

**ANALYSIS OF PHTHALATES VIA SPECTROPHOTOMETRY IN
ENVIRONMENTAL SAMPLES USING NON-IONIC SILICONE SURFACTANT
MEDIATED CLOUD POINT EXTRACTION**

By

OOI PEI WAN

Dissertation Submitted in
Partial Fulfilment of The
Requirement for the Degree of
Master of Science

UNIVERSITI SAINS MALAYSIA
2017

ACKNOWLEDGEMENT

I would like to express my special thanks of gratitude to my supervisor, Dr Nur Nadhirah Mohamad Zain as well as my co-supervisor, Dr Muggundha Raoov for their generous guidance, understanding and supports. It was a pleasure working with them and made it possible for me to work on a topic that was of great interest to me. I am also grateful to all the lecturers in Advanced Medical and Dental Institute, especially Integrative Medicine Cluster, for their support and kind guidance towards the successful completion of my studies in Universiti Sains Malaysia.

I also would like to express my appreciation to the authority of Advanced Medical and Dental Institution for given me the permission to access and carry out the study using all the facilities and equipment required. Lastly, the completion of this study could not have been possible without the assistance of many people whose names may not all be enumerated. Their contributions are sincerely appreciated and gratefully acknowledged.

TABLE OF CONTENTS

ACKNOWLEDGEMENT.....	ii
TABLE OF CONTENTS.....	iii
LIST OF TABLES.....	v
LIST OF FIGURES.....	vi
LIST OF ABBREVIATIONS.....	vii
ABSTRACT.....	ix
ABSTRAK.....	x
CHAPTER 1 INTRODUCTION.....	1
1.1 Background of study.....	1
1.2 Problem statement and significant of study.....	4
1.3 Objectives.....	5
CHAPTER 2 LITERATURE REVIEW.....	6
2.1 Phthalate.....	6
2.1.1 Exposure route of phthalates.....	9
2.1.2 Adverse effects of phthalates.....	13
2.2 Cloud point extraction.....	14
2.2.1 Surfactant.....	17
2.2.1.1 Non-ionic silicone surfactant (DC193C).....	21
2.2.2 Influential factors.....	21
CHAPTER 3 METHODOLOGY.....	23
3.1 Chemicals and material.....	23
3.2 Instrumentation.....	25
3.3 Sample preparation.....	25
3.4 Cloud point extraction.....	25
3.5 Method validation.....	27
3.6 Application on real samples.....	27
CHAPTER 4 RESULTS AND DISCUSSION.....	28
4.1 Surfactant types.....	28
4.2 Effect of concentration of DC193C surfactant.....	30
4.3 Effect of temperature and incubation time.....	32
4.4 Effect of salts.....	35
4.5 Salt concentration.....	36
4.6 Application on real samples.....	37

CHAPTER 5 CONCLUSION.....	40
5.1 Conclusion.....	40
5.2 Future Work.....	40
BIBLIOGRAPHY.....	41
APPENDICES.....	51

LIST OF TABLES

Table 2.1	Some applications of phthalates in water samples	11
Table 2.2	Comparison between other conventional extraction methods with cloud point extraction (CPE)	16
Table 2.3	Surfactants used in CPE	20
Table 4.1	Effect of Salt	36
Table 4.2	Method validation of spectrophotometric of phthalates species using CPE	39
Table 4.3	Recovery of phthalates species in spiked environmental waters	39

LIST OF FIGURES

Figure 2.1	General chemical structure of PAEs	7
Figure 2.2	Chemical Structure of (a) DEHP and (b) DBP	8
Figure 2.3	Schematic diagram of CPE	17
Figure 3.1	Chemical structure of DC193C surfactant	23
Figure 3.2	Absorption of the UV-Vis spectra for (a) DEHP and (b) DBP	24
Figure 3.3	Cloud Point Extraction Process	26
Figure 4.1	Absorbance spectrum of different types of surfactants	29
Figure 4.2	Effect of concentration of DC193C on extraction efficiency of DBP in CPE	31
Figure 4.3	Proposed mechanism between non-ionic silicone surfactant and DBP	32
Figure 4.4	Effect of temperature on extraction efficiency of phthalates in CPE	34
Figure 4.5	Effect of incubation time on extraction efficiency of phthalates in CPE	34
Figure 4.6	Effect of Salt Concentration on extraction efficiency of phthalates in CPE	37
Figure A	Sungai Derhaka Juru, Penang	52
Figure B	Industrial area next to Sungai Derhaka Juru, Penang	52
Figure C	Sungai Juru, Penang	53
Figure D	Industrial area next to Sungai Juru, Penang	53
Figure E	Sungai Nibong Kecil, Penang	54
Figure F	Sungai Ara, Penang	54
Figure G	Sungai Perai, Penang	55

LIST OF ABBREVIATIONS

BBP	Benzyl butyl phthalate
CMC	Critical micellar concentration
CO ₃ ²⁻	Carbonate ion
CPE	Cloud point extraction
CPT	Cloud point temperature
DBEP	Bis(2-n-butoxyethyl) phthalate
DBP	Dibutyl phthalate
DCHP	Dicyclohexyl phthalate
DEHA	Di-2-ethylhexyl adipate
DEHP	Diethylhexyl phthalate
DEP	Diethyl phthalate
DHP	Dihexyl phthalate
DIDP	Di-isodecyl phthalate
DINP	Di-isononyl phthalate
DMP	Dimethyl phthalate
DNA	Deoxyribonucleic acid
DnOP	Di-n-octyl phthalate
DPHP	Di-propylheptyl phthalate
DPP	Di-n-propyl phthalate
KCl	Potassium chloride
KOH	Potassium hydroxide
LLE	Liquid-liquid extraction
LODs	Limit of detection
LOQs	Limit of quantification
MBP	Monobutyl phthalate
MEHP	Mono (2-ethylhexyl) phthalate
5OH-MEHP	Mono-(2-ethyl-5-hydroxyhexyl) phthalate
5oxo-MEHP	Mono-(2-ethyl-5-oxohexyl) phthalate
5cx-MMHP	Mono-(2-ethyl-5-carboxypentyl) phthalates
2cx-MMHP	Mono[2-(carboxylmethyl)hexyl] phthalate
MEP	Mono-ethyl phthalate
Na ⁺	Sodium ion
NaCl	Sodium chloride
Na ₂ NO ₃	Sodium nitrate

