



**Development of Solid Phase Microextraction Device Based on the Use
of Pencil Lead Graphite Sorbent Modified with Nanomaterials for
the Extraction of Trace Phthalate Esters**

Nichapat Chunin

**A Thesis Submitted in Partial Fulfillment of the Requirements for the
Degree of Master of Science in Chemistry**

Prince of Songkla University

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ชื่อวิทยานิพนธ์	การพัฒนาอุปกรณ์สกัดด้วยตัวดูดซับของแข็งที่มีขนาดเล็กแบบไส้ดินสอด่ที่มี การปรับปรุงพื้นผิวด้วยวัสดุนาโนสำหรับการสกัดสารพหาลเตเอสเทอร์ ปริมาณน้อย
ผู้เขียน	นางสาวณิชาภัทร์ ชูนิรินทร์
สาขา	เคมี
ปีการศึกษา	2561

บทคัดย่อ

วิทยานิพนธ์นี้เป็นการพัฒนาเทคนิคการสกัดด้วยตัวดูดซับของแข็งที่มีขนาดเล็กแบบไส้ดินสอด่ สำหรับการวิเคราะห์สารพหาลเตเอสเทอร์ปริมาณน้อย โดยแบ่งงานออกเป็น 2 ส่วน ส่วนแรกเป็นการศึกษาถึงความเป็นไปได้ในการใช้ไส้ดินสอด่ที่มีการเพิ่มพื้นที่ผิวด้วยอนุภาคนาโนเงินร่วมกับพอลิอะนิลีนโดยการเกาะติดด้วยเทคนิคไฟฟ้าเคมีแบบชั้นตอนเดียวในการสกัดเบนซิลบิวทิลพหาลเตเอสเทอร์และได-2-เอทิลเฮกซิลพหาลเตเอสเทอร์ แล้วคายการดูดซับด้วยความร้อนบริเวณหัวฉีดของเครื่องแก๊สโครมาโทกราฟที่มีตัวตรวจวัดชนิดอิเล็กตรอนแคปเจอร์ ภายใต้สภาวะที่เหมาะสม พบว่าเทคนิคที่พัฒนาขึ้นให้ช่วงการตอบสนองเชิงเส้นสำหรับการวิเคราะห์อยู่ในช่วง 0.05-5.00 มิลลิกรัมต่อลิตร และ 0.25-5.00 มิลลิกรัมต่อลิตร และขีดจำกัดการตรวจวัดที่ 0.030 และ 0.24 มิลลิกรัมต่อลิตร สำหรับเบนซิลบิวทิลพหาลเตเอสเทอร์และได-2-เอทิลเฮกซิลพหาลเตเอสเทอร์ตามลำดับ และจากผลการสกัดสารทั้งสองชนิดที่เติมลงไปใต้น้ำปราศจากไอออนที่ความเข้มข้น 1.0 มิลลิกรัมต่อลิตร พบว่าได้ร้อยละการได้กลับคืนมาที่สูงและยังสามารถเพิ่มความสามารถของสารที่สนใจวิเคราะห์ได้ ดังนั้นเทคนิคการสกัดที่พัฒนาขึ้นจึงมีแนวโน้มที่จะสามารถสกัดสารเบนซิลบิวทิลพหาลเตเอสเทอร์และได-2-เอทิลเฮกซิลพหาลเตเอสเทอร์ได้ งานส่วนที่สองเป็นการต่อยอดความคิดจากงานแรก โดยยังคงการใช้ไส้ดินสอด่ที่มีการเพิ่มพื้นที่ผิวด้วยการเกาะติดด้วยเทคนิคไฟฟ้าเคมีแบบชั้นตอนเดียวระหว่างอนุภาคนาโนเงินร่วมกับพอลิอะนิลีนเป็นตัวดูดซับเช่นเดิม แต่ใช้ไส้ดินสอด่ที่มีขนาดใหญ่ขึ้นเพื่อเพิ่มความจุตัวอย่างและเพิ่มความคงทน นอกจากนี้ยังมีการออกแบบอุปกรณ์การสกัดให้เป็นแบบหมุนที่ใช้งานสะดวกและพกพาง่ายด้วยเครื่องพิมพ์สามมิติจากการศึกษาลักษณะโครงสร้างของตัวดูดซับที่พัฒนาขึ้นด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด พบว่าไส้ดินสอด่ที่มีการสังเคราะห์ฟิล์มของอนุภาคนาโนเงินร่วมกับพอลิอะนิลีนมีลักษณะเป็นฟิล์มที่มีรูพรุน ซึ่งจะช่วยให้เพิ่มพื้นที่ผิวในการสกัดได้เป็นอย่างดี เมื่อนำตัวดูดซับที่พัฒนาขึ้นมาประยุกต์ใช้ในการวิเคราะห์สารกลุ่มพหาลเตเอสเทอร์ 5 ชนิด ได้แก่ ไดเมทิลพหาลเตเอสเทอร์ ไดเอทิลพหาลเตเอสเทอร์ ไดบิวทิลพหาลเตเอสเทอร์ เบนซิลบิวทิลพหาลเตเอสเทอร์ และ ได-2-เอทิลเฮกซิลพหาลเตเอสเทอร์ ภายใต้สภาวะที่เหมาะสม พบว่าเทคนิคที่พัฒนาขึ้นให้ช่วงการตอบสนองเชิงเส้นสำหรับการวิเคราะห์สารพหาลเตเอสเทอร์ทั้งห้าชนิดอยู่ในช่วง 5.0 ถึง 1.0×10^3 ไมโครกรัมต่อลิตร และขีดจำกัดการตรวจวัดที่ 4.4 ± 0.91 ไมโครกรัมต่อ

ลิตร สำหรับไดเมทิลพทาเลต, 3.98 ± 0.92 ไมโครกรัมต่อลิตร สำหรับไดเอทิลพทาเลต, 3.65 ± 0.74 ไมโครกรัมต่อลิตร สำหรับไดบิวทิลพทาเลต, 4.91 ± 0.52 ไมโครกรัมต่อลิตร สำหรับเบนซิลบิวทิลพทาเลต และ 4.25 ± 0.66 ไมโครกรัมต่อลิตร สำหรับได-2-เอทิลเฮกซิลพทาเลต นอกจากนี้ยังพบว่าวิธีการเตรียมตัวดูดซับของแข็งขนาดเล็กแบบใส่ดินสอให้ค่าการทำซ้ำที่ดี (ค่าเบี่ยงเบนมาตรฐานสัมพัทธ์น้อยกว่า 6.6 เมื่อมีการเตรียมซ้ำ 6 ชุด) หลังการนั้นจึงได้ประยุกต์ใช้เทคนิคการสกัดที่พัฒนาขึ้นในการสกัดสารพทาเลตเอสเทอร์ทั้ง 5 ชนิดในตัวอย่างเวชภัณฑ์และเวชสำอาง 14 ชนิด แล้ววิเคราะห์สารที่สกัดได้ด้วยเครื่องแก๊สโครมาโทกราฟที่มีตัวตรวจวัดชนิดฟลูออโรอีนเซนชัน โดยพบการปนเปื้อนของไดเอทิลพทาเลตและได-2-เอทิลเฮกซิลพทาเลตในแอลกอฮอล์ล้างแผลเท่ากับ 7.03 ± 0.76 ไมโครกรัมต่อลิตร และ 5.89 ± 0.53 ไมโครกรัมต่อลิตร ตามลำดับ และพบการปนเปื้อนของได-2-เอทิลเฮกซิลพทาเลตในน้ำยาล้างคอนแทคเลนส์เท่ากับ 5.3 ± 1.1 ไมโครกรัมต่อลิตร ถึง 6.8 ± 1.2 ไมโครกรัมต่อลิตร โดยให้ร้อยละการได้กลับคืนของการสกัดในช่วง 81.92 ± 0.99 เปอร์เซ็นต์ ถึง 102.4 ± 1.1 เปอร์เซ็นต์ ยิ่งไปกว่านั้นอุปกรณ์สกัดที่พัฒนาขึ้นสามารถนำกลับมาใช้ซ้ำได้ถึง 18 ครั้ง โดยยังคงประสิทธิภาพที่ดี จึงสรุปได้ว่าเทคนิคการสกัดด้วยตัวดูดซับของแข็งที่มีขนาดเล็กแบบใส่ดินสอสำหรับการสกัดสารพทาเลตเอสเทอร์ที่พัฒนาขึ้นสามารถใช้งานได้ง่าย สะดวก ให้ผลการวิเคราะห์ที่แม่นยำเที่ยงตรง และมีความน่าเชื่อถือ อีกทั้งสามารถนำมาประยุกต์ใช้สำหรับการวิเคราะห์สารปนเปื้อนอื่น ๆ ได้

Thesis Title	Development of Solid Phase Microextraction Device Based on the Use of Pencil Lead Graphite Sorbent Modified with Nanomaterials for the Extraction of Trace Phthalate Esters
Author	Miss Nichapat Chunin
Major Program	Chemistry
Academic Year	2018

ABSTRACT

This thesis aimed to develop miniaturized sample preparation techniques for the determination of phthalate esters. To achieve this goal, two sub-projects were carried out. The first sub-project is the preliminary study of the extraction of benzyl butyl phthalate (BBP) and di-2-ethylhexyl phthalate (DEHP) by a silver-incorporated polyaniline film pencil lead SPME fiber (Ag/PANI SPME fiber). This fiber was used to extract trace phthalate esters and thermally desorbed at an injection port of a gas chromatograph coupled with an electron capture detector (GC-ECD). Under the optimum conditions, linearities of the determination of two phthalate esters, BBP and DEHP, were in the range of 0.05-5.00 mg L⁻¹ and 0.25-5.00 mg L⁻¹ and limits of detection of 0.030 and 0.24 mg L⁻¹ were also obtained for BBP and DEHP, respectively. A high preconcentration factor and satisfactory recoveries were achieved. This study indicated that the developed polyaniline/pencil lead SPME fiber can be used to extract BBP and DEHP. In the second sub-project, based on the results obtained and the problem found from the first sub-project, the size of the pencil lead used was enlarged to increase the sample loading capacity and to improve the robustness and the portability of the extraction technique. A screw-based portable and simple solid phase microextraction device constructed with a 3D printer was developed. The scanning electron microscope revealed the porous structure of the electrodeposited Ag/PANI film, and X-ray diffraction confirmed the presence of silver in the porous polymer film. The fiber was used to extract trace quantities of phthalate esters. The compounds of interest were dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP) and di-2-ethylhexyl phthalate (DEHP). The extracted solution was identified and quantified by a gas chromatograph equipped with

a flame ionization detector (GC-FID). Under the optimum conditions of the developed method, a good linearity was obtained in a concentration range from 5.0 to 1.0×10^3 $\mu\text{g L}^{-1}$ for all five phthalate esters with limits of detection (LODs) of 4.41 ± 0.91 $\mu\text{g L}^{-1}$ for DMP, 3.98 ± 0.92 $\mu\text{g L}^{-1}$ for DEP, 3.65 ± 0.74 $\mu\text{g L}^{-1}$ for DBP, 4.91 ± 0.52 $\mu\text{g L}^{-1}$ for BBP and 4.25 ± 0.66 $\mu\text{g L}^{-1}$ for DEHP. The developed method provided good precision when tested with standard solutions (RSD < 5.5%, n=6) and real samples (RSD < 3.4%, n=6). Good fiber-to-fiber reproducibility was also confirmed by extraction with six prepared fibers: recoveries ranged from $81.09 \pm 0.54\%$ to $92.92 \pm 0.46\%$, RSD < 6.6% (n=6). The developed method was used to determine phthalate esters in 14 cosmetic samples. In rubbing alcohol, DEP and DEHP were detected at the concentrations of 7.03 ± 0.76 $\mu\text{g L}^{-1}$ and 5.89 ± 0.53 $\mu\text{g L}^{-1}$, respectively, while in contact lens cleaner samples, DEHP was found in a concentration range from 5.3 ± 1.1 $\mu\text{g L}^{-1}$ to 6.8 ± 1.2 $\mu\text{g L}^{-1}$. No contamination was found in saline solution, eye cleaner and antibacterial disinfectant liquid samples. Recoveries in the range of $81.92 \pm 0.99\%$ to $102.4 \pm 1.1\%$ indicated the good accuracy of the developed method. It was expected that the developed method would also be applicable for the extraction and determination of other organic compounds.

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Nichapat Chunin

THE RELEVANCE OF THE RESEARCH WORK TO THAILAND

The purpose of this Master of Science Thesis in Chemistry (Analytical Chemistry) is to develop simple miniaturized sample preparation techniques, including evaluation of their performances, for the determination of phthalate esters. Two sub-projects were investigated as follow. Sub-project I: a polyaniline/pencil lead solid phase microextractor for trace phthalate esters. Sub-project II: a simple 3D printed-screw-based solid phase microextraction device with a silver decorated polyaniline pencil lead fiber for the extraction of phthalate esters in cosmeceutical products. These developed methods provide high efficiency for the extraction of target analytes. They can be applied for the quantitative analysis of trace phthalate esters in cosmeceutical products which are easy and portable. Moreover, the developed the method can be used as alternative sorbents in various governmental organizations and private sectors in Thailand.

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LIST OF ABBREVIATIONS

AOAC	Association of Analytical Communities
BBP	Benzyl butyl phthalate
CAR	Carbowax
CNTs	Carbon nanotubes
DAD	Diode array detector
DMP	Dimethyl phthalate
DEP	Diethyl phthalate
DBP	Dibutyl phthalate
DEHP	Di-2-ethylhexyl phthalate
DI-SPME	Direct immersion-solid phase microextraction
DLLME	Dispersive liquid-liquid microextraction
DVB	Divinylbenzene
ECD	Electron capture detector
FID	Flame ionization detector
FLD	Fluorescence detector
GC	Gas chromatography
HPLC	High performance liquid chromatography
HS-SPME	Headspace-solid phase microextraction
IQ	Intelligence quotient
LDR	Linear dynamic range
LLE	Liquid-liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
MCL	Maximum contaminant level
MSPE	Magnetic solid phase extraction
MS	Mass spectrometer
MWCNTs	Multiwalled carbon nanotubes
μ -SPE	Micro-solid phase extraction
PAEs	Phthalate esters
PANI	Polyaniline

LIST OF ABBREVIATIONS (Continued)

PDMS	Polydimethylsiloxane
PP	Polypropylene
PPy	Polypyrrole
RSD	Relative standard deviation
RfDs	Reference doses
Ag/PANI	Silver-incorporated polyaniline
SD	Standard deviation
SPME	Solid phase microextraction
SBSE	Stir-bar sorptive extraction
SVOCs	Semi-volatile organic compounds
SWCNTs	Single-walled carbon nanotubes
UAE	Ultrasonic-assisted extraction
UE	Ultrasonic extraction
USD	United states dollar
US EPA	United States Environmental Protection Agency
US FDA	United States Food and Drug Administration
VOCs	Volatile organic compounds

LIST OF PUBLICATIONS

This thesis is organized to contain two parts. The first part includes an introduction, some background information and conclusions obtained from this M.Sc. study and the second part consists of the following papers and petty patent from the basis of this master thesis, which are referred to in the text by their Roman

- Paper I** **Chunin, N.,** Kaewpet, M., Wattanasin, P., Kanatharana, P., Thavarangkul, P. and Thammakhet-Buranachai, C. (2018). “A polyaniline/pencil lead solid phase microextractor for trace phthalate esters” (p 146-150). In the Pure and Applied Chemistry International Conference 2018 (PACCON 2018). The 60th International Convention Center (ICC Hatvyai), Hat Yai, Songkhla, Thailand.
Proceeding
- Paper II** **Chunin, N.,** Phooplub, K., Keawpet, M., Wattanasin, P., Kanatharana, P., Thavarangkul, P. and Thammakhet-Buranachai, C. “A simple 3D printed-screw-based solid phase microextraction device with a silver decorated polyaniline pencil lead fiber for the extraction of phthalate esters in cosmeceutical products”
Manuscript submitted to Analytica Chimica Acta
- Petty Patent** จงดี บุรณชัย, ณิชากัณฑ์ ชูนิรินทร์, มรกต แก้วเพชร, พันธุ์วดี วัฒนสิน, เพรศพิชญ์ คณาธารณา, ปณิต ถาวรังกูร. 2561. อุปกรณ์สกัดสารอินทรีย์แบบหมุน (Screw-based pencils extraction device) สำหรับการสกัดสารอินทรีย์ (organic compounds) ประเทศไทย เลขคำขออนุสิทธิบัตร 1903000062

1. Introduction

1.1 Background and Rationale

Phthalates or phthalate esters (PAEs) are esters of phthalic acid, which are a class of compounds that can increase the flexibility of plastics through weak secondary molecular interactions with polymer chains. They are used as a plasticizer in about 80-85% to produce plastic containers of several personal-care products and some medications in the worldwide market, such as cosmetics, shampoos, soaps, rubbing alcohol and saline solution (Gari *et al.*, 2019; Lenoir *et al.*, 2007 and Guo and Kannan, 2012). There is no covalent bond between the PAEs and plastics, it can easily leach from plastic packaging into food and cosmeceutical products that they come into contact with (Chang *et al.*, 2019; Liu *et al.*, 2008; Balafas *et al.*, 1999). Several phthalate esters were included in the list of concerned chemicals by the United States Environmental Protection Agency (US EPA) (US EPA, 1988, 2002a, 2002b, 2009) since they can cause the allergies, endocrine disorders and alterations in the reproductive system (Weiss *et al.*, 2018; Tickner *et al.*, 2001; Arcadi *et al.*, 1998). Examples are dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP) and di-2-ethylhexyl phthalate (DEHP) (**Figure 1**) (US EPA, 1988, 2002a, 2002b). In addition, DEHP has been classified as ‘possibly carcinogenic to humans’ but there is no conclusive evidence that any phthalates cause cancer.

The toxicity of phthalate esters was studied in many cases. Phthalate esters belong to the group of endocrine disruptors (Arbuckle *et al.*, 2018; Frederiksen *et al.*, 2007), which have been shown to affect the male reproductive system in animal studies. To write the risk, US EPA has set reference doses (RfDs) for phthalate esters such as DMP, DEP, DBP, BBP and DEHP (US EPA, 1988, 2002a, 2002b). RfDs are an estimate of the daily exposure that is likely to have no appreciable risk of deleterious effects during a lifetime in human. However, a recent study shows that the phthalate esters include DEHP, DBP and BBP (US FDA, 2012; Petrović *et al.*, 2001; Tickner *et al.*, 2001; Arcadi *et al.*, 1998) have been classified as toxic to reproduction, and animal studies have now shown that the endocrine disrupting properties of these phthalate esters are what cause the damage to reproduction (Arbuckle *et al.*, 2018; Liao *et al.*, 2018). In addition, some recent studies indicate a relation between prenatal exposure to

phthalate esters and low intelligence quotient (IQ) of school age children whose mother has elevated trace of phthalate esters; these children had an average IQ around six points below their peers whose mother has lower level of phthalate esters exposure (Kim *et al.*, 2017; Factor-Litvak *et al.*, 2014). These evidences confirmed that the determination of contaminated phthalate esters is extremely important.

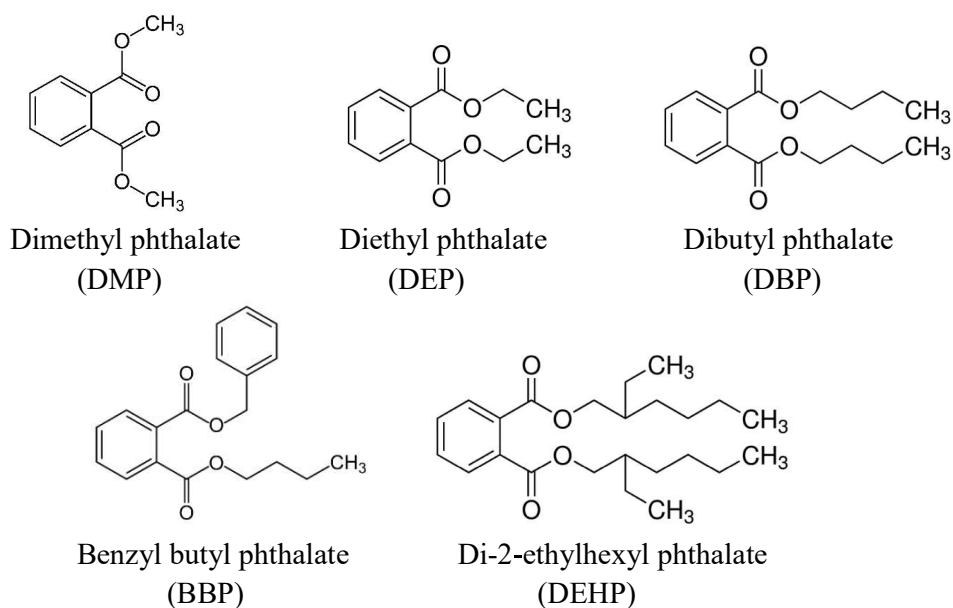


Figure 1 The chemical structure of phthalate esters molecule

There are several conventional methods used for the determination of phthalate esters such as high performance liquid chromatography (HPLC) and gas chromatography (GC). But GC coupled with electron capture detector (GC-ECD) has been defined by the United States Environmental Protection Agency (US EPA) as a standard method for the determination PAEs e.g., EPA Method 606, EPA Method 8061A because of their sensitivity and good selectivity (Jaworek *et al.*, 2014). However, the amount of the leached phthalate esters is lower than the limits of detection (LODs) of the analytical instrument, sample preparation is one of the most important steps for preconcentration of the analytes into the level that can be reliably determined by the appropriate instrument (Makkliang *et al.*, 2017; Liu *et al.*, 2008; Kueseng *et al.*,

2007). Examples are liquid-liquid extraction (LLE) (Wang *et al.*, 2019), solid-phase extraction (Puoci *et al.*, 2008; Yongfeng *et al.*, 2012; Kueseng *et al.*, 2010). These techniques require large amount of organic solvent that waste subsequently is released to the environment. Thus, miniaturized sample preparation techniques are of interest. Examples are ultrasonic-assisted extraction (UAE) or ultrasonic extraction (UE) (Chemat *et al.*, 2017), dispersive liquid liquid microextraction (DLLME) (Montevecchi *et al.*, 2017; Perez-Outeiral *et al.*, 2016; Vinas *et al.*, 2015; Farajzadeh and Khoshmaram 2015; Kanchanamayoon *et al.*, 2012), micro-solid phase extraction (μ -SPE) (Sajid *et al.*, 2016; Guo and Lee, 2013), solid phase microextraction (Liu *et al.*, 2014), stir-bar sorptive extraction (Prieto *et al.*, 2010; Xu *et al.*, 2011; Hu *et al.*, 2010) and magnetic solid phase extraction (Xu *et al.*, 2016; Liu *et al.*, 2015).

A combination of miniaturization and cost-effective sample preparations that have capability to preconcentrate trace amount of contaminants is an interesting approach. This work thus reports the development of miniaturized sample preparation techniques based on the use of pencil lead as a microextractor for the determination of trace phthalate esters. The extraction capability of silver-incorporated polyaniline film pencil lead sorbent was first evaluated as a preliminary experiment and the analysis was performed using a gas chromatograph coupled with an electron capture detector (GC-ECD). Surface characterization, optimization of adsorption and desorption times and reusability of the SPME fiber were studied. The results indicated that the proposed pencil lead microextractor is capable to extract BBP and DEHP (**Paper I**).

Although the results from the preliminary test showed a good promising of the developed SPME fiber but the fiber must be conditioned at the GC injection port at 180°C under a carrier gas stream for 2 h before use. So, it takes a long time before extraction resulting in a need to improve the extraction process to be easier to use and conveniently lead to the development of a simple 3D printing screw-based solid phase microextraction (SPME) device in the second part of this work (**Paper II**). The device was used for the extraction of DMP, DEP, DBP, BBP and DEHP. Parameters that have an effect on the extraction efficiency of the SPME fiber were optimized. Its analytical performances were studied and the method was validated before it was applied for the determination of trace phthalate esters in cosmeceutical products.

