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Optimized self-microemulsifying drug delivery systems (SMEDDS) for enhanced oral bioavailability of astilbin

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The main purpose of this research work was to design an optimized self micro-emulsifying drug delivery system (SMEDDS) to enhance the bioavailability of the poor water soluble drug, astilbin. The solubility of astilbin was evaluated in various vehicles. Pseudoternary phase diagrams were used to select the components and their ranges by evaluating the micro-emulsification area. Central composite design was applied to optimize the properties of the formulation, including particle size, polydispersity index, drug loading capacity and effective intestinal permeability. The optimized SMEDDS characteristics were investigated including the study of factors influencing particle size and showed the stability of microemulsion when varying the pH and volume of diluents. *In vitro* drug release profile study was performed using the reverse dialysis method where 95% of the drug was released after 4 h. The developed astilbin SMEDDS was subjected to bioavailability studies in beagle dogs by LC-MS and showed a significant enhancement of bioavailability, indicating the possibility of using SMEDDS as possible drug carrier for astilbin.

1. Introduction

Astilbin, a flavonoid isolated from Chinese herb *Rhizoma smilacis Glabrae,* has shown a potential pharmacological and physiological effect, such as antihypertensive activity via angiotensin converting enzyme ACE inhibition (Schulz et al. 2010), antioxidant activity due to it ROS scavenging activity (Zhang et al. 2009), contact hypersensitivity inhibition through modulation of the cytokine IL-10 (Fei et al. 2005), anti-inflammatory effect related to the inhibition of activated T lymphocytes (Yi et al. 2008), selective immunosuppressant activity by facilitating the apoptosis of interleukin-2-dependant phytohemagglutinin-activated jurkat cells and the inhibition of T lymphocytes infiltration (Ru and Qiang 2001; Qiang et al. 1999). According to these properties, astilbin can be regarded as a promising drug candidate for treating immune diseases, inflammatory diseases and liver injuries. However the poor water solubility and bioavailability after oral administration restrain its therapeutic applications.

Recently, self-microemulsifying drug delivery system (SMEDDS) has drawn much attention for the delivery of poorly water-soluble drugs, due to their ability to improve the bioavailability of lipophilic drugs; this is well illustrated by the successful marketing of several pharmaceutical products, including the potent immunosuppressant cyclosporine A, the two HIV protease inhibitors ritonavir and saquinavir (Pouton 2000; Humberstone and Charman 1997; Constantinides 1995). SMEDDS are isotropic mixtures of oil, surfactant, co-surfactant and drug, which can form oil in water microemulsion with a droplet size less than 100 nm, either under gentle agitation in aqueous media or under the motility of the gastrointestinal tract after oral administration (Gursoy and Benita 2004). SMEDDS can improve the intestinal absorption of drugs by several mechanisms including the presence of the drug in dissolved state in the GI, the small particle size which increase significantly the interfacial surface area and subsequently the interactions between the drug and its absorption sites, enhanced membrane permeability caused by the presence of surfactant, promotion of lymphatic transport, inhibiting action of surfactant on the P-gp leading to an increase in intracellular transport, opening tight junction to facilitate the paracellular transport (Humberstone and Charman 1997; O'Driscoll 2002; Swenson and Curatolo 1992; Haus et al. 1994; Holm et al. 2003; Kang et al. 2004; Khoo et al. 1998; Sha et al. 2005; Charman et al. 1992).

Table 1: Solubility of astilbin in various vehicles (mean ± S.D.; n = 3)

Component	Vehicle	Solubility (mg/g)
Oil	Labrafil M 1944 CS	0.8864 ± 0.0217
	Maisine 35-1	1.4518 ± 0.0351
	Labrafac CC	0.0123 ± 0.0121
	Miglyol N 812	0.0018 ± 0.0003
	Ethyl oleate	0.0272 ± 0.0074
	GTCC	0.0226 ± 0.0012
Surfactant	Cremophor EL	99.299 ± 1.3067
	Tween 80	103.30 ± 0.4531
Co-surfactant	Labrasol	144.62 ± 0.6926
	PEG 400	270.75 ± 21.765

The composition of the SMEDDS defines the properties of the resulting microemulsion (Zidan et al. 2007; Liu et al. 2009). In order to find the optimum formulation, it is necessary to evaluate the effect of the formulation parameters and their interactions on the properties of the final product. Response surface methodology (RSM) represents a suitable method to find a functional relationship between the formulation variables and the experimental response, and can be used to determine the optimum level of each variable to reach the required properties (McCarron et al. 1999). Box-Wilson central composite design (CCD) is an experimental design considered as a category of RSM and was successfully used to optimize different dosage forms (Zidan et al. 2007; Liu et al. 2009; McCarron et al. 1999).

