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Research Article

THE USE OF CLINOPTILOLITES AS CARRIER OF METFORMIN HYDROCHLORIDE IN DRUG DELIVERY SYSTEM: *IN VITRO* DRUG RELEASE STUDY

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ABSTRACT

Objective: As an antidiabetic drug, metformin hydrochloride (HCl) has been well known to possess low oral bioavailability and short half-life. In this study, we prepared the drug delivery system (DDS) of metformin HCl and clinoptilolite as its carrier. The *in vitro* drug release profile was further investigated.

Methods: DDS was made by encapsulating metformin HCl on clinoptilolite using the wet impregnation method at various pH and initial concentration of metformin HCl. Fourier transform infrared spectrometer (FTIR), X-ray diffractometer (XRD), and N_2 Sorption Analyzer were used to characterize the as-synthesized DDS. Drug release study was conducted by stirring the DDS in simulated gastric fluid and simulated intestinal fluid over 12 h.

Results: The encapsulation process was achieved optimally at pH 7.0 and initial concentration of metformin HCl of 300 mg/l (CLI_2-300 denoted DDS). The results of FTIR and N_2 sorption analyzer confirmed the existence of metformin HCl on clinoptilolites. Meanwhile, the XRD result showed that the crystallinity of clinoptilolites remained unchanged after the encapsulation process. The cumulative drug release in the simulated gastric fluid was found to be higher than that in the simulated intestinal fluid, which indicated the potent influence of pH on the release properties of the drugs. The drug release kinetics of metformin HCl from clinoptilolite was best fitted into the Korsmeyer-Peppas model with non-Fickian transport mechanism.

Conclusion: We found that clinoptilolite was suitable for DDS application, particularly as a carrier of metformin HCl.

Keywords: Clinoptilolites, Metformin hydrochloride, Drug delivery system.

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INTRODUCTION

Type 2 diabetes mellitus is a chronic disease caused by a combination of decreased insulin secretion and decreased insulin sensitivity [1]. The prevalence of this disease, particularly in Indonesia, has increased over the years [2]. Several complications may happen in patients with Type 2 diabetes mellitus, namely autonomic dysfunction, blindness, kidney failure, foot ulcer, amputation, and Charcot joints [3-5]. Metformin hydrochloride (HCl), a biguanide group, has been widely used to treat patients with Type 2 diabetes mellitus as well as a first-line drug for patients with obesity [6,7]. Nevertheless, due to its hydrophilic nature, this drug has low oral bioavailability (50–60%). It is also administered in high dose [8,9]. Thus, improving the drug administration property would lower the dose, as metformin HCl may contribute to Vitamin B12 and folate deficiency [10], kidney disorder [7], and lactic acidosis [11].

The drug delivery system (DDS) has been used to improve the drug properties and minimize the side effects. Moreover, the use of a carrier in the DDS has played an important role to improve the efficacy and safety of drugs by controlling the rate, time, and drug release sites in the body [12]. Various organic compounds have been used as carriers of metformin HCl, such as O-carboxymethyl chitosan nanoparticles [13], bovine serum albumin nanoparticles [14], hydroxylpropyl methylcellulose and ethyl cellulose [15], casein micelles [16], tamarind seed polysaccharide-alginate [17], and unilamellar niosome [18]. Recently, inorganic material has also been used as a drug carrier due to its unique and beneficial attributes, for example, porous material. Its nanosized pores (1–100 nm) allow drug molecules to be trapped within the pores through the interaction with the pore wall. Therefore, prolonged drug release can be achieved [19].

Due to its abundant availability in nature, clinoptilolites are promising materials as drug carrier of metformin HCl. Clinoptilolites are formed by the combination of silicon, aluminum, and oxygen atoms in three-dimensional structures. This material has channels and cages in the nano and subnanometer scales, often called micropores [20]. Moreover, clinoptilolites have been recognized for its safe property [21-23] and antidiabetic activity by forming bound to glucose in the blood [24]. The use of clinoptilolites as drug carrier has been reported by some researchers such as a carrier of ketoprofen [25], salicylate acid in aspirin [26], antibiotics [27], and diclofenac [28]. However, there were no reports on the use of clinoptilolites as a carrier of metformin HCl. Applying the hydrophilic nature and the negative surface charge [29], clinoptilolites are appropriate to be used as a carrier of hydrophilic metformin HCl. In this study, metformin HCl was encapsulated in clinoptilolites at various pH and initial concentration of metformin HCl. The *in vitro* drug release test of the DDS was performed in the simulated gastric fluid (pH 1.2) and in the simulated intestinal fluid (pH 7.4).

