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ScienceDirect
Trans. Nonferrous Met. Soc. China 19(2009) 1232−1236
Transactions of Nonferrous Metals Society of China
www.tnmsc.cn

Preparation and drug releasing property of magnetic chitosan-5-fluorouracil nano-particles

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Received 5 January 2009; accepted 4 August 2009

Abstract: In order to synthesize the targeting drug carrier system, magnetic chitosan-5-fluorouracil nano-particles were prepared by using 5-fluorouracil (5-Fu) as model drug, Fe_3O_4 nano-particles as kernel, chitosan as enveloping material and glutaraldehyde as cross linking agent through ultrasonic technique. The morphology of the magnetic chitosan-5-Fu nano-particles was observed with a transmission electron microscope(TEM). The results showed that magnetic chitosan-5-Fu nano-particles were prepared in spherical structure with a size range of 50−60 nm. The delivering capacity and drug releasing properties of magnetic chitosan-5-Fu nano-particles were investigated by UV-vis spectrum analysis. The results showed that the loading capacity was 13.4% and the cumulative release percentage in the phosphate buffer (pH=7.2) solutions was 68% in 30 h. These data indicate that the wrapped drug of magnetic chitosan-5-Fu nano-particles was slowly-released. The magnetic response of magnetic chitosan-5-Fu nano-particles was studied by UV-vis spectrometer to detect the changes of solution absorbance. Without external magnetic field, the nano-particle deposition rate was slow. When being subjected to 8 mT magnetic field, the particle sedimentation rate was increased rapidly. The results showed that magnetic chitosan-5-Fu nano-particles have a magnetic stability and strong targeting characteristics.
Key words: magnetism; chitosan; 5-fluorouracil; nano-particle; drug release

1 Introduction

5-fluorouracil(5-Fu) is an anti-cancer drug with a broad spectrum. However, its clinical application is limited because of its toxicity[1]. Paramagnetic or super magnetic Fe_3O_4 nano-particles under the effect of the external magnetic field can kill tumor cells when the temperature rises to 40−45 ℃[2]. Drug and an appropriate magnetic material can be assembled into controlled-released system of magnetic nano-particles in the blood vessels. Under the external magnetic field, it can carry the drug to a special target site and the drug can be controlled released on that spot, so that the drug is locally concentrated in the target area. Because the targeted therapy has the characteristics of driven magnetic accuracy, targeting, and high drug-capacity, it can be effective to lower toxic effects and to enhance the therapeutic effect[3−6]. Thus, it has been the focus of research and exploitation at present. The preparation of carrier material brings a large number of chemotherapeutic drugs and target-oriented, slowly-released drug is the key of new type of magnetic targeting drugs[7−8]. However, most of magnetic microsphere treatments used currently are not satisfied, because they have small magnetic flux, poor positioning, less carrying capacity, short drug time, and microsphere particles are too large to release drug, resulting in less application in the human body[9−10]. The preparation of targeting drug with high magnetic flux, large drug

Foundation item: Projects(30572455, 30670990) supported by the National Natural Science Foundation of China; Project(20060390891) supported by Postdoctor Foundation of China; Project(NCET-06-0685) supported by the Program of New Century Excellent Talent in University of Ministry of Education of China; Project(2006FJ4243) supported by Science and Technology of Hunan Province, China
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DOI: 10.1016/S1003-6326(08)60434-3

loading with small size enough has become the hot spot in current studies.

Chitosan(CS) has a good biocompatibility, and is commonly used as a drug carrier[11−13]. The present study was attempted to prepare a new targeting drug carrier system by using ultrasound technology, in which chitosan was used as enveloping material, 5-fluorouracil (5-Fu) as model drug, Fe_3O_4 nano-particles as kernel, and glutaraldehyde as cross linking agent. In addition, the morphology, carried capacity and drug releasing property of this system were determined.

2 Experimental

2.1 Instrument and reagents

Chitosan (relative molecular mass 1.38×10^6 measured with the viscosity method; degree of deacetylation 90%), Fe_3O_4 nano-particles (8−12 nm, self-made), 5-fluorouracil (Shanghai Pharmaceutical Factory, China), hydrochloric acid (AR), glutaric aldehyde (AR), and distilled water were used in this experiment.

Transmission electron microscope (H−600, Japan), laser diffraction particle size analyzer (MASTERSIZER 2000, USA), UV-visi spectrometer (UV−2100, Japan), and ultrasonic cleaner (China) were used.

