ELECTROCHEMICAL STUDY OF GLIMEPIRIDE AND ITS COMPLEXATION WITH β-CYCLODEXTRIN

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The electrochemical behavior of a hypoglycemic drug, glimepiride (GM), was studied at glassy carbon (GCE) and carbon paste (CPE) electrodes in phosphate buffer over the pH range of 2.7–11.7 using cyclic and differential pulse voltammetry. Oxidation of the drug was shown to be an irreversible and diffusion-controlled process. Using differential pulse voltammetry (DPV), the drug yielded a well-defined voltammetric peak in phosphate buffer pH 6.4 at +1.16 V and pH 7.0 at +1.07 V (vs Ag|AgCl) on glassy carbon and carbon paste electrodes, respectively. This process could be used to determine glimepiride concentrations in the range from 1.0×10^{-5} to 3.2×10^{-5} mol l^{-1} with a detection limit of 2.0×10^{-6} mol l^{-1} in case of the glassy carbon electrode and in the range of 2.0×10^{-6} to 1.5×10^{-5} mol l^{-1} with a detection limit of 7.5×10^{-7} mol l^{-1} in case of the carbon paste electrode. The method was successfully applied to the determination of the drug in a tablet dosage form. Next, the formation of an inclusion complex of glimepiride with β-cyclodextrin (β-CD) in phosphate buffer (pH 7.0):methanol (90:10 (v/v)) has been investigated by differential pulse voltammetry as well as UV spectrophotometry and its stability constant was determined by both methods to be 202.0 and 197.9 l mol^{-1}, respectively.

Keywords: Voltammetry; Cyclodextrins; Absorption spectroscopy; Supramolecular chemistry.

Glimepiride (GM) is a third generation of sulfonylurea type of oral hypoglycemic agent, which is widely used in treatment of type-2 diabetes[1]. Chemically, it is 1-[[4-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl]phenyl]sulfonyl]-3-*trans*-(4-mehtylcyclohexyl)urea (Chart 1). Preclinical investigations of glimepiride suggested a number of potential benefits over sulfonylureas currently available including lower dosage, rapid onset, longer duration of action and lower insulin C-peptide levels, possibly due to less stimulation of insulin secretion and more pronounced extrapancreatic effects[2,3].

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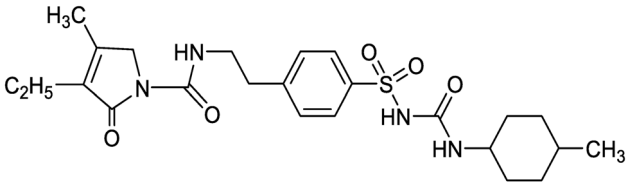

CHART 1
Chemical structure of glimepiride

Many analytical methods have been published for the determination of glimepiride based on high performance liquid chromatographic (HPLC) method with column-switching using UV detection[4], liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI/MS/MS)[5–8] and derivative UV spectrophotometry[9,10].

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[11,12]. Consequently, the interaction of glimepiride with β-CD has been studied by several techniques[13–15].

However, to our knowledge no information about the electrochemical redox properties of glimepiride and its analytical application has been appeared in the literature. The present study deals with the voltammetric oxidation behavior of glimepiride on glassy carbon (GCE) and carbon paste (CPE) electrodes and its determination by differential pulse voltammetry (DPV) in tablet dosage form. Another aim was to study the interaction of GM with β-CD by DPV and UV spectrophotometry. Furthermore, the stability constant of the GM–β-CD complex was obtained from the decrease in the peak current, or from the variation in the absorption spectra.

EXPERIMENTAL

Materials and Reagents

Glimepiride powder of pharmaceutical purity grade and Amaryl® tablets containing 2.0 mg of glimepiride (Batch No. 18E06) were a generous gift provided by Sanofi Aventis, Egypt. β-CD was purchased from Sigma Chemical Company (St. Louis, USA). Phosphate buffer solu-

tions (85% *o*-phosphoric acid, potassium dihydrogen phosphate KH_2PO_4, disodium hydrogen phosphate Na_2HPO_4, and sodium phosphate Na_3PO_4, mixed with different amounts and diluted to 200 ml with doubly distilled water to obtain the required pH) were used. Stock solutions of 1.0×10^{-3} M glimepiride were prepared daily by direct dissolution in methanol. All materials were used without any further purification and doubly distilled was were used throughout the study.

