EXTRACTION AND SEPARATION OF PARABENS IN AQUEOUS BIPHASIC SYSTEMS

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Abstract

In this study, five systems for extraction of parabens were developed, namely ionic liquid based aqueous two-phase system (IL-ATPS), ionic liquid based aqueous two-phase system with β-cyclodextrin (IL-βCD-ATPS), cloud point extraction (CPE-DC193C), cloud point extraction with β-cyclodextrin (CPE-DC193C-βCD) and cloud point extraction with β-cyclodextrin-ionic liquid (CPE-DC193C-βCD-IL). These five developed methods have been optimized in order to get the optimum conditions for phase separation of parabens in water samples. These new, green, fast and simple extraction techniques coupled with a reversed-phase high performance liquid chromatography (RP-HPLC) showed excellent results for extracting parabens from aqueous samples. β-CD and βCD-IL as modifiers improved the sensitivity of IL-ATPS and CPE-DC193C systems. The experimental results demonstrated that the method detection limits (LOD) for studied parabens using IL-βCD-ATPS were in the range of 0.022-0.075 μgmL^{-1} and CPE-DC193C-βCD-IL methods were in the range of 0.013-0.038 μgmL^{-1}. These LOD results were relatively lower compared with IL-ATPS, CPE-DC193C and CPE-DC193C-βCD methods. Addition of β-CD and βCD-IL as modifiers also improved the selectivity of the developed methods. The use of IL-βCD-ATPS reduced the matrix effect and hence, percentage of recovery of parabens extraction increased from 88.0-92.8% to 96.0-98.5%. The recoveries of parabens extraction in sea water using IL-ATPS were dramatically improved with addition of β-CD in the IL-βCD-ATPS method. The mixture of βCD-IL with the surfactant molecules and parabens in the formation of micelles produced the extra large complex formations during the CPE process. The CPE-DC193C-βCD-IL system offered an obviously lower phase volume ratio compared to CPE-DC193C-βCD and CPE-DC193C systems with the value of phase volume ratios as 0.74, 0.92 and 1.63 respectively at 30% (w/v) surfactant concentration. On the other hand, IL-βCD-ATPS system also showed a lower phase volume ratio with the value of 0.16 compared to 0.19 for IL-ATPS at 30% (w/v) ionic liquid concentration. The developed method of CPE-DC193C-βCD-IL showed the highest preconcentration factor with the values for MeP (methyl paraben), EtP (ethyl paraben), PrP (propyl paraben) and ArP (benzyl paraben) were 76, 89, 97 and 110, respectively. While, the highest preconcentration factors for IL-βCD-ATPS were 70, 86, 95 and 103 for MeP, EtP, PrP and ArP respectively. When the surfactant concentration was increased from 5% (w/v) to 60% (w/v), ArP in CPE-DC193C-βCD-IL method the measured total loss of water content was 68%. ArP lost about 50 % (w/v) water content

in IL-βCD-ATPS compared to IL-ATPS where ArP lost only 43 % (w/v) water content when the ionic liquid concentration increased. It shows that CPE-DC193C-βCD-IL is considered as the highest loss of water content compared to the CPE-DC193C-βCD, CPE-DC193C, IL-βCD-ATPS and IL-ATPS systems. The overall loss of water content for MeP was 55%, followed by EtP and PrP with 52% each in CPE-DC193C-βCD-IL. Moreover, the distribution coefficient of parabens in surfactant-rich and ionic liquid rich phase in the order of hydrophobicity of parabens is MeP<Etp<PrP<ArP. In conclusion, βCD-IL contributes to a higher distribution of parabens in surfactant-rich phase compared to the other methods.

Abstrak

Kajian ini membangunkan lima sistem pengekstrakan paraben iaitu pengekstrakan cecair ionik berasaskan sistem akueus dua fasa (IL-ATPS), cecair ionik berasaskan sistem akueus dua fasa dengan β-siklodektrin (IL-βCD-ATPS), pengekstrakan titik awan (CPE-DC193C), pengekstrakan titik awan dengan β-siklodektrin (CPE-DC193C-βCD) dan pengekstrakan titik awan dengan cecair β-siklodektrin-ionik (CPE-DC193C-βCD-IL). Sistem-sistem ini diaplikasikan bagi mendapatkan keadaan optimum untuk pemisahan fasa paraben dari sampel air. Sistem-sistem ini adalah teknik pengekstrakan yang baru, hijau, cepat, mudah dan digabungkan dengan kromatografi cecair prestasi tinggi fasa-berbalik (RP-HPLC) telah menunjukkan keputusan cemerlang untuk mengeluarkan parabens dari sampel air. β-CD dan βCD-IL sebagai ejen pengubah meningkatkan kepekaan sistem IL-ATPS dan CPE-DC193C. Keputusan eksperimen menunjukkan bahawa had pengesanan (LOD) untuk paraben yang dikaji menggunakan kaedah IL-βCD-ATPS berada dalam julat 0.022-0.075μgmL^{-1} manakala kaedah CPE-DC193C-βCD-IL berada dalam julat 0.013-0.038 μgmL^{-1}. Nilai LOD ini ialah lebih rendah dibandingkan dengan kaedah IL-ATPS, CPE-DC193C dan CPE-DC193C-βCD. Penambahan β-CD and βCD-IL sebagai ejen pengubah juga meningkat kepilihan kaedah yang dibangunkan. Penggunaan IL-βCD-ATPS mengurangkan kesan matriks, seterusnya peratusan pemulihan pengekstrakan paraben bertambah dari 88.0-92.8% hingga 96.0-98.5%. Kebolehdapatan pengekstrakan paraben dari air laut menggunakan IL-ATPS meningkat dengan dramatik dengan penambahan β-CD dalam kaedah IL-βCD-ATPS. Campuran βCD-IL dengan molekul surfaktan dan paraben dalam pembentukan misel menghasilkan formasi kompleks ekstra besar semasa proses CPE. Sistem CPE-DC193C-βCD-IL menawarkan nisbah isipadu fasa yang jauh lebih rendah berbanding sistem CPE-DC193C-βCD and CPE DC193C dengan masing-masing bernilai 0.74, 0.92 dan 1.63 pada kepekatan surfaktan 30% (w/v). Manakala sistem IL-βCD-ATPS juga menunjukkan nisbah isipadu fasa lebih rendah dengan nilai 0.16 berbanding dengan 0.19 untuk IL-ATPS pada kepekatan cecair ionik 30% (w/v). Kaedah CPE-DC193C-βCD-IL yang dibangunkan menunjukkan faktor pra-kepekatan tertinggi dengan nilai untuk metil paraben (MeP), etil paraben (EtP), propil paraben (PrP) dan benzil paraben (ArP) masing-masing ialah 76, 89, 97 dan 110. Manakala, faktor pra-kepekatan tertinggi untuk IL-βCD-ATPS ialah 70, 86, 95 dan 103 masing-masing untuk MeP, EtP, PrP and ArP. Apabila kepekatan surfaktan ditingkatkan dari 5% hingga 60% dalam kaedah CPE-DC193C-βCD-IL jumlah kehilangan kandungan air

adalah 68%. ArP kehilangan air sebanyak 50% (w/v) dalam kaedah IL-βCD-ATPS berbanding dengan kaedah IL-ATPS yang kehilangan air sebanyak 43% (w/v) sahaja apabila kepekatan cecair ionik meningkat. Peratusan ini adalah kehilangan kandungan air tertinggi berbanding dengan sistem CPE-DC193C-βCD and CPE-DC193C. Kehilangan kandungan kandungan air secara keseluruhannya adalah 55% untuk MeP, diikuti oleh EtP and PrP masing-masing dengan 52%. Pekali taburan paraben dalam cecair yang kaya dengan surfaktan dan cecair ionik fasa mengikut susunan kehidrofobisiti paraben ialah MeP<EtP<PrP<ArP. Secara kesimpulannya, βCD-IL menyumbang kepada pengagihan paraben lebih tinggi di fasa yang kaya dengan surfaktan berbanding dengan kaedah-kaedah yang lain.

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Table of Content

List of Figures

List of Tables

Abbreviations

ATPS	Aqueous two-phase system
β-CD	β-cyclodextrin
CD	Cyclodextrin
IL	Ionic liquid
βCD-IL	β-cyclodextrin functionalized ionic liquid
CPE-DC193C	Cloud point extraction using surfactant DC193C
CPE-DC193C-βCD	Cloud point extraction using surfactant DC193C-with β-cyclodextrin as a modifier
CPE-DC193C-βCD-IL	Cloud point extraction using surfactant DC193C-with β-cyclodextrin functionalized ionic liquid as a modifier
IL-ATPS	Ionic liquid based aqueous two-phase system
IL-βCD-ATPS	Ionic liquid based aqueous two-phase system with β-cyclodextrin as a modifier
MeP	Methyl paraben
EtP	Ethyl paraben
PrP	Propyl paraben
ArP	Benzyl paraben
HPLC/UV	High performance liquid chromatography
RP-HPLC	Reversed-phase high performance liquid chromatography

ABS	Aqueous biphasic system
CD-IL	Cyclodextrin-ionic liquid
RTILs	Room temperature ionic liquids
CE	Capillary electrophoresis
CP	Cloud point
CPT	Cloud point temperature
ECDs	Endocrine distrupting chemicals
IL-rich phase	Ionic liquid-rich phase
Triton X	Polyoxyethylene-(n)-octylphenyl ether
PONPE	Polyoxyethylene-(n)-nonylphenyl ether
Genapol X	Oligoethylene glycol monoalkyl ether
brij	Polyoxyethylene-10-akyl ether
[Cnmim][PF_6], n = 4, 6, 8	1-n-methyl-3-methylimidazolium hexafluorophosphate
[Cnmim][BF_4], n = 6, 8	1-n-methy-3-methylimidazolium tetrafluoroborate
[Bmim][BF_4]	1-n-butyl-3-methylimidazolium tetrafluoroborate
HP-βCD	Hydroxypropyl-β-cyclodextrin
LOD	Limit of detection
RSD	Relative standard deviation
[C_4mim][Cl]	1-butyl-3-methylimidazolium chloride

β-CDOTs	o-p-toluenesulfonyl-β-cyclodextrin
MIM	1-methylimidazole
V_s.	Volume of IL-rich phase
V_w	Volume of the aqueous phase

CHAPTER 1: Introduction

Parabens are effective preservatives in many types of formulas. They can be found in shampoos, commercial moisturizers, shaving gels, personal lubricants, topical pharmaceuticals, spray tanning solutions, cosmetics make-up (colloquially) and toothpaste. They are also used as food additives. These compounds are considered as endocrine disrupting chemicals (ECDs) because of their endocrine activity (Darbre & Harvey, 2008; Khanna & Darbre, 2012; Ramírez, Marcé, & Borrull, 2011) and have been detected in human tissues including breast tumors (Barr, Metaxas, Harbach, Savoy, & Darbre, 2012). Therefore, developing a reliable method for determining parabens in our environment should be a major concern.

Application of the cloud point extraction (CPE) in aqueous media for the analytical determination of trace organic analytes has aroused growing attention in a few years ago (Kiran et al., 2008; Man, Lam, Lam, Wu, & Shaw, 2002; Meeravali & Jiang, 2009). CPE has been demonstrated to be able to extract and preconcentrate a wide range of organic compounds from the aqueous phase (Bai, Li, Chen, & Chen, 2001; Baig et al., 2010; Fontana, Silva, Martínez, Wuilloud, & Altamirano, 2009). The CPE techniques which result in fast extraction, high preconcentration factor and avoidance of toxic and environmental unfriendly organic solvents (Khan et al., 2010; P. Liang & Yang, 2010; E. L. Silva, Roldan, & Giné, 2009) is better than other techniques.

On the other hand, the studies on CPE of non-ionic surfactant has been reported exploited only the same and similar structure of the surfactants such as Triton X (polyethylene-(n)-ocytlphenyl ether) (Citak & Tuzen, 2010; Ezoddin, Shemirani, &

Khani, 2010; R. Liang, Wang, Xu, Li, & Qi, 2009; T. Wang, Gao, Tong, & Chen, 2012), PONPE (polyoxyethylene-(n)-nonylphenyl ether) (Wuilloud et al., 2002), Genapol X (oligoethylene glycol monoalkyl ether) (Padrón Sanz, Halko, Sosa Ferrera, & Santana Rodríguez, 2004; Shi, Yan, Ma, & Zhang, 2011; J. Zhou, Sun, & Wang, 2008) and Brij (polyoxyethylene-10-cetyl ether] (Delgado, Pino, Ayala, González, & Afonso, 2004; Z. Wang, Zhao, & Li, 2003).

The major drawback with the non-ionic surfactants is that the most of these materials contain which chromophores strongly absorb ultraviolet (J.-b. Chen, Zhao, Liu, Zhou, & Yang, 2009; Filik, D.Giray, B.Ceylan, & R.Apak, 2011; Hung, Chen, & Yu, 2007). Some of these surfactants contain alkyl phenyl groups in their hydrophobic moiety, leading to some environmental concerns (Guenther, Kleist, & Thiele, 2006). This presents a major obstacle in performing analysis using ultraviolet (UV)/visible with high performance liquid chromatography (HPLC) detector (J.-b. Chen et al., 2009; Zhu, Liu, Mao, & Yang, 2008). To alleviate both problems, biodegradable surfactants, mainly ethoxylated alcohols without phenyl group and branched alkyl chains, are proposed (Haddou, Canselier, & Gourdon, 2006).

Therefore, various schemes have been developed to overcome this drawback of not using the UV detector with non-ionic surfactant (J.-b. Chen et al., 2009; Zhu et al., 2008). An electrochemical detection procedure was the suggested for the highly UV absorbing phenyl group in certain surfactant (J.-b. Chen et al., 2009). Moreover, some of the surfactants are toxic and dangerous to human and environment. Therefore, we should find a green surfactant to protect the environment and human health. Silicone non-ionic surfactant so-called DC193C which is a water soluble surfactant and is considered as a green surfactant that can be used directly to HPLC/UV without giving any obstacles to the detector is an alternative to overcome the problem from most of the

non-ionic surfactant to be used in HPLC/UV or UV instruments. Thus, CPE methods are developed using silicone non-ionic surfactant DC193C.

Aqueous biphasic systems (ABS) also known as aqueous two-phase systems (ATPS) have been widely used with ionic liquids (ILs) in separation science (J. Chen, Spear, Huddleston, & Rogers, 2005; Pan, Chiu, Lu, Lee, & Li, 2002; Willauer, Huddleston, & Rogers, 2002). ILs have been gaining great exposure due to their potential use as green solvents and possible replacements for traditional volatile organic compounds (VOCs) (Cai et al., 2007; Willauer et al., 2002). This new type of ATPS has many advantages, such as low viscosity, little emulsion formation, quick phase separation, high extraction efficiency and gentle biocompatible environment (J. Chen et al., 2004; Pei, Wang, Lui, Wu, & Zhao, 2007). In recent years, room temperature ionic liquids (RTILs) as a class of potential green solvents, have found wide application in separation study. Therefore, we are interested to develop a new method using IL in the ATPS on determination of paraben from water samples.

Cyclodextrin (CD) has been extensively applied in analytical fields such as enantiomers selector (Kewen, Jiabing, Tao, & Yongbing, 2011), as a modifier in capillary electrophoresis (Qi, Cui, Chen, & Hu, 2004), as a chiral selectors (Tan, Long, Jiao, & Chen, 2011) and as a co-modifier in mobile phase techniques (Husain, Christian, & Warner, 1995). Considering the fact that parabens are able to form an inclusion complex with β-CD (Chan, Kurup, Muthaiah, & Thenmozhiyal, 2000; de Vries & Caira, 2008; de Vries, Caira, Bogdan, Farcas, & Bogdan, 2009), we are investigating the efficiency of β-CD as a modifier in IL-ATPS and CPE for determination of parabens from environmental water samples. On the other hand, CDs functionalized to ILs are also interesting aspects to explore. This is because some studies reported that the performance of cyclodextrin functionalized ionic liquid (CD-IL) in the adsorption/removal of pollutant is very excellent strategically (Mahlambi,

Malefetse, Mamba, & Krause, 2010) Therefore, we grab this knowledge as a challenge to develop a new method on βCD-IL as modifier in CPE system.

Based on our literature search and knowledge, this research/study is the first attempt on IL-ATPS and CPE using β-CD and βCD-IL as a modifier. Because of the awareness and concern to the amount of parabens in Malaysia's water samples, this research presents the development of a simple, fast, efficient and green method to remove parabens in water samples.

1.1 Research Objective:

1. To develop ionic liquid two-phase extraction (IL-ATPS) method using ionic liquid [C_4mim][Cl] to analyse parabens in water samples.

2. To develop IL-ATPS with addition of β-cyclodextrin as a modifier (IL-βCD-ATPS) using ionic liquid [C_4mim][Cl] to analyse parabens in water samples.

3. To develop cloud point extraction (CPE) method using non-ionic surfactant DC 193C (CPE-DC193C) to analyse parabens in water samples.

4. To develop CPE method using non-ionic surfactant DC 193C with addition of β-cyclodextrin (CPE-DC193C-βCD) as a modifier to analyse parabens in water samples.

5. To develop CPE method using non-ionic surfactant DC 193C with addition of β-cyclodextrin-ionic liquid (CPE-DC193C-βCD-IL) as a modifier to analyse parabens in water samples.

6. To compare all developed methods based on the efficiency of extraction methods to analyse parabens in water samples.

CHAPTER 2: Literature review

2.1 Aqueous two-phase extraction (ATPS)

2.1.1 Principles of ATPS

ATPS are formed when a pair of solutes leads to the formation of two macroscopic liquid phases when dissolved in water above certain concentrations. This phenomenon was first observed by Beijerinck in 1896; however, it was not until 1956 that the potential use of these systems as a separation technique in biotechnology was realized (Berthod, Ruiz-Ángel, & Carda-Broch, 2008; X. Han & Armstrong, 2007).

Since the bulk of both phases comprise water, ATPS have advantages over the conventional extraction systems using organic solvents such as short processing time, low energy consumption, relative reliability in scale up and biocompatible environment (Z. Li, Pei, Wang, Fan, & Wang, 2010). They provide an economical and efficient downstream-processing method. ATPS have been widely used for the recovery and the purification of various biomolecules, for example proteins and nucleic acid (Oppermann, Stein, & Kragl, 2011; Raja, Murty, Thivaharan, Rajasekar, & Ramesh, 2011) because their versatility, high efficiency, high yield, improved purification factor, selectivity, low-cost and fast mass transfer rates are the main focus of ATPS in that area (de Brito Cardoso et al., 2013).

Nevertheless, the high viscosity of the coexisting phases led to the development of systems formed by polymers and inorganic salts such as potassium phosphate,

sodium citrate and calcium chloride (Biazus, Santana, Souza, Jordão, & Tambourgi, 2007; Souza et al., 2010). Other components like organic solvents have also been used, e.g. alcohols (Ooi et al., 2009), but the application of this type of systems is limited due to the interference of alcohols in the biological activity of several biomolecules. Several new types of ATPS have been reported, such as those containing hydrophilic organic-solvent-salt ATPS or IL-salt ATPS (X. Xie, Wang, Han, & Yan, 2011). Compared with the traditional liquid-liquid extraction, ATPS contain over 70% in recovery for IL-rich phase and a low interfacial tension between them; therefore, ATPS facilitates the separation of polar solutes without the troubles of a wide range of pH adjustment and VOCs (X. Xie et al., 2011).

2.1.2 ILs in ATPS

ILs are a broad class of salts that melt at or below 100°C and are composed of organic cations (e.g., imidazolium, pyridinium, pyrrolidinium, phosphonium and ammonium) and anions (e.g., Cl^-, PF_6^-, BF_4^-, NO_3^-, trifluoromethylsulfonate $(CF_3SO_3)^-$ and trifluoroethanoate $(CF_3CO_2)^-$). ILs sometimes called molten salts, are liquids at ambient temperatures and consist entirely ionic species. Their quite rapid emergence as alternative solvents has involved organic synthesis, chemical reactions, chemical separations and material preparations.

ILs are nonvolatile and exhibit excellent chemical and thermal stabilities. ILs have been primarily explored for applications in synthesis (Deshmukh, Qureshi, Dhake, & Bhanage, 2010; Yue, Fang, Liu, & Yi, 2011), electrochemistry (Lu, Huang, & Qu, 2011; Sun et al., 2012), catalysis (Y. Fan & Qian, 2010),chromatographic separation (Delmonte et al., 2011), extraction processes (Coll, Fortuny, Kedari, & Sastre, 2012; Marciniak, 2010), and mass spectrometry analysis (Escudero, Wuilloud, & Olsina, 2013). More recently, a new type of ATPS consisting of ILs and salts were reported for

recycle, metathesis and study of the distribution ratios of short chain alcohol (Kadokawa, Takegawa, Mine, & Prasad, 2011; Park & Bae, 2010). ILs can be hydrophobic or hydrophilic depending on the structures of the cation and/or anion (Gardas, Dagade, Coutinho, & J.Patil, 2008). Hydrophilic ILs may fully or partially dissociate into ions when mixed with water, which is similar to what is observed in regular aqueous solutions of inorganic salts. These ions are solvated in aqueous solutions causing structural changes to the aqueous environment (Gardas et al., 2008).

The designs and synthesis of functional ILs that incorporate structural or functional groups have been reported (Berthod et al., 2008). It was shown that ILs with the long alkyl chain group exhibited surface active property in their aqueous solutions and these IL surfactants have been investigated by surface tension measurements (Z. Li et al., 2010). ILs based on 1-alkyl-3-methylimidazolium cation ($[C_2mim]^+$) have received much attention and have been the most studied factor (Claudio, Ferreira, Shahriari, Freira, & Coutinho, 2011; Pei, Wang, Wu, Xuan, & Lu, 2009; Sadeghi, Ebrahimi, & Mahdavi, 2012). An interesting aspect of such ILs is that the cation ($[Cnmim]^+$) possess an inherent amphiphilic character when their alkyl group is a longer hydrocarbon chain (L. Wang et al., 2007). It has been shown that in solution, the solvation and the interactions of the ions or ion-pair with the solvent determine the unique properties of these systems. The volumetric and properties of electrolytic and non-electrolytic solutions have proved information in elucidating the solute-solute and solute-solvent interactions that exist in the solutions.

The extremely low volatility of the ILs renders them a little flammable so they become candidate to replace organic pollutant solvents (Berthod et al., 2008). Some characteristics of ILs such as electrical conductivity, viscosity, surface tension, absorbance spectrum in UV, solubility in non-aqueous solvent, solvating properties and

ability to dissolve compounds are important aspects in separation science i.e. in capillary electrophoresis (CE) (Berthod et al., 2008).

IL-ATPS have many advantages shared by ILs and ATPS, such as little emulsion formation, free of volatile organic solvent, quick phase separation, high extraction efficiency and gently biocompatible environment (J. Han et al., 2011). With the use of ATPS, one can simultaneously carry out purification, extraction and enrichment. Recently, a new kind of ATPS was reported using ILs and inorganic salts (Neves, Ventura, Freire, Marrucho, & Coutinho, 2009; Ventura et al., 2009) or saccharides (Wu, Zhang, Wang, & Yang, 2008). ILs have also been proposed as potential solvent in conventional polymer-salt-based ATPS aiming at tailoring their extraction efficiency for particular added-value compounds (Pereira, Lima, Freire, & Coutinho, 2010).

2.1.3 Application of IL based ATPS in extraction of organic pollutants

Extensive studies have been conducted for the extraction of organic compounds from aqueous phase with ILs, depending on the affinity between hydrophobic ILs and organic solutes. The extraction mechanism includes ion exchange, hydrogen bond, van der waals interaction, and so on. (Khachatryan et al., 2005) reported the extraction of phenolic compounds from aqueous solution into $[C_4mim][PF_6]$, almost quantitatively at pH<pKa. The relatively large distribution coefficient of phenolate anions indicated the ion exchange mechanism in the extraction that when phenolate anion entered into the IL phase, an equal amount of hexafluorophosphate anion was transferred to water.

The performance of a neutral IL (N-butyl-N-methyl pyrrolidinium bis(trifluoromethanesulfonyl)amide) as extractant was studied by (Vijayaraghavan, Vedaraman, Surianarayanan, & MacFarlane, 2006) for the removal of azo dyes from aqueous solutions. During the extraction, the azo dye went into the organic phase in its

ionic form, and the distribution coefficient of the dye between IL and water was about 2.0. The repeated extractions (two to three times) with fresh IL proceeded to a removal fraction about 95% of the dyes from aqueous phase into the IL phase. The ion exchange mechanism was suggested by C. P. Li, Xin, Xu, and Zhang (2007) when investigating the extraction process of acid dyes from aqueous solution into $[C_4mim][PF_6]$, in which the solvated part of IL played an important role as counter-ions for the anions of acidic dyes. (J. Fan, Fan, Wang, & Cui, 2006) investigated the suitability of imidazolium-based ILs, 1-methyl-3-alkylimidazolium hexafluorophosphate ($[Cnmim][PF_6]$, n = 4, 6, 8) and 1-methy-3-alkylimidazolium tetrafluoroborate ($[Cnmim][BF_4]$, n = 6, 8), as a substitute for volatile organic solvents in the liquid–liquid extraction of selected phenols from aqueous solutions.

The selected phenols included phenol, bisphenol A, pentachlorophenol, 4-octylphenol and 4-nonylphenol. A deep analysis of experimental results suggested the existence of strong hydrogen-bonding interaction between the anions of ILs and the phenols, which contributed to the high extraction efficiency of ILs for the phenols. As a result, the value of distribution ratio for phenol into $[Cnmim][BF_4]$ was about 10 times higher than in dichloromethane under the same conditions. The effect of aqueous phase pH on partitioning of an indicator dye and thymol blue, was studied by (Visser, Swatloski, & Rogers, 2000). A remarkable dependence of distribution ratio between $[Cnmim][PF_6]$ and water on the pH value was revealed, suggesting a possible approach of separating ILs and extract after extraction.

J. Han et al. (2011) developed a IL-ATPS method using IL (1-butyl-3-methylimidazolium chloride, $[C_4mim][Cl]$) with addition of dipotassium hydrogen phosphate salt, K_2HPO_4 . This method was successfully applied for the determination of chloramphenicol in lake water, feed water, milk and honey samples with the limit of detection (LOD) of 0.1 ng mL^{-1} and limit of quantification (LOQ) of 0.3 ng mL^{-1}. The

recovery was 97.1-101.9% from aqueous samples of environmental and food samples by the proposed method. The method was compared with the liquid-liquid extraction, solvent sublation and conventional ATPS without addition of salt, that efficiently reduce the wastage of IL. The novel technique is much simpler and more environmentally friendly and is suggested to have important applications for the concentration and separation.

The determination of trace endocrine-distrupting chemical such as chlorophenols in water sample and analyzing using HPLC was carried out by W. Liang et al. (2011). The good recovery was obtained (90.2-107%) using IL 1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim][BF_4]) and disodium dihydrogen phosphate salt $Na_2H_2PO_4$. The authors reported that their developed method of determination of chlorophenols was successfully done because it has innocuity, no pollution, quick separation, no emulsification, high sensitivity and precision.

Claudio et al. (2011) explored the proper extractive solvent and designed an optimized extraction process of vanillin, 3-methoxy-4-hydrozybenzaldehyde using improved ATPS. Vanillin or vanilla is currently used in food, beverages and pharmaceutical products to provide satisfying flavors as well as in cosmetic industry for its fragrance. The three main parameters were evaluated on study the partitioning of vanillin process which was the ionic liquid cation and anion structure, the temperature of equilibrium and the available concentration of vanillin in the global system. In all the system tested, the results gathered in this work indicate that IL-based ATPS can be further employed in the extraction and purification of vanillin from different matrices as confirmed by the large partition coefficient obtained and improved low viscosity systems.

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2.2 Cloud point extraction (CPE)

2.2.1 Principles of CPE

The traditional methods employed for the extraction of different compounds in aqueous samples such as liquid-liquid extraction, which normally requires large amounts of organic solvents to be used during a long period. The traditional method has the disadvantages such as high cost, toxical effects, long time and high dilution factor. Therefore, the development of a simple and rapid extraction method together with the use of minimal amounts of extractants have been improved. Separation procedures based on the peculiar properties of aqueous non-ionic surfactant solutions have been proposed as an alternative to the use of traditional organic solvents. Surfactants have the capability of solubilizing different kinds of solutes (Padrón Sanz, Sosa Ferrera, & Santana Rodríguez, 2002).

CPE technique was firstly introduced by Watanabe and Tanaka to preconcentrate metal ions from aqueous samples (Watanabe & Tanaka, 1978). The CPE that used surfactant is known for their capability to enhance the solubility of hydrophobic materials and environmentally benign extraction technology. It is much more attractive to analytical chemists as compared to other extraction methods. CPE has received a great attention because the procedure is simple, fast and the extraction of the analytes can be accomplished by optimizing the experimental conditions such as temperature, the addition of salts, pH, type of electrolyte and etc.

The other advantage of CPE is the preferable use of water as the solvent in the micellar solution, which is benign to the environment compared to the organic solvents still used in other preconcentration procedures. Additionally, the benefit of CPE arises from a good compatibility between surfactant-rich phase and the hygroorganic mobile

phase in liquid chromatography, which offers great convenience to the analysis of the trace of hydrophobic materials.

At cloud point temperature (CPT) usually at a higher temperature than its critical temperature, the surfactant undergoes phase separation into a surfactant-rich phase and surfactant aqueous phase. Thus, the analytes are concentrated with a high preconcentration factor (R. Liang et al., 2009; Santalad, Srijaranai, Burakham, Glennon, & Deming, 2009; J. Zhou, Wang, & Sun, 2008). CPE as an effective extraction method uses less solvent and only requires a very small amount of relatively nonflammable and nonvolatile surfactant that is environmentally friendly. Furthermore, CPE can produce high extraction efficiency, high preconcentration factor with a simple method of extraction and removal of the sample matrices all in one step (Hung et al., 2007; Jun, Yong, Lam, Lam, & Xia, 2009; Khan et al., 2010; R. Liang et al., 2009; L.-L. Wang, Wang, Zheng, & Xiao, 2010; J. Zhou, X. L. Sun, et al., 2008).

It is well known that surfactants are amphiphilic molecules which contain a polar head group and a non-polar tail. In general, the tail is a linear or branched hydrocarbon chain with different numbers of carbon atoms, and may contain aromatic rings, whereas the head is ionic or strongly polar groups. In aqueous solutions, these two moieties are hydrophobic and hydrophilic, respectively. The hydrophobic tails tend to form aggregates called micelles. Most of nonionic surfactants in aqueous solutions form two phases above the cloud point temperature; surfactant-rich phase (coacervate) and a dilute phase, in which the concentration of the surfactant is close to its critical micelle concentration (CMC) (M. F. Silva, Cerutti, & Martinez, 2006; Stalikas, 2002). Upon an appropriate alteration of the conditions such as temperature and addition of salt or additives, the solution becomes turbid at a temperature known as CP due to diminished solubility of the surfactant in water (S. Xie, Paau, Li, Xiao, & Choi, 2010).