1.2 Objective

To develop miniaturized sample preparation techniques for extraction of phthalate esters based on the use of silver-incorporated polyaniline nanoporous film pencil lead as a microextraction devices. To achieve this goal, two sub-projects were carried out as follow.

Project I: A polyaniline/pencil lead solid phase microextractor for trace phthalate esters

Project II: A simple 3D printed- screw-based solid phase microextraction device with a silver decorated polyaniline pencil lead fiber for the extraction of phthalate esters in cosmeceutical products

2. Analytical methods for phthalate esters determination

It was necessary to employ a sensitive and selective analytical method for the determination of trace organic contaminants. The most widely used techniques for the determination of phthalate esters are HPLC and GC.

2.1 High performance liquid chromatography (HPLC)

HPLC is basically a highly improved form of column liquid chromatography that analyzes non-volatile organic compounds (high molecular weight compounds). An HPLC system is shown in **Figure 2**. HPLC is an analysis method that yields high performance and high speed compared with traditional column chromatography because of the pumped mobile phase. The speed of the migrating sample component depends on whether the component has an affinity for the stationary or mobile phase. This affinity is caused by various actions such as adsorption, partition, ion exchange, etc. HPLC can be used with different detectors, such as diode array detector (DAD), fluorescence detector (FLD) and mass spectrometer (MS).

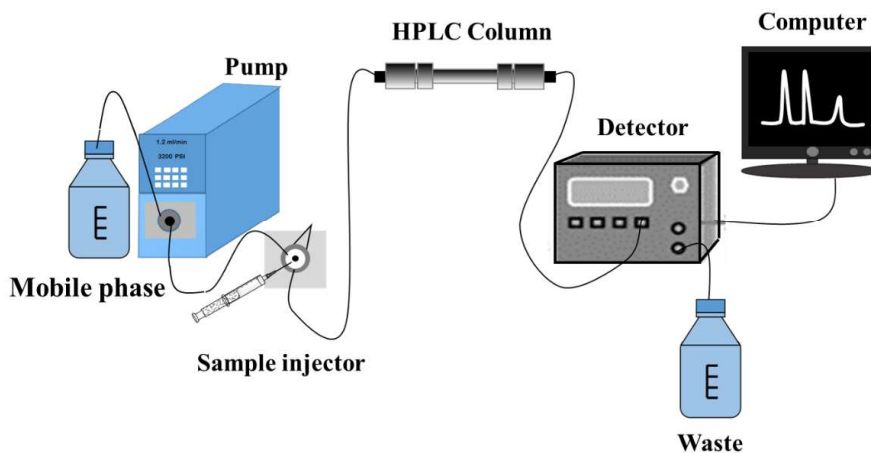


Figure 2 Schematic of an HPLC system

2.2 Gas chromatography (GC)

GC is a technique that typically analyzes volatile (VOCs) and semi-volatile organic compounds (SVOCs) specifically. A GC system is shown in **Figure 3**. The ability to quickly separate large compound groups with high resolution provides a definite advantage when analyzing complex samples. GC can be used with different detectors, such as flame ionization detector (FID), electron capture detector (ECD), mass spectrometer (MS) for the determination of VOCs and SVOCs.

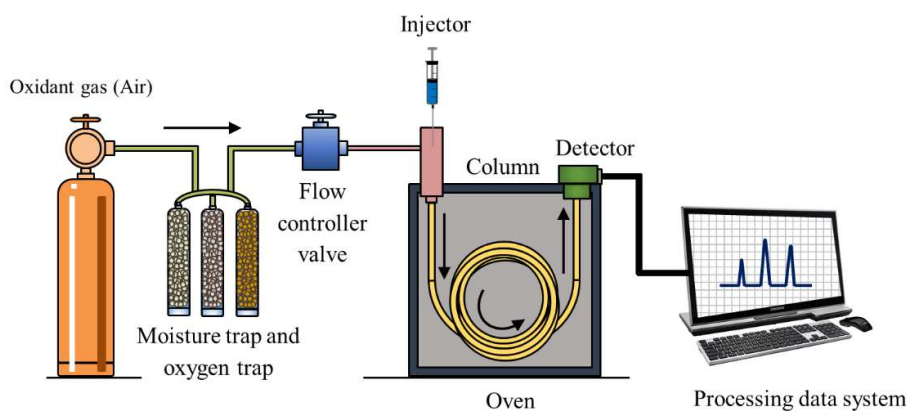


Figure 3 Schematic of a GC system

GC is one of the most suitable analytical methods for phthalate esters analysis (US EPA, 1984, 1996), and this method is recommended by US EPA as a standard method. Therefore, GC was employed in this thesis (**Papers I and II**)

3. Miniaturized sample preparation techniques

The miniaturization of the sample preparation techniques attempts to reduce the amount of sample, solvent and reagents consumed and wastes generated during the extraction process, as well as to decrease the cost and to enhance the portability. Several miniaturized sample preparation techniques have been reported such as ultrasonic extraction (UE), dispersive liquid-liquid microextraction (DLLME), micro-solid phase extraction (μ -SPE), solid phase microextraction (SPME) and stir-bar sorptive extraction (SBSE). Principles of these miniaturized extraction techniques are discussed.

3.1 Ultrasonic extraction

Ultrasonic extraction (UE) is a popular technique due to its advantages compared with other conventional techniques. The UE uses ultrasonic waves at high intensities that propagate into the liquid media resulting in alternating compression and reduction cycles, with rates depending on the frequency (**Figure 4**).

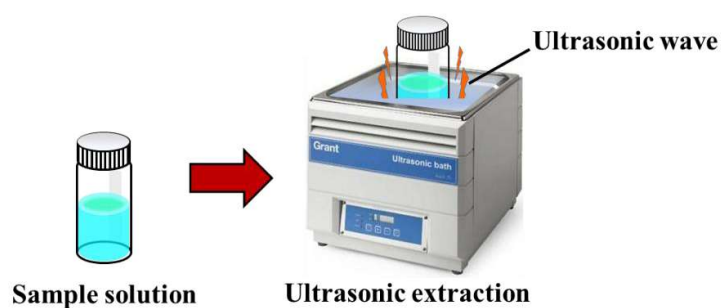


Figure 4 Ultrasonic extraction procedure

The UE has been proven to be a very useful tool for reinforcing the mass transfer process and breaking the affinity between the adsorbed analytes and the adsorbent (Tong *at et.*, 2019). Thus, this method has been employed for the primary extraction of target compounds from a variety of matrices (Staple, 2003). The advantages of

ultrasonic extraction are the lower volume of organic solvent required, shortened extraction time, and increased extraction efficiency (Ozer *et al.*, 2017).

3.2 Dispersive liquid-liquid microextraction

Dispersive liquid-liquid microextraction (DLLME) was introduced by Rezaee and co-workers in 2006 (Rezaee *et al.*, 2006). DLLME is based on formation of tiny droplets of the extraction solvent in the sample solution (**Figure 5**) by a rapid injection of a water immiscible organic solvent (extractant) dissolved in a water-miscible organic solvent and the latter acts as a dispersing medium. Extraction of the analytes from aqueous medium into the dispersed organic droplets takes place very rapidly. Thus, the extraction equilibrium is obtained rapidly. The advantages of DLLME are the use of small volume of organic solvents, simplicity of the operation, rapidity and high enrichment factor. Moreover, DLLME has been successfully used for the determination of several environmental hazardous compounds.

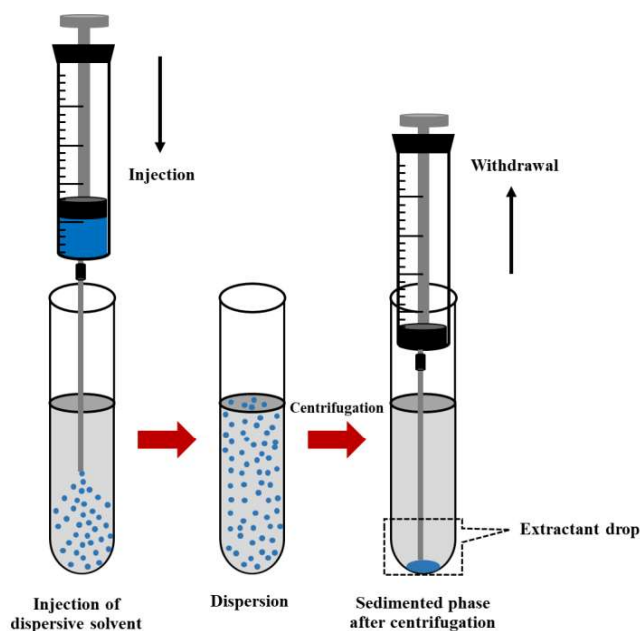


Figure 5 Schematic of a dispersive liquid-liquid microextraction

3.3 Micro-solid phase extraction

Micro-solid phase extraction (μ -SPE) has been recently developed and become very popular in a very short time. μ -SPE was first proposed by Basheer and co-workers in 2006 (Basheer *et al.*, 2006; Wang *et al.*, 2014). The technique is based on the holding of the adsorbent materials in a protective membrane, in which polypropylene (PP) is the most commonly used (**Figure 6**). Analytes diffuse freely through the membrane's pore and are retained by the solid sorbent/acceptor phase, whereas the diffusion of other components in the sample is hampered by the membrane.

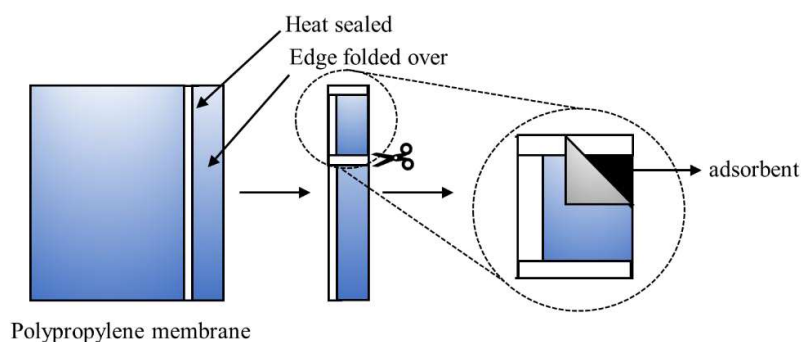


Figure 6 Schematic of a polypropylene membrane in μ -SPE

3.4 Stir bar sorptive extraction

Stir bar sorptive extraction (SBSE) is based on the partitioning of target analytes between the sample (mainly aqueous-based liquid samples) and a stationary phase-coated magnetic stir bar (**Figure 7**). SBSE was introduced by Baltussen and co-workers in 1999 for the trace enrichment of organic compounds from aqueous food, biological and environmental samples (Baltussen *et al.*, 1999). A stir bar is coated with sorbent and immersed in the sample to extract the analyte from solution. Polydimethylsiloxane (PDMS) polymeric phase is the most widely used in SBSE (Aparicio *et al.*, 2017; Si *et al.*, 2016; Serodio and Nogueira, 2006). The main problem of the conventional stir bar is that the sorbent used was coated on the surface and it is directly contacted with the bottom surface of the container during the extraction process resulting in the damage and the loss of sorbent and this leads to the decrease in the extraction efficiency as well as the reusability of the stir bar (Si *et al.*, 2016).

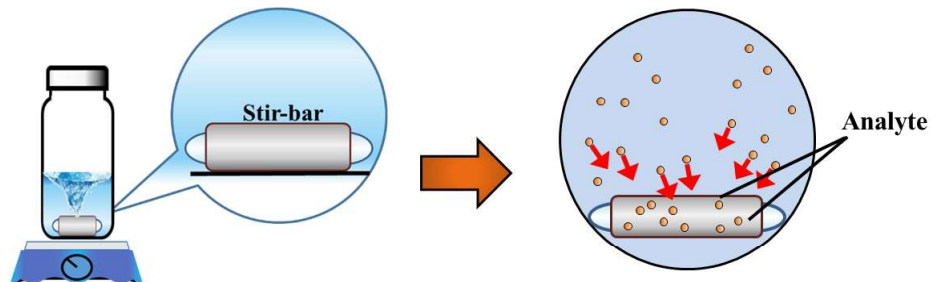


Figure 7 Schematic of an SBSE for the extraction of analytes

3.5 Magnetic solid phase extraction

Magnetic solid phase extraction (MSPE) has also been developed for the extraction and preconcentration of organic compounds (Tolmacheva *et al.*, 2016; Ibarra *et al.*, 2011; Rodriguez *et al.*, 2010). MSPE technique is based on the use of a magnetic sorbent added into the solution containing the target analytes. Under the stirring, the analytes adsorbed on the surface of a magnetic sorbent. After extraction, the analytes were desorbed from a magnetic sorbent with appropriate organic solvents and can be easily separated by an external magnet without centrifugation or filtration of the sample (Ibarra *et al.*, 2015) as shown in **Figure 8** (Makkliang *et al.*, 2015). This technique has been used for the extraction and preconcentration of target analytes from different matrices due to its simplicity, short extraction time, low consumption of organic solvents and high extraction efficiency (Sha *et al.*, 2015).

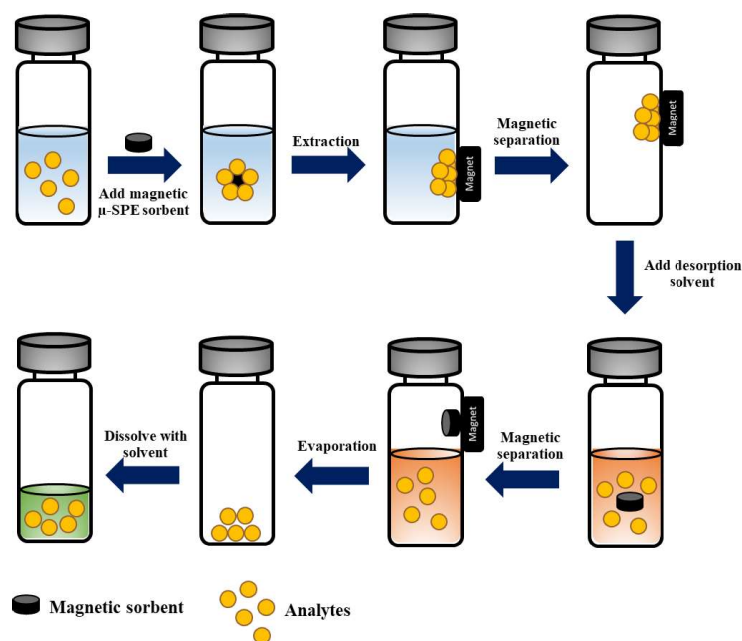


Figure 8 Schematic of the MSPE procedure for the adsorption and the desorption of analytes

3.6 Solid phase microextraction

Solid phase microextraction (SPME) technique has been widely used for the analysis of environmental pollutants and it was developed by Arthur and Pawliszyn (Arthur and Pawliszyn, 1990; Pawliszyn, 1997). Target analytes are extracted and preconcentrated simultaneously on a coated fiber. Its principle is based on the partition of analytes between sample solution and the coated fiber phase (Ouyang and Pawliszyn, 2008) It has been proven very useful for the analysis of a variety of contaminants in the environment, including volatile organic compounds (VOCs), semi-volatile organic compounds (SVOCs), pesticides and herbicides (Grafit *et al.*, 2018; Krutz *et al.*, 2003). SPME can be performed either as headspace-solid phase microextraction (HS-SPME) or direct immersion-solid phase microextraction (DI-SPME) (**Figure 9**). In spite of the wide application of SPME, the weakest aspects of SPME are fragility, low sample loading capacity, carry over effect and the expensive cost of the fiber.

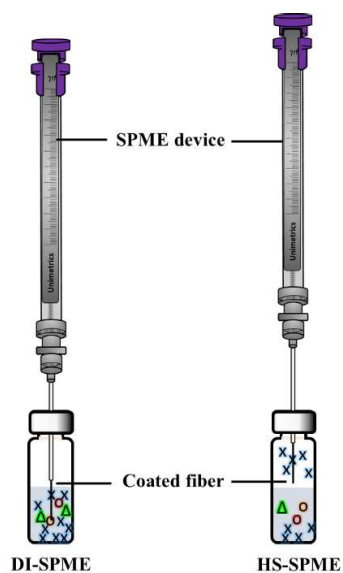


Figure 9 Schematic of a solid phase microextraction

To overcome of this drawback, we developed a novel solid phase microextraction device by the electrodeposition of silver-incorporated polyaniline (Ag/PANI) film on to the pencil lead graphite fiber for trace phthalate ester extraction and then inserted directly into the GC injector after extraction for thermal desorption (**Paper I**) and analysis with GC-ECD. This study shows the possibility and capability of the prepared Ag/PANI pencil lead SPME fiber for the extraction of BBP and DEHP. This cost effective and easy-to-prepare Ag/PANI pencil lead SPME fiber was successfully applied for the extraction of phthalate esters. In addition, the prepared fiber was reusable. Further investigation of some extraction parameters would lead to further improvement to make it more applicable for real sample analysis.

In order to increase the sample loading capacity, the size of a pencil lead used was increased. And to improve the extraction process to be simpler, more robust and more portable, the screw-based solid phase microextraction (SPME) device was constructed by a 3D printer (**Paper II**) and was efficiently applied for the determination of phthalate esters in cosmeceutical products.

3.7 Fiber core for solid phase microextraction

Generally, thermal (**Paper I**) or solvent desorption (**Paper II**) is used to desorb analytes from SPME fiber for GC analysis, so the fiber should be chemically and thermally stable. Accordingly, how to select the SPME fiber core should carefully be taken into consideration. The commercial SPME fiber core is usually made from a fused-silica fiber with a diameter of 0.55 mm, coated with a thin polymer film such as polydimethylsiloxane (PDMS), carbowax (CAR), divinylbenzene (DVB) or polyacrylate (Grafit *et al.*, 2018; Gierak *et al.*, 2006). This fused-silica fiber is fragile and has the tendency to bend and break when in physical contact with solid materials causing the fiber more expensive and less durable. Therefore, the seek of new kinds of fiber core for SPME that is robust and applicable for both thermal and chemical desorption is of interest.

Pencil lead is one of the best choices to be used as SPME fiber core since it has good mechanical rigidity, good conductivity and is very low cost (Gherghel *et al.*, 2018; Liu *et al.*, 2014; Dossi *et al.*, 2014). In addition, surface modification with appropriate sorbent can be easily performed using an electrochemical technique (**Papers I and II**). Moreover, the electrochemical technique can control layers and porosity size of thin film sorbent that coated on the pencil lead.

3.8 Coating materials for SPME fiber

3.8.1 Carbon nanomaterials

Carbon nanomaterials have long been applied in the development of analytical techniques owing to their unique chemical properties such as good chemical stability and high electrical conductivity (Wang *et al.*, 2011; Li *et al.*, 2019). Carbon nanomaterials such as graphene, carbon nanotubes and fullerenes have been widely used due to their strong adsorption ability towards target analytes via π - π interaction.

Graphene is one of the most interesting materials (Ali *et al.*, 2019; Li *et al.*, 2015), can also form a strong π - π stacking interaction with the carbon-based ring structure. Its very high surface area, vast active sites, large delocalized π -electron systems and good chemical stability make it appropriate to be used as an adsorbent material for many contaminants in the environment (Cech *et al.*, 2019).

Carbon nanotubes (CNTs) can be divided into two types, which are single-walled carbon nanotubes (SWCNTs) and multiwalled carbon nanotubes (MWCNTs) according to the carbon atom layers in the wall of nanotubes. CNTs have strong interactions with some analytes, especially the aromatic compounds since the hexagonal structure of carbon atoms in CNTs can exhibit a strong π - π interaction with the benzene ring (Mohanta *et al.*, 2019; Wang *et al.*, 2011). In addition, they can adsorb the analytes based on other weak interactions such as electrostatic forces, Van der Waals forces and hydrophobic interactions after material modification. (Makklang *et al.*, 2016; Fatoni *et al.*, 2013).

Fullerenes is also known as buckminsterfullerene (Zhou *et al.*, 2018). The first fullerene was discovered in 1985 by Kroto and co-workers (Kroto *et al.*, 1985). The large curvature of the conjugated π -electron systems of these hollow spheres has facilitated a rich chemical behavior allowing the synthesis of many different derivatives thereby making the fullerene family a versatile building block of materials of importance in physics, chemistry, and biology.

3.8.2 Conducting polymer

The number of synthesized conducting polymer has increased rapidly in the past decades due to their potential applications in many fields. They can be easily synthesized in both aqueous and non-aqueous media, by electrochemical or chemical methods. Another advantage of conducting polymers is its stability in both air and solution (Tang *et al.*, 2012). In miniaturized sample preparation, conducting polymers can be employed as sorbents due to their high surface area, ability to establish π - π interactions, excellent chemical, mechanical and thermal stability. Therefore, they are able to extract aromatic compounds through the π - π interactions and polar analytes by interactions of polar functional groups.

Polypyrrole (PPy) was one of the most popular conducting polymers used to compose with several materials. PPy is generally synthesized by either chemical or electrochemical means. Chemical synthesis is used when large quantity of material is required and involves mixing a strong oxidizing agent with a monomer solution (Armes 1987; Duchet *et al.*, 1998). It was applied to extract many groups of analytes, i.e., polar and non-polar aromatic compounds, and anionic species. Whereas the electrochemical

synthesis is more popular due to its small consumption of solvent. And surface modification with appropriate sorbent using an electrochemical technique can be easily performed and can control the layers coated on fiber.

Polyaniline (PANI) is one of the most useful conducting polymers due to its good conductivity, environmental stability, facile synthesis, redox and pH switching properties and low cost. Consequently, PANI is widely used for various applications in many works (Li *et al.*, 2019; Bhadra *et al.*, 2009; Wallace *et al.*, 2009). The remarkable properties of PANI have also made them a highly interesting materials in sample preparation technique because PANI is particularly suited for adsorption between phthalate esters and PANI by π - π interactions and hydrogen bonds (**Papers I and II**). However, the sample loading capacity of PANI is still limited through its film formation and the stability of a PANI thin film is somewhat poor (Konkol *et al.*, 2016). Some recent reports have mentioned that Ag, MnO₂ and RuO₂ can enhance the porosity of PANI film (Prasad *et al.*, 2013; Kim *et al.*, 2012). The incorporation of these metals or metal oxides to the polymer structure may help increase the surface area and thus the extraction efficiency (Sawangphruk *et al.*, 2012).

4. Analytical performances and method validation

Common validation characteristics to be considered for the development of analytical methods are linearity, limit of detection, limit of quantification, reproducibility, reusability, precision, accuracy and effect of sample matrix.

4.1 Linear dynamic range

Linearity is the ability of the method to produce responses that are proportional to the concentrations of the analyte in samples within the range of interest (working range), also known as linear dynamic range. In this thesis, the linearity of the developed SPME fiber for the determination of phthalate esters were from 5.0-1.0×10³ µg L⁻¹. This developed method showed a wide linear range with a good coefficient of determination (R² of 0.99) (**Paper II**). This linear range covered the concentration range of target analytes present in real samples confirming that the developed method can be applied for the determination of phthalate esters in cosmeceutical products.

4.2 Limit of detection and limit of quantification

Limit of detection (LOD) is the lowest quantity or concentration of an analyte that can be reliably detected with a given analytical method. And the limit of quantification (LOQ) is established as the lowest concentration of an analyte in a sample that can be quantitatively determined with acceptable precision and accuracy under the optimum conditions of the method (Gary and Long, 1983).

According to the IUPAC recommendations, the limit of detection and the limit of quantification were respectively estimated as the concentrations to 3-folds and 10-folds of the standard deviation of 20 measurements of the blank divided by the slope of the calibration curves (Gary and Long, 1983). LOD and LOQ can be calculated as follows.

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

where C_L is the limit of detection (LOD) or limit of quantification (LOQ)

S_B is the standard deviation of the response from blank (20 times)

m is the slope of calibration curve

k is the number factor chosen according of the confidence level as follows.

$k = 3$ or 3σ for the calculation of LOD

$k = 10$ or 10σ for the calculation of LOQ

Good analytical performance is indicated by the low values of LOD and LOQ that can be used to determine trace level of target analytes with high accuracy and reliability.

