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Preparation of Emamectin Benzoate·Hexaflumuron Granules Based on Response Surface Methodology

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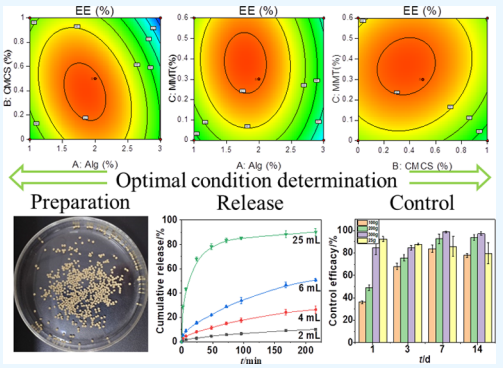
ABSTRACT: In order to obtain particles with an optimal loading rate and encapsulation efficiency and to explore the effects of sodium alginate, carboxymethyl chitosan, and bentonite on the particle loading rate and encapsulation rate, the preparation parameters of particles were optimized by the response surface method. A series of particles with constantly changing components were prepared, and the particle loading rate and encapsulation rate were determined. The release experiment of granules in different mass release media was implemented, and the optimal loading rate and encapsulation efficiency of particles were used to control the fall armyworm (FAW). The results showed that when the amount of sodium alginate was 1.83%, that of carboxymethyl chitosan was 0.41% and that of bentonite was 0.37%. The maximum theoretical value based on the response surface simulation was 92.63%, and the actual value at this ratio was 91.61%, which was 98.90% of the theoretical value. The release assay indicated that the mechanism of particle release in 2, 4, and 6 mL of the release medium was non-Fickian diffusion, and the controlled mechanism in 25 mL of the medium was Fickian diffusion. The beads were spread directly into maize leaf whorls in field production; at 14 days after application, the efficacy reached 91.28−98.82%. The combination of emamectin benzoate and hexaflumuron granules has a good control effect on the FAW.



1. INTRODUCTION

At present, in most reports of pesticide granule preparation, the preparation formulation is changed by gradually increasing or reducing the proportion of each component according to certain rules. Then, a series of tests such as drug loading rate, encapsulation efficiency, and vitro release experiments were carried out, and finally the best performing set of data was taken as the optimal preparation parameters.[1] Wang et al. reduced the quality of alginate by 0.05, 0.1, and 0.2 g, while the ratio of clay (cationic cellulose-modified bentonite) increased by 0.05, 0.1, and 0.2 g to change the particle formula; as a result, the optimal release performance of bentonite was 10%.[2] Zhang et al.[5] synthesized six BSRNFs (biochar-based slow-release nitrogen fertilizers) by changing the ratio of the carbon fertilizer and studied the nitrogen release mode and the mechanism of nitrogen release. The results showed that when the carbon fertilizer ratio was 2:1, the release effect was the best.[3] Luo et al. prepared chitosan(CS)/montmorillonite-(MMT) composite microspheres containing tanshinone IIA by changing the ratio of CS and MMT, and the samples with CS:MMT (10:2) had the highest packaging efficiency (48.18 ± 2.54%) and the slowest continuous drug accumulation release rate in phosphate buffer (pH 7.4).[4] Obviously, the optimal results of these previously published articles are artificially set sequences. In their report, they changed the granule formulation by correspondingly increasing or decreasing the content of some ingredients; for example, one component increases by 0.05, 0.1, or 0.2 g and the other component decreases by 0.05, 0.1, or 0.2 g correspondingly because this is not a continuous change process, and so there may be better results.

Response surface methodology is a widely accepted statistical method for designing experiments, evaluating the individual and interaction effects of independent variables, and optimizing the process parameters with a limited number of experiments.[6−8] It is often used for the parameter optimization of the extraction, processing, and production process. Zhu et al.[9] employed response surface methodology to optimize the extraction process of crude polysaccharides from pomegranate peel with water and obtained the best extraction conditions. Huo et al.[10] used the response surface method to optimize the preparation of microencapsulated phase change materials, which obviously improved the energy storage and temperature

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regulation of Micro-P. Qu et al.[11] optimized the preparation conditions for the calcium-modified Zn−Ce/Al_2O_3 heterogeneous catalyst by using the response surface method. Huang et al.[12] optimized the welding process in connecting borosilicate glass by picosecond laser pulses based on response surface methodology; the results indicated that the weld seam was continuous and uniform. But it is rarely reported in the preparation of pesticide granules.

