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)

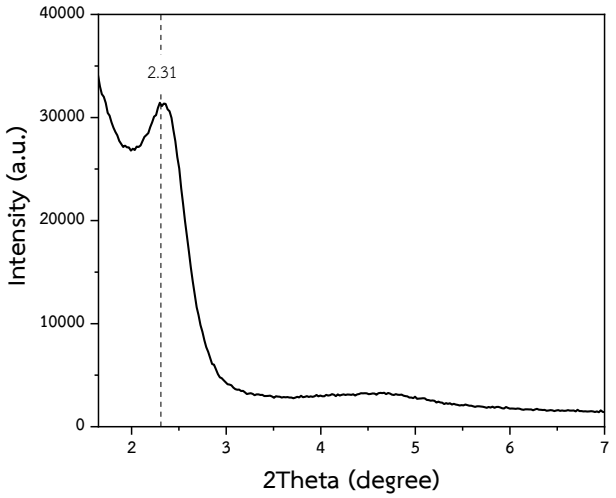

Figure 4.1 XRD pattern of MCM-41-CTAB.

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).

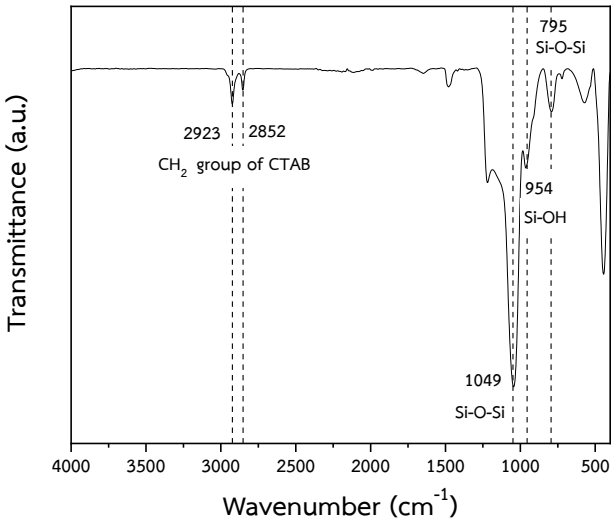

Figure 4.2 FTIR spectrum of MCM-41-CTAB.

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.

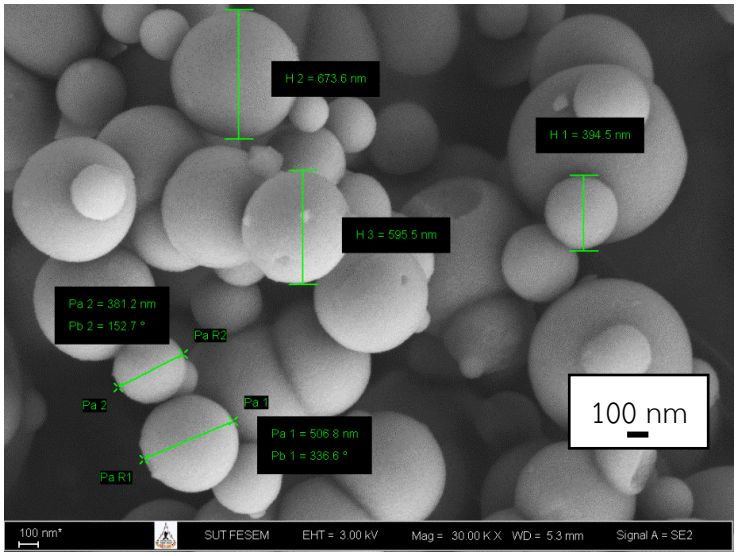

Figure 4.3 SEM images of MCM-41-CTAB.

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.

