Nanoemulsions as novel oral carriers of stiripentol: insights into the protective effect and absorption enhancement

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Abstract: Oral administration remains a significant challenge in regards to drugs with serious solubility and stability issues. This article aimed to investigate the suitability of nanoemulsions as oral carriers of stiripentol (STP), an acid-labile drug, for enhancement of stability and bioavailability. STP-loaded nanoemulsions (STP-NEs) were prepared by using a solvent-diffusion/ultrasonication technique. STP-NEs were characterized in a variety of ways such as by particle size, entrapment efficiency, in vitro drug release, and transmission electron microscopy. A bioavailability study was performed in rats after oral administration of either STP-NEs, or commercial formulation (Diacomit®). The resultant nanoemulsions were 146.6 nm in particle size with an entrapment efficiency of 99.47%. It was demonstrated that nanoemulsions significantly improved the biochemical stability and bioavailability of STP. The bioavailability of STP-NEs was up to 206.2% relative to Diacomit®. Nanoemulsions fabricated from poly(ethylene glycol) monooleate/medium-chain triglycerides exhibited excellent performance in drug stabilization and absorption enhancement. The results suggest that STP-NEs are a promising means to solve the problems associated with stability and solubility of STP.

Keywords: stiripentol, nanoemulsions, stability, intestinal permeability, bioavailability

Introduction

Stiripentol (STP) is an orphan drug that treats Severe Myoclonic Epilepsy of Infancy (SMEI) also known as Dravet syndrome.1 It has also been shown that STP could be used to treat super-refractory status epilepticus.2 Sada et al reported that STP and its analog could target lactate dehydrogenase (LDH) to potently suppress seizures of SMEI by inhibiting the metabolic pathway of lactate mediated by LDH.3 STP is highly insoluble in water, with a solubility of 49.2 μg/mL.4 It chemically possesses the property of ethylene alcohol, and is extremely unstable in acidic milieu. Poor solubility along with gastric instability largely limits its oral bioavailability and clinical efficacy. To achieve effective therapeutic concentration a high dose is required, with a maximum dose of up to 4 g adopted in clinical practice. Therefore, it is necessary to ameliorate the current plight by developing a suitable drug delivery system which is favorable in avoiding drug waste and reducing adverse reactions as a result of high dose.

The dosage forms of STP on the current market are mainly formulated in capsules and dry suspensions. Apart from convenient storage, conventional solid formulations are generally lackluster in oral delivery. To improve the dissolution and/or bioavailability, a variety of novel solid formulations have been attempted, including solid dispersions,5,6 cyclodextrin inclusion complexes,7,8 and various solidified nanocarriers.9–11 However,
these formulations possess an inferior capability of resisting drug degradation in harsh conditions, such as low gastric pH, digestive stress, and first-pass metabolism. This is because of rapid dissolution, or release from these systems, which easily result in considerable exposure of the drug to the gastrointestinal (GI) tract. For unstable drugs in the GI lumen, it is required that the delivery system protects the integrity of the drug from degradation, and in addition benefit from absorption enhancement. In this respect, some versatile nanocarriers have shown the potential of protecting labile drugs, such as PEGylated lipid nanoparticles, polymeric micelles or nanoparticles, and liposomes. Although micelles assembled from mPEG-PCL have been explored to orally deliver STP, the material used is rather expensive and the micellar system easily dissociates upon dilution of physiological fluids. To date, the protective effect of nanoemulsions on acid-labile drugs has not been fully investigated.

Nanoemulsions are oil-in-water emulsion composed of surfactant, oil, and water, with a droplet diameter at the nanoscale. Generally, a large quantity of surfactants (weight is 20% of the oil phase), is needed to obtain nano-scale emulsions, which potentially raises the safety risk of the drug delivery system, especially for long-term use. Another disadvantage held by conventional nanoemulsions is the remarkable lipolysis in the GI tract. Lipids, like oils and triglycerides, are readily digested by pancreatic lipases, resulting in drug precipitation and variable bioavailability. To enhance the oral delivery, an effective approach is to disguise the carrier with PEGylation. PEGylated materials possess several advantages, such as being less toxic, strong solvent power for solubilization, and protect the drug or carriers from degradation. Those PEGylated materials with surfactant property may be ponderable to prepare nanoemulsions instead of the common surfactants.

