Int. J. Electrochem. Sci., 16 **(2021)** Article ID: 210444, doi: 10.20964/2021.04.41

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Electrochemical Determination of Penicillin G in Cow Milk and pharmaceuticals in SDS/Acetate buffer

Joshua M. Sila[1], Peterson M. Guto[1], Immaculate N. Michira[1], Francis B. Mwaura[2], Edward K. Muge[3]

[1] Department of Chemistry, University of Nairobi, P.O. Box 30197, Nairobi, Kenya.
[2] School of Biological Sciences, University of Nairobi, P. O. Box 30197-00100, Nairobi, Kenya
[3] Department of Biochemistry, University of Nairobi, P. O. Box 30197-00100, Nairobi, Kenya
*E-mail: jaysilla@gmail.com

Received: 4 August 2020 / *Accepted:* 25 September 2020 / *Published:* 28 February 2021

Penicillin residues in animal food products like milk and meat has attracted great concern by health regulatory agencies due to their negative effects. Therefore, there is urgent need for reliable, low cost, fast and simple analytical tools/methods to monitor these penicillin residues in animal products before distribution to consumers. In our previous work, we developed a square wave voltammetric method based on bare glassy carbon electrode in SDS/ABS media to determine penicillin V and G. In this work, we apply this voltammetric method to detect penicillin G at trace levels in cow milk and selected pharmaceutical samples. Using cyclic voltammetry, the electrochemical behavior of penicillin G in both cow milk and pharmaceutical samples were obtained. The oxidation potentials were 1.65V in both samples, same as that obtained in SDS/ABS media. The diffusion coefficients were $1.494x10^{-6}cm^2$/sec in cow milk, $2.358x10^{-7}cm^2$/sec in pharmaceutical sample and $1.392x10^{-6}cm^2$/sec in SDS/ABS media. The precision for the detection of the drug was also determined and recorded as relative standard deviation (RSD). The RSD found were 4.22% and 5.51% for cow milk and pharmaceutical sample respectively. The percent recoveries for accuracy determination were found to lie between 95.8% - 103.0% for the cow milk and 92.0% - 96.0% for the pharmaceutical samples. These recovery percentages were within the recommended 90.0% - 110.0%. A detection limit of 2.5×10^3 ng/L penicillin G was achieved in cow milk samples against a maximum residue limit of 4.0×10^3ng/L set by the European Union. Overly, this method provides simple, precise and consistent results for detection and quantification of penicillin G in cow milk, pharmaceuticals and possibly other environmental and clinical samples.

Keywords: Voltammetry, precision; accuracy; Cow milk; pharmaceutical and maximum residue limit.

1. INTRODUCTION

Humans have since time immemorial sought for ways to wade off disease causing micro-organisms. Discovery of Penicillin by Flemming was a significant step in fighting these pathogens and inspired hope in medical world against life threatening infections caused by microbes. Penicillin

comprises of a number of antibacterial agents characterized by a high reactive beta-lactam ring. Penicillins work by interfering with transpeptidase, an enzyme that is involved in cross linking peptide units during cell wall synthesis. This is achieved through its active site, the beta-lactam ring. The beta-lactam ring binds irreversibly to the enzyme through covalent bonding. The resulting cell wall is structurally weak hence resulting to disentegration and ultimate cell death [1]. Since penicillin was discovered, purposeful chemical modifications have been done to improve the potency of the original molecule but still maintaining the basic penicillin structure. This was done to make it more suitable in different body environments and make it less susceptible to pencillin destroying enzyme, penicillinase produced by some bacteria. Penicillin G is naturally extracted through fermentation process and is one of the most widely used forms. Other commonly used penicillins include phenoxymethylpenicillin, amoxicillin, ampicillin, oxacillin, methicillin, oxacillin among others. Penicillins have been widely used in both humans and in animal husbandry to treat bacterial infection due to their strong antimicrobial activity [2].

A study by veterinarians in united states showed antibiotics as the drugs of choice in treatment of mastatis in lactating dairy[3]. In kenya, benzylpenicillin is one of the most commonly used antibiotic in animal husbandly. Additionally, in animal husbandry penicillin G is used for prophylactic purposes, especially in poultry farming [4]. In poultry, antibiotics have been used as feed supplements to stimulate growth, control and prevent infectious diseases [8]. It is administered in the form of soluble sodium or potassium salt or procaine penicillin G, a sustained release form [5]. Reported cases of penicillin residues in food and water samples have emerged [45]. These residues are detrimental to both human and animal health as they have been associated with proliferation of drug resistant bacteria and severe allergic reaction in humans [3, 6, 7]. To avoid the negative effects brought about by their presence in food and water, the European Union (EU) Regulation 508/1999 established a framework to minimize these negative effects. A maximum residue limit (MRL) was set for penicillin G in milk at 4×10^3 ng/L. The World Health Organization (WHO) in 2015 endorsed a worldwide action plan to reduce antimicrobial resistance by recommending improved surveillance on the antibiotics use [9, 10]. As a result of this plan, antibiotic detection methods with low detection limits are urgently needed.

