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Preparation and characterization of microspheres embedded hydrogels for controlled release of avermectin

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In this study, using sodium alginate and chitosan as carrier materials, release- controlled microspheres loaded avermectin were prepared by complex coacervation method, the drug grinding time and sodium alginate concentration were determined, and the drug loading rate and entrapment efficiency of the prepared microspheres were 30.38% and 81.47%, respectively. At the same time, the composite systems of microspheres Impregnated CS-PVA hydrogels were prepared. The influence of pH and temperature on swelling ratio of microspheres and composite systems were determined. Results showed that microspheres and composite systems have good pH and temperature responsive behavior. The release time of avermectin could be prolonged by embedding microspheres into the hydrogels, and the release mechanism of abamectin in microspheres and composite system Ⅲall fit no-Fick diffusion.

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Keywords: Abamectin, Microspheres, Hydrogels, Swelling ratio, Release mechanism

1. Introduction

Global demand for food has encouraged the maximization of agricultural production due to the increase of the world population. It is necessary that various measures are taken to ensure grain production[1,2].Among these measures, Pesticides play a very important role by effectively control the pests, because more than 30–40% of the food production is lost due to pests[3].However, this has resulted in serious environmental pollution and ecological issues since a large amount of applied pesticides often never totally reaches its intended target due to their degradation, volatilisation and leaching[4,5]. Hence, harmful effect of pesticides to the environment and health is a major limitation on their application, and safe handling of these pesticides is also very significant[6]. In recent years, new formulations have been developed， which may modify performance of active compound which have less impact on environment. Controlled release formulations (CRF) is to gradually deliver the active substance over time, and the aim is to limit the amount immediately available for transport and degradation processes. CRF also allow the extension of the substance activity, the reduction of residue amounts on food stuffs,

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savings in man power and energy by reducing the number of applications required, as well as an increased safety for those applying pesticides [7-12].

To achieve the desired controlled release characteristic, some naturally occurring, cheaply available, biodegradable, and environmentally friendly matrices have been used. Some polysaccharides such as alginate and chitosan applied employed as carriers in controlled release formulations that are used in a variety of areas, including agriculture[13,14]. Chitosan (CS), principally derived from chitin by deacetylation with an alkali, displays excellent properties such as biocompatibility, nontoxicity and biodegradability[15,16]. Alginate is a naturally occurring copolymer of 1,4-linked β-d-mannuronic acid (M) and α-l-guluronic acid (G) extracted from brown seaweeds. The character of biodegradable, low toxicity and low cost make it suitable for use in biomedical and pharmaceutical formulations[17-19]. Alginate salts are known to form a three-dimensional network structure when in contact with two valance ions such as calcium, copper, nickel ions, and this characteristic has been used to produce sustained release particulate systems for a variety of drugs, proteins, and pesticides. Furthermore, by simultaneous electrostatic interaction between protonated amino groups of CS and carboxyl groups of alginate, polyelectrolyte complex is formed[20].

Avermectin is a kind of natural product derived from streptomyces avermitilis fermentation. It is a promising biological pesticide in the prevention and control of agricultural pests. However, avermectin is prone to degrade under the influence of ultraviolet light and soil microorganism, and the control effect is reduced[21,22]. Therefore, it is helpful to maintain the stability of avermectin by preparating microspheres or microcapsules formulations.

In this paper, the AVM-SA microspheres were prepared by dropping alginate solution containing abamectin into $CaCl_2$ solution. In addition, the prepared AVM-SA microspheres were took into chitosan acetic acid aqueous solution to formed AVM-SA-CS hydrogels microspheres. At the same time, the mixed gel of chitosan and polyvinyl alcohol were prepared firstly, then the AVM-SA-CS microspheres were embedded in the CS/PVA hydrogels to form compsite systems.The effects of the influence factors such as temperature and pH on the swelling properties of the polyelectrolyte complex microspheres and compsite systems were investigated, and the release properties and drug release mechanism of abamectin from the microspheres or compsite systems were also studied. Those studies provide a promising approach of preparation of cotrolled release formulation to improve the persistence of abamectin.

