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Chitosan-Delivered Chlorantraniliprole for Pest Control: Preparation Optimization, Deposition Behavior, and Application Potential

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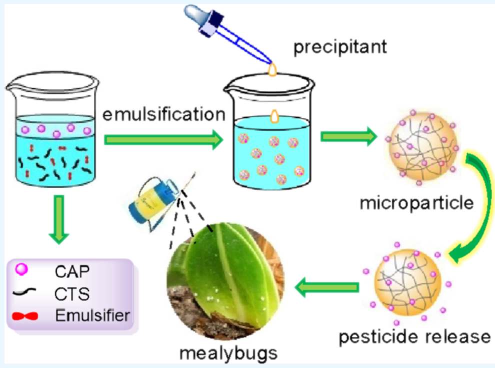
ABSTRACT: Chitosan has emerged as a promising biopolymer carrier for the sustained release of pesticides owing to its good biocompatibility, biodegradability, and bioactivity. In this work, a controlled-release formulation of insecticide chlorantraniliprole was fabricated through coprecipitation-based synchronous encapsulation with chitosan, where the optimum preparation conditions, storage stability, deposition behavior, and application potential were investigated. Preparation of optimization data from response surface methodology showed high correlation coefficient (R^2) of 0.9875 and adjusted coefficient (R_{adj}^2) of 0.9715. The resulting formulation displayed good loading content of 28.39%, high encapsulation efficiency of 75.71%, and good storage stability. Compared with the commercial suspension concentrate, the formulation exhibited better wettability and retention behaviors on plant leaves. Excitingly, effective control against one species of mealybug genus *Paraputo* Laing (outside the killing spectrum) on the *Hippeastrum reticulatum* plant was successfully achieved by spraying the controlled-release formulation at different time intervals. This work indicates the good potential of the developed formulation in expanding the application scope of chlorantraniliprole, which shows a new strategy for sustainable pest management.



1. INTRODUCTION

Nowadays, insecticides play an important role in modern agriculture to combat against pests and ensure the crop yield to meet the demands of an increasing population all over the world.[1] Current chemical insecticides mainly include organophosphorus, carbamate, neonicotinoid, pyrethroid, and diamide insecticides,[2] among which the diamide products are considered to be one of the most promising new classes due to their unique mode of action. They control the pests by activating the ryanodine receptors (RyRs) in muscles, which induces uncontrolled release of internal calcium and causes muscle disfunction and paralysis ultimately.[3−5] Both phthalic acid diamide class and anthranilic diamide class of the RyR insecticides have attracted much attention in pest control field based on their high efficiency and good selectivity to target pests.[6]

Chlorantraniliprole (CAP), a typical product of American DuPont Company, is an outstanding member of anthranilic diamides.[3,7] Its distinctive chemical structure (Figure 1) affords it excellent insecticidal activity against lepidopteran pests with low mammalian toxicity and no cross-resistance to other pesticides.[8] In addition, CAP features the advantage of long persistence, owing to its good penetration into the plant through the leaf surface. Available CAP formulations mainly focus on the conventional suspension concentrate (SC), emulsifiable concentrates (EC), and wettable powders (WP).[9] These conventional formulations usually need to be applied repeatedly at relatively high concentration to ensure the efficacy due to spray drift, rolling down, volatilization, photolysis, hydrolysis, microbial degradation, runoff, etc., which may bring a series of problems such as the development of pesticide resistance[10,11] and environmental and ecological damage.[12−15] Hence, there is an urgent need to develop environment-friendly and targeted release formulations of CAP to achieve amount reduction and efficiency improvement.

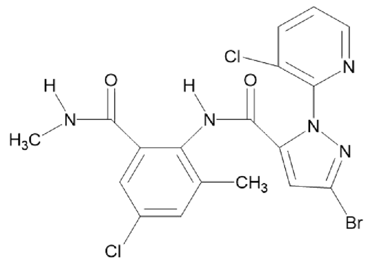


Figure 1. Chemical structural formula of insecticide CAP.

