Chinese Herbal Medicines 11 (2019) 337–343

Contents lists available at ScienceDirect

Chinese Herbal Medicines

journal homepage: www.elsevier.com/locate/chmed

Original Article

Formulation optimization, *in situ* intestinal absorption and permeability of psoralen and isopsoralen

Wan-jin Sun[a,b,1], Peng Zhang[a,b,1], Xiang-wei Qu[a,b], Li-min Xu[c], Chun-bao Yang[c], Shi-ping Gu[a,b,*]

[a] *Hubei Provincial Hospital of Traditional Chinese Medicine, Wuhan 430061, China*
[b] *Hubei Province Academy of Traditional Chinese Medicine, Wuhan 430074, China*
[c] *The 264 Hospital of the People's Liberation Army, Taiyuan 030000, China*

ARTICLE INFO

Article history:
Received 15 February 2018
Revised 20 March 2019
Accepted 21 March 2019
Available online 30 May 2019

Keywords:
desirability function
D-optimal design
isopsoralen
optimization
psoralen
self-emulsifying drug delivery system

ABSTRACT

Objective: To optimize a self-emulsifying drug delivery system (SEDDS) formulation for psoralen and isopsoralen (PSO and IPSO) isolated from *Psoraleae Fructus*.
Methods: A D-optimal design was used to investigate the influence of oil percentage, surfactant percentage and cosurfactant percentage on several properties of SEDDS including particle size, polydispersity, equilibrium solubility, *in situ* intestine absorption rate and intestinal permeability. Furthermore, the desirability function approach was applied to obtain the optimal formulation for the system.
Results: The oil percentage, surfactant percentage and cosurfactant percentage were optimized to be 53.6%, 35.7% and 10.7%, respectively, which means the model is available.
Conclusions: The D-optimal design is valuable to optimize the SEDDS formulation and understand formulation compositions' functions on SEDDS properties.

1. Introduction

Self-emulsifying drug delivery systems (SEDDSs) are isotropic mixtures of oils, surfactants, sometimes containing cosolvents, and can be used to improve the oral absorption of highly lipophilic compounds. Due to their ability to present and maintain the drug in the dissolved state all over its transit through the gastrointestinal (GI) tract, SEDDSs have gained wide interests in the design of formulation (Nikolakakis & Partheniadis, 2017; Pangeni, Choi, Jeon, Byun, & Park, 2016). It can emulsify spontaneously to produce fine oil-in-water emulsions with a particle size between 100 nm and 300 nm when being introduced into an aqueous phase under gentle agitation (Gursoy & Benita, 2004).

Both psoralen (PSO) and isopsoralen (IPSO) are furan compounds of coumarins (Fig. 1), which have the activities of antivitiligo, anti-cancer, anti-bacteria, anti-virus and may also influence drug metabolism (Liu et al., 2016). Preliminary study proved PSO and IPSO to be poorly water-soluble components, with an aqueous solubility of 233.23 μg/mL and 131.05 μg/mL at 25 °C, respectively. The inferior solubility of them may impair their GI absorption. SEDDS is usually one of choices to solve the problem (Gursoy & Benita, 2004; Li et al., 2016).

As the critical factors for the oral bioavailability of poor water-soluble drugs, *in vivo* parameters are quite needed to be taken into our consideration when makes an optimization (Zidan et al., 2007). Ying Liu and coworkers used central composite design (CCD) to optimize and characterize an oridonin self-microemulsifying drug delivery system (SMEDDS) formulation. In their research, the technique of recirculation perfusion *in situ* was utilized to investigate the *in situ* intestinal absorption which was made a response in the optimization (Liu et al., 2009). In our study, single-pass intestinal perfusion experiments in rat small intestine were performed to get intestinal absorption rate and intestinal permeability of PSO and IPSO, all of which would be involved into our following optimization. In this way, we can get the products with the needed properties and satisfactory oral absorption.

2. Materials and methods

2.1. Materials

PSO and IPSO crystals were isolated from *Psoraleae Fructus* in Hubei Province Academy of Traditional Chinese Medicine. The separation and purification of the PSO and IPSO were done before this experiment, and the purity of PSO and IPSO were 98.5% and 98.3%,

* Corresponding author.
E-mail address: 1046625326@qq.com (S.-p. Gu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.chmed.2019.03.009

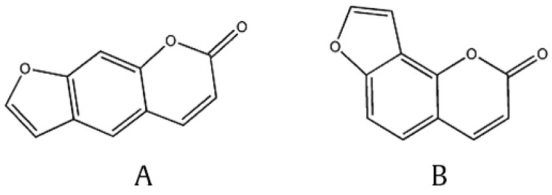

Fig. 1. Chemical structure of psoralen (A) and isopsoralen (B).

respectively. Reference standards of PSO and IPSO were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing China). Coconut oil was purchased from Jiande Qiandao Fine Chemical Industry Co., Ltd. Polysorbate 80 was purchased from Sigma-Aldrich (St. Louis, USA). Diethylene glycol monoethyl ether (Transcutol® P) was obtained from Gattefosse, France. Other chemical reagents were analytical grade.

2.2. SEDDS preparation

Solubility studies were carried out before this search, based on which, the SEDDS formulations used coconut oil as the oil phase, polysorbate 80 as the surfactant and transcutol P as the cosurfactant (Table 1). The possibility for increasing the absorption of PSO and IPSO was shown. All the formulation compositions used in the D-optimal design experiments were presented in Table 2, containing the same level of PSO and IPSO (4.0% of the vehicle, respectively), excluding studies for equilibrium solubility.