Owning to their hydrophobic nature, the analytes in the present study will exist favorably in the surfactant-rich phase, whose volume is usually smaller. The small phase volume allows us to preconcentrate and extract the analytes in one step. Compared with the traditional organic liquid-liquid extraction, CPE uses a very small amount of relatively nonflammable and nonvolatile surfactant which is easy to dispose. In addition, CPE can lead to a high recovery and preconcentration factor and can minimize losses due to the sorption of analytes onto containers (Casero, Sicilia, Rubio, & Pérez-Bendito, 1999).

Some opinions said that CPE is a mature and densely exploited technique with a very little perspective for substantial findings or for significantly new applications. Based on our literature, although CPE has been explored for 34 years with more than 500 publications but these techniques are still popular, interesting to study and receiving improvement and modification on the method from the researchers to ensure that CPE is useful to their research. Since 1999, CPE has been received great attention and huge perspective to extraction of various analytes for example PAHs (Bai et al., 2001; Casero et al., 1999; Quina & Hinze, 1999; Willauer et al., 2002), other organic compounds (Bai et al., 2001; Haddou et al., 2006; Padrón Sanz et al., 2004) and also in the systems having low concentrations of metal ions (Mesquita da Silva, Azzolin Frescura, & Curtius, 2000; L.-L. Wang et al., 2010).

We admitted that CPE offers many advantages and becomes more and more attractive (Ghouas, Haddou, Bouabdesselam, Bouberka, & Derriche, 2010; Haddou et al., 2006; Pino, Ayala, Afonso, & González, 2002). CPE has never been neglected and still continues with new applications to our environment. Nowadays, CPE has been used at ambient temperature rather than high temperature and has been combining with a various instruments such as gas chromatography flame photometric detection (GC-FPD) (Zhao et al., 2011), flow injection CPE with HPLC (FI-CPE-HPLC) (C. F. Li, Wong,

Huie, & Choi, 2008), capillary electrophoresis electrochemiluminesence (CE-ECL) (Yin, Guo, & Wei, 2010), microwave-assisted CPE (Sosa Ferrera , Padrón Sanz , Mahugo Santana, & Santana Rodríguez, 2004) and many more.

Although some reviews on CPE for analysis of metal ions, organic compounds, drugs, persistent organic pollutants and other bioactive compounds have appeared in literatures (Carabias-Martínez et al., 2000; M. F. Silva et al., 2006; Stalikas, 2002), there is no research report yet on the application of CPE in pharmaceutical and personal care products (PPCPs).

2.2.2 Proposed schematic illustration in CPE

The proposed schematic illustration of surfactant with the salt in CPE is described in schematic diagram of CPE (Figure 2.1). When a salt is dissolved in an aqueous solution, its ions are surrounded by a layer of water molecules. The formation of surfactant-salt may be considered to be competition between the hydrophilic surfactant and the inorganic ions because of their stronger affinity for the water. A similar mechanism happens in CPE-DC193C which makes surfactant-rich phase in the salted-out separate from the solution.

As a result, phase separation will be produced clearly in the solution which is known as surfactant-rich phase and aqueous phase. In the proposed schematic illustration for all the methods, it is shown that surfactant DC193C solubilises the paraben compound and bring them to the top layer of surfactant-rich phase. This surfactant-rich phase contains the paraben, the surfactant itself and some water molecules.

On the other hand, there is a migration of water molecules away from the ions of the surfactant to those of the inorganic salt, which in turn decreases the hydration and hence the solubility of the ions of the surfactant (S. Xie et al., 2010). Consequently, a

surfactant-rich phase in the salted-out separates from the solution. This means that the salting-out effect must be directly correlated to the hydration strength of the different ions of the inorganic salt (C.-X. Li et al., 2009). As a result, phase separation will be produced clearly in the solution which is known as surfactant-rich phase and aqueous phase.

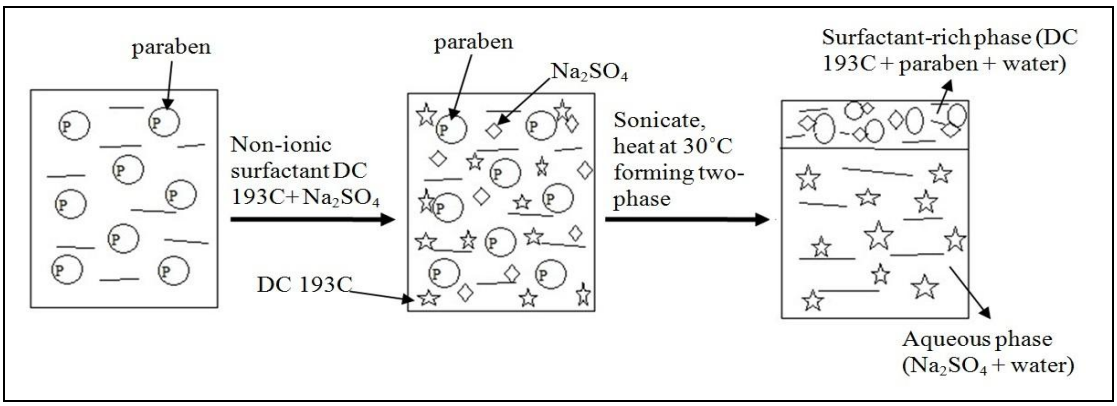


Figure 2.1 The proposed schematic illustration of CPE-DC193C.

2.2.3 Application of CPE in organic pollutants

Up to now, nonionic surfactants such as Triton X, PONPE, Genapol X and Brij are the most widely used surfactants with both hydrophilic and hydrophobic components in their molecular structures. These surfactants have been successfully applied to extract polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) polychlorinated dibenzofurans (PCDFs) and polychlorinates dibenzo-p-dioxins (PCDDs) synthetic pesticides, hydroxyaromatic compounds, vitamins, hydrophobic membrane proteins and pharmaceuticals from natural waters, soils and sediments as well as complex biological fluids (Man et al., 2002).

Besides PAHs, the determination of other organic pollutants such as PCBs, PCDDs, PCDFs, PBDEs and OCPs has also been investigated using CPE with different instruments. Fontana et al. (2009) studied PBDEs in water and soil samples using GC-MS. They extracted the target analytes from the aqueous bulk into the surfactant-rich

phase and afterwards, the analytes were ultrasound-assisted back-extracted into isooctane. The back-extracted were successfully introduced to GC-MS with good recoveries without declining the separation efficiency of the capillary column.

The PCDDs constitute a group of organochlorinated, lipophilic and bioaccumulative substances very persistent in the environment. They have become social and scientific interest over recent decades due to their high toxicity. They have been detected in a wide range of samples such as soils, sediments and water (Eljarrat, Caixach, & Rivera, 2001; Padrón Sanz et al., 2002). (Padrón Sanz et al., 2002)) have determined of PCDDs in water samples by CPE-HPLC-UV and the obtained recoveries were 70-105%. The analysis of PCDDs is complicated due to their low levels of concentration in the sample, which requires extraction and preconcentration steps prior to their determination.

Tang et al. (2010) developed a simple CPE process for determination of triazole fungicides (tricyclazole, triadimefon, tebuconazole and diniconazole) in environmental waters using nonionic surfactant, polyethylene glycol 600 monooleate (PEG600MO) and analysis using HPLC/UV. Average recovery experiments were from 82% to 92% and RSDs were from 2.8% to 7.8% for four fungicides spiked in river water and tap water. Under such conditions, the enrichment factors were higher than 60-fold for the four studied analytes and LOD were between 6.8 ngL^{-1} to 34.5 ngL^{-1}.

Padrón Sanz et al. (2004) reported the Genapol X-080 micellar extraction for eight organophosphorus pesticides in water samples before analysing using HPLC/UV. The recoveries of the studied pesticides were between 81% and 105% for most pesticides, but some studied pesticides (i.e. dimethoate and ethoprophos) showed recoveries lower than 50%. The results obtained in that study indicate that the use of Genapol X-080 provides better results than polyoxyethylene 10 lauryl ether (POLE) for

the extraction and preconcentration of organophophorus pesticides using CPE methodology.

Halko, Sanz, Ferrera, and Rodríguez (2004) studied fungicides (carbendazim, benomyl, thiabendazole and fuberidazole) in spiked water samples using Genapol X-080 and POLE with recoveries ranged between 68% and 96%. Which as observed the results of CPE for carbendazim and benomyl are slightly lower compared with other studied compounds using solid phase extraction. Pourreza and Elhami (2007) reported that malachite green was successfully detected in fish farming and river water samples using CPE with Triton X-100 and analysis using UV-Visible spectrophotometer with recoveries 95% to 102%. A successful CPE method would be that which maximizes the extraction efficiency through minimizing the phase volume ratio thus maximizing its enrichment factor.

2.2.4 Application of CPE in pharmaceutical and personal care products (PPCPs)

PPCPs are a diverse group of compounds used in soaps, lotions, toothpaste, fragrances, and sunscreens. The primary classes of PPCPs include disinfectants (e.g. triclosan), fragrances (e.g. musks), insect repellants (e.g. DEET), preservatives (e.g. parabens) and UV filters (e.g. methylbenzylidene camphor) (Mackay & Barnthouse, 2010). Unlike pharmaceuticals which are intended for internal use, PPCPs are the products intended for external use on the human body and thus are not subjected to metabolic alterations; therefore, large quantities of PPCPs enter the environment unaltered through regular usage. Many of these compounds are used in large quantities, and recent studies have indicated that many are environmentally persistent, bioactive, and have the potential for bioaccumulation(Mackay & Barnthouse, 2010). PPCPs are among the most commonly detected compounds in surface water throughout the world

(Peck, 2006). However, in comparison to pharmaceuticals, relatively little is known about PPCP toxicity (Brausch & Rand, 2011).

The most widely used methods for analyzing phthalate esters are chromatographic techniques such as gas chromatography (GC) or HPLC, but their sensitivity and selectivity limit their direct use for determination of these contaminants at a very low level of concentration in environmental samples with complex matrix. Therefore, sample pretreatment prior to chromatographic analysis such as liquid-liquid extraction and solid-phase extraction is usually necessary. Unfortunately, all of these methods are time-consuming and need a large sample volume. In particular, the traditional liquid-liquid extraction method also makes our environment toxic because of large amounts of volatile solvent used. As a result, CPE has been employed in analytical chemistry to preconcentrate organic compounds (Carabias-Martínez et al., 2000; Casero et al., 1999; Crick & Conte, 2000; Hung et al., 2007; L. Wang et al., 2007).

L. Wang et al. (2007) reported a study on di-ethyl-phthalate (DEP), 2-ethylhexyl-phthalate (DEHP) and di-cyclohexyl-phthalate (DCP) in environmental samples using HPLC/UV in spiked water samples. It showed that the recoveries of three compounds in between 85% to 103% and the enrichment factors were between 35 to 111. (Prokůpková, Holadová, Poustka, & Hajšlová, 2002) found that the recoveries of more polar phthalates (di-methyl-phthalates and di-ethyl-phthalates) were very low (32%) compared with other phthalates in their study. On the other hand, a complete extraction with good repeatability was obtained for other phthalates (moderately polar DnBP and BBP and non polar DEHP and DnOP) even at a low spiking level ($1\mu gl^{-1}$).

2.3 β-cyclodextrin (β-CD)

2.3.1 Properties of CD

The most common cyclodextrins (CDs) are made of six, seven or eight glucose units and are called α-, β- and γ-CDs, respectively (Manakker, Vermonden, Nostrum, & Hennink, 2009). Higher molecular weight CDs are popularly known, however their use is not known even in industry(Manakker et al., 2009). Figure 2.2 shows the three common types of CDs.

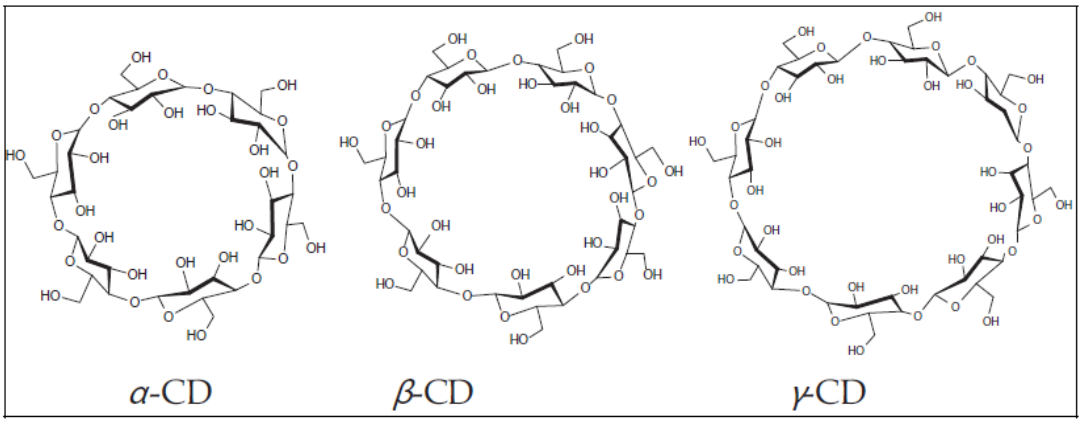


Figure 2.2 Type of cyclodextrins (Manakker et al., 2009)

They were first discovered by Villiers in 1891 (Del Valle, 2004), during the addition to reducing dextrins, a small amount of crystalline material was obtained from starch digest of Bacilus amylobacter.

According to (Del Valle, 2004)), Villiers probably used impure cultures and the CDs were produced by a Bacillus macerans contamination. Villiers named his crystalline product ‘cellulosine’(Del Valle, 2004). In 1903, Schardinger was able to isolate two crystalline products, dextrins A and B, which were described with regard to their lack of reducing power (Eastburn & Tao, 1994). The bacterial strain capable of producing these products from starch was unfortunately not maintained (Eastburn & Tao, 1994).

CD has a hydrophobic interior because of the presence of carbon and hydrogen atoms and this feature allows them to host several compounds in their cavities. Their exterior cavities are hydrophilic because of the presence of hydroxyl groups and this makes them soluble in water. Figure 2.3 shows a representation of CD moiety with a hydrophilic exterior and a hydrophobic cavity.

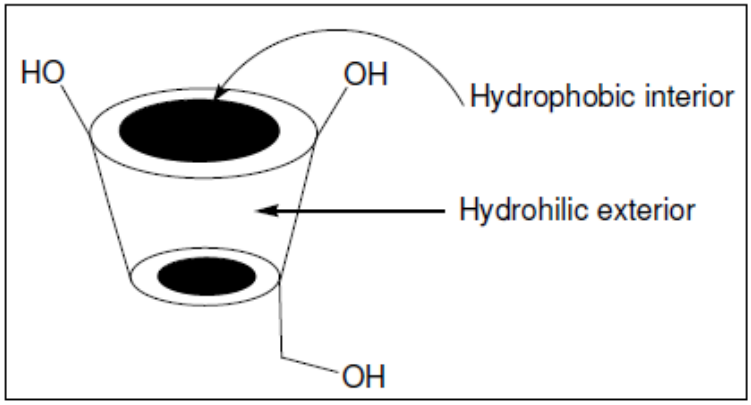


Figure 2.3 Hydrophobic interior and hydrophilic exterior of a CD (Del Valle, 2004)

CDs are frequently used as building blocks. Up to 20 substituents have been linked to β-cyclodextrin (β-CD) in a regioselective manner. The synthesis of uniform cyclodextrin derivatives requires regioselective reagents, optimisation of reaction conditions and a good separation of products. The most frequently studied reaction is an electrophilic attack at the OH-groups. The formation of ethers and esters by alkyl halides, epoxides, acyl derivatives, isocyanates, and by inorganic acid derivatives as sulphonic acid chloride cleavage of C-OH bonds has also been studied frequently, involving a nucleophilic attack by compounds such as azide ions, halide ions, thiols, thiourea, and amines; this requires activation of the oxygen atom by an electron-withdrawing group(Del Valle, 2004).

Because of their ability to link covalently or noncovalently specifically to other CDs, CDs can be used as building blocks for the construction of supramolecular complexes. Their ability to form inclusion complexes with organic host molecules offers possibilities to build supra molecular threads. In this way, molecular architectures

such as catenanes, rotaxanes, polyrotaxanes, and tubes, can be constructed. Such building blocks, which cannot be prepared by other methods can be employed, for example, for the separation of complex mixtures of molecules and enantiomers.

Each year CDs are the subject of almost 1000 research articles and scientific abstracts, large numbers of which deal with drugs and drug-related products. In addition, numerous inventions have been described which include CDs (over 1000 patents or patent applications in the past 5 years). From a regulatory standpoint, a monograph for β-CD is already available in both the US Pharmacopoeia/National Formulary (USP 23/NF 18, 1995 and the European Pharmacopoeia (3rd ed., 1997). A monograph for 2-hydroxypropyl-b-cyclodextrin is in the preparation for US Pharmacopoeia/National Formulary, and various monographs for CDs are included in compendial sources, e.g. the Handbook of Pharmaceutical Excipients (Wade & Weller, 1994). Thus, more than one century after their discovery CDs are finally, but rapidly, being accepted as 'new' pharmaceutical excipients.

2.3.2 Application of β-CD in inclusion complex

The most notable feature of CDs is their ability to form solid inclusion complexes (host–guest complexes) with a very wide range of solid, liquid and gaseous compounds by a molecular complexation (Del Valle, 2004). In these complexes, a guest molecule is held within the cavity of the CD host molecule. Complex formation is a dimensional fit between host cavity and guest molecule (Munoz-Botella, Del Castillo, & Martin, 1995). The lipophilic cavity of CD molecules provides a microenvironment into which appropriately sized non-polar moieties can enter to form inclusion complexes (Loftsson & Brewster, 1996). No covalent bond is broken or formed during the formation of the inclusion complex (Schneiderman & Stalcup, 2000). The main driving force of complex formation is the release of enthalpy-rich water molecules from the

cavity. Water molecules are displaced by more hydrophobic guest molecules present in the solution to attain an apolar–apolar association and decrease of cyclodextrin ring strain resulting in a more stable lower energy state (Del Valle, 2004).

CDs can form inclusion complexes with various compounds (guests) of low molecular weight (Mahlambi et al., 2010). The examples of the guest molecules include acids, apolar aliphatic, aromatic hydrocarbons and amines. The CD moiety harbours these small, suitably shaped organic compounds in its tubular cavities by shielding the bound species from the surrounding aqueous environment. This phenomenon is a result of the hydrophobic-hydrophobic interaction between the host CD and the organic species (N. Li et al., 2007).

The formation of inclusion complexes does not involve the formation of bonds but is an attraction between the host and guest as a result of their polarities (Chan et al., 2000). Because the lengths of the diameter of the CD vary, the organic species of compatible geometry must be able to fit (at least partly) into the CD cavity. The size of the organic compound and the type of the CD used is important for the formation of inclusion complexes.

The binding of guest molecules within the host CD is not fixed or permanent but rather is a dynamic equilibrium. Binding strength depends on how well the ‘host–guest’ complex fits together and on specific local interactions between surface atoms. Complexes can be formed either in solution or in the crystalline state and water is typically the solvent of choice. Inclusion complexation can be accomplished in a co-solvent system and in the presence of any non-aqueous solvent. CD architecture confers upon these molecules a wide range of chemical properties markedly different from those exhibited by non-cyclic carbohydrates in the same molecular weight range.

The purpose of functionalising CDs is to modify their physico-chemical properties and also to introduce groups with specific activity. The two most common types of CD substitution reactions are mono- and per-functionalisation reactions. Owing to the properties of β-CD and ILs, the functionalization of β-CD with IL has drawn our interest to prepare a new generation of material which may demonstrate interesting phenomena in extraction studies. To the best of our knowledge, the CD functionalized IL materials were widely used as chiral selectors in CE (T.-T. Ong, Wang, Muderawan, & Ng, 2008; T. T. Ong, Tang, Muderawan, Ng, & Chan, 2005; R.-Q. Wang, Ong, & Ng, 2008) and stationary phase in HPLC (Z. Zhou, Li, Chen, & Hao, 2010), while in CPE extraction it is still in the early stage. Hence, this study will serve as a preliminary work for the extraction of parabens in CPE method using modified CD as a modifier.

Herein, in this study of β-CD (CPE-DC193C-βCD) and β-CD functionalized IL (CPE-DC193C-βCD-IL) were used as a modifier in CPE system in order to determine parabens from water in a simple method, fast and efficient, using low cost experiments and contribute to green technology. The obtained results were compared with unmodified CPE-DC193C system. The developed methods will be tested in the extraction of parabens from water samples. Hence, the extraction mechanism was proposed with considering inclusion complex, hydrogen bonding and π-π interaction between β-CD and βCD-IL with the paraben molecules.

2.3.3 Application of β-CD as modifier

The usage of CDs as chiral and enantiomeric selectors for separation in CE has been reviewed many times (Morin-Crini & Crini), CDs are ideally suitable due to their well-documented ability to include in their cavity proper guest molecules. The unusual properties of CDs originate in their unique structure. (Qi et al., 2004)) reported the use of IL, 1-butyl-3-methylimidazolium tetrafluoroborate, [Bmim][BF_4] as running

electrolyte in CE with β-CD as modifier for the separation of anthraquinones extract of chinese herbs. These IL was selected because of their high conductivity and good solvating properties which were shown to improve the resolution of the analytes. The mechanism shows that the anthraquinones may associate with the imidazolium ions or with the β-CD as they may be entirely or partly embedded in the cavity of β-CD. So the association with the free imidazolium ion in the bulk solution is weaken. This association could be driven by hydrophobic, hydrogen bonding or by the ion-dipole interaction between the anthraquinones and the [Bmim][BF_4].

Kewen et al. (2011) dealt with the enantioseparation of phenylsuccinic acid (H_2A) enantiomers by liquid-liquid reactive extraction using β-CD derivatives as aqueous selectors. β-CD and its derivatives can interact with guest molecules selectively to form complexes with different stability. Kewen et al. (2011) concluded that the efficiency of the extraction depends strongly on the number of process variables including the type of organic solvents and β-CD derivatives, the concentrations of the extractants and H_2A enantiomers, pH and temperature.

Zeng et al. (2012) developed a simple method using HPLC with isocratic elution employing CDs as mobile phase additives. Various factors affecting the retention of isoflavonoids in the C_{18} reversed-phase column, such as the nature of CDs, the concentration of hydroxypropyl-β-cyclodextrin (HP-βCD) and the methanol percentage in the mobile phase were studied. The formation of the inclusion complexes between isoflavonoids and HP-β-CD explained the modification of the retention of analytes. The apparent formation of constants determined by HPLC confirmed that the stoichiometry of HP-βCD-isoflavonoid complexes was 1:1, and the stability of the complexes depended on the size and property of isoflavonoids. The optimized method was successfully applied for the simultaneous determination of major isoflavonoids in the analysis of traditional chinese herbs.

The α-CD and β-CD as well as their four methyl ether derivatives were investigated by Gubica, Pełka, Pałka, Temeriusz, and Kańska (2011) as the additives in the course of enzymatic decomposition of L-phenylalanine catalyzed by phenylalanine ammonia-lyase. In most cases CDs caused mixed inhibition, both competitive and noncompetitive, but they also acted as activators for selected concentrations. The inhibitory effect of CDs is connected with the decrease of substrate concentration and unfavourable influence on the flexibility of the enzyme molecules. All these effects are caused by the ability of the CDs to form inclusion complexes.

2.4 Paraben

2.4.1 Properties of paraben

Parabens (alkyl-p-hydroxybenzoates) are antimicrobial preservatives used in cosmetics, toiletries, pharmaceuticals, and food (Brausch & Rand, 2011). There are currently seven different types of parabens in use (benzyl, butyl, ethyl, isobutyl, isopropyl, methyl, and propyl). In 1987 over 7000 kg of parabens were used in cosmetics and toiletries alone (Soni, Carabin, & Burdock, 2005) and that number has been expected to increase over the last 20 years. Methyl- and propylparaben are the most commonly used in cosmetics and are typically co-applied to increase preservative effects (Peck, 2006).

These consumer products are used daily in various human activities. Therefore, due to their continuous release through recreational waters (liberation from the skin in swimming pools, spas, resorts, etc.) and domestic urban and industrial wastewaters, parabens might reach the aquatic media and particularly potable water sources (Blanco, Casais, Mejuto, & Cela, 2009). For this reason, starting from a few years ago the existence and transportation pathways of these compounds into the aquatic ecosystems

have been receiving great attention (Baker & Kasprzyk-Hordern, 2011; Kasprzyk-Hordern, Dinsdale, & Guwy, 2008). A growing concern and interest have arisen in relation to their potential long-term effects not only on humans but also on aquatic organisms.

2.4.2 Effect of paraben to human body

Although the acute toxicity of paraben is very low, these compounds are also referred to as endocrine-disrupting chemicals, as they all exhibit weak estrogenic activity (Blanco et al., 2009). Moreover, a relation has been proposed between the prolonged dermal exposure to paraben-containing products, such as underarm deodorants and human breast cancer (Barr et al., 2012; Khanna & Darbre, 2012) . Due to their absorption from topical application and ingestion of diverse consumer products, parabens have been detected in human breast tissue, milk, urine and serum (Canosa, Rodríguez, Rubí, & Cela, 2007; Harvey & Everett, 2012).

Studies have shown that parabens accumulate in the human body tissue over time, since they enter the bloodstream once applied to the skin. We also know that when any chemical is absorbed into the bloodstream rather than being taken orally, the human body can receive up to ten times higher concentration of that chemical (Soni, Taylor, Greenberg, & Burdock, 2002). Parabens have been shown to cause allergic reactions, such as rashes, particularly in those with sensitive skin. However, parabens have been linked to much more severe outcomes in human body, including endocrine disruptions, which contribute to certain types of cancers, the early onset of puberty, and decreased sperm production in males.

Parabens have been shown to mimic the body's naturally-occurring hormones, such as estrogen and testosterone, through the application of paraben-containing products such as a skin care cream that is consistently rubbed onto your body. The result

is an endocrine disruption, which interferes with the normal functioning of your body's endocrine system (Shanmugam, Ramaswamy, Radhakrishnan, & Tao, 2010). A rather disturbing finding regarding parabens is their correlation to the development of breast cancer in women. It has been found that low concentrations of parabens are present in breast cancer tumors, sparking controversy about the relationship between the use of paraben-containing products and the development of certain types of cancers (Shanmugam et al., 2010). It is a well-known fact that estrogen plays a role in breast cancer, and therefore a chemical ingredient which mimics estrogen (paraben) has recently come under careful scrutiny in order to determine its role in the development and progression of breast cancer.

2.4.3 Effect of paraben to our environmental

To date only a handful of studies has examined paraben concentrations in surface water. Greatest concentrations of parabens have been identified in surface water with concentrations ranging from 15 to 400 ngL^{-1} depending on paraben, whereas effluent had lower concentrations ranging from 50 to 85 ngL^{-1} (Jonkers et al., 2010). Of the seven different types of parabens currently in use, benzylparaben appears the most acutely toxic (Bazin, Gadal, Touraud, & Roig, 2010). Methyl- and ethylparaben appear to be least acutely toxic with LC50 values approximately 3 times greater than benzyl paraben for all trophic groups studied (Bazin et al., 2010).

A number of studies were conducted on investigation of persistence and partitioning of parabens in water samples. This is because some residues may well get into water after discharge from industrial effluents or pharmaceuticals and cosmetic products. Four parabens were detected to be as high as 3.3 μgL^{-1} in a Japanese river, while n-butyl paraben was detected at 0.01-0.26 μgL^{-1} in the effluent of Canadian and Spanish wastewater treatment plants (Yamamoto et al., 2007). Many cosmetics and

PPCPs such as bath gels, shampoos, deodorants, antiperspirants, skin and body creams, tanning lotions and toothpaste, among others might contribute to the direct introduction of parabens in the environment.

It has previously been reported that increasing the chain length of parabens' substituents can increase paraben acute toxicity to bacteria and this appears to be true for other trophic groups as well (Brausch & Rand, 2011). There is currently a lack of information on the chronic effects of parabens upon aquatic organisms with only a single known study examined toxicity in D. magna and Pimephales promelas (Dobbins, Usenko, Brain, & Brooks, 2009). These authors found that benzyl- and butylparaben were most toxic to invertebrates and fish whereas methyl- and ethylparaben appeared least toxic. This corresponds directly with the results of acute studies, as well as previous studies indicating the increased chain length of parabens increases toxicity. In addition to increasing the chain length, chlorination also substantially increases the toxicity of parabens to both bacteria and D. magna (Terasaki, Makino, & Tatarazako, 2009).

Based on limited environmental concentration and toxicity data, it appears benzyl-, butyl- and propylparaben could potentially cause adverse effects upon aquatic organisms. Dobbins et al. (2009) concluded parabens only pose limited hazard to aquatic organisms; however, parabens, specifically benzyl-, butyl- and propylparaben, can elicit low-level estrogenic responses. Therefore, low level exposure to parabens could potentially cause estrogenic effects upon environmentally relevant concentrations. Golden, Gandy, and Vollmer (2005) reviewed paraben's endocrine activity in rats and determined butyl-, isobutyl-, and benzylparaben demonstrate estrogenic activity although their potency is much less than estrogen itself. These results indicate that there are potential affects on aquatic organisms continually exposed to parabens. Preliminary data on environmental concentrations, however, suggest only minimal risk to aquatic

organisms as effect concentrations are generally 1000 times higher than what has been observed in surface water (Golden et al., 2005).

2.5 Theory in CPE

The phase volume ratio is defined as the ratio of the volume of the surfactant-rich phase to that of the water phase, where the volumes of the two phases were measured using graduated centrifuged tubes,

$$R_v = V_s / V_w \tag{2.1}$$

where V_s (mL) and V_w (mL) are the volume of the surfactant-rich phase and the aqueous phase, respectively (J.-L. Li & Chen, 2003).

The preconcentration factor is defined as

$$C_F = \frac{C_s}{C_o} \tag{2.2}$$

where C_s (mg/L) is the paraben concentration in the surfactant-rich phase after phase separation and C_o (mg/L) is the initial paraben concentration in the bulk solution before phase separation (J.-L. Li & Chen, 2003).

The distribution coefficient is defined as

$$K_d = \frac{C_s}{C_a} \tag{2.3}$$

where C_a referred to the paraben concentration in aqueous phase (J.-L. Li & Chen, 2003).

The percentage extraction recovery of paraben, *%R* can be characterized as the percentage of paraben extracted from bulk solution into the surfactant-rich phase

$$\%R = \frac{C_s V_s}{C_o V_t} \times 100\% \qquad (2.4)$$

where V_t is the total volume of the solution (J.-L. Li & Chen, 2003).

