International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Voltammetric Determination of Penicillin G in Sodium Dodecyl Sulfate/acetate Buffer Media on Glassy Carbon Electrode

Joshua M. Sila[a*], Peterson M. Guto[b], Immaculate N. Michira[c], Francis B. Mwaura[d]

[a,b,c]*Department of Chemistry, University of Nairobi, P. O. Box 30197-00100, Nairobi, Kenya*

[d]*School of Biological Sciences, University of Nairobi, P. O. Box 30197-00100, Nairobi, Kenya*

[a]*Email: jaysilla@gmail.com*

[b]*Email: Peterson.guto@uonbi.ac.ke*

[c]*Email: imichira@uonbi.ac.ke*

[d]*Email: fbmwaura@uonbi.ac.ke*

Abstract

The presence of residues of penicillin in food products like milk and meat of animal origin exerts negative impact on public health such as drug resistance diseases and severe allergic responses. This work reports development of a simple voltammetric method for detection of penicillin using sodium dodecyl sulfate (SDS) in acetate buffer solution (ABS) on glassy carbon electrode. Addition of SDS to the penicillin G containing acetate buffer solution (ABS) was found to enhance the voltammetric oxidation current signal by about 5 times with insignificant shift of the oxidation potentials. Using cyclic voltammetry, the oxidation potentials for penicillin G were found to be 1.65V vs. Ag/AgCl in SDS/ABS, pH 4.5 and 1.60V vs. Ag/AgCl in ABS, pH 4.5. The diffusion coefficients for penicillin G were found to be 6.01×10^{-7} cm^2/sec and 1.39×10^{-6} cm^2/sec in ABS, pH 4.5 and SDS/ABS, pH 4.5 respectively. Linear concentration range were also investigated using square wave voltammetry and found to lie in the range of 1.25 – 15µM penicillin G in SDS/ABS, pH 4.5 and 2.5 – 10µM penicillin G in ABS, pH 4.5.

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* Corresponding author.

Limits of detection were also found to be 1.25µM and 2.5µM penicillin G in SDS/ABS, pH 4.5 and ABS, pH 4.5 respectively while limits of quantitation were 3.75µM penicillin G in SDS/ABS, pH 4.5 and 7.5µM penicillin G in ABS, pH 4.5. Possible interferants like Na^+, K^+, Zn^{2+}, Ca^{2+}, Fe^{3+}, Cl^-, NO_3^-, PO_4^{3-} and SO_4^{2-} did not have any significant effect on the anodic currents and oxidation potentials of the penicillin G. These results show that the developed method is sensitive enough for use in the analysis of penicillin G in diverse real samples.

Keywords: Penicillin G; sodium dodecyl sulfate; acetate buffer; voltammetry and detection limits.

1. Introduction

Penicillin is antibacterial agent belonging to a beta-lactam group of antibiotics. Beta lactam antibiotics are characterized by presence of a high reactive four member ring, consisting of three carbons and nitrogen. Common penicillins include penicillin V, penicillin G, Amoxicillin and oxacillin among others. Due to their strong antibacterial activity, they are widely used in humans and in animal husbandry to control and manage infections caused by bacteria [1]. In animal husbandry, penicillin G is used in treatment of masitis.

Misuse of antibiotics have been a great concern, especially due to use of these drugs when they are not really needed. In some cases they are added in animal feeds to suppress microbiota in the small intestine. This ensures maximum absorption of digested food and promotes faster growth especially for food-producing animals and protects the animal from toxins produced by the microbiota [2]. Antibiotics are also used to prevent outbreaks of diseases especially when animals are known to be more susceptible.

Large percentages of the drugs are eliminated via urine and feaces unchanged in land and water sources [3]. Besides being in water sources these drug residues, have also been found in human foods especially milk products due to failure to withhold milk during withdrawal time [4, 5]. Penicillin residues are the most common and of particular concern because of adverse effects on people who are allegic to penicillins [6]. Their presence in human food and water sources has led to development of resistant microbes. Some of these resistant microbes significantly affects important proceses in production of milk products [7, 8]. Moreover allergic reactions may occur to those who are sensitive to penicillin with concentration as low as 1 ppb [9]. Worse still, some infants have displayed allergic reactions attributed to presence of penicillin in milk [10].

