

Anodic voltammetric determination of Omeprazole in Pharmaceutical formulation via pencil graphite electrode

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ABSTRACT: Background: The Omeprazole (OMZ) is a proton pump inhibitor used to treat duodenal and peptic ulcer. **Aim:** The electrochemical behaviour of Omeprazole (OMZ), which is a proton pump inhibitor, was studied using differential pulse voltammetry (DPV) on the surface of pencil graphite electrode (PGE). **Methods:** The electrochemical behaviour of Omeprazole was studied using differential pulse voltammetry (DPV) on the surface of pencil graphite electrode (PGE). Optimization of the voltammetric experimental conditions including pH, potential scan rate, and accumulation time, were carried out by cyclic and linear sweep voltammetry. **Results:** The electrochemical behaviour of Omeprazole showed a recognized oxidation peak at around 0.81 to 0.85 V, in phosphate buffer solution (pH - 7.0), and KCl 1.0 molL⁻¹ as supporting electrolyte. The results indicating a totally irreversible and diffusion controlled reaction. The Pencil graphite electrode (PGE) exhibited a linear response within the concentration range 30 to 200 µmolL⁻¹, with squared correlation coefficient 0.996 and limit of detection was 7.52 µmolL⁻¹. The electrochemical behaviour of Omeprazole exhibited good voltammetric properties such as repeatability, recovery, and robustness. **Conclusion:** Applicability of PGE was examined by determining OMZ in its pharmaceutical formulations.

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Keywords: Omeprazole, Pencil graphite electrode, Oxidation peak, Differential pulse voltammetry, Pharmaceutical formulation.

INTRODUCTIONS:

- D Duodenal ulcer or acid peptic disease represents a common medical problem that affects adults and children. Omeprazole (OMZ) (Fig 1) is proton pump inhibitors that inhibits gastric acid secretion from gastric cells, and prevent ulcer re-bleeding; it has been shown to be effective in the treatment of acid related diseases ^[1,2].
- C Chemically, OMZ is 5-Methoxy-2- [[(4-methoxy-3,5-dimethyle-2-pyridinyle) methyl] sulfinyl]-1H-benzimidazole, is an alkaline salt degrades in acidic or neutral media, and affected by heat, moisture and light ^[3].

OMZ sulphur atom is tricoordinated in a pyramidal structure, that gave OMZ its optical activity, (S) - and (R) - omeprazole, the (S) isomer has higher bioavailability than (R) isomer ^[4].

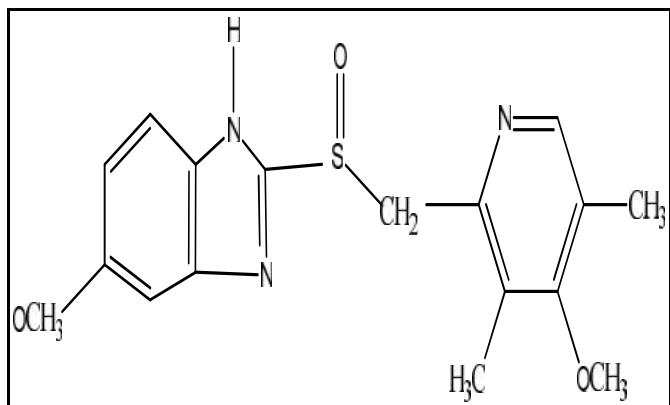


Fig 1. The chemical structure of Omeprazole.

The survey of literature revealed that many methods have been developed for quantitative and/or qualitative determination of OMZ in its Pharmaceutical formulation and in human biological fluids by different techniques, including spectrophotometric determination via nitrosation reaction ^[5] spectrofluorometric using 1,2 Naphthoquinone-4-sulphonate ^[6] high performance liquid chromatography (HPLC) determination in human plasma ^[7], RP-HPLC determination in the presence of its degradation products ^[8], simultaneous determination of OMZ in bulk powder and helicure tablets using TLC densitometric technique ^[9], and determination using potentiometric and voltammetric electrochemical methods ^[10,11].

Electrochemical techniques are powerful analytical techniques that provide results with high accuracy, precision and sensitivity, with relatively low cost instrumentation. Especially, many voltammetric analysis of electroactive samples, for instance, metals, drugs, and vitamins have been reported OMZ electrochemical behaviour has been determined, rather oxidation, reduction, or both ^[12-16]. Reduction of OMZ using polarographic technique and alternating current polarography was studied revealing that the reduction process occurred in sulphonyl group which transferred to thiol group at dropping mercury electrode (DME) ^[17].

