DEVELOPMENT OF FLUOROMETRIC METHOD FOR CARBARYL DETERMINATION USING MCM-41 CONTAINING CETYLTRIMETHYLAMMONIUM BROMIDE

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การพัฒนาวิธีวัดฟลูออเรสเซนส์สำหรับหาปริมาณคาร์บาริลโดยใช้ MCM-41 ที่มีเซทิลไตรเมทิลแอมโมเนียมโบรไมด์

นางสาวธิดา อุ่นใจดี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเคมี
มหาวิทยาลัยเทคโนโลยีสุรนารี
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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

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ธิดา อุ่นใจดี : การพัฒนาวิธีวัดฟลูออเรสเซนส์สำหรับหาปริมาณคาร์บาริลโดยใช้ MCM-41 ที่มีเซทิลไตรเมทิลแอมโมเนียมโบรไมด์ (DEVELOPMENT OF FLUOROMETRIC METHOD FOR CARBARYL DETERMINATION USING MCM-41 CONTAINING CETYLTRIMETHYLAMMONIUM BROMIDE)
อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร.สัญชัย ประยูรโภคราช, 46 หน้า.

คำสำคัญ: ซิลิกาเมโซพอร์ MCM-41 เซทิลไตรเมทิลแอมโมเนียมโบรไมด์ ฟลูออเรสเซนซ์ คาร์บาริล

งานวิจัยนี้ประสบความสำเร็จในการพัฒนาวิธีฟลูออเรสเซนซ์สำหรับตรวจวัดสารตกค้างคาร์บาริลในผัก วิธีนี้อาศัยปฏิกิริยาระหว่างคาร์บาริลกับวัสดุมีโซพอรัส Mobil Composition of Matter No. 41 (MCM-41) ที่มีเซทิลไตรเมทิลแอมโมเนียมโบรไมด์ (CTAB) ซึ่งเรียกว่า MCM-41-CTAB ทำให้เกิด 1-แนฟทอล ซึ่งสามารถตรวจวัดได้ด้วยเทคนิคสเปกโทรฟลูออโรเมตรี โดยสาร 1-แนฟทอลที่เกิดขึ้นให้สัญญาณฟลูออเรสเซนซ์ที่ความยาวคลื่น 485 นาโนเมตร เมื่อถูกกระตุ้นด้วยแสงที่ความยาวคลื่น 335 นาโนเมตร

MCM-41 สังเคราะห์ได้โดยใช้เตตระเอทอกซีไซเลนเป็นแหล่งให้ซิลิกาและ CTAB เป็นสารแม่แบบ MCM-41 ที่สังเคราะห์ได้นำมาวิเคราะห์ลักษณะด้วย เทคนิคการเลี้ยวเบนของรังสีเอ็กซ์ (XRD) เทคนิคฟูเรียร์ทรานส์ฟอร์มอินฟราเรดสเปกโทรสโกปี (FTIR) เทคนิคเอ็กซ์เรย์โฟโตอิเล็กตรอนสเปกโทรสโกปี (XPS) เทคนิคเทอร์โมกราวิเมตรี (TGA) และเทคนิคจุลทรรศน์อิเล็กตรอนแบบส่องกราด (SEM) ผลการวิเคราะห์ยืนยันว่า MCM-41-CTAB มีโครงสร้างมีโซพอรัสที่เป็นระเบียบ มีหมู่ฟังก์ชัน มีพื้นผิวของหมู่เบส และมีเสถียรภาพทางความร้อน

ปฏิกิริยาไฮโดรไลซิสระหว่างคาร์บาริลกับ MCM-41-CTAB ได้รับการปรับให้เหมาะสมอย่างเป็นระบบ พบว่าสภาวะที่มีประสิทธิภาพสูงสุดคือการใช้ MCM-41-CTAB ปริมาณ 100 มิลลิกรัม ทำปฏิกิริยากับสารละลายคาร์บาริลในเอทานอล 50% เป็นเวลา 60 นาที ภายใต้สภาวะเหล่านี้ วิธีที่พัฒนาขึ้นแสดงความสัมพันธ์เชิงเส้นในช่วงความเข้มข้นของคาร์บาริล 0.12–10 ไมโครโมลาร์ โดยมีขีดจำกัดการตรวจวัดที่ 0.11 ไมโครโมลาร์

นำวิธีที่พัฒนาขึ้นมาไปใช้กับตัวอย่างจริงเพื่อตรวจวัดสารตกค้างของคาร์บาริลในพริกและมะเขือเปราะไทย ผลที่ได้นำไปเปรียบเทียบกับผลที่ได้จากเทคนิคโครมาโตกราฟีของเหลวสมรรถนะสูงแบบอัลตร้า (UHPLC) พบว่าทั้งสองวิธีให้ผลลัพธ์ที่สอดคล้องกันเป็นอย่างดี นอกจากนี้ศึกษาการนำ MCM-41-CTAB กลับมาใช้ใหม่ พบว่าประสิทธิภาพของวัสดุลดลงตามจำนวนครั้งที่ใช้ ซึ่งเกิดจากการชะล้างของ CTAB ออกจากโครงสร้างของวัสดุ นอกจากนี้มีการศึกษาการรบกวนจากสารอื่นพบว่าไม่มีผลกระทบอย่างมีนัยสำคัญต่อสัญญาณฟลูออเรสเซนซ์ของ 1-แนฟทอล ซึ่งยืนยันความจำเพาะของ

วิธีการ ผลการศึกษาบ่งชี้ว่าวิธีที่พัฒนาขึ้นมาสามารถใช้สำหรับการตรวจวัดคาร์บาริลตกค้างในผลิตภัณฑ์ทางการเกษตรอย่างมีประสิทธิภาพและแม่นยำ

สาขาวิชาเคมี ลายมือชื่อนักศึกษา ธิดา อุ่นใจดี

ปีการศึกษา 2567 ลายมือชื่ออาจารย์ที่ปรึกษา

THIDA OUNJAIDEE : DEVELOPMENT OF FLUOROMETRIC METHOD FOR CARBARYL DETERMINATION USING MCM-41 CONTAINING CETYLTRIMETHYLAMMONIUM BROMIDE. THESIS ADVISOR : ASSOC. PROF. SANCHAI PRAYOONPOKARACH, Ph. D. 46 PP.

Keywords: mesoporous silica, MCM-41, fluorescence, carbaryl

This research successfully developed a fluorometric method to determine carbaryl residues in vegetables. The method is based on the reaction of carbaryl with Mobil Composition of Matter No. 41 (MCM-41) containing cetyltrimethylammonium bromide (CTAB) denoted as MCM-41-CTAB, which produces 1-naphthol. The generated 1-naphthol exhibited a strong fluorescence signal at 485 nm when excited at 335 nm, enabling its spectrofluorometric detection.

MCM-41 was synthesized using tetraethoxysilane as the silica source and CTAB as the template. The MCM-41 was characterized using X-ray diffraction (XRD) analysis, Fourier transform infrared (FTIR) spectroscopy, X-ray photoelectron spectroscopy (XPS), thermogravimetric analysis (TGA), and scanning electron microscopy (SEM), confirming its ordered mesoporous structure, functional groups, basic species on the as-synthesized MCM-41, and thermal stability.

The hydrolysis reaction between carbaryl and MCM-41-CTAB was systematically optimized, revealing that the most effective conditions involved 100 mg of MCM-41-CTAB in a carbaryl solution containing 50% ethanol, with a reaction time of 60 min. Under these conditions, the developed method showed a linear response for carbaryl concentration ranging from 0.12 to 10 μM, with a detection limit of 0.11 μM.

The developed method was successfully applied to real sample analysis, where carbaryl residues in chili and Thai eggplant were quantified. The results were validated against ultra-high-performance liquid chromatography (UHPLC), demonstrating strong agreement between the two methods. Additionally, the reusability of MCM-41-CTAB was investigated, showing a gradual decline in activity after multiple uses due to the leaching of CTAB. Furthermore, an interference study confirmed that the presence of tested species had negligible effects on the

fluorescence signal of 1-naphthol, highlighting the method's selectivity. The findings suggest that the developed method is a promising tool for efficiently and accurately detecting carbaryl residues in agricultural products.

School of Chemistry
Academic Year 2024

Student's Signature ธิดา อุ่นใจดี
Advisor's Signature

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The successful completion of the project on developing a fluorometric method for carbaryl determination using MCM-41 containing cetyltrimethylammonium bromide was made possible through the support of research funding, access to laboratory facilities, and the invaluable guidance of Assoc. Prof. Dr. Sanchai Prayoonpokarach, my thesis supervisor, dedicated his time to advising the research and assisting in information gathering.

I sincerely appreciate the members of the analysis and catalysis group for their valuable advice, assistance during the experiments, and support in resolving various challenges, all of which played a crucial role in the project's success.

Finally, I would like to express my gratitude for the research subsidy from the Fundamental Fund (FF66) under the Science, Research, and Innovation. I also extend my thanks to The Center for Scientific and Technological Equipment at Suranaree University of Technology, along with the laboratory staff and personnel who provided assistance and facilitated access to the instruments. I am grateful for the ongoing support provided by the School of Chemistry, Institute of Science, Suranaree University of Technology, which greatly contributed to the success of this project.

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CHAPTER I

INTRODUCTION

1.1 Significance of the study

In commercial agricultural crop production, pesticides are necessary to mitigate the impact of pests, diseases, and weeds, which can significantly reduce crop yields. Without effective management strategies, these factors may lead to extensive crop losses, resulting in decreased agricultural productivity and disruptions in the food supply chain. Therefore, the use of pesticides remains a fundamental aspect of agricultural practices.

Carbaryl, or 1-naphthyl methylcarbamate, is a carbamate insecticide. It is used to control various pests, including aphids, fire ants, and spiders, on fruits, vegetables, nuts, and other crops (Thai Agriculture Standard, TAS 9002-2016). Carbaryl contains both polar (carbamate group) and nonpolar (naphthalene ring) regions, as shown in Figure 1.1 This results in its low solubility in water, 40 mg/L at 30 °C. It is also chemically stable with a slow decomposition rate and a half-life of 1600 days in an aqueous solution of pH 5. These properties lead to carbaryl persistence in the environment and crops after harvesting, raising health concerns (Lee et al., 2018).

