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Determination of the rate constants and activation energy of acetaminophen hydrolysis by capillary electrophoresis

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Abstract

A method based on capillary electrophoresis with electrochemical detection (CE-ED) was developed for the simultaneous determination of *p*-aminophenol and acetaminophen in the hydrolysates of acetaminophen. Effects of several important factors such as the acidity and concentration of running buffer, separation voltage, injection time, and working potential were investigated to acquire the optimum conditions. The detection electrode was a 300 μm carbon disc electrode at a working potential of +0.80 V (versus SCE). The two analytes can be well separated within 6 min in a 50 cm length fused silica capillary at a separation voltage of 18 kV in a 25 mM phosphate buffer (pH 6.5). The rate constants of acetaminophen hydrolysis in 0.5 M HCl at different temperatures were determined by monitoring the concentration changes of acetaminophen. At 70, 80, 90 and 100 °C, the measured rate constants of acetaminophen hydrolysis were 5.027×10^{-3}, 8.522×10^{-3}, 18.60×10^{-3} and 32.76×10^{-3} min^{-1}, respectively. The activation energy for acetaminophen hydrolysis was calculated to be 68.13 kJ mol^{-1}, which is in good agreement with the value in the literature.

Keywords: Capillary electrophoresis; *p*-Aminophenol; Acetaminophen; Hydrolysis; Rate constant; Activation energy

1. Introduction

As an important clinical therapeutical drug, acetaminophen is a commonly used analgesic and antipyretic. It has been widely used to treat headache, arthralgia, chronic pain from cancer, postpartum pain, postoperative pain, and fever. It is the preferred alternative analgesic–antipyretic to aspirin®, particularly for patients who cannot tolerate aspirin® [1]. However, large doses (more than 15 g as a single dose) of acetaminophen may cause sever hepatic damage, even the death of patients. *p*-Aminophenol is the hydrolytic product of acetaminophen and has high toxicity. It may present in the pharmaceutical preparations of acetaminophen as a synthetic intermediate or a degradation product of acetaminophen [2]. In China, the Public Health Department requires that the content of *p*-aminophenol in acetaminophen for pharmaceutical preparations should be less than 0.005% (w/w) [3]. Hence, to establish a

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simple, economical, and accurate analytical method for the simultaneous determination of *p*-aminophenol and acetaminophen would be useful for the stability investigation of acetaminophen, pharmaceutical analysis, quality control for medicinal manufactures, etc.

To our knowledge, spectrophotometry [3,4], liquid chromatography [5–8], and capillary electrophoresis (CE) [9–14] have been widely employed for the determination of acetaminophen in body fluids and pharmaceutical preparations. Yang et al. [15] has separated *p*-aminophenol and acetaminophen by CE. The running buffer used was a phosphate buffer (pH 7.2). Actually, the two compounds have not been separated on base line because they did not optimize the acidity of the running buffer. In this study, we employed CE with electrochemical detection (CE-ED) for the determination of *p*-aminophenol and acetaminophen. It was demonstrated that the acidity of the phosphate buffer plays an important role in the separation of the two analytes by CE. Excellent resolution can be acquired when the pH values of the phosphate running buffer decrease below 6.5. Mohamed et al. [4] have developed a selective spectrophotometric method for the determination of *p*-aminophenol and acetaminophen. It is based on the reaction of *p*-aminophenol with sodium sulphide in the presence of an oxidant to produce a dye. It can be used in the selective detection of *p*-aminophenol in the presence of acetaminophen. However, *p*-aminophenol and acetaminophen cannot be determined simultaneously. Acetaminophen can be determined by this spectrophotometric method only after it is hydrolyzed to *p*-aminophenol. By comparison, the proposed CE-ED method can determine *p*-aminophenol and acetaminophen simultaneously with the advantages of minimal sample volume requirement, short analysis time, high selectivity, low expense of operation, and high separation efficiency. Under the proposed experimental conditions, the two analytes can be separated on base line within 6 min. ED typically operated in the amperometric mode can be coupled with CE to provide high sensitivity and selectivity for the determination of electroactive substances [16,17]. As *p*-aminophenol and acetaminophen are both phenolic compounds, they are electroactive at carbon electrode. ED is an ideal method for the direct detection of the two analytes after CE separation without derivation.

