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ZETA POTENTIAL ANALYSIS: EFFECT OF CPB-KAOLINITE TO THE GROWTH OF BACTERIA

[1]NUR ISTI'ANAH RAMLI, [2*]NIK AHMAD NIZAM NIK MALEK, [3]SITI AISHAH MOHD HANIM, [4]MASHITAH MAD SALIM, [5]NOR SYAFAWANI SARAH MD SAAD, [6]SALEHHUDDIN HAMDAN

Faculty of Biosciences and Medical Engineering,
UniversitiTeknologi Malaysia,
81310 UTM Johor Bahru, Johor, Malaysia

[1]nuribtr@gmail.com, [2*]niknizam@fbb.utm.my,[3]sitiaishah6@hotmail.com, [4]mashitah_madsalim@yahoo.com, [5]syafawani@fbb.utm.my, [6]saleh@fbb.utm.my,

*Corresponding author

Abstract. The formation of cetylpyridinium bromide (CPB) loaded onto natural kaolinite from Indonesia with different initial CPB concentration ($[CPB]_i$) was studied through zeta potential analysis. From the data obtained, CPB molecules have been attached to the surface of kaolinite structure indicated by the changes of the zeta potential value of the modified kaolinite with CPB from negative charge to positive charge. In detail, as the concentration of CPB achieved its critical micelle concentration (CMC) level at ~0.9 mM, the sample with $[CPB]_i$ below CMC level show negative value of zeta potential and vice versa. Interestingly, there is no growth of bacteria at sample modified with CPB concentration above CMC level of CPB. Hence, the modified kaolinite was successful inhibit growth of bacteria at a certain amount of CPB molecules attached to the kaolinite surface.

Keywords Organo-clay; adsorption; zeta potential; antibacterial agent; *Escherichia coli*

1.0 INTRODUCTION

Organo-clay is a material that consists of clay that has been modified with cationic surfactant [1]. Cationic surfactant can be attached on clay based on different charged (Positive and negative charges). Silicon and aluminium are the main elements that built the framework of clay. Uniquely, clay layer have special characteristic where its structure is negative charge and thus, having basis cation exchange capacity (CEC) [2]. In details, CEC is the capacity of clay to exchange the cation presented in the clay with other cations. Because of this behavior, the main function of clay is as adsorbent for various cations such as cationic surfactant and positively charged metal and they are located either in its layer or on its surface layer. Besides, each types of clay have different CEC value [3] and structure arrangement.

The guest cations that loaded on the structure of clay will result in different usage. In this study, cationic surfactant was used to load in clay in order to produce clay with antibacterial properties. Cationic surfactant is an organic compound that consists of hydrophilic head and hydrophobic tail resemble phospholipid molecules that build membrane cell of living organism [4].

Cetylpyridinium bromide (CPB) with formula name $C_{21}H_{38}BrN$ is one of the examples of cationic surfactant and has been used as a source of surfactant in this study because it able to inhibit the growth of bacteria. At certain concentration, cationic surfactant will form bilayer or micelles in a solution. This stage is named critical micelle concentration (CMC) [5]. For example, the CMC of CPB is at ~0.9 mM [6]. Hence, with this property, surfactant is able to function not only as excellent adsorbent but also as bactericide [7] and has been applied broadly in daily life especially in household product [8].

From previous study [9], we have shown the effect of different amount of CPB loaded on kaolinite. The CPB-loaded kaolinite with different CPB loading capacity was characterized by the elemental analysis and Fourier Transform Infrared (FTIR) spectroscopy. Results showed that the high amount of CPB adsorbed on kaolinite when there was high [CPB] in the solution. From characterization using FTIR-ATR technique, the spectra of solid sample showed the presence of two significant peaks appeared at ~2850 cm^{-1} and ~2920 cm^{-1} which represented the molecule of CPB on kaolinite. Furthermore, due to strong vibrational motion of hydrocarbon of CPB molecules, sample having high amount of CPB produce high absorbance intensity of the peaks in FTIR spectra. In summary, CPB compound have been successfully loaded on kaolinite. Therefore, as continuity from the previous research, the aim of this study was to characterize CPB-loaded kaolinite for their structure using X-Ray Diffraction (XRD), their relative charge using Zeta Potential analyzer and their antibacterial activity against *Escherichia coli* through Disc Diffusion Technique (DDT).