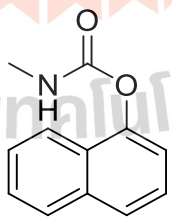

Figure 1.1 A structure of carbaryl.

Human exposure to carbaryl primarily occurs through contaminated agricultural products, with food being the dominant intake route. This pesticide can cause adverse health effects such as headaches, memory loss, muscle weakness, and anorexia due to its inhibition of acetylcholinesterase, a key enzyme that regulates neurotransmission (Yan et al., 2013; Zheng et al., 2011). Although trace levels of carbaryl have been detected in drinking water, significant contamination is uncommon. The World Health Organization (WHO) establishes a safety limit of 0.09 ppm, and general exposure through drinking water remains low except in cases of accidental over-spraying or contamination of surface water (Kanyika-Mbewe et al., 2020; WHO, 2008). Given its widespread use in agriculture, monitoring carbaryl residues in food is critical to ensuring consumer safety. The Thai Agriculture Standard (TAS 9002-2016) regulates maximum residue limits (MRLs) for carbaryl in specific fruits and vegetables. Some examples are shown in Table 1.1. Studies have reported carbaryl residues in vegetables and fruits (Fan et al., 2015). Therefore, it is important to monitor carbaryl regularly in agricultural produce to minimize exposure risks.

Table 1.1 The maximum residue limits of carbaryl in crops regulated by the National Bureau of Agricultural Commodity and Food Standards Ministry of Agriculture and Cooperatives, Thailand.

Agricultural Commodities	Maximum Residue Limits (MRL), (mg/kg)
lead tree leaves, cacao beans	0.02
grapes, chili	0.5
rambutan, rice, watermelon, coconut, mangosteen, Thai eggplant	1
peanut	2
mango	3
sweet peppers	5
apple	15
longan	20
durian	30

Carbaryl detection is crucial in environmental and food safety monitoring, requiring reliable analytical methods. Traditional techniques such as gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) are widely used for accurate quantification (Chen et al., 2022). However, these chromatographic methods involve complex sample preparation, require expensive equipment, and can be time-consuming. To address these limitations, researchers have developed alternative detection techniques that are faster and more cost-effective. Among these, spectrometric methods have gained attention due to their efficiency and ability to provide reliable results.

In addition to exploring alternative techniques, researchers often focus on detecting carbaryl's hydrolysis product, 1-naphthol, rather than the parent compound itself. This is because 1-naphthol is more stable and easier to analyze in aqueous environments, with a water solubility of 866 mg/L at 25 °C. Additionally, it exhibits

higher sensitivity in spectrophotometric and fluorometric detection methods (Massey et al., 1995), allowing for more accurate measurements.

Since 1-naphthol is the primary hydrolysis product of carbaryl, its formation is a crucial step in detection methods that rely on absorbance or fluorescence measurements. The hydrolysis process occurs in alkaline solutions, where hydroxide ions (OH^-) break the carbamate bond in carbaryl, leading to 1-naphthol formation. Studies have demonstrated that bases such as sodium hydroxide (NaOH) (Xu et al., 2025), potassium hydroxide (KOH) (Jiao et al., 2022), and tetrabutylammonium hydroxide (TBAOH) (Lee et al., 2018) can facilitate this reaction. Among these, NaOH is the preferred reagent due to its high efficiency and widespread availability.

To minimize chemical waste in laboratory analyses, this study explores an alternative approach using mesoporous materials for carbaryl detection. Instead of relying on conventional strong bases such as NaOH, this method utilizes the basicity of Mobil Composition of Matter No. 41 (MCM-41), containing cetyltrimethylammonium bromide (CTAB). The basicity of MCM-41 originates from its siloxy anion (SiO^-) groups, which interact electrostatically with the positively charged head of CTAB molecules. These siloxy anions act as basic sites for the hydrolysis of carbaryl, promoting the formation of 1-naphthol without the need for excess chemical reagents. The hydrolysis product, 1-naphthol, can then be detected fluorometrically, emitting at approximately 470 nm upon excitation at 365 nm.

1.2 Research objectives

This study aims to develop an effective method for detecting carbaryl using MCM-41 containing CTAB (MCM-41-CTAB). To achieve this, the research focuses on investigating key factors that influence the interaction between MCM-41-CTAB and carbaryl, ensuring optimal conditions for detection. Finally, the developed method will be applied to analyze carbaryl residues in vegetable and fruit samples, providing a practical approach for monitoring pesticide contamination in agricultural produce.

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CHAPTER II

LITERATURE REVIEW

2.1 Carbaryl detection

Carbaryl is widely used in agriculture, and its residues may remain in food products, posing risks if consumed above permissible limits. Monitoring carbaryl is crucial due to its potential effects on human health and the environment.

Carbaryl is typically determined using chromatographic methods, such as gas chromatography-mass spectrometry and high-performance liquid chromatography (Chen et al., 2022) with either UV/ vis absorbance or fluorescence detection (Massey et al., 1995). Despite offering robust trace analysis characterized by exceptional sensitivity and high reproducibility, these methods have some limitations. Sophisticated equipment is required, and sample preparation and purification are laborious and time-consuming. Alternative methods have also been reported, such as electrochemical methods (Kunpatee et al., 2023; Lv et al., 2023; Silva J. d. O. S. et al., 2024) and colorimetric methods (Amatatongchai et al., 2023; Raja et al., 2023). Extensive research has been devoted to developing methods for carbaryl analysis that are simple, fast, sensitive, selective, accurate, and user-friendly. Spectrometric methods are among those that serve such requirements. Some reported spectrometric methods for carbaryl determination are shown in Table 2.1.

In this study, a fluorometric method for determining carbaryl was developed based on the reaction of carbaryl with Mobil Composition of Matter No. 41 (MCM-41) containing cetyltrimethylammonium bromide (CTAB) to produce 1-naphthol. 1-Naphthol yielded a fluorescence signal at 470 nm upon excitation at 365 nm, which could be used for carbaryl determination.

Table 2.1 Some reported spectrometric methods for carbaryl determination.

Probe	Method	LOD ($\mu g\ mL^{-1}$)	Samples	Ref.
$H_{39}GFP\text{-}Cu^{2+}$	Fluorometric	7.03×10^{-6}	Apple, tomato & cucumber	(Lei et al., 2015)
CdSe–ZnS-MIP	Fluorometric	2.9×10^{-2}	Rice & Chinese cabbage	(Zhang et al., 2015)
SiQDs–AChE–ChOx	Photoluminescence	7.25×10^{-6}	Tomatoes & cucumbers	(Yi et al., 2013)
CQDs-AChE& ChOx	Photoluminescence	5.39×10^{-6}	Apples	(Li et al., 2016)
Cys-AuNPs	UV-vis	7.5	Chili	(Rujiralai et al., 2020)

2.2 Hydrolysis of carbaryl

The detection of carbaryl using spectroscopic techniques revealed that carbaryl absorbs light in the UVB range and emits light in the UVA range, producing a low signal unsuitable for quantification. Therefore, it is necessary to convert carbaryl into other substances that absorb or emit light with a sufficiently strong signal for accurate quantification.

Carbaryl can undergo a hydrolysis reaction in an NaOH solution (Shahdost-Fard et al., 2021), according to Figure 2.1, to form 1-naphthol, which can be used as an indicator for the detection and quantification of carbaryl based on absorbance or fluorescence measurement, as 1-naphthol exhibits blue fluorescence. However, a limitation of using NaOH in solution form is its inability to be separated and recovered after the reaction, resulting in waste generation and increased costs for reagent replacement. Additionally, the basic nature of NaOH may lead to undesired side reactions with other compounds in the sample, potentially interfering with fluorescence measurements. In summary, while NaOH has been traditionally used for

the hydrolysis of carbaryl in fluorescence detection methods, its limitations have prompted the exploration of alternative solid-phase reagents like MCM-41. These alternatives offer benefits in terms of reusability, specificity, and safety, making them attractive options for developing more efficient and sustainable analytical methods. In this research, MCM-41-CTAB will be investigated for its ability to hydrolyze carbaryl to produce 1-naphthol.

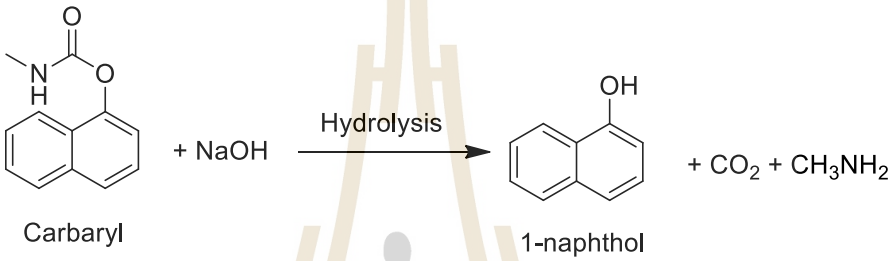


Figure 2.1 Reaction of carbaryl in NaOH solution (Lee et al., 2018).

2.3 Mobil Composition of Matter No. 41 (MCM-41)

According to the definition of the International Union of Pure and Applied Chemistry (IUPAC), porous materials can be classified into three types based on pore size, including microporous (pore size <2 nm), mesoporous (2-50 nm), and macroporous (>50 nm) materials (Chaudhary et al., 2017). MCM-41 is a mesoporous silica material used in various applications, including catalysis, chemical separation, adsorption, drug delivery, and sensors (Costa et al., 2020), due to its high surface area, tunable pore sizes ranging from 2.5 to 6 nm (Gil García et al., 2017), and significant thermal and chemical stability. Additionally, it has the ability to incorporate various metal oxides into its framework and can be easily functionalized with organic or inorganic molecules.

