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SPECTROSCOPIC STUDIES OF INCLUSION COMPLEX GLIPIZIDE AND β-CYCLODEXTRIN

(Kajian Spektroskopik Kompleks Kemasukan Glipizida dan β-siklodekstrin)

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Abstract

The complex between hypoglycemic drug, glipizide, and β-cyclodextrin (β-CD) was prepared using the kneading method with aliquot addition of ethanol. The product was characterized using Fourier Transform Infrared (FTIR) spectrometer and Thermogravimetric Analysis (TGA). ^{1}H and NOESY Nuclear Magnetic Resonance (NMR) and UV-Vis spectroscopy were used to determine the interaction involved in the formation of inclusion complex β-CD/glipizide. ^{1}H and NOESY NMR results indicated that the hydrophobic interaction occurred between glipizide and β-CD. The formation constant values of complex β-CD/glipizide at pH 9 and 4 were close to each other. The stoichiometry ratio for the inclusion complex between β-CD with glipizide was 1:1.

Keywords: β-CD, antidiabetic drug, formation constant, cavity, kneading

Abstrak

Kompleks antara ubat hipoglisemia, glipizida, dan β-siklodekstrin (β-CD) telah disediakan dengan cara menguli bersama penambahan etanol alikuot. Produk ini dicirikan menggunakan spektrometer inframerah transformasi Fourier (FTIR) dan analisis termogravimetrik (TGA). Resonan Magnetik Nuklear (NMR) ^{1}H dan NOESY dan spektroskopi UV-Vis telah digunakan untuk menentukan interaksi yang terlibat dalam pembentukan kompleks kemasukan β-CD/glipizida. Hasil NMR ^{1}H dan NOESY menunjukkan bahawa interaksi hidrofobik berlaku antara glipizida dan β-CD. Nilai pemalar pembentukan kompleks β-CD/glipizida pada pH 9 dan 4 adalah lebih kurang sama. Nisbah stoikiometri untuk kompleks kemasukan antara β-CD dan glipizida ialah 1: 1

Kata kunci: β-CD, ubat antidiabetik, pemalar pembentukan, kaviti, menguli

Introduction

β-Cyclodextrins (β-CD) are cyclic oligosaccharides that contain seven units of glucose joined through α-1,4 linkage [1]. β-CD is produced from intramolecular transglycolation reaction from the degradation of starch by CD glucanotransferase(CGTase) enzyme. β-CD has a toroidal shape with a free hydroxyl group at two rims. β-CD is referred to as an all-purpose molecular container for organic, inorganic, organometallic, and metalloorganic compounds that may be neutral, cationic, anionic or even radical. The hydrophobic cavity inside of β-CD allows them to form inclusion complexes with hydrophobic compounds in aqueous environments [2]. The inclusion complex formation affects the physical, chemical, and biological properties of guest molecules. Moreover, it improves the application characteristics of guest molecules [3]. Based on various methods construct, β-CD has

received considerable attention in the pharmaceutical field for the past few years due to its extensive use in drug delivery processes [4-9]. Smart drug carriers can act as a new weapon for treating diseases. It is difficult to build up this kind of drug carrier by general synthetic chemistry due to its complex structure. However, the appearance of β-CD supramolecular self-assembly provides a fast, convenient, and flexible way to construct a biological simulative-responsive drug carrier.

Glipizide is a second-generation sulfonylurea hypoglycemic drug that can acutely lower the blood glucose level in humans by stimulating the release of insulin from the pancreas [10]. It is typically prescribed to treat non-insulin-dependent diabetes mellitus [11]. It is a whitish, odourless powder with pKa of 5.9 and belongs to Class II of the Biopharmaceutical Classification System (BCS). It is insoluble in water, and its dissolution is a rate-determining step in its absorption from the gastrointestinal fluids [12]. This is considered to be a main factor contributing to its very limited oral bioavailability, thus resulting in delayed absorption [11]. Several studies have been developed for the enhancement of the dissolution rate, solubility, and bioavailability of glipizide through inclusion complex formation [13-17].