The voltmmetric performance of edge-plane pyrolytic graphite (EPG) electrode was investigated for study of OMZ electrochemical behaviour which revealed that oxidation of OMZ is irreversible and pH dependant process ^[18]. Redox behaviour of OMZ has been reported using glassy carbon electrode and boron- doped diamond

electrode ^[4,19], the glassy carbon study showed that the oxidation process is irreversible and undergoes in two steps, the first one including transfer of one electron and one proton, the second one include two electrons and one proton transfer, that yield non-electroactive species and the reduction process undergoes is irreversible process in one step including the same number of electrons and proton transferred ^[4]. Reversible redox behaviour of OMZ in different supporting electrolytes has been investigated recently using Boron-Doped diamond electrode, the both processes are highly pH dependant and controlled by diffusion mechanism ^[19].

An extensive attention has been attracted toward pencil graphite electrodes (PGEs), due to their advantages over other carbon based and commercial metal electrodes, represented in their low cost, availability, ease of modification, and most importantly PGEs provide renewable surface that simpler and faster than other polishing procedures ^[6].

Even though, OMZ has been studied profusely using electrochemical techniques, PGE determination of the drug has not been reported yet, therefore in this work an attempt of develop of a new sensitive, and simple method is made without using any modification, using cyclic, differential and linear sweep voltammetric technique over a wide range of pH.

MATERIALS AND METHODS:

Chemicals and Solutions:

Omeprazole standard was procured from Amipharma Laboratories Co. Ltd. An aqueous stock solution of OMZ ($1 \times 10^{-3} \text{ mol L}^{-1}$) was prepared and stored in the refrigerator ($4.0 \text{ }^\circ\text{C}$). The required concentrations of OMZ in the supporting electrolyte were then prepared by dilution of the standard stock solution. Phosphate buffer solution in the pH range 4.5 to 11.0 was prepared, containing $0.2 \text{ mol L}^{-1} \text{ KH}_2\text{PO}_4$ and $0.2 \text{ mol L}^{-1} \text{ NaOH}$ in deionised water. All chemicals and reagents used were of analytical grade and procured from authorized dealer.

Instrumentation:

Linear sweep, differential pulse and cyclic voltammetric measurements were carried out using a potentiostat - galvanostat (PGSTAT) Autolab Metrohm (Echochemie, B.V., Netherlands), connected to Nova 1.11.0 software. The electrochemical system composed of three electrodes, platinum wire (Metrohm 6.0301.100 Pt/-20 to $70 \text{ }^\circ\text{C}$) as counter electrode, Ag/AgCl/KCl (Metrohm 6.0726.100 Ag, AgCl/0 to $80 \text{ }^\circ\text{C}$) as reference electrode

and PGE (0.5 HB rotating type) as working electrode. All the electrochemical measurements were maintained at temperature $25.0 \pm 1.0^\circ\text{C}$.

Analytical procedure:

PGE preparation and pre-treatment:

Lead pencil 0.5 mm in diameter, 40 mm in length HB rotating type was purchased from a local bookshop. Electrical contact to the lead was obtained by wrapping copper wire on the top of the lead to metallic crocodile clips [11]. For each measurement 15 mm of lead immersed in the solution. Taking in the account that PGE surface needs to be pre-treated electrochemically in order to get accurate results and lengthen the lifetime of the electrode, electrochemical scanning in PBS (0.2 molL^{-1} , pH 7.0) using cyclic voltammetry in the range of (-1.5 to 1.5 V) for 3 cycles was performed after each measurement. Attention should be taken to the electrode surface structure after successive measurements (approximately 20) that may be destroyed and then the PGE should be changed.

