A Comparative Study of Labetalol *via* Electrochemical and Computational Methods

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ABSTRACT: *Normodyne is the brand name of Labetalol. It has medicinal importance and well known antihypertensive drug and is given to patients with severe hypertension conditions. It is the two-fold alpha and beta-adrenergic antagonism and has dissimilar physiological effects in acute conditions of high blood pressure. Various techniques were used to elaborate on the qualitative behavior of this drug. In the present work, Cyclic Voltammetry (CV) is used to determine the qualitative characteristics of Labetalol. The Glassy Carbon Electrode (GCE) is used as a working and Calomel as a reference electrode with supporting electrolyte (0.1M NaOH) at 30±1oC. In the case of GCE, a single anodic peak is observed which indicates that this drug showed an irreversible process with the transfer of one electron in the selected medium. In addition, different electrochemical parameters are also calculated including, Anodic peak current (Ipa), Anodic peak potential (Epa), half peak potential (Ep/2), differential peak potential ΔEp = (Epa – Ep/2), transfer coefficient (α), diffusion coefficient (D), formal potential (Eo), heterogeneous rate constant (Ko), and Gibbs free energy (ΔG). Furthermore, the adsorption process is also studied. For comparative study, computational methods are employed for finding HOMO-LUMO energies and vibrational frequencies of the Labetalol molecule. Both methods, electrochemical and computational are in good agreement and validate the irreversible oxidation of Labetalol. This study has not been reported before and it is useful for the pharmaceutical industry.*

KEYWORDS: *Cyclic Voltammetry (CV); Labetalol; Glassy Carbon Electrode (GCE); Calomel Electrode (CE); Highest Occupied Molecular Orbital (HOMO).*

INTRODUCTION

Labetalol having the chemical name 2-hydroxy-5-[1*R*)-1-hydroxy-2-[(2*R*)-4-penylbutan-2-y]amino]ethyl] benzamide is a biologically active compound (Fig. 1). Its molecular formula ($C_{19}H_{24}N_2O_3$) and molecular mass is 328.406 g/mol [1]. It is a fixed combination of hydrochlorothiazide Normodyne with a combination of alpha & beta-adrenergic antagonists and uses for the treatment of hypertension.

It blocks the adrenergic receptor which reduces peripheral vascular resistance without significantly altering heart rate or cardiac output. In hypertension, Systolic Blood Pressure (SBP) is more than 140 and Diastolic Blood Pressure (DBP) is greater than 90. It is usually happened due to elevated peripheral vascular smooth muscle tone that ultimately raises the arterial resistance and decline in the capacity of the venous system [2, 3].

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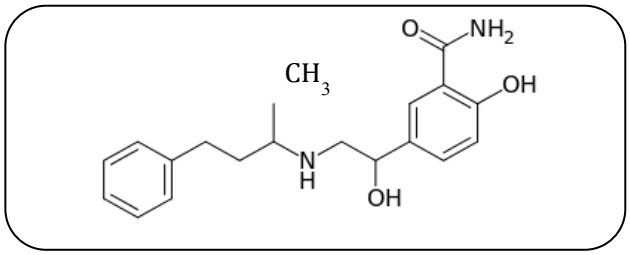


Fig. 1: Structure of Labetalol.

The voltammetry technique was established from polarography in 1922. In this technique, the current is measured as the function of applied potential where the polarization of the reference, the electrode is usually enhanced [4, 5]. CV is a sub-class of voltammetry techniques. In 1938 Randle's reported that CV is important for the analysis of the electrochemical activities of analytes. It is a delicate, multipurpose, short-time-consuming technique and useful for industrial applications, academic research, and laboratories[6-8]. It provided the facts about the electron transfer kinetics, thermodynamics of redox potential, and electrochemical reaction and governs the oxidation states of the metals. It is also used for the qualitative investigation of chemical reactions [8]**.**

Literature provided information on Labetalol regarding pharmacological aspects such as Rapid analysis of Labetalol in human plasma using liquid chromatography-tandem mass spectrometry [1]**.** Some Voltammetry studies reported Labetalol as an antihypertensive drug with Platinum (Pt) Electrodes and carbon paste electrode [3, 9]. A large number of techniques have been reported to find out the concentrations of Labetalol in biological fluids such as simple spectrophotometric [10-12], spectrofluorometric [13, 14], and selective electrodes [15]. In Colorimetric determination [16], liquid chromatography-tandem mass spectrometry was used. The dosage of Labetalol has been estimated in tablets by HPTLC method.

