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Full Paper

Kinetic Study of Paracetamol Hydrolysis: Evaluating of the Voltammetric Data

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Abstract- Hydrolysis of paracetamol in acidic media was studied using electrochemical technique in different reaction temperatures. Based on the fact that both paracetamol (PAR) and its hydrolyzed product, *p*-aminophenol (PAP) are both electroactive and their voltammetric peaks do not overlap, differential pulse voltammetry was used for the determination of the remained PAR concentration in the reaction vessel as well as produced PAP concentration. Then, the kinetic parameters of PAR hydrolysis including reaction rates and half-lives in various temperatures and the activation energy of the reaction were calculated using proposed accurate method. PAR is hydrolyzed to PAP in the presence of hydronium ion via first-order kinetic reaction with the reaction rates of 8.9×10^{-4}, 2.16×10^{-3}, 4.21×10^{-3}, 6.26×10^{-3} and 1.46×10^{-2} min^{-1} at the reaction temperatures of 50, 60, 70, 80 and 90 ˚C, respectively. The activation energy of the hydrolysis reaction was calculated to be 63.61 kJ mol^{-1}.

Keywords- Paracetamol, *p*-Aminophenol, Kinetic study, Voltammetry

1. INTRODUCTION

Paracetamol (PAR, acetaminophen) is an extensively administered antipyretic and analgesic all over the world for treating the symptoms of different painful processes [1,2]. It is also used as an intermediate for pharmaceuticals such as a precursor in penicillin [3]. *p*-Aminophenol (PAP) is the main product of the hydrolysis reaction of PAR and one of its main metabolites [4]. PAP has significant nephrotoxicity and teratogenic effects and has been detected in PAR as an impurity or synthetic intermediate. Furthermore, the industrial synthesis of PAR occurs through the acetylation of PAP with acetic anhydride [5]. The study of PAR hydrolysis rate as well as PAR degradation under abnormal conditions is important from pharmaceutical point of view. So, up to now, researchers have reported works on the hydrolysis [6], thermal degradation [7], biodegradation [8] or photo-degradation [9] of PAR and obtained kinetic or thermodynamic parameters of these reactions using chromatographic techniques. Thermal degradation of PAR was studied via conventional accelerated aging studies by initially thermally stressing the compound at temperatures between 160°C and 190°C and measuring the rate of decomposition by reversed-phase high-performance liquid chromatography [7]. Photo-degradation of PAR has also been studied by UV irradiation [9,10], as well as its photocatalytic degradation in the presence of TiO_2 [11,12] and Cu-doped TiO_2 photocatalyst [13]. Biodegradation of PAR by a combination of microbial strains of *Stenotrophomonas* and *Pseudomonas* was studied and the authors demonstrated that the degradation and mineralization of PAR was significantly improved, and PAP and hydroquinone were detected as two key metabolites of the biodegradation pathway of PAR [8]. The electrooxidation of PAR at boron-doped diamond electrode as well as Ti/SnO_2 anode in a plug-flow divided electrochemical reactor has been reported [14]. PAR oxidation led to electrochemical combustion at these electrodes, whereas at Ti/IrO_2, benzoquinone was the exclusive product except at very long electrolysis times [14]. The hydrolysis of PAR has been reported by using capillary electrophoresis and the rate constants of PAR hydrolysis at different temperatures have been determined by monitoring the concentration changes of PAR [6]. The measured rate constants were around 0.01 min^{-1} depending on the temperature of the reaction vessel.

These wide studies show the importance of PAR stability issue. Besides PAR, the kinetic study of the hydrolysis or degradation of some other important pharmaceuticals such as aesculin [15], ketoprofen [16], diclofenac [17] etc. have also been worked recently using various methods. The main reason of these studies is the close relation between the hydrolysis, photo-degradation, thermal degradation etc. and the stability of the drugs from pharmacological point of view. It is necessary to determine the rate constant of the pharmaceutical degradation for evaluating their half-life, selecting appropriate storage conditions as well as controlling drug quality.

