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# Modification of Synthetic Zeolites by Quaternary Ammonium Compounds and Its Antibacterial Activity against *Bacillus Subtilis*

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## Abstract

Quaternary ammonium compound (QAC)-zeolites A, X and Y were prepared and characterized and their antibacterial activity was studied against *Bacillus subtilis*. The presence of QAC molecules on the zeolites has been proven by Infrared (IR) Spectroscopy. The antibacterial activity of these materials was determined by Minimum Inhibition Concentration (MIC) technique. From MIC values, QAC-zeolite X has highest antibacterial activity than that of zeolites A and Y due to the higher amount of QAC adsorbed onto zeolites X. To sum up, zeolite X was found to be a good support system for antimicrobial agents of QAC against *B. subtilis*.

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**Keywords:** Synthetic Zeolites, Quaternary Ammonium Compound, Antibacterial Agent

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## 1. Introduction

In a modern world, most of the household products such as hand wash, laundry detergent, toothpaste and cosmetic contain antimicrobial agents such as Quaternary Ammonium Compound (QAC) as their additive in order to improve their personal hygiene. However, it can lead to the problem of microbial resistance to

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antimicrobial agents where the microbial strains survive when contacting with antimicrobial agent [1]. This problem creates serious issue in the treatment of microbial related infectious diseases. Therefore, it is very crucial nowadays to develop new antimicrobial agents having powerful antimicrobial activity against wide spectrum of microorganism that can be applied in household products

Zeolite, an aluminosilicate material known to have ion exchange property and hence, its cations can be exchanged with other cations such as QAC molecules. By exchanging its cations with QAC, the zeolites will have antibacterial activity since QAC are known to have antibacterial activity against wide spectrum of bacteria[2]. High ion exchange capacity of zeolites can be obtained from a zeolite framework having low Si/Al ratio. Example of zeolites having low Si/Al ratio is synthetic zeolites A, X and Y. Zeolite A is known to have the lowest Si/Al (Si/Al: 1) followed by zeolites X and Y. In the earliest invention of zeolite X and Y in 1961, Breck and his team classified zeolite as having Si/Al ratio between 1.0 to 1.5 for zeolite X and above 1.5 up to 3.0 was for zeolite Y [3]. Theoretically, when zeolite having low Si/Al ratio treated with QAC, it can adsorb a large amount of these compounds and retain in zeolite framework. Eventually, QAC-zeolites will possess antibacterial activity since their antibacterial activity depends mainly on the presence of QAC.

*Bacillus subtilis* was selected as targeted bacteria in this study due to their environmental ubiquity, adaptable in any kind of environment, causing food spoilage and also it able in forming dormant spore [4]. It is aim of this study to prepare and characterize modified zeolites with QAC using zeolites synthetic A, X and Y as support system and finally their antibacterial activity was studied against *B. subtilis*. The main purpose of this is to observe whether the Si/Al ratio of zeolites affect the antibacterial activity of the studied materials against *B. subtilis*.

## 2. Materials and Method

### 2.1. Preparation and Characterization of QAC-Zeolites

Synthetic zeolites A, X and Y was synthesized using rice husk ash as a source of silica and their preparation and characterization are described elsewhere [5]. In this study, Quaternary Ammonium Compounds namely (QAC) hexadecyltrimethyl ammonium bromide (HDTMA) and benzalkonium chloride (BKC) have been selected as modifier for zeolites external surfaces. These chemicals were purchased from Merck. Preparation of QAC-Zeolites QAC-zeolites A, X and Y were prepared by adding 200 mL QAC solution with 2.0 g of zeolites samples. The mixture was stirred using magnetic stirrer for 16 hours at room temperature. The mixture was then filtered using simple filtration technique. The solid residue was washed with distilled water twice and it was dried in an oven overnight.

Infrared (IR) Spectroscopy was used for the structural analysis and the presence of surfactant molecules on zeolites. Thermo Scientific Nicolet iS10 with Smart iTR Diamond crystal FT-IR (Fourier Transform-Infrared) spectrometer was used to obtain IR spectra. The instrument was applied for solid sample using Smart Auto Sampler. About 1-2 mg of sample was placed on sample area. FTIR spectrum was recorded using OMNIC software in the range of 4000 to 650  $\text{cm}^{-1}$ .

### 2.2. Antibacterial Assay

The antibacterial activity of the studied materials has been performed against *Bacillus subtilis* through the determination of Minimum Inhibition Concentration (MIC) values. The stock culture of *B. subtilis* has been obtained from Institute of Medical Research (IMR), Malaysia. The solution containing bacteria cells in distilled water or saline solution was added in the vessel containing different weight of solid studied samples where the concentration of the solid sample in the bacteria solution was in the range of 0.1 to 10.0 g/L (ratio

of solid samples to solution containing bacteria).

