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Optimization and *in situ* intestinal absorption of self-microemulsifying drug delivery system of oridonin

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ABSTRACT

The objective of this study was to optimize and characterize an oridonin self-microemulsifying drug delivery system (SMEDDS) formulation. A central composite design (CCD) was used to investigate the influence of factors (oil percentage and surfactant to co-surfactant ratio (Sur/Co-s ratio)) on the responses including droplet size, polydispersity, equilibrium solubility and *in situ* intestine absorption rate. Furthermore, the desirability function approach was applied to obtain the best compromise among the multiple responses. It was found that oil percentage played a significant role on the droplet size and polydispersity. The drug equilibrium solubility was mainly contributed to oil percentage and less to Sur/Co-s ratio. The *in situ* intestinal absorption was influenced by both of the two factors, whereas the oil percentage played a more important role in absorption. The practical response values under the optimized formulation were in good accordance with the predicted values. Our results demonstrate CCD is of value in optimizing the SMEDDS formulation and understanding the effects of formulation compositions on SMEDDS properties.

1. Introduction

In recent years, self-microemulsifying drug delivery systems (SMEDDS) have attracted growing interest as promising means for the delivery of poorly water-soluble drugs. SMEDDS have gained this popularity largely due to their excellent efficiency in improving the drug solubility, increasing the dissolution rate, promoting oral absorption for poorly water-soluble drugs and simplicity of preparation (Constantinides and Scalart, 1997; Hauss et al., 1998; Kommura et al., 2001; Holm et al., 2003; Wu et al., 2006a,b). SMEDDS are isotropic mixtures of oil, surfactant, co-surfactant and drug substance. Oil, surfactant, and co-surfactant are essential components in view of solubilizing the poorly water-soluble drug and forming fine microemulsion droplets after being introduced into the aqueous media under gentle agitation. Basically, type of each composition in SMEDDS formulation can be determined by solubility studies and phase behavior investigations (Kim et al., 2000; Kang et al., 2004). In addition, the weight percentage of oil in the preparations (oil percentage) and the ratio of surfactant to co-surfactant (Sur/Co-s ratio) seem to be closely related to the qualities of SMEDDS (Zidan et al., 2007). In this regard, it is necessary to know exactly how the preparation compositions determine the formulation characteristics; particularly how the formulation characteristics are influenced by the formulation factors and potential interactions between them. Therefore, an appropriate method is needed to analyze this issue and furthermore find the optimum formulation of SMEDDS achieving a required property.

Generally, the impact of each variable can be assessed by varying each variable while keeping others constant. However, it fails to take into account the interactions between these factors. Response surface methodology (RSM) is a suitable experimental design strategy to overcome this problem. Using RSM, the influence of the selected variables on the subject responses in a defined experimental region can be predicted by constructing mathematical models. The goodness of fit of the obtained mathematical models can be checked by statistical analysis. Therefore, RSM is a combination of mathematical and statistical techniques to analysis models and achieve the goal of optimizing the responses. Basically, the RSM can be classified into two categories: Box–Wilson central composite designs (CCD) and Box–Behnken designs. CCD is composed by the factorial experiment, axial points and center point. This structure makes it have a better prediction capability than the Box–Behnken design. CCD has been successfully used to optimize the technology or production conditions for drug delivery systems such as sustained-release tablets, liposomes, microspheres, nanoparticles in recent years (McCarron et al., 1999; Billon et al., 2000; Gløgård et al., 2002; Gil et al., 2006; Wu et al., 2006a,b). Furthermore, if conflict among the multiple responses occurred, it is difficult to optimize

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Table 1
Composition of preparations used in central composite design

No.	Oil percentage, X_1 (%)	Sur/Co-s ratio, X_2
1	48.41	3.56
2	48.41	1.44
3	16.59	3.56
4	16.59	1.44
5	55.00	2.50
6	10.00	2.50
7	32.50	4.00
8	32.50	1.00
9–13	32.50	2.50

Table 2
Factor levels and the correspondent values

Factor	Level				
	$-\alpha$	−1	0	+1	$+\alpha$
X_1 (oil percentage)	10.00	16.59	32.50	48.41	55.00
X_2 (Sur/Co-s ratio)	1.00	1.44	2.50	3.56	4.00

$\alpha = 1.414$.

all the responses simultaneously. A desirability function approach was commonly employed to find the best compromise condition (Ficarra et al., 2002; Pizarro et al., 2006).