MATERIALS AND METHODS

Materials

The reagents used in this study were: Metformin HCl, ammonium chloride (NH_4Cl), HCl, potassium chloride, potassium hydrogen phosphate, and sodium hydroxide. All of these reagents were analytical grade and purchased from Merck. Clinoptilolite-rich zeolites were from Padalarang, West Bandung Regency, West Java, Indonesia.

Activation of clinoptilolites

The clinoptilolite powder (passed 200 mesh sieve) was mixed with deionized water and stirred for 24 h at room temperature. Subsequently, the mixture was filtered and heated at 105°C for 3 h. It was then denoted as CLI_0. In a 500 ml of the three-necked flask, a total

of 5.000 g of dried clinoptilolites and 250 ml of 1 M NH_4Cl solution were mixed together. The formed suspension was refluxed at 90°C for 8 h. After filtered, the obtained solid was then washed with deionized water and dried in an oven at 105°C for 3 h. Furthermore, the sample was calcined at 550°C for 3 h. Activated clinoptilolite (denoted as CLI_1) was then analyzed using Fourier transform infrared spectrometer (FTIR), X-ray diffractometer (XRD), and N_2 sorption analyzer.

Effect of pH

To achieve the optimum pH value for drug encapsulation, 1.000 g of CLI_1 were mixed with 100 ml of 100 mg/l metformin HCl solution. The mixture was stirred for 24 h at room temperature and various pH (3, 5, 7, 9, and 11). The mixture was then filtered and dried in an oven at 105°C for 6 h. The amount of encapsulated metformin HCl on clinoptilolites was determined by gravimetric analysis.

Effect of initial concentration

A total of 1.000 g of CLI_1 was mixed together with 100 ml of 50 mg/l metformin HCl solution. The mixture was stirred for 24 h at room temperature. This process was conducted at optimum pH that was achieved in previous work. The mixture was then filtered and dried in an oven at 105°C for 6 h. The amount of metformin HCl encapsulated on CLI_1 was determined by gravimetric analysis. In the same way, this process was conducted with different concentrations of metformin HCl solution of 75, 100, 150, 300, 450, 750, and 900 mg/l. The final DDS with the highest amount of drugs encapsulated was denoted as CLI_2. Fourier transform infrared spectrometer (FTIR), X-ray diffractometer (XRD), and N_2 sorption analyzer were used to characterize the CLI_2.

The *in vitro* drug release study

The drug release study was performed using the dialysis method [30]. The procedure was described as follows. A total of 0.1 g of CLI_2 sample was inserted into a dialysis bag (cutoff=12 kDa) and immersed in 100 ml of buffer solution at a pH of 1.2 and 7.4. The sample was stirred at 37±0.5°C for 12 h. Every 1 h, as much as 5 ml of buffer solution was collected and replaced with the same volume of fresh buffer. The absorption of the metformin HCl in the aliquot of buffer solution was measured using a UV-Vis spectrometer (Genesys 10S, Thermo Scientific) at 232 nm. The amount of drug released from the DDS was expressed as percentage cumulative drug release.

$$\%\,\text{Cumulative drug release} = \frac{X_t}{X_0} \times 100\%$$

Where X_t is the amount of metformin HCl released over a period of time (mg) and X_0 is the amount of metformin HCl encapsulated on CLI_2 (mg).

RESULTS AND DISCUSSION

Effect of pH

Encapsulation of metformin HCl on clinoptilolites was strongly affected by the pH value (Fig. 1). It appears that the highest amount of metformin HCl encapsulated in clinoptilolites (6.674±1.209 mg/g) occurs at pH 7. In acidic solution, the clinoptilolite active sites and drug molecules are protonated so that drug-clinoptilolite interaction is very weak. The existence of pH sensitive-amine groups in metformin HCl affects its state in the solution [31]. In addition, there is a competition between metformin HCl and the solvent (water) to become bonded to clinoptilolite active sites [32].

