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ORIGINAL ARTICLE

Preparation and evaluation of a self-emulsifying drug delivery system of etoposide–phospholipid complex

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Abstract

Aim: The aim of this study was to develop a new phospholipid complex self-emulsifying drug delivery system (PC-SEDDS) to enhance bioavailability of oral etoposide, a drug with poor water solubility. *Methods*: Etoposide–phospholipid complex (EPC) was prepared by reacting etoposide and phospholipid in tetrahydrofuran and confirmed as a phospholipid compound by differential scanning calorimetry (DSC). Solubility of EPC and etoposide was determined in various vehicles. Pseudoternary phase diagrams were constructed to identify the efficient self-emulsification region of EPC-SEDDS, and the effects of oil concentration, drug loading, and aqueous media on droplet size were investigated. *Results*: The optimal formulation of EPC-SEDDS was EPC:octyl and decyl monoglyceride (ODO):Cremopher EL:PEG-400 (1:20:48:32) (w/w/w/w). Compared with etoposide–phospholipid complex suspension (EPCS) and etoposide suspension (ES), cumulative release of etoposide from EPC-SEDDS increased by 1.31 and 2.65 fold at 24 hours, respectively. Compared with ES, relative bioavailability of EPC-SEDDS, E-SEDDS, and EPCS after oral administration in rats was enhanced by 60.21-, 44.9-, and 8.44- fold, respectively. *Conclusions*: The synergistic effect between PC and SEDDS contributed to the enhanced bioavailability of etoposide. It was concluded that PC-SEDDS proved to be a potential system for delivering orally administered hydrophobic compounds including etoposide.

Key words: Bioavailability, etoposide, phospholipid complex, self-emulsifying drug delivery system

Introduction

Etoposide is a semisynthetic derivative of podophyllotoxin, a naturally occurring compound extracted from the roots and rhizomes of the plants *Podophyllum peltatum* and *Podophyllumemodi*[1]. It can increase Topo II-mediated DNA breakage primarily by inhibiting the ability of the enzyme to relegate cleaved nucleic acid molecules[2]. Now, etoposide is widely used in the treatment of patients with small-cell lung cancer, testicular tumors, Kaposi's sarcoma, lymphoma, and leukemia[3]. Etoposide dissolves easily in methanol and sparingly in water. Because of its low aqueous solubility (148.5–167.25 μg/mL, at 37°C), slow intrinsic dissolution rate (0.0094 mg/min/cm2, at 25°C), and rapid degradation at pH 1.30 (degradation half-life of 2.88 hours, at 25°C)[4], etoposide has poor oral bioavailability and high variability in absorption, which have restricted its use through oral administration. In recent years, some efforts have been made to improve the dissolution and oral bioavailability of etoposide by means of solid dispersion[5] and soft capsules[6].

Phospholipid complex (PC) could improve the therapeutic efficacy of certain drugs with poor oral absorption due to poor water solubility[7–11]. IdB 10161 is a silybin phosphatidylcholine complex, which could significantly increase the plasma level of silybin and silymarin in rats and humans[12–15].

At the same time, the self-emulsifying drug delivery system (SEDDS) is one of the most interesting approaches to improve solubility, dissolution, and oral absorption of drugs with poor water solubility[16–20]. SEDDS is an isotropic mixture of oil, surfactant–cosolvent, and drug substance. The basic principle of this system is its ability to form fine oil-in-water (o/w) microemulsions under gentle agitation following dilution by aqueous

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phases[21]. The microemulsion droplets dispersed in the gastrointestinal tract provide a large surface area and promote rapid release of the dissolved form of drug substance and/or mixed micelles containing drug substance. The main mechanisms of improving bioavailability also include increasing membrane fluidity to facilitate transcellular absorption, opening tight junction to allow paracellular transport, inhibiting P-glycoprotein (PGP) and/or CYP450 to increase intracellular concentration and residence time by surfactants, and stimulating lipoprotein/chylomicron production by lipid[22–24]. The commercial success of the SEDDS formulation Sandimmune Neoral/(cyclosporin), as well as Norvir/(ritonavir) and Fortovase/(saquinavir), has raised interest in such promising emulsion-based drug delivery systems[25].

Because of PC and SEDDS related to different absorption mechanisms, the aim of this study was to develop a new etoposide PC-SEDDS formulation with improved dissolution and bioavailability. In this study, the etoposide-PC (EPC) and the etoposide-PC SEDDS (EPC-SEDDS) were prepared and characterized. Do they have a synergistic effect between PC and SEDDS? To evaluate this, in vitro release of etoposide from EPC-SEDDS and pharmacokinetics after oral administration in rats were evaluated in comparison with the etoposide suspension (ES), the EPC suspension (EPCS), and the etoposide SEDDS (E-SEDDS).

Materials and methods

Chemicals and reagents

Chemicals and reagents used in this study included etoposide (Shanghai Modern Pharmaceutical Co. Ltd., Shanghai, PR China); soybean phospolipid (SP) (Shanghai Taiwei Co. Ltd., Shanghai, PR China); polyoxyethyleneglycerol triricinoleate 35 castor oil (CremopherEL®) (BASF, Ludwigshafen, Germany); caprylin, glyceryl caprylate-caprate, and octyl and decyl monoglyceride (ODO) (Zhejiang Quandao Fine Chemical Industry Co. Ltd., Zhejiang, PR China); soybean oil (TieLing Beiya Pharmaceutical Co., Liaoning, PR China); and ethyl oleate, 1,2-propanediol, and PEG-400 (Sinopharm Group Chemical Reagent Co. Ltd., Shanghai, PR China). All other chemicals used were of analytical grade.

