

## Research Article

# Preparation and Evaluation of Emamectin Benzoate Solid Microemulsion

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The solid microemulsions of emamectin benzoate with the same content of surfactants were prepared by a self-emulsifying method. Emulsifier 600<sup>#</sup> and emulsifier 700<sup>#</sup> (3/2, w/w) screened from eleven kinds of commonly used surfactants displayed great emulsifying properties. The redispersed solution of the solid microemulsion presented aqueous microemulsion characteristic. The mean particle size and polydispersity index were  $10.34 \pm 0.10$  nm and  $0.283 \pm 0.013$ , respectively. The solid microemulsion showed excellent storage stability and the bioassay compared with water dispersible granules against diamondback moths provided a proof of its improved biological activities. This formulation could significantly reduce surfactants and is perspective in plant protection for improving bioavailability and environmental friendliness.

## 1. Introduction

Pesticides are commonly used in agriculture to improve crop yield and quality by controlling plant diseases [1]. Most pesticides must be formulated using suitable formulations to keep bioactivity and enhance efficacy, safety, and convenience of the active ingredients while spraying. Conventional pesticide formulations mainly include emulsifiable concentrate (EC), wettable powder (WP), and suspension concentrate (SC) [2]. In the EC formulation, large amounts of organic solvents such as toluene and xylene are used as main components which are toxic, inflammable, and explosive [3]. In general, the emulsifiers in the conventional formulation compositions consist of ionic surfactants (calcium dodecylbenzenesulfonate, polycarboxylates, and so forth) and nonionic surfactants (dibenzyl phenol polyoxyethylene ether, nonylphenol ether, and so forth). The amount of surfactants is mainly equal to or higher than 8% of the total weight in these formulations [4–7]. In addition, wetting agents, such as alcohol ethoxylate, sodium dodecyl sulfate, and alkylphenol ethoxylates, are always needed to keep the stability and dispersibility of WPs and SCs besides carriers and other additives [8, 9].

Most pesticide compounds are poorly soluble in aqueous media, which limits the development of their efficient

and green formulations [1, 10]. According to the Ostwald-Freundlich equation (1), with all other factors kept constant, the solubility increases with particle size decreasing [11]. One has

$$\frac{S(d)}{S_0} = \exp \frac{\gamma V_m}{RTd}, \quad (1)$$

where  $S(d)$  is the solubility (mol/kg) of the pesticide with particle diameter  $d$  (m) at temperature  $T$  (K);  $S_0$  is the solubility of the bulk pesticide;  $V_m$  is molar volume ( $\text{m}^3/\text{mol}$ );  $\gamma$  is surface free energy ( $\text{J}/\text{m}^2$ );  $R$  is the gas constant. It means that reducing particle size of the pesticide is an effective method to enhance its dissolution rate [12, 13]. However, significant change occurs only with a particle size in the nanorange [14]. In this case, nanotechnology could be a new strategy to produce nanopesticides for improving the solubility of poorly soluble pesticides [14, 15].

Emamectin benzoate, a macrocyclic lactone insecticide (Figure 1), is a semisynthetic derivative of the avermectins [16–18]. It shows a broad spectrum, high efficiency, and low toxicity, which is further improved with thermal stability than avermectin [19]. Because its water solubility is extremely low ( $24 \text{ mg}/\text{L}^{-1}$ ), the dominant formulation of emamectin