Penicillins are easily hydrolyzed by acids and other agents to form variety of products some of which also cause allergic reactions in humans [11]. The rising cases of antimicrobial resistance has been attributed to misuse of antibiotics among other factors [12]. Penicilins bind to penicillin binding proteins(PBP). These proteins are responsible for cross linking peptide units during synthesis of peptidoglycan. The binding interferes with cell wall synthesis which results to lysis and eventual cell death [13]. Bacteria naturally develops resistance towards drugs as a mechanism of survival. Bacteria mainly resist penicillins by producing beta-lactamase hydrolytic enzymes that disrupt the amide bond of beta-lactam ring rendering the drug ineffective. However dispite development of resistant microbes penicillin G has remained to be one of the most widely used and least expensive antibiotic worldwide and it is one among the WHO list of essential drugs [14].

Several methods have been used to detect penicillins in biological samples and environmental samples. High-performance liquid chromatography coupled with other modes of detection has been the most successful due to its high sensitivity and selectivity but it is expensive and takes long time [15]. The electroanalytical chemical methods are simple, cheap, and fast and can easily be miniaturized to obtain portable sensors for on-site analysis [16]. However they have not been very successful especially for detection of penicillin due to high voltage required and resulting voltammograms are not well defined [17, 18].

Surfactants are known to modify and control properties of electrode surfaces [19]. They have been proven to be effective in electrochemical analysis of pharmaceuticals and compounds of biological origin. It was recently shown that surfactants improve accumulation of electro-active species of some compounds such as ethopropazine and protects the electrode surface from fouling [17, 35]. Entry of electro-active species in this dynamic surface film has been proven as a key step preceding electron transfer on the electrode surface [20].

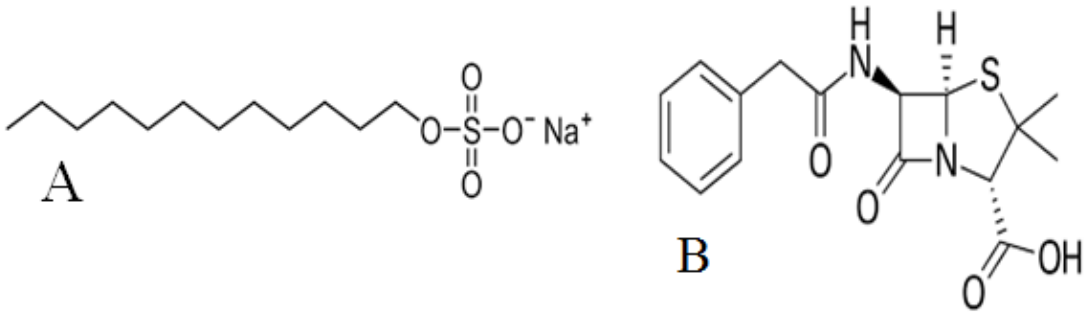

Figure 1: Structural formula of **(A)** Sodium dodecyl sulfate **(B)** Penicillin G

No work on determination of penicillin G on glassy carbon electrode in sodium dodecyl sulfate media has been published in literature. This paper demonstrates the application of sodium dodecyl sulfate (scheme 1A) containing solutions to enhance the oxidation signal and detection of penicillin G (Scheme 1B) without any chemical modification on the glassy carbon electrode surface.

2. Experimental Section

2.1. Reagents

The chemicals used were: sodium dodecyl sulfate, sodium acetate, acetic acid (glacial), all from fisher scientific. They were all of analytical grade and were used without further purification. Acetate buffer (ABS) and acetate buffer containing sodium dodecyl sulfate (SDS/ABS) were used as electrolyte. Only de-ionized water was used throughout this work. The rest of chemicals were reagent grade.

2.2. Apparatus

All the electrochemical analysis were done using CHI 1232B Electrochemical Station (CH Instruments, Inc., USA) in a three-electrode system (CH Instrument Inc., USA).The three electrode system were glassy carbon working electrode of diameter of 3 mm, a platinum wire auxiliary electrode and Ag/AgCl reference electrode.

All pH measurements were done using CyberScan pH Tutor (Eutech Instruments). All electro-analytical procedures were carried out in a 10.0mL electrochemical cell at room temperature. All data were analyzed using Kaleidagraph software, version 4.1.1.