Electrochemical measurement of OMZ:

The electrochemical behavior of OMZ at PGE was investigated by recording cyclic voltammograms in PBS in the pH range of 4.5 to 11.0. In order to indicate the selectivity of the PGE toward OMZ 2.0 ml of supporting electrolyte and appropriate amount of KCl 1.0 molL^{-1} were placed into the electrochemical cell and purged with nitrogen gas for 2 min, and after immersing the lead into the solution, the cyclic voltammogram was recorded at 100 mVs^{-1} in the range of -1.3 to 1.3 V (Fig 2). Then the cyclic voltammograms of $1.0 \times 10^{-4} \text{ molL}^{-1}$ of OMZ were recorded at the same conditions in the range of 0.05 to 1.2 V. To study the effect of the scan rate and the accumulation time, 3ml of PBS, appropriate amount of 1 molL^{-1} KCl, and $1.0 \times 10^{-4} \text{ molL}^{-1}$ of OMZ were placed into the electrochemical cell and purged for 2 min by nitrogen gas. The results were recorded using cyclic and linear sweep voltammetry respectively. The scan rate effect was recorded in the range of 10 to 100 mVs^{-1} and the effect of the accumulation time was recorded in the range of 0 to 240 s. The parameters for the analytical performance such as calibration range, limit of detection and limit of quantification, recovery and precision were investigated by recording differential pulse voltammograms.

Preparation of pharmaceutical sample:

About 10 capsules of Ami 20 mg containing OMZ were accurately weighed, and then appropriate weight was

transferred to 100 ml volumetric flask. It was dissolved in 2 ml of PBS (0.2 molL^{-1}), and the volume was adjusted up to the mark by deionized water to prepare $100 \mu\text{molL}^{-1}$ of the OMZ solution.

RESULTS AND DISCUSSIONS:

The electrode selectivity towards OMZ was examined by measuring the blank using PGE which is 25.0 ml of 0.2 molL^{-1} phosphate buffer solution pH 7.0 and 1 ml of 1.0 molL^{-1} KCl. No peak current has appeared indicating that the blank is electrochemically inactive in the potential range of PGE (Fig 2).

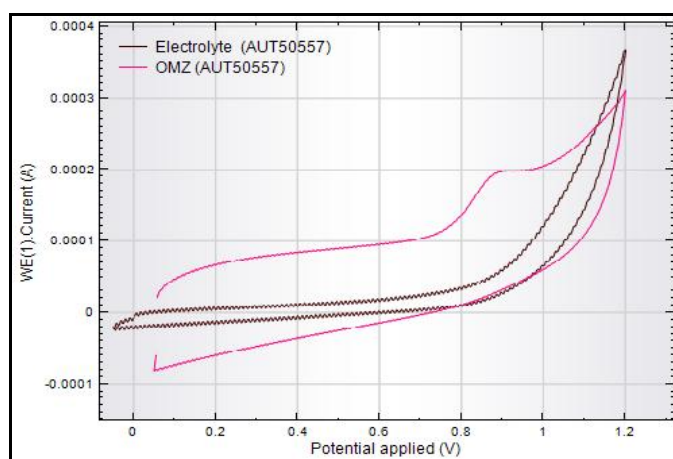


Fig 2. Shows PGE inactivity towards the matrix which contains PBS 0.2 molL^{-1} and KCl 1.0 molL^{-1} .

It is an accepted method to use potassium ferric cyanide for characterization of electrode active surface area, 1.0 molL^{-1} of ferric cyanide is used, the resultant oxidation and reduction peaks are used to determine (E_p), which is related, to surface activity, and the smaller value of (E_p) the faster rate of electron transfer hence it is more reactive. The typical value of carbon based electrode surface is near 70 mV [7], which is comparable to the calculated value (92 mV) of the PGE of our study.

The active surface area of 0.5HB PGE was calculated by Randles-Sevick equation [8] the area was investigated using cyclic voltammetry technique in $\text{K}_3\text{Fe}(\text{CN})_6$ solution (1.0 molL^{-1}) in KCl (0.1 molL^{-1}) in range of scan rate (10-100 cm/s).

$$I_p^{irrev} = \pm 0.496 (\alpha n')^{\frac{1}{2}} n F A C \left(\frac{F D \nu}{RT} \right)^{\frac{1}{2}} \dots (1)$$

There D is the diffusion coefficient of $\text{K}_3\text{Fe}(\text{CN})_6$ which is equal $7.6 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, α is electron transfer coefficient assumed to equal 0.5, n is the total number of electrons transferred per molecule, n' is the number of electrons transferred before rate determining step, A (cm^2) is the active surface area, C is the concentration of

$K_3Fe(CN)_6$ in $molL^{-1}$, $R = 8.314 J/K mol$, $T = 298 k$, $F = 96,480 Cmol^{-1}$. Plotting I_p vs $v^{1/2}$ showed a linear relationship with squared correlation coefficient 0.9953 that indicates reversibility of the reaction. The calculated active surface area is found to be $3.2 \times 10^{-8} cm^2$.