The combination of pesticides can not only solve the problem of high toxicity and the poor effect of the single pesticide but can also delay the emergence of the acting substance resistance, which has an ideal use effect.[13] The combination of emamectin benzoate (Eb) and hexaflumuron (He) is a common registered insecticide in China, which has been used in the control of the fall armyworm (FAW), beet nightworm, *Rhipicephalus sanguineus*, etc.[14] Dai et al.[15] carried out the field efficacy of 10% emamectin benzoate·hexaflumuron WDG against *Spodoptera exigua* in cabbage; the results were that the field efficacy of 10% emamectin benzoate·hexaflumuron WDG 450 g/hm^2 was significantly higher than that of the control agents (1% emamectin benzoate EC and 5% hexaflumuron EC). The efficacy of the mixture of 2.5% hexaflumuron and emamectin benzoate EC in controlling *Laphygma exigua* on cabbages was conducted by Ji et al.[16] and the results showed that 2.5% hexaflumuron and emamectin benzoate EC could control *L. exigua* well; the control effect was significantly higher than the control and the safety of crops. But its dosage form is mainly emulsion and water-dispersible granules, with a small amount of suspension and microemulsion. However, the combined use of emamectin benzoate and hexaflumuron has rarely been reported in granules.

The development and use of pesticide sustained-release agents can extend the duration of pesticides and reduce the frequency of application so as to reduce the pollution of pesticides to the environment and improve the utilization rate of pesticides.[17−19] Yang et al.[20] used a superlarge slow-release humic acid compound fertilizer in a peach orchard for a two-year field experiment of three treatments, and the results showed that the production cost was reduced, the economic benefit was improved, and the environmental nitrogen loss was effectively reduced. Our previous research has also shown that the effective time of granules for pest control is longer than reported spraying pesticide solutions, and the control efficacy can be significantly improved.[21]

In this paper, a series of particles were prepared by using alginate and carboxymethyl chitosan as the substrate and the combination of Eb and He as the model pesticide. The encapsulation rate as an evaluation index via nonlinear mathematical model fitting and the preparation process was optimized. The encapsulation rate, loading rate, and in vitro cumulative release rate were determined by HPLC. The present study aims to optimize the preparation conditions by using the response surface method to obtain the best encapsulation rate and loading rate and to determine the control effect of the optimal performance granule in the control of the FAW.

2. RESULTS AND DISCUSSION

2.1. Determination of the Drug Loading Content and Encapsulation Efficiency of Eb·He Granules. The effects of sodium alginate, carboxymethyl chitosan, and bentonite on the gel encapsulation rate are shown in Figure 1. Sodium

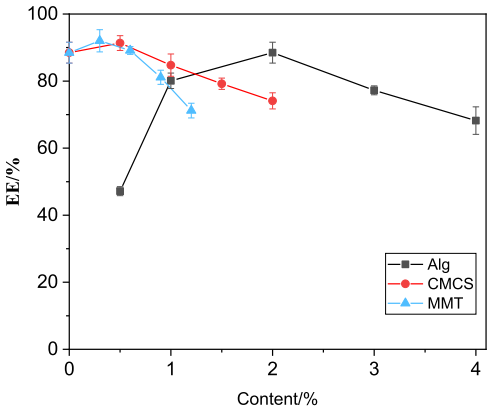

Figure 1. Influence of each component content on the granule encapsulation efficiency.

alginate can be cross-linked with Ca^{2+} to form a gel, so the amount of Ca^{2+} addition directly affects the characteristics of the gel microsphere. As can be seen from the figure, with the increase in sodium alginate, the encapsulation efficiency increases first and then decreases. When the amount of sodium alginate is 2%, the encapsulation rate reaches a maximum of 88.48%. When the amount of sodium alginate added is less than 1%, the formability is poor because the viscosity of the solution is low, resulting in a poor encapsulation effect. When the concentration of sodium alginate is too large, the solution is too viscous and leads to serious tailing, although it can form a dense network structure with calcium ions, but it also limits the loading of pesticides.[22] Therefore, the added amount of sodium alginate was fixed to 2% to study the effect of carboxymethyl chitosan and bentonite on the encapsulation efficiency.

