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Modification of Clinoptilolite with Benzalkonium Chloride as a Carrier of Metformin Hydrochloride

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Clinoptilolites have a limitation in the adsorption of drug molecules due to their fixed pore structure. In this study, we modified the clinoptilolite with benzalkonium chloride. The modification was carried out by stirring 1 g of NaCl activated clinoptilolite with 100 mL of benzalkonium chloride solution with a concentration of 0.5, 1, 5, 10 and 15 % at room temperature for 24 h. After filtration and drying process, benzalkonium chloride modified clinoptilolite was stirred with 100 mL of 300 mg/L metformin hydrochloride solution at room temperature for 24 h. The result of sample characterization using FTIR spectrometer, X-ray diffractometer and N_2 Sorption analyzer showed that clinoptilolite modification with benzalkonium chloride and the impregnation of metformin hydrochloride proceeded well. The optimal amount of metformin hydrochloride occurred in benzalkonium chloride 1 % modified clinoptilolite.

Keywords: Clinoptilolite, Benzalkonium chloride, Metformin hydrochloride, Surface modification.

INTRODUCTION

A rapid change in concentrations, notably of drugs with narrow therapeutic concentrations, usually produces adverse effects when the drug concentrations continuously increase until an effective range is reached. This has encouraged the use of inorganic materials as drug carriers. Controlled-release drug delivery allows for constant drug concentrations within a therapeutic range for an extended period of time [1]. One of inorganic materials zeolite, has demonstrated favourable performance in biomedical applications for its absorption properties, ion-exchange capacity and good catalytic properties. Clinoptilolite, a natural zeolite, is highly suitable to use as a drug carrier for its high specific surface area and adsorption capacity [2] as well as physico-chemical stability in biological environments [3]. Research results proved that natural zeolites, especially clinoptilolites are safe for the body [4-7] and inflict no biological damage to humans [3,8].

Although the use of clinoptilolites as drug carriers is highly promising, the number of drugs that can be absorbed is still very limited. The sizes of drug molecules that are greater (~ 2 nm or larger) than the sizes of clinoptilolite pores (0.3 to 1 nm) are a typical causative factor [9]. Increasing pore sizes is extremely difficult in clinoptilolites due to the fixed structure of pores (fixed pores). Additionally, modifications of clinoptilolite pore normally take place under high temperature conditions and in the presence of strong acids or bases. This leads to damages in the clinoptilolite structure and encapsulated drugs. Therefore, it is necessary to make modifications in the clinoptilolite surface without causing any damage to the structure.

Modifications on the clinoptilolite surface have been reported by a number of researchers and have opened up new opportunities in the development of drug delivery systems with slow-release properties. The use of surfactants has been proven to increase the adsorption capacity of clinoptilolites against drug molecules. Out of several surfactants, benzalkonium chloride (cationic surfactant) becomes a primary preference to researchers as it is easily adsorbed on a negatively charged clinoptilolite surface and is safe for humans. This surfactant is also used as a fast-action biocidal agent [1]. Sulfamethoxazole drug adsorption in clinoptilolite is reported to increase in the presence of benzalkonium chloride [10]. Krajisnik *et al*. [11] reported adsorption of diclofenac diethylamine, diclofenac sodium, and ibuprofen in clinoptilolites modified by benzalkonium chloride and hexadecyltrimethylammonium bromide.

The results showed that the adsorption of three drug models is highly dependent on the amount of surfactant present on the clinoptilolite surface. Jevtic *et al.* [1] studied the adsorption of salicylate anion (aspirin hydrolysis product) in clinoptilolites modified by benzalkonium chloride. As a result, the adsorption of salicylate anion takes place very well in aspirin solution. Benzalkonium chloride modified clinoptilolites have been proven to be suitable to use as carriers of some drugs, especially anionic ones. However, reports on the use of benzalkonium chloride-modified clinoptilolites as cationic drug carriers in physiological environments are still rare.

Metformin hydrochloride is a biguanide antidiabetic medication proven effective in reducing the glucose levels in patients with obesity and patients with cardiovascular diseases [12]. Being highly hydrophilic and existing as a cationic species at physiological pH, metformin hydrochloride is very difficult to diffuse across cell membranes. As a result, the bioavailability of metformin hydrochloride only reaches 50-60 % [13,14]. Besides, a high dosage of this drug (500 mg, 2 to 3 times a day) produces serious side effects such as increasing the risk of vitamin B12 and folate deficiencies [15], causing lactate acidosis [14] and increasing the risk of kidney diseases [12]. In this research, clinoptilolites were modified by benzalkonium chloride at various concentrations, followed by metformin hydrochloride loading by a wet impregnation method.