The obtained LOD and LOQ of the Ag/PANI SPME fiber for the extraction of phthalate esters (**Paper II**) were better than the recommended values by the European Union regulations (Table 1) (US EPA, 1988, 2002a, 2002b). Therefore, this method can be applied to extract and determine the concentrations of phthalate esters in cosmeceutical products.

Table 1 LODs and LOQs of the Ag/PANI SPME fiber and the recommended maximum contaminant level (MCL) values from the United States Environmental Protection Agency (US EPA, 1988, 2002a, 2002b)

Phthalate esters	LOD ($\mu\text{g L}^{-1}$)	LOQ ($\mu\text{g L}^{-1}$)	MCL ($\mu\text{g L}^{-1}$)
DMP	4.41±0.91	14.7±3.0	-
DEP	3.98±0.92	13.3±3.1	-
DBP	3.65±0.74	12.2±2.5	-
BBP	4.91±0.52	16.4±1.7	6.0
DEHP	4.25±0.66	14.2±2.2	6.0

4.3 Reproducibility

Reproducibility of the fabrication procedure of the developed sorbent is evaluated in two circumstances. One is to evaluate the reproducibility of the sorbents prepared at the same time. The other is to evaluate the reproducibility of the sorbent prepared at different times. It usually refers to the relative standard deviation (RSD). In this thesis, the reproducibility of the preparation procedure of the solid phase microextractor were investigated. The obtained RSDs were used to indicate the acceptability, which depends on analyte concentration levels (AOAC, 2016).

The reproducibility of the preparation procedure for Ag/PANI pencil lead SPME fiber was evaluated by preparing six batches of the SPME fibers (each with three fibers). Then each fiber was used to extract 1.0 mg L⁻¹ standard solution containing the five phthalate esters, followed by the analysis with GC-FID. The average extraction recoveries obtained from the six batches for the five phthalate esters were within the 81-93% range with RSDs between 0.7-6.7%. These RSDs are better than the acceptable value of 13% recommended by the AOAC (at the concentration of 1.0 mg L⁻¹), indicating that the Ag/PANI SPME fiber sorbent could be reproduced with a good precision.

To further confirm the reproducibility when the fiber was used for the extraction of real sample, another six batches of the SPME fiber were fabricated and applied to extract phthalate esters in rubbing alcohol (selected sample). The extraction recoveries

from the six batches were within the range of 81-93% with RSDs between 0.7-2.6% for the five phthalate esters. These recoveries and %RSD were also better than those recommended by AOAC.

These results indicated an excellent reproducibility of the fabrication procedure of the silver incorporated PANI SPME fiber even when it was used for the extraction of target analytes in real sample matrix. In addition, the sample matrix has no effect to the obtained recoveries.

4.4 Reusability

The reusability was investigated as the number of extraction cycles that the Ag/PANI pencil lead SPME fiber can be reused. The obtained response from the first extraction was set as 100%, then each of the sorbent was reused repeatedly with the same extraction procedure until the obtained responses gave less RSD than that recommended by AOAC (11 % at analyte concentration level of 1.0 mg L^{-1}).

In the first sub-project, the reusability was tested in only standard solution at the concentration of 1.0 mg L^{-1} of phthalate esters (BBP and DEHP). Within the reusable cycles, the average %response was in the range of 97.8 to 98.6% (RSD <3.0%) as reported in **Paper I**.

In the second sub-project, two circumstances were studied, which were standard solution and spiked samples at the same concentration (1.0 mg L^{-1} mixed standard solution of the five phthalate esters which are DMP, DEP, DBP, BBP and DEHP), as reported in **Paper II**. With the standard solution, one fiber can be used up to 18 times. The % responses average values of the five phthalate esters were in the range of 90-94% and RSDs 2.3-4.8%. These values were much better than the accepted RSD recommended by the AOAC. After 18 extraction cycles, the response decreased to less than 90 %. This was most likely due to the structural deformation of the Ag/PANI, from which some polymer could be seen strip off from the fiber core. For the spiked real samples (rubbing alcohol), one fiber can be used up to 21 times with average % responses in the range of 90-92% and RSDs 0.84-3.8%.

These two experiments revealed that the developed Ag/PANI SPME fiber provided good repeatability even when it was used for the extraction of phthalate esters in real samples indicating that the matrix in the sample did not affect the robustness of

the Ag/PANI pencil lead SPME fiber since the numbers of reusability were in the same range and the %responses were relatively stable during 18 and 21 extraction cycles.

These results also confirmed the cost-effectiveness of the fiber. The material cost of one fiber was about 0.089 USD. As it can be reused 18 to 21 times, the cost for one extraction cycle was reduced to only 0.0005 USD.

4.5 Precision

The precision of the developed method was investigated by analyzing the spiked sample solution at various concentrations (six replications at each concentration, five concentrations for one sample) and reported as the relative standard deviations (RSDs). The relative standard deviations were calculated as follows.

$$\text{RSDs} = 100s/\bar{x}$$

where s is standard deviation of sample

\bar{x} is mean of sample

The precision of the method was evaluated by spiking standard phthalate esters mixture at final concentrations of 0.050, 0.25, 0.50, 0.75 and 1.0 mg L⁻¹ into the rubbing alcohol (six replications at each concentration) (**Paper II**). The extraction and the analysis were performed under the optimal conditions. The RSDs of the recoveries for the extraction of five phthalate esters at each spiked concentration ranged from 3.1 to 5.5 %, which were better than the AOAC RSD guideline values, 11% for 1.0 mg L⁻¹. That indicated good precision achieved with the analytical scheme using the developed Ag/PANI pencil lead SPME fiber for the extraction of phthalate esters coupled to the analysis by GC-FID.

4.6 Accuracy

The accuracy of the developed method was expressed in term of the percent recovery. It was determined by comparing the concentration obtained from the spiked sample and the standard solution. In this work (**Paper II**), the percent recovery was calculated as follows.

$$(\%) \text{ Recovery} = \left(\frac{C_f - C_u}{C_a} \right) \times 100$$

where C_a is the calculated concentration of analyte added to the tested sample,
 C_f is the concentration of the fortified
 C_u is the concentration of the unfortified (AOAC, 2016)

The obtained recoveries were calculated from applying the Ag/PANI SPME fiber for the extraction of spiked standard solution of DMP, DEP, DBP, BBP and DEHP in each real samples at the concentrations of 0.0050, 0.050 0.25, 0.50 and 1.0 mg L⁻¹. Good recoveries of the five phthalate esters were obtained within the range of 82-103%, which were within the acceptable range as recommended by the AOAC (80-110% recoveries at analyte concentration level of 100 to 1000 µg L⁻¹) (AOAC 2016) indicating good accuracy of the developed method.

4.7 Effect of sample matrix

The matrix effect was first evaluated (**Paper II**) by spiking standard solutions of DMP, DEP, DBP, BBP and DEHP in the concentration range of 5.0 to 1000 µg L⁻¹ into each type of cosmeceutical product sample (matrix-matched standard solution). Standard solutions of the analytes at the same concentration range were also prepared in deionized water. Both sets were extracted and analyzed under the same conditions. Matrix-matched and standard calibration curves were plotted between the average peak area against their known concentrations. Their slopes were statistically compared by two-way ANOVA (analysis of variance).

The slopes of the matrix-matched and the standard calibration plots for contact lens cleaner and antibacterial disinfectant liquid samples were significantly different ($P < 0.05$), indicating a matrix effect. Therefore, the matrix-matched calibration plot was used for quantitative analysis of these samples. For rubbing alcohol, saline solution and eyes cleaner samples, there were no significant differences ($P > 0.05$) between the slopes of the two calibration plots, indicating no interferences from the sample matrix, and the standard calibration plot was used for quantitative analysis of this group.

4.8 Analysis of real samples

To assess the applicability of the developed method, it was used to detect five phthalate esters in 14 liquid samples (five sample groups) sold in plastic containers by local supermarkets in Hat Yai, Thailand.

DEP and DEHP were detected at $7.03 \pm 0.76 \text{ ng mL}^{-1}$ and $5.89 \pm 0.53 \text{ ng mL}^{-1}$, respectively in one of the rubbing alcohol samples. DEHP was found at $5.3 \pm 1.1 \text{ ng mL}^{-1}$ and $6.8 \pm 1.2 \text{ ng mL}^{-1}$ in two samples of contact lens cleaner solution. However, these concentrations were still lower than the MCL of $6.0 \mu\text{g L}^{-1}$ for DEHP or within the guideline values (US EPA, 1988). No phthalate esters were detectable in the samples of saline solution, eye cleaner and antibacterial disinfectant liquid.

It is noteworthy that the samples contaminated with DEP and DEHP were contained in plastic products. This is not surprising because these plasticizers are the most widely used in the world (Mortula, 2019) and are added at a content of 40% by weight during the production process (Crooker *et al.*, 2013; Erythropel *et al.*, 2014).

5. Concluding remarks

In this thesis, a novel solid phase micro-extractor using silver-incorporated polyaniline film electrodeposited on a pencil lead was successfully developed for the determination of phthalate esters in cosmeceutical products. Large surface area with porous structure of the sorption phase can improve the adsorption capacity and extraction efficiency for trace phthalate esters determination.

The results from the preliminary test indicated that the developed SPME device can be used to extract BBP and DEHP with high preconcentration factor and satisfactory recoveries but there were drawbacks of low sample loading capacity and the requirement of long conditioning time at a GC injection port before it can be used. So, the second sub-project in which the same material was used, larger size of the pencil lead coupled with the desorption using solvent was used instead. In addition, a simple 3D printed-screw-base solid phase microextraction device was also designed to improve the portability and the robustness of the extraction technique. With this modification, the developed SPME device provided high extraction efficiency when applied for the extraction of five phthalate esters in real samples. Extraction time was about 50 min and the fiber can be reused at least 18 times (average recoveries of five phthalate esters $>90.4 \pm 3.8$). Other advantages were simplicity and very low cost (0.089 USD per fiber, 0.0005 USD per extraction). Under the optimum conditions, LODs were low, good precision and relative standard deviation for fiber-to-fiber reproducibility was less than 6.6%. The recoveries and %RSD for the extraction of spiked real samples

with five phthalate esters were in the acceptable range which indicated good accuracy and precision of the method.

From all results obtained from the studies in this thesis, it was expected that the developed Ag/PANI SPME fiber can also be an alternative extraction device for the analysis of other phthalate esters or other aromatic compounds in various kinds of samples.

6. References

- Ali, I., Basheer, A., Mbianda, X., Burakov, A., Galunin, E., Burakova, I., Mkrtychyan, E., Tkachev, A. and Grachev, V. 2019. Graphene based adsorbents for remediation of noxious pollutants from wastewater. *Environment International*, 127: 160-180.
- AOAC INTERNATIONAL, Appendix F: Guidelines for Standard Method Performance Requirements, Available: https://www.eoma.aoac.org/app_f.pdf, (accessed July 2016).
- Aparicio, I. 2107. 2017. Stir bar sorptive extraction and liquid chromatography–tandem mass spectrometry determination of polar and non-polar emerging and priority pollutants in environmental waters. *Journal of Chromatography A*, 1500: 43-52.
- Arbuckle, T.E., Agarwal, A., MacPherson, S.H., Fraser, W.D., Sathyanarayana, S., Ramsay, T., Dodds, L., Muckle, G., Fisher, M., Foster, W., Walker, M. and Monnier, P. 2018. Prenatal exposure to phthalates and phenols and infant endocrine-sensitive outcomes: The MIREC study. *Environment International*, 120: 572-583.
- Arcadi F.A., Costa, C., Imperatore, C., Marchese, A., Rapisarda, A., Salemi, M., Trimarchi, G.-R. and Costa, G. 1998. Oral toxicity of bis(2-ethylhexyl) phthalate during pregnancy and suckling in the long-evans rat. *Food and Chemical Toxicology*, 36(11): 963-970.
- Armes, S.P. 1987. Optimum reaction conditions for the polymerization of pyrrole by iron(III) chloride in aqueous solution. *Synthetic Metals*, 20(3): 365-371.

- Arthur, C.L. and Pawliszyn, J. 1990. Solid phase microextraction with thermal desorption using fused silica optical fibers. *Analytical Chemistry*, 62(19): 2145-2148.
- Balafas, D., Shaw, K.J. and Whitfield, F.B. 1999. Phthalate and adipate esters in Australian packaging materials. *Food Chemistry*, 65(3): 279-287.
- Baltussen, E. 1999. Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples. *Theory and principles*, 11: 737-747.
- Basheer, C., Alnedhary, A.A., Rao, B.S.M., Valliyaveetti, S. and Lee, H.K. 2006. Development and application of porous membrane-protected carbon nanotube micro-solid phase extraction combined with gas chromatography/mass spectrometry. *Analytical Chemistry*, 78: 2853-2858.
- Bhadra, S., Khastgir, D., Singha, N.K. and Lee, J.H. 2009. Progress in preparation, processing and applications of polyaniline. *Progress in Polymer Science*, 34(8): 783-810.
- Cech, O., Klvac, O., Benesova, P., Maca, J., Cudek, P. and Vanýsek, P. 2019. Synthesizing a LiFePO₄/graphene composite with electrochemically prepared few-layer graphene. *Journal of Energy Storage*, 22: 373-377.
- Chang, W.H., Tsai, Y.S., Wang, J.Y., Chen, H.L., Yang, W.H. and Lee, C.C. 2019. Sex hormones and oxidative stress mediated phthalate-induced effects in prostatic enlargement. *Environment International*, 126: 184-192.
- Chemat, F. 2017. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. *A review. Ultrasonics Sonochemistry*, 34: 540-560.
- Christian, G.D., Dasgupta, P.K. and Schug, K.A. *Analytical Chemistry*. (seventh edition), Wiley interscience, 2013. New York, United States of America.
- Crookes, M., Larner, J., Lohmann, D.H., Maxwell, M., Sanderson, D.M. and S. Lipworth, Health and Safety Committee (EHSC) of the Royal Society of Chemistry. Should we worry about phthalates. Available: <http://www.rsc.org/ehsc>, 2013. (access June 2013).
- David, F. and Sandra, P. 2007. Stir bar sorptive extraction for trace analysis. *Journal of Chromatography A*, 1152: 54-69.

- Del Carlo, M., Pepe, A., Sacchetti, G., Compagnone, D., Mastrocola, D. and Cichelli, A. 2008. Determination of phthalate esters in wine using solid-phase extraction and gas chromatography-mass spectrometry. *Food Chemistry*, 111: 771-777.
- Dossi, N., Toniolo, R., Impellizzieri, F. and Bontempelli, G. 2014. Doped pencil leads for drawing modified electrodes on paper-based electrochemical devices. *Journal of Electroanalytical Chemistry*, 722-723: 90-94.
- Duchet, J., Legras, R. and Demoustier-Champagne, S. 1998. Chemical synthesis of polypyrrole: structure-properties relationship. *Synthetic Metals*, 98(2): 113-122.
- Erythropel, H., Maric, M., Nicell, J., Leask, R. and Yargeau, V. 2014. Leaching of the plasticizer di(2-ethylhexyl)phthalate (DEHP) from plastic containers and the question of human exposure. *Applied Microbiology and Biotechnology*, 98: 9967-9981.
- Factor-Litvak, P. 2014. Persistent associations between maternal prenatal exposure to phthalates on child IQ at age 7 years. *PLOS ONE*, 9(12): e114003.
- Farajzadeh, M.A. and Khoshmaram, L. 2015. Development of dispersive liquid-liquid microextraction technique using ternary solvents mixture followed by heating for the rapid and sensitive analysis of phthalate esters and di(2-ethylhexyl) adipate. *Journal of Chromatography A*, 1379: 24-33.
- Fatoni, A. 2013. A highly stable oxygen-independent glucose biosensor based on a chitosan-albumin cryogel incorporated with carbon nanotubes and ferrocene. *Sensors and Actuators B: Chemical*, 185: 725-734.
- Frederiksen, H., Skakkebaek, N.E. and Andersson, A.-M. 2007. Metabolism of phthalates in humans. *Molecular Nutrition & Food Research*, 51(7): 899-911.
- Gari, M., Koch, H.M., Palmke, C., Jankowska, A., Wesolowska, E., Hanke, W., Nowak, D., Bose-O'Reilly, S. and Polanska, K. 2019. Determinants of phthalate exposure and risk assessment in children from Poland. *Environment International*, 127: 742-753.
- Gary, L. and Long L., 1983: LOD A closer look at the IUPAC definition. *Analytical Chemistry*, 55: 712-724.
- Gherghel, S., Morgan, R.M., Arrebola-Liebanas, J., Romero-Gonzalez, R., Blackman, C.S., Garrido-Frenich, A. and Parkin, I.P. 2018. Development of a HS-

- SPME/GC-MS method for the analysis of volatile organic compounds from fabrics for forensic reconstruction applications. *Forensic Science International*, 290: 207-218.
- Gierak, A., Seredych, M. and Bartnicki, A. 2006. The preparation, properties and application of carbon fibers for SPME. *Talanta*, 69(5): 1079-1087.
- Grafit, A., Muller, D., Kimchi, S. and Avissar, Y.Y. 2018. Development of a solid-phase microextraction (SPME) Fiber protector and its application in flammable liquid residues analysis. *Forensic Science International*, 292: 138-147.
- Guo, L. and Lee, H.K. 2013. Vortex-assisted micro-solid-phase extraction followed by low-density solvent based dispersive liquid-liquid microextraction for the fast and efficient determination of phthalate esters in river water samples. *Journal of Chromatography A*, 1300: 24-30.
- Guo, Y. and Kannan, K. 2012. Challenges encountered in the analysis of phthalate esters in foodstuffs and other biological matrices. *Analytical and Bioanalytical Chemistry*, 404: 2539-2554.
- Hu, Y., Li, J., Hu, Y. and Li, G. 2010. Development of selective and chemically stable coating for stir bar sorptive extraction by molecularly imprinted technique. *Talanta*, 82: 464-470.
- Ibarra, I. 2015. Magnetic solid phase extraction. *Applied to Food Analysis*. 2015: 1-13.
- Ibarra, I.S. 2011. Magnetic solid phase extraction based on phenyl silica adsorbent for the determination of tetracyclines in milk samples by capillary electrophoresis. *Journal of Chromatography A*, 1218(16): 2196-2202.
- Jaworek, K., Czaplicka, M. 2014. Determination of Phthalates in Polymer Materials- Comparison of GC / MS and GC / ECD Methods. *Polímeros* 23, 718-724.
- Kanchanamayoon W., Prapatpong, P., Chumwangwapee, S. and Chaithongrat, S. 2012. Analysis of phthalate esters contamination in drinking water samples. *African Journal of Biotechnology*, 11(96): 16263-16269.
- Kim, E.-H. 2017. Exposure to phthalates and bisphenol A are associated with atopic dermatitis symptoms in children: a time-series analysis. *Environmental Health*, 16(1): 24.

- Kim, J.H., Ahn, J.H., Kang, S.W., Roh, J.S., Kwon, S.H. and Kim, J.Y. 2012. Thermal stability of RuO₂ thin films prepared by modified atomic layer deposition. *Current Applied Physics*, 12: S160-S163.
- Konkol, M., Kondracka, M., Kowalik, P., Próchniak, W., Michalska, K., Schwedt, A., Merkens, C. And Englert, U. 2016. Decomposition of the mixed-metal coordination polymer-A preparation route of the active Ag/Yb₂O₃ catalyst for the deN₂O process. *Applied Catalysis B: Environmental*, 190: 85-92.
- Kroto, H.W. 1985. C₆₀: Buckminsterfullerene. *Nature*, 318(6042): 162-163.
- Krutz, L.J., Senseman, S.A. and Sciumbato, A.S. 2003. Solid-phase microextraction for herbicide determination in environmental samples. *Journal of Chromatography A*, 999 (1-2): 103-121.
- Kueseng, P., Thammakhet, C., Thavarungkul, P. and Kanatharana, P. 2010. Multiwalled carbon nanotubes/cryogel composite, a new sorbent for determination of trace polycyclic aromatic hydrocarbons. *Microchemical Journal*, 96(2): 317-323.
- Kueseng, P., Thavarungkul, P. and Kanatharana, P. 2007. Trace phthalate and adipate esters contaminated in packaged food. *Journal of Environmental Science and Health Part B*, 42: 569-576.
- Le Noir, M, Plieva, F., Hey, T., Guieysse, B. and Mattiasson, B. 2007. Macroporous molecularly imprinted polymer/cryogel composite systems for the removal of endocrine disrupting trace contaminants. *Journal of Chromatography A*, 1154: 158-164.
- Li, L., Yu, B. and You, T. 2015. Nitrogen and sulfur co-doped carbon dots for highly selective and sensitive detection of Hg(II) ions. *Biosensors and Bioelectronics*, 74: 263-269.
- Li, Z., Wang, L., Li, Y., Feng, Y. and Feng, W. 2019. Carbon-based functional nanomaterials: Preparation, properties and applications. *Composites Science and Technology*, 179: 10-40.

- Li, Z., Zhou, X., Shi, J., Zou, X., Huang, X. and Tahir, H.E. 2019. Preparation of conducting polyaniline/protoporphyrin composites and their application for sensing VOCs. *Food Chemistry*, 276: 291-297.
- Liao, K.W., Kuo, P.L., Huang, H.B., Chang, J.W., Chiang, H.C. and Huang, P.C. 2018. Increased risk of phthalates exposure for recurrent pregnancy loss in reproductive-aged women. *Environmental Pollution*, 241: 969-977.
- Liu, H.C., Den, W., Chan, S.F. and Kin, K.T. 2008. Analysis of trace contamination of phthalate esters in ultrapure water using a modified solid-phase extraction procedure and automated thermal desorption-gas chromatography/mass spectrometry. *Journal of Chromatography A*, 1188(2): 286-294.
- Liu, Q., Cheng, M., Long, Y. and Yu, M. 2014. Graphenized pencil lead fiber: Facile preparation and application in solid-phase microextraction. *Journal of Chromatography A*, 1325: 1-7.
- Liu, X., Sun, Z., Chen, G., Zhang, W., Kong, R., Wang b, X. and Suo, b. 2015. Determination of phthalate esters in environmental water by magnetic zeolitic imidazolate framework-8 solid-phase extraction coupled with high-performance liquid chromatography. *Journal of Chromatography A*, 1409: 46-52.
- Makkliang, F., Kanatharana, P., Thavarungkul, P. and Thammakhet, C. 2015. Development of magnetic micro-solid phase extraction for analysis of phthalate esters in packaged food. *Food Chemistry*, 166: 275-282.
- Makkliang, F., Kanatharana, P., Thavarungkul, P. and Thammakhet, C. 2016. A novel miniaturized zinc oxide/hydroxylated multiwalled carbon nanotubes as a stir-brush microextractor device for carbamate pesticides analysis. *Analytica Chimica Acta*, 917: 27-36.
- Makkliang, F., Kanatharana, P., Thavarungkul, P. and Thammakhet-Buranachai, C. 2017. A polypyrrole-chitosan cryogel stir-bead micro-solid phase extractor for the determination of phthalate esters in contact lenses storage solutions and in artificial saliva in contact with baby teethers. *Analytica Chimica Acta*, 985: 69-78.

