3. 3. Effect of accumulation time and potential

The accumulation [10] of OXM at GCE surface depends on operational factors, which were precious additional investigations to ensure sensitive detections of this drug. So, the effect of accumulation time on the efficiency of the collection of 5µM OXM drug on the working electrode surface was evaluated by rising the accumulation time over the range of 0–40s. The resulting peak current- optimum accumulation time is exhibited in40s as in Fig. 5and as can be seen from this plot, a steady enhancement in the peak current was observed over the range0–40 s and after that the peak intensity nearly decreased probably due to the saturation of the GCE surface. Hence, 40s accumulation time was selected for all the future experiments. On the other hand, variance of the accumulation potential over the range from 0.15 to -0.1 V Fig. 6 at 40 s accumulation time revealed that a pre concentration potential of 0.1 V was the optimum condition.

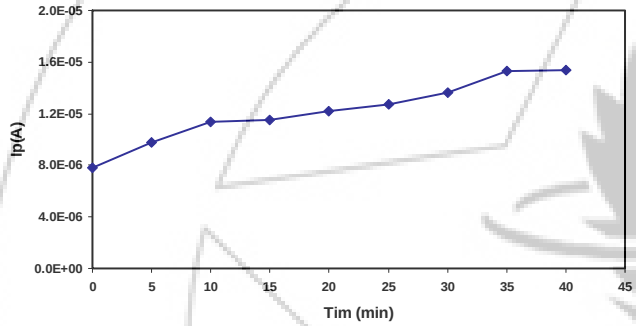

Figure 5: Effect of accumulation time (Tacc) of 5µM OXM peak current at B–R buffer, pH = 8, E= -0.1V and scan rate 100mVs^{-1}

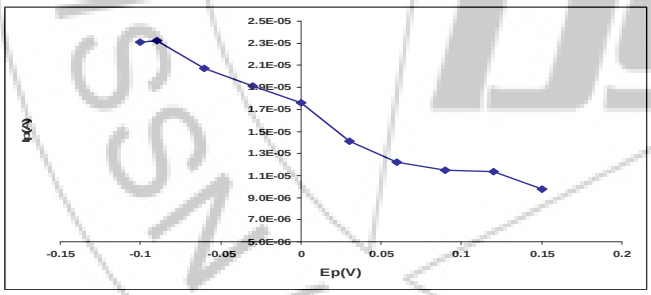

Figure 6: Effect of accumulation potential (Eac)5µM OXM peak current at B–R buffer, pH = 8, time 35s and scan rate 100mVs^{-1}

3.4. Effect of pH

pH is one of the important parameters that affected the electrode response in drug sample determination, The shape and electrochemical behavior of 20µM OXM in 0.04M B-R in the range 3.0–10.0electrolyte solution at different pH values and cyclic voltammetry was studied at GCE, the pH of the solution strongly affects the peak current and potential (Ep) as in Figs (7, 8) It can be seen that the anodic peak current of OXM reaches a maximum value at pH = 8.0, and then decreases gradually as pH increases Fig. 7. Therefore, pH = 8.0 was chosen as optimum pH for the following electrochemical detection of OXM. On the other hand the anodic peak potential of OXM at the surface of GCE shift to less positive [11-13] values linearity with increasing pH of the buffered solution as in Fig. 8.

The pH dependences of the peak potentials are expressed as follows:

E_0=-0.044pH+ 1.1362R^2= 0.9947

This slope 0.044 V/pH is indicated that which 25 °C was close to the theoretical value of −59 mVpH−1at this result was agreement with the Nernst equation for a one proton coupled reversible single electron transfer.

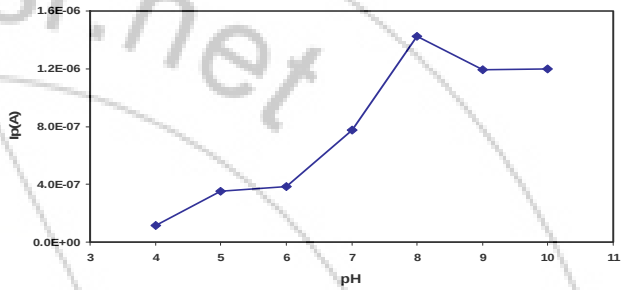

Figure 7: The relationship of anodic peak current response vs. solution pH value of 5µM OXMT solution in B-R buffer on GCE at a scan rate 100 mVs^{-1}.

