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Design and optimization of purification process of sinomenine hydrochloride

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ABSTRACT

Sinomenine hydrochloride is generally produced from *Caulis Sinomenii*. At present, the purification process in industrial production suffers from large amount of solid waste, high solvent toxicity, and low sinomenine hydrochloride yield. In this study, a new purification process for sinomenine hydrochloride was proposed by using the extract obtained from acid extraction of *Caulis Sinomenii* as the starting material. The process included the following steps: alkalization, extraction, water washing, acid–water stripping, drying, and crystallization. 1-Heptanol was used as the extractant. The distribution coefficients of sinomenine and sinomenine hydrochloride in 1-heptanol–water system were 27.4 and 0.0167, respectively. The dissociation constants of sinomenine hydrochloride were 8.27 and 11.24, respectively. Process parameters of the new purification process were optimized with experimental design. The extractant 1-heptanol and sinomenine hydrochloride in the crystallization mother solution can be recycled in the new process. The purity of the obtained sinomenine hydrochloride crystals exceeded 85%, and the yield was about 70%. Compared with current industrial processes, safer extractant, less solid waste, and higher sinomenine hydrochloride yield can be achieved using the new purification process of sinomenine hydrochloride provided in this study.

1. Introduction

Caulis Sinomenii is a medicinal herb included in the 2020 edition of Chinese Pharmacopoeia. It has the functions of dispelling wind and dampness, promoting blood circulation and detoxification [1]. Clinically, *Caulis Sinomenii* is used to treat rheumatic and rheumatoid arthritis, hyperosteogeny, and arrhythmia [2]. Sinomenine is considered as the representative active ingredient of *Caulis Sinomenii*. Pharmacological experiment results showed that sinomenine has the effects of anti-inflammation, immunosuppression, analgesia, antihypertensive, anti-arrhythmia, and so on [3]. The water solubility of sinomenine hydrochloride is higher than that of sinomenine, which is conducive to drug absorption. Therefore, sinomenine is used medicinally in sinomenine hydrochloride form.

At present, sinomenine hydrochloride is mainly prepared by direct reflux process [4–7], alkalization reflux process [1,8], and acid–water extraction process [9,10]. The direct reflux process usually uses ethanol solution as the extraction solvent, followed by concentration, pH adjustment, chloroform extraction, acid–water stripping, concentration, and drying to obtain sinomenine hydrochloride. Alkalization reflux process usually includes the following steps: slaked lime alkalization, reflux extraction with an organic solvent, drying, water dissolution, activated carbon decolorization, acidification, and crystallization. The acid–water extraction process usually includes the following steps: acid solution percolation, calcium salt precipitation, chloroform extraction, acidification, crystallization. But only about 50% of sinomenine contained in *Caulis Sinomenii* was obtained in industry. These processes suffer from toxic solvent, large amount of solid waste, or low sinomenine hydrochloride yield.

In recent years, some researchers used supercritical carbon dioxide fluid extraction technology [11–13], ionic liquid extraction technology [14,15], ultrasonic-assisted extraction technology [12,13,16,17], microwave-assisted extraction technology [18,19], catalyst-assisted extraction technology [20] to extract sinomenine. Chromatographic separation technology [17,19,21–24] and molecular imprinting polymers separation technology [25,26] were

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applied to purify sinomenine. However, due to the limitations of cost, output, or environmental protection, the above technologies have not yet been used in industry. Therefore, it is still necessary to develop a new purification process of sinomenine hydrochloride with low solvent toxicity and less solid waste.

In this study, the apparent distribution coefficient (D_{app}) of sinomenine hydrochloride in 1-heptanol–water equilibrium system under different pH values was measured. The dissociation constants of sinomenine hydrochloride were fitted. A new purification process of sinomenine hydrochloride was designed. The influences of *Caulis Sinomenii* extract properties and process parameters were investigated. Process parameters were optimized. Solvent composition of crystallization process was also optimized. Finally, 1-heptanol and sinomenine hydrochloride in the crystallization mother solution were recycled many times to verify the feasibility of the new purification process.

2. Experimental

2.1. Materials

Caulis Sinomenii (batch number yz-2103003-01) was supplied by Shupu Yulong Agricultural Development Co., Ltd. (Shupu, China). Sodium hydroxide (≥96%, analytically pure) and hydrochloric acid (36.0%–38.0%, analytically pure) were purchased from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 1-Heptanol (≥99%, analytically pure) was purchased from Shanghai Xianding Biotechnology Co., Ltd. (Shanghai, China). Ethanol (≥99.7%, analytically pure) was purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Ethylenediamine (≥99%, chromatographic purity) was purchased from the Aladdin Chemical Reagent Co., Ltd. (Shanghai, China). Sinomenine hydrochloride reference substance (batch number 200316, >98%) was purchased from Shanghai Winherb Pharmaceutical Technology Development Co., Ltd. (Shanghai, China). Methanol (≥99.9%, chromatographic purity) was purchased from Merck Chemical Technology Co., Ltd. (Darmstadt, Germany). Ultrahigh-purity water was produced using a water purification system (Milli-Q, Millipore, Burlington, MA, USA).

2.2. Preparation of Caulis Sinomenii extract

A total of 800.0 g of *Caulis Sinomenii* medicinal powder was weighed using an electronic balance (UTP, Shanghai Huachao Industrial Co., Ltd., Shanghai, China). 8 L of 0.3 mol·L^{-1} hydrochloric acid aqueous solution was added to extract the *Caulis Sinomenii* powder. Then the mixture was stirred using an electric mixer (ES-40 M, Shanghai Zhonghe Chemical Technology Co., Ltd., Shanghai, China) for more than 6 h. After filtration, about 7 L of *Caulis Sinomenii* extract was obtained.

2.3. Liquid–liquid equilibrium experiment

About 25 ml of *Caulis Sinomenii* extract was placed into each Erlenmeyer flask. Then different amounts of NaOH solution were added to adjust the pH value. After that, 25 ml of 1-heptanol was added to each Erlenmeyer flask. The mixture was then stirred using a magnetic stirrer (03–1, Hangzhou instrument Motor Co., Ltd., Hangzhou, China) at room temperature for 3 h to make it liquid–liquid equilibrium. Then the liquid–liquid equilibrium systems were centrifuged using a high-speed freezing centrifuge (5804R, Eppendorf, Hamburg, Germany) at 3200 r·min^{-1} for 20 min. After the aqueous and organic phases were taken out separately, the pH value of the aqueous phase was measured using a pH meter (S40, Mettler-Toledo, Zurich, Switzerland). The aqueous and organic phases were diluted with methanol and filtered with 0.22 μm microporous membranes. The filtrate was analyzed by a high performance liquid chromatography (HPLC) method.

