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Journal of Chromatography A, 1098 (2005) 194–198
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

Determination of the hydrolysis rate constants and activation energy of aesculin with capillary electrophoresis end-column amperometric detection

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Received 25 June 2005; received in revised form 28 September 2005; accepted 11 October 2005
Available online 8 November 2005

Abstract

Aesculetin is the product of the hydrolysis reaction of aesculin. A high sensitivity and good repeatability method based on capillary electrophoresis with amperometric detection (CE-AD) was developed for simultaneous determination of aesculin and aesculetin in the hydrolysate of aesculin. Under the optimum condition: 10 mmol/L KH_2PO_4–5 mmol/L $Na_2B_4O_7$ (pH 6.0) buffer, separation at 18 kV and +900 mV (versus Ag/AgCl) as the detection potential, the hydrolysis rate constants of aesculin hydrolysis at 25, 30, 35, 40 and 45 °C in 0.1 mol/L KOH were obtained as 1.45×10^{-2} min^{-1}, 2.01×10^{-2} min^{-1}, 2.93×10^{-2} min^{-1}, 3.76×10^{-2} min^{-1} and 5.05×10^{-2} min^{-1}, respectively. It was calculated that the activation energy for aesculin hydrolysis was 49.4 kJ/mol.

Keywords: Capillary electrophoresis; Amperometric detection; Hydrolysis rate constant; Activation energy; Aesculin; Aesculetin

1. Introduction

Cortex Fraxini is a kind of Chinese herb medicines that comes from the dry tegument of different categories of Fraxinus chinensis Roxb. It has the therapeutic function of cleaning, purging, heating, and drying dampness, nourishing the liver and improving vision [1,2]. It is also effective in the treatment of diarrhoea, cough and some gynecopathy. Furthermore, some data indicate that Cortex Fraxini has some impact on anti-cancer [3]. Aesculin and aesculetin are the main effective components of Cortex Fraxini. As reported in the textbook [4], glycoside from the crude drug tends to hydrolyze and produce aglycone. It can be judged from the structure of aesculin (shown in Section 3.1, as the equation of hydrolysis reaction) that it is a kind of glycoside, which is easy to hydrolyze producing aesculetin and glucose. Moreover, some related investigations indicated that with the increasing of the storage time of Cortex Fraxini in a damp environment, the amount of aesculin will decrease and the amount of aesculetin will increase [5]. It is described in the Pharmacopoeia of the People's Republic of China that its curative effect decreases on consumption when the content of aesculin in Cortex Fraxini was less than 1.36% [1]. Therefore, development of a simple, economical and reliable method to examine the concentration change of aesculin during the process of hydrolysis and storage, and further to obtain its kinetic parameter is very significant for the evaluation for the quality and stability of Cortex Fraxini.

As an important separation technology, capillary electrophoresis (CE) offers the advantage of minimal sample volume requirement, short analysis time and high separation efficiency, and has been a powerful tool for the drug analysis such as determination of the main component, estimation of impurities, and the separation of the chiral substance [6–12]. In addition, it was also widely used in the field of analysis of some Chinese herbal medicine [13–16]. However, the application of CE in the determination of physicochemical constant such as rate constants is very poor. The physicochemical constant is a very important property in the pharmaceutical industry [17] and phytochemistry research [18], and the rate constant is a particularly important physicochemical parameter in the study of the degradation of

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doi:10.1016/j.chroma.2005.10.029

the medicament. In the main degradation mode, for the medicament there has to be a close relation between the hydrolysis rate constant and the pharmacology of drug. However, for Chinese herb medicines, the study of the physicochemical parameter is so scarce that the research of the medicine function mechanism cannot be exhausted. The determination of hydrolysis rate constant and activation energy of Chinese herbal medicine can be done by searching pharmacology and controlling the drug quality. To our knowledge, ultraviolet (UV) spectrophotometry [19,20] is a common technique to employ for the determination of the rate constant. It is used for following a reaction in solution to monitor the changes in ultraviolet absorbance of either a reactant or a product with time. This methodology is quite complicated and the limitations are also obvious. Moreover, through the UV method it is not possible to choose an appropriate wavelength for the simultaneous determination of the reactant and its products, so it cannot be used for observing the change in the concentration of these compounds in the hydrolysis process directly. The limitation in the sensitivity of the UV method is another problem, and some substances do not even absorb UV. CE with different detectors cannot only be most effective in proving high resolution but also has more extensive analytes. The most important function of CE is that the change in concentration of both the reactant and the products with time can be directly monitored during the hydrolysis reaction. Whereas, CE method has hardly been used to study the hydrolysis of medicament, only Chen et al. had used CE-AD to study the hydrolysis of acetaminophen [21]. Although, none of the research was done to study the hydrolysis rate constant of Chinese herb medicines.

