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Short communication

Stripping voltammetric quantification of the anti-diabetic drug glipizide in bulk form and pharmaceutical formulation

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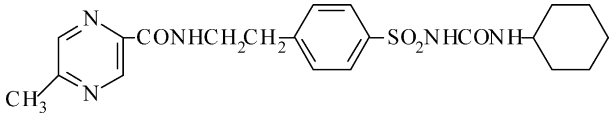
Abstract

The electrochemical behavior of glipizide at the hanging mercury drop electrode (HMDE) was studied in B–R universal buffers of pH 1.7–11. The voltammograms exhibited a well-defined 4-electron irreversible cathodic peak which attributed to reduction of the two C=N double bonds of the pyrazine ring of glipizide molecule. Glipizide was found to has an interfacial adsorptive character onto the mercury electrode surface. A monolayer surface coverage of 1.02×10^{-10} mol cm^{-2} was estimated and hence each adsorbed glipizide molecule occupied an area of 1.63 nm^2 onto the mercury electrode surface. A simple and precise square-wave adsorptive cathodic stripping (SWAdCS) voltammetric procedure was described for quantification of bulk glipizide with a limit of detection of 1.5×10^{-10} M and a limit of quantitation of 5×10^{-10} M. The proposed procedure was successfully applied for quantitation of glipizide in its pharmaceutical formulation (Minidiab tablets) without interference from excipients.

Keywords: Glipizide; Minidiab tablets; Quantification; Cyclic voltammetry; Square-wave adsorptive stripping voltammetry

1. Introduction

Glipizide, 1-cyclohexyl-3-{4-[2-(5-methylpyrazine-2-carboxamido) ethyl]-phenylsulfonyl}urea, is a second generation sulfonylurea hypoglycemic agent, which widely used in the treatment of non-insulin-dependent diabetes mellitus [1].



(Structure of glipizide molecule)

Since glipizide is given in low doses, a sensitive procedure is desired for its determination and testing content uniformity of its dosage form. Glipizide has been determined by several techniques including high-performance liquid chromatography [2–6], thin layer chromatography [7], liquid chromatography [8,9] capillary electrophoresis [10], radioimmunoassay [11] and non-aqueous titrimetry [12]. Glipizide was determined also in formulation based on its electrochemical oxidation at a carbon paste electrode [13]. To date no studies are available in literature concerning quantification of glipizide based on its electrochemical reduction.

Adsorptive cathodic stripping voltammetry has been shown to be an efficient electroanalytical technique for determination of sub-nanomolar level of a wide range of drugs which have interfacial adsorptive character onto the working electrode surface. It usually involves a simple accumulation step and most of the excipients used do not interfere in the subsequent determination of the drugs [14]. The technique is easy to use and save of both time and costs.

Here we amid to describe a fully validated simple, precise and selective square-wave adsorptive cathodic stripping voltammetric procedure for the trace quantification of glipizide in bulk form and in pharmaceutical formulation.

2. Experimental

2.1. Solutions

A standard stock solution of 1×10^{-3} M glipizide was prepared by dissolving an accurate quantity of the authentic substance in a specific volume of methanol then stored at 4 °C.

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More dilute solutions (10^{-6} to 10^{-4} M) were prepared by accurate dilution with methanol.

Solutions of minidiab tablets (Chemical Industries Development, Giza, Egypt), labeled as containing 5 mg glipizide per tablet were prepared. Twenty tablets were weighed and then the average mass per tablet was determined. The tablets were carefully grounded to a fine powder then a quantity of the homogenous powder equivalent to 25 mg of glipizide was transferred accurately with methanol into a 100 ml volume calibrated flask containing 70 ml methanol, sonicated for 15 min then completed to the volume with methanol. The desired concentrations of glipizide were obtained by accurate dilution with methanol. A Mettler balance (Toledo-AB104, Switzerland) was used for weighing the solid materials.

A series of Britton–Robinson (B–R) universal buffer (pH 1.7–11) was prepared [15] and used as a supporting electrolyte. A pH meter (Crison, Barcelona, Spain) was used for measuring the pH of the prepared buffers. The de-ionized water was supplied from a Purite-Still Plus de-ionizer connected to an Aqua-Matic double-distillation water system (Hamilton Laboratory Glass Ltd., Kent, UK).