In this study, nanoemulsions made up of poly(ethylene glycol) monooleate (PM) and medium-chain triglycerides, were designed to enhance the gastric stability of STP by masking proton attack, and enhancing the bioavailability by improving the intestinal absorption (Figure 1). The emulsion system contains no co-surfactant (e.g., glycerin) which is regularly required to reduce the interfacial tension. Furthermore, PM has the underlying ability to change the digestion of nanoemulsions. STP-loaded nanoemulsions (STP-NEs) were prepared using a solvent-diffusion/ultrasonication technique and characterized by transmission electron microscopy (TEM) (Philips Tecnai-10, Philips, Amsterdam, the Netherlands), in vitro release, and stability test. The performance of STP-NEs in bioavailability enhancement was tested in rats following oral administration with a commercial formulation (Diacomit®).

**Materials and methods**

**Materials**

STP was obtained from Haiku Pharmaceutical Science & Technology Co., Ltd. (Guangzhou, People’s Republic of China). Diclofenac sodium (internal standard) was purchased from Aladdin Industrial Inc. (Shanghai, People’s Republic of China). Medium-chain triglycerides (MCT) were kindly gifted by Gattefosse (Saint-Priest Cedex, France). PM (Mn ~1,200) was purchased from Aoke Chemicals (Shanghai, People’s Republic of China). Deionized water was prepared from Milli-Q water purifier (EMD Millipore, Billerica, MA, USA). High-performance liquid chromatograph (HPLC)-grade methanol was obtained from Merck KGaA (Darmstadt, Germany). All other chemicals were of analytical grade and used as received.

**Figure 1** Schematic illustration of the engineering of STP-NEs: the acidolytic route of STP and approach for bioavailability enhancement via nanoemulsions. **Abbreviations:** STP, stiripentol; STP-NEs, stiripentol-loaded nanoemulsions.
Preparation of STP-NEs

STP-NEs were prepared by the technique of solvent-diffusion/ultrasonic homogenization. STP, PM and MCT were dissolved in absolute ethanol and then rapidly injected into water using a syringe. The components were spontaneously assembled into emulsions upon the solvent diffusion into the aqueous phase. After this, the resultant coarse emulsions were homogenized with an ultrasonic probe for 2 minutes (400 watt) to produce the final nanoemulsions. The residual ethanol (5% v/v) was reserved in the system as a preservative. Factors affecting the properties of nanoemulsions were investigated, including the ratios of PM/MCT and drug/excipients. The produced nanoemulsions were stored in a refrigerated cabinet at 4°C ready for use.

Characterization of STP-NEs

The particle size of STP-NEs was measured by a Zetasizer Nano ZS (Malvern, UK) at 25°C. To measure the particle size, nanoemulsions were diluted and transferred into a disposable cuvette. The sample was then subjected to laser diffraction after equilibration for 120 seconds. The mean particle size was acquired using the build-in software in the Zetasizer Nano ZS based on dynamic light scattering.

TEM was used to probe the morphology of STP-NEs. STP-NEs with 50-fold dilution were dropped on a carbon-coated copper grid and fixed by vacuum drying. The anchored droplets were then subjected to TEM observation and micrographs were collected at an acceleration voltage of 100 kV.

The entrapment efficiency (EE) and drug load (DL) of STP-NEs were determined by separating the free STP from the emulsion system. Briefly, the freshly prepared nanoemulsions were centrifuged at 5,000×g to remove the free bulk drug. The refined STP-NEs then proceeded to centrifugal filtration to further remove free STP by a centrifugal filter device (Amicon Ultra-0.5, molecular weight cut-off 10 K, EMD Millipore). The concentration of STP entrapped in mixed micelles was quantified by the HPLC established below. The EE and DL of STP-NEs were calculated according to the following equations:

\[ EE(\%) = \left(1 - \frac{M_{\text{free}}}{M_{\text{tot}}}\right) \times 100\% \]  

(1)

\[ DL(\%) = \frac{M_{\text{tot}} \cdot EE}{M_{\text{tot}} + M_{\text{exc}}} \times 100\% \]  

(2)

where \( M_{\text{free}} \) and \( M_{\text{tot}} \) are the amounts of free and total STP in the nanoemulsions quantified on HPLC, respectively; \( M_{\text{exc}} \) is the amount of all excipients used in the formulation quantified on weight.

