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Voltammetric and spectrophotometric studies on the inclusion complex of glipizide with *β*-cyclodextrin

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Abstract

The formation of an inclusion complex of hypoglycaemic drug, glipizide with *β*-cyclodextrin has been investigated by cyclic, differential pulse voltammetry and UV-vis spectrophotometry. The formation constants of the complex were determined using differential pulse voltammetry and UV-vis spectrophotometry, respectively. For comparative purpose, we have also applied phase solubility study with spectrophotometric detection obtaining formation constant value of 620 M^{-1}. The phase solubility profile was classified as AL–type, indicating the formation of 1:1 stoichiometric inclusion complex of glipizide with *β*-cyclodextrin.

Keywords:

Cyclodextrin; glipizide; spectrophotometry; voltammetry

1. Introduction

Cyclodextrins are oligosacharides obtained from enzymatic hydrolysis of starch. 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. Cyclodextrins are well known in supramolecular chemistry as the most efficient molecular hosts [1-3] capable of encapsulating, with a degree of selectivity, a range of guest molecules via non covalent interactions in hydrophobic cavities. The sequestration of a hydrophobic molecule or some part of it, inside the cavity always alters the physicochemical properties of the encapsulated molecule, which is protected against the aqueous medium from light, oxidants or reactive attacks. Cyclodextrins and their derivatives have received considerable attention in the pharmaceutical field [4-9] for the past few years due to their extensive use in drug delivery processes. In addition, it can be used to enhance solubility, chemical stability, and bioavailability of the drugs.

Glipizide (GP), 1-cyclohexyl-3-[[p-[2-(5 methylpyrazinecarboxamido)ethyl] phenyl]-sulfonyl]urea, is a second generation sulfonylurea hypoglycemic agent, which is widely used in the treatment of non-insulin-dependent diabetes mellitus [10].

Glipizide has very poor aqueous solubility and resulting low bioavailability. Consequently, several studies have been developed for enhancement of the dissolution rate, solubility and bioavailability of glipizide through cyclodextrins inclusion complexes [11-15].

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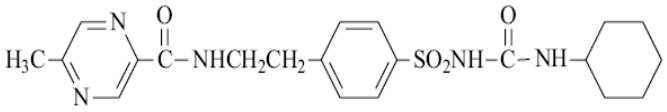

Glipizide

In the previous studies on the inclusion complex of glipizide with cyclodextrins, the general methods used were phase solubility methods, Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and powder X-ray diffraction (XRD).

In the present study, we used spectrophotometric and electrochemical methods to investigate the inclusion complexation of glipizide with β-cyclodextrin. We have taken advantage of the electroactivity of glipizide molecule to design a differential pulse voltammetric procedure in order to obtain the formation constant and the host -to- guest ratio of the inclusion complex. For comparative purpose, we have also performed a phase solubility study with spectrophotometric detection.

2. Experimental

2.1. Reagents and solutions

β-cyclodextrin was purchased from Sigma Chemical Company (St. Louis, USA). glipizide powder of pharmaceutical purity grade was obtained from Chemical Industries Development, Egypt. Phosphate buffer (0.2 mol L^{-1}, pH 7.0, from potassium dihydrogen phosphate KH_2PO_4 and disodium hydrogen phosphate Na_2HPO_4) was used. A 1×10^{-3} mol L^{-1} stock solution of glipizide was prepared in methanol. Working solutions of the drug were obtained by transferring a sample of adequate volume of stock solution into 10 mL volumetric flask containing an appropriate amount of *β*-cyclodextrin dissolved in phosphate buffer, pH 7.0. The mixed solution was diluted with phosphate buffer up to the final volume in away that the final solutions were composed of phosphate buffer: methanol, 90:10 (v/v). Then, the solution was shaken thoroughly for 20 min. and allowed for equilibration at room temperature. All materials used without any further purification and doubly distilled water were used throughout the study.

