—Articles—

Electrochemical Determination of the Antidiabetic Drug Repaglinide

Mohamed Abdel Nabi EL-RIES,*,[a] Gehad Genidy MOHAMED,[b] and Ali Kamal ATTIA[a]

[a]*National Organization for Drug Control and Research, P.O. Box 29, Cairo, Egypt, and* [b]*Chemistry Department, Faculty of Science, Cairo University, P.O. Box 12613, Giza, Egypt*

(Received June 29, 2007; Accepted September 25, 2007)

The electrochemical oxidation of repaglinide has been carried out in Britton-Robinson buffer at carbon paste and glassy carbon electrodes. Repaglinide exhibits a well-defined irreversible oxidation peak over the entire pH range (2–11). Differential pulse voltammetry was used to determine repaglinide in pure form. The peak current varied linearly in the following ranges: 8.0×10^{-7}–3.2×10^{-6} M and 4.0×10^{-7}–4.0×10^{-6} M in case of carbon paste electrode and glassy carbon electrode, respectively. In case of carbon paste electrode the limits of detection (LOD) and quantification (LOQ) were 1.348×10^{-7} M and 4.494×10^{-7} M, respectively. For glassy carbon electrode the LOD and LOQ were 1.062×10^{-7} M and 3.54×10^{-7} M, respectively. The percentage recoveries were found in the following ranges: 99.09–100.07% and 99.0–100.50% for carbon paste electrode and glassy carbon electrode, respectively. The relative standard deviations were found in the following ranges: 0.636–1.395% and 0.431–1.104% in case of carbon paste electrode and glassy carbon electrode, respectively. Differential pulse voltammetry was successfully applied for the determination of repaglinide in tablets and human serum.

Key words——repaglinide; oxidation; differential pulse voltammetry; pharmaceutical form; serum

INTRODUCTION

Repaglinide, (S)-2-ethoxy-4-[2-[[3-methyl-1-[2-(1-piperidinyl) phenyl] butyl] amino]-2-oxoethyl] benzoic acid, is a novel blood glucose lowering agent from the class of carbamoylmethyl benzoic acids. It stimulates release of insulin from the pancreatic β-cell by closure of K_{ATP} channels and is rapidly absorbed and eliminated from the body.[1] Repaglinide (Repag) is developed in attempts to overcome the adverse effects associated with existing antidiabetic compounds. These include hypoglycemia, secondary failure and cardiovascular side effects.[2] Although Repag exhibits some chemical resemblance to sulphonylurea-type antidiabetic drug glibenclamide, it differs from other sulphonylureas in both profile of action and excretion mechanism. Repag binds to different receptor sites from other sulphonylurea.[3] As a result it is three- to five- fold more potent than glibenclamide[4] and in contrast to glibenclamide it does not stimulate release of insulin in the absence of glucose.[3] Since orally and intravenously administered Repag is excreted most entirely *via* bile,[5] it is an attractive drug for diabetic patients with impaired kidney function. Studies of the therapeutic and toxic effects of drugs require sensitive methods for their determination at a trace level. All reported spectroscopic methods suffer from low sensitivity. On the other hand, HPLC methods while having the advantage of requiring minimal sample preparation are relatively slow and expensive because they require filtration, degassing, and expensive reagents and equipments. Few methods have been reported for the determination of Repag.[6–11]

Carbon-based electrodes are currently in widespread use in electroanalytical chemistry, because of their broad potential window, low cost, rich surface chemistry, low background current, and chemical inertness. Carbon paste electrode (CPE) has some special characteristics and benefits such as the ease of surface renewal, individual polarizability, and easy to apply modifications. The disadvantage of CPE is the tendency of the organic binder to dissolve in solutions containing an appreciable fraction of organic solvent. Glassy carbon electrode (GCE) is a class of non-graphitizing carbon that is widely used as an electrode material in electrochemistry. It is also known as vitreous carbon. Glassy carbon electrode is used very commonly because of its excellent mechanical and electrical properties, impermeability to gases, and extremely low porosity.[12]

