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CHITOSAN-BASED NEEM SEED EXTRACT NANOCAPSULES: A NEW APPROACH ON ENHANCING ITS EFFECTIVENESS AS AN INSECTICIDE DELIVERY AGENT

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ABSTRACT

The utilization of nanotechnology in pesticides delivery has aiming to reduce the indiscriminate use of conventional pesticides and to ensure their safe application. The focus of the present research is on the development of a nanoencapsulated neem seed extract with chitosan as an encapsulate introduced through a harmonically ultrasonic wave treatment. The verification of the presence of AI extract and chitosan in the nanoencapsulate obtained and its characterization are critical to be performed. The method advanced provides a successful insertion of AI in chitosan with no variation of its initial properties producing a nanosized material (below than 200 nm) with good size distribution. This study provides a fundamental and critical information for researchers and engineers in the field of nanotechnology especially in terms of nanoencapsulation techniques use to deliver insecticides.

Keywords: nanoencapsulation, chitosan, neem seed extract, pesticide.

INTRODUCTION

Chitosan and its derivatives receive great interest lately as an advanced material for several purposes [1]. This is in an agreement with the fact that chitosan is the second most abundant natural polymer next to cellulose with a number of advantageous properties like biocompatibility [2], biodegradability [3], easy physical and chemical modification [4]. Moreover, the hydroxyl active sites present, the long polymer chains, as well as the non-toxicity determine the choice of this material as a delivery agent for medicine, agriculture, environmental even in electronic device [5]. Chitosan is intensively improved as one of the important material for encapsulation of noxious and carcinogenic insecticides finding application in agriculture. This development referring to the controlled release and efficient and safe use of these agrochemicals is based on the utilization of small-size material platforms in diagnostics under in-vitro conditions in medicine. However, investigations on obtaining more effective insecticides are still required in view of the developing science and technology.

The increasing awareness and concern about the impact of agricultural practices on the environment, food, and fiber production promotes the concept of sustainable agriculture, thus, raising the thrust for biopesticides over synthetic pesticides.

Azadirachta indica (neem) is an evergreen tree with insecticidal qualities. It can replacement neurotoxic products such as organophosphates and carbamates in insect control. The extract itself possesses a multiplicity of components, which makes it more difficult for the insects to acquire resistance, and the concentration of the active components is high, making extraction an

easy and a low-cost process. The neem tree is a source of many triterpenoids, while azadirachtin is one of the main components of neem seeds. The extraction of the latter results in 25 wt. % raw extract. The endurance of azadirachtin structure can be enhanced through encapsulation of neem seed extract in view of botanical insectides sensitivity to light, humidity, temperature, and acid or alkaline media. The encapsulation is conducted with the participation of macromolecules and polymers like, chitosan alginate, PEG, poly(ε-caprolactone) [6 - 8]. A low diameter size of encapsulation is required to improve the stability and effectiveness of encapsulation. The design of active nano-sized ingredients provides the active compound transfer to the target organism followed by its controlled release at the site of action. This approach can also help to minimize the undesirable toxic effects on the non-target organisms, to improve the physicochemical stability and prevent degradation of the active agent by microorganisms [9 - 10]. This explains the effort to find a method of nano-size encapsulating of neem seed extract which in turn will lead to its more efficient utilization. This study focuses on nanoencapsulation of neem seed extract enclosed on chitosan using an ultrasonic environment. Succinate anhydride is added as a crosslinking agent to facilitate the controlled release. It not only keeps azadirachtin structure but also acts as an additional barrier in the controlled release of AI. The performance in respect to pod sucking bug (*Riptortus linearis*) on soybean is investigated.

Furthermore, anhydride succinate was used as crosslinked agent on the nanoencapsulation that not only support chitosan on keeping azadiracthin structure, but also as additional barrier for controlling release of AI.

EXPERIMENTAL

Material

Neem seeds were collected from neem tree growing in Surabaya. Chitosan powder, derived from shell, with viscosity of 20 mPas - 100 mPas and deacetylation degree of min 84 % was purchased from Chimultiguna Ltd. Succinate anhydride and acetic acid were purchased from Sigma Aldrich, while sodium hydroxide and hydrogen chloride were provided by Bratachem Ltd. All solvents used in this study were of an analytical grade. They were applied without any additional treatment.

