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Simultaneous Determination of Benserazide and Levodopa in Pharmaceutical Tablet, Human Serum and Urine Sample by Differential Pulse Voltammetry Using Modified Glassy Carbon Electrode

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A simple and highly sensitive method was investigated for the simultaneous determination of benserazide (BZ) and levodopa (LD) using Poly(4-(2-pyridylazo)-resorcinol) (PAR) modified glassy carbon electrodes. Both benserazide and levodopa were accumulated at the surface of the modified glassy carbon electrode (under open circuit condition for 30 s). In differential pulse voltammetry technique both BZ and LD were given sensitive oxidation peaks at 180 mV and 270 mV, respectively. Under the optimized experimental conditions (such as supporting electrolyte pH, accumulation time and scanning rate) BZ and LD were shown linear response over the range of 0.01–0.20 mM (r^2 = 0.991) and 0.025–0.4 mM (r^2 = 0.993), respectively. The lower detection limits were found to be 0.002 mM for BZ and 0.006 mM for LD. The commonly encountered excipients used in the pharmaceutical formulation showed no interference with the selective determination of BZ and LD. The investigated method showed good stability, reproducibility (1.3% (BZ) and 2.3% (LD)), repeatability (1.9%) and high recovery in pharmaceutical preparation (1.7% (BZ) and 2.7% (LD)), human serum (1.7% (BZ) and 1.9% (LD)) and urine samples (1.7% (BZ) and 1.8% (LD)).

Keywords: Voltammetry; Poly(4-(2-pyridylazo)-resorcinol); Glassy carbon electrode; Benserazide; Levodopa

1. INTRODUCTION

Levodopa, (S)-2-amino-3-(3,4-dihydroxyphenyl) propanoic acid, a metabolic precursor of dopamine used as a drug to increase dopamine levels for the treatment of Parkinson's disease [1]. Levodopa is converted in the brain through L-aromatic amino acid decarboxylase to dopamine

followed by generation of cytotoxic free radicals and other oxidizing species. Levodopa is also converted to dopamine outside the central nervous system (CNS) resulting limited drug supply to brain and peripheral side effects [2, 3]. Benserazide, 2-amino-3-hydroxy-N'-[(2,3,4-trihydroxyphenyl) methyl] propane hydrazide has been comprehensively used as an irreversible inhibitor of peripheral L-aromatic amino acid decarboxylase. This decarboxylase inhibitors drugs used in combination with LD to inhibit dopamine production outside the brain and permits direct delivery of dopamine to the brain. Therefore, BZ prevents the side effects, such as, nausea, palpitations and vomiting by blocks the conversion of LD to dopamine in the body [4, 5]. On addition, BZ at the recommended therapeutic dose cannot pass the blood-brain barrier to any significant degree and so does not affect the conversion of LD to dopamine in the brain. Thus, the combination of LD and BZ is therefore effective in the treatment of Parkinson's disease, while minimising the side effects caused by LD on the rest of the body. Also the synergistic effect of LD and BZ reduces the required dose of LD for the optimal and earlier therapeutic response [6].

A number of analytical methods including spectrophotometry [7-9], capillary electrophoresis (CE) [10-13], high performance liquid chromatography (HPLC) [14-16] and electrochemical determination [17-20] have been reported in literature for the determination of LD and BZ [21, 22] either as single analyte or in combination with other drugs in biological samples and pharmaceutical formulations. There are also few methods have been reported for the simultaneous determination of LD and BZ including spectrophotometry [23-26], capillary electrophoresis (CE) [5, 27, 28] and high performance liquid chromatography [29] in pharmaceutical preparations and biological fluids. But the polymer-modified electrodes (PMEs) prepared by electro polymerization have received extensive interest in the detection of analytes. The high selectivity and sensitivity due to the film homogeneity in electrochemical deposition, strong adherence to the electrode surface and chemical stability of the films [30, 31] are responsible for their extensive use. Fabrication of conducting polymer films is a good approach to prepare PMEs as adjusting electrochemical parameters can control film thickness, permeation and charge transport characteristics [32]. All these characteristics of PME make it suitable for the electrochemical determination of pharmaceutical preparations [33, 34]. Poly (4-(2-pyridylazo)-resorcinol) (PAR) modified electrode has also been reported for the electrochemical determination of various compounds [35-114].

Most of the reported spectrophotometric methods based on derivative technique have shown poor selectivity, sensitivity and required additional software's. Although the electrophoresis and liquid chromatographic methods have shown high sensitivities, they are expensive, involving the use of complex procedures with several sample manipulations and take longer analysis time. So the development of effective and efficient method for the simultaneous determination of BZ and LD in pharmaceutical preparation and biological fluids is an important task. To the best of our knowledge no electrochemical method reported for the simultaneous determination of BZ and LD in the literature. In the current study, PAR polymer film modified glassy carbon electrode (GCE) has been described for the simultaneous determination of LD and BZ in tablets, spiked human serum and urine samples. The proposed method showed many advantages such as low detection limit, fast response, easy to apply and economical for routine analysis. We believe that the proposed method would be a potential step forward in the simultaneous electrochemical determination of BZ and LD in biological fluids.

2. MATERIALS AND METHODS

2.1. Chemicals and reagent

All reagents were of analytical reagent grade and used without further purification. Levodopa was obtained from Roche (Germany). Benserazide hydrochloride and Poly (4-(2-pyridylazo)-resorcinol were purchased from Sigma–Aldrich (USA). The standard stock solution of 1 mM levodopa and 0.5 mM benserazide was prepared in 0.1 M phosphate buffer solution of pH 5.2. All solutions were protected from light and stored in a refrigerator at 4–5 $^{\circ}$C. Sodium dihydrogen phosphate ($NaH_2PO_4.H_2O$) and disodium hydrogenphosphate ($Na_2HPO_4.2H_2O$) (Merck, Germany) were used to prepare phosphate buffer solution. All solutions were prepared with double-distilled water. The chemical structures of Poly (4-(2-pyridylazo)-resorcinol), levodopa and benserazide hydrochloride have shown in Figure 1.

