CHAPTER 4

RESULTS & DISCUSSION

RESULTS

Adsorption capability of commercial bentonite was developed by alkaloid compound, extracted from *Coscinium fenestratum* in folk medicinal plant (Menispermaceae). Major alkaloid compound (berberine) was extracted and partial precipitated. Berberine precipitate was approach to respecting in modification of commercial bentonite clay. Investigation for increasing adsorption was determined to reduce pesticide contamination in water, comparison with modified purified berberine in bentonite clay.

4.1 Alkaloid extraction of *C. fenestratum*

The extraction of *C. fenestratum* was performed by considering based on the amount of berberine alkaloid component. Chopped the stems of *C. fenestratum* in ethanol was shown in Fig. 4.1. The clear orange-yellow was obtained. NaCl was further added in the solution to precipitate solution berberine compound in the solution.

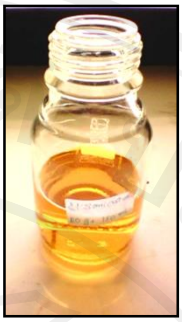



Figure 4.1 Stems of *C. fenestratum* extraction (left), and extracted solution (right)

The precipitation of the components in chloride form was an alternative process to collect the compound that can form in chloride, as berberine chloride. The polarity of berberine-precipitate was rather non-polar compound which cause when on process of removing NaCl, berberine-precipitate was not away with water dissolving, but settle down under the bottom and completely separated. Yellowish precipitate of berberine chloride was received after dryness, and yield as 236 mg (0.23% by weight) (Fig. 4.2).

Figure 4.2 Alk precipitate using precipitation of NaCl

Dragendroff's reagent was mostly used to detect alkaloid-alkaloid. Appearing of orange precipitate in the test was revealed as positive result. It is a convenient way to confirm that yellowish precipitate of extraction is possibly the berberine alkaloid compound.

4.2 Identification of *C. fenestratum* extracts using HPLC

According to the preliminary test of *Dragentdroff's* developing reagent suggested alkaloid containing in Alk was obtained. HPLC technique was concerned to estimate berberine compound in the plant extract, Standard berberine chloride compound (98%).

Optimized condition of HPLC analysis was investigated by Tungpradit [88] (Table 4.1). Both alkaloids (Alk) and berberine chloride (BbrCl) were dissolved in methanol and filtered through 0.45 μm filter paper prior to HPLC analysis.

Table 4.1 HPLC condition for determination of berberine content in Alk

HPLC Condition	Information
Mobile phase	5% Acetonitrile in 0.1% formic acid (A) 95% Acetonitrile in 0.1 % formic acid (B) (0.1% formic acid) (pH 4.5)
Solvent system / Interval time ratio	0-35-60-100-100 %B / 0-5-15-20-30 min
Total time	30 min
Flow rate	1 mL/min
Detector	UV detector at wavelength 350 nm
Inject volume	20µL

The standard BbrCl and Alk were obtained and showed in Fig. 4.3. Including of BbrCl chromatogram was represented only single signal peak at t_R = 19.596 min, see in Fig. 4.3A. Comparing retention time with sample Alk at t_R = 19.801 min were similar, with showed in Fig. 4.3B, a few second shift time for Alk was not attempt to be others. Therefore, the determination of Alk precipitate was qualitatively confirmed that berberine was demonstrated containing in Alk.

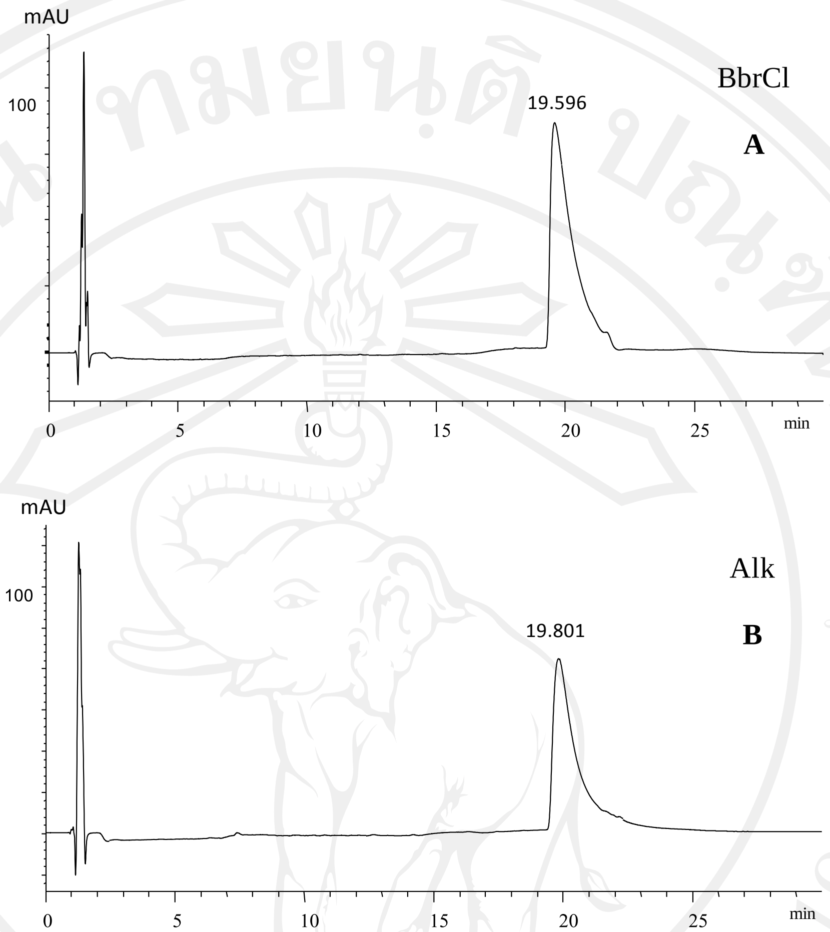


Figure 4.3 Chromatogram of berberine content in BbrCl standard **(A)**, compared with Alk extract (**B**).

