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Exploring the potential of self-assembled mixed micelles in enhancing the stability and oral bioavailability of an acid-labile drug

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Stannous octoate (PubChem CID: 9318)
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Carboxymethylcellulose sodium (PubChem CID: 23706213)
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ABSTRACT

Oral delivery of many drugs is plagued with limited solubility and/or poor stability. This paper aimed to explore the performance of polymeric mixed micelles on solubilization, stabilization and bioavailability enhancement with stiripentol as model drug. Stiripentol-loaded mixed micelles were prepared by solvent-diffusion method: rapid dispersion of an ethanol solution containing stiripentol, monomethoxy poly(ethylene glycol)-b-poly(ε-caprolactone) and sodium oleate into water. Stiripentol micelles were characterized by the particle size, entrapment efficiency, *in vitro* drug release, TEM, DSC and FTIR. The pharmacokinetic profile of stiripentol was determined in rats after oral administration of stiripentol micelles. The obtained stiripentol micelles were 44.2 nm in size with an entrapment efficiency over 90%. It was shown that micelles substantially improved the solubility and gastric stability of stiripentol. The oral absorption of stiripentol was also enhanced to a great extent with a relative bioavailability of 157% and 444% to the commercial formulation (Diacomit®) and in-house suspensions. Mixed micelles assembled by di-block copolymer/sodium oleate exhibited a good potential in the improvement of drug stability and bioavailability. It should be a promising carrier for oral delivery of therapeuticals with solubility and stability issues.

1. Introduction

The oral route is probably the most convenient way of drug delivery. However, BCS II drugs oftentimes show low and highly variable bioavailability after oral administration due to rate-limited dissolution. Although several strategies such as drug nanocrystals (Kayaert and Van den Mooter, 2012; Xu et al., 2012), solid dispersions (Tran et al., 2013; Zhang et al., 2008), and cyclodextrin inclusions (Zhang et al., 2009) have shown great potential in dissolution enhancement, they are unable to protect drugs from degradation as drug molecules are exposed to the harsh environment. In an attempt to solubilize and stabilize drugs, various alternative approaches have been explored including liposomes (Hu et al., 2013), microemulsions (Cheng et al., 2008), nanoparticles (Min et al., 2008; Tangsumranjit et al., 2006), and micelles (Manju and Sreenivasan, 2011; Opanasopit et al., 2006). Among these, micelles, especially block copolymer micelles, are becoming a powerful tool for oral delivery of insoluble and/or instable drugs (Xu et al., 2013).

Micelles are self-assembled nanoparticles formed by amphiphilic molecules with a hydrophobic core inside and a hydrophilic shell outside (Kataoka et al., 2012). The hydrophobic core serves as a reservoir to solubilize drugs with poor aqueous solubility, whereas the hydrophilic shell has the ability to protect chemically

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unstable drugs from degradation (Xu et al., 2013). Compared with micelles composed of small molecule surfactants, polymeric micelles possess excellent physiological survivability, storage stability, and biocompatibility. In general, chemical copolymerization and physical entrapment techniques are utilized to prepare drug-loaded polymeric micelles (Yokoyama et al., 1998). The former chemically links a drug with a reactive group to the hydrophobic segment of a block copolymer. The micelles were spontaneously formed in aqueous medium as the solubility of the copolymer decreases. The latter is regarded as a solvent diffusion technique by which the drugs are entrapped into the polymers due to a decrease in the level of good solvent. However, the polymeric micelles consisting of single copolymer/drug are generally larger in size with a broader distribution, if not being further processed for particle size reduction.

Stiripentol (STP) is an orphan drug used for treating severe myoclonic epilepsy in infants. STP belongs to a family of α-ethylene alcohols (Fig. 1) that show pharmacological activity on the central nervous system through a barbiturate-like effect (Chiron, 2005). It is practically insoluble in water with a log*P* of 2.94, and has not been observed to exhibit polymorphisms. STP is remarkably unstable in acidic conditions. Gastric instability and low aqueous solubility significantly limit its oral bioavailability and possible therapeutic performance. Although the effective blood concentration can be achieved by dose escalation (e.g. a dose of 100 mg/kg per day, with a maximum up to 4 g), developing appropriate drug delivery system is of great interest to enhance the oral absorption, for the purpose of avoiding drug waste and reducing adverse reactions associated with a high dose.

