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Encapsulation of a bioactive steroid in a polymer matrix

(micro-encapsulation of DI-31 in chitosan by spray drying for various purposes)

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Abstract: DI-31 is a synthetic analog of brasinosteroids (ABR), the active ingredient (PA) of Biobras, a plant growth stimulant, which has shown positive impact on Cuban agriculture, especially in rice cultivation. However, it has the drawback of having low solubility in water and being rapidly metabolized by the plants. An alternative to overcome these limitations is its micro-encapsulation in a polymer matrix. Chitosan (CHI) has been investigated as an excellent candidate for microencapsulation of DI-31. Chitosan (CHI) is a natural polysaccharide obtained from chitin extracted from exoesqueleton of crustaceans, which increases resistance of the plants to the attack of pests and promotes the yield of the crops, also possesses excellent biocompatibility, biodegradability and mucoadhesivity, also inducing the defense of the plants against the attack of different pathogens.

Keywords: POLYMER MATRIX, CHITOSAN, MICROPARTICLES, SPRAY DRYING, DI-31

Introduction

The brasinoesteroids (BR) are steroidal phytohormones with important regulatory functions in plants. These compounds determine, among other plant physiological processes, cell division and growth, seed germination, crop yield, response to different stress conditions (saline, water, thermal stress) and plant protection To different pests and diseases. The role of the same in germination of seeds and growth, in flowering, senescence, photosynthesis, chlorophyll content, plant vascular tissue differentiation and activity of some plant enzymes (carbonic anhydrase, nitrate reductase) has been extensively studied. Several brasinoesteroids (BR) and synthetic analogues of brasinosteroids (ABR) have been used for the control of insects and pests that affect crops, due to their antiecdysteroid activity[1]. The synthetic analog of brasinosteroids, DI31, synthesized at the Center for Natural Product Studies at the University of Havana (CEPN), is a commercial agrochemical widely used in many crops, being able to increase the yield of different crops between 5 and 30%. On the other hand, the low aqueous solubility thereof; as well as the need to carry out two or more applications of Biobras (commercial agrochemical based on DI-31) at different stages in the case of long cycle crops, limit the expression of their benefits in plants and make them more expensive. Chitosan (CHI) is a linear cationic polysaccharide composed essentially of 2-amino-2-deoxy-D-glucose units. This biopolymer is rarely found in nature and is mainly obtained by exhaustive deacetylation of chitin, which is the main constituent of fungal cell walls, insect cuticle and carapace of

molluscs and crustaceans[2]. Said polysaccharide is biocompatible, biodegradable, non-toxic and mucoadhesive; in addition its antifungal and antibacterial properties, antioxidants, hypocholestatic and coagulants make it very attractive for numerous applications in medicine and pharmacy. Thus there are numerous reports and patents on the preparation of microparticles, microcapsules, hydrogels, nanoparticles, films and composites of CHI and their derivatives for the controlled release of various drugs. On the other hand, CHI can induce metabolic changes in plants, with an increase in crop yield, seed germination and resistance to pests[3]. In the bibliographic review carried out by the authors for the accomplishment of this work, there was no history of obtaining systems of controlled release of steroids based on Chitosan, only systems of urea and other non-steroidal agrochemicals were found based on Said polymer. From this background, it is necessary to have controlled release systems for biologically active steroids, especially the synthetic analogue 3 of brasinosteroids (DI-31), for use in agriculture. It is possible to prepare a polymer system based on Chitosan with a biologically active steroid (microparticles) that allows its controlled dosage in plants. Therefore, in order to validate this hypothesis, the general objective of the work is to obtain and characterize physicochemically the microparticles of Chitosan loaded with DI-31, using the technique of spray drying for its application to plants[4].

Experimental Part

Chitosan used was supplied by Chitopharm (Haugesund, Norway), Mw = 129.4 KDa Degree of Acetylation 20.5%. We used DI-31 (Natural Products Center of the University of Havana) and sodium tripolyphosphate (TPP, Sigma-Aldrich).

Solubility study of the brassinosteroid analog DI-31:

A solubility study was carried out in order to evaluate the characteristics of the solvents to be used in the drying process using the spray driying. Thus, 4 solutions of ethanol and water in the proportions 80/20, 70/30, 60/40 and 50/50 were prepared with 300 rpm magnetic stirring maintained for a period of 15 minutes. In parallel, 4 more solutions were prepared in the same proportions and conditions but of tetrahydrofuran (THF) and water[5].