At pH 7.0, both the amine groups of metformin HCl and the clinoptilolite active sites become fully deprotonated, causing the drug-clinoptilolite interaction to be strong. Metformin HCl is a hydrophilic and highly soluble drug in water [33]. This drug was found forming hydrogen bonds with hydrophilic molecules such as acids and phenols [34]. Thus, possible interactions between drug molecules and clinoptilolites are hydrophilic interactions (hydrogen bond, dipole-dipole, or dipole ion). Subsequently, the amount of drug encapsulated on clinoptilolites decreases as the solution pH increase. Negatively charged sites of clinoptilolites and metformin HCl in basic solution may be the main cause of this trend.

Effect of initial concentration

The effect of initial concentration of the solution on the amount of the encapsulated drug is shown in Fig. 2. According to the figure, the amount of encapsulated metformin HCl increases as the initial solution concentration. The highest amount of the encapsulated drug (19.332±2.982 mg/g) was obtained at an initial solution concentration of 300 mg/l. However, when the initial concentration of the solution was increased to 450 mg/l, the amount of encapsulated drug was dropped dramatically to 14.304±2.441 mg/g. The decrease of the encapsulated drug amount continued to 4.289±0.723 mg/g at the initial solution concentration of 900 mg/l. This decrease is due to the active sites of clinoptilolite having been saturated with drug molecules in a high drug solution concentration, thus unable to bind the drug molecules [35].

FTIR study

The existence of functional groups of the samples was observed using a FTIR spectrometer (FTIR 8201, Shimadzu). Fig. 3 shows the FTIR spectrum of clinoptilolite (CLI_0), activated clinoptilolite (CLI_1), and DDS of metformin HCl/clinoptilolite (CLI_2). In the FTIR spectra of the natural clinoptilolite (CLI_0), a number of peaks appear on several wavenumbers. The peak at 470.63 cm^{-1} shows the bending vibration of TO_4 (T=Si and Al) tetrahedral [36,37]. The peak at 601.79 cm^{-1} shows the asymmetric stretching vibration of TO_4 (double ring external linkage). Peaks at wavenumbers of 789.96 and 1056.99 cm^{-1} show the symmetric stretching and asymmetric stretching vibrations of the external tetrahedral linkage, respectively [38,39]. The stretching vibration of –OH

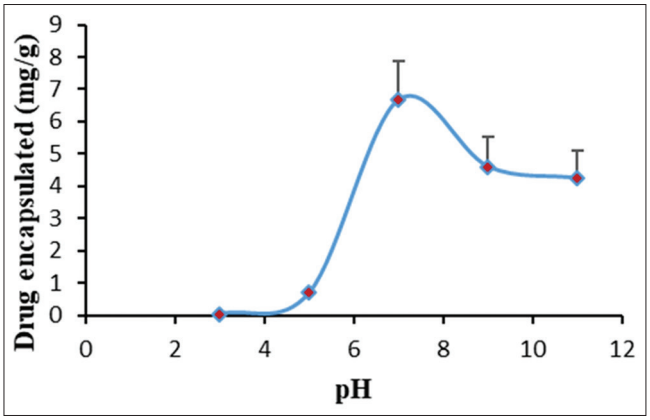

Fig. 1: The amount of drug encapsulated at various pH. Error bars represent the standard deviation (n=4)

