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The Quantitative Detection of Phenylephrine in Pharmaceutical Preparations and Spiked Human Urine by Voltammetry[1]

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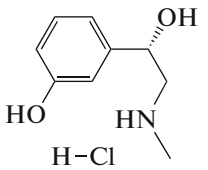
Abstract—A voltammetric method was used for the quantitative detection of phenylephrine in pharmaceutical preparations and spiked human urine. The electrochemical measurements were carried out in various buffer solutions in the pH range from 0.51 to 12.00 on ultra-trace graphite electrode (UTGE) by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. The dependence of pH on the oxidation peak current and peak potential was investigated. Britton–Robinson buffer (pH 8.02) was selected for analytical purposes. The peak was established to be diffusion controlled nature of electrode. A linear calibration curve for DPV analysis was constructed in the phenylephrine concentration range from 8×10^{-6} to 1×10^{-4} M. Limits of detection (LOD) and quantification (LOQ) were obtained as 2.07×10^{-7} M and 6.91×10^{-6} M respectively. The repeatability, accuracy and precision of the developed technique were checked in spiked urine and pharmaceutical preparations.

Keywords: phenylephrine, detection, voltammetry, pharmaceutical preparations, human urine

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INTRODUCTION

Phenylephrine (PHE) hydrochloride (Scheme 1) is a crystalline white or pale yellow salt fairly soluble in water. As a sympathomimetic agent that directly affects adrenergic receptors, it has been used in the treatment of hypertension, rhinitis and sinusitis, in the symptomatic relief of cold symptoms. It is present in the formulations of several vasopressor medicines, in eye-washes, in nasal decongestants, and in syrups. The requirement of rigorous quality control in pharmaceutical industry has led to the development of a variety of methods for the analysis of active principles in pharmaceuticals. It is reported that a lot of papers published with methods for the determination of PHE [1].



Scheme 1. Chemical structure of PHE.

It has been used the several measuring techniques such as electrochemistry, chromatography and spectrophotometry. In addition, there are still papers published to propose methods for the determination of PHE, such as flow injection analysis (FIA) [1, 2], spectrophotometry [3–6], high-performance liquid chromatography (HPLC) [7], spectrofluorimetry [8]. The electroanalytical determination based on oxidation of phenylephrine was performed with modified electrodes [9–11]. Furthermore, the electrochemical detection on the GCE and HPLC techniques were used to the determination of PHE [12]. Most of the reported methods required a long time for sample pretreatment, expensive reagents and equipment, which are not useful for routine analysis studies. Voltammetry is a practical analytical technique that offers high sensitivity, precision, and accuracy, wide linear range and low-cost instrumentation and time.

Amoung the non-modified and modified electrodes, UTGE provides greater sensitivity and selectivity in the determination of PHE. Moreover, the UTGE showed easy regeneration, good reproducibility and stability. Proposed methods can be applied to the detection of UTGE in practical drug and biological sample. So, in this study, direct and new voltammetric method was developed for the determination of PHE in pharmaceutical preparations and spiked human urine on UTGE.

[1] The article is published in the original.

EXPERIMENTAL

Apparatus

A Model Metrohm 757 VA Trace Analyzer (Herisau, Switzerland) was used for the voltammetric measurements, with a three-electrode system consisting of UTGE as working electrode (UTGE; ∅ = 3 mm, Metrohm), a platinum wire as auxiliary electrode and Ag/AgCl (KCl 3 mol/L, Metrohm) as reference electrode. The UTGE electrode was polished with alumina (prepared from ∅ = 0.01 μm aluminum oxide) on an alumina polish pad before each experiment and then rinsed with ultra-pure deionized water and ethanol. Then firstly, the deoxygenating process of the supporting electrolyte solutions was carried out with argon gas for 5 min before all experiments. The argon gas was also passed through the solutions for 60 s after the addition of each sample solution in the experiments. In each new experiment, a new bare electrode surface was used. All pH measurements were made with Model Metrohm 744 pH meter (Herisau, Switzerland) at ambient temperature of the laboratory (15 to 20°C).

