

STUDY ON PREPARATION OF SILVER NANOPARTICLES/CHITOSAN BY γ -IRRADIATION AND THEIR ANTIMICROBIAL ACTIVITY

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Abstract: Radiation-induced synthesis of colloidal silver nanoparticles (Ag-NPs) using chitosan (CTS) as a stabilizer and free radical scavenger is feasible and satisfiable for green method. The conversion dose (Ag^+ into Ag^0) was determined by UV-Vis spectroscopy and Ag-NPs size was characterized by transmission electron microscopy. The average diameter of Ag-NPs as prepared was less than 10 nm with narrow size distribution. Effect of several parameters, such as Ag^+ , CTS concentration and molecular weight of CTS on Ag-NPs size was also investigated. Ag-NPs of ~ 7 nm exhibited highly antimicrobial effect. The inhibitory efficiency of Ag-NPs for *Staphylococcus aureus* was more than 99.9% at 5 ppm and the effective dose (ED_{50}) of inhibition for *Corticium salmonicolor* was of 27.2 ppm.

Keywords: *Silver nanoparticles, chitosan, antimicrobial, γ -irradiation.*

I. INTRODUCTION

During the last decades, developments of surface microscopy, materials science, biochemistry, physical chemistry and computational engineering have converged to provide remarkable capabilities for understanding, fabricating and manipulating structures at the atomic level [1]. The study on synthesis of metal nanoparticles is of interest in both research and technology. Among metal nanoparticles, silver nanoparticles (Ag-NPs) have attracted considerable interest because of their novel properties and their potential application [2, 3]. Different methods have been used for the synthesis of Ag-NPs from Ag^+ solution, such as chemical [4, 5], photochemical reduction [6], gamma and electron beam irradiation [3, 7],... . The method for preparing Ag-NPs by exposure to ionizing rays provides several advantages, such as the manufacturing process can be carried out at room temperature, the sizes and size distribution of the particles can be easily control and purely colloidal Ag-NPs can be obtained. In addition, mass production at reasonable cost is possible [3, 8]. It is well known that Ag^+ in solution could be reduced by γ -rays to Ag atoms while they would agglomerate if there is no protective substance. Hence an effective stabilizer is the key factor to fabricate densely dispersed Ag-NPs by irradiation method [9]. Several polymers having functional groups, such as $-\text{NH}_2$, $-\text{COOH}$ and $-\text{OH}$ with high affinity for Ag atoms [2] to stabilize Ag-NPs, such as PVA, PVP [3, 4], gelatin and CMC [10], alginate [11], oligochitosan [12] and so on have been used for synthesis of Ag-NPs. Chitosan (CTS), a natural polysaccharide with excellent biodegradable, biocompatible, non-toxicity and adsorption characteristics is a renewable polymer [5, 9, 12]. Owing to the interaction with $-\text{NH}_2$ groups of CTS chain, the Ag-NPs are enveloped by CTS fragments and so the nanoparticles could be kept from agglomerating during irradiation reduction process [9, 12]. Using CTS as free radical scavenger and stabilizer for colloidal Ag-NPs prepared by γ -irradiation is appropriate to green method which should be evaluated from three aspects: the solvent, the reducing and the stabilizing agent [5, 9, 12]. In addition, Ag-NPs stabilized by CTS are positive charge enrichment in surface so that antimicrobial property is significantly improved [13, 14, 15]. Therefore, preparation of Ag-NPs/CTS by γ -irradiation and antimicrobial effect on *Staphylococcus aureus* and *Corticium salmonicolor* were carried out in this work.

II. EXPERIMENTAL

1. *Materials*: Analytical grade AgNO_3 , lactic acid and NaOH were purchased from Shanghai Chemical Reagent Co., China. Deionized water was pure product of Merck, Germany. CTS with deacetylation degree of about 70% and mass average molecular weight (M_w) from 3.5 to 460kDa was prepared at VINAGAMMA, Ho Chi Minh City. Two microorganism strains namely *Staphylococcus aureus* ATCC 6538 and *Corticium salmonicolor* were supplied by University Medicine Pharmacy, Ho Chi Minh City and Rubber Research Institute of Vietnam.