It can be seen from Figure 1(b) that with the increase of carboxymethyl chitosan, the encapsulation efficiency shows a trend of first increasing and then decreasing. Carboxymethyl chitosan is a derivative of chitosan modified by carboxymethylation with excellent hydrophilic and adsorption properties,[23] and as a polymer electrolyte, it thus has good properties of chelating metal ions.[24] Compared with chitosan, carboxymethyl chitosan has better biocompatibility, biodegradability, antibacterial activity, and moisturizing capacity.[25,26] The addition of a small amount of carboxymethyl chitosan can form hydrogen bonds with water molecules, which can improve the swelling rate and encapsulation rate of the carrier.[27] However, when the addition amount is greater than 1%, the hydrophilicity of the carrier is too high, resulting in the loss of active ingredients during the molding process, which in turn leads to a decrease in the encapsulation rate. Carboxymethyl chitosan is a carboxymethylated derivative of chitosan due to the introduction of the carboxymethyl group, has good water solubility,[28,29] and so in the process of curing, may have little carboxymethyl chitosan into the water. On the other hand, carboxymethyl chitosan can chelate with Ca^{2+}, and so it can open the granule in curing surface sodium alginate and Ca^{2+} connection,[30] leading to part of the original drug into the water, reducing the granule encapsulation rate. The nanosheet layer of bentonite contains a large number of hydroxyl groups that can also interact with sodium alginate to form hydrogen bonds to improve the carrier encapsulation rate. In this study, the encapsulation rate was the largest when the bentonite addition amount was 0.3%, which was consistent

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with the previous results.[21] However, when the amount of bentonite added exceeds 0.3%, agglomeration occurs between bentonite and sodium alginate, and the hydrogen bond force between it and sodium alginate decreases, which makes the composite carrier defective, which in turn leads to a decrease in the encapsulation rate.

2.2. Quadratic Regression Model Building and Analysis of Variance. The response surface regression program in SAS statistical analysis was used to calculate the regression of the 17 test point data and a quadratic response regression model was established (as shown in eq 1). The ANOVA analysis of the regression equation and test of the significance of the coefficients of the equation were performed (as shown in Table S1).

$$Y = 92.19 - 2.12X_1 - 1.36X_2 + 1.12X_3 - 2.01X_1X_2 - 0.4X_1X_3 + 0.55X_2X_3 - 5.33X_{12} - 2.43X_{22} - 2.27X_{23} \quad (1)$$

The regression coefficient R^2 for the above quadratic regression full-model equation is 0.9735. From the ANOVA results of the regression model shown in Table 1, it can be seen

Table 1. Analysis of Variance of the Response Surface Regression Equation

soruces of variation	free degree	square sum	F value	P value
model	9	28.94	28.61	0.0001
X_1	1	35.91	35.50	0.0006
X_2	1	14.91	14.73	0.0064
X_3	1	10.06	9.94	0.0161
X_1X_2	1	16.16	15.97	0.0052
X_1X_3	1	0.65	0.64	0. 4498
X_2X_3	1	1.21	1.20	0.3103
X_1^2	1	119.67	118.28	<0.0001
X_2^2	1	24.94	24.65	0.0016
X_3^2	1	21.62	21.37	0.0024
residual error	7	1.01		
lack of fit	3	0.25	0.16	0.9209
error	4	1.59		

that the regression equation fits well, the test error is small, and the established model can reflect the experimental data well. Under the condition of the significance level (P less than 0.05), based on the factor significance analysis of the regression equation, sodium alginate X_1, carboxymethyl chitosan X_2, and bentonite X_3 were all significant in the primary term of the encapsulation rate regression model, and X_1X_2 was significant in the quadratic term. The order of the influence of each factor on the encapsulation rate of granules is $X_1 > X_2 > X_3$. The misfitting term of the model is $P = 0.9209 > 0.05$, indicating that the misfitting is not significant, that is, the model is stable and can better predict the influence of different material additions on the encapsulation efficiency of gel particles.[31] Therefore, this model can be used for analysis and prediction.

2.3. Determination and Validation of the Optimal Ratio of Each Component. The larger the radian of the two-factor response surface, the greater is the effect of the interaction between the two factors on the response value. It can be seen from Figure 2 that the larger the radian of the response surface diagram of the interaction of each factor, the greater is the influence of the interaction between the two factors on the encapsulation efficiency of the granule. The figure shows a trend that the encapsulation efficiency increases first and then decreases, and there is a maximum value point. From the contour plot, it can be seen that the encapsulation efficiency of the granules is larger at the center of the contour line and oval in shape.

