Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 810 (2004) 165–168
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Study on the precipitation reaction between baicalin and berberine by HPLC

Li Yi, Xu Xu*

Laboratory of Analytical Chemistry, State Key Laboratory of Organometallic Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences (CAS), No. 354, Fenglin Road, Shanghai 200032, China

Received 19 May 2004; accepted 15 July 2004
Available online 13 August 2004

Abstract

The solubility product equilibrium constant for the precipitation between baicalin and berberine was investigated because of the current interest in decocting process of complex prescription of Chinese herbal medicine. HPLC was used for determining two compounds' equilibrium concentrations at different precipitate conditions to calculate thermodynamic constants and study kinetic process. The analysis was performed on a Kromasil C_{18} column with TEA-adjusted 0.02 mol/L H_3PO_4 (pH 4.82)–acetonitrile (75:25) as mobile phase at a flow-rate of 1.0 ml/min, with detection at 254 nm. According to the experiment result, the molar ratio of baicalin and berberine in precipitate is about 1:1. The experimental K_{sp} values are $(1.01 \pm 0.12) \times 10^{-9}$ mol^2/L^2 at 20 °C in 0.02 mol/L NaH_2PO_4–Na_2HPO_4 (pH 4.82), and $(3.20 \pm 0.46) \times 10^{-9}$ mol^2/L^2 at 40 °C in the same buffer. The precipitate reaction is an exothermic process and occurs immediately, even though the precipitate cannot be observed in time because the precipitate is light, yellow, flocculous and suspending in the yellow solution.

Keywords: Precipitate reaction; Baicalin; Berberine

1. Introduction

Precipitation phenomena occurred in decocting process of complex prescription of Chinese herbal medicine have been interested. Among these complex prescriptions, Xiexin Tang and Shaoyao Tang decoction were investigated frequently [1–3], in which Huanglian (*Rhizoma Coptidis*) and Huangqin (*Radix Scutellariae*) were both included in their ingredients. They can be used for purging stomach-fire, to treat such disease with difficult defection, abdominal pain and tenesmus due to damp heat as bacillary dysentery, irritable colitis and acute enteritis, etc. However, the research for the precipitate was complicated by many factors. Traditional Chinese medicine prescriptions usually consist of at least three herbs and each of them contains a great deal of components. Some of the components react with each other and result in the complex precipitate as the mixture of various compounds [4,5].

Huanglian (*Rhizoma Coptidis*) and Huangqin (*Radix Scutellariae*) is familiar medicine couple in many traditional complex prescriptions. They both belong to the species of herbal medicines whose characters are bitter and cold. They have common medicinal functions to clear away heat and toxin; to decrease blood moisture and can promote each other's medicinal efficacy release. It has been reported that their decoction can produce precipitation. There are effective components with physiological activities in the precipitate, which are proved by pharmacological experiments [5]. Our research chooses berberine and baicalin as experimental samples, because the former is main compound in Huanglian (*Rhizoma Coptidis*), the latter is a representation of flavone compounds that are the main kind compounds in Huangqin (*Radix Scutellariae*). The common analytical methods of high performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE) can be used for studying

* Corresponding author. Tel.: +86 2164163300x3426; fax: +86 2164166128.
E-mail address: xuxu@mail.sioc.ac.cn (X. Xu).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.07.007

Chinese herbal components [6–10]. The paper studied the thermodynamics and kinetics of the precipitation reaction by determining the solubility equilibrium concentrations while precipitation reaction occurs in two compounds with HPLC.

2. Experimental

2.1. Chemicals

HPLC-grade acetonitrile was from Merck (Germany). Phosphoric acid (A.R.) was purchased from Feida Co. Ltd. (Shanghai, China). Triethylamine (TEA, A.R.) was purchased from Lianchuang material Co. Ltd. (Suzhou, China). Pure distilled water was purchased from Shanghai Sparking dinking water Co. Ltd. The standard samples of baicalin and berberine hydrochloride were from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

2.2. Apparatus

The experiments were carried out with a 655 HITACHI high performance liquid chromatographic system (Tokyo, Japan), which consisted of a pump, a variable wavelength UV monitor and a 7125 injection valve with a 20 μl ration ring. The software HW-2000 (purchased from Qianpu Co. Ltd. (Shanghai, China) was used to record the detector signal. The column used in this work was a Kromasil C_{18} analytical column (150 mm × 4.6 mm i.d. 5 μm). Another two instruments in common use were a TGL-16h high speed centrifuge which was the product of Anting scientific instrument Co. Ltd. (Shanghai, China) and a $10^{-6} \times g$ M2p analytical balance (sartorius, Germany).