Inclusion complexes are a type of complex in which one compound (the host) contains a cavity or spaces where another molecular entity (the guest) is located. This is with the consideration that no covalent bonding occurs between the host and the guest. The inclusion complexation of these host-guest systems occurs through various interactions such as hydrogen bonding, Van der Waals, and electrostatic or hydrophobic interactions. Previously, the improved bioavailability of glipizide complexed with β-CD and β-CD derivatives has been documented in several articles [13, 17, 18]. However, there is no information available on the study of interactions involved in the formation of complex between β-CD and glipizide. In this study, the inclusion complex of glipizide and β-CD was evaluated using spectroscopy techniques. The result provides information on the intermolecular interaction between glipizide and β-CD.

Materials and Methods

Materials

The present study utilized β-cyclodextrin ($C_{42}H_{70}O_{35}$), glipizide ($C_{21}H_{27}N_5O_4S$), ethanol (C_2H_6O), and methanol (CH_3OH). Ultra-pure water was used to dilute the solutions.

Characterization of samples

The formation of inclusion complexes was studied *via* an analysis of the peaks. Infrared (IR) absorption spectra were determined by using Perkin-Elmer 2000 system spectrometer in the range of 4000 cm^{-1} to 600 cm^{-1}. The thermal analysis was conducted through the Thermogravimetric Analysis (TGA) using Mettler Toledo TGA/SDTA 851E Thermogravimetric Analyzer. The analysis was done over the ambient temperature range of 30 °C to 920 °C in N_2 atmosphere to understand the weight loss profile for β-CD, glipizide, and β-CD/glipizide complex. The Bruker Avance spectrometer 500 MHz was used to determine ^1H NMR spectra of the β-CD, glipizide, and β-CD/glipizide complex by employing dimethyl sulfoxide-d_6 (DMSO-d_6) as a solvent. Absorption spectra measurements were carried out with Shimadzu UV2600 spectrometer in the range of 200 nm – 800 nm.

Preparations of β-CD/glipizide complex

The complex was prepared as previously reported using the kneading method. β-CD and glipizide were put in a ceramic mortar with a molar ratio of 1:1 and grounded with additional few drops of ethanol to form a homogeneous paste [19]. This process was continued for 30 minutes and the product was placed in a desiccator to the final mass.

Determination of the formation constant of β-CD/glipizide complex

The formation constant was determined as mention in the previous procedure [20]. In 10.0 mL of volumetric flask, 2.0 mL of 0.01 mM glipizide and 3.2 mL of 0.003 M of β-CD were added to form β-CD/glipizide solution. The solution was diluted using ultra-pure water to the mark. The absorption spectra of β-CD and glipizide were recorded against a reagent blank, which was prepared with the same reagent concentration but without the addition of glipizide. The absorption spectra of β-CD and glipizide alone were also recorded. The formation constant, K of β-CD and glipizide could be obtained from the slope of Benesi-Hildebrand plot that was generated using Eqs (1)

and (2). For the formation constant curve, the concentration of glipizide was held firmly at 0.01 mM, while the concentration of β-CD was varied (0.001, 0.002, 0.003, and 0.005 M).

$$\frac{1}{A-A_0} = \frac{1}{(A'-A_0)} + \frac{1}{K(A'-A_0)[\beta-CD]} \quad (1)$$

$$K=\frac{1}{Slope(A-A_0)} \quad (2)$$

where A_0 and A are the absorbance of the free guest and β-CD, respectively. $A^{'}$ is the absorbance at the maximum concentration of β-CD.

