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Preparation and evaluation *in vitro* and *in vivo* of docetaxel loaded mixed micelles for oral administration

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ABSTRACT

A mixed micelle that comprised of monomethylol poly(ethylene glycol)-poly(D,L-lactic acid) (MPP), D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) and stearic acid grafted chitosan oligosaccharide(CSO-SA) copolymers was developed to enhance the oral absorption of docetaxel (DTX). DTX-loaded MPP/TPGS/CSO-SA mixed polymeric micelles (MPMs) were prepared with thin film hydration method and characterized in terms of morphology, size, zeta potential, encapsulation efficiency, critical micellization concentration, and *in vitro* stability in media modeling physiological conditions. The *in vitro* release of docetaxel from the mixed micelles was studied with dialysis method. The oral bioavailability studies were conducted in rats and the pharmacokinetic parameters were evaluated. The results showed that DTX-loaded MPP/TPGS/CSO-SA MPMs had a mean diameter of 34.96 nm and exhibited spherical shape under transmission electron microscopy. The drug loading of DTX in the mixed micelles was 19.15%. The critical micellization concentration of MPP/TPGS/CSO-SA copolymer was 2.11×10^{-5} M, and the size of mixed micelles in gastric fluid (pH 1.6) for 2 h and simulated intestinal fluid (pH 6.5) for 6 h showed no significant change. The *in vitro* release study showed that DTX-loaded MPP/TPGS/CSO-SA MPMs exhibited slower release characteristics compared to DTX solution. The oral bioavailability of the DTX-loaded MPP/TPGS/CSO-SA MPMs was increased by 2.52 times compared to that of DTX solution. The current results encourage further development of DTX mixed polymeric micelles as the oral drug delivery system.

1. Introduction

Docetaxel (DTX) is a potent anticancer drug used to treat various cancers including metastatic and the androgen-independent prostate cancer, breast cancer, and advanced non-small cell lung cancer [1,2]. DTX inhibits cell growth by binding to microtubules, stabilizing them, and preventing their depolymerization. However, the clinical application of DTX is limited by the poor aqueous solubility, low bioavailability and high toxicity. The currently marketed form of DTX (Taxotere®) for intravenous infusion is formulated utilizing Tween 80 and ethanol. Due to hemolysis caused by Tween 80, patients were often subjected to hypersensitivity after administration. To avoid these disadvantages, enhance the patient's convenience, and facilitate the use of more chronic treatment regimens, many studies have been directed toward developing new oral formulations of DTX [3–5].

Oral administration of anticancer agents, such as DTX, represents the easiest and the most convenient route of drug delivery. Moreover, it facilitates a prolonged exposure to the cytotoxic agent and could ease the use of more chronic regimens. Therefore, the enhancement of oral bioavailability of emerging cytotoxic agents is gaining the increasing attention for successful development of oral modes for cancer and leukemia treatment. Unfortunately, the bioavailability of DTX after oral administration is very poor. Several investigators reported that the poor bioavailability of DTX was resulted from the membrane transporter P-glycoprotein (ABCB1) [6,7] as well as poor solubility and permeability. To overcome these drawbacks, several approaches have been investigated, such as co-administration with a P-glycoprotein inhibitor [8], solubilization in self-emulsifying or self-micro-emulsifying drug delivery systems [9], formation of inclusion complexes with cyclodextrins [10], and lecithin nanoparticles [11].

Polymeric micelles (PMs) have a core-shell structure, and the inner core is the hydrophobic part of the polymer, which can incorporate poorly soluble drugs, while the outer shell or the corona

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of the hydrophilic part of the polymer protects the drug from inactivation in biological environment such as the gastrointestinal tract [12]. Due to their small particle size (<200 nm), PMs could be absorbed in their intact form *via* endocytosis which transported macromolecules through enterocyte cell membrane [13,14]. Moreover, PMs possess high loading capacity for poorly water soluble drugs, significantly lower critical micelle concentration (CMC) values and longer lifetime (indicating greater thermodynamic stability) than those of low-molecular weight surfactants [15]. All these issues related to PMs make them become ideal carriers of anticancer drugs for oral delivery. More recently, a large number of studies on mixed polymeric micelles (MPMs) have appeared because the prominent advantages for different types of copolymers concentrated in a single polymeric micellar system. Di/multifunctional PMs can be realized by preparing mixed micelles. The loading content and stability of drug in mixed micelles can be improved greatly with different kinds of polymers compared with single polymer micelles. The release and function of micelles can be modified to be desirable by forming MPMs. Therefore, MPMs would be an ideal formulation that can solubilize DTX efficiently and solve some of the aforementioned problems.

The amphiphilic block copolymer methoxy poly(ethylene glycol)-poly(lactide) (mPEG-PLA, MPP) was often used because of its excellent micelle formation capacity, drug loading capability and release behavior [16,17]. D-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS) is a PEGylated-vitamin E, which has greatly improved the pharmaceutical properties of vitamin E and thus has been widely applied in the food and drug industry. It has been found that TPGS can enhance the solubility [18], inhibit P-gp-mediated multidrug resistance [19], and increase the oral bioavailability of anticancer drugs [20]. Chitosan (CS) is a cationic polysaccharide with good safety and biocompatibility, which can open the tight junctions [21] and improve the oral uptake of hydrophilic drugs such as peptides. Despite its favorable biological properties, CS is rarely used in oral administration of drugs due to its low solubility under the physiological conditions and limited capacity for controlling the release of drugs. Chemical modification of CS was a feasible way to overcome those disadvantages, making it more suitable as oral delivery vector. Stearic acid grafted chitosan oligosaccharide (CSO-SA) is a kind of hydrophobic modification of CS with low molecular weight [22]. The CSO-SA could form micelles in the aqueous medium, and the CSO-SA micelles could be rapidly internalized into cancer cells [23]. Possessing the same merits as CS, such as biocompatibility, biodegradability and muco-adhesivity, CSO-SA could also be used as a promising oral drug carrier.

In the present study, the DTX loaded mixed micelles composed of MPP, TPGS and CSO-SA were prepared to increase the aqueous solubility and oral absorption of DTX. The physicochemical characteristics of DTX-loaded MPMs such as micro-morphology, size, zeta potential, critical micelle concentration, the *in vitro* stability in modeling physiological conditions of gastrointestinal tract and *in vitro* release were investigated. In addition, the oral bioavailability of DTX-loaded MPMs in rat was also evaluated.

2. Materials and methods

2.1. Materials and animals

DTX was purchased from Baoji Guokang Biotechnology Co., Ltd. (Shanxi, China). $mPEG_{2000}$-PLA_{2000} was obtained from Jinan Daigang Biotechnology Co., Ltd. (Shandong, China). TPGS was purchased from Eastman Co. (USA). Chitosan (M_w = 5 kDa, 85% deacetylated degree) was supplied by Haidebei Marine Biological Engineering Co., Ltd. (Shandong, China). Stearic acid was purchased from Tianjin Kemeng Chemical Industry Co., Ltd. (Tianjin, China). 1-Ethyl-3-(3-dimethylami-nopropyl) carbodiimide (EDC) was purchased from Shanghai Aladdin Co., Ltd. (China).