### 3. Results and Discussion

Synthetic zeolites A, X and Y have been modified with QAC (HDTMA (H-A, H-X and H-Y) and BKC (B-A, B-X and B-Y)) by wet technique (solution). Since the modification was through ion exchange, it is expected that it did not involve any changes in the structure of zeolites. Therefore, one of the techniques that can be used to prove this is by observation in the IR spectra at the region below  $1600\text{ cm}^{-1}$  for modified zeolites and compared it with that of parent zeolites.

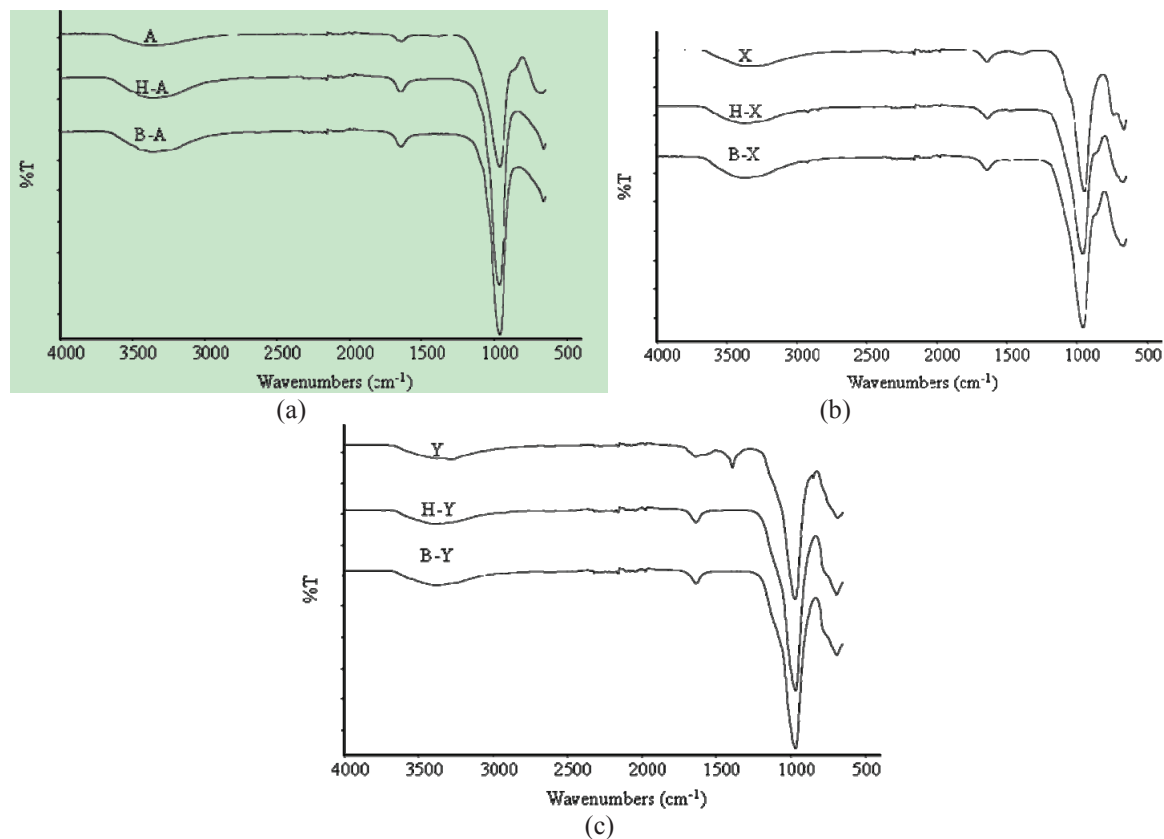


Fig. 1. FTIR spectra of zeolite A (a), X(b) and Y(c) and its modified forms

In the typical IR spectra of zeolites, bands of backbones in zeolites (Si-O-Si and Si-O-Al) can be seen in the range of  $470\text{--}1120\text{ cm}^{-1}$  associated with bending and stretching of Si-O-Si and Si-O and Al. IR spectra in Fig. 1 revealed that there was no changes of the peaks in IR spectra of modified and unmodified zeolites. This proved that the adsorbed HDTMA and BKC did not alter the structure of zeolites.

The IR spectra at a region of  $3400\text{--}2600\text{ cm}^{-1}$  for QAC-zeolites that have been compared with those respective parent zeolites can be seen in Figure 4. The two significance peaks appeared as indicator that the QAC molecules have been successfully attached on parent zeolite [6].

