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Original Article

Synthesis of Green Chitosan - Silver Nanoparticle Composite Material: Parameter Optimization and Physicochemical Characterization

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Abstract: In this study, response surface methodology (RSM) was carried out with four parameters: chitosan concentration, $AgNO_3$ concentration, ascorbic acid concentration, and time to obtain the minimum mean particle size of silver nanoparticles (response Y). The chitosan-silver nanoparticle composite material (CTS-AgNPs nanocomposite) obtained experimentally from the optimal conditions was characterized by evaluating some of their physical-chemical properties using combined techniques such as ultraviolet-visible spectroscopy (UV-Vis), dynamic light scattering (DLS), transmission electron microscopy (TEM), infrared spectra (IR) and X-ray diffraction. The optimal conditions were a chitosan concentration of 19.2%, silver nitrate of 26 mM, an ascorbic concentration of 33 mM, and a time of 4.25 hours. Spherical Ag particles obtained experimentally well dispersed in a chitosan matrix with an average diameter of around 2÷8 nm with a narrow size distribution and homogeneous dispersion.

Keywords: Response surface methodology, silver nanoparticles.

1. Introduction

Within the last decades, nanotechnology has emerged as one of the most capable and significant techniques, which has various roles in science and prospering human life. Silver nanoparticles have been widely investigated. The antimicrobial activity of silver nanoparticles (AgNPs) has been confirmed in both Gram-positive and Gram-negative bacteria as well as in fungus [1-3]. Ag nanoparticles

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have been extensively used for biomedical and agriculture applications [3]. Positive, negative, or neutral effects of AgNPs on plant development were also investigated in many studies via seed germination when the germination rate and root length are measured and via growth of seedlings, and plants, in which root elongation and dry weight are used to assess acute effects of NP form of silver on plant physiology [4, 5].

Methods of synthesis for AgNPs have gained a large of attention lately due to the need to find more efficient ways to obtain nanoparticles. Several studies optimize the synthesis parameters of AgNPs, mainly to reduce the size and improve their physicochemical properties. Green, simple and economical methods, which can finely control the size, shape, and size distribution of AgNPs, should be developed [6]. The bottom-up chemical technique is one of the most used methods in terms of nanoparticle production. This method is low-cost and has a large-scale production capacity. It may come from reducing a metal salt via a reducing agent in the presence of a protective material [7]. In particular, for the formation of Ag-based nanocomposites used reducing agents such as ascorbic acid [9-12], sodium borohydride, hydrazine, sodium citrate, and glucose [8] taking into account the potential toxicity and environmental requirements, ascorbic acid, mild reducing agents, were considered for the synthesis optimization. It is a well-known reductant in the synthesis of metallic nanoparticles.

Chitin is a nitrogen-containing polysaccharide composed of β (1-4)–linked 2-acetamido-2-deoxy-β-D glucose. Chitosan is obtained by the deacetylation of chitin. Due to the biocompatibility, biodegradability, nontoxicity, bioactivity, and easy availability of chitosan, it is accepted as the most effective and promising material for nano-biocomposite synthesis [9]. Chitosan is a suitable capping agent for the synthesis of metal nanoparticles due to both hydroxyl and amine groups in its structure [10]. Its biological activity significantly depends on the polymer properties such as molecular weight (Mw) and deacetylation degree (DD). Chitosan can form various chemical bonds with metal components, enhancing the stability of the nanoparticles.

Studies that optimize the synthesis parameters of AgNPs, mainly to reduce the size and improve their physicochemical properties, using response surface methodologies using Box-Behnken design are limited. Even if there are well-established techniques for the preparation of silver nanoparticles, it is necessary to investigate simple synthesis methods, which require short reaction times and low costs to obtain smaller-size nanoparticles [7]. It is still a challenge. Our study focused on these aspects because such research has rarely been investigated in Vietnam until now. In present reports, further attempts have been made to develop a green synthesis of chitosan-silver nanoparticle composite material. The Box-Behnken method was employed to optimize parameter reaction conditions and to find out the significant factors affecting the AgNPs' outcome. Physico-chemical properties of CTS-AgNPs nanocomposite were investigated using visible ultraviolet spectroscopy (UV -Vis), dynamic light scattering (DLS), transmission electron microscopy (TEM), and X-ray diffraction.