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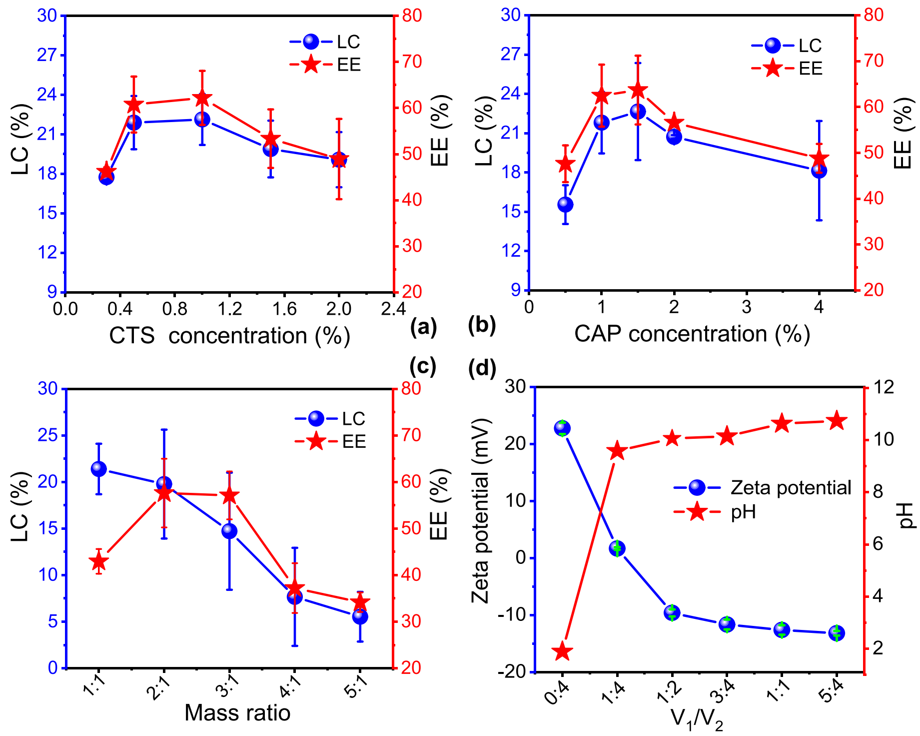

Figure 2. Effects of CTS concentration (a), CAP concentration (b), and mass ratio of CTS to CAP (c) on the LC and EE of the formulation and zeta potential and pH of the CCF filtrate at different volume ratios of precipitant (V_1) to emulsified solution (V_2) (d). Error bars of all figures in this paper represent standard deviation from the mean (n = 3).

Nanobased sustained/controlled release formulation of pesticides based on encapsulation technique features the advantages of enhanced adhesion and permeability to the plant surface, long duration, and outstanding ultraviolet shielding ability, which has shown great potential in partially alleviating the shortcomings of the conventional formulations.[16−19] It is particularly desirable for agricultural scenarios with high pest and disease incidence and long control cycles. Recently, natural polymer carriers have become popular stars owing to their good biocompatibility, biodegradability, and encapsulation capability.[20−23] Polymer-based CAP delivery systems including polylactide microcapsules[9,24,25] and chitosan-alginate microsphere floating hydrogel[26] have been fabricated. Inorganic carrier materials such as functionalized hollow mesoporous silica[27] and metal−organic framework nanohybrid material[28] have also been developed to load the insecticide CAP. Despite the advantages of environment-responsive release, prolonged efficacy, and reduced pesticide loss, most of these formulations involved complicated operation or sophisticated techniques, which may increase the costs and thereby limit their commercial application.

In our previous work, an innovative easy-to-prepare coprecipitation-based synchronous encapsulation technique has been developed to prepare the CAP controlled-release suspension,[29] where natural polymer chitosan (CTS) was adopted as the carrier owing to its excellent fungicidal activity[30,31] as well as specific insecticidal activity against some lepidopterous insects.[32] The obtained CAP/CTS controlled-release formulation (CCF) displayed obvious pH sensitivity, high cumulative pesticide release, and good insecticidal activity against *Plutella xylostella*(Lepidoptera: Plutellidae) larvae. Despite these satisfactory results, some issues related to this formulation still need to be understood, including the following: (i) Can the preparation conditions of this formulation be further optimized? (ii) What about its storage stability as well as the deposition behavior on the surface of plant leaves? (iii) As a comprehensive effect of the toxicity of insecticide and the multiple bioactivity of CTS carrier, does the developed formulation have the potential to expand the scope of insecticide application?

Aiming at solving these issues, single factor experiments and response surface methodology[33,34] were applied to optimize the preparation conditions of CAP controlled-release formulation. The storage stability of the resulting formulation under different temperature conditions was investigated. Its deposition behavior on plant leaves was also evaluated by measuring the contact angles and retentions. Especially, the insecticidal activity of CCF against one species of mealybug genus *Paraputo* Laing on the *Hippeastrum reticulatum*(Amaryllidaceae) plant was explored to check the potential of the developed formulation in expanding the application scope.