2. Experimental

2.1. Materials

Sodium alginate (SA) was purchased from Shanghai Chemical Co. Ltd (China). CS (MW is 6.0×105, degree of deacetylation is 85%) was acquired from BASF Chemical Co., Ltd (Tianjin china). Polyvinyl alcohol (PVA) and calcium chloride anhydrous were analytical grade and purchased from BASF Chemical Co., Ltd (Tianjin china). Glutaraldehyde (50%) was purchased from Tinajin zhongxin chemtical Co., Ltd. Abamectin (AVM, 96%) was provided by Jingbo Agrochemicals Technology Co., Ltd (Binzhou china). All the other chemicals and reagents used were analytical grade, and the solutions were prepared with double distilled water.

2.2. Preparation solution and abamectin suspension

Different concentration of alginate solutions were made by dispersing alginate powder into double distilled water and stirring to complete dissolution at temperature of 60℃, and overnight reserved. 2% (w/w) $CaCl_2$ aqueous solution was prepared by dissolving $CaCl_2$ into double-distilled water followed by stirring for at least 30 min. 0.6% (w/w) chitosan was obtained by taking a certain amount of chitosan into 1% (v/v) acetic acid aqueous solution to form homogeneous solutions.

Abamectin suspension was prepared by wet grinding process. Abamectin and dispersing agents were mixed to form homogeneity and milled in the ISSMJ 0.1-1 Vertical sand mill (Shenyang Research Institute of chemical industry) for certain time, then the homogeneous suspension was obtained by filtering to remove grinding media.

2.3. Preparation of AVM-SA-CS microspheres beads

The AVM-SA suspension was prepared by taking the abamectin suspension into the prepared alginate solutions at a ratio of 10% (w/w), and then gently stirred to form uniform mixing system at room temperature. The AVM-SA microspheres were formed by injecting prepared AVM-SA suspension through a 0.5 mm syringe needle into 2% $CaCl_2$ solution under gentle magnetic stirring, and spherical beads were formed instantaneously and kept crosslink with Ca^{2+} in solution for 1 h. the prepared AVM-SA microspheres were rinsed with deionized water for three times. Then, the prepared AVM-SA microspheres were took into 0.6% (w/w) chitosan acetic acid aqueous solution to formed AVM-SA-CS microspheres under gentle stirring to crosslink for 1h. AVM-SA-CS microspheres were rinsed with deionized water for three times and dried to constant weight at 54℃ for futurecharacterization and analysis.

2.4. Preparation of CS-PVA hydrogel

CS-PVA composite hydrogels were prepared according to the method of literature[23]. CS (1.0g) was dissolved in 80 mL acetic acid solution (0.1 mol/L) in a 250 mL flask, removed impurities after complete dissolution of CS, 6.0 g PVA power was added into the CS solution to dissolve completely with constant stirring at 80 ℃, then slowed down to room temperature, after degassing, the mixed solution of CS-PVA was continuous stirring for 24h to form a transparent and homogeneous solution. 20 mL glutaraldehyde aqueous solution (0.425 mol/L) was added into flask and the crosslinking reaction maintained for 20 min at 25 ℃, CS-PVA composite hydrogels were obtained after adjusting pH of the crosslinking system to 7.0 by saturated solution of sodium phosphate dibasic.

2.5. Preparation of CS – PVA hydrogel embedded microspheres

The prepared AVM-SA-CS microspheres were added to the mixed solution of polyvinyl alcohol and CS, and dispersed evenly, adding crosslinking agent glutaraldehyde to form composite systems with a certain percentage of hydrogel and microspheres Ⅰ-Ⅴ (tab1). The obtained hydrogel/ microspheres systems were rinsed with distilled water for three times, dried at room temperature for 24 h, and subsequently dried at 54 ℃in an oven to a constant weight.

Table 1.Compositions of PVA-CS hydrogel with AVM-SA-CS microspheres.