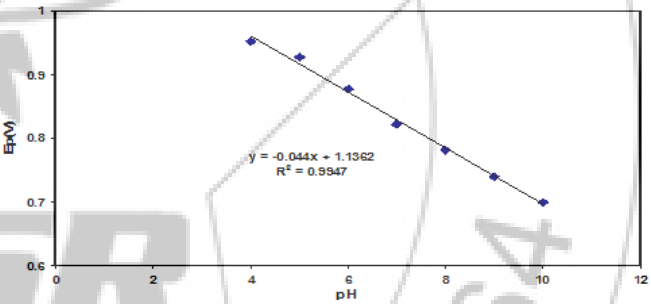

Figure 8: The relationship of Ep vs. solution pH of 5µM OXM at GCE electrode at a scan rate 100 mVs^{-1}

3.5. Analytical applications

3.5.1 Differential Pulse voltammetry

To develop a Quantitative voltammetric evaluation methodology for determining the drug is established on the linear correlation between the peak current and concentration. For analytical purposes we selected the DPV and SWV mode. Differential pulse experiments were performed on the GCE in B-R buffer solution at pH = 8 with experimental conditions were: scan rate 5 mV/s; pulse amplitude 50 mV; sample width of 40 ms; pulse width of 50 ms; and pulse period 40 ms. The potential was scanned anodically from an initial to a final potential of 500 - 1000 mV resulting voltammograms shown in Fig. 9show that while the peak potential remained almost constant at 0.809V. The DVP data for the determination of the drug under investigation in Fig. 10 shows a linear relation between the peak current (Ip) and OXM concentration (C) were found in the following range: 1.25 µM - 10µM. The calibration plots were described by the following equations:

Ip = 0.019C (µM) + 0.0355

r (Correlation coefficient) = 0.9903

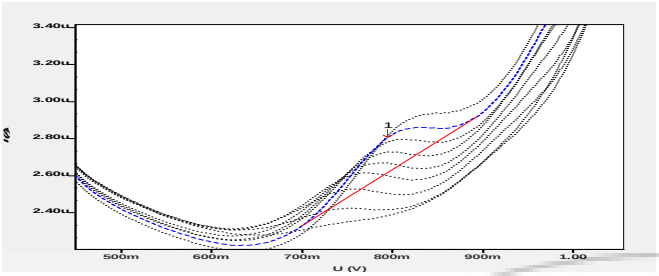

Figure 9: Background corrected DPV response for different concentrations of OXM 1.25 µM - 10µM in BR buffer solution (pH 8.0) at GCE

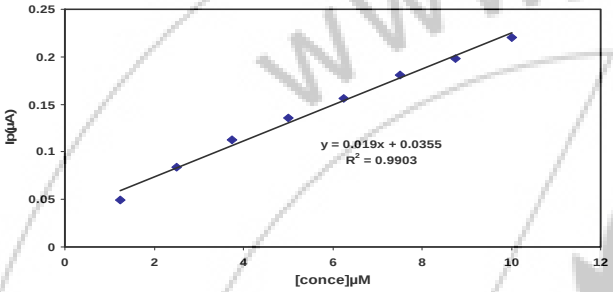

Figure 10: Defferential pulls voltammetric responses for successive additions of OXM from 1.25 µM - 10µM in BR buffer solution (pH 8.0) at GCE

3.5.2 Square wave voltammetry

SWV experiments were performed at the GCE in B-R buffer solution at pH = 8 with experimental conditions were: scan rate 100 mV/s; pulse amplitude 20 mV; , 2 mV potential step and potential range of 500 to 1000 mV and frequencies 50 Hz. The potential was scanned anodically from an initial to a final potential of 600 - 1000 mV resulting voltammograms shown in Fig. 11 shows that while the peak potential remained almost constant at 0.866V. The SWV data for the determination of the drug under investigation in Fig. 12. Shows linear relations between the peak current (Ip) and OXM concentration (C) were found in the following range: 0.37µM - 4.5µM. The calibration plots were described by the following equations:

Ip = 0.317C (µM) + 0.373
r (Correlation coefficient) = 0.9924

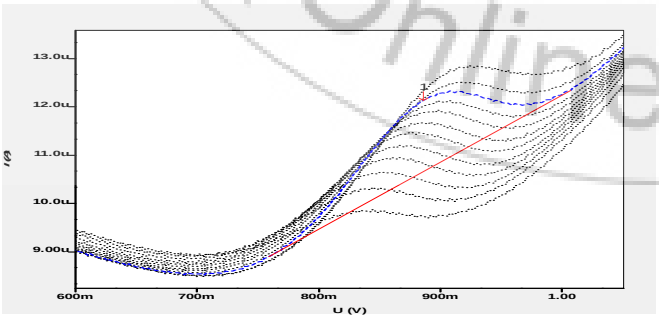

Figure 11: Background corrected SWV response for different concentrations of OXM0.37µM - 4.5µMin BR buffer solution (pH 8.0) at GCE

