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Emulsion-based synchronous pesticide encapsulation and surface modification of mesoporous silica nanoparticles with carboxymethyl chitosan for controlled azoxystrobin release

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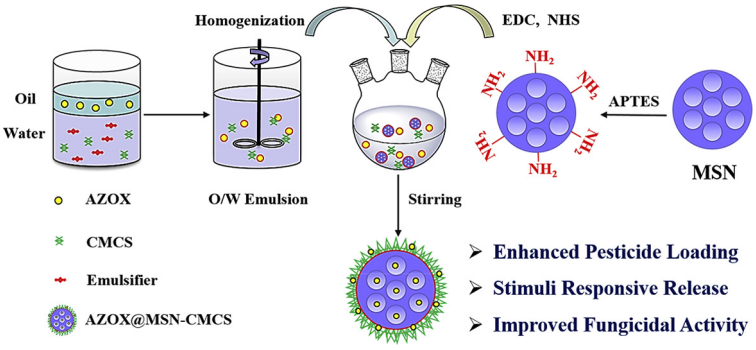
HIGHLIGHTS

- Emulsion-based synchronous pesticide loading and modification of MSN with CMCS was developed.
- Improved loading of 21% was obtained compared to that of 3.6% into pre-modified MSN-CMCS.
- Azoxystrobin-loaded nanoparticles showed pH-responsive release profiles.
- Azoxystrobin-loaded nanoparticles exhibited better bioactivity against tomato late blight.
- Uptake of the prepared nanoparticles in the target plants and fungi was confirmed by confocal microscopy.

GRAPHICAL ABSTRACT



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ABSTRACT

Controlled pesticide release in response to environmental stimuli is highly desirable for improved efficacy and reduced adverse effects. Recently, mesoporous silica nanoparticles (MSN) have become a dazzling star of nanomaterial members in smart delivery systems. In the present study, amino group-functionalized MSN and carboxymethyl chitosan (CMCS) underwent a coupling reaction to facilely afford CMCS modified MSN (MSN-CMCS) as pesticide carrier. However, the loading content of azoxystrobin (AZOX) into MSN-CMCS was only 3.6%, owing to the sharply decreased specific surface area and pore volume. To address this limitation, a novel strategy of emulsion-based synchronous pesticide encapsulation and surface modification of MSN with CMCS was developed. In this way, a satisfactory loading content (21%) can be achieved without sacrificing the pH responsive release properties controlled by gatekeeper CMCS. AZOX-loaded MSN-CMCS exhibited better fungicidal activity against tomato late blight *Phytophthora infestans* (*P. infestans*) than AZOX alone under the same doses of active ingredient applied. Fluorescein isothiocyanate-labelled MSN-CMCS were used to track the uptake and translocation of nanoparticles in the target plants and fungi. This research seeks to develop a novel nanocarrier platform for potential applications of pesticides in sustainable plant protection.

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1. Introduction

The extensive and irrational use of pesticides to control pests and diseases in agriculture has led to serious adverse effects on ecosystem balance and human health [1–3]. Therefore, demand for effective and safe pesticide use is growing. As an effective approach to address these issues, the development of controlled release formulations (CRFs) of pesticides has garnered considerable attention in recent years [4–8]. Nanoparticles have great promise for use as agrochemical carriers in modern agriculture due to their biocompatibility, high surface areas and other unique properties [9]. Various nanomaterials have been used as pesticide carriers [10–12]. Among these nanoparticles, mesoporous silica nanoparticles (MSN) have received considerable attention owing to their controllable mesoporous structure, large pore volume, easy surface modification and intrinsically large cargo loading content [13]. MSN have been widely reported as carriers to control the release of pesticides, such as uniconazole [14], imidacloprid [15], fipronil [16], 2,4-D [5], avermectin [17], chlorfenapyr [18], and pyrimethanil [19]. However, CRFs based on bare MSN release in a slow and sustained manner, and occasionally exhibit burst release [15]. To overcome this inherent limitation, functionalized MSN with diverse gatekeepers can provide an alternative for controlled pesticide release responsive to external or internal stimuli.

MSN with various gatekeepers show promising potential as scaffolds for controlled pesticide release because these gatekeepers can cap the mesoporous channels or surfaces to regulate the release of cargo molecules and avoid premature leakage during the delivery process before the target is reached. Moreover, an ideal gatekeeper can only be totally or partially removed under specific external or internal stimuli, such as temperature, pH, redox potential, light, magnetic field, and biomolecules, which is significant for potential applications in precise agriculture [13]. Cao et al. established a novel functionalized double-shelled avermectin microcapsule using silica cross-linked with chitosan, which had better controlled release properties. The prepared microcapsules can also protect avermectin against photo-and thermal degradation effectively [20]. Li et al. developed poly(diacetone acrylamide) functionalized hollow MSN, which can control the release of cyantraniliprole and enhance the adhesive property on rice leaves [21]. Yi et al. established functionalized MSN with redox-responsive short-chain decanethiol as gatekeepers for agrochemical salicylic acid (SA) delivery. The release profiles of SA under a certain amount of glutathione (GSH) were obviously high compared with that without GSH [22]. Recently, we prepared 2,4-D sodium salt-loaded positive-charge-functionalized MSN by incorporating trimethylammonium groups onto MSN via a post-grafting method. Pesticide loading and release patterns were pH, ionic strength and temperature responsive, which were mainly dominated by the electrostatic interactions [23].

