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Dual-drug delivery system based on hydrogel/micelle composites

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ABSTRACT

We present a dual-drug delivery system (DDDS) of hydrogel/polypeptide micelle composites in this work. The DDDS was constructed from aspirin (Asp) dispersed poly(vinyl alcohol) (PVA) or Chitosan (CS)/PVA hydrogel and doxorubicin (DOX) loaded poly(L-glutamic acid)-*b*-poly(propylene oxide)-*b*-poly(L-glutamic acid) (GPG) micelles. Independent release behaviors of the two drugs are observed. Asp has a short-term release while DOX has a long-term and sustained release behavior in all the DDDSs. The release of DOX from all the DDDSs is environmentally controlled due to the pH and temperature sensitivity of the GPG micelle. Asp shows the pH controlled release behavior in CS/PVA/micelle DDDS due to the pH sensitivity of CS hydrogel. The releasing profiles were analyzed using a power law equation proposed by Peppas. It reveals that the release of Asp is anomalous transport in all the hydrogel/micelle DDDSs. The release of DOX is Fickian type in PVA/micelle system, and changes to anomalous transport in CS/PVA/micelle system according to the release exponent *n*.

1. Introduction

Over the past few decades, drug delivery systems (DDSs) have been developed and studied in great depth to improve the curative effect of drugs [1–4]. DDS can ameliorate the problems of conventional administration by enhancing drug solubility, prolonging duration time, reducing side effect, retaining drug bioactivity and so on [5,6]. A variety of systems such as particulate carriers [7,8], polymer gels [9,10], lipids [11,12], etc. have been used as DDSs. At present, stimuli sensitive DDSs have been an attractive theme for controlled release. The release behaviors of drugs can be easily controlled by surrounding properties, such as pH [13,14], temperature [15,16], ionic strength [17] and electric field [18].

Hydrogels are hydrophilic three-dimensional polymer networks, which contain a large amount of water [19–21]. They are highly permeable to various drugs and the entrapped drugs can be released through their web-like structures [22]. As compared with conventional administration, drugs can prolong their duration time by hydrogel DDS. For example, Park et al. investigated biodegradable elastic hydrogel scaffolds which are based on hydrophilic poly(ethylene glycol) (PEG) and hydrophobic poly(ε-caprolactone) (PCL) as a delivery vehicle [23]. These hydrogel scaffolds can offer a suitable environment for the retention of the chondrocytic phenotype and cell therapy. When the hydrogels are stimuli sensitive, they may act as "smart" DDS [24–26]. Wu et al. designed a thermo- and pH-sensitive drug delivery hydrogel system which is composed of *N*-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride (HTCC)/glycerophosphate (GP) [26]. The polymer composition is a free flowing sol at room temperature but becomes a gel at body temperature, which makes it injectable. The hydrogel is stable at neutral and basic conditions but dissolves at acid condition, which leads to a quick releasing of drug at acid condition and a slow releasing at neutral and basic condition.

Another important DDS is polymeric micelle, which is self-assembled from amphiphilic block or graft copolymers [27–30]. These polymeric micelles show distinct stability in solution [31–33]. The core–shell structure of the micelle can improve solubility of hydrophobic drugs, and protect the incorporated drug from premature degradation [34,35]. When environmentally sensitive (pH, temperature, etc.) functional groups are introduced into these amphiphilic copolymers, "smart" micelles are formed, and they can be used as environmentally controlled drug release system [36–38]. For example, Ko et al. used methyl ether poly(ethylene glycol)–poly(β-amino ester) block copolymer micelles to encapsulate doxorubicin [38]. These micelles show noticeable pH-dependent micellization–demicellization behavior, leading to a quick release at pH 6.4 and a slow release at pH 7.4.

Recently, combined therapy with drugs of different therapeutic effects shows an effective way in treatment of diseases and tissue reborn [39,40]. In order to optimize their effects, different drugs should be used at their optimal dose and different periods in the treatment. One of the main challenges of combined therapy is to

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control the release behavior of each drug independently. However, simple drug delivery systems cannot fulfill the needs of such therapy. Therefore, developing the dual-drug delivery systems which can control the release behavior of each drug is desired. However, limited researches on the dual-drug delivery system (DDDS) are reported so far. For example, Lee et al. developed a simple dual-drug-loaded hydroxypropylmethylcellulose (HPMC) matrix tablet which simultaneously contains drug in inner tablet core and outer coated layer [41]. The obtained dual-drug-loaded HPMC matrix tablet shows biphasic release profiles, and can deliver drugs with circadian rhythmic behaviors in the body.

In this work, we present a delivery system of hydrogel/micelle composites as dual-drug release vehicle. The hydrogel is prepared from poly(vinyl alcohol) (PVA) or Chitosan (CS)/PVA. We use PVA hydrogel for its good physical–mechanical properties, and CS hydrogel for its pH sensitivity. Both hydrogels present good biocompatibility for drug delivery. The micelle is prepared from poly(L-glutamic acid)-*b*-poly(propylene oxide)-*b*-poly(L-glutamic acid) (PLGA-*b*-PPO-*b*-PLGA, abbreviated as GPG), which is pH- and thermo-sensitive. Two drugs, aspirin (Asp, water-soluble) and doxorubicin (DOX, fat-soluble) are used as model drugs. DOX is encapsulated into the GPG micelle, while Asp is directly dispersed in the hydrogel. The drug release behaviors of GPG micelle, PVA/micelle DDDS and CS/PVA/micelle DDDS were studied as functions of pH and temperature. The releasing profiles were analyzed by a power law equation to reveal the release mechanisms of drugs.

