electrolyte ($K_4[Fe(CN)_6]$) used in their studies as well as different surface area since different types of the nanocomposite were used.

# CHAPTER 6

*This chapter presents the quantitative analysis of metformin on modified and unmodified glassy carbon electrode by ultraviolet-visible spectroscopy (UV-Vis), cyclic voltammetry (CV) and scan rate voltammetry (SWV).*

## 6.1 UV-Vis of metformin

### 6.1.1 Sample preparation

Standard solutions of 0.06 M of metformin were prepared by diluting 50 $\mu$L of metformin in a 10 ml of distil water and diluted further to different concentrations. Analysis of the prepared solutions with different concentrations will be performed on a NICOLET evolution 100 Uv-vis instrument.

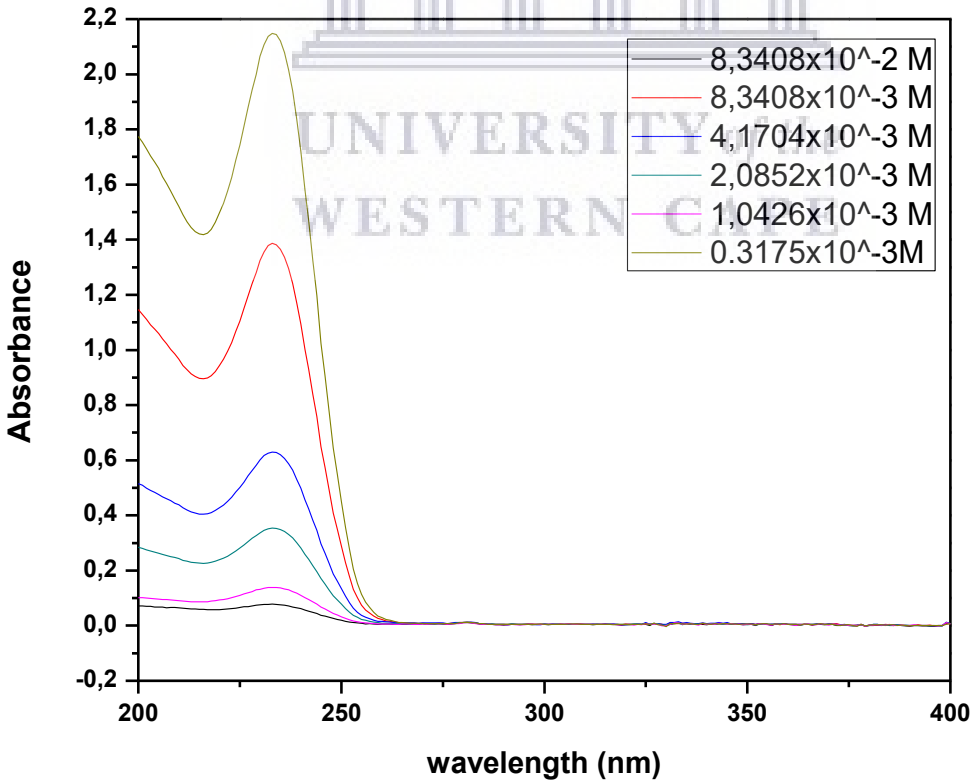

Figure 37:UV-Vis absorption spectra of metformin at λ=232nm.

Figure 37 shows the absorption spectra of metformin solution in distilled water. The absorbance spectra were recorded between the wavelength range of 200 – 400 nm. UV-Vis spectra of MET indicate one peak with a wavelength of 232 nm at maximal light absorbance.

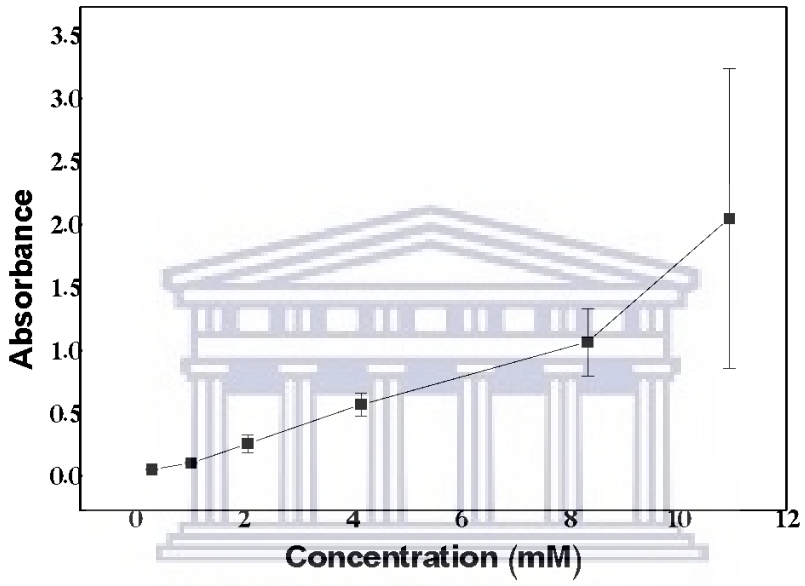


Figure38:Calibration curve for the detection of metformin (83,408 – 0.3175 mM) by UV-Vis, at maximum wavelength of 232 nm.