The literature survey revealed that no attempts

*e-mail: mohamedelries@hotmail.com

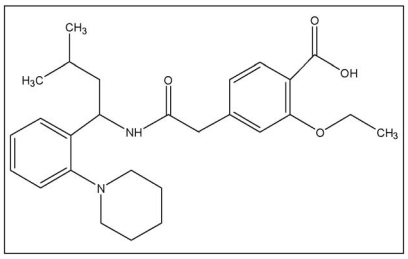

Fig. 1. Molecular Structure of Repaglinide

have been made to study the voltammetric behavior of Repag as an oxidation process and thus in continuation to our previous work,[13–16] the aim of this study is to establish and to optimize the experimental conditions for the determination of Repag in pure form, tablets, and serum by using cyclic voltammetry and differential pulse voltammetry (DPV) techniques.

EXPERIMENTAL

Apparatus Voltammetric measurements were carried out using a computer-driven AEW2 analytical electrochemical workstation with ECProg3 electrochemistry software (Sycopel, England) in combination with a C-2 stand with a three-electrode configuration: a glassy carbon disc electrode (BAS model MF-2012) or a carbon paste electrode (BAS model MF-2010) working electrode, a Ag/AgCl/3 M NaCl (BAS model MF-2063) reference electrode, and a platinum wire (BAS model MW-1032) counter electrode. Origin 7.0 software was used for the transformation of the initial signal. A cyberscan 500 (EUTECH Instruments, USA) digital pH-meter with a glass combination electrode served to carry out the pH measurements.

Reagents Repaglinide was supplied from Amoun Pharma, Egypt. Its pharmaceutical form (Novonorm tablets) was manufactured by Novo Nordisk Company, Denmark. Stock solution of Repag 1×10^{-3} M was prepared by dissolving an appropriate amount of Repag in methanol which was obtained from El-Nasr Pharmaceutical Company, Egypt. The stock solution was stored in a refrigerator. Britton-Robinson (BR) buffer was prepared by mixing the acid mixture containing phosphoric acid (0.04 M), acetic acid (0.04 M), and boric acid (0.04 M). Buffer solutions were adjusted by adding the necessary amount of 2 M NaOH solution in order to obtain the appropriate pH. Graphite powder and Nujol which is a mineral oil were supplied from Aldrich and Sigma, respectively. Serum sample, obtained from healthy volunteer, was collected and stored frozen until assay.

Preparation of the Working Electrodes The paste was prepared by mixing 0.5 g of graphite powder with 0.3 ml of Nujol in a mortar with a pestle. The carbon paste was packed into the hole of the electrode body and smoothed on a filter paper until it had a shiny appearance.

To improve the sensitivity and resolution of the voltammetric peaks, the glassy carbon electrode was polished manually with 0.5 μm alumina powder on a smooth polishing cloth prior to each electrochemical measurement. Then, it was thoroughly rinsed with methanol and double distilled water, and gently dried with a tissue paper.

Assignment of the Optimum Conditions for the Determination of Repaglinide To obtain the optimum pH, an appropriate amount of Repag working standard solution 1.0×10^{-3} M was placed in the electrolytic cell containing 5 ml of BR buffer and the cyclic voltammogram was recorded. The experiment was repeated by using buffer solutions of different pH values and the optimum pH was obtained.

To study the effect of scan rate (υ) on the peak current (Ip) of Repag, the working electrode was immersed in the optimum buffer solution containing an appropriate amount of Repag standard solution 1.0×10^{-3} M and the cyclic voltammograms were recorded at different scan rates over the scan range 10–250 mV/s. Plot log Ip *versus* log υ to know the nature of the process, diffusion controlled process or adsorption controlled process.

