Differential Pulse Voltammetric Determination of Penicillin G at Boron Doped Diamond Electrode

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Abstract: Concerns about accumulation of persistent pollutants such as penicillin G (PG) in aquatic environments results in the demand for analytical methods suitable for their fast, sensitive and simple determination. This study assesses the effectiveness of differential pulse voltammetric (DPV) method coupled with boron doped diamond electrode (BDDE) for determination of PG in aquatic samples. Britton-Robinson buffer (BRB) was used as a supporting electrolyte. Results showed that the DPV technique is suitable for the determination of PG in both drinking and river water model samples. PG gives an anodic DPV peak in the range from +800 to +1010 mV, depending on the pH of BRB. The best developed peak was obtained in BRB of pH 4, at the potential of +900 mV. Calibration curves are linear (R>0.99) in the range of 1- 9 µM of PG with the limit of quantitation (LOQ) of 1.5 µM and the limit of detection (LOD) of 0.23 µM. The method is not influenced by inorganic ions present in measured solutions, there are no problems with electrode passivation by products of PG anodic oxidation and it is accurate (RSD of 1.7-2.3%). Our developed is used as a simple, accurate, reliable and robust method for PG determination.

Keywords: Differential Pulse Voltammetry, Boron Doped Diamond Electrode, Penicillin G.

INTRODUCTION

Concerns about new emerging pollutants (NEPs) such as pesticides, drugs, dyes and endocrine disrupting chemicals in aquatic environments have recently been increasing because they have detrimental impacts on environment, including human health. They have been detected in wastewater [1,2], surface water [3,4] or even in drinking water [5,6]. Among the NEPs, drugs related compounds attract enormous attentions due to their unrestricted and diverse usages. Antibiotics such as penicillin G (PG) have several undesirable side effects ranging from hypersensitivity reaction (e.g. allergy to penicillin), soft stools or diarrhea to more serious effects such as damage for vital organs [7,8]. PG or 2S,5R,6R)-3,3-dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid is among most frequently used antibiotics for treating gram-positive bacterial infections[9,10]. Because of extensive usage, the presence of penicillins in water systems has often reported, and thus the development of electroanalytical methods for their determination is of high importance.

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Accurate and sensitive determination of PG aqueous matrices is commonly performed using advanced methods, e.g. gas chromatography coupled with mass spectrometry (GC-MS), GC coupled with tandem mass spectrometry (GC-MS/MS), liquid chromatography (LC) coupled with MS (LC-MS) and LC coupled with MS/MS (LC-MS/MS), all have been used for determination of antibiotics [11], hypertension [12] and other drugs [13]. In addition to those advanced methods, other simpler methods such as UV-VIS spectrometry [10,14], chromatography [7,15,16] and capillary electrophoresis [17,18] have also been suggested. However, they are laborious and time consuming and rather expensive. Voltammetric methods (such as differential pulse voltammetry (DPV), square wave voltammetry (SWV), and adsorptive stripping voltammetry (AdSV)) are reliable and robust for determination of organic compounds, such as pesticides [19], dyes [20,21] and pharmaceuticals [21], including penicillin [22-26]. They offer many advantages: simple operation, rapid measurement, low-cost, portable, reasonable sensitivity and selectivity, etc [27,28].

To be effective, selection of voltammetric working electrode is immensely important. Among the most promising working electrode is boron doped diamond electrode (BDDE), a novel carbon based materials, and one of the best working electrodes for voltammetric determinations. BDDE has been widely used as an effective working electrode for determination of biological active organic compounds, such as drugs [29,30], pesticides [31-37], carcinogenic compounds [21,38,39], BDDE offers many advantages: it has wide potential window (from -20V to +3.5 V) with a very low and stable background current [31,38]. A low background current enhances sensitivity and lowers limit of detection (LOD). high over-potential (for both oxygen and hydrogen) and extremely high electrochemical stability (in an alkaline and an acidic media). and a good resistance to surface fouling by adsorption of interferences and/or products of electrode reaction [31,39].