2.4. Purification process design of sinomenine hydrochloride

The new purification process of sinomenine hydrochloride in this work is shown in Fig. 1. 1-Heptanol was used as the extractant in this study. There were 6 steps in the new purification process, which were alkalization, extraction, water washing, acid–water stripping, evaporating aqueous phase, and redissolution–crystallization with ethanol, respectively. Steps 1–4 were regarded as the extraction–stripping process. Aqueous phase A_1, namely the sinomenine hydrochloride stripping solution was obtained after steps 1–4. Steps 5 and 6 were regarded as the crystallization process and sinomenine hydrochloride crystal was obtained after steps 5 and 6.

In step 7, the ethanol in the crystallization mother solution was dried and the solid was added to the sinomenine extract for recycling. In step 8, the organic phase O_3 was reused without distillation.

2.4.1. Experimental operation of extraction and stripping process

200 ml of *Caulis Sinomenii* extract was placed into an Erlenmeyer flask, and the pH value of the extract was adjusted to alkalinity with sodium hydroxide solution to obtain alkaline system E_1. 1-Heptanol was added into the alkaline system E_1 according to a certain volume ratio, and the mixture was stirred and extracted by using a magnetic mixer for more than 1 h. After that, the extraction system was centrifuged using a centrifuge (Avanti J-26XPI, Beckman, California, USA) at 4500 r·min^{-1} for 15 min to separate two phases. Then aqueous phase was discarded and the organic phase O_1 was collected. A certain volume ratio of water was added to wash organic phase O_1 in a separating funnel. After mixing and phase separation, the aqueous phase was discarded, and the organic phase O_2 was obtained. The organic phase O_2 was stripped with a hydrochloric acid solution. After phase separation, the aqueous phase A_1 and organic phase O_3 were obtained.

2.4.2. Parameter optimization of the extraction and stripping process

The parameters to be investigated of the extraction–stripping process were as follows: alkalization pH value (X_1), volume ratio of alkaline system E_1 to 1-heptanol phase (1-heptanol amount, X_2), volume ratio of 1-heptanol phase to aqueous phase during washing process (washing solution amount, X_3), volume ratio of 1-heptanol phase to aqueous phase during stripping process (stripping solution amount, X_4), stripping pH value (X_5).

The definitive screening design was adopted to arrange five process parameter values, as seen in Table 1 (Experiments 1–19). Two virtual factors were added in the experimental design, and the center point was repeated 3 times. After completing these experiments, the yield of sinomenine hydrochloride was found to be a little lower. Therefore, 1-heptanol amount was increased in the following 5 experiments (Experiments 20–24 in Table 1). The hydrochloric acid extracts obtained from 5 batches of *Caulis Sinomenii* were used as the materials in Experiments 20–24. In this study, sinomenine hydrochloride concentration (Z_1, mg·ml^{-1}), dry matter content (Z_2, mg·g^{-1}), and the purity of sinomenine hydrochloride (Z_3, %) were used as the property indicators of *Caulis Sinomenii* extracts.

2.4.3. Crystallization experiment

The sinomenine hydrochloride stripping solutions prepared in Section 2.4.1 were merged and concentrated using a rotary evaporator (Rotavapor R-200, Buchi, Zurich, Switzerland). A total of 255 ml of concentrated stripping solution was obtained. 20.5 ml

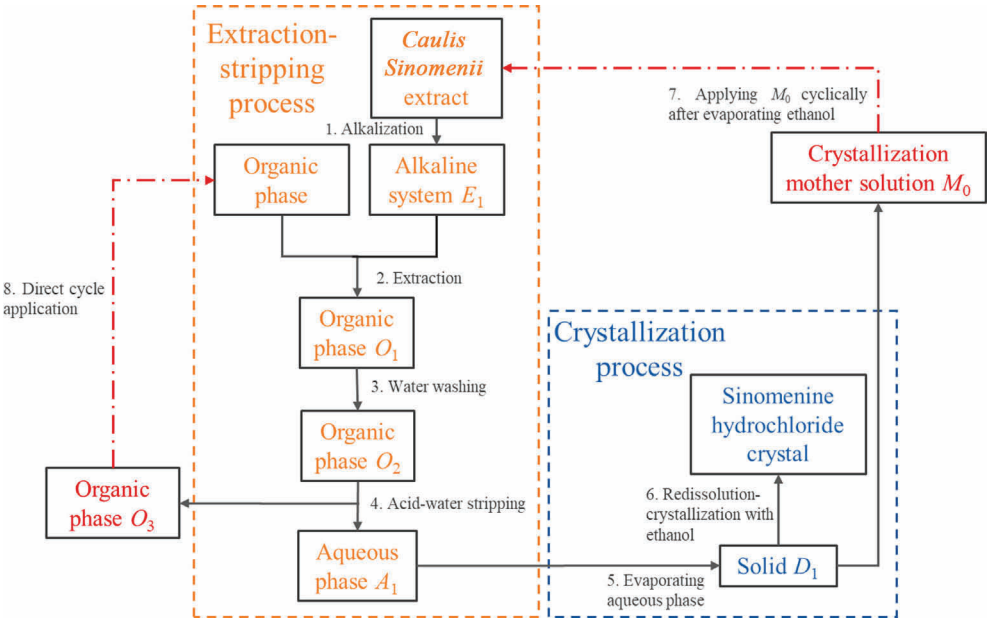

Fig. 1. Purification process flow chart of sinomenine hydrochloride.