Mesoporous silica materials can be synthesized through various methods, with the choice of method often depending on the desired structure, pore size, and application. For MCM-41, a typical synthesis method is a sol-gel process using tetraethoxysilane (TEOS) as a silica precursor and cetyltrimethylammonium bromide as a surfactant that acts as a template to create the mesoporous structure. CTAB creates spherical micelles and TEOS is used to form the mesoporous structure in MCM-41. CTAB forms spherical micelles, with the hydrophobic tails of the surfactant

molecules oriented inward and the hydrophilic heads pointing outward, in the aqueous phase, as shown in Figure 2.2. TEOS undergoes hydrolysis and condensation to form silica. The silica molecules are attracted to the hydrophilic heads of the CTAB surfactant molecules and get deposited on the surface of the micelles, leading to the formation of a mesoporous silica structure around the micelles. The silica precursor polycondenses around the micelles, forming a hexagonal arrayed structure (Hoffmann et al., 2006).

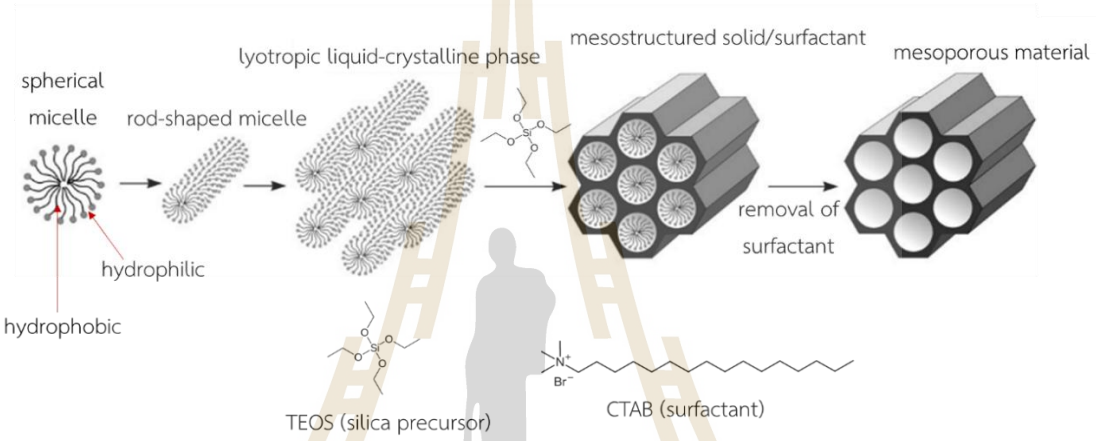


Figure 2.2 Formation of mesoporous materials (Hoffmann et al., 2006).

This study used the as-synthesized MCM-41, which still contains the surfactant within the pores, as an alternative solid-phase reagent to convert carbaryl to 1-naphthol. The basicity of MCM-41 arises from the presence of siloxy anion (SiO^-) groups, which electrostatically interacted with the positive head of the CTAB molecules. These negatively charged siloxy groups contribute to MCM-41's basicity by acting as Lewis bases, as shown in Figure 2.3. (Zapelini I. W. et al., 2018). The siloxy anions could be useful for a hydrolysis reaction of carbaryl to produce 1-naphthol, which can be monitored fluorometrically.

Figure 2.3 An illustration of the MCM-41 structure's basic siloxy site with charge compensation provided by CTA^+ or CTAB (Zapelini I. W. et al., 2018).

The basicity of MCM-41 containing CTAB has been widely utilized in various studies due to its rapid and simple synthesis, regular pore structure, uniform pore diameter, and high surface area. The as-synthesized MCM-41, with the surfactant present inside the pores, exhibits basic catalytic activity. A study conducted by Kubota et al. (2006) showed that the material exhibited exceptional performance as a basic catalyst in the Knoevenagel condensation reaction at room temperature. No activity was observed when calcined MCM-41 was used in the reaction. Characterization of the as-synthesized MCM-41 by ^{29}Si MAS NMR and O 1s XPS techniques verified that the active sites responsible for the catalysis were the basic siloxy anion, SiO^-. Because the mesopores were occluded, a reaction involving the as-synthesized MCM-41 occurred at the entrance of the pores (Martins et al., 2007). There has been no report on using MCM-41-CTAB for carbaryl detection. Therefore, in this work, MCM-41 was employed for carbaryl analysis.

MCM-41 possesses several properties that make it highly effective for adsorption applications. Its large surface area provides numerous sites for adsorption, making it ideal for capturing molecules from gases or liquids. The narrow pore size distribution allows for selective adsorption of molecules of specific sizes, improving its efficiency in separating or capturing target molecules. The pore size can be adjusted during synthesis, enabling optimization for various applications such as gas storage, pollutant removal, or selective separation. Additionally, MCM-41 has a relatively large

pore volume, which enhances its ability to adsorb significant amounts of low-molecular-weight compounds or gases. With high thermal stability (up to 800°C) and chemical stability (Kim et al., 1995), MCM-41 is well-suited for adsorbing molecules in harsh environments without degradation. The well-organized mesoporous structure also provides high accessibility for molecules interacting with the surface, making MCM-41 effective for adsorbing both small and large molecules. Beyond these advantages, MCM-41's strong adsorption capabilities make it particularly useful in analytical applications. By capturing foreign species that could interfere with fluorescence measurements, it helps improve the accuracy of fluorescence-based carbaryl detection.

CHAPTER III

EXPERIMENTAL

3.1 Chemicals

The chemicals utilized in this research are listed in Table 3.1.

Table 3.1 Chemicals used in this research.

Chemicals	Formula	Content	Supplier
1-naphthol	$C_{10}H_7OH$	99.0%	Kemaus
1-naphthyl-N-methylcarbamate (carbaryl)	$C_{12}H_{11}NO_2$	97.0%	Sigma-Aldrich
acetonitrile (for HPLC)	CH_3CN	99.8%	Supelco®
ammonia	NH_3	30.0%	Carlo Erba
calcium chloride	$CaCl_2$	95.0%	Unilab
cetyltrimethylammonium bromide (CTAB)	$C_{19}H_{42}NBr$	99.0%	Acros
ethanol	C_2H_5OH	99.9%	Carlo Erba
magnesium chloride hexahydrate	$MgCl_2 \cdot 6H_2O$	98-102%	BDH, AnalaR®
methanol	CH_3OH	99.9%	Carlo Erba
methanol (for HPLC)	CH_3OH	99.9%	Carlo Erba
methomyl	$C_5H_{10}N_2O_2S$	98.0%	Supelco®
sodium hydroxide	NaOH	99.0%	Carlo Erba
tetraethyl orthosilicate (TEOS)	$Si(OC_2H_5)_4$	98.0%	Sigma-Aldrich
zinc acetate dihydrate	$Zn(CH_3CO_2)_2 \cdot 2H_2O$	99.5%	Carlo Erba

3.2 Synthesis of MCM-41-CTAB

MCM-41-CTAB was synthesized following the procedure described in the literature (Griin et al., 1997). A sol solution was prepared by heating and stirring a mixture of 2.50 g of CTAB and 50.0 g of de-ionized (DI) water. Once the solution cooled to room temperature, 13.2 g of aqueous ammonia and 60.0 g of ethanol were added. The mixture was stirred at 250 rpm for 15 min, and then 4.67 g of TEOS was rapidly added while the stirring continued. After 2 h of stirring, the resulting white precipitate was filtered using suction, washed thoroughly with 100 mL of DI water, and rinsed with 100 mL of methanol to remove residual reactants. Finally, the solid was dried overnight at 90 °C in an air oven to obtain MCM-41-CTAB.

3.3 Characterization of MCM-41-CTAB

MCM-41-CTAB was characterized using the following techniques:

- Powder X-ray diffraction (XRD, Bruker D8 ADVANCE) to confirm the presence of a mesoporous structure. A powder sample was placed in a sample holder, and measurements were conducted using Cu $K\alpha$ radiation at 40 kV. The 2θ scan ranged from 0.5 to 10° with a step size of 0.02°.
- Field emission scanning electron microscopy (FE-SEM, Auriga Carl Zeiss) to examine the morphology of MCM-41-CTAB. The sample was dispersed onto carbon tape adhered to metal stubs and then coated with carbon.
- Fourier-transform infrared spectroscopy (FTIR, Tensor27-Hyperion, Bruker) to identify functional groups on MCM-41-CTAB. The sample was prepared by mixing it with KBr in a 1:50 weight ratio, then compressing 25 mg of the mixture at 3.5 tons for 2 min. FTIR spectra were recorded with a resolution of 4 cm^{-1}, averaging 64 scans.
- X-ray photoelectron spectroscopy (XPS, PHI 5000 VersaProbe II XPS system) to analyze the elemental composition and chemical states of surface-bound species. The sample was dispersed onto carbon tape attached to metal stubs and analyzed using Al $K\alpha$ radiation. XPS peak fitting was performed with the Multipak program, using an 80% Gaussian distribution, with the full width at half-maximum (FWHM) of

the fitted peaks around 1.80 eV. The binding energy (BE) peaks were then calibrated utilizing the C 1s peak at 284.80 eV as a standard reference (Seejandee et al., 2024).

- Thermogravimetric analysis (TGA, Mettler Toledo, TGA/DSC1) to determine the CTAB content in the MCM-41-CTAB. A 10 mg sample was placed in an alumina holder and heated from 35 °C to 700 °C at a rate of 10 °C/min under an air-zero atmosphere (O_2 + N_2) with a flow rate of 100 mL/min.

3.4 Reaction of carbaryl and MCM-41-CTAB

MCM-41-CTAB was mixed with a carbaryl solution in a 20 mL vial and stirred at 500 rpm for 1 h. After stirring, the mixture was filtered through a 0.45 µm Nylon syringe filter. The filtrate was used for fluorescent measurements with a spectrofluorometer (Sarspec, Spec res+). The effects of carbaryl concentration, solvent type, reaction time, and MCM-41-CTAB mass were investigated in this study.