- Mohanta, D. 2019. Carbon nanotubes: Evaluation of toxicity at biointerfaces. *Journal of Pharmaceutical Analysis*, 53: 456-462.
- Montevecchi, G. 2017. Determination of phthalate esters in distillates by ultrasound-vortex-assisted dispersive liquid-liquid micro-extraction (USVADLLME) coupled with gas chromatography/mass spectrometry. *Food Chemistry*, 221: 1354- 1360.
- Mortula, M. 2013. *Health Risk Assessment of PET Bottles in GCC*. 7: 267-272.
- Ozer, E.T., Osman, B. and Yazici, T. 2017. Dummy molecularly imprinted microbeads as solid-phase extraction material for selective determination of phthalate esters in water. *Journal of Chromatography A*, 1500: 53-60.
- Ouyang, G. and Pawliszyn, J. 2008. A critical review in calibration methods for solid-phase microextraction. *Analytica Chimica Acta*, 627: 184-197.
- Pawliszyn, J. *Solid Phase Microextraction: Theory and Practice*; New York: VCH, 1997. United States of America.
- Pérez-Outeiral, J., Millán, E. and Garcia-Arrona, R. 2016. Determination of phthalates in food simulants and liquid samples using ultrasound-assisted dispersive liquid- liquid microextraction followed by solidification of floating organic drop. *Food Control*, 62: 171-177.
- Petrović, M., Eljarrat, E., López de Alda, M.G. and D. Barceló. 2001. Analysis and environmental levels of endocrine-disrupting compounds in freshwater sediments. *TrAC Trends in Analytical Chemistry*, 20(11): 637-648.
- Prasad, B.B., Srivastava, A., Pandey, I. and Tiwari, M.P. 2013. Electrochemically grown imprinted polybenzidine nanofilm on multiwalled carbon nanotubes anchored pencil graphite fibers for enantioselective micro-solid phase extraction coupled with ultratrace sensing of d- and l-methionine. *Journal of Chromatography B*, 912: 65-74.
- Prieto, A. 2010. Stir-bar sorptive extraction: A view on method optimisation, novel applications, limitations and potential solutions. *Journal of Chromatography A*, 1217(16): 2642-2666.
- Puoci, F. 2008. Molecularly imprinted solid-phase extraction for cholesterol determination in cheese products. *Food Chemistry*, 106(2): 836-842.

- Rezaee, M., Assadi, Y., Hosseini, M.R.M., Aghaee, E., Ahmadi, F and Berijani, S. 2006. Determination of organic compounds in water using dispersive liquid-liquid microextraction, *Journal of Chromatography A*, 1116: 1-9.
- Rodriguez, J.A., 2010. Determination of tetracyclines in milk samples by magnetic solid phase extraction flow injection analysis. *Microchimica Acta*, 171(3): 407-413.
- Sajid, M. and Basheer, C. 2016. Stir-bar supported micro-solid-phase extraction for the determination of polychlorinated biphenyl congeners in serum samples. *Journal of Chromatography A*, 1455: 37-44.
- Sawangphruk, M. and Kaewsongpol, T. 2012. Direct electrodeposition and superior pseudocapacitive property of ultrahigh porous silver-incorporated polyaniline films. *Materials Letters*, 87: 142-145.
- Serôdio, P. and Nogueira, J.M.F. 2006. Considerations on ultra-trace analysis of phthalates in drinking water. *Water Research*, 40(13): 2572-2582.
- Sha, O. 2017. Magnetic Solid-Phase Extraction Using Fe₃O₄@SiO₂ Magnetic Nanoparticles followed by UV-Vis spectrometry for determination of paraquat in plasma and urine samples. *Journal of Analytical Methods in Chemistry*, 2017: 8704639-8704639.
- Si, Q., Li, F., Gao, C., Wang, C., Wang, Z. and Zhao, J. 2016. Detection of phthalate esters in seawater by stir bar sorptive extraction and gas chromatography-mass spectrometry. *Marine Pollution Bulletin*, 108(1-2): 163-70.
- Staple, C. 2003. Phthalate esters. (first edition). New York: Springer-Verlag Berlin Heidelberg, United States of America.
- Tang, D. 2012. Mesoporous silica nanoparticles immobilized salicylaldehyde cobalt complexes as high efficient catalysts for polymerization of 1,3-butadiene. *Journal of Colloid and Interface Science*, 369(1): 338-343.
- Tickner, J.A., Schettler, T., Guidotti, T., McCally, M. and Rossi, M. 2001. Health risks posed by use of di-2-ethylhexyl phthalate (DEHP) in PVC medical devices. *A Critical Review*, 39: 100-11.
- Tolmacheva, V.V. 2016. Facile synthesis of magnetic hypercrosslinked polystyrene and its application in the magnetic solid-phase extraction of sulfonamides from

water and milk samples before their HPLC determination. *Talanta*, 152: 203-210.

Tong, Y., Liu, X. and Zhang, L. 2019. Green construction of Fe₃O₄@GC submicrocubes for highly sensitive magnetic dispersive solid-phase extraction of five phthalate esters in beverages and plastic bottles. *Food Chemistry*, 277: 579-585.

US EPA (United State Environment Protection Agency). America's Children and the Environment: Biomonitoring Phthalates. (Third Edition), Available: <https://www.epa.gov/ace/methods-biomonitoring-phthalates.pdf>, 1984. p.169-179.

US EPA (United State Environment Protection Agency). Method 8061A: Phthalate ester by gas chromatography with electron capture detector (GC-ECD). Available:<https://www.epa.gov/sites/production/files/201512/document/8061a.pdf> (accessed July 2015), 1996.

US EPA (United States Environmental Protection Agency). EPA Announces Actions to Address Chemicals of Concern, Including Phthalates: Agency Continues Efforts to Work for Comprehensive Reform of Toxic Substance Laws. Available:https://yosemite.epa.gov/opa/admpress.nsf/a543211f64e4d199852573590040444_2/2852c60dc0f65c688525769c0068b219!OpenDocument, 2009. (accessed May 2017).

US EPA (United States Environmental Protection Agency). Integrated Risk Information System Chemical Assessment Summary: di(2-ethylhexyl) phthalate (DEHP). CASRN117-81-7. Available:https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0014_summary.pdf, 1988. (accessed July 2017).

US EPA (United States Environmental Protection Agency). Integrated Risk Information System Chemical Assessment Summary: dibutyl phthalate. CASRN84-74-2. Available:https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0038_summary.pdf, 2002a. (accessed July 2017).

US EPA (United States Environmental Protection Agency). Integrated Risk Information System Chemical Assessment Summary: diethyl phthalate.

CASRN84-66-2.

Available:https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0226_summary.pdf, 2002b. (accessed July 2017).

US FDA (United States Food and Drug Administration). Limiting the use of certain phthalates as excipients in CDER-regulated products. Available: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/limiting-use-certain-phthalates-excipients-cder-regulated-products.pdf> (accessed March 2018), 2012

Viñas, P. 2015. Determination of phthalate esters in cleaning and personal care products by dispersive liquid-liquid microextraction and liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A*, 1376: 18-25.

Wallace, G. G., Spinks, G. M., Kane-Maguire, L. A. P., Teasdale, P. R. Conductive Electroactive Polymers: Intelligent Polymer Systems. (Third edition), CRC Press: Taylor & Francis Group, 2009. Boca Raton, United States of America.

Wang, L., Zang, X., Wang, C. and Wang, Z. 2014. Graphene oxide as a micro-solid-phase extraction sorbent for the enrichment of parabens from water and vinegar samples. *Journal of Separation Science*, 37: 1525-1709.

Wang, Q., Li, Y. and Wang, Y. 2011. Optimizing the weight loss-on-ignition methodology to quantify organic and carbonate carbon of sediments from diverse sources. *Environmental Monitoring and Assessment*, 174(1): 241-257.

Wang, R., Ma, X., Zhang, X., Li, X., Li, D. and Dang, Y. 2019. C₈-modified magnetic graphene oxide based solid-phase extraction coupled with dispersive liquid-liquid microextraction for detection of trace phthalate acid esters in water samples. *Ecotoxicology and Environmental Safety*, 170: 789-795.

Weiss, J. M., Gustafsson, A., Gerde, P., Bergman, A., Lindh, C.H. and Kraus, A.M. 2018. Daily intake of phthalates, MEHP, and DINCH by ingestion and inhalation. *Chemosphere*, 208: 40-49.

Xu, M. 2016. Magnetic solid-phase extraction of phthalate esters (PAEs) in apparel textile by core-shell structured Fe₃O₄@silica@triblock-copolymer magnetic microspheres. *Talanta*, 2016. 150: 125-134.

- Xu, Z. 2011. Molecularly imprinted stir bar sorptive extraction coupled with high performance liquid chromatography for trace analysis of sulfa drugs in complex samples. *Talanta*, 85(1): 97-103.
- Yongfeng, K. 2012. Molecularly imprinted polymers of allyl- β -cyclodextrin and methacrylic acid for the solid-phase extraction of phthalate. *Carbohydrate Polymers*, 88(2): 459-464.
- Zhou, D.B., Sheng, X., Han, F., Hu, Y.Y., Ding, L. and Lv, Y.L. Song, W. 2018. Zheng, P., Magnetic solid-phase extraction based on fullerene functionalization of magnetic nanoparticles for the determination of sixteen polycyclic aromatic hydrocarbons in tea samples. *Journal of Chromatography A*, 1578: 53-60.

Appendices

Paper I

A polyaniline/pencil lead solid phase microextractor for trace phthalate esters

**Chunin, N., Kaewpet, M., Wattanasin, P., Kanatharana, P., Thavarangkul, P., and
Thammakhet-Buranachai, C.**

Proceeding



A polyaniline/pencil lead solid phase microextractor for trace phthalate esters

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Abstract:

A solid-phase microextraction (SPME) fiber was fabricated by electrodeposition of silver-incorporated polyaniline film on a pencil lead core. This fiber was used to extract trace phthalate esters and later thermally desorbed at an injection port of a gas chromatograph coupled with an electron capture detector (GC-ECD). Under the optimum conditions, linearity of two phthalate esters, benzyl butyl phthalate (BBP) and bis(2-ethylhexyl) phthalate (DEHP) were in the range of 0.05-5.00 mg L⁻¹ and 0.25-5.00 mg L⁻¹ with a coefficient of determination (R²) greater than 0.99. Limits of detection of 0.030 and 0.24 mg L⁻¹ and limits of quantification of 0.10 and 0.79 mg L⁻¹ were obtained for BBP and DEHP, respectively. The developed method was utilized for the simultaneous extraction of spiked BBP and DEHP in deionized water (1.0 mg L⁻¹). A high preconcentration factor and satisfactory recoveries were achieved. This study indicated that the developed polyaniline/pencil lead SPME fiber can be used to extract BBP and DEHP. Further optimization of the extraction and thermal desorption conditions would make this sorbent applicable for the determination of phthalate esters contaminated in food and environmental samples.

1. Introduction

Phthalate esters (PAEs) are plasticizers, a class of compounds that can increase the flexibility of plastics through weak secondary molecular interactions with polymer chains. Since there is no covalent bond between PAEs and plastics, they can easily leach from the plastic containers into the food that come into contact with.^{1,2} When exposed to PAEs in high level, it poses an effect to human body such as vertigo, nausea and vomit. It might also cause cancer or affect the human reproductive system.^{3,4} Therefore, the determination of PAEs contamination would be beneficial for human health.

High performance liquid chromatography (HPLC) and gas chromatography (GC) are the usual analytical techniques for the analysis of PAEs. GC coupled with an

electron capture detector (GC-ECD) has been recommended by the United States Environmental Protection Agency as a standard method.^{5,6} However, the amount of PAEs contaminated in the samples is in trace level and the direct injection of the sample is not possible due to the complex sample matrix. Hence, sample preparation steps are needed to preconcentrate and reduce the effect of interference in the sample.⁷

An interesting sample preparation technique is solid phase microextraction (SPME) due to its simplicity and solvent-free extraction process.⁸ However, commercially available SPME devices are relatively expensive, fragile, and have low sample capacity. This work investigated the use of pencil lead based SPME fiber to overcome the problems. Pencil lead has

good mechanical rigidity and is very low cost.⁹ Surface modification with appropriate sorbent can be easily performed using an electrochemical technique.

Conducting polymers are often used as sorbent in many sample preparation techniques, out of which polyaniline (PANI) is one of the most popular.¹⁰ It can be prepared using either chemical polymerization or direct electrodeposition onto the surface of the substrate. In addition, PANI is particularly suited for the adsorption of PAEs because it can form $\pi-\pi$ interaction and hydrogen bonding with the target analytes.¹¹ However, the sample loading capacity of PANI is still limited through its film formation and the stability of a PANI thin film is somewhat poor. Nevertheless some recent reports have mentioned that Ag, MnO₂, RuO₂ can enhance the porosity of PANI film.^{12,13} This may help increase the surface area and thus, the extraction efficiency.

Here in, we report the development of a solid phase microextractor by electrodepositing silver-incorporated polyaniline film onto the surface of a pencil lead for trace phthalate esters detection by GC-ECD.

2. Experiment

2.1 Materials

Pencil leads (type 2B, diameter 0.7 mm, length 60 mm) were from A.W. Faber-Castell (Germany) Ltd. (Thuringia, Germany). Aniline monomer (reagent grade, 99.0% purity) was from Sigma-Aldrich (St. Louis, Missouri, USA). It was distilled prior to use. H₂SO₄ were from Lab-Scan (Bangkok, Thailand). H₂O₂ (30%), KMnO₄, acetonitrile (HPLC grade) and ethyl acetate were from Merck (Darmstadt, Germany). Di-2-ethylhexyl phthalate (DEHP) (97% purity) was from Fluka (Buchs, Switzerland). Benzyl butyl phthalate (BBP) (99% purity) was from Sigma-Aldrich (St. Louis, Missouri, USA). Acetone, ethanol and methanol were from Lab-Scan (Bangkok, Thailand). Ultrapure water was from a maximum ultrapure water system

(18.2 M Ω .cm ELGA, England). All chemicals used were of analytical grade.

2.2 Instruments

All analyses were performed using a gas chromatograph (Shimadzu GC-14B) equipped with an electron capture detector (ECD) (Kyoto, Japan) and a packed column of 3% OV-3 on Supelcoport, 100/120 mesh, 2.1 m \times 3.2 mm i.d. (Bellefonte, USA). Data processing was carried out with a N2000 Chromatography Data System (Surwit Technology Inc., Hangzhou, China).

2.3 Optimization of GC-ECD

Operating conditions of the GC-ECD system for the analysis of BBP and DEHP were optimized, i.e., the carrier gas flow rate, column, injector and detector temperatures. The optimization was carried out by varying one parameter at a time while keeping the others constant. Five replications of the analysis of 1.0 μ L of 1.0 mg L⁻¹ BBP and DEHP working standard solution were performed for each tested value. The optimum conditions were obtained by considering the best resolution, the highest response and the shortest analysis time.

2.4 Preparation of pencil lead SPME fiber

A pencil lead was immersed in a mixed solution of 30 mL of conc. H₂SO₄ and 3.0 g of KMnO₄. It was then washed in sequence with 10 mL of H₂O₂ (30%) (to reduce Mn species), 10 mL of HCl (to remove Mn(II) ions) and 10 mL of water (to remove HCl and other ions) and dried at room temperature.

The Ag/PANI sorbent was electrodeposited onto the pencil lead from a solution containing 0.2 M aniline and 0.5 mM AgNO₃ in 0.2 M H₂SO₄. The three-electrode system consisted of a pencil lead a working electrode, a platinum wire counter electrode, and a Ag/AgCl reference electrode coupled to a potentiostat (ML 160, AD Instruments, Australia) connected to a computer. The electrodeposition was performed with the potential intervals of -0.2 to 0.8 V at a scan rate of 10 mV s⁻¹. The morphology of the as-prepared materials was characterized by scanning electron

microscopy (SEM) (JSM-5800LV, JEOL, Massachusetts, USA). The Ag/PANI pencil lead fiber was conditioned at the GC injection port at 180 °C under a carrier gas stream for 2 h to remove unreacted residue on the fiber before use.

2.5 Adsorption and desorption procedures

The adsorption procedure was performed by immersing the pencil lead 2 mL of 1.0 mg L⁻¹ of mixed standard solution of BBP and DEHP in a 4 mL amber vial. The mixture was stirred at 2000 rpm for 30 min at room temperature, during this time the analytes were adsorbed onto the surface of the modified pencil lead fiber. After that, the analytes were thermally desorbed at the GC injection port at 300 °C under a carrier gas stream for 30 s (Figure 1).

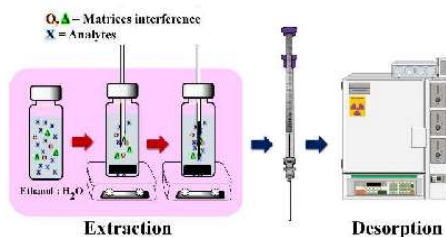


Figure 1. Schematic presentation of the extraction and desorption procedures of the Ag/PANI pencil lead SPME fiber for BBP and DEHP determination

3. Results and Discussion

3.1 Optimization of the GC-ECD operating conditions

The optimum conditions for the determination of phthalate esters are summarized in Table 1. The linear range, limit of detection (LOD) and limit of quantification (LOQ) were evaluated by the IUPAC recommended method, as 3-fold and 10-fold multiples, respectively, of the standard deviation of the ordinate intercept divided by the slope of regression line (S_a/b). Under these conditions are summarized in Table 2.

Table 1. Optimization of GC-ECD conditions, investigated and optimum values

Parameter	Investigated values	Optimum condition
Carrier gas flow rate (mL min ⁻¹)	20, 30, 40, 50	30
Column temperature		
□ Initial temperature (°C)	210, 220, 230, 240	230
□ Initial holding time (min)	3, 4, 5, 6	3
□ Ramp rate (°C min ⁻¹)	5, 10, 15, 20	10
□ Final temperature (°C)	270, 280, 290, 300	290
□ Final time (min)	2, 3, 4, 5, 6	2
Injector temperature (°C)	280, 290, 300	300
Detector temperature (°C)	280, 290, 300	290

Table 2. Analytical performances of GC-ECD for the analysis of BBP and DEHP

Analyte	Linear range (mg L ⁻¹)	LOD (mg L ⁻¹)	LOQ (mg L ⁻¹)
BBP	0.05 – 5.0	0.03	0.10
DEHP	0.25 – 5.0	0.24	0.79

3.2 Characterization of Ag/Polyaniline film on pencil lead fiber

Morphology

The SEM image of a bare pencil lead showed a smooth surface (Figure 2a). The surface became rather rough after it was immersed in acid solution, just like aged tree barks (Figure 2b). This suggests that the oxidation from the mix solution exerts a severe impact to the pencil lead. When it was modified with Ag/PANI, a good distributed porous structure film was observed along the pencil lead (Figures 2c and 2d)

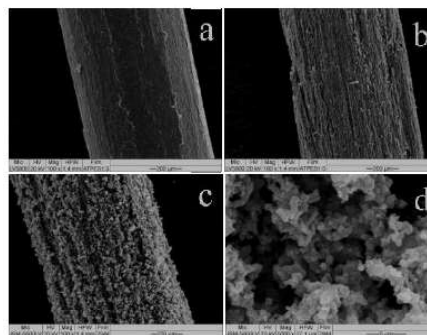


Figure 2. SEM images: surface morphologies of a bare pencil lead at magnification of 100×(a); an etching pencil lead at magnification of 100×(b); a Ag/PANI deposited on pencil lead at magnifications of 100×(c) and of 5,000×(d)

Thermal resistance

Thermal desorption at GC injection port was used in this study, thus, the thermogravimetric analysis (TGA) was first performed to investigate the thermal stability of the prepared fiber. The TGA result indicated no decomposition of the deposited Ag/PANI up to 500 °C thus, this fiber is suitable for the desorption at high temperature of the GC injection port.

3.3 Optimization of extraction conditions

Adsorption time

The adsorption time is an important parameter since it would certainly affect the extraction efficiency of the developed SPME fiber. Figure 3 shows the increase of peak area with the adsorption time from 10 to 30 minutes. The decrease at a longer time is possibly due to the back stripping of analyte from the SPME fiber into the extracting solution. Thus, 30 minutes was selected for subsequent experiments.

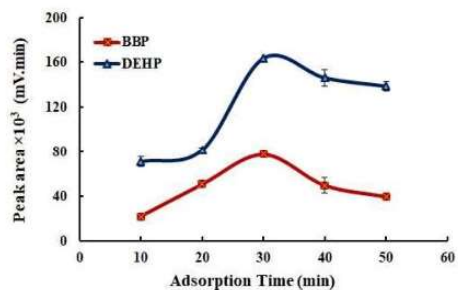


Figure 3. Effect of adsorption time on peak area of 1.0 mg L⁻¹ BBP and DEHP; desorption time of 40 second

Desorption time

The effect of desorption time is shown in Figure 4. Initially the peak area increased with time, then became stable after 30 seconds. That is, all adsorbed analytes were completely desorbed from the fiber at 30 seconds.

Under the optimum conditions, the prepared Ag/PANI pencil lead SPME fiber was used for the extraction the BBP and DEHP from the spiked standard solution in deionized water. High enrichment factor for

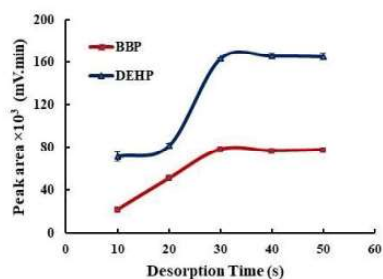


Figure 4. Effect of desorption time on peak area of 1.0 mg L⁻¹ BBP and DEHP; adsorption time of 30 minute

the extraction of DEHP was achieved (8-folds) while no enrichment for BBP was observed (Figure 5). This may be because BBP is more polar than DEHP, thus, can form a stronger $\pi - \pi$ interaction with a Ag/PANI SPME fiber making it harder to desorb. To improve the enrichment factor for BBP extraction, optimization of other affecting parameters e.g. stirring rate, salt addition, would be useful.

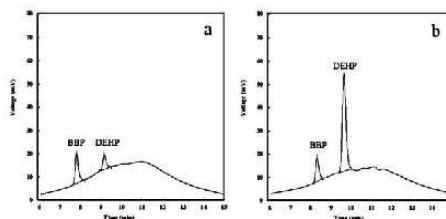


Figure 5. Chromatograms of (a) 1.0 mg L⁻¹ standard of BBP and DEHP (b) 1.0 mg L⁻¹ standard of BBP and DEHP extracted with the developed SPME fiber and thermally desorbed at the GC injection port

3.4 Reusability

A single fiber was used for the repeat extraction of 1.0 mg L⁻¹ mixed standard solution of the two phthalate esters. After the first extraction, the pencil lead SPME fiber was washed in acetonitrile and DI water, at a stirring rate of 900 rpm for 10 min before being reused. Relative peak area of subsequent extraction cycles were compared to the first one which was set as 100%.

Figure 6 shows that one fiber can be used up to 12 times) with average% responses of 97.8 ± 3.0 (RSD = 3.1%) and 98.6 ± 2.3 (RSD = 2.3%) for BBP and DEHP, respectively. These values were much better than the accepted RSD recommended by AOAC (11% at analyte concentration level of $1,000 \text{ ng mL}^{-1}$).¹⁴ Beyond these 12 extraction cycles, the response decreased to less than 90%. This may be due to structural deformation of the PANI/Ag and the leaching out of some part of it from the fiber and it can be observed by naked eyes.

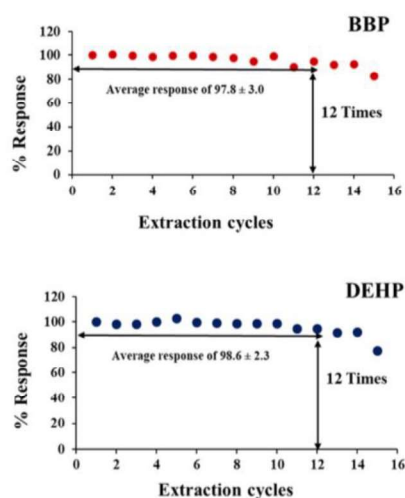


Figure 6. Reusability of Ag/PANI pencil lead fiber

Conclusions

This study shows the possibility and capability of the prepared Ag/PANI pencil lead SPME fiber for the extraction of BBP and DEHP. This cost effective and easy to prepare Ag/PANI pencil lead SPME fiber was successfully applied for the extraction of phthalate esters. In addition, the prepared fiber can be reused. Further investigation of some extraction parameters would lead to further improvement that would make it more applicable for real sample analysis.

