



**Development of Micro-Solid Phase Extraction Sorbent
for Trace Organic Compounds Analysis**

Titiwan Changsan

**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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Organic Compounds Analysis

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I hereby certify that this work has not already been accepted in substance for any degree, and is not being concurrently in candidature for any degree.

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| ชื่อวิทยานิพนธ์ | การพัฒนาตัวดูดซับขนาดเล็กสำหรับการวิเคราะห์สารประกอบอินทรีย์ปริมาณน้อย |
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บทคัดย่อ

วิทยานิพนธ์นี้เป็นการพัฒนาตัวดูดซับขนาดเล็กสำหรับการวิเคราะห์สารอินทรีย์ปริมาณน้อย โดยแบ่งงานวิจัยออกเป็น 2 ส่วน งานวิจัยส่วนแรกเป็นการพัฒนาตัวดูดซับเม็ดกลมโคโตซาน-พอลิอะนิลีนสำหรับการสกัดสารกันเสียกลุ่มพาราเบน 4 ชนิด ได้แก่ เมทิลพาราเบน เอทิลพาราเบน โพรพิลพาราเบน และบิวทิลพาราเบน ก่อนการวิเคราะห์ด้วยเทคนิคของเหลวโครมาโทกราฟีสมรรถนะสูงร่วมกับตัวตรวจวัดอัลตราไวโอเล็ต จากนั้นได้ศึกษาสภาวะที่เหมาะสมของการสกัดบางปัจจัย เช่น จำนวนตัวดูดซับเม็ดกลม ระยะเวลาในการสกัดและการคายการดูดซับ และชนิดของตัวทำละลายอินทรีย์ที่ใช้ในการคายการดูดซับ เมื่อใช้ตัวดูดซับเม็ดกลมโคโตซาน-พอลิอะนิลีนสกัดสารละลายมาตรฐานผสมของพาราเบน 4 ชนิดที่ความเข้มข้น 0.50 มิลลิกรัมต่อลิตร พบว่าให้ร้อยละการได้กลับคืนของการสกัดในช่วง 68.0 ± 7.7 ถึง 98.8 ± 3.8 ผลการทดลองนี้แสดงให้เห็นว่ามีแนวโน้มที่จะนำตัวดูดซับเม็ดกลมโคโตซาน-พอลิอะนิลีนที่พัฒนาขึ้นมาประยุกต์ใช้ในการสกัดพาราเบนในตัวอย่างจริงได้ งานวิจัยส่วนที่สองเป็นการพัฒนาตัวดูดซับแม่เหล็กพอลิไพโรลสำหรับการสกัดยาฆ่าแมลงกลุ่มคาร์บาเมต 3 ชนิด คือ คาร์บาริล คาร์โบฟูราน และเมธิโธมิล โดยใช้ในการคนด้วยเครื่องคนแม่เหล็กไฟฟ้า หลังจากนั้นจึงวิเคราะห์ด้วยเทคนิคของเหลวโครมาโทกราฟีสมรรถนะสูงร่วมกับตัวตรวจวัดชนิดไดโอดอะเรย์ ภายใต้สภาวะที่เหมาะสม พบว่าวิธีที่พัฒนาขึ้นให้ช่วงความเป็นเส้นตรงที่ดี (0.50 ถึง 1.2×10^3 , 1.0 ถึง 7.5×10^2 และ 2.5 ถึง 5.0×10^2 ไมโครกรัมต่อลิตร) และให้ขีดจำกัดการตรวจวัดที่ต่ำ (0.228 ± 0.017 , 0.78 ± 0.20 และ 2.10 ± 0.12 ไมโครกรัมต่อลิตร) สำหรับการตรวจวัดคาร์บาริล คาร์โบฟูราน และสำหรับเมธิโธมิล ตามลำดับ วิธีที่พัฒนาขึ้นให้ค่าความแม่นยำและ

ความเที่ยงที่ดี (ร้อยละการได้กลับคืนอยู่ในช่วง 81.6 ± 1.5 ถึง 108.5 ± 1.1 และค่าเบี่ยงเบนมาตรฐานสัมพัทธ์อยู่ในช่วง 0.11-4.5 เปอร์เซ็นต์) นอกจากนี้ยังพบว่าวิธีการสังเคราะห์ตัวดูดซับที่พัฒนาขึ้นให้ความสามารถในการทำซ้ำที่ดี (มีค่าเบี่ยงเบนมาตรฐานสัมพัทธ์ในช่วง 0.38-4.4 เปอร์เซ็นต์ จากการทดลองจำนวน 6 ครั้ง) และใช้ตัวดูดซับซ้ำได้ถึง 24 ครั้ง เมื่อนำวิธีที่พัฒนาขึ้นไปวิเคราะห์ยาฆ่าแมลงทั้ง 3 ชนิด พบการปนเปื้อนของคาร์บาริลในตัวอย่างแอปเปิลที่ความเข้มข้น 0.458 ± 0.038 ไมโครกรัมต่อลิตร คาร์โบฟูรานในตัวอย่างมะเขือเทศที่ความเข้มข้น 1.89 ± 0.10 ไมโครกรัมต่อลิตร และเมโทมิลในตัวอย่างแตงโมที่ความเข้มข้น 5.79 ± 0.28 ไมโครกรัมต่อลิตร

Thesis title Development of Micro-Solid Phase Extraction Sorbent for Trace Organic Compounds Analysis
Author Ms. Titiwan Changsan
Major Program Chemistry
Academic Year 2018

Abstract

This thesis aimed to develop the micro-solid phase extraction for trace organic compounds analysis. Two sub-projects were carried out. The first sub-project is the development of chitosan-polyaniline microbeads for the extraction of four parabens including methylparaben, ethylparaben, propylparaben and butylparaben before analysis by high performance liquid chromatography-ultraviolet detection (HPLC-UV). Some affecting parameters were optimized such as the number of microbeads, adsorption and desorption times and type of desorption solvent. The sorbent was used to extract four parabens at the concentration of 0.50 mg L^{-1} , the extraction recoveries in the range of 68.0 ± 7.7 - $98.8 \pm 3.8\%$ were obtained. These results indicated that the developed chitosan-polyaniline microbeads have a potential to be applied for the extraction of parabens in real samples. In the second sub-project, the polypyrrole magnetic microsphericals were synthesized and used as a sorbent to extract carbamate pesticides (carbaryl, carbofuran and methomyl) before the extractant was analyzed by high performance liquid chromatography-diode array detector (HPLC-DAD). Under the optimum conditions, the developed method provided wide linear ranges (0.50 to $1.2 \times 10^3 \text{ } \mu\text{g L}^{-1}$, 1.0 to $7.5 \times 10^2 \text{ } \mu\text{g L}^{-1}$ and 2.5 to $5.0 \times 10^2 \text{ } \mu\text{g L}^{-1}$) with low limits of detection ($0.228 \pm 0.017 \text{ } \mu\text{g L}^{-1}$, $0.78 \pm 0.20 \text{ } \mu\text{g L}^{-1}$ and $2.10 \pm 0.12 \text{ } \mu\text{g L}^{-1}$) for carbaryl, carbofuran and methomyl, respectively. Good accuracy (recoveries in the range of $81.6 \pm 1.5\%$ to $108.5 \pm 1.1\%$) and good precision (RSDs in the range of 0.11 - 4.5%) were also presented. In addition, this developed sorbent provided good reproducibility (RSDs in the range of 0.38 - 4.4% for 6 batches) and good reusability (24 extraction cycles). Using the developed method to measure three carbamate pesticides in fruit and vegetable samples, carbaryl was detected in apple at the concentration of $0.458 \pm 0.038 \text{ } \mu\text{g L}^{-1}$, carbofuran was found in tomato at the concentration of 1.89 ± 0.10

$\mu\text{g L}^{-1}$ and methomyl was found in watermelon at the concentration of 5.79 ± 0.28
 $\mu\text{g L}^{-1}$.

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Titiwan Changsan

The Relevance of the Research Work to Thailand

The purpose of this Master of Science Thesis in Chemistry (Analytical Chemistry) is to develop a novel simple micro-solid phase extraction sorbent for the extraction of trace organic compounds and analyzed by high performance liquid chromatography. This developed sorbent provided high extraction efficiency and it can be used for determination of parabens and carbamate pesticides in real samples *e.g.*, environmental, food and cosmetic samples. This method can also be applied for the determination other trace organic compounds in real samples by several governmental organizations and private sectors in Thailand.

Contents

| | Page |
|--|-------------|
| List of Figures | xiii |
| List of Tables | xiv |
| List of Abbreviation | xv |
| List of Publication | xvii |
| 1. Introduction | 1 |
| 1.1 Background and Rationale | 1 |
| 1.2 Objectives | 2 |
| 2. Analytical methods for parabens and carbamate pesticides determination | 3 |
| 2.1 Gas chromatography (GC) | 3 |
| 2.2 High performance liquid chromatography (HPLC) | 3 |
| 3. Sample preparation with micro-solid phase extraction (μ -SPE) techniques | 4 |
| 3.1 Dispersive micro-solid phase extraction (D- μ -SPE) | 5 |
| 3.2 Pipette-tip micro-solid phase extraction (PT- μ -SPE) | 6 |
| 3.3 Membrane supported micro-solid phase extraction (membrane- μ -SPE) | 6 |
| 3.4 Magnetic-micro-solid phase extraction (M- μ -SPE) | 8 |
| 4. Coating nanomaterial-polymer for micrometer sorbent | 9 |
| 4.1 Graphene and graphene oxide (GO) | 9 |
| 4.2 Multi-walled carbon nanotubes (MWCNTs) | 10 |
| 4.3 Polyaniline (PANI) | 10 |
| 4.4 Polypyrrole (PPy) | 11 |
| 5. Analytical performance of method validation | 11 |
| 5.1 Linearity | 11 |
| 5.2 Limit of detection (LOD) and limit of quantification (LOQ) | 12 |
| 5.3 Reproducibility | 13 |
| 5.4 Reusability | 14 |
| 5.5 Precision | 15 |
| 5.6 Accuracy | 15 |
| 5.7 Effect of sample matrix | 16 |
| 5.8 Analysis of real samples | 16 |

Contents

| | Page |
|-----------------------|-------------|
| 6. Concluding remarks | 17 |
| 7. References | 18 |
| Appendices | 26 |
| Paper I | 27 |
| Paper II | 33 |
| Vitae | 69 |

List of Figures

| Figure | | Page |
|---------------|--|-------------|
| 1 | A schematic of GC system | 3 |
| 2 | A schematic of HPLC system | 4 |
| 3 | A schematic of the D- μ -SPE procedure for the adsorption and the desorption of analytes | 5 |
| 4 | A schematic of the PT- μ -SPE | 6 |
| 5 | A schematic of the membrane- μ -SPE preparation | 7 |
| 6 | A schematic of the membrane- μ -SPE for the extraction of analytes | 8 |
| 7 | A schematic of the M- μ -SPE procedure for the adsorption and the desorption of analytes | 9 |

List of Tables

| Table | | Pages |
|--------------|--|--------------|
| 1 | | 12 |
| 2 | | 13 |

List of Abbreviations

| | |
|----------------------|---|
| ACFS | Agricultural Commodity and Food Standards |
| AOAC | Association of Analytical Communities |
| BP | Butylparaben |
| CNT | Carbon nanotube |
| DAD | Diode array detector |
| D- μ -SPE | Dispersive micro-solid phase extraction |
| ECD | Electron capture detector |
| EP | Ethylparaben |
| FID | Flame ionization detector |
| FLD | Fluorescence detector |
| GC | Gas chromatography |
| GO | Graphene oxide |
| HPLC | High performance liquid chromatography |
| LOD | Limit of detection |
| LOQ | Limit of quantification |
| Membrane- μ -SPE | membrane supported micro-solid phase extraction |
| MRL | Maximum residue limit |
| MS | Mass spectrometer |
| μ -SPE | Micro-solid phase extraction |
| M- μ -SPE | Magnetic-micro-solid phase extraction |
| MP | Methylparaben |
| MWCNTs | Multiwalled carbon nanotubes |
| PANI | Polyaniline |
| PP | Propylparaben |
| PPy | Polypyrrole |
| PT- μ -SPE | Pipette tip micro-solid phase extraction |
| RSD | Relative standard deviation |
| SB- μ -SPE | Stir-bar supported micro-solid phase extraction |
| SBSE | Stir-bar sorptive extraction |
| SPE | Solid phase extraction |

List of Abbreviations

| | |
|--------|---------------------------------|
| SPME | Solid phase micro extraction |
| SVOCs | Semi-volatile organic compounds |
| UV | Ultraviolet |
| UV-Vis | Ultraviolet-Visible |
| VOCs | Volatile organic compounds |

List of Publications

This thesis contains two parts. The first part includes an introduction, some background information, experiments, results and conclusions obtained from this M.Sc. study and the second part (Appendices) consists of the following papers from the basis of this master thesis, which are referred to in the text by their roman number.

Paper I **Changsan, T.**, Kanatharana, P., Thavarungkul P., and Thammakhet-Buranachai, C. (2018). “A polyaniline-chitosan bead sorbent for parabens extraction” (p 161-165). In the Pure and Applied Chemistry International Conference 2018 (PACCON 2018). The 60th International Convention Center (ICC Hat yai), Hat Yai, Songkhla, Thailand.

Proceeding

Paper II **Changsan, T.**, Wannapob, R., Kaewpet M., Wattanasin P., Mak, W. C., Kanatharana, P., Thavarungkul P. and Thammakhet-Buranachai, C., “A novel polypyrrole microspherical magnetic sorbent for the extraction of trace carbamate pesticides in fruits and vegetables”

Manuscript submitted to Food Chemistry

1. Introduction

1.1 Background and Rationale

Sample preparation procedures are generally required for the determination of trace organic compounds to preconcentrate the concentration of target analyte, to reduce and clean-up the sample matrix and to change the analyte into a suitable form for the selected analytical instruments (Chen et al., 2018B; da Silva et al., 2018; Filippou et al., 2017). The commonly used sample preparation techniques are solid phase extraction (SPE), solid phase microextraction (SPME) and stir-bar sorptive extraction (SBSE). However, they have some drawbacks, for example, SPE is a time-consuming extraction technique and requires large amount of sample and toxic organic solvent (Sánchez-González et al., 2015) whereas SPME is limited by the high price and the fragile fiber with long sampling/adsorption times (Li et al., 2014). SBSE is prone to have a problem from the loss of the sorbent during the extraction due to the direct contact between the coating phase of the stir-bar and the bottom of the sample container sometimes induce an un-repeatable extraction (Ghani, 2019; Scherer et al., 2018).

The interesting method is a micro-solid phase extraction (μ -SPE). It is a relatively simple, fast sampling technique that requires minimal amounts of solvent, sample, and sorbent (Lashgari et al. 2015; Kanimozhi et al., 2011). There are several μ -SPE techniques that have been currently developed such as dispersive micro-solid phase extraction (D- μ -SPE) (Khezeli & Daneshfar, 2017), pipette tip micro-solid phase extraction (PT- μ -SPE) (Seidi et al., 2019), membrane supported micro-solid phase extraction (membrane- μ -SPE) (Tanimu et al., 2019) and magnetic-micro-solid phase extraction (M- μ -SPE) (Chen et al., 2018A).

One of the most remarkable method is a magnetic solid-phase extraction (M- μ -SPE) because the extraction can be performed very easily by adding the magnetic sorbent into a sample solution, the sorbent can be rapidly isolated using an external magnet. (Chen et al., 2018A; Lian et al., 2018). In addition, the M- μ -SPE also uses less volume of sample or standard solution and organic solvent and less amount of sorbent resulting in the decreasing of potential harmfulness to user and the decreasing of waste generation to the environment (Nasir et al., 2019).

To further improve the extraction efficiency, nanomaterials and polymers were introduced. Nanomaterials such as graphene (Ghodrati & Ghomashchi, 2019) and

multi-walled carbon nanotubes (MWCNTs) (Joseph et al., 2019; Kumar et al., 2019; Okil et al., 2019) have large surface area and stronger π - π interactions with organic compounds while polymers have large surface area, high porosity and can interact with organic compounds via hydrophobic, hydrogen bonding, dipole-dipole forces and π - π interactions such as polyaniline (Wójciak-Kosior et al., 2019; Sowa et al., 2015) and polypyrrole (Devasurendra et al., 2018; Li et al., 2016). Therefore, polyaniline and polypyrrole were selected as a sorbent for trace organic compound determination.