In HPLC techniques microbore column is used in combination with reversed-phase ion-pair and Labetalol detection limits (5 ng/ml) were found by fluorescence and UV detection [17]. In 2008, the detection limit of Labetalol and the complex between zinc (II) and eosin was found by simple spectrofluorimetric methods. The investigation shows that the proposed method is relatively simple, precise, accurate, and has fewer chances of any interference [18]. *Nafisur* and *Manirul* quantify Labetalol in urine samples by High-Performance Liquid Chromatography (HPLC) technique with a combination of the amperometric method. They found that the proposed method is accurate and can be useful for pharmacokinetic studies of urine samples of humans [19]. Later on, one of the well-known techniques i.e. Chromatography in combination with fluorimetric detection is used for the analysis of detection limits in the urine sample. It was found that the beta-blockers detection limit is in the range of 0.5-28-ng/mL. A further study evaluated that this method is sensitive and can detect other drugs 24-72h time frame after ingestion [20, 21].

In 2009, *Hadi Beitollahi et al.* worked on the development of a sensitive electrochemical device that is based on a modified graphite electrode with nanoparticles of tungsten. The modified electrode was used with Phosphate Buffer Solution (PBS) (pH 7.0) for the determination of electron mediating behavior of epinephrine and their results prove that the modified nanoparticles electrode is found promising application and it further can be used for the study of biochemical species [22]. In 2017, *Hadi Mahumodi et. al.* used a modified Carbon Paste Electrode (CPE) with graphene quantum dots to investigate the interaction between ds-DNA and topotecan. The results indicated that the modified electrode is useful and showed high selectivity and sensitivity in measurements [23]. In 2018, *Mariani A. Ciciliati et. al*. studied the thermal behavior of Labetalol by differential thermal gravimetry, DSC, and other techniques to investigate the complete mechanism of Labetalol decomposition. The experiment was performed in a nitrogen and air atmosphere till 450 °C, in the first step loss of water takes place followed by the release of isocyanic acid and which further decomposes into carbon dioxide and ammonia, resulting in the loss of carbonaceous material by oxidative burning till no residue left at the end of the run. The proposed work explored the thermal mechanism of Labetalol [24]. *Hadi Mahmoudi Moghaddam el. al.* in 2019 used carbon paste electrodes with nanorods of zinc oxide (ZnO) to study the oxidation behavior of droxidopa by cyclic voltammetry, square wave voltammetry, and chronoamperometry. The results indicate that peak current (oxidation peak) showed linear relation with the concentration of droxidopa and the modified electrode can be used for the simultaneous determination of compounds [25]. In 2020, *Pradeep Singh Rawat et. al* used a bioanalytical method to determine drug concentrations of Labetalol and Nebivolol both in aqueous humor and plasma. The liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed

to study nebivolol and labetalol by simultaneous quantification using nebivolol-d4 and metoprolol, respectively, as internal standards in aqueous humor and plasma. Reverse phase Zorbax SB-C18 column (4.6×100 mm, 3.5 µm) was used for Chromatographic separation and the result showed linear relation with a concentration range of 0.43- 750 ng/mL for nebivolol and 0.39-668 ng/mL for labetalol. They further noticed that during the study of inter-day no degradation was observed in both drug and both were stable for 18 h and 8 h, respectively. Their study further can be applied to the determination of pharmacokinetic parameters of drugs [26]. *Hadi Beitollahi et. al* used a modified electrochemical sensors electrode to determine the amount of dopamine as a neurotransmitter. The author used a modified carbon paste electrode to study the redox process of dopamine in buffer solution by Cyclic voltammetry, their result showed that oxidation peak current has a linear relation with dopamine concentration and it further helps to determine pharmaceutical preparation [27]. Similarly, the current study used a modified carbon electrode for the characterization of Labetalol as in the previous study.

However, in the current study characterization of Labetalol is done by using Cyclic Voltammetry with GCE. This instrument has drawn the attention of worldwide chemists and biologists for a variety of purposes. The goal of this learning focused on the application of Cyclic Voltammetry for the determination of the electrochemical nature of Labetalol as a biologically active drug. It was also confirmed via computational chemistry.

EXPERIMENTAL SECTION

Apparatus and Chemicals

The Cyclic Voltammetry (CHI-700d) was used for the electrochemical study of Labetalol. It possesses three electrodes i.e. Glassy Carbon Electrode as working, platinum wire as counter, and Calomel as a reference electrode. To record pH of the solution, a pH meter (JENWAY - 3510) was used. The electrochemical balance (DENVER- TP-214) was used for weighing chemicals. These instrumental facilities are available in the Department of the Chemistry Federal Urdu University of Arts Science and Technology Gulshan-e-Iqbal Campus Karachi Pakistan. All the chemicals used were analytical grade. A solution of 5mM Labetalol (E. Merck) was prepared in 0.1 M NaOH (E. Merck) which was used as a supporting electrolyte. Solutions to all these chemicals were prepared in deionized water.