The aim of the present study was to design a SMEDDS of the poorly water-soluble drug astilbin with the most appropriate components by screening based on solubility and pseudoternary phase diagrams. CCD was applied to obtain a formulation possessing the desired properties, namely: a small particle size in order to increase the interfacial area for drug absorption, a small polydispersity index, a large drug loading capacity that could contain an amount which meet the medical use and an enhanced rat intestine effective permeability in order to improve the oral bioavailability of astilbin.

2. Investigations and results

2.1. Screening of SMEDDS

2.1.1. Solubility studies

Solubility studies were aimed to identify the oils, surfactant and co-surfactant with the maximal solubilizing potential to ensure a sufficient drug loading capacity and minimize solvent capacity loss after dilution of the formulation (Pouton 2000). Solubility of astilbin in various vehicles is expressed in Table 1.

Surfactant and co-surfactant showed the best solubility, while the oils showed a very poor solubility with a maximum of 1.45 mg/g obtained in Maisine 35-1. Regarding to its high solubility in surfactants and co-surfactant, the screening of oil will depend on its micro-emulsifying ability rather than the drug solubility.

2.1.2. Construction of pseudo- ternary phase diagram

The construction of pseudo-ternary phase diagram was an operation of great importance in the selection of SMEDDS components.

During the screening of oils, Labrafil M 1944 CS showed a better disposition to form microemulsion with both Cremophor EL and Tween 80, while Maisine 35-1 has a poor ability to form microemulsion with both of them. The larger microemulsion area is observed in the Labrafil M 1944 CS and Cremophor El diagram (Fig. 1). During the screening of co-surfactants, Labrasol and PEG 400 were respectively combined with Cremophor EL at fixed Km, an increase in microemulsion area associated with a reduction in liquid crystal area were observed, the most satisfying result being obtained with Labrasol (Fig. 1).

As a result of this study, Labrafil M1944 CS, Cremophor EL and Labrasol were respectively chosen as oily phase, surfactant and co-surfactant.

2.2. Experimental design and optimization of the formulation

Four responses were detected, including the particle size (PS), the polydispersity index (PI), the drug loading capacity (DLC) and the effective permeability in rat intestine (P_{eff}). The collected experimental results by CCD are listed in Table 2. The multi linear regression produced several polynomial models of different orders. Models with the highest R-squared value were selected as the fitting model. F-values indicated that all models are significant.

Table 2: Results of central composite design

No.	PS (nm)	PI	DLC (mg/g)	$P_{eff} \times 10^5$ (cm/s)
1	21.0	0.0459	69.54	4.8748
2	19.1	0.0360	71.12	3.5367
3	13.57	0.0673	97.08	2.7329
4	17.6	0.0490	78.53	3.8825
5	14.4	0.0803	90.09	4.6966
6	17.5	0.0457	80.47	3.6757
7	22.7	0.0838	68.35	5.2929
8	15.6	0.0589	90.52	2.4465
9	16.5	0.0485	79.30	4.8518
10	17.4	0.0433	82.87	3.3328
11	17.1	0.0478	84.09	3.4967
12	19.4	0.0401	80.83	5.2283
13	17.0	0.0443	78.37	3.3759

2.2.1. Experimental factors impact on particle size

Oil weight percentage (X_1) and surfactant/co-surfactant ratio (X_2) also known as Km were designated as experimental factors considering their impact on the SMEDDS properties (Zidan et al. 2007; Liu et al. 2009). Quadratic model best fitted PS response (Y_1) and were expressed as Eq. (1) and represented by the predictive response surface on Fig. 2 (a).

$$Y_1 = 17.32 + 2.79X_1 - 1.11X_2 - 0.60X_1X_2 + 0.066X_1^2 + 0.40X_2^2 \quad (1)$$

$$(F = 137.71,\ R^2 = 0.9899)$$

Both oil weight percentage and surfactant/co-surfactant ratio are significant model terms, lowering the oil weight ratio level and increasing Km have a reducing effect on PS.

2.2.2. Experimental factors impact on polydispersity index

PI response (Y_2) was fitted to quadratic model and expressed as equation (2). However a significant lack of fit and a relatively low R-squared value attest to the unreliability of the model as response predictor.

$$Y_2 = 0.046 - 0.0062X_1 - 0.0018X_2 - 0.017X_1X_2 + 0.0088X_1^2 + 0.0027X_2^2 \quad (2)$$

$$(F = 4.66,\ R^2 = 0.7690)$$

None of experimental factors are significant model terms according to their respective coefficients values. However, a high level of oil weight percentage associated to a low Km seems to reduce the PI as observed in the predicted response surface in Fig. 2b).