3.5 Effect of solvent on the reaction between carbaryl and MCM-41-CTAB

Carbaryl has low solubility in water (~40 mg/L) at 30 °C, but its solubility varies under different conditions. Since the efficiency of the reaction between carbaryl and MCM-41-CTAB depends on carbaryl's solubility, this study examined the influence of different solvents on its dissolution. To investigate solvent effects, carbaryl was dissolved in various ethanol-to-DI water co-solvent mixtures with ethanol volume percentages of 0, 5, 10, 25, 33, and 50%. The ethanol solutions were mixed with MCM-41-CTAB at a given time. After that the mixtures were filtered. The obtained solutions were used for fluorescence measurements.

3.6 Calibration study

Under the optimized reaction conditions determined in Sections 3.4 and 3.5, MCM-41-CTAB was mixed with a series of carbaryl solutions of varying concentrations. Fluorescence signals were measured at an emission wavelength of 485 nm. The resulting data were used to construct a calibration graph for carbaryl.

3.7 Real sample analysis

To assess the practicality of the developed method, real samples of chili and Thai eggplant were collected from local markets near Suranaree University of Technology and analyzed for carbaryl. Sample preparation and carbaryl extraction were carried out by soaking the vegetable sample (25 g Thai eggplant, 3.5 g chili) in 40 mL of methanol for 30 min with sonication. The obtained liquid was evaporated at 50 °C for 10 min or until the liquid volume was reduced to 1-2 mL. After that, the remaining liquid was transferred into a 25 mL volumetric flask, and the solution was made up to the volume using a 50% ethanol solution. Similar sample solutions were also prepared with the addition of carbaryl for the recovery study. An aliquot of 5 mL of 0.0497 mM carbaryl was added to the sample solutions to obtain a carbaryl added concentration of 10 μM.

For the fluorescence measurements, a 20 mL sample solution was mixed with MCM-41-CTAB under the optimized experimental conditions. For method validation, the carbaryl concentration in the samples was determined using ultra high performance liquid chromatography (UHPLC, Agilent Technologies, 1290) with an Agilent ZORBAX SB C18 Rapid Resolution HD column (2.1 × 150 mm, 1.8 μm). The mobile phase was a mixture solution of water and acetonitrile at a 55:45 (v/v) ratio and the mobile phase flow rate was 0.3 mL/min. The sample injection volume was 2.000 μL. The eluate detection was performed using a diode-array detector (DAD). The results obtained from the chromatographic analysis were compared with those obtained from the fluorescence experiments.

3.8 Interferent study

The influence of foreign species on the reaction between carbaryl and MCM-41-CTAB was investigated. To ensure accurate analysis of the target species, this study evaluated the method's selectivity in the presence of other pesticides (e.g., methomyl) and metal cations (e.g., Mg^{2+}, Ca^{2+}, Zn^{2+}) that could be commonly found in real samples due to irrigation water. The experiments were conducted under the optimized reaction conditions. MCM-41-CTAB was mixed with carbaryl solutions of fixed concentrations with/without the addition of potential interfering species. Methomyl and metal cations were introduced at the concentration of 30 μM, and their effects on

the fluorescence signal were evaluated by comparing signal intensities to those obtained from carbaryl-only solutions.

3.9 Reusability of MCM-41-CTAB

MCM-41-CTAB was studied for its reactivity after being used in the reaction. Following each reaction cycle, the material was collected and washed thoroughly with 20 mL of DI water to remove residual carbaryl. The washed MCM-41-CTAB was then dried overnight at 60 ℃ in an air oven before reuse. To assess its reusability, the MCM-41-CTAB was mixed with a carbaryl solution of fixed concentration under the optimized reaction conditions, and fluorescence signals were measured.

CHAPTER IV
RESULTS AND DISCUSSION

This chapter presents the characterization and performance evaluation of MCM-41-CTAB as a reactant in the hydrolysis of carbaryl. The structural and chemical properties of MCM-41-CTAB were examined using various analytical techniques, including XRD, FTIR, XPS, and FE-SEM, to confirm its mesoporous structure, functional groups, elemental composition, and morphology. The reactivity of MCM-41-CTAB was investigated by studying its interaction with carbaryl under different conditions, highlighting its basicity in converting carbaryl to 1-naphthol. The influence of solvent composition, MCM-41-CTAB quantity, and reaction time on the hydrolysis efficiency was systematically explored to optimize reaction parameters. Furthermore, the reliability of the developed method was validated through fluorescence spectroscopy and UHPLC, demonstrating high reproducibility and accuracy in detecting carbaryl in real samples, such as chili and Thai eggplant extracts. The findings support the potential of MCM-41-CTAB as an effective base for pesticide degradation, with implications for environmental remediation and food safety analysis.

4.1 Characterization of MCM-41-CTAB

The structural characteristics of MCM-41-CTAB were analyzed XRD. MCM-41-CTAB was synthesized with TEOS as the silica source and CTAB as the template. The XRD pattern, as shown in Figure 4.1, exhibits a prominent diffraction peak at 2.3° (2θ), which corresponds to the ordered mesoporous structure of MCM-41-CTAB. This observation is consistent with previous findings reported by Grün et al. (1997)

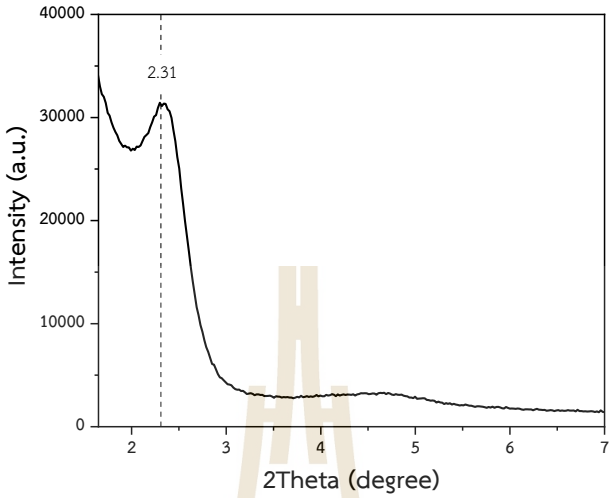


Figure 4.1 XRD pattern of MCM-41-CTAB.

The functional groups of the as-synthesized MCM-41-CTAB were analyzed using FTIR. The results are shown in Figure 4.2. The wavenumbers at 2923 and 2852 cm^{-1} correspond to the -CH_2 groups of CTAB, while the peaks at 1049, 954, and 795 cm^{-1} are characteristic of silica (Farrokhzadeh et al., 2019).

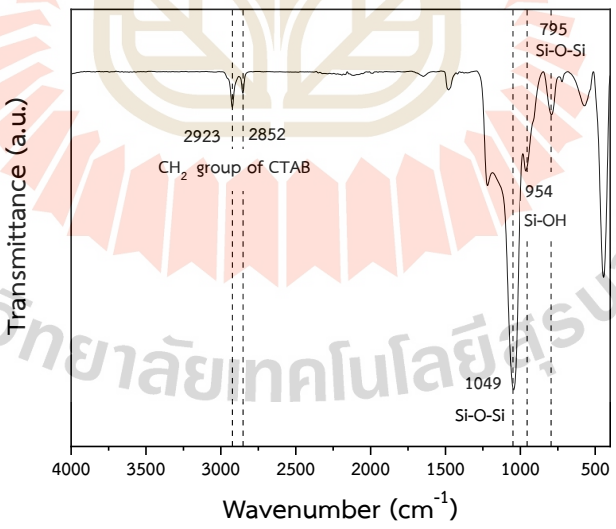


Figure 4.2 FTIR spectrum of MCM-41-CTAB.

The morphology of MCM-41 was characterized by SEM. The results are shown in Figure 4.3. The SEM images reveal that most particles have spherical shape, though

some agglomeration is present. The particles exhibit a range of sizes from 400 to 1100 nm, with an average diameter of 620 nm.

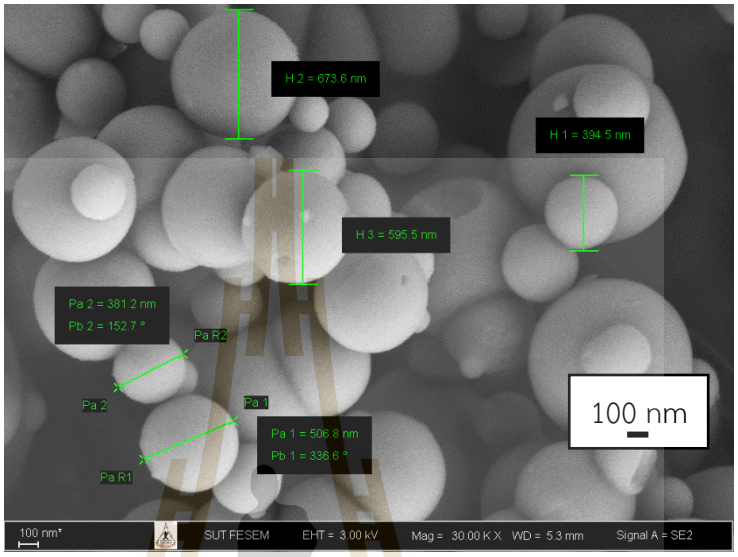


Figure 4.3 SEM images of MCM-41-CTAB.

4.2 Reaction of carbaryl and MCM-41-CTAB

The synthesized MCM-41-CTAB was mixed with a carbaryl solution in ethanol inside a tightly sealed polypropylene (PP) bottle. The mixture was stirred magnetically for 1 h and then filtered. The fluorescence signal of the solution was then measured using a spectrofluorometer (Horiba, Fluoromax-Plus), and the spectra obtained are shown in Figure 4.4. Before mixing with MCM-41-CTAB, the carbaryl solution exhibited an emission peak at 335 nm under excitation at 290 nm, which is characteristic of carbaryl. After mixing with MCM-41-CTAB, a new fluorescence peak appeared at 462 nm upon excitation at 335 nm, indicating the presence of 1-naphthol. Similar fluorescence spectra of 1-naphthol were also reported for the reaction of carbaryl and sodium hydroxide solution (Lee et al., 2018; Shahdost-Fard et al., 2021). These results suggest that MCM-41-CTAB facilitates the hydrolysis of carbaryl to form 1-naphthol.