Results and Discussion

FT-IR

The FT-IR spectra and main frequencies of β-CD, glipizide, and complex of β-CD/glipizide are reported in Figures 1 (a)-(c) and Table 1, respectively. The frequencies for β-CD were observed at 3292.81 cm^{-1}, 2924.94 cm^{-1}, and 1152.37 cm^{-1}, which corresponded to the broad peaks of OH, CH_2, and -C-C, respectively. The spectrum of glipizide (Figure 1 (b)) showed absorption bands at 1687.51 cm^{-1} (for C=O stretching vibration), 1331.94 cm^{-1} (for -C-N stretching vibration), and 1484.95 cm^{-1} (for aromatic C=C groups stretching vibration).

In the spectrum of β-CD/glipizide complex (Figure 1 (c)), the frequencies of S=O (1157.21 cm^{-1}) for glipizide and C-O group (1334.23 cm^{-1}) disappeared, whereas the frequency of C-N, $-CH_3$, and -C=O shifted from 1331.94 cm^{-1} to 1332.52 cm^{-1}, 2944.00 cm^{-1} to 2927.26 cm^{-1} and 1687.51 cm^{-1} to 1689.38 cm^{-1} respectively. Besides, a broad hydroxyl band of pure β-CD at 3292.81 cm^{-1} was found to be narrowed in the spectrum of the β-CD/glipizide complex (Figure 1 (c)), which was a good indication of the formation of the inclusion complex. This is a common phenomenon observed by many researchers in synthesizing the inclusion complex between β-cyclodextrin as host and guest molecules [21-24]. The changes in the FT-IR spectrum of complex shown in Figure 1 (c) were due to the change of microenvironment with the formation of inclusion complex. Thus, the result of FT-IR spectroscopy indicated that the inclusion complex of glipizide with β-CD was formed.

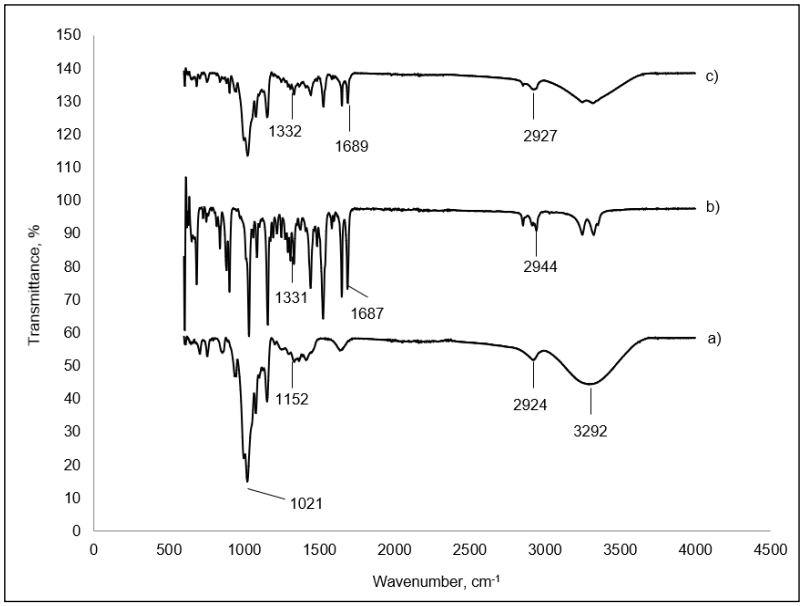


Figure 1. FTIR spectrums of a) β-CD b) glipizide c) β-CD/glipizide.

Table 1. Main FTIR frequencies for β-CD, glipizide and β-CD/glipizide

Wavenumber (cm^{-1})	**Assignments**	**β-CD**	**Glipizide**	**β-CD/glipizide**
3292.81	O-H stretch	√		
2944.00	C-H stretch		√	
2927.26				√
2924.94		√		
1689.38	C=O stretch			√
1687.51			√	
1484.95	C=C stretch		√	
1332.52	C-N stretch			√
1331.94			√	
1157.21	S=O		√	
1152.37	C-C	√		