Based on the above analysis of the response surface and the software simulation, when the amount of sodium alginate added is 1.83%, that of carboxymethyl chitosan is 0.41%, and that of bentonite is 0.37%, the granule encapsulation efficiency reached a maximum of 92.63%. To test the accuracy of the model predictions, three replicates were performed in this study. The encapsulation efficiency of the prepared granules was 91.93, 90.80, and 92.11%, and the average value was 91.61%, which was 98.90% of the theoretical value. The experimental values were basically consistent with the model optimization simulation values, which indicates that the optimization model had good reliability. Response surface method optimization determined the quality of each factor affecting the granule encapsulation efficiency, and the granule formulation optimized by the response surface method was significantly improved compared to the conventional artificial formulation.[21,32−36] Typical photographs of Eb·He-loaded composite beads are shown in Figure 3. The beads obtained were generally spherical in shape and the size varied in the range from 1.1 to 1.5 mm for the composites.

2.4. Eb·He Particles Released in Different Masses of Methanol−Water. As shown in Figure 4, the release rate of Eb·He particles was closely related to the environmental medium, and the release rate was significantly accelerated compared with alginate-bentonite particles. Due to the abundance of hydrophilic groups in CMCS, hydrogen bonds are formed with water molecules, and the swelling of the carrier is improved, which is conducive to drug release;[37] this is consistent with the results presented in Section 2.1. In addition, the results showed that when the mass ratio of CMCS to alginate is between 1:3−1:4, it has a higher water absorption swelling rate.[38]

To better understand the release properties of polymer gel particles, the Ritger−Peppas[39] equation and the Higuchi[40] equation were fitted using eq 2 and 3.

$$\frac{M_t}{M_\infty} = k \times t^n \quad (2)$$

$$\frac{M_t}{M_\infty} = k \times t^{1/2} \quad (3)$$

where $\frac{M_t}{M_\infty}$ is the fraction of the active ingredient released at time t, n denotes the diffusional exponent and the release mechanism, and k is a characteristic constant. The cumulative release of the pesticide from granules in different mass release media was nonlinear fitted by the least-squares method, and the k and n values of the pesticide released from granules were calculated. Both emamectin benzoate and hexaflumuron are soluble in the organic solvent methanol and slightly soluble in water, so methanol was added in the release process to accelerate the release rate. When methanol (V):water (v) = 50:50, the release was too fast, and when methanol (V):water (v) = 20:80, the release rate of the pesticide from the granule was slower. Methanol (V):water (v) = 30:70 was therefore selected as the release medium (Table 2).

The diffusion coefficient n is the basis for analyzing the mechanism of drug release. For spherically homogeneous

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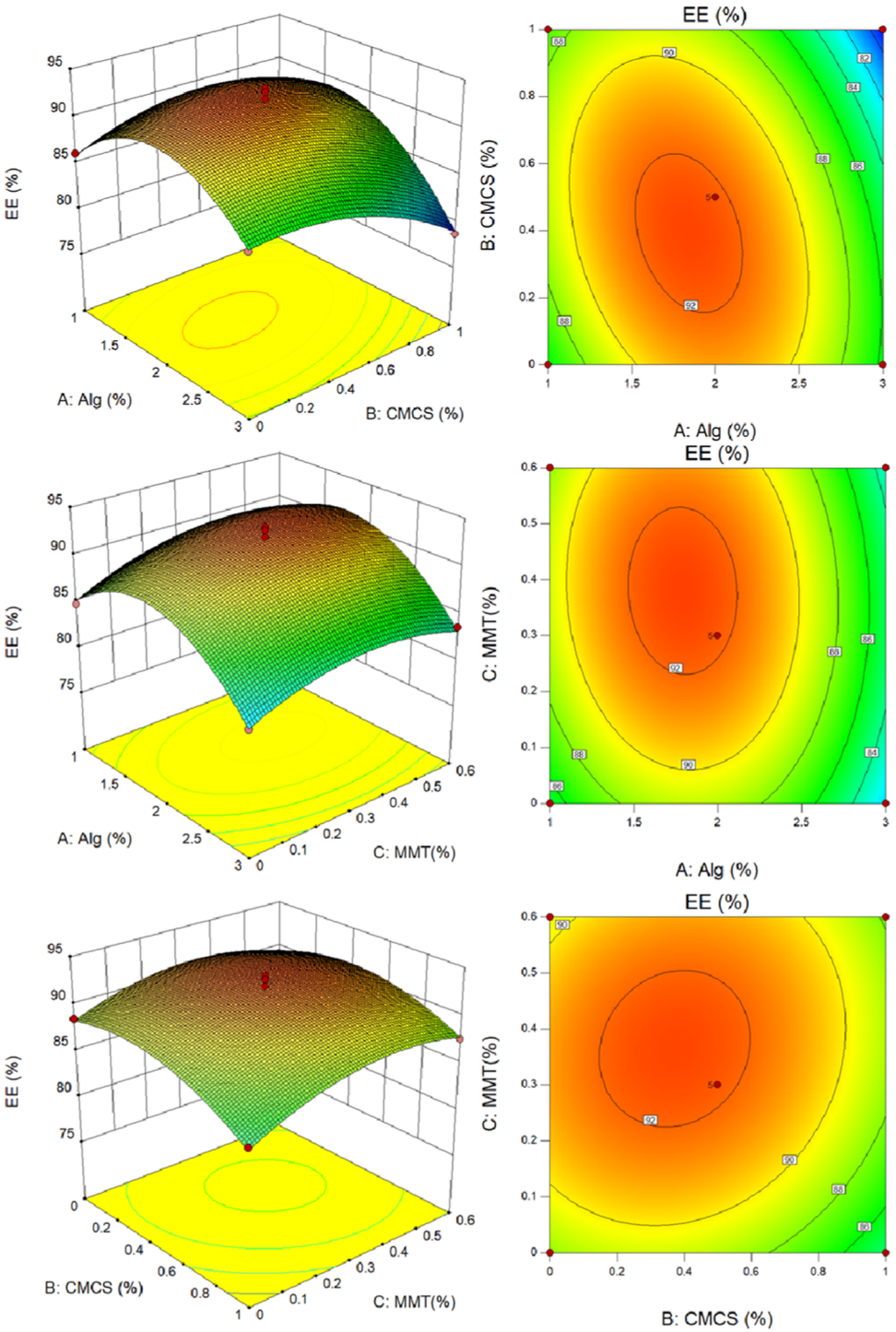

