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.