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Determination of deferasirox (anti-thalassemia drug) in serum and urine: cyclic voltammetry study

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***Abstract*---**The purpose of this project is to examine cyclic voltammetry (CV) analytical technique for anti_thalassemia drug of deferasirox by modified multi-walled carbon nanotubes (MWCNTs) on glassy carbon electrod (GCE) was described. The electrochemical performance of deferasirox was studied by cyclic voltammetry technique. The ability of the electrode for the determination of deferasirox under Optimize condition in pH 13.8~14, scan rate 100mv/s, temperature 30 ^{0}C and interference that have been studied. Where found the results that calibration curve of deferasirox was linear in the concentrations 13.4×10^{-4}-2.6×10^{-2} M, its detection limit was 8.46×10^{-11} M and LOQ was 2.82×10^{-10} M. The enthalpy ΔH was calculated to be (6.736 kJ. mol^{-1}), and the entropy can be calculated to be (213.8 J. K^{-1} mol^{-1}). The area of electrode was calculated to be 0.314 cm^2 and also the diffusion coefficient was 3.154×10^{-4} cm^{2}sec^{-1}. RSD% for bulk and form was less than 0.3% while serum and urine less than 2.5% and recovery in all close to 100.1%. The voltammogram for deferasirox give irreversible process with diffusion–controlled process. Finally, this technique has been applied for deferasirox on pharmaceutical formulations and biological samples (serum and urine).

***Keywords*---**Electrochemistry, Cyclicvoltametery, Glassy carbon electrod, MWCNTs, Defeasirox and Thalassema.

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Introduction

Thalassemias are hereditary anemias that are caused by changes in the globin chain ,which is a protein component of hemoglobin. These hereditary anemias are produced by reducing and lacking creation of one type of globin chain either α or β globin chain. The disorders range from asymptomatic to intense anemia which can result in considerable morbidity and death (Bahadur Kc *etal.* 2018). In 71 percent of 229 countries, hemoglobin diseases are a major public health concern, and these 71 percent of countries represent for 89 percent of all births worldwide.

One of most essential techniques aimed at drug quality control is drug analysis because there are many types of drugs and a large number of manufacturers(Moffat, Osselton, and Widdop 2011). Therefore it is highest important to determine the action of drug and their pharmacokinetic and pharmacodynamics studies in pharmaceutical formulations (Chou and Talalay 1984).

Deferasirox ($C_{21}H_{15}N_3O_4$) (advertised as Exjade or Desferal) is an orally administered iron chelator that has been shown to be effective in the treatment of thalassemia in children. Its main purpose is to decrease chronic iron excess in patients who need long-term blood transfusions for illnesses includes beta-thalassemia and added to chronic anemias (Parvizi Fard and Emamali Sabzi 2018).

Electrochemistry is a branch of chemistry that was founded in the late 19th and early 20th centuries to describe the relationship between electrical and chemical effects(Scholz 2015). Electrochemical techniques are strong and efficient analytical techniques that provide great sensitivity, accuracy, precision with a wide linear dynamic range, where using comparatively low-cost instrumentation(Farghaly, Abdel Hameed, and Abu-Nawwas 2014; Zadeh et al.,2022). A typical electrochemical detection systems consists of three electrodes: a working electrode (WE), a reference electrode (RE), and a counter electrode (CE)(Bontidean *etal.* 1998; Huldani et al.,2022). Multi-walled carbon nanotubes (MWCNTs) are modified as working electrode because its capability to stimulate electron transmission, improve sensitivity and chemical inertness(Zare and Nasirizadeh 2010; Ansari et al.,2022). To enhance the detection limit, a glassy carbon electrode modified with multi-walled carbon nanotubes (GCE-MWCNTs) was developed in this study (Jain Rajeev and Rather 2011; Mohammed and Qasim, 2021). One of the types of voltammetry is Cyclic voltammetry(Kounaves 1997; Hafsan et al.,2022). Cyclic voltammetry is very suitable in determining the mode of transport for the system which is designed for two possible modes of transport, adsorption and diffusion (Nicholson 1965; Bokov et al.,2022).

UV spectroscopic method (Chaitanya and Prasanna 2013), high performance liquid chromatography HPLC (Sagiroglu, Onal, and Evrim Kepekci Tekkeli 2020), and cyclic voltammetry and Electrochemical methods(Hajjizadeh *etal.* 2008) are some of the methods used to determine deferasirox in pharmaceutical formulations that have been published.

Materials and Methods

Chemicals and reagents

Deferasirox was provided by interpharmachem of China. Where all of solutions were made with deionized water. Drug-free serum forms were taken from healthy volunteers and stored icy till the analyze. All solutions of this drug were prepared freshly at 0.01 M concentration, the stock solution was prepared at 0.027 M concentration by dissolved in 50 ml of Sodium hydroxide that it was standardization by titrating it with HCl at a concentration of 0.1M to study the (effects of scan rate, pH, temperature, concentration, analytical application, and interferences). Finally, the samples were kept in a dark place at 4 ^{0}C till study because the concentrations of deferasirox solutions remained constant through time.