2.3. Polishing the glassy carbon working electrode

A glassy carbon serving as working electrode with total surface area of 0.071cm^2, was polished on wet silicon carbide paper (600 grit, Buehler) [21, 26] and rinsed in water. The working electrode was then polished thoroughly with aluminum oxide slurry of decreasing size to remove redox active products from the electrode surface and other possible contaminants [22, 26]. This procedure was repeated before every use.

2.4. Voltammetry

All electrochemical procedures were done in the cyclic (CV) and square wave (SWV) voltammetric modes. For both CV and SWV, the potentials were scanned between 1.0V and 2.0V.

2.5. Preparation of the acetate buffer

Acetate buffer was prepared by dissolving 1.5g of sodium acetate and 1ml of ethanoic acid in de-ionized water and made up to 500ml. The resulting solution pH was adjusted to 4.5 using ethanoic acid.

2.6. Preparation of sodium dodecyl sulfate in acetate buffer Solution

A solution of sodium dodecyl sulfate (SDS) in acetate buffer was prepared by dissolving 25g of SDS in 100ml acetate buffer prepared above and made up to 250ml mark using the acetate buffer. The pH of the resulting SDS-Acetate buffer was adjusted accordingly using the acetic acid.

2.7. Linearity, Limit of Detection and Limit of Quantitation

Measurements were made for the detection of penicillin G with varying concentrations of penicillin G both in the acetate buffer and in the SDS containing acetate buffer on GC electrode. The linearity of the method was evaluated by using calibration curve to calculate coefficient of correlation, slope and intercept values. Based on three times the standard deviation of the baseline (*equation 1*) [23, 24, 25, 26], the limits of detection (LOD) were estimated for penicillin G.

$$C_{LOD} = \frac{3.s}{m} \qquad (1)$$

The limit of quantitation (LOQ) was calculated using the standard deviation of the response and the slope method expressed as shown in equation 2 [23, 24, 25, 26]:

$$C_{LOQ} = \frac{10.s}{m} \quad (2)$$

2.8. Effect of Impurities in the determination of penicillin G

The effect of interferences were explored by adding the mostly expected possible interferants like Na^{+}, K^{+}, Zn^{2+}, Ca^{2+}, Fe^{3+}, Cl^{-}, NO_3^{-}, PO_4^{3-} and SO_4^{2-}. These ions are known to be present in both penicillin G drugs and environmental and clinical samples. Their effects on the electrochemical oxidation currents and oxidation potentials of penicillin G were monitored by square wave voltammetry.

3. Results and Discussion

In this work, pH 4.5 and 0.347M SDS in acetate buffer solution were found to provide the highest voltammetric signal, consistent with our previous work [26] with penicillin V.