2.2. Apparatus

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, Φ = 3 mm) working electrode, an Ag/AgCl/3 mol L^{-1} 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 0.05μm alumina slurries before each measurement.

The UV spectra were performed by the Perkin Elmer UV–VIS double beam spectrophotometer equipped with a PC for data processing (UV WinLab-ver 2.80.03, Perkin Elmer, USA). 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.

2.3. Procedures

Current titrations were carried out by keeping constant concentration of glipizide while varying concentration of *β*-cyclodextrin. The current titration equation was described as follows [16,17]:

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

where, CCD is the molar concentration of *β*-CD, Kf is the apparent complex formation 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 CCD is much larger than the total concentration of the drug 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.

Absorption spectra were recorded in the range of 200-350 nm, and for the calculation of stability constant, the change of absorption of glipizide was measured at 275 nm as a function of *β*-CD concentration. The concentration of glipizide was fixed at 1×10^{-5} M and the *β*-CD concentration was changed from 0 to 4×10^{-4} M. The formation constant can be evaluated spectrophotometrically according to the following equation [18,19]:

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

where Ao and A are the absorbances of the free guest and the apparent one, εG and εCD–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 glipizide with *β*-CD is a 1:1 association complex.

Solubility diagrams were obtained according to Higuchi and Connors [20]. Briefly, excess amounts of solid glipizide (10 mg) were added to 10 ml aqueous solutions (phosphate buffer pH 7.0 : methanol, 90:10 (v/v)) containing various concentrations of *β*-CD ($0 - 6\times10^{-3}$ M). The suspensions were shaken in screw-capped vials for three consecutive days at 25 ^{o}C., when the equilibrium had been reached, the contents of each vial were centrifuged, filtered through a 0.45μm membrane filter and suitably diluted and the glipizide concentrations in the filtrates were measured by a UV-Vis spectrophotometric method at 275 nm. The formation constant, Kf, was calculated from the phase solubility diagrams according to the equation:

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

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

Calibration curve of glipizide was constructed using a series of standard solutions in the range of ($1\times10^{-5} - 5\times10^{-5}$ mol L^{-1}) prepared by appropriate dilutions of the stock solution of glipizide with phosphate buffer pH 7.0 in away that the final solutions were composed of phosphate buffer: methanol, 90:10 (v/v).

3. Results and discussion

3.1. Electrochemical results

The electrochemical oxidation behaviour of glipizide on carbon paste electrode has been previously reported [21]. But, no previous electrochemical data were available concerning the electrochemical oxidation behaviour of glipizide on a glassy carbon electrode.

As can be seen in Fig. 1, the cyclic voltammetric behaviour of 4×10^{-5} mol L^{-1} glipizide in the absence *β*-CD yielded one oxidation process in phosphate buffer pH 7.0, probably by the formation of a cation radical at the nitrogen of the amide group. On the reverse sweep, no distinct reduction wave was observed, indicating that the drug is irreversibly oxidized at the glassy carbon electrode. The free glipizide gave anodic peak potential at 1.28 V. The addition of *β*–CD to the solution of glipizide causes two main changes in the voltammogram. Firstly, the anodic peak potential (*E*P) shifted to a more positive direction and secondly, the peak current (*I*P) decreased and the peak became less distinct. These results indicate the formation of inclusion complex with *β*–CD according to the equilibrium:

$$GP + \beta\text{-CD} \rightleftharpoons GP/\beta\text{-CD}$$

Wherein GP/*β*-CD means the inclusion complex between GP and *β*-CD. The change in the *E*P reveals that the glipizide molecules were oxidized with more difficulty, when they were included in the *β*-CD cavity. On the other hand, the decrease of the peak current is due to the decrease in the diffusion coefficient of the glipizide included in the complex with *β*-CD, when compared with the apparent diffusion coefficient of glipizide alone.

