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Self-double-emulsifying drug delivery system (SDEDDS): A new way for oral delivery of drugs with high solubility and low permeability

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ABSTRACT

Water-in-oil-in-water (w/o/w) double emulsions are potential for enhancing oral bioavailability of drugs with high solubility and low permeability, but their industrial application is limited due to the instability. Herein, we developed a novel formulation, self-double-emulsifying drug delivery systems (SDEDDS) by formulating mixtures of hydrophilic surfactants and water-in-oil (w/o) emulsions, which were easier to be stable through formulations optimization. SDEDDS can spontaneously emulsify to water-in-oil-in-water (w/o/w) double emulsions in the mixed aqueous gastrointestinal environment, with drugs encapsulated in the internal water phase of the double emulsions. We employed SDEDDS to improve the oral absorption of pidotimod, a peptide-like drug with high solubility and low permeability. The optimized pidotimod-SDEDDS were found to be stable up to 6 months under 25 °C. Plasma concentration–time profiles from pharmacokinetic studies in rats dosed with SDEDDS showed 2.56-fold ($p < 0.05$) increased absorption of pidotimod, compared to the pidotimod solution. Histopathologic studies confirmed that SDEDDS exerted absorption promoting effect without serious local damages. These studies demonstrate that SDEDDS may be a promising strategy for peroral delivery of peptide and peptidomimetic drugs.

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1. Introduction

Oral administration is the most favorable route of drug delivery for both patients and drug manufacturers. Nevertheless, many potential hydrophilic drugs, such as protein and peptide drugs, administrated orally exhibit low oral bioavailability mainly due to their low intestinal permeability. For this kind of drugs, defined as 'high solubility/low permeability class' or biopharmaceutics classification system (BCS) class III, gastrointestinal permeation is the rate-controlling step in the absorption process (Amidon et al., 1995). Many approaches such as absorption enhancers (Miyake et al., 2003; Takatsuka et al., 2006), chemical modification (Asada et al., 1995; Wang et al., 2003) and pharmaceutical means were used to enhance the oral bioavailability of those drugs. Among these approaches, water-in-oil-in-water (w/o/w) double emulsions show great potential (Koga et al., 2010; Shima et al., 2006).

Water-in-oil-in-water (w/o/w) double emulsions are complex systems consisting of aqueous droplets dispersed within larger oil droplets, which are themselves dispersed in an aqueous continuous phase. The internal aqueous droplets encapsulated by the oil membrane can be seen as a storage chamber for hydrophilic drugs. This structure could protect the drug dissolved in the internal aqueous phase and have shown great promise for enhancing oral bioavailability of low-permeability compounds (Koga et al., 2010). However, the industrial application of double emulsions is limited mainly due to their instability against heat, organic solvents and pH changes. Although many efforts have been done, no pharmaceutical double emulsions have overcome the research phase and been marketed (Benichou et al., 2007; Su et al., 2006).

Herein, we developed a novel formulation design, self-double-emulsifying drug delivery system (SDEDDS), which are the formulated mixtures of water-in-oil (w/o) emulsions and hydrophilic surfactants. Generally, w/o/w double emulsions are prepared by a modified two-step emulsification method. SDEDDS changed the process of the second emulsification step, which can self-emulsify to w/o/w double emulsions due to the gastrointestinal peristaltic movements in vivo instead of artificial emulsification in vitro. The concept of SEDDS was employed to realize this idea.

Self-emulsifying drug delivery systems (SEDDS) are a vital tool with great promise in enhancing the oral bioavailability of poorly water-soluble drugs (Atef and Belmonte, 2008; Julianto et al., 2000; Setthacheewakul et al., 2010; Zhao et al., 2010). As isotropic mixtures of drug, oils and surfactants, these systems rapidly disperse in gastrointestinal fluids following their oral administration, yielding micro- or nanoemulsions containing the solubilized drug (Gursoy and Benita, 2004; Kohli et al., 2010; Singh et al., 2009).

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Similar to SEDDS, SDEDDS can spontaneously emulsify in the mixed aqueous gastrointestinal environment. But the formed emulsions are water-in-oil-in-water (w/o/w) double emulsions not o/w emulsions, and drugs are encapsulated in the internal water phase of the double emulsions. Compared to conventional thermodynamically unstable double emulsions, SDEDDS are stable formulation systems. In addition, SDEDDS can be filled directly into soft or hard gelatin capsule which are easy to administration and storage.

The main aims of this study were to develop and characterize SDEDDS formulations. Pidotimod, a peptide-like drug, was chosen as a model drug of high solubility/low permeability class (Riboldi et al., 2009). Pseudo-ternary phase diagrams were constructed to identify the efficient self-double-emulsification region. The developed formulations were characterized by assessing self-double-emulsification performance, viscosity, double emulsions droplet size analysis, confocal scanning laser microscopy studies, in vitro drug release characteristics, and formulation stability studies. At last, the oral absorption of pidotimod was evaluated in rats for the pidotimod-SDEDDS and pidotimod aqueous solution.

Table 1
Solubility of pidotimod in various vehicles.

