Formulation and in Vitro Characterization of a Novel Solid Lipid-Based Drug Delivery System

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The liquid self-emulsifying drug delivery system (L-SEDDS), commonly used to deliver effective but poorly water-soluble oleanolic acid (OA), has many limitations such as high manufacturing costs, few choices of dosage forms, risk of leakage from hard gelatin capsules, low stability, limited portability, incompatibility with capsule materials, and relatively restricted storage conditions. Thus the main purpose of our study was to develop a promising solid lipid-based drug delivery system (S-SEDDS) for OA. The S-SEDDS, prepared from wet granulation with an optimized L-SEDDS formulation and mannitol, was characterized by particle size analysis, scanning electron microscopy, differential scanning calorimetry, and X-ray powder diffraction. Finally, the solubility of the OA-loaded S-SEDDS was compared with that of OA powder in the dissolution assay. Our new S-SEDDS for OA was developed from the optimum L-SEDDS with ethyl oleate (oil phase), Labrasol (surfactant), and Transcutol P (cosurfactant) at a volume ratio of 15:71:14 with 1.5% w/v OA and mannitol. The dissolution of OA was improved by 60% compared with that of the pure OA powder. All the problems associated with the L-SEDDS were resolved. The methodologies we developed for OA delivery could also be utilized for the delivery of other drugs with the S-SEDDS.

Key words self-emulsifying drug delivery system; solid dosage form; mannitol; wet granulation; oleanolic acid

Oleanolic acid (OA), a naturally-occurring pentacyclic triterpene, is widely distributed in many medicinal herbs such as Olea europaea, Viscum album, Aralia chinensis, Mile swertia and Ligustrum lucidum. OA has many drug activities including hepatoprotective, antitumor, antinociceptive potential, and anti-inflammatory effects. In China, OA has been used as an oral drug for human liver disorders in a conventional tablet or capsule formulation. However, OA has various disadvantages such as poor water solubility (4.61 µg/mL) and low dissolution rate, thus leading to low absorption and bioavailability. Due to these limitations, chemically modified derivatives have been developed to improve water solubility. Chemical modification can be effective, however it is an expensive and time-consuming process and may not be applicable to all natural compounds. Various formulations such as solid dispersions and cyclodextrin inclusions for the oral delivery of OA have also been explored to improve its bioavailability; however, these commonly used approaches failed to show significant improvements in the solubility and dissolution of OA. In recent years, much attention has been given to the liquid self-emulsified drug delivery system (L-SEDDS) to improve the low water solubility, dissolution rate, and bioavailability characteristics of poorly water-soluble drugs. L-SEDDS is defined as an isotopic and thermodynamically stable mixture of oil, surfactant, co-surfactant, and drug substance that instantaneously forms oil-in-water emulsions when mixed with aqueous media (gastrointestinal fluids) under gentle stirring. Self-emulsifying formulations spread readily in the gastrointestinal tract, and the digestive motility of the stomach and the intestine provides the necessary agitation for the spontaneity of emulsion formation. The L-SEDDS formulation typically produces emulsion with a droplet size ranging from 100 to 300 nm. The droplet size of the emulsion is a critical factor for self-emulsification. The smaller droplet size provides the larger interfacial surface area for the stability of the emulsion, which increases the rate and extent of oral absorption of drugs. The preparation of L-SEDDS involves solubilization of drug in oil and surfactant/co-surfactant as a liquid dosage form. This process involves a number of limitations such as high manufacturing costs, few choices of dosage forms, risk of leakage from hard gelatin capsules, low stability, limited portability, incompatibility with capsule materials, and relatively restricted storage conditions.

