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

Xingchu Gong, Shichao Huang, Jianyang Pan, Haibin Qu

PII: S1004-9541(16)30127-6
DOI: doi: 10.1016/j.cjche.2016.06.005
Reference: CJCHE 607

To appear in:

Received date: 1 March 2016
Revised date: 3 June 2016
Accepted date: 8 June 2016

Please cite this article as: Xingchu Gong, Shichao Huang, Jianyang Pan, Haibin Qu, Modelling of degradation kinetics of Salvianolic acid B at different temperatures and pH values, (2016), doi: 10.1016/j.cjche.2016.06.005

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

Xingchu Gong, Shichao Huang, Jianyang Pan, Haibin Qu*

Pharmaceutical Informatics Institute, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China

*Corresponding Author

E-mail: quhb@zju.edu.cn, Tel: 86-571-88208428, Fax: 86-571-88208428

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.

Keywords: Danshen; modelling; hydrolysis; Salvianolic acid B

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 form 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.

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.

MATERIALS AND METHODS

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.

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

NO.	T/ ^{o}C	pH values	Average absolute deviation value (mmol/L)		
			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

Procedures

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. 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 phosphoric acid solution was used to acidify the samples. After that, samples were kept in ice bath before HPLC analysis.

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 um of particle size) purchased from Agilent (Santa Clara, CA, USA). The injection volume was 10 µL and flowrate was 0.5 mL/min. The column temperature was maintained at 35 ^{o}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 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.

Data processing

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.

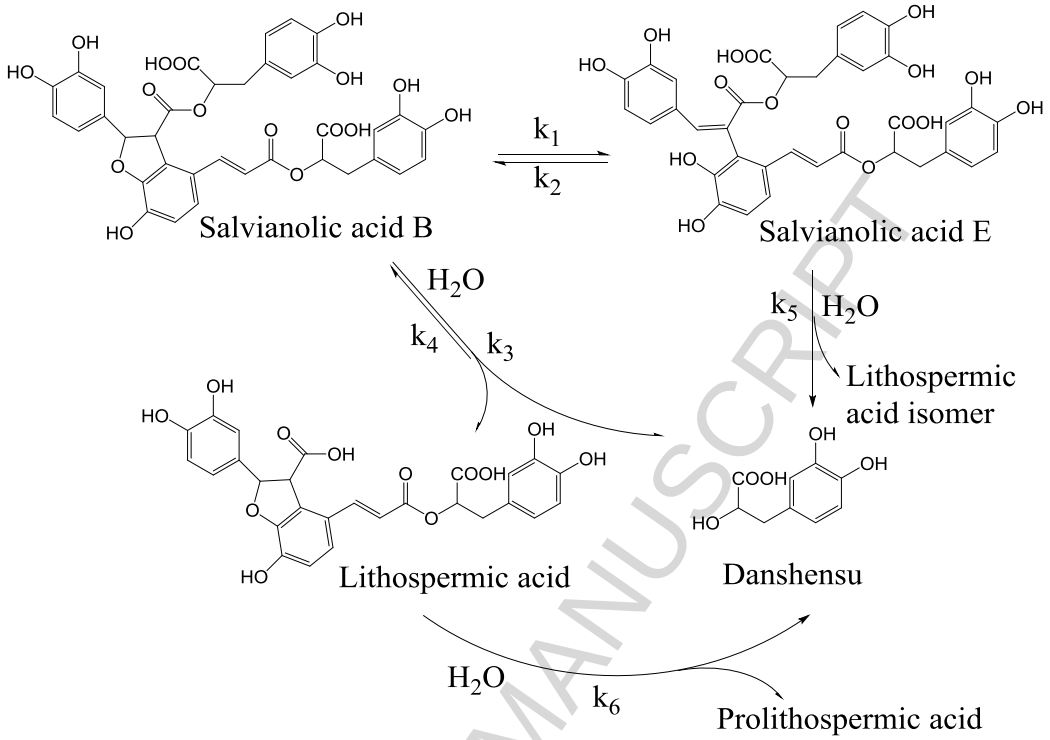


Fig. 1 Simplified degradation reactions of Salvianolic acid B

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. Equation 5 then is used to model the effects of pH value and temperature on k value.

$$\log k = a + b \cdot pH + \frac{c}{T} \quad (5)$$

where T is thermodynamic temperature; *a*, *b*, and *c* are coefficients. The values of *a*, *b*, and *c* were calibrated by minimizing the following objective function.

$$F = \sum_{j}\sum_{k}\sum_{l}\left|C_{j,k,l}^{\exp} - C_{j,k,l}^{pre}\right| \quad (6)$$

where *exp* and *pre* represent experimental value and calculated value, respectively; *j, k,* and *l* are different phenolic acids, data sets, and sampling time points, respectively. The concentration data of Salvianolic acid B, lithospermic acid, and Danshensu were used in parameter calibration. The ordinary equations were solved using fourth-order Runge-Kutta integration method. All the calculations were carried out using a self-written program of Matlab (2014b, MathWorks, USA).