Vehicles	Solubility of pidotimod (mg/ml)
Oils	
MCT	0.72 ± 0.01
Oleic acid	0.39 ± 0.02
Soybean oil	0.38 ± 0.01
Olive oil	0.36 ± 0.01
Surfactants	
PGPR	0.21 ± 0.01
Span 80	0.90 ± 0.01
Span 85	1.90 ± 0.02
Tween 80	0.82 ± 0.01
Aqueous buffer solutions	
pH = 2.0	26.73 ± 0.26
pH = 3.0	33.49 ± 3.83
pH = 4.0	34.09 ± 0.96
pH = 5.0	67.45 ± 3.52
pH = 6.0	47.00 ± 1.79
pH = 7.0	51.38 ± 3.80
pH = 8.0	54.67 ± 3.37
pH = 9.3	28.47 ± 1.85
pH = 10.0	31.86 ± 0.88

2. Materials and methods

2.1. Materials

Pidotimod (99.6% purity) was supplied by the Wuzhong Medicine Co., Ltd. (Suzhou, China). Bean phospholipids were purchased from Taiwei Co., Ltd. (Shanghai, China). Tween 80, Span 80, Span 85, polyglycerol ester of polyricinoleic acid (PGPR), medium chain triglycerides (MCT), oleic acid, soybean oil and olive oil were obtained from Well Chemical Co., Ltd. (Nanjing, China). Methanol of HPLC grade was obtained from Yuwang Chemical Reagents Co. (Shandong, China). Perchloric acid was purchased from Linfeng (Shanghai, China). Isopropanol, sodium dihydrogen phosphate, alcohol, sodium hydroxide and gelatin were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Water was purified by redistillation and filtered through a 0.22 μm membrane filter (Zongxing Filter Co., Ltd., China) before use. All other chemicals were of analytical grade.

2.2. Solubility studies

Solubility of pidotimod in various vehicles, including oils (MCT, oleic acid, soybean oil, olive oil), surfactants (Span 80, Span 85, PGPR, Tween 80) and various aqueous buffer solutions were studied by the shake flask method. The following buffers were used: acetate (pH 4–5), phosphate (pH 2–3 and pH 6–8) and carbonate (pH 9–10). An excess amount of pidotimod (approximately 1 g) was added to each cap vial containing 1 ml of the vehicles (Table 1). After sealing, the mixture was vortexed using a mixer (XW-80A, Shanghai Medical Instrument Factory, China) at a maximum speed for 10 min and kept for 48 h at 25 °C in a shaking water bath (SHY-2A, Jintan Experimental Instrument Factory, China) to facilitate the solubilization. The samples were centrifuged (Sigma 3K30, Harz, Germany) at 3000 × *g* for 15 min to remove the undissolved pidotimod. The supernatant was taken and diluted with methanol for quantification of pidotimod by HPLC. Each value represents the mean ± SE ($n = 3$).

The quantitative determination of pidotimod was performed on a Shimadzu LC-20AT (Shimadzu Cooperation, Tokyo, Japan) with a C18 column (Phenomenex® ODS, 250 mm × 4.6 mm i.d., 5 μm). The mobile phase was composed of 0.01 M sodium dihydrogen phosphate–methanol–isopropanol (97:2:1, v/v/v), with the flow rate of 1.0 ml/min. Detection was recorded at wavelength of 210 nm by a Shimadzu SPD ultraviolet detector, with a sample injection volume of 20 μl by a manual injection valve (7725i, Rheodyne, USA). The pidotimod stock solution was diluted to working solutions ranging from 0.1 to 20 μg/ml, and the standard calibration curves were constructed by plotting concentrations against peak areas. A good linearity was achieved with a correlation coefficient of 0.9999 over the concentration range of 0.1–20 μg/ml. The RSDs of inter- and intra-day precision and accuracy were all less than 2% at low, medium and high concentrations.

2.3. Construction of pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams were constructed by using the titration method, with the oil phase being replaced by water-in-oil (w/o) emulsion. The w/o emulsions was developed by one step emulsification procedure. Pidotimod was dissolved in distilled water when adjusting the pH to 6.0 ± 0.5 by a pH meter (PHS-25, Magnetic, Shanghai, China), and 0.5% gelatin solution was added to the solution. Then, the pidotimod aqueous solution was added to the oil phase which consisted with MCT, oleic acid, Span 80 and bean phospholipids under moderate magnetic stirring (Magnetic Stirrer with Hot Plate, USA). The course w/o emulsion was homogenized by a rotor–stator system at 9500 rpm for 3 min (CAT X620, Zipperer GmbH, Staufen, Germany). The chosen w/o emulsion formulation was contained 0.4 g pidotimod, 0.8 g bean phospholipids, 1.75 g Span 80, 3 g oleic acid, 1 g MCT, 0.25 g of 0.5% gelatin solution and 1 g water.

A series of mixtures that consisted with w/o emulsion and aqueous phase were made at certain weight ratios (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 0:10). Each mixture was accurately weighed into glass vials and mixed homogeneously under moderate magnetic stirring at room temperature. Tween 80 was then added into each mixture drop-by-drop by a dropper quantitatively. During the titration process, samples were stirred and observed by optical microscopy. At proper concentration of Tween 80, the structure of double emulsions would appear. The concentration of Tween 80 at which double-emulsions-formation and double-emulsions-disappearance transition occurred was obtained by the weight measurements. These values were then used to determine the boundaries of the double emulsion regions, which is corresponding to the selected optimum ratios of combination vehicles for developing pidotimod-SDEDDS formulations.

Table 2
Composition (mg/capsule) of pidotimod-SDEDDS formulations F1–F5 with 40 mg pidotimod in 830 mg of mixture of oil phase and surfactants phase per capsule.

Compositions (mg/capsule)	Formulations (mg/capsule)				
	F1	F2	F3	F4	F5
Pidotimod	40	40	40	40	40
Oil phase					
Bean phospholipids	0	20	40	80	100
Span 80	175	175	175	175	175
Oleic acid	380	360	340	300	280
MCT	100	100	100	100	100
0.5% gelatin solution	25	25	25	25	25
H_2O	100	100	100	100	100
Surfactant phase					
Tween 80	50	50	50	50	50

2.4. *Formulation and preparation of pidotimod-SDEDDS*

The formulated SDEDDS were mixtures of a hydrophilic surfactant and w/o emulsions. Five formulations of SDEDDS (F1–F5) containing a fixed proportion of pidotimod (4.60%, w/w) and different amount of phospholipids were prepared. A typical formulation (e.g. F4) contained 4 g of pidotimod, 8 g of bean phospholipids, 17.5 g of Span 80, 30 g of oleic acid, 10 g of MCT, 2.5 g of 0.5% gelatin solution, 10 g of water and 5 g of Tween 80.