Berberine-containing yield in Alk was obtained approximately 70% (See in Appendix A) which suggested using NaCl to settle Alk precipitate can provide pleasantly berberine compound in Alk. Contribution of time shift in Alk chromatogram result was supported. Otherwise, contamination in Alk was optically assumed by color, which suggested by purified BbrCl exhibited bright yellow precipitate, but Alk turned darker yellow than BbrCl, as see in Fig. 4.4.

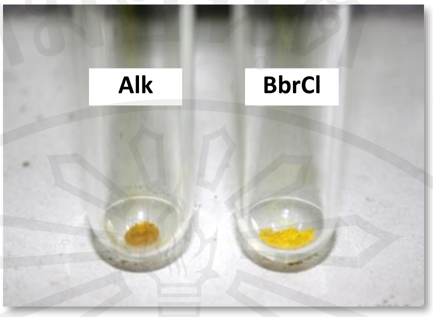


Figure 4.4 Difference color property of Alk (left) compared with BbrCl (right)

4.3 Saturated Na-Bentonite preparation

Commercial bentonite was commonly identified as calcium-bentonite (Ca-Bent), knew as non-swelling bentonite. The converting to Na-Bent and exhibited swelling bentonite properties by process "ion-exchange" was normally examined before experiments; this means commercial bentonite would change to Na-Bent and allowed for ions exchange to take place. In this bentonite preparation, NaCl was applied to enhanced and uses of bentonite. The ion-exchange Na^+ ion process, standing out of bentonite clay in NaCl solution was inquired. Swelling properties of 2nd time NaCl solution was observed after hours (Fig. 4.5), its seem moderately adsorption increasing, in assuming that Na^+ ion was displaced into clay-interlayer to exchange the metals in commercial bentonite and produced Na-Bent. Clay product was sieved before kept in desiccator so as obtain to the same rate of particle size.

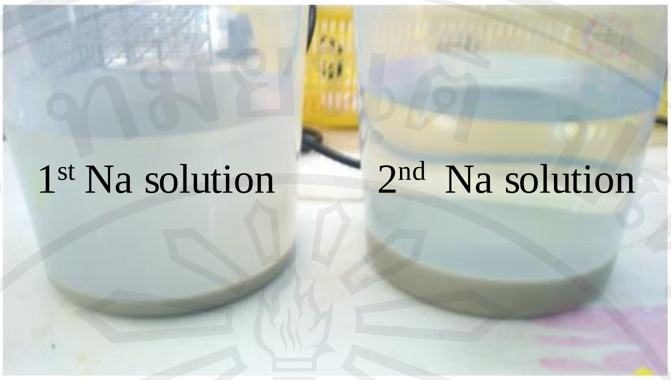
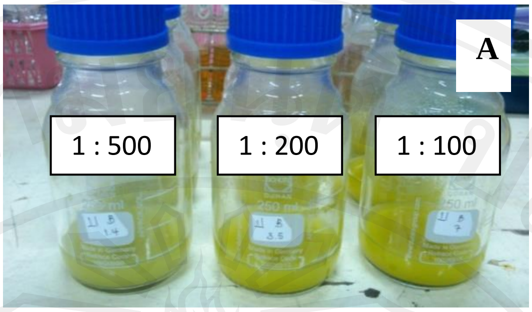


Figure 4.5 Comparison the saturated bentonite-swelling between 1st NaCl solution and 2nd NaCl solution.

4.4 Experimental of organoclay

From previous study, increasing adsorption property of Na-Bent by modification with organic or inorganic compound was applied bentonite to greater adsorbent. Berberine is once organic compound, normally used as a modifier to make Na-Bent undergoes on organoclay, as denoted as organobentonite, since modify with berberine in Alk and purified BbrCl in aqueous solution. Physical properties of aqueous solution for both treatments were shown in Fig. 4.6.



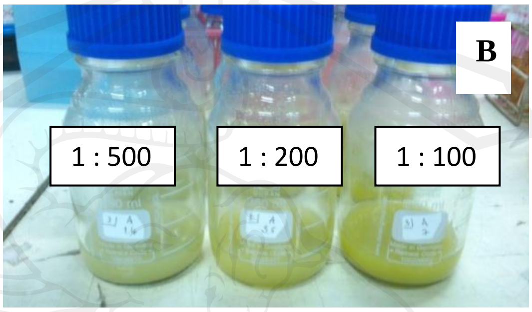


Figure 4.6 Suspension samples after treatment of Na-Bent with BbrCl (**A**), from left to right; BbrCl-Bent$_{1:500}$, BbrCl$_{1:200}$, BbrCl$_{1:100}$ and Na-Bent with Alk precipitate (**B**), from left to right; Alk-Bent$_{1:500}$, Alk-Bent$_{1:200}$, Alk-Bent$_{1:100}$.

Considering of solution color, each of Alk-Bent and BbrCl-Bent were illustrated different color levels, classified the organobentonite color was described by naked eye. BbrCl-Bent and Alk-Bent were compared as the highest yellow level series, as 1:100 > 1:200 > 1:500, respectively. Supernatant was removed by centrifuged and maintained only organobentonite. Both types of organobentonites mass were pound to smaller size particle by mortar (Fig. 4.7).

Figure 4.7 Physical property for organobentonite after modified with berberine (**A**), Na-bentonite (**B**) and commercial bentonite without any treatment (**C**)

4.5 Adsorption of carbamate pesticide

Organobentonite were prepared for the adsorption experiment with carbamate pesticides. Adsorptions of each carbamte were treated as the same condition. Analysis of this experiment was used aqueous solution from supernatant to determine remains of pesticide after sorption bythe activation of modified bentonite. Fig. 4.8 was shown the supernatant which was collected after centrifugation. Clear yellow solution still maintained organobentonite particle, since they had been observed by standing overnight, and the yellow dust colloid would settle at the bottom.

