Journal of Solid State Chemistry 183 (2010) 1811–1816

Contents lists available at ScienceDirect

Journal of Solid State Chemistry

journal homepage: www.elsevier.com/locate/jssc

Synthesis and release behavior of composites of camptothecin and layered double hydroxide

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ARTICLE INFO

Article history:
Received 2 December 2009
Received in revised form
25 May 2010
Accepted 30 May 2010
Available online 8 June 2010

Keywords:
Camptothecin
Layered double hydroxides
Nanohybrids
Controlled release
Composites

ABSTRACT

A simple method, reconstruction of calcinated layered double hydroxides (CLDH) in an organic (ethanol)–water mixed solvent medium containing drug, was developed to intercalate partially a non-ionic and poorly water-soluble drug (camptothecin) into the gallery of layered double hydroxides (LDHs) to form the drug–LDH composites. The purpose of choosing organic–water mixed solvent is to increase remarkably the solubility of camptothecin (CPT) in the reconstruction medium. A probable morphology of CPT molecules in the gallery of LDHs is that CPT molecules arrange as monolayer with the long axis parallel to the LDH layers. The *in vitro* drug release from the composites was remarkably lower than that from the corresponding physical mixture, which shows these drug–inorganic composites can be used as a potential drug delivery system.

1. Introduction

Layered double hydroxides (LDHs), or the so-called hydrotalcite-like compounds (HTlc), are a family of layered inorganic materials with structurally positively charged layers and interlayer balancing anions [1,2]. LDHs may be represented by the general formula $[M^{II}_{(1-x)}M^{III}_x(OH)_2][A^{n-}]_{x/n}\cdot mH_2O$, where M^{II} is a divalent cation, M^{III} is a trivalent cation, A^{n-} is a gallery anion, x is the molar ratio of $M^{III}/(M^{II}+M^{III})$, and m is the number of moles of co-intercalated water per formula weight of the compound. The interlayer region (or gallery) of LDHs may be considered as a microvessel in which drug molecules may be stored, *i.e.*, some drug molecules may be intercalated into the gallery of LDHs to form drug-LDHs nanohybrids [3–14]. The drug-LDHs nanohybrids may evidently inhibit the release of drug molecules stored in LDHs; therefore, LDHs may be potentially used in drug controlled-release systems.

Choy et al. [3] first developed the DNA-LDHs nanohybrids for efficient gene delivery, and it was shown that the DNA molecules could be easily intercalated into LDHs by anion exchange. To date, many methods for synthesis of drug-LDHs nanohybrids were developed, such as coprecipitation [11], ion exchange [3,4] and reconstruction [15]. For anionic drugs, the nanohybrids are usually synthesized simply by the above mentioned methods, while for charge-neutral and poorly water-soluble drugs, it is usually needed to modify LDHs with surfactants to form a hydrophobic region in the gallery of LDHs, and then target drug molecules are intercalated into the hydrophobic region of LDHs. Tyner et al. [9] developed a novel method to synthesize charge-neutral and poorly water-soluble drugs (camptothecin)-LDHs nanohybrids; camptothecin was first incorporated into micelles derived from negatively charged surfactants, and the negatively charged micelles were then encapsulated in nanoparticles of Mg–Al LDHs by an ion exchange process.

In this present study, a simple method, reconstruction in a mixed organic–water solvent medium containing camptothecin (CPT), was used to synthesize CPT–LDHs composites. Camptothecin is a typical model of charge-neutral and poorly water-soluble drugs, and shows significant antitumor activity in nude mice bearing human lung, ovarian, breast, pancreas and stomach cancers [16]. We found the intercalation of CPT into the gallery of Mg–Al LDHs was not available using routine coprecipitation, ion exchange and reconstruction methods in water medium, which may be because of the too low solubility of CPT in water. First CPT was dissolved in NaOH solution, it was found that the CPT–LDHs nanohybrids might be synthesized by coprecipitation method with the co-precipitant of NaOH solution containing CPT [14]. A disadvantage of this method is that the lactone ring (E-ring) of CPT is unstable in the strong basic medium (NaOH