We hypothesized that incorporation of a liquid self-emulsification formulation into a solid oral dosage form such as granule or pellet might overcome these inevitable problems associated with liquid formulation described above. Solid carriers have become increasingly important in the development of a desirable formation of solid lipid-based drug delivery system (S-SEDDS). Mannitol is an excellent excipient, which is water soluble and non-hazardous with a semi-sweet, smooth and cool taste. An acceptable daily intake of mannitol has not been specified by the World Health Organization since the amount consumed as a sweetening is not considered to be hazardous to health. Mannitol is classified as a sugar alcohol found in almost all plants and vegetables. It is widely used in pharmaceutical formulations, food and other industries, but is mainly used as a diluent (10–90%, w/w) in tablet formulations. In addition, mannitol is less hygroscopic and compatible with most active pharmaceutical ingredients. Thus we decided to utilize mannitol as an alternative solid carrier. The aim of this study was to develop a novel OA-
loaded S-SEDDS with mannitol as a solid carrier. Both spray-drying technique and wet granulation method have been commonly employed to prepare conventional S-SEDDS.\textsuperscript{23,24} However, the spray-drying technique, a costly and complicated method, requires a large amount of organic solvent to dissolve the drug and a hydrophilic polymer. Organic solvents cause environmental pollution and are toxic due to the residual solvents.\textsuperscript{24} The wet granulation method using mannitol as a solid carrier, with low cost and simple process advantages, has been preferred to be employed to formulate a solid dosage form of a drug.\textsuperscript{24} Wet granulation is a size enlargement process of converting small-diameter solid particles (typically powders) into larger diameter agglomerates to generate a specific size, to improve flow ability and to produce a powder with specific properties such as dissolution rates, granule strength and apparent bulk density.\textsuperscript{25}

It is easy to mix L-SEDDS and solid carrier to form solid dosage formulation by wet granulation method. Thus the S-SEDDS formulation of OA was prepared by wet granulation with L-SEDDS and mannitol. Its physicochemical property was examined by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and X-ray powder diffraction (XRPD). Reconstitution properties of the granules were also investigated. Finally, in vitro dissolution rate of OA-loaded S-SEDDS was evaluated compared to OA powder.

**Experimental**

**Materials** OA was supplied by Zelang Pharmaceutical Corp. (Nanjing, China). Labrafac CC, Labrasol, Transcutol P, and Capryol 90 were obtained from Gatrefosse (Saint-Priest Cedex, France). Isopropyl myristate, ethyl olate, OP-10, and Cremophor EL were purchased from Aladdin Chemical Corp. (Shanghai, China). Tween 20 was purchased from Solarbio Science & Technology Co., Ltd. (Beijing, China). Acetonitrile (HPLC grade) was purchased from Kemiou Chemical Reagent Science & Technology Co., Ltd. (Tianjin, China). All other chemicals used were of analytical grade.

**Methods: Solubility Studies** The objective of the solubility study was to identify the appropriate oil, surfactant, and co-surfactant that have good solubilizing capacity for OA. An excess amount of OA was applied to 2 mL of each vehicle and was mixed by a vortex mixture for 5 min to facilitate the solubilization. The mixture was kept at room temperature for 72 h to achieve equilibrium.\textsuperscript{26} Then each sample was centrifuged at 9400 \( \times g \) for 10 min at room temperature. The supernatant was passed through a 0.22 \( \mu \)m polytetrafluoroethylene membrane filter, followed by dilution with acetonitrile. The quantification of OA was done by a Waters HPLC System (Waters, U.S.A.) with a Symmetry C18 column (4.6 x 150 mm, 5 \( \mu \)m) at 35°C. Acetonitrile and 0.1% phosphoric acid solution (95:5, v/v) was used as the mobile phase at a flow rate of 0.5 mL/min with a run time of 10 min as described by Tian et al.\textsuperscript{27} with minor modifications. The injection volume was 10 \( \mu \)L and the UV detection was set at 210 nm. The concentration of dissolved OA in the different vehicles was quantified by this HPLC method.

**Compatibility Tests** Compatibility tests were conducted to evaluate the spontaneous emulsifying ability of a certain combination of surfactant and oil according to the method described by Cui et al.\textsuperscript{28} A series of formulations with different oils (Labrafac CC, isopropyl myristate, and ethyl olate) and surfactant (Labrasol) at the volume ratio of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 was mixed for 5 min using a vortex mixer. 50 \( \mu \)L of the formulation was applied to 50 mL of distilled water (37°C) under constant magnetic stirring. Then the progress of forming the emulsion droplets and the spontaneous emulsification tendency were visually assessed according to the five-grading system.\textsuperscript{16,29} As the compatibility tests, optimum oil, surfactant, and co-surfactant were selected to establish the ternary phase diagram. All studies were repeated at least three times.