Quantification of STP

The concentration of STP in nanoemulsions and other samples was determined by Agilent 1100 series HPLC system (Santa Clara, CA, USA) equipped with a quaternary pump, a degasser, an autosampler, a column heater, and an ultraviolet detector. STP was separated by a C18 column (Agilent-Bonus-RP, 5 μm, 4.6 mm × 150 mm) guarded with a precolumn at 40°C. The injection volume was 20 mL, and the eluates were monitored at 254 nm. The mobile phase consisted of 78% methanol and 22% water pumped at a flow rate of 1.0 mL/min.

Acidolytic destabilization experiment

Simulated gastric fluid (ie, 0.1 M HCl with no pepsin) was used to check the acidolysis of free STP and STP-NEs. An aliquot of STP methanol solution or STP-NEs, equal to 0.5 mg STP, was introduced into 10 mL simulated gastric fluid and maintained at 37°C. At the time of 0.25, 0.5, 1, 1.5, 2, 3, and 4 hours, 1 mL of sample solution was withdrawn and immediately replenished by the same volume of fresh medium. The concentration of retained STP was analyzed by HPLC.

In vitro release study

The release of STP from nanoemulsions was investigated using the dialysis method. Aliquots of STP-NEs containing 250 mg STP from a dialysis bag (molecular weight cut-off 14,000) were added into 900 mL of pH 6.8 phosphate buffer, or 0.1 M HCl solution, in which 0.25% (w/v) sodium dodecyl sulfate was involved as solubilizers. At predetermined time points, 5 mL of the sample was withdrawn and immediately replenished by the same volume of fresh medium. The concentration of retained STP was determined by HPLC as described in the Quantification of STP section, and the percentage of drug release was calculated as mean ± SD (n=3).

Stability test of STP-NEs

For the evaluation of stability, STP-NEs were stored in ambient temperature for 1 month. The changes in particle size and drug content as a function of time were observed. STP-NEs encapsulated in a penicillin bottle were placed on the experiment desk under ambient temperature. The particle size of STP-NEs were monitored every day for 8 days, and then every other day thereafter. Three batches of STP-NEs, treated as particle size sample, were used to quantify the drug content at day 30 with the original value (day 0) as reference. The stability of STP-NEs was outlined by particle size distribution and the percentage of the remaining drug.
Bioavailability study
All animal experiments were conducted according to the protocols for animal experiments launched by the Experimental Animal Ethical Committee of Shandong University, which reviewed and approved the study. Sprague Dawley rats (220 g ±20 g) were fasted overnight (12 hours) before drug administration, but were allowed free access to water. Rats were randomly grouped into STP-NEs (n=5), and the commercial formulation group (Diacomit®), (n=5). The rats were dosed with the two preparations, STP-NEs and the conventional suspensions prepared from the commercial formulation, by gavage at the dose of 27 mg/kg. Blood was gathered into the heparinized tubes from the jugular vein at 0.5, 1, 2, 4, 6, 8, and 12 hours after administration. The blood samples were then centrifuged at 5,000× g for 5 minutes to collect the plasma.

To quantify the plasma STP, a deproteinization procedure was used to recover STP from the plasma. Briefly, 100 μL of plasma was mingled with 400 μL of methanol, and supplemented with 10 μL of diclofenac sodium (100 μg/mL), as an internal standard. After alternate vortex (1 minute) and sonication (15 minutes), the samples were centrifuged at 10,000× g for 10 minutes to precipitate the insoluble components. The extraction step was performed twice. The supernatant fraction was transferred into new tubes followed by evaporation at 30°C for 5 hours under vacuum to remove the solvent using a Concentrator Plus (Eppendorf, NY, USA). The residue were reconstituted in 100 μL of 78% methanol for HPLC analysis.