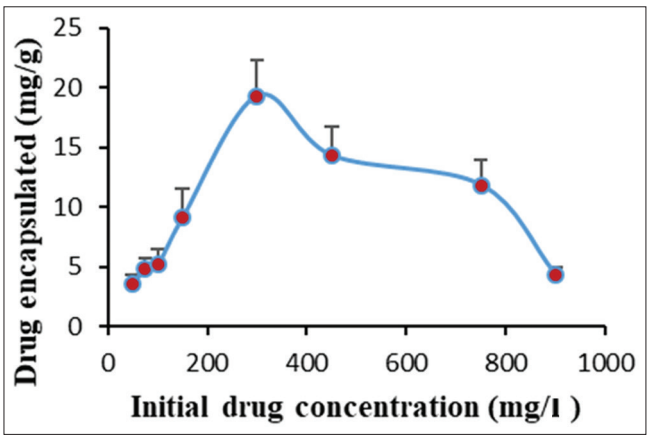

Fig. 2: The amount of drug encapsulated at various initial drug concentration. Error bars represent the standard deviation (n=4)

from silanol or aluminol groups (Si–O(H)–Al bridging hydroxyls) appears at 3649.32 cm^{-1}. This vibration is related to Brønsted's acidity. The bending and stretching vibrations of the -OH group of absorbed water molecules are shown at 1635.64 and 3448.72 cm^{-1}, respectively [40,41].

The FTIR spectra of activated clinoptilolites (CLI_1) show that the activation of natural clinoptilolite with NH_4Cl has an effect of peak shifting at some wavenumbers. The peak shifts occurred at 3448.72, 1056.99, and 470.63 cm^{-1} to 3433.29, 1064.71, and 462.92 cm^{-1}, respectively. These are related to the calcination process of clinoptilolites at 550°C. According to Farías *et al*. [42], the heat treatment applied on clinoptilolites may affect the state and placement of aluminum in clinoptilolites.

In the FTIR spectra of the DDS (CLI_2), several shifts are observed at 3649.89, 1064.71, 601.76, and 462.92 cm^{-1} to 3633.89, 1072.42, 609.51, and 455.20 cm^{-1}, respectively. These shifts do not seem too wide, indicating the interaction between clinoptilolites and drug molecules is weak. Furthermore, the absorption peaks of metformin HCl are not observed in the spectra, suggesting that the amount of encapsulated drug molecules is too small. Thus, their appearance is disrupted by the absorption peaks of clinoptilolites [43].

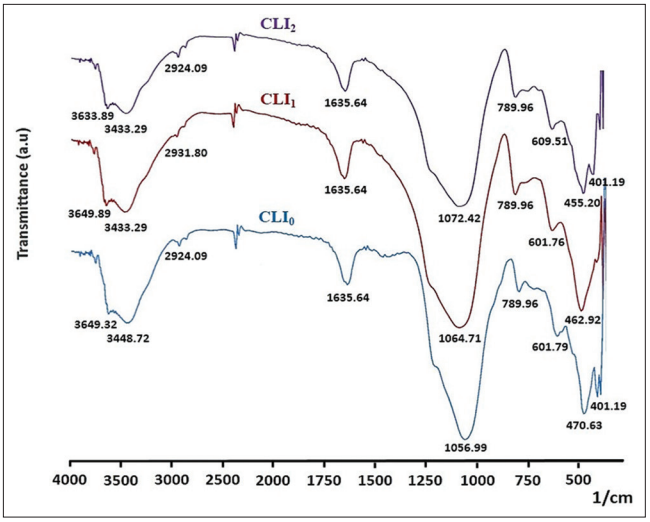

Fig. 3: Fourier transform infrared spectrometer spectra of clinoptilolites (CLI_0), activated clinoptilolites (CLI_1), and metformin HCl encapsulated clinoptilolite (CLI_2)

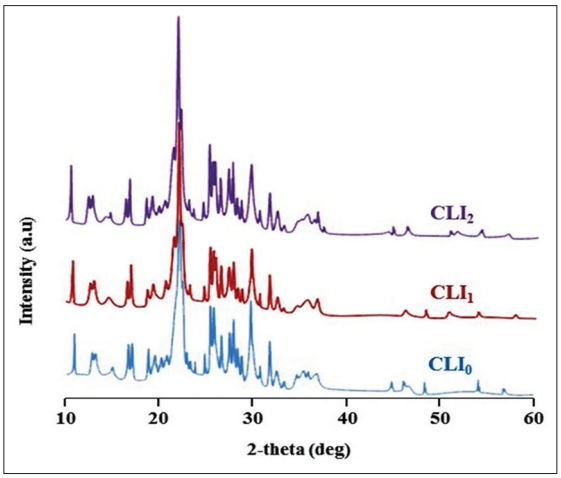

Fig. 4: Diffractogram of clinoptilolites (CLI_0), activated clinoptilolites (CLI_1), and metformin HCl encapsulated clinoptilolite (CLI_2)