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References

- Jen, J. F.; Liu, T. C. *J. Chromatogr. A.* **2006**, *1130*, 28–33.
- Balafas, D.; Shaw, K. J.; Whitfield, F. B. *Food Chem.* **1999**, *65*, 279–287.
- Le Noir, M.; Plieva, F.; Hey, T.; Guieysse, B. *J. Chromatogr. A.* **2007**, *1154*, 158–164
- Guo, Y.; Kannan, K. *Anal. Bioanal. Chem.* **2012**, *404*, 2539–2554.
- http://www.epa.gov/oppt/existingchemicals/pubs/actionplans/phthalates_actionplan_revised_2012-03-14.pdf.
- <http://www3.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/8061a.pdf>.
- Kueseng, P.; Thavarungkul, P.; Kanatharana, P. *J. Environ. Sci. Health B.* **2007**, *42*, 569–576.
- Sungkaew, S.; Thammakhet, C.; Thavarungkul, P.; Kanatharana P. *Anal. Chim. Acta* **2010**, *664*, 49–55.
- Heydari, A and Gharibi, H. *J. Power Sources* **2016**, *325*, 808–815.
- Bhadra, S.; Khastgir, D.; Singha, N. K.; Lee, J. H., *Prog. Polym. Sci.* **2009**, *34*, 783–810.
- Prasad, B.; Srivastava, A.; Pandey, I.; Tiwari, M. *J. Chromatogr.* **2013**, *912*, 65–74.
- Kim, J.; Ahn, K.; WonKang, S.; SungRoh, J.; HunKwon, S.; YongKim, J. *Curr. Appl. Phys.* **2012**, *12*, 160–163.
- Sawangphruk, M.; Kaewsongpol, T. *Materials Letters* **2012**, *87*, 142–145.
- <http://www.eoma.aoac.org/appf.pdf>

Paper II

A simple 3D printed-screw-based solid phase microextraction device with a silver decorated polyaniline pencil lead fiber for the extraction of phthalate esters in cosmeceutical products

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A simple 3D printed- screw-based solid phase microextraction device with a silver decorated polyaniline pencil lead fiber for the extraction of phthalate esters in cosmeceutical products

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Highlights

- Microextraction device made with 3D printer uses SPME pencil lead fiber.
- Pencil lead fiber was modified with silver-incorporated polyaniline film SPME.
- SPME fiber was highly efficient, inexpensive and robust.
- Fiber can be reused up to 18 times for the extraction of phthalate esters.
- The developed microextraction device was applied to detect trace phthalate esters.

Abstract

A screw-based portable and simple solid phase microextraction device was constructed with a 3D printer and used in combination with a solid-phase microextraction (SPME) fiber fabricated by electrodeposition of silver-incorporated porous polyaniline film onto a pencil lead core (Ag/PANI SPME fiber). The scanning electron microscope revealed the porous structure of the electrodeposited Ag/PANI film and X-ray diffraction confirmed the presence of silver in the porous polymer film. The fiber was used to extract trace quantities of phthalate esters. The compounds of interest were dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP) and di-2-ethylhexyl phthalate (DEHP). The extracted solution was identified and quantified by a gas chromatograph equipped with a flame ionization detector (GC-FID). Under the optimum conditions of the developed method, a good linearity was obtained in a concentration range from 5.0 to 1000 $\mu\text{g L}^{-1}$ for all five phthalate esters with limits of detection (LODs) of $4.41\pm 0.91 \mu\text{g L}^{-1}$, $3.98\pm 0.92 \mu\text{g L}^{-1}$, $3.65\pm 0.74 \mu\text{g L}^{-1}$, $4.91\pm 0.52 \mu\text{g L}^{-1}$ and $4.25\pm 0.66 \mu\text{g L}^{-1}$ for DMP, DEP, DBP, BBP and DEHP, respectively. The developed method provided good precision when tested with standard solutions (RSD < 5.5%, n=6) and real samples (RSD < 3.4%, n=6). Good fiber-to-fiber reproducibility was also confirmed by extraction with six prepared fibers: recoveries ranged from $81.09\pm 0.54\%$ to $92.92\pm 0.46\%$, RSD < 6.6%. The developed method was used to determine phthalate esters in 14 cosmeceutical samples. In rubbing alcohol, DEP and DEHP were detected at $7.03\pm 0.76 \mu\text{g L}^{-1}$ and $5.89\pm 0.53 \mu\text{g L}^{-1}$, respectively, while in contact lens cleaner, DEHP was found in a concentration range from $5.3\pm 1.1 \mu\text{g L}^{-1}$ to $6.8\pm 1.2 \mu\text{g L}^{-1}$. No contamination was detectable in saline solution, eye cleaner and antibacterial disinfectant liquid. Recoveries in the range of $81.92\pm 0.99 \%$ to $102.4\pm 1.1\%$ indicated the good accuracy of the developed method.

Keywords: 3D printed, screw-based solid phase microextraction device, silver decorated polyaniline, pencil lead fiber, cosmeceutical products

1. Introduction

Phthalate esters are plasticizers, a class of compounds that increase the flexibility of plastics through weak secondary molecular interactions with polymer chains. Since there is no bonding between phthalate esters and plastics, they can easily leach from plastic packaging into food and cosmeceutical products that they come into contact with [1, 2]. Exposure to high levels of phthalate esters can lead to endocrine disruption [3], reproductive mutation [4, 5] and carcinogenic effects in humans [6, 7]. The intelligence quotient (IQ) of children might also be affected [8]. The United States Environmental Protection Agency (US EPA) has classified some phthalate esters as priority pollutants [9-11]. Examples include dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), di-2-ethylhexyl phthalate (DEHP) and di-*n*-octyl phthalate (DNOP). In this study, DMP, DEP, DBP, BBP and DEHP were chosen as compounds of interest because of their common use in personal-care products and medication containers [12, 13]. In addition, DEHP has been classified as ‘possibly carcinogenic to humans’ although there is no conclusive evidence that any of the phthalates causes cancer [14]. Therefore, the detection and determination of phthalate ester contamination is beneficial for human health.

Typically used analytical techniques are high performance liquid chromatography (HPLC) and gas chromatography (GC) [15-17]. However, the trace level of phthalate esters contamination and the complexity of the sample matrix make it impractical to directly inject the sample. Hence, sample preparation steps are needed to reduce the effect of interferences in the sample [16, 18]. An interesting technique is solid phase microextraction (SPME), a simple solvent-free or solvent-reduced process with high extraction efficiency [19-21]. There are many commercially available SPME fibers but they are relatively expensive, quite fragile and have low sample capacity [22, 23]. To address these issues, a pencil lead-based SPME fiber is presented. The benefits of a pencil lead are its very low cost, good mechanical rigidity and conductivity [24]. Therefore, it should be a good candidate for simple electrochemical surface modification with an appropriate sorbent [25].

A number of conducting polymers have been employed as sorbents in sample preparation techniques. Polyaniline (PANI) has been one of the most popular [26-29].

It can be prepared by either chemical polymerization or direct electrodeposition onto the surface of a substrate. PANI is particularly suited for the adsorption of phthalate esters because it can form π - π interaction and hydrogen bonding with the target analytes [29-31]. However, the sample loading capacity of PANI is still limited through its film formation and the stability of a PANI thin film is somewhat poor [30]. Nevertheless, some recent reports have suggested that Ag, MnO₂ and RuO₂ can enhance the porosity of PANI film [32-34]. The incorporation of these metals or metal oxides into the polymer structure may help increase the surface area and thus, the extraction efficiency.

An extraction device was developed based on a 3D-printed screw that holds a solid phase microextraction fiber fabricated by electrodepositing silver-incorporated polyaniline nanoporous film onto the surface of a pencil lead (Ag/PANI SPME fiber). This device was used to extract trace phthalate esters for analysis by gas chromatograph equipped with a flame ionization detector (GC-FID). The large surface area of silver-incorporated polyaniline facilitated mass transfer of the target analytes. This improved the loading capacity of the analytes and increased the efficiency of their extraction. The affecting parameters were optimized and the method was validated before it was applied for the determination of phthalate esters leached from plastic containers into cosmetic products.

2. Experiment

2.1 Materials

Pencil leads (type 2B, diameter 2.0 mm, length 120 mm) were from Tianzhuo (China) Ltd. (Hangzhou, China). Acetonitrile (HPLC grade) and ethyl acetate were from Merck (Darmstadt, Germany). Di-2-ethylhexyl phthalate (DEHP) (97% purity) was from Fluka (Buchs, Switzerland). Dibutyl phthalate (DBP) (99% purity), dimethyl phthalate (DMP) (99% purity), diethyl phthalate (DEP) (99.5% purity) and benzyl butyl phthalate (BBP) (99% purity), silver nitrate and aniline monomer (reagent grade, 99.0% purity) were from Sigma-Aldrich (St. Louis, Missouri, USA). The monomer was distilled prior to use. Acetone, ethanol, hexane and methanol were from Lab-Scan (Bangkok, Thailand). Ultrapure water was from a maximum ultrapure water system (18.2 M Ω .cm ELGA, England). All chemicals were of analytical grade.

2.2 Instrumentation

Analyses were performed using a gas chromatograph (Shimadzu GC-2010 plus) equipped with a flame ionization detector (FID) (Shimadzu, Kyoto, Japan), and a DB-5 fused silica capillary column (30 m length, 0.25 mm I.D. and 0.25 μm film thickness; J&W Scientific, California, USA). Data processing was carried out with a LabSolutions data system (Shimadzu Corporation, Kyoto, Japan). To obtain the shortest analysis time, the highest response and a good separation, during analysis of the five phthalate esters (DMP, DEP, DBP, BBP and DEHP) the GC-FID was first calibrated by optimizing the flow rates of the carrier (He), make up (N_2), fuel (H_2) and oxidant (air) gases, and the temperatures of the column, injector and detector.

Morphological characterization of the Ag/PANI SPME fiber and elemental analysis of the Ag/PANI nanocomposite were performed on an FEI Quanta 400 scanning electron microscope coupled with energy-dispersive X-ray spectroscopy (SEM/EDX-Quanta) (Brno, Czech Republic).

The resin SPME device (Fig. 1A) was produced by a 3D printer-form 2 (Formlabs, USA) controlled by an SLA (stereolithography) system.

2.3 Preparation of the Ag/PANI nanoporous film pencil lead SPME fiber

The bare pencil lead was immersed in ethanol, acetonitrile and DI water to remove any interferences and ions from the fiber core and dried at 60°C. Silver and PANI were simultaneously deposited onto a 1.5 cm length of the pencil lead as an Ag/PANI nanoporous film by electrodeposition in a solution of 0.20 M aniline and 0.50 mM AgNO_3 in 0.20 M H_2SO_4 . The three-electrode system consisted of a pencil lead working electrode, a platinum wire counter electrode, and an Ag/AgCl reference electrode coupled to a potentiostat (ML 160, AD Instruments, Australia) connected to a data acquisition system (EChem Electrochemistry software running with an e-corder, eDAQ Pty Ltd., Australia). Electrodeposition was achieved by potential cycling between -0.20 and 1.0 V at a scan rate of 10 mV s^{-1} for 10 scans. The Ag/PANI SPME fiber was inserted into a cylindrical hole within the shaft inside the SPME device (Fig. 1B).

2.4 Adsorption and desorption

The modified part of the SPME fiber was dipped into 1.5 mL of a 1.0 mg L⁻¹ mixed standard solution of the five phthalate esters in a 2.0 mL amber glass vial. The solution was stirred at 600 rpm for 30 min at room temperature. The adsorbed target analytes were desorbed with a solvent and the obtained solution was evaporated to dryness at 60°C, redissolved in ethanol and 1.0 µL of the extract was injected into the GC-FID for analysis (Fig. 2).

To optimize the affecting parameters, consideration was given to the adsorption and desorption times and stirring rates, the number of scans for the electrodeposition step, and the type of desorption solvent used.

The initial extraction conditions in 1.5 mL of standard solution (1.0 mg L⁻¹) or sample solution were: 30 min stirring at 600 rpm for adsorption and 20 min at 400 rpm for desorption, 10 scans for the electrodeposition process and 1.5 mL of acetonitrile as the eluting solvent. Optimization was carried out by varying one parameter at a time, keeping the others constant, over three replications for each condition. The optimal value provided the highest recovery and the shortest analysis time. The obtained optimum was then fixed for the optimization of the next parameter.

2.5 Real sample analysis

The method was applied to extract DMP, DEP, DBP, BBP and DEHP in five groups of liquid samples sold in plastic containers: rubbing alcohol (3 samples), contact lens cleaner (4 samples), saline solution (4 samples), eye cleaner (2 samples) and antibacterial disinfectant liquid (1 sample). Without any pretreatment, 1.5 mL of each sample was extracted in three replications with the SPME fiber, as described in section 2.4.

3. Results and discussion

3.1 Optimization of GC-FID conditions

For the analysis of the five phthalate esters, the optimum conditions were flow rates of 1.0, 30, 300 and 30 mL min⁻¹ for the carrier, make up, oxidant and fuel gases, respectively, and temperatures of 300 and 260°C for the detector and injector, respectively. The temperature of the column was initially set at 110°C and held for 0.5

min before it was increased to 280°C with a temperature ramp rate of 20°C min⁻¹. Finally, the temperature was held at 300°C for 5 min before it was cooled down to the initial temperature for the next analysis. The chromatogram of DMP ($t_R = 5.64$ min), DEP ($t_R = 6.48$ min), DBP ($t_R = 8.51$ min), BBP ($t_R = 10.63$ min) and DEHP ($t_R = 11.78$ min), showed good peak separation with good resolution ($R_s > 1.5$) and good peak shape.

3.2 Characterization of the Ag/PANI nanoporous film on pencil lead fiber

The smooth surface of the bare pencil lead was revealed under the SEM (Fig. 3A). The homogenous Ag/PANI polymer film modification of the pencil lead fiber core (Fig. 3B) exhibited a nanoporous structure under high magnification (Fig. 3C). The large surface area of this nanoporous film of Ag/PANI indicated a high adsorption capacity towards the extracted phthalate esters.

The EDX spectrum (Fig. 4) presents peaks for carbon (C) and nitrogen (N), components of both the pencil lead core and polyaniline. The two tiny peaks of Ag confirm that Ag was successfully decorated on the PANI film. Oxygen (O), aluminium (Al) and sulfur (S) are components of the pencil lead.

3.3 Optimization of adsorption and desorption conditions

3.3.1 Adsorption time

Adsorption time is an important factor in the extraction process. It directly affects the amount of analyte adsorbed on the sorbent. The effect of time on adsorption of the five phthalate esters was evaluated from 10 to 60 min in increments of 10 min. Between 10 and 30 min, recovery increased from 40.4±1.3% to 83.1±2.9% (Fig. 5A). Over longer adsorption times, recovery decreased. The reduction in recovery was possibly due to the back stripping of target analytes from the SPME fiber into the extracting solution [35]. Thus, 30 min, the shortest time with the highest recovery, was selected as the optimum condition for subsequent experiments.

3.3.2 Desorption time

Desorption time was also varied to determine the shortest time that provided the highest desorption efficiency. The maximum recoveries were obtained at a desorption

time of 20 min: $82.7\pm 2.0\%$ for DMP, $80.86\pm 0.66\%$ for DEP, $79.8\pm 1.7\%$ for DBP, $81.4\pm 1.0\%$ for BBP and $73.95\pm 0.56\%$ for DEHP (Fig. 5B). A possible explanation for the lower recovery at desorption times longer than 20 min is that analytes can be extracted back from the desorption solution onto the surface of the SPME fiber [35]. For further optimization of affecting parameters, 20 min was the desorption time used.

3.3.3 *Stirring rates*

The stirring rate of the sample solution during adsorption helps to accelerate the diffusion and mass transport of analytes onto the sorbent and thus reduce the equilibration time. To evaluate the effect of this parameter, the standard solution of phthalate esters was stirred at 200, 400, 600 and 800 rpm during the 30 min adsorption step. Between 200 and 600 rpm, recovery of the target analytes increased with the stirring rate from $56.0\pm 1.1\%$ to a maximum $91.1\pm 3.2\%$ for DMP and DEP (Fig. 5C). But, when stirred at 800 rpm, the solution formed a vortex, contact with the SPME fiber in the middle of the vial was reduced and recovery decreased. Also, vortex forces may strip adsorbed phthalate esters from the fiber [11]. For the adsorption step, 600 rpm was selected as the optimum stirring speed.

The rate of stirring during the desorption step is equally important. The recoveries of target analytes increased from $46.3\pm 3.5\%$ at 200 rpm to a maximum of $92.6\pm 1.2\%$ for DEP at 400 rpm, were constant at 400 and 600 rpm and then decreased at 800 and 1000 rpm (Fig. 5D). This behavior might possibly be explained by the effect of the vortex force. The optimum stirring rate for desorption was determined to be 400 rpm.

3.3.4 *The number of scans for Ag/PANI electrodeposition*

Extraction efficiency is affected by the electrodeposited adsorbent layer. To improve extraction efficiency of the target analytes, the modification of the developed Ag/PANI SPME fiber was optimized over 5, 10, 15 and 20 cycles of electrodeposition of silver-incorporated PANI film. The highest recoveries from the standard solution at the tested concentration were obtained with a fiber modified over 10 scans. These recoveries were $91.5\pm 1.6\%$ for DMP, $92.9\pm 2.0\%$ for DEP, $82.7\pm 4.2\%$ for DBP,

82.2±1.6% for BBP and 77.5±1.5% for DEHP (Fig. 5E). Therefore, 10 electrodeposition cycles were used to modify the developed SPME fiber.

3.3.5 Type of desorption solvent

The type of desorption solvent used is one of the most important parameters in any sorbent-based extraction technique. The solvent used in the present technique must efficiently desorb phthalate esters that bond to the Ag/PANI SPME fiber by hydrogen bonding and π - π interaction. Therefore, selection of the desorption solvents for study was based on their polarity. The selected solvents were acetonitrile (polarity index of 5.8), ethyl acetate (polarity index of 4.4), ethanol (polarity index of 5.2) and acetone (polarity index of 5.1).

For most of the phthalate esters, the lowest recoveries, in the range of 39.7±3.2 to 55.01±0.92%, were obtained after desorbing with ethanol and acetone (Fig. 5F). Ethyl acetate and acetonitrile provided better results (with recoveries in the range of 74.8±1.6 to 91.1±3.2%). This could be explained by the rule of “like dissolves like”. Ethyl acetate and acetonitrile may have polarities closer to those of the five phthalate esters than ethanol and acetone.

However, the recoveries of some compounds using a single solvent were still not acceptable, especially for DMP, DEP, DBP with ethyl acetate and DEHP with acetonitrile. To improve desorption efficiency, an acetonitrile-ethyl acetate mixture was considered an interesting option. Various ratios were investigated (Fig. S1, Supplementary data). At a 4:1 volume ratio (Fig. 5E), extraction efficiency, especially of DMP and DEP, was significantly improved. Recoveries of DMP, DEP, DBP, BBP and DEHP were respectively 91.7±1.2%, 92.6±1.2%, 82.8±2.6%, 83.98±0.33% and 83.89±0.68%, which are acceptable levels by AOAC standards (below 13% at the concentration of 1.0 mg L⁻¹ and 80-110% recoveries at analyte concentration levels of 100 to 1000 µg L⁻¹) [36].

These results can be explained by considering the polarity of the target analytes and the polarity of the desorption solvent. DMP, DEP and DBP are slightly polar compounds whereas BBP and DEHP are non-polar compounds. Ethyl acetate is a less polar solvent with a polarity index of 4.4 and acetonitrile is a more polar solvent with a polarity index of 5.8. Combining the two solvents helps adjust the polarity of the

desorption solvent, making it suitable for the desorption of both groups of phthalate esters from the Ag/PANI SPME fiber. Therefore, in further experiments, a mixture of ethyl acetate and acetonitrile at the volume ratio of 4:1 was used for desorption.

3.4 Extraction efficiencies of fibers

To assess the extraction performance of the modified fiber, 1.0 mg L⁻¹ mixed standard solutions of the five phthalate esters were extracted for 50 min with a bare, a PANI, and an Ag/PANI pencil lead SPME fiber. The recoveries of phthalate esters by these fibers clearly differed (Fig. 6). Since pencil lead contains graphite and its carbon atoms are arranged in a hexagonal structure, it can interact with the target analytes by π - π interactions [37]. Modification with PANI improved extraction efficiency from about 40% to about 60% because the functional group in the PANI moiety can interact with the target analytes via both π - π interactions and hydrogen bonding. The Ag/PANI-modified pencil lead achieved the highest extraction recoveries of 88.2±1.4% for DMP, 91.6±1.5% for DEP, 82.8±2.6% for DBP, 81.6±1.5% for BBP and 85.5±1.8% for DEHP. This overall improvement is probably caused by the increased surface area of the sorbent resulting from the one-step electrodeposition of silver with PANI. The electrodeposition process is likely initiated by the formation of [Ag-aniline]⁺ complex ions which undergo polymerization involving both complex ions and pristine aniline monomer [16, 34].

3.5 Analytical performances and method validation

3.5.1 Linearity, limit of detection and limit of quantification

After extraction under the optimal conditions and analysis by GC-FID, response points from the determination of all the phthalate esters were linear in the concentration range of 5.0 to 1000 µg L⁻¹.

The limits of detection (LODs) and limits of quantification (LOQs) were evaluated by the IUPAC recommended method, as 3-fold and 10-fold, respectively, of the standard deviation (SD) of 20 blank measurements divided by the slope of the calibration plot [38, 39]. They are summarized in Table 1.

Table 1 Analytical performances of Ag/PANI fiber for the analysis of the five phthalate esters

	Linear range ($\mu\text{g L}^{-1}$)	Linear equation	LOD ($\mu\text{g L}^{-1}$)	LOQ ($\mu\text{g L}^{-1}$)
DMP	5.0 – 1000	$y = (17.39 \pm 0.70)x + (0.80 \pm 0.39)$, $R^2 = 0.9935$	4.41 \pm 0.91	14.7 \pm 3.0
DEP	5.0 – 1000	$y = (19.28 \pm 0.94)x + (0.37 \pm 0.53)$, $R^2 = 0.9905$	3.98 \pm 0.92	13.3 \pm 3.1
DBP	5.0 – 1000	$y = (20.99 \pm 0.85)x + (0.39 \pm 0.47)$, $R^2 = 0.9935$	3.65 \pm 0.74	12.2 \pm 2.5
BBP	5.0 – 1000	$y = (15.61 \pm 0.58)x + (0.81 \pm 0.32)$, $R^2 = 0.9946$	4.91 \pm 0.52	16.4 \pm 1.7
DEHP	5.0 – 1000	$y = (18.05 \pm 0.82)x + (0.80 \pm 0.46)$, $R^2 = 0.9917$	4.25 \pm 0.66	14.2 \pm 2.2

These LODs are lower than the recommended maximum contaminant level (MCLs) of $6.0 \mu\text{g L}^{-1}$ for BBP and DEHP, adopted by the US Environmental Protection Agency [40]. Thus, the developed method can certainly be used for the determination of phthalate esters in real samples. However, no regulatory limits have yet been set by any organization on the contamination levels in the five sample groups studied in this work. So, monitoring the leaching of these phthalate esters from plastic containers may raise awareness of contamination from these compounds in the kinds of samples studied in this work.

3.5.2 Fiber-to-fiber reproducibility

To investigate the preparation procedure reproducibility of the fabricated SPME fiber, six batches of three SPME fibers were prepared. Each fiber was used to extract 1.0 mg L^{-1} standard solution containing the five phthalate esters, followed by analysis with GC-FID. The average extraction recoveries obtained from the six batches for the five phthalate esters were within the 81 to 93% range with RSDs between 0.7 and 6.7% (Fig. S2, Supplementary data). These RSDs are better than the acceptable value of 13% recommended by the AOAC (at the concentration of 1.0 mg L^{-1}) [36], indicating that the Ag/PANI SPME fiber sorbent could be reproduced with good precision.

To confirm the reproducibility of the fiber in real world application, another six batches of the SPME fiber were fabricated and applied to extract phthalate esters in rubbing alcohol. The extraction recoveries from the six batches were within the range

of 81 to 93% with RSDs between 0.7 and 2.6% for the five phthalate esters (Fig. S3, Supplementary data). These recoveries and %RSD were also better than those recommended by the AOAC [36].