Na ₂ SO ₄	Sodium sulfate
PAEs	Phthalates
PEG	Polyethylene glycol
PVC	Polyvinyl chloride
R and R'	Functional group
SDS	Sodium dodecyl sulfate
SLE	Solid-liquid extraction
SPE	Solid-phase extraction
SPME	Solid phase microextraction
SO ₄ ²⁻	Sulfate ion
TSCA	Toxic Substances Control Act
UV	Ultraviolet
VOCs	Volatile organic compounds

ABSTRACT

Analysis of Phthalates via Spectrophotometry in Environmental Samples using Non-ionic Silicone Surfactant Mediated Cloud Point Extraction

Determination of phthalates in environmental matrices become crucial in recent years due to the growing international concern about the health effects of phthalates. In the present study, a greener method based on cloud point extraction was developed for the extraction of selected phthalates in environmental samples using non-ionic silicone surfactant of DC193C. The parameters affecting the extraction efficiency such as the surfactant concentration, salt types, salt concentration, temperature, and incubation time were evaluated and optimized. Good linearity with R^2 in the range of 0.9963 – 0.9988 for all calibration curves were obtained. The LODs were $0.95 \mu\text{g L}^{-1}$ (diethylhexyl phthalate) and $0.72 \mu\text{g L}^{-1}$ (dibutyl phthalate) and the LOQs were $3.16 \mu\text{g L}^{-1}$ (diethylhexyl phthalate) and $2.42 \mu\text{g L}^{-1}$ (dibutyl phthalate), respectively. The proposed method was successfully applied in extracting the diethylhexyl phthalate and dibutyl phthalate in selected environmental samples under optimized conditions with satisfactory recoveries in the range of 82 – 98 %.

ABSTRAK

Analisis ftalat dengan spektrofotometri dalam sampel sekitaran menggunakan pengesktrakan titik awan dengan surfaktan silikon bukan ion

Penentuan ftalat dalam matriks sekitaran menjadi satu isu yang penting beberapa tahun ini disebabkan peningkatan kesedaran antarabangsa mengenai kesan-kesan kesihatan bagi ftalat. Dalam kajian ini, suatu kaedah hijau berdasarkan pengesktrakan titik awan telah dibangunkan untuk mengekstrak ftalat terpilih di dalam sampel sekitaran dengan menggunakan surfaktan silikon bukan ion DC193C. Faktor-faktor yang mempengaruhi kecekapan pengesktrakan seperti kepekatan surfaktan, jenis garam, kepekatan garam, suhu dan masa pengeraman telah dinilai dan dioptimumkan. Kelinearan yang bagus dengan R^2 julat dalam 0.9963 – 0.9988 telah dicapai untuk semua keluk tentukan. LODs masing-masing adalah $0.95 \mu\text{g L}^{-1}$ (dietilheksil ftalat) dan $0.72 \mu\text{g L}^{-1}$ (dibutil ftalat) manakala LOQs adalah $3.16 \mu\text{g L}^{-1}$ (dietilheksil ftalat) and $2.42 \mu\text{g L}^{-1}$ (dibutil ftalat). Kaedah yang dicadangkan telah berjaya digunakan untuk pengesktrakan dietilheksil ftalat dan dibutil ftalat di dalam sampel sekitaran terpilih di bawah keadaan optimum dengan pengambilan yang memuaskan dalam julat 82 – 98 %.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Plastic have been used massively in the last decades for manufacture of wide range of products due to its characteristics of lightweight, strong, durable and cheap (Derraik, 2002). Different types of plastics have been applied in medical devices (Kostić, Anđelković, Anđelković, Cvetković, & Pavlović, 2016), food containers (Raharjo, Kartika, & Handajani, 2016; M. Zhang *et al.*, 2013) and plastic film in many agricultural sectors (Wang *et al.*, 2015; J. Wang *et al.*, 2013). As a result, many researches have reported that the accumulation and fragmentation of plastics in the environmental compartments has become one of the most ubiquitous and changes to the surface of planet (Barnes, Galgani, Thompson, & Barlaz, 2009). The threat of plastic to the environment, especially to the marine environment where most of the debris accumulated, is mostly due to the environmental exposure to its toxic components such as bisphenol A and phthalates (PAEs) (Mariana, Feiteiro, Verde, & Cairrao, 2016).

PAEs which also known as phthalic acid esters, are a class of chemical compounds used widely in industrial and domestic applications to increase the flexibility and durability of the product (Pérez-Outeiral, Millán, & Garcia-Arrona, 2016). Since PAEs are not covalently attached, significant amount have leached into the environmental compartment during the process of their production, usage and disposal, mainly via natural and anthropogenic combustion processes (Lifei Zhang *et al.*, 2012). Several researches have found that exposure to these compounds may lead to carcinogenic effects, endocrine disruption, respiratory disorders and afflict the development of reproductive system (Gani & Kazmi, 2016b). As reported by Morét-Ferguson *et al.* (2010), plastic can also release PAEs when exposed to sunlight and direct leaching which then may disrupt the endocrine system of mammals (Morét-Ferguson *et al.*, 2010). As a result of these harmful effects, PAEs have been considered as

one of the major environmental pollutants. Thus, it is very crucial to develop a simple and efficient method in extraction of PAEs in order to monitor their presence in the environment.

