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Eco-Friendly Chitosan Nanoparticles Cross Linked with Genipin: Basis to Develop Control Release Nanofertilizer

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Abstract: Synthesis of nanoparticles has become a matter of great interest in recent years due to their so many functional properties and applications in a variety of fields. Nanoparticle mediated control release fertilizer is one of the applications which has potential to enhance plant growth and yield while minimizing serious environmental impacts due to excessive use of conventional bulk fertilizers. Nevertheless, many of the research work carried out in relation to synthesis of nanoparticles have used synthetic constituents which are being considered as harmful to the human health and environment. Investigations have also indicated that certain engineered nanomaterials can lead to unforeseen environmental, health and safety risks. The aim of the present study was to produce biodegradable and biocompatible nanoparticles in an eco-friendly manner originated from locally available raw materials and natural excipients addressing the said risks which will ultimately lead to development of eco-friendly nanofertilizers to release nutrients gradually in a controlled manner. Chitosan, a natural biocompatible and biodegradable polymer, was synthesized from chitin which was extracted from exoskeleton of black tiger shrimp (*Penaeus monodon* Linn). A natural cross linker, 'Genipin' was extracted from tender fruit of Gardenia (*Gardenia jasminoides* Linn). Chitosan nanoparticles were synthesized using Genipin' (Fig. 1) as the cross linking agent with ionotropic gelation method. Fourier transform infra-red (FTIR) spectroscopic analysis confirmed structure of the synthesized chitosan. Average size of the synthesized chitosan nanoparticles is 90 nm (Fig. 2) which can be tuned by controlling the p^H, dose of the cross linker and chitosan concentration. Innovative and promising results of this study will pave pathway to achieve green nanopartciles.

Keywords: Biocompatible, Biodegradable, Chitosan, Cross linking, Genipin, Ionotropic gelation, Natural polymer.

I. INTRODUCTION

Nanotechnology involves creating and using structures, devices, and systems at the atomic, molecular, or macromolecular levels, 1-100 nanometer (nm) range at least in one dimension.

Nanotechnology will leave no field untouched including agriculture by its ground breaking scientific innovations. Demand for agricultural produce is increased eventually but natural resources such as land, water and soil fertility are limited. Cost of production inputs like chemical fertilizers and pesticides is expected to increase at an alarming rate.

The rapidly growing world population is projected to reach 9.6 billion by the year 2050 (UN, 2015). Food and Agriculture Organization of United Nations predicts that the global grain production is required to increase by 70 % by 2050 to meet the demand for foods. Total global macronutrient fertilizer (nitrogen, phosphorous and potassium) usage was 175.7 million tonnes in 2011 and it is projected to increase to 263 million tonnes in 2050 (Alexandratos, 2011). However, one of the global issues in agriculture today faces is sustainable use of resources and ensuring that agricultural practices do not have an adverse impact on the environment (e.g. accumulation of pesticides and fertilizers). Due to the low efficiency (30-50 %) and excessive application of these macronutrient fertilizers, a significant amount is translocated into surface and groundwater bodies, disrupting aquatic ecosystems and threatening health of human and aquatic life. Therefore, there is an imperative research need to develop innovative fertilizers to increase crop yields, enhance efficiencies of plant nutrient utilization while minimizing the environmental disruptions to ensure sustainable development. Like other technologies, low-cost nanomaterials and field application technologies are needed for their applications in agriculture (Khot *et al.,* 2012).

Nanoparticles have great potential to be used as delivery systems aimed at specific targets in living organisms and is being used in medical sciences. In plants, the same principles can be applied for a broad range of uses, particularly to tackle phytopathological infections, nutrition supplement and as growth adjuvant. Nanoparticles can be tagged into agrochemicals or other substances as delivery agent to plant system and tissues for controlled release of chemicals.

A nanotechnology approach, namely "nano-encapsulation" protect the active ingredient from the adverse environmental conditions and promote persistence. Encapsulated nanoparticles in the form of fertilizer allow proper absorption of the nutrients into the plants for a longer period hence synchronizing release of nutrients with plant's demand. Compared with the conventional bulk chemical fertilizers, nanofertilizers are expected to significantly improve crop growth and yields, enhance the efficiency of fertilizer use, reduce nutrient losses and minimize the adverse environmental impacts (Liu and Lal, 2015). Development of nitrogen and phosphorous macronutrient nanofertilizers is a high research and development priority for both food production and environmental protection (FAO, 2012).