To study the effect of accumulation time (t_{acc}), the working electrode was immersed in the optimum buffer solution containing an appropriate amount of Repag standard solution 1.0×10^{-3} M for selected times with stirring at 1200 rpm at open circuit condition. After accumulation, the cyclic voltammograms were recorded then plot the peak current *versus* time to obtain the optimum accumulation time.

The optimum instrumental conditions for the determination of Repag by using DPV method were chosen from the study of the variation of the peak current with pulse amplitude, pulse width, and scan rate. During the study, each parameter was changed while the others were kept constant: pulse amplitude over the range of 25–100 mV, pulse width 30–90 ms, and scan rate 10–50 mV/s.

General Procedure for the Determination of Repaglinide in the Pure Form Voltammetric analyses were performed in 5 ml of BR buffer. The solution was continuously stirred at 1200 rpm when accumulation potential (usually open circuit condition) was applied for a certain time to the working electrode. At the end of accumulation period, the stirrer was stopped and a 5 s. rest period was allowed for the solution to become quiescent. Then the drug accumulated at the working electrode was removed from its surface by anodic stripping by using DPV method. Aliquots of the drug solution 1×10^{-3} M were introduced into the electrolytic cell and the procedures were repeated. The voltammograms were recorded. The peak current was evaluated as the difference between each voltammogram and the background electrolyte voltammogram. All measurements were carried out at the room temperature.

Determination of Repaglinide in Tablets Twenty tablets of Novonorm were weighed and the average mass per tablet was determined, then these tablets were powdered. A portion of the powder needed to obtain 1×10^{-3} M drug solution was accurately weighed and transferred into a 100 ml volumetric flask which contains 70 ml of methanol. The content of the flask was sonicated for about 15 min and then made up to the volume with methanol. The solution was filtered to separate the insoluble excipients. Aliquots of the drug solution were introduced into the electrolytic cell and the general procedure was carried out.

Determination of Repaglinide in Serum After gentle thawing, 0.1 ml of serum sample was transferred into a 10 ml volumetric flask and a suitable volume of the drug solution was added. The volume was completed to the mark with methanol. After separation of proteins, the supernatant was taken carefully, an appropriate volume of supernatant liquor was transferred to 10 ml volumetric flask, diluted to the volume, and the obtained solution was used for voltammetric determination by using DPV method under the previous conditions.

RESULTS AND DISCUSSION

To elucidate the electrode reaction of Repag, the cyclic voltammograms at carbon paste and glassy carbon electrodes were recorded at different pH values and at different scan rates. As an example, Fig. 2 shows the cyclic voltammograms of 4.0×10^{-5} M Repag solution in BR buffer at pH 6 in case of CPE and at pH 7 in case of GCE at a scan rate of 100 mV/s. Each voltammogram exhibits one well-defined anodic peak, with no peak on the reverse scan, suggesting the irreversible nature of the electrode reaction.

Effect of pH Repaglinide exhibits two pK_a values of 4.19 and 5.78, and being a weakly acidic compound, the drug is ionized at higher pH values, owing to its higher aqueous solubility at higher pH values.[17] The influence of pH on Repag at carbon paste and glassy carbon electrodes was studied. Figure 3 shows the plot of peak current (Ip) *vs.* pH. It is obvious from the figure that the peak current

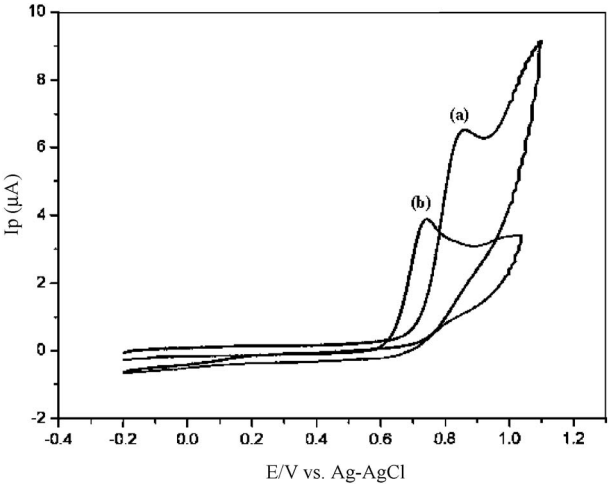

Fig. 2. Cyclic Voltammograms of 4.0×10^{-5} M Repaglinide Solution in BR Buffer of pH 6 for CPE (a) and pH 7 for GCE (b). Scan Rate 100 mV/s.

