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ORIGINAL ARTICLE

Voltammetric and spectrophotometric study on the complexation of glibenclamide with β-cyclodextrin

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Abstract The formation of an inclusion complex of glibenclamide (GL) with β-cyclodextrin (β-CD) in an aqueous ethanolic buffer solution of pH 7.0 has been investigated by UV spectrophotometry and differential pulse voltammetry and its stability constant is determined to be 855 and 354.15 M^{-1}, respectively. The phase solubility profile, based on the spectrophotometric absorbance's variations, was classified as A_L-type, indicating the formation of 1:1 stoichiometric inclusion complex of glibenclamide with β-CD with a stability constant value, K_S, of 846 M^{-1}.

Keywords Cyclodextrin · Glibenclamide · Spectrophotometry · Voltammetry

Cyclodextrins are cyclic organic compounds obtained by enzymatic transformation of starch. Among the class of "host" molecules, the β-cyclodextrin (β-CD) is one of the most abundant natural oligomers and corresponds to the association of seven glucose units with cavity which exhibits a hydrophobic character whereas the exterior is strongly hydrophilic. In pharmaceutical industries, the inclusion process of pharmaceutical molecules with β-CD led to important modifications of pharmaceutical properties of guest molecules, to enhance solubility, chemical stability, and bioavailability of the substance [1, 2].

Glibenclamide (GL) (1-[[*p*-[2-(5-chloro-*o*-anisamido)ethyl] phenyl]-sulfonyl]-3-cyclohexylurea; glyburide) is a potent, second generation oral sulfonylurea antidiabetic agent widely-used to lower blood glucose levels in patients with type II non-insulin-dependent diabetes mellitus. It acts mainly by stimulating endogenous insulin release from beta cells of the pancreas [3]. Glibenclamide has extremely poor aqueous solubility and resulting low bioavailability. Consequently, an improved biological performance of this drug through enhancing its solubility and dissolution rate by complexation with β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin has been reported [4–6], and the more recently; a study of the ability of hydroxybutenyl β-cyclodextrin (HBenβCD) to form complexes with GL with enhanced solubility has been reported [7].

In the present paper, the interaction of GL with β-CD has been studied using voltammetric and spectrophotometric methods. Furthermore, the stability constant of the GL-β-CD complex was obtained from the decrease in the peak current, or from the variation in the absorption spectra and from the phase solubility diagram.

Experimental

Materials and reagents

GL powder of pharmaceutical purity grade was a generous gift provided by Sanofi Aventis, Egypt. β-CD was purchased from Sigma Chemical Company (St. Louis, USA). Phosphate buffer (0.2 M, pH 7.0, from potassium dihydrogen phosphate KH_2PO_4 and disodium hydrogen phosphate Na_2HPO_4) was used. For the preparation of standard GL stock solution (1.0×10^{-3} M), 5 mg of GL was accurately weighted, dissolved in ethanol and then adjusted to 10 mL with ethanol. All materials used without any further purification and doubly distilled water were used throughout the study.

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Apparatus

The UV spectra were performed by a Perkin Elmer UV–VIS double beam spectrophotometer equipped with a PC for data processing UV WinLab-ver 2.80.03, Perkin Elmer, USA). The spectra were recorded over the wavelength range from 200 to 350 nm at a scan speed of 240 nm min^{-1}. A quartz cell with a 1.0 cm path length was used. All pH measurements were performed on a CG 808 (Schott Gerate, Germany) digital pH-meter with glass combination electrode.

The voltammetry experiments were performed using CHI610C Electrochemical Analyzer controlled by CHI Version 9.09 (USA). A three-electrode system was composed of a glassy carbon (BAS model MF-2012, $\Phi = 3$ mm) working electrode, an Ag/AgCl/3 M KCl (BAS model MF-2063) reference electrode and a platinum wire (BAS model MW-1032) counter electrode. The working electrode surface was polished with 0.3 and 0.05 μm alumina slurries before each measurement.

Methods

Cyclic voltammetry and differential pulse voltammetry experiments were performed for 7.0×10^{-5} M of GL in phosphate buffer (0.2 M, pH 7.0): ethanol, 90:10 (v/v) containing various concentrations of β-CD ($0.0–5.0 \times 10^{-3}$ M). The current titration-equation was described as follows [8]:

$$1/C_{CD} = K_S \frac{(1-A)}{1 - i/i_o} - K_S \quad (1)$$

where C_{CD} is the concentration of β-CD, K_S is the apparent stability constant, i_o and i are the peak current without and with β-CD. A is the proportional constant. The condition of using this equation is that a 1:1 association complex is formed and C_{CD} is much larger than the total concentration of GL in solution. In other words, if Eq. 1 corresponds well to the experimental data, this may suggest that the complex of GL with β-CD is a 1:1 association complex.