Electrochemical studies:

Electrochemical oxidation of OMZ ($1.0 \times 10^{-4} molL^{-1}$) was investigated by cyclic voltammetry at PGE surface, one main anodic peak was appeared at potential +0.87 V, in the negative scanning no reduction peak was observed indicating the irreversibility of the oxidation process.

Effect of pH of supporting electrolyte:

The use of pH measurement scan gives insights into mechanism of the reaction between the electrode and the analyte. The influence of pH on the peak current of OMZ ($1 \times 10^{-4} molL^{-1}$) at PGE surface was studied by measuring the current response (peak height) using cyclic voltammetry mode at the pH range 4.0 to 11.0. The results revealed that the OMZ degrades in acidic solution, and undergoes oxidation in the basic pH's, the anodic peak current appeared the highest at pH= 7 (Fig 3A) and decreased gradually with increasing of pH value. The pKa value of OMZ is nearly 9 (8.7 as reported in) [21], which indicates its protonation at pH lower than 7, and make its reaction at electrode surface is favourable, and by increasing of the pH value the protonated form of OMZ decreases which results in lessen of peak current.

In addition, the potential value became less positive with the increase of the pH, since the reaction of OMZ at the electrode surface is more favorable, also the plot of the peak potential V/s. pH gives information on the number of electrons and protons transferred in the electrochemical process. In the curve between E_p and pH the potential varies according to pH by $-2.303 \frac{mRT}{nF}$ per pH unit and the deviation of this linear relationship is due to the pKa of the OMZ [8]. Practically, plotting of E_p V/s. pH (Fig 3B) showed two linear relationships the first one is in the range of 5.0 to 9.0 with slope = -55 mV/pH which is close to the Nernstian value (= 59 mV/pH) indicates that the same number of protons and electrons was involved in this electrode process. The second one between the range of 8.0 to 10.0 with slope = -33 mV/pH which is very close to Nernstian value of two electrons one proton transferred process. Since the maximum peak appeared in pH = 7.0 it assumed that electrochemical reaction includes the same number of electrons and protons. The calculated ratio between the number of

proton (m) and the number of electrons per molecule that involved in the electrochemical oxidation process (n) is found to be 1:1.07.

Effect of Scan rate:

The effect of the scan rate on the peak current of $1.0 \times 10^{-4} molL^{-1}$ of OMZ in $0.2 molL^{-1}$ PBS (PH =7.0) was investigated by cyclic voltammetry technique in the range of 10 - 100 mV/s, the results showed that increasing of peak current with increasing of scan rate (Fig 4A). Hence scan rate can be used to determine if the reaction is diffusion or adoption controlled.

Plotting peak current (I_p) with scan rate (SR) a linear relationship is obtained as follows:

$$I_p = 0.2285 v + 5.4669 \quad r^2 = 0.9962 \quad \dots\dots (2)$$

By plotting $\log I_p$ and $\log v$ the linear relationship was observed, and expressed as follows:

$$I_p = 0.5641 \log v + 0.3 \quad r^2 = 0.9901 \quad \dots\dots (3)$$

The slope value is 0.5641 which is close to the theoretical value (0.5) of purely diffusion controlled [9]. For more characterization of OMZ using scan rate, it has been found that the peak potential grows linearly with scan rate in the range 10 to 70 mVs^{-1} , at higher scan rates a slight dropping was observed (Fig 4B), making the relationship nonlinear. The number of electrons involved in the electrochemical reaction can be calculate from the slope of E_p V/s. curve by estimating that charge transfer coefficient, = 0.5, and it found to be 1.071. To calculate the standard heterogeneous rate constant (K^0) for irreversible reaction lavinor equation was used [10]:

$$E_p = E^0 + \left(\frac{2.303RT}{anF}\right) \log\left(\frac{RTK^0}{anF}\right) + \left(\frac{2.303RT}{anF}\right) \log v \quad \dots\dots (4)$$

Where, E_p is peak potential, E^0 is the formal redox potential (872.36 mV which is calculated from the curve of E_p V/s. $\log v$ by extrapolating to the vertical axis when $v = 0$), $R = 8.314 J/K mol$, $T = 298 k$, $F = 96,480 C mol^{-1}$, and K^0 is the standard heterogeneous rate constant, standard heterogeneous rate constant can be calculated from the intercept of E_p V/s. $\log v$ curve which is found to be $19.05 \times 10^3 s^{-1}$.

