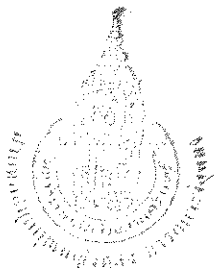
Development of Analytical Techniques for Trace and Ultra-Trace Analysis



Chongdee Thammakhet

A Thesis Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Chemistry

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Thesis Title	Development of Analytical Techniques for Trace and Ultra-Trace Analysis
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Major Program	Chemistry

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ชื่อวิทยานิพนธ์	การพัฒนาเทคนิคเพื่อการวิเคราะห์สารปริมาณน้อยและน้อยมาก
ผู้เขียน	นางสาวจงดี ธรรมเขต
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บทคัดย่อ

วิทยานิพนธ์นี้ศึกษาการพัฒนาเทคนิคการเตรียมตัวอย่างและเทคนิคการวิเคราะห์แบบง่าย ราคาถูก ใช้แรงงานและเวลาน้อย และสามารถใช้งานแบบออนไลน์ (on-line) เพื่อประยุกต์ใช้ในการวิเคราะห์สารปริมาณน้อยและน้อยมาก

เทคนิคแรกที่พัฒนาเป็นอุปกรณ์เก็บตัวอย่างอากาศแบบพาสซีฟ (passive sampler) ที่มีราคาถูก ใช้งานง่าย และเหมาะสำหรับการวิเคราะห์สารอินทรีย์ระเหยง่ายในอากาศ โดยตัวอย่างของสารอินทรีย์ระเหยง่ายที่เลือกศึกษา คือ เบนซีน โทลูอีน และไซลีน อุปกรณ์เก็บตัวอย่างอากาศแบบพาสซีฟที่พัฒนาขึ้นทำจากขวดแก้วฝาเกลียวขนาดเส้นผ่านศูนย์กลางภายใน 10.6 มิลลิเมตร สูง 67.6 มิลลิเมตร บรรจุด้วยตัวดูดซับทีแนกซ์ ทีเอ (Tenax TA) 75 มิลลิกรัม โดยก่อนนำอุปกรณ์เก็บตัวอย่างอากาศไปประยุกต์ใช้กับตัวอย่างจริงได้มีการสอบเทียบมาตรฐาน (calibration) ของระบบด้วยสารมาตรฐานที่ทราบปริมาณของเบนซีน โทลูอีนและไซลีน เมื่อได้รับความร้อนเบนซีน โทลูอีนและไซลีนที่ถูกดูดซับไว้ที่ผิวของทีแนกซ์ ทีเอ (Tenax TA) จะถูกปล่อยออกมาและส่งต่อไปยังเครื่องแก๊สโครมาโตกราฟ ที่มีตัวตรวจวัดชนิด เฟลมไอออไนเซชัน ด้วยระบบเพิร์จแอนด์แทรป (Purge and Trap) ซึ่งต่อพ่วงกับแซมปลิงวาล์ว ที่ทำหน้าที่ฉีดสารเข้าเครื่องแก๊สโครมาโทกราฟ ทั้งนี้ได้ศึกษาสภาวะเหมาะสมของปัจจัยต่าง ๆ ที่ส่งผลต่อประสิทธิภาพในการวิเคราะห์ เช่น เวลาที่ใช้เพื่อคายการดูดซับ อัตราไหลของแก๊สที่ใช้เพื่อเพิร์จ สารที่ต้องการวิเคราะห์ไปยังแก๊สแซมปลิงวาล์ว และสภาวะของเครื่องแก๊สโครมาโทกราฟ เพื่อให้ระบบวิเคราะห์ที่พัฒนาขึ้นมีความไวในการวิเคราะห์สูงที่สุด โดยมีประสิทธิภาพในการแยกดี และใช้เวลาในการวิเคราะห์น้อย จากผลการทดลองพบว่าระบบการวิเคราะห์ให้ค่าการตอบสนองเชิงเส้นดีมาก โดยมีค่าสหสัมพันธ์เชิงปริมาณ (coefficient of determination, R^2) มากกว่า 0.99 สำหรับสารทั้งสามชนิด นอกจากนี้ระบบวิเคราะห์ยังมีค่าการตอบสนองที่เป็นเส้นตรงในช่วงกว้างคือ 0.20-13,254 3.6-5,104 และ 7.0-1,962 ไมโครกรัมต่อลูกบาศก์เซนติเมตร และมีขีดจำกัดการตรวจวัดต่ำคือ 0.31 0.24 และ 0.73 ไมโครกรัมต่อลูกบาศก์เซนติเมตร สำหรับเบนซีน โทลูอีน และ ไซลีนตามลำดับ จากนั้นจึงนำ

อุปกรณ์เก็บตัวอย่างอากาศแบบพาสซีพอย่างง่ายไปแขวนไว้ในบริเวณจุดเก็บตัวอย่าง เพื่อตรวจสอบและติดตามปริมาณเบนซีน โทลูอีนและไซลีนที่ปนเปื้อนในอากาศบริเวณสถานีเติมน้ำมัน 10 สถานี ในเขตพื้นที่ อ .หาดใหญ่ จ. สงขลา พบว่าความเข้มข้นของเบนซีน โทลูอีนและไซลีน อยู่ในช่วงที่ไม่สามารถตรวจวัดได้-19 12-200 และ 23-200 ไมโครกรัมต่อลูกบาศก์เมตร ตามลำดับ ซึ่งค่าความเข้มข้นดังกล่าวต่ำกว่าเกณฑ์มาตรฐานที่กำหนดไว้โดยสมาคมส่งเสริมความปลอดภัยและอนามัยในการทำงาน (The Occupational Safety and Health Administration, OSHA) และสถาบันส่งเสริมความปลอดภัยและอนามัยในการทำงาน (The National Institute of Occupational Safety and Health, NIOSH)

ในลำดับต่อมาได้พัฒนาเทคนิคออนไลน์ไมโครแทรป เพื่อลดปัญหาที่เกิดจากการล่าช้าระหว่างขั้นตอนการเก็บตัวอย่างและขั้นตอนการวิเคราะห์ เทคนิคดังกล่าวถูกนำมาใช้เพื่อวิเคราะห์สารอินทรีย์ที่มีมวลโมเลกุลต่ำ โดยเลือกใช้มีเทนเป็นสารตัวอย่างในการพัฒนาเทคนิคการวิเคราะห์ โดย ไมโครแทรปทำขึ้นจากท่อซิลิโคสตีล (silicosteel tubing) ที่มีขนาดเส้นผ่านศูนย์กลางภายใน 1.02 มิลลิเมตร เส้นผ่านศูนย์กลางภายนอก 1.59 มิลลิเมตร ยาว 15 เซนติเมตร บรรจุด้วยคาร์โบสเฟียร์ (Carbosphere) ขนาด 80/100 เมช (mesh) ซึ่งใช้เป็นตัวดูดซับ ไมโครแทรปจะถูกนำมาต่อไว้ระหว่างแหล่งกำเนิดแก๊สมาตรฐานและตัวตรวจวัดชนิดเฟลมไอออไนเซชัน เพื่อทำหน้าที่เป็นทั้งอุปกรณ์เพิ่มความเข้มข้นและอุปกรณ์ฉีดสาร เพื่อให้ไมโครแทรปมีประสิทธิภาพในการดูดซับและการคายการดูดซับสูงที่สุด จึงมีการศึกษาสภาวะที่เหมาะสมของปัจจัยต่าง ๆ ที่ส่งผลต่อสัญญาณการตอบสนอง ในช่วงอุณหภูมิ -50 ถึง 25 องศาเซลเซียส พบว่า -50 องศาเซลเซียสเป็นอุณหภูมิที่เหมาะสมที่สุดสำหรับการดูดซับ และระบบออนไลน์ที่พัฒนาขึ้นให้ผลการทดลองภายในระยะเวลาเพียงไม่กี่วินาทีหลังจากที่ทำการคายการดูดซับ โดยไมโครแทรปสามารถเพิ่มสัญญาณการตอบสนองได้สูงถึง 260 เท่า เมื่อเปรียบเทียบกับการผ่านสารเข้าตัวตรวจวัดโดยตรง นอกจากนี้ระบบวิเคราะห์แบบออนไลน์ไมโครแทรปให้ค่าสหสัมพันธ์เชิงปริมาณ (coefficient of determination, R^2) มากกว่า 0.99 และให้ขีดจำกัดการตรวจวัดต่ำ (28.3 ส่วนในพันล้านส่วนโดยปริมาตร) และมีความเสถียรสูงต่อการทำงานติดต่อกันเป็นระยะเวลานาน (ค่าเบี่ยงเบนมาตรฐานสัมพัทธ์น้อยกว่า 5 เปอร์เซ็นต์) ดังนั้นระบบวิเคราะห์แบบออนไลน์อย่างง่ายที่พัฒนาขึ้นจึงมีความเหมาะสมสำหรับการวิเคราะห์แก๊สมีเทนปริมาณน้อยรวมทั้งสารที่มีมวลโมเลกุลขนาดเล็กอื่น ๆ ทั้งในสิ่งแวดล้อมและในแก๊สที่มีความบริสุทธิ์สูงได้

เทคนิคสุดท้ายที่พัฒนาขึ้นเป็นเทคนิคการวิเคราะห์ที่สามารถใช้งานได้ง่ายเช่นกัน แต่จะมีความจำเพาะเจาะจงสูงขึ้น เทคนิคนี้อาศัยหลักการดูดซับแบบแอฟฟีนิตี (affinity adsorption) โดยได้มีการสังเคราะห์และเคลือบโคพอลิเมอร์ของ กรด3-อะคริลามิโดฟีนิลโบโรนิคอะคริลาไมด์

(3-acrylamidophenylboronic acid-acrylamide) ไว้บนแผ่นกระจก ซึ่งผลิตภัณฑ์ที่ได้จากการสังเคราะห์มีลักษณะเป็นเจลกึ่งโปร่งใส (semitransparent gel) เมื่อนำมาวิเคราะห์หาปริมาณกลูโคส พบว่าระบบการวิเคราะห์ดังกล่าวให้ค่าการตอบสนองเชิงเส้นดีมาก (ค่าสหสัมพันธ์เชิงปริมาณ coefficient of determination, R^2) มากกว่า 0.99 (โดยมีช่วงความเป็นเส้นตรง 1-60 มิลลิโมลาร์ และขีดจำกัดการตรวจวัดที่ 1 มิลลิโมลาร์ และเมื่อใช้เจลอย่างต่อเนื่องเป็นเวลา 42 ครั้ง ค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ที่ได้มีค่าน้อยกว่า 3 เปอร์เซ็นต์ ซึ่งสามารถแสดงถึงเสถียรภาพในการใช้งานของเจล นอกจากนี้ยังพบว่าเจลที่สังเคราะห์ขึ้นยังมีสมบัติที่ดีมากคือสามารถผ่านการฆ่าเชื้อ ที่อุณหภูมิ 121 องศาเซลเซียส และความดัน 1.2 บาร์ เป็นเวลา 10 นาที ด้วยสมบัติข้อนี้จึงทำให้สามารถประยุกต์ใช้เจลที่สังเคราะห์ได้กว้างขวางขึ้น โดยเฉพาะอย่างยิ่งสำหรับการตรวจสอบและติดตามกระบวนการทางชีววิทยา

Thesis Title	Development of Analytical Techniques for Trace and Ultra-Trace Analysis
Author	Ms. Chongdee Thammakhet
Major Program	Chemistry
Academic Year	2007

Abstract

This thesis focuses on the development and evaluation of simple, economical, less laborious, less time consuming, and on-line techniques for trace and ultra-trace analysis.

The first developed technique is a laboratory-built passive sampling coupled with a laboratory-built purge and trap system to be used as a simple and economical airborne sampling technique for volatile organic compound analysis. Benzene, toluene and xylene were used as representative components. A simple passive sampler was a screw capped glass bottle (67.6 mm high and 10.6 mm I.D.) packed with 75 mg of activated Tenax TA. The passive sampling system was calibrated with benzene, toluene and xylene standard gases. After exposed to a known amount of standard, the adsorbent was desorbed using a laboratory-built thermal desorption device connected with purge and trap system. The analytes were purged to fill a sampling loop and then injected by a gas sampling valve to a gas chromatograph connected with a flame ionization detector (GC-FID). All parameters, *i.e.,* desorption time, purge flow rate and gas chromatograph conditions, were optimized to obtain the highest sensitivity, the highest resolution and the shortest analysis time. The system provided excellent linearity (R^2>0.99) for all three compounds. It also provided wide linear dynamic ranges (0.20-13,254, 3.6-5,104, 7.0-1,962 μg m^{-3}) with low detection limits (0.31, 0.24 and 0.73 μg m^{-3}) for benzene, toluene and xylene, respectively, for the 3 week sampling time. The developed method was implemented for the monitoring of benzene, toluene and xylene at 10 gasoline stations in Hat Yai city, Songkhla, Thailand. The concentrations were found in the range of N.D.-19, 12-200 and 23-200 μg m^{-3} for benzene, toluene and xylene, respectively which were still under the limited concentration set by the Occupational Safety and Health

Administration (OSHA) and the National Institute of Occupational Safety and Health (NIOSH).

The second development aimed to overcome the errors occurring from the delay time between sampling and analysis steps. On-line microtrap was developed to be used as an on-line system for real-time monitoring of low molecular weight hydrocarbons; methane was chosen as a target analyte. Silicosteel tubing, 15 cm long, 1.02 mm I.D. and 1.59 mm O.D. was packed with Carbosphere 80/100 mesh and placed in between a standard gas source and a flame ionization detector to be used as a preconcentrator and an injector. To achieve the highest adsorption and desorption efficiencies, parameters affecting the response of the system were optimized. The chromatogram was obtained in a few seconds after the desorption using a laboratory-built thermal desorption device controlled by a timer. Excellent enhancement as high as 260 times (at -50°C, its optimum adsorption temperature) was obtained comparing to that obtained from direct-FID. The on-line microtrap system showed excellent linearity ($R^2 > 0.99$), low detection limit (28.3 ppbv) and excellent long term stability (RSD of less than 5.0 %). Therefore, this simple device is suitable for on-line analysis of trace methane and similar small molecules in the environment and highly pure gases.

The last project was the development of a simple and selective analytical system based on affinity adsorption. 3-Acrylamidophenylboronic acid-acrylamide copolymer was synthesized and coated on the glass slide. The resultant semitransparent gel was then used as a simple affinity sensor for the determination of glucose. Excellent linearity between optical density change and glucose concentrations was obtained over a wide concentration range of 1-60 mM with 1 mM detection limit. The system showed excellent repeatability with a relative standard deviation of less than 3% during the 42 consecutive tests. Interestingly, the synthesized gel can be sterilized (121°C, 1.2 bar, 10 min), making it more applicable particularly in bioprocess monitoring.

Acknowledgements

The completion of this thesis would be impossible without the help of many people, whom I would like to thank.

I am very grateful to Associate Professor Dr. Proespichaya Kanatharana and Associate Professor Dr. Panote Thavarungkul for allowing me to work on very interesting and challenging projects, for all the help and guidance over the past years, for putting up with me for so long, for never failing to answer those annoying questions I kept asking and for all the excellent teaching and supervision.

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Chongdee Thammakhet

The Relevant of the Research Work to Thailand

The purpose of this Doctor of Philosophy thesis in chemistry (analytical chemistry) is to develop and evaluate the performance of sample preparation and analytical techniques for the analysis of organic compounds at trace and ultra-trace levels. These developed methods are simple, economical and easy to operate.

The first developed sample preparation technique, passive sampling, can be applied to monitor volatile organic compounds in both out door and indoor air. This will be useful for the evaluation of air quality in Thailand where, volatile organic compounds are not currently monitored. Furthermore, the developed passive sample is cheap and will be suitable tool for large scale sampling.

The second method is the on-line microtrap that provides real-time data suitable for process control of many industries in Thailand using high purity gases. By changing the adsorbent inside the microtrap this technique can also be used for real-time monitoring of different gaseous samples (depending on the adsorbent used) to evaluate air quality particularly inside the building.

The last developed method is a simple glucose sensor. This is useful for the monitoring of glucose in the fermentation process or bioprocess monitoring because of the duration of the synthesized gel.

These techniques can be applied for quantitative analysis of trace amount of target analyte by several governmental and private organizations in Thailand which are, the Ministry of Public Health, the Ministry of Industry, the Ministry of Environment and the Ministry of Education.

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List of Abbreviations

BTEX	Benzene, Toluene, Ethyl benzene, Xylene
BTX	Benzene, Toluene, Xylene
BTV	Breakthrough Volume
CFME	Continuous Flow Microextraction
CE	Capillary Electrophoresis
CE/UV	Capillary Electrophoresis/Ultraviolet Detector
CI	Color Index
DI-SDME	Direct Immersion Single Drop Microextraction
DDT	Dichloro-diphenyl-trichloroethane
EE	Extraction Efficiency
ECD	Electron Capture Detector
μECD	micro-Electron Capture Detector
FIE	Flow Injection Extraction
FPD	Flame Photometric Detector
FID	Flame Ionization Detector
GC	Gas Chromatography
GC/FID	Gas Chromatography/Flame Ionization Detector
GC/μFID	Gas Chromatography/micro Flame Ionization Detector
GC/IT/MS	Gas Chromatography/Ion Trap/Mass Spectrophotometry
GC/NPD	Gas Chromatography/Nitrogen Phosphorus Detector
GC/MS	Gas Chromatography/Mass Spectrophotometry
GC/PDHID	Gas Chromatography/Pulsed Discharge Helium Ionization Detector
GPE	Gum Phase Extraction
HPLC	High Performance Liquid Chromatography
HPLC/DAD	High Performance Liquid Chromatography/Diode Array Detector
HPLC/UV	High Performance Liquid Chromatography/Ultraviolet Detector
HPLC/PAD	High Performance Liquid Chromatography/Photodiode Array

List of Abbreviations (Continued)

HPLC/MS	High Performance Liquid Chromatography/ Mass Spectrophotometry
HPLC/MS/MS	High Performance Liquid Chromatography/Tandem Mass Spectrophotometry
HS-SDME	Head Space Single Drop Microextraction
IUPAC	The International Union of Pure and Applied Chemistry
I.D.	Inner Diameter
K_D	Distribution coefficient
LC/UV	Liquid Chromatography/Ultraviolet Detector
LPME	Liquid Phase Microextraction
MISPE	Molecular Imprinted Polymer Solid Phase Extraction
MMLLE	Microporous Membrane Liquid-Liquid Extraction
MS	Mass Spectrophotometry
MCC/UV/IMS	Multi Capillary Column/Ultraviolet/Ion mobility spectrometry
NAAPBA	*N*-acryloyl-*m*-aminophenylboronic acid
NIOSH	The National Institute of Occupational Safety and Health
OSHA	Occupational Safety and Health Administration
OTT	Open Tubular Trapping
O.D.	Outer Diameter
ppm	Parts per million
ppb	Parts per billion
ppt	Parts per trillion
PAHs	Polycyclic Aromatic Hydrocarbons
PDMS	Polydimethylsiloxane
PEEK	Polyetheretherketone
PELs	Permissible Exposure Limits
PTFE	Polytetrafluoroethylene
R^2	Coefficient of determination
RSD	Relative Standard Deviation

List of Abbreviations (Continued)

SBSE	Stir Bar Sorptive Extraction
SDME	Single Drop Microextraction
SLME	Supported Liquid Membrane Extraction
SPME	Solid Phase Microextraction
SFE/HPLC/UV	Supercritical Fluid/High Performance Liquid Chromatography/ Ultraviolet Detector
STELs	Short-Term Exposure Limits
THMs	Trihalomathanes
TEMED	*N,N,N',N'*-tetramethylethylenediamine
TWA	Time Weighted Average
US EPA	Unite State Environmental Protection Agency
VOCs	Volatile Organic Compounds

CHAPTER 1

Introduction

1.1 Background and rationale

One of the challenges in modern analytical chemistry is the development of new analytical techniques that have a capability to determine exotic organic contaminants at trace and ultra-trace levels (Wise *et al.*, 2006). These components are relevant in many areas such as the production of high purity materials, genetic engineering and biotechnology and environmental protection (Namieśnik, 2002). The contamination of these compounds even at trace level can severely affect the quality of the products such as those in the wafer fabrication industry resulting in the rejection of the products (Pepponi *et al.*, 2003). Trace level contaminants of organic compounds can also have some adverse effect on human and a number of these have been classified as mutagenic and carcinogenic compounds (Jones, 1999; US EPA, 2000b). Therefore, the development of analytical techniques for trace and ultra-trace analysis of organic contaminants is necessary to provide the useful analytical results that are not only beneficial to the assessment of environmental quality but also for the quality control of products or production processes.

Trace component is defined by the International Union of Pure and Applied Chemistry (IUPAC) as a compound that has an average concentration less than about 100 parts per million (ppm) or less than 100 $\mu g\ g^{-1}$ (IUPAC, 1997). Determination of contaminated organic compounds at trace level generally needs sample preparation step. This is to achieve two main objectives, one is to isolate the target analytes from sample matrix or *vice versus* to avoid the interferences from the matrix that can give the negative impact to the results. The other is to preconcentrate or enrich the concentration of the analytes to the level that can be quantitatively detected by the appropriate techniques such as gas chromatography, high performance liquid chromatography or spectrophotometry. The latter objective plays a

very important role in trace and ultra-trace analysis since without this step the analysis at this level could not be performed (Hajšlová and Zrostlíková, 2003). The requirement of sample preparation techniques for each sample depends on the matrix in the sample and the level of the analytes concentration in the sample (Ridgway *et al.*, 2007). Typically sample preparation techniques, starting from sampling until the final analysis, consist of several steps, and these might be the sources of errors that will certainly affect the reliability of the results as well as the cost of the analysis (Hajšlová and Zrostlíková, 2003; Ridgway *et al.*, 2007). Consequently, the development of simple yet cost effective sample preparation techniques is gaining a lot of attention (Hajšlová and Zrostlíková, 2003) because the time taken can be shorten as well as the potential source of the error can be avoided. Among these, on-line analysis that combined the step of isolation of the target analyte from sample matrix, the step of preconcentration and the analysis into one step is now widely investigated in the development of sample preparation. Not only it can overcome the above problems but it can also provide more reproducible results than the manual techniques (Ridgway *et al.*, 2007).

This thesis focuses on the development of simple, cost effective and on-line sample preparation and analytical techniques that can achieve the analysis at the trace and ultra-trace concentration levels of organic compounds contaminating in real samples.

1.2 Objective of the research

The aim of this study is the development of the sample preparation and analytical techniques for trace and ultra-trace analysis of organic compounds and their implementation in real samples. To reach this objective three subprojects emphasizing on sample enrichment techniques were carried out as follows;

1.2.1 Cost effective passive sampling for monitoring of volatile organic compounds

Simple and cost effective passive sampling device was developed to be used as a sample preparation technique that included the step of sampling and isolation of analyte from gaseous matrix (indoor and outdoor air). An effective laboratory-built thermal desorption device was then used to desorb the analytes after sampling. After desorption, the analytes were injected to a gas chromatograph connected with flame ionization detector by injection valve that connected to the passive samplers. This developed off-line passive sampling system will be described further in Chapter 5.

1.2.2 On-line system for trace organic compound analysis

A simple sample preparation technique based on the adsorption on a suitable adsorbent was developed. An automatic and easy operated laboratory-built heating device was used to desorb the analytes after allowing them to be adsorbed for a period of time. By the combination of the adsorption, desorption device and an appropriate detector, a simple on-line analytical technique, which combined sample preparation and detection in one step, for real-time monitoring of trace organic compounds was obtained and the detail will be presented in Chapter 6.

1.2.3 Simple system for monitoring of glucose and other carbohydrates

To obtain a specific sample preparation technique as well as a specific analytical technique, phenylboronate containing polymer was employed as an affinity binding ligand since it can bind to *cis*-diol functional group compounds and glucose is one of the examples. After binding, the optical density of polymer changes and this effect can be developed into an optical sensor for monitoring of glucose using a spectrophotometer. The detection principle and the operation will be discussed further in Chapter 7.

1.3 Benefits

It is expected that the developed sample preparation and analytical techniques will become alternative approaches that can be implemented to detect trace and ultra-trace amount of organic compounds. Since they are not only simple and cost effective techniques but also provide high precision, repeatability, sensitivity and reusability.

CHAPTER 2

Enrichment Techniques for Trace Organic Compound Analysis

2.1 Introduction

Organic contaminants are often present in the sample at very low concentration, trace or ultra-trace level (Ridgway *et al.*, 2007; Sun *et al.*, 2002; Wan *et al.*, 1998). In many cases direct analysis of trace organic compounds in samples is not possible since most instruments can not directly handle the sample matrix (Jinno, 2002) making sample preparation necessary for the isolation of the desired components. Sample preparation is also known as preconcentration or enrichment, the analytes are concentrated into a suitable level that can be measured by the chosen method of analysis (Jinno, 2002). Several techniques, classical conventional and newly developed methods, have been used to enhance the response of the instrument for trace and ultra-trace organic compounds determination. Some of the commonly used techniques are summarized in this chapter.

2.2 Conventional liquid-liquid extraction

Liquid-liquid extraction is a classical conventional enrichment technique that is widely used as prescribed by many standard analytical methods (Psillakis and Kalogerakis, 2003a; Zhao and Lee, 2002). The basis of this approach is to take a large volume of the sample and the compounds of interest will then effectively partition into a small volume of an immiscible organic solvent causing the enrichment of the analyte. In addition, liquid-liquid extraction can be used to clean up the analyte from its matrix. It is therefore a very useful technique in trace analysis.

The extraction is based on the establishment of an equilibrium in which the solutes or analytes are partition between two immiscible liquids. The analytes are partitioning from one liquid phase, usually water, to another immiscible liquid phase, usually organic solvent, in contact with it (Meloan, 1999). The ratio of

amount extracted in organic phase to the amount remaining in aqueous phase is known as the distribution coefficient, K_D as shown in equation (2.1).

$$K_D = \frac{C_{o,eq}}{C_{aq,eq}} \tag{2.1}$$

Where $C_{o,eq}$ and $C_{aq,eq}$ are the concentrations of the analyte in organic and aqueous phases at equilibrium, respectively. The concentration in the organic phase at equilibrium is given in equation (2.2).

$$C_{o,eq} = K_D C_{aq,eq} = \frac{K_D C_{aq,initail}}{1 + K_D V_o / V_{aq}} \tag{2.2}$$

Where $C_{aq,initial}$ is the initial concentration of the analyte in aqueous phase; V_o and V_{aq} are the volumes of organic and aqueous phases, respectively. The equilibrium can be reached quickly by shaking or stirring the aqueous phase containing the analytes, with the organic phase. The extraction efficiency (EE) for one step extraction can be written as in equation (2.3) (Mol *et al.*, 1995).

$$EE = 1 - \frac{1}{1 + K_D\left(\frac{V_o}{V_{aq}}\right)} \tag{2.3}$$

The extraction efficiency, can be enhanced by several methods such as increasing of organic solvent volume to improve phase ratio (V_o / V_{aq}), changing the type of organic solvent to improve the distribution coefficient (K_D) or repeating the extraction.

The most common approach for conventional liquid-liquid extraction is the use of a separatory funnel. Aqueous sample is introduced into the separatory funnel with a Teflon stopcock and then an appropriate volume of a suitable organic

solvent is added. The funnel is then sealed with a stopper, and shaken vigorously, either manually or mechanically for a period of time. This shaking process allows thorough interspersion between the two immiscible solvents, therefore maximizing the contact between the two solvent phases, assisting mass transfer, and allowing efficient partitioning to occur. This technique can offer high reproducibility and high sample capacity and, therefore, has been widely used as a sample enrichment method. However, some disadvantages are also expressed, *e.g.,* time consuming, tedious, multistage operation and poor potential for automation because of the formation of an emulsion (He and Lee, 1997; Psillakis and Kalogerakis, 2002; Psillakis and Kalogerakis, 2003a; Qian and He, 2006; Zhao and Lee, 2002). Moreover, it requires the use of large amounts of potentially toxic organic solvents that can be harmful to the laboratory personel and results in the production of hazardous laboratory waste, the analysis cost will also increase by the cost for waste treatment (Psillakis and Kalogerakis, 2003a).

2.3 Flow injection extraction (FIE)

Flow injection extraction (FIE) was independently developed to overcome the problem of large solvent consumption and poor automation in 1978 by Karlberg and coworkers (Psillakis and Kalogerakis, 2002). In conventional FIE procedures, an aqueous sample is injected into an aqueous carrier stream where organic segmented are continuously introduced into this stream. After the segmented stream passes through a coil, where the extraction occurs, organic phase is separated from the aqueous phase and leads through a flow cell for measurement. Comparing with traditional liquid-liquid extraction FIE offers the advantages of low consumption of reagent, sample, and organic solvent, high speed, and low cost. However, the consumption of organic solvent was still at the level of several hundred microliters per analysis (He and Lee, 1997; Liu and Dasgupta, 1996; Psillakis and Kalogerakis, 2002).

2.4 Liquid-liquid microextraction

The drawbacks described previously have driven the development of a new liquid-liquid extraction technique. Solvent microextraction or liquid phase microextraction (LPME) was introduced as extraction techniques that use small volume of organic solvent (Liu and Lee, 2000).

2.4.1 Theory of liquid phase microextraction (LPME)

The extraction process relies on the analyte concentration gradient between aqueous and organic phases. Mass transfer from aqueous phase to organic phase is continuing until the equilibrium is reached or the extraction is terminated so, the initial amount of the analyte in the sample is equal to the analyte left in the aqueous phase plus the amount of the analyte in the organic phase (Jeannot and Cantwell, 1996; Psillakis and Kalogerakis, 2002) as shown in equation (2.4).

$$C_{aq,initial}V_{aq} = C_oV_o + C_{aq}V_{aq} \tag{2.4}$$

Where C_o and C_{aq} are the concentrations of the analyte in organic and aqueous phases at time *t*, respectively and V_o and V_{aq} are the volumes of the organic and aqueous phases, respectively. The general rate equation of the analyte in the solvent is given in equation (2.5), where A_i is the interfacial area and $\overline{\beta}_o$ is the overall mass transfer coefficient of the analyte with respect to the organic phase (cm s^{-1}).

$$\frac{dC_o}{dt} = \frac{A_i}{V_o}\overline{\beta}_o(K_DC_{aq} - C_o) \tag{2.5}$$

In the case where the transfer across the liquid-liquid interface is rapid, the overall mass transfer can be written as in equation (2.6).

$$\frac{1}{\overline{\beta}_o} = \frac{1}{\beta_o} + \frac{K_D}{\beta_{aq}} \tag{2.6}$$

Where β_o and β_{aq} are the mass transfer coefficients of organic and aqueous phases, respectively. Substitution of equation (2.4) into equation (2.5), equation (2.7) is obtained by assuming that the volume of solvent remains constant during the extraction occur, resulting in a constant A_i therefore, the concentration of the analyte in organic phase is a function of time (equation (2.7)).

$$C_o = C_{o,eq}(1 - e^{-kt}) \tag{2.7}$$

where k is the rate constant (s^{-1}), and can be written as

$$k = A_i \overline{\beta}_o \left(\frac{K_D}{V_{aq}} + \frac{1}{V_o} \right) \tag{2.8}$$

The important characteristic of microextraction is the absolute amount of the analyte extracted from the sample to the small amount of solvent is negligible if compared to its total amount in a bulk sample solution (Liu and Lee, 2000). Therefore, high concentration of analyte at equilibrium and fast extraction are expected. From equations (2.6) and (2.8), to obtain rapid analysis, the parameters needed to be maximized are A_i and $\overline{\beta}_o$ while V_{aq} and V_o should be minimized. The increasing of $\overline{\beta}_o$ can be obtained by increasing the value of β_o and β_{aq} since β_o is much larger than β_{aq} so, the mass transfer in the aqueous side control the overall mass transfer rate and this can be increased by increasing the stirring rate in sample vial (Psillakis and Kalogerakis, 2002). From kinetic consideration, increasing a convection of the analyte from aqueous phase through the organic phase by stirring and decreasing the volume of sample and organic phase can also shorten the analysis time. To obtain high extraction yield, the same parameters as in conventional liquid-liquid extraction should be investigated. Techniques for solvent microextraction or liquid phase

microextraction (LPME) which have been recently developed are single drop microextraction and liquid phase membrane extraction (Zhao and Lee, 2002).

2.4.2 Single drop microextraction (SDME)

SDME is one of the novel microextraction techniques that combines the classic extraction by liquid and microextraction in a stationary phase. The extraction process occurs at a drop of water immiscible solvent hanging on the tip of Teflon rod or a conven-tional microsyringe and immersed in a water containing sample or exposed to its headspace. The organic contaminants are transferred from the aqueous to the organic phase and after sampling for a set period of time, a concentrated analyte in a microdrop is retracted into the syringe and transferred to the analytical system for further analysis (Wardencki *et al.*, in press).

2.4.2.1 Direct immersion single drop microextraction (DI-SDME)

DI-SDME is first reported in 1996 by Liu and Dasgupta, 1.3 μL of chloroform was suspended into a larger volume of flowing aqueous drop to accomplish the extraction process (Fig. 2.1). After extracting for a period of time, the aqueous layer was replaced by a clear wash solution leaving an organic drop colored by the analyte and the absorbance signal was monitored by light emitting diode based absorbance detector, then the next cycle of the extraction could be performed after the organic phase was pumped away (Liu and Dasgupta, 1996). At almost the same time, Jeannot and Cantwell introduced a new solvent microextraction technique in which a drop (8 μL) of 1-octanol hang at the tip of a Teflon rod and suspended in a stirred aqueous sample solution (Fig. 2.2) (Jeannot and Cantwell, 1996). After allowing the extraction process to occur for a period of time, the Teflon rod was taken out from the aqueous solution, then a microsyringe was used to withdraw the organic phase from the end of the Teflon rod and injected into a gas chromatograph for quantitative analysis. Since the extraction and injection have to be performed separately and using different devices, it becomes the disadvantage of this technique.

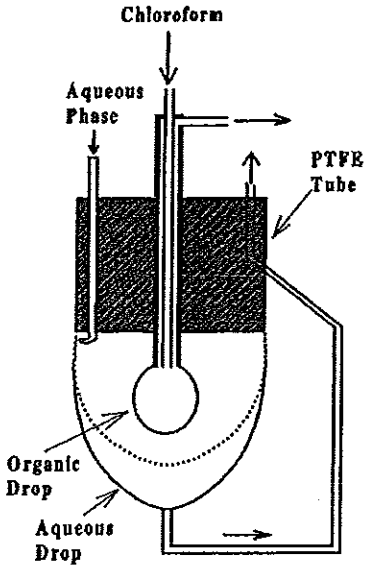


Fig. 2.1 Schematic diagram of the drop head developed by Liu and Dasgupta, 1996.

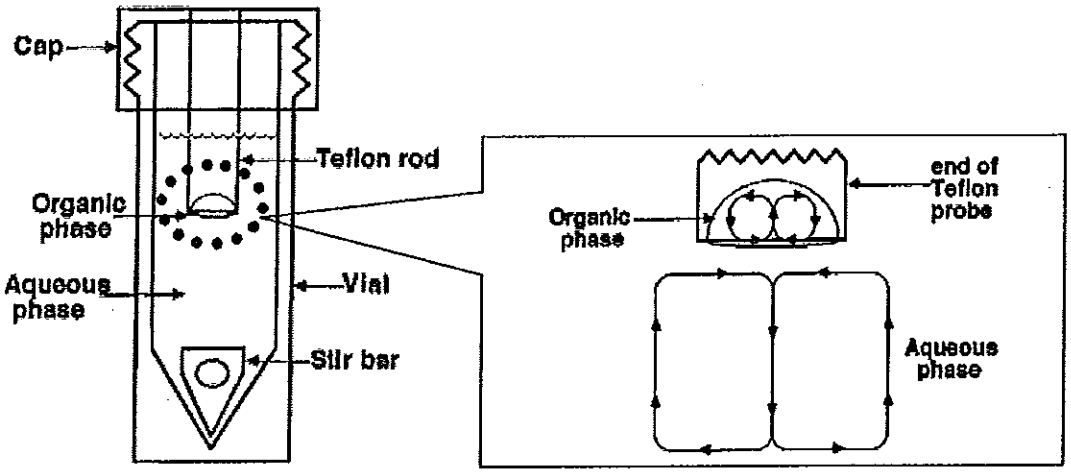


Fig. 2.2 Side view illustration of the solvent microextraction system, magnetic stirrer not shown, where the extraction process occurs was magnified (Jeannot and Cantwell, 1996).

In 1997, Jeannot and Cantwell introduced a conventional microsyringe as the organic solvent holder instead of a Teflon rod (Jeannot and Cantwell, 1997b). A 1 μL of organic solvent was first withdrawn into a microsyringe, then the needle of

the microsyringe was passed through the sample vial septum and immersed in the liquid sample. A droplet of organic solvent was suspended at the tip of the syringe needle under a stirred aqueous sample, as shown in Fig. 2.3. After extraction, the organic phase was withdrawn back into the microsyringe and directly injected into a GC system. In this case, the microsyringe was used as both solvent holder and sample injector. Therefore, the extraction and sample injection were carried out using only one device.

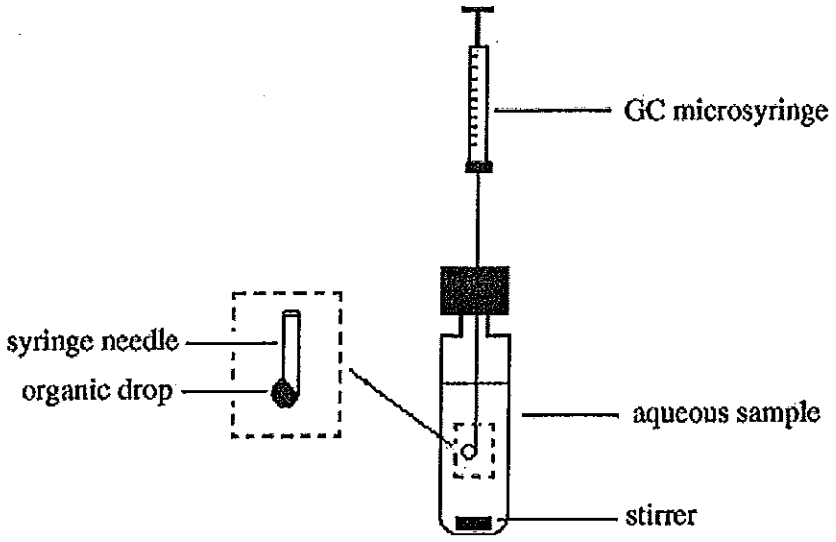


Fig. 2.3 Direct immersion single drop microextraction (DI-SDME) (Xu *et al.*, 2007).

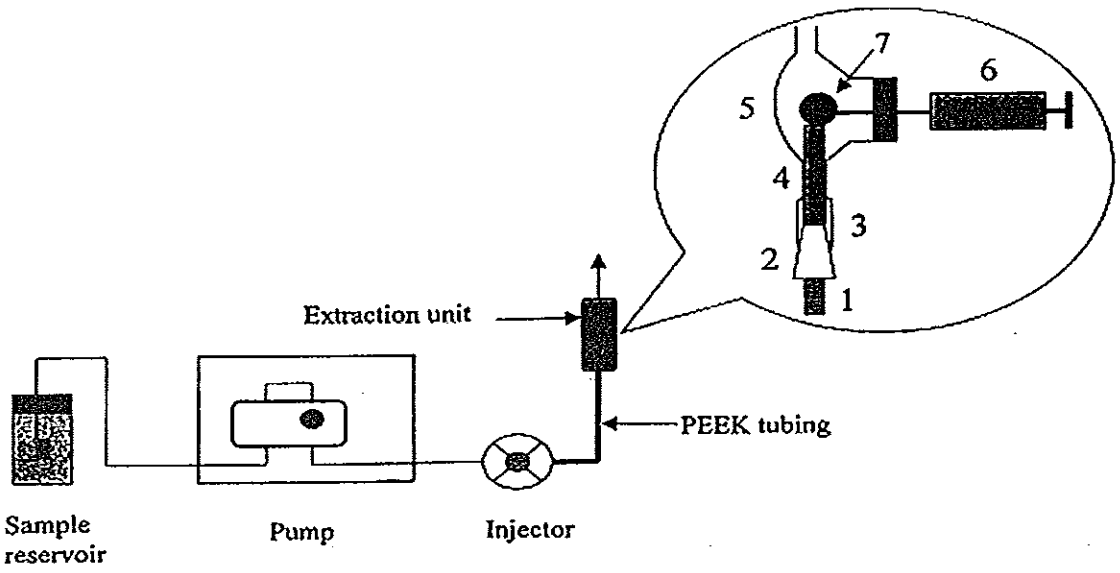


Fig. 2.4 Assembly of continuous-flow microextraction system, (1) connecting Polyetheretherketone (PEEK) tubing, inserted into the extraction chamber; (2) modified pipet tip; (3) o-ring; (4) inlet of extraction chamber; (5) extraction chamber; (6) microsyringe; (7) solvent drop (Liu and Lee, 2000).

In 2000 Liu and Lee introduced the continuous flow microextraction (CFME) (Fig. 2.4) to enhance the extraction efficiency of DI-SDME technique. The sample was continuously pumped through the extraction chamber *via* PEEK connection tube, after the extraction chamber was partially filled with the aqueous sample, the organic solvent was injected (1-5 μL) at the injector. The solvent plug moved together with the sample solution to the outlet of PEEK tubing and the extraction plug formed a drop after reaching the outlet of the tubing. Then it was ejected through the end of the tube and transferred to the injection port of gas chromatograph by microsyringe. Since the drop of solvent was fully and continuously contacted with a fresh and flowing sample solution, an enrichment factor up to 1000 times can be achieved for extraction of nitroaromatic compounds (Liu and Lee, 2000). However, the DI-SDME has some limitations, such as high stirring speeds could not be used even it can increase the extraction efficiency because the organic drop will break off from the syringe tip (Xu *et al.*, 2007). The matrix interference is also a

major problem for DI-SDME since the drop contacts directly to the aqueous sample so, other non-volatile compounds can also diffuse into the organic drop (Xu *et al.*, 2007).

2.4.2.2 Head space single drop microextraction (HS-SDME)

HS-SDME was introduced in 2001 by Theis and coworkers to avoid the problems obtained from using DI-SDME. Instead of immersion the suspended organic drop into the aqueous sample, it was held at the headspace of the sample as shown in Fig. 2.5 (Theis *et al.*, 2001).

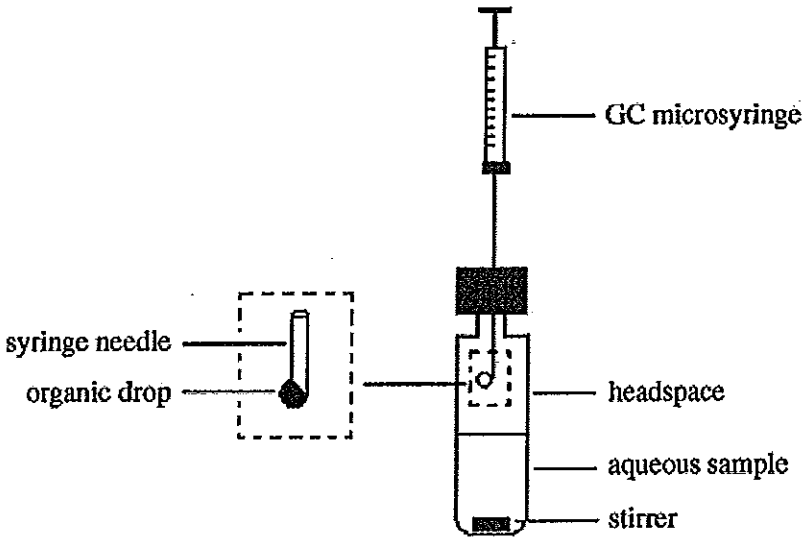


Fig. 2.5 Headspace single drop microextraction (HS-SDME) (Xu *et al.*, 2007).

Since the organic drop used for extraction is in the headspace of the sample, the distribution of the analytes involved three phases, water sample, headspace and organic drop. Therefore, the extraction theory is slightly different from the one previously described in section 2.4.1. The equilibrium distribution coefficients can be written as shown in equations (2.9), (2.10) and (2.11).

$$K_{h,aq} = C_h / C_{aq} \tag{2.9}$$

$$K_{o,h} = C_o / C_h \tag{2.10}$$

$$K_{o,aq} = C_o / C_{aq} = K_{h,aq} K_{o,h} \tag{2.11}$$

Where $K_{h,aq}$ is the headspace-aqueous distribution constant, $K_{o,h}$ is the organic drop-headspace distribution constant, and $K_{o,aq}$ is the (overall) organic drop-aqueous distribution constant. A dynamic equilibrium was finally established between the concentration of the analytes in headspace and those of analytes in the organic solvent drop. The amount of the analyte (n) extracted by the microdrop at equilibrium is described in equations (2.12) and (2.13).

$$n = \frac{K_{o,h} V_o C_{initial} V_{aq}}{K_{o,h} V_o + K_{h,aq} V_h + V_{aq}} \tag{2.12}$$

$$\frac{1}{n} = \frac{1}{C_{initial}} \left[\frac{1}{V_{aq}} + \frac{1}{K_{o,aq}} \left(\frac{K_{h,aq} V_h}{V_{aq}} + 1 \right) \right] \tag{2.13}$$

It was reported that mass transfer in the headspace is assumed to be a fast process, because diffusion coefficients in the gas phase are typically about 10^4 times greater than the diffusion coefficients in condensed phases. Therefore, the rate of extraction is limited by slow mass transfer in aqueous phase or slow mass transfer in the organic phase or both (Theis *et al.*, 2001).

2.4.2.3 Mass transfer characteristics of SDME

There are two theoretical models involved in the mechanistic interpretation of mass transfer coefficient in aqueous (β_{aq}) which are known as the controlling part for the overall mass transfer, film theory and penetration theory.

Film theory was first proposed by Nernst in 1904 and further developed by Lewis and Whitman in 1924. They assumed that immediately after the solution contacted to the interface, no movement occurred. Then the vigorousness of convection of solution located far away from the interface was gradually increased. This condition is approximated in film theory by assuming that uniform, instantaneous, and complete convection mixing exists in the bulk solution to some distance (δ, cm) away from the liquid-liquid interface. The thickness of liquid layer called Nernst diffusion film is postulated to be completely stagnant and nonconvecting, therefore, the sample molecules cross through this film by pure diffusion only. At the steady state, the aqueous mass transfer coefficient is given as shown in equation (2.14).

$$\beta_{aq} = D_{aq} / \delta_{aq} \tag{2.14}$$

where D_{aq} is the diffusion coefficient in aqueous phase. At high speed of stirring rate, β_{aq} increases because of the decreasing of δ_{aq} and it is proportional to D_{aq} (Jeannot and Cantwell, 1997a).

Another theory is penetration theory. It was first reported by Higbie and later developed by Danckwerts (Jeannot and Cantwell, 1997a). This theory assumes that the fluid convection is right up to the interface. At the interface, a small fluid volume element of one phase is momentarily in contact with the other phase for some exposure time, t_e, after which the volume element is mixed back into the bulk fluid. Mass transfer of solute occurs *via* unsteady state semi-infinite linear diffusion for the time increment, t_e. The resulting expression for the aqueous phase mass transfer coefficient is shown equation (2.15).

$$\beta_{aq} = 2\sqrt{D_{aq} / \pi t_e} \tag{2.15}$$

Where t_e is constant at a constant stirring rate. The faster stirring rate, the smaller t_e value. In contrast to the linear dependence predicted from film theory, a square-root dependence of β_{aq} on D_{aq} is predicted for penetration theory.

The values of β_{aq} and D_{aq} can be independently measured by different types of experiments and their functional relationship can be experimentally obtained. The appropriate theoretical model (*i.e.*, film theory or penetration theory) for β_{aq} in the single drop microextraction can be determined.

The plot between concentration of the analyte in the organic phase at time t (C_o) (M) *versus* time (s) is perfectly fitted with equation (2.7), the concentration of the analyte in organic phase at equilibrium ($C_{o,eq}$) and rate constant (k) can be obtained from the fitted curve. These values can then be used to calculate the distribution coefficient (K_D) from equation (2.16) which is adapted from equations (2.1) and (2.4).

$$K_D = \frac{C_{o,aq}V_{aq}}{C_{aq,initial}V_{aq} - C_oV_o} \tag{2.16}$$

The overall mass transfer rate ($\overline{\beta}_o$) can be later obtained using equation (2.8) since A_i can be estimated with a reasonable accuracy. In the case of a large distribution coefficient (K_D), equation (2.6) can be written as shown in equation (2.17).

$$\beta_{aq} = K_D\overline{\beta}_o \tag{2.17}$$

In order to know which theoretical model is suited with the designated single drop microextraction system, the curve between the mass transfer coefficient for aqueous (β_{aq}) and the diffusion coefficient of each analyte (D_{aq}) is plotted as shown in Fig. 2.6. It clearly shows that the single drop microextraction used by Jeannot and Cantwell (1997) is an excellent fit to the linear dependent model (film

theory) with R^2=0.997 while nonlinear fit (penetration theory) is very poor (R^2=0.750). The investigation is under the normal system, 1 µL of *n*-octanol to extract 4 compounds, progesterone, malathion, 4-methyl acetophenone and 4-nitrotoluene. Therefore, if the mass transfer model is known, it will help the operator to get an idea of how to improve the extraction efficiency of this technique.

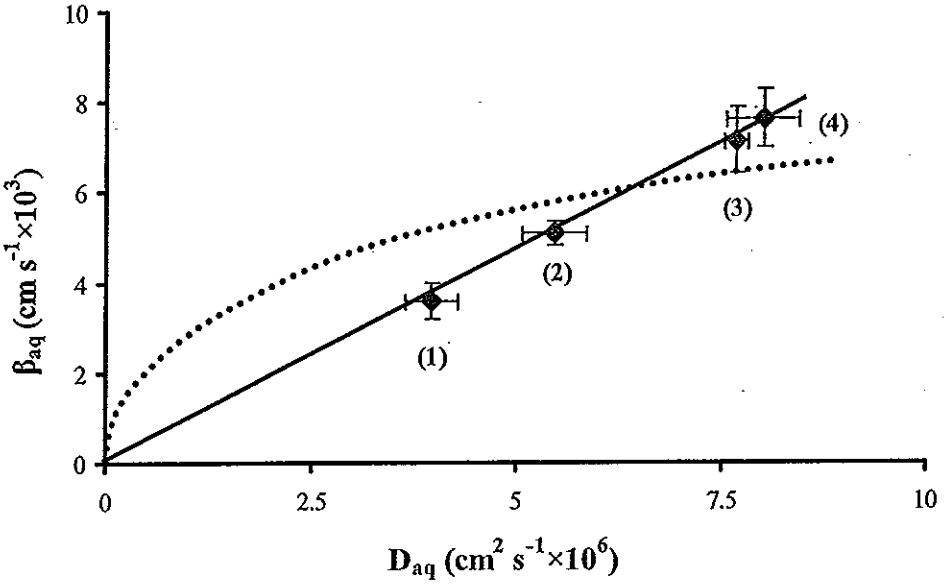


Fig. 2.6 Plot of aqueous mass transfer coefficient β_{aq} *versus* aqueous diffusion coefficient D_{aq} for four compounds: (1) progesterone; (2) malathion; (3) 4-methyl acetophe- none; and (4) 4-nitrotoluene. Points indicate experimental data. Solid line is a fit to the film model, equation (9). Dashed line is a fit to the penetration model, equation (10). The errors from the estimation are indicated with the error bars (Jeannot and Cantwell, 1997).

2.4.2.4 Parameters affecting SDME

Selection of extracting solvent is one of the most important parameters that should be considered to achieve a good selectivity of the target analyte. As in liquid-liquid extraction, the selection of extraction solvent is based on the principle of "like dissolves like". The choice of extracting solvent is based on the following

consideration. First, it should have low solubility in aqueous solution in order to limit its dissolution. Second, it should provide high solubility for target analyte. However, other parameters must also be considered for the selection of the extracting solvent, *i.e.*, selectivity, extraction efficiency, incidence of drop loss, rate of drop dissolution and level of toxicity (Psillakis and Kalogerakis, 2002; Qian and He, 2006; Ye *et al.*, 2007; Zhao *et al.*, 2004).

Stirring rate of magnetic stirrer can affect the thickness of the Nernst diffusion film (δ) as shown in equation (2.14), the film thickness decreases with increasing stirring rate. A thin Nernst diffusion film can speed up the diffusion of the analyte into the organic drop and shortened the time to achieve the equilibrium concentration. However, when the drop is directly immersed into the aqueous solution, there is a limit of maximum stirring rate that causes dislodgement or dissolution especially for prolonged extraction time (Psillakis and Kalogerakis, 2002; Qian and He, 2006; Ye *et al.*, 2007; Zhao *et al.*, 2004).

