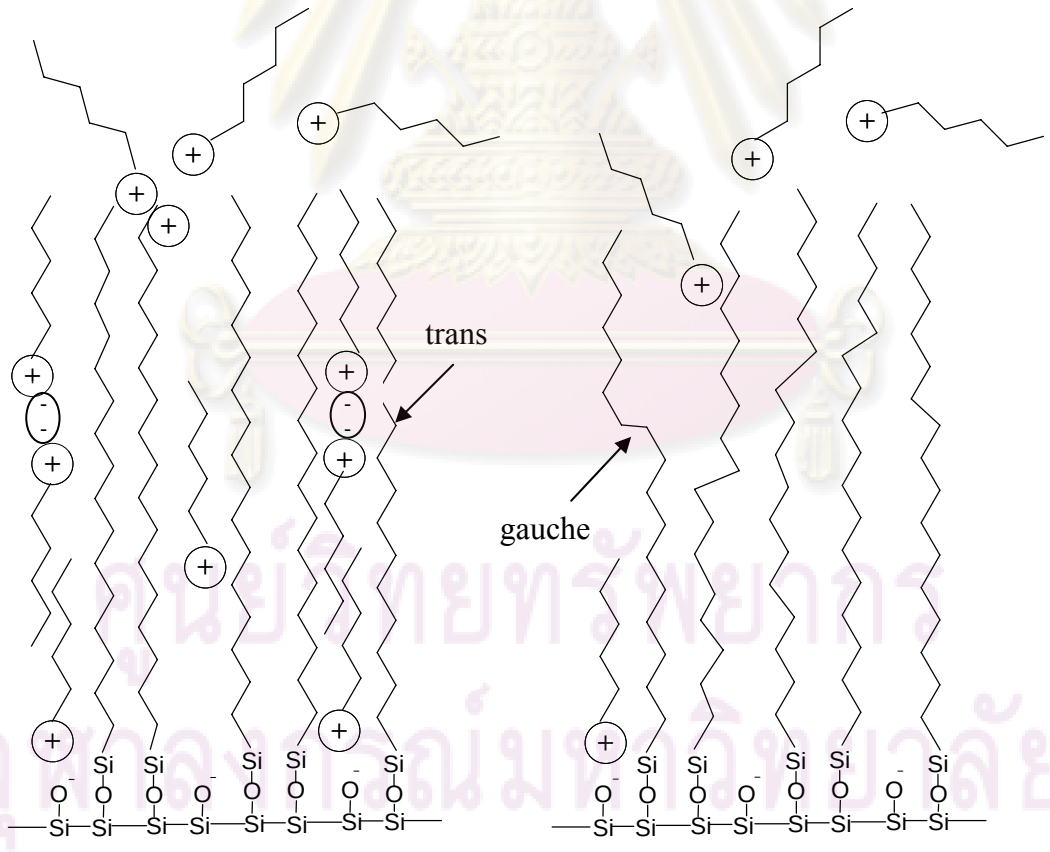


Figure 4.3 Stationary phase modified with surfactants at (a) low and (b) high temperatures. Adapted from ref. [16] and [24].

4.2.2.2 Type of surfactant and temperature

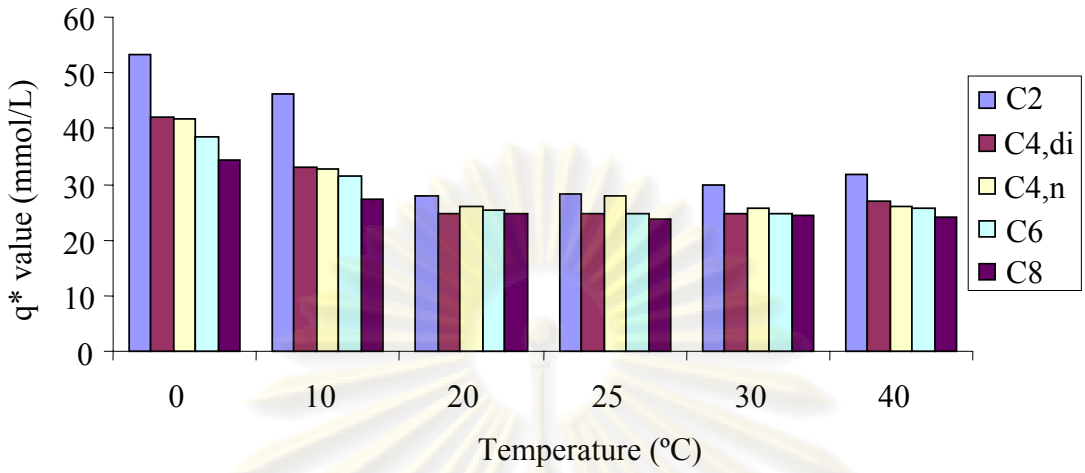


Figure 4.4 Amount of surfactant adsorbed onto the stationary phase (q*) using various types of surfactant and temperature at 4mM for all surfactant. C2: ethylammonuimchloride, C4, di: diethylamine hydrochloride, C4, n: butylamine, C6: hexylamine and C8: octylamine.

In Figure 4.4 the amount of surfactant adsorbed onto the stationary phase dependencies of the shape and chain length of surfactants is shown. The q* values of ethylammonuimchloride, which is the smallest molecule, were higher than the other surfactants at all temperatures. At low temperatures (0 and 10 ºC), the q* value of Ethylammonuimchloride showed a significant difference when compare to the other surfactant. To explain this phenomenon, the molecular size of ethylammoniumchloride is smallest then it can be adsorbed on the stationary phase easiest compare to the longer molecules.

The octylamine, which is the longest alkyl chain length, has the largest steric effect when compared to the other surfactants. Therefore the least amount of octylamine can be adsorbed on to the stationary phase resulting in smaller magnitude in the change of morphology rigidity. The steric hindrance of octylamine even showed a significant effect on q* value at low temperature (0 and 10 ºC).

At temperature above 20 ºC, all surfactants were observed to give similar q* values. This result showed that the amount of surfactant adsorbed onto the stationary phase was independent of the shape and chain length of surfactants at

higher temperature range. The effect of temperature and q* value was previously discussed in Section 4.2.2.1 and it consistent with these results.

4.2.3 Precision of the amount of surfactant adsorbed on the stationary phase

Precision is subdivided into repeatability (within day precision) and reproducibility (between day precision). Which was studied by the performance method using the same laboratory and the same equipment. The frontal analysis curves were recorded for each condition and only one condition was tested for precision test due to the large consumption of surfactant amount and mobile phase. The frontal analysis curves were recorded at different wavelengths (200, 210, 220 and 254 nm) to maximize the accuracy of the measurement.

4.2.3.1 Repeatability

The closeness of the agreement was determined by the percent of RSD. Three replicates at 1, 2, 3, 4 and 5mM butylamine (25 ºC) were presented as the %RSD ranging from 0.00 to 4.72.

4.2.3.2 Reproducibility

Between day precision was determined by the comparison between the q* value of the first and second day. Three replicates at 1, 2, 3, 4 and 5mM butylamine (25 ºC) were presented as the %RSD ranging from 0.22 to 4.68.

Table 4.3 %RSD of 1, 2, 3, 4 and 5mM butylamine (25 ºC)

Concentration (mM)	%RSD	
	Repeatability	Reproducibility
1	1.96	2.94
2	0.00	2.97
3	2.77	3.12
4	4.72	4.68
5	0.22	0.22

This work giving %RSD not exceeding 10% [20].

4.3 Effect of surfactants on the retention of PAHs

The retention factor (k'), the selectivity (α) and the resolution (R_s) on the separation of polycyclic aromatic hydrocarbons (PAHs) were calculated by Equation 2.1, 2.2 and 2.3, respectively.

The elution order of triphenylene ($C_{18}H_{12,}$ L/B=1.119), benz[a]anthracene ($C_{18}H_{12,}$ L/B=1.599), benzo[k]fluoranthrene ($C_{20}H_{12,}$ L/B=1.474) and dibenz[a,c]anthracene ($C_{22}H_{14,}$ L/B=1.238) can be explained in terms a hydrophobicity and L/B ratio. Considering L/B ratio of structural isomer (triphenylene and benz[a]anthracene), benz[a]anthracene has a L/B ratio higher than triphenylene, it would be able to fit more easily into the narrow slot and interaction strongly with bonded alkyl chain. Therefore benz[a]anthracene eluted after triphenylene. Benzo[k]fluoranthrene and dibenz[a,c]anthracene can be explained by hydrophobicity. Dibenz[a,c]anthracene has a more hydrophobic character than benzo[k]fluoranthrene, thus it will be eluted after benzo[k]fluoranthrene, although it has small L/B ratio.

All retention factors, resolution and selectivity reported are based on the average of three measurements, with relative standard deviation (%RSD) less than 1.0% for all cases.

To compare the trends of retentions with surfactant concentration, we used the normalized retention parameter *K* of the PAHs defined as

$$K = \frac{k'}{k'_0} \qquad (4.1)$$

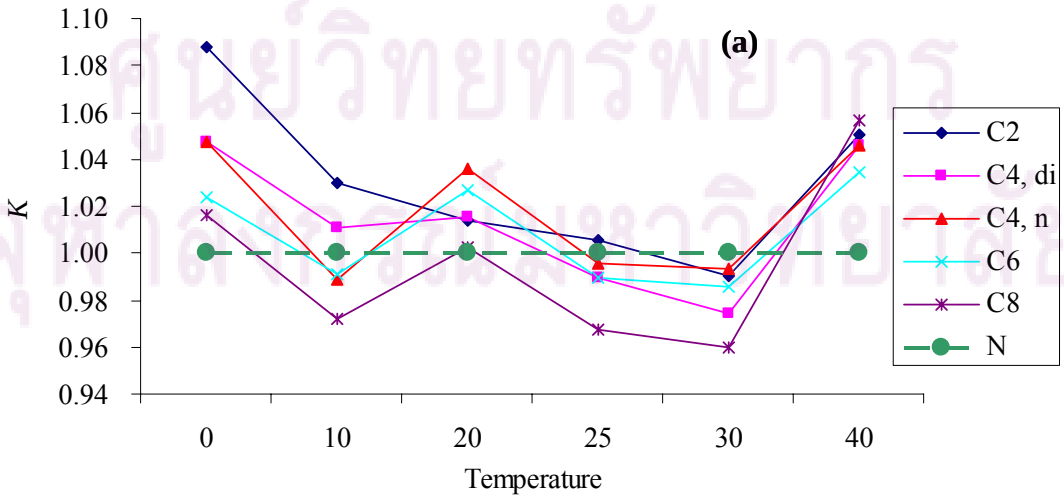
Where; k' is the retention factor of PAHs in system with the surfactant.

k'_0 is the retention factor of PAHs in system without the surfactant.

If $K > 1$, it means that the retention factor obtained from the system with surfactant is greater than the system without surfactant.

4.3.1 Temperature and type of surfactant

In general, the retention factor of PAHs increases with increasing bonding density [4, 6]. When the surfactant adsorbed onto the stationary phase, it could increase the stationary phase density resulted in the increases of phase rigidity. Consequently, the retention factors for PAHs should be improved.



