



Polarographic and Spectrophotometric Determination of the Complexation of Rutin with Pb(II) ion at different temperature

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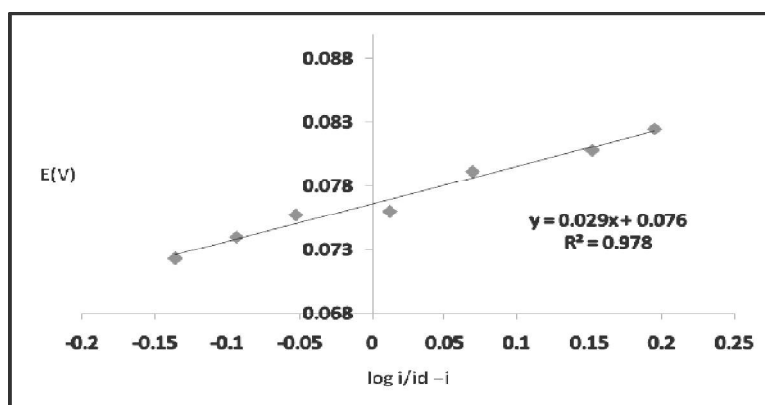
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ABSTRACT

The medical properties of naturally occurring compounds such as flavonoids have been well known for many years. Flavonoids can be effective drugs because of their potent chelating or radical scavenging and anti-inflammatory properties. In this paper, the formation of Pb(II) complex with rutin has been studied spectrophotometrically at an absorption maximum of 398 nm. The data shows that Pb(II) and rutin combine in the mole ratio of (1: 1). The equilibrium constant of the complex were calculated to be $6.11- 6.62 \times 10^5$ by continuous variation method at (293- 313K). The thermodynamic parameters of complex also have been calculated at different temperatures. Polarographic measurements were used to determine the coordination number, stability constant, and the kinetics parameters such as K^0_{fh} , an using Meites- Israel method. The (K^0_{fh} , an) values for the kinetics of the electrode processes show that the electrode processes were irreversible and diffusion controlled.

Graphical Abstract



Relation between concentration and Peak current of Rutin at 298K.

Keywords: Flavonoids, Rutin, Pb(II)-Rutin complex, Polarographic, Spectrophotometric.

INTRODUCTION

Rutin (3, 4,5,7- tetrahydroxy flavone - 3 β -D- rutinose) is a bioactive flavonoid. The structure of rutin (Figure 1), consists of an a glycon quercetin and a disaccharide rutinose bound to quercetin at position 3, ring C [1]. Rutin is usually found in buckwheat and apple peels. Rutin may have antioxidant activity [2], anti- inflammatory activity in some animals and in vitro model [3, 4]. It increases the strength of the walls of blood capillaries, regulates their permeability and can therefore reduce the symptoms of many other capillary diseases. In addition , the antioxidant activity of rutin can be enhanced by complexation with transition metals like Cu⁺², Zn⁺² [5]. Characterization of these complexes was accomplished by IR [6, 7], UV-Visible spectroscopy [8, 9], ¹H-NMR [10], Raman [11]. Lead is naturally occurring bluish-gray metal found in earth's crust. Lead can combine with other chemicals to form what are known as Lead salts. These compounds are water – soluble, while elemental lead is not [12]. The propensity for lead to catalyze oxidation reactions and generate reactive oxygen species has been demonstrated in multiple studies. These reactive oxygen species (ROS) inhibit the production of sulfhydryl antioxidants, inhibit enzyme reactions impairing heme production, cause inflammation in vascular endothelial cells, damage nucleic acids and inhibit DNA repair, and initiate peroxidation in cellular membrane [13, 14]. It is plausible that impaired oxidant / antioxidant balance can be partially responsible for the toxic effect of lead. Several studies have been done to determine the effect of antioxidant supplementation following lead exposure, the higher intake of antioxidant (ex: ascorbic acid or flavonoids) may be effective in preventing lead toxicity, they can functions as chelators.