Male Wistar rats (250 ± 20 g) were supplied by Laboratory Animals Center of Shandong University, Jinan, China. The animals were used following the guidelines of the Ethical Committee for Animal experiments of Shandong University. The animals were acclimatized at a temperature of 25 ± 2 °C and a relative humidity of 70 ± 5% under natural light/dark conditions for at least 24 h before dosing.

2.2. Synthesis and characterization of CSO-SA.

The CSO-SA copolymer was synthesized *via* the reaction of carboxyl groups of SA and amine groups of CSO in the presence of EDC, as described in previous report [24]. Briefly, 0.4460 g of CSO was dissolved in 20 mL of distilled (DI) water and heated up to 60 °C. SA and EDC with the molar ratio of 1–10 were dissolved in 10 mL of an ethanol/acetone mixture (ethanol/acetone = 2/5, *v*/*v*). After stirring for 1 h at 400 rpm, 60 °C, the solution was added into CSO aqueous solution, followed by stirring for another 24 h. Then, the reaction solution was dialyzed against DI water using a dialysis membrane with MWCO of 3.5 kDa (Solarbio, China) for two days, and then the reaction solution was lyophilized. Then the lyophilized product was further purified with ethanol to remove the byproduct. Finally, the ethanol was evaporated to obtain the CSO-SA product.

2.3. Preparation of micelles

MPP/TPGS/CSO-SA MPMs were prepared by thin film hydration method as described earlier [25]. Briefly, CSO-SA (10 mg) was dissolved in 3 mL water. Acetonitrile solution of MPP and TPGS with different weight ratio (the total amount of MPP and TPGS was 30 mg) was evaporated by rotary evaporation at 37 °C to obtain a solid polymer matrix. Residual acetonitrile remaining in the film was removed under vacuum overnight at room temperature. Then, the film was dispersed with CSO-SA solution at 60 °C and vortexed for 5 min, the mixture was then centrifuged to obtain a clear micellar solution. DTX-loaded MPP/TPGS/CSO-SA MPMs were also prepared as described above, except for the addition of DTX in acetonitrile.

DTX-loaded or blank MPP/TPGS MPMs were prepared by replacing the CSO-SA solution with water.

2.4. Characterization of the mixed micelles

Transmission electron microscope (TEM, JEM-1200EX, JEOL, Tokyo, Japan) was performed to evaluate the surface morphology of micelles after negative staining with phosphotungstic acid solution (2%, *w*/*v*). The mean particle size and zeta potential of the micelles were determined by dynamic light scattering (DLS, DELSA^{TM}NANO particle size and zeta potential analyzer, Beckman Coulter Inc., USA). All measurements were performed at 25 °C. Experimental values were calculated from the measurements performed at least in triplicate.

2.5. Determination of docetaxel content in mixed micelles

To determine drug loading content (DL), encapsulation efficiency (EE) and precipitated drug percentage (PD) of micelles, the DTX-loaded MPMs was dissolved with acetonitrile properly and vortexed to get a clear solution, after which the concentration of DTX was measured by high performance liquid chromatography (HPLC). The DL, EE and PD were calculated using the following equations:

$$\mathrm{DL}\% = \frac{\text{weight of the drug in micelles}}{\text{weight of the feeding polymer and drug}} \times 100\% \quad (1)$$

$$EE\% = \frac{\text{weight of the drug in micelles}}{\text{weight of the feeding drug}} \times 100\% \quad (2)$$

$$PD\% = \frac{\text{drug content of micelles} - \text{drug content of micelles after storage}}{\text{drug content of micelles}} \times 100\% \quad (3)$$

In formula (3), the drug content of micelles means the weight of DTX in freshly prepared micelles, and the drug content of micelles after storage means the weight of DTX in micelles which have been stored at 4 °C for 24 h.

2.6. Critical micelle concentration determination

Critical micelle concentration (CMC) of the mixed micelles was determined by fluorescence spectroscopy using pyrene as fluorescence probe [26]. Upon formation of micelles, pyrene would move inside micelles from the aqueous phase, which results in an alteration in the intensity ratio of I_{372}/I_{383}. Fluorescence spectrum was recorded on a F-2500 fluorescence spectrophotometer (Hitachi, Japan) at room temperature. Solutions of the mixed copolymer with different concentrations were prepared by diluting MPP/TPGS or MPP/TPGS/CSO-SA mixed copolymer in distilled water. The mixed copolymer solution were added and mixed with pyrene, allowed to stand to equilibrate for 24 h at room temperature. The copolymer concentration in these experiments ranged from 6.67×10^{-4} to 3.34×10^{-7} M and 7.3×10^{-4} to 3.65×10^{-7} M for MPP/TPGS and MPP/TPGS/CSO-SA, respectively. The final pyrene concentration was 6.0×10^{-7} M, slightly below the saturation concentration of pyrene in water at 25 °C. All samples were excited at 334 nm, and fluorescence spectra were recorded between 350 and 500 nm. The excitation and emission slit widths were set at 5 nm.

2.7. Stability in media modeling physiological conditions of gastrointestinal tract

The stability of the DTX-loaded MPP/TPGS/CSO-SA MPMs dispersions was evaluated after dilution with simulated gastric fluid without pepsin (SGF, pH 1.6) and simulated intestinal fluid without trypsin (SIF, pH 6.5), respectively [27]. SGF was composed of 0.2% sodium chloride (NaCl), 0.25% Sodium dodecyl sulfate (SDS), and 0.7% hydrochloric acid (HCl) in water, and the pH was adjusted to 1.6 by adding concentrated HCl. SIF was composed of 0.3% dipotassium hydrogen phosphate (K_2HPO_4) with 0.77% potassium chloride (pH adjusted to 6.5 using NaOH), 3 mM sodium taurocholate and 0.75 mM lecithin. The DTX-loaded MPP/TPGS/CSO-SA MPMs dispersions were mixed with appropriate volumes of the media (SGF or SIF) to dilute the samples 10 times. The diluted samples were incubated at 37 °C. Samples were taken to determine the particle size at 2, 4, 6, 8, 12 h. The experiments were repeated three times, and the average values are reported ($n = 3$).

2.8. In vitro release studies

In vitro DTX release test was investigated according to the reported procedure with modifications [28]. Briefly, 200 μL of DTX-loaded MPMs was introduced into a dialysis membrane bag ($M_{w\ cut\text{-}off}$ = 3.5 kDa, Solarbio) and the end-sealed dialysis bag was incubated in 50 mL release media at 37 ± 0.5 °C and shaken at a speed of 100 rpm. SGF, SIF, and phosphate-buffered saline (PBS, pH 7.4) with 0.5% Tween 80 were used as release media, respectively. After 1 h incubation of DTX micelles in 50 mL SGF, the bags were transferred into 50 mL SIF up to 48 h. Sink conditions were achieved by the addition of sodium dodecyl sulfate (SDS) in SGF, lecithin/sodium taurocholate in SIF and Tween 80 in PBS. At predetermined time intervals, 0.5 mL of the release media was withdrawn and replaced with an equal volume of the fresh solution (SGF, SIF or PBS). The *in vitro* release behavior of DTX-loaded MPMs was measured and compared with that of DTX solution (2 mg/mL, DTX was dissolved in the solution containing 520 mg of Tween 80 and 13% ethanol). Into each sample, 0.5 mL of acetonitrile was added and stored till HPLC analysis. All assays were performed in triplicate. Release profiles were expressed in terms of cumulative release of DTX in percentage and plotted *vs.* time.

