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Square Wave Voltammetric Determination of Penicillin V in Sodium Dodecyl Sulfate Containing Media on Glassy Carbon Electrode

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Abstract

The effect of adding sodium dodecyl sulfate (SDS), a surface-active agent to acetate buffer solution containing penicillin V was investigated. The voltammetric responses of penicillin V on glassy carbon electrode was a function of the concentration of penicillin V, surfactant and pH. Addition of SDS to the penicillin V containing acetate buffer solution (ABS) was found to enhance the voltammetric oxidation current signal by about 10 times with insignificant shift of the oxidation potentials. With this electrochemical method, the optimal pH and SDS concentration were found to be pH 4.5 and 0.347M respectively. Using cyclic voltammetry, the oxidation potential for penicillin V were found to be 1.61V vs. Ag/AgCl in SDS/ABS, pH 4.5 and 1.55V vs. Ag/AgCl in ABS, pH 4.5. Linear concentration range were also investigated using square wave voltammetry and found to lie in the range of 0.04 – 34.6µM penicillin V in SDS/ABS, pH 4.5 and 3.5 – 14.0µM penicillin V in ABS, pH 4.5. Limits of detection were also found to be 0.04µM penicillin V in SDS/ABS, pH 4.5 and 3.5µM penicillin V in ABS, pH 4.5 and limits of quantitation were 0.12µM penicillin V in SDS/ABS, pH 4.5 and 14µM penicillin V in ABS, pH 4.5.

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Foreign substances like Na^+, K^+, Mg^{2+}, Zn^{2+}, Ca^{2+}, Fe^{3+}, Cu^{2+}, Cl^-, NO_3^-, PO_4^{3-} and SO_4^{2-} did not have any significant effect on the voltammetric currents of penicillin V. These results confirm that this electrochemical method is sensitive enough to be used in the determination of penicillin V in diverse environmental and clinical samples.

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

1. Introduction

Penicillins are antibiotics with strong antibacterial activity. They are widely used for clinical purposes and animal breeding practices to eliminate infection-causing bacteria [1]. Common types of penicillins include amoxicillin, ampicillin, carbenicillin, dicloxacillin, oxacillin among others. Flemming warned that misuse of penicillin would lead to development of resistant microbes. The widespread and uncontrolled use of penicillin drugs over years has led to slow but steady growth of resistant microbes, one of the world's greatest health challenge. Antimicrobial resistance (AMR) is not restricted to penicillin drugs alone but has also been observed in other antibiotics. The greatest challenge is that the resistance is developing faster than discovery of better antibiotics. Over time currently manageable infectious conditions such as pneumonia, gonorrhea, and diarrhoea among others may be life-threatening if antibacterial resistance is not arrested [2].

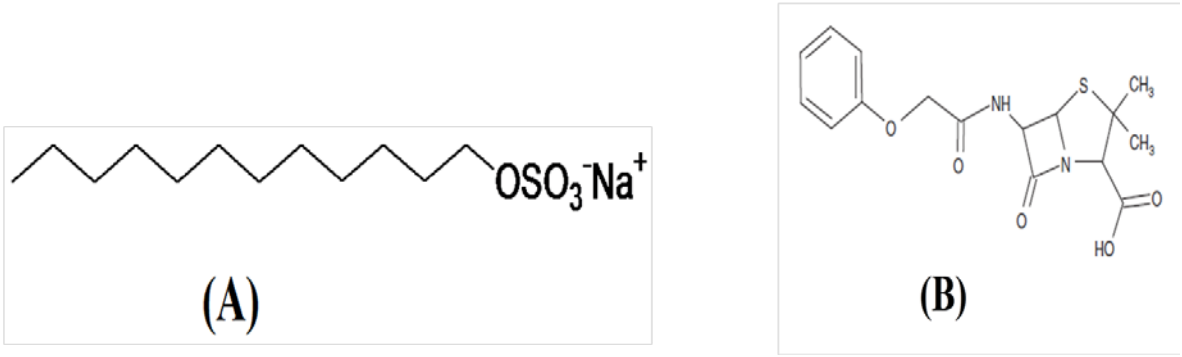
Moreover some patients are known to be allergic to penicillin sometimes with fatal results [3]. Penicillin is also known to reduce the efficacy of some birth control pills and is antagonistic when co-administered with drugs such as methotrexate and probenecid among others. In various situations like when dealing with children or the elderly, patients may not be well informed of the medication they are under. Furthermore for unconscious patients the doctor may not know the medical history of the patient. This calls for a simple, more sensitive and onsite analytical methods or tools to determine levels of penicillin in clinical and environmental samples.