As an important degradation mode, there is a close relation between the hydrolysis and the stability of acetaminophen. It is necessary to determine the rate constants and activation energy of acetaminophen hydrolysis for evaluating half life, selecting appropriate storage conditions, controlling drug quality, etc. Koshy et al. have investigated the degradation of acetaminophen in aqueous solution at various pH values [18]. They determined the rate constants and activation energy of acetaminophen hydrolysis based on monitoring the acetaminophen content in the hydrolysates by spectrophotometry. Unfortunately, the hydrolysis product, *p*-aminophenol interfered with the determination of acetaminophen severely. So, *p*-aminophenol had to be separated from acetaminophen by ion exchange chromatography before analysis. The analysis procedures employed were rather complicated and time-consuming. In this work, the rate constants and activation energy of acetaminophen hydrolysis have been successfully determined by CE-ED. It is simple, sensitive, efficient, and selective providing an alternative method for the kinetic investigations of acetaminophen hydrolysis.

2. Experimental

2.1. Reagents

Acetaminophen and *p*-aminophenol were both obtained from Shanghai No. 3 Chemical Reagent Factory (Shanghai, China). All aqueous were made up in doubly distilled water (Medical Center of Fudan University, Shanghai, China). Other chemicals were all analytical grade.

Stock solution of acetaminophen (0.1 M) was prepared in doubly distilled water. *p*-Aminophenol was dissolved in 0.05 M HCl aqueous solution to reach the final concentration of 0.01 M, as it is fairly stable in strong acidic medium [18]. Both stock solutions were kept in a 4 °C refrigerator and were stable for at least 1 month. Samples were made by diluting stock solutions with an

appropriate amount of running buffer prior to use. The running buffer used as the separation medium of CE was 25 mM NaH_2PO_4–Na_2HPO_4 buffer (PB, pH 6.5), unless indicated otherwise.

2.2. Apparatus

The hydrolysis was carried out in a 501 constant temperature water bath (Shanghai Experiment Instrument Factory, Shanghai, China) with an immersion heater, a circulator, a regulator, and a thermometer calibrated to 0.1 °C. A laboratory-built CE-ED system used for analysis has been described previously [16,17]. Briefly, a ± 30 kV high-voltage dc power supply (Shanghai Institute of Nuclear Research, Shanghai, China) provided a separation voltage between the ends of the capillary. The inlet end of the capillary was held at a positive potential and the outlet end of capillary was maintained at ground. The separations proceeded in a 50 cm length of 25 μm i.d. and 360 μm o.d. fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA).

A three-electrode electrochemical cell consisting of a 300 μm diameter carbon disc working electrode, a platinum auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode, was used in combination with a BAS LC-4C amperometric detector (Bioanalytical Systems Inc., West Lafayette, IN, USA). The filter of the detector was set at 0.1 Hz. The working electrode was positioned carefully opposite the outlet of the capillary with the aid of a micro-manipulator (CORRECT, Tokyo, Japan) and arranged in a wall-jet configuration [19]. The distance between the tip of the working electrode and the capillary outlet was as close as possible so that the CE effluent directly impinged upon the electrode surface. The electropherograms were recorded using a LKB·REC 1 chart record (Pharmacia, Sweden). An YS 38-1000 220V alternate constant-voltage power supply (Shanghai Instrumental Transformer Factory, Shanghai, China) was employed to suppress the voltage fluctuation of the power line. The whole system was assembled in a 10 m^2 Faraday room that was air-conditioned at 20 °C in order to minimize the effects of external noise sources.

2.3. Analysis procedures

Before use, the carbon disc electrode was successively polished with emery paper and alumina powder, and then sonicated in doubly distilled water. CE was performed at the separation voltage of 18 kV. The potential applied to the working electrode was $+0.8$ V (versus SCE). Samples were injected electrokinetically at 18 kV for 6 s. Moreover, sample solutions, standard solutions, and the running buffer were all filtered through a syringe cellulose acetate filter (0.22 μm) prior to their use.