This study evaluates electrochemical behaviours of PG using DPV at BDDE as and then utilizes those behaviours to sense PG in drinking and river water samples. The basic structure of PG and PV is indeed similar, both share the beta-lactam-ring but differ in the side change. However, it is well known that Penicillin V analysed [22] and PG have very different behavior with respect to pH. Penicillin V is a more acid stable and thus typically administered orally. On the other hand, PG is very sensitive to pH and thus administered intravenously or intramuscularly. At least, the discrepancy in pH sensitivity is expected to lead to different optimum sensing conditions. To our best knowledge, this is the first study to assess the effectiveness of BDDE as working electrode for determination of PG using DPV technique. Firstly, a proper buffer and appropriate pH was found. Afterwards, the influence of the initial potential (E_i) was investigated to find the best developed peak. The PG accumulation on the BDDE surface was also evaluated by assessing the influence of accumulation potential (E_{acc}) and accumulation time (t_{acc}). Limit of quantification (LOQ) and limit of detection (LOD) were evaluated. Lastly, the effect of ionic interferences was evaluated and direct measurement of PG in model samples of drinking and river water samples was performed.

MATERIALS AND METHODS

Chemicals and Reagents

PG (98% purity) was obtained from BIO BASIC, Ontario, Canada. Glacial acetic acid, ortophosphoric acid and sodium hydroxide were purchased from Merck, Darmstadt, Germany and Lachema, Brno, Czech Republic. The Britton-Robinson buffers (BRBs) were prepared using 0.04 mol/L phosphoric acid, acetic acid, and boric acid. BRBs were adjusted to appropriate pH by adding precise amount of 0.2 M NaOH. A stock solution of 0.1 M PG was prepared by dissolving 0.713 g of PG sodium salt in 250 mL of deionized water (DW). Diluted PG solutions were prepared by precise serial dilutions. All chemicals used were of reagent grade quality.

Instrumentation

The voltammetric measurements (both CV and DPV) were performed using Eco-Tribo Polarograph (Polaro-Sensors, Prague, Czech Republic). The three electrode system used Ag/AgCl (3 M KCl) (Monokrystaly, Turnov, Czech Republic) as reference electrode and a platinum wire as counter electrode (Monokrystaly, Turnov, Czech Republic). The applied working electrode was BDDE in polyether ether ketone body (Winsdor Sciencetific Ltd., United Kingdom). It has inner diameter of 3 mm, resistivity of 0.075 Ω cm and boron doping level of 1000 ppm. Nitrogen gas (Linde, Prague) was used to remove dissolved oxygen from a water sample by purging it for 5 mins (before first measurement) or for 30 s (between individual measurements). The pH of BRB was measured using pH meter 3510 (Jenway, Chemsford, UK) with combined glass electrode.

Procedures

20 mL of BRB solutions (pH 2-12) was measured into electrochemical cell, and then a calculated volume of the stock solution (different concentrations) of PG was added and corresponding voltammograms (CV, DPV or DPAdSV) of those solutions were recorded. Each measurement was repeated at least three times to estimate the reproducibility of the results. For DPV, the pulse height, pulse width and scan rate were set at 50 mV, 0.1 s, and 50 mVs^{-1}, respectively. The calibration curve was constructed from measurements of PG in the range of 1-9 μM in measured solution. The BDDE was rinsed with DW before each measurement. The pre-treatment was carried out by immersing the electrode in 1 M H_2SO_4 for 5 mins, followed by cathodic scanning from -2 to +2 V to clean the surface of the electrode. In all measurements, Ag/AgCl (3 M KCl) was used as the reference electrodes. DPV peaks were evaluated from the straight line connecting the minima before and after the peak. The calibration curves were constructed to calculate the LOQ and the LOD using OriginPro 8.5.1.

Sample Preparation

Drinking water samples were taken from the public water pipeline in the Faculty of Science building, Charles University in Prague. The river water samples were taken from Vltava River in the centre of Prague. These samples were spiked with appropriate amounts of PG stock solutions. For DPV determination of PG, 18.0 mL of a spiked model sample was filled to 20.0 mL with BRB of appropriate pH.