2.0 EXPERIMENTAL

2.1 Materials

The source of clay used in this study was natural kaolinite (NK) obtained from Bandung, Indonesia. The CEC of NK was determined as 8.51 meq/100g calculated from the method of saturation of NH_4^+ and the analysis of released of NH_4^+ was performed by UV-vis spectrophotometer (Macherey-Nagel). Surfactant compound named N-Cetylpyridinium bromide monohydrate (CPB) was purchased from Merck, Germany with structural formula of $C_{21}H_{38}BrN.H_2O$ and the molecular weight (MW) is 402.46 g/mol. Nutrient agar, sodium chloride (NaCl) and barium chloride were purchased from Merck. Sulfuric acid (H_2SO_4) 95-97% was purchased from QRëC company. The *E. coli* species was obtained from Microbiology Laboratory, Faculty of Biosciences and Medical Engineering, UTM Skudai, Johor, Malaysia. All chemical reagents were of analytical grade and all aqueous solutions were prepared with distilled water (D-H_2O).

2.2 Sample preparation

A series of organo-kaolinite were prepared according to previous paper [9] where the kaolinite was mixed with CPB solution having concentration at a range of 0.01 mmol/L to 4.0 mmol/L.

2.3 Characterization Techniques

The dried organo-kaolinite powders were characterized by XRD (X-ray Diffraction) instrument (Bruker, D8 advance). The XRD patterns were recorded with a CuKα radiation at λ = 1.5406 Å at 40 kV and 20 mA in the range of $2\theta = 5^o$ to 35^o with a scanning speed of 0.05^o per second. The zeta potential analysis of organo-kaolinite was determined using ZEECOM, ZP analyzer from Microtec Co., Ltd. Company. The sample was prepared by mixed up 0.01g of sample with 50 g of distilled and the mixture was shaken vigorously to make sure the organo-kaolinite evenly dispersed in the solution. The sample was analyzed and the data was taken via ZEECOM software.

2.4 Antibacterial Assay

The disc diffusion method was chosen for the determination of the antibacterial activity of the samples. The bacterial colonies of *E. coli* were cultured on NA plates for 24 hrs at 37 °C. About 3 to 5 *E. coli* colonies from cultured plates were diluted in the 0.9% NaCl solution and the turbidity needed to be matched with 0.5 McFarland's turbidity (1.5×10^8 CFU/ml). The bacteria suspension was inoculated with sterile cotton bud on the surface of NA plates evenly by rotating 4 times at every 60° of the plates. The round pellet of dried organo-kaolinite powder that were pressed using hydraulic jack at a pressure of 3000 psi were located on the surface of the agar plates and incubated overnight at 37 °C. The zones of inhibition (total zone of inhibition - sample area) were measured and compared with control sample (without surfactant).

3.0 RESULTS AND DISCUSSION

3.1 Characterization of CPB-Loaded Kaolinite: XRD and Zeta Potential Analysis

Based on previous result [9], it has been found that there was a good correlation between the amounts of CPB molecules adsorbed on the structure of kaolinite with the initial concentration of CPB. Characterization by FTIR spectroscopy concluded the existence of CPB molecules in organo-kaolinite samples. Hence, further characterization of organo-kaolinite sample by XRD and ZP analysis may give more evidence regarding the attachment of surfactant on kaolinite and at the same time provide more other information in understanding about the structure of unmodified kaolinite and organo-kaolinite.

XRD is a method to identify the crystalline mineral phase of a material. In this study, it is hard to obtain accurate knowledge about the composition of natural kaolinite. In natural kaolinite, there are many species other than clay that might present together in its structure. Therefore, a careful examination needs to be done in order to compare between various kaolinite group mineral and also its impurities phases such as illite. The XRD pattern of all samples including the unmodified natural kaolinite are shown in Figure 3.1.