Based on the fact that both aesculin and aesculetin were electroactive compounds, a sensitive, selective and low-cost capillary electrophoresis system coupled with end-column amperometric detection was used to determine the hydrolysis kinetic parameter of aesculin in this paper. Under optimum conditions, aesculin, and its hydrolysate aesculetin, could be completely separated within 8 min, and the experimental results indicated that this method was simple, intuitive and efficient for the determination of the hydrolysis rate constant and activation energy.

2. Experimental

2.1. Chemicals

Aesculin and aesculetin were obtained from the Chinese Institute of Biological Products Control (Beijing, China). All chemicals were of analytical reagent grade. All solutions were freshly prepared with doubly distilled water and passed through a 0.22 μm membrane filter before use.

Stock solution of aesculin (3.5 mmol/L) and aesculetin (3.9 mmol/L) were prepared by using 5% (v/v) methanol–buffer solution (10 mmol/L KH_2PO_4–5 mmol/L $Na_2B_4O_7$ pH 6.0) mixture and diluted to the desired concentration with the running buffer just prior to use. Both solutions were kept in a refrigerator at 4 °C and were stable for at least 2 months.

Buffer solution was prepared from a mixture of 0.1 mol/L KH_2PO_4 and 0.05 mol/L $Na_2B_4O_7$. Then the desired concentrations of buffers were obtained by diluting with water. The pH values of buffers were adjusted precisely by 0.1 mol/L H_3PO_4 or 0.1 mol/L KOH. The running buffer used for electrophoresis was 10 mmol/L KH_2PO_4–5 mmol/L $Na_2B_4O_7$ (pH 6.0) buffer unless indicated otherwise.

2.2. Apparatus

In this work, a laboratory-built CE-AD system has been constructed for analysis [22]. A ±30 kV high-voltage dc power supply (Shanghai Institute of Nuclear Research, Shanghai, China) provided the separation voltage between the ends of capillary. A 30 cm length of 25 μm i.d., 360 o.d. μm uncoated fused-silica capillary was used (Yongnian Optical Fiber Factory, Hebei, China). The capillary had been flushed with 0.1 mol/L sodium hydroxide solution for 4 h before use, then rinsed with 0.1 mol/L HCl and doubly distilled water for 10 min each. Between each run, the capillary was rinsed with 0.05 mol/L sodium hydroxide solution, water and running buffer for 2 min, respectively.

A three-electrode electrochemical cell consisting of a 300 μm diameter carbon disc working electrode, a platinum auxiliary electrode, and an Ag/AgCl (saturated KCl) electrode as reference electrode, was connected to a BAS LC-4C amperometric detector (Bioanalytical Systems Inc., West Lafayette, IN, USA). The data were recorded by the TL9902 analytical system of chromatogram.

The hydrolysis was carried out in a constant temperature water bath (Medical Treatment Instrument Factory, Jiangsu, China).

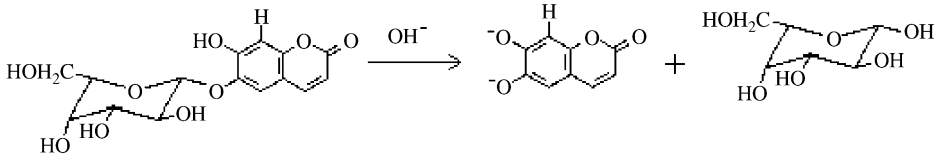
2.3. Hydrolysis procedures

The constant temperature water bath was adjusted to the required temperatures at which the hydrolysis was carried out (25, 30, 35, 40 and 45 °C, respectively). Both tubes with 600 μL of 0.35 mmol/L aesculin solution and the same volume of 0.1 mol/L KOH solution, respectively, were placed into the constant temperature water bath at the stated temperature for 20 min. Then, 600 μL of 0.1 mol/L KOH solution was added to the above-mentioned tube of aesculin mixed by shaking the tube and the time was noted simultaneously. In a certain time interval, an accurate volume of 100 μL of hydrolysates was transferred to another tube, and then 100 μL of 0.1 mol/L of H_3PO_4 solution was added in order to terminate the hydrolysis reaction. Finally, the solution was determined by CE-AD, and every determination had been repeated for at least three times. The hydrolysis times ranged from 1 to 90 min.

3. Results and discussion

3.1. Kinetic equations

As was been mentioned in Section 1, aesculin can hydrolyze to produce aesculetin and glucuronic acid in alkaline aqueous solution.