Neem sees were initially oven-dried at 60°C until a constant weight and then finely ground (one kilogram was collected). Ethanol was added to the powder and the mixture was stirred two days until it became uniform. It was then subjected to filtration. The solid mass obtained was washed with chloroform which was then evaporated. The resulting powder was named as neem seed extract. It was further used.

Two grams of chitosan were dissolved in acetic acid solution (5 %). Then 160 mL of methanol were added. The succinate anhydride used was initially dissolved in 30 mL of acetone. It was added to the chitosan solution

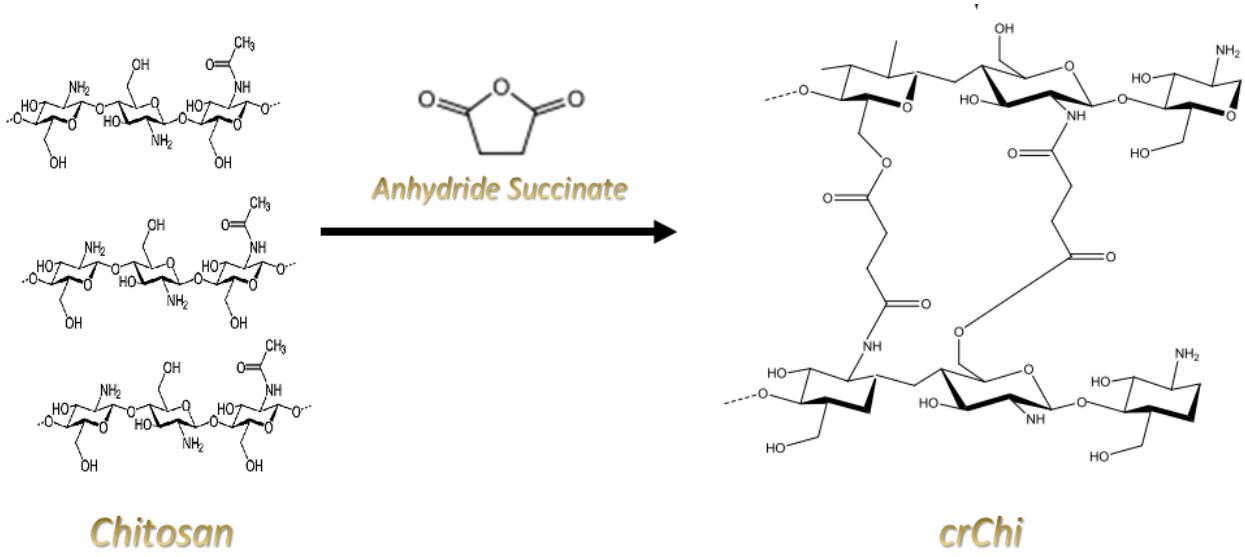


Fig. 1. A crosslinking reaction between succinate anhydride and chitosan.

and vigorously stirred for 24 h. Succinate anhydride takes part in two simultaneous reactions, namely amidation and esterification (Fig. 1) because of its structure. This facilitates its action as interconnector.

