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Quantification of sub-nanomolar levels of Penicillin G by differential pulse adsorptive stripping voltammetry

S. Abbasi,[a]* K. Khodarahmian[a] and A. Farmany[b]

A novel selective and sensitive method is developed for determination of Penicillin G by Differential Pulse Adsorptive Stripping Voltammetry (DPAdSV). Penicillin G gave well-resolved diffusion-controlled cathodic peaks at −0.42 and −0.584 V, respectively (*vs* Ag/AgCl) in pH 7.50 of borate buffer. Optimal conditions were obtained as pH 7.50, accumulation potential of −0.2 V (*vs* Ag/AgCl), accumulation time of 120 s, and scan rate of 100 mV/s. Under the optimized conditions, a linear calibration curve was established for the concentration of Penicillin G in the range of 0.007–2.13 μg/ml with a detection limit of 0.000717 μg/ml. The procedure was successfully applied to the determination of Penicillin G in various medicine and biological samples. The relative standard deviation of the method at 0.05 and 0.5 μg/ml Penicillin G, for 10 runs, was 2.55% and 2.06%, respectively.

Keywords: Penicillin G; differential pulse adsorptive stripping voltammetry; medicine and biological samples

Introduction

Penicillin is a group of antibiotics derived from Penicillium fungi. Penicillin antibiotics are historically significant because they are the first drugs that were effective against many serious diseases, such as syphilis and Staphylococcus infections. Penicillins are still widely used today, though many types of bacteria are now resistant. All penicillins are Beta-lactam antibiotics and contain bulky side-chains and a carboxylic grope (Figure 1) which are used in the treatment of bacterial infections caused by susceptible, usually Gram-positive, organisms.

Penicillins are among the oldest and most frequently prescribed natural antimicrobial. These compounds have high selectivity and low toxicity because they are adiaphorous to human cells which do not have a cell wall. Owning to the extensive application of these drugs in clinic,[1–3] the toxic side-effects from these drugs are generally minimal.[4–6] Because antibiotics are frequently administered in large doses, it is sometimes important to be able to monitor their concentrations in serum, especially in patients with impaired renal function. Several reports for the determination of penicillin antibiotics have been developed, including chemiluminescence,[7,8] potentiometry and amperometry using biosensors,[9–18] potentiometric ion-selective electrodes based on anion exchangers,[19,20] capillary electrophoresis,[21] spectrophotometry,[22–25] liquid chromatography,[26,32] high performance liquid chromatography (HPLC),[28,29] direct current and differential pulse polarography (DPP),[30] tandem mass spectrometry,[27,32] *etc*. HPLC was very useful for the determination of trace penicillin antibiotics but it needed complex pretreatments. Although some other reported methods have their respective advantages, they also have some deficiencies in the sensitivity, selectivity, simplicity, and unsuitability for automatic or continuos analysis. So, it is necessary to develop a simpler, more sensitive, and selective method for the determination of penicillin antibiotics.

At this time there is no report on the differential pulse adsorptive stripping voltammetry determination of Penicillin G. In this paper we report a Differential Pulse Adsorptive Stripping Voltammetry (DPAdSV) procedure for determination of Penicillin G. The method is applied to the determination of Penicillin G in various medicine and biological samples with satisfactory results.

Experimental

Apparatus

DPAdSV measurements were made using a 746 VA-Trace Analyzer, (Metrohm, Switzerland Herisau) connected to an electrode stand, 747 VA-Stand, (Metrohm, Switzerland Herisau) The three-electrode configuration was used comprising a Metrohm multimode electrode (MME) in hanging mercury drop electrode (HMDE) state as working electrode, a double junction Ag/AgCl (3M KCl, saturated AgCl, and 3M KCl in the bridge) reference electrode and a Pt wire auxiliary electrode. All potentials quoted are relative to the Ag/AgCl reference electrode. A rotating Teflon rod stirred solutions in the voltammetric cell. The mercury was triple-distilled quality, and the medium drop size of the HMDE was selected. All experiments were done at room temperature. pH measurements were made with a Metrohm pH meter model 780 (Switzerland Herisau). Eppendorf reference variable micropipettes (10–100 and 100–1000 μl) were used to pipette microlitre volume of solutions. All glassware and storage bottles were soaked in 10% HNO_3 overnight and thoroughly rinsed with water prior to use.