The schematic diagram above indicates a good linear response of metformin at low concentrations as well as a plateau being reached at high concentrations. Limit of detection (LOD) and limit of quantification (LOQ) was calculated using the following equation:

$$\mathbf{LOD} = 3\times\frac{SD}{Slope} \ldots . \boldsymbol{Equation\ 2}$$

$$\mathbf{LOQ} = 10\times\frac{SD}{Slope} \ldots . \boldsymbol{Equation\ 3}$$

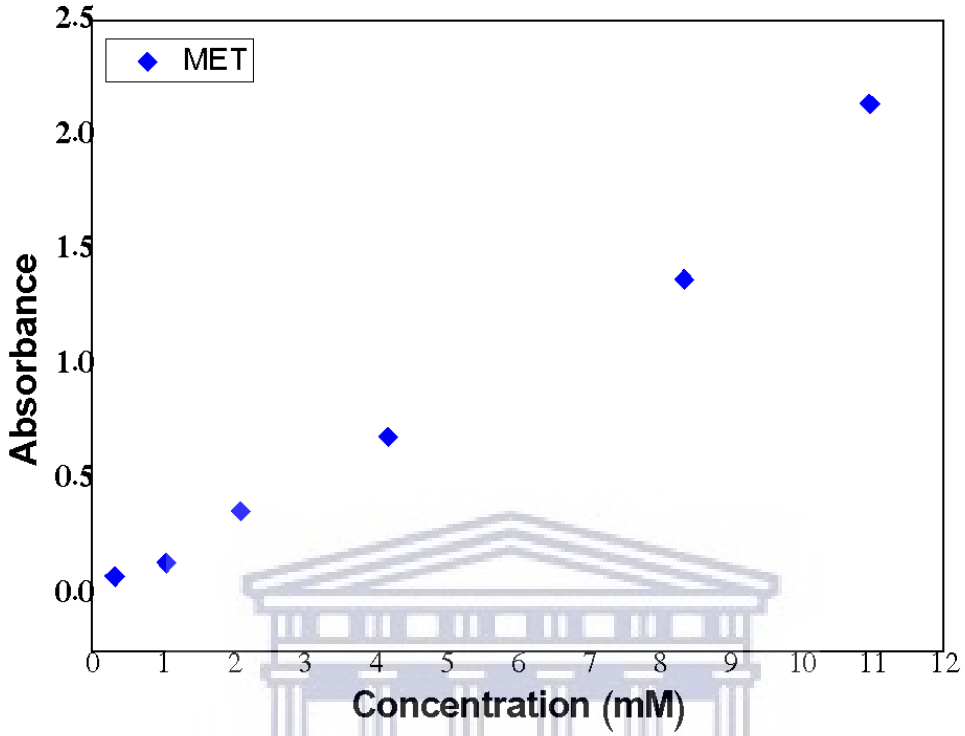


Figure 39:Full calibration curve of UV-Vis for different concentrations of metformin at maximum wavelength of 232 nm.



The calculated concentration in the range of (0.3175 mM- 8.341 mM) was made in use to construct the calibration curve in Figure 38. In this concentration range, Beer's law is obeyed. The determination coefficient value was calculated ($r^2$ = 0.9828, $y = 0.1880x - 0.0462$). Limit of detection was calculated to form the linear range of $5.2660 \times 10^{-3} M$ and the limit of quantification (LOQ) were 0.016 M (Figure 39). The UV/vis method sensitivity for the detection of metformin in a solution using standard solutions was found to be 0.1880 mA/M.

### 6.2 Electrochemical detection of metformin

The standard solutions of metformin were prepared prior the experiments as indicated above. The standard solution of metformin was added in increments of 10 $\mu$L in an electrochemical cell. The

electrochemical detection of metformin was then studied using cyclic voltammetry and square wave voltammetry (SWV) at a bare electrode as well as at a modified electrode in 0.1 M HCl within the potential window of -0.4 V to 1.0 V, scan rate of 50 mV/s.