Major efforts have been done in the past 50 years in identification of carcinogens, toxins and toxic compounds in the environment. The United State Environmental Protection Agency (EPA) has identified an initial list of chemicals, which include PAEs, into a program under the Toxic Substances Control Act (TSCA) (Schechter *et al.*, 2013). Even though there is no criterion have been set for saltwater aquatic life, the water quality criteria for dibutyl phthalate (DBP) and diethylhexyl phthalate (DEHP) are determined to be 5 mg L⁻¹ and 10 mg L⁻¹ respectively by United State Environmental Protection Agency for protection of human health (Regulations, Criteria, & Division, 1980). However, direct determination of these environmental pollutants is difficult due to the extremely low concentration and complex matrix (Z. Wang *et al.*, 2013). Generally, liquid-liquid extraction (LLE), solid-phase extraction (SPE) and solid phase microextraction (SPME) were employed for preconcentration purpose. However, these methods have many limitations and disadvantages such as the usage of organic solvents, expensive and time consuming (Cravotto, Binello, & Orio, 2011; Yue, Li, Xu, & Jiang, 2016). Volatile organic compounds (VOCs), which well-known with high toxicity, highly flammable and detrimental effects to the environment, have been used as organic phase solvents in these techniques (Płotka-Wasyłka, Rutkowska, Owczarek, Tobiszewski, & Namieśnik, 2017).

A few researchers have discovered about the principles and advantages of cloud point extraction (CPE) as the new extraction method especially in improving the sensitivity in determination of trace elements (Gürkan, Kır, & Altunay, 2015). According to Souza, Teixeira and Bezerra (2016), CPE is a technique that use surfactant and manipulate temperature to induce formation of micellar environment (Souza, Teixeira, & Bezerra, 2016). Surfactants are amphiphilic molecules that organized and assembled in various shapes, such as micelles and

bilayer lamellae, in a solution depending on its structure and concentration (Zana & Talmon, 1993). When the concentration of surfactant is above the critical micellar concentration (CMC), the non-ionic surfactants in aqueous solution will form micelles and become turbid when heated to their respective cloud point temperature (CPT) (de Andrade, de Andrade, Felsner, Quinária, & dos Anjos, 2017). Once reach above the temperature, the solution will separate into two immisible phases which are the surfactant rich phase and aqueous phase. The analytes of interest will be solubilised and concentrated in the surfactant rich phase, which has smaller volume as compared to the aqueous phase.

As compared to other conventional extraction techniques, CPE can be classified as a “green chemistry” principle as it is simple, highly efficient and has lower toxicity as compared to other extraction techniques. According to Chemat, Vian and Cravotto (2012), the term “green chemistry” is generally used to define the invention, design and application of chemical products and processes to decrease or to eliminate the use and generation of hazardous substances (Chemat, Vian, & Cravotto, 2012). The extraction solvent required in CPE method is diluted solutions of non-ionic surfactants in small quantities. This unique characteristic of CPE is cost-effective and resulted in few laboratory species which fulfilled the approach of green chemistry on optimal consumption of raw materials and solvents (Armenta, Garrigues, & de la Guardia, 2015). Other than that, it is also reported that the non-ionic surfactant are non-volatile, non-flammable and has lower toxicity as compared to those organic solvents used in other extraction techniques such as liquid-liquid extraction (Ghasemi & Kaykhaii, 2016).

Nowadays, there are many types of surfactants that have been employed as an extraction solvent such as Triton X series, Tween series, Tergiol and PONPE series in CPE. Triton X-114 is well known with their micelle forming ability with varies analytes such as insecticides

(Santalad, Burakham, Srijaranai, Srijaranai, & Deming, 2012), heavy metals (Gürkan *et al.*, 2015) and dyes (Ghasemi & Kaykhaii, 2016). However, aromatic chromophore in this surfactant has strong ultraviolet (UV) absorbance or fluorescence signals which becomes obstacles in UV and fluorescence detector (Yao & Yang, 2008). As solution for this limitation, non-ionic DC193C was used as it has more flexible polysiloxane chains without any aromatic structure (Bosman *et al.*, 2009). Despite of all the advantages of CPE over the other extraction methods, factors affecting the extraction process will be evaluated according to the properties of analyte in interest in order to obtain high extraction efficiency (Phasukarratchai, Damrongsiri, & Tongcumpou, 2017). Thus, in this study, factors influence the efficiency were optimized according to the properties of PAEs which is the analyte of interest in this study. The optimized and developed method was applied for the extraction of PAEs in environmental samples.

1.2 Problem statement and significant of study

Many different PAEs have exist with different properties, uses and health effects. They are produced in high quantity annually and have been used in all kinds of household products and personal care products. Although exposure to PAEs can produce a variety of effects in laboratory animals, certain PAEs are found to have adverse effects on the development of reproductive system. People are mostly exposed to PAEs through eating and drinking foods that have been in contact with products and containers containing PAEs since significant amount of PAEs have leached along their production and usage cycle. Due to the high toxicity and widespread in variety environmental compartments, the concentration of PAEs in the environment need to be monitor to ensure the public safety. However, direct determination of PAEs is difficult due to low concentration and presence of complex matrices, it is crucial to develop a simple and efficient method for the extraction of PAEs from the environmental matrices (Lifei Zhang *et al.*, 2012). CPE is a suitable extraction technique for this purpose

since this technique is safer and eco-friendly. The developed method in this study will be useful in future for the determination of phthalate in environmental water.

1.3 Objectives

The objective of this study is to develop an environmentally and greener extraction method of cloud point extraction combined with spectrophotometer using non-ionic silicone surfactant as the extraction solvent to extract PAEs in environmental water sample.

