

ELECTROSPUN NYLON 6,6 NANOFIBERS FUNCTIONALIZED WITH β -CYCLODEXTRIN
AS SOLID PHASE EXTRACTION SORBENT FOR DETERMINATION OF PARABENS



A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Petrochemistry and Polymer Science

Field of Study of Petrochemistry and Polymer Science

FACULTY OF SCIENCE

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เส้นใยระดับนาโนเมตรอิเล็กทรอนิกส์แบน 6,6 ดัดแปรหมู่ฟังก์ชันด้วยปีตาไซโคลเดกซ์ทรินเพื่อ
เป็นตัวดูดซับในการสกัดด้วยวิทยาของแข็งสำหรับการตรวจวัดพาราเบน



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PHASE EXTRACTION SORBENT FOR DETERMINATION
OF PARABENS

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In this study, electrospun nanofibers of nylon 6,6 functionalized with β -cyclodextrin (nylon 6,6/ β -CD) were fabricated and applied as a sorbent in solid-phase extraction (SPE) for determination of parabens in water. The electrospinning parameters such as polymer concentration, nylon 6,6/ β -CD ratio, and electric field strength were investigated in order to obtain uniform nanofibers. The morphology and physical properties of obtained fibers were characterized by scanning electron microscope (SEM), Fourier transforms infrared spectroscopy (FTIR), and BET surface area analysis. The electrospun nylon 6,6/ β -CD nanofibers were achieved with diameter in the range of 120 - 150 nm. The additional of β -CD slightly increased the fiber diameter but decreased the surface area. Then, the nylon 6,6/ β -CD nanofibers were examined as a sorbent in solid-phase extraction of parabens in water. The optimal condition is 10.0 ± 0.5 mg of nanofibers as a sorbent, 15 mL of sample volume, and 200 μ L of methanol for elution solvent. Under the optimal conditions, the linear range of parabens analysis was in the range from 25-200 μ g.L⁻¹ with a detection limit of 10 μ g.L⁻¹ and a limit of quantitation of 25 μ g.L⁻¹. The results of the validation method had a correlation coefficient greater than 0.9997, with %recovery of 40.6%, 41.5%, 81.4%, and 61.3% for iso-butyl paraben, n-butyl paraben, benzyl paraben, and pentyl paraben, respectively. Finally, the developed method is used for the determination of parabens in the drinking water sample.

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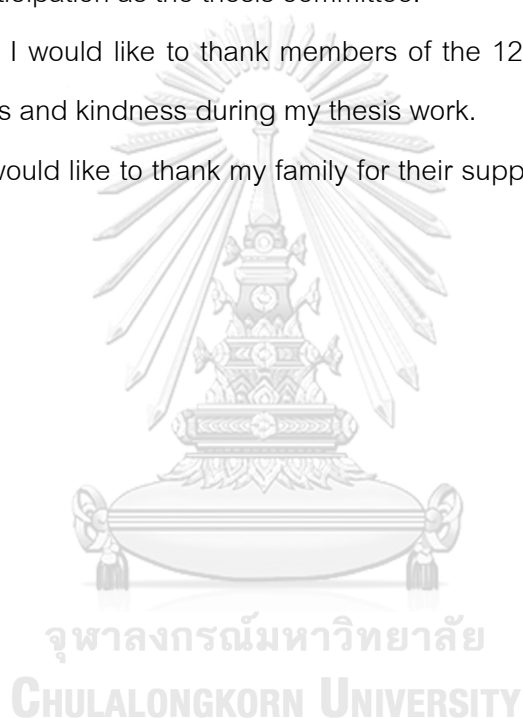


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CHAPTER I INTRODUCTION

1.1 Statement of purpose

Parabens are alkyl esters of p-hydroxybenzoic acid (PHBA) that widely used as preservatives in foods, pharmaceuticals, cosmetics, beverages, and personal care products (PCPs) to prolong shelf life and maintain sterility of the products. Parabens are good preservatives due to their broad-spectrum antimicrobial properties, capability, highly stable in regard to variation in pH, lack of distinguishable odor, taste, discoloration, non-volatility, good stability, low cost, and relatively safe to use (Table 1.1) [1-3]. The general structure of paraben is shown in Figure 1.1. The antimicrobial activity of parabens increases with an increase in the length of the alkyl chain of the ester group [4]. However, esters with a long alkyl chain are of limited applications due to their lower solubility in water [5].

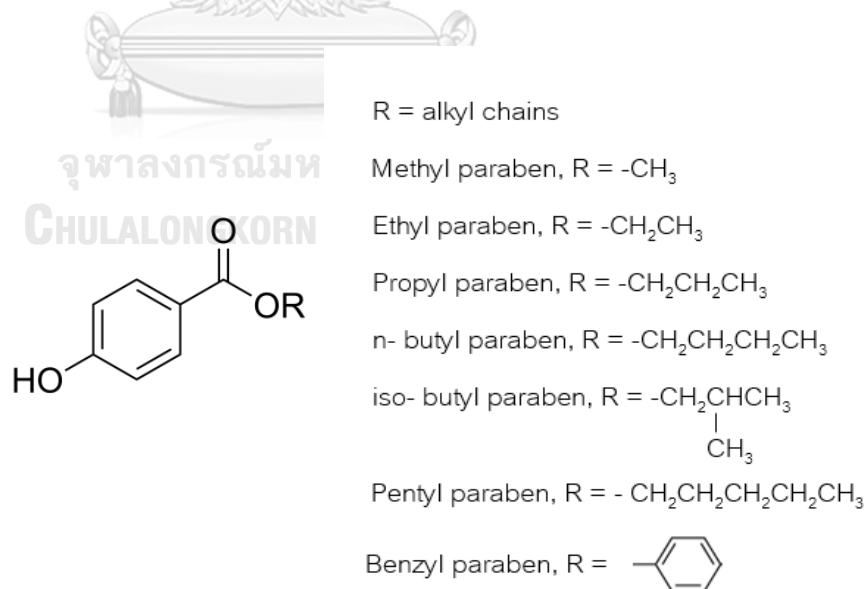
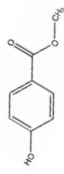
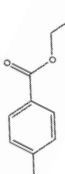
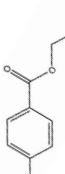
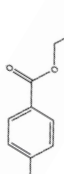
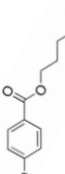
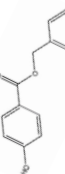


Figure 1.1 Chemical structures of paraben compounds.

Table 1.1 Chemical and physical properties of parabens [1].

| Compound | Methyl paraben (MP) | Ethyl paraben (EP) | Propyl paraben (PP) | Butyl paraben (BuP) | Pentyl paraben (PeP) | Benzyl paraben (BzP) |
|--------------------------|---|---|---|--|---|---|
| Structure |  |  |  |  |  |  |
| Physical form | Powder | Crystals or powder | Crystals or powder | Crystals or powder | Crystals or powder | Crystals or powder |
| Color | Colorless or white | Colorless or white | Colorless or white | White | White to off white | White |
| Molecular formula | $C_8H_8O_3$ | $C_9H_{10}O_3$ | $C_{10}H_{11}O_3$ | $C_{11}H_{14}O_3$ | $C_{12}H_{16}O_3$ | $C_{14}H_{12}O_3$ |
| Molecular weight (g/mol) | 152.16 | 166.18 | 180.23 | 194.23 | 208.25 | 228.25 |
| Solubility(g/L) | 2.00 | 0.86 | 0.30 | 0.15 | - | 0.05 |
| pKa | 8.17 | 8.22 | 8.35 | 8.37 | 8.22 | 8.18 |
| Log P | 1.66 | 2.19 | 2.17 | 3.24 | - | 3.56 |

Recently, several studies shown that parabens are involved in the occurrence of breast cancer in females [2], shown endocrine disrupting effects and related with male infertility. When parabens enter to human body, they work like estrogen hormones and hinder the function of hormones that produced by the endocrine glands. As a result, disorders of various systems or organs in the body that works under the control of hormones from the endocrine glands, occur [3-5].

Furthermore, parabens are able to bind with estrogen and androgen receptor, activate estrogen-responsive genes, stimulate cellular proliferation, and increase levels of estrogen receptor protein [6]. The European Food Safety Authority (EFSA) established an acceptable daily intake (ADI) of 0-10 mg/kg (body weight) bw-day for the sum of methyl paraben, ethyl paraben and their sodium salts. Regarding penthyl paraben and butyl paraben, the EFSA has not estimated an ADI [7]. In the same way, there are currently no regulations regarding the presence of parabens in the environment or, in particular, in drinking water or wastewater [8]. Eventhough, there are very few data on the presence of paraben in drinking water. But recently, methyl paraben (40 ng.L^{-1}), propyl paraben (23 ng.L^{-1}) were detected in bottled water [9]. Therefore, the study of parabens detection at trace level (ng.L^{-1}) is required.

Reversed-phase HPLC (RP-HPLC) is a preferable technique for analysis of parabens because its complementary, and suitable for nonvolatile compound. However the analysis at low concentration requires the extraction and preconcentration steps before analysis by HPLC. Among the extraction methods, solid phase extraction (SPE) is the most attention owing to its advantages like low cost, convenience, simplicity, and time savings [10]. The common sorbents used in SPE are activated carbon, polydimethylsiloxane (PDMS), divinylbenzene (DVB) and octadecyl silica (ODS), which have specific selections for analyte polarity [11]. However, the major drawback of these sorbents is the low selectivity for difference kinds of analytes and high backpressure during the extraction procedure resulting from their particles format and affected to the flow rate. Furthermore, the adsorbents are less resistant to the organic solvent, expensive, and difficult to reuse.

A sorbent that modify with β -CD has a remarkable capacity to select certain analytes because of its hollow truncated cone structure with a hydrophobic cavity and hydrophilic wall, allowing it to trap and hold targets of a certain size, with polarity in the cavity generating invertible and noncovalent inclusion complexes [12].

In research by Chan et al. [13], parabens are able to form an inclusion complex with beta-cyclodextrin (β -CD). They were investigation the complex formation of β -CD with *p*-hydroxybenzoic esters (parabens) by mixing β -CD with methyl, ethyl, propyl and butyl parabens in aqueous solutions. Different process of mixing is used such as shaking, ultrasonification, autoclaving, and freeze-drying. The obtained results showed that the difference in the method of processing did not affect to the degree of interaction. On the other hand, increase of the content of β -CD and the size of the alkyl group significantly enhanced the extent of interaction.

In research conducted by Chin et al. [14], used β -CD polymer that cross-linked with different cross-linkers, toluene-2,6-diisocyanate (TDI) and hexamethylene diisocyanate (HMDI) were used to remove of four parabens, methyl-, ethyl-, propyl-, and benzyl-paraben from aqueous solution. This study indicates that β -CD polymer which cross-linked with TDI was more adsorb than HMDI and suitability to be used as adsorbent for the removal of parabens from aqueous solution. The β -CD polymers have high reusability, simple regeneration procedure, and show a positive result when tested on real samples.

Cano et al. [15], studied the formation of inclusion complexes of cyclodextrin with various substances and found that the hydroxyl groups of cyclodextrin were active sites to react with other substances, including with polymers. The inclusion complex formation of β -CD and polymer resulting in change the physical and chemical properties of the polymer that can used as adsorbents in various fields such as pharmacology, nutrition, environment and industry.

Electrospinning has emerged as the most versatile technique for nanofiber fabrication. Owing to their large surface area to volume ratio, electrospun nanofibers have the potential to serve as a good sorbent material for solid phase extraction (SPE) based

techniques. The ability to incorporate a variety of functionalities prior or post-electrospinning presents a platform to tune the sorbents for specific applications [16].

In research conducted by Hakova et al. [17], the nylon 6 nanofibers that created by needleless electrospinning were tested for their ability to serve as a sorbent for solid phase extraction. Absorption of parabens, steroids, flavonoid, insecticides and hydroxylamine group were studied. The results showed that the nylon 6 nanofibers with high surface area density are more sorptive than low surface and more selective to parabens than other groups.

In a recent study by Kayaci et al. [18], the electrospun nylon 6,6 nanofibers functionalized with cyclodextrins were for used removal of toluene vapor. The nylon 6,6/CD nanofibrous membranes entrapped higher amount of toluene when compared to pristine nylon 6,6 nanofibers due to the inclusion complex formation capability of the surface associated cyclodextrin molecules. The entrapment efficiency of toluene vapor was found to be better for nylon 6,6/ β -CD nanofibers when compared to nylon 6,6/ α -CD and nylon 6,6/ γ -CD nanofibers possibly due to better fit and size match between β -CD cavity and toluene.