2.2.3. Influence of experimental factors on drug loading capacity

Linear model showed the best correlation between the formulation variables and the DLC (Y_3), it was expressed as Eq. (3).

$$Y_3 = 80.86 - 10.01X_1 + 0.022X_2 \quad (3)$$

$$(F = 83.44,\ R^2 = 0.9435)$$

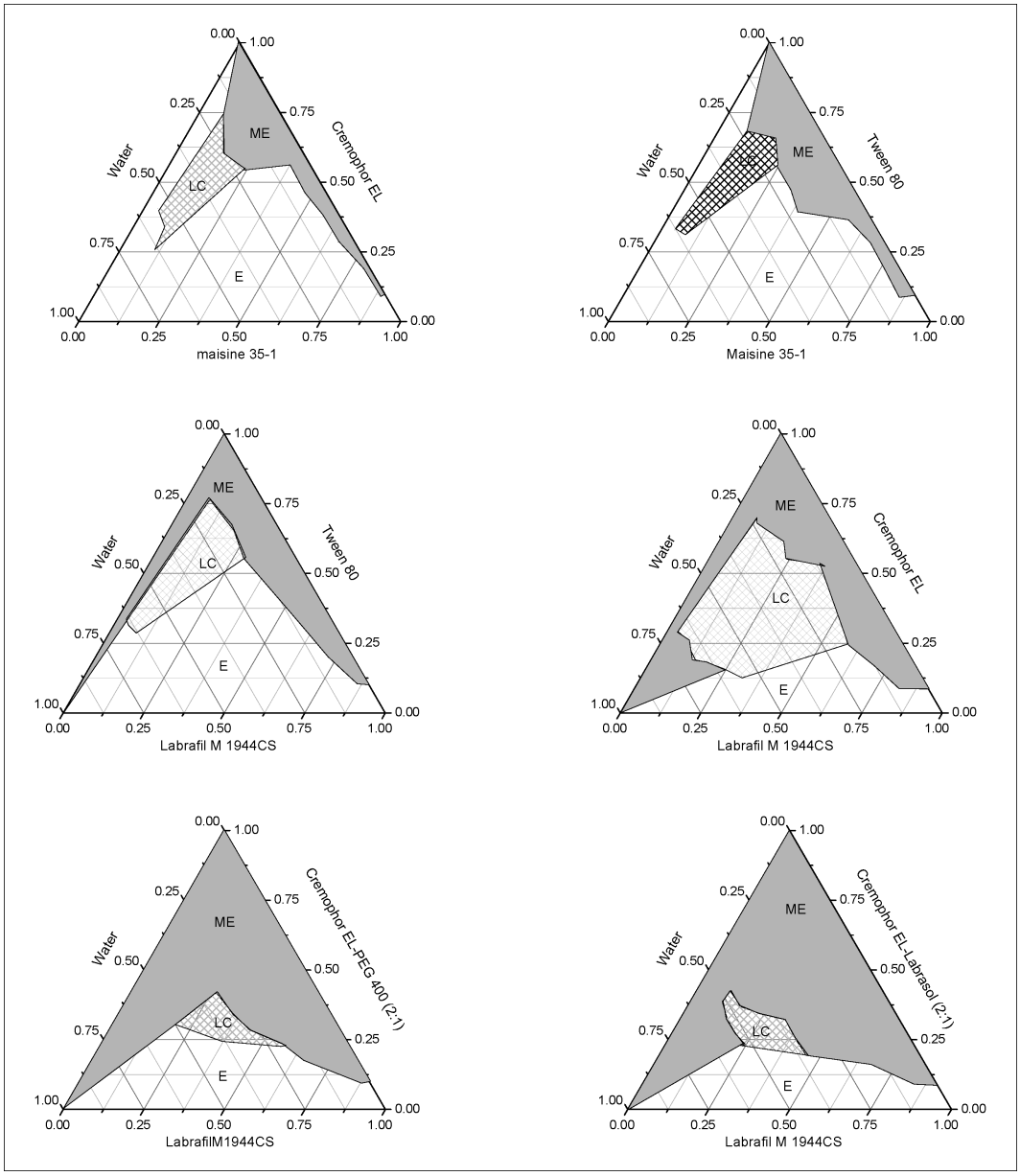

Fig. 1: Pseudo ternary phase diagrams of oils and surfactant co-surfactant mixtures

According to its coefficient value, oil percentage appears as significant factor, while Km does not influence the response. An increase of oil amount decreases DLC of the formulation and *vice versa*, this due to the poor solubility of the drug in the oily phase and its high solubility in the surfactant/co-surfactant pair, the described effect obviously appears on the predicted response surface on Fig. 2c).