2. *Methods*: CTS solution was prepared by dissolving 3 g CTS in 100 ml lactic acid 2% (v/v) solution and stored overnight. Then the pH of CTS solution was adjusted to about 6 by NaOH 2M solution. CTS solution after mixing with desired content of AgNO_3 was poured into glass tubes and irradiated by gamma Co^{60} radiation with dose rate of 1.3 kGy/h under ambient conditions at VINAGAMMA Center, Ho Chi Minh City. UV-Vis spectra of Ag-NPs solution which was diluted by water to 0.1mM calculated as Ag^+ concentration were recorded on an UV-2401PC, Shimadzu, Japan. The size of Ag-NPs was characterized by TEM images on a JEM 1010, JEOL, Japan, operating at 80 kV and statistically calculated using Photoshop software [3]. The X-ray diffraction (XRD) of Ag-NPs/CTS powder was taken on an ADVANCE 8-Brooker, Germany with $\text{CuK}\alpha$ radiation. The antimicrobial activity of Ag-NPs was tested against *S. aureus* and *C. salmonicolor* by culture medium toxicity method [13, 14]. The Luria Bertani or Malt Extract agar plate containing the test sample and control was incubated at 37°C for *S. aureus* and 27°C for *C. salmonicolor*. The antibacterial effect (*S. aureus*) was calculated using the equation: $\eta(\%) = (N_0 - N) \times 100/N_0$, where N_0 and N are the survival number of bacteria in the control and studied samples, respectively. The antifungal effect (*C. salmonicolor*) was evaluated by measuring diameter of colony growth and calculated as follows: Inhibition, % = $100 \times d/d_0$, where d_0 and d are the diameter of the colony of the control and studied samples, respectively.

III. RESULTS

CTS has been used as a very effective reducing/stabilizing agent for preparation of colloidal silver or gold nanoparticles by chemical method [5] and as a stabilizing/scavenging agent by ionizing irradiation method [9, 12, 15]. So in all these experiments, the external agent to scavenge $\cdot\text{OH}$ free radical which arising from radiolysis of water is not employed. According to Chen et al. [9], stabilization of CTS for Ag-NPs is due to their interaction with $-\text{NH}_2$ groups of CTS chain and the Ag-NPs are enveloped by CTS fragments. Concurrently, in aqueous solution the $-\text{NH}_2$ groups of CTS are protonated to $-\text{NH}_3^+$ ions and so the Ag-NPs could be kept from agglomerating through static repulsions between each other. However, the $\cdot\text{OH}$ radical can oxidize nascent metallic silver into silver ion that impacting on the formation of Ag-NPs. Fortunately, CTS scavenges $\cdot\text{OH}$ radical via hydrogen abstraction and the newly formed CTS radical that itself can also reduce Ag^+ to Ag^0 as described by Long et al. [12].

1. Influence of Ag^+ concentration

Table 1. The characteristics of colloidal Ag-NPs from CTS (120kDa) 1%/ Ag^+ 1-20 mM

Samples	OD	λ_{max} (nm)	d (nm)
$[\text{Ag}^+] 1 \text{ mM}$	1.33	397.0	4.6 ± 1.0
$[\text{Ag}^+] 5 \text{ mM}$	1.17	400.5	7.3 ± 1.4
$[\text{Ag}^+] 10 \text{ mM}$	1.01	403.0	10.4 ± 4.5
$[\text{Ag}^+] 20 \text{ mM}$	0.90	410.0	11.4 ± 1.6

As known from the Mie theory for the optical absorption bands of small metal particles, the size and amount of nanoparticles affect both the absorption wavelength and the intensity of the plasmon absorption band [16, 17]. Generally, colloidal metal nanoparticles solution with small sizes and high content of particles has high intensity at maximum absorption band and the maximum absorption wavelength (λ_{\max}) shifts to shorter wavelength [2, 3, 5]. The results in Fig. 1A showed that optical density (OD) values of irradiated Ag^+ solutions were increased up to a maximum at dose of 8 - 40 kGy for Ag^+ concentration from 1 to 20 mM. Those doses were defined as conversion doses to reduce Ag^+ into metallic silver completely [3, 7]. The influence of Ag^+ concentration on OD, λ_{\max} and size of Ag-NPs (d) is manifested in Table 1. The results showed that OD decreased from 1.33 to 0.90 and λ_{\max} shifted towards longer wavelengths for Ag^+ concentration from 1 to 20 mM. It means that the higher the Ag^+ concentration (1 - 20 mM), the larger the particles size (5 - 11 nm) would be formed.

