Cellular and Molecular Biology

Journal homepage: www.cellmolbiol.org

Preparation and characterization of chloridazon-loaded alginate/chitosan nanocapsules

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ARTICLE INFO

Original paper

Article history:
Received: December 01, 2021
Accepted: March 04, 2022
Published: March 31, 2022

Keywords:
Herbicide, Chloridazon, Nanocapsule, Alginate, Chitosan, Ionic gelation

ABSTRACT

Herbicides contaminate soil, atmosphere, groundwater aquifers and runoff water while threatening human health. Nano-encapsulation of herbicides used in agriculture is one of the strategies to reduce the impact of such widely used agrochemicals. Nano-encapsulated herbicides are controlled membrane systems in which the active ingredient is coated with semi-permeable membranes, which may be organic or inorganic polymers. In our study, chloridazon herbicide was selected as the active ingredient to be nano-encapsulated. The ionic gelation method was used to synthesize nanocapsules consisting of alginate and chitosan for chloridazon encapsulation. Alginate-chitosan nanoparticles were prepared in a two-step process involving the ionotropic pre-gelation of an alginate core and then the formation of a chitosan polyelectrolyte complex. The alginate-chitosan nanocapsules containing chloridazon were synthesized at a size of 253 nm with a polydispersity index (PDI) of 0.266 and a zeta potential of -1.43 mV. The loading capacity and entrapment efficiency of these nanocapsules were 14% and 57%, respectively. The monitoring of chloridazon release from formulated alginate-chitosan nanocapsules was performed using dialysis tube testing and UV spectroscopy. The results indicated the slow release of chloridazon from loaded alginate-chitosan nanocapsules. controlled release and increasing the duration of action of chloridazon, along with reducing the required dose, is promising in reducing the adverse effects of chloridazon. Overall, the synthesized alginate-chitosan nanocapsules, as nanocarriers, have effective properties such as controlled release, prolonged action time, and reduced dose, which are expected to reduce the adverse effects of chloridazon on health and the environment, and improve precision agriculture.

DOI: http://dx.doi.org/10.14715/cmb/2022.68.3.5

Introduction

Weeds can represent a major constraint to crop production in agricultural fields and should be managed to ensure crop productivity and high quality of the harvest Chemical weed control remains the most common method of weed control due to its high cost/efficacy ratio. However, the over-use of herbicides has led to serious human health and environmental concerns (1). Nano-encapsulation of herbicides by increasing the effectiveness of herbicides can reduce their usage amount (2).

Nanotechnology refers to the research and development of technology at the atomic, molecular, and macromolecular scales that lead to controlled manipulation and the study of structures and devices with at least one longitudinal scale in the range of 1 to 100 nm. Nanotechnology has become an important field of research in the early 21st century, as scientists make different uses for the unique properties of nanometer-scale materials (3). Efforts to use nanotechnology in agriculture began with the growing awareness that conventional agricultural technologies are unable to increase productivity and repair damaged ecosystems of existing technologies. Therefore, nanotechnology with its many applications can be very helpful in improving agriculture and reducing the side effects of chemicals used in agriculture (4). Nanotechnology helps to improve agricultural production by increasing the efficiency of inputs and minimizing the associated losses. Indeed, nanomaterials provide a more specific surface area for fertilizers, pesticides and herbicides. In addition,

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nanomaterials, as unique carriers of agricultural chemicals, improve controlled delivery and release of active ingredients to increase productivity and crop protection. (5)

One of the main differences between nanoparticles and bulk materials is that there is a high ratio of atoms of a nanoparticle on the surface. In fact, nanomaterials have a higher surface-to-volume ratio. Compared to macroparticles of the same substance, nanoparticles may have different surface compositions, different types, and different densities. Also, they show different reactivity due to processes such as adsorption and redox reactions, which can be useful in the synthesis of nanomaterials and nanostructures, including nanocapsules, for use in agriculture (6, 7).