CHAPTER 3: Ionic Liquid Based Aqueous Two-phase System (IL-ATPS) and Ionic Liquid Based Aqueous Two-Phase System with β-cyclodextrin as a Modifier (IL-βCD-ATPS)

3.1 Introduction

Aqueous two-phase systems (ATPS) have been widely used with ionic liquids (ILs) in separation science (J. Chen et al., 2005; Pan et al., 2002; Willauer et al., 2002). Typical aqueous two-phase system (ATPS) is generated by mixing aqueous solutions with two structurally different polymers, mixing one polymer with certain salts or mixing two surfactants. However, most of phase-formation polymers in conventional ATPS form an opaque solution with high viscidity during phase separation, which might make the following determination difficult. Due to that, room temperature ionic liquid (RTILs) has received attention for their potential use as green solvent in ATPS and possible replacement to the traditional organic solvent (J. Chen et al., 2004; Pei et al., 2007; Rahim, Mohamad, Alias, & Sarih, 2012). Several study reported that hydrophilic ionic liquid form ATPS when contacting with the concentrated solutions of water-structuring salts. Ionic liquid received a great exposure in ATPS because it has many advantages due to its unique characters (S. Li, He, Liu, Li, & Liu, 2005).

In this chapter, the study was carried out to optimize two methods which are ionic liquid [C_4mim][Cl] based aqueous two-phase extraction (IL-ATPS) and ionic liquid based aqueous two-phase extraction with the presence of β-CD. The optimization parameters for the developed methods of IL-ATPS and IL-βCD-ATPS will be

discussed. The investigation on the performance of β-CD as a modifier in the IL-βCD-ATPS method will be compared with the IL-ATPS method. Then, the optimum condition was applied in real water samples. In order to investigate the mechanism of extraction techniques, inclusion complex method was carried out to support the obtained results.

3.2 Experimental

3.2.1 Reagents and standards

Ionic liquid (1-butyl-3-methylimidazolium chloride), [C_4mim][Cl] (99.5%) was purchased from Merck (Germany). B-cyclodextrin (β-CD), potassium phosphate tribasic anhydrous, K_3PO_4, potassium carbonate, K_2CO_3, potassium hydroxide, KOH, dipotassium hydrogen phosphate, K_2HPO_4 were purchased from Acros Organics (USA). Methylparaben (MeP), ethylparaben (EtP), propylparaben (PrP) and benzylparaben (ArP) were purchased from Sigma Aldrich (Germany). Acetonitrile (HPLC grade) was purchased from Merck (Germany). Stock solutions of parabens at a concentration of 1000 mg/L each were prepared in acetonitrile. Working standard solutions were prepared by step-wise dilution with deionized water of the stock solutions. The pH of the samples solution was adjusted with diluted hydrochloric acid or diluted sodium hydroxide solutions.

3.2.2 Instrumentation

The separation and quantification of the tested parabens were carried out on Shimadzu HPLC system consisting of a pump, degasser, auto injector, column oven, ultraviolet detector, guard column, Chromolith C_{18} column (100 mm x 4.6 mm, Merck, Germany). HPLC gradient conditions were used to separate the analytes using

acetonitrile and deionized water, flow rate of 0.7 mL/min and detection at 254 nm. The gradient elution was performed as follows: 30% acetonitrile (0-2 min), ramped to 40% acetonitrile (2-5 minutes) and then ramped to 30% acetonitrile (5-8 min).

3.2.3 Preparation of phase diagrams

A series of salt solution (K_3PO_4, K_2CO_3, KOH and K_2HPO_4) were tested for the formation of ATPS with 1.0 g ionic liquid [C_4mim][Cl]. The known concentration of salt was added dropwise to the test tube until the solution became turbid and a two-phase system was formed. The composition of this mixture was noted. Then, water was added dropwise to the test tube to get a clear one-phase system and more salt solution was added again to afford a two-phase system. The molalities of salt and ionic liquid were calculated according to equation 3.1 and 3.2 to plot a graph between molality of ionic liquid and molality of salt.

$$Molality\ \ of\ \ IL = \frac{number\ of\ \ moles\ \ IL}{weight\ \ of\ \ water\ \ in\ \ kg} \qquad (3.1)$$

$$Molality\ \ of\ \ salt = \frac{number\ of\ \ moles\ \ of\ \ salt}{weight\ \ of\ \ water\ \ in\ \ kg} \qquad (3.2)$$

3.2.4 Preparation of ionic liquid aqueous two-phase system (IL-ATPS)

1.0 mL of 5% (w/v) [C_4mim][Cl] solution, 1.0 mL of the standard solution of parabens and 0.5 mL of 1.5 M K_3PO_4 was added into 15 mL centrifugal tube. The pH was adjusted with dilute acid or dilute alkaline solution until the desired pH was obtained. The mixture was then sonicated to ensure the solution was thoroughly mixed, and two phases were formed in about two minutes. The top layer of the phase was separated and put into vials and a 20 µl portion was directly injected into the HPLC system for analysis.

3.2.5 Preparation of ionic liquid aqueous two-phase system with β-CD as a modifier (IL-βCD-ATPS)

1.0 mL of 5% (w/v) [C_4mim][Cl] solution, 1.0 mL of the standard solution of parabens, 1.0 mL of β-CD (10 ppm) and 0.5 mL of 1.5 M K_3PO_4 were added into a 15 mL centrifugal tube. The pH was adjusted with dilute acid or dilute alkaline solution until the desired pH was obtained. The mixture was sonicated to ensure the solution was thoroughly mixed until two phases were formed in about two minutes. The top layer of the phase was separated into vials and a 20 µl portion was directly injected into the HPLC system for analysis.

3.2.6 Determination of parabens in real samples using IL-ATPS and IL-βCD-ATPS techniques

Tap water samples were collected from the laboratory, University of Malaya, Malaysia. River water samples were collected from Bahau, Negeri Sembilan Malaysia (geographical coordinate 3˚1'44"N 102˚22'1"E), while treated water samples were collected from a wastewater treatment plant in Kuala Lumpur, Malaysia (geographical coordinate 3˚7'25"N 101˚39'12"E) and sea water samples were collected from Perak, Malaysia (geographical coordinate 4˚13'0"N 100˚34'0"E). All the water samples were filtered by using a 0.45 µm nylon membrane filter to remove suspended particulate matter and then stored at 4˚C in the dark. Then, 1.0 mL of water samples was added into centrifugal tube for ATPS preparation (See Section 3.2.4 and Section 3.2.5).

3.2.7 Optimization of Parameters for Paraben Extraction

3.2.7.1 Effect of salt concentration

A series of anhydrous potassium phosphate tribasic, K_3PO_4 solution (0.5 M, 1.0 M, 1.5 M and 2.0 M) were prepared with other parameter kept constant.

3.2.7.2 Effect of ionic liquid concentration

A series of ionic liquid (1-butyl-3-methylimidazolium chloride), [C_4mim][Cl] were prepared at various concentrations (5% (w/v) to 60% (w/v)) using deionized water with other parameter kept constant.

3.2.7.3 Effect of pH

A series of sample at different pH (2, 4, 7, 8, 9, 10, 11 and 12) was adjusted with dilute acid or dilute alkaline solution to get the desired pH solution with other parameter kept constant.

3.2.7.4 Effect of extracting temperature

The extraction of paraben was carried out in a water bath in order to ensure the desired temperatures (30˚C to 60˚C) were achieved with other parameter kept constant.

3.2.7.5 Study on water content in IL-rich phase

To measure the water content, the ionic liquid-rich phase was dried at 75˚C until no loss of mass was observed. The drying method was used in this research because this technique is easy, simple and fast to measure the water content in IL-rich phase. The accuracy of this method is within ±0.5% (J.-L. Li & Chen, 2003). The volume of the water content was obtained by the weight difference of the ionic liquid-rich phase before and after drying. All the data given were the average of triplicate measurements. The experiments were repeated similar as above method with addition of 1.0 mL of β-CD (10 ppm) for IL- βCD-ATPS method.

3.2.7.6 Effect of phase volume ratio

A series of IL concentrations (5% (w/v) to 60% (w/v)) was studied in order to get the optimum phase ratio by calculating the volume of ionic liquid rich phase to the volume of aqueous phase with other parameter kept constant.

3.2.8 Preparation and characterization of inclusion complex of β-CD, IL and ArP

In order to investigate the mechanism of extraction between β-CD, IL and ArP, the inclusion complex method was carried out to support the obtained results. The inclusion complex of β-CD with ionic liquid [C_4mim][Cl] and ArP was prepared using the conventional kneading method. Equimolar amounts of βCD, [C_4mim][Cl] and ArP were kneaded with mortar and pestle in minimal ethanol to form a homogeneous paste. The complex was kneaded for approximately 30 minutes and dried to constant mass. After drying, a white powder (βCD-IL-ArP complex) was obtained. ^{1}H NMR and 2D NOESY spectra were recorded on AVN 600 MHz and DMSO-d6 used as solvent.

3.3 Results and discussion

3.3.1 Equilibrium phase diagram

Phase diagrams are used to characterize the phase systems and to select the suitable salt for phase forming. Herein different salts are tested for the formations of the ATPS with [C_4mim][Cl] as shown in Figure 3.1. Among the salts that can cause phase separation, four types are chosen to determine the phase diagrams of IL-salt-water system. The tendency of salts to form ATPS in the mixtures with [C_4mim][Cl] is related to the cations and anions of the salts, which implies the phase forming [C_4mim][Cl]-salt-two-phase system.

As shown in the results, the abilities of the salts studied for phase separation followed the order of K_3PO_4 > K_2CO_3 > K_2HPO_4 > KOH, in accordance with the salting-out ability of anion. Here, K_3PO_4 was then chosen for the following studies because K_3PO_4 led to an effective phase isolation between [C_4mim][Cl] and salt enriched solutions. Another factor is that K_3PO_4 shows the lowest molality ratio and has a good phase separation.

The phosphate ion, PO_4^{3-} shows the lowest value in phase diagram compared to the other salts because the triple charge and the ability of PO_4^{3-} ion to act as hydrogen acceptor towards water, and hence certainly has an important contribution in extensive formation of complexes with water (Mourão, Cláudio, Boal-Palheiros, Freire, & Coutinho, 2012). The competition for water molecules will lead to the hydration of IL ions which releases water molecules and increases of the surface tension of the IL. Therefore, PO_4^{3-} is a strong salting-out species and leads to the liquid-liquid demixing of a wide range of aqueous two-phase extraction(Mourão et al., 2012).

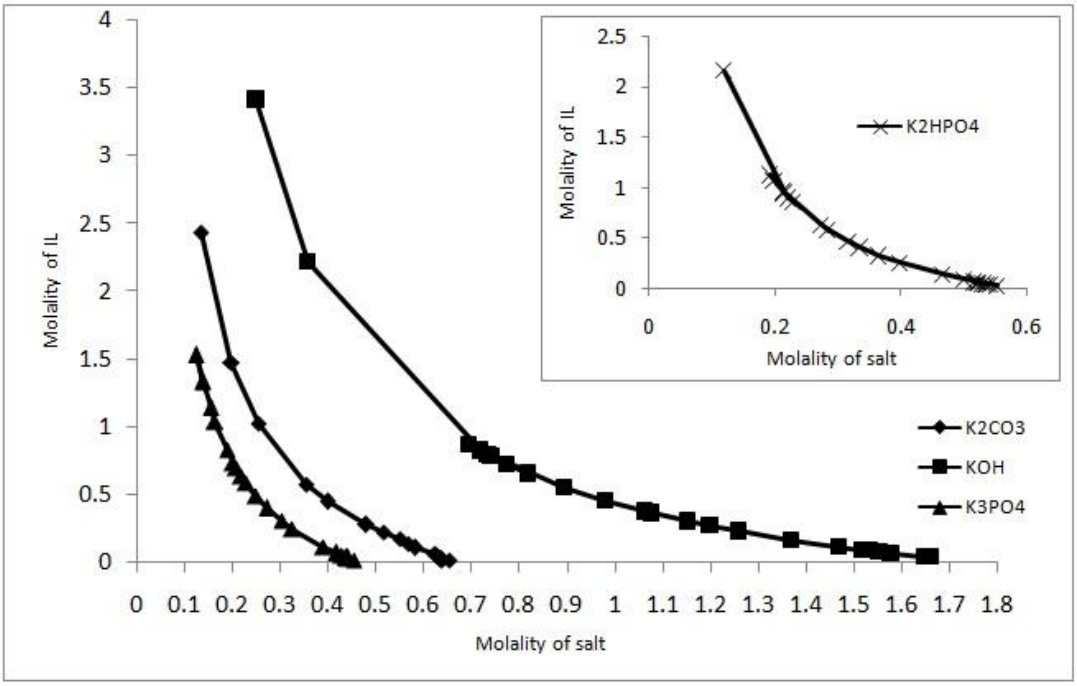


Figure 3.1 Phase diagrams for the [C_4mim][Cl]/salt/water systems at room temperature

The Results show that ATPS can only be formed by adding appropriate amount of KOH, K_3PO_4, K_2HPO_4 and K_2CO_3 to aqueous [C_4mim][Cl], while other salts i.e. KCl, NaCl and K_2SO_4 cannot cause phase separation. According to Gutowski et al. (2003), this phenomenon is probably a solvophobic one. The kosmotropic ions, i.e. HPO_4^{2-}, SO_4^{2-}, OH^-, CO_3^{2-} and PO_4^{3-} which exhibit stronger interaction with water molecule than that between water molecules are beneficial to the ATPS formation (Z. Li et al., 2010). However, the chaotropic ions i.e. Cl^-, NH_4^+, K^+, $H_2PO_4^-$ have the opposite

effect because of their weaker interactions with water. It is therefore easier to form ATPSs by adding kosmotropic salts rather than chaotropic salts. The salting-out ability may also be related to the Gibbs energy of hydration of the ions (Z. Li et al., 2010; X. Xie et al., 2011). The more negative the value of Gibbs energy of hydration, the better effect of salting-out of the ions. Based on Table 3.1 and Table 3.2, although PO_4^{3-} has its solubility the lowest in water their Gibbs energy of hydration value is the highest compared with other salt studied. Thus, K_3PO_4 was chosen in this study based on the ease of forming phase separation in the water system.

Table 3.1 Solubility of studied salts in the water

Name of salts	Solubility of salt in the water (g/L)
KOH	112
K_2CO_3	109
K_2SO_4	111
K_2HPO_4	150
K_3PO_4	92.3

Table 3.2 The Gibbs energy of hydration for selected anions and cations

Name of anion / cation	Gibbs energy of hydration, ΔG_{hyd} (kJ/mol)
OH^-	-430
HPO_4^{2-}	-1125
CO_3^{2-}	-1315
PO_4^{3-}	-2765
K^+	-295

3.3.2 Effect of salts concentrations on the recoveries of parabens

Ionic strength is often an important factor for the extraction and the enrichment performance of phase separation. The effect of ionic strength on the salting out was surveyed by adding different salt concentrations to the ATPS solution. In this experiment, concentrations of K_3PO_4 were optimized in the range of 0.5 M to 2.0 M for IL-ATPS and IL-βCD-ATPS. As can be seen from the Figure 3.2, the percent recovery of the all studied analytes for both compositions increased when the salt concentrations were increased and attained optimum value at 1.5 M.

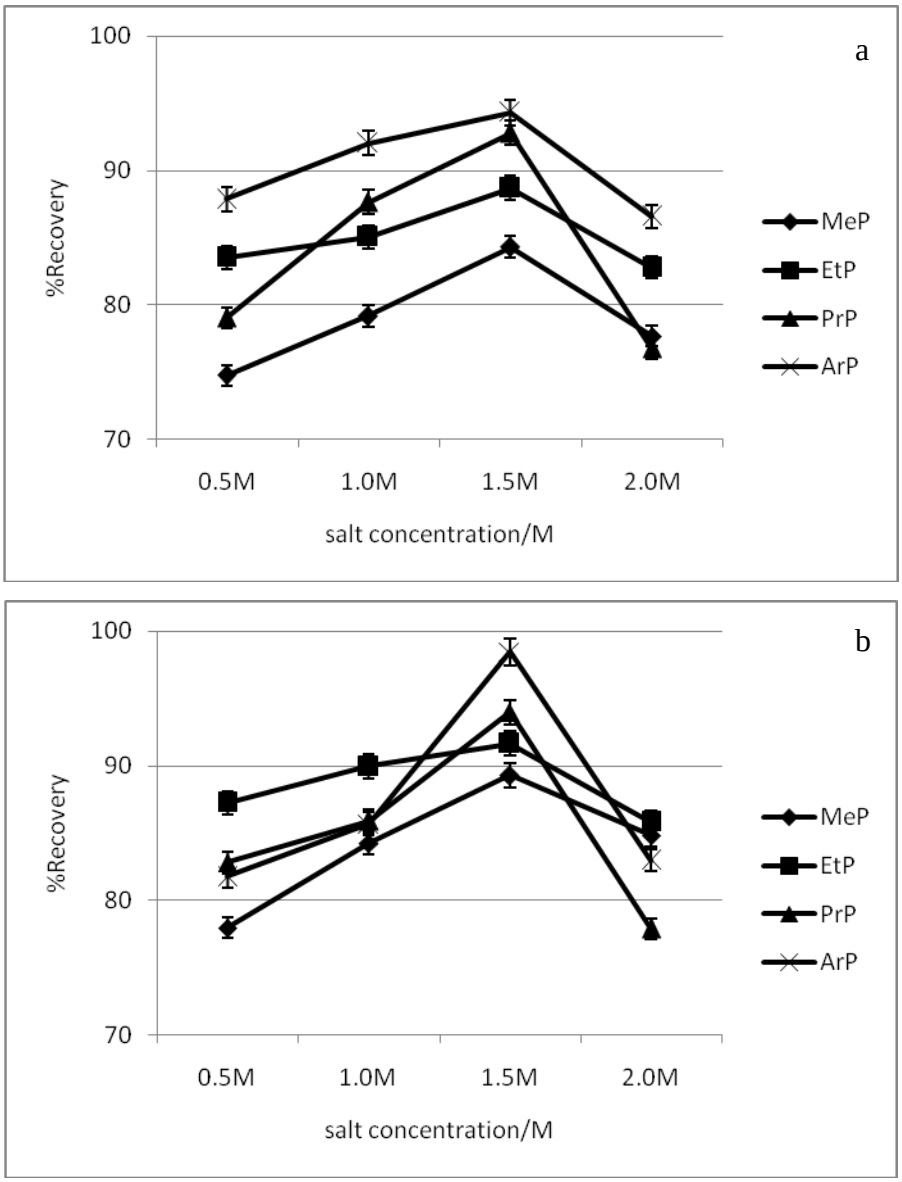


Figure 3.2 Effect of salts concentration on percentage recoveries of parabens using (a) IL-ATPS and (b) IL-βCD-ATPS

The percentage recoveries decreased dramatically at salt concentration of 2.0 M for both methods. In addition, the phase separation is difficult to form when the salt concentration of 2.0 M was used. It is because at high concentration of salt precipitates appeared at the bottom of the centrifuge tube. Therefore, 1.5 M salt concentration was selected for the following experiments for both studies.

The amount of salt is important to induce the formation of phase but the excess of salt would interfere the phase forming ability and hence the extraction process is reduced. Accordingly, the percentage recoveries of extraction also decreased. Some studies reported that the amount of salt used in the system produces constant or lower percentage recoveries of extraction because the salting-out effect has reached a maximum degree, so it was not able to improve the partition coefficient of analyte studied (J. Han et al., 2010).

3.3.3 Effect of ionic liquid concentrations on the recoveries of parabens

The effect of the concentrations of the added ionic liquid in the system on the extraction recoveries of parabens has been investigated for IL-ATPS and IL-βCD-ATPS. The results are illustrated in Figure 3.3. In this study, we focused on the ionic liquid concentration from 5% (w/v) to 60% (w/v) in order to evaluate the percentage recoveries of parabens extraction by both methods. Apparently both methods show similar trend that is when the IL concentration is increased the percentage recovery of parabens also increased. From the graph, it is clear that IL-βCD-ATPS method gives better percentage recoveries for all the studied parabens compared to IL-ATPS method.

IL-ATPS method shows that when 5% (w/v) IL concentrations was used, the percentage recoveries were low (below 70%) for all the studied parabens. This low recovery was continuously obtained until the IL concentration was increased to 20% (w/v). The percentage recoveries were achieved more than 80% for all the studied

parabens when 30% (w/v) IL concentration was used. Then, the percentage recoveries for all the studied parabens increased dramatically achieving more than 90% recoveries at 60% (w/v) IL concentration.

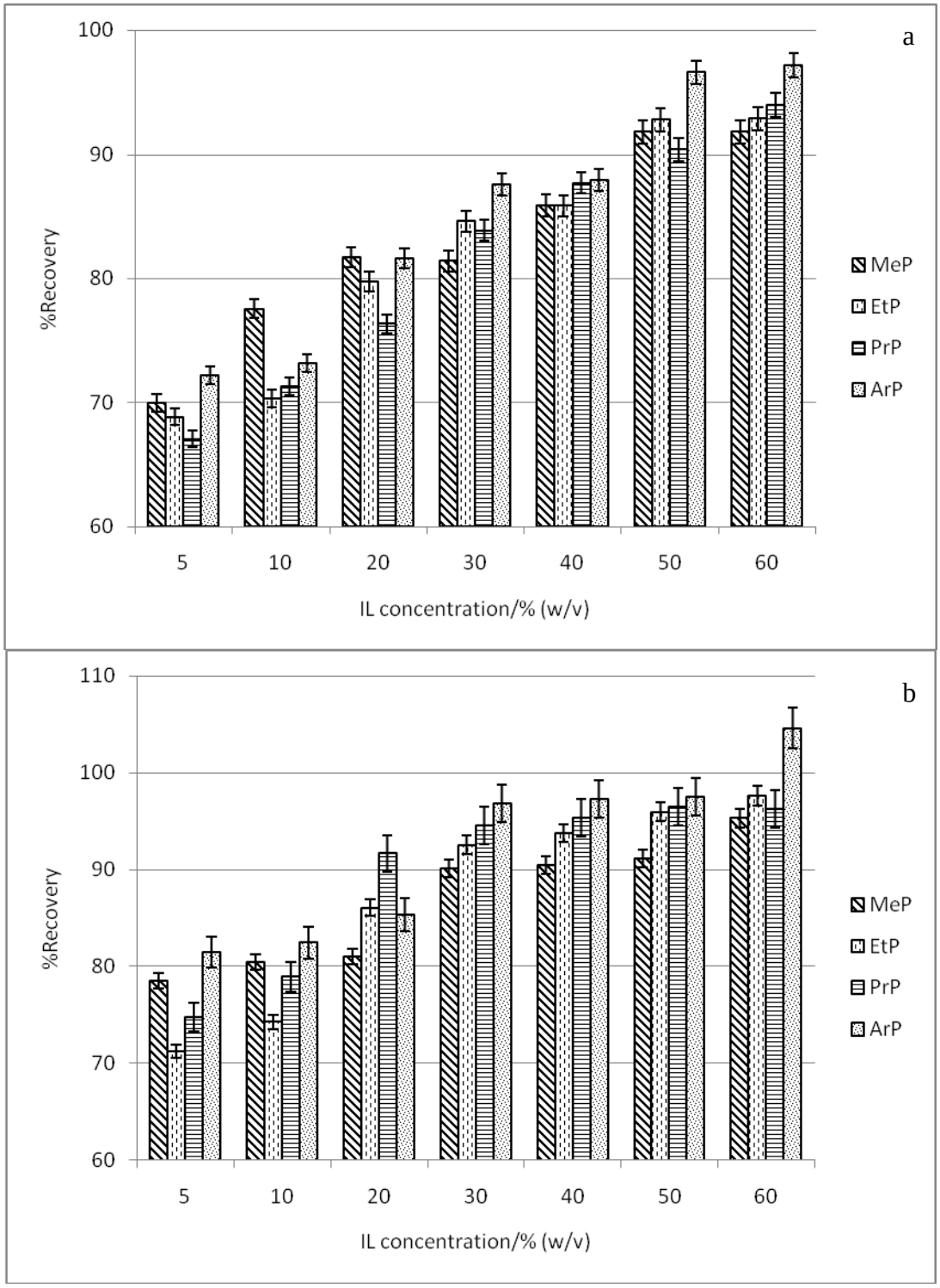


Figure 3.3 Effect of ionic liquid concentrations on percentage recoveries of parabens extraction using (a) IL-ATPS and (b) IL-βCD-ATPS.

For IL-βCD-ATPS, the percentage recoveries for all parabens increased until it reached 30% (w/v) IL concentration. Then, the percentage recoveries became constant until it reached IL concentration of 60% (w/v). All the studied parabens had achieved more than 90% recoveries at 30% (w/v) IL concentration for the IL-βCD-ATPS while the IL-ATPS method showed 90% recoveries at 50% (w/v) IL concentration.

IL concentration has an important influence on the extraction recoveries of parabens. Therefore, 30% (w/v) IL concentration has been selected for both methods for the following studies because at this concentration, all the studied parabens attained equilibrium with higher percentage recoveries. This is because in both methods, the amount of hydrophilic ionic liquid is getting higher in the solution and the solute-solute interaction between ionic liquid itself has a greater contribution than the solute-water interaction. Therefore, molecule of ionic liquid would be more favourable to interact with the same ionic liquid rather than interact with the water molecules resulting in higher amount of IL in the IL-rich phase. Thus, higher percentages of parabens are extracted in the IL-rich phase. The higher recoveries obtained for IL-βCD-ATPS are due the presence of β-CD in the system making the formation of phase easier compared to the IL-ATPS method.

3.3.4 Effect of pH on the recoveries of parabens

As generally known, compounds exist as different states in a different pH environment and the sample enrichment is related directly to the present state of the compounds in most cases (Q. Zhou, Bai, Xie, & Xiao, 2008). In proposing a new extraction procedure, the pH of sample solution would give an important impact on the preconcentration of analytes, in this case parabens. The effect of sample pH to the recoveries of paraben was optimized over the range of 2-12. The results are exhibited in Figure 3.4.

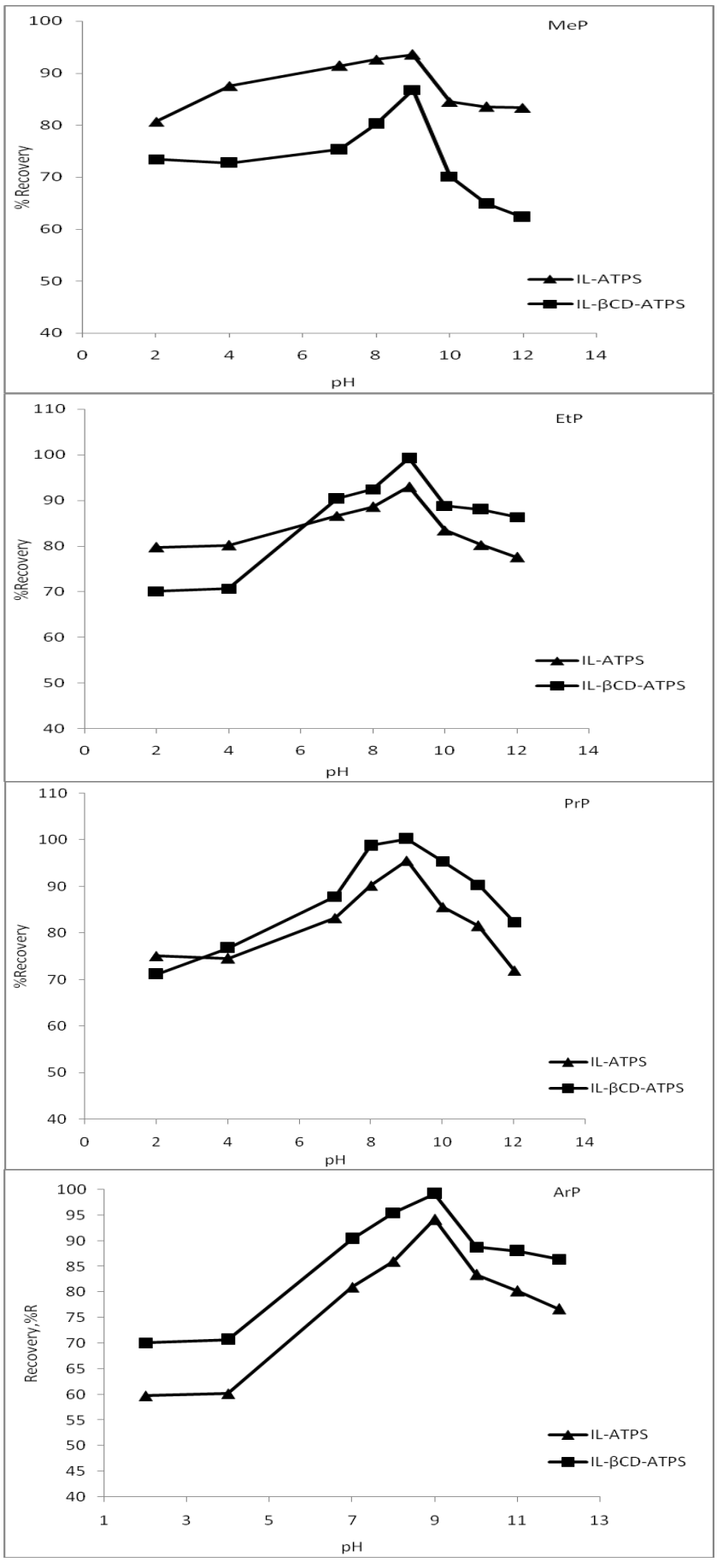


Figure 3.4 Effect of pH on percentage recoveries of paraben extraction using IL-ATPS and IL-βCD-ATPS

It was found that both methods show that the extraction performance reached the highest level at pH 9. There are significant differences between the percentage recovery for parabens extracted by IL-ATPS and IL-βCD-ATPS methods. However, the trend of pH shows an insignificant difference by both methods. The different in the extraction recoveries at various pH can be explained by considering the change in the charge of the paraben. At below pH 3, paraben is present in protonated form. The extraction recovery of paraben in this form is low because of the electrostatic repulsion between protonated paraben and the positive charge from imidazolium group of ionic liquid. While at pH 4-6.5, paraben exists mainly in neutral form. There is a slight increase in the extraction recovery of paraben in this region as paraben loses its net positive charge due to the deprotonation of the parabens hydroxyl group.

At pH values greater than 7 until pH 9, paraben exists mostly in a negatively charged form because the hydroxyl group is now fully deprotonated. In this region, percentage recovery is increased dramatically and the maximum percentage recovery is achieved at pH 9. The percentage recovery is increased because of the electrostatic attraction between deprotonated paraben and the positive charge of imidazolium ion of ionic liquid.

When the pH was adjusted to pH 10, the percentage recovery decreased. The decrease in percentage recoveries continued until pH 12. This is because of above pH 8, alkaline hydrolysis of parabens had taken place, leading to the formation of the corresponding alcohol and hydroxybenzoic acid (Angelov, Vlasenko, & Tashkov, 2007). This might be the reason for the decrease of the percentage recoveries of parabens starting from pH 10.