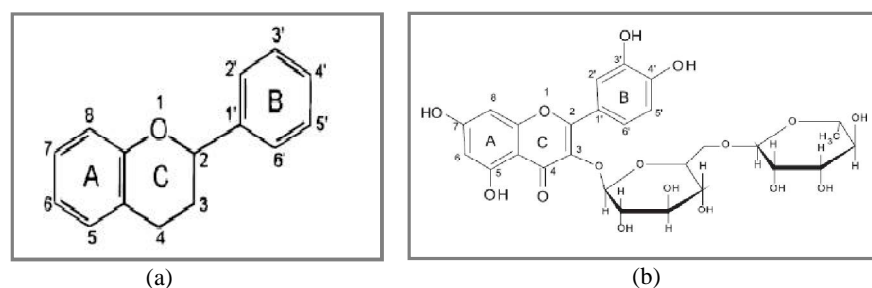


Figure 1. (a) Base structure of flavonoids (b) Rutin.

In this work, the interaction of Rutin and Pb(II) ions was studied using UV-Visible spectroscopy and electrochemically by differential pulse polarographic technique , the stability constant for the formation of the complexes were determined at different temperature .

MATERIALS AND METHODS

Chemicals and solutions: Rutin was obtained from Aldrich chemical company, Methanol from scarlet, Lead nitrate from Himedia Laboratories, Hydrochloric acid from BDH, Sodium hydroxide, and Potassium chloride from GCC-England.

Rutin solution: The stock solution of rutin (10^{-3} M) was prepared by dissolving 61 mg of rutin in 100mL volumetric flask using 50% methanol/water mixture as a solvent. All other chemicals were prepared using double distilled water.

Pb(II) solution: A stock solution of Pb (II) (10^{-3} M) was prepared by dissolving (33.1mg) of Pb (NO_3)₂ in 100mL volumetric flask using double distilled water .

Absorption spectroscopy: All spectral measurements were recorded on a double beam UV-Visible spectrophotometric, Shimadzu- model-160A, using a 1cm path length quartz cell. Absorbance value of Rutin in the presence and absence of Pb(II) solution were made in the range of (200-600nm).

Polarographic measurements: Electrochemical experiments, differential pulse polarography were carried out using a par model 797Va polarographic analyzer equipped version 1.2. The current voltage curves were measured manually and performed with three electrode system consisting of a medium size hanging mercuric drop electrode (HMDE) as the working electrode, a platinum wire counter electrode and an Ag/AgCl (saturated KCl) reference electrode was used.

Electrochemical measurements were performed in (15 mL) accurately measured solution placed in a polarographic cell and deoxygenated with pre-purified nitrogen for at least 5 minutes prior to each experimental and the nitrogen atmosphere was maintained thereafter. The operating parameters were pulse amplitude 50mv, pulse time 0.04sec, scan rate 15mv, initial potential (-1.0V), end potential (+1.0V), drop size 9 mm³, voltage step 0.006V, voltage step time 0.4 sec, deposition time 60sec, equilibrium time 5 sec.

Stoichiometric analysis: The stoichiometry of the complexation of Rutin with Pb(II) ion were determined by continuous variation method (Jobs method) [15]. Equimolar concentrations (10⁻⁴M) of Rutin and Pb(NO₃)₂ were prepared, Job method was applied by placing 1 to 9 mL of (10⁻⁴M) rutin solution into series of 10 mL flask, was followed by placing 9 to 1 mL of (10⁻⁴M) Pb(NO₃)₂ solution, and the absorbance were measured of the maximum wave length of 398nm.

RESULTS AND DISCUSSION

Absorption spectroscopy: The optimized solvent mixtures (Methanol/water) were obtained by measuring the UV-VIS absorption spectra of rutin in various mixture composition as shown in table 1. This table shows that Rutin exhibit two absorption band, band I (358nm), band II (256 nm). The two bands does not exhibit any significant changes in λ max with the variant of solvent composition, whereas the absorbance does. The maximum absorbance required at 50% methanol/ water mixture, figure 2.