For this reason, it is our interest to study and develop nanofibers as a sorbent for SPE application that have high and fast adsorption ability, large specific surface area, small fiber diameter, good mechanical and chemical stability, versatility, inexpensive, and reusability. Polyamides (nylon) is great interest polymer to fabricate the nanofibers due to their extensive applications, good mechanical and chemical stability, a cost-effective sorbent, high reusability, and specific to the phenolic group. In addition, in order to increase specific for parabens analysis, functional groups on nylon 6,6 fibers are modified with β -CD that have hydrophobic interior and hydrophilic exterior can form inclusion complexes with hydrophobic molecules same as parabens. Therefore, the purpose of the present work was to prepare nylon 6,6/ β -CD nanofibers by electrospinning technique and

applied for the trace analysis of parabens in water. The effect of β -CD content in nylon 6,6 nanofibers was studied and optimized. Then, the application of parabens extractions in water was performed.

1.2 Objective of this research

1. To prepare nylon 6,6 nanofiber functionalized with β -CD by electrospinning technique.
2. To study the use of nylon 6,6/ β -CD as a sorbent in solid phase extraction for determination of parabens in water sample.

1.3 Scopes of this research

1. Preparation of nylon 6,6/ β -CD nanofibers by varying parameters including concentration of nylon 6,6, β -CD content, voltage, and flow rate.
2. Characterization of obtained nanofibers in terms of morphology, functional group, and chemical analysis.
3. Evaluation of the extraction efficiency of nylon 6,6/ β -CD nanofibers as a sorbent for extraction parabens in water sample.

1.4 Benefits of the research

The objective of this research is to obtain an electrospun of nylon 6,6/ β -CD nanofibers which can be an alternative sorbent for extraction of parabens in water samples.

CHAPTER II

THEORY AND LITERATURE REVIEWS

2.1 Nylon 6,6

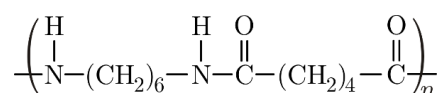


Figure 2.1 Structure of nylon 6,6

Nylon 6,6 (polyhexamethylene diamine adipamide) is a polyamide made from adipic acid and hexamethylenediamine by polycondensation, two monomers each containing 6 carbon atom, which give nylon 6,6 its name as shown in Figure 2.1. DuPont researchers led by Dr. Wallace Carothers, invented nylon 6,6 polymer in the 1930s. Nylon, the generic name for a group of synthetic fibers, was the first of the “miracle” yarns made entirely from chemical ingredients through the process of polymerization. Nylon 6,6 is semicrystalline polymer which has good thermal stability, high mechanical strength, great rigidity, good stability, insoluble in solvents, and resistant to acids or alkalis. Such advantages have made nylon 6,6 attractive for various biomedical and environmental applications as a functional polymer [20-21]. It is an important engineering thermoplastic [22-23]. This polymer is suitable for electrospinning processing due to its poly-electrolytic behavior in acid solution and has sufficient stability of the melt and adequate viscosity [22]. Electrospun nylon 6,6 nanofibrous membranes have been indicated as a suitable material for filtration application in the literature [16-18].

2.2 β -cyclodextrin (β -CD)

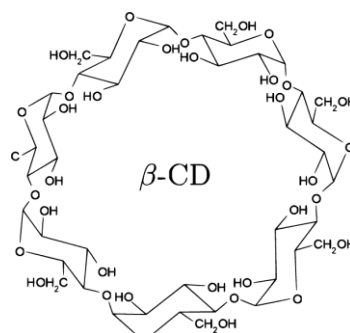


Figure 2.2 Structure of β -cyclodextrin.

Beta-cyclodextrin (β -CD) is a cyclic oligosaccharide composed of seven units of glucose (α -D-glucopyranose) unit linked by alpha-(1,4) type bonds as shown in Figure 2.2. An important feature of cyclodextrin is presents a hydrophilic external surface and a hydrophobic internal cavity. This structure enables cyclodextrins to form inclusion complexes with non-polar molecules into the hydrophobic cavity [18] or partial inclusion complexes with macromolecules, here improving the physicochemical and biological properties of the complexed compound [19-21].

2.3 Nanofibers by electrospinning technique

Electrospinning has been known for over 60 years in the textile industry for manufacturing non-woven fiber fabrics. In recent years, there has been an increasing interest in exploiting this technology to produce nanoscale fibers [5]. The nanofibers from these electrospun polymer solutions have been used in various applications such as, tissue engineering scaffolds, filtration membranes and in various biomedical applications [2]. It is a simple, versatile, and cost-effective technique to produce nanofibers from a wide variety of polymers.

Electrospinning is a spinning technique that a unique approach using electrostatic forces to produce ultra-fine fibers and nonwoven fabrics from applying high voltage to a polymeric solution in order to induce electrical charges within the fluid. In the

electrospinning process, a polymer solution held by its surface tension at the end of a capillary tube is subjected to an electric field and an electric charge is induced on the liquid surface due to this electric field. When the electric applied reaches a critical value, the repulsive electrical forces overcome the surface tension forces. Eventually, a charged jet of the solution is ejected from the tip of the Taylor cone and an unstable band rapid whipping of the jet occurs in the space between the capillary tip and collector which leads to evaporation of the solvent, leaving a polymer behind [4].

Nanofibers obtained from electrospinning technique have an extremely high surface-to-volume ratio, high aspect ratio, large specific surface area, high sorption capacity, high porosity, and high stability in liquid media, malleability to conform to a wide variety of sizes and shapes and ability to control the nanofiber composition to achieve the desired results from its properties and functionality. Electrospinning is conducted at room temperature with atmosphere conditions. The typical set up of electrospinning apparatus is shown in Figure 2.3.

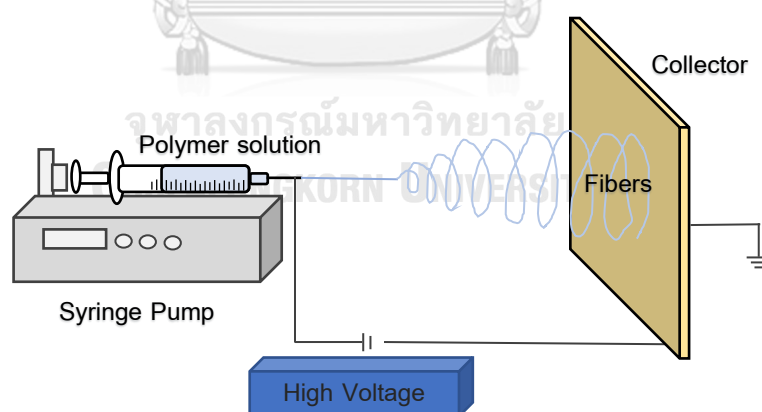


Figure 2.3 Schematic diagram of set up of electrospinning apparatus.

Basically, an electrospinning system consists of three major components: a high voltage power supply, a spinneret and a grounded collecting plate and utilizes a high

voltage source to inject charge of polarity into a polymer solution, which is then accelerated towards a collector of opposite polarity [3].

There are many parameters which influence the morphology and diameter of electrospun nanofibers. Generally, the influential parameters on electrospinning, classified broadly into solution parameters, process parameters, and ambient parameter. First, solution parameters include viscosity, conductivity, molecular weight, surface tension and process parameters include applied electric field, distance from tip to collector and flow rate. The ambient parameters include temperature and humidity of the surroundings while spinning.

2.3.1 Solution parameters in electrospinning technique

2.3.1.1 Viscosity

In the electrospinning, viscosity of polymer solution plays an important role in determining the range of concentrations from which continuous fibers can be obtained. For solution of low viscosities, surface tension is the dominant factor and just beads or beads fibers are formed while above a critical concentration, a continuous fibrous structure is obtained and its morphology is affected by the concentration of the solution [8].

2.3.1.2 Polymer concentration

In the electrospinning process, for fiber formation to occur, a minimum solution concentration is required. The concentration of solution was optimized in the electrospinning process because at low concentrations beads can formed and at high concentrations of solution the formation fibers are restricted due to the invalidity to keep the flow of the solution at the tip of the needle as a result the formation of larger fibers [6].

2.3.1.3 Molecular weight of polymer

Molecular weight of the polymer has a significant effect on electrical properties and rheological such as viscosity, surface tension, dielectric strength and conductivity [7]. Molecular weight of the polymer reflects the number of entanglements of polymer chains in a solution, thus solution viscosity. Chain entanglement plays an important role in the processing of electrospinning that affects the morphology of electrospun fiber. For low a molecular weight solution tends to form beads rather than fibers, so generally high molecular weight polymer solutions have been used in electrospinning because they can provide the desired viscosity for the fiber generation [2].

2.3.1.4 Conductivity

Polymers are mostly conductive, the charge ions in the polymer solution are highly influential in jet formation. Normally, when increase of electrical conductivity of the solution, there is a significant decrease in the diameter of the electrospun nanofibers whereas with low conductivity of the solution, there results insufficient elongation of a jet by electrical force to produce uniform fiber, and beads may also be observed [9].

2.3.1.5 Surface tension

The surface tension of the polymer solution plays an important role in the electrospinning process. Different solvents may contribute different surface tensions. Generally, when reducing the surface tension of a nanofiber's solution, fibers can be obtained without beads and at the high surface tension of a solution inhibits the electrospinning process because of instability of the jet and the generation of sprayed droplets [10].

2.3.2 Processing parameters in electrospinning technique

2.3.2.1 Applied voltage

Applied voltage to the polymer solution is a crucial element in the electrospinning process. When higher voltages are applied, polymer is more ejection and facilitates the formation of a larger diameter fiber [11-12]. A higher voltage causes greater stretching of the solution due to the greater columbic forces in the jet as well as a stronger electric field and these effects lead to reduction in the fiber diameter and also rapid evaporation of solvent from the fibers results. At a higher voltage there is also greater probability of beads formation [13-14].

2.3.2.2 Tip to collector distance

The distance between the tip and the collector should be optimum distance between the tip and collector which favors the evaporation of solvent from nanofibers [2]. A minimum distance is required to give the fibers sufficient time to dry before reaching the collector, otherwise with distances that are either too close or too far, beads have been observed [15].

2.3.2.3 Flow rate of solution

The flow rate of the polymer from the syringe is an important process parameter as it influences the jet velocity and the material transfer rate. A lower feed rate is more desirable as the solvent will get enough time for evaporation [16]. At high flow rates result in beaded fibers due to unavailability of proper drying time prior to reaching the collector [17].

2.3.3 Temperature and Humidity in electrospinning technique

Apart from solution and processing parameters, ambient parameters also affect the electrospinning process. The effect of temperature on electrospinning process is an inverse relationship between viscosity and temperature. When temperature increase, the viscosity of polymer solutions is decreased resulting in diameter of fibers are decreased while spinning. For the humidity, when increasing humidity, there is an

appearance of small circular pores on the surface of the nanofibers because of increasing the humidity leads to the pores coalescing [18]. At very low humidity, a volatile solvent may dry rapidly as the evaporation of the solvent is faster. Sometimes the evaporation rate is so fast than compared to the removal of the solvent from the tip of the needle and this would create a problem with electrospinning.

2.3.4 Application as a sorbent in solid-phase extraction

SPE is one of the most extensively used sample preparation technique for environmental samples. The advantages of SPE include simplicity, flexibility, high selectivity, automation, rapidity, higher enrichment factors, and use of different sorbents [22]. Nanomaterials (NMs) have been used as sorbents for the qualitative and quantitative analysis of a wide variety of analytes. The nanomaterials are applied as sorbents because of their advantageous properties such as large surface area and specific affinity towards ultra-trace-level target analytes [10].

2.4 Solid-phase extraction (SPE)

Solid-phase extraction (SPE) is an extraction method that uses a solid phase and a liquid phase to isolate analyte from a solution. SPE procedures are used not only to extract traces of organic compound from environmental samples but also to remove the interfering components of the complex matrices in order to obtain a cleaner extract containing the analytes of interest before using chromatographic [23]. SPE is an important and widespread method for the separation and preconcentration of trace quantities of analytes in environmental sample with complex matrices because of its notable advantages, such as speed, simplicity, low cost, reusability of the adsorbent, an environmentally friendly and high enrichment factors [24]. The steps of the solid phase extraction process are shown in Figure 2.4. First step is condition the SPE, second is loading the sample solution and, washing away undesired components, finally elute the compounds of interest.