Apparatus

Apparatus A DY2100 series potentiostate with glassy carbon (3mm) diameter was modified with MWCNTs electrode as working electrode, Ag/AgCl as reference electrode, and platinum electrode as auxiliary electrode was used to measure volumetrically. The primary transformation signal was Windows 10 (64 bit). A (Hanna- instrumental) was used as digital pH meter a glass combination electrode served to carry out the pH measurements.

Preparation of MWCNTs suspension on glassy carbon electrode

To obtain a stable suspension, 10 mg MWCNTs were dissolved in 10 mL N, N-dimethylformamide (DMF) and it is placed for 30 minutes in an ultrasonic bath. The surface of the glassy carbon electrode (GCE) was mechanically prepared by polishing it with 0.3–0.05 mm alumina in water slurry onto microcloth pads. By washing the electrode surface with double distilled water, any adherent Al_2O_3 particles were removed. The GCE surface is then sonicated in a 50:50 methanol:water (v/v) solution and washed with double distilled water until being dried in a hot air stream (40 ^{0}C). The GCE-MWCNTs were formed by using a micropipette to apply an 8 μL MWCNTs suspension on the GCE and allowing it to dry at room temperature (Jain and Sharma 2012).

Analysis of human serum and urine samples

The supporting electrolyte was diluted (1:25) with serum samples, and the diluted solutions were immediately analyzed using the cyclic voltammetry technique, 0.1M NaOH solution was used to dilute urine samples (1:25) collected from a healthy volunteer. The cyclic voltammetry technique was used to examine the resultant solution.

Results and Discussion

The purpose of this study was to use cyclic voltammetry and calculate the active area of the electrode surface to determine deferasirox in pharmaceutical (Exjade). In summary, we investigated the effects of scan rate, pH, temperature, concentration, and interferences.

Active Area of GCE-MWCNTs electrode

The active area of the electrode in Fig.1(A) was measured using the cyclic voltammetric technique with a sample of 5×10^{-3}mM $K_3Fe(CN)_6$ at various scan rates. The Randles–Sevcik formula can be used to determine a reversible process (Rezaei and Damiri 2008).

$$I_p = 268600\, n^{3/2} A D^{1/2} C \upsilon^{1/2} \quad \text{---------- (1)}$$

For 5×10^{-3} mM $K_3Fe(CN)_6$ in 50ml of deionize water, at room temperature T = 298 K, n = 1, D = 7.6 × 10^{-6} cm ^{2} s $^{-1}$, then the slope of the scheme of Ipa vs. υ ^{½}. Shown in Fig.1(B) that the slope was 1.1 × 10^{-3} A (V s^{-1}) $^{1/2}$ and the area of electrode was 0.314 cm ^{2}.