2. Methodology

2.1. Materials

Chitosan with a low molecular weight of 5kDa was prepared in our laboratory as described previously [11]. Silver nitrates ($AgNO_3$), L-ascorbic acid (99%), and acetic acid were supplied by Sigma-Aldrich. All other reagents were used as received.

2.2. Methods

Preparation of CTS-AgNPs nanocomposite:

Chitosan solutions (10 to 30 mg/ml) were firstly prepared by dissolving chitosan in 0.5% (v/v) acetic acid followed by stirring to achieve complete dissolution. Ascorbic acid (0.02÷0.04M) and chitosan solutions were mixed in a 1:1 volume ratio with continuous stirring followed by the drop-wise addition of

silver nitrate solution (0.02÷0.04M). The mixtures were kept stirring for 2÷6 hours. The color of the solution was changed from colorless to light yellow and finally to yellowish brown, indicating the formation of Ag NPs.

Experimental design:

The parameters were optimized by response surface methodology. A three-level, four-variable Box-Behnken factorial design (BBD) was adopted to determine the best combination for silver nanoparticle size. Chitosan concentration (X_1), $AgNO_3$ concentration (X_2), ascorbic acid concentration (X_3), and time (X_4) were chosen as independent variables. The range and central point values of four independent variables presented in Table 1 were based on the results of our preliminary single-factor experiments. The particle size was selected as the response (Y).

The behavior of the system was explained by the following quadratic equation {1}:

$$Y = b0 + \sum_{i=1}^{4} biXi + \sum_{i=1}^{4} biiXi^2 + \sum_{i=1}^{3}\sum_{j=i+1}^{4} bijXiYj \quad \{1\}$$

Table 1. Independent variables and their levels

Independent variables	Level		
	-1	0	+1
X_1: Chitosan concentration (mg/ml)	10	20	30
X_2: $AgNO_3$(M)	0.02	0.03	0.04
X_3: ascorbic acid concentration (M)	0.02	0.03	0.04
X_4: Time (hours)	2	4	6

Where Y is the dependent variable; b0, bi, bii, and bij are coefficients estimated by the model, Xi and Xj are the levels of the independent variables. They represent the linear, quadratic, and cross product effects of the X_1, X_2, X_3, and X_4 factors on the response, respectively.

2.2.3. Measurements

The plasmon surface resonance was determined using UV-vis SP-3000 nano. Particle size and PDI were determined by dynamic light scattering technique using Litesizer 500 (Anton Paar GmbH). The morphological characteristics of NPs were observed using a transmission electron microscopy JEOL - JEM 1010, National Institute of Hygiene and Epidemiology. FT-IR spectra were recorded using a Perkin Elmer FT-IR spectrometer in the range of 500-4000 cm^{-1}, Institute of Chemistry. The XRD patterns were recorded using D8 ADVANCE X-Ray (Bruker). Analysis of the experimental design and calculation of predicted data were carried out using Design Expert Software (version 11.0).

3. Results and Discussion

3.1. Statistical Analysis and the Model Fitting

A 27-run BBD with four factors and three levels was used to fit a second-order response surface to optimize the extraction conditions. Table 2 shows the process variables and experimental data.