Electrochemical Study of Labetalol

To fulfill experimental requirements following parameters were adjusted i.e. the current-voltage time parameters, initial potential, potential scan rate, and current sensitivity. The instrument (CHI 700d) analyzer was attached to the computer and operated according to their manual instructions [28], when the system gets ready; it recorded the voltammogram of the particular supporting electrolyte. After each scanning, the surface of the test electrode was refreshed (polished with aluminum powder 0.5 micron solution (E.Merck)), rinsed with double distilled water, and dried. This was done to elude impurity from the test electrode surface to maintain reproducibility in results [29]. Initially, the baseline of supporting electrolytes was recorded (0.1 M NaOH). After the collection of the baseline, the electrochemical cell was filled with the solution of β-blockers (Labetalol) that has been prepared in the same supporting electrolyte. Nitrogen gas was purged for five minutes to get an inert atmosphere and adjusted the electrode assembly in the cell. Voltammograms were recorded with β-blockers (Labetalol) at different scan rates i.e. 100, 150, 200, 250, 300, 350, 400, and 450 mV/s (Fig. 3).

In silico protocols

In Silico method is used for calculations of the electronic properties; Gaussian 16 software [30] is used to calculate HOMO-LUMO energies and oscillator strengths at B3LYP/6-31G level of theory. Initially, Labetalol is optimized in the gas phase by Density Functional Theory (DFT) on a computer with a 1.80 GHz processor. In IR spectroscopy transitions are induced at vibrational levels of the molecule in the region of 700-4000 cm^{-1}. For the estimation of Raman frequency B3LYP/6-31G(d) level of theory was used, and similarly, optimized geometry was used for the estimation of HOMO/LUMO potentials to reveal the mechanism of proton transfer [30].

RESULTS AND DISCUSSION

Electrochemical oxidation of Labetalol at GCE

In this work, the potential range +0.0 to +0.8 V was selected at all scan rates 100, 150, 200, 250, 300, 350, 400, and 450mV/s. The CV profiles of Labetalol revealed that a single well-defined anodic peak appeared. Reversed peak is not observed in this potential range representing that Labetalol did not give the reversible process in this selected system and it gave an irreversible response [31].

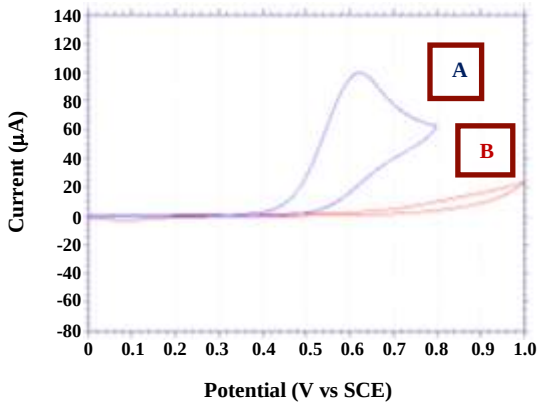


Fig. 2: (A) Cyclic Voltammogram of Labetalol in 0.1M NaOH Solution at 100 mV/s scan rate (B) Baseline (0.1 M NaOH) at 100mV/s scan rate using GCE at 30±1°C.

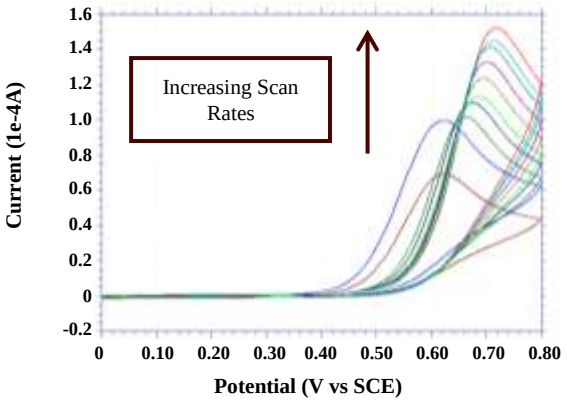
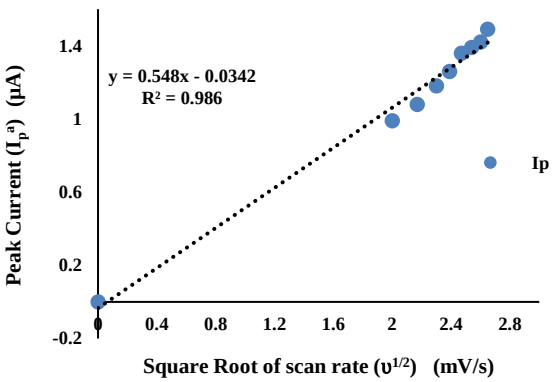


Fig. 3: Cyclic voltammogram of Labetalol at pH=8 in 0.1M NaOH used as supporting electrolytes at different scan rates (a)100, (b)150, (c) 200,(d) 250, (e) 300,(f) 350, (g) 400 (h) 450 mV/s.



