Synthesis of hydroxyapatite nanoparticles from eggshells combined to chitosan nanoparticles and its application for urea fertilizer loading

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Abstracts: In this study, the preparation of hydroxyapatite nanoparticles (HA NPs) from eggshells were carried out using chitosan nanoparticles (CS NPs) for the incorporation of urea fertilizers. The interaction and stability of HA NP/CS NP suspensions containing urea fertilizer were evaluated by FTIR spectroscopy, X-Ray diffraction (XRD), particle size and shape analysis by transmission electron microscopy (TEM) and composition by EDX. The synthesized HA NPs/CS NPs and HA NPs/CS NPs loaded with urea fertilizer after dissolved in water for 6 h with sizes as small as respective ~30-55 nm and ~50-90 nm were achieved. The obtained nanoparticles had a homogeneous morphology showing a quite uniform particles size distribution with a spherical shape. The mean diameter increase of the HA NPs/CS NPs in suspension with the addition of urea indicated that the elements are being aggregated and loaded on the surface of urea fertilizers.

Keywords: Hydroxyapatite nanoparticles (HA NPs)/chitosan nanoparticles (CS NPs), Hydroxyapatite nanoparticles (HA NPs)/chitosan nanoparticles (CS NPs) loaded with urea fertilizer; controlled release fertilizer (CRF); hydroxyapatite nanoparticles (HA NPs) from eggshells.

1. INTRODUCTION

In recent years, the development of functional nanomaterials open up new trends in agriculture and biotechnology. The use of slow release fertilizer has become a new trend to save fertilizer consumption and to minimize environmental pollution [1]. Due to its polymeric cationic, biodegradable, bioabsorbable, and bactericidal characteristics, chitosan nanoparticle (CS NP) is an interesting material for use in controlled release systems [2-5]. However, there are no attempts to explore the potential of chitosan nanoparticles combined to hydroxyapatite nanoparticles as controlled release for urea fertilizers. They have received scientific interest because combinations of nanocomponents give rise to multifunctional properties due to synergistic effects from interfacial particle−particle interactions [6]. The

usage of natural polysaccharides in the preparation of nanoparticles has attracted attention because of their biodegradability and hydrophilic characters which are favorable characters in multiple applications [7, 8]. Chitosan nanoparticles emerged due to their unequalled properties [9-12]. Fertilizers are chemical compounds applied to enhance plant growth [13, 14].

 Usually, fertilizers are applied either through the soil or by foliar spreading. Artificial fertilizers are inorganic compounds devised in appropriate concentrations and combinations to supply three main nutrients: nitrogen, phosphorus and potassium (N, P and K) for multiple crops and growing conditions [14]. N (nitrogen) induces leaf growth and forms proteins and chlorophyll. P (phosphorus) enhances root, flower and fruit development. K (potassium) induces stem and root growth and synthesis of proteins [15]. Naturally occurring polymers, such as chitosan, have been extensively studied as carriers for therapeutic protein and gene delivery systems . Besides, Hydroxyapatite $[(Ca_{10}(PO_4)_6(OH)_2]$ nanoparticles (HA NPs) and their hybrids have been extensively studied because of their relevance in material science, biology, and medicine in last years [16-18].

In developing countries, the cost of fertilizers can be significant and is often the limiting factor for food supply. Thus, it is important to develop technologies that minimize the cost of fertilizers through efficient and targeted delivery. Urea is a rich source of nitrogen and therefore a commonly used fertilizer. HA is used traditionaly as a phosphorus fertilizer but has low solubility [19, 20]. Therefore, there is also increased possibility of P solubility to be achieved through a NP formulation. Furthermore, from a scientific point of view, the reactive surface functional groups on the HA NPs offer rich opportunities for surface modification through immobilization of strategic chemicals for creation of nanohybrids with multifunctional properties [21-23].