These results indicate an excellent reproducibility of the fabrication procedure of the silver-incorporated PANI SPME fiber even when used for the extraction of target analytes in a real sample matrix. In addition, the sample matrix had no effect on the obtained recoveries.

3.5.3 Reusability

To establish the number of extraction cycles that the Ag/PANI pencil lead SPME fiber can effectively perform, a mixed standard solution of the five phthalate esters at 1.0 mg L^{-1} was extracted in standard solutions and spiked samples. Before reuse after each extraction, the SPME fiber was washed for 5 min in acetonitrile, DI water and acetone, at a stirring rate of 400 rpm. The relative peak area of the first extraction cycle was considered as 100% against which the peak area of each subsequent cycle was calculated.

In the standard solution, one fiber could be used up to 18 times. The average % responses of the five phthalate esters were about 90 to 94% and RSDs were 2.3 to 4.8% (Fig. S4, Supplementary data). These values were much better than the accepted RSD recommended by the AOAC (11 % at an analyte concentration level of 1.0 mg L^{-1}) [36]. Beyond 18 extraction cycles, the response decreased to less than 90 %. This was most likely due to structural deformation of the Ag/PANI film, where some polymer had been stripped from the fiber core.

In the spiked real sample (rubbing alcohol), one fiber could be used up to 21 times with average % responses of about 90 to 92% and RSDs 0.84 to 3.8% (Fig. S5, Supplementary data).

These two experiments indicated that the developed Ag/PANI SPME fiber provided good repeatability. When used for the extraction of phthalate esters in real samples, the similarity in the response percentages indicated that the matrix in the sample did not affect the robustness of the Ag/PANI pencil lead SPME fiber..

These results also confirmed the cost-effectiveness of the fiber. The material cost of one fiber is about 0.089 USD and since the fiber can be reused 18 to 21 times, the cost of one extraction cycle is only 0.0005 USD.

3.5.4 Precision

The precision of the SPME fiber was evaluated by spiking rubbing alcohol with standard solutions of the phthalate esters at final concentrations of 0.050, 0.25, 0.50, 0.75 and 1.0 mg L⁻¹ (six replications at each concentration). The extraction and the analysis were performed under the optimal conditions. The RSDs of the recoveries for the extraction of five phthalate esters at each spiked concentration ranged from 3.1 to 5.5 %. These values were better than the AOAC RSD guideline value of 11% for 1.0 mg L⁻¹ [36]. Therefore, the extraction and analysis of phthalate esters with the developed Ag/PANI pencil lead SPME fiber coupled with GC-FID achieved good precision.

3.6 Analysis of real samples

To assess the applicability of the developed method, it was used to detect five phthalate esters in five liquid sample groups (14 samples in total) sold in plastic containers by local supermarkets in Hat Yai, Thailand.

The matrix effect was first evaluated by spiking standard solutions of DMP, DEP, DBP, BBP and DEHP in the concentration range of 5.0 to 1000 µg L⁻¹ into each type of cosmeceutical product sample (matrix-matched standard solution). Standard solutions of the analytes at the same concentration range were also prepared in deionized water. Both sets were extracted and analyzed under the same conditions. Matrix-matched and standard calibration curves were plotted of the average peak area against their known concentrations. Their slopes were statistically compared by two-way ANOVA (analysis of variance).

The slopes of the matrix-matched and the standard calibration plots for contact lens cleaner and antibacterial disinfectant liquid samples were significantly different ($P < 0.05$), indicating a matrix effect. Therefore, the matrix-matched calibration plot was used for quantitative analysis of these samples. For rubbing alcohol, saline solution and eyes cleaner samples, there were no significant differences ($P > 0.05$) between the slopes

of the two calibration plots, indicating no interferences from the sample matrix and the standard calibration plot was used for quantitative analysis of this group.

DEP and DEHP were detected at 7.03 ± 0.76 ng mL⁻¹ and 5.89 ± 0.53 ng mL⁻¹, respectively in one of the rubbing alcohol samples. DEHP was found at 5.3 ± 1.1 ng mL⁻¹ and 6.8 ± 1.2 ng mL⁻¹ in two samples of contact lens cleaner solution (Table S1, Supplementary data). However, these concentrations are still lower than the MCL of 6.0 µg L⁻¹ for DEHP or within the guideline values [40]. No phthalate esters were detectable in the samples of saline solution, eye cleaner and antibacterial disinfectant liquid.

It is noteworthy that the samples contaminated with DEP and DEHP were contained in plastic products. This is not surprising because these plasticizers are the most widely used in the world [41] and are added at a content of 40% by weight during the production process [12, 42].

The developed method was further validated by the obtained recoveries from the extraction of spiked standard solutions of DMP, DEP, DBP, BBP and DEHP in each real sample at concentrations of 0.005, 0.050, 0.250, 0.500 and 1.0 mg L⁻¹. Good recoveries of the five phthalate esters within the range of 82-103% (Table S2, Supplementary data) were obtained. These values are within the acceptable range as recommended by AOAC (80-110% recoveries at analyte concentration level of 100 to 1000 µg L⁻¹) (AOAC 2016) [36] indicating good accuracy of the developed method.

Conclusions

A novel solid phase micro-extractor was successfully developed using silver-incorporated polyaniline film electrodeposited on a pencil lead. It is easy to prepare, and provided high extraction efficiency when applied for the extraction of five phthalate esters in real samples. Extraction time is about 50 min and the fiber can be reused at least 18 times (average recoveries of five phthalate esters $>90.4 \pm 3.8$). Other advantages are simplicity and economy (0.089 USD per fiber, 0.0005 USD per extraction). Under the optimum conditions, LODs were low, precision good and relative standard deviation for fiber-to-fiber reproducibility was less than 6.6%. The recovery range of the developed method for extraction of five phthalate esters in real samples indicated good accuracy and precision. This developed Ag/PANI SPME fiber can also be an

alternative extraction device for the analysis of other phthalate esters or other aromatic compounds in various kinds of samples.

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References

1. J.F. Jen and T.C. Liu, *Determination of phthalate esters from food-contacted materials by on-line microdialysis and liquid chromatography*. Journal of Chromatography A, 2006. 1130(1): p. 28-33.
2. D. Balafas, K.J. Shaw, and F.B. Whitfield, *Phthalate and adipate esters in Australian packaging materials*. Food Chemistry, 1999. 65(3): p. 279-287.
3. A. Lenoir, V. Cuvillier-Hot, S. Devers, J.P. Christidès, and F. Montigny, *Ant cuticles: A trap for atmospheric phthalate contaminants*. Science of The Total Environment, 2012. 441: p. 209-212.
4. Y. Guo, Q. Wu, and K. Kannan, *Phthalate metabolites in urine from China, and implications for human exposures*. Environment International, 2011. 37(5): p. 893-898.
5. M. Petrović, E. Eljarrat, M.J. López de Alda, and D. Barceló, *Analysis and environmental levels of endocrine-disrupting compounds in freshwater sediments*. TrAC Trends in Analytical Chemistry, 2001. 20(11): p. 637-648.
6. J. A. Tickner, T. Schettler, T. Guidotti, M. McCally, and M. Rossi, *Health risks posed by use of Di2-ethylhexyl phthalate (DEHP) in PVC medical devices: A critical review*. Vol. 39. 2001. 100-11.
7. F. A. Arcadi, C. Costa, C. Imperatore, A. Marchese, A. Rapisarda, M. Salemi, G.R. Trimarchi, and G. Costa, *Oral toxicity of bis(2-ethylhexyl) phthalate during*

- pregnancy and suckling in the long–evans rat*. Food and Chemical Toxicology, 1998. 36(11): p. 963-970.
8. J.H. Kim, S.H. Kim, S.S. Oh, H.J. Ihm, H.D. Chae, C.H. Kim, and B.M. Kang, *In vitro effects of phthalate esters in human myometrial and leiomyoma cells and increased urinary level of phthalate metabolite in women with uterine leiomyoma*. Fertility and Sterility, 2017. 107(4): p. 1061-1069.e1.
 9. J. J. Rios, A. Morales, and G. Márquez-Ruiz, *Headspace solid-phase microextraction of oil matrices heated at high temperature and phthalate esters determination by gas chromatography multistage mass spectrometry*. Talanta, 2010. 80(5): p. 2076-2082.
 10. M. Coquery, A. Morin, A. Bécue, and B. Lepot, *Priority substances of the European Water Framework Directive: analytical challenges in monitoring water quality*. TrAC Trends in Analytical Chemistry, 2005. 24(2): p. 117-127.
 11. F. Makkliang, P. Kanatharana, P. Thavarungkul, and C. Thammakhet-Buranachai, *A polypyrrole-chitosan cryogel stir-bead micro-solid phase extractor for the determination of phthalate esters in contact lenses storage solutions and in artificial saliva in contact with baby teethers*. Analytica Chimica Acta, 2017. 985: p. 69-78.
 12. M. Crookes, J. Larner, D.H. Lohmann, C. Maxwell, D.M. Sanderson, and S. Lipworth, *Health and Safety Committee (EHSC) of the Royal Society of Chemistry. Should we worry about phthalates*. Available: <http://www.rsc.org/ehsc>, 2013. (access June 2013).
 13. US EPA (United State Environment Protection Agency). *America's Children and the Environment: Biomonitoring Phthalates. (Third Edition)*. Available: <https://www.epa.gov/ace/methods-biomonitoring-phthalates.pdf>, p. 169-179.
 14. T. Nakajima, N.B. Hopf, and A. Paul, *Schulte Di(2-ethylhexyl) phthalate (DEHP)*. IARC Monographs 2000. 77: p. 183-196.
 15. F. Makkliang, P. Kanatharana, P. Thavarungkul, and C. Thammakhet, *Development of magnetic micro-solid phase extraction for analysis of phthalate esters in packaged food*. Food Chemistry, 2015. 166: p. 275-282.
 16. P. Kueseng, C. Thammakhet, P. Thavarungkul, and P. Kanatharana, *Multiwalled carbon nanotubes/cryogel composite, a new sorbent for determination of trace*

- polycyclic aromatic hydrocarbons*. *Microchemical Journal*, 2010. 96(2): p. 317-323.
17. US EPA (United State Environment Protection Agency). *Method 8061A: Phthalate ester by gas chromatography with electron capture detector (GC-ECD)*. Available: <https://www.epa.gov/sites/production/files/2015-12/document/8061a.pdf> (accessed July 2015), 1996.
 18. H.C. Liu, W. Den, S.F. Chan, and K.T. Kin, *Analysis of trace contamination of phthalate esters in ultrapure water using a modified solid-phase extraction procedure and automated thermal desorption-gas chromatography/mass spectrometry*. *Journal of Chromatography A*, 2008. 1188(2): p. 286-294.
 19. W.K. Meng, L. Liu, X. Wang, R.S. Zhao, M.L. Wang, and J.M. Lin, *Polyphenylene core-conjugated microporous polymer coating for highly sensitive solid-phase microextraction of polar phenol compounds in water samples*. *Analytica Chimica Acta*, 2018. 1015: p. 27-34.
 20. E. Ghasemi and H. Farahani, *Head space solid phase microextraction based on nano-structured lead dioxide: Application to the speciation of volatile organoselenium in environmental and biological samples*. *Journal of Chromatography A*, 2012. 1258: p. 16-20.
 21. N. Campillo, R. Peñalver, L. López-García, and M. Hernández-Córdoba, *Headspace solid-phase microextraction for the determination of volatile organic sulphur and selenium compounds in beers, wines and spirits using gas chromatography and atomic emission detection*. *Journal of Chromatography A*, 2009. 1216(39): p. 6735-6740.
 22. Sigma-Aldrich Co. *Solid Phase Microextraction (SPME)*. Available: <https://www.sigmaaldrich.com/analytical-chromatography/sample-preparation/spme.pdf>, 2019.
 23. Sigma-Aldrich Co. *SPME for GC Analysis*, Available: <https://www.getting started with solid phase microextraction.com/analytical-chromatography.pdf>. 1997. (access February 2018).
 24. Q. Liu, M. Cheng, Y. Long, M. Yu, T. Wang, and G. Jiang, *Graphenized pencil lead fiber: Facile preparation and application in solid-phase microextraction*. *Journal of Chromatography A*, 2014. 1325: p. 1-7.

25. N. Dossi, R. Toniolo, F. Terzi, and G. Bontempelli, *Pencil leads doped with electrochemically deposited Ag and AgCl for drawing reference electrodes on paper-based electrochemical devices*. *Electrochimica Acta*, 2014. 146: p. 518-524.
26. M. Wójciak-Kosior, I. Sowa, S. Dresler, J. Kováčik, M. Staniak, J. Sawicki, S. Zielińska, R. Świeboda, M. Strzemiński, and R. Kocjan, *Polyaniline based material as a new SPE sorbent for pre-treatment of Chelidonium majus extracts before chromatographic analysis of alkaloids*. *Talanta*, 2019. 194: p. 32-37.
27. Z. Li, X. Zhou, J. Shi, X. Zou, X. Huang, and H.E. Tahir, *Preparation of conducting polyaniline/protoporphyrin composites and their application for sensing VOCs*. *Food Chemistry*, 2019. 276: p. 291-297.
28. S. Bhadra, D. Khastgir, N.K. Singha, and J.H. Lee, *Progress in preparation, processing and applications of polyaniline*. *Progress in Polymer Science*, 2009. 34(8): p. 783-810.
29. G.G. Wallace, G.M. Spinks, L.A.P. Kane-Maguire, and P.R. Teasdale, *Conductive Electroactive Polymers: Intelligent Polymer Systems, 3rd edition*. *CRC Press: Taylor & Francis Group*, 2009. 2009.
30. M. Konkol, M. Kondracka, P. Kowalik, W. Próchniak, K. Michalska, A. Schwedt, C. Merckens, and U. Englert, *Decomposition of the mixed-metal coordination polymer—A preparation route of the active Ag/Yb₂O₃ catalyst for the deN₂O process*. *Applied Catalysis B: Environmental*, 2016. 190: p. 85-92.
31. B.B. Prasad, A. Srivastava, I. Pandey, and M.P. Tiwari, *Electrochemically grown imprinted polybenzidine nanofilm on multiwalled carbon nanotubes anchored pencil graphite fibers for enantioselective micro-solid phase extraction coupled with ultratrace sensing of d- and l-methionine*. *Journal of Chromatography B*, 2013. 912: p. 65-74.
32. J.H. Kim, J.H. Ahn, S.W. Kang, J.S. Roh, S.H. Kwon, and J.Y. Kim, *Thermal stability of RuO₂ thin films prepared by modified atomic layer deposition*. *Current Applied Physics*, 2012. 12: p. S160-S163.
33. X. Wang, L. Liu, A. Liu, Q. Liu, X. Du, and G. Jiang, *Preparation and evaluation of mesoporous cellular foams coating of solid-phase microextraction fibers by*

- determination of tetrabromobisphenol A, tetrabromobisphenol S and related compounds.* Analytica Chimica Acta, 2012. 753: p. 1-7.
34. M. Sawangphruk and T. Kaewsongpol, *Direct electrodeposition and superior pseudocapacitive property of ultrahigh porous silver-incorporated polyaniline films.* Materials Letters, 2012. 87: p. 142-145.
35. M. Dent, D.U. Verica, M. Penić, M. Brncic, T. Bosiljkov, and B. Levaj, *The Effect of Extraction Solvents, Temperature and Time on the Composition and Mass Fraction of Polyphenols in Dalmatian Wild Sage (Salvia officinalis L.) Extracts.* Vol. 51. 2013. 84-91.
36. AOAC INTERNATIONAL, *Appendix F: Guidelines for Standard Method Performance Requirements, Available: https://www.eoma.aoc.org/app_f.pdf, (access July 2016).*
37. M.E. Pérez and N. Martín, *π - π interactions in carbon nanostructures.* Chemical Society Reviews, 2015. 44(18): p. 6425-6433.
38. *Chronicles of Young Scientists. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Available: <https://www.cyonline.org> on Thursday.pdf, (access November 2016), 2016. 2(1): p.21-25.*
39. J.N. Miller., J.C. Miller, ed. *Statistics and Chemometrics for Analytical Chemistry. (Sixth Edition)*, Pearson Education Limited, 2010. United Kingdom, p. 124.
40. *US EPA U.S. Environmental Protection Agency. Integrated Risk Information System Chemical Assessment Summary: Di(2-ethylhexyl)phthalate (DEHP). CASRN 117-81-7. Available:http://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0014_summary.pdf, 1988. (access July 2017).*
41. M. Mortula, *Health Risk Assessment of PET Bottles in GCC.* Vol. 7. 2013. 267-272.
42. H. Erythropel, M. Maric, J. Nicell, R. Leask, and V. Yargeau, *Leaching of the plasticizer di(2-ethylhexyl)phthalate (DEHP) from plastic containers and the question of human exposure.* Vol. 98. 2014.

Figure Captions:

- Fig. 1 Photo of the SPME device (A) and schematic of the Ag/PANI SPME device (B)
- Fig. 2 Schematic presentation of the extraction and desorption procedures of the Ag/PANI pencil lead SPME fiber for the five phthalate esters
- Fig. 3 SEM images: surface morphologies of a pencil lead at magnification of 50×(A); and Ag/PANI deposited on pencil lead at magnifications of 50× (B) and 10,000× (C)
- Fig. 4 EDX spectrum of silver-incorporated polyaniline film on a pencil lead core
- Fig. 5 The five phthalate esters (n=3) in solution at 1.0 mg L⁻¹ were extracted with the developed device. The charts show the effects on recovery of adsorption time (A), desorption time (B), stirring rate of adsorption (C), the stirring rate of desorption (D), number of scans for electrodeposition of Ag/PANI (E) and type of desorption solvent (F)
- Fig. 6 Extraction recoveries of bare, PANI and Ag/PANI coated pencil leads

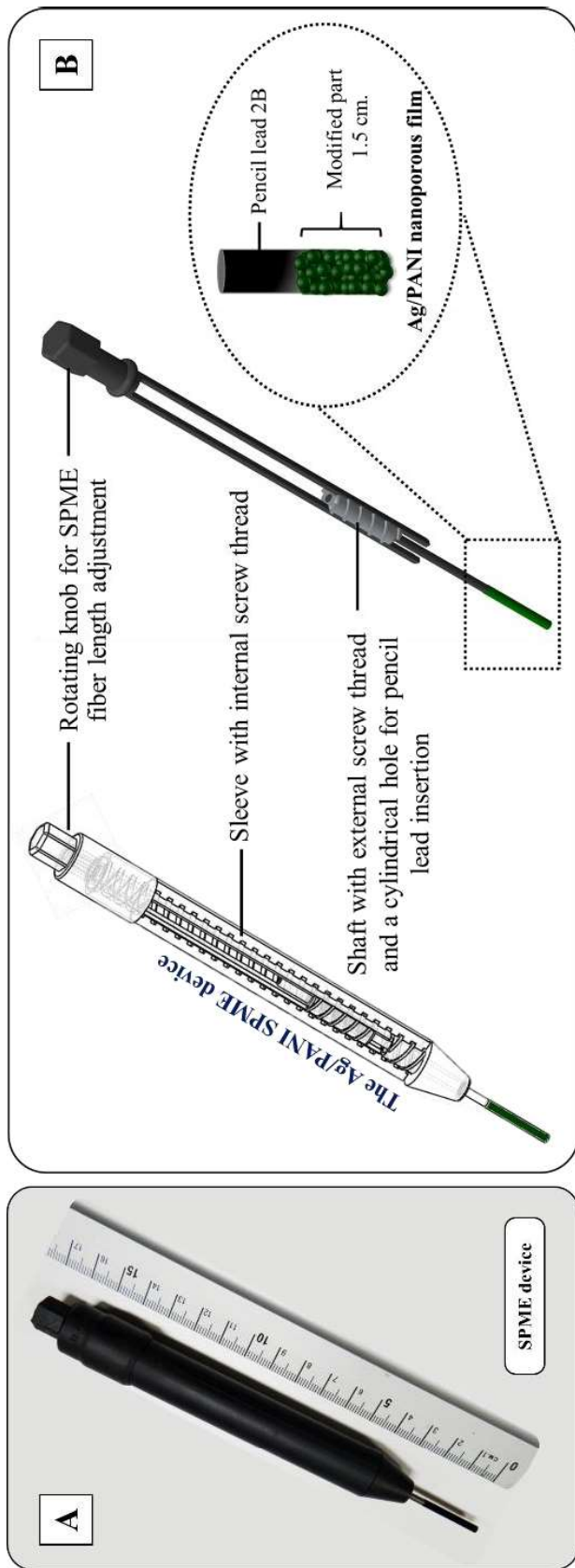


Fig. 1 Photo of the SPME device (A) and schematic of the Ag/PANI SPME device (B)

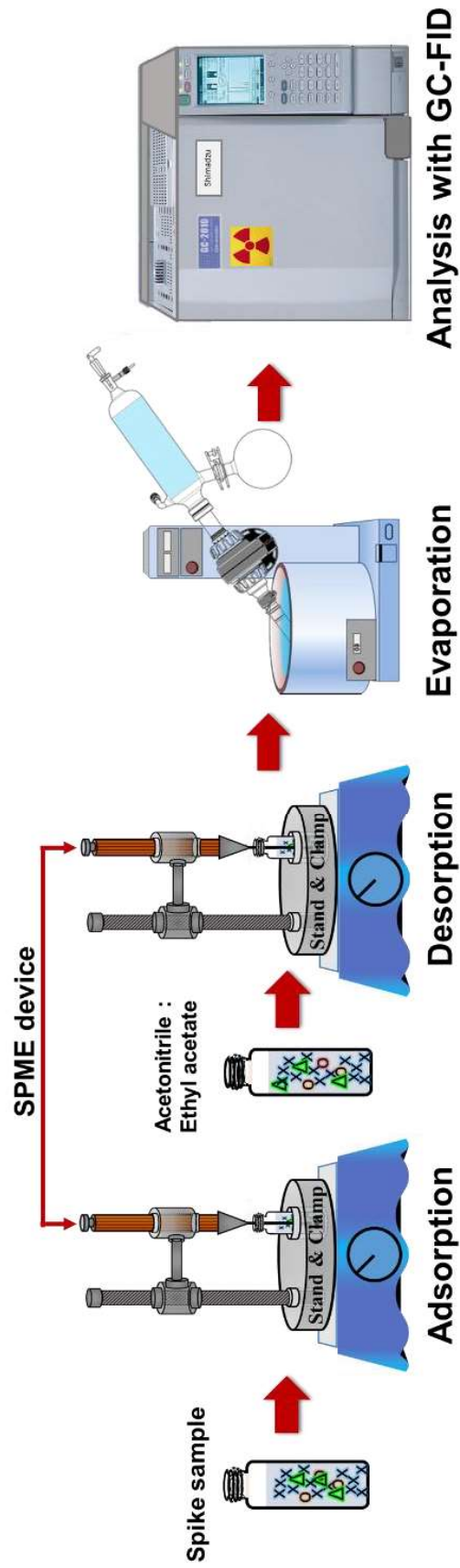


Fig. 2 Schematic presentation of the extraction and desorption procedures of the Ag/PANI pencil lead SPME fiber for the five phthalate esters