Table 1. Absorption spectra of (2x10⁻⁵M) Rutin in various methanol/water mixtures

Sample. No.	Methanol/ water mixture	Wave length(nm)	Absorbance
1	30%	256	0.459
		355	0.395
2	50%	256	0.563
		358	0.424
3	70%	256	0.456
		359	0.380
4	90%	257	0.394
		359	0.342
5	100%	257	0.481
		356	0.387

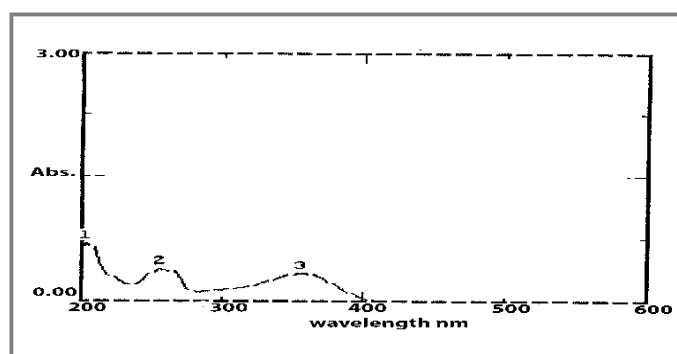


Figure 2. UV- Visible absorption spectra of (2x10⁻⁵M) Rutin in 50% methanol water mixed solvent.

Upon addition of lead (II) solution to (1×10^{-4} M) rutin solution, significant changes are observed in the electronic spectra, [figure 3](#).

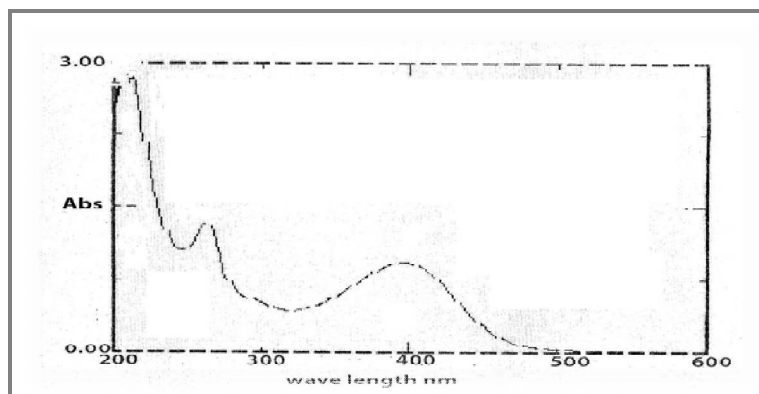


Figure 3. Electronic spectra of Lead (II) Rutin complex in 50% methanol/ water mixed solvent.

Complexation usually be detected because of the difference in their absorption spectra from those of pure components, the electronic spectra of the mixture of Rutin –Pb (II) shows a bathochromic shift (red shift) in band (I) which attributed to $n \rightarrow \pi^*$ transition to the extent of ($\Delta\lambda = 40$ nm) and a shift in band (II) which corresponding to $\pi \rightarrow \pi^*$ transition by ($\Delta\lambda = 7$ nm). The changes in λ_{\max} and the changes in the intensities of the bands were due to the coordination between rutin molecules and Pb (II) ion, these results were presented in [table 2](#).

Table 2. Electronic spectra of (10^{-4} M) rutin and (1:1) Pb- Rutin complex

Compound	λ_{\max} , nm	Absorbance	assignment
Rutin	358	1.666	$n \rightarrow \pi^*$
	256	2.960	$\pi \rightarrow \pi^*$
Pb(II) - Rutin	398	0.932	$n \rightarrow \pi^*$
	263	1.321	$\pi \rightarrow \pi$

Stoichiometry of the formed complex: The stoichiometric ratio of Pb(II) to rutin in the complex was determined by Jobs method of equimolar solutions. The curve displayed maxima absorbance at mole fraction $X_{\max} = 0.5$, which indicates the formation of complex with metal ion to ligand ratio (1 : 1), [figure 4](#). $n = X_{\max}/1 - X_{\max}$, n represent coordination number of the complex, X_{\max} represent mole fraction corresponding to the maxima absorbance.