For the analytical application, the following parameters were employed: pulse amplitude 50 mV; pulse time 0.04 s, voltage step 0.009 V, voltage step time 0.04, potential step 10 mV (DPV); with the scan rate in the range from 10 to 1000 mV s^{-1} (CV).

Reagents and Materials

PHE was kindly supplied by Ibrahim Etem Ulagay (Istanbul, Turkey). A stock solution of 1.0×10^{-2} M of PHE was prepared by dissolving an accurate mass of the drug in an appropriate volume of ultra-pure-deionized water. The working solutions for the voltammetric investigations were prepared by dilution of the stock solution. All solutions were protected from light and were used within 24 h to avoid decomposition. 0.5 M H_2SO_4 (pH 0.51) solution, 0.067 M phosphate buffer (pH 4.40 to 7.28), 0.2 M acetate buffer (pH 3.51 to 5.62) and 0.04 M Britton–Robinson (BR) buffer (pH 2.02 to 12.00) were used for the supporting electrolyte solutions. Ultra-pure water (UPW) obtained from Sartorius Arium model Ultra-Pure Water Systems was used to prepare supporting electrolytes. All chemicals used were of analytical-reagent grade.

Calibration Graph for Quantitative Determination

The stock solution of PHE was diluted with UPW to obtain different PHE concentrations. Using the optimum conditions described in the experimental section, a linear calibration curve for DPV analysis was constructed in the phenylephrine concentration range from 8×10^{-6} to 1×10^{-4} M. The repeatability, accuracy and precision were checked.

Working Voltammetric Procedure for Spiked Tablet Dosage Forms

Ten tablets were weighed and ground to a fine powder. An adequate amount of this powder, corresponding to a stock solution of concentration 1×10^{-2} M, was weighed and transferred to a 10 mL calibrated flask and the volume was adjusted with UPW. The contents of the flask were centrifuged for 20 min at 4000 rpm to ensure complete dissolution and then diluted to volume with the UPW. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting with UPW. Each solution was transferred into the voltammetric cell. The nominal content of the corresponding regression equations was compared with previously plotted calibration plots.

Working Voltammetric Procedure for Spiked Human Urine

Urine obtained daily from a volunteer was diluted 1 : 9 with UPW. Firstly, 9.4 mL of 0.04 M BR buffer (pH 8.02) was put into the voltammetric cell and its voltammogram was taken as the blank. Then, 600 μL of the diluted urine solution was added to this solution and its voltammogram was taken as the urine blank. Subsequently, to obtain a 4×10^{-5} M cell concentration, 40 μL of urine sample (1 mL urine + 8 mL ultrapure water + 1 mL of 1×10^{-1} M PHE stock solution) was added into the voltammetric cell and its voltammogram was recorded. Then, 20 μL of 1×10^{-2} M PHE standard solution was added four times and the voltammograms of the resulting solutions were individually recorded after each addition. The calibration curve was plotted using obtained results.

RESULTS AND DISCUSSION

Electrochemical Oxidation Behavior of Phenylephrine

In order to obtain the optimum experimental conditions, some variables affecting the peak current and peak potential, which are pH and the species of supporting electrolyte (pH 0.51 to 12), were studied for a PHE solution of 5×10^{-5} M on the UTGE by the proposed voltammetric technique.

When studied the effect of pH on the peak current, the high peak current and shape peak was observed at pH 8.02 in 0.04 M BR buffer. Therefore, this pH value and buffer solution were chosen for the electroanalytical studies (Fig. 1). Consequently, DPV technique and BR buffer (pH 8.02) were preferred for further work. The voltammetric response was strongly pH dependent.