Table 2. Box-Behnken experimental design with the independent variables

No	X_1	X_2	X_3	X_4	Y (nm)
1	-1	-1	0	0	18.5
2	+1	-1	0	0	10.01
3	-1	+1	0	0	23.25
4	+1	+1	0	0	28.8
5	0	0	-1	-1	19.25
6	0	0	+1	-1	11.55
7	0	0	-1	+1	12.25
8	0	0	+1	+1	10.81
9	-1	0	0	-1	20.5
10	+1	0	0	-1	19.5
11	-1	0	0	+1	16.2
12	+1	0	0	+1	14.1
13	0	-1	-1	0	16.5
14	0	+1	-1	0	25.15
15	0	-1	+1	0	8.68
16	0	+1	+1	0	20.15
17	-1	0	-1	0	21.15
18	+1	0	-1	0	19.25
19	-1	0	+1	0	13.75
20	+1	0	+1	0	12.56

No	X_1	X_2	X_3	X_4	Y (nm)
21	0	-1	0	-1	13.65
22	0	+1	0	-1	26.86
23	0	-1	0	+1	9.84
24	0	+1	0	+1	19.01
25	0	0	0	0	6.22
26	0	0	0	0	6.68
27	0	0	0	0	6.62

The following quadratic model explains the experimental data:

$$Y= 6.51- 0.76X_1 + 5.50X_2 - 3.00X_3 - 2.43X_4 + 3.51X_1X_2 + 0.70X_2X_3 - 0.18X_1X_3 + 1.01 X_2X_4 + 1.57X_3X_4 + 6.81X_1^2 + 7.16X_2^2+3.49X_3^2+3.80X_4^2 \quad \textbf{\{2\}}$$

The fit of the model was checked by determination of the coefficient R^2, which was calculated to be 0.9906, indicating that 99.9% of the variability in the response of Y can be explained by the model equation {2}. The Predicted R^2 of 0.9460 is in reasonable agreement with the Adjusted R^2 of 0.9795.

The Model F-value of 89.92 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case X_1, X_2, X_3, X_4, X_1X_2, X_2X_4, X_3X_4, X_1^2, X_2^2, X_3^2, X_4^2 are significant model terms. The Lack of Fit F-value of 15.14 implies that there is a 6.35% chance that a Lack of Fit F-value this large could occur due to noise. As indicated in equation **{2}** the silver concentration had a highly significant effect on the Y. The silver particle diameter increased when increasing the silver concentration. Based on 100 solutions the parameters selected in ranges (-1 to +1), the optimal conditions extracted by Design Expert Software for the minimum value of the response (Y) were: chitosan concentration of 19.2%, silver nitrate of 26 mM, ascorbic concentration of 33 mM, and time of 4.25 hours. Under these conditions, value Y of 4.86 nm was obtained (CTS-AgNPs-1).

An additional experiment was conducted under the predicted optimal conditions to confirm the validity of the suggested mathematical model. The minimum Y obtained experimentally was found to be 5.5 nm (assigned CTS-AgNPs-2). It is obviously in close agreement with the model prediction. We could synthesize ultra-small silver nanoparticles by applying the BBD model for parameter optimization. It is much smaller than that in previous publications (mean particle size of 15.8 nm) [8], or without using microwave heating [12].

3.2. Structural Analysis of Chitosan-silver Nanoparticle Composite Material (CTS-AgNPs-2)

The minimum Y obtained experimentally was chosen for physicochemical characterization using combined techniques including UV-Vis, IR DLS, TEM, and X-Ray diffraction.

Silver ions underwent reduction by both chitosan functional groups and ascorbic acid. The change of color of the solution from colorless to yellow and finally to yellowish brown indicated the generation of AgNPs. Generation of AgNPs was identified from the surface plasmon band in UV–Vis spectra. In the present case, the peak due to AgNPs is found in the 400-450 nm regions. Such absorption bands presumably correspond to AgNPs smaller than 60 nm [13]. According to Mie's theory [13, 14], the plasmon absorption maximum shifts to a longer wavelength with increasing NP size. As shown in Figure 2, the characteristic absorbance peak was observed at about 405 nm indicating an ultra-small particle size formation.

