

# The Enhanced Carbamate Adsorption of Modified Bentonite with *Coscinium fenestratum*

S. Tha-in, H. A. Dau, and K. Dumri

**Abstract**—Pesticides daily pollutes soil and water in farmland and environment in ASEAN agricultural developing countries. In this study, pesticide adsorption was studied by bentonite modification, targeting the “organoclay” adsorbent in comparison to native bentonite, which was probably reported to adsorb agricultural pesticide. Commercial bentonite was modified with the berberine containing natural extract from *Coscinium fenestratum*; the local folk medicine in Thailand. The modified clay by extracted berberine was able to adsorb carbamate pesticide carbaryl with significant rate. HPLC analysis of residue carbaryl after adsorption process showed that it was up to 80% carbaryl adsorption when bentonite was modified by extracted berberine with ratio 1:100 (w/w), whereas commercial bentonite has showed at 70%. Modified bentonite by *C. fenestratum* extract and berberine revealed by scanning electron microscopy showed that the different transform levels of this clay surface, herein, the highest carbaryl adsorption occurred when berberine/bentonite ratio for modification process was 1/200 (w/w). SEM results also demonstrated that the amount of berberine should be taken into consideration in term of bentonite modification for pesticide adsorption. *Coscinium fenestratum* plant will be further studied for its contribution to pesticide adsorption material in Thailand.

**Index Terms**—Bentonite, berberine, modification, carbaryl, adsorption, *Coscinium fenestratum*.

## I. INTRODUCTION

The contamination of water and soil in farmland and surrounding environment by carbamate pesticide has been raising since it has become popular by its actual low mammalian oral and dermal toxicity [1]. Carbamates are easy to be washed out from leaves and soil, therefore, they may affect to the structure and functioning of the whole ecosystem [2].

Clay minerals have a great adsorption capability for cation and intensively specific surface areas involved with their small particles size. Previous reports have shown that, the modified clay minerals was used as an adsorbent for many different branches of industry e.g. in fluids [3], cement or

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drilling; in pharmaceutical e.g. nanocomposite [4],[5], removal pathogenic, virus [6], and toxin including heavy metal from aqueous solution [7], or an adsorbent for many hazardous pesticides and herbicides for eliminate pollutant from water and entirely systems [8],[9].

Bentonite is a type of clay minerals composes of mainly mineral of the smactite (Montmorillonite) group. It's hold the excellent plasticity, lubricity, high dry-bonding strength, low permeability and low compressibility makes bentonite important. The abundance of bentonite spreads in all continents and its low-cost makes it a strong alternative adsorbent for removal of pollutants. There are two types of bentonite; swelling-type or sodium bentonite and non-swelling-type or calcium bentonite [10], [11].

In this study, berberine (5,6-dihydro-9,10-dimethoxybenzo[g]-1,3 ben-zodioxolo[5,6-a] quinolizinium) ( $C_{20}H_{18}NO_4$ )<sup>+</sup> (Bbr) was used to modify bentonite. Bbr is the major pharmaceutical component in root and stem of the Thai “Ham” plant *Coscinium fenestratum* (Gaertn.) Colebr. (Menispermaceae) [12]. *C. fenestratum* extract has antifungal, antibacterial, antiyeast, and antiproliferative activities [13-15]. Berberine is the main isoquinoline alkaloid that was presented the natural organic monovalent cation in *C. fenestratum*, and involves the minor alkaloids such palmatine, tetrahydroplamatine, cerbanine and jatrorrhizine, etc [16].

Recent studies have shown the reaction of berberine, (organic cation component), with commercial bentonite was suitable for the pretreatment of industrial effluents e.g. winery effluents and brine effluents. The expanding of the basal spacing of the smectite [17] and the adsorption modification of anthocyanins [18]. The combination between berberine and bentonite might be promising for environmental applications such as elimination of pesticides or herbicides [19].