2. RESULTS AND DISCUSSION

2.1. Single Factor Experiments for CCF Preparation. As shown in Figure 2a, both loading content (LC) and encapsulation efficiency (EE) of CAP/CTS controlled-release microparticles increased first and then declined gradually with the increase of CTS concentration in the range of 0.3 to 2.0%, and the optimum values were observed at 1.0%. As the concentration of CTS elevated slightly, the content of CTS in the outer aqueous layer of the O/W emulsion would increase, which is beneficial to form a stable coating layer of insecticide during synchronous precipitation and thus can give rise to the improved LC and EE of the formulation. When the CTS concentration was too high, the polymers were prone to

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aggregate together to form flocculent precipitate, leading to the reduced amount of the O/W emulsion droplets and thereby the decreased LC and, ultimately, EE. A similar trend has also been found for the change of LC and EE with the increasing CAP concentration, where a point of 1.5% CAP illustrated the highest LC and EE values (Figure 2b). As for the effect of mass ratio of CTS to CAP (Figure 2c), a lowest EE of 42.95% was obtained at a ratio of 1:1 due to the relatively small amount of CTS in the coating layer. The dosage of CTS at ratio of 2:1 or 3:1 may be appropriate with high EE of around 57% observed. A further increase in the ratio gave rise to a continued decline of EE, where a large amount of CTS polymers aggregated together without the encapsulation of insecticide under alkaline conditions. Differently, values of LC always exhibited a relatively rapid downward trend with the increasing ratio, which can be explained by the increased weight of the controlled release microparticles due to the improved proportion of CTS. Based on a combined consideration of EE and LC, a mass ratio of 2:1 was selected for the preparation.

Based on the results of single factor experiments, the volume ratio of precipitant to emulsified solution in the preparation process was determined by measuring both zeta potential and pH of the CCF filtrate. As can be seen from Figure 2d, the presence of H^+ (from HCl) and $-NH_3^+$ (from CTS) in the filtrate gave rise to a high positive zeta potential of 22.8 mV and a low pH of 1.9 before the precipitant was added. With the dropwise addition of the precipitant, the anions (OH^-) from the precipitant interacted with the cations in the system through electrostatic interactions, resulting in a rapid increase in pH and a sharp decline in zeta potential. When the volume ratio reached 1:4, the zeta potential was close to zero. As the system was electrostatically neutral at this time, the precipitate needed to be added quickly under stirring conditions to avoid aggregation. A further increase of the precipitant led to a gradual increase in pH but a decrease in potential. Actually, both pH and zeta potential changed slowly after a volume ratio of 3:4, indicating a relatively stable state of the suspension. Therefore, the optimal volume ratio of the precipitant to emulsion is adopted to be 3:4.

2.2. Preparation Optimization with Response Surface Methodology. Based on the results of single factor experiments, the variables CTS concentration X_1 (0.5, 1.0, and 1.5%), CAP concentration X_2 (1.0, 1.5, and 2.0%), and mass ratio of CTS to CAP X_3 (1:1, 2:1, and 3:1) were further optimized using the Box–Behnken design[35] of response surface methodology. The specific experimental design and results are shown in Table 1. The resulting regression equation between EE and the variables is shown as follows:

$$\mathrm{EE}(\%) = -58.26 + 101.56X_1 + 78.28X_2 + 31.82X_3 - 2.04X_1X_2 + 3.71X_1X_3 - 4.72X_2X_3 - 59.23X_1^2 - 21.92X_2^2 - 7.89X_3^2 \quad (1)$$

The F-test was adopted to examine the significance level of each variable on EE in the regression model, and the related coefficients were also calculated to indicate the effect of interactions between the three variables. As can be seen from the ANOVA data shown in Table 2, the model featured a high F-value of 61.53 and a low P-value (probability value) of less than 0.0001, indicating the reasonable design of the polynomial regression model to determine the optimum conditions for the encapsulation of the formulation. Furthermore, a high correlation coefficient (R^2) of 0.9875 and adjusted coefficient (R_{adj}^2) of 0.9715 were obtained, suggesting the reliable results of response surface design and a good fitting degree of the model, respectively.