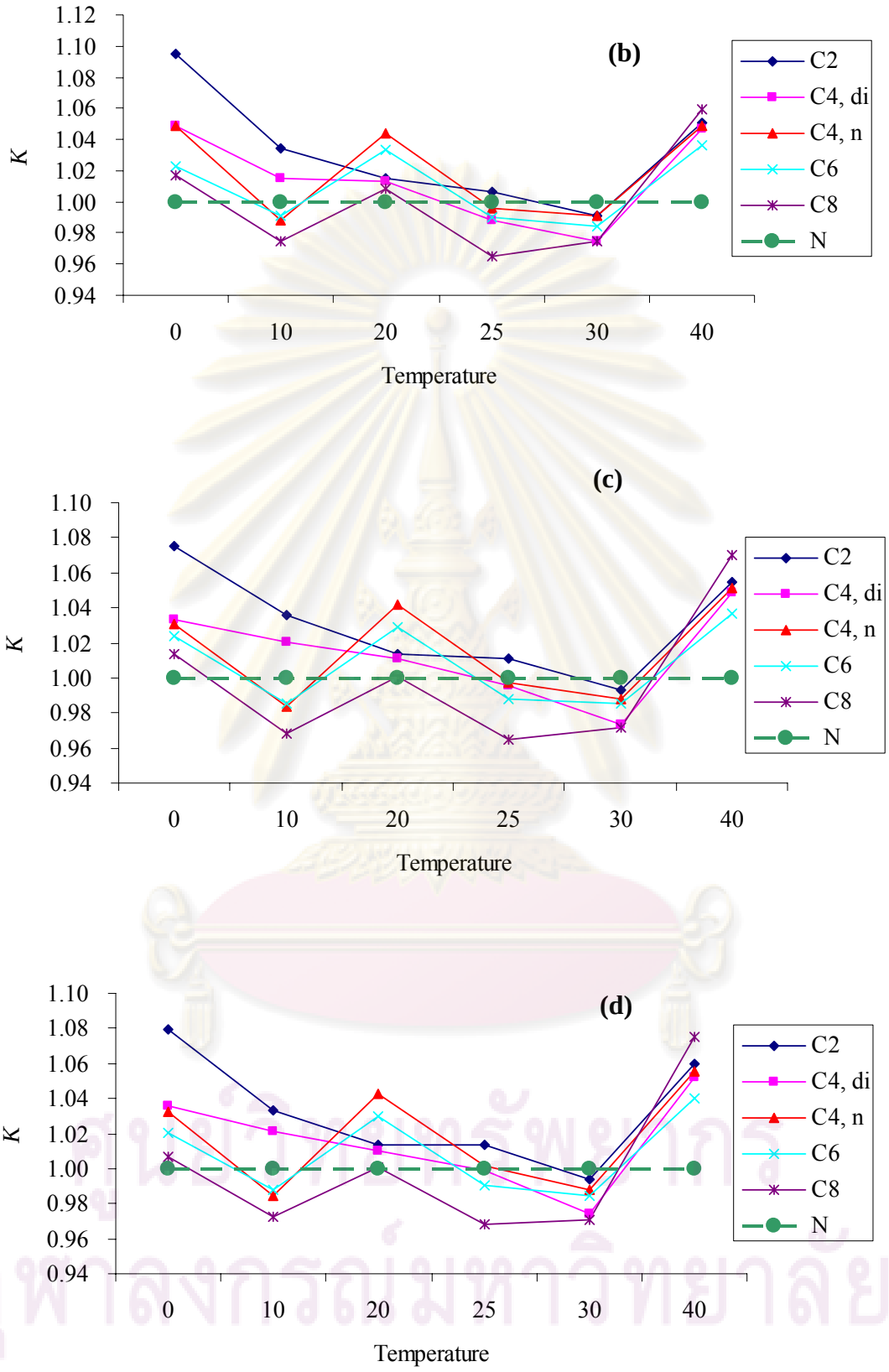


Figure 4.5 The plot of *K* values for four test solute probes versus temperatures: (a)triphenylene, (b)benz[a]anthracene, (c)benzo[k]fluoranthene and

(d)dibenz[a,c]anthracene at various temperature and surfactants; C2: ethylammoniumchloride; C4, di: diethylamine hydrochloride; C4, n: butylamine; C6: hexylamine; C8: octylamine and N: no surfactant.

Table 4.4 List of concentration offering the highest *K* value for four PAHs used for plotting the plot in Figure 4.5 and 4.7.

Surfactant	Concentration (mM)					
	0 ºC	10 ºC	20 ºC	25 ºC	30 ºC	40 ºC
Ethylammoniumchloride	5	5	4	4	1	1
Diethylamine hydrochloride	8	7	4	4	1	1
Butylamine	5	5	4	4	1	1
Hexylamine	5	5	4	4	1	1
Octylamine	4	4	4	4	1	1

In Figure 4.5 (a), (b), (c) and (d), to compare the system with surfactant and without surfactant at low temperature (0 ºC), system with surfactant gave the *K* value larger than system without surfactant. The *K* values of PAHs were improved at 0 ºC and insignificantly changed at temperatures above 10 ºC. Similar trends were observed for all surfactants.

From our study, the higher *K* values were obtained at low temperatures, which were consistent with the measured q* values. As we have discussed previously at low temperature the alkyl chains anchored on the stationary phase have less mobility so the surfactant molecules can penetrate between the C_{18} alkyl chains easier compared to the higher temperatures resulting in high q* values. Therefore, it led to in higher magnitude of interaction between stationary phase and solutes (higher retention factors). On the other hand, the alkyl chains bonded on the

stationary phase have more mobility at high temperatures. Therefore, a lower amount of surfactants was adsorbed onto the stationary phase, so we observed a decrease of the *K* value of PAHs with increasing temperatures which is consistant with observed q* values. At the higher temperatures, as reported in section 4.2.1, there is no significant change of q* value at temperatures above 20 ºC for any surfactants.

Surprisingly, at 40 ºC, the *K* values were increased again, though all surfactant gave the similar q* value for high temperature (20-40 ºC) as show in Figure 4.2. This indicated that the adsorbed surfactant on the stationary phase retained the solute more. Similar trends were observed for all surfactants. This could be explained by the following thermodynamics aspect:

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \qquad (4.2)$$

$$\Delta G^0 = -RT \ln K \qquad (4.3)$$

Where: ΔG^0 is Gibbs free energy.

ΔH^0 is standard enthalpy.

ΔS^0 is the standard entropy.

R is the gas constant.

T is the temperature studies.

K is the retention equilibrium

From Equation 4.2 increasing column temperature increases the influence of ΔS^0 result to the increasing of ΔG^0. According to Equation 4.3, the retention equilibrium constant (*K*) increase with increasing of ΔG^0, when the retention equilibrium constant (*K*) increase affect to increasing the retention factor (*K*).

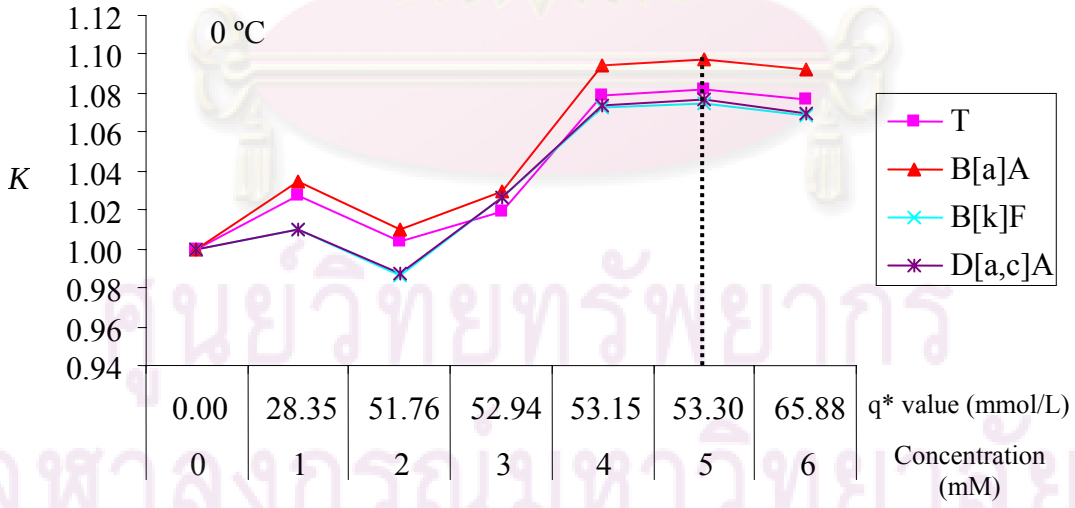
Retention factors obtained from using ethylammoniumchloride as the rigidity inducer were higher than the diethylamine hydrochloride, butylamine, hexylamine and octylamine. Because ethylammoniumchloride molecules are the

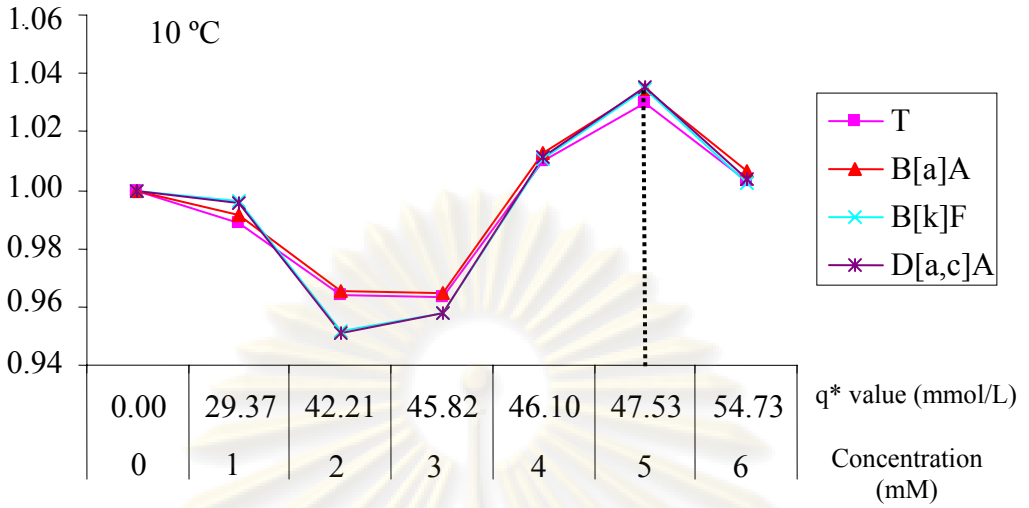
smallest, they can be adsorbed on the stationary phase more easily when compared to longer molecules (discussed in section 4.2.2). We suggested that the longer alkyl chain length possesses greater steric effect, therefore less amount can be adsorbed onto the stationary phase resulted in smaller magnitude in the change of morphology rigidity.

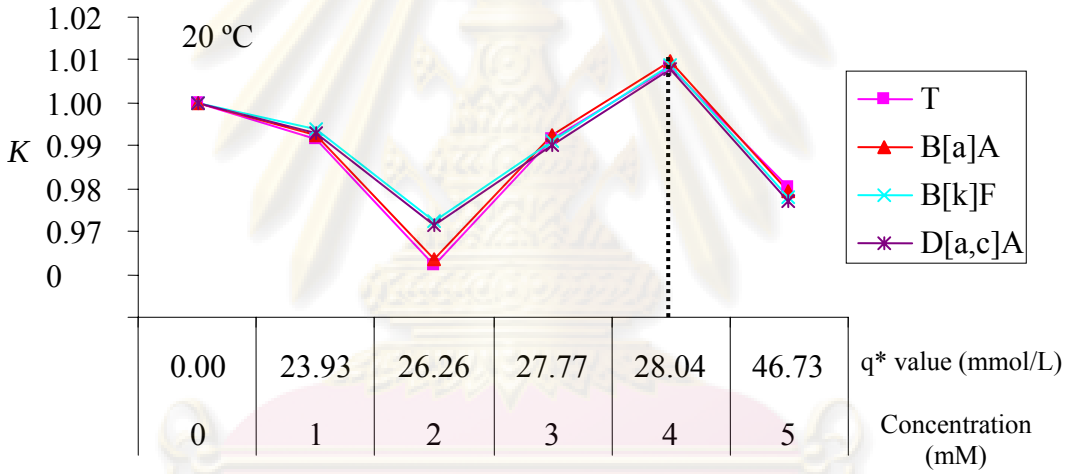
4.3.2 Concentration of surfactant

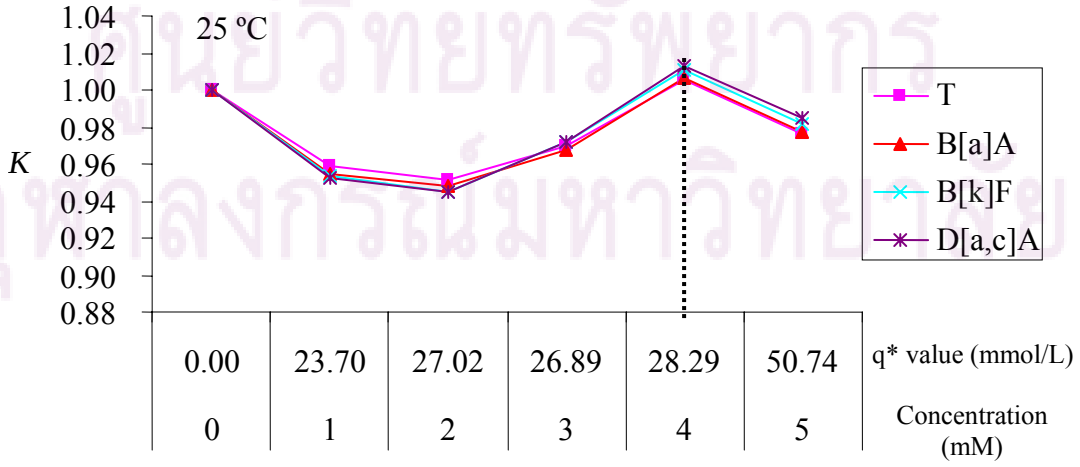
The separation in liquid chromatography is **based on the differences in partitioning** of solute between the stationary phase and the mobile phase. The compound partitions into the stationary phase more than into the mobile phase so it interacts more strongly with stationary phase. Therefore, the compound would be retained longer in stationary phase.

Adding the surfactants in mobile phase could increase the stationary phase and the interaction between solutes and stationary phase resulting in the longer retention. However, it also increased the elution strength of the mobile phase, which would shorten the solute retention time [35].