3.1. SWV of penicillin G in presence and absence of Sodium dodecyl sulfate

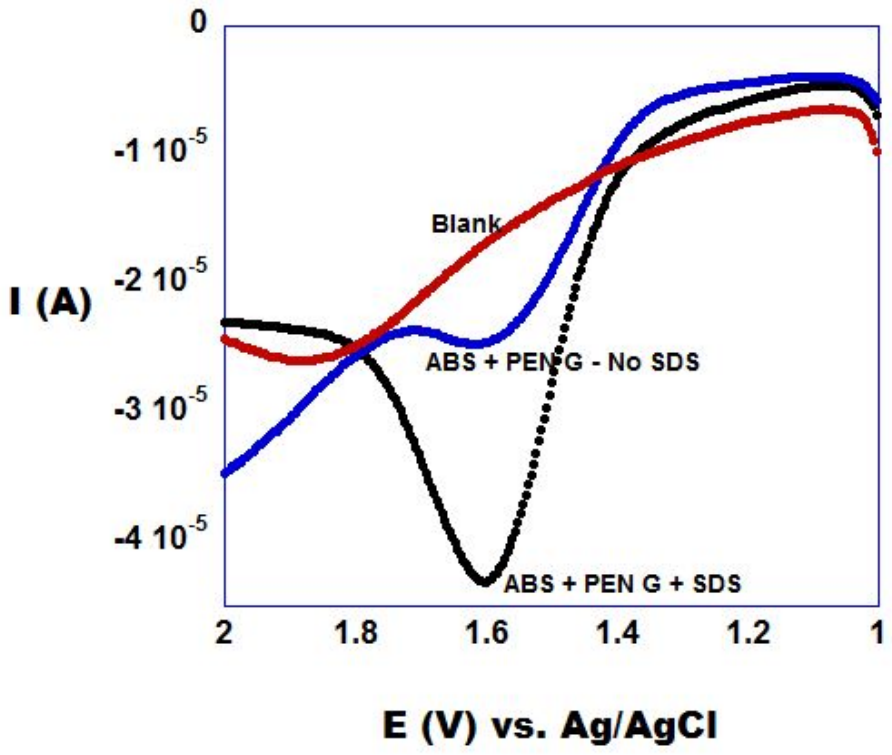

Figure 2: Square wave voltammograms of 2.0mM Penicillin G in ABS, pH 4.5 (**Blue**) and 0.1M Penicillin G in 0.347M SDS/ABS, pH 4.5 (**Black**) at GC electrode. The amplitude and frequency were 0.025V and 15Hz respectively.

Figure 1 shows the square wave voltammetric responses of 2.0mM penicillin G at the GC electrode in ABS, pH 4.5 and SDS/ABS, pH 4.5. In SDS/ABS, pH 4.5 the voltammetric current peaks for 2.0mM penicillin G are remarkably higher compared to ABS, pH 4.5 under similar conditions. This further indicates that adding 0.347M SDS to ABS, pH 4.5 facilitated easier and faster charge transfer at the electrode surface hence significantly increasing the sensitivity for the determination of penicillin G.

3.2. Cyclic voltammetry of penicillin G

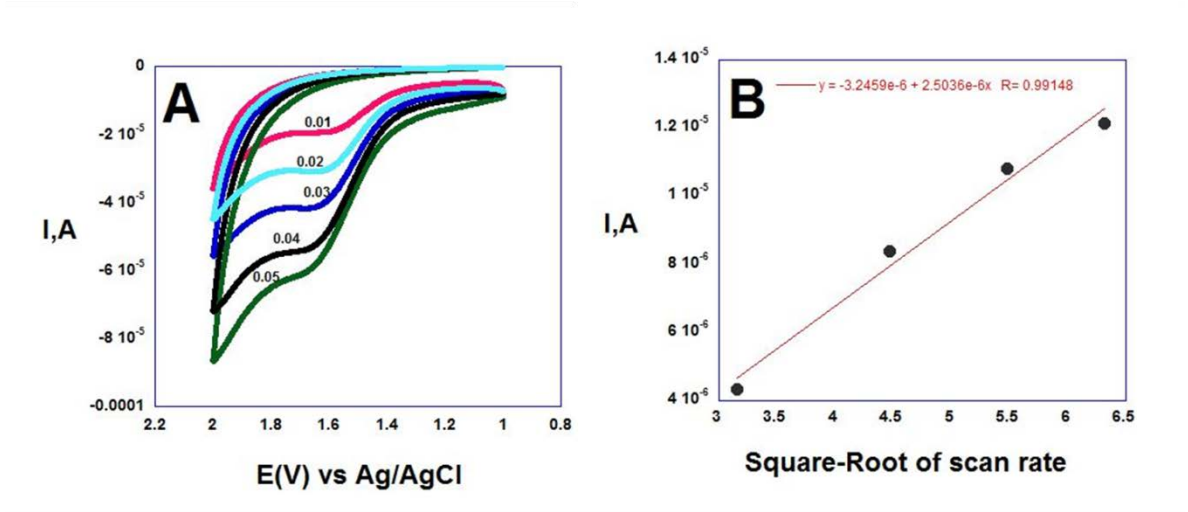

Figure 3: (A) Cyclic voltammogram of 2.0mM penicillin G in ABS, pH 4.5 on glassy carbon electrode. Initial potential: 1.0V; high potential: 2.0V; low potential: 1.0V; sample interval: 0.001V, quiet time: 0.1sec. Scan rates ranging from 50mV/s to 10mV/s **(B)** Plot of oxidation peak currents against square root of scan rate.