Table 1. Response Surface Test Design and Results

	independent variables			actual values
batch	X_1 (%)	X_2 (%)	X_3	EE (%)
1	0.5	1.5	1:1	64.39
2	1.5	1.5	1:1	45.64
3	1.0	1.0	3:1	59.36
4	1.0	2.0	3:1	56.91
5	1.0	1.5	2:1	74.72
6	1.0	1.5	2:1	75.43
7	1.0	1.5	2:1	74.72
8	1.0	2.0	1:1	67.54
9	0.5	1.5	3:1	54.18
10	1.5	1.0	2:1	49.75
11	1.0	1.0	1:1	60.56
12	1.5	1.5	3:1	42.85
13	0.5	2.0	2:1	59.62
14	1.0	1.5	2:1	72.02
15	0.5	1.0	2:1	58.78
16	1.0	1.5	2:1	75.43
17	1.5	2.0	2:1	48.55

As can be seen from the effects of these three variables shown in the three-dimensional response surfaces of Figure 3, all the values of EE increased initially and decreased subsequently with the increase of variables, and the optimum value of EE could be obtained at an appropriate location of each response surface. Meanwhile, it was found that the EE changed sharply with the increase of CTS concentration, indicating that the CTS concentration played an important role in the CAP encapsulation. This was consistent with the data listed in Table 2, where the significant effects on the encapsulation were found in the order of CTS concentration, mass ratio, and CAP concentration. Different from the approximately circular curves in Figure 3b,d, an elliptical contour was observed in Figure 3f, indicating that the combined effect of mass ratio and CAP concentration is significant.[36,37] From the above experimental design, the optimal conditions of the three factors for the formulation preparation should be 0.89% CTS, 1.55% CAP, and a mass ratio of CTS to CAP of 1.76:1. Under these conditions, the weight of controlled release microparticles in CCF prepared from 0.15 g of CAP and 0.26 g of CTS was about 0.40 g. The LC and EE of the resulting formulation were determined to be about 28.39 (s = 0.39%, n = 3) and 75.71% (s = 1.03%, n = 3), respectively. Here, the experimental value of EE is found to be very close to the predicted value (75.57%) calculated by eq 1, confirming the reliable optimization result of the response surface method.

2.3. Stability Evaluation. The long-term storage stability is very important for the practical application of pesticide formulations in agriculture as delamination, agglomeration, and even degradation of active ingredients may occur during the period. In our present work, all the CAP suspensions stored at 0 ± 1, 25 ± 1, and 54 ± 1 °C kept their initially milky white color (Figure 4a). Phenomenon of precipitation or delamination was not observed at 0 and 25 °C. Although the delamination event occurred during the thermal storage at

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Table 2. Analysis of Variance (ANOVA) for the Designed Model

source	sum of squares	degrees of freedom	mean square	F-value	P-value	significant[a]
model	1864.37	9	207.15	61.53	<0.0001	yes
X_1	314.87	1	314.87	93.52	<0.0001	yes
X_2	2.16	1	2.16	0.64	0.4490	
X_3	77.00	1	77.00	22.87	0.0020	yes
X_1X_2	1.03	1	1.03	0.31	0.5967	
X_1X_3	13.79	1	13.79	4.09	0.0827	
X_2X_3	22.26	1	22.26	6.61	0.0369	yes
X_1^2	923.51	1	923.51	274.29	<0.0001	yes
X_2^2	126.49	1	126.49	37.57	0.0005	yes
X_3^2	262.02	1	262.02	77.82	<0.0001	yes
residual	23.57	7	3.37			
lack of fit	15.62	3	5.21	2.62	0.1877	
pure error	7.95	4	1.99			
total	1887.94	16				

[a]$P < 0.05$ means significant, and $P < 0.01$ means highly significant.