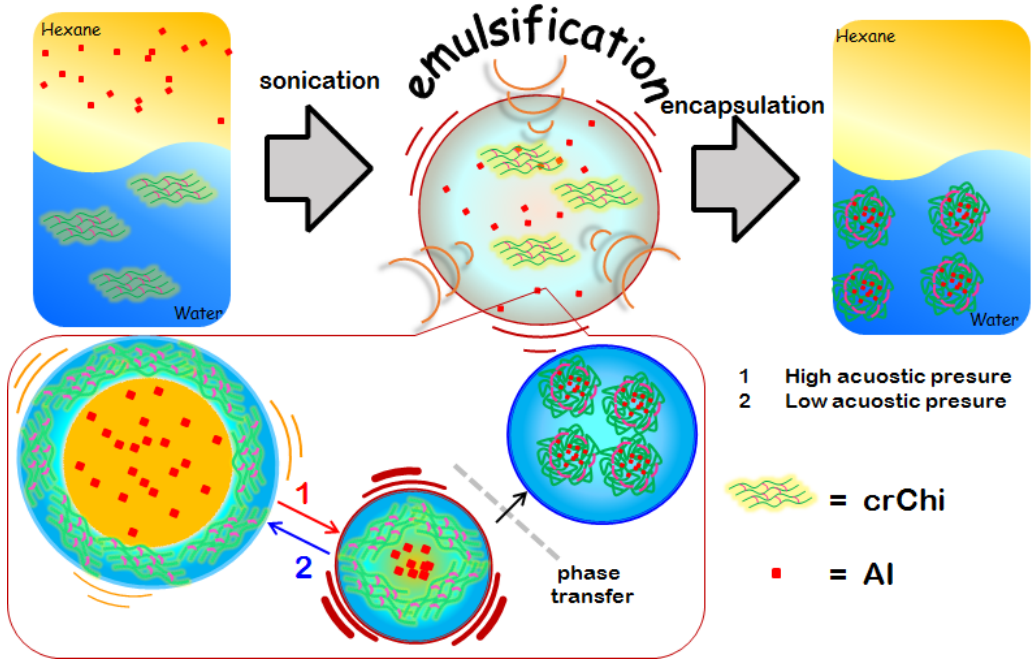
Then an ultrasonication wave (20 Hz, 80 %) was applied for 30 min aiming homogenization of the mixture. The latter was conditioned to pH of 10 by addition of NaOH. After that it was dialyzed using a dialysis membrane (MWCO 50 kDa) to remove the unreacted chitosan.

The encapsulation process described in ref. [11] was applied with some modifications. Ten mg of crChi were initially dissolved in 10 mL of acetic acid (10 %) solution and then mixed with 0.5 mL of neem seed extract solution (0.5 g in hexane). This two-phase mixture (the neem seed extract was the upper phase) was further treated with an ultrasonic wave (20 Hz, 80 %) for 20 min aiming homogenization. The wave treatment illustrated in Scheme 1 generates cavitation (hexane-in-water) bubbles in the medium via a series of compression and relaxation waves. The high dimension of crChi provides the capsulation of small molecules, present even in a difference phase. Once the hexane-in-water bubble is small enough, because of the harmonic compression resulting from the ultrasonic wave, crChi catches adequately AI and drives on the water phase.

The sample was then cured for 24 h and then centrifuged (6000 rpm, 20 min) to separate the water phase containing the neem seed extract. This step was followed by dialysis using a dialysis membrane (MWCO 50 kDa, 24 h). The product obtained was kept for further processing.

Species of Riptortus were collected from the soybean field at Mojosari, Mojokerto. Then the insects were reared in a big screen cage with a long bean as its feed. The feed was replaced in every 3 - 4 days. The rearing described was conducted in the Protection Laboratory of the Agriculture Faculty at University of Wijaya Kusuma Surabaya, Indonesia. The adults of the insects were in a stage of a life cycle corresponding to an insecticidal bioassay.

The efficacy of neem seed extract nanoencapsulate was tested by soaking the feed for 15 min in the nanoencapsulate solution. The concentration of solution referred to 0 % (control), 15 %, and 20 % (v/v). Each of treatment was carried out using six replicates. The long bean hanging at a cord functioned as a frame of the screen cage. There twenty adult riptortus were introduced. The insects' mortality was checked after 24 h. The data was analyzed by using a one-way ANOVA.



Scheme 1. AI encapsulation process using crosslinking chitosan.