In a previous study, we developed a SMEDDS for delivering oridonin, an active compound isolated from the Chinese herb *Raddosia rubescens* (*Hamsl.*) *Hara*. The formulation consisted of Maisine 35-1 and Labrafac CC (1:1, w/w), Cremophor EL and Transcutol P. The system exhibited the potential for increasing the bioavailability of oridonin, which provided a promising approach to the delivery oridonin by the oral route (Zhang et al., 2008).

The aim of the present study was to acquire a clear understanding of the influence of formulation compositions on the properties of SMEDDS and obtain an optimal formulation for oridonin SMEDDS. CCD was used to study the effect of formulation variables (oil percentage and Sur/Co-s ratio) on the response variables including droplet size, polydispersity, equilibrium solubility and intestinal absorption rate. Furthermore, the desirability function approach was used to simultaneously optimize the responses. The optimal preparation was characterized by morphological observation and *in vitro* release test besides the evaluation of properties shown in CCD experiments.

2. Materials and methods

2.1. Materials

Oridonin (purity 98.2%) was purchased from Nanjing Qingze Medical Technology Development. Co., Ltd. (Nanjing, China). Polyoxyethyleneglycerol triricinoleate 35 caster oil (Cremophor® EL) was a gift from BASF, Germany. Glyceryl monolinoleate (Maisine® 35-1), caprylic/capric triglyceride (Labrafac CC), and diethylene glycol monoethyl ether (Transcutol® P) were supplied by Gattefosse, France. All other chemicals used were of analytical grade.

2.2. Preparation of SMEDDS

Table 1 presents all of the formulation compositions used in the central composite design experiment. All the formulations contain the same level of oridonin (0.5% (w/w) of the vehicle) except those for equilibrium solubility studies. The oridonin SMEDDS was prepared as described previously (Zhang et al., 2008). Briefly, each formulation was prepared by dissolving oridonin in the mixture of Transcutol P and Maisine 35-1 at 50 °C in an isothermal water bath, followed by the addition of Cremophor EL and Labrafac CC. Then, the components were mixed by gentle vortexing until a transparent preparation was obtained. An optimized formulation was prepared with the same method.

2.3. Experimental design

2.3.1. Central composite design

The oil percentage or content in the formulation (oil%, w/w), the ratio of surfactant to co-surfactant (Sur/Co-s ratio) as well as the drug content were reported to affect the properties of SMEDDS (Wu et al., 2006a,b; Zidan et al., 2007). The drug content in this study is capable of meeting the needs of medical use, therefore we kept the drug content as a fixed concentration. Based on the preliminary experiments and our previous studies, two formulation parameters, the oil percentage and Sur/Co-s ratio, were identified as key factors responsible for the properties of SMEDDS. In view of the feasibility of SMEDDS formation at the extreme values, the ranges of the two factors were determined as follows: oil percentage (X_1): 10–50%; Sur/Co-s ratio (X_2): 1–4. Four responses include droplet size (Y_1), polydispersity index (PI) (Y_2), equilibrium solubility (Y_3), and intestinal absorption rate (Y_4) since they are generally regarded as significant factors for assessing the qualities of SMEDDS. A two-factor, five-level CCD was undertaken to investigate the main effects and the interactions of the two factors on the four responses (Table 2). The design consists of 9 runs (4 factorial points, 4 star points and 1 center point) and 4 replicated runs (center points) yielding 13 experiments in total (Table 3). The purpose of the replication was to estimate experimental error and increase the precision.

The data obtained for the four responses in each trial were fitted to classical second-order polynomial model and third-order quadratic model. The mathematical models were expressed as

Table 3
Experimental responses and result of central composite design

No.	Particle size (nm)	Polydispersity index (PI)	Solubility (mg/ml)	Intestinal absorption rate constant (h^{-1})	D
1(+1, +1)	34.2	0.018	15.28	0.177	0.180
2(+1, −1)	42.1	0.089	22.66	0.256	0.241
3(−1, +1)	20.3	0.084	35.94	0.199	0.383
4(−1, −1)	22.3	0.127	39.56	0.562	0.545
5(+α, 0)	42.0	0.057	9.87	0.390	0.141
6(−α, 0)	19.0	0.144	36.56	0.388	0.230
7(0, +α)	25.5	0.027	23.74	0.274	0.500
8(0, −α)	29.7	0.033	24.89	0.587	0.670
9(0, 0)	25.9	0.060	20.43	0.651	0.629
10(0, 0)	25.5	0.043	20.35	0.671	0.667
11(0, 0)	26.5	0.017	18.48	0.638	0.653
12(0, 0)	25.9	0.032	19.01	0.622	0.642
13(0, 0)	26.8	0.020	18.04	0.635	0.638

follows:

second-order polynomial model :