A number of techniques have been used to determine penicillins in various matrices including biological, pharmaceutical, and others have been documented in literature. High-performance liquid chromatography [4, 5] and spectrophotometric [6] techniques have been popularly used for the detection and quantification of penicillins because they are among the most sensitive and selective techniques. However, these techniques are known to be time consuming, expensive and sometimes too laborious, especially when they involve derivatization, extraction and purification procedures.

The electrochemical techniques are known to be superior in the sense that they are simple, low-cost and easily miniaturized. These properties eliminate limitations found in other analytical techniques [7]. On the context of literature survey there is limited information on the electroanalysis of penicillins compared to other drugs. This can be explained by the fact that oxidation of penicillins normally yields poorly defined voltammograms at a very high positive potential. This potentially affects the extent of the potential window from the anodic side of the voltammogram [8, 9].

Surfactants are extensively utilized in chemistry, especially in numerous electrochemical processes [10]. The electrochemical applications include; electroplating [11], corrosion [12], fuel cells [13], electrocatalysis [14],

and electroanalysis [15]. Recently it was demonstrated that surfactants excellently stabilizes the electrochemical response of serotonin by preventing the electrode surface from fouling [8]. It has also been shown that anionic surfactants enhance the accumulation of ethopropazine at gold electrodes [16]. Ascorbic acid and dopamine [17], catechol and hydroquinone [18] have been simultaneously determined in micelles [19]. It's prudent to mention that carbon paste electrodes whose surfaces are known to have hydrophobic characters normally interact with surfactants through surface adsorption. Hence this nature of interaction has made it possible for these carbon paste electrodes modified with surfactants to be used for the determination of diverse number of both inorganic and biological compounds [20, 21].



Scheme 1: Structural formula of **(A)** Sodium dodecyl sulfate **(B)** penicillin V

This work demonstrates for the first time the square wave voltammetric determination of penicillin V in sodium dodecyl sulfate solutions on bare glassy carbon electrode. It's important to mention that there are no reports on this yet published in literature. The results presented shows that presence of sodium dodecyl sulfate (scheme 1A) in the working electrolyte solutions enhances the voltammetric current signals of penicillin V (Scheme 1B) without any chemical modifications and/or electrochemical pretreatment of the glassy carbon electrode surface.

2. Experimental Section

2.1 Chemicals and Solutions

All the chemicals used were of analytical grade quality and were used without further purification. The chemicals used included; sodium dodecyl sulfate, sodium acetate, acetic acid (glacial), acetonitrile, sodium hydroxide and hydrochloric acid all from fisher scientific. For voltammetry, the solutions were acetate buffer (ABS) and acetate buffer containing sodium dodecyl sulfate (SDS/ABS). All other chemicals were reagent grade.

2.2 Apparatus

All the electrochemical experiments were performed with a CHI 1232B Electrochemical Station (CH Instruments, Inc., USA). A three-electrode system (CH Instrument Inc., USA) consisted of a glassy carbon

working electrode with diameter of 3 mm, a platinum wire auxiliary electrode and Ag/AgCl reference electrode. A pH meter Bench – Model CyberScan pH Tutor (Eutech Instruments) was used for all pH measurements. All experiments were carried out in a 10.0mL electrochemical cell at room temperature [26]. All data were analyzed using Kaleidagraph software, version 4.1.1.