* *Correspondence to: S. Abbasi, Department of Chemistry, Ilam University, Ilam, Iran. E-mail: s.abbasi@mail.ilam.ac.ir abosina2000@yahoo.com*

a *Department of Chemistry, Ilam University, Ilam, Iran*

b *Islamic Azad University, Young Researchers Club, Hamedan Branch, Hamedan, Iran*

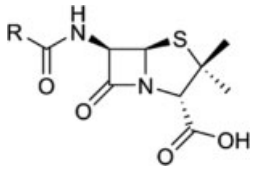

Figure 1. Structure of Penicillin G.

Reagents and solutions

All solutions were prepared with doubly distilled water. The stock solutions of 1.0×10^{-3} M Penicillin G (Merck D-6100 Darmestadt, Germany) was prepared by dissolving 0.0356 g of the compound in 100.0 ml of water. Borate buffer solutions (pH 5.5–9.0) were prepared by mixing different amounts of boric acid, 0.1 M, and NaOH, 0.1 M, in a 100.0 ml volumetric flask.

Procedures

The supporting electrolyte solution (10 ml of 0.1 M $NaOH/H_3BO_3$ buffer solution, pH 7.50) containing 6.0×10^{-6} M Penicillin G was transferred into the electrochemical cell and purged with nitrogen for at least 1 min. The accumulation potential (−0.2 V *vs* Ag/AgCl) was applied to a fresh mercury drop while the solution was stirred for a period of 120 s. After 120 s of accumulation time, the stirring was stopped and voltammograms were recorded from −0.2 V to −1.00 V with a potential scan rate of 100 mV/s and pulse amplitude of 50 mV. All data were obtained at room temperature.

Sample preparation and determination

In order to demonstrate the application of the reported method in practical analysis, the procedure was employed to detect Penicillin G in tablet, dose, vial, and urine samples that were prepared as follows:

Determination of Penicillin G in tablet

Five tablets (500 mg per tablet) were powdered and a quantity of the powder was dissolved, filtrated, and transferred into a 100.0-ml volumetric flask. The flask was diluted to the mark and mixed thoroughly. Then, the above solution was diluted to the working solution. The concentration of Penicillin G in the working solution was determined according to the general procedure under the optimum conditions by DPAdSV method. The results of determination of tablet are listed in Table 1.

Table 1. Determination of Penicillin G in vial, tablet, and dose samples

Sample	Added (μg/ml)	Found (μg/ml)	Recovery (%)	Reference method
Vial	0	0.833	–	0.829
	0.05	0.882	98	0.884
	1	1.834	100.1	1.831
Tablet	0	0.634	–	0.638
	0.05	0.682	97.8	0.675
	1	1.633	99.97	1.62
Dose	0	0.468	–	0.471
	0.05	0.517	98.2	0.52
	1	1.468	100.06	1.46

Table 2. Determination of Penicillin G in urine samples

Sample	Added (μg/ml)	Found (μg/ml)	Recovery (%)	Reference[a] method
Urine	0	0.436	–	0.44
	0.05	0.486	100.02	0.479
	1	1.415	97.9	1.4

[a] Differential pulse polarography method

Determination of Penicillin G in dose

Dose (250 mg) was dissolved, filtrated, and transferred into a 100.0-ml volumetric flask. The flask was diluted to the mark and mixed thoroughly. Then, the above solution was diluted to the working solution. The concentration of Penicillin G in the working solution was determined according to the general procedure under the optimum conditions by DPAdSV method. The results for the determination of dose are listed in Table 1.

Determination of Penicillin G in vial

Vial (1200 mg) was dissolved, filtrated, and transferred into a 100.0-ml volumetric flask. The flask was diluted to the mark and mixed thoroughly. Then, the above solution was diluted to the working solution. The concentration of Penicillin G in the working solution was determined according to the general procedure under the optimum conditions by DPAdSV method. The results for the determination of vial are listed in Table 1.

Determination of Penicillin G in urine

The fresh urine sample was taken. Deproteinization of the sample was achieved by adding 2 ml of 10% trichloroacetic acid and centrifuging the mixture at 4500 rpm for 20 min. Then 5.0 ml aliquot of the supernatant fluid was taken into a 10.0-ml calibrated flask for determining Penicillin G concentration according to the general procedure. The accuracy was tested by a standard addition method. The results of the determination of urine are listed in Table 2.

