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One-pot β-cyclodextrin-assisted extraction of active ingredients from Xue–Zhi–Ning basing its encapsulated ability

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ABSTRACT

Xue–Zhi–Ning (XZN) is a traditional Chinese medicine formula, containing active ingredients with poor solubility in water, which has been demonstrated to be helpful for patients with hyperlipidemia. One-pot β-cyclodextrin (β-CD)-assisted extraction of active ingredients from XZN has been carried out to develop an efficient and eco-friendly extraction process. Five active compounds—rubrofusarin gentiobioside, 2,3,5,4′-tetrahydroxy-stilbene-2-O-β-D-glucoside, emodin, nuciferine and quercetin—were identified by UPLC/DAD/MS and used as indexes to evaluate the process optimized by an orthogonal test. The results showed that addition of β-CD significantly enhanced the extraction ratios of all five components. The enhancement of extraction ratios was positively correlated with the apparent formation constants between β-CD and the compounds. The study also showed that the stabilities and dissolution rates of the active ingredients were improved in the presence of β-CD. This one-pot β-cyclodextrin-assisted extraction has the potential to be applied in pharmaceutical preparations directly.

1. Introduction

Traditional Chinese medicine (TCM) is famous for satisfactory treatment with few side effects. The most widely used forms of TCM are those involving multiple herbs combined under the guidance of TCM theories, known as formulae. It is widely accepted that the therapeutic effects of TCM are based on the synergistic effects of multiple herbs, each of which contains active chemical constituents affecting multiple targets (Jiang, 2005; Normile, 2003). Traditionally, patients drink an aqueous solution of decocted herbs. Modern forms of TCM have been developed including pills, oral liquids, granules or capsules, prepared using modern technology after organic solvent extraction. Xue–Zhi–Ning (XZN) is a TCM formula recorded in the Pharmacopeia of the People's Republic of China (China, 2010a). It is composed of four food homology Chinese herbs, *Semen Cassiae*, *Radix Polygoni Multiflori Preparata*, *Folium Nelumbinis* and *Fructus Crataegi*, at a ratio of 3:2:1.5:1. The combination of multiple herbs in XZN has been clinically applied in the treatment of hyperlipidemia (Chen et al., 2011; Du et al., 2010; Ko et al., 2011; Wang, Zhao, Wang, Mao, & Yu, 2012; Xie, Zhao, & Du, 2012).

Naturally occurring cyclodextrins (CDs) are cyclic oligosaccharides containing six, seven, or eight D-(+)-glucopyranose units (α-, β-, and γ-cyclodextrin) connected by α-1,4 glycosidic bonds (Harada, 1997; Liu, Han, Qi, & Chen, 1997; Tafazzoli & Ghiasi, 2009). CDs have a hydrophilic exterior and an internal hydrophobic cavity and can act as host molecules that form inclusion complexes with lipophilic guest molecules (Irie & Uekama, 1997). β-Cyclodextrin (β-CD) has been used to form inclusion complexes with

Abbreviations: β-CD, β-cyclodextrin; TCM, traditional Chinese medicine; XZN, Xue–Zhi–Ning; RG, rubrofusarin gentiobioside; TSG, 2,3,5,4′-tetrahydroxy-stilbene-2-O-β-D-glucoside; OAD, orthogonal array design; AHP, analytic hierarchy process.

