Preparation and characterisation of poly(butylene succinate) microcarriers containing pesticide

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Microcarriers containing pesticide have been designed to improve drug stability and increase release efficiency, which is favourable to the environment and agriculture development. In this work, biodegradable poly(butylene succinate) microspheres containing pesticide, λ-cyhalothrin, as an active agent are prepared by the solvent evaporation induced phase separation method. The microspheres show a uniform morphology and a narrow particle size distribution. The pesticide content is measured by high-performance liquid chromatography. The prepared microcarriers demonstrate high-loading capacity, encapsulation efficiency and long release time, which may find potential applications as new pesticide formulations.

1. Introduction: Pesticides are compounds or mixtures that are used to prevent, destroy, or control any pest or undesired species of plants or animals [1]. There is an increasing demand for pesticide with the development of agricultural production [2]. However, traditional pesticides have inevitable limitations when put into wide use. The most prominent one is their powerful toxicity, which is not conducive to human health [3, 4]. The unnecessary waste will be generated due to inappropriate use, thus causing environmental pollution [5, 6]. Therefore, new types of pesticides with high efficiency, low toxic and environmental friendliness are in demand.

Microencapsulation is a process in which some materials including solids, liquids or gases are encapsulated with a hard shell or soft film to form microcapsules [7]. It is mainly used to increase the stability and lifetime of the core material, to manipulate and control its release time, and to be isolated from its surroundings. The applications of the microcapsules are numerous, such as anti-corrosive coatings [8], optimised conductive materials [9], pharmaceuticals [10], phase change materials [11], catalysts [12], powder perfume, self-healing coatings [13], protection of bioactive compounds [14], and so on. Owing to its long residual activity, controlled release as well as safety and low toxicity to mammals, microencapsulation plays a more and more important role in pesticide formulations [15].

A variety of works have been proposed to meet the different needs of users [16]. Pioneering researchers focused on the encapsulation of the pesticide and the polymer shell [17]. Both biological and chemical pesticides were achieved by encapsulation into starch matrices [18]. Researchers subsequently developed coating methods under different reaction conditions [19, 20]. Li and Xu [21] developed porous hollow silica nanoparticles synthesised by direct coating of inorganic nano-templates, as a novel controlled release carrier for controlled drug delivery. Mohammad and Fariba [22] created a two-step pre-polymer preparation to produce microspheres containing theophylline in polyurethane. More studies have been done to explore the drug release mechanisms [23, 24]. Recently, the issue of environmental protection has been taken seriously. Biodegradable polymers are a specific type of polymer that could degrade after service. They are widely used in drug research. By using biodegradable materials as polymer matrices, we can reduce environmental pollution, improve service safety, and make residues harmless. For example, nanoparticles of poly (lactide-co-glycolide) were used as the carrier for pulmonary drug delivery [25]. However, because of the high price and complex preparation of the shell materials they are often applied in human drug delivery or oral administration [26]. Poly(lactic acid) (PLA) microspheres have been used extensively for the encapsulation of a variety of pharmaceutical compounds [27]. However, PLA is a typical hydrolytically degradable polymer material and therefore needs a harsh dry storage environment. Poly(butylene succinate) (PBS) material is easier to store than PLA because it only degrades when coming in contact with enough microorganisms in soil or natural water. Therefore, PBS is a kind of biodegradable polymer with suitable characters and is expected to work as a novel biodegradable carrier material for pesticides.

In this work, we aim to produce pesticide-containing microspheres using biodegradable PBS as the carrier matrix and cyhalothrin as the active ingredient. The higher encapsulation efficiency of solvent evaporation method [28] reduces the amount of pesticide usage. At the same time, the package in the polymer prolongs the duration of the pesticide. Furthermore, the good adhesion behaviour of PBS microcapsules is demonstrated by the leaf experiment, which helps reduce the drug loss and increase the utilisation rate of pesticide.