Table 1
Solubility of PSO and IPSO in different organic solvents ($n = 3$).

Organic solvents	Solubility / (mg·mL^{-1})	
	PSO	IPSO
Oil phase		
Ethyl oleate	32.92	29.20
Coconut oil	28.15	57.77
Peanut oil	21.10	24.76
Soybean oil	10.15	22.65
Labrafac	20.49	41.63
Surfactant		
Tween 80	80.62	117.24
Tween 85	52.38	91.06
Solutol HS 15	59.88	99.07
Cremophor EL	38.87	77.48
Cosurfactant		
PEG 400	109.92	187.41
Propylene glycol	18.93	30.54
Labrafil M 1944	11.69	46.65
Glycerine	2.08	3.51
Transcutol P	107.03	210.31

Table 2
Different compositions for preparations used in D-optimal design.

No.	Variable factors		
	Oil percentage (X_1)	Surfactant percentage (X_2)	Cosurfactant percentage (X_3)
1	0.698	0.204	0.098
2	0.648	0.205	0.147
3	0.700	0.245	0.055
4	0.642	0.273	0.085
5	0.599	0.273	0.128
6	0.528	0.322	0.149
7	0.545	0.354	0.101
8	0.578	0.371	0.051
9	0.500	0.421	0.079
10	0.400	0.452	0.148
11	0.450	0.434	0.116
12	0.450	0.492	0.058
13	0.400	0.493	0.107

Firstly, PSO and IPSO were dissolved in the mixture of transcutol P and coconut oil under an isothermal water bath at 37 °C, followed by the addition of polysorbate 80. Continually, the components were mixed by gentle vortexing until a transparent solution was formed. The same procedure was used to prepare the optimized formulation. The prepared formulations were stored at ambient conditions until further use.

2.3. Measurement of particle size and polydispersity

Before the measurement of particle size and polydispersity, each SEDDS (200 mg) sample was diluted with 200 mL of distilled water and gently stirred at 50 rev/min for 2 min using the dissolution tester (Gao, Sun, & Zhang, 2011) (Sotax A7 Dissolution Apparatus: Sotax Ltd, London, UK). The particle size/distribution and polydispersity of the emulsions was determined by photon correlation spectroscopy using a Zetasizer 3000 (Malvern Instruments, United Kingdom) able to measure sizes between 10 nm and 5000 nm. Light scattering was monitored at 25 °C at a 90° angle after external standardization with spherical polystyrene beads (220 ± 6 nm).

2.4. Equilibrium solubility

In Table 2, the preparation of the blank SEDDS with the composition was shown, which was absence of PSO and IPSO. Excess PSO and IPSO were added to each blank SEDDS and the mixtures were stirred for 48 h in a thermostatically controlled water bath (37 °C) until equilibrium. Preliminary study showed that the two compounds were stable in the mixture. The suspensions were centrifuged at 10000 × *g* for 15 min using a refrigerated table top centrifuge (Sigma 1-15 K, Sigma, Germany). The supernatant was filtered through a 0.45 μm membrane filter. The concentrations of PSO and IPSO were determined by HPLC after appropriate dilution with methanol.

2.5. In situ single pass intestinal absorption study

An *in situ* single pass intestinal perfusion technique was used for the absorption studies. The study was approved by the Ethical Committee of China Pharmaceutical University. Male Sprague-Dawley rats, weighing (180–220) g, were obtained from experimental Animal Center of Hubei Province (Animal certification number was SCXK: 2008-0005) and were divided into 13 groups with six in each group, three for PSO and the same for IPSO. All animals used in this study were handled in accordance with the guidelines of the Principles of Laboratory Animal Care.

All rats were fasted overnight and permitted free access to drinking water. Then, the rats were anesthetized by intraperitoneal injection of ethylcarbamate (100 mg/100 g body weight) and placed on a thermostatic surface maintained at 37 °C. To expose the abdominal content, about 3 cm incision was made. The intestine segment in perfusion was also exposed and incised at both sides of the segment. Then, it was rinsed with physiological saline, purged by air, and connected with the catheters to the perfusion system. The perfusate was prepared by dispersing the SEDDS containing PSO and IPSO in the Krebs-Ringer's solution (200 mL). The perfusion was started with the rate of 5 mL/min for 10 min to equilibrate the intestinal segment. Then, the rate was reduced to 0.2 mL/min. Samples were collected at an interval of 15 min in a pre-weighed glass tubes. All the samples were weighed and filtered through the 0.45 μm membrane filter, followed by the concentration detection of PSO and IPSO. At the end of the experiment, the rats were euthanized with a cardiac injection of saturated potassium chloride solution, and the intestine was removed and the length (cm) and diameter (mm) of the intestines were measured with digital

calipers (Miles, Lynch, & Sikes, 2015; Pires, Honda, & Cardoso, 2004).

After obtaining the weight, length and radius of intestine and the concentrations of PSO and IPSO of each sample, the absorption rate (K_a) was calculated. The whole small intestine (from duodenum to ileum) was investigated to all formulations in D-optimal design as the intestinal absorption behavior.

