A method for preparation PCL/CoFe$_2$O$_4$ magnetic composite microspheres and the properties

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Abstract: This study developed a facile and accurate approach to synthesis a poly($\varepsilon$-caprolactone) (PCL) magnetic composite microspheres with 5-Fluorouracil (5-Fu) as a new drug-loaded magnetic functional material, which was considered as an effective anti-cancer drug model. The obtained samples were characterized by FTIR, XRD, UV-vis spectrum and SEM scan. The drug-loading quantity was changed with the increase of the amount of 5-Fu, and the encapsulation efficiency was gradually decreased. The important data were that saturation magnetization of the composite microspheres was 29.91 emu/g, showed good superparamagnetic properties. When the dosage of 5-Fu was added to 0.3g, the encapsulation efficiency was 27.5%, reached the best encapsulation efficiency. The released properties of the composite microspheres were also investigated, and it was found that the release behavior of 5-FU showed a sustained and controlled.

1. Introduction

Currently, cancer is one of the world community’s major killers. There were several proposed anticancer drug delivery systems including: conjugates and implants polymer, colloidal systems (nanoparticles emulsions, and liposomes). Controlled release of drugs$^{[1-3]}$ from nanostructured functional materials, especially nanoparticles (NPs), is attracting increasing attention because of the opportunities in cancer therapy and the treatment of other ailments.

Poly $\varepsilon$-caprolactone (PCL) was a well-known biodegradable linear aliphatic flexible polyester$^{[4-6]}$, which was widely being used in drug delivery strategies. The main structure of PCL is made up of five aliphatic chain (-CH$_2$-) and a polar ester group, which makes it easy to be biodegradable and processed. PCL were one of the most widely used biodegradable polymers due to its good drug biocompatibility$^{[7,8]}$. Meanwhile, the PCL nanoparticles with magnetic drug-loaded microspheres had been used in various drugs including anti-cancer drug such as tamoxifen, which have good performance of biocompatibility biodegradability and targeting$^{[9,10]}$.

Magnetic drug targeting should be safe and effective, i.e. particles capable of carrying the desired and releasing drug at a controlled rate. In various drug delivery systems, polymers and magnetic carriers are the most representative$^{[11,12]}$. Scientist paid more attention to the way the drug could be delivered to the target cells and the subsequent release of the drug. In particular, cobalt ferrite magnetic particles (CoFe$_2$O$_4$) were chosen both to serve as the magnetic nuclei and to set the size of the particles, which were explored for biomedical applications due to its large magnetostriction, high Curie temperature, effective anisotropy and moderate saturation magnetization. The model drug 5-Fu, it is a widely used in cancer treatment of anti-drug metabolism through the inhibition of thymidylate synthase and inhibition of DNA synthesis$^{[13]}$.

In our work, PCL/CoFe$_2$O$_4$/5-Fu magnetic targeted drug delivery microspheres were polymerized
by solvent evaporation emulsion method\(^{14,15}\), in which PCL was used as the enveloping matrix, CoFe\(_2\)O\(_4\) nanoparticles as magnetic kernel and doxorubicin hydrochloride (DOX) as anti-cancer model drug. We systematically studied their magnetic properties, drug loading, encapsulation efficiency and drug release properties.

2. Materials and methods

2.1 Materials

CoFe\(_2\)O\(_4\) and 5-Fu were purchased from Solvay(USA). PCL\((M_w=4,000)\), PVA Ethyl Alcohol and EDTA (ethylene diamine tetraacetic acid) were obtained from Guangzhou Chemical Reagents Wholesale (China) without further purification. Other solvents were offered from Tianjin Kermel Chemical Reagent Company(China).

2.2 Preparation of CoFe\(_2\)O\(_4\) magnetic nanoparticles

A co-precipitation method was used to synthesize magnetic nanomaterials. Compared with other reported skills, this technique was easier to operate. In a typical experiment, an aqueous solution containing Co\(^{2+}\) and Fe\(^{3+}\) were mixed with thoroughly stirring at 80\(^\circ\)C for 60 min. The black precipitate was collected by magnetic separation and washed three times with distilled water. Then, the product was dried in a vacuum for 24 hours to obtain magnetic nanoparticles. Here is the ionic reaction equation: Co\(^{2+}\)+2Fe\(^{3+}\)+8OH\(^-\)=CoFe\(_2\)O\(_4\)+4H\(_2\)O

2.3 Preparation of 5-Fu/CoFe\(_2\)O\(_4\)/PCL microspheres

Magnetic drug loaded microspheres were prepared through a modified oil-in-water emulsion solvent-evaporation method\(^{15}\). In short, a calculated CoFe\(_2\)O\(_4\) and PCL were dissolved in CH\(_2\)Cl\(_2\), to make a uniformly dispersed oil phase. The 5-Fu powder of PVA water solution were dropwise added with stirring at a certain speed. After getting the colostrum emulsion for 10 minutes, the obtained colostrum was slowly added to the PVA solution. It was stirring for 24 hours at room temperature. The resulted product was washed, filtered and dried to a constant weight. The typical procedure was shown as Fig. 1.