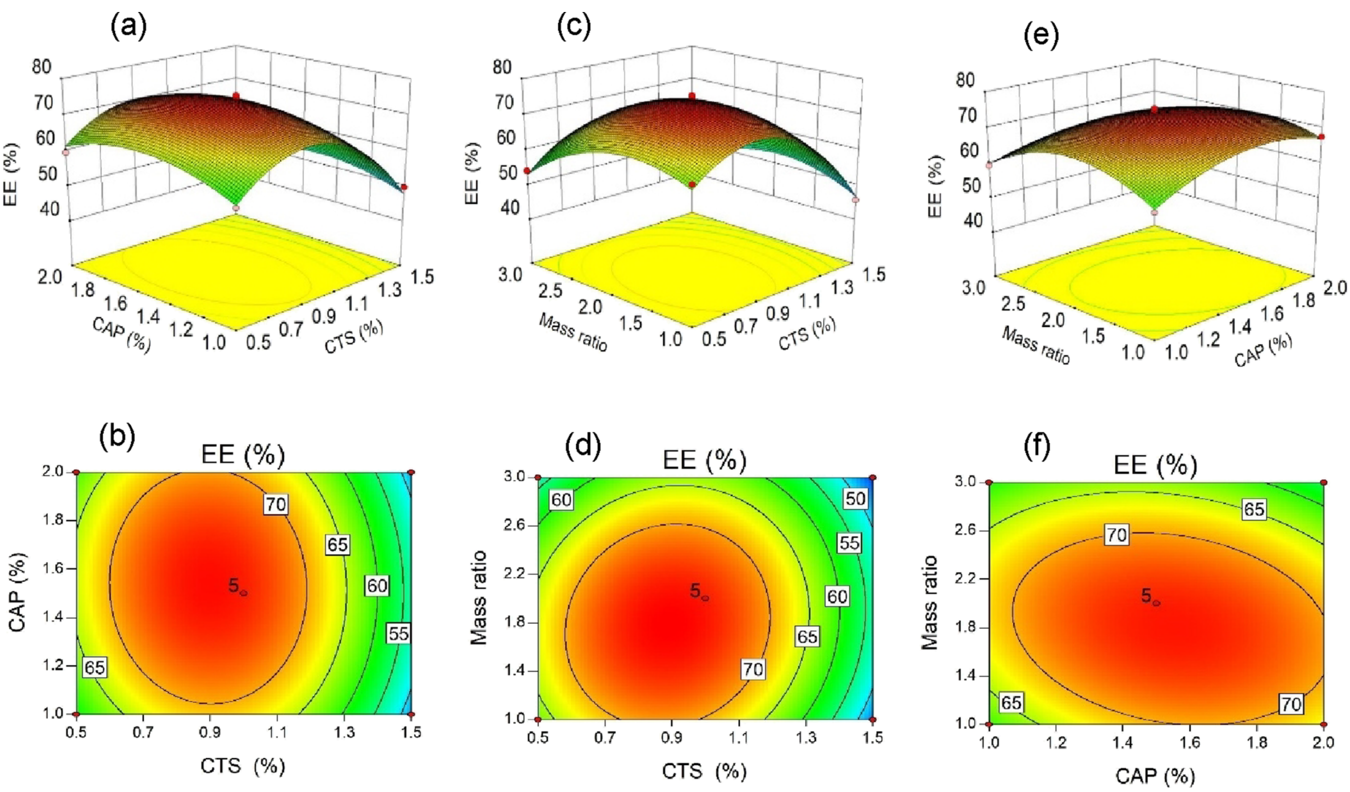

Figure 3. Response surfaces (a−e) and contour plots (b−f) showing the effects of different parameters on EE.

54 °C, the suspension exhibited good redispersibility after being shaken gently by hand. The changes in EE of CCF stored at the three temperatures for different days can be seen from Figure 4b. Values of EE remained almost unchanged when stored at 0 and 25 °C for 7 days and even 14 days, indicating the stable state of controlled release microparticles in the suspension. With respect to the hot storage environment at 54 °C, the value decreased slightly from the initial 75.71% on 0 day to 71.43% on 7 days and then to 69.48% on 14 days. The relatively high temperature accelerated the swelling of polymer CTS, which may lead to the release of small amounts of CAP from the controlled-release microparticles into the suspension. According to the data shown in Figure 4, the calculated stability degree of the insecticide loaded microparticles was around 100% at 0/25 °C and about 91.77% at 54 °C after 14 days of storage, revealing the good storage stability of the prepared CCF.

2.4. Deposition Behavior. The wettability of liquid pesticide formulations on the surfaces of plant leaves is one of the key factors that influence the utilization of pesticides. The droplet contact angle,[38,39] which is formed between the tangent to droplet and the leaf surface, is usually measured to evaluate the wettability of pesticide droplets. In the present work, contact angles of the prepared controlled release suspension and commercial CAP suspension concentrate (CSC) on the leaves of Chinese cabbage and *Hippeastrum reticulatum*plants were determined. From the data listed in Table 3, it can be seen that both CSC and CCF displayed contact angles for Chinese cabbage smaller than those of the *Hippeastrum reticulatum*plant, revealing a more hydrophilic

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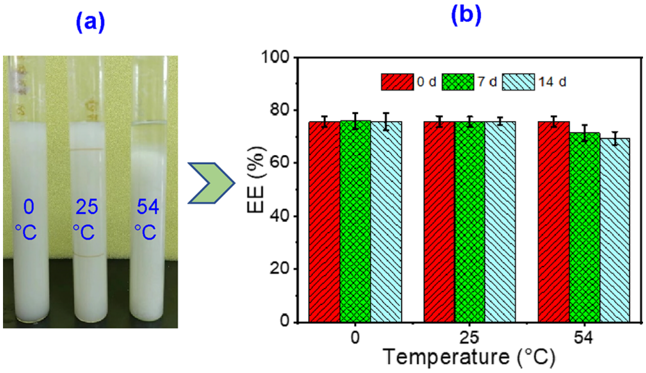

Figure 4. Photo of CCF after being stored at different temperatures for 14 days (a) and its EE at different storage periods (b).

surface of the cabbage leaves. Compared with commercial CSC formulation, the CCF exhibited comparatively low contact angles on the leaves of both plant species, indicating the good wettability of the prepared controlled-release formulation of CAP.