In situ single-pass intestinal perfusion
To investigate the effect of nanoemulsions on membrane permeability of STP, in situ single-pass intestinal perfusion was performed as described in previous studies. Sprague Dawley rats were fasted for 12 hours (free access to water) prior to the experiment. The rats were sacrificed by an intraperitoneal injection of 20% urethane (1.0 g/kg). The intestines were exposed by a midline abdominal incision. The segments of duodenum, jejunum, and ileum were identified and cannulated with silicone tubes (Ø 2.5 x 4 mm). Krebs–Ringer buffer (pH 7.4) was gently injected through the inlet tube to wash the intestinal contents. Perfusion solutions, equivalent to 45 μg/mL of STP, were prepared by diluting STP-NEs or STP ethanol solution into Krebs–Ringer buffer. After pre-perfusion for 30 minutes, the perfusate (approximately 3 mL) were collected every 15 minutes, up to 120 minutes. Finally, the diameter and length of the intestinal segments were measured. Net water flux was calibrated by weight with the sham-operated group in the perfusion experiment.

The effective permeability coefficient \( P_{\text{eff}} \) was calculated based on the inlet and outlet concentrations according to the equation below:

\[
P_{\text{eff}} = -\frac{Q}{2\pi r L} \ln \left( \frac{C_{\text{out}}}{C_{\text{in}}} \right)\]

where Q denotes the flow rate (0.2 mL/min), r is the radius of the intestine (cm), L is the length of the perfused intestinal segment (cm), and \( C_{\text{in}} \) and \( C_{\text{out}} \) are the inlet and outlet concentrations of STP, respectively.

Cytotoxic evaluation
The cytotoxicity of nanoemulsions was checked by assessing the viability of Caco-2 cells in the presence of different concentration of nanoemulsions using MTT assay. Cell culture followed the same method as described in a previous study. Caco-2 cells were cultured for 48 hours and were triply washed with PBS (pH 7.4). STP-NEs with various PM levels were then introduced to the cells and incubated for 24 hours at 37°C. After that time, 20 μL of MTT solution (5 mg/mL) was dripped thoroughly into each well and continued to incubate for 4 hours. To dissolve the purple formazan crystals, 200 μL of dimethyl sulfoxide was used. The ultraviolet absorbance of each well was measured at 490 nm. The cell viability was calculated according to the equation:

\[
\text{Cell viability} (\%) = \left( \frac{A_{\text{tri}}}{A_{\text{con}}} \right) \times 100\%
\]

where \( A_{\text{tri}} \) was the absorbance of the cells treated with STP-NEs, and \( A_{\text{con}} \) was the absorbance treated with the culture medium.

Results and discussion
Preparation and characterization of STP-NEs
It was convenient and economical to prepare STP-NEs by solvent diffusion/homogenization. The procedure of preparation was rather straightforward. All ingredients (i.e., drug, PM, and MCT) were dissolved in ethanol and then rapidly injected into water, followed by an ultrasonic processing. The particle size of carriers can reflect the formulation performance. It was demonstrated that smaller nanoparticles are more easily taken up by the absorptive epithelia. The factors affecting the particle size of STP-NEs included the ratios (w/w) of PM/MCT and drug/excipients. The influence of these variables on the particle size are shown in Figure 2. The ratio of PM/MCT had a certain effect on the droplet diameter of emulsions. In the case of blank formulation,
A high ratio of PM resulted in smaller nanoemulsions (Figure 2A), which conformed to the rule that more surfactant is favorable to the reduction of particle size. Otherwise, the incorporation of drug exerted negative effect on particle size. The particle size of STP-NEs decreased with the ratio of drug/excipients (Figure 2B). This could be explained by the increased viscosity or consistency of the system, due to more drug incorporation into the oil phase, thereby enhancing the interfacial tension upon emulsifying. Of note, most of formulations possessed an acceptable polydispersity index (PDI) below 0.3, indicating good suitability of the preparation process.