2. Experimental

2.1. Materials

Tetrahydrofuran (THF), hexane, 1,4-dioxane were refluxed with sodium and distilled immediately before use. Doxorubicin hydrochloride (DOX-HCl) was obtained from Zhejiang Hisun Pharmaceutical Co., Ltd. Aspirin was supplied by Huayin Jinqiancheng Pharmaceutical Co., Ltd. Poly(vinyl alcohol) (PVA, $M_w = 1750$) was purchased from Shanghai Tianlian Industry of Fine Chemicals Co., Ltd. Chitosan (CS, degree of deacetylation $\geq 90\%$) was obtained from Sinopharm Chemical Reagent Co., Ltd. α,ω-Amino poly(propylene oxide) (NH_2–PPO–NH_2, $M_w = 4000$) was purchased from Sigma–Aldrich Co., Inc. NH_2–PPO–NH_2 was dissolved in toluene in a flame-dried reaction bottle, followed by removing the toluene in high vacuum to obtain the initiator used for copolymerization. Cellulose membrane dialysis bag (3500 molecular weight cut-off) was provided by Serva Electrophoresis GmbH. All other reagents are of analytical grade and used without further purification.

2.2. Synthesis and characterization of triblock copolymer

Poly(γ-benzyl-L-glutamate)-*b*-poly(propylene oxide)-*b*-poly(γ-benzyl-L-glutamate) (PBLG-*b*-PPO-*b*-PBLG) triblock copolymer was synthesized by ring-opening polymerization of γ-benzyl-L-glutamate-*N*-carboxyanhydride (BLG-NCA) initiated by terminal amino groups of NH_2–PPO–NH_2 [42,43]. NH_2–PPO–NH_2 (0.2 g; 0.05 mmol) and BLG-NCA (0.71 g; 2.7 mmol) were dissolved in dioxane separately in two flame-dried reaction bottles, then, BLG-NCA solution was added to the solution of NH_2–PPO–NH_2 via a transfer cannula. The reaction was performed at 15 °C under a dry nitrogen atmosphere. After 72 h stirring, the reaction mixture became a viscous liquid and was poured into a large volume of anhydrous ethanol. The precipitation product was collected and dried under vacuum. The product was purified twice by repeated cycle of dissolution (in chloroform) and precipitation (in anhydrous ethanol) of the product. PLGA-*b*-PPO-*b*-PLGA triblock copolymer was prepared by hydrolyzation of PBLG-*b*-PPO-*b*-PBLG with potassium hydroxide (KOH) [44]. As a brief, 1 g PBLG-*b*-PPO-*b*-PBLG was dissolved in 40 mL THF. In a separate step, a concentrated aqueous solution of KOH (3 mol equivalence with respect to benzyl group) was prepared and added to the solution of the polymer. After 4 h stirring, the mixture was acidulated with excessive HCl, and dialyzed against distilled water for 3 days to remove organic solvents and other small impurities. The product was finally freeze-dried to get PLGA-*b*-PPO-*b*-PLGA powder.

The molecular weight of the block copolymer before hydrolysis was estimated using ^{1}H NMR measurements (Avance 550, Bruker). Since the degree of polymerization (DP) of the PPO block is known (69), the total molecular weight of the triblock copolymer PBLG-*b*-PPO-*b*-PBLG can be calculated by the peak intensities of the methylene proton signal (5.1 ppm) of PBLG and the methylene proton signal (3.6 ppm) of PPO in the ^{1}H NMR spectrum. The calculation shows that the molecular weight of the original copolymer is 15,800 and the DP of PBLG is 27. The disappearance of methylene proton peak (5.1 ppm) of PBLG segments in the ^{1}H NMR spectrum provides the evidence of deprotection of benzyl group from PBLG-*b*-PPO-*b*-PBLG copolymer to form PLGA-*b*-PPO-*b*-PLGA copolymer. The molecular weight of PLGA-*b*-PPO-*b*-PLGA copolymer is calculated to be 10,900 after hydrolysis.

2.3. Preparation of drug-loaded delivery systems

To prepare drug-loaded micelle, 3 mg DOX-HCl and 6 mg PLGA-*b*-PPO-*b*-PLGA (GPG) were first dissolved in 10 mL DMF/DMSO mixed solvent (v/v = 4/1). One drop of triethylamine (ca. 0.05 mL) was added to the solution to remove hydrochloride. After stirring at room temperature overnight, the mixed solution was dialyzed against distilled water for 72 h at 20 °C to form the DOX-loaded GPG micelle. The distilled water was replaced every 3–4 h. The obtained drug-loaded micelle solution was diluted, and the final concentration of GPG is 0.3 mg/mL.