**Construction of Ternary Phase Diagram** A ternary phase diagram was constructed to identify the self-emulsifying region and the concentration range of formulation containing an oil/surfactant/co-surfactant. A series of self-emulsifying formulations were prepared with different concentrations of ethyl olate (oil phase, 5–40% v/v), Labrasol (surfactant, 40–95% v/v), and Transcutol P (co-surfactant, 0–25% v/v), which were selected based on the results obtained from the solubility and compatibility tests. Under a gentle constant magnetic stirring, the formulation (50 \( \mu \)L) was added into a glass beaker containing 50 mL of 37°C water. The performance of self-emulsifying formulation was visually observed and evaluated based on the progress of forming the emulsion droplets and the spontaneous emulsification tendency. If the droplets spread out easily in water and formed a fine milky emulsion in clear or slightly opaque appearance, the performance to form an emulsion was judged as “good.” If the emulsion formation was poorly developed with coalescent oil droplets, the corresponding performance was judged as “bad.”\textsuperscript{15,21} After identifying the good self-emulsifying region in the phase diagram, the proper ratio of surfactant to oil/co-surfactant was further determined. All studies were repeated at least three times.

**Preparation of L-SEDDS** Based on the results of the ternary phase diagram, a series of formulations were finally confirmed and prepared with varying proportions of Labrasol to ethyl olate/Transcutol P. Subsequently, the formulation was diluted 1000 times with water and examined for signs of turbidity or phase separation before the determination of droplet size. The droplet size of the emulsion was measured by a Malvern Zetasizer Nano ZS 90 (Malvern, U.K.). 10 mg, 15 mg, and 20 mg of OA were individually dissolved in 1 mL of the optimized formulation containing ethyl olate, Labrasol and Transcutol P. The final self-emulsifying formulation was mixed by a vortex mixer until a clear solution was obtained.

**Preparation of S-SEDDS** Mannitol was used as the solid adsorbents to load L-SEDDS.\textsuperscript{29} The mixture of 1 mL OA-L-SEDDS, 3 g mannitol, and 200 \( \mu \)L ethanol was loaded into a granulation bowl and mixed for 3 to 5 min. The granules were sized by manual sieving with 16-mesh metal screen. The obtained granules were dried with ventilated dryer at 60°C for 5 h.

**Characterization of the S-SEDDS: Morphological Analysis of S-SEDDS** The morphological features of OA powder, mannitol and OA-loaded S-SEDDS formulation were investigated using a scanning electron microscope (Hitachi S-4800, Japan) with an image analysis system (Image inside Ver 2.32). The samples were fixed to a brass specimen club by coating in a vacuum (6 Pa) with platinum (6 nm/min) using an Eiko Ion Sputter (IB-3) for 180 s at 66 mA.
Differential Scanning Calorimetry (DSC) The physical state of OA in S-SEDDS was characterized by the DSC thermogram analysis (DZ 3335, China). An empty aluminum pan was used as the control. The samples (about 10mg) were placed in standard aluminum pans under an inert environment using nitrogen. The temperature was gradually increased from 50°C to 350°C at a pre-programmed heating rate of 10°C/min.

X-Ray Powder Diffraction (XRPD) The crystallinity changes of the S-SEDDS formulation components were assessed by the XRPD (Rigaku D/max-2500/PC, Japan). The detection was performed at room temperature using a 35kV and a 50mA with copper (Cu) as the tube anode material. The solids were exposed to a Cu-K radiation over 2θ ranges from 5° to 50°, at a rate of 2° (2θ)/min, a sampling interval of 0.02°.

Reconstitution Properties of S-SEDDS A 2.67mL of L-SEDDS and 10.7g of S-SEDDS (equivalent to 40mg of OA) prepared as described above were added into 200mL and 500mL of distilled water (37°C), respectively. They were mixed gently for 30s using a magnetic stirrer to form a fine emulsion, then they were kept at 25°C for 30min. The mean droplet size, size distribution, and polydispersity index of emulsions from both L-SEDDS and S-SEDDS were determined by a Malvern Zetasizer Nano ZS 90 (Malvern). All studies were repeated at least three times.