This work thus reports the development of micro-solid phase extraction techniques for the determination trace organic compounds. The extraction capability of synthesized polyaniline-chitosan beads was preliminary studied with parabens (methylparaben, ethylparaben, propylparaben and butylparaben) (**Paper I**), some affecting parameters were optimized. The results indicated that there was a potential to apply polyaniline-chitosan beads for the extraction of these compounds. The second part of the thesis is the development of polypyrrole microspherical magnetic sorbent (**Paper II**). It was used for the extraction carbamate pesticides (carbaryl, carbofuran and methomyl). Parameters that have an effect on the extraction efficiency of polypyrrole magnetic microsphericals were also optimized. The analytical performances were studied before polypyrrole magnetic microsphericals were applied as a sorbent for the determination of carbamate pesticides in fruit and vegetable samples.

1.2 Objectives

To develop a simple, inexpensive and highly efficient micro-solid phase extraction techniques for the extraction of trace organic compounds. To achieve this goal, two sub-projects were carried out as follows.

Project I: A polyaniline-chitosan bead sorbent for parabens extraction

Project II: A novel polypyrrole microspherical magnetic sorbent for the extraction of trace carbamate pesticides in fruits and vegetables.

2. Analytical methods for parabens and carbamate pesticides determination

The most widely used techniques for the determination of parabens and carbamate pesticides are gas chromatography (GC) and high performance liquid chromatography (HPLC) due to their high sensitivity and selectivity.

2.1 Gas chromatography (GC)

GC (Fig. 1) is a technique used to analyze volatiles (VOCs) and semi-volatile (SVOCs) organic compounds in complex mixture. It can be coupled with different detectors, such as flame ionization detector (FID), electron capture detector (ECD) and mass spectrometer (MS). For the interesting organic compound (parabens and carbamate pesticides), sometimes if higher sensitivity was needed, the derivatization before the separation and the analysis with GC is necessary inducing long time and complicated step to the method. (Gu et al., 2016; Carro et al., 2013). So, GC might not be a suitable choice of instrument.

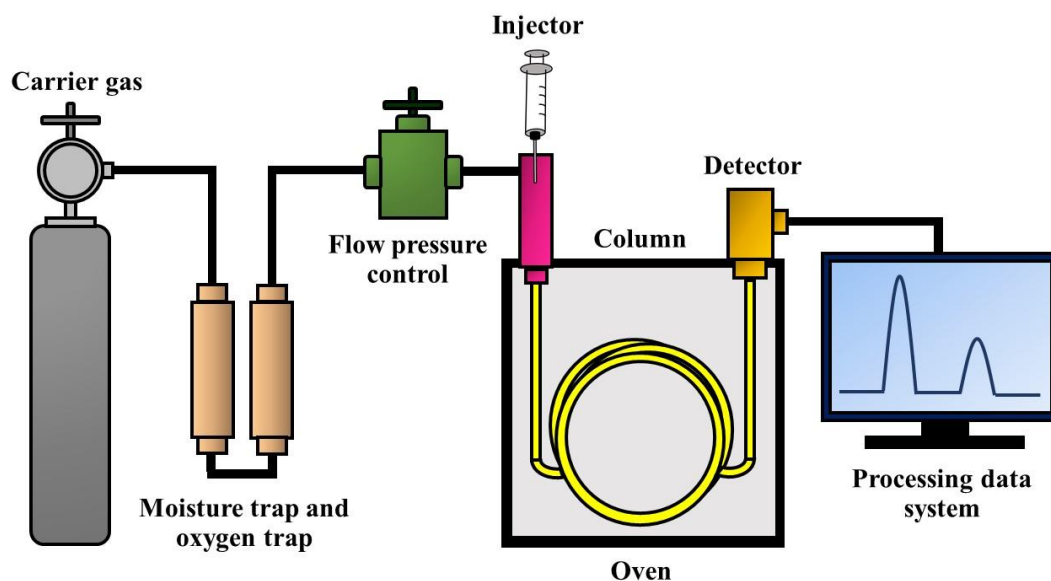


Fig. 1 A schematic of a GC system

2.2 High performance liquid chromatography (HPLC)

HPLC is a widely used technique for analysis of non-volatile organic compounds. A HPLC system is shown in Fig. 2. HPLC uses pumps to pass a pressurized solution of mobile phase and a sample mixture through a column filled with stationary

phase, leading to the separation of the compounds (Valkó, 2016). HPLC can be used with different detectors like in GC, such as ultraviolet-visible (UV-Vis) (**Paper I**), diode array detector (DAD) (**Paper II**), fluorescence (FLD) and mass spectrometry (MS). The determination of parabens and carbamate pesticides can be achieved without the requirement of other additional steps. So, it was used in this study in both sub-projects.

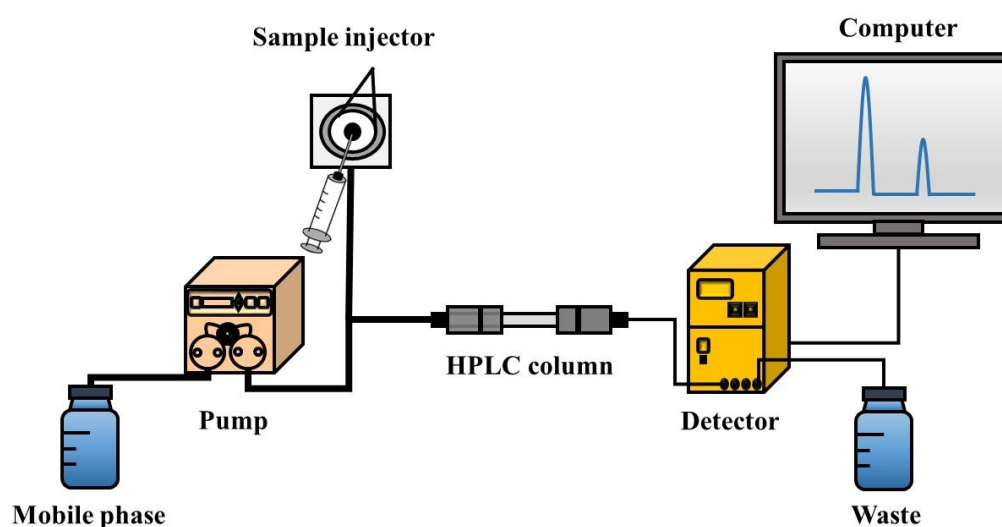


Fig. 2 A schematic of an HPLC-DAD system

3. Sample preparation with micro-solid phase extraction (μ -SPE) techniques

Micro-solid phase extraction (μ -SPE) was developed in terms of sorbent phase extraction in 2006 (Basheer et al., 2006). This technique is simple and rapid for the extraction, preconcentration and clean-up of target analytes. This μ -SPE method was developed to overcome the large volume of organic solvent, large amount of sorbent and time consumption (Khezeli et al., 2017; Sae-Khow & Mitra, 2009). In μ -SPE process, the target analytes were adsorbed on the surface of the sorbent, and then the suitable organic solvent was used to elute the target analytes from the sorbent using centrifugation, magnetic stirrer, vortex mixing and sonication (Yahaya et al., 2014; Fu et al., 2012). Finally, the target analytes was analyzed with the suitable analytical instruments.

Several μ -SPE techniques have been reported in recent years such as dispersive micro-solid phase extraction (D- μ -SPE), pipette tip micro-solid phase extraction (PT- μ -SPE), membrane supported micro-solid phase extraction (membrane- μ -SPE) and magnetic-micro-solid phase extraction (M- μ -SPE). The principle, benefits and drawbacks of each technique are discussed as follows.

3.1 Dispersive micro-solid phase extraction (D- μ -SPE)

D- μ -SPE is a rapid and simple extraction technique, a small amount of sorbent is dispersed into the sample solution for trapping analytes. The sorbent is then separated by filtration or centrifugation before the analytes are desorbed using a suitable solvent for further analysis (Hamidi et al., 2019; Nascimento et al., 2019) (Fig. 3). It has many advantages such as simple, short time requirement, high extraction efficiency and less volumes of organic solvent and amounts of sorbent (Du et al., 2018). However, the isolation of the sorbent from the aqueous solution might generate the loss of the sorbent and analyte which is the main drawback of this extraction technique. In the first sub-project, D- μ -SPE of polyaniline-chitosan microbeads was applied as a tool for the extraction for parabens (**Paper I**) and due to its larger size (approximately 2 mm) than that of the sorbent usually used in D- μ -SPE, and no filtration or centrifugation was needed.

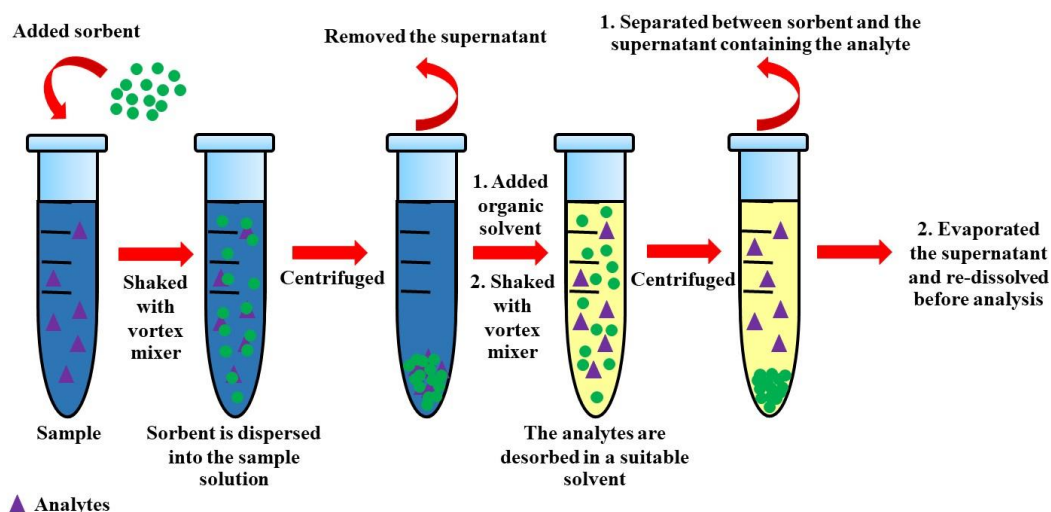


Fig. 3 A schematic of the D- μ -SPE procedure for the adsorption and the desorption of analyte

3.2 Pipette-tip micro-solid phase extraction (PT- μ -SPE)

Pipette-tip micro-solid phase extraction (PT- μ -SPE) is an extraction technique in which a very small amount of sorbent is inserted in a disposable pipette tip, which is held by two frits (Aqda et al., 2019) (Fig. 4). The extraction process was based on the sorption equilibrium of analytes between the sample solution and the sorbent. PT- μ -SPE required several repeated aspirating/dispensing cycles to compel the entire procedure (Wang et al., 2014, Kumazawa et al., 2006). PT- μ -SPE procedure made it easy for sample pretreatment, saved time, used less volume of solvent and was inexpensive compared to conventional SPE (Yan et al., 2019). However, the clog of the sorbent during the extraction and the backpressure limitations of the device were usually observed and this will certainly affect the extraction efficiency of the method (Seidi et al., 2019).

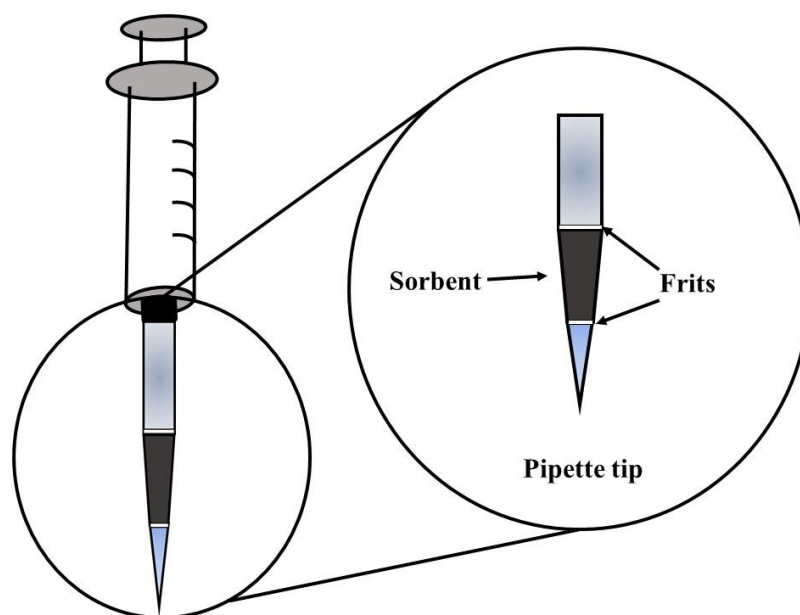


Fig. 4 A schematic of the PT- μ -SPE

3.3 Membrane supported micro-solid phase extraction (membrane- μ -SPE)

Porous membrane protected micro-solid phase extraction was first introduced by Basheer and coworkers in 2006 (Basheer et al., 2006). In membrane- μ -SPE, low amount of sorbent is packed inside a sheet of porous polymer membrane and edges of this sheet were heat sealed to convert it into a small bag as shown in Fig. 5 (Pelden et al., 2014). The extraction was performed by adding the sorbent with the

protected membrane into the sample solution, the analytes were extracted under the magnetic stirrer, vortex mixing or sonication. After extraction, the sorbent containing bag was taken out of the sample solution and the target analytes were then desorbed into a suitable desorption solvent by help of ultrasonic bath or magnetic stirrer (Sajid & Basheer, 2016).

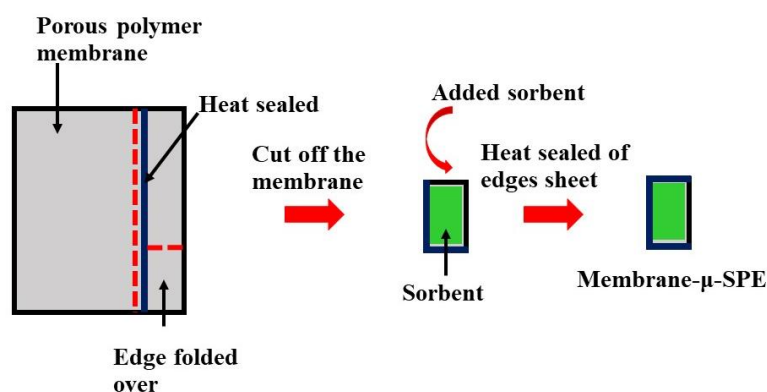


Fig. 5 A schematic of the membrane- μ -SPE preparation

One of the main problems found with this method was the floating of the enveloped membrane sorbent over the sample surface. So, a mini-stir-bar or metal rod was inserted inside the membrane envelop together with the sorbent (Tanimu et al., 2019) (Fig. 6) in order to help the membrane sinking into the solution (Sajid & Basheer, 2016). In addition, the mini-stir-bar also helped to enhance the interaction of sorbent in the membrane and the analytes in the sample solution through a continuous stirring and a rotation, which in turn increased the extraction efficiency (Jillani et al., 2019).

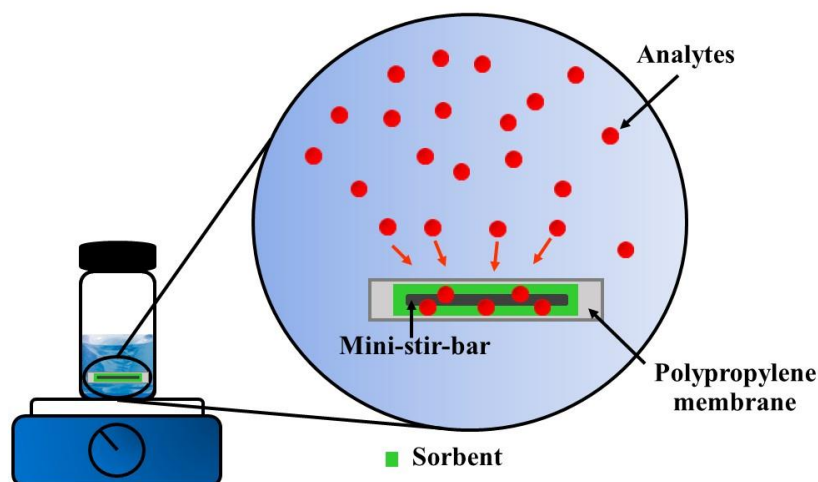


Fig. 6 A schematic of the membrane- μ -SPE for the extraction of analytes

3.4 Magnetic-micro-solid phase extraction (M- μ -SPE)

Magnetic-micro-solid phase extraction (M- μ -MSPE) was first introduced in 1999 (Safariköková & Safarik, 1999). In the process (Fig. 7), the target analytes were adsorbed on the surface of magnetic sorbent, then the magnetic sorbent was isolated from the sample solution using an external magnet. After that, the target analytes were eluted from the magnetic sorbent with an appropriated organic solvent of a small volume. Finally, the magnetic sorbent was separated from the extracted analytes by an external magnet again. The M- μ -MSPE technique has several advantages such as reduced time consumption, a rapid and simple method, high selectivity, high sensitivity and high extraction efficiency for the separation of target analytes from the samples without the centrifugation and filtration (Li et al., 2016).