Nano-encapsulation is defined as the packaging technology of solid, liquid, or gas nanoparticles, also known as the core or active ingredient, in a secondary material called the matrix or shell to form nanocapsules. The core of a nanocapsule contains the active ingredient (e.g., drugs, biocides, etc.) while the shell of a nanocapsule separates the core from the surrounding environment and protects it. This protection can be permanent or temporary. In the temporary state, the core is usually released by diffusion or in response to an environmental stimulus, such as shell decomposition, pH, or enzymatic action. Due to this issue, controlled and scheduled delivery and release of the active ingredient to the desired location is possible (8-10).

Nano-encapsulation of herbicides is done with the aim of slow-release so that the active ingredients are released and act in lower doses and with a suitable time to receive the desired response in weed control without receiving a negative response from crops. In nano-encapsulated herbicides, the active ingredient is coated with water-soluble semi-permeable membranes (matrix) and the active ingredients are released by diffusion, osmotic pressure, ion exchange or destruction of the matrix (11,12).

Chloridazon is a pyridazinone (13) that primarily inhibits plant growth and survival (14, 15). This herbicide is an active ingredient that is rapidly absorbed by the roots and transported to all parts of the plant (16, 17). Chloridazon is currently used for selective control in sugar beet fields. But like many agricultural herbicides, the main problem with this herbicide is its environmental pollution and adverse health effects (18).

One of the most widely used types of nanomaterials in nanotechnology applications is polymeric nanoparticles, such as sodium alginate, chitosan, gelatin, albumin, etc. These nanomaterials have significant advantages such as biocompatibility, biodegradability and renewable sources. Polymeric nanoparticles have been increasing in recent years due to their clinical use in the diagnosis, therapeutic, and drug delivery systems as well as biotechnological applications such as coating of cells, tissue, proteins and DNA, vaccines production, fabrication of Nano-fertilizers, nano-pesticides and refining of heavy metals from water (19-21).

Carbohydrate polymers of natural origin have been used in biotechnology applications for many years. In the meantime, alginic acid, also called algin is of special importance. Alginate (salts of alginic acid) is a water-soluble polysaccharide that has excellent biocompatibility and biodegradability (22, 23). Alginate (AG) is an anionic polysaccharide of natural origin and abundant, which is an attractive source for the production of degradable polymer nanoparticles. These types of polymers have two main sources, which include bacteria and brown algae. Alginate is obtained from brown algae species such as *Laminaria hyperborea*, *Ascophyllum nodosum* and *Macrocystis pyrifera*. Alginate can easily form polymer nanoparticles due to its ability to form hydrogels when crosslinked (24). Various properties such as biocompatibility, non-toxicity, biodegradability and low cost, have made alginate a suitable candidate for biotechnological applications. Alginic acid is a linear copolymer with homopolymeric blocks of (1→4)-linked β-D-mannuronate (M) and α-L-guluronate (G) residues, respectively, covalently linked together in different sequences or blocks. (25)

Chitosan (CS) is a chitin-derived cationic polymer produced by the removal of acetate from chitin. In general, chitin with a degree of deacetylation above 70 % is considered as chitosan (26). Chitin and chitosan are natural amino polysaccharides with unique structures, multidimensional properties, highly advanced functions and wide applications in biomedical and other industrial fields (27, 28). Chitin-derived chitosan is a linear polysaccharide composed of randomly distributed β-linked D-glucosamine and

N-acetyl-D-glucosamine (29). Chitosan is a non-toxic and degradable cationic polymer with high molecular weight and is widely used in many fields of biotechnology. (30, 31).

Among these natural polysaccharides, two selected in our study, chitosan and alginate, are polyelectrolyte polymers with opposite charges (32). These polymers are highly promising in biotechnological applications due to their biologically desirable properties and have been widely used in the delivery of controlled release materials (33, 34).