3.3.5 Effect of extracting temperature on the recoveries of parabens

The experiments on the optimization of temperature were carried out in order to investigate the importance of temperature on the efficiency of the developed method, IL-ATPS and IL-βCD-ATPS. The obtained results are depicted in Figure 3.5. A previous study (X. Xie et al., 2011) had reported that an increase in temperature would affect the transfer of water from the bottom phase to upper phase. Therefore, the IL-rich phase concentration increases in the upper phase (X. Xie et al., 2011). As a result, higher concentration of analyte will be extracted.

In this experiment, the temperature was maintained between the desired temperatures 30-60˚C using a ultrasonic waterbath with temperature controller. The results show that temperature is less pronounced on the paraben extraction using IL-ATPS and IL-βCD-ATPS. Within 30-60˚C, the extraction recoveries of parabens fell in the narrow range of 87-93.5% for IL-ATPS indicating that temperature had no significant influence on the distribution behavior of parabens. When the temperature increment exceeded 60˚C, the phase separation was difficult to form under these circumstances and this is due to the capability of phase separation of ATPS which is decreasing with increasing the temperatures (C.-X. Li et al., 2009).

IL-βCD-ATPS method shows that temperature has little influence on the recovery where there were slightly increased at 30˚C to 60˚C with the value of 88-97%. These results proved that temperature does not affect the dehydration of the micelle and the volume of IL-rich phase indicating that IL can be dehydrated at room temperature. Therefore, the volume ratio and preconcentration factor also are not enhanced dramatically by varying the temperature.

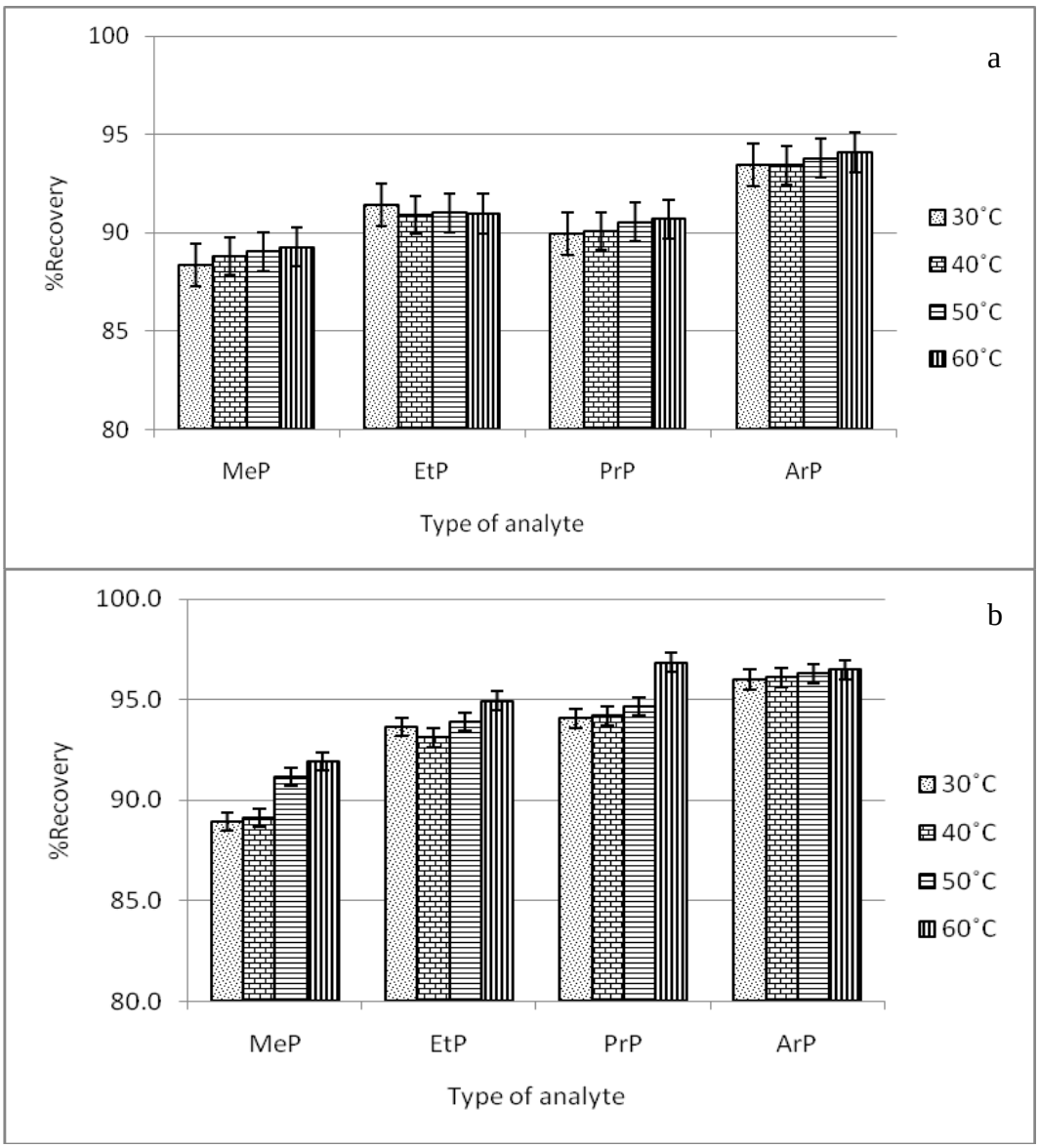


Figure 3.5 Effect of temperature on percentage recovery of parabens using (a) IL-ATPS and (b) IL-βCD-ATPS method

The study on the effect of temperature has been done by Pei et al. (2007). They demonstrated that the binodal curve was not sensitive to increasing the temperature. A possible reason is that the hydration of ionic liquid is not sensitive to temperature. Similar results have been reported by He, Li, Liu, Li, and Liu (2005) indicating that temperature had no significant influence on the distribution behavior of steroids. Thus, this method provides a relatively wide range of temperature that may be used to study on the extraction behavior of parabens. Therefore, we conducted the experiment using 30˚C for the following studies. This lower temperature is important to ensure the

efficient separation of phases is achieved. It is better and efficient/economical as well as the green extraction can be performed successfully at a lower temperature.

3.3.6 Effect of water content in IL-rich phase

Some water will always remain in the IL-rich phase after separation. The water content in the layer of surfactant-rich phase, W_s, is commonly around 80% (J.-L. Li & Chen, 2003; Yao & Yang, 2008). High W_s will limit the performance of extraction method to a large extent and causes difficulty to further increase of the preconcentration factor, C_F, or distribution coefficient, K_d.

Based on Figure 3.6, ArP lost about 50 % (w/v) water content in IL-βCD-ATPS compared to IL-ATPS where ArP lost only 43 %(w/v) water content when the ionic liquid concentration increased. The use of β-CD showed the highest lost of water because β-CD has the ability to form inclusion complex with the paraben as shown in Figure 3.126. It is because β-CD has the hydroxyl groups at the outer surface of the molecule, with the primary hydroxyls at the narrow side and secondary hydroxyls at the wider side, hence makes β-CD as water soluble but simultaneously generates an inner cavity that is relatively hydrophobic. Thus, β-CD can either partially or entirely accommodate suitable size of molecules that are hydrophobic. Then, the deprotonated ArP had electrostatic interaction with the positive charge of the ionic liquid $[C_4mim]^+$ at the outside space of the β-CD. This mechanism has been confirmed using 1H NMR spectra in Section 3.3.10.

Both methods show decreasing trend in water content when the ionic liquid concentration is increased (Figure 3.6). This is because when the amount of hydrophilic ionic liquid is getting higher in the solution, the solute-solute interaction between ionic liquid itself has a greater contribution than the solute-water interaction. Therefore, molecule of ionic liquid would be more favourable to interact with the same ionic liquid

rather than interact with the water molecules resulting in less amount of water can be detected in the IL-rich phase. This interaction has been verified by some thermodynamic studies (Gardas et al., 2008; Sadeghi et al., 2012).

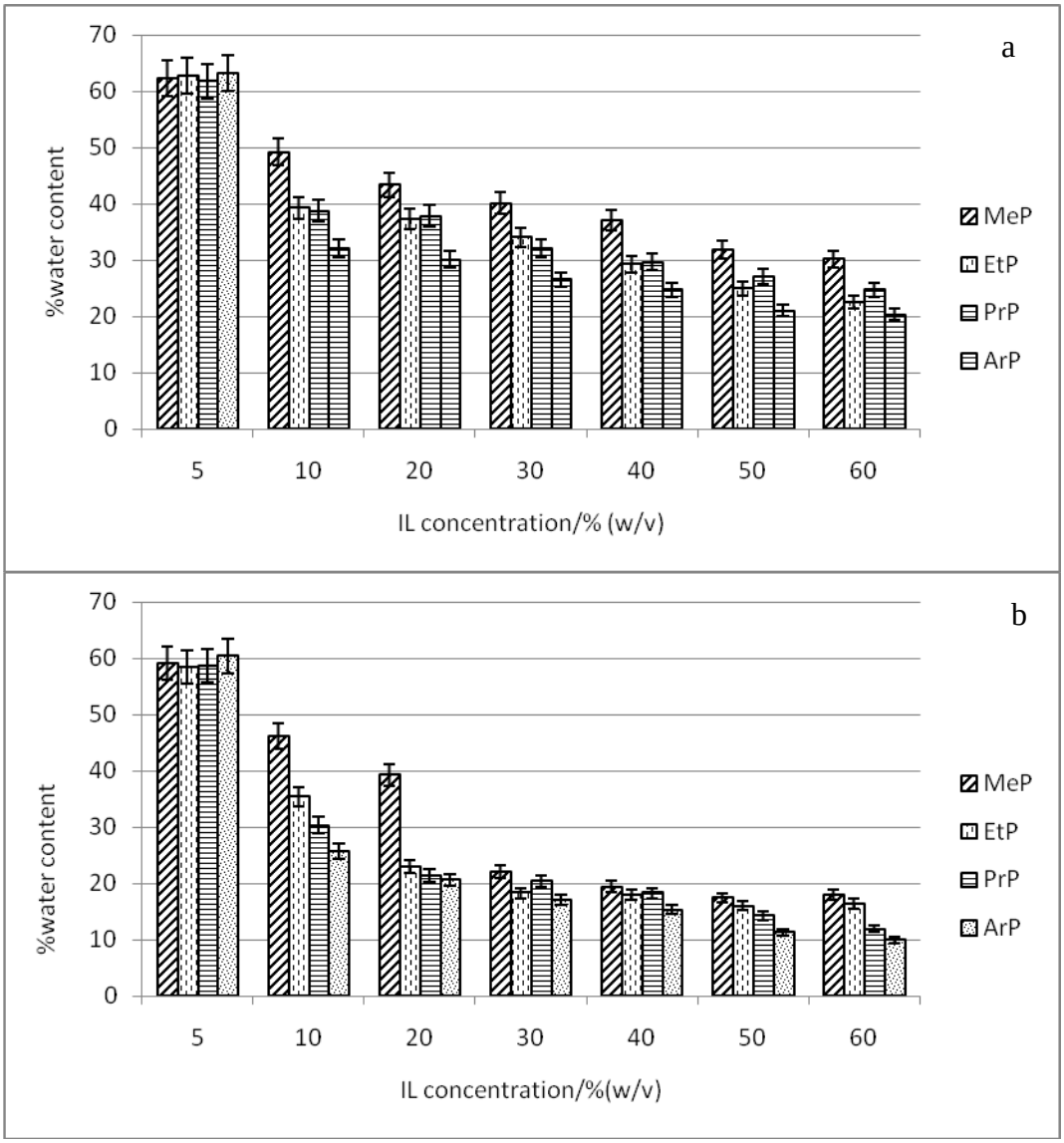


Figure 3.6 Percentage water content of IL-rich phase for parabens applies at (a) IL-ATPS and (b) IL-βCD-ATPS at different ionic liquid concentrations

This phenomenon was also described by Zafarani-Moattar and Hamzehzadeh (2009). They studied the interaction between different ionic liquids such as $[C_2mim][Br]$ and $[C_4mim][Cl]$ with salt or water. They concluded that solute-solute interaction between the studied ionic liquids was greater compared with the interaction between ionic liquid and salt or water.

IL-βCD-ATPS method shows higher loss of water content due to the complex formation between IL and the β-CD molecules and paraben in the IL-rich phase which is thought to be present in the form of micelles during the aqueous two-phase system process. So the spaces remained for water inside or among the micelles were efficiently compressed. Therefore, the amount of water that has been extracted in the IL-rich phase is reduced compared to the IL-ATPS. The proposed structure will be discussed in more details in Section 3.3.10.

3.3.7 Distribution Coefficient

The distribution coefficient, K_d, is the ratio of the concentration of parabens in the IL-rich phase to the concentration of parabens in the aqueous phase. The distribution coefficients of the four parabens studied by both methods are shown in Figure 3.7. The results obtained using the IL-ATPS method show only a slight difference in the distribution coefficients for all the studied analytes compared to the IL-βCD-ATPS method. The latter method shows increase of the K_d when the hydrophobicity of parabens increased. The values of K_d in the IL-βCD-ATPS for the four parabens used in this study increased in the order of MeP< EtP< PrP< ArP. The highest result obtained was for ArP because this analyte could be easily adsorbed into the cavity of β-CD which is relatively hydrophobic as ArP is the least polar and the least soluble in water (Chin, Mohamad, & Abas, 2010). Therefore we can conclude that the distribution of parabens in IL-rich phase for IL-βCD-ATPS method depends on hydrophobicity of the parabens whereas in the IL-ATPS method hydrophobicity is not an important factor that affects the extraction of parabens.

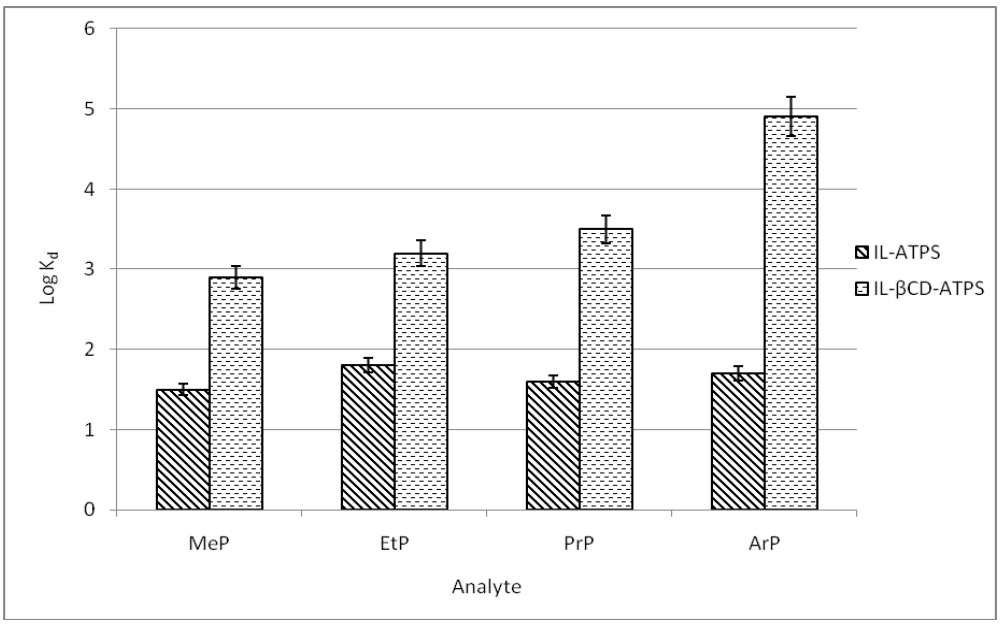


Figure 3.7 Distribution coefficients (K_d) obtained by the developed method of IL-ATPS and IL-βCD-ATPS for studied parabens

3.3.8 Effect of preconcentration factor and phase volume ratio on the recoveries of parabens

The phase ratio is the ratio of volume of IL-rich phase, V_s, to the volume of the aqueous phase, V_w. The plot of V_s/V_w against various concentrations of IL is given in Figure 3.8. This plot is important in determining the optimum volume of IL-rich phase when ATPS are performed. At lower volume of V_s, the system will produce higher concentration of parabens extracted in the IL-rich phase resulting in higher preconcentration factor. But taking into account that if the volume of V_s is too low, it is getting difficult to extract the parabens from the aqueous phase since the amount of IL-rich phase is inadequate to work as an efficient extractant.

Based on Figure 3.8, both methods show a similar trend that is when the IL concentration is increased the phase volume ratio also increased. This is because when the amount of IL is increased in the solution, the volume of IL-rich phase also increased; resulting in clear separation between the IL-rich phase and aqueous phase. Phase volume ratio of IL-βCD-ATPS shows the lowest value compared with IL-ATPS.

This is because of the fact that addition of β-CD in the system makes the phase formation easier. The experimental observation showed that when 30% (w/v) IL concentration was used, the phase separation between the IL-rich phase and the aqueous phase was clearly seen in the centrifuge tube. This IL concentration produced low V_s and was sufficient to extract parabens from the aqueous phase. Sufficient and small volume of V_s was required to produce good extraction recoveries of paraben in real samples.

If 20% (w/v) IL concentration or lower were used in the IL-βCD-ATPS method, the volume of V_s was too small and difficult to separate from the aqueous phase. If the 40% (w/v) IL concentration and more were used in the same method, the amount of V_s is too large making the volume of IL-rich phase bigger and hence less effective in extracting parabens from aqueous phase. It was important to select the optimum phase ratio to ensure that V_s was sufficient and produced higher percentage recoveries for parabens extraction. Therefore, 30% (w/v) IL concentration was chosen for the following experiments.

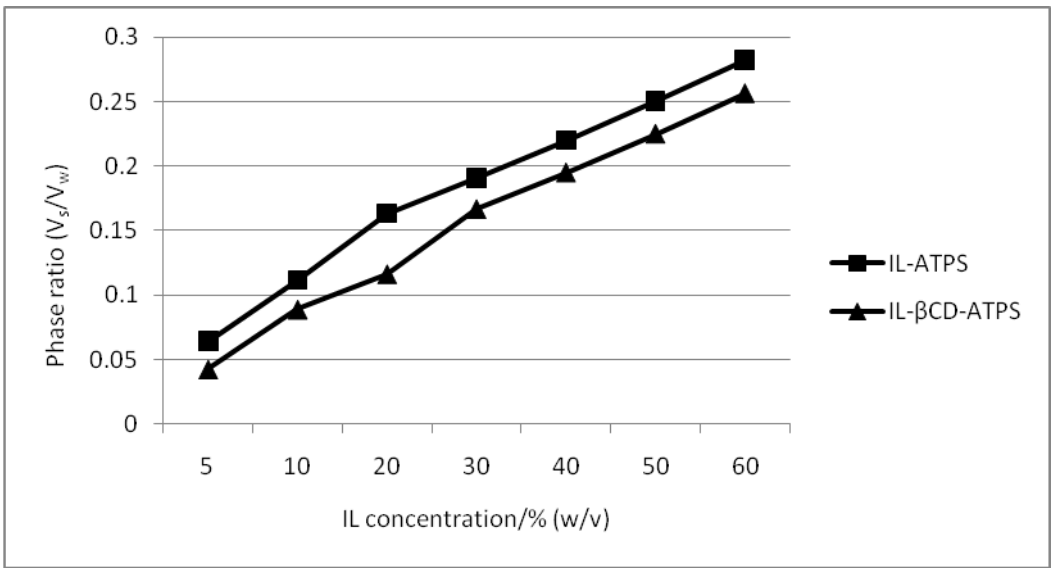


Figure 3.8 Phase ratios of against IL concentration for IL-ATPS and IL-βCD-ATPS methods

Figure 3.9 illustrates the preconcentration factor of the four selected parabens at different IL concentrations. The results clearly show that the highest preconcentration factor in these two methods is for ArP, followed by PrP, EtP and lastly MeP. IL-βCD-ATPS shows the highest preconcentration factor with values for MeP, EtP, PrP and ArP as 70, 86, 95 and 103 respectively. The results are higher than the preconcentration factors obtained with IL-ATPS which are 48, 78, 85 and 95 for MeP, EtP, PrP and ArP. The excellent results obtained by IL-βCD-ATPS were due to low water content in IL-rich phase, V_s, which reduced the volume of final IL-rich phase.

The higher preconcentration factor is attributed to the smaller phase volume of the IL-rich phase. Thus, the volume of sample injected in HPLC was highly concentrated and produced good results (Delgado et al., 2004; Yao & Yang, 2008). In order to get an acceptable preconcentration factor and a low V_s as possible (taking into consideration the cost of IL used), a 30% IL concentration was used throughout this experiments.

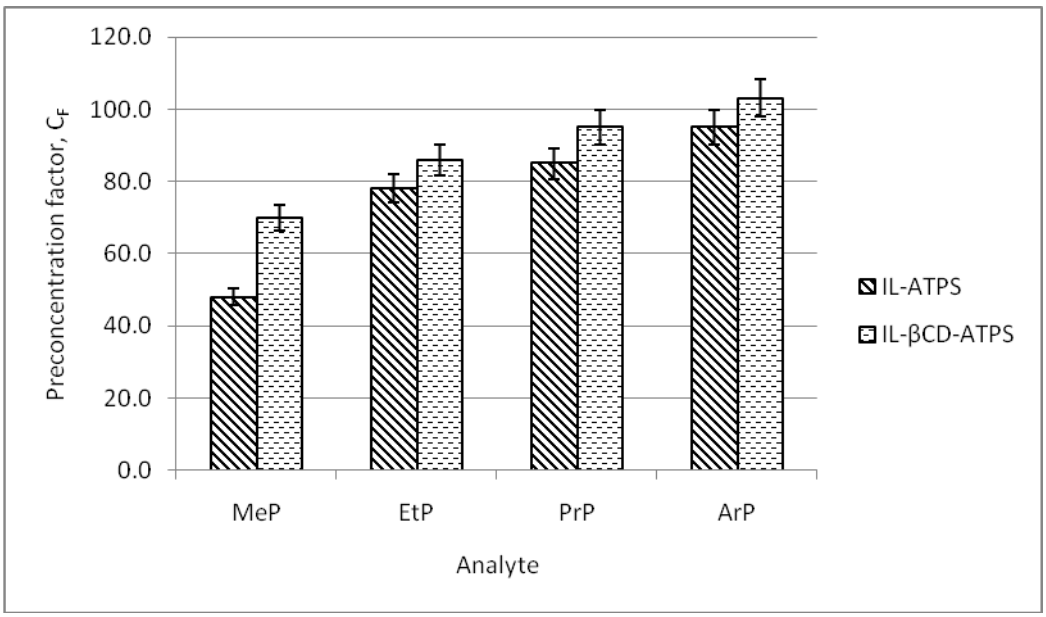


Figure 3.9 Preconcentration factor of studied paraben using IL-ATPS and IL-βCD-ATPS methods.

3.3.9 Method Validation

The parameters such as precision, linearity and limit of detection (LOD) for four parabens were determined from spiked solution of analytes under the above mentioned optimized condition. The results are listed in Table 3.3. Linear ranges of parabens were obtained from 0.01-0.10 μgmL^{-1} respectively. The detection limits of proposed methods were obtained based on a signal to noise ratio (S/N) of three extractions using double distilled water spiked with the studied parabens at three concentrations. The experimental results demonstrated that the detection limits for the studied parabens using IL-βCD-ATPS method were lower compared to IL-ATPS method. The addition of β-CD improves the sensitivity of the developed method due to higher distribution coefficient and preconcentration factor compared to IL-ATPS method.

Table 3.3 Relative standard deviations, coefficient of determination and limits of detection of developed method for the determination of parabens from aqueous solution

Analyte	Precision, coefficient of determination and limit of detection	IL-ATPS	IL-βCD-ATPS
MeP	RSD (% n =3)	1.1	0.3
	Coefficient of determination, R^2	0.994	0.994
	LOD (μg/mL)	0.24	0.075
EtP	RSD (% n =3)	1.1	0.6
	Coefficient of determination, R^2	0.995	0.991
	LOD (μg/mL)	0.51	0.055
PrP	RSD (% n =3)	1.0	0.2
	Coefficient of determination, R^2	0.992	0.995
	LOD (μg/mL)	0.27	0.048
ArP	RSD (% n =3)	0.3	0.2
	Coefficient of determination, R^2	0.993	0.997
	LOD (μg/mL)	0.17	0.022

It can be concluded that our proposed method gives similar and some higher limit of detection as compared to the other alternative procedures (J. Han et al., 2010; He et al., 2005; S. Li et al., 2005). Furthermore, the cost of acquisition, total extraction time and solvent consumption for the present method are lower than those methods. Moreover, the complexity of the analytical systems employed (Zhang, Lian, Liu, & Cui, 2005) and sample pretreatment required (Blanco et al., 2009; Labat, Kummer, Dallet, & Dubost, 2000) in those procedures are comparable with the developed method.

The developed method was applied for the determination of the recoveries of parabens from various water samples such as river, treated, sea and tap water. The results are summarized in Table 3.4. The spiked parabens concentrations in the real samples for IL-βCD-ATPS method and IL-ATPS method are 0.02 μgmL^{-1} and 0.1 μgmL^{-1} respectively. Percentage recoveries using IL-βCD-ATPS are between 91.3% and 99.9% with relative standard deviations (RSD) of less than 4%. While the percent recoveries using IL-ATPS are between 88% and 96.1%. This shows that the addition of β-CD improves the selectivity of the developed method. The matrix effect is totally reduced by the addition of βCD in the IL-βCD-ATPS method.

Table 3.4 Percent recoveries of parabens from spiked water samples using the developed methods.

Method	Analyte	River water	Tap water	Treated water	Sea water
		%Recovery (RSD%)	%Recovery (RSD%)	%Recovery (RSD%)	%Recovery (RSD%)
IL-ATPS	MeP	88.6 (0.24)	90.1 (0.50)	91.7 (0.14)	87.2 (0.40)
	EtP	89.1 (0.26)	89.5 (0.12)	94.0 (0.30)	88.0 (0.10)
	PrP	90.3 (0.16)	92.7 (0.27)	94.6 (0.35)	88.5 (0.43)
	ArP	91.7 (0.12)	95.8 (0.36)	96.1 (0.55)	92.8 (0.30)
IL-βCD-ATPS	MeP	97.2 (0.25)	94.4 (0.40)	96.0 (0.20)	97.3 (1.40)
	EtP	96.5 (0.42)	97.1 (1.20)	99.9 (0.40)	98.5 (0.30)
	PrP	95.3 (0.21)	94.3 (0.90)	98.0 (0.50)	96.2 (0.70)
	ArP	91.3 (0.17)	99.2 (0.60)	98.8 (0.30)	96.0 (0.90)

The above results show that the developed method has a satisfactory recovery for the determination of the parabens from aqueous solution. These results have shown that the method developed is feasible to be used for monitoring parabens compound in environmental water samples.

3.3.10 Extraction behavior of ArP, IL[C_4mim][Cl] and β-CD

The analysis of inclusion complex between modified β-CD, ArP and ionic liquid [C_4mim][Cl] is very crucial in this work since the cavity of β-CD is maintained during the extraction process. Furthermore the findings supported that the inclusion complex formation is one of the main interactions that take place between β-CD, ArP and [C_4mim][Cl] in the extraction process. In order to evaluate the geometry of inclusion formation of β-CD, ArP and [C_4mim][Cl], ^{1}H NMR (Figure 3.10) and 2D NOESY measurements (Figure 3.11) (DMSO-D6, 25°C, 600 MHz) were performed on an AVN600 spectrometer. The obvious upfield shifts of the protons on the inner cavity of β-CD (H3, 3.5572 ppm and H5, 3.5353 ppm) were observed. This change indicates that the IL or ArP has entered to the cavity of CD.

Based on the results obtained (Table 3.5) the protons of IL and ArP were found to be shifted upon the formation of inclusion complex (βCD-IL-ArP). The presence of ^{1}H signals belonging to both β-CD, ArP and IL molecules could be observed in ^{1}H NMR spectrum of βCD-IL-ArP which strongly suggests that the new inclusion complex has been formed. Since, there are two guest compounds (IL and ArP) in this study, it is necessary to investigate further with 2D NMR in order to predict which one enters into the cavity of CD. There are a few NMR techniques that can provide supporting evidence for specific structures in CD complexes. 2D-NOESY and 2D-ROESY experiments give rise to cross peaks between dipolarly coupled spins (Neuhaus & Williamson, 2000; Sanders & Hunter, 1993), in order to indicate the close proximity

between atoms in the two components of the complex. In addition, 2D NOESY and 2D ROESY experiments provide an upper limit (ca. 5Å) on the distance between protons that produce cross peaks under favorable conditions.