To produce PVA hydrogel/GPG micelle DDDS, 1 mg Asp was dissolved in 10 mL PVA aqueous solution (PVA: 50 mg/mL), and then mixed with 10 mL pre-made DOX-loaded GPG micelle solution. The PVA hydrogel/GPG micelle DDDS was then prepared by a freezing–thawing cycle (freezing at −20 °C for 24 h and thawing at room temperature for 3 h) in special moulds [45]. The CS/PVA/micelle mixture was prepared as follows: CS (100 mg/mL, dissolved in 2 vol% acetic acid) and PVA (100 mg/mL) aqueous solutions were mixed with various CS/PVA volume ratios (1/1, 2/3, 1/3 and 1/6). Therefore, the total polymer concentration of all the CS/PVA solutions is 100 mg/mL. And then 1 mg Asp and 10 mL pre-made DOX-loaded GPG micelle solution were added to 10 mL CS/PVA solution. The CS/PVA/micelle solution also went through the freezing–thawing cycle (the same as those applied to the PVA/micelle system) to form the hydrogel/micelle DDDS. All the hydrogel/micelle DDDS samples are cylindrical in shape (ca. 28 mm thick, and 30 mm in diameter).

2.4. Characterization of drug delivery systems

The amount of DOX encapsulated in the GPG micelles was analyzed by an ultraviolet–visible spectrometer (UV/vis, Unico UV2102). 8 mL DMF was introduced into 2 mL DOX-loaded micelle solution, the micelles were broken up and the DOX was dissolved in the solution. The characteristic absorbance of DOX at 485 nm was recorded and compared with a standard curve generated from a DMF/H_2O (v/v = 4/1) mixture with DOX concentrations varying from 0 to 100 μg/mL.

The morphologies of DOX-loaded GPG micelles both in solution and in PVA/micelle DDDS were examined using transmission electron microscope (TEM, JEM-1200-EXII), operated with an accelerating voltage of 120 kV. Drops of DOX-loaded GPG micelle solution were placed on a carbon film-coated copper grid and stained by phosphotungstic acid aqueous solution (0.5 wt%). DOX-loaded GPG micelle/PVA solution was pre-stained in solution and dropped to carbon film-coated copper grid, followed by a freezing–thawing cycle. All the samples were dried at room temperature.

2.5. Swelling properties

Swelling degrees (SDs) of hydrogels were measured at 37 °C. The fresh made samples (wet) were weighted and immersed in buffer solutions with different pH values. These samples were gently wiped with filter paper to remove the surface solution when taken out from the solutions, then weighted and returned to the same container at pre-determined time intervals. The SD was calculated as follows:

$$\mathrm{SD}\ (\%) = \left(\frac{W_t}{W_0}\right) \times 100 \quad (1)$$

where W_0 is the weight of the original hydrogel and W_t is the weight of hydrogel at various swelling times.

2.6. In vitro drug release study

A fixed volume of DOX-loaded micelle solution was suspended in dialysis bag, and placed into 10 mL buffer solution with various pH values. PVA/micelle and CS/PVA/micelle DDDS were directly immersed in 10 mL buffer solutions. All the samples were then laid in a shaking bath at 90 rpm, with constant temperature of 20, 30, or 37 °C respectively. The buffer solution was replaced periodically. UV/vis absorbance was recorded at 296 nm (Asp) and 485 nm (DOX); the concentrations of Asp and DOX in buffer solutions were determined according to the standard curves of each drug at corresponding buffer solutions. And then the release amount of the drugs can be calculated.

3. Results and discussion

This paper consists of three sections: In the first section, dual-drug delivery system was prepared from PVA hydrogel/GPG micelle composites. Asp is dissolved in the hydrogel, while DOX is encapsulated in the micelle. We investigated the drug release profiles of GPG micelle and PVA/micelle DDDS as functions of pH and

temperature. In the second section, in order to make the DDDSs have environmental sensitivity of Asp release, CS was introduced into the hydrogel. We studied the drug release profiles and the swelling behavior of the CS/PVA/micelle DDDS at various environmental conditions. In the last section, a power law equation was used to analyze the drug release mechanism of PVA/micelle and CS/PVA/micelle DDDS.

3.1. In vitro drug release behaviors of dual-drug-loaded PVA/micelle system

We first examined the release behavior of DOX from GPG micelle. Fig. 1a shows the release profiles at 37 °C as a function of pH. It reveals that the release rate of DOX from the GPG micelle is markedly influenced by the pH value. When the pH is higher (pH = 8.4), the release rate is slower. As the pH value is decreased, the release rate is accelerated. The total release amount of DOX is up to 96% at pH = 4.0 within 72 h. In addition, the influence of temperature on the release behavior of DOX from GPG micelle is also studied. Fig. 1b shows the release profiles of the DOX at pH = 7.4 as a function of temperature. The release rate is accelerated obviously with increasing temperature from 20 °C to 37 °C.

GPG micelle is sensitive to both pH value and temperature. PLGA blocks take a coil conformation under basic condition and transforms to α-helix when the pH value decreases (meanwhile the solubility drops) [46–50]. At low pH values, the PLGA segments shrink and generate a stress on the core of micelles, which gives rise to a core distortion [49]. Subsequently, DOX leaks from the micelles.