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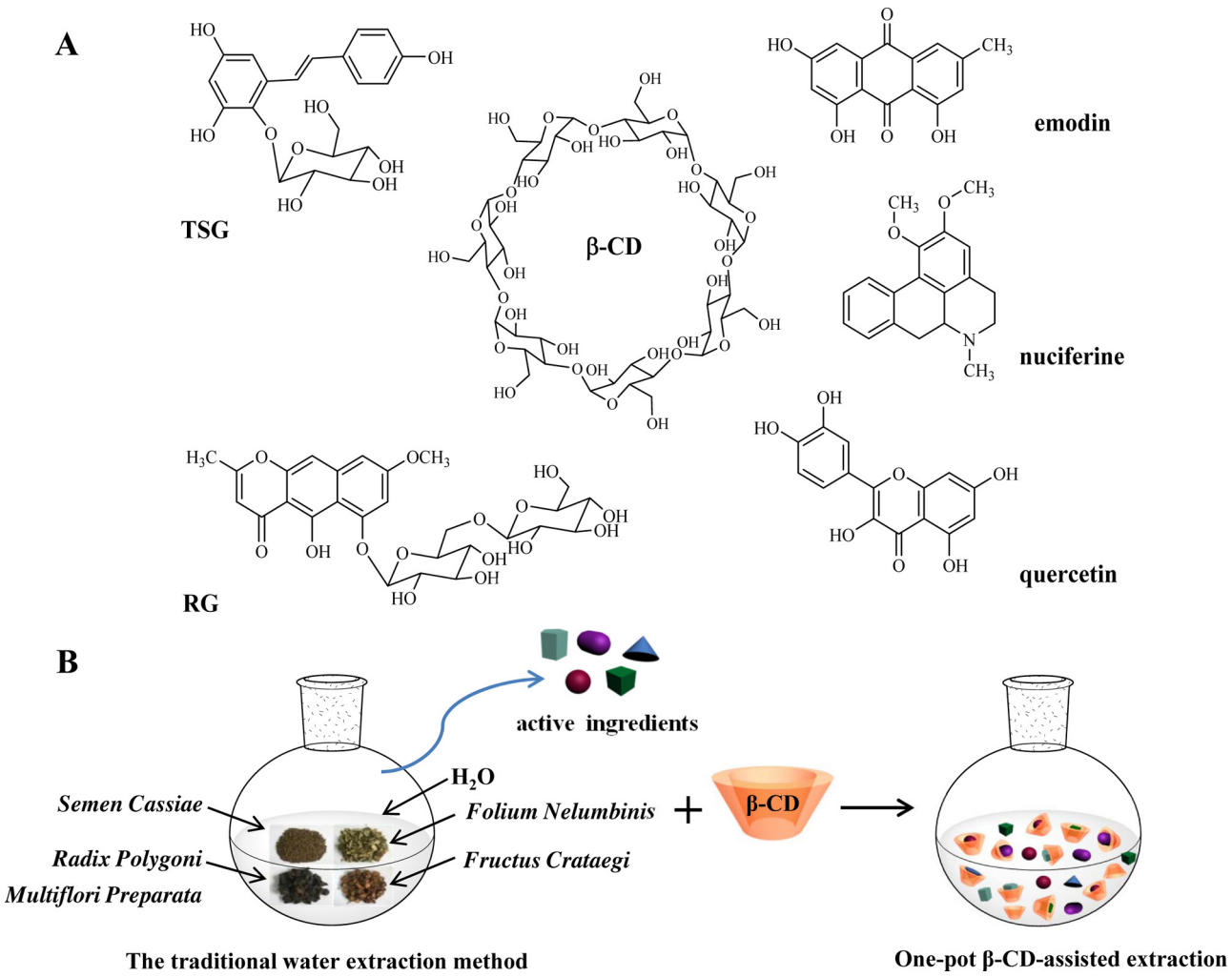

Fig. 1. (A) Structures of β-CD, RG, TSG, emodin, nuciferine and quercetin; (B) schematic of β-CD-assisted extraction.

bioactive compounds, which can improve the solubility, stability and bioavailability of the guest molecule. As a consequence, the use of β-CD in the food and pharmaceutical industries is increasingly common (Pinho, Grootveld, Soares, & Henriques, 2014; Szente & Szejtli, 2004). Recently, studies have demonstrated that herbal extracts containing multiple ingredients can be complexes with CDs to enhance their solubility and bioactivity (Hsu, Yu, Tsai, & Tsai, 2013). Of particular interest, other scholars studies have reported that β-CD aqueous solution can be used to extract polyphenols from grape pomace, apple pomace or *Polygonum cuspidatum* (Mantegna et al., 2012; Parmar, Sharma, & Rupasinghe, 2014; Ratnasooriya & Rupasinghe, 2012). Instead of organic reagents, this method is a way to use water as a solvent in the process of extraction, which is safer and more environmentally friendly.

To the best of our knowledge, β-CD has never been applied to the extraction of formulae. In this study, the encapsulation ability of β-CD has been used to assist the extraction of bioactive ingredients. Our previous research showed that the hypolipidemic effect of a β-CD extract in rats was better than that of an aqueous extract (Yang et al., 2015). In this work, the system of ultra performance liquid chromatography coupled with diode-array detector-tandem mass spectrometry (UPLC/DAD/MS) has been used to identify the active ingredients of XZN and a UPLC–DAD analysis method was established for the determination of active ingredients. The process of β-CD extraction was optimized using an orthogonal experiment design. The apparent formation constants and stoichiometries of inclusion complexes formed between β-CD and each active ingredient have been determined, in addition to the stability and dissolution rate of extract *in vitro* (Fig. 1).