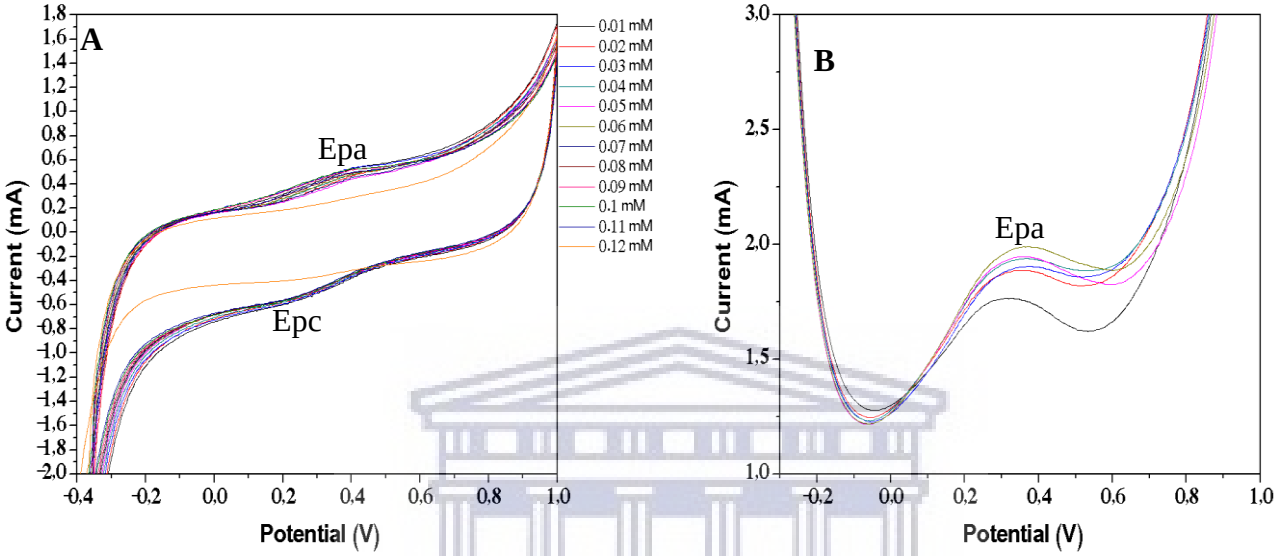


Figure 40: Concentration-dependent cyclic voltammogram (A) and square wave voltammogram (B) of metformin at a bare GCE in 0,1 M HCl, scan rate of 50 mV/s.

The electrochemical behaviour of metformin was measured by cyclic voltammetry (CV) at GCE/MET in 0,1M HCl at a scan rate of 50 mV/s. The cyclic voltammogram demonstrated above indicates an increase in current response with every increase of metformin (Figure 40). Figure 40 shows that as the concentration of metformin increases no peaks of metformin are observed and that means the activity of the electrode towards metformin is very low are a bare electrode. The oxidative square wave voltammetry of metformin at a bare glassy carbon electrode confirmed the increasing current with concentration behaviour observed with cyclic voltammetry with improved resolution of the oxidation peak at 0.41V (vs Ag/AgCl).

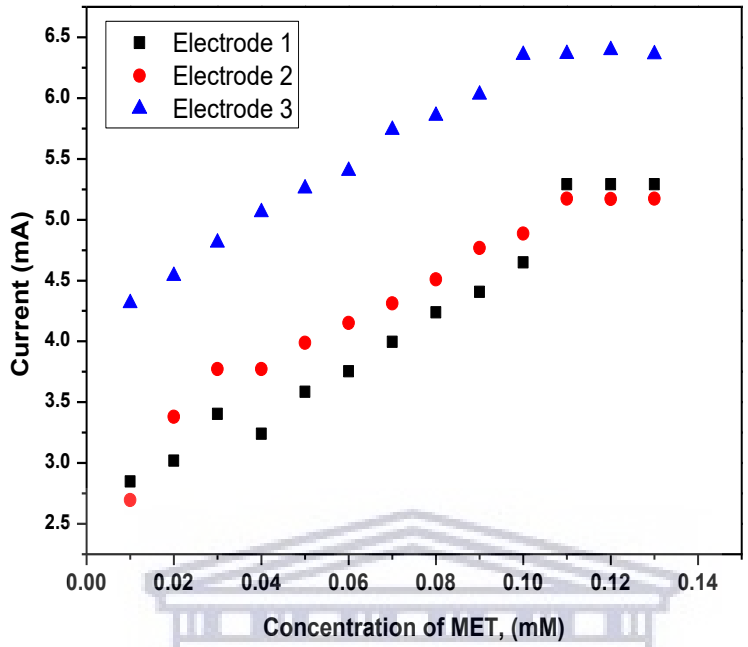


Figure 41: Calibration curve for the detection of metformin (0.01 - 0.13 mM) at bare GC electrodes, at a potential between -0.4 to 1.0 V (Ag/AgCl), 50 mV/s in 0.1 M HCl.

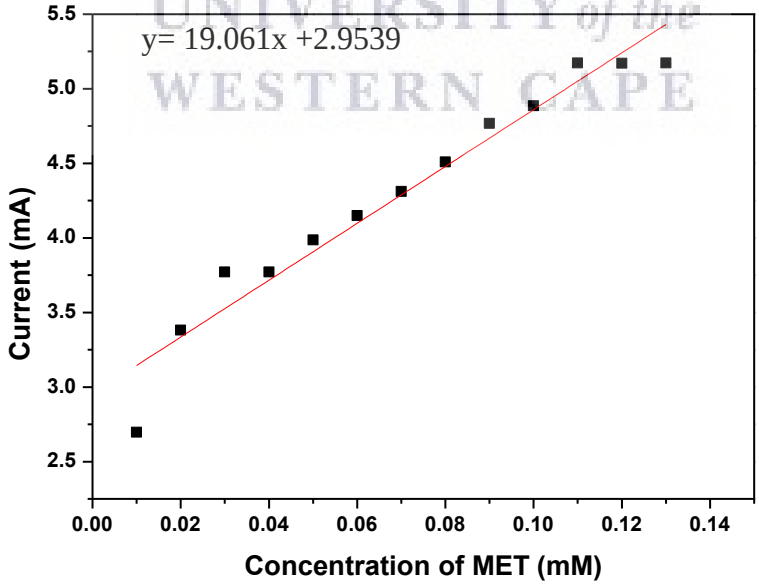


Figure 42:Linear plot of the current response of metformin within the concentration range of 0.01- 0, 13 mM at GCE in HCl, scan rate of 50 mV/s.