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 electrode (BAS model MF-2012, ϕ = 3 mm) or home-made carbon paste electrode (ϕ = 3 mm) as working electrodes, an Ag|AgCl|3 M KCl (BAS model MF-2063) reference electrode and a platinum wire (BAS model MW-1032) counter electrode. The glassy carbon electrode surface was polished with 0.3 and 0.05 μm alumina slurries before each measurement. The carbon paste was prepared in the usual way by hand mixing of graphite powder (Aldrich, Milwaukee (WI), USA; ϕ = 1–2 μm) and 1.8 ml of Nujol (Sigma; d = 0.84 g ml^{-1}). The ratio of graphite powder to mineral oil was 70:30.

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 a glassy combined electrode.

Voltammetric Procedure

For voltammetric measurements, 10 ml of the electrolyte solution and the appropriate amount of glimepiride solution were added to the cell. The anodic potential sweep was carried under different operational parameters. To provide a reproducible active surface of CPE and to improve the sensitivity and resolution of the voltammetric peaks, the working electrode was transferred to a blank electrolyte solution and three cyclic scans from 0 to 1.0 V at v = 100 mV s^{-1} were sufficient for total leaching of glimepiride into the solution and reaching a voltammogram corresponding to the residual current. All data were obtained at ambient temperature.

Analysis of Tablets

Ten tablets were totally weighed and powdered. An amount of this powder corresponding to 1.0×10^{-3} mol l^{-1} stock solution of glimepiride was accurately weighed and transferred into a 10-ml volumetric flask, 5 ml methanol were added and the flask was sonicated for 15 min and shaked for 20 min, then completed to the mark with methanol. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting them with phosphate buffer solution. Each solution was transferred to a voltammetric cell and the differential pulse voltammogram was subsequently recorded following the optimized conditions. The content of the drug in tablet was determined referring to the calibration graph or regression equation.

Procedures for Calculating Stability Constant (K_S)

Differential pulse voltammetry experiment was performed for 4.0×10^{-5} mol l^{-1} glimepiride in phosphate buffer (pH 7.0):methanol (90:10 (v/v)) containing various concentrations of β-CD ($0.0–1.0 \times 10^{-3}$ mol l^{-1}). The current titration equation was described as follows[16]

$$1 / C_{CD} = K_S \frac{1 - A}{1 - i / i_0} - K_S \qquad (1)$$

where, C_{CD} is the concentration of β-CD, K_S is the apparent stability constant, i_0 and i are the peak currents without and with β-CD, respectively, and 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 GM in solution. In other words, if Eq. (*1*) corresponds well to the experimental data, this may suggest that the complex of GM 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 GM was measured at 227 nm as a function of β-CD concentration. The concentration of glimepiride was fixed at 2.5×10^{-5} mol l^{-1} and the β-CD concentration was changed from 0.0 to 5.5×10^{-4} mol l^{-1}. The stability constant can be evaluated spectrophotometrically according to the following equation[17,18]

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

where A_0 and A are the absorbances of the free guest and the apparent one, respectively, and ε_G and $\varepsilon_{CD\text{-}G}$ 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 GM with β-CD is a 1:1 association complex.

RESULTS AND DISCUSSION

Voltammetric Behavior of Glimepiride

A typical cyclic voltammogram of 4.0×10^{-5} mol l^{-1} glimepiride at GCE at pH 6.4 is shown in Fig. 1. In the forward scan, one well-defined anodic peak is observed. In the reverse sweep, no cathodic peak is observed which indicates that the glimepiride oxidation is irreversible.