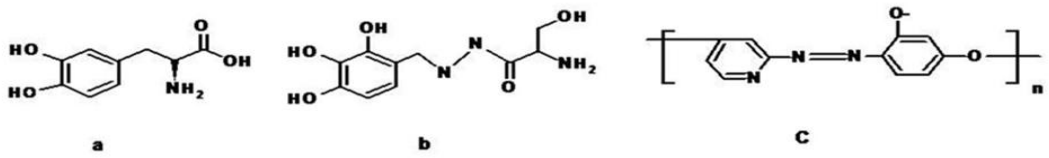

Figure 1. Chemical structure of (a) Levodopa, (b) Benserazide and (c) Poly (4-(2-pyridylazo) - resorcinol).

2.1.1. Pharmaceutical sample solution preparation

10 Madopar tablets (Roche South Korea) containing 25 mg of BZ and 100 mg of LD were finely powdered in a mortar with pestle. Calculated amounts of the tablets required for 0.5 mM of BZ and 1.0 mM LD were separately transferred into 25 mL volumetric flask and were dissolved in PBS of pH 5.2. The content of the flask were sonicated for 5 min to obtain complete dissolution of the analytes. The sample solution was filtered and suitable aliquot of the clear filtrate was collected and stored in the refrigerator for further use.

2.1.2. Serum sample preparation

Serum sample was prepared according to our previously reported method [115]. Drug-free human blood, obtained from healthy volunteers (after obtaining their written consent), was centrifuged at 4000 rpm for 30 min at room temperature. A 1.2 mL of acetonitrile was added to a 2 mL serum sample to remove serum protein, followed by fortification with BZ and LD dissolved in PBS to achieve the final concentrations of LD and BZ of 1 mM and 0.5 mM respectively. After vortexing for 45 seconds, the mixture was centrifuged for 10 min at 10000 rpm to remove serum protein residues. Supernatant was taken carefully and appropriate volumes of this supernatant were transferred into the electrochemical glass cell and diluted up to the volume with the PBS of pH 5.2. The concentration of

LD and BZ were varied in the range of 60 μM to 180 μM and 30 μM to 90 μM respectively in human serum samples.

2.1.3. Urine sample preparation

The urine sample was taken from healthy individuals immediately before the experiments. Spiked urine samples were obtained by treating 2.0 mL aliquots of urine with appropriate amount of standard solutions of BZ and LD to obtain the final concentration 0.5 mM and 1 mM, respectively. An aliquot of spiked urine was diluted with PBS of pH 5.2 in the range of calibration curve without any pre-treatment, and transferred into the voltammetric cell.

2.2. Apparatus

All electrochemical measurements were carried out using computer-controlled portable Potentiostat (RS-PDA1, Palm Instruments, Netherland). A conventional three-electrode system was used throughout the experiments. The voltammetric experiments were performed in an electrochemical cell that contains bare or PAR modified GCE as a working electrode, platinum wire as an auxiliary electrode and Ag/AgCl as a reference electrode. All potentials mentioned in this paper were referred to this reference electrode. Magnetic stirrer was used for the convective transport of the analyte. A centrifuge instrument (Model Eppendorf-5417 C) was used to separate the precipitated protein before analysis of the human serum. Ultra sonic bath (Bransonic 5210, USA) was used for dissolution of sample and cleaning the electrode surface. pH meter (Mettler Toledo MP220, UK) was used for pH measurement.

2.3. Preparation of PAR modified GCE

The bare GCE surface was initially polished with fine emery paper followed by 0.3 μm and 0.05 μm alumina powder to mirror finish and sonicated with Milli-Q water for 3 min. The modification of the GCE surface was performed according to the previously reported method [35, 36]. Before electro polymerization of the GCE surface, the polished electrode was electrochemically pretreated in 0.1 M PBS solution (pH 5.0) at a scan rate of 100 mV/s using 20 times cyclic potential sweeps in the range of –0.5 V to 1.8 V. For the deposition of PAR–film on GCE surface 20 times voltammetric sweeps in PAR solution (1 mM PAR) was performed utilizing the conditions of the pretreated procedure. Finally, the PAR film modified GCE was washed with distilled water and used for further experiments. All sample solutions were deoxygenated by purging N_2 gas before carried out each experiment.

2.4. General procedure

The PAR-film coated GCE was first activated in pH 5.2 phosphate buffers by cyclic voltammetric sweeps between 0.0 and 0.6 V until stable cyclic voltammograms were obtained. After obtaining the stable cyclic voltammogram the PAR-film coated GCE was transferred into new cell containing pH 5.2 phosphate buffers and certain concentration of analyte. After 20 seconds of open-circuit accumulation, the differential pulse voltammogram from 0.0 to 0.6 V at a scan rate 30 mV/s was recorded for the analyte. Upon using the DPV technique the peak position for BZ and LD appeared at 180 and 270 mV, respectively. After every measurement the PAR-film modified GCE was dipped into 0.1 M NaOH solution for few seconds to remove the adsorbed substances from the electrode surface. But the electrode was dipped in 1 M HCl for 30 seconds followed by dipping for few seconds in 0.1 M NaOH solution after the measurement of analytes was performed in serum sample. The reproducibility of the PAR–film modified GCE surface was achieved by applying five successive cyclic voltammetric sweeps in pure phosphate buffer solution of pH 5.2 before performing each individual experiment.

3. RESULTS AND DISCUSSION

3.1. Deposition of PAR film on the GCE surface

The GC electrode surface was electrochemically pretreated prior to polymerization process. The pretreatment process causes the formation of some functional groups on the electrode surface as well as it helps to increase the surface roughness of the GCE [116]. For the deposition of PAR solution on GCE surface cyclic voltammogram was scanned in PAR solution at a scan rate of 100 mV/s using 20 times cyclic potential sweeps in the range of –0.5 V to 1.8 V. The resulted cyclic voltammogram agrees closely with previous reports [35]. A new redox couple was appeared at about 0.5 V in cyclic voltammogram and the increments of peak currents with successive scanning confirms the formation of PAR film on the surface of GCE. Finally, a uniform adherent deep yellow film was formed at the electrode surface which was stable in common solvents and solutions.