Preparation of EPC

The complex was prepared with etoposide and phospholipids at a molar ratio of 1:1.2. Weighed amount of etoposide and phospholipids were taken in a 250-mL round-bottom flask and 100 mL of tetrahydrofuran was added[26]. The mixture was stirred at a 35°C for 1 hour. The resultant clear solution was vacuum-evaporated at 40°C. The dried residues were collected, placed in desiccators overnight, and powdered. The resultant EPC was transferred into a glass bottle, flushed with nitrogen, and stored at room temperature.

Characterization of EPC

Differential scanning calorimetry analysis

Samples sealed in aluminum crimp cells were heated at the rate of 10°C/min from 0°C to 250°C in the atmosphere of nitrogen (DSC Q100, TA, USA). Peak transition onset temperature of etoposide, SP, the physical mixture of etoposide and SP (1:1.2, mol/mol), and EPC (1:1.2, mol/mol) was monitored.

Solubility study

Solubility of etoposide and EPC in various solvents, surfactants, and cosolvents were determined. An excess amount of etoposide or EPC was added in 2 mL of each selected vehicle in cap vial. After the cap was sealed, the mixture was shaken at 37°C to facilitate the solubilization. The mixture was equilibrated and then centrifuged at 1007 × *g* for 10 minutes to remove the undissoved drug. The supernatant was collected and diluted with methanol or alcohol for quantification of etoposide by high-performance liquid chromatography (HPLC).

Pseudoternary phase diagram construction

Pseudoternary phase diagrams of oil, surfactant–cosolvent, and water were developed using water titration method: the mixture of oil and surfactant–cosolvent at certain weight ratios was diluted with water in a dropwise manner. For each phase diagrams at a specific ratio of surfactant–cosolvent, 1:2, 2:3, 1:1, 3:2, and 2:1 (w/w), transparent and homogenous mixtures of oil and drug were formed under the mixing, and each mixture was titrated with water. After equilibrium was achieved, the mixture was visually observed for phase clarity and flow ability. With identification of the emulsion region in the phase diagrams, emulsion formulations were selected at desired component ratios.

Preparation of SEDDS

EPC-SEDDS consisted of EPC:ODO:Cremopher EL: PEG-400 (1:20:48:32) (w/w/w/w), and E-SEDDS consisted of etoposide:ODO:Cremopher EL:PEG-400 (0.4:20:48:32) (w/w/w/w). The formulations were prepared by taking the formulation amount of the drugs in ODO at 40°C. Cremopher EL and PEG-400 were then added. This mixture was stirred until a transparent preparation was obtained. As above, E-SEDDS was preparation.

In addition, ES and EPCS were prepared. Briefly, 1.00 g of EPC or 0.35 g of etoposide were suspended in 0.1% CMC-Na solution and mixed thoroughly.

SEDDS characterization

Morphological characterization

SEDDS was diluted with distilled water (1:100) and mixed under gentle shaking. A drop of SEDDS was placed onto a carbon-coated copper grid. The excess was drawn off with filter paper. Subsequently, it was stained in 1% phosphotungstic acid solution for 30 seconds. The morphology of SEDDS was observed with transmission

electron microscopy (TEM) (JEM-2010, JEOL, Tokyo, Japan).

Determination of droplet size and ζ-potential

SEDDS (50 μL) was diluted with water, 0.1 N HCl, and pH 6.8 phosphate buffer solution (PBS) (50 mL), and gently mixed. The efficiency of self-emulsification was assessed by the previous methods[27]. The droplet size distributions of resultant emulsions were determined with Zetasizer (Nano series, Malvern, UK). Each sample was analyzed in triplicate.

pH Stability

EPC-SEDDS was diluted with 0.1 N HCl and PBS at a final concentration of 60 μg/mL as etoposide, and the solutions were maintained at 37°C. At various time intervals, the samples were taken and quantified by HPLC.

In vitro release

In vitro release of etoposide from EPC-SEDDS, ES, EPCS, and E-SEDDS containing the same quantity of drug was tested with the modified method[28–30]. After the formulation containing about 12 mg dose of etoposide was instilled into the dialysis bag (MWCO 10000, Spectrum Medical Industries Inc., Houston, Tx, USA), the bag was firmly sealed and then placed in 250 mL PBS at 37°C. The paddle revolution speed was set at 100 rpm. At the predetermined intervals, 1 mL of sample was withdrawn and the same volume of fresh dissolution medium was replenished. The amount of etoposide was analyzed with HPLC.

Pharmacokinetic study

All experimental procedures were reviewed, approved by the institutional animal ethical committee, and performed in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. The oral pharmacokinetic study was performed in male Sprague–Dawley (SD) rats (230–250 g) (Laboratory Animal Center of the Second Military Medical University, Shanghai, PR China). Rats were randomized into four groups and fasted for 12 hours prior to the experiment, with free access to water. After oral administration of the four formulations with a 140 mg/kg dose of etoposide, 0.5 mL blood was collected from the retroorbital plexus in a heparinized tube at 0, 5, 15, 30, 60, 120, 240, 360, 480, 720, and 1440 minutes. The blood samples were centrifuged at 4028 × *g* for 5 minutes. The serum samples were collected and stored at –20°C until analysis.