Among various methods, chromatographic techniques have been extensively used in these studies. However, other analytical techniques have their own limitations. Because of the potential spectral overlapping of pharmaceuticals with their hydrolysis or degradation products, spectrophotometric methods without separation processes cannot be used successfully. Electrochemical methods can only be a useful technique for the kinetic studies of hydrolysis or degradation reaction of the electroactive pharmaceuticals. Based on the fact that both paracetamol and its hydrolyzed product, *p*-aminophenol are both electroactive compounds, electrochemical techniques can be used for the investigation of the hydrolysis reaction of PAR. Both of these species are electroactive and have quasi-reversible behavior on solid electrodes such as boron-doped diamond electrode [18] or carbon electrodes [19,20].

In this work, the hydrolysis of paracetamol was studied by following the concentration changes of PAR and PAP in solution using differential pulse voltammetry (DPV) based on the fact that the DPV peaks of PAR and PAP do not overlap. The rate constants of PAR hydrolysis at various temperatures, half-lives and the activation energy of reaction have been calculated. The proposed method is accurate, simple and selective, providing an alternative method besides chromatographic techniques for the kinetic study of the hydrolysis or degradation reaction of electroactive pharmaceuticals.

2. EXPERIMENTAL

2.1. Chemicals

All chemicals were of analytical reagent grade and double distilled water was used throughout. Hydrochloric acid solution (1.0 M) was prepared by dilution of its concentrated solution (Fluka). NaOH solution (1.0 M) was prepared by dissolving 4.00 g of NaOH (Merck) in a 100-mL flask and diluting to the mark. The stock solution of PAR (500 µg mL^{-1}) was prepared in a 100-mL flask by dissolving 50.0 mg of paracetamol (Merck) in water and diluting with water to the mark. The stock solution of PAP (500 µg mL^{-1}) was also prepared in a 100-mL flask by dissolving 50.0 mg of *p*-aminophenol (Fluka) in water and diluting with 0.010 M HCl to the mark. These solutions (PAR and PAP) are fairly stable at least for a month in refrigerator. PAP solution in water (not in acidic medium) is stable for few days in refrigerator. So, it was preferred to prepare its stock solution in acidic medium.

2.2. Electrochemical measurements

The electrochemical measurements were carried out using a galvanostat/potentiostat Autolab PGSTAT101 instrument and a three electrode electrochemical cell. The reference electrode was Ag/AgCl (KCl, 3 M). A platinum rod electrode was used as auxiliary electrode and the working electrode was a glassy carbon electrode.

2.3. Hydrolysis procedure

All experiments were done in a water bath (Precision Scientific (PS) Co., USA) for achieving the desired constant temperatures at which the hydrolysis was carried out (50, 60, 70, 80, and 90 ˚C). An accurate volume of 50.0 mL of PAR (500 µg mL^{-1}) was transferred into a flask and preheated to the desired temperature for 15 min in the water bath. The stock solution of 1.0 M HCl was also preheated in the same way. An aliquot of 50.0 mL of HCl solution was added to the PAR solution and mixed immediately by shaking the flask. The time when almost half of HCl solution was added was considered as initial time of hydrolysis.

In a certain time intervals of 15, 30, 45, 60, 75, 90 and 120min, an accurate volume of 2.5 mL of hydrolysates was transferred to the electrochemical cell and immediately 2.5 mL of NaOH solution (1.0 M) was added in order to terminate the hydrolysis reaction. Then, 5.0 mL of phosphate buffer solution pH 7.0 was added to cell and differential pulse voltammetry (DPV) of the solution was carried out using glassy carbon electrode (GCE) as a working electrode.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behaviors of PAR and PAP