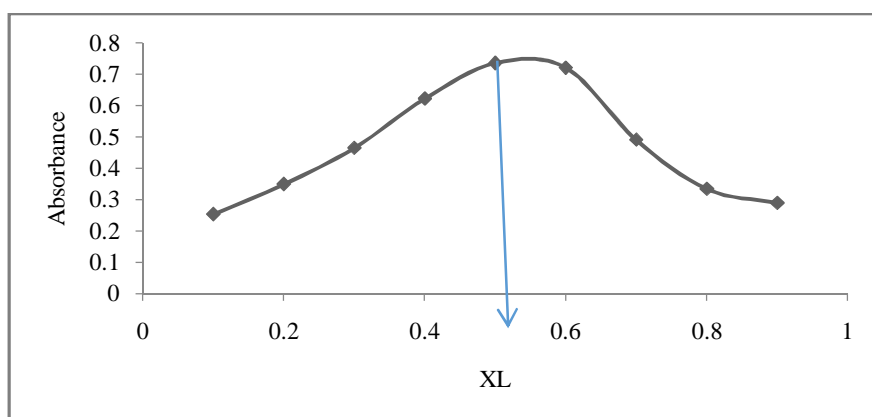


Figure 4. Job's plot for the composition of Lead (II)- Rutin complex at $\lambda = 398$ nm.

Stability constant (K_{eq}): The equilibrium constant can be calculated using the continuous variation method [17] .



$$K_{eq} = \frac{[A_{\max} / \epsilon l]}{[C \text{ Pb} - A_{\max} / \epsilon l] [C \text{ Rut} - A_{\max} / \epsilon l]} \dots(1)$$

A_{\max} = the maximum absorbance of the complex, ϵ = molar absorptivity of the complex ($\text{L. mole}^{-1} \cdot \text{cm}^{-1}$), l = path length. cm. CPb = Concentration of the metal ion at equilibrium, CRur = Concentration of Rutin at equilibrium.

The molar absorptivity of the complex was calculated by recording the absorbance of various concentration of the (1:1) complex. Plotting of the absorbance of the complex against concentration given a straight line with the slope equal to (ϵ). The values of K_{eq} obtained by the continuous variation method were determined in five temperatures (293-313K) as shown in table 3.

Table 3. Metal- Ligand equilibrium constant from Continuous variation method at different temperature .

Sample . No.	Temp.(K)	keq
1	293	6.11×10^{-5}
2	298	6.19×10^{-5}
3	303	6.37×10^{-5}
4	308	6.46×10^{-5}
5	313	6.62×10^{-5}

Thermodynamic parameters: Thermodynamic parameters the enthalpy ΔH^0 , entropy ΔS^0 , free energy ΔG^0 and their relation to equilibrium of studied Pb-Rutin complex calculated as follows:

K_{eq} were determined from the concentration of all components at equilibrium as in previous section which then allows us to calculate ΔG^0 [18].

$$\Delta G^0 = - RT \ln K_{eq} \dots(2)$$

The enthalpy of reaction can be determined [19] by measuring the equilibrium constant for a system at different temperature. The enthalpy of the complex can calculated from the slope [$-\Delta H^0 / R$] of the resulting linear Van't Hoff of $\ln K_{eq}$ versus ($1/T$), figure 5.

Entropy change can then calculate from equation -3, Table 4.

$$\Delta G^0 = \Delta H^0 - T \Delta S^0 \dots\dots\dots (3)$$

Table 4. Thermodynamic parameters for Pb-Rutin complex, at PH= 7.40.