A liquid-liquid phase extraction procedure with chloroform was used for the extraction of etoposide from plasma[31]. For each 0.2 mL plasma sample, 3.0 mL chloroform was added. After the mixture was vortexed and centrifuged at 363 × *g* for 5 minutes, 2.0 mL organic layer was transferred to a tube and evaporated at 40°C. The residue was dissolved in 100 μL mobile phase solution and 20 μL was injected into the HPLC system.

In the concentration range of 0.25–20 μg/mL, the linear regression equation of the calibration graph of etoposide was $C = 0.087A - 0.1862$ (correlation coefficient $r = 0.9996$, $n = 6$). Limit of detection ($S/N > 3$) was 0.063 μg/mL and that of quantification ($S/N > 10$) was 0.20 μg/mL. At concentrations of 0.5, 5, and 20 μg/mL, the spiked recovery of etoposide from rat plasma was 102.54%, 96.17%, and 100.80%, respectively; the intra-day precision was 4.27%, 2.84%, and 3.82%; the inter-day precision was 3.02%, 2.87%, and 3.68%. Data were analyzed using standard noncompartment analysis. The peak plasma concentrations (C_{max}) and the time for their occurrence (T_{max}) were noted directly from the individual plasma concentration versus time profiles. The area under the plasma concentration–time curve ($AUC_{0\rightarrow t}$) from zero to the last time point was estimated by the linear trapezoidal method. The relative bioavailability (F) was calculated using the following equation:

$$F = \frac{AUC_{test}}{AUC_{reference}} \times 100\%$$

HPLC analysis

The Agilent 1100 series HPLC system (Agilent Technologies Inc., Wilmington, DE, USA) consisted of a quaternary pump, a degasser, an autosampler, a column heater, and a tunable ultraviolet detector. An ODS column (Kromasil C_{18}, 250 × 4.6 mm, 5 μm, Dikma Technology, Shanghai, PR China) was used as the analytical column. The mobile phase was composed of acetonitrile:acetic acid (60:40, v/v) at a flow rate of 1.0 mL/min. A 20 μL volume was injected onto the column and the column temperature was kept at 25°C. The detector was set at 254 nm.

Statistical analysis

Statistically significant differences were determined by one-way classification with an unpaired *t*–test (Student–Newman–Keuls method) for multiple comparisons at a significance level of α=0.05. The results were presented as mean ± their standard deviations (± SD).

Results and discussion

Preparation of EPC

The physical–chemical and biological properties of a drug may undergo a significant change after formation of PC, including improved therapeutic effects, extended reaction time, and reduced adverse effects. For this reason, PC has been widely studied, such as IdB 1016[12–15,32], amphoteriein B[33–35], curcumin[36], and ursodeoxycholic acid[11,37]. Etoposide is a kind of saponin drug and can easily combine with the polarity group and unsaturated portion of the phospholipids to form PC. In the preliminary trials, the reaction conditions including the solvent, time, and temperature were optimized by singal factor exploration[26], and then EPC was prepared based

on different quantity ratios of drugs and phospholipids, such as 0.8:1, 1:1, 1:1.2, and 1:1.6 (mol/mol). The results showed that the resultant materials at the ratio of 1:1.6 appeared viscous and were not easily prepared to other preparations, but the reaction of EPC was incomplete at the ratio of 0.8:1. For the purpose of getting the optimal quality, using less quantity of phospholipids, and ensuring full complexity of drugs, EPC with the quantity ratio 1:1.2 (mol/mol) was prepared, in which the content of etoposide was about 35% (w/w).

Characterization of EPC

DSC analysis

Differential scanning calorimetry (DSC) analysis was widely used for identifying PC. Figure 1 showed the DSC curves of etoposide, SP, a physical mixture of etoposide and SP, and EPC. In the DSC curve of the physical mixture of etoposide and SP, there were some small, sharp endothermal peaks between 150.78°C and 222.93°C. The exothermic peak at 209.40°C of etposide and the endothermal peak at 222.93°C of SP were shifted to 180.58°C and 233.14°C, respectively. These findings might imply that when the mixture was grinded, etoposide and SP partly interacted to make thermodynamic variation which had some differences from the individual components[38]. The DSC curve of the EPC showed that the original peaks of etoposide and SP disappeared between 160°C and 250°C. It was evident that etoposide and SP exhibited some interactions, such as the formation of hydrogen bonds and van der Waals forces. After the combination of etoposide and the phospholipid molecule polarity parts, the carbon-hydrogen chain in phospholipids could turn freely and enwrap the phospholipids molecule polarity parts, which made the sequence to decrease between phospholipids aliphatic hydrocarbon chains, the second endothermal peak of phospholipids to disappear, and the phase transition temperature to depress[11,38–40].

Solubility studies

It is believed that a self-emulsifying formulation should consist of a suitable surfactant, cosolvent, and oil, and possess good solubility for drugs. The solubility of etoposide and EPC in various media is presented in Table 1. ODO provided the best solubility for etoposide and EPC (3.806 and 8.988 mg/g, respectively) among the oils studied. Because the solubility of etoposide and EPC in Cremopher EL was higher than that in Tween-80, Cremopher EL should be the desirable surfactant. In addition, among the cosolvents, PEG-400 exhibited the best solubility for etoposide (53.839 mg/g) and EPC (62.788 mg/g). Based on these findings, ODO, Cremopher EL, and PEG-400 were, respectively, selected as the oil, surfactant, and cosolvent for the optimal SEDDS formulation, which was shown to improve drug-loading capability significantly.