Herein, we have successful developed and efficient used hydroxyapatite nanoparticles (HA NPs) generated from eggshells combined with chitosan nanoparticles (CS NPs) for covering of urea fertilizer. As known as, the synthesized HA NPs/CS NPs@urea as the first material used for loading of urea fertilizer due to this material has not previous reported and published yet.

2. MATERIALS AND METHODS

a. Materials

Ethanol (C_2H_5OH; 96%), sodium hydroxide (NaOH; 99%), phosphoric acid (H_3PO_4; 98%), acetic acid (CH_3COOH; 99%) were bought from Sigma-Aldrich. Amoniac solution (NH_3; 25%), was purchased from Xilong, China. Eggshells were obtained from food waste at Can Tho city. Sodium tripolyphosphat (STPP, 99%) were purchased from HiMedia, Mumbai, India. Chitosan was bought from Vietnam's company. All solutions were prepared with deionized water from a MilliQ system.

b. Preparation of hydroxyapatite nanoparticles

Eggshells powder were poured into a beaker with 20 mL deionized water. After that, the mixture was stirred well during 15 min to create a suspension solution. Then, EDTA (2.4 g)

and urea (1.1 g) were added directly to this beaker and homogenized carefully by a magnetic stirrer in 15 min until both of them dissolved absolutely manual. Next step, 20 mL of H_3PO_4 0.3 M was dropped one by one into the suspension at 95°C. The medium reaction was maintained stably at pH = 10 by NH_3 solution. The mixture solution was kept stirring for 4 h. When completed process, the milky solution was aged 24 h to stabilize structure. The white sedimentation was centrifuged and neutralized many times by deionized water. The opalescent crystals were carried out in order to react to a solvent. The precipitate was separated by a centrifugation and washed with deionized water. Finally, the mixture was re-dispersed in deionized water and stored at 8°C for the next step.

c. Preparation of chitosan nanoparticles

Chitosan (0.1 g) was dissolved in 100 mL of 2% acetic acid to prepare chitosan solution. Chitosan nanocomposites (CS NCPs) were synthesized by a simple and rapid method using STPP as a reducing agent. In a typical synthesis, 2 mL of STPP (1 mg in 1 mL DI H_2O) was added to 40 mL of chitosan solution (10 mg/mL in acetic acid solution of 2%) and stirred for 30 min at room temperature. The solution was then centrifuged (15000 rpm; 20 min) and washed with deionized water (DI water) to remove excess and then redispersed in DI water. Chitosan nanoparticle was stored at 8ºC for next steps.

d. Synthesis of hydroxyapatite nanoparticles (HA NPs)/chitosan nanoparticles (CS NPs)

In a typical synthesis, various volumes (6 mL, 10 mL and 14 mL) of hydroxyapatite nanoparticles (HA NPs) solution were respective added into 40 mL of chitosan nanoparticles (CS NPs) solution and stirred for 30 min at room temperature corresponding to H1, H2 and H3, respectively. And then, the mixtures solution were centrifuged and re-dispersed in DI water to use for next steps. The synthesized HA NPs/CS NPs obtained with sizes as small as ~30-55 nm.

e. Preparation of HA NPs/CS NPs loaded with urea fertilizer

Preparing three glassy petries plates cleaned by ethanol, each one contains 6 g of urea fertilizer distributed well. Then, we carried out spraying HA NPs/CS NPs solution on their surface. These particles covered by HA NPs/CS NPs were dried 20 min at 60°C to get HA NPs/CS NPs loaded with urea fertilizer – see sample (H4). Two remain plates were carried out spraying two and three times HA NPs/CS NPs solution and dried in the same with condition (H4) assigned in (H5) and (H6), respectively.

f. Studying of durable urea fertilizer covered by shells of HA NPs/CS NPs in water

The HA NPs/CS NPs@urea fertilizer samples (H1, H2, H3 grouped No.1 and H4, H5, H6 grouped No.2) were poured six glassy beakers containing 100 mL deionized water, respectively. After that, the time to release completely quantity of urea fertilizer was recorded.