XRD study

The crystallinity of clinoptilolites (CLI_0), activated clinoptilolites (CLI_1), and metformin HCl encapsulated clinoptilolite (CLI_2) were determined using XRD (Philips X'Pert Graphics and Identify). The result is shown in Fig. 4. The clinoptilolites diffraction pattern (CLI_0) show strong peaks at 2θ=11.151° (d=7.28), 17.29° (d=5.124), 22.385° (d=3.9683), 28.058° (d=3.1777), and 29.900 (d=2.9859). Based on the Mineral Powder Diffraction File, JCPDS (25–1349), these peaks are characteristic of clinoptilolite mineral and indicate that clinoptilolites are the main mineral of natural zeolites [38,43,44].

In the activated clinoptilolites (CLI_1) pattern, there are no changes observed in the diffraction peaks. Likewise, in the metformin HCl encapsulated clinoptilolite (CLI_2) pattern, the diffraction peaks are maintained. This indicates that the crystallinity of clinoptilolites is not impaired by activation with NH_4Cl nor with the encapsulation of the metformin HCl molecules [45].

Brunauer-Emmett-Teller (BET) surface area measurement

The surface area of clinoptilolites (CLI_0), activated clinoptilolites (CLI_1), and metformin HCl encapsulated clinoptilolite (CLI_2) was measured using N_2 Sorption Analyzer (TriStar II 3020, Micromimetics). The calculation was based on BET method. The results of the BET surface area measurement are shown in Table 1. It can be seen that the surface area of clinoptilolites (CLI_0) is 48.0731±0.6032 m^2/g. This value increases drastically to 110.2712±2.4540 m^2/g after activation with NH_4Cl and calcination. It is due to the calcination process caused the increase of surface area, total pore volume, and micropore volume of clinoptilolites [46]. At high temperature (500°C), dehydroxylation was occurred in clinoptilolites which lead to the formation of one Lewis site from two Bröensted sites [42].

Encapsulation of metformin HCl in CLI_1 results in drastic reduction in the surface area. When loaded with 50 mg/l of metformin HCl, the surface area of activated clinoptilolites (CLI_2-50) decreases to 54.0341±0.9724 m^2/g. Furthermore, the increase of metformin HCl concentration to 300 mg/L makes the surface area of activated clinoptilolites (CLI_2-300), decreases to 34.8993±0.3870 m^2/g. The decrease of the surface area indicates that the encapsulation process has proceeded well. Some researchers have also confirmed this result. Łukarska *et al*. [47] showed a very drastic reduction in surface area when they encapsulated fluorescein in zeolite Y. Putra and Mustika [48] also reported the same result, that the encapsulation of solasodine drastically lowered the surface area of clinoptilolites.

Drug release study

Drug release study was performed *in vitro* in the simulated gastric fluid (pH 1.2) and the simulated intestinal fluid (pH 7.4) for 12 h. Fig. 5 shows the release pattern of metformin HCl from CLI_2. In the lower pH (1.2), the release of metformin HCl was observed reaching 83.36% within 7 h. Furthermore, the release of metformin HCl took place slowly until it reaches 85.32% in 12 h. In the higher pH (7.4), the drug release occurred very fast until the first 2 h. This is due to the weak interaction between drug molecules and the surface of clinoptilolites. In addition, the hydrophilic properties of metformin HCl also have an effect on this stage [49]. Subsequently, drug release occurred quite slowly (71.10% of drug release in 12 h). The drug release from clinoptilolites was begun with the diffusion of the

Table 1: BET surface area of the samples (n=3)

Sample codes	BET surface area (m^2/g)±SD
CLI_0	48.0731±0.6032
CLI_1	110.2712±2.4540
CLI_2-50	54.0341±0.9724
CLI_2-300	34.8993±0.3870