2.2.4. Formulation variables effect on effective intestinal permeability

P_{eff} response (Y_4) was fitted to the quadratic model and expressed as Eq. (4). F value indicated that the model is significant and can be used as a predictive tool.

$$Y_4 = 45.29 + 26.15X_1 - 6.96X_2 - 48.25X_1X_2 + 6.26X_1^2 + 36.47X_2^2 \quad (4)$$
$$(\mathrm{F} = 20.01,\ R^2 = 0.9346)$$

Oil percentage is considered as the most influent experimental factor, the influence of Km appears less important. The predicted response surface on Fig. 2 (d) showed that increasing oil percentage combined to a low Km level enhances the intestinal permeability.

2.2.5. Selection of the optimal formulation

Data generated by the above analysis are used to define the design space, which represents the limits of variables values and provides the most accurate predictions. After assigning optimization objectives for each response, including the minimization of PS and PI, and the maximization of DLC and P_{eff}, the response prediction ability of each model has been used to generate potential solutions.

To reach the best compromise between the four responses, a combination parameter D was applied (Liu et al. 2009), the definition was listed in. Eq. (5)

$$D = \left(\prod_{i=1}^{n} d_i^{ri}\right)^{\sum \frac{1}{n}} \quad (5)$$

Where D is the global desirability, d is the individual desirability of each response, n is the number of the response and r is the importance; its value varies from 1 to 5.

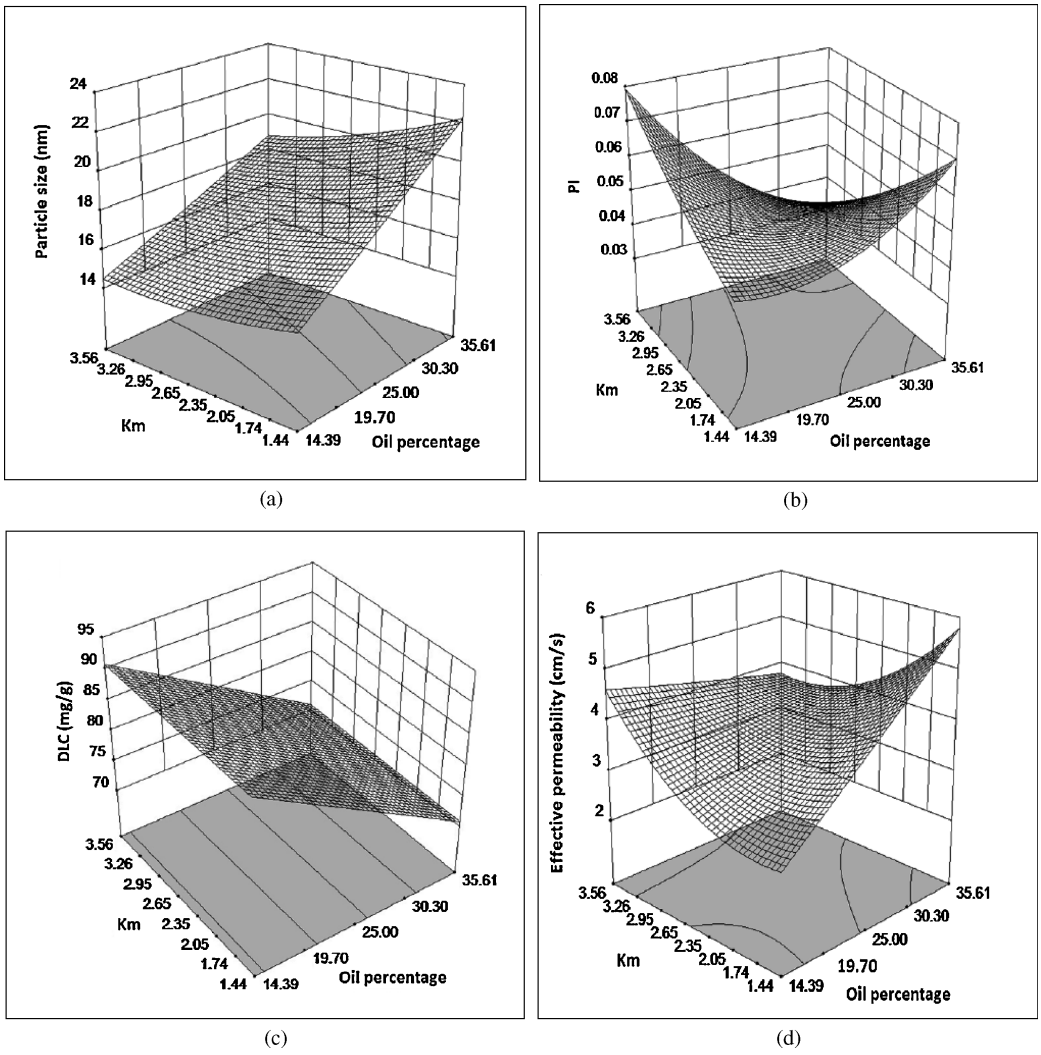

Fig. 2: Predicted response surface.