The absorption spectra were recorded in the range of 200–350 nm, and for the calculation of stability constant, the change of absorption of GL was measured at 302 nm as a function of β-CD concentration. The concentration of glibenclamide was fixed at 1.0×10^{-5} M and the β-CD concentration was changed from 1.0×10^{-4} to 5.0×10^{-4} M. The stability constant can be evaluated spectrophotometrically according to the following equation [9, 10]:

$$\frac{A_0}{A - A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} \frac{1}{K_S C_{CD}} \quad (2)$$

where A_0 and A are the absorbances of the free guest and the apparent one, ε_G and ε_{CD-G} are the absorption coefficients of the guest and complex, respectively. Thus, if Eqs. 1 and 2 fit the experimental data, this may suggest that the complex of GL with β-CD is a 1:1 association complex.

The solubility diagram was obtained according to Higuchi and Connors [11]. Briefly, excess amounts of solid GL (40 mg) were added to 10 mL flasks of aqueous solutions (phosphate buffer pH 7.0: ethanol, 90:10 (v/v) containing various concentrations of β-CD, $0.0 - 6.0 \times 10^{-3}$ M). The flasks were sealed and the suspensions were shaken for 24 h at 25 °C. After equilibrium attainment for one weak, the samples were filtered through a 0.45 μm membrane filter and suitably diluted. The GL concentration in the filtrate was spectrophotometrically analyzed at 302 nm. The stability constant, K_S, was calculated from the phase solubility diagrams according to the equation:

$$K_S = \frac{\text{Slope}}{S_o \times (1 - \text{Slope})} \quad (3)$$

where S_o is the solubility of glibenclamide in the absence of β-CD and the slope means the corresponding slope of the phase solubility diagrams, i.e., the slope of the GL concentration versus β-CD concentration graph. The glibenclamide concentration was obtained using calibration curve constructed in the same experimental conditions.

The calibration curve of GL was constructed using a series of standard solutions in the range of ($1.0 \times 10^{-5} - 5.0 \times 10^{-5}$ M) prepared by appropriate dilutions of the stock solution of GL with phosphate buffer pH 7.0 in away that the final solutions were composed of phosphate buffer: ethanol, 90:10 (v/v).

Results and discussion

Electrochemical results

The electrochemical oxidation behaviour of glibenclamide on a bare carbon paste electrode (CPE) and a Sephadex-modified carbon paste electrode (SMCPE) has been previously reported [12], the cyclic voltammetric behavior of the compound yielded one oxidation process with one electron-one proton transfer, probably by the formation of a cation radical at the nitrogen of the amide group. However, no previous electrochemical data were available concerning the electrochemical oxidation behaviour of glibenclamide on a glassy carbon electrode.

As can be seen in Fig. 1, the cyclic voltammetric behaviour of 7.0×10^{-5} M glibenclamide in the absence and presence of β-CD yielded one oxidation process in phosphate buffer pH 7.0. On the reverse sweep, no distinct reduction wave was observed, indicating that the drug is irreversibly oxidized at the glassy carbon electrode. The free glibenclamide gave anodic peak potential at 1.26 V, which reflects the oxidation of the amide group, the most probable

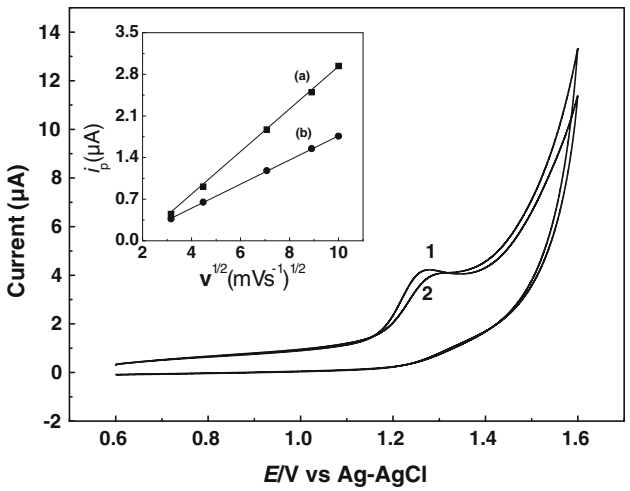


Fig. 1 Cyclic voltammograms for 7.0×10^{-5} M glibenclamide solution obtained in phosphate buffer (pH 7.0) using a scan rate of 50 mVs^{-1}. (1) Without β-CD (2) with 5.0×10^{-3} M β-CD. Inset is the plot of i_p versus $v^{1/2}$ (*a*) without β-CD, (*b*) with 5.0×10^{-3} M β-CD