Sample .No.	Temp.(k)	ΔG^0 (KJ/mole)	ΔH^0 (KJ/mole)	ΔS^0 (KJ/mole)
1	293	- 32.454	+ 3.086	+ 0.12129
2	298	- 33.043	+3.086	+ 0.12123
3	303	- 33. 670	+ 3.086	+0.12130
4	00308	- 34. 262	+ 3.086	+ 0.12125
5	313	- 34. 880	+ 3. 086	+ 0.12129

Gibbs free energy is very importance in deciding the direction of process and position of equilibrium. The negative values of Gibbs free energy for these interaction indicates the spontaneous process in the direction of equilibrium and increase with the increase in temperature. The enthalpy of interaction has a positive value indicating that formation of Rutin-Pb complex is endothermic. The positive value of enthalpy and entropy change also refers to the type of interaction between Rutin and Lead (II) ion which are electrostatic in nature.

Polarographic study

Supporting electrolyte: The goal of the preliminary studies is the choice of a supporting electrolyte which is made such that investigated compound is stable for at least 30 Min, and yields a well developed wave separated from waves of other components present in the sample and well separated from the current of the supporting electrolyte together with the range of potentials in which the electrolysis occurs.

A variety of supporting electrolyte can be used in determination of organic compounds. There are usually buffers or solutions of strong acids or strong bases ,the concentrations of them is kept at least 20 times higher than concentration of the electroactive species. The polarographic measurements for Rutin in several supporting electrolytes such as KCl, HCl, NaOH were performed. It was found that HCl was the most suitable supporting electrolyte. The results obtained are summarized in [table 5](#), and the differential puls polarogram are shown in [figure 5](#).

Table 5. Peak potential and peak current of 1.2×10^{-4} M Rutin in different supporting electrolyte

Concentration of supporting electrolyte	Potassium chloride		Sodium hydroxide		Hydrochloric acid	
	Ep(v)	Id(μ A)	Ep(v)	Id(μ A)	Ep(v)	Id(μ A)
1M	0.0415	2.50	- 0.214	2.31	0.0774	21.3
0.1M	0.0417	5.22	-0.015	2.80	0.0770	18.1
0.01M	0.0421	8.38	0.0294	2.85	0.0655	14.7

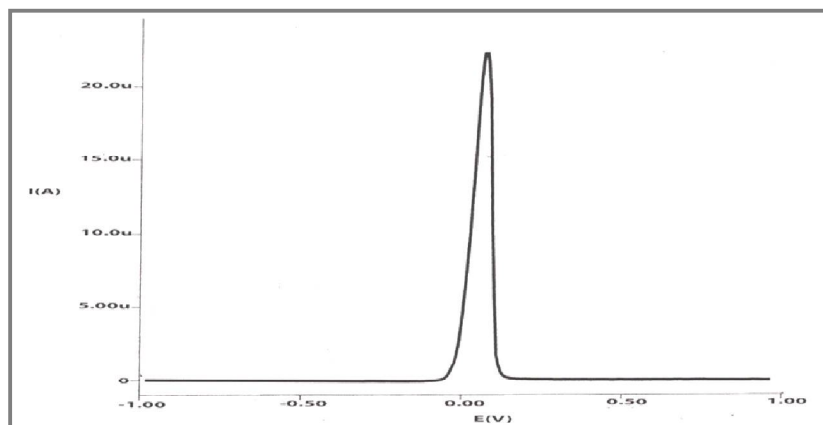


Figure 5. Differential pulse polarogram of 1.2×10^{-4} M Rutin in 1M HCl.

Polarographic wave of Lead (II)ion and Rutin: A well defined two- electron reversible reduction and diffusion controlled wave of Pb^{+2} was observed in 1M HCl solution , the half wave potential or peak potential (Ep) for Pb^{+2} was (-0.387V) VS. Ag/AgCl electrode, [figure 6](#).

Rutin reduction at hanging mercury drop electrode (HMDE) gave one polarographic wave with a peak potential (Ep) of (+0.0774V) IN 1M HCl VS. Ag/Agcl electrode, (Ep) is independent of concentration. The limiting current (Id) is controlled by diffusion and is proportional to the

concentration of Rutin. An increase in the wave high (I_d) with the increase in concentration which understood from Ilkovic equation (4) [20].