In our study, the ionic gelation method was used to synthesize nanocapsules consisting of alginate and chitosan. Ionic gelation is simply the interaction of an ionic polymer with oppositely charged ions to initiate cross-linking. In the meantime, the three-dimensional structure and the presence of other groups will affect the ability of cations or anions to combine with anionic (or cationic) functions (35).

Therefore, our study aimed to develop alginate-chitosan nanocapsules for the controlled release of chloridazon. This type of formulation has the potential to reduce the side effects of chloridazon on the environment and health along with increasing the productivity of this herbicide in sustainable agriculture. The different stages of the nanocapsule preparation process were examined by microscopic and spectroscopic techniques and the controlled release test was performed in an aqueous medium.

Materials and Methods

Technical chloridazon was a gift from Bisetoon Kermanshah Chemical Complex Company, (Moshabak CO Kermanshah, Iran) to conduct this research. Sodium alginate and chitosan (medium molecular weight) and other chemicals used in this study were purchased from Merck KGaA, Darmstadt, Germany.

Preparation of chloridazon-loaded AG/CS nanocapsules

alginate-chitosan nanoparticles were prepared based on the modified method of S. Kumar et al 2015. Alginate-chitosan nanoparticles were prepared in a two-step process involving the ionotropic pre-gelation of an alginate core and then the formation of a chitosan polyelectrolyte complex. (36)

First, an aqueous solution of sodium alginate (0.06 % m/v) was prepared and then stirred (Uniequip. Germany) at room temperature for 24 h. Chitosan solution (0.05 % m/v) was also diluted in acetic acid (1 %) and then stirred at room temperature for 24 h. The alginate and chitosan solutions were then filtered. To load the chloridazon (26 mg), it was sonicated (Bandelin Sonopuls. Germany) with 100 ml of prepared alginate solution for 15 min. Then, to perform the cross-linking reaction, 20 ml of calcium chloride solution (0.06 7%) was added dropwise to the alginate-chloridazon solution and stirred continuously for 30 min at room temperature. Chitosan solution (15 ml) was added dropwise to the above solution and stirring continued for 30 min. The pH of the chloridazon-loaded alginate-chitosan solution was adjusted to 4.7 using hydrochloric acid (1 %) and 2 normal NaOH solutions. Finally, the synthesized nanocapsules were separated by centrifugation (Hettich Universal 320R) at 140,000 rpm for 25 min at 4 °C. The isolated nanocapsules were freeze-dried (Christ. alpha 2-4 LD plus) to continue the studies.

Determining the size and surface charge

The mean size of chloridazon-loaded alginate-chitosan nanocapsules was determined using zeta sizer (Malvern. Zetasizer Nano S90). And Zeta potential was used to evaluate the surface charge of chloridazon-loaded alginate-chitosan nanocapsules.

Morphology study

To determine the morphology of chloridazon-loaded alginate-chitosan nanocapsules, an SEM microscope (FEI quanta 450) was used by dropping a drop of the aqueous solution of nanocapsules on aluminum foil and drying at room temperature aeration.

Components and molecular structures

To determine the components and molecular structures of the substances involved in the prepared nanocapsules and also to determine the presence of chloridazon in the structure of the loaded alginate-chitosan nanocapsules, samples including alginate, chitosan, chloridazon, blank alginate-chitosan nanocapsules and chloridazon-loaded alginate-chitosan nanocapsules were examined by FTIR

(Shimadzu irprestige-21). Characteristic peaks were recorded for the mentioned samples.

The efficiency of encapsulation and controlled release of chloridazon in water

To determine the encapsulation efficiency of chloridazon in alginate-chitosan nanocapsules, it was necessary to determine the standard line equation of chloridazon. To do this, different concentrations of chloridazon were prepared and UV spectroscopy (Shimadzu. UV 2450) was performed in the range of 200 nm to 800 nm. After determining the adsorbents at each concentration, a standard graph was drawn. Using the T-test, adsorption at concentrations that caused problems in the standard graph was removed. The standard line equation was then calculated.