Thermal analysis

TGA analyses were performed on the β-CD, glipizide, and β-CD/glipizide complex in the temperature range of 30 ^{o}C - 900 ^{o}C. Based on the thermogram shown in Figure 2, there was an initial loss of weight at temperature below 100 ^{o}C for β-CD, glipizide, and β-CD/glipizide complex due to the loss of water molecules. The weight losses of β-CD starting from 328.90 ^{o}C to 800 ^{o}C were attributed to the decomposition of β-CD, while glipizide started to decompose around 220 ^{o}C. The weight loss for β-CD/glipizide complex that occurred at the range of 210 ^{o}C - 400 ^{o}C could be related to the decomposition of organic moieties at the surface. The result showed that the β-CD/glipizide complex had the least pronounced weight loss than β-CD and glipizide. Apart from that, the high stability of β-CD/glipizide could be due to the strong hydrophobic interaction between β-CD and glipizide, which enabled this material to be used in high-temperature applications. The temperature of weight loss with detailed assignment is shown in Table 2.

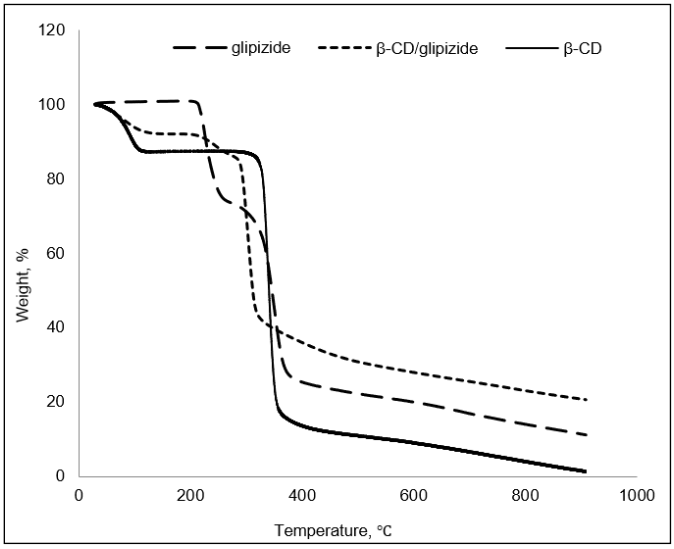


Figure 2. TGA for β-CD, glipizide and β-CD/glipizide

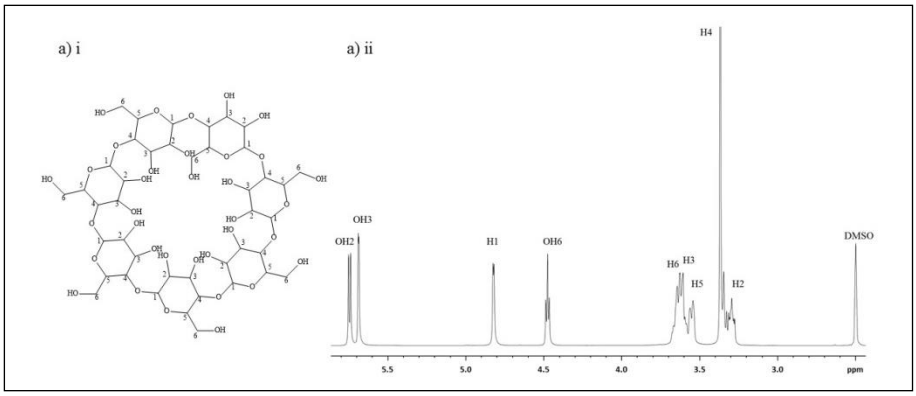
Table 2. The assignment for temperature of weight loss