Cyclic voltammograms of 50 µg mL^{-1} of PAR and 50 µg $mL^{-1}$of PAP in pH 7.0 phosphate buffer solution on GCE are shown in Fig. 1. The quasi-reversible peaks for PARcorresponds to the oxidation of PAR to N-acetyl-p-benzoquinone-imine (NAPQI) in positive direction of potential scan and the reduction of NAPQI to PAR in negative direction of potential scan via a two-electron process [19]. At GCE, PAR has a quasi-reversible behavior with anodic peak potential of 0.51 V and the cathodic peak potential of -0.08 V with ΔE_p of about 0.59 V *vs.* Ag/AgCl (KCl 3 M). For PAP, however, the anodic and cathodic peak potentials appear in 0.29V and 0.14 V *vs.* Ag/AgCl (KCl 3 M), respectively, with ΔE_p of about 0.15 V. The electrochemical kinetic parameters of PAR and PAP on carbon electrodes such as carbon paste electrode (CPE) and carbon ionic liquid electrode (CILE) was studied and reported recently by our group [20].

Differential pulse voltammogram of GCE in a solution containing 5.0 µg mL^{-1} of PAR and 1.0 µg mL^{-1} of PAP in pH 7.0 was carried out in the potential range of 0.2 to 0.7 V *vs.* Ag/AgCl (Fig. 2). Two well-defined peaks at about 0.35 V and 0.52 V were observed corresponding to the differential pulse voltammograms of PAP and PAR, respectively. The oxidation peak separation of 0.17 V for PAR and PAP allows us to determine their concentrations in hydrolyzed solution containing remained PAR and produced PAP. Relative standard deviations (%RSD) for 10 determinations of PAR (5.0 µg mL^{-1}) and PAP (1.0 µg mL^{-1}) using DPV were 2.13, 1.91%, respectively. PAR and PAP can be determined in the

ranges of 1.0 µg mL^{-1} to 300 µg mL^{-1} and 0.2 µg mL^{-1} to 250 µg mL^{-1}, with the detection limits of 0.25 µg mL^{-1} and 0.08 µg mL^{-1} (calculated by 3σ), respectively, using DPV.

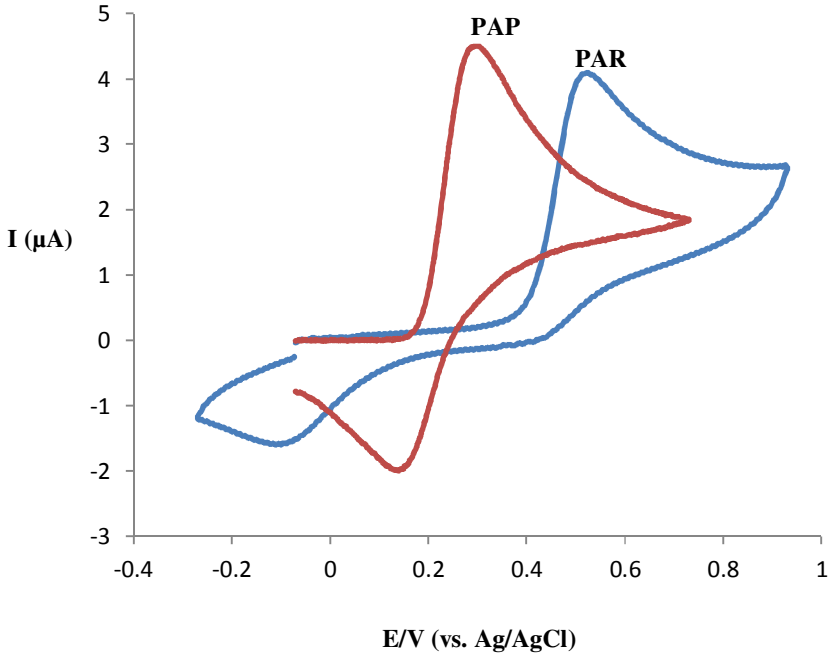


Fig. 1. Cyclic voltammograms of (a) PAR (50 µg mL^{-1}) and (b) PAP (50 µg mL^{-1}) in pH 7.0 phosphate buffer solution on GCE