In this study, the solubility of EPC in various vehicles was higher than that of etoposide, suggesting that PC improved the lipophilic and hydrophilic capability of the drug. When etoposide was combined with phospholipids,

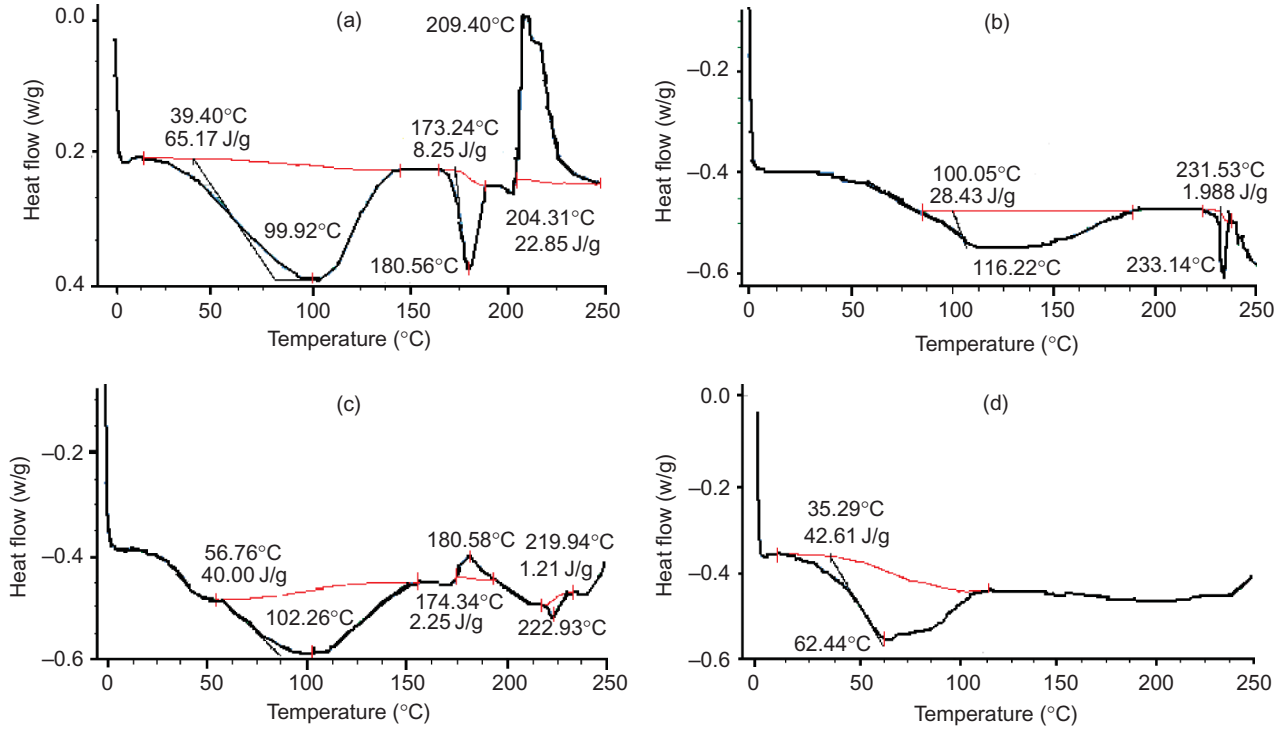

Figure 1. DSC spectra of EPC and its individual components. (a) etopside, (b) soybean phospolipid, (c) physical mixture of etoposide and soy phospholipids (1:1.2, mol/mol), and (d) EPC (1:1.2, mol/mol).

Table 1. Solubility of etoposide and EPC in various media at 25°C ($n = 3$).

Media	Etoposide (mg/g)	EPC (mg/g)
Soybean oil	0.03 ± 0.00	0.12 ± 0.01
Ethyl oleate	0.02 ± 0.00	0.88 ± 0.03
Caprylin	0.74 ± 0.16	3.60 ± 0.29
Glyceryl caprylate-caprate	0.27 ± 0.02	2.01 ± 0.18
ODO	3.81 ± 0.38	8.99 ± 0.33
Cremopher EL	30.63 ± 2.73	36.48 ± 2.89
Tween-80	26.33 ± 2.60	33.56 ± 3.29
PEG-400	53.84 ± 1.73	62.78 ± 5.89
1,2-Propanediol	3.71 ± 0.37	4.88 ± 0.21
Alcohol	0.55 ± 0.02	1.52 ± 0.02
Water	0.13 ± 0.01	0.21 ± 0.02
1-Octanol	0.20 ± 0.02	0.95 ± 0.10
Blank-SEDDS	40.88 ± 1.67	44.77 ± 0.63

Blank-SEDDS consisted of 20% ODO, 48% Cremopher EL, and 32% PEG-400 (w/w).

its solubility in the oil was increased due to polarity change of EPC[7–9] and its amorphous state in the EPC that illustrated in the DSC thermograms (Figure 1).