In the following, UV spectroscopy was used in the range of 200 to 400 nm from chloridazon-loaded nanocapsules to determine the absorbance in the range of 285 nm. The loaded nanocapsule suspension was centrifuged at 14,000 rpm for 25 min at 4 °C. The obtained supernatant was then diluted up to two times. And samples were examined for spectroscopy. According to the standard line equation of chloridazon and the absorption of 285 nm of nanocapsules loaded with chloridazon, the percentage of active ingredient loading and entrapment efficiency (EE) were calculated using the following formulas:

$$\%Entrapment\ efficiency = \frac{Weight\ of\ initial\ active\ ingredient - Weight\ of\ free\ active\ ingredient}{Weight\ of\ initial\ active\ ingredient} \times 100$$

$$\%Active\ ingredient\ loading = \frac{Amount\ of\ entrapped\ active\ ingredient\ in\ nanocapsules}{Total\ weight\ of\ nanocapsules} \times 100$$

The dialysis tube method was used to evaluate the controlled release of chloridazon from loaded nanocapsules in water. Dialysis tubing cellulose membrane (Sigma-Aldrich) used had a cut-off: 12400 daltons. First, 7 ml of chloridazon-loaded alginate-chitosan nanocapsules were isolated and poured into a dialysis tube. The dialysis tube was then placed in a container containing 200 ml of distilled water (pH 7.5). For sampling, a time interval of 0 to 48 h was considered and sampling was performed at 7 specific times (h: 0, 1, 2, 3, 4, 22, and 48). At each sampling, equal to the volume of the sample taken, distilled water was added to the container containing the dialysis tube. The samples were examined by UV spectroscopy in the range of 200 nm to 400 nm. Finally, a release graph was drawn.

Results and discussion

Characterization

Characterization of chloridazon-loaded alginate-chitosan nanocapsules was performed using zeta-sizer and zeta-potential. The average size of the synthesized nanocapsules was 253 nm, which together with the obtained polydispersity index (PDI) of 0.266 (0.5>) indicates a colloidal suspension and a good homogenous size distribution. (Figure 1). Due to the spontaneous diffusion of the polymer solution in the aqueous phase, nanoparticles are formed rapidly in an attempt to avoid water molecules, and as the solvent diffuses out of the nanoparticles, the polymer precipitates in the form of nanocapsules. (37)

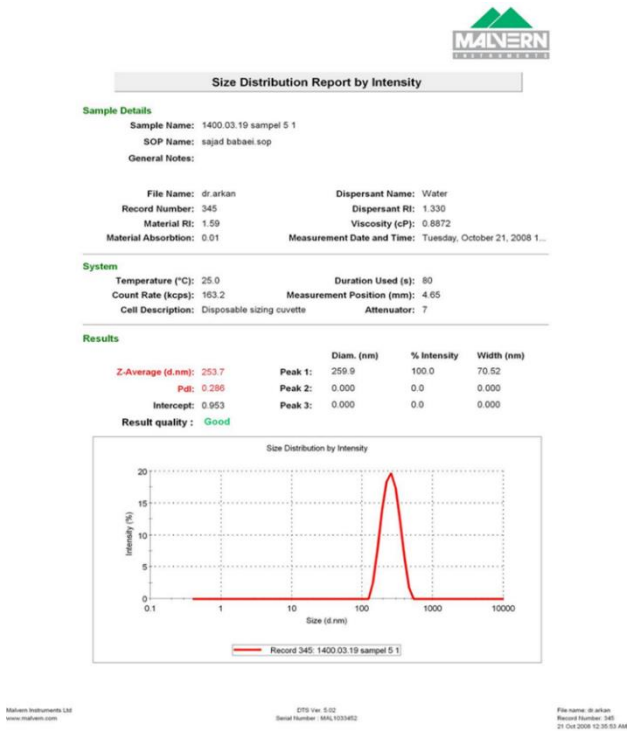

Figure 1. Size of chloridazon-loaded AG/CS nanocapsules

The zeta potential value of -1.43 mV was obtained, which indicates the acceptable stability of the formulated nanocapsules. (Figure 2) The value of negative zeta potential indicates the presence of a free –COO group on the surface, which can be the result of higher concentrations of alginate than chitosan because the carboxyl group is present on the alginate. Carboxylic acid groups attribute negative charges to alginate and therefore can interact electrostatically with positively charged molecules to form gels, which is the strong point of alginate in combination with chitosan. (38)

