

Antibacterial activity of CTAB-modified zeolite NaY with different CTAB loading

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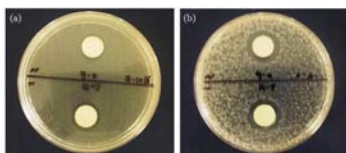
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GRAPHICAL ABSTRACT



ABSTRACT

The antibacterial activity of cetyltrimethyl ammonium bromide (CTAB) – modified Zeolite NaY against *Escherichia coli* and *Staphylococcus aureus* was examined in agar disk diffusion (Kirby-Bauer) method. The diameter of the inhibition zones for *E. coli* and *S. aureus*, increased from 1.5 to 1.7 cm and 1.9 to 2.0 cm, respectively, with the increasing concentrations of CTAB adsorbed on Zeolite NaY from percent coverage of the External Cation Exchange Capacity of Zeolite NaY: 0.5 to 5.0. CTAB-modified Zeolite NaY showed effective antimicrobial activity against *S. aureus* compared to *E. coli*.

Keywords: Cetyltrimethyl ammonium bromide (CTAB), Zeolite NaY, Cation Exchange Capacity, *Escherichia coli*, *Staphylococcus aureus*

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1. INTRODUCTION

Due to the outbreak of the infectious diseases caused by different pathogenic bacteria and development of antibiotic resistance, pharmaceutical companies and researchers are searching for new antibacterial agents. Immobilized antimicrobial agents on inert materials such as zeolites and clays gave a great interest as they have some advantages; (1) preventing patient exposure to biocides solutions, (2) lowering their irritant properties, and (3) prolong the duration of antibacterial efficacy [1].

Zeolites are crystalline hydrated aluminosilicate with their structure is based on an infinitely extended three-dimensional network consisting of $[AlO_4]^{5-}$ and $[SiO_4]^{4-}$ connected mutually via oxygen atoms. The substitution of Al^{3+} for Si^{4+} in zeolite framework results in negatively charged aluminosilicate lattice. Hence, inorganic metal cations such as Na^+ , K^+ , Ca^{2+} and Mg^{2+} are presented in the zeolite framework to neutralize the aluminosilicate lattice of zeolite. The cations are movable and exchangeable by other cations [2] and thus, zeolite having low Si/Al ratio resulted in high cation exchange capacity [3].

Quaternary ammonium compounds (QACs) are kind of biocides which are used in household products. Due to its broad spectrum antibacterial characteristics, they also have been applied in pharmaceutical, water treatment and antifungal treatment for crops [4]. In general, QACs are chemically stable within pH 3 to pH 9 [5] and show antimicrobial activity with chain length composed of 8 to 18

carbon atoms [6]. Since cetyltrimethyl ammonium bromide (CTAB) has 16 carbon atoms on its hydrocarbon tail, it was used to modify Zeolite NaY surfaces in this study.

It is well known that QAC functions as a cationic surface-active agent having antimicrobial activity due to cellular disruption of the cell membrane [6, 7, 8]. QAC displays antimicrobial activity even after being covalently immobilized on inert supports [9]. Immobilized surfactant (CTAB) on natural zeolite has been shown to inactivate viruses and bacteria [10]. Preliminary results for the combination of QAC and zeolite have shown promise for their combined antimicrobial efficacy [11]. QAC as a cationic surfactant reduces the surface tension at interfaces and is attracted to negatively charged surfaces, including zeolites and microorganisms. Quaternary ammonium ions act by penetrating the cell membrane causing leakage of cell components, disruption of the bacterial metabolism, inhibition of cell growth, and cell death [12].

Staphylococcus aureus is one of the most common causes of foodborne disease and it is also an infectious pathogen. Staphylococci are frequently found on surfaces in hospitals, households, and in the food industry. *S. aureus* present on surfaces or equipment may be a source of cross-contamination of food. As other Gram positive bacteria, staphylococci are relatively tolerant to desiccation and may survive on open surfaces for long periods [13]. *E. coli* as a representative of fecal, facultative anaerobic microorganism was used to represent Gram negative bacteria.