Various analytical methods have been proposed to detect antibiotic residues in food samples. Some of these methods include Delvotest T [11], Charm II [12], LacTek and Penzyme [13], HPLC-DAD [14] and the conventional microbial inhibition assays. A major limitation of these methods is that they do not fully identify and quantify individual residues. Other documented methods include the immunoassay method like the enzyme-linked immunosorbent assays (ELISA). Unfortunately, this method requires specific antibodies, it's costly and tedious on large scale application. The above mentioned methods are powerful tools for detecting antimicrobial residues especially in food samples. Some of these methods are fast but they require specialized instruments or may involve inhibitory tests which take longer time or may not work in all matrixes [11].

Electrochemical methods tend to be simple, fast and low-cost alternative for penicillins detection. Comparatively, they offer more advantages than other methods. These include high sensitivity and selectivity and short time of analysis because of fewer analytical steps. Furthermore, they offer a possibility of miniaturization to achieve portable tools for *in situ* analysis [15]. The electrochemical methods so far known for the detection of penicillins include direct detection [16, 17], amperometric

biosensors [18, 19] and immunosensors [20, 21]. Direct detection has always remained one of the best options for most electroactive molecules because it provides quick and reproducible results. A key reason for this is that there is no electrode modification required [22, 23, 24].

In our previous work, a simple voltammetric method was developed for the determination of penicillin V and G using square wave method [25, 26]. This method involved the use of sodium dodecyl sulfate in acetate buffer (SDS/ABS) media on bare highly polished glassy carbon electrode. The method proved highly sensitive and reproducible with very low detection limits for penicillin V and G in the SDS/ABS media. It is also worth mentioning that the method was very simple because it did not involve electrode modification which is a tedious process and most often produces uneven electrode surface which reduces reproducibility of the analytical tool. This work evaluates the suitability of this improved square wave voltammetric method [25, 26] in determination of penicillin G in cow milk and pharmaceuticals samples.

2. EXPERIMENTAL

2.1. Reagents

The chemicals used were sodium dodecyl sulfate, sodium acetate, acetic acid (glacial), Penicillin-G ((2S,5R,6R)-3,3-dimethyl-7-oxo-6-(2-phenylacetamido)-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic acid) all from fisher scientific. Pen-strep, a common penicillin drug used to treat mastitis was bought over the counter. All these chemicals were of analytical grade. Acetate buffer (ABS) containing sodium dodecyl sulfate (SDS) was used as the electrolyte. Only de-ionized water was used throughout this work.

2.2. Apparatus

All electrochemical analysis was done using CHI 1232B Electrochemical Station (CH Instruments, Inc., USA) using a three-electrode system (CH Instrument Inc., USA). All pH measurements were done on a pH meter Bench – Model CyberScan pH Tutor (Eutech Instruments). A 10.0mL electrochemical cell was used for all the electrochemical procedures at room temperature. The cow milk samples were centrifuged at 10,000 rotations per minute (rpm) using MSE 869-Minor centrifuge. De-ionized water was prepared using B114 Elga-Star wall mounted De-ionizer with disposable cartridges. 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) [27, 28] 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 [27, 28]. This procedure was repeated before every use.

2.4. Preparation of the acetate buffer

A solution of acetate buffer was prepared by dissolving 1.5g of sodium acetate and 1ml of concentrated acetic acid in de-ionized water and made up to 500ml. The pH of the resulting acetate buffer (ABS) was adjusted accordingly using 1M hydrochloric acid or concentrated acetic acid. The pH of the final solution was 4.5.

2.5. Preparation of sodium dodecyl sulfate -acetate buffer (SDS-ABS) Solution

A solution of sodium dodecyl sulfate in acetate buffer solution, pH 4.5 was prepared by dissolving 25g of sodium dodecyl sulphate in acetate buffer solution and made up to 250ml using the same solution. The resulting mixture, sodium dodecyl sulfate- Acetate buffer solution (SDS-ABS) was stirred over warm water for 30 minutes until a homogenous solution was formed. SDS-ABS solution was left to cool down. The pH of the resulting SDS-Acetate buffer was adjusted accordingly using the acetic acid.

2.6. Sample treatment

A sample of cow milk was spiked with known concentration of penicillin G. Acetonitrile was added under constant stirring for 20 minutes to coagulate and deproteinize the milk [7, 29]. The resulting mixture was filtered using a filter paper (whatman, 125mm) and the supernatant recovered. The supernatant was centrifuged for 30 minutes at 10,000rpm using MSE 869-Minor centrifuge to remove any suspended matter. The resulting supernatant was kept at -20^{o}C when not in use.

2.7. 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. The sample interval for all cyclic voltammogram studies was 0.001V and a quiet time of 0.1 seconds. Amplitude and frequency of the square-wave method were 0.025V and 15Hz respectively. All electro-analytical work was done using a three-electrode system in a 10.0ml electrochemical cell.

2.8. Scan rate studies

Scan rate studies were done by spiking the support electrolyte, SDS-ABS with 200µl of 0.1M penicillin G containing supernatant (cow milk and pharmaceutical samples) and potential scanned from 1.0V to 2.0V at different scan rates ranging from 0.01 V/s to 0.1V/s using cyclic Voltammetry method.