The specific objectives of this study include:

1. To optimize the conditions of CPE method using non-ionic silicone surfactant, DC193C, towards the extraction of PAEs.
2. To develop and validate of CPE method combined with spectrophotometry for PAEs analysis.
3. To apply the developed method of CPE combined with spectrophotometry to the determination of PAEs in environmental water samples.

CHAPTER 2

LITERATURE REVIEW

2.1 Phthalates

Since the early of twenty century, PAEs have been used extensively in a wide range of industrial and domestic applications (Schechter *et al.*, 2013). They are esters manufactured through reaction of phthalic anhydride with oxo alcohol through alcoholysis and followed by esterification (Mariana *et al.*, 2016). They are mainly used as plasticizers to increase the products' flexibility, transparency, durability and longevity through weak secondary molecular interactions with polymer chains (Pérez-Outeiral *et al.*, 2016; J. Yang *et al.*, 2015). They have been used in polyvinyl chloride (PVC) products, additives in personnel care products, flooring, paints, car coating and wall covering (Gani & Kazmi, 2016b).

The type of PAEs added to the products is depending on their function such as insulation, heat resistance and weatherproofing (B. Chen & Zhang, 2013). The chemical and physical properties of the individual PAEs is depending on the functional group (R) which can be in linear, branched, linear/branched or cyclic ring form. During the application of same molecular weight of PAEs, branched molecules are more effective in plastic processing as compared to linear one but the linear arrangement has better advantages in processing at relatively low temperature (Rivera-Utrilla, Ocampo-Pérez, Méndez-Díaz, & Sánchez-Polo, 2012). The general chemical structure of PAEs, where R and R' are C_nH_{2n+1} , is shown in **Figure 2.1**.

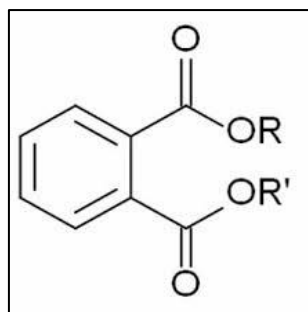


Figure 2.1: General chemical structure of PAEs

The term PAEs or phthalate esters is referring to a large group of chemically similar compounds but each of them have own unique physical and chemical properties (Kamrin, 2009). PAEs have been divided into two group based on their size, where PAEs with 6 or less carbon chain is known as low molecular weight PAEs and those with more than 6 are known as high molecular weight PAEs (Sohn, Kim, Koschorreck, Kho, & Choi, 2016). The most commonly used high molecular weight PAEs are di-isononyl phthalate (DINP), di-isodecyl phthalate (DIDP) and di-propylheptyl phthalate (DPHP). They are mostly used in the PVC products and automobile applications.

On the other hand, di-(2-ethylhexyl) phthalate (DEHP) and dibutyl phthalate (DBP) are the most commonly used low molecular weight PAEs. The chemical structure of both DEHP and DBP were shown in **Figure 2.2**. These PAEs are commonly used in medical devices, general purpose PVC, adhesives and inks. Among all the PAEs, DEHP is the most popular and extensively produced PAEs, which accounts for more than 50 % of the total PAEs (J. Yang *et al.*, 2015). This is supported by the result obtained by Zhang *et al.* (2012), which has reported that DEHP and DBP are the main PAEs found in the Yangtze river delta, China. DEHP, with the chemical formula of $C_{24}H_{38}O_4$, is the phthalate ester of the 2-ethylhexanol alcohol, which is normally manufactured by the dimerisation of butyraldehyde (Lifei Zhang *et al.*, 2012).

Meanwhile, DBP is a phthalate with the same core structure as DIDP and DINP but only has two shorter side chains with four carbon atoms each.

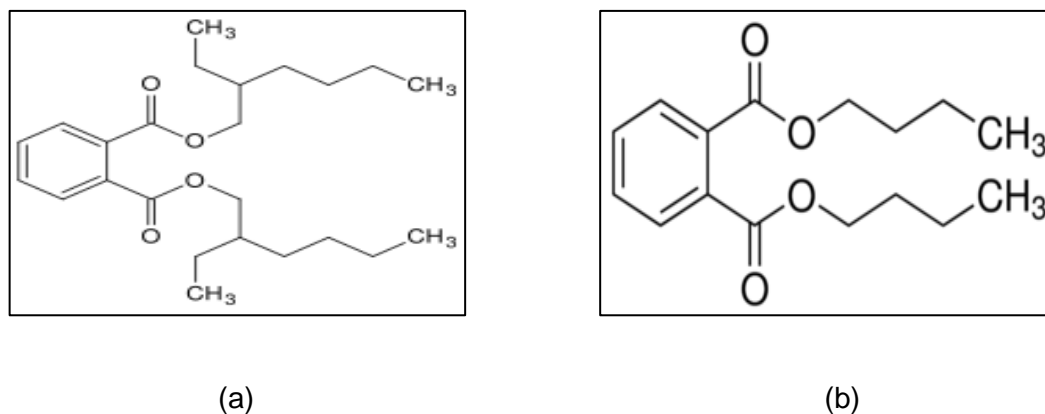


Figure 2.2: Chemical Structure of (a) DEHP and (b) DBP

Plastics have become one of the most important materials in sustaining living of the society, such as food packaging even though there are alternatives. However, since PAEs are only reversibly and physically attached to the plastic components, significant amount of them can leached into the environmental compartment during the process of their production, manufacture, usage and disposal (Ling *et al.*, 2007; Mariana *et al.*, 2016). At room temperature, most of the PAEs are colourless and odorless. PAEs have been detected throughout the worldwide environmental compartments, including solid waste, atmospheric air and environmental water (Net, Delmont, Sempéré, Paluselli, & Ouddane, 2015). People are exposed to PAEs through ingestion of food and drinking water that have in contact with these contaminated matrices. Thus, increasing risk of this compounds to the environment and its impact on human health has attracted many researchers all over the world to alleviating this pollutants (Benjamin, Kamimura, Takahashi, & Masai, 2016).