Extraction time is also another important parameter for this technique. It is not only to achieve good extraction efficiency but also to obtain a reasonable sample throughput. Typically, the amount of the extracted analyte increases with the extraction time, however, too long extraction time, a small throughput will be obtained. Therefore, the consideration of the optimum extraction time needs to compromise between analytical sensitivity and chromatographic running time (Psillakis and Kalogerakis, 2002; Qian and He, 2006; Ye *et al.*, 2007; Zhao *et al.*, 2004).

The volume of organic drop can certainly affect the enrichment factor since the extraction efficiency can be enhanced by increasing of drop volume however, large organic drop is difficult to manipulate and less reliable. Besides, a large injection volume leads to peak broadening in capillary GC. Normally, 1 μL of the organic drop is frequently used for good stability and reproducibility. It also allows fast stirring rate, although it suffers a lost of some sensitivity when compared with a large volume. (Psillakis and Kalogerakis, 2002; Qian and He, 2006; Ye *et al.*, 2007; Zhao *et al.*, 2004).

The addition of salt into the sample has been used universally in liquid-liquid extraction since it can increase ionic strength of the solution resulting in the

decreasing of the solubility of target analytes in aqueous sample and enhancing of their partition into an organic drop (salting-out effect). The salting-out effect might play the important role in low salt concentration, while Nernst diffusion film might be changed and caused the restriction in a high salt concentration (Psillakis and Kalogerakis, 2002; Qian and He, 2006; Ye *et al.*, 2007; Zhao *et al.*, 2004).

The other parameter is the headspace volume where HS-SDME was performed. As indicated in equation (2.13), the volume of headspace can affect the extracted amount. To obtain the highest sensitivity of HS-SDME, the volume of gas phase should be minimized (Zhao *et al.*, 2004).

The drop based microextractions provide high extraction speed which can be indicated by high sample throughput, furthermore this technique is extremely simple and only one microsyringe is used to perform the extraction. Sensitivity of this technique can be enriched by affecting parameters that have been previously mentioned. The applications of SDME, either DI-SDME or HS-SDME, are summarized in Table 2.1.

Table 2.1

Applications of SDME (modified from Xu *et al.*, 2007).

Compound	Real sample	Extraction solvent	Extraction mode	GC detector	Reference
Organophosphorus pesticides	Farm water	Carbon tetrachloride	DI-SDME	FPD	(Ahmadi *et al.*, 2006)
	Surface water	Toluene	DI-SDME	MS	(Lambropoulou *et al.*, 2004)
	Lake water and fruit juice	Toluene	DI-SDME	FPD	(Xiao *et al.*, 2006)
	Tap water and reservoir water	*n*-Hexane	DI-SDME	ECD	(Zhao and Lee, 2001)
Organochlorine pesticides	Teas	*n*-Hexane:ethyl acetate (2:1, v/v)	DI-SDME	ECD	(Qian and He, 2006)
	Natural, tap and river water	Toluene	DI-SDME	μECD	(Zhao *et al.*, 2006)
Chloroacetanilide herbicides	Traditional Chinese Medicines	*n*-Dodecane	HS-SDME	MS	(Cao *et al.*, 2006)
74 volatile compounds	Pharmaceutical products	*n*-Octanol	HS-SDME	FID	(Wang *et al.*, 2006)
Volatile solvents	Traditional Chinese Medicines	Benzyl alcohol	HS-SDME	MS	(Fang *et al.*, 2006)
Volatile compounds BTEX	Water and Engine oils	*n*-Hexadecane	HS-SDME	FID	(Kokosa and Przyjazny, 2003; Przyjazny and Kokosa, 2002)
	Solid, polystyrene	Buthyl acetate	HS-SDME	MS	(Hansson and Hakkarainen, 2006)
Styrene	Water	1-Butanol	HS-SDME	FID	(Shariati-Feizabadi *et al.*, 2003)
PAHs	Drinking water	*n*-Octanol	HS-SDME	ECD	(Zhao *et al.*, 2004)
Trihalomethanes	Tap , sea and waste waters	Dodecane	HS-SDME	ECD	(Khajeh *et al.*, 2006)
Chlorobenzene	Tap and well water	Toluene	HS-SDME	MS	(Vidal *et al.*, 2005)
	Human blood	Decane	HS-SDME	MS	(Li *et al.*, 2005)
Aldehydes	Beer	*n*-Octanol	HS-SDME	MS	(Saraji, 2005)
Alcohols	River water	Hexyl acetate	DI-SDME	MS	(Saraji and Bakhshi, 2005)

Compound	Real sample	Extraction solvent	Extraction mode	GC detector	Reference
Phenols		Butyl acetate	DI-SDME	MS	(Bagheri *et al.*, 2004)
	River water	Toluene	DI-SDME	MS	(Kawaguchi *et al.*, 2006)
Bisphenol A					
	Color sample	Benzyl alcohol	HS-SDME	FID	(Yamini *et al.*, 2004)
2-Butoxyethanol	Traditional Chinese Medicines	*n*-Octanol	HS-SDME	MS	(Deng *et al.*, 2005a)
Panaxynol	Traditional Chinese Medicines	*n*-Octanol	HS-SDME	MS	(Deng *et al.*, 2006)
Paeonol	Blood	Decane	HS-SDME	MS	(Dong *et al.*, 2006)
Acetone	Human blood	*n*-Octanol	HS-SDME	MS	(Li *et al.*, 2005)
Carbonyl compounds	Tap and surface water	*iso*-Octane	DI-SDME	ECD	(López-Blanco *et al.*, 2003)
Endosulfan	Ground water	Toluene	DI-SDME	MS	(Psillakis and Kalogerakis, 2001)
Nitroaromatic explosives	Water	Dichloromethane:tetrachloro-methane (3:1, v/v)	DI-SDME	ECD	(Palit *et al.*, 2005)
Chemical warfare agents					
	Traditional Chinese Medicine	Cyclohexane	HS-SDME	MS	(Deng *et al.*, 2005b)
Essential oil	Water and soil	Decane	HS-SDME	MS	(Colombini *et al.*, 2004)
Organotins	Pharmaceuticals, iodized salt, milk powder and vegetables	*iso*-Octanol	DI-SDME	MS	(Das *et al.*, 2004)
Iodine					

2.4.3 Liquid phase membrane extraction

Membrane based liquid-liquid extraction is a powerful technique that offers a great number of advantages compared to other extraction methods (Jönsson and Mathiasson, 1999). The types of membrane mostly used is hollow fiber membrane. Comparing to other type of membrane, it has high surface area and is easy to handle. Before used hollow fiber membrane needs to be immersed in an organic solvent for a couple of seconds for impregnation of its pores (Psillakis and Kalogerakis, 2003a; Zhao and Lee, 2002). Then the extraction can either be done with a two-phase or a three-phase systems (Jönsson and Mathiasson, 1999; Jönsson and Mathiasson, 2000; Psillakis and Kalogerakis, 2003a).

2.4.3.1 Two-phase sampling mode

Two-phase sampling mode or microporous membrane liquid-liquid extraction (MMLLE) involved an aqueous-organic extraction, is suitable for non-polar organic compounds. In this mode, the organic solvent (acceptor phase) immobilized in the pores of the membrane by capillary forces is sandwiched by two aqueous phases (donor phase). The inside of the hollow fiber (lumen) was filled with the same immobilized organic solvent to act as an acceptor phase. The analytes will be extracted from aqueous sample outside the hollow fiber through the organic solvent in the pores of the membrane into the same organic solvent (Fig. 2.7 (a)). The acceptor phase (organic phase) can be stagnant or flowing. If the acceptor is stagnant, the only driving force for the mass transfer is the attainment of distribution equilibrium between the aqueous and organic phases, and the efficiency will then be higher if the partition coefficient is large (*i.e.,* the hydrophobic property of the analyte is large). Mass transfer can be improved if the acceptor phase is continuously moving causing the extracted analytes to move with the acceptor (Jönsson and Mathiasson, 1999). The extraction process in a two-phase liquid microextraction (membrane based) can be written as follows;

$$C_d \Leftrightarrow C_a$$

Where C_d and C_a are the concentrations of the analyte in donor and organic phases (receptor), respectively. The initial amount of analytes ($n_{initial}$) is equal to the summation of the individual amount of analyte presented in two phases during the whole extraction process;

$$n_{initial} = n_d + n_a \tag{2.18}$$

Where n_d and n_a are the amounts of the analyte in donor and acceptor phases, respectively. At equilibrium equation (2.18) can be written as:

$$C_{initial}V_d = C_{d,eq}V_d + C_{a,eq}V_a \tag{2.19}$$

Where $C_{initial}$ is the initial concentration of the analyte in the sample, $C_{d,eq}$ and $C_{a,eq}$ are the concentrations of the analyte in donor and organic or acceptor phases at equilibrium respectively, V_d and V_a are the sample (donor phase) and acceptor volumes, respectively. At equilibrium, the amount of analyte extracted into the acceptor phase ($n_{a,eq}$) can be expressed as;

$$n_{a,eq} = \frac{K_{a,d}V_a C_{initial}V_d}{K_{a,d}V_a + V_d} \tag{2.20}$$

Where $K_{a,d}$ is the distribution coefficient, therefore, the recovery of the analyte (R) can be calculated by equation (2.21) and the enrichment (E) can be obtained from equation (2.22)

$$R = \frac{100n_{a,eq}}{C_{initial}V_d} = \frac{K_{a,d}V_a}{K_{a,d}V_d + V_d} \times 100 \tag{2.21}$$

$$E = \frac{C_a}{C_d} = \frac{V_d R}{100 V_a} \tag{2.22}$$

The fraction of the acceptor phase impregnated in its pore is not available for further analysis and only those in lumen of the fiber can be collected, therefore, the recovery of two-phase sampling mode calculated from equation (2.17) will be higher than the actual recovery (Ho *et al.*, 2002). This mode of sampling is suitable for extraction of hydrophobic compounds, since the analyte will finally be extracted into the organic solvent, therefore, it is perfectly suited for further analysis with both gas and liquid chromatography (Barri *et al.*, 2004; Sarafraz-Yazdi and Es'haghi, 2006; Zhao and Lee, 2002).

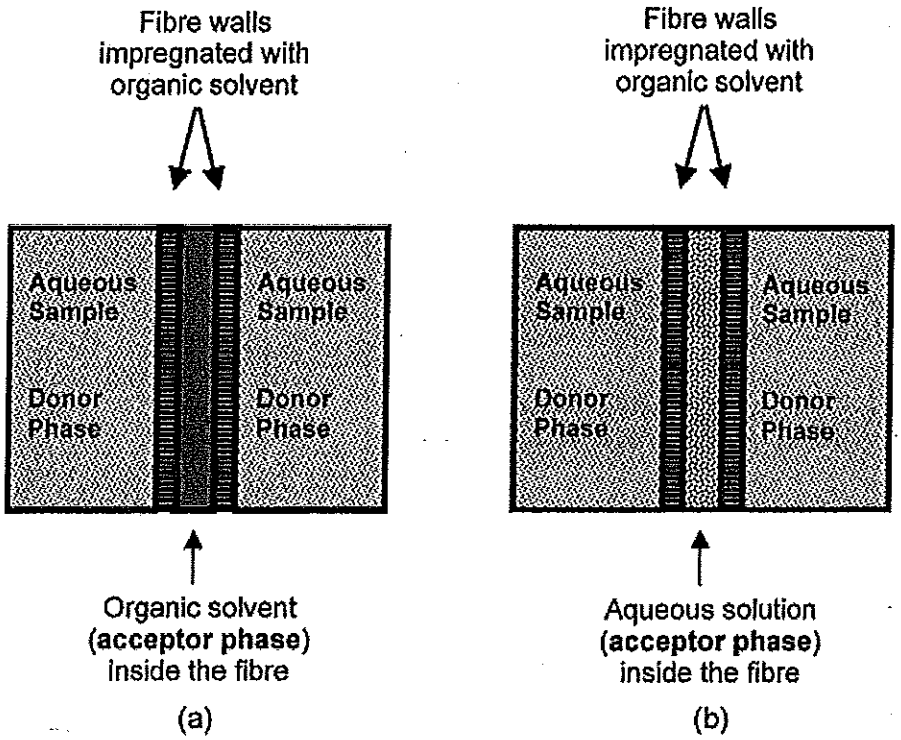


Fig. 2.7 Cross section of the hollow fiber inside the aqueous sample, (a) two phase sampling mode; (b) three phase sampling mode (adapted from Psillakis and Kalogerakis, 2003).

2.4.3.2 Three-phase sampling mode

Three-phase sampling mode or supported liquid membrane extraction (SLM), organic solvent in the pores of the fiber act as only a barrier between donor and acceptor to prevent the mixing of these two phases (Jönsson and Mathiasson, 1999; Psillakis and Kalogerakis, 2003a). In this case, both donor and acceptor phases are aqueous solution. The analytes are extracted from one aqueous solution (donor phase) through the organic solvent immobilized in the pores of the hollow fiber membrane (organic phase) into another aqueous phase (acceptor phase) filled inside the lumen of the hollow fiber as shown in Fig. 2.7 (b). Three phase extraction process is given as follows (Psillakis and Kalogerakis, 2003a);

$$C_d \Leftrightarrow C_o \Leftrightarrow C_a$$

Where C_d, C_o and C_a are the concentrations of the analyte in the donor phase, organic phase and acceptor phase, respectively. In a three-phase system, the initial amount of analyte (n_i) is equal to the summation of the amounts of analyte in donor (n_d), organic (n_o) and acceptor phases (n_a), as shown in equation (2.23) (Ho *et al.*, 2002)

$$n_i = n_d + n_o + n_a \tag{2.23}$$

Furthermore, equation (2.23) at equilibrium can be re-written as in equation (2.4).

$$C_{initial}V_d = C_{d,eq}V_d + C_{o,eq}V_o + C_{a,eq}V_a \tag{2.24}$$

Where $C_{initial}$ is the initial concentration of the analyte in the sample, $C_{d,eq}$, $C_{o,eq}$ and $C_{a,eq}$ are the concentrations of the analyte in donor, organic and accept phases at equilibrium, respectively, V_d, V_o and V_a are the volumes of donor, organic and acceptor phases, respectively. Two partition coefficients are involved in this three-

phase system. First is the partition coefficient between the organic phase and donor phases ($K_{o,d}$) and the second is that between the acceptor and the organic phases ($K_{a,o}$). The partition coefficient between the acceptor and the donor phases ($K_{a,d}$) can be expressed as;

$$K_{a,d} = \frac{C_{a,eq}}{C_{d,eq}} = K_{o,d} \times K_{a,o} \tag{2.25}$$

The recovery (R) and enrichment (E) can be calculated by the following equations (Ho *et al.*, 2002);

$$R = \frac{100 n_{a,eq}}{C_{initial} V_d} = \frac{100 K_{a,d} V_a}{K_{a,d} V_a + K_{o,d} V_o + V_d} \tag{2.26}$$

$$E = \frac{C_a}{C_d} = \frac{V_d R}{100 V_a} \tag{2.27}$$

In a three-phase sampling mode large $K_{a,d}$ will be obtained when the analytes in the accepter phase are converted to be a species that can not be extracted back into the organic phase and the recovery in this case is different from a two-phase system since the whole volume of acceptor phase can be used for further analysis. The actual recovery is therefore equal to those obtained from equation (2.26). Since the final extracted solution is in aqueous thus, this mode of sampling is usually combined with an HPLC or a capillary electrophoresis (CE) (Psillakis and Kalogerakis, 2003a).

To obtain high extraction yields, the nature of the membrane and analytes need to be carefully considered and selected. The hollow fiber membrane should be hydrophobic and compatible with the organic solvent being used and the organic solvent must be able to impregnate and becomes immiscible within the pores of the hollow fiber to enhance the analytes transfer into the organic phase since the extraction occurs on the surface of the immobilized organic solvent (Psillakis and Kalogerakis, 2003b). In the case of a two-phase liquid membrane extraction, a very

good organic solvent for analyte immiscible with water should be selected to maximize the partition coefficient between acceptor phase (organic solvent) and donor a phase (sample phase), $K_{a,d}$. While in a three-phase system, the organic solvent should be selected in order to ensure both high $K_{o,d}$ and $K_{a,o}$, especially the latter because with high $K_{a,o}$ a low amount of the analyte will be left in the organic phase which means that most of analyte will diffuse into an acceptor phase and high enrichment will be obtained (Ho *et al.*, 2002). In addition, others parameters such as the stirring of sample, extraction time, volume of organic phase and the addition of salt must also be optimized to achieved high enrichment. However, with the liquid membrane extraction technique, higher speed of stirring than SDME can be applied since the organic phase was protected inside the pore and the lumen of hollow fiber membrane (Psillakis and Kalogerakis, 2003b). Liquid membrane extraction can be used in a wide range of applications for environmental, biomedical or food analysis. They have important advantages over the classical extraction techniques such as fast, effective, inexpensive and can be claimed as solvent free technique. Furthermore, there is the possibility to perform automated extraction system. The applications of this technique are summarized in Table 2.2.

Table 2.2

Applications of liquid membrane extraction (modified from Psillakis and Kalogerakis, 2003a).

Application	Compound	Sampling mode	Donor phase	Solvent	Acceptor phase	Analytical method	Reference
Drug analysis	Diazepam	Two-phase	1.5 mL plasma	*n*-Octanol	15 μL *n*-octanol	GC/NPD	(Rasmussen *et al.*, 2000)
	Methamphetamine	Three-phase	2.5 mL urine or plasma (made alkaline)	*n*-Octanol	25 μL aqueous (acidic)	CE/UV	(Pedersen-Bjergaard and Rasmussen, 1999)
	Cocaine and cocaine metabolites	Two-phase	2 mL saliva (made in alkaline)	Chloroform	10 μL chloroform	GC/PDHID	(de Jager and Andrews, 2002)
	Amino alcohol	Three-phase	3.5 mL water or urine (made in alkaline)	*n*-Octanol	10 μL aqueous (acidic)	CE/UV	(Hou *et al.*, 2002)
Environmental Analysis	Polybrominated diphenyl ethers	Two-phase	100 mL water	*n*-Undecane	22 μL *n*-undecane	GC/MS	(Fontanals *et al.*, 2006)
	Organochlorine pesticides	Two-phase	5 mL sea water	Toluene Octane	5 μL toluene	GC/MS	(Basheer *et al.*, 2002)
	Polycyclic aromatic hydrocarbon (PAHs)	Two-phase	1 g soil, 7 mL acetone and 15 mL water	Toluene	8 μL octane	GC/FID	(King *et al.*, 2002)
	Phthalates	Two-phase	5 mL water	Undecane	3 μL toluene	GC/MS	(Psillakis and Kalogerakis, 2003b)
	Dinitrophenolic compounds	Three-phase	200 mL water	Dihexyl ether	200 μL $NaHCO_3$	HPLC/ DAD	(Berhanu *et al.*, 2006)

Application	Compound	Sampling mode	Donor phase	Solvent	Acceptor phase	Analytical method	Reference
	Aromatic amine	Three-phase	4 mL water or sea water 20% NaCl, 2% acetone	Dihexyl ether	4 µL aqueous (acidic) 18-crown-6-ether	HPLC/UV	(Zhao *et al.*, 2002)
	Aniline derivatives	Three-phase	3 mL water	Benzyl alcohol: Ethyl acetate (80:20,v/v)	3 uL of organic solvent	HPLC/UV	(Shariati-Feizabadi *et al.*, 2003)
Food Analysis	Triazine herbicides	Two-phase	3 mL water or slurry 10% NaCl	Toluene	3 µL toluene	GC/MS	(Shen and Lee, 2002)
	Phenoxy herbicides	Three-phase	8 mL bovine milk	*n*-Octanol	7 µL aqueous (basic) NaOH-KCl pH 13	HPLC/UV	(Zhu *et al.*, 2002)
	Vanillin	Three-phase	1 g vanilla sugar, chocolate biscuit and chocolate in 1 mL of 0.2 M H_2SO_4	di-*n*-Hexyl ether		Ampero-metric detection	(Luque *et al.*, 2000)

2.5 Adsorption on solid sorbents

Extraction or separation by adsorption on a solid sorbent is a very important technology and widely used in trace analysis. It can be applied with various types of sample such as, air, water, soil and food. Therefore, this enrichment technique becomes more and more popular as can be seen in many publications in this area in only one year. The adsorption is a surface phenomenon, in which molecules of the analytes (adsorbate) accumulate on the surface of the solid (adsorbent) (Harper, 2000) as a result of two main processes, physical and chemical adsorptions (Webb, 2003).

Physical adsorption is the result of relatively weak van der Waals forces between the solid surface and the adsorbate therefore this kind of adsorption can be easily reversed. All molecules of the adsorbed analytes can be evacuated at the same temperature at which the adsorption occurred. Elevated temperature of the adsorbent bed by heating will stimulate the desorption process since it makes readily available to the adsorbed molecules the energy necessary to escape the adsorption site. Unlike the physical adsorption, the chemical adsorption is difficult to reverse since molecules are strongly adsorbed on the surface of the adsorbent through chemical bonding (sharing of electrons between the adsorbate and the solid surface). A larger quantity of energy than physical adsorption is required to clean the surface of chemically adsorbed molecules. Therefore, the understanding of the nature of the adsorbent, adsorbate and their interaction is necessary (Webb, 2003). Main characteristics and the applications of some commonly used adsorbents are shown in Table 2.3 (Alltech, 2005; Camel and Caude, 1995; Grob and Barry, 2004; Harper, 2000; Supelco, 2005/2006).

Table 2.3

Main characteristics and the applications of adsorbent used for preconcentration of trace organic volatiles.

Adsorbent	Surface area (m^2g^{-1})	Temperature limit (°C)	Application
Graphitized carbon blacks: non porous, non specific, extremely hydrophobic and high surface homogeneity and suitable for aqueous injection			
Carbotrap	100	400	Airborne C_4-C_8 compounds and heavier
Carbotrap C	10	400	compounds
Carbopack B	100	500	C_1 to C_{10} compounds including alcohols, free
Carbopack C	10	>400	acids, amines, ketones, phenols and aliphatic
Carbopack F	5	>400	hydrocarbons, depending on its surface area
Carbon molecular sieve: abundance of micropores in a size range of molecular dimension, the extraction mechanism using sieve effect			
Carbosieve G	910	225	Small airborne molecule such as C_2
Carbosieve S-III	820	400	hydrocarbon and smaller molecules
Carboxen 563	510	400	C_2-C_5 volatile organic compounds
Carboxen 564	400	400	C_2-C_5 volatile organic compounds with higher capacity than Carboxen 563
Carboxen 569	485	400	C_2-C_5 with highest capacity for volatile organic compounds and lowest capacity for water
Carboxen 1000	1200	400	Permanent gases and light hydrocarbons
Carboxen 1004	1100	225	
Porous polymer: Tenax			
Tenax TA (GC)	35	350	High boiling point compounds, amines, alcohols, C_6-C_9 hydrocarbons
Tenax GR	24	350	Low molecular mass organic compounds

Adsorbent	Surface area (m^2g^{-1})	Temperature limit (°C)	Application
Porous polymer: Chromosorb			
Chromosorb 101	350	275	Fatty acids, alcohols, glycols, ester, ketones, aldehydes, ethers and hydrocarbons
Chromosorb 102	350	250	Volatile organics and permanent gases
Chromosorb 103	350	275	Basic compounds, such as amines and ammonia; useful for separation of amides, hydrazines, alcohols, aldehydes and ketones
Chromosorb 104	100-200	250	Nitriles, nitroparaffins, hydrogen sulfides, ammonia, sulfur dioxide, carbon dioxide, vinylidene chloride, vinyl chloride, trace water content in solvent
Chromosorb 105	600-700	250	Formaldehydes, acetylene
Chromosorb 106	700-800	225	C_2-C_5 alcohols; C_2-C_5 fatty acids from corresponding alcohols
Chromosorb 107	400-500	225	Formaldehydes, sulfur gases, and various classes of compounds
Chromosorb 108	100-200	225	Gases and polar species such as water, alcohols, aldehydes, ketones, glycols
Porous polymer: Porapak			
Porapak N	225-350	190	Ammonia, carbon dioxide, water, and separation of acetylene from other C_2 hydrocarbons,
Porapak P	100-200	250	Wide variety of alcohols, glycols and carbonyl compounds
Porapak Q	500-600	250	Hydrocarbons, organic analytes in water, and oxides of nitrogen
Porapak R	450-600	250	Ether and ester
Porapak S	300-450	250	Alcohol (normal and branched)
Porapak T	250-350	190	Highest polarity of Porapak, formaldehyde, other polar compounds

Adsorbent	Surface area (m^2g^{-1})	Temperature limit (°C)	Application
Porous polymer: HayeSep			
HayeSep A	526	165	Permanent gases including hydrogen, nitrogen, oxygen, argon, CO and NO at ambient temperature, C_2 hydrocarbon, hydrogen sulfide and water at elevated temperature
HayeSep B	608	190	C_1-C_2 amines, trace amount of ammonia in water
HayeSep C	442	250	Polar gases such as HCN, ammonia, hydrogen sulfide and water
HayeSep D	795	250	Light gases, CO, CO_2 from room air at ambient temperature
HayeSep N	405	165	Acetylene, ethylene
HayeSep P	165	250	Ammonia, alcohol in water
HayeSep Q	582	275	General, hydrocarbon, sulfur gases
HayeSep R	344	250	Light hydrocarbon, chlorinated compounds
HayeSep S	583	250	C_2-C_3 hydrocarbon, polar compounds
HayeSep T	250	165	Light hydrocarbons, formaldehydes

Some applications for these adsorbents will be explained further in Chapters 5 and 6. However, when dealing with adsorption, the parameter which requires carefully considered is the breakthrough volume of the adsorbent.

2.5.1 Breakthrough characteristic of the adsorbent

Breakthrough volume is a crucial parameter for adsorption. It is the loading capacity of an adsorbent. It is usually assumed as the volume of gas, containing analyte, that can be flowed through the adsorbent bed until its concentration at the outlet reaches some fraction (generally 5%) of its inlet concentration (Harper, 2000; Poole and Schuette, 1984). Therefore, the additional

back-up adsorbent bed must be placed to adsorb the excess amount from the front bed, and this is a normal method used for guarding of the saturation of the adsorbent. The acceptable percentage found in the back-up section should be less than 15% of the total concentration. This can then be combined to the concentration obtained from the front section and used as the results. The sampling using both front and back-up beds is limited and inefficient when the sample found in back-up section exceeds 20-25% of the total sample when this is more than 33%, saturation has occurred (Fig. 2.8) (Harper, 2000).

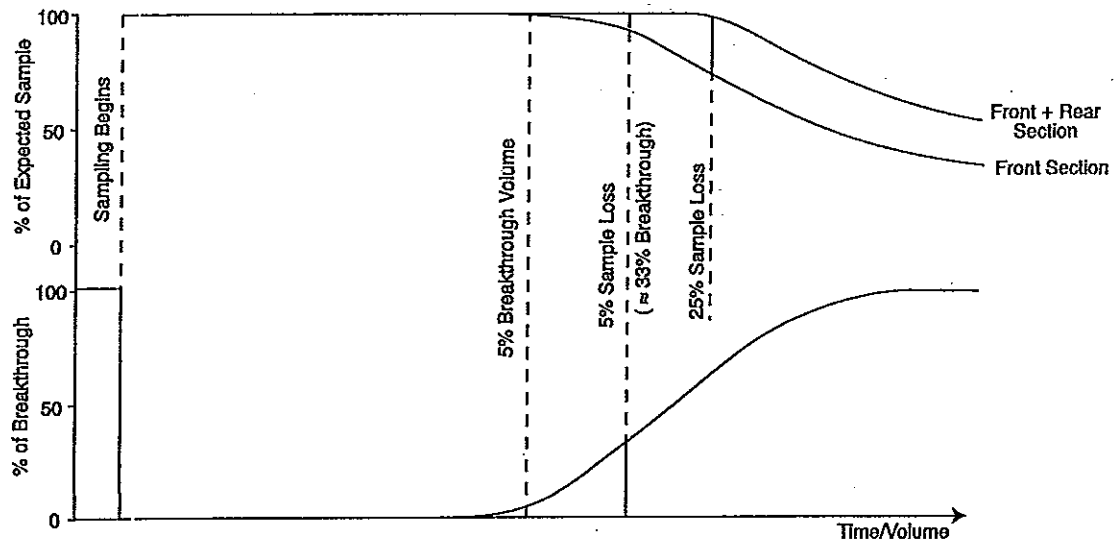


Fig. 2.8 Relationship of breakthrough to sample loss in a typical two-section sample tube sampling an atmosphere of constant sorbate concentration, showing the difference between 5% breakthrough volume and 5% sample loss to the back-up section. Actual shape of the curves will vary with sorbent, sorbate, concentration, temperature and the presence of other sorbates (Harper, 2000).

Using both front and back-up beds is one of several methods used for determination of breakthrough volume of the adsorbent. Loading the trap with known amount of analyte is another method that can be performed, after loading the trap then purging with a volume of gas, the breakthrough can be obtained by the determination of the remaining amount of the analyte. Another method that can be used is the

estimation of the measured chromatographic retention volume data (Manura, 1995-2005; Poole and Schuette, 1984). The operating system is shown in Fig. 2.9, the inlet of the adsorbent bed is connected to the injection port of a gas chromatograph and the outlet is connected to the detector. A known amount and known concentration of the analyte was injected at the injection port under the known continuous flow of nitrogen. The analyte was adsorbed and carried further along the adsorbent bed until it reached to the detector and the retention time recorded. The breakthrough volume of the adsorbent of the injected analyte can be calculated by equation (2.28).

$$B_V = \frac{t_R \times F}{W_A} \tag{2.28}$$

Where B_V is the breakthrough volume (L g^{-1} of adsorbent), t_R is the retention time (min), F is the flow rate of the carrier gas (mL min^{-1}) and W_A is the adsorbent weight (g).

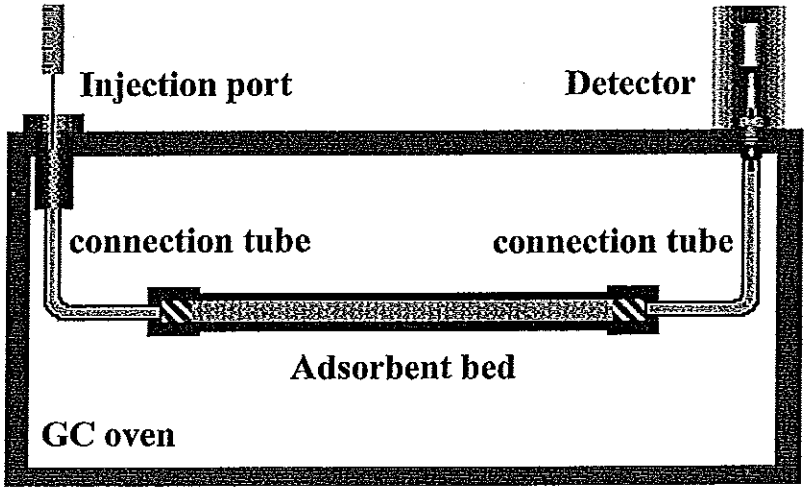


Fig. 2.9 The system used for breakthrough volume determination, the adsorbent bed can be placed either in or out sides the GC oven.

Another alternative approach for the determination of breakthrough volume has similar principle to the previous method except that the same

concentration of analyte is continuously flowed through the adsorbent bed, so the effluent was directly detected by the detector of a gas chromatograph. The breakthrough curve was plotted between the concentration at the effluent and time as shown in Fig. 2.10. The breakthrough volume is indicated by the stable effluent which means that the adsorbent already reached its full adsorption capacity (Peters and Bakkeren, 1994).

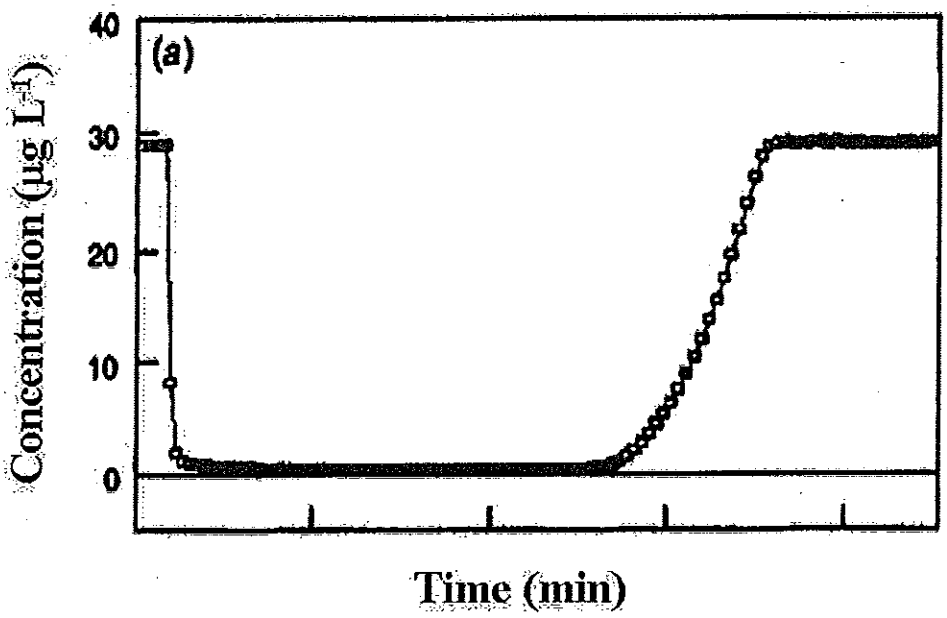


Fig. 2.10 Breakthrough curve of *m*-xylene on 250 mg of Tenax GR at air flow rate of 50 mL min^{-1}.

Breakthrough volume is influenced by several factors, one of which is the concentration of analyte in the sample, the higher the sample concentration, the lower the breakthrough volume. When sampling at high concentration, either shorten sampling time or low sampling flow rate should be used to avoid the breakthrough. The temperature is another parameter that affects the breakthrough, high temperature resulting in low breakthrough volume. At low temperature, the analyte can be retained longer than at high temperature which can be explained by Van't Hoff-type relationship (Simon *et al.*, 1995). In addition, the presence of other species in the atmosphere (Harper, 2000) also give a severe problem on the breakthrough volume. For example, in the collection of toluene on coconut shell charcoal the breakthrough

volume was reduced by 50% when the relative humidity was increased from 0 to 80% at a flow rate of 1 L min^{-1}(Saalwaechter *et al.*, 1975). Therefore, these factors should be taken into account for the determination of breakthrough. This breakthrough can however be partially avoided by carefully choosing the most adequate adsorbent and working conditions.

2.5.2 Sampling methods

General techniques used for sampling with adsorbent are active and passive sampling. Both techniques usually combine the sampling, analyte isolation and preconcentration into one single step.

2.5.2.1 Active sampling

Active sampling devices consist of a sampling pump and a collecting medium. Sample was drawn by the sampling pump and the analyte was adsorbed on the active surface of the adsorbent packed in the sample tube (Fig. 2.11). In this method, a flow meter for measuring the flow rate and volume of sample is necessary for the calculation of concentration of the analyte after the analysis by suitable instruments. The analysis will give a first result as a mass of analyte per tube in the unit of micrograms per tube, the defined volume of sample that pumped through the sample tube in unit of cubic meter (m^3) was used to divide from mass per tube, then the concentration of analyte in the unit of micrograms per cubic meter will be obtained (mg m^{-3}).

One important thing on sampling by active method is the adsorbent tube that will be connected to the sampling pump, mostly preferring prepared by using two sections, front and back-up bed to prevent of the analyte lost because of the breakthrough. Mostly, the amount of adsorbent packed in the front bed is double of those packed in the back-up bed as shown in Fig. 2.12.

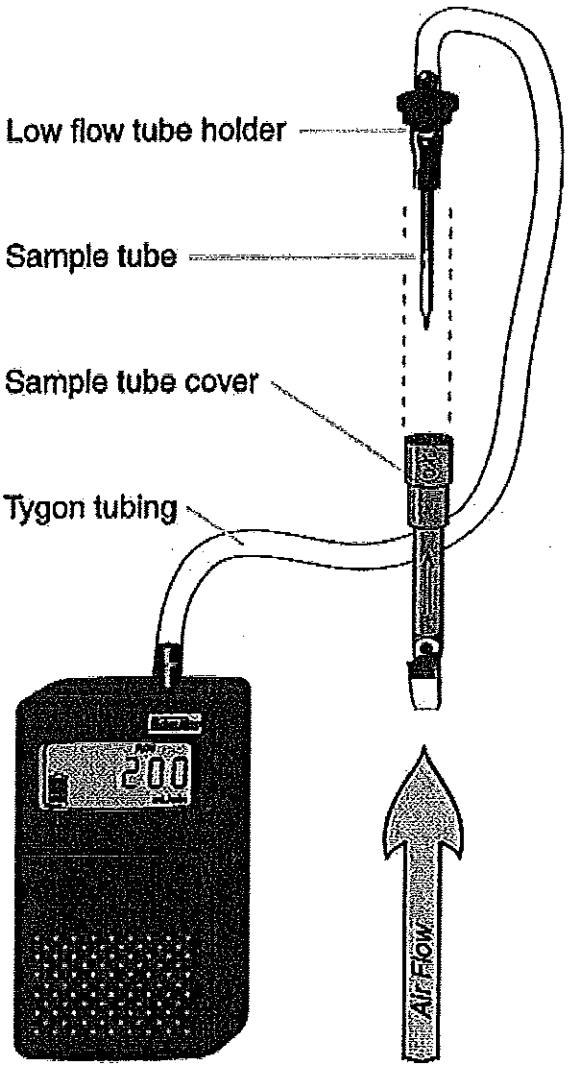


Fig. 2.11 Active sampling technique, the sample was pumped through the sample tube containing an adsorbent by air pump that the accurate flow rate can be controlled (Salter, 2005).

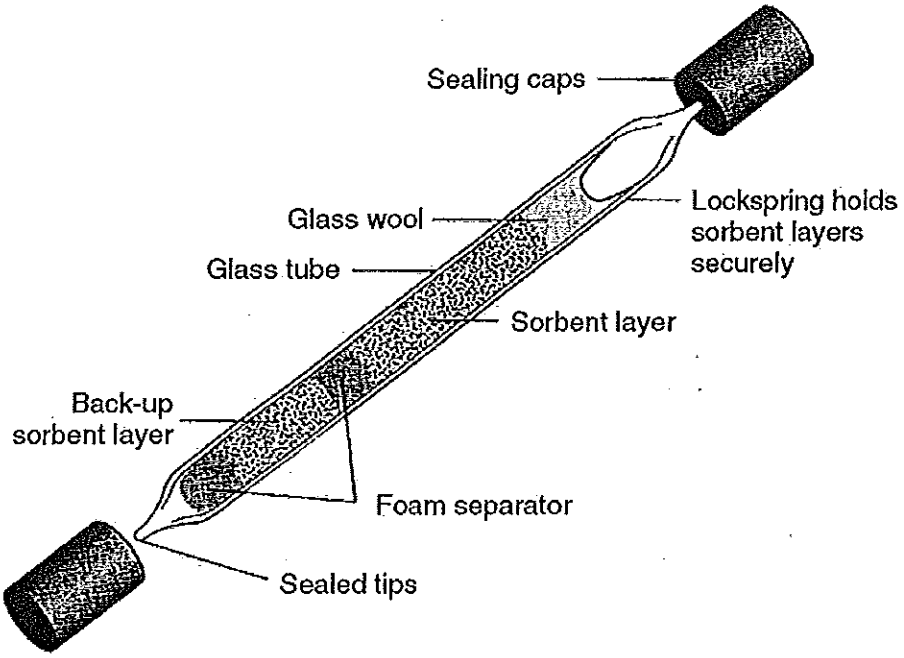


Fig. 2.12 A typical adsorbent tube (Dean, 2003).

The main disadvantage for active sampling is its unsuitability for the sampling in high risk area since it might be harmful to the operator. Moreover, it is limited by the requirement of the power. Therefore, passive sampling technique is more appropriate in many cases.

2.5.2.2 Passive sampling

Passive sampling is defined as a technique based on free flow of the analyte molecules from the sample medium to the collecting medium, as a result of different concentrations of the analyte between two media. The mass transfer can be explained by Fick's first law of diffusion (equation (2.29).

$$Q = \frac{AD(C_1 - C_0)t}{L} \qquad (2.29)$$

Where Q is the uptake amount of the analyte (μg) transported to the sampler in time t, A is the cross section of the diffusion path (cm^2), D is the molecular diffusion coefficient of the analyte ($cm^2\ s^{-1}$), C_1 and C_0 are concentrations of the analyte in medium examined ($mg\ m^{-3}$) and the sampler surface (normally is zero), respectively and L is the total length of diffusion path (cm). The analyte collected by this technique depends on its concentration in the sample medium and the exposure time and more details on this sampling technique will be expressed in Chapter 5.

2.5.3 Desorption methods

The desorption of analyte from solid sorbent can be performed by 2 methods, thermal and solvent desorptions. Thermal desorption (followed by GC) is more popular than solvent desorption since it can provide the analysis of 100% of the trap content (instead of an aliquot part), there is no solvent peak, no waste and no contamination from solvent. Moreover, the desorption using an elevated temperature can easily be automated (Pillonel *et al.*, 2002). However, when the analytes are too strongly adsorbed (this frequently occurs with polar solutes and strong adsorbents such as activated carbon), thermal desorption is not suitable for recovering of compounds due to the very high temperature required. Too high temperature will entail the thermal degradation of the solutes or/and the sorbent bed (Camel and Caude, 1995; Pillonel *et al.*, 2002). In this case, it is more convenient to apply liquid extraction. For liquid extraction, the adsorbent is extracted with a low-boiling point solvent, such as carbon disulphide, dichloromethane, benzene or pentane, mostly with Soxhlet type extraction procedures. Solvent extraction allows longer sorbent beds, as well as higher flow-rates and larger total sample volumes than thermal desorption. Moreover, the sample obtained is in the solution from that can be analysed using different techniques not only by GC, and the results are high accurate (Camel and Caude, 1995).

Adsorption on solid adsorbent has some crucial problems, *e.g.*, the occurrences of artifacts due to catalytic activity of the adsorbent, discrimination and displacement effects due to selective adsorption and irreversible adsorption of high

molecular weight and/or polar molecules (Pillonel *et al.*, 2002). Some of these can be reduced by using the sorption techniques.

2.6 Sorptive extraction

Sorption, absorption or dissolution is distinguished from adsorption by the fundamental of mechanism occurred in the extraction medium. In the case of absorption, the analytes are sorbed (dissolved) into the bulk of liquid phase, while adsorption, the analytes will remain on the surface of porous material (Baltussen *et al.*, 2002; Pillonel *et al.*, 2002), as can be simply described with Fig. 2.13.

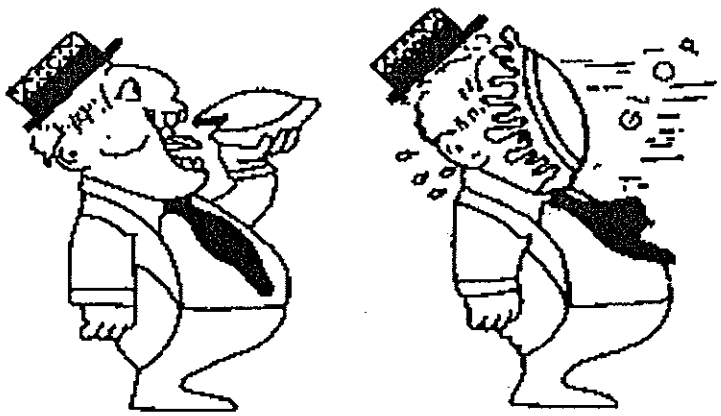


Fig. 2.13 The different behaviors between the adsorption and the absorption phenomena (Rouessac and Rouessac, 2000).

Sorptive extraction by nature is an equilibrium technique, for water samples the extraction of solutes from the aqueous phase into the extraction medium is controlled by the partitioning coefficient of the solutes between the silicone and the aqueous phases (David *et al.*, 2003). The process for sorption is similar to liquid-liquid extraction without using solvents and recent studies have correlated this partition coefficient with octanol-water distribution coefficient (K_{ow}). Although it is

not fully correct but at least it gives some idea for how well solute can be extracted with sorptive materials. The molecular interaction between the solute and sortive material is much weaker than in the case of adsorption, causing the reduction of retention power and enrichment capacity. On the other hand, the release of the analytes by thermal sorption can be performed at much lower temperature than the adsorption mode. Sorptive extraction techniques that have been reported are open tubular trapping (OTT), gum phase extraction (GPE), solid phase microextraction (SPME) and stir bar sorptive extraction (SBSE) (Baltussen *et al.*, 2002). Among these, SPME and SBSE are two of the most popular techniques which will be described.

2.6.1 Solid phase microextraction (SPME)

SPME is a powerful, innovative and rapid extraction/ enrichment technique (Pillonel *et al.*, 2002). The equilibrium occurs in SPME extraction process can be explained by equation (2.30).

$$K_{f,s} = \frac{C_f}{C_s} = \frac{(n/V_f)}{C_{initial} - (n/V_s)} \tag{2.30}$$

Where $K_{f,s}$ is the distribution coefficient of the analyte between fiber coating of SPME and sample, $C_{initial}$ is the initial concentration of the analyte in sample, C_f and C_s are the concentrations of the analyte in fiber coating and sample, respectively, V_f and V_s are the volumes of the fiber coating and sample, respectively and n is the amount of the analyte absorbed on the fiber. By solving equation (2.30), equation (2.31) can be obtained.

$$n = \left[\frac{K_{f,s}V_fV_s}{V_s + K_{f,s}V_f}\right]C_{initial} \tag{2.31}$$

Since the volume of fiber coating is much less than the volume of sample ($V_s >> K_{f,s}V_f$), therefore,

$$n = K_{f,s}V_f C_{initial} \tag{2.32}$$

This equation indicates that the amount of extracted analyte is independent of V_s, as long as $V_s >> K_{f,s}V_f$ and it is proportional to the initial concentration of the analyte in the sample and the volume of fiber coating (Pawliszyn,1999; Loconto, 2001). SPME can be devided into two types, in-tube and fiber SPMEs..

2.6.1.1 In-tube solid phase microextraction (In-tube SPME)

In-tube SPME or OTT is the oldest technique that uses a thick film capillary GC column (10-100 μm or even thicker at 165 μm) for sampling or extracting the analytes from either gaseous or liquid samples. The samples are pumped to the capillary tube mostly coated with 100% polydimethylsiloxane (PDMS) (Pillonel *et al.*, 2002). The trapped analytes can be released either by thermal desorption or liquid extraction. The benefit of PDMS coated trap is that it provides a very rapid desorption, shows good blanks (the degradation products formed from PDMS can easily be identified by MS-detector), does not subject to the formation of artifacts (no catalytic activity) and has a flow compatibility with capillary columns, furthermore, water uptake of PDMS is so low so, no additional water management is needed. Recently the in-tube SPME has been applied successfully as an in line monitoring for the simultaneous determination of 6 semivolatile organic compounds, *e.g.*, hexachlorobutadiene, dieldrin, 4,4'-DDT, benzo[b]fluoranthene, benzo[a]pyrene and dibenzo[a,h]an-thraxcene (Peeraprasompong *et al.*, 2006).

2.6.1.2 Fiber solid phase microextraction (Fiber SPME)

The extraction of SPME uses the same mechanism as in tube capillary microextraction, it can be performed either by directly immersed the fiber in the aqueous solution or in its headspace. The liquid phase was coated out side the fiber mounted on a gas tight syringe (Zhang and Pawliszyn, 1996) or the syringe liked device (Fig. 2.14). The fiber is attached to the syringe plunger, so the fiber will be easily exposed to the samples or to perform a desorption which is usually done at the heating zone (injection port) of a gas chromatograph. The sampling and the analysis are done in only one step and with only one device which is an important advantage of fiber solid phase microextraction. The comparison of in tube and fiber SPMEs was illustrated in Table 2.4.

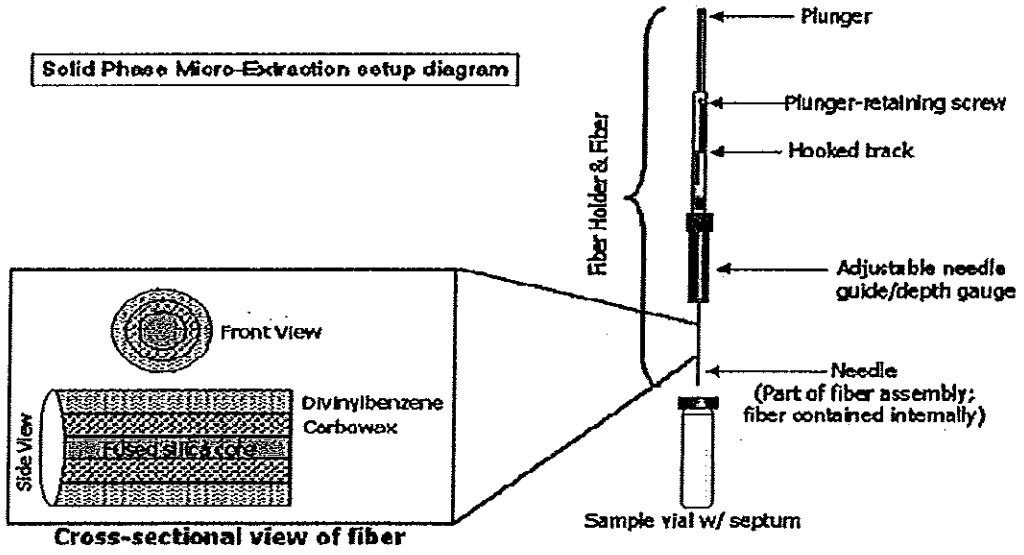


Fig. 2.14 SPME device.

Table 2.4

Comparison of in tube and fiber SPME techniques (modified from Kataoka, 2002).

Parameter	In-tube SPME	Fiber SPME
SPME device	Commercial capillary columns with a vast array of stationary phase is available	Commercially available SPME fibers are limited
Field	Inner surface of capillary column	Outer surface of the fiber
Extraction	Repeatedly drawn the sample into the capillary column	Immerse fiber in the sample solution or in its headspace
Equilibration time	10-15 min (depending on compounds)	30-60 min (depending on compounds)
Desorption	Draw a desorption solvent or mobile phase into a capillary column, or using thermal desorption	Expose fiber in the desoption chamber filled with mobile phase or heated zone of GC or immerse it into the extraction solvent
Carry over	Negligible	~10% (depending on compounds)
Applicable sample	Clear sample only	Clear and cloudy sample
Operation	Automation	Manual
Precaution	Sample solution must be miscible with mobile phase and not contain insoluble matters because the flow line is prone to stop form the clogging by particles in the sample	Fiber must be carefully handle because the coating is prone to strip off from the needle during insertion to the desorption point

Table 2.5

SPME phase coating thickness affects analyte recovery (Pawliszyn, 1999).

Analyte	% Relative recovery for PDMS at film thickness		
	100 μm	30 μm	7 μm
Benzene	2	1	<1
Toluene	5	1	<1
Chlorobenzene	6	2	<1
Ethylbenzene	3	4	1
1,3-Dichlorobenzene	17	5	2
1,4-Dichlorobenzene	15	5	1
1,2-Dichlorobenzene	15	4	1
Naphthalene	13	4	1
Acenaphthylene	19	8	3
Fluorene	29	18	8
Phenanthrene	37	27	16
Anthracene	49	38	32
Pyrene	69	54	47
Benze[a]anthracene	105	91	96
Chrysene*	100	100	100
Benzo[a]fluoranthene	104	111	120
Benzo[k]fluoranthene	111	124	127
Benzo[a]pyrene	119	127	131
Indeno[1,2,3-*cd*]pyrene	61	140	148
Benzo[ghi]perylene	61	117	122

Note; * Reference value

SPME: fiber immersed in 15 min rapidly stirring sample

The extraction efficiency of solid phase microextraction can be increased by increasing the volume of the sorptive phase (as shown in equation (2.32)) which can be increased by increasing the film thickness of the SPME fiber. The coating thickness affects the retention of the analyte, it will increase the analyte capacity and selectivity. Since the film is thick, the appropriate extraction time and

the desorption time are also increased. The affects of the SPME film thickness is shown in Table 2.5.

A thick fiber coating will extract more of a given analyte than a thin coating. Consequently, a fiber with a thicker coating is used to retain volatile compounds and transfer to the GC injection port without loss as shown with the recovery in Table 2.5 but a thin coating is used to ensure fast diffusion and release of high boiling point compounds during the desorption with high temperature at GC injection port. However, thick coating SPME will effectively extract high boiling point compounds from the sample matrix, but it requires longer desorption time, and the analytes could be carried over to the next extraction. Therefore, successfully application of SPME depends primarily on the selection of a suitable fiber for a particular analysis. Examples of SPME applications are summarized in Table 2.6.

Table 2.6

Applications of SPME (modified from Kataoka, 2002).