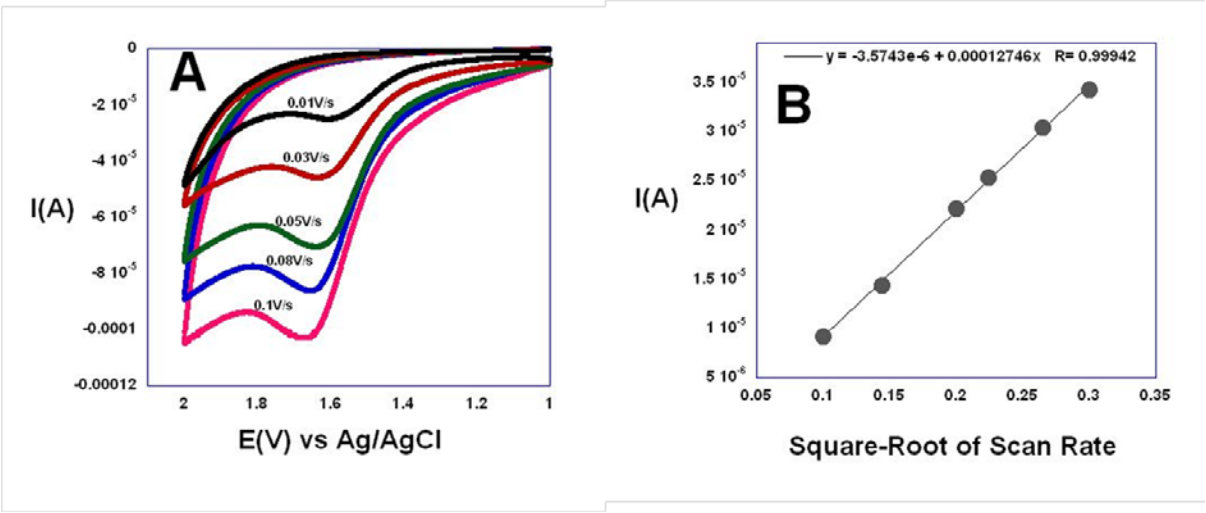

Figure 4: (A) Cyclic voltammogram of 2.0mM penicillin G in 0.347M SDS in ABS, pH 4.5 on glassy carbon electrode. Initial potential: 1.0V; high potential: 2.0V; low potential: 1.0V; sample interval: 0.001V, quiet time: 0.1sec at different scan rates.**(B)** Plot of oxidation peak currents against square root of scan rate.

Cyclic voltammograms of penicillin G at a polished GC electrode gave a well defined irreversible peak at very

high positive potential of 1.6 V versus Ag/AgCl in ABS, pH 4.5 and 1.65V versus Ag/AgCl in SDS/ABS, pH 4.5 (*figures 2A and 3A*). The oxidation potential of penicillin G in presence of SDS (SDS/ABS, pH 4.5) compares relatively well with the potential in absence of SDS (ABS, pH 4.5) on GC electrodes as shown in figures 2A and 3A. The slight difference particularly with respect to the shape of the voltammogram can be attributed to the slight change in the chemistry of the electrolyte. Plots of oxidation peak currents against square root of scan rates are linear in both cases indicating that the electrochemical reaction for the oxidation of penicillin G on glassy carbon electrode is dorminantly diffusion controlled.

We made plots of oxidation currents (i_{pa}) versus the square root of scan rates ($v^{1/2}$) between 0.01 and 0.1 V/s as provided by the Randles–Sevcik equation (3) [27, 28]. We obtained linear plots as shown in figures 2B and 3B. The diffusion coefficients of penicillin G in both acetate buffer solutions and SDS/acetate buffer media were obtained from the slopes of these linear plots.

$$i_{pa} = (2.69x10^5)n^{3/2}C^*AD^{1/2}v^{1/2} \qquad (3)$$

where i_{pa} is the diffusion anodic peak current, n is the number of electrons exchanged in the oxidation process, C^* is the concentration of penicillin G, A is the electrode area, D is the diffusion coefficient, and v is the scan rate. The linear plots obtained at low scan rates indicate that the electrode processes for the penicillin G are diffusion controlled. Accordingly, $1.392x10^{-6}$ cm^2/sec and $6.01x10^{-7}$ cm^2/sec values were obtained in SDS/ABS, pH 4.5 and ABS, pH 4.5 respectively on highly polished glassy carbon electrodes. We speculate a possible interaction between sodium dodecyl sulfate and penicillin G.