2.2. Apparatus

The computerized Electrochemical Analyzers Models 273A and 263A-PAR were used for the voltammetric measurements. An electrode assembly (303A-PAR) with a micro-electrochemical cell of three electrode system configuration comprising of a hanging mercury drop electrode (HMDE) as a working electrode (area: 0.026 cm^2), an Ag/AgCl (saturated KCl) reference electrode and a platinum wire auxiliary electrode, was used. All the measurements were automated and controlled through the programming capacity of the apparatus.

2.3. General analytical procedure

For stripping voltammetric analysis of glipizide, the analyte solution was introduced into a 10 ml volume calibrated flask and then completed to the volume with a B–R universal buffer of pH 6. The solution was then transferred into the micro-electrochemical cell and a pure nitrogen stream was passed for about 10 min in the first cycle and for 30 s in each successive cycle, while the nitrogen gas was kept over surface of the solution during the measurements. An accumulation potential of −0.6 V was then applied to the hanging mercury drop for an accumulation duration of 20–25 s while the solution was stirred at 400 rpm. At the end of the accumulation duration, the stirring was stopped and a 5 s rest period was allowed for the solution to become quiescent. The voltammograms were then recorded by scanning the potential towards the negative direction using the square-wave mode.

3. Results and discussion

3.1. Cyclic voltammetry studies

Cyclic voltammograms of glipizide at a hanging mercury drop electrode (HMDE) in the B–R universal buffers of pH 1.7–11 exhibited a well-defined 4-electron irreversible cathodic peak which may be attributed to reduction of the two C=N double bonds of the pyrazine ring of the target molecule. The peak potentials (E_p) shifted to more negative values upon rise either the pH of solution (1.7–11) and the scan rate v (25–500 mV/s) which confirmed, respectively, the involvement of protons in the electrode reaction [16] and the irreversible nature of the reduction process [17].

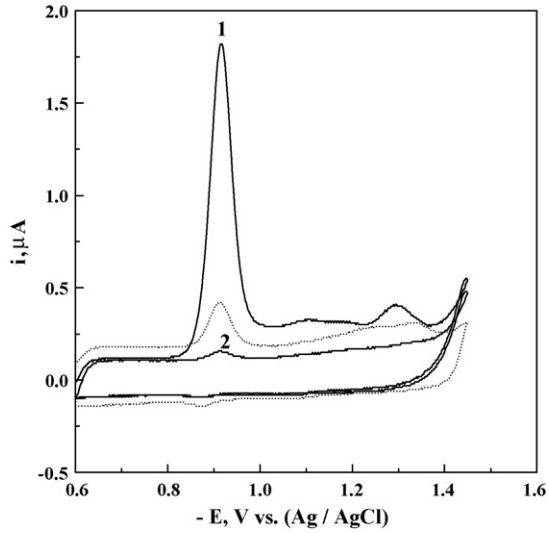

Fig. 1. Cyclic voltammograms of 1×10^{-6} M bulk glipizide in a B–R universal buffer of pH 6 following its accumulation onto the HMDE: at open circuit (dashed scan), at $E_{acc.} = -0.6$ V for 30 s (scan 1) and the repetitive scan (scan 2); scan rate = 200 V s^{-1}.

The interfacial adsorptive character of glipizide onto the HMDE was identified by recording the cyclic voltammograms of 1×10^{-6} M glipizide in a B–R universal buffer of pH 6 following its accumulation onto the HMDE at open circuit (Fig. 1, dotted curve) and at −0.6 V for 30 s (Fig. 1, curve 1). A much developed peak current intensity was achieved following accumulation of glipizide onto the HMDE surface at −0.6 V whereas its second cycle at the same mercury drop (curve 2) exhibited a lower peak current intensity. This behavior indicated the interfacial adsorptive character of glipizide onto the mercury electrode surface.