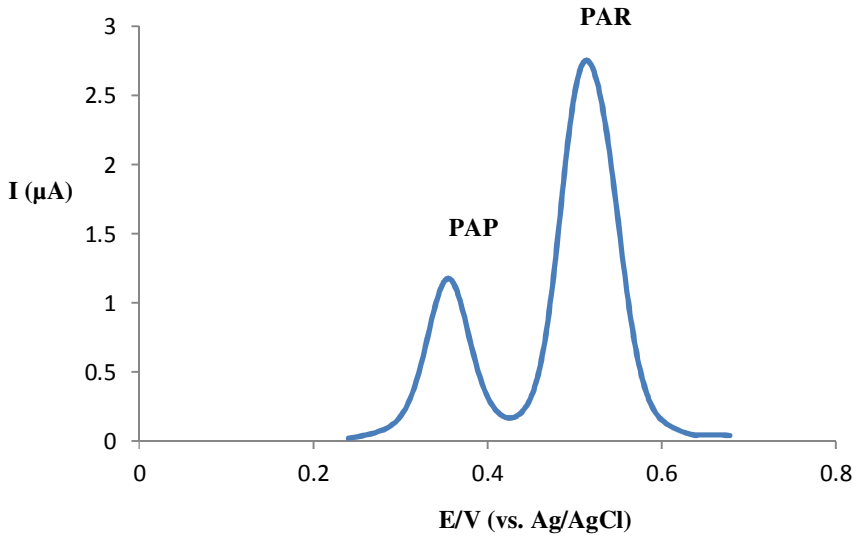
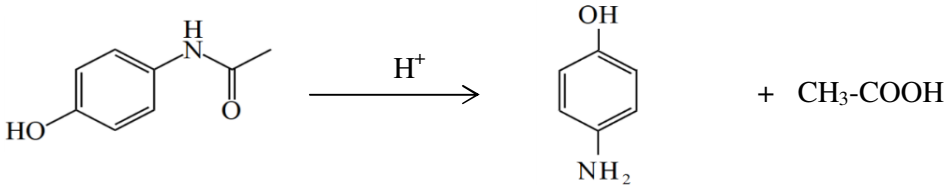


Fig. 2. Differential pulse voltammogram of GCE in a solution containing PAR (5.0 µg mL^{-1}) and PAP (1.0 µg mL^{-1}) in pH 7.0 on GCE

3.2. The hydrolysis reaction of paracetamol and the determination of hydrolysis rate constants

As an important degradation mode, there is a close relation between the hydrolysis and the stability of paracetamol. Thus, it seems necessary to determine the rate constants and activation energy of PAR hydrolysis for evaluation of half-life, selecting appropriate storage conditions, controlling drug quality, etc. by using simple, appropriate and accurate methods. PAR is hydrolyzed to produce PAP and acetic acid in acidic solution (Scheme 1) via first-order kinetic reaction [6].



Scheme 1. The hydrolysis reaction of paracetamol

So, the kinetic equation will be:

$$\frac{-dC}{dt} = kC \quad (1)$$

Where C is PAR concentration, t the time of the reaction and k the hydrolysis reaction rate constant. The equation (1) can be expressed as:

$$ln\left(\frac{C}{C_0}\right) = -kt \quad (2)$$

So, a plot of the logarithm of PAR concentration ($ln\ C/C_0$) versus reaction time (t) gives a straight line with the slope of rate constant, k.