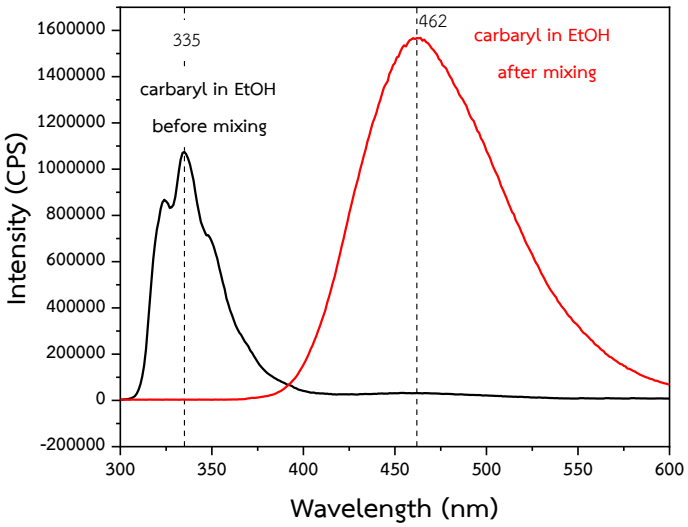

Figure 4.4 Fluorescence spectra of the carbaryl solution in ethanol before and after mixing with MCM-41-CTAB.

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.

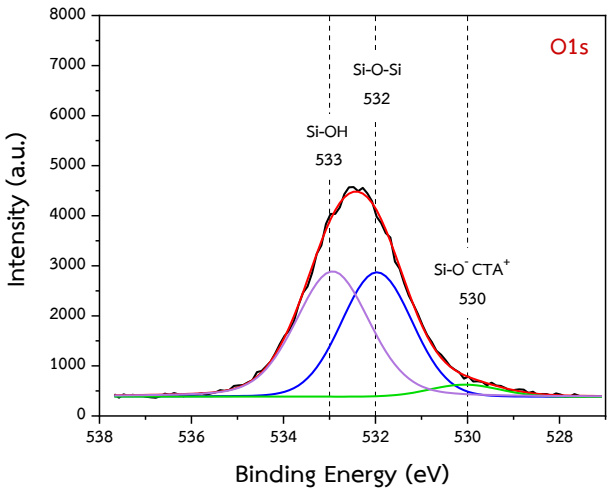

Figure 4.5 XPS spectra of oxygen 1s of MCM-41-CTAB.

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.

