Preparation and Properties of Pesticide-loaded Carboxymethyl Chitosan Microsphere

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Abstract. Controlled release system has been widely developed and used in agriculture to improve efficacy and minimize environmental pollution. Here, the pesticide-loaded carboxymethyl chitosan microspheres were constructed by suspension-crosslinking method. Carboxymethyl chitosan was used as a carrier to formulate microsphere to load neonicotinoids with a mixture of calcium chloride and glutaraldehyde as double-component cross-linking agent. The resulting microsphere processed high drug loading and encapsulation efficiency with a mean diameter of 1 mm. The UV detection method of pesticide was established, which was convenient and quick. The pesticides release properties of the microspheres were investigated, and the controlled release time was more than 6 days, which achieves the objective of improving the efficacy of the pesticides. This work would promote the development of controlled-release pesticide systems.

1. Introduction

The conventional pesticide formulations are facing the biggest challenge for the sustainable development of the modern green agriculture due to the problems of quickly releasing, low efficiency and serious environmental pollution. Controlled release pesticide technologies can effectively resolve these problems. Pesticide controlled release system can not only improve the utilization efficiency of its active ingredients, reduce its application amount and frequency of application, improve application efficiency, thereby reducing environmental pollution, reducing side effects, and saving costs; moreover, the controlled release system can also reduce the loss of volatilization, flow loss, and biochemical decomposition of effective components, increase its physical and chemical stability, and is easy to preserve. Compared with traditional application methods, the controlled-release active substance can be kept within the effective concentration range for a long time, prolong its action time and improve its effect [1].

Carboxymethyl chitosan, as a derivative of chitosan, makes up for the deficiency of chitosan to a large extent. The biggest advantages of using carboxymethyl chitosan as a carrier to prepare the controlled release agent are biodegradability, water solubility, biocompatibility and natural non-toxic. It has special spongy structure and swelling property. Through appropriate cross-linking reaction and preparation method, the release of active substances can be controlled to the ideal release rate, the solubility and absorption of drugs can be improved, and the bioavailability of active substances can be improved. Carboxymethyl chitosan itself has biological activity and can be used as biological pesticide and plant growth agent [2].

Acetamiprid and imidacloprid are neonicotinoid insecticides developed in the 1980s and commercialized in the 1990s. Their chemical names are 1-(6-chloro-3-pyridyl)-N-nitroimidazole-2-imine, (E)-N-{[6-chloro-3-pyridine)methyl]-N'-cyano-N-methylacetamidine, respectively (Figure 1).
They can block the normal conduction of insect central nervous system by selectively controlling nicotinic acetylcholinesterase receptor in insect nervous system, which leads to paralysis and death of insect pests. Acetamiprid and imidacloprid have the characteristics of broad-spectrum, low toxicity (for mammals), and high efficiency. They are good substitutes for highly toxic pesticides and have been widely used in agricultural production [3].

![Structural formula of acetamiprid and imidacloprid](image)

**Figure 1.** Structural formula of acetamiprid (Light) and imidacloprid (Right).

In this paper, we prepared neonicotinoid-loaded carboxymethyl chitosan microspheres by suspension-crosslinking method, and discussed the effects of different crosslinking agents on the preparation of gel microsphere. The UV detection method of pesticide was established, and the release of pesticides was investigated. This work provides a kind of pesticide controlled release system with simple preparation and convenient detection, which has broad application prospects.

2. Experimental

2.1. Materials and Methods
Carboxymethyl chitosan was prepared by ourselves according to reference 4. Glutaraldehyde, calcium chloride and all other chemical reagents used in this paper were obtained from commercial sources and were of the highest purity available.

UV-Vis absorption spectra were recorded using a Lambda 35 UV-Vis spectrophotometer (PerkinElmer, US). Deionised water was obtained from a Milli-Q Element water purification system (Millipore Co., Billerica, MA, USA). Morphology was observed by electron microscope (Nikon, Japan).

2.2. Preparation of Microspheres
Gel microspheres were prepared by suspension crosslinking method using calcium chloride and glutaraldehyde as double-component crosslinking agent. The microspheres containing pesticides were prepared by mixing carboxymethyl chitosan in 30 mL deionised water and drugs (acetamiprid and imidacloprid) in 5 mL dichloromethane. Then, the mixture was stirred at 40 °C for 2 h. Subsequently the suspension was slowly dropped into the calcium chloride glutaraldehyde mixed crosslinking solution with a 5 mL injector, and crosslinked 1 h. The crosslinking liquid was removed by filtration. The gel microspheres were washed with deionized water for several times, and dried in a vacuum drying oven.

2.3. Observation of Diameter and Morphology of Microspheres
A few of the microspheres were placed on the glass slide. The morphology and dispersibility of the microspheres were observed by electron microscope with micrometer. A representative area was selected and no less than 200 particles were determined. The particle size range was divided into several units. The average particle size was obtained by statistical treatment.

