Egypt. J. Chem. **Vol. 64,** No. 4 pp. 2013 - 2024 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Electro-reduction, Quantification and Pharmacokinetic Studies for the Anti-Hyperglycemic Drug Glibenclamide Using Stripping Voltammetric method: Development and Validation

M. A. El-Attar[1]*, M. M. Ghoneim[2]

[1]*High Institute of Engineering & Technology, 31739, Tanta, Egypt*

[2]*Analytical Chemistry Research Unit, Chemistry Department, Faculty of Science, Tanta University, 31527, Tanta, Egypt*

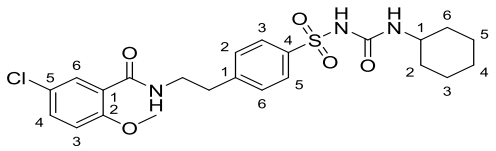
Abstract

The electrochemical behavior of anti-hyperglycemic (glibenclamide, GBC) was investigated and discussed at HMDE in Britton-Robinson universal buffer using cyclic voltammetry. Its voltammogram at pH values 6-11 displayed a quasi-reversible redox couple one-electron peak corresponding to a catalytic hydrogen process. Based on this voltammetric peak, a validated and sensitive SW-AdCS voltammetric method was depicted for determination of GBC in the bulk form, diabetic drug (Daonil® 5 mg) and in human serum. The (LOD) of 6×10^{-9} M (2.964 ng mL^{-1}) and (LOQ) of 2×10^{-8} M (9.88 ng mL^{-1}) bulk GBC were achieved, respectively, While for protein-free GBC-spiked human serum were (LOD) of $1.5x10^{-8}$ M (7.41 ng mL^{-1}) and limit of quantification (LOQ) of $5x10^{-8}$ M (24.7 ng mL^{-1}), respectively. The described analytical method was utilized to estimate the pharmacokinetic parameters of GBC in plasma of hospitalized volunteers after the administration of a single oral dose of Daonil® 5 mg ($p > 0.05$).

Keywords: Glibenclamide; Voltammetry; Quantification; Pharmacokinetics; SW-AdCS; Statistical analysis.

1. Introduction

The differences between substituted arylsulfonylureas were clarified in the species of substitutions at the two ends of the molecule. Anti-hyperglycemic GBC is a cardinal drug in the remediation of type II diabetes mellitus, which is a common illness particularly in patients with ischaemic heart disease. GBC has been described to stimulate predominately the late second phase of the insulin response and to have no clear effect on the first phase response. Also, GBC is defined as (glyburide, GB), 1-{4-[2-(5-Chloro-2-methoxybenzamido)ethyl] benzenesulfonyl}-3-cyclohexylurea, (Scheme 1).



Scheme 1. Chemical structure of GBC molecule.

It is a potent second generation sulphonylurea with similar action of glipizide (GP) [1] (previous our reported work), which an orally active hypoglycemic agent that reduces the blood-sugar concentration in patients with kind II non-insulin based on diabetes mellitus for long acting. GBC has a greater effect in lowering fasting blood glucose and elevating fasting plasma insulin levels than GP. In contrast, GP caused a greater increase in plasma insulin and a lesser glycemic rise after meals than GBC. So that, the main difference between GBC and GP is that the former is more effective in patients who are fasting while, the latter is more effective in those who are eating. The second generation of sulfonylurea (glyburide, glipizide, gliclazide, and glimperide) hypoglycemic agents have stronger therapeutic effect is up to 200 times than the first generation (tolbutamide, chlorpropamide, tolazamide and acetohexamide). Therefore, they should be prescribed in small doses. This efficiency is because of the greater intrinsic hypoglycemic potency of the molecule rather than to

*Corresponding author e-mailmel_attar@yahoo.com.; (Mona El-Attar).

Receive Date: 07 November 2020, Revise Date: 11 December 2020, Accept Date: 23 December 2020

DOI: 10.21608/EJCHEM.2020.49056.3004

a prolonged biological half-life [2]. GBC is rapid completely absorbed after oral administration in the gastrointestinal tract. As there is no significant first pass metabolism, 100% of the oral dose is bioavailable [3]. Various analytical procedures have been notified in the literature for determination of GBC in bulk form, pharmaceutical formulation and biological fluids. These methods include, micellar electrokinetic chromatography [4], HPLC [5-11], RP-HPLC [12, 13], liquid chromatography (LC/APCI-MS/MS) [14, 15], LC/ESI/MS [16], TLC [17], gas chromatography (GC) [18], radioimmunoassay [19], spectrophotometry and voltammetry [20-23]. Most of the previously reported techniques used to determination of GBC are based on a derivatization prior to the analysis due to the high polarity of its chemical structure and the low concentration of GBC found in blood, rarely beyond 300 ng ml^{-1} [10, 11]. Also, after administration of therapeutic doses of GBC [16] they usually have shortage in the efficiency and sensitivity for determination of pharmacokinetic data in human plasma. The most confirmed method for determination of GBC is dependent on the derivatization with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole prior to HPLC analysis with fluorescence detection [10]. As no studies are available in the literature to date concerning the quantification of GBC in bulk form, diabetic drug and human serum based on its electrochemical reduction. It is aimed in this assay to demonstrate the redox pathway of GBC in buffered solutions at HMDE using cyclic voltammetry. Moreover, a square-wave adsorptive cathodic stripping (SW-AdCS) voltammetric procedure for quantification of GBC in bulk form and its pharmaceutical dosage form (Daonil® 5 mg) has been developed. Also, because of low therapeutic dose of the second generation of sulfonylureas and the shortness of the serum half-life, the expected low serum levels required a specific analytical method with good accuracy and sensitivity. Therefore, the proposed high sensitivity SW-AdCSV method has been used for determination of the lowest possible concentration of GBC in protein-free spiked human serum samples without requirement for derivatization and / or consuming time in extraction steps prior to the analysis and to estimate its pharmacokinetic parameters in plasma of two healthy male volunteers, each of them administrated a single oral dose of (Daonil® 5 mg).

2. Experimental

2.1 Apparatus

The electrochemical experiments, weighing the solid materials, the used deionized water and convective transport during the preconcentration step were performed using the same equipment that described in the refs. [1, 24-26]. An automatic micropipette (Eppendorf-Multipette® plus) was used to transferring analyte solutions. Centrifuge Model 5417C (Eppendorf AG, Hamburg, Germany). Vortex (Autovortex Mixer SA2, UK). Deep Freez -80 ^{o}C Model WUF-300 (Wise Cryo - Korea). A pH-meter Model HI8014 (Hanna Instruments, Italy) accurate to ± 0.01 pH units was used for the pH measurements.