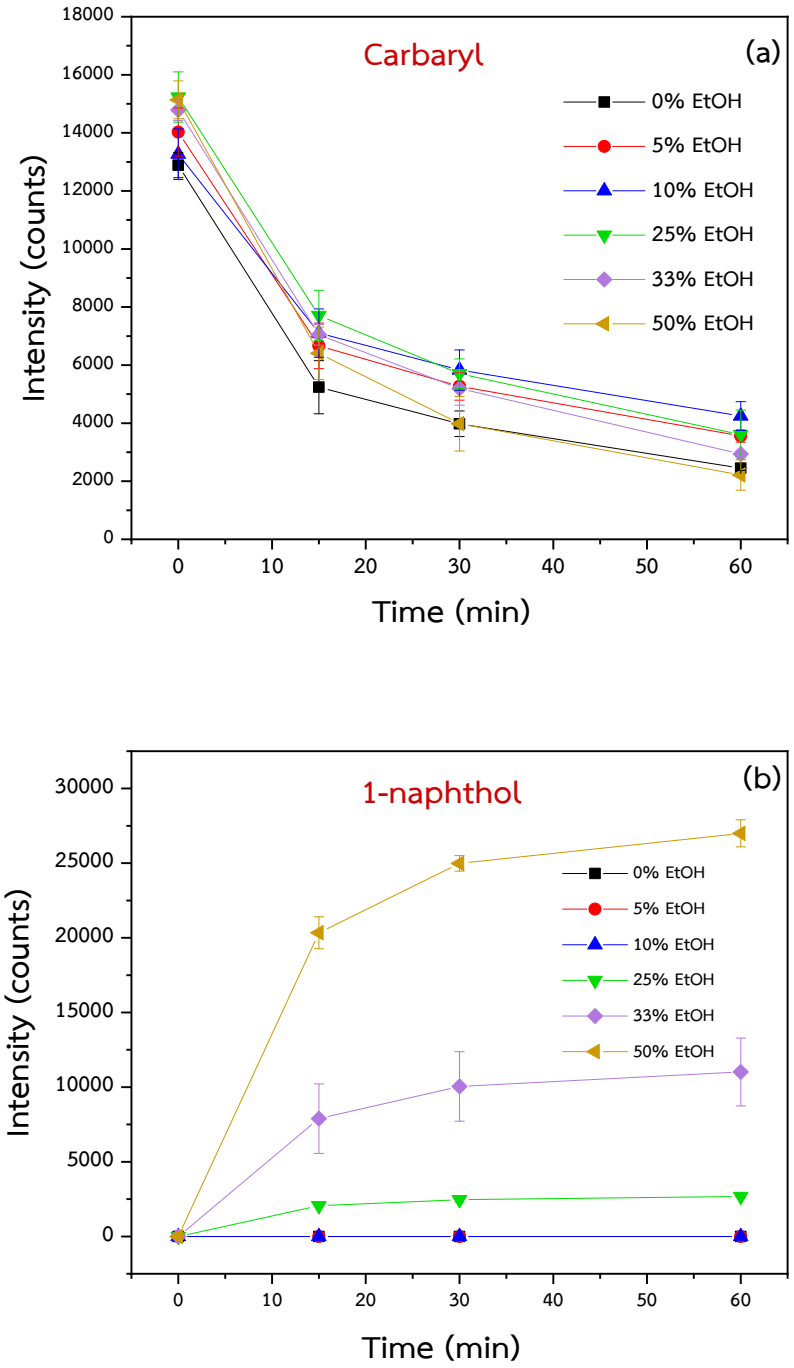

Figure 4.6 Time profiles of fluorescence signals of a) carbaryl and b) 1-naphthol in solvents with various % ethanol. The carbaryl concentration was 0.0497 mM.

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.