2.9. Precision study

To test the precision of the proposed penicillin G method, current response of ten samples of the supernatant obtained from milk samples were obtained using square wave voltammetry. The average current, standard deviation and relative standard deviation (RSD) were calculated using equation 1 [30] from the resulting current.

$$\mathbf{RSD\% = (\frac{standard\ deviation}{mean})x\ 100\%} \quad (1)$$

2.10. Accuracy/Recovery

Accuracy of this method in determining penicillin G present in a sample was done by addition of known concentration of penicillin G. This was done by adding 50%, 100% and 150% standard solutions of the expected working sample concentration. The current response after every addition was monitored using square wave voltammetric method. The current response was compared with the current expected from working sample concentration of similar concentration as shown in equation 2 [30] to obtain the percent (%) recovery.

$$\%\ recovery = \frac{\mathbf{obtained\ current}}{\mathbf{expected\ current}} \times 100 \quad (2)$$

3. RESULTS AND DISCUSSION

3.1. Cyclic Voltammetry of Penicillin G in Cow Milk and Capsules

Cyclic voltammetry is widely used to study redox systems because of its relative simplicity, high performance and ability to quickly establish the formal potentials of redox-active samples [31, 32, 33]. Cyclic voltammetry of penicillin G in cow milk and pharmaceutical samples gave only one irreversible oxidation peak around 1.65V versus Ag/AgCl (*figure 1A and 2A*). The oxidation peak was well defined.

The irreversible nature of electrode reaction is mainly attributed to a possible sluggish charge transfer across the electrode/solution interface. The reverse reduction peak is absent probably because the product formed during oxidation scan is not redox active or it might have formed at a potential outside the reported potential window. It is interesting to note that the observed oxidation potentials for the penicillin G compares relatively well with those obtained for penicillin G in SDS/ABS media [26]. The slight difference particularly with respect to the shape of the voltammogram can be attributed to the additional components from cow milk and pharmaceutical samples that were not separated during sample treatment.

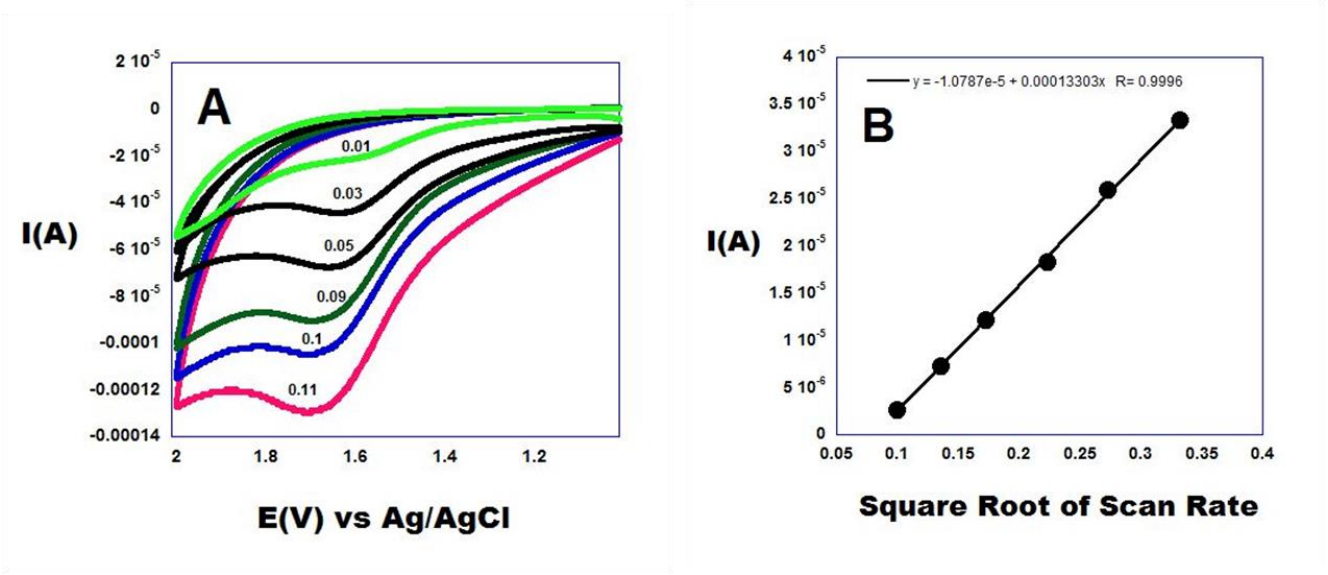

Figure 1. (A) An overlay of cyclic voltammograms of 0.1M penicillin G in cow milk, pH 4.5 on GCE at different scan rates of 0.01V/s, 0.03V/s, 0.05V/s, 0.09V/s 0.1V/s and 0.11V/s **(B)** A plot of anodic peak currents against square root of scan rate.