Considering the particle size and drug load, the formulation was finally fixed to PM/MCT at 1:3 and drug/excipients at 1:7, respectively, which consisted of 100 mg STP, 250 mg PM, and 450 mg MCT. The above components were dissolved in 1 mL absolute ethanol and then formulated into 20 mL water. The resulting STP-NEs had a particle size of 142.5 nm with a PDI value of 0.190 (Figure 3A). The ζ potential was determined to be −33.7 mV, a hallmark of stable colloidal systems. STP-NEs showed an excellent entrapment efficiency of 99.47%, and the drug load was up to 12.43%. STP-NEs appeared opalescent and exhibited blue light scattering upon dilution (Figure 3B). In addition, the prepared STP-NEs were near-spherical as revealed by TEM (Figure 3C). The particle size gauged from the scale bar (0.5 μm) was consistent with the hydrodynamic size measured by the Nano ZS analyzer.

**Anti-acid stability of STP-NEs**

Figure 4 shows the survival profiles of free STP and entrapped STP in nanoemulsions in simulated gastric fluid. It could be seen that significant acidic hydrolysis took place for free STP due to direct exposure to H⁺. Although the transformation was decelerated after 0.5 hour as a result of H⁺ depletion, the cumulative loss of the parent drug was more than 45% after 4 hours. However, STP-NEs almost did not undergo acidolysis, and the retained percentage of STP was kept well above 98%. The minor loss came from the destabilization of free STP that intrinsically dissolved in the external aqueous phase of emulsions. It was clear that the protective effect of nanoemulsions on STP was evident. The encapsulation of STP into the inner oil phase of nanoemulsions effectively shielded the attack of the proton, suggesting that nanoemulsions are suitable carriers for oral delivery of STP.

**Drug release**

The release of STP from nanoemulsions is shown in Figure 5. The release increased as a function of time both in pH 6.8 buffer, and in 0.1 M HCl. STP could be continuously released into the media in a mode of slowness, which observed the principle of passive diffusion. The release pattern of STP-NEs was also consistent with those from previous studies.27,28 However, the release of STP-NEs was somewhat different in pH 6.8 buffer and in 0.1 M HCl. The accumulative release of STP was 11.56% in pH 6.8 buffer and 5.23% in 0.1 M HCl within 24 hours. Decreased release in 0.1 M HCl than in pH 6.8 buffer might be ascribed to the chemical conversion of a portion of STP. High lipophilicity (logP 2.94) should be responsible for the insignificant release of STP. From the release kinetics, it can be observed that the majority of STP was maintained in nanoemulsions in the first 4 hours. This nature of release allowed the STP-NEs to survive in the harsh gastric environment.
**Figure 3** Characterization of sTP-Nes.
Notes: (A) size distribution, (B) appearance, and (C) morphology observed by TEM.
Abbreviations: sTP-Nes, stiripentol-loaded nanoemulsions; TEM, transmission electron microscopy.

**Figure 4** Acidic destabilization curves of free sTP and sTP-Nes in simulated gastric fluid indicated by the retained percentage of STP (n=3, mean ± SD).
Abbreviations: SD, standard deviation; STP, stiripentol; STP-Nes, stiripentol-loaded nanoemulsions.

**Stability of STP-Nes**

Figure 6 shows the changes of particle size plus PDI of STP-Nes as a function of storage time. There were no significant differences in particle size between various appointed time and the origin (paired t-test, $P>0.05$). Although the PDI fluctuated somewhat day to day, the value was below 0.3, demonstrating acceptable physical stability. The drug content in nanoemulsions at day 30 was determined to be $99.22\%\pm0.65\%$ of the initial level, which was indicative of good chemical stability. Taken together, STP-Nes showed satisfactory physicochemical stability.