2.1.1 Exposure route of phthalates

Chemical pollutants can be persistent or non-persistent in the environment based on their biodegradability (Le Magueresse-Battistoni, Vidal, & Naville, 2015). According to Koch and Calafat (2009), PAEs are considered as non-persistent chemical and only have half-lives of hours in the human body, where complete excretion can be done by a day or two (Koch & Calafat, 2009). However, due to the intensive and continuous release of these pollutants into the environments, they are capable to caused adverse effects within the body and they are found to be toxic to many species including protozoan, fishes and invertebrates (X. Chen *et al.*, 2014). There are many potential pathways for the exposure of PAEs including ingestion, inhalation, intravenous injection tubing and solutions as well as through skin absorption.

When ingested, PAEs are often converted to their metabolites. Metabolism of DEHP in human body is complex and usually yield mono (2-ethylhexyl) phthalate (MEHP) and some oxidative metabolites. This is because the MEHP can be further metabolized into either mono-(2-ethyl-5-hydroxylhexyl) phthalate (5OH-MEHP), mono-(2-ethyl-5-oxohexyl) phthalate (5oxo-MEHP), mono-(2-ethyl-5-carboxypentyl) phthalate (5cx-MMHP), and mono[2-(carboxymethyl)hexyl] phthalate (2cx-MMHP), mainly through the ω -oxidation at the terminal carbon of the alkyl ester side chain (Hopf *et al.*, 2014). According to Wittassek and Angerer (2008), the 5cx-MEPP is the primary metabolites of DEHP in human, which contributed more than 25% of the total PAEs. On the other hand, metabolism of diethyl phthalate (DEP) will yield mono-ethyl phthalate (MEP) while metabolism of DBP will produce monobutyl phthalate (MBP) (Wittassek & Angerer, 2008).

The major routes for most of the PAEs exposure is through diet and followed by the use of personal care products (Koch *et al.*, 2013). It is found that children are more susceptible to get exposed to PAEs since polymer toys often use PAEs to increase their elasticity and longevity

(Schettler, 2006). Other than toy, medical devices with DEHP also caused exposure to highly susceptible subpopulation of individuals such as kids and elderly people. PAEs can also migrate from tableware to drinking water where Li *et al.* (2016) reported the amount of DBP and DEHP of drinking water in paper bowls and plastics cups have exceeded the limitation levels for drinking water regulated by some international agencies (C. Li, Xu, Chen, & Xiao, 2016). There are many researches have investigated on the determination of PAEs present in the all kinds of water samples, such as drinking water, tap water, river and ocean, as some of the researches have been summarized in **Table 2.1**.

Researchers also found that PAEs are ubiquitous components of the indoor environment since they are semi-volatile organic compounds (Geiss, Tirendi, Barrero-Moreno, & Kotzias, 2009). Thus, inhalation is another exposure route especially those with a lot of polymer and plastics in their daily routine. Oven baking of polymer clays may cause short-term, high-level inhalation exposures to higher molecular weight PAEs (Schettler, 2006). Bamai *et al.* (2016) also reported the high exposure of PAEs through the house dust especially to the housewife and children who spend more time at house (Bamai *et al.*, 2016). At the same time, Bi *et al.* (2015) has reported that the positive relationship between the plastic flooring material and the application of chemical floor care products with the concentration of total phthalate in floor dust (Bi, Yuan, Pan, Winstead, & Wang, 2015).

Table 2.1: Some applications of phthalates in water samples

Extraction methods	Phthalates Compounds	Samples	References
Solid phase microextraction	Di-(2ethylhexyl) phthalate (DEHP); Di-n-butyl phthalate (DBP); Benzyl butyl phthalate (BBP); Di-2-ethylhexyl adipate (DEHA); Di-n-octyl phthalate (DnOP)	Tap water, barreled drinking water, pond water	(Song <i>et al.</i> , 2016)
Coacervate extraction	Benzyl butyl phthalate (BBP); Di-n-butyl phthalate (DBP); Di-(2ethylhexyl) phthalate (DEHP); Dicyclohexyl phthalate (DCHP)	Drinking water	(C. Li <i>et al.</i> , 2016)
Solid phase microextraction	Di-n-propyl phthalate (DPP); Di-n-butyl phthalate (DBP); Di-(2ethylhexyl) phthalate (DEHP); Di-2-ethylhexyl adipate (DEHA)	Tap water, tanked water, bottled mineral water	(Amanzadeh, Yamini, Moradi, & Asl, 2016)
Ozone Microbubbles	Diethyl phthalate (DEP)	Water in a pilot plant	(Jabesa & Ghosh, 2016)
Solid phase microextraction	Dimethyl phthalate (DMP); Dibutyl phthalate (DBP); Diisononyl phthalate (DINP); Diethyl phthalate (DEP); Benzyl butyl phthalate (BBP); Di-(2ethylhexyl) phthalate (DEHP); Di-(n-octyl) phthalate (DnOP); Diisodecyl phthalate (DIDP)	Drinking water	(Lirio, Fu, Lin, Hsu, & Huang, 2016)