2.2. Solutions and methodology

(i) The same volumes of 0.04 M of each of acetic, boric and *o*-phosphoric acids were mixed and then solutions of various (B-R) universal buffer (pH 2-11) were specified at the required pH with 0.2 M sodium hydroxide solution [27]. As well, 0.1 M sodium carbonate and 0.1 M sodium hydroxide were used as supporting electrolytes. Deionized water and analytical grade chemicals were used to prepare the solutions.

(ii) GBC (Egypt S.A.E., sanofi-aventis under license from Germany) was supplied as a gift sample from department of analytical chemistry, faculty of pharmacy, Tanta University. A stock standard solution of 1×10^{-3} M bulk GBC was prepared in methanol (Merck) and stored at 5°C. Working solutions (10^{-5} to 10^{-4} M) were prepared workaday by adequate dilution for the stock standard solution with methanol directly before used.

(iii) Weigh twenty tablets Daonil® 5 mg (Egypt S.A.E., sanofi-aventis under license from Germany), each tablet Daonil® as active ingredient contains 5 mg GBC was grinded well in an agate mortar, after that the average mass per tablet was calculated. An amount of the fine powder equivalent to 1×10^{-3} M formulation was transferred exactly into 50 mL measuring flask and diluted to volume with methanol, then sonicated for 15 min. The solution was then filtrated through a 0.45 mm milli-pore filter (Gelman, Germany), that to separate out the insoluble excipients, rejecting the first portion of the filtrate. The required more diluted concentrations of the prepared solution (10^{-5} to 10^{-4} M) were achieved by precise dilution with methanol.

(iv) Aliquots of serum samples from a healthy volunteer were preserved in centrifugation tubes, then fortified with different concentrations of GBC (10^{-5} to 10^{-4} M) and then mixed with 1.0 mL of methanol to denature and precipitate proteins. The precipitated

proteins after vortexing for 2 min were discarded by centrifugation for 3 min at 14000 r.p.m using an (Eppendorf AG). Protein-free GBC-spiked human serum samples were obtained from the filtration of the clear supernatant layer using a 0.45 μm pore size Millipore filter (Gelman, Germany).

(v) The validated SW-AdCSV procedure was applied on human plasma of two healthy volunteers (male) with a mean age 37.5 years for GBC determination. Caffeine-containing beverages, smoking and alcohol were not allowed for at least 12 hours before the administration of the drug. The protocol of the study was formally accepted by the Department of Internal Medicine of the Ramadan Specialized Hospital, Tanta City, Egypt. A written approval was obtained from the two volunteers prior to anticipating in the study. The whole study was performed following all applicable guidelines for good clinical practices. Each volunteer after an overnight fasting received a single 5 mg oral dose of the tested product (Daonil® 5 mg) in the morning. An indwelling venous cannula was inserted into a forearm vein for blood sampling. Venous blood samples were taken instantly after the oral administration of (Daonil® 5 mg), 1 mL for each pre-dose (0 h) and for the following post-dose intervals within 0.5, 1.0, 1.5, 2, 3, 4, 6, 8, 12 and 24 h, then the blood samples were centrifuged immediately at 3000 rpm for 15 min. Following separation, each plasma sample was transferred to a coded polypropylene tube and frozen immediately at -20 °C until analyzed.

(vi) The contents of the electrochemical cell were appropriate concentration for each of bulk GBC, Daonil® 5 mg, proteins free-solution in case of human serum or plasma samples and the optimal buffered solution of pH = 9.7, then the analyte solution was deoxygenated with stream of pure N_2 gas for about 10 min and then maintaining it over the solution during performing the measurements. The voltammograms were performed using the square-wave potential waveform. All experiments were recorded at room temperature.

(vii) Statistical analysis of the present analytical results was conducted using the paired non-parametric Wilcoxon Signed Rank and Friedman Tests using Statistical Package for Social Science program (SPSS 16) [28]. The level of significance was taken as (Probability value; $p <$ 0.05). All values were reported as mean ± relative standard deviation (% R ± R.S.D.).

HMDE is the most considerably used with high renewable and smooth surface, low consuming of mercury and the probability of adsorptive or electrolytic accumulation to the analytes on its surface [29, 30]. The use of Hg was facing a restriction in some countries. Actually, we live in an age of irrational mercurophobia. This situation involves psychology rather than science. Moreover, mercury is usually stored under water and cannot be volatized as vapor at room temperature. Mercury salts have been used in medicine for centuries, with no symptoms of the ill health. It is only the organomercury compounds (not formed under controlled laboratory conditions) are toxic [31]. There are many works have been announced for the authors based on the use of Hg electrodes [1, 24-26, 29]. Also, Petr Zuman [31] worked on daily with Hg for many years with no ill health effects, but he met during his life at least 500 polarographers who lived and worked in laboratories. None of them reported any ill effects or exhibited elevated levels of Hg in the blood.

3. Results and discussion

No previous voltammetric methods were available concerning the electroreduction behavior, electrode reaction mechanism and adsorption of GBC at the Hg electrode.

3.1. Electrochemical behavior

The redox behavior of GBC was identified by cyclic voltammetry of $1x10^{-4}$ M at HMDE in B-R universal buffer solutions of pH ≥ 6 at sweep rate 300 mV s^{-1} and displayed one redox couple (Fig. 1, curves a-d). In analogy to similar works this peak attributed to the catalytic reaction of the liberation of hydrogen from depolarizer molecule (Scheme 2, reaction 3). [32, 33]. The peak current (i_p) of the GBC was pH-dependent in solution of pH 6-11. The dependence of i_p on pH in this range has the shape of a dissociation curve (with Z-shape) of one inflection point at pH 7.3 which may correspond to the pK_a of GBC (Fig. 1; Inset, plot e). The peak potential of the redox couple shifted toward less negative potential at pH 6-11 (Fig. 1, Inset; plot f), confirming that this peak is mainly a catalytic hydrogen peak.