The influence of the scan rate on the cyclic voltammogram of glimepiride was then investigated in the range of 10–150 mV s^{-1}. The data showed a positive shift in the peak potential, confirming the irreversible nature of the electrochemical process, with simultaneous increase in peak current (i_p) when the scan rate was increased. The linear relationship existing between log i_p and log v gave a slope of 0.49 with a correlation coefficient of 0.9905 (inset of Fig. 1), which predict a diffusion-controlled regime over the stud-

ied scan rate[19]. Moreover, the current function ($i_p/v^{1/2}$) decreases with scan rate, which is characteristic of a coupled chemical reaction following the electron transfer (EC mechanism)[20]. This type of mechanism occurs quite frequently in organic compounds.

In order to obtain information on the rate determining step, the αn_a value (where α is the charge transfer coefficient and n_a is the number of electrons involved in the rate determining step) determined from Tafel slope expression ($b = 2.303RT/\alpha n_a F$)[21] where Tafel slope (b) can be obtained using the following equation for totally irreversible diffusion controlled process[22].

$$E_p = \frac{b \log v}{2} + \text{Constant}$$

When E_p was plotted versus log v for scan rates in the range of 10–150 mV s^{-1}, a straight line was observed with a slope of 0.06 V, so, Tafel slope (b) was 2 × 0.06 = 0.12 V. The αn_a value was estimated as 0.53.

Effect of pH

The influence of pH on glimepiride at glassy carbon and carbon paste electrodes was studied over the pH range of 2.7–11.7, all the peaks were ob-

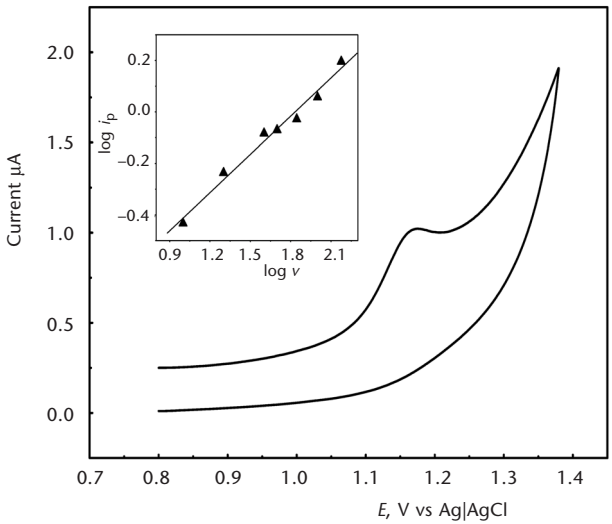

FIG. 1
Cyclic voltammogram of 4.0×10^{-5} mol l^{-1} glimepiride at GCE in phosphate buffer pH 6.4 and at scan rate 10 mV s^{-1}. Inset: the plot of log i_p vs log v

served within the pH range of 4.4–9.1. Figure 2a shows the plot of peak potential versus pH. The E_p varied with pH obtaining two linear ranges, the first between pH 4.4 and 7.0, and the second between pH 5.6 and 7.0 at GCE and CPE, respectively. The equations obtained were the following:

$$E_p \text{ (V)} = 1.63–0.073 \text{ pH}; r = 0.9999 \text{ (GCE)}$$

$$E_p \text{ (V)} = 1.69–0.080 \text{ pH}; r = 0.9996 \text{ (CPE)}$$

In the two pH ranges, the potential shifted to less positive values, and in the second one, the slope was higher than in the first one. The αn_a and the slope of E_p–pH plots most likely correspond to one electron–one proton transfer involved in the rate determining step. The intersection point of the E_p–pH plot at about 7.0 may be correlated to the pK_a value of the amide