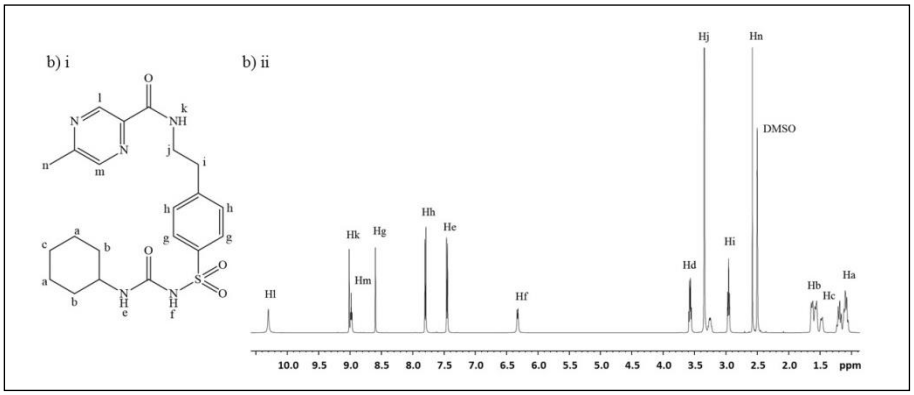
Samples	Region (°C)	Weight Loss (%)	Assignment
β-CD	<100	29.46	Water loss
	328.9-800	81.35	β-CD decomposition
glipizide	<100	29.52	Water loss
	220	96.96	Glipizide decomposition
β-CD/glipizide	<100	29.45	Water loss
	210-400	91.73	Glipizide decomposition

^{1}H NMR analysis

^{1}H NMR spectroscopy was introduced to study the inclusion complex formation. The deduced structures of β-CD and glipizide with the spectra of ^{1}HNMR are shown in Figure 3. Chemical shift (δ) variations provided evidence for the formation of inclusion complex. The values of chemical shifts obtained from ^{1}H NMR for each β-CD, glipizide, and β-CD/glipizide are listed in Table 3. The method relied on changes in chemical shift caused by the glipizide (guest) and β-CD (host) of one another. The difference in chemical shift for the protons of β-CD in the presence or absence of glipizide was defined as induced shift (Δδ). In this case, the induced shift was calculated using Eq 3:

$$\Delta\delta=\delta \text{ (complex)}-\delta \text{ (free)} \quad (3)$$





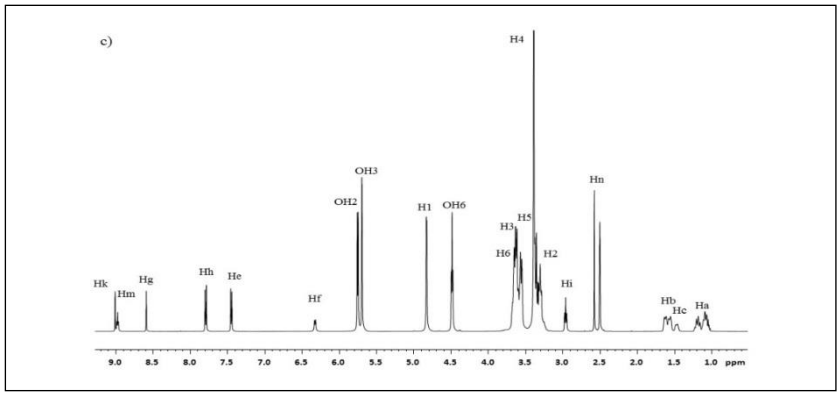


Figure 3. [1] H NMR spectra of a) β-CD, b) glipizide and c) β-CD/glipizide

Table 3. Chemical shift corresponding to β-CD and glipizide

Proton	β-Cyclodextrin δ	Glipizide δ	Inclusion Complex δ	Induced Shift Δδ
H1	4.826		4.829	0.003
H2	3.295		3.299	0.004
H3	3.606		3.610	0.004
H4	3.367		3.384	0.017
H5	3.542		3.563	0.021[a]
H6	3.643		3.629	-0.014
Ha		1.097	1.092	-0.005
Hb		1.554	1.551	-0.003
Hc		1.462	1.459	-0.003
Hd		3.565	-	-
He		7.461	7.458	-0.003
Hf		6.319	6.317	-0.002
Hg		8.597	8.593	-0.004
Hh		7.790	7.785	-0.005
Hi		2.961	2.958	-0.003
Hj		3.346	-	-
Hk		9.017	9.012	-0.005
Hl		10.307	-	-
Hm		8.983	8.978	-0.005
Hn		2.579	2.575	-0.004