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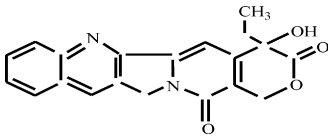
doi:10.1016/j.jssc.2010.05.035

solution) and may undergo spontaneous hydrolysis to yield the inactive carboxylate form [17,18]. Malherbe et al. [19] had reported that calcinated LDHs (CLDH) might be reconstructed in a series of organic–water media to form LDHs with modified surface and porosity properties. In this study, the ethanol–water mixed solvent containing CPT was chosen for reconstruction medium of CLDH to synthesize CPT–LDHs nanohybrids, this is because CPT has an remarkably high solubility in ethanol–water mixture. It was found that Mg–Al CLDH immersed in ethanol–water mixed solvent containing CPT could induce successful intercalation of CPT into the gallery of LDH through the reconstruction process, showing a good CPT loading amount.

2. Experimental

2.1. Materials

CPT (99.3% purity) was purchased from Chinese Huangshi Feiyun Pharmaceutical Co. Ltd. and used as received. Its structure is



The other chemicals and solvents of analytical grade were purchased from Chinese National Medicines Co. Ltd. and used without further purification. Deionized water from which carbon dioxide was removed by boiling under nitrogen was used in all experiments.

2.2. Preparation of CPT–LDH composites

2.2.1. Preparation of pristine LDH and calcinated LDH

The pristine Mg–Al–Cl LDH was prepared by coprecipitation method from a mixed solution of magnesium and aluminum chloride hexahydrates. Under a N_2 atmosphere, the mixed salt solution of $MgCl_2 \cdot 6H_2O/AlCl_3 \cdot 6H_2O$ at a 2:1 molar ratio was prepared with a total metal ion concentration of $\sim$0.5 mol L^{-1}. Then the coprecipitation agent, diluted ammonia water (6 wt%), was added to the mixed salt solution under stirring at a speed of 25 mL/min till the final pH value reached 9.5. The precipitate was aged for 1.5 h in the mother solution at room temperature and then filtered and washed with deionized water to remove NH_4Cl and the excess ammonia. The filter cake held in a glass bottle was peptized at a constant temperature of 80 °C in an oven for about 24 h, to obtain Mg–Al–Cl LDH sol sample.

The LDH sol sample obtained was first dried at about 105 °C in an oven, and then the dried LDH sample was calcinated at 500 °C for 5 h in Muffle furnace to obtain the calcinated LDH (CLDH) sample [15].

The metal contents of the dried LDH sample were determined by inductively coupled plasma mass spectrometry (ICP-MS), and the content of carbon element was analyzed with a desert analytics instrument; meanwhile Cl^- was analyzed by chloride ion-selective electrode. The results of elemental analyses showed that the chemical composition of the pristine LDH sample might be expressed as $[Mg_{0.64}Al_{0.36}(OH)_2]\ Cl_{0.15}\ (OH)_{0.21}$.

2.2.2. Preparation of CPT–LDH composites

0.025 g of the CLDH sample was dispersed in 50 mL of ethanol–water solution with a desired volume ratio (R_v) of ethanol/water containing a known concentration of CPT. This mixture was stirred for a desired time at a desired temperature. After that, the mixture was centrifuged and then the precipitate was washed with distilled water once and then with ethanol twice, and finally dried at 60 °C in an oven to obtain CPT–LDH composite sample.

2.3. Characterization

Powder X-ray diffraction (PXRD) patterns were obtained on a D/max-rA model diffractometer with Cu$K\alpha$ radiation (40 kV and 80 mA). The morphologies of the products were observed using a JEM-100cxII model transmission electron microscope (TEM).

2.4. Determination of camptothecin loading

The amount of CPT loaded into the composites (A_{in}) was determined by UV–vis spectroscopy using the following method [9]. A known weight of the composite sample was placed in a 10 mL volumetric test tube. 5 mL of 1 mol L^{-1} HCl was added to dissolve the inorganic layers and then ethanol was used to fill the balance. Then the concentration of CPT in solution was determined by monitoring the absorbance at λ_{max}=370 nm with an SP-1105 model UV–vis absorption spectrophotometer, and the concentration was calculated by regression analysis according to the standard curve obtained from a series of standard solution of CPT. The final value was an average of measurements of three parallel samples. The A_{in} value was obtained according to the concentration of CPT in the solution and the used weight of the composite sample.