Preparation of microparticles of chitosan and chitosan/DI-31 by spray-drying:

To obtain the chitosan microparticles loaded with DI-31, a solution of CHI at 1% (m/V) was started. DI-31 (as active ingredient to be encapsulated) and TPP (as crosslinking agent) were added to the chitosan solution by 0.25 and 0.01 respectively, relative to the polymer mass. The resulting suspension was sprayed in a mini Spray-Dryer B-290 (Büchi Labortechnik AG, Flawil, Switzerland) with 0.7 µm nozzle at an inlet temperature of 90 ° C with a feed liquid flow at 5 mL / min and An outlet temperature of 115 °C[6].

Obtaining the microparticles:

The microparticles of Chitosan loaded with DI-31 were obtained by spray drying of the BUCHI brand from Germany. The process passed through successive stages, the first of which involved the formation of an oil-water emulsion (O / W). An aqueous / ethanol solution (70/30%) was used as the aqueous phase, while a solution of DI-31 (active ingredient) was used as the organic phase. In parallel, a solution of Chitosan in both acetic acid was prepared at 1%. The resulting formulation was suctionally introduced into the apparatus having an inlet temperature of 105 degrees Celsius and an outlet temperature of 90. Microparticles of sizes between 1 and 4 micrometers were obtained[7].

Physico-chemical characterization of microparticles obtained:

The microparticles of CHI/DI-31 were characterized by FTIR spectroscopy using a Perkin-Elmer FTIR (Italy) spectrophotometer with 32 scans and 4 cm^{-1} resolution. Samples were prepared by the Potassium Bromide tablet method. The size of microparticles was determined using a Nikon Eclipse E-400 optical microscope (Mexico D.F.). The morphology of the microparticles was studied by scanning electron microscopy (SEM) with a TESCAN 5130 SB microscope (Czech Republic). Samples were coated with Au-Pd using a POLARON SC 7620 (UK).

Determination of Encapsulation Efficiency:

25 mg of CHI/DI-31 microparticles, and CHI (placebo) microparticles were shaken at 40 ° C in 0.1 N acetic acid for 24 h. The resulting solutions were filtered and the amount of encapsulated steroids was estimated by absorbance determined by UV at 245 nm using an Ultrospec 2100 Pro UV spectrophotometer. The concentration of the solution was determined from a previously obtained calibration curve. The amount of encapsulated compound was expressed as percent loading (g of Brasinoesteroides in 100 g of microparticles). The reported loading rate is expressed as the mean standard deviation ± SD of four experiments[7,8].

In vitro drug release study of microparticles:

In vitro release of DI-31 from the CHI microparticles was studied by measuring the corresponding profiles using UV detection at a specific wavelength. 25 mg of CHI/DI-31 microparticles were placed in a volumetric flask containing phosphate buffer and sodium PBS (pH 6.0) and 70% ethanol solution in parallel to a total volume of 25 ml and incubated at 30 ° C with constant stirring at 100 rpm. A 1ml aliquot was periodically taken from the flask and replaced with fresh solution to maintain a constant volume. Through the value of the UV absorbance of the solution, the concentration of the solution was determined by extrapolation of the curve and with the slope value. These studies were done in triplicate for each sample.

Results

Figure 1 shows the electron micrograph of the microparticles of CHI/DI-31 + TPP obtained by spray-drying; the microparticles have a morphology and size characteristic of the used drying

process as described in the literature for the equipment employed. The images exhibit a spherical morphology of the microparticles, attributable to the use of the spray drying.

The morphology of CHI / DI-31 microparticles can be seen in Figure 2. In addition, a speckle is observed on the surface thereof, characteristic of the insolubility in water of the encapsulated active principle.

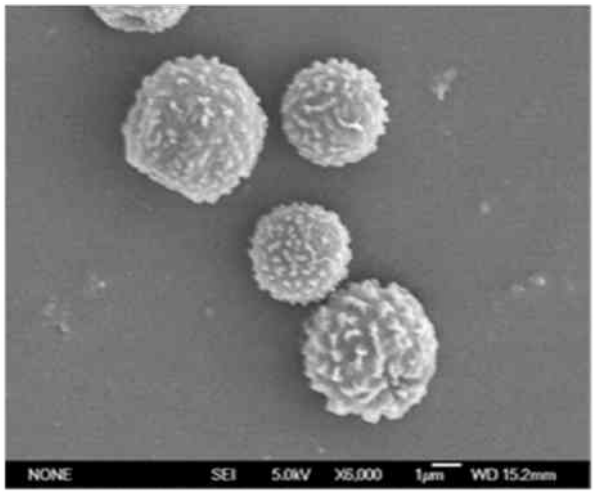

Figure 1. SEM micrograph of the CHI/DI-31 + TPP microparticles obtained by spray driying.