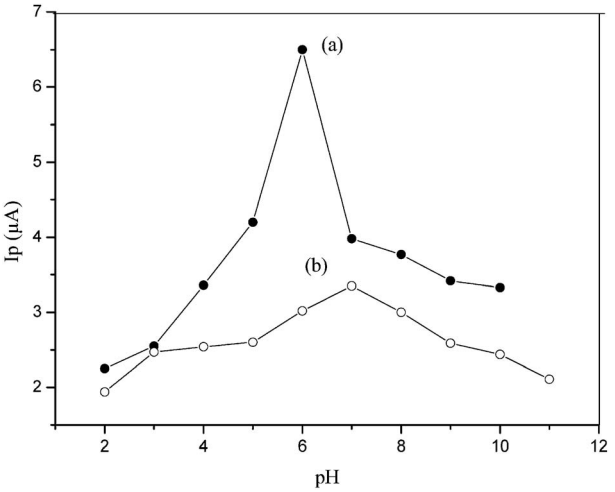

Fig. 3. Effect of pH on Peak Current of 4.0×10^{-5} M Repaglinide Solution in BR Buffer at CPE (a) and GCE (b). Scan Rate 100 mV/s.

reaches its maximum value at pH 6 in case of CPE and at pH 7 in case of GCE, *i.e.*; faint acidic or neutral medium is the suitable medium for the determination of Repag by using DPV technique.

Effect of Scan Rate The effect of scan rate (υ) on the peak current (Ip) of Repag was shown in Fig. 4. Linear relationships were observed between log Ip and log υ over the scan range 10–250 mV/s and correspond to the following equations: log Ip$=-0.81+0.63$ log υ and log Ip$=-0.63+0.43$ log υ in case of CPE and GCE, respectively. The slope of 0.63 indicates to diffusion controlled process with some adsorption character and the slope of 0.43 is close to the theoretically expected value of 0.50 for a diffusion controlled process.[18]

Effect of Accumulation Time The effect of accumulation time on the anodic peak current (Ip) of Repag at pH 6 was studied at CPE at open circuit condition and the results were shown in Fig. 5. It was concluded that increasing peak currents were obtained up to accumulation time 90 s. Hence 90 second was chosen as the optimum accumulation time.

Effect of Instrumental Parameters It was found that the peak current was increased with the increasing pulse amplitude and scan rate, while it decreased with the increasing pulse width. To obtain relatively high and narrow peaks the values of 50 mV, 30 ms and 20 mV/s were finally chosen for pulse amplitude, pulse width and scan rate, respectively.

Determination of Repaglinide in the Pure Form On the basis of the electrochemical oxidation of Repag at CPE and GCE, analytical method was developed involving DPV method for the determination of the drug under investigation. Linear relations between the peak current (Ip) and Repag concentration (C) were found in the following ranges: 8.0×10^{-7}–3.2×10^{-6} M and 4.0×10^{-7}–4.0×10^{-6} M in case of CPE and GCE, respectively. The calibration plots were described by the following equations:

$$\text{Ip } (\mu\text{A}) = 0.222\ \text{C } (\mu\text{M}) + 1.013 \quad r = 0.9999 \quad \text{for CPE} \tag{1}$$

$$\text{Ip } (\mu\text{A}) = 0.117\ \text{C } (\mu\text{M}) + 1.557 \quad r = 0.9999 \quad \text{for GCE} \tag{2}$$