One of the main disadvantages of magnetic sorbent is the agglomeration of the magnetic particles, which affects the decreasing of the surface area resulting in the decreasing of the extraction efficiency (Yamini et al., 2018). Herein we present (**Paper II**) a new polypyrrole microspherical magnetic sorbent synthesized by a time-dependent template polymerization that provided nice and single spherical particle with rough surface and high porosity. It was used as an M- μ -MSPE for the extraction of three

carbamate pesticides (carbaryl, carbofuran and methomyl) in fruit and vegetable samples.

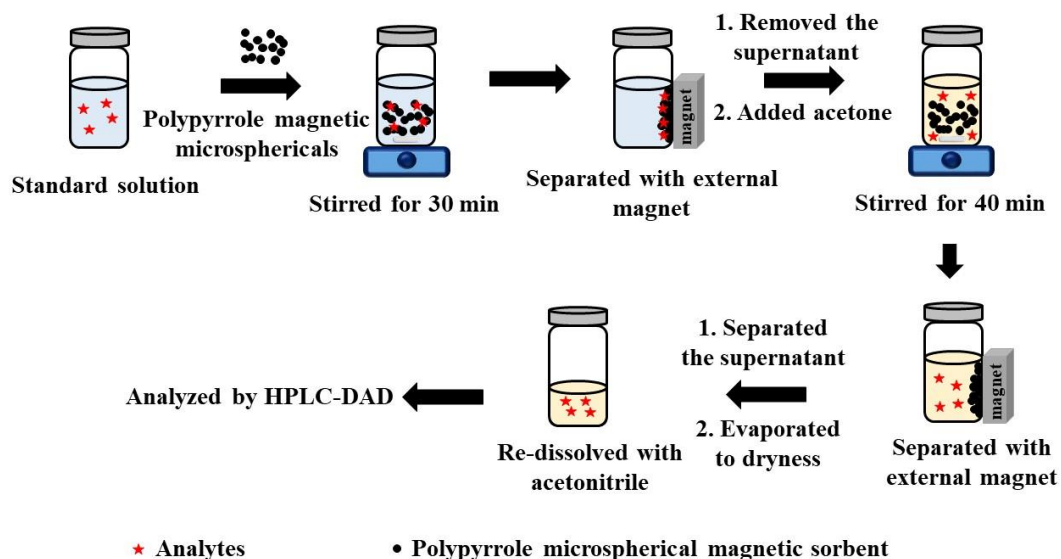


Fig. 7 A schematic of the M-μ-SPE procedure for the adsorption and the desorption of analytes

4. Coating nanomaterial-polymer for micrometer sorbent

To improve the performance of the developed sample preparation technique, various nanomaterials and polymers have been used to increase the extraction efficiency for the determination of contaminated organic compounds in various samples. Nanomaterials such as graphene and multi-walled carbon nanotubes (MWCNTs) have been widely used due to their strong adsorption ability towards target analytes via π - π interactions and some types of polymers such as polyaniline (PANI) and polypyrrole (PPy) were the most two conducting polymers that have been widely used since they are easy to synthesized by either chemical or electrochemical polymerization, in addition, they can also interact with target analytes via π - π interaction and hydrogen bonding.

4.1 Graphene and graphene oxide (GO)

Graphene, a single layer of carbon atoms, is considered as a structural monomer of graphite and carbon nanotube (CNT) (Cui et al., 2019; Sun et al., 2019).

Graphene has a strong interaction with the aromatic compounds since its hexagonal arrays of carbon atom can exhibit strong π - π interactions with benzene ring of target analytes (Ghodrati et al., 2019). It is applicable to use for the adsorption of non-polar compounds. To improve the extraction efficiency in the case of polar target analytes, it has been applied in the form of graphene oxide (GO) by using concentrated acid to induce oxygen functional group in order to enhance the extraction yield and selectivity for specific analytes (Karimiyan et al., 2019; Mateos et al., 2019). GO can form both π - π interactions and H-bonding with polar target compound (Li et al., 2019).

4.2 Multi-walled carbon nanotubes (MWCNTs)

MWCNTs are one of the most widely used sorbent for the development of sample preparation technique due to their high surface area, high ability for π - π interactions, relatively low cost, wide selectivity toward organic compounds since they can be functionalized with various chemical groups (Joseph et al., 2019; Kumar et al., 2019; Okil et al., 2019).

4.3 Polyaniline (PANI)

PANI is one of the best known conjugated conducting polymers because of its low cost, easy to synthesize, good electrochemical activity and high oxidation and corrosion resistance (Jin et al., 2019). The interesting properties of PANI is π -conjugated structure, hydrophobicity, polar groups, ion exchange and easy to composite with other materials (Wójciak-Kosior et al., 2019; Sowa et al., 2015). Therefore, its application as micro-solid phase extraction sorbent for parabens extraction was presented in **Paper I**.

The incorporation of polyaniline powder was incorporated into the chitosan solution before the mixture was used to form polymer microbeads with an average size of 2.0 mm. These beads were used to extract four types of parabens (methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP) and butyl paraben (BP) under stirring with a vortex mixer via π - π interactions between the benzene rings of parabens and polyaniline and hydrogen bonding between the hydroxyl group of parabens and amino group of polyaniline. In this study, some affecting parameters were optimized, *i.e.*, the

number of chitosan-polyaniline microbeads, adsorption and desorption times and desorption solvent.

4.4 Polypyrrole (PPy)

PPy is one of the most useful conducting polymers due to its easyness to polymerize from organic or aqueous media at neutral pH by electrochemical or chemical method, high conductivity, and environmental stability (Li et al., 2016). It has been attracted much attention as a material for sample preparation techniques because of its unique properties such as large surface area, accelerated π - π interactions and hydrogen bonding between the adsorbent and the target analytes resulting in increasing the adsorption capacity (Devasurendra et al., 2018). The second part of this thesis focused on the application of this polymer as a sorbent for the extraction of carbamate pesticides in fruit and vegetable samples (**Paper II**).

A new magnetic PPy sorbent synthesized by a time-dependent template polymerization is presented. The PPy particles were synthesized by loading of magnetic nanoparticles and monomer into porous spherical templates in subsequently, incubating for a certain time before adding the oxidant solution to initiate the polymerization reaction. The polymer was formed from outside to inside of the porous spherical template (Vagin et al., 2016; Wannapob et al., 2015). With this synthesis procedure, the well-disperse of single yet porous spherical polymer particles with magnetic properties were successfully achieved.

5. Analytical performance and method validation

To demonstrate and validate the applicability of the method for the determination of target organic compounds, their analytical performances were evaluated under the optimum conditions as follows.

5.1 Linearity

The linearity of the method is the concentration range that provided the coefficient of determination (R^2) greater than or equal to 0.99. A calibration curve was plotted between concentration of analytes (x axis) and peak area (y -axis) (Long & Winefordner, 1983). In sub-project I, only the linearity of the instrument was reported

since the experiment was done till the optimization only. The linear range of the HPLC-UV for the determination of four parabens were from ranging 0.050 - 60 mg L⁻¹ (**Paper I**). Whereas in sub-project II, completed work were carried out and the linearity of the developed method for the determination of three carbamate pesticides were ranging from 0.50–1.2×10³ µg L⁻¹ for carbaryl, from 1.0–7.5×10² µg L⁻¹ for carbofuran and from 2.5–5.0×10² µg L⁻¹ for methomyl with coefficient of determinations (R²) greater than 0.995 (**Paper II**). This linear range covered the concentration range of target analytes presented in real samples confirming that the developed method can be applied for the determination of carbamate pesticides in fruits and vegetables.

5.2 Limit of detection (LOD) and limit of quantification (LOQ)

According to the IUPAC recommendations, LODs are usually defined as the lowest quantity or concentration of a component that can be reliably detected with a given analytical method. LOQs are established as the lowest concentration of the analytes in the sample that can be determined with an acceptable precision and accuracy under the optimum conditions of the method (Swartz & Hrull, 1997).

The LOD and LOQ of the HPLC-UV used in the first sub-project (**Paper I**) were determined based on the use of signal-to-noise ratio. The concentrations provided a signal-to-noise ratio equal or higher than 3 (S/N ≥ 3) and 10 (S/N ≥ 10) were the LODs and LOQs of the instrument (ICH, 1996). The obtained LODs and LOQs of the instrument for the determination of four parabens are summarized in **Table 1**.

Table 1 LODs and LOQs of the instrument performance

| Parabens | LODs (mg L ⁻¹) | LOQs (mg L ⁻¹) |
|--------------------|----------------------------|----------------------------|
| Methylparaben (MP) | 0.025 | 0.075 |
| Ethylparaben (EP) | 0.025 | 0.075 |
| Propylparaben (PP) | 0.025 | 0.075 |
| Butylparaben (BP) | 0.050 | 0.25 |

In the second sub-project (**Paper II**), LODs and LOQs were estimated as the concentration to 3-folds and 10-folds of the standard deviation of 20 measurements

of the blank divided by the slope of the calibration curves (Long & Winefordner, 1983). LODs and LOQs 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

The obtained LODs and LOQs of the polypyrrole magnetic microsphericals for the extraction of three carbamate pesticides coupled with the analysis by HPLC-DAD (**Paper II**) after converted into the mass unit are summarized in **Table 2**. These values were better than the maximum residue limits (MRLs) values recommended by the National Bureau of Agricultural Commodity and Food Standards (ACFS), Ministry of Agriculture and Cooperative, Thailand. Therefore, this method can be applied to extract and determine the carbamate pesticides contaminated in fruits and vegetables.

Table 2 LODs and LOQs of the developed methods and the MRLs recommended values depending on fruit and vegetable samples

| Carbamate pesticides | LODs ($\mu\text{g kg}^{-1}$) | LOQs ($\mu\text{g kg}^{-1}$) | MRLs ($\mu\text{g kg}^{-1}$) |
|----------------------|--------------------------------|--------------------------------|-------------------------------------|
| Carbaryl | 0.228±0.017 | 0.761±0.057 | $2.0 \times 10^1 - 3.0 \times 10^4$ |
| Carbofuran | 0.78±0.20 | 2.61±0.68 | $1.0 \times 10^1 - 2.0 \times 10^3$ |
| Methomyl | 2.10±0.12 | 6.95±0.32 | $2.0 \times 10^1 - 2.0 \times 10^3$ |

5.3 Reproducibility

The reproducibility for the preparation of polypyrrole magnetic microsphericals was evaluated in terms of the percentage relative standard deviation

(%RSD) from each set of the prepared magnetic sorbent. The RSDs of the response from each extraction was used to indicate the reproducibility of developed sorbent. In this study, six sets of polypyrrole magnetic microsphericals were prepared using the same chemicals. Each of these sorbents was used to extract the carbamate pesticides and the eluted target analytes were determined by analytical instrument. The acceptable RSDs was based on the Association of Analytical Communities (AOAC) guideline (AOAC, 2016).

The average extraction recoveries obtained from the six batches of the polypyrrole magnetic microsphericals for extraction of the three carbamate pesticides were within 83-100% with RSDs less than 5%. Both recoveries and RSDs values were in the acceptable range recommended by the AOAC (at the concentration of 1.0 mg L^{-1} in which recoveries and RSDs must be 80-110% and below 16%, respectively). These results indicated that the preparation procedure of polypyrrole microspherical magnetic sorbent proposed in this work can be reproduced with good reproducibility.

5.4 Reusability

One of the most important parameters is reusability, which is the number of times of the polypyrrole magnetic microsphericals which can be reused. Each of three batches of the prepared sorbent was used to extract ultrapure water spiked with mixed carbamate pesticides standard solution at the concentration of 0.20 mg L^{-1} . The obtained % response from the first extraction was set as the 100% response and the extraction procedures were repeated until the response gave unacceptable %RSD recommended by AOAC (11 % at analyte concentration level of 1.0 mg L^{-1}) (AOAC, 2016) (**Paper II**).

The results showed that the polypyrrole microspherical magnetic sorbent can be reused up to 24 times with the average response percentages in the range of 84-99% and RSDs in the range of 0.99-1.2% for three carbamate pesticides. These were better than those of the AOAC guideline indicating the good reusability of the prepared sorbent.

5.5 Precision

The precision of the developed method was investigated by analyzing the spiked sample solution (guava sample) containing standard solution at the concentration of 0.0050, 0.010, 0.050 and 0.10 mg L⁻¹ (six replications at each concentration). The extraction and the analysis were performed under the optimum condition. The precision was evaluated from the obtained relative standard deviations (RSDs) of the extraction efficiency at each concentration. The acceptable RSDs of precision was based on AOAC guideline (AOAC, 2016). The RSDs were calculated as follows.

$$RSDs = 100 \frac{S}{\bar{X}}$$

where S is standard deviation of sample

\bar{X} is mean of sample

The obtained RSDs from the determination of carbaryl, carbofuran and methomyl were in the range of 0.11 to 4.5%. These values were better than the RSD limits recommended by the AOAC (11% at the analyte concentration of 1.0 mg L⁻¹). These results indicated that good precision of the method was achieved.

5.6 Accuracy

Accuracy is another important parameter that used to demonstrate the validity of the developed method. The accuracy of the method was evaluated by analyzing of the spiked sample solution at different concentration levels. In this thesis (**Paper II**) the accuracy of the developed method was reported in term of % recovery and the AOAC guideline was used as a tool to assess the method accuracy (AOAC, 2016). The % recovery is calculating as

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

Where C_a is the calculated concentration of analyte add to the test sample.

C_f is the concentration of the fortified.

C_u is the concentration of the unfortified.

The developed polypyrrole microspherical magnetic sorbent was used for the extraction of spiked standard solution of carbaryl, carbofuran and methomyl in

guava samples at the concentrations of 0.0050, 0.010, 0.050 and 0.10 mg L⁻¹ and the analysis was performed using HPLC-DAD. The recoveries in the ranges of 91.1±2.8% to 108.3±2.2% for carbaryl, 90.7±6.0% to 108.5±1.1% for carbofuran and 81.6±1.5% to 108.0±1.0% for methomyl were obtained. These recovery values were in an acceptable range of the AOAC guideline (40-120% at the analyte concentration of 1.0 µg L⁻¹, 60-115% at the analyte concentration of 10 µg L⁻¹ and 80-110% at the analyte concentration of 10²-10³ µg L⁻¹) indicating the good accuracy of the developed method.

5.7 Effect of sample matrix

The matrix effect of the sample on the extraction efficiency was studied by spiking standard solution of target analytes at various concentrations into real sample (matrix matched solution). Another set of standard solutions with the same concentration range was also prepared in ultrapure water (standard solution). These two sets of standard solutions were extracted and analyzed under the optimum conditions and the slopes obtained from both set of standard plots were compared using two-way ANOVA (analysis of variance). If the statistical test showed significantly different between the two slopes ($P < 0.05$), the sample matrix interfered the accuracy of the developed method, the matrix matched calibration plot was then used for the quantitative analysis. The results from this study showed that the slopes of the standard and matrix matched calibration plots were significantly different for all samples. Therefore, the matrix matched calibration plot of each sample was used to determine the concentration of analytes (**Paper II**).

5.8 Analysis of real samples

The developed method was used to extract and analyzed carbamate pesticides in five types of fruits (guava, rose apple, apple, orange and watermelon) and five types of vegetables (tomato, Thai basil, cabbage, cucumber and lettuce) bought from fresh market in Hat Yai, Songkhla (**Paper II**).

Carbaryl was found at the concentration of 0.458±0.038 µg L⁻¹ in apple, carbofuran was found at 1.89±0.10 µg L⁻¹ in tomato and methomyl was found at 5.79±0.28 µg L⁻¹ in watermelon. These values were then converted to the concentration unit of µg kg⁻¹ and these following values were obtained; 0.458±0.038 µg kg⁻¹ for

carbaryl, $1.89 \pm 0.10 \mu\text{g kg}^{-1}$ for carbofuran and $5.79 \pm 0.28 \mu\text{g kg}^{-1}$ for methomyl. They were much lower than the MRL values recommended by the Agricultural Commodity and Food Standards (ACFS) (**Table 2**), however, these compounds can be accumulated in the fat tissue of the human body (Yan et al., 2019; Wei et al., 2018) so these fruits and vegetables were considered to “safe to consume” after proper cleaning.