In Vitro Dissolution Tests Dissolution tests were carried out using a dissolution apparatus (RC-6, China) according to Appendix XC of Chinese Pharmacopoeia (2010 version, vol. II). The 10mg of OA powder, the OA-loaded L-SEDDS and S-SEDDS formulations (equivalent to 10mg of OA) were placed individually into the 900mL water with 0.5% sodium dodecyl sulfate as the dissolution medium. The temperature was maintained at 37°C and the revolution speed of the basket was maintained at 100rpm. At predetermined intervals (5, 10, 20, 30, 45 and 60min), an aliquot of sample (2mL) was withdrawn and filtered through a 0.22µm membrane filter. The quantification procedure of OA was determined using the HPLC method as described above in Methods: Solubility Studies in Experimental. The withdrawn sample was replaced with 2mL of fresh dissolution medium to maintain the volume constant.

Statistical Analysis All data shown as mean±S.D. in figures and tables were statistically analyzed by Student’s t-test using SPSS 16.0 software. Significant difference was defined as p<0.05.

Results and Discussion

Solubility Studies The optimal SEDDS formulation consisted with oil, surfactants, co-surfactants, and drug should appear to be a clear and monophasic liquid at ambient temperature when applied to aqueous phase. It is also desirable to dissolve higher concentration of drug in the mixture. Therefore, the components used in the system should have good solvent properties to achieve optimum drug loading while maintaining an excellent emulsifying performance. The solubility of OA in various vehicles is shown in Table 1. All three oils such as Labrafac CC, isopropyl myristate, and ethyl oleate were able to dissolve OA at very similar levels (2–3mg/mL). Among surfactants Labrasol, a medium length alkyl chain surfactant with HLB 14, together with OP-10 showed relatively high drug solubility (16mg/mL). In addition, Labrasol is known to enhance the intestinal absorption of drugs. Thus we selected Labrasol as the surfactant. Among the co-surfactants tested in this study, Transcutol P presented the highest drug solubility (Table 1). It was also reported that mixing Transcutol P with Labrasol generally helps to improve the emulsifying ability of Labrasol. Thus, we selected Transcutol P as the co-surfactant.

Compatibility Tests Compatibility tests play a critical role in the self-emulsifying formulation design. Not all of the oils/surfactants with good solubility possess excellent emulsifying performance for the drug. Based on the compatibility tests, the emulsifying performance of the mixtures with various oils and surfactants was observed to evaluate the compatibility with each other, which facilitates the optimization of the combination of an oil and surfactant. As shown in Table 2, ethyl oleate showed the best emulsifying performance with Labrasol when compared to Labrafac CC and isopropyl myristate. A visual grading of “A” was observed from the mixture of Labrasol and ethyl oleate. Thus ethyl oleate was selected as the oil phase.

Construction of Ternary Phase Diagram To identify the
self-emulsifying region and to optimize the concentrations of oil, surfactant and co-surfactant in the SEDDS, ternary phase diagram was constructed according to the self-emulsifying characteristics of a series of SEDDS formulations. The phase diagram of the delivery system containing ethyl oleate, Labrasol and Transcutol P as the oil, the surfactant and the co-surfactant, respectively, was shown in Fig. 1. Shaded area in the phase diagram corresponding to ethyl oleate (less than 30%), Labrasol (between 55–95%), and Transcutol P (between 0–25%) in the SEDDS gave us efficient spontaneous emulsion formation. A desired ratio of components was further determined by investigating the droplet size distribution.