2.4. Hydrolysis procedures

The electric constant temperature water bath was adjusted to the needed temperatures at which the hydrolysis was carried out (70, 80, 90 and 100 °C, respectively). Both 0.1 M acetaminophen and 1.0 M HCl aqueous solution were preheated to the same temperature for 10 min in the constant temperature water bath. A 10 ml aliquot of 0.1 M acetaminophen was transfer to a 50 ml round flask that had been preheated at the same water bath. And then, an aliquot of 10 ml of 1.0 M HCl was added and mixed by shaking the flask. The time when half HCl solution was added was the initial time of hydrolysis. An accurate volume of 1.0 ml of hydrolysates was taken out and transferred to a 100 ml volumetric flask at the hydrolysis times of 5, 15, 30, 50 and 75 min, respectively. An aliquot of 0.5 ml of 1.0 M NaOH was subsequently added to the sample solution to terminate the hydrolysis reaction. Finally, the mixture solutions were diluted to 100 ml with the running buffer for analysis.

3. Results and discussion

3.1. Effect of the potentials applied to the working electrode

Fig. 1A illustrates the cyclic voltammograms of *p*-aminophenol and acetaminophen at the carbon

disc electrode used. It is apparent that both analytes can be oxidized electrochemically at moderate potentials. Therefore, electrochemical detection was employed for the detection of the two analytes in this work. Hydrodynamic voltammograms (HDVs) of *p*-aminophenol and acetaminophen are shown in Fig. 1B. When the applied potential exceeds +0.20 V (versus SCE) for *p*-aminophenol and +0.50 V (versus SCE) for acetaminophen, the peak current of both analytes increases rapidly. However, the current response levels off when the potential passes above +0.60 V (versus SCE) for *p*-aminophenol and +0.80 V (versus SCE) for acetaminophen, respectively. Although an applied potential greater than +0.80 V (versus SCE) results in higher peak currents, both the baseline noise and the background current increase substantially, resulting in an unstable baseline. The applied potential of the working electrode was, therefore, maintained at +0.80 V (versus SCE), under which condition the background current was not too high and the S/N ratio was the highest. At the optimum potential, the working electrodes showed good stability and the reproducibility was high.

3.2. Effects of the pH and concentration of the running buffer

The acidity of the running buffer affect the zeta potential, and the electroosmotic flow (EOF), as well as the overall charge of the analytes, which determine the migration time and the separation of the analytes [20]. The electropherograms of *p*-aminophenol and acetaminophen in 25 mM PBs at pH values of 6.0, 6.5, 7.0, and 7.6 were exhibited in Fig. 2. The resolution of the two analytes is poor at pH 7.0 and 7.6. When the running buffer pH value decreased below 6.5, the resolution was improved dramatically. Fig. 3A shows the effect of the running buffer concentration on the migration time of the analytes. It can be seen that the migration time and the resolution increases with increasing buffer concentration. However, higher buffer concentrations (>25 mM) also have a negative effect on the detection limits because the peak currents of both analytes decrease and the effect of Joule heat becomes more pronounced. So 25 mM PB (pH 6.5) is chosen as the running buffer in this work in considering the peak current, resolution, analysis time, and buffer capacity.

