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Preparation of drug-loaded chitosan microspheres repair materials

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Abstract. – OBJECTIVE: With the development of society and the progress of science and technology, microspheres, as a new polymer material, have been applied to all aspects of human beings. Microspheres can play a huge role in food safety, electronic technology, sewage treatment, biomedicine, etc., and are non-toxic or harmless. There are three main types of substrates for the preparation of microspheres: natural polymers, semi-synthetic polymer materials, and synthetic polymer materials.

MATERIALS AND METHODS: In this study, the inorganic material kaolin was modified by the emulsification-crosslinking method with chitosan and composite microspheres with large interlayer spacing were prepared, which were characterized by Fourier Transform Ioncyclotron Resonancel (FTIR) analysis and Scanning Electron Microscope (SEM). The prepared kaolin/chitosan microspheres were then placed in different amounts of aspirin and the optimal dose was investigated by encapsulation efficiency and drug loading rate. The drug release rate of 0.5 h, 1 h, 1.5 h, 2 h, 4 h, 6 h, and 12 h was then determined by simulating the human colon to determine the performance of the sustained-release drug.

RESULTS: The experimental results showed that after the prepared composite microspheres were loaded with aspirin drug, we got the optimal dosage of 0.1 g by discussing the encapsulation efficiency and drug loading rate of the drug-loaded microspheres, and the encapsulation efficiency reached 80.80%, while the drug loading rate was 24.40%, the drug release capacity reached about 83% in about 12 hours.

CONCLUSIONS: The research shows that the kaolin/chitosan drug-loaded microspheres prepared by the emulsification and cross-linking method are excellent drug-loading materials.

Key Words:
Chitosan, Drug loading, Microspheres, Slow release.

Introduction

In recent years, polymer material microspheres have been applied in many fields, such as electronic technology, food safety field, environmental water treatment, biomedicine, and so on. As early as the 20th century, foreign countries took the lead in researching microspheres as a new drug delivery material. The particle size of the microspheres is generally between 1 and 250 μm, and the drugs can be dissolved or adsorbed in the polymer matrix to form tiny spheres. The main functions are:

1. Mask[1] the bad smell of some drugs and improve the compliance of patients with medication.
2. Improve the stability of the drug.
3. Reduce the irritation and side effects to the human stomach.
4. Solidify the liquid medicines.
5. Reduce the change in the compatibility of two different drugs.
6. Slow-release and controlled-release drugs.

Method for Preparing Chitosan Microspheres

Chitosan is abundant in nature and has good biocompatibility and antibacterial properties[2]. However, pure chitosan microspheres have poor hydrophilicity and a low degree of deacetylation[3], and their applications are limited. Therefore, a variety of materials are used to prepare composite microspheres.

The main methods are the emulsification cross-linking method, ion gel method, and spray drying method, among others.

Emulsification Cross-Linking Method

The emulsification cross-linking method[4] is one of the simplest and most commonly used methods. It involves mixing the water and oil phases, then emulsifying the mixture after stirring. During the stirring process, the polymer solution will form emulsion droplets, and then the chemical cross-linking agent will be added. The aldehyde group in the cross-linking agent will undergo a condensation reaction with the amino group of chitosan to obtain solid microspheres.

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Ion Gel Method

The ion gel method uses an anionic gel solution as a physical cross-linking agent and acetic acid as the preparation system, in which the negative charge is combined with the positive charge of chitosan gels chitosan to form microspheres.

Spray Drying Method

Spray drying technology is a method of dissolving and dispersing chitosan in an acidic solution, and spraying the emulsion in a hot air stream, so that the solvent quickly evaporates into a gas to form chitosan microspheres[5].

Other Methods

In addition, other available methods are the spray drying method, solvent evaporation method, etc. The principle is to mix chitosan with an organic solvent to obtain O/W or W/O type emulsion, and then atomize or distill, and then evaporate to obtain a solid micro-emulsion ball.