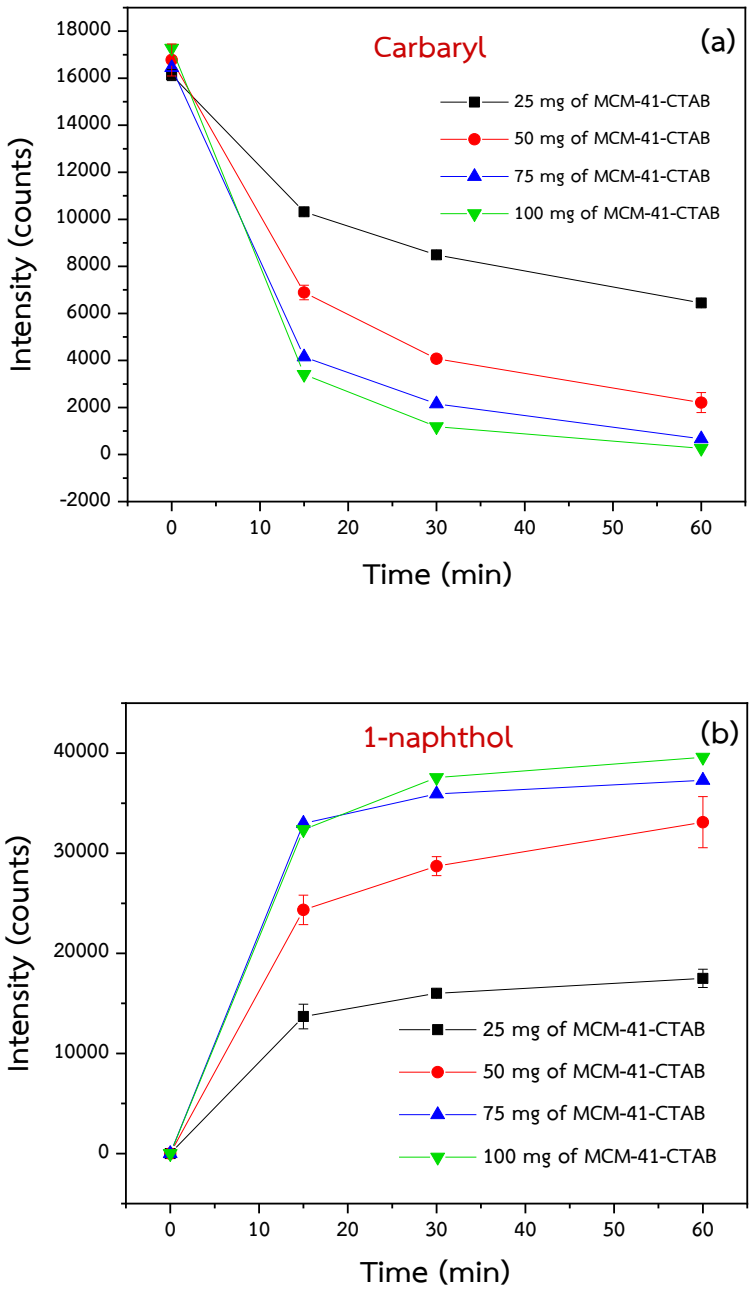

Figure 4.7 Time profiles of fluorescence signals of a) carbaryl and b) 1-naphthol from the reaction with various MCM-41-CTAB mass.

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.

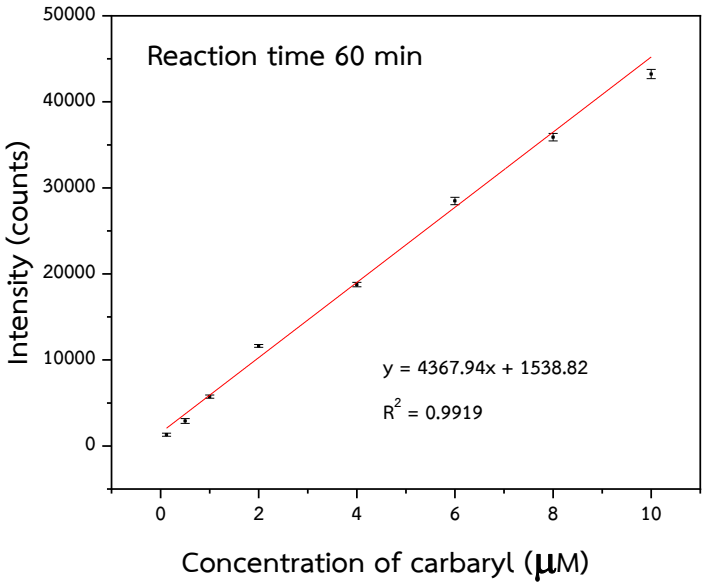

Figure 4.8 Calibration curve for carbaryl at the reaction 60 min.

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-	30	36702	1445	3.94
20240108	60	38333	1805	4.71
MCM-	30	39011	129	0.33
20240502	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.

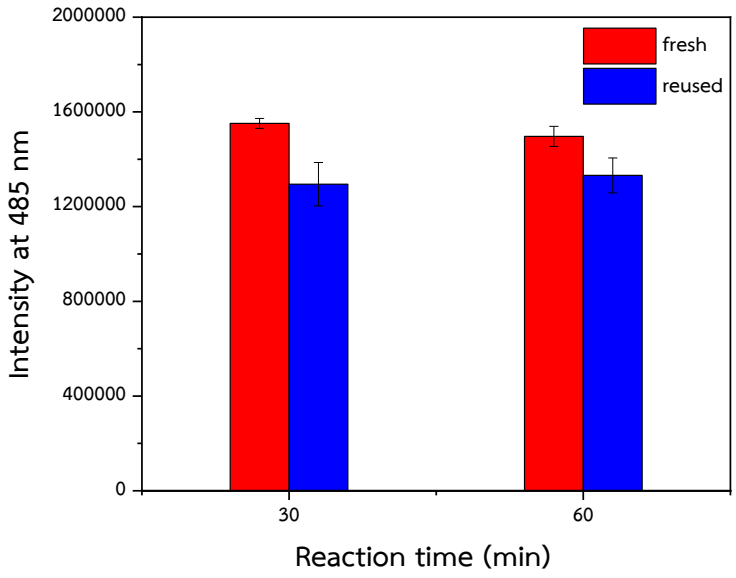

Figure 4.9 Comparison of the fluorescence signals of 1-naphthol from the reaction with fresh and reused MCM-41-CTAB samples.