Figure 2. Response profile plot of two-factor interactions to the encapsulation efficiency.

systems, the control mechanism is Fickian diffusion when diffusional exponent $n \leq 0.43$, the diffusional exponent $n \geq 0.85$ when the drug is released according to the "Case II" transport mechanism, and its non-Fickian diffusion when n is between 0.43 and 0.85.[39] The granules prepared in this paper can be regarded as a spherical homogeneous system. The fitting results showed that when the amount of methanol−water is 2−6 mL, the pesticide diffusion index n is between 0.43−0.85, indicating that Eb·He release is non-Fickian diffusion, and the release process is controlled by polymer swelling and relaxation. When the amount of methanol−water added is 25 mL, $n < 0.43$, the release of active ingredients is mainly controlled by the Fickian diffusion, that is, the whole release process can be seen as, when the polymer gel particles absorb water, the pesticide molecules dispersed in the gel network are dissolved and released into the environment through solution diffusion.

The Higuchi model is established for the evaluation of the release mechanism, which is the restricted form of the Ritger−Peppas model.[21] The correlation coefficient R^2 for the cumulative release rate of Eb·He and time was fit by using eq 3 (Table 2). As shown in Table 2, the correlation coefficient

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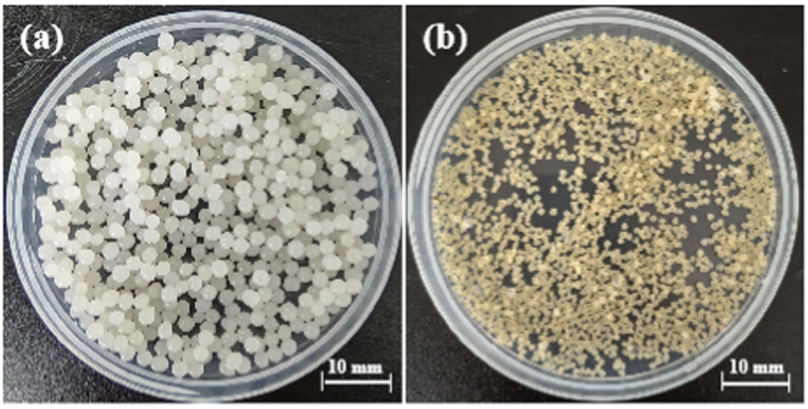

Figure 3. Images of alginate-carboxymethyl chitosan-bentonite gel particles (a) before and (b) after drying.