As water is superfluous during the hydrolysis, its concentration can be considered to be constant in the reaction. So, the hydrolysis of aesculin is a first-order reaction and the kinetic equation is as follows:

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

The equation also can be expressed as,

$$\ln C = -kt + \ln C_0 \quad (2)$$

where C_0 is the initial concentration of aesculin, C the concentration of aesculin at a hydrolysis time t which can be determined by CE-AD. If the kinetic reaction is of first-order, when $\ln C$ is plotted against t, and a straight line can be obtained. The slope of the line is the first-order rate constant k.

As demonstrated in Arrhenius equation, the relationship between k and the absolute temperature of the reaction T can be described as follows:

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

where A is the apparent frequency factor, E the activation energy of the reaction and R is the mole gas constant ($8.314\,JK^{-1}\,mol^{-1}$).

Eq. (3) can also be expressed as,

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

When $\ln k$ is plotted to T^{-1}, a straight line (slope = $-E/R$) is obtained. So the activation energy E can be calculated according to the slope.

3.2. The optimum conditions for electrophoresis

To determine the optimum conditions for the separation of aesculin and aesculetin, the composition of the buffers and the voltage were varied to optimize the conditions for the separation.

In this study, some kinds of single buffers were firstly considered, but poor Rs were obtained. Then different mixture buffers were tested and satisfying separation and good sensitivity could be obtained with the KH_2PO_4–$Na_2B_4O_7$ buffer solution.

The pH of the buffer was studied in the pH range of 5.8–6.8. When the pH of the buffer was 6.0, the value of Rs between the two analytes was the best and good response current was obtained at the same time. Furthermore, buffers with five different concentrations were tried out respectively. When the concentration of the mixture buffers was 10 mmol/L KH_2PO_4–5 mmol/L $Na_2B_4O_7$ (pH 6.0), it can obtain good Rs and short migration time.

Increasing the voltage would give shorter migration time for all compounds, but it also increase the baseline noise, resulting in poorer detection limits. In our experiment, when the voltage was less than 18 kV the current in the capillary was only 4–6 μA. But when it was greater than 20 kV the current would increase to 22 μA, and this may result in a more prominent effect of Joule heat. So 18 kV was chosen as the working voltage.

3.3. Amperometric detection

As demonstrated in the literature [9], aesculin and aesculetin could easily be oxidized at the carbon disc electrode. The cyclic voltammograms [9] of aesculin and aesculetin indicate that the oxidation is irreversible at the potential of +0.85 and +0.50 V (versus Ag/AgCl), respectively. The oxidation peaks are probably caused by the oxidation of the phenolic hydroxyl groups. Because one of the hydrogen atoms of the hydroxyl group of aesculetin is substituted for glucose, aesculin has a larger overpotential than aesculetin.

For CE-AD method, the potential applied to the working electrode directly affects the sensitivity and detection limit. Fig. 1 shows the hydrodynamic voltammograms of two analytes. From the figure, it can be seen that the peak current of two analytes increased with potential accreted. But when the potential was higher than 900 mV, the basic current rose sharply. The experiment indicated that under the condition of 900 mV, high response current could be obtained; so 900 mV was selected as the optimal potential.

The typical electropherogram of a standard mixture solution of aesculin (4.0×10^{-5} mol/L) and aesculetin (1.2×10^{-4} mol/L) was obtained under the above-mentioned optimum conditions, and as Fig. 2 illustrates, two analytes are completely separated within 8 min.

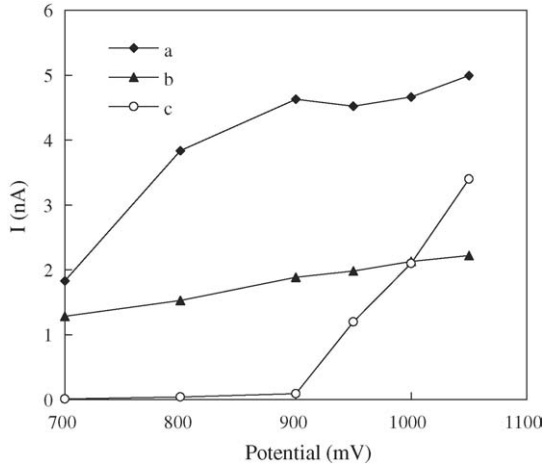


Fig. 1. The hydrodynamic voltammograms of aesculin and aesculetin (a) aesculin; (b) aesculetin; (c) basic current. Working electrode: 0.3 mm carbon disc electrode; fused-silica capillary: 30 cm × 25 μm; injections: 15 kV × 10 s; separation voltage: 18 kV; running buffer: 10 mmol/L KH_2PO_4–5 mmol/L $Na_2B_4O_7$ (pH 6.0); concentration of aesculin and aesculetin: 6.7×10^{-5} mol/L and 2.0×10^{-4} mol/L.