Among the suggested solutions, the formulation presenting the highest overall desirability value ($D = 0.73$) was selected with $X_1 = 25\%$ and $X_2 = 3.56$, which means that the optimized formulation contained 25% Labrafil M 1944 CS, 58.5% Cremophor EL and 16.5% Labrasol.

The optimal SMEDDS properties were then assessed to examine the correlation between the predicted and experimental values of the responses (Table 3). The observed values showed the reliability of the model as response predictor concerning PS, DLC and P_{eff}. As expected, a poor correlation existed between the predicted PI and its experimental value.

2.3. Characterization of the optimized SMEDDS

2.3.1. Factors affecting PS and PI

PS and PI of blank and astilbin loaded SMEDDS dispersed in different media are given in Table 4. Variation of pH and drug loading did not significantly affect the PS and PI, a slight increase was observed in most cases. PS stability in media with differing pH is due to the use of non-ionic surfactant which is not affected by pH variations.

Dilution rate influence on PS is also studied. PS slightly increased from 16.5 to 20 nm with dilution volume, negligible difference (< 2 nm) between 10 fold diluted and 1000 diluted SMEDDS was observed, confirming the robustness of the resulting microemulsion upon dilution.

Table 3: Comparison of predicted and experimental response values of the optimal SMEDDS formula

Response	PS (nm)	PI	DLC (mg/g)	$P_{eff} \times 10^5$ (cm/s)
Predicted value	16.6	0.0469	80.07	4.18
Experimental value	16.9	0.0744	80.80	4.45
Bias (%)	1.8	58.6	0.9	6.5

2.3.2. Morphology study

The morphology of the microemulsion was examined with a transmission electron microscope. The droplet on the microemulsion appears in a spherical shape with narrow size range (Fig. 3).

2.3.3. In vitro drug release study

Reverse dialysis method was carried out to study the release characteristics of astilbin from the SMEDDS after dilution. The result is illustrated in Fig. 4. During the first hour, 65% of the drug was released from the SMEDDS. The cumulative drug released reached 96% after 4 h, after what it remained the same until 12 h.

2.4. Bioavailability study

The plasma concentration profile and pharmacokinetic parameters of astilbin following oral administration of a single dose

Table 4: PS and PI of blank and drug loaded SMEDDS at different pH (n = 3) A: blank SMEDDS, B: 5% astilbin loaded SMEDDS

Dissolution medium	Distilled water		HCl 0.1 mM		PBS pH 6.8	
	A	B	A	B	A	B
PS (nm)	16.9 ± 0.26	18.7 ± 0.46	18.3 ± 0.15	20.1 ± 0.23	19.6 ± 0.1	19.8 ± 0.1
PI	0.074 ± 0.009	0.097 ± 0.024	0.078 ± 0.012	0.119 ± 0.012	0.094 ± 0.002	0.087 ± 0.010

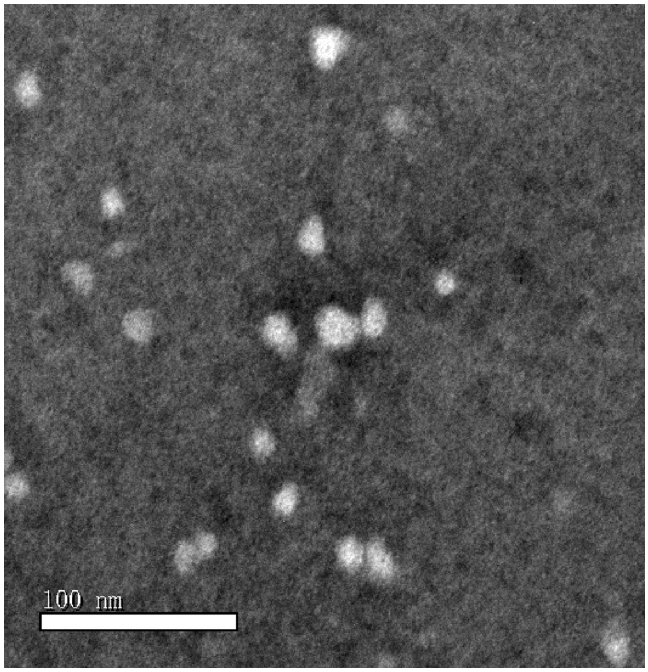

Fig. 3: TEM photo of astilbin microemulsion

(15 mg/kg) of SMEDDS formulation and astilbin suspension were expressed in Fig. 5 and Table 5, respectively. A significant improvement of drug absorption was observed with SMEDDS compared to the suspension. The relative bioavailability of the SMEDDS calculated on AUC $_{0\text{-}8h}$ was 5.59 fold to suspension. Relative bioavailability enhancement of astilbin can be attributed to several mechanisms. Besides the improved drug release profile of SMEDDS, some other factors may contribute to the improvement of drug oral absorption (Wu et al. 2006).