After the determination of a, b, and c, the deviation between calculation results and prediction results were calculated. The average absolute deviation (AAD) values were calculated using the following equation.

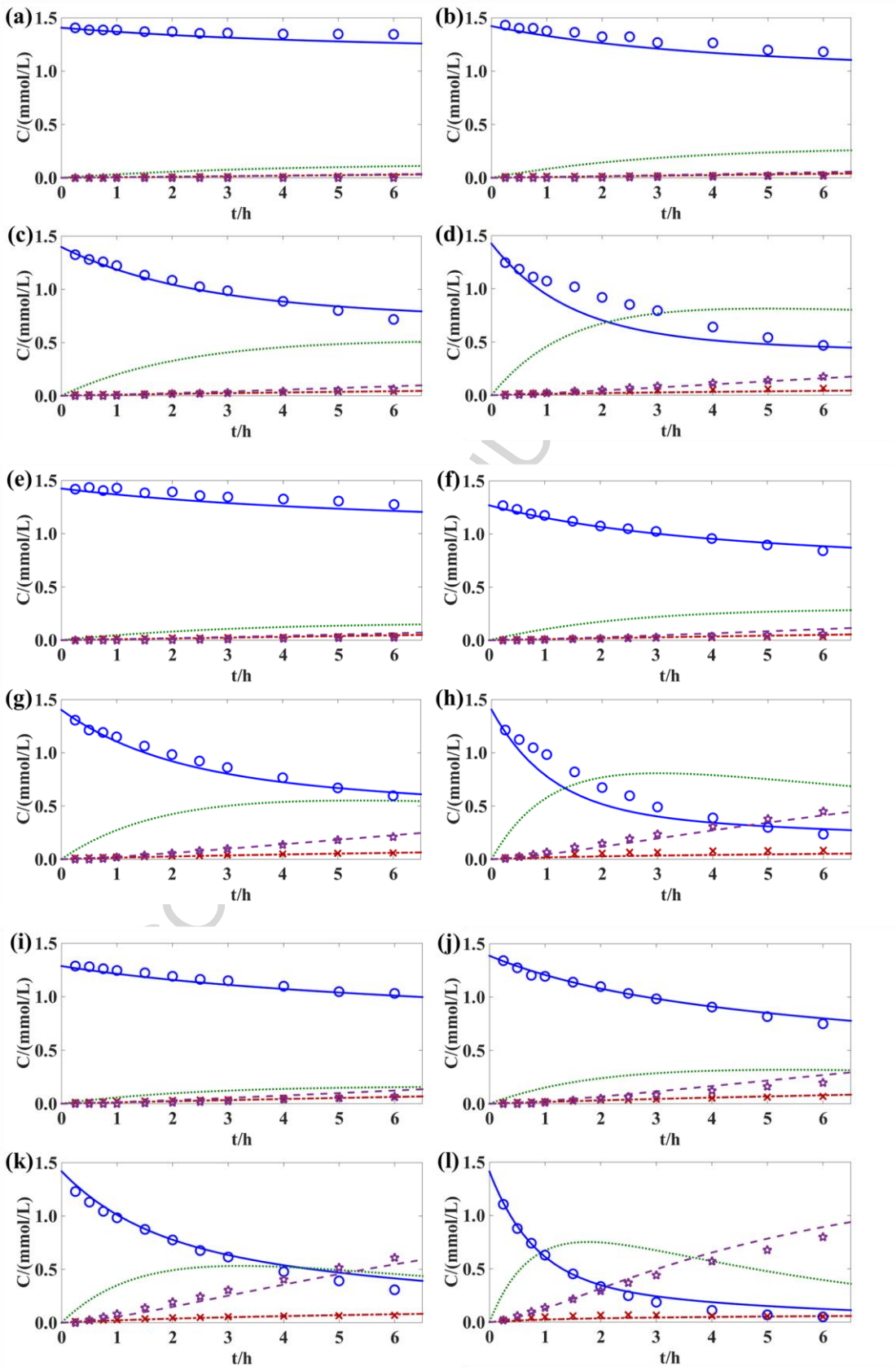
$$AAD = \frac{\sum_{l=1}^{n}\left|C_l^{exp} - C_l^{pre}\right|}{n} \quad (7)$$

where *n* is the number of experimental points, which is 11.