g. Characterizations of samples

The morphology and size, and compositions of samples were examed by transmission electron microscope (HR-TEM, Tecnai G20, FEI) supported with an energy dispersive X-ray

(EDX) unit was used for the sake of imaging and crystal structure revelation. The structure of the prepared sorbents was identified using X-ray diffraction (XRD), which operated at 45 kV and 30 mA using filtered Cu Ka radiation (k =1.5406 A) in the 2θ range from 10º to 80º and high score plus software. In order to investigate the sorbent' functionality, Fourier transform infrared spectroscopy (FTIR, Jasco 6100-Japan) was conducted in KBr pellet at room temperature over the range from 500 to 4000 cm^{-1}.

3. RESULTS AND DISCUSSION

The electron diffraction pattern (Figure 1(a)) of HA NPs showed polycrystalline diffraction rings corresponding to the crystallographic planes (002), (102), (130), (202) and (211) of HA NPs crystals. The XRD pattern (Figure 1) of HA NPs s, HA NPs/CS NPs and HA NPs/CS NPs loaded with urea fertilizer agreed well with the typical one of HA (JCPDS no. 01-073-8417) with feature diffraction peaks at 2θ = 25.88º, 31.88º, 32.28º, 34.08º, 39.78º, 49.58º assigning as facets of (002), (102), (130), (202); and (213). Alternatively, two well-defined signals have been appeared for CS NPs at 2θ= 26.08º and 10.58º. The signals appeared at HA NPs/CS NPs were very much similar to those of HA NPs except in peak height and width. The disappearance of signals characterizing CS NP in HA NPs/CS NPs was attributed to the low doping level of CS NP in the composite, which agreed with previous expectation for certain HA/CS ratios [24-26]. The diffraction peaks of HA NPs/CS NPs loaded with urea fertilizer for the maxium intensity of diffraction peak at 2θ = 23.04º, while other diffraction peaks were disappeared – see in Figure 2(c). It indicated that amino groups of urea fertilizer linked to the surface of HA NPs and CS NPs composites. Thus, it confirmed that HA NPs/CS NPs loaded with urea fertilizer successfully.
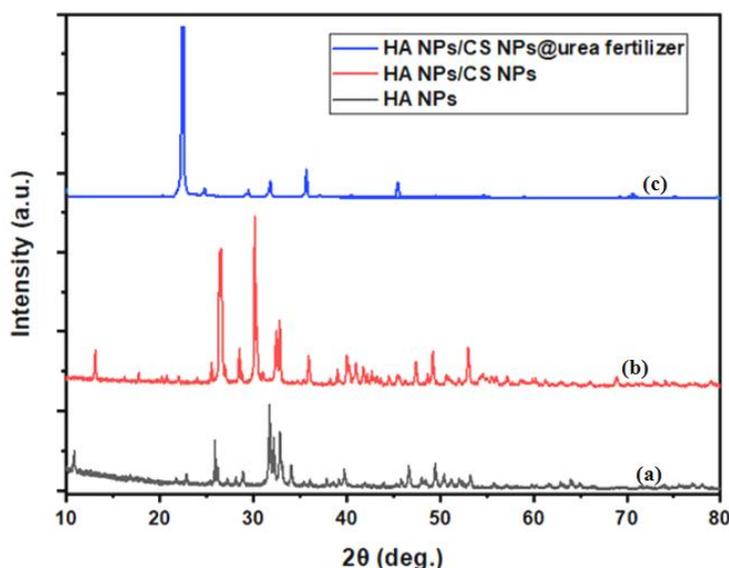

Figure 1. XRD patterns of (a) HA NPs; (b) HA NPs/CS NPs; and (c) HA NPs/CS NPs loaded with urea fertilizer, respectively.

Figure 2 comparing the FTIR spectra of CS NPs, HA NPs/CS NPs and HA NPs/CS NPs loaded with urea fertilizer, interestingly, reveals a direct evidence confirming the formation of HA NPs/CS NPs composite [26].