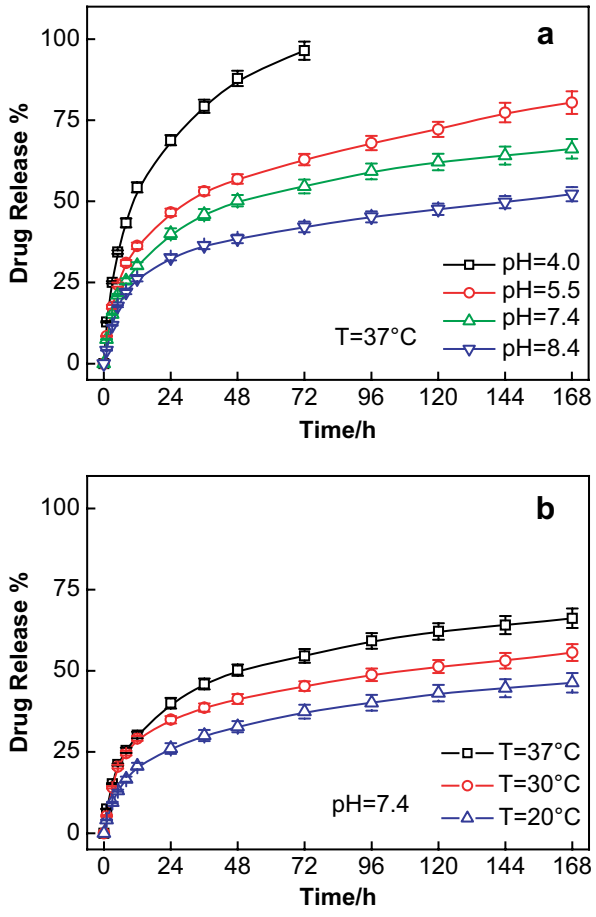

Fig. 1. Release of DOX from GPG micelles: (a) $T = 37$ °C, pH = 4.0, 5.5, 7.4 and 8.4, respectively; (b) pH = 7.4, $T = 20$ °C, 30 °C and 37 °C, respectively.

As a result, rapid drug release behavior can be found when pH value is lower. On the other hand, the propylene units of PPO are highly hydrated at low temperature, and gradually dehydrated with increasing temperature [51–54]. The micelle core becomes more compact and contains less water at higher temperature [53,54]. Thus, the DOX releases quickly from GPG micelle at higher temperature.

The release behaviors of DOX and Asp from PVA hydrogel/GPG micelle dual-drug delivery system (DDDS) were further examined. Fig. 2 shows typical release profiles of the two drugs from the DDDS as functions of pH value (Fig. 2a) and temperature (Fig. 2b). The insets in Fig. 2a and b show enlarged release profiles of Asp. Despite of the change of pH value and temperature, Asp shows a similar short-term release behavior in 8 h. However, the release behavior of DOX from the DDDS presents a sustained long-term release and is markedly dependent on environmental pH value and temperature. Fig. 2a shows that the release rate of DOX is accelerated by reducing the pH value from 8.4 to 4.0. Increasing the temperature gives rise to an acceleration of DOX releasing as shown in Fig. 2b. Comparing with the DOX release profiles in Figs. 1 and 2, we find that the release behaviors of DOX are very similar in the two systems, indicating that the DOX release rate in PVA/micelle composite system is mainly controlled by the diffusion from GPG micelle. In other words, the release behavior of DOX is controlled by the micelle carriers. However, as shown in the inset profiles of Fig. 2, Asp has a short-term release behavior within 8 h, and its pH and temperature sensitivity is less pronounced in PVA/micelle DDDS.

The morphologies of DOX-loaded GPG micelles in solution and in PVA hydrogel were examined by TEM. As shown in Fig. 3, spherical morphologies are observed in both cases. The diameter of the micelle is about 170 nm in aqueous solution (Fig. 3a), and about 130 nm in PVA hydrogel (Fig. 3b). The micelles are well dispersed in the PVA hydrogel without aggregation. Note that the diameter of the GPG micelles in hydrogel is slightly smaller than that in aqueous solution from TEM observations. This could be caused by the shrinkage of PVA network during the freezing–thawing cycle in the formation of hydrogel.

Based on the experimental observations, we proposed the mechanism of the different release behaviors of Asp and DOX in the PVA/micelle DDDS. Hydrogel has a network structure and contains a large amount of water, thus the water-soluble Asp can be dissolved directly in the water phase of PVA hydrogel. The DOX-loaded GPG micelles can be well distributed in the water phase of the hydrogel due to the hydrophilic shell. The resulting structure of the DDDS is the PVA hydrogel containing DOX-loaded micelles. When the drugs are released, Asp diffuses directly from the hydrogel, and DOX must first move out of GPG micelles and then diffuse from PVA hydrogel. As a result, the release rate of DOX is much slower than that of Asp. Since the release behavior of DOX is mainly controlled by GPG micelles, the DOX release shows pH and temperature sensitivity.