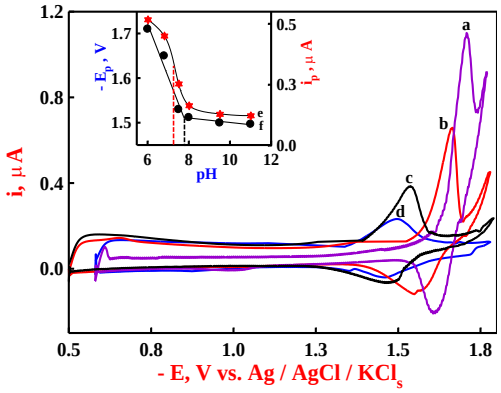
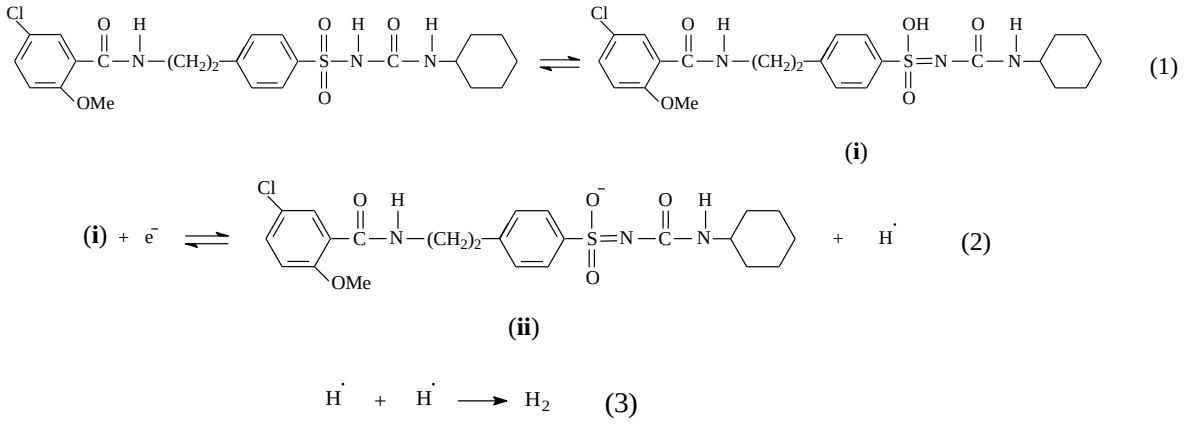


Fig. 1. Cyclic voltammograms of 1×10^{-4} M bulk GBC at v = 300 mV s^{-1} in B-R universal buffer of various pH values: (a) 6, (b) 6.8, (c) 7.5 and (d) 11. Inset: (e) i_p and (f) E_p *vs* pH plots.

The peak observed at less negative potential because the rate of elimination of proton from the molecule increased by increasing the pH of solution which is facilitated by tautomeric equilibrium of ionizable sulfonamide NH group (Scheme 2, reaction 1) [33] The E_p-pH plot of the cathodic peak in the pH range 6-11 gave two straight lines with intersection at pH = 7.75, which may coincided with the acid-base constant (pK_a) of GBC as shown in i_p - pH plot (Fig. 1; Inset, plots e and f) [21]. This behavior can be assigned to change in the nature of GBC on increasing pH values. The linear E_p-pH plot (pH 6.0-7.5) was obtained at sweep rate of 300 mV s^{-1} (E_p (V) = 0.12 pH + 2.4; r = 0.99 and n = 3). From its slope (0.059 P / α n_a), value of αn_a was calculated as: αn_a = 0.059 P / 0.12 = 0.5 and symmetry transfer coefficient α was estimated and found to equal 0.5 at the ratio p/n_a = 1. Where n_a and p are the numbers of electrons and protons participating in the rate determining step. The i_{pc} increases with the increasing of $v^{1/2}$ at various pH values, resulting linear correlations with little deviation from the origin, confirming controlled diffusion of the electrode process with some adsorption contribution. The peak separation between anodic and cathodic peak potentials ($\Delta E_p = E_p^a - E_p^c = 0.059/n$, V) varies from 0.05 to 0.11 V and increases with increasing (v), and the calculated number of electrons consumed per one reactant molecule GBC were found to be one (n = 1 electron). The peak potential (E_p) changing slightly with increasing of the sweep rate (25-500 mV s^{-1}) as expected for a quasi-reversible process. The ratio of (i_p^a/i_p^c) peak current tends to unity with dependent on sweep rate used, which is a support for an *EC* mechanism (slow chemical reaction). Also, the current function ($i_p/v^{1/2}$) depends on the sweep rates. These data are compatible with a quasi-reversible one-electron step followed by slow chemical reaction ($E_{qui\text{-}rev}C$). Finally, the electrode reaction mechanism of GBC can be suggested as following scheme:



Scheme 2. Electrode reaction pathway of GBC at HMDE.

Accordingly, the occurrence of the above electrode reaction pathway of GBC at HMDE mentioned that the tautomeric sulfur with the elimination of proton from the enolic form (Scheme 2, reactions 1 and 2), this produces a catalytic hydrogen wave (Scheme 2, reaction 3), which was observed previously during the formation of transition metal ion complexes with some sulfonamide derivatives [33].

3.2. Adsorptive character of GBC onto HMDE

Adsorption of GBC onto the HMDE surface was confirmed by recording cyclic voltammograms in a B-R universal buffer of pH 9.7 at various scan rates (25-300 mV s^{-1}) after adsorptive accumulation at -0.3 V for 30 s. A straight line following the equation: *log* i_p *(μA)* = 0.83 *log v* (mV s^{-1}) + 0.094 (n = 7 and r = 0.999) was attained. The slope value of 0.83 indicated that the electrode process is surface-adsorbed species [34]. Also, CV of $5x10^{-6}$ M GBC was recorded at sweep rate of 100 mV s^{-1}, following its deposition onto the HMDE at open circuit and at -0.6 V for 30 s. As well, its repetitive cycle measurement at the same Hg drop was recorded, significant enhancement of the i_p was achieved onto the HMDE at -0.6 V for 30 s compared to that in the subsequent scan at the same Hg drop or to that recorded under open circuit conditions, confirming adsorption of GBC onto the mercury electrode surface. The electrode surface coverage (Γ^o *mol* cm^{-2}) was estimated using the relation: $\Gamma^o = Q / n F A$, where (Q) is the quantity of exhaust charge in μC via surface area under CV peak current, n = 1 electron consumed in the overall reduction process per GBC molecule and A is the Hg electrode surface area (0.026 cm^2). The determined number of coulombs transferred (Q) was 0.847 μC at pH 9.7 and the calculated adsorbed GBC molecule was occupied an area of 0.492 nm^2.

3.3. Square-wave adsorptive stripping voltammetric (SW-AdCSV) studies

3.3.1. Optimization of supporting electrolyte and instrumental conditions

The recorded SW-AdCS voltammograms of $1x10^{-6}$ M GBC following accumulation onto the HMDE at -0.3 V for 30 s in different supporting electrolytes such as B-R buffer of pH ≥ 6 (Fig. 2; curves a, b and c), NaOH (0.1 M) (Fig. 2, curve d) or Na_2CO_3 (0.1 M) (Fig. 2, curve e) solutions, displayed a well single cathodic peak. A sharper, a well defined and reproducible cathodic i_p was obtained in B-R universal buffer of pH 9.7 (Fig. 2, curve b). So, it was found to be the most appropriate alkaline supporting electrolyte for measurements of the acidic drug GBC in the present analytical study.