6. Concluding Remarks

In this thesis, the development of micro-solid phase extraction techniques for trace organic compounds analysis were presented. The work was divided into two sub-projects, In the first sub-project, chitosan-polyaniline microbeads were synthesized and used for the extraction of parabens using vortex mixer before the extractant were analyzed by high performance liquid chromatography-ultraviolet detector (HPLC-UV) (**Paper I**). The extraction recoveries in the range of 68.0 ± 7.7 - $98.8 \pm 3.8\%$ were obtained. The results indicated that the developed chitosan-polyaniline beads can be potentially useful for the extraction of parabens in real samples such as cosmetics, food, drinks and drugs.

In the second sub-project (**Paper II**), a new polypyrrole microspherical magnetic sorbent was synthesized and used for the extraction of carbamate pesticides under the magnetic stirrer before analysis by high performance liquid chromatography-diode array detector (HPLC-DAD). A fast, simple and highly efficient extraction method was obtained with good linearity, low LODs and LOQs, good reproducibility with 24 times reusability. In addition, developed sorbent was successfully used to extract carbamate pesticides contaminated in fruit and vegetable samples with good extraction recoveries indicating good accuracy of the developed method.

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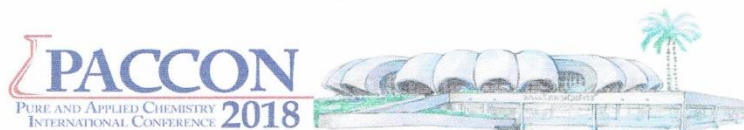
Appendices

Paper I

A polyaniline-chitosan bead sorbent for parabens extraction

Changsan, T., Kanatharana, P., Thavarungkul P., and Thammakhet-Buranachai, C.

Proceeding



A polyaniline-chitosan bead sorbent for parabens extraction

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Abstract:

Polyaniline-chitosan beads were synthesized by incorporating polyaniline powder into chitosan solution. The mixture was added dropwise into a sodium hydroxide solution to form beads. These non-toxic and low cost sorbent beads were applied to extract parabens (methylparaben, ethylparaben, propylparaben and butylparaben) under stirring. The extractants were analyzed by high performance liquid chromatography. The system provided good linearity for the four parabens in the range of 0.050 mg L⁻¹ to 60 mg L⁻¹. The limits of detection and limits of quantitation were 0.025 mg L⁻¹ and 0.075 mg L⁻¹ for methylparaben, ethylparaben and propylparaben and 0.050 mg L⁻¹ and 0.25 mg L⁻¹ for butylparaben, respectively. The extraction recoveries were in the range of 68-99% (n=4). The results indicated that the developed polyaniline-chitosan beads can potentially be useful for the extraction of parabens in real samples such as cosmetics, food, drinks and drugs.

1. Introduction

Parabens, a group of alkyl esters of *p*-hydroxybenzoic acid, are commonly used as preservatives in daily products such as cosmetics, foods, drinks, and drugs.¹⁻³ They were found to influence endocrine system and development of human breast cancer.⁴ The European Economic Community (EEC) law thus regulated parabens at a maximum concentration of 0.4% (w/w) each and a total maximum concentration of 0.8% (w/w) in cosmetic products.⁵

In real samples, the matrix is complex. Thus, sample preparation procedures are generally required to reduce interferences and to preconcentrate the analyte. The commonly used sample preparation techniques are liquid liquid extraction (LLE), solid phase extraction (SPE) and solid phase microextraction (SPME). However, they have some drawbacks, for example, LLE and SPE are time-consuming extraction techniques and require large amount of sample and toxic organic solvent^{6,7} whereas SPME is limited

by the high price and fragile fiber with long sampling/adsorption times.^{8,9} An alternative interesting approach is micro-solid phase extraction (μ -SPE). It is a relatively simple, fast sampling technique that requires minimal amount of solvents, sample, and sorbent.^{10,11}

This work aims to develop a μ -SPE technique by incorporating polyaniline powder into chitosan solution before the mixture was used to form polymer microbeads. The interactions between the sorbent and parabens are π - π interaction via the benzene rings of parabens and polyaniline and hydrogen bonding between the hydroxyl group of parabens and amine group of polyaniline.¹² These microbeads were applied to extract parabens under stirring with a vortex mixer. Optimization of the analytical performances and validation of the method were carried out before applying for the extraction of the target parabens. Methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP) and butyl paraben (BP) were detected since they are

the most widely used and found in real samples.¹³⁻¹⁵

2. Materials and Methods

2.1 Chemicals and reagents

MP, EP, PP, BP were from Sigma-Aldrich (St. Louis, Missouri, USA). Stock parabens standard solutions were prepared in acetonitrile (HPLC grade, Sigma-Aldrich, St. Louis, Missouri, USA). Series of working standard solutions were diluted from stocks with ultrapure water and stored at 4 °C.

Chitosan from shrimp shell (low viscosity, < 200 mPas), aniline monomer (reagent grade, ≥99.5% purity) and ammonium perchlorate were from Sigma-Aldrich (St. Louis, Missouri, USA). Hydrochloric acid, acetic acid, ethyl acetate and sodium hydroxide were from Merck (Darmstadt, Germany). Ammonia, acetone and methanol were from Lab-Scan (Bangkok, Thailand).

2.2 Instruments

Chromatographic studies were performed using a high performance liquid chromatography with an ultraviolet detector (HPLC-UV). Separation of the four parabens was carried out using a Vertical™ UPS C18 column (5 µm particle size, 150 mm × 4.6 mm I.D.) (Vertical Chromatography Co., Ltd, Bangkok, Thailand). Data acquisition and evaluations were performed using eDAQ data acquisition system with PowerChrom software (eDAQ Pty, Australia).

2.3 Optimization of HPLC system

Parameters of the HPLC-UV for the analysis of MP, EP, PP and BP were optimized, *i.e.*, detection wavelength, ratio and flow rate of mobile phase. The optimization was carried out by varying one parameter at a time while keeping the others constant. The optimum conditions were obtained by considering the best resolution, the highest response and the shortest analysis time.

2.4 Synthesis of polyaniline-chitosan bead

Polyaniline was first synthesized by a previously reported oxidative polymerization method¹⁶, *i.e.*, 4.0 g of ammonium persulphate

dissolved in 100 mL of ultrapure water was added dropwise into 2.0 mL of aniline in 100 mL of 1.0 M HCl under vigorous stirring. The solution was continuously stirred for 2 h and then kept in a refrigerator for 4 h for the complete polymerization of aniline. The obtained dark green precipitate was treated with 20 mL of 0.50 M of ammonia for 4 h and cleaned with water before it was dried at 60°C in an oven.

Chitosan, 2.0 g, was dissolved in 60 mL of 5% (v/v) acetic acid aqueous solution and stirred at 600 rpm.¹⁷ Then, 2.0 g of polyaniline powder was added and stirred at 600 rpm to obtain the homogeneous mixture. This polyaniline-chitosan mixture was added dropwise into 300 mL of 0.50 M NaOH solution to form polyaniline-chitosan beads (Figure 1).

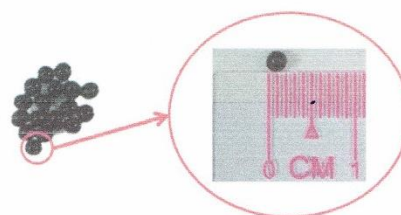


Figure 1. Photos of the prepared polyaniline-chitosan beads with scale for size indication

2.5 Adsorption and desorption procedure

Twenty polyaniline-chitosan beads were added in 2.0 mL of 0.10 mg L⁻¹ mixed MP, EP, PP and BP standard solution with in a 5.0 mL glass vial. The extraction was performed using a vortex mixer for 30 min to allow the adsorption of target analytes by the polyaniline-chitosan beads. After the solution was rinsed out, 2.0 mL of acetonitrile was added as the elution solvent. Vortex mixing was then applied for 30 min. Beads were removed before the extractant was evaporated to dryness using a rotary evaporator (Buchi, Rotavapor, R-200, Flawil, Switzerland) and re-dissolved with 1.0 mL of acetonitrile before being analyzed with the HPLC-UV system.

3. Results and Discussion

3.1 Optimization of the HPLC-UV system

The detection wavelength for parabens determination was varied between 250 and 260 nm with a 2 nm interval. The maximum peak areas of the four compounds were obtained at 256 nm and this was fixed for subsequent investigation.

The ratio of the mobile phase was studied by varying the volume of acetonitrile from 40% to 80% (v/v in water). 60% was found to be the best ratio since it provided the acceptable peak resolution with the shortest analysis time.

The flow rate of the mobile phase was another main factor affecting the column efficiency. It was investigated from 0.20 to 1.0 mL min⁻¹. The optimum flow rate was achieved by considering the lowest height equivalent to a theoretical plate (HETP) of a van Deemter plot (the plot between mobile phase flow rate and the HETP). 0.6 mL min⁻¹ was found to be the optimized value since it provided the lowest HETP, good peak resolution for the separation of four parabens ($R_s > 1.5$) and the total analysis time was only 10 min. The chromatogram under the optimum conditions is shown in Figure 2.

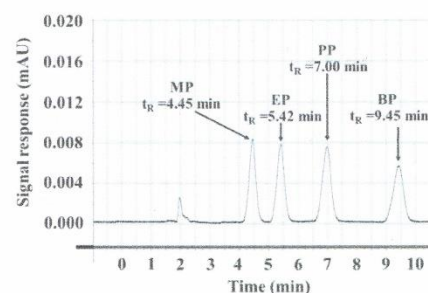


Figure 2. Chromatogram of standard mixture of MP, EP, PP and BP at the concentration of 0.50 mg L⁻¹ each under the optimum conditions

A good linearity in the concentration range of 0.050 mg L⁻¹ to 60 mg L⁻¹ was obtained for all four parabens. The LODs

(S/N ≥ 3) of 0.025 mg L⁻¹ for MP, EP and PP and 0.050 mg L⁻¹ for BP with the LOQs (S/N ≥ 10) of 0.075 mg L⁻¹ for MP, EP and PP and 0.25 mg L⁻¹ were obtained. These values were lower than the allowable maximum concentration in cosmetic products.⁵ Therefore, the developed method has a great potential to be a useful tool for the determination of these parabens in real samples.

3.2 Amount of sorbent

The effect of the amount sorbent on the adsorption efficiency toward target analytes was investigated by varying the number of the polyaniline-chitosan beads from 10 to 40 beads. The extraction recovery only increased slightly (7%) from 10 to 20 beads. Further increase number of beads did not have significantly effect on the extraction efficiency (Figure 3). Consequently, 20 beads of polyaniline-chitosan were then used.

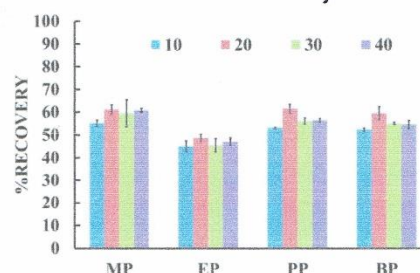


Figure 3. The effect of the number of polyaniline-chitosan beads on the extraction efficiency

3.3 Adsorption time and desorption time

The effect of adsorption time was studied at an interval of 10 min from 10 to 50 min. When the adsorption time was longer than 20 min, there were no significantly change of the recovery for EP and BP and only a small change for PP (3%). However, there was an obviously increase of the MP recovery between 20 and 30 min (Figure 4). Therefore, 30 min was selected for subsequent experiments.

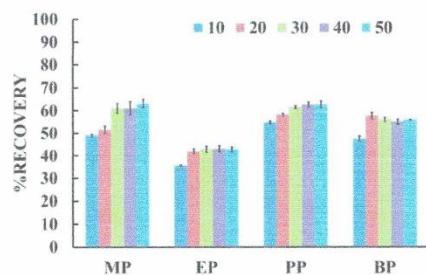


Figure 4. The effect of adsorption time on the extraction efficiency of four parabens

When the desorption time was varied from 10 to 50 min, the recoveries of the four parabens increased with the desorption time till 30 min. There was no significant change thereafter (Figure 5), therefore, 30 min was chosen.

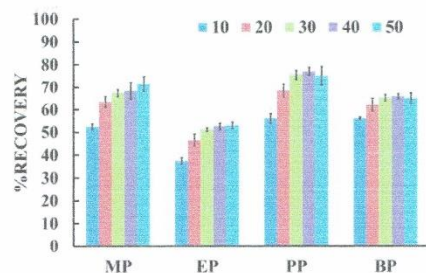


Figure 5. The effect of desorption time on the extraction efficiency of four parabens

3.4 Type of desorption solvent

Desorption of the four parabens from the sorbent beads were studied with acetonitrile, ethyl acetate, methanol and acetone, chosen because of their different polarities. The results are shown in Figure 6. The highest recoveries were obtained from acetonitrile (polarity index = 5.8) while methanol and acetone (polarity index = 5.1) provided lower recoveries. The lowest recoveries were obtained from ethyl acetate (polarity index = 4.4). This might be because polar analytes could not be easily desorbed using slightly polar desorption solvents. Therefore, acetonitrile was chosen for this experiment.

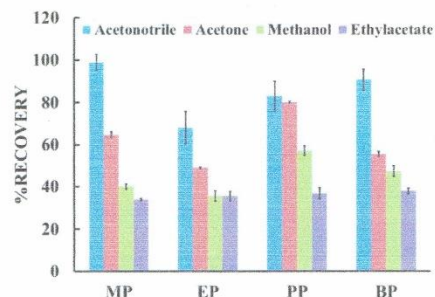


Figure 6. The effect of desorption solvent on the extraction efficiency of four parabens

The recoveries under optimal conditions are shown in Table 1. These extraction recoveries can still be further improved by investigating additional affecting parameters such as the speed of vortex mixer and the volume of desorption solvent.

Table 1. Extraction recoveries of four parabens under the optimal conditions

| Analytes | %Recovery |
|----------|------------|
| MP | 98.8 ± 3.8 |
| EP | 68.0 ± 7.7 |
| PP | 82.9 ± 7.1 |
| BP | 90.7 ± 4.8 |

4. Conclusion

Non-toxic and low cost polyaniline-chitosan sorbent beads were simply synthesized. When applied for the extraction of parabens (MP, EP, PP and BP) under stirring by a vortex mixer, relatively good recoveries were obtained. Further improvement through additional optimization of affecting parameters is highly possible. This study indicates that the use of these beads as extracting sorbent is promising.

Acknowledgments

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Paper II

A novel polypyrrole microspherical magnetic sorbent for the extraction of trace carbamate pesticides in fruits and vegetables

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Manuscript submission to Food Chemistry

A novel polypyrrole microspherical magnetic sorbent for the extraction of trace carbamate pesticides in fruits and vegetables

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Abstract

A new polypyrrole microspherical magnetic sorbent synthesized by a time-dependent template polymerization provided large surface area and high porosity. It was used for the extraction of carbaryl, carbofuran and methomyl under the stirring before being analyzed by a high performance liquid chromatography with diode array detector (HPLC-DAD). Under the optimum conditions, good linearity for the analysis of three carbamate pesticides in the ranges of $0.50\text{-}1.2\times 10^3 \mu\text{g L}^{-1}$, $1.0\text{-}7.5\times 10^2 \mu\text{g L}^{-1}$ and $2.5\text{-}5.0\times 10^2 \mu\text{g L}^{-1}$ with the limits of detection of $0.228\pm 0.017 \mu\text{g L}^{-1}$, $0.78\pm 0.20 \mu\text{g L}^{-1}$ and $2.10\pm 0.12 \mu\text{g L}^{-1}$ were obtained for the determination of carbaryl, carbofuran and methomyl, respectively. Good reproducibility (RSDs < 5%) and good accuracy were achieved with 24 cycles of reusability. After validation, the developed method was applied for the determination of carbaryl, carbofuran and methomyl in fruit and vegetable samples.

Keywords: polypyrrole microsphericals; magnetic sorbent; time-dependent template polymerization; high performance liquid chromatography; carbamate pesticides analysis; fruit and vegetable samples

Highlights

- Cheap yet efficient polypyrrole microspherical magnetic sorbent was synthesized.
- Single particle with controllable size of the sorbent was achieved.
- High recovery and reusability for carbamate pesticides extraction were obtained.