oxidation centre in the glibenclamide molecule. This oxidation potential is in agreement with the reported oxidation potentials of propanil and related *N*-substituted amides [13], which confirms that oxidation occurs at the nitrogen of the amide group as previously reported at the carbon paste electrode [12]. The addition of β-CD to the solution of glibenclamide causes two main changes in the voltammograms. Firstly, the anodic peak potential (E_p) shifted to a more positive direction and secondly, the peak currents (i_p) decreased. These results can be ascribed to the formation of the inclusion complexes according to the equilibrium:

$$\mathrm{GL} + \beta\text{-CD} \rightleftharpoons \mathrm{GL}/\beta\text{-CD}$$

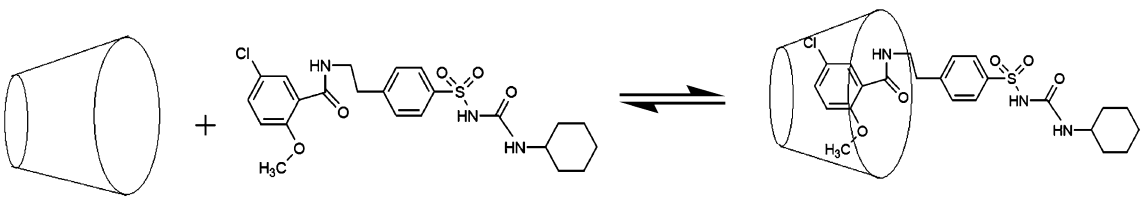
wherein GL/β-CD means the inclusion complex between GL and β-CD. The positive shift in the E_P reveals that the amide groups in the glibenclamide molecules were oxidized with more difficulty, suggesting that they were included in the β-CD cavity, while the sulphonylurea groups remain out side (Scheme 1). Otherwise, if the other side of the glibenclamide was included in the β-CD cavity there would not be any change in the peak potential. However, the proposed inclusion mechanism is in agreement with that reported for the inclusion complex of glibenclamide with 2-hydroxypropyl-β-cyclodextrin, in which the observations suggest that the complex is formed by inclusion of the 3-chloro-6-methoxyphenyl residue of glibenclamide molecule in the 2-hydroxypropyl- β-cyclodextrin cavity, while the sulphonylurea group remains out side [6]. On the other hand, the decrease of the peak current can be ascribed as a diminution of the apparent diffusion coefficient of the glibenclamide included in the complex with β-CD, when compared with the apparent diffusion coefficient of glibenclamide alone.

The CV peak currents were proportional to the square root of scan rates in the range of 10–100 mVs^{-1} both without and with β-CD. The slope of the linear plot of i_p versus $v^{1/2}$ without β-CD (0.3651 μA mV$^{-1/2}$ s$^{1/2}$) was more than that with β-CD (0.20293 μA mV$^{-1/2}$ s$^{1/2}$) as shown in Fig. 1 as inset, suggesting that the diffusion coefficient of the free form of glibenclamide was larger than that of the complexed form of glibenclamide with β-CD.

The inclusion phenomenon of glibenclamide with β-CD was also studied by differential pulse voltammetry, which is a more sensitive electrochemical method (Fig. 2). The differences in the peak currents using DPV are more pronounced, obtaining more accurate results for calculation of the stability constant. According to the decrease of peak currents with increasing concentration of β-CD, the following equation was obtained: $1/C_{CD} = 134.3/(1 - i/i_o) - 354.15$ with a linear correlation coefficient (r) of 0.9996. This revealed that the inclusion complex of glibenclamide with β-CD was a 1:1 association complex and the stability constant (K_S), which is usually used to characterize the inclusion phenomena of cyclodextrin systems [14], was 354.15 M^{-1} as calculated from the y-intercept.