This report purposed the production of low-cost “organoclay” adsorbents to enhance the adsorption of carbamate pesticide carbaryl; *C. fenestratum* extract was prepared to modify commercial bentonite in comparison to standard berberine-modified bentonite. High performance liquid chromatography (HPLC) was used to determine the residue of carbaryl after adsorption process and scanning electron microscope of modified bentonite was studied.

## II. PROCEDURE

### A. Materials

The bentonite powder was purchased from Union Science Co., LTD. (Chiang Mai, Thailand). Sodium bentonite was produced by ion-exchange of calcium-bentonite (Ca-Bent) with sodium chloride (NaCl). Na-Bent product was washed

by deionized water for few times and dried to eliminate Cl<sup>-</sup> at 105°C. Dried stems of *C. fenestratum* were provided by herbal medicine store in Chiang Mai, Thailand. Berberine chloride (BbrCl) was purchased from Sigma-Aldrich (Bangkok, Thailand). Standard carbamate pesticide carbaryl was kindly supplied by Pesticide Residue Analytical Laboratory (Royal Project Foundation, Chiang Mai, Thailand).

### B. Sample Extraction and Precipitation

Stems of *C. fenestratum* were chopped to small pieces about 0.5-1 cm. Maceration extraction was performed in vase by adding 100 g of *C. fenestratum* into 300 ml ethanol for 3 days by still not disturb. The extract was filtered to remove the plant residue following by addition of NaCl 1 g to precipitate the alkaloid content. It was dried at 60°C for a day to eliminate the ethanol. The remaining NaCl in the precipitate was removed by washing with deionized water. Precipitate (Alk) was collected by centrifugation at 4000 rpm, for 10 min, at room temperature. It was dried at 60°C overnight and kept in desiccators.

### C. Determination of Berberine in Alk Extract

Alk precipitate was firstly identified as an alkaloid by using *dragendorff's* reagent [20]; HPLC was used to confirm the Alk precipitate that contains berberine compound in extract. HPLC analysis was performed by Agilent 1100 HPLC system equipped with a various wavelength detector (VWD). The separation was used on a HP hypersil C18 column (125 mm x 4.6 mm i.d., 5 µm) (Agilent Technologies, USA). All of determination was followed the method of R. Tunpradit [15]. The mobile phases, which consist of 5% acetonitrile, 0.1% formic acid (A) and 95% acetonitrile in 0.1% formic acid in aqueous solution (B), which formic solution was adjusted pH at 4.5 using NaOH, after that all solvents were filtered through a 0.45 µm Milipore filter and degases before use. The HPLC condition was used the gradient program 0-35% B from 0-5 min, 36-60% B from 6-15 min and 61-100% B from 16-20 min after that, constantly 100% B for 10 min, total time was 30 min. The injection volume was 20 µL at flow rate of 1 ml·min<sup>-1</sup>. The Alk solution was monitored by measuring its absorption at 350 nm.

### D. Preparation of Modified Bentonite

The Na-Bent was prepared by dispersing into bottle with deionized water. The dispersion was modified by weight mixing with BbrCl or Alk by ratio (100:1, 200:1 and 500:1, %w/w) in 25 ml of aqueous solution, respectively. The mixtures were shaken continuously in room temperature at 160 rpm. After 24 h, the resulting "organoclay" dispersion which was identified as BbrCl-Bent<sub>1:500,1:200</sub> and 1:100 and Alk-Bent<sub>1:500,1:200</sub> and 1:100, the remains of BbrCl and Alk were washed by centrifuge at 25°C, 4000 rpm for 30 min. The supernatant was removed gently and sediment was dried at 105°C overnight. The dried sediment was grinded to powder and sieved using a 300-mesh sieve (Gallenkamp, London, US). Organoclays samples were kept in desiccators.