The oxidation peak potential is shifted linearly to more negative values with increasing pH (Fig. 2). That

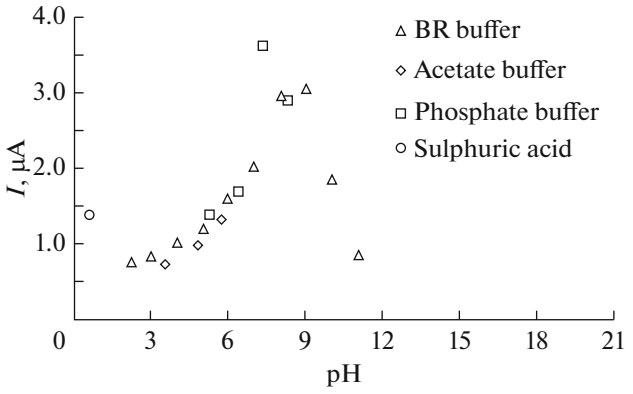


Fig. 1. The effect of pH on the peak current of 5×10^{-5} M PHE in different buffer solutions by DPV technique.

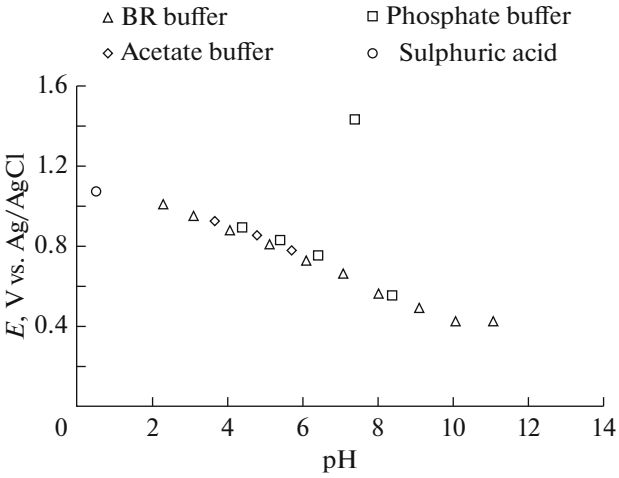


Fig. 2. The effect of pH on peak potential of 5×10^{-5} M PHE in different buffer solutions by DPV technique.

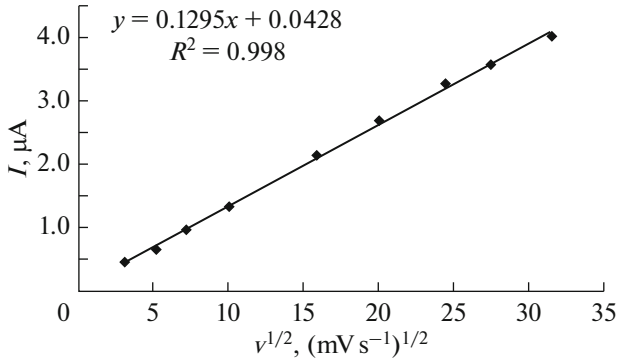


Fig. 3. Plot of square root of scan rate versus peak current.

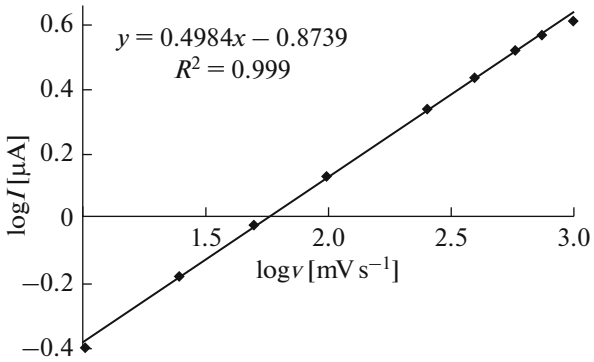


Fig. 4. Plot of logarithm of peak current versus logarithm of scan rate.

indicates participation of proton transfer in the electrode reaction.

The effects of the scan rates from 10 to 1000 mV s^{-1} on the peak potential and the PHE peak current were evaluated. The electrochemical oxidation process was carried out using CV techniques.

The correlation coefficient of peak current and square root of the scan rate 0.998 ($I_p/(\mu A) = 0.061v^{1/2} + 0.0625$) and 0.4984 slope of plot of logarithm of peak current versus logarithm of scan rate indicated that the oxidation process is predominantly diffusion-controlled (Figs. 3 and 4) [13–15].