The calibration curve of the bare electrode at different concentrations of metformin in Figure 41 showed a linear relationship in the range (0.01 mM- 0.11 mM) between peak current and metformin concentration (Figure 42). Figure 41 confirms the experiment to be repeated three times at three different electrodes, meaning there are 3 limits of detections (LOD) calculated from the experiments conducted. The limit of detection is a measure of the smallest concentration of an analyte an electrochemical sensor can detect. The calculated limit of detections were $6{,}36 \times 10^{-3}$ M, $6.4726 \times 10^{-3}$ M, $7.0 \times 10^{-3}$M respectively with a relative standard deviation of 7.1418 %. Additionally, the average limit of detection was calculated to be $6.6109 \times 10^{-3}M$ with a sensitivity of 19,06 mA/M. The analytical performance of metformin on a bare GCE was poorly observed with low sensitivity similar to the sensor reported by Hadi, et al, 2016. Thus, modifying the electrode was of interest, in order to increase sensitivity and selectivity.

Table 10: Comparison of the analytical performance of the GCE/MET.

| Electrode | Sensitivity (M) | LOD (mM) | LOQ (M) | R-Square |
|---|---|---|---|---|
| GCE- Met 1 | 21,7230 | 6,3610 | 0,0192 | 0,9613 |
| GCE-Met 2 | 19,0680 | 6,4726 | 0,0196 | 0,9416 |
| GCE-Met 3 | 18,4793 | 7.0000 | 0,0202 | 0,9574 |

### 6.3 Electrochemical detection of metformin on PPy/GCE

The electroanalytical response of metformin was studied using cyclic voltammetry (CV) as well as the square wave voltammetry (SWV) with the electrode modified with polypyrrole in 0.1 M HCl at 50 mV/s.

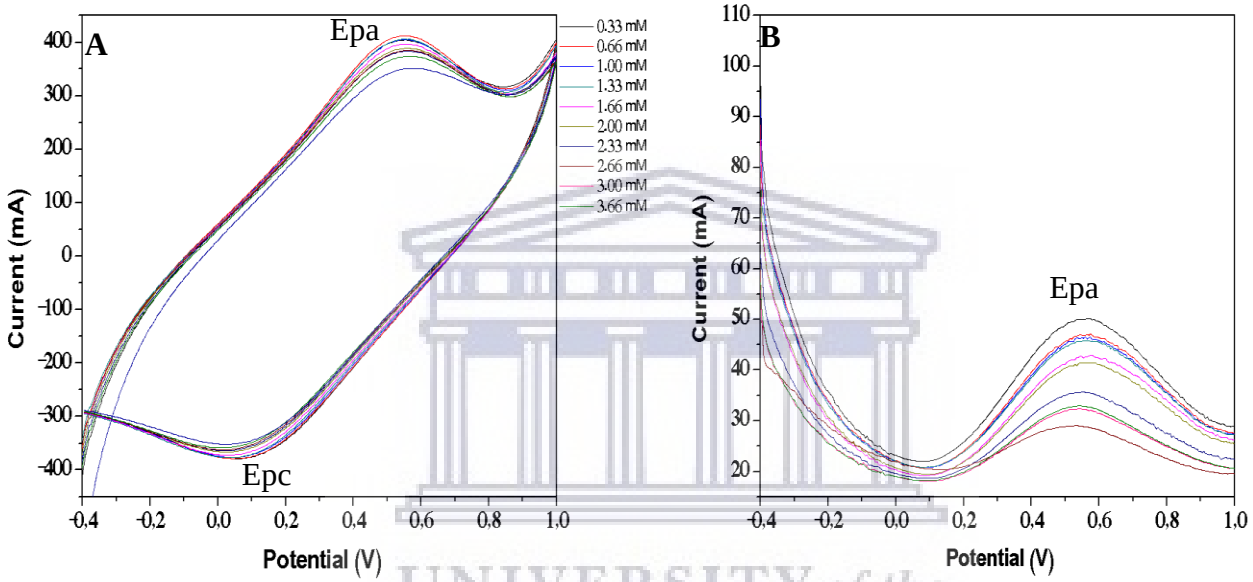




Figure 43:Concentration dependent cyclic voltammogram (A) of metformin on PPy/ GCE in 0,1 M HCl at 50 mV/s.

Figure 43 shows the detection of metformin on polypyrrole modified electrode in HCl electrolyte. The analytical performance of the sensor was investigated with CV (A) and SWV (B). The above figures indicate the current response to increase as the concentration of metformin increases. Upon the addition of 10 uL of metformin in the cell, oxidation and subsequent reduction peaks appeared at 0.55 V and 0.06 V respectively, indicating a quasi-reversible electrode process which is probably due to the diffusion barrier of the polymeric film. Comparing the concentration profile of metformin at bare electrode and at PPy modified electrode, the peak potential of the modified electrode shifted to the right and started to increase with the increasing concentration of metformin.