2.3 Polishing the glassy carbon working electrode

Glassy carbon disk electrodes (3 mm diameter) were abraded on wet silicon carbide paper (600 grit, Buehler) **[22]**. Rinsed in water, then polished thoroughly with 0.05 micron micropolish (CH Instruments) slurry on a soft cloth then rinsed in de-ionized water to remove particles and other possible contaminants [23, 26]. The actual surface area was 0.071cm^2. This procedure was repeated before every use.

2.4 Voltammetry

All voltammetric measurements were carried out 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 acetic acid in de-ionized water and made up to 500ml. This buffer contained both water and acetonitrile in the ratio of 8:2. The acetonitrile was included to dissolve the penicillin V. The pH of the resulting acetate buffer was adjusted accordingly using the hydrochloric acid or sodium hydroxide solutions.

2.6 Preparation of sodium dodecyl sulfate in acetate buffer Solution

After preparing the acetate buffer (section 2.5), 25g of sodium dodecyl sulphate was dissolved in prepared acetate buffer and made up to 250ml mark using the acetate buffer. The pH of the resulting SDS-Acetate buffer was adjusted accordingly using the hydrochloric acid or sodium hydroxide solutions.

2.7 Effect of sodium dodecyl sulfate (SDS) concentration

To study the effect of concentration of sodium dodecyl sulfate (SDS), different SDS concentrations ranging from 0.0M to 0.0052M were used at pH 4.5 and a scan rate of 0.1V/s. The scanning potential was from 1.0V to 2.0V in acetate buffer solution. The voltammetric current responses were monitored to establish the optimum amount of SDS required.

2.8 Effect of Buffer pH

A series of buffer pH were prepared by mixing appropriate ratios of acetic acid and sodium acetate. Effects of varying pH on the voltammetric peak currents of penicillin V at the glassy carbon electrode (GCE) were monitored by square wave voltammetry. To determine the optimal buffer pH, a graph of voltammetric current against pH, was plotted.

2.9 Linearity, Limit of Detection and Limit of Quantitation

Detection of penicillin V was measured with varying concentrations of penicillin V both in the acetate buffer, pH 4.5 and in the SDS/acetate buffer, pH 4.5 on GC electrode. The linearity of the method was evaluated by making calibration curves to calculate the coefficient of correlation, slope and intercept values. Based on three times the standard deviation of the baseline (*equation 1*) [24, 25, 26], the limits of detection (LOD) were estimated for penicillin V.

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

Where s is the standard deviation and m is the slope of the related calibration line. The limit of quantitation (LOQ) was obtained following equation 2 [24, 26]:

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

2.10 Effect of Impurities in the determination of penicillin V

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

3. Results and Discussion

3.1 Effect of SDS amount

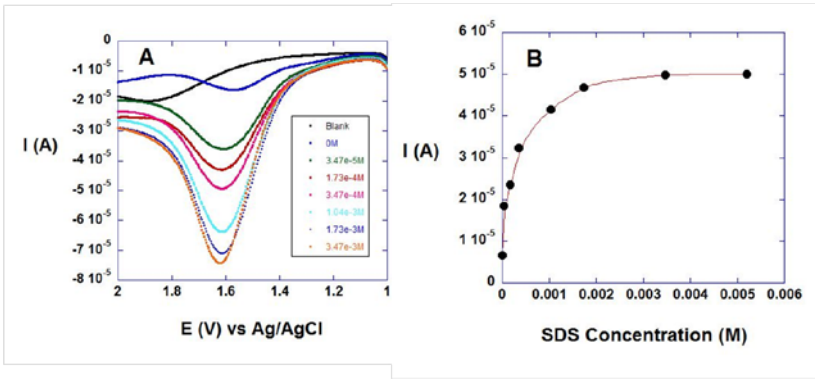


Figure 1: (A) Effect of successive addition of sodium dodecyl sulfate (SDS) on the voltammetric response of 0.14mM Penicillin V in ABS, pH 4.5 at GC electrode. The amplitude and frequency were 0.025V and 15Hz respectively (B) Plot of varying SDS concentrations versus voltammetric current responses.