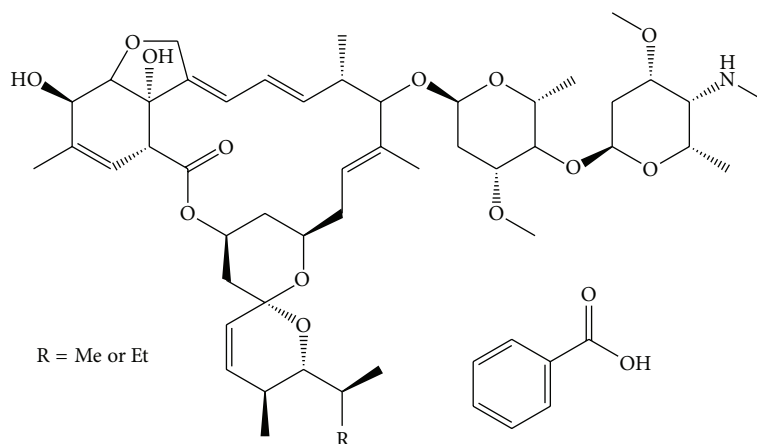


FIGURE 1: Chemical structure of emamectin benzoate.

benzoate is still EC at present with low pesticide content and a large amount of organic solvent [20]. Though microemulsion of emamectin benzoate has emerged to reduce organic solvent, few studies were reported, especially the solidified microemulsion.

In this study, we prepared emamectin benzoate solid microemulsions (SMEs) using a self-emulsifying method. The solid nanoformulation presented excellent dispersibility, stability, and efficiency. In addition, the surfactant content in the composition was equal to that of pesticide which was much lower than most other self-emulsified systems. The solid microemulsion provides a novel strategy to develop a solid nanoformulation with enhanced environmental friendliness and safety and may become a desired alternative for conventional formulations.

## 2. Experimental Methods

**2.1. Materials.** Emamectin benzoate (95%) was purchased from Hebei Veyong Bio-Chemical Co., Ltd. Styryl phenol polyoxyethylene ether (emulsifier 600<sup>#</sup>), alkylphenol formaldehyde resin polyoxyethylene ether (emulsifier 700<sup>#</sup>), phenylethyl phenol polyoxyethylene polyoxypropylene ether (emulsifier 1601<sup>#</sup>), Silwet 408, and polyoxyethylene (40) castor oil ether (EL-40) were purchased from Cangzhou Hongyuan Agrochemical Co., Ltd. Polyoxyethylene sorbitan monooleate (Tween 80), polyethylene glycol mono-4-nonylphenyl ether (PGME), sodium dodecylbenzenesulfonate (SDBS), and calcium dodecylbenzenesulfonate (CDBS) were purchased from J&K Chemical. Maleic rosin-polyoxypropylene-polyoxyethylene ether sulfonate (MRES) and polycarboxylate were provided by Jiangsu Sinvochem S&D, Ltd. Two emamectin benzoate water dispersible granules were purchased from Shaanxi Sunger Road Bio-Science Co., Ltd. (WDG-A), and Qingdao Star Cropscience Co., Ltd. (WDG-B), respectively. HPLC grade methanol, acetonitrile, and ammonium acetate were purchased from Fisher. Milli-Q water (18.2 MΩ·cm, TOC ≤ 4 ppb) was used in all analytical experiments.

**2.2. Preparation of Emamectin Benzoate SMEs.** Considering the formulation composition changed with different pesticide contents, the preparation process of 2.8% emamectin benzoate SME was taken as an example to be described in detail as follows. 2.95 g emamectin benzoate was dissolved in ethyl acetate and mixed with 2.8 g surfactants. Then 94.25 g sodium benzoate as carrier was added to adsorb the solution. After stirring evenly, the mixture was dried using an oven (DHG-9070A, Shanghai Yiheng Scientific Instrument Co., Ltd.) at 40°C for 1 h to obtain emamectin benzoate SMEs.

**2.3. Particle Size of Nanoparticles.** The mean particle size and polydispersity index (PDI) of the redispersed aqueous microemulsion were measured by dynamic light scattering (DLS) with a Zetasizer Nano ZS90 (Malvern Instruments, UK) at 25°C. The measurement was carried out in triplicate for each sample.

**2.4. Morphology Characterization of Nanoparticles.** The morphology of emamectin benzoate nanoparticles was characterized by scanning electron microscopy (SEM) (JSM-7401F, JEOL, Japan) with an accelerating voltage of 3 kV. The samples were dropped on freshly cleaned silicon slice, dried naturally, and coated with a thin layer of platinum for 30 s with ETD-800 sputter coater (Beijing Elaborate Technology Development Ltd., China).