As shown in Table 2, the w/o emulsions were prepared by one step emulsification procedure as described in Section 2.3. Then the w/o emulsions were mixed with Tween 80 using a magnetic stirrer until pidotimod-SDEDDS was obtained. All the formulations were left for 24 h at room temperature. Hard gelatin capsules (size 00) were manually filled with 870 ± 13 mg of each formulation (F1–F5), resulting in each capsule containing 40 mg pidotimod. The pidotimod-SDEDDS capsules were stored in air-tight glass containers at room temperature until required for analysis.

2.5. *Physical characterization of SDEDDS formulations*

2.5.1. *Confocal scanning laser microscopy*

Confocal scanning laser microscopy (CSLM) (Leica TCS SP2, Leica Microsystems, Germany) was used to observe the freshly prepared pidotimod-SDEDDS (F4) to estimate the size of the water droplets in F4. SDEDDS (1 ml) was diluted with 2 ml MCT and stained for fat by thoroughly mixing with 2 μl fluorescein (0.1%, w/v); 10 μl of sample was then dropped on a plain glass slide, covered by a cover slip and examined using an Ar/Kr laser with an excitation line of 494 nm; the resolution of this techniques is >0.2 mm.

2.5.2. *Viscosity analysis of SDEDDS formulations*

The rheological measurements of the SDEDDS formulations were performed with a programmable rheometer (DVIII; ULTRA Premium Oilfield Services, Ltd., Brookfield, Ohio) operating with cone plate geometry (cone diameter 60 mm, angle 1°, and gap 0.058 mm). Samples (about 5 ml) were transferred to the instrument and allowed to equilibrate to 25 ± 1 °C for 10 min prior to the measurement. The apparent viscosity was measured over a shear rate range of 0.1–300 s^{-1}. Viscosities (mPa s) of each SDEDDS formulations were measured at different shear rates; the mean constant shear viscosity was determined from the data obtained at 300 s^{-1}. Six replicate analyses were carried out for each formulation, and data presented as means ± SD.

2.5.3. *Assessment of self-double-emulsification performance of SDEDDS formulations*

The self-double-emulsification performance of the formulations was visually assessed after 120 min according to the grading system that has previously been reported (Ruan et al., 2010; Setthacheewakul et al., 2010). Three replicate assessments were performed for each formulation in each of the media. One capsule of each of the pidotimod-SDEDDS formulations (F1–F5) was placed separately in 900 ml of simulated gastric fluid (SGF, pH 1.2) without pepsin. Gentle agitation was provided by a paddle rotating at 37 ± 0.5 °C and a rotating speed of 75 rpm.

Moreover, CSLM was used to observe the self-double-emulsification performance/behavior/process of the optimized pidotimod-SDEDDS formulation (F4). One capsule of the previously stained formulation (as described in Section 2.5.1.) was added to 200 ml of purified water at 37 °C. Gentle agitation was provided by a standard stainless steel dissolution paddle rotating at 60 rpm. Immediately, 1 min after the addition, the stained samples was withdrawn and dropped on a glass slide to observe the formation of w/o/w emulsions.

2.5.4. *Emulsion droplet size analysis*

The formulations (F1–F5) droplet size distribution of double emulsions of were measured by dynamic light scattering using a Malvern Particle Size Analyzers (Mastersizer 2000S, Malvern Instruments, Ltd., Malvern, Worcestershire, UK) equipped with a He–Ne laser (623 nm). Pidotimod-SDEDDS (0.87 g equivalent to pidotimod 40 mg) were mixed with distilled water (200 ml) and stirred at mild agitation (75 rpm) in a magnetic stirrer for 5 min at room temperature, forming the double emulsions. The particle size distribution of the double emulsions was determined after presentation. The refractive index of dispersed phase and continuous phase were 1.600 and 1.333, respectively, and the absorbance value of the emulsion droplets was 0.1. The results are reported as the volume average diameter (d_{43}). Three replicate analyses were carried out for each formulation, and data presented as means ± SD.

2.6. *Release of pidotimod from SDEDDS formulations in vitro*

Release profiles from pidotimod-SDEDDS (F1–F5) filled in capsules were performed using the USP30 rotating paddle apparatus (Hanson Research Corporation, USA) with 900 ml of simulated gastric fluid (SGF, pH 1.2) without pepsin as the medium at 37 ± 0.5 °C. The speed of the paddle was adjusted to 75 rpm. Samples (5 ml) were withdrawn and replaced with fresh media after 5, 15, 30, 60, 120, 240, 360 and 480 min. Samples were filtered using a 0.45 μm filter and analyzed using an HPLC assay. Three replicate analyses were carried out for each formulation, and data presented as means ± SD. The quantification of pidotimod by HPLC is shown in Section 2.2.

2.7. *Stability studies*

Stability study was performed by storing the ready-to-use pidotimod-SDEDDS (F4) in the sealed amber glass vials at 25 °C. The stability was evaluated by monitoring the time-dependent change in appearance, viscosity, self-emulsifying properties and double emulsion droplet size of the SDEDDS formulation within the capsules at 0, 3, and, 6 month.

2.8. *In vivo absorption studies*

Male Sprague–Dawley rats (250 ± 20 g) were obtained from Laboratory Animal Center of China Pharmaceutical University (Nanjing, China). The rats were housed under normal laboratory conditions of temperature, relative humidity and light, and had free access to standard rodent diet and water before the experiment. The animal experiments were approved and supervised by the Animal Experimental Ethical Committee of China Pharmaceutical University (Nanjing, China).