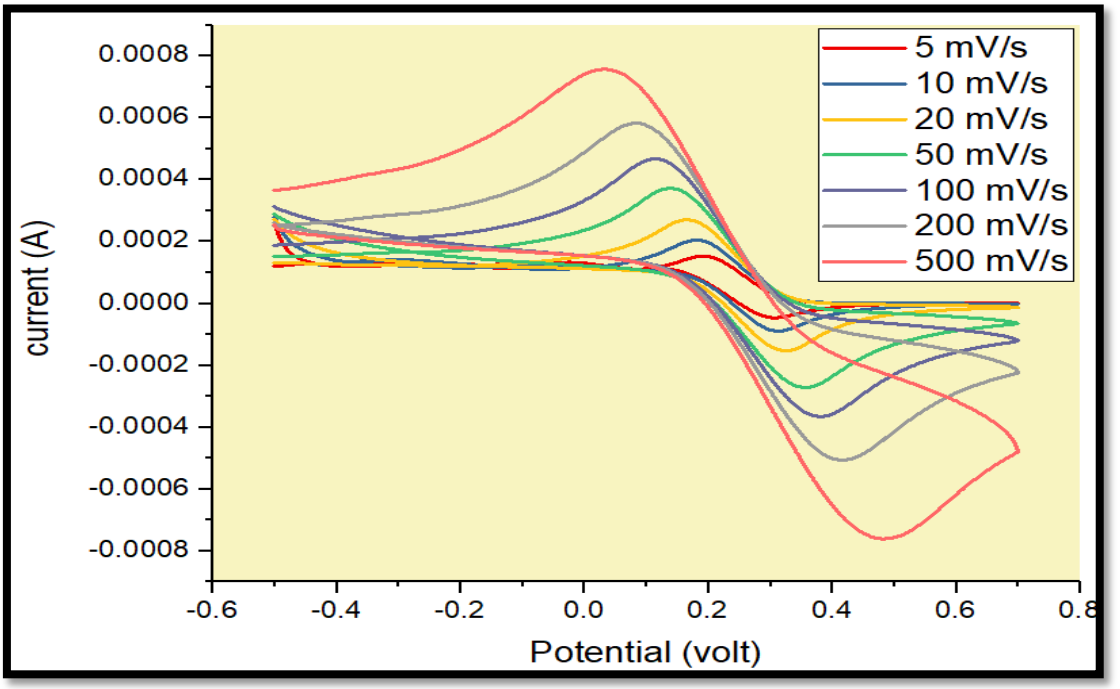

Fig.1 (A) Cyclic voltammograms of 5×10^{-3} mM K_3 [Fe (CN) $_6$] at scan rates of (1)5, (2)10, (3)20, (4)50, (5) 100 (6)200 (7)500 mV/s.

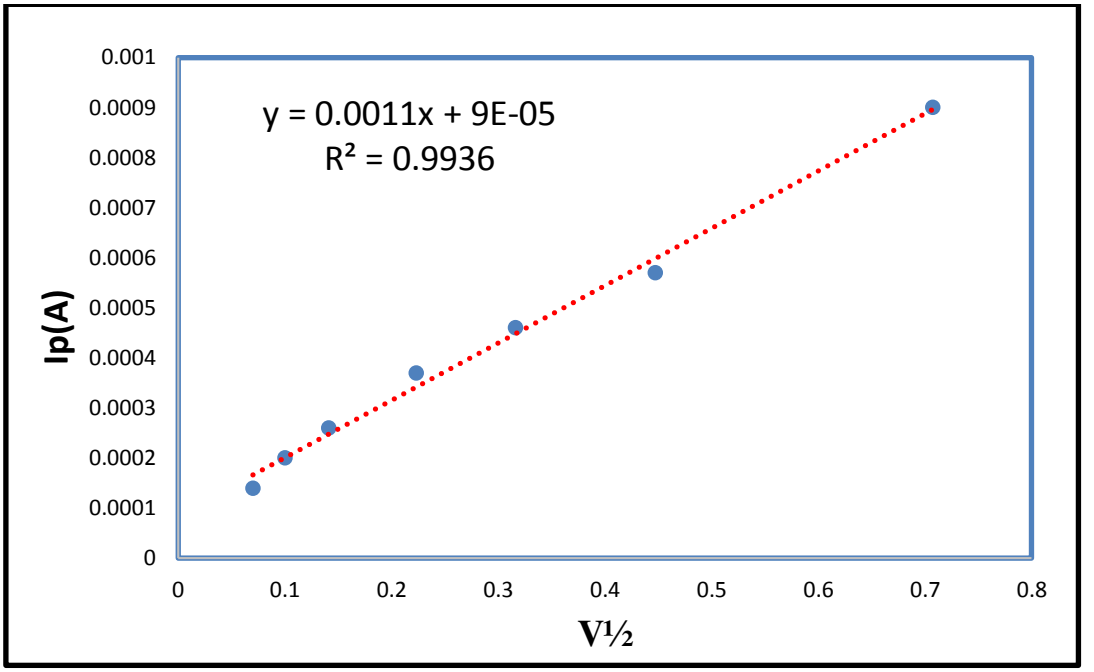

Fig.1 (B) Relationship between anodic peak current and square root of the scan rate

Parameter Optimization for Deferasirox
Effect of scan rate

Using the CV technique in 0.1M sodium hydroxide solution (pH 12.5), the effect of the potential scan rate on the GCE-MWCNTs was investigated at various scan rates (5, 10, 20, 50, 100, 200, 500 and 1000 $mV.s^{-1}$). The best scan rate according to Fig.2, was (100 $mV.sec^{-1}$).