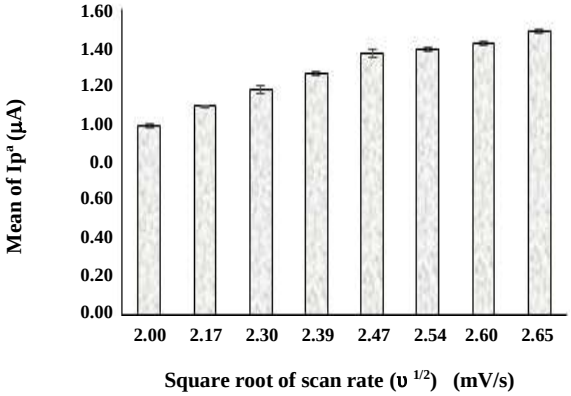


Fig. 4: a) Plot between Anodic Peak current (I_p^a) vs. square root of different scan rates (υ). B) Bar Error plot between anodic Peak current (Ip^a) vs. Square root of different Scan rates(U).

The voltammogram of Labetalol indicated one anodic peak at +0.67 V with an anodic peak current 91.6 µA which is shown in Fig. 2. One electron was transferred in the oxidation of Labetalol, slope value (0.567) closely matched with theoretical values which confirmed that this process is diffusion controlled. Thus the value of α_n, formal potential (E^o), heterogeneous rate constant (K^o), and Gibbs free energy were obtained 0.75, 0.63 V, 1.46 x 10^{-4}cm$S^{-1}$and -607.95 coulomb/mole respectively.

Effect of scan rate on peak current

The effect of different scan rates was examined at pH 8 and it is clearly shown that the intensity of peak current depends on the scan rate which is shown in Fig. 3. The linear relationship was observed between the I_p^a and square root of scan rate (Fig. 4) and showed good correlation coefficient (r^2= 0.986). It reflected that area under peak getting narrow at a high scan rate. The plot of peak current $v_s.\upsilon^{1/2}$ gives a slope value of 0.548 (100 to 450 mV/s) and it is shown in Fig. 4a. While the Error bar graph shown (Fig. 4b)

$$I_{pa} = 0.548\,\upsilon^{1/2} - 0.0342 \quad \left(r^2 = 0.986\right) \qquad (1)$$

Further, the linear relationship is confirmed by the plot of log I_p and log of scan rates which are shown (Fig. 5a), The slope value (0.561) obtained shows a small margin to the theoretical values i.e. (0.5) and it confirmed that the process is diffusion controlled rather than adsorption-controlled [32, 33]**.** While the Error bar graph between the average anodic peak current against the log of Scan rate is shown in (Fig. 5b)

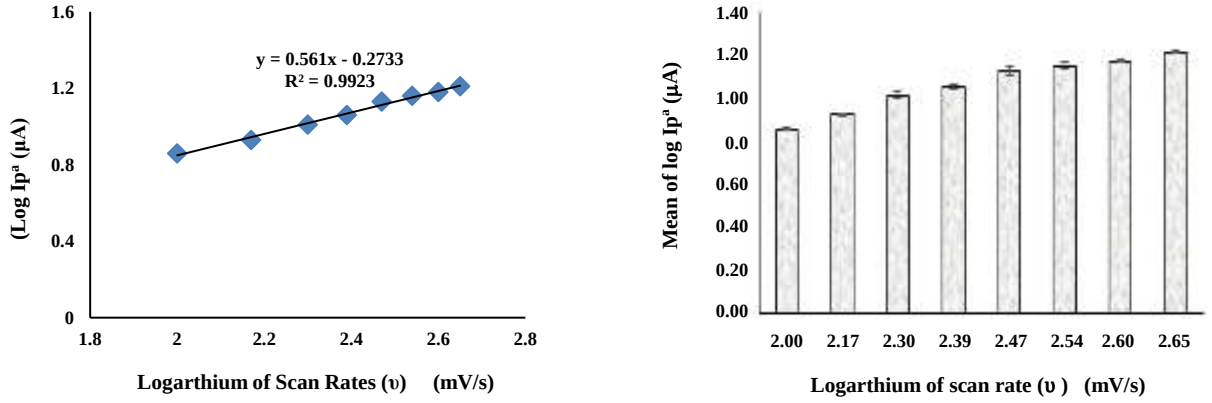


Fig. 5: a) Plot between log of Anodic Peak current (I_p^a) vs. log of different scan rates (υ). b) Plot between Average log of Anodic Peak current (I_p^a) vs. log of different scan rates (υ).