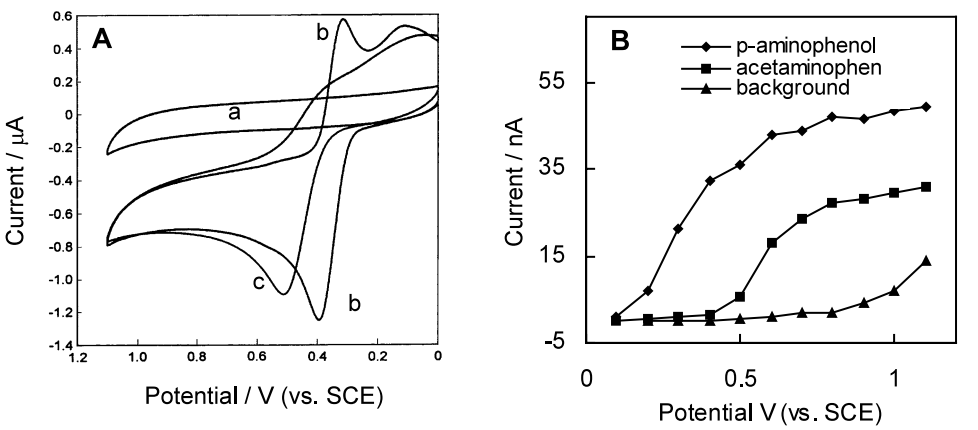

Fig. 1. (A) Cyclic voltammograms for *p*-aminophenol and acetaminophen at a 300 μm diameter carbon disc electrode in 25 mM NaH_2PO_4–Na_2HPO_4 buffer (pH 6.5). Reference electrode, SCE; auxiliary electrode, platinum wire. (a) Blank solution; (b) 1.0×10^{-3} M *p*-aminophenol; (c) 1.0×10^{-3} M acetaminophen. Scan rate, 100 mV s^{-1}. (B) Hydrodynamic voltammograms (HDVs) for 5.0×10^{-4} M of *p*-aminophenol and acetaminophen in capillary electrophoresis. Fused-silica capillary, 25 μm i.d. × 50 cm; the three-electrode system and the running buffer are the same as (A); separation voltage, 18 kV; electrokinetic injection, 6 s (at 18 kV).

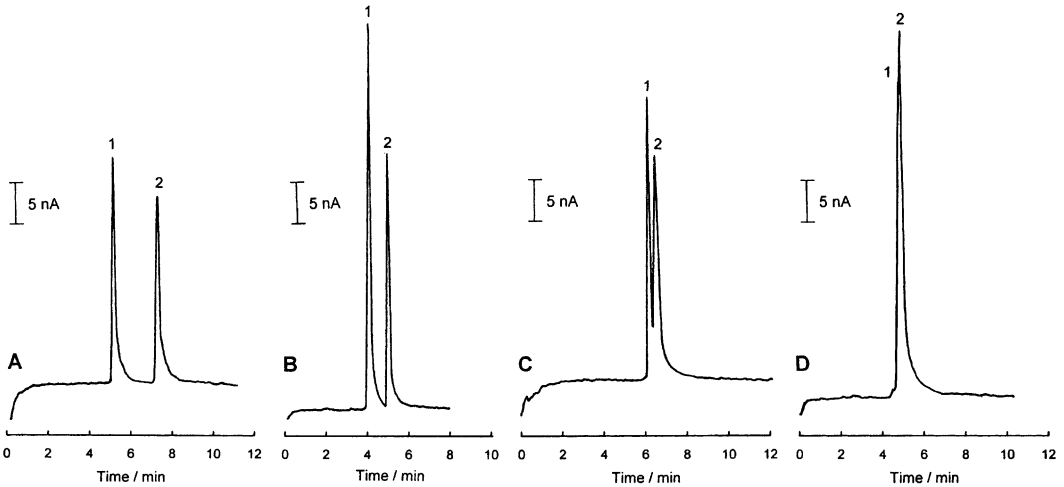

Fig. 2. Typical electropherograms for 5.0×10^{-4} M of *p*-aminophenol and acetaminophen in 25 mM NaH_2PO_4–Na_2HPO_4 buffer at pH of (A) 6.0, (B) 6.5, (C) 7.0, and (D) 7.6. Working potential, +0.8 V (versus SCE); other conditions as in Fig. 1B.