**2.5. Crystalline State Analysis of Nanoparticles.** The crystalline state of emamectin benzoate nanoparticles was characterized by an X-ray diffractometer (Bruker AXS Inc., D8 Advance, Germany) with Cu Kα radiation generated at 40 kV voltage and 40 mA current. Samples were analyzed in a 2θ range of 5–50°, with a step size of 0.02° and a time step of 0.1 s.

**2.6. HPLC Analysis.** The emamectin benzoate content was analyzed by high performance liquid chromatography (HPLC) (WAT035876, Waters Co., Milford, USA) using a C18 column (5 μm, 4.6 mm × 250 mm, Shiseido, Japan) and 245 nm UV

TABLE 1: The influence of single surfactant on the mean particle size and PDI of SMEs.

Surfactant	Mean particle size (nm)	PDI
Emulsifier 600 <sup>#</sup>	21.11 ± 0.68 <sup>h</sup>	0.716 ± 0.032 <sup>bc</sup>
Emulsifier 700 <sup>#</sup>	55.47 ± 3.93 <sup>gh</sup>	0.406 ± 0.096 <sup>d</sup>
Emulsifier 1601 <sup>#</sup>	33.94 ± 9.71 <sup>h</sup>	0.535 ± 0.165 <sup>cd</sup>
Silwet 408	249.30 ± 5.75 <sup>d</sup>	0.456 ± 0.015 <sup>d</sup>
Tween 80	418.87 ± 9.88 <sup>c</sup>	0.745 ± 0.051 <sup>b</sup>
EL-40	638.00 ± 61.28 <sup>b</sup>	0.171 ± 0.075 <sup>e</sup>
PGME	122.43 ± 6.33 <sup>f</sup>	0.950 ± 0.039 <sup>a</sup>
MRES	36.02 ± 4.27 <sup>h</sup>	0.969 ± 0.044 <sup>a</sup>
Polycarboxylate	89.45 ± 1.32 <sup>fg</sup>	0.742 ± 0.046 <sup>b</sup>
SDBS	440.07 ± 5.54 <sup>c</sup>	0.535 ± 0.061 <sup>cd</sup>
CDBS	187.67 ± 3.71 <sup>e</sup>	0.497 ± 0.111 <sup>d</sup>

Different letters at each data value indicate significant differences according to Duncan's multiple range test at  $P < 0.05$ .

detector. The mobile phase was a mixture of methanol, acetonitrile, and 0.02% aqueous ammonium acetate (42 : 42 : 16, v/v) and the flow rate was 1.0 mL/min.

**2.7. Statistical Analysis.** In the surfactant screening process, the particle sizes were expressed as the mean ± standard deviation (SD) and the statistical significance of the difference was examined using one-way analysis of variance (ANOVA) and Duncan's multiple range test. Results with a probability ( $P$ ) of less than 0.05 were considered to be statistically significant.

### 3. Results and Discussion

#### 3.1. The Influence of Surfactant on the Particle Size and Dispersibility of SMEs

**3.1.1. The Influence of Single Surfactant.** Table 1 shows the mean particle sizes and PDIs of SMEs which were stabilized with eleven single surfactants, including eight nonionic type surfactants and four anionic type surfactants. The mean sizes of particles prepared with emulsifier 600<sup>#</sup>, emulsifier 700<sup>#</sup>, emulsifier 1601<sup>#</sup>, MRES, and polycarboxylate were less than 100 nm. The PDI value less than 0.3 reflects a good uniformity of particle diameter and can be used to depict the stability of the system [21, 22]. However, all the PDIs of the above SMEs were more than 0.4, indicating a wide size distribution. Among the eleven surfactants, EL-40 had significant effects in decreasing PDI. In order to obtain a stable solid nanoformulation, emulsifier 600<sup>#</sup>, emulsifier 700<sup>#</sup>, emulsifier 1601<sup>#</sup>, MRES, polycarboxylate, and EL-40 were chosen for further investigation.