Majority of the polymer materials developed and available are synthetic polymers and therefore, polymers used for encapsulation / coating nanofertilizers are also synthetic of which monomers will not be environmental friendly. There is a growing concern over the synthetic polymers due to environmental issues and importance of use of natural polymers has been highlighted. Accordingly, natural polymers are gaining considerable acceptance over synthetic polymers as controlled-release devices because of their eco-friendly nature, cost effectiveness, easy availability, and biodegradability. It is worth noting that researchers ought to design control release nanofertilizers by using natural excipients to develop efficient, effective, reliable and cost-effective control release nanofertilizers formulations addressing the prevailing resource limitations thereby minimizing food crisis and other challenges facing crop production (Sempeho *et al*., 2014). More than two decades ago, two eminent toxicologists advised that "it would be prudent to examine and address environmental and human health concerns before the widespread adoption of nanotechnology (Oberdörster *et al*., 2005). It is also important to mention that the bioaccumulation, bio-magnification and biotransformation of engineered nanoparticles in food crops are still not well understood (Rico *et al*., 2011). Therefore, development of reliable and eco-friendly approach for synthesis of nanoparticles is an important milestone prior to developing nanofertilizers and application in the field of agriculture. Use of natural polysaccharide in nanoparticle preparation has attracted much attention due to its biocompatibility and biodegradability and non-toxicity. Chitosan is a biopolymer and chitosan nanoparticles have many desirable characteristics such as biocompatibility, biodegradability, thermal stability, film forming ability, gelatinous, hydrophilic and bio-adhesion. So far, the use of nanotechnology in agriculture has been mostly theoretical, but it has begun to have a significant effect in the main areas of the food industry. The full potential of nanotechnology in the agricultural and food industry is yet to be realized and is gradually moving from theoretical knowledge towards the application regime *(*Agrawal and Pragya*,* 2014*)*.

One of the relatively new cross-linking agents is the naturally occurring substance genipin (Fig. 1). It is an excellent cross-linker for polymers containing amino groups upon spontaneous reaction with amino groups (Jin and Song, 2006). It has been found that plant extracts of *Genipa americana* and *Gardenia jasminoides* contains genipin. Accordingly, genipin has potential to be used for synthesizing nanopartciles from chitosan. Nevertheless, optimization of parameters such as p^H, chitosan and cross linker concentration need to be explored.

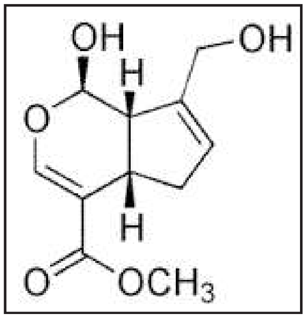

Fig. 1: Structure of Genipin

II. Materials and Methods

A. Synthesis Chitosan from Chitin Extracted from Shrimp Exoskeleton

The black tiger shrimps (*P. monodon* L.) were obtained from supermarket. Shell and operculum were removed from the animal, thoroughly washed with water to remove any foreign materials. A known quantity of shrimp exoskeletons were oven-dried at 65 °C until obtain a constant weight. The dried exoskeletons were crushed into smaller pieces (0.5-3 mm) using a motar and pestle. Crushed shrimps exoskeletons were placed in a 500 ml beaker and soaked in boiling sodium hydroxide (2 % w/v) for one hour in order to dissolve the proteins and sugars thus isolating the crude chitin. After the samples are boiled in the sodium hydroxide, the beaker containing the shrimp shell was removed from the hot plate, and allowed to cool for 30 minutes at room temperature. The supernatant was discarded and then the sample was washed with distilled water.

The sample was soaked in 1 % HCl three times its quantity for 24 hrs to remove minerals mainly the calcium carbonate (Puvvada *et al.*). The demineralized sample was then treated for one hour with adequate volume of 2 % NaOH solution to decompose the albumen into water soluble amino-acids. The remaining chitin was washed with distilled water. The chitin was further converted into chitosan by the process of deacetylation.

The chitosan was synthesiszed from chitin as described by (Puvvada *et al.,* 2012). The deacetylation process is carried out by adding 50 % NaOH and then boiled at 100 °C for 2 hrs on a hot plate. The samples are then cooled for 30 min at room temperature. Afterwards, the samples were washed continuously with the 50 % NaOH and filtered in order to retain the solid matter, which is the chitosan. The samples were then left uncovered and oven dried at 110 °C for 6 h. The chitosan obtained was in a creamy-white form.

Characterization of synthesized chitosan was done using Fourier Transform Infrared spectroscopy (FTIR). Synthesis of chitosan nanoparticles was carried out using ionotropic gelation method and characterization of nanoparticles was done with particle size analyzer (*FRITSCH* Analysette 22 Nano Tec plus).