Absorption rate constant K_a and $C_{out(corr)}$ were calculated from Eqs. (1) and (2):

$$C_{out\,(corr)} = C_{out}\frac{Q_{out}}{Q_{in}} \tag{1}$$

and

$$K_a = \left(1 - \frac{C_{out\,(corr)}}{C_{in}}\right)\frac{Q}{\pi r^2 l} \quad V = \pi r^2 l \tag{2}$$

where C_{out} is the drug concentration in the exiting perfusate at the steady state.

Q_{out} is the measured flow rate (mL/min) of exiting intestinal perfusate at the specified time interval, calculated from the actual intestinal perfusate density (g/mL). The density of collected samples was determined by weighing the content using an electronic weighing balance of a known volume of perfusate.

Q_{in} is the flow rate (mL/min) of the perfusion solution entering the intestinal segment, $C_{out(corr)}$ is the corrected drug concentration in the exiting perfusate at the steady state.

The water flux corrected concentration of the compound measured in the exiting perfusate at the specified time interval (45, 60, 75, 90, 105 and 120 min); C_{in} is the drug concentration in the inlet of the perfusate entering the intestinal segment. Q is the perfusion rate (0.2 mL/min); r is the radius of the intestine (cm), and l is the length of the perfused intestinal segment (cm).

The single pass intestinal perfusion is based on reaching steady state with respect to the diffusion of compound across intestine. Steady state is confirmed by plotting the ratio of the outlet to inlet concentrations (corrected for water transport) *versus* time.

The rat single pass intestinal perfusion technique is a widely accepted method for the determination of intestinal drug permeability. Permeability calculations across rat's small intestine (P_{eff}) were performed from intestinal perfusate samples collected over 45–120 min (steady state). P_{eff} of PSO and IPSO was calculated using Eq. (3):

$$P_{eff} = \frac{\left\lfloor -Q_{in}\ln\left(C_{out\,(corr)}/C_{in}\right)\right\rfloor}{2\pi r l} \tag{3}$$

where Q_{in} is the flow rate (mL/min) of entering perfusate, $C_{out(corr)}$ is the water flux corrected concentration of the permeant in the exiting perfusate, r is the radius of the intestine (cm), and l is the length of the perfused intestinal segment (cm).

The statistical significance of the differences among group means were assessed using the one-way unweighted means analysis of variance (ANOVA) with the least significant difference (LSD) test and a value of $P < 0.05$ was considered statistically significance.

2.6. Analysis of PSO and IPSO

In this study, a modified HPLC/UV method was employed to simultaneously determine the concentration of PSO and IPSO in samples using a reversed phase HPLC (Waters 600, Waters Corporation, US). PSO and IPSO were separated by a C_{18} column (Shimadzu VP-ODS column, 250 mm × 4.6 mm) guarded with a precolumn (Shimadzu) and detected at 245 nm. The mobile phase consisted of methanol and water in a volume ratio of 55/45. The mobile phase was pumped at a flow rate of 1.0 mL/min. The column temperature was 30 °C. Totally, 20 μL of perfusate supernatant was injected into the HPLC system for analysis as described above.

Quantification of PSO and IPSO was based on peak areas. PSO and IPSO were separated with retention time of 8.9 min and 9.9 min, respectively. The resolution between them was about 2.8. In the concentration range of 0.8236–32.94 μg/mL (for PSO) and 1.140–45.60 μg/mL (for IPSO), peak area ratio correlated well to concentration (X): $Y = 3.2095 \times 10^4\ X - 8.4798 \times 10^3$, ($r = 0.9999$, $n = 6$) (for PSO) and $Y = 3.3592 \times 10^4\ X - 7.6896 \times 10^3$, ($r = 0.9999$, $n = 6$) (for IPSO). The limit of detection for PSO and IPSO were 0.6177 ng/mL and 0.912 ng/mL, respectively.

2.7. Experimental design

As a robust computer-aided design, D-optimal design was used in the study to optimize the formulation of SEDDS containing PSO and IPSO. In the three-component system, coconut oil (X_1), polysorbate-80 (X_2), and transcutol P (X_3) determined their mixtures' ability to form a self-emulsifying system and were used as the three formulation variables of the experimental design.

To refine the region of variables in the design, the following range of each component was selected based on preliminary investigations:

$40\% \le X_1 \le 70\%$
$20\% \le X_2 \le 50\%$
$5\% \le X_3 \le 15\%$

A design expert software package Design-Expert (version 7.0, Stat-Ease, Inc) was used to generate the design. The software selected a set of experimental points as a basic design consisted of 13 runs. The authentic experimental percentage of each component was recounted according to the actual weighing and was listed in Table 2. The observed responses of all candidate formulations were shown in Fig. 2. The fitting of a full quadratic model on the eight responses of the design was operated by Design-Expert 7.0.