Generally, all signals of HA NPs/CS NPs coincided to a great extent with the major signals of HA NPs – see in Figure 2 and Table 1. The featuring vibrations in range 2900 – 3444 cm^{-1} are assigned to intermolecula. However, the signals at 3420 cm^{-1} corresponding to (-NH) stretching group appeared only in CS NPs and HA NPs/CS NPs loaded with urea fertilizer but disappeared from HA NPs/CS NPs. Moreover, the signals at 3318-3444 cm^{-1} and 1664-1728 cm^{-1} corresponding to (-OH) stretching and (-OH) bending groups only in HA NPs/CS NPs and HA NPs/CS NPs loaded with urea fertilizer but disappeared from CS NPs., which verify the HA NPs/CS NPs loaded with urea fertilizer complex formation.
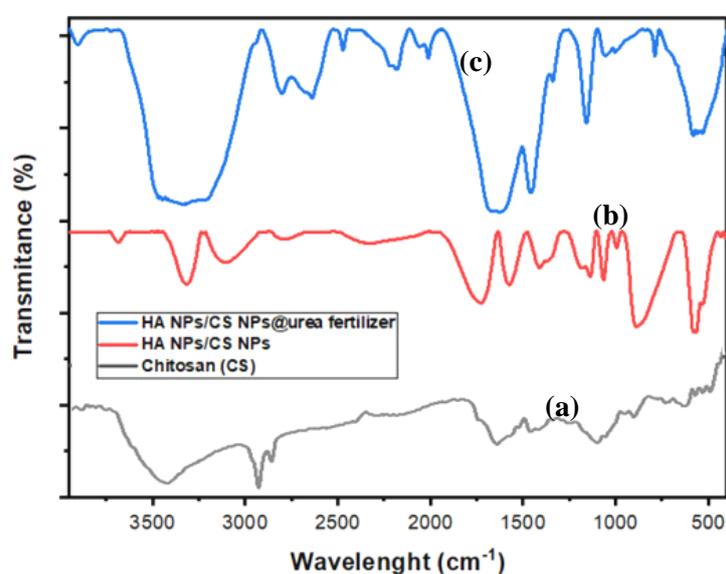

Figure 2. FTIR spectras of (a) Hydroxyapatite nanoparticles (HA NPs), (b) Hydroxyapatite nanoparticles/chitosan nanoparticles (HA NPs/CS NPs), and (c) HA NPs/CS NPs loaded with urea fertilizer, respectively.

Table 1. Characteristic FTIR wavenumbers of HA NPs, HA NPs/CS NPs and HA NPs/CS NPs loaded with urea fertilizer.

Characteristic group	CS NPs (cm^{-1})	HA NPs/CS NPs (cm^{-1})	HA NPs/C NPs loaded with urea fertilizer (cm^{-1})
-NH (stretching)	3421	-	3420
-CH (stretching)	2900	2900	2900
-CH (bending)	1388	1388	1388
-CN	1643	-	1664
C – O – C (stretching)	1039	1039	1039
-OH (stretching)	-	3318	3444
-OH (bending)	-	1728	1664
-PO$_4$	-	1066	1054
	-	888	788