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1^2 + b_4X_2^2 + b_5X_1X_2 \quad (1)$$

where X_1 and X_2 correspond to the studied factors, Y is the measured response, b_0 is an intercept, b_1–b_5 are the regression coefficients.

third-order quadratic model :

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1^2 + b_4X_2^2 + b_5X_1X_2 + b_6X_1^2X_2 + b_7X_1X_2^2 \quad (2)$$

where X_1, X_2, Y, b_0, and b_1–b_7 represent the same meaning with those shown at Eq. (1).

Data was analyzed by nonlinear estimation using STATISTICA 6.0 software. *F*-test was used to evaluate lack of fit within each model and identify the fitting model. Response surface delineation was performed according to the fitting model. Graphs of surface responses were plotted with the response variation against the two factors. Further optimization was carried out by the following desirability function.

2.3.2. Desirability function

All of the four responses in the study should be evaluated in the optimization of oridonin SMEDDS. However, it is almost impossible to optimize all the objectives simultaneously because they do not coincide with each other and conflict may occur between them. The optimum condition reached in one response may have an opposite influence on another response. For the purpose of finding the best compromising formulation for all responses, the multicriteria problem can be treated as single criterion problem by using the desirability function approach. The desirability function for the response to be minimized can be defined as:

$$d_i = \frac{Y_{max} - Y_i}{Y_{max} - Y_{min}} \quad (3)$$

where Y_{min} represents the lowest possible value; Y_{max} represents the highest possible value and Y_i indicates the experimental value. In addition, 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 $d_i = 0$. On the contrary, for a response to be maximized, the desirability function is defined as:

$$d_i = \frac{Y_i - Y_{min}}{Y_{max} - Y_{min}} \quad (4)$$

where Y_{min}, Y_{max} and Y_i represent the values as the same as those expressed in Eq. (3). 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 $d_i = 1$. According to the observed response values, the limits were selected for Y_1: $Y_{max} = 45$ (largest tolerable droplet size) and $Y_{min} = 18$ (desired droplet size); Y_2: $Y_{max} = 0.145$ (largest tolerable dispersity) and $Y_{min} = 0.015$ (desired dispersity); Y_3: $Y_{max} = 40$ (highest solubility) and $Y_{min} = 9.5$ (lowest tolerable solubility); Y_4: $Y_{max} = 0.68$ (the largest absorption rate) and $Y_{min} = 0.17$ (lowest absorption rate). Among these objectives, Y_1 and Y_2 were minimized, while Y_3 and Y_4 were maximized.

After obtaining the individual desirability for each response, an overall desirability (D), a global desirability function, is calculated by combining the individual desirability using the geometric mean as shown in Eq. (5).

$$D = \left(\prod_{i=1}^{k} d_i\right)^{1/k} \quad (5)$$

where k is the number of the responses.

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.

2.4. Characterization of SMEDDS

2.4.1. Droplet size and polydispersity

Prior to the droplet size and polydispersity measurement, each oridonin SMEDDS (200 mg) was diluted with distilled water (20 ml) and gently stirred. The droplet size/distribution and polydispersity were determined with Nicomp™ 380 ZLS Zeta Potential/Particle sizer (PSS Nicop, Santa Barbara, CA, USA). The measuring conditions were: He–Ne laser; angle, 90°; temperature, 23 °C; reflection index, 1.333; wavelength, 635 nm. Each sample was analyzed in triplicate.

2.4.2. Equilibrium solubility

The blank SMEDDS (absence of drug) was prepared with the composition shown in Table 1. Excess oridonin was added to each blank SMEDDS and the mixtures were stirred for 48 h at 37 °C in a thermostatically controlled water bath until they reached equilibrium. The samples were centrifuged at 2500 × *g* for 20 min. The supernatant was filtered through a membrane filter (0.45 μm). The obtained filtrate was diluted with methanol. The concentration of oridonin was determined by HPLC (Zhang et al., 2008). The calibration curve was linear in the range of 2–100 μg/ml ($r = 0.9999$).