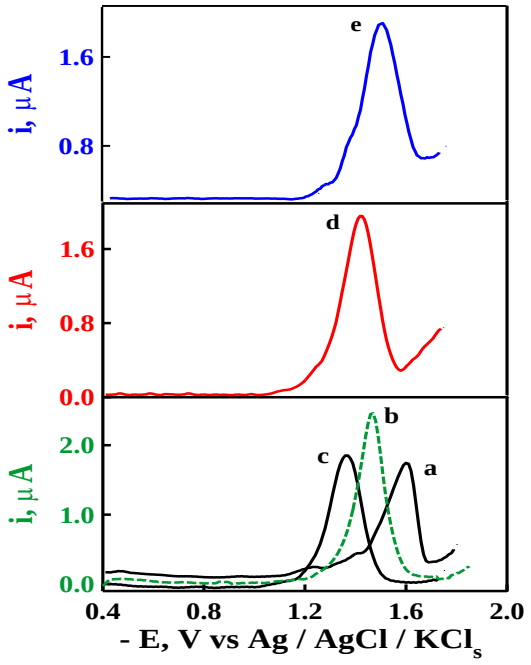


Fig. 2. SW-AdCSVs for 1×10^{-6} M bulk GBC in a B-R buffer of pH: 6.8 (a), 9.7 (b) & 10.5 (c), 0.1 M NaOH (d) and 0.1 Na_2CO_3 solutions (e); E_{acc} = -0.3 V, t_{acc} = 30 s; f = 100 Hz, ΔE_s = 8 mV and a = 60 mV.

The influence of instrumental parameters scan increment (ΔE_s), frequency (f), and pulse-amplitude (a) to achieve the optimum conditions for determination of $1x10^{-6}$ M bulk GBC by SW-AdCSV in B-R buffer of pH 9.7 following adsorptive accumulation onto the HMDE at -0.3 V for 30 s. By changing pulse parameters, f: 10-120 Hz, ΔE_s: 2-10 mV and a: 10-100 mV. A sharper and much advanced i_p of bulk GBC was obtained at f = 100 Hz, ΔE_s = 8 mV and a = 80 mV.

3.3.2. Preconcentration conditions

The effect of adsorptive accumulation potential ($E_{acc.}$) on the voltammetric i_p intensity of $1x10^{-6}$ M GBC onto the HMDE for 30 s was also examined over the potential range -0.2 to -0.7 V (*vs* Ag/AgCl/KCl_s). A larger and enhanced i_p was realized at $E_{acc.}$ of -0.6 V. As well, the SW-AdCSVs of different concentrations of bulk GBC solutions ($1x10^{-8}$, $3x10^{-7}$ and $1x10^{-6}$ M) were determined under the obtained optimal experimental operational

conditions at adsorptive E_{acc} = -0.6 V for the increasing the adsorptive duration ($t_{acc.}$) (Fig. 3). The i_p magnitude is dependent linearly on both the GBC concentration and accumulation duration up to $t_{acc.} \leq$ 50 s as shown in Figure 3. In the present electro-analytical procedure the $t_{acc.}$ 50 s was used.

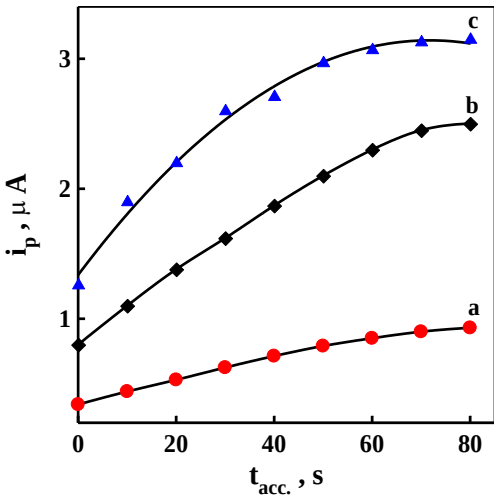


Fig. 3. Influence of preconcentration duration ($t_{acc.}$) on the SW-AdCS voltammetric i_p; (a) 1x10^{-8}, (b) 3x10^{-7} and (c) 1x10^{-6} M GBC in a B-R buffer of pH 9.7; $E_{acc.}$ = -0.6 V, f = 100 Hz, ΔE_s = 8 mV and a = 80 mV.

3.4. Method validation

Validation of the SW-AdCSV method was examined with respect to standard curve, recovery, accuracy, precision, selectivity, robustness and intermediate precision [35].

3.4.1. Standard curve, linearity and sensitivity

The SW-AdCSVs of various concentrations (C) of GBC under the described conditions were carried out at the HMDE (Fig. 4, curves b-g). Standard curve plot of the linear variation of peak current (i_p) versus (C) of bulk GBC was rectilinear over the extent of 2x10^{-8} to 1x10^{-6} M (Fig. 4; inset) following the linear regression equation: i_p (μA) = 1.89 C (M) + 0.81, (r = 0.99 and n = 8), as the equation $y = ax + b$, where y was peak current i_p (μA), x was the concentration C (M) of GBC solutions, b was the intercept and a was the slope of the regression line. The unknown concentrations of the GBC samples were calculated from the regression equation of the standard curve (x = y-b/a). The limit of detection (LOD) of 6×10^{-9} M (2.964 ng mL^{-1}) and limit of quantification (LOQ) of 2×10^{-8} M (9.88 ng mL^{-1}) were achieved, respectively, using the relation k.SD / a [35], where k = 10 for (LOQ) and 3 for (LOD), (S.D.) is the standard deviation of the blank (S.D. = 0.0038), and (a) is the slope of the linear plot (a = 1.89). These obtained data approved the sensitivity of the proposed SW-AdCSV for determination of GBC under the optimal conditions.