The rats were deprived of food but had free access to water 24 h before the experiment. Rats were divided randomly into two groups with six rats each. One group was administered orally pidotimod aqueous solution (control group), another was administered pidotimod-SDEDDS formulation (F4) at a pidotimod dose of 80 mg/kg body weight (Mailland et al., 1994). Sample of pidotimod powder (400 mg), pidotimod-SDEDDS (F4) (8.7 g equivalent to pidotimod 400 mg) were weighed accurately and separately dispersed into distilled water (50 ml) by mixing homogeneously for 30 s prior to dosing. Each formulation (2.5 ml) was given orally using feeding sonde. Under ether anesthesia, blood samples (0.5 ml) were collected from the eye ground vein into heparinized plastic centrifuge tubes using 0.8–1.1 mm capillary glass tube, at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 24 h after intragastric administration. The samples were centrifuged for 15 min at 2600 × *g* in an Nr.12154 rotor (Sigma 3K30, Harz, Germany). 200 μl of plasma were uptaken by pipettor (Eppendorf, Germany) and mixed with the same volume of 10% perchloric acid solution in order to precipitate proteins. The mixture was stirred on a vortex mixer for 90 s and centrifuged at 9000 rpm for 10 min. Then, 20 μl of supernatant was analyzed by a HPLC method. The column was an Aminex Ion Exclusion HPX 874 with a PRP precolumn, the mobile phase was 0.05% sulfuric acid–acetonitrile (88:12, v/v), the flow rate was 0.6 ml/min and the detection wavelength was 210 nm (Dal Bo et al., 1993). After validation of specificity, linearity, recovery, precision, accuracy and stability, the method has been successfully applied in rat plasma.

The pharmacokinetic parameters were computed by DAS 2.0 software (Mathematical Pharmacology Professional Committee of China, China) using the trapezoidal rule (for AUC calculation).

2.9. Histopathologic evaluation

Male Sprague–Dawley rats (Laboratory Animal Center of China Pharmaceutical University, Nanjing, China), weighing 200–250 g, were fasted for about 20 h. At 2 h after the intragastric administration of 0.5 ml pidotimod-SDEDDS formulation (F4) and 5.0% glucose solution (control), the colonic lumen were carefully excised and then washed out with 10 mM Tris/HCl buffer (pH 7.4). All the dissected intestines were cut into 5 mm pieces and placed in a fixative solution (4.0% paraformaldehyde containing 1.0% glutaraldehyde dissolved in PBS, pH 7.4) for 4 h at room temperature and agitated constantly. Cross-sections were prepared and stained with hematoxylin–eosin, and were examined by a Nikon model Eclipse T*i* optical microscope equipped with Nikon Digital Camera model DS-Ri 1 and Nikon NIS-Elem F 3.0 (Nikon, Tokyo, Japan). As a positive control for mucosal damage, a mixture of N-acetyl-L-cysteine (NAC) and Triton X-100 was also administered to rats according to the method of Takatsuka et al. (2006).

2.10. Statistical analysis

Results are expressed as mean ± SD of more than three experiments. Analysis of variance (ANOVA) was used to test the statistical significance of differences among groups. Statistical significance in the differences of the means was determined by Dunnet's method or Student's *t*-test.

3. Results and discussion

3.1. Solubility studies

The self-double-emulsifying formulations consisted of water, oil, surfactants and drug should be a clear mixture and should have good solvent properties to allow presentation of the drug in the mixture. The solubility results (Table 1) revealed that pidotimod had high aqueous solubility, and it was therefore solubilized in the internal aqueous core of w/o emulsions, which were the major component of self-double-emulsifying systems. All the oils and surfactants showed poor solubility of the drug. Among the oils tested in this study, MCT was selected as oil phase for its better solubility than others. Oleic acid was also added because it was reported for its enhanced intestinal absorption of drugs (Morishita et al., 1998; Onuki et al., 2004). Even though Span 85 showed better solubility for pidotimod than other surfactants, Span 80 was selected as a hydrophobic surfactant due to its good emulsion-forming ability. Furthermore, Tween 80 was selected as a hydrophilic surfactant for its good compatibility with the w/o emulsions.

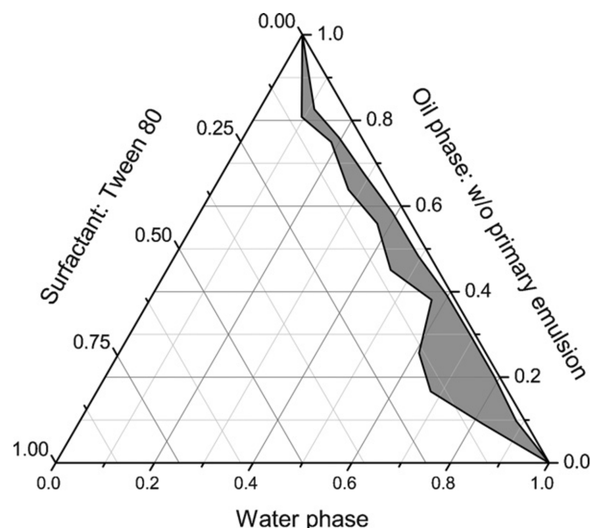

Fig. 1. Pseudo-ternary phase diagram showing double emulsion region (oil: w/o emulsions; SA: Tween 80 and distilled water) at room temperature.

3.2. Pseudo-ternary phase diagrams

Pseudo-ternary phase diagram was constructed to identify the self-double-emulsifying regions for the selected vehicle (w/o primary emulsion and Tween 80). As shown in Fig. 1, the shadow region represents the double emulsion region. It is important to determine this area in order to ensure successful conversation of pidotimod-SDEDDS to double emulsion by dilution with distilled water. Combined with surfactant (<15%, w/w), different ratios of w/o emulsion to water (from 1:9 to 9:1) could spontaneously form water-in-oil-in-water (w/o/w) double emulsions to develop a SDEDDS formulation.