Pseudoternary phase diagram study

Nonionic surfactants were used in most investigations about SEDDS because they are less toxic and less affected by pH and ionic strength, and are known to improve bioavailability through various mechanisms[20,29]. Moreover, addition of cosolvents had been shown to increase the extent of the emulsion region in lecithin–triglyceride systems[41]. Series SEDDS were prepared and their self-emulsifying properties were observed visually. Pseudoternary phase diagrams at the ratio of surfactant–cosolvent (Cremopher EL/PEG-400), 2:1, 3:2, 1:1, 2:3, 1:2 (w/w), were constructed to identify the self-emulsifying regions and obtain the optimal ratio of surfactant-to-cosolvent (Figure 2). The maximum fields of self-emulsion were obtained at the ratio of Cremopher EL/PEG-400 1:2, and the larger region was at the ratio of Cremopher EL/PEG-400 2:3. However, stability of the self-emulsifying droplets at the ratio of Cremopher EL/PEG-400 1:2 and 2:3 (w/w) was decreased because of precipitation after a few hours. Furthermore, when SEDDS dispersed in an acidic medium, an increase in the cremopher EL concentration led to an improvement in clarity of the emulsions formed upon dissolution and a corresponding decrease in droplet size date42, the ratio of Cremopher EL/PEG-400 2:1 or 3:2 might be selected. But excessive amounts of the surfactant may cause irritation to the gastrointestinal tract[18,19]. Therefore, the optimal surfactant-to-cosolvent ratio was selected as 3:2. Based on our results, a three-component SEDDS formulation was established: 20% ODO as the oil, 48% Cremopher EL as the surfactant, and 32% PEG-400 as the cosolvent.

SEDDS characterization

Morphological characterization

The E-SEDDS and EPC-SEDDS turned into emulsion when diluted with 0.1N HCl, water, and PBS. The efficiency of self-emulsification of E-SEDDS and EPC-ESDDS was assessed. The results demonstrated E-SEDDS and EPC-ESDDS rapidly formed (within 1 minute) emulsion, which was clear or slightly bluish in appearance. The TEM picture was shown in Figure 3. Spherical emulsion droplets of E-SEDDS and EPC-SEDDS were observed. In terms of appearance, there was no significant difference except droplet size.

Droplet size analysis

It was known that the droplet size distribution was one of the most important characteristics of emulsion for its stability evaluation and in vivo fate of emulsion[43,44]. The smaller the droplet size, the larger the interfacial surface area, which would be suitable for drug absorption[18,19]. In this study, the effects of the oil and drug concentration on the droplet size were investigated in water and 0.1N HCl. As shown in Figure 4, there was a slight increase in mean droplet size of the SEDDS when the oil concentration increased from 20% to 40%. However, as for the 20% oil, the mean droplet sizes of blank-SEDDS or EPC-SEDDS were almost same in water and in 0.1N HCl, which was slightly affected by pH and 20% oil was selected in the formulation.

The effect of drug loading on droplet size in 0.1N HCl and water is presented in Figure 5. In different media, the mean size remained almost unchanged when drug loading increased from 40 to 100 mg. When drug loading increased to 120 mg, the droplet size increased greatly. Hence, the EPC loading was selected to be 100 mg in the formulations.

The effect of media on droplet size was also investigated. Table 2 indicated the results of SEDDS dispersion in distilled water, 0.1N HCl, and PBS. There was no significant difference among the three media, indicating that the formulation was not affected by pH.

In addition, the stability of droplet size in 0.1N HCl was investigated. The results showed that the droplet size unchanged during 3 hours (data not shown).

ζ-Potential analysis

Generally, the electrostatic repulsive forces between emulsion droplets affect the stability of emulsion and oral bioavailability. Table 2 indicated the zeta potential of SEDDS diluted with distilled water, 0.1N HCl, and PBS. There was a significant difference in the absolute ζ-potential value among the three media. But it was interesting that the surface charge was positive (+4.97 ± 0.75 mV) for EPC-SEDDS droplets in 0.1N HCl, indicating that this formulation would reach a positive zeta potential at physiological pH, which might be beneficial to improving bioavailability[18,19].

