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Short communication

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

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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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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.