2. Experimental

2.1. Materials: PBS was kindly provided by Xinjiang Blue Ridge Tunhe Polyester Co. Ltd. It was dissolved in the solvent as the encapsulation shell material. Poly(vinyl alcohol) (PVA, 1788, M_w = 70,000–80,000, hydrolysis degree = 87∼89%) was purchased from Aladdin Industrial Corporation and was dissolved in deionised water to use as the emulsifier. λ-Cyhalothrin and methanol (chromatographically pure) used for high performance liquid chromatography (HPLC) were purchased from J&K Chemicals. Dichloromethane and chloroform (analytically pure) were purchased from Beijing Chemical Co. Ltd and used as the solvent. Deionised water (resistivity > 18.25 MΩ cm) was produced in the laboratory and used as the aqueous medium.

λ-cyhalothrin is the raceme. There is no difference between the two different configurations of cyhalothrin in physical and chemical properties. However, they exhibit different bioactivity, toxicity as well as metabolism behaviour in the environment [29]. The pesticide was purchased as a mixture of two different configurations and used directly, so we use cyhalothrin in the following sections.

2.2. Preparation of the microcarriers: The solvent evaporation method was applied to prepare the microcarriers. PBS (10 g) and

cyhalothrin (10 g) were fully dissolved independently in 100 ml solvent (dichloromethane or chloroform). PVA was dissolved in deionised water to form a 0.5% PVA aqueous solution. Then 10 ml of the solution with different ratios of PBS/cyhalothrin (10/0, 9/1, 8/2, 7/3, 6/4, 5/5) and 40 ml PVA aqueous solution were mixed and emulsified with a high speed (6–7 kr/min) stirrer for 10 min to obtain the oil-in-water emulsion. Then the emulsion was transferred into a 100 ml three flasks with a stirring paddle and was stirred at the room temperature for solvent evaporation. After 6 h, microemulsion droplets were solidified and precipitated gradually with the evaporation of the solvent. Then the emulsion was centrifuged and washed with deionised water three times to remove emulsifier on the surface of microspheres. Finally, dried microspheres containing pesticides were obtained by lyophilisation.

2.3. Characterisations: The morphology of the microspheres was observed directly by using a scanning electron microscope (SEM, JSM 7401F, JEOL, Japan) with an accelerating electronic voltage of 10–15 kV. The particle size distribution was measured by using a laser particle size meter (laser particle size analyser, Mastersizer 3000, Malvern, UK).

2.4. Exploration of the HPLC measurement: To determine the drug loading capacity and encapsulation efficiency in microspheres and the release profile of pesticide, we obtain a fast and accurate method to measure the pesticide content by exploring the different test conditions of HPLC (Shimadzu, CTO/SPD/LC-16, Japan).

The optimisation of HPLC conditions: the column type was SB-C18, and the wavelength of the fluorescence detector (230 nm or 245 nm), temperature (25°C or 30°C), flow rate (1.25 or 1.00 ml/min) and flow phase ratio (methanol/water = 9/1 or 8/2) were used in the HPLC system and the injection amount was chosen as 20 µl. The analysis conditions were optimised to obtain the optimal working curve [30].

A standard curve of cyhalothrin: standard solutions of cyhalothrin (concentration of 1000, 100, 10, 1, and 0.1 µg/ml) were prepared in methanol. The standard solutions were detected under the conditions described above. By comparing the linear regression analysis of the area and height data at each condition, we got the standard working curve.

2.5. Loading capacity and encapsulation efficiency: A certain quantity of the microsphere was accurately weighed after grinding into powder. Then 1 ml methanol together with microspheres was added to a test tube. The samples were treated with ultrasonication for 10 min and then put in a shaking table overnight. The obtained suspensions were filtrated to remove any precipitate before analysis. With the optimal conditions of the working curve, the cyhalothrin concentrations in the microspheres were quantified by comparing the peak areas of the samples with those of cyhalothrin standards. After the weight of cyhalothrin was obtained, the loading capacity and encapsulation efficiency can be calculated by the following formula:

$$\mathrm{LC_T(the\ theoretical\ loading\ capacity)} = W_{\mathrm{DT}}/W_{\mathrm{M}},$$
$$\mathrm{LC_E(the\ experimental\ loading\ capacity)} = W_{\mathrm{DE}}/W_{\mathrm{M}},$$
$$\mathrm{EE(encapsulation\ efficiency)} = W_{\mathrm{DE}}/W_{\mathrm{DT}} = \mathrm{LC_E}/\mathrm{LC_T},$$

where W_{M} is the weight of microspheres and W_{D} is the weight of the drug. W_{DT} is the weight of drug used in the experiment and W_{DE} is the weight of drug tested and calculated by HPLC.