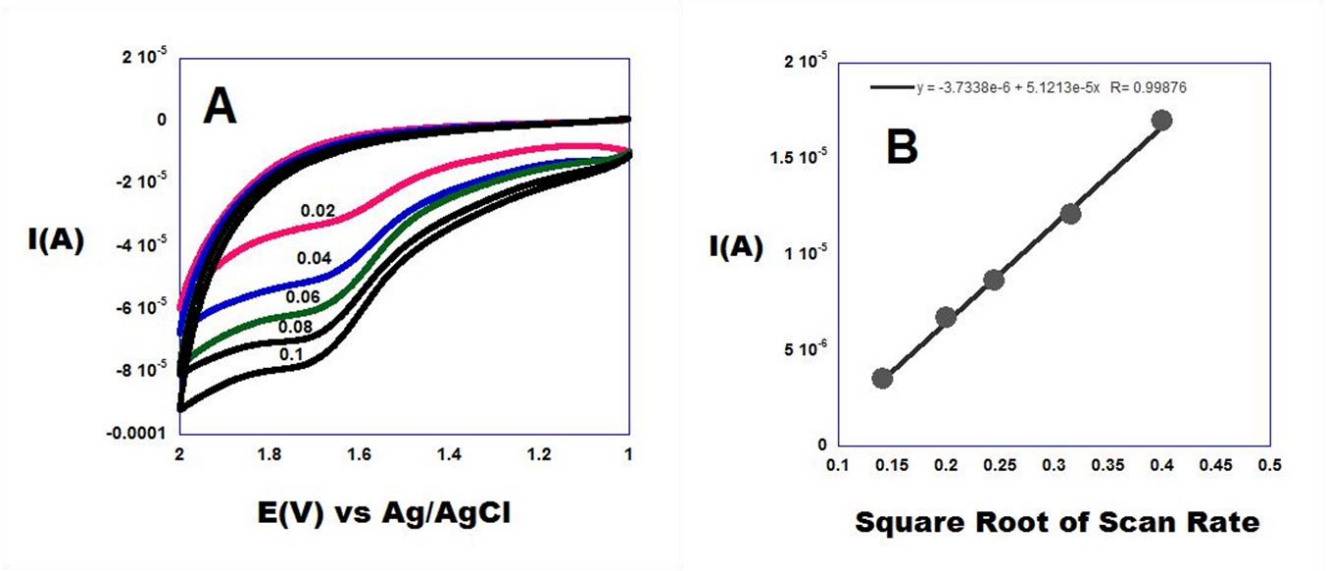

Figure 2. (A) An overlay of cyclic voltammograms of 0.1M penicillin G in drug, pH 4.5 on GCE at different scan rates of 0.02V/s, 0.04V/s, 0.06V/s, 0.08V/s and 0.1V/s **(B)** A plot of anodic peak currents against square root of scan rate.

Additionally, the results for the oxidation of penicillin G in both samples also compares favorably with the results of Freier *et al* [10], who found out that penicillin G on boron doped diamond electrode oxidized at 1.6V versus Ag/AgCl. They attributed this oxidation potential response to the presence of penicillin G. The resulting current peak at this potential is possibly caused by oxidation of penicillin G to its sulfoxide.

The oxidation currents for the penicillin G were found to increase with increasing scan rate as shown in figure 1A and 2A. The number of electrons (n) involved in the oxidation of penicillin G was calculated from the slope of the plots of potential, E, versus log[i/i_d-i] as provided in equation 3 below.

$$E = E_{1/2} - \frac{0.0591}{n}\log\left(\frac{i}{i_d - i}\right) \quad (3)$$

Where $E_{1/2}$ is the half-wave potential and E is the potential at any point on the wave. i_d is the peak current while i is the current at any point on the wave and n is the number of electrons exchanged in the penicillin G oxidation process [31]. n was found to have a value of 2 for penicillin G in all the samples.

When the peak oxidation currents (i_{pa}) were plotted against the square root of scan rates ($\nu^{1/2}$), a linear plot was obtained [31, 40] for scan rates between 0.01V/s to 0.09V/s as shown in figures 1B and 2B. Using Randles-Sevcik equation 4, diffusion coefficients (D_o) of penicillin G were obtained.

$$i_{pa} = (2.69\times10^5)n^{3/2}C^*AD^{1/2}\nu^{1/2} \quad (4)$$

Where i_{pa} is the peak oxidation current, n is the number of electrons while C^* is the concentration of penicillin G. A is the surface area of electrode and D is the diffusion coefficient while ν is the scan rate. Diffusion coefficient is a measure of how fast electrons are transfered to the electrode surface. Linear plots were obtained as shown in figures 1B and 2B which indicates that oxidation of penicillin G at the glassy carbon electrode is predominantly diffusion controlled. However, the none zero y-intercept implies that other modes of mass transport were involved in transfering penicillin G to the electrode surface but in a smaller extent. The table below gives a summary of the electrochemical properties of penicillin G on glassy carbon electrode.

Table 1. Electrochemical properties of Penicillin G in cow milk and pharmaceutical samples.

No	Media	D_o, cm^2/sec	$E_{oxidation}$, V	n
1.	Pen G in cow milk	1.494×10^{-6}	1.65	2
2.	Pen G in pen-strep	2.358×10^{-7}	1.65	2
3.	Pen G in ABS-SDS	1.392×10^{-6}	1.65	2

D_o: *diffusion coefficient obtained from the Randles-Sevcik equation,* n: *number of electrons exchanged during the oxidation process.*

From table 1, the Diffusion coefficient (D_o) for penicillin G in cow milk and pharmaceuticals were found to be 1.494×10^{-6} cm^2/s and 2.358×10^{-7} cm^2/s, respectively. This difference can be ascribed to the differences in sample components which possibly interacted with penicillin G differently.