[a] Values in bold refer to the highest induced shift of that particular proton

In the structure of β-CD (Figure 4), the protons H3 and H5 were located inside the cavity. Meanwhile, the other protons (H1, H2, and H4) were located at the exterior of the cavity. Normally, the inclusion of the non-polar region of an analyte into hydrophobic cavity would affect the inner protons of the glucose units of β-CD, which were H3 and H5 [19-20, 25]. For β-CD/glipizide complex, there was a significant shift at H5 (Table 3). However, there was

no significant change of chemical shift for H3. This revealed that there was a partial penetration of glipizide into the cavity of β-CD. 2D NMR, which is NOESY, was studied to obtain detailed information of the interaction involved. The cross peak (Figure 5) between Hm (proton of glipizide) and H5 (proton of β-CD) confirmed the aromatic moiety penetration of glipizide into the β-CD cavity.

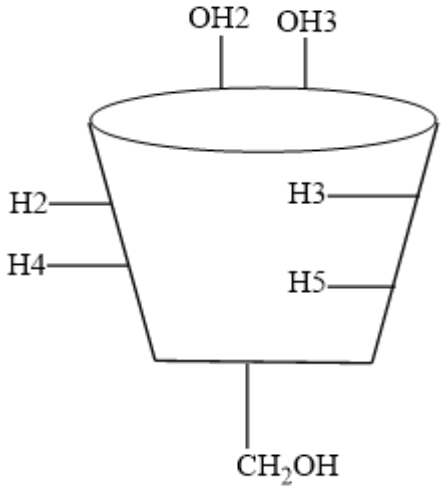


Figure 4. β-CD with proton numbering

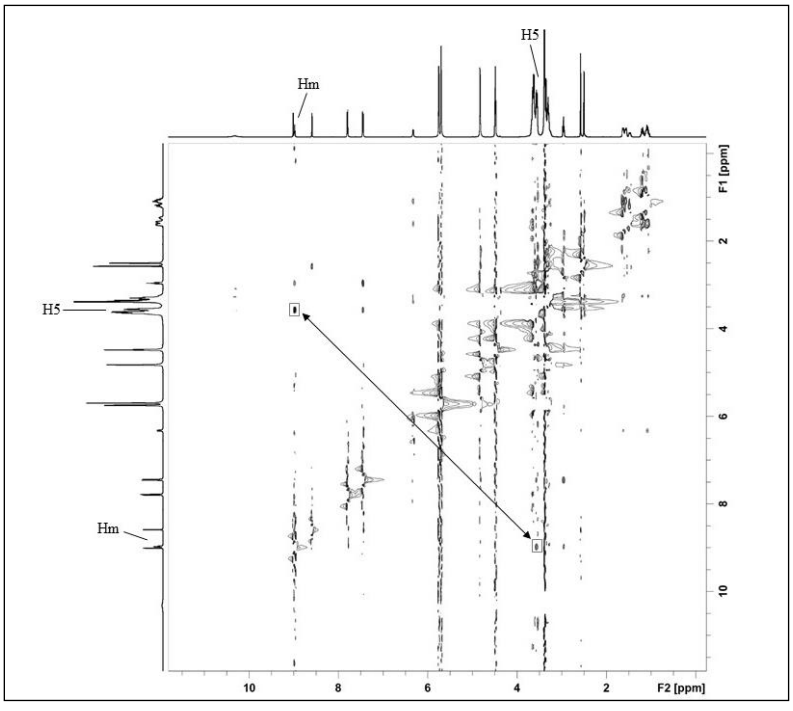


Figure 5. 2D NOESY spectra of complex β-CD/glipizide

UV-Vis