2.9. Pharmacokinetics studies

Before the experiment, 20 rats were kept overnight with free access to water and randomly divided into four groups. Four groups of rats were either injected a diluted DTX injection (2 mg/mL, DTX was dissolved in the solution containing 520 mg of Tween 80 and 13% ethanol) at the dose of 10 mg/kg through the caudal vein, or orally administered DTX-loaded MPP/TPGS/CSO-SA MPMs, DTX-loaded MPP/TPGS MPMs and DTX solution at a dose of 20 mg/kg respectively. The whole blood samples were collected by sinus jugularis puncture from each rats in every group into heparinized tubes at 0.167, 0.25, 0.5, 1, 2, 4, 8 and 12 h following oral administration and at 0.083, 0.25, 0.5, 1, 2, 4, 8 and 12 h following intravenous administration. Blood samples were centrifuged for 5 min at 4000 rpm, and the plasma was collected and stored at −20 °C. 300 μL of plasma samples were combined with 600 μL acetonitrile, followed by vortexing for 3 min and centrifugation for 10 min at 12 000 rpm, then the supernatant were transferred to new tubes and dried under nitrogen gas stream at 40 °C. The residue was dissolved in 100 μL acetonitrile and vortexed for 5 min. The solution was centrifuged at 10 000 rpm for 5 min, and the supernatant was injected into the HPLC system.

The pharmacokinetic parameters of each formulation were calculated using the DAS (version 2.0). The area under the curve and the mean residence time were determined by standard methods applying the linear trapezoidal rule. The maximum plasma concentration and time taken to reach the maximum plasma concentration were determined by a visual inspection of the experimental data. The absolute bioavailability of docetaxel after oral administration compared to the intravenous administration was calculated as follows:

$$F\% = \frac{AUC_{oral}}{AUC_{iv}} \times \frac{i.v._{dose}}{Oral_{dose}} \times 100\%$$

F represents the absolute bioavailability, AUC represents the area under the curve, and *i.v.* stands for intravenous.

2.10. HPLC analysis

HPLC method was used for the analysis of docetaxel concentration in all samples. The HPLC system (Agilent 1200 liquid chromatograph, USA) was equipped with a UV detector (Agilent G1314-60100) and reversed phase column (Elite ODS C-18, 4.6 mm × 250 mm, Dalian Elite Analytical Instruments Co., Ltd., China). The wavelength of the UV detector was set at 230 nm [29]. A degassed mixture of acetonitrile and water (45/50, *v*/*v*) was used as the mobile phase and the mobile phase was pumped at a flow rate of 1.0 mL/min. The column temperature was maintained at room temperature. Sample solution was injected at a volume of 20 μL.

2.11. Statistical analysis

The statistical significance of differences among more than two groups was determined by one-way ANOVA. A value of $P < 0.05$ was considered to be significant.

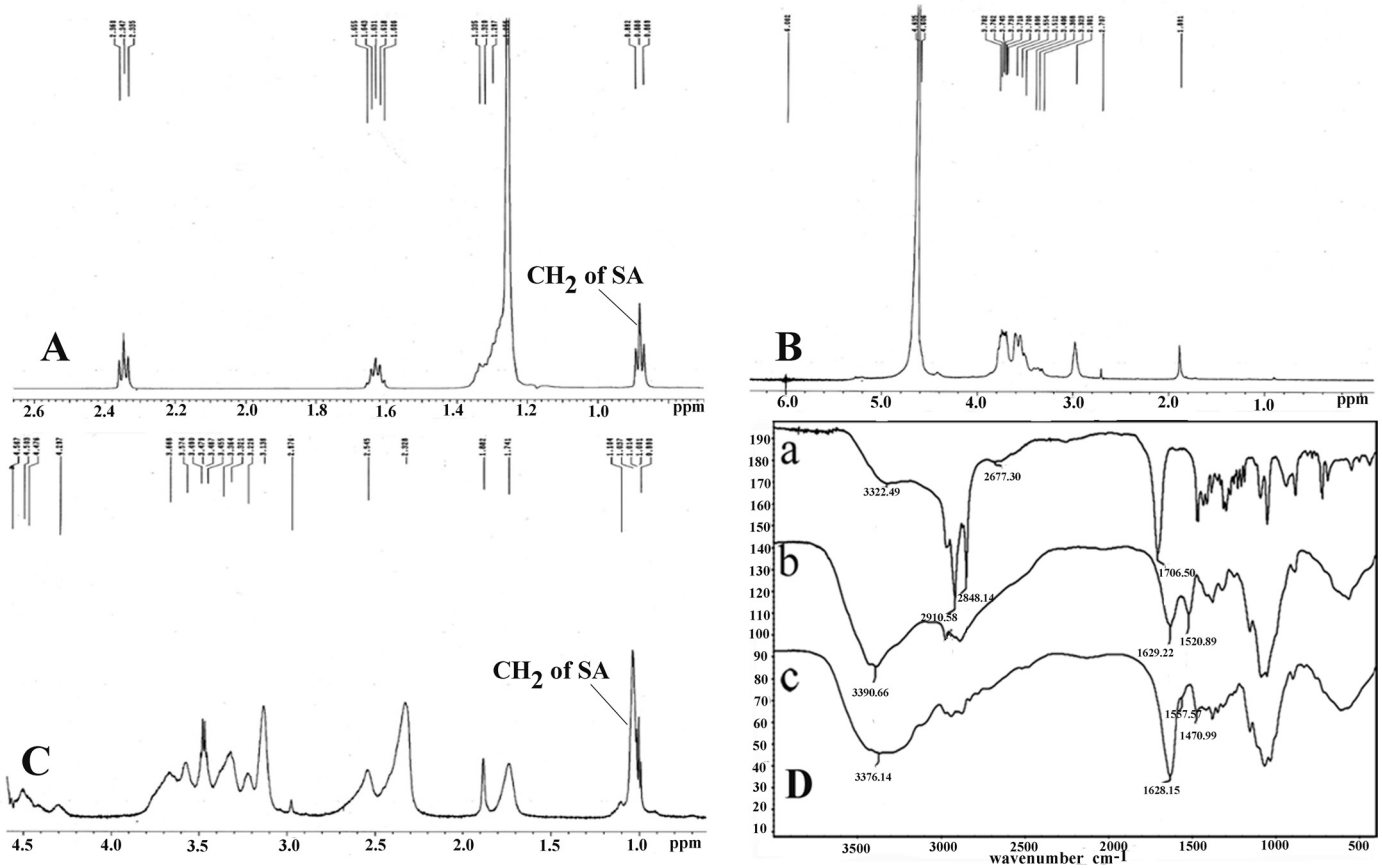

Fig. 1. ^{1}H NMR spectra of SA (A), CSO (B) and CSO-SA(C). Infrared spectroscopy (D) of SA (a), CSO (b), CSO-SA (c).