To investigate the effect of the SDS amount on the square wave voltammetric current responses, different amounts of the SDS were added to the ABS, pH 4.5. Voltammograms in figure 1 shows the effects of varying SDS concentrations on voltammetric peak currents of 0.14mM penicillin V. As the amount of SDS increased, the voltammetric current increased linearly up to 0.347M SDS, whereas beyond 0.347M SDS the voltammetric currents remained the same possibly due to saturation. Similar observation was made by Galal, A and his colleagues [27] in electrolyte containing sodium dodecyl sulfate when they were determining terazosin, an antihypertensive drug on glassy carbon electrode. To determine amount of SDS that enables the electrode to give maximum voltammetric current, current readings were taken from the voltammograms in figure 1A and plotted against the concentration of SDS (*figure.1B*). Therefore, 0.347M of the SDS in ABS, pH 4.5 was used in this study in all subsequent work.

3.2 Effect of pH on the electrochemical response of penicillin V

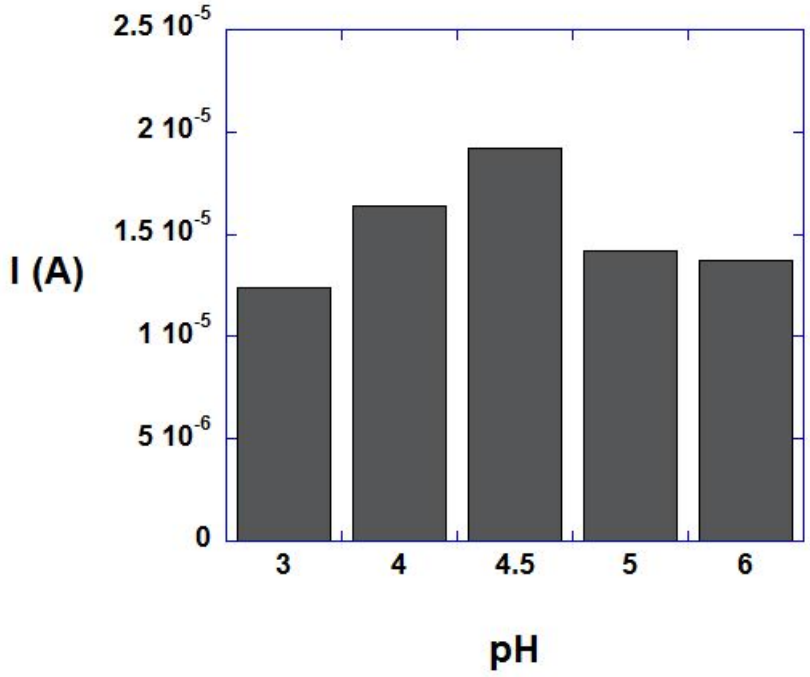


Figure 2: Effect of pH on the square wave voltammetric peak currents of 0.14mM Penicillin V at the GC electrode in acetate buffer. The pH values considered were 3, 4, 4.5, 5 and 6.

The electrochemical responses of 0.14mM penicillin V in acetate buffer with different pH values were studied by square wave voltammetry. We plotted the voltammetric currents against buffer pH and the histogram (*figure 2*) was used to determine the optimal buffer pH for the proposed method. Figure 2 shows that the highest voltammetric peak current responses for 0.14mM penicillin V were at pH 4.5. Below and above this pH, the current responses are lower. Consequently, a pH 4.5 was selected for further work. Note that the highest voltammetric current corresponds to highest sensitivity. This therefore means that this method is most sensitive at pH 4.5.