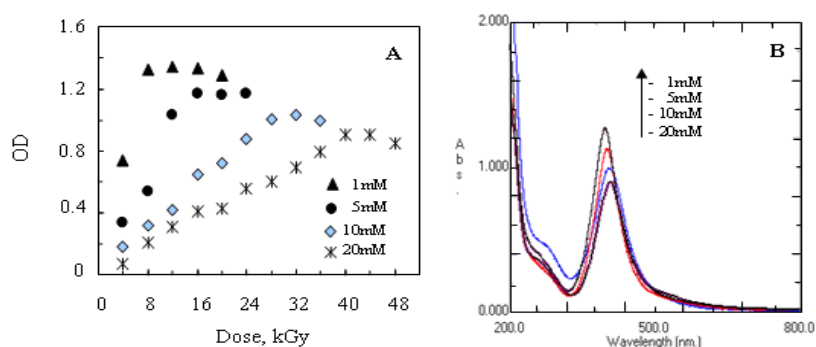


Figure 1. The relationship of OD and dose (A) and UV-Vis spectra of colloidal Ag-NPs at conversion dose (B) from CTS (120kDa) 1%/ Ag^+ 1-20 mM

The Ag-NPs obtained were mainly quasi-spherical in shape and Gaussian type in size distribution (Fig. 2a). The XRD pattern of CTS and Ag-NPs/CTS shown in Fig. 2b was also to confirm the existence of Ag-NPs. There was only one peak at 20° for CTS, while four intense peaks at 38.1° , 44.3° , 64.3° and 77.2° for Ag-NPs/CTS were observed. These peaks pertain to 111, 200, 220 and 311 faces of crystal Ag-NPs with typically face-centered cubic (fcc) lattice of silver [9, 11, 12].

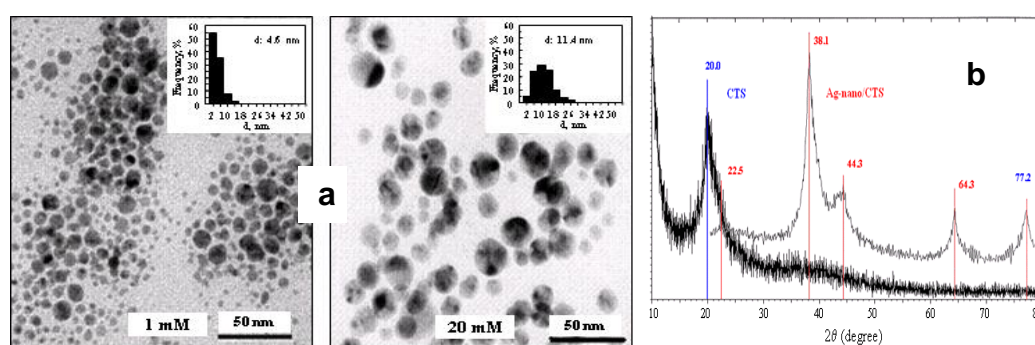


Figure 2. Typical TEM images and histograms of size distribution of Ag-NPs from CTS 1%/ Ag^+ 1 & 20 mM (a), XRD patterns of CTS (lower line) and Ag-NPs/CTS (upper line) (b)

In comparison with the particles size of 6 - 12 nm prepared by our group using the same Ag^+ concentration (1 - 20 mM) and PVP as stabilizer [3], the particles size of 5 - 11 nm (Table 1) obtained in this work was slightly smaller. The reason may be explained by the contribution of electrostatic repulsion of protonated amine groups on CTS chains that cannot

be attained in case of PVP. Long et al. [12] studied to prepare Ag-NPs by gamma irradiation using oligochitosan as stabilizer, the particles size was of 5 - 15 nm for Ag^+ concentration from 0.12 to 1 mM. Thus, the particles size of 15 nm was threefold larger than our result (5 nm) at the same Ag^+ concentration (1 mM). The difference may be due to low pH (3) and oligochitosan with low molecular weight (17kDa) in their experiments. In addition, the concentration of oligochitosan was of 0.03% compared to 1% CTS in our work.