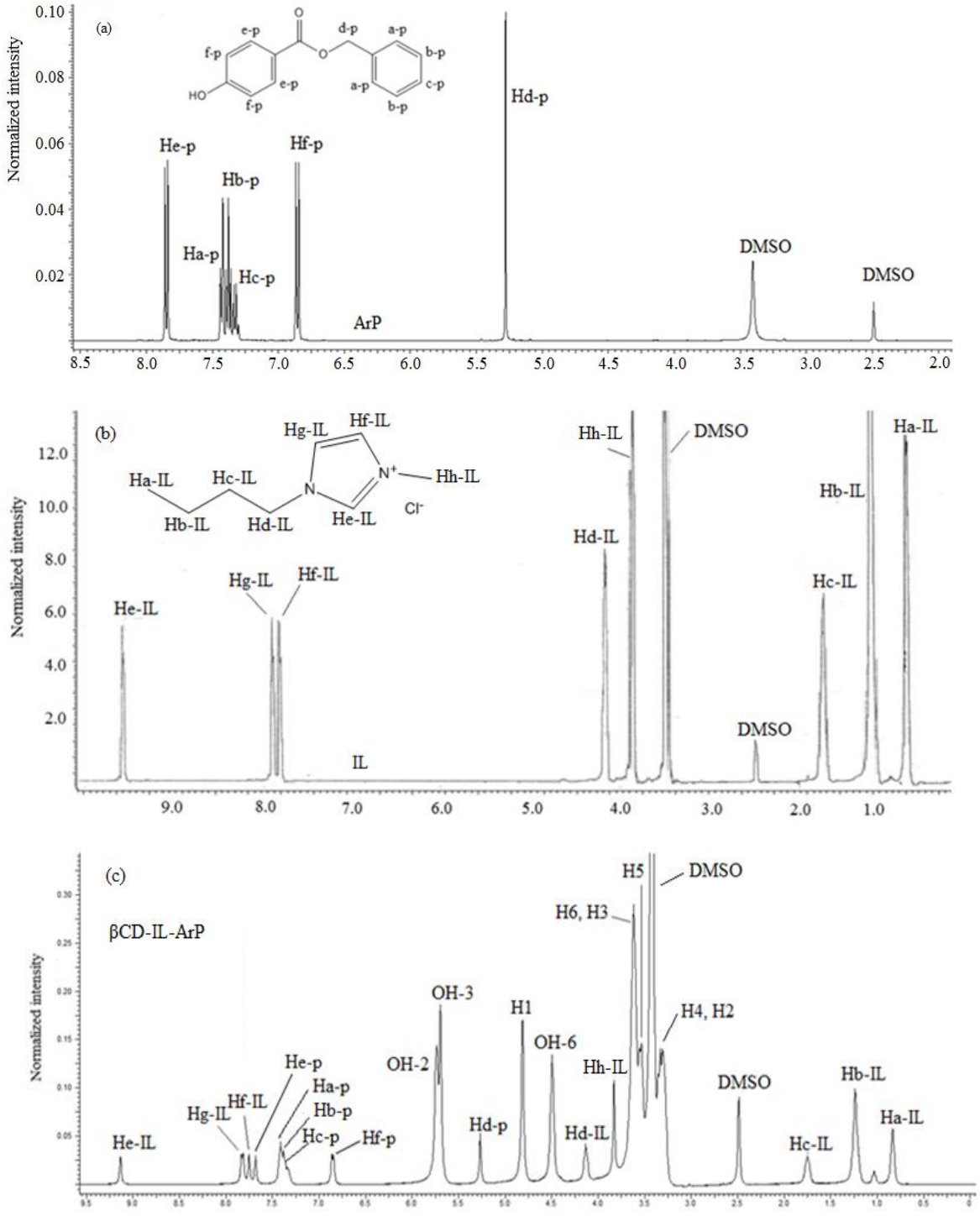


Figure 3.10 NMR spectrum of a) ArP b) IL c) βCD-IL-ArP

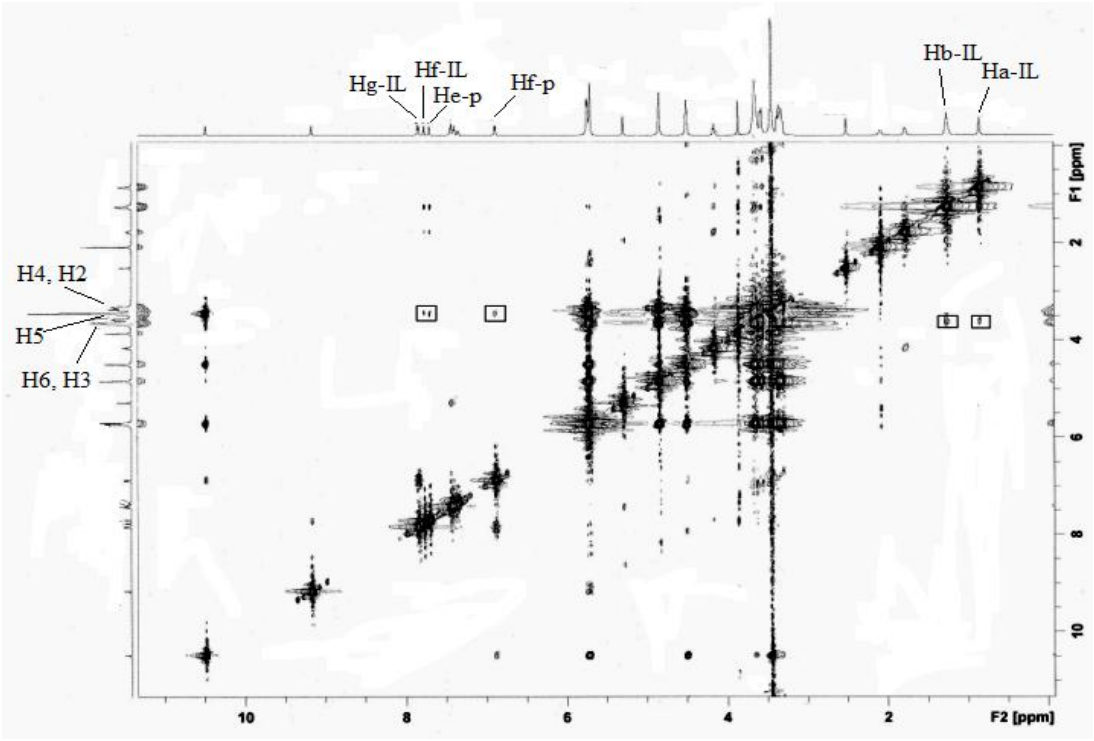


Figure 3.11 Two-dimensional NOESY spectrum of βCD-IL-ArP complex in DMSO-D6

The formation of inclusion complex was further proven by the 2D-NOESY analysis (Figure 3.11) since 2D NMR is a powerful tool for investigating intermolecular interactions and gaining more information on the conformation of the inclusion complex (J. Li, Ni, Zhou, & Leong, 2003). The cross-peaks in the spectra, indicated in Figure 3.11, originate from the interaction of the protons of IL, ArP and βCD. The cross peaks of βCD (3.5–3.6 ppm, H-3, H-5) and IL (Ha-IL, Hb-IL, Hf-IL, Hd-IL) demonstrate strong intensity. Hence, from the 2D NOESY spectra, we can conclude that IL has been accommodated in the β-CD cavity and may be within less than 5 Å apart from H3 and H5 of CD. Apart from that, 2D NOESY also shows interactions between β-CD and ArP. The cross peak (He-p, Hf-p) shows an interaction with β-CD (3.5–3.6 ppm, H-3, H-5) and it further supports that ArP has been accommodated in the cavity of CD.

Table 3.5 ^{1}H NMR chemical shift(§) of β-CD, ArP and βCD-IL-ArP

	β-CD	ArP	βCD-ArP-IL	
	δ	δ	δ	Δδ
H1	4.8191		4.8136	-0.0055
H2	3.3119		3.3089	-0.0030
H3	3.5987		3.5572	**-0.0415**
H4	3.3656		3.3553	-0.0103
H5	3.5517		3.5353	**-0.0164**
H6	3.6176		3.6195	+0.0019
Ha-p		7.4200	7.4124	-0.0076
Hb-p		7.3770	7.3783	+0.0013
Hc-p		7.3370	7.3398	+0.0028
Hd-p		5.2780	5.2676	-0.0104
He-p		7.8530	7.6815	**-0.1715**
Hf-p		6.8730	6.8425	**-0.0305**
		IL	βCD-ArP-IL	
		δ	δ	Δδ
Ha-IL		0.7949	0.8364	**+0.0415**
Hb-IL		1.2019	1.2403	**+0.0384**
Hc-IL		1.5747	1.7517	**+0.1770**
Hd-IL		4.1705	4.1333	**-0.0372**
He-IL		9.5469	9.1326	**-0.4143**
Hf-IL		7.8018	7.7505	**-0.0513**
Hg-IL		7.8976	7.8140	**-0.0836**
Hh-IL		3.9905	3.8312	**-0.1593**

Hence the possible formations of the inclusion complex structure of the complexation of ArP and [C_4mim][Cl] with β-CD shown in Figure 3.12 and Figure 3.13 have been proposed by taking into account the electrostatic attraction between imidazolium ring and ArP, also the inclusion complex between β-CD with [C_4mim][Cl] and β-CD with ArP and the possible formations for the structure of complexation of deprotonated ArP with IL are shown in Figure 3.14. The possible interaction between the deprotonated ArP with IL is electrostatic interaction. It is clearly shown that the method with β-CD as a modifier shown triple interactions which are π-π attraction, electrostatic attraction and inclusion complex formation. While in method IL-ATPS without β-CD as a modifier has shown double interactions which are π-π attraction and electrostatic attraction. These triple interactions make the stronger attractions between ArP, β-CD and [C_4mim][Cl] and consequently higher distribution coefficients and preconcentration factor of parabens obtained in IL-rich phase. These triple interactions also contribute to a sensitive developed method in extraction of paraben in aqueous samples.

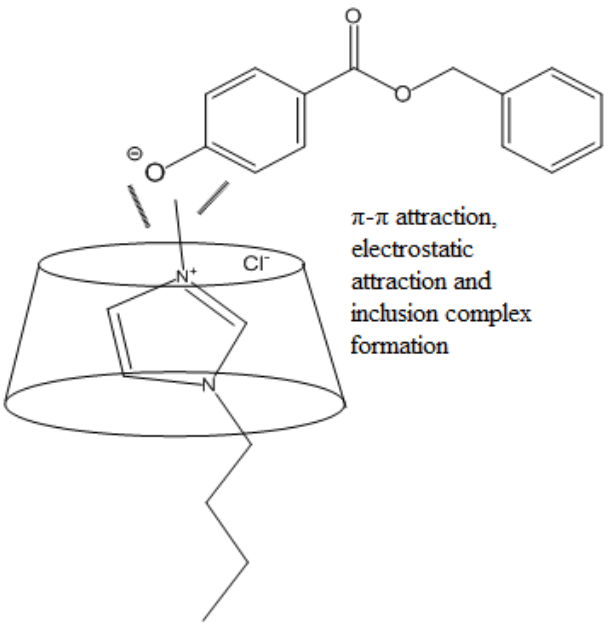


Figure 3.12 Schematic illustration of the complexation of ArP and IL with β-CD

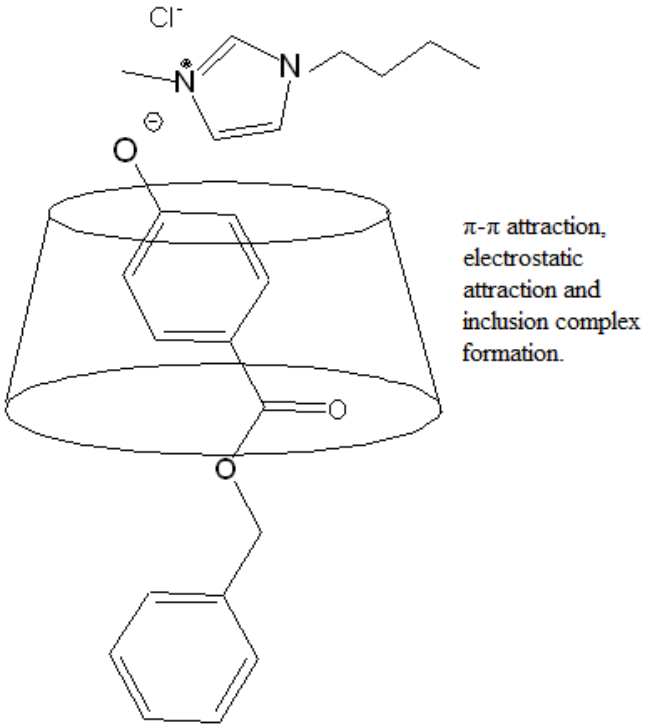


Figure 3.13. Schematic illustration of the complexation of ArP and IL with β-CD

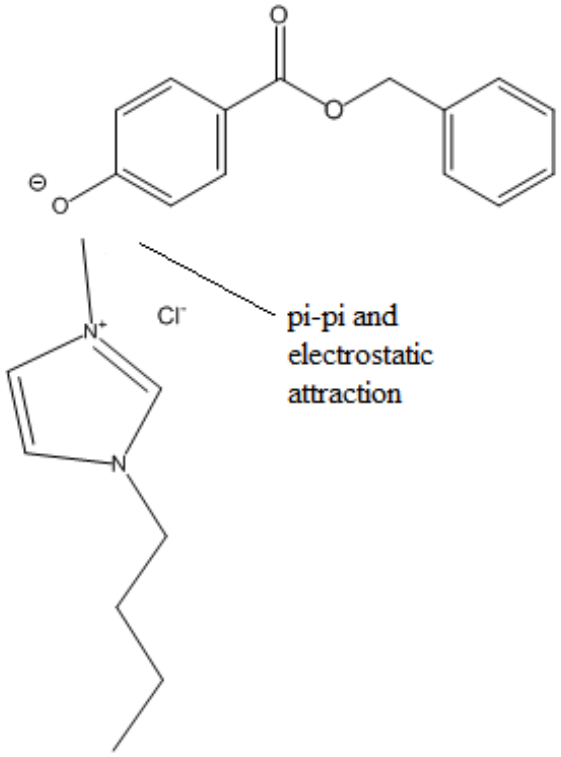


Figure 3.14 Schematic illustration of the complexation of ArP with IL without β-CD.

3.4 Conclusions

This study demonstrates that IL-βCD-ATPS consisting of [C_4mim][Cl] and β-CD as a modifier were excellent strategies for the extraction of parabens from various water samples. The water content loss in IL-βCD-ATPS and also lowers compared with IL-ATPS. This is because complex formations of IL with the β-CD molecules and paraben in the IL-rich phase were thought to be present in the form of micelles during the ATPS process. So the spaces remained for the water inside or among the micelles were efficiently compressed. Therefore, the amount of water that has been extracted in the IL-rich phase is reduced compared to the IL-ATPS. On the other hand, it is shown that β-CD gives effect on giving a higher distribution of parabens in IL-rich phase compared to IL-ATPS. On the other hand, the distribution coefficient of paraben in IL-rich phase of IL-βCD-ATPS method also affected by the hydrophobicity of parabens. β-CD as modifier makes improvement to selectivity and sensitivity of IL-ATPS. The experimental results demonstrated that the detection limits for studied paraben using IL-βCD-ATPS method were lower compared with IL-ATPS method. Addition of β-CD also improves the selectivity of the developed method. For examples the matrix effect is totally reduced where low recoveries of paraben extraction in sea water using IL-ATPS are dramatically improved with addition of β-CD in the IL-βCD-ATPS method. By comparing the two methods i.e IL-βCD-ATPS and IL-ATPS, can be concluded that is economically viable to use β-CD because it improves the performance of ATPS method dramatically as well as this chemicals are cheap and not toxic to our environment.

CHAPTER 4: Cloud Point Extraction of DC193C (CPE-DC193C), Cloud Point Extraction with β-cyclodextrin as Modifier (CPE-DC193C-βCD) and Cloud Point Extraction with β-cyclodextrin-Ionic Liquid as Modifier (CPE-DC193C-βCD-IL)

4.1 Introduction

Surfactant-based extraction methods to preconcentrate trace solutes of grave environmental concerns from aqueous solutions have become more and more attractive, as water is often utilized as the main solvent in such methods, in contrast to the massive use of volatile organics in the conventional liquid-liquid extraction techniques. Among these surfactant-based extraction techniques, the one based on the clouding phenomenon of surfactants and is called the cloud point extraction method is the most popular. Based on the promising results obtained for IL-βCD-ATPS using β-CD as a modifier, further study was conducted to investigate the performance of β-CD and βCD-IL as modifiers in CPE system of DC 193C surfactant (Figure 4.1). The study on the performances of βCD-IL was carried out to investigate the effect of β-CD derivatives compared with native β-CD on improving the efficiency of CPE method for the extraction of parabens from real water samples. In order to investigate the mechanism of extraction techniques, the inclusion complex method was carried out to support the obtained results.

4.2 Experimental

4.2.1 Reagents and Standards

Silicone-ethyleneoxide copolymer (DC193C) was manufactured by Dow Corning (Shanghai, China) and supplied by Dow Corning Malaysia (Figure 4.1). Unfortunately, no information was available on the detailed molecular structure, the values of x, y and molecular weight of this compound by the manufacturer. Methylparaben (MeP), ethylparaben (EtP), propylparaben (PrP) and benzylparaben (ArP) were purchased from Sigma Aldrich (Germany). β-CD was purchased from Acros Organics (USA). Acetonitrile (HPLC grade) was purchased from Merck (Germany). Deionized water used in mobile phase had its conductivity at 18 MΩ cm. Sodium sulfate (Na_2SO_4) was obtained from Merck (Germany). Stock solutions of parabens were at a concentration of 1000 mg/L was prepared in acetonitrile.

Working standard solutions were prepared by step-wise dilution of the stock solution with deionized water. pH of the samples was adjusted with either concentrated hydrochloric acid or concentrated sodium hydroxide solutions. β-CD is commercially available and was purchased from Acros (Hungary) (99%).1-methylimidazole was supplied by Sigma Aldrich. N,N-Dimethylformamide (DMF) and anhydrous hexane were purchased form Merck. p-toluene sulfonic chloride, p-toluene sulfonic acid were purchased from sigma aldrich and was used without further purification. p-Toluene sulfonic anhydride was prepared according to a literature procedure (Qian, Guan, & Xiao, 2008), and was used without further purification.

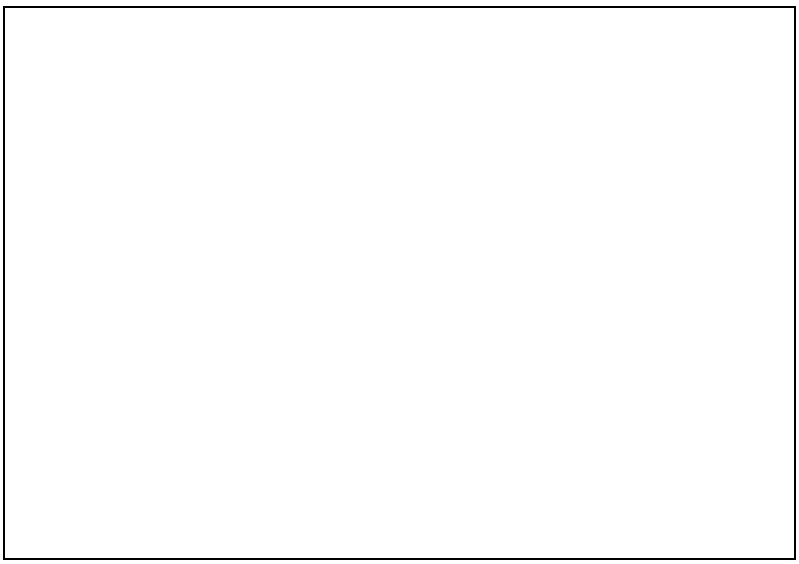


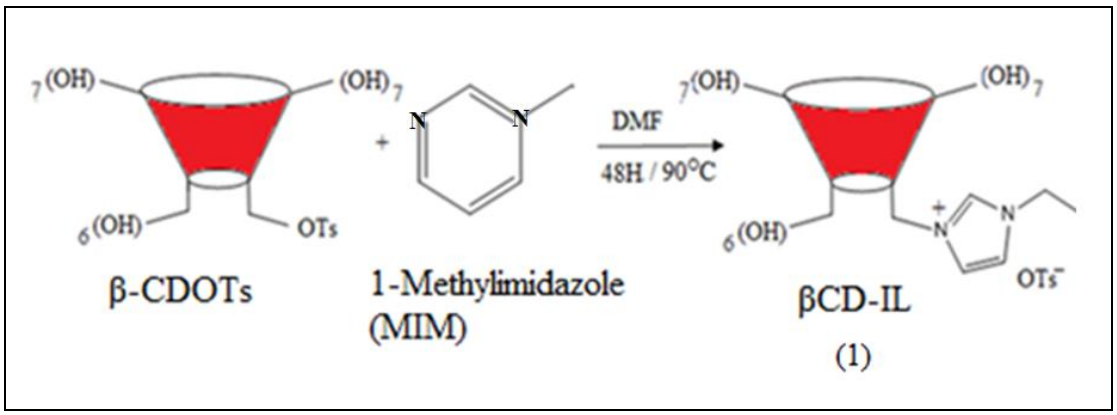
Figure 4.1 Structure of non-ionic surfactant DC 193C

4.2.2 Synthesis of β-cyclodextrin functionalized ionic liquid (βCD-IL) (1)

βCD-IL was prepared by reacting o-p-toluenesulfonyl-β-cyclodextrin (β-CDOTs) with 1-methylimidazole (MIM). β-CDOTs were prepared according to Zhong, Byun, and Bittman (1998). Since o-p-toluenesulfonyl OR tosyl is a good leaving group, imidazole can easily undergo nucleophilic substitution. The reaction was carried out in DMF solvent since β-CDOTs and MIM form a homogenous solution. The preparation of mono-functionalized βCD with MIM (βCD-IL) was done according to the reported procedure (T. T. Ong et al., 2005) and is shown in Scheme 4.1: where freshly dried CDOTs (1.00g, 78mmol) and appropriate amount of MIM (10 mole equivalent in excess amount) were dissolved in anhydrous DMF (40ml) and the solution was stirred at 90°C in an inert atmosphere. After two days, the resultant solution was cooled to room temperature and slowly added to acetone. Then the mixture was stirred for 30 minutes and thereafter filtered and washed in excess of acetone. The obtained product was recrystallized three times from hot water to get the final product (1) as a white precipitate. Figure 4.2(c) shows ^{1}H NMR spectrum of compound 1 (βCD-IL) in DMSO solvent. The formed product was soluble in water and several organic solvents (DMF, DMSO and ethanol). New peak was observed in proton (H6*, 3.86 ppm) and carbon

signal (C6*, 49.8 ppm), belonged to a substituted CD. OH_7 in Scheme 4.1 represents 7 hydroxyl units at primary, secondary and tertiary position of β-CD while OH_6 is the remaining hydroxyl group since IL reacts with only one hydroxyl group out of 7 units of β-CD.

IR/cm^{-1} 3301(OH), 2919(C-H), 1650(C=C), 1152(C-N). **^{1}HNMR/ppm,** H_b (9.0,s), H_d (7.93,s), H_c (7.67,s), H_a (2.27,s), H_8 (7.47, d), H_9 (7.11, d), OH_2-OH_3 (5.5-6.0,m),H_1 (4,81,s),OH_6(4.4-4.6,m), H_6* (3.86,s), H_3,H_5,H_6 (3.5-3.6,m),H_2-H_4 (3.3-3.4,m), H_{11} (2.07,s). **^{13}CNMR/ppm,DMSOD$_6$** C_b(137),C_d (123.4),C_c (123),C_a(30.87),C_7 (145.4),C_{10} (137.7),C_9 (128.05),C_8 (125.4), C_1 (101.64), C_4 (81.5), C_2 (73.3), C_3 (72.6), C_5 (72.1), C_6 (59.9), C_6* (49.8), C_{11}(21.9). **CHNS/%** C (37.4), H (7.51), S (0.54), N(1.13). Percentage yield (90%).



Scheme 4.1 Preparation of βCD functionalized IL (βCD-IL)

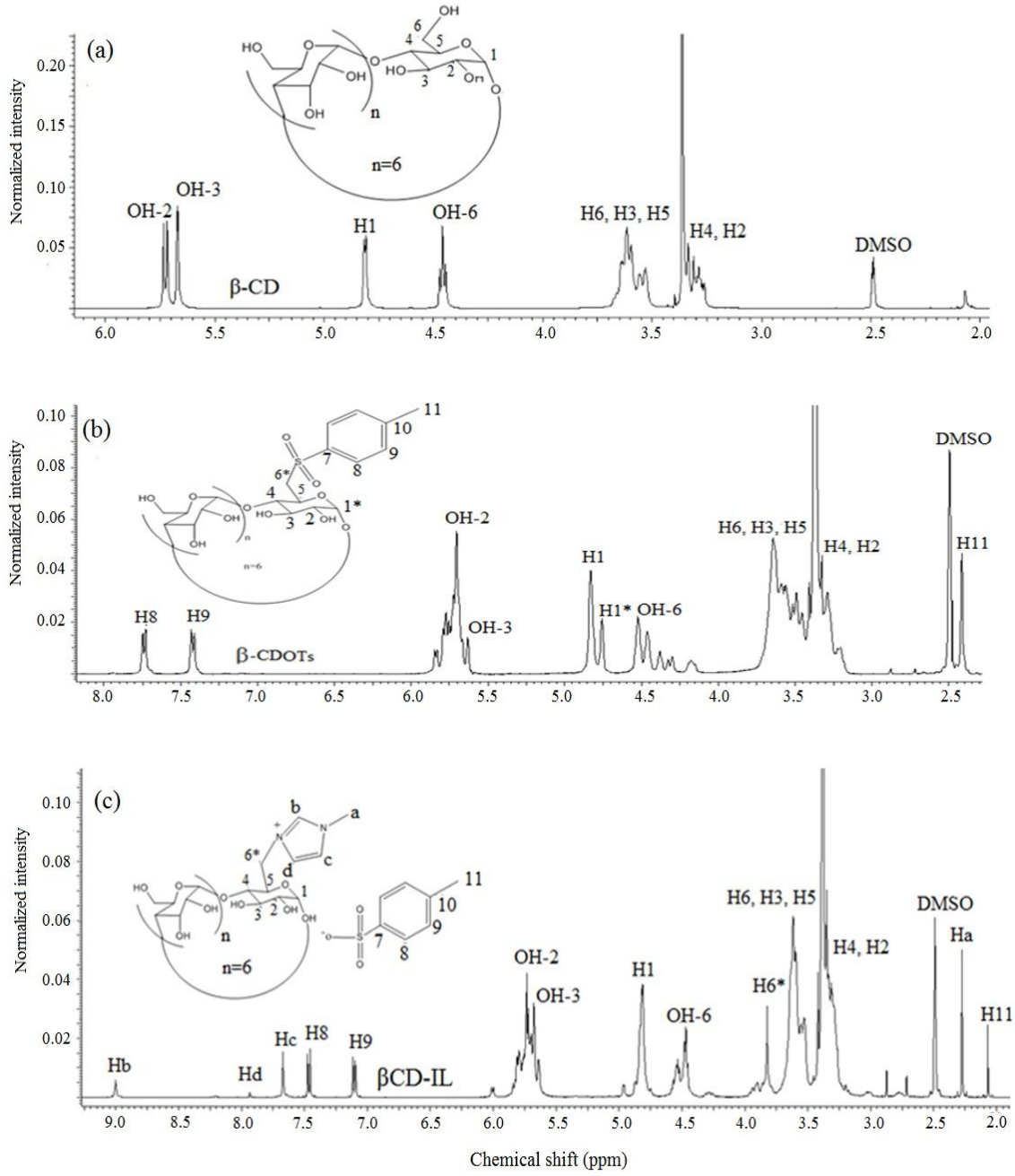


Figure 4.2 NMR spectrum of a) βCD, b) βCD-OTs, c) βCD-IL (1)

4.2.3 Instrumentation

The separation and quantification of the tested parabens were carried out on the Shimadzu HPLC system. The system consisted of a pump, degasser, auto injector, column oven, ultraviolet detector, guard column, Chromolith C_{18} column (100 mm x 4.6 mm, Merck, Germany). HPLC gradient conditions were used to separate the analytes using acetonitrile and deionized water, flow rate of 0.7 mL/min and detection at 254

nm. The gradient elution was performed as follows: 30% acetonitrile (0-2 min), ramped to 40% acetonitrile (2-5 minutes) and then ramped to 30% acetonitrile (5-8 min).

4.2.4 Cloud-Point Temperature Determination

To measure the cloud point of the solution, the surfactants, analyte, and salt were transferred to a test tube with a thermometer and then the test tube was placed in an isothermal water bath. The solution was gradually heated until turbidity was observed and at this point the temperature was measured using a thermometer. The temperature gradually dropped until the solution became clear. These experiments were repeated using different salt concentrations.

4.2.5 Preparation for Cloud Point Extraction of DC 193C (CPE-DC193C), Cloud Point Extraction with β-cyclodextrin as Modifier (CPE-DC193C-βCD) and Cloud Point Extraction with β-cyclodextrin-Ionic Liquid (CPE-DC193C-βCD-IL) as Modifier.

In the CPE-DC193C experiments, a desired aqueous solution was obtained by mixing 30% (w/v) surfactant aqueous solution, 1 mL of stock solution of parabens and 0.5 mL of 1.5 M sodium sulfate solution in an ultrasonic waterbath at 30˚C for 5 min. Meanwhile for the CPE-DC193C-βCD method and CPE-DC193C-βCD-IL method the solutions were obtained by mixing 1.0 mL of 30% (w/v) surfactant aqueous solution, 1.0 mL of β-CD (10 ppm) or 1.0 mL of βCD-IL (10 ppm) and 0.5 mL solution of 1.5 M sodium sulfate solution. The pH of the solution was adjusted in a glass centrifuge tube prior to the blending process. Subsequently, separation of the phases was achieved by centrifugation for 10 min at 4000 rpm or otherwise kept overnight to ensure separation between surfactant rich phase and aqueous phase was achieved. Then, the volumes of surfactant-rich phase and aqueous were measured. The top layer of the phase was

separated to vials and a 20 µl of top layer was directly injected into the HPLC system for analysis.

4.2.6 Preparation for CPE of real samples

Tap water samples were collected from the laboratory. River water samples were collected from Bahau, Negeri Sembilan, Malaysia (geographical coordinate 3˚1'44"N 102˚22'1"E), while treated water samples were collected from a wastewater treatment plant in Kuala Lumpur, Malaysia (geographical coordinate 3˚7'25"N 101˚39'12"E) and sea water samples were collected from Perak, Malaysia (geographical coordinate 4˚13'0"N 100˚34'0"E). All water samples were filtered using a 0.45 µm nylon membrane filter to remove suspended particulate matters and then stored at 4˚C in the dark. Then 1.0 mL of water sample was added to the centrifugal tube for CPE preparation (as mentioned above in 4.2.5) and analyzed using HPLC-UV.

4.2.7 Optimization of Parameters for Paraben Extraction

4.2.7.1 Effect of salt concentration

A series of sodium sulfate solutions at various concentrations (0.5 M, 1.0 M, 1.5 M and 2.0 M) were prepared with other parameters which are kept constant.

4.2.7.2 Effect of surfactant DC 193C concentration

A series of surfactant DC 193C were prepared at various concentrations (5% (w/v) to 60% (w/v)) using deionized water with other parameters kept constant.

4.2.7.3 Effect of pH

The pH of sample solutions (2, 4, 7, 8, 9, 10, 11 and 12) was adjusted with either dilute acid or dilute alkaline solution to get the desired pH solution with other parameters which were constant.

4.2.7.4 Effect of CD-IL concentration

A series of CD-IL solutions was prepared at various concentrations (10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm) in deionized water with other parameters kept constant.

4.2.7.5 Effect of extracting temperature

The extraction of paraben was carried out in a sonicator with the temperature adjusted to the desired temperatures (room temperature, 30˚C, 40˚C, 50˚C, and 60˚C) with other parameters kept constant.

4.2.7.6 Study on water content in surfactant-rich phase

To measure the water content of the surfactant-rich phase, the surfactant-rich phase was dried at 75˚C until no loss of mass was observed and water content was obtained by calculating the weight difference of the surfactant-rich phase before and after drying. All the data given were the average of triplicate measurements.

4.2.7.7 Effect of phase volume ratio

A series of surfactant DC193C at various concentrations (5% (w/v) to 60% (w/v) was studied to get the optimum phase ratio by calculating the volume of surfactant-rich phase to the volume of aqueous phase with other parameters kept constant.

4.2.8 Preparation and characterization of inclusion complex of β-CD or βCD-IL, surfactant DC193C and ArP

In order to investigate the mechanism of extraction between surfactant DC193C, β-CD or βCD-IL and ArP, the inclusion complex method was carried out to support the obtained results. The inclusion complex of βCD-IL with non-ionic surfactant, DC 193C and benzyl paraben (ArP) was prepared using the conventional kneading method in basic condition (Cwiertnia, 1999). Equimolar amounts of βCD or βCD-IL, surfactant

DC193C and ArP were kneaded with mortar and pestle in minimal ethanol to form a homogeneous paste. The complex was kneaded for approximately 30 minutes and dried to constant mass. After drying, a white powder of βCD-surfactant DC193C-ArP complex and βCD-IL-surfactant DC193C-ArP complex were obtained. ^{1}H NMR and 2D NOESY spectra were recorded on AVN 600 MHz and DMSO-d6 was used as solvent.