The aim of this work was to prepare CTAB-modified Zeolite NaY with different CTAB loading and to observe the effects of different CTAB loading on Zeolite NaY on the antibacterial activity against *E. coli* and *S. aureus*. In this study, a good carrier system from Zeolite NaY was used because of its relatively high ion exchange capacity and lattice stability [14]. Zeolite NaY (SiO₂/Al₂O₃ ratio: 5.1) was loaded with CTAB, a QAC by the ion exchange method. Zeolites having a specific surface area at least 150 m²/g with Si/Al ratio below 14 are suitable to be used as a carrier for antibacterial agents [15]. Because of its large size, the cationic surfactant was unable to penetrate into the zeolite framework, thus the surfactant molecules were occupied on the surface of Zeolite NaY by ion exchange with the Na⁺ on zeolite surface. Increasing the concentrations of CTAB in the solution will increase the amount of cationic surfactant loaded onto Zeolite NaY.

2. EXPERIMENTAL

2.1 Materials

The commercial Zeolite NaY was supplied by Zeolyst International (CBV 100) with a SiO₂/Al₂O₃ mole ratio 5.1; nominal cation in sodium form (Na₂O weight (%): 13.0); while the unit cell size 24.65 Å and surface area 900 m²/g. The external cation exchange capacity (ECEC) of Zeolite NaY was 0.53 mequiv/g [16]. Cetyltrimethyl ammonium bromide (CTAB) was supplied by Merck-Schuchardt.

2.1.1 Modification of Zeolite NaY with CTAB

Eight series of CTAB-modified Zeolite NaY were prepared by reacting zeolite with CTAB in aqueous solutions. CTAB was added in an amount equal to 0.5, 0.7, 1.0, 1.5, 2.0, 3.0, 4.0 and 5.0 of ECEC of zeolite. This experiment was designed to study the effect of different surface coverage of CTAB onto the zeolite; in the antibacterial activity against pathogenic bacteria of *E. coli* and *S. aureus*. For example, the preparation of CTAB-modified Zeolite NaY (ECEC: 0.5) needed 0.1932 g of CTAB to saturate 50 % of the ECEC Zeolite NaY (2 g). The calculation of the amount of CTAB needed was shown below:

ECEC of Zeolite NaY = 53.0 meq/100 g.

Molecular weight of CTAB = 364.46 g/mol.

For 1 meq/100 g = 6.02×10^{20} adsorption sites.

= 1 mmol/100 g.

= 0.01 mmol/g = 1.0×10^{-5} mol/g.

Hence, 1 g of Zeolite NaY with ECEC = 53 meq/100 g can be satisfied with 5.3×10^{-4} mol of cations to achieve 100 % satisfaction.

Amount of CTAB needed:

= $0.50 \times 2 \text{ g} \times 5.3 \times 10^{-4} \times 364.46 \text{ g/mol}$.

= 0.1932 g CTAB.

CTAB solution was prepared by dissolving an appropriate amount of CTAB in double distilled water and was heated gently until all CTAB was dissolved. After that, the CTAB solutions were mixed with zeolite and the mixture was stirred using a magnetic stirrer for 16 hours at room temperature. The mixture was then filtered by Whatman (No. 2) filter paper and the solid samples were dried at 70 °C overnight. The resultant products were readily used for antibacterial test.

2.1.2 Determination of adsorbed CTAB on zeolite

The CTAB concentrations in the solution were determined by UV-Visible Spectrophotometer. However, prior to the determination of CTAB concentrations, the solution needs to be complexed by acid orange 7 (orange II) dye since CTAB alone cannot be detected by the UV-Visible Spectrophotometer [17]. The acidic condition is needed in order for the CTAB molecules to bind to the dye forming CTAB-dye and these compounds are soluble in chloroform. The acid orange dye alone was not soluble in chloroform, but soluble in water. About 25 ml of distilled water (DW), 5 ml of sulfuric acid (H₂SO₄, 2M) (QRec) and 10 ml of chloroform (QRec) were mixed in the separating funnel. About 2 ml of acid orange 7 (orange II, 1 mM) was then added to the previous mixture and shaken. Then, 1 ml of the solution sample was added, vigorously shaken and chloroform layer was extracted and ready for the next analysis. The standard solutions of CTAB were prepared in the range of 0.2 to 1.0 mM. The extracted chloroform from the samples was measured by UV-Vis at absorbance $\lambda_{487 \text{ nm}}$ using the Vis Spectrophotometer (NANOCOLOR VIS, Macherey Nagel).