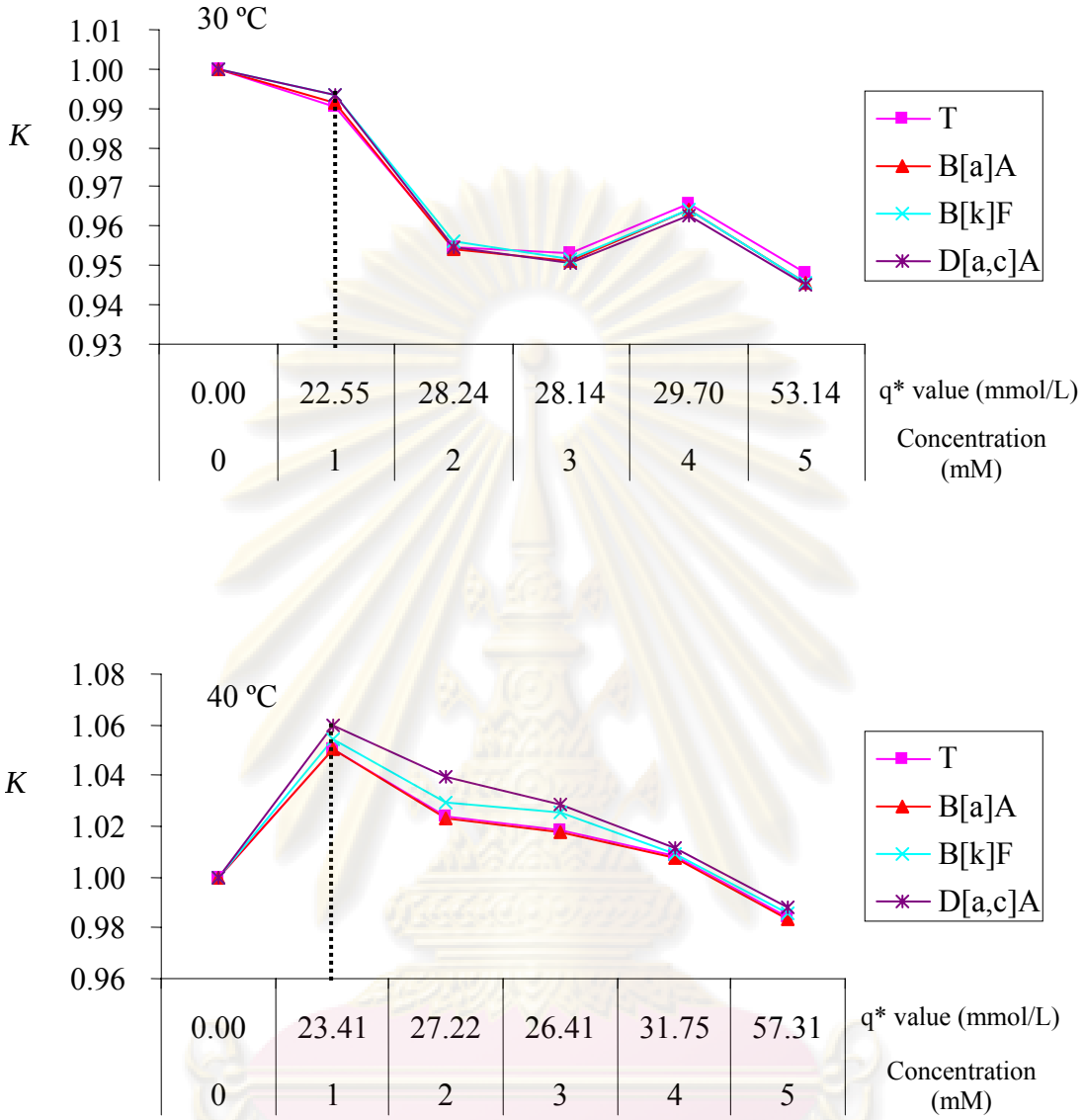


Figure 4.6 The effect of ethylammoniumchloride concentrations in the mobile phase on retention parameter (*K*) of four PAHs at 0, 10, 25, 30 and 40 ºC, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene. (·····) Dash line indicated the surfactant concentration giving the best *K* values.

The relationship between of surfactant concentration and retention factors was found to be similar for all surfactants. Herein, we chose ethylammoniumchloride solute probe as an example for discussion. The results from

Figure 4.6, show that at temperature below 30 ºC the retention factors of all PAHs slightly decreased for low surfactant concentration range (1- 3mM). This result, implied that the a lower of surfactant could be adsorbed onto the stationary phase (smaller q* values), so the increase in the rigidity of stationary phase was insignificant compare to the increases in the elution strength of mobile phase. Also, the interactions between the solute and mobile phase were larger than those between solute and stationary phase, so the *K* value of PAHs decreases at this concentration range.

At high concentration, the *K* value was drastically increased with increasing of concentration of surfactant (large q* values) because the larger amount of surfactant was adsorbed onto the stationary phase, so the bonding density of stationary phase increased. The interactions between solute and stationary phase were higher than those between solute and mobile phase. When the concentrations of surfactant were above CMC, the *K* values decreased again. At this high concentration, the surfactant formed micelle, which could act as a pseudo stationary phase in mobile phase and increase the elution strength [42-43].

Considering at high temperatures (30-40 ºC), an increase in surfactant concentration resulted in the decrease of retention factor which might be the result of the decrease in the q* value compare to the high temperature at the same concentration (as show in Figure 4.2). At high temperature, a lower amount of surfactant could be adsorbed onto the stationary phase (smaller q* values). When the surfactant concentration increase affects to increasing the elution strength of mobile phase resulting in a decreases of the *K* value.

4.4 Effect of surfactants on selectivity of PAHs

The selectivity factor (α) of a separation is defined as a ration of retention factor for two adjacent compounds that can be calculated from the equation

$$\propto = \frac{k_2'}{k_1'} \qquad (2.2)$$

4.4.1 Temperature and type of surfactants

The detailed temperature effect on the selectivity of four PAHs has been investigated and the results are summarized in Figure 4.7 (a) and (b).

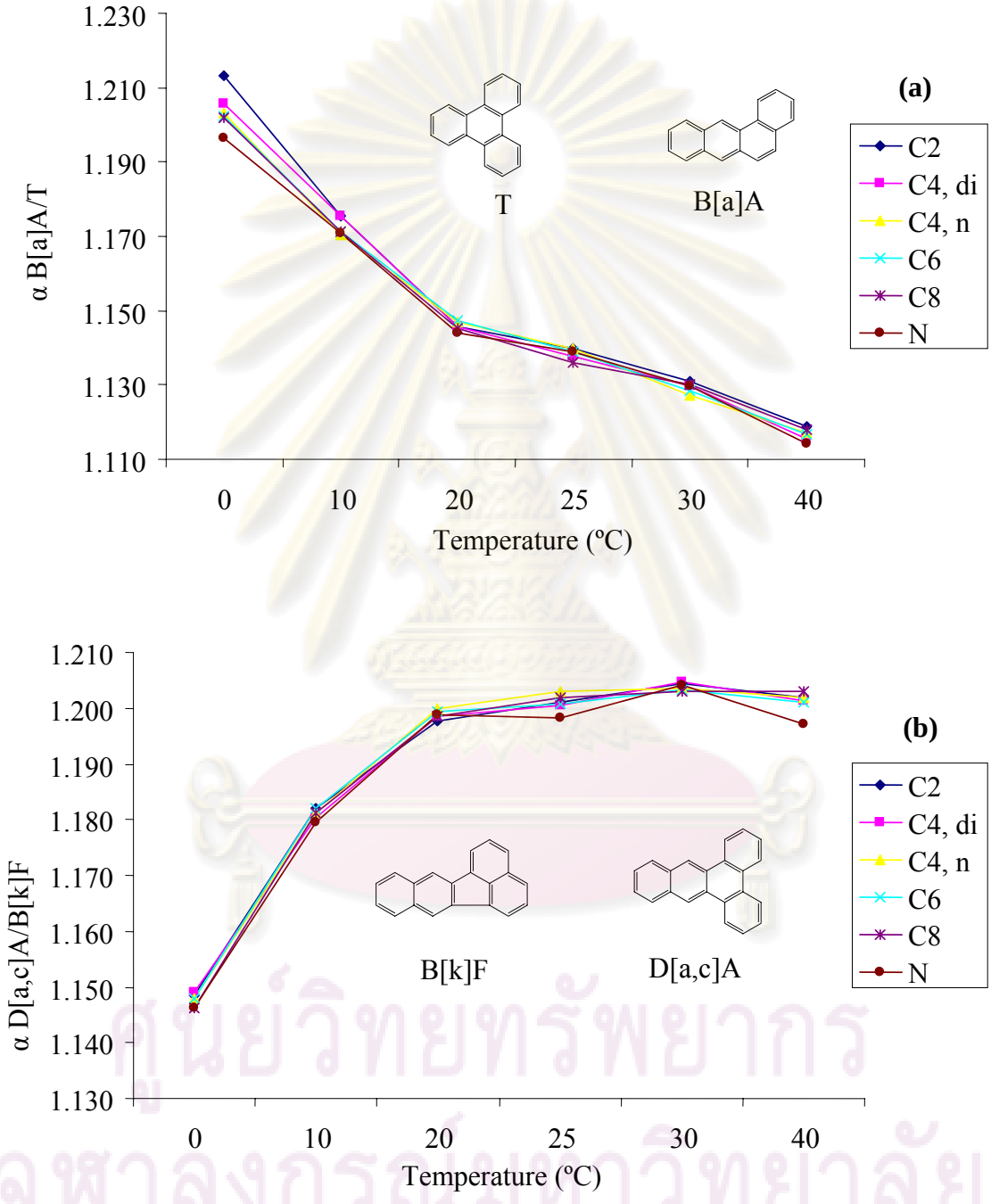


Figure 4.7 Temperature dependence of the selectivity factors between (a) B[a]A/T and (b) D[a,c]A/B[k]F with surfactants. C2: ethylammoniumchloride; C4, di:

diethylamine hydrochloride; C4, n: butylamine; C6: hexylamine; C8: octylamine and N: no surfactant.

For each type of surfactants, the selectivity of benz[a]anthracene/triphenylene (α B[a]A/T) increased with the decrease of temperatures. As previous studies, the decrease in temperature would increase the selectivity of the shape selectivity structural isomer [6, 10-12]. This result could be explained by at low temperature the stationary phase have less mobility so the surfactant molecules should be able to penetrate between the C_{18} alkyl chains easier compared to the higher temperatures resulted in increase in the rigidity of stationary phase. However, at temperature above 10 ºC, there were insignificantly differences in selectivity observed between two systems, with and without the surfactant added. It means that the increasing in rigidity by adsorption of surfactant might not be an important factor as much as the temperature effect.

Surprisingly, at 0 ºC ethylammoniumchloride gave a significant increase in the selectivity toward benz[a]anthracene and triphenylene compounds to the values obtained from the other surfactants as show in Figure 4.7 (a). This result could be explained base on the size of ethylammoniumchloride which is smallest then it can be adsorbed on the stationary phase more easily when compare to longer molecule (larger q* value), so the bonding density of stationary phase increased resulted in larger *K* value and larger selectivity of benz[a]anthracene/ triphenylene.

From Figure 4.7 (b), the relations between selectivity factors of dibenz[a,c]anthracene/benzo[k]fluoranthrene and temperatures was obtained. In general, the carbon atom of dibenz[a,c]anthracene (L/B=1.238, $C_{22}H_{14}$) more than of benzo[k]fluoranthrene(L/B= 1.474, $C_{20}H_{12}$), thus dibenz[a,c]anthracene will be eluted after benzo[k]fluoranthrene. However, at low temperature, the stationary phases posses trans conformation more than gauche conformation. Spaces between bonded alkyl chains on the stationary phase are viewed as a slot into which the solute molecules can penetrate. The benzo[k]fluoranthrene (L/B= 1.474) is more planar and is able to fit between chains deeper than at high temperature. Therefore

benzo[k]fluoranthrene were retained longer resulted in the decrease of selectivity towards dibenz[a,c]anthracene and benzo[k]fluoranthrene.

However, when comparing the system with surfactant and without surfactant, the selectivity of dibenz[a,c]anthracene/benzo[k]fluoranthene insignificantly changed when density of stationary phase was enhanced. Because the effect of temperature for these compounds has more effect to their retentions than the improvement of stationary phase rigidity (improve the shape selectivity of column). Thus the increasing of q* value has no affect to retention of these compounds.