2.8. Desirability function

Responses for SEDDS can be classified into responses to be minimized and responses to be maximized. The desirability function for the response to be minimized can be defined as Eq. (4):

$$d_i = \frac{Y_{\max} - Y_i}{Y_{\max} - Y_{\min}} \tag{4}$$

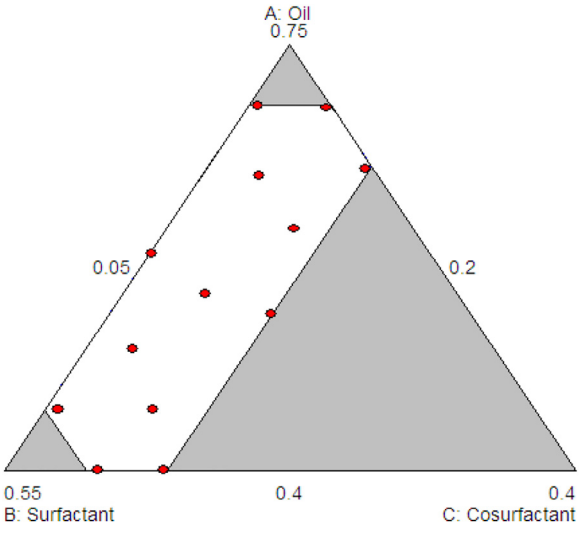

Fig. 2. Schematic presentations of experimental domain (white area) with domain of ternary diagram, constraints, and conducted experiments.

where Y_{max} is the maximum desired value for the response, Y_{min} is the minimum desired value for the response, Y_i is the experimental value. If Y_i is equal to or below Y_{min}, then d_i =1. If Y_i is higher than or equal to Y_{max}, then $d_i = 0$.

Inversely, for responses to be maximized, the desirability function is shown as Eq. (5):

$$d_i = \frac{Y_i - Y_{\min}}{Y_{\max} - Y_{\min}} \quad (5)$$

Y_{min}, Y_{max} and Y_i represent the values the same as in Eq. (4). If Y_i is equal to or below Y_{min}, then $d_i = 0$. If Y_i is higher than or equal to Y_{max}, then $d_i = 1$.

After obtaining the individual desirability for each response, the overall desirability function (D), defined as the geometric mean of all the d_i, is calculated as shown in Eq. (6):

$$D = \sqrt[n]{d_1 \cdot d_2 \cdot d_3 \cdot \ldots \cdot d_n} \quad (6)$$

where n is the number of the responses.

Based on the requirements of SEDDS, particle size and polydispersity index were minimized. The solubility, the intestinal absorption rate constant, and the intestinal permeability constant were maximized.

To obtain the condition on the design variables that maximize D, a three-dimensional graph of the response against the two factors (X_1, X_2) was plotted, from which the region corresponding to optimum values for D was yielded (Cruz Bournazou et al., 2017).

3. Results and discussion

3.1. Fitting of model to data

The experimental results were presented in Table 3. Quadratic model and cubic model were fitted to eight different responses.

Table 3
Results for experimental responses and D-optimal design.

No.	Particle size/ nm	Polydispersity index (PI)	Solubility (mg•mL^{-1})		Intestinal absorption rate constant / (min^{-1})		Intestinal permeability constant / (cm•min^{-1})	
			Psoralen	Isopsoralen	Psoralen	Isopsoralen	Psoralen	Isopsoralen
1	406.8	0.028	47.4	77.8	0.0416	0.0538	0.004	0.0052
2	449.5	0.035	44.8	75.4	0.0577	0.0622	0.0056	0.0061
3	472.4	0.034	49.6	78.6	0.0471	0.0485	0.0049	0.0046
4	335.1	0.087	58.2	91.2	0.0911	0.1013	0.0103	0.0107
5	295.3	0.091	63	95.6	0.0969	0.1013	0.0092	0.0095
6	254.6	0.102	67.4	99.2	0.1108	0.1195	0.0102	0.0108
7	239	0.114	70.4	104.2	0.1201	0.1302	0.0116	0.0128
8	252	0.107	67.2	96.4	0.1194	0.1144	0.0107	0.0111
9	191	0.127	79.6	113	0.1088	0.1178	0.0106	0.0115
10	178.7	0.136	90.2	118.8	0.0361	0.0463	0.0032	0.0044
11	167.1	0.13	85.6	117.8	0.0872	0.099	0.0083	0.0097
12	195.2	0.147	84.4	109.8	0.0694	0.0633	0.0068	0.0061
13	181	0.132	93.4	122.8	0.0398	0.0482	0.0038	0.0047

And the model was validated by analysis of variance (ANOVA). In each response, the model with a higher F-value was taken for the fitting model. It was shown that quadratic model was better for the fitting of all responses. The resultant equations for the eight responses were presented below:

$$\text{Particle size(nm)} = 1387.33\ X_1 + 1251.09\ X_2 + 13864.52\ X_3 - 3858.73\ X_1X_2 - 17876.25\ X_1X_3 - 18292.49 X_2X_3 \quad (7)$$

$$\text{Polydispersity index(PI)} = -0.30\ X_1 - 0.20\ X_2 - 2.19\ X_3 + 1.47\ X_1X_2 + 3.28\ X_1X_3 + 3.02\ X_2X_3 \quad (8)$$

$$\text{Solubility of psoralen (mg/mL)} = 0.1680\ X_1 + 95.25\ X_2 - 1121.90\ X_3 + 71.18\ X_1X_2 + 1338.12\ X_1X_3 + 1804.64\ X_2X_3 \quad (9)$$

$$\text{Solubility of isopsoralen(mg/mL)} = -6.53X_1 + 30.61X_2 - 2236.24X_3 + 266.67X_1X_2 + 2787.45X_1X_3 + 3365.90X_2X_3 \quad (10)$$

$$\text{Intestinal absorption rate of Psoralen}\left(\text{min}^{-1}\right) = -0.57X_1 - 1.19X_2 - 3.30X_3 + 3.79X_1X_2 + 5.52X_1X_3 + 4.18X_2X_3 \quad (11)$$