The electrode surface coverage Γ_0 (amount of reactant adsorbed onto the mercury electrode surface, mol cm^{-2}) was calculated using the equation $\Gamma_0 = Q/nFA$, where Q is the amount of charge consumed by the surface process as calculated by the integration of the area under peak of the cyclic voltammogram (for 1×10^{-6} M glipizide at pH 6) corrected to residual current [18], n the total number of electrons consumed in the reduction process ($n = 4$), F the Faraday's constant and A is the mercury electrode surface area (0.026 cm^2). On dividing the number of coulombs transferred by the conversion factor (nFA) a monolayer surface coverage of 1.02×10^{-10} mol cm^{-2} was obtained. Each adsorbed glipizide molecule therefore occupied an area of 1.63 nm^2.

3.2. Square-wave stripping voltammetry studies

3.2.1. Optimization of an analytical procedure

Square-wave adsorptive cathodic stripping (SWAdCS) voltammograms for 1×10^{-7} M glipizide in the B–R universal buffers of pH 1.7–11 following its accumulation onto the HMDE for 30 s exhibited a single irreversible cathodic peak over the entire pH range. The peak current intensity was much developed in a B–R universal buffer of pH 6. So it was chosen as a supporting electrolyte for the rest of the present work.

The peak current for 1×10^{-7} M glipizide in a B–R universal buffer of pH 6 following its accumulation onto the HMDE at −0.3 V for 30 s was optimized by changing the pulse-amplitude (a), frequency (f), scan increment (ΔE_s) within the ranges 10–50 mV, 10–200 Hz and 2–10 mV, respectively. Although the SWAdCS voltammetric peak current intensity of glipizide was almost directly proportional to each of a, f and ΔE_s, however a sharper peak and a much developed peak current intensity were obtained under the following conditions $a = 30$ mV, $f = 160$ Hz and $\Delta E_s = 6$ mV.

The effect of varying the accumulation potential ($E_{acc.}$) from 0.0 to −0.8 V on the peak current intensity of the SWAdCS voltammogram for 1×10^{-7} M glipizide in a B–R universal buffer of pH 6 following its accumulation onto the HMDE for 30 s was also evaluated. The peak current intensity was practically independent of the accumulation potential up to −0.8 V (Fig. 2). Therefore an accumulation potential of −0.6 V was chosen for the rest of the present measurements. SWAdCS voltammograms for 1×10^{-9} and 1×10^{-8} M glipizide, were recorded at increased accumulation duration. As shown in Fig. 3, the peak current intensity is a linear relationship with the accumulation duration up to 20–25 s.

Accordingly, the optimal conditions of the proposed SWAdCS voltammetric procedure for quantification of glipizide were: a B–R universal buffer of pH 6 as a supporting electrolyte,

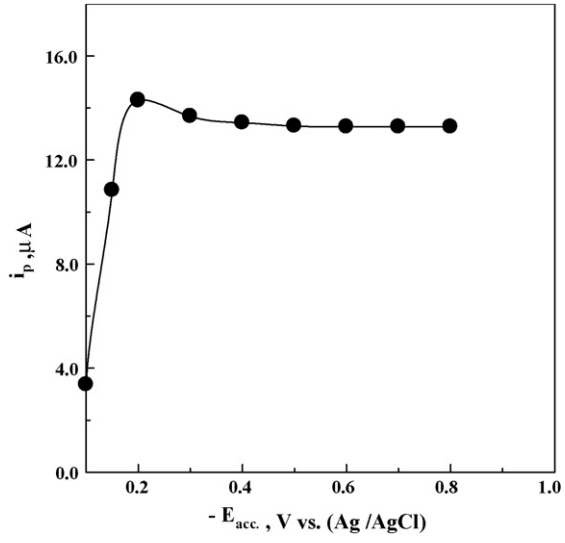

Fig. 2. Effect of accumulation potential ($E_{acc.}$) on the SWAdCS voltammetric peak current intensity (i_p) for 1×10^{-7} M bulk glipizide following its accumulation onto the HMDE for 30 s in a B–R universal buffer of pH 6; $f = 160$ Hz, $\Delta s = 6$ mV and $E_{sw} = 30$ mV.