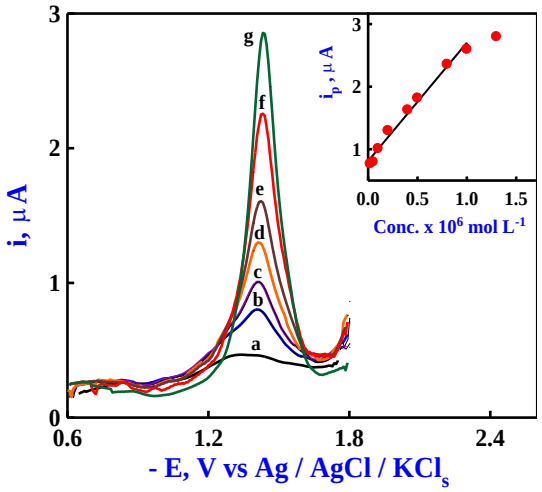


Fig. 4. SW-AdCSVs in B-R buffer of pH 9.7 for various concentrations of GBC; (a) blank solution, (b) 2x10^{-8}, (c) 5x10^{-8}, (d) 1x10^{-7}, (e) 2x10^{-7}, (f) 5x10^{-7} and (g) 1x10^{-6} M at $E_{acc.}$ = -0.6 V for $t_{acc.}$ = 50 s; f = 100 Hz, ΔE_s = 8 mV and a = 80 mV. Inset: calibration plot of bulk GBC.

3.4.2. Recovery

Recovery of the proposed SW-AdCSV procedure was identified from repeated measurements for the concentrations 1x10^{-7}, 5x10^{-7} and 8x10^{-7} M GBC (n = 4 at each concentration) [35]. The mean percentage recoveries (% R) and their relative standard deviations (R.S.D. %) calculated in the described SW-AdCSV method were 99.00 ± 1.82, 101.00 ± 1.76 and 100.7 ± 1.8, respectively, which were insignificantly different (Friedman test: p = 0.085; p > 0.05 & Chi-Square = 4.9). The obtained results indicated the sensitivity and accuracy of the represent SW-AdCSV procedure for quantification of GBC.

3.4.3. Precision and accuracy

The data evaluated within five days validation using the described SW-AdCSV procedure. The samples were left at ambient temperature during performed the measurements. Two concentration levels were chosen from the standard curve (1x10^{-7} and 8x10^{-7} M GBC) to analysis at each of day of validation, (n = 4 at each concentration). Mean percentage recoveries (%R) for intra- and inter-day precisions (R.S.D. %) and accuracy (Bias %) [35] were obtained with insignificant differences between the amounts taken

and found of GBC (Table 1). The statistical comparison of the results was done using Friedman analysis and no difference was found, see Table 1, which indicated the reproducibility precision and accuracy of the present stripping voltammetric assay for assay of GBC.

Table 1. Mean recoveries (R %), precision (R.S.D. %) and accuracy (Bias %) data of assay of bulk GBC within and between-day at two concentration levels by SW-AdCSV (n = 4).

Day	Intra-day (Repeatability)			inter-day (reproducibility)		
	Mean R %	R.S.D. %	Bias %	Mean R %	R.S.D. %	Bias %
$1x10^{-7}$ M						
1	101.3	1.28	1.3	101.00	0.95	1.00
3				101.73	1.45	1.73
5				101.04	1.69	1.04
(Friedman test: p = 0.64; p > 0.05 & Chi-Square = 1.7						
$8x10^{-7}$ M						
1	101.1	1.1	1.1	100.70	1.44	0.70
3				101.02	1.32	1.02
5				100.64	1.05	0.64
(Friedman test: p = 0.62; p > 0.05 & Chi-Square = 1.8						

3.4.4. Selectivity

The selectivity of the SW-AdCSV method was tested by analysis of $1x10^{-7}$ M GBC (which contains no excipients) and presence of excipients (Lactose monohydrate, maize starch, pregelatinized maize starch, talc, aerosol 200 and magnesium stearate) usually present in 1×10^{-7} M of drug formulation (Daonil® 5 mg), following accumulation of GBC onto the HMDE at -0.6 V for 50 s in both cases. No significant differences in the recoveries or the relative standard deviations were obtained in the absence (99.00 ± 1.79) and presence of excipients (98.4 ± 1.5) (Wilcoxon test: p = 0.285; p > 0.05, n = 3). Subsequently, the proposed SW-AdCSV procedure can be considered specific [35].

3.4.5. Ruggedness and inter-laboratory precision

Analysis of 5×10^{-7} M GBC by the proposed SW-AdCSV procedure was examined by studying the effect of minor variations of some important procedural conditions such as pH (9.5 to 10.5), accumulation potential (-0.55 to -0.65 V) and accumulation duration (45 to 55 s). The statistical comparison of the results was done with Friedman analysis. The obtained mean percentage recoveries (R %) and relative standard deviations (R.S.D. %) based on four replicate measurements in the described SW-AdCSV method were 99.75 ± 1.40 to 101.2 ± 1.37 (Friedman Test: P = *0.39*, P > 0.05 & Chi-Square = 1.86), 100.72 ± 1.34 to 101.6 ± 1.02 (Friedman Test: P = *0.47*, P > 0.05 & Chi-Square = 1.5) and 98.97 ± 1.15 to 100.92 ± 0.97 (Friedman Test: P = *0.19*, P > 0.05 & Chi-Square = 3), respectively. The mean percentage recoveries (R %) and relative standard deviations (R.S.D. %) were not significantly affected, consequently the optimized SW-AdCSV procedure was reliable for assay of GBC and could be considered robust [35]. The inter-laboratory precision of measurements was evaluated by applying the proposed SW-AdCSV procedure to assay of GBC using two different models; 273-PAR (Lab. 1) and 394-PAR (Lab. 2) potentiostats at different times [35]. The mean percentage recoveries and relative standard deviations (% R ± R.S.D.) achieved in Lab. (1) and Lab. (2) for the described method were 102.42 ± 1.43 and 101.7 ± 1.7 (Wilcoxon test: p = 0.72; p > 0.05, n = 4), respectively. The differences observed in the mean percentage recoveries (R %) and relative standard deviations (R.S.D. %) under the studied variable conditions were insignificant (p > 0.05), confirming the accuracy and reproducibility of the analysis.

3.4.6. Stability of solutions

The stability of two concentration levels ($5x10^{-8}$ and $5x10^{-7}$ M GBC) were chosen from the standard curve (n = 4 at each concentration) to test by the described SW-AdCSV method at the optimized procedural conditions after being stored for 1 month (long term stability), while the samples were left at the place of storage samples at ambient temperature, after being freeze and thaw (stored in a refrigerator at -2 ºC) for 1 day (short term stability) and for 1 month (long term stability). The frozen solutions were left to thaw at ambient temperature, and then analyzed. As illustrated data in Table 2, there is no statistically significant differences were found in the recoveries (R %), precision (R.S.D. %) and accuracy (Bias %), indicating that the described procedures were considered precise and reliable for GBC solution at varies interval times at different concentrations.