Table 1
Experimental conditions and results

No.	Process parameters								Process evaluation indicators	
	Z_1/mg·ml^{-1}	Z_2/mg·g^{-1}	Z_3/%	X_1	X_2	X_3	X_4	X_5	Yield/%	Purity/%
1	0.7908	8.019	11.22	11.0	6.0	4.0	4.0	2.0	63.49	74.56
2	0.7908	8.019	11.22	11.0	4.0	4.0	2.0	2.0	68.24	80.43
3	0.7908	8.019	11.22	10.0	6.0	3.0	3.0	4.0	66.59	76.22
4	0.7908	8.019	11.22	9.0	6.0	2.0	2.0	6.0	59.28	78.16
5	0.7908	8.019	11.22	9.0	8.0	2.0	4.0	6.0	41.60	80.87
6	0.7908	8.019	11.22	11.0	8.0	2.0	4.0	2.0	55.54	77.58
7	0.7908	8.019	11.22	9.0	8.0	4.0	3.0	2.0	54.40	72.10
8	0.7908	8.019	11.22	10.0	6.0	3.0	3.0	4.0	61.57	78.25
9	0.7908	8.019	11.22	11.0	4.0	2.0	3.0	6.0	64.97	84.90
10	0.7908	8.019	11.22	9.0	4.0	4.0	2.0	6.0	62.52	83.34
11	0.7908	8.019	11.22	11.0	8.0	4.0	2.0	6.0	48.09	87.28
12	0.7908	8.019	11.22	9.0	8.0	3.0	2.0	2.0	53.91	71.91
13	0.7908	8.019	11.22	10.0	8.0	4.0	4.0	6.0	39.17	83.19
14	0.7908	8.019	11.22	9.0	4.0	2.0	4.0	2.0	61.44	73.52
15	0.7908	8.019	11.22	11.0	4.0	3.0	4.0	6.0	59.16	89.17
16	0.7908	8.019	11.22	10.0	4.0	2.0	2.0	2.0	71.92	71.72
17	0.7908	8.019	11.22	10.0	6.0	3.0	3.0	4.0	65.31	78.52
18	0.7908	8.019	11.22	9.0	4.0	4.0	4.0	4.0	63.73	75.59
19	0.7908	8.019	11.22	11.0	8.0	2.0	2.0	4.0	59.41	76.70
20	1.484	17.26	9.873	10.0	1.0	2.0	3.0	2.0	87.22	74.94
21	1.412	17.17	9.250	11.0	1.0	2.0	1.0	6.0	82.24	75.11
22	1.523	14.08	12.29	10.0	3.0	2.0	1.0	2.0	77.63	80.53
23	1.536	15.52	11.10	10.5	2.0	2.0	2.0	4.0	79.20	73.21
24	1.482	17.21	9.576	11.0	3.0	2.0	3.0	6.0	58.55	74.09

of concentrated stripping solution was evaporated to obtain the solid D_1 using a rotary evaporator. Ethanol solution was added to dissolve the solid D_1 with heating. The ratio of ethanol volume to solid D_1 mass was 10:1 ml·g^{-1}. Then the ethanol solution was cooled and placed in a 4 °C refrigerator (HLR-310FL, Qingdao Haier Special Electric Appliance Co., Ltd., Qingdao, China) to crystallize for more than 24 h. The crystallization mother solution M_0 and sinomenine hydrochloride crystal were both obtained by filtration. A small amount of ethanol was used to wash the crystal. After that, sinomenine hydrochloride crystal was dried. Ethanol solutions with different ethanol volume fractions of 87.5%, 90%, 92.5%, and 95% were used in the crystallization process. The experiments were performed in parallel for 3 times.

2.5. Recycling of 1-heptanol and sinomenine hydrochloride in the crystallization mother solution

400 ml of *Caulis Sinomenii* extract were treated with the operations described in Sections 2.4.1 and 2.4.3 each time to get sinomenine hydrochloride crystal. The organic phase O_3 and crystallization mother solution M_0 obtained in the purification process were collected. The organic phase O_3 was reused as the extrac-

tant in step 2. The crystallization mother solution M_0 was evaporated and dissolved with 400 ml of *Caulis Sinomenii* extract as the material for the next purification in step 7. The above operations were repeated for seven times.

2.6. Analytical method

2.6.1. Determination of the content of sinomenine hydrochloride

The content of sinomenine hydrochloride was determined with an HPLC method. Preparation procedure of reference substance solution and test sample solution are shown in Sections S1.1 and S1.2 of the Supplementary Materials. The method validation procedure and results are also shown in Sections S1.3 and S1.4.

An HPLC (1100, Agilent, Santa Clara, USA) system with a UV detector and Agilent ChemStation (B.04.03, Agilent) was used. Analyses were conducted on a ZORBAX SB-C18 column (4.6 mm × 250 mm × 5 mm) with the column temperature controlled at 25 °C. The injection volume was 10 μl. The detection wavelength was 262 nm. The mobile phase consisted of 0.25% ethylenediamine–water (A) and 0.25% ethylenediamine–methanol (B) using (50/50, volume ratio) gradient program. The flow rate of solvent was maintained at 1.0 ml·min^{-1}. Representative chromatograms of sinomenine hydrochloride reference substance and samples are shown in Fig. 2.

2.6.2. Determination of the dry matter content

The dry matter content was determined by a gravimetric method [27]. The weighing bottle with sample was placed in an electric heating constant temperature blast drying oven (DHG-9146A, Shanghai Jinghong Experimental Equipment Co., Ltd., Shanghai, China) at 105 °C till a constant mass was obtained. After weighing, the dry matter content of the sample was calculated.

2.7. Data analysis

Eq. (1) was used to calculate the apparent distribution coefficient (D_{app}) of sinomenine in 1-heptanol–water system.

$$D_{app} = \frac{C_{org}}{C_{aq}} \tag{1}$$

where C is the content of sinomenine in the sample, and the subscripts org and aq refer to the organic phase and the aqueous phase, respectively.

The dissociation constant pK_a of sinomenine was calculated by the liquid–liquid equilibrium method [28]. Eq. (2) was used in fitting.

$$\lg(D_{app}) = \lg\left(\frac{10^{d_0+pK_{a,1}-pH} + 10^{d_1} + 10^{d_2+pH-pK_{a,2}}}{10^{pK_{a,1}-pH} + 1 + 10^{pH-pK_{a,2}}}\right) \tag{2}$$

where p$K_{a,1}$ and p$K_{a,2}$ are dissociation constants of sinomenine amino dissociation and phenol hydroxyl dissociation, respectively, d_0, d_1, and d_2 are the base 10 logarithms of the distribution coefficient of sinomenine hydrochloride, sinomenine, and sinomenine with phenol hydroxyl ionized.

The yield of sinomenine hydrochloride stripping solution and crystal were calculated in Eqs. (3) and (4), respectively.

$$\text{Stripping solution yield} = \frac{C_1 M_1}{C_0 V_0} \tag{3}$$

$$\text{Crystal yield} = \frac{C_2 M_2}{C_0 V_0} \tag{4}$$

where C is the content of sinomenine hydrochloride in the sample, M is mass, V is volume, and the subscripts 0, 1 and 2 refer to the *Caulis Sinomenii* extract, sinomenine hydrochloride stripping solution, and sinomenine hydrochloride crystal, respectively.