Preparation of Liquid and Solid SEDDS  
In SEDDS, the primary way of self-emulsifying assessment is visual evaluation.\textsuperscript{19} The efficiency of self-emulsification could be estimated by determining the droplet size of emulsion. As shown in Fig. 2, the mean droplet size of the formed emulsion was found to decrease dramatically with an increase in the ethyl oleate concentrations from 5% to 15% in SEDDS formulation, and the smallest emulsion droplet size (146±2.4 nm) appeared at 15% of ethyl oleate concentration; however, the emulsion droplet size increased significantly when the oil concentration became more than 15%. The SEDDS prepared with 15% ethyl oleate and 71% Labrasol gave the smallest droplet size (Fig. 3). The surfactant forms a layer around oil globule in such a way that polar head lies toward aqueous and nonpolar tail pulls out oil and thereby reduces surface tension between oil phase and aqueous phase. Increasing oil concentrations (from 5% to 15%, v/v) in the SEDDS formula decreased the droplet size of the emulsion. The increased surfactants could decrease the interface tension of oil–water phase and stabilize the oil–water interface, resulting in a decrease in droplet size. Furthermore, the decrease in the droplet size reflects the formation of a better closely-packed film of the surfactant at the oil-water interface, thereby stabilizing the oil droplets. However, the droplet size slightly increased above 15% with ethyl oleate because the insufficient concentration of surfactant could not reduce the interface tension and formed the micelle. When the co-surfactant was applied to the formulation, the co-surfactant molecules which were embedded in the surfactant molecules reduced the interfacial tension between the oil and water interface and increased the membrane fluidity to form smaller droplet size of emulsion. However, an excessive amount of co-surfactant will make the system less stable due to its high solubility and lead to the increased droplet size as a result of the expanding interfacial film.\textsuperscript{22} Maximum of 15 mg of OA was dissolved in 1 mL of the above mixture to obtain a clear solution. Thus, we concluded that ethyl oleate/Labrasol/Transcutol P (15/71/14, v/v/v) with 1.5% w/v OA was the optimal L-SEDDS formulation. Subsequently, the S-SEDDS was developed from wet granulation with the optimized L-SEDDS formulation (1 mL) and mannitol (3 g). The final OA content in the S-SEDDS was 0.5% w/w ratio. Forty milligrams of OA in 10.7 g of the S-SEDDS is recommended as one dose, which is generally acceptable amount for some drug granules.

Characterization of OA-Loaded S-SEDDS by SEM  
The SEM images of OA powder, mannitol and S-SEDDS were shown in Fig. 4. OA powder showed smooth-surfaced crystalline with rectangular shape (Fig. 4a). The SEM image of
mannitol exhibited irregular shape (Fig. 4b). In general, the L-SEDDS was absorbed or coated inside the pores of the inert solid carrier, which resulted in the rough-surfaced particles that appeared in the conventional S-SEDDS formulation. The surface of OA-loaded SEDDS prepared with mannitol appeared rough, irregular and agglomerate particles, which indicated that the L-SEDDS with OA formed a microcapsule with mannitol (Figs. 4c, d).

The physical state of drug in the S-SEDDS was investigated since it would have an important influence on the in vitro and in vivo release characteristics. OA’s status in this S-SEDDS was also investigated here. DSC curves of pure OA, mannitol, physical mixture and S-SEDDS were presented in Fig. 5. The physical mixture was prepared by simply mixing oleanolic acid and mannitol (1:20, w/w). Pure OA exhibited a melting endothermic peak at about 310°C (Fig. 5a), which indicated that OA was crystalline. Mannitol (Fig. 5b) exhibited three peaks with gradually increased temperatures from 50°C to 350°C. The melting point, which was shown in the OA endothermic peak (Fig. 5a), was absent in the physical mixture (Fig. 5c). No obvious endothermic peak of the OA powder was detected in the S-SEDDS formulation (Fig. 5d). It is indicated that the granulation procedure using lipid surfactants and solid carrier influenced the melting characteristics of OA and inhibited its crystallization.

The internal physical state of OA powder in the S-SEDDS was investigated since it would have an important influence on the in vitro and in vivo release characteristics. OA’s status in this S-SEDDS was also investigated here. DSC curves of pure OA, mannitol, physical mixture and S-SEDDS were presented in Fig. 5. The physical mixture was prepared by simply mixing oleanolic acid and mannitol (1:20, w/w). Pure OA exhibited a melting endothermic peak at about 310°C (Fig. 5a), which indicated that OA was crystalline. Mannitol (Fig. 5b) exhibited three peaks with gradually increased temperatures from 50°C to 350°C. The melting point, which was shown in the OA endothermic peak (Fig. 5a), was absent in the physical mixture (Fig. 5c). No obvious endothermic peak of the OA powder was detected in the S-SEDDS formulation (Fig. 5d). It is indicated that the granulation procedure using lipid surfactants and solid carrier influenced the melting characteristics of OA and inhibited its crystallization.