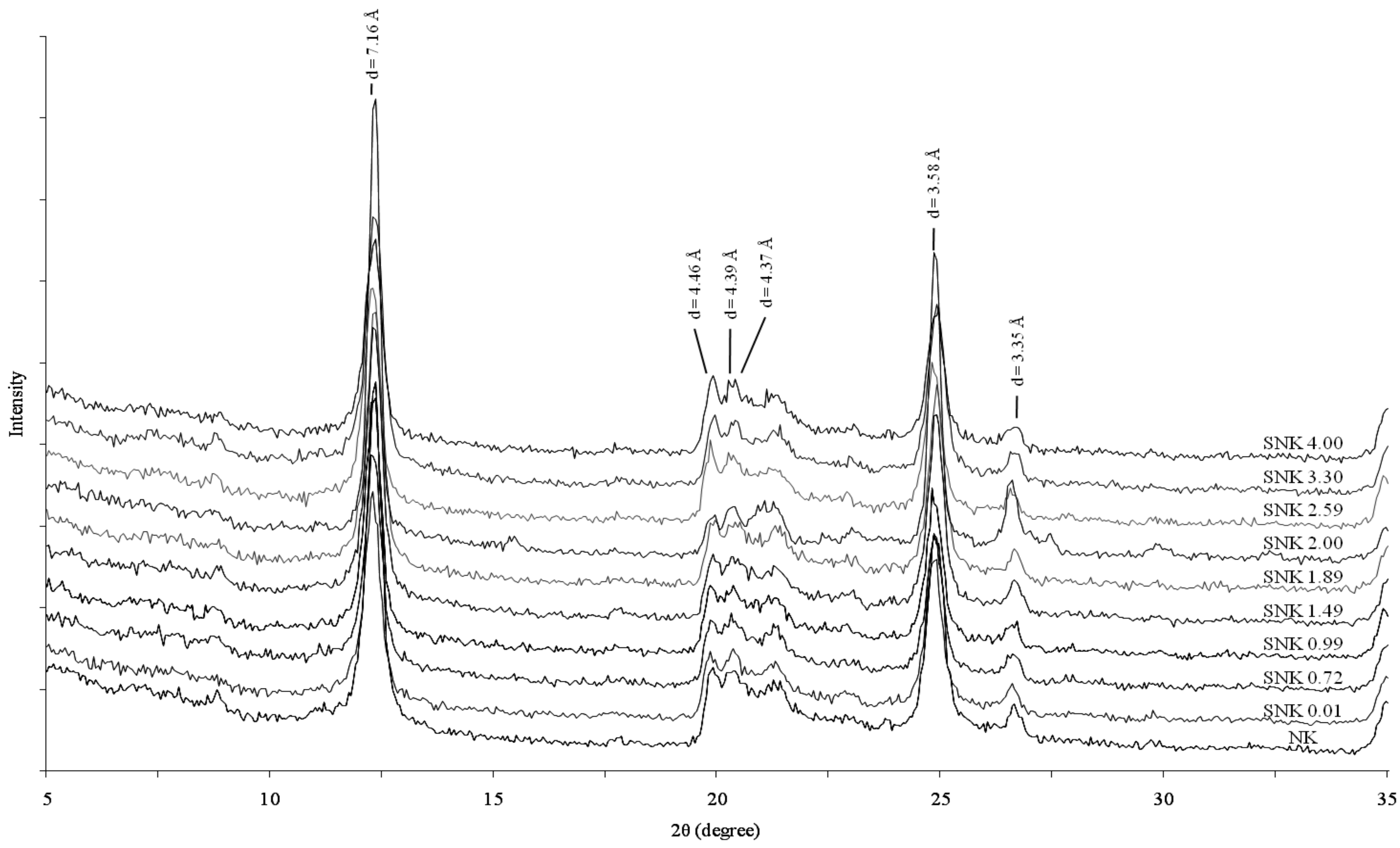

Figure 3.1: XRD diffractogram of organo-kaolinite with different surfactant loading

The XRD pattern of untreated kaolinite possess some strong peaks at 7.16 and 3.58 Å identical to those for kaolinite [10-12] while the other small peaks at 4.46 and 4.39 Å might contribute to the presence of illite, halloysite or kaolinite itself. Other impurities that are shown at ~4.37 and ~3.35 Å are probably the quartz element [10]. XRD pattern of the unmodified kaolinite is similar to that of the entire organo-kaolinite samples (labelled from NK 0.01- SNK 4.0) because there are no changes occurred after attachment with surfactant molecules since only cation exchange process between the CPB and kaolinite structure had been occured [13-14].

To confirm the changes in the surface properties of organo-kaolinite, zeta potential (ZP) analysis was applied for the organo-kaolinite with different surfactant loading. It was an easy method to observe the surface properties of organo-kaolinite particles since it can determine the electrical potential at the solid-liquid interface in response to relative movement of particles. The kaolinite itself has negative value of zeta potential because of the structure of kaolinite that composed of aluminosilicate structure [15]. Figure 3.2 shows the ZP value of kaolinite modified with different CPB loading.