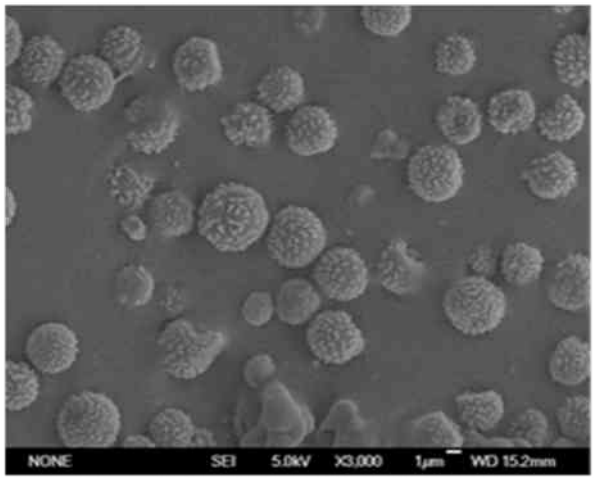

Figure 2. SEM micrograph of the microparticles of CHI/DI-31 obtained by spray drying.

Infrared spectroscopy to Fourier transform

The FTIR, Chitosan, CHI/ DI-31 microparticles(MDI-31) and the TPP cross-linked microparticles (MDI-31+TPP) are shown in Fig. 3. Also included is the placebo spectrum (M) of the microparticles for comparison of the characteristic bands.

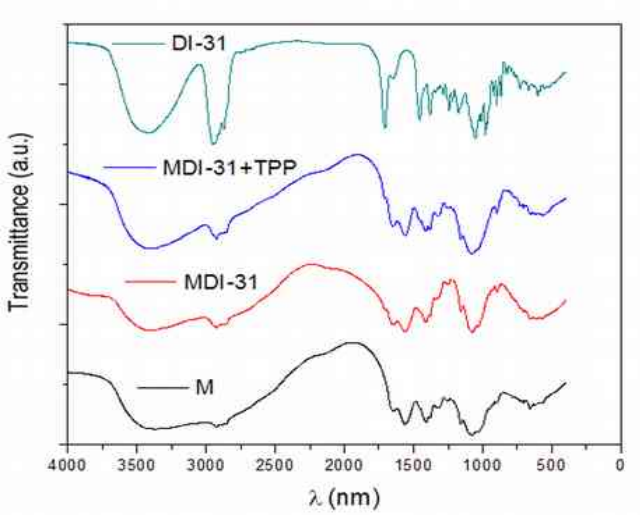

Figure 3. FTIR spectra of CHI / DI-31 + TPP microparticles

Release study of CHI/ DI-31 microparticles in ethanol / water and buffer-phosphate solution (PBS)

The release behavior of the DI-31 studied from the microparticles was evaluated by performing in vitro release experiments at pH 6 (PBS) and by mixing water and ethanol to simulate the conditions of the current agrochemical formulations. In all cases a sustained release of the steroid was obtained, characterized by an almost constant release rate (zero order kinetics) during the first 24 h. As expected, the release was always higher in ethanol / water than in PBS, due to the higher solubility of the steroid in said organic solvent.