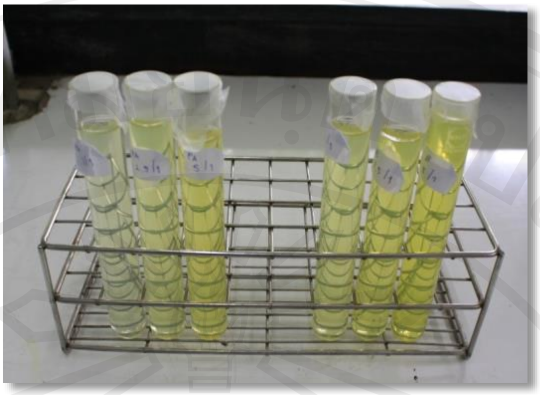


Figure 4.8 Clear yellow solutions were collected after centrifugation at 4,000 rpm speed, these solutions still have particle of organobentonite

Obviously, crystal-clear samples were represented by naked eye. Finally, all samples would approach to analysis the carbamate residue using HPLC. Samples preparation by filtration with 0.45 µm filter paper and stored at 4°C were employed for experimental.

4.6 Adsorption analysis

According to HPLC condition in Table 4.1, gradient profile was activated on this experiment. To determine pesticide residue, carbaryl was analyzed the residue. Fig. 4.9A showed the chromatogram standard of carbaryl. Single signal at t_R = 9.770 min was occupied, certainly identified as carbaryl compound. Chromatogram in Fig. 4.9B was possessed results from organobentonite experiment, at t_R = 9.828 min was shown carbaryl residue identity in adsorption experiment.

Occurring methomyl in chromatogram was signalized at t_R = 4.431 min identified as standard and only one peak was notable in Fig. 4.10A. Unexpected circumstance was occurred in result chromatogram of methomyl. 2 signals were displayed in results of organobentonite samples, including, first dominantly signal t_R = 3.334 min (2) and t_R = 4.438 min (1), which showed in Fig. 4.10B. Mainly signal at t_R = 4.438 min was verified such methomyl residue in sorption sample. And other signal at t_R = 3.334 min happened just in sample chromatogram but disappear on standard chromatogram was identified as unknown product. Look forward, remaining of organobentonite was cause of chemical reaction to receive new unknown product which indicate as same wavelength with pesticide.

HPLC analysis was suggest the standard chromatogram was pronounced at t_R = 5.200 min (1) as methiocarb, shown in Fig. 4.11A. In part of result, organobentonite chromatograms were signalized for 2 times. First, retention time at 3.396 min (2), and second signal was on retention time at 5.275 min (1). Methiocarb residue was identified at 5.275 min and other peak at 3.396 was identified as unknown product. Note that, this unknown signal was occurred in similar time as methomyl experiment results as in Fig. 4.11B. Assuming in adsorption of both organobentonite which occupy in methomyl and methiocarb adsorption experiments was estimated a chemical reaction between pesticide and organobetonite in solution. Allowing product for chemical reaction illustrated that, the reaction was occurred for experimental with organobentonite in methomyl and methiocarb adsorption reaction.

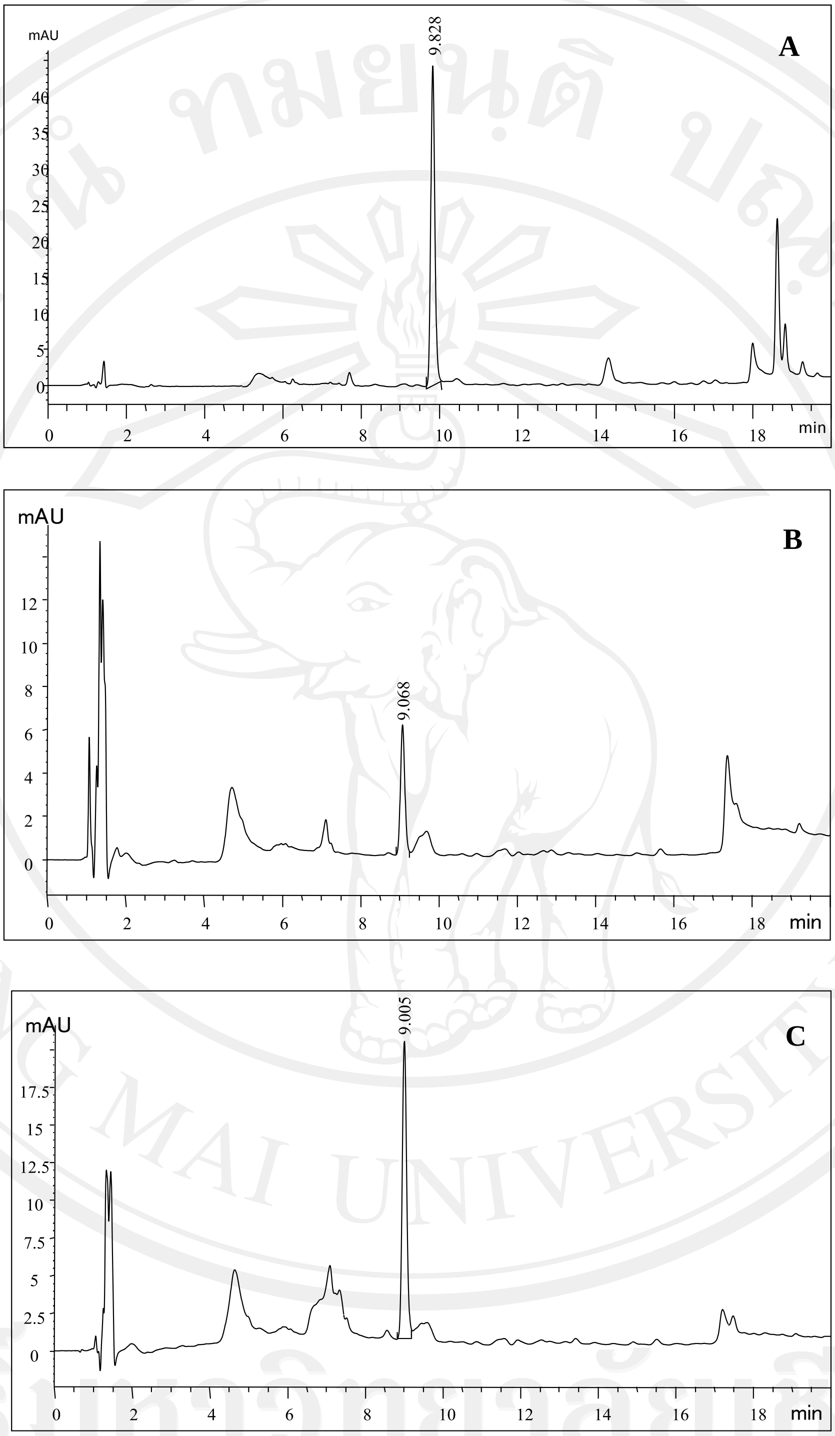


Figure 4.9 Examples of carbaryl chromatogram obtained from cabaryl standard (**A**), BbrCl-Bent$_{1:200}$ (**B**) and Alk-Bent$_{1:200}$ (**C**)