3. Results and discussion

3.1. Synthesis and characterization of the CSO-SA

The CSO-SA with 50% theoretical charged amount of SA was synthesized by the coupling reaction between the carboxyl groups of SA and primary amino groups of CSO with average molecular weight of 5 kDa in the presence of EDC. The structure of synthesized CSO-SA chemical conjugate was confirmed by the ^{1}H NMR spectrum and infrared spectroscopy (IR) (Fig. 1). ^{1}H NMR spectrum showed that there are proton characteristic peaks of $-CH_2$ belonging to steric acid in the spectroscopy of SA and CSO-SA, not shown in that of CSO. In infrared spectroscopy, it was clear that the peaks of amide bands I and II of CSO appeared at 1629 cm^{-1} and 1521 cm^{-1}, respectively. The new peaks of amide band were observed at 1632 cm^{-1} and 1560 cm^{-1} in the spectra of CSO-SA, respectively. The changed peaks of amide band were caused by the amide band between CSO and SA. On the other hand, no peak for the carboxyl groups of SA at 1700 cm^{-1} was found in the IR spectra of CSO-SA. Both the ^{1}H NMR spectrum and infrared spectroscopy indicate the success conjugation of CSO and stearic acid.

3.2. Optimization of micelle composition and characterization of mixed micelles

Many studies have reported that the major factor influencing both the loading capacity and encapsulation efficiency of polymeric micelles is the compatibility between the solubilizate (drug) and core-forming block [30]. In pre-experiments, it was found that CSO-SA had less impact on the encapsulation of DTX (data not shown), however, considering the merits of CSO-SA in oral absorption, the weight of CSO-SA was fixed at 10 mg. To investigate the impact of different proportion of TPGS in copolymers on the EE of DTX in mixed micelles, the EE of DTX was determined in the mixed micelles with varied weight ratio of MPP to TPGS with a fixed amount of DTX (27%) (Fig. 2). The amount of DTX (27%) was chosen because preliminary experiments showed that at this level, the difference of EE values of micelles composed of MPP/TPGS/CSO-SA

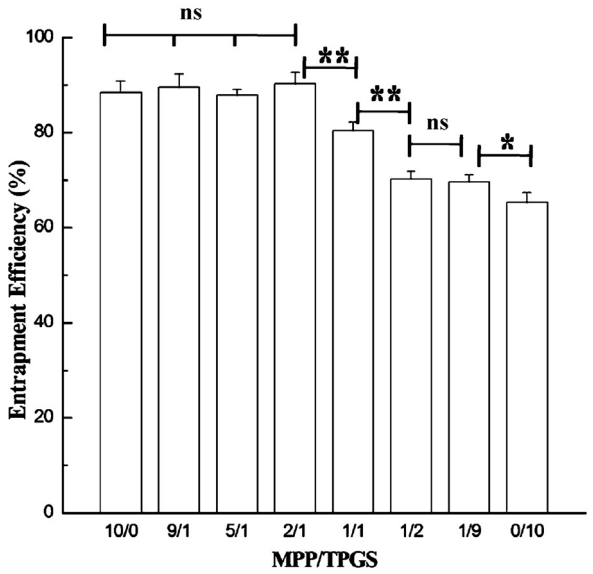

Fig. 2. The influence of TPGS weight ratio on the entrapment efficiency ns: $P > 0.05$ between any two groups; $^{**}P < 0.05$; $^{*}P < 0.01$.

Table 1
The effect of the amount of docetaxel on loading content and precipitated drug.

Theoretical loading content (%)	Practical loading content (%)	Precipitated drug (%)
11	10.89 ± 0.68	0.23 ± 0.02
20	19.15 ± 0.72	0.35 ± 0.01
27	22.95 ± 0.89	8.95 ± 0.65

with different weight ratio of TPGS was more obvious compared to that of micelles containing lower amount of DTX (11% and 20%), which is helpful to choose the proportion of TPGS with best drug loading capacity. The results indicated that the EE of DTX had no obvious change ($P > 0.05$) when the weight ratio of TPGS less than 50% of total weight of MPP and TPGS. In comparison, as TPGS content increased to 50%, the EE of DTX showed obvious decrease ($P < 0.01$). This could be attributed to the fact that the higher affinity of drug to the relatively more hydrophobic PLA of MPP copolymer than vitamin E of TPGS. Although MPP possesses better ability of encapsulating DTX, owing to the inhibition of TPGS on P-gp efflux pump, we fixed the weight ratio of MPP to TPGS at 2:1 to obtain suitable mixed micelles for oral administration.

The effect of amount of docetaxel used to prepare mixed micelles on the EE and precipitated drug percentage was studied in the theoretical loading content of DTX at 11–27%, and the results were profiled in Table 1. It was observed that the more the drug amount used, the higher precipitated drug percentage of MPP/TPGS/CSO-SA mixed micelles was. This could be due to the fact that the drug super-saturation was formed when the dosage of docetaxel exceeded the loading capacity of mixed micelles. Stored at 4 °C for 24 h, the micelles would attain the balance state and the excess docetaxel would precipitate [31]. This leads to the assumption that micelle formulations could enhance the solubility of poorly soluble drugs but to a maximum limit after which any increase in the drug concentration can bring about drug precipitation.

It was also shown in Table 1 that there was no significant change in docetaxel loading content and precipitated drug percentage when the amount of docetaxel was lower than 20%, suggesting that the weight ratio of docetaxel should be less than 20% to acquire the best docetaxel solubilization. Thus the final optimized preparation of docetaxel-loaded mixed micelles applied in the following evaluation had an ideal composition of MPP:TPGS:CSO-SA:DTX at the weight ratio of 2:1:1:1.

3.3. Characterization of mixed micelles

Fig. 3 showed the TEM image of DTX-loaded MPP/TPGS and MPP/TPGS/CSO-SA mixed micelles, which indicated that the self-assembled micelles are well dispersed as individual particles with spherical shape. The rough surface and increased size following the addition of CSO-SA was confirmed by Fig. 3b. TEM image showed that the size of DTX-loaded MPP/TPGS/CSO-SA mixed micelles was about 50 nm.