![Image](image)

Figure 1. The preparation of 5-Fu/CoFe2O4/PCL microspheres

2.4 Determination of the properties of drug-loaded microspheres

A 0.1mol/L 5-Fu dilute HCL CH\(_2\)Cl\(_2\) solution was measured with UV spectrophotometer to determine the absorbance of 265nm. The entrapment efficiency (EE) and drug-load quantity of magnetic drug-loaded microspheres(DL) were calculated as follow formula:

\[
EE\ (%)=\frac{W_0-W_C}{W_0} \times 100\%
\]

(1)

\[
DL\ (%)=\frac{W_0-W_m}{W_m} \times 100\%
\]

(2)

Where, \(W_0\) is the total amount of 5-Fu which was added in microspheres, \(W_C\) represents the amount of 5-Fu in the supernatant solution, \(W_m\) means the qualities of microspheres.

We conducted dynamic dialysis taking 20mg magnetic drug-loaded microspheres. Dialysis bag is sealed and put in a conical flasks with 40ml phosphate buffer. Make the flask in constant temperature water bath at 37\(^\circ\)C. Suck up 10ml solution at regular time, at the same time, added 10ml phosphate
buffer in a flask. The absorption data was determined by UV spectrophotometer at 265nm wavelength. The amount of drug release was tested by standard curve. The cumulative release dosage of 5-Fu was calculated in the following formula.

\[
\text{Drug Release Rate \% } = \sum \frac{M_t}{M_0} \times 100 \% \tag{3}
\]

Where, \(M_t\) is the release amount of 5-Fu in buffer solution at some time \(t\), and \(M_0\) is the amount of 5-Fu microsphere in test.

3. Results and discussions

3.1 X-ray diffraction analysis

The crystal structure of 5-Fu/CoFe\(_2\)O\(_4\)/PCL, PCL/CoFe\(_2\)O\(_4\) and 5-Fu magnetic microspheres was investigated by XRD, as shown in Fig. 2. 5-Fu diffraction diagram had a strong characteristic peaks at 2\(\theta\)=29°. PCL/CoFe\(_2\)O\(_4\) and 5-Fu/PCL/CoFe\(_2\)O\(_4\), have the basic same obvious diffraction peaks at 2\(\theta\)=22°. No other diffraction peaks of 5-Fu and CoFe\(_2\)O\(_4\) were shown in 5-Fu/PCL/CoFe\(_2\)O\(_4\), which may be attributed to the thick layer of PCL matrix and stronger interaction between PCL. The crystallization behavior of 5-Fu and CoFe\(_2\)O\(_4\) became weakened, showing only the diffraction peak location of PCL. The detectability limit of XRD testing should be also one of the cause.

![Figure 2. XRD patterns of 5-Fu/CoFe\(_2\)O\(_4\)/PCL, PCL/CoFe\(_2\)O\(_4\) and 5-Fu magnetic microspheres.](image)

3.2 FTIR analysis

As seen in Fig. 3, the peak at 3134cm\(^{-1}\) is the broad stretching vibration peak of N-H in(-NH-CO-); and the 1667cm\(^{-1}\) absorb is mainly the C=O and C=C stretching vibration. It is also found that the bending vibration of C-H of (-CF-CH-) is at 1430cm\(^{-1}\) and the stretching vibration peak of C-N exist at 1250cm\(^{-1}\). From the infrared spectrum of PCL/CoFe\(_2\)O\(_4\), the peak at 1730cm\(^{-1}\) is the characteristic stretching vibration absorption of ester bond (-CO—O-), and the strong peak of 3440cm\(^{-1}\) is another expansion vibration absorption peak of -OH. Compared the FTIR of 5-Fu/CoFe\(_2\)O\(_4\)/PCL and PCL/CoFe\(_2\)O\(_4\), both of them had the same characteristic peaks. The characteristic peak of magnetic material and the medicine are not obvious found.
Figure 3. FTIR spectra of (a) 5-Fu drug, PCL/CoFe₂O₄ microspheres and 5-Fu/CoFe₂O₄/PCL drug carried magnetic microspheres

3.3 SEM observation
From Fig. 4, we found that magnetic drug-loaded microspheres had regular, relatively smooth spherical surface. The average particle size of magnetic drug-loaded microspheres was larger than that of polymer microspheres under the certain agitation speed. It was also found that there were some microholes on the surface of the particles, which could be generated by continuous phase evaporation. It was good to provide a channel for the release of the drug from the internal of microspheres and improved the drug release time. From the scanned figures, we can also see some smaller particles at the larger’s surface, this should be a part of the 5-Fu or magnetic material adhesion in the surface of microsphere ball, which were not in the inner.

Figure 4. SEM images of 5-Fu/CoFe₂O₄/PCL drug-loaded magnetic microspheres: (a) 3.0k, (b) 1.8k, (c) 4.0k, (d) 15k.