Composite system	AVM/SA/CS microspheres (g)	PVA/CS hydrogel (L)
I	0	1
II	10	1
III	20	1
IV	30	1
V	40	1

2.6. Drug loading content of microspheres

A total of 0.05g (accurate to 0.0002g) of dried microspheres were added to the amount of methanol, after ultrasonic treatment, the supernatant of sample was obtained after centrifugation with 4000 r/min for 15 min, and this supernatant was fixed with methanol to 100mL, the avermectin content determined using high performance liquid chromatography method. The drug loading content (L%) of dried microspheres are calculated using the Eq. (1):

$$L\% = =(Wa/Wt)\times 100 \quad (1)$$

where Wa is the weight of avermectin in microcapsules mixed system and Wt is weight of microcapsules.3. Results and discussion.

2.7. Measurement of swelling ratio

Dried samples were carefully weighed and immersed in a definite volume of water at definite pH and temperature and taken out at predetermined time intervals. The swollen samples were blotted with filter paper to remove excess water, and then weighed on sensitive balance. Swelling ratio (SR) of the sample was defined as grams of water absorbed by per gram of sample and calculated according to the Eq. (2):

$$SR= (\%) = ((Ws-Wd)/ Wd)\times 100 \quad (2)$$

where Ws and Wd is the weight of the swollen beads and dry beads, respectively.

2.8. Morphological characterization

Micrographs of the external surface of the samples was obtained by scanning electron microscopy (JEOL, JSM-7500F, Japan). Prior to observation all dried samples were coated with a thin (2 nm) layer of gold.

2.9. The cumulative release properties of avermectin

About 50 mg of dried samples with were put in conical flasks containing 500 mL dissolution media (distilled water) and incubated in a shaking water bath at 25 ℃ under 50 rpm. At specific time intervals, 5mL of the solution was withdrawn and replaced with an equal amount of fresh dissolution media to maintain a constant volume. Abamectin content of collected solution was determined by UV-visible spectrometer at 245 nm. From the absorbance values, the cumulative percent released was determined.

3. Results and discussion

3.1. The effect of milling time on the particle of abamectin

According to stokes' formula, the particle size is related to the stability of the suspension, and small particle size has high suspension ability within certain range[24]. Good dispersion and suspension rate of drug could ensure the drug content of microspheres. In this study, the effects of milling time on the particle of abamectin were investigated (showed in the Fig.1). The result showed that the particle size of abamectin decreased with milling time extending. However, when the milling time reached 120 min，the particle size of abamectin had no significant change. Therefore, considering the grinding effect and energy loss, the grinding time was set to 120 min.

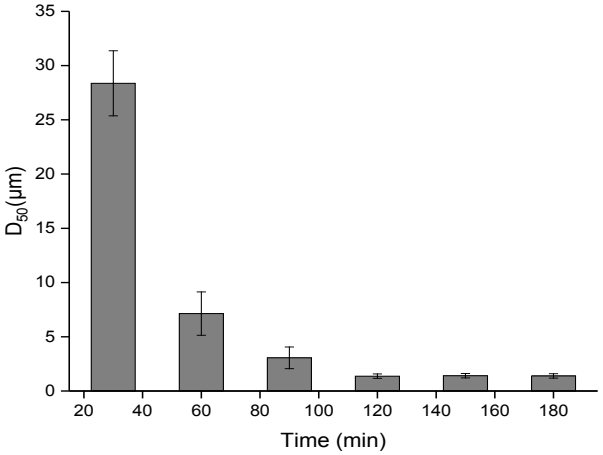

Fig. 1. The effect of milling time on D_{50} of avermectin suspension.

Table 2 showed that the effect of different mass fraction of sodium alginate on the formation of AVM/SA/CS microspheres. With the increase of the mass fraction of sodium alginate, the formation effect was better, but when the mass fraction was more than 3%, the viscosity of the mixed system was too large to prepare microspheres.

Table 2. Effect of the mass fraction of sodium alginate on the formation of microspheres.