Spectrophotometric studies

The formation of inclusion complex between GL and β-CD could be further confirmed by a spectroscopic experiment. The absorption spectra of GL in the absence, and presence, of β-CD are shown in Fig. 3. It is noticed that upon addition of β-CD, the absorption maximum of glibenclamide at 302 nm has a hypsochromic shift (i.e., shifted towards lower wavelength). However, the UV–vis absorbance decreased with increasing concentration of β-CD. The spectral data further proved the formation of the inclusion complex of GL with β-CD, and the stability constant, K_S, of this complex can be determined according to Benesi–Hildebrand equation [15], under the condition of the 1:1 inclusion complex formation



Scheme 1 The proposed reaction sketch of the inclusion complex of glibenclamide with β-CD

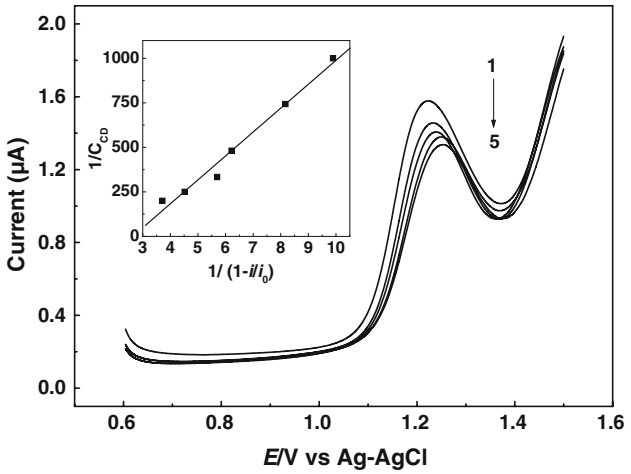


Fig. 2 DPV curves for 7.0×10^{-5} M glibenclamide solution obtained in phosphate buffer (pH 7.0) in absence (1) and presence of (2) 1.0×10^{-3}, (3) 3.0×10^{-3}, (4) 4.0×10^{-3}, (5) 5.0×10^{-3} M β-CD. Inset is the plot of $1/C_{CD}$ versus $1/(1 - i/i_o)$

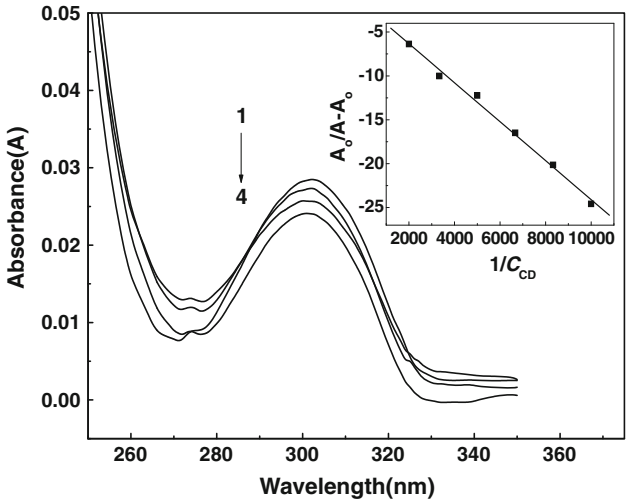


Fig. 3 Absorption spectra of glibenclamide (1.0×10^{-5} M) in the absence and presence of various concentrations of β-CD: (1) 0, (2) 1.0×10^{-4}, (3) 3.0×10^{-4}, (4) 5.0×10^{-4} M. Inset is the plot of $A_o/(A - A_o)$ versus $1/C_{CD}$

equilibrium with an excess concentration of β-CD compared to the total concentration of GL. According to Eq. 2, from an $A_o/(A - A_o)$ versus $1/C_{CD}$ plot (inset of Fig. 3), the following equation was obtained: $A_o/(A - A_o) = -1.89873 - 0.00222/C_{CD}$ with a linear correlation coefficient (r) of 0.996. The ratio of the intercept to the slope gives the value of stability constant of 855 M^{-1}.

Phase solubility studies

Figure 4 shows the absorption spectra of glibenclamide without β-CD and shows as inset the calibration curve with a linear regression equation of $A = -0.00901 + 3553.3C$ (M) and correlation coefficient of 0.998 and Fig. 5 shows the phase solubility diagram of GL with β-CD with a linear regression equation of $C(M) = 4.239 \times 10^{-5} + 0.03478C_{CD}$, and correlation coefficient of 0.991.