3.2. Electrochemistry of BZ and LD on PAR modified GCE

Initially the cyclic voltammogram was investigated for the poly-PAR modified GCE in pure phosphate buffer solution of pH 5.2 (blank) as shown in Figure 2, curve a. It is apparent from curve a (Fig. 2), within the potential window from 0.0 to 0.60 V, no observable redox peaks were appeared in the voltammogram. However, a well-shaped and highly sensitive oxidation peak was appeared at 210 mV upon adding 50 μM of BZ solution to the blank (Fig. 2, curve b). On the reverse potential scan in the potential window 0.6 to 0.0 V, no corresponding reduction peak were appeared which suggests that the electrode reaction of BZ at the poly-PAR modified electrode is totally irreversible. Similarly the potential electro catalytic activity of PAR modified GC electrode on different concentration of LD was

carried out through CV technique in PBS supporting electrolyte at pH 5.2. The oxidation peak at 330 mV was observed for LD (100 μM) when used as single analyte (Fig. 2, curve c).

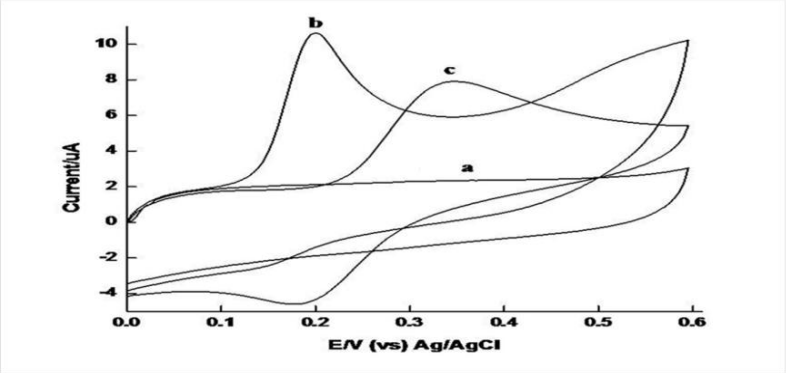

Figure 2. Cyclic voltammogram of (a) blank solution, (b) BZ (50 μM) and(c) LD (100 μM) in PBS at PAR modified GCE.

It was observed that the intensity of oxidation peak currents for both BZ and LD in the second cyclic voltammetric sweep were decreased as compared to the first voltammetric sweep confirms the adsorption of BZ and LD on the surface of PAR–film modified GCE. Therefore, the electrode surface was cleaned by washing with distilled water followed by dipping for few seconds in 0.1 M NaOH solution to remove the adsorbed substances to obtain the reproducible results.

In order to show the sensitivity effect of the PAR–film, the electrochemical properties of BZ in the presence of LD were compared to two different working electrodes (i.e., bare GCE, and poly-PAR-modified GCE). It was obvious that no oxidation peaks were observed upon using the bare GCE to the blank solution (curve a Fig. 3). Meanwhile, BZ and LD were also not given any oxidation peak at applied potential window after open-circuit accumulation of the analytes to the bare electrode surface (Fig. 3, curve b).

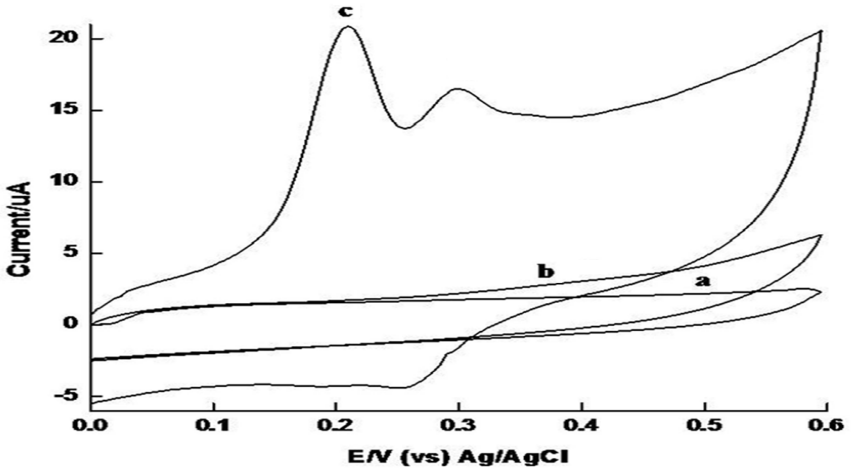

Figure 3. Cyclic voltammogram of (a) blank solution (b) mixture of BZ and LD using bare GCE and (c) mixture of BZ and LD using PAR modified GCE in PBS. Concentration of BZ = 50 μM and LD = 100 μM; E_{ads} = 0.0V; t_{ads} = 30; scan rate = 100 mV/s.

However, the response was considerably improved at the poly-PAR-modified GCE as shown in Figure 3(c). It was obvious from the obtained results that the peaks are sharper and current intensity is increased significantly compared to bare GCE. This remarkable current enhancement can undoubtedly be attributed to the unique structure and properties of PAR–film, including very large specific area, strong adsorptive ability, and subtle electronic properties. In short, a poly-PAR modified GCE was greatly improved the determination sensitivity of BZ and LD in mixed solution.

3.4. Simultaneous determination of BZ and LD on PAR modified GCE

Figure 4 exhibits the differential pulse voltammograms (DPVs) obtained for BZ and LD mixture on PAR–film modified GCE in PBS by synchronously changing the concentrations of BZ and LD. The current responses due to the oxidations of BZ (at 180 mV) and LD (at 270 mV) with the peak difference of 90 mV were observed to increase linearly with a correlation coefficient (r^2) of 0.991 and 0.993, respectively. Whereas no oxidation peaks for BZ and LD were observed on bare electrodes under the same condition. The peak positions for BZ and LD in mixture might be attributed to the different electrochemical activity of their function groups [117]. The mechanism behind the oxidation of BZ and LD at separated potential on the modified electrodes may be due to the electrostatic interaction of the electrode surface and the analyte. The PAR modified electrode contains negative charge in its backbone and acts as both electron source and reaction site with multivalent nature. Therefore, it is capable of reducing itself and to oxidize the LD and BZ with respect to their oxidation potential. In solution LD exists as negatively charged species whereas BZ exists as positively charged one. This difference in the nature of the charge between LD and BZ may be responsible for appearance of the oxidation peak at different potential [36, 118].