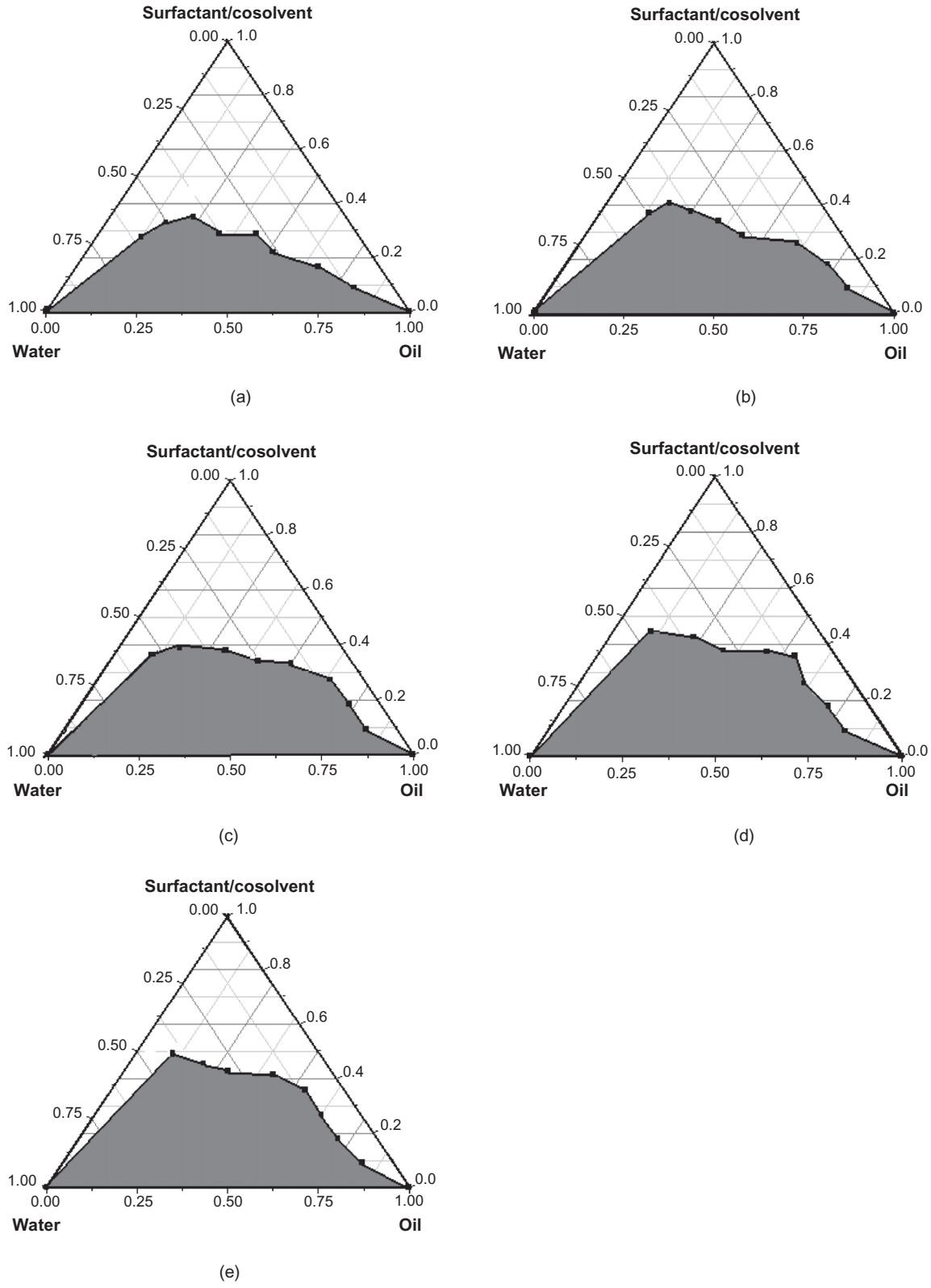

Figure 2. Pseudoternary phase diagrams of the formulations composed of ODO, and various surfactant/cosolvent ratios as dispersed with distilled water at 25°C as follows: (a) Cremopher EL:PEG-400 (2:1), (b) Cremopher EL:PEG-400 (3:2), (c) Cremopher EL:PEG-400 (1:1), (d) Cremopher EL:PEG-400 (2:3), and (e) Cremopher EL:PEG-400 (1:2). The shadow area represented the emulsion region.

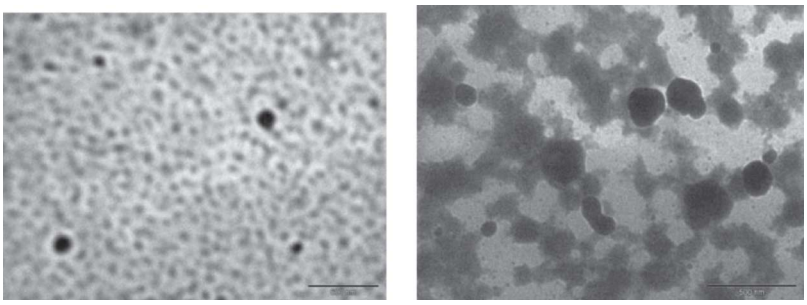

Figure 3. TEM photos of (a) E-SEDDS and (b) EPC-SEDDS. The E-SEDDS and EPC-SEDDS were diluted with water.

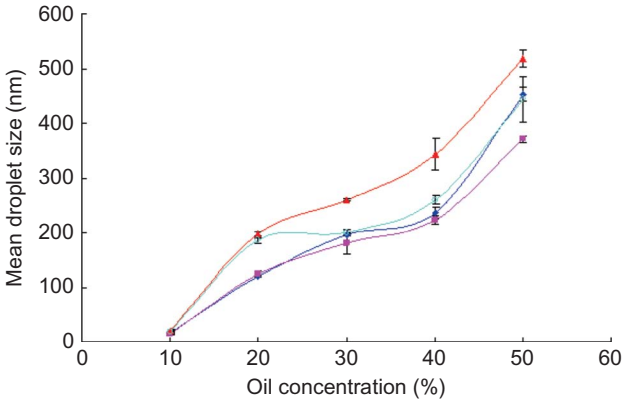

Figure 4. Effect of the oil concentration on mean emulsion droplet size (n=3) (◆, blank-SEDDS in 0.1 N HCl; ■, blank-SEDDS in water; ▲, EPC-SEDDS in 0.1 N HCl; ❍, EPC-SEDDS in water). The blank-SEDDS formulations were composed of various ratios of ODO at the ratio of surfactant/cosolvent of 3:2. The EPC-SEDDS was prepared by adding EPC (40 mg) to blank-SEDDS.

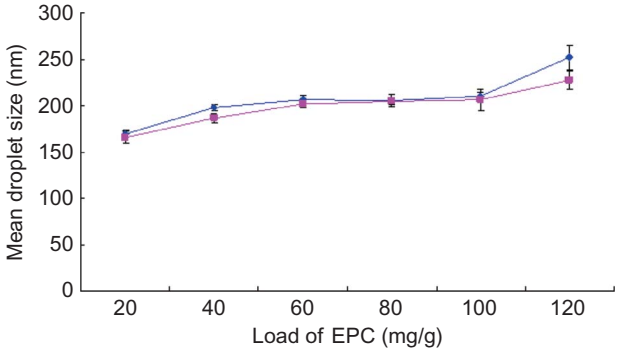

Figure 5. Effect of drug loading on mean emulsion droplet size in 0.1 N HCl and water (n = 3) (◆, 0.1N HCl; ■, water. The ratio of surfactant/cosolvent was 3 : 2.).

pH stability

In 0.1N HCl, about 7% and 12% of etoposide in the EPC-SEDDS degraded for 1 and 2 hours, respectively. However, EPC-SEDDS had a stability in PBS in 24 hours (data not shown), which was in accordance with the results that etoposide had a stability at pH 5.0–7.4[4].