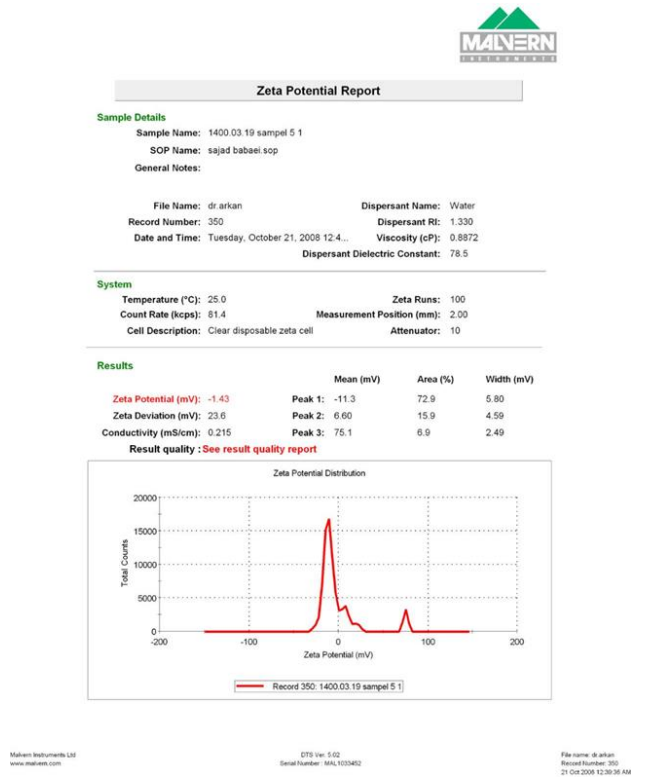

Figure 2. Surface charge of chloridazon-loaded AG/CS nanocapsules

Morphology

SEM images showed separate, solid nanocapsules in the size range of 253 nm. Figure 3 shows the surface topography of synthesized nanocapsules containing chloridazon at different magnifications. It seems that the amino group of chloridazon has been effective in the shape of nanocapsules in interaction with alginate and chitosan polymers. On the other hand, due to water retention and high hygroscopic properties of chitosan and hydrogen bonding, it can be expected that chitosan mechanical properties have changed and it has been effective in the shape of nanocapsules.

FTIR spectroscopy

The FTIR spectroscopy was used to accurate alginate-chitosan nanocapsules formation and verify chloridazon loading in these nanocapsules (Figure 4). The peaks of the alginate spectrum close to 1610 cm^{-1} and 1417 cm^{-1} are associated with asymmetric and symmetric tensile vibrations of the -COO alginate groups, respectively. Given that the peak has changed from 1420 to 1419 cm^{-1}, and this occurs after the formation of a complex between alginate and chitosan, so the formation of alginate-chitosan nanocapsules can also be confirmed. The presence of chloridazon in alginate-chitosan nanocapsules can be confirmed by the peak in 1618 cm^{-1}, which is related to the tensile vibration of the C=N bond. Due to the hydrogen bonding between the COOH of alginate and NH_2 of chitosan, the peak expands from about 3400 to 3500 cm^{-1} (36)