RESULTS AND DISCUSSION

Degradation of Salvianolic acid B in buffer solution

Fig. 2 shows the degradation results of Salvianolic acid B. Salvianolic acid B concentration decreased in all the experiments, which means that the degradation of Salvianolic acid B occurred. Danshensu concentration always increased. More Danshensu can be obtained when more Salvianolic acid B degraded. It indicates that Danshensu is a relative stable degradation product of Salvianolic acid B. The increase of lithospermic acid concentration is very slow because it is also easy to degrade [11]. The degradation rate of Salvianolic acid B increased as the pH value increased or temperature increased. Similar results were reported by Guo et al. [19].



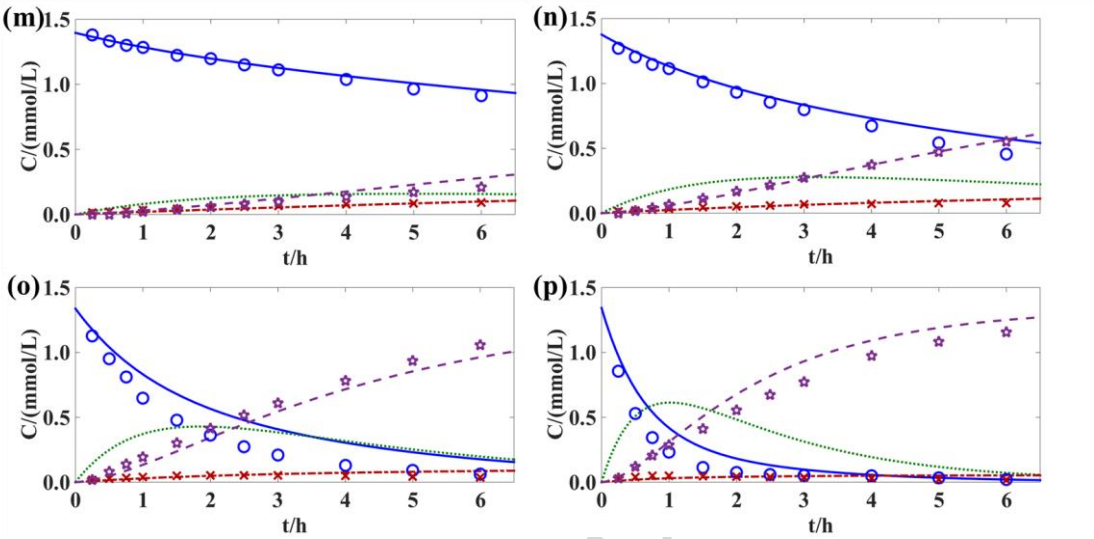


Fig. 2 Degradation kinetics of Salvianolic acid B

Blue open cycle: experimental results of Salvianolic acid B; red cross: experimental results of lithospermic acid; purple open pentagram: experimental results of Danshensu; blue solid line: prediction results of Salvianolic acid B; red dash dot line: prediction results of lithospermic acid; purple dash line: prediction results of Danshensu; green dot line: calculation results of Salvianolic acid E; (a)-(p): Experiment 1 - Experiment 16.

Modeling of Salvianolic acid B degradation kinetics

The parameters of a, b, and c were determined by fitting the experimental data to proposed ordinary differential equations. The effects of pH value and temperature on k_2, k_4, and k_6 were found to be very small in our experiments. Therefore the corresponding b values and c values were assumed to be zero. The calibration results were tabulated in Table 2. Calculated concentrations of different phenolic acids are shown in Fig. 2. For most experiments, satisfactory calculation results are obtained.

The average absolute deviation (AAD) values were calculated and listed in Table 1. The AAD values of Danshensu, lithospermic acid, and Salvianolic acid B were lower than 0.09, 0.03, and 0.16 mmol/L, respectively. Small AAD values also indicate a close agreement between experimental results and calculation results.