4.3 Results and Discussion

4.3.1 Effect of salt concentration on cloud-point temperature

In order to determine the effect of different salts towards lowering the cloud point temperature of surfactant for the extraction process, several electrolytes like NaOH, K_3PO_4, Na_2CO_3, NaCl, KI, K_2SO_4 , NaF were tested. The obtained results were not very good. There were a few problems such as the phase separation was not achieved and some salts took so long to form two layers. Therefore their results are not reported in this section. The ability of the inorganic salts studied to enable phase separation in CPE-DC193C is related to the kosmotropic and chaotropic ions (Hung et al., 2007; Z. Li et al., 2010). These kosmotropic and chaotropic ions effect has been discussed in details in Section 3.2.7.1. Based on Table 4.1, although sulfate ion SO_4^{2-} has the lowest solubility on water their Gibbs energy of hydration value is higher compared to the other salts studied. The salting-out ability may also be related to the Gibbs energy of hydration of the ions (Z. Li et al., 2010; X. Xie et al., 2011).

Table 4.1 Solubility of the studied salts in the water

Name of salts	Solubility of salt in the water
NaOH	111 g/100 mL
Na_2CO_3	215 g/L
Na_2SO_4	42.7 g/100 mL

Table 4.2 shows the Gibbs energy of hydration for the studied salts and the highest value is for phosphate ion. The sulfate ion shows a high value of Gibbs energy of hydration, whilst sodium ion also shows the highest value compared to potassium ion. Thus, Na_2SO_4 was chosen in these three methods based on its performance that is, the ease of phase separation in the water system.

Table 4.2 The Gibbs energy of hydration for selected anions and cations

Name of anion / cation	Gibbs energy of hydration, ΔG_{hyd} (kJ/mol)
Cl^-	-340
I^-	-342
F^-	-465
SO_4^{2}	-1266
Na^+	-365

Figure 4.3 shows the effects of salts concentration on (A) CPE-DC193C (B) CPE-DC193C-βCD (C) CPE-DC193C-βCD-IL. These results show that the cloud point temperature for the phase separation decreases with increasing the salt content up to 60% (w/v) for all methods. The Na_2SO_4 concentration was prepared up to 2.0 M because at lower salt concentrations such as below 0.5 M, the solutions did not produce phase separation. While at higher salts concentration i.e., 2.0 M and above, the solution produce precipitate at the bottom of the centrifuges tube. This precipitate will be interrupted in phase separation.

Results in Figure 4.3 show that the salt as an electrolyte in the phase separation has the ability to lower the cloud-point temperature for all methods and the two phases remain separated at room temperature after centrifugation. Based on the results, the cloud point temperature for all methods decreases significantly when the salts

concentrations is increased. In addition, the cloud point temperature also decreases when surfactant concentration is increased for CPE-DC193C-βCD and CPE-DC193C-βCD-IL methods while in CPE-DC193C it is constant. This is because as surfactant concentration increases, the intermicellar attractive forces between surfactant with βCD-IL and βCD increases and hence, the cloud point temperature is decreased (Taechangam, Scamehorn, Osuwan, & Rirksomboon, 2009). Since cloud point is the temperature at which micelles agglomerate or coagulate into a surfactant-rich phase(Taechangam et al., 2009), the addition of βCD-IL and βCD will reduce the cloud point temperature of DC193C.

When salt concentration at 1.5 M was used in the CPE-DC193C system, the cloud point temperature decreased to 29˚C, while the CPE-DC193C-βCD system is decreased to 25˚C and 22˚C for CPE-DC193C-βCD-IL system. This results show that βCD-IL and βCD decrease the cloud point temperature of CPE-DC193C because in CPE-DC193C-βCD-IL and CPE-DC193C-βCD systems, the proposed mechanism involves complex formation, so spaces remained for the water inside or among the micelles were efficiently compressed. Therefore the amount of water is reduced and the phase separation between surfactant-rich phase and aqueous phase is easier to form. The details on the proposed mechanism will be discussed further in Section 4.2.8.

At the same time, the cloud point temperature of CPE-DC193C-βCD-IL system is the lowest compared with other two methods. Thus, the concentration of 1.5 M Na_2SO_4 enables the extraction of parabens at lower temperature (Noorashikin, Mohamad, & Abas, 2013). Therefore 1.5 M Na_2SO_4 has been chosen as the optimum condition and this concentration will be used for the following optimization procedures for all methods.

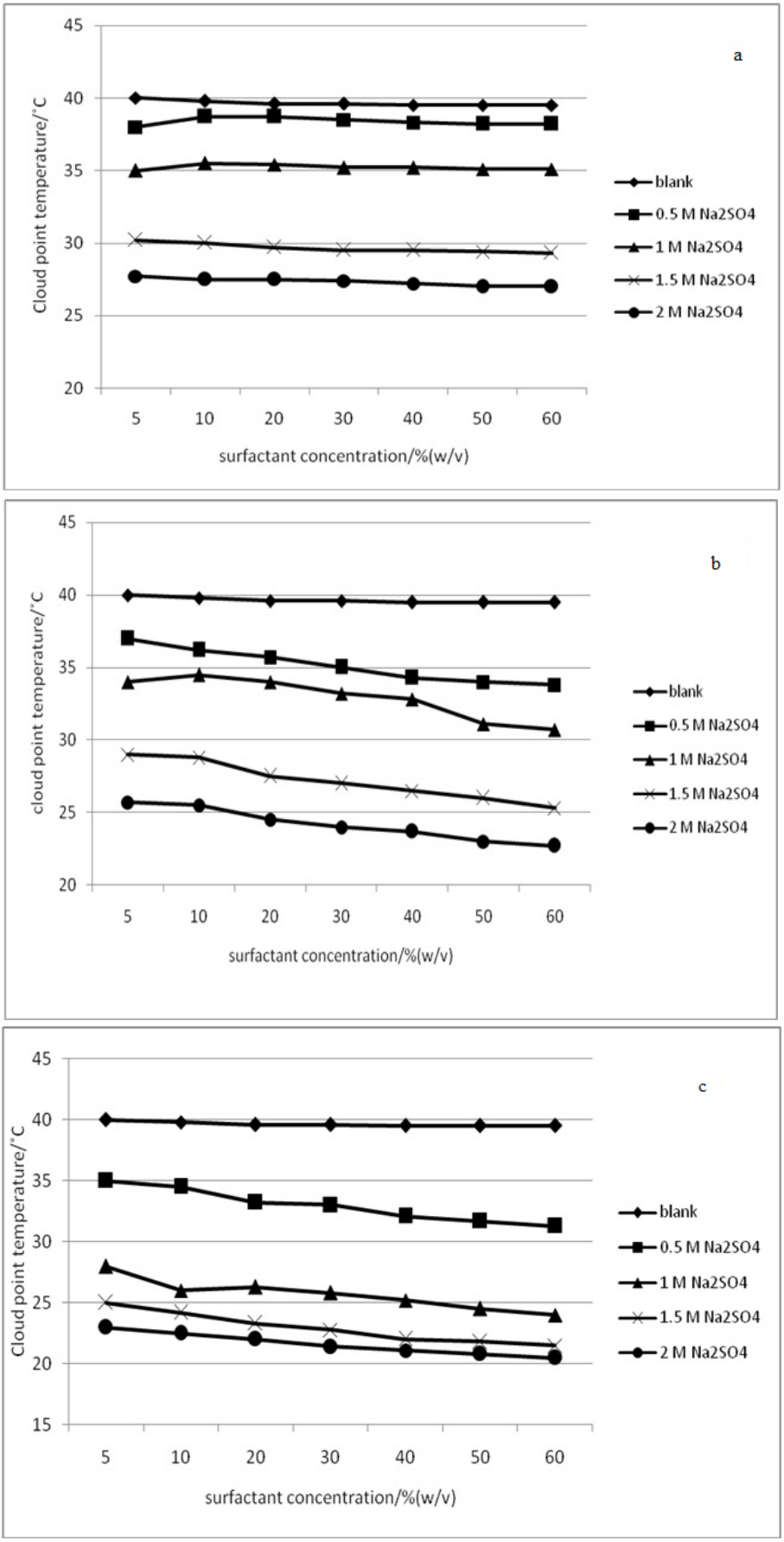


Figure 4.3 Effects of salt concentrations on (a) CPE-DC193C (b) CPE-DC193C-βCD and (c) CPE-DC193C-βCD-IL

4.3.2 Effect of surfactant concentrations on the recoveries of parabens

The effect of surfactant concentrations on paraben extraction recoveries was studied by using a series of parabens solutions in the presence of Na_2SO_4 (1.5 M) at different concentrations of surfactant DC193C ranging from 5 to 60% (w/v). The results in Figure 4.4 show similar behavior for all methods whereby the percentage recoveries increase dramatically when the DC193C concentration is increased from 5-30% (w/v), and remain practically constant or slightly increased from 40-60% (w/v). The increase in percentage recoveries when the surfactant DC193C increased (from 5-30% (w/v) is due to the increase in viscosity of the surfactant-rich phase when the concentration of surfactant is increased. Therefore, the decrease in the volume of surfactant-rich phase leads to a higher percentage of parabens extracted. This study indicates that modifiers β-CD and βCD-IL did not affect the percentage recoveries of parabens when the surfactant concentration was increased.

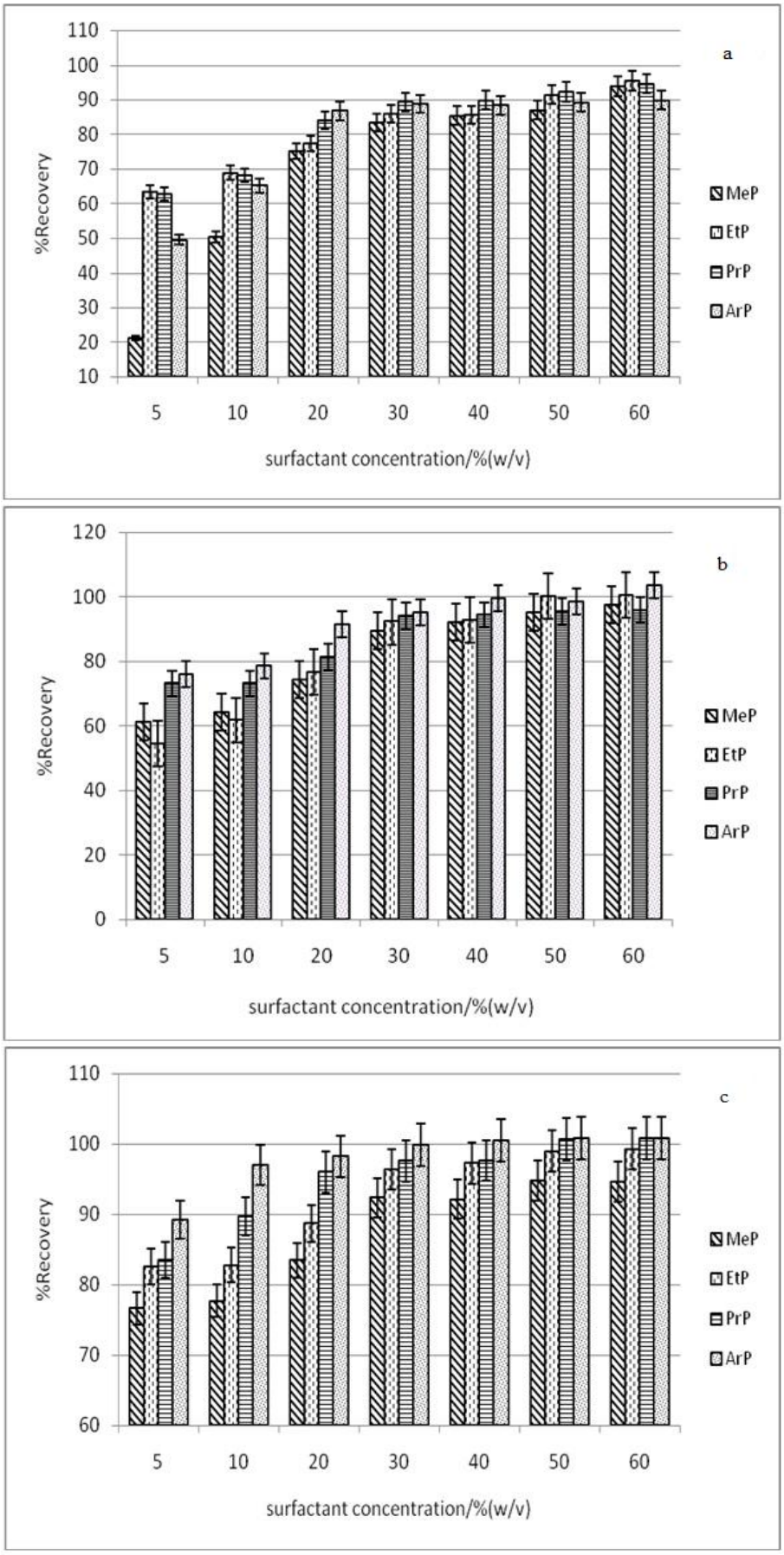


Figure 4.4 Effect of surfactant concentrations on percentage recoveries of parabens extraction using (a) CPE-DC193C, (b) CPE-DC193C-βCD and (c) CPE-DC193C-βCD-IL method

4.3.3 Effect of pH on the recoveries of parabens

The effect of pH on CPE depends on the characteristics of both surfactants and analytes. In most studies, the influence of pH on extraction recoveries is not crucial for those neutral or non-ionized compounds such as PAHs, PCBs, PCDFs and PCDDs, however a few notable exceptions have been reported (S. Xie et al., 2010). For analyte possessing an acidic or basic moiety, pH plays an important role in their extraction in CPE system. The ionic form of a molecule formed upon deprotonation of a weak acid or protonation of a weak base normally does not interact and bind as strongly as its neutral form with the surfactant aggregate. As a result, the lesser ionized form of an analyte is extracted.

The effect of sample pH on the recoveries of paraben for all the methods was optimized over the range of 2-12. The results are exhibited in Figure 4.5(A-D). It is found that all the methods shown the extraction performance reached optimum at pH 9 for all the parabens used. The change in the extraction recoveries can be explained by considering the change in the charge of the parabens. As can be seen from Figure 4.6, at below pH 3, paraben is in protonated form and the extraction recovery of paraben in this form is low. While at pH 3-6.5, paraben exists mainly in neutral form. There is a slight increase in extraction recovery of paraben in this region as paraben loses its net positive charge due to the deprotonation of hydroxyl group. At the pH values greater than 7 up to pH 9, paraben exists mostly in a negatively charged form because the hydroxyl group is now fully deprotonated. In this region, percentage recovery is increased dramatically and maximum percentage recovery is achieved at pH 9. When the pH is adjusted to pH 10, the percentage recovery decreases again. These low values of percentage recoveries are consistently observed until pH 12. This is because at pH above 8 parabens undergo alkaline hydrolysis, leading to the formation of corresponding alcohol and hydroxybenzoic acid (Angelov et al., 2007).

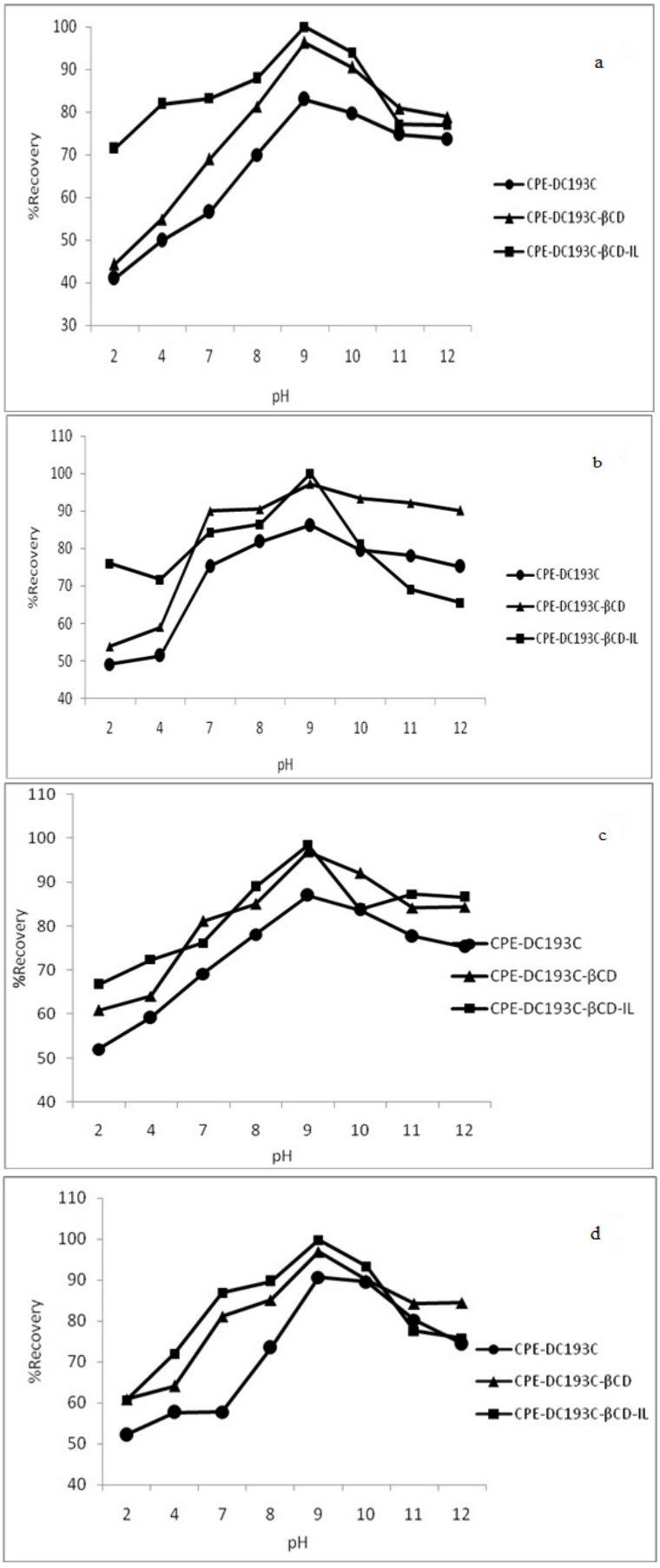


Figure 4.5 Effect of pH on percentage recoveries of parabens extraction (a) MeP, (b) EtP, (c) PrP and (d) ArP using CPE-DC193C, CPE-DC193C-βCD and CPE-DC193C-βCD-IL method

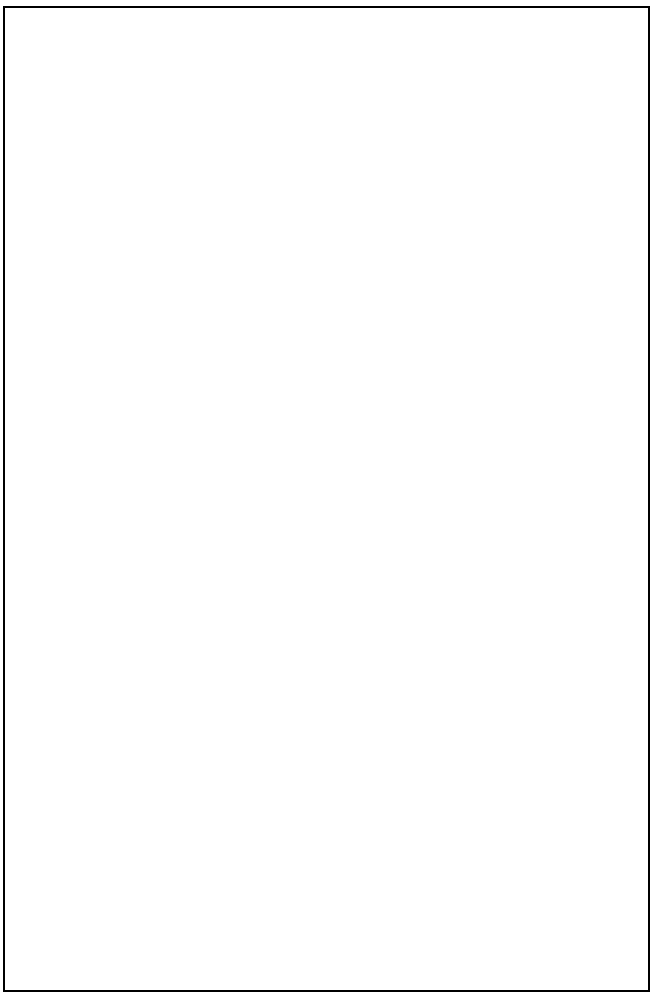


Figure 4.6 Schematic diagram of ArP in various pH solutions

All methods, the hydrogen bonding, electrostatic attraction and π-π interaction could be the main interaction when the paraben starts to be deprotonated by the surfactant DC193C, βCD-IL and β-CD. This proposed interaction will be discussed further in Section 4.3.9. As a result, a higher concentration of parabens could be extracted into the surfactant-rich phase. The results prove that pH play an important role in determining the optimum condition for paraben extraction.

4.3.4 Effect of extracting temperature on the recoveries of parabens

The effect of extraction temperature on the recoveries of parabens was studied over various temperatures ranging from room temperature to 60˚C. Figure 4.7 shows that the percentage recoveries of all the studied parabens do not follow any trend when

the temperatures were increased from room temperature to 60˚C in all the methods. The percentage recovery in CPE-DC193C system increases slightly when the temperature increases form room temperature to 40˚C. Then, percentage recoveries were almost constant at higher temperatures, i.e. 50˚ to 60˚C. The percentage recovery for PrP in CPE-DC193C-βCD system is decreased slightly from 92% to 90% when the temperature increases form room temperature to 30˚C. The percentage recoveries were almost constant at higher temperatures, i.e. 40˚ to 60˚C.

In CPE-DC193C-βCD-IL system, the percentage recoveries do not increase consistently for most of the analytes when the temperature is increased. A PrP recovery are lower at room temperature and 30˚C but gradually increases to 90% at 40˚C and becomes practically inconsistent in the range of 95% to 94%. While ArP shows that the percentage recoveries increased dramatically from room temperature to 30˚C from 91% to 97% and decreased at higher temperatures 50˚C and 60˚C. The results of ArP in CPE-DC193C-βCD system also show that temperature has a little influence on the recovery whereby there was a slight increases from room temperature to 50˚C with the value of 84.4% to 94.0%. These results indicate that temperature is not a significant factor in increasing the percentage recoveries of parabens extraction for all the developed methods.

All the methods show that temperature has only a little influence on the parabens extraction recoveries where there was a slight increase and shows inconsistency at room temperature to 60˚C. These results proved that temperature does not have significant effect on the dehydration of the micelle and volume of surfactant-rich phase. It shows that surfactant DC193C can undergo dehydration at room temperature. Therefore, the volume ratio and preconcentration factor are also not affected by changing of the temperature. It can also be observed from the results that modifiers βCD-IL and β-CD

do not have significant effect on the percentage recoveries of parabens extraction when the temperature is increased.

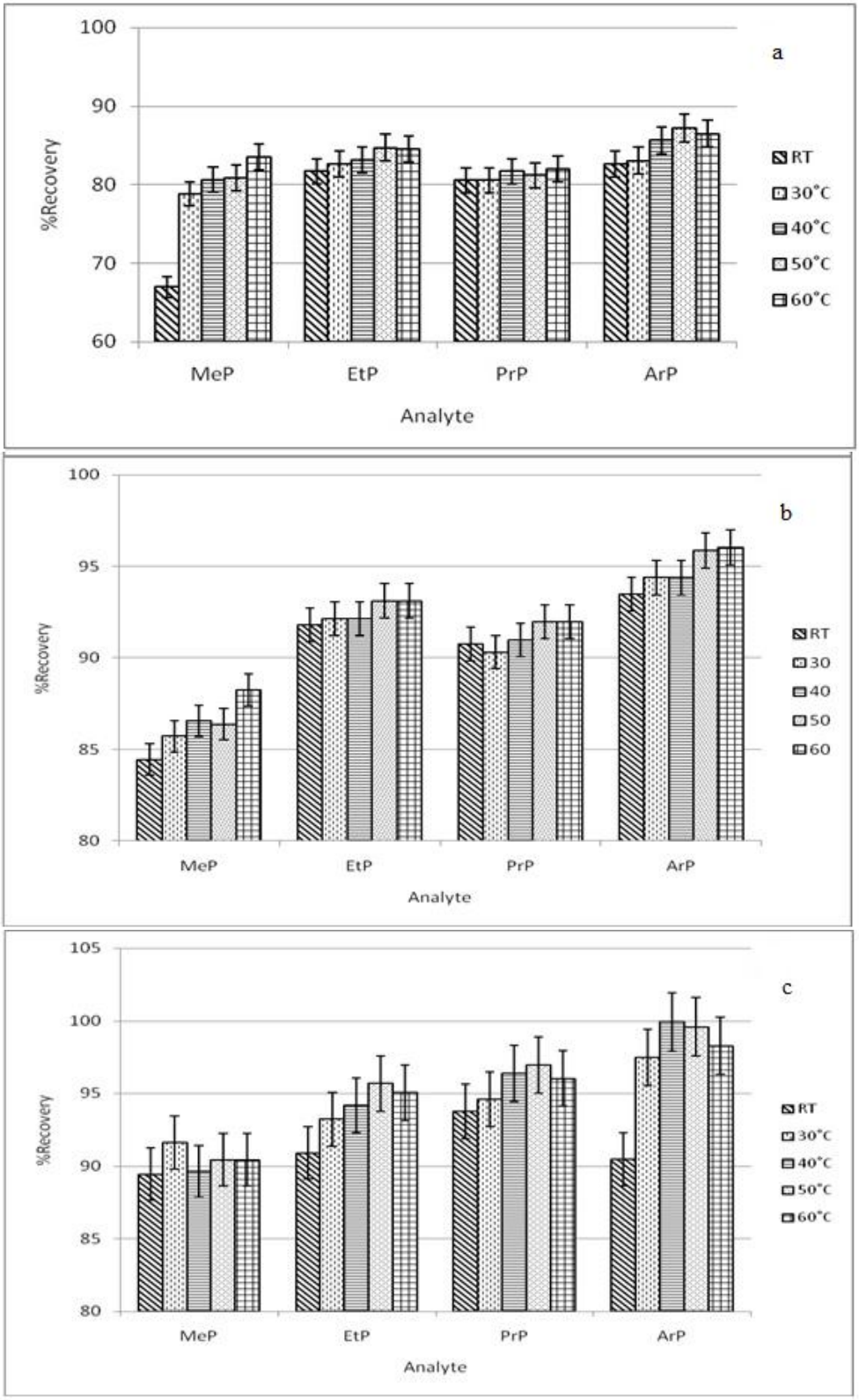


Figure 4.7 Effect of temperature on extraction recoveries of paraben using method (a) CPE-DC193C, (b) CPE-DC193C-βCD and (c) CPE-DC193C-βCD-IL

The equilibrium temperature was set at 30˚C for all the methods by taking into account the cost for using higher temperature more than 50˚C and procedure need to be conducted to stabilize the temperature throughout the experiment. This lower temperature was important to ensure the efficient separation of phases was achieved as temperature does not have a significant effect on the performance of extraction for all systems. A similar study conducted by He et al. (2005) have also indicated that temperature was not a significant factor on the distribution behavior of steroids.

Theoretically, the optimal equilibrium temperature of CPE occurs when the temperature is between 15-20˚C higher than the cloud point temperature of the surfactant (S. Xie et al., 2010). If the temperature is lower than the cloud point temperature, the two phases cannot be formed. But an excessive temperature may lead to decomposition of analytes. It has also been demonstrated that the preconcentration factor and recovery in the CPE increase as the equilibrium temperature for phase separation progressively increased to above the cloud point temperature. This study shows that at 30˚C or even at room temperature the two phases are formed successfully. This proved that at lower temperature the phase separation can still be formed without having difficulty to separate this surfactant-rich phase and aqueous phase.

A similar study was conducted by Ezoddin et al. (2010) who reported that room temperature, 25˚C was the equilibrium temperature the CPE of chromium from water samples. Zhao et al. (2011) had shown by experiments that the stability of organophosphorus pesticides was reduced with the rise of temperature. Hence, room temperature was determined as adequate for the CPE procedures in this study.

4.3.5 Water content in surfactant-rich phase

It is necessary to study the percentage of water content in the surfactant-rich phase during CPE process. This is because the amount of water in the surfactant-rich

phase will have an effect on the preconcentration factor as well as the recovery of the analyte extracted. The higher the amount of water in the surfactant-rich phase, the lower the concentration of analyte extracted. Therefore this study aims to get the lowest amount of water in the surfactant-rich phase when conducting the CPE.

Based on Figure 4.8, the extraction of ArP by the CPE-DC193C method procedured the highest amount of water at the beginning of experiment with almost 61% of water content in surfactant-rich phase at 5% (w/v) surfactant concentration. This percentage was rapidly reduced to 28% of water content at 60% (w/v) surfactant concentration. A reduction of 33% was measured for ArP when the surfactant concentration was increased from 5% (w/v) to 60% (w/v) using the CPE-DC193C method. The overall reduction of water content for MeP was 13.3%, followed by EtP with 24%, EtP with 27% and ArP with 33%.

CPE-DC193C-βCD method shows a similar trend as CPE-DC193C because the water content was reduced from almost 58% (w/v) to 16% (w/v) for ArP, when the surfactant concentration was increased. The highest reduction of water content was for ArP with 40%, followed by PrP with 34%, EtP 29% and lastly MeP 20%. For the extraction of ArP with the CPE-DC193C-βCD-IL method the highest amount of water was observed at the beginning of the experiment with almost 68% (w/v) of water content in surfactant-rich phase at 5% (w/v) of surfactant concentration. This percentage was rapidly reduced to 0% (w/v) water content at 60% (w/v) of surfactant concentration. A total loss of 68% was measured when the surfactant concentration was increased from 5% (w/v) to 60% (w/v) in CPE-DC193C-βCD-IL method. This is considered the highest loses of water content when compared with the CPE-DC193C-βCD method. The overall loses of water content for MeP was 55%, followed by EtP and PrP with 52% each.