In Ulubay's paper, similar redox peaks were obtained at lower potentials of 0.24 (oxidation) and 0.16 V (reduction). The peak characteristics of the above diagram clearly indicate an electrocatalytic oxidation of metformin at PPy modified electrode. Comparing the electrochemical behaviour of metformin on a bare electrode and on a polypyrrole modified electrode, the polypyrrole modified electrode indicated an active surface area by increasing the analytical determination sensitivity of metformin. Metformin was further characterised by SWV. The oxidative square wave voltammetry of metformin at PPy modified glassy carbon electrode confirmed the increasing current as a function of metformin concentration behaviour with improved resolution of the oxidation peak at 0.55 V (vs Ag/AgCl).

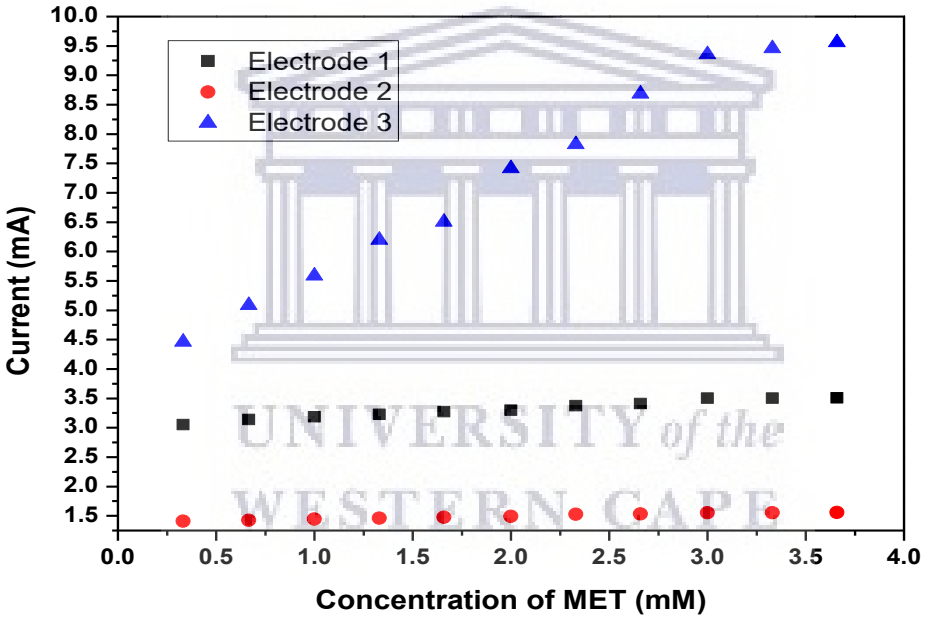


Figure 44:Calibration curve for the detection of metformin (0.33 – 3.66 mM) at PPy/GCE electrodes, at a potential range of -0.4 to 1.0 V (Ag/AgCl), 50 mV/s in 0.1 M HCl.

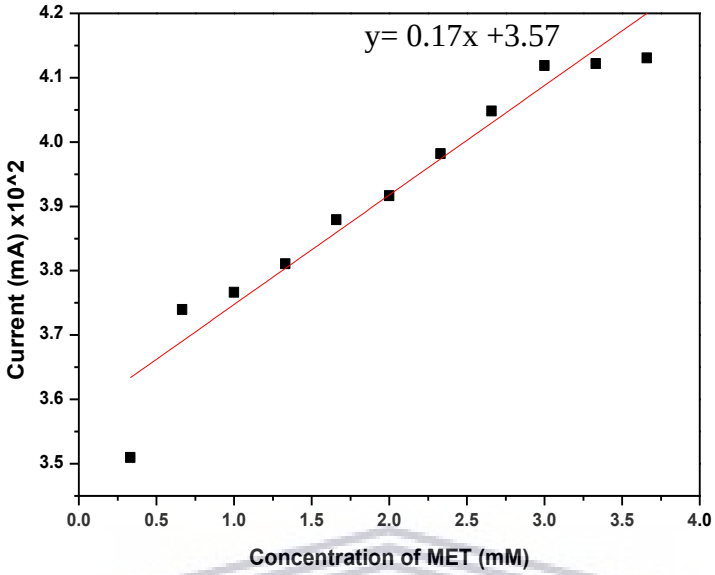


Figure 45: Linear plot of the current response of metformin within the concentration range of 0.33-3.66 Mm at PPy/GCE in HCl, scan rate of 50 mV/s.

The calibration curve of PPy/GCE electrodes correspond to the current response of metformin at different concentrations (0.01 mM- 0.11 mM). Electrode 3 indicated a high current background compared to the other two electrodes and that could be influenced by electrode surface as well as faradaic processes. Electrode 1 was indicated to have a better linearity compared to other electrodes. The experiments were repeated three times; hence three limits of detections were calculated from the experiments conducted. The limit of detection was calculated using equation 2. The calculated limit of detections was $2.0431 \times 10^{-2}$ M, $7.1466 \times 10^{-2}$ M, $1.7279 \times 10^{-2}$ M respectively with a relative standard deviation of 7.1418 %. The average limit of detection was calculated to be $3.6392 \times 10^{-2} M$ with a sensitivity of 0.17 mA/M. The detection limit was obtained to be higher than the one in the previously reported studies (Ulubay et al, 2010).

### 6.4 Electrochemical detection of metformin on CuNP-PPy/GCE

The electrochemical study of MET standard solution in 0,1 M HCl at CuNP-PPy/GCE was performed using cyclic voltammetry and square wave (Figure 41) at 50 mV/s.