**3.1.2. The Influence of Complex Surfactants.** Five complex surfactants were used to produce emamectin benzoate SMEs. Considering that emulsifier 600<sup>#</sup> was the most efficient to reduce the particle size, it was fixed (60%, w/w) to mix with the other surfactants (40%, w/w). As shown in

TABLE 2: The influence of complex surfactants on the mean particle size and PDI of SMEs.

Surfactant	Mean particle size (nm)	PDI
600 <sup>#</sup> + 1601 <sup>#</sup>	69.74 ± 20.01 <sup>ab</sup>	0.638 ± 0.166 <sup>a</sup>
600 <sup>#</sup> + MRES	51.38 ± 10.32 <sup>bc</sup>	0.456 ± 0.042 <sup>bc</sup>
600 <sup>#</sup> + polycarboxylate	97.54 ± 26.30 <sup>a</sup>	0.429 ± 0.072 <sup>bc</sup>
600 <sup>#</sup> + 700 <sup>#</sup>	10.34 ± 0.10 <sup>d</sup>	0.283 ± 0.013 <sup>c</sup>
600 <sup>#</sup> + EL-40	80.72 ± 0.52 <sup>ab</sup>	0.558 ± 0.001 <sup>ab</sup>

Different letters at each data value indicate significant differences according to Duncan's multiple range test at  $P < 0.05$ .

TABLE 3: The influence of different ratios of emulsifier 600<sup>#</sup> to emulsifier 700<sup>#</sup> on the mean particle size and PDI of SMEs.

Ratio of emulsifier 600 <sup>#</sup> to emulsifier 700 <sup>#</sup>	Mean particle size (nm)	PDI
3/2	10.34 ± 0.10 <sup>b</sup>	0.283 ± 0.013 <sup>b</sup>
2/3	19.83 ± 1.14 <sup>a</sup>	0.739 ± 0.046 <sup>a</sup>
1/1	19.68 ± 0.61 <sup>a</sup>	0.760 ± 0.031 <sup>a</sup>

Different letters at each data value indicate significant differences according to Duncan's multiple range test at  $P < 0.05$ .

Table 2, the combination of emulsifier 600<sup>#</sup> and emulsifier 700<sup>#</sup> (3/2, w/w) reduced the mean particle size and PDI to 10.34 ± 0.10 nm and 0.283 ± 0.013, respectively, which were smaller than other combinations. Emamectin benzoate is positively charged after being dispersed in water; anionic surfactants may induce particle aggregation via electrostatic interaction, so the optimized surfactant was of nonionic type. In addition, the polymeric surfactants adsorbing on the surface of emamectin benzoate can also afford steric barrier between particles. Therefore, the combination of emulsifier 600<sup>#</sup> and emulsifier 700<sup>#</sup> was chosen to prepare the emamectin benzoate SMEs.

Table 3 shows the effect of different ratios of emulsifier 600<sup>#</sup> to emulsifier 700<sup>#</sup> on the particle size and distribution of SMEs. When the ratio was 3/2, the mean particle size and PDI were the smallest. Though both emulsifier 600<sup>#</sup> and emulsifier 700<sup>#</sup> belong to polyoxyethylene ethers surfactants, the differences in structure make emulsifier 600<sup>#</sup> more effective in decreasing particle size and improving uniformity ( $P < 0.05$ ). Then the 3/2 ratio with higher content of emulsifier 600<sup>#</sup> was chosen.