NO.	SA/%	microspheres formulation
1	1	no form microspheres
2	2	formed irregular microspheres
3	3	easy to shape, and microspheres with smooth surface
4	4	the viscosity of the system was too large to form microspheres

Based on the above conditions, the alginate-chitosan microspheres and polyvinyl alcohol -chitosan hydrogel were further investigated. The SEM images of microspheres and PVA/CS hydrogel is showed in fig. 2. As shown in Figure 2a, the microspheres had regular shapes, and the surface of microspheres was smooth without adhesion. The entrapment efficiency and drug loading rate of microspheres prepared under optimal conditions were 81.47% and 30.38%

respectively. At the same time, as can be seen from Figure 2b, chitosan and polyvinyl alcohol formed interpenetrating network hydrogel with dense porous structure, uneven surface and large surface area. The release time of the drug can be controlled effectively by embedding the microspheres in those composite gels.

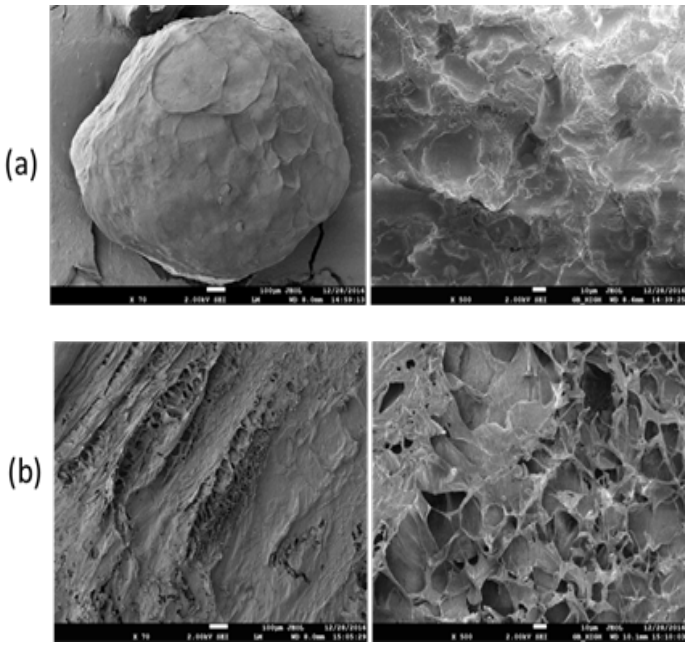

Fig. 2. SEM images of microspheres and PVA-CS hydrogel. (a) SEM images of microspheres; (b) and SEM images of PVA-CS hydrogel.

3.3. Effects of pH and temperature on swelling ratio of microspheres and composite systems

Swelling behavior is very important property of microspheres because they have great influences on controlled drug delivery behavior[20]. Chitosan and alginate gels have attracted much attention due to their sensitivity to temperature and pH[25,26]. The change of swelling characteristics of the hydrogel microspheres with the temperature and pH can control the release of the drug. The effect of pH and temperature on swelling ratio of microspheres and composite systems were showed in fig.3 and fig. 4.

As can be seen from fig. 3a, the swelling ratio of AVM-SA-CS microspheres and CS-PVA hydrogels increases sharply with increasing of temperature from 20 to 40℃, and then decreases with further increase of temperature. In addition, the results showed that the temperature has a significant effect on the swelling ratio of AVM-SA-CS microspheres than CS-PVA hydrogels. Similar results were also shown in the figure 3b, the results showed that with the increasing of pH, the swelling ratio of AVM-SA-CS microspheres increased first and then decreased. However, the swelling ratio of CS-PVA hydrogels increased continuously with the increase of pH. The characteristic for variation of swelling ratio with pH and temperature illustrates that AVM-SA-CS microspheres and CS-PVA hydrogels had good pH and temperature responsive behavior, the release of avermectin could be achieved by controlling temperature and pH.