2.5. Determination of release rate

The drug release examinations were performed at 37 °C in pH 4.8 and 7.2 buffer solution (0.1 M). The phosphate–citrate buffer solution was used to achieve a pH 4.8 environment, and phosphate to get a pH 7.2 environment. 2.5 mg of the composite sample was placed into 250 mL of either a pH 4.8 or pH 7.2 buffer solution and the suspensions were stirred at 37 °C. 4 mL of solutions was withdrawn at predetermined time intervals and filtered through a 0.45-μm syringe filter. The absorbances were measured at λ_{max}=370 nm by UV–vis absorption spectrophotometer to obtain the CPT concentrations of solutions, in turn to calculate the release amounts (q_t) and the percent releases (X_t) of CPT from the composite. The tests were made in triplicate and the final values were an average of measurements. The percent release values of CPT from the composites were plotted versus time (t) to examine the release rates of the drug from the controlled release system.

To compare the release rate of CPT from the composites with that from the physical mixture of CPT and the pristine LDH, 2.5 mg of the physical mixture of CPT (0.2 mg) and the pristine LDH (2.3 mg) replaced the composite sample to perform the same drug release experiments.

3. Results and discussion

3.1. The amount of CPT loaded into CPT–LDH composites

The effects of the volume ratio (R_v) of ethanol to water, initial CPT concentration (C_{CPT}), temperature and contact time on the

amount of CPT loaded into CPT–LDH composites (A_{in}) in the reconstruction process were investigated.

Fig. 1 shows the effects of contact time and temperature on the A_{in} value at C_{CPT}=200 μg/mL and R_v=7:3. It can be seen from Fig. 1 that within the initial 10 h, the A_{in} value increases rapidly with the increase of contact time; after 24 h the A_{in} value reaches a plateau, *i.e.*, an intercalation equilibrium is obtained. With the increase in temperature from 25 to 40 °C, the A_{in} value increases obviously; meanwhile further increasing the temperature to 60 °C induces a very little increase of the A_{in} value.

Fig. 2 shows the effects of C_{CPT} and R_v on the A_{in} value at 60 °C. It can be seen from Fig. 2 that the A_{in} values increase gradually with the increase of C_{CPT} from 20 to 200 μg/mL, which is in accordance with the case in the similar reconstruction process [15]. Among the three R_v values (1:0, 7:3 and 1:1), the maximum A_{in} value (about 14%) was obtained at R_v=7:3 under the same C_{CPT} value; the increase or decrease of R_v value all induced the decrease of the A_{in} value, which might be relative to the maximum CPT solubility (200 μg/ml) at R_v=7:3 among the ethanol–water solutions with three R_v values studied.

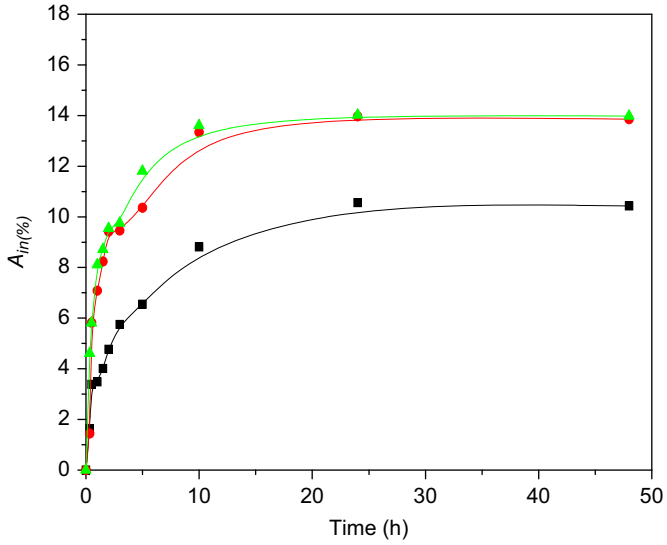


Fig. 1. Effects of contact time and temperature in reconstruction process of CLDH on the amount of CPT loaded into CPT–LDH composite at R_v=7:3 and C_{CPT}=200 μg/mL. (■) room temperature, (●) 40 °C, (▲) 60 °C.