2.3. Chromatography

TEA-adjusted 0.02 mol/L H_3PO_4 (pH 4.82) with 25% acetonitrile was chose as mobile phase. The flow-rate was 1.0 ml/min. Signals were detected with UV detector at 254 nm. In each injection, 25 μl sample solution was injected manually and actual injection volume was controlled by 20 μl ration ring.

3. Results and discussion

3.1. The choice of sample solvent

Since baicalin is a weak acidic compound with several reaction points, its reaction mechanisms are different at different pH values [10]. It is very unstable in the solution tending to alkaline, and its stability decreases slightly when the pH value of the solution is more than 3.0. Experimental data indicate that baicalin does not decompose obviously in 70 h at 50 °C when solution pH value is in the range of 3.0–5.0

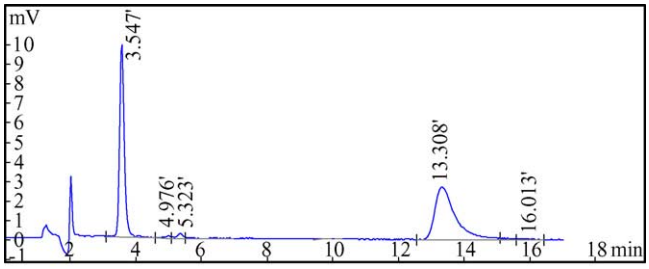

Fig. 1. Chromatogram of baicalin (3.5 min, 15.35 μmol/L) and berberine (13.3 min, 9.47 μmol/L) on 5 μm Kromasil C_{18} column dimensions, 150 mm × 4.6 mm i.d.; mobile phase, acetonitrile–phosphatic buffer (25:75); flow-rate, 1 ml/min; UV 254 nm.

[10]. It also be reported that the precipitate of baicalin and berberine can dissolve partially in dilute acid [5]. In view of all mentioned factors, the finally selected sample solvent was phosphate buffer (pH 4.82) whose concentration was 0.02 mol/L.

3.2. HPLC analysis of baicalin and berberine

Under the chosen HPLC conditions, retention times for baicalin and berberine are around 3.5 min and 13.3 min, respectively (Fig. 1). Two peaks appearing in the neighborhood of 5 min were verified impurities taken from baicalin standard sample. Besides them, there are no new peaks appearing in the chromatogram. Therefore, it was briefly considered that no new compounds come into being in the mixed solution besides the precipitate.

3.3. The determination of standard work curves and detection limits

The 0.649 mg baicalin and 0.501 mg berberine were precisely weighed and put into 25 ml volumetric flask respectively. Then they were dissolved with 0.02 mol/L NaH_2PO_4–Na_2HPO_4 buffer (pH 4.82) to obtain 58.16 × 10^{-6} mol/L baicalin reserve solution and 53.90 × 10^{-6} mol/L berberine reserve solution. 5, 4, 2, 1, 0.5, and 0.25 ml of reserve solution was taken out and put into 5 ml volumetric flask in turn, which was diluted to the scale with the same buffer. By this means two series of different concentration (x, mol/L) of sample solutions were made up, which was injected into the column one by one to obtain y (peak area). Calibration graphs were plotted by linear regression of peak area with concentration. As the result, the regression equation of baicalin was $y = 6359.4x + 3393.4$, in which the linear range of x (concentration) was within 2.908 × 10^{-6} to 58.16 × 10^{-6} mol/L. The regression equation of berberine was $y = 11652x + 3753.5$, in which the linear was within the range of 2.695 × 10^{-6} to 53.90 × 10^{-6} mol/L. The correlation coefficient of the former was 0.9999, and the latter was 0.9998. The 0.23 pmol of baicalin and 39 pmol of berberine standard samples were prepared to determine detection limits. By extrapolating measured signals, when the signal-to-noise ratio

Table 1
K_{sp} value of precipitation between baicalin (Bai) and berberine (Ber) at 20 °C determined by HPLC