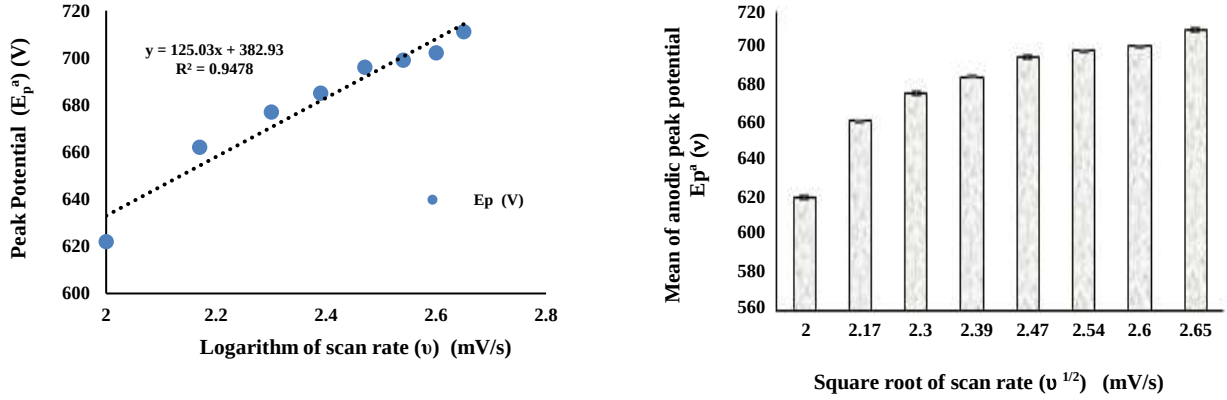


Fig. 6: a) Plot of (E_p^a) vs. Logarithm of scan rate (υ). b) Plot of Mean Anodic Peak Potential (E_p) vs. Logarithm of scan rate (υ).

$$\text{Log } I_{pa} = 0.561 \log \upsilon - 0.2733 \quad \left(r^2 = 0.9923\right) \qquad (2)$$

Effect of scan rate on peak potential

It is observed that the peak potential E_p^a (oxidation peak) is shifted with increasing the scan rates, it is shown in (Fig. 3). The linear relationship is observed between peak potential (E_p) vs. log of scan rates with correlation coefficient (r^2= 0.9478) and it is represented in Fig. 6a. It is also confirmed from the Bar Error plot between the Mean of anodic peak potential against log of Scan rates is reflected in Fig. 6b.

$$E_{pa} = 125.03 \log \upsilon + 382.93 \quad \left(r^2 = 0.9478\right) \qquad (3)$$

The constant variation in peak potential is indicated that the electrode reaction is coupled with an irreversible process [34].

Study of transfer coefficient (α)

The transfer coefficient (α) is commonly employed in the kinetics investigation of the electrode process. This factor is originally presented in the field of electrochemistry by Buttler and EradyGruz [35]**.** It explores the fraction of electrostatic potential energy affecting the reduction in an electrode reaction with the remaining fraction (1- α) affecting the corresponding oxidation rate.

The transfer coefficient (α) value was calculated by *Bard* and *Faulkner* using the following equation (4)

$$\alpha = \frac{47.7}{E_p - E_{p/2}} \qquad (4)$$

Where $E_{p/2}$ is the half-peak potential at which current is halved. The transfer coefficient (α) obtained is 0.5. However, the number of electron transfers in the electro-oxidation process of Labetalol was found to 1.2 ≈1, thus the linear relationship between I_p^a vs. scan rate reflects

Table 1. The values of Anodic Peak potential (E_p^a), Half peak potential ($E_{p1/2}$), Anodic Peak current(I_p^a), Transfer coefficient (α), and Diffusion coefficient(D) from the cyclic voltammograms of 5mM Labetalol at GCE in the presence of B-R buffer (pH =8) used as supporting electrolytes with different scan rates at 30±1°C.

S. No	Scan rate(mV/s)	pH = 8					
		Anodic peak					
		E_{pa} (mV)	$E_{p1/2}$ (mV)	I_{pa} (μA)x10^6	E_p-$E_{p/2}$ (mV)	A	[b] D ×10^4 (cm^2/s)
1	100	622±1	535±1	0.99±0.01	91±1	0.524±0.01	0.19±0.00
2	150	66.2±2	591±1	1.08±0.02	71±2	0.671±0.01	1.67±0.00
3	200	677±1	603±2	1.18±0.02	74±2	0.644±0.02	1.69±0.01
4	250	685±3	614±4	1.26±0.02	71±3	0.671±0.02	1.96±0.01
5	300	696±4	621±2	1.36±0.03	75±3	0.636±0.02	1.96±0.02
6	350	704±2	627±1	1.39±0.03	77±4	0.619±0.00	2.4±0.02
7	400	708±1	632±1	1.42±0.01	76±1	0.627±0.01	1.25±0.03
8	450	711±1	637±1	1.50±0.01	74±1	0.644±0.00	4.76±0.01

that the charge transfer is under diffusion control and the adsorption process [21, 31]. The value of Formal Potential +0.63V was calculated by extrapolating the graph between log E_p^a Vs. log υ^2. Gibbs free energy was calculated by using equation (6).

$$\Delta G = -nFE^o \quad (5)$$

Here, the number of the proton was also calculated by applying equation (6) only one proton was transferred during this electrochemical process.

$$\left(\Delta E_p / pH\right) = \left(0.059 / \alpha_n\right).\, p \quad (6)$$

The values of heterogeneous rate constant (K^o) = 1.46 x 10^{-4}cm/S were obtained from Eq. (7) which was also reported by Reinmuth

$$K^o = Ip / C^o nFA \quad (7)$$

Here K^o is the standard heterogeneous rate constant, C^o is concentration and another symbol has its usual meaning.