Sample	Analyte	Coating phase	Mode of extraction	Final analysis	Reference
SPME					
Water	Substituted benzene	100 μm PDMS	DI	GC/IT/MS	(Kastner and Neubert, 1992)
Water, sand, clay	Volatile and semivolatile compounds	PDMS	HS	GC/FID, GC/MS	(Zhang and Pawliszyn, 1993)
Soil solution	Organochlorine pesticides	100 μm PDMS	DI	GC/ECD, GC/MS	(Popp *et al.*, 1994)
Water	Organophosphorus pesticides	85 μm PA	DI	SFE/HPLC/UV	(Salleh *et al.*, 2001)
Fish, river water	Methylmercury	100 μm PDMS	HS	GC/MS	(Cai and Bayona, 1995)
Water	Explosives	50 μm CW/TPR, 60 μm PDMS/DVB, 85 μm PA	DI	HPLC/UV	(Monteil-Rivera *et al.*, 2004)
Ground and surface water	BTEX, naphthalene, chlorinated alkaline, chlorinated benzenes	100 μm PDMS, 85 μm PA	HS	MCC/UV/IMS	(Walendzik *et al.*, 2005)
Water	BTEX	65 μm PDMS/DVB	HS	GC/μFID	(Ji *et al.*, 2006)
Water	*N*-nitrosamine	CAR/PDMS	HS	GC/NCD, GC/NPD, GC/MS	(Grebel *et al.*, 2006)
Industrial effluent	Maladorous sulfur compounds	PDMS/DVD, PDMS/Carboxen	HS	GC/MS	(Lestremau *et al.*, 2004)
Water	Benzyl chloride, benzyldichloride, benzyl-trichloride	Activated carbon fiber (ACF)	HS	GC/MS	(Sun *et al.*, 2005)

Sample	Analyte	Coating phase	Mode of extraction	Final analysis	Reference
Water	Organichlorine pesticides, triazine hesbicides, alkylphenol, bisphenol-A	Sol/gel coated oligomers	DI	GC/MS	(Basheer *et al.*, 2005)
Air	Chlorobenzene	Carbowax-PDMS, PDMS-DVD, Carbowax-DVB	HS	GC/MS, GC/ECD	(Barro *et al.*, 2004)
In-tube SPME					
Water	Phenylureas and carbamates	Omegawax 250	-	HPLC-UV	(Eisert and Pawliszyn, 1997)
Tap water, surface water	Carbamates	Omegawax 250	-	HPLC-UV	(Gou and Pawliszyn, 2000)
Water	Organophosphorus pesticides	95% PDMS, 5%phenyl-substituted backbone	-	LC/UV	(Cháfer-Pericás *et al.*, 2007)
Fish muscles	Tetracyclin antibiotics	Poly(MAA–EGDMA) monolithic column	-	HPLC/PAD	(Wen *et al.*, 2006a)
Water	Fluoroquinones	Carboxen 1010 porous layer open tubular (PLOT) capillary column	-	HPLC/MS/MS	(Mitani and Kataoka, 2006)
Tap water, lake water	PAHs, aromatic amines	PPY-coated	-	HPLC/UV	(Wu and Pawliszyn, 2001)
Water	PAHs, fatty acids, alcohols, phenols	Sol–gel CN-PDMS	-	GC-FID	(Kulkarni *et al.*, 2006)
Liquid medicine	Endocrine disruptor	Supel-Q Plot	-	HPLC/UV	(Mitani *et al.*, 2003a)
	Endocrine disruptor	Poly(AA-VP-bis) monolith	-	HPLC/MS	(Wen *et al.*, 2006b)

Sample	Analyte	Coating phase	Mode of extraction	Final analysis	Reference
Urine	Amphetamines	Poly(MAA-EGDMA) monolith	-	HPLC/UV	(Fan *et al.*, 2005)
Soybean foods	Daidzein and genistein	Supel-Q Plot	-	HPLC/DAD	(Mitani *et al.*, 2003b)
Meat	Heterocyclic amines	Omegawax 250	-	LC/MS	(Kataoka and Pawliszyn, 1999)

2.6.2 Stir bar sorptive extraction (SBSE)

Stir bar sorptive extraction is an enrichment technique that is very useful for extraction of analytes from aqueous samples (Tienpont *et al.*, 2002). Moreover, this technique can overcome the problem of low sample capacity loading from SPME technique since the liquid phase was coated on a magnetic stir bar encapsulated in a glass jacket (larger surface area than SPME fiber). The sample is extracted by stirring of a coated stir bar (typically PDMS) for a period of time, the analytes are absorbed on the liquid phase and then desorbed either by thermal desorption following with GC or by liquid to improve the selectivity. The extracted solution can also be injected into the HPLC system as an alternative instrument.

It is very important to realize that the equilibrium of sorptive is also dependent on the phase ratio and thus on the amount of polydimethylsiloxane coated on the glass bar. This relationship can be written as;

$$K_{o,w} = K_{PDMS/W} = \frac{C_{PDMS}}{C_W}$$

$$= \left(\frac{m_{PDMS}}{m_W}\right)\left(\frac{V_W}{V_{PDMS}}\right)$$

$$= \beta\left(\frac{m_{PDMS}}{m_W}\right) \qquad (2.33)$$

The distribution coefficient between octanol (organic phase) or PDMS and and water ($K_{o,w}$ or $K_{PDMS/W}$) is defined as the ratio between the concentration of a solute in the PDMS phase (C_{PDMS}) over the concentration in the water (C_W) at equilibrium. This ratio is equal to the ratio of the mass of the solute in the PDMS phase (m_{PDMS}) over the mass of the solute in the aqueous phase (m_W) times the phase ratio (β, where $\beta = V_W / V_{PDMS}$). The amount of analyte extracted into the PDMS coated on the glass bar in term of the recovery can be calculated by equation (2.34) with known partition coefficient and phase ratio;

$$\frac{m_{PDMS}}{m_{initial}} = \frac{\left(\frac{K_{PDMS/W}}{\beta}\right)}{1+\left(\frac{K_{PDMS/W}}{\beta}\right)} \tag{2.34}$$

From the above equation, the extraction efficiency increases with the increase of $K_{PDMS/W}$ and the decrease of β or phase ratio. The higher amount of PDMS coating, the lower β and the higher extraction efficiency. The amount of PDMS can be increased by increasing of the thickness. However, the thick coating requires longer time for both absorption and desorption steps than the thin coating due to the resistance to mass transfer in the coating. The speed of extraction (or the required equilibration time) is also an important factor to consider. Due to the thickness of the coating, it is assumed that all resistance to mass transfer is in the coating and that the sample is perfectly stirred. For this situation, the time required to reach 95% extraction (t_{95}) can be calculated by (Pawliszyn, 1997);

$$t_{95} = \frac{d_{PDMS}^2}{2D_{PDMS}} \tag{2.35}$$

where d_{PDMS} is the thickness of the PDMS coating (cm) and D_{PDMS} is the diffusion coefficient of the analyte under investigation in PDMS, in $cm^2\ s^{-1}$. From equation (2.35), the time to reach 95% of the extraction efficiency increase with the thickness of coating layer, therefore the compromise between the extraction efficiency, extraction time and the thickness is needed to obtain SBSE that can provide high recovery in a short time.

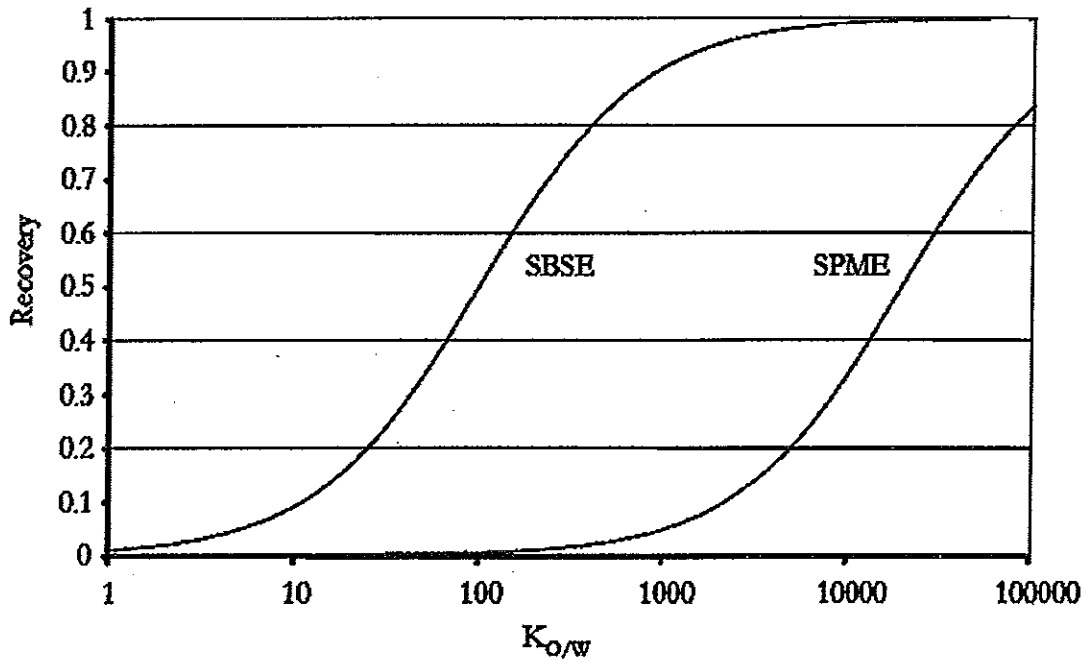


Fig. 2.15 Theoretical recovery of analytes in SBSE and SPME from a 10 mL water sample as a function of their octanol-water partitioning coefficient. Volumes of PDMS on SPME fiber and SBSE are 0.5 μL and 100 μL, respectively (David *et al.*, 2003).

The comparison of recovery between SBSE and SPME can be expressed in Fig. 2.15. In SPME, the maximum volume of PDMS coated onto the fiber is approximately 0.5 μL (100 μm film thickness). For a typical sample volume of 10 mL, the phase ratio is equal to 2×10^4. This results in poor recovery for solutes with low $K_{o,w}$. While in SBSE, a stir bar coated with 100 μL PDMS can easily be used to extract 10 mL of water since phase ration is reduced down to 100, 200 times less than that for SPME. Assume both techniques are applied for the same sample and target anayte having $K_{o,w}$ = 1000. SPME provides less than 10% recovery while the recovery for SBSE is closed to 100% (Fig. 2.15), because SPME has a larger phase ratio than SBSE.

2.7 Conclusions

The most commonly methods used for enrichment in sample were discussed in this chapter. The benefits as well as the limitations of each method are also detailed. In order to obtain the highest extraction efficiency, a suitable enrichment technique should be selected. Most of the extraction techniques mentioned in this chapter are still being investigated and improved to obtain the highest efficiency to apply for various types of samples. In addition, trace and ultra-trace analysis of analytes in the environment, foods, biological samples or even in the industrial raw material, *etc.* are a good driving force for the development of new enrichment techniques that can enhance the concentration of the analytes in matrix sample into the measurable level of the analytical instrument.

CHAPTER 3

Adsorption Based Sample Preparation

3.1 Introduction

According to various estimations, sampling and sample preparation typically account for 70-90% of analysis time. It is quite obvious that very often greater gains in the overall response time of the analytical system can be realized by reducing the time necessary to process the samples. Thus, a great effort is going into the development of reliable sampling/sample preparation procedures characterized by the simplicity of both the operation and the devices involved in the process. One of the prevailing trends is the combination of several steps into one, such as sampling and sample preparation. Elimination or significant reduction of the amount of solvents used during sample preparation is also a priority, as it can reduce the cost of analysis, as well as the amount of time and labor required to process a sample. In addition, solventless technique is one of the cornerstones of so-called "green analytical chemistry", a concept gaining the importance very rapidly (Górecki and Namieśnik, 2002).

The sampling/sample preparation method based on the use of solid adsorbent fulfills many of the above requirements, because it usually combines sampling, analyte isolation and preconcentration into one single step. Furthermore, using solid adsorbent has advantages in that fast direct determination can be carried out, sampling is simple and the sample are easily stored and transported to the laboratory and furthermore, solid adsorbent has higher sample capacity than the sorptive technique. Consequently this technique continues to be popular (Churáček, 1993).

Adsorption based sample preparation is based mainly on the difference between the adsorption affinity of the analytes in the sample to the active surface of solid adsorbent. It can be classified into two groups, the first group after sampling is storage the samples before analysis in the laboratory is named as an "off-line sample preparation" whereas another is an "on-line sample preparation" which is the method that eliminates the error associated from the delay between sampling and analysis because the sample preparation step is connected directly to the analysis system.

3.2 Off-line adsorption based sample preparation

There are two off-line adsorption based sample preparation methods based on how the sampling/preconcentration is processed, *i.e.,* dynamic and static modes.

3.2.1 Dynamic mode

Sample preparation based on the dynamic mode is a method in which some forces are used to drive the sample through the adsorbent bed. This include purge and trap and active sampling techniques.

3.2.1.1 Purge and trap

Purge and trap is a dynamic preconcentration technique using an inert gas to force the analyte to flow through and accumulate in the adsorbing tube. This technique has been developed by Unite State Environmental Protection Agency (US EPA) since early 1970s (Loconto, 2001). It is perfectly suited with aqueous, water miscible liquid, soil and waste samples and it has been as approved as standard US EPA methods, method 5030C (aqueous sample) and method 5035 (soil sample) (US EPA, 1996; US EPA, 2003). A simple purge and trap system is illustrated in Fig. 3.1.

To operate the purge and trap preconcentration system, the sample is placed in the tube as shown in Fig. 3.1. Inert gas is purged through a portion of the aqueous/solid sample at an ambient temperature or an elevated temperature depending on the desired target analytes. The analytes, mostly volatile components, are efficiently transferred from the aqueous phase or solid phase to the vapor phase. The vapor is swept through the adsorbing tube where the analytes are adsorbed. After purging is completed, another inert gas line might be used to dry the adsorbent before doing the desorption. There are two ways to perform the desorption. The first one is that the adsorbing tube is automatically heated with the automated thermal desorption system, back flushed and analytes are then transferred directly to the detection system which mostly is the gas chromatograph equipped with the appropriate detector

(Demeestere *et al.*, 2007; Loconto, 2001; Meloan, 1999). Another way is disconnecting the adsorbing tube from the purge and trap system, and desorbing the analytes using suitable solvent or placing it in a suitable thermal desorption system.

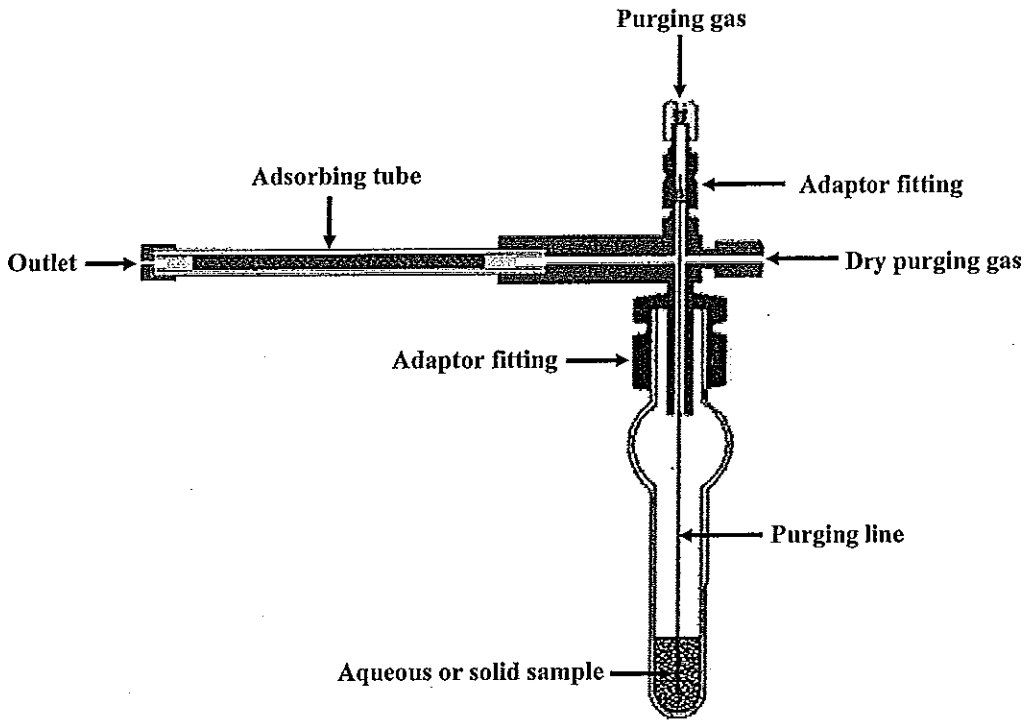


Fig. 3.1 Simple setup for purge and trap system.

This purge and trap system can be used in both off-line and on-line modes. In most cases it is performed off-line especially for solid samples. The sample needs to be collected and stored before doing the preconcentration and the analysis. Examples of the use of this technique are the determination of *tert*-butyl ether, its degradation products and other gasoline additives in soil samples (Rosell *et al.*, 2006), the speciation of organotin compounds in water and marine sediments (Campillo *et al.*, 2004), the use of needle concentrator for determination of benzene, toluene and

xylenes in aqueous samples (Kubinec *et al.*, 2004), *etc.* Precise and accurate results were obtained from these applications with excellent detection limits.

In this technique, the purging efficiency depends on several parameters, one of which is the vapor pressure of the analyte, the higher the vapor pressure, the higher the purging efficiency. In contrast, the greater the solubility of the analyte in the sample matrix, the harder it is to purge the compounds of interest. In addition, temperature, sample size, purge volume and purging method also have some effect on the purging efficiency (Meloan, 1999). Therefore, to obtain the highest performance of the purge system, the parameters mentioned above must be optimized.

Besides the purging system, the trap system also needs to be taken into account. The following is a requirement list for good trapping materials, (i) retain analytes of interest, (ii) allow O_2, CO_2 and H_2O to pass, (iii) release the analytes quickly and easily upon heating, (iv) remain stable and do not contribute contaminant volatile compounds, (v) operate without causing catalytic reaction, (vi) be of reasonable price (Meloan, 1999). The adsorbents suited for some applications are given in Table 2.3 in Chapter 2. However, the followings are the most commonly used in the trap system: Tenax, silica gel, coconut charcoal, 3% OV-1 on Chromosorb WHP, molecular sieve and Carbotrap (Meloan, 1999). Tenax adsorbs weak polar compounds and no water. Silica gel adsorbs water so tightly while charcoal adsorbs mostly organic compounds, but with the charcoal, the desorption can not be done at moderate temperature. OV-1 is similar to Tenax whereas molecular sieve shows high efficiency for trapping O_2, CO_2 and H_2O (Grob and Barry, 2004; Meloan, 1999). Therefore, sometimes the combination of the adsorbents, known as multibed adsorbent can be used as an efficient way for trapping the analytes of interest or removing the contaminants. After the analytes are desorbed from the trap, the system can be combined with a cryogenic focusing trap at the GC column head to refocus the analyte band (Grob and Barry, 2004).

3.2.1.2 Active sampling

Active sampling is defined as a collection of analytes using a sampling pump to draw air through an appropriate adsorbent as shown in Fig. 2.11 in Chapter 2.

It is the sampling and preconcentration method that has been evaluated and approved by the National Institute of Occupational Safety and Health (NIOSH) and the Occupational Safety and Health Administration (OSHA) for collecting most hazardous gases and vapors from the air, for example OSHA method 12 and OSHA method 1005 which are the sampling and analytical methods for the determination of benzene (OSHA, 2006). By using a pump to draw the gas sample through a bed of adsorbent, not only the sensitivity can be increased but also the sampling period required to trap a given mass of analyte can be reduced (Mendham *et al.*, 2000). The simplest system, applicable when high level of analyte is expected, uses a simple hand-operated bellows-type pump which forces a few liters of gas through a tube packed with adsorbent material (Mendham *et al.*, 2000). In sampling process, a sample is collected by an opened adsorbing tube connecting to the sampling pump. Airborne chemicals are trapped on the active sites of the adsorbent surface while the pump is drawing the sampled air through the tube. After sampling, the tube needs to be sealed and/or frozen during the storage and transportation for further analysis. The desorption can be done by both thermal and solvent desorptions depending on the adsorbent used and also the available and designable machine and devices.

The federal law has established permissible exposure limits (PELs) for workers exposures to a variety of airborne chemical hazards. These limits are specified in several ways: eight hour time weighted average (TWA), short-term exposure limits (STELs) and ceiling values. Eight-hour TWA limits are specified for full-time exposures. STELs are usually issued as 15 minute exposure limits and ceiling values are issued as peak levels not to be exceeded at any time during the working day. Therefore, the monitoring of the volatile organic compounds in workplace is necessary to evaluate the effect of these pollutants to the workers.

An adsorbent tube has been established as a reliable tool for each of these sampling requirements and the selection of the good adsorbent will provide good adsorption efficiency. Personal charcoal tube samplers using battery-powered pumps require specially trained personnel in order to obtain valid results and do not fulfill the mobility and sterility requirements in operating rooms. Their main disadvantages and limitations of capability are relatively high unit cost, necessity of periodic replacement or repair of pumps (each of which usually has a relatively short

service period/life time), sampling time limited by the battery life time and worker reluctance towards wearing active units through the whole working day, due to their ease of use, weight, and the noise generated by the pump (Fig. 3.2). In addition, this type of sampling is not suitable to perform in high risk area since it will be harmful to the operator (Namieśnik, 2000).

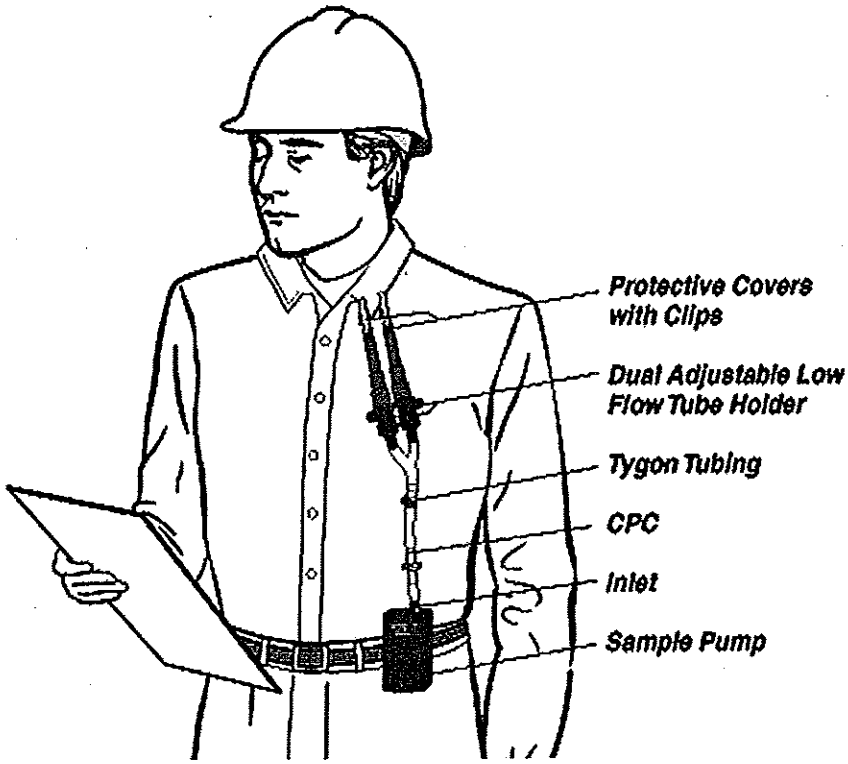


Fig. 3.2 Illustration of how to use an active sampling, the volunteer has to carry the device including of air personal pump connected to the adsorbing tubes for workplace air sampling (Galson Laboratory).

3.2.2 Static mode-passive sampling

The adsorption based static sample preparation is functioned by free flow of the sample containing the molecules of analyte through the adsorbent bed.

There is only one sampling technique, passive sampling, that can be categorized in this mode.

Passive sampling, also called diffusive sampling, is defined as a technique capable of taking samples of gas or vapor pollutants from the atmosphere, at the rate controlled by physical process but it does not involve an active movement of air through the samplers (Namieśnik, 2000). It can also be defined as a sampling technique based on free flow of analyte molecules (according to Fick's first law of diffusion) from the sample medium to a collecting medium, resulting from a difference in chemical potentials of the analyte between two media (Namieśnik *et al.*, 2005). Net flow of analyte molecules from one medium to another continues until equilibrium is established in the system (at maximum sampling time corresponding to the sampler capacity), or the sampling session is terminated by the user. This concentration can then be determined based on the ratio of analyte distribution between the two media involved or experimental calibration of the device. When sampling is terminated by the user, the amount of analyte collected by the sampler depends on both its concentration in the sampled medium and the exposure time. If the relationship between the sampling rate and analyte concentration is known, TWA analyte concentration can be easily determined (Górecki and Namieśnik, 2002).

In this method, the receiving medium (absorbent) acts as a so called zero sink meaning that it should not let the trapped molecules released even if the concentration of the analyte around the sampler decreases to zero. The sampling rate (the amount of analyte collected by the sampler per unit time at a constant concentration in the surrounding medium) must remain constant throughout the sampling session. This can be easily accomplished when the analyte is absorbed (for example into a liquid receiving phase) or chemisorbed, but it can be problematic when physical adsorption is responsible for analyte collection. In this case, only the linear portion of the adsorption isotherm should be utilized throughout the entire sampling process. Typically, this problem is solved by using high-capacity sorbents (*e.g.* Tenax TA) at low mass loading (Górecki and Namieśnik, 2002).

The use of passive samplers (dosimeters) is one of the modern approaches to the analysis of atmospheric air, indoor air, and work place atmosphere pollution. Recently such devices have become predominant in pollution monitoring.

This is because they are simple in design and in use and are relatively cheap compared to active sampling. Moreover, they do not require any power supply and make possible simultaneous detection of vapors of many compounds. Passive samplers, therefore, offer the most attractive alternative to sampling technique since the use of passive samplers to control workplace airborne health hazards can substantially reduce the cost of analyses (Namieśnik, 2000). The advantages, limitations and main applications of passive sampling are summarized in Tables 3.1 and 3.2.

Table 3.1

Basic advantages and shortcomings of the use of passive sampler (Namieśnik, 2000).

Advantage	Disadvantage
Passive sampler is simple to operate; no moving parts	They can produce only integrated concentration values
These instruments are relatively inexpensive (both in purchase and analytical costs) as long as they are used in large quantities	Some systems experience interference problems from other pollutants
They can be used by inexperienced people (occupants of buildings) and are suitable for mail-out monitoring program and the TWA can be performed	Results may be affected by air current and temperature

Table 3.2

Main applications of passive sampling.

Medium	Sample type	Measurement goal
Gas	Atmospheric air	Determination of TWA concentration over long period of time
	Indoor air	Determination of TWA concentration
		Determination of momentary concentration
	Workplace air	Determination of 8-h TWA concentration
		Determination of personal exposure (personal dosimeter)
Liquid	Surface water	Determination of TWA concentration over long period of time
		Determination of momentary concentration
Solid	Soil	Determination of volatile organic compounds

The geometry of the sampler is also very important. There are two general designs of passive samplers, diffusive or permeable, and they can be prepared as tubes or badges. These two main designs are the basis of all further modifications, which are performed to improve the efficiency, reduce the sensitivity to air mass fluctuations, and simplify the analyte desorption.

3.2.2.1 Tube-type passive sampler

Tube-type samplers are usually characterized by a long, axial, diffusion path length and a low cross-section area, resulting in a relatively low sampling rate. Fig. 3.3 shows an example of long diffusion path passive sampler which employed thermal desorption and is an air monitoring product of Markes International (Markes International Limited, 2007). This passive sampler is designed to use the inert coated tube as the sampler. Diffusion caps are required at both ends of the tube to ensure a fix diffusive path length, a critical value for quantitative diffusive monitoring.

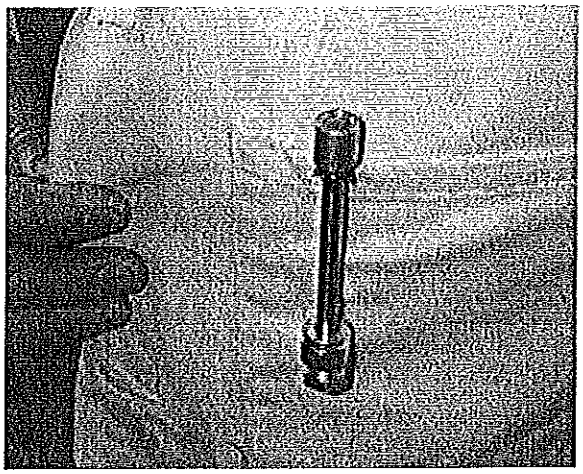


Fig. 3.3 Tube-type passive sampler with long diffusion path length (Markes International Limited, 2007).

To improve the sampling rate, an axial diffusion passive sampler is designed as shown in Fig. 3.4. This tube-type passive samples, named *Radiello* passive sampler, has been introduced to the market and patented by the Fondazione Salvatore Maugeri of Padova, Italy (Namieśnik *et al.*, 2005). Its cylindrical outer surface acts as a diffusive membrane. The gaseous molecules move axially parallel towards an adsorbent bed which is also cylindrical and coaxial to the diffusive surface. When compared to the badge-type sampler (see section 3.2.2.2), *Radiello* shows a much higher diffusive surface without the increase of adsorbing material. Even if the adsorbing surface is smaller than the badge-type, each point of the diffusive layer faces the diffusion barrier at the same distance (Radiello®). This sampler is light (less than 15 g) and small (7 cm long and 1 cm internal diameter). The system is included in ISO 16200-2 for sampling and analysis of volatile organic compounds, and conforms to CEN/TC 264 WG11 standard (Namieśnik *et al.*, 2005).

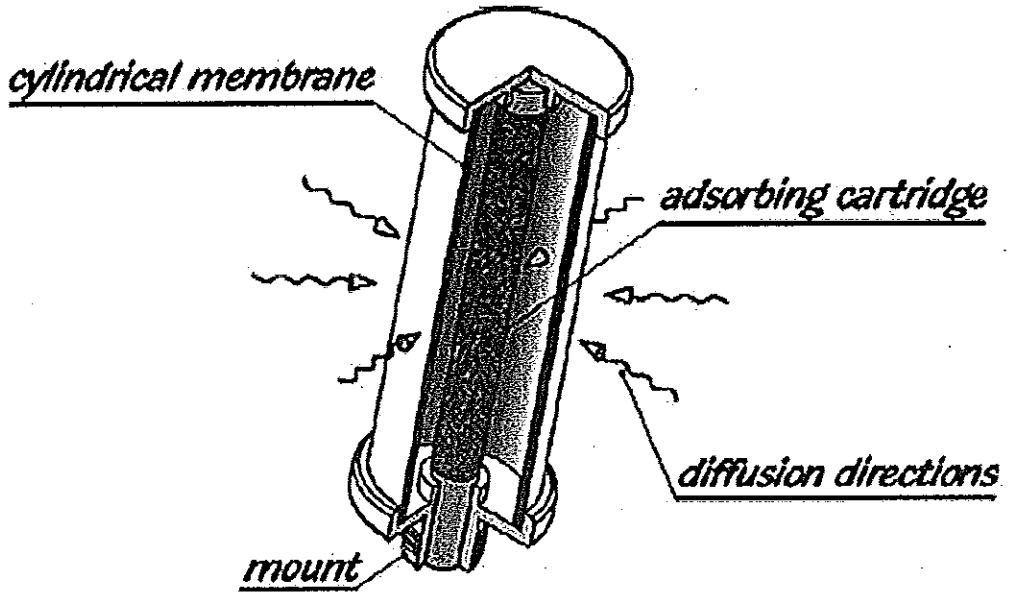


Fig. 3.4 Design of tube-type *Radiello* passive sampler with an axial diffusion (Namieśnik *et al.*, 2005).

3.2.2.2 Badge-type passive sampler

The badge design is dictated by the need to have a large surface area to achieve the useful sampling rate since with a shorter diffusion path length and a greater cross-section area, uptake rates are typically higher. Fig. 3.5 is an example of the badge-type passive sampler which is commercially available from SKC Inc. SKC Ultra Passive (diffusive) Samplers are small badges that provide a reliable collection of low ppb-level of volatile organic compounds (VOCs). Individual chemicals simply diffuse from the atmosphere into the sampler at a fixed rate (©SKC Gulf Coast Inc.).

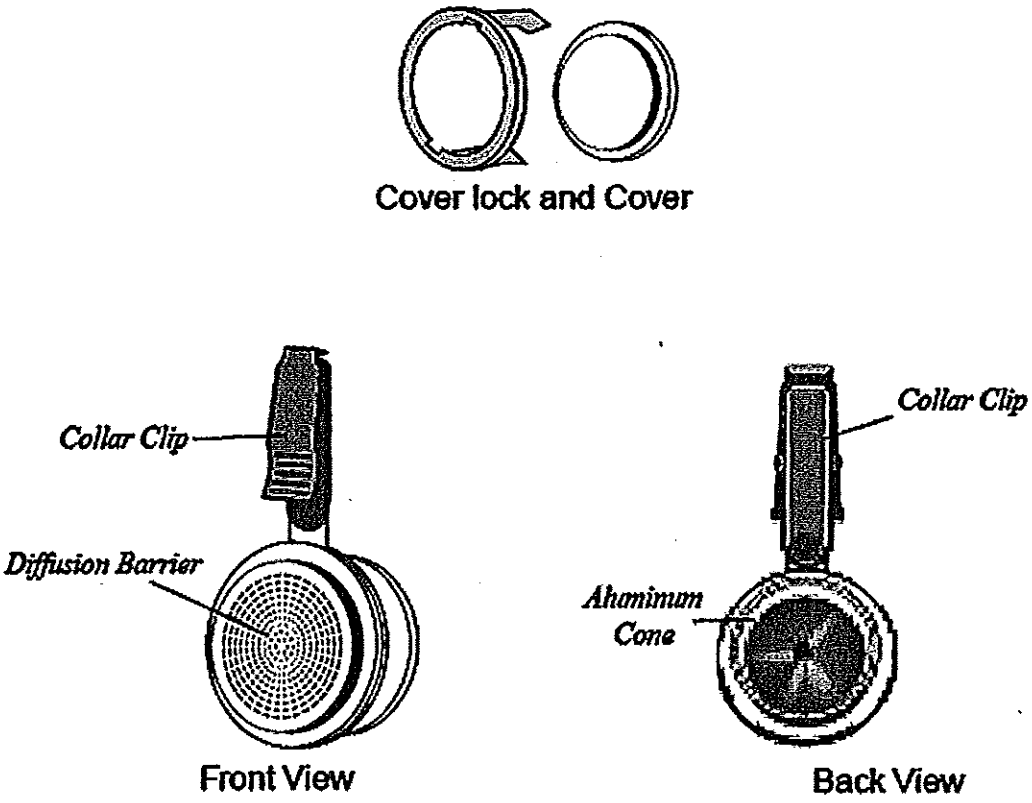


Fig. 3.5 Badge-type passive sampler from SKC Inc.

This type of samplers is mostly applied to monitor hazardous compounds in workplace because of higher sampling rate compared to the tube-type passive sampler, so, the shorter sampling period is possible. Importantly, the sampling rate is not linear even with longer sampling periods and in particular with high substance concentrations, so the constancy of the sampling rate must be checked (Giese *et al.*, 2006).

Although there already are a number of passive sampler designs, both commercially available and laboratory-built samplers, but the development of this kind of samplers are continually under investigation due to their benefits, *e.g.*, simple, low cost, easy to operate and able to collect simple even in high risk area. Besides the passive sampling many advantages it is based on free flow of the analyte, so there are the limitations that may sometimes be difficult to overcome, the most important of which is the possible effect of environmental conditions such as temperature, air

movement, and humidity on the analytes uptake. Despite such concern, many users find passive sampling an attractive alternative to more established sampling procedures. One of the developments of simple and low cost passive sampler will be presented in Chapter 5.

3.3 On-line adsorption based sample preparation

Modern analytical techniques allow the acquisition of reliable results that provide the information necessary for proper evaluation of the degree of pollution in different parts of the environment, such as air, water, or soil similar to the techniques which are previously explained in sections 3.2.1.1, 3.2.1.2 and 3.2.2. However, the use of such techniques are often time and labor consuming, expensive, and requires highly qualified personnel. There is a growing need in everyday analytical practice for rapid and more specific methods, which would allow field measurements (*in situ*) in the on-line mode (Namieśnik, 2000).

The main difference of off-line and on-line adsorptions based sample preparation is how to sample as well as how to perform the analysis. In case of on-line adsorption the sample flows directly to the adsorbent bed by means of the pump or the pressure driven the sample of interest which can be used in on-line system is limited only gaseous and water samples. The following are two most useful on-line adsorption techniques.

3.3.1 On-line adsorption trap

The adsorption tube can not only be operated in an off-line mode (section 3.2) but also in an on-line mode. Illustration of an on-line adsorption trap is shown in 3.6. Valves V_1 and V_2 are used to control the gas flow direction through the trap tube. The solid line from the sample source to the vacuum pump shows sample gas flow during preconcentration, and the dashed line shows carrier gas flow during desorption (Sanchez and Sacks, 2003).

This multibed adsorbent trap consists of 4 different adsorbents symbolized by "B", "C", "X" and "Y" in Fig. 3.6 and these are referred to Carbopack

B Carboxen1000, Carbopack X and Carbopack Y, respectively. With this multibed adsorbent, a wide range of organic compounds can be trapped and determined. After adsorption, this multibed adsorbent is back flushed, desorbed by electric current and directly flowed into a gas chromatograph equipped with flame ionization detector for the analysis (Sanchez and Sacks, 2003).

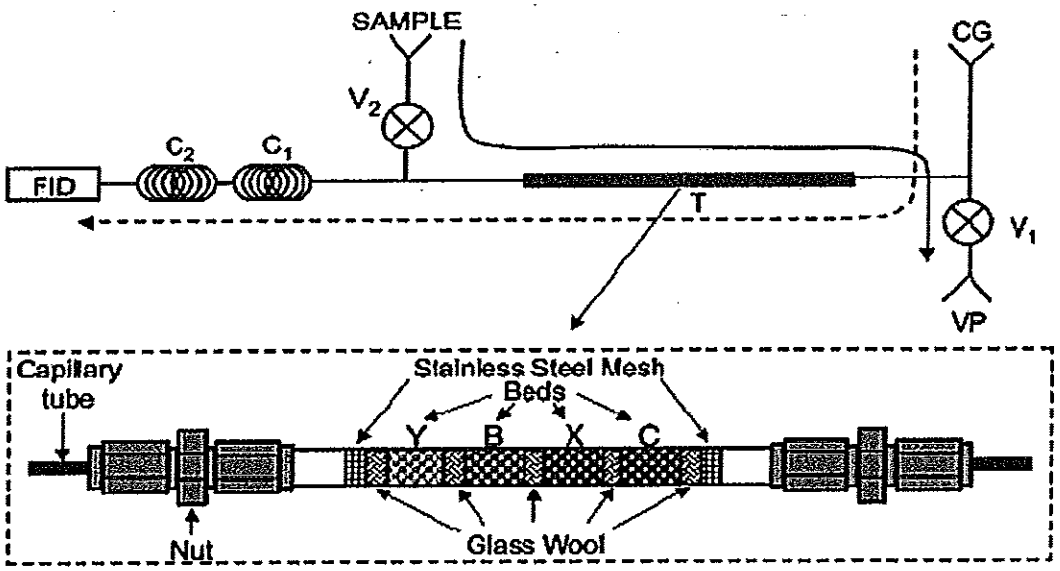


Fig. 3.6 Experimental system used to evaluate performance of the multibed on-line sorption trap (Sanchez and Sacks, 2003)

Purge and trap system which is previously mentioned in off-line mode (section 3.2.1.1) can also be operated in an on-line mode. In this case the sample is pumped directly from the sampling site, passed through the adsorption bed and then directly detected by an appropriate detector. A recent work using an on-line purge and trap system based on the use of an adsorbing tube is presented in Fig. 3.7. This system is used to monitor trihalomathanes (THMs) in drinking water. Water sample is pumped from the water tap by peristaltic pump to the gas extraction cell. THMs in water sample are carried to Traps 1 and then 2 in which are packed with Tenax®-GR

in the loading step. The trap is then heated and the valve is switched to the inject position to allow the desorbed THMs to be detected (Brown *et al.*, 2007).

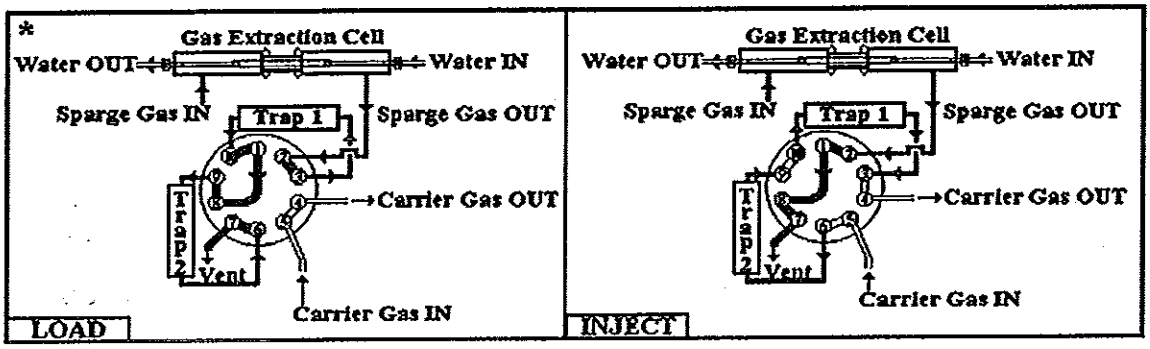


Fig. 3.7 On-line purge and trap system.

Even though the adsorption based sample preparation can be performed on-line in many cases (not only in purge and trap technique but also another type of sampling which based on the use of adsorbent), when compared to cryogenic sample preconcentration, the main problem which is the significant thermal resistance with rather long diffusion path sill occurred, resulting in slow desorption and necessity to refocus the analyte prior to the separation to obtain high peak resolution. Therefore, miniaturization of the adsorbent trap is an important issue since the refocusing step can be eliminated whereas it can maintain the good peak resolution (Demeestere *et al.*, 2007).

3.3.2 On-line microtrap

The microtrap was introduced as an on-line technique by Phillips and coworkers in 1985 by the name of modulator. Modulator is a short length thin tubing containing an adsorbent or short segment of fused silica column (Mitra and Phillips, 1988; Phillips *et al.*, 1985). It was further developed by the same group and has been named "microtrap" since 1993 (Mitra and Yun, 1993).

Microtrap is a small capillary tubing packed with an adsorbent, the typical size of microtrap is 0.5 mm inner diameter (I.D.) (Feng and Mitra, 1998). Due

to its small size, it has low heat capacity and can be heated and cooled very rapidly. Since the applied heat to perform the desorption has to migrate from external heated wall into the sorbent therefore, a larger diameter trap takes a longer time and the desorption of organic compounds is slower. Fast desorption is essential for generating a narrow injection band so that high resolution separation can be achieved. However, the breakthrough of the trap depends on the amount of the adsorbent packed inside, the smaller tubing, and the lower breakthrough volume (Feng and Mitra, 1998). Therefore, a compromise between these two parameters needs to be considered.

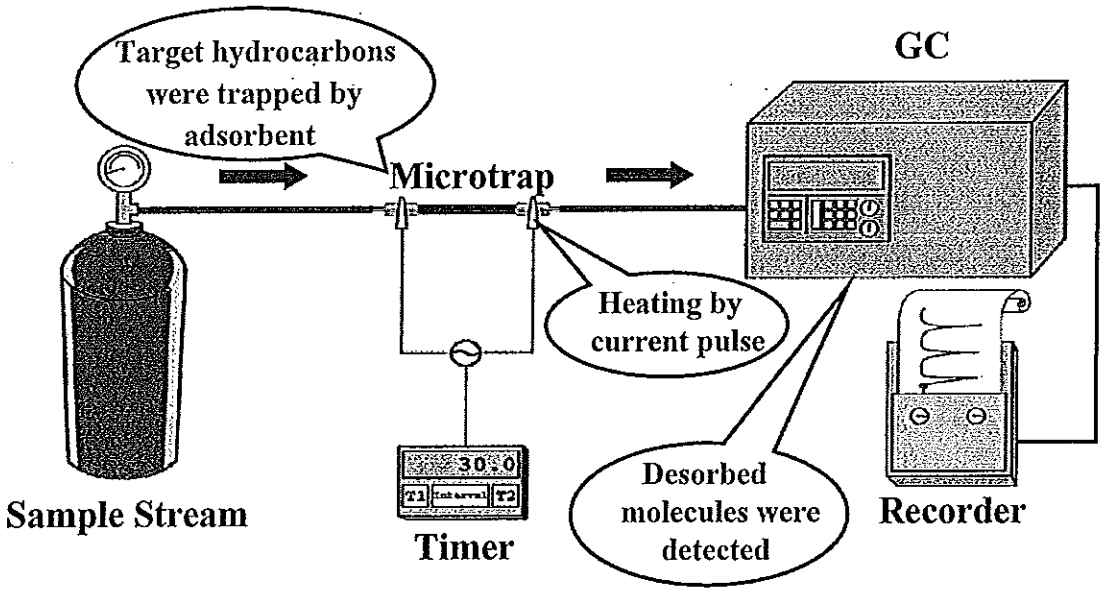


Fig. 3.8 Schematic diagram of typical on-line microtrap system.

Fig. 3.8 shows a schematic diagram of an analysis system with an on-line microtrap. In this system, the microtrap is placed in between the sample source and the detection system. It is used as both a preconcentrator and an injector in the sense that when the sample is flowed through the microtrap the analyte of interest will be trapped by the suitable adsorbent packed inside the microtrap while other gases act as background. In this step, it is used as a preconcentrator. Then it is heated by

applying a current, the analytes are desorbed and injected directly to the gas chromatographic column. In this step, the microtrap is used as an injection device.

In most of the on-line microtrap system development, the analytes of interest are VOCs (Feng and Mitra, 1998; Mitra *et al.*, 1999; Mitra *et al.*, 1996a; Mitra *et al.*, 1996b; Mitra and Yun, 1993). To the best of our knowledge, the on-line microtrap system is applicable for the analysis of small molecules has not been investigated. Therefore, the development of on-line microtrap system for real-time monitoring of small molecules will be reported in Chapter 6.

3.4 Conclusions

The adsorption based sample preparation is very useful for the determination of a wide range of compounds. A wide variety of the adsorbents can be selected for proper application. Most of the adsorption based sample preparation combines sampling, isolation as well as preconcentration of an analytes into one single step. Since, there are several sample preparation methods that use the adsorbent and each technique has its own benefits and limitations, it is necessary to carefully consider the suitability of the technique.

CHAPTER 4

Sample Preparation based on Affinity Adsorption

4.1 Introduction

To increase the selectivity of the analytical techniques, sometimes multistage sample preparation or the use of high selective detector could be performed (Vessman *et al.*, 2001). However, the trend of analytical techniques is to reduce complicated steps, the former is not preferable, and the latter can not be used in every case. One of the widely used sample preparation technique to improve the selectivity that is rapid and less laborious is based on affinity interaction (Tsikas, 2001). Many types of affinity interactions can be utilized such as chemical reaction (Rosenfeld, 1999), associate formation (Kormos *et al.*, ; Small *et al.*, 1982; Wang *et al.*, 2002), biochemical principle (Fujihara *et al.*, 2006; Heegaard *et al.*, 1998; Hermanson *et al.*, 1992) and adsorption to surfaces (Bossi *et al.*, 2007; Pichon, 2007; Pichon and Haupt, 2006; Puoci *et al.*, 2005). Affinity adsorption is one of the most promising methods which can be used in both sample preparation and analytical techniques.

Affinity chromatography is a type of liquid chromatography that makes use of biological or biological-like interactions for the separation and specific analysis of sample components (Hage, 2000). The concept of an affinity separation results from a naturally occurring phenomenon existing within all biological macromolecules. Each biological macromolecule contains a unique set of inter-molecular binding forces, existing throughout its internal and external structures. When alignment occurs between specific sites of these forces in one molecule with the site of a set of forces existing in another (different) molecule, an interaction can take place between them. This recognition is highly specific to the pair of molecules involved.

By this high selective specification, affinity chromatography provides a unique and powerful role in separation technology as the only technique enabling

the purification of an analyte on the basis of biological function or individual chemical structure (Tsikas, 2001).

To understand the principle of affinity adsorption or affinity chromatography, the common parameters involving are summarized.

4.2 Ligand for affinity adsorption

Affinity ligands are species that bind to a solid support to form the stationary phase which can be used either in the step of sample preparation or in the analysis step.

These affinity ligands may be specific (bind only one or a few very closely related molecules) in the sense that an antibody binds to only one solute or epitope (Mikkelsen and Cortón, 2004; Raez *et al.*, 2007), or the molecular imprinted polymer specifically binds with only its template molecule (Bossi *et al.*, 2007; Breton *et al.*, 2007; Pichon, 2007; Rajkumar *et al.*, 2007; Tamayo *et al.*, 2007). On the other hand, these ligands may be general, that is they bind a family or a class of related molecules such as dye anologues or lectins that bind to certain groups of solutes (De-Simone *et al.*, 2006; Helmholz *et al.*, 2003; Mikkelsen and Cortón, 2004; Monzo *et al.*, 2007). Examples of affinity ligands which have been used in affinity adsorption are listed in Table 4.1 (Hermanson *et al.*, 1992).

Appropriate selection of a ligand which can selectively recognize the target analyte will give high purification and high enrichment. However, in order to obtain the highest efficiency for selective binding, the ligand must be in a suitable form or suitable orientation. The first feature to be recognized in ligand binding is that a correct orientation of ligand to target compounds has to be found, this can be demonstrated in the case of dye analogues as the ligand. The affinity of Color Index (CI) Reactive Blue 2 derivatives for adenine nucleotide binding proteins was influenced by the point of dye attachment (Jones, 1991). Most ligands, themselves needed to be immobilized on the suitable support materials to obtain the highest benefit from the specificity of the affinity ligands for the separation or sample preparation of target molecules, rather than using free ligands. The immobilized ligands are more helpful in term of increasing the application fields.

Table 4.1

Examples of biological and non-biological affinity ligands and their binding partners used for affinity adsorption (Hermanson *et al.*, 1992; Jones, 1991; Jones, 2000).

Ligand	Binding partner
Biological ligand	
Hormone	Receptor
Enzyme	Co-factor, inhibitor, substrate analogue
Antibody	Antigen, protein A or β-galactosidase fusion products, virus, cell
Bacterial Ig Fc receptor	Immunoglobulin
Lectins: concanavalin A, lentil lectin, wheat germ lectin configurations	Glycoprotein, cell surface receptor, membrane protein, cell
Nucleic acids, nucleosides or nucleotides	Nucleases, polymerases, nucleic acids
Carbohydrate	Lectin, enzyme or other sugar binding protein
Biotin	Avidin or streptavidin
Vitamin	Carrier protein
Trypsin inhibitor, methyl ester of amino acids, or *D*-amino acids	Protease
Protein A	Immunoglobulin, binding through Fc fragment
Non-biological ligand	
Phenylboronic acid	Species containing *cis*-diol groups, such as glycosylated hemoglobins, sugars, and nucleic acids
Cibacron blue F3GA, Procion Red HE3B	Enzymes and other proteins (albumin, interferon, blood coagulation factor II and IX and plasminogen)
Molecular Imprinted Polymers (MIPs)	Cavities formed by polymerization of gel monomers around the printed molecule

4.3 Affinity matrix

A matrix is any material in which the affinity ligands may be covalently attached (Hermanson *et al.*, 1992). The matrix must play no part in the separation and be inert, insoluble in the solvent or buffer that may be employed, mechanically and chemically stable with good flow properties and easily coupled to ligand or to spacer arms onto which ligand may be attached. In addition, the matrix has high surface area, high porosity will provide high binding capacity resulting in the high accessibility to biological ligand of biomolecules which needed to be purified or separated (Jones, 1991; Scouten, 1981). Up to now, there is neither "perfect" nor "best" matrix material which is suitable for every application and possesses all of the desired characteristics, so, in order to select the right matrix for the proposed work, it would be good to consider the following useful information (Hermanson *et al.*, 1992; Jones, 1991; Jones, 2000).

Firstly, the matrix should be commercially available since it will provide more reproducibility and be more economically than the individual production of matrix by the investigators in their own laboratory (Hermanson *et al.*, 1992; Jones, 1991; Jones, 2000).

Secondly, their functional group should easily form the derivatization. The preferable functional group of the matrix for subsequent activation and attachment of an affinity ligand is the primary hydroxyl group, since this group is amenable to a wide assortment of activation procedures but does not contribute to non-specific binding of non- target molecules. Support with an abundance of primary amines or carboxylic acids are also easily derivatizable but resulting support may contain residual non-specific ion exchange effect (Hermanson *et al.*, 1992; Jones, 1991; Jones, 2000).

Furthermore, the matrix should be able to resist mechanical and chemical changes and these parameters are the most severely challenge during the chemical activation and ligand coupling procedure. The matrix should stand with the solvent, oxidizing condition, pH variation from 3-11, moderate mixing operation, high pressure and flow rate without disintegrating and becoming deformed and it should

resist to abrasion which can be tested easily by using the finger crashing to its surface (Hermanson *et al.*, 1992; Jones, 1991; Jones, 2000).