The adsorption study was carried out by batch method. The amount of CTAB adsorbed on Zeolite NaY was calculated from the difference in concentrations of CTAB before and after the adsorption on zeolite. The adsorbed amounts were determined by using the following equation:

$$Q = ((C_i - C_{eq})V)/m$$

Where Q is the adsorbed amount in mmol/kg, C_i is the initial concentration of CTAB before the adsorption in mmol/L, C_{eq} is the equilibrium concentration of CTAB in mmol/L, V is the volume of the solution in L, and m is the amount of Zeolite NaY.

2.1.3 FTIR characterization of CTAB-modified Zeolite NaY

FTIR Spectroscopy was used to examine the presence of surfactant (CTAB) molecules on zeolite. Thermo Scientific Nicolet iS5 with Smart iTR Diamond crystal FT-IR (Fourier Transform-Infrared) spectrometer was used to obtain IR spectra. The FT-IR spectra of the samples were obtained from 550 to 4000 cm⁻¹ by

accumulating 16 scans at a resolution 4 cm^{-1} by KBr method and FTIR spectrum was recorded using OMNIC software.

2.1.4 Antibacterial activity testing

The antibacterial activity of the CTAB-modified Zeolite NaY was tested against *E. coli* and *S. aureus* by slightly modified the disk diffusion test according to the National Committee for Clinical Laboratory Standards [18]. The stock culture of both bacteria has been obtained from Institute of Medical Research (IMR), Malaysia. The tests were accomplished in duplicate. The bacteria were cultured on Nutrient Agar (NA) at $37\text{ }^{\circ}\text{C}$ for 24 h; three to five colonies were diluted with sterile saline solution (0.9 %) and the suspension was adjusted to 0.5 McFarland turbidity (1.5×10^8 CFU). A sterile cotton swab was used to inoculate the surface of another NA plate, rotating the plate every 60° to ensure homogenous growth. The synthetic and treated samples (0.3 g), with and without CTAB, were pressed into pellets (13 mm diameter) and placed over the surface of the agar plates. The width of inhibition was measured after 24 h of incubation at $37\text{ }^{\circ}\text{C}$.

3 RESULTS & DISCUSSION

3.1 Adsorption of CTAB on Zeolite NaY with Different CTAB Loading

The external cation exchange capacity (ECEC) is a measurement of the negative charges on zeolite external surface. Because of the large molecular size of surfactant molecules (CTAB), these molecules cannot enter the angstrom sized pore of the zeolite, instead will exchange with Na^+ on the external surface of the Zeolite NaY. Different concentrations of surfactant molecules in aqueous solution will give different ECEC coverage of the surfactant molecules on zeolite surface. Fig. 1 shows the amount of CTAB adsorbed on Zeolite NaY with different CTAB loading.

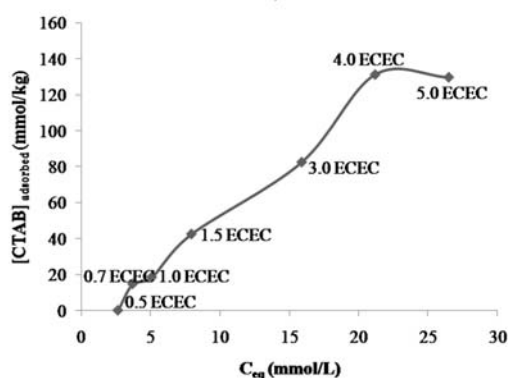


Fig. 1 Adsorption of CTAB on Zeolite NaY from aqueous solution.

According to Fig. 1, an increase in the equilibrium concentration of CTAB in solution from surfactant coverage

0.5 to 4.0 (2.65-21.20mmol/L); would increase the amount of surfactant molecules adsorbed onto Zeolite NaY. The steep slope obtained reflects the high affinity of adsorbate (surfactant molecules) adsorbed onto adsorbent (Zeolite NaY). Based on theory, at the ECEC coverage of 0.5-0.7, surfactant cations were in the form of partial-monolayer on zeolite surface, at the ECEC coverage of 1.0, surfactant cations were in the form of complete-monolayer, at the ECEC coverage of 1.5, surfactant cations were in the form of partial-bilayer and at the ECEC coverage of 2.0, surfactant cations were in the form of complete-bilayer [19]. At low concentration of surfactant (ECEC: < 1.0), the cationic surfactant molecules were adsorbed on negative charges of zeolite surface by ion-exchange, while increasing the concentration of surfactant (ECEC: > 1.0), the cationic surfactant molecules were adsorbed on the zeolite surface by hydrophobic bonding to the alkyl chain of the first layer of surfactant molecules [19]. At surfactant coverage more than twice ECEC (ECEC > 2.0), the adsorption of surfactant molecules on zeolite surface create hemimicelle aggregates more than as bi-layer [20]. There are variety of sizes and shapes of the aggregates such as patchy bi-layer, small spherical micelle as well as multi-layer [21]. At this point, the adsorption of surfactant molecules on Zeolite NaY are unstable as it is loosely bound to Zeolite NaY surface and tend to release back into the solution [16]. In this study, the adsorption of surfactant molecules on the Zeolite NaY continuing increase above surfactant coverage (ECEC: > 2.0) until it reaches surfactant coverage (ECEC: 4.0). The graph reached saturation at the surfactant coverage of ECEC 4.0 to 5.0 (21.20 - 26.51mmol/L). Therefore, increase the amount of surfactant molecules in working solution does not increase the amount of surfactant loaded onto Zeolite NaY.