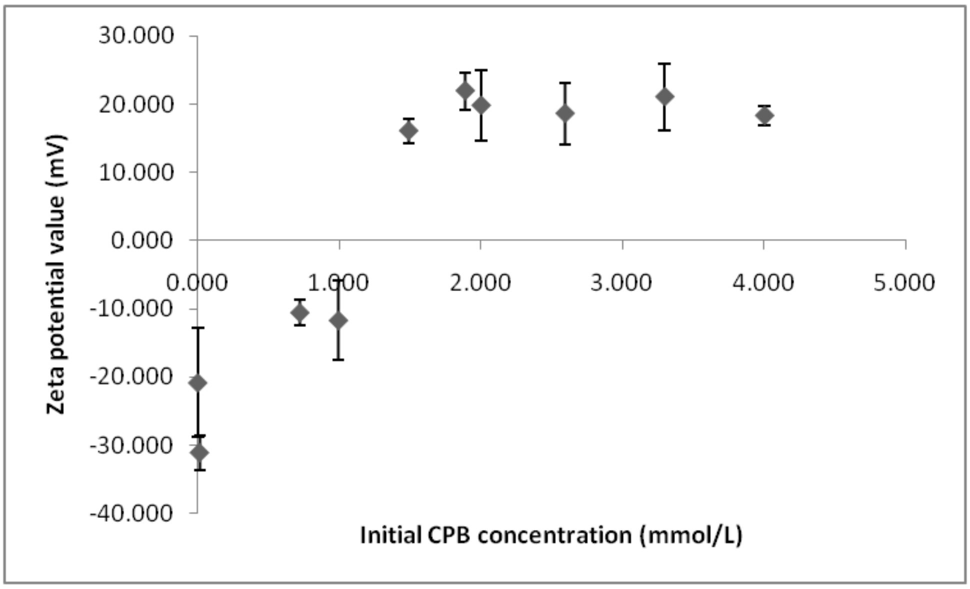

Figure 3.2: Zeta potential value of organo-kaolinite with different amount of surfactant loading.

The ZP value obtained as shown in Figure 3.2 demonstrated that the unmodified kaolinite and organo-kaolinite with $[CPB]_i$ at 0.01 (lower CMC level), 0.72 mmol/L (near CMC level) and 0.99 mmol/L (at CMC level) show negative zeta potential (negative charge). On the other hand, modified kaolinite with $[CPB]_i$ at above CMC level (from 1.488 – 4.00 mmol) show positive zeta potential (positive charge). To understand more on these phenomena, the possible mechanism of surfactant molecules attached on kaolinite is illustrated in Figure 3.3.

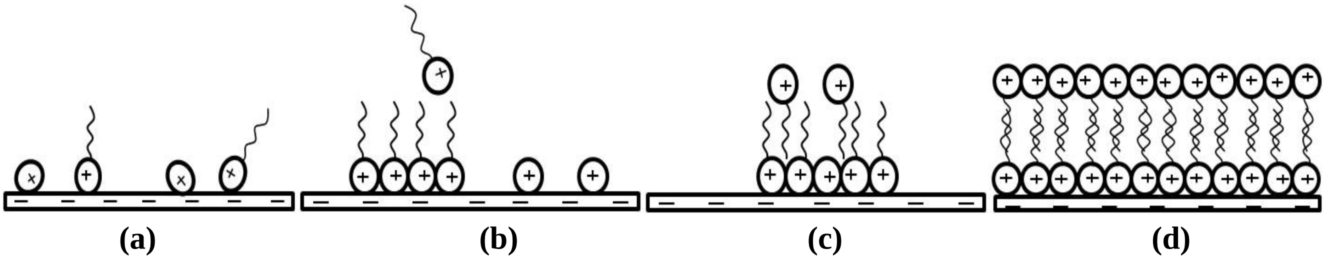

Figure 3.3: Mechanism of the attachment of surfactant molecules on kaolinite with different initial concentration of CPB. Notes: Organo-kaolinite layer; **(a-b)** below CMC level, **(c)** near CMC level, **(d)** at/above CMC level.