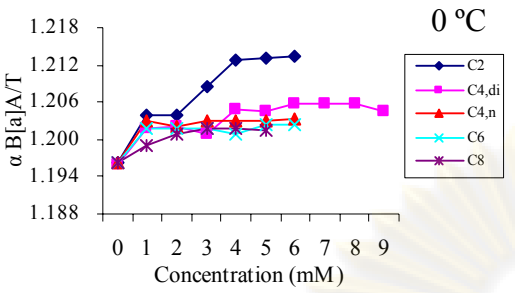
In comparison to monomeric C18 modified with 5mM ethylammonuimchloride and polymeric C18 stationary phase for selectivity of benz[a]anthracene/triphenylene at 10 ºC, it appears that the monomeric C18 modified with 5mM ethylammonuimchloride gave a better selectivity of benz[a]anthracene/triphenylene than polymeric C18 stationary phase. This result as show in Table 4.5

Table 4.5 Show the selectivity of benz[a]anthracene/triphenylene for monomeric modified with 5mM ethylammonuimchloride and polymeric C_{18} stationary phase at 10 ºC polymeric C18 stationary phase at 10 ºC

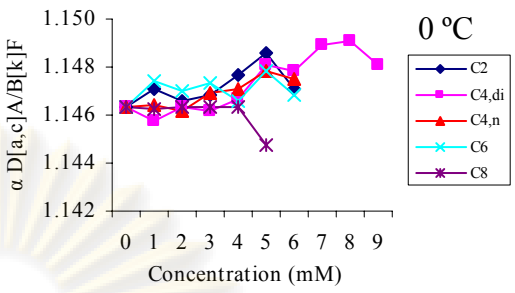
Type of stationary phase	Selectivity of B[a]A/T at 10 ºC
Monomeric C_{18} stationary modified with 5mM ethylammonuimchloride	1.176
polymeric C_{18} stationary phase	1.053[a]

[a] ref.[30]

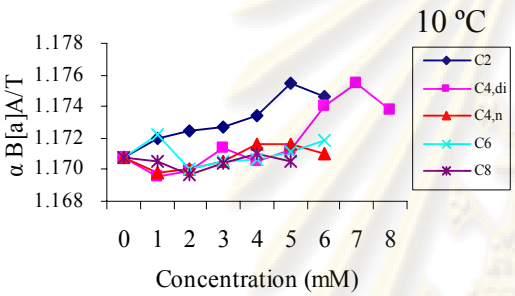
4.4.2 Concentration of surfactants



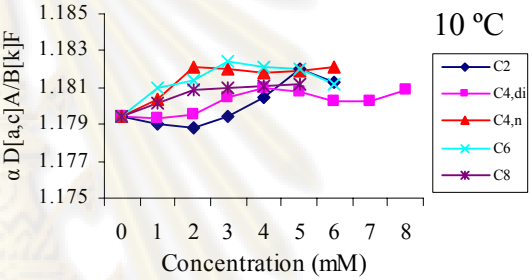
(a)



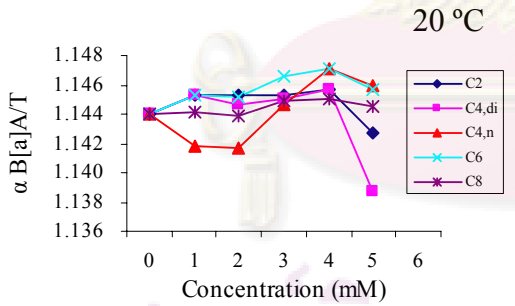
(b)



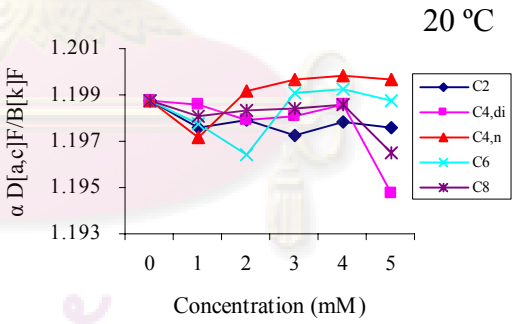
(a)



(b)



(a)



(b)

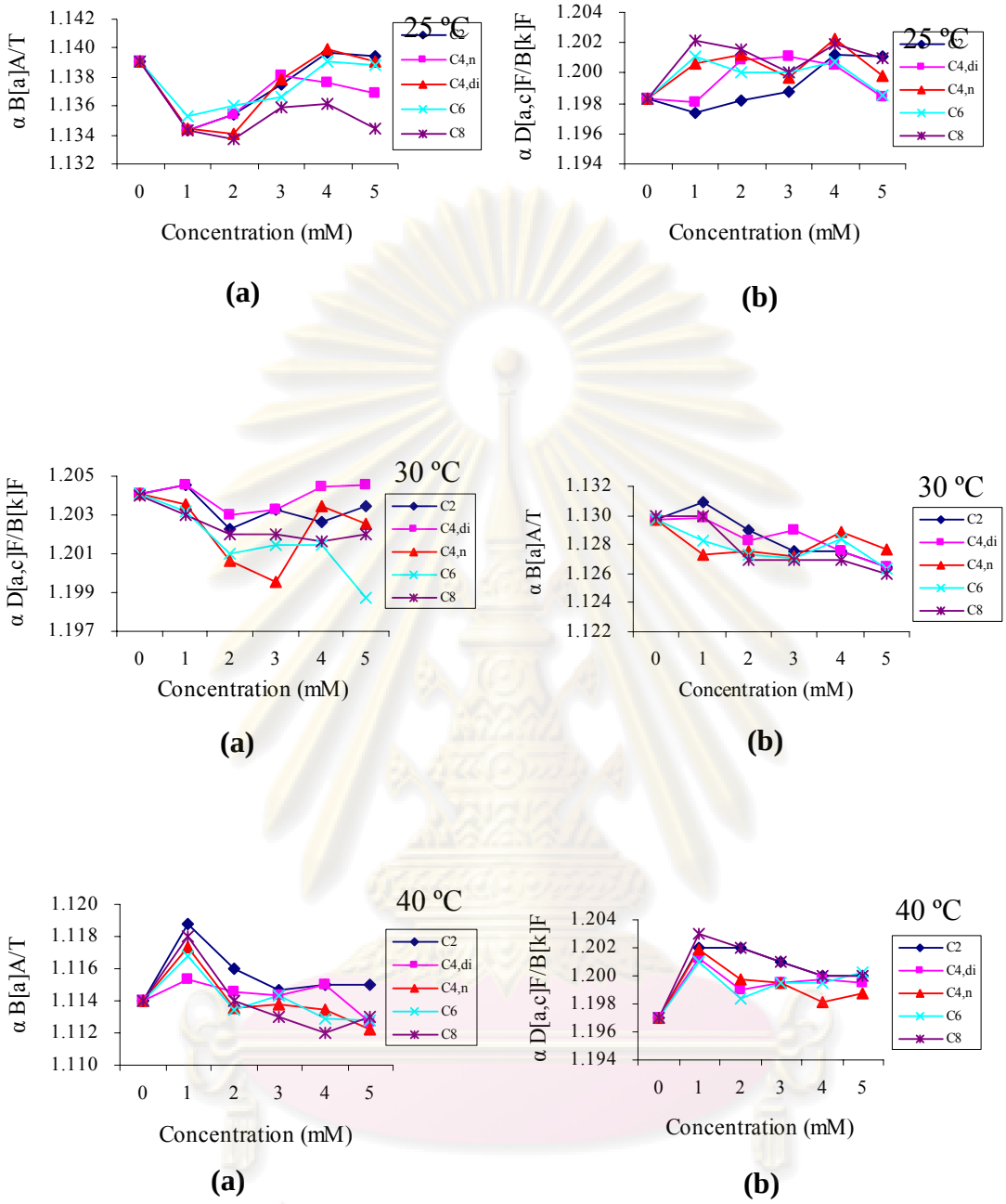


Figure 4.8 Plot of selectivity of four PAHs versus surfactant concentration for all temperatures; (a)selectivity of benz[a]anthracene/triphenylene, (αB[a]A/T) and (b)benzo[k]fluoranthrene/dibenz[a,c]anthracene, (αB[k]F/D[a,c]A). C2: ethylammonuimchloride; C4, di: diethylamine hydrochloride; C4, n: butylamine; C6: hexylamine; C8: octylamine and N: absence surfactant.

From Figure 4.8, the selectivity of PAHs obtained from the added surfactant system were larger than the system without surfactant added. Which

implied the adsorbed surfactant can enhance the shape selectivity due to the increase in the bonding density of stationary phase [6, 12, 28]. However, the selectivity dramatically changed with the increase in surfactant concentrations.

At 0 ºC, ethylammoniumchloride gave the clear relation of the surfactant concentration and selectivity. The selectivity factors of PAHs were increase with increasing surfactant concentration, due to the increase in q* values (increase rigidity of stationary phase) results in an increase in the retention and selectivity of PAHs [4, 6, 30].

Considering at high temperatures (30-40 ºC), an increase in surfactant concentration resulted in decrease in selectivity factors. This phenomenon can be explained in a same way as in retention factor data (section 4.3.2) that high temperature, the less amount of surfactant could be adsorbed onto the stationary phase. When the surfactant concentration increases effects to increasing the elution strength of mobile phase more than increase the rigidity of stationary phase resulted in decreases of the selectivity value.

4.5 Effect of adsorbed surfactants on the resolution of PAHs

In HPLC, the resolution (R_s) of two solutes is given by

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'}{1+k'}\right) \qquad (4.4)$$

Where N is the average number of theoretical plates, α is selectivity which is defined as the ration of the retention factor and *k´* is retention factor.

From Equation 4.4, to obtain the better resolution, the large values of α and N and small value of k are needed.

From the experiments, the added surfactant system can improve the resolution of PAHs. Observing the q* value and the resolution, the conditions giving the best resolution for PAHs and the highest q* value were different. This might be the result of the loss of separation efficiency (N). As shown in equation 4.4, R_s relates

to retention factor (*k'*), selectivity (α) and efficiency (*N*). When the surfactant adsorbed onto the stationary phase it could increase the phase density and retention factors. However the solutes retained in column for a longer time (higher *k'*), peak width of a solute became wider (smaller *N*). The resolution depending on this parameter opposite effects as we observed in our study. The similar result was found in previous work [44].

4.6 Application

We chose to work at 0 ºC with all surfactants as shown in Table 4.5 to improve the separation of mixture II (5-methoxyflavone and 6-methoxyflavone).

Table 4.6 Concentration of surfactant used in this study section used for plotting the plot in Figure 4.10

Surfactant	Concentration (mM)
Ethylammonuimchloride	5
Diethylammonium hydrochloride	8
Butylamine	5
Hexylamine	5
Octylamine	4

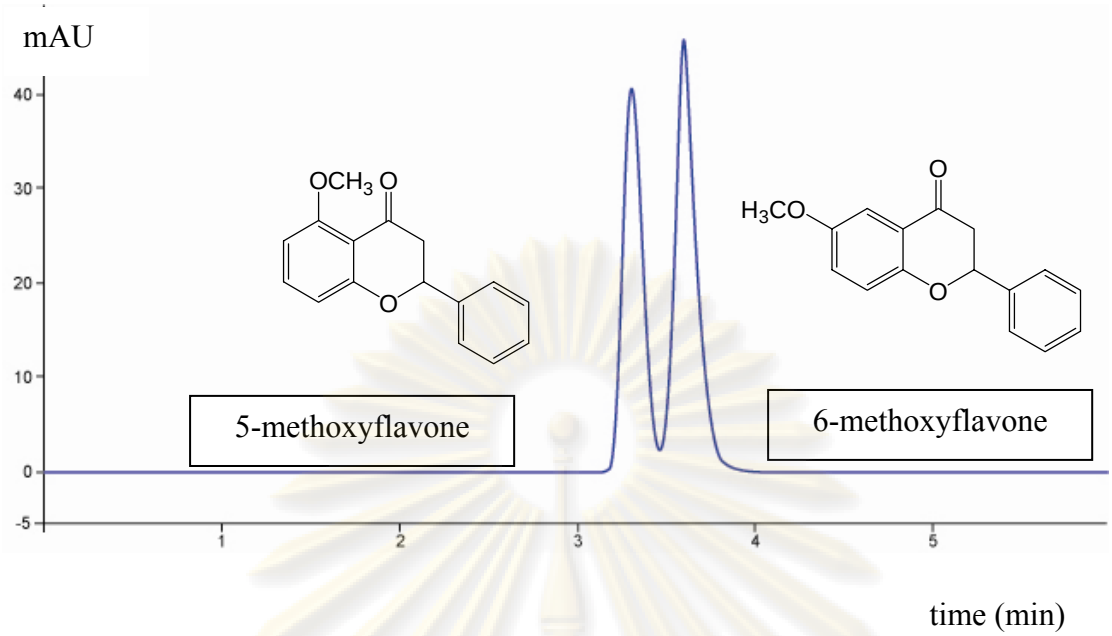
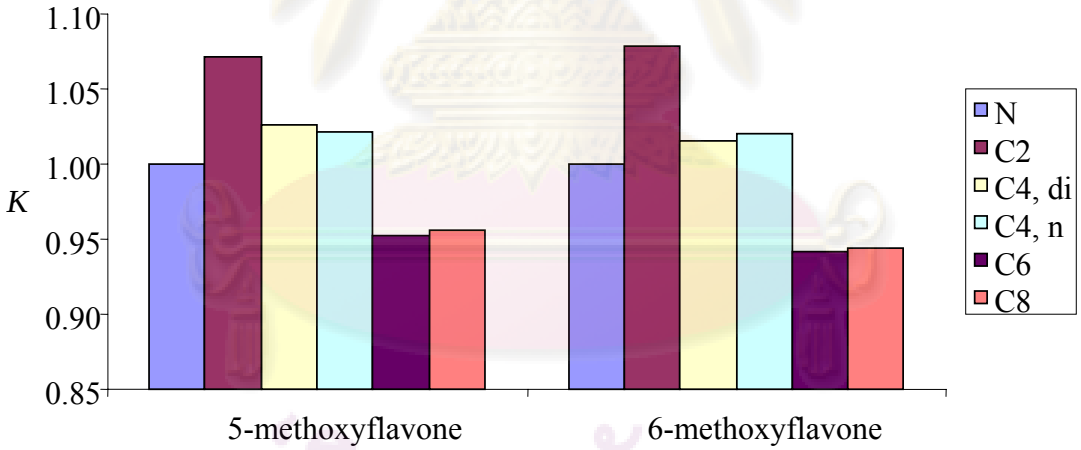
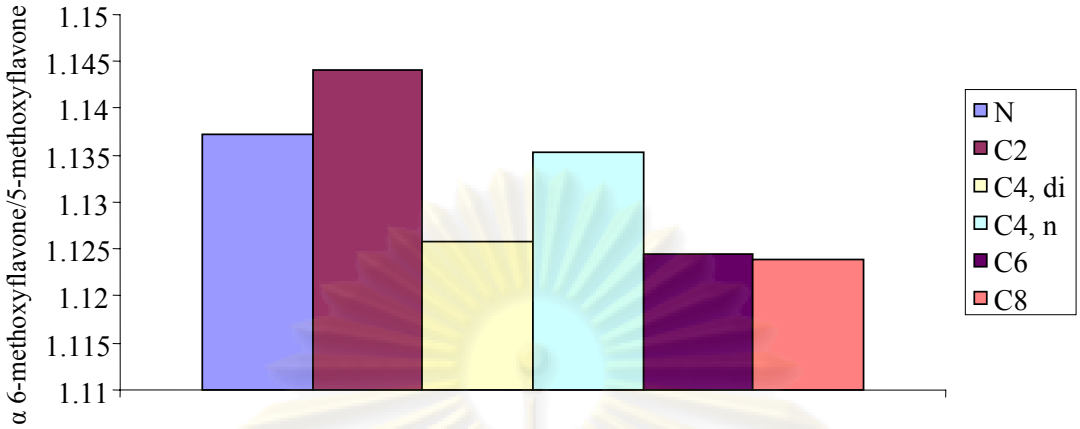