The hydrolysis of PAR was carried out in a constant temperature water bath in various temperatures including 50, 60, 70, 80 and 90˚C, as indicated in details in Section 2.3. Fig. 3 shows the voltammograms of PAR and the hydrolysate PAP in the hydrolysis times of 0, 15, 30, 45, 60, 75, 90 and 120 min at the reaction temperature of 80 ˚C.As it is clear, the peak current corresponding to PAR at 0.52 V is decreased gradually with the hydrolysis time, while the peak current of PAP is increased at the same time at 0.35 V. The change in the peak currents corresponding to PAR and PAP demonstrates the change of their concentrations during the hydrolysis. Also, as Fig. 3 shows, it is not detected any additional peak corresponding to hydrolyzed solution. It indicates that the only electroactive product of PAR hydrolysis is PAP. The voltammograms of PAR and gradual change in PAR and PAP peak

currents at 50, 60, 70 and 90 ˚C are the same as their behavior at 80 ˚C (as depicted in Fig. 3) except in the amount of PAR and PAP peak currents, indicating the variation of reaction rate with temperature.

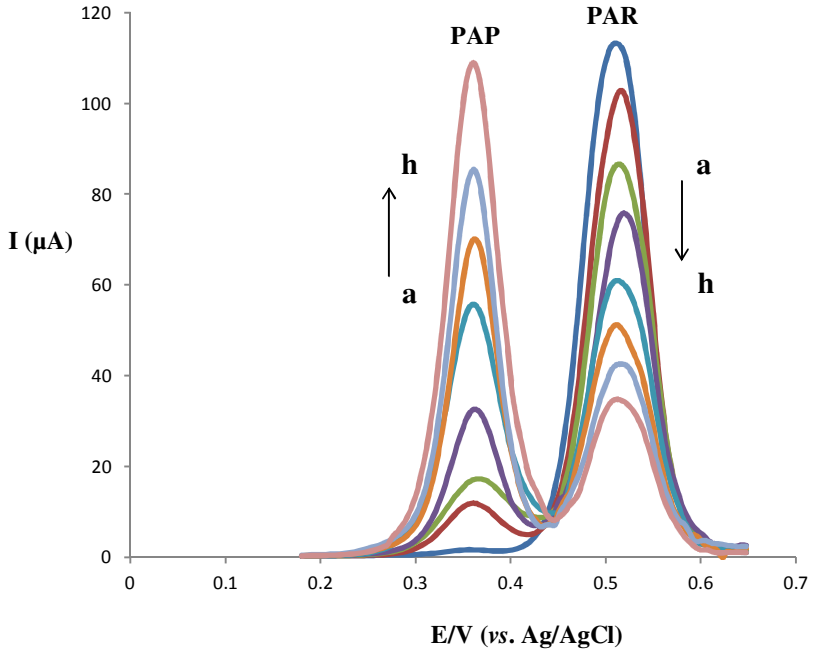


Fig. 3. Differential pulse voltammograms of PAR hydrolyzed solution in the hydrolysis times of (a) 0, (b) 15, (c) 30, (d) 45, (e) 60, (f) 75, (g) 90 and (h) 120 min at the reaction temperature of 80 ˚C. The initial concentration of PAR was 250 µg ml^{-1}

For calculating the reaction rate of PAR hydrolysis, the data corresponding to the logarithm of PAR concentration ($ln\ C/C_0$) should be plotted versus reaction time (t). The slope of such plots gives the rate constants, k, at the desired temperatures. Fig. 4 shows the ($ln\ C/C_0$) $-t$ curves for PAR hydrolysis calculated from the experimental data at various temperatures of 50, 60, 70, 80 and 90 ˚C. As it is clear, there is a linear relationship between the logarithm of PAR concentration and the reaction time at various temperatures. The resulted rate constants and half-lives are given in Table 1. The Arrhenius plot i.e. the plot of lnk versus the reciprocal of the absolute temperature (T^{-1}) was plotted in the range of the working temperatures (Fig. 5).The resulted curve exhibits a linear relationship between lnk and $T^{-1}$with the correlation coefficient of 0.9883. This indicates that the term lnk directly depends on T^{-1} in the temperature range of 50 to 90 ˚C and the activation energy (E_a) is constant over this range of T, i.e. E_a is independent of T. To obtain the activation energy of the PAR hydrolysis from the Arrhenius plot, the equation of the resulted straight line was obtained:

$$lnk = -7820.4T^{-1} + 17.252 \quad (3)$$

From this equation (eq. 3), and its comparison with Arrhenius equation (eq. 4), the activation energy (E_a) was calculated to be 63.61 kJ mol^{-1}.