Diffusion coefficient (D)

The diffusion coefficient (D) is one of the valuable factors of the analytes, it helps to explain the diffusional transportation of the species [35]**.** The above mention parameters were estimated by using the Cyclic Voltammetry techniques which were evaluated from the theoretical relationship by Randles- Sevcikequation which is given below.

$$I_p = -2.6\times10^5 n^{3/2} AD^{1/2} C\upsilon^{1/2} \quad (8)$$

Where

I_p = peak current (μA), A = area of the electrode surface (0.702 cm^2), D = diffusion co-efficient of the electroactive specie (cm^2/s), C = concentration (mol/cm^3) and υ = scan rate (V/s)

The value of diffusion coefficient (D) was found to be increased at all scan rates which is given in Table 1. It reflected that increasing the applied potential the diffusion transportation of species increases [36]**.** This factor was calculated at different scan rates which are represented in Table 1. These parameters also pointed out the mass transfer process in an electrochemical system. In this technique, the reacting species diffused slowly from the bulk of the solution toward the electrode surface. The rate of dispersal of analytes depends upon the surface area of the electrode. So the diffusion rate of the macro-electrode was found to be higher than the microelectrode (Fig. 7).

Determination of the adsorption process

CV technique is a less time-consuming electrochemical process. It helps in determining the interfacial attitude of electro-active species. The entire chemical process of reactant and product is involved in the adsorption and desorption process [33]**.** Recently, the adsorption process was found in the presence of supporting electrolyte, where no pre and post-peak appeared at all successive scan rates.

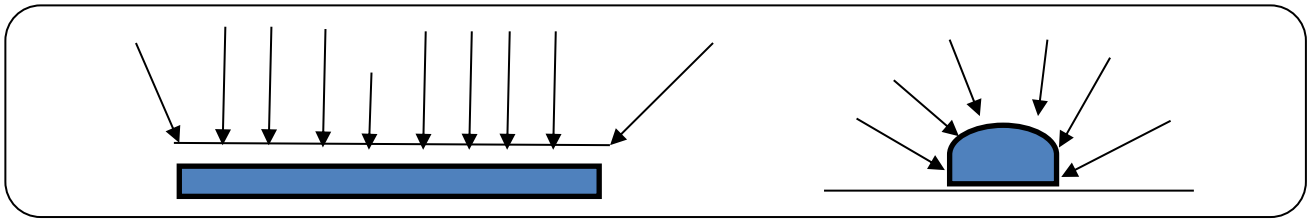


Fig. 7: (a) Diffusion phenomena at Macro-electrodes (b) Diffusion process at Micro-electrodes.

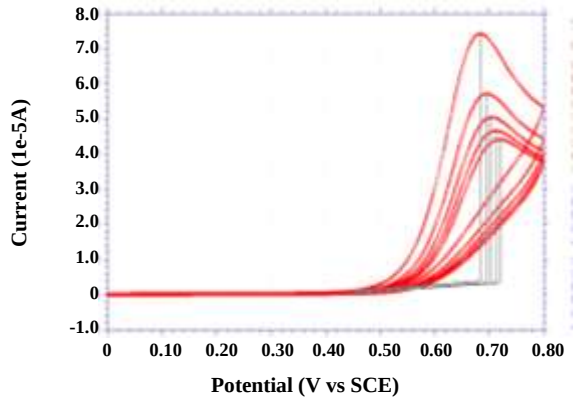


Fig. 8: Repeated cyclic voltammogram of Labetalol in 0.1M NaOH at 100mV/s at30 ± 1°C.

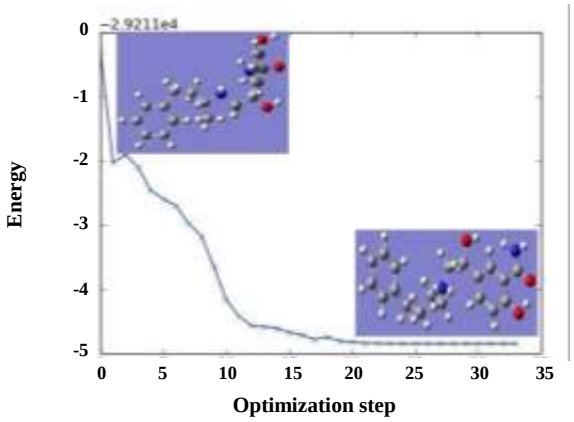


Fig. 9: Optimization of Labetalol, a plot showing un-optimized and optimized geometry of labetalol.

It was noticed that the oxidation peak gradually decreased at 100mV/s which indicated that some adsorption occurs at the surface of the Glassy Carbon Electrode which is shown (Fig. 8).

Analytical test for irreversibility

An investigative test was applied for confirmation of the chemical behavior of Labetalol in a basic medium (NaOH). The first criterion of the diagnostic test of the irreversible process at 25 ± 1°C is that there should be no reverse peak, similarly same in the case of Labetalol only an anodic peak is observed which favors the first diagnostic test for irreversibility [21, 33].

