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Development and evaluation of alginate–chitosan nanocapsules for controlled release of acetamiprid

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ABSTRACT

Smart formulations based on nanomaterials have the capability to reduce the consumption of hazardous pesticides and their impact on human health and environment. Nanoformulations of agrochemicals have the potential to improve food productivity without compromising with the ecosystem. In the present work, controlled release nanocapsules containing acetamiprid were prepared by polyelectrolyte complexation of two natural macromolecules, i.e. alginate and chitosan. The size, morphology and chemical interaction studies of the prepared nanocapsules were investigated by Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), and Fourier Transform Infrared Spectroscopy (FTIR). The zetapotential studies revealed stability of the nanocapsules. TEM results show spherical morphology of the nanocapsules. The encapsulation efficiency was found to be 62% as quantified by Ultra High Pressure Liquid Chromatography (UHPLC). Nanocapsules were analysed for controlled release *in vitro* at three different pH. Maximum release was observed at pH 10 followed by pH 7 and 4, respectively. A non-Fickian release mechanism was found to be followed by the nanoformulation. A controlled release pattern was also found from nanoformulation as compared to commercial formulation in soil. Thus this formulation can reduce the frequency of application of pesticides by controlling the release and will subsequently reduce their side effects.

1. Introduction

The production of quality food to feed growing population is the major challenge faced by agricultural scientists. Excessive and repetitive use of pesticides is a matter of major environmental concern as numbers of pesticides are identified as noxious or carcinogenic and can adversely affect human health and ecosystem. Ineluctable use of pesticides in modern agricultural practices has motivated researchers worldwide to focus attention in developing smarter formulations that can minimize use of such hazardous agrochemicals. The utilization of nanoplatforms in diagnostics and medicine under *in-vitro* conditions has generated interest in agri-nanotechnology for site specific and controlled release of various macromolecules enabling efficient use and safer handling of these agrochemicals. Controlled release formulations (CRFs) are emerging continuously to combat the issues associated with pesticides. Controlled release formulations of pesticides are emerging continuously to decrease the consumption and related side effects of pesticides. Controlled release formulations are reported to be superior to conventional formulations as they extend activity of pesticide [1], prevent pollution by reducing leaching [2] and volatilization [3], minimize residues on food stuffs, reduce health problems by decreasing dermal and inhalation toxicity [4,5], minimize cost involved with the production of pesticide in bulk and lastly but not the least, CRFs also reduce the harm caused to human health directly involved in handling of pesticides or indirectly through environment. Thus, the challenge of agricultural sustainability and food security can be addressed by encapsulating active ingredients such as herbicides, fungicides, fertilizers, in controlled release matrices, for decreasing toxicity and providing ecoprotection.

Nanotechnology based controlled release formulations are finding application in drug delivery [6,7] for improving healthcare and has been investigated extensively by researchers across the globe. A carrier must possess high drug loading and should prevent any premature release before reaching the intended site. The combination of nanotechnology and polymer science has resulted in the development of several novel formulations of existing compounds with improved characteristics. Polymeric nanocapsules have been frequently used as drug-delivery systems [8,9] but only a few reports

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are available on pesticide or herbicide release [10–12]. Nanocapsules are solid hollow particles which are used actively in controlled release formulation as these use little amount of pesticide compared to conventional formulation. Nanocapsules have small size and high surface area which helps them deposit on the plant leaves and thus reduces pesticide waste [10].

In the present study, acetamiprid was chosen as the active ingredient for encapsulation in nanocapsules. Acetamiprid, (E)-N-1-[(6-chloro-3-pyridyl)methyl]-N-2-cyano-N1-methylacetamidine is a neonicotinoid insecticide, used on vegetables, fruits and tea crops for control of insects of Hemiptera, mainly aphids, Thyasnoptera and Lepidoptera sps, [13]. The major problem associated with acetamiprid is its high water solubility that allows it to enter the natural water resources and poses threat to environment. Thus its great demand for a variety of crops and environmental concern prompted us to encapsulate it in nanocapsules which can provide a physical barrier as well as control its release.

A variety of natural polymers such as sodium alginate, chitosan, gelatin, albumin, etc., are used to develop nanocapsules as these are non-toxic, biodegradable and inexpensive. The alginate–chitosan system has been studied widely for drug delivery [14–24]. Alginate is an anionic biopolymer of α-L-guluronic acid and β-D-mannuronic acid units linked together by 1,4-glycosidic bonds [25]. Nanocapsules formed by an alginate polymer are found to have low stability that results in loss of the encapsulated materials. Cationic polymers such as chitosan have been employed with alginate for overcoming limitations associated with swift release of encapsulated material [26–28].