Results and discussion

Figure 2 shows the differential pulse adsorptive stripping voltammograms of Penicillin G at pH 7.50 (borate buffer), after accumulation at −0.2 V for 120 s on an HMDE. The sample solution containing the Penicillin G shows two peaks at −0.42 and −0.584 V in pH of 7.50. These peak currents increased with increasing accumulation time before the potential scan. The effects of the potential scan rate on the current showed that the cathodic peak increase with increasing the scan rate from 20 to100 mV/s, thereafter the peak height decreased.

Effects of variables

To obtain the best sensitivity in determination of Penicillin G, the influence of different parameters such as pH, deposition time and potential and scan rate were investigated.

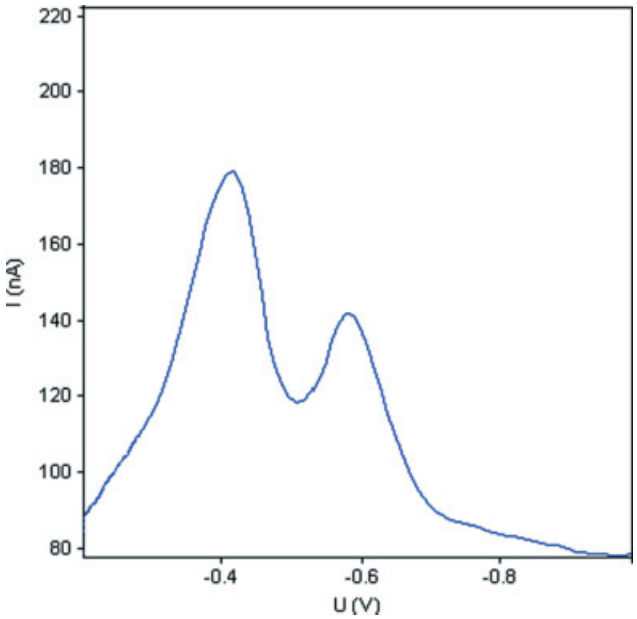

Figure 2. Deferential pulse adsorptive stripping voltammograms of Penicillin G; conditions: Penicillin G, 2.13 µg/ml, pH, 7.8; accumulation potential, −0.2 V; accumulation time, 120 s; scan rate, 100 mV/s.

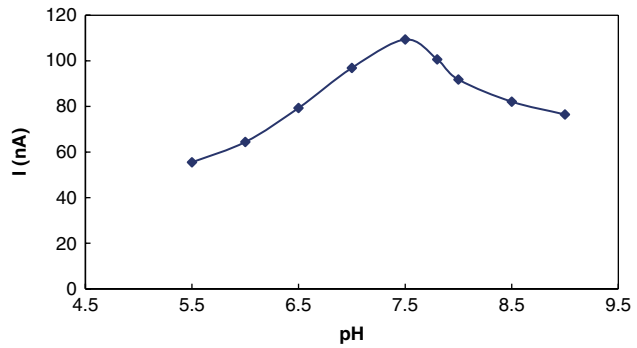

Figure 3. Effect of pH on the peak currents; conditions: Penicillin G 2.13 µg/ml, accumulation potential, −0.1 V; accumulation time, 50 s; scan rate, 60 mV/s.

Influence of supporting electrolyte and pH

Preliminary experiments were carried out with different types of buffers such as acetate, phosphate, citrate, borate, phthalate, Britton-Robinson, ammonia-ammonium, and TRIS. The results showed that the peaks shape for Penicillin G was improved in the presence of borate buffer solution. Therefore, borate buffer was used for optimization of pH. The influence of pH on the cathodic stripping peak currents of Penicillin G was studied in the pH range of 5.5–9.0 of borate buffer (t_{acc} = 50 s. and E_{acc} = −0.1 V). The results are shown in Figure 3. The results show that the peak currents for Penicillin G increase with increasing the pH to about 7.50. Thus, pH of 7.50 was adopted for further studies.

Influence of accumulation potential

The effect of the accumulation potential on the peak heights of Penicillin G was studied in the range of 0.0 to −0.5 V (t_{acc} = 50 s). As shown in Figure 4, the accumulation potential of −0.2 V has better sensitivity for Penicillin G. So an accumulation potential of −0.2 V was used for the optimized analytical procedure.