BET: Brunauer-Emmett-teller, SD: Standard deviation, CLI_0: Clinoptilolites, CLI_1: Activated clinoptilolites, CLI_2-50: Activated clinoptilolite-metformin HCl 50 mg/l, CLI_2-300: Activated clinoptilolite-metformin HCl 300 mg/l

Table 2: Release kinetics data of metformin HCl

Release media	Zero-order	First-order	Higuchi	Korsmeyer-Peppas	
	R^2	R^2	R^2	R^2	n
Buffer pH 1.2	0.7723	0.4416	0.9363	0.9776	0.69
Buffer pH 7.4	0.5038	0.3028	0.7677	0.9119	0.64

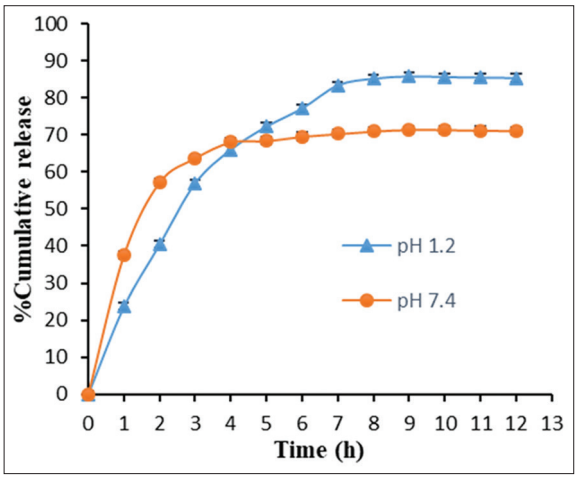

Fig. 5: Release pattern of CLI_2 in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4). Error bars represent the standard deviation (n=4)

simulated fluid into the pores of the clinoptilolites and dissolves the trapped metformin HCl molecules. The dissolved drug molecule then diffuses out of pores [50,51].

The release of metformin HCl from clinoptilolites in the simulated gastric fluid is higher than that in the simulated intestinal fluid. This indicates that the positively charged protons (H^+) in the solution force the drugs to diffuse out through cation exchange [52]. However, this condition seems to be difficult to happen in a simulated intestinal fluid, since the concentration of H^+ is decreased at higher pH. The release kinetics data of metformin HCl from clinoptilolites were fitted into four kinetic models, namely zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. As seen in Table 2, the release kinetics data of metformin HCl from clinoptilolites were best fitted into Korsmeyer-Peppas model with R^2 value of 0.9776 (with n=0.69) in pH 1.2 and of 0.9119 (with n=0.64) in pH 7.4. The n value of 0.69 and 0.64 ($0.45<n<0.89$) suggested the non-Fickian transport mechanism [53].

CONCLUSION

Encapsulation of metformin HCl in clinoptilolite has been successfully conducted using the wet impregnation method. The FTIR spectra confirmed the existence of metformin HCl by absorption peak shifts of clinoptilolite functional groups. The XRD results showed that there were no changes in clinoptilolite crystallinity when metformin HCl was encapsulated on clinoptilolites. BET surface area of clinoptilolites was found to decrease as the metformin HCl encapsulated. The highest amount of drugs encapsulated on clinoptilolites was occurring at pH 7.0 and initial concentration of 300 mg/l. The cumulative release of metformin HCl from clinoptilolites in the simulated gastric fluid was higher than that in a simulated intestinal fluid, which indicated the strong influence of pH on the release properties of the drug. Surface modification is needed to increase the amount of metformin HCl encapsulated on clinoptilolite. The drug release kinetics of metformin HCl from clinoptilolite was best fitted into the Korsmeyer-Peppas model with n value of 0.69 (pH 1.2) and 0.64 (pH 7.4) suggested non-Fickian transport mechanism.

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AUTHORS' CONTRIBUTIONS

I Made Wisnu Adhi Putra, has provided research conception and method, conducted the experiment in the laboratory, and authored the manuscript. I Gusti Ayu Wita Kusumawati has conducted experiment in the laboratory and analyzed the obtained data.

CONFLICTS OF INTEREST

All authors have none to declare.

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