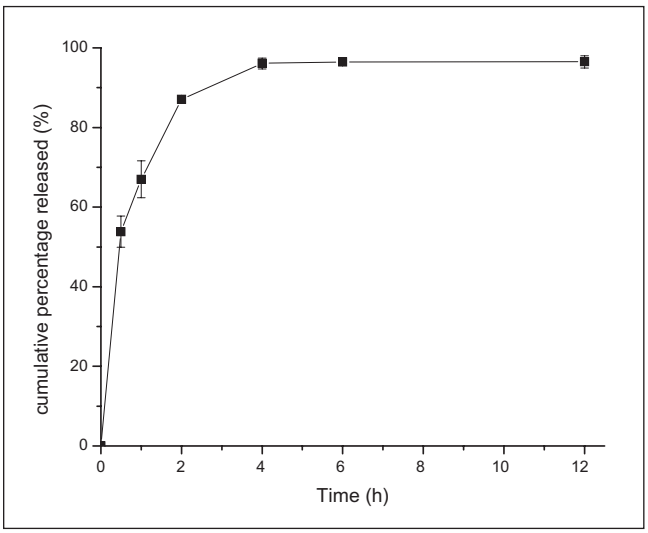

Fig. 4: *In vitro* release profile of astilbin microemulsion

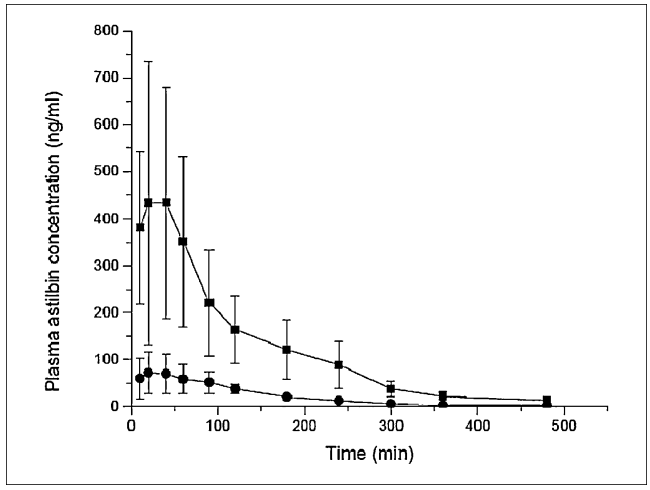

Fig. 5: Plasma concentration profiles in beagle dogs after oral administration of SMEDDS (square) and suspension (round)

The presence of the drug in a dissolved state can explain its faster absorption, the small droplet size of the formed microemulsion increase the interfacial area and consequently the interaction of the drug with its absorption sites. Other mechanisms such as the lymphatic transport, the promotion of para-cellular transport and the improvement of the trans-cellular due to the surfactant action may be involved in the enhancement of the oral bioavailability.

3. Discussion

A SMEDDS formulation for astilbin was developed. The ranges of the components were optimized taking into consideration their impact on formulation properties. The study demonstrated the suitability of the central composite design in optimizing the compositions for SMEDDS. The optimal formulation consisted of 25% Labrafil M 1944 CS, 58.5% Cremophor EL and 16.5% Labrasol, its properties were in accordance with the predicted values of particle size, drug loading capacity and rat intestine effective permeability and achieved the required properties showing the reliability of the central composite design as predictive tool. The objective concerning the minimization of the PI did not meet the expected value, we assume that the polydispersity index variation is not significantly affected by the ratio of the formulation components. The developed SMEDDS showed

Table 5: Pharmacokinetic parameters of oral administration of astilbin (mean ± SD, n = 6)

Parameters	SMEDDS	Suspension
C_{max} (ng/ml)	506.8 ± 268.9	85.65 ± 44.52
t_{max} (min)	26.66 ± 19.67	36.67 ± 28.76
$t_{1/2}$ (min)	105.2 ± 40.68	178.4 ± 133.7
MRT (min)	139.6 ± 15.68	173.2 ± 47.21
AUC $_{0\text{-}8h}$ (ng.min/ml)	59374.88 ± 28138.18	10613.71 ± 3587.12
AUC $_{0-\infty}$ (ng.min/ml)	61585.77 ± 28574.21	11570.50 ± 4116.09

Table 6: Levels of formulation variables

Factor	Level				
	$-\alpha$	-1	0	$+1$	$+\alpha$
X_1 (oil percentage)	10.00	14.39	25.00	35.61	40.00
X_2 (Km)	1.00	1.44	2.50	3.56	4.00

$\alpha = 1.414$.

a stable particle size over pH variation and increasing dilution volume.

The developed formulation significantly enhanced the intestinal absorption with a relative bioavailability 5.59 fold to API. These suggested its use as a vehicle for of the delivery of astilbin by oral route.