2. Experimental

2.1. Materials

β-CD was provided by Huaxing Biological Chemical Co., Ltd. (Mengzhou, China). Deionized water was supplied by Hangzhou WAHAHA Group Co., Ltd. (Hangzhou, China). 2,3,5,4′-Tetrahydroxy-stilbene-2-O-β-D-glucoside (TSG), emodin, nuciferine and quercetin reference substances were purchased from the National Institutes for Food and Drug Control (Beijing, China). Rubrofusarin gentiobioside (RG) was purchased from Tuohai Biological Technology Co., Ltd. (Nanjing, China). Methanol (HPLC grade) and formic acid (HPLC grade) were purchased from Sigma–Aldrich (USA). Dried *Semen Cassiae*, *Radix Polygoni Multiflori Preparata*, *Folium Nelumbinis* and *Fructus Crataegi* were purchased from a Chinese medicine store (Chang'an, Anguo) and authenticated by pharmacognosists at Tianjin University of Traditional Chinese Medicine.

2.2. Materials and sample preparation

For the calibration curves of RG, TSG, emodin, nuciferine and quercetin, standard solutions at different concentrations were prepared in methanol and a 3 μL sample of each was injected for UPLC/DAD analysis. The linear ranges were: 0.21–13.40 μg/mL for RG, 1.59–101.50 μg/mL for TSG, 0.04–4.92 μg/mL for emodin, 0.07–4.20 μg/mL for nuciferine and 0.10–3.14 μg/mL for quercetin.

A 1 mL β-CD extract or aqueous extract at room temperature was diluted with methanol in a 10 mL volumetric flask (final volume 10 mL) and the solution subjected to ultrasound irradiation for 30 min to precipitate β-CD. A 2 mL sample was filtered using 0.22 μm nylon filters and 3 μL aliquots of the filtrate were used for UPLC analysis.

2.3. UPLC/DAD/MS analysis

Chromatographic analysis was performed on a Waters ACQUITY UPLC™ system (Waters Co., USA) equipped with binary solvent manager, sample manager, column oven and diode array detector (DAD). The software used was Mass Lynx V4.1. The column used for analyses was an ACQUITY UPLC BEH shield RP18 (2.1 mm × 100 mm, 1.7 μm; Waters Co., USA) at 45 °C. For the mobile phase, 0.1% aqueous formic acid (A) and methanol (B) were used. The gradient program was 10–25% B from 0 to 2 min, 25–35% B from 2 to 9 min, 35–40% B from 9 to 12 min, and 40–90% B from 12 to 40 min. The flow rate was 2 μL/min. Detection wavelengths were changed at different time intervals as follows: 0.00–4.00 min, 270 nm; 8.01–10.50 min, 320 nm; 10.51–19.00 min, 277 nm; 19.01–30.00 min, 360 nm; 30.01–40.00 min, 254 nm.

Waters LC-MS/MS (Waters ACQUITY UPLC™ system tandem Waters Quattro Premier XE MS, software version: Mass Lynx V4.1) was used for the qualitative analysis. The conditions were the same as those used by Zhang et al. (2012).

2.4. Optimization of extraction by orthogonal array design (OAD)

OAD is a systematic method for optimizing experimental parameters (Zhang et al., 2013; Ai et al., 2013). In our work, an $L_9(3)^4$ orthogonal matrix was applied to optimize four factors: the ratio of β-CD to XZN (%), time (h), number of extractions and the ratio of solid to liquid. Each of these factors was varied at three levels: β-CD to XZN ratios of 5%, 10% and 15%; times of 1, 1.5, and 2 h; number of extractions of 1, 2 and 3; solid/liquid ratios of 1/15, 1/20 and 1/25. All nine experimental runs were carried out in round-bottomed flasks, and the reflux extraction was conducted after XZN had soaked for 1 h. If the extraction was processed more than once, the aqueous extracts were combined and filtered. Filtrates were prepared as described in Section 2.2 and analyzed using UPLC–DAD. Using the extraction ratios of RG, TSG, emodin, nuciferine and quercetin as indexes, the weighting coefficients of these components were determined by an analytic hierarchy process (AHP) (Ren, Lu, Tian, & He, 2008; Xie et al., 2014). For evaluation of the OAD, the following weighting coefficients of the components were used: RG 0.3544, TSG 0.3544, emodin 0.1441, nuciferine 0.0898 and quercetin 0.0574. Composite scores were calculated using Eq. (1):

$$Y = \left(0.3544\frac{A_i}{A} + 0.3544\frac{B_i}{B} + 0.1441\frac{C_i}{C} + 0.0898\frac{D_i}{D} + 0.0574\frac{E_i}{E}\right)\% \quad (1)$$

where Y is the composite score; A_i, B_i, C_i, D_i, and E_i are the extraction ratios of RG, TSG, emodin, nuciferine and quercetin, respectively; A, B, C, D, and E are the maximum extraction ratios of RG, TSG, emodin, nuciferine and quercetin, respectively.