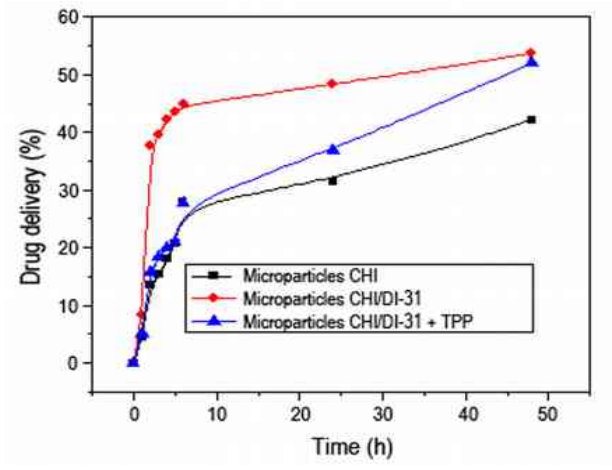

Figure 4. Microparticle release profile in ethanol / water

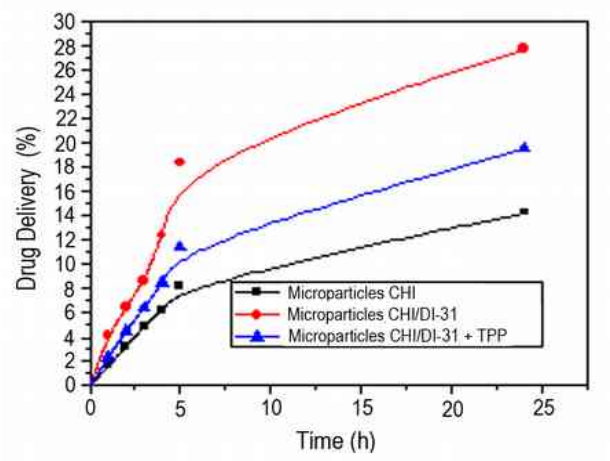

Figure 5. Release profile of the microparticles in PBS (pH 6)

Discussion

As shown in Figure 1, the CHI/DI31 particles exhibit a collapsed appearance, whereas in the CHI/DI31 + TPP particles the predominant shape is spherical, further exhibiting a characteristic mottling on its surface. The round shape of the particles of CHI/DI31 can be attributed to the drying process due to the evaporation of the solvent that generates an external crust, and once formed this is that the solvent still present in its interior evaporates resulting in a partial shrinkage of the particle[9,10]. In the case of the microparticles of CHI/DI31 + TPP, the shape is markedly spherical, some with some roughness, but not collapsed, which is a consequence of the presence of TPP in the atomized mixture.

Depending on the equipment used that has a nozzle with internal diameter of 0.7 µm, the particle size should be less than 10 µm. Taking into account the scale of the micrograph, the particle size oscillates around 4 µm, which is in agreement with the nozzle used and with the results reported by Desai et al.[11] in a study of obtaining chitosan microparticles with TPP in which the particle size obtained varies from 3.1-10.1 µm.

As for the yield of the process, 69 ± 1% was obtained for CHI /DI31 microparticles and 54.4 ± 0.8% for CHI / DI31 + TPP microparticles. Other authors report similar results and associate the loss of mass to the material adhered to the walls of the chamber and the cyclone of the spray, which is inherent in the technological process when working at a bank scale[12]. Efficiency of encapsulation reached was 46.8 ± 0.7%. This result is due, among other factors, to the known fact that when a suspension is atomized, some of the drug crystals are left out of the microdroplets and therefore are not encapsulated[6]. This low encapsulation efficiency could be improved from a technological view from a study of the concentrations of polymer, drug and crosslinking agent in the mixture to be atomized, in addition to the optimization of the drying parameters.

The IR spectrum of CHI / DI-31 microparticles shows absorption bands at 2942-2784 cm^{-1} (C-H stretching aliphatic band), 1657 cm^{-1} (amide I), and 1.597 cm^{-1} (-NH 2) flexure (amide III). The absorption bands at 1154 cm^{-1} (anti-symmetrical stretching of the C-O-C bridge), 1082 and 1032 cm^{-1} (skeletal vibrations involving C-O stretching) are characteristic of their saccharide structure[8]. The CHI/ DI-31 microparticle spectra are dominated by the CHI peaks due to the excess CHI on the DI-31 in the microparticles. The spectrum of DI-31 shows a more intense and narrow band at 1700 cm^{-1}, which is absent in the spectrum of empty microparticles. These bands overlap, the Amide I and the -NH2 bands at 1650 and 1597 cm^{-1}, respectively, producing a broadband ranging from 1700 to 1500 cm^{-1}. The observed hypsochromic change of these peaks is probably the result of an interaction of the hydroxyl groups and / or carbonyl groups of the DI-31 with the amine group of CHI molecules.

The ABR DI-31 was loaded into TPP and Chitosan forming microparticles. The highest loading percentages were obtained using ethanol solution of the steroid. Although in both cases the load never exceeded 50%, a remarkable increase in the load percentage is obtained when the DI-31 is only in the system. Sustained release profiles were obtained for all 3 cases studied. The results indicate that when introducing a novel process of obtaining by driying spray it would be possible to design an efficient system of supply based on chitosan for the sustained release of brasinoesteroids for its application as agrochemicals.

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