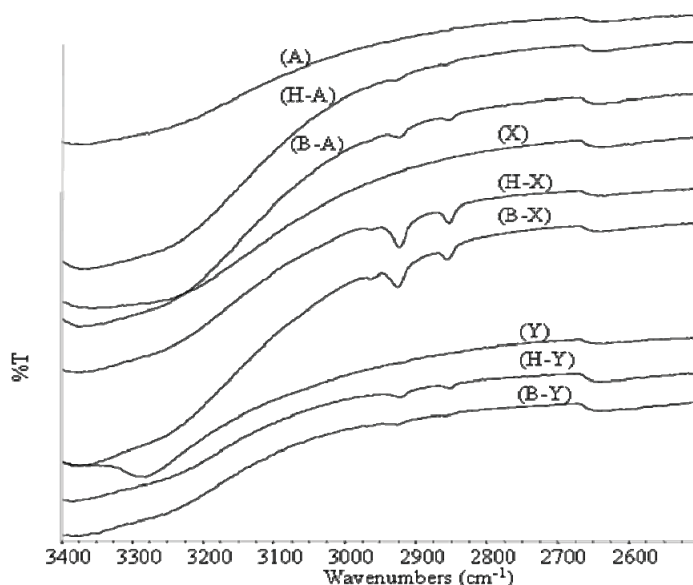


Fig. 2. Comparison of IR spectra of QAC-zeolites and parent zeolites

IR spectra of QAC-zeolites showed the emergence of 2 additional peaks between 2800 and 2900  $\text{cm}^{-1}$  but absence in IR spectra of parent zeolites. This indicates the presence of QAC molecules on the zeolites surfaces. QAC molecules (HDTMA and BKC) comprise of two parts; positive charge of ammonium hydrophilic head and another one is long chain of hydrocarbon tail. The positive charge head can be attached on the negative sites of zeolites framework leaving the outer layer with long chain hydrocarbon. Hence, two additional peaks that were observed in IR spectra are assigned for the C-H bonding of long chain hydrocarbon tail. This result is in agreement with other papers where peaks around 2849 and 2916  $\text{cm}^{-1}$  was assigned to the symmetric and asymmetric stretching mode of C-H in each QAC [6]. Therefore, IR spectra in Figure 4 revealed the successful attachment of QAC molecules on each zeolites. In addition, it was found that the IR for QAC-zeolite X (H-X and B-X) at the studied region is more prevalent than QAC-zeolites A and Y. This result indicates that the amount of HDTMA and BKC adsorb on zeolites X was much higher than that in zeolites A and Y since the absorbance intensity is in accordance with the amount of surfactant molecules [7].

The antibacterial activity was determined by minimum inhibitory concentration (MIC) values that represents the minimum concentration of antimicrobial at which there is complete inhibition of the growth of *B.subtilis* [8]. Table 1 gives the result of MIC of parent zeolites and their modified form

Table 1. MIC values (g/L) of studied samples against *B. subtilis*.

Solution	Samples								
	A	H-A	B-A	X	H-X	B-X	Y	H-Y	B-Y
Distilled Water	>10	>10	>10	>10	4	4	>10	>10	>10
Saline Solution	>10	8	6	>10	6	4	>10	>10	>10

Table 1 revealed that the antibacterial activity of QAC modified zeolite X has the highest antibacterial activity compared to that of zeolites Y and A. QAC modified zeolite Y do not show any antibacterial activity

even at high dosage of the studied sample and the MIC value was similar to that unmodified zeolites. QAC modified zeolite X has higher antibacterial activity because there was high amount of QAC adsorbed on zeolite X as shown in IR spectra at the region between 2800 and 2900  $\text{cm}^{-1}$  (Fig. 2). The antibacterial activity of these zeolite is contributed from the presence of QAC since QAC is known as disinfectant.

Zeolite having lower Si/Al ratio tends to adsorb high amount of cationic compound (QAC) since one Al will give one exchange sites. The higher amount of QAC on zeolite, the better the antibacterial activity since the antibacterial activity depends on the QAC molecules. However, we found that zeolite X which has higher Si/Al ratio resulted in higher adsorption capacity for QAC and has shown to have higher antibacterial activity compared to that of zeolite A. This is certainly due to the porosity characteristics between zeolites A and X. Zeolite X which in Faujasite family is known to have large pore among other zeolites while zeolite A (LTA) composed of the sodalite cage that form a rigid framework structure [3]. The larger pore volume of zeolite allows QAC molecules that having high molecular mass to exchange with cations located inside the framework of zeolite. There are more exchange sites for QAC in zeolite X compared to that of zeolite A because of this phenomenon. As compared to zeolite Y, zeolite X showed higher antibacterial agent because it can adsorb high amount of QAC than zeolite Y due to the lower Si/Al ratio for zeolite X than zeolite Y. This result is in accordance from the previous works [5].

#### 4. Conclusion

From this study, it is shown that the best antibacterial agents was found for zeolite X modified with QAC molecules compared to that zeolite A and Y. Here, we have prove that the antibacterial activity of zeolite comes from the presence of QAC and the attachment of QAC depended on the characteristics of zeolites such as lower Si/Al ratio and larger pore volume of zeolites. It is utmost important to know the characteristic of zeolite used to become support system for antibacterial agents since it can affect the ability of zeolite to attach antibacterial agents and finally, to give higher antibacterial activity.

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