Effect of Accumulation time:

Accumulation time is effective for increase the electrode sensitivity for determination, due to the analyte accumulation in the surface of the electrode. The effect of accumulation time on the anodic peak current of $1.0 \times 10^{-4} mol L^{-1}$ OMZ at PGE was investigated by linear sweep voltammetry in the range of 0 to 240 s. The peak current increased gradually as accumulation time

increased (0 to 180) and decreased after that (180 to 240), due to the electrode saturation. Considering time consumed, sensitivity and efficiency, the accumulation time was set at 180 s (Fig 5).

Mechanism:

Based on the electrochemical data from pH, and scan rate effects, the number of electron transferred is equal 1.0. As reported in [4] according to OMZ resonance structure, and the benzimidazole moiety is planner the favourable position of attack is ortho in the ring, because the radical formed is stabilized by resonance, the attack is preferable in the aromatic ring due to the greater conjugation, making the loss of the electron easier, the removal of the electron followed by deprotonation of the cation radical form to hydroxylated species (Fig 7).

Quantitative analysis of Omeprazole:

Differential pulse voltammetry technique was used to measure the peak currents of OMZ which increases linearly with concentration increase. To verify this linear relationship a calibration curve was constructed under the optimized experimental conditions, other validation characters have been determined.

Linear range and detection limit:

Calibration curve was obtained in the optimum parameters, the potential range 0.05 to 1.2V, PBS 0.2 molL⁻¹, 7.0 pH, accumulation time 180s and the scan rate is 100 mV/s, the voltammetric response of PGE toward different working concentrations were recorded, in the range 10 to 200 μ molL⁻¹, R² = 0.9964 (Fig 6), the linear regression equation is:

$$I_p = 0.0421C_{OMZ} + 1.1222R^2 = 0.9964 \dots\dots (5)$$

Limit of detection (LOD), and limit of quantization (LOQ) of the method were calculated, by the equation kSD/m, k = 3.0 for LOD, and 10 for LOQ, SD is the standard deviation of the intercept; m is the slope of the calibration curve (Table 1).

Repeatability:

All experiments of repeatability have been performed for three successive measurements for each concentration selected (100 μmolL⁻¹ and 180 μmolL⁻¹ of OMZ), the standard deviation was calculated and the precision was low for higher concentration making the estimation of low charge transfer process of OMZ on PGE surface in all cases.

Robustness:

The robustness of the analytical method is the measure of the method constancy toward small variations in method

parameters, the robustness of this new method has been measured by small changing in pH, Scan rate, and Accumulation time showing that there are no significant changes in the peak current (Table 2).

Table 1. The regression parameters obtained from the calibration curve of Ip V/s. OMZ.

Parameters	Values
Peak potential (V)	0.81- 0.85
Linear range (MoleL ⁻¹)	30×10 ⁻⁶ to 200 ×10 ⁻⁶
Slope	0.0421×10 ⁻⁶
Intercept± SD	1.1222×10 ⁻⁶ + 1.05 ×10 ⁻⁷
Square co-relation coefficient (r ²)	0.9964
Limit of detection (LOD) molL ⁻¹	7.52 ×10 ⁻⁶
Limit of Quantification (LOQ) molL ⁻¹	25.08×10 ⁻⁶
Standard deviation	2.436
Relative standard deviation	0.439

Table 2. Effect of the changes in the experimental conditions on the voltammetric response of the electrode.

Robustness parameter	Peak height (μA)			SD
	-2	optimum	+2	
pH	9.7898	11.175	11.945	1.0921
Scan rate mVs ⁻¹	10.292	11.175	13.993	1.9329
Accumulation time (s)	12.878	11.175	0.98957	1.4962

Analysis of pharmaceutical formulation:

Applicability of PGE was investigated to determine OMZ in pharmaceutical formulation, differential pulse voltammograms are obtained under the optimized conditions that described earlier showed acceptable recovery (Table 3), indicating that PGE could effectively be used to determine low concentrations of OMZ.