1. Introduction

Sample preparation plays an important role for trace analysis of toxic compounds since it helps isolating and cleaning up the interesting target analyte from the sample matrix, reducing the effect of interferences in real samples and preconcentrating the analyte concentration up to the level that can be accurately determined by the suitable and available instruments (Fan et al., 2018; Háková et al., 2018; Mateos et al., 2017). The time consumed in the sample preparation step takes almost 70% of the total analysis time (Ouertani et al., 2016), the development of simple, rapid and efficient sample preparation techniques is thus very important.

Magnetic solid phase extraction (MSPE) has become one of the most popular sample preparation techniques because the MSPE used a small amount of sorbent with very small volumes of both sample and organic solvent coupled with a simple and rapid extraction process (Nasir et al., 2019; Ma et al., 2018). The extraction is based on the dispersion of the magnetic sorbent into the sample or standard solution then the sorbent was separated from the sample solution using an external magnetic field. The eluting solvent was then added to desorb the extracted analytes, and again the magnet was used to isolate the magnetic sorbent before the extract was subjected to qualitative and/or quantitative determination by the appropriate instruments (Chen, He, et al., 2018; Lian et al., 2018).

There have been many reports on the development of magnetic particles applied in solid phase extraction, most of them focused on the functionalization with various types of polymers and chemicals to improve the efficiency and selectivity of the extraction (Noormohamadi et al., 2018; Xia et al., 2018; Yavuz et al., 2018; Qiao et al., 2016; Hosseini et al., 2015; Li et al., 2014). Nevertheless, one of the main disadvantages of magnetic sorbent is the agglomeration of the magnetic particles, which affects the decreasing of the surface area resulting in the decreasing of the extraction efficiency (Yamini et al., 2018) and only a few researchers have paid attention on this issue.

This work thus presents a new magnetic sorbent synthesized by a time-dependent template polymerization in which the polymer particles will be synthesized by loading of monomer into porous spherical templates, incubating for a certain period of time before adding the oxidant solution to initiate the polymerization reaction.

The polymer was formed from outside to inside of the porous spherical template. The characteristics of hollow or dense porous polymer particles obtained depend on polymerization time (Vagin et al., 2016; Wannapob et al., 2015). With this synthesis procedure, the well-disperse of single yet porous spherical polymer particles were achieved.

To combine the benefits of simple extraction procedure using magnetic sorbent and large surface area of well-disperse spherical polymer particles, magnetic nanoparticles were first synthesized (Ghasemzadeh et al., 2013) before they were incorporated in the time-dependent template polymerization procedure. Not only single and spherical porous polymer particles will be obtained but the magnetic property of such polymer sphericals can also be achieved and this will facilitate the extraction process.

Polypyrrole was chosen as the sorbent phase because the synthesis can be performed very easily, the polymer itself is environmentally safe and chemically stable. In addition, it can interact with the interested target analytes, carbamate pesticides, via π - π interactions and hydrogen bonding (Chougule et al., 2011). Carbaryl, carbofuran and methomyl were selected as target analytes since they are the most widely used carbamate pesticides in agriculture for insect protection due to their high effective and short-lived in the environment (Luo et al., 2017; Ai et al., 2015; Hanot et al., 2015; Tien et al., 2013).

Here in, the extraction of carbamate pesticides using a new polypyrrole microspherical magnetic sorbent, coupled with the analysis by a high performance liquid chromatography- diode array detector (HPLC-DAD) is presented. Optimization of the analytical performances and validation of the method were carried out before applying the developed method for the determination of the target carbamate pesticides (carbaryl, carbofuran and methomyl) in fruit and vegetable samples.

2. Experiment

2.1 Chemicals and materials

Methomyl and carbofuran, both with 98% purity were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Carbaryl with 96% purity was the product of Supelco (Bellefonte, Pennsylvania, USA). Stock standard solutions of each

carbamate pesticide at the concentration of 1000 mg L⁻¹ were prepared in acetonitrile (HPLC grade, Sigma-Aldrich, St. Louis, Missouri, USA). Series of working standard solutions were diluted from the stocks with acetonitrile and stored at 4°C.

Calcium chloride (CaCl₂), copper (II) perchlorate, sodium carbonate (Na₂CO₃), ethylenediamine-tetraacetic acid (EDTA), iron (III) chloride hexahydrate, iron (II) chloride tetrahydrate and pyrrole monomer (reagent grade, 98% purity) were from Sigma-Aldrich (St. Louis, Missouri, USA) and the monomer was distilled prior to use. Hydrochloric acid, ethyl acetate and 2-propanol were from Merck (Darmstadt, Germany). Ethanol, acetone, methanol and sodium hydroxide were from Lab-Scan (Bangkok, Thailand). Ultrapure water with the resistivity of 18.2 MΩ.cm was from a Milli Q system (Merck, Germany).

2.2 Instruments

Chromatographic studies were performed using an HPLC-DAD (Hitachi HPLC, Japan). Data analysis was performed using LaChrom Elite HPLC (Merck-Hitachi HPLC, Japan). The separation of the three carbamate pesticides were carried out using a VerticalTM UPS C18 column (5 μm particle size, 150 mm × 4.6 mm I.D.) (Vertical Chromatography Co., Ltd, Bangkok, Thailand). The extraction of three carbamate pesticides using polypyrrole microsphericals magnetic sorbent were carried out under a stirring by a magnetic stirrer (COLOR SQUID, IKA, Germany). The solution was evaporated to dryness by a rotary evaporator (Buchi, Rotavapor, R-200, Flawi, Switzerland). Their surface morphology was characterized using a scanning electron microscope (FE-SEM, Apreo, FEI, the Netherlands). Energy dispersive X-ray (EDX) was recorded using an SEM, Quanta 400 (FEI, the Netherlands) and the functional groups of the synthesized product were characterized with a Fourier Transform infrared (FTIR) spectroscopy (BX FTIR spectroscope, PerkinElmer, USA).

2.3 Preparation of polypyrrole microsphericals magnetic sorbent

The synthesis of polypyrrole microspherical magnetic sorbent was carried out in two steps. First, magnetic powder was synthesized, 6.0 g of iron (III) chloride hexahydrate and 2.5 g of iron (II) chloride tetrahydrate were dissolved in 15 mL of ultrapure water. The mixture was added dropwise into 1.0 mL of concentrated

hydrochloric acid before it was dropped into 250 mL of 0.5 M sodium hydroxide solution to form magnetic nanoparticles. The precipitate was rinsed with ultrapure water before dried at 70°C in an oven (Ghasemzadeh et al., 2013).

Next (Fig. 1), the polypyrrole microspherical magnetic sorbent was synthesized by a time-dependent template polymerization (Wannapob et al., 2015), 1.0 mL of 0.50 M CaCl₂ and 1.0 mL of 0.50 M Na₂CO₃ were mixed for 1.0 min to produce calcium carbonate (CaCO₃) spherical template with an average size of 2-3 μm. Then the mixture of 0.10 g of magnetic nanoparticles dispersed in 0.50 mL of water was added into 2.0 mL of suspended CaCO₃ and incubated for 30 min to allow the magnetic nanoparticles to absorb and to penetrate onto the surface and inside the pores of the CaCO₃ microspherical templates. The CaCO₃ microspherical magnetic templates were washed with water, ethanol, and 2-propanol by centrifugation. After that 0.020 mL of distilled pyrrole monomer was added into the CaCO₃ microspherical magnetic templates and the mixture was incubated for another 30 min. The pyrrole-loaded CaCO₃ microspherical magnetic templates were centrifuged at 6000 rpm for 1.0 min and the supernatant was discarded to eliminate the unabsorbed pyrrole monomer.

Polymerization reaction was initiated by adding 1.0 mL of 1.0 M copper (II) perchlorate into the solution containing pyrrole-loaded CaCO₃ microspherical magnetic template and the reaction was terminated after 6 h. The polypyrrole-loaded CaCO₃ microspherical magnetic templates were then successively washed with 2-propanol, ethanol, and water by centrifugation. The CaCO₃ microspherical template was then removed by the addition of 1.0 mL of 0.20 M EDTA solution and incubated for 1 h at room temperature (Hanafy et al., 2015). Finally, polypyrrole microspherical magnetic sorbent were washed with ultrapure water.

2.4 Adsorption and desorption procedures

To a 1.2 mL solution of 0.20 mg L⁻¹ mixed standard of carbaryl, carbofuran and methomyl in ultrapure water was added 150 mg of polypyrrole microspherical magnetic sorbent. The extraction was performed by stirring for 30 min to allow target analytes to adsorb onto the surface of the polypyrrole microspherical magnetic sorbent, an external magnet was used to separate the magnetic sorbent from the solution before the aqueous solution was discarded. Then 1.4 mL of acetone was added as the

desorption solvent, the stirring was performed for 40 min before polypyrrole microspherical magnetic sorbent were isolated from the extractant. The extracting solution was then evaporated to dryness using a rotary evaporator and re-dissolved with 0.30 mL of acetonitrile before being analyzed with the HPLC-DAD system.

To obtain the highest extraction efficiency, the adsorption and desorption conditions were optimized, *i.e.*, adsorption and desorption times, amount of sorbent, type and volume of desorption solvent, rate of stirring in the desorption step.

The initial conditions for the optimization of the extraction were as follows: the concentration of 0.20 mg L⁻¹ and the volume of 1.2 mL of carbamate pesticide standard solution, 30 min of adsorption time at a stirring rate of 500 rpm, 100 mg of polypyrrole microspherical magnetic sorbent, 1.2 mL of acetonitrile as desorption solvent and 30 min of desorption time with a stirring rate of 500 rpm. The optimization was carried out by varying one parameter at a time and keeping other parameters constant. Three replications were done at each tested condition. The optimum value of each condition was chosen from the one providing high response and short analysis time.

2.5 Analytical performance and method validation

The linearity of the method is the concentration range that provided the coefficient of determination (R^2) greater than or equal to 0.99. 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) from 20 blank measurements divided by the slope of the calibration curve (Long & Winefordner, 1983).

The precision of the developed method was evaluated by extracting spiked fruit and vegetable sample solution with standard solutions of carbamate pesticides at the concentrations of 5, 10, 50 and 100 µg L⁻¹ (six replications at each concentration), then 150 mg of polypyrrole microspherical magnetic sorbent was added. The extraction was performed under the optimum conditions before the extractant was analyzed with HPLC-DAD. The precision was evaluated from the obtained %RSD of the extraction efficiency at each concentration.

Accuracy is another important parameter that used to demonstrate the validity of the developed method. The accuracy of the method was evaluated by analyzing of the spiked fruit and vegetable sample solution at different concentration levels (5, 10, 50 and 100 $\mu\text{g L}^{-1}$ of carbamate pesticide standard solution). In this work, the accuracy of the developed method was reported in term of recovery and the AOAC guideline was used as a tool to assess the method accuracy.

To further validate the developed method, six batches of polypyrrole magnetic microsphericals were synthesized under the same conditions. Each batch, polypyrrole magnetic microsphericals were used to extract the analytes from the solution of ultrapure water spiked with mixed standard solution at the concentration of 0.20 mg L^{-1} (three replicates), followed by analysis with the HPLC-DAD system. The reproducibility was evaluated from the %RSD of the extraction efficiency obtained from the six batches.

In addition, the reusability of the prepared sorbent was also investigated using three batches. Each of them was used to extract the analytes from the solution of ultrapure water spiked with mixed standard solution at the concentration of 0.20 mg L^{-1} (three replicates). After the first and all subsequent extraction cycles, the polypyrrole microspherical magnetic sorbent were washed in acetone and ultrapure water before being reused. The reusability was evaluated from %RSD together with %recovery obtained from each of the three batches.

2.6 Analysis of real samples

Five types of fruits (guava, rose apple, apple, orange and watermelon) and five types of vegetables (tomato, Thai basil, cabbage, cucumber and lettuce) were bought from fresh market in Hat Yai, Songkhla, Thailand. 200 g of each sample was cut and 200 mL of ultrapure water was added into the sample. They were homogenized with a blender for 3 min, and centrifuged for 15 min before 150 mL of the supernatant was filtered through a Whatman GF/F filter paper (0.25 μm pore size; Maidstone, England) to remove the solid part out of the solution (Makkliang et al., 2016). 1.2 mL of the filtrate was used as a sample. The extraction was then performed with the described procedure in section 2.4.

3. Results and discussion

3.1 HPLC-DAD conditions

The composition and flow rate of the mobile phase and detection wavelength of the HPLC-DAD were optimized by injecting 20 μL of 0.20 mg L^{-1} mixed standard solution. The conditions of HPLC-DAD for the determination of carbaryl, carbofuran and methomyl that provide high peak area, good peak shape and short analysis time are as follow. Acetonitrile and ultrapure water at the ratio of 55:45 %v/v was used as the mobile phase at the flow rate of 0.60 mL min^{-1} . The wavelengths of the diode array detector at 236, 198 and 218 nm were used for the detection of carbaryl, carbofuran and methomyl, respectively. At these conditions, the obtained chromatogram exhibits good peak shape and good peak resolution with analysis time of 9 min ($t_{\text{R(methomyl)}} = 3.60$ min, $t_{\text{R(carbofuran)}} = 7.90$ min and $t_{\text{R(carbaryl)}} = 8.20$ min).

3.2 Characterization of polypyrrole magnetic microsphericals

Fig.2 shows a well-dispersed synthesized polypyrrole magnetic microsphericals in water. The sorbents were easily and completely separated from the solution using external magnet within only 15 s.

The morphology of polypyrrole magnetic microsphericals was observed under a scanning electron microscope (SEM). The polypyrrole magnetic microsphericals synthesized by the time-dependent template polymerization showed a nice and single spherical particle with an average size of $2.81 \pm 0.25 \mu\text{m}$ ($n=30$). No agglomeration with rough surface and porous of microsphericals was observed (Fig. 3a), resulting in larger surface area and possibly higher extraction efficiency compared to those of the magnetic sorbent using the normal synthesis method (Vagin et al., 2016).

The EDX spectrum (Fig. 3b) shows C and N atoms which come from the polypyrrole whereas Fe and O atoms from the magnetite adsorbed in the polypyrrole microsphericals. Cu and Cl atom come from the oxidant (copper (II) perchlorate) used for the synthesis that might still remain on the surface of polypyrrole microsphericals. In addition, the molecular structure of the polypyrrole microspherical magnetic sorbent was also characterized by FTIR spectroscopy (Fig. 4). The spectrum reveals the presence of magnetic particles that incorporate in the polypyrrole microsphericals as can be seen from the absorption peaks of Fe-O bond at 627 cm^{-1} . The vibration bands

from the polypyrrole core structure were also observed, i.e., N-H stretching at 3522 cm^{-1} , C=C stretching at 1569 cm^{-1} , C-C stretching at 1313 cm^{-1} , C-N stretching at 1212 cm^{-1} , pyrrole ring at 1540 cm^{-1} , and pyrrole C-H in-plane and C-H out-of-plane at 1049 and 964 cm^{-1} , respectively. All of these characteristic bands indicated that the polypyrrole magnetic microsphericals were successfully synthesized.

It was expected that the polypyrrole magnetic microsphericals can be used as a sorbent to extract three carbamate pesticides by π - π interactions between aromatic rings of polypyrrole of the sorbent and analytes and hydrogen bonding between NH groups of polypyrrole and carbamic groups of carbamate pesticides.

3.3 Optimization of extraction conditions

3.3.1 Adsorption time

The extraction is the kinetic process which depending on time, *i.e.*, the longer time, the more extraction efficiency, until reaching the extraction equilibrium. To obtain the shortest time with the highest extraction efficiency, the time used in the adsorption step was studied by varying from 10 to 50 min (Fig. 5a). It was clearly observed that the adsorption time did not affect much on the recoveries of carbaryl (from $71.2 \pm 2.2\%$ at 10 min to $76.8 \pm 1.3\%$ at 50 min) but it played an important role on the extraction of both carbofuran and methomyl. When the adsorption time was increased from 10 to 30 min, the recoveries increased from $49.3 \pm 1.2\%$ to $58.1 \pm 2.0\%$ for carbofuran and from $35.0 \pm 3.3\%$ to $41.4 \pm 1.2\%$ for methomyl. For longer adsorption times (40 and 50 min), the recoveries decreased, possibly due to too long adsorption time, which carbofuran and methomyl were stripped from the sorbent back into the aqueous solution. Therefore, 30 min was chosen as the optimum adsorption time.