Extraction ratios were calculated using Eq. (2):

$$R = \frac{cv}{mw}\% \quad (2)$$

where R is the extraction ratio of the component; c (mg/mL) is the concentration of the component determined by UPLC–DAD analysis in the orthogonal tests; v (mL) is the volume of the sample in the orthogonal test; m (g) is the mass of the medicinal materials that the component is obtained from; w (mg/g) is the content of the component in the medicinal material it is obtained from, measured and calculated according to the China Pharmacopeia (China, 2010b) and related reports (Dong, Zhang, & Li, 2010; Sun et al., 2009). The process derived from the orthogonal experiment was compared with the traditional extraction method that uses distilled water as the extraction solvent.

2.5. Apparent formation constants and stoichiometries of β-CD for each active ingredient

2.5.1. UV–vis spectrophotometry

UV–vis spectrophotometry was used to determine the stoichiometric ratios and binding constants of inclusion complexes formed between β-CD and all five ingredients. Absorption spectra were recorded on a TU-1901 spectrophotometer (Beijing Purkinje General Instrument Co., Ltd). A fixed concentration of guest was added into a series of aqueous solutions containing increasing amounts of β-CD. The mixtures were treated with ultrasound at 25 °C and protected from light. The absorbance of each solution was measured at the maximum absorption wavelength of the guest against a reagent blank that was prepared using an identical reagent concentration but without guest. The wavelengths used for UV–visible analysis were: 277 nm for RG, 319 nm for TSG, 296 nm for emodin, 270 nm for nuciferine and 254 nm for quercetin. The stoichiometric ratios and the apparent formation constants (K) for the complexes were calculated using the Benesi–Hildebrand method (Benesi & Hildebrand, 1949). If the stoichiometric ratio of the inclusion complex between host and guest is 1:1, a good linear relationship will be obtained between $1/\Delta A$ and $1/[CD]_0$ according to the following equation:

$$\frac{1}{\Delta A} = \frac{1}{\Delta\varepsilon[G]_0} + \frac{1}{\Delta\varepsilon[G]_0 K[CD]_0} \quad (3)$$

where $[G]_0$ is the initial concentration of the guest, $[CD]_0$ is the initial concentration of β-CD, ΔA is the change in the absorbance of the guest after addition of β-CD, $\Delta\varepsilon$ is the difference in the molar absorptivities of the complex and free guest, and K is the binding constant, calculated from the slope of the equation.

2.5.2. Phase solubility analysis

Phase solubility studies were conducted in distilled water at 25 °C based on the method reported by Higuchi and Connors (1965). An excess of emodin or quercetin was added to 10 mL of distilled water containing increasing concentrations of β-CD. The suspensions were equilibrated in a thermostatic shaking water bath for 24 h at 25 °C protected from light. After equilibrium was achieved, the supernatants were filtered through 0.45 μm nylon filters. The concentration of dissolved emodin or quercetin was assayed using HPLC (Waters, USA). Phase solubility diagrams were plotted using CD concentration as the X-axis and guest concentration as the Y-axis. Apparent formation constants were calculated from the phase solubility diagram using the Higuchi–Connors equation:

$$K = \frac{\text{slope}}{s_0(1 - \text{slope})} \quad (4)$$

where K is the apparent formation constant and s_0 is the solubility of guest in the absence of β-CD.

2.6. In vitro *stability of the active ingredients in β-CD and aqueous extracts*

XZN was extracted with water or β-CD solution using the process established in the orthogonal test (ratio of β-CD to XZN, extraction time, number of extractions and ratio of solid/liquid). The aqueous extracts were concentrated by rotary evaporation and dried under vacuum. Equal masses of each dried, powdered sample were added to distilled water in 10 mL brown volumetric flasks (final volume of 10 mL), then placed in water baths at 25, 37, and 90 °C. Separately, equal masses of each dried, powdered sample were added to buffer solution at varying pH values (1.2, 6.8, and 8.3) in 10 mL brown volumetric flasks (final volume of 10 mL), then placed in a water bath at 25 °C. Finally, powdered extracts were added to distilled water in transparent volumetric flasks (final volume of 10 mL) and stored at room temperature under natural light. At predetermined time intervals, aliquots (1 mL) were processed as described in Section 2.2 for UPLC determination of RG, TSG, emodin, nuciferine and quercetin (Ren, Wang, Wang, Ou-Yang, & Qi, 2011).