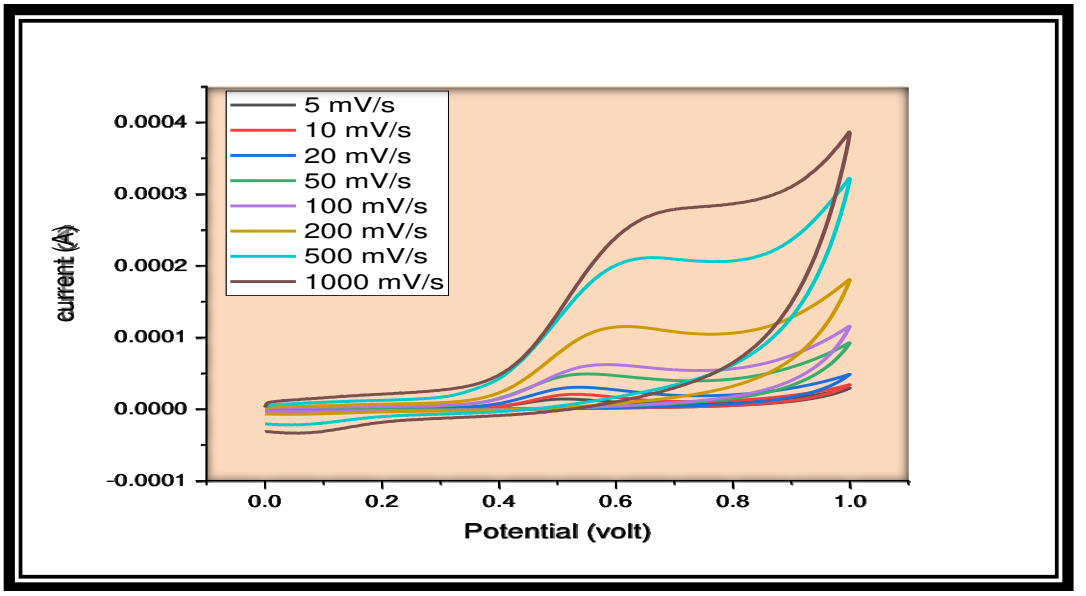

Fig. 2 cyclic voltammogram showed the effect of scan rate at MWCNTs electrode

Fig.3 shows that the potential applied to deferasirox dissolved in 0.1 M NaOH solution increases as the scan rate increases, this indicating that diffusion takes place on the electrode surface (Li *etal.* 2019). The relationship shows in Fig.4 between logarithm of current density for anodic peak and logarithm of scan rate, due to the Randles-Sevick equation in a linear diffusion controlled process ($J \alpha v^{1/2}$), for the adsorptive process ($J \alpha v$) ($\log J \alpha \log v$) (Morya *etal.* 2013). which conformed the following equation:

$(R^2 = 0.9937)$ .... (2) $\text{Log } J = 0.5727 \log v + 1.2264$

If the slope is near to 0.5, the diffusion-controlled process is described, whereas the adsorption-controlled process is described by the slope is near to 1.0 (Chrzescijanska *etal.* 2014). The slope in this study is 0.5727, indicating a diffusion-controlled process.

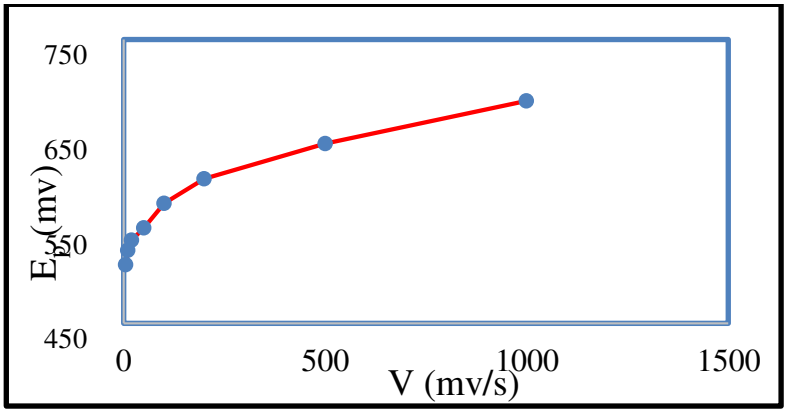

Fig.3: Relationship between (E_p) versus scan rate at GCE-MWCNTs electrode

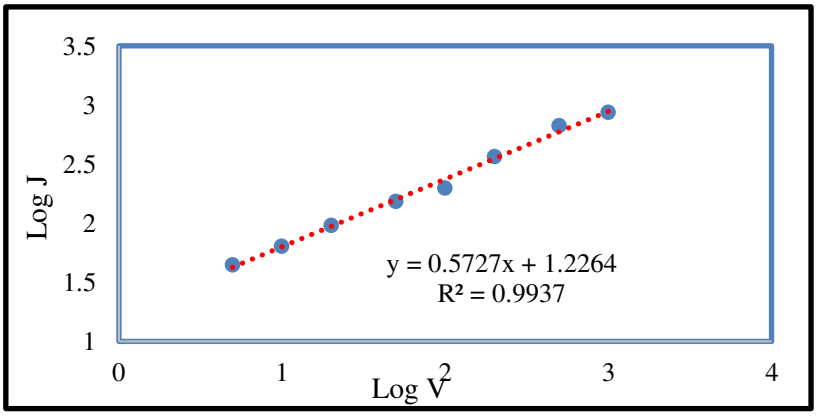

Fig.4: The effect of log current density vs log v at GCE-MWCNTs electrode

In case of irreversible process (Ep) peak potential is defined by Laviron (Shetti *etal.* 2019) and expressed in equation (3)

$$E_p = E^{\circ} + \left[\frac{2.303RT}{\alpha nF}\right] \log \left[\frac{RTK_0}{\alpha nF}\right] + \left[\frac{RT}{\alpha nF}\right] \log \upsilon \ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