The extent and rate of particle absorbed in gastrointestinal tract are mainly dictated by the size and surface properties of the carrier [32]. Thus the effects of DTX and CSO-SA on particle size and zeta potential were investigated. The mean sizes of DTX loaded MPP/TPGS and MPP/TPGS/CSO-SA mixed micelles are 21.03 ± 1.18 and 34.96 ± 0.51 nm, respectively. The increased micellar size indicated the insertion of CSO-SA into micelles. Moreover, a decrease in the average size after DTX loading was observed for both micelles with or without CSO-SA copolymer, changing from 44.96 ± 1.82 to 34.96 ± 0.51 nm and 30.7 ± 1.13 to 21.03 ± 1.18 nm, respectively. All the micelles showed a relatively narrow polydispersity index, which is close to 0.3.

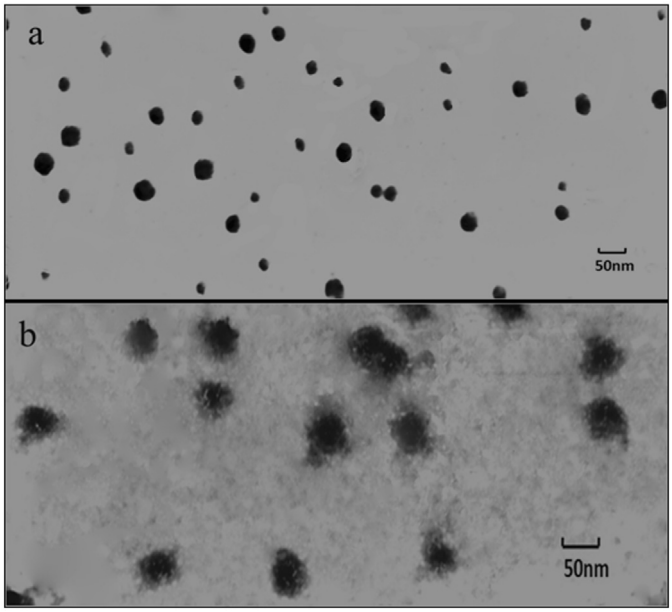

Fig. 3. Transmission electron micrograms of DTX-loaded MPP/TPGS micelles (a) and DTX-loaded MPP/TPGS/CSO-SA micelles (b).

A hydrophobic drug can be encapsulated into micelles by chemical conjugation or physical entrapment. The physical entrapment of drug in micelles mainly results from hydrophobic interactions, hydrogen bond and van der Waals forces. In the DTX-loaded mixed micelles, the decrease of micelle size could be attributed to the effect of hydrogen bonds between the hydroxy groups of MPP/TPGS and DTX. Furthermore, the enhanced cohesive force of the hydrophobic interaction was another reason.

The zeta potential of both unloaded and DTX-loaded MPP/TPGS/CSO-SA MPMs was 27.12 mV and 29.87 mV, obviously increased compared with 2.32 mV and 6.69 mV, zeta potential of both unloaded and DTX-loaded MPP/TPGS MPMs. The increase of zeta potential value might be attributed to the cationic amino groups of CSO-SA.

3.4. Critical micelle concentration determination

CMC is an important parameter for the stability of drug-loaded micelles, both *in vitro* and *in vivo*. In this study, pyrene was used as a hydrophobic probe to monitor the formation of mixed micelles. As a small hydrophobic molecule, pyrene prefers to enter the hydrophobic microenvironment of copolymers, so that the solubility of the compound in the detergent phase is significantly increased compared to its solubility in pure water. The CMC values of MPP/TPGS and MPP/TPGS/CSO-SA mixed copolymers were obtained by plotting the ratio of I_{372}/I_{383} of the emission spectra profile *vs.* the concentration of copolymers as shown in Fig. 4a and b. The CMC values for MPP/TPGS and MPP/TPGS/CSO-SA micellar solution were as low as 5.51×10^{-5} M and 2.11×10^{-5} M. The low CMC values indicated that the mixed micelles composed of MPP/TPGS or MPP/TPGS/CSO-SA should show high stability and ability to maintain integrity even upon extreme dilution in gastrointestinal tract.

3.5. Stability in media modeling physiological conditions of gastrointestinal tract

During the experiment, the optimal formulation was stored at room temperature for one week, and 99.2 ± 0.6% of the drug content in MPP/TPGS/CSO-SA/DTX MPMs remained after one week' storage. In addition, there was also no obvious change in particle

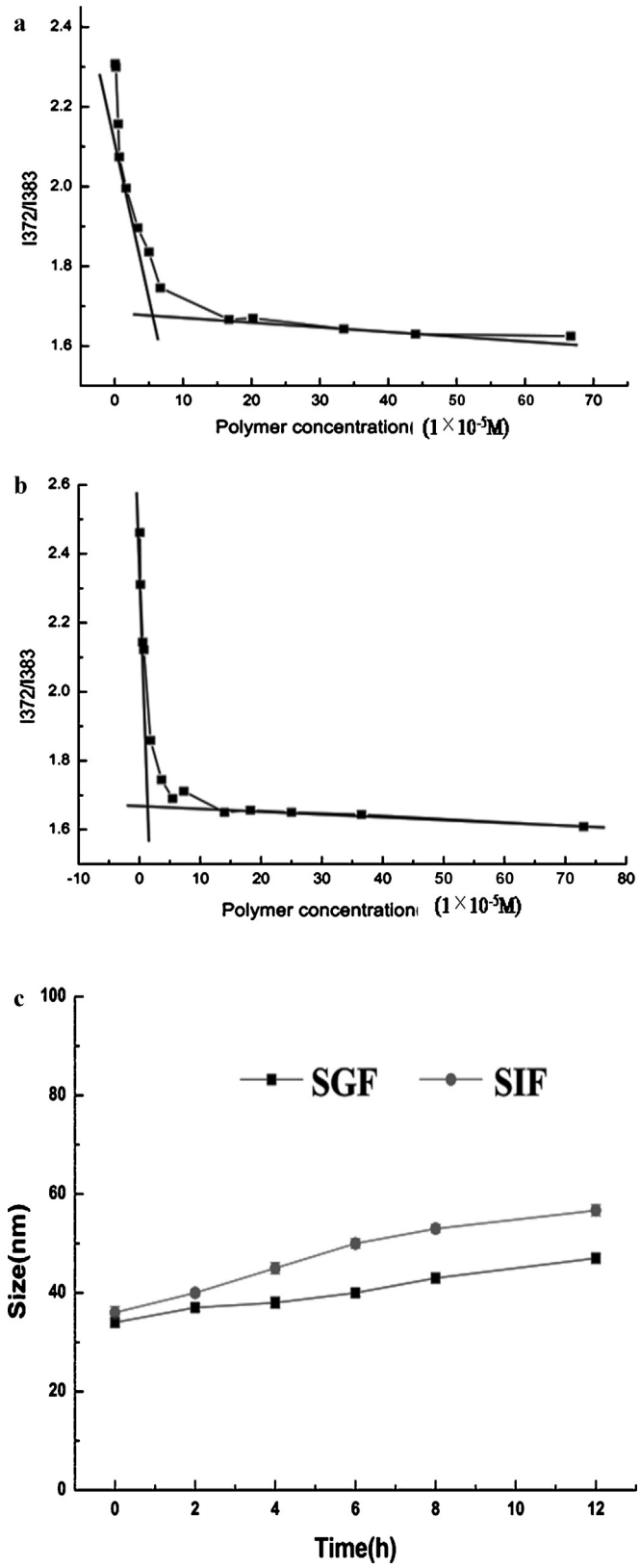

Fig. 4. (a) Plot of I_{372}/I_{383} *vs.* concentrations of MPP/TPGS in water; (b) plot of I_{372}/I_{383} *vs.* concentrations of MPP/TPGS/CSO-SA in water. The CMC value was related to the ratio of I_{372}/I_{383} of the emission spectra profile. (c) Stability of the DTX-loaded MPP/TPGS/CSO-SA MPMs in SGF (pH 1.6), SIF (pH 6.5) (mean ± SD, $n = 3$).