RESULT & DISCUSSION

The Influence of pH on Cyclic Voltammetry

The PG voltammetric behaviours are shown by participation of protons in electrode process within pH range 2-12 from CV data (**Fig. 1A**). PG showed well-developed anodic peaks between pH 3 and 7, and at potentials between 800 and 1010 mV (**Fig. 1B**). No anodic peak was observed when pH was increased from 8 to 12. Similarly, no cathodic peak was observed at any pH on the reverse scan, which confirmed irreversible charge transfer process on a bare BDDE. The highest CV peak for PG was found at pH 4 and at a potential of + 900 mV (**Fig. 1C**). This finding suggests that the pH of supporting electrode significantly affects the peak potential and peak current, for detection and quantification of biologically active compounds. The CV was performed by measuring influence of pH on the PG voltammetric behavior (at PG of 0.5 mM) and potential range of -2000 to +2000 mV.
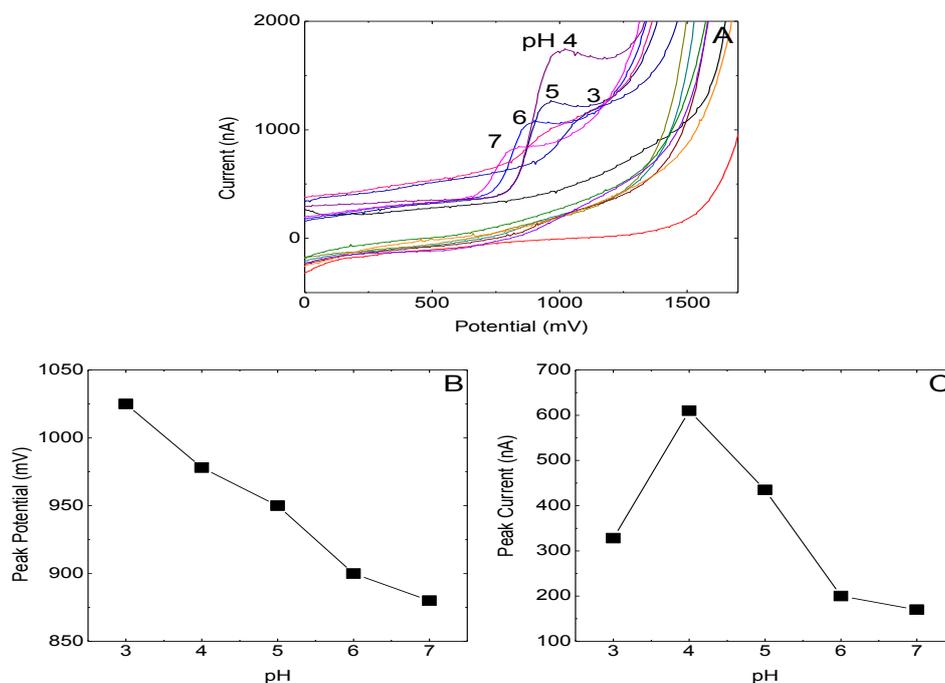

Fig. 1: (A) Cyclic voltammograms of 0.5 mM penicillin G at BDDE in BRB at various pH denoted by number above individual curves. (B) Dependence of anodic CV peak potential of 0.5 mM penicillin G on pH. (C) Dependence of anodic CV peak current of 0.5 mM penicillin G on pH.

The CV peaks of PG shift to the left (towards less positive) with increase of pH from 4 to 7 (**Fig. 1A,** notice the peak position at pH of 4). The same pH was found optimum in other studies summarized in **Table 2**. It follows the relationship of peak potential (E_p, mV) = -52.8 pH + 1130.6 (correlation coefficient of r = 0.99). This strong relationship (showed by a high r-value) demonstrates the involvement of protons during PG oxidation.

Fig. 1 also shows the dependence of PG oxidation peak current on pH in BRB. The highest, best developed and most obvious peak was obtained at pH 4 (using peak potential of +900 mV and peak current of 622 nA), which was later taken as the optimum pH and used during optimization of other voltammetric parameters.

Mechanism of Oxidation of PG

Oxidation of PG is suggested to be on the β-lactam backbone, not from the chain side because of the presence of the phenol group in the side chain (such as in the oxidation of amoxicillin), as also suggested elsewhere [22]. The proposed PG oxidation reaction is presented in the inset of **Fig. 2**: sulfide moiety of the β-lactam ring is oxidized into a sulfoxide derivative by the involving water, two protons and transfer of two electrons. Based on the slope of plot between E_p and pH, the number of proton transferred in the reaction (**Fig. 1B**), the slope equals 2.303 RT/αnF, where α is the electron transfer coefficient (with a value of approximately 0.5 for a totally irreversible electrode process), n is the number of electrons involved in the reaction and the value of RT/F in room conditions is 0.02693 mV. The value of n was then estimated to be 2.2, which is approximately 2. This finding is in agreement with others [22].
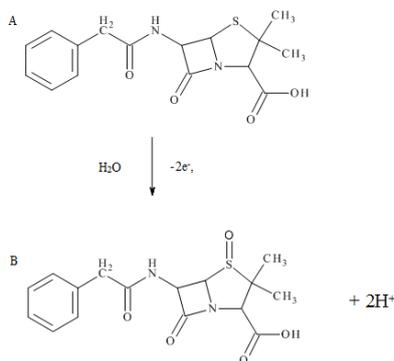