B. Preparation of Genipin Extract

A known quantity of tender fruit of Gardenia was crushed into smaller pieces (0.5 - 1 mm) with distilled water using a motar and pestle. Ice was added to control the temperature generated due to grinding process. The extract was filtered and added distilled water to make the final volume as 20 ml which was used as the working solution.

C. Synthesis of Chitosan Nanoparticles with Ionotropic Gelation Method

Before taking into cross linking process, p^H of both chitosan and genipin was adjusted to 5.0.

Then chitosan solution was added into the genipin solution drop wise using a syringer while sonication. Sonication was continued for a period 45 minutes. A turbidity was observed in the genipin solution.

III. RESULTS AND DISCUSSION

Physical and chemical properties of chitosan synthesized from shrimp exoskeleton depend on the degree of deacetylation and molecular weight which will ultimately depend on species, locality and even country of origin. The extent of deacetylation, the content of impurities and the distribution of the molar mass of chitosan depends on the natural source of the primary material as well as the preparation method (Senel and McClure, 2004). In this context it is important to develop a procedure to synthesize and characterization of chitosan nanoparticles from shrimp exoskeleton in the Sri Lankan context which will lead to develop environment friendly control release nanofertilizers for socio-economic advantage.

The process of synthesizing chitosan has several steps which involves various chemicals. Extraction of the chitin from the shrimp exoskeleton involves removal of the proteins from shells followed by demineralization to remove carbon and other salts (mainly $CaCO_3$) present in the crude. Chitosan is synthesized by the process of deacetylation of the chitin. Producing pharmaceutical grade chitosan at commercial scale involves further steps to purify chitosan. As per the literature, 34 % yield of chitosan has been obtained after the purification process (Puvvada *et al*., 2012). However, the chitosan yield obtained during the present study is 22 %. This may be due to difference in characteristics such as feed conversion ratio of the local shrimp.

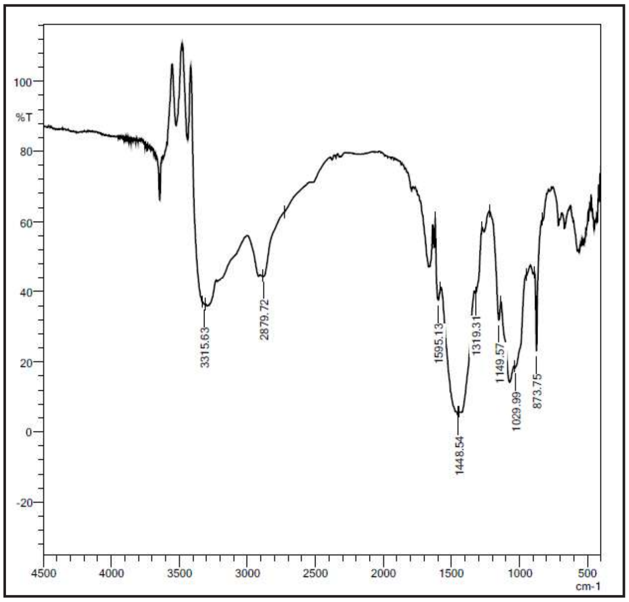

Fig. 2: The FTIR Spectrum of the Synthesized Chitosan from Shrimp (*Penaeus monodon)*

The FTIR spectrum of the synthesized chitosan showed five major peaks at a range of 873-3315 cm^{-1} wave numbers. A major absorption band is observed at 1029 cm^{-1} which represents the stretching of C-O group. The peak at 3315 cm^{-1} represents the stretching of alcoholic group (-OH). The absorbance bands of 1448 cm^{-1} and 2879 cm^{-1} indicated the CH3 deformation and CH stretching respectively. Ring stretching indicated by the peak at 873 cm^{-1}. Major peaks and corresponding wave numbers obtained for the chitosan synthesized in the present study are almost identical to the peaks of standard chitosan which confirms the structure of chitosan.

TABLE I: EFFECTS OF GENIPIN DOSE ON SIZE OF NANOPARTICLES

Chitosan concentration (mg/ml)	*p^H*	*Volume of Genipin extract (ml)*	*Sonication*	*Minimum particle size achieved (nm)*
2.0	5.0	2.0	yes	490
2.0	5.0	5.0	yes	90
2.0	5.0	7.5	yes	600

The cross-linking mechanism (Fig. 3) involves a nucleophilic attack by the amino group of chitosan on to the olefinic carbon atom at C-3 of genipin, followed by the opening of the dihydropyran ring. The formation of a secondary amide and a heterocyclic amino linkage leads to the cross-linking of chitosan.