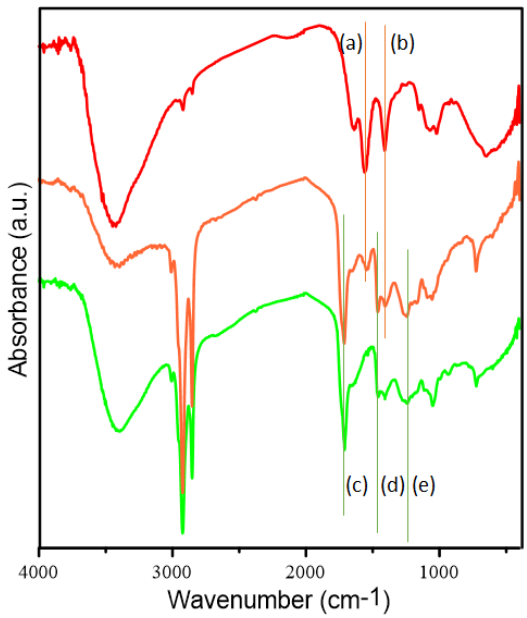


Fig. 2. FTIR spectra on a neem seed extract (red), a neem seed extract /crChi (orange), and a crChi (green).

The zeta potential (ζ) and physicochemical analysis values were obtained using dynamic light scattering (DLS). They were collected by Zetasizer Nanoseries 3000 HS Malvern instrument. The Fourier transform infrared (FTIR) spectra was recorded by a FTIR spectrometer (Shimadzu, Japan), while the corresponding absorption spectra – by an UV spectrophotometer UV-1800 (Shimadzu, Japan).

RESULTS AND DISCUSSION

The nanoencapsulate formation is verified by FTIR (Fig. 2). As expected chitosan presence is outlined through the strong and broad absorbance band referring to the axial O-H and N-H stretching bonds centered at 3440 cm^{-1}, the C-H stretching bonds registered at 2870 cm^{-1} - 2880 cm^{-1}, and the absorption centered at 1655 cm^{-1} attributable to the axial C=O stretching bond of the acetamido groups (named amide I). The vibration band at 1580 cm^{-1}(a) is attributed to the angular deformation of N-H bonds of the amino groups, while that recorded at 1420 cm^{-1} - 1477 cm^{-1} (b) results from the coupling of C-N axial stretching and N-H angular deformation. They are well outlined in FTIR spectra of neem seed extract /crChi. The presence of neem seed extract in the nanocapsulate is additionally verified by the bands at 1723 cm^{-1} (c), 1493 cm^{-1} (d), and 1250 cm^{-1}(e). They refer to the carbonyl stretching, C-C stretching vibration, and CH_2 asymmetric deformation, respectively. This phenomenon strongly indicates that neem seed extract is successfully encapsulated.

The characteristics of neem seed extract/crChi are identified in the course of the physicochemical investigation carried out. The data obtained is listed in Table 1 and illustrated in Fig. 3. The comparison with unloaded chitosan shows that chitosan nanoparticles slightly increase their diameter size in presence of neem seed extract. The zeta potential values support the data pointed above referring to neem seed extract encapsulation on chitosan. Because of its abundant hydroxyl and carboxylate functional group the neem seed extract determines the negative zeta potential values, whereas the introduction of chitosan to neem seed extract in neem seed extract /crChi shifts the zeta potential to more positive values mainly determined by chitosan amine groups.

The further investigation is addressed to evaluate the stability of the nanoencapsulate obtained against UV light and varied pH. Neem seed extract has a specific UV absorption at 276 nm due to the n-π transition in the keto-enol group. This peak is further correlated to neem seed extract concentration. Hence, AI concentration can be traced by this peak intensity.

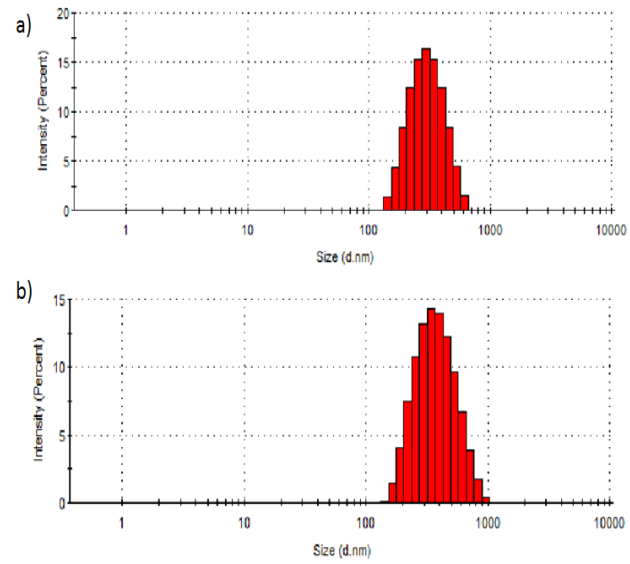


Fig. 3. A size distribution graph of crChi (a) and neem seed extract/crChi nanoencapsulate (b).