No.	[Bai]/[Ber]	Bai or Ber	$(1/2)C_0$ ($\times 10^{-6}$ mol/L)	$(1/2)C_D$ ($\times 10^{-6}$ mol/L)	$[(1/2)C_0 - (1/2)C_D]$ ($\times 10^{-6}$ mol/L)	[Bai]/[Ber] in precipitate	K_{sp} ($\times 10^{-10}$ mol^2/L^2)
1	5:2	Bai	56.90	40.61	16.29	1.07	11.79
		Ber	22.47	7.26	15.21		
2	2:1	Bai	54.15	34.81	19.34	0.98	11.24
		Ber	27.76	8.07	19.69		
3	3:2	Bai	50.73	26.83	23.90	0.94	9.64
		Ber	34.37	8.98	25.39		
4	1:1	Bai	45.24	15.34	29.90	0.95	8.21
		Ber	44.95	13.38	31.57		
5	2:3	Bai	38.39	8.67	29.72	0.94	9.19
		Ber	58.17	26.51	31.66		
6	1:2	Bai	33.59	6.39	27.20	0.98	10.11
		Ber	67.42	39.54	27.88		
7	2:5	Bai	30.16	5.43	24.73	1.00	10.67
		Ber	73.95	49.11	24.84		
				Mean		0.98 ± 0.05	10.12 ± 1.23

C_0: Initial concentrations of Bai and Ber calculated by mixed ratio and their concentrations before mixed.; C_D: determined concentrations of Bai and Ber was unprecipitated concentration in mixed solution determined by HPLC.

is 3:1 (S/N = 3), the detection limit is 0.13 pmol for baicalin, 15 pmol for berberine.

3.4. The K_{sp} value determination of baicalin–berberine precipitate

Respectively prepared 0.1371 mmol/L baicalin standard solution and 0.2644 mmol/L berberine standard solution with pH 4.82, 0.02 mol/L NaH_2PO_4–Na_2HPO_4 buffer for using in each precipitation experiment. Different volumes of two standard solutions were taken out in proportion to prepare series of mixed solutions. After they were placed in a thermostatic water bath for 2 or 3 days, these mixed solutions were centrifuged at 4800 × g for 10 min. Then 0.5 ml upper pellucid liquid was taken out and diluted by 0.5 ml phosphate buffer (pH 4.82, 0.02 mol/L).

The results of baicalin–berberine precipitate in different concentrations at 20 °C for 3 days were listed in Table 1. When initial concentration ratio was in the range of 5:2 to 2:5, the composing ratio of baicalin to berberine in the precipitate was [Bai]/[Ber] = 0.98 ± 0.05, in other words, the two compounds precipitated in roughly equal quantity. K_{sp} =

Table 2
K_{sp} value of precipitation between baicalin (Bai) and berberine (Ber) at 40 °C determined by HPLC

No.	[Bai]/[Ber]	Bai or Ber	$(1/2)C_0$ ($\times 10^{-6}$ mol/L)	$(1/2)C_D$ ($\times 10^{-6}$ mol/L)	$[(1/2)C_0 - (1/2)C_D]$ ($\times 10^{-6}$ mol/L)	[Bai]/[Ber] in precipitate	K_{sp} ($\times 10^{-9}$ mol^2/L^2)
1	5:2	Bai	57.44	53.01	4.43	1.03	3.90
		Ber	22.68	18.38	4.30		
2	2:1	Bai	55.36	19.00	7.72	1.01	3.62
		Ber	26.68	38.89	7.68		
3	3:2	Bai	51.21	38.89	12.33	0.92	3.32
		Ber	34.68	21.33	13.35		
4	1:1	Bai	45.24	24.67	20.57	1.05	2.50
		Ber	44.90	25.29	19.61		
5	2:3	Bai	38.39	19.55	18.84	1.02	3.10
		Ber	58.10	39.62	18.48		
6	1:2	Bai	33.59	15.01	18.58	1.01	2.94
		Ber	67.35	48.94	18.41		
7	2:5	Bai	30.16	13.24	16.92	1.07	3.08
		Ber	73.95	58.10	15.85		
				Mean		1.02 ± 0.05	3.20 ± 0.46

Table 3
Free concentrations of baicalin (Bai) and berberine (Ber) in mixed solution determined by HPLC in different time

No.	Time (min)	(1/2)C_D ($\times 10^{-6}$ mol/L)		[Bai][Ber] ($\times 10^{-10}$ mol^2/L^2)
		Bai	Ber	
1	0	23.25	21.41	19.91
2	44	19.04	17.59	13.40
3	110	19.19	16.52	12.68
4	220	18.02	15.93	11.48
5	340	16.75	15.51	10.39
6	460	16.03	14.20	9.11
7	610	15.13	12.63	7.64
8	1426	15.50	13.89	8.61

For baicalin, (1/2)C_0 = 45.25 × 10^{-6} mol/L. For berberine, (1/2)C_0 = 44.95 × 10^{-6} mol/L.

[Bai][Ber], so $K_{sp} = (1.01 \pm 0.12) \times 10^{-9}$ mol^2/L^2, log K_{sp} = −8.99. There was an interesting phenomenon that K_{sp} value decreased slightly and then came back along with the decrease of [Bai]/[Ber]. It also appeared in other several repeated experiments.