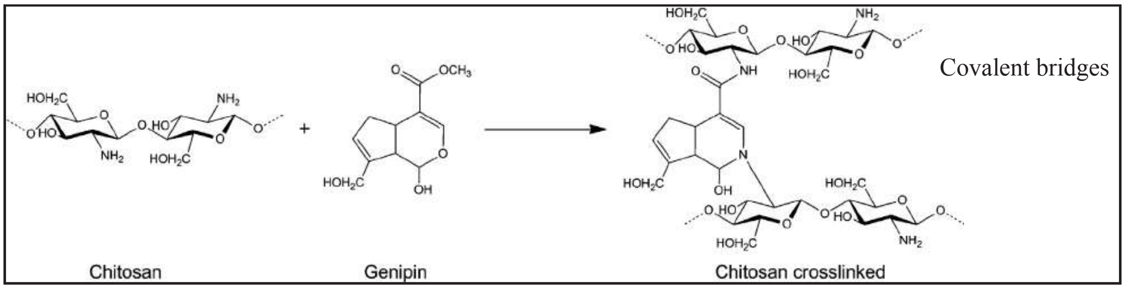

Fig. 3: Representation of Crosslinking Reaction between Chitosan and Genipin

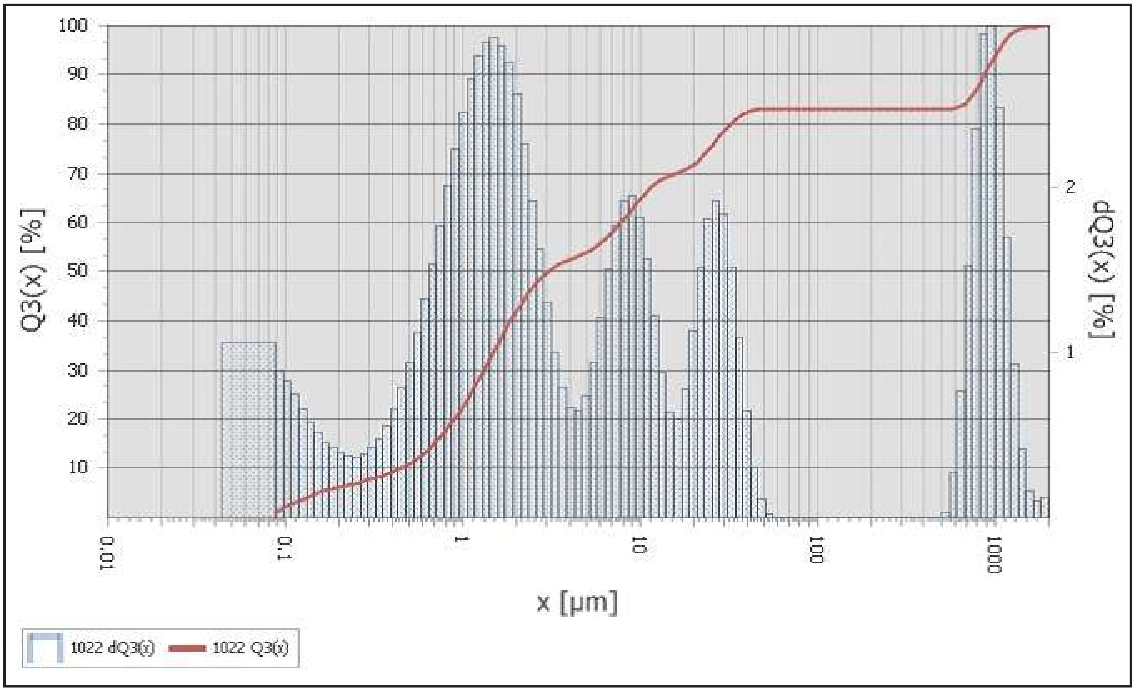

Fig. 4: Particle Size Distribution of Chitosan Nanoparticles Achieved with Genipin at Concentration Level of 1 mg/ml at p^H 5.0

Particle analysis studies indicated that optimum p^H and chitosan concentration for synthesis of chitosan nanoparticles are 5.0 and 2 mg/ml respectively. Average size of nanoparticles achieved with these conditions is 90 nm (Table I and Fig. 4).

IV. Conclusions

Extraction of chitin from shrimp shell can be achieved with the methods described in literature. Chitosan nanoparticles can be synthesized based on chitin extracted from shrimp shells using genipin, a naturally originated cross linking agent. The size of the nanoparticles is depend on p^H, chitosan concentration, cross linker concentration. Size of the chitosan nanoparticles can be tuned by changing orthogonal factors hence enabling an eco-friendly pathway to synthesize chitosan nanoparticles. The present study will not only provide a basis for preparation of environment friendly control release nanofertilizer but also enable value addition to by product of local shrimp and crab industry harnessing nanotechnology.

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