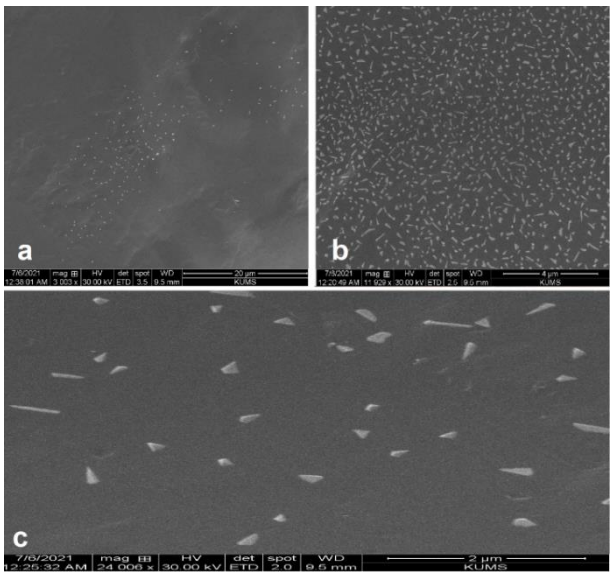

Figure 3. Scanning electron microscope images of chloridazon-loaded AG/CS nanocapsules: (a): ×3000 ; (b): ×12000 ; (c): ×24000 . The bars represent the scale of each image.

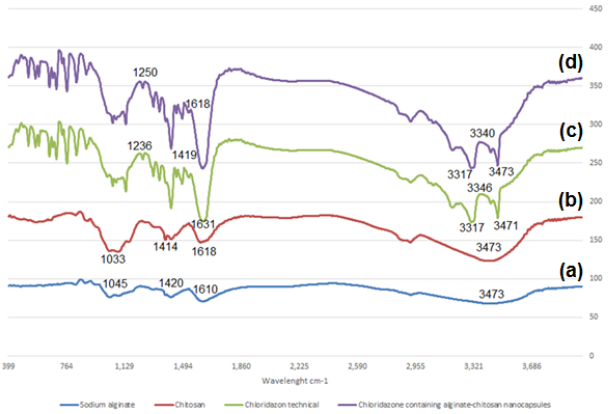

Figure 4. Fourier transforms infrared spectroscopy analysis of the herbicide, polymers and nanoparticles. The samples were (a) sodium alginate; (b) chitosan; (c) technical chloridazon; (d) chloridazon-loaded AG/CS nanocapsules.

Encapsulation efficiency and release in water

The percentage of chloridazon loading in the Nano formulation was 14 % and the encapsulation efficiency was 57 %. The calculations of these two parameters were obtained based on the standard line equation of chloridazon. (Figure 5)

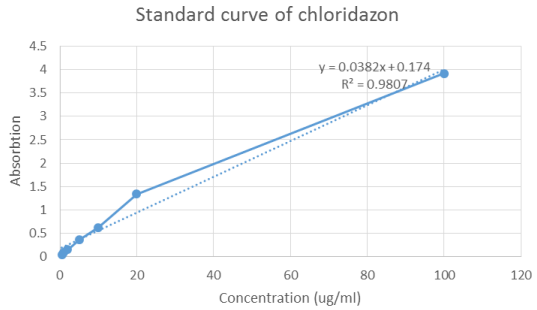

Figure 5. Chloridazon standard line equation in 285nm UV spectrum

The study of chloridazon release from formulated alginate-chitosan nanocapsules was performed using a dialysis tube test and UV spectroscopy. The highest amount of release (65 %) occurred after the first 5 h and then the release reached a stable level during the next 43 h. (Figure 6)

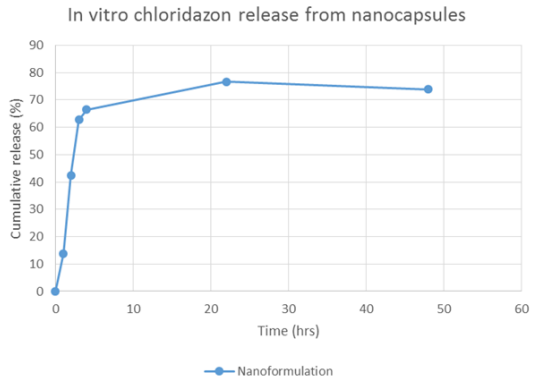

Figure 6. Release diagram of chloridazon from chloridazon-loaded AG/CS nanocapsules

The release rate of the active ingredient from the polymeric nanocapsules depends on two factors, which include the solubility of the encapsulated active ingredient and the properties of the polymer used (36). It seems that the relatively rapid release of chloridazon at pH 7.1 solution is due to the greater solubility of chloridazon at this pH and the simultaneous dissolution of alginate, which has a relatively higher solubility rate compared to chitosan in water. The results showed that chloridazon nanoformulation required more time to release the herbicide than its commercial formulation.