EXPERIMENTAL

The materials used in this study were clinoptilolite zeolites purchased from Padalarang, Bandung, Indonesia. The chemicals benzalkonium chloride, metformin hydrochloride, sodium chloride and silver nitrate were procured from Merck. All of the reagents used in this study were analytical grade quality. X-ray diffractometer (Philips X'Pert Graphics & Identify), FTIR spectrophotometer (Shimadzu 8201) and N_2 sorption analyzer (Quantacrome Nova) were used to characterize the drug delivery systems. To obtain the amount of drug encapsulated, the UV spectrophotometer (Thermo Fisher Scientific, Genesys 10S) was used.

Activation of clinoptilolites: In initial stage, clinoptilolite powder was sieved using a 200 mesh sieve. The sieved clinoptilolite was mixed with distilled water and stirred for 24 h at ambient temperature. The mixture was then filtered and dried at 105 ºC in an oven for 3 h. 10 g of dried clinoptilolite was added to 1000 mL of 1 mol/L NaCl solution. The suspension formed was stirred for 24 h at ambient temperature. The sample obtained was washed with distilled water until it became completely Cl^- ion-free (test with $AgNO_3$) and dried at 105 ºC in an oven for 3 h. Afterwards, the activated clinoptilolite was calcinated at 500 ºC for 3 h. The activated clinoptilolite (Na-CLI) was then analyzed using FTIR, XRD and N_2 sorption analyzer.

Modification of clinoptilolite with benzalkonium chloride: 1 g of clinoptilolite was mixed with 100 mL of 0.5, 1, 5, 10 and 15 % benzalkonium chloride solutions. The mixtures were stirred for 24 h at ambient temperature. After filtering, the residues were dried at 60 ºC for 6 h. The final products were labelled CLI-$BKC_{0.5}$, CLI-BKC_1, CLI-BKC_5, CLI-BKC_{10} and CLI-BKC_{15}, with the numbers showing the initial concentrations of benzalkonium chloride solutions. The clinoptilolite modified by benzalkonium chloride was then analyzed using FTIR, XRD and N_2 Sorption Analyzer.

Metformin hydrochloride loading on BKC-modified clinoptilolite: 1 g of CLI-$BKC_{0.5}$ was mixed with 100 mL of 300 mg/L metformin hydrochloride solution. The mixture was stirred for 24 h at ambient temperature and pH of 7. The mixture was then filtered and dried at 105 ºC in an oven for 6 h. The amount of metformin hydrochloride loaded on clinoptilolite was determined by UV-visible spectrophotometer. In the same manner, metformin hydrochloride was subjected to loading processes on CLI-BKC_1; CLI-BKC_5, CLI-BKC_{10} and CLI-BKC_{15}.

Characterization of drug delivery systems: The sample functional group of drug delivery systems was determined by Shimadzu FTIR-8201 infrared spectrophotometer by mixing 2 mg of sample with 200 mg of KBr, which later was formed into pellets. Measurement was conducted within the wavenumber range 4000-400 cm^{-1}. An analysis of mineral type using X-ray diffraction was conducted at 2θ = 4-70º using Phillips X'Pert Graphics & Identity X-ray diffractometer with a CuK_α = 1.54 Å light operated at 40 kV with electric current of 35 mA. The surface area of the drug delivery systems was determined using N_2 sorption analyzer.

RESULTS AND DISCUSSION

FTIR analysis: The analysis using an FTIR spectrometer aimed to figure out the chemical interactions occurring between clinoptilolites, benzalkonium chloride and metformin hydrochloride. Fig. 1 shows the FTIR spectra of activated clinoptilolite (Na-CLI), clinoptilolite-BKC (CLI-BKC) and clinoptilolite-BKC-metformin hydrochloride (CLI-BKC-MH).

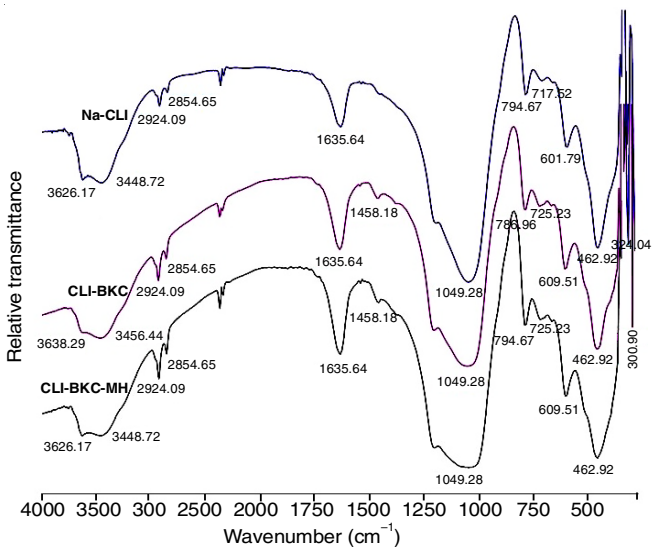

Fig. 1. FTIR spectra of Na-clinoptilolite (Na-CLI), clinoptilolite-benzalkonium chloride (CLI-BKC) and clinoptilolite-benzalkonium chloride-metformin hydrochloride (CLI-BKC-MH)