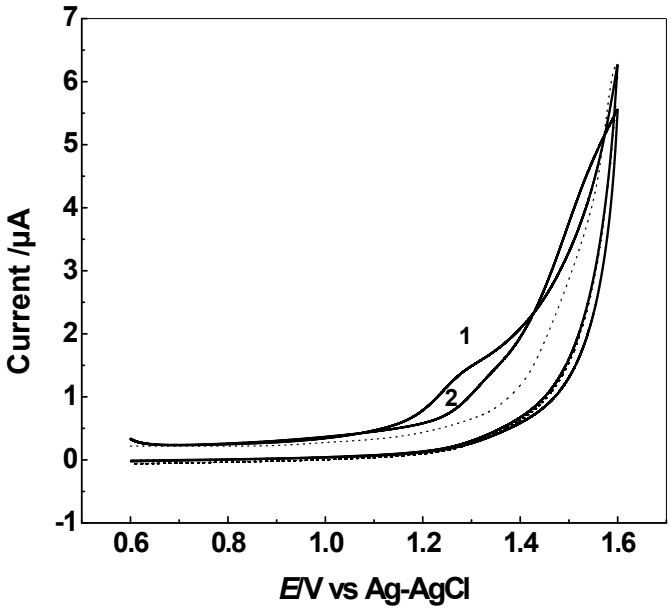

Fig.1 Cyclic voltammograms for 4×10^{-5} mol L^{-1} glipizide solution obtained in 0.2 mol L^{-1} phosphate buffer pH 7.0 : methanol, 90:10 (v/v). Using a scan rate of 10 mVs^{-1}. (1) without β-CD, (2) with 4×10^{-3} mol L^{-1} β-CD. The dotted line represents the background voltammogram

The inclusion phenomenon of glipizide was also studied by differential pulse voltammetry (Fig. 2). According to the decrease of peak currents with increasing concentration of *β*-CD, the following equation was obtained: $1/C\text{CD} = 835.8/(1–i/i\text{o}) – 533.6$ with a linear correlation coefficient (r) of 0.994. This revealed that the inclusion complex of

glipizide with *β*-CD was a 1:1 association complex and the formation constant (Kf) was 533.6 mol^{-1} L^{-1} as calculated from the y-intercept.

3.2. Spectrophotometric studies

The formation of inclusion complex between GP and *β*-CD could be further confirmed by a spectroscopic experiment. The absorption spectra of GP in the absence, and presence, of *β*-CD are shown in Fig. 3. It is noticed that upon addition of *β*-CD, the absorption spectra showed a decrease in the peak intensities and the wavelengths of the absorption bands remain practically unaltered. The formation constant, Kf, of GP/*β*-CD complex can be determined according to Eq. 2, from an Ao/(A−Ao) versus 1/*C*CD plot (inset of Fig. 3), the following equation was obtained: Ao/(A−Ao)= −0.7696−0.00139/*C*CD with a linear correlation coefficient (r) of 0.997. The ratio of the intercept to the slope gives the value of formation constant of 553.6 mol^{-1} L^{-1}.