3.3.2 Amount of polypyrrole microspherical magnetic sorbent

To use the least amount of polypyrrole microspherical magnetic sorbent for the extraction of the three carbamate pesticides, their weight was varied from 50 to 200 mg. The results showed that increasing the amount of sorbent from 50 to 150 mg led to decreasing the extraction recoveries (Fig. 5b). This was possibly because at high amount of sorbent with the fixed volume of desorption solvent would induce the incomplete desorption. This hypothesis was confirmed by the analysis of the three

pesticides in the spiked ultrapure water after extraction at each amount of sorbent used. The result of %remaining were shown in Fig. 5c, the higher the amount of sorbent, the lower the %remaining of the three pesticides in water after extraction and this %remaining is stable at 150 mg (almost 0% for carbaryl, $4.45\pm 0.10\%$ for carbofuran and $7.56\pm 0.77\%$ for methomyl). These results indicated that at 150 mg of sorbent provide the highest adsorption efficiency but the reason behind the lower recoveries than those at 50 mg might be because the desorption conditions were still not suitable to completely desorb the target pesticides from the sorbent.

3.3.3 Volume of desorption solvent

The volume of desorption solvent was varied to improve the desorption efficiency of the adsorbed analytes from the surface of the polypyrrole microspherical magnetic sorbent. The least volume that can completely desorb the studied carbamate pesticides was chosen. The volumes of acetonitrile were varied between 1.0 to 1.6 mL. The higher the volume of desorption solvent (from 1.0 to 1.4 mL), the higher the recoveries (from $58.3\pm 2.9\%$ to $81.47\pm 0.82\%$ for carbaryl, from $35.3\pm 1.6\%$ to $72.24\pm 0.68\%$ for carbofuran and from $13.7\pm 1.4\%$ to $58.2\pm 1.3\%$ for methomyl) indicating that better eluting power were obtained with larger volume of acetonitrile (Fig. 5d). Further increase of the volume to 1.6 mL, the recoveries remain unchanged. Thus, the least volume that provided the highest extraction efficiency was 1.4 mL.

3.3.4 Desorption time

The effect of desorption time was also studied to improve the extraction efficiency within a short analysis time. It was investigated from 10 to 50 min (Fig. 5e). The results showed a similar trend with the adsorption time in which the time in the desorption step did not play an important role on the extraction recoveries of carbaryl because the obtained recoveries remained almost stable ($81.45\pm 0.30\%$ at 10 min and $82.072\pm 0.025\%$ at 50 min). Whereas the recoveries of carbofuran and methomyl increased from $63.4\pm 2.5\%$ to $81.26\pm 0.22\%$ and from $29.00\pm 0.43\%$ to $66.5\pm 1.1\%$, respectively, when the desorption time was increased from 10 to 40 min before remaining constant at longer time. Therefore, 40 min was chosen as an optimum desorption time.

3.3.5 Stirring rate

Stirring rate has an enormous effect on both adsorption and desorption steps since it helps to facilitate the mass transfer of the target analytes to the sorbent surface (adsorption) as well as from the sorbent surface back to the eluting solvent (desorption). In this study, only the stirring rate in the desorption step was studied because the adsorption of the target analyte from the aqueous solution onto the surface of the sorbent reaches almost 100% at the initial used stirring rate at 500 rpm in the adsorption step. The portion of the mixed standard that still remained in the spiked ultrapure water after extraction was less than 8%. The reason that the extraction recoveries are still lied in un-acceptable ranges because of an incompleated desorption. Therefore only stirring speed in the desorption step was optimized to improve the extraction recoveries.

The stirring rate in the desorption step was varied from 250 to 2000 rpm (Fig. 5f). Increasing the stirring rate from 250 to 500 rpm resulted in higher extraction recoveries from $72.23 \pm 0.61\%$ to $83.1 \pm 2.4\%$ for carbaryl, from $68.2 \pm 3.4\%$ to $82.5 \pm 1.4\%$ for carbofuran and from $64.5 \pm 2.9\%$ to $69.98 \pm 0.46\%$ for methomyl. The speed less than 500 rpm was not fast enough to increase the migration of the target analytes from the sorbent surface to the desorption solvent. However, too fast stirring rates (1000 to 2000 rpm) did not significantly affect the recoveries of carbaryl and carbofuran but it showed the negative effect with methomyl. This might be possibly because the interaction between methomyl and the polypyrrole microsphericals magnetic sorbent was hydrogen bonding which was stronger than π - π interactions of carbaryl and carbofuran, so at too high stiring speed the contact time between the sorbent and the eluting solvent decreased and this affected the elution power. So, 500 rpm of stirring speed was used for the next optimization.

3.3.6 Type of desorption solvent

Type of desorption solvent is another important parameter that has an effect on the desorption efficiency. Acetonitrile (polarity index=5.8), methanol (polarity index=5.1), acetone (polarity index=5.1) and ethanol (polarity index=4.3) were chosen in this investigation based on their polarity were similar to those of the three carbamate pesticides. All three compounds were desorbed with the highest

desorption efficiency when acetone was used as the desorption solvent with the recoveries of $103.5\pm 2.4\%$, $93.81\pm 0.99\%$ and $88.4\pm 1.7\%$ for carbaryl, carbofuran and methomyl, respectively (Fig. 5g).

If the polarity index was the only parameter that was taken into account, acetonitrile should be the solvent type that provided the highest and ethanol was the one that provided the lowest eluting power to desorb carbamate pesticides from the polypyrrole microspherical magnetic sorbent since it has the highest and the lowest polarity index among the studied solvents. However, the interaction between desorption solvent and target analytes must also be considered since it directly affected the eluting power of the analytes from the sorbent surface.

All studied solvents can form hydrogen bonding with carbaryl, carbofuran and methomyl, so all of them have the ability to dissolve target analyte out from the polypyrrole microspherical magnetic sorbent. Acetone has two lone pairs electron while acetonitrile has only one and this will affect the solvating power of solvent towards the target analyte, acetone thus provide the highest extraction efficiency (Rathinavel et al., 2019). As a result, acetone was selected for desorption solvent to eluting the analytes from the sorbent.

All optimum parameters for the extraction using polypyrrole microspherical magnetic sorbent are summarized in Table S1. Under the optimum conditions, the extraction recoveries of $99.2\pm 1.2\%$ for carbaryl, $90.5\pm 2.4\%$ for carbofuran and $85.4\pm 4.0\%$ for methomyl were obtained.

3.3.7 Extraction efficiency of magnetic nanoparticles and polypyrrole magnetic microsphericals

The efficiencies of magnetic nanoparticles and polypyrrole magnetic microsphericals were compared (Fig. S1). 150 mg of each material was used to extract 0.20 mg L^{-1} mixed standard solution of carbaryl, carbofuran and methomyl under the optimum conditions. The recoveries in the range of $81.6\pm 1.8\%$ to $94.2\pm 6.6\%$ were achieved with the use of polypyrrole microspherical magnetic sorbent while less than $9.27\pm 0.18\%$ of recoveries were obtained with only magnetic nanoparticles. These results indicated that the main function of the magnetic nanoparticles in the polypyrrole microspherical magnetic sorbent is to add up the magnetic property of the sorbent only,

it can extract very little amount of carbamate pesticides via hydrogen bonding. Most extraction efficiency of the polypyrrole microspherical magnetic sorbent is from the polypyrrole itself since polypyrrole polymer backbone can form π - π interactions and hydrogen bonding with these three carbamate pesticides.

3.4 Analytical performances

3.4.1 Linearity, limits of detection (LODs) and limits of quantification (LOQs)

The analytical performances of the polypyrrole microspherical magnetic sorbent coupled with HPLC-DAD were observed. Good linearity was obtained in the concentration range from 0.50 to $1.2 \times 10^3 \mu\text{g L}^{-1}$ for carbaryl, from 1.0 to $7.5 \times 10^2 \mu\text{g L}^{-1}$ for carbofuran and from 2.5 to $5.0 \times 10^2 \mu\text{g L}^{-1}$ for methomyl with coefficient of determinations (R^2) greater than 0.995. LODs and LOQs were calculated using the IUPAC recommendation of $3S_B/m$ and $10S_B/m$, respectively (S_B is the standard deviation of 20 blank responses; m is the slope of the calibration curve) (Long & Winefordner, 1983). The LODs and LOQs were $0.228 \pm 0.017 \mu\text{g L}^{-1}$ and $0.761 \pm 0.057 \mu\text{g L}^{-1}$ for carbaryl, $0.78 \pm 0.20 \mu\text{g L}^{-1}$ and $2.61 \pm 0.68 \mu\text{g L}^{-1}$ for carbofuran and $2.10 \pm 0.12 \mu\text{g L}^{-1}$ and $6.95 \pm 0.32 \mu\text{g L}^{-1}$ for methomyl, respectively.

The developed method provided a wide linear range for the detection of all three compounds, in addition the obtained LODs and LOQs were lower than the allowable maximum concentration of these pesticides in fruits and vegetables. The National Bureau of Agricultural Commodity and Food Standards (ACFS), Ministry of Agriculture and Cooperative, Thailand has regulated maximum residue limits (MRLs) for carbamate pesticides as follow: carbaryl in the range of 2.0×10^1 to $3.0 \times 10^4 \mu\text{g kg}^{-1}$, carbofuran in the range of 1.0×10^1 to $2.0 \times 10^3 \mu\text{g kg}^{-1}$ and methomyl in the range of 2.0×10^1 to $2.0 \times 10^3 \mu\text{g kg}^{-1}$ depending on the types of fruits and vegetables (TAS9002-2016, 2016).

We have converted the values and concentration unit for the three carbamate pesticides obtained from our developed method to the same of those recommended by the ACFS based on the weight of sample that have been used in this study. It was found that our LODs and LOQs values in the unit of $\mu\text{g kg}^{-1}$ were $0.228 \pm 0.017 \mu\text{g kg}^{-1}$ and $0.761 \pm 0.057 \mu\text{g kg}^{-1}$ for carbaryl, $0.78 \pm 0.20 \mu\text{g kg}^{-1}$ and

$2.61 \pm 0.68 \mu\text{g kg}^{-1}$ for carbofuran and $2.10 \pm 0.12 \mu\text{g kg}^{-1}$ and $6.95 \pm 0.32 \mu\text{g kg}^{-1}$ for methomyl, respectively. These LODs and LOQs values were much lower than the MRLs values provided by the ACFS. All these performances of the developed method confirmed that it can be applied for the extraction and analysis of carbaryl, carbofuran and methomyl in fruit and vegetable samples.

3.4.2 Reproducibility

The reproducibility for the preparation of polypyrrole microspherical magnetic sorbent was studied with the procedure mentioned in section 2.5. The recoveries and %RSD obtained from the extraction using polypyrrole microspherical magnetic sorbent prepared from each of six batches were as follows (Fig. S2), $84.22 \pm 0.31\%$ to $98.0 \pm 1.8\%$ with RSDs $< 1.9\%$ for the 1st batch, $86.2 \pm 1.8\%$ to $98.6 \pm 1.2\%$ with RSDs $< 2.1\%$ for the 2nd batch, $85.6 \pm 1.6\%$ to $98.9 \pm 3.9\%$ with RSDs $< 3.9\%$ for the 3rd batch, $83.4 \pm 2.1\%$ to $100.1 \pm 1.4\%$ with RSDs $< 2.5\%$ for the 4th batch, $87.1 \pm 3.8\%$ to $99.6 \pm 1.4\%$ with RSDs $< 4.4\%$ for the 5th batch and $84.3 \pm 1.9\%$ to $98.1 \pm 1.6\%$ with RSDs from $< 2.3\%$ for the 6th batch.

Both recoveries and %RSDs values were in the acceptable range recommended by the AOAC (at the concentration of 1.0 mg L^{-1} in which recoveries and RSDs must be 80-110% and below 16%, respectively). These results indicated that the preparation procedure of polypyrrole microspherical magnetic sorbent proposed in this work can be reproduced with good reproducibility.

3.4.3 Reusability

To study the number of extraction cycles that polypyrrole microspherical magnetic sorbent can be reused. The reusability was studied (section 2.5). The average %response from three prepared batches were plotted against the extraction cycles. It was found that the polypyrrole microspherical magnetic sorbent can be reused up to 24 times (Fig. S3) and the average %responses were $99.9 \pm 1.2\%$ for carbaryl, $94.8 \pm 1.2\%$ for carbofuran and $84.42 \pm 0.97\%$ for methomyl. The %RSDs were in the range of 0.99-1.2% were achieved and they were better than the acceptable values recommended by the AOAC (%RSD less than 11% at the concentration of 1.0 mg L^{-1}) indicating the good reusability of the prepared sorbent. The material cost of 150 mg of

sorbent was only 0.38 USD and after it was divided by 24 times reusable, the cost for one extraction was further reduced to only 0.016 USD compared to conventional SPE sorbent of C₁₈ that can be used only once (150 mg of C₁₈ SPE Packing ca. 3.29 USD from Sigma-Aldrich, Singapore) (Sigma-Aldrich, 2019).

3.4.4 Precision

To further validate the developed method, the precision was then investigated by analysis of the spiked known concentration of carbamate pesticides in guava sample. The extraction and the analysis were performed under the optimum conditions (n=6). The %RSDs obtained from the determination of carbaryl, carbofuran and methomyl were in the range of 0.11 to 4.5%. These values were better than the RSD limits recommended by the AOAC (11% at the analytes concentration of 1.0 mg L⁻¹). These results indicated that good precision was achieved with the analytical scheme using the developed polypyrrole microspherical magnetic sorbent to extract carbamate pesticides and subsequently analyze using HPLC-DAD.

3.5 Analysis of real samples

The matrix effect on the extraction efficiency was first studied by spiking standard solution of carbamate pesticides in real samples. For the standard curve, standard solutions were prepared in ultrapure water. These two sets of standard solutions were extracted and analyzed under the optimum conditions and the slopes obtained from both set of standard plots were compared using two-way ANOVA (analysis of variance). If the statistical test showed significantly different between the two slopes ($P < 0.05$), this indicated that the sample matrix interfered the accuracy of the developed method. The results from this study showed that the slopes of the standard and matrix matched calibration plots were significantly different for all samples. Therefore, the matrix matched calibration plot of each sample was used to determine the concentration of analytes.

The developed polypyrrole microspherical magnetic sorbent was applied to detect carbaryl, carbofuran and methomyl in 10 fruit and vegetable samples and the samples were prepared before proceeding to the extraction process with the procedure mentioned in section 2.6. Carbaryl was detected in apple at the concentration of

$0.458 \pm 0.038 \mu\text{g L}^{-1}$, carbofuran was found in tomato at the concentration of $1.89 \pm 0.10 \mu\text{g L}^{-1}$ and methomyl was found in watermelon at the concentration of $5.79 \pm 0.28 \mu\text{g L}^{-1}$ (Table 1). These values were then converted to the concentration unit of $\mu\text{g kg}^{-1}$ and these following values were obtained; $0.458 \pm 0.038 \mu\text{g kg}^{-1}$ for carbaryl, $1.89 \pm 0.10 \mu\text{g kg}^{-1}$ for carbofuran and $5.79 \pm 0.28 \mu\text{g kg}^{-1}$ for methomyl. They were much lower than the MRLs values recommended by the ACFS, however, these compounds can be accumulated in the fat tissue of the human body (Yan et al., 2019; Wei et al., 2018) so these fruits and vegetables were considered to “safe to consume” after proper cleaning.

The accuracy of the method was reported in term of the extraction recoveries, the extraction and the analysis of all spiked samples were in the ranges of $91.1 \pm 2.8\%$ to $108.3 \pm 2.2\%$ for carbaryl, $90.7 \pm 6.0\%$ to $108.5 \pm 1.1\%$ for carbofuran and $81.6 \pm 1.5\%$ to $108.0 \pm 1.0\%$ for methomyl (Table 1). These recovery values were in acceptable range of the AOAC guideline (40-120% at the analyte concentration of $1.0 \mu\text{g L}^{-1}$, 60-115% at the analyte concentration of $10 \mu\text{g L}^{-1}$ and 80-110% at the analyte concentration of 10^2 - $10^3 \mu\text{g L}^{-1}$) (Latimer, 2016) which confirmed the good accuracy of the developed method.