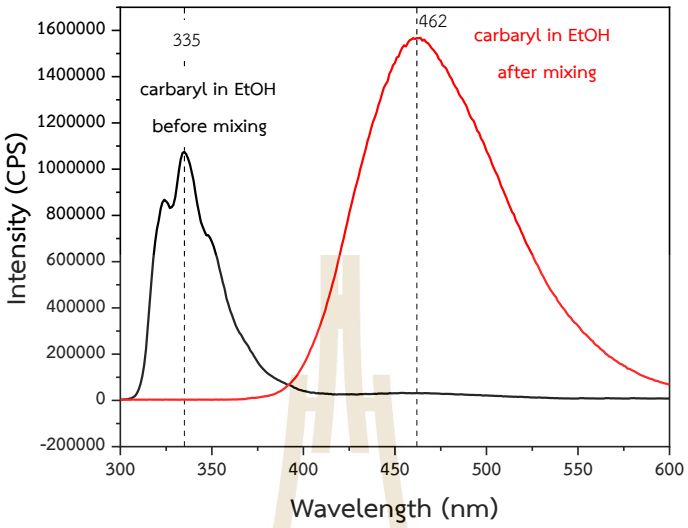


Figure 4.4 Fluorescence spectra of the carbaryl solution in ethanol before and after mixing with MCM-41-CTAB.

MCM-41 with CTAB retained within its pores exhibits significant basicity. This basicity originates from siloxy anion (SiO^-) groups, which electrostatically interact with the positively charged head of CTAB molecules, thereby stabilizing the basic sites (Zapelini et al., 2018). To confirm the presence of these basic sites, an XPS analysis was conducted. The XPS spectra of oxygen 1s in Figure 4.5 exhibit a peak at 530 eV, characteristic of SiO^- groups, confirming the basic nature of MCM-41-CTAB. These basic sites are likely responsible for facilitating the hydrolysis of carbaryl, enabling its conversion into 1-naphthol.

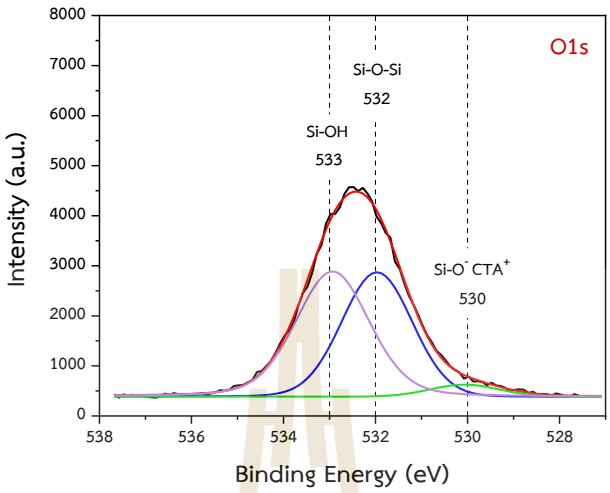


Figure 4.5 XPS spectra of oxygen 1s of MCM-41-CTAB.

4.2.1 Effect of solvent on the reaction between carbaryl and MCM-41-CTAB

Carbaryl dissolves readily in ethanol but has limited solubility in water, approximately 40 mg/L at 30 °C (Derbalah et al., 2020). To optimize ethanol usage while maintaining sufficient carbaryl solubility, experiments were conducted to determine an ethanol-to-water ratio that facilitates its reaction with MCM-41-CTAB. Six solvent mixtures with ethanol concentrations of 0%, 5%, 10%, 25%, 33%, and 50% by volume were tested, and their fluorescence signals were measured using a spectrofluorometer (Sarspec, spec res+).

The results, shown in Figure 4.6, indicate that the reaction between MCM-41-CTAB and carbaryl proceeded efficiently in solvents with 25%, 33%, and 50% ethanol, as evidenced by the fluorescence signal of 1-naphthol. However, in the solvents containing 0%, 5%, and 10% ethanol, no detectable 1-naphthol fluorescence was observed. The fluorescence signal of carbaryl decreased over time under all experimental conditions, suggesting that carbaryl or 1-naphthol may have been adsorbed onto MCM-41-CTAB in solutions with lower ethanol content.

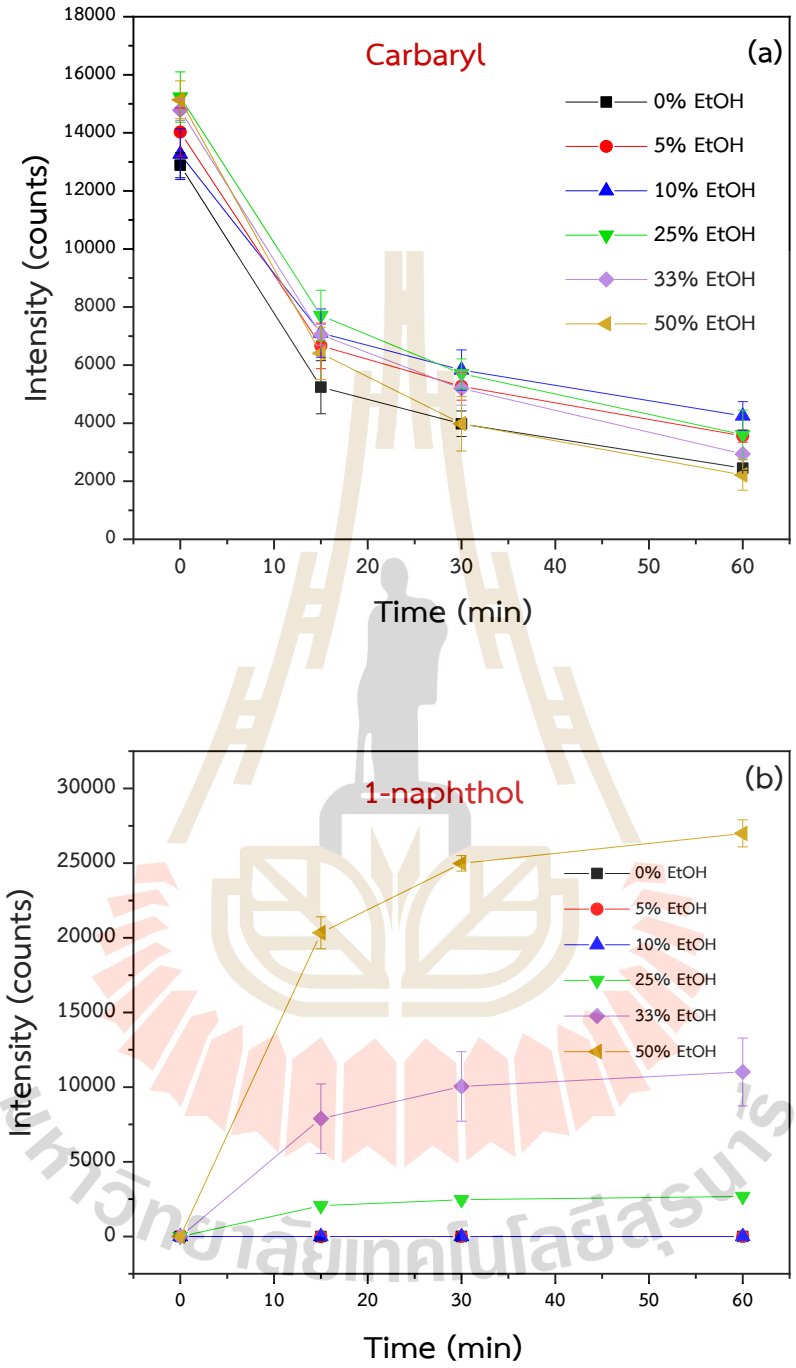


Figure 4.6 Time profiles of fluorescence signals of a) carbaryl and b) 1-naphthol in solvents with various % ethanol. The carbaryl concentration was 0.0497 mM.

To quantify the reaction efficiency, the percentage of unreacted carbaryl was calculated using equation (4.1), and the results are shown in Table 4.1.

$$\%\text{carbaryl remaining in the solution} = \frac{I}{I_0} \times 100 \quad (4.1)$$

where I_0 is the fluorescence signal of the carbaryl solution before adding MCM-41-CTAB, and I is the fluorescence signal of the carbaryl solution after the reaction at different times. The data in Table 4.1 show a clear trend in the effect of solvent composition on reaction efficiency. Carbaryl hydrolysis proceeded most effectively in the solution containing 50% ethanol, where the lowest percentage of unreacted carbaryl was recorded after 60 min. In contrast, reactions in 0%, 5%, and 10% ethanol solutions exhibited limited hydrolysis, likely due to poor carbaryl solubility and increased adsorption onto MCM-41-CTAB. Based on these results, 50% ethanol was identified as the most suitable solvent composition for further experiments, as this condition yielded the highest fluorescence signal of 1-naphthol.

Table 4.1 The content of carbaryl remained in the solutions with various percentages of ethanol after the reaction.

Reaction time (min)	%Carbaryl remained in the solutions with various contents of EtOH					
	0% EtOH	5% EtOH	10% EtOH	25% EtOH	33% EtOH	50% EtOH
15	40.7	47.5	53.6	50.6	47.6	42.3
30	30.9	37.6	43.9	37.5	35.1	26.3
60	19.0	25.4	32.0	23.7	19.9	14.6

4.2.2 Effect of the amount of MCM-41-CTAB on the reaction with carbaryl

The effect of MCM-41-CTAB dosage on the hydrolysis of carbaryl to 1-naphthol was investigated by varying its mass in a 0.0497 mM carbaryl solution (50% ethanol) and measuring the fluorescence signal using a spectrofluorometer (Sarspec, spec res+). The results, shown in Figure 4.7, demonstrated that the fluorescence signal of carbaryl decreased and the fluorescence signal of 1-naphthol increased, corresponding to the increasing amount of MCM-41-CTAB. This behavior is attributed to the increasing amount of base associated with a higher mass of MCM-41-CTAB, which facilitates more efficient hydrolysis of carbaryl to 1-naphthol. The results indicate that 100 mg of MCM-41-CTAB provides the highest 1-naphthol fluorescence signal at 60 min, slightly higher than 75 mg. However, the mass of 100 mg was chosen for further study to ensure the highest carbaryl conversion to 1-naphthol.