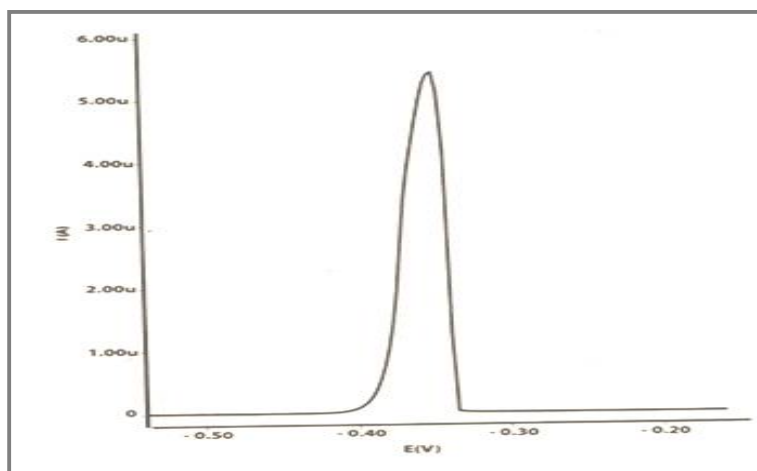


Figure 6. DPP of 1×10^{-4} M Pb^{2+} ion in 1M HCl.

$$I_d = 706 \cdot n \cdot D^{1/2} \cdot m^{2/3} \cdot t^{1/6} \cdot C \dots(4)$$

Where, I_d is the average diffusion current in microampere during the life of the drop, n is the number of electrons consumed in the reductive of one molecule of the electroactive species, D is the diffusion coefficient of the reducible or oxidisable substance expressed as $cm^2/sec.$, m is the rate of flow of mercury from the dropping electrode expressed in $mg/sec.$, t is the drop time in sec., and C is the concentration in $mmole L^{-1}$, the constant 706 is a combination of natural constants.

Linear relation between concentration and diffusion current (I_d) was obtained with the value of correlation coefficient (r^2) near to one, this has been proved statistically by applying straight line equation, Figure 7.

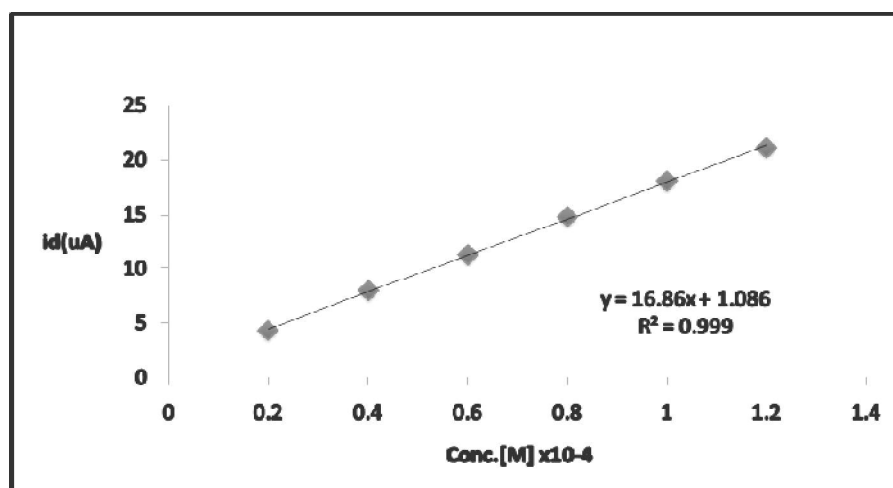


Figure 7. Relation between concentration and Peak current of Rutin at 298K.

The electron transfer is a reversible process as a linearity is observed in the plot (E) vs. $\log(i/i_d - i)$, for each polarographic measurement the Heyrovsky–Ilkovic equation-5 [21] Was used to calculate the number of electron (n) required for the reduction.

$$E = E_{1/2} - 0.0591/n \cdot \log i/id - i \dots(5)$$

Thus the value of (n) was obtained from the slope of the straight line corresponding to (E) vs. $\log i/id - i$. The whole number value of (n) is taken as a sign of reversible reduction, Figures 8.