$$ln\, k = -\frac{E_a}{RT} + \ln A \quad (4)$$

where, E_a is the activation energy of the reaction (J), R the molar gas constant (8.314 J mol^{-1} K^{-1}), T, the absolute temperature (K) and A, the frequency factor. The frequency factor (A) was also obtained as 3.11×10^7 min^{-1}. The obtained reaction rates and activation energy for the hydrolysis of PAR in acidic medium are all in good agreement with the values reported earlier using capillary electrophoresis [6].

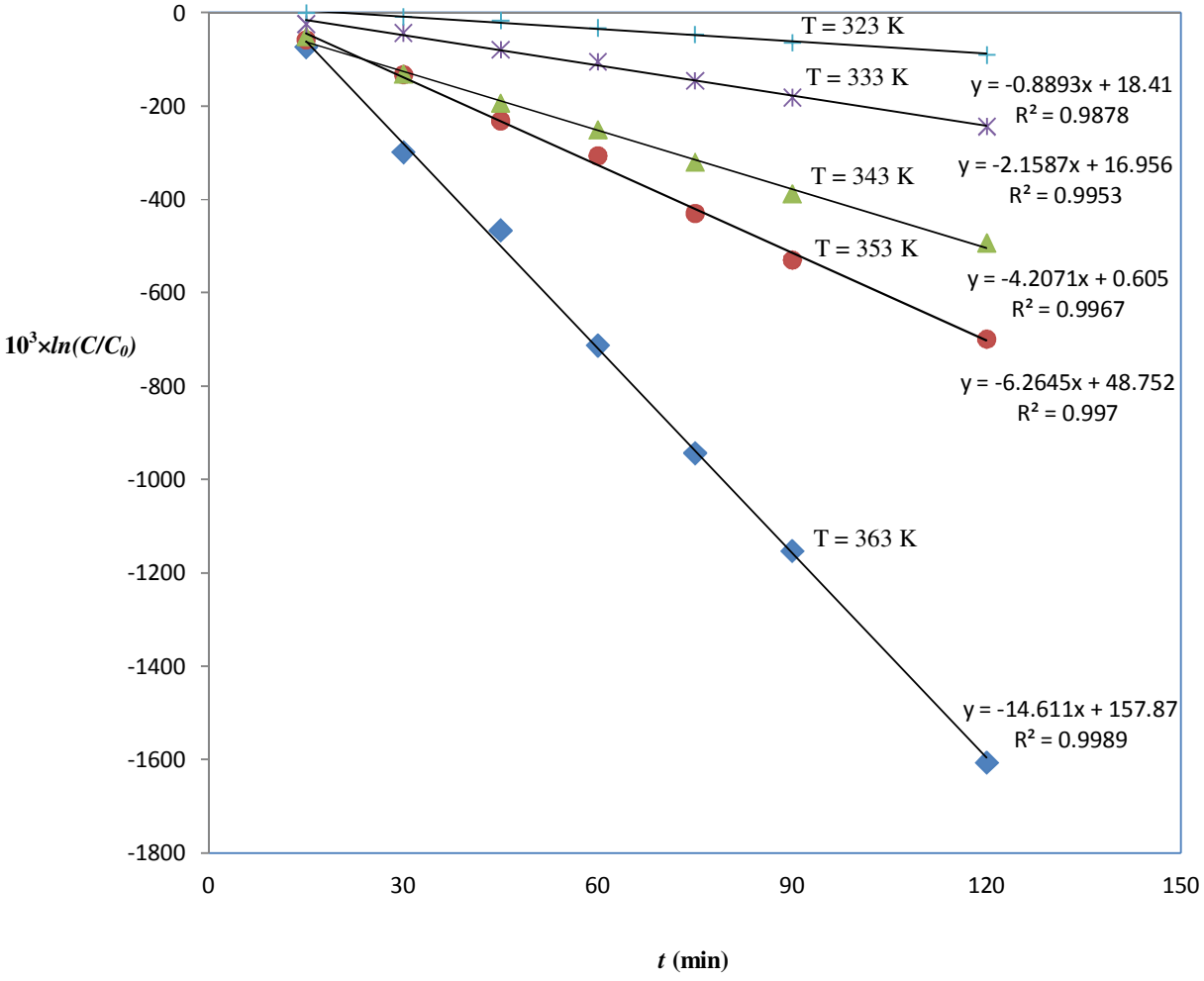


Fig. 4. The plot of $ln(C/C_0)$ *vs.* reaction time *t* for PAR hydrolysis at various temperatures of 50, 60, 70, 80 and 90˚C

Table 1. Reaction rates, correlation coefficients and half-lives obtained from related curves (Fig. 4)

Temperature (K)	Correlation coefficient	Rate constant ($\times 10^{-3}$ min^{-1})	Half-life (min)	Reported rate constant ($\times 10^{-3}$ min^{-1})*
323	0.9878	0.89	779.4	-
333	0.9953	2.16	321.1	-
343	0.9967	4.21	164.8	5.027
353	0.9970	6.26	110.6	8.522