Table 1. Physicochemical characteristics of neem seed extract /crChi and its constituent raw materials.

Sample	Size (nm)	PDI	Zeta Potential (mV)
neem seed extract	-	-	- 35.6
crChi	272.7	0.13	- 0.02
neem seed extract /crChi	293.4	0.53	- 20.3

Table 2. Larva immortality data versus the adjusted concentration of neem seed extract and neem seed extract/crChi nanoencapsulate.

Sample	AI Concentration (%)	Larva immortality (%)
Neem seed extract	15	94.0
	20	88.3
Neem seed extract /crChi	15	75.2
	20	66.7

Fig. 4 illustrates the stability of both neem seed extract and neem seed extract /crChi against UV radiation (254 nm). It also indicates the degradation of neem seed extract structure starting after 40 min of UV treatment. Unlike it, the structure of neem seed extract/ crChi nanoencapsulate maintains its stability up to 50 min. This fact shows that the encapsulation process increases the stability of neem seed extract in respect to UV treatment. The effect of pH varying is also illustrated in Fig. 4.

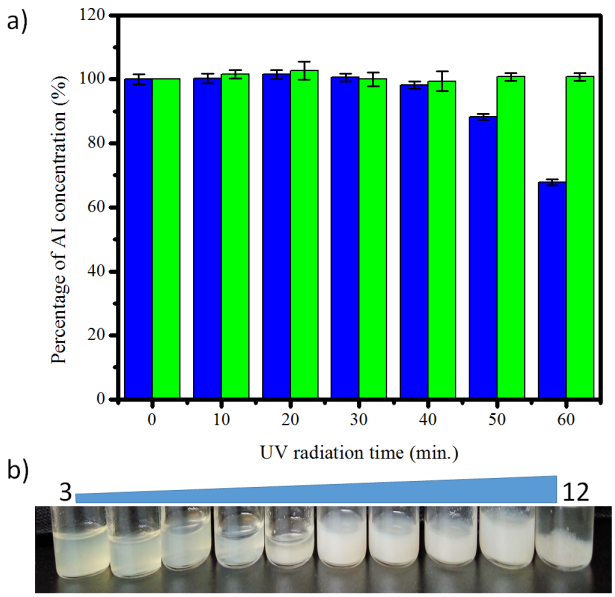


Fig. 4. (a) Percentage of neem seed extract concentration in absence (blue) and presence of encapsulation (green) after UV treatment. (b) neem seed extract /crChi nanoencapsulate in case of varying pH.

Finally, the insecticide capability of the neem seed extract/crChi nanoencapsulate obtained is evaluated on the ground of the percentage of *Riptortus linearis* mortality. The comparison with the effect of the neem seed extract (Table 2) shows that the encapsulate investigated exhibits greater activity against *R. linearis*, i.e. it is more effective in pod sucking bugs killing. This data verifies that the encapsulation process is a promising way of enhancing the capability of neem seed extract as an organic insecticide.

CONCLUSIONS

This study concludes that chitosan is an ideal crosslinking material in encapsulating neem seed extract. The encapsulation process is successfully carried out in an ultrasonication-wave environment giving quite precisely nano-sized capsule particles. The encapsulation

process increases the stability of AI against UV exposure and varying pH. The bioassay investigation based on counting the number of dead larva shows that neem seed extract/crChi has better anti-feeding properties than the neem seed extract even at higher concentrations, which in turn provides to conclude that the encapsulation results in increase of its effect as an insecticide.

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