3.3 Comparison of the SWV of penicillin V in presence and absence of SDS surfactant

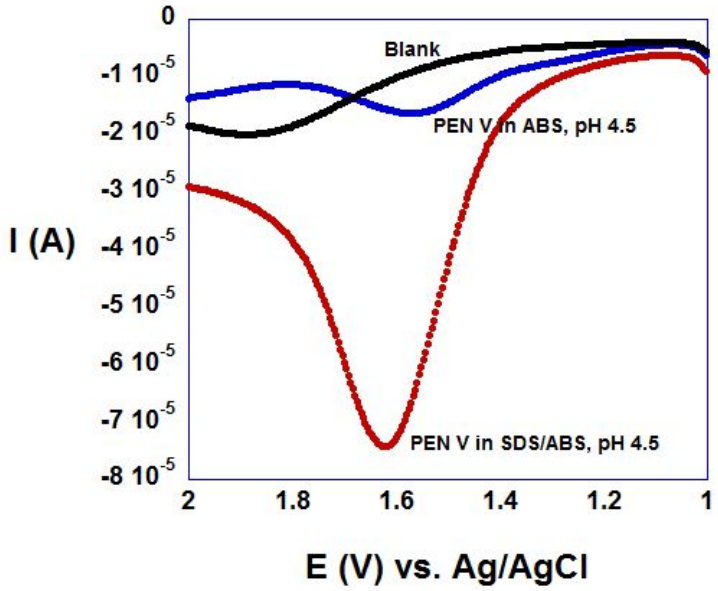


Figure 3: Square wave voltammograms of 0.14mM Penicillin V in ABS, pH 4.5 (**Blue**) and 0.14mM Penicillin V in 0.347M SDS/ABS, pH 4.5 (**Red**) at GC electrode. The amplitude and frequency were 0.025V and 15Hz respectively.

Figure 3 shows the square wave voltammetric responses of 0.14mM penicillin V 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 0.14mM penicillin V 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 V. This behavior is consistent with earlier observations by Galal, A. and his colleagues [27].

3.4 Cyclic voltammetry of penicillin V

Cyclic voltammograms of penicillin V at a polished GC electrode gave a well defined irreversible voltammetric peak at very high positive potential of 1.55 V versus Ag/AgCl in ABS, pH 4.5 and 1.61V versus Ag/AgCl in SDS/ABS, pH 4.5 (*figures 4A and 5A*).

These peak potentials are within the potential range observed by Ľubomír and his colleagues [28] for penicillin V on bare boron-doped diamond electrode using differential pulse voltammetric method.