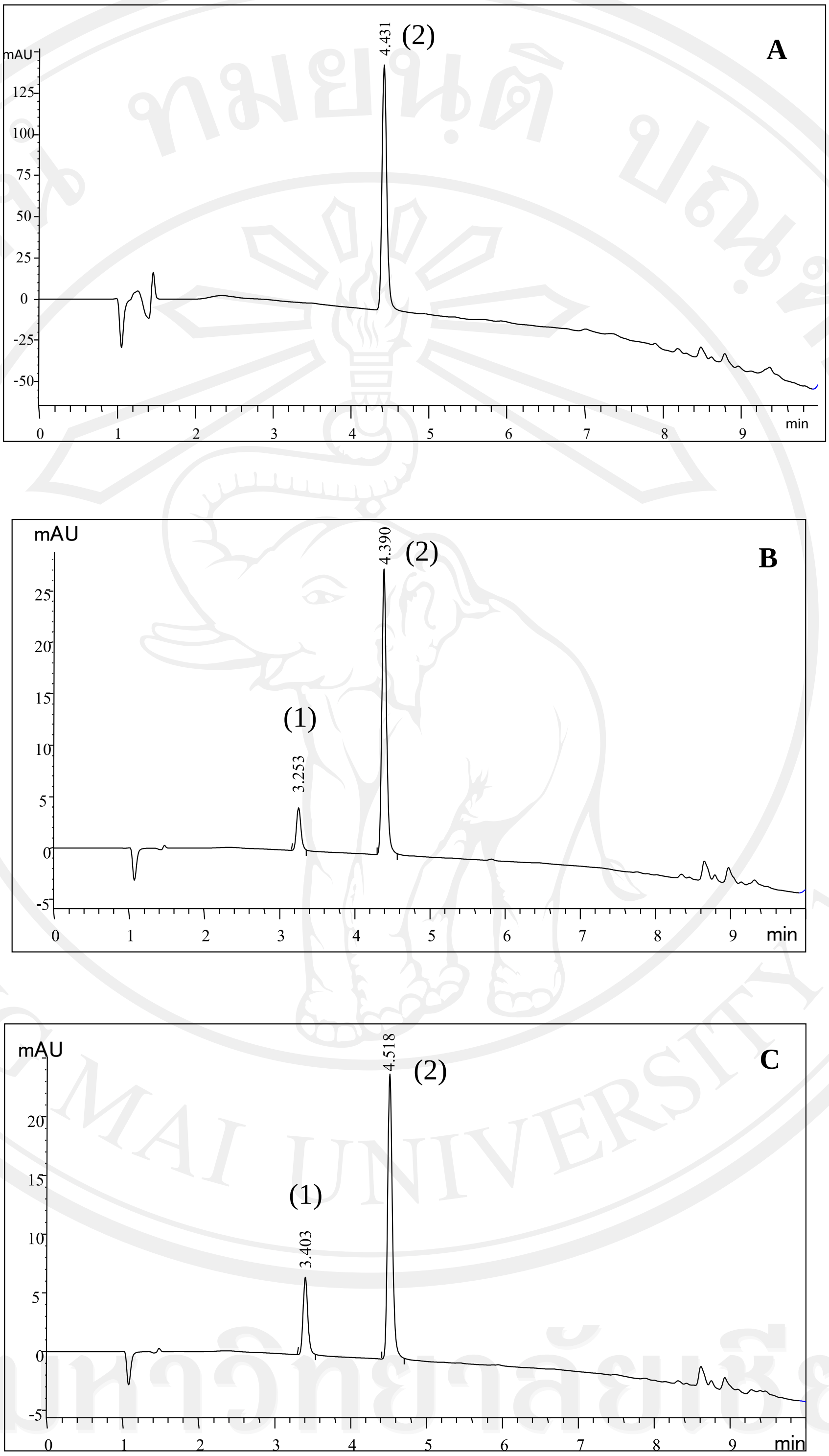


Figure 4.10 Examples of methomyl chromatogram obtained from methomyl standard (**A**) BbrCl-Bent$_{1:200}$ (**B**) and Alk-Bent$_{1:200}$ (**C**)