2.7. In vitro*dissolution rates*

Dissolution rate studies were carried out using the dissolution test method II described in the China Pharmacopoeia (China, 2010c). Dissolution flasks were immersed in a water bath at 37.0 ± 0.5 °C. Phosphate buffer (pH 6.8, 900 mL) or aqueous hydrochloric acid (pH 1.2, 900 mL) were used as the dissolution media and the stirring rate was set at 100 rpm. At the beginning of the tests, extracts were added to the media. Samples (5 mL) were withdrawn and replaced with 5 mL fresh medium after 5, 10, 20, 40, 60 and 90 min. The dissolution samples were processed as described in Section 2.2 then analyzed using UPLC–DAD. The dissolution rate was determined by plotting the cumulative amount of dissolved solute against time. Rates were calculated according to Eq. (5).

$$D = \frac{C_n V_n + V_n \sum_{i=1}^{n-1} C_n}{M} \times 100\% \quad (5)$$

where D is the dissolution rate, C_n is the concentration of ingredients at each time interval, V_n is the sample volume at each time interval, and M is the content of the active ingredient in the powder extract determined by UPLC/DAD.

3. Results and discussion

3.1. UPLC/DAD/MS analysis

In recent years, the major constituents in each herb of XZN have been studied. The results suggest that RG, TSG, emodin, nuciferine and quercetin, as analyzed in this work, are the active components of XZN (Jung et al., 2010; Lee, Jang, Lee, Kim, & Kim, 2006; Lin, Kuo, Lin, & Chiang, 2009; Lin et al., 2015; Wang et al., 2012, 2014).

The UPLC/MS analyses of aqueous and β-CD extracts are shown in Fig. S1, in which nuciferine (m/z = 296, retention time = 5.86 min), TSG (m/z = 407, retention time = 10.49 min), RG (m/z = 597, retention time = 12.63 min), quercetin (m/z = 303, retention time = 21.27 min) and emodin (m/z = 271, retention time = 33.71 min) are marked. It can be seen that RG, TSG, emodin, nuciferine and quercetin are all present in both extracts. Considering the maximum absorption wavelength of each component, the detection wavelengths were changed at different time intervals as described in Section 2.3.

Table 1
Factors and levels for the orthogonal test.

Variable	Levels		
	1	2	3
(A) Ratio of β-CD to XZN (%)	5	10	15
(B) Extraction time (h)	1	1.5	2
(C) Number of extractions (n)	1	2	3
(D) Ratio of solid to liquid (n)	1/15	1/20	1/25

3.2. Optimization of the extraction process by OAD

Various parameters play a significant role in the development of a solvent extraction method. In this work, single factor experiments were conducted to establish the factors and their levels for OAD. The ratio of solid to liquid, extraction method, extraction time (h), number of extractions and the ratio of β-CD to XZN (%) were studied in single factor experiments. The results indicated that a reflux process should be used, and four factors needed to be optimized in the orthogonal test as described in Section 2.4. Details of the single factor experiments are presented in the supplementary materials (Liang, 2008). The investigated levels of independent variables are listed in Table 1. All selected factors were explored using an orthogonal $L_9(3)^4$ test design. The total evaluation index was used for statistical analysis and the results of the orthogonal test and extreme difference analysis are presented in Table 2. The extraction ratios of the five active ingredients were predetermined and the composite score for each sample was calculated according to the methods discussed in Section 2.4. The results indicated a maximum score of 97.34 and suggested that a further orthogonal analysis was warranted. Statistical software was employed to calculate the values of K and R as shown in Table 2. As can be seen from the results, the influence of factors on the composite scores decreased in the order $C > B > D > A$, according to the R values. The maximum score was obtained when the ratio of β-CD to XZN (%), time (h), number of extractions and ratio of solid to liquid were 15%, 2 h, 3 and 1/25, respectively. According to the R value, time (h), number of extractions and ratio of solid to liquid were more important than the ratio of β-CD to XZN (%). To minimize cost of production and avoid excess extraction agent, the optimum process was selected as follows: β-CD to XZN (%), time (h), number of extractions and ratio of solid to liquid of 5%, 2 h, 3 and 1/25, respectively.

A confirmatory experiment was conducted to verify the process suggested by the orthogonal experiment and demonstrate whether the addition of β-CD could increase the extraction ratio of active ingredients or not. Time (h), number of extractions and ratio of solid to liquid were set at 5%, 2 h, 3 and 1/25, respectively, and run in the presence or absence of β-CD. β-CD-assisted and aqueous extractions were both performed in triplicate and the

Table 2
Analysis of $L_9(3)^4$ test results.