The pesticide retention, another factor closely related to pesticide utilization in spray application, was also measured (Table 3). For the Chinese cabbage, retention of CCF increased from 9.86 mg/cm^2 at 20 mg/L to 15.04 mg/cm^2 at 100 mg/L, after which the values remained almost unchanged. Similar trend was also found for the *Hippeastrum reticulatum*-plant, where the datum of 11.04 mg/cm^2 at 100 mg/L was very close to the value of 11.66 at 200 mg/L. Under the same concentration conditions, CCF exhibited relatively higher retention than that of CSC. It may be attributed to the high viscosity of CTS in the sustained release formulation, which improves the adhesion of CCF to the leaves and thus increases the amount of CAP on their surfaces. Based on the combined results of contact angle and retention measurements, CCF has good wettability and deposition behaviors on plant leaf surfaces, which helps to improve its application efficiency and reduce environmental pollution. This finding is consistent with the good insecticidal activity of CCF against *Plutella xylostella*larvae in our previous work.[29]

2.5. Control Effect on Species of *Paraputo* Laing. The control effect of CCF on the destructive mealybugs in our laboratory was explored, with the growth process of *Hippeastrum reticulatum* recorded. As shown in Figure 5, the *Paraputo* Laing species features a white color, oval shape, and small size (2−3 mm in length,1−2 mm in width), which is very similar to the mealybug *Acanthococcus kaki* (*Kuwana*) (Homoptera: pseudococcidae) common on persimmons. Both of them were found to damage plants by inserting their threadlike mouthparts into any part of the plants and then sucking out the sap. Before the pesticide treatment, the *Hippeastrum reticulatum*has been infested seriously with the pests, where mature leaves became yellow and even died. There were more than one hundred mealybug pests observed on the leaves, most of which were located behind the leaves or on the surface of relatively young leaves.

After the CCF (about 5 mL) was sprayed for the first time, obvious dehydration and inflexibility occurred to the body of mealybug pests on the second day (Figure 5a). On the fourth day, a large number of pests crawled on the wall of the flowerpot and lost their feeding ability (Figure 5b). Meanwhile, the plant began to grow taller. Excitingly, the population reduction rate of pests exceeded 90% on the ninth day (Figure 5c). In order to track the remaining mealybugs, CCF was sprayed on the leaves for the second time. Only a very small number of pests were observed on the leaf surface on the fourth day, with lots of carcasses appearing around the flowerpot (Figure 5d). The plants were also found to grow faster. Afterward, single-digit mealybug pests could still survive, which might come from the eggs under the soil. Thus, the third and fourth pesticide sprayings were carried out 15 days apart to further control the pests. To our surprise, the mealybugs disappeared completely after the four consecutive treatments of CCF. Colorful buds and flowers can be seen from Figure 5e,f at 84 and 91 days after the fourth pesticide spraying, respectively.

To the best of our knowledge, there are few reports related to the controlling of mealybug pests using the formulations of pesticide CAP. Control effects of some insecticides against the third or second instar nymphs of mango mealybug *Drosicha mangiferae*(Green) were once evaluated.[40,41] They found that the selected suspension concentrate of CAP did not show contact toxicity and effective control to *Drosicha mangiferae*. Different from the mango *Drosicha mangiferae*, a high reduction rate of pest population (90%) was observed on the ninth day after the first pesticide treatment in our present work, which may be attributed to the combined results of both contact toxicity and stomach toxicity. With respect to the complete disappearance of these *Paraputo* Laing pests, the good sustained-release performance and spray deposition behavior of CCF (Table 3) as well as the penetration of CAP into the *Hippeastrum reticulatum*plant may play an

Table 3. Contact Angles and Retentions of CCF and CSC at Different Concentrations on Leaves of Chinese Cabbage and *Hippeastrum reticulatum*