Fig. 2: Mechanism of electro-oxidation of (A) Penicillin G to (B) Beta-sulfoxide at BDDE

The Influence of pH on DP Voltammograms

Based on the results obtained from CV data, DPV was conducted on a series of PG of 1-5 μM, with an E_i of 0.5 V and scan rate of 20 mV/s in BRB of pH 4 (**Fig. 3A**). A range of E_i of 0-0.5 V was tested to see its effect on the peak. Increasing E_i slightly increased peak height, with the highest value of 37.8 nA for E_i of 0.5 V (**Fig. 3B**). Hence, E_i of 0.5 V was taken as the optimum potential.

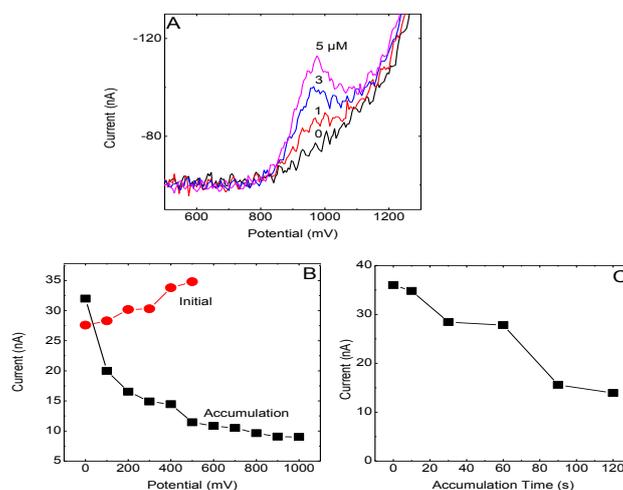
Fig. 3: (A) DP voltammograms of penicilin G at BDDE in BRB pH 4 PG is denoted by numbers above the voltammograms. (B) Effect of initial and accumulation potential on peak current (C) Effect of accumulation time on peak current. The tests were performed at scan rate of 50 mV/s and PG of 5 μM.

The LOD and LOQ of PG using BDDE as working electrode is 0.23 µM and 1.5 µM, respectively. This value was obtained from voltammograms and analysis of a calibration curve (**Fig. 4,** detailed analysis on the curve is presented in **Table 1**). From calibration curve data, LOD is calculated based on 3s/m definition, while LOQ was measured based on 10 s/m, in which s is standard deviation of the signal and m is the slope of calibration curve. The curve was constructed by measuring the oxidation peak of PG solution in a range of 1-9 µM (five replicates). The peak current measurements were performed under optimum parameters obtained earlier (Section 3.1: BRB of pH 4, E_i = 0.5 V, E_{final} = 1.5 V, pulse width of 0.1 s and pulse amplitude of 0.05 V).
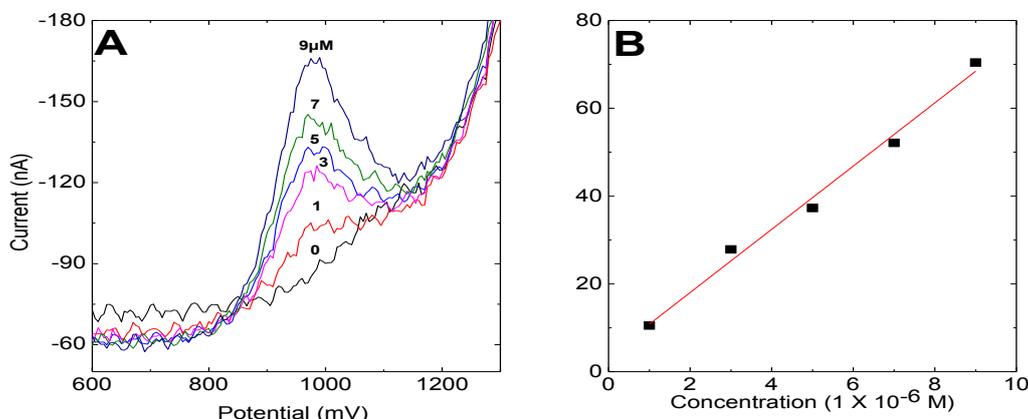

Fig. 4: (A) DP voltammograms of Penicillin G at BDDE in BRB pH 4 at different penicilin G concentrations (given above the curves). (B) The Corresponding Calibration Straight lines (r=0.99).