3.3. The development and characterization of the pidotimod-SDEDDS

3.3.1. Development of the pidotimod-SDEDDS

The physical properties of different SDEDDS formulations containing pidotimod 40 mg per capsule are summarized in Table 3, and the release profiles of formulations are shown in Fig. 2. The SDEDDS systems composed with different amount of phospholipids and Span 80 became bright white after diluted by aqueous medium (visual grading system, see Table 3 footnote). Using phospholipids (F2–F5) could result in the increased viscosity, but the decreased dissolutions of those formulations over time (Fig. 2). In addition, the formulation (F4) showed slower dissolution of pidotimod than the other formulations and provided sustained release up to 480 min. It was also noted that the amount of phospholipids had no significant influence on the mean droplets size of double emulsions (F1–F5), which could not explain the sustained release effect of phospholipids. Besides, as a polymeric surfactant, phos-

Table 3
Physical properties of different pidotimod SDEDDS formulations. Data represents the mean ± SD (n = 3 or 6).

Physical properties	Formulations				
	F1	F2	F3	F4	F5
Appearance	Clear orange-yellow liquid	Clear orange-yellow liquid	Clear orange-yellow liquid	Clear orange-yellow liquid	Clear orange-yellow liquid
Visual grading[a]	C	C	C	C	C
Viscosity (mPa s)	51.66 ± 0.46	58.16 ± 1.32	73.68 ± 0.83	105.70 ± 1.88*	108.17 ± 1.85*
Particle size (μm)	13.86 ± 1.80	20.86 ± 1.40	19.73 ± 1.69	21.83 ± 1.03	23.44 ± 1.16
$T_{50\%}$[b] (min)	0.94 ± 0.15	5.10 ± 0.68	5.60 ± 0.71	30.55 ± 1.84**	15.35 ± 1.34*

* $p < 0.05$ versus bean phospholipids none group by Tukey test.
** $p < 0.01$ versus bean phospholipids none group by Tukey test.
[a] Visual grading system: (A) denoting a rapidly forming (within 1 min) microemulsion that was clear or slightly bluish in appearance; (B) denoting a rapidly forming, slightly less clear emulsion that had a bluish white appearance; (C) denoting a bright white emulsion (similar in appearance to milk) that formed within 2 min; (D) denoting a dull, grayish white emulsion with a slightly oily appearance that was slow to emulsify (longer than 2 min); and (E) denoting a formulation that exhibited either poor or minimal emulsification with large oil droplets present on the surface.
[b] Time point corresponding to 50% of drug release.

pholipids are known to increase the interfacial coverage during emulsification, and that could improve the encapsulation efficiency and control release of the addenda entrapped in the internal core of the double emulsions (Fechner et al., 2007). In this case, phospholipids molecules supplied the powerful protection to entrapped substance to form a comparatively hard polymeric film with the oil phase and control the pidotimod release as a barrier. Based on the results, F4 presented a system in self-double-emulsification performance studies. The synergistic effect of a blend of phospholipids and Span 80 afforded pidotimod-SDEDDS which provided sustained release of pidotimod. F4 was therefore selected as the optimum formulation and used for stability studies, and for in vivo absorption studies.

3.3.2. The optimum formulation (F4) of the pidotimod-SDEDDS

Pidotimod-SDEDDS (F4) (Table 2), a clear orange-yellow liquid, could form fine water-in-oil-in-water double emulsions rapidly with a bright white appearance when introduced into aqueous media. It did not show any signs of phase separation and drug precipitation, even after 8 h. The droplets size of this formulation was 21.83 ± 1.03 μm, with a narrow distribution size distribution. CLSM micrographs (Fig. 3A) revealed that the water droplets in the pidotimod-SDEDDS (F4) were macroscopically homogeneous with smooth surfaces. CLSM micrographs (Fig. 3B) showed that the SDEDDS could form fine w/o/w double emulsions upon dilution with the dispersion medium at gentle stirring condition within 1 min, and the shape of the obtained double emulsions were spherical mostly, with structure of dispersed oil droplets contain smaller dispersed aqueous droplets. The release profile (Fig. 2) of F4 shows that the SDEDDS has significantly decreased the dissolution of pidotimod and provided a sustained release of pidotimod up to 8 h in SGF, when compared to formulation without phospholipids.

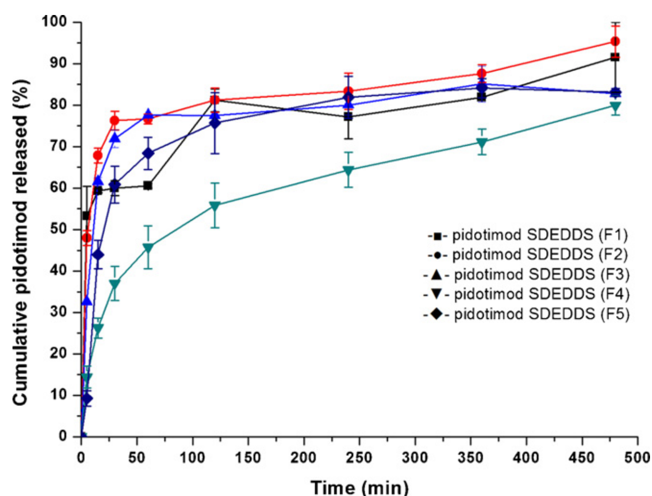

Fig. 2. Release profiles of pidotimod from SDEDDS (F1–F5) in simulated gastric fluid (SGF, pH 1.2) without pepsin. Data represent the means ± SD (n = 3).