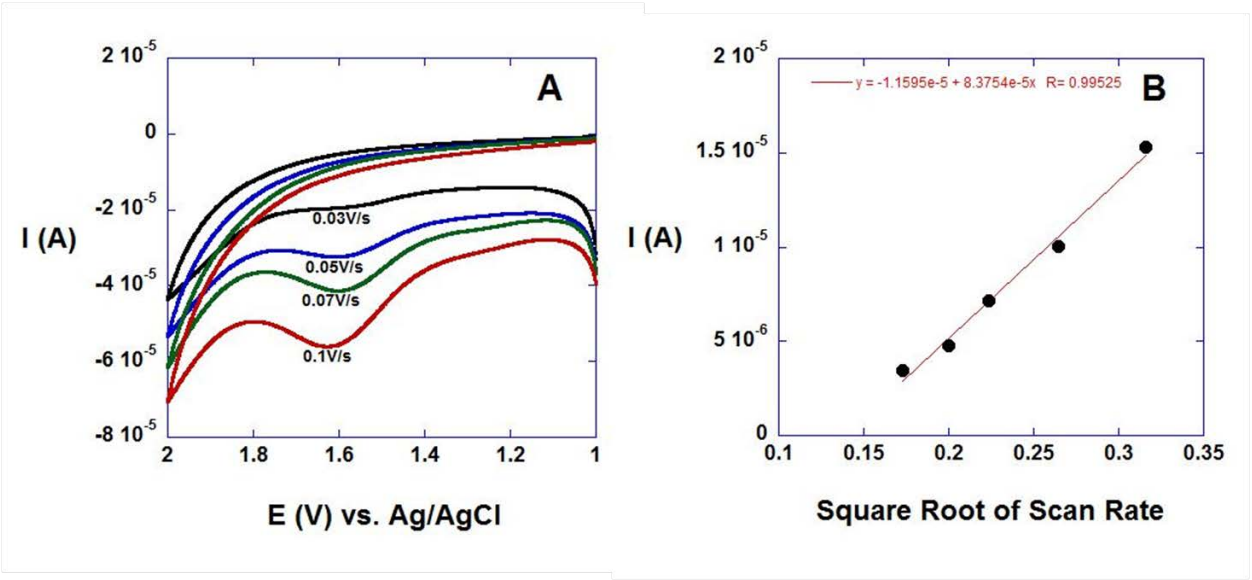


Figure 4: (A) Cyclic voltammograms of 0.14mM penicillin V in ABS, pH 4.5 on glassy carbon electrode. Initial potential: 1.0V; high potential: 2.0V; low potential: 1.0V; sample interval: 0.001V, quiet time: 0.1sec. Scan rates were varied from 30mV/s to 100mV/s. (B) Calibration plot of anodic peak currents versus square root of scan rate.

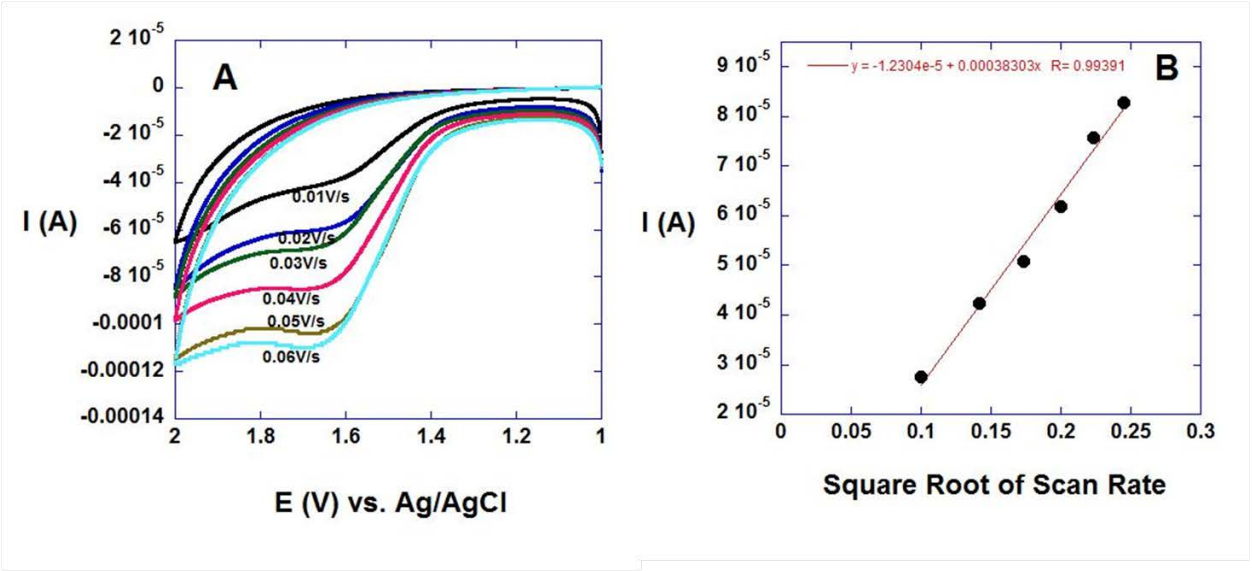


Figure 5: (A) Cyclic voltammograms of 0.14mM penicillin V in 0.347M SDS in ABS, pH 4.5 on glassy carbon electrode. Initial potential: 1.0V; high potential: 2.0V; low potential: 1.0V; sample interval: 0.001V, quiet time: 0.1sec. Scan rates were varied from 10mV/s to 60mV/s. (B) Calibration plot of anodic peak currents versus square root of scan rate.