2.2 Preparation of Fe_3O_4 nano-particles

According to Ref.[14], Fe_3O_4 nano-particles with the size of 8−12 nm were prepared. The preliminary studies showed that they had good magnetic properties.

2.3 Preparation of magnetic chitosan-5-fluorouracil nano-particles

0.1 g (dry mass) Fe_3O_4 nano-particle mud paste, 0.1 g 5-fluorouracil and 100 mL 0.1% chitosan solution (containing 1% acetic acid) were mixed for 1 h with intense stirring, and then the mixture was installed in the drip funnel.

In a 250 mL three-neck flask, 80 mL olive oil and a small amount of anionic dispersing agent were added under ultrasound; then the above mixture was slowly dripped under severe agitation; at last 1 mL 50% glutaraldehyde was added and reacted at 60−90 ℃ for 1−2 h. After holding for 12 h, the precipitates of nano-particles were washed by petroleum ether, water and ethanol, then magnetically separated and purified.

2.4 Size and morphology of magnetic chitosan-5-fluorouracil nano-particles

Transmission electron microscopy(TEM) was used for observation of the size and morphology of magnetic chitosan-5-Fu nano-particles. The obtained nano-particles were diluted with ethanol, then vibrated with ultrasound for 30 min, and dripped on the carbon-coated copper for TEM observation.

2.5 Determination of drug load rate and package rate

5-fluorouracil (10 mg) was dissolved in 0.1 mol/L HCl and diluted to 100 mL. 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 mL solution were used and diluted to 25 mL, respectively. UV-vis spectra were used to measure 265 nm absorbance frequency for the standard curve.

The content of 5-Fu in the magnetic chitosan-5-fluorouracil nanoparticles was measured by spectroscopy. Because 5-Fu has a maximum absorption at 265 nm, and chitosan and hydrochloric acid do not, the content of 5-Fu can be calculated. The test process is as follows. Magnetic chitosan-5-Fu microspheres were ground, added with 100 mL 0.1 mol/L hydrochloric acid, then heated. After refluxing for 8 h, the microspheres were dissolved. The solution was diluted in a 250 mL flask. Then, 2 mL solution was taken out and diluted 100 times for UV-vis spectrometer measuring, and compared with the standard content curve. The formulae for calculating the drug load rate(R_l) and drug package rate(R_p) are as follows:

$$R_l=m_c/m_t\times100\% \quad (1)$$

$$R_p=m_c/m_0\times100\% \quad (2)$$

where m_0 is the total mass of drugs, m_c is the mass of 5-fluorouracil on the microsphere, and m_t is the total mass for the chitosan microspheres, 5-fluorouracil and Fe_3O_4.

2.6 Determination of drug releasing curve

10 mg magnetic chitosan-5-Fu nano-particles was put into a d1 cm dialysis bag, was added with 10 mL, pH 7.2 phosphate buffer solution, then was kept at temperature of (37.0±0.5) ℃. The oscillation frequency was 1.33 Hz. 5 mL solution was regularly checked for UV absorbance and at the same time fresh buffer solution was added. The cumulative release rate—time curve was measured as mentioned above.

3 Results and discussion

3.1 Morphology of magnetic chitosan-5-fluorouracil nano-particles

The morphology of magnetic chitosan-5-fluorouracil nano-particles is shown in Fig.1.

From Fig.1, the magnetic chitosan-5-fluorouracil nano-particles are regularly spherical, and the range of particle size is from 50 to 60 nm.

The laser diffraction analysis result showing the particle-size distribution is shown in Fig.2. The average size of the chitosan-5-fluorouracil nano-particles is 60 nm from Fig.2. It is closed to the result of the electron microscope observation.