4. Conclusions

A new developed magnetic sorbent was successfully synthesized by a time-dependent template polymerization. Polypyrrole magnetic sorbent was obtained in a spherical shape in micrometer size with large surface area and high porosity. This will induce the sorbents to exhibit good adsorption and desorption efficiencies towards the interested target analytes, since the aqueous sample solution can easily solvate around the polypyrrole magnetic microsphericals. In addition, the magnetic sorbent was environmentally friendly and inexpensive compared with the C₁₈ commercial sorbent (about 9 times at the same sorbent weight of 150 mg and more than 200 times cheaper in case of 24 times reusability of the synthesized magnetic sorbent). This polypyrrole magnetic microsphericals can play a vital role as a sorbent material to interact with carbamate pesticides via π - π interactions and hydrogen bonding with easy extraction method. The developed method was used to detect the residual pesticides in fruit and vegetable samples, and the detected amount were less than the recommended maximum residue levels (MRL) recommended by the National Bureau of Agricultural Commodity and Food Standards (ACFS), Ministry of Agriculture and Cooperative, Thailand. However, these compounds can accumulate in the fatty tissue of the body, continuous consuming without proper cleaning may be harmful to human body. In addition, good accuracy indicated by the acceptable recoveries was achieved, expecting that the developed method can be applied for the extraction of other organic compounds in various kinds of samples.

Acknowledgments

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Figure Captions:

Fig. 1 Preparation procedure of polypyrrole magnetic microsphericals

Fig. 2 Photo of polypyrrole microspherical magnetic sorbent (a) dispersed in water solution and (b) separated from solution with an external magnet

Fig. 3 (a) SEM image of polypyrrole microspherical magnetic sorbent surface with magnification at 90000× (b) EDX spectrum of polypyrrole microspherical magnetic sorbent

Fig. 4 FTIR spectrum of polypyrrole magnetic microsphericals recorded in the frequency region of 400–4000 cm^{-1}

Fig. 5 Optimization of type of (a) extraction time (b) amount of sorbent (c) %remaining of the three pesticides in the spiked ultrapure water after extraction (d) volume of desorption solvent (e) desorption time (f) stirring rate of desorption and (g) type of desorption solvent.

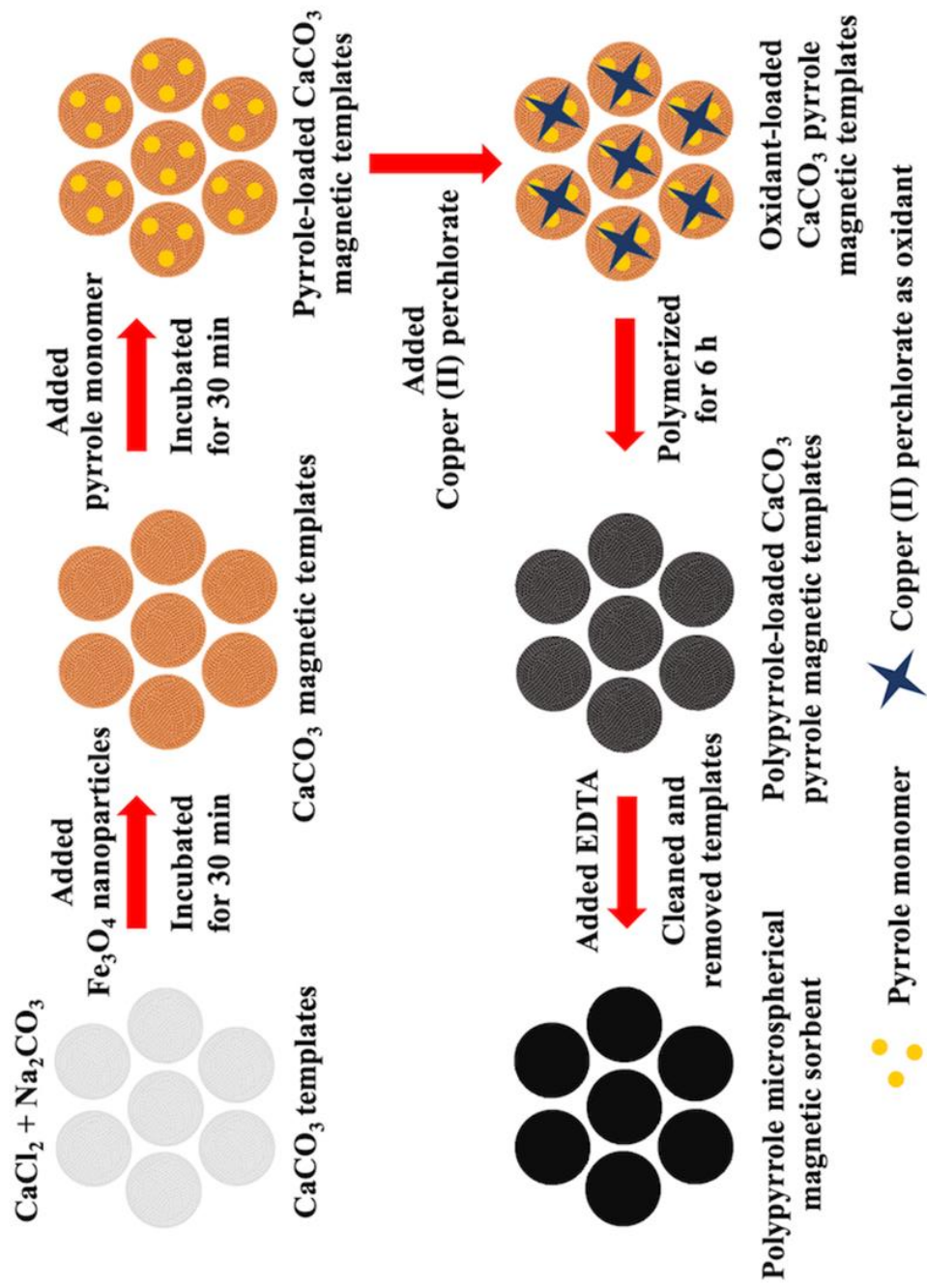


Fig. 1 Preparation procedure of polypyrrole magnetic microsphericals

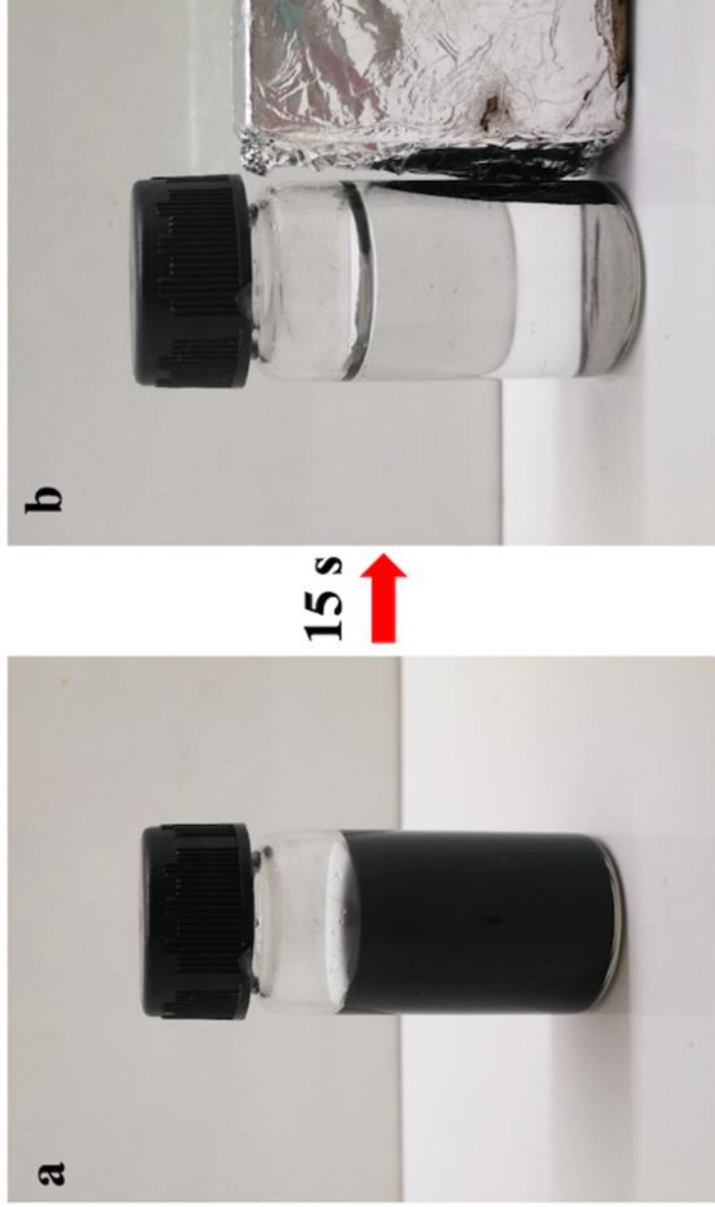


Fig. 2 Photo of polypyrrole microspherical magnetic sorbent (a) dispersed in water solution and (b) separated from solution with an external magnet

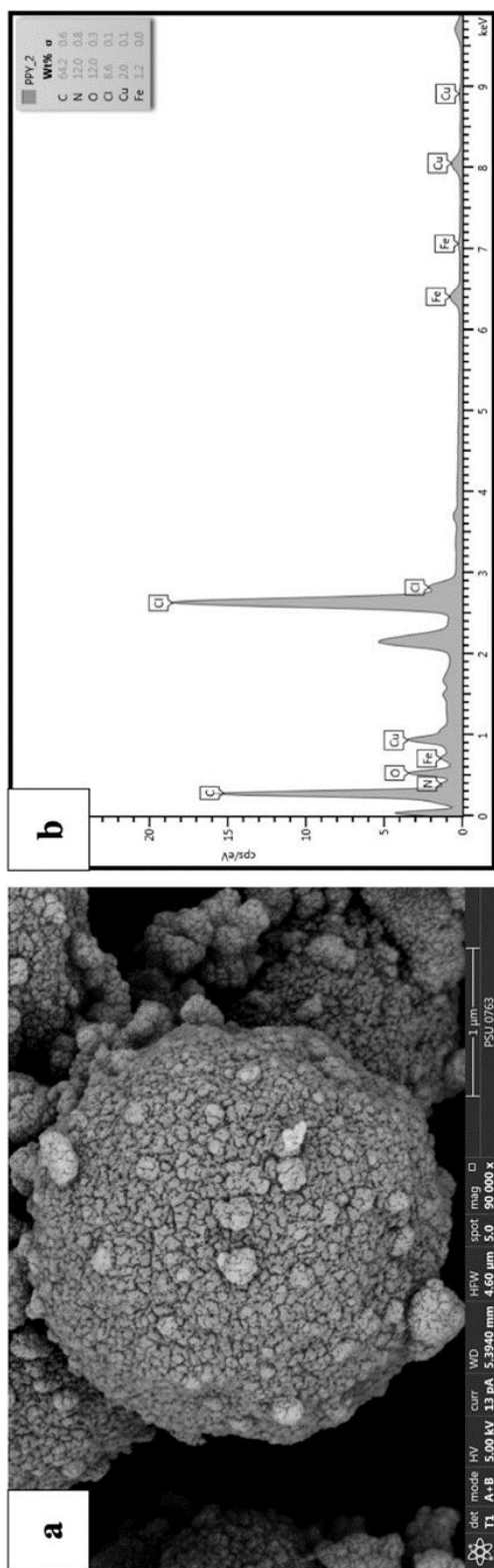


Fig. 3 (a) SEM image of polypyrrole microspherical magnetic sorbent surface with magnification at 90000×
 (b) EDX spectrum of polypyrrole microspherical magnetic sorbent

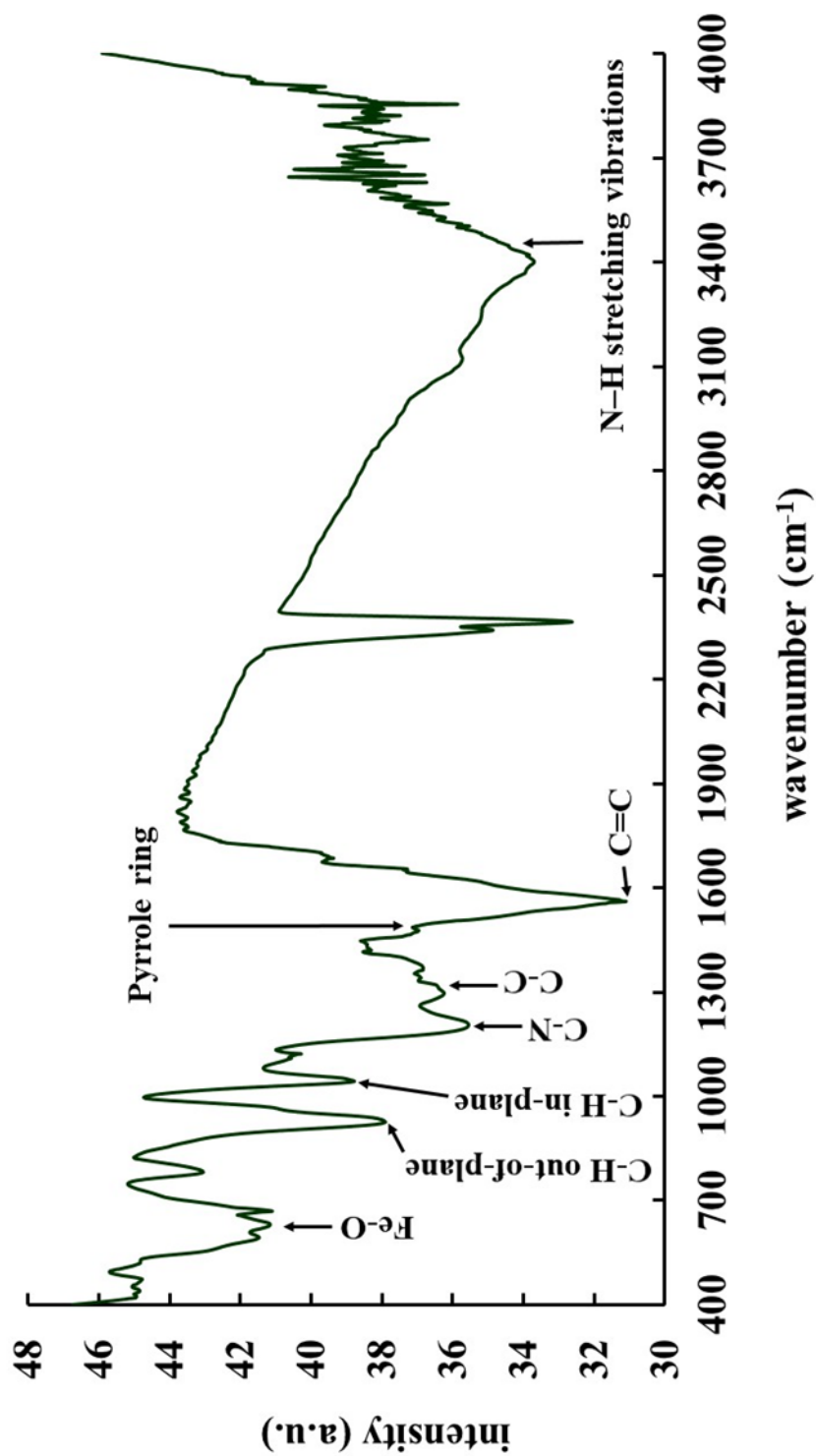


Fig.4 FTIR spectrum of polypyrrole magnetic microsphericals recorded in the frequency region of 400–4000 cm⁻¹