4. Experimental

4.1. Materials

Astilbin crude extract was provided from Professor Qiang Xu in Nanjing University with a purity of 90.05%, Cremophor-EL was obtained as a free sample from BASF (Germany). Maisine 35-1, Labrafac CC, Labrafil 1944 CS and Labrasol were a kind gift from Gattefosse (France). Miglyol N812 was purchased from Sasol (Germany). Ethyl oleate, PEG 400, GTCC and Tween 80 were purchased from Sinopharm Group Chemical Reagent Co., Ltd (Shanghai, China). All other chemicals were analytical grade.

4.2. Screening of SMEDDS

4.2.1. Solubility studies

The solubility of astilbin in various oils, surfactants and co-surfactants was determined as follows: an excess amount of the drug was added to each vehicle and the mixture was magnetically stirred for 48 h at 30 °C, the speed revolution was set at 200 rpm. After the equilibrium was reached, each sample was centrifuged at 12000 rpm for 10 min, and the supernatant was successively diluted with methanol and mobile phase (water, methanol, acetic acid 55:45:0.165 volume ratios). The concentration of astilbin was determined by high performance liquid chromatography (HPLC).

4.2.2. Construction of pseudo ternary phase diagrams

Pseudo ternary phase diagrams were constructed by the water titration method, briefly: mixtures of oil, surfactant and co-surfactant at fixed Km were prepared at different weight ratio as 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9 by magnetic stirring until a clear mixture was obtained. Distilled water was added to each sample in dropwise manner at ambient temperature under gentle stirring, and the mixtures were visually assessed for their clarity and flow ability. The isotropic clear and slightly bluish solution were considered as microemulsion, gel and gel-like mixtures were considered as liquid crystals, and turbid or milky solution were defined as emulsions.

4.3. Experimental design and optimization of the formulation

The ranges of the factors values were defined during preliminary experiments and are summarized in Table 6. Four responses were considered being the most significant properties of a SMEDDS, including the PS (Y_1), PI (Y_2), the DLC (Y_3) and P_{eff} (Y_4). The experimental design was carried out using Design-Expert 8.0 software (Stat-Ease Corporation, USA). A two factor and five levels central composite design under the criteria of rotatability was applied and required 13 runs listed in Table 7 (4 factorial points, 4 star points and 1 center point being replicated 5 times to increase the precision and estimate the variability of the inherent experiments).

4.3.1. PS and PI

SMEDDS pre-concentrate was diluted to 100 fold with distilled water at 37 °C under gentle stirring prior to the globule size analysis. The mean PS and PI of the resulting microemulsion were determined by photo correlation spectrometer (Zetasizer 3000HS, Malvern Instruments Corp., U.K.).

4.3.2. DLC

An excess amount of astilbin was added to each formulation and the mixture was magnetically stirred under a speed of 200 rpm at 30 °C for 48 h. Every mixture was centrifuged at 12000 rpm during 10 min, the supernatant was diluted successively with methanol and mobile phase and the concentration was determined by HPLC.

Table 7: Matrix of central composite design

No.	Oil percentage,x_1 (%)	Km,x_2
$1(+\alpha, 0)$	40.00	2.50
$2(+1, +1)$	35.61	3.56
$3(-\alpha, 0)$	10.00	2.50
$4(0, 0)$	25.00	2.50
$5(-1, +1)$	14.39	3.56
$6(0, 0)$	25.00	2.50
7(+1, -1)	35.61	1.44
$8(-1, -1)$	14.39	1.44
$9(0, +\alpha)$	25.00	4.00
10(0, 0)	25.00	2.50
11(0, 0)	25.00	2.50
$12(0, -\alpha)$	25.00	1.00
13(0, 0)	25.00	2.50

4.3.3. P_{eff}

Single-pass perfusion technique was performed using established methods as previously described (Sinko et al. 1995; Cook and Shenoy 2003; Laurent et al. 2003; Yao et al. 2008). Briefly, male Sprague-Dawley rats (200–240 g) were fasted over night with free access to water. The rats were anesthetized with an intraperitoneal injection of urethane solution (1 g/kg) and placed under a surgical lamp to maintain the body temperature. A midline incision of approximately 4 cm was made and the small intestine segment of interest was exposed and canulated with plastic tubing (from the beginning of duodenum 2-3 cm after pillory to the extremity of ileum 2-3 cm before the colon). The intestinal segment was rinsed with isotonic saline at 37 °C using an infusion pump, then it was perfused at a flow rate of 0.4 mL/min during 30 min with blank Krebs–Ringer's buffer (NaCI 115 mM, KCl 5.9 mM, $MgCl_2$ 1.2 mM, NaH_2PO_4 1.2 mM, $CaCl_2$ 2.5 mM, $NaHCO_3$ 2.5 mM, D-glucose 10 mM) and purged by air. Plastic tubes were then recorded to the reservoir containing the perfusion solution at 37 °C at the flow rate of 0.4 mL/min. Perfusion solution was a dispersion of 5% astilbin loaded SMEDDS in Krebs-Ringer's buffer containing phenol red (20 μg/ml) as a marker to correct water flux (Sutton et al. 2001). Samples were collected in the following intervals: 0–30, 30–60, 60–90, 90–120, 120–150 and 150–180 min and kept at −20 °C until analysis by HPLC.