**3.1.3. The Influence of Surfactant Content.** The surfactant content plays an important role in stabilizing nanoparticles, especially in self-emulsified microemulsions [23]. As shown in Figure 2, the mean particle size of SMEs decreased with increasing surfactant concentration from 0.5-fold to 4-fold that of the active ingredient. When the surfactant content was the same as pesticide, the particle size and PDI decreased sharply to 30 nm and 0.221, respectively. However, there was no significant change in particle size and dispersibility when increasing the ratio of surfactant to pesticide from 4

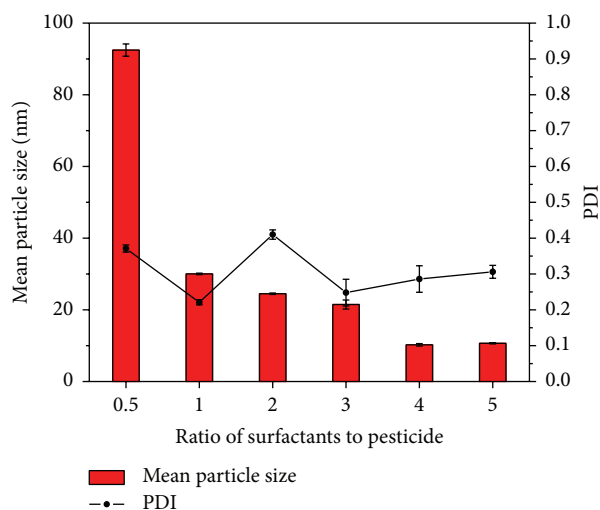


FIGURE 2: The influence of surfactant content on the mean particle size and PDI of SMEs.

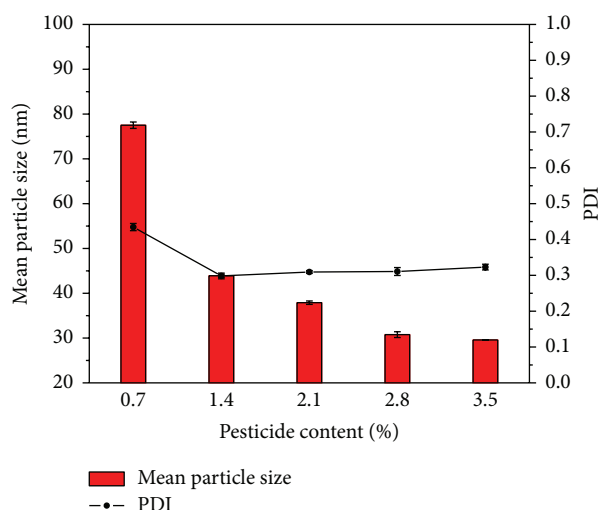


FIGURE 3: The influence of pesticide content on the mean particle size and PDI of SMEs.

to 5. Above all, the 1:1 ratio was used for the preparation of emamectin benzoate SMEs. In order to achieve self-emulsifying property, the surfactant contents in aqueous microemulsions are usually larger than twice that of the active ingredient even in the presence of cosolvents [20, 24, 25]. Furthermore, they are normally more than 5 times in solid self-emulsifying formulations [26–28]. In contrast, the composition of emamectin benzoate SME significantly decreased the surfactant amount and revealed great advantages in environmental friendliness and production cost.

**3.2. The Influence of Pesticide Content on the Particle Size and Dispersibility of SMEs.** The emamectin benzoate SMEs with different pesticide concentrations have been prepared. Figure 3 shows that the mean particle size decreased from 77.51 nm to 29.58 nm as emamectin benzoate concentration

increased from 0.7% (w/w) to 3.5% (w/w). During the preparation of SMEs, ethyl acetate dissolving pesticide increased with increasing emamectin benzoate content. When the amount of solution was beyond the absorption capability of sodium benzoate carrier, the mixture could not form solid state and the particle size became larger after drying. Therefore, the 2.8% emamectin benzoate SME was chosen for the detailed characterization.