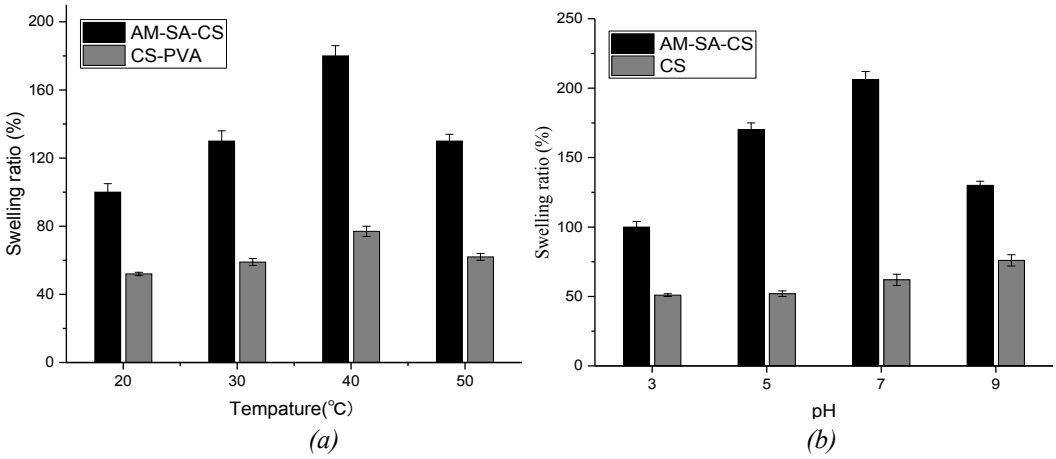

Fig. 3. Swelling property of AVM-SA-CS microspheres beads and CS-PVA hydrogels with different pH and temperature Error bars represent standard deviation from the mean (n = 3).

The trends of swelling ratio of composite systems of AVM-SA-CS microspheres in CS-PVA hydrogels with temperature and pH were showed in Fig.4. It can be seen from Fig. 4a, the swelling ratio of five composite systems increased firstly and then decreased with increasing of temperature.When the temperature was reached to 40℃, the swelling ratio reached the highest. fig. 4b showed that the swelling ratio of composite systems increased with increasing of pH from 3 to 7, and then decreased with further increase of pH exceptcomposite system I.

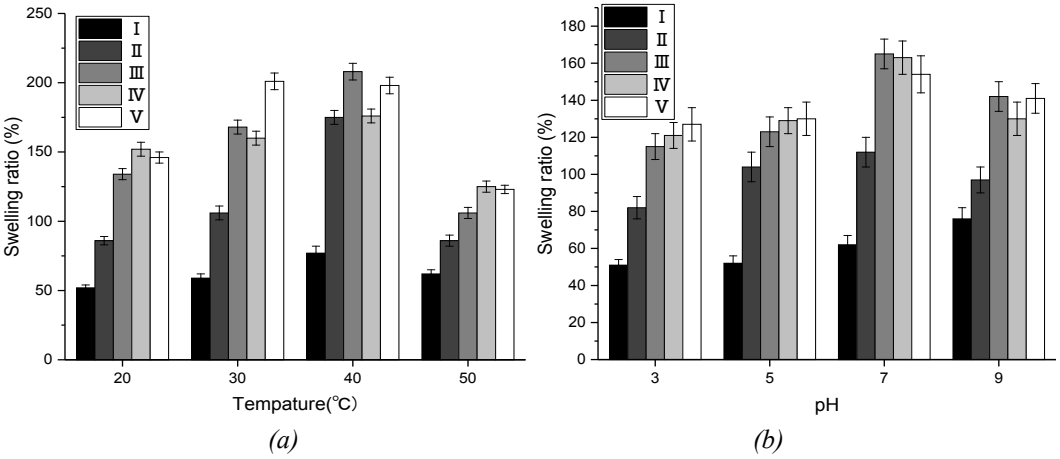

Fig. 4. Swelling property of five Composite systems with different pH and temperature Error bars represent standard deviation from the mean (n = 3).

Overall, the curves in the fig.4 indicated the different proportions of composite systems have influence on swelling rate at different temperature and pH, and when the amount of AVM-SA-CS microspheres in CS-PVA hydrogels was 30 g/L (composite system III), the swelling behavior was most obvious under 40℃and pH 7.

3.4 Controlled release behaviors of microspheres and composite system III

Figure 5 shows the release of avermectin from AVM-SA-CS microspheres and composite system III (AVM-SA-CS microspheres in CS-PVA hydrogels was 30 g/L). It can be seen that the AVM-SA-CS microspheres had more obvious burst release phenomena relative to the composite system III. The burst release phenomena indicate that there were a amount of avermectin on the surfaces of mircrospheres by weak interaction forces between carriers and avermectin[27]. Accordingly, the composite system III entrapped microspheres into polyvinyl alcohol and reduced the release rate of drugs on the microspheres surface. Within 240 min, the amount of avermectin released from AVM-SA-CS microspheres was 65.16%, and it was higher than the amount of avermectin from the composite system III. It also reveals the relationship between cumulative release percentage and swelling rate. The relatively high release amount of the studied microspheres should be related to the high degree of swelling ratio of the microspheres.