Samples were filtered through a membrane filter (0.45 μm) and diluted with mobile phase, and then the remaining drug was measured by HPLC. Phenol red concentration was determined by UV-spectrophotometer at 428 nm. The effective permeability was calculated using following Eq. (6) (Yao et al. 2008):

$$P_{eff} = \frac{Q \ln(C_{in}/C_{out})}{2\pi r l} \quad (6)$$

where Q is the flow rate in mL/min, r the radius of the intestine (0.18 cm in rat), L is the length of the perfused intestinal segment (cm) and (C_{in}/C_{out}) is the concentration ratio corrected for the fluid flux.

4.4. Characterization of the optimized SMEDDS

4.4.1. Effects of pH and drug loading on PS and PI

0.5 mL of blank SMEDDS and 5% drug loaded SMEDDS were dispersed in 50 mL of distilled water, 0.1 mol/L hydrochloride acid, and pH 6.8 phosphate buffer saline respectively at 37 °C and magnetically stirred for 20 min. The droplet size and PI of the resultant microemulsion were analyzed with the method described in section 2.3.1.

4.4.2. Microemulsion droplet size at different dilution rates

In order to evaluate the effect of dilution upon particle size of microemulsion, the SMEDDS pre-concentrate containing astilbin (5% w/w) were diluted to 10, 20, 50, 100, 200, and 1000 fold respectively, the droplet size of resulting microemulsion was measured according to the method described above.

4.4.3. Morphological study

The morphology of SMEDDS was investigated by a HITACHI H-7650 transmission electron microscope (TEM) (Hitachi High-Technologies Cor-

poration, Japan). Drug loaded SMEDDS was diluted with distilled water at 1:100 under mild agitation. A drop of the diluted microemulsion was deposited on copper grids coated with a porous polymer support. Excess sample was blotted away with filter paper. The sample was negatively stained with 2% phosphotungstic acid for 3 min and observed.

4.4.4. In vitro drug release study

In vitro drug release from SMEDDS was evaluated by reverse dialysis method that better simulate the *in vivo* conditions (15, 27) (Kang et al. 2004; Thao et al. 2009). Six dialysis bags (10000 MWCO) were filled with 1 mL of dissolution medium (hydrochloric acid 0.1 mol/L) and sealed firmly with clamps; the bags were placed into beaker containing 500 mL of dissolution medium at 37 °C 1 h prior to the test. The stirrer was set at 200 rpm. 5% loaded SMEDDS was accurately weighted and introduced into the dissolution medium. Dialysis bag were withdrawn individually at 0.5, 1, 2, 4, 6, and 12 h respectively, and 1 mL of fresh dissolution medium was added at same time to maintain the volume and sink conditions. The content of each bag was filtrated through 0.45 μm membrane filter and the drug concentration was analyzed by HPLC.

4.5. Bioavailability study

Six healthy male beagle dogs (9.1 ± 0.4 kg) used in this study were provided by the experimental animal center of Southeast University. They were fasted 12 h prior to the dosing with water available at libidum. The dogs were administered SMEDDS (15 mg/kg) and astilbin suspension (15 mg/kg). The washout periods between administrations were 7 days. Blood samples (3 mL) were collected through forelimb vein at 10, 20, 40, 60, 90, 120, 180, 240, 300, 360 and 480 min after oral administration. Blood samples collected in heparinized tubes were then centrifuged at 3000 rpm for 10 min; plasma samples were taken and stored at −20 °C. 0.1 mL of plasma was accurately added in a 2 mL centrifugation tube with 10 μL of internal standard and 1 mL of ethyl acetate, mixed for 5 min by vortex and centrifuged at 12000 rpm for 5 min. 0.75 mL of supernatant was dried under nitrogen stream at 37 °C water bath, the residue was diluted with 100 μL of methanol, vortexed for 3 min, centrifuged at 12000 rpm for 5 min and 20 μL supernatant were analyzed by LC-MS to determine astilbin and its major metabolite 3'-o-methylastilbin.

From the inspection of the plasma concentration versus time profiles of astilbin and its metabolite, the maximum plasma concentration (C_{max}) and its corresponding time (t_{max}) were determined. The area under curve of the plasma concentration versus time ($AUC^{0-\infty}$) was calculated using trapezoidal rule.

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