2.6. Release behaviour: Dried microspheres in a dialysis bag were immersed in a 100 ml mixture solution with a ratio of methanol/water = 1/1. The bottles were put in a temperature controlled shaker at 35°C and the samples were collected every 2 h (within one day) or 24 h (after one day). The amount of drug release was determined by measuring the concentration of cyhalothrin dissolved in the mixture solution by HPLC.

2.7. Contact angle of the emulsion on the leaf: The adhesion properties of the pesticide containing microspheres were examined by the comparison of the contact angle of ordinary water and the microsphere suspension fluid on the crops. The water and the microsphere suspension fluid were dripped on the surface of corn and rice leaves, respectively, and contact angles between leaves and the testing liquid were measured after 15 s.

The distribution of microspheres on the leaves was observed as well. The microsphere suspension fluid was sprayed on the leaves of corn and rice. After dried in the air, washed with water and stored in vacuum overnight, the microcarriers were observed under an SEM (JSM 7401F, JEOL, Japan). The microcarriers were prepared as described in Section 2.2 using chloroform as a solvent and the ratio of PBS/cyhalothrin was 8/2.

3. Results and discussion

3.1. Characterisation of microspheres: In this work, microspheres were prepared using two different co-solvents. The morphology and particle size distributions of the microspheres prepared from the two co-solvents are shown in Fig. 1. In both solvent systems of chloroform and dichloromethane, the microspheres show a certain size distribution (Figs. 1*c* and *d*). It can be seen that the particle size distribution in chloroform (Fig. 1*c*) is narrower than that in dichloromethane (Fig. 1*d*). The particle size of microspheres approximately ranges from 5 to 50 µm for chloroform, and 10 to 110 µm for dichloromethane, respectively. The laser scattering results are in accordance with the SEM images in Figs. 1*a* and *b*. In both solvents, the surfaces of the microspheres have wrinkles and pores (Figs. 1*a* and *b*). Furthermore, the microspheres produced from chloroform are spheres with some small folds and pores, while the microspheres from dichloromethane show the deeper wrinkles with an irregular shape. From the inserted picture in Fig. 1*a*, it is

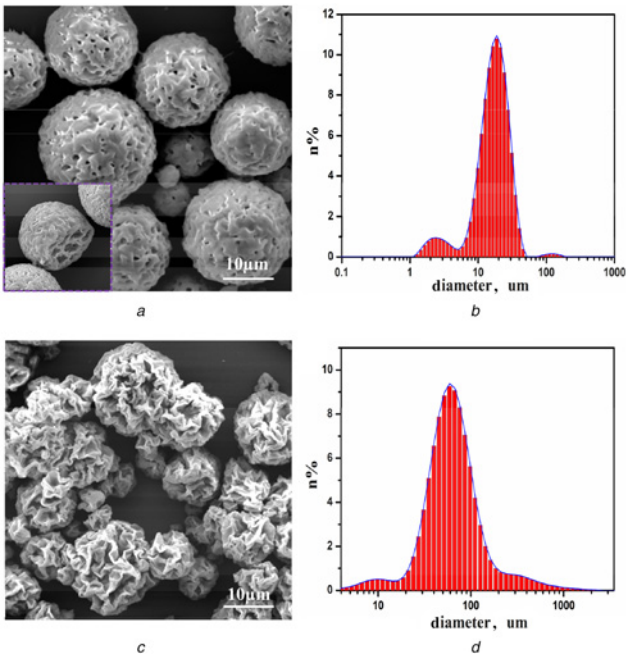

Fig. 1 *SEM images and particle size distribution of microspheres prepared by different solvents*
a, c SEM images
a, b Microspheres prepared by chloroform
c, d Microspheres prepared by dichloromethane
Inset in a presents the internal morphology of microspheres

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clearly shown that the internal morphology of the microspheres is highly porous with no distinct hollow interiors.

