Preparation and Drug Release Properties of Fe₃O₄/Chitosan Magnetic Microspheres

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Abstract. Fe₃O₄/chitosan(CS) magnetic microspheres were prepared by emulsion crosslinking method, in which 5-fluorouracil (5-Fu) was loaded as model drug. The preparation conditions were optimized by orthogonal experiment with the the package rate as an index. The structure and morphology of the magnetic microspheres were characterized by scanning electron microscopy(SEM). The drug release properties of the microspheres in vitro were investigated. The optimal preparation conditions of drug loaded magnetic microspheres were as follows: 0.3 g 5-Fu, 5 mL glutaraldehyde, 3 h crosslinking time and 0.2 g/ml acetic acid concentration. Under these conditions, the drug loading efficiency was up to 6.35% as well as the package rate was up to 35.2%. The Fe₃O₄/chitosan magnetic microspheres also displayed an excellent drug release behavior under the experimental conditions of simulated intestinal fluid. The releasing content reached a peak in 10 h.

1. Introduction
The clinical applications of some anti-cancer drug is limited because of its toxicity, such as 5-fluorouracil(5-Fu)[1]. Drug and an appropriate magnetic material can be performed into controlled drug-released magnetic microspheres. 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 dosage and toxic effects of drugs are both significantly reduced, and the treatment efficiency can be enhanced[2-3]. However, most of magnetic microsphere treatments used currently are not satisfied, due to their small magnetic flux, less carrying capacity and short drug time[4]. The preparation of drug-released magnetic microspheres with high magnetic flux, large drug loading as well as small size has become the hot spot in current studies.

Chitosan(CS), a kind of natural polysaccharide, is widely used as drug carrier, due to its good biocompatibility and biodegradability. Paramagnetic or super magnetic Fe₃O₄ nanoparticles under the effect of the external magnetic field can kill tumor cells when the temperature rises to 40 °C-45 °C[5]. This paper was attempted to prepare a drug carrier system, in which chitosan was used as enveloping material, 5-Fu as model drug, Fe₃O₄ as kernel, and glutaraldehyde as crosslinking agent. The preparation conditions were optimized by drug loading efficiency and package rate. In addition, the morphology and drug release properties were determined.
2. Materials and methods

2.1. Materials and Instrument
5-Fu (purity>98%, Zhourui Chem. Shanghai, China), chitosan (purified, Haiyun Chem. Dalian, China), Fe₃O₄ nanoparticles (self-made), glutaraldehyde (AR, Hangzhou Luer Tech Co., Ltd. Hangzhou, China), acetic acid (AR, Tianjin Kemiou Chemical Reagent Co., Tianjin, China), Phosphate buffer solution (PBS, pH 6.86, Tianjin Damao Chemical reagent Factory, Tianjin, China), dialysis bag (Beijing JingKeHongDa, Beijing, China), and distilled water were used in this experiment. Ultraviolet spectrometer (UV2100, Unico, USA), scanning electron microscopy (SU3500, Hitachi, Ltd, Japan).

2.2. Preparation of Fe₃O₄ nanoparticles
According to Ref.[6-7], Fe₃O₄ nanoparticles were prepared. The preliminary studies showed that they had good magnetic properties.

2.3. Preparation of 5-Fu loaded Fe₃O₄/chitosan magnetic microspheres
0.1 g (dry mass) Fe₃O₄ nanoparticles mud paste, 5-Fu and 0.6 g chitosan were mixed in 30 mL acetic acid solution for 1 h with intense stirring, and then the mixture was installed to remove the foam generated by the agitation. In a 250 mL three-neck flask, 50 mL Liquid paraffin was mixed with 1.5 mL span80, which was used as dispersing agent; then the above mixture was slowly dripped under severe agitation; at last 1 mL 50% glutaraldehyde was added and reacted at 35 ℃ for a period of time. After holding for 12 h, the precipitates of nanoparticles were washed by petroleum ether, ethanol and distilled water for three times, respectively. Then 5-Fu loaded Fe₃O₄/chitosan magnetic microspheres were obtained after centrifugation and drying in a vacuum oven.

2.4. Morphology of 5-Fu loaded Fe₃O₄/Chitosan magnetic microspheres
Scanning electron microscopy(SEM) was used for observation of the size and morphology of the magnetic microspheres. The obtained microspheres were vibrated with ultrasound for 30 min, and treated by spray-gold for SEM observation.

2.5. Determination of drug loading efficiency and package rate
5-Fu (10 mg) was dissolved in phosphate buffer solution (pH 6.68) and diluted to 100 mL. 1.0, 2.0, 3.0, 4.0, and 5.0 mL solution were used and diluted to 50 mL, respectively. After 1 h lasting ultrasonic treatment, UV-vis spectrometer were used to measure 265 nm absorbance frequency for the standard curve. 5-Fu loaded Fe₃O₄/chitosan magnetic microspheres were measured, added with phosphate buffer solution (pH 6.68). After the microspheres were dissolved, the solution was diluted for UV-vis spectrometer measuring, and compared with the standard content curve. The formulas of the drug loading and package rate are as follows:

\[
\text{Drug loading} (\% \text{ w/w}) = \frac{\text{mass of } 5\text{-Fu in microspheres}}{\text{mass of microspheres recovered}} \times 100\% \quad (1)
\]

\[
\text{Package rate} (\% \text{ w/w}) = \frac{\text{mass of } 5\text{-Fu in microspheres}}{\text{total mass of } 5\text{-Fu}} \times 100\% \quad (2)
\]