3.4. Stability studies

The formulation of pidotimod-SDEDDS (F4) was kept at 25 °C. The stability data are summarized in Table 4. The selected formu-

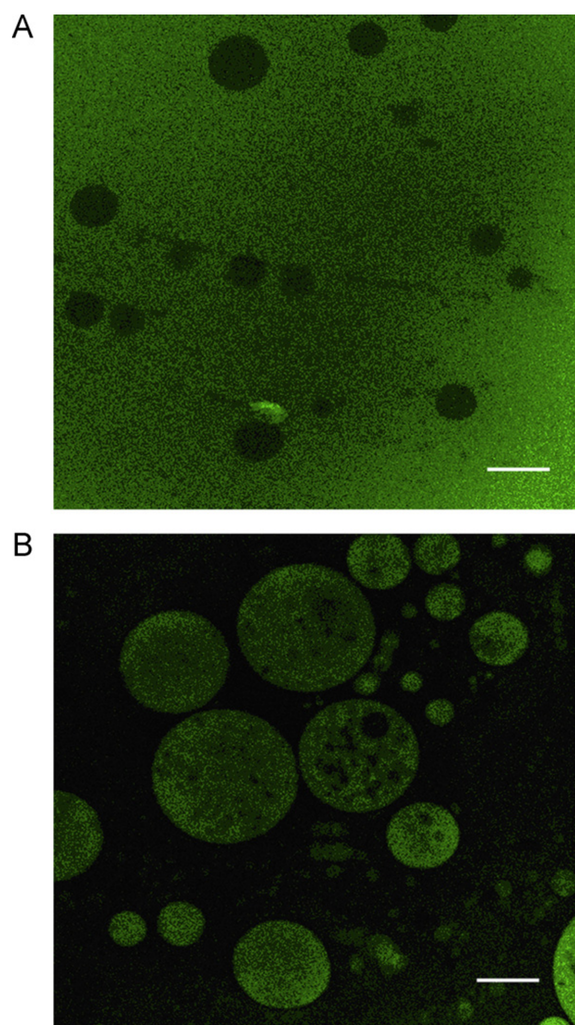

Fig. 3. Confocal microscopy images of (A) freshly prepared pidotimod-SDEDDS (F4) and (B) formulated fine w/o/w double emulsions of F4 after 1 min dilution with the dispersion medium. Scale bar represents 10 μm.

Table 4
Stability data of capsules filled with liquid pidotimod-SDEDDS (F4). Data reported are means ± SD (n = 3).

Sampling time	Appearance	Viscosity (mPa s)	Visual grading[a]	Droplets size (μm)
0 month	Clear orange-yellow liquid	108.17 ± 0.58	C	24.55 ± 1.77
3 month	Clear orange-yellow liquid	110.50 ± 1.03	C	25.30 ± 2.49
6 month	Clear orange-yellow liquid	111.76 ± 2.06	C	20.82 ± 1.34

[a] Visual grading system: (A) denoting a rapidly forming (within 1 min) microemulsion that was clear or slightly bluish in appearance; (B) denoting a rapidly forming, slightly less clear emulsion that had a bluish white appearance; (C) denoting a bright white emulsion (similar in appearance to milk) that formed within 2 min; (D) denoting a dull, grayish white emulsion with a slightly oily appearance that was slow to emulsify (longer than 2 min); and (E) denoting a formulation that exhibited either poor or minimal emulsification with large oil droplets present on the surface.

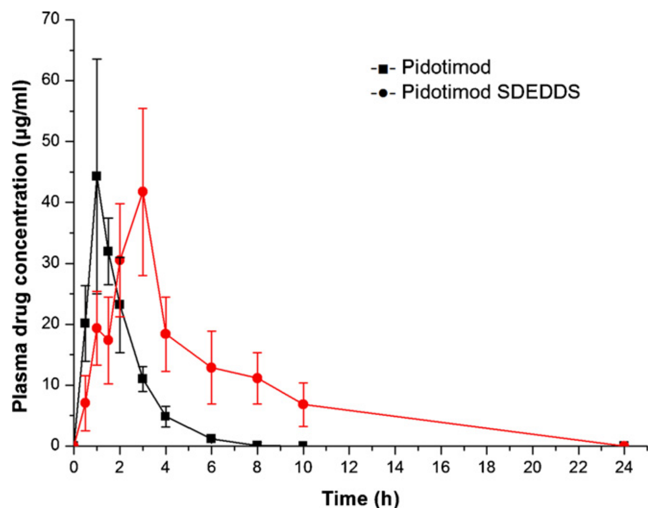

Fig. 4. Plasma concentration versus time profiles after oral administration of pidotimod formulated as SDEDDS, compared with pidotimod pharmacokinetics after dosing aqueous solutions (dose 80 mg/kg). All values reported are means ± SD (n = 6).

lation (F4) was found to be stable, without significant changes in viscosity, self-emulsification properties and particle size distribution under this condition up to 6 months. In addition, no physical phase separation was observed, which showed a good stability.

3.5. In vivo absorption study

The plasma concentration of pidotimod versus time profiles in rats following oral administration of F4 and unformulated pidotimod are presented in Fig. 4. The pharmacokinetic parameters

Table 5
Pharmacokinetic parameters of pidotimod after oral administration of pidotimod-SDEDDS and unformulated pidotimod (dose 80 mg/kg). All values reported are means ± SD (n = 6).

Parameter	Pidotimod	Pidotimod-SDEDDS
C_{max} (μg/ml)	47.1 ± 19.2	43.2 ± 13.6
T_{max} (h)	1.20 ± 0.13	2.90 ± 0.27*
$AUC_{(0-24)}$ (μg h/ml)	83.2 ± 18.1	213.1 ± 27.7*
$t_{1/2}$ (h)	0.947	2.65*

* $p < 0.05$ versus control group by Tukey test.

are summarized in Table 5. Results showed that the $AUC_{(0-24)}$ of pidotimod in SDEDDS increased by 2.56-fold compared to the unformulated pidotimod. T_{max} increased from 1.20 h to 2.90 h. C_{max} (43.2 μg/ml) of pidotimod-SDEDDS was the same as the C_{max} of aqueous solutions (47.1 μg/ml).