The stoichiometric ratios and formation constants describing the extent of formation of the complexes were obtained by measuring the changes in UV-Vis absorbance of the substrates, in the presence of increasing concentrations of β-CD. Figure 6 showed the absorbance increased by increasing the β-CD concentrations at pH 4 and pH 9. The absorption spectra of β-CD, glipizide, and β-CD/glipizide complexes are shown in Figure 7. The results indicated that β-CD/Glipizide had λ_{max} in the range of 250 nm – 300 nm. The absorbance of β-CD/glipizide complex underwent the hyperchromic effect, which increased the value of absorbance. The effect observed was due to π-π^* transition of dipole-dipole moments of aromatic ring. The formation constant, K values are shown in Table 4. The formation constant was carried out at two different pH values to find out whether the strength of binding between glipizide and β-CD was affected in acidic or basic condition. However, it was found that the binding strength was not affected in both acidic and basic conditions. The double reciprocal plot shown in Figure 8 clearly indicated that the stoichiometry ratio for the inclusion formation between glipizide and β-CD was 1:1.

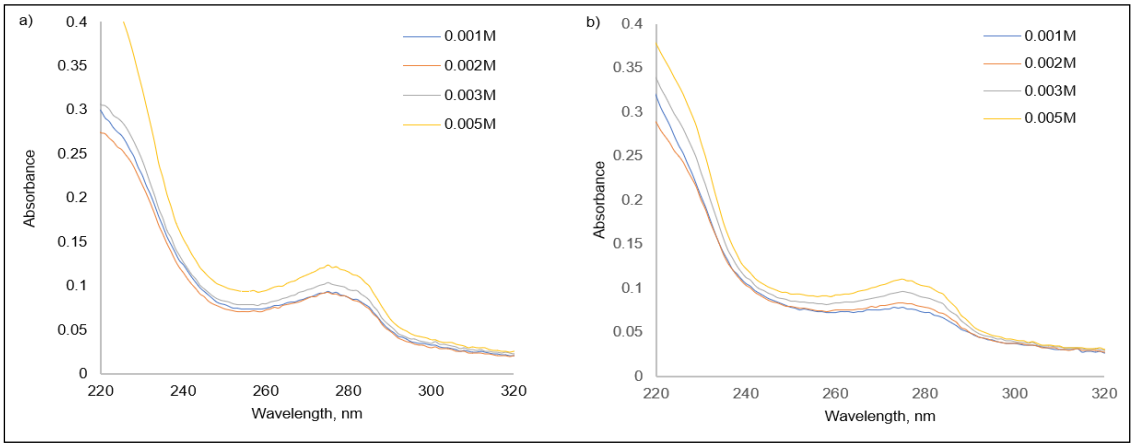


Figure 6. Absorption spectra of glipizide in the presence of increasing concentration of a) β-CD (0.001-0.005M) pH 4 and b) β-CD (0.001-0.005M) pH 9