It is interesting to note that the oxidation potentials in ABS, pH 4.5 compares relatively well with those taken in SDS/ABS, pH 4.5 on GC electrodes as shown in figures 4A and 5B. 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. Now using the 0.14mM penicillin V in ABS, pH 4.5 and SDS/ABS, pH 4.5, current readings were taken from the voltammograms in figures 4A and 5A and then plotted against the square root of the scan rate. The plots are linear in both cases indicating that the electrochemical reaction for the oxidation of penicillin V on glassy carbon electrode is dorminantly diffusion controlled.

3.5 Validation of the proposed method for penicillin V determination

The applicability of the proposed SWV procedure as analytical method for the determination of penicillin V was examined by measuring the oxidation voltammetric peak current as a function of penicillin V concentration for at least three times under the optimized operational parameters (*figure 6*). The calibration plot of the peak current versus the penicillin V concentration was found to be linear over the range 34.6 µM – 0.04µM penicillin V in SDS/ABS, pH 4.5. The linear regression plots (*figure 6B*) showed that there is a linear dependence of the voltammetric current intensity on the penicillin V concentration in SDS/ABS, pH 4.5 over the range 34.6 µM – 0.04µM penicillin V (*table 1*).

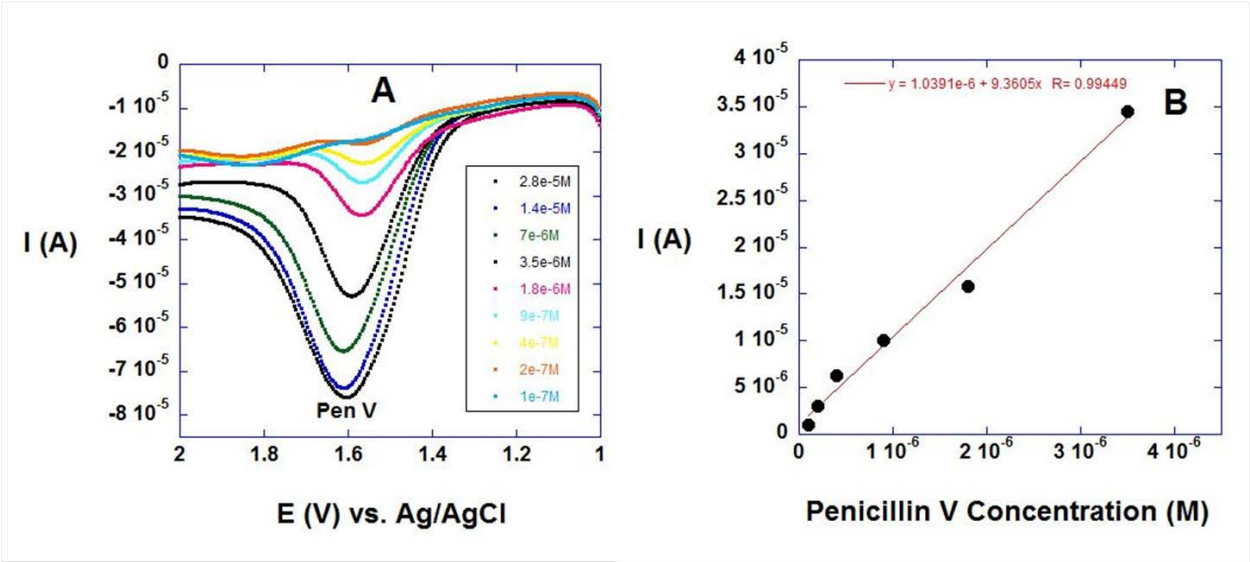


Figure 6: (A) Square wave voltammetry of different concentrations of Penicillin V in the SDS/ABS, pH 4.5; frequency: 15Hz and amplitude 0.025V. (B) Calibration plot of concentration of penicillin V versus voltammetric current.

Table 1: The linear concentration range (LCR), limit of detection (LOD), limit of quantitation (LOQ) and the regression equation of Penicillin V in Acetate buffer, pH 4.5 and SDS/Acetate buffer, pH 4.5 on bare GC electrode.