		Chinese cabbage		*Hippeastrum reticulatum*	
formulation	concentration (mg/L)	contact angle (°)	retention (mg/cm^2)	contact angle (°)	retention (mg/cm^2)
CCF	20	76.02 ± 2.52	9.86 ± 2.01	91.81 ± 2.42	4.96 ± 1.54
	60	71.40 ± 2.73	12.22 ± 2.42	89.72 ± 2.91	8.69 ± 1.16
	100	70.14 ± 2.97	15.04 ± 2.47	88.42 ± 0.71	11.04 ± 2.76
	150	66.17 ± 2.27	15.15 ± 1.78	85.65 ± 1.49	11.32 ± 1.88
	200	57.37 ± 2.36	15.53 ± 3.46	81.96 ± 1.36	11.66 ± 2.46
CSC	20	76.67 ± 1.23	7.67 ± 2.05	93.77 ± 0.34	3.54 ± 2.85
	60	73.65 ± 2.92	9.76 ± 2.88	91.90 ± 3.37	5.16 ± 1.97
	100	72.87 ± 2.74	10.93 ± 2.51	90.93 ± 0.90	7.16 ± 1.20
	150	68.64 ± 2.81	12.37 ± 2.82	88.87 ± 1.32	9.17 ± 2.25
	200	63.45 ± 2.29	12.53 ± 2.78	86.16 ± 3.17	9.66 ± 1.07

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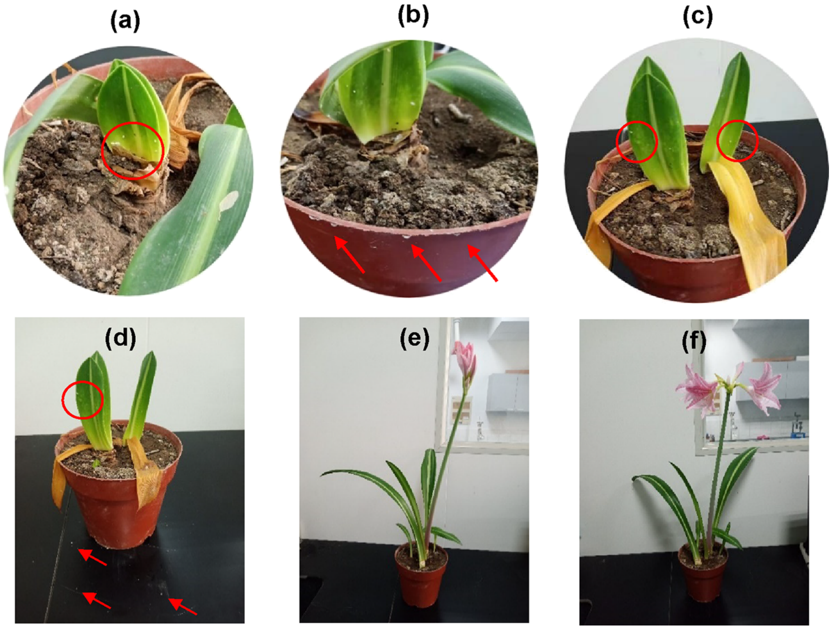

Figure 5. Photographs of the pot of *Hippeastrum reticulatum*infested with mealybugs for 1 (a), 3 (b), and 8 days (c) after the first pesticide spraying, 3 days after the second pesticide spraying (d), 84 (e), and 91 (f) days after the fourth pesticide spraying.

important role here. On the other hand, the antifungal and insecticidal activities of CTS help to reduce the egg hatching rate of mealybugs under the soil.[42,43]

Overall, effective control of one species of mealybug genus *Paraputo* Laing has been successfully achieved through four consecutive sprayings of CCF at different time intervals, which provides a new window for the CAP application in mealybug control. Combined with the good insecticidal activity of CCF against *Plutella xylostella*larvae in our previous work,[29] it can be found that the innovative easy-to-prepare CCF not only can be used as a formulation with high efficiency and long duration on target pests within the killing spectrum but also has good potential for controlling some destructive pests outside the spectrum, which shows a new strategy for sustainable pest management.

3. CONCLUSIONS

In this work, we optimized the preparation of CCF by means of single factor experiments, response surface methodology, and zeta potential analysis. The obtained optimum conditions were 0.89% CTS, 1.55% CAP, a mass ratio of CTS to CAP at 1.76:1, and a volume ratio of precipitant to emulsion solution at 3:4. The formulation prepared under these conditions exhibited good LC (28.39%), high EE (75.71%), excellent storage stability, and better wettability and retention behaviors on plant leaves than the commercial CSC. As a comprehensive effect of the toxicity of insecticide and the multiple bioactivity of CTS carrier, destructive pests of one species of mealybug genus *Paraputo* Laing on the *Hippeastrum reticulatum*plant were effectively controlled by spraying the CCF at different time intervals, indicating that the encapsulation of insecticide in versatile polymer CTS may have the potential to expand the scope of insecticide application. This work contributes to the development of potential pesticide formulations for pest control, especially using natural polymer materials as pesticide carrier systems.