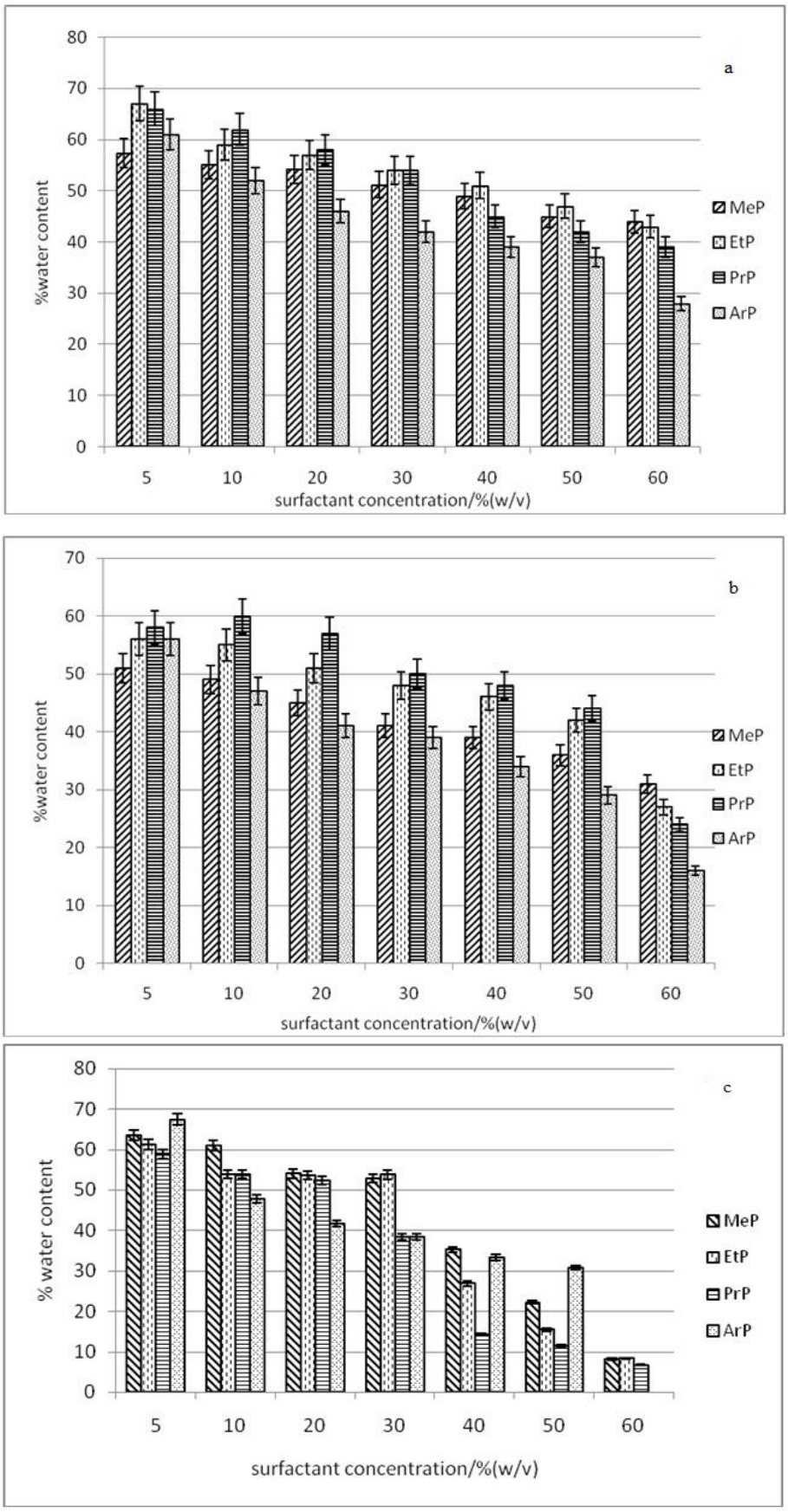


Figure 4.8 Water content in surfactant-rich phase between two methods (a) CPE-DC193C, (b) CPE-DC193C-βCD and (c) CPE-DC193C-βCD-IL

This result clearly shows that CPE-DC193C-βCD-IL method produced the highest reduction of water in the surfactant-rich phase. This amount of water loses is lower compared to the other reported studies (J.-L. Li & Chen, 2003). All methods show decreasing trend in water content when surfactant concentration is increased. This is because the solute-solute interaction between surfactant itself has a greater contribution than the solute-water interaction when the amount of hydrophilic surfactant is getting higher in the solution. Therefore, molecule of surfactant would be more favourable to interact with the same surfactant rather than interact with the water molecules resulting in less amount of water detected in the surfactant-rich phase. This interaction has been verified by some thermodynamic studies (Gardas et al., 2008). The same phenomenon was also reported by Sadeghi et al. (2012). They studied the interaction between polymer surfactant polyethylene glycol with salt or water. They concluded that solute-solute interaction between studied polymer surfactant was greater than the interaction between surfactant with salt or water.

The method using modifier of β-CD or βCD-IL shows the higher loss of water compared with the method without the presence of modifier because β-CD has the ability to form inclusion complex with the surfactant DC193C. This is because eventhough CD is water soluble it simultaneously generates an inner cavity that is relatively hydrophobic (Manakker et al., 2009). Thus, CD can either partially or entirely accommodate suitable size of molecules that are hydrophobic.

In this study, the hydrophobic tail of surfactant DC193C is the most favourable to accommodate in the inner cavity compared to the studied parabens. In addition, the complex conformation between surfactant with modifiers βCD, βCD-IL and parabens make the spaces remain for the water inside or among the micelles are efficiently compressed. Therefore, the amount of water in surfactant-rich phase in CPE-DC193C-βCD-IL and CPE-DC193C-βCD is less than that in CPE-DC193C method.

Moreover, the presence of IL which made the complex conformations between surfactant with βCD-IL and parabens forms a multiple interactions. Therefore water content loss in CPE-DC193C-βCD-IL is the highest compared with CPE-DC193C-βCD and CPE-DC193C methods. This result indicates that the addition of modifiers will improve the performance of CPE-DC193C method efficiently. The details about these proposed mechanisms will be discussed in Section 4.3.9.

4.3.6 Distribution Coefficient

During the phase separation, the solubilized parabens moved with the surfactant micelles and were enriched in the surfactant-rich phase. However, there is still a certain amount of parabens left in the aqueous phase. Figure 4.9 illustrates the distribution coefficient of parabens in the two phases after phase separation. The values of K_d corresponding to the four parabens used are in the order of MeP< EtP< PrP< ArP. This behavior can be explained by considering the interaction between parabens solubilization and aggregation of DC193C micelles with β-CD and βCD-IL.

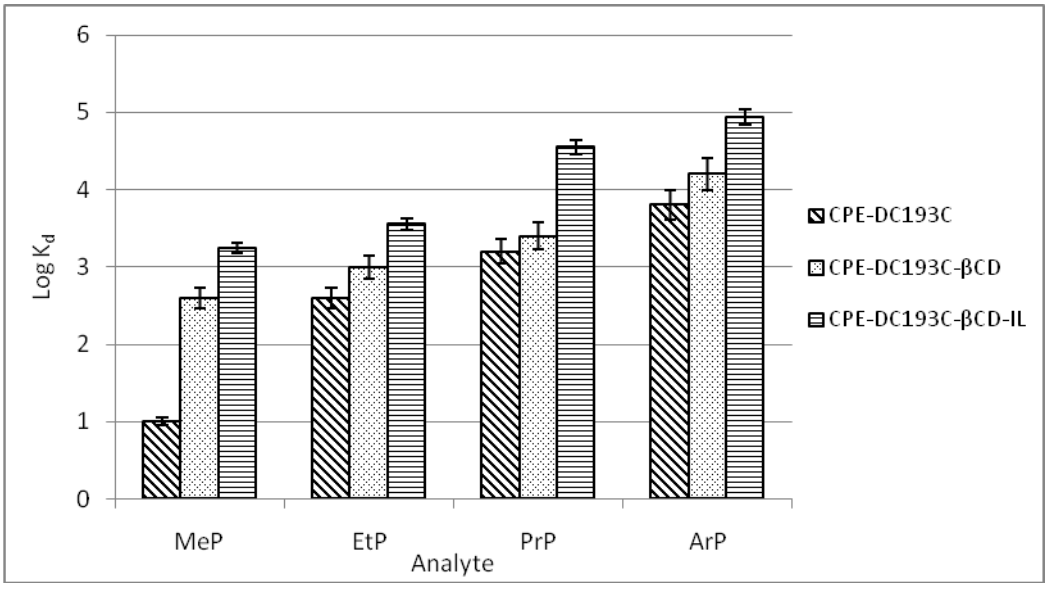


Figure 4.9 Distribution coefficients of parabens studied using CPE-DC193C, CPE-DC193C-βCD and CPE-DC193C-βCD-IL systems.

The obtained results using all the methods show a significantly difference in the distribution coefficient for all the studied analytes. All the methods show the value of log K_d increased when the hydrophobicity of parabens increased, the trend is MeP<EtP<PrP<ArP. It can be explained clearly that the longer the chain the higher the hydrophobicity. Therefore, more hydrophobic analyte can be detected in surfactant-rich phase. MeP shows the lowest value of log K_d for all the methods, because this molecule is less hydrophobic compared with the aromatic ArP. The value of log K_d for method with the presence of β-CD and βCD-IL as modifiers are higher than method without modifier because of the presence of β-CD in the complex. β-CD has the ability to form inclusion complex with the paraben because β-CD has the hydroxyl groups at the outer surface of the molecule, with the primary hydroxyls at the narrow side and secondary hydroxyls at the wider side. Hence it makes β-CD water soluble but simultaneously, it generates an inner cavity that is relatively hydrophobic.

Thus, β-CD can either partially or entirely accommodate suitable size of molecules that are hydrophobic. In this situation, parabens form inclusion complex with the inner part of β-CD. Thus, the capability of β-CD or βCD-IL as modifiers to extract paraben from aqueous phase into surfactant-rich phase is higher. Therefore, the higher percentage recovery of parabens can be detected at the surfactant-rich phase when the phase separations are successfully formed. ArP shows the highest value of log K_d because ArP is easily adsorbed into the cavity of β-CD that is relatively hydrophobic. ArP is the least polar and least soluble in water, therefore ArP can easily adsorb into the cavity of β-CD (Chin et al., 2010). These findings were further correlated with the NMR result where it proves that ArP has been accommodating into the β-CD cavity and this will be discussed in detail in Section 4.3.9.

4.3.7 Phase volume ratio and preconcentration factor on the recoveries of parabens

This study was conducted in order to investigate the effect of phase volume ratio on the preconcentration factor for all the studied systems. Figure 4.10 shows the phase volume ratios for various surfactant concentrations ranging from 5% (w/v) to 60% (w/v). The aim of this study was to get lower phase volume ratio and adequate volume of surfactant-rich phase. It is because lower phase volume ratio will produce a higher preconcentration factor by reducing the final surfactant-rich phase. Accordingly, the higher recoveries of parabens extraction will be obtained.

The CPE-DC193C-βCD-IL system offers an obviously lower phase volume ratio compared to CPE-DC193C-βCD and CPE-DC193C systems with the value of phase volume ratio 0.74, 0.92 and 1.63 respectively at 30% (w/v) surfactant concentration. This is because the amount of water in surfactant-rich phase for CPE-DC193C-βCD-IL is getting smaller when the surfactant concentration is increased due to complex conformation produced between surfactant with modifier CD-IL and parabens. From the experiment, we obtained that at 30% (w/v) surfactant concentration produced a sufficient volume of surfactant-rich phase and this amount is adequate for analyzing using HPLC/UV.

If higher than 30% (w/v) surfactant concentration are used, the volume of surfactant-rich phase is insufficient for analysis using HPLC/UV. This is because at higher concentrations of surfactant, the viscosities of surfactant is also higher thus producing a small volume of surfactant-rich phase. This small volume of surfactant-rich phase is not sufficient to run the sample in HPLC/UV. In addition, the phase separation is also difficult to be observed. If lower than 30% (w/v) surfactant concentrations are used, the volume of surfactant-rich is sufficient for HPLC preparation but not producing

higher percentage recoveries. In this study a 30% (w/v), surfactant concentration was chosen and considered to give sufficient volume of surfactant rich phase and surfactant concentration that can produce higher preconcentration factor.

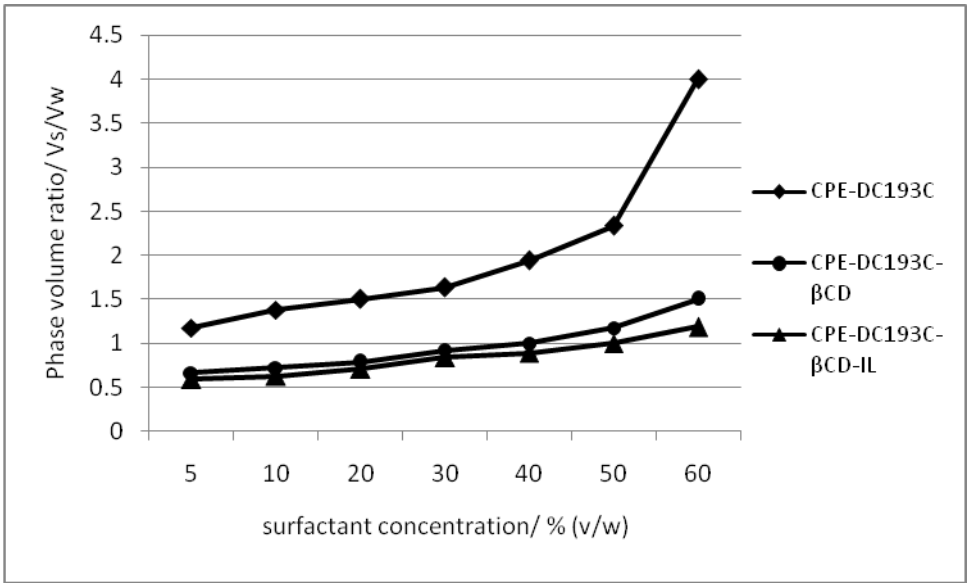


Figure 4.10 Phase volume ratio of MeP using CPE-DC193C, CPE-DC193C-βCD and CPE-DC193C-βCD-IL

Figure 4.11 shows the preconcentration factor of parabens for all systems used. All the methods show an increase in the preconcentration factor with the decrease in the polarity of analyte. The developed method of CPE-DC193C-βCD-IL shows the highest preconcentration factor with the values for MeP, EtP, PrP and ArP are 76, 89, 97 and 110 respectively. The CPE-DC193C-βCD method shows the preconcentration factor with the values for MeP, EtP, PrP and ArP as 74, 82, 87 and 107 respectively. While the preconcentration factor for the developed method with CPE-DC193C as 57, 63, 78 and 85 respectively. Since a higher preconcentration factor is attributed to the smaller phase volume of the surfactant-rich phase, the volume of sample injected in HPLC is highly concentrated and produces good results. It can be concluded that the presence of modifiers will increase the preconcentration factor and hence, produce a more sensitive method on extraction of parabens in aqueous samples.

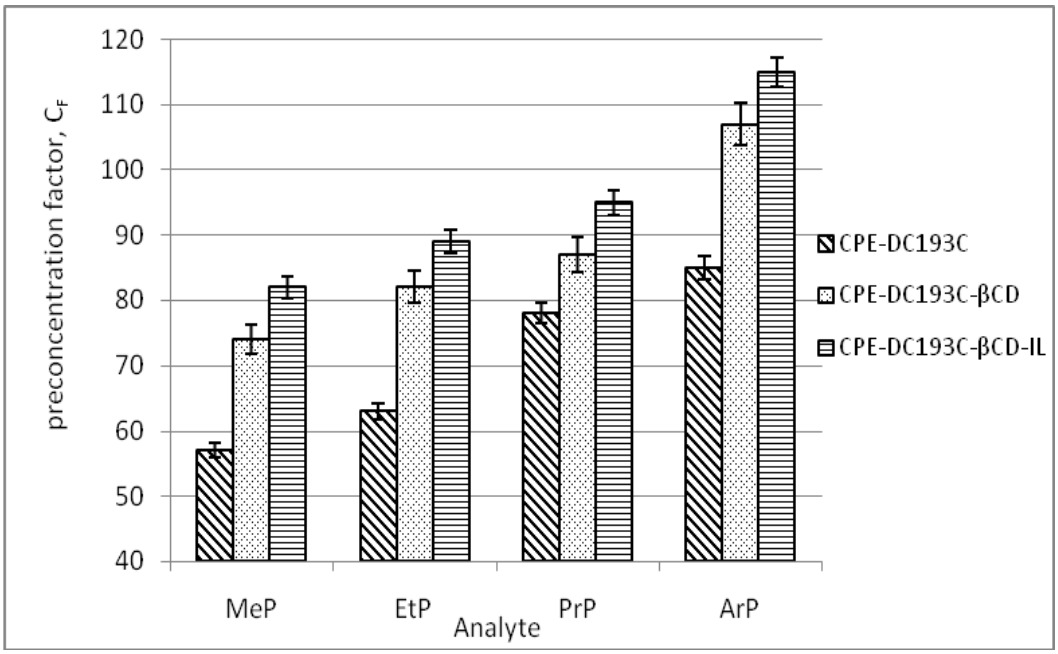


Figure 4.11 Preconcentration factor of paraben studied using CPE-DC193C, CPE-DC193C-βCD and CPE-DC193C-βCD-IL

4.3.8 Method Validation

Based on the method development described above, the performances of all the methods were tested by using real water samples. The relative standard deviations, the coefficient of determination and the limits of detection are shown in Table 4.3 Linear ranges of parabens for CPE-DC193C-βCD and CPE-DC193C-βCD-IL methods were 0.010-0.10 µg mL^{-1}. While linear ranges for CPE-DC193C method were 0.10-1.0 µg mL^{-1}. The addition of βCD-IL system improves the sensitivity of the developed method. For example the LOD of CPE-DC193C-βCD-IL system is lower compared with the CPE-DC193C and CPE-DC193C-βCD method. On the other hand, the preconcentration factor for CPE-DC193C-βCD-IL system is also higher compared with the other two systems. It shows that CPE-DC193C-βCD-IL method has a very high sensitivity and stability and it would have a tremendous potential to be widely used for the analysis of such parabens at trace level.

Table 4.3 Relative standard deviations, coefficient of determination and limits of detection of developed method on determination of parabens from aqueous solution

		CPE-DC193C	CPE-DC193C-βCD	CPE-DC193C-βCD-IL
MeP	RSD (%)(n =3)	0.17	0.6	0.28
	Coefficient of determination, R^2	0.998	0.998	0.99
	LOD (μg/mL)	0.29	0.051	0.038
EtP	RSD (%)(n =3)	0.45	0.44	0.86
	Coefficient of determination, R^2	0.991	0.996	0.991
	LOD (μg/mL)	0.23	0.027	0.026
PrP	RSD (%)(n =3)	0.66	1.4	0.36
	Coefficient of determination, R^2	0.986	0.972	0.993
	LOD (μg/mL)	0.21	0.024	0.016
ArP	RSD (%)(n =3)	0.47	0.76	0.13
	Coefficient of determination, R^2	0.993	0.992	0.991
	LOD (μg/mL)	0.14	0.017	0.013

The proposed CPE-DC193C-βCD-IL method gives better recoveries and almost similar limit of detection as compared to the other procedures (Márquez-Sillero, Aguilera-Herrador, Cárdenas, & Valcárcel, 2010; Núñez, Tadeo, García-Valcárcel, & Turiel, 2008; Núñez, Turiel, Martin-Esteban, & Tadeo, 2010; Ramírez et al., 2011). The cost of acquisition, total extraction time and solvent consumption of the present method are however, lower than those methods. Moreover, the complexity of the analytical systems employed (Zhang et al., 2005) and sample pretreatment required (Blanco et al.,

2009; Labat et al., 2000) in those procedures are comparable with the developed CPE-DC193C method.

The developed systems are applied to the determination of the recoveries of parabens from various of water samples i.e. river, treated, sea and tap water. The results are summarized in Table 4.4. The spiked parabens concentrations in the real samples for CPE-DC193C-βCD and CPE-DC193C-βCD-IL methods are 0.01 μgmL^{-1} and CPE-DC193C method is 0.1 μgmL^{-1}. The percentage recoveries using CPE-DC193C are between 71.2% and 97.7% with relative standard deviation (RSD) less than 1.0%. While the recovery rates for CPE-DC193C-βCD are between 90.3% and 98.9%. Whereas, the recovery rates using CPE-DC193C-βCD-IL are between 91.2% and 100%. The following results show that the developed method of CPE-DC193C-βCD-IL has a satisfactory recovery for the determination of parabens from aqueous solutions. Thus it is feasible to be used for monitoring parabens compound in environmental water samples.

A lower percentage of recovery was obtained for sea water sample when using CPE-DC193C method compared to the other water samples. This is probably due to the electrolyte factor (salt concentration) from sea water interrupting the CPE-DC193C method. Fortunately, this matrix effect only involved two analytes, MeP and EtP. This problem has been solved by using CPE-DC193C-βCD and CPE-DC193C-βCD-IL systems where the results show that recovery rate of parabens extraction in sea water samples is higher compared with CPE-DC193C. Thus, the presence of modifiers βCD-IL and β-CD is significant in improving the CPE systems investigated.

Table 4.4 Recovery of developed methods of parabens in spiked water samples

Method	Analyte	River water Recovery,% RSD,%	Tap water Recovery,% RSD,%	Treated water Recovery,% RSD,%	Sea water Recovery,% RSD,%
CPE-DC193C	MeP	96.2 (0.47)	83.8(0.59)	85.9 (0.2)	72.1 (0.62)
	EtP	93.8 (0.15)	96.3 (0.76)	87.7 (0.30)	71.2 (0.55)
	PrP	97.7 (0.63)	93.3 (0.26)	94.3 (0.40)	87.9 (0.23)
	ArP	89.5 (0.29)	80.6 (0.67)	85.6 (0.34)	85.8 (0.40)
CPE-DC193C-βCD	MeP	98.9 (0.70)	90.9 (0.60)	91.8 (0.63)	90.5 (0.17)
	EtP	92.1 (0.60)	97.9 (0.80)	90.3 (0.90)	91.3 (0.60)
	PrP	96.0 (0.30)	94.5 (0.90)	95.2 (0.40)	93.7 (0.90)
	ArP	90.5 (0.27)	91.3 (0.60)	94.9 (0.65)	92.5 (1.30)
CPE-DC193C-βCD-IL	MeP	97.5 (0.35)	92.3 (0.26)	97.8 (0.22)	96.2 (0.32)
	EtP	98.9 (0.80)	94.9 (0.83)	92.9 (0.96)	91.2 (0.72)
	PrP	97.4 (0.15)	97.8 (0.42)	96.1 (0.48)	93.2 (0.64)
	ArP	97.6 (0.63)	95.5(0.34)	100.0 (0.49)	98.2 (0.68)

4.3.9 Extraction behavior of ArP and DC193 C with βCD and βCD-IL

The analysis of inclusion complexes between β-CD, βCD-IL, ArP and surfactant DC193C is studied in this work since the cavity of β-CD was maintained during the extraction process. Furthermore the findings suggest that the inclusion complex is one of the main interactions that take place between β-CD, βCD-IL, ArP and DC193C in the extraction process. The evaluation geometry of inclusion formation of β-CD, ArP and DC193C, ^{1}H NMR (Figure 4.12) and 2D NOESY measurements (Figure 4.13) (DMSO-D6, 25°C, 600 MHz) were performed. The obvious upfield shifts of the protons on the inner cavity of β-CD (H3, 3.5349 ppm and H5, 3.5200) were observed. This change indicates that the DC193C or ArP has been entered deeply into the cavity of CD. Based on the results obtained (Table 4.5) the protons of DC193C and ArP were found to be shifted upon the formation of inclusion complex (βCD-DC193C-ArP). Meanwhile, H5 proton of β-CD changes from doublet to singlet upon the formation of inclusion complex as shown in Figure 4.12.

The presence of ^{1}H signals belonging to both β-CD, ArP and DC193C molecules could be observed in ^{1}H NMR spectrum of βCD-DC193C-ArP which strongly suggests that the new inclusion complex has been formed. Since in this study there are two guest compounds (DC193C and ArP), it is necessary to investigate further with 2D NMR in order to predict which one enters into the cavity of CD. There are a few NMR techniques that can provide supporting evidence for specific structures in cyclodextrin complexes. 2D-NOESY and 2D-ROESY experiments give rise to cross peaks between dipolarly coupled spins (Neuhaus & Williamson, 2000; Sanders & Hunter, 1993), in order to indicate the close proximity between atoms in the two components of the complex. In addition, 2D NOESY and 2D ROESY experiments provide an upper limit (ca. 5Å) on the distance between protons that produce cross peaks under favorable conditions.

The formation of inclusion complex was further proven by the 2D-NOESY analysis (Figure 4.13) since 2D NMR is a powerful tool to investigate intermolecular interactions and to gain more information on the conformation of the inclusion complex (J. Li et al., 2003). The cross peaks in the spectra, indicated in Figure 4.13 originate from the interaction of the protons of DC193C, ArP and β-CD. The cross peaks of β-CD (3.5–3.6 ppm, H-3, H-5) and DC193C (Ha-s, 0.4621 ppm, Hb-s, 0.0508 ppm, Hc-s, 0.8058 ppm, Hd-s, 1.4625) demonstrate a strong intensity. Hence, from the 2D NOESY spectra, we can conclude that DC193C has been accommodated in the β-CD cavity and may be within less than 5 Å apart from H3 and H5 of CD. Apart from that, 2D NOESY also shows interactions between β-CD and ArP. The cross peak (Ha-p, Hb-p, Hc-p, He-p, Hf-p) shows an interaction with β-CD (3.5–3.6 ppm, H-3, H-5) and it further supports that ArP has been accommodated in the cavity of CD. Hence the possible formations of the inclusion complex structure of complexation of deprotonated ArP and DC193C with

β-CD are shown in Figure 4.14 and Figure 4.15 and have been proposed by taking account of the hydrogen bonding between DC193C and deprotonated ArP.

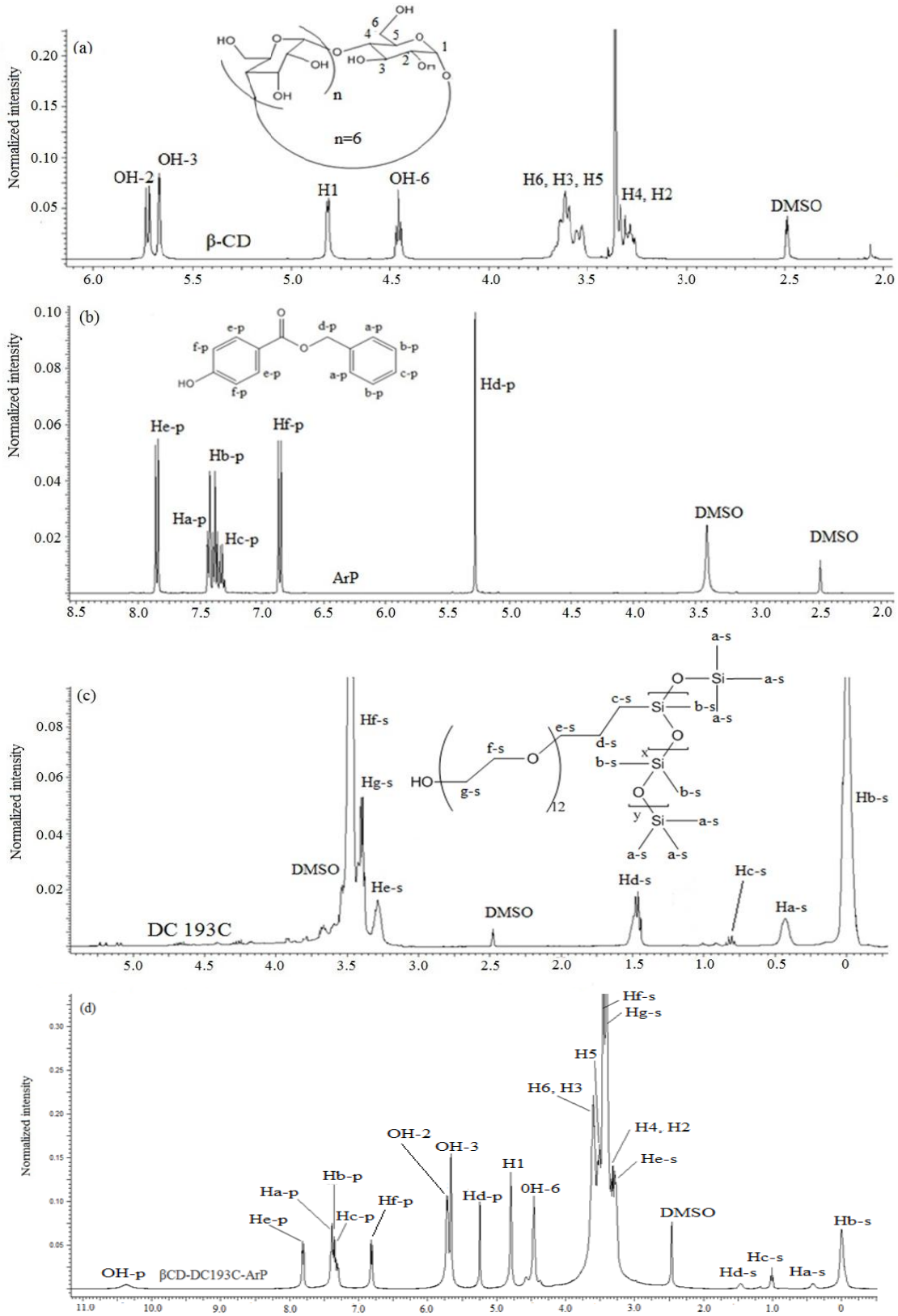


Figure 4.12 NMR spectrum of a) β-CD, b) ArP, c) DC193C and d) βCD-DC193C-ArP

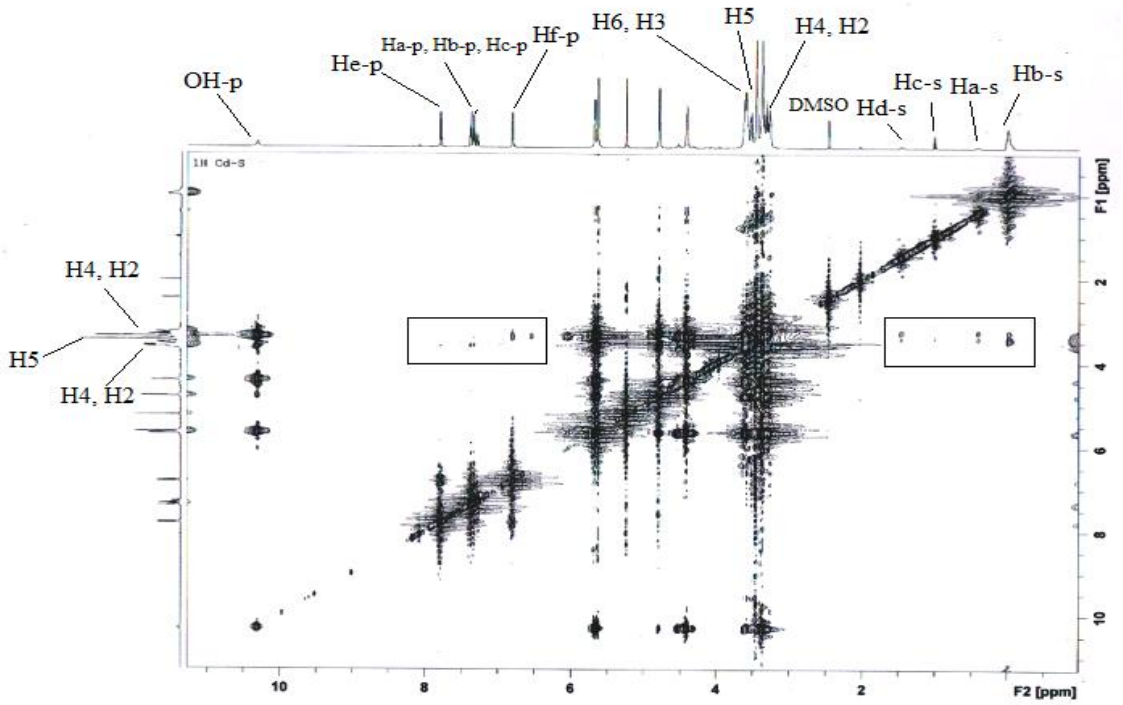


Figure 4.13 Two-dimensional NOESY spectrum of βCD-DC193C-ArP complex in DMSO-D_6

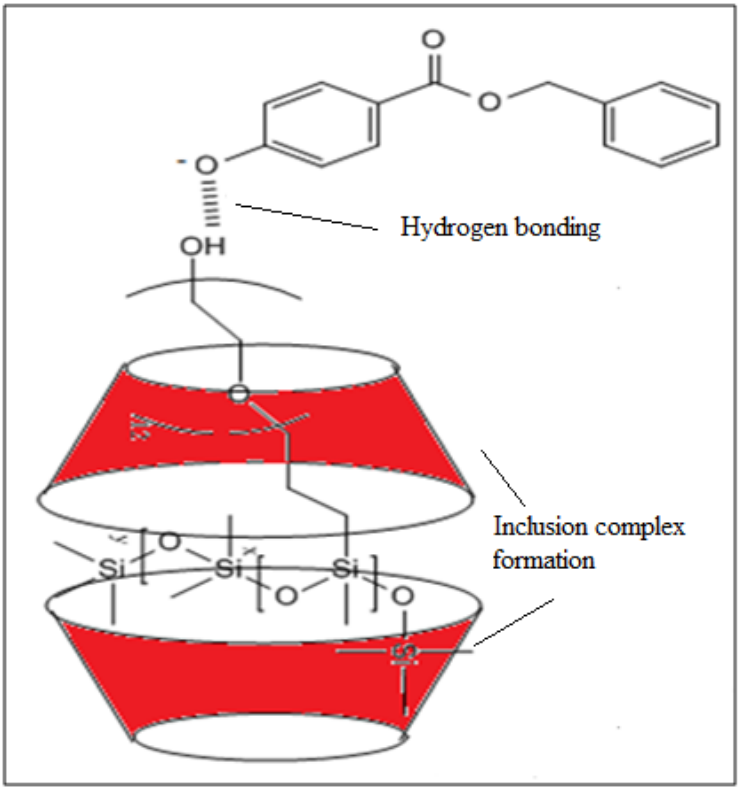


Figure 4.14 Schematic illustration of the complexation of ArP and DC193C with β-CD