Good capacity for target molecule is another important parameter for matrix selection. The word capacity means how much the target molecule can be selectively bound per unit volume of supported material, typically expressed as milligrams of the target molecule per milliliter of swollen beaded support or per area of matrix surface. Since the capacity of support refers to the accessibility of matrix surface and effective ligand density attached to that surface, high capacity will cause high purification or high enrichment of target molecules which is the main objective of the sample preparation (Hermanson *et al.*, 1992; Jones, 1991; Jones, 2000).

When an affinity column is used, the stability of the carrier and sample flow also has to be considered since the flow can affect the separation efficiency. The flow characteristic of the matrix depends on particle size, particle size distribution and rigidity. Representatives of common matrices which have been used in affinity adsorption are summarized in Table 4.2.

Table 4.2

Examples of material used as support matrix (Jones, 1991).

Material	pH working range
Agarose (cross linked)	2-14
Cellulose	1-14
Dextran	2-14
Silica	<8
Glass	<8
Polyacrylamide	3-10
Hydroxyethyl methacrylate	2-12
Oxirane-acrylic polymer	0-12
Stylene-divinylbenzene polymer	1-13
Polyvinyl alcohol	1-14
N-acroyl-2-amino-2-hydroxymethyl-1,3-propane diol	1-11
Polytetrafluoroethylene (PTFE)	unaffected by pH

Once the matrix to be used has been selected, the investigator must decide on the method of attaching the affinity ligand to the matrix. The ligands can be attached directly to the matrix but in some cases, the matrix sterically hinders the target molecules from binding with the ligand. To allow the ligand to reach into and bind to the active site of target molecules, spacer arms are sometimes necessary (Fig. 4.1) (Scouten, 1981).

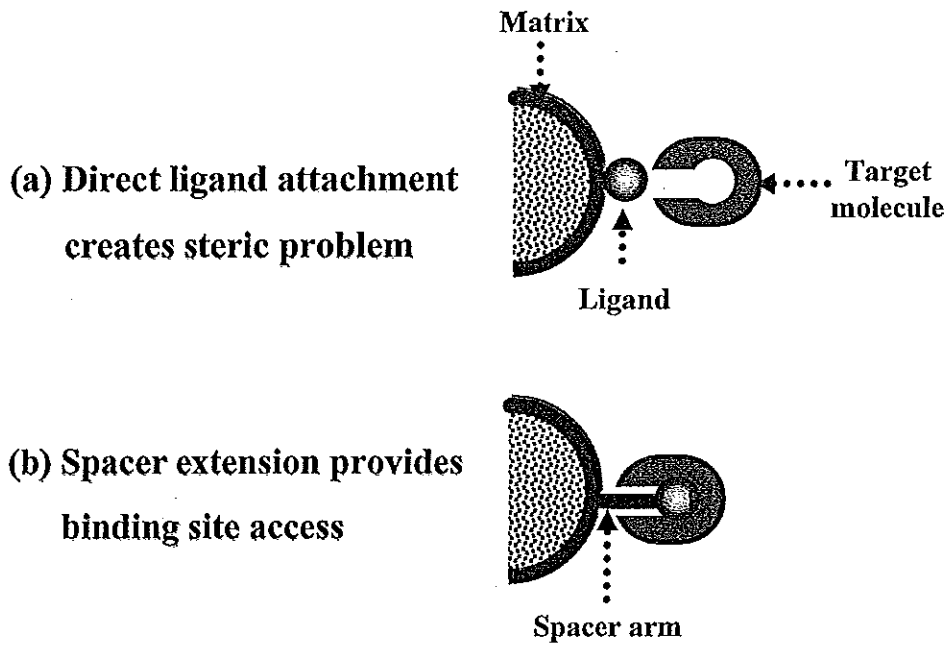


Fig. 4.1 Effect of spacer arm on the binding between the immobilized ligand and target molecule: (a) direct attachment of ligand to the matrix; (b) attachment through spacer arm.

4.4 Spacer arms

Spacer arms are low molecular weight molecules that are used as intermediary linkers between a supported material and an affinity ligand. Usually spacers consist of linear hydrocarbon chains with functionalities on both ends for easy coupling to the support and ligand. First one end of spacer is chemically attached to the matrix using traditional immobilization technique and another end is subsequently

connected to the ligand using a secondary coupling procedure. The result is an immobilized ligand that sticks out from the matrix backbone by a distance equal to the length of spacer arms chosen. Fig. 4.2 illustrates the example of the use of oxirane group as spacer arm, one side of its chain is attached to agarose gel which is used as matrix and another side is attached with the appropriate ligand.

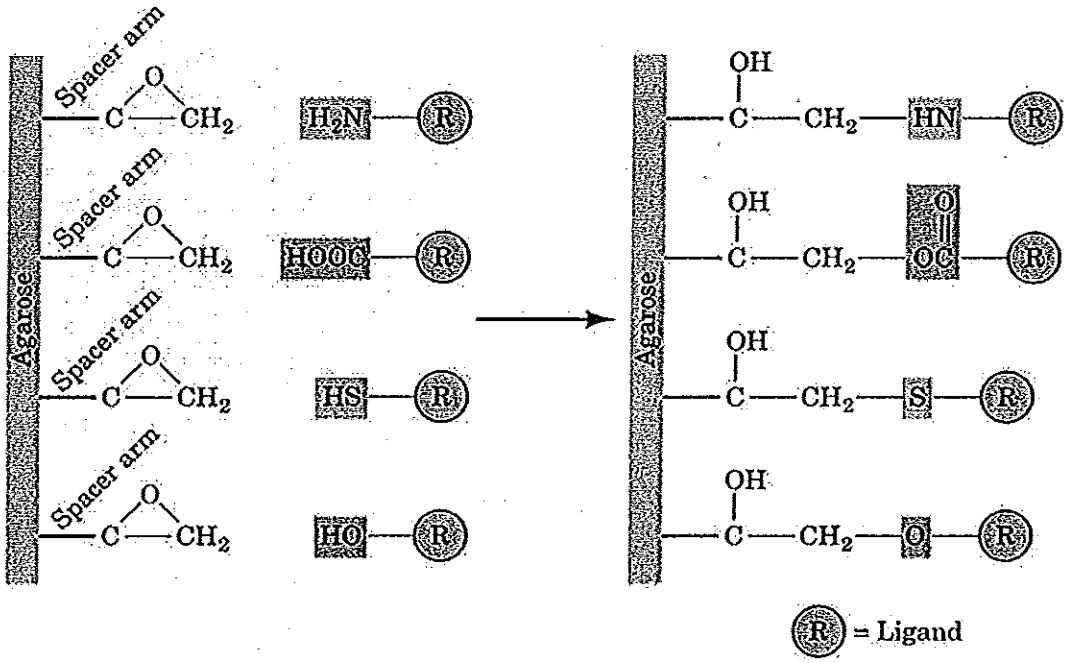


Fig. 4.2 Attachment of oxirane group to the agarose gel for the immobilization of affinity ligand.

A ligand that is attached directly to a polymeric support material may not protrude far enough from the matrix surface to reach the level of binding site on an approaching protein molecule. The result may be a weakened interaction or no binding at all (Hermanson *et al.*, 1992; Scouten, 1981). The principle advantage of using a spacer arm is that it provides ligand accessibility to the binding site of target molecule. When the target molecule is a protein with a binding site somewhat beneath its outer surface, a spacer is essential to extend ligand out far enough from the matrix to allow the interaction and the common spacer molecules are shown in Table 4.3. However, the length of the spacer must be chosen carefully since if too long, it may

contain potential internal hydrophobic interaction sites and this may well fold back on itself, making the effective length of the spacer much shorter than it really is as shown in Fig. 4.3 (Scouten, 1981).

Table 4.3

Some common spacer molecules used to extend a ligand out from the matrix surface.

Name	Structure
Diaminodipropylamine (DADPA)	NH_2–CH_2–CH_2–CH_2–NH–CH_2–CH_2–CH_2–NH_2
6-Aminocaproic acid (6-AC)	NH_2–CH_2–CH_2–CH_2–CH_2–CH_2–COOH
1,6-Diaminohexane (DAH)	NH_2–CH_2–CH_2–CH_2–CH_2–CH_2–CH_2–NH_2
Succinic acid	HOOC–CH_2–CH_2–COOH
1,3-Diamino-2-propanol	NH_2–CH_2–CHOH–CH_2–NH_2
Ethylenediamine	NH_2–CH_2–CH_2–NH_2

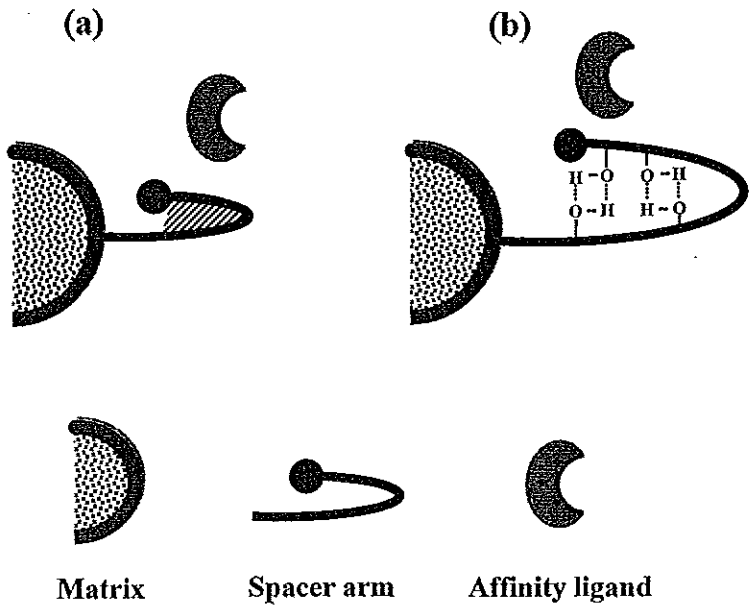


Fig. 4.3 Ligands buried by folding of spacer arm on itself: (a) hydrophobic folding of spacer arm; (b) hydrogen bonding of spacer arm.

4.5 Buffer or solvent system

4.5.1 Binding buffer or solvent

The binding conditions can have a dramatic effect on the separation potential of an affinity adsorption since it can establish a favorable affinity environment. The binding buffer/solvent should be used to initially wash and equilibrate the immobilized support in order to reach two aims: (i) remove the retained and unwanted impurity before loading the sample through the affinity matrix and (ii) adjust the matrix surface into a suitable form for binding with the target compound. If the parameters of binding buffer/solvent (concentration, polarity, and pH) are not at the optimum condition such as, the affinity interaction might be weaker than expected or might not occur at all (Hermanson *et al.*, 1992). The example of the effect of binding buffer/solvent condition is shown in Fig. 4.4. In this figure, the effect of pH on the interaction of immobilized protein A, protein G and protein A/G with the corresponding immunoglobins is presented. In case of protein A, the binding performance increases with increasing of pH and the optimum pH for protein A-immunoglobins complexes is 8, while the binding between protein G and protein A/G with their corresponding immunoglobins are almost constant over the pH range of 4.5-8 (Hermanson *et al.*, 1992).

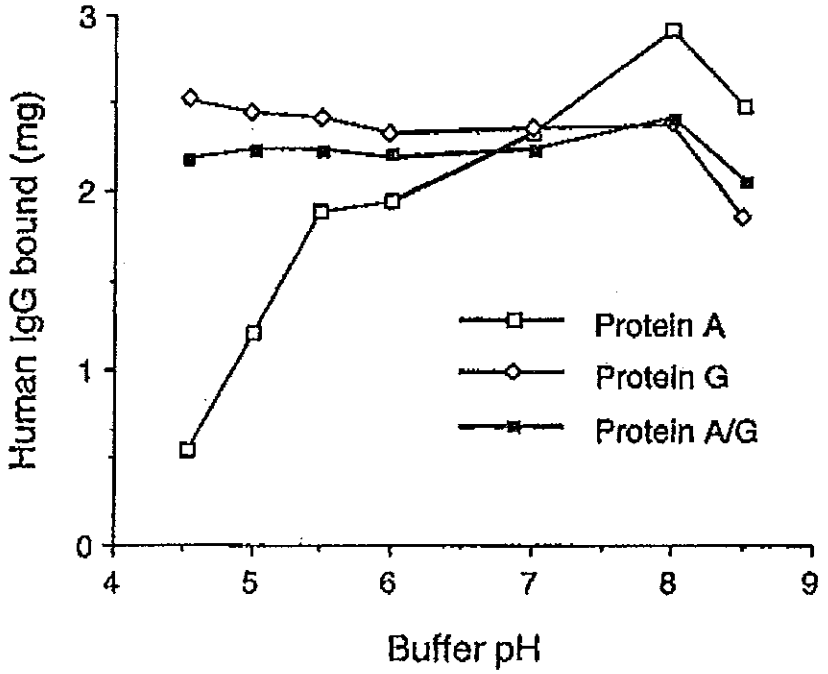


Fig. 4.4 The effect of pH on the binding of immunoglobins to immobilized protein A, immobilized protein G, and immobilized protein A/G (Hermanson *et al.*, 1992).

After the high efficiency binding is obtained from the optimization of the buffer or solvent condition then the remaining non-specific compounds must be washed out before moving to the next step which is the elution of the adsorbed molecules out of the affinity matrix for further analysis.

4.5.2 Eluting buffer or solvent

The choice of eluting buffer or eluting solvent used for breaking the affinity interaction is as important to the purification or separation of target molecules as binding buffer. The appropriate eluting buffer or solvent will liberate the bound compounds whereas it still maintains the activity of the affinity ligand. In order to break the interaction, the environment from the binding buffer must be changed to activate the liberating of target molecule from the affinity ligand.

Displacement of the bound material by adding of a low molecular weight inhibitor or counter-ligand which causes the competitive binding is the most

basic approach for the eluting step (Hermanson *et al.*, 1992; Mikkelsen and Cortón, 2004). For example, the binding between phenylboronic acid (target compound) with Sepharose CL-6B (affinity matrix) may be reversed by the addition of fructose, a strong competitor for sugar binding to boronate causing the release of borate molecule from the Sepharose CL-6B through the stronger binding between boronate and fructose (Kuzimenkova *et al.*, 2006). The former is the displacement between the affinity matrix and the added compound, since the affinity of a target compound, phenylboronic acid, to fructose higher than that to Sepharose CL-6B. However, not all cases can use the displacement as an elution technique since the competitive ligands could not be found.

In the case that the affinity interaction is pH dependent, the changing of buffer pH is an alternative method that can be used to break the interaction. For example, most antigen-antibody interaction can be eliminated at low (pH 2.3) or high pH (pH 10-11) value depending on the type of interaction couples (Hermanson *et al.*, 1992).

The changing of the composition of the buffer or solvent can also be used for the elution of target molecules from the affinity matrix. Since the change in the composition causing the change in the polarity or the strength of the buffer or solvent, which is totally different from the optimal binding condition, this change will break the affinity bonds and therefore, the adsorbed molecules will be eluted from the affinity matrix. Alternatively, the eluting agent can be used in a gradient mode from low to high effective elution such as concentration, ionic strength or polarity index in order to elute a wide range of compounds that can be bound with the general affinity ligands. In this case, the one with the weakest affinity interaction will be the first eluted and the last one will be the one with the strongest binding interaction.

Each method has its own benefit but the important factors for the eluting buffer or eluting solvent are that it should only elute the target compounds without changing their properties and by the use of the selected eluting buffer the affinity of the immobilized ligand should remain constant (no lost of active binding sites).

4.6 Modes of affinity adsorption and their applications

Affinity adsorption can be divided by the origin of ligands into two categories, bio- and non-bioselective adsorptions.

4.6.1 Bioselective adsorption

Biological macromolecules contain a unique set of intermolecular binding forces, existing throughout their internal and external structures. When the alignment occurs between specific sites of these forces in one molecule with the site of a set of forces existing in another molecule, an interaction can take place between them. This recognition is specific to the pair of molecules involved.

Basic principle of biological ligands is their ability to recognize and bind specifically to the other molecule. Biological recognition reflects the degree of fitting and the summation of various molecular interactions (electrostatic, hydrophobic, hydrogen bonding and van der Waals) that exist when target molecule bind to ligand (Jones, 1991). The selectivity of ligands depends on the number of interactions involved in the binding.

Firstly one of biologically functional pair of molecules, biological ligand, is immobilized on the inert solid supports, which can be called matrix, then the affinity matrix is packed into the column and this can be used as an affinity column for bioseparation. The sample to be purified is passed through the column and the complementary molecule from the sample is adsorbed onto the affinity matrix, then the affinity matrix is washed thoroughly to remove all compounds which have no interaction with the ligand and all remaining non-specific bound compounds. The bound molecules are then desorbed from the column by a solution of a soluble affinity ligand or more frequently, by changing the solvent composition such as pH, ionic strength or temperature, or alternatively, by a solution containing chaotropic agent such as urea, guanidine, thiocyanate, or detergents. It is important to use the conditions which promote the dissociation of the complex without destroying either the biomolecule to be purified or the ligand. Finally the affinity matrix is regenerated and re-equilibrated to prepare it for reuse in another cycle of operation (Hage, 1999).

An example of the simplest and most common operating scheme for bioselective adsorption is shown in Fig. 4.5.

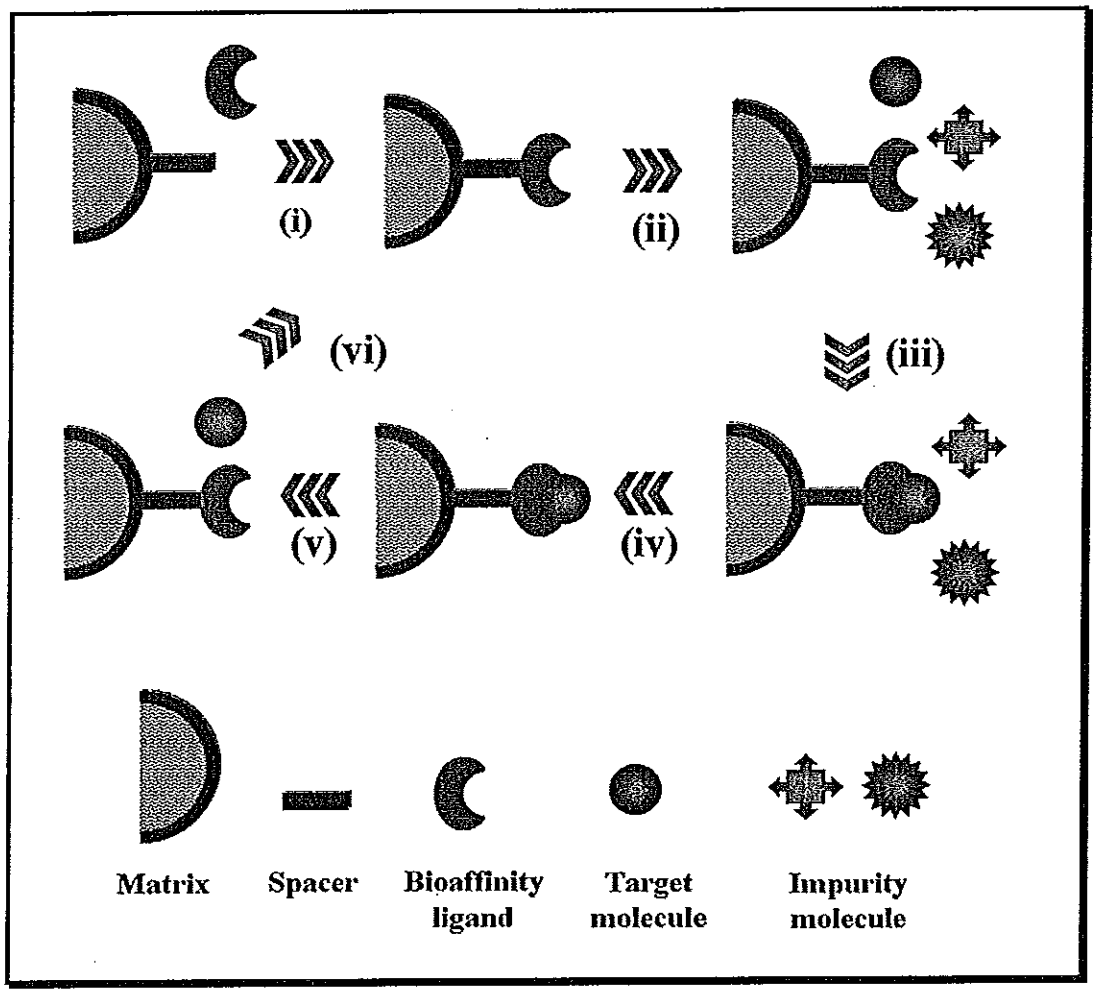


Fig. 4.5 Bioselective adsorption process. (i) Ligand is immobilized on the matrix; (ii) sample is passed through the affinity matrix; (iii) target molecule is bound with the affinity ligand; (iv) unbound compounds are removed by washing step; (v) target molecule is desorbed from the affinity matrix by the regeneration step; (vi) affinity matrix is ready to use for the next cycle.

As a result of the process shown in Fig. 4.5, one of the simplest applications is the purification of fibrinogen in human plasma (Fig. 4.6). The other examples of biological ligands and their complementary compounds are already given in Table 4.1.

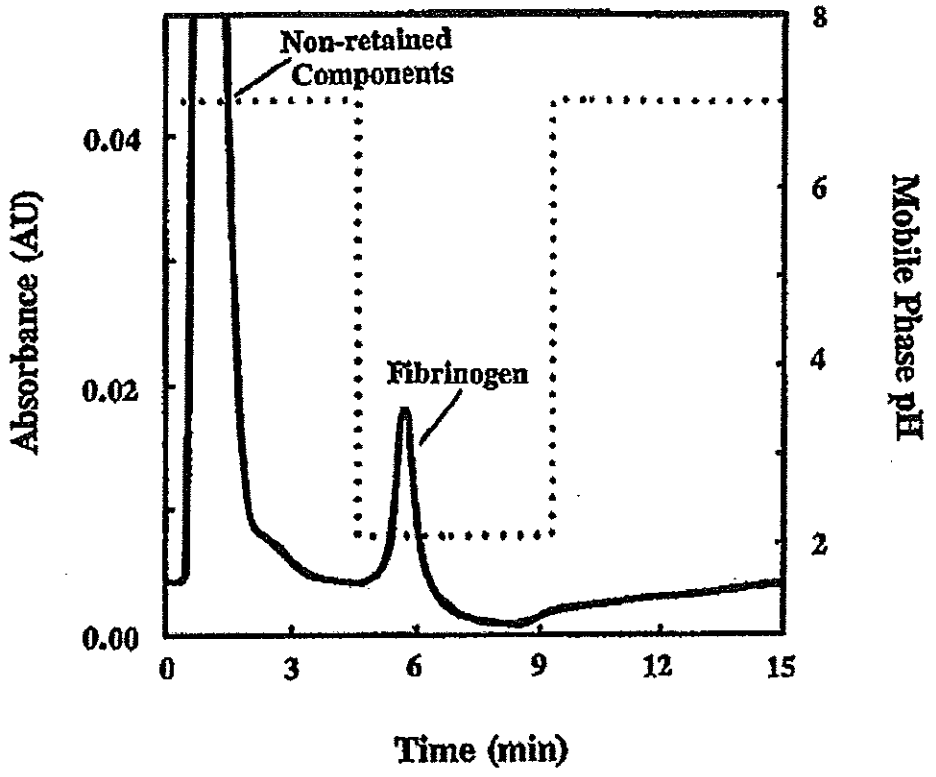


Fig. 4.6 Typical operating scheme for affinity chromatography, as illustrated by the determination of fibrinogen in human plasma, using an anti-fibrinogen immobilized antibody column and high performance immunoaffinity chromatography (HPIAC). The dotted line indicates the times during which the application buffer (pH 7.0) and elution buffer (pH 2.1) were passed through the column (McConnell and Anderson, 1993).

Another application using the bioselective ligand as an affinity column is the use of lectins which are non-immune system proteins having the ability to recognized and bind certain types of carbohydrate residues. Two certain lectins that are often placed into affinity column are concanavalin A, which binds to α-*D*-glucose residues, and wheat germ agglutinin, which binds to *D*-N-acetylglucosamine. These ligands are commonly used for the isolation of many carbohydrate containing compounds (Hage, 1999). Recently, immobilized surface of porous polymer monolith with concanavalin A (conA) has been used for selective separation of glycopeptides (Bedair and Oleschuk, 2006). In their work, monolith is used as the matrix and

concanavalin A is an affinity ligand. Bovine pancreatic ribonuclease B (RNase B) is chosen as a model for glycoprotein to digest and preconcentrate its glycopeptides on the monolithic lectin sprayer since it is known to have high affinity to concanavalin A (Bedair and Oleschuk, 2006).

However, because this mode of affinity adsorption is dealing with the use of the biological elements, poor stability and high cost often occur as the problematic of this technique (Ouyang *et al.*, 2007). Furthermore, the bioselective ligands could not be applied to every analyte, in some applications, the target molecule could not bind to the biological ligands, and therefore non-biological interactions are more preferable.

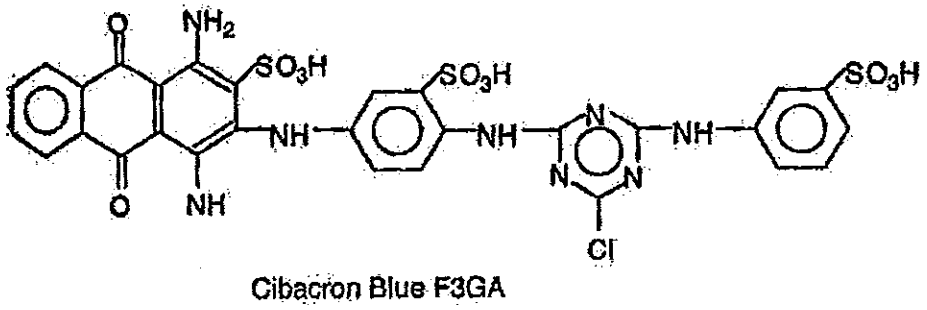
4.6.2 Non-bioselective adsorption

Non-bioselective adsorption is based on the use of non-biological ligand as a material for affinity adsorption. The examples of the most commonly used non-biological ligand are previously summarized in Table 4.1.

4.6.2.1 Dye analogue affinity

Textile dyes had already been proved to be suitable ligands for protein separation. However, textile dyes are bulk chemicals, most of which contain many by-products, co-produced at every stage of the dye manufacturing process. This fact alone makes reproducibility problematic for using this kind of affinity ligands. Despite this limitation, it was recognized that dye-like structures had a powerful ability to separate or purify a very diverse range of proteins (Jones, 2000).

Cibacron Blue F3GA (or closely related Procion Blue MX-3G and MX-R) is the most popular dye used in the affinity technique for purification of proteins (Small *et al.*, 1982). It contains an anthraquinone (Fig. 4.7) that successfully mimics the structure of certain enzyme substrates. Cibacron Blue F3GA can bind enzymes that require adenylic cofactors, such as NAD^+, $NADP^+$, and even ATP. Therefore, this dye is the excellent general ligand for the purification of many dehydrogenases and kinases (Hermanson *et al.*, 1992).



Cibacron Blue F3GA

Fig. 4.7 Principle structure elements of anthraquinone dye, Cibacron Blue F3GA

4.6.2.2 Molecular imprinted polymers (MIPs)

MIPs are the synthetic biomimetic receptors that are capable of binding a target with high affinity and specificity. Molecular imprinting technique is based on the use of molecular imprinting for the formation of specific recognition sites in the synthetic polymers through the use of template or imprinted molecule.

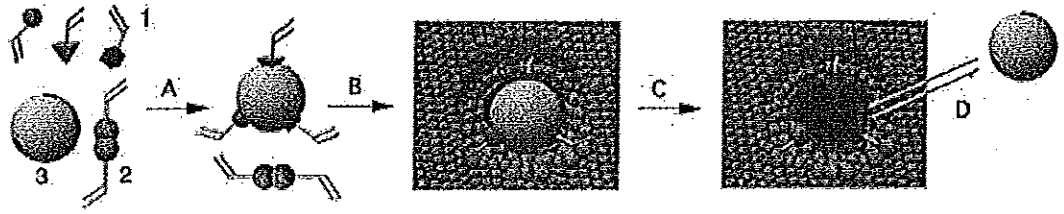


Fig. 4.8 The molecular imprinting principle, 1: functional monomers; 2: cross linkers; 3: template molecule (Pichon and Haupt, 2006).

Molecular imprinting is a process in which functional and cross linking monomers are copolymerized in the presence of target compounds (Fig 4.8, step A) which is used as template or imprint molecule and following the polymerization, these functional group are held in the position by highly cross linked polymeric structure (Fig 4.8, step B). Subsequent removal of the imprint molecule provides the binding

sites which is complementary in size and shape to the desired target or analyte (Fig 4.8, steps C and D) (Pichon and Haupt, 2006). The complexes between the monomers and imprinted molecule can be produced through reversible covalent or non-covalent interactions. The latter is more preferable since it is more flexible with regard to the choice of functional monomer, possible target molecules and use of imprinted materials. In addition, after polymerization the template can be removed by simple solvent extraction technique whereas the covalent imprinting needs to be removed by the chemical cleavage which is more difficult (Pichon and Haupt, 2006).

Most reported MIPs were prepared in bulk which is the most widely used method for MIPs preparation due to its simplicity. To obtain the particles MIPs suitable for each application, the bulk MIPs was then crushed, ground and sieved to get the optimal particle sizes. The last step is the removal of too fine particles to avoid the clogging of the cartridge or column when using it as packing material. To use the MIPs particles in a solid phase microextraction, the suitable particle size is in the range of 25-50 μm and this technique is named molecular imprinted solid phase extraction (MISPE). This MISPE can be used in various applications in the sample preparation step for selectively extract only the target molecule that have been printed as a template (Puoci et al., 2007; Puoci et al., 2005; Yin et al., 2006; Zhu et al., 2005). One of the applications is the use of MISPE for selective extraction of cholesterol in yolk sample. The sample was passed through MISPE after performing the saponification to remove fat and extract nonsaponified chemicals (including cholesterol) from the samples. The extractant was then analyzed by gas chromatograph with flame ionization detector (Shi *et al.*, 2006). The chromatograms obtained from the analysis of cholesterol in yolk samples without any clean up procedure or using C18 or MISPE cartridge for the purification of the extracted are shown in Fig. 4.9.

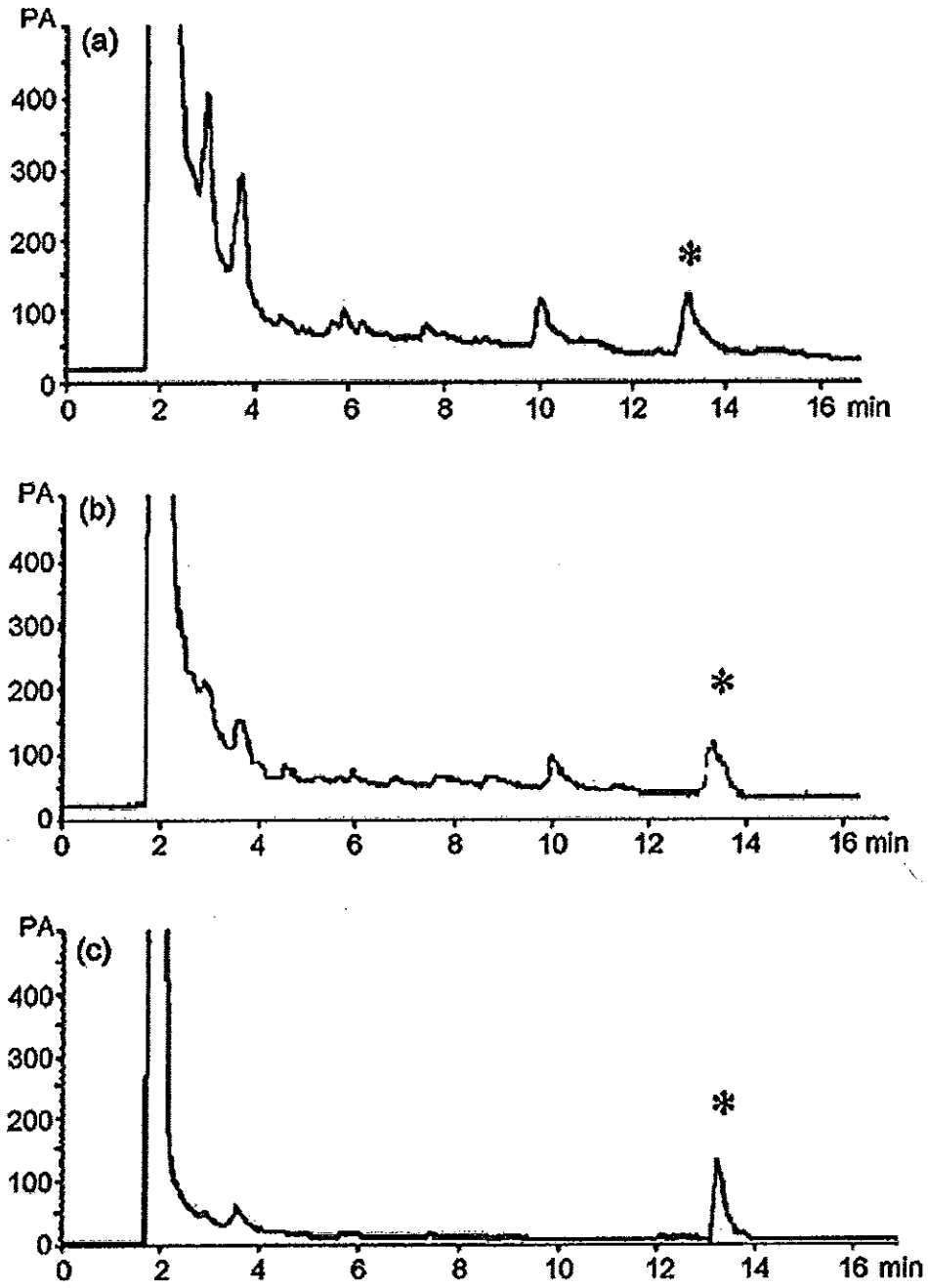


Fig. 4.9 Chromatograms from the analysis of cholesterol in yolk sample by gas chroma-tography after: (a) saponification; (b) saponification followed by C18 SPE; (c) saponification followed by MISPE. Spiked concentration: 50 μg mL^{-1}. (Shi *et al.*, 2006).

From Fig. 4.9, the obvious advantage of MISPE over the normal C18 cartridge is expressed. By the use of C18 SPE cartridge, some impurities are still present after purification, whereas no impurity or contaminant occurs in the case of using MISPE because of its selectivity.

In addition, MIPs particles can be packed in a small column (20-50 mm × 2-4 mm I.D.) to be coupled on-line with liquid chromatography (Caro *et al.*, 2003; Pichon, 2007) or some other techniques (Figueiredo *et al.*, 2007) which is called on-line MISPE. This small column can be placed before the analytical column (in case of liquid or gas chromatography) or the analysis system (spectrophotometer) to provide the on-line sample preparation/preconcentration before analysis.

4.6.2.3 Boronate affinity adsorption

Boronic acids and their derivatives are capable of forming covalent linkages with 1,2- and 1,3-*cis*-diol moieties with high affinity through reversible ester formation as shown in Fig. 4.10 (Foettinger *et al.*, 2005; Kataoka *et al.*, 1998; Liu *et al.*, 2005; Potter *et al.*, 2006; Springsteen and Wang, 2002).

The binding process of boronic acid with *cis*-diol can be explained as follows, in an aqueous solution, boronic acid can exist in either the neutral trigonal planar (Fig. 4.10, **1**) or tetrahedral anionic forms (Fig. 4.10, **2**) but at low pH, boronic acid exists in an uncharged form (Fig. 4.10, **1**), which is not ready to bind with *cis*-diols although it can result in the formation of a strained molecule (Fig. 4.10, **3**) which can easily be hydrolyzed. However, the strained complex **3** can react with OH^- to form the more stable tetrahedral molecule (Fig. 4.10, **4**) which possesses a negative charge. At pH value above the pKa~8.8 (Liu and Scouten, 1994), boronic acid exists in the form of tetrahedral state (Fig. 4.10, **2**) which binds with *cis*-diol more readily. Consequently, in most applications of these boronate analogues, the pH must be in the basic range, *i.e.*, higher than 8, for the effective binding of compounds containing target molecules (Lee *et al.*, 2004). From the binding principle between boronate and *cis*-diols, these ligands have been widely used in a sample preparation step for numerous dihydroxy compounds (Tsikas, 2001).

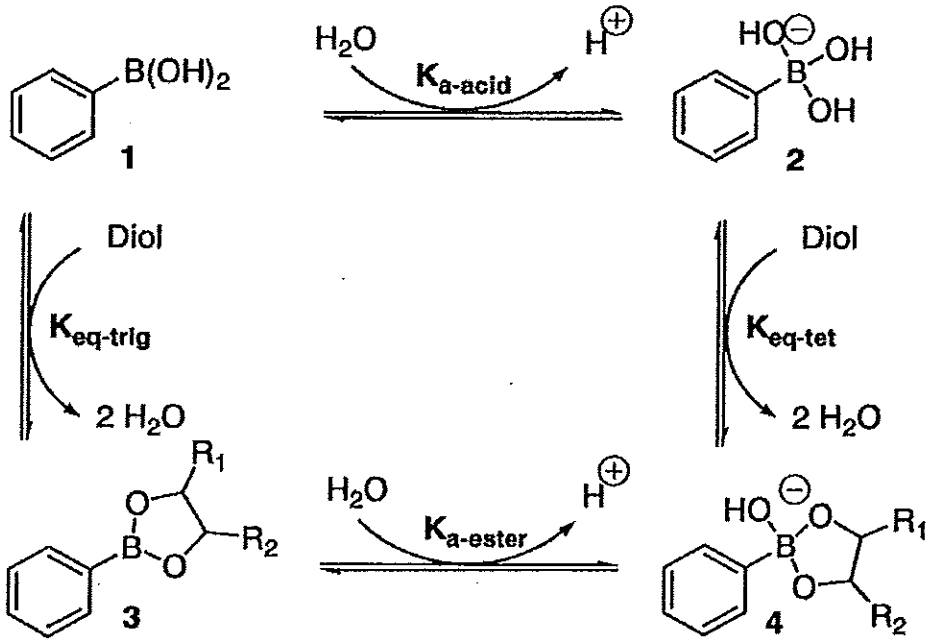


Fig. 4.10 The overall binding process between phenylboronic acid and a diol.

One of the applications is the use of the boronate ligand as an affinity column which was first introduced by Mallia and coworkers in 1981 for the determination of glycohemoglobin. An agarose gel was used as the matrix for the immobilization of the boronate ligand, the bound and unbound hemoglobin fractions in human hemolysate samples were detected by using the ultraviolet/visible detection at 414 nm. The elution was performed by passing through the column a soluble diol-containing agent such as sorbitol that displaced the retained glycohemoglobin from the column (Mallia *et al.*, 1981).

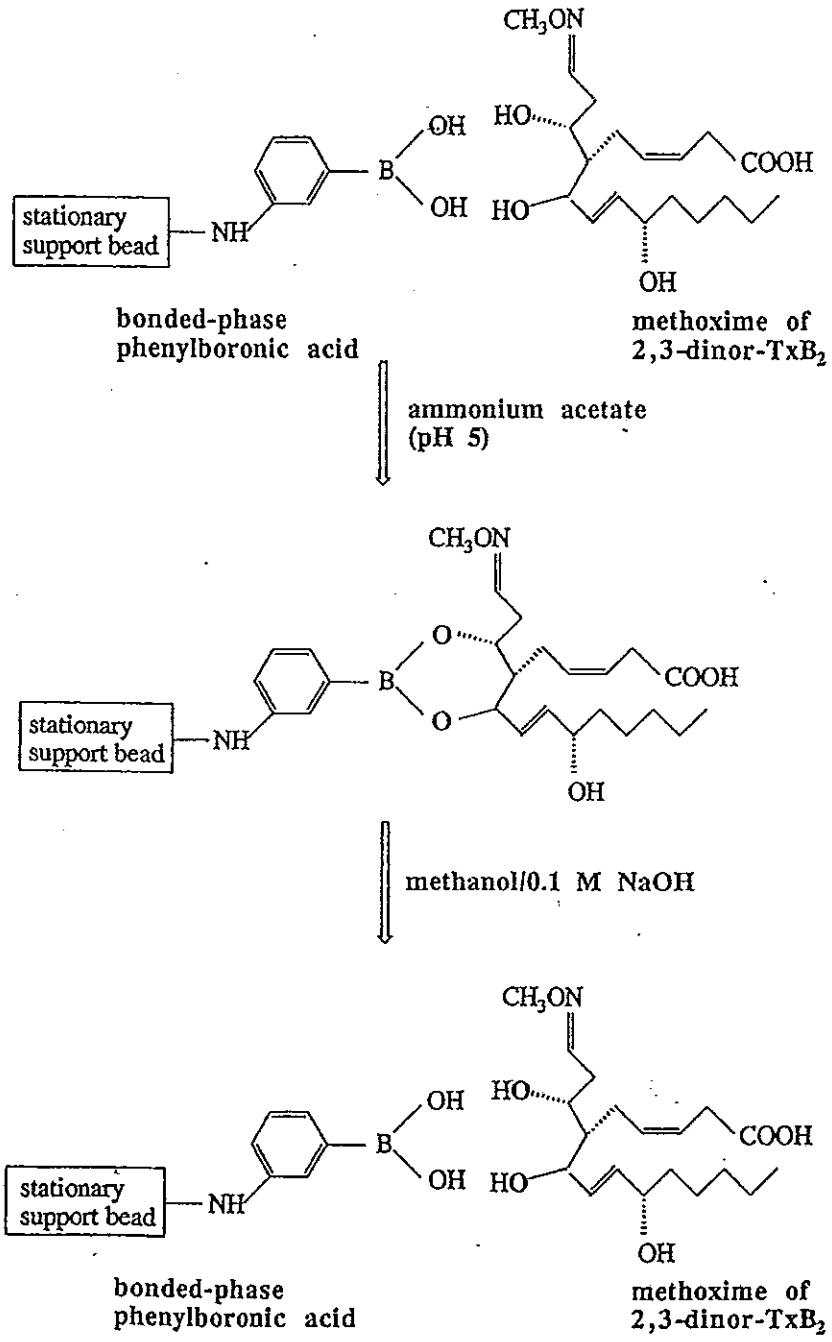


Fig. 4.11 The binding affinity of phenylboronic acid with 2,3-dinor-thromboxane B_2 (Lawson *et al.*, 1985).

Another application is the use of boronate affinity adsorption for solid phase extraction to clean up the sample and preconcentration of the target compounds before second analysis. An example is the use of boronate affinity for

preconcentration of thromboxane B_2 (TxB_2) and its major metabolites which are 2,3-dinor-TxB_2 and 11-dehydro-TxB_2 (Lawson *et al.*, 1985; Tsikas, 2001). All 1,3-diols were found to bind with the bonded phase phenylboronic acid as shown in Fig 4.11 (only 2,3-dinor-TxB_2 is shown). This interaction can be explained as a result of the tendency of the planar phenyl group to orient themselves so that their π orbitals align, thereby forcing the boronic acid groups too close together to admit sterically fixed cyclic 1,3-diols (Tsikas, 2001).

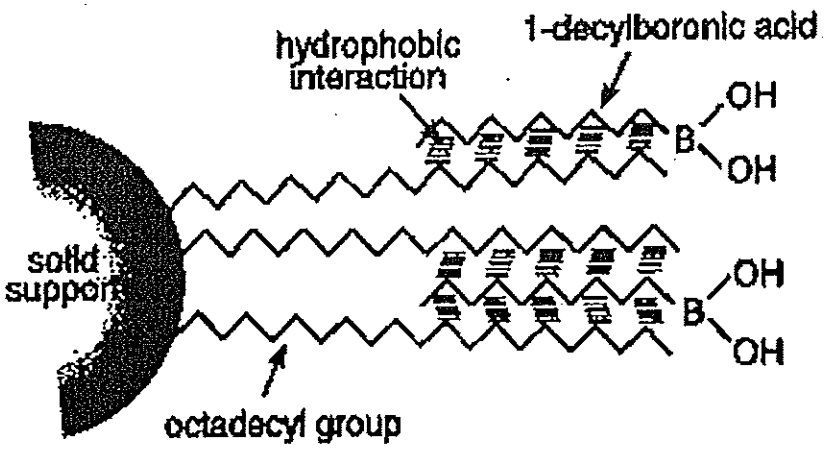


Fig. 4.12 Immobilization of an alkylboronic acid on the support surface *via* hydrophobic interaction.

According to a good binding of boronate to *cis*-diols which is present in some sugars, several works involved in the use of boronate ligands for selective binding to sugar have been published, for instance in 2002, Soh and coworkers used the stationary phase modified with alkylboronic acid as a separation column in liquid chromatography for the separation of six monosaccharides (glucose, mannose, galactose, arabinose, sorbose, and fructose). Fig. 4.12 showed the immobilization of 1-decylboronic acid on the column support (Soh *et al.*, 2002). Because of the high affinity interaction of boronate to *cis*-diol of sugars, the work using this benefit will be discussed in Chapter 7.

4.7 Conclusions

From the principle of affinity sample preparation mentioned above, the important parameters needed to be considered have been stated in this chapter. Affinity adsorption provides more advantageous than normal adsorption since in this mode, high selectivity to the target compounds was obtained, resulting in high purity of the target compounds, and consequently high enrichment factor.

Even though there are lots of affinity ligands available for a wide range of applications but the development of this technique is still growing and becoming accessible to a constantly increasing number of analysts all over the world (Tsikas, 2001). The main aim of this technique is to develop new ligands and affinity matrix which are more selective, robust and repeatable for the target molecule.

CHAPTER 5

Cost Effective Passive Sampling Device for Volatile Organic Compound Monitoring

5.1 Introduction

VOCs are commonly found in the environment, work place and consumer products (Begerow *et al.*, 1996; Schneider *et al.*, 2001; Zabiegała *et al.*, 2002). As a result human are easily exposed to these chemicals through skin, breathing and eating. Intake of these compounds, even at low concentration, can lead to long term health risks (Shojania *et al.*, 1999). Evidence on animal studies indicates that some of these VOCs have carcinogenic or mutagenic effects on tissue development. Benzene is one of these VOCs which is classified by US EPA as a human carcinogenic compound group A (US EPA, 2000b). Benzene, toluene and xylenes, known as BTX, are also used as markers for human exposure to VOCs. Therefore, the monitoring of these three compounds is necessary in order to evaluate the risk to human health (Begerow *et al.*, 1996).

Analysis of VOCs in ambient air with the concentrations in the range of hundreds of ppb to tens of ppt, required a preconcentration step to improve the sensitivity as well as the limit of detection of the method (Wang *et al.*, 1999). Most of the preconcentration steps were performed after sampling causing the error of the results from the delay between these two steps. Therefore, the sampling and at the same time preconcentration on solid sorbent was introduced.

Sampling of VOCs on solid sorbents was done by either active or passive sampling techniques (Batterman *et al.*, 2002; Gouin *et al.*, 2005; Periago *et al.*, 1997; Prado *et al.*, 1996). In active sampling, a device such as a pump is required to force the flow of the sample through the medium. A flow meter measuring the flow rate and volume of air is also necessary. In passive sampling, the analytes flow through the collecting medium without any force follows Fick's first law (Górecki and Namieśnik, 2002). The main sampling process occurs from the concentration

gradient between two media (sample and collecting media). Therefore, the equipment uses for sampling is not complicated as in active sampling. Furthermore, passive sampling can provide results which are as accurate as active sampling (Zabiegała *et al.*, 2002) with much more advantages. It is simple and cost effective, can be put on high risk area and several samples can be collected at the same time. Consequently, passive sampling becomes more and more attractive and acceptable (Zabiegała *et al.*, 2002).

Since commercial passive samplers are still quite expensive, for example one package of SKC Ultra I Passive Sampler (5 items) costs $180 (©SKC Gulf Coast Inc.). Therefore, this work focused on the development of a simple and economical laboratory-built passive sampling system for air monitoring. The developed system was evaluated and then applied to real air monitoring for BTX in gasoline stations in Hat Yai, a major city in Southern Thailand.

5.2 Methods

5.2.1 Laboratory-built passive sampler and thermal desorption device

Tenax TA was used as the adsorbent because it has been shown to adsorb BTX (Wideqvist *et al.*, 2003; Zabiegała *et al.*, 2002). The special properties of Tenax TA are suitable for purging and trapping of VOCs from high moisture content samples including the analysis of compounds in water (Scientific Instrument Service Inc.), making it suitable for Hat Yai, a city with high humidity (The Weather Underground, Inc., 2005). Before used, Tenax TA was activated, through the clean-up process, by heating at 300°C for 2 hours under purging of pure nitrogen gas. This process was to remove contaminants from the adsorbent before applying it to standards or samples (Scientific Instrument Service Inc.).

Glass bottles, (10 ml, 67.6 mm high, 10.6 mm I.D.) with screw caps, filled with 75 mg of activated Tenax TA (60/80 mesh, Supelco, USA) were used as passive samplers. The adsorbent was retained in the bottle by placing a stainless steel net on its top. When sampling, four bottle samplers which hung inside an up-side down open box (Fig. 5.1) were placed at each site. The box was used to protect the

passive samplers from rain and dust. On the box, there was a label informing people of its purpose.

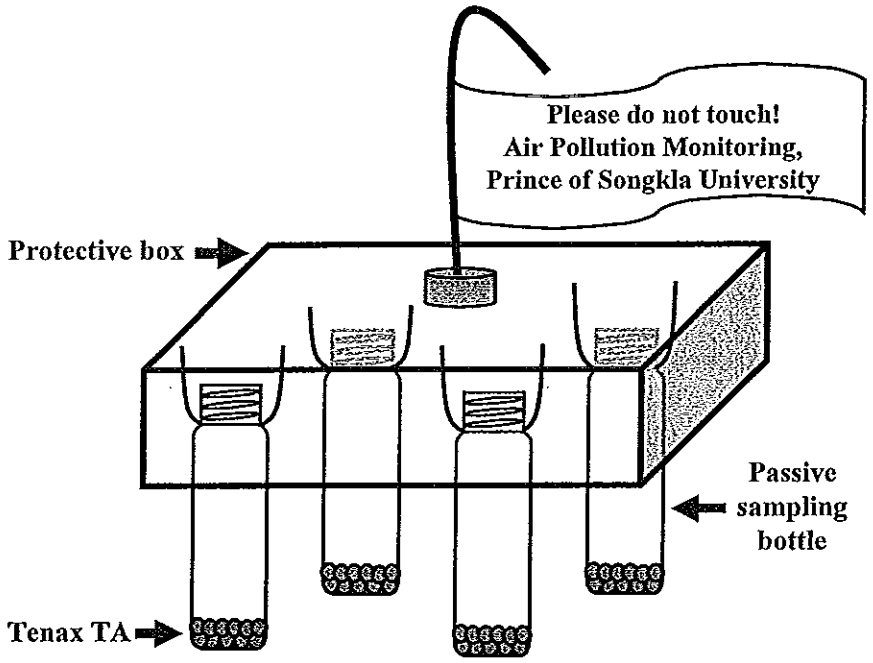


Fig. 5.1 Laboratory-built passive sampler.

A simple and effective thermal desorption device (Fig. 5.2) was constructed using a block of brass (2.9 cm I.D., 1.3 cm high) and a soldering iron (ERSA 30, Germany). The soldering iron was inserted into a tightly fitted hole, drilled into the bottom of the brass block, to generate heat. The temperature of the brass block, acting as a heating plate, was calibrated and monitored by a thermocouple-multimeter (DL 297T digital multimeter, Universal Enterprises, Inc. Korea). The temperature could be as high as 250°C.

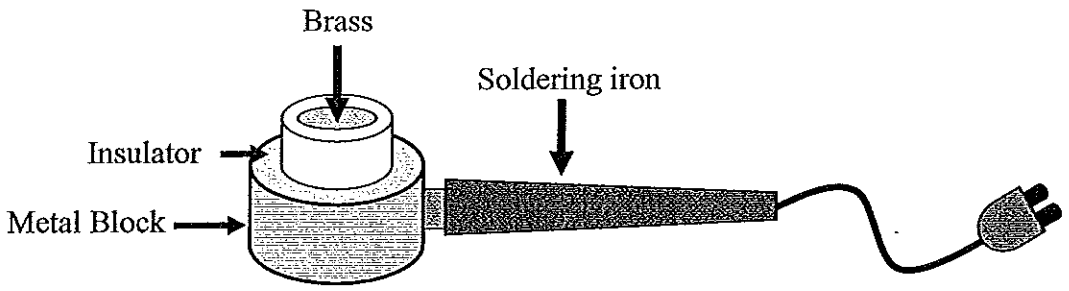
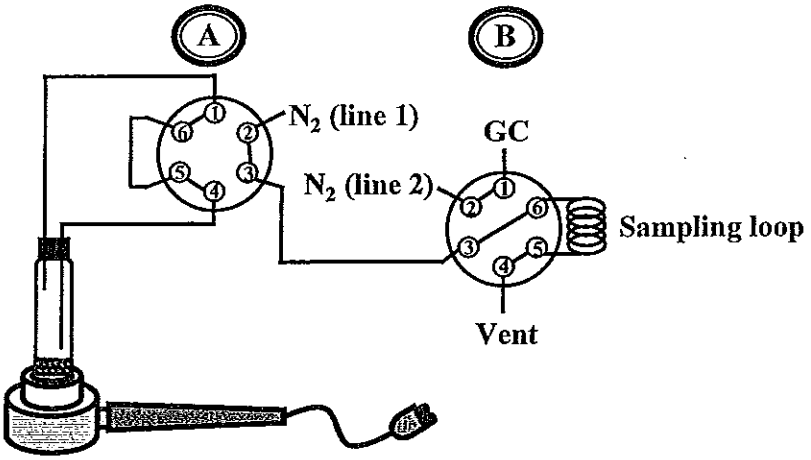


Fig. 5.2 Laboratoty-built thermal desorption device.