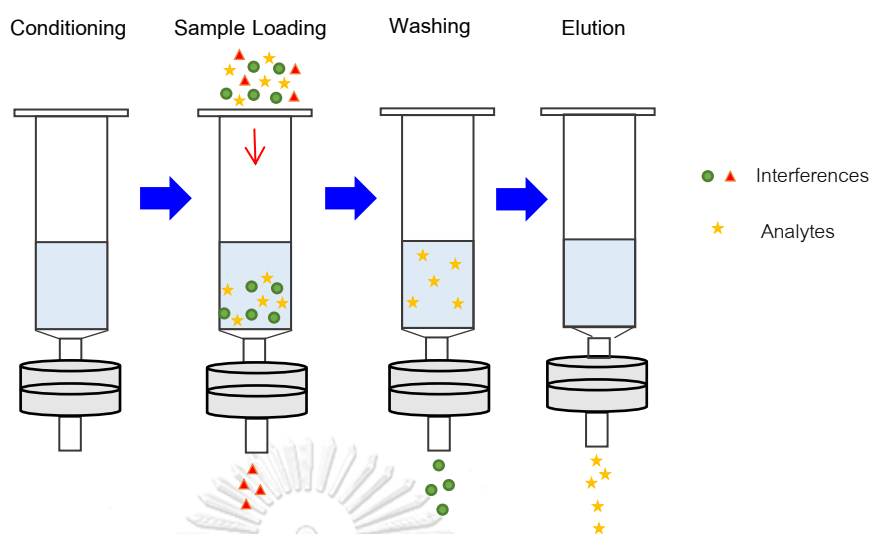


Figure 2.4 Procedure of solid phase extraction (SPE).

SPE is the very popular technique currently available for rapid and selective sample preparation. It involves partitioning between a liquid (sample matrix or solvent with analytes) and a solid (sorbent) phase. The selection of an appropriate SPE extraction sorbent depends on understanding the mechanism of interaction between the sorbent and analyte of interest. The most common retention mechanisms in SPE are based on van der Waals forces (non-polar interactions), hydrogen bonding, dipole-dipole forces (polar interactions) and cation-anion interactions (ionic interaction) [23].

A separation/enrichment step is necessary prior to the measurements to resolve these limitations by increasing the sensitivity and enhancing the accuracy of estimation. Enrichment factor (EF) was calculated using the following equation [25]:

$$EF = \frac{C_{a,final}}{C_{s,initial}}$$

Where $C_{a,final}$ and $C_{s,initial}$ are the final and initial concentrations of the analyte in the receiving and source phases, respectively.

CHAPTER III

EXPERIMENTAL

3.1 Chemicals

3.1.1 Preparation of polymer fibers by electrospinning technique

- 1) Nylon 6,6, MW: 262.35 g.mol⁻¹ (ALDRICH, USA)
- 2) β -cyclodextrin, 98% (ACROS ORGAICS, CHINA)
- 3) Formic acid, 85% (CARLO ERBA, France)

3.1.2 Parabens

- 1) Methyl 4-hydroxybenzoate, 99% (C₈H₈O₃) (Alfa Aesar, Britain)
- 2) Ethyl 4-hydroxybenzoate, 99% (C₉H₁₀O₃) (Alfa Aesar, USA)
- 3) n-Propyl 4-hydroxybenzoate, 99% (C₁₀H₁₂O₃) (Alfa Aesar, China)
- 4) n-Butyl 4-hydroxybenzoate, 99% (C₁₁H₁₄O₃) (Alfa Aesar, Britain)
- 5) Isobutyl 4-hydroxybenzoate, 97% (C₁₁H₁₄O₃) (Sigma-Aldrich, USA)
- 6) n-Pentyl 4-hydroxybenzoate, 98% (C₁₂H₁₆O₃) (Alfa Aesar, China)
- 7) Benzyl 4-hydroxybenzoate, 99% (C₁₄H₁₂O₃) (Sigma-Aldrich, USA)

3.1.3 Extraction of parabens in water

- 1) Methanol (Merck, Germany)
- 2) Acetonitrile (Merck, Germany)
- 3) Ethanol (Merck, Germany)
- 4) Hexane (Mallinckrodt, USA)
- 5) Sodium Chloride (Sigma-Aldrich, USA)

6) Ultrapure water was produced on a Milli-Q water purification system (Millipore SAS, Molsheim, France)

3.2 Instrument and Apparatus

The instruments and apparatus used in this research were listed in Table 3.1.

Table 3.1 Instrument and apparatus.

| Instrument | Model | Manufacture |
|------------------------------------|------------------|-------------------|
| Swinnex Filter Holder | - | Merck |
| Hotplate and Stirrer | HS7 | IKA®C-MAG |
| pH meter | FiveEasy Plus | Mettler Toledo |
| Micropipette(100-1000µl) | Volumate | Mettler Toledo |
| High voltage | Series 230 | BERTAN |
| Syring pump | NE-100 | Prosense |
| Analytical balance | AT200 | Mettler |
| HPLC | Agilent 1260 | Agilent |
| Scanning electron microscopy (SEM) | JSM-IT100 | JEOL |
| BET analysis | Bel Sorp mini II | Bel-Japan |
| FT-IR analysis | Nicolet 6700 | Thermo Scientific |
| Chemical Duty Pump | WP6122050 | Merck |

3.3 Preparation of nylon 6,6/ β -CD nanofibers by electrospinning technique

3.3.1 Preparation of spinning solution

Nylon 6,6 solution was first prepared by dissolving 15%(w/v) nylon 6,6 in formic acid. Different amount of β -CD was also dissolved in formic acid. Then, nylon 6,6 solution (15%,w/v) was added to β -CD solution and stirred for 8 hr (overnight) at 35 °C to obtain homogeneous and clear solutions. 3 mL of this solution was applied for the fabrication of electrospun nanofibers. Different ratios of nylon 6,6: β -CD were prepared as listed Table 3.2.

Table 3.2 Ratio of nylon 6,6: β -CD ratio to prepare solution for the fabrication of electrospun nanofibers.

| Nanofibers | Nylon 6,6 : β -CD ratio (by weight) | Weight (g) | | Formic acid (mL) |
|---------------------------|---|------------|-------------|------------------|
| | | Nylon 6,6 | β -CD | |
| Nylon 6,6 | 1 : 0 | 1.50 | - | 10 |
| Nylon 6,6/ 25 β -CD | 1 : 0.25 | 1.50 | 0.375 | 10 |
| Nylon 6,6/ 50 β -CD | 1 : 0.50 | 1.50 | 0.750 | 10 |
| Nylon 6,6/ 75 β -CD | 1 : 0.75 | 1.50 | 1.125 | 10 |

3.3.2 Fabrication of nylon 6,6/ β -CD nanofibers

The electrospinning experiment was set-up as shown in Figure 3.1. The distance between needle tip-to-collector is 15 cm. The flow rate of polymer solution was $0.3 \text{ mL}\cdot\text{hr}^{-1}$ and the applied voltage was 29 kV. The nanofiber production was carried out under temperature and humidity in range 25-29 °C and 30-40 %RH, respectively.

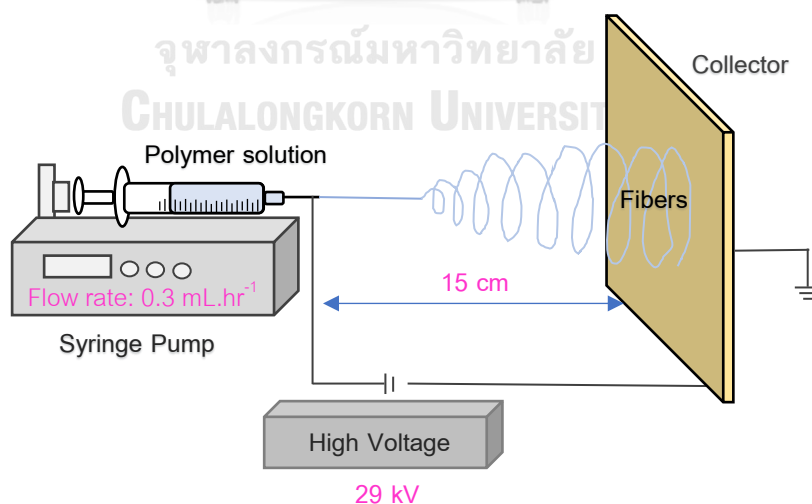


Figure 3.1 Electrospinning process setup.

3.4 Characterization of nylon 6,6/ β -CD nanofibers

3.4.1 Scanning Electron Microscopy (SEM)

The morphology and surface of nylon 6,6/ β -CD electrospun nanofibers were characterized by JEOL scanning electron microscope (SEM; model JSM-IT100 Series, Japan). The fiber diameter was reported as the average values with standard deviation ($n=30$) measured using the ImageJ 1.52a software (LOCI, University of Wisconsin).

3.4.2 Fourier-Transform Infrared Spectroscopy (FT-IR)

The FT-IR was used to evaluate the functional groups in nylon 6,6 nanofiber. Infrared spectra were recorded by Attenuated Total Reflectance (ATR) and KBr technique using FT-IR Spectrometer (Nicolet 6700 model, U.S.A) at the wavenumber range of 500-4000 cm^{-1} using the absorbance mode with 32 scans for ATR and 16 scans for KBr and the wavenumber resolution of $\pm 4 \text{ cm}^{-1}$.

3.4.3 Nitrogen adsorption-desorption analysis

The specific surface area and porous structures of electrospun nylon 6,6 and nylon 6,6/ β -CD nanofibers were determined using the Brunauer-Emmett-Teller (BET) method by a surface area analyzer (BELSORP Mini, BEL, Japan). The Barrett-Joyner-Halenda model (BJH) was also calculated to evaluate the pore diameters of nylon 6,6/ β -CD electrospun nanofibers.

3.5 Nylon 6,6/ β -CD as a sorbent in solid phase extraction (SPE)

3.5.1 Preparation of standard solutions

Stock standard solutions of parabens in methanol were monthly prepared at 1000 $\text{mg}\cdot\text{L}^{-1}$. The mixture of seven parabens which are methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP), iso-butyl paraben (iso-BuP), n-butyl paraben (n-BuP), pentyl paraben (PeP) and benzyl paraben (BzP) were prepared. A set of working standards solution at different concentration was freshly prepared by dilution of the stock

solution with methanol. The stock solutions of the standards were stored in a sealed amber bottle at 4 °C to prevent degradation.

3.5.2 Solid phase extraction (SPE) procedure

The nylon 6,6/ β -CD electrospun nanofibers were cut into a circle with diameter of 13 mm. and assembled in the syringe filter holder (Figure 3.2). In SPE process, the nylon 6,6/ β -CD sorbent was first conditions with 3.0 mL of methanol, followed by 3.0 mL of water. Then, 15.0 mL of sample were passed through the SPE tube with the flow rate of 1.0 mL.min⁻¹. Then, the nylon 6,6/ β -CD sorbent was dried under vacuum (20 mmHg) for 10 min. For desorption, 200 μ L of methanol were passed through the dry sorbents in order to elute the parabens. 10 μ L of this solution were injected to HPLC-DAD for quantitative analysis.

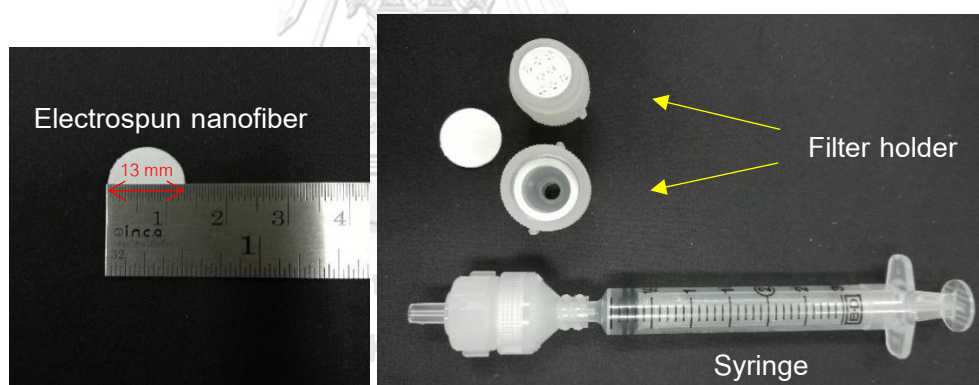


Figure 3.2 The electrospun nanofibers in the syringe filter holder.