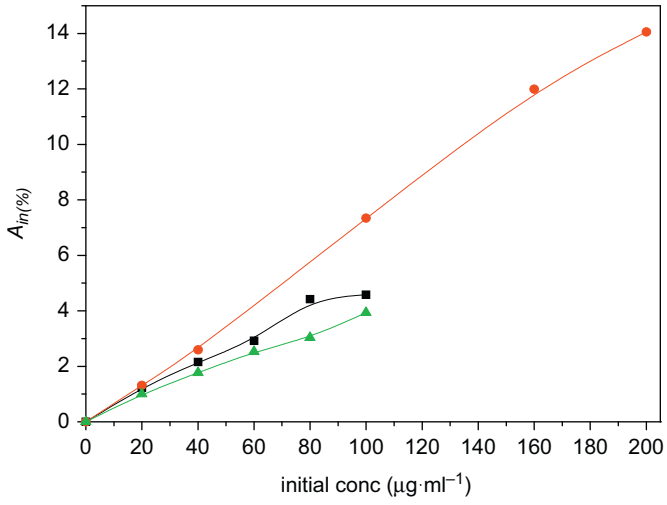


Fig. 2. Effects of initial CPT concentration and the ethanol/water volume ratio in reconstruction process of CLDH on the amount of CPT loaded into CPT–LDH composite at 60 °C. (■) R_v=1:1, (●) R_v=7:3, (▲) R_v=1:0.

3.2. Characterization of CPT–LDH composites

The PXRD patterns of the pristine LDH, CLDH and CPT–LDH composite with A_{in}=14% are shown in Fig. 3. The PXRD patterns of the pristine LDH sample (Fig. 3B) exhibit characteristic diffractions of hydrotalcite (JCPDS card No. 51-1528), indicating the pristine LDH sample has a well-crystallized structure. The basal spacing (d_{003}) of 0.78 nm corresponding to Cl^- ions between the layers was expected. The PXRD patterns of the CLDH sample (Fig. 3A) only show two peaks corresponding to MgO diffractions [15], indicating the layered structure of the pristine LDH has been completely destroyed during the calcinations and it seems to be converted to a mixture of crystalline MgO and amorphous Al_2O_3. When the CLDH sample was dispersed into the ethanol–water solution containing CPT, the layered structure of the sample was reconstructed (Fig. 3C). However, the peaks were broadened and their intensities decreased in comparison with those of the pristine LDH, indicating some reductions in crystallinity following the calcination and re-hydration or intercalation of CPT. After the reconstruction, the basal spacing (d_{003}) was increased to 0.81 nm. The increase of the basal spacing indicates that some CPT molecules were indeed intercalated into the gallery of LDH. Given that the thickness of the brucite-like layer of LDHs is about 0.48 nm [1], the gallery height of the LDH intercalated by CPT is about 0.33 nm. The length, breadth and height of the CPT molecule calculated by the method of molecular mechanics [20] are about 1.4, 0.72 and 0.34 nm, respectively. According to the size of CPT molecule and the gallery height of the composite, a probable morphology of CPT molecules in the gallery of LDHs may be proposed, *i.e.*, CPT molecules arrange as monolayer with the long axis parallel to the LDH layers. These PXRD results of the CPT–LDH composite obtained by reconstruction method are different from those of the CPT–LDH composite obtained by the coprecipitation method with co-precipitant NaOH solution containing CPT [14]. For the CPT–LDH composite obtained by coprecipitation method, it was observed that the 003 basal reflection pattern of d_{003}=0.88 nm corresponding to pristine Mg–Al–NO_3 LDH was divided into two patterns with d_{003}=3.49

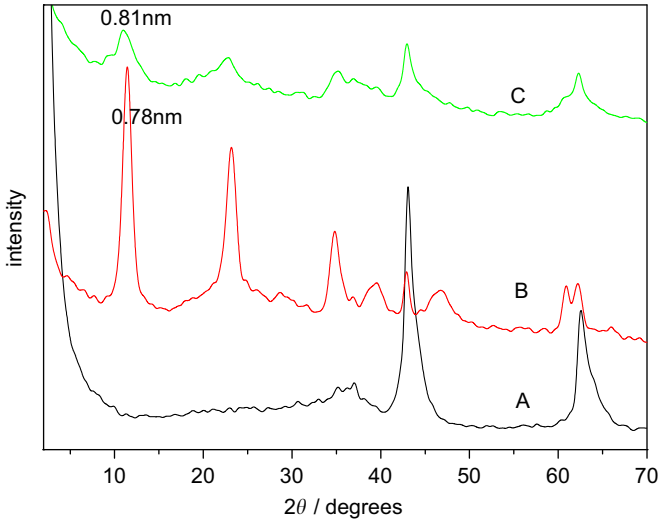


Fig. 3. PXRD patterns of (A) CLDH, (B) pristine LDH and (C) CPT–LDHs composite with A_{in}=14%.