No.	*A*	*B*	*C*	*D*	Composite scores
1	5	1	1	1:15	39.19
2	5	1.5	2	1:20	77.85
3	5	2	3	1:25	97.34
4	10	1	2	1:25	71.96
5	10	1.5	3	1:15	85.57
6	10	2	1	1:20	62.44
7	15	1	3	1:20	75.50
8	15	1.5	1	1:25	57.22
9	15	2	2	1:15	87.65
K1	71.46	62.22	52.95	70.80	
K2	73.33	73.55	79.15	71.93	
K3	73.46	82.48	86.14	75.51	
R	2.00	20.26	33.19	4.70	

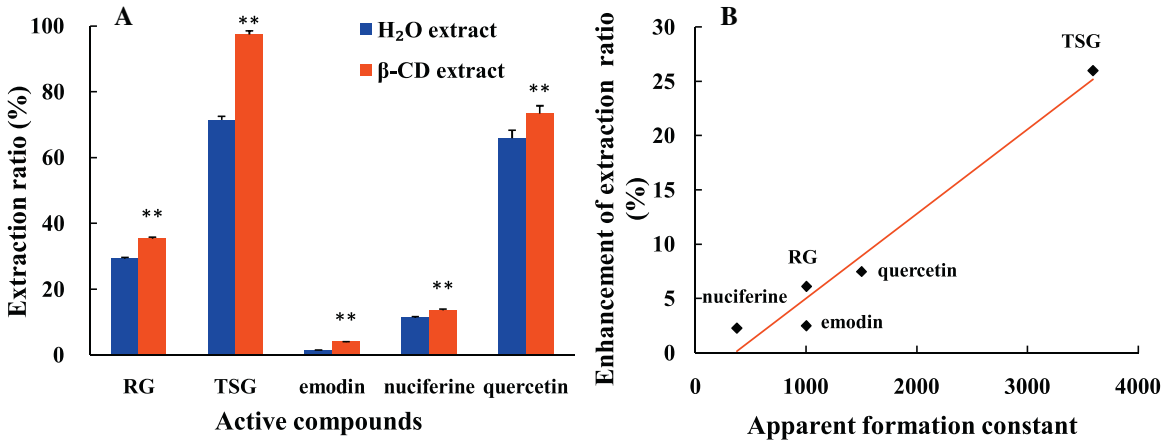

Fig. 2. (A) Effect of β-CD on the extraction ratios of active compounds. Significance was evaluated using Student's *t*-test (**$P < 0.01$); (B) Linear correlation between the enhancement of extraction ratio and apparent formation constant.

results are shown in Fig. 2(A) (details are reported in Table S1). In the confirmatory test, a high composite score of 94.48 ± 0.52 was obtained. The extraction ratios of the active ingredients in β-CD-assisted and aqueous extractions were calculated. Statistical analysis was performed using ANOVA and Student's *t*-test. A least significant difference (LSD) test with a confidence interval of 99% was used to compare the means. As shown in Fig. 2(A), β-CD-assisted extraction significantly enhanced the extraction ratios of RG, TSG, emodin, nuciferine and quercetin, compared with aqueous extraction ($P < 0.01$), particularly those of TSG and emodin.

3.3. *Apparent formation constants and stoichiometries of β-CD complexes*

The assisted extraction effect of β-CD may be mainly due to association of β-CD with the compound, so apparent formation constants for the formation of β-CD inclusion complexes were determined by UV–vis spectrophotometry or phase solubility analysis. Both methods are widely accepted for the determination of binding constants in CD complexation. The changes of absorbance value indicate that the formation of inclusion complex between β-CD and guest. Binding constants were calculated as described in Section 2.5.1, and are listed in Table 3. Because of the poor solubilities of emodin and quercetin, phase solubility experiments have been carried out. In the phase solubility experiments, the solubilities of emodin and quercetin increased linearly with increasing β-CD concentration, giving A_L-type phase-solubility diagrams with the formation of 1:1 complexes (Higuchi & Connors, 1965) (Fig. S8). The binding constants of the complexes were calculated as described in Section 2.5.2, and are presented in Table 3.

In general, the range of *K* values for CDs or their derivatives with most drugs is 100–20,000 L/mol (Stella & Rajewski, 1997). Higher values of K indicate enhanced complexation between CDs and guest drugs, resulting in higher solubility (Banerjee, Chakraborty, & Sarkar, 2004). The results for the five active ingredients show that they form inclusion complexes with β-CD at a molar ratio of 1:1. We suspect that this is one of the reasons that their extraction ratios were significantly increased in the presence of β-CD. Table 3 shows that the binding constants for complexes with RG, TSG, emodin and quercetin were distinctly higher than that for nuciferine, consistent with the observed improvements in extraction ratios. The enhancement of extraction ratios by β-CD was positively correlated with apparent formation constants as shown in Fig. 2(B). The linear equation was $y = 0.0078x - 2.7821$, and the correlation coefficient was 0.9803. The curve was fitted on the apparent formation constants of inclusion complexes between β-CD and RG, TSG, emodin, nuciferine and quercetin, which had been determined using the same method.