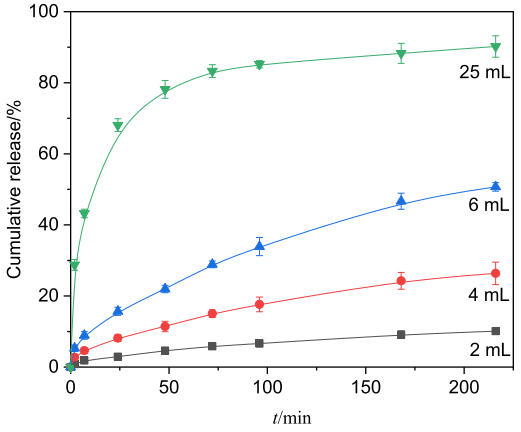

Figure 4. Cumulative release of Eb·He from 1.00 g of granules in different volumes of methanol−water.

of the Ritger−Peppas model is larger than that of the Higuchi model, indicating that the control mechanism of Eb·He release from granules is more consistent with the Ritger−Peppas model. By fitting the data on the release of fluramide from the granule with the two release models, the results $R_{\text{Ritger−Peppas}}^2 > R_{\text{Higuchi}}^2$ depicted in Table 2 showed that the release of emamectin benzoate and hexaflumuron from the granule is more consistent with the Ritger−Peppas model, with the release rate being fast and then slow, and the small release medium being 2, 4, and 6 mL. In field applications, dry and rainless weather is generally selected for application, so the water content in the corn horn is relatively small, and a small amount of the release medium is conducive to reducing the release rate of the granule and prolonging the holding period of the granule.

2.5. Control Effect of Eb·He Granules against *Spodoptera frugiperda* in Field Trials. Field experiments showed that the 1:10 combination of Eb and He had good insecticidal and egg-killing activity. In our previous agent screening test, the 72 h insect population reduction rate treatment by 11% Eb·He WG was 100%, and the prevention effect was as high as 91.39% in 14 days. In this study, the field doses of Eb·He granules were 100, 200, and 300 g/667 m^2, respectively. 11% Eb·He WG (commercial drug) was used as a control at an amount of 25 g/667 m^2. As shown in Table 3, after commercial drug treatment, the 3-day insect population reduction rate reached 90.24%. It had a good field insecticidal activity. However, newly hatched larvae were found in maize plants after 7 days. The 14-day insect population reduction rate was only 67.07%, and the spray application was ineffective. In polymer gel particle processing, the treatment plot with a dose of 100 g/667 m^2 had a poor control efficacy, and the insect population reduction rate was only 37.50−83.33% within 14 days, and the average control efficacy was only 34.86−86.86%. Newly hatched larvae were observed at 14 days. Due to the environment, the rapidity of the gel treatment group was poor at three doses; however, the larvae of the fall armyworm in the treated plots showed an obvious refusal to feed. With the continuous release of pesticides, the gel treatment with dosages of 200 and 300 g/667 m^2 showed a good effective period, and the average control effect was 91.28−98.82% in 14 days.

3. CONCLUSIONS

The gel particles were prepared by the sol−gel method. The effect of the ratio of sodium alginate, carboxymethyl chitosan, and bentonite on the drug encapsulation rate was studied. Therefore, the response surface method was used to optimize the preparation process of polymer gel particles and the optimal preparation process was screened by merging the nonlinear mathematical model. The results showed that when the ratio of sodium alginate was 1−3%, that of carboxymethyl chitosan was 0−0.5% and that of bentonite was 0−0.6%. The measured value of the established response surface model has a small error with the predicted value. The correlation is preferable, and the model is stable and reliable, which is

Table 2. Mathematical Model Fitting of Release Results

	Ritger−Peppas			Higuchi	
release medium/mL	n	k	R^2	k	R^2
2.0	0.56 ± 0.02	1.30 ± 0.07	0.9978	1.54 ± 0.01	0.9783
4.0	0.55 ± 0.02	3.89 ± 0.27	0.9959	4.46 ± 0.06	0.9667
6.0	0.54 ± 0.03	7.20 ± 0.62	0.9933	8.14 ± 0.08	0.9378
25.0	0.28 ± 0.02	31.13 ± 1.91	0.9842	15.20 ± 1.18	0.8043

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Table 3. Control Effect of Eb·He Granules against *Spodoptera frugiperda*

insecticide	treatment g/667 m^2	population density	after 1 d		after 3 d		after 7 d		after 14 d	
			decline rate/%	control efficacy/%	decline rate/%	control efficacy/%	decline rate/%	control efficacy/%	decline rate/%	control efficacy/%
Eb·He granules (GR)	100	72	37.50	36.03 ± 1.17	73.61	67.92 ± 2.85	83.33	83.57 ± 3.29	68.06	77.83 ± 1.83
	200	52	51.92	48.91 ± 2.36	82.69	75.51 ± 2.88	94.23	92.59 ± 4.34	84.62	93.85 ± 2.57
	300	72	80.56	84.58 ± 6.18	87.18	84.68 ± 2.12	97.22	98.55 ± 0.85	93.06	97.20 ± 1.62
11% Eb·He (WG)	25	40	92.50	92.39 ± 2.34	90.24	87.63 ± 0.72	81.82	85.70 ± 8.87	67.07	79.55 ± 9.50
CK	water	59	3.39		18.64		−27.12		−61.02	

expected to provide a reference for the preparation of high-encapsulation gel sustained-release particles.