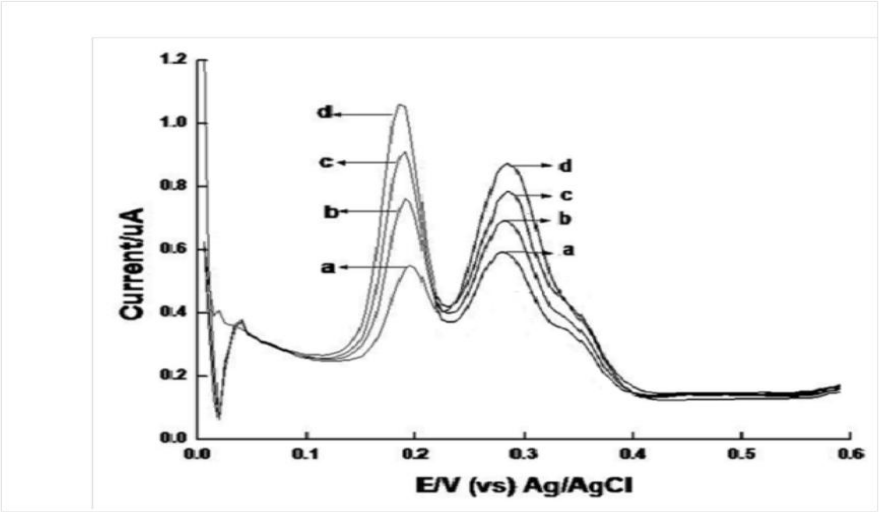

Figure 4. DPVs for the mixture containing BZ and LD with different concentrations at PAR modified GCE, BZ concentrations: (a) 0.01 mM, (b) 0.05 mM, (c) 0.1 mM, (d) 0.2 mM; LD concentrations: (a) 0.05 mM, (b) 0.1 mM, (c) 0.2 mM, (d) 0.4 mM; scan rate: 30mV/s, pH 5.2.

The separation between the two oxidation peaks potential is sufficiently enough (90 mV) for the simultaneous determination of BZ and LD using PAR modified GCE. Based on the above proposed mechanism the PAR–film coated electrode discretely separate BZ and LD and can detect BZ at its low concentration (e.g., 0.007 mM) in the presence of 5 times higher concentration of LD which is applicable to biological and pharmaceutical samples.

3.5. Parameter optimization for LD and BZ on PAR modified GCE

The optimization of experimental conditions such as supporting electrolyte, pH value, accumulation time of the analyte, and scan rate have been carried out to get the maximum response for the investigated method. Initially the accumulation time was optimized individually for LD and BZ. In case of individual BZ and LD solutions, the accumulation time showed no obvious effect on the oxidation peak current of BZ and LD. However, for the mixed solution of LD and BZ after 2 min of the accumulation time the response was poor therefore the accumulation time ranging from 0 to 50 seconds was optimized again for the mixed solution. As can be observed (Fig. 5) the current intensity increases up to 50 seconds for BZ and 30 seconds for LD, however, after 30 seconds decrease in the current response was observed for LD. Therefore, the adsorption equilibrium was estimated to reach t_{ads} in 50 seconds and 30 seconds for BZ and LD, respectively. A little difference in the adsorption kinetic for these species is reasonable due to the little difference in molecular structure of BZ and LD. The remarkable increase in peak current with an increase in the accumulation time (t_{ads}) is owing to the adsorption kinetics of BZ and LD on PAR modified GCE. In this study, an accumulation time of 30 s was chosen as optimum for the mixture of LD and BZ to obtain stable peak with higher sensitivity and shorter analysis time.

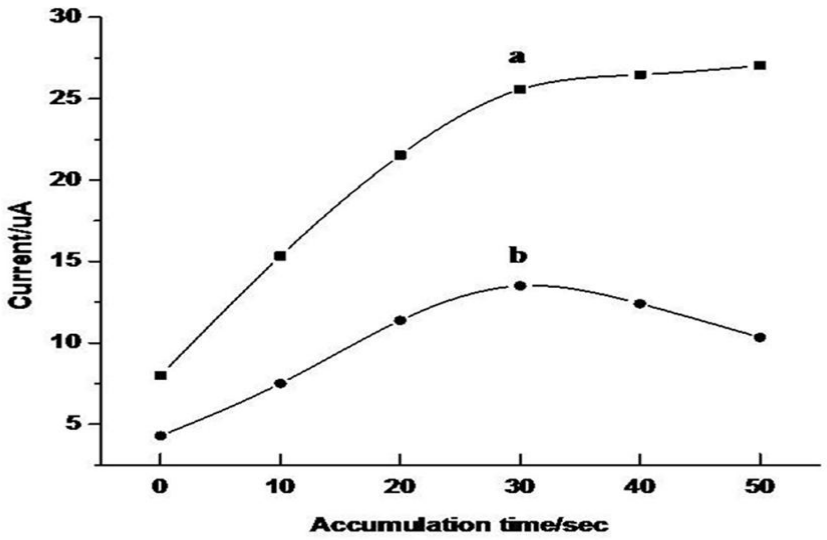

Figure 5. Optimization of open circuit accumulation time (seconds) on PAR modified GCE for (a) BZ (50 μM) and (b) LD (100 μM) using CV technique in 0.1 M PBS (pH 5.2) and 100 mV/s scan rate.

The influence of electrode potential (E_{ads}) plays an important role for optimal accumulation of species on the electrode surface. Experimental results showed that peak currents decreased slightly when the accumulation potential shifted from 0.0 to 0.60 V. Hence the potential around 0 V was considered as favorable for obtaining the maximal peak currents for both the species in pH 5.2. Thus, the accumulation of LD and BZ at the modified GCE was performed under open-circuit condition. The DPV measurements were also carried out by transferring PAR modified GCE into a blank electrolyte solution after the surface accumulation. Similar DPV peaks with only a very small decrease in peak current was observed for the medium exchange, which proved that the interaction of BZ and LD with PAR modified GCE was strong, and the DPV peaks were mainly contributed by the species pre-accumulated at the electrode surface.

The effect of scan rate for CV and DPV techniques on the anodic peak current of BZ and LD was also studied. The optimum scan rate for CV and DPV was 100 and 30 mV/s, respectively. The electrochemical oxidation behavior of BZ and LD in various media, such as phosphate buffer, sodium citrate–HCl buffer, Britton–Robinson buffer, were compared by CV and DPV. The best oxidation response and peak resolution were obtained in PBS. As the pH of medium was increased the peaks for both BZ and LD were shifted to more positive potential side along with little affect on the resolution of peaks. The maximum peak current for both CV and DPV were obtained at pH 5.2. Therefore, in this study PBS of pH 5.2 was chosen as the determining medium.