### E. Adsorption of Carbamate by Modified Bentonite

200 ppm of carbaryl was used in overall treatments. The experiments were carried out in shaker at 160 rpm, for 24 h. under room temperature. After equilibration, the dispersions

were centrifuged at 4°C, 4000 rpm for 30 min and collected the supernatant for next experiment. The amount adsorbed was calculated from the different concentration between initial and the equilibrium solution. Triplicates of each adsorption were used in all series of experiments. The concentration of carbaryl was determined by HPLC method.

### F. HPLC Analysis of Carbaryl

Supernatant of carbaryl was collected, after centrifuge at 4000 rpm for 30 min at room temperature, after that the residue was centrifuge again at 12,000 rpm for 5 min to removed the dedicated particle. Solution samples were diluted for 4 times by deionized water and filtered +all samples with 0.45µm filter paper for preparing HPLC technique. The profile system for analysis of carbaryl was followed by R. Tunpradit method, the observation wavelength was on 280 nm.

### G. Scanning Electron Microscopy Measurement

The surface morphology of Ca-Bent, Na-Bent, BbrCl-Bents and Alk-Bents were observed under scanning electron microscopy (SEM) instrument (JEOL, JSM 5910 LV, Techniques LTD., Japan). Samples were coated by gold as a conductive material. Accelerating voltage at 15 kV and 10,000 time magnification were applied.

## III. RESULTS AND DISCUSSIONS

### A. Alkaloid Berberine Extraction from *C. Fenestratum*

The yellowish-brownish precipitate which collected from centrifuge after dryness was 236 mg (0.23% weight) as in Fig. 1. In order to screen for alkaloid, the precipitate was analyzed by TLC. The orange-brown zone was occurred on paper that corresponded to alkaloid compound in precipitate could be observed.



Fig. 1. Alkaloid extract from *C. fenestratum*

After precipitate was responded positively results for developing reagent, Alk precipitate was continued to quantified berberine compound by HPLC. BbrCl was used as a standard compound to compare the content of berberine in the Alk precipitate.

### B. Confirmation of Berberine in Alkaloid Extract

The confirmation of berberine content by HPLC in Alk precipitate is illustrated in Fig. 2. The signal was indicated only one major peak in both chromatograms. The retention time ( $t_R$ ) of standard BbrCl was 16.990 min (Fig. 2a) and quite similar peak at retention time 17.058 was belongs to Alk precipitate in methanol (Fig. 2b). Comparison the retention time between BbrCl and alk precipitate chromatograms were showed non-significantly difference time shift, assume that Alk was contained berberine compound about 70%. The other peaks after these could not

be identified, probably using high performance techniques to carried out.

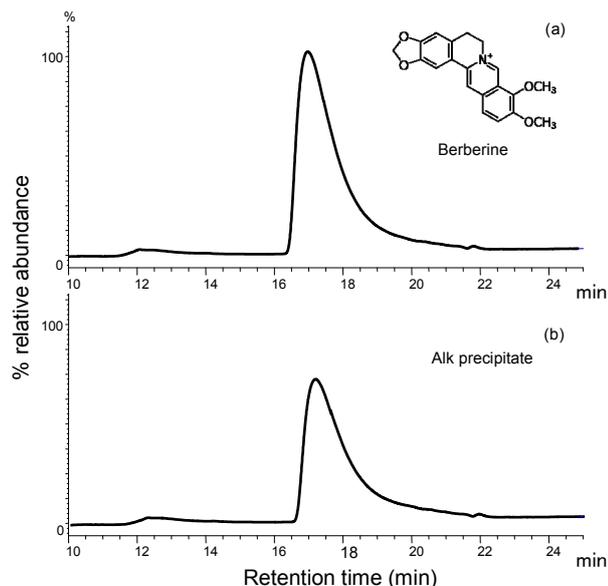


Fig. 2. HPLC chromatograms of standard BbrCl (a) compared with berberine content in Alk precipitate. (b) The HPLC elution profile was recorded at 350 nm.