2.4.3. In situ intestinal absorption study

An *in situ* recirculation perfusion technique was used and performed as the methods reported previously (Rubinstein et al., 1991; Yuan et al., 2004; Wang et al., 2007). Male Sprague–Dawley rats (180–220 g) were supplied by the Laboratory Animal Center of Shanghai University of Traditional Chinese Medicine. The experimental procedures were approved by the institutional animal ethical committee and were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Rats were fasted overnight and permitted free access to drinking water. The rat was anesthetized by intraperitoneal injection of ethylcarbamate (100 mg/100 g body weight) and placed on a thermostatic surface maintained at 37 °C. An incision approximately 3 cm was opened through a midline to expose the abdominal content. The intestine segment to be in perfusion was exposed and incisions were made at both sides of the segment. Then, it was rinsed with physiological saline, and purged by air, followed by being connected with the catheters to the perfusion system. The perfusate was prepared by dispersing oridonin SMEDDS in the Krebs–Ringer's solution (200 ml) containing phenol red (20 μg/ml) at 37 °C. The perfusion was started by recirculation with the rate of 5 ml/min for 10 min. Then, reduced the rate to 2.5 ml/min. Samples (5 ml) were taken at 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 h, and the same amount (5 ml) of Krebs–Ringer's solution containing phenol red (20 μg/ml) was added to the perfusate. The sample was filtered through the membrane filter (0.45 μm), followed by the concentration detection of oridonin and phenol red. For the purpose of correcting the perfusion volume, phenol red was used. The concentration of phenol was determined by UV detection at 558 nm. After obtaining the remaining drug at each time point, the absorption rate (K_a) was calculated from the slope of a plot of the log of remaining drug versus time. The intestinal absorption behavior of all formulations in CCD was comparatively studied at the whole small intestine (from duodenum (2 cm from the pyloric sphincter) to ileum (immediately proximal to the cecum)).

2.4.4. Morphology observation

The morphology of Oridonin SMEDDS prepared under the optimum condition was observed by transmission electron microscope (TEM) (PHILIPS TECNAI 12, The Netherlands). The sample preparation method was the same with the previous report (Zhang et al., 2008).

2.4.5. Self-microemulsifying time

1.0 g of each formulation was individually introduced to 100 ml purified water at 37 °C with the stirring rate of 50 rpm. Aliquots of 2 ml were taken at the time intervals of approximately 25 s and the absorbance was measured at 400 nm immediately (UV754N, Lengguang, China). The time when the absorbance was equal to that determined at 20 min was defined as self-microemulsifying time. Each sample was analyzed in triplicate.

2.4.6. In vitro dissolution study

Reverse dialysis method was reported to be a suitable method to evaluate SMEDDS *in vitro* dissolution behavior. Compared with ultrafiltration method and dialysis method, it can better simulate the circumstance *in vivo* (Levy and Benita, 1990; Chen et al., 2008). Therefore, reverse dialysis technique was applied to investigate *in vitro* dissolution of oridonin SMEDDS in this study. The medium was 250 ml phosphate buffer saline (PBS, pH 6.8) without enzymes. The stirring rate was 50 rpm. The temperature was maintained at 37 °C. Six dialysis bags with the same size were prepared and soaked in purified water for 12 h to be ready for use. The bags were sealed closely by clamps after 2 ml of PBS solutions were dropped into each bag. And then, bags were immersed in the medium to equilibrium for 12 h at 37 °C. A mass of each formulation was accurately weighed and introduced into the dissolution medium. Dialysis bag was taken out individually at 10, 30, 60, 90, 120 min and 12 h, respectively. At the same time, 2 ml PBS (37 °C) was added to the curette. The sample was carefully withdrawn from the dialysis bag, and 20 μl was injected into HPLC.

3. Results and discussion

3.1. Central composite design

The experimental results are reported in Table 3. All the data were computed by STATISTICA 6.0 software. The four responses were individually fitted to second-order polynomial model and third-order quadratic model. Each obtained model was validated by ANOVA. For each response, the model, which generated a higher *F*-value, was identified as the fitting model. The fitting model for each response and statistical evaluation results are shown in Table 4.