Figure 4.9 Chromatogram of the separation of 5-methoxyflavone and 6-methoxyflavone using the column modified with 5mM ethylammoniumchloride; temperature: 0 ºC, detection: UV at 254 nm.



(a)



(b)

Figure 4.10 Figure show (a) the *K* value and (b) the selectivity of of 5-methoxyflavone/6-methoxyflavone for all surfactants. C2: ethylammoniumchloride, C4, di: diethylamine hydrochloride, C4, n: butylamine, C6: hexylamine, C8: octylamine and N: absence surfactant.

In Figure 4.10, it can be seen that the separations of 5-methoxyflavone and 6-methoxyflavone could be improved by adding ethylammoniumchloride into the systems. The ethylammoniumchloride gave the greater *K* and selectivity values than other surfactants, due to the higher q* values. Diethylamine hydrochloride, butylamine, hexylamine and octylamine could not improver the retention and selectivity of 5-methoxyflavone and 6-methoxyflavone. We cannot explain this result.

However, a small change in the retention and selectivity of 5-methoxyflavone and 6-methoxyflavone was obtained in ethylammonuimchloride system. Because the structure of these compounds has the smaller constrain structures thus the increasing of q* value has less affect to the retention and selectivity of these compound.

This study has shown a simple method to enhance the shape selectivity of monomeric C_{18} by adding the surfactant onto the stationary phase to induce more rigidity of alkyl chains.

CHAPTER V

CONCLUSION AND FUTURE WORK

In this work, monomeric C_{18} column was developed for separation of PAHs and flavonoid by adding a surfactant into the mobile phase with a concentration lower than its critical micellar concentration (CMC).

In general, the separation of structural isomer is based on the shape selectivity, shape selectivity of these compound increases with increasing bonding density. When the surfactant adsorbed onto the stationary phase, it could increase the phase density resulted in the increases of phase rigidity and which should improve the retention factors and selectivity for structural isomer (planar and non planar PAHs).

The amounts of surfactant adsorbed on the column were determined by frontal analysis. Effects of the following parameters on the retention factor (k'), selectivity (α) and resolution (R_s) of PAHs were investigated: q* value, surfactant concentration, surfactant type and temperature.

In comparison with added surfactant and without surfactant system, added surfactant system gave the better retention factor (k'), selectivity (α) and resolution (R_s) of PAHs than without surfactant system.

Frontal analysis was chosen to determine the amount of surfactant adsorbed on the stationary phase, q* value. At below CMC, the q* value increases with increased the concentration of surfactants and the q* value increases drastically just above CMC.

Focusing on the concentration of surfactant, when increases in the surfactant concentration resulted in increase q* values, which enhance the phase rigidity. This should improve the retention factor (k'), and selectivity (α) for PAHs.

However, retention of PAHs decreased at above CMC. At this high concentration, the surfactant form micelle which could act as a pseudo stationary phase present in mobile phase and increase the elution strength.

For the study of the temperature effect, the temparature was controlled at 0, 10, 20, 25, 30 and 40 ^{0}C. The decrease of the column temperatures resulted in the decrease of gauche conformation of C_{18} alkyl chains anchored on the stationary phase and the increase in the rigidity property of stationary phase (trans structure). Therefore, at low temperature surfactant molecules can penetrate between C_{18} alkyl chains easier compared to higher temperatures resulted in higher q* value. Then, it gave higher retention factors for PAHs. Therefore, at 0 °C, the better retention of PAHs can be obtained.

In comparisons with ethylammonuimchloride, diethylamine hydrochloride, butylamine, hexylamine and octylamine used as modifier for C_{18} column. Ethylammonuimchloride can be adsorbed onto the stationary phase higher than the other surfactant, it gave the larger q* value. Because the size of ethylammonuimchloride is smallest then it can be adsorbed on the stationary phase easiest compare to longer molecules. This causes the increase in the retention factor (k') and selectivity (α). We suggested that the bulky molecule (diethylamine hydrochloride) and longer alkyl chain length (butylamine, hexylamine and octylamine) possesses greater steric effect, therefore less amount can be adsorbed onto the stationary phase resulted in small magnitude in the change of morphology rigidity.

From experiment, the condition gave highest *K* value was giving the best selectivity of PAHs. From equation 2.2, selectivity is the ratio of retention factors of two adjucent peaks.

$$\propto = \frac{k_2'}{k_1'} \qquad (2.2)$$

When the surfactant adsorbed onto the stationary phase, it could increase the phase density and retention factor (higher *k'*) so it increase the selectivity of PAHs also.

In comparison to monomeric C18 modified with 5mM ethylammonuimchloride and polymeric C18 stationary phase for selectivity of benz[a]anthracene/triphenylene at 10 ºC, it appears that the monomeric C18 modified with 5mM ethylammonuimchloride gave the selectivity of benz[a]anthracene/triphenylene more than polymeric C18 stationary phase.

As we have known that resolution, R_s, is the function of retention factor (*k'*), selectivity (α) and efficiency (*N*) as described in this equation.

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'}{1+k'}\right) \qquad (4.2)$$

The relation of resolution (R_s) and retention factor (*k'*), from experiment,

We observed that the condition giving the highest *K* value and the condition giving the best resolution of PAHs were two different points. It is due to the loosing separation efficiency. When the surfactant adsorbed onto the stationary phase (q* value), it could increase the phase density and retention factor. When the solutes retained longer in column (higher *k'*), peak width of a solute became wider (smaller *N*) result in decrease resolution (R_s). This affected the R_s of PAHs.

We have adapted our best condition to improve the separation of more isomeric polar compounds; 5- and 6-methoxyflavone. We found that the separations of 5-methoxyflavone and 6-methoxyflavone can be improved by adding the ethylammonuimchloride into the systems, it gave the greater *K* and selectivity values than other surfactants.

The new method development can be released for separation of PAHs and flavonoids with good method precision (% RSD < 1.0%), simple method to enhance the shape selectivity of monomeric C_{18} column, cheap and easy. Further work should be focus on the developing the best coniditon for more complex PAHs mixture. This method can be improve the efficiency (shape selectivity) of monomeric C_{18} stationary phase is equal to the polymeric C_{18} stationary phase.

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APPENDICES

APPENDIX A

Table A1 The values of q*, k', R_s and α of ethylamine hydrochloride at 0 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/ B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.64	10.49	12.55	23.23	26.63	3.22	2.19	1.196	1.146
1	28.35	1.56	10.78	12.98	23.45	26.9	3.61	2.65	1.204	1.147
2	51.76	1.59	10.53	12.68	22.92	26.28	3.58	2.59	1.204	1.147
3	52.94	1.62	10.69	12.92	23.84	27.34	3.92	2.64	1.209	1.147
4	53.15	1.64	11.32	13.73	24.92	28.60	3.37	2.42	1.213	1.148
5	53.30	1.61	11.35	13.77	24.97	28.68	3.35	2.29	1.213	1.149
6	65.88	1.63	11.30	13.71	24.82	28.47	3.31	2.29	1.213	1.147

[A]: acetylsalicylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A2 The values of q*, k', R_s and α of ethylamine hydrochloride at 10 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/ B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.41	8.96	10.49	17.39	20.51	3.56	3.83	1.171	1.179
1	29.37	1.32	8.86	10.40	17.32	20.42	3.59	3.89	1.172	1.179
2	42.21	1.31	8.64	10.13	16.55	19.51	3.60	3.87	1.172	1.179
3	45.82	1.39	8.63	10.12	16.66	19.65	3.57	3.89	1.173	1.179
4	46.10	1.41	9.05	10.62	17.57	20.74	3.67	3.88	1.173	1.180
5	47.53	1.41	9.23	10.85	17.97	21.24	3.63	3.78	1.176	1.182
6	54.73	1.39	8.99	10.56	17.43	20.59	3.57	3.83	1.175	1.181

[A]: acetylsalicylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A3 The values of q*, k', R_s and α of ethylamine hydrochloride at 20 ºC

Concentration (mM)	Q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.20	7.15	8.18	12.63	15.14	3.42	4.76	1.144	1.199
1	23.93	1.18	7.09	8.12	12.55	15.03	3.39	4.79	1.145	1.198
2	26.26	1.20	6.88	7.88	12.28	14.71	3.40	4.80	1.145	1.198
3	27.77	1.19	7.09	8.12	12.52	14.99	3.39	4.79	1.145	1.197
4	28.04	1.20	7.21	8.26	12.74	15.26	3.40	4.80	1.146	1.198
5	46.73	1.19	7.01	8.01	12.35	14.79	3.41	4.72	1.143	1.198

[A]: acetylsalicylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A4 The values of q*, k', R_s and α of ethylamine hydrochloride at 25 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.18	6.83	7.78	11.75	14.08	3.13	4.63	1.139	1.198
1	23.70	1.12	6.55	7.43	11.20	13.41	3.18	4.99	1.134	1.197
2	27.02	1.13	6.50	7.38	11.10	13.30	3.10	4.83	1.135	1.198
3	26.89	1.16	6.62	7.53	11.42	13.69	3.14	4.64	1.137	1.199
4	28.29	1.14	6.87	7.83	11.88	14.27	3.08	4.58	1.140	1.201
5	50.74	1.14	6.67	7.60	11.54	13.86	3.17	4.64	1.139	1.201

[A]: acetylsalicylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A5 The values of q*, k', R_s and α of ethylamine hydrochloride at 30 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.16	6.17	6.97	10.29	12.39	3.16	5.11	1.130	1.204
1	22.55	1.12	6.11	6.91	10.22	12.31	3.14	5.06	1.131	1.205
2	28.24	1.10	5.89	6.65	9.84	11.83	3.10	4.97	1.129	1.202
3	28.14	1.10	5.88	6.63	9.79	11.78	3.08	4.99	1.128	1.203
4	29.70	1.10	5.96	6.72	9.92	11.93	2.91	4.85	1.128	1.203
5	53.14	1.09	5.85	6.59	9.73	11.71	2.88	4.80	1.126	1.203

[A]: acetylsalicylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A6 The values of q*, k', R_s and α of ethylamine hydrochloride at 40 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.04	4.97	5.54	7.87	9.42	2.65	4.73	1.114	1.197
1	23.41	1.03	5.22	5.84	8.30	9.98	2.81	5.05	1.119	1.202
2	27.22	1.03	5.09	5.67	8.10	9.79	2.59	4.56	1.116	1.202
3	26.41	1.02	5.06	5.64	8.07	9.69	2.57	4.60	1.115	1.201
4	31.75	1.01	5.01	5.58	7.94	9.53	2.52	4.47	1.115	1.200
5	57.31	1.01	4.89	5.45	7.76	9.31	2.56	4.50	1.115	1.200