$$\text{Intestinal absorption rate of isopsoralen}\left(\text{min}^{-1}\right) = -0.58X_1 - 1.28X_2 - 6.20X_3 + 3.83X_1X_2 + 8.97X_1X_3 + 8.33X_2X_3 \quad (12)$$

$$\text{Intestinal permeability of Psoralen(cm/min)} = -0.054X_1 - 0.114X_2 - 0.492X_3 + 0.356X_1X_2 + 0.738X_1X_3 + 0.618X_2X_3 \quad (13)$$

$$\text{Intestinal permeability of isopsoralen(cm/min)} = -0.057X_1 - 0.125X_2 - 0.732X_3 + 0.368X_1X_2 + 1.026X_1X_3 + 0.967X_2X_3 \quad (14)$$

where X_1 is the fraction of Coconut oil in the formulation, X_2 is the fraction of polysorbate-80, and X_3 is the fraction of transcutol P. Table 4 listed the model summary statistics for the eight responses variables.

Table 4
Summary statistics of model.

Response		R^2	F	P
Particle size		0.9682	42.57	<0.0001
Polydispersity index		0.9714	47.60	<0.0001
Solubility	Psoralen	0.9951	283.10	<0.0001
	Isopsoralen	0.9947	263.97	<0.0001
Intestinal absorption rate constant	Psoralen	0.9924	182.94	<0.0001
	Isopsoralen	0.9824	78.01	<0.0001
Intestinal permeability constant	Psoralen	0.9821	76.89	<0.0001
	Isopsoralen	0.9764	58.02	<0.0001

3.2. Influence of formulation composition factors on particle size

As we known, the size of particle is an important value for assessing SEDDS. The smaller particle size was made, the bigger interfacial surface area for drug absorption was obtained. In addition, the smaller particle size could permit a faster release rate.

A mean particle size between 167 nm and 472 nm was obtained with these test formulations, which exhibited the formation of fine emulsions upon dilution. Visually, all the formulations appeared a bluish white emulsion within 1 min, indicating the rapid formation of emulsions. Based on the calculated model for the mean particle size (Table 3), Fig. 3 showed the contour plot of mean particle size. The oil and surfactant provided a larger contribution to the particle size, and the cosurfactant had less effect. The best optimized domain was obtained (the blue area in Fig. 3) when the oil percentage was less than about 61%, and meanwhile the surfactant percentage should be larger than about 29%.

3.3. Influence of formulation composition factors on polydispersity

The polydispersity index is the crucial parameter to distinguish the diameters, which spread in a multimodal distribution generated by SEDDS dispersion in aqueous media. A mean polydispersity index from 0.028 to 0.147 was obtained with these test formulations. Sensitivity between the formulation components and the polydispersity index was obtained. Based on the calculated model for the mean polydispersity index (Table 3), the contour plot is shown in Fig. 4. The result showed that the highest sensitivity of the obtained polydispersity index originated from variations in the fraction of the oil and the surfactant, otherwise the cosurfactant had less effect. Although the best optimized domain (the blue area in Fig. 4) was extremely small, nearly all the design points in the range explored can be accepted as proper polydispersity index for SEDDS.

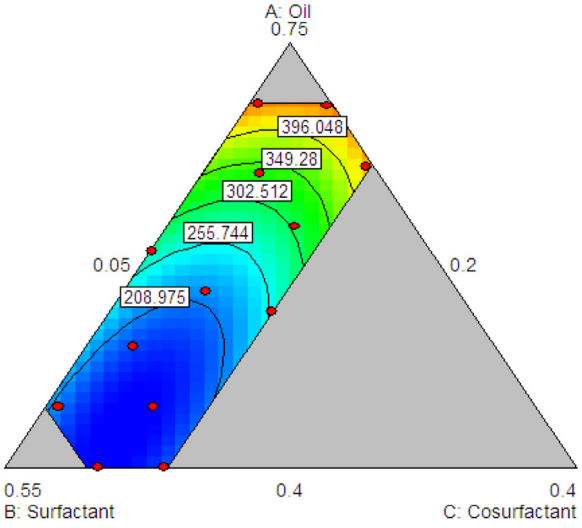

Fig. 3. Mixture's 2-D contour plot of mean particle size (nm).

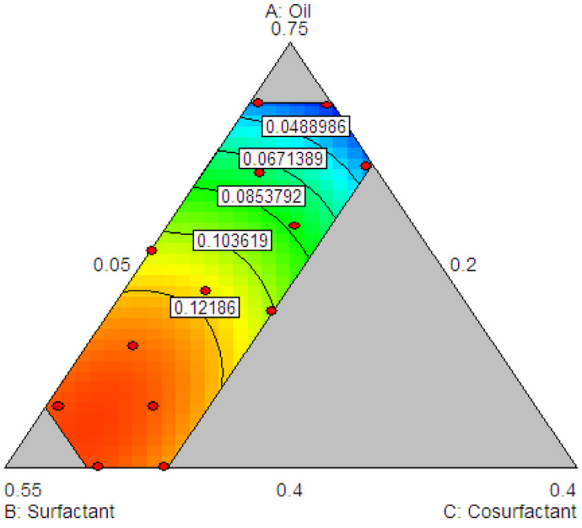

Fig. 4. Mixture's 2-D contour plot of mean polydispersity index (PI).