3.2 Characterization of CTAB-Modified Zeolite NaY by FTIR

The FTIR spectra at a region of 3500 to 2500 cm^{-1} for CTAB-modified Zeolite NaY with different ECEC coverage (0.5, 0.7, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0) that have been compared with that of parent Zeolite NaY can be seen in Fig. 2.

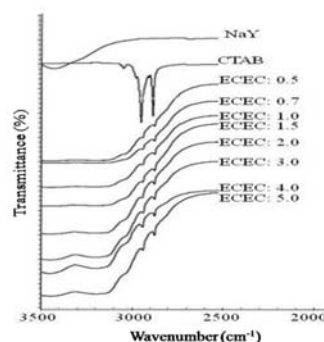


Fig. 2 FTIR spectra of CTAB-modified Zeolite NaY with different CTAB coverage of ECEC Zeolite NaY.

The two significance peaks appeared as an indicator that the CTAB molecules have been successfully attached on parent zeolite [22]. FTIR spectra of CTAB-modified

Zeolite NaY showed the emergence of 2 additional peaks between 2800 and 2900 cm^{-1} but absent in the FTIR spectra of the parent zeolite. This indicates the presence of CTAB molecules on the zeolite surface. CTAB comprises of two parts; positive charge of ammonium hydrophilic head and another one is a long chain of hydrocarbon tail. The positive charge head can be attached to the negative sites of zeolite frameworks leaving the outer layer with long chains of hydrocarbon tail. Hence, two additional peaks that were observed in FTIR spectra were assigned to the C-H bonding of long chain hydrocarbon tail.

This result is in agreement with other papers where peaks in the FTIR spectra around 2849 and 2916 cm^{-1} were assigned to the symmetric and the asymmetric stretching mode of C-H in CTAB molecules [23]. Therefore, FTIR spectra in Fig. 2 revealed the successful attachment of CTAB molecules on Zeolite NaY.

3.3 Antibacterial Activity of CTAB-Modified Zeolite NaY

The antibacterial activities of CTAB-modified Zeolite NaY with different CTAB coverage of Zeolite NaY was carried out by using disk diffusion (Kirby-Bauer) method where the diameter of the zone of inhibition was measured to determine their antibacterial activities. The bacteria *E. coli* and *S. aureus* were used to represent the Gram negative and Gram positive bacteria, respectively. An example of the optical image of the bacteria plate containing disks of CTAB-modified Zeolite NaY after incubation for 24 hours can be seen in Fig. 3 while the diameter of inhibition zone against the coverage of CTAB according to ECEC of Zeolite NaY is given in Fig 4.

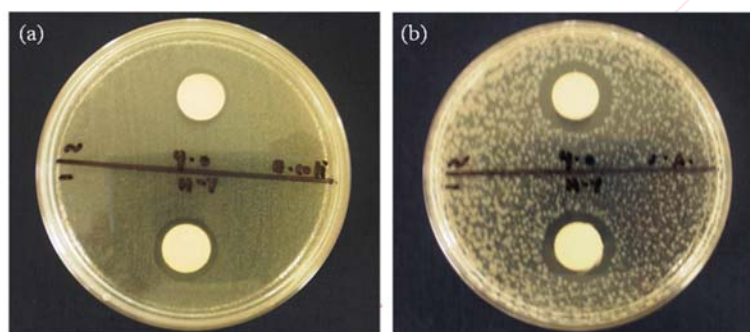


Fig. 3 Optical images of (a) CTAB (ECEC: 4.0)-modified Zeolite NaY on *E. coli* and (b) CTAB (ECEC: 4.0)-modified Zeolite NaY on *S. aureus* streaked agar plates after 24 h incubation.