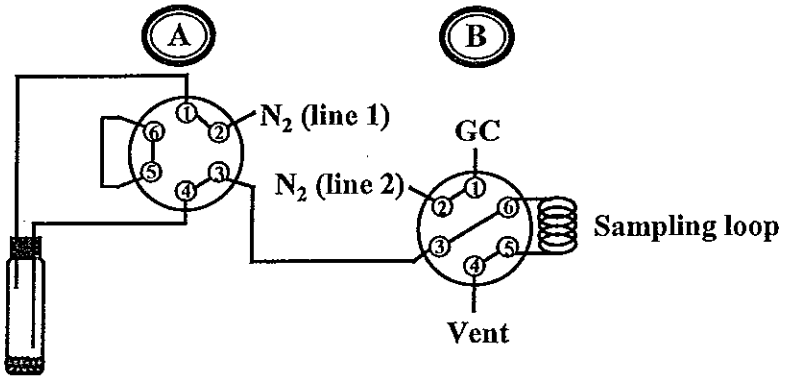
5.2.2 Chromatographic analysis

All analyses were performed using a gas chromatograph, Shimadzu GC-14A (Shimadzu, Japan) with a 30 m, 0.53 mm and 1.00 μm, VB-WAX capillary column (ValcoBond, USA) equipped with a flame ionization detector. High purity nitrogen was used as carrier gas (99.99%, Thai Industrial Gases Public Company Limited, TIG, Thailand). Data processing was carried out with a C-R4A Chomatopac (Shimadzu, Japan). The gas chromatographic conditions were first optimized by varied only a single parameter while other parameters were kept constant and the criteria used for consideration of the optimum condition were high response, short analysis time and good separation.

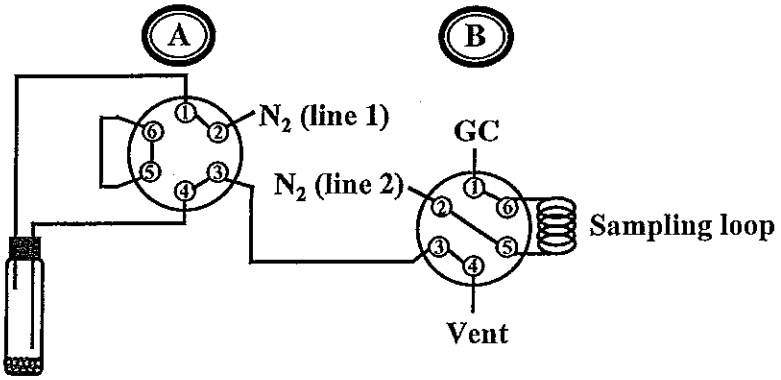
Standards of benzene, toluene and xylene used for the optimization were prepared by introducing 5 mL of each pure compound into 3 different 60 mL of headspace vials. It was sealed immediately with a rubber stopper and an aluminum crimp top cap. Each standard was allowed to vaporize at room temperature for 30 minutes. Vapor phase of 10 μL of benzene and toluene and 50 μL of xylene were collected from the vial and injected into the passive sampling bottles and left to stand for 5 minutes at room temperature (25°C). The amounts of the injected analytes were calculated using ideal gas law. The adsorbed analytes were then thermally desorbed for 1 minute by the laboratory-built thermal desorption device (Fig. 5.2) and purged with nitrogen gas at flow rate 60 mL min^{-1} for 5 seconds and consequently injected into the gas chromatograph. This optimization was performed to achieve the best analysis performance.



a. Heating



b. Purging



c. Injecting

Fig. 5.3 Schematic diagrams showing the operation of thermal desorption and purge system.

5.2.3 Laboratory-built purge and trap system

In the purge system (Fig. 5.3) two six port valves, A and B, were used to switch nitrogen gas between the purge line (to the passive sampling bottle) and the carrier line (to GC-FID). The operation of this system was carried out in three steps.

Step I: heating, valve A was in the position that allowed nitrogen (line 1) to flow through the sampling loop to the vent line while valve B allowed nitrogen (line 2) to pass directly to GC-FID. In this step, the passive sampling bottle was heated by the laboratory-built thermal desorption device without purging.

Step II: purging, valve A was then switched to allow nitrogen (line 1) to pass through the passive sampling bottle. The analytes were purged and carried to a 1.0 ml sampling loop in valve B.

Step III: injection, in this step valve B was switched to allow nitrogen (line 2) to carry the analytes from the sampling loop and injected to GC-FID.

The optimum desorption parameters such as desorption time, purging flow rate, purging time were investigated with the same concentration as mentioned in section 5.2.3.

5.2.4 Calibration

To calibrate the system, appropriate volume of benzene, toluene and xylene vapor phases from the standard vial prepared as mentioned in section 5.2.2 were injected into the bottles containing activated Tenax TA. The injected amounts were in the range of 0.01-640, 0.14-225 and 0.2-7.3 μg for benzene, toluene and xylene, repectively. Each bottle was then connected to the purge system shown in Fig. 5.3. The analytical procedure followed the three steps described in section 5.2.3 and the analysis was carried out by GC-FID. Calibration curves were obtained by plotting the responses *versus* the injected amounts of analytes.

5.2.5 Validation of the laboratory-built passive sampling bottles

Since the sampling bottle has a neck with a smaller cross section than the body and this may affect the diffusion process, an experiment was set up to test the system in a room where the temperature was controlled at 25°C. Toluene is used as a representative standard gas because the values of its boiling point, vapor pressure, and diffusion coefficient are between the other two compounds (benzene and xylene). Toluene standard gas was generated from its liquid form using the diffusion cell which was connected to a glass chamber where the analyte was diluted by incoming air from an air pump (Fig. 5.4). The air flow rate was 1.35 L min^{-1}. Laboratory-built passive samplers were placed at the end of the chamber to collect the analytes that diffused into the bottles and adsorbed on the surface of Tenax TA. After 3 hours the passive sampling bottles were removed from the chamber, each bottle was connected to the purge system in Fig. 5.3 and the analysis was carried out by GC-FID. From the response the uptake amount of toluene could be obtained from the calibration curve done prior to the experiment. Validation was done by comparing the uptake amount of toluene obtained experimentally to the value determined theoretically.

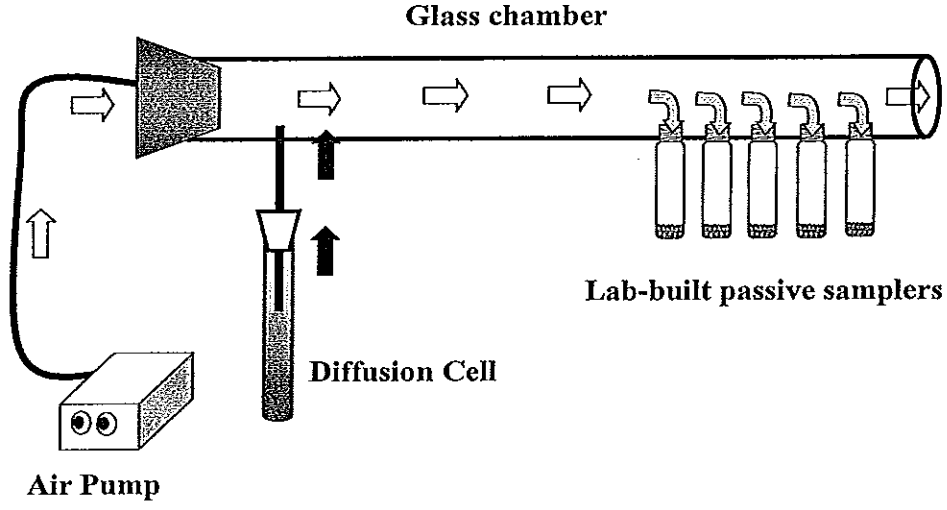


Fig. 5.4 System used to validate passive sampling bottles. Solid arrows show the route of standard gas that diffused to the chamber and was diluted by air from the air pump. The analyte was then collected in passive sampling bottles.

5.3 Results and discussion

5.3.1 Optimum conditions for GC-FID and purge and trap system

First, gas chromatographic conditions were optimized to obtain high response, short analysis time and good peak separation.

Initially a sampling loop was not used in the system (shown in Fig. 5.3) and the analytes released from the adsorbent were continuously flowed through the GC. Although the responses were high but the chromatograms gave poor resolutions. Therefore, a sampling loop was incorporated into the system to allow the "plug" injection of the analytes and sharp chromatograms were obtained.

For desorption, optimization of heating time was necessary to provide a high desorption efficiency without the degradation of the adsorbent and adsorption performance. The flow rate of purge gas and the time for purging must also be considered to minimize the loss of the analytes. If the time used in purging step was too long, the analytes might be lost through the vent and if it was too short, only a small amount of the analytes would be injected to GC-FID, resulting in low sensitivity. These parameters and the optimum purging and desorption conditions were investigated and summarized in Table 5.1. These conditions would be used for further studies.

Table 5.1

Optimum gas chromatographic, desorption and purge conditions for benzene, toluene and xylene (BTX).

Parameter	Optimized range	Optimum value
Carrier gas (N_2)	1-6 mL min^{-1}	4 mL min^{-1}
Fuel gas (H_2)	10-50 mL min^{-1}	30 mL min^{-1}
Oxidant gas (Air)	100-400 mL min^{-1}	300 mL min^{-1}
Column temperature program		
Initial temperature	30-60°C	40°C
Initial holding time	0-3 min	0 min
Ramp rate	2-14°C min^{-1}	4°C min^{-1}
Final temperature	90-120°C	110°C
Final holding time	2 min	2 min
Detector temperature	180-220°C	200°C
Heating or desorption time	30-120 s	60 s
Purging flow rate	40-80 mL min^{-1}	50 mL min^{-1}
Purge time	2-10 s	6 s

5.3.2 Validation of the passive sampling bottles

The theoretical uptake amount of toluene in the passive sampling bottle can be calculated using the following equation (Batterman *et al.*, 2002; Górecki and Namieśnik, 2002);

$$Q = \frac{AD(C_1 - C_0)t}{L} \qquad (5.1)$$

Where Q is the uptake amount of the analyte in the bottle (μg), A is the cross section area of the sampler (1.62 cm^2), D is the diffusion rate of analyte which is toluene in this case (8.5×10^{-2} cm^2 s^{-1} at 25°C) (Danish Ministry of the Environment, 2002), C_1 and C_0 are the concentrations of the analyte in the chamber (9.46×10^{-7} g L^{-1}) and at

the surface of the sampler (0 g L^{-1}), respectively, t is exposure time (10800 s) and L is the diffusion length (6.47 cm).

Concentration of toluene in the chamber, C_1, was obtained as follows. First, the concentration in the test tube of the diffusion cell (C_d) was calculated using ideal gas law (equations (5.2) and (5.3)).

$$C_d = \frac{n}{V} \tag{5.2}$$

$$C_d = \frac{P}{RT} \tag{5.3}$$

Where C_d is the concentration in the test tube of the diffusion cell (mol L^{-1}), P is the vapor pressure of toluene at 25°C (atm), R is the ideal gas constant (L atm mol K^{-1}) and T is the absolute temperature (K). Using equation (5.3), the concentration in the test tube of the diffusion cell was found to be 1.53×10^{-3} mol L^{-1}. Then the mass transfer rate (diffusion rate, Q/t) between the test tube and the chamber through the capillary connecting tube was obtained using Fick's first law as shown in equation (5.4).

$$\frac{Q}{t} = \frac{DA_c(C_d - C_1)}{L_c} \tag{5.4}$$

Where $\frac{Q}{t}$ is the mass transfer rate or diffusion rate (mol s^{-1}), D is the diffusion coefficient of toluene, A_c and L_c are the cross section area and length of the capillary tube of the diffusion cell, C_d and C_1 are the concentrations of toluene in the test tube and the chamber, respectively. The results showed that mass transfer rate is 2.31×10^{-10} mol s^{-1}. Knowing the air flow rate the concentration of toluene in the chamber, C_1, could be calculated from the mass transfer rate. Using equation (5.1), the uptake amount of toluene (Q) obtained from the calculation was 0.22 μg.

To obtain Q experimentally, a calibration curve was first established as described in section 5.2.5. After 3 hour exposure time, each passive sampling bottle was connected to the system in Fig 5.3. Peak heights obtained from the GC-FID were converted to the amount of toluene, Q in equation (5.1), using the linear equation of the calibration curve done prior to the experiment. The amount of 0.21 ± 0.02 μg of toluene was obtained for 5 passive sampling bottles. The results showed that there was no difference between the two values indicating that the laboratory-built passive samplers can certainly be used for monitoring of benzene, toluene and xylene in the environment.

5.3.3 Performance of the system

Linear dynamic range of an analytical method is the ability to obtain test results of variable data which are directly proportional to concentration or the amount of analyte in the sample. It was investigated by using different volumes of vapor phase of standard solution that could be converted into the amount of the analytes. Linearity is achieved when the coefficient of determination (R^2) is equal or greater than 0.99 (Chung Chaw, 2004). This system provided wide linear range as shown in Fig. 5.5.

Linear dynamic ranges and linear equations of the system were shown in Table 5.2. The linearity of xylene was less than benzene and toluene. Xylene is the last compound coming out from the chromatographic column and its peak shape is broader than that of the other two compounds due to its high molecular weight and low volatility. Consequently, peak heights of xylene increased only slightly with concentration. In contrast benzene and toluene which have sharper peaks provided better results (wider linear dynamic range).

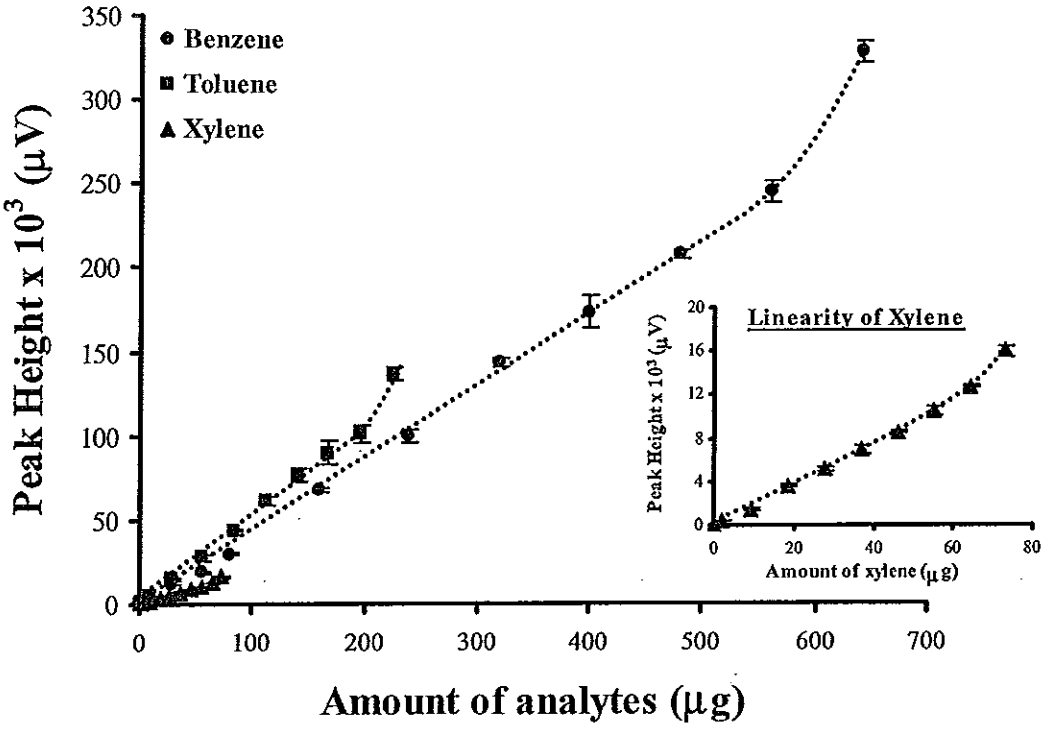


Fig. 5.5 Linear dynamic range of benzene, toluene and xylene.

Table 5.2

The linear equation of benzene, toluene and xylene obtained from the laboratory-built passive sampling system.

Compound	Linear dynamic range (μg)	Equation	R^2
Benzene	0.01-560	y = 0.4378x - 1.9802	0.9991
Toluene	0.14-197	y = 0.5259x + 0.0569	0.9983
Xylene	0.23-64	y = 0.2075x - 0.3525	0.9901

The values reported in the Table 5.2 are mass uptake (Q). Using 3 weeks of sampling time C_1 could be obtained from equation (5.1). In term of concentration the linearity of the three compounds are shown in Table 5.3.

Table 5.3

Linear dynamic ranges and working calibration curves of the system in term of mass uptake and concentration.

Compound	Linear dynamic range		Working calibration curve		Reported concentration** ($\mu g\ m^{-3}$)
	Mass uptake (μg)	Concentration ($\mu g\ m^{-3}$) *	Mass uptake (μg)	Concentration ($\mu g\ m^{-3}$) *	
Benzene	0.01-560	0.20 -13,254	0.01-80	0.20-1,893	15.1-50.2
Toluene	0.14-197	3.6-5,104	0.14-85	3.6-2,201	25.8-131
Xylene	0.23-64	7.0-1,962	0.23-64	7.0-1,962	-

*Based on 3 weeks of sampling time

** Muttamara, S. and Leong, S. T. 2000. Monitoring and Assessment of Exhaust Emission in Bangkok Street air. *Environmental Monitoring and Assessment* **60**: 163-180.

The research work that was carried out in Bangkok, Thailand (a city which is much more traffic than Hat Yai), reported that, the concentration of benzene and toluene in ambient air were found in the range of 15.1-50.2 and 25.8-131 $\mu g\ m^{-3}$, respectively (Muttamara and Leong, 2000). Therefore, the linear concentration ranges obtained in this work covered these reported concentrations.

For the analysis of real samples, working calibration curves of benzene, toluene and xylene were prepared using 6 to 9 concentrations which covered the concentration that have been reported in Bangkok (Table 5.3). Each concentration was performed for 5 replications. Very good coefficients of determination (higher than 0.99) were obtained for all three compounds (Fig. 5.6).

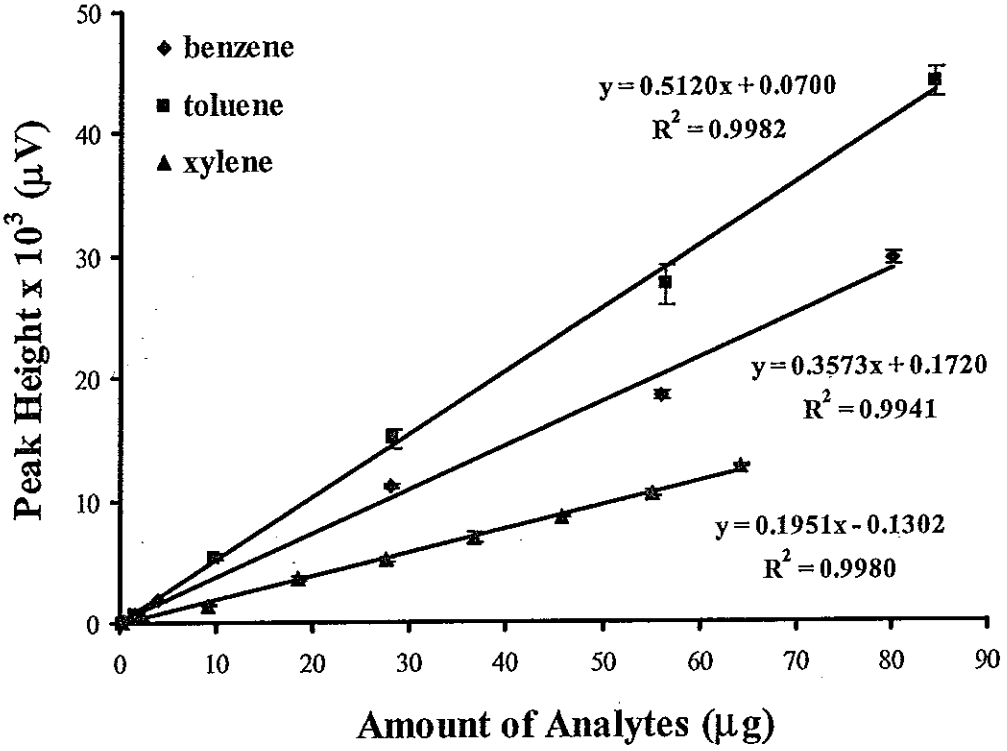


Fig. 5.6 Calibration curve of benzene, toluene and xylene in term of mass uptake or the amount of the analytes which have been adsorbed by the adsorbent in the laboratory-built passive sampler.

Average peak area from 20 blank injections was used to calculate the limit of detection using the IUPAC method (Long and Winefordner, 1983). Mean value of blank response, $\overline{X}_B$, and standard deviation (S_B) were calculated using these following equations;

$$\overline{X}_B = \frac{\sum_{j=1}^{n_B} X_{Bj}}{n_B} \qquad (5.5)$$

$$S_B = \sqrt{\frac{\sum_{j=1}^{n_B} \left(X_{Bj} - \overline{X}_B\right)^2}{n_B - 1}} \tag{5.6}$$

In defining the smallest detectable signal (X_L), IUPAC states that

$$X_L = \overline{X}_B + kS_B \tag{5.7}$$

Where k is a numerical factor chosen in accordance with the confidence level desired and the accepted value is 3 at a confidence level of 99.86%, S_B is the standard deviation for 20 times of injections. C_L was then obtained as a function of X_L.

$$C_L = \frac{\left(X_L - \overline{X}_B\right)}{m} \tag{5.8}$$

Where m is the analytical sensitivity (slope of the calibration curves), and C_L is the smallest concentration that can be detected with reasonable certainty for a given analytical procedure. Because the mean blank reading, $\overline{X}_B$, is not always 0 the signal must be background corrected. Equation (5.9) was obtained after substitution equation (5.7) into (5.8).

$$C_L = \frac{kS_B}{m} \tag{5.9}$$

From the above equation and the data in Table 5.4, limit of detections for benzene, toluene and xylene were found to be 0.31, 0.24 and 0.73 μg m^{-3}, respectively (refer to 3 week sampling time), indicating that this technique is sensitive to detect trace amount of BTX in the environment (Muttamara and Leong, 2000).

5.3.4 Reusability

Before sampling, reusability and storage stability of the samplers were studied. The idea of reusability came from the fact that after desorption, there should be no analytes on the adsorbent, and therefore, it can be reactivated and reused and this would help to reduce cost.

To evaluate how long Tenax TA could maintain the adsorption efficiency, the number of time the adsorbent could be reactivated and reused was studied. Fig. 5.7 shows the example result for benzene. Between 1 and 12 times the response reduced only slightly after each use (1.4%). Afterwards the response showed significant reduction (13%). Similar results were obtained for toluene and xylene which showed that they can be used up to 14 times. Even the results showed that the adsorption efficiency of Tanax TA reduced with using time, however, if the calibration was done with the same adsorbent, this would not be any problem. Consequently, the laboratory-built passive sampler could be used until the adsorbent has no adsorption efficiency, while the cartridge from Radiello® Passive sampling system is not reusable (Radiello®). Therefore, these laboratory-built passive samplers are much cheaper since it costs less than 10 bahts for 1 sampler.

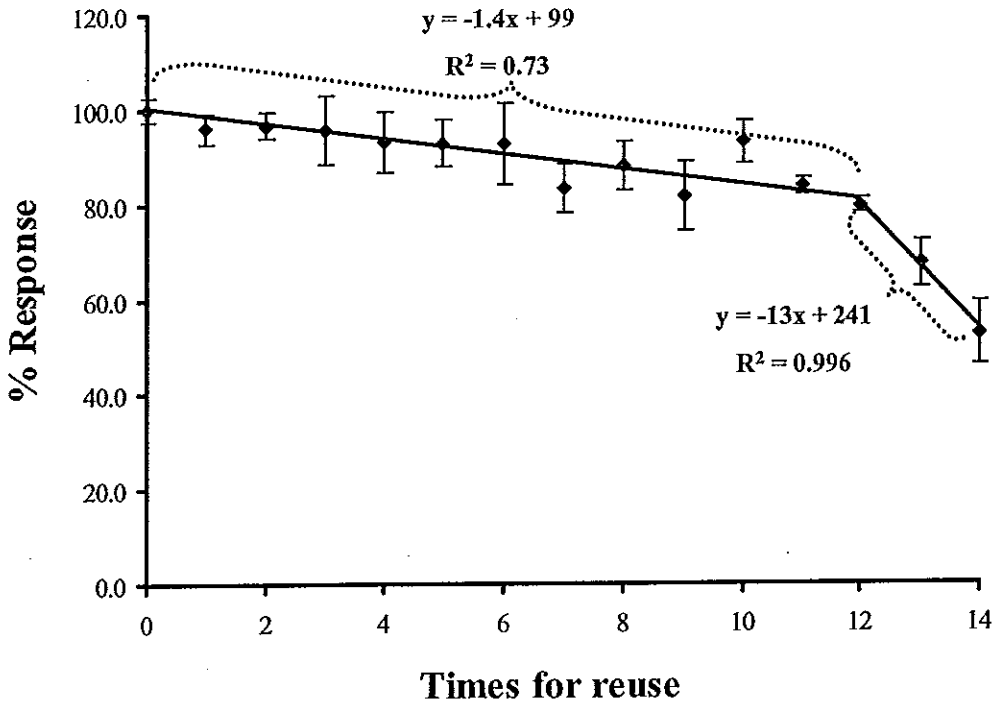


Fig. 5.7 Response of benzene obtained from the laboratory-built passive sampler after the adsorbent that was reactivated and reused.

5.3.5 Storage stability

Storage time of the sampler was studied in the case where the analysis could not be done immediately after the samplers were collected and also to ensure the sample life time before analysis. This was done by placing 45 samplers, with the concentration of the three analytes at 100 times the limit of detection of each compound, in the desiccator at 25°C. At the end of each week, five samplers were analysed and the relationship between the percentages of responses and the storage time was investigated. The responses decreased by 0.52%, 2.7% and 1.3% per week for benzene, toluene and xylene, respectively (Fig. 5.8).

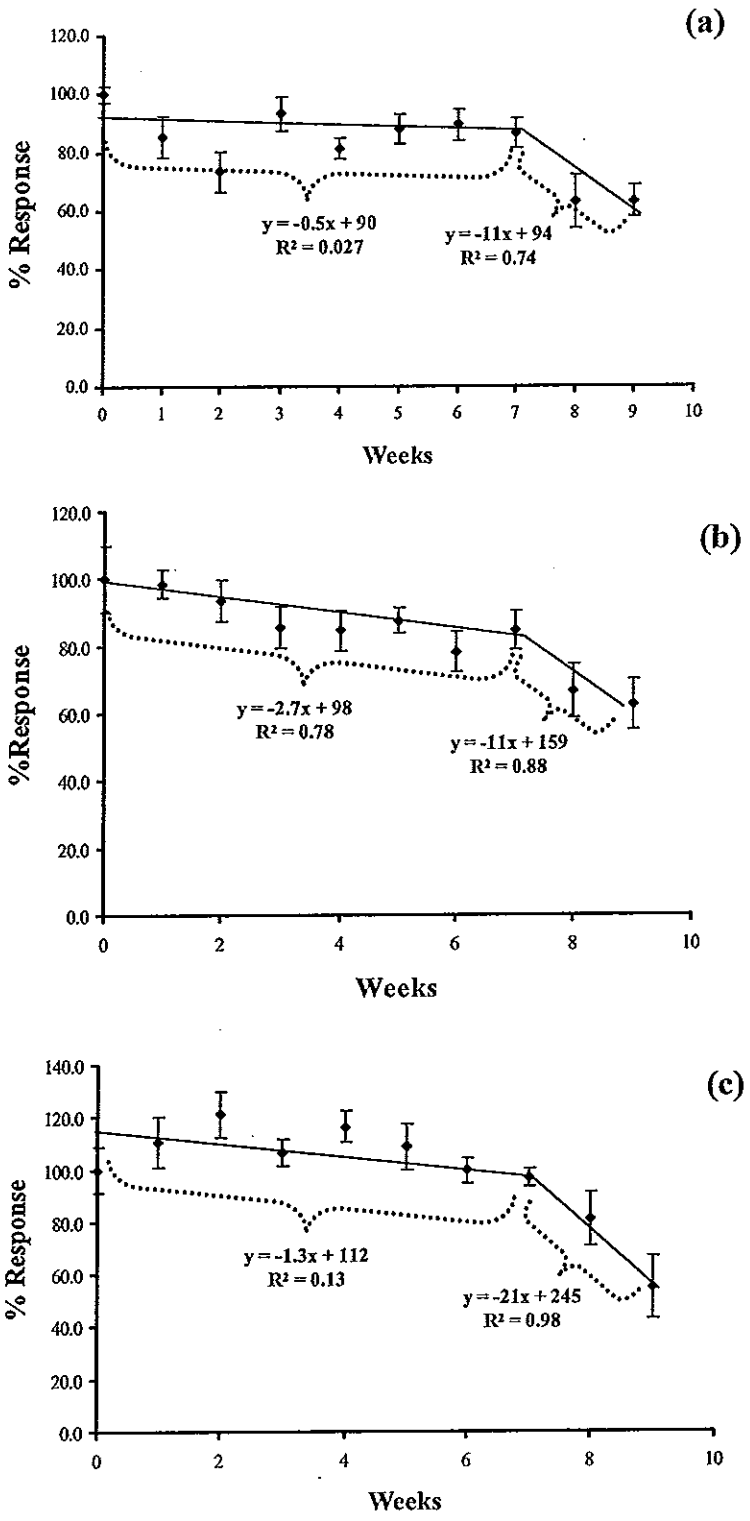


Fig. 5.8 Response of (a) benzene, (b) toluene and (c) xylene obtained from the laboratory-built passive sampler after kept in the desiccator for a period of time.

For storage stability, NIOSH recommends that the average quantitative measurements of the samplers should not differ from the analysis on day 0 by more than 10% (Volden *et al.*, 2005). Using this criterion the laboratory-built passive samplers can be kept in the desiccator at room temperature (25°C) for up to 3 weeks (2.7% per week × 3 weeks = 8.1% for toluene) before being analysed. This is the same storage time recommended by SKC Inc. for their commercial passive sampler that needs to be stored at 4°C (SKC® Inc.) while the storage time for Radiello® is up to three months at 4°C after exposure (Radiello®). Another advantage is the laboratory-built passive sampler has no shelf life. It only needs an activation step to remove the contaminants before sampling. However, before exposure, the commercial passive samplers have the shelf life of 30 days and 18 months for SKC and Radiello® passive samplers for VOCs sampling, respectively.

5.3.6 Sampling time

Sampling time was another parameter investigated. Three groups of gasoline stations were studied. They were classified by their activities. Gasoline stations located far away from the city were classified as low activity. Those located in the city were medium activity, and gasoline stations located in the city near shopping centers were classified as high activity.

Optimization was done by varying the sampling time between 1 and 4 weeks at two gas stations that were selected as the representative of medium and high activity. Four boxes of sampling devices were hung at each sampling site. One box, with 4 passive samplers, was collected and analysed at the end of each week. The results showed that the responses of benzene, toluene and xylene increased as the sampling time increased and started to level off at 1 week for the high activity gasoline station and 3 weeks for the medium one. It means that at these times, the adsorbent capacity was reached or the retro-diffusion phenomenon occurred. Therefore, the sampling time used for high activity gasoline stations was one week, while medium and low activity was three weeks.

5.3.7 Vertical and horizontal distance concentration profiles

To study the change of concentration with distance, one gasoline station was selected as the emission source. The samplers were placed at the gasoline station and at a few sites further away in both directions along the road. The sampling time was three weeks. The effect of distance on the responses is shown in Fig. 5.9 and indicated that the concentration was highest at the gasoline station and decreased with distance except at +80 m. This was because at this point the samplers were placed near the main road and the exhaust was probably contributed to the high value.

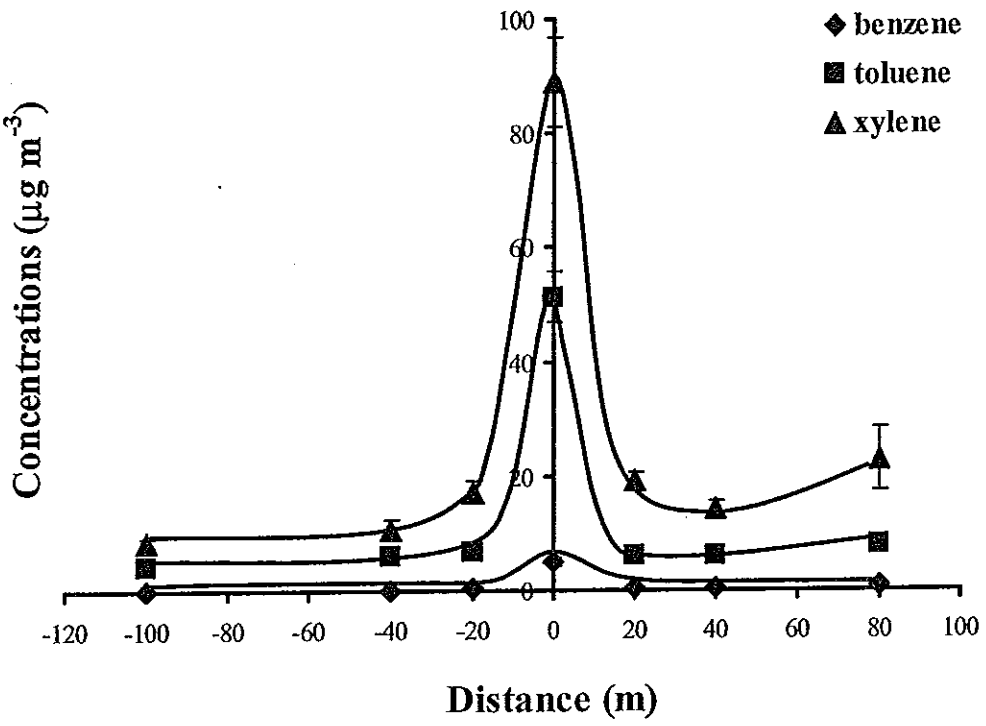


Fig. 5.9 The effect of distance on the concentration distribution.

For the concentration profile in the Y axis, the samplers were placed at the gasoline station at 0.5, 1.0, 1.5, 2.0 and 2.5 meters high from ground level (Fig. 5.10). The highest response was obtained at 0.5 meter since it was the level of the

exhaust pipe of the vehicles. However, the height of 1.50 meters was used for further sampling since it is the average breathing height of Thai people.

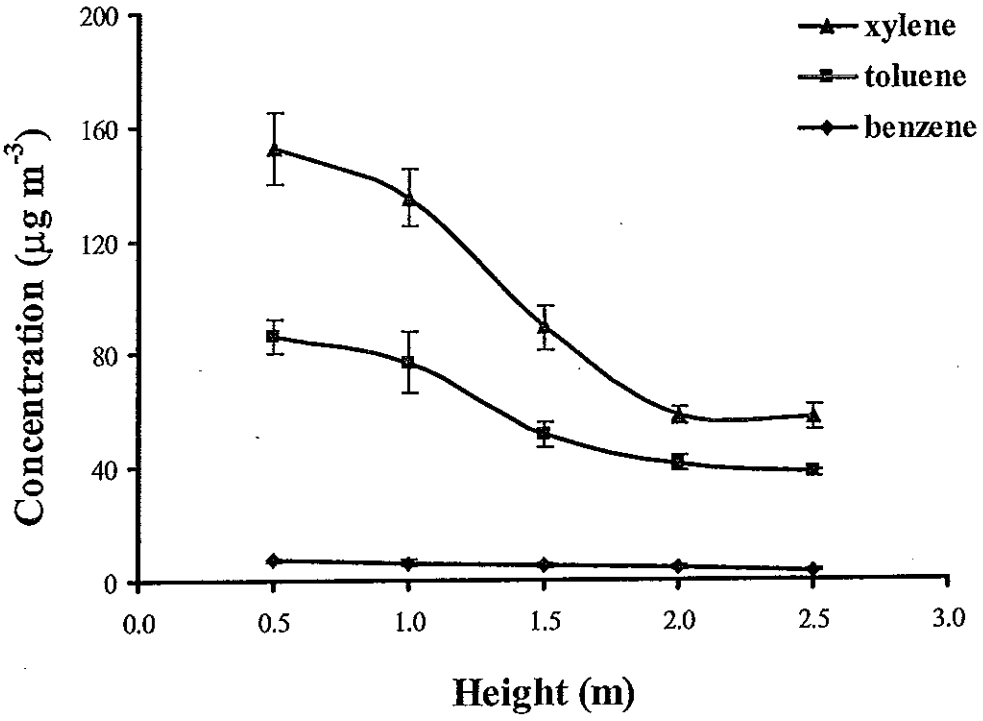


Fig. 5.10 The effect of vertical distance on the concentration distribution.

5.3.8 Applications

Ten gasoline stations located around Hat Yai city, Songkhla, Thailand were selected as the sampling sites since they are possible benzene, toluene and xylene emission sources. 3, 3 and 4 gasoline stations of low, medium and high activity, respectively were chosen as case study. The sampling was done by placing the passive samplers at a height of 1.5 meters for three weeks for low and medium activity and one week for high activity. At all sampling sites, blanks were collected by opened and then rapidly closed the screw caps of the samplers. After sampling time

was terminated, the samplers and blanks were collected and analysed in the laboratory.

The concentration ranges of benzene, toluene and xylene are shown in Table 5.4 and the relative standard deviations of these laboratory-built passive samplers (at the same sampling site) were less than 22%, better than the acceptable value of 25% (NIOSH protocol) (Myers *et al.*).

Table 5.4

The average concentrations of benzene, toluene and xylene from 10 gasoline stations (classified into three groups) compared with the guideline value from OSHA and NIOSH.

Compound	Concentration [μg m^{-3} (%RSD)]			OSHA ($mg\ m^{-3}$)	NIOSH ($mg\ m^{-3}$)
	Low Activity	Medium Activity	High Activity		
Benzene	N.D. – 2.0(5)	2.7(4) – 4.5(7)	5(19) – 19(2)	3.2	0.3
Toluene	12(7) – 17(13)	19(9) – 36(9)	31(7) – 76(10)	375	375
Xylene	23(9) – 31(18)	50(8) – 196(4)	81(22) - 198(3)	435	435

The concentrations of benzene, toluene and xylene at all 10 gasoline stations were lower than the guideline from OSHA and NIOSH (US EPA, 2000a; US EPA, 2000c; US EPA, 2000d). Although the concentrations are still low, the number of vehicles is rapidly increased and this is one of the main sources that contribute these toxic compounds to the environment. Currently these compounds are not monitored by the Pollution Control Department, Ministry of Natural Resource and Environment, Thailand (Pollution Control Department, 2007) but there is a need to monitor these compounds, especially in a big city.

5.4 Conclusions

The laboratory-built passive sampling device coupled with the purge and trap system can be used to monitor BTX in air with better precision (RSD<22%) than the acceptable value of 25% for passive sampling (Myers *et al.*). The sampler is easily prepared by filling a screw capped bottle with Tenax TA. During sampling no additional device is required, and this helps to reduce cost. After analysis the sampler can be reactivated and reused to a total of 12 times and this helps to reduce cost even further. The use of a screw cap bottle makes it easy to rapidly start and stop the sampling. Unlike some commercial sampler where the adsorbent needs to be transferred to another container for analysis, the analysis of the developed passive sampler is done using the same bottle and this helps to reduce analysis error. In the case where analysis can not be done immediately after sampling, the sampler can be kept for up to 3 weeks before being analysed. The wide linear ranges make this system suitable for monitoring a wide scale of concentration of BTX and possibly for other VOCs. This certainly is useful for monitoring and assessing air quality.

CHAPTER 6

Microtrap Modulated Flame Ionization Detector for On-Line Monitoring of Methane

6.1 Introduction

Natural gas contains 75% of methane, the smallest hydrocarbon and most abundance molecule. As a consequent, methane contamination can easily occur even in high purity gases (Medici, 1974; Xu *et al.*, 2003). These gases are generally used for calibrations as well as running or producing many analytical instruments or high quality products such as in the semiconductor fabrication. Methane contamination can become a critical issue since it can lead to a negative impact on production yields. Therefore, measurements and control of the impurities play an important role in high purity gases research applications (Tigeroptics, 2003).

Many methods have been used to determine methane concentration, most of which required two main steps, *i.e.,* sample collection and sample analysis. In a typical situation, samples were collected from the sites and later analysed by injected into a gas chromatograph with a syringe or a gas sampling valve (Panda *et al.*, 1995). Since a large sample injection would cause band broadening and the degradation of the chromatograph resolution so only a small amount of sample (a few microliters to a couple milliliters) was injected and analysed but this will limit the sensitivity of the detector. Therefore, a preconcentration step was needed to provide a higher sensitivity of the analytical method.

The method that is often used to preconcentrate VOCs in air or gas matrix is adsorption on solid adsorbents, such as Tenax TA, Carbopack C and activated charcoal. The desorption can be either by solvent or thermal desorption techniques. For solvent desorption, only a small amount of the desorbed analyte is injected into a gas chromatograph and most of the solvents used are toxic. Therefore, thermal desorption is more widely used since it provides advantages over solvent

desorption, *i.e.,* all desorbed analyte can be injected to the detector, the sensitivity is higher and there is no toxic solvent wastes (Pillonel *et al.*, 2002).

Although sensitivity of the analysis can be increased by a preconcentration step but an error due to the delay between sampling and analysis can still occur (Feng and Mitra, 1998; Mitra *et al.*, 1999; Mitra *et al.*, 1996a) and this would certainly affect the analysis of trace methane in high purity gases. The small size and very high vapor pressure of methane molecule also make it difficult to adsorb on the surface of the adsorbent and suitable adsorbent that can provide high adsorption efficiency was rarely found. Therefore, there is a clear need for effective on-line trap for small molecules.

One development was the use of cryogenic trap. By cooling a short section of the column, the sample can be focused in a narrow band at the head of the column. The trap is designed for rapid cooling and efficient re-heating, ensuring the reproducibility and optimum peak shape. The advantage of this technique is that certain molecules can be preferentially collected before analysis. However, there is one major problem, if the source has a lot of water, it will freeze and plug the tube and will stop the collection process (Rafson, 1998). The cryogenic system is also quite complicated to apply, since some part of the instruments must be modified. Recently, a microtrap was introduced as an alternative.

A microtrap is an automatic, simple sampling and injection device for continuous, on-line GC analysis of organic compounds (Chen *et al.*, 2002; Guo and Mitra, 1999; Mitra *et al.*, 1996b; Mitra and Yun, 1993). It is a small capillary tubing, with a common size in the range of 0.5-1.1 mm. I.D, packed with an small amount of the absorbent. The organic molecules are trapped by the adsorbent while the carrier gas flows through it acted as the background. The retained organics are rapidly desorbed by resistive heating with pulse of electric current which make an injection to the GC for analysis. The main advantage of microtrap as an injection device over a sample valve is that it also serves as a sample preconcentrator which allows the larger sample volume to be analyzed for trace components.

In this work, the development of an on-line microtrap that is interfaced directly to the flame ionization detector for the determination of methane is reported.

6.2 Methods

6.2.1 Instrumentation

All experiments were carried out by a GC equipped with a conventional FID and the data were integrated by C-R4A Integrator (GC-14A and C-R4A Integrator, Shimadzu, Japan). Methane standard concentration of 11.6 ppmv (Thai Industrial Gases Public Company Limited, TIG, Thailand) was used to study the characteristics of the microtrap packed with a suitable adsorbent. By considering the appropriate pore size and surface area, Carbosphere 80/100 mesh (Alltech, IL, USA) was selected as the suitable adsorbent. It was packed in a 15 cm long, 1.02 mm I.D., and 1.59 mm O.D., silicosteel welded/drawn 304 grade stainless steel tubing as shown in Fig. 6.1 (Restek Co., Bellefonte, PA, USA).

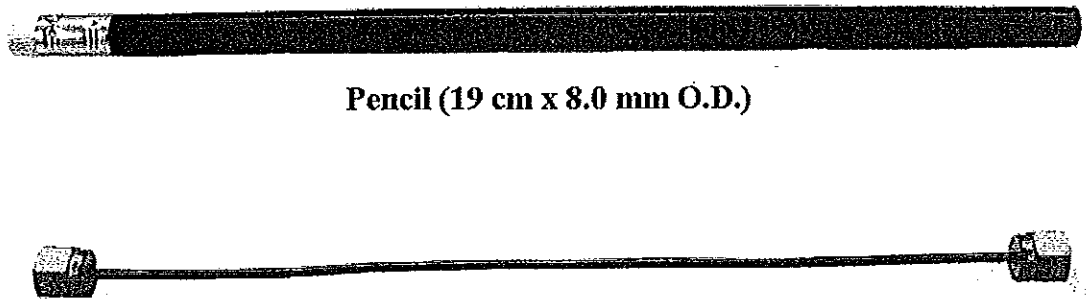


Fig. 6.1 Relative size of a microtrap comparing to a pencil.

6.2.2 On-line microtrap for trace methane analysis

Schematic diagram of the on-line system used for methane monitoring is shown in Fig. 6.2. The microtrap was placed in-line of the stream of the standard gas and in front of the detector to act as a trap/preconcentrator as well as an injector. The microtrap was rapidly heated by applying a current pulse from a custom-built heating system and a temperature as high as 250-300°C was reached within a few

milliseconds. This was to desorb and to inject the analyte into the FID. The effect of heating was similar to using an injection port in term of retention time, peak height, band duration and terminal band length (Mitra *et al.*, 1999). A microprocessor-based timer (GraLab 451 timer, USA) was used to control the interval between the adsorption and desorption times and the duration for which the current was applied.

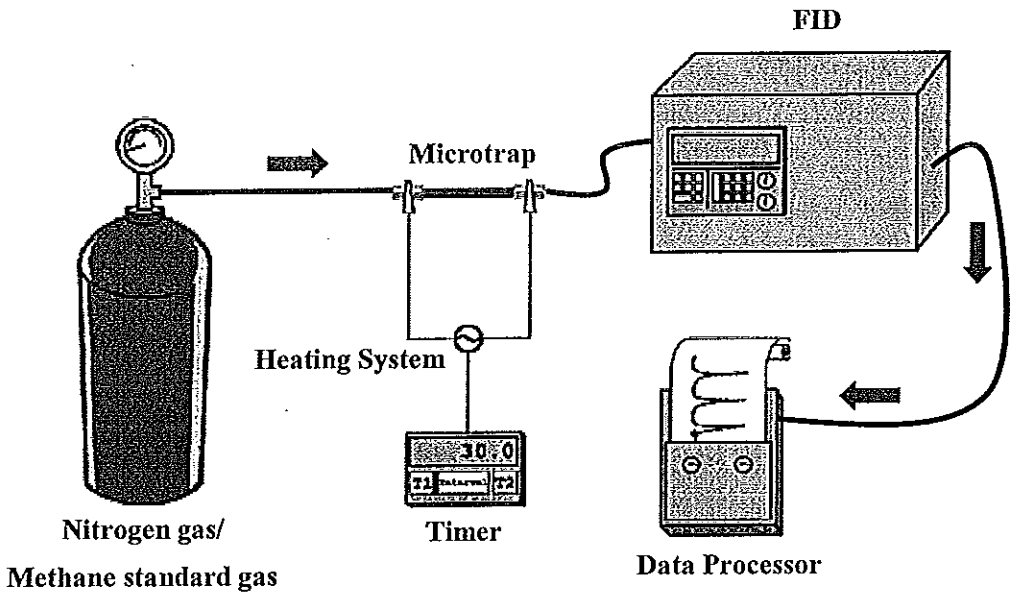


Fig. 6.2 Microtrap interfaced to flame ionization detector for on-line monitoring of methane.

6.2.3 Breakthrough characteristics

To determine breakthrough of this microtrap, the method of using the variation in microtrap response as a function of injection interval was implemented (Panda *et al.*, 1995). The capacity, in term of breakthrough, was studied by passing a stream of standard methane gas (11.6 ppmv) through the microtrap. Methane molecules were adsorbed while the carrier gas served as a background stream. The system was first investigated at room temperature (25°C). Desorption voltage and time were optimized by varying them in the range of 10-50 volts and 1-4 seconds, respectively. The optimization was done by varying only the studied parameter and

kept other parameters constant. The optimum desorption voltage and time were then used to determine the breakthrough by varying the adsorption time until stable response peaks were obtained.

6.2.4 Enhancement of microtrap capacity

The most important factor for trace quantity analysis is the enhancement. An increasing of the enhancement (or enrichment) factor can be obtained by decreasing the microtrap temperature. A microtrap was placed inside an insulating foam box, surrounded by the dry ice. Temperatures were varied from 25°C (room temperature) to -50°C by varying the amount of dry ice. At each adsorption temperature, the optimized desorption voltage and time were investigated to obtain the maximum analyte desorbed from the microtrap. The adsorption time at each temperature was then varied to determine the breakthrough time. The responses were also used to calculate the enhancement.

6.2.5 Performance of the microtrap

Linearity of the calibration curve is another important factor affecting the performance of a quantitative analysis system. To study the linear response of the on-line microtrap system, a series of standard methane concentrations, 11.6, 5.8, 3.9, 1.1 and 0.2 ppmv, was prepared by dynamic dilution method (Grob and Barry, 2004) using nitrogen gas (99.99% Thai Industrial Gases Public Company Limited, TIG, Thailand) as a dilutor. Each of the concentration of methane was continuously passed through the microtrap. The injections were made at 6 minute intervals using a pulse time of 3.5 seconds at 40 volts for five replications at -50°C (optimum adsorption temperature in section 6.2.4). The signals and concentrations were plotted for the calibration curve.

High purity nitrogen gas (99.99%) was used as blank. It was continuously applied through the microtrap. The desorptions, *i.e.,* injections were done every 6 minutes and the obtained data were used to calculate the limit of detection based on IUPAC method (Long and Winefordner, 1983).

6.2.6 Contaminant trap

A contaminant trap was placed in front of the microtrap to remove the contaminants that might interfere with the on-line trace methane analysis system (Fig. 6.3a). A suitable adsorbent was evaluated and the best was selected for further use. The adsorbents were activated charcoal (Palm Shell), coated Carbopack B, and Carbopack C, selected by considering their surface area, and pore size. Each of these adsorbents was packed in a stainless steel tube (1/4" O.D. × 4 mm I.D. × 16 cm).

The experiments consisted of two parts. The first part was to test the trapping efficiency of the contaminant trap. This was done by placing the contaminant trap between the standard hexane gas (from diffusion cell), with nitrogen as a dilutor, and the microtrap packed with coated Carbopack B (Fig. 6.3a). The injection was automatically performed every one minute, therefore hexane that could not be adsorbed by the contaminant trap can be monitored on-line by the microtrap.

The second part was to test the effect of contaminant trap on the response of methane (Fig 6.3b). The diffusion cell was removed and standard methane gas was used instead of nitrogen gas and flowed through the contaminants trap before passing through the microtrap packed with Carbosphere. The signals obtained with and without the contaminants trap were compared.