These results reveal that pidotimod-SDEDDS can improve the bioavailability of pidotimod with distinct delay in T_{max}. It can be envisaged that after oral administration, the pidotimod-SDEDDS would disperse and form a w/o/w double emulsions spontaneously in the gastrointestinal fluid, with the active components entrapped in the oil droplets as internal reservoirs. Thus, the prolonged absorption and elimination of pidotimod may be explained by the function of the oil coating which can postpone the drug release into the continuous phase, in accordance with the sustained drug release in vitro (Benichou et al., 2004). It is well known that phospholipids can act as a penetration enhancer for topically applied substances and can facilitate the transport of molecules into cells (Kato et al., 1987). On the other hand, there are reports that medium chain fatty acids enhanced the intestinal membrane permeability of hydrophilic compounds via the paracellular route (Cano-Cebrian

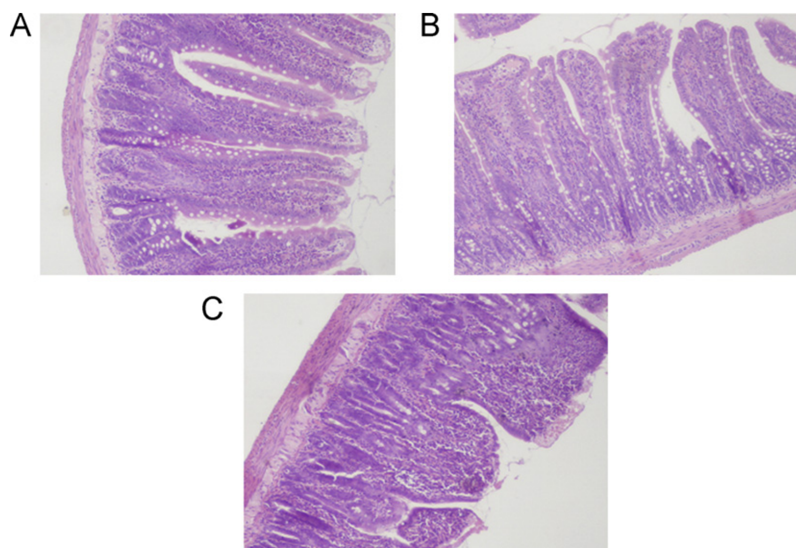

Fig. 5. Representative histological sections of intestinal segments treated according to (A) control, (B) pidotimod-SDEDDS formulation, and (C) positive control conditions (H&E stain, ×100).

et al., 2005). Therefore, the absorption-enhancing effect of SDEDDS on the intestinal absorption of pidotimod may be partly ascribed to the phospholipids and medium chain fatty acids contained in the formulation. Besides, the lymphatic transport of double emulsions through the transcellular pathway may also contribute to the increased bioavailability (Gershanik and Benita, 2000). Oral lipid-based drug-delivery systems, including various types of lipid suspensions, solutions and emulsions, have shown great potential to increase the extent of transportation via the intestinal lymphatic system and bypass the hepatic first-pass effect, thereby augmenting drug absorption from the GI tract (Buyukozturk et al., 2010; Charman et al., 1993; Humberstone and Charman, 1997; Porter and Charman, 2001). For BCS class III drugs, only when the drugs are loaded inside the oil droplets, can the absorption be significantly increased (Rao et al., 2008).

3.6. Histopathologic evaluation

Certain absorption enhancers including surfactants were reported to cause the intestinal mucosal damage, although they showed the higher intestinal absorption enhancement (Sakai et al., 1998). Therefore, we evaluated the extent of intestinal mucosal damage caused by the emulsifiers of SDEDDS through histopathologic analyses. Fig. 5A and B shows images of intestinal segments 2 h after administration of 5.0% glucose solution (control), and after intragastric administration of pidotimod-SDEDDS (F4) containing Span 80, Tween 80 and phospholipids. As a positive control of mucosal damage, the image of intestinal segment 2 h after intragastric administration of solution containing NAC and Triton X-100 was observed as a decidual alteration in the epithelial membranes (Fig. 5C). On the other hand, the epithelial membrane treated with the pidotimod-SDEDDS was free of gross damage, such as abrasion and atrophy of the epithelium, strongly suggesting that the absorption-enhancing effect of SDEDDS formulation does not cause mucosal damage.

4. Conclusion

The present studies have clearly demonstrated the potential utility of SDEDDS for formulating pidotimod with sustained release in vitro and improved oral bioavailability in vivo. The optimal formulation of the pidotimod-SDEDDS (F4) was successfully developed. The SDEDDS readily released the lipid phase to form fine water-in-oil-in-water double emulsions, with a sustained release of pidotimod. Pharmacokinetic studies in rats revealed that the absorption of pidotimod from SDEDDS showed 2.56-fold increase in bioavailability as compared to the same oral dose (80 mg/kg) of the pidotimod aqueous solution. Moreover, the SDEDDS were found to be stable over a period of 6 months under 25 °C. Our studies illustrated the potential use of novel self-double-emulsifying drug delivery systems for oral delivery drug with high solubility and low permeability.

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References

Amidon, G.L., Lennernas, H., Shah, V.P., Crison, J.R., 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 12, 413–420.

Asada, H., Douen, T., Waki, M., Adachi, S., Fujita, T., Yamamoto, A., Muranishi, S., 1995. Absorption characteristics of chemically modified-insulin derivatives with various fatty acids in the small and large intestine. J. Pharm. Sci. 84, 682–687.

Atef, E., Belmonte, A.A., 2008. Formulation and in vitro and in vivo characterization of a phenytoin self-emulsifying drug delivery system (SEDDS). Eur. J. Pharm. Sci. 35, 257–263.

Benichou, A., Aserin, A., Garti, N., 2004. Double emulsions stabilized with hybrids of natural polymers for entrapment and slow release of active matters. Adv. Colloid Interface Sci. 108–109, 29–41.

Benichou, A., Aserin, A., Garti, N., 2007. W/O/W double emulsions stabilized with WPI–polysaccharide complexes. Colloid Surf. A 294, 20–32.

Buyukozturk, F., Benneyan, J.C., Carrier, R.L., 2010. Impact of emulsion-based drug delivery systems on intestinal permeability and drug release kinetics. J. Control. Release 142, 22–30.

Cano-Cebrian, M.J., Zornoza, T., Granero, L., Polache, A., 2005. Intestinal absorption enhancement via the paracellular route by fatty acids, chitosans and others: a target for drug delivery. Curr. Drug. Deliv. 2, 9–22.