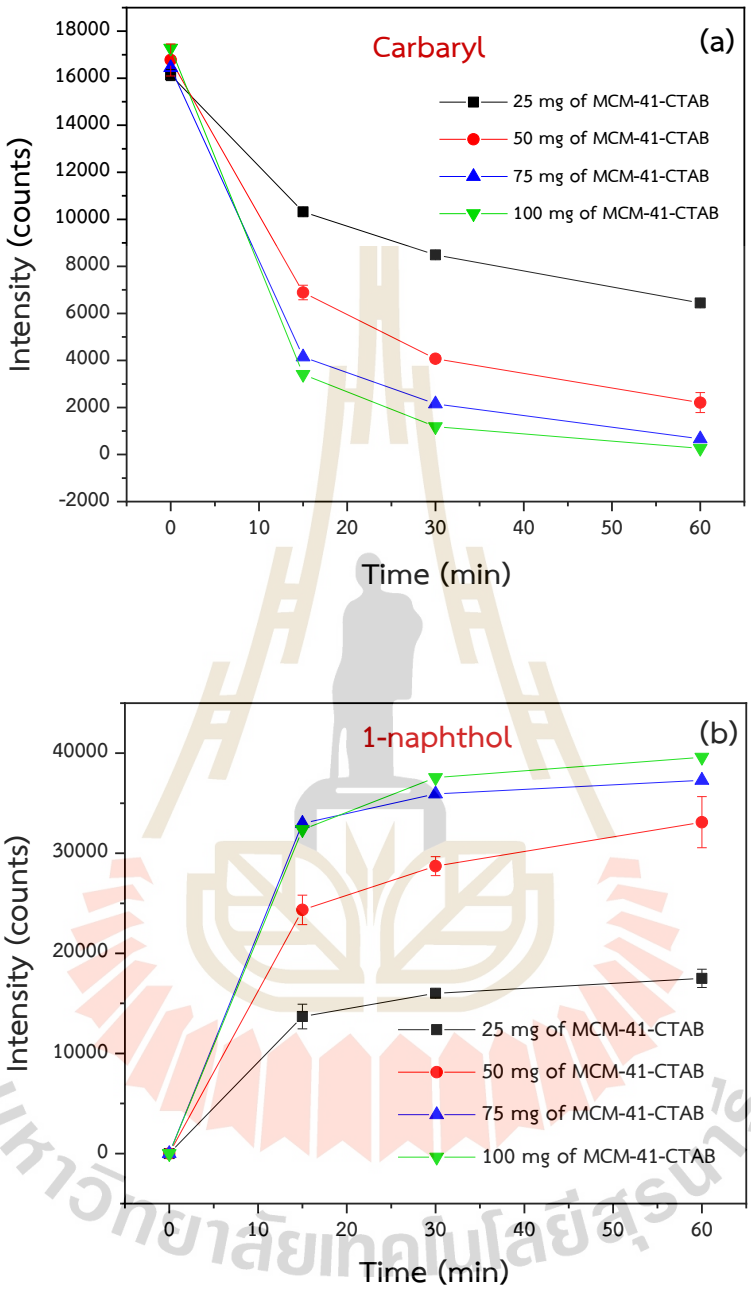


Figure 4.7 Time profiles of fluorescence signals of a) carbaryl and b) 1-naphthol from the reaction with various MCM-41-CTAB mass.

4.3 Calibration study

Carbaryl solutions with concentrations ranging from 0.12 to 10 μM were prepared. The experiment involved mixing carbaryl solutions with MCM-41-CTAB for 60 min, followed by filtration and fluorescence signal measurement using a spectrofluorometer (Sarspec, spec res+). As carbaryl concentration increased, the fluorescence signal of 1-naphthol also increased, indicating enhanced hydrolysis, as shown in Figure 4.8. The linear equation for carbaryl at the reaction time of 60 min is y = 4367.94x+ 1538.82, with an R^2 value of 0.9919.

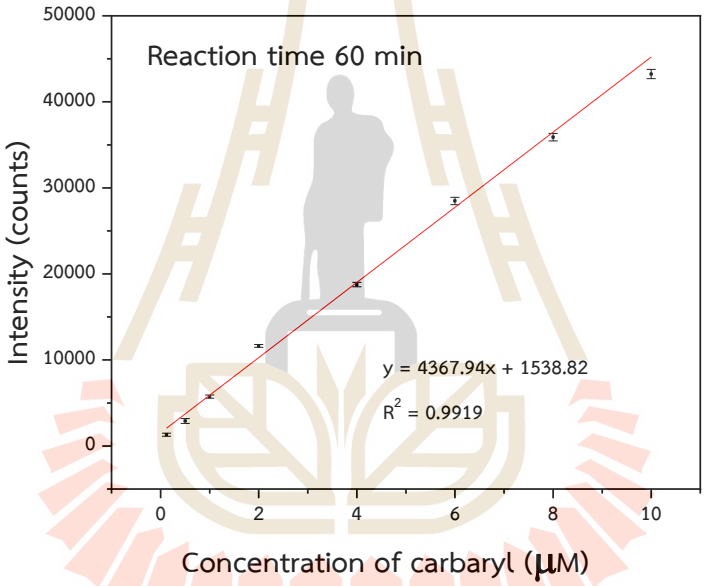


Figure 4.8 Calibration curve for carbaryl at the reaction 60 min.

The detection limit for carbaryl is 0.11 μM calculated from equation (4.2)

$$DL=3\frac{s_{bk}}{m} \quad (4.2)$$

where DL is the detection limit, s_{bk} is the standard deviation of a blank, and m is the slope of the calibration curve.

4.4 Reproducibility study

In this study, the reproducibility of MCM-41-CTAB synthesis and its reactivity with carbaryl was evaluated by conducting identical experiments on two separate occasions. Two samples of MCM-41-CTAB synthesized on January 8, 2024 (MCM-20240108) and May 2, 2024 (MCM-20240502), were used in the study. A 0.0497 mM carbaryl solution in 50% ethanol and 100 mg of MCM-41-CTAB were mixed in a vial and stirred magnetically and fluorescence signals were measured using a spectrofluorometer (Sarspec, spec res+). The fluorescence signals of carbaryl and 1-naphthol were measured at the reaction times of 30 and 60 min. As shown in Table 4.2, the results from the experiment conducted on May 2, 2024 were similar to those from January 8, 2024 (20240108), confirming the reproducibility of both the synthesis of MCM-41-CTAB and the reaction between carbaryl and MCM-41-CTAB.

Table 4.2 Comparison of fluorescence signals of carbaryl and 1-naphthol from the reaction using two batches of MCM-41-CTAB. Each condition was conducted in three replicates.

Sample	Reaction time (min)	Intensity at 485 nm	S.D.	%RSD
MCM-20240108	30	36702	1445	3.94
	60	38333	1805	4.71
MCM-20240502	30	39011	129	0.33
	60	39301	79	0.20

4.5 Reusability of MCM-41-CTAB

In this study, MCM-41-CTAB was first used in a reaction with carbaryl, then collected, washed thoroughly with DI water, and dried overnight at 60 °C before being subjected to a second reaction cycle under identical conditions. Three batches of each fresh and reused MCM-41-CTAB sample were employed in the study. The fluorescence signals of 1-naphthol were measured at 30 and 60 min of the reaction time to determine whether the reused material retained its reactivity using a spectrofluorometer (Horiba, Fluoromax-Plus). The results are presented in Figure 4.9. The fluorescence signals obtained from the reaction with the reused MCM-41-CTAB were lower than those observed with fresh MCM-41-CTAB. The results suggest a reduction in reactivity, which could be from the deactivation of siloxy anion to some extent from the first reaction cycle.

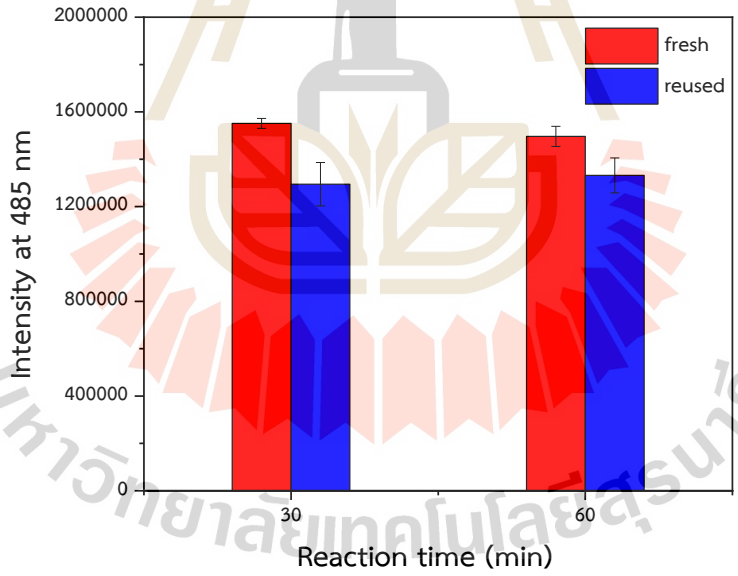


Figure 4.9 Comparison of the fluorescence signals of 1-naphthol from the reaction with fresh and reused MCM-41-CTAB samples.

4.5.1 Thermal analysis of reused MCM-41-CTAB

The amount of CTAB in MCM-41-CTAB before and after reacting with carbaryl was determined using thermogravimetry (Mettler Toledo, TGA/DSC1). The results are shown in Figure 4.10. The DTG curves in Figure 4.10(a) reveal that surfactant decomposition occurred within the temperature range of 136–416 °C. Weight loss below 136 °C is attributed to the removal of physically adsorbed water, while at the temperature above 416 °C the weight loss corresponds to the dehydroxylation of structural silanol groups in the material. Furthermore, a reduction in CTAB content, shown in Figure 4.10(b), was observed after each reaction involving carbaryl, likely due to the leaching of CTAB into the carbaryl solution during the reaction (Deekamwong et al., 2017; Oliveira et al., 2024; Zapelini Iago W. et al., 2018). The loss of CTAB could lead to lower basic sites for carbaryl reaction, resulting in a lower fluorescence signal of 1-naphthol obtained using the reused MCM-41-CTAB.