3.4. In vitro *stability of active ingredients in β-CD and aqueous extracts*

Thermal stabilities of RG, TSG, emodin, nuciferine and quercetin were conducted at 25, 37 and 90 °C. At 25 °C, the compounds from both β-CD and aqueous extracts were mostly unchanged after 120 h. The addition of β-CD increased *in vitro* stability of TSG (aqueous extract = 69.44% vs. β-CD extract = 86.60%) and nuciferine (aqueous extract = 39.76% vs. β-CD extract = 84.63%) after 120 h at 37 °C (Fig. S9). The same effect of β-CD was observed for RG (aqueous extract = 66.91% vs. β-CD extract = 74.83%), TSG (aqueous extract = 84.28% vs. β-CD extract = 91.81%), nuciferine (aqueous extract = 60.01% vs. β-CD extract = 76.74%) and quercetin (aqueous extract = 66.90% vs. β-CD extract = 84.01%) after 6 h at 90 °C (Fig. S10). The results show that addition of β-CD can improve the stability of the active ingredients at high temperature, reducing losses during the reflux extraction process. We speculate that this is another reason for the increased extraction ratios of the active ingredients in β-CD-assisted extraction.

At pH 1.2, 6.8 and 8.3 (simulated gastric juice, small intestine and colon pH environments, respectively), there were different degrees of degradation after 120 h, and stabilities were improved by β-CD: RG (pH1.2, aqueous extract = 84.73% vs. β-CD extract = 87.46%; pH 6.8, aqueous extract = 92.38% vs. β-CD extract = 99.46%; pH 8.3, aqueous extract = 38.45% vs. β-CD extract = 50.32%), TSG(pH1.2, aqueous extract = 89.30% vs. β-CD extract = 91.88%; pH 6.8, aqueous extract = 79.85% vs. β-CD extract = 88.13%; pH 8.3, aqueous extract = 10.71% vs. β-CD extract = 19.67%), emodin (pH1.2, aqueous extract = 61.17% vs. β-CD extract = 74.37%; pH 6.8, aqueous extract = 99.71% vs. β-CD extract = 100.00%; pH 8.3, aqueous extract = 99.95% vs. β-CD extract = 99.38%), nuciferine (pH1.2, aqueous extract = 79.09% vs. β-CD extract = 89.54%; pH 6.8, aqueous extract = 76.96% vs. β-CD extract = 83.87%; pH 8.3, aqueous extract = 86.85% vs. β-CD extract = 89.74%) and quercetin (pH1.2, aqueous extract = 99.59% vs. β-CD extract = 98.85%; pH 6.8, aqueous extract = 100.00% vs. β-CD extract = 100.00%; pH 8.3, aqueous extract = 100.00% vs. β-CD extract = 99.20%) (Figs. S11–S13).

Cumulative light exposure was measured at the same time in the light stability test. β-CD in the extract also increased the light stability of emodin (aqueous extract = 18.65% vs. β-CD extract = 44.19%)

Table 3
Apparent formation constants and stoichiometries of inclusion complexes.

Compound	Method	Stoichiometry	Apparent formation constant (L/mol)	Coefficient of determination (R^2)
RG	UV–vis spectrophotometry	1:1	1005	0.9972
TSG	UV–vis spectrophotometry	1:1	3591	0.9895
Nuciferine	UV–vis spectrophotometry	1:1	376	0.9981
Quercetin	UV–vis spectrophotometry	1:1	1502	0.9976
	Phase solubility analysis	1:1	1033	0.9939
Emodin	UV–vis spectrophotometry	1:1	1003	0.9896
	Phase solubility analysis	1:1	1362	0.9959