The purity of sinomenine hydrochloride of stripping solution and crystal were calculated in Eqs. (5) and (6), respectively.

$$\text{Purity}_s = \frac{C_1}{M_d} \tag{5}$$

$$\text{Purity}_c = C_2 \times 100\% \tag{6}$$

where M_d is the dry matter content of the sample; the subscripts s and c refer to the sinomenine hydrochloride of stripping solution and crystal, respectively.

The experimental results of extraction–stripping process were analyzed with Design Expert software (11.0.0, Stat-Ease, Inc., Minneapolis, USA). Multiple linear regression method was used to establish quantitative models by Eq. (7).

$$Y = a_0 + \sum_{i=1}^{5} b_i X_i + \sum_{k=1}^{3} c_k Z_k \tag{7}$$

where Y is a process evaluation indicator, which is the sinomenine hydrochloride yield or sinomenine hydrochloride purity; a_0 is a constant; b_i and c_k are the partial regression coefficients; X_i (i = 1–5) is a process parameter; and Z_k (k = 1–3) is a material attribute. The backward elimination method was used to simplify the models, and the significance level of removing terms were 0.05. The remaining terms in the models were considered to be critical material attributes (CMAs) and critical process parameters (CPPs).

Eq. (8) was used to establish the quantitative models between process evaluation indicators and CMAs and CPPs [29,30].

$$Y = b_0 + \sum_{i=1}^{n} b_i X_{cp,i} + \sum_{i=1}^{n} b_{ii} X_{cp,i}^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} X_{cp,i} X_{cp,j} + \sum_{k=1}^{m} c_k Z_{cm,k} \tag{8}$$

where b_0 is a constant; n is the number of CPPs; m is the number of CMAs; b_i, b_{ii}, b_{ij} and c_k are the partial regression coefficients; X_{cp} refer to a CPP; Z_{cm} represent a CMA. The models were simplified by stepwise regression, and the significance levels of adding terms and removing terms were both set to 0.05.

Matlab (Version 9.8, MathWorks Inc., MA, USA) was used to calculate the operation space of extraction–stripping process using the probability of reaching the standard method based on an exhaustive search-Monte Carlo method [31,32]. The calculation steps length of alkalization pH value, 1-heptanol amount, stripping solution amount, and the stripping pH value were 0.02, 0.016, 0.016 and 0.04, respectively. The minimum acceptable probability of the operation space was 0.90. The simulation was performed 1000 times to calculate reliable probability values.

3. Results

3.1. D_{app} and pK_a of sinomenine hydrochloride

D_{app} of sinomenine hydrochloride in 1-heptanol–water extraction system at different pH values was determined, and the results are shown in Fig. 3 and Table S2. The results showed that when the pH value of the system was less than 10, the D_{app} value increased as the pH value increased. When the pH value of the system was greater than 10, the D_{app} value decreased as the pH value increased. In the 1-heptanol–water system, the D_{app} of sinomenine could be more than 25, which showed that 1-heptanol had a large extraction capacity for sinomenine.

The experimental results were fitted using Origin (2018, OriginLab, MA, USA), and the fitting results are shown in Fig. 3. The

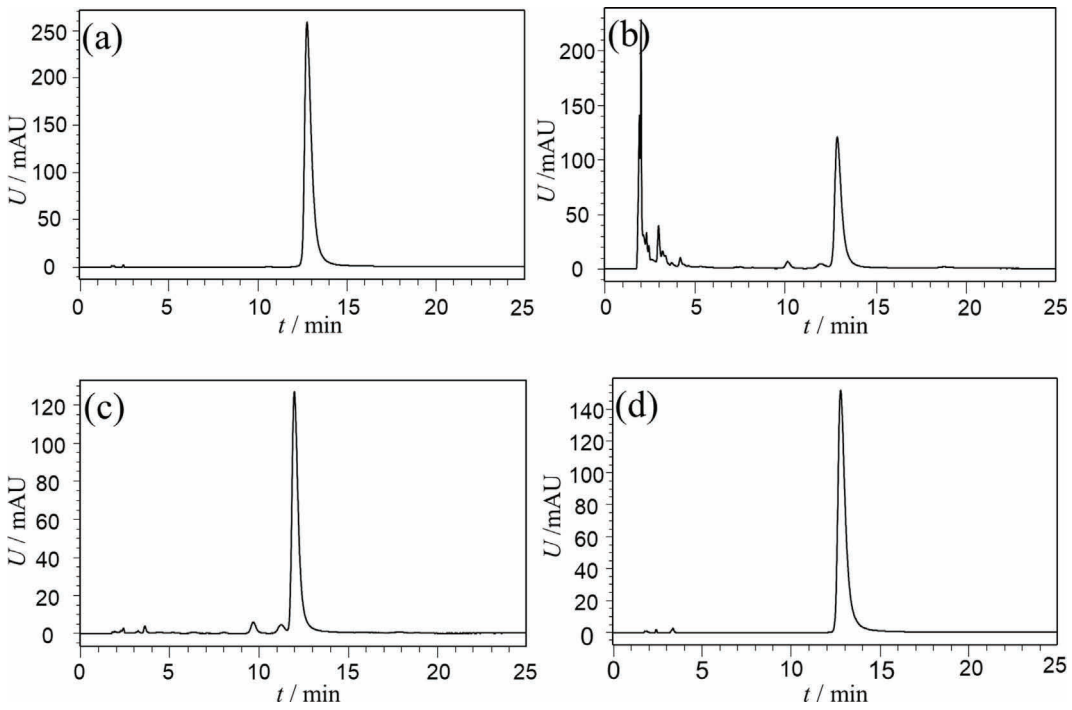

Fig. 2. Representative chromatograms: (a) sinomenine hydrochloride reference substance; (b) *Caulis Sinomenii* extract; (c) sinomenine hydrochloride stripping solution; (d) sinomenine hydrochloride crystal.