Table 2. Mean recoveries (R %), precision (R.S.D. %) and Accuracy (Bias %) data of stability of bulk GBC solutions at two concentration levels by SW-AdCSV (n = 4).

stability analysis	**Conc. $1x10^{-7}$ (M)**	**Conc. $8x10^{-7}$ (M)**
At room temperature	**Long term (one month)**	
Recovery (R %)	100.9	102.20
Precision (R.S.D. %)	0.65	0.37
Accuracy (Bias %)	0.90	2.20
Freeze and thaw	**Short term (one day)**	
Recovery (R %)	101.32	101.40
Precision (R.S.D. %)	1.73	1.18
Accuracy (Bias %)	1.32	1.40
Freeze and thaw	**Long term (one month)**	
Recovery (R %)	100.12	101.21
Precision (R.S.D. %)	1.22	1.87
Accuracy (Bias %)	0.12	1.21
Friedman test:	$p = 0.47$; $p > 0.05$ & Chi-Square = 1.5	$p = 0.78$; $p > 0.05$ & Chi-Square = 0.5

4. Applications

4.1. Assessment of GBC in pharmaceutical formulation

The proposed SW-AdCSV procedure was successfully applied for estimation of $5x10^{-7}$ M GBC in its pharmaceutical formulation (Daonil® 5 mg) onto the HMDE at -0.6 V for 50 s, with no requirement for pretreatments or any time-consuming extraction concerning the drug analysis. The mean percentage recoveries and relative standard deviations (% R ± R.S.D.) of GBC in its pharmaceutical formulation (Daonil® 5 mg), were achieved with the average of five replicate measurements using both calibration plot and standard addition procedures as presented in Table 3.

Table 3. Recovery (%R) and relative standard deviation (±R.S.D.) values of $5x10^{-7}$ M GBC in its pharmaceutical products (Daonil® 5 mg) by the proposed SW-AdCSV procedures and the reported TLC method [17]. n = 5, ($P > 0.05$).

Claimed value; 5 mg/tablet	**Proposed methods (SW-AdCSV)**	**The reported TLC method.**
The proposed calibration curve method. (%R ±R.S.D.)	98.86 ± 1.06 $P = 0.96$	99.8 ±1.75 $P = 0.98$
The proposed standard addition method. (%R ±R.S.D.)	99.38 ± 1.11 $P = 0.98$	

The proposed SW-AdCSV results were statistically matched with those obtained by a reported TLC method [17]. The statistical analysis of the results obtained were performed using Friedman Test, there was significant agreement between the proposed stripping voltammetric procedures and the reported TLC method [17] (Friedman Test: $P = 0.5$, $P > 0.05$ & Chi-Square = 1.2), confirming the reproducibility of the described methods.

4.2. Assay of GBC in spiked human serum

Protein-free GBC-spiked human serum samples (prepared as clarified in the experimental section, iv) were analyzed using the proposed SW-AdCSV procedure. Figure 5 illustrated the recorded voltammograms of various concentrations of GBC using the effective procedural conditions. The linear variation of i_p with C of GBC was represented within the range $5x10^{-8}$-$1x10^{-6}$ M (Fig. 5; inset) following the linear regression equation: i_p (µA) = 2.04 C (M) + 0.73 (r = 0.98 and n = 7), the standard deviation of the blank (S.D. = 0.0102). (LOD) of $1.5x10^{-8}$ M (7.41 ng mL^{-1}) and (LOQ) of $5x10^{-8}$ M (24.7 ng mL^{-1}) of protein-free GBC-spiked human serum were realized [35]. Based on the average of four replicate measurements for the analysis of $2x10^{-7}$, $8x10^{-7}$ and $1x10^{-6}$ M protein-free GBC-spiked human serum, the mean percentage recoveries were identified without pretreatment or extraction prior to the analysis and were found to equal 99.82 ± 1.54, 98.95 ± 1.75 and 98.0 ± 1.6, respectively, which were insignificantly different (Friedman test: $p = 0.14$; $p > 0.05$ & Chi-Square = 4). The obtained results indicated the sensitivity and accuracy of the proposed SW-AdCSV procedure for quantification of GBC in protein-free spiked human serum.

4.3. Pharmacokinetic measurement

The described SW-AdCSV method was applied for the selective determination of plasma GBC concentration without interferences. This distinctive property was used for studying the pharmacokinetics of GBC in plasma of two healthy male volunteers (a and b) following the administration of therapeutic single oral dose of (Daonil® 5 mg). The obtained mean plasma (± SD) GBC concentration-time curve profiles of the two subjects (a and b) performed by SW-AdCSV method is illustrated in Figure 6.

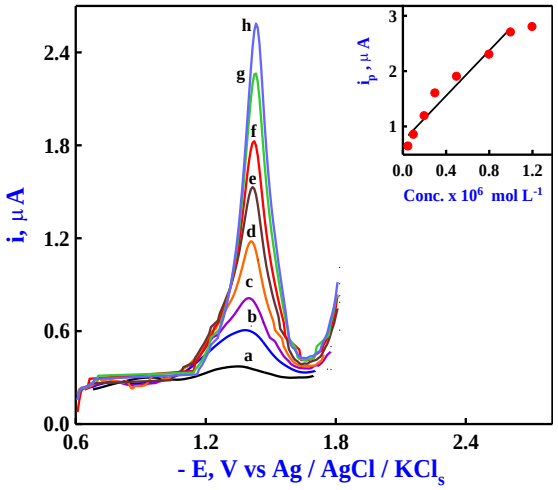


Fig. 5. SW-AdCSVs in B.R. buffer of pH 9.7 for various concentrations of GBC spiked in human serum; (a) blank solution, (b) $5x10^{-8}$, (c) $1x10^{-7}$, (d) $2x10^{-7}$, (e) $3x10^{-7}$, (f) $5x10^{-7}$, (g) $8x10^{-7}$ and (h) $1x10^{-6}$ M, at $E_{acc.}$ = -0.6 V for $t_{acc.}$ = 50 s; f = 100 Hz, ΔE_s = 8 mV and a = 80 mV. Inset: calibration curve of GBC spiked in human serum.