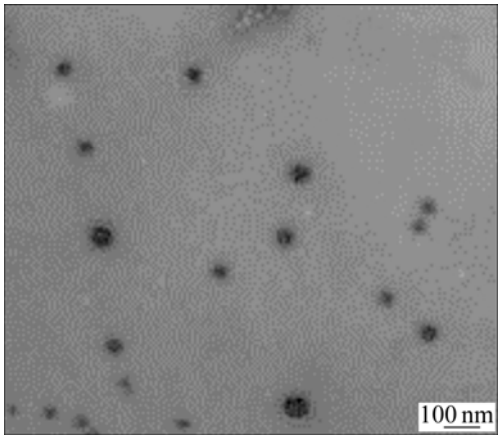

Fig.1 TEM image of magnetic chitosan-5-fluorouracil nano-particles

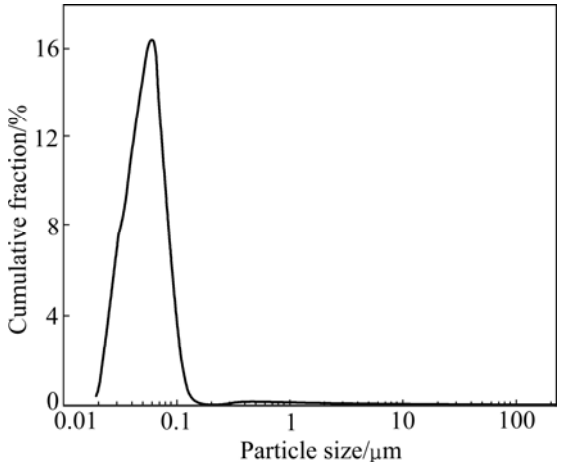

Fig.2 Particle-size distribution of magnetic chitosan-5-fluorouracil nano-particles

3.2 Drug load rate and package rate

The relationship of 5-fluorouracil concentration and extinction specification was analyzed by linear regression as follows:

C=33.47A−0.436

where A is the extinction value, and C is mass concentration of 5-fluorouracil (μg/mL).

By calculating the above equation, the drug load rate of chitosan-5-fluorouracil nano-particles is 13.4%, while the drug package rate is 25.8.%.

3.3 Drug releasing property in phosphate buffer solution

The comparison of 5-Fu release condition between 5-Fu and magnetic chitosan-5-fluorouracil nano-particles is shown in Fig.3.

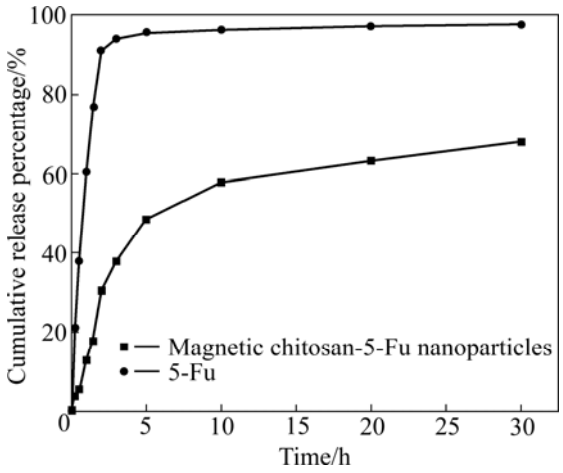

Fig.3 5-Fu release curve in 5-Fu and in magnetic chitosan-5-fluorouracil nano-particles (medium is phosphate buffer solution, pH=7.2)

As seen in Fig.3, the basic release of 5-Fu is finished in 2 h. Magnetic chitosan-5-fluorouracil nano-particles also have a quick release within 5 h and the whole releasing quantity is achieved to be about 48.6%. But the release rate becomes more and more slowly, and the release rate is about 68% in 30 h (32% is still remained). Thus, these data indicate that the magnetic chitosan-5-fluorouracil nano-particles have a better releasing performance. Both of 5-Fu and magnetic chitosan-5-fluorouracil nano-particle have a quicker release in the beginning and a slower release in the late releasing process. In contrast to 5-Fu, the release speed of 5-fluorouracil from magnetic chitosan-5-fluorouracil nano-particle is much slower. The slower releasing property may be not only due to the concentration difference, but also the action between 5-Fu and chitosan. The atoms (O, N and F) with strong negativity in 5-Fu have formed quite strong hydrogen bond with the hydrogen of —OH in chitosan, or the hydrogen of —NH— in 5-Fu so that the connection between 5-Fu and chitosan in magnetic chitosan-5-fluorouracil nano-particles is much stronger. Thus, the 5-Fu is hard to be released from the nano-particles. But under unceasing function of the hydrone, the hydrogen bond is destroyed, and 5-Fu is slowly released from the nano-particles until the end of the process.