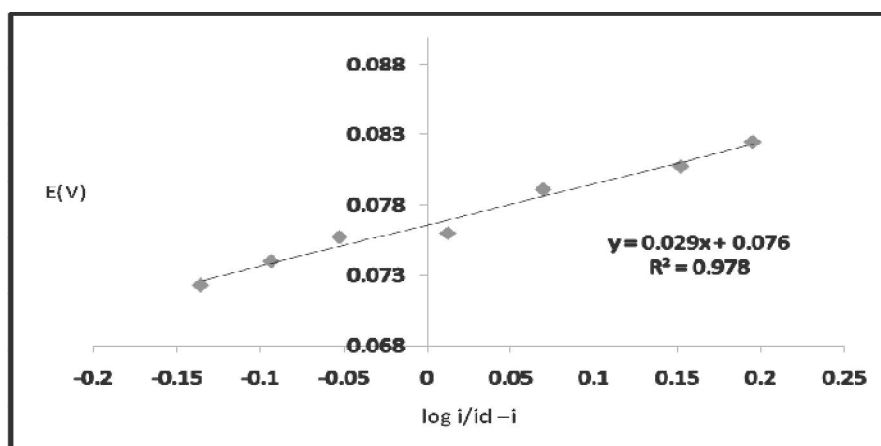


Figure 8. Heyrovsky – Ilkovic plots of 1.2×10^{-4} M Rutin.

Rutin - Pb^{+2} complexation: Addition of a different concentration of Quercetin in a range of 2×10^{-5} - 1×10^{-4} M to a solution of a constant concentration 1×10^{-4} M of Pb^{+2} ion, show the appearance of a new peak in a more negative potential (-0.459V) than the $Pb(II)$ peak with a gradual decrease in a peak current of $Pb(II)$ ions which suggests the complex formation between them, Figure 9.

The peak potential of the complex formed shifts cathodically with the increase in ligand concentration due to increasing the activation energy involved in reduction [22].

Stoichiometry and stability constant: The stoichiometry and the stability constant of lead(II) complex with Quercetin using a polarographic method were calculated by Lingane equation (6) [23].

$$\Delta E_{1/2} = (E_{1/2})_C - (E_{1/2})_M = - (0.0591/n) \log KML - (0.0591/n) \cdot P \cdot \log [L] \dots[6]$$

This method is based on the observation that the complexation of a metal by a ligand will cause a shift towards more negative potentials in the polarographic reduction wave of the metal ion, the magnitude of the shift is directly related to the thermodynamic stability of the complex and the concentration of the complexing ligand.

A straight line was obtained by the application of Lingane equation (3), as shown in Figure (10) and table 6. The slope of line allows the determination of the coordination number P, $P=1$, which implies that the stoichiometric ratio of $Pb(II)$ – Rutin complex is 1:1, and the stability constant were determined from the intercept $k_{ML} = 1.28 \times 10^7$.

Table 6. Application of lingane equation in Pb – Rutin complex at 298K, 1×10^{-4} M $Pb(II)$ ion.

No.	Conc. of [L], M	Log [L]	$\Delta E_{1/2}$, (V)
1	2×10^{-5}	- 4.69	-0.048
2	4×10^{-5}	- 4.39	-0.056
3	6×10^{-5}	- 4.22	-0.063
4	8×10^{-5}	-4.09	-0.068
5	1×10^{-4}	- 4.00	-0.072

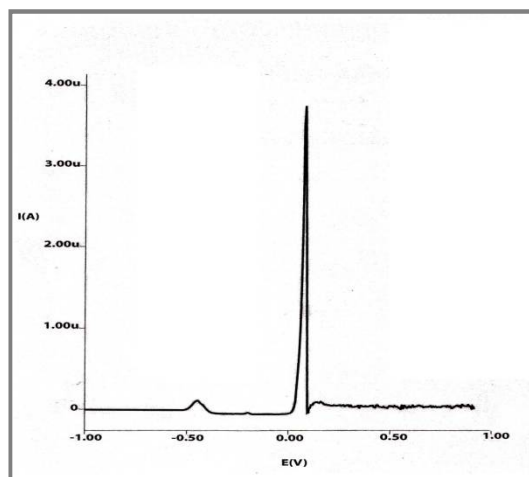


Figure 9. Differential pulse polarogram of Rutin-Pb system in 1M HCl.