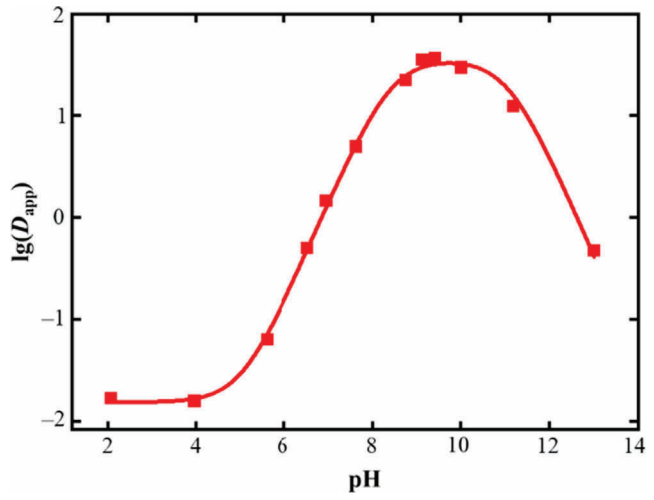

Fig. 3. Measured values and the fitting curve of D_{app} values (■: measured values; solid line: fitting values).

distribution coefficient of sinomenine salt after ionization of the phenolic hydroxyl group was too small, thus, d_2 was set to –20 in the fitting. The R^2_{adj} value obtained by fitting was 0.998, indicating that the fitting results were in good agreement with the measured values. The d_0 value obtained by fitting was –1.81 ± 0.04, and the d_1 value was 1.43 ± 0.03.

In Fig. 3, when pH value was lower than 8, the proportion of sinomenine in the molecular form gradually increased as pH increased. Accordingly, D_{app} value gradually increased. When pH value was higher than 10, the phenol hydroxyl of sinomenine ionized and form sinomenine salt, which resulted in a decrease of D_{app} value. According to the fitting results, the distribution coefficient of sinomenine hydrochloride in 1-heptanol–water extraction system was about 0.0167, which indicated that 1-heptanolcan hardly extract sinomenine hydrochloride. The predicted maximum distribution coefficient of sinomenine in 1-heptanol–water system was 27.4. In the measurement of this experiment, the pK_a value of sinomenine conjugated acid was 8.27 ± 0.04 and the pK_a value of sinomenine phenol hydroxyl dissociation was 11.24 ± 0.06, which were very close to the results reported by Zhu [33], which were 8.02 and 11.24, respectively.

It can be concluded that 1-heptanol is a potential extractant for sinomenine at a pH range of 9–11. A pH range of 2–6 will be suitable for the stripping of sinomenine from 1-heptanol phase.

3.2. Identification of CPPs and CMAs of the extraction–stripping process

The experimental phenomenon of the extraction–stripping process is shown in Fig. 4. It can be seen that the color of extract was dark, but the color of the drug solution obtained after extraction–stripping was light yellow. Eq. (7) was used to process the experimental results to identify the CMAs and CPPs in the extraction–stripping process, and the partial distribution coefficients and variance analysis of the multiple linear regression models are shown in Table S3. The significance level p value of each model was less than 0.0001, indicating that the models were significant. According to the results of models, the CMAs and CPPs in the extraction–stripping process were the purity of sinomenine hydrochloride in *Caulis Sinomenii* extract (Z_3), alkalization pH value (X_1), 1-heptanol amount (X_2), stripping solution amount (X_4), and the stripping pH value (X_5).

Eq. (8) was used to establish mathematical models between process evaluation indicators and CMAs and CPPs, the partial distribution coefficients and variance analysis results of each model are shown in Table 2. The yield of sinomenine hydrochloride increases with the amount of 1-heptanol during extraction and the amount of water during stripping. The distribution coefficient of 1-heptanol was almost a constant when pH value was fixed. Accordingly, sinomenine yield could only be improved by increas-

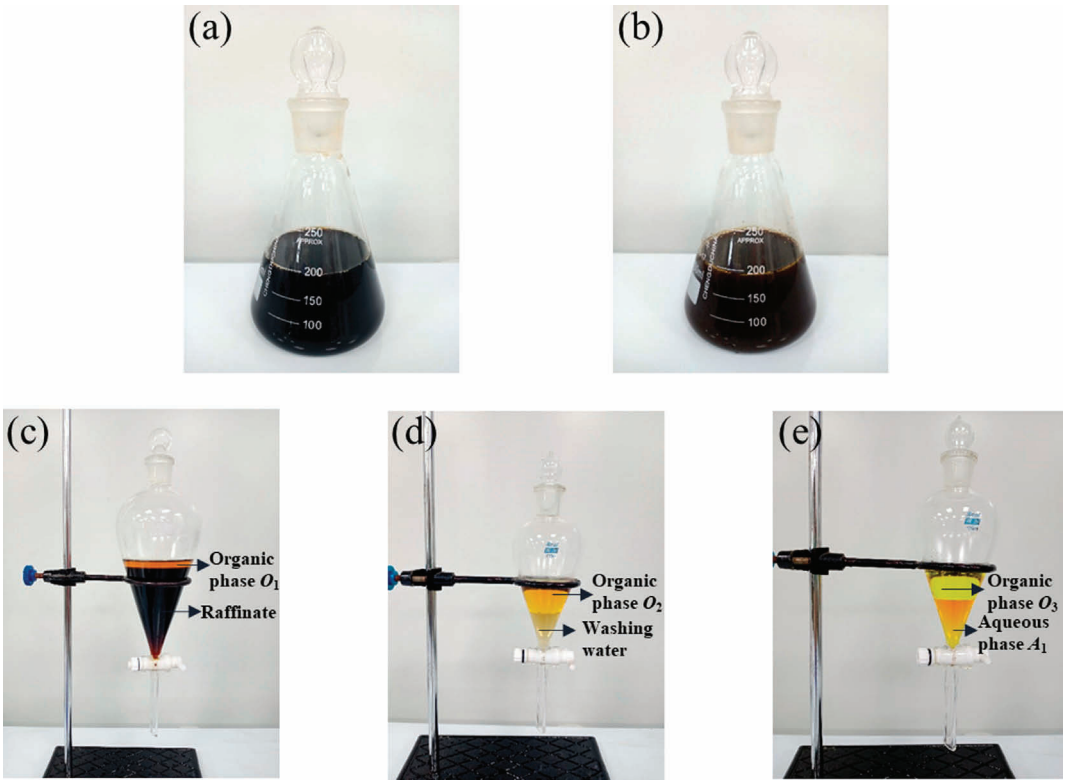

Fig. 4. Extraction–stripping process: (a) hydrochloric acid extract of *Caulis Sinomenii*; (b) alkaline system E_1; (c) extraction and phase separation; (d) phase separation after water washing; (e) phase separation after stripping.