and 0.79 nm, respectively, by the intercalation of CPT, and a probable morphology of CPT molecules in the CPT–LDH composite was proposed that the horizontal-arranged monolayer and vertical-arranged bilayer of CPT molecules coexisted in the interlayer region of LDH [14]. The reason why non-ionic CPT molecules could be stabilized in the gallery of LDHs is perhaps because of the hydrogen bonds between hydrogen of hydroxyl OH, belonging to the brucite-type layers, and oxygen or nitrogen of the CPT molecules intercalated.

The FT-IR spectra of pristine LDHs, CPT–LDH composite and CPT are shown in Fig. 4. As can be seen, the three characteristic peaks at 1655 (stretching vibration of C=O ketone group), 1591 and 1497 cm^{-1} (skeletal vibrations of phenyl rings) of CPT appear in the FT-IR spectrum of the CPT–LDH composite, indicating that CPT molecules were loaded on LDHs. However, the characteristic peak at 1740 cm^{-1} corresponding to C=O stretching vibration of lactone ring disappears in the FT-IR spectrum of the CPT–LDH nanohybrid; maybe this is because the lactone ring of CPT molecule undergoes spontaneous hydrolysis to yield the carboxylate form [17,18]. In addition, an intense absorption band at about 1364 cm^{-1} may be assigned to the υ_3 mode asymmetric stretching of CO_3^{2-} in the interlayer, indicating that some CO_3^{2-} ions existed in the gallery of the LDH sample. However, the content of carbonate in the sample was so little that no carbon was detected in C elemental analyses.

It is needed to note that maybe not all CPT molecules loaded on LDHs were intercalated into the gallery of LDHs, but only part of them was intercalated into the gallery of LDHs and another part was adsorbed on the surface of LDHs. However, we cannot yet distinguish the relative amount of the above two morphologies of CPT molecules according to our information available.

Fig. 5 shows the TEM images of the pristine LDH and CPT–LDH composite. It can be seen that the pristine LDH consisted of the typical thin, hexagonal plate-like crystals with 100–200 nm in size (Fig. 5a), while the composite showed very thin crimpled pieces (Fig. 5b), which is similar to the TEM image of glycine intercalated LDHs shown in the previous literature [21]. The obvious aggregation may arise from the intercalation of CPT molecules.

3.3. Camptothecin release from CPT–LDH composites

The release profiles of CPT from the CPT–LDH composite with A_{in}=14% and the physical mixture of CPT and pristine LDH are shown in Fig. 6. It can be seen from Fig. 6 that the physical mixture of CPT and pristine LDH exposed to either a pH 4.8 or pH 7.2 environment released CPT quickly, the release being complete within 10 min. The release rate of CPT from the composite is obviously lower than that from the physical mixture, indicating

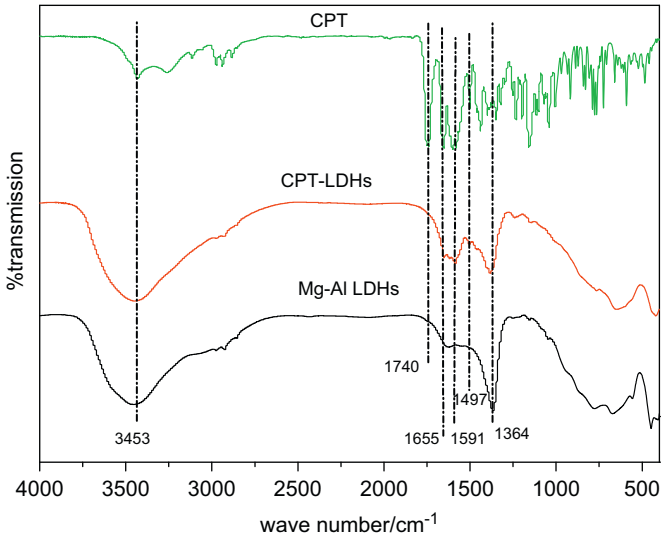


Fig. 4. FT-IR spectra of pristine LDHs, CPT–LDH composite and CPT.