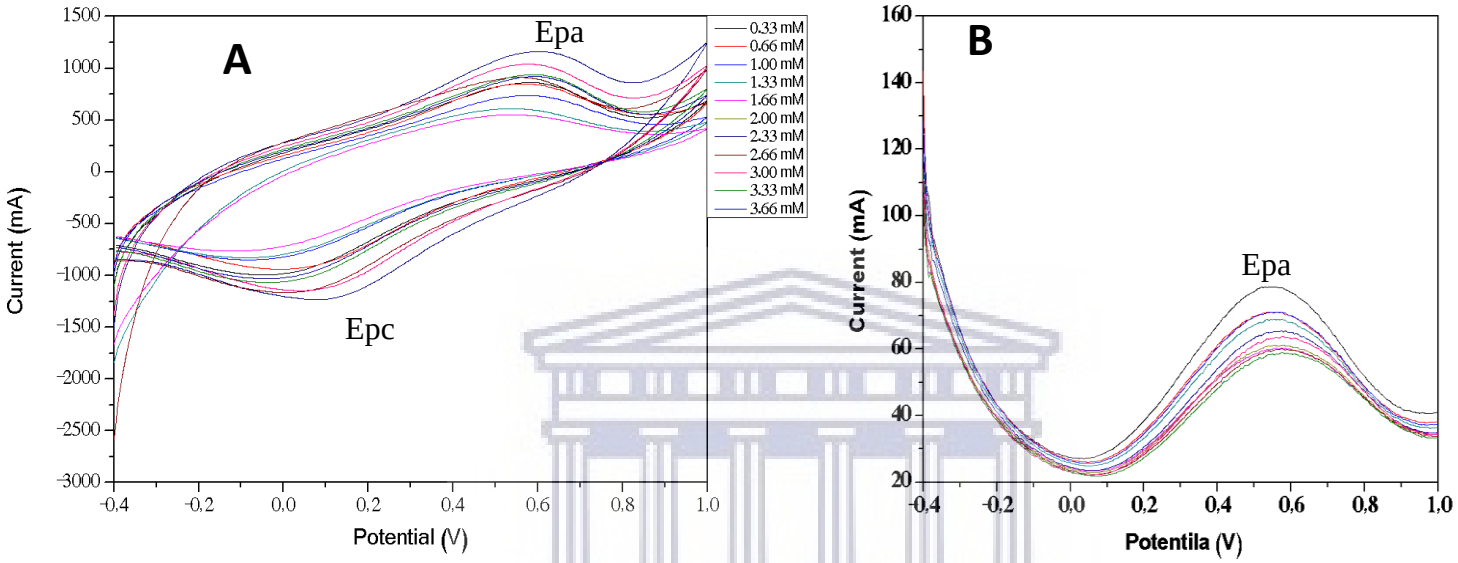


Figure 46: Cyclic voltammogram (A) and oxidative square wave voltammogram (B) of concentration dependent metformin analysis at Cu-PPy/ GCE composite in 0,1 M HCl at 50 mV/s.



The analytical performance of CuNP-PPy chemical sensor was evaluated with CV (Figure 46, A) and SWV (Figure 46, B). To date, not much has been reported on metformin electrochemistry on this platform. Nonetheless, CuNP-PPy chemical sensor was comparable to the reported studies. The redox couple of Epa at 0.61 V (Ag/AgCl) and Epc at 0.1 V oxidation of the amino group (NH) and reduction of N-carbonyl-guanidine. The cyclic voltammogram (A) indicates an enhancement and shift of oxidative peak form low potentials to high potential compared to the bare GC electrode. However, increasing metformin concentrations presented that the CuNP-PPy thin films peaks indicated a decreasing trend. A more sensitive electrochemical technique (SWV) was applied and well define peaks indicating current response as a function of metformin concentration at a modified electrode is demonstrated at Figure 46 B. Improvement of peak potential is due to

strong chelating of $Cu^{2+}$ which is present in CuNP towards metformin (Hadi, et al, 2016). The modified electrode showed a high electron transfer rate which indicated improve sensitivity.