However, functional group modification for MSN prior to cargo loading may lead to the blockage of pore channels, thereby generally resulting in decreases in surface area, pore volume, and subsequent loading content for guest molecules [24]. Therefore, it is highly desirable and beneficial to develop synchronous cargo encapsulation/MSN modification approaches by which the satisfactory loading content can be maintained without sacrificing the on-command release controlled by gatekeepers. For this purpose, the choice of corresponding gatekeeper compounds and preparation method are crucial.

Research has recently shown that certain responsive compounds can be anchored onto the surfaces or pore channels of MSN [25]. Natural poly- and oligosaccharides, such as chitosan [26], alginate [27], α-cyclodextrin [28], and glycyrrhizic acid [29], with their inherent biocompatibility, low toxicity and biodegradable properties, are widely used as novel delivery systems for plant protection compounds. Carboxymethyl chitosan (CMCS) (Fig. 1A), one derivate of chitosan, is an ideal material to modify MSNs owing to its unique properties of favorable biodegradability, nontoxicity, good water-solubility and antibacterial activity [30]. In addition, the carboxyl and amine groups in CMCS can be protonated at pHs less than 7.2 and 3.4, respectively [31], which indicates a possible way to use CMCS as smart molecular devices that can be responsive to an external pH stimulus [32,33]. Moreover, the carboxyl group in CMCS can be covalently bonded with other functional groups in MSN to afford functionalization.

In the present work, azoxystrobin (AZOX) (Fig. 1B), which is a broad-spectrum and low-toxicity novel strobilurin fungicide [34], was selected as a model pesticide to explore the feasibility of CMCS-modified MSN as controlled release carriers. The advantages of synchronous AZOX encapsulation/MSN modification with CMCS were clearly demonstrated. The controlled and sustained pesticide release properties under different aqueous solutions were determined in vitro. Moreover, the fungicidal activity of AZOX-loaded CMCS-modified MSN against the fungus tomato late blight (*P. infestans*) was explored, and visual observation in mycelium and tomato plant with confocal microscopy was also studied. This research seeks to develop a novel nanocarrier platform for potential applications of pesticides in sustainable plant protection.

2. Materials and methods

2.1. Materials

Tetraethylorthosilicate (TEOS, 99%) was purchased from Fluorochem Ltd. (Hadfield, UK). Cetyltrimethylammonium bromide (CTAB, 99%), 3-aminopropyltriethoxysilane (APTES, 98%), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC, 98%), N-hydroxy succinimide (NHS, 98%) and fluorescein isothiocyanate (FITC, 95%) were purchased from J&K Scientific Ltd. (Beijing, China). Carboxymethyl chitosan (CMCS) was purchased from Beijing HWRK Chen Co., Ltd. (Beijing, China). Azoxystrobin (AZOX, 98.5%) was generously provided by Jiangsu Lianyungang Liben Crop Science and Technology Co., Ltd. The tomato late blight (*P. infestans*) strain was supplied by Pesticide Bioassay Lab in Institute of Plant Protection, Chinese Academy of Agricultural Sciences (Beijing, China). Deionized water was obtained from a Milli-Q water purification system from Millipore, USA. All other chemicals and reagents were commercially available and used as received without further purification.

2.2. Synthesis of the nanoparticles

2.2.1. Synthesis of MSN

MSN were prepared following a sol-gel method reported by Radu with minor modifications [35]. CTAB and TEOS were used as the structure-directing agent and silica source, respectively. Briefly, 3 g of CTAB was dissolved in 2000 mL of deionized water under constant stirring with a rate of 800 rpm (rpm) at room temperature, followed by the addition of 10.5 mL of 2 M sodium hydroxide solution. The mixture was heated to 80 °C in an oil bath, and 15.0 mL of TEOS was later added dropwise. The solution was stirred vigorously at 80 °C for 6 h. The resultant white precipitate was collected by vacuum filtration, washed several times with ethanol and water, and dried at 80 °C overnight in an oven. The removal of the surfactant was carried out by calcining the as-synthesized white powder at 550 °C for 5 h.

2.2.2. Synthesis of amino-functionalized MSN (MSN-NH_2)

Amino-functionalized MSN (MSN-NH_2) were synthesized by a post-grafting method according to Hikosaka with slight modification [36]. Specifically, 1 g of pristine MSN was suspended in 80 mL of anhydrous toluene, followed by the addition of 2.0 mL of APTES after vigorous stirring for 20 min. The reaction mixture was refluxed for 4 h under vigorous stirring. The resultant samples were collected by centrifugation at 10,000 rpm for 5 min and washed with ethanol and water three times. The obtained nanoparticles were dried at 80 °C overnight.