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Modeling of degradation kinetics of Salvianolic acid B at different temperatures and pH values☆

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ABSTRACT

In this work, the effects of degradation time, temperature, and pH value on the degradation of Salvianolic acid B in aqueous solution were determined. Higher pH values, higher extraction temperature, and longer extraction time led to more degradation of Salvianolic acid B. Danshensu concentration increased as Salvianolic acid B degraded. A mechanism model was developed considering the degradation of Salvianolic acid E and lithospermic acid, which were two degradation products of Salvianolic acid B. The reverse reactions of Salvianolic acid B degradation were also considered. Degradation kinetic constants were calibrated. The degradation kinetics of Salvianolic acid B, lithospermic acid, and Danshensu in a *Salvia miltiorrhiza* extract aqueous solution were predicted using the mechanism model. The predicted concentrations agreed well with the experimental results. This model was developed using degradation data obtained from simple composition systems, but it can be applied in a complex botanical mixture with high prediction accuracy.

1. Introduction

Salvia miltiorrhiza, Danshen in Chinese, is a medicinal and edible plant in China. It is the material of healthy foods with many forms in China, such as Sanqi Danshen Capsule, Danshen Juhua Tea, Juqi Danshen Tablet, Danshen Wine, and Shouwu Danshen Granule. Phenolic acids extracted from *Salvia miltiorrhiza*, such as Danshensu and Salvianolic acid B, are usually considered as quality control components of these healthy foods because they possess more activities other than antioxidant activity [1]. For example, Salvianolic acid B possesses antihypertensive effect [2], antifibrotic effect [3], and neuroprotective effect [4], and inhibition effect on HIV-1 replication [5].

However, phenolic acids extracted from *Salvia miltiorrhiza* easily degrade during processing. The degradation of Salvianolic acid B in aqueous solution was investigated by many researchers [6–14]. Danshensu, lithospermic acid, Salvianolic acid A, protocatechuic aldehyde, caffeic acid, Salvianolic acid E, Salvianolic acid D, and many other phenolic compounds are found to be the degradation products of Salvianolic acid B [7–12,15]. Lithospermic acid will degrade and form Danshensu, Salvianolic acid A, protocatechuic aldehyde [11]. Salvianolic acid A is easily oxidized and forms Salvianolic acid C, iso-Salvianolic acid C, and other compounds [13]. For a food supplier, keeping batch-to-batch consistency of products is very important to maintain brand equity. The degradation of phenolic acids during processing makes it difficult to keep batch-to-batch consistency of healthy foods made from *Salvia miltiorrhiza*.

Recently, Quality by design (QbD) concept based on knowledge management and risk management was generally adopted in industry [16]. In the implementation of QbD concept, it is necessary to gain more knowledge on physical and chemical changes during processing [17]. Control strategy aiming at improving batch-to-batch consistency then can be developed based on available knowledge [18]. Because Salvianolic acid B is the most abundant phenolic acid in *Salvia miltiorrhiza*, it is an urgent task to know degradation products and degradation kinetics of Salvianolic acid B.

Published works on degradation kinetics of Salvianolic acid B are much less than those on the identification of degradation products. Till now, only first-order irreversible reaction kinetics model was adopted to quantitatively describe the degradation of Salvianolic acid B [6,10,19]. However, remarkable deviation can be observed when degradation time was long [6]. The predicted Salvianolic acid B concentration value was lower than experimental results, which means that reverse reactions of Salvianolic acid B degradation cannot be neglected. The degradation reactions of Salvianolic acid E and lithospermic acid, which are two of Salvianolic acid B's degradation products, were also reported [6,11]. These reactions also need to be considered in the modeling of degradation kinetics of Salvianolic acid B to accurately calculate the effects of reverse reactions of Salvianolic acid B degradation.

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In this work, degradation kinetics of Salvianolic acid B were determined at different temperatures and pH values. A mechanical model for Salvianolic acid B degradation was built considering the effects of the reverse reactions of Salvianolic acid B degradation, degradation of Salvianolic acid E, and degradation of lithospermic acid. Degradation kinetic constants were fitted. The model was verified using the degradation of a *Salvia miltiorrhiza* extract.

2. Materials and Methods

2.1. Materials and chemicals

Standard substances including Danshensu (>98%), lithospermic acid (>98%), and Salvianolic acid B(>98%) were obtained from Shanghai Winherb Medical Science Co., Ltd. (Shanghai, China). Disodium hydrogen phosphate dodecahydrate (>99.0%) and citric acid monohydrate (>99.5%) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Methyl silicone was purchased from Zhejiang Rongcheng Silicone Material Co., Ltd. (Anji, Zhejiang, China). Ammonium formate (>99%) was purchased from Alfa Aesar (Tianjing, China). HPLC grade phosphoric acid (85%–90%) was obtained from Sigma-Aldrich Corporate (St. Louis, MO, USA). HPLC grade acetonitrile (>99.9%) was purchased from Merck (Darmstadt, Germany). HPLC grade formic acid (>99%) was purchased from Roe Scientific Inc. (Newark, DE, USA). Ultrahigh-purity water was produced using a Milli-Q academic water purification system (Milford, MA, USA). All materials were used as received without any further purification.

2.2. Experimental design

Full factor design was applied to investigate the effects of temperature and pH value on the degradation process of Salvianolic acid B. The values of pH values and temperatures are listed in Table 1. According to industrial experiences, pH values were between 5 and 8.