The internal physical state of OA powder in the S-SEDDS was further evaluated by X-ray powder diffraction (Fig. 6). All of the major peaks between 10° and 15° in the mannitol
Dissolution Profiles of the OA Powder, OA-Loaded Liquid SEDDS and Solid SEDDS Formulations Were Performed at 37°C Using the Basket Method at 100 rpm with 900 mL Water Containing 0.5% Sodium Dodecyl Sulfate as the Dissolution Medium

The concentration of released OA in the different times (5, 10, 20, 30, 45 and 60 min) was quantified by HPLC method. Each value represents the mean±S.D. (n=6).

Reconstitution Properties of S-SEDDS In the present study, mannitol was used as a carrier to prepare S-SEDDS. The mean droplet size and polydispersity index of the reconstituted emulsions in different dilution volume were presented in Tables 3 and 4. The mean droplet sizes for L-SEDDS and S-SEDDS systems in 500 mL water were 162.7 nm and 157.0 nm, respectively (Table 3). Although the L-SEDDS was transformed into a solid state by wet granulation, the mean droplet size of the emulsion remained unchanged. Meanwhile, the S-SEDDS formulation showed a similar size distribution compared to the L-SEDDS formation (Table 3). However, the mean droplet size of the S-SEDDS in 200 mL water was significantly larger than that of the L-SEDDS (p<0.05), which may be due to the absence of the dispersion effect from the mannitol in 200 mL water than in 500 mL water. The mean droplet size of L- and S-SEDDS was decreased when the dilution volume was increased from 200 mL to 500 mL. It seemed that dilution volume had an effect on the droplet size of the SEDDS.

In Vitro Dissolution Tests The formation of an emulsion needs very low free energy in the self-emulsifying systems, thereby an interface could be formed spontaneously between the oil droplets and aqueous phase. It could be suggested that the oil/surfactant/co-surfactant and aqueous phase effectively decrease the droplet size of emulsion and subsequently increase the dissolution rate. As shown in Fig. 7, the dissolution characteristics of the drug from the L- and S-SEDDS formulations were compared with those of OA powder. The L-SEDDS formulation showed higher dissolution rates than the S-SEDDS. In particular, dissolution and release of the SEDDS from the adsorbent was found to be dependent on the area of contact between the formulation and the adsorbent. Increase in the area of contact between the formulation and the surface of the adsorbent decreases the extent of drug release. Therefore, with mannitol as a solid carrier, a greater portion of the liquid OA-SEDDS is in direct contact with the adsorbent, which leads to precipitation and subsequently lower dissolution rates. The release rate of S-SEDDS reached 72% within 5 min, which was much higher than that of the OA powder (32%), mainly due to the faster spontaneous emulsion formation and the reduced droplet size. The dissolved OA from the S-SEDDS reached 81% after 60 min, which was 1.6 times higher than that of OA powder (Fig. 7). Our dissolution result is similar to the reports by Xi et al. and Yang et al. where they reported the dissolutions of OA rapidly released from their L-SEDDS to be 75% and 85%, respectively. Thus, our S-SEDDS prepared with mannitol had a comparable in vitro dissolution of the OA drug to L-SEDDS. Furthermore, our S-SEDDS has many advantages such as low production costs, increased stability and storage time, superior portability, easy storage conditions, and increased safety compared to the L-SEDDS. The faster dissolution in S-SEDDS formulation may be due to the fact that drug is in a solubilized form with a small droplet size after forming an emulsion in the dissolution medium. Additionally, the increased solubility of OA in S-SEDDS contributed to the higher dissolution rate.

Conclusion

First, a novel L-SEDDS formulation consisting of ethyl oleate, Labrasol and Transcutol P as an oil phase, a surfactant and a co-surfactant, respectively, was determined. Second, the S-SEDDS by wet granulation technique using mannitol as a solid carrier was developed.

Our newly developed S-SEDDS preserved the self-emulsification performance of the L-SEDDS and enhanced the dissolution rate of OA. Considering the limitations associated with L-SEDDS, a solid formulation should be a more acceptable form. Taken together, our in vitro results strongly suggest that the S-SEDDS can be a promising method for the oral delivery
of poorly soluble lipophilic drugs. We plan to investigate the efficacy of this method using an in vivo system.

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