Generally, both the O-H and N-H functional groups have a strong affinity towards silver ions. The difference in electronegativity between O and N atoms plays an important role as it dictated the de-protonation site which can favor the binding of free electrons to the metal. As shown in Figure 3, the chitosan (A) showed the characteristic band at 3409cm^{-1} for overlapped -N-H and –O-H stretching vibrations. The FTIR spectra of CTS-AgNPs-2 (B) gave a broader peak at 3390 cm^{-1} which was indicative of more prevalence of the O-H group as the N-H group is involved in binding

to the silver metal. This was further indicated by a reduction in the intensity of–N-H stretching vibration is also affected, as the position shifts from 1593 cm^{-1} to 1552 cm^{-1} with reduced intensity. Other bands including -C=O (amide-I) at 1640 cm^{-1},–C-H group at 2912 cm^{-1}, amide I group at 1643 cm^{-1}, -OH group at 1410 cm^{-} and -CH_2 wagging vibration at 1326 cm^{-1} were almost unchanged, suggesting that these transmittance bands are not sensitive to metal nanoparticle surface reduction.

The mean particle size distribution by number (Figure 4) was 5.5 nm (area 100%), and PDI 0.24 indicated a homogeneous dispersion of silver nanoparticles in the chitosan matrix.

TEM imaging is widely used to investigate NPs morphology, as well as their size. TEM photographs of CTS-AgNPs-2 (Figure 5) showed that spherical Ag particles were well dispersed in the chitosan matrix with an average diameter of around 2÷8 nm and a narrow size distribution. It is a good approximation to the size parameters obtained by DLS-number.

XRD patterns of CTS-AgNPs-2 (Figure 6) showed peaks at 2θ values of 38.2°, 44.5°, and 64.6° that correspond to the (111), (200), (220) planes of face-centered cubic for Ag° [15].

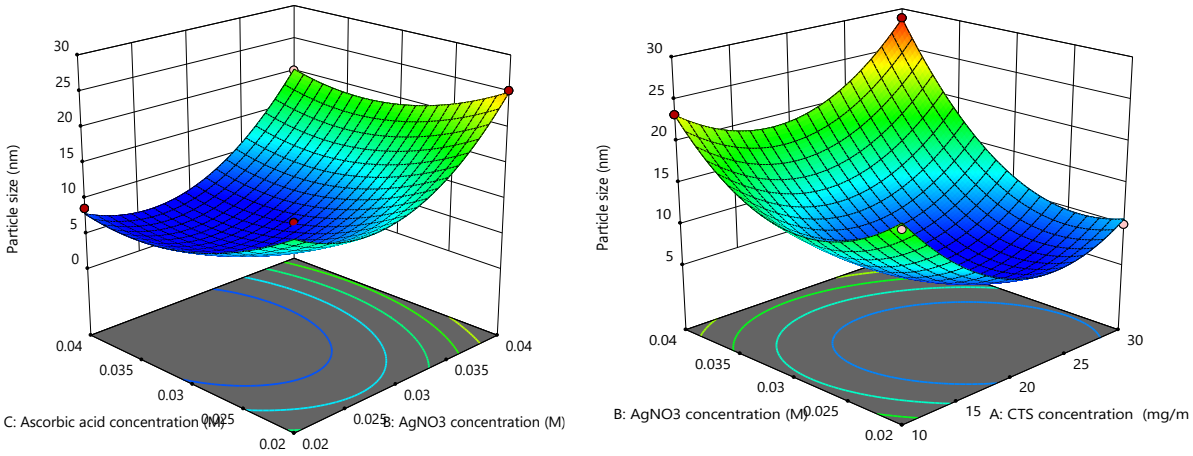

Figure 1. Response surface (3-D) showing the effect of parameters on the response Y.

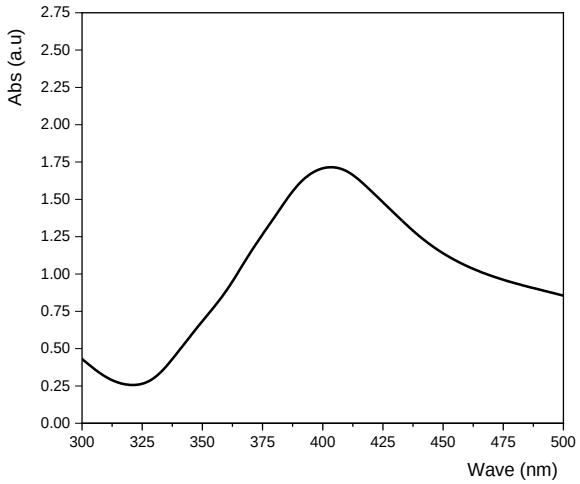

Figure 2. UV–vis spectra of CTS-AgNPs-2.