	Solvent	LCR	LOD	LOQ	RE	R^2
1.	ABS, pH 4.5	28.0 – 3.5µM	3.5µM	14.0µM	y=-1.0e-7+0.804x	0.98771
2.	SDS in ABS, pH 4.5	34.6 –0.04µM	0.04µM	0.12µM	y=1.0391e-5+9.3605x	0.99449

Limit of detection (LOD) of this method is typically determined to be in the region where the signal to noise ratio is greater than three [29 – 32]. Voltammetric measurements were made for the detection of varying concentrations of penicillin V 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 0.04µM in SDS/ABS, pH 4.5 while the limit of quantitation (*equation 2*) was found to be 0.12µM.

3.6 Effect of Interferants

Natural samples normally contain enormous number of easily oxidizable/reducible substances. Serious interference in penicillin V determination can occur if there is competitive adsorption of these substances/interferants into the electrode surface at uncontrolled concentrations. Serious interferences arise when these substances reduce and/or oxidize at potentials close to the analyte ions and/or due to poor specificity of the electrode surface and the type of electrolyte. The effects of Na^+, K^+, Mg^{2+}, Zn^{2+}, Ca^{2+}, Fe^{3+}, Cu^{2+}, Cl^-, NO_3^-, PO_4^{3-} and SO_4^{2-} possible interferants were investigated in the determination of penicillin V. These substances did not have any significant effect on the oxidation potential of penicillin V. Moreover, most of these substances like Fe^{2+} enhanced the oxidation current by about 10% while others had insignificant effect on the voltammetric currents. Incase of trace levels of these interferants in less contaminated real samples, their interfering effect(s) will pose insignificant consequence to the general analysis [33].

3.7 Comparison of this proposed method with other electrochemical methods

Table 2 provides a comparison between the analytical performance of the proposed method in SDS/ABS, pH 4.5 on bare GC electrode and some reported voltammetric methods for penicillins analysis. With this proposed method for determining penicillin V, the calculated limit of detection is pretty much lower than those obtained by modified electrodes or biosensors (*table 2*) [28, 34 - 38]. Moreover, this method is simple and straight forward compared to modifying electrodes which is a time consuming process.

Table 2: Comparison of the proposed voltammetric method with some previously reported electrochemical methods for determination of Penicillin V.

No.	Electrode	Support Electrolyte	Technique	LR (µM)	LOD (µM)	Ref.
1.	BDD	ABS, pH 4.0	DPV	0.5 - 40	0.25	[28]
2.	Au	ABS, pH 4.7	PAD	-	0.4	[34]
3.	HMT probe Pt	PBS with NaCl	AD	4 - 200	4	[35]
4.	PGA acid/GCE	ABS, pH 5.2	SWV	2 - 25	0.92	[36]
5.	[VO(salen)]CPE	-	DPV	-	16.6	[37]
6.	Pt DEN-modified SPCEs	Penicillinase enzyme in 100 mM NaCl	-	0.1–500µM	0.1	[38]
8.	GCE	SDS/ABS, pH 4.5	SWV	0.04–34.6µM	0.04	This work

SDS: sodium dodecyl sulfate; ABS: acetate buffer solution and SWV: square wave voltammetry.

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 V. Addition of SDS to the penicillin V containing acetate buffer solution was found to enhance the oxidation current signal by about 10 times with insignificant shifts of the oxidation potentials. With this electrochemical method, the optimal pH and SDS amount which gave the highest voltammetric currents were found to be pH 4.5 and 0.347M respectively. Linear concentration range for this method was found to be 0.04 – 34.6µM, limit of detection 0.04µM and limit of quantitation 0.12µM penicillin V in SDS/ABS, pH 4.5 solution. Foreign substances like Na^{+}, K^{+}, Mg^{2+}, Zn^{2+}, Ca^{2+}, Fe^{3+}, Cu^{2+}, Cl^{-}, NO_3^{-}, PO_4^{3-} and SO_4^{2-} were found to have insignificant effect on the voltammetric currents and oxidation potentials of the penicillin V. This electrochemical method is sensitive enough for real sample analysis.

Acknowledgement

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