3.6. Quality parameters

Stability of PAR modified GCE was tested by keeping the electrode in PBS of pH 5.2 for 2 month, after this time period the CVs were recorded and compared with CVs obtained before immersion. The results indicated that peak current decreased only slightly for PAR modified GCE, which indicated that PAR modified GCE has good stability. A relative standard deviation (R.S.D.) of 1.3% and 2.1% for 10 measurements of 0.5 M and 1.0 M of BZ and LD, respectively suggested that PAR modified GCE has higher reproducibility. Three PAR modified GCE fabricated independently, were used to determine 1.0 M LD and the R.S.D. was 3.0%, revealing an excellent repeatability of the electrode preparation procedure. To establish the repeatability of the electrode, 5 measurements of 1.0M LD was carried out using PAR modified GCE at the intervals of 1 h. The R.S.D. value was found to be 2.2%, which indicated that the modified GCE has good repeatability.

3.6. Analytical applications

3.6.1. Calibration curve

Initially the CV technique was applied for the confirmation that PAR modified electrode have sufficient response for the LD and BZ determination. Later on the DPV technique was applied for application to real sample due to good discrimination against background current and low detection limits. Figure 6 (I) exhibits the DPVs that were obtained in different concentrations (0.01–0.20 mM) of BZ in the presence of 0.125 mM LD on PAR modified GCE. The voltammetric peak corresponding to

the oxidation of BZ was found to increase linearly in consonance with the increase in bulk concentration of BZ, whereas the peak current for oxidation of LD remain the same as the number of cycles increased. Figure 6 (II) exhibits the DPVs that were obtained in different concentrations (0.025–1.0 mM) of LD in the presence of 0.2 mM BZ on PAR modified GCE. The voltammetric peak corresponding to the oxidation of BZ was found to increase linearly in consonance with the increase in bulk concentration of LD, whereas the peak current for oxidation of BZ remain the same as the number of cycles increased. The inset figures (Fig. 6) are showing the calibration plots of the DPV peak currents versus the increasing concentrations of BZ and LD, respectively. The regression equation for calibration curve of BZ was y = 0.398 + 0.1682x (r^2 = 0.991), where x is the concentration of BZ (mM) and y is current intensity (μA). For LD the regression equation was y = 0.4147 + 0.0.201x (r^2 = 0.993), where x is the concentration of LD (mM) and y is current intensity (μA). At higher concentration the response of LD was suppressed by BZ probably due to more affinity of BZ towards PAR film which resulting loss of linearity. Limit of detection (LOD) was found to be 0.002 mM for BZ and 0.006 mM for LD. Similarly, the limit of quantification for BZ and LD was 0.007 and 0.02 mM, respectively. Both LOD and LOQ values confirmed the sensitivity of the proposed methods. These results indicated that the PAR modified GCE is sensitive towards BZ in the presence of high concentration (up to 0.4 mM) of LD and towards LD in the presence of high concentration (upto 0.2 mM) of BZ.

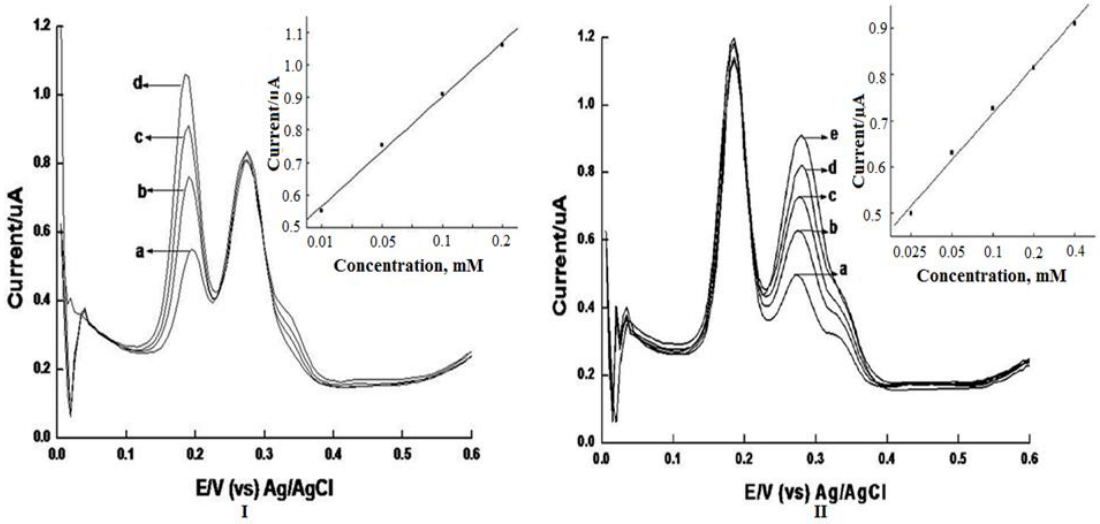

Figure 6. (I) DPVs obtained for BZ in the presence of 0.125 mM of LD at PAR modified GCE in 0.1 M PBS of pH 5.2. Concentration of BZ: (a) 0.01 mM, (b) 0.05 mM, (c) 0.1 mM, (d) 0.2 mM and (II) DPVs obtained for LD in the presence of 0.2 mM of BZ at PAR modified GCE in 0.1 M PBS of pH 5.2. Concentration of LD: (a) 0.025 (ab) 0.05 mM, (c) 0.1 mM, (d) 0.2 mM, (e) 0.4 mM.

3.6.2. Determination of DA and AP in tablets, human serum and urine

The validity of the proposed method for the determination of LD and BZ in pharmaceutical preparation (madopar tablets), human serum and urine samples was investigated. After sample

preparation and adequate dilution steps as described earlier, the DPV method was applied to the direct determination of LD and BZ in madopar tablets. The summarized results for the analysis are shown in Table 1. The mean results of the five determinations for the pharmaceutical preparations of BZ and LD were close to the values declared on the labels. To check the accuracy of the investigated method in pharmaceutical preparations and its applicability to the determination of BZ and LD in the human serum and urine sample, the recovery studied were carried out. The obtained recovery results in Table 1 and 2 indicates that PAR modified GCE can be successfully used for the determination of BZ and LD in pharmaceutical preparations, human serum and urine samples.