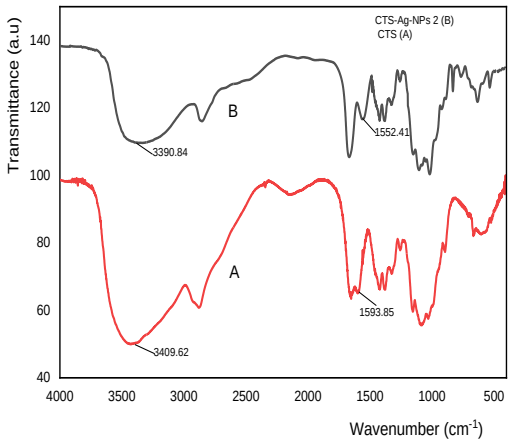

Figure 3. The FTIR spectra of chitosan (A) and CTS-AgNPs-2 (B).

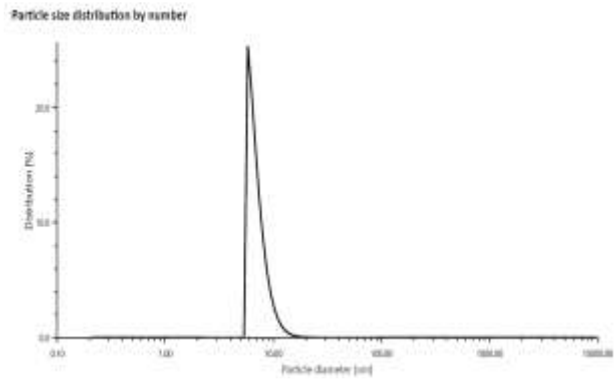

Figure 4. Size distribution of CTS-AgNPs-2.

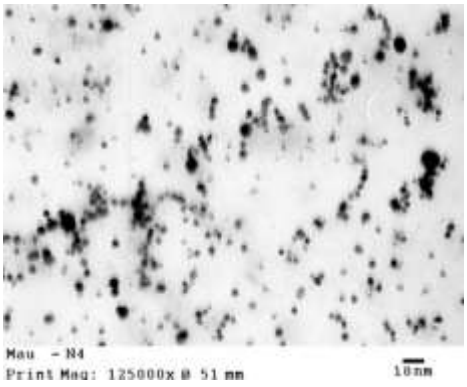

Figure 5. TEM of CTS-AgNPs-2.

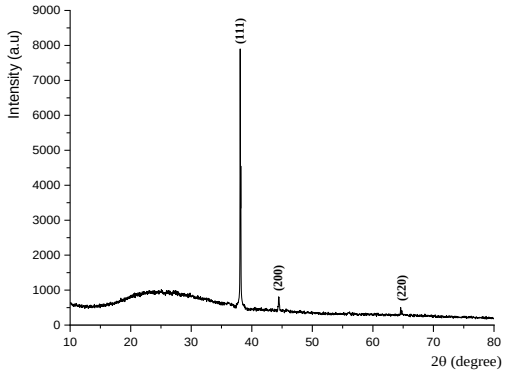

Figure 6. XRD patterns of CTS-AgNPs-2.

4. Conclusion

Box-Behnken design through response surface methodology (RSM) was successfully applied for parameter optimization of the green chemical reduction synthesis of chitosan-silver nanoparticle composite material. The model validation provided good agreement between the experimental results and the predicted response. The face-centered cubic form of metallic silver was observed. Spherical Ag nanoparticles with an average diameter of around 2÷8 nm and a narrow size distribution were successfully obtained.

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