3.4. Influence of formulation composition factors on equilibrium solubility

SEDDS formulations should have good solvent properties to allow the existence of drug in solution. Both oil percentage, surfactant percentage and the cosurfactant percentage influence the solubility of two drugs in SEDDS to some extent. Based on the calculated model for the mean solubility (Table 3), the results for the contour plot were shown in Fig. 5. The oil and surfactant provided larger contribution to the solubility of PSO and IPSO than cosurfactant.

3.5. Influence of formulation composition factors on in situ intestinal absorption

Till now, the *in situ* single pass intestinal perfusion technique had been widely used in the research of drug intestinal absorption behavior, particularly at the primary stage of drug development. In this study, the influence of oil percentage, surfactant percentage and the cosurfactant percentage for the intestinal absorption were investigated. In Table 3, the results of intestinal absorption rate constant were shown, and the contour plot was shown in Fig. 6. It showed that oil percentage and surfactant percentage both had important effects on the intestinal absorption rate compared with cosurfactant percentage.

From the prediction data in model graphs generated by Design Expert 7.0, we got the information that intestinal absorption rate of psoralen rised up when the oil percentage increased from 46% to 62%, and it was decreased when the oil percentage was larger than 62%. It was supposed that when the oil percentage increased to a proper range (up to 62% in this study), more oil can be employed to form emulsion; More particles can be generated. On the other hand, because of more unnecessary amount of oil and less of surfactant, the enhancement of intestinal absorption may be weakened, corresponding to the decreased absorption rate. As to isopsoralen, the situation was almost the same.

3.6. Influence of formulation composition factors on intestinal permeability

The results of intestinal permeability were shown in Table 3, and the contour plot was shown in Fig. 7. It showed that oil percentage and surfactant percentage had a more important effect on the intestinal permeability compared to the cosurfactant percentage, and we got the information from the figures that the best optimized domain for intestinal permeability of PSO and IPSO (the red area in Fig. 7) obtained when the oil percentage was in range from about 50% to about 65%, and meanwhile the surfactant percentage should be in range from 28% to 41%. By single pass intestinal perfusion, we determined the drug permeability of both PSO and IPSO. As drugs with low solubility, both PSO and IPSO belonged to the second group of BCS (Mohsin et al., 2016). It is a must that we spare no effort to improve the bioavailability of this kind of drugs which is conformed to our objective in this study.

3.7. Optimization by desirability function

The aim of the optimization for pharmaceutical formulation is to find the levels of the variables that affect the chosen responses

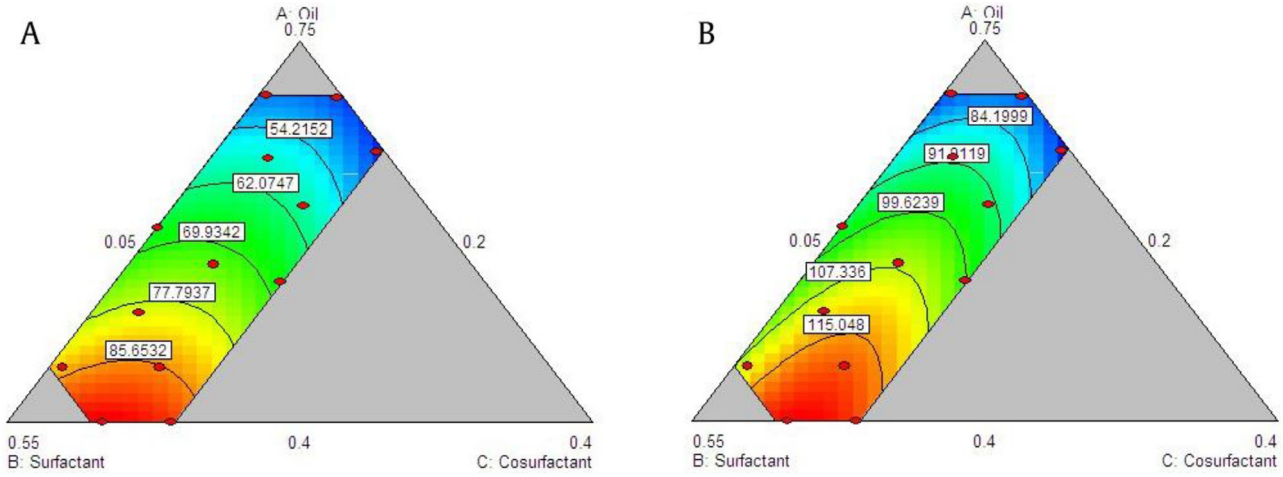

Fig. 5. Mixture's 2-D contour plots of psoralen solubility (A) and isopsoralen solubility (B).