Research Progress

According to the data reviewed, Shi et al[6] used sodium alginate, berberine sulfate, and distilled water to prepare a mixed solution, added liquid paraffin, span-80, Tween-80, and propylene glycol for stirring and emulsification, and then obtained the oil-in-water type. The latex is added to the calcium chloride solution, stirred, reacted, filtered to obtain microspheres, and finally added to the chitosan solution to obtain chitosan-sodium alginate drug-loaded microspheres.

Zhou et al[7] prepared chitosan drug-loaded microspheres with uniform particle size and good spherical morphology by glutaraldehyde cross-linking by emulsification and cross-linking method.

Zhang et al[8] dissolved chitosan in acetic acid solution by ion gel method, then stirred at room temperature and then added sodium tripolyphosphate solution dropwise, cooled and dried to obtain chitosan nanoparticles.

Abdellatif et al[9] used chitosan (CH) and Pluronic F 127 (PL F 127) to cross-link to form polymer-based hydrogels for targeted drugs.

Iezzi et al[10] used degradable starch microspheres to treat advanced hepatocellular carcinoma.

Shuang et al[11] researched the effect of injectable chitosan demineralized bone matrix (DBM) on bone repair.

Research Content

The preparation of drug-loaded chitosan microspheres repair materials adopts the emulsification and cross-linking method and selects chitosan, a natural high-analytical material. Micron-scale spheroids wrapped in liquid or solid substances with chitosan are chitosan microspheres[12].

Chitin is a natural polysaccharide that widely exists in crustacean shells and insect shells[13-14]. Chitosan is the deacetylation product of chitin[12], which is non-toxic and has good biocompatibility, biodegradability, and antibacterial properties. Therefore, chitosan is widely used in cosmetics production, food safety, medical dressings, and other fields.

Chitosan is insoluble in water, only soluble in acid, and has a low degree of deacetylation. Only when the degree of deacetylation of chitosan reaches more than 70% will it have real use value, while the degree of deacetylation of pure chitosan is about 55%. Therefore, in the process of preparing microspheres from chitosan, it needs to be modified by some methods or by adding other materials to prepare more hydrophilic and more water-soluble microspheres.

In this study, kaolin, a natural porous material, was selected to prepare drug-loaded microspheres with a sustained-release effect, and to measure the sustained-release effect. Kaolin is a white powdery porous material mainly composed of kaolinite clay minerals. It contains large amounts of Al_2O_3, SiO_2, a small amount of Fe_2O_3, TiO_2, k_2O, Na_2O, CaO, MgO[13], which create good plasticity, and chemical stability. The principle is to load pure chitosan in kaolin. Because kaolin has a flat and compact layered planar structure, chitosan molecules can enter the interlayer of kaolin molecules, thereby further increasing the interlayer spacing of kaolin molecular structure. The molecular structure of the prepared microspheres also has a larger interlayer spacing, so that the drug to be loaded can be more fully adsorbed during the drug-loading process.

In the experiment, the inorganic material kaolin was modified by crosslinking with chitosan to prepare composite microspheres, and then the composite microspheres were analyzed by Fourier Transform Ioncyclotron Resonancel (FTIR) and Scanning Electron Microscope (SEM). The prepared kaolin/chitosan microspheres were then put into different amounts of aspirin, and the optimal dosage was found by measuring the encapsulation rate and drug loading rate. Then, it was tested by simulating the human colon (phosphate buffer solution with pH 7), and the drug release rate at 0.5 h, 1 h, 1.5 h, 2 h, 4 h, 6 h, and 12 h was measured to determine the performance of the sustained-release drug.

Materials and Methods

Preparation of Kaolin/Chitosan Microspheres Main instruments and reagents

Analytical balance, digital constant temperature water bath, PH meter, vacuum drying oven, UV spectrophotometer, scanning electron microscope (SEM), Fourier Transform Ioncyclotron Resonancel (FTIR), chitosan, kaolin, acetic acid solution, liquid paraffin, 5% Span-80, pentylene Dialdehyde, petroleum ether, sewage ethanol are all the necessary instruments and reagents used in the experiment.

Experimental Method

1) 3.00 g of chitosan was put in a beaker with about 25 ml of acetic acid solution and stirred gently with a glass rod to slowly dissolve the chitosan in the acetic acid solution.