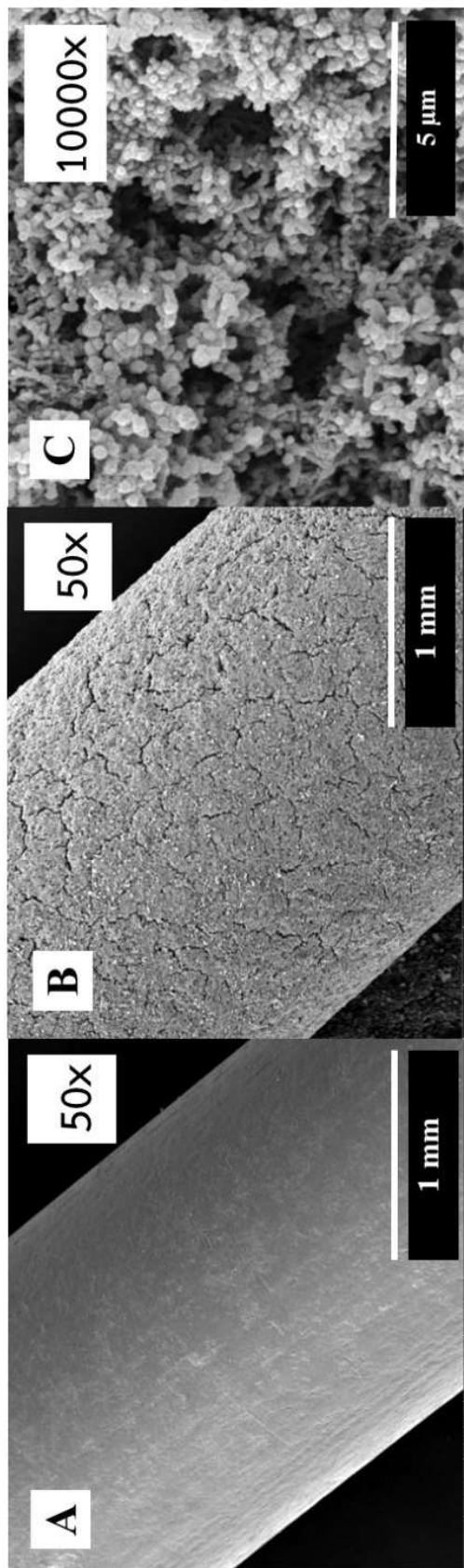


Fig. 3 SEM images: surface morphologies of a pencil lead at magnification of 50 \times (A); a Ag/PANI deposited on pencil lead at magnifications of 50 \times (B) and of 10,000 \times (C)

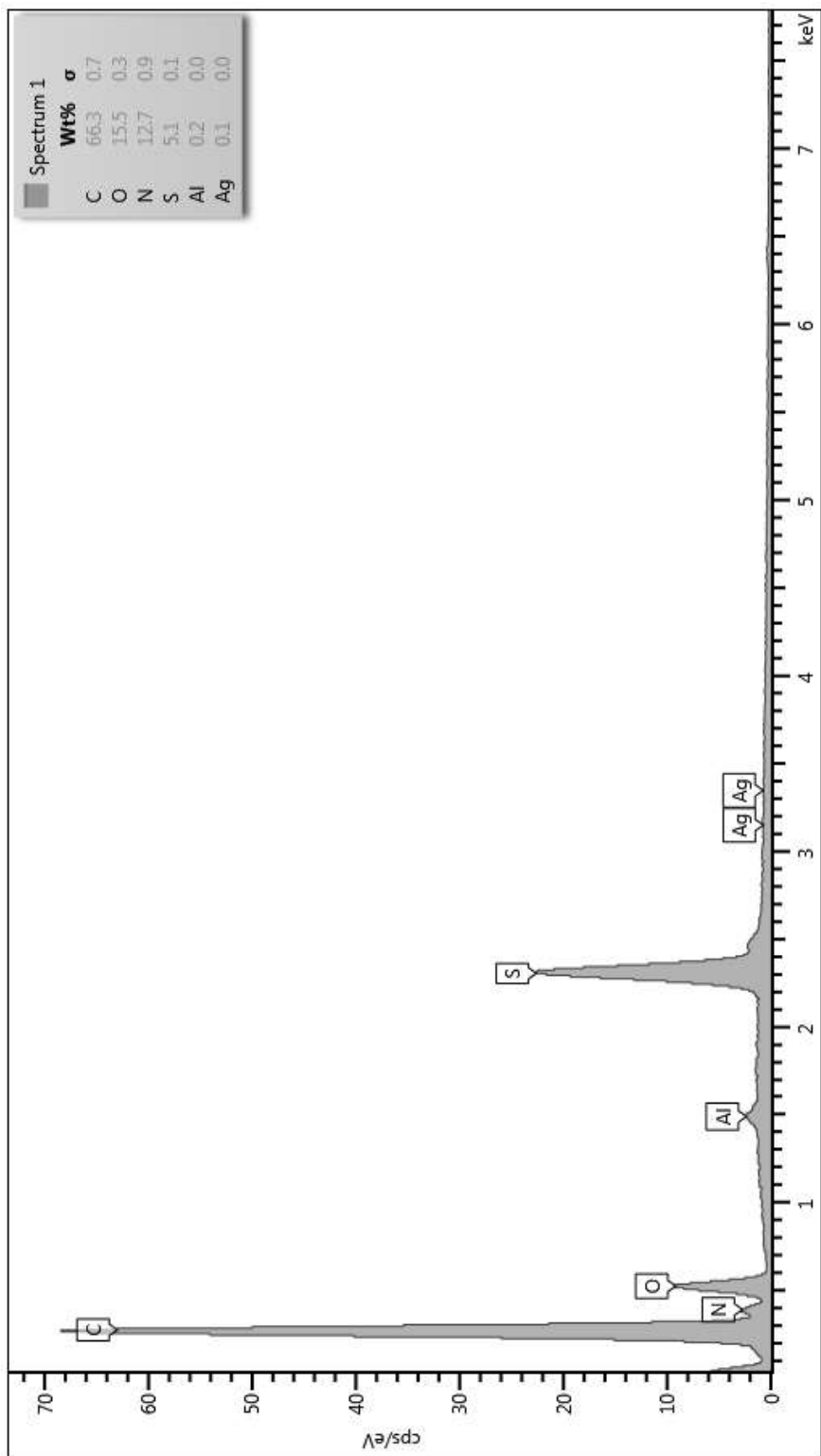


Fig. 4 EDX spectrum of silver-incorporated polyaniline film on a pencil lead core

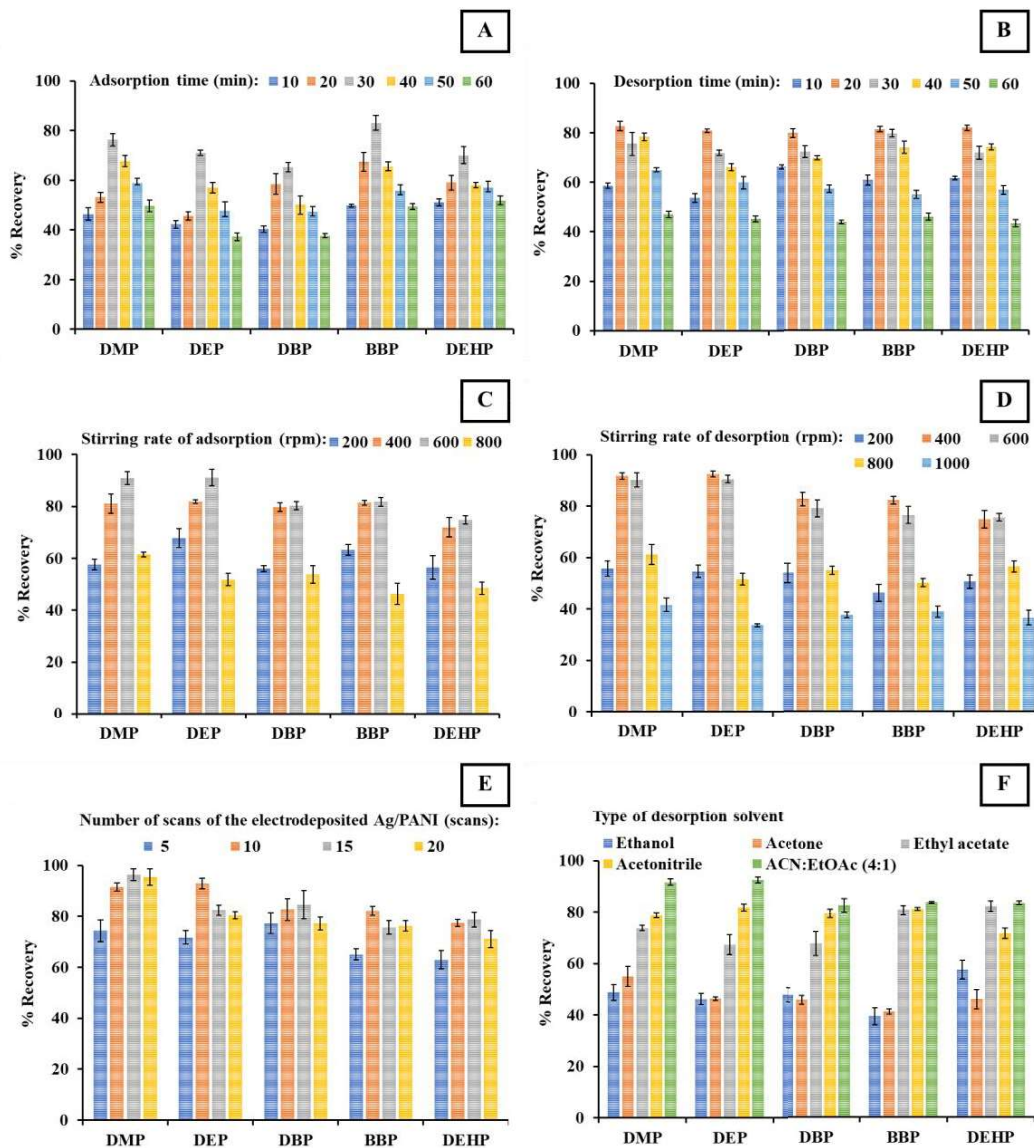


Fig. 5 The effects on the extraction recovery of 1.0 mg L^{-1} of the five phthalate esters ($n=3$) of (A) adsorption time, (B) desorption time, (C) stirring rate of adsorption, (D) the stirring rate of desorption, (E) number of scans of the electrodeposited Ag/PANI and (F) type of desorption solvent

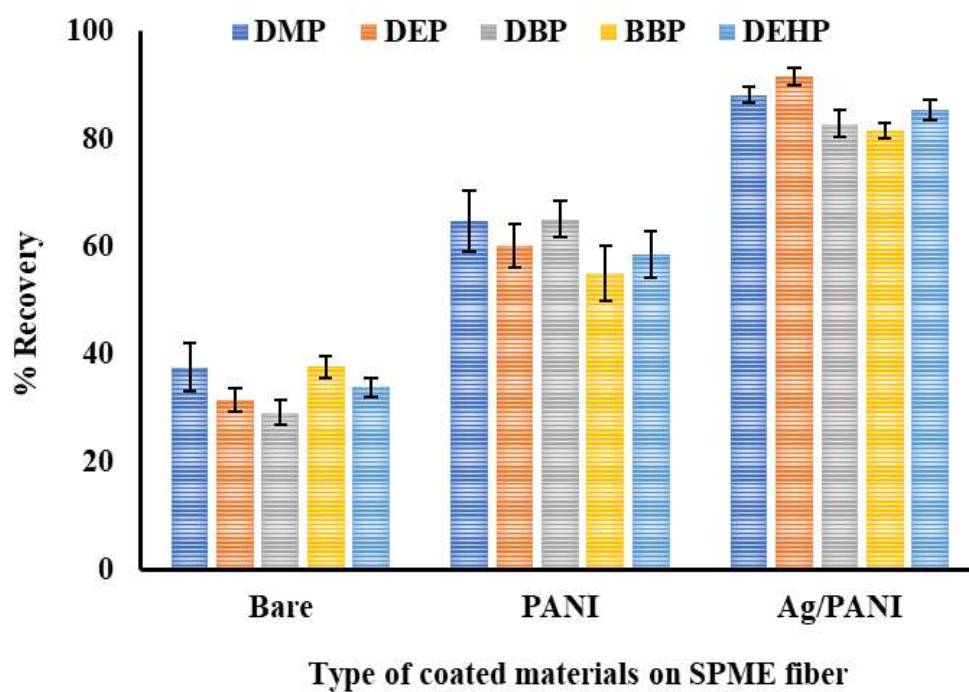


Fig. 6 Extraction recoveries of bare, PANI and Ag/PANI coated pencil leads for the extraction of a 1.0 mg L^{-1} of the five phthalate esters standard solution under optimal conditions ($n=3$)

Supplementary Data

A simple 3D printed-screw-based solid phase microextraction device with a silver decorated polyaniline pencil lead fiber for the extraction of phthalate esters in cosmeceutical products

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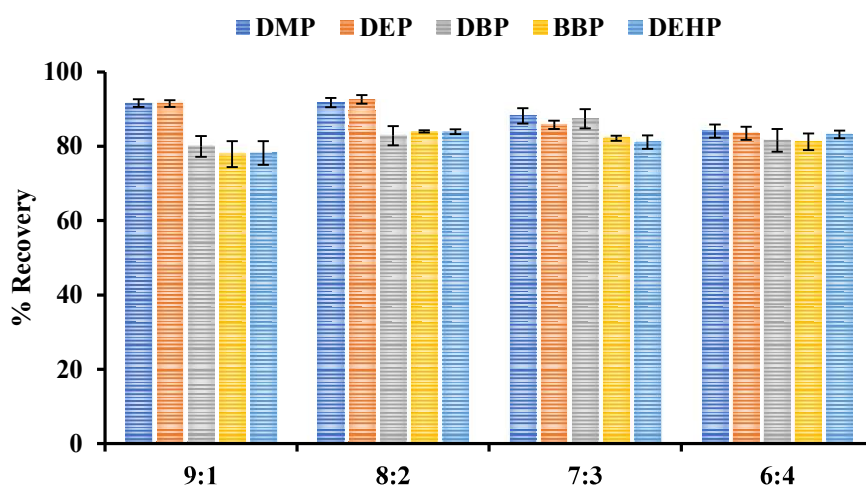


Fig. S1 Effect of acetonitrile and ethyl acetate ratio on the extraction recoveries of the phthalate esters at 1.0 mg L^{-1} ($n=3$)

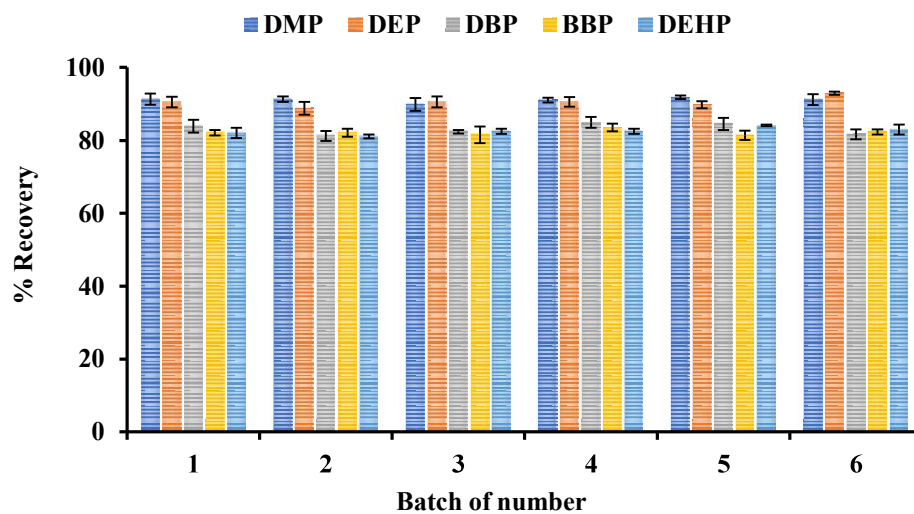


Fig. S2 Fiber-to-fiber reproducibility as indicated by the extraction recoveries of the phthalate esters at 1.0 mg L^{-1} ($n=3$)

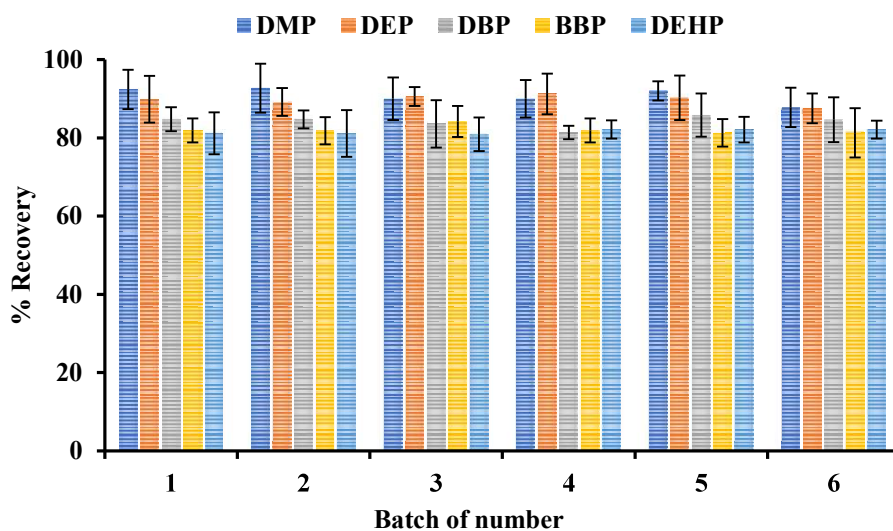


Fig. S3 Fiber-to-fiber reproducibility as indicated by the extraction recoveries of the phthalate esters at 1.0 mg L^{-1} in real samples ($n=3$).

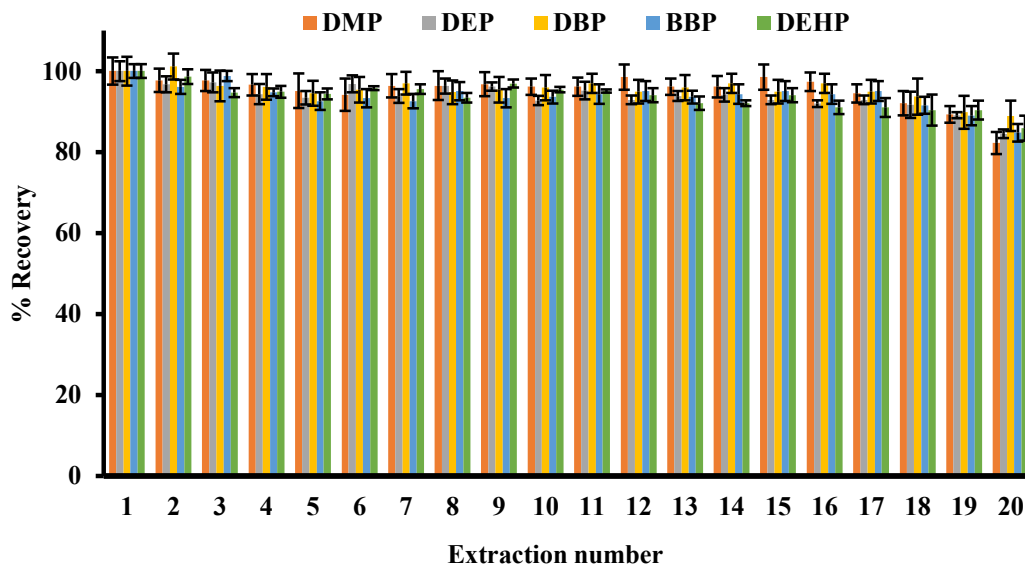


Fig. S4 Recoveries from repeated extractions of the phthalate esters at 1.0 mg L^{-1} ($n=3$)

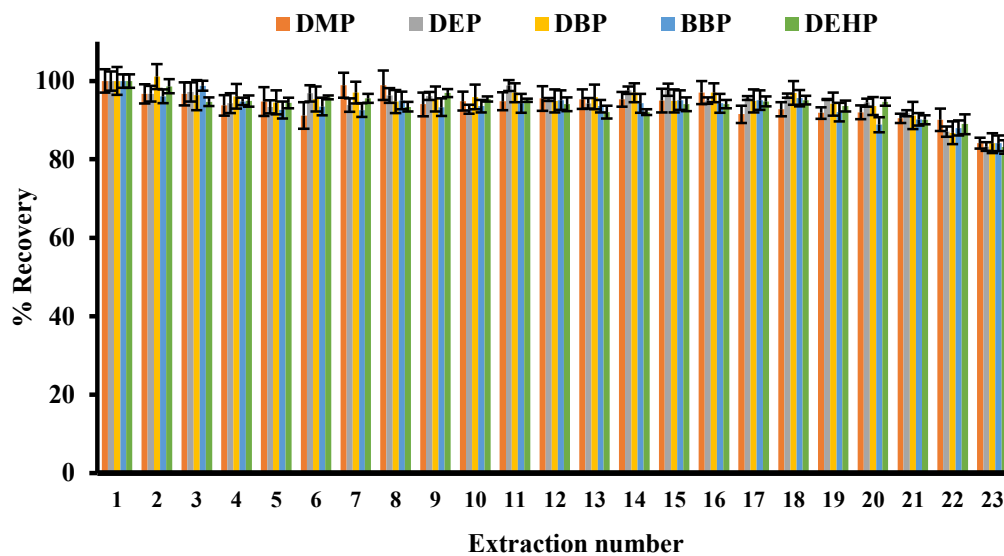


Fig. S5 Recoveries from repeated extractions of the phthalate esters at 1.0 mg L^{-1} in real samples ($n=3$)

Table S1 Concentration of phthalate esters in real samples

Samples	Concentration of phthalate esters ($\mu\text{g L}^{-1}$)				
	DMP	DEP	DBP	BBP	DEHP
Rubbing alcohol					
RA-1	ND	ND	ND	ND	ND
RA-2	ND	7.03 \pm 0.76 ^a	ND	ND	5.89 \pm 0.53 ^a
RA-3	ND	ND	ND	ND	ND
Contact lens cleaner					
CLC-1	ND	ND	ND	ND	ND
CLC-2	ND	ND	ND	ND	6.8 \pm 1.2 ^a
CLC-3	ND	ND	ND	ND	5.3 \pm 1.1 ^a
CLC-4	ND	ND	ND	ND	ND
Saline solution					
SS-1 (external use drug)	ND	ND	ND	ND	ND
SS-2 (external use drug)	ND	ND	ND	ND	ND
SS-3 (intravenous injection)	ND	ND	ND	ND	ND
SS-4 (intravenous injection)	ND	ND	ND	ND	ND
Eye cleaner					
EC-1	ND	ND	ND	ND	ND
EC-2	ND	ND	ND	ND	ND
Antibacterial disinfectant liquid					
ADL -1	ND	ND	ND	ND	ND

RA = rubbing alcohol, CLC = contact lens cleaner, SS = saline solution, EC = eye cleaner,

ADL = antibacterial disinfectant liquid

ND is non-detectable (<LOD)

^a Detectable, not determined (>LOD, <LOQ)

Table S2 Recoveries of phthalate esters at various concentrations (n=6) (I)