Table 2
Partial distribution coefficients and variance analysis of the multiple quadratic regression model

Parameter	Yield		Purity	
	Coefficient	*P* value	Coefficient	*P* value
Constant	82.190	–	6.760	–
Z_3	–	–	4.718	0.0001
X_1	–	–	2.071	0.0087
X_2	–3.865	<0.0001	–	–
X_4	–2.948	0.0026	–	–
X_5	8.544	0.0001	–3.990	<0.0001
X_5^2	–1.340	0.0075	0.7743	0.0335
Model *p* value	<0.0001		<0.0001	
R^2	0.9114		0.7416	

ing 1-heptanol volume. Sinomenine hydrochloride purity of the stripping solution increases with alkalization pH value and the purity of sinomenine hydrochloride in *Caulis Sinomenii* extract. With the decrease of pH value, the yield of sinomenine hydrochloride increases first and then tends to be stable, while the purity of sinomenine hydrochloride decreases first and then tends to be stable. It indicates that some impurities are easier to dissolve in the aqueous phase with low pH value.

3.3. Optimization and validation of extraction–stripping process parameters

An exhaustive search-Monte Carlo method was used to calculate the operation space. The lowest standard probability value was set at 90%, and the lower limits of yield and purity of sinomenine hydrochloride stripping solution were set to 70% and 75%, respectively. The probability of reaching the standards was calculated for different material properties and CPPs. Then, the optimized operation space was calculated, as shown in Fig. 5(a)–(f). It can be seen that the shape of the optimized operation space was irregular. In order to facilitate the actual process operation, the recommended operation range of CPPs were: 10.0–11.0 of alkalization pH value, 2.0–1.0 of 1-heptanol amount, 2.0–3.0 of stripping solution amount, and 4.0–5.0 of stripping pH value.

In order to verify the reliability of the operation space, a point inside and a point outside the operation space were selected for the verification of the operation space, as shown in Fig. 5(e) and (f). The calculation method of the average relative deviation (ARD) between the experimental value and the predicted value is shown in Eq. (9).

$$\mathrm{ARD} = \frac{|V_m - V_p|}{V_m} \times 100\% \tag{9}$$

where V_m is the measured value and V_p is the predicted value.

Three parallel experiments were performed for the point inside operation space (V_1, V_2 and V_3) and the point outside operation space (V_4, V_5 and V_6). The validation experimental results are shown in Table 3. According to the verification results, the

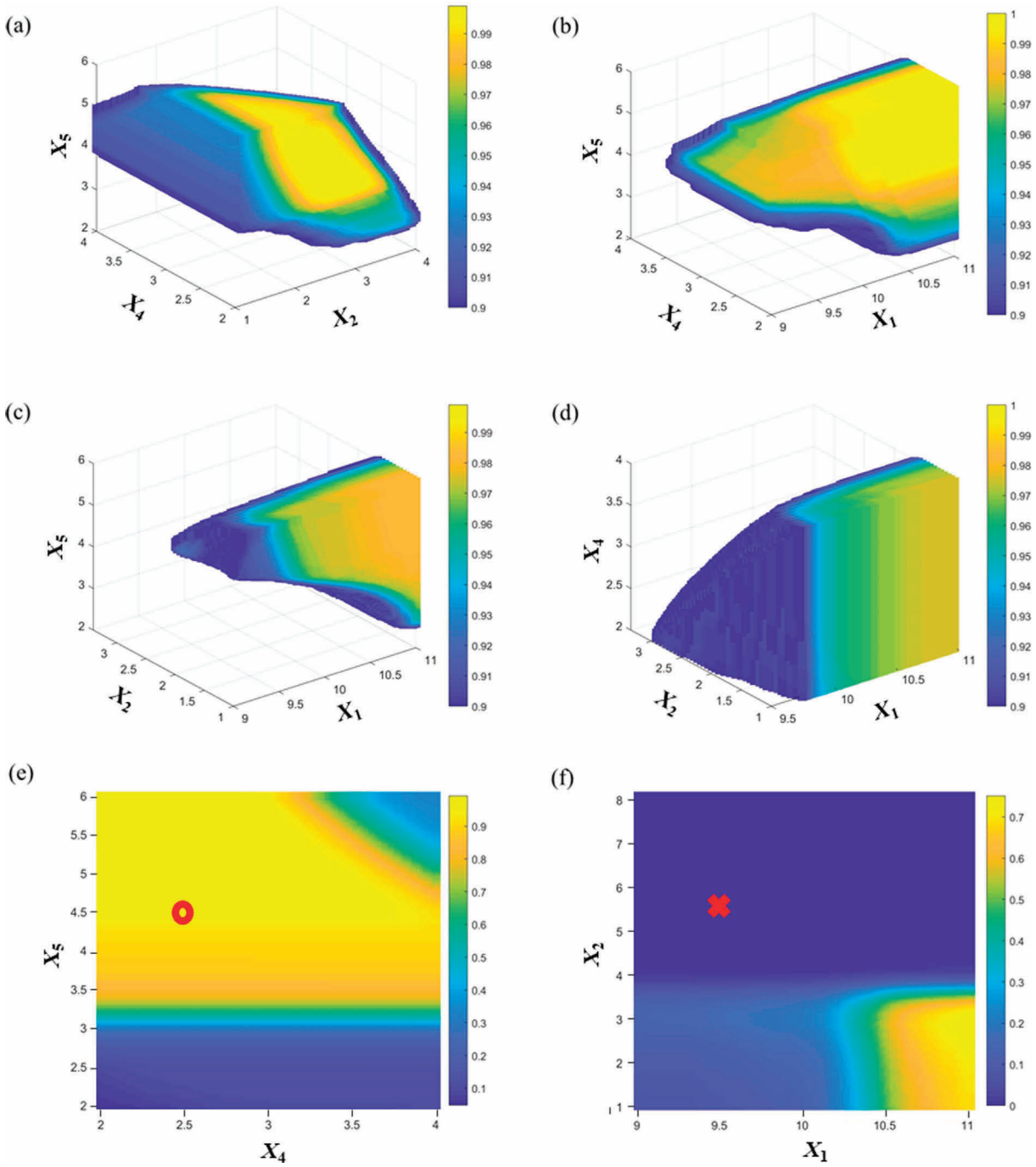

Fig. 5. Operation space and verification points (○ represents the verification points in the design space. × represents the verification points outside the design space): (a) Z_3 = 11.22%, X_1 = 2.0; (b) Z_3 = 11.22%, X_2 = 2.0; (c) Z_3 = 11.22%, X_4 = 3.0; (d) Z_3 = 11.22%, X_5 = 4.0; (e) Z_3 = 10.72%, X_1 = 10.5, X_2 = 1.5; (f) Z_3 = 9.98%, X_4 = 3.5, X_5 = 2.5.