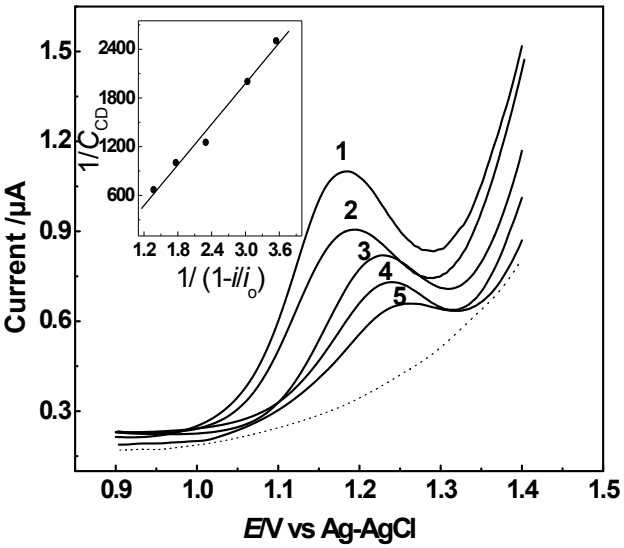

Fig. 2. DPV curves for 4×10^{-5} mol L^{-1} glipizide solution obtained in in 0.2 mol L^{-1} phosphate buffer pH 7.0 : methanol, 90:10 (v/v) in absence (1) and presence of (2) 4×10^{-4}, (3) 8×10^{-4}, (4) 1×10^{-3}, (5) 1.5×10^{-3} mol L^{-1} *β*-CD. Inset is the plot of 1/*C*CD versus 1/(1–*i*/*i*o). The dotted line represents the background voltammogram.

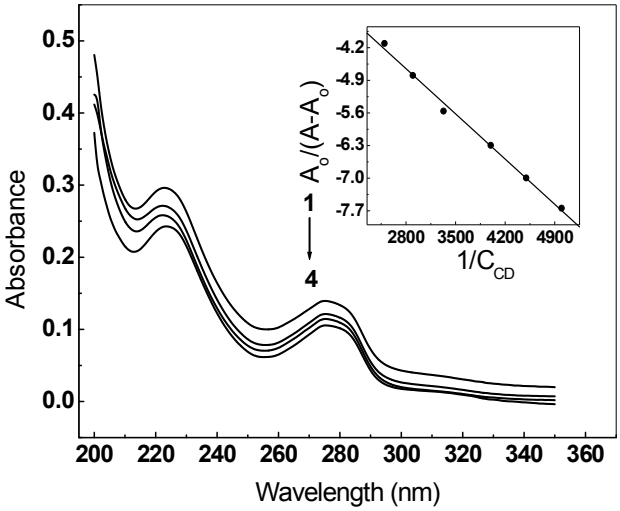

Fig. 3. Absorption spectra of glipizide (1×10^{-5} mol L^{-1}) in 0.2 mol L^{-1} phosphate buffer pH 7.0: methanol, 90:10 (v/v) in the absence and presence of various concentrations of β-CD: (1) 0, (2) 2×10^{-4} mol L^{-1}, (3) 3×10^{-4} mol L^{-1}, (4) 4×10^{-4} mol L^{-1} β-CD. Inset is the plot of Ao/(A−Ao) versus 1/*C*CD.

3.3. Phase solubility studies

Fig 4. shows the absorption spectra of glipizide without *β*-CD and shows as inset the calibration curve with a linear regression equation of A= –0.0206+14980*C* (mol L^{-1}) and correlation coefficient of 0.9991 and Fig. 5. shows the phase solubility diagram of glipizide with *β*-CD with a linear regression equation of C (mol L^{-1})= 6.124×10^{-5}+0.03658 *C*CD, and correlation coefficient of 0.9995.

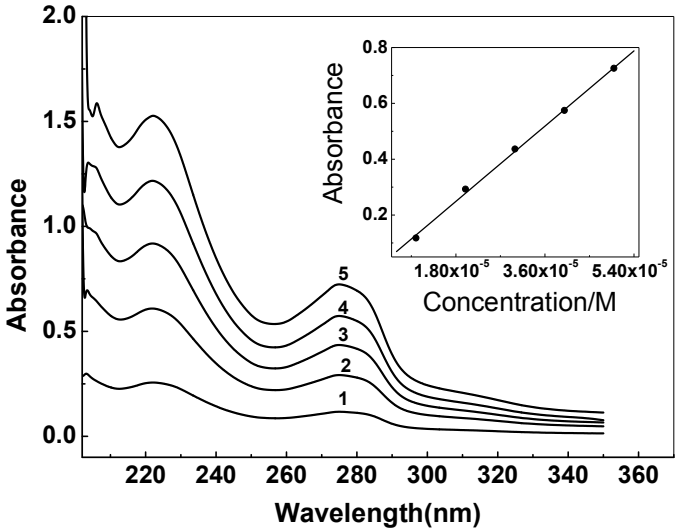

Fig. 4. Absorption spectra of various concentrations of glipizide under the conditions of phosphate buffer pH 7.0 : methanol, 90:10 (v/v). Inset is the calibration curve.