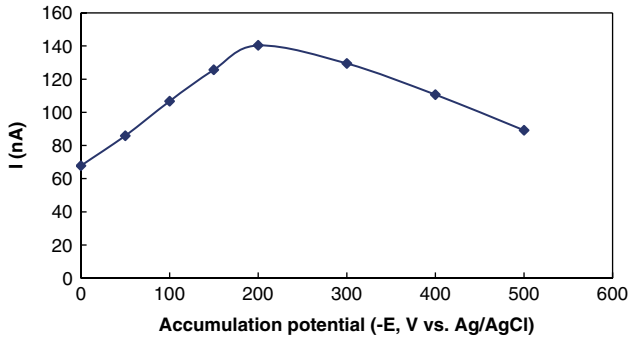

Figure 4. Effect of accumulation potential on the peak currents; conditions: Penicillin G, 2.13 µg/ml, pH, 7.8; accumulation time, 50 s; scan rate, 60 mV/s.

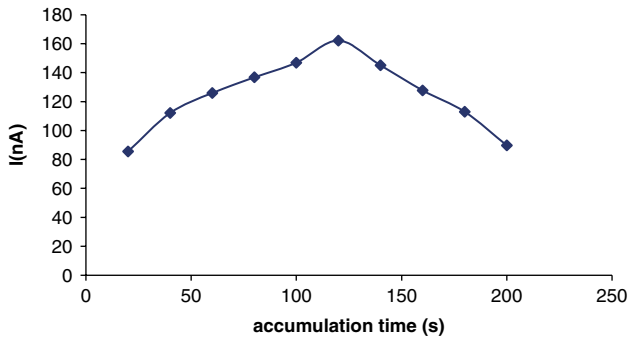

Figure 5. Effect of accumulation time on the peak currents; conditions: Penicillin G 2.13 µg/ml, pH, 7.8; accumulation potential, −0.2 V; scan rate, 60 mV/s.

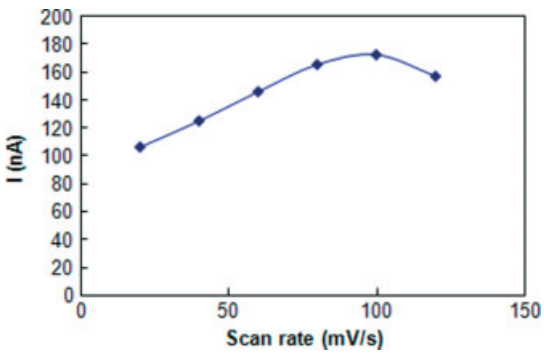

Figure 6. Effect of scan rate on the peak currents; conditions: Penicillin G 2.13 µg/ml, pH, 7.8; accumulation potential, −0.2 V; accumulation time, 120 s.

Influence of accumulation time

The effect of the accumulation time on the stripping peak currents of Penicillin G in the range 20–200 s (E_{acc} = −0.2 V) is illustrated. As shown in Figure 5, the peak currents increased initially with increasing pre-concentration time, indicating that before adsorptive equilibrium is reached, the longer accumulation time, the more Penicillin G was adsorbed and thus the peak currents become larger. However, after a specific period of accumulation time, the peak currents tend to level off slowly as the equilibrium surface concentration of the adsorbed Penicillin G was approached. Therefore, an accumulation time of 120 s was selected for further investigations.

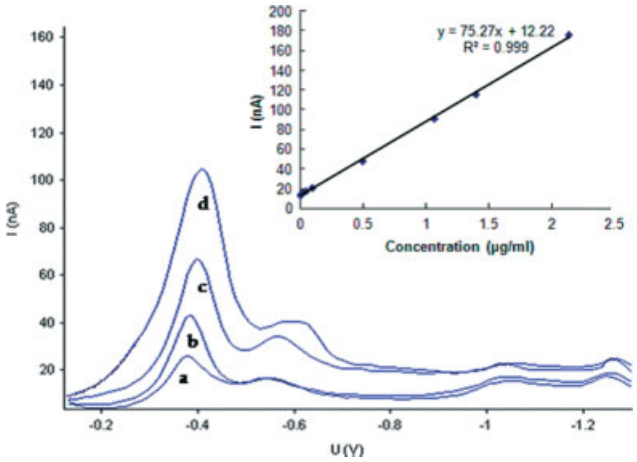

Figure 7. Typical voltammograms for determination of Penicillin G under optimum conditions. (a) 0.007 µg/ml Penicillin G. (b) 0.017 µg/ml Penicillin G. (c) 0.05 µg/ml Penicillin G. (d) 0.1 µg/ml Penicillin G.