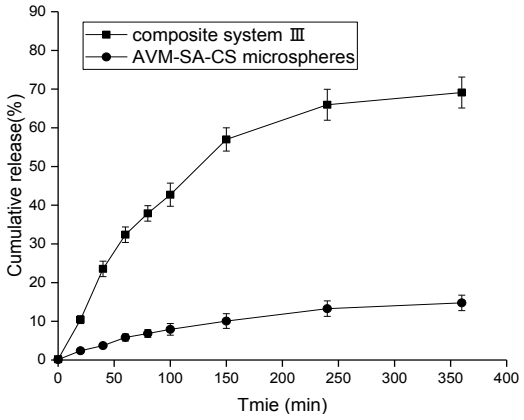

Fig. 5. Cumulative release of composite system III and AVM-SA-CS microspheres Error bars represent standard deviation from the mean (n = 3).

Drug release kinetics was analyzed by fitting with mathematical models first-order, Higuchi, zero-order and Riger-Peppas equations to explain the drug release mechanism. The release parameters of 'n', 'k' and 'n' are summarized in Table 2. According to regression coefficient 'R^2' analysis, the drug release mechanisms of AVM-SA-CS microspheres and the composite system III are more in line with the Riger-Peppas model. The Ritger–Peppas[28] equation is as follows:

$$M_t/M_\infty=kt^n$$

where M_t and M_∞ are the amount of avermectin released at time t and at equilibrium, respectively, n is the diffusional exponent, and k is a proportional constant. In general, when $n \leq 0.43$, the drug release mechanism is Fickian. If $n \geq 0.85$, Case II transport is detected and the zero-order release. When $0.43 < n < 0.85$, anomalous (non-Fickian) transport occurred[29]. Fitting of the microspheres and the composite system III to the Ritger–Peppas model shows n values between 0.43 and 0.89 (Tab.3). This indicates that the release mechanism of abamectin in microsphere and composite system Ⅲ is no-Fick diffusion.

Table 3. Release models and release parameters.

samples	Zero order		First order		Higuchi		Riger-Peppas		
	k	R^2	*k*	R^2	*k*	R^2	*k*	*n*	R^2
AVM-SA-CS	0.1614	0.8212	0.0031	0.9040	4.1228	0.9344	2.0540	0.6386	0.9645
III	0.0361	0.9164	0.0004	0.9258	0.8957	0.9639	0.3689	0.6504	0.9896

4. Conclusions

In this study, AVM-SA-CS microspheres loaded avermectin and CS-PVA hydrogels were successfully prepared. The prepared microspheres were embedded in CS-PVA hydrogels to form composite systems (Ⅰ-Ⅴ). The effect of milling time and sodium alginate concentration on the particle of abamectin and AVM/SA/CS microspheres were determined. Various factors influencing the swelling degree of microspheres and composite systems were investigated. When the milling time and the alginate concentration were 120 min and 3%, the shape of microspheres were regular, diameter was about 0.7 mm, and the entrapment efficiency and embedding rate of microspheres was 81.47% and 30.38%, respectively.

The effect of pH and temperature on swelling ratio of microspheres and composite systems were showed that AVM-SA-CS microspheres and composite systems have good pH and temperature responsive behavior. It is also observed that abamectin release from the AVM-SA-CS microspheres was much higher than the composite system Ⅲ, and the release time of composite system Ⅲ is prolonged Consequently, the burst effect of the abamectin is reduced. These obtained results illustrate that composite system formed by embedding SA-CS microspheres into PVA-CS hydrogel may offer suitable approaches for the preparation of new controlled drug delivery systems for agrochemical applications to improve pesticide stability and persistence, and reduce pollution to the environment.

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