The results of our study provide information on the encapsulation of chloridazon by alginate-chitosan as a nanocarrier. The synthesized alginate-chitosan nanocarriers have the potential to become commercial formulations for chloridazon encapsulation.

Herein, alginate and chitosan were used as selected polymers for encapsulation of chloridazon due to their positive properties. In fact, the biggest advantage of alginate is the behavior of its liquid gel in aqueous solutions. When monovalent ions (e.g., sodium in sodium alginate) are exchanged with divalent ions (especially Ca^{2+}), the reaction proceeds almost immediately, transforming from a low-viscosity solution into a gel structure. In addition, large numbers of free hydroxyl and carboxyl groups are distributed along the alginate polymeric backbone. They are highly reactive and modifiable to chemical changes. Alginate, on the other hand, is a relatively inexpensive polymer and can easily form nanoparticles in different sizes using the ion gelation (IG) method. (39-41)

Chitosan, on the other hand, while insoluble in water at neutral pH, can be dissolved in dilute acids due to the protonation of its free amine groups. Chitosan is very sensitive to environmental conditions, especially moisture, due to its high hygroscopic properties. Water retention on chitosan occurs by hydrogen bonding and has been reported to have the ability to alter the mechanical properties of chitosan and cause a partial loss of its adhesion properties. Chitosan-based nanosystems can be used as nanocarriers due to their significant physical, chemical and biological properties, due to their ability to change the loading of the active ingredient and adjust the value of each parameter during preparation. (42-43)

The amine and carboxyl groups of chitosan and alginate, respectively, can create a rapid electrostatic interaction through the ionotropic gelation (IG) technique, resulting in the formation of a complex polyelectrolyte nanocomposite. Ionotropic gelation consists of the crosslinking of both biopolymers in the presence of polyvalent ionic compounds. However, the polycationic and polyanionic nature of these two biopolymers may spontaneously form a set of polyelectrolytes in an aqueous solution. Therefore, the combination of both biopolymers has been shown to be more effective than chitosan or alginate separately and improves bonding performance and stability in acidic and alkaline environments. It also enables controlled delivery and release of active ingredients due to external stimuli (including temperature and ion resistance. (44)

Conclusions

Synthesized chloridazon-loaded alginate-chitosan nanocapsules had an average size of 253 nm with a negative surface charge, indicating the acceptable status of these nanocapsules for use as a nanocarrier in precision agriculture. Due to the fact that alginate has a high solubility in water, in combination with chitosan, whose solubility is lower at neutral pH, they have the ability to create a suitable matrix for nanocarriers. The 57 % encapsulation efficiency of the synthesized nanocapsules is promising for the development of alginate-chitosan-chloridazon nanoformulation. On the other hand, chloridazon has a good relationship with the surrounding alginate-chitosan nanocapsules based on the characterization of the nanoformulation and the release test results. Controlled release and increased duration of action of chloridazon, along with dose reduction, are promising in reducing the adverse health and environmental effects of chloridazon. To the best of our knowledge, this is the first report of chloridazon nano-encapsulation (alginate/chitosan/Chloridazon) which provided a suitable background for investigating its impact on agricultural environments and further studies.

Acknowledgments

The authors would like to thank the Bisetoon Kermanshah Chemical Complex Company (Moshabak CO Kermanshah, Iran) who provided the technical chloridazon as an active ingredient for this research.

Interest conflict

The authors declare that they have no conflict of interest.

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