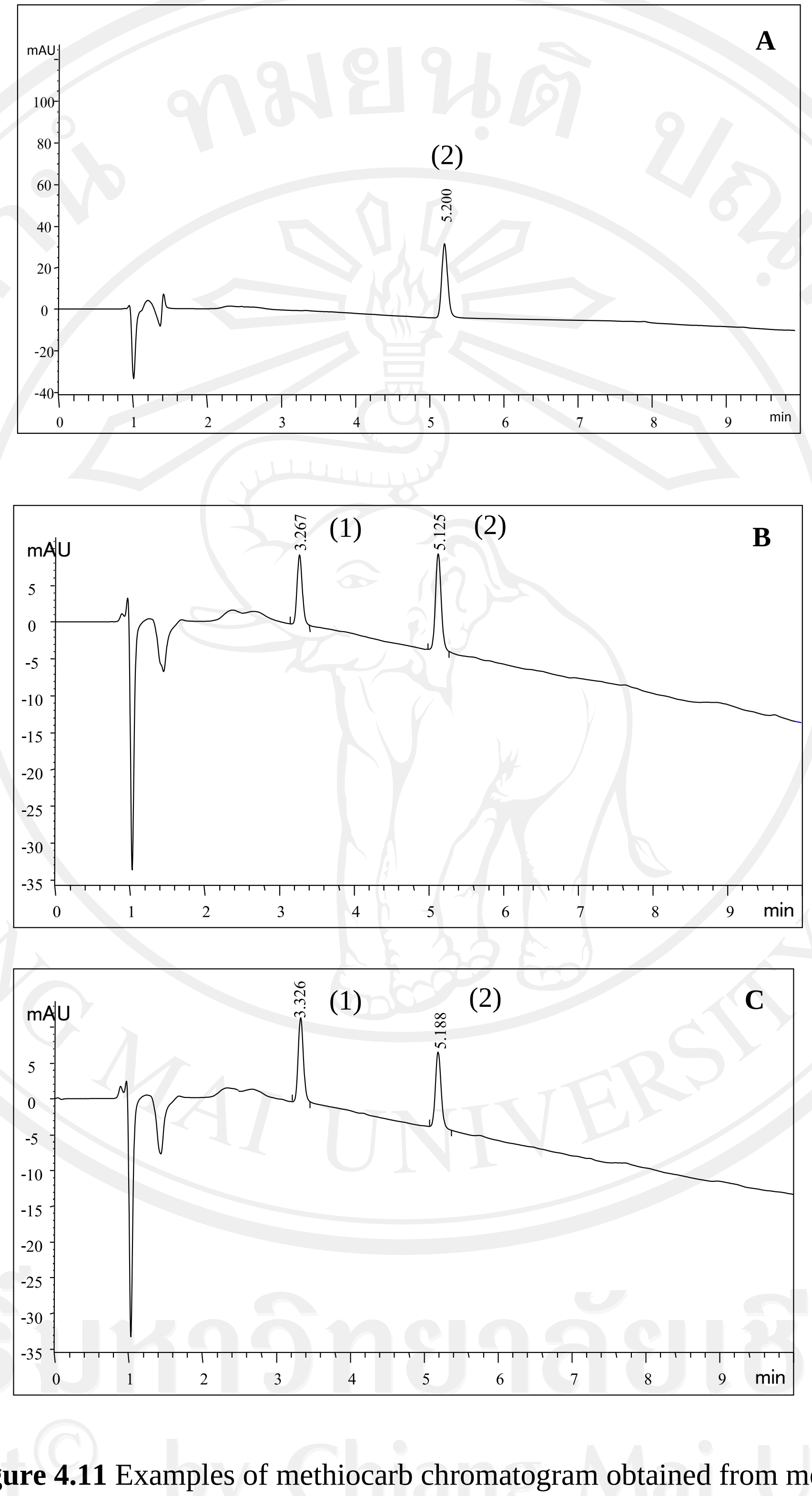


Figure 4.11 Examples of methiocarb chromatogram obtained from methiocarb standard (**A**) BbrCl-Bent$_{1:200}$ (**B**) and Alk-Bent$_{1:200}$ (**C**)

Quantitation of pesticide residue was determined the concentration in sample remain in aqueous solution. Decreasing of pesticide concentration was computed to adsorption percentage which showed in Table 4.2. BBrCl-Bent was indicated the lowest contamination of carbaryl pesticide in aqueous solution. But in part of methomyl, the adsorption results revealed non-difference between modified and non-modified bentonite (Na-Bent). Both of organobentonite represented similar result, and also Na-Bent was maintained the lowest value of adsorption concentration. In case of methiocarb, BbrCl-Bent at ratio 1:100 was determined least of pesticide-containing concentration in experiment and the lowest contamination of pesticide in Alk-Bent was on ratio 1:500. All of these concentration would further calculation and represented in Table 4.3.

Table 4.2 Pesticide residue concentration for carbamate pesticide (mean ± SD, n=3)

Organobentonite[a]	**Concentration of pesticide residue (ppm)**		
	Carbaryl	**Methomyl**	**Methiocarb**
Control (Na-Bent)	44.0 ± 4.0	10.2 ± 1.2	1.62 ± 0.2
Alk-bent$_1$ (1:500)	39.5 ± 6.0	11.7 ± 1.1	3.17 ± 0.1
Alk-bent$_2$ (1:200)	38.2 ± 6.0	12.1 ± 0.9	3.71 ± 0.2
Alk-bent$_3$ (1:100)	34.8 ± 4.4	12.0 ± 0.2	6.06 ± 0.7
BbrCl-bent$_1$ (1:500)	15.1 ± 2.0	12.2 ± 1.3	2.35 ± 0.1
BbrCl-bent$_2$ (1:200)	6.9 ± 0.9	12.6 ± 1.3	3.06 ± 0.3
BbrCl-bent$_3$ (1:100)	44.6 ± 7.1	12.1 ± 0.2	2.27 ± 0.3

([a]Alk-bent$_1$ (1:500) in ratio of Alk 1.0 mg in potion of bentonite 500 mg; Alk-bent$_2$ (1:200) ratio of Alk 2.5 mg in portion of bentonite 500 mg; Alk-bent$_2$ (1:100) in ratio of Alk 5.0 mg in portion of bentonite 500 mg; Bbr-bent$_2$ (1:500) ratio of standard BbrCl 1.0 mg in portion of 500 mg; Bbr-bent$_2$ (1:200) ratio of standard BbrCl 2.5 mg in portion of bentonite 500 mg; Bbr-bent$_2$ (1:100) ratio of stand BbrCl 5.0 mg in portion of 500 mg.