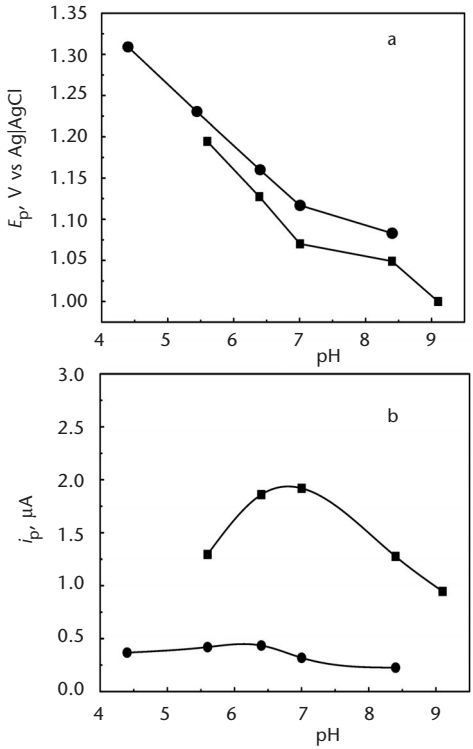

FIG. 2
Effect of pH on peak potential (a) at GCE (●) and CPE (■), effect of pH on peak current (b) at GCE (●) and CPE (■), in phosphate buffer using DPV for 2.0×10^{-5} mol l^{-1} glimepiride, pulse amplitude 50 mV, pulse width 0.2 s, sample width 0.02 s and pulse period 0.5 s

group of glimepiride. Figure 2b shows the plot of peak current versus pH. The peak current reached its maximum value at pH 6.4 in the case of GCE and at pH 7.0 in the case of CPE. Thus, these supporting electrolytes were selected as the optimum values for quantitative analysis.

Analytical Application

Differential pulse mode of glimepiride yielded a voltammogram in which the peak current was found higher than the values obtained by cyclic voltammetry at the experimental conditions of pulse amplitude 50 mV, pulse width 0.2 s, sample width 0.02 s and pulse period 0.5 s. Figure 3 shows the dependence of the peak current on glimepiride concentration at GCE and CPE. Using the conditions described above, the peak currents increased linearly with increasing amounts of glimepiride. The characteristics

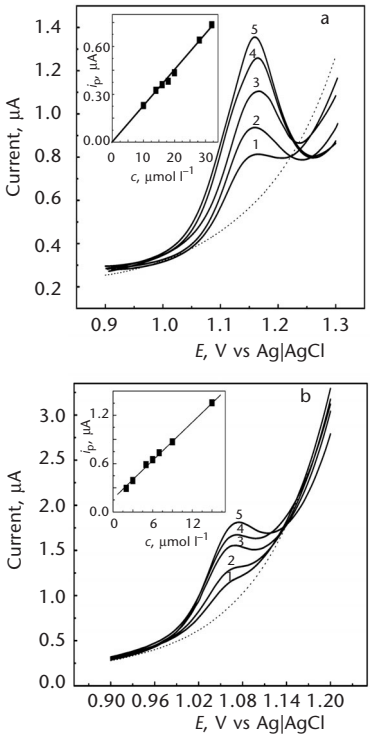

FIG. 3

DPVs at GCE (a) for increasing concentration of glimepiride: 1.0×10^{-5} (1), 1.6×10^{-5} (2), 2.0×10^{-5} (3), 2.8×10^{-5} (4), 3.2×10^{-5} (5) mol l^{-1}. DPVs at CPE (b) for concentration of glimepiride: 2.0×10^{-6} (1), 3.0×10^{-6} (2), 6.0×10^{-6} (3), 7.0×10^{-6} (4), 1.0×10^{-5} (5) mol l^{-1}

of the calibration plots and the detection (LOD) limits of the procedure are listed in Table I. The detection limits estimated as LOD = $3S_{y/x}/b$ (ref.[23]), where $S_{y/x}$ is the standard deviation of *y*-residuals and *b* is the slope of the calibration plot.

Accuracy and Precision

In order to determine the accuracy and precision of the method, five replicate measurements for the concentrations of 2.0×10^{-5} and 9.0×10^{-6} mol l^{-1} glimepiride were analyzed at GCE and CPE. The relative standard deviations (RSD) of 2.58 and 1.30% and mean recovery of 98.6 and 101.2% were achieved, respectively, that indicated good accuracy and precision of the proposed procedure.

Interference Studies

In order to investigate the selectivity of this method, the effect of the excipients present in the dosage form was examined by carrying out the determination of 1.0×10^{-5} mol l^{-1} glimepiride in the presence of each of the different excipients at concentrations that can be found in the tablet dosage form. A deviation of more than 2% from the peak current of the solution containing no interfering additives was taken as a sign of interference. These studies showed that none of the excipients at the concentration level existing in the dosage form caused a positive or a negative error indicating that there were no significant interferences to the method.