2) 1.00 g of kaolin was weighed and placed in a beaker, then distilled water was added and stirred gently to obtain a kaolin suspension.

3) The chitosan solution obtained by the initial dissolution was added dropwise to the kaolin clay suspension using a pipette[7].

4) Liquid paraffin, of which 5% Span-80, was added. Span-80 is a water-in-oil emulsifier, so a water-in-oil emulsion formed after adding Span-80, kaolin and chitosan existed in the water phase. At this time, the pH of the solution can be adjusted to neutrality. It is important not to adjust the pH to alkaline because, as mentioned above, chitosan is soluble in water. Under an acidic environment, if the alkalinity is too high, chitosan will precipitate out in the solid form.

5) 5 mL of 2.5% glutaraldehyde was added for cross-linking reaction, and the cross-linking time was 1.5 h while the cross-linking temperature was 70°C.

6) After the cross-linking, the previous water-in-oil type milk was cross-linked into solid milk. At this time, it was poured into a beaker for cooling. During this process, the solid milk, which is the prepared microspheres, was slowed down. After the slow sedimentation was completed, successively, the excess crosslinking agent with petroleum ether and anhydrous ethanol was washed.

7) Finally, a suction filter bottle was connected under the filter funnel, and a vacuum pump was connected next to the suction filter bottle to filter the solution.

8) This solid was obtained by drying the material by filtration and then named kaolin/chitosan microspheres.

Analysis of Results

The prepared material was solid and pale yellow to the naked eye. Fine round particles could be seen after close observation, and the prepared microspheres appeared as solid mud after drying.

Scanning Electron Microscopy Characterization

There was no "ball" shape appearing in the obtained microsphere, but we can more clearly recognize the morphological characteristics of the microsphere through the scanning electron microscope. We first cleaned the sample stage of the scanning electron microscope, then pasted the double-sided conductive adhesive we prepared on the sample plate, put a little of our prepared microspheres on the conductive adhesive, and used the ear-washing balls. The excess is blown away in one direction and then placed in a scanning electron microscope for scanning. We characterize the two main materials, chitosan, kaolin, and the final composite microspheres. Finally, Figure 1 was obtained by scanning electron microscope (SEM).

Figure 1 shows that the prepared kaolin/chitosan microspheres have a relatively full spherical shape in the SEM scanning results. Compared with the small spheres of chitosan, the composite microspheres had no adhesion and aggregation was evident, while the structure was more stable.

Discussion on the Optimal Dosage of Aspirin Loaded with Kaolin/Chitosan Microspheres

Aspirin standard curve

According to a literature review[14], aspirin has a corresponding ultraviolet absorbance between 240 nm and 330 nm and has the largest absorption peak at the wavelength of 276 nm. Therefore, in this experiment, a UV spectrophotometer (Shanghai

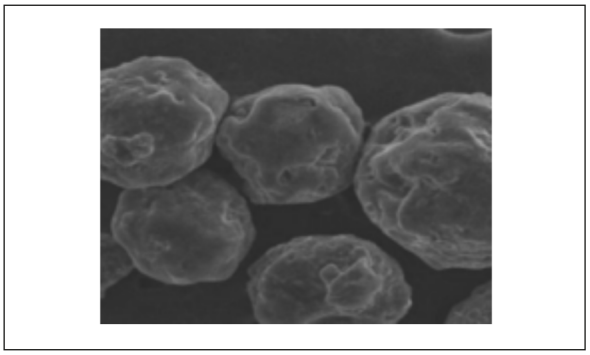

Figure 1. Scanning electron microscope image of Kaolin/chitosan (Magnification x1,000).

instrument Co.Ltd., Shanghai City, China) with a wavelength of 276 nm was used to determine aspirin absorbance. Specific steps were followed:

1) 250 mg[15] of aspirin was put in a 250 mL volumetric flask and ethanol was added to dissolve it, then, it was shaken well to obtain a 1 mg/mL aspirin solution.