Where **E°** is the standard redox potential, **n** is the number electron transferred in the reaction, $\mathbf{k_0}$ is the standard rate constant of the reaction, $\boldsymbol{\upsilon}$ is the scan rate, $\boldsymbol{\alpha}$ the electron transfer coefficient, can be calculated from the difference between the peak potential (E_p) and the half wave potential ($E_{p/2}$) and the frequency can be calculated from the equation (4). The other symbols were used as R=8.314 $JK^{-1}mol^{-1}$, T=298 K°, and F= 96485C.mol^{-1}. The value of E^0 can be determined from equation (3), The value of E^0 is 0.744v and the value of K_0 is 0.447 s^{-1}. For irreversible electrode reaction the value of $\boldsymbol{\alpha}$ is (0.426) which in the number of electron for oxidation peak 1.95 ≈ 2 have been observed which showed the transfer of two electrons in electro oxidation process of the deferasirox. The value of D_0 was (3.154×10^{-4} cm^2sec^{-1}) in scan rate 100 mv/s.

$$\alpha = 47.7\ E_p - E_{p/2}\ mV \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

Effect of pH:

The electrochemical study was explamined of deferasirox in NaOH solution (0.1M) as a supporting electrolyte at various pH values (7-13.8), the measurement was made at 0.01 M using (GCE-MWCNTs) electrode, pH 13.8 ~14 was obtained the maximum peak current for deferasirox at GCE-MWCNTs in scan rate (100 mv/s) that shows in Fig.5 and change was detected in the oxidation peak currents with increasing pH values as ascertained in measurements with deferasirox. Furthermore the E_p can be calculated form the following equation for a diffusion-controlled electrode(Ortaboy and Atun 2015) :

$$E_p = \frac{-0.05915\ P}{\alpha n}\ pH \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

The p value at the GCE-MWCNTs electrode can be calculated at 0.556 using equation (6) or the relationship between Ep and pH. Because the p value is less than 1, this value indicates no protonation process. At pH levels below7, the deferasirox solution became turbid, probably because of the low solubility of deferasirox at these pH values. At pH levels above 10, the currents of oxidation peaks of deferasirox were increased. Thus at pH =11 the value of the potential increases with pH as we notice this in the Fig.6.

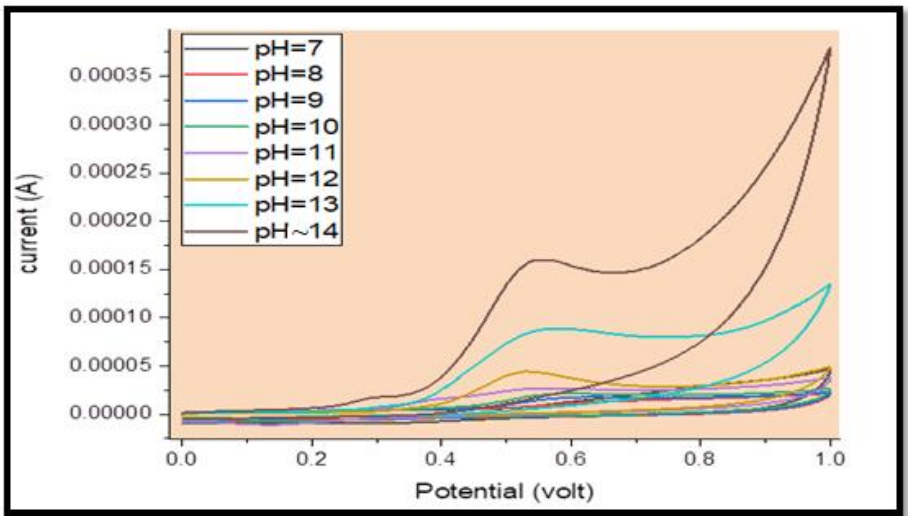

Fig.5: The effect of pH at GCE-MWCNTs electrode for scan rate100mv/s

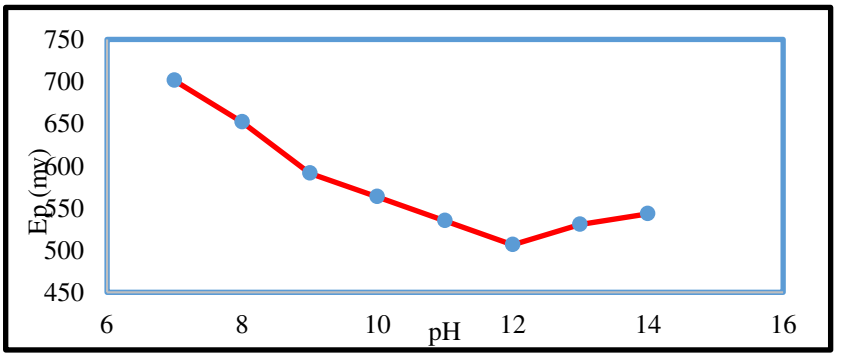

Fig.6: Effect of pH in the peak potential at GCE- MWCNTs in *1*00mv/s

So that the suggestion of electro oxidation mechanism of reaction can be illiterate in Fig.7