Chitosan, a naturally occurring linear polysaccharide consisting of copolymers of D-glucosamine and N-acetyl-D-glucosamine units joined through β-(1-4)-glycosidic bonds, has emerged as an exciting prospect for efficient delivery of micronutrients and agrochemicals [29]. Chitosan, due to its cationic character forms complex with negatively charged polymers such as alginate, sodium tripolyphosphate (STPP), TPP, xanthan gum, carrageenan, etc., and has been examined thoroughly for active ingredient delivery owing to its cost effectiveness, biodegradability, high permeability and low toxicity. In the present work alginate–chitosan nanocapsules were prepared by two step procedure, first the formation of pregel on addition of calcium chloride to sodium alginate and the second step involved formation of polyelectrolyte complex between carboxyl group of alginate and amine group of chitosan. A pregel nucleus forms upon interaction of alginate and Ca^{2+} at certain ion concentration on stirring [17]. The addition of chitosan solution into the pregel forms a polyelectrolyte complex which stabilizes the pregel into separate sponge-like nanoparticles [30]. The polyelectrolyte complex protects the encapsulated active ingredient, and limits its release more effectively than matrix formed from alginate or chitosan alone [31].

Thus, the present study was taken up with the aim to develop alginate–chitosan nanocapsules for controlled release of acetamiprid. These kinds of formulations are capable of providing alternative strategies for pest control in agriculture along with reducing dependency on synthetic pesticides and pesticide residue problems. The various process steps have been examined by microscopic and spectroscopic techniques. The release studies were performed at different pH ranges and in soil.

2. Experimental

2.1. Materials

Chitosan was purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Sodium Alginate was purchased from Sisco Research laboratories Pvt. Ltd. (Mumbai, India) Dichloromethane (DCM), glacial acetic acid and acetone were of analytical reagent quality and purchased from Merck. Methanol and water for HPLC were HPLC grade. Acetamiprid technical (96.5%) was obtained as a gift from Nagarjuna Agrichem chemicals pvt ltd. Ultra high pressure liquid chromatogram (UHPLC) from Thermo Fisher Scientific was used to estimate the amount of active ingredient in nanoformulation.

2.2. Preparation of acetamiprid containing alginate–chitosan nanocapsules

Alginate–chitosan nanocapsules were prepared by ionic pregelation and polyelectrolyte complexation method [22]. 0.06% Sodium alginate aqueous solution and 0.05% chitosan solution in 1% acetic acid were prepared and kept overnight. Thereafter, alginate and chitosan solutions were filtered and their pH was adjusted to 4.9 and 4.6, respectively. Acetamiprid (25 mg) was ultrasonicated with 100 mL of alginate solution for 15 min. Then 20 mL of 0.067% calcium chloride solution was dropped slowly to the above solution and stirred continuously for 30 min. Chitosan solution (15 mL) was added dropwise into the above and stirred further for 30 min which resulted in a colloidal suspension (pH 4.7). Nanocapsules were recovered by centrifugation at 14,000 rpm for 30 min at 4 °C.

3. Characterization

3.1. Size estimation and stability study

The average particle size of the acetamiprid loaded alginate–chitosan nanocapsules was determined using the Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). Dynamic light scattering (DLS) technique was used to measure the average size of nanocapsule and size distribution (polydispersity index), while zeta potential was studied for determining stability of the prepared nanocapsules which depends on electrophoretic movement of nanocapsules in the solution.

3.2. Transmission electron microscopy (TEM)

The morphology of acetamiprid loaded alginate–chitosan nanocapsules was determined by JEOL's JEM 1011 transmission electron microscope by taking drop of aqueous solution of nanocapsules on carbon coated Cu grid.

3.3. Fourier transform infrared spectroscopy (FTIR)

Alginate–chitosan nanocapsules separated from suspensions were lyophilized and their FTIR spectra were obtained using a Bruker's alpha spectrophotometer. Pellet was made by grinding 1% (w/w) of sample, with respect to potassium bromide (KBr) and compressed into KBr disc under a hydraulic press at 10,000 psi. Samples were scanned over a wavenumber region of 600–4000 cm^{-1}. The characteristic peaks were recorded for different samples.

3.4. Encapsulation efficiency

Acetamiprid content was estimated by UHPLC (Fig. 6). For this, nanoformulation was centrifuged and the pellet obtained was washed twice with distilled water to remove any untrapped pesticide. The pellet was dried and ultrasonicated with methanol, filtered through 0.22 μ filters into vials and kept in UHPLC apparatus. The syringe itself takes the sample (1 μL) from vials which are sequenced in the software. Methanol and water (50:50, v/v) was used as mobile phase at a flow rate of 0.1 mL/min. The absorbance was taken at 254 nm. The total analysis time for one sample was 10 min with elution of acetamiprid at 4.8 min (retention time).