In vitro release

To understand the characteristics of drug release from SEDDS, an in vitro release study was carried out, for which sink conditions were provided during the whole release test. The profiles of drug release were shown in Figure 6. The results indicated that the release of four formulations followed first-order kinetics and was typical of sustained characteristics. At 24 hours, the release of ES was not more than 26.5%, whereas EPCS was 34.7%, E-SEDDS was 70.3%, and EPC-SEDDS was 77.6%. The release of EPCS increased by 1.31-fold as compared to that of ES, which indicated PC could enhance drug release. Moreover, the release of SEDDS increased by more 2.65-fold as compared with that of ES. It is thought that SEDDS could be easily dispersed to form emulsion, and dissolved molecules could permeate out of the dialysis bag easily, so that release of etoposide can be greatly enhanced by SEDDS. It was found in our study that drug release from EPC-SEDDS was slightly faster than that from E-SEDDS, though the difference is not statistically significant.

Pharmacokinetic study

The experiment was carried out to compare the pharmacokinetics of EPC-SEDDS, E-SEDDS, EPCS, and ES in rats. Figure 7 showed the plasma profiles of etoposide after oral administration of the four preparations. The pharmacokinetic parameters are given in Table 3.

Table 2. The mean droplet size and zeta potential of EPC-SEDDS and E-SEDDS in various aqueous media at 25°C (n = 3).

	EPC-SEDDS		E-SEDDS	
Media	Droplet size(nm)	Zeta potential (mV)	Droplet size (nm)	Zeta potential (mV)
Distilled water	204.1 ± 10.2	−18.60 ± 0.55	49.4 ± 2.5	−12.93 ± 0.74
0.1N HCl	208.1 ± 7.4	+4.97 ± 0.75	48.6 ± 3.2	−0.35 ± 0.26
pH 6.8 PBS	206.7 ± 9.6	−1.98 ± 0.60	48.9 ± 3.4	−1.48 ± 0.18

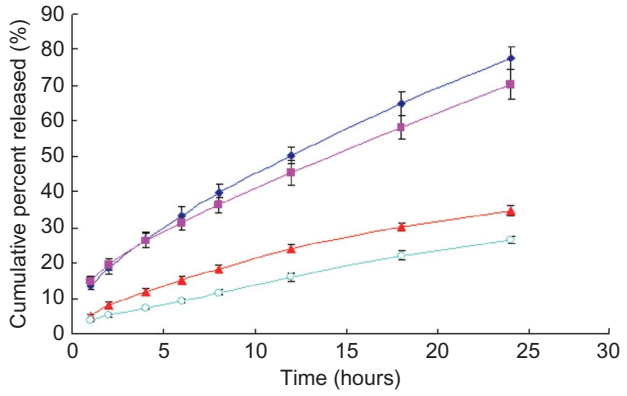

Figure 6. Release profiles of etoposide from several vehicles as determined with a dialysis method in pH 6.8 PBS ($n = 6$). (◆, EPC-SEDDS; ■, E-SEDDS; ▲, EPCS; ❍, ES).

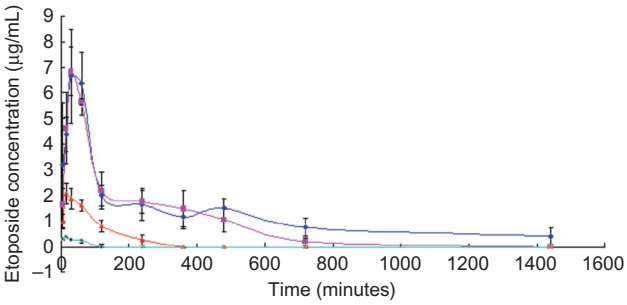

Figure 7. Mean plasma etoposide concentration-time plots after a single oral dose of etoposide in four formulations to rats ($n = 6$) (◆, EPC-SEDDS; ■, E-SEDDS; ▲, EPCS; ❍, ES).

First, as shown in Figure 7, the plasma concentration of EPCS was much higher than that of ES. The peak plasma concentration reached 0.39 μg/mL rapidly within 15 minutes when ES was administered, whereas the peak concentration (2.17 μg/mL) appeared at 22.5 minutes for EPCS. In addition, $AUC_{0 \to 24}$ of EPCS was significantly higher than that of ES, by approximately 8.44-fold. EPC could improve etoposide absorption and enhance bioavailability, which was in agreement with that of Curcumin[39] and Puerarin[8,9]. This might be due to greater solubility of EPC in water than etoposide (Table 1), which was beneficial to drug absorption. Phospholipid is a component of the cell membrane with strong affinity with the cell surface, thus it can stimulate etoposide to integrate with the cells and improve the absorption and bioavailability[8,9,11]. High level of phospholipids in EPC facilitated the formation of micelles in the intestine when they were mixed with bile salt, thus increasing drug solubility and preventing drug precipitation. As a result, drug absorption was increased[45].