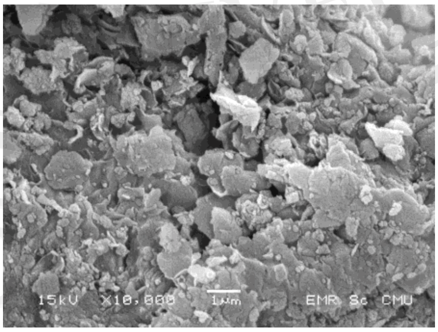
Table 4.3 Adsorption capability of organobentonite after experiment

Organobentonite	% Adsorption capability for pesticide in organobentonite sample		
	Carbaryl	Methomyl	Methiocarb
Control (Na-Bent)	78 ± 2.0	95 ± 0.8	98 ± 0.8
Alk-bent$_1$ (1:500)	81 ± 1.9	94 ± 0.5	96 ± 0.3
Alk-bent$_2$ (1:200)	80 ± 1.5	94 ± 0.4	96 ± 0.6
Alk-bent$_3$ (1:100)	83 ± 4.4	94 ± 0.1	94 ± 2.8
BbrCl-bent$_1$ (1:500)	92 ± 1.0	94 ± 0.6	97 ± 0.2
BbrCl-bent$_2$ (1:200)	97 ± 0.5	94 ± 0.7	97 ± 1.3
BbrCl-bent$_3$ (1:100)	78 ± 3.5	94 ± 0.1	98 ± 1.1

The results indicated the adsorption containing in modified organobentonite. Concerning of Alk-Bent, the best of adsorption result of carbaryl for Alk-Bent was organobentonite ratio 1:500, 89% of carbaryl adsorption, even though Bbr-Cl-Bent was showed at 96% of adsorption for carbaryl. In initially research, increasing adsorption of pesticide in solution was satisfied. In addition, experiment of methomyl and methicarb were described the similarity of pesticide adsorption. Capability of organobentonite sorption were non-significantly difference (in range 92-98%), including of non-modified bentonite (Control) determined the highest adsorption of pesticides. And both Alk-Bents and BbrCl-Bents which modified received the closely numbers and not effective with methomyl and methiocarb pesticide. Suggested that the modification of organobentonite with berberine compound was usable for carbaryl pesticide, but not for methomyl and methiocarb. May be the unpredictable of these results was regarding with the chemical reaction which happened between organobentonite and pesticide in adsorption experiment.

4.7 Scanning electron microscopy (SEM)

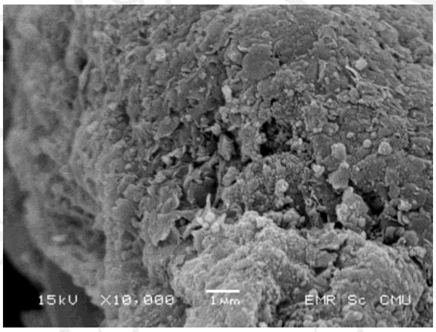
Morphology of bentonite was defined using SEM technique. 10,000 times was magnified and 15 kV was applied to analysis physical structure of each bentonite. The results were represented the compact structure of layer sheet. Commercial bentonite without treatments was described the layer of sheets (Fig. 4.12). Bentonite sheet did not pack tightly from each other. Distribution of layer sheet indicated that large metal or ions was contained in layer sheet. Sorption of this clay by water or fluid could identified this bentonite was non-swelling bentonite.



Commercial-Bentonite

Figure 4.12 Physical structure of commercial Bentonite

After preparation of Na-Bent using NaCl protocol, Fig. 4.13, instructed Na-Bent increasing swelling properties, notice on layer sheet was compacted closer each sheet by sheet. And when Na-Bent swelled, liquid or water can adsorb much higher than commercial bentonite, which normally call, swelling bentonite.



Na-bentonite

Figure 4.13 Physical structure of Na-bentonite

Modification of bentonite using berberine from 2 sources was verified. The parameter for this observation described as ratio of berberine. In Fig. 4.14, identification for organobentonite structures were demonstrated reforming of Alk-Bent and BbrCl-Bent completely difference by Na-Bent structure. From Fig. 4.14A-F, the porous was expanding, non-rearrangement for all ratio of organobentonite appeared, each sheet of bentonite layers were contained larger area for support water or fluid. Increasing free space around bentonite surface was determined the