Herein, a nanosized delivery vehicle of mixed micelles (MMs), assembled by monomethoxy poly(ethylene glycol)–b-poly(ε-caprolactone) (mPEG–PCL) and sodium oleate, was proposed for oral delivery of STP. The polymer is neutrally charged and possesses a very high molecular weight that make them less cytotoxic and unable to be absorbed through gastrointestinal epithelia into the body circulation, thus showing a low toxicity. Sodium oleate is also a safe excipient that has been approved as food additive in USA and as injectable ingredient in China. These biocompatible excipients confer MMs an excellent safety profile. The STP-loaded mixed micelles were readily prepared by the solvent-diffusion method. Use of sodium oleate facilitated the formation of smaller and more uniform micelles, avoiding an additional homogenization or ultrasound process. The micellized nanoparticles exhibited great potential in solubilization, resisting acid-induced degradation and enhancing oral bioavailability of STP.

2. Materials and methods

2.1. Materials

Stiripentol, poly(ethylene glycol) methyl ether (mPEG, Mn = 5000), ε-caprolactone, stannous octoate ($Sn(Oct)_2$), and sodium oleate were purchased from Sigma–Aldrich (Shanghai, China). Deionized water was prepared by a water purifier (Chengdu, China). HPLC-grade methanol was obtained from Mreda technology Inc. (MA, USA). All other chemicals were of analytical grade and used as received.

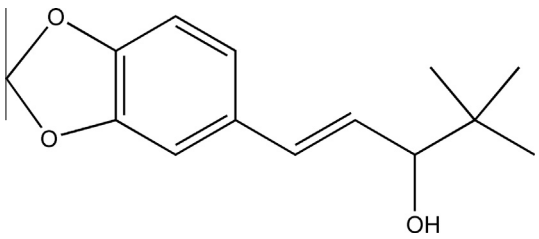

Fig. 1. Chemical structure of stiripentol.

2.2. Synthesis of mPEG–PCL

Di-block copolymer of mPEG–PCL was synthesized by ring–opening polymerization method with a minor modification (Shen et al., 2008). Briefly, mPEG and ε-caprolactone (weight ratio = 1:1) were introduced into a dry glass flask followed by the addition of 0.5% $Sn(Oct)_2$ (w/w). The flask was sealed and kept at 130 °C to polymerize under agitation atmosphere. The polymerization was terminated by cooling the product to room temperature after reaction for 24 h. The resultant mPEG–PCL copolymer was first dissolved in dichloromethane and re-precipitated from the filtrate using excessive cold diethyl ether. Afterward, the mixture was filtered and dried to constant weight under vacuum. The molecular weight of purified mPEG–PCL copolymer was determined by gel permeation chromatography (GPC) using a Polymer PL-GPC 50 system equipped with an RI detector (Polymer Lab., UK).

2.3. Preparation of STP-MMs

STP-MMs were prepared using the solvent-diffusion technique. Briefly, STP (100 mg) and the excipients (sodium oleate 200 mg and mPEG–PCL 600 mg) were dissolved in 3.5 mL ethanol–water solution (80/20, v/v) and then rapidly injected into 20 mL water with a syringe. The materials were spontaneously assembled into micelles upon the solvent diffusion into the aqueous phase. Subsequently, the residual ethanol was removed under reduced pressure by a rotatory evaporator until the nanosuspensions were condensed to an appropriate volume. Particle size and entrapment efficiency (EE) as indexes were adopted to optimize the formulation of STP-MMs using the ratio of drug/excipients and sodium oleate/PEG–PCL as formulation variables.

2.4. Characterization of STP-MMs

The particle size of STP-MMs was determined by dynamic light scattering using Zetasizer Nano ZS (Malvern, Worcestershire, UK) at 25 °C. To measure the particle size, the sample of 0.1 mL STP-MMs was diluted with deionized water to 1 mL and then subjected to laser diffraction. The data was analyzed with the build-in software for the calculation of particles size.

The morphology of STP-MMs was observed by transmission electron microscopy (TEM). STP-MMs was dropped on a carbon-coated copper grid and then anchored to the supporter. The residual water was evaporated under a warming lamp. The fixed nanoparticles was stained with a drop of 1% phosphotungstic acid for 120 s. The pigmented particles were allowed to dry at ambient atmosphere and photographed with TEM (Philips, Tecnai 10, Netherlands) at an acceleration voltage of 100 kV.

EE of STP in MMs was determined by separating free STP from STP-loaded MMs using a centrifugal filter device (Amicon® Ultra-0.5, MWCO 5000, Millipore, USA). The samples were subjected to a centrifugal force of 10,000*g* for 10 min. The concentrations of free STP (M_{fre}) in the filtrate and initial STP in micelles were quantified by HPLC. The EE was defined as the ratio of MMs-entrapped STP (M_{ent}) to total STP (M_{tot}) and was calculated according to the equation of EE (%) = $(1 - M_{fre}/M_{tot}) \times 100\%$. To avoid the deviation from membrane absorption or arrestment, we validated the method by assessing the changes of concentration of free STP in cosolvent (ethanol/PEG400/water = 10/20/70) with three levels before and after ultrafiltration.