TABLE I
Characteristics of glimepiride calibration plot at glassy carbon and carbon paste electrodes

Parameter	CPE	GCE
Linearity range, mol l^{-1}	2.0×10^{-6}–1.5×10^{-5}	1.0×10^{-5}–3.2×10^{-5}
Slope, μA l μmol^{-1}	0.080	0.023
Intercept, μA	0.157	0.004
Correlation coefficient	0.9986	0.9971
SD of slope	2.0×10^{-3}	7.8×10^{-4}
SD of intercept	0.015	0.016
LOD, mol l^{-1}	7.5×10^{-7}	2.0×10^{-6}

Determination of Glimepiride in Tablets

The proposed voltammetric method was applied to the determination of glimepiride in Amaryl® tablets. Each tablet was labelled to contain lactose, sodium starch glycolate, polyvidone 25000, microcrystalline cellulose, magnesium stearate, iron oxide yellow (E172) and indigo carmine aluminium lake (E132). There is no need for any extraction procedure before voltammetric analysis. The mean recoveries (101.3 and 98.5%) and relative standard deviations (1.73 and 3.55%) were obtained for the analysis of glimepiride at CPE and GCE, respectively, indicating adequate precision and accuracy of the proposed method. The results were compared with those obtained by a spectrophotometric method[9]. The results of the student's *t*-test and variance ratio *F*-test show that there are no significant differences between the techniques with regard to accuracy and precision (Table II).

TABLE II
Application of the proposed voltammetric method to the determination of glimepiride in Amaryl® tablets (2.0 mg per tablet)

Parameter	Proposed method		Reference method[9]
	CPE	GCE	
Labeled amount, mg	2.0	2.0	2.0
n	7	7	7
$\overline{X}$	2.026	1.970	2.037
SD	0.035	0.070	0.045
RSD, %	1.73	3.55	2.21
t-Test of significance	0.51	2.13	{*t*(P = 0.05)} = 2.18
F-Test of significance	0.60	2.45	{*F*(P = 0.05)} = 4.28

Complexation of Glimepiride with β*-Cyclodextrin*

Electrochemical Results

The inclusion complex of glimepiride with β-CD was studied by differential pulse voltammetry. As can be seen in Fig. 4, the differential pulse voltammetric behavior of 4.0×10^{-5} mol l^{-1} glimepiride in the absence of β-CD

yielded one oxidation process in 0.2 mol l^{-1} phosphate buffer (pH 7.0):methanol (90:10 (v/v)). The addition of β-CD to the solution of glimepiride led to shift in the anodic peak potential (E_p) to a more positive direction, and as the concentration of the β-CD increased, the peak current (i_p) decreased. These results indicate the formation of inclusion complex with β-CD. The change in the E_p reveals that the glimepiride molecules were oxidized with more difficulty, when they were included in the β-CD cavity. On the other hand, the decrease in the peak current can be due to the decrease in the diffusion coefficient of the glimepiride included in the complex with β-CD as previously reported with different inclusion complexes of drugs with β-CD[16,24–28].

According to the decrease of peak currents with the concentrations of β-CD, the following equation was obtained

$$1/C_{CD} = 169.7/(1 - i/i_0) - 197.9$$

with a linear correlation coefficient (r) of 0.9999. This revealed that the inclusion complex of glimepiride with β-CD was a 1:1 association complex and the stability constant (K_S) was 197.9 l mol^{-1} as calculated from the y-intercept.