This results in a possible pre-concentration of penicillin G on the surface of the electrode. Consequently, a higher diffusion coefficient of penicillin G develops in the vicinity of the electrode surface. Further work is underway to elucidate the actual mechanism leading to the increase of the diffusion coefficient in the presence sodium dodecyl sulfate.

Labomir and his colleagues [15] have demonstrated that the electrochemical oxidation of penicillin requires participation of two protons and two electrons. Both these protons and electrons are involved in this oxidation process. If the site of oxidation of penicillin G is within the β-lactam backbone then the sulfide moiety of the β-lactam ring might be oxidized into a sulfoxide derivative of penicillin G. This involves a water molecule, two protons and transfer of two electrons as shown in Scheme 2.

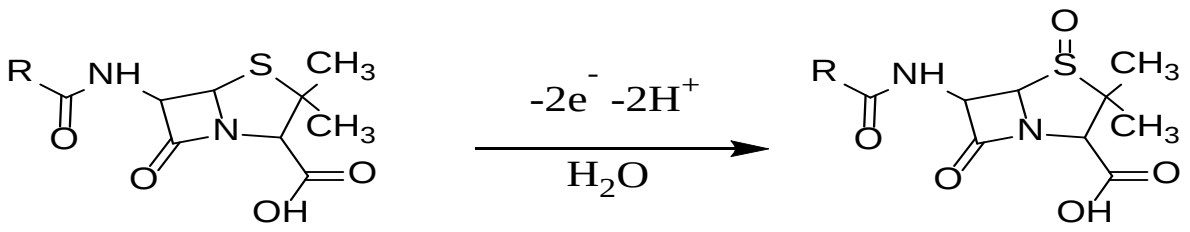

Figure 5: Proposed mechanism of the electrochemical oxidation of penicillin G

3.3. Validating the Proposed Method for penicillin G Detection

To test suitability of penicillin G detection using the proposed square wave voltammetric (SWV) method, the variation in peak current was monitored against the concentration of the penicillin G for at least three times under the optimized conditions (*figure 4A*). The calibration plot of the anodic peak current versus the drug concentration (*figure 4B*) was found to be linear over the range 15.0–1.25µM penicillin G in SDS/ABS, pH 4.5 and linear regression equations are expressed in table 1. There is a clear linear dependence of the current intensity on the penicillin G concentration in SDS/ABS, pH 4.5 over the range 15.0–1.25µM (table 1) as supported by the regression plots.