3.4 Magnetic response of magnetic chitosan-5-fluorouracil nano-particles

If the targeted drug has a strong magnetic response, the majority of drug particles may remain in the target

site under the magnetic field applied. The drug concentration of the target site may be increased, significantly reducing the killing effect to normal cells. Therefore, the magnetic properties of nano-particles are essential. The sedimentation method was used to test the magnetic chitosan-5-fluorouracil nano-particles response to the magnetic field in order to detect the magnetic properties.

As a result of the precipitation of nano-particles in suspension, the absorbance of the solution is reduced. In the same magnetic field, the stronger the magnetism of the magnetic nano-particles, the greater the attraction of the magnetic field, and the faster the particle sedimentation, leading to the phenomenon that the rate of absorbance is reduced fast. Changes in absorbance of magnetic nano-particles in the magnetic field may reflect their magnetic properties. The sedimentation of magnetic chitosan-5-fluorouracil nano-particles in the gravitational field and magnetic field is shown in Fig.4.

As shown in Fig.4, without external magnetic field, the absorbance of suspension changes slowly, meaning that nano-particles only precipitate slowly under gravity.

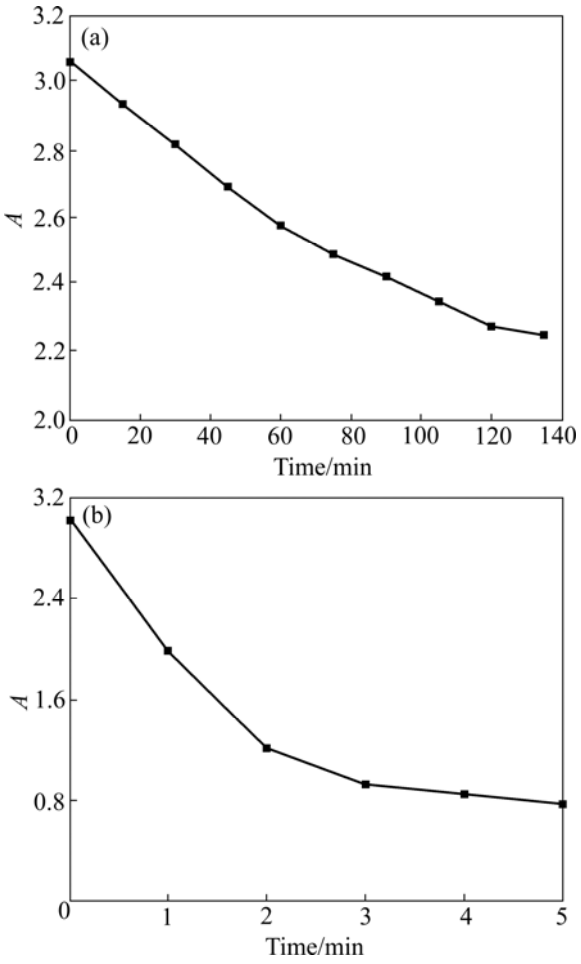

Fig.4 Sedimentation curves of magnetic chitosan-5-fluorouracil nano-particles: (a) In absence of magnetic field; (b) In the presence of 8 mT magnetic field

When magnetic field of 8 mT is imposed, the absorbance is lowered fast, and the sedimentation rate of nano-particles increases rapidly. This shows that magnetic chitosan-5-fluorouracil nano-particles have a good response to the magnetic field. It is also found that change in the mass ratio of chitosan to Fe_3O_4 can significantly change the magnetic properties of magnetic chitosan-5-Fu nano-particles. The larger the mass ratio of chitosan to Fe_3O_4 is, the more the chitosan is coated on Fe_3O_4 nano-particles, the greater the size is, and the faster the sedimentation rate is under the gravity field, however, the worse the magnetic response is.

4 Conclusions

1) The magnetic chitosan-5-Fu nano-particles are successfully prepared and the size range of the spherical particle is from 50 to 60 nm

2) The drug load rate of the nano-particles is 13.4% and the drug package rate is 25.8%.

3) In the phosphate buffer solution at pH=7.2, the accumulation medicine release rate of magnetic chitosan-5-fluorouracil nano-particles in 30 h is 68%, showing a certain sustained release effect.

4) Without external magnetic field, the nano-particle sedimentation rate is slow. While being subjected to 8 mT magnetic field, the particle sedimentation rate is increased rapidly. The results show that magnetic chitosan-5-Fu nano-particles have a good magnetic stability and strong targeting characteristics.

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(Edited by YANG Bing)