The results of baicalin–berberine precipitate in different concentrations at 40 °C for 2 days were showed in Table 2. In the precipitate, [Bai]/[Ber] = 1.02 ± 0.05, $K_{sp} = (3.20 \pm 0.46) \times 10^{-9}$ mol^2/L^2, log K_{sp} = −8.49. The relationship between K_{sp} value and [Bai]/[Ber] also existed.

3.5. Thermodynamic and kinetic of baicalin–berberine precipitate

From Table 2, K_{sp} value at 40 °C is about 3.20 × 10^{-9} mol^2/L^2 (log K_{sp} = −8.49), which is slightly more than 1.01 × 10^{-9} mol^2/L^2 (log K_{sp} = −8.99) at 20 °C. According to van't Hoff equation: $\log(K_2/K_1) = (\Delta H/2.303R)((1/T_1) - (1/T_2))$ the dissolution reaction's enthalpy was estimated, $\Delta H \approx 42.42$ kJ mol^{-1} > 0. Therefore, dissolution reaction is an endothermic process, while precipitation reaction is an exothermic process. In the same way, the K_{sp} value at 100 °C was also estimated as log K_{sp} = −7.31, which showed that temperature has significant effect on the precipitate reaction.

0.1371 mmol/L baicalin standard solution and 0.2644 mmol/L berberine standard solution were took out, respectively in proportion of 1:1 to prepare eight same mixed solutions. They were placed in a 20 °C thermostatic water bath. Experiments began from the time when the mixed solutions were prepared. Each time one solution was used. Before injected it was centrifuged at 4800 × g for 8 min and diluted to double volume. Once the mixed solution was tried to be centrifuged at 540 × g, yet the precipitate was so light that it could not gather at the bottom of the centrifugal tube thoroughly. So, a bigger centrifugal force of 4800 × g was necessary for the experiment.

Free concentrations of baicalin and berberine in mixed solution in different time were showed in Table 3. The distinct precipitate could not be observed immediately at the time when two standard solutions were mixed but the mixed solution seemed to be turbid. However, yellow flocculous precipitate could appear distinctly as long as the mixed solution was centrifuged or it was placed for a long time. The data in Table 3 verified the precipitation process that the reaction occurred immediately as long as two compounds were mixed, and then the precipitate particles grew and gathered gradually.

4. Conclusions

The actual state of available effective composition in traditional Chinese herbal medicine when it was taken is absorbing more and more interest in pharmaceutical industry. Those flavone's compounds and alkaloids whose structures are analogous to baicalin and berberine may precipitate in the similar way. Qiao et al. [5] have proved that the precipitate contains palmatine, jalrorrhizing, eipberberine, copticine and wogonoside besides baicalin and berberine. Combining the experimental data with literature reported data [11], one can roughly estimate out the state change of main components in Xiexin decoction, which is a typical Chinese herbal medicine.

Acknowledgements

The work is supported by Innovation Fund of Chinese Academy of Sciences.

References

[1] Q.M. Sun, G.Q. Li, Chin. Pharm. Bull. 16 (1981) 40.
[2] T. Tomimori, M. Yoshimoto, Jpn. J. Pharmac. 34 (1980) 138.
[3] Y. Xue, T.H. Zhou, X.M. Wang, Chin. J. Pharm. Anal. 19 (1999) 224.
[4] G.X. He, W.L. Jiang, Y.B. Feng, D.P. Li, Chin. J. Chin. Mater. Med. 23 (1998) 432.
[5] L. Qiao, J.R. Peng, X.S. Pu, Chin. J. Chin. Mater. Med. 24 (1999) 352.
[6] G.H. Zhang, Y.Z. Wang, C.Y. Zhang, L. Wang, Chin. J. Chromatogr. 13 (1995) 247.
[7] H.M. Liebich, R. Lehamann, C. Di Stefanno, H.U. Haring, J. Chromatogr. A 795 (1998) 388.
[8] Z.Y. Cheng, F.M. Han, M. Cai, Y. Chen, Acta Pharmac. Sinica 34 (1999) 854.
[9] Y.L. Zheng, T.R. Xie, L.H. Jiang, S.L. Wei, Fenxi Ceshi Xuebao 20 (2001) 21.
[10] B.T. Yu, Z.R. Zhang, W.S. Liu, P. Wang, T. Yang, Chin. Trad. Herbal Drugs 33 (2002) 218.
[11] X. Xu, X.W. Dong, P. Mao, Acta Pharmac. Sinica 38 (2003) 779.