The combination of emamectin benzoate and hexaflumuron granules has a good control effect on the FAW. The beads were spread directly onto maize leaf whorls in field production; at 14 days after application, the efficacy reached 91.28−98.82%.

4. MATERIALS AND METHODS

4.1. Materials. Emamectin benzoate (Eb, 73.5%) and hexaflumuron (He, 98.3%) were obtained from Guizhou Daoyuan Biotechnology Co., Ltd. (Guizhou, China). Carboxymethyl chitosan and Ca-bentonite were purchased from Shandong Yousuo Chemical Technology Co., Ltd. (Shandong, China). Sodium alginate ((10g/L, 20 °C)/(Pa·s) ≥ 0.02) and calcium chloride were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Acetonitrile and methanol (HPLC grade) were obtained from Anhui Tedia High Purity Solvents Co., Ltd. (Anhui, China). Ammonia was obtained from Chongqing Chuandong Chemical Co., Ltd. (Chongqing, China). Deionized water was prepared in the laboratory, and all experimental samples were prepared to be ready-to-use. All of the above chemicals were used as required.

4.2. Preparation of Eb·He Granules. A certain amount of sodium alginate, carboxymethyl chitosan, and Ca-bentonite were added in a conical flask with 100 mL of deionized water (Table 4). The mixtures were then stirred at a speed of 500 r·min^{-1} at 60 °C until they were homogeneous. Subsequently, 0.15 g of the active ingredient (Eb:He = 1:10) was dissolved in methanol to mix a solution at a concentration of 0.15 g·mL^{-1}, and then the speed was adjusted to 1500 r·min^{-1} and the solution was dropped slowly into the conical flask and stirred for 2 h until evenly mixed. The mixture was slowly dropped into a 0.2 M $CaCl_2$ solution with a 2.5 mL syringe (needle size, 1.45 mm) under constant slow stirring. After stirring for 30 min, the obtained product was filtered, washed with deionized water, and then dried to constant weight at 45 °C. The products obtained were labeled as S_x, S_xC_y, and S_xB_y for the sodium alginate hydrogel containing only active ingredients and the composites without bentonite or without carboxymethyl chitosan, whereas x was the weight percentage of sodium alginate and y was the weight percentage of carboxymethyl chitosan or bentonite.

4.3. Response Surface Test Design. According to the design principle of the Box−Behnken test, considering sodium alginate, carboxymethyl chitosan, and bentonite as three factors affecting the encapsulation rate, they were recorded as X_1, X_2, and X_3, respectively. Three levels were established for each factor, denoted as −1, 0, and 1 (as shown in Table 5). The paper designed the response surface analysis test of 17 test points (Table 6), of which 12 factorial point experiments and 5 zero-point experiments were used for estimating errors. A series of particles were prepared as described in Section 2.2.

4.4. Determination of Eb and He Loading Content and Encapsulation Efficiency. 0.50 g of Eb·He loaded composite beads were added to a volumetric flask containing 40 mL of methanol and dissolved by ultrasound for 2 h and then diluted to 50 mL with methanol. The resulting solution was filtered with a 0.22 μm organic filter. The concentrations of Eb and He in solutions were determined on an Agilent 1260 HPLC equipped with a UV−vis detector set at 245 nm. The column was a C18 column (150 mm × 4.6 mm), and the mobile phase was methanol:acetonitrile:ammonia (ammonia:-

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Table 4. Particle Formulations

formulation	sodium alginate/g	carboxymethyl chitosan/g	bentonite/g	active ingredient/g
$S_{0.5}$	0.50	0	0	0.15
S_1	1.00	0	0	0.15
S_2	2.00	0	0	0.15
S_3	3.00	0	0	0.15
S_4	4.00	0	0	0.15
$S_2C_{0.5}$	2.00	0.50	0	0.15
S_2C_1	2.00	1.00	0	0.15
$S_2C_{1.5}$	2.00	1.50	0	0.15
S_2C_2	2.00	2.00	0	0.15
$S_2B_{0.3}$	2.00	0	0.30	0.15
$S_2B_{0.6}$	2.00	0	0.60	0.15
$S_2B_{0.9}$	2.00	0	0.90	0.15
$S_2B_{1.2}$	2.00	0	1.20	0.15

Table 5. Factor Level of Gel Granules

factor level	sodium alginate/%	carboxymethyl chitosan/%	bentonite/%
−1	1.00	0	0
0	2.00	0.25	0.30
1	3.00	0.50	0.60

water = 1:300) = 42:42:16 at a flow rate of 1.5 mL·min^{-1}. The measurement was performed in triplicate.