ARD values of both process evaluation indicators were less than 6%. The measured values were close to the predicted values, indicating that the prediction performance of the models was good. The process evaluation indicators of the experimental point in the operation space all met the preset standards, while the two process evaluation indicators of the experimental point outside the operating space could not reach the standards. It showed that the established operation space was reliable, and operating in the operation space could ensure the process quality of extraction–stripping process.

3.4. Influence of ethanol content on crystallization

The ethanol volume content was changed during crystallization process, and the experimental results are shown in Fig. 6 (a) and (b), respectively. With the increase of ethanol volume fraction, the crystal purity gradually improved, which indicated that the increase of ethanol content was conducive to the removal of impurities in the crystallization process. It can be seen from Fig. 6(b) that more than 50% of the sinomenine hydrochloride was still dissolved in crystallization mother solution M_0.

Table 3
Verification experimental results

CPPs		Inside the design space			Outside the design space		
		V_1	V_2	V_3	V_4	V_5	V_6
Z_3/%		10.72	10.72	10.72	9.98	9.98	9.98
X_1		10.549	10.532	10.515	9.530	9.495	9.501
X_2		1.5	1.5	1.5	6.5	6.5	6.5
X_4		2.5	2.5	2.5	3.5	3.5	3.5
X_5		4.501	4.492	4.322	2.461	2.514	2.546
Yield/%	V_m	78.51	78.39	77.02	59.33	57.79	59.67
	V_p	80.34	80.37	80.93	59.66	59.76	59.82
	ARD/%	2.33	2.53	5.08	0.56	3.41	0.25
Purity/%	V_m	77.12	78.33	76.43	65.12	66.24	66.02
	V_p	76.28	76.22	75.75	68.26	68.18	68.18
	ARD/%	1.09	2.69	0.89	4.82	2.93	3.27

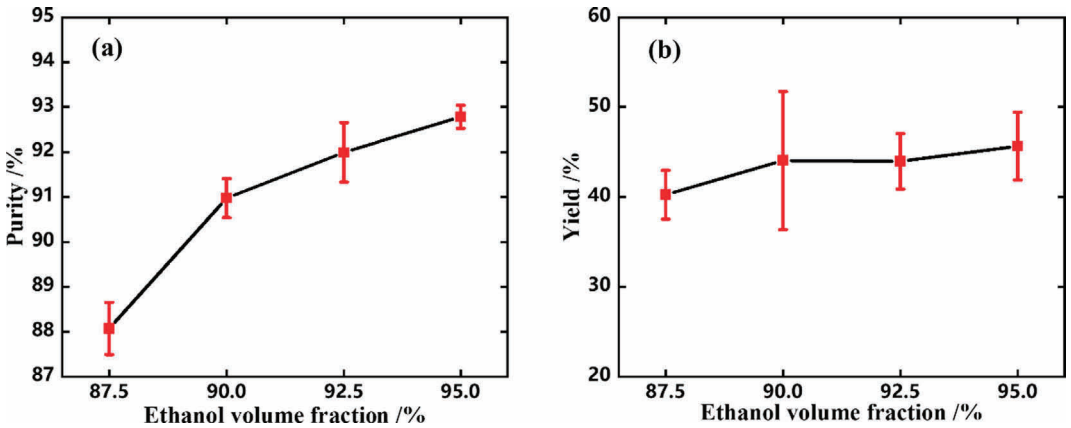

Fig. 6. The influence of ethanol volume fraction on the crystal purity and yield: (a) purity; (b) yield.

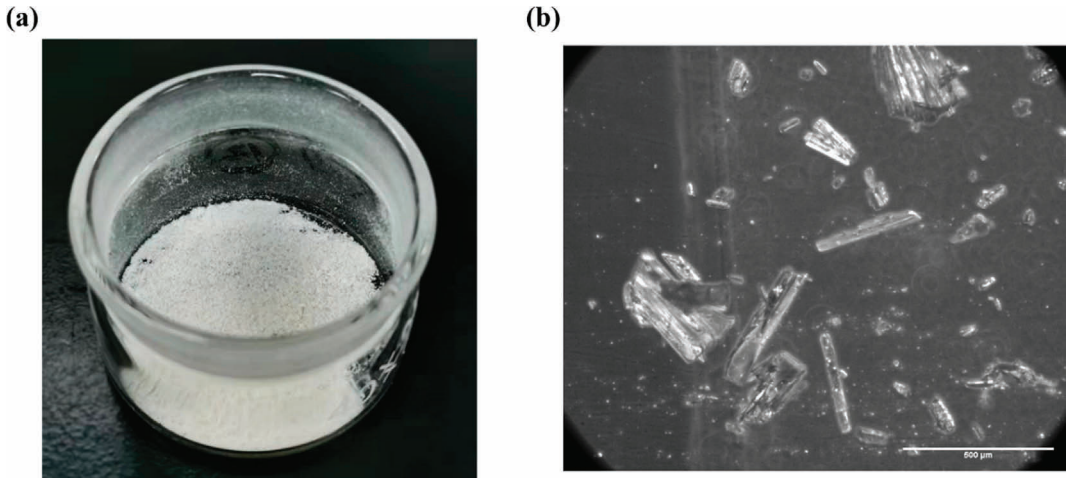

Fig. 7. Purified sinomenine hydrochloride crystal: (a) crystal; (b) micrograph.

Although the experimental RSD value was relatively large, the increase of the ethanol volume fraction still showed a tendency to increase the yield. Pure ethanol was not tried in this work due to the considerations of cost. In industry, ethanol needs to be recycled and it is expensive to obtain pure ethanol. Therefore, it was recommended to use ethanol with a volume fraction of 95% during the crystallization process.

In Fig. 7(a), sinomenine hydrochloride crystal prepared in the crystallization process was white. After the crystal was magnified ten times under an inverted fluorescence microscope (DMI3000B, Leica, Solms, Germany), rod-like structure could be seen, as shown in Fig. 7(b). The HPLC chromatogram of sinomenine hydrochloride crystal is shown in Fig. 2(d). It can be seen that the number of impurity peaks in the chromatography were significantly less than that in the chromatogram of *Caulis Sinomenii* extractor sinomenine hydrochloride stripping solution. It showed that the purification process could effectively remove impurities and prepare high purity sinomenine hydrochloride.