**3.3. Morphology of Nanoparticles.** In the SEM imaging, sodium benzoate as carrier formed large crystals during the drying process and made it difficult to observe the small nanoparticles, so the aqueous dispersion without carrier was prepared and imaged. The pesticide nanoparticles presented irregular shape as observed in Figure 4(a). The particle size based on SEM imaging was from 20 nm to 50 nm, which was well in agreement with the result measured by DLS (Figure 4(b)).

**3.4. Crystalline State of Nanoparticles.** The crystalline state of nanoparticles has significant impact on the solubility, stability, and bioavailability of nanoformulations [29]. Figure 5 shows the X-ray powder diffractogram profiles of emamectin benzoate, emulsifier 600<sup>#</sup>, emulsifier 700<sup>#</sup>, sodium benzoate, and emamectin benzoate SME. As shown in the patterns, emamectin benzoate, emulsifier 600<sup>#</sup>, and emulsifier 700<sup>#</sup> were of amorphous state. Emamectin benzoate SME presented similar crystalline structure as sodium benzoate because most part in the nanoformulation composition was sodium benzoate. This crystal structure is beneficial to maintaining the formulation stability during storage compared with the amorphous form [29, 30].

**3.5. Stability of SME.** Changes of particle size and distribution of the SMEs during storage at room temperature are shown in Figure 6. The mean size of nanoparticles increased from 10.34 nm to 55.37 nm during 14 days of storage. This slight aggregation phenomenon is difficult to avoid, especially for nanoparticles [31]. However, the particle size still kept at about 64.27 nm after 30 days of storage, and PDI remained at around 0.35. In addition, the redispersed microemulsion also presented colorless and transparent appearance. These results suggested the excellent physical stability of emamectin benzoate SME.

**3.6. Biological Activity.** The biological activities of emamectin benzoate SME and two kinds of WDGs against diamondback moths (*Plutella xylostella* L.) were compared in Table 4. The median lethal concentration (LC 50) of the emamectin benzoate SME was lower and the data showed that its toxicity was 1.3-fold that of the other two WDGs. As reported, the larvicidal effect enhanced with droplet size of pesticide decreasing and the above results are consistent with the literature [32]. Nanopesticides could be more potent to target organisms than bulk form and conventional formulations of pesticide because they have larger specific surface area which could increase the accumulation and uptake of the active ingredient by the pest [32, 33].

TABLE 4: Bioassay results of emamectin benzoate SME and water dispersible granules.

Formulation	Toxicity regression equation	Correlation coefficient	LC 50 ( $\mu\text{g/mL}$ )	95% confidence limit
SME	$Y = 6.8779 + 1.5677x$	0.9387	0.0634	0.0012–0.2057
WDG-A	$Y = 6.7968 + 1.6220x$	0.9778	0.0830	0.0037–0.2188
WDG-B	$Y = 7.0744 + 1.9262x$	0.9755	0.0838	0.0028–0.2075