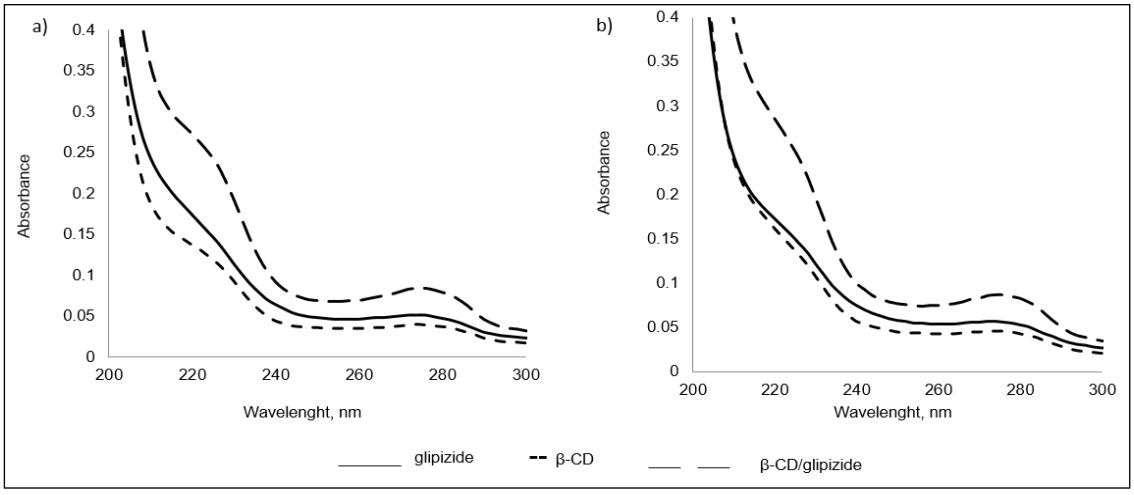


Figure 7. Absorption spectra of β-CD, Glipizide and β-CD/Glipizide at a) pH 4 and b) pH 9

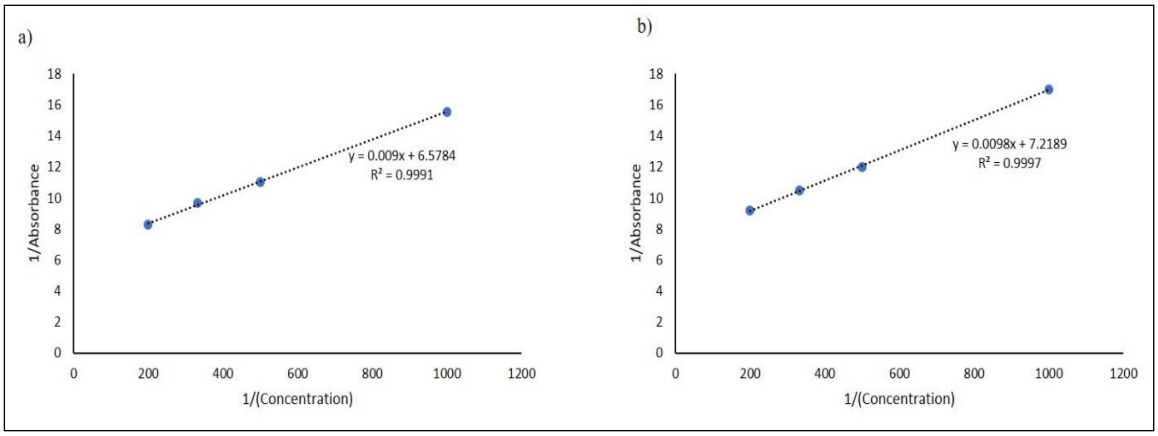


Figure 8. The double reciprocal plot of $\frac{1}{A}$ against $\frac{1}{[\beta-CD]}$ in a) pH 4 and b) pH 9

Table 4. Formation constant (K) values for β-CD/glipizide at differents pH

pH	Formation Constant, K
4	730.93
9	743.47

Conclusion

The inclusion complex between β-CD and glipizide was prepared using the kneading method and the determinations of formation constant were studied at pH 4 and 9. The results obtained from FT-IR and TGA proved that β-CD formed the inclusion complex with glipizide. From ^{1}H NMR determination, a large shift was observed at H5 proton of β-CD, proving the partial penetration of glipizide into the cavity of β-CD. This was strengthened by the cross-peak proton of aromatic glipizide with inner protons of β-CD from the NOESY experiment. β-CD/glipizide complex performed 1:1 host-guest interaction at pH 4 and 9 with apparent formation constants of 730.93 and 743.47, respectively, calculated from the Hildebrand-Benesi equation. This integrated experimental method revealed the molecular inclusion mechanism of β-CD with glipizide that would benefit the future formulation development of the drug carrier system.

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