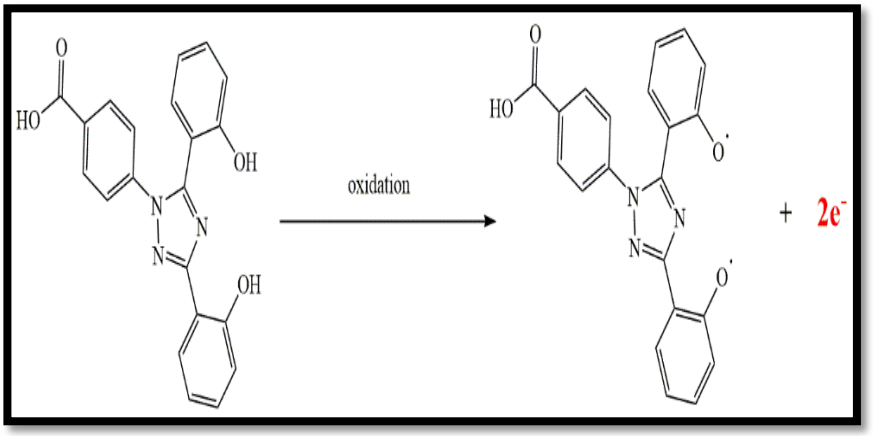

Fig.7 Scheme Mechanism of electrochemical oxidation reaction of deferasirox (Society *etal.* 2006)

The results can be attributed to the presence of two phenolic groups in the deferasirox structure, which create radical cations during the electro-oxidation process. The carbon is then powerfully and irreversibly adsorbed by these radical cations (Longo-Mbenza *etal.* 2004).

Effect of temperature

The electrochemical behavior of deferasirox on the GCE-MWCNTs electrode at varying temperature (5, 10, 20, 30, 40) ^{0}C was shown in Fig.8. We noticed a gradual increase in the current with temperature when the temperature reached 30 ^{0}C maximum this indicates that the best temperature was 30 ^{0}C for GCE-MWCNTs electrode.

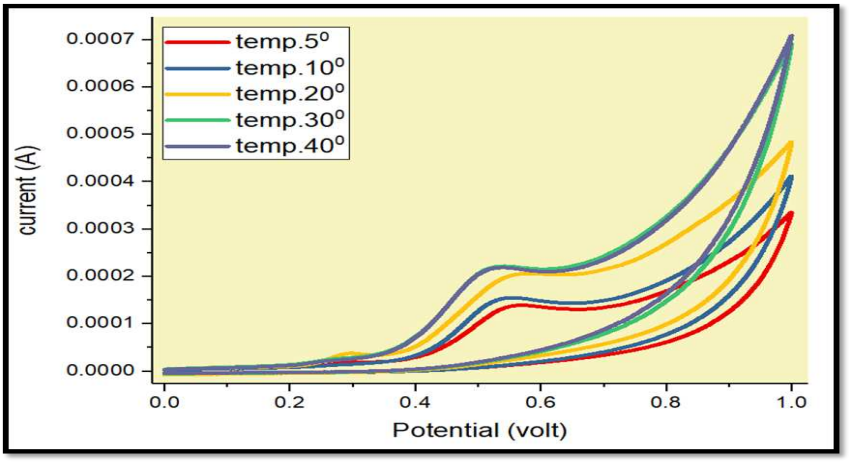

Fig.8: The effect of temperature at GCE-MWCNTs electrode for scan rate 100mv/s

The activation energy was calculated to be (535.5 kJ. mole^{-1}) for GCE-MWCNTs electrode. The activation energy obtained is large, which indicates that the electrochemical reaction is more temperature dependent.

From the slope in Fig.9 the enthalpy ΔH of electrochemical reaction if deferasirox is calculated to be (6.736 kJ. mol^{-1}), and from the intercept the entropy can be calculated to be (213.8 J. K^{-1} mol^{-1}). The positive value of ΔH refers to endothermic reaction and the positive value of ΔS refers to that the disorder is increasing from reactants to products(Molina *etal.* 2020). So that the change in Gibbs free energy can be simply calculated to from the equation (6) to be (-58.04 kJ.mol^{-1}) for GCE-MWCNTs electrode.