3.5.3 Paraben analysis by HPLC

The separation and quantification of the tested parabens were performed by Agilent 1260 Infinity II HPLC system equipped with an automated sample injector, a quaternary pump and a Diode Array Detector (Agilent Technologies, USA). The eclipse XDB-C18 column, 5.0 μ m (4.6x150 mm) was used to resolve parabens and the temperature of the analytical column was maintained at 27 °C (\pm 1°C). The gradient elution

mode was chosen with a flow rate of $1.5 \text{ mL}\cdot\text{min}^{-1}$. A mixture of water (A) and methanol (B) was used as mobile phase. The elution profile was showed in Table 3.3. The detection wavelength was set at 256 nm. The total chromatographic run time was 30 min. ChemStation software was used to collect and analyze data.

Table 3.3 Mobile phase gradient program of HPLC-DAD (A: water and B: methanol).

| Time (min) | % Water (A) | % Methanol (B) |
|------------|-------------|----------------|
| 0.00 | 45 | 55 |
| 8.00 | 55 | 45 |
| 23.00 | 55 | 45 |
| 30.00 | 10 | 90 |

3.5.4 Optimization of the SPE extraction

The parameters that can affect the extraction efficiency were studied including β -CD content in nylon 6,6/ β -CD nanofibers, desorption solvent, desorption volume, sample volume, and pH of sample water. The best sorbent material was chosen and we evaluated the influences of sample pH, desorption solvent, sample volume and ionic strength. The sample pH values were evaluated at 3-9 ; the desorption solvents were tested for methanol, acetonitrile, ethanol, acetone, and hexane; the effect of sample volume on extraction efficiency in the range 5-20 mL were evaluated ; the influence of the ionic strength was evaluated by the addition of 0-5% NaCl. All parameters were optimized using deionized water as a sample solvent, spiked with $100 \mu\text{g}\cdot\text{L}^{-1}$ of parabens and carried out in triplicate.

3.5.5 Method validation

To ensure for intend purposes, method validation was evaluated for linearity range, accuracy and precision. Linearity and range of the method was displayed by using the correlation coefficient (r^2) value of the plot between concentrations of standard solutions and their corresponding peak area. Correlation coefficient value

greater than 0.99 was approved for linearity. Accuracy of the method was performed by spiking method at 2 times of LOQ with three replicates of standard were evaluated. Accuracy of the method was expressed in term of %recovery of amount quantity of standard found and amount quantity of standard added. Precision experiments were performed for repeatability and intermediate precision. The HorRat test were used to assess precision of the method. The HorRat test was the ratio of experiment %RSD and predicted %RSD which was calculated by using Horwitz equation($0.66 \times 2 \times C^{-0.1505}$). Acceptable precision is the HorRat test value less than 2.

HorRat ratio was calculated by the following equation[26].

$$\text{HorRat} = \text{RSD}/\text{PRSD}$$

Where; RSD = %RSD of laboratory data

PRSD = Calculated %RSD from Horwitz equation

3.5.6 Application to real sample

The analytical performance and possible application of the method in real sample analysis was investigated under optimal condition. The drinking water samples were purchased from local supermarkets at different sources in Thailand. The water samples were filtered through a 0.45 μm nylon membrane filter (Millipore, Bedford, MA, USA) and stored at 4 $^{\circ}\text{C}$ until analysis. Quantification of the analytes in the sample was performed by spiked water sample with 50 $\text{ng}\cdot\text{mL}^{-1}$ of parabens standard. The %recovery of all parabens from these real samples used to assessment of the method to the determination of parabens in drinking water sample.

CHAPTER IV

RESULTS AND DISCUSSION

This chapter is divided into four major sections. First, preparation of nylon 6,6/ β -CD nanofibers by electrospinning technique. Then, the characterization of the electrospun nylon 6,6/ β -CD nanofibers were demonstrated. After that, the optimization of the electrospun nylon 6,6/ β -CD nanofibers as a sorbent in solid-phase extraction of parabens was studied. Finally, the nylon 6,6/ β -CD nanofibers were applied for determination of parabens in water samples.

4.1 Preparation of nylon 6,6/ β -CD nanofibers by electrospinning technique

The electrospun nylon 6,6 and nylon 6,6 / β -CD nanofibers were processed under optimized condition of electrospinning as shown in Table 4.1. Smooth and no bead formation on the fibers were obtained. Also, adding the β -CD on nylon 6,6 nanofibers was not affected the morphology of electrospun nanofiber.

Table 4.1 Condition for electrospinning processes.

| Parameter | Optimized condition |
|------------------------|-------------------------|
| Flow rate | 0.3 mL.hr ⁻¹ |
| Voltage | 29 kV |
| Tip-collector distance | 15 cm |
| Volume | 3.0 mL |
| Temperature | 25-29 °C |
| Relative humidity | 30-40 %RH |

4.2 Characterizations of nylon 6,6/ β -CD nanofibers

4.2.1 Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR was used to evaluate the functional groups in nylon 6,6/ β -CD nanofibers. The spectrum of nylon 6,6 nanofibers exhibited main characteristic peak at 3301 cm⁻¹ (N-H stretching vibration), 2934 cm⁻¹ (-CH₂ stretching vibration), 2860 cm⁻¹ (CH-

symmetric stretching vibration), 1637 cm^{-1} (C=O stretching vibration), and 1536 cm^{-1} (N-H bending vibration) as shown in Figure 4.1(a).

The spectrum of nylon 6,6/ β -CD nanofibers (Figure 4.1(b)) exhibited the main characteristic peaks of nylon 6,6 at 3301 cm^{-1} , 2934 cm^{-1} , 2860 cm^{-1} , 1637 cm^{-1} , 1536 cm^{-1} , and β -CD at 1027 cm^{-1} (-OH bending vibration), 1150 cm^{-1} assigned to the antisymmetric stretching vibration of the C-O-C glycosidic bridge. In addition, there is a peak at 1715 cm^{-1} (C=O stretch dimer H-bonded) which was the H-bonding interaction between β -CD and nylon 6,6.

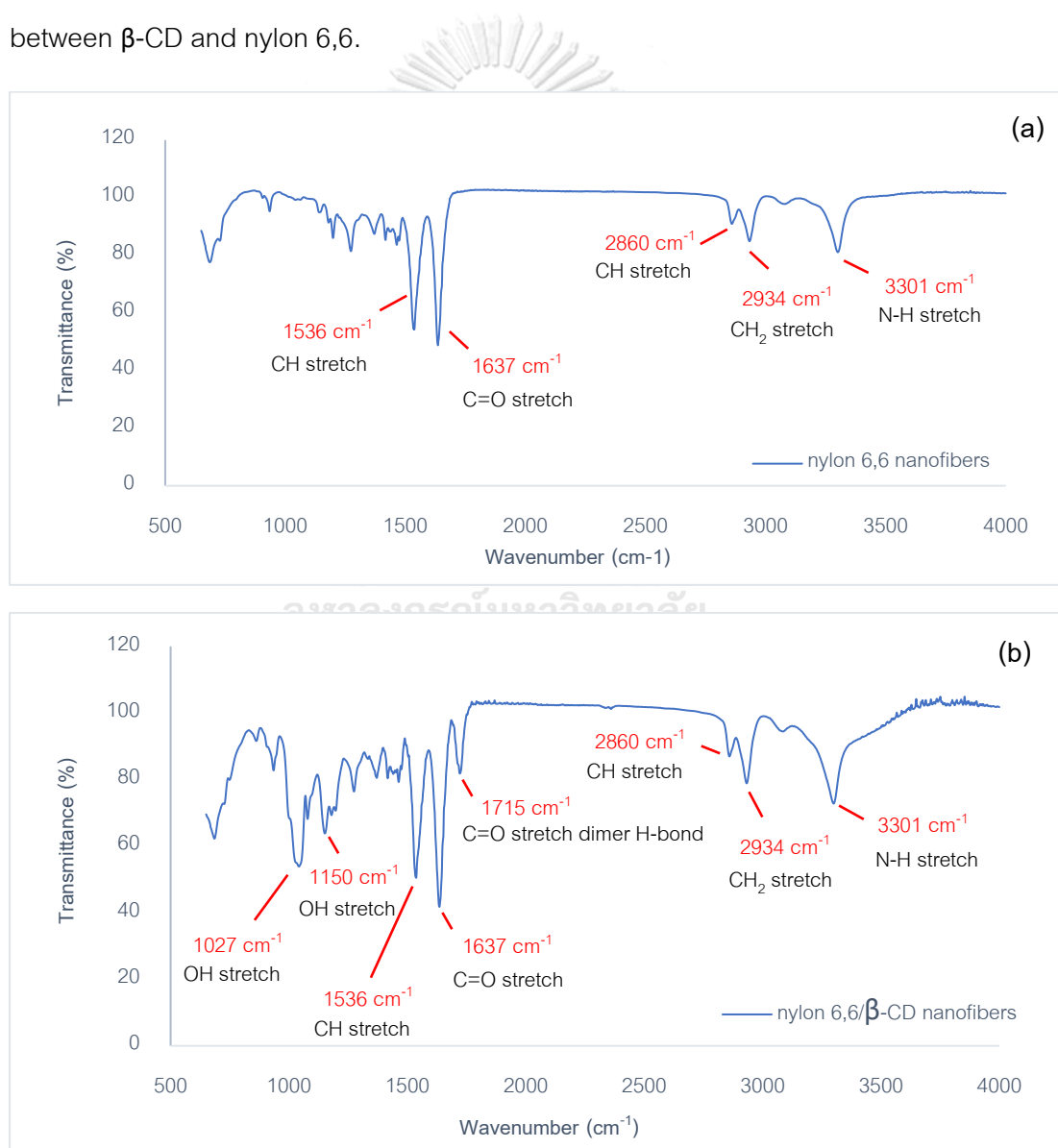


Figure 4.1 FTIR spectra of (a) nylon 6,6 nanofibers, (b) nylon 6,6/ β -CD nanofibers.

4.2.2 Morphology of nylon 6,6/ β -CD nanofibers

Scanning electron microscopy (SEM) was used to investigate the morphology of the nanofibers and determine a diameter of the nanofibers. The morphology of the electrospun nylon 6,6 nanofibers at various addition of β -CD was shown in Figure 4.2.

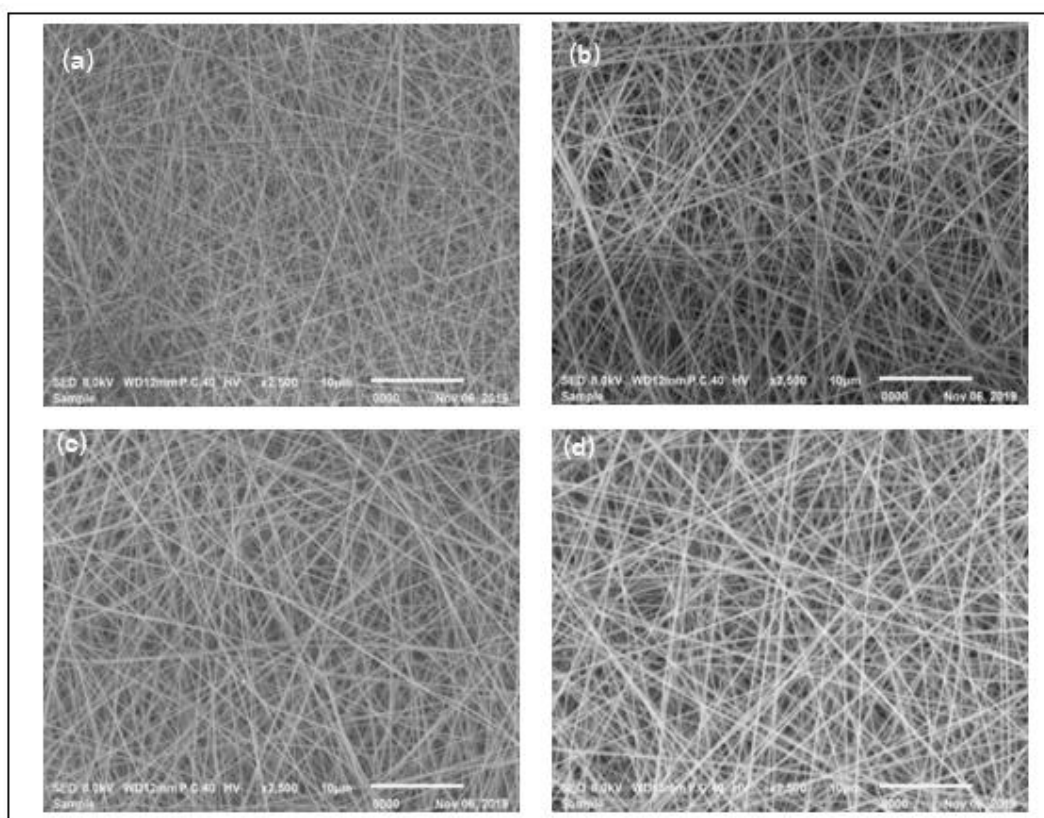


Figure 4.2 SEM images of electrospun nanofibers (a) nylon 6,6 (15%w/v) (b) nylon 6,6/25 β -CD (c) nylon 6,6/50 β -CD and (d) nylon 6,6/75 β -CD.