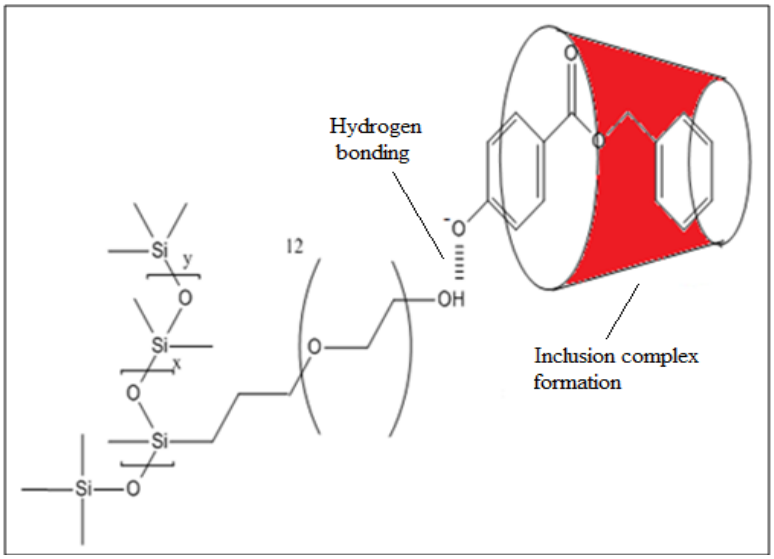


Figure 4.15 Schematic illustration of the complexation of ArP and DC193C with β-CD

Table 4.5 ^{1}H NMR chemical shift (§) of β-CD, ArP and βCD-DC193C-ArP

	B-CD	ArP	βCD-DC193C-ArP	
	δ	δ	δ	Δ δ
H1	4.8191		4.7967	-0.0224
H2	3.3119		3.3161	-0.0042
H3	3.5987		3.5349	-0.0638
H4	3.3656		3.3744	-0.0088
H5	3.5517		3.5200	-0.0317
H6	3.6176		3.6039	-0.0137
Ha-p		7.4200	7.3823	-0.0377
Hb-p		7.3770	7.3441	-0.0329
Hc-p		7.3370	7.2895	-0.0475
Hd-p		5.2780	5.2717	-0.0063
He-p		7.8530	7.8090	-0.0440
Hf-p		6.8730	6.8021	-0.0709
		DC193C	βCD-DC193C- ArP	
		δ	δ	Δδ
Ha-s		0.4320	0.4621	+0.0301
Hb-s		0.0305	0.0508	+0.0203
Hc-s		0.8262	0.8058	-0.0202
Hd-s		1.4401	1.4625	+0.0224
He-s		3.3780	3.2573	-0.1207
Hf-s		3.5257	3.3744	-0.1513
Hg-s		3.4037	3.4588	+0.0551

In order to evaluate the geometry of inclusion formation of βCD-IL, ArP and DC193C, ^{1}H NMR (Figure 4.16) and 2D NOESY measurements (Figure 4.17) (DMSO-D6, 25°C, 600 MHz) were performed on an AVN600 spectrometer. The obvious upfield shifts of the protons on the inner cavity of βCD-IL (H3, 3.5483 ppm and H5, 3.4848) were observed. This change indicates that the DC193C or ArP has been entered deeply into the cavity of CD. Based on the results obtained (Table 4.2) the protons of DC193C and ArP were found to be shifted upon the formation of inclusion complex (βCD-IL-DC193C-ArP). Meanwhile, H5 proton of βCD-IL changes from doublet to singlet upon the formation of inclusion complex as shown in Figure 4.16.

The presence of ^{1}H signals belonging to both βCD-IL, ArP and DC193C molecules could be observed in ^{1}H NMR spectrum of βCD-IL-DC193C-ArP which strongly suggests that the new inclusion complex has been formed. Since in this study there are two guest compounds (DC193C and ArP), it is necessary to investigate further with 2D NMR in order to predict which one enters into the cavity of CD. The formation of inclusion complex was further proven by the 2D-NOESY analysis (Figure 4.17) since 2D NMR is a powerful tool for investigating intermolecular interactions and to gain more information on the conformation of the inclusion complex (J. Li et al., 2003). The cross-peaks in the spectra, indicated in Figure 4.17 originate from the interaction of the protons of DC193C, ArP and βCD-IL. The cross peaks of βCD-IL (3.5–3.6 ppm, H-3, H-5) and DC193C (Ha-s, 0.4314 ppm, Hb-s, 0.0001 ppm, Hd-s, 1.4795 ppm) demonstrate strong intensity. Hence, from the 2D NOESY spectra we can conclude that DC193C has been accommodated in the β-CD cavity and may be within less than 5 Å apart from H3 and H5 of CD. Apart from that, 2D NOESY also shows interactions between βCD-IL and ArP. The cross peak (Hd-p, 5.2623 ppm; Hf-p, 6.8256 ppm) shows an interaction with βCD-IL (3.5–3.6 ppm, H-3, H-5) and it further supports that ArP has been accommodated in the cavity of CD.

Apart from that, the cross peak around 6-8 ppm which belongs to βCD-IL and ArP shows that there is a interaction between the imidazolium ring and ArP (Figure 4.18) and this could be due to the π-π interaction and electrostatic attraction. Hence the possible formations of the inclusion complex structure of the complexation of ArP and DC193C with βCD-IL are shown in Figure 4.18 and Figure 4.19 and have been proposed by taking account of the hydrogen bonding between DC193C and deprotonated ArP, pi-pi attraction, electrostatic attraction between imidazolium ring and ArP, also the inclusion complex between βCD-IL with DC193C and βCD-IL with ArP. While the possible formations for the structure of complexation of deprotonated ArP with DC193C are shown in Figure 4.20. The possible interaction between the deprotonated ArP with DC193C is hydrogen bonding.

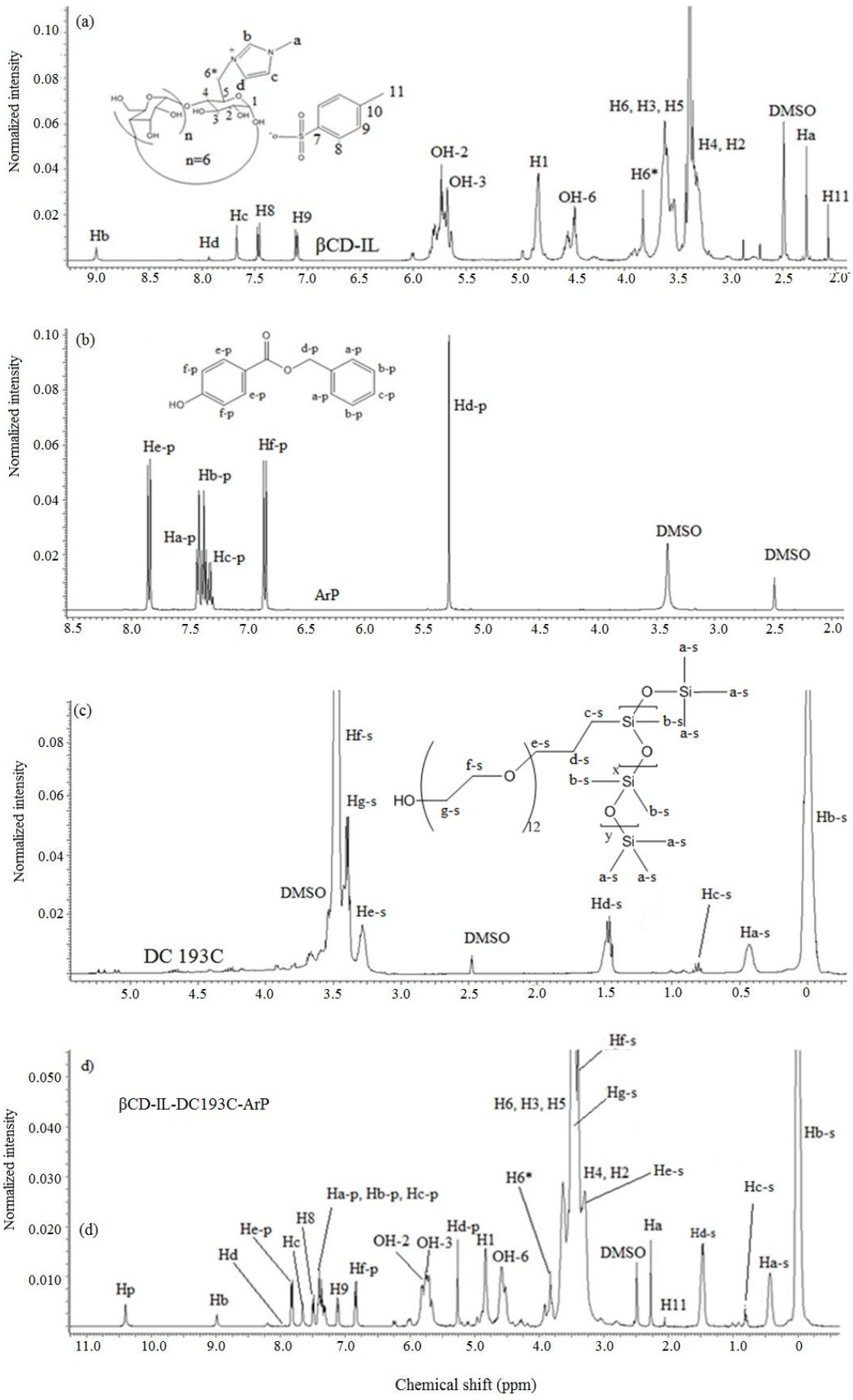


Figure 4.16 NMR spectrum of a) βCD-IL, b) ArP, c) DC193C d) βCD-IL-DC193C-ArP

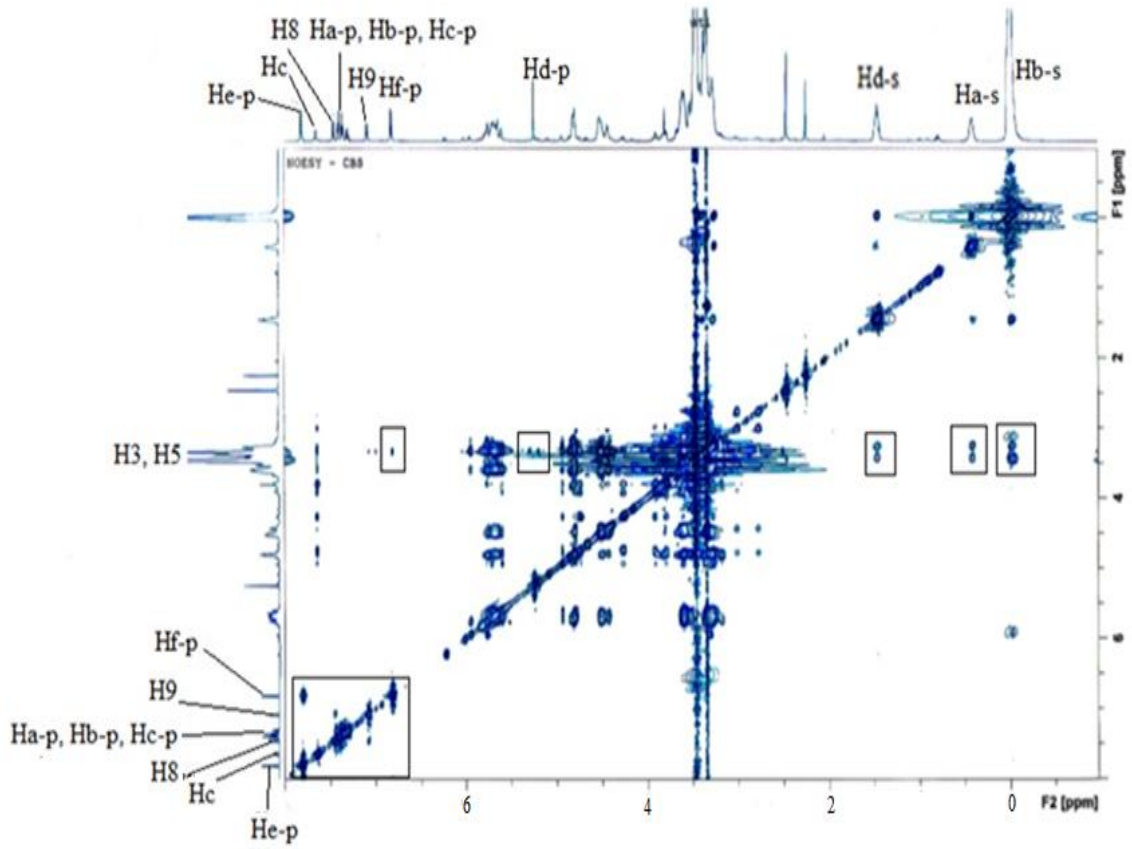


Figure 4.17 Two-dimensional NOESY spectrum of βCD-IL-DC193C-ArP complex in DMSO-D6

Table 4.6 ^{1}H NMR chemical shift (§) of βCD-IL, ArP and βCD-IL-ArP

	βCD-IL	ArP	βCD-IL-ArP-DC193C	
	δ	δ	δ	Δδ
H1	4.8191		4.8315	+0.0124
H2	3.3119		3.3445	+0.0326
H3	3.5987		3.5483	**-0.0504**
H4	3.3656		3.3945	+0.0289
H5	3.5517		3.4848	**-0.0669**
H6	3.6176		3.6331	+0.0155
H8	7.4545		7.4847	+0.0302
H9	7.0939		7.1057	+0.0118
H11	2.0677		2.0594	-0.0083
Ha	2.2752		2.2760	+0.0008
Hb	9.0063		8.9827	-0.0236
Hc	7.6730		7.6586	-0.0144
Hd	7.9300		7.9500	+0.0200
Ha-p		7.4200	7.4023	-0.0177
Hb-p		7.3770	7.3669	-0.0101
Hc-p		7.3370	7.3272	-0.0098
Hd-p		5.2780	5.2623	-0.0157
He-p		7.8530	7.8154	**-0.0376**
Hf-p		6.8730	6.8256	**-0.0474**
		DC193C	βCD-IL-ArP-DC193C	
		δ	δ	Δδ
Ha-s		0.4320	0.4314	-0.0006
Hb-s		0.0305	0.0001	**-0.0304**
Hc-s		0.8262	0.7951	-0.0238
Hd-s		1.4401	1.4795	**-0.0384**
He-s		3.3780	3.2938	**-0.0842**
Hf-s		3.5257	3.4848	**-0.0414**
Hg-s		3.4037	3.3945	-0.0092

It is clearly shown method with β-CD as a modifier shown double interactions which are hydrogen bonding and inclusion complex formation. While in method with βCD-IL as a modifier has shown multiple interaction which are hydrogen bonding, pi-pi attraction, electrostatic attraction and inclusion complex formation. On the other hand, methods without modifier show only a single attraction which is hydrogen bonding. This multiple interaction make the stronger attractions between ArP, βCD-IL and surfactant hence higher distribution coefficients and preconcentration factor of parabens obtained in surfactant-rich phase. These multiple interactions also contribute to a sensitive developed method in extraction of paraben in aqueous samples.

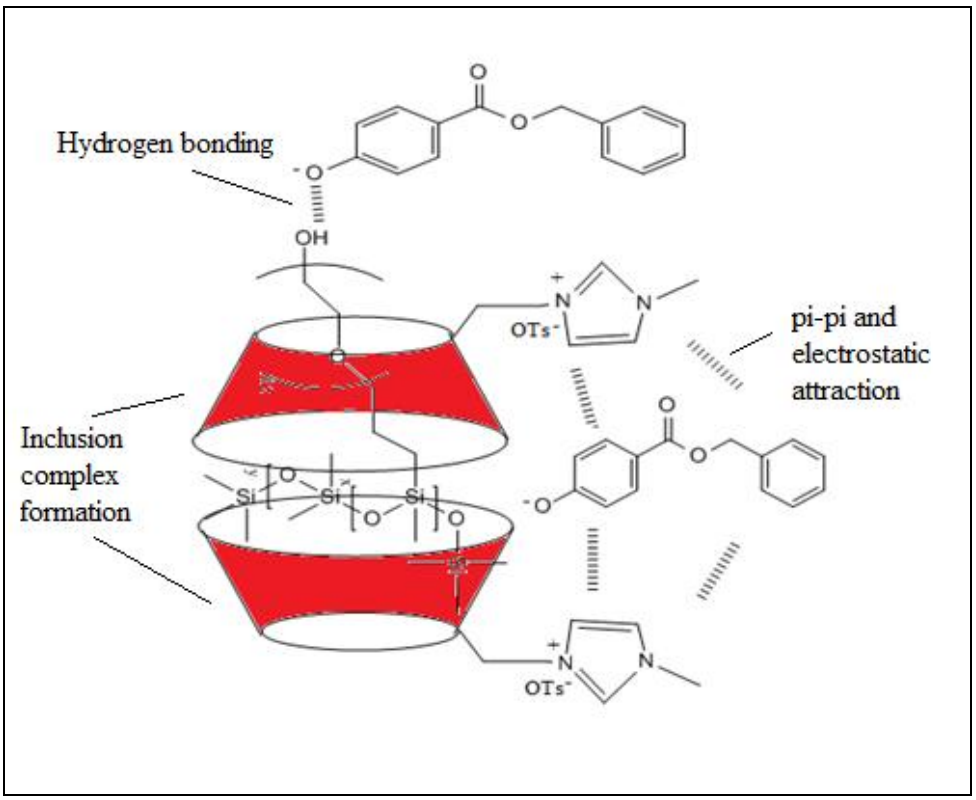


Figure 4.18 Schematic illustration of the pH-dependent complexation of ArP and DC193C with βCD-IL

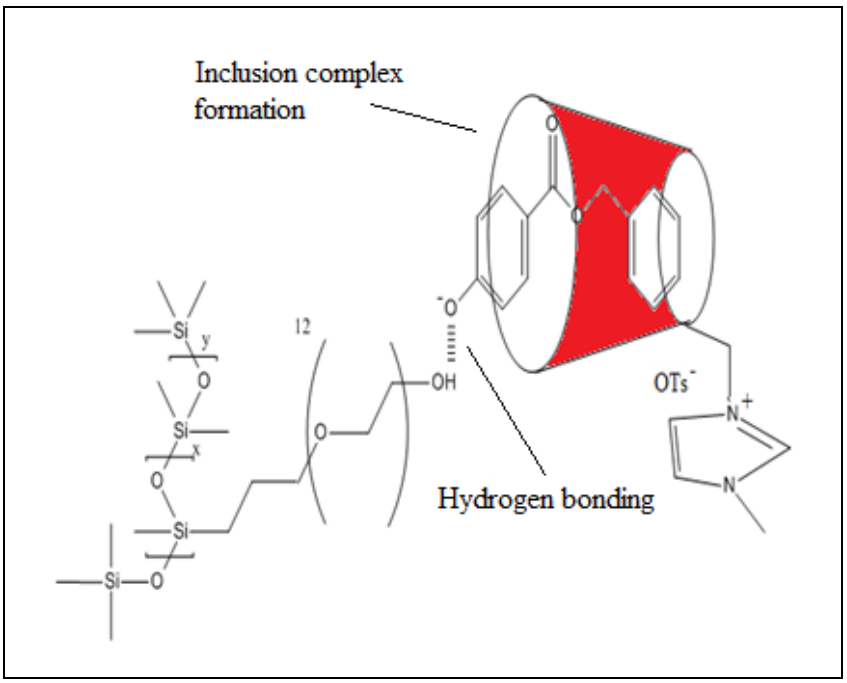


Figure 4.19 Schematic illustration of the complexation of ArP and DC193C with βCD-IL

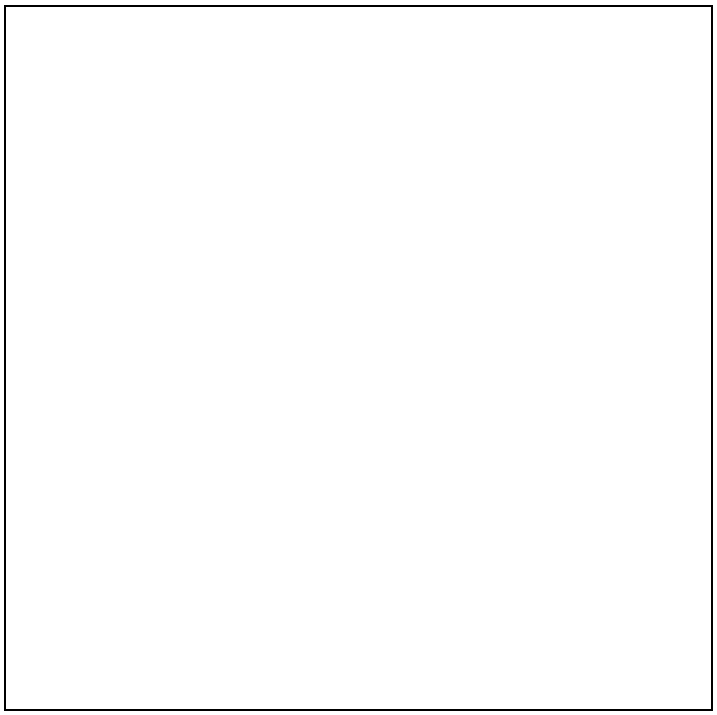


Figure 4.20 Schematic illustration of the complexation of ArP with DC193C

4.4 Conclusions

This study demonstrates that the CPE-DC193C-βCD-IL consisting of βCD-IL as a modifier is suitable for the extraction of parabens from various water samples compared with the other two developed methods. βCD-IL and βCD as modifiers improved the selectivity and sensitivity of CPE-DC193C however βCD-IL shows a more excellent modifier in these CPE-DC193C methods. The addition of βCD-IL improves the sensitivity of the developed methods because the experimental results demonstrated that the detection limits for studied paraben using CPE-DC193C-βCD-IL method were lower compared with CPE-DC193C-βCD and CPE-DC193C methods. In addition, the preconcentration factor and water content loses of CPE-DC193C-βCD-IL also higher compared with the other two methods. This is because the complex formations of βCD-IL with the surfactant molecules and paraben in the surfactant-rich phase are extra large in the formation of micelles during the CPE process. So the spaces remained for the water inside or among the micelles were efficiently compressed. Therefore, the amount of water content that has been extracted in the surfactant-rich phase is reduced compared to the other two methods. On the other hand, the addition of βCD-IL also improves the selectivity of the developed method. For examples the matrix effect is totally reduced in the CPE-DC193C method when low recoveries of parabens in sea water using CPE-DC193C are dramatically improved with the addition of βCD-IL in the CPE-DC193C-βCD-IL method. Moreover, the distribution coefficient of paraben in surfactant-rich phase also followed the hydrophobicity of parabens. It is show that βCD-IL plays an important role in giving a higher distribution of parabens in surfactant -rich phase. By taking into account the results obtained for the CPE-DC193C-βCD-IL and CPE-DC193C-βCD systems, it can be concluded that it is an economically viable to use βCD-IL because it improve the performance of CPE method dramatically as well as this chemical is cheap and not toxic to our environment.

CHAPTER 5: Conclusions

In this research, an extraction system for parabens has been investigated. They are ionic liquid-aqueous two phase system (IL-ATPS), ionic liquid-aqueous two phase system with addition of β-cyclodextrin as modifier (IL-βCD-ATPS), cloud point extraction using non-ionic surfactant (CPE-DC193C), cloud point extraction with β-cyclodextrin as modifier (CPE-DC193C-βCD) and cloud point extraction with β-cyclodextrin functionalized of ionic liquid as modifier (CPE-DC193C-βCD-IL). These systems were applied on the extraction of four parabens, i.e. MeP, EtP, PrP and ArP from water samples. A few parameters, i.e. salt concentration, surfactant DC193C concentration, IL concentration, β-CD concentration, βCD-IL concentration, temperature and pH of the solution were evaluated to get the optimum conditions for phase separation of parabens. The optimum separation of the five systems were successfully obtained and applied on the spiked parabens in water samples. Four types of water samples were collected from various places in Malaysia which are river water, sea water, treated water and tap water.

These new, green, fast and simple extraction techniques coupled with a reversed-phase high performance liquid chromatography (RP-HPLC), showed excellent results in extracting parabens from aqueous samples. Water content in IL-βCD-ATPS method showed higher loses of water content due to the complex formation between IL, the β-CD molecules and paraben in the IL-rich phase which was thought present in the form of micelles during the aqueous two-phase system process. Thus, the spaces available for water within or among the micelles were efficiently compressed.

Therefore, the amount of water that has been extracted in the IL-rich phase was reduced compared to the IL-ATPS.

Other than that, distribution coefficient results using the IL-ATPS method showed only a slight difference for all the studied analytes compared to the IL-βCD-ATPS method. The latter method showed a mark increase in the distribution coefficients when the hydrophobicity of parabens increased. The values of K_d in the IL-βCD-ATPS for the studied parabens increased in the order of MeP< EtP< PrP< ArP. The highest result was obtained for ArP because this analyte could be easily adsorb into the cavity of β-CD which is relatively hydrophobic and ArP is the least polar and the least soluble in water. The results showed that the distribution of parabens in IL-rich phase for IL-βCD-ATPS method depended on hydrophobicity of the parabens whereas in the IL-ATPS method hydrophobicity was not an important factor that affects the extraction of parabens.

Phase volume ratio of IL-βCD-ATPS showed the lowest value compared to IL-ATPS. This was due to the addition of β-CD in the system that made the phase formation become easier. Experimental observation showed that when 30% (w/v) IL concentration was used, the phase separation between the IL-rich phase and the aqueous phase was clearly seen in the centrifuge tube. It was clearly shown that the highest preconcentration factor in these two methods is for ArP, followed by PrP, EtP and lastly MeP. IL-βCD-ATPS obtained the highest preconcentration factor with values for MeP, EtP, PrP and ArP were 70, 86, 95 and 103 respectively. The results were higher than the preconcentration factors obtained with IL-ATPS which were 48, 78, 85 and 95 for MeP, EtP, PrP and ArP respectively. The excellent results obtained by IL-βCD-ATPS were due to low water content in IL-rich phase, V_s, which reduced the volume of final IL-rich phase.

In CPE-DC193C-βCD-IL method, the measured total loss of water content was 68% when the surfactant concentration was increased from 5% (w/v) to 60% (w/v). This is considered as the highest loses of water content compared to the CPE-DC193C-βCD and CPE-DC193C systems. The overall loses of water content for MeP was 55%, followed by EtP and PrP with 52% each. The CPE-DC193C-βCD-IL system offered an obviously lower phase volume ratio compared to CPE-DC193C-βCD and CPE-DC193C systems with the value of phase volume ratios were 0.74, 0.92 and 1.63 respectively at 30% (w/v) surfactant concentration. The developed method of CPE-DC193C-βCD-IL showed the highest preconcentration factor with the values for MeP, EtP, PrP and ArP were 76, 89, 97 and 110 respectively. Moreover, the distribution coefficient of parabens in surfactant-rich in the order of hydrophobicity of parabens was MeP<Etp<PrP<ArP. Again, the highest distribution coefficient values for all studied parabens were using CPE-DC193C-βCD-IL system. In conclusion, βCD-IL as modifier contributes to a higher distribution of parabens in surfactant-rich phase compared to the other methods.

By comparing CPE-DC193C-βCD-IL and IL-βCD-ATPS systems, the good and efficient system is CPE-DC193C-βCD-IL. This is because in CPE-DC193C-βCD-IL system has shown multiple interactions which are hydrogen bonding, pi-pi attraction, electrostatic attraction and inclusion complex formation. This multiple interaction in CPE-DC193C-βCD-IL method make the stronger attractions between ArP, βCD-IL and surfactant hence higher distribution coefficients and preconcentration factor of parabens can be obtained in surfactant-rich phase. While IL-βCD-ATPS method shows only double interactions which are electrostatic attraction and inclusion complex formation.

At last, we can conclude that βCD-IL as modifier improves the performance of parabens extraction from water samples. This is because additions of βCD-IL as modifier in the CPE system improves the water content in the surfactant-rich phase,

lower phase volume ratio, higher preconcentration factor and higher distribution coefficient.

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