3.1.1. Influence of formulation composition factors on the droplet size

Droplet size is a critical value for assessing SMEDDS. The smaller droplet size provides a larger interfacial surface area for drug absorption. In addition, it was suggested that the smaller droplet size permit a faster release rate (Kang et al., 2004). From the levels of significance for regression coefficients shown in Table 4, it was deduced that the oil percentage contributed to the regression model. Fig. 1(a) shows the response surface for droplet size and illustrated the relationship between the droplet size and the two

Table 4
Regression coefficients and statistical analysis

Model fitting	Factor	Factor coefficient	*P*-value	ANOVA
Third-order quadratic model (droplet size)	Intercept	27.2165	0.0084	$F = 3408.43$, $R^2 = 0.9968$
	Oil content	−0.3737	0.2902	
	Sur/Co-s ratio	−3.8323	0.4360	
	(Oil content)^2	0.0186	0.0074	
	(Sur/Co-s ratio)^2	0.2747	0.7572	
	(Oil content)(Sur/Co-s ratio)	0.0706	0.6894	
	(Oil content)^2(Sur/Co-s ratio)	−0.0037	0.0776	
	(Oil content)(Sur/Co-s ratio)^2	0.0164	0.5412	
Third-order quadratic model (PI)	Intercept	0.4344	0.0661	$F = 23.75$, $R^2 = 0.9214$
	Oil content	−0.0246	0.0422	
	Sur/Co-s ratio	−0.0551	0.6892	
	(Oil content)^2	0.0004	0.0245	
	(Sur/Co-s ratio)^2	−0.0074	0.7703	
	(Oil content)(Sur/Co-s ratio)	0.0046	0.3763	
	(Oil content)^2(Sur/Co-s ratio)	−0.0001	0.0952	
	(Oil content)(Sur/Co-s ratio)^2	0.0003	0.7263	
Second-order polynomial model (solubility)	Intercept	71.1438	0.0002	$F = 161.31$, $R^2 = 0.9379$
	Oil content	−1.2551	0.0099	
	Sur/Co-s ratio	−16.0088	0.0257	
	(Oil content)^2	0.0124	0.0242	
	(Sur/Co-s ratio)^2	3.2664	0.0120	
	(Oil content)(Sur/Co-s ratio)	−0.0557	0.5346	
Second-order polynomial model (intestinal absorption rate)	Intercept	−0.0612	0.8292	$F = 93.65$, $R^2 = 0.9071$
	Oil content	0.0266	0.0252	
	Sur/Co-s ratio	0.3548	0.0486	
	(Oil content)^2	−0.0006	0.0010	
	(Sur/Co-s ratio)^2	−0.1193	0.0023	
	(Oil content)(Sur/Co-s ratio)	0.0042	0.1014	
Third-order quadratic model (D)	Intercept	0.7671	0.0733	$F = 382.48$, $R^2 = 0.9890$
	Oil content	0.0258	0.1813	
	Sur/Co-s ratio	−0.7653	0.0236	
	(Oil content)^2	−0.0010	0.0068	
	(Sur/Co-s ratio)^2	0.1354	0.0280	
	(Oil content)(Sur/Co-s ratio)	0.0271	0.0271	
	(Oil content)^2(Sur/Co-s ratio)	1.600×10^{-5}	0.8591	
	(Oil content)(Sur/Co-s ratio)^2	−0.0053	0.0098	