3.2. Precision in Determination of Pen G in Cow Milk and in Capsules

Precision is the closeness of a series of data points obtained from multiple sampling of the same sample under similar analytical conditions. Precision was established by carrying out analysis of ten samples of the analyte using the same analytical machine. Moreover, the analysis was done by the same analyst.

Table 2. Experimental results showing mean, standard deviation and relative standard deviation of current response of penicillin G in cow milk and Pharmaceutical samples.

	Calculated statistical parameter	Pen G in cow milk	Pen G (pen-strep)
1.	Number of replicate Sample	10	10
2.	Average Value	1.92×10^{-5}	8.56×10^{-6}
3.	Standard Deviation (SD)	8.12×10^{-7}	4.7142×10^{-7}
4.	RSD%	4.22%	5.51%

The percent relative standard deviation for penicillin G in cow milk was found to be 4.22% while in pharmaceuticals was found to be 5.51%. The recommended maximum RSD% is 10%. Therefore, the obtained RSD% for this analysis were within the acceptable range [34 – 38, 41].

3.3. Accuracy in Determination of Pen G in Cow Milk and in Capsules

Table 3. The percent recovery of penicillin G in cow milk and Pharmaceutical samples.

	Sample	Original (mM)	Current (A)	Added (mM)	Current (A)	Found (A)	Recovery, %
1.	Pen G (Cow milk)	0.01	8.01×10^{-6}	0.005	1.20×10^{-5}	1.15×10^{-5}	98.5%
		0.01	8.01×10^{-6}	0.01	1.602×10^{-5}	1.66×10^{-5}	103.0%
		0.01	8.01×10^{-6}	0.015	2.003×10^{-5}	2.03×10^{-5}	101.0%
2.	Pen G (Pharm)	0.004	5.83×10^{-6}	0.002	0.874×10^{-5}	0.84×10^{-5}	96.0%
		0.004	5.83×10^{-6}	0.004	1.17×10^{-5}	1.08×10^{-5}	92.0%
		0.004	5.83×10^{-6}	0.006	1.46×10^{-5}	1.38×10^{-5}	94.0%

Accuracy is a measure of agreement between the experimental results and the reference true value or conventional value [34 - 39]. Typically, accuracy is represented and determined using percent recovery as shown in table 3. The support electrolyte was spiked with increasing concentration of penicillin G (50%, 100%, and 150%). Current readings were done in triplicates for the three different

concentrations as recommended by ICH and the average of each concentration recorded as shown in the table 3 below.

The Percentage recovery of penicillin G from the sample was obtained as shown in equation 5.

$$\%\, recovery = \frac{obtained\ current}{expected\ current} \times 100 \qquad (5)$$

The percent recoveries for accuracy determination (*table 3*) were found to lie between 95.8% - 103.0% for the cow milk and 92.0% - 96.0% for the pharmaceutical samples. These recovery percentages were within the recommended 90.0% - 110.0% range [34 - 39].

When the voltammograms of penicillin G were overlaid, it was observed that there was increase in peak currents as the concentration of penicillin G increased at constant potential peaked at 1.6V as shown in figure 3A and 4A. Moreover, a plot of current versus concentration was linear as shown in figure 3B and 4B. This implies that an increase in concentration of penicillin G results into a corresponding increase in voltammetric current.