3.2. In vitro drug release behaviors of dual-drug-loaded CS/PVA/micelle system

The above PVA/GPG micelle DDDS shows an insensitive release behavior of Asp to environmental stimulus. In order to make the DDDS to have environmental sensitivity of Asp release, we introduce Chitosan (CS) into the hydrogel. CS is a typical cationic hydrogel, and it protonates and swells in low pH condition [55]. Fig. 4a presents the release profiles of Asp and DOX from CS/PVA/micelle DDDS (CS/PVA, w/w = 1/3) at four pH values, and the measurements were carried out at 37 °C. The enlarged release curves of Asp are given in the inset of Fig. 4a. It reveals that Asp has

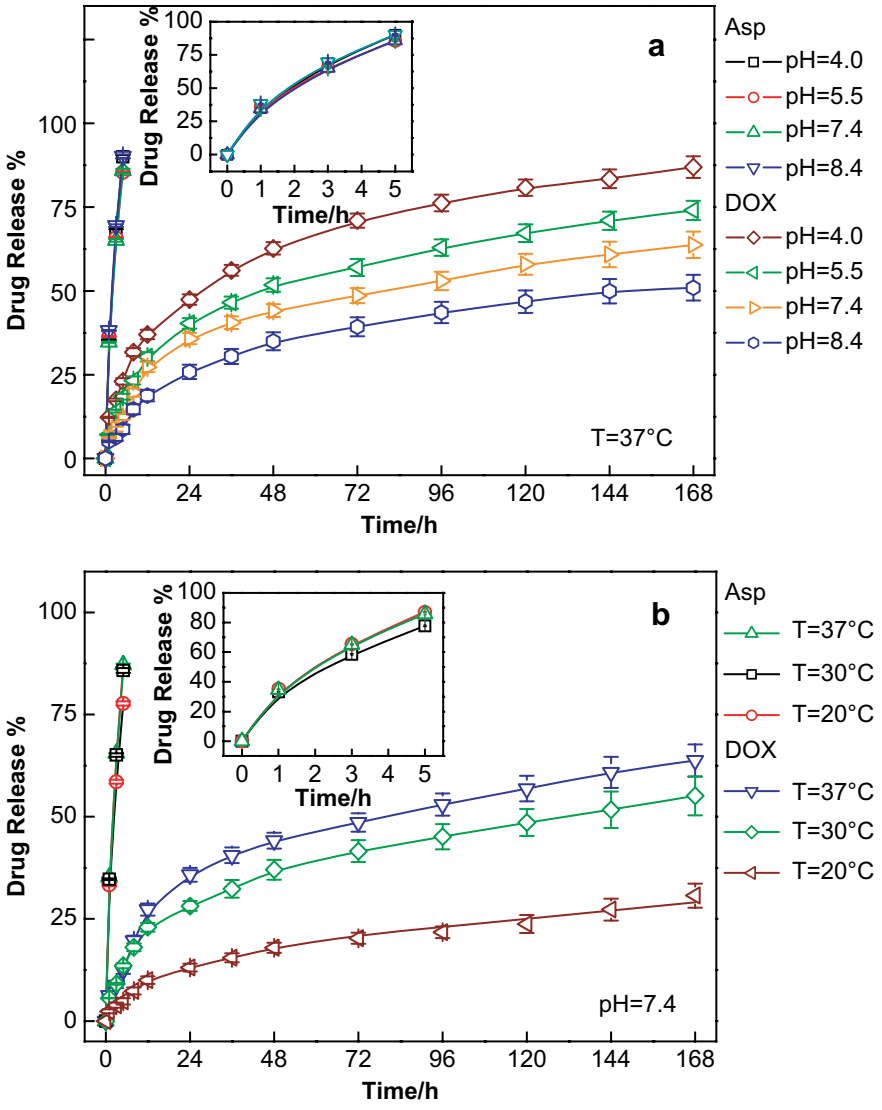

Fig. 2. Release of Asp and DOX from PVA/micelle DDDS: (a) $T = 37\ ^{\circ}C$, pH = 4.0, 5.5, 7.4 and 8.4, respectively; (b) pH = 7.4, $T = 20\ ^{\circ}C$, 30 °C and 37 °C, respectively.

a quick release within 12 h while DOX has a sustained and long-term release for a week. Different from the PVA/GPG micelle DDDS, the release behavior of Asp in this system shows pH sensitivity. The release rate of Asp increases when the surrounding pH value is increased. As for the DOX, the release rate is accelerated when pH value drops from 8.4 to 4.0. This shows the same trend as that of PVA/micelle DDDS. Temperature controlled release of DOX from this DDDS is also observed as shown in Fig. 4b, but the release of

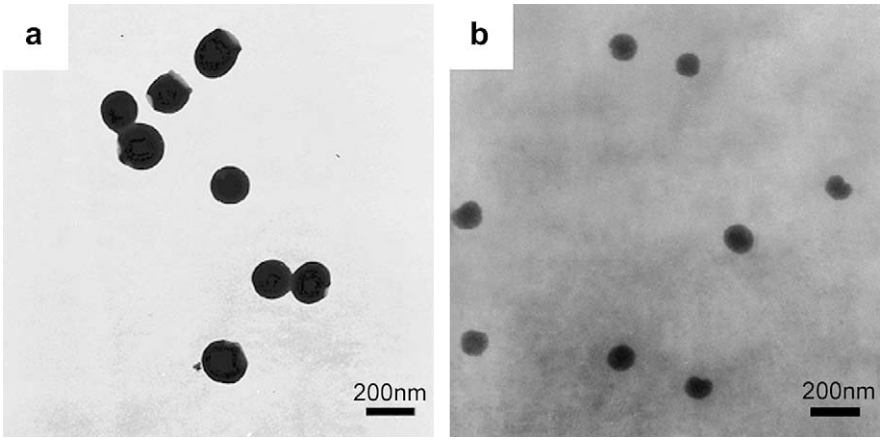

Fig. 3. TEM images of DOX-loaded GPG micelles: (a) in aqueous solution, (b) in PVA hydrogel; pH = 7.4, $T = 25\ ^{\circ}C$.