$$\Delta G = \Delta H - T\Delta S \quad \text{..... (6)}$$

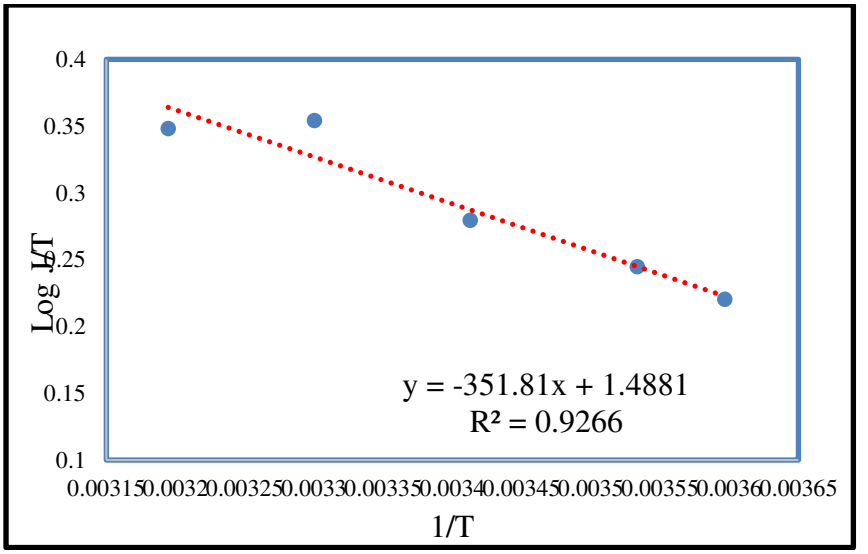

Fig.9: Relationship between log J/T, and 1/T at GCE-MWCNTs for scan rate 100mv/s

Calibration curve

The effect of the concentration of deferasirox versus current density on the GCE-MWCNTs electrode was investigated using CV method under the optimum condition (pH 13.8 ~14), (υ 100 mV.sec^{-1}), (T 30 °C), the calibration plot was describing by the following equation:

$$J(\mu A) = 0.1273\ \text{Conc.} + 98.685 \quad R^2 = 0.9854 \quad \text{..... (7)}$$

A linear calibration plot was obtained for deferasirox Fig.10, the properties of plot graph for deferasirox is in the range (13.4×10^{-4}-2.6×10^{-2} M). The LOD is calculated to be (8.46×10^{-11} M) from 3σ /m, and also LOQ calculated to be (2.82×10^{-10} M) from 10σ /m where **m** is the slop. The characteristics of deferasirox listed in table (1).

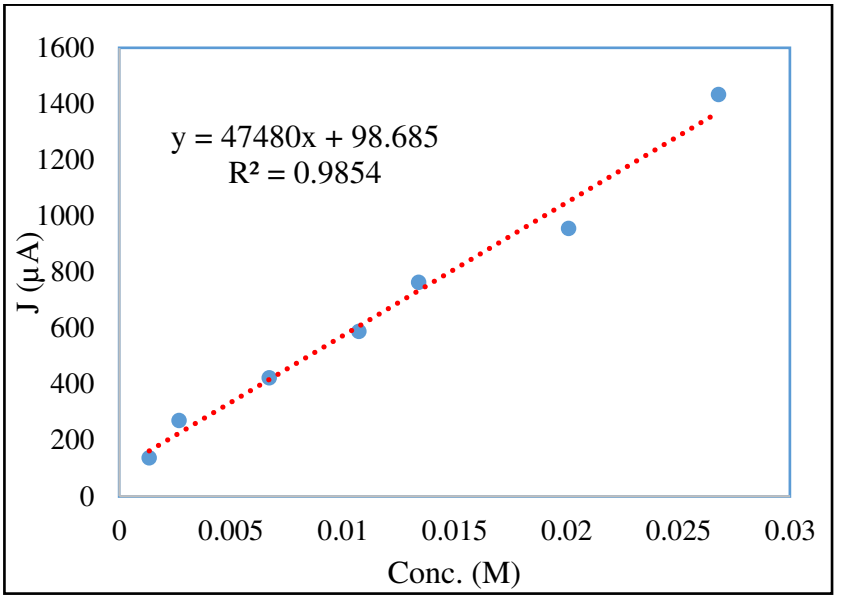

Fig.10 Relationship between J, and conc. at GCE-MWCNTs for scan rate 100mv/s

Table (1)
Analytical parameters of the calibration plot

parameter	GCE-MWCNTs
Linearity range (M)	13.4×10^{-4}-2.6×10^{-2}
Slope	47480
Intercept (µA)	98.685
Correlation coefficient (R^2)	0.9854
LOD (M)	8.46×10^{-11}
LOQ (M)	2.82×10^{-10}
SD (M)	1.349×10^{-6}
RSD%	3.372×10^{-9}

Accuracy and precision in analytical applications

For order to assess the method's precision and accuracy, five duplicate measurements for the added weights 25-500 mg in analytical applications, were analyzed at GCE-MWCNTs. The method validity was applied by taken two types are Exjade and Ipijade which available from local pharmacies were analyzed deferasirox content for their labeled content. The recoveries and relative standard deviation are labeled and according to the following values **in table 2.** this indicates good accuracy and precision for the suggestion method.