From Figure 4.2, when β -CD was added into nylon 6,6 solution, the morphology of electrospun nanofibers was not significantly changed. Smooth and uniform nanofibers were obtained. The average diameters of nylon 6,6 with 25% wt. β -CD (nylon 6,6/25 β -CD), 50% wt. β -CD (nylon 6,6/50 β -CD) and 75% wt. β -CD (nylon 6,6/75 β -CD) were 132 ± 2 nm, 136 ± 3 nm and 143 ± 1 nm, respectively (Table 4.2). The addition of β -CD from 25% to 75% w/w was slightly increased the electrospun nanofiber diameter. This caused

by the increase of solution viscosity and the interaction (H bonds) between the β -CD molecules and nylon 6,6 polymer chains [14].

Table 4.2 Diameter of nylon 6,6/ β -CD electrospun nanofibers.

| Sorbent | Diameter (nm) (\pm SD) |
|--------------------------|---------------------------|
| Nylon 6,6 | 125 \pm 3 |
| Nylon 6,6/25 β -CD | 132 \pm 2 |
| Nylon 6,6/50 β -CD | 136 \pm 3 |
| Nylon 6,6/75 β -CD | 143 \pm 1 |

4.2.3 Specific surface area

The specific surface area and porous structures of electrospun nylon 6,6 and nylon 6,6/ β -CD nanofibers were determined using the Brunauer-Emmett-Teller (BET) method. The nylon 6,6 nanofibers yielded a surface area of $18.37 \pm 0.39 \text{ m}^2 \cdot \text{g}^{-1}$. Nevertheless, when functionalized nylon 6,6 with β -CD, the surface area of the nanofibers was decreased. This caused by the existence of CD which is a non porous material on the surface of the nylon 6,6/CD nanofibers. Barrett-Joyner-Halenda model (BJH) was also calculated to evaluate the pore diameters of nylon 6,6/ β -CD electrospun nanofibers as shown in Table 4.3 indicating a mesoporous structure as defined by IUPAC definition, which classifies the mesoporous materials to have a diameter of 2-50 nm.

Table 4.3 Specific surface area (BET) and pore size of nylon 6,6/ β -CD electrospun nanofibers.

| Nanofiber | surface area ($\text{m}^2 \cdot \text{g}^{-1}$) | pore diameter (nm) |
|--------------------------|---|--------------------|
| Nylon 6,6 | 18.37 \pm 0.39 | 7.64 \pm 0.46 |
| Nylon 6,6/25 β -CD | 12.58 \pm 0.49 | 7.02 \pm 0.39 |
| Nylon 6,6/50 β -CD | 10.73 \pm 0.53 | 6.93 \pm 0.62 |
| Nylon 6,6/75 β -CD | 9.77 \pm 0.59 | 6.65 \pm 0.69 |

4.3 Nylon 6,6/ β -CD as a sorbent for solid phase extraction of parabens.

In order to comprehensively assess the performance of nylon 6,6/ β -CD nanofibers, important factors that influence the extraction efficiency, such as β -CD content, type and volume of desorption solvent, sample pH and sample volume had been investigated. The extraction of seven parabens, which are methyl paraben(MP), ethyl paraben(EP), propyl paraben(PP), iso-butyl paraben(iso-BuP), n-butyl paraben (n-BuP), benzyl paraben(BzP),and pentyl paraben(PeP), in water were evaluated.

4.3.1 Effect of β -CD content

The extraction efficiencies of nylon 6,6 and nylon 6,6/ β -CD nanofibers with different mass content of β -CD were compared in Figure 4.3. The extraction efficiency of alkyl parabens were decreased with the increase of β -CD content in nylon 6,6 nanofibers. The nylon 6,6 nanofibers provided a higher interaction with parabens via H-bonding than β -CD. Moreover, the β -CD cover the surface of nylon 6,6 resulting in less surface area of nylon 6,6/ β -CD to extract parabens. Therefore, nylon 6,6/ β -CD nanofiber gave the lower extraction efficiency than nylon 6,6 nanofibers. Interestingly, the extraction of benzyl paraben by nylon 6,6/ β -CD was better than nylon 6,6 nanofibers. This might be the presence of benzene ring instead of the alkyl chain. Therefore, the inclusion of paraben in the hydrophobic cavity of the β -CD was enhanced. As a result, the nylon 6,6/25 β -CD nanofibers, which provided the comparable extraction efficiency to nylon 6,6 nanofibers was chosen further for study and evaluation for the extraction of parabens.

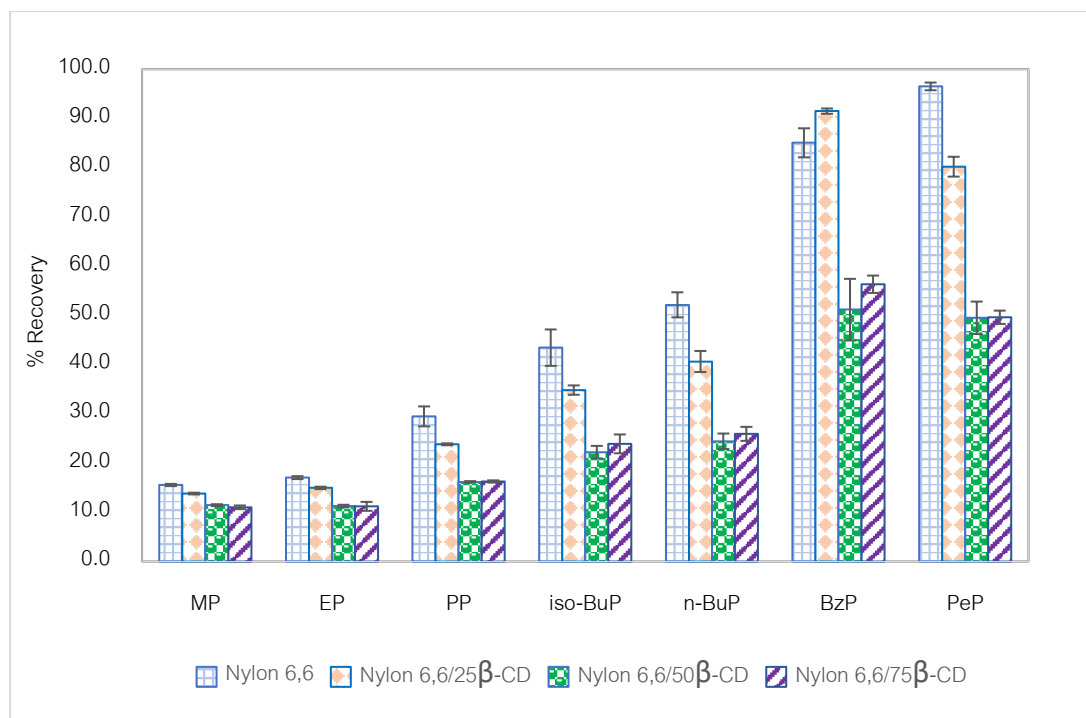


Figure 4.3 Effect of sorbent type on the extraction efficiency.

4.3.2 Effect of desorption solvent

Desorption solvents have a significant role in ensuring a high extraction efficiency. Methanol, ethanol, acetonitrile and hexane were examined as a desorption solvent for this study. The water sample spiked with parabens at 100 ng.mL^{-1} were used to evaluate the desorption solvent. Parabens have a polar interaction with nylon 6,6/ β -CD nanofibers. Therefore, a high polarity solvent is required for sufficient elution strength to elute the parabens from the sorbent. The results showed in Figure 4.4. Methanol gave the highest desorption activity due to its polar characteristic. Therefore, methanol was selected as the desorption solvent for further study of the extraction of parabens from water samples.

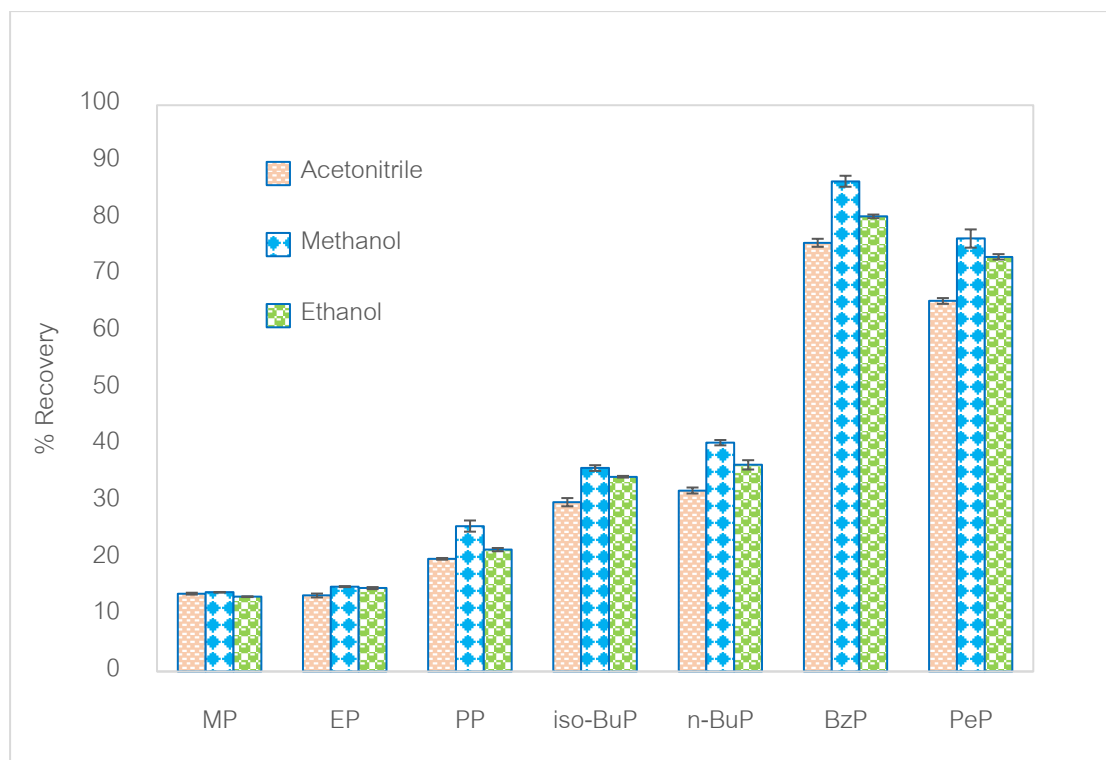


Figure 4.4 Effect of types of desorption solvent on the extraction efficiency.

4.3.3 Effect of desorption volume

The effect of desorption volume was studied using methanol as desorption solvent in the range of 150-350 μL . It is well known that with increasing in the volume of extraction solvent can increase %extraction. According to the obtained results, %extraction were increased with increase of desorption solvent volume (Figure 4.5). Noticably, %recovery of benzyl paraben and pentyl paraben were steady when using the desorption solvent more than 200 μL and %recovery of methyl paraben, ethyl paraben, propyl paraben, iso-butyl paraben and n-butyl paraben were slightly increased after 200 μL of desorption solvent. However, increasing desorption volume will lower the enrichment factors (EFs) as shown in Table 4.4. Therefore, 200 μL of methanol was chosen as a desorption volume for this study as an optimized volume to get a high EFs and sufficient desorption parabens from adsorbent.