[A]: acetylsaliccylic acid, T: triphenylene B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A7 The values of q*, k', R_s and α of diethylamine hydrochloride at 0 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.64	10.48	12.6	23.24	26.64	3.22	2.19	1.196	1.146
1	24.02	1.51	10.60	12.74	23.12	26.49	3.70	2.73	1.202	1.146
2	26.51	1.52	10.19	12.25	22.14	25.38	3.71	2.73	1.202	1.146
3	31.60	1.45	9.95	11.95	21.75	24.93	3.62	2.66	1.201	1.146
4	34.70	1.51	10.49	12.64	23.32	26.74	2.93	2.24	1.205	1.147
5	41.13	1.52	10.56	12.72	23.23	26.67	2.93	2.20	1.205	1.148
6	43.58	1.51	10.59	12.77	23.47	26.94	2.68	1.90	1.206	1.148
7	43.45	1.56	10.79	13.01	23.84	27.39	2.59	1.84	1.206	1.149
8	47.60	1.50	10.98	13.24	24.01	27.59	3.00	2.15	1.206	1.149
9	69.99	1.49	10.85	13.07	23.90	27.44	2.76	1.97	1.205	1.148

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A8 The values of q*, k', R_s and α of diethylamine hydrochloride at 10 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.41	8.96	10.49	17.39	20.51	3.56	3.83	1.171	1.179
1	21.27	1.34	8.79	10.28	17.06	20.12	3.56	3.92	1.170	1.179
2	25.87	1.32	8.77	10.26	17.04	20.10	3.58	3.94	1.170	1.180
3	30.15	1.33	8.52	9.98	16.29	19.23	3.91	4.02	1.171	1.180
4	33.16	1.38	9.03	10.57	17.63	20.82	3.39	3.47	1.171	1.181
5	39.84	1.38	9.05	10.6	17.65	20.84	3.29	3.43	1.171	1.181
6	43.58	1.38	9.08	10.66	17.81	21.02	3.40	3.49	1.174	1.180
7	45.91	1.37	9.06	10.65	17.75	20.95	3.41	3.52	1.175	1.180
8	65.55	1.36	8.86	10.4	17.19	20.30	3.53	3.88	1.174	1.181

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A9 The values of q*, k', R_s and α of diethylamine hydrochloride at 20 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/B[a]A	B[k]F/ D[a, c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.20	7.15	8.18	12.63	15.14	3.42	4.76	1.144	1.199
1	21.84	1.17	7.09	8.12	12.49	14.97	3.18	4.96	1.145	1.199
2	24.91	1.14	7.05	8.07	12.43	14.89	3.25	4.74	1.145	1.198
3	26.60	1.19	7.10	8.13	12.52	15.00	3.36	4.73	1.145	1.198
4	27.13	1.18	7.21	8.26	12.69	15.21	3.35	4.76	1.146	1.199
5	47.37	1.17	6.99	7.96	12.17	14.54	3.40	4.71	1.139	1.195

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A10 The values of q*, k', R_s and α of diethylamine hydrochloride at 25 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.18	6.83	7.78	11.75	14.08	3.13	4.63	1.139	1.198
1	21.17	1.15	6.55	7.43	11.26	13.49	3.18	4.96	1.134	1.198
2	25.04	1.18	6.50	7.38	11.10	13.33	3.14	4.87	1.135	1.201
3	25.06	1.16	6.52	7.42	11.19	13.44	3.04	4.54	1.138	1.201
4	24.70	1.16	6.76	7.69	11.72	14.07	3.08	4.65	1.138	1.201
5	41.77	1.16	6.50	7.39	11.19	13.41	2.92	4.50	1.137	1.198

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A11 The values of q*, k', R_s and α of diethylamine hydrochloride at 30 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.16	6.17	6.97	10.29	12.39	3.16	5.11	1.130	1.204
1	19.09	1.10	6.01	6.79	10.02	12.07	3.15	5.08	1.130	1.205
2	25.29	1.06	5.69	6.42	9.46	11.38	3.07	5.00	1.128	1.203
3	25.25	1.08	5.89	6.65	9.79	11.78	3.06	4.91	1.129	1.203
4	24.70	1.09	5.96	6.72	9.93	11.96	2.36	4.43	1.128	1.204
5	44.49	1.09	5.85	6.59	9.73	11.72	2.34	4.40	1.126	1.205

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A12 The values of q*, k', R_s and α of diethylamine hydrochloride at 40 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.04	4.97	5.54	7.87	9.42	2.65	4.73	1.114	1.197
1	20.75	1.02	5.20	5.80	8.25	9.91	2.79	5.03	1.115	1.201
2	24.01	1.02	4.89	5.45	7.74	9.28	2.73	4.96	1.115	1.199
3	24.58	0.99	4.90	5.46	7.77	9.32	2.76	4.95	1.114	1.199
4	27.01	1.02	4.88	5.45	7.76	9.31	2.51	4.61	1.115	1.200
5	47.85	1.00	4.88	5.43	7.72	9.26	2.32	4.32	1.113	1.199

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A13 The values of q*, k', R_s and α of butylamine at 0 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.64	10.49	12.55	23.23	26.63	3.22	2.19	1.196	1.146
1.0	27.93	1.58	10.58	12.77	23.43	26.86	3.68	2.46	1.203	1.146
2.0	29.78	1.45	10.53	12.65	23.12	26.50	3.46	2.48	1.202	1.146
3.0	36.40	1.54	10.60	12.74	23.21	26.62	3.41	2.47	1.203	1.147
4.0	41.74	1.54	10.82	13.02	23.66	27.14	3.48	2.37	1.203	1.147
5.0	45.29	1.57	10.98	13.21	23.95	27.49	3.52	2.46	1.203	1.148
6.0	78.38	1.56	10.87	13.08	23.79	27.30	3.51	2.48	1.203	1.148

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A14 The values of q*, k', R_s and α of butylamine at 10 ºC

Concentration (mM)	q*	k'					R_s		A	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/ B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.41	8.96	10.49	17.39	20.51	3.56	3.83	1.171	1.179
1	21.04	1.30	8.54	9.99	16.52	19.5	3.51	3.82	1.170	1.180
2	27.73	1.37	8.47	9.91	16.20	19.15	3.44	3.8	1.170	1.182
3	30.73	1.31	8.50	9.95	16.37	19.35	3.52	3.81	1.171	1.182
4	32.77	1.37	8.80	10.31	17.00	20.09	3.68	3.94	1.172	1.182
5	39.68	1.36	8.86	10.38	17.1	20.21	3.66	3.93	1.172	1.182
6	78.38	1.35	8.77	10.27	16.91	19.99	3.69	3.94	1.171	1.182

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A15 The values of q*, k', R_s and α of butylamine at 20 ºC

Concentration (mM)	q*	k'					R_s		A	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.20	7.15	8.18	12.63	15.14	3.42	4.76	1.144	1.199
1.00	21.94	1.18	6.98	7.97	12.12	14.51	3.32	4.70	1.142	1.197
2.00	26.00	1.18	7.06	8.06	12.30	14.75	3.27	4.69	1.142	1.199
3.00	26.02	1.19	7.12	8.15	12.57	15.08	3.40	4.76	1.145	1.200
4.00	26.11	1.19	7.41	8.50	13.16	15.79	3.47	4.78	1.147	1.200
5.00	52.66	1.18	7.06	8.09	12.42	14.90	3.38	4.73	1.146	1.200

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A16 The values of q*, k', R_s and α of butylamine at 25 ºC

Concentration (mM)	q*	k'					R_s		A	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.18	6.83	7.78	11.75	14.08	3.13	4.63	1.139	1.198
1.0	22.23	0.93	5.80	6.58	9.92	11.91	3.15	4.91	1.134	1.201
2.0	26.00	1.08	5.89	6.68	10.09	12.12	3.10	4.85	1.134	1.201
3.0	26.12	1.15	6.53	7.43	11.17	13.40	3.25	4.93	1.138	1.200
4.0	27.90	1.16	6.79	7.76	11.72	14.10	3.31	5.10	1.143	1.203
5.0	45.45	1.15	6.68	7.63	11.56	13.87	3.30	4.86	1.142	1.200

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A17 The values of q*, k', R_s and α of butylamine at 30 ºC

Concentration (mM)	q*	k'					R_s		A	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/ B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0.0	0.00	1.16	6.17	6.97	10.29	12.39	3.16	5.11	1.130	1.204
1.0	21.01	1.13	6.13	6.91	10.17	12.24	3.12	5.10	1.127	1.204
2.0	26.32	1.06	5.41	6.10	8.82	10.59	2.91	4.94	1.128	1.201
3.0	26.69	1.06	5.66	6.38	9.37	11.24	3.08	4.95	1.127	1.200
4.0	25.72	1.08	5.90	6.66	9.83	11.83	2.98	4.97	1.129	1.203
5.0	48.50	1.06	5.72	6.45	9.48	11.40	2.90	4.88	1.128	1.203

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A18 The values of q*, k', R_s and α of butylamine at 40 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/ B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.04	4.97	5.54	7.87	9.42	2.65	4.73	1.114	1.197
1	23.86	1.02	5.20	5.81	8.27	9.94	2.61	4.63	1.117	1.202
2	25.55	1.02	4.93	5.49	7.81	9.37	2.67	4.90	1.114	1.200
3	25.54	1.01	4.92	5.48	7.77	9.32	2.72	4.88	1.114	1.199
4	25.98	1.01	4.85	5.4	7.67	9.19	2.71	4.86	1.113	1.198
5	48.82	1.00	4.9	5.45	7.75	9.29	2.52	4.70	1.112	1.199

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A19 The values of q*, k', R_s and α of hexylamine at 0 ºC

Concentration (mM)	Q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/ B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.64	10.49	12.55	23.23	26.63	3.22	2.19	1.196	1.146
1	27.67	1.51	10.51	12.63	23.06	26.46	3.57	2.58	1.202	1.147
2	29.07	1.47	10.31	12.39	22.58	25.90	3.37	2.41	1.202	1.147
3	35.25	1.53	10.56	12.69	23.14	26.55	3.27	2.31	1.202	1.147
4	38.41	1.40	10.65	12.79	23.19	26.59	3.25	2.26	1.201	1.147
5	42.57	1.56	10.73	12.90	23.68	27.18	3.19	2.23	1.202	1.148
6	74.15	1.52	10.67	12.83	23.36	26.79	3.37	2.34	1.202	1.147

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A20 The values of q*, k', R_s and α of hexylamine at 10 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/ B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.41	8.96	10.49	17.39	20.51	3.56	3.83	1.171	1.179
1	19.73	1.28	8.71	10.21	16.69	19.71	3.47	3.78	1.172	1.181
2	24.46	1.29	8.41	9.84	16.15	19.08	3.44	3.77	1.170	1.181
3	30.06	1.31	8.50	9.95	16.50	19.51	3.47	3.83	1.171	1.182
4	31.49	1.35	8.73	10.22	16.86	19.93	3.68	3.90	1.171	1.182
5	41.93	1.37	8.88	10.40	17.14	20.26	3.69	3.92	1.171	1.182
6	63.39	1.32	8.61	10.09	16.67	19.69	3.69	3.88	1.172	1.181

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A21 The values of q*, k', R_s and α of hexylamine at 20 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/ B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.2	7.15	8.18	12.63	15.14	3.42	4.76	1.144	1.199
1	21.20	1.19	7.02	8.04	12.39	14.84	3.39	4.74	1.145	1.198
2	25.87	1.18	6.89	7.89	12.17	14.56	3.43	4.76	1.145	1.196
3	25.44	1.20	7.30	8.37	12.96	15.54	3.33	4.71	1.147	1.199
4	25.47	1.18	7.34	8.42	13.00	15.59	3.46	4.81	1.147	1.199
5	76.69	1.19	7.21	8.26	12.88	15.44	3.41	4.75	1.146	1.199

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A22 The values of q*, k', R_s and α of hexylamine at 25 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/ B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.18	6.83	7.78	11.75	14.08	3.13	4.63	1.139	1.198
1.0	22.23	0.93	5.80	6.58	9.92	11.91	3.15	4.91	1.134	1.201
2.0	26.00	1.08	5.89	6.68	10.09	12.12	3.10	4.85	1.134	1.201
3.0	26.12	1.15	6.53	7.43	11.17	13.40	3.25	4.93	1.138	1.200
4.0	27.90	1.18	6.79	7.74	11.72	14.09	3.31	5.10	1.140	1.202
5.0	45.45	1.15	6.69	7.62	11.56	13.87	3.30	4.86	1.139	1.200

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A23 The values of q*, k', R_s and α of hexylamine at 30 ºC

Concentration (mM)	q*	k'					R_s		A	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/ B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.16	6.17	6.97	10.29	12.39	3.16	5.11	1.130	1.204
1	21.36	1.12	6.08	6.86	10.14	12.20	3.14	5.10	1.128	1.203
2	24.20	1.10	5.89	6.64	9.80	11.77	3.20	5.01	1.127	1.201
3	25.16	1.09	5.90	6.65	9.83	11.81	3.10	5.09	1.127	1.201
4	24.70	1.09	5.92	6.68	9.83	11.81	3.12	5.01	1.128	1.201
5	42.89	1.09	5.86	6.60	9.71	11.64	2.92	4.90	1.126	1.199