Three replicate calibration curves were obtained over the concentration ranges 8.0×10^{-7}–3.2×10^{-6} M in case of CPE and 4.0×10^{-7}–4.0×10^{-6} M in case of GCE. The LOD and LOQ were calculated by using the following equations: LOD$=3$ SD/m and LOQ$=10$ SD/m, where ''SD'' is the standard deviation of the intercept of the calibration curve and ''m'' is the slope of the calibration curve.[19] The LOD and LOQ were 1.348×10^{-7} M and 4.494×10^{-7} M, respectively in case of CPE. For GCE, the LOD and LOQ were 1.062×10^{-7} M and 3.54×10^{-7} M, respectively.

Accuracy and precision of the proposed method were determined by replicate analyses of standard solutions of the used drug, the results were given as shown in Table 1. The recovery (%R) was in the range of 99.09–100.70% and the relative standard deviation (RSD) was in the range of 0.63–1.395% in case of CPE.

For GCE, the recovery was in the range of 99.0–100.5% and the relative standard deviation was in the

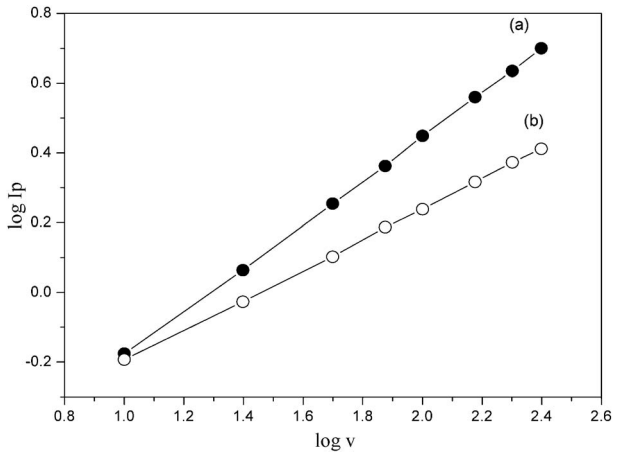

Fig. 4. Anodic Peak Current Response of 4.0×10^{-5} M Repaglinide Solution as a Function of Scan Rate (υ) in BR Buffer of pH 6 for CPE (a) and pH 7 for GCE (b).

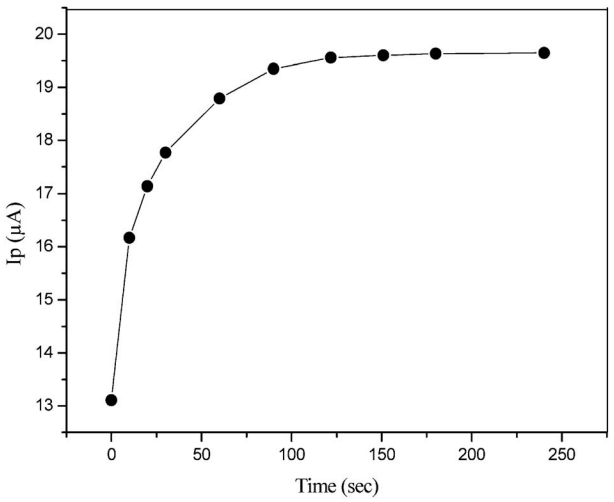

Fig. 5. Effect of Accumulation Time on the Peak Current of 4.0×10^{-5} M Repaglinide Solution in BR Buffer of pH 6 at CPE.

range of 0.431–1.104%. The values of the recovery and the relative standard deviations indicate to adequate accuracy and precision of the proposed method.

The results obtained by using the proposed method were compared with those obtained by using the official HPLC method.[10] The chromatographic method resulted in an average value of 99.5% with a relative standard deviation of 2.0%. These results show no significant difference between the performance of the two methods regarding the accuracy and precision, respectively. Moreover, the proposed method is more simple, rapid and inexpensive.