The cyclic voltammogram of PHE exhibited only one anodic peak, with no peak on the reverse scan, indicating the totally irreversible nature of the electrode reaction. In addition, for an irreversible oxidation process, the peak potential, E_p shifts to more positive values with the increasing scan rate. Therefore, the oxidation process of PHE was proved to be irreversible [13].

Validation of the Proposed Voltammetric Technique

DPV technique was used to develop a voltammetric methodology for determination of the drug in pharmaceutical form. Under the optimized experimental conditions, a linear relationship between the oxidation peak current of PHE at UTGE and concentration can be established in the range from 8×10^{-6} to 1×10^{-4} M (Fig. 5).

The equation of the linear regression plots was $I_p/(\mu A) = 22880$ (mol/L)–0.1027; correlation coefficient, $r = 0.9971$; $n = 5$ repeat measurements. Standard deviations for intercept and slope of the calibration curve are given in Table 1.

Validation of the procedure for the quantitative detection of PHE was examined via evaluation of the limit of detection (LOD), limit of quantification (LOQ), repeatability, reproducibility (Table 1).

LOD and LOQ were calculated on the oxidation peak current using the following equations: LOD = 3 s/m, LOQ = 10 s/m (s is the standard deviation of the peak currents six runs, m is the slope of the calibration

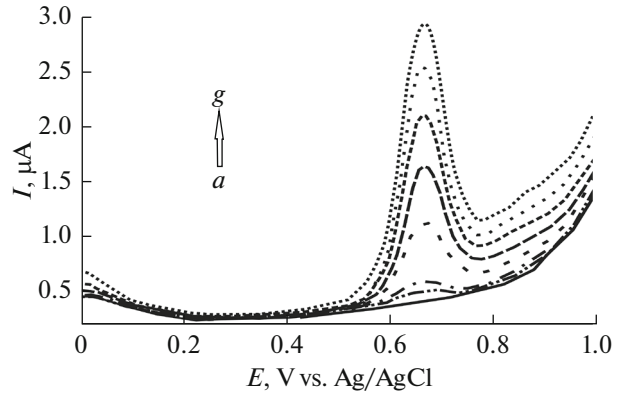


Fig. 5. The calibration voltammograms at different concentrations of PHE in BR buffer (pH 8.02) at UTGE by DPV (*a*) 8×10^{-6}; (*b*) 1×10^{-5}; (*c*) 3×10^{-5}; (*d*) 5×10^{-5}; (*e*) 7×10^{-5}; (*f*) 9×10^{-5}; (*g*) 1×10^{-4} M.

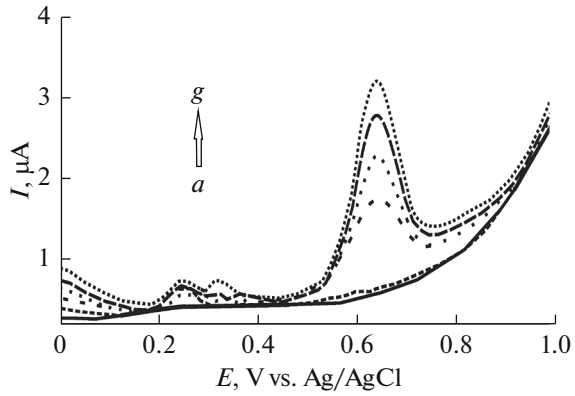


Fig. 6. DPV voltammograms of PHE in spiked human urine; (*a*) blank (0.04 M BR buffer (pH 8.02); (*b*) blank + 600 μL urine (1 : 9); (*c*) 4×10^{-6} M PHE with urine; (*d*) 2×10^{-6}; (*e*) 4×10^{-6}; (*f*) 6×10^{-6} and (*g*) 8×10^{-6} M PHE.

curve). The LOD and LOQ were calculated as 2.07×10^{-7} and 6.91×10^{-6} M respectively. A good repeatability and reproducibility of the peak current and potential were calculated from five independent measurements (Table 1).