3.4 VSM analysis
The magnetic properties of CoFe₂O₄ nanoparticles and 5-Fu/CoFe₂O₄/PCL microspheres were studied by VSM analysis from -20000 Oe to 20000 Oe, as shown in Fig. 5. The specific saturation magnetization of above two samples was 43.438 emu/g, and 29.91 emu/g, which is smaller than that of the bulk nanoparticles, probably due to their smaller particle size. It could be seen from the curves that the coercivity of the hysteresis loops was 0, which indicated that the magnetic microspheres had super paramagnetic properties. The two samples showed typical superparamagnetic effect, having very small
remanence and coercivity.

![Graph showing magnetic curves of 5-Fu/CoFe2O4/PCL and CoFe2O4 magnetic microspheres.](image)

Figure 5. Magnetic curves of 5-Fu/CoFe2O4/PCL and CoFe2O4 magnetic microspheres.

### 3.5 Determination of 5-Fu standard curve

![Graph showing the standard curve of 5-Fu in 0.1 mol/L HCL solution.](image)

Figure 6. The standard curve of 5-Fu in 0.1 mol/L HCL solution.

![Graph showing the standard curve of 5-Fu in PBS solution.](image)

Figure 7. The standard curve of 5-Fu in PBS solution.

We studied UV curves of 5-Fu in different solutions in 0.1 mol/L HCL solution and PBS solution. As seen in Fig. 6 and Fig. 7. The absorbance curve of 5-Fu was at a good linear relationship in the concentration range of 0.004-0.024mg/mL both of the two solutions. Linear regression equations (linear regression with c-A) in HCl solution is:

$$A=58.84C+0.019 \ , \ r^2=0.999 \qquad (4)$$

In PBS solution, Linear regression equations is

$$A=49.17C+0.025 \ , \ r^2=0.999 \qquad (5)$$
In the form: The linear range is 4~24 mg/ml, A represents the absorbance, C represents the drug concentration (mg/ml).

3.6 Drug loading and release properties in vitro
The in vitro cytotoxicity of 5-Fu microspheres was assessed. Put 0.1, 0.2, 0.3, 0.4g of 5-Fu into 10ml 0.1mol/L PVA solution and ultrasonic for 60min. Then, slowly drop the PVA solution into CH2Cl2 solution by stirring, which had dissolved quantitative PCL and CoFe2O4. After the oil water emulsion was formed, put it into the PVA emulsion solution, in the same time not stop stirring. After, emulsifying 30min, ionized water was added. It was also evaporated solvent overnight, and making filtrating, washing and drying.

| Quantity of 5-Fu(g) | EE(%) | DL(%) |
|---------------------|-------|-------|
| 0.1                 | 14.6% | 10.7% |
| 0.2                 | 21.7% | 18.5% |
| 0.3                 | 27.5% | 29.6% |
| 0.4                 | 10.3% | 8.8%  |

The data in table 1 showed the effect of 5-Fu to the envelopment efficiency and drug-loading rate. With the increase of the amount of 5-Fu, the drug loading was also increased before the 0.3g of 5-Fu dosage and decreased after that. The encapsulation efficiency was reduced gradually. Normally, the more 5-Fu content in the ratio of raw materials could improve the drug loading, however, it did not increase the encapsulation efficiency of the drug nanoparticles. When the amount of 5-Fu was up to 0.4g, the encapsulation efficiency and drug loading had the minimum, which could be attributed to excessive drug dosage. Another reasons was the poor solubility of the drug in the solution. We could find the drug loading and entrapment efficiency of magnetic drug-loaded microspheres were the maximum when the amount of 5-Fu was at 0.3g. By compared with our work of the magnetic chitosan microspheres prepared before, the 5-Fu drug loading rate and encapsulation efficiency have obviously been improved.

The results also showed that the PCL as the substrate material could be a good drug substance. For magnetic drug-loaded microspheres, PCL loaded magnetic drug microspheres not only contained drugs, but also contained magnetic material. It mean that the amount of 5-Fu in the microspheres was relatively small, which could lead to lower drug loading rate.

4. Conclusions
In this work, a facile strategy has been reported to prepare PCL composite microspheres loading magnetic drug loaded microspheres by the method of double emulsion solvent evaporation, taking the CoFe2O4 as magnetic core, the PCL as the matrix material, and 5-Fu as drug model.

The main results of the research were evaluated with magnetic properties, encapsulation efficiency, drug loading rate and drug release rate. The superparamagnetic composite microspheres demonstrated typical negative contrast enhancement effect. The specific saturation magnetization of 5-Fu/CoFe2O4/PCL was 29.911emu/g. Compared with the added equal amount magnetic materials of the magnetic microspheres, it were reduced. The coercivity of the hysteresis loops was 0, which told us the superparamagnetism. In magnetic drug nanoparticles loaded microspheres, when the amount of 5-Fu was added to 0.3g, encapsulation efficiency and the drug loading of magnetic drug-loaded microspheres were the maximum. Magnetic drug-loaded microspheres showed good release behavior in vitro. At last, according to the results of encapsulation efficiency and drug loading, 0.3g 5-Fu was selected for the best drug concentration.

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