2. Effect of CTS concentration

The particle size of Ag-NPs usually varies with both precursor ion and stabilizer concentration [2, 3, 11]. The change of particles sizes on CTS concentration in the range of 0.5-3.0% was studied. Results in Table 2 demonstrated that the λ_{max} shifted from 414.0 nm to 403.5 nm and the particles size decreased from 11.3 to about 7.0 nm with the increase in CTS concentration from 0.5% to 1.0%. This indicated that the higher the CTS concentration, the smaller the particles size was attained. A similar trend was also observed by Yoksan et al. [15]. The obtained particle size was of 23 nm for 0.1% CTS and 14 nm for 0.5% CTS in solution containing 0.1 mM Ag^+ concentration. This may be due to larger number of CTS chains enveloping the Ag-NPs surface to limit collision among particles.

Table 2. The characteristics of colloidal Ag-NPs from Ag^+ 5 mM/CTS 0.5-3.0%

Samples	OD	λ_{max} (nm)	d (nm)
CTS 0.5%	0.94	414.0	11.3 ± 2.0
CTS 1.0%	1.06	403.5	7.3 ± 1.4
CTS 2.0%	1.12	408.5	7.2 ± 1.3
CTS 3.0%	1.25	405.5	6.6 ± 3.0

Results in Table 2 indicated no obvious changes in particles size (~7 nm) for the CTS concentration from 1 to 3%. It was also found in our previous work that PVA concentration of 2-3% was a critical range for Ag^+ concentration of 20 mM in order to obtain the smallest Ag-NPs (~10 nm) [18]. Thus, we speculated that there will be an optimal stabilizer concentration for certain precursor Ag^+ concentration to obtain the smallest Ag-NPs by γ -irradiation method. Based on our results, the critical concentration of CTS was 1% for Ag^+ 5 mM.

3. Effect of CTS molecular weight

Table 3. The characteristics of colloidal Ag-NPs from Ag^+ 5 mM/CTS 1% with M_w 3.5-460kDa

Samples	OD	λ_{max} (nm)	d (nm)
CTS 3.5kDa	0.82	410.5	15.5 ± 1.6
CTS 60kDa	1.03	409.5	8.4 ± 1.3
CTS 120kDa	1.06	403.5	7.3 ± 1.4
CTS 460kDa	1.20	399.5	5.0 ± 1.7

The influence of molecular weight of CTS on characteristics of colloidal Ag-NPs is shown in Table 3. The λ_{max} values of colloidal Ag-NPs appeared in the range 399–410 nm, that is the specific surface plasmon resonance band of Ag-NPs [15, 16]. It was also obvious in Table 3 that the higher the M_w of CTS, the shorter the λ_{max} and the smaller the particles size of Ag-NPs. The reason for that may be due to the cumbersomeness of high M_w CTS which could enhance the antiagglomeration among Ag clusters to form small Ag-NPs. Similar

results were reported by Du et al. [3] for PVP K90 (1,100kDa) and PVP K30 (50kDa) in the synthesis of Ag-NPs by γ -irradiation. Yin et al. [11] also concluded that PVP with a short polyvinyl chain was unfavorable for the electrochemical synthesis of Ag-NPs. Temegire and Joshi [17] prepared Ag-NPs by γ -irradiation using PVA as stabilizer, the particles sizes obtained were of 18.6, 19.4 and 21.4 nm for PVA 125kDa, PVA 30kDa and PVA 14kDa, respectively. In addition, results of Huang et al. [16] confirmed that the diameter of Ag-NPs prepared by UV irradiation in carboxyl methyl CTS (0.8kDa) was larger than that in carboxyl methyl CTS (31kDa).