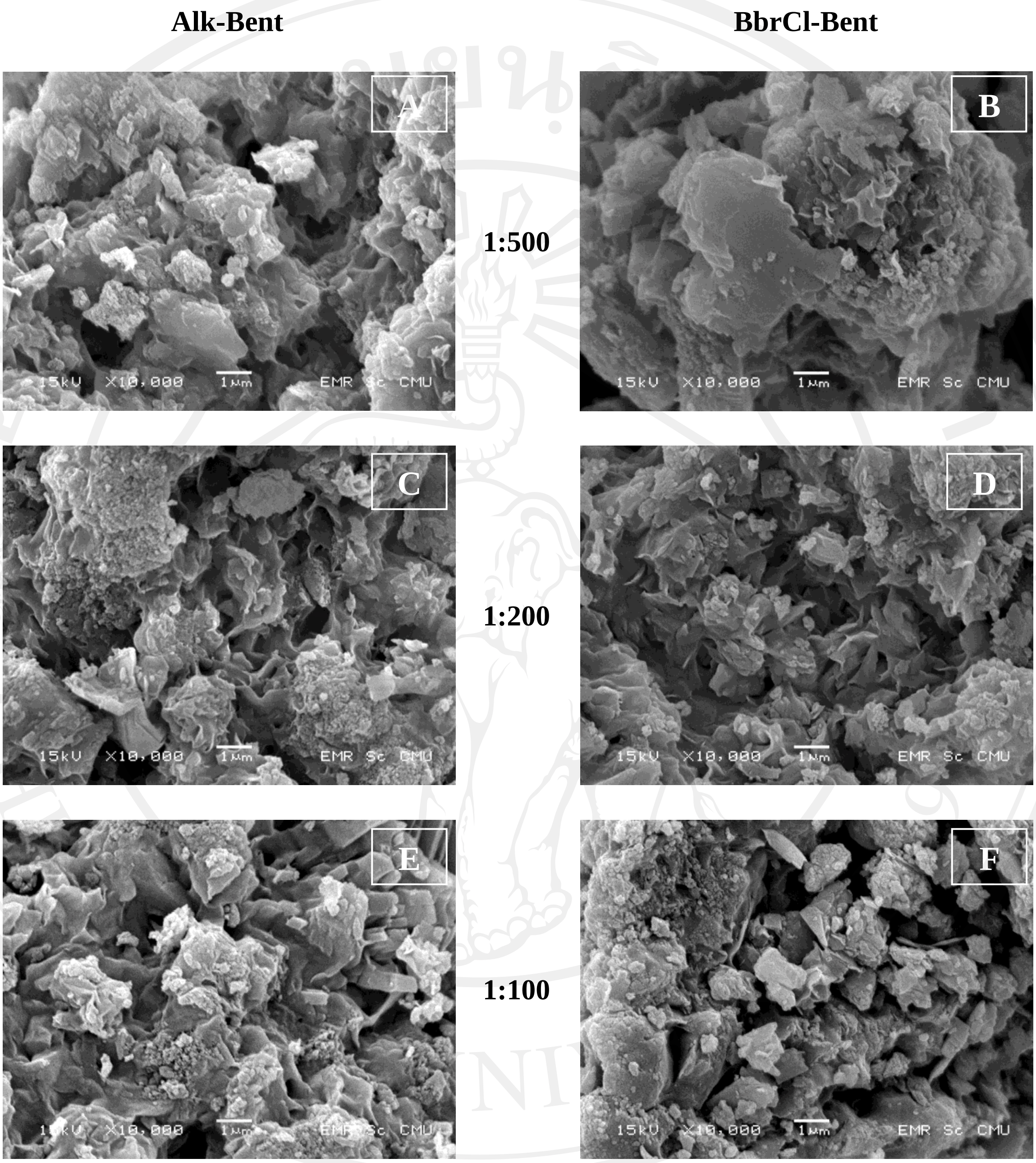


Figure 4.14 Physical structures of modified Alk-Bents and BbrCl-Bents by SEM technique. Alk-Bent (A, C, E) and BbrCl-Bent (B, D, F) were arranged by ratio 1:500, 1:200, and 1:100 respectively

DISCUSSION

Pharmaceutical study showed that *C. fenestratum* has antifungal, antiyeast, antibacterial, hypertensive and antiproliferative activities. The major components in stem or root of *C. fenestratum* are isoquinoline alkaloids such as berberine, palmatine, crebanine, and jatrorhizine, while berberine is found to be major and active constituent [49]

Usually, acid-base extraction would rather for alkaloid isolation, but, this is not specifying for berberine compound. Maceration with ethanol was reported and effective for extraction main compound as berberine [59]. In addition, berberine was noticed to precipitate in chloride salt form. NaCl; a simple chemical which harmless, low-cost and chloride dissociable was on the top of berberine for separation and initial purification in *C. fenestratum*. Since, addition of NaCl was preceded in solution, including of evaporation ethanol was processed the mixture would be separated berberine precipitate by dissolve in NaCl solution. Completely NaCl removal was released yellow precipitate on the bottom of a centrifuge tube. Berberine in chloride form was non-polar compound; the rule “like dissolve like” was responded for separation from water. Yellow precipitate was noticed and optically observed by naked eyes after dried in oven all night.

Determination of alkaloid quality in yellow precipitate using *Dragendroff’s* reagent was once method for alkaloid identify (See in appendix C). Few drops of reagent were reacted with sample in ethanol solution, orange precipitate was settled down immediately drop. That's mean it is positive results for further berberine identification by HPLC.

Most of active adsorbent studies were performed with purified clays of specific sources. Although such purifications were essential for research purposes, they are generally not performed for commercial or industrial uses, due to high cost. Thus, there is interest in studying and understanding interactions between organic modifiers and raw commercial bentonite in order to explore their applicability for producing low-cost adsorbent, since in most cases the cost of the prepared organobentonite would be crucial in the decision whether to use this material [26].

Bentonite is a 2:1 layer clay mineral and has two silica tetrahedral (T) sheets bonded to a central alumina octahedral (O) sheet. Considering only two most common bentonite varieties, sodium bentonite expand or swell in aqueous suspensions more than the calcium bentonite. A comparison of the calcium and sodium bentonite showed the at the calcium variety has fewer, more tightly bound water layers associated with it, whereas the sodium variety has many water layer, but bound less tightly [29].

Commercial bentonite was used to perform Na-Bent by NaCl solution. Exchanging of Na^+ ions into inorganic interlayer surface of bentonite would release any inorganic or matrix contamination. Calcium bentonite corresponding to the exchangeable cation in sodium Na^+ ion gave higher extending space. Na-Bent was preferable use in several treatments or act as active adsorbents, that's because their less tightly bound and higher quality of adsorption.

Bentonite known to be an effective adsorbent for water systems, however bentonite has limited use as coagulant to remove organic pollutants from both drinking water and wastewater [29]. The increased treated of the environmental pollutant by difference organic compounds and especially pesticides promoted investigation so to modify of bentonite on the use as sorbents for organic compounds. Instead of activated carbon used before [1].

In this report, commercial bentonite was perform to Na-Bent by costless and continued to modify with berberine. It's an organic compound acted as organic ammonium salt. In generally, call organobentonite. Bentonite obtained by exchange of organic cations for inorganic interlayer cations with salt. In both purified and extracted berberine sources.

Several investigations have been conducted; the surface properties of bentonite were modified greatly by replacing natural inorganic exchangeable cations by large ammonium cations [26]. The metal cations are leased into solution and the intercalated organic cation act as pillars which hold the 2:1 layers permanently apart [29]. The physical observation was noticed the change of color into berberine color like. The grinded mass was easier than Na-Bent which was assuming the expanding interlayer porous.

Large quaternary ammonium cations of organic cation, including of berberine compound improved adsorptive capability of organobentonite for the organic compound. This type of organobenonite, quaternary ammonium cations are separate from each other and function as pillar to permanently open the interlayers of bentonite

. Identification of structure changing of organobentonite after modified with berberine was monitored by SEM technique which was further discussed in their topic [14, 27].