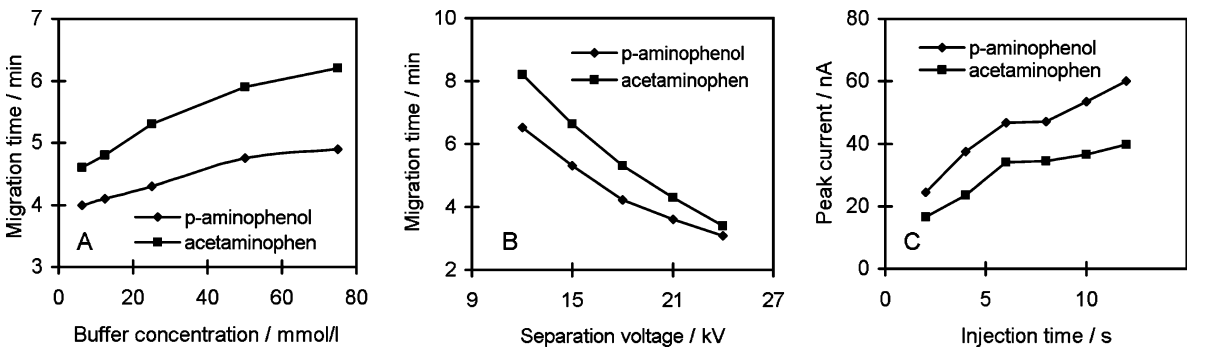

Fig. 3. Effect of (A) concentration of running buffer, (B) separation voltage on the migration time, and (C) effect of injection time on the peak current of the analytes. Working potential, +0.8 V (versus SCE); other conditions as in Fig. 1B.

3.3. Effect of separation voltage and injection time

The influence of separation voltage on the migration time of the analytes is exhibited in Fig. 3B. Increasing the voltage gives shorter migration time for both analytes, but also increases the baseline noise, resulting in poorer detection limits. It is found that higher separation voltages are not beneficial to the resolution. Moreover, higher separation voltages may result in higher Joule heat that directly affects the separation efficiency of this method. However, too low separation voltages will increase the analysis time considerably, which in turn cause peak broadening. Based on experiments, 18 kV was chosen as the optimum voltage to accomplish a good compromise.

The effect of injection time on CE separation was investigated by changing the sampling time (2, 4, 6, 8, 10, and 12 s at a voltage of 18 kV, as shown in Fig. 3C). It was found that both peak current and peak width increase with increasing sampling time. When injection time exceeds 6 s, the peak current increases slowly and peak broadening becomes more severe. In this experiment, 6 s (at 18 kV) is selected as the optimum injection time in considering resolution and sensitivity.

Through the experiments above, the optimum conditions for determining *p*-aminophenol and acetaminophen are acquired. The typical electropherogram for a standard mixture solution is shown in Fig. 2B. Baseline separation for both analytes can be achieved within 6 min.

3.4. Reproducibility, linearity and detection limits

A standard mixture solution of 5.0×10^{-4} M of *p*-aminophenol and acetaminophen were analyzed seven times to determine the reproducibility of the peak current and migration time of both analytes under the optimum conditions. The relative standard deviations (RSDs) of peak current and migration time are 2.85 and 0.79% for *p*-aminophenol, 1.65 and 1.33% for acetaminophen, respectively.

A series of the standard mixture solutions of *p*-aminophenol and acetaminophen with concentrations ranging from 1.0×10^{-6} M to 2.0×10^{-3} M were tested to determine the linearity for both analytes at the carbon disc electrode used in this method. The results of regression analysis on calibration curves and detection limits are presented in Table 1. The determination limits are evaluated on the basis of a single-to-noise ratio of 3. The calibration curves exhibit satisfactory linear behavior over the concentration range of about three orders of magnitude with satisfactory detection limits.

3.5. Kinetics equations

Acetaminophen can be hydrolyzed to produce *p*-aminophenol and acetic acid in acidic aqueous solution [18]. As the concentration of water can be considered to be constant during the hydrolysis, the hydrolysis of acetaminophen is first-order reaction. So, the hydrolysis rate is proportional to the instantaneous concentration of acetaminophen, so that

$$-\mathrm{d}c/\mathrm{d}t = kc \tag{1}$$

Separating the variables and integrating, the following equation can be obtained.