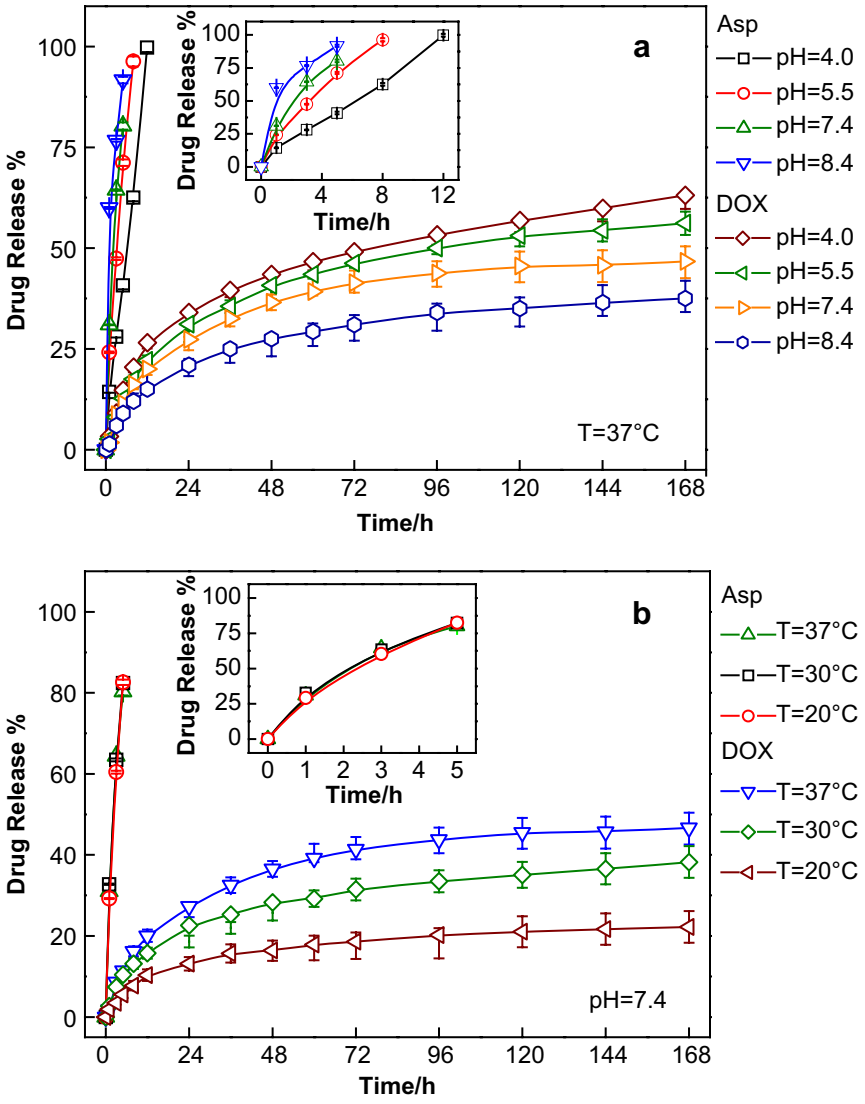

Fig. 4. Release profiles of Asp and DOX from CS/PVA/micelle DDDS (CS/PVA, w/w = 1/3): (a) $T = 37\ ^{\circ}C$, pH = 4.0, 5.5, 7.4 and 8.4, respectively; (b) pH = 7.4, $T = 20\ ^{\circ}C$, 30 °C and 37 °C, respectively.

Asp does not show the temperature dependence (see the inset of Fig. 4b).

To explain the environmental sensitivity of drug release behaviors in CS/PVA/GPG micelle DDDS, swelling behaviors of CS/PVA hydrogels at different pH values are examined. Fig. 5 shows the typical profiles of the shrinking ratio of CS/PVA (w/w = 1/3) hydrogel against time at various pH values. At pH = 4.0, the CS/PVA hydrogel has an initial swelling process in the first 8 h and then shrinks during the next 160 h. Similar swelling behavior of CS/PVA hydrogel is observed at pH 5.5. However, at pH = 7.4 and 8.4, the hydrogels shrink from beginning to end. These results reveal that the CS/PVA hydrogel shrinks markedly and excludes large amount of water from its network, when the surrounding pH value is increased. This can explain why the release rate of Asp has a pH dependence and Asp can be released fast at higher pH as shown in Fig. 4.

We further examined the influence of CS/PVA ratio of hydrogel on the release rates of the drugs, a series of CS/PVA/GPG micelle DDDSs with various CS/PVA ratio were studied. Fig. 6 presents the release profiles of CS/PVA/micelle systems with various CS contents (pH = 7.4, $T = 37\ ^{\circ}C$). As shown in Fig. 6, the release rates of Asp and DOX are both accelerated with increasing the content of CS in the

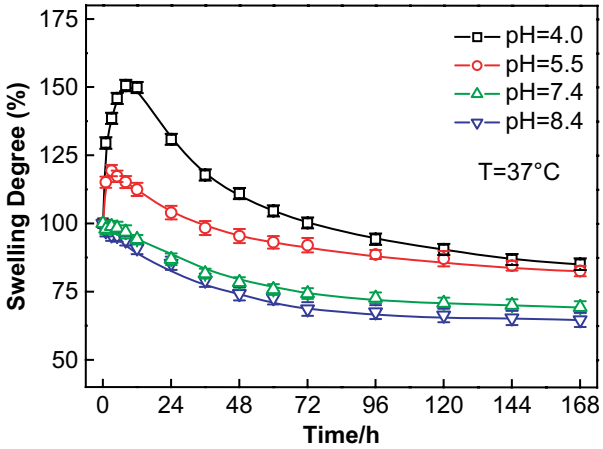

Fig. 5. Swelling degree of CS/PVA (w/w = 1/3) hydrogels. $T = 37\ ^{\circ}C$, pH = 4.0, 5.5, 7.4 and 8.4, respectively.