### C. Modification of Commercial Bentonite

In the part of modification into Ca-Bent and Na-Bent, the morphology of both Ca-Bent and Na-Bent was observed using SEM in Fig. 3 (x10,000). The Ca-Bent layer before modification demonstrated the wild plate form and less basal space as in Fig. 3a. The sheets of Na-Bent after modified with NaCl was exhibited more delicate than Ca-Bent, the space and layer sheets were increased which mean that Na-Bent should be adsorb water or liquid higher than Ca-Bent, follow by their called Na-Bent is swelling-clay.

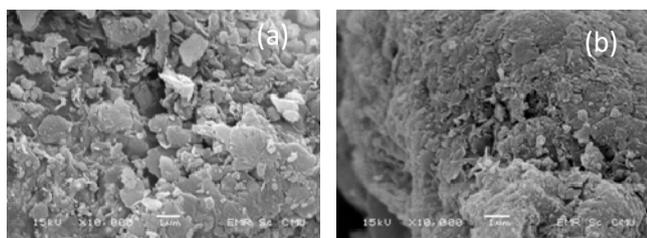


Fig. 3. SEM image showed the morphological surface of Ca-Bent before modification with NaCl (a) and Na-Bent which morphology was changed after modification with NaCl (b).

### D. Determination of Carbamate of Clay Modification

The triplicate dispersions of each organoclay; BbrCl-Bent and Alk-Bent were exhibited changing physical properties after modification treatment with BbrCl and Alk precipitate for 24 h was finished. The color of dispersion which was changed from natural gray to gray-yellowish that belongs to BbrCl and Alk intercalation colors. The strongest yellowish was appeared on BbrCl-Bent<sub>1:100</sub> in Fig. 4a, BbrCl-Bent<sub>1:200</sub> and BbrCl-Bent<sub>1:500</sub> and the Alk-Bent samples were indicated same ratio which was 1:100, 1:200 and 1:500, respectively (Fig. 5b). After drying process overnight, all organoclays was observed as still gray-yellowish and easier to grinded to powder, that could explain the intercalation of BbrCl or Alk into space of bentonite, and expanding the inter space of bentonite comparing with Na-Bent before treatments.

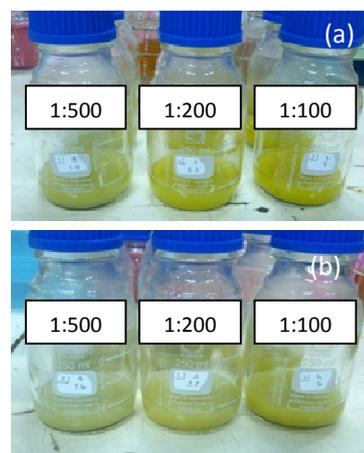


Fig. 4. Suspension samples after treatment of Na-Bent with BbrCl (a), from left to right; BbrCl-Bent<sub>1:500</sub>, BbrCl<sub>1:200</sub>, BbrCl<sub>1:100</sub> and Na-Bent with Alk precipitate (b), from left to right; Alk-Bent<sub>1:500</sub>, Alk-Bent<sub>1:200</sub>, Alk-Bent<sub>1:100</sub>.