2) 2.0 ml, 3.0 ml, 4.0 ml, 5.0 ml, and 6.0 ml of aspirin solution, respectively, were aspirated and placed in a 50 mL volumetric flask, then ethanol was added and diluted to the mark to obtain 0.04 mg/mL, 0.06 mg/mL, 0.08 mg/mL, 0.10 mg/mL, 0.12 mg/mL reference solution.

3) Ethanol solution was used as a blank solution. The zero-adjustment test should be carried out before the test; that is, the No. 1 cuvette tank and the No. 2 cuvette tank were put into the blank solution for zero adjustment.

4) After the zero adjustment, the blank solution in the cuvette tank No. 1 was taken out, and the aspirin control of 0.04 mg/mL, 0.06 mg/mL, 0.0 8 mg/mL, 0.10 mg/mL and 0.12 mg/mL were put in accordingly. The blank solution was taken in the cuvette tank No. 2 as the reference solution, and then the violet spectrophotometer at the wavelength of 276 nm should be used to measure the absorbance of aspirin with different mass concentrations, and thus the standard working curve of aspirin was obtained.

Based on the data obtained by measuring the absorbance of different concentrations of aspirin, the Aspirin standard working curve is shown in Figure 2.

Experimental Method

0.05 g, 0.10 g, 0.15 g of aspirin were weighed after grinding into powder, respectively. Then, the same amount of 0.25 g of microspheres was placed in three glasses, and the weighed aspirin was poured into the three beakers, 50 mL of warm water was added, stirred, and allowed to stand.

Analysis of Results

Aspirin standard curve: y=0.96x+0.0274.

The data obtained from the experiment are shown in Table I.

According to the information available:

Encapsulation efficiency= (total amount of drug-drug content in supernatant solution)/total amount of drug×100%.

Drug loading rate=(total amount of drug-drug content in supernatant solution)/(total amount of carrier+total amount of drug-drug content in supernatant solution)×100%.

The encapsulation rate and drug loading rate of different drug doses are shown in Table II.

From Table II, it can be seen that with the increase of the dosage, the encapsulation first increases and then decreases, and the drug loading rate keeps increasing. The permeability difference between the inside of the microspheres and the external water phase gradually increases,

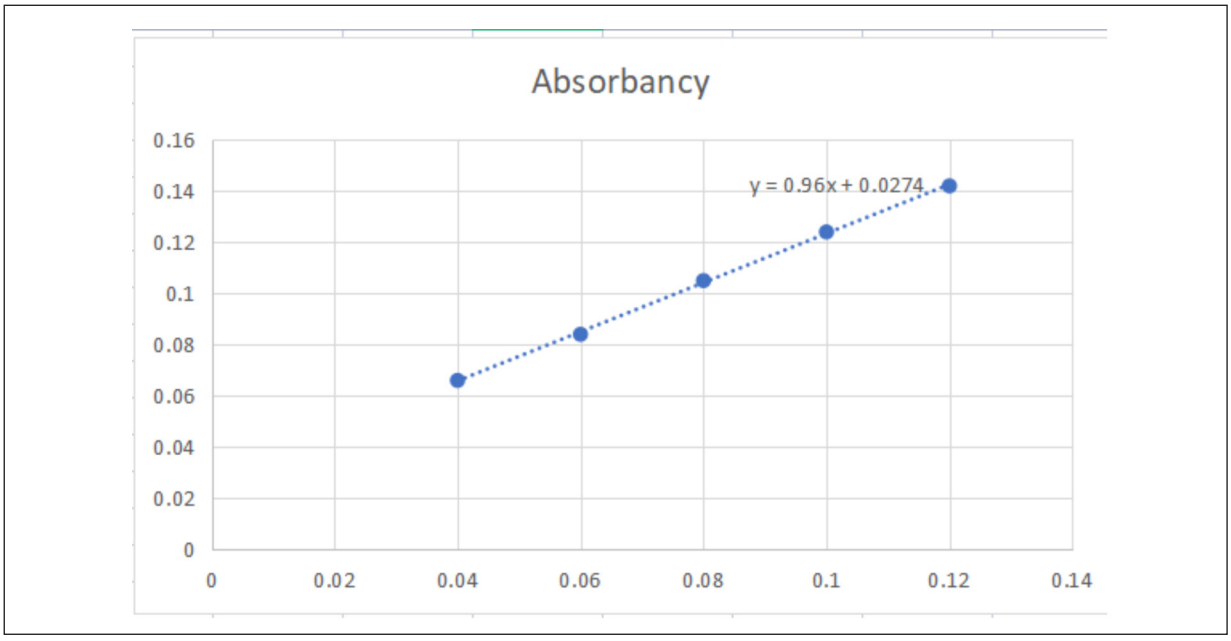

Figure 2. Aspirin standard working curve.