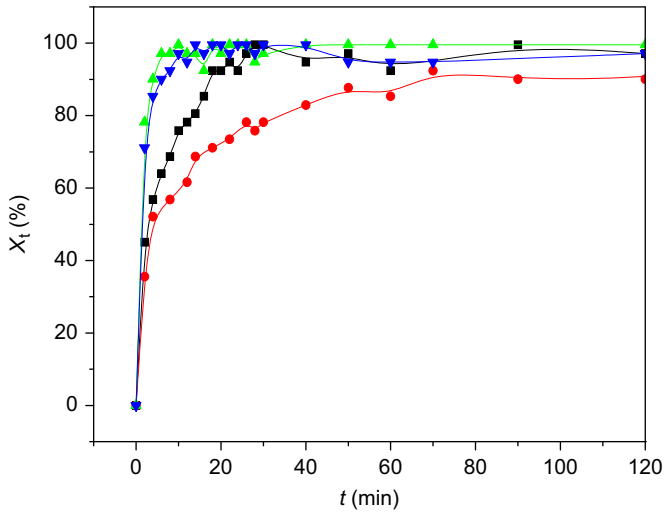


Fig. 6. Release profiles for CPT from the CPT–LDH composite with A_{in}=14% and the physical mixture at pH 4.8 and pH 7.2. (■) composite, pH=4.8; (●) composite, pH=7.2; (▲) physical mixture, pH=4.8; (▼) physical mixture, pH=7.2.

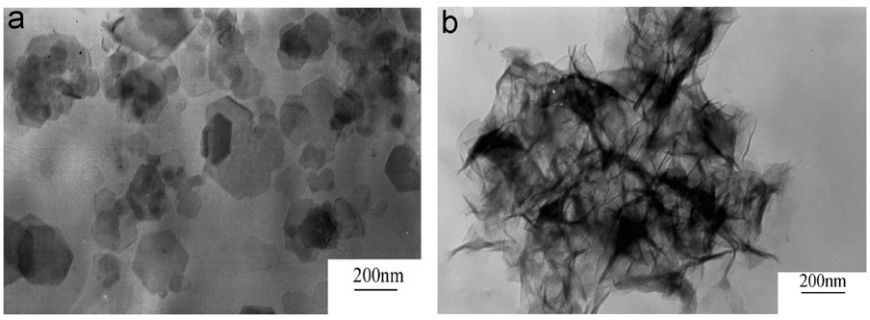


Fig. 5. TEM images of (a) pristine LDH and (b) CPT–LDH composite with A_{in}=14%.

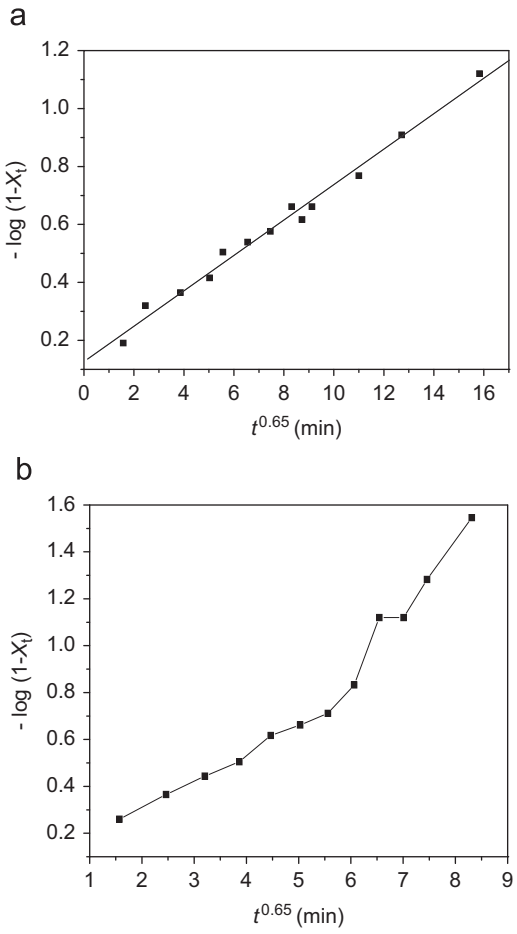


Fig. 7. Release of CPT from the CPT–LDH composite with A_{in}=14% as a function of $t^{0.65}$ at pH 7.2 (a) and pH 4.8 (b).