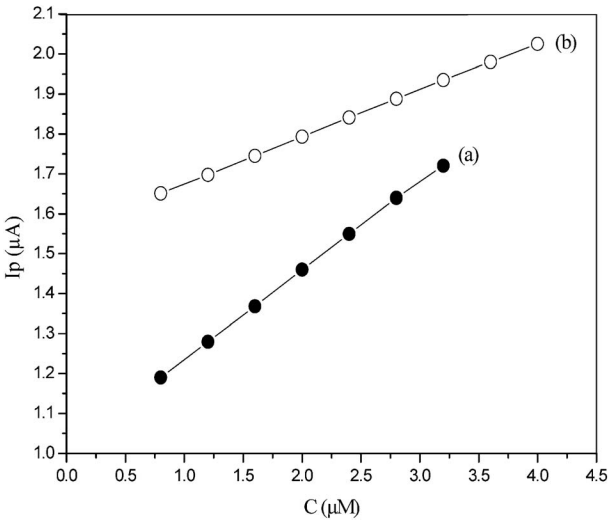

Fig. 6. Calibration Curve of Repaglinide at CPE (a), and GCE (b) by using DPV Method, Pulse Amplitude=50 mV and Scan Rate=20 mV/s.

Determination of Repaglinide in Tablets The proposed method was successfully applied to determine Repag in dosage form (Novonorm tablets) without interference from some common excipients used in pharmaceutical preparations such as starch, magnesium stearate and microcrystalline cellulose. Replicate analyses of standard solutions have been carried out to obtain the accuracy and precision of the proposed method, the results were given as shown in Table 2. The linearity range was 8.0×10^{-7}–3.2×10^{-6} M with mean recovery of 99.86% and mean relative standard deviation of 0.743% in case of carbon paste electrode. In case of glassy carbon electrode the linearity range was 4.0×10^{-7}–4.0×10^{-6} M with mean recovery of 100.13% and mean relative standard deviation of 0.559%.

Repag was determined by using the chromatographic method with UV detection[7] in the following range 2.2×10^{-7}–1.1×10^{-6} M. By comparing the results obtained by using the proposed method with those obtained by using the HPLC method we found that our method shows a wider range than this method.

The proposed voltammetric method was successfully used for the determination of Repag in Novonorm tablets. The results were compared with those ob-

Table 1. Analytical Parameters of the Calibration Plots for the Determination of Repag

Parameter	Carbon paste electrode (CPE)	Glassy carbon electrode (GCE)
Linearity range	8.0×10^{-7}–3.2×10^{-6} M	4.0×10^{-7}–4.0×10^{-6} M
Slope	0.222	0.117
Intercept	1.013	1.557
Correlation coefficient (r)	0.9999	0.9999
Limit of detection (LOD)	1.348×10^{-7} M	1.062×10^{-7} M
Limit of quantification (LOQ)	4.494×10^{-7} M	3.54×10^{-7} M
Relative standard deviation (RSD)	0.636–1.395%	0.431–1.104%
Recovery (R)	99.09–100.7%	99.0–100.5%

Table 2. Determination of Repag in Tablets Compared with Reference Chromatographic Method[7]

Parameter	Linearity range	Relative standard deviation (RSD)	Recovery (R)
Carbon paste electrode (CPE)	8.0×10^{-7}–3.20×10^{-6} M	0.431–0.981%	99.06–100.83%
Glassy carbon electrode (GCE)	4.0×10^{-7}–4.0×10^{-6} M	0.352–0.810%	99.50–101.67%
Reference HPLC method[7]	2.20×10^{-7}–1.10×10^{-6} M	0.480–1.010%	98.40–99.72%

tained with the approved reference potentiometric titration method.[11] The student *t*-test and variance ratio *F*-test excluded any significant differences between both methods with respect to accuracy and precision. The results were shown in Table 3.