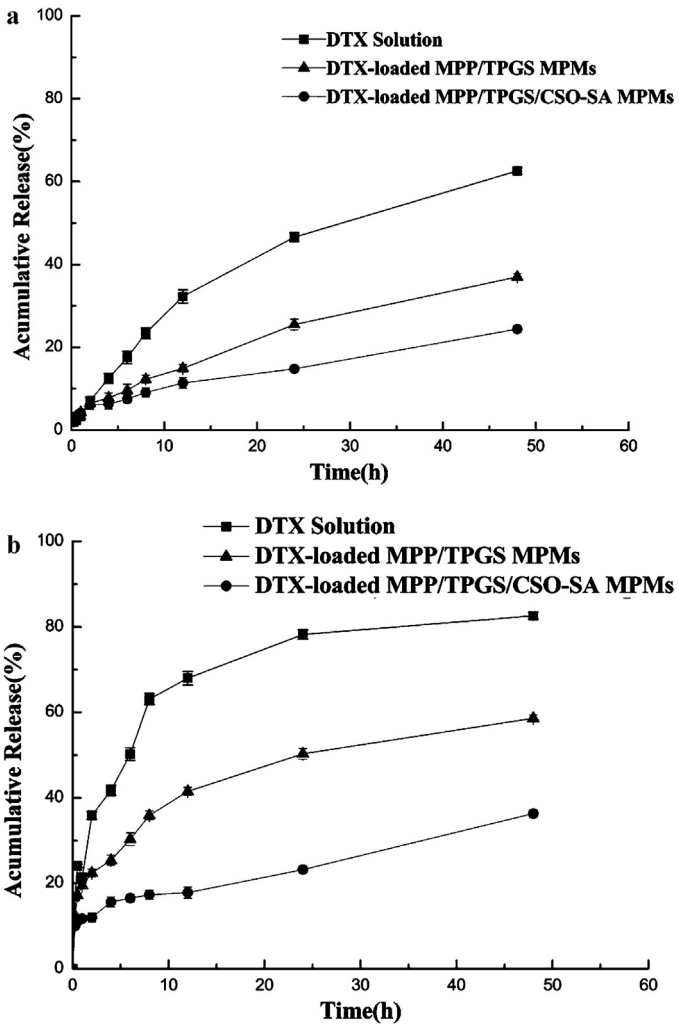

Fig. 5. (a) Cumulative released amount of DTX as a function of time in SGF (0–1 h) and in SIF (1–48 h). (b) Cumulative released amount of DTX as a function of time in PBS (pH = 7.4).

size (36.21 ± 0.74 nm after storage). These results indicated that the micelles were of satisfactory physical stability.

In order to ensure delivery of the carried drug to its site of absorption, the micellar carrier must be able to resist rapid dissociation upon dilution and exposure to the harsh conditions of the gastrointestinal tract. Thus, the stability of the DTX-loaded MPP/TPGS/CSO-SA MPMs was investigated at 37 °C in SGF (pH 1.6), SIF (pH 6.5) for 12 h (Fig. 4c). As presented in Fig. 4c, in SGF, the size of micelles showed little change over 2 h. In SIF, the size of micelles slightly increased from 36.11 to 45.08 nm over 6 h. Owing to the retention time of micelles in intestine was less than 6 h, therefore, the DTX-loaded MPP/TPGS/CSO-SA MPMs should not show significant change in the course of absorption of DTX in the gastrointestinal tract.

3.6. *In vitro release of DTX*

In order to simulate *in vivo* biological environment, the *in vitro* release experiments were conducted in a pH gradient condition simulating the pH evolution of the gastrointestinal tract (SGF, pH = 1.6; SIF, pH = 6.5), while PBS (pH = 7.4) was used to simulate the blood circulation.

Fig. 5a and b showed the release profile of DTX in SGF/SIF and in PBS (pH 7.4), respectively. The release percentages of DTX from

Table 2
Pharmacokinetic parameters after intravenous or oral administration of docetaxel formulations in rats.

	Intravenous DTX solution	Oral administration		
		DTX mixed micelles with CSO-SA	DTX mixed micelles without CSO-SA	DTX solution
Dose (mg/kg)	10	20	20	20
T_{max} (h)	0.083	0.25	0. 25	1
C_{max} (ng/mL)	–	168.13 ± 8.52**	123.86 ± 15.90*	62.15 ± 5.84
AUC (h ng/mL)	4512.70 ± 25.96	889.59 ± 13.98	764.25 ± 5.34	353.63 ± 21.46
MRT (h)	2.81 ± 0.34	4.93 ± 0.23	4.971 ± 0.88	3.61 ± 0.56
F (%)	–	9.86**	8.46*	3.92

** $P < 0.01$ compared DTX solution (oral).
* $P < 0.05$ compared to DTX-loaded MPP/TPGS/CSO-SA MPMs.

DTX-loaded MPP/TPGS MPMs were about 37% in SGF/SIF and 58% in PBS within 48 h, faster than that of DTX-loaded MPP/TPGS/CSO-SA MPMs (24% in SGF/SIF and 36% in PBS). However, both DTX-loaded MPMs possessed a lower drug release rate than DTX solution. And the slower release of DTX from DTX-loaded MPMs compared to DTX solution in SGF/SIF solution, could prevent the rapid leakage and precipitation within the gastrointestinal lumen during the drug delivery. It also can be seen from Fig. 5a and b that the release of DTX from the 3 formulations in PBS solution (pH 7.4) was higher than that from SGF/SIF solution, which might be related with pH value. The larger the pH value, the faster the drug release. The reason might be that swelling and erosion of the polymers could be inhibited in lower pH value. And similar result of pH effect on the drug release *in vitro* was reported [33].

Normally, three basic mechanisms, namely swelling/erosion, diffusion and degradation are present for the release of the loaded drug from polymeric particles [34]. Any or all of these mechanisms may occur in a given release system. The hydrophilicity of the polymer would determine the uptake speed of water during the course of release. The difference between the release behavior of docetaxel from the solution and DTX-loaded mixed micelles might be attributed to the fact that the drug was encapsulated into the core of micelles. With the uptake of water, the micelle particles would swell and allow the drug within to diffuse through the pores. However, the MPMs core with high hydrophobic character could retard the diffusion of water into the core, and the disintegration of the micelles after dilution was a relatively slow process. As a result, the diffusion of DTX was prevented subsequently, which could be concluded from the sustained release behavior of DTX loaded MPP/TPGS. In addition, the slow release of DTX from MPP/TPGS/CSO-SA MPMs could be attributed to the swelling of CSO-SA which would inhibit the diffusion of water into the core of micelles.