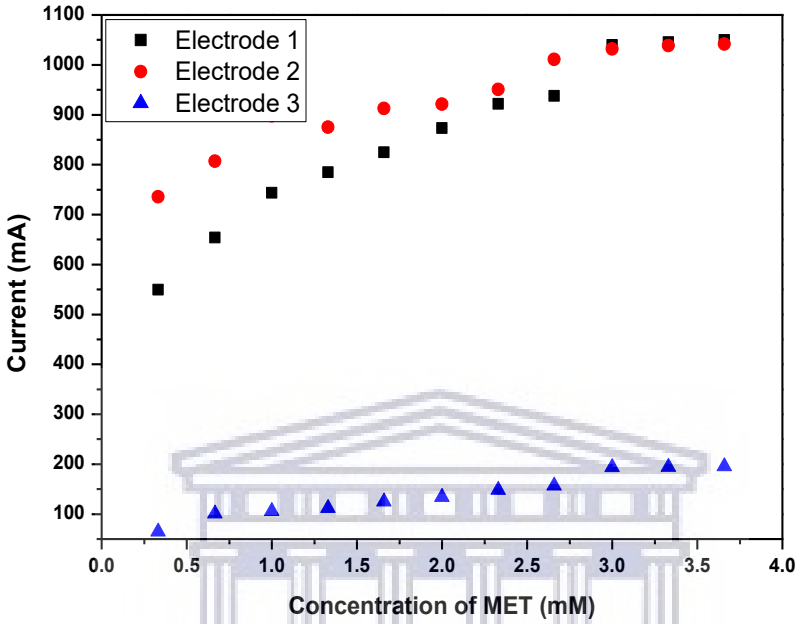


Figure 47:Calibration curve for the detection of metformin (0.01 - 0.13 mM) at CuNP-PPy/GCE, at a potential between -0.4 to 1.0 V (Ag/AgCl), 50 mV/s in 0.1 M HCl.



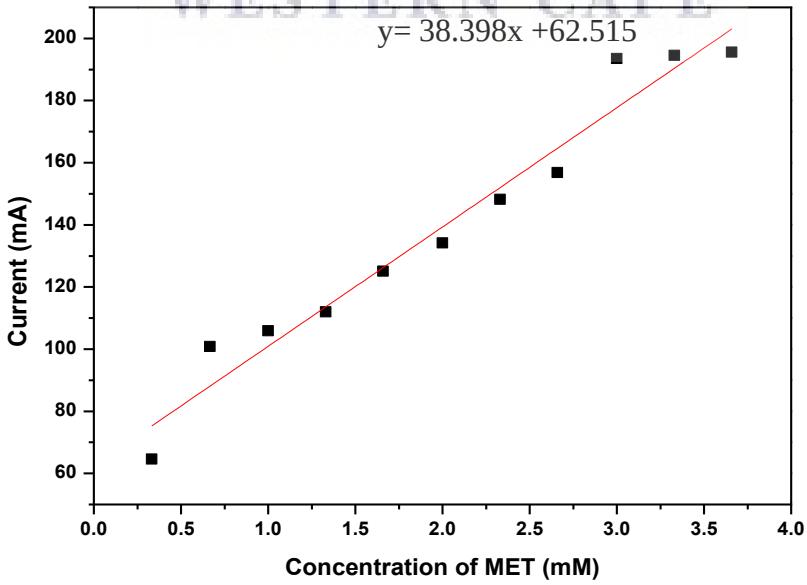

Figure 48: Linear plot of the current response of metformin within the concentration range of 0.33- 3,66 Mm at CuNP-PPy/GCE in 0.1 M HCl, scan rate of 50 mV/s.

Figure 47, demonstrate the calibration curves of CuNP-PPy/GCE at different concentrations of metformin in 0.1 M HCl, at scan rate of 50 mV/s. The calibration curves indicated a linear relationship as metformin concentrations increased from 0.01 mM- 0.11 mM. The electrochemical analysis of metformin in aqueous solution was performed n=3 by cyclic voltammetry (Figure 48). The current response of two electrodes were observed to be higher than the current response previously reported in literature. The processing factors such as temperature and time may be the contributing factors to the high background current of the electrodes. The linear plot in figure 43 was used to investigate the analytical performance of CuNP-PPy/GCE composite in 0.1 M HCl at 50 mV/s for the detection of metformin in aqueous solution. Thus, the calculated limit of detections from the experiments was $9.0454\ x10^{-5}\ M$, $4.0142\ x10^{-5}M$ and $2.3749\ x10^{-5}M$ respectively. The average LOD for CuNP-PPy/GCE sensor towards the detection of metformin was found to be $5.1448\ x10^{-5}\ M$ and the sensitivity of the sensor 38.3900 mA/M. Thereafter, the analytical performance of the electrochemical sensor was compared with the nanomaterial-based sensors in literature.



Table 11: Analytical performance of CuNP-PPy/GCE sensor compared to published data for MET detection.

| Electrode | LOD | Author |
|---|---|---|
| PPy-RGO-(AuNPs-GOD) n/GCE | $5.6000\ x10^{-6}M$ | Wu, 2018 |
| $Fe_3O_4$NP/MWCNT nanocomposite | $3.0000\ x10^{-9}M$ | Shahrokhian et al, 2012 |
| CuNP-PPy/GCE | $2.3749\ x10^{-5}M$ | This study |

The analytical performance of CuNP-PPy/GCE sensor was compared with the work in literature, the CuNP-PPy/GCE chemical sensor was discovered to have comparable LOD which proves that

the CuNP-PPy/GCE electrochemical sensor is a stable, low cost, environmentally friendly and easy alternative method in detecting metformin in an aqueous medium (Table 11).