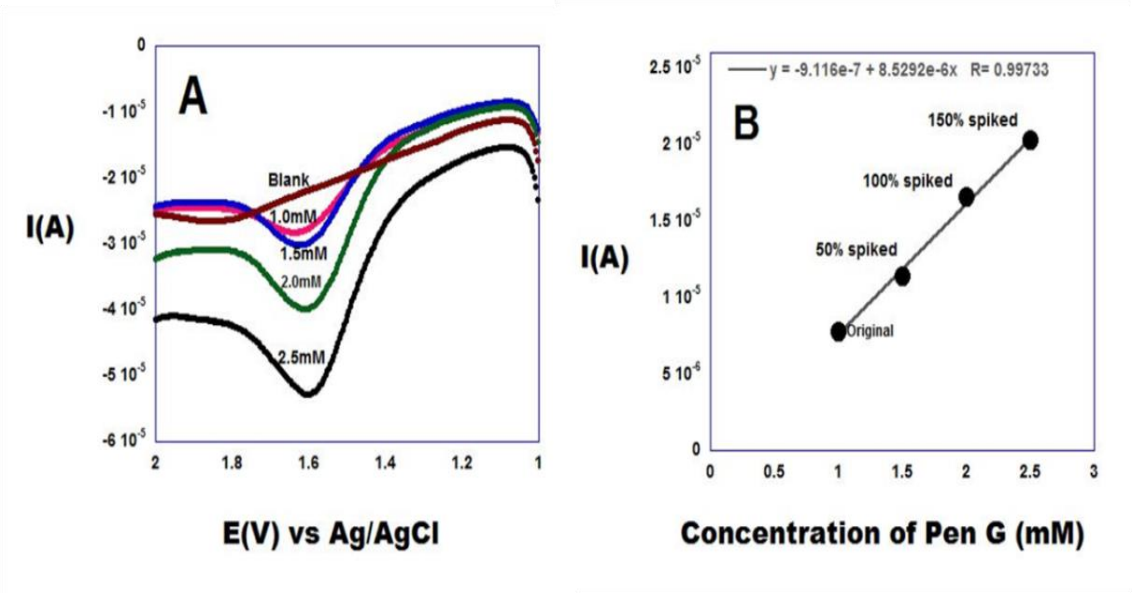

Figure 3. (A) An overlay of Penicillin G current response in cow milk spiked with 50%, 100% and 150% concentrations of penicillin G at pH 4.5 on glassy carbon electrode **(B)** Plot of current versus concentration of Penicillin G.

Similar behavior is observed when a drug sample (Pen-strep) containing penicillin G is tested using square wave voltammetry under similar conditions.

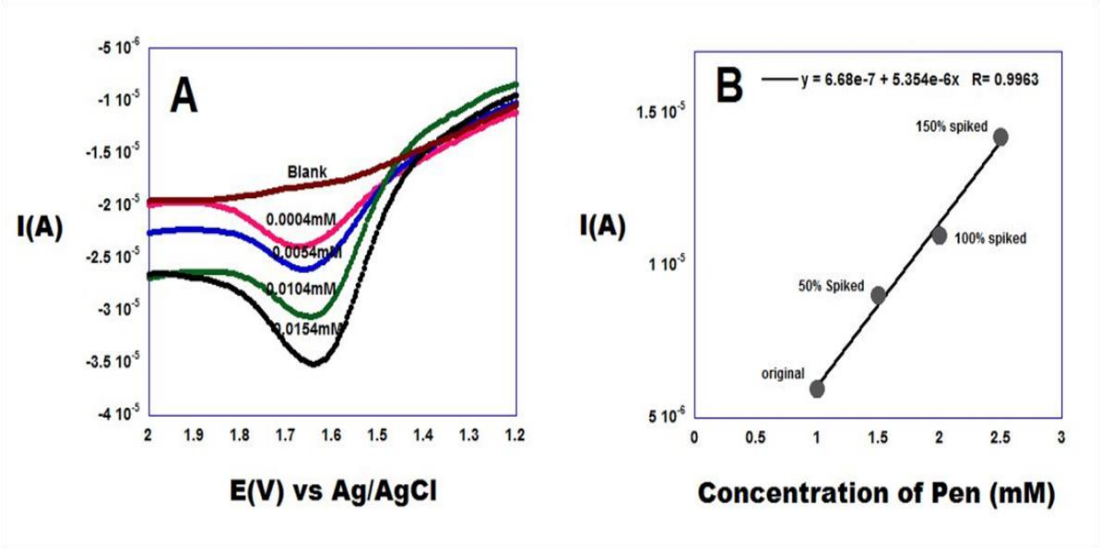

Figure 4. (A) An overlay of Penicillin G current response in drug sample spiked with 50%,100% and 150% concentrations of penicillin G at pH 4.5 on glassy carbon electrode **(B)** Plot of current versus concentration of Penicillin G.

3.4. Detection Limit of Penicillin G in Cow Milk

Limit of detection is the lowest concentration level that can be determined to be statistically different from an analyte blank with 99% confidence level [10]. Limit of detection (LOD) of this method was generally determined to be in the region where the signal to noise ratio is greater than three [34, 37, 38]. To determine the lowest detection limit, subsequent dilution of 2mM Penicillin G solution was monitored by square wave voltammetry as shown in figure 5A.