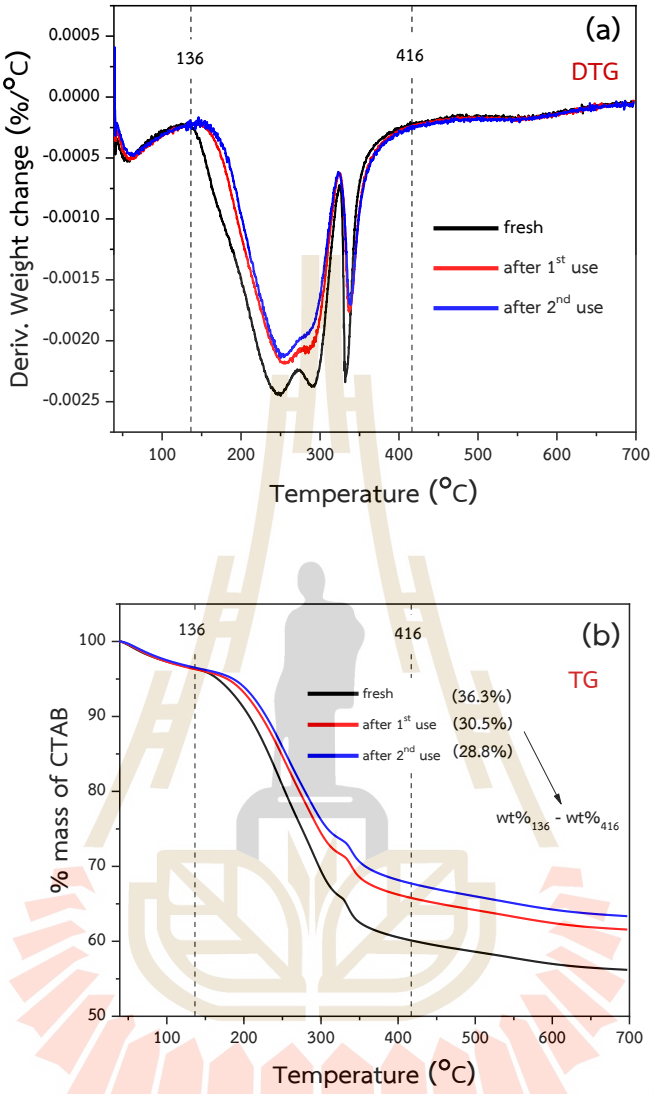


Figure 4.10 Thermograms of MCM-41-CTAB before and after use in the reaction a) derivative weight change and b) %weight loss.

4.5.2 Phase identification analysis of MCM-41-CTAB by XRD

Figure 4.11 shows that after using MCM-41-CTAB in the reaction, the peak of the as-synthesized material shifted from 2.3 to 2.4. Lost the template molecules could lead to the shrinkage of the pore structure, resulting in the peak shift at the higher 2θ indicating a decrease in the % mass of CTAB in the MCM-41 structure. (Deekamwong and Wittayakun, 2017; Silva D. P. S. et al., 2021; Zapelini Iago W. et al., 2018).

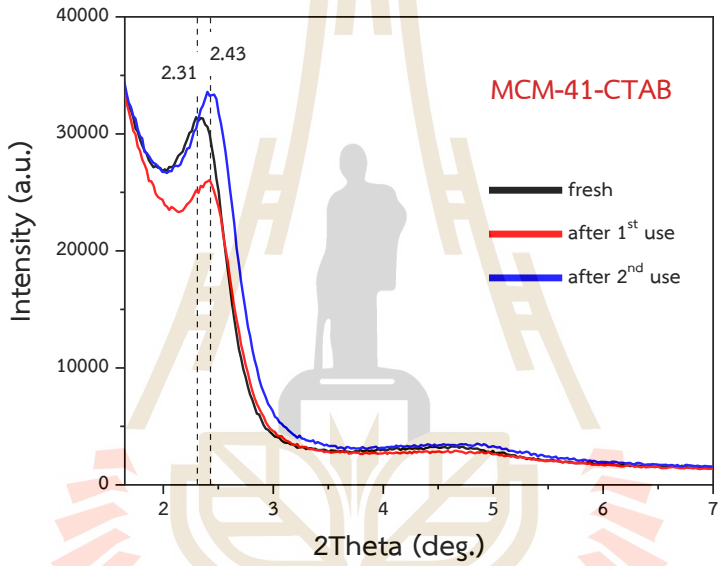


Figure 4.11 XRD pattern of MCM-41-CTAB.

4.5.3 Functional group analysis of MCM-41-CTAB by FTIR

The functional groups of synthesized MCM-41-CTAB after reacting with carbaryl (fresh), after its first use in the reaction with carbaryl (after 1st use), and after its second use in the reaction with carbaryl (after 2nd use) were analyzed using FTIR. A sample was prepared by mixing the MCM-41-CTAB sample and KBr in a 1:50 weight ratio. Then, 25 mg of the mixture was pressed at 3.5 tons for 2 min. Figure 4.12 shows that all sample has similar FTIR spectra. The data obtained from FTIR measurements were calculated to confirm that the CTAB peak decreased after reacting with carbaryl, as hypothesized, with a reduction corresponding to the number of reaction cycles.

The intensity of silica was compared to the intensity of CTAB, as shown in Tables 4.3 and 4.4. Examining the silica peak at 1067 and 795 cm^{-1}, it was observed that the ratio increased with the number of times MCM-41-CTAB was used in the reaction cycle. This suggests that with each reaction cycle, CTAB could be released from the structure, allowing the basic sites in MCM-41-CTAB to interact and hydrolyze carbaryl to form 1-naphthol.

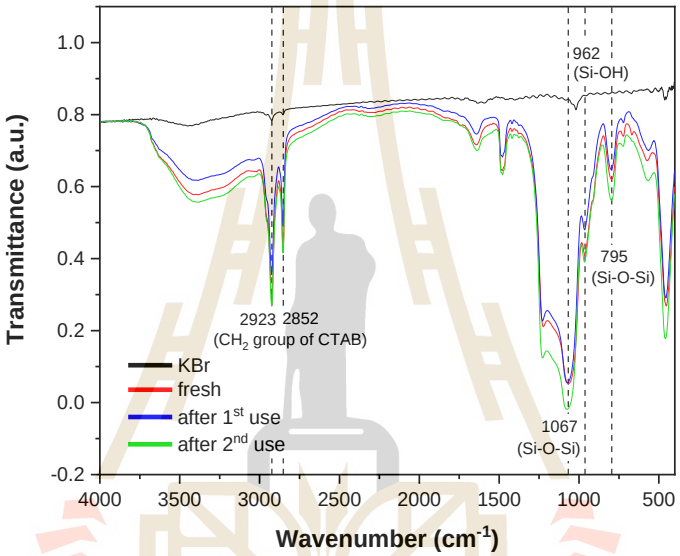


Figure 4.12 FTIR spectrum of MCM-41-CTAB.

Table 4.3 The ratio of the intensity of CTAB and the intensity of silica at 1067 cm^{-1}.

Sample	Intensity of Si-O-Si at 1067 cm^{-1}	Intensity of CTAB at 2923 cm^{-1}	Intensity of CTAB at 2852 cm^{-1}	Ratio of 1067/2923	Ratio of 1067/2852
Fresh	0.948	0.715	0.583	1.33	1.63
After 1st use	0.942	0.645	0.509	1.46	1.85
After 2nd use	0.976	0.692	0.544	1.41	1.79

Table 4.4 The ratio of the intensity of CTAB and the intensity of silica at 795 cm^{-1}.

Sample	Intensity of Si-O-Si at 795 cm^{-1}	Intensity of CTAB at 2923 cm^{-1}	Intensity of CTAB at 2852 cm^{-1}	Ratio of 795/2923	Ratio of 795/2852
Fresh	0.380	0.715	0.583	0.33	0.65
After 1st use	0.351	0.645	0.509	0.54	0.69
After 2nd use	0.398	0.692	0.544	0.58	0.73

4.6 Interferent study

The effect of foreign species, Mg^{2+}, Ca^{2+}, Zn^{2+}, and methomyl, on the fluorescence signal measurement of 1-naphthol was investigated using a 1:5 molar ratio between carbaryl and the foreign species. The concentration of carbaryl was 6 µM, while the foreign species was 30 µM. The experiment included four conditions: (1) a control condition with no foreign species and (2–5) individual reactions in which 6 µM carbaryl was mixed with 30 µM of either Mg^{2+}, Ca^{2+}, Zn^{2+}, or methomyl. Each solution was then combined with MCM-41-CTAB, stirred for 1 h, filtered, and measured fluorescence signal at 485 nm using a spectrofluorometer (Sarspec, spec res+).

The results in Figure 4.13 illustrate the fluorescence signal of 1-naphthol derived from the hydrolysis of carbaryl in the presence of the tested foreign species. The presence of Mg^{2+}, Ca^{2+}, Zn^{2+}, and methomyl caused a deviation in the 1-naphthol signal of less than 5% as shown in Table 4.5. %Error was calculated using equation (4.3). Deviations in the signal below 5% are considered insignificant, the results confirm that Mg^{2+}, Ca^{2+}, Zn^{2+}, and methomyl do not interfere with the fluorescence signal measurements of 1-naphthol.

$$\%\text{error} = \frac{I_{carbaryl} - I_{mix}}{I_{carbarly}} \cdot 100\% \quad (4.3)$$

where $I_{carbaryl}$ is the signal measured from the carbaryl solution, and I_{mix} is the signal measured from the carbaryl solution mixed with the investigated species.