Determination of Repaglinide in Spiked Human Serum The applicability of the proposed DPV method for the determination of Repag in spiked human serum was investigated. Figures (7 and 8) illustrate the differential pulse voltammograms for different concentrations of Repag in serum samples. The linearity range was 1.2×10^{-6}–3.2×10^{-6} M with mean recovery of 99.82% and mean relative standard deviation of 0.650% in case of CPE. In case of GCE the linearity range was 8.0×10^{-7}–4×10^{-6} M with mean recovery of 100.11% and mean relative standard deviation of 0.766%. The results were given as shown in Table 4.

CONCLUSION

The proposed DPV method could be used success-

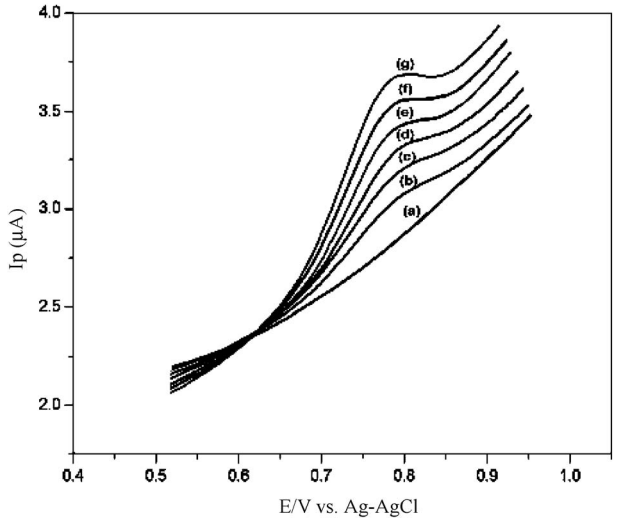

Fig. 7. Differential Pulse Voltammograms for Different Concentrations of Repaglinide in Serum Samples at CPE, Pulse Amplitude=50 mV, t_{acc}=90 s and Scan Rate=20 mV/s. Blank (a), 1.2 (b), 1.6 (c), 2 (d), 2.4 (e), 2.8 (f) and 3.2 μM (g).

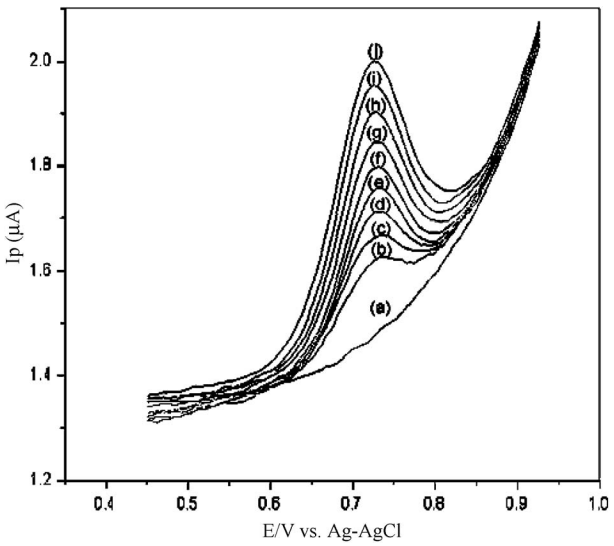

Fig. 8. Differential Pulse Voltammograms for Different Concentrations of Repaglinide in Serum Samples at GCE, Pulse Amplitude=50 mV and Scan Rate=20 mV/s. Blank (a), 0.8 (b), 1.2 (c), 1.6 (d), 2 (e), 2.4 (f), 2.8 (g), 3.2 (h), 3.6 (i) and 4 μM (j).