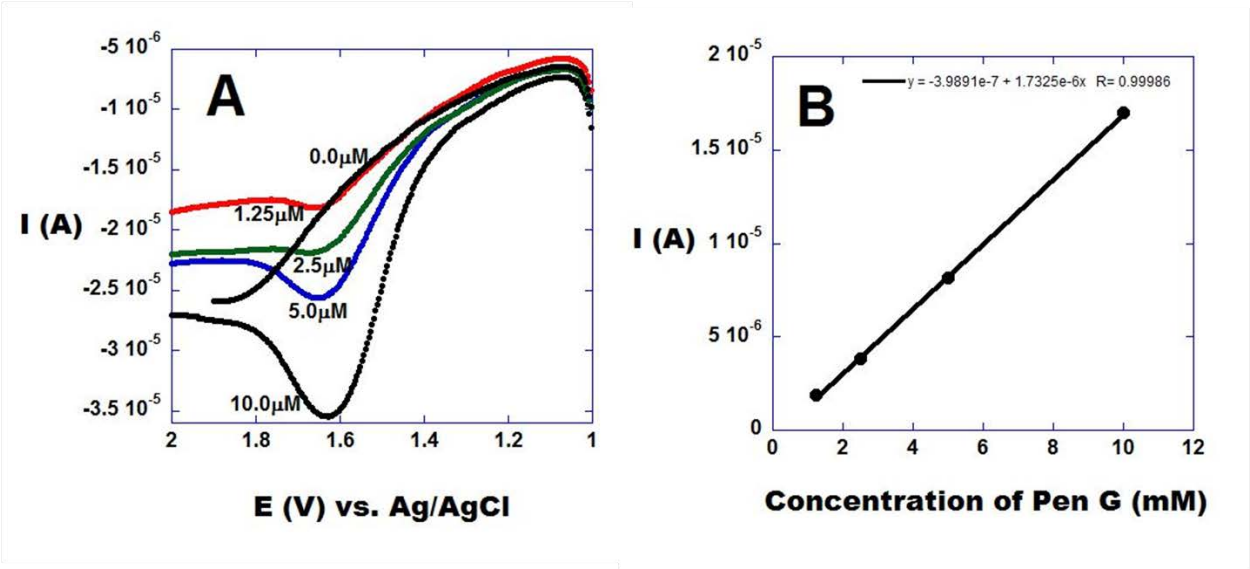

Figure 6: (A) Square wave voltammograms of penicillin G at varying penicillin G concentrations in 0.347M SDS in ABS, pH 4.5; frequency: 15Hz and amplitude 0.025V. **(B)** A plot of concentration of penicillin G versus voltammetric current.

Table 1: LCR, LOD, LOQ, R and regression equation for Penicillin G in ABS, pH 4.5 and ABS/SDS, pH 4.5 on bare Glassy Carbon electrode

	Solvent	LCR	LOD	LOQ	RE	R^2
1.	ABS, pH 4.5	10.0 – 2.5µM	2.5µM	7.5µM	y=-3.2e-6+2.5e-6x	0.99148
2.	SDS in ABS, pH 4.5	15.0–1.25µM	1.25µM	3.75µM	y=-3.99e-6+1.73e-6x	0.99986

LCR = Linear concentration range, LOD = Limit of detection, LOQ = Limit of quantitation, RE = Regression equation.

Limit of detection (LOD) is defined as the lowest concentration level of an analyte that can be determined to be statistically different from a blank and with 99% confidence level [29 – 34]. In this method the LOD was determined to be within the region where the signal to noise ratio is equal or greater than three [29 – 34].

Voltammetric measurements were made for the detection of varying concentrations of penicillin G in the SDS/ABS, pH 4.5. Based on three times the standard deviation of the baseline (*equation 1*), the limits of detection (*table 1*) were estimated to be 1.25µM in SDS/ABS, pH 4.5 while the limit of quantitation (*equation 2*) was found to be 3.75µM. The proposed method is simple, reproducible and less laborious since no surface modification of the electrode is required.

3.4. Interferance Studies

Natural samples like milk, meat and water normally contain quite a number of easily oxidizable and/or reducible substances. Serious interference in penicillin G determination can occur if there is competitive adsorption of these substances onto the electrode surface at uncontrolled concentrations. These interferences arise when these substances reduce and/or oxidize at potentials close to the penicillin G and/or due to poor specificity of the electrode surface and the type of electrolyte. The effects of Na^+, K^+, Zn^{2+}, Ca^{2+}, Fe^{3+}, Cl^-, NO_3^-, PO_4^{3-} and SO_4^{2-} possible interferants were investigated in the determination of penicillin G. These substances did not have any significant effect on the oxidation potential of penicillin G. Further, no interfering peaks were also recorded around the peak potentials of penicillin G in 50-fold excess of these substances. Moreover, most of these substances like Fe^{3+} enhanced the oxidation current by about 20% while others had insignificant effect on the voltammetric currents. Similar signal enhancement by Fe^{3+} was observed in our previous work on penicillin V [26]. Its notable to mention that for trace levels of these interferants in less contaminated real samples, their interfering effect will not pose any significant consequence to the their analysis [36].

4. Conclusion

A simple square wave voltammetric method based on SDS/ABS, pH 4.5 using bare GC electrode has been developed for the determination of penicillin G. The voltammetric current signal due to the penicillin G oxidation process was a function of the amount of penicillin G, pH of the medium and amount of surfactant. Addition of SDS to the penicillin G containing acetate buffer solution was found to enhance the oxidation current signal by about 5 times with insignificant shifts of the oxidation potentials. Linear concentration range for this method was found to be 1.25µM – 15.0µM, limit of detection 1.25µM and limit of quantitation 3.75µM penicillin G in SDS/ABS solution. Possible interferants like Na^+, K^+, Zn^{2+}, Ca^{2+}, Fe^{3+}, Cl^-, NO_3^-, PO_4^{3-} and SO_4^{2-} did not have any significant effect on the anodic currents and oxidation potentials of the penicillin G.

Acknowledgement

This work was supported by the research grants from The Third World Academy of Sciences (TWAS), Italy, Deans' Research Grant (DRG), University of Nairobi and Kenya/south Africa collaborative grant, ref NCST/5/003/2/53.

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