Table 2
The analytical characteristics of deferasirox

	Bulk	Form	Urine	Serum
Added (mg)	125 250	500 500	25 50 100	25 50 100
Found (mg)	124.8 250.1	490 492	25.9 50.5 98.5	24.0 49.5 100.5
N	2	2	3	3
Average recovery %	99.840 100.040	98.000 98.400	103.600 101.000 98.500	96.000 99.000 100.500
Mean S.D RSD %	99.940 0.1414 0.1410	98.200 0.2828 0.2879	101.033 2.5501 2.5240	98.500 2.2912 2.3260

Interferences

The interference of many possible potentially co-interfering compounds on the oxidation of deferasirox was performed. Interferences oxidation response does not interfere with deferasiroxs oxidation signal. Fig.11 show the interferences study of deferasirox at 1.34×10^{-3} M concentration, was established by adding several potentially co-interfering compound in to 0.1M of NaOH solution. The formulation of the pharmaceutical (Povidone, Microcrystalline cellulose, silica colloidal anhydrous, Magnesium stearate, and Lactose). These compounds solutions were produced fresh at pH 13.8~14 and 30 ^{0}C in (25ml) methanol except for silica that dissolves in 25 mL of (0.1M) sodium hydroxide solution scan rate of 100 mV.sec^{-1}. The interferences effect on the peak current show in Fig.12.

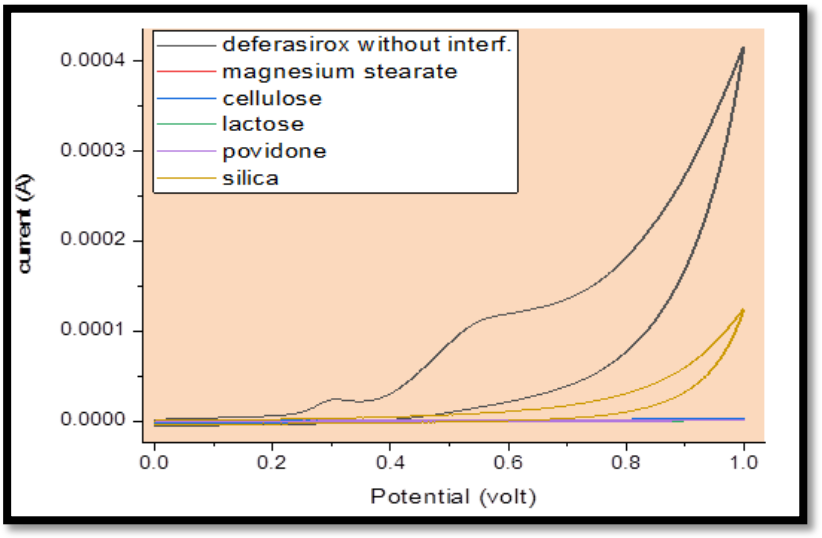

Fig.11: the effect of interferences on deferasirox in 4000ppm

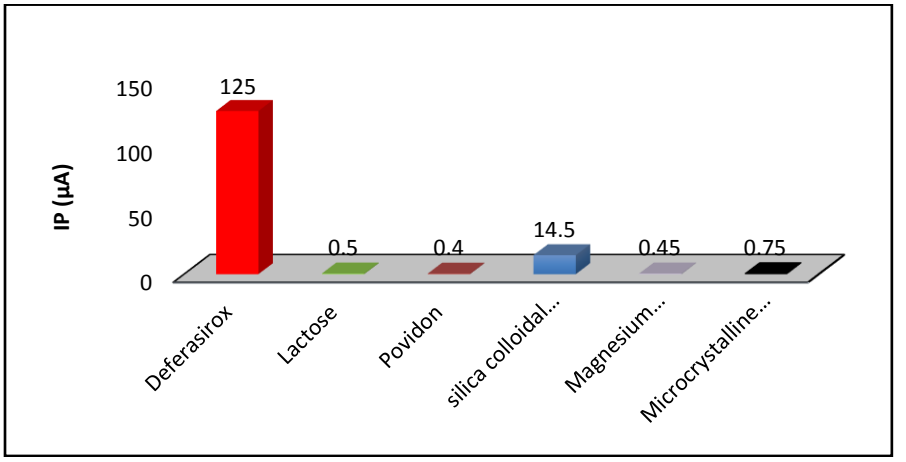

Fig.12 The effect of interferences on the peak current

Conclusions

The electrochemical study of deferasirox at GCE-MWCNTs electrode was described and discussed by cyclic voltammetry, based on the diffusion behavior of deferasirox onto the GCE-MWCNTs surface. This technique a fully validated, simple, sensitive, selective, fast and low-cost for determination of deferasirox in bulk form, urine and serum.

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