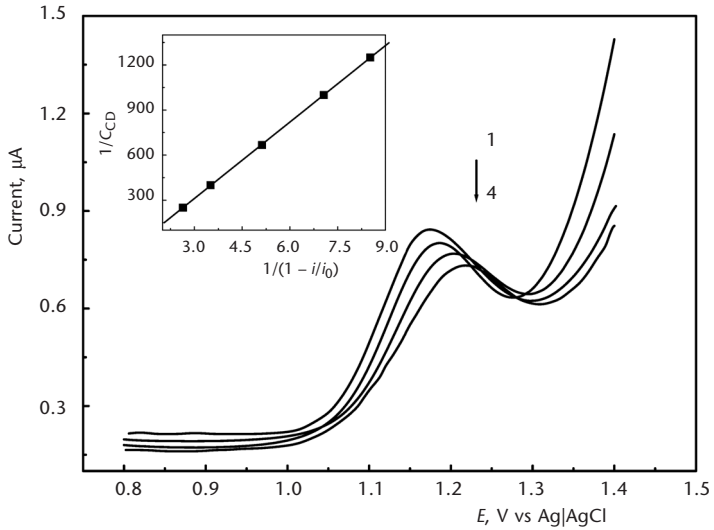

FIG. 4

DPV curves for 4.0×10^{-5} mol l^{-1} glimepiride solution obtained in phosphate buffer pH 7.0 in absence (1) and presence of 8.0×10^{-4} (2), 1.0×10^{-3} (3), 2.5×10^{-3} (4) mol l^{-1} β-CD. Inset: the plot of $1/C_{CD}$ vs $1/(1 - i/i_0)$

Spectrophotometric Results

The formation of inclusion complex between GM and β-CD could be further confirmed by a spectroscopic experiment. The absorption spectra of glimepiride in 0.2 mol l^{-1} phosphate buffer (pH 7.0):methanol (90:10 (v/v)) in the absence and presence of β-CD are shown in Fig. 5. It is noticed that upon addition of β-CD, the wavelength of the absorption bands remain practically unaltered. While, the UV-vis absorbance decreased with increasing concentration of β-CD. The spectral data proved the formation of the inclusion complex of glimepiride with β-CD and the stability constant (K_S) of this complex can be determined according to Eq. (*2*), from an $A_0/(A - A_0)$ versus $1/C_{CD}$ plot. The following equation was obtained

$$A_0/(A - A_0) = -1.414 - 0.007/C_{CD}$$

with a linear correlation coefficient (r) of 0.991. The ratio of the intercept to the slope gives the value of stability constant of 202.0 l mol^{-1}.

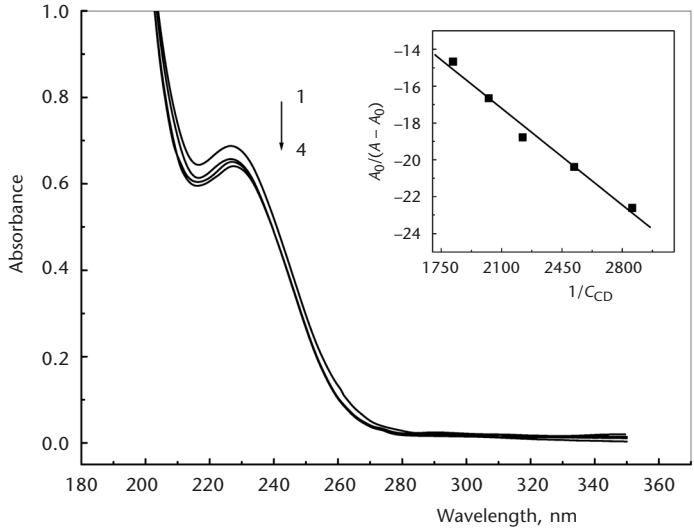

FIG. 5
Absorption spectra of 2.5×10^{-5} mol l^{-1} glimepiride in 0.2 mol l^{-1} phosphate buffer pH 7.0: methanol (90:10 (v/v)) in absence (1) and presence of 3.5×10^{-4} (2), 4.5×10^{-4} (3), 5.5×10^{-4} (4) mol l^{-1} β-CD. Inset: the plot of $A_0/(A - A_0)$ vs $1/C_{CD}$

CONCLUSION

Glimepiride is irreversibly oxidized at glassy carbon and carbon paste electrodes. Application of the DPV method to determination of glimepiride at pharmaceutical preparation is possible after a simple dilution step without interference from the ingredients of tablet matrix. The proposed DPV method is simple, inexpensive, selective and precise, and does not require any complex pretreatment.

From the voltammetric and spectrophotometric results, it may be concluded that β-CD forms 1:1 type inclusion complexes with glimepiride, and the obtained stability constants were 202.0 and 197.9 l mol^{-1}, respectively.

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