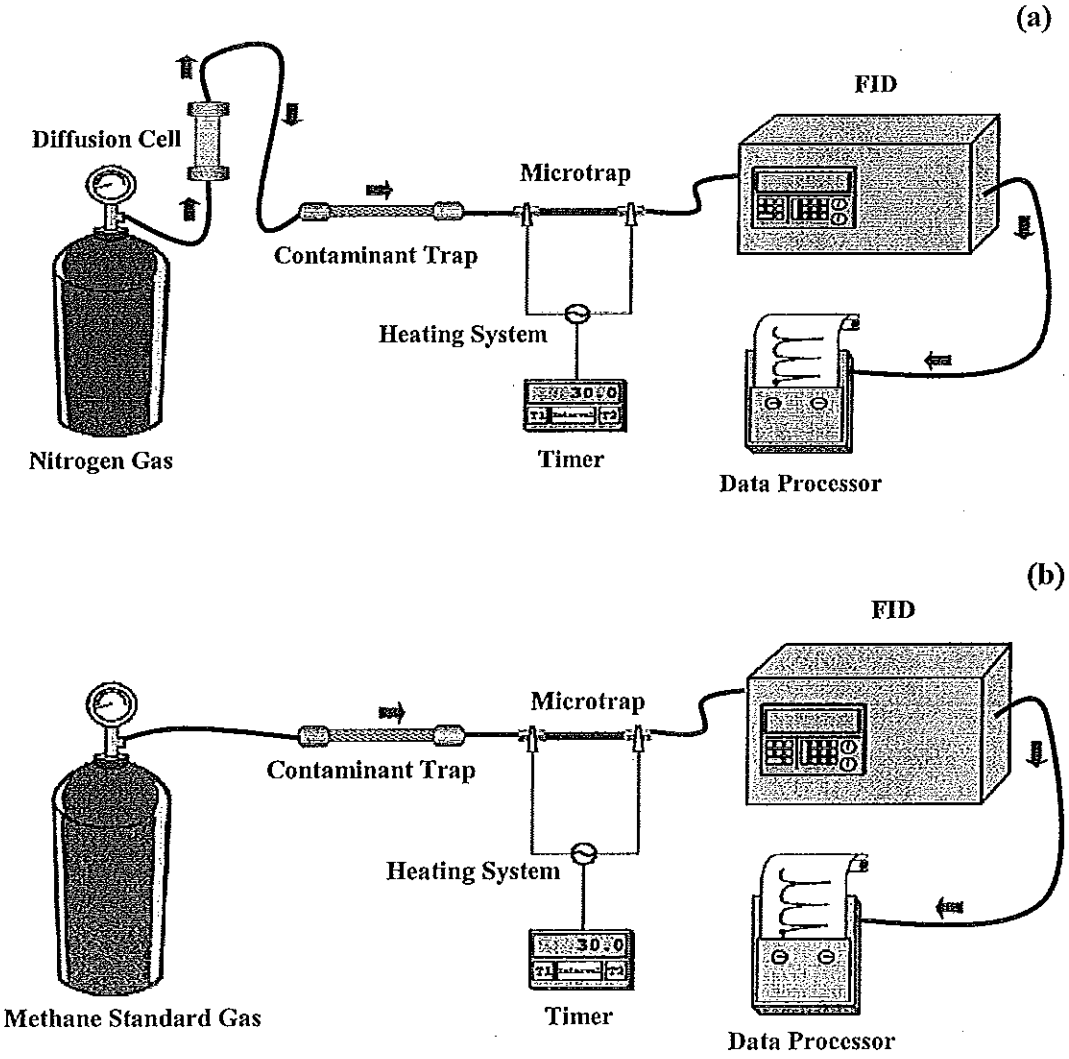


Fig. 6.3 On-line system to test the performance of the contaminant trap

(a) system to monitor contaminant (hexane) using microtrap packed withcoated Carbopack B

(b) system to test the effect of contaminant trap on the response of methane using microtrap packed with Carbosphere.

6.3 Results and discussion

6.3.1 Desorption conditions

To obtain the highest desorption efficiency, the voltage and time used to apply the current to the microtrap were first investigated at room temperature (25°C). Desorption voltage was increased from 10 volts to 30 volts and desorption time was fixed at 2.0 seconds. The peak height increased with increased voltage and then became constant after 15 volts (Fig. 6.4). That is, the voltage at 15 volts is high enough to desorb all adsorbed methane from the microtrap. The desorption time was then varied from 1.0 second to 4.5 seconds. The peak height also increased when increasing desorption time. After 2.5 seconds the peak height was stable (Fig. 6.5) and this was the optimum desorption time.

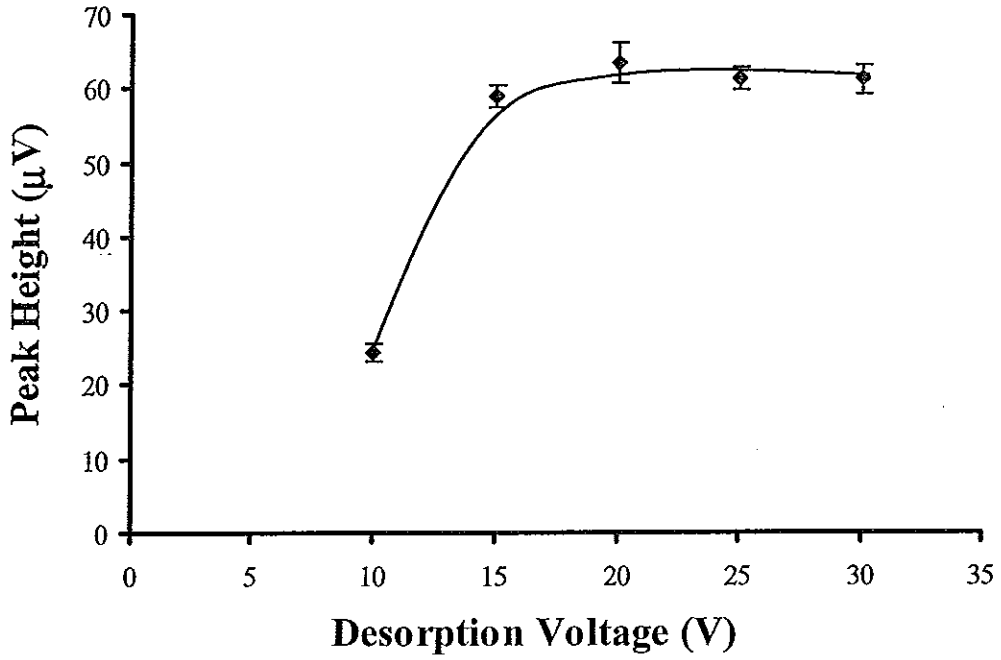


Fig. 6.4 Optimization of desorption voltage at 25°C.

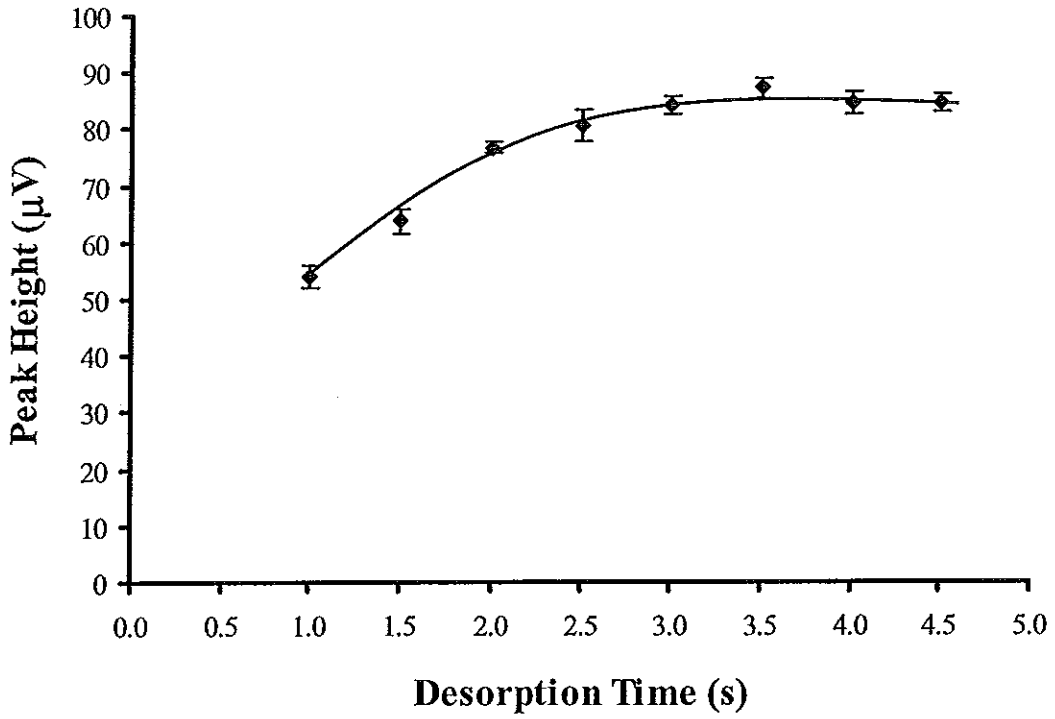


Fig. 6.5 Optimization of desorption time at 25°C.

6.3.2 Breakthrough characteristics of the microtrap

Breakthrough is an important parameter since it can indicate the capacity of the microtrap in term of the volume or the time that the microtrap can retain the analytes without loosing them. The adsorption time of the microtrap was varied from 0.5 to 4 minutes, and then desorbed by applying the current to the microtrap at optimum condition, at room temperature (25°C), 15 volts, 2.5 seconds (section 6.3.1). The response increased with adsorption time (Fig. 6.6) up to 2.0 minutes, and then became constant and this was the breakthrough time. Two minutes was then used as the optimum adsorption time for each analysis cycle.

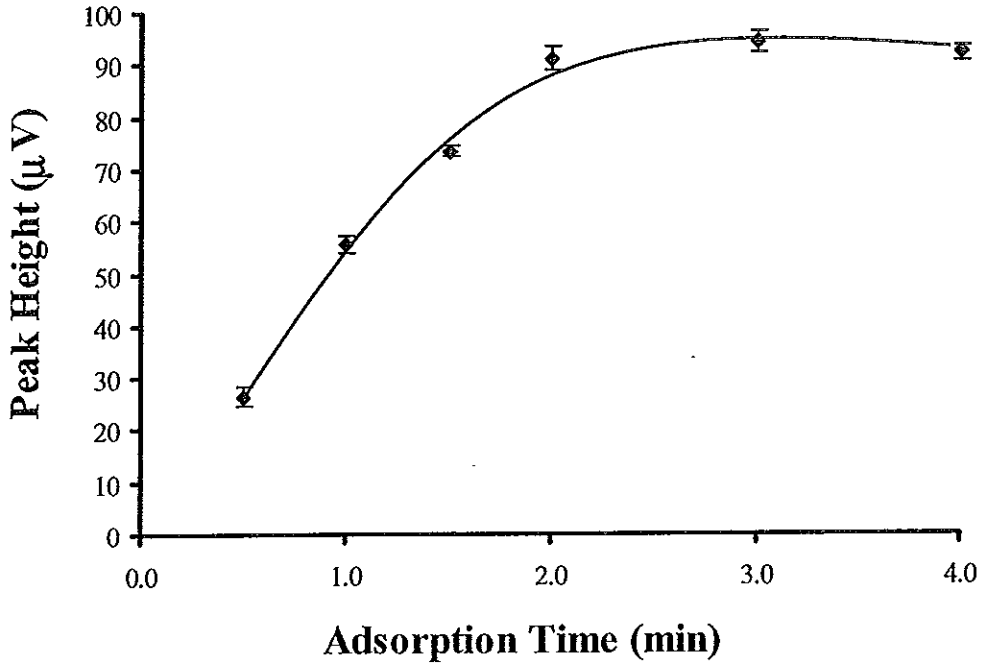


Fig. 6.6 Response at different adsorption times used to determine breakthrough time of the microtrap at room temperature (25°C). The microtrap was heated at desorption voltage of 15 volts for 2.5 seconds.

Optimum conditions at room temperature provided chromatograms with an average peak height of 90 μV for 11.6 ppmv of methane. With this response, the on-line system could not be used to detect trace methane in high purity gas which are in the range of 0.1 to 5.0 ppmv (Matheson Tri Gas). Therefore, the sensitivity of the on-line microtrap must be enhanced to meet the high purity gas specification.

6.3.3 Relationship between breakthrough volume and temperature

Adsorption is an exothermic phenomenon and the logarithm of the breakthrough volume (BTV) is inversely proportional to the temperature as shown in following equations (Atkin, 1994; Simon *et al.*, 1995);

$$\frac{d[\log(BTV)]}{d\left(\frac{1}{T}\right)} = -\frac{\Delta H_{ad}}{2.3R} \tag{6.1}$$

Where ΔH_{ad} is the adsorption enthalpy, R is the gas constant and T is the absolute temperature. Considering ΔH_{ad} as a constant, equation (6.1) can be re-written as

$$\log(BTV) = -\frac{\Delta H_{ad}}{2.3RT} + K \tag{6.2}$$

or more generally,

$$\log(BTV) = a + \frac{b}{T} \tag{6.3}$$

From the relationship in equation (6.3) the less the adsorption temperature, the higher the response and the breakthrough.

Table 6.1 shows optimum desorption conditions that provide the highest desorption efficiency (the trend of the results are similar to those from Figs. 6.4 and 6.5). For all temperatures the thermal desorption could be done by heating the microtrap for a few seconds and the analytes were carried to the detector as a plug and obtained very sharp peaks (Fig. 6.7).

Table 6.1

Optimum desorption conditions and optimum adsorption time (breakthrough time) at various adsorption temperatures.

Adsorption temperature (°C)	Optimum desorption condition		Optimum adsorption time or breakthrough time (min)
	Voltage (V)	Duration of pulse (s)	
25	15	2.5	2.0
-10	20	1.5	3.0
-20	30	2.5	4.0
-30	35	2.5	4.0
-40	40	2.5	5.0
-50	40	3.5	6.0

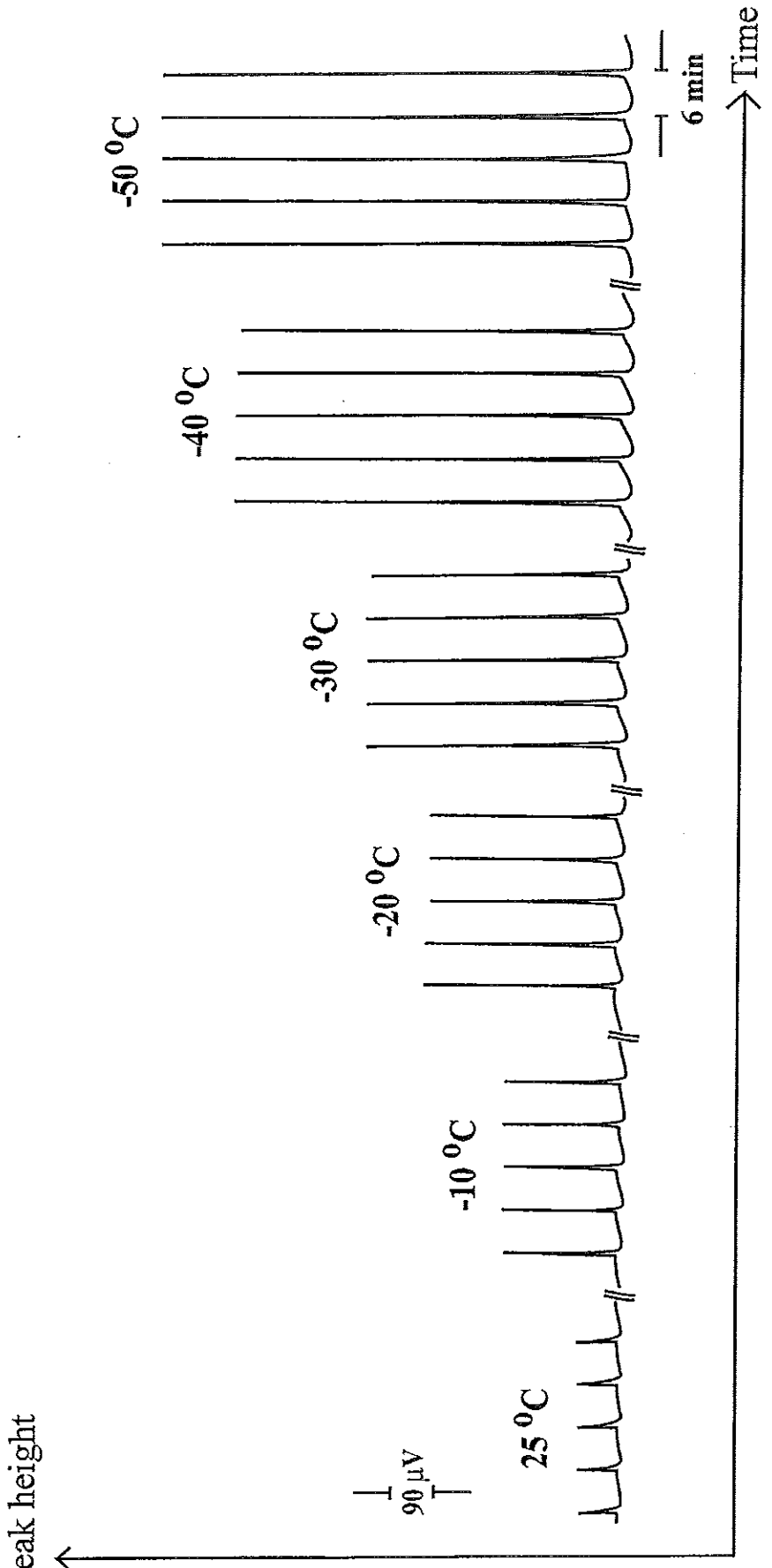


Fig. 6.7 Chromatograms of the on-line microtrap at various temperatures.

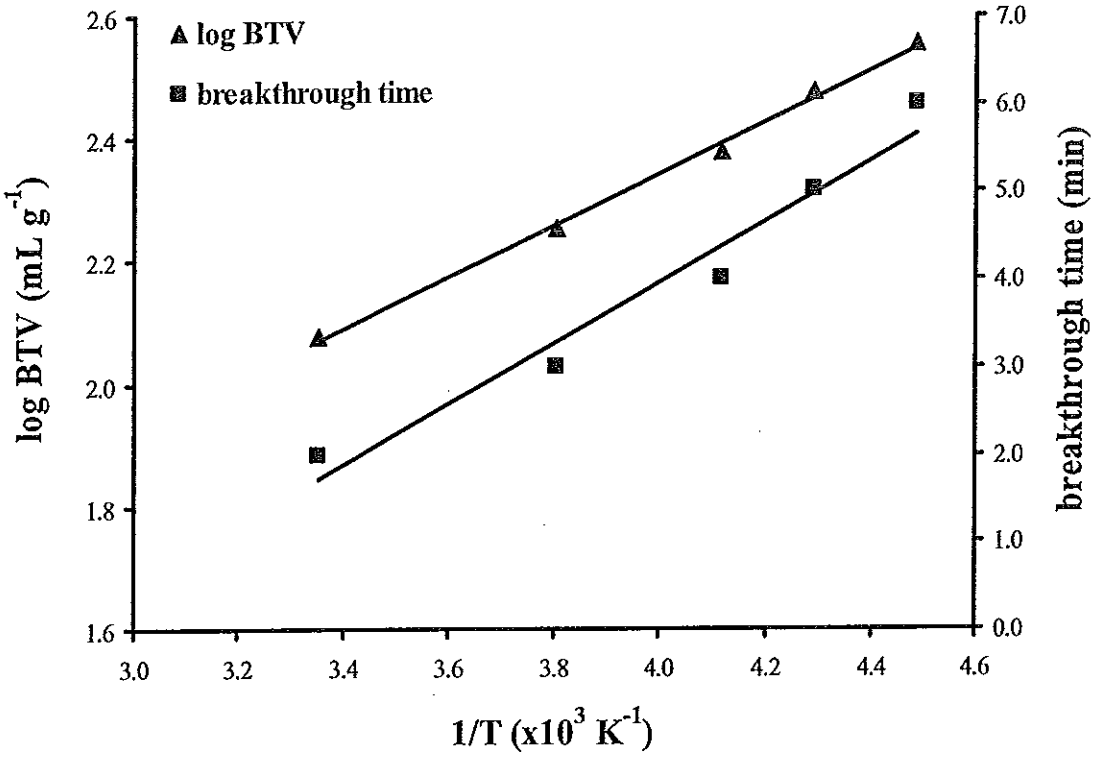


Fig. 6.8 Relationship between the breakthrough volume (BTV) and breakthrough time of the microtrap and sampling temperature.

The results confirmed the Van't Hoff-type relationship (Atkin, 1994; Simon *et al.*, 1995), that is, the lower the adsorption temperature, the higher the breakthrough volume (Fig. 6.8). This is because when the temperature decreased, the average time of molecules resided on a surface increased. Therefore, more methane can accumulate on the surface of the adsorbent which caused the increase of the breakthrough of the microtrap. The linear relationship is log (BTV) = 0.42×10^3 (1/T) + 0.65, and this can be used to calculate the breakthrough volume of methane on Carbosphere. The adsorption enthalpy (ΔH_{ad}) of methane on Carbosphere can also be calculated from the slope $\left(-\frac{\Delta H_{ad}}{2.3R}\right)$ (Simon *et al.*, 1995) and this is equal to -8.1 kJ mol^{-1}. Since the maximum observed enthalpy reported for physical adsorption was -21 kJ mol^{-1} while the enthalpy for chemisorption was in the region of -200 kJ mol^{-1}

(Atkin, 1994). The result indicated that, methane was adsorbed on the Carbosphere by physical adsorption.

6.3.4 Enhancement

The enhancement at various sampling temperatures is the ratio of the response from the on-line microtrap system (Fig. 6.9a) to the direct-FID (Fig. 6.9b). When the temperature decreased, the enhancement increased (Fig. 6.10). At -50°C the enhancement factor reached 260, *i.e.*, the response increased tremendously from 3.99 μV obtained from direct-FID to 1040 μV using a microtrap and this makes it very suitable for trace methane analysis.

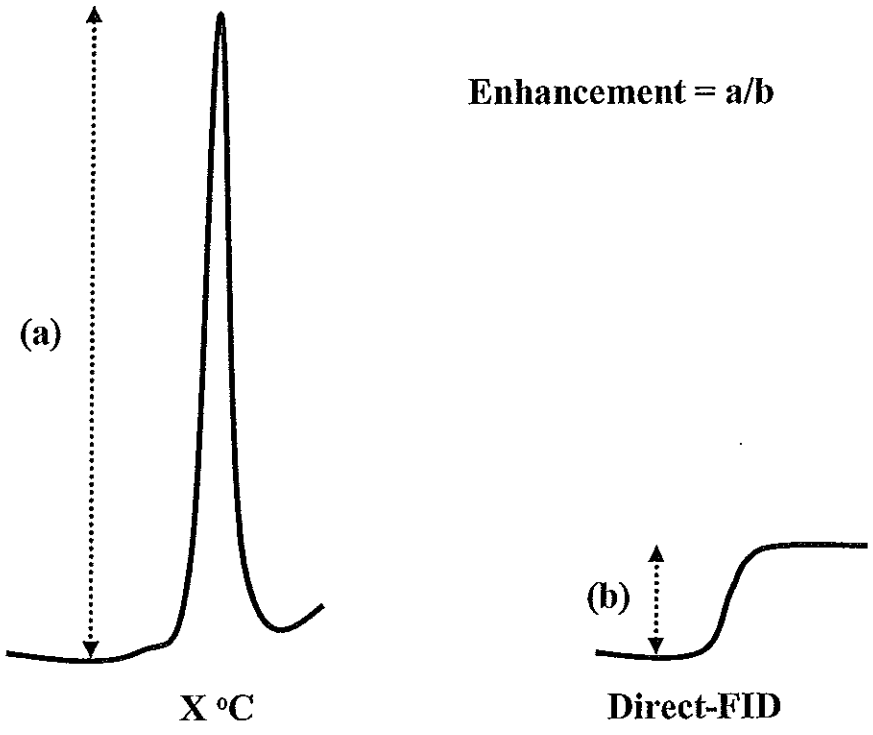


Fig 6.9 The enhancement factor of the microtrap was calculated by dividing the peak height of the chromatogram at any temperature (x°C) (a) and by the value from direct-FID at room temperature (b).

Due to the limitation of the laboratory-built cooling box, the lowest tempera-ture that could be reached was -50°C. However, at this temperature, the signal for 11.6 ppmv of methane had already increased from 90 μV at room temperature to 1040 μV and this is high enough to detect trace methane contaminated in high purity gases which are in the range of 0.1-5.0 ppmv (Matheson Tri Gas) and this observation will be confirmed and supported in section 6.3.4.

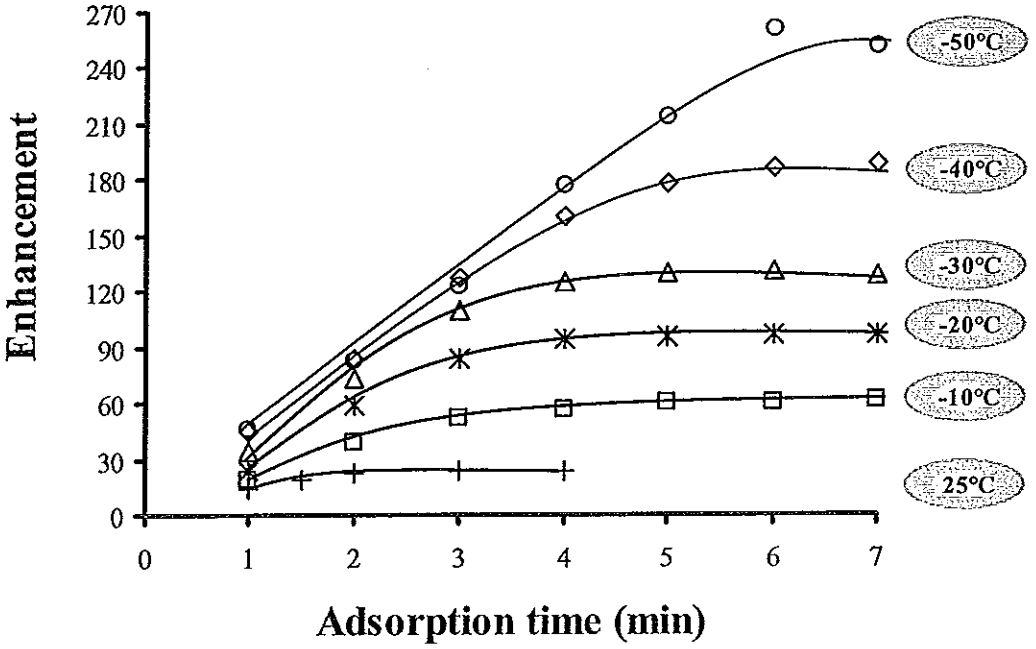


Fig. 6.10 Enhancement at various sampling temperatures and adsorption times (injection intervals)

6.3.5 Performance of the on-line microtrap

The linear relationship between the peak height and the concentration of methane standard gas was investigated between 0.2 to 11.6 ppmv. A linear calibration curve from the microtrap was obtained as shown in Fig. 6.11. That is, linearity was good enough and this system can certainly be applied for quantitative analysis of the trace methane.

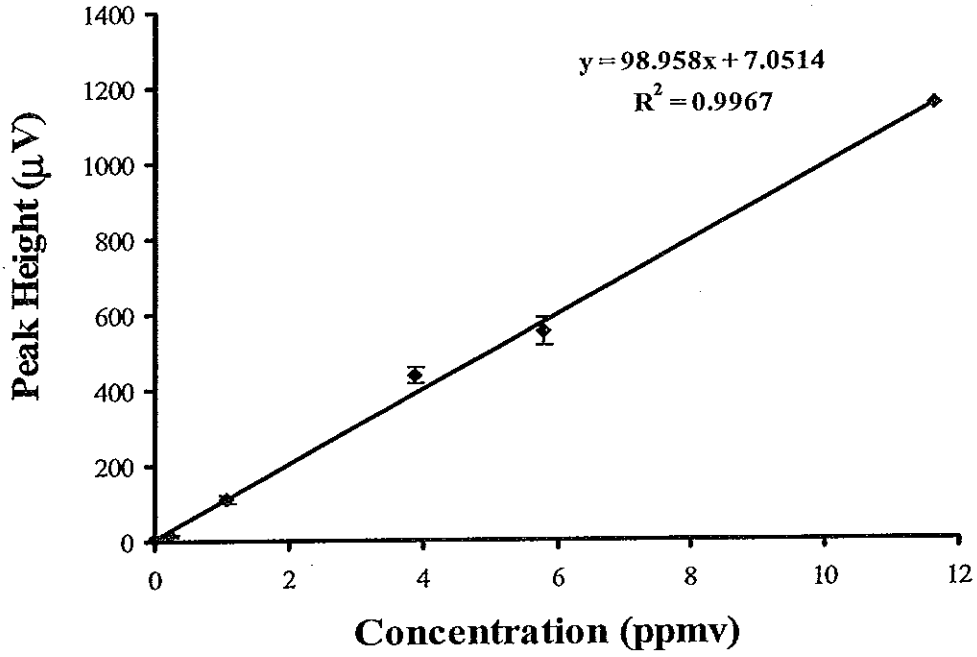


Fig. 6.11 Calibration curve of methane obtained from on-line microtrap system.

The limit of detection was also studied to ensure that this system is sensitive enough to determine the concentration of methane in high purity gas. Blank injection was performed 20 times in order to determine the limit of detection as recommended by IUPAC method (Long and Winefordner, 1983) that previously described in section 5.3.3 in Chapter 5, the obtained data are shown in Table 6.2.

Using the experimental data from Table 6.2 and the analytical sensitivity from Fig. 6.11, the limit of detection was found to be 28.3 ppbv. This is much better than the 300 ppbv determined without preconcentration reported by Kamiński *et al.* (Kamiński *et al.*, 2003). Therefore, this proposed system is more suitable to be used as an on-line device to be placed in line of the production processes for the continuous monitoring of the pure and research purity gases where the concentration of methane must not be higher than 0.1 ppmv (Matheson Tri Gas).

Table 6.2

The data from 20 times injections of blank obtained from on-line microtrap system.

Injection time	Peak Height (μV)	Injection time	Peak Height (μV)
1	7	11	5
2	6	12	5
3	7	13	4
4	6	14	5
5	6	15	5
6	5	16	6
7	7	17	5
8	6	18	5
9	5	19	4
10	5	20	4
$\overline{X}$			**5.4**
S_B			**0.94**

6.3.6 The contaminant trap

To further increase the selectivity of a microtrap, a contaminant trap was applied to the system to remove other compounds, except methane, before reaching the microtrap. The results from three different contaminant traps are shown in Fig. 6.12, indicating that all of the studied adsorbents could adsorb the impurity/contaminant (hexane was used as the representative of the impurity at 500 ppmv) with efficiencies of higher than 99%. So, the effect of contaminant trap on methane signal and the lifetime of the trap were used as the parameters for adsorbents selection. Fig. 6.12 shows that activated charcoal had a much greater lifetime (>9000 minutes) compared to coated Carbopack B and Carbopack C (120 minutes). However, it also trapped some methane on its active surface (Fig. 6.13). Therefore, either coated

Carbopack B or Carbopack C would be a suitable adsorbent for contaminants trap since they showed very high removal efficiency and had no effect on methane signal.

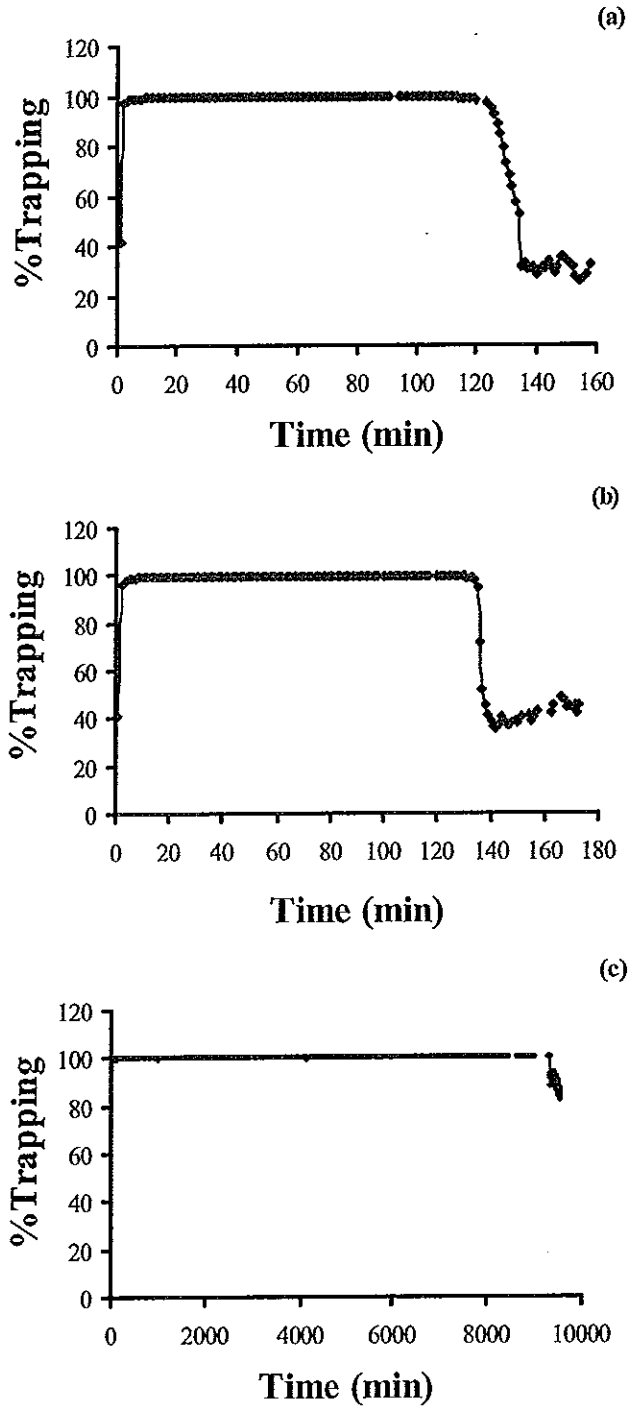


Fig. 6.12 Life time of the contaminant trap packed with various adsorbents; (a) coated Carbopack B, (b) Carbopack C, (c) activated charcoal.

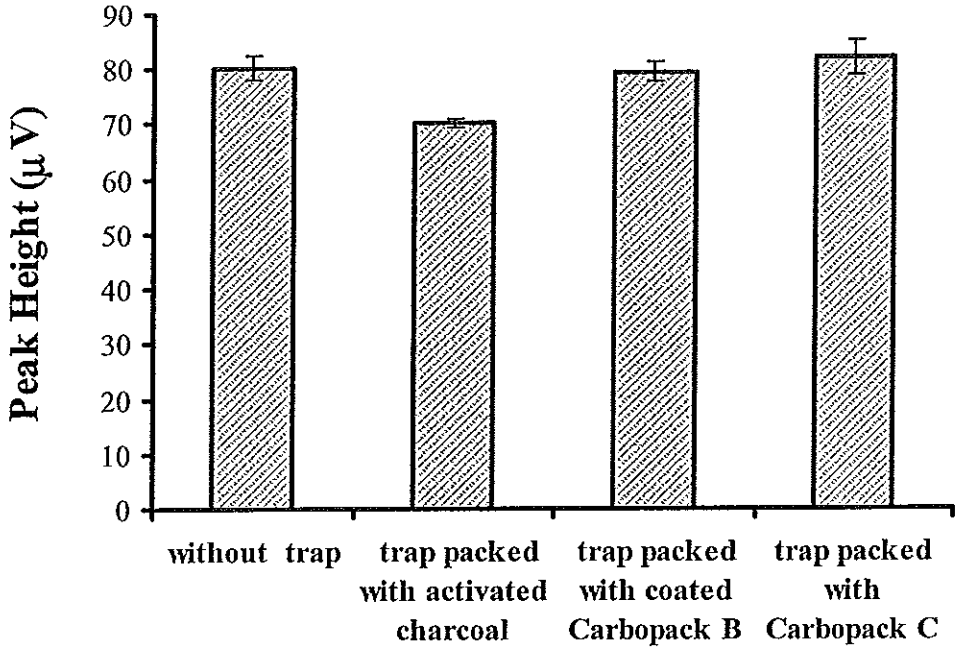


Fig. 6.13 Effect of contaminants trap on methane signal.

6.3.7 Long term stability

Stability of this system was evaluated by flowing 11.6 ppmv of methane standard continuously with a rate of 5.0 mL min^{-1} at room temperature. Nearly 2,000 injections were made by applying current through the wall of the microtrap every two minutes for 64 hours (Fig. 6.14). The average response was 89.2 ± 3.8 μV (relative standard deviation (RSD) < 5%). Since -50°C was the optimum adsorption temperature, the stability of the on-line system at this temperature was also studied. It was tested for a work day period, which is 8 hours. The injections were made every 6 minutes with desorption voltage and time of 40 volts and 3.5 seconds, respectively. The average peak height was 1037 ± 51 μV (RSD < 5%) as shown in Fig. 6.15. The results indicated that the system was very stable. It also showed that the adsorbed analytes were efficiently desorbed from the adsorbent without any memory effect.

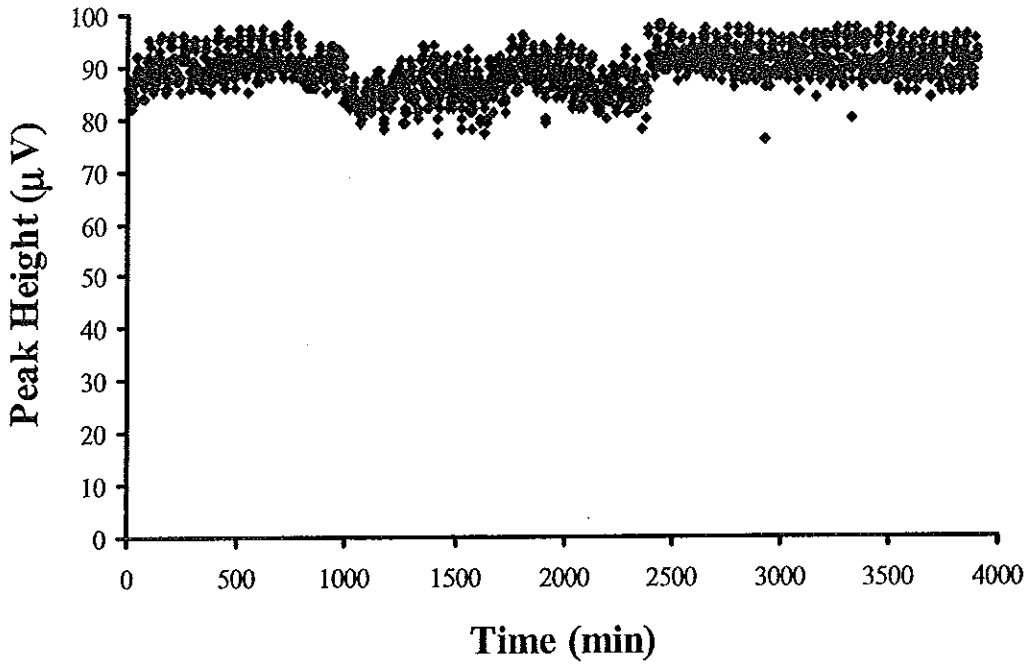


Fig. 6.14 The stability of the microtrap at room temperature (25°C).

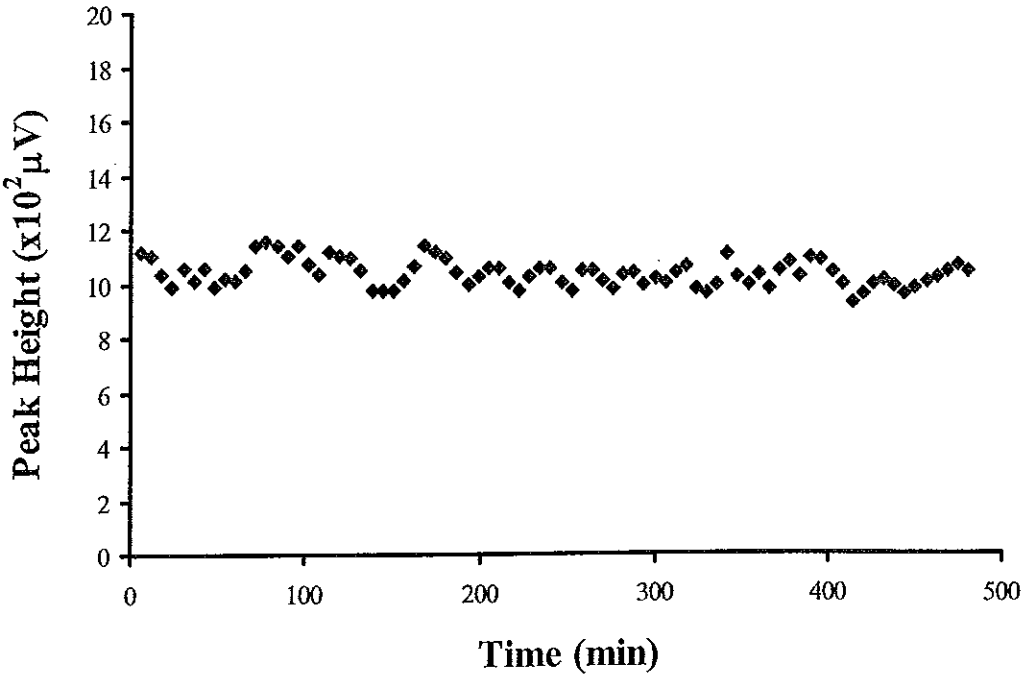


Fig. 6.15 The stability of the microtrap at -50°C.

6.4 Conclusions

The results demonstrated that the on-line microtrap can provide reliable data and good repeatability. It can be operated over a very long period of time with good precision and without the degradation of the adsorbent. An enhancement of up to 260 times could be obtained when using a simple, cost effective cooling system. Therefore, this microtrap can certainly be applied to the gas production industries to check their production quality, not only for checking the methane contamination but also for determination of methane concentration required by the customers.

CHAPTER 7

Affinity Phenylboronic Acid-containing Polymer Gel for Sugar Sensing

7.1 Introduction

The determination of sugars is very important in diagnostic analysis and process control of food industries. One of the most widely used techniques is based on the use of enzyme based sensor since its high selectivity to particular sugar. This property is well suited for particular sugar determination of the mixture of isomers and even in the biological fluids such as blood and urine. However, some disadvantages of enzyme based sensors are poor stability as a result of long term contact with media and the enzymes activity usually degrades gradually day by day so requiring a frequent calibration before use. For this reason, the development of alternative specific sensor which used non-natural (non-enzymatic) or synthetic component has been an important subject in bioanalytical science and technology (Takahashi and Anzai, 2005).

There are many recent reports describing the performance of polymeric gels which change their physicochemical properties with external stimuli such as, temperature (Ivanov *et al.*, 2005), light (Suzuki and Tanaka, 1990), pH (Annaka and Tanaka, 1992) and the change in concentration of particular molecule (Kataoka *et al.*, 1998). These have been the subjects of great interest because of their potential utility to be used in diverse fields including controlled drug release, analytical and preparative separations and sensor technologies (Kataoka *et al.*, 1998). Hence these polymeric gels are named as "intelligent material".

A well-known synthetic ligand exhibiting specificity to sugars is phenylboronic acid. It has attracted much attention in both chemistry and biology since they can form reversible bonds with *cis*-diol to generate five- or six-membered cyclic complexes by affinity interaction under mild and easy controllable reaction condition. Furthermore, there is a report mentioned that the binding of sugar to

phenylboronic acid can decrease its pKa and when it was used as the buffer, addition of diols would lower the pH of the solution due to complex formation (Lorand and Edwards, 1959). Therefore the combination of the intelligent polymer (which changes the physiological properties with the external stimuli) with the affinity binding ligand (phenylboronic acid) will provide a specific sensor for the determination of sugars.

Various sensors based on the use of phenylboronic containing polymer have been developed such as optical sensors (Deng *et al.*, 1994; Yoon and Czarnik, 1992), gel sensitive sugar sensors (Alexeev *et al.*, 2003; Gabai *et al.*, 2001; Lee *et al.*, 2004; Matsumoto *et al.*, 2004a; Matsumoto *et al.*, 2004b; Takahashi and Anzai, 2005). Most of these developed sensors did not provide a linear response of dimensional or electrical characteristic of the gels to sugar concentration. Gels properties such as transparency, porosity and permeability for sugars are considered important for the character and need more study.

Therefore, a simple analytical system based on the affinity binding of the highly durable semitransparent phenylboronic acid-containing gels with *cis*-diol functional group of sugar is presented in this chapter.

7.2 Methods

7.2.1 Materials

3-Aminophenylboronic acid, sodium hydroxide and hydrochloric acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acryloyl chloride, *D*-(+)-mannose and α-lactose were purchased from Fluka Chemie AG (Buchs, Switzerland). Acrylamide (AA), *N,N,N',N'*-tetramethylethylenediamine (TEMED), ammonium persul-phate, *D*-(+)-glucose, *N*-acetyl-*D*-glucosamine, *D*-(-)-fructose and sucrose were purchased from Aldrich (Steinheim, Germany). Sodium dihydrogen phosphate monohydrate and di-sodium hydrogen phosphate anhydrous were products from Merck (Darmstadt, Germany). *D*-galactose was purchased from BDH (Poole, England) and *N*-acryloyl-*m*-aminophenylboronic acid (NAAPBA) was prepared as described in section 7.2.2.

7.2.2 Synthesis of *N*-acryloyl-3-aminophenylboronic acid (NAAPBA)

3-Aminophenylboronic acid (2.74 g, 20 mmol) was dissolved in 2.0 M NaOH (40 mL, 80 mmol) and the solution was cooled in an ice bath. Then acryloyl chloride (3.2 mL, 40 mmol) was added dropwise to the solution with intensive magnetic stirring for 15 min followed by dropwise adding of 2.0 M hydrochloric acid to the mixture to adjust the pH to approximately 1. The mixture was filtered through a sintered glass filter (Scott, Duran, No. 3), the precipitate was collected and washed on the filter by cold distilled water (50 mL). Then, the precipitate was dissolved in 80 mL of distilled water at 60°C. The residual insoluble impurities were filtered off and the light violet needles were obtained by crystallization of the solution overnight in a refridgerator. The crystals were filtered off on paper filter, washed with cold distilled water, and dried under vacuum in a desiccator over anhydrous calcium chloride where 35% yield of NAAPBA was obtained (Shiomori *et al.*, 2004).

7.2.3 Synthesis of 3-acrylamidophenylboronic acid acrylamide copolymer (NAAPBA-co-AA) gel on the glass plate

The glass plates (36 mm × 9 mm) were cleaned with normal detergent to remove the dust and oil from their surface then, dried at room temperature. NAAPBA-co-AA gel was prepared by dissolving 48 mg (0.25 mmol) of NAAPBA and 153 mg (2.2 mmol) of acrylamide in 2.5 mL distilled water at 50°C. The mixture was gently agitated by manual shaking for dissolution. Ten microliters of TEMED was added and gently agitated by manual shaking again and then degassed. Ammonium persulphate, 19.9 mg, was dissolved in 0.5 mL of distilled water in a separate vial. To obtain the copolymer gel, 100 µL of the monomer mixture was dropped on the surface of the glass plate followed by 50 µL of the ammonium persulphate solution. The solutions were mixed on the surface of the glass plate where the copolymer gel was formed, indicated by an occurring of a semitransparent white gel (Fig. 7.1). The gel coated glass slides were then kept in desiccators, containing a

water beaker, for 2 h to allow the complete and stable formation of the gel, after that kept in distilled water prior to use.

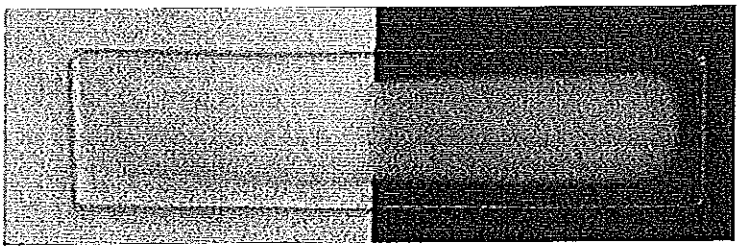


Fig. 7.1 NAAPBA-co-AA gel coated on the glass plate.

7.2.4 pH dependence of NAAPBA-co-AA gel

To investigate the sensing property of the synthesized copolymer gel, its optical density at 500 nm was investigated using Ultraspec® 1000, UV/Visible Spectrophotometer (Pharmacia Biotech, Buckinghamshire, UK). The coated glass plate with NAAPBA-co-AA, 0.35 mm thick, was equilibrated in 50 mM sodium phosphate buffer for 8 h then immersed in the same buffer in a 3.0 mL optical glass cuvette. Optical density of buffer at each pH was monitored until obtained the stable signal then, the solution was changed to 40 mM of glucose in the buffer and its optical density was recorded. The investigated pHs were 6.5, 7.0, 7.3, 7.5 and 8.0.

7.2.5 Optical density changes of NAAPBA-co-AA gel as a function of glucose concentration

The coated glass plate with NAAPBA-co-AA gel, 0.35 mm thick, was equilibrated in 50 mM sodium phosphate buffer for 8 h then it was immersed in the buffer in a 3.0 mL optical glass cuvette. The optical density of the copolymer gel was monitored at 500 nm every 5 minutes using Ultraspec® 1000, UV/Visible Spectrophotometer (Pharmacia Biotech, Buckinghamshire, UK) until a stable optical density was obtained, the optical density at this stage was used as base line of the

sensor system. The solution in the cuvette was changed to glucose solution in the buffer and the optical density was recorded at 5 minute intervals until the equilibrium was reached (constant optical density), the optical density at this step was recorded as a signal. To obtain the optical density change, the signal was subtracted from base line. The optical density change as a function of glucose concentration was then investigated in the concentration range of 1-90 mM to observe the sensor property of this copolymer gel after binding with glucose.

7.2.6 The effect of gel thickness on the optical density and diffusion coefficient of glucose

NAAPBA-co-AA gel on glass plates prepared as described in section 7.2.3 was used in this study but the volumes of monomer mixture and ammonium persulphate were varied to obtain various gel thicknesses as shown in Table 7.1. Three glass plates were prepared for each thickness.

Table 7.1

The amount of monomer mixture and ammonium persulphate used for preparation of different gel thicknesses.

Gel no.	Acrylamide (mg)	NAAPBA (mg)	Water (mL)	TEMED (μL)	Monomer mixture (μL)	0.175 M ammonium persulphate (μL)
1-3	153	48	2.5	10	200	100
4-6	153	48	2.5	10	150	75
7-9	153	48	2.5	10	100	50
10-12	153	48	2.5	10	75	37.5
13-15	153	48	2.5	10	50	25

Each glass plate with different thicknesses was alternately immersed into 50 mM of sodium phosphate buffer pH 7.3 and 40 mM of glucose in the buffer; the optical density was monitored as a function of time until the equilibrium was reached. The ratio between optical density change at time t (ΔOD_t) and optical

density change at equilibrium (ΔOD_∞) were plotted against $t^{1/2}$. The diffusion coefficient was obtained from this following equation (Aminabhavi *et al.*, 1997).

$$F = \frac{\Delta OD_t}{\Delta OD_\infty} = \frac{4}{h}\left(\frac{Dt}{\pi}\right)^{1/2} \tag{7.1}$$

$$slope = \frac{4}{h}\left(\frac{D}{\pi}\right)^{1/2} \tag{7.2}$$

Where F is a fractional attainment of equilibrium, ΔOD_t is an optical density at time t, ΔOD_∞ is an optical density at equilibrium, h is the thickness of the gel and D is the diffusion coefficient of substance.

The thickness of the gel was determined following this procedures, each glass plate was divided into 2 to 5 sections depending on the shape of the coated copolymer gel. The length and the width of each section were measured by ruler in order to calculate the surface area of the gel on the glass plate, and then the glass plate was weighted with a 4 digit balance. The copolymer gel was scratched out of the glass plates using a spatula to obtain the copolymer gel weight. Since the main content of this copolymer gel is water so, the density of the gel was assumed to be unity. Therefore, the thickness of the gel can be obtained as follows.

$$Density = \frac{Mass}{Volume} \tag{7.3}$$

$$Density = \frac{Mass}{Width \times Length \times Height} \tag{7.4}$$

Thickness of the gel is the height in the above equation (equation (7.4)) and it was used as h for calculation of diffusion coefficient using equation (7.2).

7.2.7 The effect of sterilization on the sensitivity of NAAPBA-co-AA gel to glucose

To investigate the possibility of sterilization of NAAPBA-co-AA gel coated on the glass plate, the gel was autoclaved. The copolymer gel, 0.35 mm thick, was alternately immersed in 50 mM sodium phosphate buffer and various concentrations of glucose in the buffer to obtain the sensitivity (slope of calibration curves) of the copolymer gel before autoclaving. Then, the gel coated glass plate in water was autoclaved at 121°C, 1.2 bars for 10 min followed by cooling and equilibrating in 50 mM sodium phosphate buffer, pH 7.3 and the sensitivity of the copolymer gel to glucose was measured and compared with the sensitivity before autoclaving.

7.2.8 Specificity of NAAPBA-co-AA gel with different sugars

The coated glass plate with NAAPBA-co-AA gel, 0.35 mm thick, was alternately immersed in 50 mM sodium phosphate buffer and each standard sugar in the buffer. The 7 different sugars to be investigated were glucose, fructose, mannose, galactose, sucrose, lactose and *N*-acetylglucosamine. The optical density change of each sugar at each concentration was investigated to obtain the specificity of the copolymer gel to these sugars.