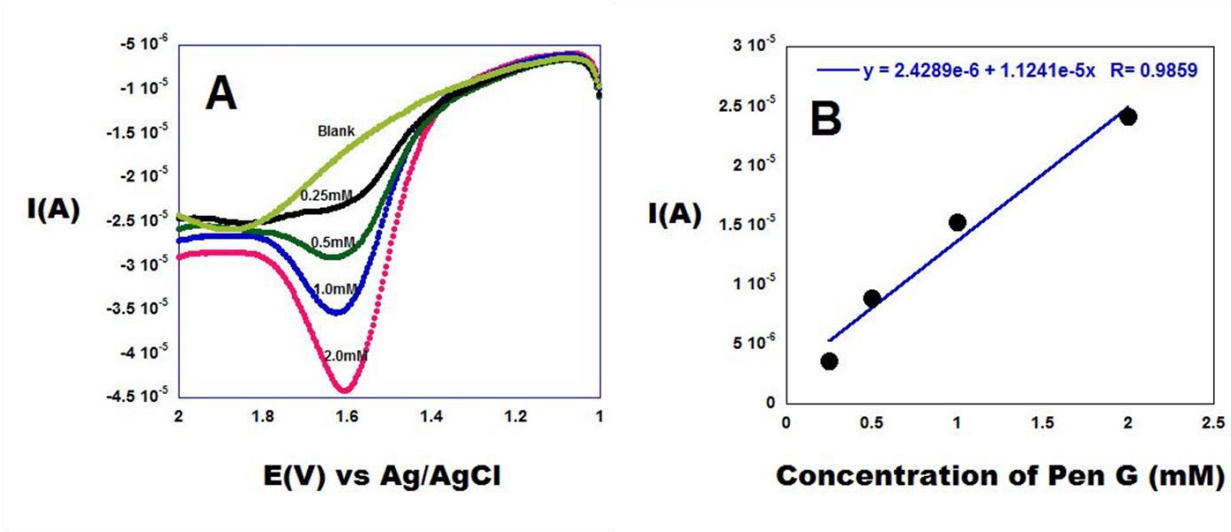

Figure 1. (A) Square wave voltammetry of different concentrations of Penicillin G in cow milk on plain glassy carbon electrode. The concentrations were 0.25mM, 0.5mM, 1.0mM and 2.0mM **(B)** Linear plot of current (A) against concentration (mM) of penicillin G in cow milk.

Table 4. Comparison of the LOD obtained in the SDS/ABS media with some previously reported electrochemical methods for the determination of Penicillin G in milk.

No.	Electrode	Support Electrolyte	Technique	LOD (M)	Ref.
1.	CSP-MB/AuE aptasensor	PBS	SWV	1.7nM	[42]
2.	MHM/MGCE/Pen-X	PBS	DPV	$2.655x10^{-7}$ mg/mL	[43]
3.	BDDE	(BRB)	DPV	0.23µM	[44]
4.	NiNPs/APTES/SPCE	PBS	DPV	0.001µM	[45]
5.	AuE bio-chip/TGA/Pen-X	PBS	CV	1.26nM	[46]
6.	AP/AuNPs/s-BLM/GCE	KCl solution	EIS	$2.7x10^{-4}$ng/L	[47]
7.	GCE plain	SDS/ABS	SWV	$2.5x10^{3}$ng/L	This work

A plot of peak currents against concentration were linear from $2.5x10^{3}$ng/L to $2.0x10^{4}$ng/L with R^2 at 0.9859 for penicillin G in milk. The detection limit for penicillin G in milk was found to be 2.5×10^{3}ng/L where the signal-to-noise ratio was greater than three. Table 4 provides a comparison between the analytical performance of the SDS/ABS, pH 4.5 on bare GC electrode method and some reported voltammetric methods for penicillin G analysis in milk samples. With this method the calculated limit of detection is pretty much similar or lower than those obtained by modified electrodes or biosensors (*table 4*) [42 – 47]. The European Union has set the maximum residue limit (MRL) of penicillin G in food samples at 4×10^{3} ng/L [7, 47]. Therefore, this method is sensitive enough for the analysis of penicillin G in milk samples. Moreover, it's simple and straight forward compared to modifying electrodes which is a time consuming process.

4. CONCLUSIONS

In this study we have successfully applied the square wave voltammetric method based on SDS/ABS on bare PG electrode to determine penicillin G at trace level in cow milk and pharmaceuticals. The electrochemical behavior of penicillin G in these two samples were similar to those obtained in the SDS/ABS media. This method selectively detects penicillin G in cow milk to a very low detection limit of 2.5×10^{3} ng/L against maximum residue limit of 4×10^{3} ng/L set by the European Union (EU). The method provides accurate, reliable and consistent results for the rapid on-site detection of penicillin G in cow milk, pharmaceuticals and possibly other environmental and clinical samples.

CONFLICTS OF INTEREST
The authors declare that there are no conflicts of interest.

ADDITIONAL INFORMATION
No additional information is available for this paper.

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. We also acknowledge the department of Biochemistry, University of Nairobi for providing the centrifuge.