Second, C_{max} and $AUC_{0 \to 24}$ of E-SEDDS were proximately 17.56- and 44.94-fold compared to that of ES, and T_{max} rose from 15 minutes to more than 30 minutes, which demonstrated that SEDDS could greatly enhance etoposide bioavailability. Therefore, SEDDS might be

a promising approach to oral delivery of etoposide. As to SEDDS,

some factors demonstrated that the drug absorption behavior could be facilitated by the formation composition, especially by the surfactant, which could reduce the interfacial surface tension and enhance penetration of the drug to epithelial cells[45]. The surfactant also dispersed the lipid formulation in the gastrointestinal tract into small droplets with a large interfacial surface area, which might also be responsible for oral absorption[28,47,48]. The cosurfactants enabled dissolution of large quantities of the hydrophilic surfactant or the drug in the lipid base. In addition, the medium-chain triglycerides with bile salts formed lipophilic particles and overcame the barrier of aqueous diffusion layer in the gastrointestinal (GI) tract[49–51]. At the same time, the medium-chain fatty glyceride had an enhanced effect on the intestinal cells to allow the lipid particles to pass across the cell layer[52–54], which may reduce the opportunity for the hepatic first-pass metabolism and, therefore, enhance bioavailability of drugs[45].

Third Table 3 showed that relative bioavailability of EPC-SEDDS was enhanced by 1.34- and 7.13-fold as compared to E-SEDDS and EPCS, respectively. There might be a synergistic effect between PC and SEDDS, which we thought that might be related to some different absorption mechanisms between them. In additional, some factors might be beneficial to enhance the relative bioavailability. Etoposide was a substrate for P-glycoprotein,

Table 3. Relative bioavailability and pharmacokinetic parameters of etoposide after oral administration of EPC-SEDDS, E-SEDDS, EPCS, and ES to rats ($n = 6$).

	EPC-SEDDS	E-SEDDS	EPCS	ES
C_{max} (μg/mL)	7.16 ± 1.21	6.85 ± 0.94	2.17 ± 0.36	0.39 ± 0.05
T_{max} (minutes)	45 ± 17.3	30 ± 0	22.5 ± 8.7	15 ± 0
$AUC_{0 \to 24}$ (μg/mL min)	1819.48 ± 173.64	1385.97 ± 99.51	255.05 ± 56.53	30.22 ± 11.47
F1 (%)	6021.26	4493.97	844.06	
F2 (%)	713.37	532.43		
F3 (%)	133.96			

The data were mean ± SD ($n = 6$). C_{max}, peak plasma concentrations; T_{max}, peak time; AUC_{0-t}, area under the concentration–time curve. The relative bioavailability (F) was calculated using the following equation: $F = AUC_{test}/AUC_{reference} \times 100\%$
For F1, $AUC_{reference}$ refers to AUC of ES, and AUC_{test} refers to AUC of EPC-SEDDS, E-SEDDS, and EPCS; for F2, $AUC_{reference}$ refers to AUC of EPCS, and AUC_{test} stands for AUC of EPC-SEDDS and E-SEDDS; and for F3, $AUC_{reference}$ refers to AUC of E-SEDDS, and AUC_{test} stands for AUC of EPC-SEDDS.

and phospholipids in EPC-SEDDS inhibited drug-mediated efflux by P-glycoprotein[45], resulting in enhanced bioavailability. Table 1 shows that EPC had higher solubility in blank-SEDDS than etoposide, so that EPC-SEDDS had high solvent capacity to prevent the drug from precipitating when diluted with gastrointestinal fluid. This might also be responsible for better bioavailability of EPC-SEDDS. As shown in Section 'pH stability', only 10% etoposide in the EPC-SEDDS degraded in 0.1N HCl within 2 hours, which made most of etoposide reach the intestine. This might also account for the enhanced oral bioavailability for EPC-SEDDS. The higher bioavailability of EPC-SEEDS compared to E-SEDDS might arise from the increase in the duration of action. It is surprising that the plasma concentration profile of EPC-SEDDS had a small peak at 480 minutes, which the mechanisms should be concerned for the following studies.

In this study, rats were used due to their low cost and relatively facile accessibility. However, one of the problems that may arise from the rat model was that the amount of fluid present in the rat stomach might not be large enough to emulsify the administered dose of SEDDS and what the absorption mechanisms of EPC-SEDDS were. Therefore, the influence of this animal model on bioavailability was therefore needed to be further investigated.

Conclusions

EPC was prepared by reacting etoposide and phospholipid in tetrahydrofuran at a ratio of 1:1.2 (mol/mol). DSC curves showed that the drug and phospholipids combined through hydrogen bonds or van der Waals force. The solubility of EPC in various solvents was higher than that of etoposide. The components and their ratios for SEDDS formulation were obtained according to pseudoternary phase diagram construction. The optimal formulation of SEDDS consisted of 20% ODO, 48% Cremopher EL, and 32% PEG-400, which had sufficient drug loading, rapid self-emulsification in aqueous media, and availability of forming droplet size in the range of emulsion. The vitro release studies revealed that the release of etoposide from EPCS and EPC-SEDDS was faster than that of ES. Compared to ES, relative bioavailability of EPC-SEDDS, E-SEDDS, and EPCS after oral administration in rats was enhanced by 60.21-, 44.9-, and 8.44-fold, respectively. The higher bioavailability of EPC-SEDDS was compared to EPCS and E-SEDDS. It was concluded that the synergistic effect between PC and SEDDS contributed to the enhanced bioavailability of etoposide. These results suggested that the combined use of PC and SEDDS was a potential approach for oral administration of etoposide.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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