-	602	580
-	566	558

The morphology of HA NPs/CS NPs and HA NPs/CS NPs loaded with urea fertilizer are shown in Figure 3. TEM image (Figure 3(a)) displayed that the HA NPs/CS NPs is spherical with average particle size of ~30–55 nm, which are distributed homogeneous and uniform. Besides, the HA NPs/CS NPs loaded with urea fertilizer after dissolving in water for 6 h is spherical nanoparticles which is the range size ~50 – 90 nm. It indicated that urea fertilizer loaded by HA NPs/CS NPs, leading to the average particle size of HA NPs/CS NPs loaded with urea fertilizer increased and appeared agglomeration between particles of HA NPs/CS NPs and urea fertilizer in the water solution.
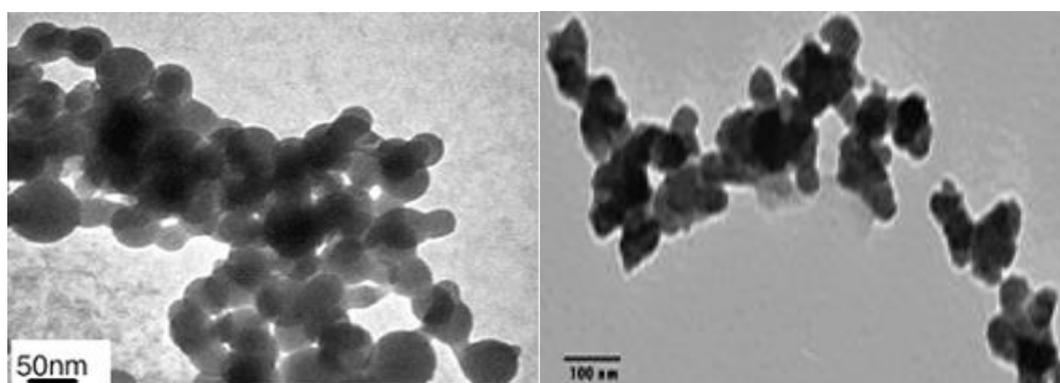

Figure 3. TEM images of (a) Hydroxyapatite nanoparticles (HA NPs)/chitosan nanoparticles (CS NPs) and (b) HA NPs/CS NPs loaded with urea fertilizer disolved in water after 6 h, respectively.

EDX which is attached to FESEM was used for elemental analysis of hydroxyapatite nanoparticles (HA NPs) from eggshells. In EDX spectra (Figure 4), there are peaks of carbon, oxygen, sodium, phosphorus and canxium. HA NPs compose of 19.18% (P) và 32.07% (Ca) impurities of phosphorus and canxium, with ratio of Ca:P = ~1.67, respectively. It demonstrated that obtained HA NPs with Ca and P contents in eggshells are agreed to ratio value of Ca:P in the hydroxyapatite material.

Element	Weight%	Atomic%
C	2.87	5.44
O	45.24	59.81
Na	0.64	0.64
P	19.18	14.09
Ca	32.07	20.03
Totals	100.00	

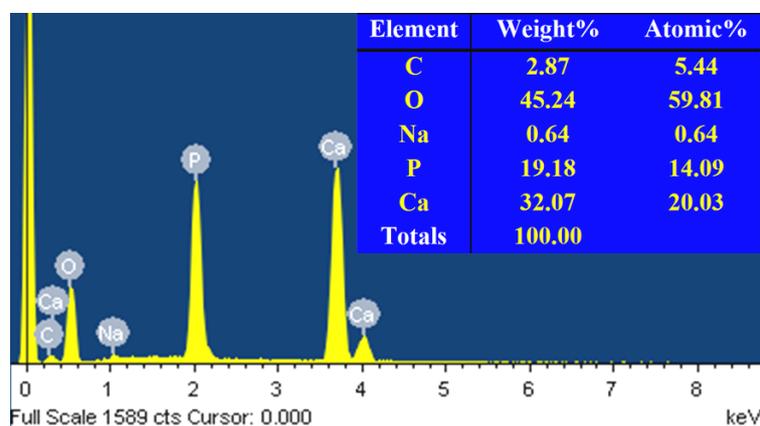Full Scale 1589 cts Cursor: 0.000

Figure 4. EDX spectrum of hydroxyapatite nanoparticles (HA NPs).