$$\ln c = -kt + \ln c_0 \tag{2}$$

where c_0 is the initial concentration of acetaminophen, c is the concentration of acetaminophen at the hydrolysis time t and can be determined by CE-ED, and k is the rate constant of hydrolysis. If $\ln c$ is plotted against t, a straight line should be obtained if the reaction kinetics is first-order. The slope of the line is the first-order rate constant. According to Arrhenius equation [21], the relationships between k and absolute temperature T can be expressed as follows,

$$k = Ae^{-E/(RT)} \tag{3}$$

The parameter E is activation energy of the reaction, R is the mole gas constant (8.314 J K^{-1} mol^{-1}), and A is called frequency factor. Eq. (3) can also be written as

$$\ln k = -E/(RT) + \ln A \tag{4}$$

A plot of the logarithm of the rate constant $\ln k$ versus the reciprocal of the absolute temperature T^{-1}, should be a straight line provided that the influence of T on E is not too large. The slope of the line is $-E/R$, and the intercept of the line extrapolated to $T^{-1} = 0$ gives $\ln A$.

Table 1
The results of regression analysis on calibration curves and the detection limits[a]

Compound	Regression equation $y = a + bx$[b]	Correlation coefficient	Linear range ($\times 10^{-3}$ M)	Detection limit[c] ($\times 10^{-6}$ M)
p-Aminophenol	$y = 0.6287 + 91.712x$	0.9998	0.0025–2.0	1.09
Acetaminophen	$y = 0.3997 + 59.098x$	0.9996	0.0025–2.0	1.69

[a] Working potential is +0.8 V (versus SCE). Other CE-ED conditions are the same as in Fig. 1B.
[b] Where the y and x are the peak current (nA) and concentration of the analytes (mM), respectively.
[c] The detection limits corresponding to concentrations giving signal to noise ratio of 3.

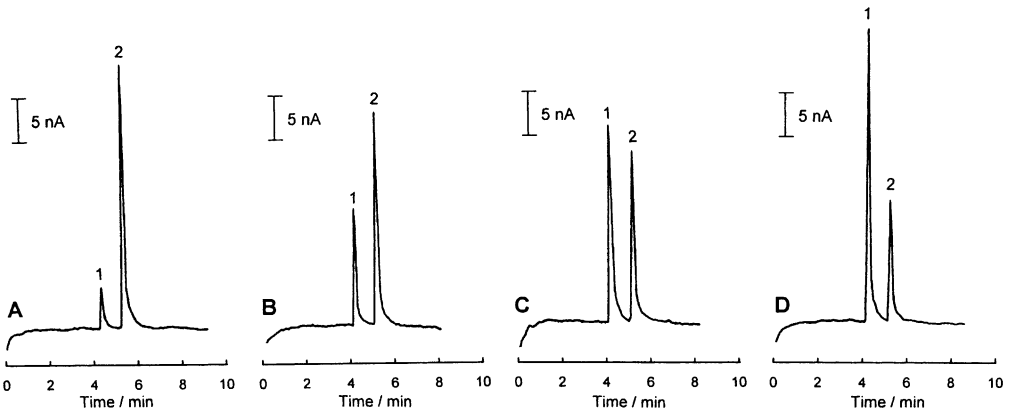

Fig. 4. Electropherograms for the diluted hydrolysates of acetaminophen (hydrolyzed at 90 °C) in the running buffer at a ratio of 100 (1:100) when the hydrolysis times were (A) 5, (B) 15, (C) 30 and (D) 50 min, respectively. Working potential, + 0.8 V (versus SCE); other conditions as in Fig. 1B.