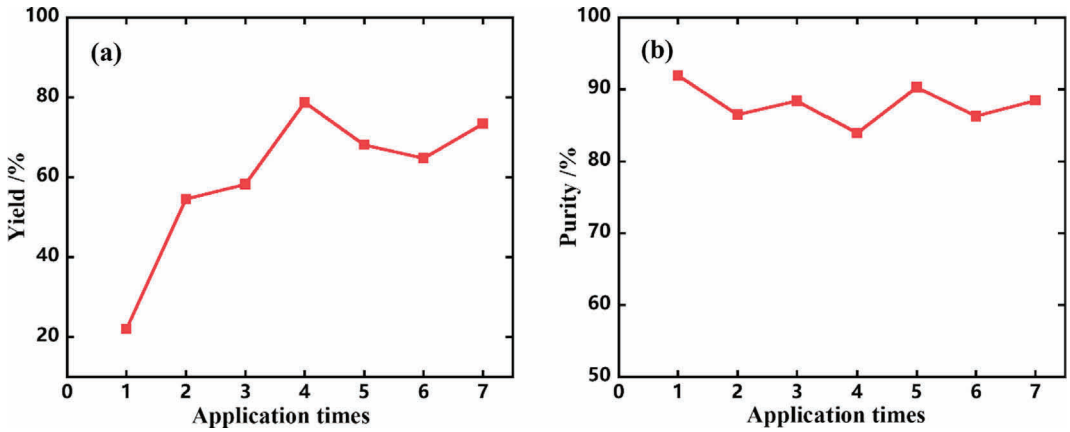

Fig. 8. The change of yield and purity of sinomenine hydrochloride crystal in cycle application study: (a) yield; (b) purity.

3.5. Recycling of 1-heptanol and sinomenine hydrochloride in the crystallization mother solution

In Section 3.4, more than 50% of sinomenine hydrochloride was left in the crystallization mother solution M_0. In order to obtain more sinomenine hydrochloride crystal product, step 7 in Fig. 1 was applied. In order to reduce the energy consumption of regenerating 1-heptanol, organic phase O_3 was used to extract a new batch of alkaline system E_1. Process parameters and material attributes were fixed in these recycling experiments as follows: X_1 = 10.5, X_2 = 1.5, X_3 = 3.0, X_4 = 2.5, X_5 = 4.5, X_6 = 95%, Z_1 = 3.150 mg·ml^{-1}, Z_2 = 32.75 mg·g^{-1}, and Z_3 = 10.72%.

The experimental results of recycling experiments are shown in Fig. 8. As can be seen from Fig. 8(a), with the increase of recycling times, the crystal yield of sinomenine hydrochloride increased gradually from about 20%, and finally tended to fluctuated at about 70%. It indicating that step 7 could significantly improve the yield of sinomenine hydrochloride.

It can be seen from Fig. 8(b) that after the optimization of the process parameters, the purity of sinomenine hydrochloride crystal (primary crystallization) reached more than 85%. With the increase of 1-heptanol recycling times, the purity of the crystal did not change significantly. These results indicated that recycling of 1-heptanol and sinomenine hydrochloride in the crystallization mother solution is feasible.

4. Discussions

In this study, 1-heptanol was used as the extractant, mainly due to the following considerations. 1-Butanol and 1-octanol were used to extract sinomenine in previous work [28]. These alcohols both showed large extraction capacity for sinomenine. However, the solubility of 1-butanol in water at 20 °C is 7.8% [34], which easily cause dissolution loss of 1-butanol. The boiling point of 1-octanol is 195 °C, which may cause high energy consumption in the recovery of 1-octanol. The solubility of 1-heptanol in water at 25 °C is about 0.1% [35], which is much lower than that of 1-butanol. The boiling point of 1-heptanol is 176.5 °C, which is significantly lower than that of 1-octanol. 1-Heptanol is also a low toxicity solvent [34].

Compared with the current industrial process, the new purification process of sinomenine hydrochloride proposed in this work showed several advantages. Firstly, sodium hydroxide was used instead of calcium hydroxide for alkali adjustment, which led to less solid waste. Secondly, 1-heptanol is a safer solvent compared with chloroform. Thirdly, the yield of the current process was about 70%, which was much higher than that of industrial process.

In Section 3.5, about 30% of sinomenine hydrochloride was lost during the purification process, which was due to four possible reasons. Firstly, about 12% sinomenine hydrochloride was lost due to dissolution in aqueous phase in steps 2 and 4. Secondly, about 3% sinomenine hydrochloride remained on the container wall during material transfer. Thirdly, about 5% of 1-heptanol containing sinomenine was lost after extraction and phase separation due to emulsification phenomena. Fourthly, less than 10% of sinomenine hydrochloride loss may be attributed to its degradation under alkaline conditions. Some researchers have confirmed that sinomenine will undergo oxidative polymerization under alkaline conditions and form bissinomenine [36].

In this work, 1-heptanol and sinomenine hydrochloride in the crystallization mother solution were recycled for seven times. The purity and yield of sinomenine hydrochloride seemed to be stable. If more recycling times were carried out, some impurities may accumulate in the system and result in a lower sinomenine hydrochloride purity value. At that time, it is suggested to regenerate 1-heptanol by distillation.

5. Conclusions

In this work, the D_{app} of sinomenine in the 1-heptanol–water system increased first and then decreased as equilibrium pH value increased. The first and second dissociation constants of sinomenine hydrochloride was calculated by fitting the D_{app} data, which were 8.27 and 11.24, respectively. The distribution coefficients of sinomenine and sinomenine hydrochloride in the 1-heptanol–water system were 27.4 and 0.0167, respectively.

A new purification process of sinomenine hydrochloride was designed. The new purification process included the following steps: alkalization, extraction, water washing, acid–water stripping, drying, and crystallization. 1-Heptanol was used as the extractant. Sinomenine hydrochloride purity in stripping solution increased with the increase of alkalization pH value, stripping pH value, and the purity of the sinomenine hydrochloride in *Caulis Sinomenii* extract. Sinomenine hydrochloride yield in stripping solution increased with the increase of 1-heptanol amount and stripping water amount, and decreased with the increase of the stripping pH value. Operation space was calculated with a method based on the probability to reach preset process standards. According to the verification experiments, the operation space was reliable. The recommended operating range of the CPPs was: alkalization pH value was 10.0–11.0, 1-heptanol amount was 2.0–1.0, stripping solution amount was 2.0–3.0, and stripping pH value was 4.0–5.0. The purity and yield of sinomenine hydrochloride crystals both increased as ethanol volume fraction increased

in crystallization process. Therefore, 95% ethanol was recommended to use in the crystallization process.

It was feasible to apply 1-heptanol and sinomenine hydrochloride in the crystallization mother solution cyclically. Compared with the current process in industry, the new purification process proposed in this study uses safer extractant, reduces solid waste, and increases sinomenine hydrochloride yield.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary Material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cjche.2022.04.022.

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