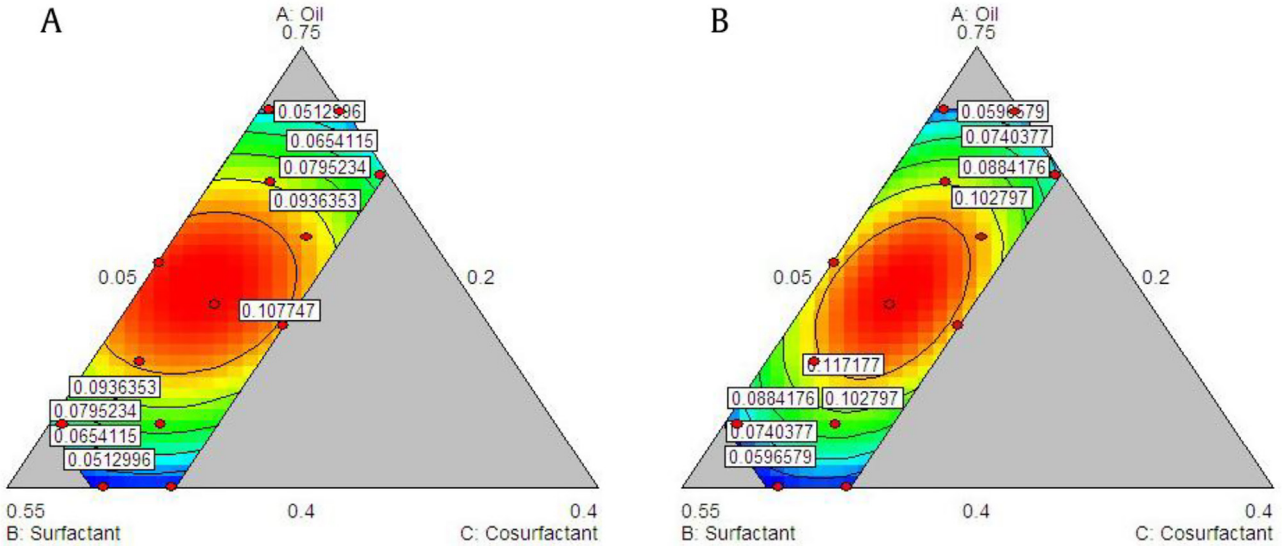

Fig. 6. Mixture's 2-D contour plots of intestinal absorption rate of psoralen (A) and isopsoralen (B).

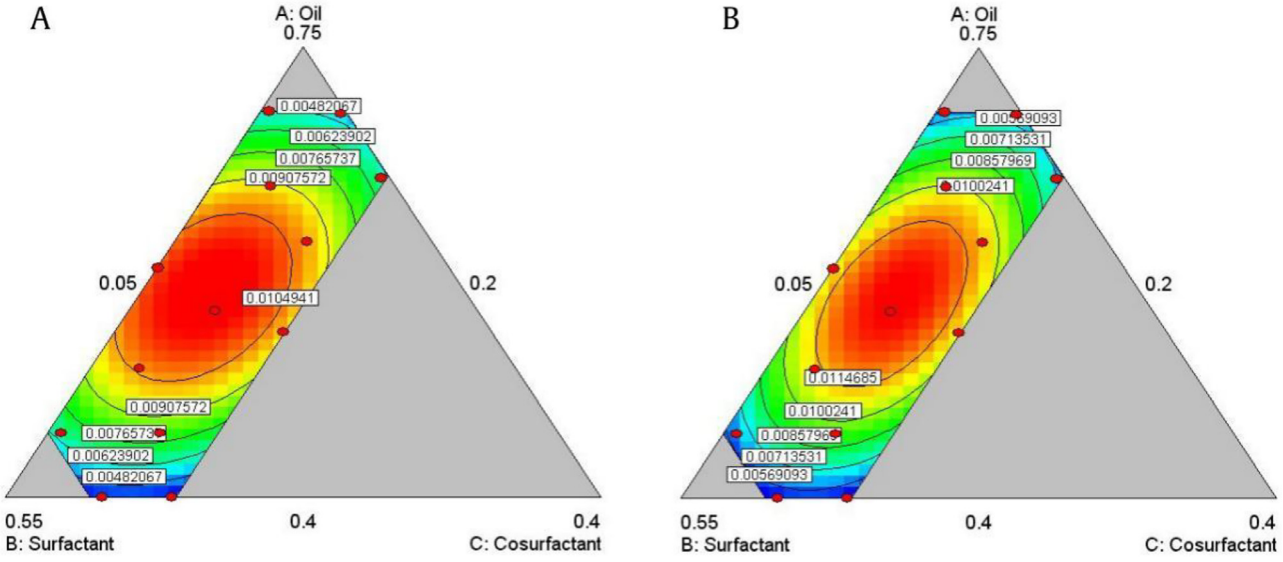

Fig. 7. Mixture's 2-D contour plots of intestinal permeability of psoralen (A) and isopsoralen (B).

and determine the levels of the variables. All the measured responses that may affect the quality of the product should be taken into consideration. Some of these responses must be minimized, such as the particle size and polydispersity index in this study. Some responses, such as the solubility, the intestinal absorption rate constant (K_a), the intestinal permeability constant of PSO and IPSO (P_{eff}), have to be maximized in order to produce a product of desired characteristics. Using the desirability function, all the defined responses can be combined into one overall response, the overall desirability (D). It was calculated by Eq. (6). The optimum formulation was obtained by the desirability function with the maximum value of D (0.730). In order to confirm the model adequacy for prediction, three batches of formulations under the optimum composition were prepared, and the eight responses were also evaluated respectively for each batch. The results were shown in Table 5. The results found that the model was validated since a fine agreement existed between the predicted and observed results. Thus, the oil percentage (X_1), surfactant percentage (X_2) and cosurfactant percentage (X_3) were optimized to be 53.6%, 35.7% and 10.7%, respectively.

Table 5
Predicted values and experimental results of SEDDS prepared under optimum conditions.