7.3 Results and discussion

7.3.1 Effect of pH on NAAPBA-co-AA gel

To investigate the feasibility of the synthesized NAAPBA-co-AA gel as glucose sensor, its optical density in both 50 mM sodium phosphate buffer as well as in the glucose standard, 40 mM, was investigated and the results are as shown in Fig. 7.2. The optical density decreased when pH increased especially in the present of sugar as can be seen in the gap between two curves. This is because phenylboronic

acid compounds in water exists in the equilibrium between the uncharged form, I, (relatively hydrophobic), and charged forms (charged phenyl borates), II, (relatively hydrophilic) as shown in Fig. 7.3 and only charged borates can form stable complexes with glucose in aqueous medium (Kataoka *et al.*, 1998; Matsumoto *et al.*, 2004a; Matsumoto *et al.*, 2004b) while the complexes with uncharged form in water is unstable due to its high susceptibility to hydrolysis (Matsumoto *et al.*, 2004b). Therefore, the equilibrium was shifted in the direction of increasing charged phenyl borates (II+III) when pH was increased and glucose was added. The formation of anionic form of the gel caused the different between ions in the gels and those in the surrounded solution resulting in Donnan potential which changes the chemical potential of water and caused an osmotic pressure which turns the gel swell and decrease its optical density (Reese *et al.*, 2001). The difference between the curves indicated that it has a potential for detection of glucose in the pH between 6.5 and 8.0.

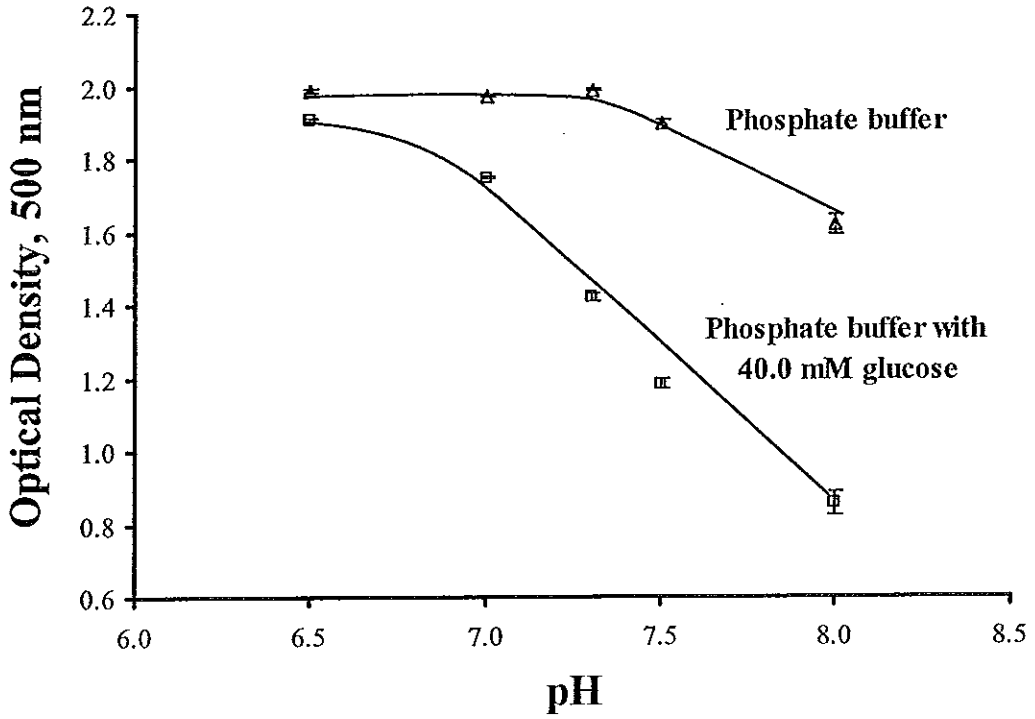


Fig. 7.2 Optical density of the NAAPBA-co-AA gel as a function of pH. The error bars indicate the standard deviation for three replications. Gel thickness was 0.35 mm.

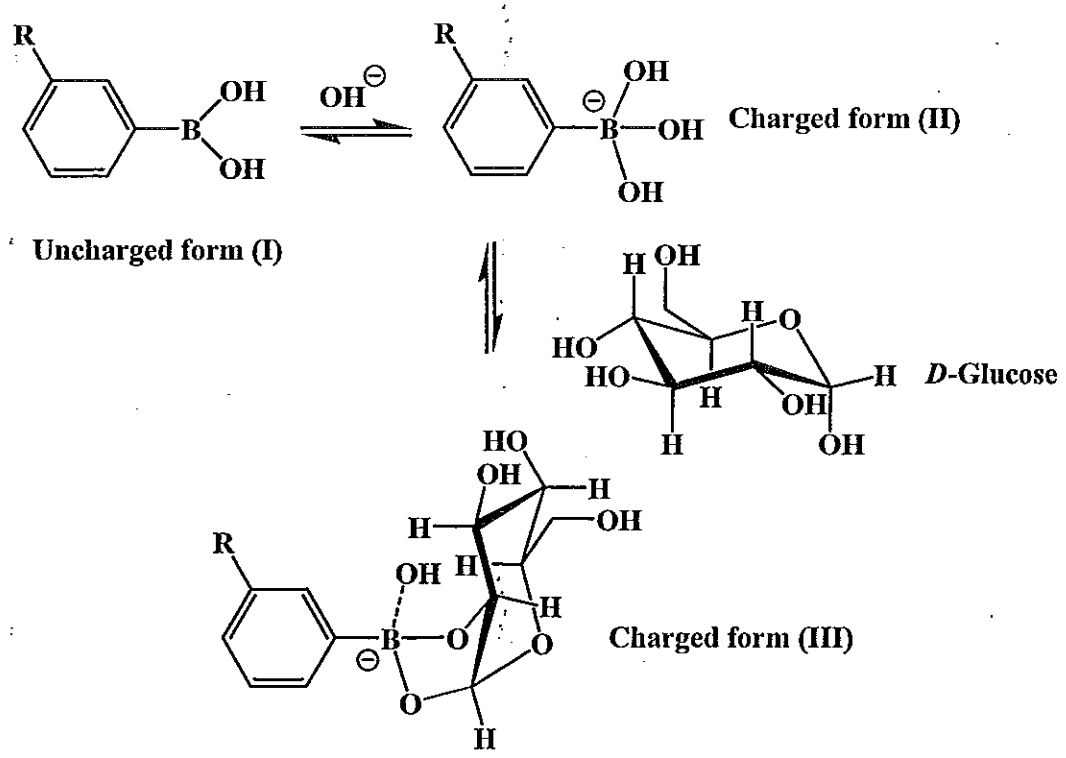


Fig. 7.3 Equilibrium between the phenylboronic acid derivatives and the OH^- and/or α-*D*-glucose in aqueous media.

7.3.2 Linearity of the optical density change of the gel as a function of glucose concentration

The relationship between the gel optical density change and the concentration of glucose was investigated and found that its optical density decreased with increasing concentration of glucose as shown in Fig. 7.4. This can be confirmed by the result as shown in Fig. 7.5. The higher the pH, the higher the sensitivity and pH 7.3 was selected for further studied.

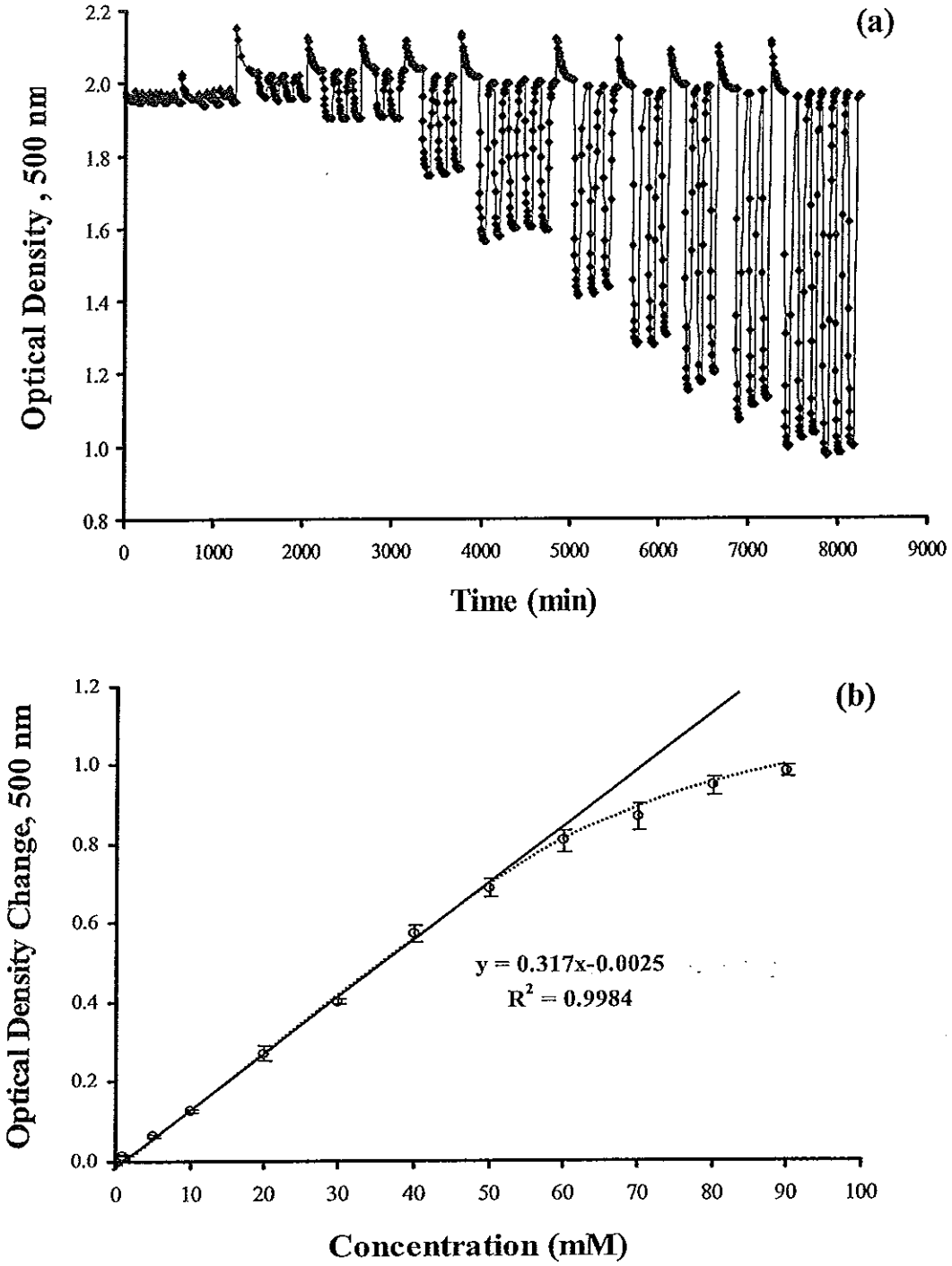


Fig. 7.4 Optical density of NAAPBA-co-AA gel in 50 mM sodium phosphate buffer pH 7.3 at 500 nm at various concentrations of glucose (a). Linearity of the optical density changes of the gel (b).

The changes in the optical density at each concentration of glucose were well repeatable and showed complete reversibility of the original optical density of the gel after the depletion of glucose. The linear dynamic range was obtained from 1 to 60 mM with R^2 greater than 0.99 and the limit of detection was 1 mM.

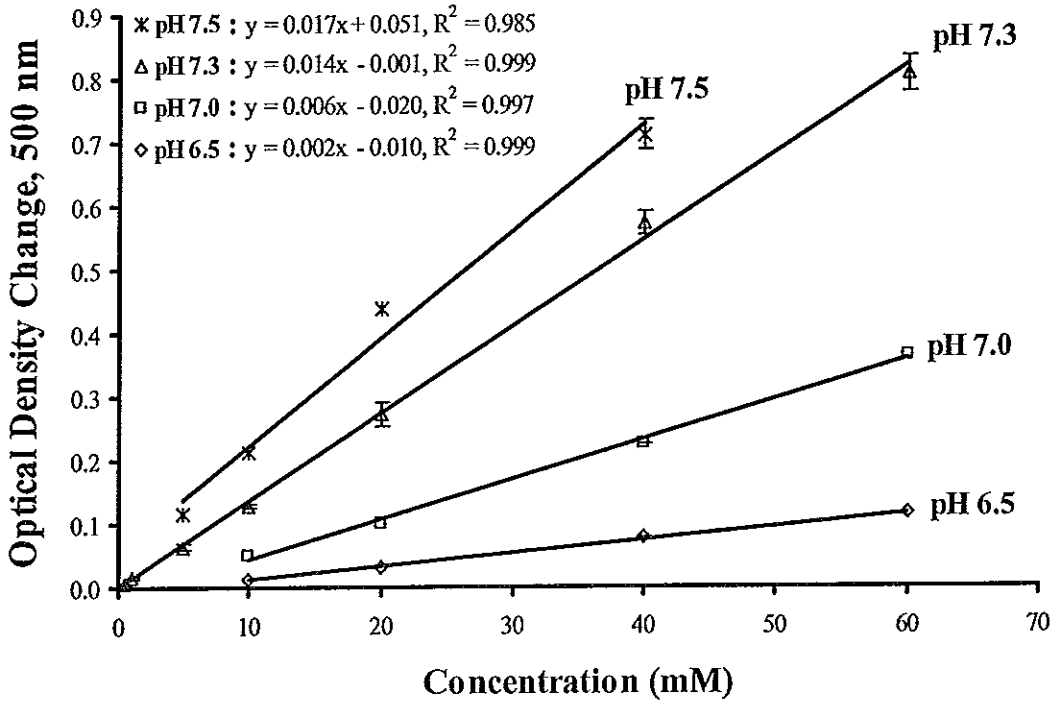


Fig. 7.5 The effect of pH on the sensitivity of glucose detection with NAAPBA-co-AA gel: the optical density change of the gel as a function of glucose concentration. The error bars were used to indicate the standard deviation of three replications. The thickness of the gel was 0.35 mm.

7.3.3 Effect of gel thickness on the changes of the optical density and the diffusion coefficient of glucose

The thicknesses of the synthesized copolymer gels prepared with various volumes of monomer mixture were obtained (Table 7.2). Thicker gels were obtained with larger volume of monomer mixture.

Table 7.2

Thicknesses of gels and diffusion coefficient of glucose from various volumes of monomer mixture.

Gel No.	Monomer mixture (μL)	Ammonium persulphate (0.0175 M)	Surface area (cm^2)	Weight (g)	Thickness (mm)	Diffusion coefficient ($cm^2\ s^{-1}$)
1-3	200	100	2.67±0.12	0.268±0.057	1.0±0.2	$(2.9\pm1.3)\times10^{-7}$
4-6	150	75	2.56±0.02	0.212±0.018	0.83±0.1	$(2.1\pm0.4)\times10^{-7}$
7-9	100	50	1.95±0.01	0.068±0.01	0.35±0.07	$(2.6\pm1.0)\times10^{-7}$
10-12	75	37.5	1.89±0.16	0.065±0.005	0.34±0.1	NC
13-15	50	25	1.29±0.07	0.012±0.003	0.13±0.1	NC

NC: not calculated

The binding of glucose with the boronic acid in the thin gel is faster than that in thick gel therefore thin gel needed less equilibration time than the thick gel causing shorter analysis time (Fig. 7.6). However, the change in the optical density was also decreased when the thickness was decreased because of the decreasing of the content of phenylboronic acid compounds in the copolymer gel except in case of the gels were too thick. Therefore, the thickness of 0.35 mm was the optimum and selected for further studied.

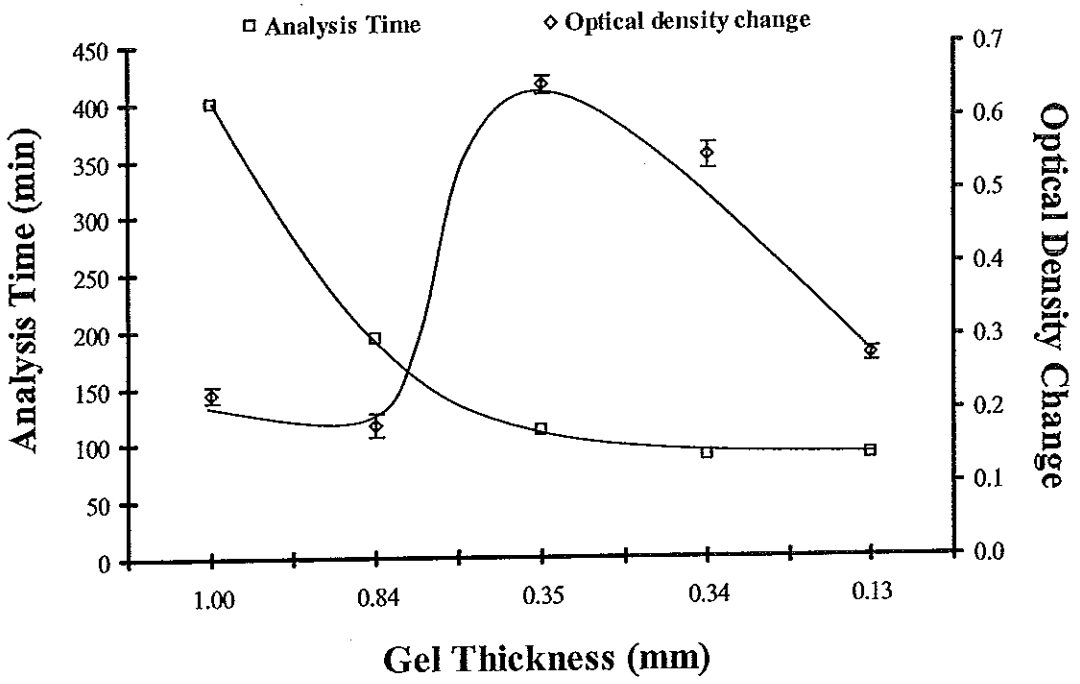


Fig. 7.6 Relationship between optical density changes and analysis time for detection of 40.0 mM of glucose with different thicknesses of NAAPBA-co-AA gel.

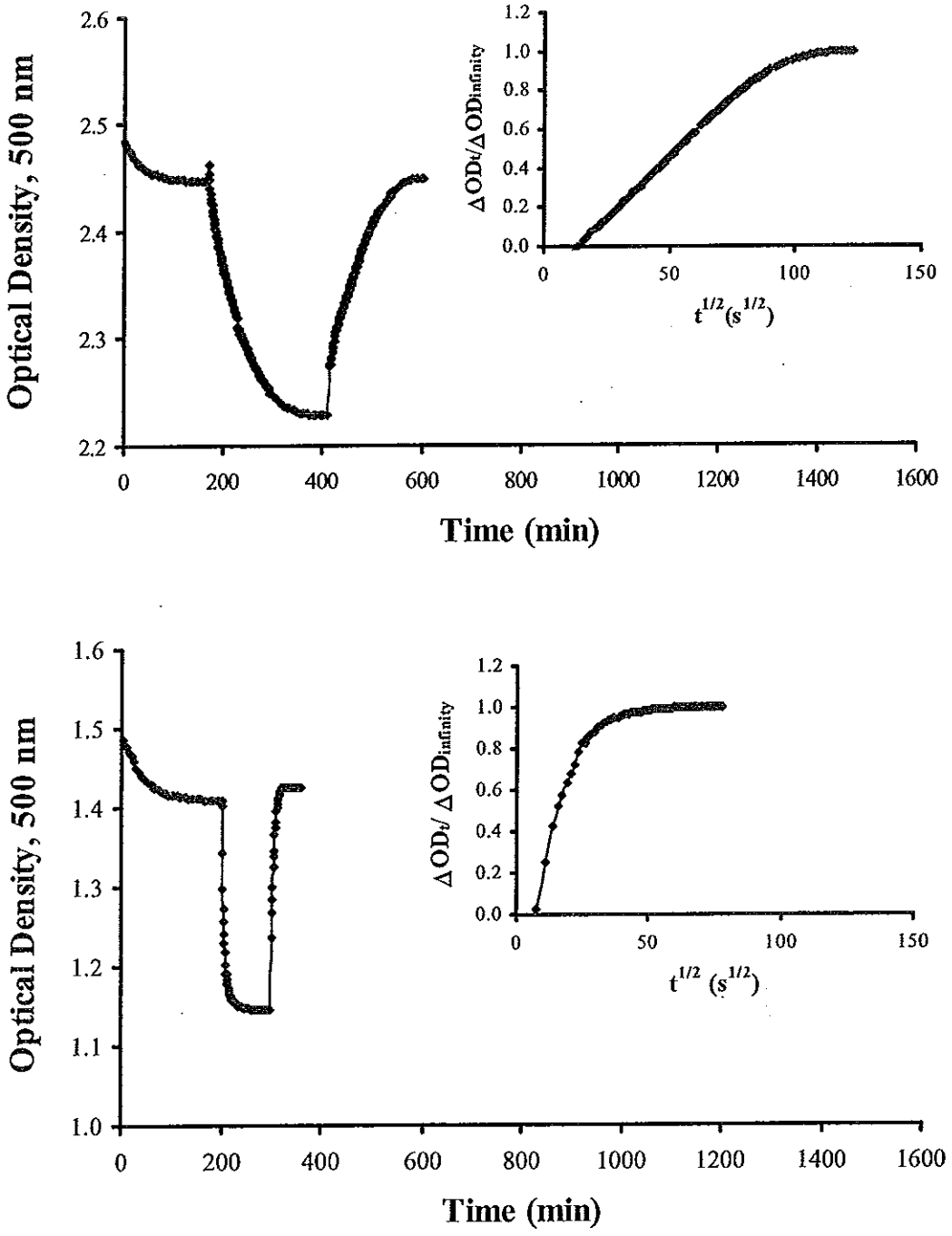


Fig. 7.7 Diffusion behaviors of glucose through the NAAPBA-co-AA gel in 50 mM sodium phosphate buffer pH 7.3 at two different thicknesses. Downward arrows indicate immersion of the gel into 40 mM of glucose in the buffer.

The diffusion profiles of glucose plotted between the optical density and time were compared. To obtain a clear different image in the diffusion profile so only those from the thickest (1.0 mm) and thinnest (0.13 mm) gels are shown (Fig. 7.7). This figure gives a clear image of how different glucose diffuses through the thick and thin gels. In addition, to obtain the diffusion coefficient of glucose through the gels, the ratios of the optical density change at time t and at the equilibrium, F or $\left(\frac{\Delta OD_t}{\Delta OD_\infty}\right)$, were plot against the square root of time ($t^{1/2}$) (Fig. 7.7, inset) and the slopes of the curve were used to calculate the diffusion coefficient of glucose as mentioned in equation (7.2) and the values obtained were summarized in Table 7.2.

The dependences of F on $t^{1/2}$ were linear in the range of F value from 0 to 0.7 for the gel thicknesses of 0.35 mm and thicker but for thinner gel, the relationship of F and $t^{1/2}$ were not linear since the response of glucose through the gel is very fast and the equilibrium was reached with the time less than 20 minutes compared to 1300 minutes for thick gel (Fig. 7.7). Therefore, the thickness lower than 0.35 mm could not be fit with the model in equation (7.2) and the diffusion coefficient of glucose at 0.34 and 0.13 mm was not calculated. It was found that the obtained diffusion coefficients were independent on the gel thickness and the average diffusion coefficient (0.35 ± 0.07, 0.83 ± 0.1 and 1.0 ± 0.2 mm thick) was (2.5 ± 0.9) $\times 10^{-7}$ cm^2 s^{-1}.

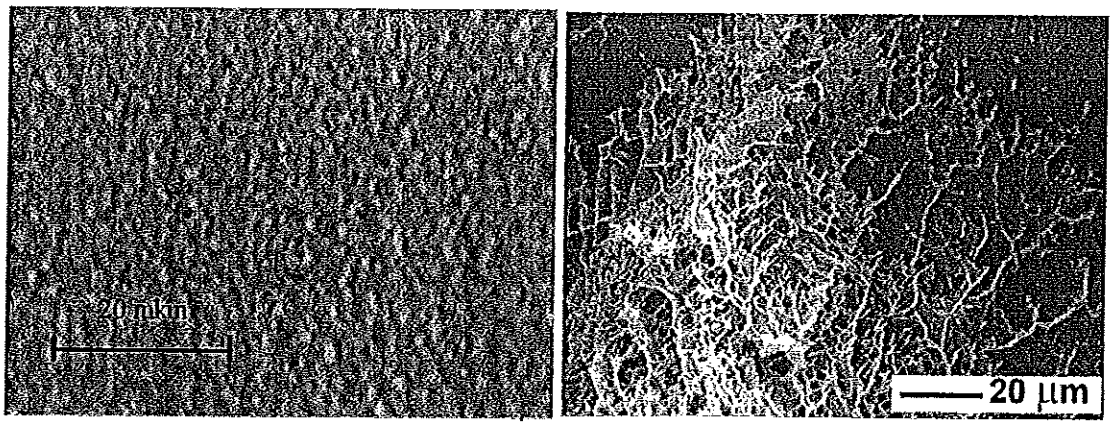


Fig. 7.8 (a) Light microscopy photograph of an NAAPBA-co-AA gel in distilled water; (b) Electron microscopy of a dry NAAPBA-co-AA gel; the photograph of a crack edge has been taken.

Obviously, the diffusion was somewhat hindered by the gel network, as the diffusion coefficient of glucose in pure water is 6.7×10^{-4} cm^2 s^{-1} (Lide and Fredrikse, 1994). On the other hand, the obtained diffusion coefficient is 5 orders of magnitude higher than that reported for poly(aniline boronic acid) (2×10^{-12} cm^2 s^{-1}) (Shoji and Freund, 2002), most likely due to highly porous structure of the NAAPBA-co-AA gels as shown in Fig. 7.8.

7.3.4 Repeatability of gel coating

Three different coated gels on glass plates synthesized from the same monomer mixture were used to investigate the repeatability of the coating. The calibration curves of glucose were performed with each gel in the concentration ranged from 1 to 60 mM (Fig.7.9).

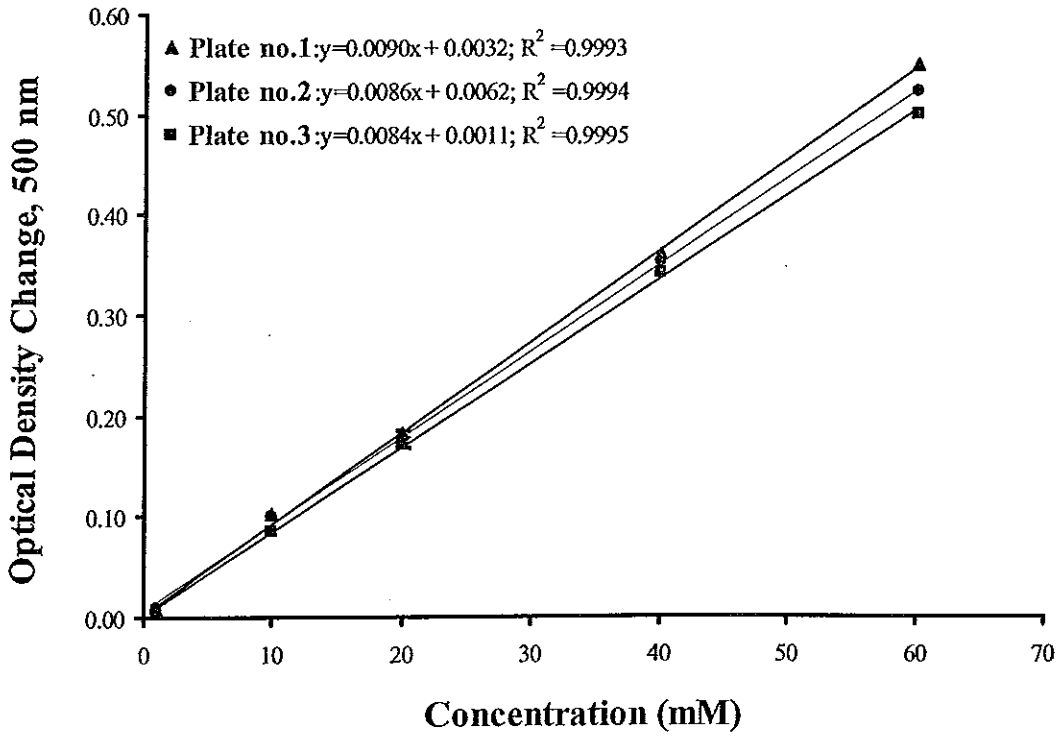


Fig. 7.9 Calibration curves from three different gel coated glass plates prepared from the same monomer mixture.

To test whether the differences among the three different coated gels on the glass plates are significant or whether they can be accounted for merely by random variations, a statistical test known as a significance test was applied. Significant test is widely used in the evaluation of experimental results. Different concentrations are used as controlled factors since the concentrations are chosen randomly by the researcher. The curves on which the experiments are performed introduce uncontrolled variation. The slopes of the three curves were tested using two-way ANOVA (analysis of variance).

In making a significant test, the truth of a hypothesis known as a null hypothesis, denoted by H_0, is that the interaction of each pair of the slopes is not significant, and an alternative hypothesis (H_1), the interaction of each pair of the slopes is significant. If the P value is less than α (level of significance), then the null hypothesis was rejected at the significant level. This P value was calculated by R software (R development Core Team, 2006). The results from significant tests of the comparison of the three calibration curves are shown in Table 7.3.

Table 7.3

Statistical values for the comparison between the slopes of the calibration curve of each pair of three different gel coated glass plates using two-way ANOVA by R software.

Pair of plates being Tested	D_f	Sum Sq	Mean Sq	F	P
1 and 2	4	1.8×10^{-4}	0.5×10^{-4}	1.1645	3.8×10^{-1}
2 and 3	4	4.8×10^{-4}	1.2×10^{-4}	1.4829	2.8×10^{-1}
1 and 3	4	1.2×10^{-4}	2.9×10^{-4}	3.0542	6.9×10^{-2} .

Significant codes: '.' (α=0.1), '*' (α=0.05), '**' (α=0.01)

Where D_f : Degree of freedom

Sum Sq : Sum square

Mean Sq : Mean square

F : Ratio of two variances

P : Probability

The slope of regression line of the calibration curves from gel coated glass plate no. 2 was not significantly different from gel no. 1 and 3 but the slopes of the regression line of the calibration curve from gel coated glass plate no. 1 and 3 were significantly different ($P<0.1$). This means that the forming of gels from the same lot of monomer mixture on different glass plates were not repeatable. This might be because they were manually coated by the experimenter. Therefore, in the case where the results needed to be compared *i.e.* during optimization and the analysis of real sample, the experiments should be done using a single coated gel glass plate in order to obtain reliable results. Hence, the reusability of a single gel was tested with 30 mM of glucose in phosphate buffer pH 7.3. It was found that for 42 consecutive analyses (25 days), the average signal was 0.321 ± 0.009 with relative standard deviation only 2.9% (Fig. 7.10) which indicate excellent reusability of the NAAPBA-co-AA gel.

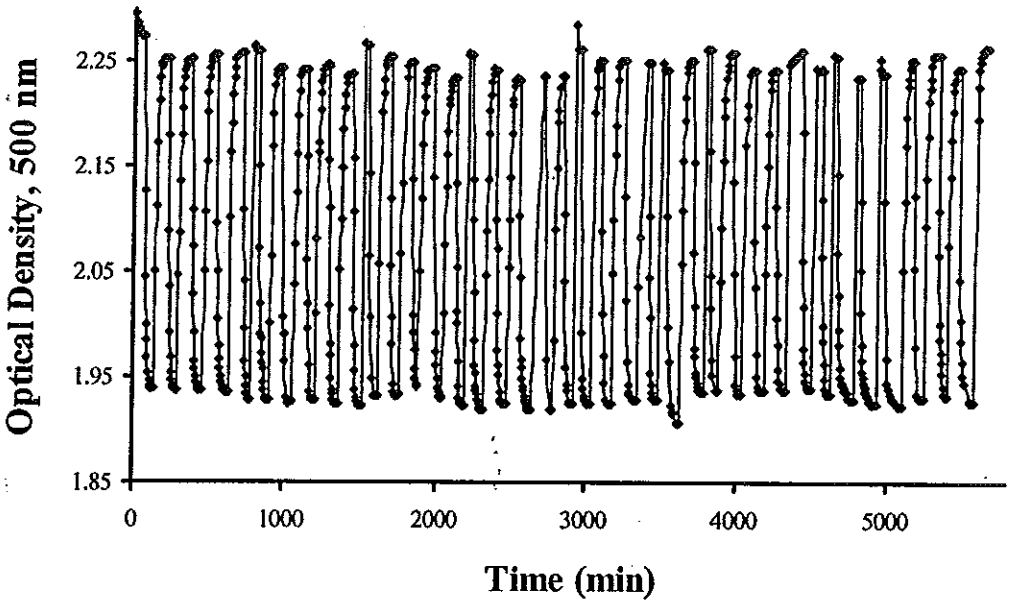


Fig. 7.10 Repeatability of a single coated gel plate tested with 30 mM of glucose in phosphate buffer pH 7.3.

7.3.5 The effect of the gel sterilization

Calibration curves of glucose before and after autoclaving are shown in Fig. 7.11 indicating that the autoclaving of the glass plates with the attached gel (10 min, 121°C, 1.2 bar) followed by cooling and equilibrating in 50 mM sodium phosphate buffer, pH 7.3 gave almost unchanged sensitivity to glucose as can be seen with the coincided within the limits of the experimental errors (Fig. 7.11). To confirm this, the significant test was performed using R software as mentioned in section 7.3.4 and the result is shown in Table 7.4. The null hypothesis (H_0) is that the interaction of both curves (before and after autoclaving) is significant; and an alternative hypothesis (H_1) is that the interaction is significant. The significance test also confirmed that the autoclaving did not have any effect on the sensitivity of the gels (at all levels of significance). Therefore, instability of enzymatic biosensors during sterilization can be overcome by using the NAAPBA-co-AA gels, making them more applicable.

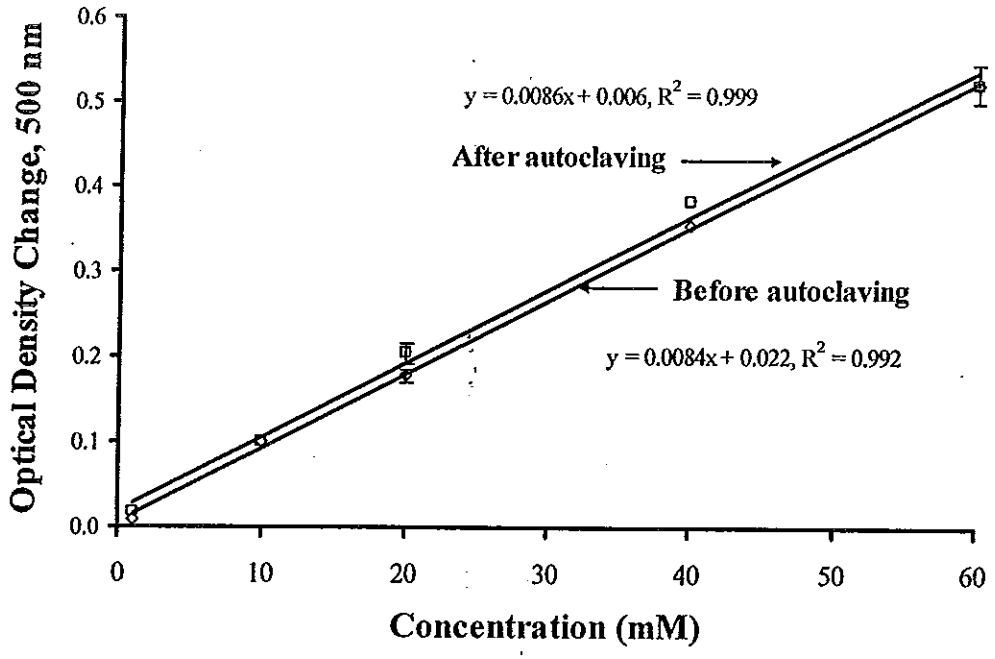


Fig. 7.11 Sensitivity of NAAPBA-co-AA gel to glucose in 50 mM sodium phosphate buffer pH 7.3 before and after autoclaving.

Table 7.4

Statistical values for the comparison between the slopes of the calibration curve before and after autoclaving of the coated gel using two-way ANOVA by R software.

Pair of plates being Tested	D_f	Sum Sq	Mean Sq	F	P
Before and after autoclaving	4	5.7×10^{-4}	1.4×10^{-4}	1.8541	1.9×10^{-1}

Significant codes: '.' (α=0.1), '*' (α=0.05), '**' (α=0.01)

Where D_f : Degree of freedom

Sum Sq : Sum square

Mean Sq : Mean square

F : Ratio of two variances

P : Probability

7.3.6 Sugar specificity of NAAPBA-co-AA gel

Since boronic acid is known to bind a wide variety of *cis*-diols (Lee *et al.*, 2004; Lorand and Edwards, 1959; Wang *et al.*, 2002). Any sensor system utililizing boronic acid ligands would be susceptible to have the interference from completing *cis*-diol containing compounds in the sensing environment. The response to various mono- and disaccharides was studied to investigate the cross-reactivity of the NAAPBA-co-AA gel with other sugars. Mannose and galactose (two monosaccharides which have similar structures to that of glucose), *N*-acetyl-glucosamine (monosaccharide derivative of glucose), fructose (monosaccharide which has a different structure from glucose), lactose and sucrose (two disaccharides) were selected for the testing and their chemical structures are shown in Fig. 7.12. The calibration curves of each sugar were performed in the concentrations range of 1 to 60 mM as shown in Fig. 7.13.

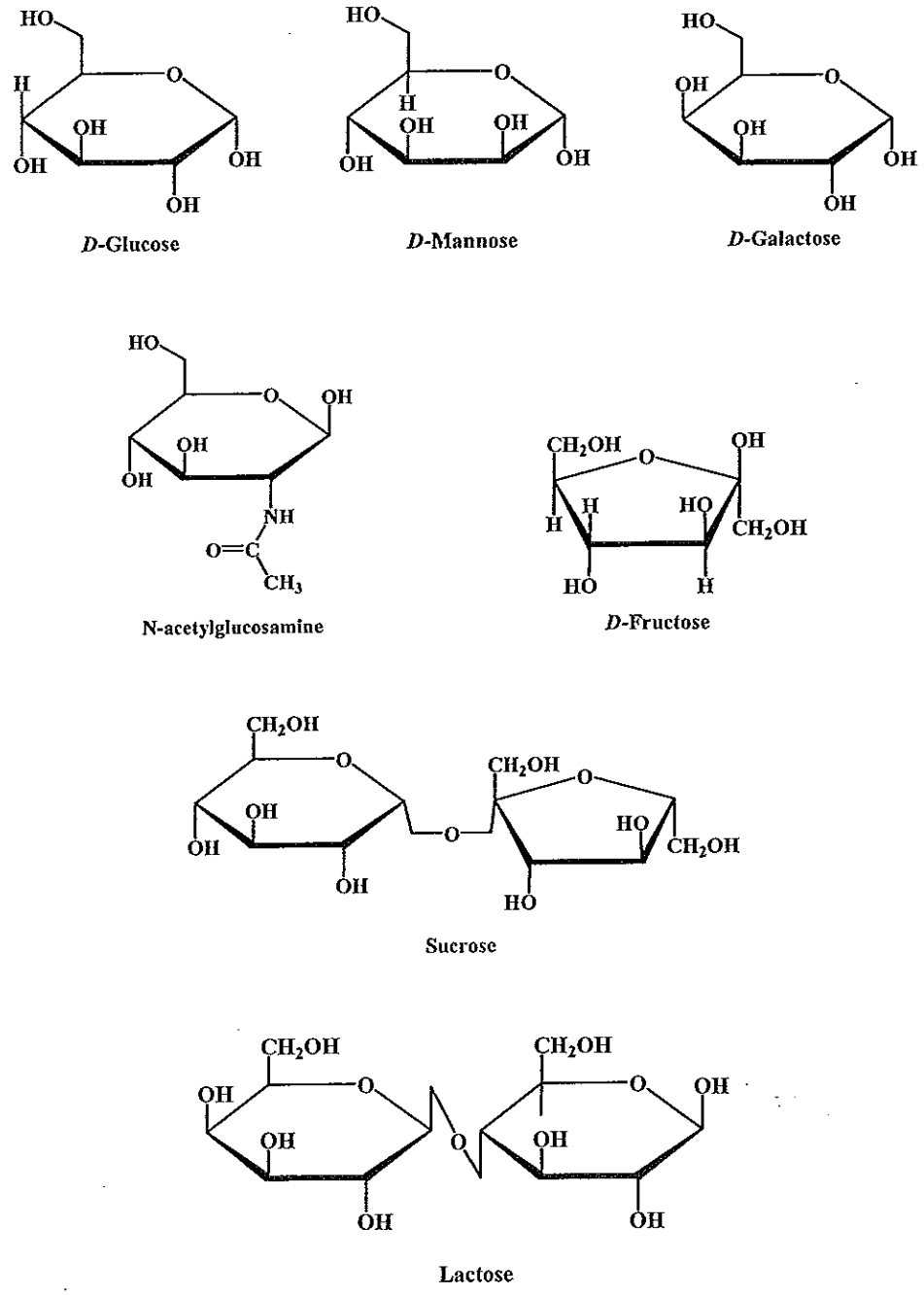


Fig. 7.12 Chemical structures of 4 monosaccharides: glucose, mannose, galactose and fructose, 1 glucose derivative: *N*-acetylglucosamine and 2 disaccharides: sucrose and lactose.

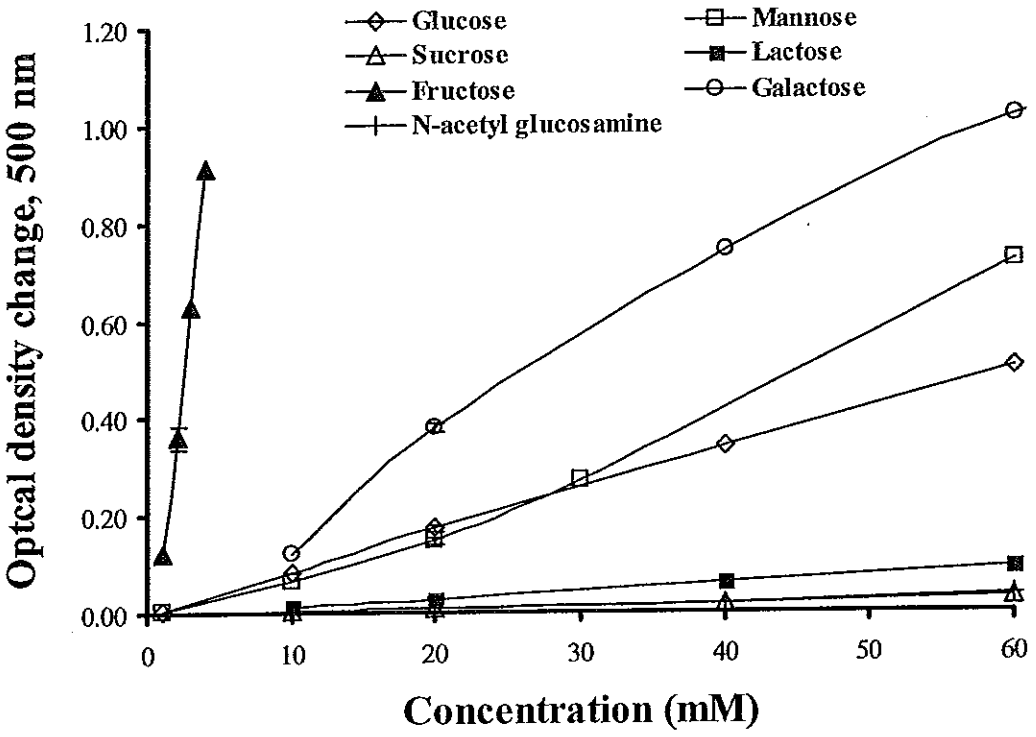


Fig. 7.13 Calibration curves of each sugar in phosphate buffer pH 7.3.

As mentioned earlier that the binding between phenylboronic acid and sugars is known to occur only with suitably configure *cis*-diols, consequently the position of hydroxyl groups on different sugars plays an important role to the changes in the optical density of this sensor. By considering the chemical structure showed in Fig. 7.12, the number of cis-diols moieties in the saccharide and its derivative is 0 for *N*-acetylglucosamine and sucrose, 1 for glucose (1,2-position), mannose (2,3-position) and fructose (1,2-position) and 2 for galactose (1,2- and 3,4-positions).

The sensitivity of the gels to fructose was found to be the highest followed by galactose, glucose~mannose, lactose and sucrose~*N*-acetylglucosamine (Fig. 7.13). This is because five-membered furanose rings in fructose offer the most suitable positioning of diols for binding to boronic acid and it has been suggested by van den Berg and van Bekkum that the *cis*-1,2 diol configuration on the furanose ring produces a very stable borate ester with a low ring strain and a decrease in entropy of the ligand (van den Berg *et al.*, 1994). And it can be seen very clear with Fig. 7.14

that the sensitivity of the gel to fructose is much higher than to galactose. To obtain the same optical density change, fructose needed only 2 mM while the concentration of galactose needed to be 10 times higher.

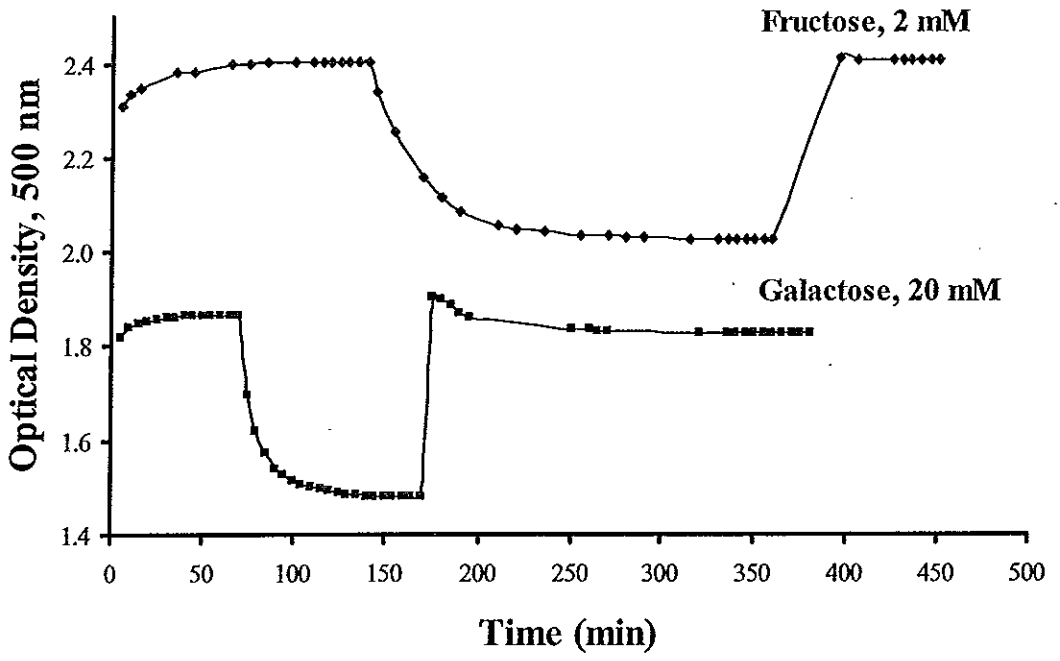


Fig. 7.14 Optical density of NAAPBA-co-AA gel in 2 mM of fructose and 20 mM of galactose in phosphate buffer pH 7.3.

In the case of galactose, glucose and mannose which are six-membered pyranose rings, the sensitivity of galactose was higher than that of glucose and mannose because of its 2-*cis*-diol moieties in the structure while glucose and mannose showed quite similar sensitivity. Disacharides, lactose and sucrose, and glucose derivative, *N*-acetylgluco- samine showed poor sensivity, probably due to steric hindrance cuased by the glycosidic bond and the fact that certain diol groups were no longer available for binding (Lee *et al.*, 2004). The order of binding affinities from this sensor agrees with the data obtained by using a holographic sensor that measuring the change in diffraction wavelength of a hologram (Lee *et al.*, 2004), glucose sensing photonic crystal measuring the shift of diffraction wavelength (Alexeev *et al.*, 2003), the stationary modified with alkylboronic acid by means of a liquid chromatography

(Soh *et al.*, 2002) and using a competitive fluorescence assay (Springsteen and Wang, 2002).

7.4 Conclusions

A simple affinity optical sensor based on the use of thin and high durable semitransparent gels of NAAPBA-co-AA coated glass plates was obtained. The gels are specifically responsive to *cis*-diol sugars which caused the change in their volume through swelling resulted in the change of their optical density. These have been utilized to develop sugar recognition sensor which have been reported in this chapter. The gel not only provides very good repeatable results (at least 42 times with RSD less than 3%) but it can also be sterilized which is an important property that makes this gel more applicable. Although the gel does not display unique specificity to a particular sugar but glucose is normally the predominant free sugar used in majority of cell growth media as the primary carbon source therefore, in case of the target analyte is glucose, this would be one suitable application of this developed sensor.

On the other hand, instead of using this synthesized polymer gel as sugar sensor, it can be used as packing material in a separation column or a cartridge of SPME for the separation or preconcentration of sugar prior to analysis or *vice versus* (extract sugars out from the analysis of some other organic compounds).

The results reported in this subproject are initial investigations of the use of NAAPBA-co-AA gel mostly involved qualitative analysis (behavior of the synthesized gels, how does the gels response to each sugar). Consequently, further study/development for quantitative study of this sensor needed to be investigated. These include several performance parameters such as linear dynamic range, limit of detection of each sugar and its recovery. The developed sensor also needed to be validated with standard method. This simple affinity optical sensor can then be used for quantitative analysis of sugars in real sample.

To obtain more benefit from this simple sensor, flow system is one of the most interesting and this will be a future subproject after the preliminary investigation of the feasibility of this sensor in batch system. With flow system, an on-line monitoring

of sugars can be performed resulting in the useful data in bioprocess or fermented process monitoring.

CHAPTER 8

Conclusions

The development and evaluation of the performance of sample preparation and analytical techniques for trace and ultra-trace analysis have been described in this thesis. All developed methods attempted to improve the complicated analytical methods by making them simpler, more economical, less laborious, less time consuming, miniaturizable and/or on-line.

The first development was the use of a screw capped glass bottle packed with Tenax TA as a passive sampler coupled with a laboratory-built thermal desorption device which was connected directly to the gas chromatograph for the determination of volatile organic compounds (VOCs). Three toxic compounds, benzene, toluene and xylene, were chosen as target analytes. The proposed system showed excellent linearity over wide ranges of 0.20-13,254, 3.6-5,104 and 7.0-1,962 $\mu g\ m^{-3}$ for benzene, toluene and xylene, respectively. The laboratory-built passive sampler can be reused up to 12 times and can be stored in the desiccator at room temperature for 3 weeks before the analysis can be performed. In addition, the validation of the passive sampling bottle demonstrated a good agreement between theoretically and experimentally uptake mass values. This method was successfully applied for the simultaneous analyses of VOCs in gaseous samples. Table 8.1 illustrated the comparison of analytical features of the developed passive samplers with some other works. The developed passive sampler gave similar detection limits to the commercial ones whereas it provided an excellent benefit, it can be reused up to 12 times. This will make the developed passive sampler much cheaper than to the commercial one (< 10 bahts/sampler).

Even though the laboratory-built passive sampling technique was very useful for the monitoring of VOCs in the ambient air, it would be better if the analysis could be performed in an on-line system to obtain the data for real-time monitoring. So, the second development using on-line microtrap was subsequently carried out. This was the use of microtrap packed with Carbosphere (a suitable adsorbent for

methane adsorption as methane was chosen as a target analyte), and then placed it in between the gas cylinder and the flame ionization detector to perform as both a preconcentrator and an injector. The chromatogram was obtained in a few seconds after the desorption using a laboratory-built thermal desorption device controlled by a timer. The system illustrated excellent repeatability when operated at both room temperature and -50°C (its optimum adsorption temperature) with the relative standard deviation less than 5% for 64 hours and 8 hours automated continuous operating periods, respectively. The 260 times enhancement was obtained at the optimum condition comparing to direct-FID signal. The detection limit was at 28.3 ppbv which was low enough to detect trace methane in high purity nitrogen gas. During operation a contaminant trap could also be inserted in front of the microtrap in order to increase the selectivity of the microtrap by removing other hydrocarbon compounds before they reached the microtrap. The comparison of the analytical features of some methods used for methane determination is summarized in Table 8.2. The on-line microtrap not only showed excellent limit of detection but also provided very short analysis time comparing to other methods.

The third investigation was the development of a simple and selective analytical system based on affinity binding. Glucose and phenylboronic acid were used as a case study binding pair. Copolymer of 3-acrylamidophenylboronic acid-acrylamide was synthesized and coated on the glass slide. The resultant semitransparent gel was then used as a simple affinity sensor for the determination of glucose. The proposed system showed excellent linearity of the optical density change over a wide concentration range (1-60 mM) of glucose with the limit of detection at 1 mM. Furthermore, this gel demonstrated an excellent repeatability with a relative standard deviation less than 3% during 42 consecutive run and could be sterilized (10 min, 121°C, 1.2 bar) making this gel more applicable. Table 8.3 summarized the analytical feature of some methods used for the determination of glucose. Although the developed sensor based on the use of phenylboronic acid-containing polymer gel in the combination with spectrophotometry showed higher limit of detection than that of the enzymatic system (Method I) but it was in the same range as other two methods using phenylboronic acid containing polymer modified electrode in cyclic voltammetry (Method G) and phenylboronic acid containing polymer-polyvinyl

acohol membrane complex modified electode in the measurement of current change (Method H). However, since the developed glucose sensor consists of only a glass slide coated with the synthesized copolymer gel, which is easily performed under mild conditions, and a spectrophotometer therefore, this glucose sensor is simpler than the enzymatic glucose sensor.