The different morphologies of the two types of microspheres originate from the preparation process. During the evaporation process of the solvent, the polymer concentration increases in the oil phase. Due to the phase separation of polymer and solvent, the organic solvent assembles and evaporates to become channels or voids and finally leads to a porous structure. It is well known that the porosity of microspheres is determined by many factors, such as polymer molecular weight, solvent removal rate, dispersed phase to continuous phase ratio as well as the preparation method. In our study, the solubility of PBS in chloroform is much higher than in dichloromethane. During precipitation, the large amount of PBS in the chloroform system can form more compact spheres with a porous internal morphology (inset in Fig. 1*a*), while the amount of polymers in dichloromethane is too low to maintain the shell, which makes the microspheres irregular with a wider size distribution and rougher surface.

Moreover, the boiling point of the solvent also plays a critical role to determine the morphology of microspheres. The boiling point of dichloromethane is much lower than that of chloroform (39.7 and 61.3°C, respectively). As the evaporation progresses, the polymer and pesticide condense together to form the emulsion droplets. The faster evaporation rate results in a rougher surface morphology. While the surface is generally smooth when the polymer precipitates slowly due to slow removal of the organic solvent. The type of solvent determines the rate of volatilisation in a similar trend to the effect of temperature on the rate of volatilisation.

The surface and internal morphology of the microspheres are expected to have a considerable effect on the loading capacity, encapsulation efficiency, release behaviour of the loaded small molecules and even the adhesion properties. These will be discussed in the following sections.

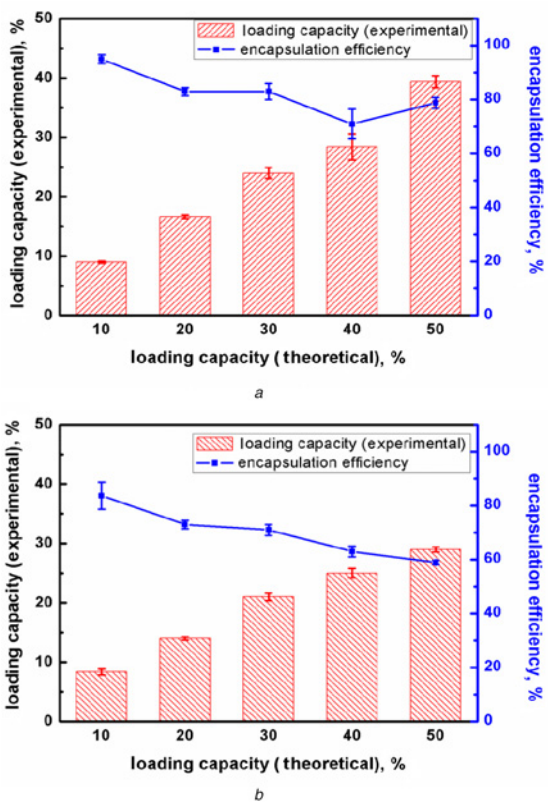

Fig. 2 *Loading capacity and encapsulation efficiency of microspheres prepared by different solvents*
a Chloroform
b Dichloromethane

3.2. Optimisation of HPLC conditions: To determine the drug loading capacity and encapsulation efficiency in microspheres and the release profile of pesticide, we followed a fast and accurate method to measure the pesticide content by exploring the different test conditions of HPLC.

The HPLC system used the SB-C18 column. The column temperature was 30°C, the flow rate was 1.00 ml/min, the detector wavelength was 245 nm, and the flow phase ratio was alcohol/water = 8/2. Under the above optimal condition, the corresponding working curve of cyhalothrin concentration could be achieved. The linear regression equations were established as follows: $Y = 39375X + 33326$, where Y and X are a concentration of pesticide and curve area, respectively.

3.3. Loading capacity and encapsulation efficiency: The loading capacity and encapsulation efficiency of pesticides in the microspheres have an important effect on the overall performance of the pesticide delivery system. The test method of HPLC was applied to examine the pesticide microspheres obtained in the experimental part.

From Fig. 2, the loading capacity and encapsulation efficiency have a similar trend in the two systems. It is clear that the experimental loading capacity increases with the increase of the theoretical loading capacity (Fig. 2*a*). However, the encapsulation efficiency decreases with the increase of the feeding (Fig. 2*b*). It is reasonable that polymers could not completely encapsulate the pesticide with the increase of the drug/polymer ratio. Therefore, the corresponding capacity becomes lower.