The pharmacokinetic parameters (mean ± SD) were estimated from the plasma concentration of GBC (Table 4). The following mean plasma pharmacokinetic parameters considered for the period of 0-24 h were: area under the plasma concentration-time curves from zero to the last measurable sample time (AUC_{0-24}) and to infinity ($AUC_{0-\infty}$);

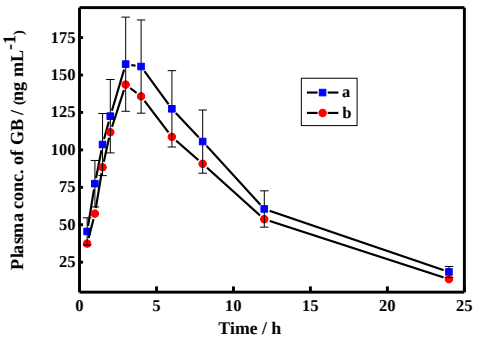


Fig. 6. Mean plasma concentration-time curve profiles obtained for two healthy male volunteers: (a and b) following an oral administration of a single oral dose Daonil® 5 mg, applying the SW-AdCSV method.

observed maximum peak plasma concentration of GBC (C_{max}); sampling time of the maximum plasma concentration (t_{max}); elimination rate constant (K_e) and terminal elimination half-life time ($t_{1/2}$). These pharmacokinetic parameters obtained for the two volunteers (a and b) were in good compatible with

those presented in references [2, 5, 21]. The differences observed in the mean percentage recoveries (% R) and relative standard deviations (R.S.D. %) of the achieved plasma pharmacokinetic parameters for the two healthy male volunteers (a and b) under the studied variable conditions were insignificant ($p > 0.05$), confirming the accuracy and reproducibility of the presented SW-AdCSV procedure (Table 4). Based on the previous results, this method could be used to as a standard method for the evaluation of plasma concentration of GBC, avoiding time consuming, sample pretreatment, expensive equipments and reagents.

5. Conclusion

In this study, the redox behavior of GBC in B-R universal buffered solutions pH values ≥ 6 was investigated and discussed at HMDE using cyclic voltammetry. Based on a catalytic hydrogen process a sensitive SW-AdCSV method was developed for determination of GBC in bulk form and in commercially available diabetic drug (Daonil® 5 mg).

Table 4. Mean pharmacokinetic parameters estimated for two healthy male volunteers (a and b) following an oral administration of a single dose (Daonil® 5 mg). n = 3, (P > 0.05).

Parameter/unit	**R ± R.S.D. %**	**R ± R.S.D. %**
	Subject (a)	**Subject (b)**
C_{max} (ng mL^{-1})	157.23 ± 18.91 *P* = 1.0	143.58 ± 10.72 *P* = 1.0
t_{max} (h)	3 ± 0.2 *P* = 1.0	3 ± 0.15 *P* = 1.0
AUC_{0-24} (ng h mL^{-1})	1535.58 ± 34.97 *P* = 0.98	1307.11 ± 31.58 *P* = 1.0
$AUC_{0-\infty}$ (ng h mL^{-1})	1615.71 ± 40.48 *P* = 0.97	1351.14 ± 30.95 *P* = 1.0
K_e (h^{-1})	0.23 ± 0.02 *P* = 0.99	0.31 ± 0.05 *P* = 0.99
$t_{1/2}$ (h)	3.01 ± 0.48 *P* = 1	2.24 ± 0.44 *P* = 1

The validated SW-AdCSV produce was linear response up to 1x10^{-6} M. The (LOD) and (LOQ) were found to be 6x10^{-9} M (2.964 ng mL^{-1}) and 2x10^{-8} M (9.88 ng mL^{-1}) bulk GBC, respectively. Recovery and precision for different chosen concentrations from the standard curve were 99.00 ± 1.82 to 101.00 ± 1.76 ($p > 0.05$). Statistical analysis of the present analytical results was performed by the paired non-parametric Wilcoxon Signed Rank and Friedman Tests using (SPSS 16) program, ($p < 0.05$). Also, the presented SW-AdCSV method was applied for determination of protein-free GB-spiked human serum solutions without the necessity for samples derivatization or any time-consuming extraction steps prior to the analysis. Pharmacokinetic parameters of GBC were also estimated in mean human plasma ± SD using the validated described voltammetric procedure ($p > 0.05$). The SW-AdCSV procedure is simple, fast, precise, robust, reproducible, cheap and could be recommended for analysis of GBC in clinical laboratories.

6. Conflicts of interest

"There are no conflicts to declare"

7. Acknowledgement

The authors express their deep thanks to Ramadan Specialized Hospital's staff (Tanta City, Egypt) for

providing the great facilities for collection and treatments of plasma samples required for the pharmacokinetic studies.

8. References

[1] E. Ghoneim, M. El-Attar, E. Hammam, P. Khashaba, Stripping voltammetric quantification of the anti-diabetic drug glipizide in bulk form and pharmaceutical formulation, Journal of pharmaceutical and biomedical analysis 43(4) (2007) 1465-1469.

[2] A.C. Moffat, M.D.Osselton, B. Widdop, "Clarke's Analysis of Drugs and Poisons "in pharmaceuticals, body fluids and postmortem material", 4ed ed., Pharmaceutical Press, London, Chicogo 2011.

[3] G. Neugebauer, G. Betzien, V. Hrstka, B. Kaufmann, U. Abshagen, Absolute bioavailability and bioequivalence of glibenclamide (Semi-Euglucon N), International journal of clinical pharmacology, therapy, and toxicology 23(9) (1985) 453-460.

[4] V. Maier, J. Znaleziona, D. Jirovský, J. Skopalová, J. Petr, J. Ševčík, Determination of antihyperglycemic drugs in nanomolar concentration levels by micellar electrokinetic chromatography with non-ionic surfactant, Journal of Chromatography A 1216(20) (2009) 4492-4498.

[5] I. Niopas, A.C. Daftsios, A validated high-performance liquid chromatographic method for the determination of glibenclamide in human plasma and its application to pharmacokinetic studies, Journal of Pharmaceutical and Biomedical analysis 28(3-4) (2002) 653-657.

[6] J. Shaodong, W.J. Lee, J.W. Ee, J.H. Park, S.W. Kwon, J. Lee, Comparison of ultraviolet detection, evaporative light scattering detection and charged aerosol detection methods for liquid-chromatographic determination of anti-diabetic drugs, Journal of pharmaceutical and biomedical analysis 51(4) (2010) 973-978.

[7] S. AbuRuz, J. Millership, J. McElnay, The development and validation of liquid chromatography method for the simultaneous determination of metformin and glipizide, gliclazide, glibenclamide or glimperide in plasma, Journal of Chromatography B 817(2) (2005) 277-286.

[8] J. Khatri, S. Qassim, O. Abed, B. Abraham, A. Al-Lami, S. Masood, A novel extractionless hplc fluorescence method for the determination of glyburide in the human plasma: application to a bioequivalence study, J. Pharm. Pharm. Sci 4(2) (2001) 201-206.