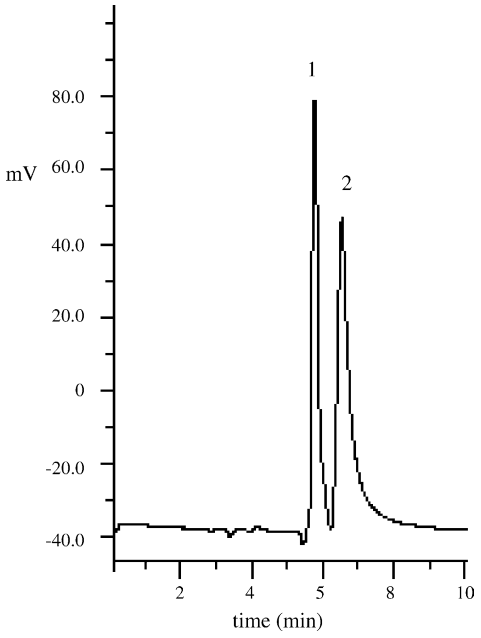


Fig. 2. The electropherograms of standard mixture (1) aesculin (4.0×10^{-5} mol/L) and (2) aesculetin (1.2×10^{-4} mol/L). Working electrode: 0.3 mm carbon disc electrode +900 mV (vs. Ag/AgCl); other conditions were the same as Fig. 1.

Table 2
Determination results of the recovery for this method ($n = 3$)[a]

Compound	Added (μmol/L)	Found (μmol/L)	Recovery (%)	R.S.D (%)
Aesculin	6.0	6.5	108	3.3
	12.0	13.6	113	2.6
Aesculetin	10.8	11.5	106	2.7
	21.6	24.0	111	2.3

[a] Conditions same as in Fig. 2.

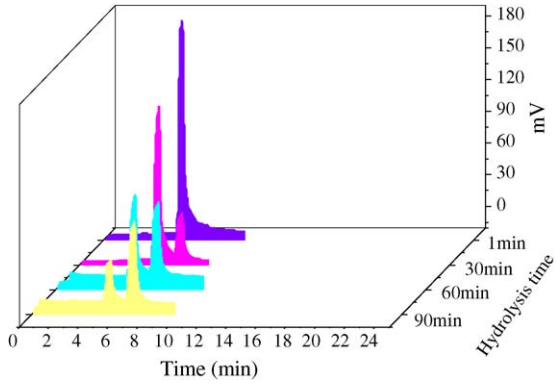


Fig. 3. Electrophorogram showing the hydrolysis of aesculin. The conditions were the same as in Fig. 2.

3.4. Detection limit, linearity and repeatability

Under the above-mentioned optimum conditions, a series of different concentrations of the standard mixture solutions of aesculin and aesculetin (110, 80, 60, 40, 20, 0.16 μmol/L for aesculin and 390, 236, 177, 118, 59, 0.46 μmol/L for aesculetin, respectively.) were analyzed to determine the linearity between the analytical concentration and current response. To check out the precision, every concentration was determined three times, respectively. Following the IUPAC recommendations (LOD = $3\delta_b/s$, where "δ_b" is the relative standard deviation (RSD) of the blank, "s" is the slope of the regression equation), low detection limits of 0.19 and 0.05 μmol/L were obtained for aesculin and aesculetin, respectively. The results of the regression equation, linear and detection limits are shown in Table 1. The calibration curves exhibited an excellent linear behavior ($R^2 = 0.9998$, 0.9979 for aesculin and aesculetin, respectively) over the concentration range, which were achieved to about three orders of magnitude.

The repeatability of the peak current and migration time of aesculin and aesculetin were tested by repeated ($n = 5$) injection of a standard mixture solution. The RSD of aesculin and aesculetin were obtained as 4.5 and 2.0% for peak current, 0.51 and 0.62% for migration time. It demonstrated that this method was precise.

Furthermore, ensure accuracy of the method, the recovery of a spiked sample was determined. A standard mixture solution of 2.6×10^{-5} and 4.7×10^{-5} mol/L of aesculin and aesculetin, respectively, was selected as the spiked sample. Then two different concentrations of standard mixture solution were added to the spiked sample, and the obtained recoveries of aesculin and aesculetin were shown in Table 2.