At the same time, there is a slight difference in the two solvent systems. All the loading capacity and encapsulation efficiency in chloroform are higher than those in dichloromethane. The encapsulation efficiency of the microspheres is influenced by many factors, such as polymer solubility, solvent evaporation rate, water to oil ratio and drug concentration [31]. In the present study, the solubility difference of polymer in different solvents results in the different surfaces and internal morphologies. The porous structure might let drug leak out in the process of solidification and washing. Therefore, the different microsphere morphologies affect the drug loading capacity and encapsulation efficiency of the pesticide.

3.4. Release behaviour of the pesticide from the microcarriers: The release profiles of pesticide in different microspheres are presented in Fig. 3. From the curves, there appear to be two distinct kinds of pesticide release. The release curves of the pesticide in the microspheres fabricated from chloroform exhibit steady release of pesticide over time. In contrast, in the system of the microspheres precipitated from dichloromethane, there is a great deal of drug released on the first day, and the release rate slows down later.

The difference in the release behaviour can be attributed to the different structural characteristics of the microcarriers. Larger holes on the microspheres might contribute to a sudden release. Another possible reason is that the microspheres produced from chloroform demonstrate a slower release rate due to their smaller surface areas for drug diffusion. A slow release represents drug release by diffusion through the polymer matrix. This release pattern is consistent with many matrix-type drug delivery systems.

At the same time, the encapsulation rate of the drug may affect the drug release rate. Due to the fact that these microspheres are mainly prepared through a solvent evaporation process, they have dense and tight skin structures. Therefore, the release of embedded drug from the inner to outer becomes difficult. The initial release rate depends on the diffusion of cyhalothrin in PBS, which may be the determining factor. It can be seen in both Fig. 3*a* and *b* that the slowest release rate of cyhalothrin from the microsphere was at 50%, while the fastest was at 20%. It seemed that the

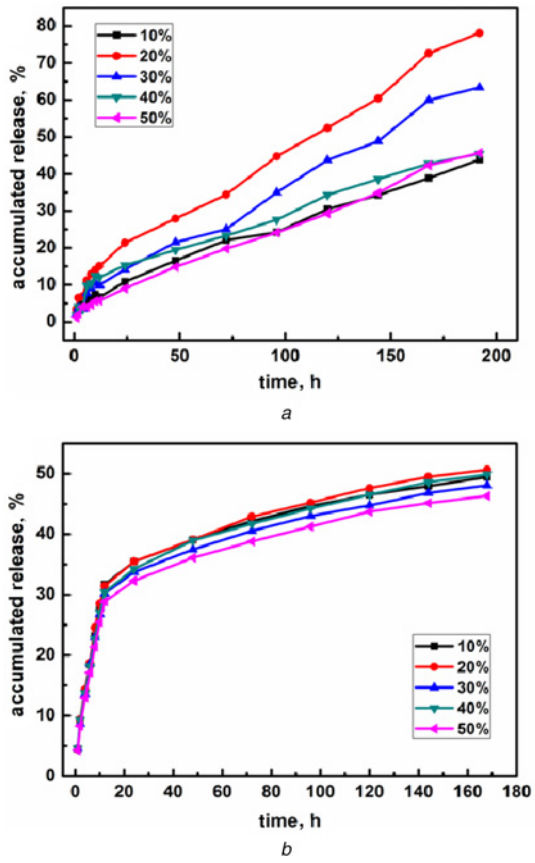

Fig. 3 *Release behaviour of microspheres prepared by different solvents*
a Chloroform
b Dichloromethane

higher concentration of the polymer in solvent results in the accelerated rate of solidification, the increasing amount of the encapsulated pesticide, and also the decreased release rate. Based on the above-mentioned results, it is likely that the polymer/pesticide ratio affects both the encapsulation efficiency and the release behaviour.

3.5. Adhesion properties of the pesticide microcarriers: To observe the distribution and adhesion of microspheres on the leaf surface of crops, the SEM was used to observe the original plant leaves. The microsphere suspension fluid was sprayed on the leaves, and the SEM was used for observation after dried in the air and washed with water. The leaf surface of the crop is not smooth. Some surfaces of leaves have villi and some have oil layers. These structures will help plant adhere to some suspended particles, such as dust. In this case, because of the low particle density and larger particle surface area, the present microsphere can be adsorbed effectively on the leaf surface. In SEM micrographs (Fig. 4), we can see that the microspheres have a relatively uniform distribution on the leaves.