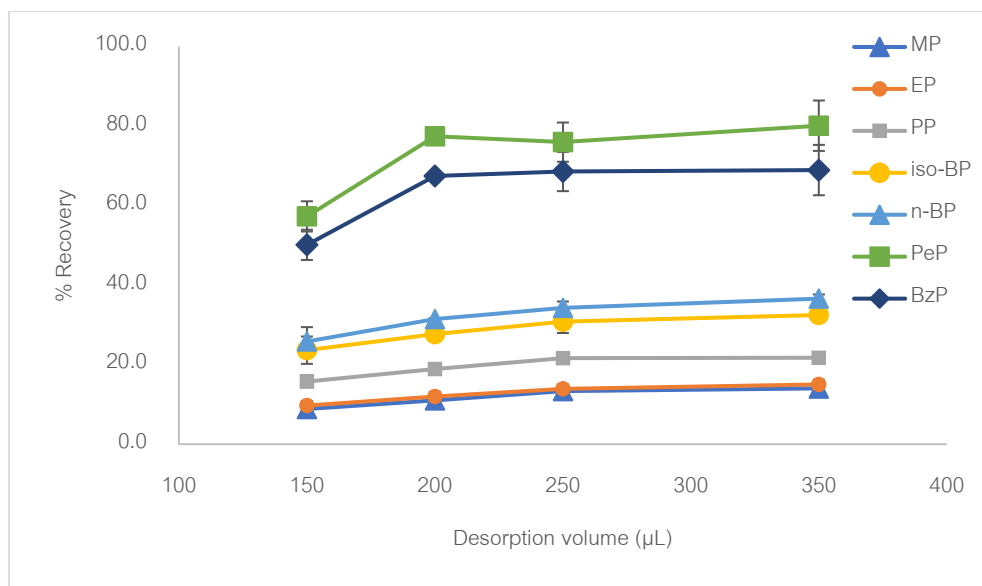


Figure 4.5 Effect of desorption volume on the extraction efficiency. Experiments were carried out by triplicate, using $100 \mu\text{g}\cdot\text{L}^{-1}$ of parabens under optimized condition.

Table 4.4 Enrichment factor (EF) of parabens in different desorption volume.

| Desorption volume (μL) | Enrichment factor (EF) |
|-------------------------------------|------------------------|
| 150 | 100 |
| 200 | 75 |
| 250 | 60 |
| 350 | 43 |

4.3.4 Effect of pH of sample solution

The pH of sample solution affects the existing forms of the parabens and alters the interaction with nylon 6,6/ β -CD sorbent. To evaluate the effect of solution pH on the extraction efficiency, solution pH was adjusted in the range of 3-9 by 0.1 M HCl or NaOH.

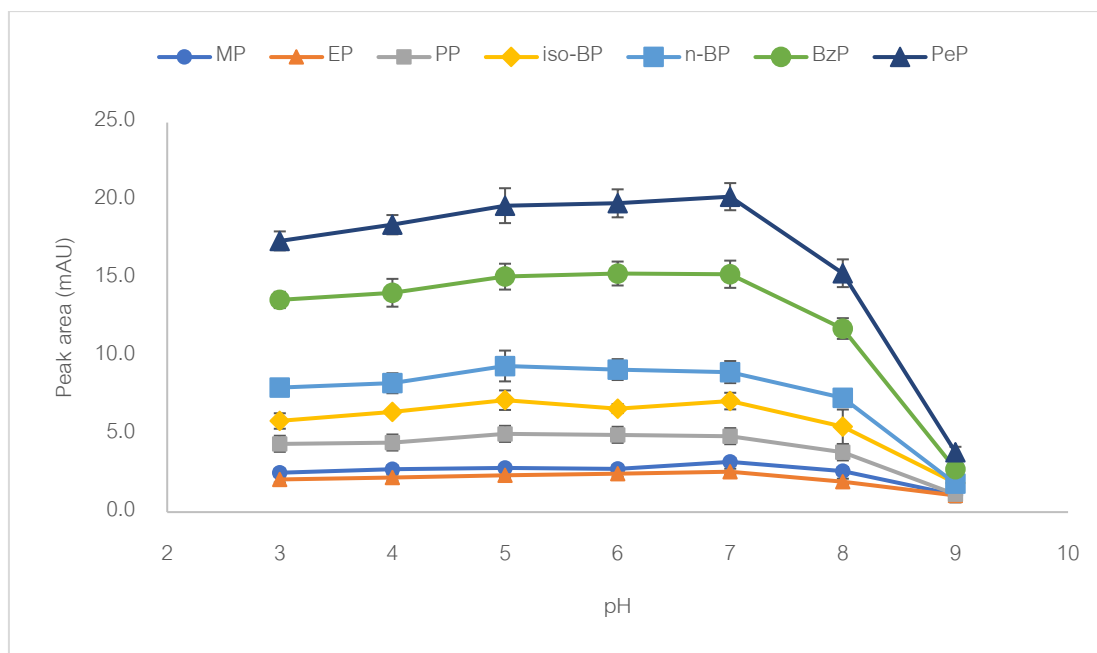


Figure 4.6 Effect of solution pH on the extraction efficiency

From Figure 4.6, the highest extraction efficiency were obtained for all of parabens (at pH 7). Due to at pH 7, the parabens were in the neutral form that led to efficient hydrophobic interaction between the parabens and the nylon 6,6/ β -CD nanofibers resulting in higher extraction. At pH lower than 7, the peak areas of paraben were slightly decreased since the oxygen atom of the ester group (C=O) of parabens was protonated. On the other hand, when increasing pH to 9, the peak areas of paraben were significantly decreased as a result of parabens exist in the anionic form because the hydroxyl group of parabens was deprotonated. Moreover, at pH above 9 a process of alkaline hydrolysis of the parabens takes place, leading to the corresponding alcohol and *p*-hydroxybenzoic acid. This reduced the extraction efficiency. Based on the obtained results, pH 7 was selected as the suitable pH for extraction. Nevertheless, the sample waters have commonly an optimum pH value in the range 5-7, thus pH adjustment was not necessary.

4.3.5 Effect of sample volume

The mass of nylon 6,6/ β -CD nanofibers used as a sorbent was 10.0 ± 0.5 mg. The volume of sample can affect the extraction performance caused by the breakthrough effect. Volume of sample was evaluated in the range of 5-20 mL with the same mass loading of $0.50 \mu\text{g}$. As a result shown in Figure 4.7, the mass of MP, EP, PP, iso-BuP, n-BuP were decreased when sample volume increasing while the mass of BzP, PeP were constant from 5-15 mL and slightly decreased after 15 mL. It probably due to BzP, PeP are more hydrophobicity than MP, EP, PP, iso-BuP, and n-BuP resulting in a strong interaction of analyte with the sorbent more than MP, EP, PP, iso-BuP, and n-BuP.

In real samples, the concentrations of parabens in water were much lower than the detection limit of analytical instrument hence it is necessary to enrich the parabens by increasing volume of samples to get a higher enrichment factor as shown in Table 4.5. Therefore, 15 mL of sample solution was chosen as the best volume as the best preconcentration factor with the sufficient extraction of parabens.

Table 4.5 Enrichment factor (EF) of parabens in different sample volume.

| Sample volume (mL) | Enrichment factor (EF) |
|--------------------|------------------------|
| 5 | 25 |
| 10 | 50 |
| 15 | 75 |
| 25 | 100 |

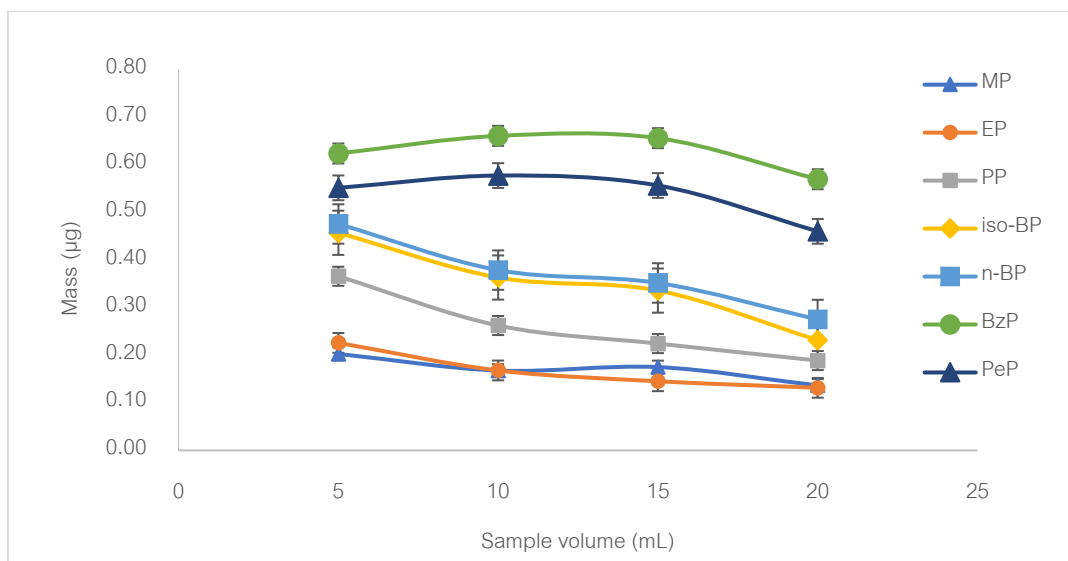


Figure 4.7 Effect of sample volume on the extraction efficiency.

4.3.6 Effect of salt addition

Normally, addition of salts can reduce the solubility of the target analytes in aqueous sample solutions and thus enhance their partitioning into the organic phase and can improve the extraction efficiency. In this work, the addition of NaCl concentration was studied in the range of 0-5%(w/v). The obtained results showed that the increase of NaCl from 0 to 5 %(w/v) has no significant effect on the extraction. As a result, salt addition was not necessary in all subsequent experiments.

According to the results, the optimized β -CD content: nylon 6,6 was 1:4 (by weight), desorption solvent was 200 μ L of methanol with 15 mL sample volume and the optimal area of pH is in range 5-7. Under optimized conditions, the acceptable %recovery was obtained for long alkyl chain parabens (iso-butyl paraben, n-butyl paraben, pentyl paraben) and a benzyl chain paraben (benzyl paraben) as shown in Table 4.6. Due to the hydrophobic properties of these parabens are more than a short alkyl chain leading to strong hydrophobic interaction with sorbent. For trace analysis, %recovery above 40% is recommended to get a good performance for quantitative

method [27]. Thus, iso-butyl paraben, n-butyl paraben, benzyl paraben, and pentyl paraben were further evaluated to validate the method.

Table 4.6 %Recovery of parabens under optimize conditions.

| Compounds | %recovery | %RSD |
|-------------------|-----------|------|
| Methyl paraben | 18.2 | 1.36 |
| Ethyl paraben | 16.4 | 0.60 |
| Propyl paraben | 25.5 | 1.40 |
| iso-butyl paraben | 40.6 | 1.55 |
| n-butyl paraben | 41.5 | 0.30 |
| Benzyl paraben | 81.4 | 1.43 |
| Pentyl paraben | 61.3 | 1.10 |

4.4 Validation of SPE method

The analytical performance of the purposed method for iso-butyl paraben, n-butyl paraben, benzyl paraben, and pentyl paraben were evaluated under the optimized condition in terms of linear range, limit of detection (LOD), limit of quantification (LOQ), extraction recovery (ER) and precision.

4.4.1 Linearity

Linearity was also assessed using seven calibration curves. The curves were determined by analyzing a linear range of 25 – 250 $\mu\text{g.L}^{-1}$ at seven standard mixtures with concentrations of 25, 50, 75, 100, 125, 150, 200, 250 $\mu\text{g.L}^{-1}$, added to the blank samples. Linearity of the method was showed by using the correlation coefficient (r^2) value of the plot between concentrations (X-axis) of standard solutions and their corresponding peak area values (Y-axis). The obtained results are listed in Table 4.7. The calibration curves showed a good linearity on the concentration range of 25-200 $\mu\text{g.L}^{-1}$ with satisfactory correlation coefficients ($r^2 > 0.9977$).

4.4.2 Limit of detection (LOD), and limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) values were calculated based on the International Union of Pure and Applied Chemistry (IUPAC) recommendation from the linear calibration curve of parabens [28]. The limit of detection (LOD) values were $10 \mu\text{g.L}^{-1}$ and the limit of quantitation (LOQ) values were found $25 \mu\text{g.L}^{-1}$ for all of paraben.