Table 1. Determination of BZ and LD in pharmaceutical preparations

Tablets	Sample	Labeled (mg)		Found (mg)		Recovery (%)		RSD[a] (%)	
		BZ	LD	BZ ± SD[a]	LD ± SD[a]	BZ	LD	BZ	LD
Madopar	1	25	100	25.2±0.7	101.2±1.2	100.8	101.2	0.91	1.22
	2	25	100	25.3±0.5	101.9±2.0	101.2	101.9	1.02	1.51
	3	25	100	25.1±0.6	100.9±1.7	100.4	100.9	0.95	1.35

[a]Mean values of 5 determinations.

Table 2. Determination of BZ and LD in human serum and urine samples

Sample	Added (mM)		Found (mM)		Recovery (%)		[a]RSD (%)	
	BZ	LD	BZ ± SD[a]	LD ± SD[a]	BZ	LD	BZ	LD
Human Serum	0.1	0.2	0.102±0.003	0.203±0.004	100.8	101.5	1.71	2.01
	0.2	0.4	0.203±0.005	0.410±0.008	101.5	102.5	1.72	2.07
Urine	0.1	0.2	0.103±0.002	0.205±0.003	103.0	102.5	1.72	2.11
	0.2	0.4	0.206±0.004	0.412±0.007	103.0	103.0	1.70	2.15

[a]Mean values of 5 determinations.

4. CONCLUSION

An electrochemical method was developed and successfully applied for the simultaneous determination of benserazide (BZ) and levodopa (LD) using poly(4-(2-pyridylazo)-resorcinol) (PAR) modified glassy carbon electrode. A pre-concentration step was established for the accumulation of these species at the electrode obtaining well separated voltammetric peaks for sensitive and selective determination of BZ and LD. In the anodic sweep from 0.0 to 0.6V, both BZ and LD adsorbed at the PAR polymer film modified electrode surface and oxidized at 180 and 270 mV respectively using DPV method. The experimental conditions like supporting electrolyte, accumulation time and scan rate

were optimized for the simultaneous determination of BZ and LD using the DPV technique. The commonly encountered excipients used in the pharmaceutical formulation showed no interference with the selective determination of BZ and LD. The investigated method showed good stability, reproducibility (1.3% (BZ) and 2.1% (LD)), repeatability (2.2%) and high recovery in pharmaceutical preparation (0.91% (BZ) and 1.22% (LD)), human serum (1.71% (BZ) and 2.01% (LD)), and urine (1.72% (BZ) and 2.11% (LD)). The proposed method is simple, sensitive, easy to apply and economical for routine analysis, which could be used not only in evaluating the quality of some medicines in marketplaces but also in investigating their contents in human bodies.

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References

1. K. Kitahama, M. Geffard, S. Araneda, R. Arai, K. Ogawa, I. Nagatsu and J.M. Pequignot, *Brain Res.* 1167 (2007) 56.
2. C.W. Olanow, J.A. Obeso and F. Stocchi, *Lancet Neurol.* 5 (2006) 677.
3. M. Gordon, J. Markham, J.M. Hartlein, J.M. Koller, S. Loftin and K.J. Black, *J. Neurosci. Meth.* 159 (2007) 300.
4. V.K. Gupta, A.K. Jain, R. Ludwig and G. Maheshwari, *Electrochim. Acta* 53 (2008) 2362.
5. S. Fanali, V. Pucci, C. Sabbioni and M.A. Raggi, *Electrophor.* 21 (2000) 2432.
6. S.A. Treseder, S. Rose, L. Summo and P. Jenner, *J. Neural. Transm.* 110 (2003) 229.
7. V.K. Gupta, A.K. Jain and P. Kumar, *Electrochim. Acta* 52 (2006) 736.
8. M. Chamsaz, A. Safavi and J. Fadaee, *Anal. Chim. Acta* 603 (2007)140.
9. T. Madrakian and M. Mohammadnejad, *Chem. Pharm. Bull.* 55 (2007) 865.
10. S. Zhao, W. Bai, B. Wang and M. He, *Talanta* 73 (2007) 142.
11. W. W. He, X. W. Zhou and J. Q. Lu, *Chinese Chem. Lett. 18* (2007) 91.
12. J. Wang, Y. Zhou, A.F. Wang, S.H. Zhang, L. Ning, P.G. He and Y.Z. Fang, *Anal. Bioanal. Chem.* 382 (2005) 198.
13. P.T.T Ha, A.V. Schepdael, T. Hauta-Aho, E. Roets and J. Hoogmartens, *Electrophor.* 23 (2002) 3404.
14. F. Chen, Z. Zhang, Y. Zhang and D. He, *Anal. Bioanal. Chem.* 382 (2005) 211.
15. I. Baranowska and J. Plonka, *J. Chromatogr. Sci.* 46 (2008) 30.
16. E. Nalewajko, A. Wiszowata and A. Kojlo, *J. Phar. Biomed. Anal.* 43 (2007) 1673.
17. Y. Tu, Q. Xu, Q.J. Zou, Z.H. Yin, Y.Y. Sun and Y.D. Zhao, *Anal. Sci.* 23 (2007) 1321.
18. S. Viswanathan, W.C. Liao, C.C. Huang, W.L. Hsu and J.A. Ho, *Talanta* 74 (2007) 229.
19. C. Xiang, Y. Zou, J. Xie and X. Fei, *Anal. Lett.* 39 (2006) 2569.
20. M.C. Blanco-Lopez, M.J. Lobo-Castanon, A.J.M. Ordieres and P. Tunon-Blanco, *Electroanal.* 19 (2007) 207.
21. J. B. Raoof, R. Ojani, M.A. Aref and M. Baghayeri, *Sensors and Actuators B: Chem.* 166–167 (2012) 508.
22. C. Z. Urzua, M. P. Ortiz, M. Bravo, A.C. Olivieri and A. A. Lueje, *Talanta,* 82 (2010) 962.
23. E. Dinc, S. Kaya, T. Doganay and D. Baleanu, *J. Pharm. Biomed. Anal.* 44 (2007) 991.
24. M. Pistonesi, M.E. Centurion, B.S.F. Band, P.C. Damiani and A.C. Olivieri, J. *Pharm. Biomed. Anal.* 36 (2004) 541.