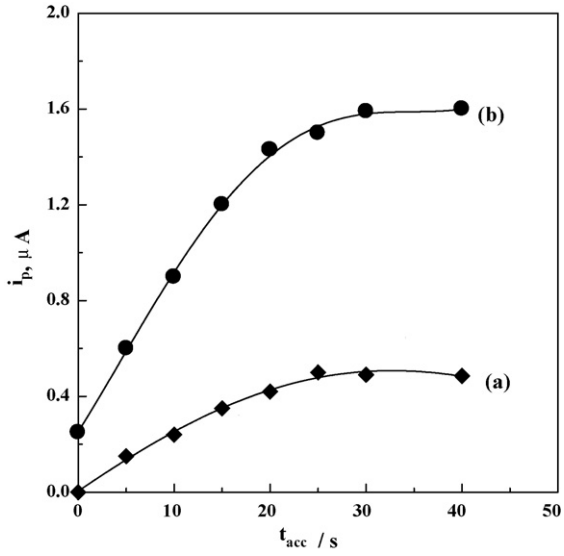

Fig. 3. Effect of accumulation duration ($t_{acc.}$) on the SWAdCS voltammetric peak current intensity (i_p) for (a) 1×10^{-9} M and (b) 1×10^{-8} M bulk glipizide in a B–R universal buffer pH 6; $E_{acc.} = -0.6$ V, $f = 160$ Hz, $\Delta s = 6$ mV and $E_{sw} = 30$ mV.

pulse-amplitude (a) = 30 mV, frequency (f) = 160 Hz, scan increment (ΔE_s) = 6 mV, accumulation potential $E_{acc.} = -0.6$ V and accumulation duration = 20–25 s.

3.2.2. Method validation

Validation of an analytical method is the procedure by which it is established that performance characteristics of the method meet the requirements for the extended analytical applications. This was examined via evaluation of limit of detection (LOD), limit of quantitation (LOQ), repeatability, reproducibility, precision, selectivity, robustness and intermediate precision. From the recorded SWAdCS voltammograms of various concentrations of bulk glipizide under the optimized procedural conditions (Fig. 4), a linear calibration curve was constructed over the concentration range 5×10^{-10} to 1×10^{-8} M. Limits of detection (LOD) and quantitation (LOQ) of glipizide in the analyzed samples were estimated using the relation [19]: $S.D._a/b$, where $k = 3$ for LOD and 10 for LOQ, $S.D._a$ is the standard deviation of the intercept and b is the slope of the calibration curve and were found to equal 1.5×10^{-10} and 5×10^{-10} M, respectively.

The repeatability of results using the proposed SWAdCS voltammetric procedure was examined by performing three replicate measurements for 5×10^{-8} M bulk glipizide following its accumulation onto the HMDE for 20 s on the same day using the same standard solution of glipizide and over 3 days using different standard solutions of glipizide. The recovery results (Table 1) confirmed the good precision and accuracy of the proposed procedure and the stability of analyte solutions.

The selectivity of the described SWAdCS voltammetric procedure was tested by analysis of 5×10^{-8} M bulk glipizide solution (the excipients were absent) and a standard tablet (Minidiab) solution containing 5×10^{-8} M glipizide (the excipients were present), following accumulation of the analyte onto the HMDE at −0.6 V for 20 s in both cases. No significant differences in

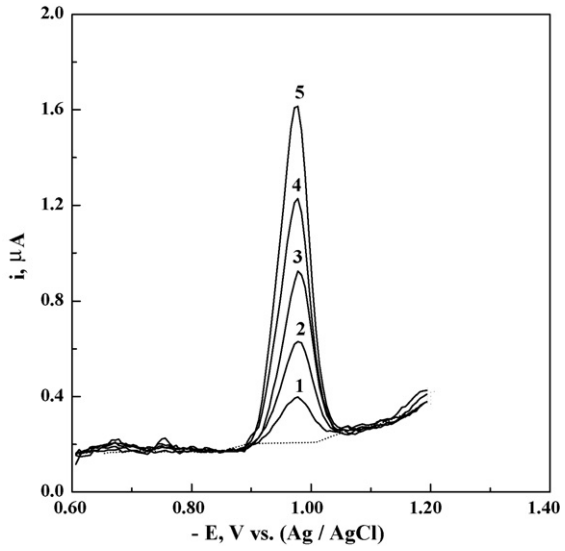

Fig. 4. SWAdCS voltammograms for various concentrations of bulk glipizide in a B–R universal buffer of pH 6. The dotted line represents the blank solution: (1) 1×10^{-9} M, (2) 3×10^{-9} M, (3) 5×10^{-9} M, (4) 7×10^{-9} M and (5) 9×10^{-9} M bulk glipizide; $E_{acc.} = -0.6$ V, $t_{acc.} = 20$ s, $f = 160$ Hz; $\Delta s = 6$ mV and $E_{sw} = 30$ mV.