Table 4.7 Linearity data for the determination of parabens by HPLC-DAD.

| Compound | Linearity range ($\mu\text{g.L}^{-1}$) | r^2 | LOD ($\mu\text{g.L}^{-1}$) | LOQ ($\mu\text{g.L}^{-1}$) |
|-------------------|--|--------|------------------------------|------------------------------|
| iso-butyl paraben | 25 – 200 | 0.9989 | 10 | 25 |
| n-butyl paraben | 25 – 200 | 0.9977 | 10 | 25 |
| Benzyl paraben | 25 – 200 | 0.9999 | 10 | 25 |
| Pentyl paraben | 25 – 200 | 0.9992 | 10 | 25 |

4.4.3 Accuracy and precision

Accuracy and precision of the method based on the percentage recovery and HorRat test were determined at a fortification level of $50 \mu\text{g.L}^{-1}$ of analytes under optimized condition. The results were listed in Table 4.8. The recovery values for intra-day evaluation were in range of 38.8-43.8%, 36.1-41.6%, 71.1-82.5%, and 60.4-63.2% of iso-BuP, n-BuP, BzP, and PeP, respectively. The overall average recovery for three days were 41.1%, 39.5%, 77.2 % and, 61.6% of iso-BuP, n-BuP, BzP, and PeP, respectively. The accuracy of the method was in the acceptable range. Precision experiments were performed for repeatability and intermediate precision at concentration at $50 \mu\text{g.L}^{-1}$ of standard. From Table 4.8, the experimental %RSD was in the range of 0.30-3.90% for intra-day precision. Inter-day precision was evaluated by pool data of all three days and the experimental %RSD was 3.67, 5.48, 4.69 and 1.47 for iso-BuP, n-BuP, BzP, and PeP, respectively. The HorRat Test of inter-day precision was in the range 0.02-0.24, which illustrated a good precision of the method with the HorRat values was less than 2.

Table 4.8 Results of accuracy and precision study of iso-butyl paraben, n-butyl paraben, benzyl paraben and, pentyl paraben using the optimum condition.

| Compounds | Iso-Butyl paraben | | | n-Butyl paraben | | | Benzyl paraben | | | Pentyl paraben | | |
|-----------------------------|-------------------|------|------|-----------------|------|------|----------------|------|------|----------------|------|------|
| | Day1 | Day2 | Day3 | Day1 | Day2 | Day3 | Day1 | Day2 | Day3 | Day1 | Day2 | Day3 |
| %recovery | 42.7 | 40.0 | 40.6 | 40.2 | 36.8 | 41.5 | 74.4 | 75.7 | 81.4 | 62.6 | 61.0 | 61.3 |
| %RSD _{exp} | 2.88 | 2.66 | 1.55 | 1.29 | 2.14 | 0.30 | 3.90 | 1.54 | 1.43 | 0.74 | 0.96 | 1.09 |
| Overall %recovery | 41.1 | | | 39.5 | | | 77.2 | | | 61.6 | | |
| Overall %RSD _{exp} | 2.36 | | | 1.24 | | | 2.29 | | | 0.93 | | |
| Horrrat | 0.17 | 0.16 | 0.09 | 0.08 | 0.13 | 0.02 | 0.24 | 0.09 | 0.09 | 0.04 | 0.06 | 0.07 |

4.5 Application to real sample

The method has been applied for simultaneous separation and determination of iso-butyl paraben, n-butyl paraben, benzyl paraben and pentyl paraben in drinking water. The drinking water samples were purchased from local supermarkets in Pijit, Nakhon Si Thammarat, and Ayutthaya province to demonstrate the analytical performance on real samples. All the samples were collected and filtered by 0.45 μm membrane filters and stored in amber glass containers at 4 °C until analysis. The water samples were extracted by nylon 6,6/25 β -CD nanofibers under optimized condition. All parabens were not detected in real water sample and no interference from sample was observed as shown in Figure 4.9(a). Therefore, the real drinking water samples were spiked at 50 $\mu\text{g}\cdot\text{L}^{-1}$ parabens for validation of the method as shown in Figure 4.8(b). The obtained results, showed good recoveries, no sample matrix effect, and %RSD of three analyses (n=3) are in the acceptable range (Table 4.9).

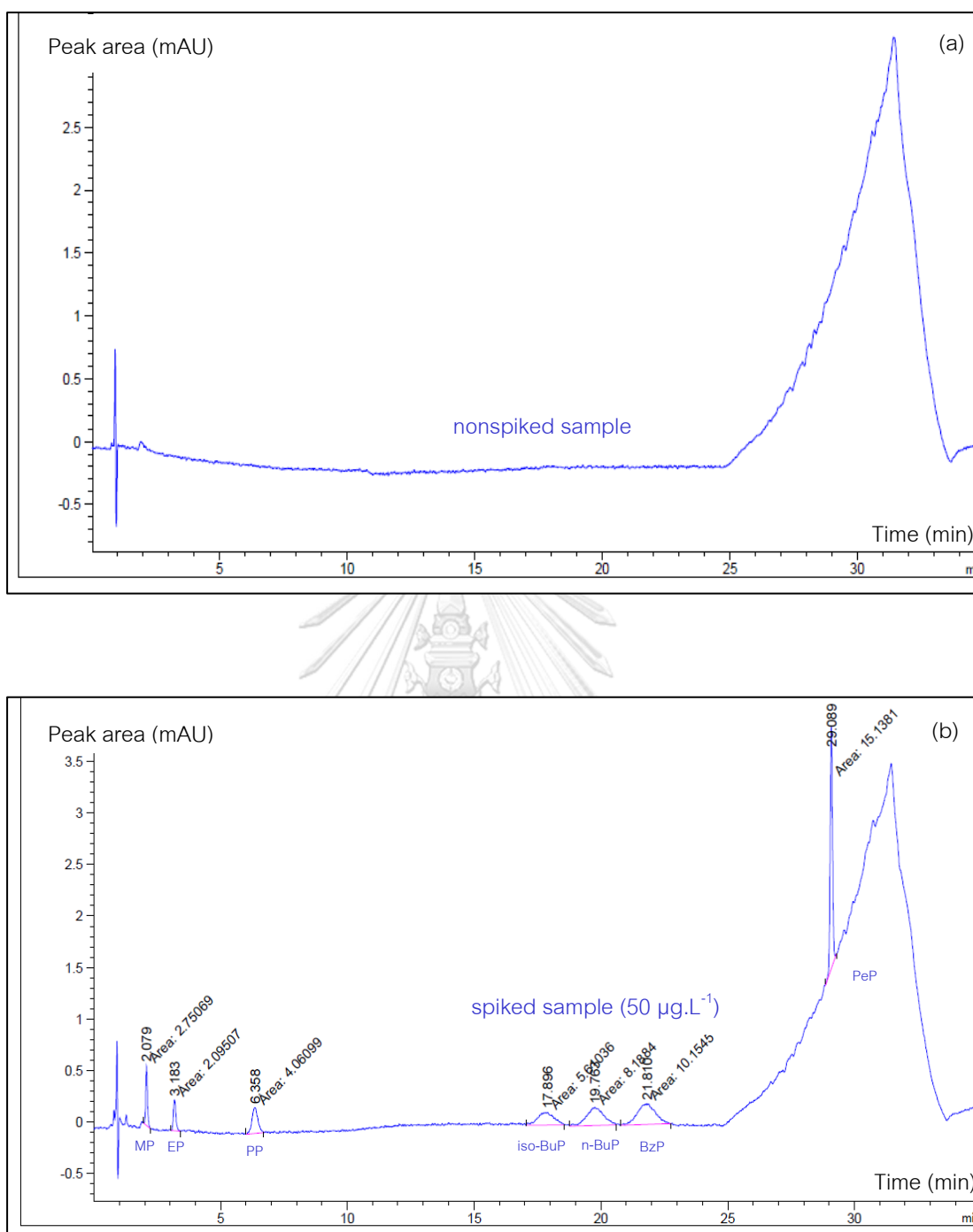


Figure 4.8 Chromatograms of (a) a nonspiked sample and (b) spiked sample ($50 \mu\text{g.L}^{-1}$).

Table 4.9 %Recoveries and %RSD of the extraction of spiked drinking water at $50 \mu\text{g.L}^{-1}$ parabens (n=3).

| Sample | iso-butyl paraben | | n-butyl paraben | | Benzyl paraben | | Pentyl paraben | |
|---------|-------------------|------|-----------------|------|----------------|------|----------------|------|
| | %recovery | %RSD | %recovery | %RSD | %recovery | %RSD | %recovery | %RSD |
| Brand 1 | 33.6 | 1.71 | 40.2 | 4.52 | 81.1 | 3.00 | 73.7 | 3.01 |
| Brand 2 | 42.9 | 6.41 | 48.7 | 4.02 | 87.9 | 2.37 | 86.9 | 4.20 |
| Brand 3 | 43.7 | 5.27 | 52.3 | 2.12 | 89.5 | 2.77 | 89.5 | 0.87 |

4.6 Reusability

The reusability of the nylon 6,6/ β -CD sorbent was evaluated to observe the reusability of the adsorbent. As shown in Figure 4.9, %recovery slightly increases during the cycles from 1 to 4, and then stabled at cycle 5-10. After 10 cycles, %recovery of all parabens were decreased. Therefore, the nylon 6,6/25 β -CD nanofiber could be reused after 10 times without a significant reduction in recoveries.

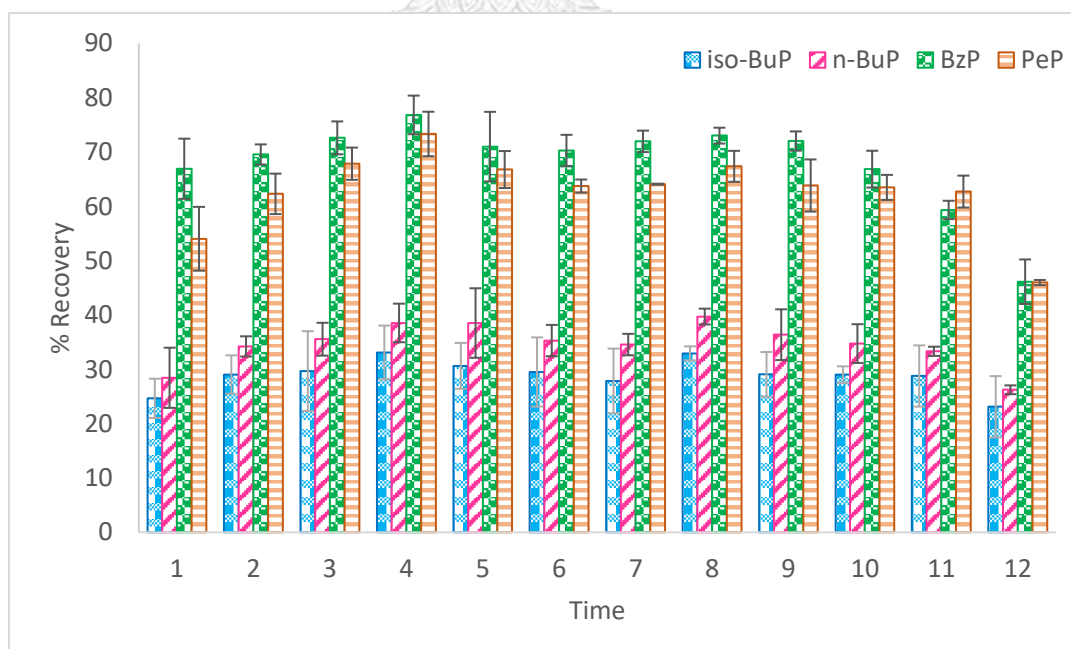


Figure 4.9 Reusability of nylon 6,6/25 β -CD nanofiber for adsorption of iso-BuP, n-BuP, BzP and PeP.

4.7 Comparison of proposed method with other methods

The efficiency of the proposed method was compared with the previously reported method. The comparison are summarized in Table 4.10. Comparing the analytical method based on SPE with HPLC-UV analysis, the proposed method improved the sensitivity in relation to linearity, LOD and LOQ and lower the organic solvent consumption. Moreover, extraction time in the proposed method is shorter than other SPE method. That caused from the mass transferring processing of analytes from aquatic phase to organic phase was improved. However, the recoveries of iso-BuP, n-BuP and PeP in this proposed method were lower than those of the method [25, 29-33]. This may be caused by the alkyl chain in structure of iso-BuP, n-BuP and PeP are not specific with β -CD while BzP that have aromatic in its structure leading to good extraction efficiencies. Despite this, the method is very simple, does not need any complex or expensive instrument and requires a small amount of sample. Thus, the proposed method is a promising sensitive, rapid, repeatable and suitable alternative to the traditional techniques for sample preparation when analyzing parabens.