CONCLUSION:

In the present work, we studied the behaviour of OMZ in the pre-treated PGE surface, in neutral PBS pH = 7.0 using DPV. The results showed that OMZ adsorb on electrode surface under diffusion controlled process, and then an appropriate mechanism of the OMZ reaction was proposed. Many parameters were studied to optimize the determination condition and the method is validated to some validation characters such as linearity, sensitivity, robustness and recovery. This method found to be simple

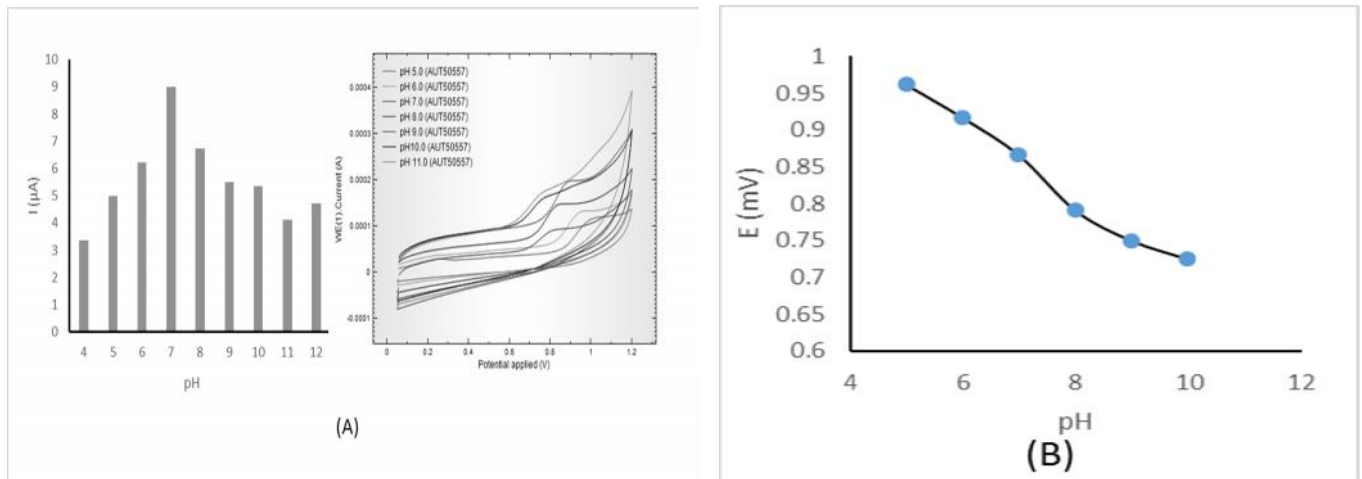


Fig 3. (A) Shows the highest peak current achieved at the pH =7.0, (B) Shows the dependence of peak potential on the pH value change.