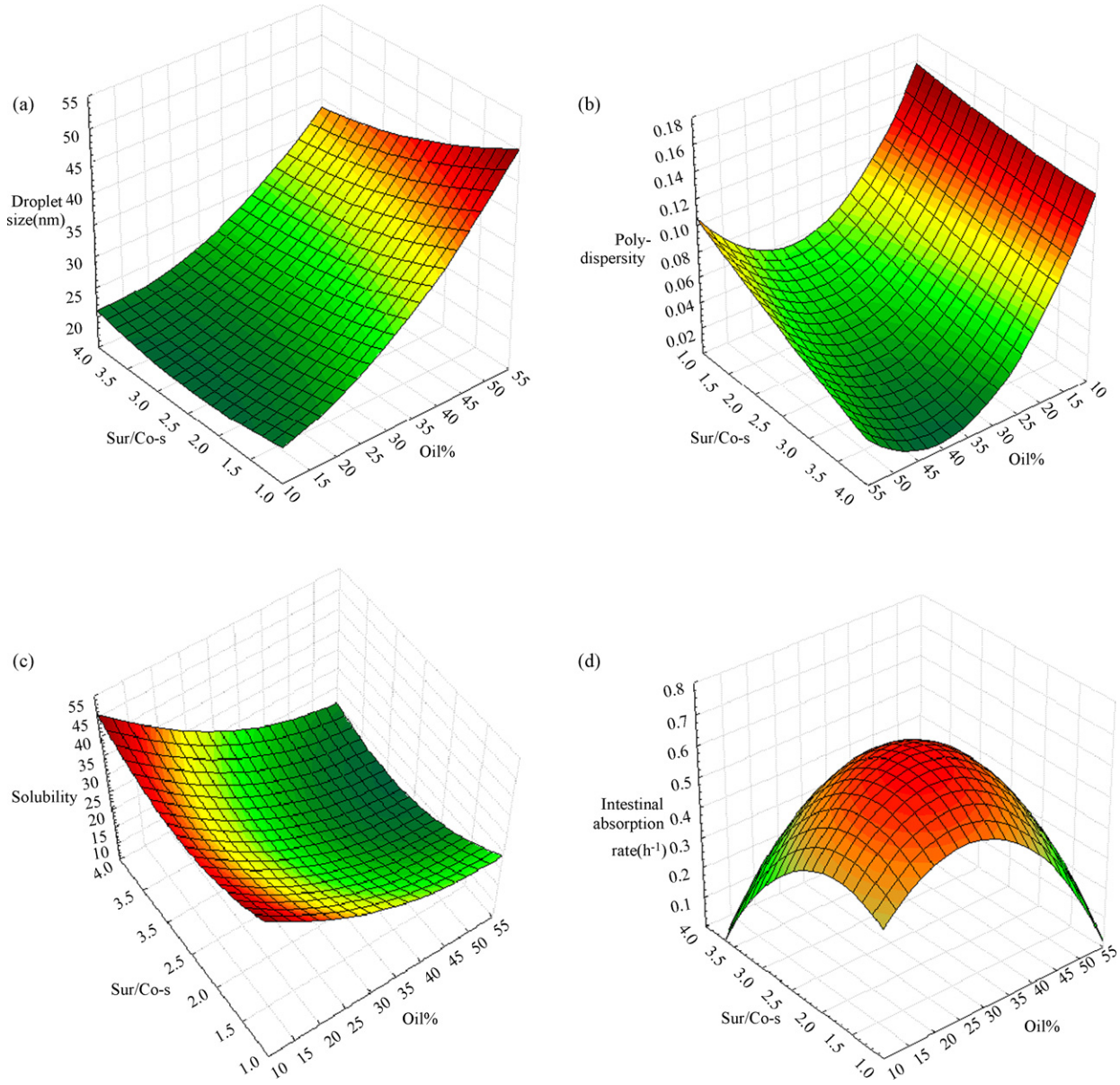

Fig. 1. Response surface for (a) droplet size; (b) polydispersity (PI); (c) equilibrium solubility; (d) intestinal absorption rate as a function of oil% and Sur/Co-s ratio.

factors (oil percentage and Sur/Co-S ratio). A droplet size of approximately 20–30 nm can be seen at the domain with oil percentage less than 35% and Sur/Co-S ratio varying from 1.5 to 3.5.

3.1.2. Influence of formulation composition factors on polydispersity

The polydispersity index is a very important parameter to evaluate the diameter spread in a multimodal distribution generated by SMEDDS dispersion in aqueous media. PI was significantly influenced by the oil percentage ($P < 0.05$), whereas Sur/Co-s ratio was of low significance (Table 4). Fig. 1(b) shows an optimal range of the oil percentage for achieving a minimum PI is around 30–40% within the whole Sur/Co-s ratio range.

3.1.3. Influence of formulation composition factors on equilibrium solubility

SMEDDS formulations should have good solvent properties to allow presentation of drug in solution. Both oil percentage and Sur/Co-s ratio influence the solubility of drug in SMEDDS to some extent. The effect of oil percentage on the oridonin solubility in the formulation was highly significant, whereas Sur/Co-s ratio played a less role. In addition, variables including (oil percentage)^2 and (Sur/Co-s ratio)^2 were also significant (Table 4). As shown in Fig. 1(c), an increase in oil percentage led to a decrease of solubility when the Sur/Co-s ratio maintained constant. Based on the previous study, it was found the sequence of oridonin solubility was Maisine 35-1/Labrafac CC(1:1) < Cremophor EL < Transcutol P. Therefore, it is reasonable to surmise that the content of Transcutol P may be mainly responsible for the solubilizing capability of the SMEDDS. Correspondingly, increasing the oil percentage will lower the drug solubility.