Table 1: Parameters applied for preparing calibration curve for penicillin G determination (deionized water). The data for drinking and river water samples were from testing PG in real samples.

Matrix samples	C (µM)	Slope (nA µM)	Intercept (nA)	r	LOD (µM)	LOQ (µM)
Deionized water	1.00-9.00	7.20	4.03	0.99	0.23	1.5
Drinking water	0.90-8.10	6.78	3.14	0.99	0.38	1.8
River water	0.90-8.10	7.20	3.63	0.99	0.48	1.9

C: concentration; *r*: correlation coefficient; LOD: limit of detection

Adsorptive Stripping Voltammetry (AdSV) Optimization of PG on BDDE

To evaluate the AdSV, the effect of E_{acc} and t_{acc} were evaluated. We performed the tests using constant PG of 5 µM and constant scan rate of 50 mV/s. The E_{acc} parameter was varied within 0-1 V. PG does not accumulate on the surface of electrode at low E_s because as E_{acc} increases, it rapidly decreased the peak currents (**Fig. 3B**).

A series of time dependant experiments (0-120 s) were performed to evaluate the effect of t_{acc} and (if present) to envisage the optimum time for the analyte to accumulate and adsorbed on the surface of BDDE. The peak current decreased continuously as t_{acc} increases, with non-linear trend (**Fig. 3C**). The enhanced peak current at t_{acc}=0 indicates that the process is independent of applied potential and deposition occurs by adsorptive phenomenon. Overall results suggest that despite depositing on the BDDE surface, PG does not accumulate. This finding shows that BDDE is a suitable working electrode for determination of PG in combination with DPV technique.

Interference of Ions

Addition of selected ions into the PG solution samples did not interfere the measurements at all ranges (data not shown). This finding demonstrates the reliability of this developed method. Five types of cations and three types of anions were used to observe their interference effect on the peak current. Cations of Fe^{3+}, Al^{3+}, Ca^{2+}, Mg^{2+}, Na^+ and anions of NO_3^-, CO_3^{2-} and SO_4^- are commons in river water and were thus applied. Those ions were loaded up to a fixed concentration of 5 µM in the sample solutions containing 5 µM of PG.

The resistance of BDDE from interference ions was expected since BDDE is a highly selective electrode, in this case with respect to PG. The finding also demonstrates the capability of this method to potentially measure PG in saline water such as sea water not only in surface water as tested in this study. However, interference might be occurred when PG exist with other similar electro-active compound and this should be confirmed in a further study. This can be connected with one of the advantages of the

boron-doped diamond electrode surface which is a good resistance to surface fouling due to weak adsorption [40,41].

Assessments of the Proposed Method

Table 2 compares the results of this study with other reported results for determination of penicillin groups. Three working electrodes have proven effective: gold electrode, HMDE (hanging mercury drop electrode) and BDDE, in combination with either CSV or SWV techniques. Only the gold electrode in combination with CSV technique has been used for determining PG [22]. In contrast to this study CSV technique showed a wider linear range of up to 100 µM. We deliberately select a smaller range (up to 9 µM) for anticipating a low concentration of PG commonly found in water sample. Such low concentrations require a very low LOD too. The narrower the range, the lower the "s" resulting is a low LOD, as also demonstrated elsewhere [24].

Table 2: Comparison of voltammetric methods for determination of penicillin.

Woking Electrode	Techniques	Type of Penicillin	Linear range (µM)	Limit of Detection (M)	Ref
BDDE	DPV	G	1.00-9.00	0.23	This study
BDDE	SWV	V	0.40 – 100.00	0.32	[22]
Au Electrode	CSV	G	100.00	-	[23]
HMDE	CSV	Four types of penicillins	0.001-0.09	0.07	[24]

BDDE : boron doped diamond electrode; HMDE: hanging mercury drop electrode; DPV: differential pulse voltammetry; CSV: cathodic stripping voltammetry; SWV: square wave voltammetry, Au: Gold

Measurement in Drinking and River Water Samples

In order to evaluate the applicability of the developed method for the determination of PG in drinking and river water, known amount of PG were added into both samples (**Table 3**) and then subjected the measurement according to the developed method. The measured voltammograms are shown in **Fig. 5**.