Table 1
Experimental conditions and average absolute deviation values

			Average absolute deviation value/mmol·L^{-1}		
No.	T/°C	pH values	Danshensu	Lithospermic acid	Salvianolic acid B
1	60	5	0.013	0.005	0.033
2	60	6	0.015	0.006	0.059
3	60	7	0.012	0.004	0.031
4	60	8	0.007	0.016	0.121
5	70	5	0.018	0.005	0.051
6	70	6	0.018	0.006	0.019
7	70	7	0.006	0.005	0.039
8	70	8	0.026	0.025	0.108
9	80	5	0.024	0.006	0.028
10	80	6	0.029	0.005	0.018
11	80	7	0.036	0.006	0.044
12	80	8	0.040	0.019	0.036
13	90	5	0.026	0.011	0.017
14	90	6	0.007	0.010	0.050
15	90	7	0.059	0.015	0.152
16	90	8	0.081	0.017	0.097

2.3. Procedures

2.3.1. Degradation of Salvianolic acid B

To determine the effects of degradation pH value and temperature, 50 mg of Salvianolic acid B was dissolved in a 50 ml buffer solution composed of disodium hydrogen phosphate dodecahydrate and citric acid monohydrate. The pH value of Salvianolic acid B solution was measured with a pH meter (S40, Mettler-Toledo Instruments Co., Ltd.). Salvianolic acid B solution then was transferred into a jacketed glass tank in which the air was removed by pumping high purity argon for 2 min with a flowrate of 0.16 m^3·h^{-1}. After that, 10 ml of methyl silicone was added into the tank to prevent Salvianolic acid B solution from contacting the air during degradation experiments. The jacketed glass tank was heated with a thermostat bath (ZCY-15B, Ningbo Tianheng Instrument Factory). The solution was stirred using a magnetic stirring apparatus (85-2, Hangzhou Instrument Motor Co., Ltd.). Samples were collected at different time intervals with a volume of 200 μl. Then 200 μl of 0.3 mol·L^{-1} phosphoric acid solution was used to acidify the samples. After that, samples were kept in ice bath before HPLC analysis.

2.4. Analytical methods

An HPLC-UV method was used to determine the concentrations of Danshensu, lithospermic acid, and Salvianolic acid B [20]. HPLC system of HP 1100 series (Agilent Technologies, Waldbronn, Germany) was equipped with a Chemstation Software (Agilent Technologies). All the separations were carried out on an Eclipse plus C_{18} column (100 mm × 4.6 mm i.d., 1.8 μm of particle size) purchased from Agilent (Santa Clara, CA, USA). The injection volume was 10 μl and flowrate was 0.5 ml·min^{-1}. The column temperature was maintained at 35 °C and detection wavelength was set at 280 nm. Eluent A was composed of 0.4% (v/v) aqueous formic acid containing 0.01 mol·L^{-1} ammonium formate, and eluent B was a solution of acetonitrile and 0.1% (v/v) formic acid. The gradient elution was as follows: 2%–13% B at 0–15 min; 13%–20% B at 15–30 min; 20%–25% B at 30–40 min; 25%–40% B at 40–45 min; 40%–90% B at 45–50 min; 90%–90% B at 50–55 min. Salvianolic acid E concentration was not determined in this work because of the difficulties in the separation of Salvianolic acid E and its isomer.

2.5. Data processing

2.5.1. Modeling of Salvianolic acid B degradation

In industry, extraction process of *Salvia miltiorrhiza* is usually carried out in stainless steel extraction tanks. The weight ratio of Salvianolic acid B degradation products generated from oxidation is usually small in an aqueous extract. Therefore, no oxidation products of Salvianolic acid B is considered in model building. In previous work, it is found that hydrolysis reactions of Salvianolic acid B and Salvianolic acid E are main degradation reactions [6]. Therefore, the degradation of Salvianolic acid B was simplified, as shown in Fig. 1. Only three main degradation products of Salvianolic acid B were considered, which were Danshensu, lithospermic acid, and Salvianolic acid E.

Water activity was assumed to be a constant. The hydrolysis reactions of Salvianolic acid E and lithospermic acid were assumed to be irreversible. According to the law of mass conservation, ordinary differential equations can be obtained as follows:

$$\frac{dC_1}{dt} = -(k_1 + k_3)C_1 + k_2C_2 + k_4C_3C_4 \quad (1)$$

$$\frac{dC_2}{dt} = k_1C_1 - k_2C_2 - k_5C_2 \quad (2)$$

$$\frac{dC_3}{dt} = k_3C_1 - k_4C_3C_4 - k_6C_3 \quad (3)$$

$$\frac{dC_4}{dt} = k_3C_1 - k_4C_3C_4 + k_5C_2 + k_6C_3 \quad (4)$$

where C_1, C_2, C_3, and C_4 are the concentrations of Salvianolic acid B, Salvianolic acid E, lithospermic acid, and Danshensu, respectively; t is degradation time; k_i (i = 1 to 6) is a degradation kinetic constant. The value of k_i was assumed to be affected by both pH value and temperature. According to Arrhenius equation, k value increases when temperature increases. According to Guo *et al.*'s work [19], we assume that k value increases exponentially as pH value increases. Eq. (5) then