Table I. Absorbance values measured by drug-loaded microspheres with different dosages.

Dosage	Absorbance measured Y value (A)	The mass concentration corresponding to the X value (mg/mL)
0.05	0.220	0.2006
0.10	0.397	0.3850
0.15	0.676	0.6756

as well as the drugs diffused from the inside of the microspheres to the outside, resulting in a decrease in the encapsulation efficiency. In terms of drug loading rate, the hydrophobic space of the microspheres is limited. If the drug loading rate is too large, the microspheres may not be able to perform effective hydrophobicity, which may lead to drug leakage. Therefore, the dosage was selected with less drug loading rate and a better encapsulation rate. In this experiment, the optimal dosage is 0.1 g.

FTIR Analysis of Drug-Loaded Microspheres

The blank kaolin/chitosan microspheres and the aspirin-loaded kaolin/chitosan microspheres were separately analyzed by FTIR in Figure 3.

From the infrared spectrum of the 4,000-400 cm^{-1} wavenumber in Figure 3, the upper one is KL/CS, namely kaolin/chitosan blank composite microspheres, and the lower one is KL/CS/APC, namely kaolin/ peak changed to a certain extent after adding the drug, which may be caused by the interaction between the amino group of chitosan and the carboxyl group of aspirin, and the overall comparison. The characteristic peaks of the spectra of the two microspheres are basically the same; that is to say, the internal structure of the blank microspheres is not destroyed to a great extent after adding the drug.

SEM Characterization of Drug-Loaded Microspheres

According to the method for detecting kaolin, chitosan, and blank composite microspheres, the SEM characterization of the drug-loaded microspheres is continued as shown in Figure 4.

Comparing Figure 4 with the SEM images of the blank microspheres in Figure 1, it can be observed that the microspheres after drug loading are still spherical, and no major structural changes, such as spherical collapse, have occurred. In addition, we can also observe that the microspheres after drug loading are brighter than the blank microspheres, which is due to the formation of drug crystals loaded on the surface of the microspheres. The surface structure of the drug-loaded microspheres is similar to that of the blank microspheres and still has tiny holes to facilitate drug release

Table II. Encapsulation efficiency and drug loading efficiency of microspheres with different dosages.

Dosage	Encapsulation rate	Drug loading rate
0.05	79.90%	13.80%
0.10	80.80%	24.40%
0.15	77.50%	31.70%

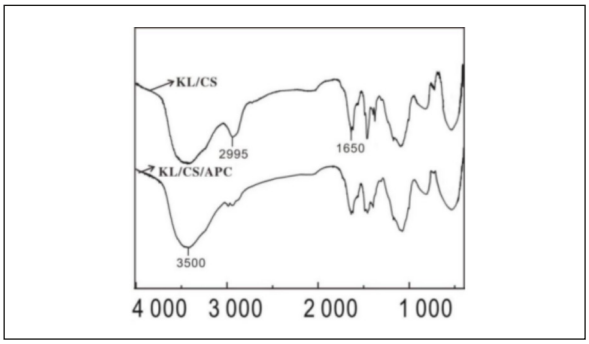

Figure 3. FTIR plots of KL/CS and KL/CS/APC.