Table 12: Comparison of the analytical performance of the CuNP-PPy/GCE.

| Electrode | Sensitivity (mA/mM) | LOD (M) | LOQ (M) | R-Square |
|---|---|---|---|---|
| CuNP-PPy/GCE 1 | 38.3900 | $9.0454 X10^{-5}$ | $2.7410 x10^{-4}$ | 0,9565 |
| CuNP-PPy/GCE 2 | 86.5250 | $4.0142 x10^{-5}$ | $1.2164 x10^{-4}$ | 0,9141 |
| CuNP-PPy/GCE 3 | 146.2580 | $2.3749 x10^{-5}$ | $7.1965 x10^{-5}$ | 0,9558 |

An electroanalytical voltammetric method was described for determination of MET in bulk drug, based on its redox reaction at glassy carbon electrode. The commercial metformin showed a good response on the electrode modified with nanocomposite material compare to a polymer electrode and bare GC electrode. This is confirmed by low limit of detection for CuNP-PPy compared to PPy and bare GC electrode. Furthermore, it was discovered that CuNP-PPy electrode is more sensitive compared to bare glassy carbon electrode and that is confirmed by lower limit of detection for CuNP-PPy than bare electrode. That means CuNP-PPy can detect the analyte at lower concentration.

# Conclusion

Metformin is classified as the best recommended anti-diabetic drug, particularly for individuals with type 2 diabetes mellitus disease which is considered a worldwide epidemic. The wide use of this anti-diabetic drug with bioavailability below 60% for a broadening variety of health problems globally, result to it being regarded as an emerging pollutant found in large quantities in wastewater. Previously, different methods were utilized for the early detection of metformin in wastewater, however they proved to suffer from several disadvantages such as long chromatographic run times, low sensitivity, and complicated sample preparation procedures before analysis. Developing a sensitive electrochemical sensor will make it easy to quantify metformin concentration in aqueous systems.

In this work, the first part focused on synthesising metformin derivatives with better bioavailability. However, the full synthesis was not completed since it was costly and complicated. Nonetheless, the starting material, 2-Amino-3-methylbutanoic acid **$A_1$** (DL-Valine) was synthesised following **Scheme 2**. Characterisation of 2-Amino-3-methylbutanoic acid **$A_1$** (DL-Valine) with column chromatography, thin layer chromatography, confirmed the starting material to be synthesized successfully. The chemical structure of the above-mentioned compound was fully elucidated by analysis of spectroscopic data with $^{1}$H NMR and $^{13}$C NMR. Notwithstanding of the incomplete organic synthesis of metformin derivatives, the main aim of this study was to evaluate the electroanalytical behaviour as well as the concentration profile of metformin in aqueous matrices. Thus, a commercial metformin drug was made in use to proceed with the experiment.

Metformin solutions were prepared from the commercial tablets (Glucophage, 500mg) and were characterised using cyclic voltammetry (CV) square wave voltammetry (SWV). For comparison purposes MET was also analysed using a standard analytical method, Ultraviolet Visible spectroscopy (UV-Vis). The limit of detection determined from UV-Vis for metformin was 5.2660 $\times 10^{-3} M$. A conductive polymer, polypyrrole was synthesised electrochemically following Bozzini's methods for some nitigrities and was characterized using Cyclic Voltammetry (CV) and Square Wave Voltammetry (SWV). Cyclic voltammograms confirmed synthesis to be a success

since the peak potential observed agreed with literature. The calculated limit of detection for PPy/GCE was $2.0431 \times 10^{-2} M$ which is high compared to the ones previously reported in literature.

Copper nanoparticles were synthesized using chemical reduction method. Copper nanoparticles were characterized by Scanning Electron Microscopy (SEM), Fourier Transform infrared spectroscopy (FTIR), Transform Electron Microscopy (TEM). The SEM was used to study the morphology of copper nanoparticles, the SEM image of CuNP, a typical clustered structure confirmed the successful synthesis of the nanoparticles. Fourier transform infrared spectroscopy (FTIR) confirmed the functional groups associated with copper nanoparticles. Moreover, Small Angled X-ray Scattering (SAXS) was utilized for the size of nanoparticles, which was found to be 37 nm. The composite of CuNP-PPy/GCE was formed by combining copper nanomaterial and polypyrrole. CuNP-PPy/GCE was then characterised using Cyclic voltammetry (CV). The main purpose of the composite was to increase sensitivity of the sensor. Cyclic voltammograms observed confirmed a success synthesis of the composite by studying its electrochemical behaviour. Literature agreed with the electrochemistry of composite.

Metformin was successfully detected by the sensor designed, with the average limit of detection of $5.1448\, x10^{-5}\, M$. Meaning the designed sensor can detect low concentrations of metformin in the presence of the 0,1m Hydrochloric acid as an electrolyte. This was demonstrated by the small standard deviation per sample set (n=3) with a relative standard deviation (RSD) of 47%. Even though the electrochemistry of metformin on Cu-PPy/GCE is limited, the analytical performance of the Cu-PPy/GCE sensor in this work in terms of LOD, LOQ and sensitivity illustrates that the sensor provides a cost-effective, reproducible and is a fast analytical tool for the detection of metformin in waste water.

# Future Works

The motivation of this work was synthesis metformin analogues since metformin's bioavailability was 60%, with large amounts of it being excreted in wastewater unchanged. Due to time and cost effectiveness of the process we could not finish. Thus, in future we will focus on synthesizing metformin analogues with high bioavailability in human system such that small amounts of this anti-diabetic drug are excreted in wastewater. Additionally, in the future we will focus more on applying the novel electrochemical sensor to real sample analysis and develop a portable monitoring tool to evaluate the concentration levels of metformin in wastewater. Collaboration with water and sanitation department form the municipality will be a necessity to develop sampling protocols and evaluating on site analysis efficiency and reliability.

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