Liquid-liquid extraction	Bis(2-n-butoxyethyl) phthalate (DBEP); Bis-(2-n-ethylhexyl) phthalate (DEHP); Butyl benzyl phthalate (BBP); Di-butyl phthalate (DBP); Diethyl phthalate (DEP); Dihexyl phthalate (DHP); Dimethyl phthalate (DMP); Di-(n-octyl) phthalate (DnOP); Dinonyl phthalate (DINP)	Bottled water	(Otero <i>et al.</i> , 2015)
Liquid extraction	Di-butyl phthalate (DBP); Diethyl phthalate (DEP); Bis-(2-n-ethylhexyl) phthalate (DEHP); Butyl benzyl phthalate (BBP)	Wastewater system treatment	(Gani & Kazmi, 2016a)
Nanofiltration (NF) extraction	Dimethyl phthalate (DMP); Diethyl phthalate (DEP); Di-butyl phthalate (DBP); Di-(n-octyl) phthalate (DnOP); Bis-(2-n-ethylhexyl) phthalate (DEHP)	Unknown source of water samples	(X. Wei <i>et al.</i> , 2016)
Solid phase extraction	Di-butyl phthalate (DBP)	Both river and drinking water	(Domínguez-Morueco, González-Alonso, & Valcárcel, 2014)
	Dimethyl phthalate (DMP); Diethyl phthalate (DEP)	Only drinking water	

2.1.2 Adverse effects of phthalates

Exposure to different PAEs will have different harmful effects, such as irritation on the skin, cancer and endocrine disruption, depending on their chemical properties. This is because different functional groups have different ability to interfere with hormone homeostasis and bind to molecular targets when they enter the body (Gani & Kazmi, 2016b; Mariana *et al.*, 2016). Even though PAEs will breakdown and excreted quickly through urine after they enter the body, the biomonitor studies show widespread exposure of PAEs in human urines (Komada, Gendai, Kagawa, & Nagao, 2016). According to Gomez-Hens and Aguilar-Caballos (2003), low molecular weight PAEs are more likely to caused irritation to eye, nose and throat while high molecular weight PAEs are suspected to be carcinogenic and might interfere with the development as demonstrated in many animal studies, mainly using rats and mice (Gomez-Hens & Aguilar-Caballos, 2003). However, dosage and exposure period have affected the consequences brought by the phthalate. Yang *et al.* (2015) has found that some high molecular weight PAEs, such as DINP and DIDP, have no observed effects on development and reproductive system even though the exposures are at 5% body weight for 260 days (J. Yang *et al.*, 2015).

According to Howdeshell *et al.* (2008), the main concern on the adverse effects of PAEs exposure to human and wildlife are the effects on the reproductive system (Howdeshell, Rider, Wilson, & Gray, 2008). Kamrin (2009) has reported the decreased in testicular weight and seminiferous tubular atrophy in the rat after PAEs exposure at dosage of DEHP of at least 14 mg/kg/d (Kamrin, 2009). Meeker *et al.* (2009) has also reported damaging effect of PAEs on sperm deoxyribonucleic acid (DNA) and count in male adults (Meeker, Calafat, & Hauser, 2009). Meanwhile, Meeker *et al.* (2009) has reported on the decreased of steroid hormone level in adult men with detected DEHP in their urine test (Meeker *et al.*, 2009). This harmful effect may due to the ability of elicitation of agonistic androgen receptors activity by some PAEs, such as DEHP, DIDP and DBP, and the ability to enhance the effects of other inducer

of androgen receptors, such as BBP (Krüger, Long, & Bonefeld-Jørgensen, 2008). However, PAEs are not only affecting males, but also females. Many researches have also found that phthalate has pose the highest toxicity and endocrine disruptive threat to human race, especially those in high vulnerable period such as infants, pregnant and nursing mothers (Colacino *et al.*, 2011; Zia *et al.*, 2013). Examples of the effects on female included increased risk of altered breast development, premature puberty, breast cancer and prostate changes (Colacino *et al.*, 2011; López-Carrillo *et al.*, 2010).

Other than affecting reproductive and endocrine system, PAEs in house dust are also considered to increased the risk of allergies as animal studies have indicated some PAEs may enhance the allergic responses through acting as adjuvants for the production of cytokines and immunoglobins which are responsible for the allergic sensitization (Koike *et al.*, 2010). At the same time, Benson (2009) has reported that irritation to eyes, nose and throat have been resulted from the exposure some molecular PAEs (Benson, 2009). Based on all these toxicological evaluation, some of the low molecular weight PAEs, such as DEP, DBP, BBP and DEHP, have been declared as one of the ubiquitous environmental pollutants by a number of health monitoring agencies all around the world. For example, the daily intake of DEHP should be below 20 µg/kg of body weight/day while the maximum admissible concentration in water should be below 6 µg/L water as specified by United State Environmental Protection Agency (US EPA) (Amanzadeh *et al.*, 2016; J. Yang *et al.*, 2015).

2.2 Cloud point extraction

Identification and quantification of trace compounds in complex matrices and low concentration often required pretreatment of the samples to pre-concentrate and clean-up the sample prior of determination with instrumentation. There are many benefits of using extraction-separation technique for pretreatment purposes since it allowed the detection of

low levels analytes by enrichment and isolation of the analytes in interest from the matrix solution and other possible interference substances which will affect the result during determination process (Paleologos, Giokas, & Karayannis, 2005). This will then lead to lower limits of quantification and greater sensitivity for better determination and quantification process (Souza *et al.*, 2016). Other than that, choosing an extraction method with massive use of solvent and volatile compounds are not encouraged as these substances are not environmentally friendly (Hung, Chen, & Liya, 2007). Thus, the development of simpler, greener and more effective extraction method is crucial for better determination and quantification of trace compounds in environmental samples.