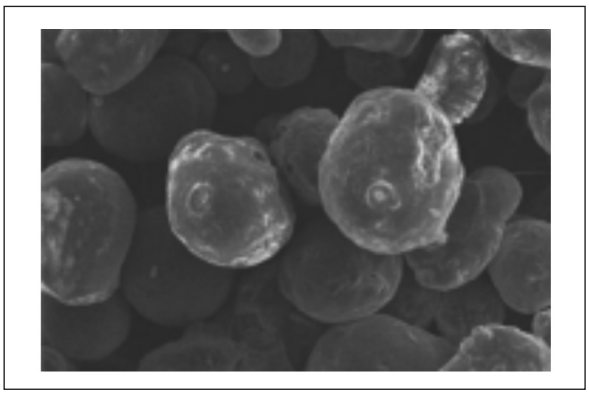

Figure 4. Scanning electron microscope (SEM) of drug-loaded composite microspheres (Magnification x1,000).

Discussion

Experimental Method on Drug Release Properties of Kaolin/Chitosan-Loaded Aspirin Microspheres

50 mg of the prepared drug-loaded microspheres were taken, 100 mL of pH=7.4 phosphate buffer solution (simulating human colon environment) was prepared, and then 0.1 g of aspirin-loaded drug-loaded microspheres was added to pH=7.4 phosphate buffer in the solution. The solution was stirred at a speed of 70 r/min and heated in a water bath at a temperature of 37°C (human body temperature). Then, after adding the drug-loaded microspheres, the drug will be released into the solution. By measuring the absorbance value in the solution at a wavelength of 276 nm, the previously obtained aspirin standard curve is used to calculate the amount of drug released in the solution.

After placing the drug-loaded microspheres in the simulated environment, 0.5 h, 1 h, 1.5 h, 2 h, 4 h, 6 h, and 12 h, 4 mL of each solution were taken to measure the absorbance value. Then 4 mL of phosphate buffer solution was added to ensure the accuracy of the next measurement.

For each calculation, the calculated drug release was added to the previous calculated drug release to obtain the cumulative drug release, then it was divided by the total drug load to obtain the cumulative drug release rate.

Analysis of Results

The above experimental data are recorded in Table III.

It can be seen from Table III that the drug-loaded microspheres just entered the phosphate buffer solution simulating the human colon environment within the first two hours, the drug release rate was relatively fast, and after entering the simulated solution for two hours, the drug release rate slowly decreased. Noticeable slow dosing performance can be seen, which to some extent reduces the possible side effects of rapid dosing. After nearly 12 hours, its drug release ability reached about 83%, indicating that the drug-loaded microspheres not only have relatively good sustained-release performance but also can release the loaded drug more entirely, thereby exerting the drug's effect.

Table III. Absorbance values of drug-loaded microspheres at different times.

Time (h)	Absorbency
0.5	0.172
1.0	0.127
1.5	0.147
2.0	0.060
4.0	0.193
6.0	0.214
12.0	0.252

Conclusions

In this experiment, kaolin/chitosan composite drug-loaded microspheres were prepared and characterized by FTIR analysis and SEM. The composite microspheres were put into an aspirin solution with mass concentrations of 0.04 mg/mL, 0.06 mg/mL, 0.08 mg/mL, 0.10 mg/mL, and 0.12 mg/mL, respectively. The optimal dosage of aspirin was explored through the two indexes of sealing rate and loading rate. Experiments show that the dosage is 0.1 g, the encapsulation rate reaches 80.80%, and the drug loading rate is 24.40%.

The experiment was carried out by simulating the human colon with phosphate buffer solution with pH-7, and the drug release rate at 0.5 h, 1 h, 1.5 h, 2 h, 4 h, 6 h and 12 h was respectively measured to determine the sustained release performance. The drug release rate was fast in the first two hours of the experiment, reaching 33.73%, and the drug release capacity reached about 83% in about 12 hours. It shows that the kaolin/chitosan drug-loaded microspheres prepared by the emulsification and cross-linking method can exert excellent performance in the drug sustained-release system.

Authors' Contributions

Keyong Tang and Xu Luo selected the topic for the thesis. Yangyang Wang and Xu Luo conducted experiments and obtained data. Keyon Tang, Xu Luo, and Yangyang Wang analyzed and processed the obtained data. All authors read and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Informed Consent

Not applicable.

Ethics Approval

Not applicable.

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Availability of Data and Materials
The data generated in this study were all derived from experiments and shared by all authors of this paper.

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