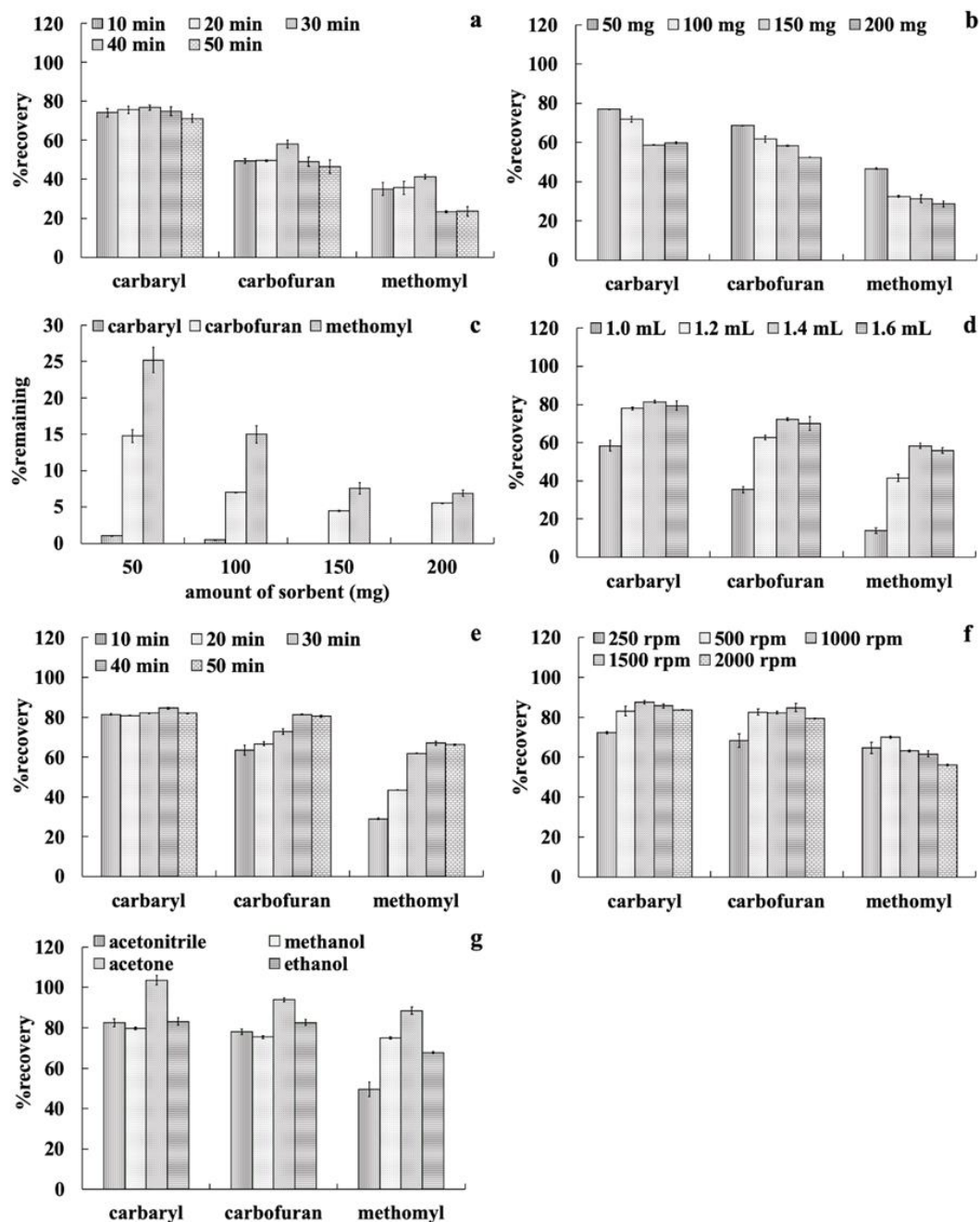


Fig. 5 Optimization of type of (a) extraction time (b) amount of sorbent (c) %remaining of the three pesticides in the spiked ultrapure water after extraction (d) volume of desorption solvent (e) desorption time (f) stirring rate of desorption and (g) type of desorption solvent.

Table 1 Recovery percentages of methomyl, carbofuran and carbaryl from spiked standard solution in real sample

| Sample | Carbaryl | | | Carbofuran | | | Methomyl | | |
|------------|---|---|-------------|---|---|-------------|---|---|-------------|
| | Detected concentration ($\mu\text{g L}^{-1}$) | Spiked concentration ($\mu\text{g L}^{-1}$) | %Recovery | Detected concentration ($\mu\text{g L}^{-1}$) | Spiked concentration ($\mu\text{g L}^{-1}$) | %Recovery | Detected concentration ($\mu\text{g L}^{-1}$) | Spiked concentration ($\mu\text{g L}^{-1}$) | %Recovery |
| Guava | ND ^a | 5 | 103.9±1.9 | ND ^a | 5 | 94.4±4.3 | ND ^a | 5 | 83.3±1.7 |
| | | 10 | 99.25±0.13 | | 10 | 93.1±1.7 | | 10 | 81.6±1.5 |
| | | 50 | 101.51±0.49 | | 50 | 94.35±0.55 | | 50 | 82.4±1.5 |
| Apple | 0.458±0.038 ^b | 100 | 99.83±0.16 | ND ^a | 100 | 97.75±0.10 | ND ^a | 100 | 82.6±1.4 |
| | | 5 | 98.2±1.6 | | 5 | 98.9±2.9 | | 5 | 99.2±3.1 |
| | | 10 | 100.41±0.27 | | 10 | 98.7±1.2 | | 10 | 99.8±1.9 |
| Rose apple | ND ^a | 50 | 100.29±0.49 | | 50 | 100.9±1.1 | | 50 | 102.0±2.9 |
| | | 100 | 99.98±0.10 | | 100 | 100.22±0.22 | | 100 | 100.26±0.63 |
| | | 5 | 108.2±2.8 | ND ^a | 5 | 108.1±1.0 | ND ^a | 5 | 107.6±0.81 |
| Orange | ND ^a | 10 | 108.3±2.2 | | 10 | 106.8±3.3 | | 10 | 101.6±2.0 |
| | | 50 | 108.1±1.2 | | 50 | 108.5±1.1 | | 50 | 103.9±4.3 |
| | | 100 | 105.2±2.5 | | 100 | 104.8±1.5 | | 100 | 105.6±2.9 |
| Watermelon | ND ^a | 5 | 91.1±2.8 | ND ^a | 5 | 92.8±2.1 | ND ^a | 5 | 91.1±7.2 |
| | | 10 | 101.8±2.1 | | 10 | 97.0±1.5 | | 10 | 99.1±2.3 |
| | | 50 | 102.9±5.5 | | 50 | 101.24±0.85 | | 50 | 102.9±1.8 |
| Tomato | ND ^a | 100 | 99.2±6.9 | | 100 | 101.03±0.19 | | 100 | 99.27±0.36 |
| | | 5 | 100.61±0.87 | ND ^a | 5 | 90.7±6.0 | 5.79±0.28 ^b | 5 | 108.0±1.0 |
| | | 10 | 98.3±3.1 | | 10 | 104.1±1.8 | | 10 | 102.2±7.7 |
| Thai Basil | ND ^a | 50 | 104.4±3.8 | | 50 | 100.4±2.7 | | 50 | 107.0±1.7 |
| | | 100 | 105.3±1.6 | | 100 | 99.3±1.8 | | 100 | 98.7±1.2 |
| | | 5 | 95.42±0.75 | 1.89±0.10 ^b | 5 | 94.1±2.4 | ND ^a | 5 | 98.42±0.28 |
| Cabbage | ND ^a | 10 | 102.0±1.9 | | 10 | 102.2±1.1 | | 10 | 103.0±1.9 |
| | | 50 | 102.9±1.0 | | 50 | 105.64±0.77 | | 50 | 104.0±1.1 |
| | | 100 | 98.8±3.1 | | 100 | 99.6±2.7 | | 100 | 98.8±2.3 |
| Cucumber | ND ^a | 5 | 93.51±0.75 | ND ^a | 5 | 94.1±2.4 | ND ^a | 5 | 93.61±2.3 |
| | | 10 | 97.7±1.9 | | 10 | 97.9±1.1 | | 10 | 97.0±2.1 |
| | | 50 | 103.2±1.1 | | 50 | 99.76±0.77 | | 50 | 103.2±1.1 |
| Lettuce | ND ^a | 100 | 99.9±4.6 | | 100 | 97.7±2.5 | | 100 | 98.1±3.2 |
| | | 5 | 97.7±1.3 | ND ^a | 5 | 93.49±0.58 | ND ^a | 5 | 98.2±1.3 |
| | | 10 | 101.1±1.7 | | 10 | 103.2±1.4 | | 10 | 99.3±1.7 |
| Guava | ND ^a | 50 | 101.01±0.95 | | 50 | 102.0±2.1 | | 50 | 96.1±3.0 |
| | | 100 | 100.2±4.6 | | 100 | 99.9±2.8 | | 100 | 98.8±1.8 |
| | | 5 | 104.67±0.87 | ND ^a | 5 | 105.14±0.60 | ND ^a | 5 | 107.3±7.5 |
| Apple | ND ^a | 10 | 104.1±3.1 | | 10 | 99.8±1.6 | | 10 | 104.6±2.8 |
| | | 50 | 100.6±3.8 | | 50 | 100.3±2.7 | | 50 | 106.8±2.8 |
| | | 100 | 107.4±5.9 | | 100 | 100.4±1.8 | | 100 | 101.1±1.5 |
| Orange | ND ^a | 5 | 102.1±3.4 | ND ^a | 5 | 101.06±0.60 | ND ^a | 5 | 104.6±7.5 |
| | | 10 | 99.8±2.3 | | 10 | 98.2±3.1 | | 10 | 101.9±5.1 |
| | | 50 | 106.69±0.71 | | 50 | 99.4±1.9 | | 50 | 101.5±4.5 |
| Watermelon | ND ^a | 100 | 108.07±0.90 | | 100 | 100.4±1.1 | | 100 | 100.83±0.93 |

ND^a is non-detectable (<LOD); ^bdetectable; not determined (>LOD, <LOQ)

Supplementary data

A novel polypyrrole microspherical magnetic sorbent for the extraction of trace carbamate pesticides in fruits and vegetables

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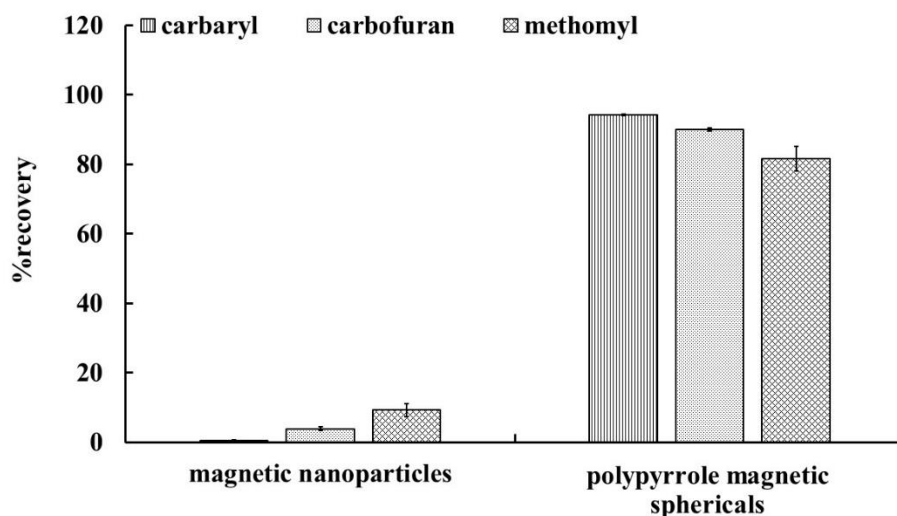


Fig. S1 Extraction recoveries of magnetic nanoparticles and polypyrrole magnetic microsphericals for the extraction of three carbamate pesticides at the concentration of 0.20 mg L^{-1} in water; sample volume: 1.2 mL; amount of sorbent: 150 mg; extraction time: 30 min; stirring rate: 500 rpm; desorption solvent: 1.4 mL acetone; desorption time: 40 min

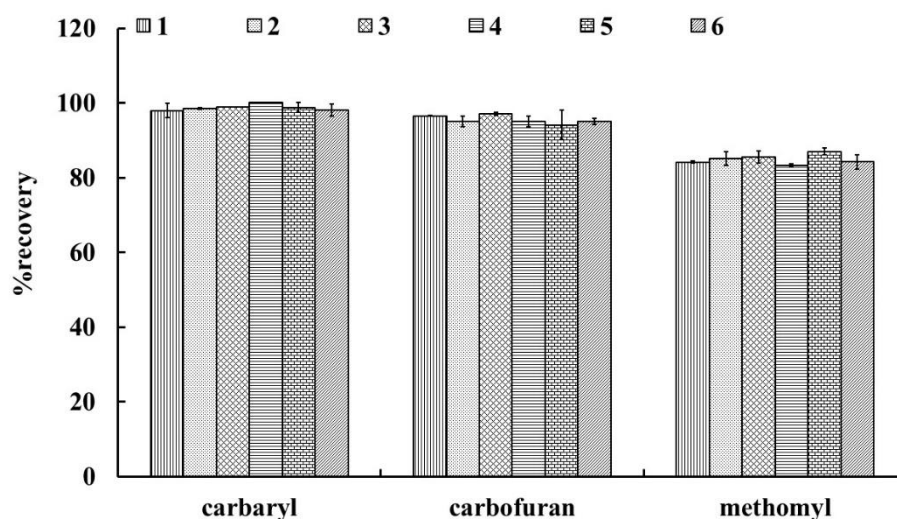


Fig. S2 Reproducibility of polypyrrole magnetic microsphericals for the extraction of three carbamate pesticides at the concentration of 0.20 mg L^{-1} in water; sample volume: 1.2 mL; amount of sorbent: 150 mg; extraction time: 30 min; stirring rate: 500 rpm; desorption solvent: 1.4 mL acetone; desorption time: 40 min

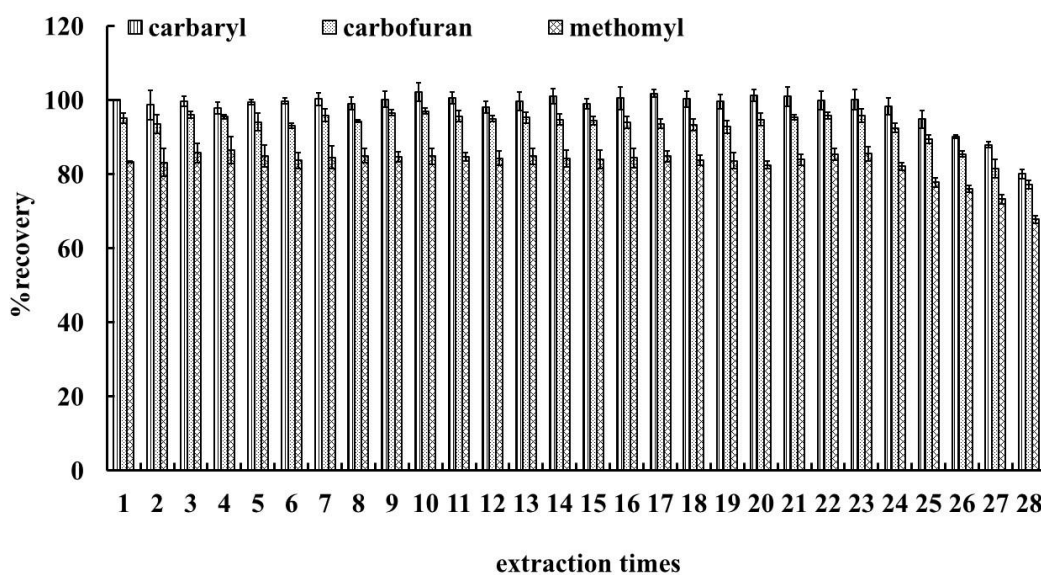


Fig. S3 Reusability of polypyrrole magnetic microsphericals for the extraction of three carbamate pesticides at the concentration 0.20 mg L^{-1} in water; sample volume: 1.2 mL; amount of sorbent: 150 mg; extraction time: 30 min; stirring rate: 500 rpm; desorption solvent: 1.4 mL acetone; desorption time: 40 min

Table S1 Summary of optimum parameters for the extraction of carbamate pesticides using polypyrrole microspherical magnetic sorbent with HPLC-DAD

| Parameters | Investigated values | Optimum conditions |
|------------------------|---|--------------------|
| Extraction time (min) | 10 – 50 | 30 |
| Amount of sorbent (mg) | 25 – 200 | 150 |
| Desorption volume (mL) | 1.0 – 1.6 | 1.4 |
| Desorption time (min) | 10 – 50 | 40 |
| Stirring rate (rpm) | 250 – 2000 | 500 |
| Desorption solvent | acetonitrile, methanol, acetone and ethanol | acetone |

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Scholarship Awards during Enrolment

The Faculty of Science Research Fund, Prince of Songkla University, Contract no. 1-2559-02-008. The Center of Excellence for Innovation in Chemistry (PERCH-CIC) and Graduate School. The Center of Excellence for Trace Analysis and Biosensor (TAB-CoE).

List of Poster presentations and Publication

Changsan, T., Wannapob, R., Kanatharana, P., Thavarungkul P., and Thammakhet-Buranachai, C. "Magnetite microparticles for solid phase extraction of pesticides analysis". 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.

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Manuscript submission to Food Chemistry