PAEs are usually present in trace amount in the environmental compartment, where many sample pretreatment methods have been employed to enrichment them for further analysis. Examples of commonly used conventional extraction methods in enrichment of PAEs include liquid-liquid extraction (LLE) (Lifei Zhang *et al.*, 2012), solid-liquid extraction (SLE) (B. Chen & Zhang, 2013; H.-L. Li *et al.*, 2016), solid-phase extraction (SPE) (Domínguez-Morueco *et al.*, 2014), membrane filtration (G. C. Yang, Chen, Yang, & Yen, 2016), coacervate extraction method (C. Li *et al.*, 2016) as well as cloud point extraction (CPE) (Ling *et al.*, 2007). Among these methods, LLE method is the method certified by Environmental Protection Agency in isolating the organic pollutants from environmental samples (Noorashikin, Raoov, Mohamad, & Abas, 2013). However, over the past few years, CPE is now in interest of many researchers because of its wide applications and advantages over the other methods as summarized in **Table 2.2** (Ling *et al.*, 2007; Noorashikin *et al.*, 2013; Pongpiachan, 2009; N. Zain, Abu Bakar, & Mohamad, 2016; N. N. M. Zain, Raoov, Abu Bakar, & Mohamad, 2016).

Table 2.2: Comparison between other conventional extraction methods with cloud point extraction (CPE)

Other conventional extraction methods	Cloud point extraction (CPE)
Time consuming	Less time needed
High cost due to large amount of organic solvent	Low cost
Large sample volume	Smaller sample volume
Not environmental friendly as large volume of volatile organic solvent used.	Environmental friendly as only used very small amount of non-flammable and non-volatile surfactant that is low toxicity.
Caused analyte loss during solvent concentration process.	No solvent concentration which cause loss of analyte.

CPE is a simple, rapid, efficient and easy to operate method and thus it gained popularity among analytical chemistry researchers since the first report in 1977 (Ghasemi & Kaykhaii, 2016). This process has been widely used in the isolation, purification and enrichment of a variety of sample, including metal ions and organic compounds, in many different matrices (S. Li, Wang, Zhong, Zhang, & Yang, 2015). CPE is a separation and pre-concentration technique based on the use of a surfactant to induce the formation of a micellar environment and phase separation when reaches the cloud point temperature (CPT) (Souza *et al.*, 2016). The analytes will then be concentrated in a lower volume of surfactant rich phase. In the other words, CPE is done within three simple steps (as shown in **Figure 2.3**), which are adding surfactant into sample, formation of micelle at CPT and finally separating the enriched micellar phase from the aqueous phase (Ghasemi & Kaykhaii, 2016). This method is based on the principle of decreasing in solubility of the surfactant in the water at that certain temperature and thus emerged into two phases (Santalad *et al.*, 2012).

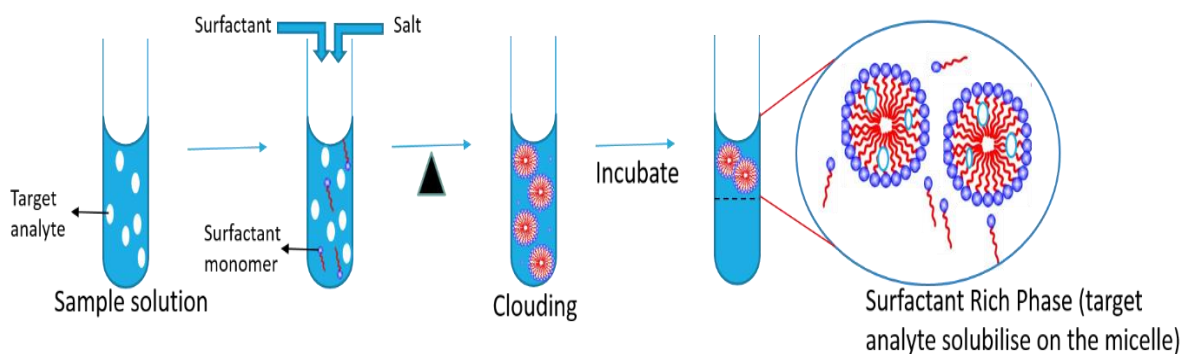


Figure 2.3: Schematic diagram of CPE

2.2.1 Surfactant

Surfactants are used in CPE because of their ability to entrap and isolate the hydrophobic substance. Surfactants are amphiphilic molecules where they have two distinct structure moieties (N. Zain, Bakar, Mohamad, & Saleh, 2014). In the other words, they have extended polar layer, which also known as hydrophilic head, and a non-polar core, which is the hydrophobic tails comprising of hydrocarbon chain (N. Zain *et al.*, 2016). The ability to entrap the hydrophobic substances in the sample is depending on this hydrocarbon chain, which can be linear, branched or contain aromatic rings. Some of the surfactants that have been used in CPE have been categorized in **Table 2.3**. These commonly used surfactants shared some similar characteristics which are non-toxic and have low cloud point temperature. Generally, surfactants can be categorized based on their characteristics or their uses. However, some of the surfactants have been used for more than one application. Thus, the most acceptable and scientifically sound classification of surfactants is based on their dissociation in water. Thus, the surfactants can be categorized into four groups, which are non-ionic, cationic, anionic and amphoteric (Badera, Edbeya, & Telghederb, 2014).

Among these surfactants, anionic surfactants have accounted for approximate 50 % of world production. These surfactants are dissociated in water into amphiphilic anion and cation, which generally an alkaline metals (such as Na^+ and K^+) or a quaternary ammonium. Examples of anionic surfactants commonly used in industry include alkylbenzene sulfonates (detergents), soaps, lauryl sulfate (foaming agent) and di-alkyl sulfosuccinate (wetting agents). Non-ionic surfactants come as second widely used surfactants which accounted for around 45 % of the overall industrial production. Since the hydrophilic groups of this surfactant are non-dissociable type, such as alcohol, phenol, ether, ester or amide, they do not ionize in aqueous solution. Commonly used non-ionic surfactants series are Triton X-114 (Ghasemi & Kaykhaii, 2016; Kazi & Tuzen, 2016; S. Li *et al.*, 2015; Pongpiachan, 2009) and Tween series (Candir, Narin, & Soylak, 2008; Phasukarratchai *et al.*, 2017). On the other hand, cationic surfactants are those dissociated in water into an amphiphilic cation and an anion, which often is halogen type. The production and application of these surfactants are less as their production require a high pressure hydrogenation reaction. When a single molecule exhibit both anionic and cationic dissociations, it is known as amphoteric or zwitterionic. Even though amphoteric surfactants are known with their high biological compatibility and low toxicity, they are generally more expensive as compared to other surfactants. Some amphoteric surfactants are insensitive to pH whereas others are cationic at low pH and anionic at high pH, with amphoteric behaviour at intermediate pH.