Figure 5 as shown clearly the effect various volumes of HA NPs solution on diffusion in the water of covered urea fertilizer particles. In a detail way, (H3) and (H4) samples demonstrate considerably properties than (H1) sample and a blank sample. According to (H1) sample, due to depriving of HA NPs. Hence, they can not cover entirely the core fertilizer. So that, the time order to diffusion is only 43 min more than 4 min of the blank sample. Besides, while (H2) sample upholds 85 min in water; while, (H3) sample just is able to stabilize in 71 min. The reason why since the moisture dissolves the fertilizer while drying. Therefore, the 10 mL of HA NPs solution was selected as optimal paramters for other experiments next steps.
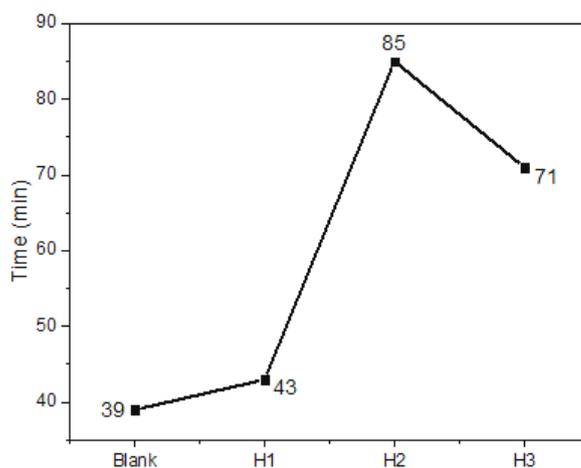

Figure 5. Effect various volumes of HA NPs/CS NPs solution covered urea fertilizer particles for diffusion in the water.

Figure 6 demonstrates the influence of the number of covering layers to the degradable ability of urea fertilizer covered by HA NPs/ CS NPs. The sample with two layers (H5) iillustrates perfectly resistant water. The fertilizer core is protected 118 min; while soaking in deionized water. According to the samples possess a covering layer or three ones or without (H4, H6 and blank samples) respective to the time being ~85 min, 76 min and 36 min. When HA NPs/CS NPs is overloaded, the moisture inside each fertilizer particle is so high. Therefore, the fertilizer was dissolved significantly whereas drying. However, if the HA NPs/CS NPs layer covered on the surfaces of urea fertilizer is thin, the shells will absorb easily water. Since, the most optimum condition to control HA NPs/CS NPs layer cover on the urea fertilizers' surfaces with the speed realizing fertilizer is two layers.
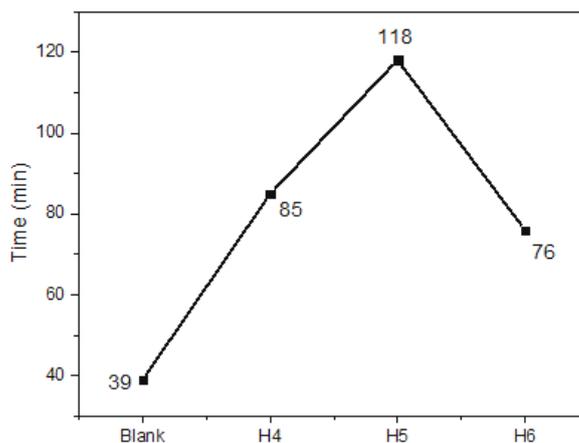
Figure 6. Effect various layer thicknesses of HA NPs/CS NPs solution covered on the urea fertilizers' surfaces to dissolve time of the urea fertilizer in the water.

4. CONCLUSIONS

Herein, we have successfully fabricated hydroxyapatite nanoparticles (HA NPs) from eggshells by a simple and environment friendly method at low temperature. Moreover, the generated HA NPs have also successful combin with chitosan nanoparticles (CS NPs) with their average particle size obtained ~30-55 nm, the morphology of spherical and uniform distribution. In addition, the HA NPs/CS NPs loaded with urea feritlizer obtained with average particle size in the range of ~50-90 nm. They are carried out to explore the optimum conditions of HA NPs/CS NPs layers to time release of urea fertilizer in the water being two layers of HA NPs/CS NPs covered on the urea fertilizer's surfaces. Thus, the synthesized HA NPs/CS NPs also loaded with urea fertilizer using as a nanomaterial potential and promise applied in the controlled release fertilizer (CRF) in the field of agriculture at the current time and in future.

5. REFERENCES

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