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.

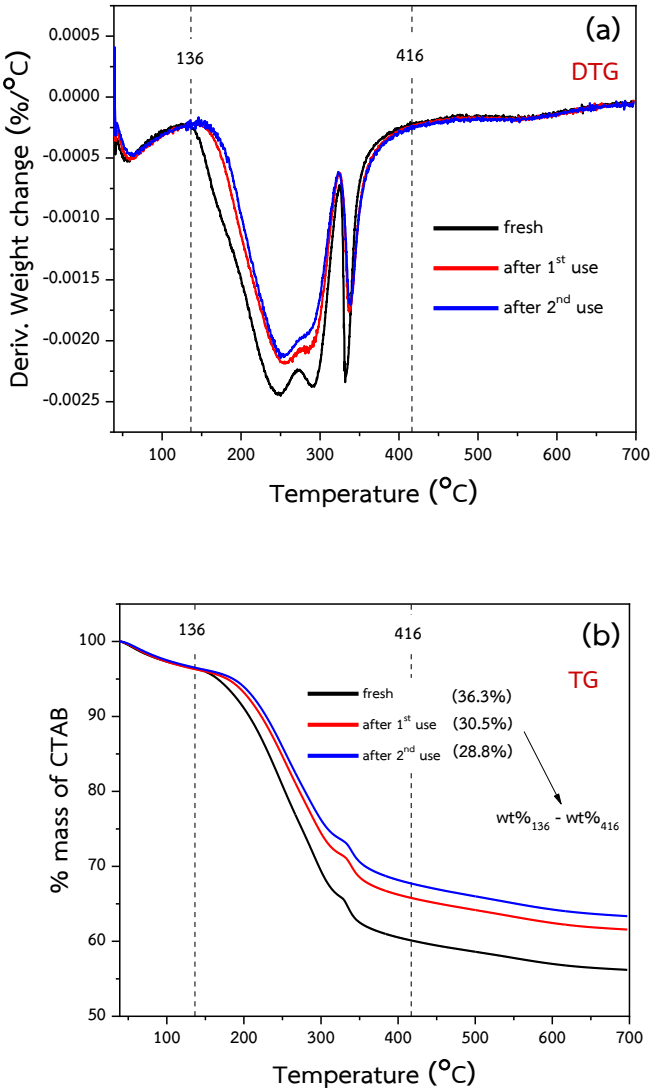

Figure 4.10 Thermograms of MCM-41-CTAB before and after use in the reaction a) derivative weight change and b) %weight loss.

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).

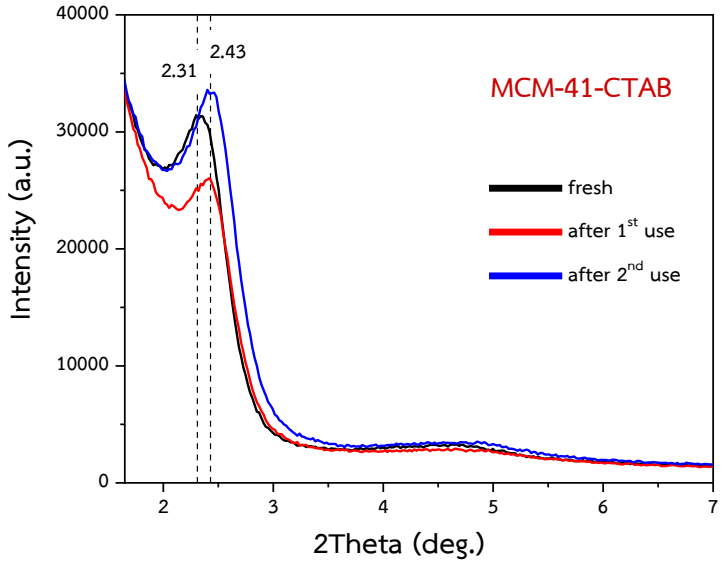

Figure 4.11 XRD pattern of MCM-41-CTAB.