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A24 The values of q*, k', R_s and α of hexylamine at 40 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/ B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.04	4.97	5.54	7.87	9.42	2.65	4.73	1.114	1.197
1	21.65	1.04	5.14	5.74	8.16	9.80	2.77	4.92	1.117	1.201
2	24.46	1.00	4.85	5.40	7.66	9.18	2.66	4.79	1.113	1.198
3	24.96	0.89	4.81	5.36	7.62	9.14	2.67	4.84	1.114	1.199
4	25.72	0.99	4.96	5.52	7.92	9.50	2.56	4.75	1.113	1.199
5	46.09	0.95	4.79	5.33	7.59	9.11	2.42	4.50	1.113	1.200

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A25 The values of q*, k', R_s and α of octylamine at 0 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/ B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.64	10.49	12.55	23.23	26.63	3.22	2.19	1.196	1.146
1	25.56	1.48	10.42	12.52	23.04	26.41	3.29	2.4	1.199	1.146
2	27.79	1.47	10.26	12.32	22.42	25.70	3.50	2.81	1.201	1.146
3	33.04	1.53	10.56	12.69	23.10	26.48	3.54	2.49	1.202	1.146
4	34.18	1.55	10.65	12.80	23.44	26.87	3.67	2.66	1.202	1.146
5	64.04	1.50	10.38	12.47	22.59	25.86	3.42	2.49	1.201	1.145

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A26 The values of q*, k', R_s and α of octylamine at 10 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/ B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.41	8.96	10.49	17.39	20.51	3.56	3.83	1.171	1.179
1	17.36	1.32	8.68	10.16	16.76	19.78	3.59	3.88	1.171	1.180
2	25.42	1.28	8.31	9.72	15.92	18.80	3.52	3.95	1.170	1.181
3	25.44	1.32	8.57	10.03	16.41	19.38	3.59	3.97	1.170	1.181
4	27.39	1.34	8.71	10.2	16.84	19.89	3.62	3.99	1.171	1.181
5	59.55	1.33	8.62	10.09	16.61	19.62	3.60	3.92	1.171	1.181

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A27 The values of q*, k', R_s and α of octylamine at 20 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/ B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.20	7.15	8.18	12.63	15.14	3.42	4.76	1.144	1.199
1	22.55	1.19	7.08	8.1	12.57	15.06	3.3	4.69	1.144	1.198
2	24.85	1.15	6.88	7.87	12.15	14.56	3.26	4.64	1.144	1.198
3	24.10	1.18	7.04	8.06	12.45	14.92	3.42	4.71	1.145	1.198
4	24.83	1.19	7.17	8.21	12.64	15.15	3.40	4.77	1.145	1.199
5	51.70	1.18	7.06	8.08	12.47	14.92	3.40	4.75	1.144	1.196

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A28 The values of q*, k', R_s and α of octylamine at 25 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/ B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.18	6.83	7.78	11.75	14.08	3.13	4.63	1.139	1.198
1	19.12	0.85	6.48	7.35	11.03	13.26	3.18	4.96	1.134	1.202
2	23.12	0.82	6.21	7.04	10.57	12.70	3.11	4.88	1.134	1.202
3	22.75	1.09	6.33	7.19	10.85	13.02	3.19	4.83	1.136	1.200
4	23.80	1.15	6.61	7.51	11.34	13.63	3.25	4.94	1.136	1.202
5	52.66	1.11	6.32	7.17	10.80	12.97	3.19	4.88	1.134	1.201

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A29 The values of q*, k', R_s and α of octylamine at 30 ºC

Concentration (mM)	q*	k'					R_s		A	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/ B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.16	6.17	6.97	10.29	12.39	3.16	5.11	1.130	1.204
1	18.96	1.11	5.92	6.69	10.00	12.03	3.09	5.01	1.130	1.203
2	23.44	1.04	5.73	6.46	9.56	11.49	3.00	4.93	1.127	1.202
3	24.29	1.06	5.76	6.49	9.59	11.53	3.04	4.93	1.127	1.202
4	25.98	1.07	5.82	6.56	9.62	11.56	3.05	4.93	1.127	1.202
5	46.41	1.09	5.91	6.67	9.80	11.78	3.08	4.95	1.126	1.202

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

Table A30 The values of q*, k', R_s and α of octylamine at 40 ºC

Concentration (mM)	q*	k'					R_s		α	
		acetyl[A]	T	B[a]A	B[k]F	D[a,c]A	T/ B[a]A	B[k]F/ D[a,c]A	B[a]A/ T	D[a,c]A/ B[k]F
0	0.00	1.04	4.97	5.54	7.87	9.42	2.65	4.73	1.114	1.197
1	21.59	1.04	5.25	5.87	8.42	10.13	2.79	4.96	1.118	1.203
2	23.12	0.95	4.74	5.28	7.53	9.05	2.59	4.77	1.114	1.202
3	23.23	0.95	4.78	5.32	7.56	9.08	2.56	4.62	1.113	1.201
4	24.06	1.02	4.91	5.46	7.76	9.31	2.71	4.84	1.112	1.200
5	51.70	1.00	4.87	5.42	7.69	9.23	2.66	4.79	1.113	1.200

[A]: acetylsaliccylic acid, T: triphenylene, B[a]A: benz[a]anthracene, B[k]F: benzo[k]fluoranthene, D[a,c]A: dibenz[a,c]anthracene.

APPENDIX B

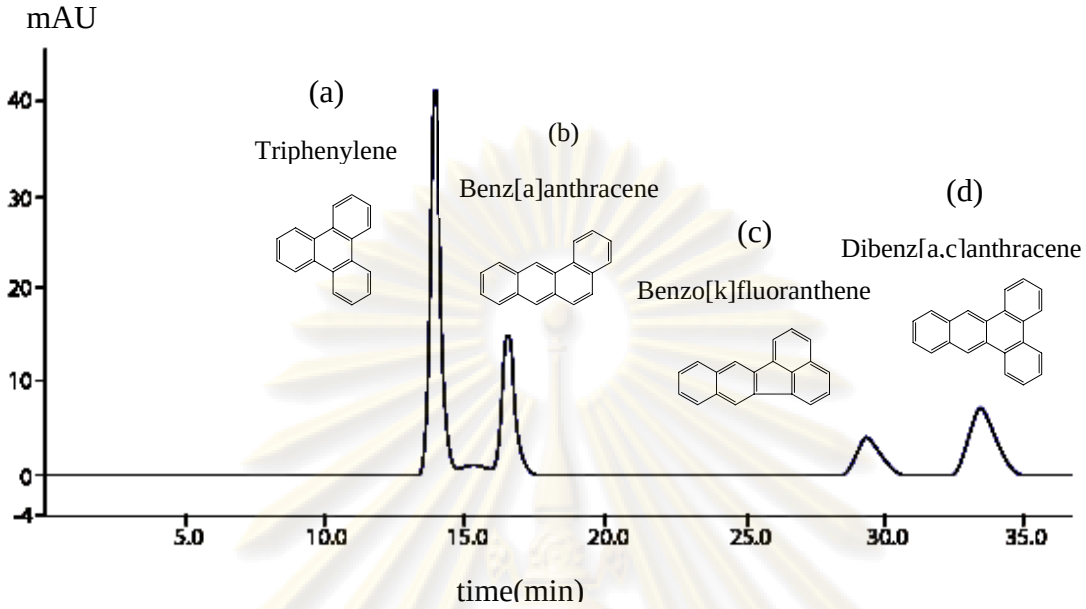

Figure B1. Chromatogram of separation of four PAHs, mobile phase: 40mM carbonate buffer/ACN, column temperature: 0 ºC.

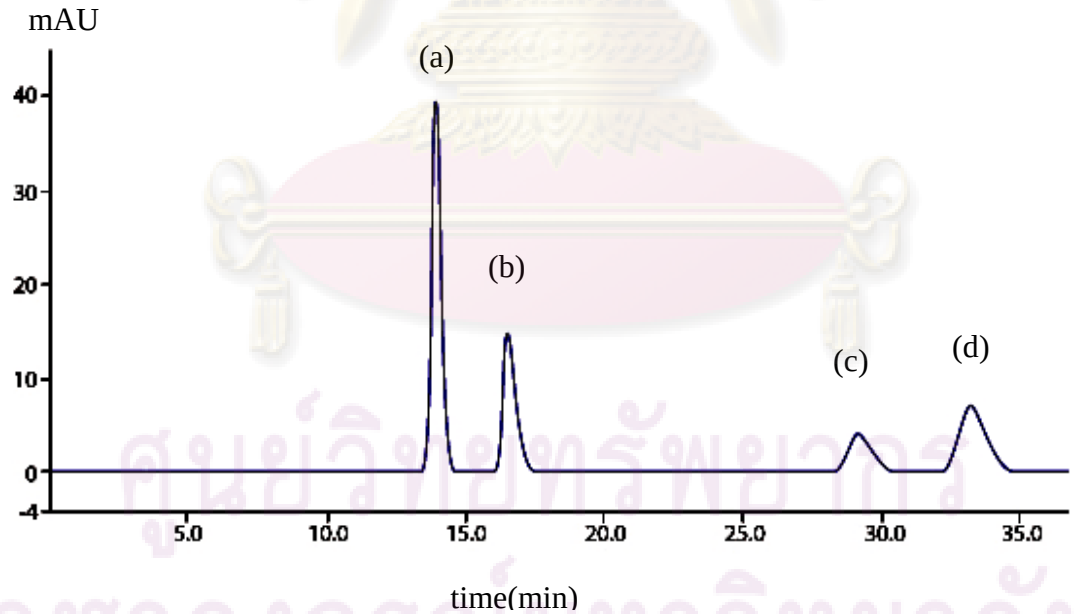

Figure B2. Chromatogram of separation of four PAHs, modified column with 5mM ethylammonuimchloride, column temperature: 0 ºC, detection: UV 254 nm.

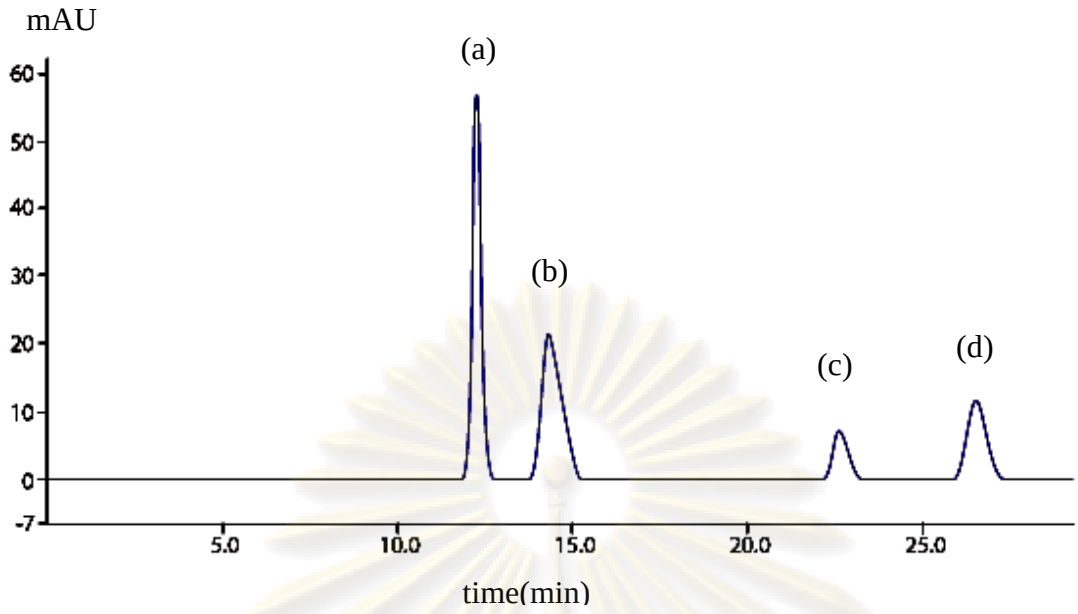

Figure B3. Chromatogram of separation of four PAHs, modified column with 5mM ethylammonuimchloride, column temperature: 10 ºC, detection: UV 254 nm.

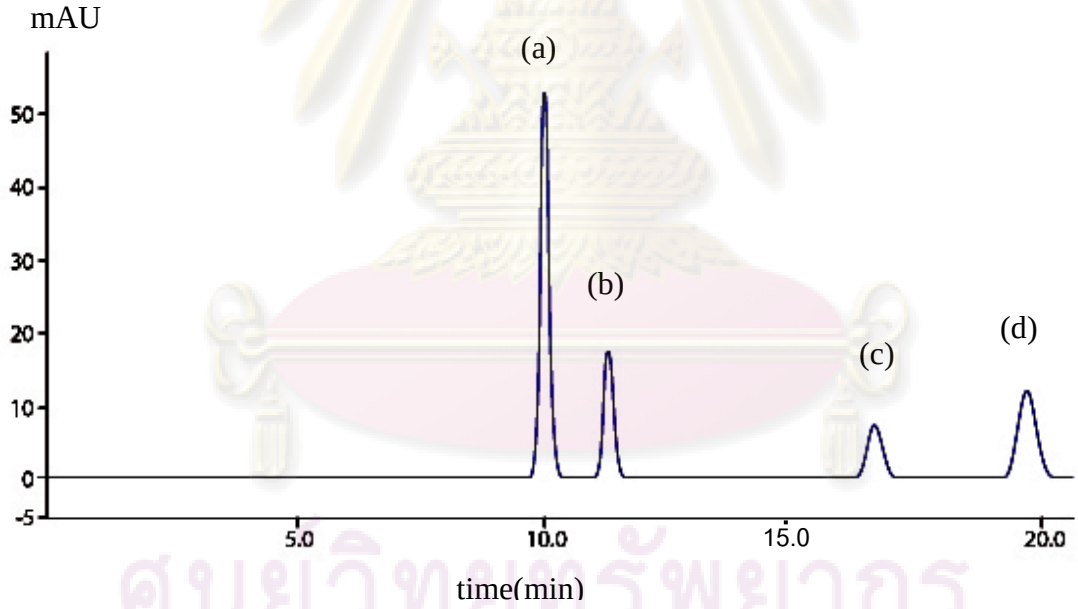

Figure B4. Chromatogram of separation of four PAHs, modified column with 4mM ethylammonuimchloride, column temperature: 20 ºC, detection: UV 254 nm.