In the activated clinoptilolite FTIR spectra (Na-CLI), absorption peaks were observed to occur at some wavenumbers. The peak at 462.92 cm^{-1} showed bending vibrations of bonds within tetrahedral TO_4 (T = Si and Al) [16,17]. The peak at 601.79 cm^{-1} showed asymmetric stretching vibrations of TO_4

(external tetrahedral double ring). The peaks at wavenumbers 794.67 and 1049.28 cm^{-1} showed symmetric and asymmetric stretches of tetrahedral external linkages, respectively [18,19]. The -OH stretching vibrations of silanol or aluminol groups [bridging hydroxyls Si-O(H)-Al] occurred at wavenumber 3626.17 cm^{-1}. These vibrations are associated with Brønsted acidity. Bending and stretching vibrations of -OH groups of water molecules absorbed by natural clinoptilolites are shown at wavenumbers 1635.64 and 3448.72 cm^{-1}, respectively [20,21].

The FTIR spectra of BKC-modified clinoptilolite (CLI-BKC) showed broadening of absorption peaks at wavenumber 1049.28 cm^{-1}. This indicates the existence of a bond between clinoptilolite and benzalkonium chloride. This result is consistent with the research by Hassan *et al*. [22], which showed that caffeine encapsulation in chitosan nanoparticles broadened -OH absorption peaks. CLI-BKC FTIR spectra shows that BKC absorption in Na-CLI results in a shift in absorption peak from 3448.72 cm^{-1} to 3456.44 cm^{-1}, showing an interaction between -OH group and benzalkonium chloride molecules. In the FTIR spectra of drug delivery system (CLI-BKC-MH), absorption peak broadening was observed at wavenumber 1049.28 cm^{-1} to be ongoing and increase. This shows that the bond was formed not only between clinoptilolite and benzalkonium chloride, but also between clinoptilolite and metformin hydrochloride. Furthermore, the absorption peaks of metformin hydrochloride molecules were not observed within the spectra showing that the number of drug molecules adsorbed was very small, causing their occurrence to be disturbed by clinoptilolite absorptions [9].

X-ray diffraction: The crystallinity of Na-CLI, CLI-BKC and CLI-BKC-MH was measured by X-ray diffrac-tometer. The activated clinoptilolite diffraction pattern (Na-CLI) (Fig. 2) shows strong peaks at 2θ = 11.151º (d = 7.28), 17.29º (d = 5.124), 22.385º (d = 3.9683), 28.058º (d =3.1777), and 29.900º (2.9859). Based on the matching with the standard diffraction peaks in Mineral Powder Diffraction File, JCPDS (25-1349), these peaks are characteristic of clinoptilolite mineral peaks and indicate that clinoptilolites are the primary component of natural zeolites [9,18,23]. In clinoptilolite modification with BKC (CLI-BKC), there are no changes observed in the clinoptilolite diffraction peaks. Similarly, in clinoptilolite loaded with metformin hydrochloride (CLI-BKC-MH), clinoptilolite diffraction peaks are maintained. This indicates that the crystallinity of clinoptilolites is neither disturbed by modifications with benzalkonium chloride nor metformin hydrochloride molecule loading [24].

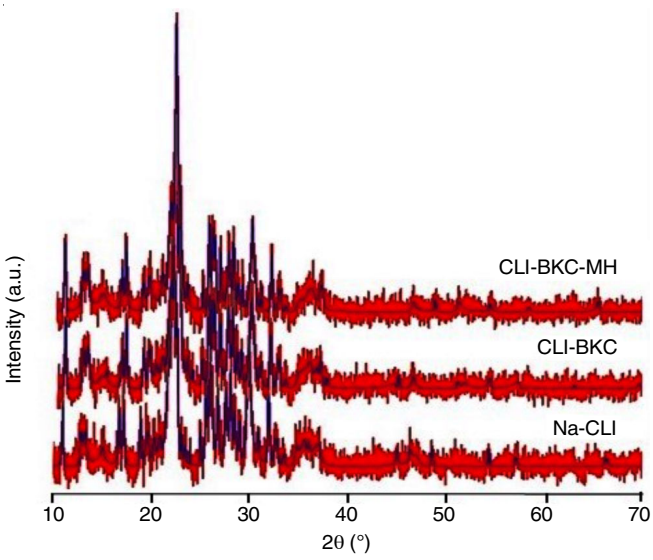

Fig. 2. XRD pattern of Na-clinoptilolite (Na-CLI), clinoptilolite-benzalkonium chloride (CLI-BKC) and clinoptilolite-benzalkonium chloride-metformin hydrochloride (CLI-BKC-MH)

BET Surface area: Sample measurements using N_2 sorption analyzer aimed to figure out the effect of clinoptilolite modifications with benzalkonium chloride and drug loading process on the clinoptilolite suface area. The principle of calculation of clinoptilolite surface area was based on the Brunauer-Emmett-Teller (BET) method.