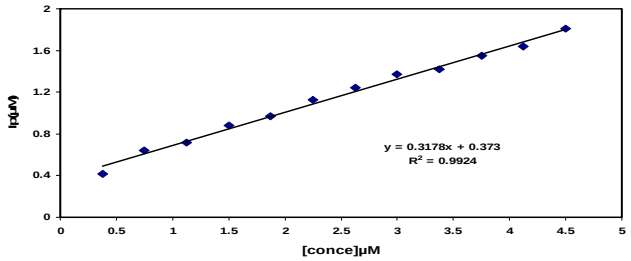

Figure 12: Square wave voltammetric responses for successive additions of OXM from 0.37µM - 4.5µM in BR buffer solution (pH 8.0) at GCE.

3.6. Validation of the Analytical Procedure

The linearity of the calibration curve was obtained for both DPV and SWV techniques as in above these concentration range sand the loss of linearity was probably due to the adsorption of OXM on the electrode surface. The characteristics of these graphs are given in Table1. The precision of the method was investigated by repeatedly (n = 5) measuring peak potential and peak current of OXM within a day and over three consecutive days for both techniques. LOD and LOQ were calculated as (3 s/m) and (10 s/m), respectively where s is standard deviation of response (five runs) and m is the slope of the calibration curve. LOD and LOQ values confirmed the sensitivity of the proposed methods, These results demonstrated good precision and accuracy [14, 15].

Table 1: Characteristics of PNT calibration plots using proposed voltammetric methods

Parameters	DPV	SWV
Slope (mV decade^{-1})	0.019	0.317
Intercept (mV)	0.0355	0.373
Correlation coefficient	0.9903	0.9924
Detection limit (µmol L^{-1})	0.365	0.053
Limit of quantitation (µmol L^{-1})	1.219	0.177
Working pH	8	8
Concentration range, µmol L^{-1}	0.37 - 4.5	0.37 - 4.5
Average recovery (%)	99.85-99.95	99.84-99.95
RSD% a	0.021	0.0079

3.7. Application to Analysis of Pharmaceuticals

On the aim of these results, both proposed methods (DPV and SWV) were applied to the direct determination of OXM in syrup [16, 17], using the related calibration curve of the straight lines without sample preparation and after an adequate dilution Table 2.The proposed analysis procedure was successfully applied for the assay of OXM in its pharmaceutical formulations. As far as we know, there is no official method in any pharmacopoeias related to pharmaceutical preparations of OXM. For this reason, the HPLC method [18] was used for compare son and for the reliability of the developed procedures. The results obtained for the formulation are listed in Table 2 and compared with the HPLC. The recovery studies were carried out by adding the known amount of the pure drug to the earlier analyzed pharmaceutical formulations of OXM. The recovery [19] of the drug was calculated by comparing the concentration

obtained from the spiked mixtures with those of the pure drug. Table 2 shows a good result demonstrates the selectivity of the proposed method for the determination of OXM in commercial tablet forms.

Table 2: Evaluation of the accuracy and precision of the proposed and official methods for the determination of OXM In its pharmaceutical forms at GCE

Pantoloc	[Drug]	Proposed method ±%RSD, n=5	Official method ±%RSD, n=5	F-test	T-test
DVP	33mg	100.02 ± 0.12	99.91± 1.3	1.13	2.04
SWV	33mg	100.01 ± 0.11	100.05± 1.08	1.22	2.06

4. Conclusion

DPV and SWV methods have been developed for the determination of OXM in pure form and pharmaceutical formulation. The principal advantage of the proposed method over the reference HPLC method is sensitivity and specificity. The proposed voltammetric technique has the advantages of being simpler, faster, more selective and more cost-effective than other technique.DPV and SWV methods are rapid, requiring about 5 min to run the sample. The possibility of monitoring the compound in pharmaceutical formulation makes the voltammetric method useful for pharmacodynamic purposes.

Acknowledgement

The author gratefully acknowledges the financial support of the Faculty of Pharmacy, October 6 University for helping to execute this work.

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