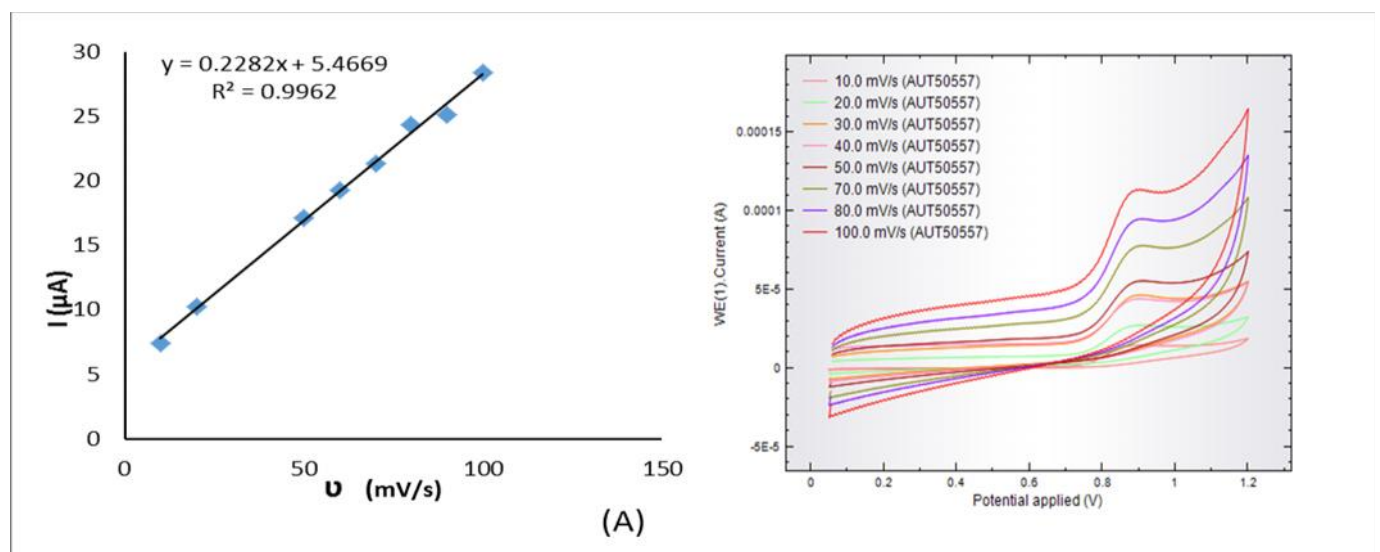


Fig 4. (A) $1.0 \times 10^{-4} \text{ molL}^{-1}$ OMZ in 0.2 molL^{-1} PBS, accumulation time = 5 s was measured at different scan rates (10 to 100 mV/s), (B) relationship between the $\log I$ and $\log \nu$.

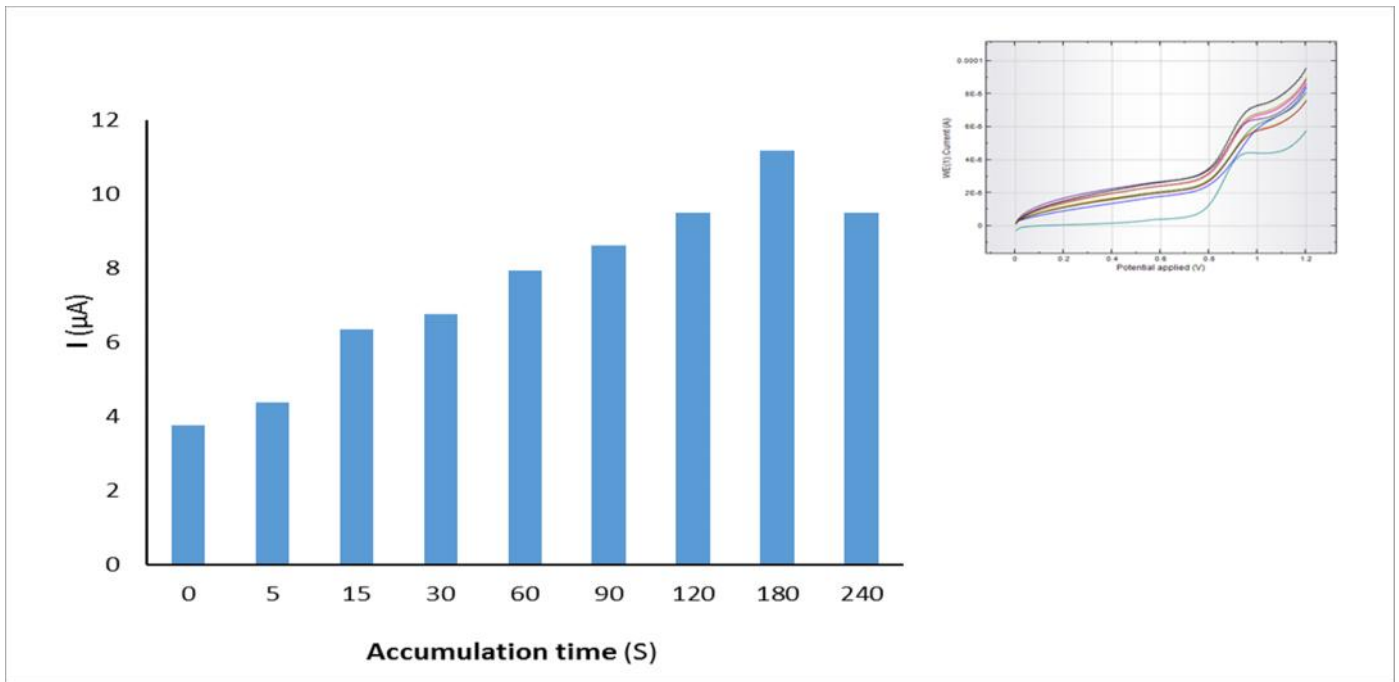


Fig 5. Effect of the accumulation time on the peak current of $1.0 \times 10^{-4} \text{ molL}^{-1}$ OMZ in 0.2 molL^{-1} PBS, and scan rate 100 mV/s .