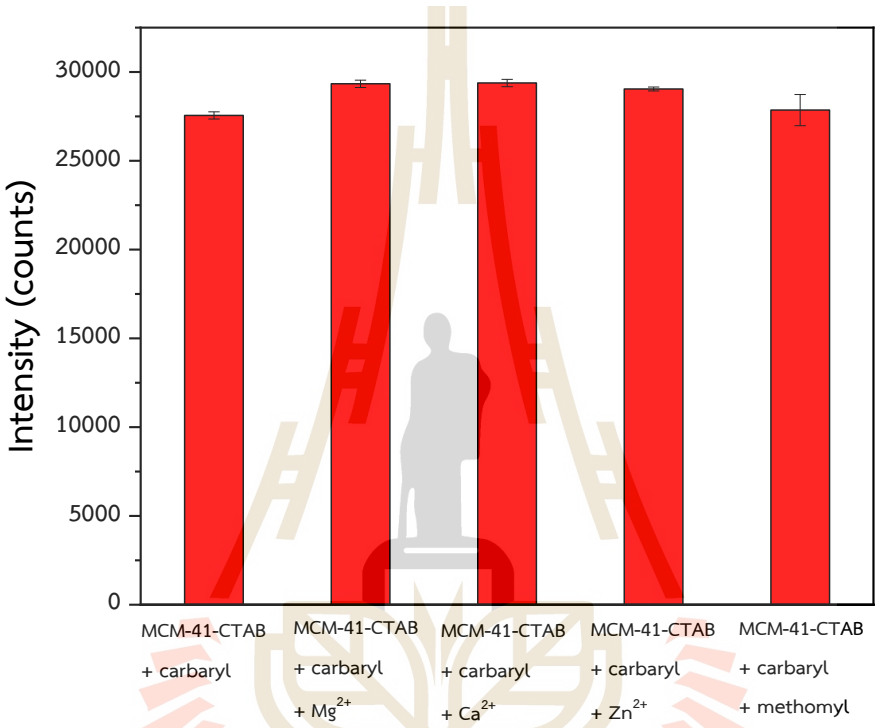


Figure 4.13 The fluorescence signals obtained from a solution containing carbaryl and MCM-41-CTAB and solutions of carbaryl with MCM-41-CTAB mixed with investigated species.

Table 4.5 The deviation in the fluorescence signal of 1-naphthol in solutions with tested species.

Tested species	%Error
Mg^{2+}	2.86
Ca^{2+}	3.02
Zn^{2+}	1.82
methomyl	1.08

4.7 Real sample analysis

The applicability of the developed method was demonstrated through the quantification of carbaryl in real samples, which are chili and Thai eggplant, using a spectrofluorometer (Sarspec, spec res+). The results are shown in Table 4.6. Carbaryl was not detected in unspiked chili and Thai eggplant 1 sample. For the Thai eggplant 2 sample, a residual carbaryl was found which was calculated to be 0.32 mg carbaryl/kg sample. For spiked samples, the method exhibited recovery values of 81.2% in chili and 83.4% in Thai eggplant, demonstrating acceptable accuracy for pesticide analysis. The relative standard deviation (%RSD) values remained below 1%, confirming good precision.

Table 4.6 Determination of carbaryl in chili and Thai eggplant samples.

Sample	Spiked (μM)	Found (μM)[a]	%Recovery	%RSD
Chili	0	ND[b]	-	-
Chili	10	8.12±0.06	81.2	0.76
Thai eggplant 1	0	ND[b]	-	-
Thai eggplant 1	10	8.34±0.07	83.4	0.79
Thai eggplant 2	0	1.56±0.04	-	-

[a] The reported values were the average ± standard deviation (n = 3). [b] ND = not detected

Besides, UHPLC was also used to analyze the samples and compare them to the results obtained from the developed method. The results are shown in Table 4.7.

Table 4.7 Comparison of carbaryl determination using the developed method and UHPLC method.

Sample	Carbaryl concentration (μM)[a]	
	Developed method	UHPLC
10 μM carbaryl solution	10.86±0.24	10.36±0.04
Chili (spiked with carbaryl)[b]	8.12±0.06	8.33±0.03
Thai eggplant 1 (spiked with carbaryl)[b]	8.34±0.071	8.31±0.05
Thai eggplant 2	1.56±0.04	1.71±0.03

[a] The reported values were the average ± standard deviation (n = 3). [b] The spiked samples contained an added carbaryl concentration of 10 μM.

The difference in carbaryl concentrations measured by fluorescence and UHPLC techniques was evaluated using the paired t-test. A t value was calculated according to equation (4.4). This method was chosen because both techniques were

applied to the same set of samples. Based on the concentrations obtained from all four samples, the calculated t value is 0.2605, which is less than the critical t value, 3.182 (95% confidence level, 3 degrees of freedom). This suggests that the results obtained from the developed method are not significantly different from those obtained from the UHPLC method. The developed method, therefore, could be used to accurately determine carbaryl concentrations in real samples with high reliability.

$$t = \frac{|\bar{D}|}{SD}\sqrt{n} \tag{4.4}$$

where $\bar{D}$ is the mean of the differences between the paired samples. SD is the standard deviation of the differences, and n is the number of sample pairs.

CHAPTER V
CONCLUSIONS

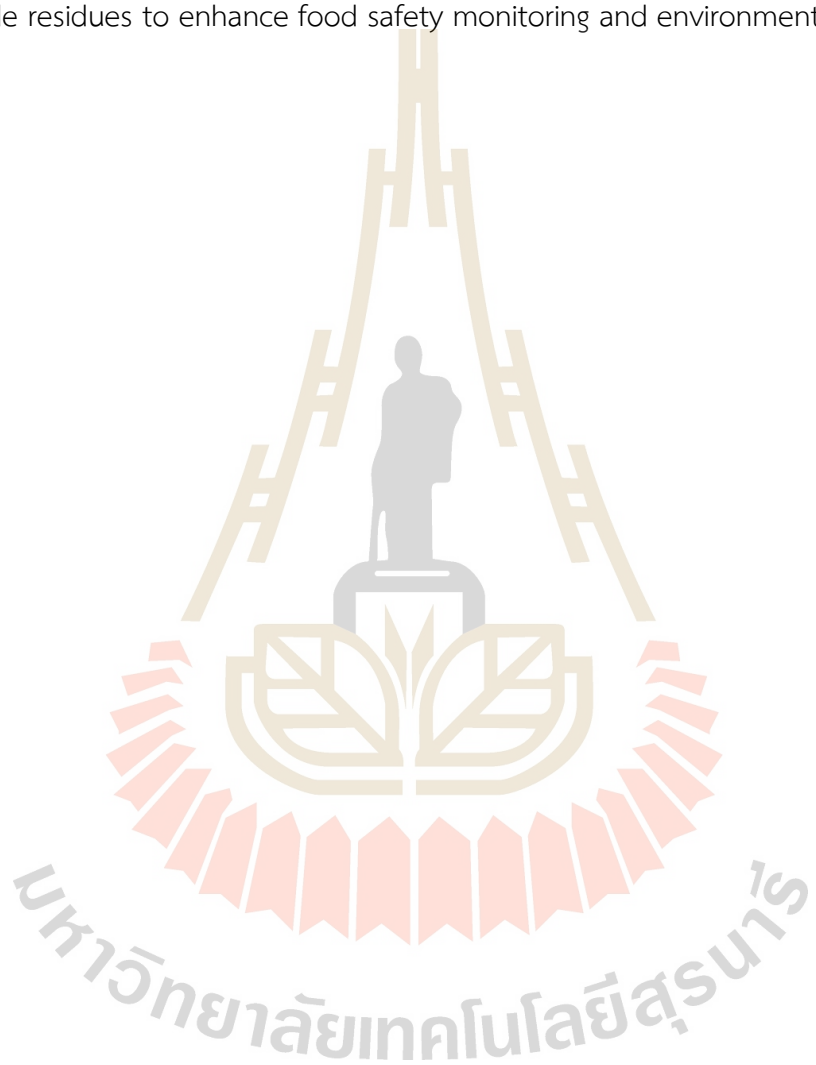
This research successfully developed a fluorometric method for detecting carbaryl residues in agricultural products, utilizing MCM-41-CTAB as a solid base reagent. The study demonstrated that MCM-41-CTAB provides a viable alternative to conventional strong alkaline hydrolysis, enabling the transformation of carbaryl into 1-naphthol, which exhibits strong fluorescence at 485 nm when excited at 335 nm. This method allows for sensitive, selective, and efficient carbaryl detection without harsh chemical reagents.

The synthesis and characterization of MCM-41-CTAB confirmed its highly ordered mesoporous structure. The basicity of MCM-41-CTAB, derived from its siloxy anion (SiO^-) groups, played a crucial role in the hydrolysis reaction. The optimal reaction conditions were identified as 100 mg of MCM-41-CTAB reacting with carbaryl in 50% ethanol solution for 60 min, yielding a linear calibration curve for carbaryl concentrations ranging from 0.12 to 10 µM, with a detection limit of 0.11 µM.

The method was successfully applied to real sample analysis, specifically for quantifying carbaryl residues in chili and Thai eggplant. The results obtained from the fluorometric method were validated against ultra-high-performance liquid chromatography, demonstrating strong agreement between the two techniques. Additionally, an interference study confirmed that the method remained highly selective, as the presence of common metal ions (Mg^{2+}, Ca^{2+}, Zn^{2+}) and another pesticide (methomyl) had negligible effects on the fluorescence signal of 1-naphthol.

The reusability study revealed that MCM-41-CTAB could be reused, but its efficiency gradually declined due to CTAB leaching after multiple reaction cycles. Thermal analysis showed a significant decrease in the CTAB content, while XRD and FTIR analyses indicated structural shrinkage and a reduction in functional groups after repeated use, explaining the observed decrease in the material activity.

Overall, this study provides a simple and environmentally friendly method for detecting carbaryl residues in agricultural products. By eliminating the need for strong alkaline hydrolysis and complex sample preparation, this method offers a practical alternative to conventional chromatographic techniques. Future research could further improve the reusability of MCM-41-CTAB and extend its application to other pesticide residues to enhance food safety monitoring and environmental protection.



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