2.6. In Vitro experiment of drug release
10 mg 5-Fu loaded Fe₃O₄/chitosan magnetic microspheres was put into a dialysis bag, added with 10 mL phosphate buffer solution (pH 6.68), then was kept at temperature of 37 ℃. 5 mL solution was regularly measured for absorbance and at the same time 5 mL fresh buffer solution was added. The release amount of 5-Fu via time curve was measured as mentioned above.
3. Results and discussion

3.1. The standard curve of 5-Fu

The relationship of 5-Fu concentration with absorbance was analyzed by linear regression. With the correlation coefficient $R^2=0.9999$ as follows:

$$A = 0.0521C + 0.0229$$

(3)

$A$ is absorbance value, whereas $C$ is mass concentration of 5-Fu (μg/mL).

![Figure 1. The standard curve of 5-Fu.](image)

3.2. Optimization of the preparation conditions

The optimal preparation conditions were selected by orthogonal experiment with the package rate as an index. The 5-Fu dosage, glutaraldehyde dosage, crosslinking time and acetic acid concentration were estimated as four major factors due to the pre-experiments. The experimental design is shown in Table 1.

The results of orthogonal experiment is shown in Table 2. According to these data, sorted in order of size, the Ranges of the four factors were $R_D > R_A > R_B > R_C$, which indicated that 5-Fu dosage was the most important factor affecting the encapsulation rate of the preparation of magnetic microspheres, whereas other influencing factors were acetic acid concentration, glutaraldehyde dosage, and crosslinking time in order. No. 3 experiment $A_1B_3C_3D_3$ displayed the best performance. The optimal preparation conditions were as follows: 0.2 g/ml acetic acid concentration, 5 mL glutaraldehyde, 3 h crosslinking time and 0.3 g 5-Fu. Under these conditions, the package rate was up to 35.2%, as well as the drug loading efficiency was up to 6.35%.

| Factor design of the orthogonal experiment |
|-------------------------------------------|
| Factor A: acetic acid concentration (g/mL) | Factor B: glutaraldehyde dosage (mL) | Factor C: crosslinking time (h) | Factor D: 5-Fu dosage (g) |
| 1  | 0.2 | 2 | 2 | 0.15 |
| 2  | 0.4 | 4 | 2.5 | 0.2   |
| 3  | 0.6 | 5 | 3  | 0.3   |

Table 1. Factor design of the orthogonal experiment

| Analysis of the orthogonal experiment |
|--------------------------------------|
| No. | Factor A | Factor B | Factor C | Factor D | the package rate (%) |
|-----|----------|----------|----------|----------|---------------------|
| 1   | 1        | 1        | 1        | 1        | 10.3                |
| 2   | 1        | 2        | 2        | 2        | 23.6                |

Table 2. Analysis of the orthogonal experiment
3.3. Morphology of 5-Fu loaded Fe₃O₄/chitosan magnetic microspheres

The morphology of 5-Fu loaded Fe₃O₄/chitosan magnetic microspheres (experiment No.3) is shown in Fig.2.

As seen in Fig.2, the magnetic microspheres were regularly spherical, and surface was smooth. They were homogeneous in size, and the range of microspheres size is from 5 to 10 μm.

3.4. Drug releasing property

The releasing property of 5-Fu loaded Fe₃O₄/chitosan magnetic microspheres (experiment No.3) is shown in Fig.3.

As seen in Fig.3, 5-Fu loaded Fe₃O₄/chitosan magnetic microspheres hold a quick release within 6 h and the whole releasing quantity was achieved to be about 74.2%. Then the release rate became more slowly, and the release rate is about 85.6% in 24 h. Thus, the release curve, which had a quicker release in the beginning and a slower release in the late, showed that 5-Fu loaded Fe₃O₄/chitosan magnetic microspheres had a good releasing performance.

|    | 3  | 1  | 3  | 3  | 3  | 35.2 |
|----|----|----|----|----|----|------|
| 4  | 2  | 1  | 2  | 3  | 35.0|
| 5  | 2  | 2  | 3  | 1  | 23.4|
| 6  | 2  | 3  | 1  | 2  | 32.2|
| 7  | 3  | 1  | 3  | 2  | 30.6|
| 8  | 3  | 2  | 1  | 3  | 30.8|
| 9  | 3  | 3  | 2  | 1  | 25.9|
| K₁ | 23 | 25.3| 24.4| 19.9|
| K₂ | 30.2| 25.9| 28.2| 28.8|
| K₃ | 29.1| 31.1| 29.7| 33.7|
| R  | 7.2| 5.8 | 5.3 | 13.8|

Figure 2. SEM image of 5-Fu loaded Fe₃O₄/chitosan magnetic microspheres
Figure 3. The releasing property of 5-Fu loaded Fe₃O₄/chitosan magnetic microspheres in phosphate buffer solution (pH 6.68)

4. Conclusion
In this work, the 5-Fu loaded Fe₃O₄/chitosan magnetic microspheres were successfully prepared. The preparation conditions were optimized by orthogonal experiment, and the experiment No.3 possessed the best performance in both of package rate and drug loading efficiency, which was 35.2% and 6.35%, respectively. In the phosphate buffer solution (pH 6.68), the accumulation 5-Fu release rate of magnetic microspheres in 24 h is 85.6%, showing a obvious sustained release effect.

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