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.

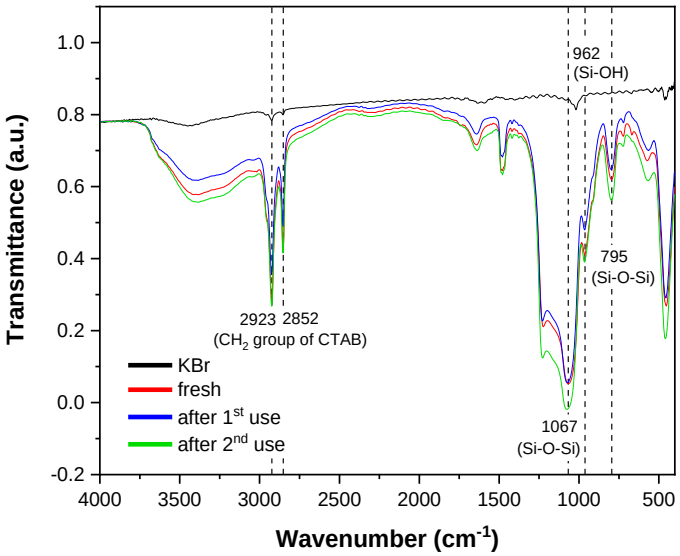

Figure 4.12 FTIR spectrum of MCM-41-CTAB.

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.

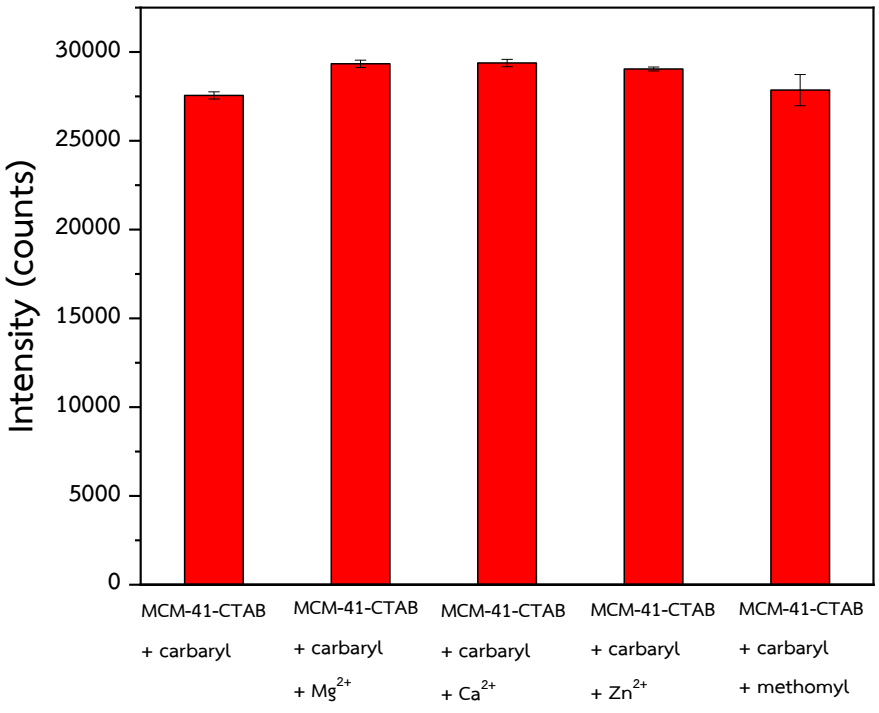

Figure 4.13 The fluorescence signals obtained from a solution containing carbaryl and MCM-41-CTAB and solutions of carbaryl with MCM-41-CTAB mixed with investigated species.

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.