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Development of chitosan nanocapsules for the controlled release of hexaconazole

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ABSTRACT

Accelerated use of pesticides in cutting edge agriculture prompted us to explore smart nanoformulations to subside the consumption of these perilous chemicals. Polymer nanocapsules carrying a fungicide, hexaconazole were developed through ionotropic gelation method utilizing chitosan and tripolyphosphate (TPP). The nanocapsules were characterized by photon correlation spectroscope (PCS), transmission electron microscope (TEM), and Fourier transform infra-red (FTIR) spectroscope. Nanocapsules were optimized for size and high encapsulation efficiency using central composite design (CCD) software. The encapsulation efficiency of nanocapsules for hexaconazole was 73% as assessed by gas chromatography (GC). Nanocapsules were analysed and compared with commercial formulation for controlled release in vitro at three different pH values. Release of hexaconazole from nanocapsules was fastest at pH 4 in comparison to pH 7 and pH 10. Release study in soil was also conducted and revealed a controlled pattern for nanoformulation. The fungicidal activity of the prepared nanoformulation was evaluated against *R. solani* and was compared with commercial formulation of hexaconazole. The cytotoxicity assay performed on vero cell lines by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay confirmed that nanoformulation is less toxic than commercial formulation of pesticide.

1. Introduction

One of the prime concerns for the scientific community is to increase crop production. Pesticides, which include fungicides, insecticides, and herbicides, have the capability of saving crops from pests, although most of an applied pesticide is lost to the environment. As such, the incessant application of pesticides worsens environmental pollution. Additionally, pesticides show toxic effects on non-target organisms through the environment or from residues in crops. Hexaconazole [(*R*,*S*)-2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazole-1-yl)hexane-2-ol] is a systemic fungicide that is extensively used for controlling fungal pathogens on various crops [1]. Besides its use as a fungicide, hexaconazole has some pernicious effects including reduction in shoot length, leaf area, and whole dry weight in the case of some Plectranthus sps. Its residues are found in tea leaf extracts and it also inhibits the dehydrogenase system of nitrogen fixing bacteria in soil [2].

Controlled release formulations (CRFs) are capable of mitigating issues associated with the use of pesticides such as degradation, leaching, evaporation, amount of pesticide used, number of applications, and toxic side effects [3]. Nanotechnology has been predicted to revolutionize the agriculture sector. Controlled release nanoformulations (CRNFs) could be extremely useful due to their small size and high surface area, which can augment the effectiveness, solubility, and stability of agrochemicals.

Polymeric nanoparticles have been adopted in the formulation of therapeutic drugs due to their controlled release properties and nanometer dimensions [4,5]. Chitosan is synthesized from naturally occurring chitin by partial *N*-deacetylation [6]. A number of advantageous properties (e.g., biodegradability, biocompatiblility, ready availability, and inexpensive cost) of this polymer make it a suitable candidate for numerous research-based applications. There has been substantial interest in chitosan-based nanoformulations due to their facile and moderate production procedures. These formulations are used as controlled-release drug delivery

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vehicles and have been extensively exploited in the pharmaceutical industry [7–15]. Chitosan nanoparticles (NPs) can be prepared by different methods, such as the emulsion-droplet coalescence coacervation/precipitation, ionic gelation, and reverse micellar methods [16]. Chitosan is positively charged due to the presence of amine groups that can form complexes with polyanions such as TPP, alginate, carrageenan, and xanthan gum. The ionotropic gelation technique for chitosan nanoparticle development used in the present study, based on electrostatic interactions between amine groups of chitosan and negatively charged phosphate groups of TPP, has been widely examined. Chitosan undergoes ionic gelation due to complexation between oppositely charged species and results in formation of spherical nanoparticles.

Extensive research has been performed on nanoparticle based drug delivery, but there are only a few reported studies on agrochemicals [17–19]. In this work, the fungicide hexaconazole was encapsulated in chitosan nanocapsules to assess the potential of a CRNF to curtail various risks involved in its application. The prepared nanocapsules containing hexaconazole were characterized by PCS, TEM, and FTIR. The encapsulation efficiency and controlled release in different pH buffers and soil were also measured using gas chromatography-flame ionization detection (GC-FID). The fungicidal activity in vitro was observed against *R. solani* by the poisoned food technique. Toxicity of the nanoformulation on non-target organisms was evaluated by an MTT assay with the vero cell line.