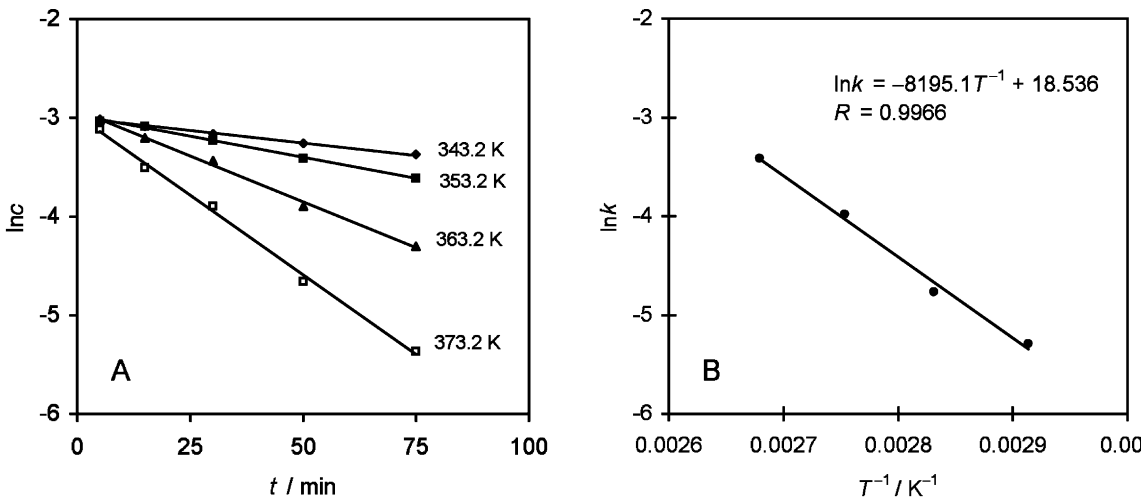

Fig. 5. (A) Plots of the logarithm of acetaminophen concentration ($\ln c$) versus time (t) for the hydrolysis of acetaminophen at various temperatures (70, 80, 90, and 100 °C). (B) A plot of the logarithm of the rate constant ($\ln k$) against the reciprocal of the absolute temperature (T^{-1}) showing the temperature dependence of the rate constant of acetaminophen hydrolysis.

3.6. Determination of the rate constants for acetaminophen hydrolysis

Fig. 4 shows the typical electropherograms of the diluted hydrolysates of acetaminophen (hydrolyzed at 90 °C) in the running buffer at a ratio of 100 (1:100) when the hydrolysis times were 5, 15, 30 and 50 min, respectively. In this study, the acetaminophen content in its hydrolysates was all determined by CE-ED.

Fig. 5A shows the relationships between the logarithm of acetaminophen concentration $\ln c$ and hydrolysis time t. It can be concluded that the hydrolysis of acetaminophen in 0.5 M HCl (at 70, 80, 90 and 100 °C, respectively) was first-order since straight line relationships were obtained when $\ln c$ was plotted as a function of t. The regression equations for $\ln c$–t, correlation coefficients, rate constants, and half lives at the four temperatures were presented in Table 2. The

Table 2
Influence of temperature on the rate constant and half life of acetaminophen hydrolysis

Temperature (K)	Regression equation $\ln c = \ln c_0 - kt$	Correlation coefficient	Rate constant ($\times 10^{-3}$ min^{-1})	Half life (min)
343.2	$\ln c = -3.003 - 5.027 \times 10^{-3} t$	0.9976	5.027	137.88
353.2	$\ln c = -2.976 - 8.522 \times 10^{-3} t$	0.9987	8.522	81.340
363.2	$\ln c = -2.922 - 18.60 \times 10^{-3} t$	0.9981	18.60	37.266
373.2	$\ln c = -2.979 - 32.76 \times 10^{-3} t$	0.9985	32.76	21.158

Arrhenius plot of $\ln k$ against T^{-1} was found to give a straight line (Fig. 5B). The regression equations for $\ln k - T^{-1}$ is $\ln k = -8195.1 T^{-1} + 18.536$ with the correlation coefficient of 0.9966. The slope of the straight line is -8195.1 K, so that, the activation energy $E = 8.314$ (J K^{-1} mol^{-1}) $\times$ 8195.1 (K) = 68.13 kJ mol^{-1}, which is in good agreement with the value reported for the hydrolysis of acetaminophen in aqueous solution at pH value of 2 ($E = 16.69$ kcal mol^{-1} = 69.83 kJ mol^{-1}) [18]. The intercept is at $\ln A = 18.536$, so that $A = 1.122 \times 10^8$ min^{-1}. For the hydrolysis reaction of acetaminophen in 0.5 M HCl, therefore, Arrhenius equation (3) is $k = 1.122 \times 10^8 e^{-8195.1/T}$ min^{-1}. The fact that the data in Fig. 5B fall on a straight line implies that E is almost a constant which is independent of T in the investigated temperature range.

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