25. B. Uslu and S.A. Ozkan, *Anal. Lett.* 35 (2002) 303.
26. J. Coello, S. Maspoch and N. Villegas, *Talanta* 53 (2000) 627.
27. E. Dinç, S. Kaya, T. Doganay and D. Baleanu, *J. Pharm. and Biom. Ana. 44* (2007) 991.
28. W.W. He, X.W. Zhou and J.Q. Lu, *J. Chromatogr. A* 1131 (2006) 289.
29. F. Bugamelli, C. Marcheselli, E. Barba and M.A. Raggi, *J. Pharm. and Biom. Ana.* 54 (2011) 562.
30. M.R. Nateghi and M. Borhani, *React. Funct. Polym.* 68 (2008) 153.
31. S.A. Kumar and S.M. Chen, *J. Mol. Catal.* A 278 (2007) 244.
32. A.A. Wassil, A. El-Ftaah Bastawy Farag and F.A. Moukdad, *Ind. Pharm.* 33 (2007) 57.
33. S. Sadeghi, F. Fathi and J. Abbasifar, *Sens. Actuat. B* 122 (2007) 158.
34. J.B. Raoof, R. Ojani and F. Chekin, *Electroanal.* 19 (2007) 1883.
35. J. Chen, J. Zhang, Q. Zhuang, S. Zhang and X. Lin, *Talanta* 72 (2007) 1805.
36. J. Chen, J. Zhang, X. Lin, H. Wan and S. Zhang, *Electroanal.* 19 (2007) 612.
37. M. Lapkowski, J. Zak and J. W. Strojek, *J. Electroanal. Chem. Inter. Electrochem. 145* (1983) 173
38. A. K. Jain, V. K. Gupta and L. P. Singh, Analytical *Proceedings including Analytical Communications*, 32 (1995) 263.
39. A. K. Jain, V. K. Gupta, B. B. Sahoo and L. P. Singh, *Analytical Proceedings including Analytical Communications*, 32 (1995)99.
40. S. K. Srivastava, V. K. Gupta and S. Jain, *Analyst*, 120 (1995) 495.
41. S. K. Srivastava, V. K. Gupta, M. K. Dwivedi and S. Jain, Caesium, *Analytical Proceedings including Analytical Communications*, 32 (1995) 21.
42. A. K. Jain, V. K. Gupta and L. P. Singh, *Bull. Electrochem.* 12(1996)418
43. S. K. Srivastava, V. K. Gupta and S. Jain, *Electroanalysis*, 8 (1996) 938
44. S. K. Srivastava, V. K. Gupta and S. Jain, *Anal. Chem*. 68(1996)1272.
45. A. K. Jain, V. K. Gupta, L. P. Singh and U. Khurana, *Electroanalysis*, 9(1997)1360.
46. V. K. Gupta, A. K. Jain, L. P. Singh and U. Khurana, *Anal. Chim. Acta*, 355(1997)33.
47. A. K. Jain, V. K.Gupta, U. Khurana and L. P. Singh, *Electroanalysis*, 9 (1997) 857.
48. A. K. Jain, V. K. Gupta, L. P. Singh and U. Khurana, *Analyst*, 122(1997) 583.
49. V. K. Gupta, S. Jain and U. Khurana, *Electroanalysis*, 9 (1997) 478
50. V. K. Gupta and P. Kumar, *Anal. Chim. Acta*, 389(1999) 205
51. V. K. Gupta, A. K. Jain, L. P. Singh and U. Khurana, *Sens. Actuators B,* 55(1999)201
52. V.K. Gupta, R. Mangla, U. Khurana and P. Kumar, *Electroanalysis*, 11 (1999)573
53. V. K. Gupta, R. Prasad, R. Mangla, and P. Kumar, *Anal. Chim. Acta*, 420(2000)19
54. V.K.Gupta, Rajni Mangla and S. Agarwal, *Electroanalysis*, 14, (2002) 1127
55. V. K. Gupta, R. Prasad, A. Kumar, *J. Appl. Electrochem.*, 33(2003)381.
56. V. K. Gupta, R. Prasad, A. Kumar, *Talanta*, 60(2003)149.
57. V.K Gupta, S. Jain, S. Chandra, *Anal. Chim. Acta* , 486(2) (2003)199.
58. V. K. Gupta, R. Prasad and Azad Kumar, *Talanta*, 63(2004)1027
59. R. Prasad, V. K. Gupta and Azad Kumar, *Anal. Chim. Acta* , 508(2004)61
60. R.N. Goyal, V.K. Gupta, M. Oyama and N. Bachheti, *Electrochem. Commun.*, 7 (2005)803
61. R.N. Goyal, V.K. Gupta, A. Sangal and N. Bachheti, *Electroanalysis*, 17 (2005)2217.
62. V. K. Gupta, S. Chandra and H. Lang, *Talanta*, 66(2005)575
63. V. K. Gupta, R. Ludwig and S. Agarwal, *Anal. Chim. Acta*, 538 (2005)213
64. A. K. Jain, V. K. Gupta, L. P. Singh, P. Srivastava and J. R. Raisoni, *Talanta*, 6(2005)716
65. A.K. Jain, V.K. Gupta, S. Radi, L.P. Singh, J.R. Raisoni, *Electrochim. Acta*,51(2006) 2547
66. V. K. Gupta, A. K. Singh and Barkha Gupta, *Anal. Chim. Acta*, 575 (2006)198
67. R.N. Goyal, V.K. Gupta, A. Sangal and N. Bachheti, *Electrochem. Commun.*,8 (2006)65
68. A.K. Singh, V. K. Gupta and Barkha Gupta, *Anal. Chim. Acta*, 585(2007)171
69. V. K. Gupta, A. K. Jain and G. Maheshwari, *Int. J. Electrochem. Sci.*, 2 (2007) 102.
70. V. K. Gupta, A. K. Singh, M. Al Khayat, B. Gupta, *Anal. Chim. Acta*, 590(2007)81