The second criterion of the diagnostic test is that peak current I_p must be directly proportional to the square root of scan rate $\upsilon^{1/2}$, in the case of Labetalol it also fulfills the second criterion (Fig. 4).

A third criterion is that E_p shifts negatively for each decades increases in υ , where Labetalol shows a similar pattern and validated the third criterion (Fig. 3). The fourth criterion of the irreversible diagnostic test is the difference between Peak potential and half wave peak potential is equal to a constant value, so Labetalol satisfies fourth criteria in supporting electrolyte and Glassy Carbon Electrode (Table. 1). Therefore, it is concluded that Labetalol follows irreversible behavior in the selected supporting electrolytic system [21, 33].

In Silico Analysis

Initially, the structural geometry of the compound is evaluated to energy-minimum and molecular geometries were located by minimizing energy, with respect to all geometrical coordinates without imposing any symmetrical constraints (Fig. 9).

Computational findings proposed that Labetalol is oxidized by donating an electron from its Highest Occupied Molecular Orbital (HOMO) and didn't accept an electron reversibly as the energy of LUMO is greater than the energy of HOMO (Fig 10).

The correlation between the calculated energies of the HOMO and the half-wave potential at different scan rates of Labetalol is shown in Fig. 11. Simple relations have been found between the energy of the HOMO and half-wave potential ($E_{1/2}$) and larger regression coefficient (r^2 = 0.9347) showing rapid loss of an electron from Labetalol (Fig. 11). In the case of LUMO linear relationship between $E_{1/2}$ and LUMO demonstrates that they have fewer chances to gain electron back and undergo a reversible reaction (r^2 = 0.6237) (Fig. 12). The greater correlation coefficient

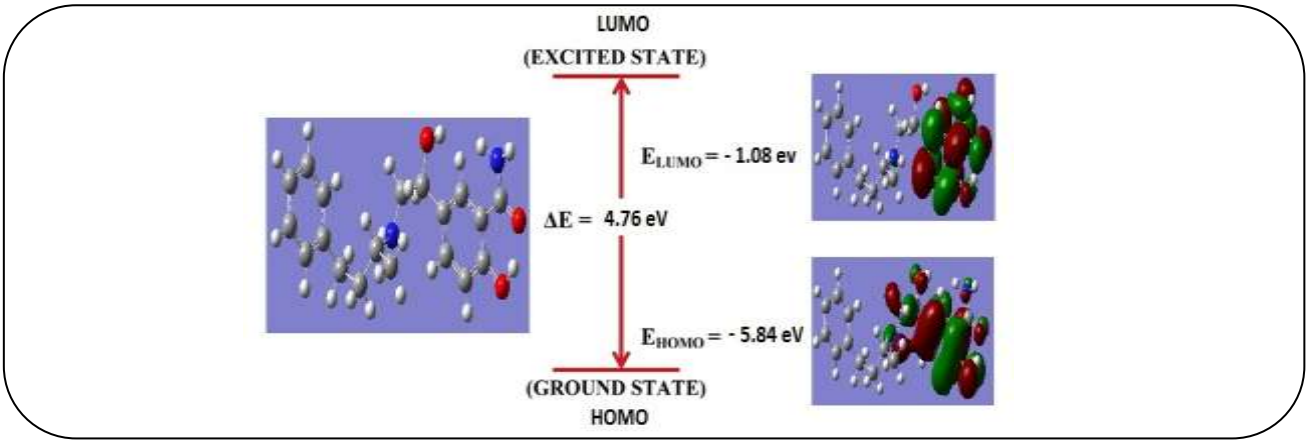


Fig. 10: Energy diagram of Labetalol showed ground state HOMO energy and excited state LUMO energy.

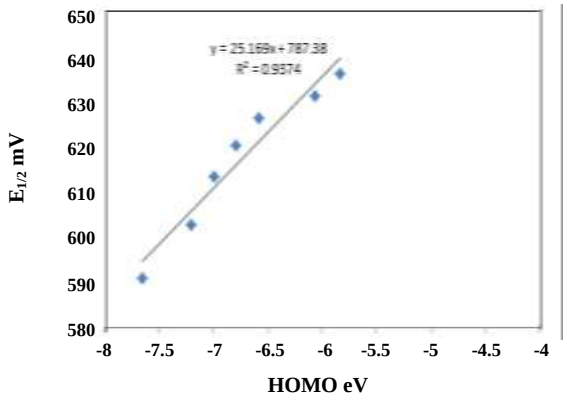


Fig. 11: Half-wave potentials ($E_{1/2}$) of Labetalol vs. HOMO energies at scan rate (150, 200, 250, 300, 350, 400, 450 mV/s.).