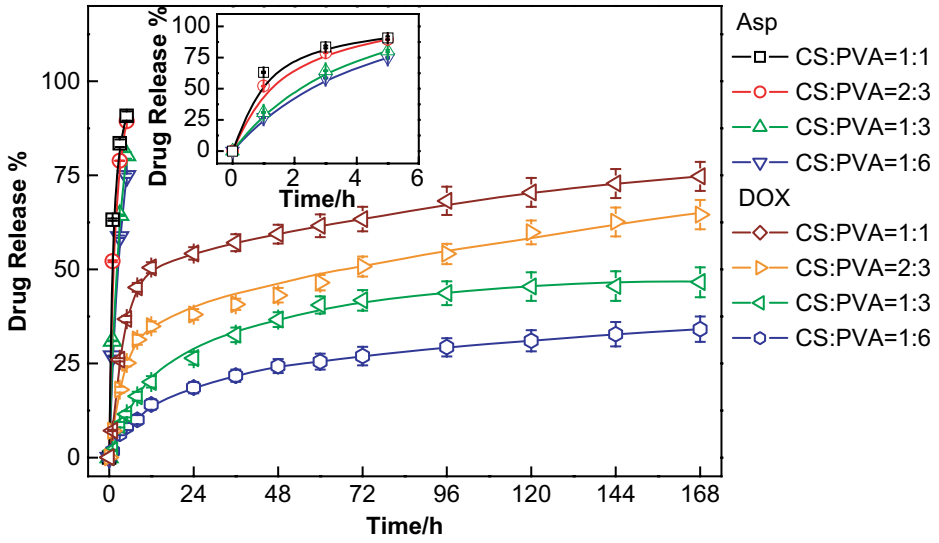

Fig. 6. Release profiles of Asp and DOX from CS/PVA/micelle DDDS with various CS/PVA ratios ($w/w = 1/6$, 1/3, 2/3 and 1/1); pH = 7.4, $T = 37\ ^\circ C$.

hydrogel. Since the hydrogel network is mainly crosslinked by the PVA chains, the crosslink density of CS/PVA hydrogel becomes lower when reducing the PVA content [45]. In other words, the crosslink density is lower when the CS/PVA ratio is higher (the total concentration of polymers in the hydrogels is the same). The retardation of the molecule diffusion tends to be weak. As a result, the release rates of both Asp and DOX are accelerated when increasing CS/PVA ratio as shown in Fig. 6.

3.3. Release mechanism studies

To explain the nature of the drug release behaviors, Peppas et al. have proposed an empirical power equation [56,57]. The equation is written as:

$$\frac{M_t}{M_\infty} = kt^n \tag{2}$$

$$\log\left(\frac{M_t}{M_\infty}\right) = n\log t + \log k \tag{3}$$

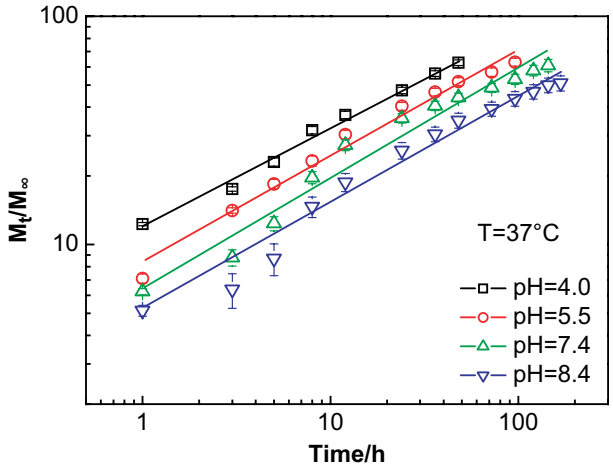

Fig. 7. Plots of $\log(M_t/M_\infty)$ against $\log t$ for DOX release from the PVA/micelle DDDS, at various pH values, $T = 37\ ^\circ C$.

where M_t and M_∞ are the absolute cumulative amount of drug released at time t and infinite time, respectively, k is a constant incorporating structural and geometric characteristics of the device, and n is the release exponent, indicative of the mechanism of drug release.

According to Peppas's equation, there are two distinct physical realistic meanings in the two special cases of $n = 0.45$ (indicating diffusion-controlled drug release) which is called Fickian diffusion, and $n = 0.89$ (indicating swelling-controlled drug release) which is called case-II transport [58]. When n is between 0.45 and 0.89, the drug release behavior can be regarded as the superposition of both phenomena, which is called anomalous transport.