[9] W.W. Mohamad, A.T. Fizi, R. Ismail, M. Mafauzy, Efficacy and safety of single versus multiple daily doses of glibenclamide in type 2 diabetes mellitus, Diabetes research and clinical practice 49(2-3) (2000) 93-99.

[10] F. Susanto, H. Reinauer, Glibenclamide in serum: comparison of high-performance liquid chromatography using fluorescence detector and liquid chromatography/mass spectrometry with atmospheric-pressure chemical-ionization (APCI LC/MS), Fresenius' journal of analytical chemistry 356(5) (1996) 352-357.

[11] J.V. Santurio, E.G. Porto, Determination of glibenclamide in human plasma by solid-phase extraction and high-performance liquid chromatography, Journal of Chromatography B: Biomedical Sciences and Applications 682(2) (1996) 364-370.

[12] N. Haq, F.K. Alanazi, I.A. Alsarra, F. Shakeel, Rapid Analysis of glibenclamide using an environmentally benign stability-indicating RP-HPLC method, Iranian journal of pharmaceutical research: IJPR 13(3) (2014) 863.

[13] S. Edla, B.S. Sundhar, New analytical method development and validation for the simultaneous estimation of Metformin and Glibenclamide in bulk and tablet dosage form using RP-HPLC, Rasayan journal of chemistry 7(1) (2014) 55-63.

[14] F. Albu, C. Georgiţă, V. David, A. Medvedovici, Determination of glibenclamide in human plasma by liquid chromatography and atmospheric pressure chemical ionization/MS-MS detection, Journal of Chromatography B 846(1-2) (2007) 222-229.

[15] L. Ramos, R. Bakhtiar, F. Tse, Application of liquid chromatography atmospheric pressure chemical ionization tandem mass spectrometry in the quantitative analysis of glyburide (glibenclamide) in human plasma, Rapid communications in mass spectrometry 13(24) (1999) 2439-2443.

[16] B.-M. Chen, Y.-Z. Liang, F.-Q. Guo, L.-F. Huang, F.-L. Deng, X. Chen, Y.-L. Wang,

Rapid, simple, specific liquid chromatographic-electrospray mass spectrometry method for the determination of glibenclamide in human plasma, Analytica chimica acta 514(2) (2004) 185-191.

[17] N.M. El Kousy, Stability-indicating densitometric determination of some antidiabetic drugs in dosage forms, using TLC, Microchimica Acta 128(1-2) (1998) 65-68.

[18] D. Castoldi, Tofanetti, O., Gas chromatographic determination of glibenclamide in plasma, Clinic. Chim. Acta 93 (1979) 195-198.

[19] W. Heptner, M. Badian, S. Baudner, C. Hellstern, R. Irmisch, W. Rupp, K. Weimer, H. Wissmann, A Radioimmunoassay for Determination of Glibenclamide and Other Sulfonylureas, Pharmaceutical research 1(5) (1984) 215-220.

[20] A.-E. Radi, S. Eissa, Voltammetric and spectrophotometric study on the complexation of glibenclamide with β-cyclodextrin, Journal of Inclusion Phenomena and Macrocyclic Chemistry 68(3-4) (2010) 417-421.

[21] A. Radi, Voltammetric study of glibenclamide at carbon paste and Sephadex-modified carbon paste electrodes, Analytical and bioanalytical chemistry 378(3) (2004) 822-826.

[22] P.M. Jahani, S. Tajik, H. Beitollahi, S. Mohammadi, M.R. Aflatoonian, Voltammetric detection of gliclazide and glibenclamide with graphite screen-printed electrode modified with nanopetal-structured MoWS 2, Research on Chemical Intermediates 46(1) (2020) 837-852.

[23] E. Pourtaheri, M.A. Taher, G.A. Ali, S. Agarwal, V.K. Gupta, Electrochemical detection of gliclazide and glibenclamide on ZnIn2S4 nanoparticles-modified carbon ionic liquid electrode, Journal of Molecular Liquids 289 (2019) 111141.

[24] M.A. El-Attar, Stripping voltammetric determination of anticoccidial drug Diclazuril in the bulk form, poultry feed additive and eggs, Chemia Analityczna 54(5) (2009) 857-870.

[25] A.M. Beltagi, M.A. El-Attar, E.M. Ghoneim, Adsorptive stripping voltammetric determination of the anti-inflammatory drug tolmetin in bulk form, pharmaceutical formulation and human serum, Central European Journal of Chemistry 5(3) (2007) 835-845.

[26] E. Hammam, M. El-Attar, A. Beltagi, Voltammetric studies on the antibiotic drug cefoperazone: Quantification and pharmacokinetic studies, Journal of pharmaceutical and biomedical analysis 42(4) (2006) 523-527.

[27] H.T.S. Britton, Hydrogen Ions, Chapman & Hall: London, 1952, p. 113.

[28] S.B.S. Green, N. J., Using SPSS for Window and Macintosh: Analyzing understanding data, Upper Saddle River, NJ: Pearson Prentice Hall2008.

[29] M.A. El-Attar, I.M. Ismail, M.M. Ghoneim, Synthesis, electrochemical, spectrophotometric and potentiometric studies of two azo-compounds derived from 4-amino-2-methylquinoline in ethanolic-aqueous buffered solutions, Journal of the Brazilian Chemical Society 23(8) (2012) 1523-1535.

[30] P. Zuman, Electrolysis with a dropping mercury electrode: J. Heyrovsky's contribution to electrochemistry, Critical reviews in analytical chemistry 31(4) (2001) 281-289.

[31] P. Zuman, Polarography in solution of some problems in organic chemistry: recent applications, Microchemical journal 72(3) (2002) 241-250.

[32] I. Bulut, Study of binary complexes of nickel (II), copper (II), and vanadium (V) with acetazolamide in aqueous medium by voltammetry, Turkish Journal of Chemistry 33(4) (2009) 507-520.

[33] R.M.A. Issa, F. A.; Abdel-Gawad F. M.; El-Ries, M. A., Spectrophotometric Studies of Multidentate Dianils, Communications, Series B: Chemistry and Chemical Engineering (Fac. Sci. Ankara Univ.) 31 (1985) 289-294.

[34] E. Laviron, L. Roullier, C. Degrand, A multilayer model for the study of space distributed redox modified electrodes: Part II. Theory and application of linear potential sweep voltammetry for a simple reaction, Journal of Electroanalytical Chemistry and Interfacial Electrochemistry 112(1) (1980) 11-23.

[35] M. Thompson, S.L. Ellison, R. Wood, Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report), Pure and Applied Chemistry 74(5) (2002) 835-855.