4.5. Eb·He Release in Different Masses of Methanol−Water. The paper conducted a release test of the release medium of different masses at room temperature. 1.00 g of composite beads were spread evenly flat into the glass dish with filter paper at the bottom, and then the release medium methanol−water (methanol:water = 30:70) was dropped evenly on the filter paper and sealed with plastic wrap, and the contents of the release medium were set as 2, 4, 6, and 25 mL separately. At the point of the designed time interval, the granules were transferred to another glass dish with filter paper, which contained the same volume of the release medium. Then, methanol−water was added to fill up to 6.0 mL to wash the glass dish after the particles were transferred and ultrasonicated to make the active ingredient homogeneously distributed. The above steps were repeated at the appropriate time according to the number of points designed. Then, the solutions were filtered and analyzed by HPLC. Additionally, the amounts of Eb and He released at intervals were calculated according to the following formula and the release curve was drawn. Experiments were conducted in duplicate.

$$M_t(\%) = \frac{V_0 \sum_{i=0}^{n-1} C_i \cdot V_0 C_n}{M_E} \times 100 \quad (4)$$

where M_t is the cumulative release (%) of Eb and He, V_0 is the constant volume after sampling (mL) at a predetermined time interval (V_0 = 6 mL), C_n (mg·mL^{-1}) is the Eb and He concentration in the release medium at time t, and M_E (mg) is the total amount of pesticide encapsulated in the composite beads. These measurements were performed in triplicate.

4.6. Control Efficacy of Eb·He Granules against *Spodoptera frugiperda* in Field Trials. The maize variety "Qiannuo 938" was planted in 6 m × 5 m plots on May 20, 2022 in Qingzhen, Guizhou, China. This field experiment was conducted in July 2022. All plots were subjected to the same water and fertilizer management. While the maize plants were in the seedling stage, plots with a serious occurrence of the FAW were chosen as test plots. The experiment involved three treatments (200 g/667 m^2, 300 g/667 m^2, and 500 g/667 m^2) and one blank control, and the experiments were repeated four times.

Table 6. Box−Behnken Test Design

number	sodium alginate/%	carboxymethyl chitosan/%	bentonite /%	loading content/%
1	1.00	0.50	0.30	0.15
2	2.00	0.50	0	0.15
3	3.00	0.25	0.60	0.15
4	1.00	0.25	0.60	0.15
5	2.00	0.25	0.30	0.15
6	2.00	0	0.60	0.15
7	2.00	0.25	0.30	0.15
8	3.00	0.25	0	0.15
9	3.00	0.50	0.30	0.15
10	3.00	0	0.30	0.15
11	2.00	0.25	0.30	0.15
12	1.00	0.25	0	0.15
13	1.00	0	0.30	0.15
14	2.00	0.25	0.30	0.15
15	2.00	0	0	0.15
16	2.00	0.25	0.30	0.15
17	2.00	0.50	0.60	0.15

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To ensure a uniform dosage, the amount of application in each corn plant was weighed and put into a 2 mL test tube before the test, and the dosages for each of the three treatments were 0.034 0.067, and 0.1 g. The insect population density was investigated before the application of composite granules. The general survey method was applied to investigate and record the number of larvae in each plot. The number of live larvae was recorded on the first, third, fifth, seventh, and 14th days after the granule application, respectively. The control efficacy was calculated using the decline rate (eq 5) and control efficacy (eq 6).

$$K(\%) = \frac{N_b - N_a}{N_b} \times 100 \quad (5)$$

where K is the decline rate, N_b is the number of live larvae before treatment, and N_a is the number of live larvae after treatment.

$$E(\%) = \frac{K - K_c}{1 - K_c} \times 100 \quad (6)$$

where E is the control efficacy, K is the decline rate of the blank control, and K_c is the decline rate of the treatment.

4.7. Data Processing. Statistical analysis was performed using Origin Lab 2019b software to process and map the release data of Eb and He granules in different release medium contents.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c09280.

Box−Behnken test design and results (Table S1) (PDF)

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Notes

The authors declare no competing financial interest.

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