Based on Figure 3.3 and ZP values, the ZP for unmodified kaolinite and organo-kaolinite attached with $[CPB]_i$ 0.01 mmol/L showed negative charge because of the framework structure of kaolinite itself is negatively charge. The concentration of CPB (0.01 mmol/L) is very low in solution and only a few molecules will adsorb on kaolinite surface as can be seen in Figure 3.3 (a). Sample at $[CPB]_i$ from 0.72 and 0.99 mmol/L showed negative value of ZP eventhough the $[CPB]_i$ was increase probably due to the little amount of surfactant adsorbed on kaolinite. Only a little surfactant molecules have been distributed on the external surfaces of the kaolinite particle at the equilibrium and ZP being sensitive only to the composition of the external surface. In fact, the significant mechanisms of cationic surfactant adsorption onto clay are through ion exchange and hydrophobic interaction. For that reason, the CPB concentration range from 0.72 and 0.99 mmol/L is expected to be dominated by ion exchange mechanism where it occurs in an equivalent amount of cations in order to maintain total electroneutrality which requires the ZP to remain constant through the exchange process [16].

A sharp increase of ZP with the decreased [CPB] (from 0.99 to1.48 mmol/L) was due to the dramatic change in the composition of the external surface of sample because of CPB adsorption. The surface of kaolinite particle has at least some of CPB molecules that adsorbed via hydrophobic bonding were distributed on its external surface. The positive charge is able to disaggregate the cluster of kaolinite's platelets and increase the degree of sample dispersion if it have sufficient amount in the sample interlayer. The sample at higher $[CPB]_i$ (> 1.48 mmol/L) with almost constant positive value that show only little change in ZP as the CPB loading increase might be because of the disaggregation of the kaolinite's platelets surface that bonding together caused by surfactant adsorption through hydrophobic bonding in the interlayer space of kaolinite [16].

3.2 Antibacterial activity

The antibacterial activity of organo-kaolinite with different CPB-loading was studied by Disk Diffusion technique against *E. coli*. The zone of inhibition (mm) from Disc Diffusion Technique is given in Table 1.

Table 1: Zone of inhibition after applied with different surfactant loading on kaolinite

Sample	Zone of inhibition (mm)
SNK 0.011	0.00
SNK 0.720	0.00
SNK 0.992	1.47 ± 0.03
SNK 1.488	1.48 ± 0.03
SNK 1.888	1.50 ± 0.01
SNK 2.000	1.44 ± 0.02
SNK 2.592	1.45 ± 0.04
SNK 3.296	1.46 ± 0.02
SNK 4.000	1.51 ± 0.06

Generally, organo-kaolinite seems to have antibacterial activity when kaolinite was modified with initial concentration of CPB of 0.992 mM and higher. As the amount of CPB in the sample of organo-kaolinite increase, the formation of inhibition zone becomes higher [17]. Report from Özdemir and his group showed that the antibacterial activity occurs at the surface of organo-clay between the surfactant molecules and the bacteria's cell wall. When the bacteria cell attached to the surface of organo-clay, moieties of the surfactant penetrate the cell membrane and interrupt the normal function of the cell by causing leakage of the cell components, disrupt the cell metabolism and cell growth inhibition leading to cell death. [18]. Therefore, it is suggested that organo-kaolinite with high amount of CPB molecules showed good antibacterial effect.

Another study suggested that the 'tail' of surfactant molecules penetrate into the membrane of bacteria and consequently destroyed the membrane layer leading to death of bacteria as the negatively charged bacteria's membrane make contact with the 'head' of the surfactant molecules [19]. Another study show that the intercalation of cationic surfactants not only changes the surface properties of organo-clay but also increases the anion adsorption capacity especially when the surfactant loading beyond the clay CEC. As a result, the adsorption of surfactant molecules through hydrophobic bonding and the cationic surfactant head will attract anions. Hence, the condition subsequently changes the pearmeability of cellular membrane leading to the diffusion of intercellular ions and low molar mass metabolites out of the bacteria's cell [17].

4.0 CONCLUSIONS

The structure of organo-kaolinite was confirmed by XRD and ZP analysis proved the charge of organo-kaolinite at different surfactant loading. The result shows that there is no change of the kaolinite structure after attachment with surfactant. Also, the relationship between the amounts of CPB in organo-kaolinite is directly proportional to the value of zeta potential and the antibacterial effect.

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