References

1. S. T. Odonkor and K. K. Addo, *Int. J. Biol. Med. Res.*, *2*(4) (2011) 1204.
2. Z. Astrid, S. Eric, F. Jean-Marie, C. Paulette, L. André, *Molecules*, 17 (2012) 12478.
3. J. M. Mitchell, M. W. Griffiths, S. A. Mcewen, W. B. Mcnab and A. J. Yee, *J. Food Protection*, *61*(6) (1998) 742.
4. E. Mitema, G. Kikuvi, H. Wegener and K. Stoh. *J. Vet. Pharmacol. Ther.,* 24 (2001) 385.
5. W. Wachira, A. Shitandi and R. Ngure, *Int. Food Res. J.,* 18(3) (2011) 1203.
6. F. M. Aarestrup, *Int. J. Antimicrob. Agents,* 12 (1999) 279.
7. H. Li, B. Xu, D. Wang, Y. Zhou, H. Zhang, W. Xia, S. Xu and Y. Li, *J. Biotechnol.,* 203 (2015) 97.
8. J. Adla and E. Nada, *Antibiotics*, 8(2) (2019) 58.
9. World Health Organization. *Global Action Plan on Antimicrobial Resistance*, 2015. http://www.wpro.who.int/entity/drug_resistance/resources/global_action_plan_eng.pdf
10. B. Feier, I. Ionel, C. Cristea, R. Săndulescu, *New J. Chem.*, *41*(21) (2017) 12947.
11. C. Bion, A. B. Henzelin, Y. Qu, G. Pizzocri, G. Bolzoni and E. Buffoli, *Food Addit. Contam: Part A*, 33(1) (2016) 54.
12. R. Salter, *Apiacta*, 38 (2003) 198.
13. E. N. Escobar, *14th Annual Goat Field Day,* (1999.) 115. Oklahoma: Langston University.
14. K. Eftychia, C. Sofia, I. Maria, P. Evdoxios and S. Georgios, *Foods*, 7(6) (2018) 82.
15. O. A. Farghaly, R. S. Abdel Hameed and H. Abd-Alhakeem, *Int. J. Electrochem. Sci.*, 9 (2014) 3287.
16. Ľ. Švorc, J. Sochr, M. Rievaj, P. Tomčík and D. Bustin, *Bioelectrochemistry*, *88* (2012) 36.
17. L. Švorc, J. Sochr, P. Tomčík, M. Rievaj and D. Bustin, *Electrochimica Acta*, 68 (2012) 227
18. B. Chen, M. Ma and X. Su, *AnalyticaChimicaActa*, 674 (2010) 89.
19. L. M. Gonçalves, W. F. A. Callera, M. D. P. T. Sotomayor and P. R. Bueno, *Electrochem. Commun.,* 38 (2014) 131.
20. A. Pollap and J. Kochana, *Biosensors, 9(2)* (2019) *61*.
21. N. A Karaseva, T. N. Ermolaeva, *Talanta*, 120 (2014) 312.
22. Š. Ľubomír, S. Jozef, R. Miroslav and T. Peter, *Electrochimica Acta*, 68 (2012) 227.
23. J. H. Luong, K. B. Male and J. D. Glennon, *Analyst*, 134 (2009) 1965.
24. J. V. Macpherson, *Phys. Chem. Chem. Phys.,* 17 (2015) 2935.
25. J. M. Sila, P. M. Guto, I. N. Michira and F. B. Mwaura, *Int. J. Sci.: basic appl. Res., 40(1) (2018) 220.*
26. J. M. Sila, P. M. Guto, I. N. Michira and F. B. Mwaura, *Int. J. Sci.: basic appl. Res., 42(4) (2018) 144.*
27. P. M. Guto and J. F. Rusling, *J. Phys. Chem. B.,* 109 (2005) 24457.
28. P. M. Guto and G. N. Kamau, *Electroanalysis*, 22(11) (2010) 1186.
29. E. Kirchmann, R. L. Earley and L. E. Welch. *J. Liq. Chromatogr.*, 17(8) (1994) 1755.
30. M. Gumustas and S. Ozkan, The Open Anal. Chem. J., 5 (2011) 1.
31. A. J. Bard and L. R. Faulkner, *Electrochemical methods: fundamentals and applications. John Wiley & Sons, New York, 2nd Edition*, (2004).
32. J. F. Rusling and S. L. Suib, *Adv. Mater.*, 6(12) (1994) 922.
33. E. Noémie , J. R. Kelley , D. M. Brian, S. R. Eric, T. E. Thomas and L. D. Jillian, *J. Chem. Educ.*, 95(2) (2018) 197.
34. ICH Guideline Q2A (RI), Validation of Analytical Procedures: Text and methodology, (2005).
35. C. M. Riley and T. W. Rosanske, Eds; Development and Validation of Analytical Methods, Pergamon Press; Oxford (1996).

36. C. C. Chan, H. Lam, T. C. Lee and X. M. Zhang, Eds; Analytical Method Validation and Instrument Performance Verification, Wiley Interscience Pub.; New jersey (2004).
37. P. De Bièvre and H. Günzler, Validation in chemical measurement (Vol. 549). Springer (2005).
38. M. Thompson, S. L. Ellison and R. Wood, *Pure Appl. Chem.*, *74*(5) (2002) 835.
39. P. Ravisankar, C. N. Navya, D. Pravallika and D. N. Sri, *J. Pharm.*, *5*(10) (2015) 7.
40. P. M. Guto, *Int. J. Chem. Kinet.,* 49(8) (2017) 596.
41. M. O. Oyagi, J. O. Onyatta, G. N. Kamau and P. M. Guto, *Int. J. Electrochem. Sci.*, 11 (2016) 3852.
42. Z. Yu, P. Cui, Y. Xiang, B. Li, X. Han, W. Shi, H. Yan and G. Zhang, *Microchem. J.,* 157 (2020) 105077.
43. L. Wang, Y. Xiu, B. Han, L. Liu, X. Niu and H. Wang, *J. Food Sci.*, In Press. doi: 10.1111/1750-3841.15328
44. M. D. H. Wirzal, A. R. M. Yusoff, M. S. Qureshi, R. Javeria, M. R. Bilad, G. Z. Memon and A. O. Solak, *Eurasian J. Anal. Chem.,* 14 (1) (2019) 12.
45. S. Salihu, N. A. Yusof, F. Mohammad, J. Abdullah and H. A. Al-Lohedan, *J. Nanomater.*, 2019 (2019) 1.
46. M. M. Rahman and A. M. Asiri, *Biomed. Microdevices,* 17 (2015) 9.
47. H. Li, B. Xu, D. Wang, Y. Zhou, H. Zhang, W. Xia, S. Xu, Y. Li, *J. Biotechnol.,* 203, (2015) 97.