To fulfill the requirements of analytical techniques further developments could still be performed. In the case of laboratory-built passive sampler, the compactable of the passive sampling unit could be improved to make it more suitable not only for using as sampling tools for the target analytes in the environment but also as sampling tool for evaluation of the worker exposure of the target analytes from indoor air quality. Another technique which was the on-line microtrap system could also be further developed, either by using more selective adsorbent to improve its selectivity or improving design of the on-line system to it more attractive and compact. For the last developed method which was the affinity sensor. The improvement can be performed by adding a dialysis membrane to exclude high molecular weight compounds, such as polysaccharides which also have *cis*-diol functional groups, before they reach the gel since the dialysis membrane allows only the compounds with lower molecular weight than its molecular weight cut off. Other low molecular weight compounds can still diffuse through the dialysis membrane and interfere with the overall optical density changes indicating that this affinity sensor is not selective. However it can be used as a screening technique for the monitoring of monosaccharides containing *cis*-diols. In addition, the use of flow system combined with the high sensitive and selective detector are also the important aim for further development.

For any further development quality assurance, quality control and method validation must always be peformed to confirm that the developed methods can be applied for the analysis of real samples.

Table 8.1

Comparison of the analytical features of passive samplers for the determination of benzene, toluene and xylene.

Analytical feature	Present work	Method A	Method B	Method C
Passive sampler type	Tube-type	Tube-type	Badge-type (commercial)	Badge-type (commercial)
Adsorbent used	Tenax TA	Molecular sieve	Charcoal	Charcoal
Desorption method	Thermal	Solvent (carbon disulfide)	Solvent (carbon disulfide)	Solvent (carbon disulfide)
Limit of detection ($\mu g\ m^{-3}$)	Based on 3 weeks	Not mentioned	Based on 4 weeks	Based on 2 h
Benzene	0.3	-	0.1	0.4
Toluene	0.2	-	0.2	0.8
Xylene	0.7	-	0.4	2.0
Reusability	Yes (12 times)	No	No	No
Storage stability	3 weeks at 25°C	1 week at 25°C	6 weeks at 4-8°C	Not mentioned

Method A: Evaluation of a small prototype passive sampler for airborne volatile organic compounds (Otson and Cao, 1998)

Method B: Screening method for the determination of 28 volatile organic compounds in indoor and outdoor air at environmental concentrations using dual-column capillary gas chromatography with tandem electron-capture-flame ionization detection (Begerow *et al.*, 1996)

Method C: Determination of benzene, toluene, ethylbenzene and xylenes in indoor air at environmental levels using diffusive samplers in combination with headspace solid-phase microextraction and high-resolution gas chromatography-flame ionization detection (Elke *et al.*, 1998)

Table 8.2

Comparison of the analytical features of 4 different methods for methane determination.

Analytical feature	Present work	Method D	Method E	Method F
Limit of detection	28.3 ppbv	300 ppbv	300 ppbv	130 ppmv
Linear range	0.2-11.6 ppmv*	0.6 -3,800 ppmv	0.3-300 ppmv	0.1-5 %
Analytical time (min)	< 1	~4	~5	>10
Applicability for on-line determination	Yes	No	Yes	Yes

* the linear range was limited by the concentration of standard gas available in the laboratory.

Method D: A simple modification of gas chromatograph equipped with flame ionization detector for carbon monoxide, methane and carbon dioxide determination (Kamiński *et al.*, 2003)

Method E: Evaluation of the pulsed discharge helium ionization detector for the analysis of hydrogen and methane in breath (Roberge *et al.*, 2004)

Method F: Determination of methane and other small hydrocarbons with a platinum–Nafion electrode by stripping voltammetry (Jacquinot *et al.*, 2001)

Table 8.3

Comparison of the analytical features of 4 diferent methods for glucose determination.

Analytical feature	Present work	Method G	Method H	Method I
Limit of detection (mM)	1	3	Not mentioned	1.4×10^{-3}
Linear range (mM)	1-60	3-300	0-17	1.4×10^{-3} -5.5×10^{-2}
Enzymatic system	No	No	No	Yes
Working pH	7.3	7.6	7.4	6
Reusability	Yes	Yes	Yes	Yes
Sterilizability	Yes	Not mentioned	Not mentioned	No

Method G: Phenylboronic acid monolayer-modified electrodes sensitive to sugars (Takahashi and Anzai, 2005)

Method H: Glucose-sensing electrode coated with polymer complex gel containing phenylboronic acid (Kikuchi *et al.*, 1996)

Method I: Direct glucose determination in blood using a reagentless optical biosensor (Sanz *et al.*, 2007)

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Appendices

Appendix A

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JOURNAL OF CHROMATOGRAPHY A
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Microtrap modulated flame ionization detector for on-line monitoring of methane

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Abstract

A microtrap for on-line analysis of trace methane was developed. Silicosteel tubing, 15 cm long, 1.02 mm i.d. and 1.59 mm o.d. was packed with Carbosphere 80/100 mesh and placed between the standard gas source and the flame ionization detector (FID) to be used as a preconcentrator and an injector. The sample stream was passed continuously through the microtrap that was heated at a fixed interval by an electric pulse. To achieve the highest adsorption and desorption efficiency, parameters affecting the response of the system were optimized, and adsorption temperature in the range of 25 to −50 °C was studied. The response from the microtrap as compared to that from direct-flame ionization detector was enhanced by as much as 260 times. The on-line microtrap system showed good linearity ($r^2 > 0.99$), low detection limit (28.3 ppbv) and good long term stability (relative standard deviation, RSD of less than 5.0%). Therefore, this simple device is suitable for on-line analysis of trace methane and similar small molecules in the environment as in high purity gases.

Keywords: Methane; Microtrap; Carbosphere; High purity gases

1. Introduction

Methane, due to its abundance, can occur even in high purity gases which are crucial for research and development applications and this contamination can have a negative impact on the process [1]. Therefore, measurements and control of the impurities play an important role in high purity gases research applications.

To determine methane concentration, samples are generally collected before injected into a gas chromatograph [2]. A small injection volume is preferred but this will limit the sensitivity of the detector and a preconcentration step is generally needed. Even when the sensitivity is increased by a preconcentration step, there is always the delay between sampling and analysis [3–5]. This is clearly not acceptable for continuous, on-line monitoring, but also adds error to the measurement process. One approach is using an on-line cryogenic trap to focus the sample in a narrow band at the head of the column. The trap rapid cooling and re-heating, ensuring the reproducibility and optimum peak shape. However, if the source has a lot of water, it freezes and plugs the sorbent trap [6]. It is also quite complicated to apply, therefore, a microtrap was introduced as an alternative.

A microtrap is a small capillary tubing packed with a small amount of adsorbent. Sample containing the analytes continuously flow into the microtrap, and the organics are trapped by the sorbent while the gas flows through. The retained organics are rapidly desorbed by resistive heating with pulse of electric current which make an injection for GC separation.

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The main advantage of microtrap as an injection device over a sample valve is, it also serves as a sample preconcentrator, which allows the larger sample volume to be analyzed for trace components [7–10].

In this paper, the development of an on-line microtrap that is interfaced directly to the flame ionization detector (FID) for the determination of methane is reported.

2. Experiment

2.1. Instrumentation

Fig. 1a shows the schematic diagram of the on-line system used for methane monitoring. All experiments were carried out by a gas chromatograph equipped with a conventional FID and the results were integrated by CR-4A Integrator (GC-14A and CR-4A Integrator, Shimadzu, Japan). Methane standard concentration of 11.6 part per million by volume (ppmv) (TIG, Thailand) was used to study the characteristics of the microtrap packed with a suitable adsorbent.

2.2. Microtrap

By considering the suitability of pore size and surface area, Carbosphere 80/100 mesh (Alltech, IL, USA) was selected as the adsorbent. It was packed in a 15 cm long, 1.02 mm i.d., and 1.59 mm o.d., silicosteel welded/drawn 304 grade stainless steel tubing (Restek Co., Bellefonte, PA, USA). The microtrap was placed in-line of the stream of the standard gas and in front of the detector to act as a trap/concentrator as well as an injector (Fig. 1a). The microtrap was rapidly heated by applying a current pulse from a lab-built heating system and a temperature as high as 250–300 °C was reached within a few milliseconds. This was to desorb and to inject the analyte into the FID. The effect of heating was similar to using an injection port in term of retention time, peak height, band duration and terminal band length [7]. A microprocessor-based timer was used to control the interval between the adsorption and desorption times and the duration for which the current was applied.

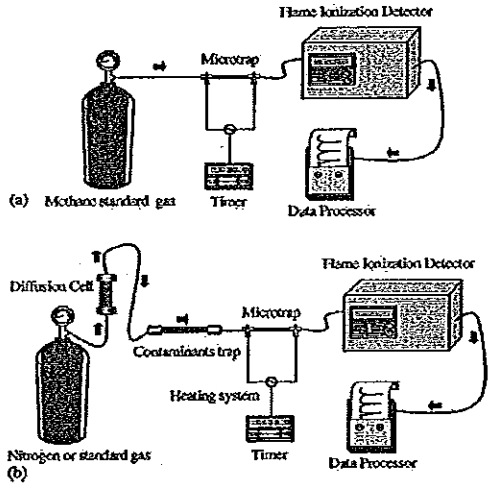

Fig. 1. Schematic diagram showing the analysis system. (a) microtrap interfaced to flame ionization detector for on-line determination of methane. (b) Diffusion cell was used to introduce organic in the N_2 stream to test the ability of the contaminants trap to remove the organic.

2.3. Breakthrough characteristics

To determine breakthrough in a microtrap, the method using the variation in microtrap response as a function of injection interval [4] was implemented. The capacity, in term of breakthrough, was studied by passing a stream of standard methane gas (11.6 ppmv) through the microtrap. Methane molecules were adsorbed while the background stream served as a carrier gas. The system was first investigated at room temperature (25 °C). Desorption voltage and time were varied in the range of 10–50 V and 1–4 s. The optimum desorption voltage and time were then used to determine the breakthrough by varying the adsorption time until stable response peaks were obtained.

2.4. Enhancement of microtrap capacity

The most important factor for trace quantity analysis is enhancement. A higher enhancement (or enrichment) factor can be obtained by decreasing the microtrap temperature. The microtrap was placed inside an insulating foam box, surrounded by dry ice. The temperatures were varied from 25 °C (room temperature) to −50 °C by varying the amount of dry ice. At each adsorption temperature, the optimized desorption voltage and time were investigated to obtain the maximum desorbed analyte from the microtrap. The adsorption time at each temperature was then varied to determine the breakthrough time. The responses were used to calculate enhancement.

2.5. Performance of the microtrap

To study the linear response of the on-line microtrap system, a series of standard gas concentrations, 11.6, 5.8, 3.9, 1.1 and 0.2 ppmv, was prepared by dynamic dilution method [11] using nitrogen gas (99.99% TIG, Thailand) as the dilutor. Each concentrations of methane were continuously passed through the microtrap. The injections were made at 6 min interval using a pulse time of 3.5 s at 40 V for five replications at −50 °C. The signals were used to plot the standard curve.

High purity nitrogen gas (99.99%) was used as blank. It was continuously applied through the microtrap. The desorptions, i.e., injections were done every 6 min and the obtained data were used to calculate the limit of detection based on IUPAC method [12].

2.6. Contaminants trap

A contaminants trap was placed in front of the microtrap to remove the contaminants that might interfere with the on-line trace methane analysis system (Fig. 1b). A suitable adsorbent was evaluated. These included activated charcoal (Palm Shell), coated Carbopack B, and Carbopack C, selected by considering their surface area, and pore size. Each of these adsorbents was packed in a stainless steel tube (1/4″ o.d. × 4 mm i.d. × 16 cm).

The experiments consisted of two parts. First was to test the trapping efficiency of the contaminants trap. It was placed between the standard hexane gas (from diffusion cell) and the microtrap packed with coated Carbopack B (Fig. 1b). This microrap was used to monitor hexane that could not be adsorbed by the contaminants trap. The second part was to test the effect of contaminants trap on the response of methane. The diffusion cell was removed and standard methane gas was flowed through the contaminants trap before passing through the microtrap packed with Carbosphere. The signals obtained with and without the contaminants trap were compared.

3. Results and discussion

3.1. Breakthrough characteristics of the microtrap

Breakthrough is an important parameter since it can indicate the capacity of the microtrap in term of the volume or the time that the microtrap can retain the analytes without loosing them. The adsorption time of the microtrap was varied from 0.5 to 4 min, and then desorbed by applying the current to the microtrap at optimum conditions, 15 V, 2.5 s, at room temperature (25 °C). The response increased with adsorption time (Fig. 2) up to 2.0 min, and then became constant and this was the breakthrough time. Two minutes was then used as the optimum adsorption time for each analysis cycle.

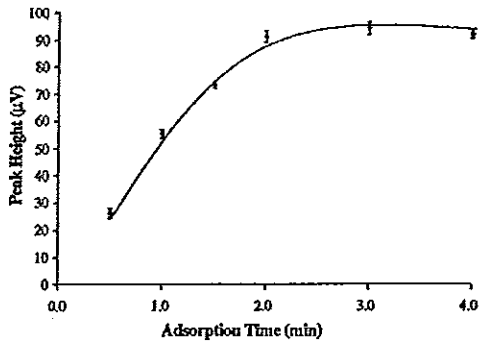

Fig. 2. Response at different adsorption times used to determine breakthrough time of the microtrap at room temperature (25 °C). The microtrap was heated at desorption voltage of 15 V for 2.5 s.

Optimum conditions at room temperature provided chromatograms with an average peak height of 90 μV for 11.6 ppmv of methane. With this response, the on-line system could not be used to detect trace methane in high purity gas which are in the range of 0.1–5.0 ppmv [13]. Therefore, the sensitivity of the on-line microtrap must be increased.

3.2. Relationship between breakthrough volume and temperature

Adsorption is an exothermic phenomenon and the logarithm of the breakthrough volume (BTV) is inversely proportional to the temperature [14,15]. To increase the response and the breakthrough, the adsorption temperature was decreased. Table 1 shows optimum desorption conditions that provide the highest desorption efficiency. For all temperatures the thermal desorption could be done by heating the microtrap for a few seconds and the analytes were carried to the detector as a plug resulting in very sharp peaks (Fig. 3).

The results confirmed the Van't Hoff-type relationship [14,15], that is, the lower the adsorption temperature the higher the breakthrough volume (Fig. 4). This is because when the temperature decreased, the average time molecules resided on a surface increased. Therefore, more methane can accumulate on the surface of the adsorbent which caused the increase of the breakthrough of the microtrap. The linear relationship is $\log(\mathrm{BTV}) = 0.42 \times 10^3(1/T) + 0.65$, and this can be used to calculate the breakthrough volume of methane on Carbosphere. The slope of the curve is, $-8.1\ \mathrm{kJ\,mol^{-1}}$, the adsorption enthalpy (ΔH_{ad}) [14]. Since the maximum observed enthalpies reported for physical adsorption was $-21\ \mathrm{kJ\,mol^{-1}}$ while the enthalpies for chemisorption were in the region of $-200\ \mathrm{kJ\,mol^{-1}}$ [15]. The result indicates that, methane was adsorbed on the Carbosphere by physisorption.

3.3. Enhancement

The enhancement at various sampling temperatures is the ratio of the response from the on-line microtrap system to the direct-FID. When the temperature decreased, the enhancement increased (Fig. 5). At −50 °C the enhancement factor reached 260, i.e., the response increased tremendously from

Table 1
Optimum desorption conditions and optimum adsorption time (breakthrough time) at various adsorption temperature

Adsorption temperature (°C)	Desorption conditions		Optimum adsorption time or breakthrough time (min)
	Voltage (V)	Duration of pulse (s)	
25	15	2.5	2.0
−10	20	1.5	3.0
−20	30	2.5	4.0
−30	35	2.5	4.0
−40	40	2.5	5.0
−50	40	3.5	6.0

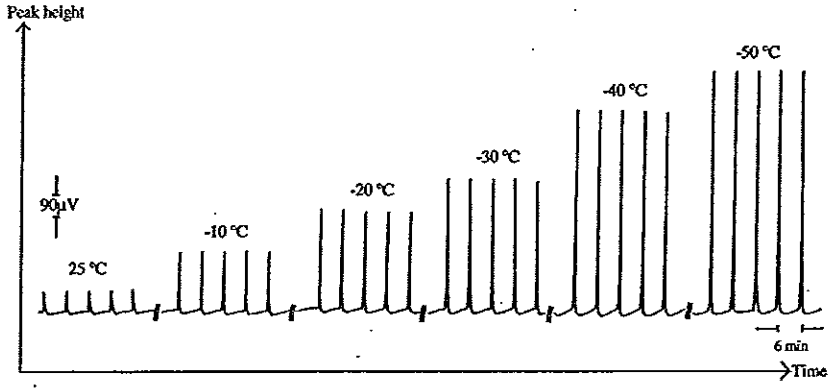

Fig. 3. Chromatograms of the on-line monitoring microtrap at various temperatures.

3.99 μV obtained from direct-FID to 1040 μV using a microtrap and this makes it very suitable for trace methane analysis.

Due to the limitation of the lab-built cooling box, the lowest temperature that can be obtained was −50 °C. However, at this temperature, the signal for 11.6 ppmv of methane had already increased from 90 μV at room temperature to 1040 μV and this should be high enough to detect trace methane contaminated in high purity gases which are in the range of 0.1–5.0 ppmv [13] and this is confirmed in 3.4.

3.4. Performance of the on-line microtrap

The linear relationship between the peak height and the concentration of methane standard gas was investigated between 0.2 and 11.6 ppmv. A linear calibration curve from the microtrap was obtained as peak height (μV) = 99.9 concentration (ppmv) + 11.7 (r^2 = 0.995). That is, a very good linearity was obtained and this system can certainly be applied for quantitative analysis of methane.

The limit of detection was also studied to ensure that this system is sensitive enough to determine the concentration of methane in high purity gas and it was found to be 28 ppbv. This is much better than the 300 ppbv determined without preconcentration reported by Kamiński et al. [16]. Therefore, this proposed system is more suitable to be used as an on-line device to be placed in line of the production processes for the continuous monitoring of the pure and research purity gases where the concentration of methane must not be higher than 0.1 ppmv [13].

3.5. The contaminants trap

To further increase the selectivity of a microtrap, a contaminant trap can be applied to the system to remove other compounds, except methane, before reaching the microtrap. The results from three different contaminants traps are shown in Fig. 6, indicating that all of the studied adsorbents could adsorb the impurity/contaminant (hexane was used as the representative of the impurity at 500 ppmv) with efficiencies of higher than 99%. So, the effect of contaminant trap on methane signal and the lifetime of the trap were used as the parameters for adsorbents selection. Fig. 6 shows that activated charcoal had a much greater lifetime (>9000 min) com-

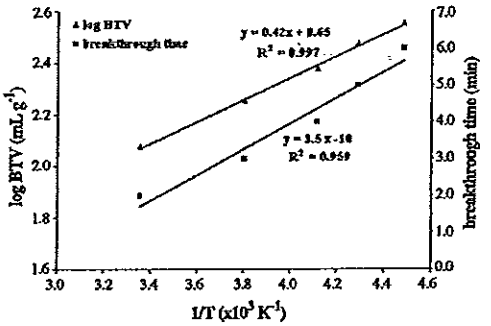

Fig. 4. Relationship between the breakthrough volume (BTV) and breakthrough time of the microtrap and sampling temperature.

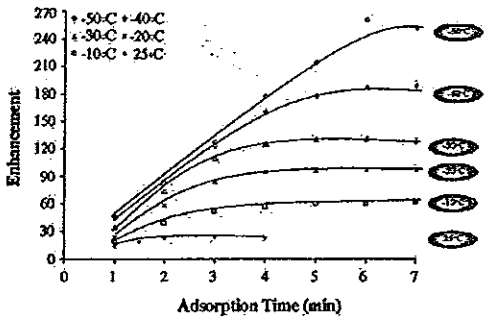

Fig. 5. Enhancement at various sampling temperature and adsorption time (injection interval).

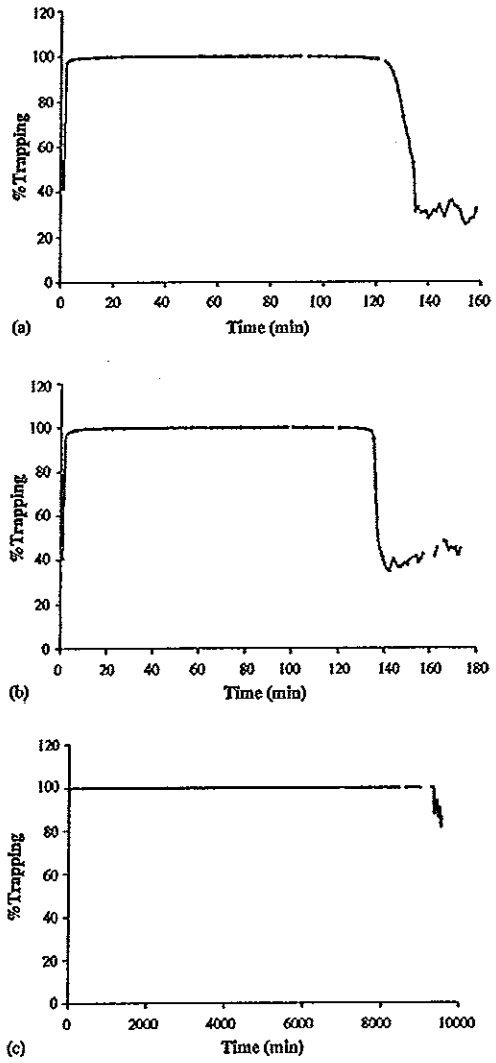

Fig. 6. Life time of the contaminants trap packed with various adsorbent; (a) coated Carbopack B, (b) Carbopack C, (c) activated Charcoal.

pared to coated Carbopack B and Carbopack C (120 min). However, it also trapped some methane on its active surface (Fig. 7). Therefore, either coated Carbopack B or Carbopack C would be a suitable adsorbent for contaminants trap since they showed very high removal efficiency and had no effect on methane signal.

3.6. Long term stability

Stability of this system was evaluated by continuously flowing 11.6 ppmv of methane standard at room temperature

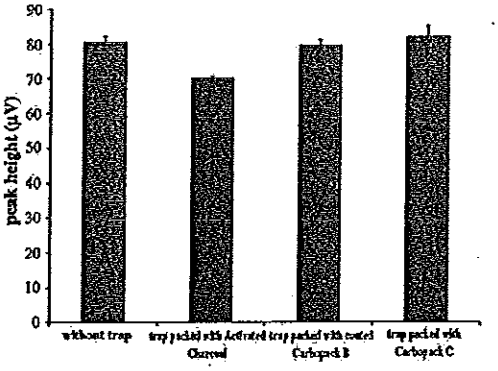

Fig. 7. Effect of contaminants trap on methane signal.

with a rate of 5.0 mL min^{-1}. Nearly 2000 injections were made by applying current through the wall of the microtrap every 2 min for 64 h. The average response was 89.2 ± 3.8 μV (relative standard deviation, RSD <5%). Since −50 °C was the optimum adsorption temperature, the stability of the on-line system at this temperature was also studied. It was tested for a work day period, which is 8 h. The injections were made every 6 min with desorption voltage and time of 40 V and 3.5 s, respectively. The average peak height was 1037 ± 51 μV (RSD <5%).

The results indicated that the system was very stable. It also showed that the adsorbed analytes were efficiently desorbed from the adsorbent without any memory effect.

4. Conclusions

The results demonstrated that the on-line microtrap can provide reliable data and good reproducibility. It can be operated over a very long period of time with good precision and without the degradation of the adsorbent. An enhancement of up to 260 times could be obtained when using a simple, cost effective cooling system. Therefore, this microtrap can certainly be applied to the gas production industries to check their production quality, not only for checking the methane contamination but also for determination of methane concentration required by the customers.

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Appendix B

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ATMOSPHERIC ENVIRONMENT
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Cost effective passive sampling device for volatile organic compounds monitoring

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Abstract

A laboratory-built passive sampler was developed as a simple and cost effective device for monitoring volatile organic compounds (VOCs) such as benzene, toluene and xylene (BTX). Common glass bottles (screw cap, 10 ml, 67.6 × 10.6 mm ID), packed with 75 mg of activated Tenax TA, were used as passive samplers. After exposed to real sample, the adsorbent was desorbed using a laboratory-built thermal desorption device. The analytes were purged to fill a sampling loop and then injected by a gas sampling valve to a gas chromatograph with a flame ionization detector (FID). All parameters, i.e., desorption time, purge flow rate, gas chromatograph conditions were optimized to obtain high sensitivity, resolution and short analysis time. The system was calibrated by BTX standard gas and the linear regression coefficient of greater than 0.99 was obtained with detection limits 0.3, 0.2 and 0.7 $\mu g m^{-3}$ for benzene, toluene and xylene, respectively. The proposed method was implemented for the monitoring of BTX at 10 gasoline stations in Hat Yai, Thailand. The concentrations were found in the range of N.D.–19, 12–200 and 23–200 $\mu g m^{-3}$ for benzene, toluene and xylene, respectively.

Keywords: Laboratory-built; Passive sampling; VOCs; BTX

1. Introduction

Volatile organic compounds (VOCs) are commonly found in the environment, work place and consumer products (Begerow et al., 1996; Schneider et al., 2001; Zabiegała et al., 2002). Human are, therefore, easily exposed to these chemicals through skin, breathing and eating and even at low concentration these present long term health risks (Shojania et al., 1999). There is some evidence on animal studies indicated that some of these VOCs have carcinogenic or mutagenic effects on tissue development. Benzene is one of these chemicals which are classified by US EPA as a human carcinogenic compound group A (EPA[1], 2005). Moreover, benzene, toluene and xylene, known as

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BTX, are the markers for human exposure to VOCs; therefore, the monitoring of these three compounds is necessary in order to evaluate the risk to human health (Begerow et al., 1996).

Analysis of VOCs in ambient air, which has the concentration in the range of hundreds of ppb to tens of ppt, required a preconcentration step to improve the sensitivity as well as the limit of detection of the method (Wang et al., 1999). Therefore, the sampling and at the same time preconcentration on solid sorbents was introduced.

Sampling of VOCs on solid sorbents was done by either active or passive sampling (Prado et al., 1996; Periago et al., 1997; Batterman et al., 2002; Guoin et al., 2005). In active sampling, a device such as a pump is required to force the flow of the sample through the medium. A flow meter measuring the flow rate and volume of air is also necessary. In passive sampling, the analytes flow through the collecting medium without any force follows Fick's first law (Górecki and Namieśnik, 2002). The main sampling process occurs from the concentration gradient between two media (sample and collecting media). Therefore, the equipment uses for sampling is not complicated as in active sampling. Furthermore, passive sampling can provide results which are accurate as active sampling (Zabiegała et al., 2002) while it provides much more advantages such as, it is a simple and low cost technique, it can be put on high risk area and several samples can be collected at the same time. Therefore, passive sampling becomes more attractive and acceptable (Zabiegała et al., 2002).

Since commercial passive samplers are quite expensive, for example one package of SKC Ultra I Passive Sampler (five items) costs $180 (©SKC Gulf Coast Inc., 2005). Therefore, this work focused on the development and evaluation of a simple and economical laboratory-built passive sampling system for air monitoring. The developed system was then applied to real situation by using it to sampling BTX in gasoline stations in Hat Yai, a major city in Southern Thailand.

2. Methodology

2.1. Laboratory-built passive sampler and thermal desorption device

Tenax TA was used as the adsorbent because it has been shown to adsorb BTX (Zabiegała et al., 2002, Wideqvist et al., 2003). It is especially useful for purging and trapping of volatile organic compounds from high moisture content samples including the analysis of compounds in water (Scientific Instrument Services, Inc.), making it suitable for Hat Yai high humidity (The Weather Underground, Inc., 2005). Before used, Tenax TA was activated, through the clean-up process, by heating at 300 °C for 2 h under purging of pure nitrogen gas. This was to remove the contaminants from the adsorbent before applying it to the standards or samples (Scientific Instrument Services, Inc., 2005).

Glass bottles, 10 ml (67.6 mm height, 10.6 mm ID) with screw caps, filled with 75 mg of activated Tenax TA (60/80 mesh, Supelco, USA) were used as passive samplers. The adsorbent was retained in the passive sampling bottle by placing a stainless steel net on its top. When sampling, four bottle samplers, hung inside an up-side down open box (Fig. 1), were placed at each site. The box was used to protect the passive samplers from rain and dust. On the box, there was a label informing people of its purpose.

A simple yet effective thermal desorption device was constructed using a block of brass (∅ 2.9 cm, 1.3 cm height) and a soldering iron (ERSA 30, Germany). The soldering iron was inserted into a tightly fitted hole, drilled into the bottom of the brass block, to generate heat. The temperature of the brass block, acting as a heating plate, was calibrated and monitored by a thermocouple-multimeter (DL 297T digital multimeter, Universal Enterprises, Inc. Korea). The temperature could be as high as 250 °C.

2.2. Laboratory-built purge and trap system

Fig. 2 shows the schematic diagrams of the purge system where two six port valves, A and B, were used to switch nitrogen gas between the purge line (to the passive sampling bottle) and the carrier line (to GC-FID). The operation of this system was carried out in three steps.

Step I: heating, valve A was in the position that allowed nitrogen (line 1) to flow through the sampling loop to the vent line while valve B allowed nitrogen (line 2) to pass directly to GC-FID. In this step, the passive sampling bottle was heated by the laboratory-built thermal desorption device without purging.

Step II: purging, valve A was switched to allow nitrogen (line 1) to pass through the passive

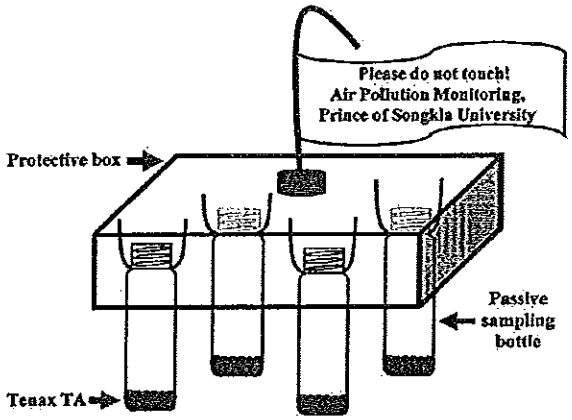

Fig. 1. Laboratory-built passive sampler.

sampling bottle. The analytes were purged and carried to a 1.0 ml sampling loop in valve B.

Step III: injection, in this step valve B was switched to allow nitrogen (line 2) to carry the analytes in the sampling loop and injected to GC-FID.

The optimum desorption parameters such as desorption time, purging flow rate, purging time were investigated.

2.3. Gas chromatographic system

All analyses were performed using a Shimadzu GC-14A (Shimadzu, Japan) with a 30 m, 0.53 mm and 1.00 μm, VB-WAX capillary column (Valco-Bond, USA) equipped with a flame ionization detector. High purity nitrogen was used as carrier gas (99.99%, TIG, Thailand). Data processing was carried out with a C-R4A Chomatopac (Shimadzu, Japan). The gas chromatographic conditions were also optimized to achieve the best performance.

2.4. Calibration

To calibrate the system, known amounts of standard BTX were injected into the bottles containing activated Tenax TA. Each bottle was then connected to the purge system in Fig. 2. The analytical procedure followed the three steps described in Section 2.2 and the analysis was carried out by GC-FID. Calibration curves were obtained by plotting the responses versus the injected amounts.

2.5. Validation of the laboratory-built passive sampling bottles

Since the sampling bottle has a neck with a smaller cross section than the bottle and this may affect the diffusion process, an experiment was set up to test the system in a room where the temperature was controlled at 25 °C. Toluene is used as a representative standard gas because the values of its boiling point, vapor pressure, and diffusion coefficient are between the other two compounds (benzene and xylene). Toluene standard gas was generated from its liquid form using the diffusion cell which was connected to a glass chamber where the analyte was diluted by incoming air from an air pump (Fig. 3). The air flow rate was 1.35 L min^{-1}. Laboratory-built passive samplers were placed at the end of the chamber to collect the analytes that diffused into the bottles and adsorbed on the surface of Tenax TA. After 3 h the passive sampling bottles were removed from the chamber, each bottle was connected to the purge system in Fig. 2 and the analysis was carried out by GC-FID. From the response the uptake amount of toluene could be obtained from the calibration curve done prior to the experiment. Validation was done by comparing the uptake amount of toluene obtained experimentally to the value determined theoretically.

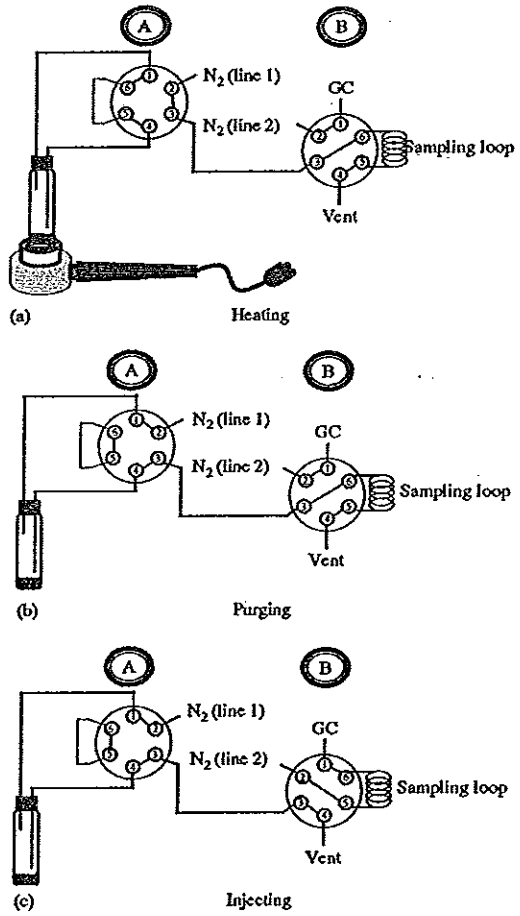

Fig. 2. Schematic diagrams showing the operation of thermal desorption and purge system.

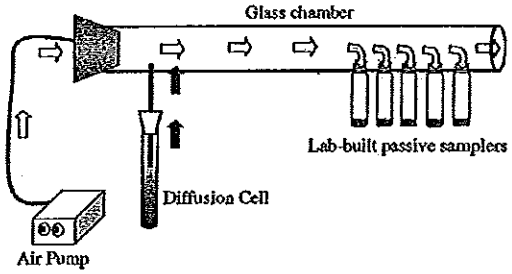

Fig. 3. System used for validation of passive sampling bottles. Solid arrows show the route of standard gas that diffused to the chamber and is diluted by air from the air pump. The analyte was then collected in passive sampling bottles.

3. Results and discussion

3.1. Optimum conditions for GC-FID and purge and trap system

Gas chromatographic conditions were optimized by considering two parameters, that is, high response and short analysis time. The optimum conditions are shown in Table 1.

Initially a sampling loop was not used in the system (shown in Fig. 2) and the analytes released from the adsorbent were continuously flowed through the GC. Although the responses were high the chromatograms gave poor resolutions. Therefore, a sampling loop was incorporated into the system to allow the "plug" injection of the analytes and sharp chromatograms were obtained.

For desorption, optimization of heating time was necessary to provide a high desorption efficiency without degradation of the adsorption performance of the adsorbent. The flow rate of purge gas and the time for purging must also be considered to minimize the loss of the analytes. If the time used in purging step was too long, the analytes might be lost through the vent and if it was too short, only a small amount of the analytes would be injected to GC-FID, then low sensitivity would be obtained. These parameters were investigated and the optimum purging and desorption conditions were found as shown in Table 1. These conditions were used for further studies.

Table 1
Optimum gas chromatographic, desorption and purge conditions for benzene, toluene and xylene (BTX)

Parameters	Optimized values	Optimum
Carrier gas (N_2)	1–6 mL min^{-1}	4 mL min^{-1}
Fuel gas (H_2)	10–50 mL min^{-1}	30 mL min^{-1}
Oxidant gas (Air)	100–400 mL min^{-1}	300 mL min^{-1}
Column temperature program		
Initial temperature	30–60 °C	40 °C
Initial holding time	0–3 min	0 min
Ramp rate	2–14 °C min^{-1}	4 °C min^{-1}
Final temperature	90–120 °C	110 °C
Final holding time	2 min	2 min
Detector temperature	180–220 °C	200 °C
Heating or desorption time	30–120 s	60 s
Purging flow rate	40–80 mL min^{-1}	50 mL min^{-1}
Purge time	2–10 s	6 s

3.2. Validation of the passive sampling bottles

The theoretical uptake amount of toluene in the passive sampling bottle can be calculated using the following equation (Batterman et al., 2002; Górecki and Namieśnik, 2002):

$$Q = \frac{AD(C_1 - C_0)t}{L}, \quad (1)$$

where Q is the uptake amount of the analyte in the bottle (μg); A the cross section area of the sampler = 1.62 cm^2; D the diffusion rate of the analyte = 8.5×10^{-2} $cm^2 s^{-1}$ at 25 °C (Danish EPA, 2002); C_1 the concentration of the analyte in the chamber = 9.46×10^{-7} $g L^{-1}$; C_0 the concentration of the analyte at the surface of the sampler = 0 $g L^{-1}$; t the exposure time = 10 800 s; L the diffusion length = 6.47 cm.

The concentration of toluene in the chamber, C_1 (9.46×10^{-7} $g L^{-1}$), was obtained as follows. First, the concentration in the test tube of the diffusion cell was calculated using ideal gas law and was found to be 1.53×10^{-3} mol L^{-1}. Then the mass transfer rate (diffusion rate—Q/t) between the test tube and the chamber through the capillary connecting tube was obtained using Fick's first law ($Q/t = 2.31 \times 10^{-10}$ mol s^{-1}). Knowing the air flow rate the concentration of toluene in the chamber could be calculated from the mass transfer rate.

Using Eq. (1), the uptake amount of toluene (Q) obtained from the calculation was 0.22 μg. To obtain Q experimentally, a calibration curve was first established as described in Section 2.4. After 3 h exposure time, each passive sampling bottle was connected to the system in Fig. 2. Peak heights obtained from the GC-FID were converted to the amounts of toluene, Q in Eq. (1), using the linear equation of the calibration curve done prior to the experiment. The amount of 0.21 ± 0.02 μg of toluene was obtained for five passive sampling bottles. The results showed that there was no difference between the two values. This experiment indicated that the laboratory-built passive samplers can certainly be used for monitoring of BTX in the environment.

3.3. Performance of the system

Calibration of the system was investigated as described in Section 2.4. The calibration curve of BTX were carried out using six to nine concentrations, five replications for each concentration. Very good correlation coefficients were obtained for all three compounds (> 0.99). The linear dynamic ranges were found to be 0.01–560, 0.14–197 and 0.23–64 μg for benzene, toluene and xylene, respectively. These values are mass uptake (Q). Using 3 weeks of sampling time C_1 could be obtained from Eq. (1). Therefore, in term of concentration the linearity of the three compounds were in the ranges 0.20 $\mu g m^{-3}$–13 $mg m^{-3}$, 3.6 $\mu g m^{-3}$–5.1 $mg m^{-3}$ and 7.0 $\mu g m^{-3}$–2.0 $mg m^{-3}$, respectively. From the research work that have been carried out in Bangkok, Thailand (a city which much more traffic than Hat Yai), the concentration of benzene and toluene in ambient air were found in the range of 15.1–50.2 and 25.8–131 $\mu g m^{-3}$, respectively (Muttamara and Leong, 2000). Therefore, the linear concentration ranges obtained in this work covered the concentrations that have been reported.

Average peak area from 20 blank injections was used to calculate the limit of detection using the IUPAC method (Long and Winefordner, 1983). Limit of detections for benzene toulene and xylene were found to be 0.31, 0.24 and 0.73 $\mu g m^{-3}$, respectively (refer to 3 weeks of sampling time) and these are sensitive to detect trace amount of BTX in the environment (Muttamara and Leong, 2000).

3.4. Reused

Before sampling, reusability and storage of the samplers were studied. The idea of reusability came from the fact that after desorption, there should be no analytes on the adsorbent, and therefore, it can be reactivated and reused and this would help to reduce cost.

To evaluate how long Tenax TA could maintain the adsorption efficiency, the number of time the adsorbent could be reactivated and reused was studied. Fig. 4 shows the results for benzene. Between 1 and 12 times the response reduced only slightly after each use (1.4%). After that the response showed significant reduction (13%). Similar results were obtained for toluene and xylene which showed that they can be used up to 14 times while the cartridge from Radiello® Passive sampling system is not reusable (Radiello's manual full version, 2004). Therefore, these laboratory-built passive samplers are much cheaper.

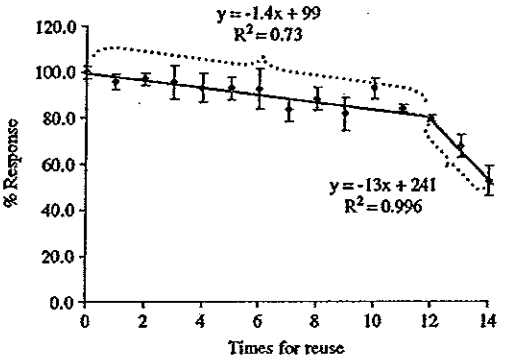

Fig. 4. Response of benzene obtained from the adsorbent that was reactivated and reused.

3.5. Storage stability

Storage time of the sampler was studied in the case where the analysis could not be done immediately after the samplers were collected and also to ensure the sample life time before analysis. This was done by placing 45 samplers, with the concentration of the three analytes at 100 times the limit of detection of each compound, in the desiccator at 25 °C. At the end of each week, five samplers were analysed and the relationship between the percentage of responses and the storage time was investigated. The responses decreased by 0.52%, 2.7% and 1.3% per week for benzene toluene and xylene, respectively. For storage stability, the National Institute for Occupational Health and Safety (NIOSH, USA) recommends that the average quantitative measurements of the samplers should not differ from the analysis on day 0 by more than 10% (Volden et al., 2005). Using this criterion the laboratory-built passive samplers can be kept in the desiccator at room temperature (25 °C) for up to 3 weeks (2.7% per week × 3 weeks = 8.1% for toluene) before being analysed. This is the same storage time recommended by SKC Inc. for their commercial passive sampler that needs to be stored at 4 °C (SKC®, 2005) while the storage time for Radiello is up to 3 months at 4 °C after exposure (Radiello's manual full version, 2004). Another advantage is the laboratory-built passive sampler has no shelf life. It only needs an activation step to remove the contaminants before sampling. However, before exposure, the commercial passive samplers have the shelf life of 30 days and 18 months for SKC and Radiello® passive samplers for VOCs sampling, respectively.

3.6. Sampling time

Sampling time was another parameter investigated. Three groups of gasoline stations were studied. They were classified by their activity. Gasoline stations located far away from the city were classified as low activity. Those located in the city were medium activity, and gasoline stations located in the city near shopping centers were classified as high activity.

Optimization was done by varying the sampling time between 1 and 4 weeks at two gas stations that were selected as the representative of medium and high activity. Four boxes of sampling devices were hung at each sampling site. One box, with four passive samplers, was collected and analysed at the end of each week. The results showed that the responses of BTX increased as the sampling time increased and started to level off at 1 week for the high activity gasoline station and 3 weeks for the medium one. It means that at these times, the adsorbent capacity was reached. Therefore, the sampling time used for high activity gasoline stations was 1 week, while medium and low activity was 3 weeks.

3.7. Vertical and horizontal distance concentration profile

To study the change of concentration with distance, one gasoline station was selected as the emission source. The samplers were placed at the gasoline station and at a few sites further away in

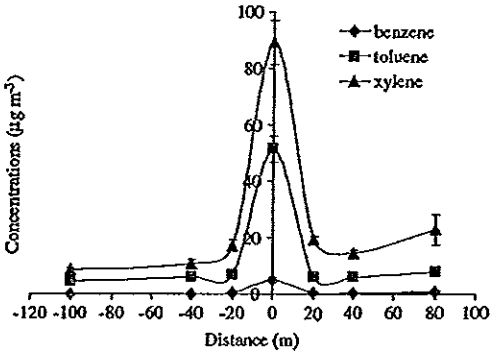

Fig. 5. The effect of distance on the concentration distribution.

Table 2
The average concentrations of benzene, toluene and xylene from 10 gasoline stations (classified into three groups) compared with the guideline value from OSHA and NIOSH

Compounds	Concentration [$\mu g\,m^{-3}$ (%RSD)]			OSHA ($mg\,m^{-3}$)	NIOSH ($mg\,m^{-3}$)
	Low activity	Medium activity	High activity		
Benzene	N.D.–2.0(5)	2.7(4)–4.5(7)	5(19)–19(2)	3.2	0.3
Toluene	12(7)–17(13)	19(9)–36(9)	31(7)–76(10)	375	375
Xylene	23(9)–31(18)	50(8)–196(4)	81(22)–198(3)	435	435

OSHA: Occupational Safety and Health Administration; NIOSH: National Institute of Occupational Safety and Health.

both directions along the road. The sampling time was 3 weeks. The effect of distance on the responses is shown in Fig. 5 and indicated that the concentration was highest at the gasoline station and reduced with distance except at +80 m. This was because at this point the samplers were placed near a main road so the exhaust probably contributed to the high value. For the concentration profile in the *Y*-axis, the samplers were placed at the gasoline station at 0.5, 1.0, 1.5, 2.0 and 2.5 m from ground level. The highest response was obtained at 0.5 m since it was the level of the exhaust pipe of the vehicles. However, the height of 1.50 m was used for further sampling since it is the average breathing height of Thai people.

3.8. Applications

Sampling sites were selected from possible BTX emission sources. Ten gasoline stations located around Hat Yai city, Songkhla, Thailand were selected as the sampling sites. There are three, three and four gasoline stations of low, medium and high activity, respectively. The sampling was done by placing the passive samplers at a height of 1.5 m for 3 weeks for low and medium activity and 1 week for high activity. At all sampling sites, blanks were collected by opened and then rapidly closed the screw caps of the samplers. After sampling time was terminated, the samplers and blanks were collected and analysed in the laboratory.

The concentration ranges of BTX are shown in Table 2 and the relative standard deviations of these laboratory-built passive samplers (at the same sampling site) were less than 22%, better than the acceptable value of 25% (NIOSH protocol) (Myers et al., 2005).

The concentrations of benzene at most gasoline stations, either low, medium or high activity, were higher than the guideline from the National Institute of Occupational Safety and Health (NIOSH) (EPA[1], 2005; EPA[2], 2005; EPA[3], 2005). Furthermore, benzene concentrations in medium and high activity gasoline stations were also more than the guideline from the Occupational Safety and Health Administration (OSHA) (EPA[1], 2005). On the other hand, the concentrations of the other two compounds were less than the values from both guidelines. This may be the result of the high content of benzene, 3–5%, in the gasoline used in Thailand while in Europe it is just 1% (Pryor, 2001).

4. Conclusions

The laboratory-built passive sampling device coupled with the purge and trap system can be used to monitor BTX in air with better precision (RSD<22%) than the acceptable value of 25% for passive sampling (Myers et al., 2005). The sampler is easily prepared by filling a screw cap bottle with Tenax TA. During sampling no additional device is required, and this helps to reduce cost. After analysis the sampler can be reactivated and reused to a total of 12 times and this helps to reduce cost even further. The use of a screw cap bottle makes it easy to rapidly start and stop the sampling. Unlike some commercial sampler where the adsorbent needs to be transferred to another container for analysis, the analysis of the developed passive sampler is done using the same bottle and this helps to reduce analysis error. In the case where analysis cannot be done immediately after sampling, the sampler can be kept for up to 3 weeks before being analysed. The wide linear ranges make this system suitable for monitoring a wide scale of concentration of BTX and possibly for other VOCs. This certainly is useful for monitoring and assessing air quality.

Acknowledgments

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Vitae

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2. The Royal Golden Jubilee Ph.D. Program (RGJ) of the Thailand Research Fund (TRF)
3. The Center for Innovation in Chemistry: Post Graduate Education and Research Program in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education and Graduate School

List of Publications and Proceedings

Publications

1. Thammakhet, C., Villeneuve, T., Munisawang, V., Thavarungkul, P. and Kanatharana, P. 2004. Monitoring of BTX by Passive Sampling in Hat Yai, *Songklanakarin Journal of Science and Technology,* **26** (Suppl.1) Environmental & Hazardous Management: 151-160.

2. Thammakhet, C., Thavarungkul, P., Brukh, R., Mitra, S. and Kanatharana, P. 2005. Microtrap Modulated Flame Ionization Detector for On-Line Monitoring of Methane, *Journal of Chromatography A* **1072**: 243-248.

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Presentations

Oral presentations

1. Thammakhet, C., Kanatharana, P., Mosbæk, H., and Tjell, J. C. "Development and Evaluation of Passive Sampling as a Method to Measure Volatile Aromatic Hydro-carbons" Workshop on Chemical Risk Assessment of the Environment, Chaing Mai, Thailand, August, 2003.

2. Thammakhet, C., Kanatharana, P., Thavarungkul, P., Mosbæk, H., and Tjell, J. C. "A Lab Built Passive Sampling for Volatile Aromatic Hydrocarbons" The 2nd PERCH Annual Scientific Congress (PERCH Conference II), Pattaya, Thailand, May, 2003.

3. Thammakhet, C., Kanatharana, P., Thavarungkul, P., Mosbæk, H. and Tjell, J. C. "Passive Sampling (Laboratory Built) for Volatile Organic Compounds" International Conference on Environmental Management and Technology: A Clean Environment Towards Sustainable Development, Malaysia, August, 2003.

4. Thammakhet, C., Villeneuve, T., Munisawang, V., Thavarungkul, P. and Kanatharana, P. "Monitoring of BTX by Passive Sampling in Hat Yai" The 3rd National Environmental Conference, Hat Yai, Thailand, January, 2004.

5. Thammakhet, C., Thavarungkul, P., Brukh, R., Mitra, S. and Kanatharana, P. "On-Line Microtrap for Trace Methane Analysis" RGJ Seminar Series XXX: Biosciences and Biotechnology for the Development of Southern Thailand, Prince of Songkla University, Hat Yai, Thailand, August, 2004.

6. Thammakhet, C., Munisawang, V., Thavarungkul, P. and Kanatharana, P. "Cost Effective Passive Sampling Device for BTX Monitoring" The 2nd Asian International Conference on Ecotoxicology and Environmental Safety, Songkhla, Thailand, September, 2004.

7. Thammakhet, C., Thavarungkul, P., Mitra S. and Kanatharana, P. "Fast and Simple On-Line System for Trace Methane Analysis" RGJ-Ph.D. Congress VI, Pattaya, Thailand, April, 2005.

8. Thammakhet, C., Thavarungkul, P., Mitra S. and Kanatharana, P. "On-Line Signal Enhancement for Trace Methane Analysis" The 4th PERCH Annual Scientific Congress (PERCH Congress IV), Pattaya, Thailand, May, 2005.

9. Thammakhet, C., Munisawang, V., Thavarungkul, P. and Kanatharana, P. "Cost effective Passive Sampler for BTX monitoring in Gasoline Stations" RGJ Seminar Series XLV: Innovation of Agricultural Resources, Prince of Songkla University, Hat Yai, Thailand, September 2006.

Poster presentations

1. Thammakhet, C., Brukh, R., Mitra, S., Kanatharana, P. and Thavarungkul, P. "A Microtrap Modulated FID for On-Line Monitoring of Light Hydrocarbon in High Purity Gases" RGJ-Ph.D. Congress IV, Pattaya, Thailand, April, 2003.

2. Thammakhet, C., Thavarungkul, P., Mitra, S. and Kanatharana, P. "Development of On-Line Impurities Removal Trap for Methane Analysis" The 3rd PERCH Annual Scientific Congress (PERCH Congress III) Pattaya, Thailand, May, 2004.

3. Thammakhet, C., Villeneuve, T., Munisawang, V., Thavarungkul, P. and Kanatharana, P. "The Use of Lab-Built Passive Samplers for BTX Monitoring in Hat Yai, Songkhla, Thailand" International Workshop on MARNURE: Management of Resources in urban areas and industries, focus on Nutrient Recycling, Suratthani, Thailand June, 2004.

4. Thammakhet, C., Thavarungkul, P., Brukh, R., Mitra, S. and Kanatharana, P. "On-Line System for Nano Level Analysis of Methane" The 2005 International Congress of Pacific Basin Societies (Pacifichem 2005), Honolulu, Hawaii, USA, December, 2005.

5. Teeparuksapun, K., Thammakhet, C., Kanatharana P. and Thavarungkul, P. "PDMS Microfluidic Chip for Amperometric Glucose Biosensor" The 5th PERCH-CIC Annual Scientific Congress (PERCH-CIC Congress V), Pattaya, Thailand, May 2007.