### E. Determination of HPLC Analysis

The carbaryl pesticide adsorption by organoclays after modifies with BbrCl and Alk were determined by using HPLC technique. The HPLC graphs demonstrate the residue of carbaryl which could not be adsorb by organoclay treatments. The retention time of carbaryl and standard carbaryl were occurred as the same retention time at ~9.0 min (data not shown). Fig. 5 illustrates the adsorption capabilities of different modified bentonite samples. Data showed the higher adsorption of modified bentonite Alk precipitate, the increasing at 7% of Alk-Bent compared with reaction control at the adsorption percentage at 77% after treatment on ratio 1:100 was the highest data among Alk-Bent samples. However, the ratio is not quite difference with the Alk-Bent<sub>1:500</sub> at 5% and the Alk-Bent<sub>1:200</sub> were demonstrated the lowest value of adsorption pesticide at 2% in total reaction. On the other hand, bentonite which modified with BbrCl was carried out the most increasing percentage adsorption at 24% belongs to BbrCl-Bent<sub>1:200</sub> after with BbrCl-Bent<sub>1:500</sub> and BbrCl-Bent<sub>1:100</sub> at 19% and 3% respectively. The excess obtaining of BbrCl or Alk precipitate in the reaction can decrease the adsorption capability because the berberine was supposed to be saturated in the space of clay may block the carbaryl sorption process.

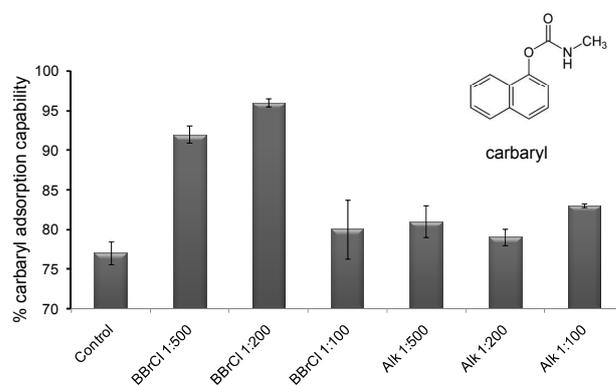


Fig. 5. The carbaryl adsorption capability of modified BbrCl-Bentonite and Alk-Bentonite.

### F. Electron Microscopy

The morphology of non-swelling bentonite or Ca-Bent was not changed after modification to swelling Na-Bent (Fig. 3). New reflection appeared after modification, by BbrCl and

Alk. The distance between blocks was expanded between the smectite layers. The surface of organoclays in Fig. 6a-b is differed from the original bentonite. The micrographs of modified samples reveal another deposited phase on the grinded of original minerals which explain both berberine standard compound and natural berberine extracted can intercalate into the space of smectite interlayer and change their surface morphology of modified organoclays.

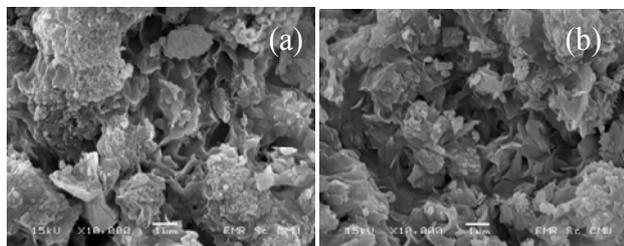


Fig. 6. Scanning electron microscopy images of BbrCl-Bent<sub>1:200</sub>(a), Alk-Bent<sub>1:100</sub> (b), the maximum capability of adsorption after modified with BbrCl and Alk precipitate.

#### IV. CONCLUSION

This study investigated the ability modified bentonite with berberine to adsorb carbamate pesticide; carbaryl. The bentonite modifications by natural extract from *C. fenestratum* containing berberine can change the morphology of commercial bentonite, and enhance the carbaryl adsorption of the “organoclay” berberine-sodium-bentonite. SEM analysis of this material showed the expanding basal space onto interlayer of clay and maximum ratio of BbrCl-Bent at 1:200 and Alk-Bent at 1:500 that related to the bentonite modification and its higher adsorption to carbaryl. The higher ratio might cause the saturated of BbrCl or Alk precipitate in the layer of clay and occurring the decreasing the adsorption capability.

Taken into account, modified bentonite by natural alkaloid such as berberine could generate a new ideal material to adsorb carbamate pesticide effectively. It could be applied to clean-up pesticide residue in agricultural water zone and products. The new material based on the combination between berberine and bentonite is not only easy to produce but also brings a low-cost material in term of economy.

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