Table 1. Analytical parameters of PHE obtained in BR buffer (pH 8.02) by DPV technique

Parameters	Results
Measured potential, V	0.635
Linear concentration range, M	8×10^{-6}–1×10^{-4}
Slope, μA M^{-1}	22880
SD of slope	2.74
Intercept, nA	0.1027
SD of intercept	0.8
Correlation coefficient, *r*	0.9971
LOD, M	2.07×10^{-7}
LOQ, M	6.91×10^{-6}
Repeatabilty of peak current (RSD, %)	0.67 for 9×10^{-5} M
Repeatabilty of peak potential (RSD, %)	0.78 for 9×10^{-5} M
Reproducibility of peak current (RSD, %)	0.55 for 9×10^{-5} M
Reproducibility of peak potential (RSD, %)	0.02 for 9×10^{-5} M

Determination of Phenylephrine in Pharmaceutical Preparations by Voltammery Techniques

The amount of PHE in Corsal commercial tablets was calculated by reference to the appropriate calibration plots. The results obtained are given in Table 2.

To detect whether excipients in the tablets interfered with the analysis, the accuracy of the proposed methods was evaluated by recovery tests after the addition of a certain amount of pure drug to pre-analyzed formulations of PHE (Table 2). The results showed the validity of the proposed techniques for the quantitative determination of PHE in tablets.

Application to Human Urine Samples

The possibility of applying the voltammetric procedure to the quantitative detection of PHE in spiked human urine was also successfully tested by standard additions of PHE as described in the experimental section. The voltammograms for PHE in spiked human urine were given in Fig. 6.

The found amount of PHE in human urine was calculated from the related linear regression equations. The results of these analyses are summarized in Table 3.

Interference Studies PHE Was Formulated Either in Pure Form or with Some Additives in Drug Tablets

In present study, PHE tablet contained only 25 mg PHE. Therefore, we observed only one peak without interferences. So, in order to investigate the effect of co-formulated substances such as glucose, starch, citric acid and magnesium stearate on the voltammetric response of PHE was carried out. Differential-pulse voltammetric experiments were carried out 1×10^{-6} M

Table 2. Application of the DPV technique for the assay of PHE in Pharmaceutical Preparations

Parameters	Results
Labeled PHE , mg	5
Amount found, mg	5
Relative Standard deviation (RSD, %)	0.324
Bias, %	4.8
PHE spiked, mg	1
Found, mg	0.96
Average recovery, %	96
Relative standard deviation of recovery (RSD, %)	4.77
Bias, %	4

Table 3. Application of the DPV technique to the quantitative determination of PHE in spiked human urine samples with recovery results

Parameters	Results
Phenylephrine, M	4×10^{-6}
Phenylephrine found, M	3.93×10^{-6}
Number of measurements, N	5
Averagerecovery, %	98.25
RSD, %	1.32
Bias, %	2.84

Table 4. Influence of potential interferents on the voltammetric responses of 1×10^{-6} M PHE

Interferent	Concentration, M	Current signal change, %
Glucose	1×10^{-4}	−1.32
Starch	1×10^{-4}	−1.70
Citric acid	1×10^{-4}	+ 1.21
Magnesium stearate	1×10^{-4}	+ 1.31

PHE in the presence of 1×10^{-4} M of each of the interferents (concentration ratios, 1 : 100) are given Table 4.

CONCLUSIONS

A simple, sensitive, selective DPV technique for the quantitative detection of PHE based on the electrochemical oxidation at an UTGE was established. From the CV and DPV measurements, it is understood that the electrode reaction process is irreversible and pH dependent. PHE was successfully determined in BR buffer (pH8.02) in Pharmaceutical Preparations by DPV technique.

The principal advantage of the DPV technique over the other techniques is that it may be applied directly to the analysis of pharmaceutical dosage form without the need for extensive sample preparation, since there was no interference from the excipients and endogenous substances. Another advantage is that the developed DPV technique is rapid, requiring about 5 min to run any sample and involves no sample preparation other than dissolving, diluting, precipitating, centrifuging and transferring an aliquot to the supporting electrolyte.

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