SGF/SIF could successfully produce a sink condition (relatively high solubility of DTX in the aqueous solution of lecithin/sodium taurocholate, 193.4 μg/mL), but appeared to hardly interfere with the micelle structure, which could be concluded from stability and *in vitro* release experiments. However, the physiological condition cannot be perfectly mimicked by any simulated fluid. It is unknown whether the orally administered micelles can be significantly affected by constituents of the fluid in the gastrointestinal tract. Thus, the bioavailability of DTX formulated in the micelles should be further examined by *in vivo* experiments.

3.7. Pharmacokinetic studies

The pharmacokinetic parameters of the DTX-loaded mixed micelles and DTX solution are displayed in Table 2. The pharmacokinetic study of free DTX through intravenous route (*i.v.*) was conducted to calculate the absolute bioavailability of oral formulations. The absolute bioavailability obtained by calculating data obtained under same experiment condition would be more convincing, compared to the calculated absolute bioavailability according to literature data. As shown in Table 2, both the T_{max} value of DTX-loaded MPMs with or without CSO-SA were smaller than that of the DTX solution by oral administration. Moreover, both mixed micelles yielded higher plasma concentrations than DTX solution did, with 168.13 ng/mL and 123.86 ng/mL for DTX-loaded MPMs with and without CSO-SA, respectively, compared to the DTX solution (62.15 ng/mL). The plasma $AUC_{(0-t)}$ of DTX-loaded MPP/TPGS/CSO-SA MPMs and DTX-loaded MPP/TPGS MPMs was 889.59 h ng/mL and 764.25 h ng/mL, while 353.63 h ng/mLfor the DTX solution. From the above results, it is clear that DTX-loaded MPMs can significantly improve the oral absorption of DTX. Moreover, DTX-loaded MPP/TPGS/CSO-SA micelles showed the highest absolute oral bioavailability (9.86%) compared to that of DTX-loaded MPP/TPGS micelles (8.46%) and DTX solution (3.92%). The plasma concentration–time curves of the DTX-loaded MPMs and DTX solution are shown in Fig. 6.

Although both DTX-loaded MPMs exhibited slow release behavior *in vitro*, the earlier T_{max} of DTX-loaded mixed micelles suggested a rapid absorption of DTX. Owing to the reason that conditions which bring about micelle dissociation and drug release upon dilution *in vivo* cannot be readily simulated *in vitro*, it was accepted that the *in vivo* data was discordant with *in vitro* behavior [35]. And the difference between the *in vitro* and *in vivo* behavior of DTX-loaded MPMs might be attributed to the more complicated gastric and intestinal environment, such as the existence of bile acid and digestive enzyme, which would accelerate the release of DTX. In addition, a rapid interaction between micelles and the gastrointestinal surface could result in a rapid absorption of DTX.

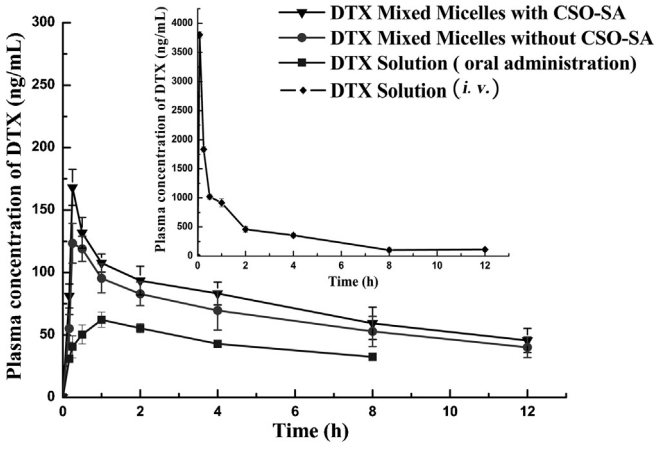

Fig. 6. Plasma concentration-time profiles of DTX solution after *i.v.* (10 mg/kg), oral administration of DTX solution and DTX mixed micelles in rats (20 mg/kg) (mean ± SD, $n = 5$).

The improved oral bioavailability of DTX in the DTX-loaded MPMs with and without CSO-SA might be attributed to the following effects. Firstly, the polymeric micelles increased the solubility of drug and the high drug concentration could be helpful to the passive diffusion of drug into the gastrointestinal membrane. Secondly, the potent ability of inhibiting the P-gp efflux pump of TPGS and CSO-SA copolymer could protect the drug from the P-gp efflux pump, improving the absorption and maintaining higher concentration of the drug in the plasma [36]. In addition, as a nano-sized drug delivery system (less than 100 nm), the micelles could be absorbed by the adsorptive endocytosis pathway [37] and fluid-phase pinocytosis, which results in the membrane invagination and vesicle formation [38]. And the higher C_{max} and oral bioavailability of DTX-loaded MPP/TPGS/CSO-SA MPMs compared with DTX-loaded MPP/TPGS MPMs, might be related to the ability of bio-adhesion and opening the tight junctions of micelles caused by the existing of CSO-SA. Zhang et al. reported that after modification with chitosan, the poly(D,L-lactide-co-glycolide) nanoparticles could provide a great many of advantages, such as a positive charge, mucosal adhesion, and improved absorption [39]. Yuan et al. demonstrated that apical administration of CSO-SA micelles in the Caco-2 cell monolayer would induce an increase in permeability coefficient and an immediate and notable decrease in TEER values [36]. Therefore, the presence of CSO-SA in micelles could enhance the absorption of DTX.

Compared to other oral formulations of DTX, the bioavailability of DTX-loaded MPP/TPGS/CSO-SA MPMs was 1.12-fold that of DTX-lecithin nanoparticles (8.75%) [11]. While in comparison of DTX-Cremophor EL/Transcutol/Capryol 90 microemulsion (34.42%) [3], it was lower. However, the high concentration of surfactant and cosurfactant used in microemulsion has stimulating effect to gastrointestinal mucosa, as well as chronic toxicity effect to the whole body. The DTX-loaded MPP/TPGS/CSO-SA MPMs is a system prepared with safe and biocompatible polymers, thus the DTX-loaded MPP/TPGS/CSO-SA MPMs have less side effects than microemulsion.

4. Conclusion

In this paper, mixed micelles containing sparingly soluble drug docetaxel with combinations of safe excipients MPP, TPGS and CSO-SA at weight ratio of 2:1:1 were formulated successfully by thin film hydration method. The prepared mixed micelles had a mean diameter of 34.96 ± 0.51 nm with drug loading capacity of $19.15 \pm 0.72\%$. The CMC value for MPP/TPGS/CSO-SA micellar solution was as low as 2.11×10^{-5} M. The *in vitro* release studies of DTX-loaded MPP/TPGS/CSO-SA MPMs demonstrated slow-release property both in SGF/SIF and PBS. In addition, the absolute bioavailability of DTX-loaded MPP/TPGS/CSO-SA MPMs was increased by 2.52 times compared to DTX solution with less side effect. These results indicate that DTX-loaded MPP/TPGS/CSO-SA MPMs are valuable as drug delivery carrier to enhance the oral absorption of docetaxel.