According to the surface area data as presented in Table-1, natural clinoptilolite (CLI) had a surface area of 48.0731 ± 0.6032 m^2/g. After activation with NaCl and calcination (Na-CLI), clinoptilolite surface area increased dramatically to 107.2572 ± 2.4540 m^2/g. This is because the calcination process undertaken after a cation exchange process causes an increase in the surface area, pore total volume and micropore volume of clinoptilolites [25,26]. Farías *et al*. [27] stated that at 500 ºC, dehydroxylation takes place in clinoptilolites, causing the formation of one Lewis site from two Brøensted acid sites. This process results in a structural gap in clinoptilolites.

TABLE-1
BET SURFACE AREA OF THE SAMPLES (n = 3)

Samples	BET surface area (m^2/g) ± SD
CLI	48.0731 ± 0.6032
Na-CLI	107.2572 ± 2.4540
CLI-BKC	43.0521 ± 0.8613
CLI-BKC-MH	36.7729 ± 0.5271

BET = Brunauer-Emmett-Teller, SD = standard deviation, Na = natrium, CLI = clinoptilolite, BKC = benzalkonium chloride, MH = metformin hydrochloride

Loading of benzalkonium chloride in Na-CLI produces a decrease in the surface area to 43.0521 ± 0.8613 m^2/g (CLI-BKC) (Table-1). Furthermore, metformin hydrochloride loading causes the surface area of CLI-BKC decrease to 36.7729 ± 0.5271 m^2/g. This decrease in the surface area indicates that the loading process of benzalkonium chloride and metformin hydrochloride took place well. Some research studies have confirmed this result, one of which is the study by Lukarska *et al*. [28] stated that a dramatic decrease in the surface area was reached when fluorescein is encapsulated in zeolite Y. Putra and Mustika [29], similarly reported that loading of the anticancer active compound solasodin decreased the surface area of clinoptilolite sharply.

Loading of metformin hydrochloride on BKC-modified clinoptilolites: This process was undertaken at pH 7 and ambient temperature for 24 h. The initial concentration of metformin hydrochloride solution used was 300 mg/L. According to Fig. 3, metformin hydrochloride was loaded optimally in clinoptilolite modified by 1 % benzalkonium chloride. Metformin hydrochloride is hydrophilic and highly soluble in water [30]. This drug readily forms hydrogen bonds with molecules such as acids and phenol [31]. The same applies to benzalkonium

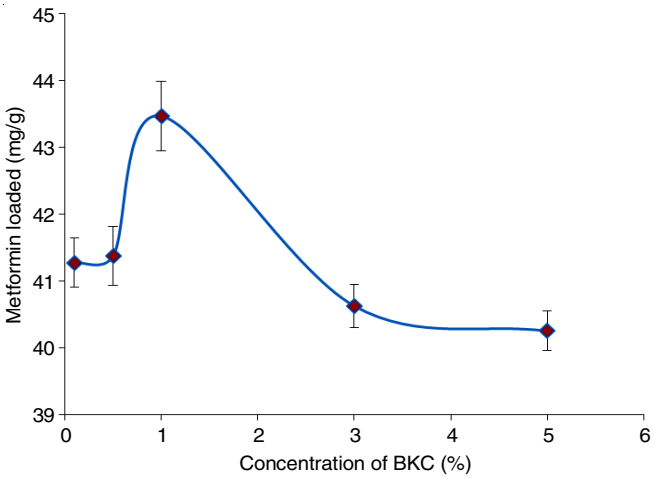

Fig. 3. Amount of metformin hydrochloride loaded on BKC modified clinoptilolite. Error bars show the standard deviation (n = 4)

chloride, which is a polar compound (soluble in water). Hence, the interactions possibly formed between drug molecules and benzalkonium chloride molecules attached to clinoptilolites are hydrophilic interactions (hydrogen bonding, dipole-dipole or ion-dipole).

Conclusion

The loading of benzalkonium chloride and metformin hydrochloride in clinoptilolite was successfully performed. This was proven by the broadening of clinoptilolite absorption peaks in FTIR spectra and the decrease in the clinoptilolite surface area. The XRD diffractogram of drug delivery systems showed that the crystallinity of clinoptilolites did not face any alteration, even after drug molecule loading. Metformin hydrochloride was loaded optimally in the clinoptilolites modified by 1 % benzalkonium chloride. The results of this research open up opportunities for benzalkonium chloride-modified clinoptilolite applications for drug delivery systems.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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