71. V.K. Gupta, A.K. Jain, M. Al Khayat, S. K. Bhargava, J.R. Raisoni, *Electrochim. Acta*, 53(2008)5409
72. R.N. Goyal, V.K. Gupta and S. Chatterjee, *Electrochim. Acta*, 53 (2008)5354
73. R.N. Goyal, V.K. Gupta, N. Bachheti and R.A. Sharma, *Electroanalysis*, 20 (2008)757.
74. V. K. Gupta, R. N. Goyal, and R. A. Sharma, *Int. J. Electrochem. Sci.* 4 (2009) 156.
75. V. K. Gupta, M. Al Khayat, A.K. Singh and Manoj. K. Pal, *Anal.Chim. Acta*, 634(2009)36
76. R.N. Goyal, V.K. Gupta and S. Chatterjee, *Biosens. Bioelectron.*, 24 (2009)1649
77. Vinod K. Gupta, Rajeev Jain, Manoj K. Pal, *Int. J. Electrochem. Sci.*, 5 (2010) 996
78. V. K. Gupta, A. J. Hamdan, R. Jain, S. Agarwal, A. K. Bharti, *Anal. Chim. Acta*, 681(2010) 27
79. R. Jain, V.K. Gupta, N. Jadon and K. Radhapyari, *Anal. Biochem.*, 407 (2010)79.
80. V.K. Gupta, *The Arabian Journal for Science and Engineering A-Science 35(2A*) (2010) 7
81. R.N. Goyal, V.K. Gupta and S. Chatterjee, *Sens. Actuators B*, 149 (2010)252
82. V. K. Gupta, R. Jain, K. Radhapyari, N. Jadon, Shilpi Agarwal, *Anal. Biochem*,408(2011)179
83. V.K. Gupta, S. Agarwal, B. Singhal, *International Journal of Electrochemical Science*,6(2011) 3036-3056
84. V.K. Gupta, A. Nayak, S. Agarwal, B. Singhal, *Combinatorial Chemistry and High Throughput Screening*,14(2011)284-302
85. V.K. Gupta, R. Jain, M.M. Antonijevic, H. Khani, M.N. Siddiqui, A. Dwivedi, R. Mishra, S. Agarwal, *International Journal of Electrochemical Science*,6(2011)37-51
86. V.K. Gupta, I. Ali, *Separation and Purification Technology* 18 (2000)131-140
87. V.K.Gupta, D. Mohan, S. Sharma, *Separation Science and Technology* 33(1998)1331-1343
88. A. Mittal, L. Kurup, V.K.Gupta, *Journal of Hazardous Materials*,117(2005)171-178
89. V.K.Gupta, I. Ali, *Environmental Science and Technology*,42(2008)766-770
90. V.K.Gupta, A. Rastogi, *Colloids and Surfaces B: Biointerfaces*, 64 (2008)170-178
91. V.K.Gupta, A.Rastogi, *Journal of Hazardous Materials*, 154 (2008)347-354
92. V.K.Gupta, A. Rastogi, M.K. Dwivedi, D. Mohan, *Separation Science and Technology*, 32 (1997), 2883-2912
93. V.K. Gupta, A. Mittal, V. Gajbe, J. Mittal, *Industrial and Engineering Chemistry Research*, 45 (2006)1446-1453
94. V.K.Gupta, I. Ali, V.K. Saini, *Journal of Colloid and Interface Science*,315 (2007)87-93
95. V.K. Gupta, R. Jain, S. Varshney, *Journal of Hazardous Materials*,142(2007)443-448
96. V.K. Gupta, A. Rastogi, *Journal of Hazardous Materials*,153(2008)759-766
97. V.K. Gupta, R. Jain, A. Mittal, M. Mathur, S. Sikarwar, *Journal of Colloid and Interface Science*, 309 (2007)464-469
98. V.K. Gupta, A. Rastogi, *Journal of Hazardous Materials*,163(2009)396-402
99. V.K. Gupta, A. Mittal, L. Kurup, J. Mittal, *Journal of Colloid and Interface Science*, 304 (2006)52-57
100. V.K. Gupta, R. Jain, S. Varshney, *Journal of Colloid and Interface Science*, 312 (2007)292-296
101. V.K.Gupta, I. Ali, V.K. Saini, *Water Research*, 41(2007)3307-3316
102. V.K. Gupta, R.N. Goyal, R.A. Sharma, *Analytica Chimica Acta*, 647 (2009) 66-71
103. V.K. Gupta, A. Rastogi, A. Nayak, *Journal of Colloid and Interface Science*,342(2010)135-141
104. V. K. Gupta, I. Ali and Shilpi Agarwal, *Int. J. Electrochem. Sci.*, 6(2011) 5639-5648.
105. V. K. Gupta, M. R. Ganjali, Hadi Khani, P. Norouzi, A. Nayak, Shilpi Agarwal, , *Critical Reviews in Analytical Chemistry*, 41, (4) (2011)1-32.
106. V.K. Gupta, A. Nayak, *Journal of Colloid and Interface Science* 342 (2010) 533-539.
107. R.N. Goyal, V.K. Gupta, M. Oyama and N. Bachheti, *Talanta* 71 (2007) 1110-1117.
108. R.N. Goyal, V.K. Gupta, M. Oyama and N. Bachheti, *Talanta* 71 (2007) 976-987.
109. V. K. Gupta, D. Mohan and S. Sharma, *Sep. Sci. Technol*., 33(1998), 1331- 1343.
110. V. K. Gupta and I. Ali, *Water Res*., 35(2001), 33-40.
111. V. K. Gupta, D. Mohan, S. Sharma and K. T. Park, *The Environmentalist*, 19(1999)129-136.

112. V. K. Gupta, A. Mittal , L. Krishnan and J. Mittal, *J. Colloid Interface Sci*., 293(2006)16-26.
113. A.K.Jain, V.K.Gupta, Shubhi Jain, and Suhas, *Environ. Sci. Technol*., 38, (2004) 1195-1200.
114. V.K. Gupta, AK.Singh, B. Gupta, *Analytica Chimica Acta* 583 (2007) 340-348.
115. Z.A. Alothman, N. Bukharia, S.M. Wabaidur and S. Haider, *Sens. Actuators B-Chem.* 146 (2010) 314.
116. H.T. Xu, F. Kitamura, T. Ohsaka, K. Tokuda, D. Kagaku, *Elctrochem.* 60 (1992) 1068.
117. X. Q. Lin, J. B. Hea and Z.G. Zhab, *Sens. Actuators B. Chem*. 119 (2006) 608.
118. P.R. Roy, T. Okajima and T. Ohsaka, *Bioelectrochem.* 59 (2003) 11.