Charman, W.N., Rogge, M.C., Boddy, A.W., Berger, B.M., 1993. Effect of food and a monoglyceride emulsion formulation on danazol bioavailability. J. Clin. Pharmacol. 33, 381–386.

Dal Bo, L., Broccali, G.P., Silingardi, S., Coppi, G., 1993. A new HPLC method for pidotimod plasma levels determination. Boll. Chim. Farm. 132, 126–128.

Fechner, A., Knoth, A., Scherze, I., Muschiolik, G., 2007. Stability and release properties of double-emulsions stabilised by caseinate–dextran conjugates. Food Hydrocolloid 21, 943–952.

Gershanik, T., Benita, S., 2000. Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs. Eur. J. Pharm. Biopharm. 50, 179–188.

Gursoy, R.N., Benita, S., 2004. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. Biomed. Pharmacother. 58, 173–182.

Humberstone, A.J., Charman, W.N., 1997. Lipid-based vehicles for the oral delivery of poorly water soluble drugs. Adv. Drug Deliv. Rev. 25, 103–128.

Julianto, T., Yuen, K.H., Noor, A.M., 2000. Improved bioavailability of vitamin E with a self emulsifying formulation. Int. J. Pharm. 200, 53–57.

Kato, A., Ishibashi, Y., Miyake, Y., 1987. Effect of egg yolk lecithin on transdermal delivery of bunazosin hydrochloride. J. Pharm. Pharmacol. 39, 399–400.

Koga, K., Takarada, N., Takada, K., 2010. Nano-sized water-in-oil-in-water emulsion enhances intestinal absorption of calcein, a high solubility and low permeability compound. Eur. J. Pharm. Biopharm. 74, 223–232.

Kohli, K., Chopra, S., Dhar, D., Arora, S., Khar, R.K., 2010. Self-emulsifying drug delivery systems: an approach to enhance oral bioavailability. Drug Discov. Today.

Mailland, F., Coppi, G., Silingardi, S., 1994. Pharmacokinetics and oral bioavailability of pidotimod in humans. Arzneimittelforschung 44, 1465–1469.

Miyake, M., Oka, Y., Minami, T., Toguchi, H., Odomi, M., Ogawara, K., Higaki, K., Kimura, T., 2003. Combinatorial use of sodium laurate with taurine or L-glutamine enhances colonic absorption of rebamipide, poorly absorbable antiulcer drug, without any serious histopathological mucosal damages. J. Pharm. Sci. 92, 911–921.

Morishita, M., Matsuzawa, A., Takayama, K., Isowa, K., Nagai, T., 1998. Improving insulin enteral absorption using water-in-oil-in-water emulsion. Int. J. Pharm. 172, 189–198.

Onuki, Y., Morishita, M., Takayama, K., 2004. Formulation optimization of water-in-oil-water multiple emulsion for intestinal insulin delivery. J. Control. Release 97, 91–99.

Porter, C.J., Charman, W.N., 2001. Intestinal lymphatic drug transport: an update. Adv. Drug Deliv. Rev. 50, 61–80.

Rao, S.V., Yajurvedi, K., Shao, J., 2008. Self-nanoemulsifying drug delivery system (SNEDDS) for oral delivery of protein drugs: III. In vivo oral absorption study. Int. J. Pharm. 362, 16–19.

Riboldi, P., Gerosa, M., Meroni, P.L., 2009. Pidotimod: a reappraisal. Int. J. Immunopathol. Pharmacol. 22, 255–262.

Ruan, J., Liu, J., Zhu, D., Gong, T., Yang, F., Hao, X., Zhang, Z., 2010. Preparation and evaluation of self-nanoemulsified drug delivery systems (SNEDDSs) of matrine based on drug–phospholipid complex technique. Int. J. Pharm. 386, 282–290.

Sakai, M., Imai, T., Ohtake, H., Otagiri, M., 1998. Cytotoxicity of absorption enhancers in Caco-2 cell monolayers. J. Pharm. Pharmacol. 50, 1101–1108.

Setthacheewakul, S., Mahattanadul, S., Phadoongsombut, N., Pichayakorn, W., Wiwattanapatapee, R., 2010. Development and evaluation of self-microemulsifying liquid and pellet formulations of curcumin, and absorption studies in rats. Eur. J. Pharm. Biopharm..

Shima, M., Tanaka, M., Fujii, T., Egawa, K., Kimura, Y., Adachi, S., Matsuno, R., 2006. Oral administration of insulin included in fine W/O/W emulsions to rats. Food Hydrocolloid 20, 523–531.

Singh, B., Bandopadhyay, S., Kapil, R., Singh, R., Katare, O., 2009. Self-emulsifying drug delivery systems (SEDDS): formulation development, characterization, and applications. Crit. Rev. Ther. Drug Carrier Syst. 26, 427–521.

Su, J., Flanagan, J., Hemar, Y., Singh, H., 2006. Synergistic effects of polyglycerol ester of polyricinoleic acid and sodium caseinate on the stabilisation of water–oil–water emulsions. Food Hydrocolloid 20, 261–268.

Takatsuka, S., Morita, T., Koguchi, A., Horikiri, Y., Yamahara, H., Yoshino, H., 2006. Synergistic absorption enhancement of salmon calcitonin and reversible mucosal injury by applying a mucolytic agent and a non-ionic surfactant. Int. J. Pharm. 316, 124–130.

Wang, J., Chow, D., Heiati, H., Shen, W.C., 2003. Reversible lipidization for the oral delivery of salmon calcitonin. J. Control. Release 88, 369–380.

Zhao, Y., Wang, C., Chow, A.H., Ren, K., Gong, T., Zhang, Z., Zheng, Y., 2010. Self-nanoemulsifying drug delivery system (SNEDDS) for oral delivery of Zedoary essential oil: formulation and bioavailability studies. Int. J. Pharm. 383, 170–177.