**Enhanced bioavailability**

The plasma STP concentration versus time plots are presented in Figure 7 with pharmacokinetic parameters, processed by the WinNonlin software (Certara USA, Inc., Princeton, NJ, USA).
Nanoemulsions as carriers of stiripentol

are summarized in Table 1. For the commercial formulation (dry suspensions), the oral absorption was significantly lower than the home-made nanoemulsions. The maximum concentration ($C_{\text{max}}$) and area under the plasma STP concentration–time curve ($\text{AUC}_{0-t}$) were 2.02 $\mu$g/mL and 10.21 $\mu$g-h/mL, respectively. In comparison with the commercial formulation, STP-NEs produced higher STP levels at various time points with the $C_{\text{max}}$ of 6.16 $\mu$g/mL and $\text{AUC}_{0-t}$ of 21.06 $\mu$g-h/mL. Both the absorption rate and extent of STP were enhanced after oral administration of STP-NEs. Analysis of variance (ANOVA) showed that there were significant differences ($P<0.05$). The relative bioavailability of STP-NEs was up to 206.2% compared to the conventional suspensions. In terms of peak time ($T_{\text{max}}$) and plasma half-time ($T_{1/2}$), STP-NEs and Diacomit® possessed similar values of approximately 1 and 2.5 hours, respectively, indicating that STP could be rapidly absorbed and eliminated following administration.

By contrast with the micellar system, our prepared nanoemulsions yielded higher blood STP concentrations and bioavailability. As known, lipid-based formulations have many advantages in enhancing the oral bioavailability of poorly soluble drugs by several mechanisms, such as solubilizing drug in the intestinal milieu, increasing intestinal lymphatic transport of drug (thereby reducing first-pass effect), and altering the drug disposition by enterocytes. It was reported that PEG materials having a molecular weight over 2,000 are difficult to absorb by the epithelial membrane through paracellular transport or macropinocytosis. Thus, micelles entering into the absorptive epithelia, as intact nanoparticles, are less feasible. To date, the absorption mechanism of micelles has been elusive. The digestion–absorption route is considered as the essential mechanism of nanoemulsions absorption, by which the lipid/oil constituents of nanoemulsions are digested

Table 1 Main pharmacokinetic parameters of STP in Sprague Dawley rats after oral administration of commercial formulation (Diacomit®) and STP-NEs

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Diacomit®</th>
<th>STP-NEs</th>
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<tbody>
<tr>
<td>$C_{\text{max}}$ ($\mu$g/mL)</td>
<td>2.02±0.47</td>
<td>6.16±0.53*</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>0.94±0.32</td>
<td>1.18±0.46</td>
</tr>
<tr>
<td>$\text{AUC}_{0-t}$ ($\mu$g-h/mL)</td>
<td>10.21±0.53</td>
<td>21.06±0.69*</td>
</tr>
<tr>
<td>$T_{1/2}$ (h)</td>
<td>2.59±0.78</td>
<td>2.70±0.52</td>
</tr>
<tr>
<td>RBA (%)</td>
<td>N/A</td>
<td>206.2</td>
</tr>
</tbody>
</table>

Notes: Data shown as mean ± SD (n=5). *Significantly different from the commercial formulation using ANOVA ($P<0.05$).

Abbreviations: $\text{AUC}_{0-t}$, area under the plasma STP concentration–time curve; $C_{\text{max}}$, maximum concentration; h, hour; RBA, relative bioavailability calculated based on $\text{AUC}_{0-t}$; SD, standard deviation; STP, stiripentol; STP-NEs, STP-loaded nanoemulsions; $T_{1/2}$, plasma half-time; N/A, not applicable; $T_{\text{max}}$, peak time.
by pancreatic lipases on the apical side of enterocytes and taken up by enterocytes subsequently.\textsuperscript{31,32} In addition, the uptake of nanoemulsions into the enterocytes can be fulfilled via clathrin- or caveolae-mediated endocytosis and macropinocytosis.\textsuperscript{33} Besides, MCT used in the nanoemulsions formulation has been proved to be able to enhance the permeability of the intestinal membrane.\textsuperscript{34} These advantages render nanoemulsions more suitable to orally deliver the highly lipophilic STP.