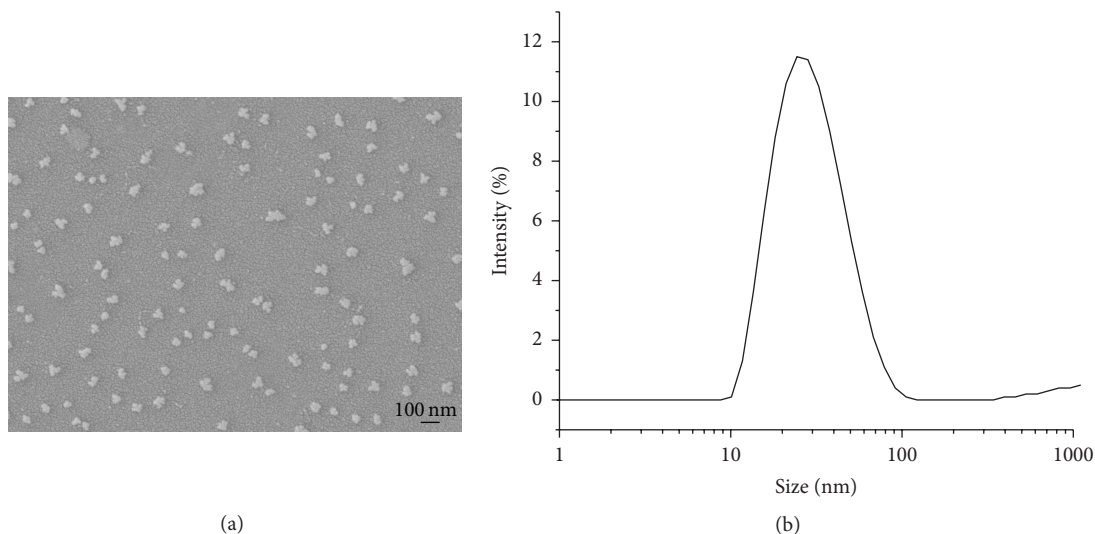


FIGURE 4: (a) SEM image of emamectin benzoate nanoparticles and (b) particle size distribution of emamectin benzoate SME measured by DLS.

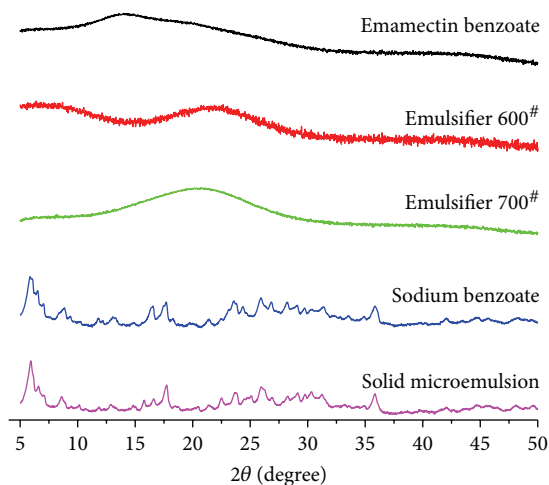


FIGURE 5: X-ray diffraction patterns of emamectin benzoate SME and related ingredients.

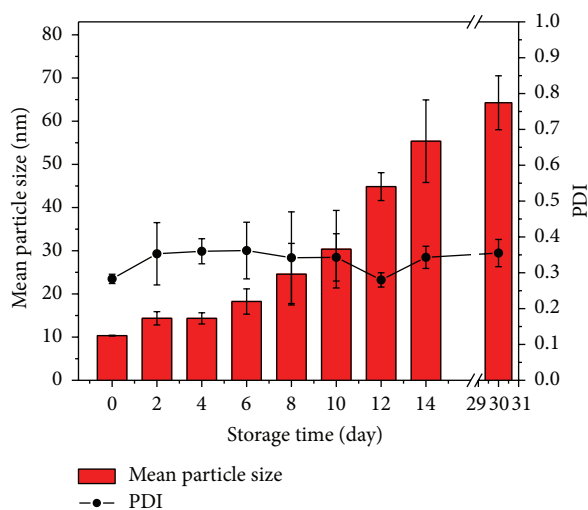


FIGURE 6: The changes of particle size and PDI of the emamectin benzoate SME during storage.

#### 4. Conclusion

In summary, the solid microemulsions of emamectin benzoate were prepared by a self-emulsifying method. Emulsifier 600<sup>#</sup> and emulsifier 700<sup>#</sup> (3/2, w/w) were optimized from eleven kinds of surfactants, and the content the same as pesticide was enough to exhibit excellent self-emulsifying feature. The solid microemulsion changed into aqueous microemulsion after redispersing by water and the mean

particle size was 10.34 nm. In addition, the solid microemulsion had good stability and improved biological activity compared to conventional solid formulations. This research provides a simple and convenient method to produce solid nanoformulations and the solid microemulsion is perspective in plant protection for improving bioavailability and reducing environmental pollution.



## Competing Interests

The authors declare that they have no competing interests.

## Authors' Contributions

Lei Feng and Bo Cui contributed equally to this work.

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