Other than the type of surfactants, the concentration of surfactant used is also a very crucial factor as they affecting the extraction efficiency and enrichment factor (Ghasemi & Kaykhaii, 2016). Badera *et al.* (2014) has reported that the structure of surfactant molecules may become spherical, rod or lamellar-shaped micelles when the concentration of surfactant increase (Badera *et al.*, 2014). At the first critical micellar concentration (CMC), spherical micelles will be formed while conversion from spherical micelles to rod-like micelle will occur at second CMC. The CMC is a crucial characteristics of a surfactant as this value indicates

the amount of surfactant required to reach maximum surface tension reduction. In the other word, CMC plays crucial roles in colloidal stability, binding affinity, nucleation process and reaction kinetics. When they reach the CMC, the molecules in the surfactant tend to self-associate with hydrophobic tails aggregating inward into an assembled micellar structure with an outward hydrophilic head that is favoured thermodynamically in polar aqueous solution (Bhaisare, Pandey, Khan, Talib, & Wu, 2015). Paleologos *et al.* (2005) has also reported that maximum extraction efficiency and analytical signal can be obtained when the surfactant concentration used is within the narrow optimal range of the easy phase separation (Paleologos *et al.*, 2005).

Table 2.3: Surfactants used in CPE

Group of surfactants	Examples of surfactants	Chemical Formula
Non-ionic	polyoxyethylene octyl phenyl ether (Triton X-100)	$C_{14}H_{22}O(C_2H_4O)_n$, where $n = 9$ or 10
	Octylphenol ethoxylate (Triton X-114)	$C_{14}H_{22}O(C_2H_4O)_n$, where $n = 7$ or 8
	Polyethylene glycol dodecyl ether (Brij® 35)	$C_{12}H_{25}(OCH_2CH_2)_{23}OH$
	Polyoxyethylated lauryl ether (Brij 30)	$C_{12}H_{25}(OCH_2CH_2)_4OH$
	Polyoxyethylene (20) sorbitan monolaurate (Tween 20)	$C_{58}H_{114}O_{26}$
Cationic	Cetrimethylammonium bromide (CTAB)	$(C_{16}H_{33})N(CH_3)_3Br$
	Dodecyltrimethylammonium bromide (DTAB)	$CH_3(CH_2)_{11}N(CH_3)_3Br$
	Isopropyl-N-(3-chlorophenyl)carbamate (CICP)	$C_{10}H_{12}ClNO$
Anionic	sodium lauryl sulfate (SDS)	$CH_3(CH_2)_{11}SO_4Na$
	Dihexadecyl phosphate (DHF)	$[CH_3(CH_2)_{15}O]_2P(O)OH$
	Diocetyl sulfosuccinate sodium salt (Aerosol OT)	$C_{20}H_{37}NaO_7S$
Amphoteric	Dodecyldimethyl(3-sulfopropyl)ammonium hydroxide (SB-12)	$C_{17}H_{37}NO_3S$
	Dodecylammonium acetate butyrate (DAB)	$C_{18}H_{37}NO_2$

2.2.1.1 Non-ionic silicone surfactant (DC193C)

Silicone or siloxane surfactants are polyether modified polydimethylsiloxanes based amphiphilic molecules where the polyether part can be oxyethylene units or oxypropylene units (Sastry, Singh, Thummar, Verma, & Hassan, 2017). In recent years, silicone surfactant has gained attention since they not only equipped with the common feature of conventional nonionic surfactants but also possess some unique properties. Silicone surfactants are known to be equally surface active in both water and non-aqueous solvents besides their ability to remain as liquids even with high molecular weights (Soni, Panjabi, & Sastry, 2011). In this study, non-ionic silicone Dow Corning 193C (DC193C) fluid has been used. Since non-ionic silicone of DC193C surfactant is a surfactant based on silicone (PEG) copolymer, this surfactant is also referred as polyethylene glycol (PEG), PEG-12 Dimethicone and polydimethylsiloxane copolymer polyols (J. Chen, Spear, Huddleston, & Rogers, 2005; N. Zain *et al.*, 2014). Non-ionic silicone of DC193C surfactant has wide range of applications in personal care products as well as in automotive and household cleaning products. Even though this surfactant is yet to be fully studied for its application in CPE, this surfactant has the potential to be a green alternative as their safety to human and environmental friendly has been recognized (Bingjia, Li, Qiong, & Shigendo, 2007).

2.2.2 Influential factors

There are many other parameters that also affecting the extraction efficiency in CPE such as pH, temperature and incubation time as well as type, amount and concentration of salt used other than the selection of surfactant. During cloud point phenomenon at cloud point temperature, the liquid will separated into two phases in equilibrium. In the micellar system, pH will be the crucial factor in regulating the partitioning of the target molecules (N. Zain *et al.*, 2014) while the additives such as salt will then affect the cloud

point of the surfactants (Badera *et al.*, 2014). Generally, CPE can be carried out without the addition of additives. However, most studies have shown that the addition of salts can facilitate the phase separation and improve the extraction efficiency (Santalad *et al.*, 2012). This is because salt has salting out effect which will decrease the solubility of surfactant in water. This will then lead to the easier formation of two phases, which are surfactant rich phase and aqueous phase.