Table 3. Interference study for Penicillin G determination

Species	Tolerance limit $W_{ion}/W_{Penicillin\ G}$
Na(I), K(I)	500
ClO_3^-, IO_3^-,	200
BrO_3^-, I^-, Cl^-, F^-, SCN^-, CN^-	100
Al(III), Fe(III)	50
Cd(II), Zn(II), Ni(II), Co(II)	20
Pb(II), Cu(II)	10

Influence of scan rate

Figure 6 depicts the effect of scan rate on the stripping peaks of Penicillin G in the optimal conditions described above. The results show that the peak height for Penicillin G increase nearly from 20 to100 mV/s and in larger scan rates the sensitivity decreases. Therefore, the scan rate of100 mV/s was selected.

Linear range, detection limit, and precision

To verify the linear relationship between peak currents and Penicillin G concentrations, a calibration graph was plotted under optimum conditions (pH 7.50, a deposition potential of −0.2 V and 120 s deposition time) and is shown in Figure 7. The calibration equation, obtained by least-squares method, is: I = 75.275C(µg/ml) +12.224 (r^2 = 0.9991), where I is the peak current (nA). The stripping peak current of Penicillin G was found to be directly proportional to the Penicillin G concentration in the range of 0.007–2.13 µg/ml. The relative standard deviation for 10 replicate analyses of a solution containing 0.05 µg/ml and 0.5 µg/ml Penicillin G was 2.55% and 2.06%, respectively. A detection limit of 0.000717 µg/ml of Penicillin G was estimated from 10 replicate determinations of blank solution under optimum conditions.

Interference study

Possible interference of other species in the adsorptive stripping voltammetric determination of Penicillin G was studied by addition of the interfering ion to a solution containing 30 µg/ml of Penicillin G using the optimized conditions (the criterion for interference was a 5% error in the peak heights of Penicillin G). The results of this study are summarized in Table 3. It was found that most of the foreign ions did not interfere in the Penicillin G determination.

Table 4. Comparison between the detection limit of the proposed method with the other reported methods

No.	Method	LOD	Reference
1	Chemiluminescence	0.01 µg/ml	[7]
2	Flow-injection-chemiluminescence	0.07 µg/ml	[8]
3	Capacitive Penicillin G sensor	50 µg/ml	[9]
4	Pyrolysis-negative ion mass spectrometry	0.01 µg/ml	[10]
5	Continuous-flow/stopped-flow system	0.343 µg/ml	[11]
6	ISFET-based Penicillin G sensor	1.715 µg/ml	[12]
7	Amperometric pH-sensing sensors	722 µg/ml	[18]
8	Spectrophotometry	0.45 µg/ml	[22]
9	Spectrophotometry	0.06 µg/ml	[23]
10	Spectrophotometry	0.6 µg/ml	[24]
11	tandem mass spectrometry	0.002 ppm	[27]
12	HPLC	0.008 µg/ml	[28]
13	HPLC	8.9–11.1 µg/kg	[29]
14	Direct current polarography†	8–200 µg/ml	[30]
15	Differential pulse polarography	2–160 µg/ml	[30]
16	Liquid chromatography	1.1 µg/ml	[31]
17	Liquid-liquid extraction method for HPLC-DAD	0.05 µg/ml	[32]
18	Flow-injection analysis-solid phase extraction (FIA-SPE)	0.012 µg/ml	[33]
19	Liquid chromatography-electrospray ionization tandem mass spectrometry	0.002 µg/ml	[34]
20	DPAdSV	0.000717 µg/ml	This work

† 8–200 µg/ml and 2–160 µg/ml are linear range no detection limit.

Application

To investigate the applicability of the proposed method for the determination of Penicillin G, the method was applied to the determination of Penicillin G in various medicine samples (tablet, vial, and dose) and biological samples (urine) by standard addition method. The results are given in Tables 1 and 2.

Comparison of the sensitivity of the proposed method and other previously reported detection methods

Table 4 compares the detection limit of the proposed method with the other reported methods. As shown in Table 1, the detection

limit of the reported method is lower than the best previously reported methods.

Conclusion

A novel method is developed for the determination of trace amount of Penicillin G by DPAdSV. The proposed method is sensitive, precise, selective, and simple for determination of Penicillin G.

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