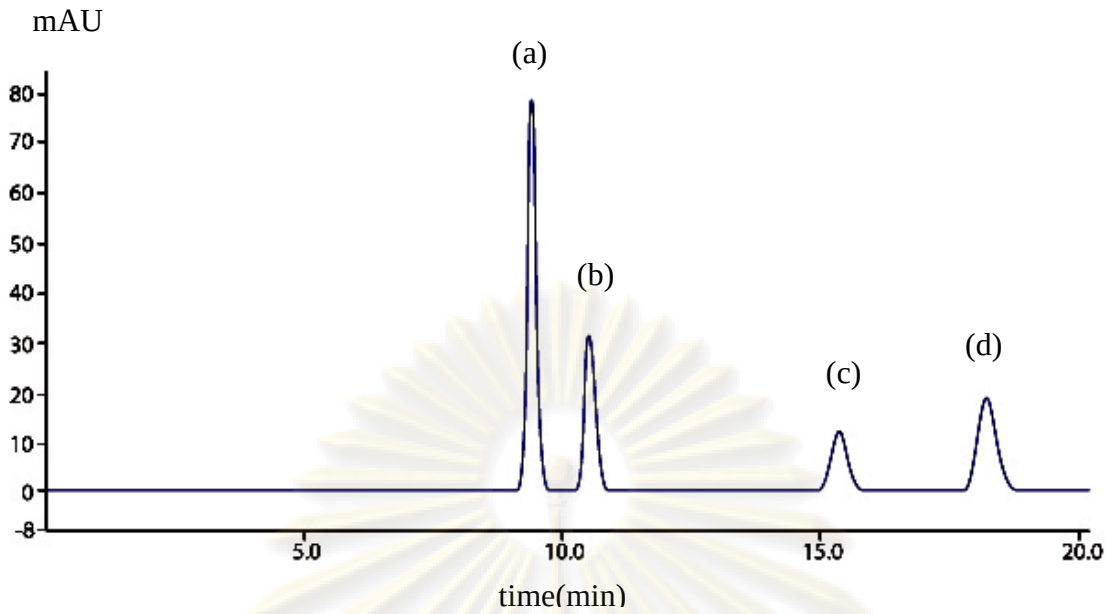

Figure B5. Chromatogram of separation of four PAHs, modified column with 4mM ethylammonuimchloride, column temperature: 25 ºC, detection: UV 254 nm.

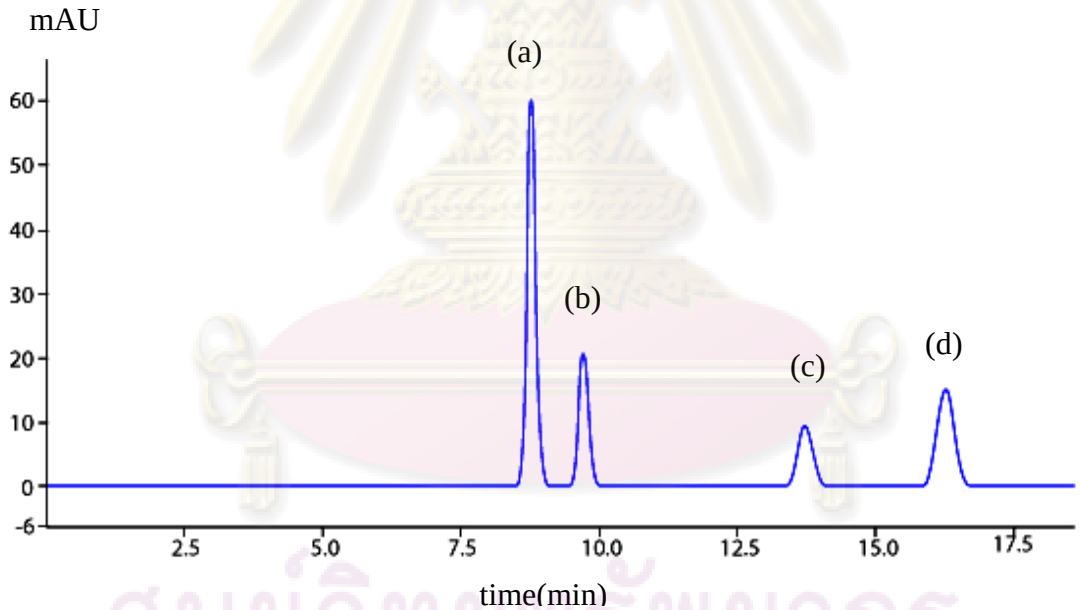

Figure B6. Chromatogram of separation of four PAHs, modified column with 1mM ethylammonuimchloride, column temperature: 30 ºC, detection: UV 254 nm.

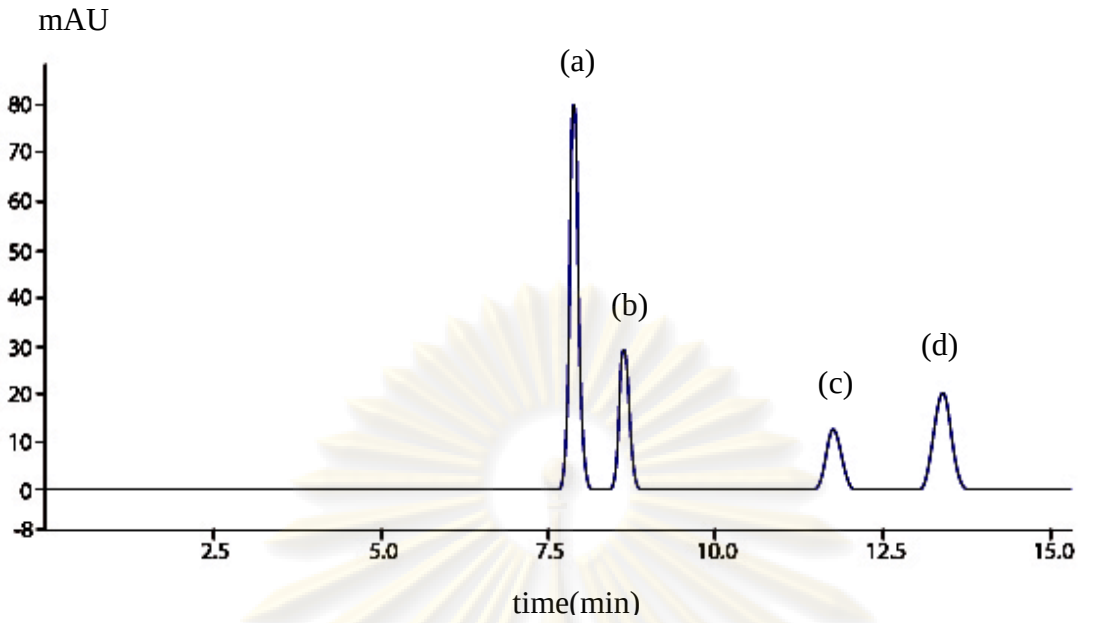

Figure B7. Chromatogram of separation of four PAHs, modified column with 1mM ethylammonuimchloride, column temperature: 40 ºC, detection: UV 254 nm.

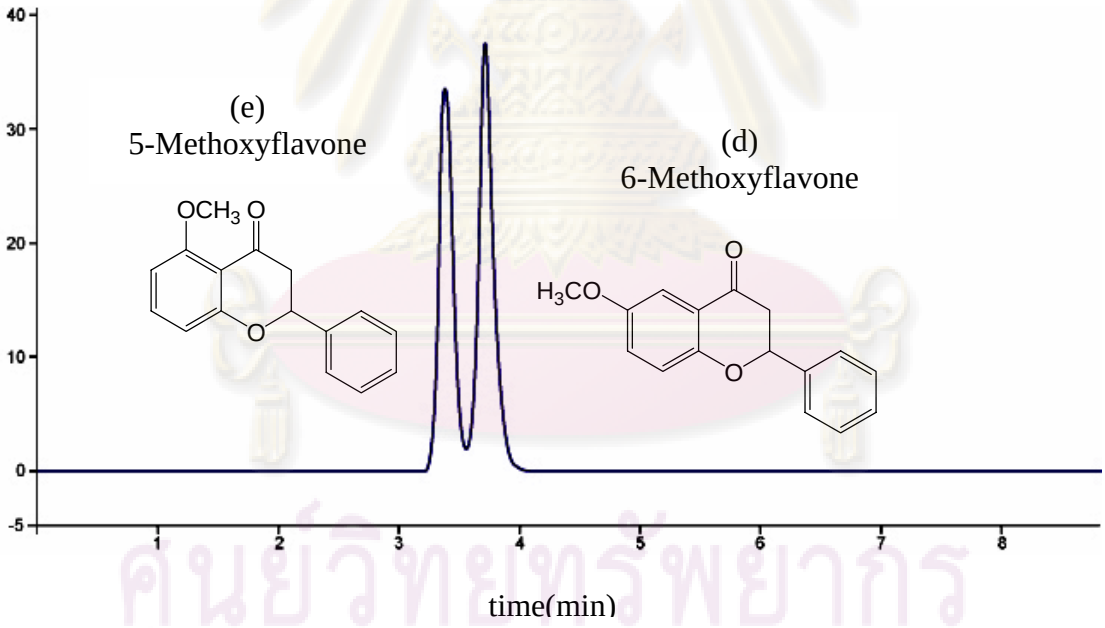

Figure B8. Chromatogram of separation of 5- and 6-methoxyflavone, modified column with 40mM carbonate buffer, column temperature: 0 ºC, detection: UV 254 nm.

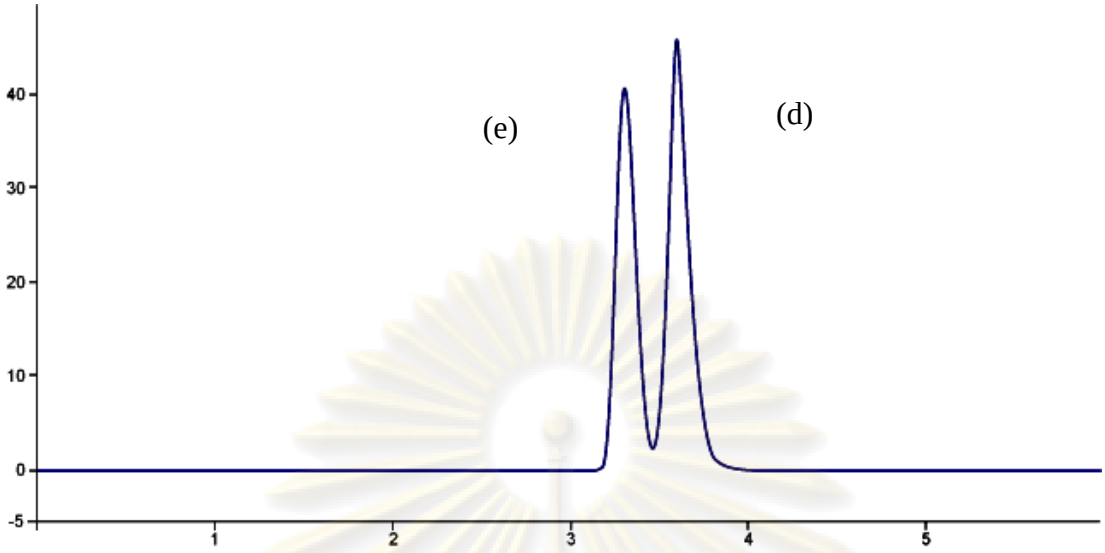

Figure B9. Chromatogram of separation of 5- and 6-methoxyflavone, modified column with 5mM ethylammonuimchloride, column temperature: 0 ºC, detection: UV 254 nm.

VITA

Miss Anongnart Rotjanakorn was born on April 6, 1982, in Kalasin, Thailand. She received a Bachelor Degree in Chemistry from the Faculty of Science, Chulalongkorn University in 2004. After that, she continued study for a M. Sc. program in Analytical Chemistry, Department of Chemistry, Faculty of Science, Chulalongkorn University and will received her Master of Science Degree in 2008.