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

The *in situ* recirculation perfusion technique was widely used in studying the drug intestinal absorption behavior particularly at

the primary stage of drug development. In this study, it was used to evaluate the influence of oil percentage and Sur/Co-s ratio on the intestinal absorption. The results of intestinal absorption rate constant are shown in Table 3. As shown in Table 4, both oil percentage and Sur/Co-s ratio had an important effect on the intestinal absorption rate ($P < 0.05$). As shown in Fig. 1(d), the intestinal absorption rate boosted when the oil percentage increased from 10% to approximately 25%, while it decreased when the oil percentage was larger than 25%. It is assumed that with the oil percentage increasing in a proper range (up to 25% in this study), more oil can be employed to form microemulsion; more droplets can be generated. However, the enhancement of intestinal absorption may be weakened with more unnecessary amount of oil and less of surfactant and co-surfactant, corresponding to the decreased absorption rate.

3.2. Optimization by desirability function

A further optimization process was undertaken with desirability function to optimize the four responses simultaneously. The responses: droplet size (Y_1), polydispersity index (Y_2), equilibrium solubility (Y_3) and absorption rate in rat intestine (Y_4) were transformed into the desirability scale d_1, d_2, d_3 and d_4, respectively. Among them, Y_1, Y_2 had to be minimized, while Y_3, Y_4 had to be maximized. The overall objective function (D) was calculated by Eq. (4) and the results were shown in Table 3. The model was fitted with a second-order polynomial expression and a third-order polynomial expression. The higher coefficient of determination and F value in terms of the third-order polynomial expression indicated the goodness of fit (shown in Table 4). Fig. 2(a) shows the response surface for D holding variable X_1 and X_2. The region where optimization was studied was the gray domain marked at Fig. 2(a). We sought the maximum value of D in this region (X_1: 20–38; X_2: 1.0–3.3). In detail, computer script calculation was performed by Visual Basic language with step width of 0.1. The functional expression used in script calculation was the regression equation shown in Table 4 (D). After 4140 run, the maximum function value was obtained at X_1: 24.2 and X_2: 1. The corresponding D value was 0.7126, which is higher than the maximum experimental value (0.6773). To confirm the model adequacy for prediction, five batches of formulations under the optimum composition were prepared, and the four responses were evaluated respectively for each formulation. The results were shown in Table 5. The model was proved to be validated since a fine agreement existed between the predicted and observed results. Thus, the oil percentage (X_1) and Sur/Co-s ratio (X_2) were optimized to be 24.2% and 1, respectively. Namely, the optimal formulation of SMEDDS containing oridonin was: Maisine 35-1 and Labrafac CC (1:1, w/w) of 24.2%, Cremophor EL of 37.9% and Transcutol P of 37.9%.

3.3. Evaluation of the optimized formulation

The morphology was shown in Fig. 3. The droplets were shown to be spherical with narrow droplet size dispersion. The results of other evaluations including droplet size, PI, the equilibrium solu-

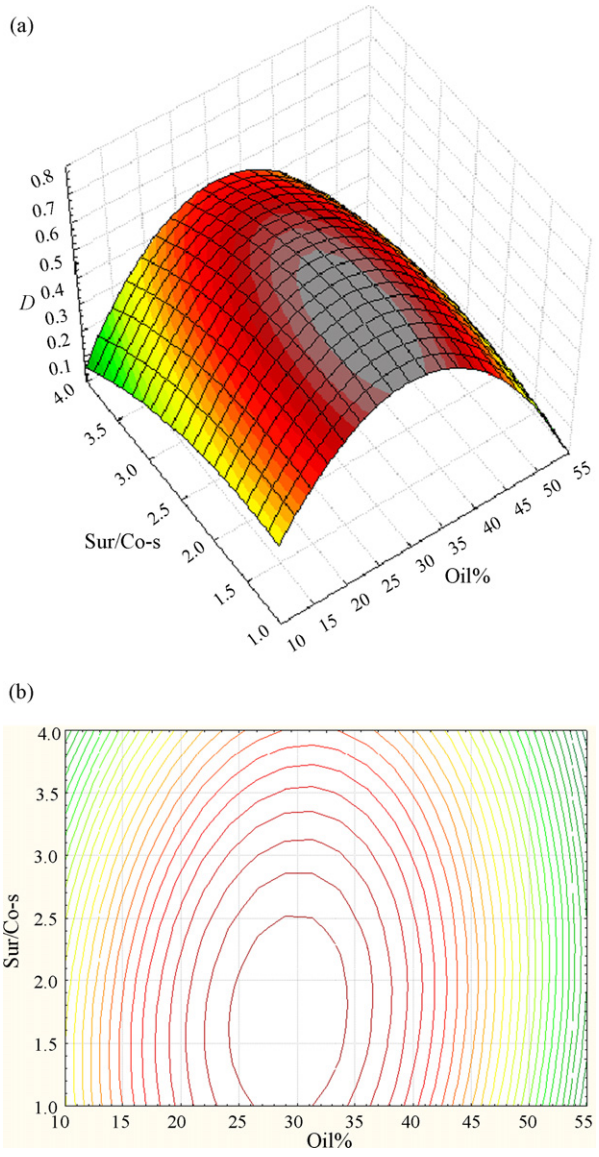

Fig. 2. Response surface (a) and contour plot (b) for overall desirability (D) as a function of oil% and Sur/Co-s ratio.