2.4. Drawing of Standard Curves
A certain amount of pesticide standard was dissolved in an appropriate amount of solvent, and the maximum absorption wavelength of pesticide was obtained by UV scanning. In the same way, a series of pesticide solutions with different concentrations were prepared, and were measured the absorbances at the maximum absorption wavelength. The standard curve of absorbance Vs pesticide concentration was drawn with pesticide concentration as X-axis and absorbance as Y-axis.
2.5. Determination of Drug Loading and Encapsulation Efficiency
Weigh an appropriate amount of fully ground microsphere powder, and add an appropriate amount of solvent. After the drug was fully dissolved, centrifugation was performed. Take an appropriate amount of the supernatant and dilute to a constant volume. The absorbance value Y was determined at the maximum absorption wavelength. According to the standard curve of absorbance Vs pesticide concentration, the drug concentration X was obtained. The drug loading and encapsulation efficiency of the microspheres were calculated according to the following formula:

\[
\text{Drug loading} = \left( \frac{\text{mass of drug in microspheres}}{\text{mass of weighed microspheres}} \right) \times 100\%
\]

\[
\text{Encapsulation efficiency} = \left( \frac{\text{actual drug content}}{\text{theoretical drug content}} \right) \times 100\%
\]

2.6. Drug Release Properties of Drug Loaded Microspheres
A certain amount of drug loaded gel microspheres were put into 100 mL deionized water and released at 30 °C. Samples were taken at regular intervals and the same volume of fresh deionized water was added. The absorbance of the sample was measured at the maximum absorption wavelength, and the drug release rate was calculated according to the standard curve.

2.7. Statistical Analysis
In this study, all data are expressed as mean ± SD. Differences between groups were evaluated by one-way ANOVA followed by LSD-test, and P < 0.05 was considered statistically significant.

3. Result and Discussion

3.1. Preparation of Microspheres
The formation of gel microspheres prepared by formaldehyde, glutaraldehyde and calcium chloride as crosslinking agents was investigated, respectively. It was found that the forming conditions of gel spheres prepared by different crosslinking agents were quite different. The formaldehyde molecule was too short to form effective crosslinks due to the steric effect. When glutaraldehyde was used alone, due to the swelling effect, none of them could form a ball, but only a disc with strong swelling. Calcium chloride solution alone could form white spheres, but the strength of the spheres was very low and it was easy to break into powder. When glutaraldehyde was mixed with calcium chloride, it could form elastic ball with high strength. As shown in figure 2, the pesticide / carboxymethyl chitosan gel microspheres was prepared by using calcium chloride and glutaraldehyde two-component crosslinking agent. Most of the gel microspheres were spherical with a small amount of tailing. The color of microspheres prepared by different pesticides was slightly different.

![Figure 2. Gel microspheres prepared by suspension crosslinking method.](image)

3.2. Observation of Diameter and Morphology of Microspheres
Figure 3 showed the picture of gel microspheres through electron microscope. It could be seen from the figure that the size of the gel microspheres was uniform, and the appearance was a smooth and regular spherical shape with a particle size of around 1 mm. The inside of the gel microspheres was an interwoven network, and the drug was evenly embedded in the grid.
3.3. Drawing of Standard Curves
Using ultraviolet method, the standard curve of absorbance Vs pesticide concentration was drawn. The interference of the adjuvant in the microspheres on the absorption of the pesticide was subtracted. As shown in Table 1, the standard curve equations of acetamiprid and imidacloprid had good linear relationships between absorbances and mass concentrations. Compared with the HPLC detection method reported in the literature [5, 6], this method is more convenient and quick.

Table 1. The standard curve equation of pesticide by ultraviolet method.

| Pesticide  | Wavelength (nm) | Calibration curve         | $R^2$  | Linear range(ug/mL) |
|------------|-----------------|---------------------------|--------|---------------------|
| Acetamiprid | 250             | $Y=0.1921X-0.1864$        | 0.9997 | 1-8                 |
| Imidacloprid| 270             | $Y=0.0757X-0.0.0369$      | 0.9933 | 0-22                |

3.4. Determination of Drug Loading and Encapsulation Efficiency
The drug loading and encapsulation efficiency of the carboxymethyl chitosan microsphere on acetamiprid and imidacloprid were shown in Table 2. The drug loading and encapsulation efficiency of imidacloprid were higher than that of acetamiprid, which were higher than those reported in the literature [7, 8]. It was related to the physicochemical properties of the pesticides themselves and the structural characteristics of the carrier [9, 10]. It might be that imidacloprid interacted more easily with carboxymethyl chitosan, and its molecular size is easier to be embedded in the grid of net-like gel microspheres.

Table 2. The drug loading and encapsulation efficiency of microspheres.

| Item                | Acetamiprid | Imidacloprid |
|---------------------|-------------|--------------|
| Drug loading / %    | 25.40       | 39.28        |
| Encapsulation efficiency / % | 58.56 | 77.45     |

3.5. Drug Release Properties of Drug Loaded Microspheres
The release of drugs from carboxymethyl chitosan cross-linked gel system was studied. As shown in Figure 4, it can be seen from the cumulative release-time curve that the drug release rate is slow, there is no obvious sudden release phenomenon, and the sustained release time can reach more than 6 days, indicating that the pesticide-loaded carboxymethyl chitosan microspheres have good sustained-release properties. It also can be seen from Figure 4 that before 72 hours, the release of imidacloprid by carboxymethyl chitosan microspheres was faster, and after that, just the opposite. On the 6th, the release of acetamiprid is about to be completed, while the release of imidacloprid is less than 90%. This may be related to the loading capacity of carboxymethyl chitosan microspheres for imidacloprid and acetamiprid and the properties of the drug itself, such as water solubility and molecular structure characteristics. The mechanism of drug release needs further study.
4. Conclusion
In this paper, the pesticide-loaded carboxymethyl chitosan microspheres with smooth and regular appearance were prepared by emulsion crosslinking method. The method is simple and easy to operate. The experiment used UV to detect pesticide content, which was convenient and quick. The prepared sustained-release microspheres have high drug loading, encapsulation efficiency and good sustained-release performance. The sustained-release time could reach more than 6 days. This study would further promoted the application of carboxymethyl chitosan in pesticide controlled release agents.

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