The phase solubility diagrams of GL in aqueous solutions of β-CD obtained at 25 °C shows that the solubility of GL increased linearly as a function of β-CD concentration and over the range of concentrations studied showed the features of an A_L-type following Higuchi and Connors' classification [11]. The increase in the solubility can be attributed to the formation of inclusion complexes between GL and the β-CD characterized by greater solubilities than that of GL alone. Fig. 5 shows that in the absence of β-CD, the solubility of GL

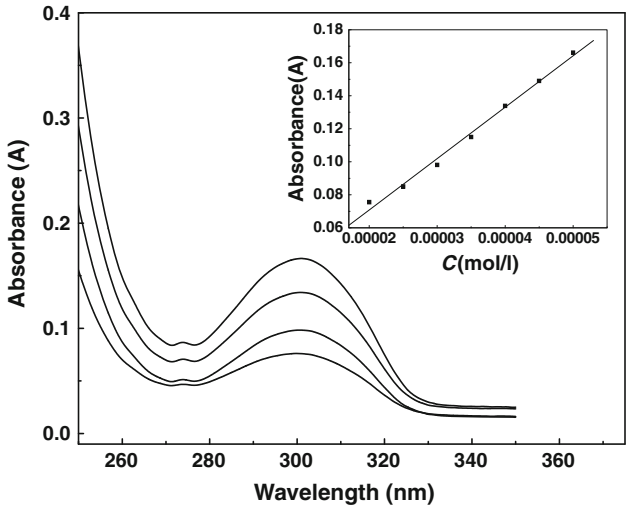


Fig. 4 Absorption spectra of various concentrations of glibenclamide under the conditions of phosphate buffer pH 7.0: ethanol, 90:10 (v/v). Inset is the calibration curve

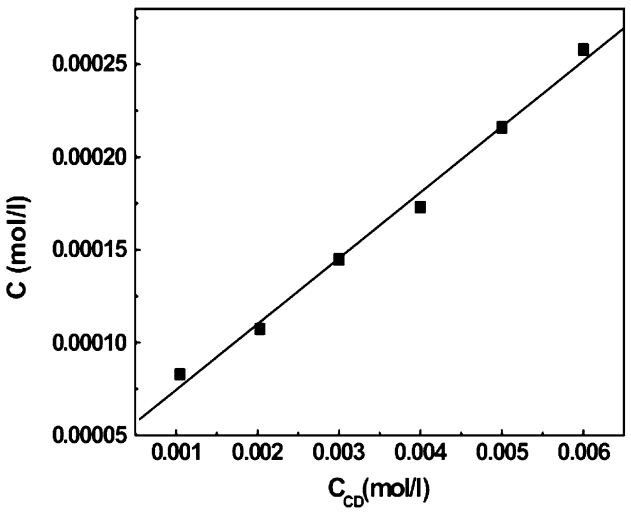


Fig. 5 Phase solubility diagrams for glibenclamide with increasing concentration of β-CD established by UV–Vis method under the condition of phosphate buffer pH 7.0: ethanol, 90:10 (v/v)

is 4.24×10^{-5} M while in presence of β-CD, the solubility increases to 2.6×10^{-4} M at the maximum concentration of the β-CD studied. As the slope of the solubility curves is less than unity, it can be assumed that the stoichiometry of inclusion complexes is 1:1. The stability constants of the inclusion complexes were calculated from the straight-line diagram according to the Eq. 3. We have obtained a K_S value of 846 M^{-1} for the inclusion complex between GL and β-CD, suggesting a relatively stable complex. The obtained K_S value was lower than the published value [16], perhaps due to addition of ethanol, which led to decrease in the polarity of the medium, and this is unfavorable for the hydrophobic interaction between GL and β-CD.

In our experiments, we can notice that the electrochemical and spectrophotometric methods do not give close results for the stability constants. The probable reason could be the use of different techniques for the calculation of the stability constant as it is a fact that the stability constants of the host–guest molecules significantly depends upon the technique used for their evaluation [17–19]. The spectrophotometric and the phase solubility methods depending on the same technique give the close results for the stability constant (855 and 846 M^{-1}), while the voltammetric method depending on measuring another experimental property (changes of the peak current) doesn't give the same result (354 M^{-1}).

Conclusion

The effect of β-CD on the voltammetric behavior of glibenclamide showed a positive shift in the anodic peak potential and a decrease in the peak current. From these changes, we can assume that the amide group on the glibenclamide molecule was located inside the cavity of β-CD and a diminution of the peak current due to a diminution in the diffusion coefficient of glibenclamide as a consequence of the formation of an inclusion complex with β-CD. The phase solubility diagrams of GL in aqueous solutions of β-CD showed that the solubility of GL increased linearly as a function of β-CD concentration. The increase in solubility can be attributed to the formation of inclusion complexes between GL and the β-CD characterized by greater solubilities than that of GL alone. From the voltammetric, spectrophotometric and phase solubility results, it may be concluded that β-CD forms 1:1 type inclusion complexes with glibenclamide and the obtained stability constants were 354, 855 and 846 M^{-1}, respectively.

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