The voltammograms correspond well with the standard addition of PG in the tested sample, both river and drinking water. The peaks increase proportionally to an increase in PG loading with a high accuracy (**Table 1**). The relative standard deviations (RSD) between the known and measured concentrations were lower than 2.3% with very narrow (95%) confidence interval. These findings demonstrate the accurate and precise measurement, thanks to the high selectivity of the BDDE (**Table 3**). The overall results demonstrate the effectiveness of PVD technique coupled with BDDE as working electrode to determine PG in water samples.
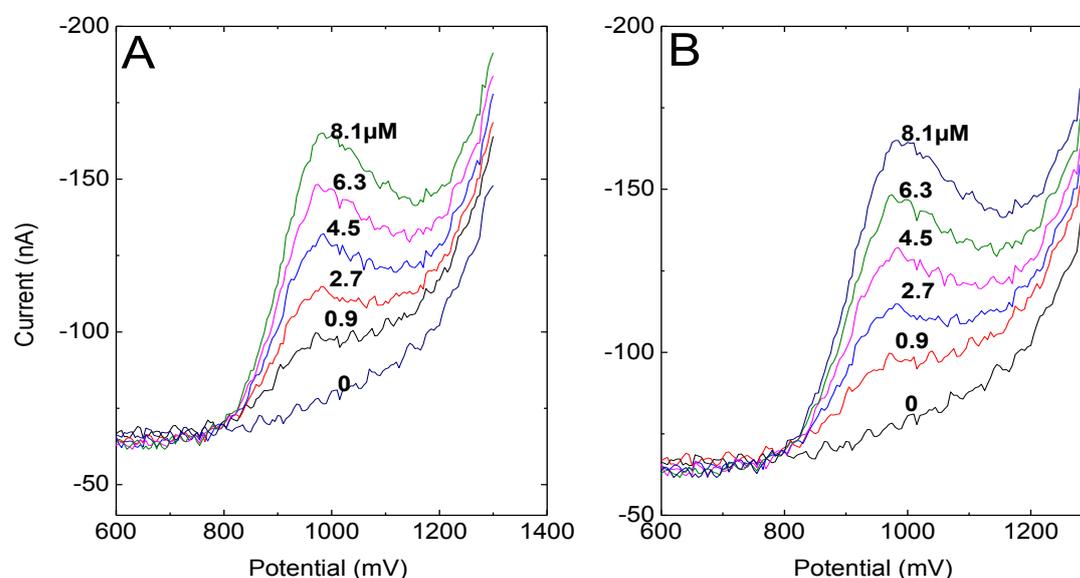

Fig. 5: DP voltammogram of penicillin G at BDDE in BRB pH 4. (A) Drinking water sample. (B) river water samples. 9 ml of the water sample were mixed with 1 ml of BRB. The numbers above the curves correspond to the penicilin G concentration in the water sample.

Table 3: Comparison between known and measured penicillin G concentrations in river and drinking water samples.

Matrix sample	Known PG concentration (μM)	Detected PG concentrations (μM)	Recoveries (%)	RSD (%) n=3	CI μM)
River water	0.90	0.91	101	1.20	0.91 ± 2
	2.70	2.74	101	2.30	2.74 ± 2
	4.50	4.35	97	1.34	4.35 ± 2
	6.30	6.36	100	1.18	6.36 ± 2
	8.10	8.05	99	0.70	8.05 ± 2
Drinking water	0.90	0.88	98	1.42	0.88 ± 2
	2.70	2.76	102	2.14	2.76 ± 2
	4.50	4.45	99	1.68	4.45 ± 2
	6.30	6.31	100	0.42	6.31 ± 2
	8.10	8.05	99	0.52	8.05 ± 2

RSD: Relative standard deviation. CI: the confidence intervals were calculated on the level of significance (α) = 0.05

CONCLUSION

This study demonstrates a simple yet effective DPV at BDDE determination of PG in drinking and river water model samples with high accuracy, low LOD and high reproducibility. This method gives linear calibration curve in the concentration range from 1μM to 9μM at potential of +0.9V. The optimum conditions for gaining reliable results are pH of BRB 4 and at E_i of 0.5 V, The practical applicability of these methods was confirmed by the determination of PG in model samples proving that the newly developed method is rapid and robust alternative method.

ACKNOWLEDGMENT

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