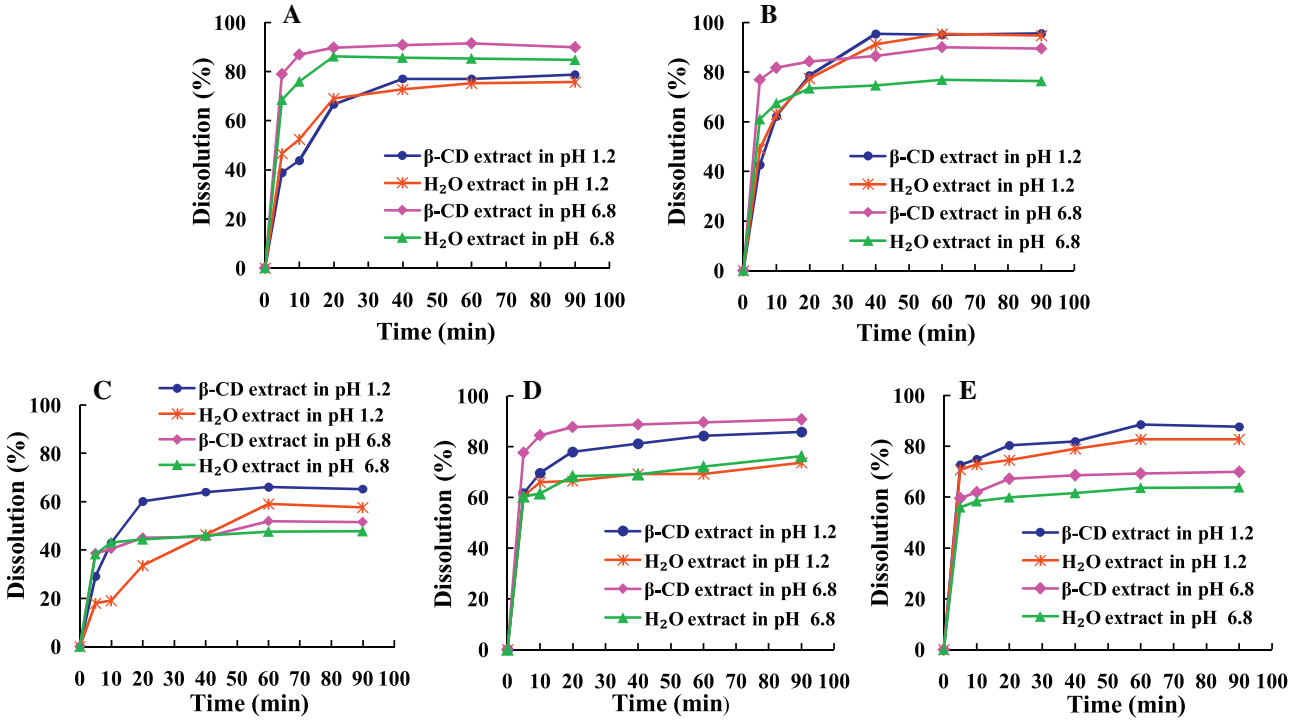

Fig. 3. Dissolution curves of RG (A), TSG (B), emodin (C), nuciferine (D) and quercetin (E).

and quercetin (aqueous extract = 80.44% vs. β-CD extract = 91.23%) after 37,079 lux h (Fig. S14).

3.5. In vitro *dissolution rates*

The dissolution profiles of β-CD and aqueous extracts were studied by UPLC/DAD. The profiles for RG, TSG, emodin, nuciferine and quercetin in both β-CD and aqueous extracts are shown in Fig. 3. After 90 min, the amounts of dissolved active ingredients were determined as follows: RG (aqueous extract = 75.85% vs. β-CD extract = 78.70% at pH 1.2; aqueous extract = 84.87% vs. β-CD extract = 89.96% at pH 6.8), TSG (aqueous extract = 94.81% vs. β-CD extract = 95.55% at pH 1.2; aqueous extract = 76.32% vs. β-CD extract = 89.50% at pH 6.8), emodin (aqueous extract = 57.64% vs. β-CD extract = 65.27% at pH 1.2; aqueous extract = 47.75% vs. β-CD extract = 51.54% at pH 6.8), nuciferine (aqueous extract = 73.60% vs. β-CD extract = 85.72% at pH 1.2; aqueous extract = 76.16% vs. β-CD extract = 90.66% at pH 6.8) and quercetin (aqueous extract = 82.77% vs. β-CD extract = 87.73% at pH 1.2; aqueous extract = 63.86% vs. β-CD extract = 70.00% at pH 6.8). It was evident that emodin and nuciferine in the β-CD extracts exhibited higher dissolution rates than the aqueous extracts at pH 1.2. The total dissolution rates for RG, TSG, nuciferine and quercetin from the β-CD extracts were higher at pH 6.8. Cyclodextrin extraction improves the dissolution of some of the active ingredients. We presume that β-CD-assisted extraction can also improve dissolution of some ingredients *in vivo* to some extent.

4. Conclusion

In this study, a new eco-friendly extraction method has efficiently been applied to the extraction of a variety of active ingredients from XZN, a formula of TCM, by using OAD. UPLC/DAD/MS results showed that both aqueous and β-CD extracts contained the active ingredients, RG, TSG, emodin, nuciferine and quercetin. β-CD-assisted extraction significantly enhanced the extraction ratios of RG, TSG, emodin, nuciferine and quercetin, compared with aqueous extraction ($P < 0.01$). Apparent formation constants showed that β-CD could form inclusion complexes with the active ingredients. A speculation that the enhancement of extraction ratios by β-CD was positively correlated with the apparent formation constants between β-CD and the compounds was proposed. In addition, the presence of β-CD in the final extract may improve the stability and dissolution of the active ingredients. In conclusion, β-CD can assist the extraction process of Chinese medicine formulae without organic solvents and the powder extract containing β-CD can be used in pharmaceutical preparations directly. This β-CD-assisted extraction technology could provide a new eco-friendly and efficient alternative to traditional extraction methods for TCM formulae.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.carbpol.2015.06.072

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