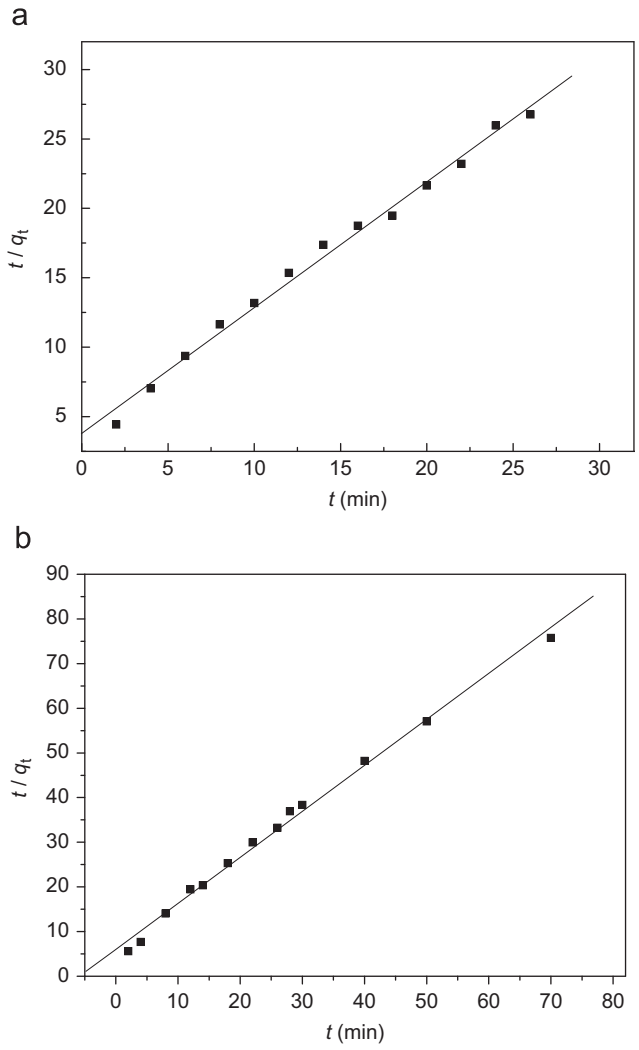


Fig. 8. Linear regression curves of release data fitting with pseudo-second-order kinetic model at pH 4.8 (a) and pH 7.2 (b).

that the CPT–LDH composites are a potential drug controlled release system, which may be attributed to the restricted motion of CPT molecules arising from steric effect of LDH and the hydrogen bonds between layers of LDHs and CPT molecules intercalated.

In addition, the release rate of CPT from the composite is obviously dependent on pH, and the release rate at pH 7.2 is remarkably lower than that at pH 4.8. The percent release of CPT from the composite reaches about 100% within about 40 min when exposed to a pH 4.8 environment; meanwhile it reaches only about 90% within about 70 min when exposed to a pH 7.2 environment. The time taken for 100% of CPT to be released from the composite at pH 7.2 is longer over 120 min. Such a discrepancy of the release rate at pH 4.8 and pH 7.2 may be due to a possible difference in mechanism for the release of CPT from the composite [9]. At acidic pHs, LDHs may be dissolved. This would indicate that the release of the interlayer molecules might occur through the removal of LDHs layers. At and above pH 7, LDHs should be more stable, and as a result, release would occur primarily through a diffusion out of drug in the gallery of LDHs. That is to say, the mechanism of release in the pH 4.8 environment should be through both the dissolution of LDH layers and the diffusion; however for the pH 7.2 release, the mechanism should be primarily through the diffusion.