Response	Predicted value	Experimental value	Bias /%
Y_1, Droplet size (nm)	211.7	196.8 ± 14	7.6
Y_2, PI	0.119	0.108 ± 0.006	10.2
Y_3, Solubility of Psoralen (mg mL^{-1})	73.3	74.6 ± 2.5	1.7
Y_4, Solubility of Isopsoralen (mg mL^{-1})	107.6	103.6 ± 6.5	3.9
Y_5, Intestinal absorption rate of Psoralen (min^{-1})	0.1197	0.1282 ± 0.0012	6.6
Y_6, intestinal absorption rate of Isopsoralen (min^{-1})	0.1308	0.1314 ± 0.0014	0.5
Y_7, Intestinal permeability of Psoralen (cm min^{-1})	0.0117	0.0108 ± 0.0009	8.4
Y_8, Intestinal permeability of Isopsoralen (cm min^{-1})	0.0128	0.0121 ± 0.0011	5.8

4. Conclusion

In the studies performed to improve the solubility and bioavailability of poorly water-soluble drugs using SEDDS, the crucial factors for SEDDS properties and *in vivo* performances (Li et al., 2016) include the weight percentage of oil in the preparations (oil percentage) and the ratio of surfactant to cosurfactant (Sur/Co-s ratio). However, it is essential to find out how the formulation factors and the potential interactions between them affect the formulation characteristic. Therefore, an appropriate optimization technique is required to solve this problem and even to find the optimum formulation of SEDDS which can achieve the required physicochemical properties.

Since empirical approach through trial-and-error is time consuming, it is necessary to use more efficient ways to find out optimal formulation variables of SEDDS in cost-effective manner. Mathematical optimization in computer aid is suggested to shorten the experimental time and save resource.

In this study, the optimization of PSO and IPSO SEDDS formulation were investigated by D-optimal design combined with desirability function. The effects of oil percentage, surfactant percentage and the cosurfactant percentage on the particle size, polydispersity, equilibrium solubility, intestinal absorption rate and intestinal permeability were also carried out as well. The observed responses for the optimum formulation fairly agreed with the predicted values, which meaning the optimization procedure's excellent predictability. The study also showed that D-optimal design was really efficient for the modeling and optimization of PSO and IPSO SEDDS as well as the formulation factors influence to the properties of SEDDS.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgment

This work was funded by general project on PLA (CBJ15J002).

References

Cruz Bournazou, M. N., Barz, T., Nickel, D. B., Lopez Cardenas, D. C., Glauche, F., Knepper, A., et al. (2017). Online optimal experimental re-design in robotic parallel fed-batch cultivation facilities. *Biotechnology and Bioengineering, 114*(3), 610–619.

Gao, Y., Sun, W., & Zhang, J. (2011). Optimization of preparation and property studies on glycosylated albumin as drug carrier for nanoparticles. *Die Pharmazie, 66*(7), 484–490.

Gursoy, R. N., & Benita, S. (2004). Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. *Biomedicine and Pharmacotherapy, 58*(3), 173–182.

Li, C. C., Wang, T. L., Zhang, Z. Q., Yang, W. Q., Wang, Y. F., Chai, X., et al. (2016). Phytochemical and pharmacological studies on the genus *Psoralea*: A mini review. *Evidence-Based Complementary and Alternative Medicine, 6*(43), 1–17.

Liu, W., Pan, H., Zhang, C., Zhao, L., Zhao, R., Zhu, Y., et al. (2016). Developments in methods for measuring the intestinal absorption of nanoparticle-bound drugs. *International Journal of Molecular Sciences, 17*(7), 1–20.

Liu, Y., Zhang, P., Feng, N., Zhang, X., Wu, S., & Zhao, J. (2009). Optimization and *in situ* intestinal absorption of self-microemulsifying drug delivery system of oridonin. *International Journal of Pharmaceutics, 365*, 136–142.

Miles, F. L., Lynch, J. E., & Sikes, R. A. (2015). Cell-based assays using calcein acetoxymethyl ester show variation in fluorescence with treatment conditions. *Journal of Biological Methods, 2*(3), e29.

Mohsin, K., Alamri, R., Ahmad, A., Raish, M., Alanazi, F. K., & Hussain, M. D. (2016). Development of self-nanoemulsifying drug delivery systems for the enhancement of solubility and oral bioavailability of fenofibrate, a poorly water-soluble drug. *International Journal of Nanomedicine, 11*, 2829–2838.

Nikolakakis, I., & Partheniadis, I. (2017). Self-emulsifying granules and pellets: Composition and formation mechanisms for instant or controlled release. *Pharmaceutics, 9*(4), 50.

Pangeni, R., Choi, S. W., Jeon, O. C., Byun, Y., & Park, J. W. (2016). Multiple nanoemulsion system for an oral combinational delivery of oxaliplatin and 5-fluorouracil: Preparation and *in vivo* evaluation. *International Journal of Nanomedicine, 11*, 6379–6399.

Pires, A. E., Honda, N. K., & Cardoso, C. A. (2004). A method for fast determination of psoralens in oral solutions of phytomedicines using liquid chromatography. *Journal of Pharmaceutical and Biomedical Analysis, 36*, 415–420.

Zidan, A. S., Sammour, O. A., Hammad, M. A., Megrab, N. A., Habib, M. J., & Khan, M. A. (2007). Quality by design: Understanding the product variability of a self-nanoemulsified drug delivery system of cyclosporine *A. Journal of Pharmaceutical Sciences, 96*, 2409–2423.