4. MATERIALS AND METHODS

4.1. Materials. The polymer CTS (degree of deacetylation <90%; viscosity <100 cps) was purchased from Shanghai Lan-Ji Biotechnology Development Co., Ltd., China, and the insecticide CAP was supplied by FMC Corporation, USA. Commercial formulation of CSC was provided by FMC Corporation, Singapore. Acetonitrile of chromatographic grade was used for the mobile phase of high-performance liquid chromatography (HPLC). All the other reagents were used at the purity of analytical reagent grade, which were provided by Sinopharm Chemical Reagent Co., Ltd., China. Deionized water was used throughout the experiments.

4.2. Preparation and Optimization of CCF. The preparation process of CCF was carried out according to the coprecipitation method in our previous work.[29] In brief, emulsifier tween 80 was put into the mixture of CTS solution (in 0.1 M HCl) and CAP solution (in *N*,*N*-dimethylformamide) to generate an O/W (oil in water) emulsion by shear emulsification. An alkaline precipitant of a mixture of 3% ammonia and isopropanol was subsequently added. The CTS in the outer aqueous layer of emulsion droplets would precipitate synchronously with the inner CAP molecules to form the CAP loaded controlled release microparticles ultimately. In order to obtain the optimum conditions, single factor experiments were first adopted to examine the effects of CTS concentration, CAP concentration, and a mass ratio of CTS to CAP on LC and EE. Subsequently, the volume ratio of precipitant to emulsified solution was determined by analyzing the zeta potential and pH of the CCF filtrate. Based on the results of the above experiments, the response surface methodology was then applied to further optimize the preparation conditions using the software of Design-Expert 8.0.6. The independent variables were set according to the Box-Behnken design, and the significance of the effect of each variable on the pesticide encapsulation was evaluated by the *F*-test method.

4.3. Stability of CCF. The storage stability of the CCF prepared under the optimum conditions was examined by

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keeping it in glass tubes at different temperatures of 0 ± 1, 25 ± 1, and 54 ± 1 °C for 7 and 14 days. The changes of apparent morphology were observed, and the EE of CAP in the formulation was determined by the HPLC method.[29] The experiments were repeated at least three times with the average values adopted. The stability degree of the insecticide loaded microparticles was calculated according to eq 2:

$$\text{stability degree} = EE_t/EE_0 \times 100\% \quad (2)$$

where EE_0 and EE_t stand for the values of EE before and after storage, respectively.

4.4. Wettability on Leaves. Fresh leaves of Chinese cabbage and *Hippeastrum reticulatum*plants were cut into rectangular strips with a length of about 4 cm and fixed on the glass slide with double-sided tape to avoid bending. Pesticide droplets (3 μL) were added to the surface of leaves with the help of a microsyringe. The contact angles were then measured using a DSA25 contact angle measuring instrument (Kruss, Germany).

4.5. Retention on Leaves. Retention of CCF and CSC on plant leaves was measured by the *Wilhelmy* immersing method.[44] Briefly, fresh round leaves with a diameter of about 2 cm were dipped vertically into the pesticide solution for 30 s. The weights of the leaf pieces before and after the immersion were recorded, and the retention R (mg/cm^2) can be calculated by the following equation:

$$R = (W_2 - W_1)/S \quad (3)$$

where W_1 (mg) and W_2 (mg) are the weights of round leaf before and after the immersion, respectively, and S (cm^2) is the area of the round leaf.

4.6. Controlling of One Species of Genus *Paraputo* Laing. The *Hippeastrum reticulatum*(Amaryllidaceae) plant in the laboratory, which was cultivated without any agrochemical treatment, has been found to be seriously infested with pests belonging to the species of mealybug genus *Paraputo* Laing (Homoptera: Coccoidea: pseudococcidae). As exploratory experiments of CAP efficacy, the CCF at a concentration of 100 mg/L was sprayed on the plant four times at different time intervals to control the pests. The mortality and population reduction rate of pests as well as the plant growth were observed and recorded at different times after the CCF application.

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Author Contributions

[#]Conceptualization and funding acquisition were done by X.P.K. and J.W. Experimentation was performed by J.H.L., Y.M.W., X.Y.Q., and Y.J.F. Data analysis and diagrams were done by J.H.L., Y.M.W., X.P.K., and J.W. Manuscript writing was done by X.P.K., J.W, L.L., and Y.J.F. Manuscript revision and approval was performed by X.P.K., J.W., and L.L. Here, J.H.L. and Y.M.W. contributed equally to this work.

Notes

The authors declare no competing financial interest.

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