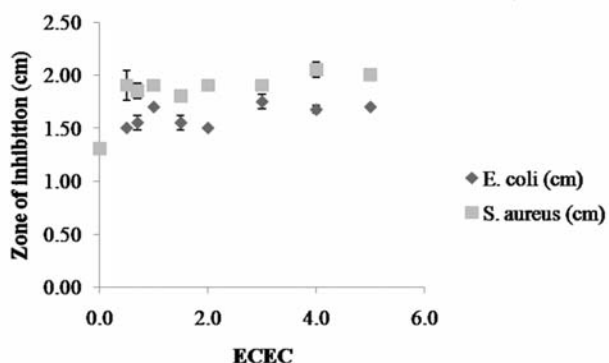


Fig. 4 Inhibition zone values as a function of CTAB-modified Zeolite NaY concentrations against *E. coli* and *S. aureus*. Each point represents an average of duplicate measurements.

The results of the antibacterial activity tests indicated that parent Zeolite NaY has very low antibacterial activity against both *E. coli* and *S. aureus* whereas antibacterial activities of CTAB-modified Zeolite NaY were effective against both bacteria. Generally, increased in the ECEC coverage of CTAB-modified Zeolite NaY (0.5, 0.7, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0) would increase the diameter of inhibition

zones of the samples thus reflect the increased of the antibacterial activity of the samples. However, compared to *E. coli* (inhibition zone: 1.5 to 1.7 cm), *S. aureus* (inhibition zone: 1.9 to 2.0 cm) were more susceptible to CTAB-modified Zeolite NaY. A fecal, facultative anaerobic microorganism of *E. coli* was used to represent Gram negative bacteria. The Gram negative bacteria (*E. coli*) contains more complex cell wall compared to the Gram positive bacteria (*S. aureus*) as it has an outer membrane which functions to preserve the bacteria cell from an attack of intrude compounds such as CTAB-modified Zeolite NaY. The outer membrane is located at the outside layer of peptidoglycan layer and it consists of lipopolysaccharides and phospholipids [24]. Thus, the lower sensitivity of *E. coli* towards CTAB-modified Zeolite NaY is mainly due to the presence of the outer membrane.

Based on Fig. 4, it is shown that the antibacterial activity of the corresponding CTAB-modified Zeolite NaY shows antibacterial effect to both bacteria, although it was not significantly different with different CTAB loading on Zeolite NaY. It might be due to the CTAB molecules that could dissociate from the CTAB-modified Zeolite NaY surface and directly exerts its antibacterial effect on the bacteria on the NA plates [25]. At surfactant coverage of

ECEC 0.5, 1.0, 1.5, 2.0 respectively surfactant coverages on Zeolite NaY surface were in the formed of partial-monolayer, complete-monolayer, partial-bilayer and complete-bilayer as mentioned before [19]. However, at higher CTAB levels (ECEC: > 2.0), CTAB molecules that loosely bound from admicelles on the CTAB-modified Zeolite NaY was released into the aqueous solution [16] containing bacterial cell culture. This behavior has been indicative of the adsorption of CTAB cationic surfactant through hydrophobic interactions since adsorption through ion exchange (ECEC: \leq 1.0) is almost irreversible [12]. Thus, the antibacterial activity of CTAB-modified Zeolite NaY with cationic surfactant coverage of 0.5 to 1.5 as shown in Fig. 4 was due to the localized of bacterial cells on CTAB-modified Zeolite NaY whilst at surfactant coverage of CTAB-modified Zeolite NaY ECEC \geq 2.0, the antibacterial activity was due to the released CTAB molecules onto the NA plates containing colonies of bacteria (*E. coli* and *S. aureus*).

4. CONCLUSIONS

- (1) For all samples, the amount of CTAB adsorbed increased with increasing initial concentration of the surfactant. At low concentrations, the adsorption process is driven primarily by cation exchange. At high concentrations, surfactant cations are adsorbed via Van der Waals interactions and by the interaction of the hydrocarbon chain with adsorbed CTAB on external surface of the Zeolite NaY.
- (2) Generally, the increasing of the ECEC of CTAB-modified Zeolite NaY (0.5-5.0) increased the inhibition zones for both of the bacteria (*E. coli* and *S. aureus*). However, compared to *E.coli*, *S. aureus* was more susceptible to CTAB-modified Zeolite NaY.

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