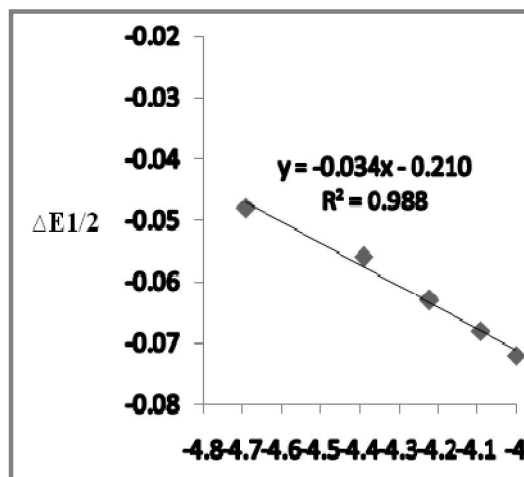


Figure 10, Lingane plot of $\Delta E_{1/2}$ of Pb- Rutin vs $\log [L]$.

Kinetic parameters: kinetic parameters ($K^0_{fh, \alpha n}$) were calculated by Meites & Israel's method [24]:-

$$E = E_{1/2} - 0.0542/\alpha n \cdot \log i/i_d - i \dots (7)$$

$$E_{1/2} = 0.0591/\alpha n \cdot \log 1.349 K^0_{fh} \cdot t^{1/2} / D^{1/2} \dots (8)$$

Where, K^0_{fh} = formal rate constant for forward reaction, D = Diffusion coefficient, αn = transfer coefficient

E and $E_{1/2}$ were determined with respect to Ag/AgCl electrode, the values of αn were obtained by equation (7) and K^0_{fh} by equation (8). The value of diffusion coefficient (D) were determined by using Ilkovic equation(4).

Table 7. Electrochemical reduction of [1:1] Pb^{+2} – Rutin system at HDME in 1M HCl at various temperatures

T (K)	(E_p)c (v)	I_d (μA)	αn	$D^{1/2}$ (cm^2)	k^0_{fh} (cm/s)
293	-0.454	0.112	2.004	0.3294	4.415×10^{-17}
298	-0.484	0.148	1.914	0.4352	5.780×10^{-18}
303	-0.511	0.240	1.968	0.7058	1.112×10^{-18}
308	-0.527	0.313	1.993	0.9205	4.345×10^{-19}
313	-0.546	0.443	2.028	1.3029	1.336×10^{-19}

A gradual change in diffusion current and half wave potential was observed when the solution temperature was increased from 20-40 °C (Table 8). When we increase the temperature of solution the half wave potential of system becomes more negative.

The value of K^0_{fh} at various temperatures comes out to be of the order of $10^{-18 \pm 1}$ which indicates irreversible nature of reaction. The value of αn implies that transfer of electrons becomes difficult as temperature was elevated.

Further the value of K^0_{fh} decreases with increase in temperature which suggests that irreversibility increase with increase in temperature. This implies that reduction products.

CONCLUSION

Pb(II) ions and Rutin form a (1:1) complex in 50% methanol. Determination the complexation properties of the complex investigated by UV-Visible spectrophotometry and electrochemically. A spectrophotometric method for determination of Rutin is proposed, optimal conditions for rutin determination by this method are at $\lambda=358\text{nm}$ and 256 nm respectively in 50% methanol, while for complex was $\lambda=398\text{nm}$. Electrochemical study show that the hydrochloric acid was the most suitable supporting electrolyte for the reduction of Rutin by differential pulse polarography, the presence of a new peak in the more negative potential and a decrease in the current peak confirms the complex formation between Lead (II) ion and Rutin.

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