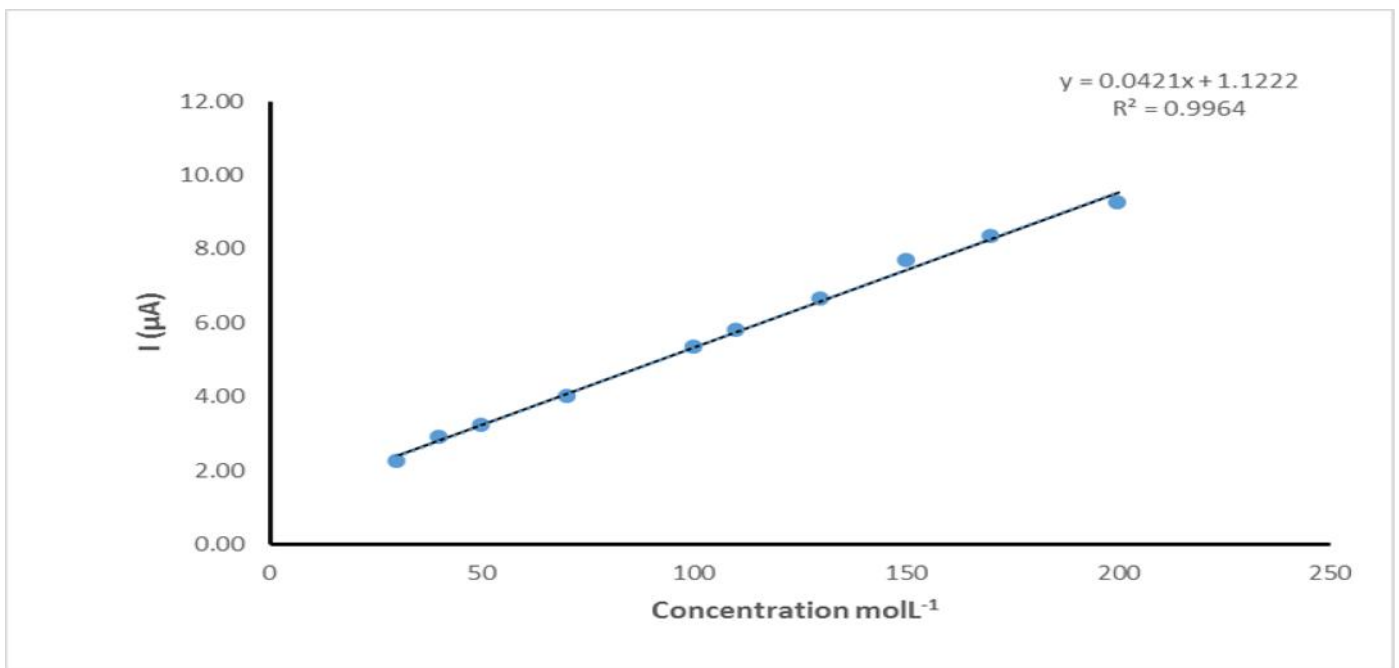


Fig 6. The calibration curve constructed in the optimum voltammetric conditions showing linear dependence of peak current on OMZ concentration, with squared correlation equal to 0.9964.

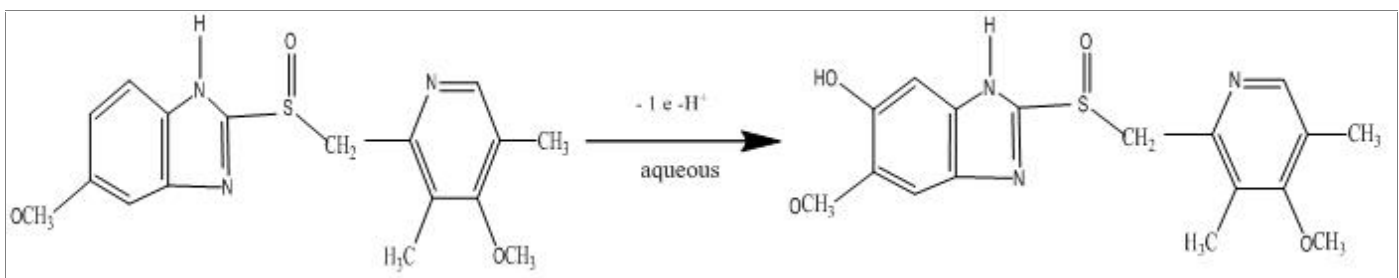


Fig 7. Proposed oxidation mechanism of OMZ at the surface of PGE.

and sensitive for determination of OMZ in pure and dosage form and could be alternative for OMZ determination because it is simple, fast, and inexpensive, and it could be adopted for pharmacokinetic studies.

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