Samples	Spiked concentration ($\mu\text{g mL}^{-1}$)	% Recoveries (n=6)				
		DMP	DEP	DBP	BBP	DEHP
Rubbing alcohol						
RA-1	0.005	83.36±1.2	85.0±1.3	84.60±0.71	83.75±0.79	90.29±0.87
	0.050	86.5±1.9	85.9±1.5	85.76±0.83	82.7±1.1	86.11±0.59
	0.250	88.6±1.4	84.71±0.23	86.72±0.96	85.5±1.2	82.7±1.2
	0.500	91.9±1.2	87.5±1.0	92.8±1.4	84.66±0.61	86.28±0.15
	1.0	90.1±2.2	87.52±0.71	88.7±1.5	100.75±3.4	87.5±2.1
RA-2	0.005	90.09±0.99	85.4±1.2	87.28±0.87	81.92±0.99	92.3±1.1
	0.050	94.4±2.2	91.62±0.99	88.9±1.2	87.7±1.3	88.05±0.85
	0.250	86.8±3.5	85.9±2.9	86.3±1.7	85.7±1.7	82.5±3.0
	0.500	90.7±1.2	87.5±1.0	92.8±1.4	84.66±0.61	86.29±0.15
	1.0	89.6±2.4	88.11±0.44	92.1±2.0	97.0±3.2	90.4±2.1
RA-3	0.005	92.6±1.0	89.4±1.2	90.2±1.2	81.92±0.71	92.6±1.0
	0.050	99.3±1.9	96.3±1.3	90.1±1.3	91.39±0.63	92.2±1.4
	0.250	84.7±2.9	87.4±2.0	86.7±1.7	86.4±1.6	82.5±1.2
	0.500	88.9±0.9	91.34±0.91	101.0±1.0	86.61±0.30	92.2±1.2
	1.0	92.5±2.0	89.29±0.76	88.9±1.5	96.5±3.5	89.4±1.5
Contact lens cleaner						
CLC-1	0.005	91.87±0.69	91.7±1.2	94.75±0.62	81.0±1.2	84.6±1.7
	0.050	98.1±1.3	99.66±0.96	93.3±1.6	93.39±0.88	95.1±1.4
	0.250	84.4±2.0	88.3±1.2	87.2±1.4	86.0±1.5	83.1±1.6
	0.500	89.61±0.86	97.34±0.72	99.43±0.64	87.87±0.38	93.91±0.88
	1.0	92.5±1.9	88.7±1.2	89.5±2.0	93.6±1.9	88.5±2.2
CLC-2	0.005	96.36±0.62	93.7±1.7	97.0±1.0	88.3±1.1	90.4±1.7
	0.050	96.8±1.3	100.10±0.84	95.3±1.4	95.38±0.61	92.2±1.3
	0.250	84.1±2.1	87.1±1.7	86.3±3.1	85.0±2.5	84.9±1.0
	0.500	92.5±1.0	98.30±0.24	100.61±0.81	87.48±0.50	95.29±0.55
	1.0	101.2±1.4	88.1±1.9	89.5±2.6	91.7±1.7	89.9±1.1
CLC-3	0.005	94.1±1.2	92.4±1.8	101.5±1.3	95.6±1.7	96.3±1.5
	0.050	89.0±1.1	94.4±1.6	93.20±0.84	90.39±0.98	89.3±1.4
	0.250	82.22±0.97	88.1±2.1	89.1±1.6	84.3±1.3	85.83±0.61
	0.500	92.86±0.64	97.8±0.23	97.68±0.76	88.65±0.59	96.28±0.44
	1.0	92.52±0.98	89.3±1.8	88.3±2.5	91.2±1.9	92.8±1.3
CLC-4	0.005	85.13±0.90	85.9±1.3	99.2±1.1	86.5±1.0	92.4±1.5
	0.050	84.4±1.3	95.5±1.5	93.20±0.84	91.4±1.7	91.3±1.4
	0.250	87.42±2.6	88.6±2.4	89.8±1.7	86.58±0.67	90.1±0.61
	0.500	102.6±1.5	99.02±0.39	91.5±1.6	90.70±0.35	98.96±0.44
	1.0	94.0±1.0	92.9±1.2	89.46±0.52	92.2±1.9	95.3±1.3


Table S2 Recoveries of phthalate esters at various concentrations (n=6) (II)

Samples	Spiked concentration ($\mu\text{g mL}^{-1}$)	% Recoveries (n=6)				
		DMP	DEP	DBP	BBP	DEHP
Saline solution						
SS-1 (external use drug)						
	0.005	89.47±0.93	91.4±1.5	102.4±1.1	86.5±1.4	96.3±1.2
	0.050	91.3±1.0	92.1±1.8	89.0±1.6	91.4±2.0	89.3±1.3
	0.250	87.6±2.6	88.6±1.8	91.2±1.8	87.5±1.2	91.3±1.2
	0.500	100.9±1.6	100.46±0.49	92.9±1.4	89.43±0.32	98.36±0.35
	1.0	94.0±2.1	92.3±0.82	91.8±1.2	92.7±2.4	92.0±1.2
SS-2 (external use drug)						
	0.005	93.8±1.1	100.5±1.0	91.25±0.99	93.4±1.1	102.15±0.93
	0.050	91.29±0.67	96.7±1.4	94.26±0.68	93.4±2.0	91.27±0.95
	0.250	87.0±2.9	90.5±1.2	89.3±2.7	87.2±1.0	90.9±1.1
	0.500	93.3±1.2	96.02±0.39	103.4±2.0	88.36±0.25	97.4±0.31
	1.0	99.7±1.5	96.01±0.93	87.7±1.1	94.1±1.6	96.27±0.95
SS-3 (intravenous injection)						
	0.005	96.20±0.63	95.8±1.1	100.2±1.1	86.5±1.5	99.2±1.7
	0.050	93.6±1.4	92.1±0.98	94.26±0.98	93.4±1.2	91.3±1.3
	0.250	85.5±3.4	90.5±2.6	88.4±2.1	86.2±1.2	90.3±1.4
	0.500	98.5±1.1	96.38±0.63	97.6±1.1	88.75±0.33	96.38±0.65
	1.0	96.1±1.1	89.9±1.3	94.7±1.7	92.7±2.4	95.3±1.1
SS-4 (intravenous injection)						
	0.005	102.8±1.1	91.2±1.1	100.60±0.80	92.0±2.1	98.2±1.3
	0.050	95.81±0.58	96.7±1.4	94.26±0.71	95.4±1.4	94.17±0.84
	0.250	88.5±2.7	90.2±2.5	91.9±1.54	88.3±1.1	89.9±2.0
	0.500	98.3±1.4	100.90±0.31	96.39±0.80	89.04±0.47	97.27±0.52
	1.0	92.5±1.9	89.89±0.82	92.35±0.95	93.6±3.1	93.9±1.2
Eye cleaner						
EC-1						
	0.005	98.3±1.3	91.5±1.6	91.67±0.75	86.5±1.8	92.38±0.99
	0.050	89.0±1.0	93.3±2.1	86.9±1.5	89.4±2.0	92.2±1.0
	0.250	85.5±4.0	91.6±1.5	92.1±2.5	87.0±1.9	88.0±3.0
	0.500	99.7±1.5	97.1±1.0	96.39±0.40	85.83±0.77	95.29±0.35
	1.0	91.8±1.3	86.3±1.6	91.2±1.4	95.1±1.9	94.83±0.61
EC-2						
	0.005	93.80±0.53	85.0±1.2	93.91±0.73	86.5±1.8	102.2±1.0
	0.050	87.5±1.7	100.1±1.5	90.0±1.5	92.4±2.1	92.2±2.1
	0.250	90.4±2.8	91.4±3.1	93.8±1.4	88.1±1.4	90.7±1.9
	0.500	98.0±1.4	99.4±1.3	96.4±1.3	86.1±1.2	97.47±0.42
	1.0	96.12±0.95	91.1±1.0	90.0±1.0	92.7±3.8	93.4±2.3
Antibacterial disinfectant liquid						
ADL-1						
	0.005	87.06±0.57	84.98±0.97	98.4±1.2	86.5±1.8	96.3±1.3
	0.050	90.8±1.0	99.0±1.6	94.3±1.6	92.4±2.1	90.3±2.3
	0.250	88.5±2.4	92.2±3.4	93.8±2.2	88.5±1.7	90.0±1.9
	0.500	96.50±0.93	97.0±1.6	101.07±0.68	87.68±0.84	95.49±0.60
	1.0	92.5±1.5	86.9±2.5	85.4±1.6	93.14±0.80	91.0±2.9

Petty Patent

อุปกรณ์สกัดสารอินทรีย์แบบหมุน (Screw-based pencils extraction device) สำหรับการ
สกัดสารอินทรีย์ (organic compounds)

จงดี บุรณชัย, นิชาภัทร์ ชูรินทร์, มรกต แก้วเพชร, พันธุ์วดี วัฒนสิน, เพรศพิชญ์ คณาธารณา,
ปณต ถาวรังกูร.

 คำขอรับสิทธิบัตร/อนุสิทธิบัตร		สำหรับเจ้าหน้าที่	
<input type="checkbox"/> การประดิษฐ์ <input type="checkbox"/> การออกแบบผลิตภัณฑ์ <input checked="" type="checkbox"/> อนุสิทธิบัตร ข้าพเจ้าผู้ลงลายมือชื่อในคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้ ขอรับสิทธิบัตร/อนุสิทธิบัตร ตามพระราชบัญญัติสิทธิบัตร พ.ศ. 2522 แก้ไขเพิ่มเติมโดยพระราชบัญญัติสิทธิบัตร (ฉบับที่ 2) พ.ศ. 2535 และ พระราชบัญญัติสิทธิบัตร (ฉบับที่ 3) พ.ศ. 2542		วันรับคำขอ ๑๑ มี.ค. ๒๕๖๓	เลขที่คำขอ 1903000062
		วันยื่นคำขอ A ก.ค. 2561	
สัญลักษณ์จำแนกการประดิษฐ์ระหว่างประเทศ			
ใช้กับแบบผลิตภัณฑ์ ประเภทผลิตภัณฑ์			
		วันประกาศโฆษณา	เลขที่ประกาศโฆษณา
		วันออกสิทธิบัตร/อนุสิทธิบัตร	เลขที่สิทธิบัตร/อนุสิทธิบัตร
ลายมือชื่อเจ้าหน้าที่			
1. ชื่อที่แสดงถึงการประดิษฐ์/การออกแบบผลิตภัณฑ์ อุปกรณ์สำหรับการสเก็ตสารถือ			
2. คำขอรับสิทธิบัตรการออกแบบผลิตภัณฑ์นี้เป็นคำขอสำหรับแบบผลิตภัณฑ์อย่างใดอย่างหนึ่งและเป็นคำขอลำดับที่ ในจำนวน <input type="text"/> คำขอ ที่ยื่นในคราวเดียวกัน			
3. ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร <input type="checkbox"/> บุคคลธรรมดา <input type="checkbox"/> นิติบุคคล <input checked="" type="checkbox"/> หน่วยงานรัฐ <input type="checkbox"/> มูลนิธิ <input type="checkbox"/> อื่นๆ ชื่อ มหาวิทยาลัยสงขลานครินทร์ ที่อยู่ 15 ถนนกาญจนวนิชย์ ตำบล/แขวง คอหงส์ อำเภอ/เขต หาดใหญ่ จังหวัด สงขลา รหัสไปรษณีย์ 90110 ประเทศ ไทย อีเมล sawarod49@gmail.com			3.1 สัญชาติ ไทย 3.2 โทรศัพท์ 0-7428-9337 3.3 โทรสาร 0-7428-9339
<input type="checkbox"/> เลขประจำตัวประชาชน <input type="checkbox"/> เลขทะเบียนนิติบุคคล <input checked="" type="checkbox"/> เลขประจำตัวผู้เสียภาษีอากร <input type="text"/> 0 9 9 4 0 0 0 5 8 0 8 6 0 <input type="checkbox"/> เพิ่มเติม (ตั้งแบบ) ในกรณีที่กรมา สื่อสารกับท่าน ท่านสะดวกใช้ทาง <input type="checkbox"/> อีเมลผู้ขอ <input checked="" type="checkbox"/> อีเมลตัวแทน			
4. สิทธิในการขอรับสิทธิบัตร/อนุสิทธิบัตร <input type="checkbox"/> ผู้ประดิษฐ์/ผู้ออกแบบ <input checked="" type="checkbox"/> ผู้รับโอน <input type="checkbox"/> ผู้ขอรับสิทธิโดยเหตุอื่น			
5. ตัวแทน (ถ้ามี) ชื่อ นางสาวเพชรดา สัตยากุล ที่อยู่ ศูนย์ทรัพยากรพันธุศาสตร์ อุทยานวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ 15 ถนนกาญจนวนิชย์ ตำบล/แขวง คอหงส์ อำเภอ/เขต หาดใหญ่ จังหวัด สงขลา รหัสไปรษณีย์ 90110 ประเทศ ไทย อีเมล phetrada.sk@gmail.com เลขประจำตัวประชาชน <input type="text"/> 1 9 2 0 6 0 0 1 0 4 8 1 8 <input checked="" type="checkbox"/> เพิ่มเติม (ตั้งแบบ)			5.1 ตัวแทนเลขที่ 2440 5.2 โทรศัพท์ 0-7428-9337 5.3 โทรสาร 0-7428-9339
6. ผู้ประดิษฐ์/ผู้ออกแบบผลิตภัณฑ์ <input type="checkbox"/> ชื่อและที่อยู่เดียวกับผู้ขอ ชื่อ นางจงดี ปุณชัย ที่อยู่ คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ตำบล/แขวง คอหงส์ อำเภอ/เขต หาดใหญ่ จังหวัด สงขลา รหัสไปรษณีย์ 90110 ประเทศ ไทย อีเมล chongdee.t@psu.ac.th เลขประจำตัวประชาชน <input type="text"/> 3 9 4 0 4 0 0 0 0 3 1 6 0 <input checked="" type="checkbox"/> เพิ่มเติม (ตั้งแบบ)			
7. คำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้แยกจากหรือเกี่ยวข้องกับคำขอเดิม ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร ขอให้ถือว่าได้ยื่นคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้ ในวันเดียวกับคำขอรับสิทธิบัตร เลขที่ <input type="text"/> วันยื่น <input type="text"/> เพราะคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้แยกจากหรือเกี่ยวข้องกับคำขอเดิมเพราะ <input type="checkbox"/> คำขอเดิมมีการประดิษฐ์หลายอย่าง <input type="checkbox"/> ถูกคัดค้านเนื่องจากผู้ขอไม่มีสิทธิ <input type="checkbox"/> ขอเปลี่ยนแปลงประเภทของสิทธิ			
หมายเหตุ ในกรณีที่ไม่มีอาชญากรรมและยึดได้ครบถ้วน ให้จัดทำเป็นเอกสารแนบท้ายแบบพิมพ์นี้โดยระบุหมายเลขกำกับข้อและหัวข้อที่แสดงรายละเอียดเพิ่มเติมดังกล่าวด้วย สำหรับเจ้าหน้าที่			
จำนวนประเภทสิทธิบัตร/อนุสิทธิบัตร <input type="checkbox"/> กลุ่มวิศวกรรม <input type="checkbox"/> กลุ่มเคมี <input type="checkbox"/> สิทธิบัตรการออกแบบ <input type="checkbox"/> อนุสิทธิบัตร สิทธิบัตรการประดิษฐ์ (วิศวกรรม) <input type="checkbox"/> สิทธิบัตรการประดิษฐ์ (เคมีเทคนิค) <input type="checkbox"/> สิทธิบัตรการออกแบบ (ออกแบบผลิตภัณฑ์ 1) <input type="checkbox"/> อนุสิทธิบัตร (วิศวกรรม) สิทธิบัตรการประดิษฐ์ (ไฟฟ้า) <input type="checkbox"/> สิทธิบัตรการประดิษฐ์ (ปิโตรเคมี) <input type="checkbox"/> สิทธิบัตรการออกแบบ (ออกแบบผลิตภัณฑ์ 2) <input type="checkbox"/> อนุสิทธิบัตร (เคมี) สิทธิบัตรการประดิษฐ์ (ฟิสิกส์) <input type="checkbox"/> สิทธิบัตรการประดิษฐ์ (เทคโนโลยีชีวภาพ) <input type="checkbox"/> สิทธิบัตรการออกแบบ (ออกแบบผลิตภัณฑ์ 3)			

8. การยื่นคำขออนุญาตราชอาณาจักร <input type="checkbox"/> PCT <input type="checkbox"/> เพิ่มเติม (ดังแนบ)				
วันยื่นคำขอ	เลขที่คำขอ	ประเทศ	สัญลักษณ์จำแนกการประดิษฐ์ระหว่างประเทศ	สถานะคำขอ
8.1				
8.2				
8.3				
8.4 <input type="checkbox"/> ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตรขอสิทธิให้ถือว่าได้ยื่นคำขอในวันที่ได้ยื่นคำขอรับสิทธิบัตร/อนุสิทธิบัตรในต่างประเทศเป็นครั้งแรกโดย <input type="checkbox"/> ได้ยื่นเอกสารหลักฐานพร้อมคำขอนี้ <input type="checkbox"/> ขอยื่นเอกสารหลักฐานหลังจากวันยื่นคำขอนี้				
9. การแสดงการประดิษฐ์หรือการออกแบบผลิตภัณฑ์ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตรได้แสดงการประดิษฐ์ที่หน่วยงานของรัฐเป็นผู้จัด วันแสดง 4 - 7 กรกฎาคม 2561 วันเปิดงานแสดง 4 กรกฎาคม 2561 ผู้จัด ศูนย์ความเป็นเลิศด้านวิศวกรรมทางเคมี				
10. การประดิษฐ์เกี่ยวกับจุลชีพ				
10.1 เลขทะเบียนฝากเก็บ		10.2 วันที่ฝากเก็บ		10.3 สถาบันฝากเก็บ/ประเทศ
11. ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร ขอยื่นเอกสารภาษาต่างประเทศก่อนในวันยื่นคำขอนี้ และจะจัดยื่นคำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้ที่จัดทำเป็นภาษาไทยภายใน 90 วัน นับจากวันยื่นคำขอนี้ โดยขอยื่นเป็นภาษา <input type="checkbox"/> อังกฤษ <input type="checkbox"/> ฝรั่งเศส <input type="checkbox"/> เยอรมัน <input type="checkbox"/> ญี่ปุ่น <input type="checkbox"/> อื่นๆ				
12. ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร ขอให้อธิบายรายละเอียดคำขอรับสิทธิบัตร หรือรับจดทะเบียน และประกาศโฆษณาอนุสิทธิบัตรนี้ หลังจากวันที่ <input type="checkbox"/> ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตรขอให้ใช้รูปเขียนหมายเลข ในการประกาศโฆษณา				
13. คำขอรับสิทธิบัตร/อนุสิทธิบัตรนี้ประกอบด้วย				
ก. แบบพิมพ์คำขอ	3	หน้า		
ข. รายละเอียดการประดิษฐ์ หรือคำพรรณนาแบบผลิตภัณฑ์	4	หน้า		
ค. ข้อถ้อยสิทธิ	1	หน้า		
ง. รูปเขียน	1	รูป	1	หน้า
จ. ภาพแสดงแบบผลิตภัณฑ์		รูป		หน้า
<input type="checkbox"/> รูปเขียน		รูป		หน้า
<input type="checkbox"/> ภาพถ่าย		รูป		หน้า
ฉ. บทสรุปการประดิษฐ์	1	หน้า		
14. เอกสารประกอบคำขอ				
<input type="checkbox"/> เอกสารแสดงสิทธิในการขอรับสิทธิบัตร/อนุสิทธิบัตร				
<input checked="" type="checkbox"/> หนังสือรับรองการแสดงการประดิษฐ์/การออกแบบผลิตภัณฑ์				
<input type="checkbox"/> หนังสือมอบอำนาจ				
<input type="checkbox"/> เอกสารรายละเอียดเกี่ยวกับจุลชีพ				
<input type="checkbox"/> เอกสารการขอนับวันยื่นคำขอในต่างประเทศเป็นวันยื่นคำขอในประเทศไทย				
<input type="checkbox"/> เอกสารขอเปลี่ยนแปลงประเภทของสิทธิ				
<input checked="" type="checkbox"/> เอกสารอื่นๆ				
15. ข้าพเจ้าขอรับรองว่า				
<input checked="" type="checkbox"/> การประดิษฐ์นี้ไม่เคยยื่นขอรับสิทธิบัตร/อนุสิทธิบัตรมาก่อน				
<input type="checkbox"/> การประดิษฐ์นี้ได้พัฒนาปรับปรุงมาจาก				
16.ลายมือชื่อ				
<input type="checkbox"/> ผู้ขอรับสิทธิบัตร/อนุสิทธิบัตร <input checked="" type="checkbox"/> ตัวแทน				
(<u>นางสาวเพชรดา สัตยากุล</u>)				

หมายเหตุ บุคคลใดยื่นขอรับสิทธิบัตรการประดิษฐ์หรือการออกแบบผลิตภัณฑ์ หรืออนุสิทธิบัตร โดยการแสดงข้อความอันเป็นเท็จแก่พนักงานเจ้าหน้าที่ เพื่อให้ได้ไปซึ่งสิทธิบัตรหรืออนุสิทธิบัตร ต้องระวางโทษ
จำคุกไม่เกินหกเดือน หรือปรับไม่เกินห้าพันบาท หรือทั้งจำทั้งปรับ

ใบต่อแนบท้าย _____

5. ตัวแทน

ชื่อ นายจิตติยุทธ เขี่ยมยกกุล หมายเลขตัวแทน 2266

ที่อยู่ อุทยานวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ตำบลคอหงส์ อำเภอหาดใหญ่ จังหวัดสงขลา 90110 ประเทศไทย

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เลขประจำตัวประชาชน 3 9599 00384 18 7

6. ผู้ประติษฐ์/ผู้ออกแบบผลิตภัณฑ์

2. นางสาวณิชาภัทร์ ชูรินทร์ หมายเลขบัตรประชาชน 1 7099 00826 50 9

ที่อยู่ คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ถนนกาญจนวนิชย์ อำเภอคอหงส์

จังหวัดสงขลา 90110

3. นางสาวปอเตธ ถาวรังกูร หมายเลขบัตรประชาชน 3 1014 01033 66 4

ที่อยู่ คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ถนนกาญจนวนิชย์ อำเภอคอหงส์

จังหวัดสงขลา 90110

4. นางสาวเพริศพิชญ์ คุณาธารณา หมายเลขบัตรประชาชน 3 1002 00250 37 3

ที่อยู่ คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ถนนกาญจนวนิชย์ อำเภอคอหงส์

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5. นางสาวมรกต แก้วเพชร หมายเลขบัตรประชาชน 3 8498 00005 58 4

ที่อยู่ คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ถนนกาญจนวนิชย์ อำเภอคอหงส์

จังหวัดสงขลา 90110

6. นางสาวพันธุ์วดี วัฒนสิน หมายเลขบัตรประชาชน 1 9299 00101 00 6

ที่อยู่ คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ ถนนกาญจนวนิชย์ อำเภอคอหงส์

จังหวัดสงขลา 90110

VITAE

Name Miss Nichapat Chunin

Student ID 5910220032

Educational Attainment

Degree	Name of Institution	Year of Graduation
Bachelor of Science (Chemistry)	Prince of Songkla University	2016

Scholarship Awards during Enrollment

Prince of Songkla University granting number of SCI610440S, Scholarship for Outstanding GPA, The Center of Excellence for Innovation in Chemistry (PERCH-CIC), The Ministry of Higher Education, Science, Research and Innovation (MHESI), The Center of Excellence for Trace Analysis and Biosensor (TAB-CoE). Scholarship for Support Exchange Students and International Credit Transferred Through ASEAN Community.

List of Oral and Poster Presentations and Publication

Oral presentation

Chunin, N., Kanatharana, P., Kaewpet, M., Wattanasin, P., Thavarangkul, P. and Thammakhet-Buranachai, C. “A silver incorporated polyaniline SPME fiber for trace analysis of phthalate esters in cosmeceuticals products”. The 55th Japanese Association for Flow Injection Analysis (JAFIA), November 16, 2018, Shibaura Institute of Technology (SIT), Toyosu campus, Tokyo, Japan.

Poster presentation

Chunin, N., Kanatharana, P., Teshima, N., Murakami, H., Kaewpet, M., Wattanasin, P., Thavarangkul, P. and Thammakhet-Buranachai, C. “A silver incorporated polyaniline SPME fiber for trace analysis of phthalate esters in cosmeceuticals products”. The 55th Japanese Association for Flow Injection

Analysis (JAFIA), November 16, 2018, Shibaura Institute of Technology (SIT), Toyosu campus, Tokyo, Japan.

Chunin, N., Kanatharana, P., Kaewpet, M., Wattanasin, P., Thavarangkul, P. and Thammakhet-Buranachai, C. "A polyaniline/pencil lead solid phase microextractor for trace phthalate esters". PERCH-CIC Congress X; 2018 International Congress for Innovation in Chemistry "Contributing Expertise for Thailand 4.0", July 4-7, 2018, Jomtien Palm Beach Hotel & Resort, Pattaya, Chonburi, Thailand.

Proceeding

Chunin, N., Kaewpet, M., Wattanasin, P., Kanatharana, P., Thavarangkul, P.; and Thammakhet-Buranachai, C. "A polyaniline/pencil lead solid phase microextractor for trace phthalate esters" (p 146-150). The Pure and Applied Chemistry International Conference 2018 (PACCON 2018), February 7-9, 2018, International Convention Center (ICC Hat Yai), Hat Yai, Songkhla, Thailand.