### Intestinal permeability

The $P_{\text{eff}}$ of free STP and STP-NEs are given in Table 2. STP itself has good permeability in various intestinal segments with $P_{\text{eff}} > 1 \times 10^{-3}$ cm/s, a value implying good intestinal absorption.\textsuperscript{35} There were no significant differences in $P_{\text{eff}}$ of free STP among three intestinal segments. However, the entrapment of STP into nanoemulsions not only failed to improve the $P_{\text{eff}}$ of STP, but also decreased it to some extent. This can be accounted for by the complete drug molecule exposure to the intestine in the case of solution formulation and encapsulation effect of the drug in the case of nanoemulsions. The concentration of STP for perfusion was lower, close to the saturated concentration (49.2 $\mu$g/mL), which allowed STP molecules to be fully accessible to the intestinal mucosa. For STP-NEs, the drug release can be negligible relative to STP solution, causing the STP to be unable to be absorbed quickly in the form of molecules. Moreover, the ligation of intestinal segments blocked the entry of digestive enzymes. These factors rendered STP-NEs transport across the intestine slower than free STP. Free STP appeared to be more permeable in the intestinal epithelia than STP-NEs. This seemed to be in conflict with the significantly enhanced bioavailability of STP-NEs. It was suggestive that other factors should be involved in the overall absorption of STP-NEs, such as pre-enterocyte digestion, post-enterocyte processing of nanoemulsions, and first-pass metabolism of the drug. Interestingly, the $P_{\text{eff}}$ of STP-NEs decreased from the proximal intestine to the distal intestine. The underlying reason for such a decrease was possibly related to the differential permeability of intestinal segments to MCT. Although the permeability of STP was not improved by STP-NEs, the oral bioavailability has been substantially enhanced. The perfusion experiment reversely demonstrated the positive role of nanoemulsions in enhancing the oral absorption of STP.

### Cytotoxicity

For pediatric medication, a major concern is the safety of preparations. The toxicity of nanoemulsions generally comes from the use of surfactants in large quantity. In this study, PM is the exclusive substance of surfactant, and thus a cytotoxic test was performed on the variable of PM concentration. Figure 8 shows the cell viability of Caco-2 cells after treatment with STP-NEs. There was no obvious cytotoxicity observed for STP-NEs at various concentrations of PM. The cell viability remained above 96% after 24 hours incubation, demonstrating the low cytotoxicity of STP-NEs. Emulsions are a favorite dosage form that children like to take. The low toxicity and popularity make STP-NEs rather applicable as a pediatric formulation.

### Conclusion

In this study, a novel nanoemulsions formulation based on the excipients of MCT and PM was developed for oral delivery of STP. The nanoemulsions prepared by solvent-diffusion/ultrasonic homogenization possessed a small particle size (<200 nm) and slow drug release, both in pH 6.8 buffer and 0.1 M HCl. STP-NEs exhibited good protection of STP from acidic destabilization and had acceptable physiochemical stability. The bioavailability of STP was significantly enhanced through the nanoemulsion system. Furthermore, the nanoemulsions were fairly safe owing to the use of PM, a low-toxic surfactant excipient. This article demonstrated the suitability of nanoemulsions as oral delivery carriers of STP.

### Table 2 The effective intestinal permeability ($P_{\text{eff}}$) of STP and STP-NEs evaluated by the in situ single-pass intestinal perfusion (n=3)

<table>
<thead>
<tr>
<th>Intestinal segment</th>
<th>$P_{\text{eff}}$ Free STP</th>
<th>$P_{\text{eff}}$ STP-NEs</th>
</tr>
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<tbody>
<tr>
<td>Duodenum</td>
<td>$2.393 \pm 0.107$ E-4</td>
<td>$1.529 \pm 0.128$ E-4</td>
</tr>
<tr>
<td>Jejunum</td>
<td>$1.918 \pm 0.171$ E-4</td>
<td>$1.189 \pm 0.093$ E-4</td>
</tr>
<tr>
<td>ileum</td>
<td>$2.175 \pm 0.084$ E-4</td>
<td>$0.882 \pm 0.030$ E-4</td>
</tr>
</tbody>
</table>

**Note:** One-way ANOVA, *significantly different from free STP ($P<0.05$).

**Abbreviations:** STP, stiripentol; STP-NEs, stiripentol-loaded nanoemulsions; ANOVA, analysis of variance.
Acknowledgments
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Disclosure
The authors report no conflicts of interest in this work.

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