3.5. Determination of the hydrolysis rate constants of aesculin

According to Section 2.3, the hydrolysis of aesculin was carried out in a constant temperature water bath (25 °C). Fig. 3 shows the typical electropherograms of aesculin and the hydrolysate aesculetin when the hydrolysis times were 1, 30, 60, and 90 min, respectively. The change of the peak current of peaks 1 and 2 demonstrates the change of concentrations of aesculin and aesculetin during the process of hydrolysis. It can be seen from Fig. 3 that the peak current of aesculin decreased gradually

Table 1
The regression equations and the detection limits[a]

Compounds	Regression equation ($Y = a + bX$[b])	Correlation coefficient	Linear range (μmol/L)	Detection limits[c] (μmol/L)
Aesculin	$Y = -0.0395 + 15.78(X)$	0.9998	0.16–110	0.19
Aesculetin	$Y = -0.0962 + 3.709(X)$	0.9979	0.46–390	0.05

[a] Conditions are the same as in Fig. 2.
[b] Where Y is the peak current (in 1 nA) and X is the compound concentration (in μmol/L).
[c] The detection limits were calculated according to IUPAC recommendations (LOD = $3\delta_b$/s).

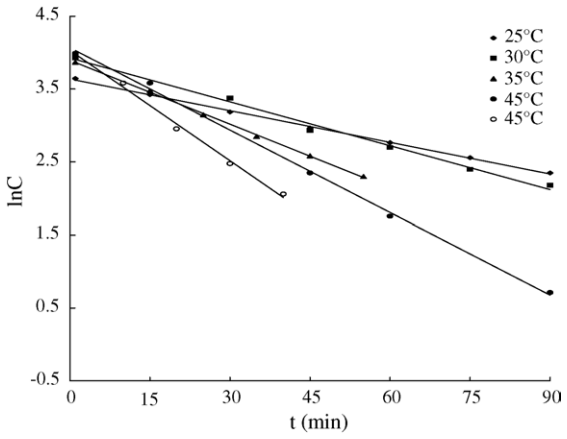


Fig. 4. Relationship between ln C and t for aesculin hydrolysis reaction at various temperatures.

with the hydrolysis time, while the peak current of aesculetin was increased at the same time. The hydrolysis of aesculin at 30, 35, 40 and 45 °C was then carried out in the same way. The experiments indicated that the hydrolysis velocity increased quickly with augmentation of temperature. At 25 °C the hydrolysis had to be finished about at 3 h, when temperature increased to 45 °C the hydrolysis time reduced as little as 1 h.

3.6. Determination of the activation energy of aesculin

On the basis of Eq. (2), when ln C was plotted against t, a straight line was obtained and the slope of the line was the hydrolysis rate constants $-k$. Fig. 4 shows the relationship between ln C and t for aesculin hydrolysis reaction at various temperatures (at 25, 30, 35, 40 and 45 °C), and it exhibits a satisfactory linearity. The correlation coefficients and rate constants at the five temperatures are presented in Table 3. Then, the regression equation of ln k versus $1/T$ is shown as follows:

$$\ln k = \frac{-5944.4}{T} + 15.719$$

The slope of the line is −5944.4 K, so the activation energy is 49.4 kJ mol^{-1}. With the correlation coefficient of 0.9991, the good linearity between k and absolute temperature T is approximately in accordance with the Van't Hoff Rule.

Table 3
Influence of temperature on rate constant of aesculin hydrolysis[a]

Temperature (K)	Regression equation	Correlation coefficient	Rate constant (10^{-2}/min^{-1})
298	ln C = −0.0145 t + 3.6374	0.9994	1.45
303	ln C = −0.0201 t + 3.927	0.9961	2.01
308	ln C = −0.0293 t + 3.8946	0.9996	2.93
313	ln C = −0.0376 t + 4.0656	0.9991	3.76
318	ln C = −0.0505 t + 4.0296	0.9976	5.05

[a] Conditions were the same as in Fig. 2.

As demonstrated in the literature [23], the activation energy of this type of chemical reaction should be in the range of 40–60 kJ/mol. The good straight line illustrates that E is approximately a constant, which is independent of T in the tested temperature range.

4. Conclusions

This study shows the applicability of capillary electrophoresis with amperometric detection to determine the hydrolysis rate constant of aesculin. Compared with the traditional measurements of physicochemical constant, this proposed method is simpler, more rapid and economical. Furthermore, the method also promises to be used for the study of physicochemical constant for Chinese herb medicines and related substances.

Acknowledgements

This project was financially supported by the National Nature Sciences Funding of China (20377007), the International Corporation Program of Science and Technology Department of Fujian Province, China (2004I015) and the Key Program of Science and Technology Department of Fujian Province, China (2004Y003).

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