As the drug release is due to the diffusion, once the drug molecules diffuse through the LDH particle and then through the diffusion layer. Thus the drug release could be controlled by the diffusion through the LDH particle, or by the diffusion through the solution layer surrounding the particle. The release rate of drug molecules would be determined by the slower step of these two processes [22]. Bhaskar et al. [22] developed a simple procedure to establish whether the diffusion through the particle was the rate limiting step. For a particle diffusion-controlled release, Bhaskar et al. [22] obtained the following equation:

$$\ln(1-X_t) = -1.59(6/d_p)^{1.3} D^{0.65} t^{0.65}$$

where d_p is particle diameter, and D is the diffusivity. This suggests that particle diffusion control can be tested by simply testing for linearity between $\log(1-X_t)$ and $t^{0.65}$. This method was applied to the experimental data, and a good linear relationship (correlation coefficient r^2=0.9936) was obtained for the pH 7.2 release (see Fig. 7a), while in the pH 4.8 environment such a line cannot be obtained (see Fig. 7b), indicating for the pH 7.2 release the diffusion through the LDH particle is the rate limiting step. Similar results were obtained for the CPT–LDH composite synthesized by the coprecipitation method with co-precipitant NaOH solution containing CPT [14].

3.4. Release kinetics of CPT from CPT–LDH composites

Usually, the release process of drug molecules from drug-LDH composites may be described with pseudo-first-order kinetic or pseudo-second-order kinetic equations.

Pseudo-first-order kinetic equation may be represented in the linear form as

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (1)$$

where q_e and q_t are the equilibrium release amount and the release amount at any time (t), respectively, and k_1 is the rate constant of pseudo-first-order release kinetics. If the pseudo-first-order kinetics is applicable, the plot of $\ln(q_e - q_t)$ vs. t will be linear, and the k_1 value can be obtained from the slope of the linear plot.

Pseudo-second-order kinetic equation may be represented in the linear form as

$$t/q_t = 1/(k_2 q_e^2) + t/q_e \quad (2)$$

where k_2 is the rate constant of pseudo-second-order release kinetics. If the pseudo-second-order kinetics is applicable, the plot of t/q_t vs. t will be linear, which allows computation of k_2.

With the simulation of the above two kinetic models for the release kinetic data, it was found that the pseudo-second-order model is more satisfactory for describing the release kinetic processes of CPT from the CPT–LDH composites. Fig. 8 shows the plots of t/q_t vs. t for the release of CPT at pH 4.8 and 7.2 environments, respectively, and as can be seen, fair straight lines were obtained. For the pH 4.8 release, the correlation coefficient (r^2) and k_2 values are 0.9961 and 0.26 min^{-1}, respectively, and for the pH 7.2 release, they are 0.9970 and 0.16 min^{-1}, respectively. This release kinetic result is not similar to the CPT–LDH composite synthesized by the coprecipitation method with co-precipitant NaOH solution containing CPT, for which it was found that the pseudo-first-order model is more satisfactory [14].

4. Conclusions

The ethanol–water mixed solvent containing drug was chosen for reconstruction medium, the non-ionic and poorly water-soluble drug, camptothecin (CPT), was successfully intercalated into the gallery of the LDH to obtain the CPT–LDH composites by reconstruction of CLDH. The purpose of choosing organic–water mixed solvent is to increase remarkably the solubility of CPT in the reconstruction medium. A probable morphology of CPT molecules in the gallery of LDHs is that CPT molecules arrange as monolayer with the long axis parallel to the LDH layers. The amount of CPT loaded into CPT–LDH composites (A_{in}) is dependent on the volume ratio (R_v) of ethanol to water, initial CPT concentration (C_{CPT}), temperature and contact time in the reconstruction process. The maximum A_{in} value may reach about 14% (w/w) under studied conditions. The release rate of CPT from the composites was obviously lower than that from the physical mixture of CPT and the pristine LDH sample at either a pH 4.8 or 7.2 environment, showing the CPT–LDH composites might be a potential drug controlled release system. In addition, the release rate of CPT from the composite at pH 7.2 is remarkably lower than that at pH 4.8, which is due to a possible difference in the release mechanism. For the pH 7.2 release, the mechanism is primarily through diffusion; however for the pH 4.8 release, it is through both the dissolution of LDH layers and diffusion. Kinetic analysis showed that the release kinetics of CPT from the composites obeyed the pseudo-second-order kinetic model.

Acknowledgment

This research was supported by the National Natural Science Foundation of China (No. 50772062) and the Natural Science Foundation of Shandong Province of China (No. Z2008B08) and Taishan Scholar Foundation of Shandong Province of China (No. ts20070713).

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