363	0.9989	14.61	47.4	18.60

*From Ref. [6]

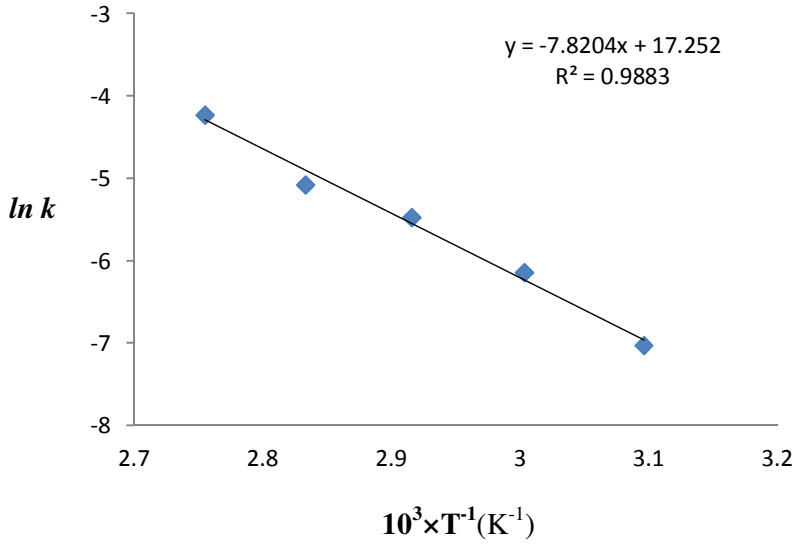


Fig. 5. The plot of the logarithm of the rate constants (*ln k*) *vs.* the reciprocal of absolute temperature (T^{-1}, K^{-1})

4. CONCLUSIONS

This study shows the applicability of electrochemical techniques as simple, accurate and selective methods for the hydrolysis studies of electroactive pharmaceuticals such as paracetamol and the determination of the rate constants of these reactions. Compared with separation techniques such as capillary electrophoresis and liquid chromatography, the proposed method is simpler and economical. The proposed method has wide linear ranges for the determination of remained PAR and produced PAP in hydrolyzed solution with improved

limits of detection. The obtained reaction rates are in the range of 0.89×10^{-4} min^{-1} for 50 ˚C to 1.461×10^{-4} min^{-1} for 90 ˚C and are in good agreement with the values reported in literature.

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