the recoveries or the relative standard deviations were achieved in the absence ($100.7 \pm 0.26\%$) and presence ($102.8 \pm 0.86\%$) of excipients. Thus, the proposed procedure can be considered selective.

The robustness of measurements using the proposed SWAdCS voltammetry procedure was examined by studying the effect of small variation of some important procedural conditions, e.g. pH (6 ± 0.5) and accumulation potential (-0.6 V ± 0.05) on the recovery of 5×10^{-8} M bulk glipizide. The obtained recoveries ($98.98 \pm 0.95\%$ to $100.7 \pm 0.26\%$) were not significantly affected and consequently the optimized procedure was reliable for assay of bulk glipizide and it could be considered robust.

The intermediate precision of measurements using the proposed SWAdCS voltammetric procedure was examined by the assay of glipizide using the two potentiostats 273A-PAR (Lab. 1) and 263A-PAR (Lab. 2). The recovery obtained due to Lab.1 ($100.7 \pm 0.26\%$) or Lab. 2 ($98.9 \pm 0.90\%$) was found reproducible, since there was no significant difference between the recoveries or the standard deviations values.

Table 1
Mean recovery (%*R*), precision (R.S.D.%) and accuracy (R.E.%) of assay of 5×10^{-9} M glipizide by the proposed SWAdCS voltammetric procedure following accumulation onto the HMDE for 20 s, $n = 4$

Day	%*R*	(R.S.D.%)	(R.E.%)
Intra-assay			
1	101.2	0.26	0.60
2	101.7	0.38	0.55
Inter-assay			
1	101.6	0.26	0.80
2	101.7	0.37	0.60
3	101.5	0.52	1.40

Table 2
Assay of glipizide in its pharmaceutical formulation (Minidiab® tablets) by the proposed SWAdCS voltammetric procedure and a reported TLC method [7]

Claimed (mg/tablet)	5
Recovery (%$R \pm$ R.S.D.) by the proposed method (calibration curve method)	102.84 ± 0.86
Recovery (%$R \pm$ R.S.D.) by the proposed method (standard addition method)	100.40 ± 0.86
Recovery (%$R \pm$ R.S.D.) by the reported TLC method [7] (calibration curve method)	101.7 ± 0.45
F-value	3.65
t-Test	2.35

Theoretical value of *F* and *t*-test at 95% confidence limit (for $n_1 = 4$ and $n_2 = 4$) are 6.60 and 2.45, respectively.

3.2.3. Analysis of minidiab tablets

The proposed square-wave adsorptive cathodic stripping (SWAdCS) voltammetric procedure was successfully applied for the determination of glipizide in its pharmaceutical formulation (Minidiab tablets) without interference from excipients. Recovery of glipizide in its pharmaceutical formulation (Minidiab tablets), based on the average of four replicate measurements is reported in Table 2. The results obtained were statistically compared with those obtained by a reported TLC method [7]. Values of *F*-calculated, *F*-theoretical, *t*-calculated and *t*-theoretical are also included in Table 2. Since the calculated value of *F* (3.65) does not exceed the theoretical value (6.6), which means there is no significant difference between the proposed and reported method with respect to reproducibility [19]. Also, no significant difference was noticed between the two methods regarding accuracy and precision as revealed by *t*-value, 2.35 [20]. The accuracy of the proposed procedure was also judged by applying the standard addition method [21] (Table 2).

3.3. Conclusion

A fully validated simple, fast, sensitive and precise square-wave adsorptive cathodic stripping voltammetric procedure was described for quantification of glipizide in bulk form and in its pharmaceutical formulation (Minidiab tablets) without interference from excipients. The procedure is simple, sensitive, precise and could be used in trace analysis laboratories.

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