Table 4.10 Comparison of the proposed method (SPE/HPLC-DAD) with other methods used in determination of parabens in water.

| Method | Sample | Analytes | Linearity | LOD | LOQ | Sample amount (mL) | Recovery | EF | Extraction time | RSD (%) | | Ref. | |
|------------------|---------------------------|----------|-----------|---------|---------|--------------------|---|-----|-----------------|-----------|-----------|------------|-----|
| | | | range | (ng/mL) | (ng/mL) | | % | | (min) | Intra-day | Inter-day | | |
| | | | (ng/mL) | (ng/mL) | (ng/mL) | | | | | | | | |
| DLLME/LC-UV | tap water | MP | 1-500 | 0.046 | 0.15 | 5 | 25 | 27 | 1 | 7.8 | - | [25] | |
| | | EP | 1-500 | 0.043 | 0.14 | | 48 | | | 50 | 9.3 | | - |
| | | PP | 1-500 | 0.022 | 0.07 | | 67 | | | 70 | 8.9 | | - |
| | | BuP | 1-500 | 0.021 | 0.07 | | 86 | | | 90 | 4.1 | | - |
| DES/HPLC-DAD | tap water, waste water | MP | 3-1000 | 0.8 | 3.1 | 8 | 69 | - | - | 4.6 | 2.5 | [29] | |
| | | EP | 3-1000 | 0.6 | 2.5 | | 76 | | | 5.4 | 3.5 | | |
| | | PP | 3-1000 | 0.6 | 2.5 | | 78.5 | | | 3.4 | 7.2 | | |
| | | BuP | 3-1000 | 0.8 | 3.1 | | 72.2 | | | 1.1 | 3.6 | | |
| DLLME/GC-FID | tap water, river water | MP | 2-5000 | 0.5 | - | 5 | 51 | 128 | 10 | 8.1 | 9.2 | [30] | |
| | | EP | 2-5000 | 1.0 | - | | 55 | | | 137 | 7.6 | | 7.8 |
| | | PP | 2-5000 | 0.5 | - | | 59 | | | 147 | 7.8 | | 9.4 |
| SPE/LC-QTOF-MS | waste water | MP | 5-500 | 0.0040 | - | 200 | 99 ± 4 | - | 30 | 3.9 | - | [31] | |
| | | EP | | 0.0014 | | | 104 ± 4 | | | 2.0 | | | |
| | | iso-P | | 0.0016 | | | 99 ± 2 | | | 3.2 | | | |
| | | n-P | | 0.0013 | | | 103 ± 5 | | | 2.1 | | | |
| | | iso-BuP | | 0.0022 | | | 108 ± 8 | | | 1.6 | | | |
| | | n-BuP | | 0.0022 | | | 103 ± 7 | | | 2.1 | | | |
| | | BzP | | 0.0019 | | | 102 ± 8 | | | 3.4 | | | |
| SPE/LC/ESI-MS/MS | Water | MP | - | 0.7 | - | 200 | > 85 % (River water) > 65% (waste water) | 50 | 30 | 2.5 | - | [32] | |
| | | EP | | 0.6 | | | | | | 4.9 | | | |
| | | iso-P | | 0.6 | | | | | | 4.2 | | | |
| | | n-P | | 0.6 | | | | | | 4.3 | | | |
| | | iso-BuP | | 0.6 | | | | | | 3.4 | | | |
| | | n-BuP | | 0.6 | | | | | | 4.7 | | | |
| | | BzP | | 0.2 | | | | | | 2.7 | | | |
| SPE/HPLC-UV | Water | MP | 300-5000 | 100 | - | 2 | 92.0 | 10 | - | < 6.6 % | - | [33] | |
| | | EP | 300-5000 | 100 | | | 88.0 | | | | | | |
| | | PP | 300-5000 | 100 | | | 86.0 | | | | | | |
| | | iso-BuP | 600-5000 | 200 | | | 91.0 | | | | | | |
| | | n-BuP | 600-5000 | 200 | | | 89.0 | | | | | | |
| SPE/HPLC-DAD | Water | iso-BuP | 25-200 | 10 | 25 | 15 | 40.6 | 75 | 15 | 1.6 | 3.7 | This study | |
| | | n-BuP | | | | | 41.5 | | | 0.3 | 5.5 | | |
| | | BzP | | | | | 81.4 | | | 1.4 | 4.7 | | |
| | | PeP | | | | | 61.3 | | | 1.1 | 1.5 | | |

CHAPTER V

CONCLUSION

5.1 Conclusion

The electrospun nylon 6,6/ β -CD nanofiber as a sorbent in SPE were successfully fabricated. In this research, the diameter of electrospun nylon 6,6/ β -CD nanofibers were unaffected with the additional of β -CD. The diameter of the obtained nanofibers were in the range 120-150 nm. However, the specific surface area of nylon 6,6/ β -CD nanofibers was decreased when the content of β -CD in the fibers increases due to the covering of β -CD on the surface of nylon 6,6 nanofibers. The presence of β -CD on the surface nylon 6,6 nanofiber is able to form an inclusion complex with benzyl paraben (hydrophobic interaction) leading to good extraction efficiency. For alkyl parabens, they prefer to have hydrogen bonding interaction with nylon 6,6 nanofiber resulting in higher extraction efficiencies than nylon 6,6 nanofibers. Finally, the electrospun nylon 6,6/25 β -CD which has the largest surface area among nylon 6,6/25 β -CD was applied as a sorbent in extraction of parabens in water sample prior to HPLC with DAD analysis. In order to achieve the best extraction efficiency, the variables affecting the extraction was optimized. As a result, the optimized conditions were summarized in Table 5.1. Under the optimized conditions, the method showed very low detection limits, relatively good precision, short extraction time, and low organic solvent and sample consumption. Therefore, nylon 6,6/25 β -CD nanofiber could be used as an alternative sorbent for extraction of parabens from environmental water samples.

Table 5.1 Optimization of the solid-phase extraction method for determination parabens.

| Parameter | Optimized condition |
|--------------------------|---------------------|
| Sorbent dosage | 10.0 ± 0.5 mg |
| β-CD content : nylon 6,6 | 1 : 4 (by weight) |
| Desorption solvent | Methanol |
| Desorption volume | 200 μL |
| pH sample solution | 5-7 |
| Sample volume | 15 mL |

5.2 Suggestion of future work

The electrospun nylon 6,6/β-CD nanofibers were a good material for extraction of parabens. However, the selectivity of nylon 6,6/β-CD nanofibers to parabens could be improved. It could interact with various forces e.g. pi-pi interaction, hydrogen bonding, and electrostatic force. Moreover, this material could be applied for extraction of other analysts with specified of the sorbent properties leading to improving the good extraction efficiency.

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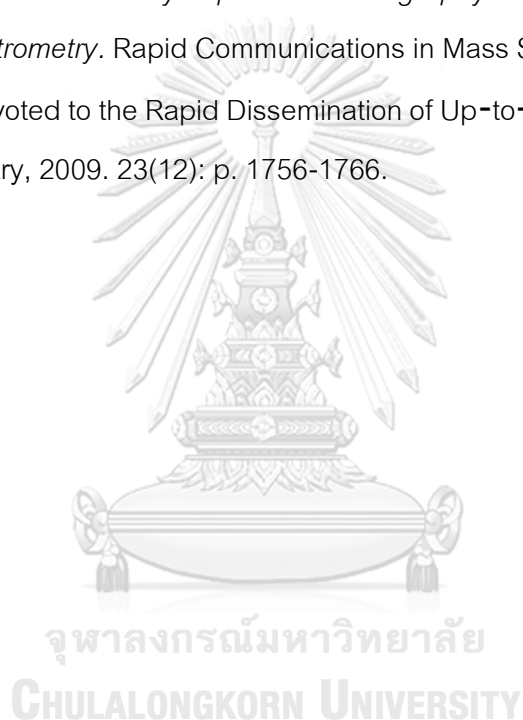
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APPENDIX

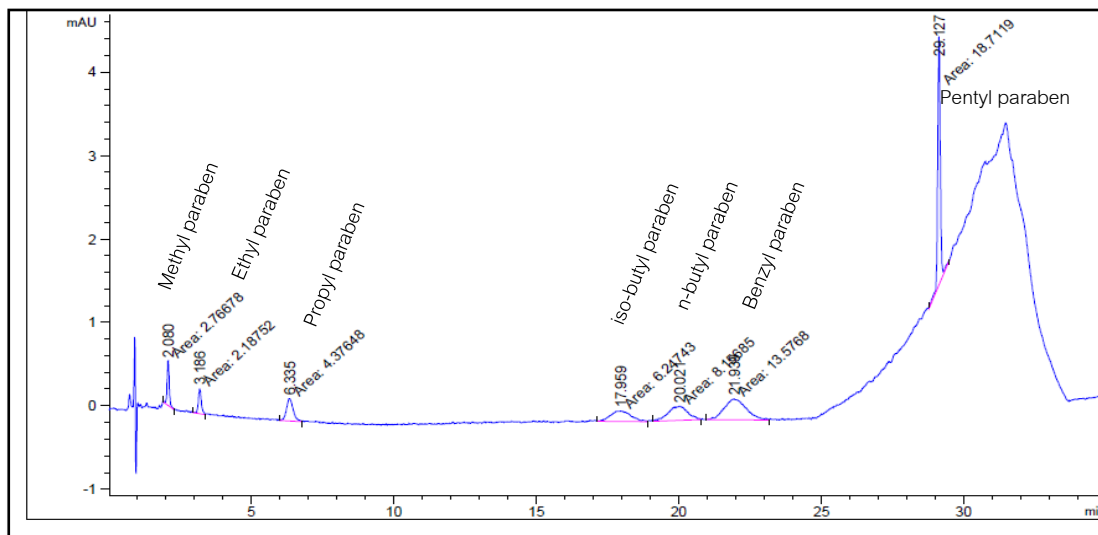


Figure A.1 SPE-HPLC-DAD chromatograms of parabens in water sample (spiked $100 \mu\text{g.L}^{-1}$) under optimized experimental conditions.

Table A.1 Results of accuracy and precision study of iso-butyl paraben, n-butyl paraben, benzyl paraben and, pentyl paraben using the optimum condition (n=3).

| No. | % Recovery | | | | | | | | | | | |
|---------------------|--------------------|-------|-------|-----------------|-------|-------|----------------|-------|-------|----------------|-------|-------|
| | iso -Butyl paraben | | | n-Butyl paraben | | | Benzyl paraben | | | Pentyl paraben | | |
| | Day 1 | Day 2 | Day 3 | Day 1 | Day 2 | Day 3 | Day 1 | Day 2 | Day 3 | Day 1 | Day 2 | Day 3 |
| 1 | 43.8 | 38.8 | 41.1 | 40.1 | 37.6 | 41.6 | 76.5 | 74.6 | 80.2 | 63.2 | 61.0 | 61.8 |
| 2 | 41.4 | 40.9 | 40.9 | 39.7 | 36.5 | 41.5 | 75.5 | 75.7 | 82.5 | 62.3 | 61.6 | 61.6 |
| 3 | 43.0 | 40.3 | 39.9 | 40.7 | 36.1 | 41.3 | 71.1 | 76.9 | 81.3 | 62.4 | 60.4 | 60.6 |
| Mean | 42.7 | 40.0 | 40.6 | 40.2 | 36.7 | 41.5 | 74.4 | 75.7 | 81.4 | 62.6 | 61.0 | 61.3 |
| SD | 1.23 | 1.07 | 0.63 | 0.52 | 0.79 | 0.12 | 2.90 | 1.17 | 1.17 | 0.47 | 0.59 | 0.67 |
| %RSD _{exp} | 2.88 | 2.66 | 1.55 | 1.29 | 2.14 | 0.30 | 3.90 | 1.54 | 1.43 | 0.74 | 0.96 | 1.09 |
| HorRat | 0.17 | 0.16 | 0.09 | 0.08 | 0.13 | 0.02 | 0.24 | 0.09 | 0.09 | 0.04 | 0.06 | 0.07 |

$$*PRSD = \pm 0.66 \times 2 \times C^{-0.1505} = 16.572$$

Table A.2 Optimized chromatographic conditions of HPLC-UV

| Parameter | Conditions |
|----------------------|---|
| Column | Eclipse XDB-C 18, 5 μ m (4.6x150mm) |
| Mobile phase | Gradient mode: (A)%water, (B)%Methanol 0.0 min – 55%B 8.0-23.0 min – 45%B 30 min – 90%B |
| Flow rate | 1.5 mL/min |
| Detection wavelength | 256 nm |
| Injection volume | 10 μ L |
| Column temperature | 27 $^{\circ}$ C |
| Run time | 30 min |

VITA

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|----------------|--|
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