Table 2 Calculated values of coefficients

Degradation constant	a	b	c (K)
k_1	4.269	0.9473	-4.206×10^{3}

k_2	-1.425	0	0
k_3	9.380	0.3360	-5.555×10^{3}
k_4	-32.87	0	0
k_5	30.86	0.3200	-1.230×10^{4}
k_6	-11.73	0	0

Model verification

A *Salvia miltiorrhiza* extract powder was prepared using water decoction, concentration, column chromatography, and freezing-drying. The contents of Danshensu, lithospermic acid, and Salvianolic acid B were determined and shown in Table 3. In the verification experiment, 278.3 mg of the *Salvia miltiorrhiza* extract powder was dissolved in a 50 mL buffer solution with a pH value of 7.0. In order to obtain a relatively fast degradation of Salvianolic acid B, the solution was kept at 90 ^{o}C for 12 h. The concentrations of phenolic acids at different degradation time were determined and shown in Figure 3. Salvianolic acid B concentration decreased and Danshensu concentration increased. The degradation kinetics of these phenolic acids were also predicted using the model presented in this work. The prediction results are shown in Figure 3. The AAD values are listed in Table 3. It can be concluded that predicted values agreed well with the experimental results, which means that the present model was predictive. Though this model was developed using degradation data determined from simple composition systems, it can be applied in a botanical mixture with a more complicated composition.

Table 3 The contents and AAD values of phenolic acids in the *Salvia miltiorrhiza* extract powder

Phenolic acids	Danshensu	Lithospermic acid	Salvianolic acid B
Contents (mg/g)	141	28.0	189
AAD (mmol/L)	0.237	0.139	0.0936

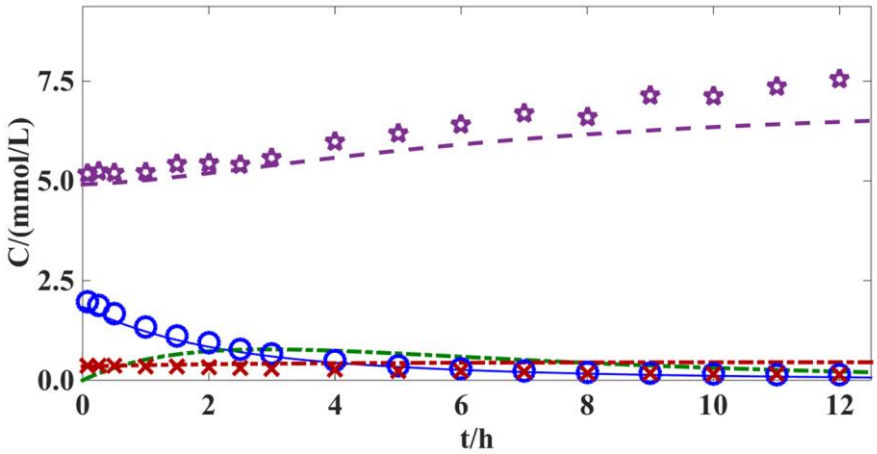


Fig. 3 Degradation kinetics of phenolic acids in a *Salvia miltiorrhiza* extract

Blue open cycle: experimental results of Salvianolic acid B; red cross: experimental results of lithospermic acid; purple open pentagram: experimental results of Danshensu; blue solid line: prediction results of Salvianolic acid B; red dash dot line: prediction results of lithospermic acid; purple dash line: prediction results of Danshensu; green dot line: calculation results of Salvianolic acid E.

CONCLUSIONS

In present work, the degradation of Salvianolic acid B at different degradation time points, degradation temperatures, and pH values were determined. Lower pH values, lower extraction temperature, and shorter extraction time led to less degradation of Salvianolic acid B. Danshensu concentration increased as Salvianolic acid B concentration decreased. Mechanism model 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 average absolute deviations for Danshensu, lithospermic acid, and Salvianolic acid B of degradation kinetics model were less than 0.09 mmol/L, 0.03 mmol/L, and 0.16 mmol/L, respectively. It means that satisfactory calibration results were obtained. The degradation kinetics of Salvianolic acid B, Danshensu, and lithospermic acid in a *Salvia miltiorrhiza* extract solution were predicted using the mechanism model. The predicted values agreed well with the experimental results, which means that mechanism model was predictive.

ACKNOWLEDGMENTS

The authors like to acknowledge the supports of National Natural Science Foundation of China (No. 81273992), and Public Service Technology Research and Social Development Project of Science Technology Department of Zhejiang Province of China (2015C33128) on this work.