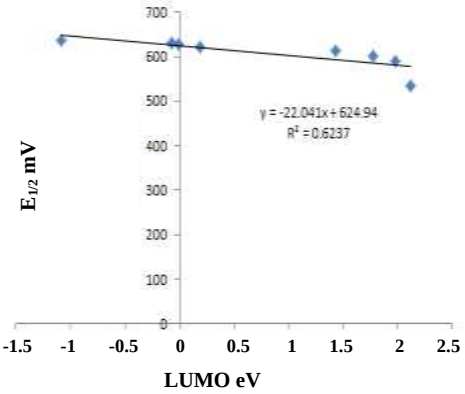


Fig. 12: Half-wave potentials ($E_{1/2}$) of Labetalol vs. LUMO energies at various scan rates.

between the measured potentials ($E_{1/2}$) and the calculated HOMO energies evidenced that there is a single electron transfer reaction.

As Table 2 shows, the regression coefficient (r^2) significantly increases by removing the lowest scan rate i.e. 100 mV/s. r^2 is goodness-of-fit and shows good relation between half-wave potential ($E_{1/2}$) and energy of the Highest Occupied Molecular Orbital of the molecule.

Comparison of experimental and computational results of IR Spectra.

To interpret the computational results of IR, an experimental Labetalol IR spectrum [37] is used and a comparison is made between computational and experimental results (Table 3). An optimized geometry structure was used for the calculation of vibrational frequencies and IR intensities by using the B3LYP/631G basis set. The Computational IR plot represents predicted peaks and their intensities in spectra (Fig 13). Further correlation of vibrational frequencies between experimental and computational methods is evaluated and regression value (R^2 = 0.9985) evidenced that computational results are reliable and comparable to experimental results (Fig. 14).

CONCLUSIONS

- The current finding concluded that Labetalol is a biologically active compound and can be used for the preparation of medicinal drugs.
- The results indicated that Labetalol undergoes oxidation reaction by transfer of one electron with supporting electrolyte (0.1M NaOH) at GCE. In addition, the Cyclic Voltammetry technique explores that it owns only irreversible reaction in a selected medium with high sensitivity and extensive linear range.

Table 2: The relations between $E_{1/2}$ of Labetalol and HOMO in various scan rates.

Equation	Equation R^2
E1/2 = 40.55 HOMO + 885.38	0.6828
E1/2[a] = 25.169 HOMO + 787.38	0.9374

a) Scan rate 200mV/s is removed from the data

Table 3: Comparison of IR characteristic peak of Labetalol by Experimental and Computational method.

Functional groups	Standard peaks	Observed peaks by experimental IR	Observed peaks by computational IR
OH-Stretching	3100-3600	3356	3263.2949
NH-Stretching	3100-3500	3188	3119.0730
Aromatic –CH	2900-3100	2982	2973.1422
Aliphatic-CH	2850-2960	2810	Not observed
C=O Stretching	1650-1700	1673	1736.6140
C=C Stretching	1620-1680	1640	1646.3803

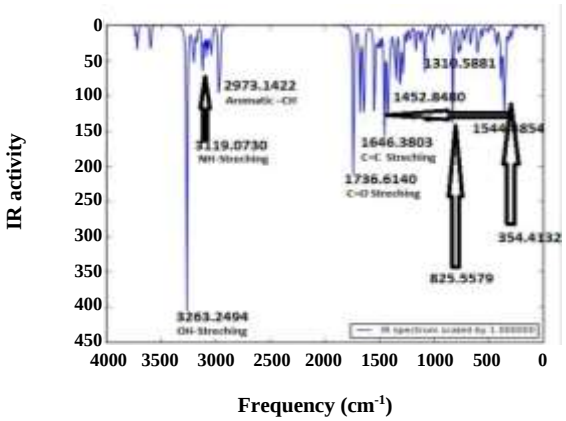


Fig. 13: Computational IR spectra of Labetalol.

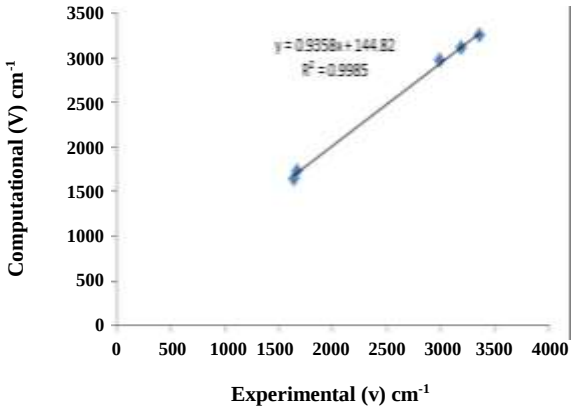


Fig. 14: Plot of experimental vibrational frequencies vs computational vibrational frequencies.

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- Furthermore, electrochemical diagnostic parameters of Labetalol validate the irreversible behavior of Labetalol and it also confirms by computational methods.
- So it can be used to reduce acute high blood pressure.
- This study provides the applicability of CV technique on medicinal drugs and this approach provides benefits to pharmaceutical aspects for evaluation of the chemical nature of drug candidates.

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