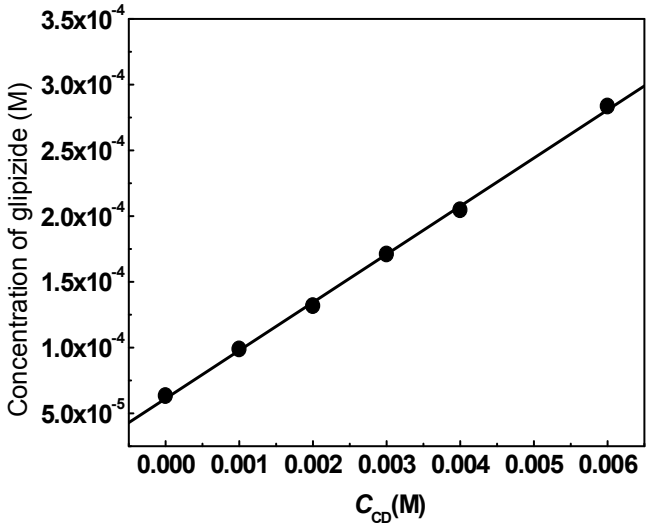

Fig. 5. Phase solubility diagrams for glipizide with increasing concentration of *β*-CD established by UV-Vis method under the condition of phosphate buffer pH 7.0: methanol, 90:10 (v/v)

The phase solubility diagram of glipizide in aqueous solutions of *β*-CD obtained at 25°C shows that the solubility of glipizide increased linearly as a function of *β*-CD concentration and over the range of concentrations studied showed the features of an AL type following Higuchi and Connors' classification [20]. The increase in solubility can be attributed to the formation of inclusion complexes between glipizide and the *β*-CD characterized by greater solubilities than that of glipizide alone. Fig. 5 shows that in absence of *β*-CD, the solubility of glipizide is 6.12×10^{-5} mol L^{-1} while in presence of *β*-CD, the solubility increases to 2.8×10^{-4} mol L^{-1} 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 formation constant of the inclusion complex was calculated from the straight-line diagram according to Eq. 3. We have obtained a Kf value of 620 M^{-1} for the inclusion complex between glipizide and *β*-CD. The stability formation between the range of 100 and 1000 mol^{-1} L^{-1} is considered as an ideal value, smaller values indicate weak interaction between drug and cyclodextrin, while large value indicate incomplete drug release from the inclusion complex [22].

The electrochemically and spectrophotometrically determined formation constants are close to each other, validating our results. Consequently, the voltammetric approach can be recommended as a good alternative for determination of inclusion complexes with *β*-CD.

The kind of electrolyte has a significant influence on the formation constants of inclusion complexes [23]. Complexation of alkali metal cations is not as obvious as that of other inorganic anions [24]. It was found that $[C1O_4]^-$, $[NO_3]^-$, SCN^- and I^- are the most strongly anions complexed by CD while $C1^-$, $[SO_4]^{2-}$, $[PO_4]^{3-}$, $[H_2PO_4]^-$ and $[HPO_4]^{2-}$ did not seem to be complexed by CD. The concentration of electrolyte also affects the formation constants [25]. Experimental results showed that in the phosphate buffer, the values of the formation constants of inclusion complexes in 0.5 M were about 70 % greater than that in 0.2 mol L^{-1} solution. In this report, all of the experiments were performed in 0.2 M (KH_2PO_4-Na_2HPO_4) buffer (pH 7.0).

4. Conclusion

We have demonstrated by three independent methods that glipizide forms a 1:1 inclusion complex with *β*-CD. Furthermore, we have calculated formation constants values of 533.6, 553.6 and 620 M^{-1} from electrochemical, spectrophotometric and phase solubility measurements, respectively. The solubility of glipizide was increased about five times due to the formation of inclusion complexes with *β*-CD.

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