REFERENCES

[1] Y.A. Zhang, X. Li, Z.Z. Wang, Antioxidant activities of leaf extract of Salvia miltiorrhiza Bunge and related phenolic constituents, *Food. Chem. Toxicol.*, 48 (2010) 2656-2662.
[2] T.O. Cheng, Cardiovascular effects of Danshen, *Int. J. Cardiology.*, 121 (2007) 9-22.
[3] F. Duval, J.E. Moreno-Cuevas, M.T. Gonzalez-Garza, C. Rodriguez-Montalvo, D.E. Cruz-Vega, Protective mechanisms of medicinal plants targeting hepatic stellate cell activation and extracellular matrix deposition in liver fibrosis, *Chinese Medicine*, 9 (2014) 11.
[4] C.S. Liu, N.H. Chen, J.T. Zhang, Protection of PC12 cells from hydrogen peroxide-induced cytotoxicity by salvianolic acid B, a new compound isolated from Radix Salviae miltiorrhizae, *Phytomedicine*, 14 (2007) 492-497.
[5] I.S. Abd-Elazem, H.S. Chen, R.B. Bates, R.C.C. Huang, Isolation of two highly potent and non-toxic inhibitors of human immunodeficiency virus type 1 (HIV-1) integrase from Salvia miltiorrhiza, *Antiviral Research*, 55 (2002) 91-106.
[6] J.Y. Pan, X.C. Gong, H.B. Qu, Quantitative H-1 NMR method for hydrolytic kinetic investigation of salvianolic acid B, *J. Pharmaceut. Biomed. Anal.*, 85 (2013) 28-32.
[7] X. Zheng, H. Qu, Characterisation of the Degradation of Salvianolic Acid B Using an On‐line Spectroscopic Analysis System and Multivariate Curve Resolution, *Phytochem. Anal.*, 23 (2012) 103-109.
[8] H.J. Lee, J.Y. Cho, J.H. Moon, Chemical conversions of salvianolic acid B by decoction in aqueous solution, *Fitoterapia*, 83 (2012) 1196-1204.
[9] Y.X. Guo, Z.L. Xiu, D.J. Zhang, H. Wang, L.X. Wang, H.B. Xiao, Kinetics and mechanism of degradation of lithospermic acid B in aqueous solution, *J. Pharmaceut. Biomed. Anal.*, 43 (2007) 1249-1255.
[10] L.N. Zhou, X. Zhang, W.Z. Xu, X.N. Ma, Z. Jia, Y.M. Zheng, S. You, Studies on the stability of salvianolic acid B as potential drug material, *Phytochem. Anal.*, 22 (2011) 378-384.
[11] Y. Wang, J. Zhu, S. Fu, L. Zhu, Y. Zhang, Study on chemical changes of salvianolic acid B and lithospermic acid aqueous under conditions of high temperature and high pressure, *China Journal of Chinese Materia Medica*, 36 (2011) 434-438.
[12] S.S. Liu, F. Han, H. Xu, K. Liu, Isolation and identification of chemical constituents from thermal decomposition extract of salvianolic acid B, *Chinese Traditional and Herbal Drugs*, 42 (2011) 247-250.
[13] X. Zheng, X. Gong, Q. Li, H. Qu, Application of multivariate curve resolution method in the quantitative monitoring transformation of salvianolic acid A using online UV spectroscopy and mass spectroscopy, *Ind. Eng. Chem. Res.*, 51 (2012) 3238-3245.
[14] M. Xu, J. Han, H.-f. Li, L. Fan, A.-h. Liu, D.-a. Guo, Analysis on the stability of total phenolic acids and salvianolic acid B from Salvia miltiorrhiza by HPLC and HPLC-MS(n), *Nat. Prod. Commun.*, 3 (2008) 669-676.
[15] M. Xu, J. Han, H. Li, L. Fan, A. Liu, D. Guo, Analysis on the stability of total phenolic acids and salvianolic acid B from Salvia miltiorrhiza by HPLC and HPLC-MS (n), *Nat. Prod. Commun.*, 3 (2008) 669-676.
[16] L.X. Yu, Pharmaceutical quality by design: Product and process development, understanding, and control, *Pharm. Res.-Dordr.*, 25 (2008) 781-791.
[17] L.X. Yu, G. Amidon, M.A. Khan, S.W. Hoag, J. Polli, G.K. Raju, J. Woodcock, Understanding Pharmaceutical Quality by Design, *AAPS J.*, 16 (2014) 771-783.
[18] K. Li, D.W. Li, Y.G. Xi, D.B. Yin, Model Predictive Control with Feedforward Strategy for Gas Collectors of Coke Ovens, *Chinese J. Chem. Eng.*, 22 (2014) 769-773.
[19] Y.X. Guo, D.J. Zhang, H. Wang, Z.L. Xiu, L.X. Wang, H.B. Xiao, Hydrolytic kinetics of lithospermic acid B extracted from roots of Salvia miltiorrhiza, *J. Pharmaceut. Biomed. Anal.*, 43 (2007) 435-439.
[20] S. Huang, Study on the effect of sodium salt of Danshensu and protocatechuic aldehyde on the

degradation of Salvianolic acid B, *Bachelor thesis*, Zhejiang University, 2013.