4. The antimicrobial effect

The colloidal Ag-NPs/CTS of about 7 nm exhibited highly antimicrobial activity with $\eta \sim 99.99\%$ corresponding to decrease of 4 logCFU/ml at 5 ppm Ag-NPs. Sanpui [14] also reported that the antibacterial activity of Ag-NPs/CTS for *E. coli* was significantly improved. The reason might be attributed to the positive charge of CTS fragments on surface of Ag-NPs that increases the attachable ability to bacteria cells. This is so-called synergistic effect of CTS and Ag-NPs. In addition, results of Cho et al. [13] indicated clearly that the antibacterial effect of Ag-NPs/PVP on *S. aureus* and *E. coli* was as much as of 99.99% while Ag-NPs/SDS did not show any growth inhibition at the same Ag-NPs concentration (10 ppm). The reason was due to the surface negative charge of SDS interferes with attachment of Ag-NPs to microbial cells. In this work, it was found that the antibacterial activity of Ag-NPs/CTS increased with the increase of Ag-NPs concentration and η values reached to 39.80, 64.29, 98.98 and 99.99% for Ag-NPs 1, 2, 3 and 5 ppm, respectively (Fig. 3). The antifungal effect of Ag-NPs/CTS also increased with the increase in Ag-NPs concentration. After incubation for 8 days, the growth inhibition was of 35.4, 68.6, 76.7, 79.7 and 81.9% for Ag-NPs 20, 40, 60, 80 and 100 ppm, respectively. Based on the results in Fig. 4, ED₅₀ value (effect dose for 50% inhibition) of Ag-NPs on *C. salmonicolor* was found to be 27.2 ppm.

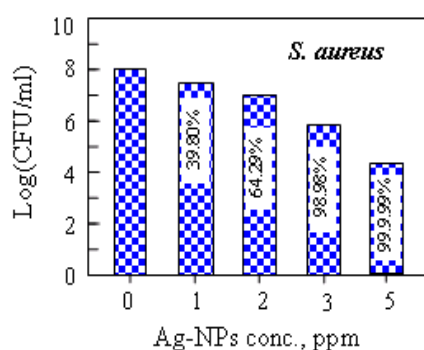


Figure 3. The antibacterial effect of Ag-NPs/CTS on *S. aureus*

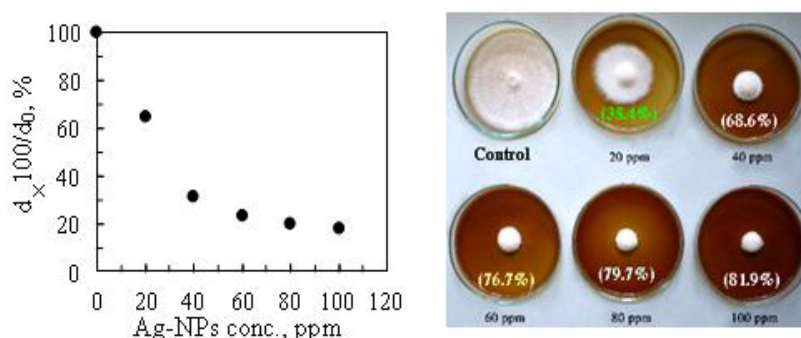


Figure 4. The antifungal effect of Ag-NPs/CTS on *C. salmonicolor*

Thus, Ag-NPs/CTS showed higher antibacterial activity (*S. aureus*) compared to antifungal activity (*C. salmonicolor*). The antimicrobial effect of nanosized silica-silver on several pathogenic strains in plant was also investigated by Park et al. [8]. Their results revealed that the inhibitory effect on bacteria (*B. subtilis*, *P. syringae*,...) was also higher in comparison to that on fungi (*M. grisea*, *B. cinerea*,...). This may be because of differences in organization, structure and function of their cells [19, 20].

IV. CONCLUSIONS

Colloidal Ag-NPs were prepared by γ -irradiation using CTS as a stabilizer and free radical scavenger. The particles size was in the range of 4.6-11.4 nm with narrow size distribution for Ag⁺ concentration from 1 to 20 mM and feasibly controllable by selecting concentration and/or molecular weight of CTS. Ag-NPs of ~7 nm showed strongly inhibition effect against *S. aureus* bacteria with η of 99.99% at 5 ppm and fungal *C. salmonicolor* with ED₅₀ of 27.2 ppm. The synthesis method, γ -irradiation might be useful for mass production of Ag-NP/CTS for application in different fields, especially in biomedicine.

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