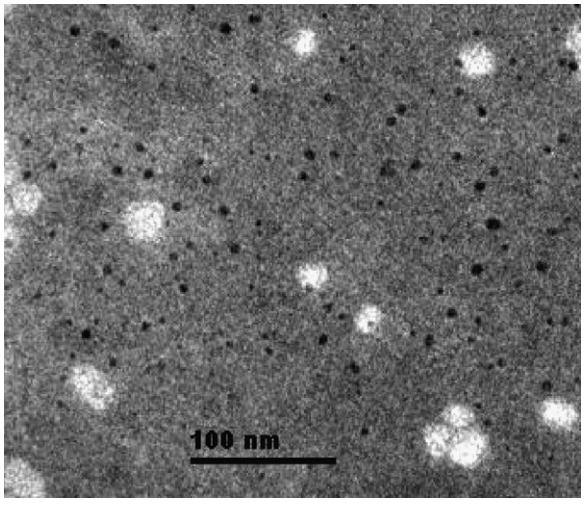

Fig. 3. TEM photo of oridonin microemulsion (×105,000).

Table 5
The predicted values and the experimental results of oridonin SMEDDS prepared under the optimum conditions

Response	Predicted value	Experimental value	Bias (%)
Y_1, droplet size (nm)	25.4	28.0	10.2
Y_2, PI	0.066	0.071	7.6
Y_3, solubility (mg/ml)	33.9	31.0	−8.6
Y_4, intestinal absorption rate (h^{-1})	0.5630	0.5877	4.4

Bias (%) = (predicted value − observed value)/observed value × 100.

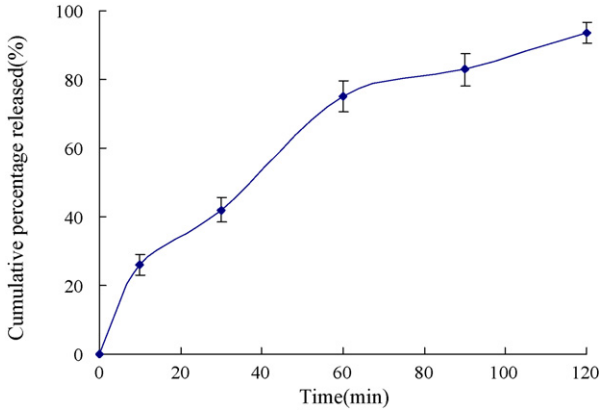

Fig. 4. *In vitro* release profile of oridonin from SMEDDS.

bility and the absorption rate in rat intestine are shown in Table 5. In addition, the characteristics on the self-microemulsifying time and *in vitro* dissolution were investigated as well. The self-microemulsifying time of the preparation was approximately 2.18 min, indicating the excellent efficiency of emulsification.

The profile of oridonin release from the optimized formulation was illustrated in Fig. 4. A rapid release up to 26% occurred in the first 10 min. The accumulated amount of drug released in 120 min was approximately 94%. The kinetics of drug release from oridonin SMEDDS was investigated using various models (zero-order, first-order, Higuchi and Hixson–Crowell equations). It was found that the drug release from SMEDDS best fitted with Hixson–Crowell equation: $(Q_\infty - Q_t)^{1/3} = -0.0214t + 4.4099$, $r = 0.9917$.

4. Conclusion

The optimization of oridonin SMEDDS formulation was carried out by central composite design-response surface methodology combined with desirability function. The effects of oil percentage and Sur/Co-s ratio on the droplet size, polydispersity, equilibrium solubility and intestinal absorption rate were investigated as well. The observed responses for the optimum formulation were in close agreement with the predicted values, indicating the excellent predictability of the optimization procedure. The optimized formulation displayed a rapid release with approximately 26% released at the first 10 min. It was proved that central composite design was efficient for the modeling and optimization of oridonin SMEDDS as well as for understanding how the formulation factors influence the properties of SMEDDS.

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