Table 3. Determination of Repag in Tablets Compared with the Potentiometric Titration Method[11]

Parameter	Carbon paste electrode (CPE)	Glassy carbon electrode (GCE)	Official titrimetric method[11]
Recovery (%) ± SD[a]	100.20±0.80	100.50±0.65	99.65±0.92
F-test	1.45	1.70	6.39[b]
t-test	1.56	1.81	2.31[b]

a) Averaged from five separate determinations.
b) Tabulated *F* and *t* values at p=0.05.[19]

Table 4. Determination of Repag in Spiked Human Serum

Parameter	Carbon paste electrode (CPE)	Glassy carbon electrode (GCE)
Linearity range	1.20×10^{-6}–3.20×10^{-6} M	8.0×10^{-7}–4.0×10^{-6} M
Limit of detection (LOD)	3.98×10^{-7} M	3.30×10^{-7} M
Limit of quantification (LOQ)	1.33×10^{-6} M	1.10×10^{-6} M
Relative standard deviation (RSD)	0.359–0.834%	0.559–1.113%
Recovery (R)	99.25–100.50%	99.25–100.94%

fully to determine repaglinide in pure form, pharmaceutical forms and serum. It compares reasonably with the reported chromatographic and potentiometric methods. It is a good alternative for the analytical determination of repaglinide because it is simple, low cost, sensitive, accurate and precise. The proposed procedure showed clear advantages such as short period of real time of drug analysis and no pretreatment or time consuming extraction steps were required prior to the analysis. Although CPE and GCE give acceptable results in the analysis of repaglinide, we prefer GCE for biological analysis due to its high sensitivity.

REFERENCES

1) Oliver S., Windfeld K., Hatorp V., *Diabetes*, **46**, 331 (1997).
2) Groop L. C., "Textbook of Diabetes," Vol. 1, Chap. 38, Blackwell, Oxford, 1996, 1–17.
3) Fuhlendorff J., Rorseman P., Kofod H., Brand C. L., Rolin B., Mackay P., Shymko R., Carr R. D., *Diabetes*, **47**, 345–351 (1998).
4) Balfour J. A., Faulds D., *Drugs Aging*, **13**, 173–180 (1998).
5) Heiningen P. N., Hatorp V., Lier J. J., *Diabetes*, **47**, 355 (1998).
6) Gumieniczek A., Hopkala H., Berecka A., Kowalczuk D., *J. Planar. Chromatogr. -Mod. TLC.*, **16**, 271–275 (2003).
7) Ganhimathi M., Ravi T. K., Renu S. K., *Anal. Sci.*, **19**, 1675–1677 (2003).
8) Gumieniczek A., Hopkala H., Berecka A., *J. Liq. Chromatogr. Related Technol.*, **27**, 2057–2070 (2004).
9) Ho E. N., Yiu K. C., Wan T. S., Stewart B. D., Watkins K. L., *J. Chromatogr. B*, **811**, 65–73 (2004).
10) "The United States Pharmacopoeia," (28), 2005, p. 1710.
11) "The British Pharmacopoeia," Her Majesty's Stationary Office, London, 2005, p. 1719.
12) Uslu, B., Ozkan, S. A., *Anal. Lett.*, **40**, 817–853 (2007).
13) El-Ries M. A., Wassel A. A., Abdel Ghani N. T., El-Shall M. A., *Anal. Sci.*, **21**, 1–6 (2005).
14) Radi A., El-Ries M. A., Kandil S., *Anal. Bioanal. Chem.*, **381**, 451–455 (2005).
15) Ghoneim M. M., El-Ries M. A., Hammam E., Beltagi A. M., *Talanta*, **64**, 703–710 (2004).
16) Ghoneim M. M., El-Ries M. A., Hassanein A. M., Abd-Elaziz A. M., *J. Pharm. Biomed. Anal.*, **41**, 1268–1273 (2006).
17) Purvis T., Mattucci M. E., Crisp M. T., Johnston K. P., Williams R. O., *AAPS Pharm. Sci. Tech.*, **8**(3), 1–9 (2007).
18) Gosser, D. K., "Cyclic Voltammetry Simulation and Analysis of Reaction Mechanism," VCH, New York, 1994, p. 43.
19) Miller, J. C., Miller, J. N., "Statistics for Analytical Chemistry," Ellis Horwood Series, Prentice Hall, New York, 1993, p. 119.