2. Materials and methods

2.1. Chemicals

Chitosan, sodium tripolyphosphate (STPP), & EZcount™ MTT cell assay kits were purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Chitosan from shrimp shells with a degree of deacetylation ≥75%, viscosity 780mPas (1% wt at 27 °C), and molecular weight 1129 KDa was used in the present study. The STPP used had following specifications i.e. molecular weight = 367.86, density(g/cm^3)= >1.5, melting point = 622 °C, pH value = 9.5–10.3.was used in the present study. Dichloromethane (DCM), sodium hydroxide, glacial acetic acid, and acetone were procured from Merck. Technical grade hexaconazole (92%) and a commercial formulation (5% EC) were procured from Tata Rallis Ltd. GC-FID (Varian 3900) was utilized to determine the quantity of hexaconazole in nanoformulation.

2.2. Quality assurance parameters

The concentration of active ingredient in the sample was determined by GC-FID with a minimum limit of detection of 2.5 ppm. Experiments were carried out in triplicate in the range of 10–50 ppm with an R^2 value > 0.99.

2.3. Preparation of nanocapsules

Nanocapsules of chitosan were developed through ionotropic gelation of chitosan with TPP [20]. Different concentrations of chitosan (0.05%–0.15%) and TPP (0.05%–0.10%) were taken. Chitosan solutions were prepared by dissolving it in 1% (v/v) acetic acid solution. The pH of the chitosan solution was raised to 4.6–4.8 with 1 N NaOH. Tween 80 (0.5%) was added to the chitosan solution to prevent particle aggregation. All of the solutions were filtered through Whatman filter paper (125 mm diameter). Next, 20 mg of hexaconazole in ethanol were added to 100 mL of chitosan solution. A further 25 mL of TPP solution was slowly added to the chitosan solution under continuous magnetic stirring. Chitosan-TPP (CS-TPP) nanocapsule formation started spontaneously via the TPP-initiated ionic gelation mechanism. Nanocapsules were centrifuged at 21,952*g* for 30 min. The supernatant was discarded, and the hexaconazole containing chitosan nanocapsules were then freeze-dried before further analysis.

Table 1
Optimization of nanocapsules size and EE by CCD software.

Exp. No.	Conc. of chitosan (%)	Conc. of TPP (%)	Particle size (nm) Y1	EE (%) Y2	pdi (polydispersity index)
1	0.10	0.08	100	73	0.325
2	0.05	0.10	602	50	0.536
3	0.15	0.05	186	65	0.539
4	0.10	0.08	100	73	0.325
5	0.15	0.10	122	69	0.287
6	0.10	0.08	100	73	0.357
7	0.04	0.08	365	56	0.295
8	0.10	0.04	131	67	0.428
9	0.10	0.08	100	73	0.316
10	0.10	0.08	100	73	0.329
11	0.16	0.08	154	66	0.402
12	0.05	0.05	230	58	0.587
13	0.10	0.11	215	60	0.609

2.4. Optimization of nanocapsules

CS-TPP nanocapsules containing hexaconazole were optimized for size and encapsulation efficiency (EE) through central composite design (CCD) using the Design Expert 8 software (version 8.0.4, Stat-Ease Inc., Minneapolis, MN). Chitosan and TPP concentrations were taken as independent variables while nanocapsule size and encapsulation efficiency were studied as response variables. Table 1 shows the experimental results (n = 13) with different combinations of polymer concentrations utilized for optimization.

2.5. Techniques used for characterization

Properties of the developed nanocapsules, including size, stability, shape, interaction of polymers with pesticide, and encapsulation efficiency, were examined by PCS, TEM, FTIR, and GC.

A Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) was used to analyze the size of hexaconazole entrapped CS-TPP nanocapsules at 25 °C. The phenomena of dynamic light scattering (DLS) was utilized to determine the average size of the nanocapsules and their polydispersity index. The stability of nanocapsules was determined from the zeta potential value, which is based on the electrophoretic mobility of the nanocapsules in aqueous suspension.

The morphology of hexaconazole entrapped chitosan nanocapsules was examined using a JEOL JEM 1011 TEM.

After centrifugation, CS–TPP nanocapsules were lyophilized, and the obtained sample was used for FTIR analysis (Bruker alpha spectrophotometer). Pellets of the sample and potassium bromide (KBr) were obtained by grinding and then compressing at 10,000 psi with a hydraulic press. Each KBr disc was scanned over the wave number region of 600–4000 cm^{-1}, and characteristic peaks were recorded for each sample.

After centrifugation of the nanocapsule suspension, the pellet was twice washed with DW to remove unentrapped fungicide. The supernatant was ultrasonicated with 100 mL of 10% sodium chloride solution, and the resulting mixture was extracted with dichloromethane (50 mL × 3). The dichloromethane extract was evaporated using a rotary evaporator, and the residue was dissolved in acetone and hexane in 1:4 ratio. A 1 μL aliquot of this sample was injected into the GC. The amount of active ingredient in the sample was determined by GC-FID. The EE% of nanocapsules was