The presence of pesticides in water has raised concern for the protection of the environment. Using of clay adsorbent for adsorb pesticide was studied by modified with berberine. This change can be used to enhance pesticide adsorption. HPLC is considered an instrumental technique of analytical chemistry. Due to the small sample amount can separate and HPLC columns are made with smaller sorbent particle, comparable with other technique, which is why it is a popular chromatographic technique. Several pesticides studies were analyzed with this HPLC, either.

Three pesticides were treated with organobentonite; the results reveal the difference adsorption property in clay. All adsorption experiments with organobentonite were showed that more than 80% of pesticides were adsorb. In level of carbaryl residue in aqueous sample from difference ratio was determined by HPLC analysis. Highly adsorption capacity of carbaryl by organobentonite, suggested the less toxicity of pesticide which can spread on the environment. In part of clay, modified bentonite with BbrCl gave higher adsorptive results than Alk adsorption. Portion between berberine: bentonite at ratio 1:200 may be estimated as the best condition for adsorption of carbaryl adsorption determination. Therefore, this modify organobentonite was helpful for enhanced carbaryl adsorption pesticide.

A part of methomyl, quantitative determination described the similar results of all experiments including control, Alk-Bent and BbrCl-Bent. Percentages of each organobentonite were not different value, even though, modified bentonite experiment. Explanation for these results was the pesticide property of degradation. The mechanism of two pesticides was rapid degradation itself. Thus, the traceable of both pesticides were difficult [83, 89].

In addition, methiocarb experimental was also occurred as same as methomyl results, which degradation reaction was occupy on the HPLC analysis process. The degradation product explained the degradation on the sample adsorption chromatograms. Finally, the product cause of degradation of these two pesticides might be other degraded compound which was occurred, that couldn't certainly identified in this experiment was 100% be sure. Meaning rational for undetectable in the HPLC analysis in this experiment. The other suitable techniques may be given the crystal clear results for further identification the degradation product in these pesticides adsorption by modification of bentonite with berberine. The degradation product in Fig 5.1 was explained the adsorption results of methomyl and methiocarb. Because of organobentonite surface was rich of oxygen atom, which belongs to anion surrounding on the surface that can adsorb cation from solution. But methomyl oxime and methiocarb phenol; the degradation products contained non-cation which hardly stayed in anion surface. The similar anion for both organobentonite surface and degradation product was repulsing each other. That's mean possibility of degradation adsorption in organobentonite clay was negative and also released in solution [90].

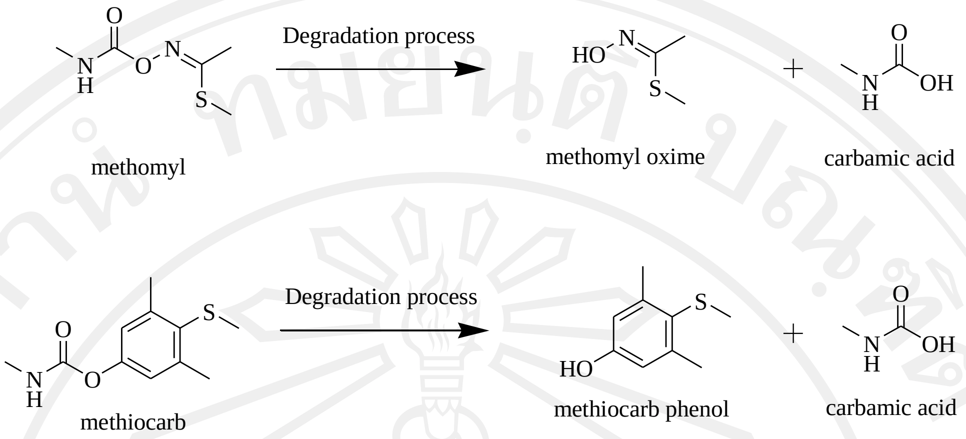


Figure 5.1 Degradation mechanisms of methomyl and metiocarb

Explanation of this situation that according to modification clay with quaternary ammonium cation, berberine could cause the opening permanently apart of organobentonite interlayer and make tightly bond. Other small molecules of pesticides from degradation product which can unbind in the clay surface because of they are smaller than pillars and not suitable for clay would be non-adsorb and still contaminate in water system. Thus, for methomyl adsorption experiment, also in the methiocarb degradation, comparable of adsorption of pesticide with degradation product was demonstrated the relevant of adsorption. Similarity with methomyl, the degradation product was effective with Na-Bent with berberine, their hydrolysis reaction; methiocarb was degraded and dissolved in water approximately 43% as methiocarb phenol and carbamic acid solution.

The modification of bentonite using purified berberine and extracted berberine with *C. fenestratum* were discriminate the results, such in carbaryl experimental, positively results reveal the higher adsorption pesticide property after modification with berberine. The situation was monitored when experimental of methomyl and methiocarb was proceeding, because of the other properties of degradation of pesticide which can be rapid for degradation in soil. Meaning, this modification was not suitable for metomyl and methiocarb, because of they can easy and give unpredictable results.

Even though, the degradation of methomyl and methiocarb was caused the contamination of degradation product of both pesticide above, but it's seem to prove that berberine compound in modification with bentonite clay helps much to collect pesticides inside and not allowed them to contaminate environment.

Only carbaryl pesticide was suitable with modified bentonite with berberine, and other carbamate pesticide; methomyl and methiocarb were not perfect for adsorption experiment with this organobentonite modification.

Na-Bent showed the compacted layers between interlayer of bentonite. Na^{+} ion exchanged into layers was affected to clay and made Na-Bent increased the swelling. Notice on commercial bentonite, the tightly bound of Ca^{2+} ions between interlayer responded less swelling of water system and showed the morphology that larger impact than Na-Bent.

From previous study, the organobentonite prepared by smaller group of organic ammonium cation showed the very weak adsorptive capability. And large group of ammonium cation can cause of separate the interlayer apart and permanently. Berberine is a large and fixable molecule; the modification with bentonite would rather show the changing of organobentonite morphology, after the modification. The intercalated into layers of bentonite with berberine make the porous of organobentonite larger, compared with physical structure of modified Na bentonite and commercial bentonite.