We plotted $\log(M_t/M_\infty)$ against $\log t$ of the experimental data according to Eqs. (2) and (3). Fig. 7 shows a typical plot of $\log(M_t/M_\infty)$ versus $\log t$ at various pH values for DOX in PVA/micelle DDDS at 37 °C. Good linearity is shown, indicating that the Peppas's equation is applicable to the present systems. By these plots, the release exponents of n, rate constant of $\log k$ and the correlation coefficient R^2 from two DDDSs were obtained and they are listed in Tables 1 and 2. Table 1 shows the values of n and $\log k$ at various pH values for the PVA/micelle DDDS at 37 °C. Values of n for Asp are all greater than 0.45 and much smaller than 0.89, indicating that the release of Asp is anomalous transport. For the DOX release, the n values are very close to 0.45, which corresponds to Fickian diffusion. The different release mechanism of Asp and DOX suggests that the hydrogel has a more marked influence on the Asp than DOX in their release process [59]. This is well in line with the fact that the release of Asp is determined by the PVA hydrogel and DOX release is mainly controlled by the GPG micelle in the PVA/micelle DDDS (see Fig. 2). As can be seen from Table 1, the values of $\log k$ for Asp

Table 1
Release exponent (n), rate constant ($\log k$), and correlation coefficient (R^2) for PVA/micelle DDDS; $T = 37\ ^\circ C$.

Drug	pH	$\log k$	n	R^2
Asp	4.0	1.55	0.58	0.99
	5.5	1.56	0.53	0.99
	7.4	1.54	0.56	0.99
	8.4	1.58	0.53	0.99
DOX	4.0	1.07	0.43	0.99
	5.5	0.91	0.48	0.99
	7.4	0.81	0.48	0.98
	8.4	0.72	0.46	0.99

Table 2
Release exponent (n), rate constant ($\log k$), and correlation coefficient (R^2) for CS/PVA/micelle DDDS (CS/PVA, w/w = 1/3); $T = 37\ ^\circ C$.

Drug	pH	$\log k$	n	R^2
Asp	4.0	1.14	0.69	0.99
	5.5	1.38	0.61	0.99
	7.4	1.49	0.66	0.99
	8.4	1.67	0.65	0.99
DOX	4.0	0.71	0.53	0.96
	5.5	0.64	0.54	0.97
	7.4	0.59	0.55	0.95
	8.4	0.53	0.52	0.94

are almost the same at all four pH values, while the values of $\log k$ for DOX decrease when pH is increased. This indicates that the release rate of Asp has no pH sensitivity and the release of DOX decreases with increasing pH.

Values of n at various pH values in the CS/PVA/micelle DDDS (CS/PVA, $w/w = 1/3$) at 37 °C are shown in Table 2. The values of n for Asp and DOX both increase obviously in comparison with the PVA/micelle DDDS. The values of n for Asp are much greater than 0.45 and n for DOX turns to be greater than 0.45. These results indicate that the release behavior of Asp tends to be swelling-controlled, while DOX changes from Fickian diffusion to anomalous transportation when introducing the CS component. It is mainly because CS/PVA hydrogel is environmentally sensitive and has a stronger influence on drug release comparing with the PVA/micelle DDDS. Table 2 also shows that the value of $\log k$ for Asp increases while $\log k$ for DOX decreases with increasing pH, suggesting that the release rate of Asp increases while the DOX release rate decreases when pH is increased.

In the present work, to the best of our knowledge, we report a first example of dual-drug delivery systems which are based on hydrogel/micelle composites. Such delivery systems display some unique advantages for clinical applications: 1) combined drug delivery: the dual-drug delivery system can overcome the poor therapeutic effect of single drug delivery system; 2) independent drug release: in our research, the required independent different release period of each drug in the DDDS is obtained by the designed hydrogel/micelle structure; 3) targeting effect: in PVA/CS/GPG micelle DDDS, the release behavior of DOX is pH and temperature controlled and the release behavior of Asp is pH controlled; 4) convenience: the hydrogel/micelle DDDS is easy to prepare and the release behavior of each drug can be easily tuned by choosing different kinds of the hydrogels or micelles. We anticipate that this hydrogel/polypeptide micelle system can be a promising candidate as dual-drug carrier with controlled release behavior of each drug in the application of combined therapy.

4. Conclusions

We prepared a dual-drug delivery system (DDDS) from hydrogel/polypeptide micelle composites. The resulting structure of the DDDS is hydrogel network containing GPG micelles. Asp is dissolved directly in the water phase of hydrogel, while DOX is encapsulated in GPG micelles. Different release behaviors of the two drugs in DDDS are achieved. Asp shows a short-term release while DOX shows a sustained long-term release in all the DDDSs. Moreover, the release behavior of DOX shows pH and temperature sensitivity, which is attributed to the environmental sensitivity of GPG micelles. The release rate of DOX from the DDDS is significantly accelerated by decreasing pH or increasing temperature. With adding CS to the system, the pH controlled release behavior of Asp can be observed. The obtained release profiles are well fitted by the classical empirical power law. According to the release exponent n, the release of DOX follows Fickian diffusion and Asp follows anomalous transport in PVA/micelle DDDS. However, the release of DOX changes to anomalous transport in CS/PVA/micelle DDDS. The results gained through both experiments and theoretical analysis reveal that the release behavior of Asp can be tuned by modifying the hydrogels, and the release behavior of DOX is mainly controlled by the micelles in the DDDS.

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