Also, the contact angles of water and microsphere dispersion on leaves surface were measured The contact angles of water on the corn and rice leaves are 107° and 93°, respectively (Fig. 5), while the contact angles of microsphere dispersion on the corn and rice leaves are 85° and 77°, respectively. The microsphere suspensions have better wetting property than water (Fig. 5).

4. Conclusion: In this study, an environmentally friendly pesticide containing microcarriers was prepared through the solvent evaporation-induced phase separation method, using biodegradable PBS as the carrier matrix and cyhalothrin as the active ingredient. The SEM and light scattering results have confirmed the wrinkled

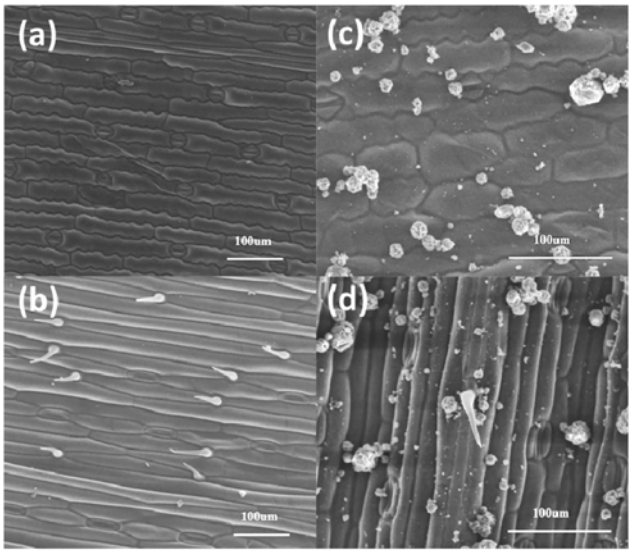

Fig. 4 *SEM images and microspheres of corn and rice leaves*
a, b SEM images of corn and rice leaves
c, d Microspheres of corn and rice leaves

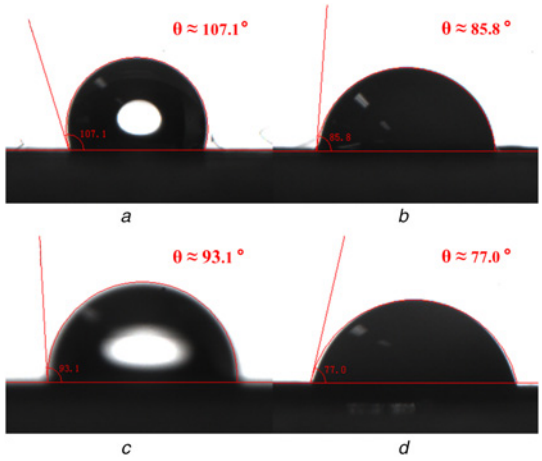

Fig. 5 *Contact angle of water and microsphere dispersion on*
a, b Corn leaves
c, d Rice leaves

surface and narrow size distribution of the microspheres. The column temperature 30°C, the flow rate 1.00 ml/min, the detector wavelength 245 nm, and the solvent ratio of alcohol/water = 8/2 in the eluent, were recommended as an optimal condition for HPLC. Under these conditions, we obtained the drug loading capacity and encapsulation efficiency. Also, the drug release behaviour was also determined by HPLC.

The solubility difference of the polymer in different solvents results in the different surfaces and internal morphologies. Therefore, the different microsphere morphologies affect the drug loading capacity, encapsulation efficiency and release behaviour of the pesticide. Comparing the two different solvent systems, a good solubility of the polymer in chloroform means a better entrapment of the pesticide so as to reduce initial burst release than that in dichloromethane. Meanwhile, the adhesion properties demonstrate a good prospect in agricultural applications. When microcarriers are produced on a large scale, they can be stored and transported for spraying. After spraying, the microcarriers will adhere to the leaves, and the pesticide will be released gradually as well as the PBS slowly degraded.

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