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References

[1] B.R. Goldspiel, Pharmacotherapy 17 (1997) 110.
[2] A.T. van Oosterom, D. Schrijvers, D. Schrijvers, Anticancer Drugs 6 (1995) 356.
[3] Y.M. Yin, F.D. Cui, C.F. Mu, M.K. Choi, J.S. Kim, S.J. Chung, C.K. Shim, D.D. Kim, J. Control. Release 140 (2009) 86.
[4] E. Lee, H.J. Kim, I.H. Lee, S.Y. Jon, J. Control. Release 140 (2009) 79.
[5] J.J. Moesa, S.L.W. Koolena, A.D.R. Huitemaa, J.H.M. Schellens, J.H. Beijnen, B. Nuijena, Int. J. Pharm. 420 (2011) 244.
[6] T.M. Sissung, C.E. Baum, J. Deeken, D.K. Price, J.A. Ching, S.M. Steinberg, W. Dahut, A. Sparreboom, W.D. Figg, Clin. Cancer Res. 14 (2008) 4543.
[7] P. Wils, V. Phung-Ba, A. Warnery, D. Lechardeur, S. Raeissi, I.J. Hidalgo, D. Scherman, Biochem. Pharmacol. 48 (1994) 1528.
[8] Y.D. Yan, N. Marasini, Y.K. Choi, J.O. Kim, J.S. Woo, C.S. Yong, H.G. Choi, Eur. J. Drug Metab. Pharmacokinet. 37 (2012) 217.
[9] Q.Z. Quan, D.W. Kim, N. Marasini, D.H. Kim, J.K. Kim, J.O. Kim, C.S. Yong, H.G. Choi, J. Microencapsul. (2012) (Epub ahead of print).
[10] J. Wu, Q. Shen, L. Fang, Drug Dev. Ind. Pharm. (2012) (Epub ahead of print).
[11] K.L. Hu, S. Cao, F.Q. Hu, J.F. Feng, Int. J. Nanomed. 7 (2012) 3537.
[12] U. Kedar, P. Phutane, S. Shidhaye, V. Kadam, Nanomedicine 6 (2010) 714.
[13] C. Allen, Y.Y. Yu, A. Eisenberg, D. Maysinger, Biochim. Biophys. Acta 1421 (1999) 32.
[14] Y.S. Nam, H.S. Kang, J.Y. Park, T.G. Park, S.H. Hana, I.S. Chang, Biomaterials 24 (2003) 2053.
[15] N. Nishiyama, K. Kataoka, Adv. Polym. Sci. 193 (2006) 67.
[16] H.M. Burt, X.C. Zhang, P. Toleikis, L. Embree, W.L. Hunter, Colloids Surf. B – Biointerfaces 16 (1999) 161.
[17] E. Blanco, E.A. Bey, Y. Dong, B.D. Weinberg, D.M. Sutton, D.A. Boothman, J.M. Gao, J. Control. Release 122 (2007) 365.
[18] Z.P. Zhang, S.W. Tan, S.S. Feng, Biomaterials 33 (2012) 4889.
[19] J.M. Dintaman, J.A. Silverman, Pharm. Res. 16 (1999) 1550.
[20] L.Y. Zhao, S.S. Feng, J. Pharm. Sci. 99 (2010) 3552.
[21] T.H. Yeh, L.W. Hsu, M.T. Tseng, P.L. Lee, K. Sonjae, Y.C. Ho, H.W. Sung, Biomaterials 32 (2011) 6164.
[22] F.Q. Hu, M.D. Zhao, H. Yuan, J. You, Y.Z. Du, S. Zeng, Int. J. Pharm. 315 (2006) 158.
[23] Y.Z. Du, P. Lu, J.P. Zhou, H. Yuan, F.Q. Hu, Int. J. Pharm. 391 (2010) 260.
[24] Q. Li, Y.Z. Du, H. Yuan, X.G. Zhang, J. Miao, F.D. Cui, F.Q. Hu, Pharm. Acta Helv. 41 (2010) 498.
[25] X.R. Li, P.Z. Li, Y.H. Zhang, Y.X. Zhou, X.W. Chen, Y.Q. Huang, Y. Liu, Pharm. Res. 27 (2010) 1498.
[26] I. Astafieva, X.F. Zhong, A. Eisenberg, Macromolecules 26 (1993) 7339.
[27] F.Z. Dahmani, H. Yang, J.P. Zhou, J. Yao, T. Zhang, Q. Zhang, Eur. J. Pharm. Biopharm. 47 (2012) 179.
[28] S. Kim, J.Y. Kim, K.M. Huh, G. Acharya, K. Parka, J. Control. Release 132 (2008) 222.
[29] M.S. Muthu, S.A. Kulkarni, J.Q. Xiong, S.S. Feng, Int. J. Pharm. 421 (2011) 332.
[30] C. Allen, D. Maysinger, A. Eisenberg, Colloids Surf. B – Biointerfaces 16 (1999) 3.
[31] P. Balakrishnan, S. Shanmugam, W.S. Lee, W.M. Lee, J.O. Kim, D.H. Oh, D.D. Kim, J.S. Kim, B.K. Yoo, H.G. Choi, J.S. Woo, C.S. Yong, Int. J. Pharm. 377 (2009) 1.
[32] T. Jung, W. Kamm, A. Breitenbach, E. Kaiserling, J.X. Xiao, T. Kissel, Eur. J. Pharm. Biopharm. 50 (2000) 147.
[33] Z.P. Zhang, S.S. Feng, Int. J. Pharm. 324 (2006) 191.
[34] L. Mu, M.M. Teo, H.Z. Ning, C.S. Tan, S.S. Feng, J. Control. Release 103 (2005) 565.
[35] F.Z. Dahmani, H. Yang, J.P. Zhou, J. Yao, T. Zhang, Q. Zhang, Eur. J. Pharm. Sci. 47 (2012) 179.
[36] H. Yuan, L.J. Lu, Y.Z. Du, F.Q. Hu, Mol. Pharmacol. 8 (2010) 225.
[37] M. Guan, Q.L. Zhu, Y. Liu, Y.Y. Bei, Z.L. Gu, X.N. Zhang, Q. Zhang, Int. J. Nanomed. 7 (2012) 1921.
[38] L. Luo, J. Tam, D. Maysinger, A. Eisenberg, Bioconjug. Chem. 13 (2002) 1259.
[39] X.Y. Zhang, M.Z. Sun, A.P. Zheng, D.Y. Cao, Y.Q. Bi, J.X. Sun, Eur. J. Pediatr. Surg. 45 (2012) 632.