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Radiation-induced synthesis of colloidal silver nanoparticles (Ag-NPs) using chitosan (CTS) as a stabiliser and free radical scavenger is feasible and satisfiable to green method. The conversion dose (Ag^+ into Ag^0) was determined by UV-Vis spectroscopy and Ag-NPs size was characterised by transmission electron microscopy. The average diameter of Ag-NPs was smaller than 10 nm with narrow size distribution and the colloidal Ag-NPs have good stability for a long time of storage. The effect of several parameters, such as pH, Ag^+ and 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

1. Introduction

During the past 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. The rapid evolution of this new science and the opportunities for application promise that nanotechnology will become one of the dominant technologies of the twenty-first century [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], electrochemical [6], photochemical reduction [7], ultrasonic spray pyrolysis [8], gamma and electron beam irradiation [3,9]. The method for preparing

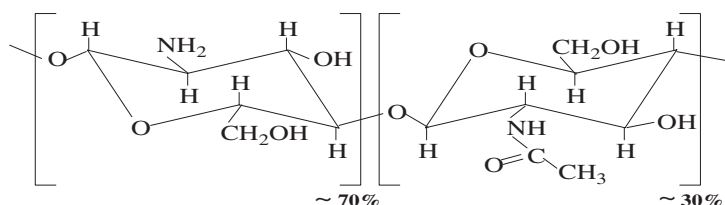*Corresponding author. Email: hien7240238@yahoo.com

Figure 1. The molecular structure of CTS with deacetylation degree of about 70%.

Ag-NPs by exposure to ionising 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 controlled and purely colloidal Ag-NPs can be obtained. In addition, mass production at reasonable cost is possible [3,10]. 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 stabiliser is the key factor to fabricate densely dispersed Ag-NPs by irradiation method [11]. Several polymers having functional groups, such as $-NH_2$, $-COOH$ and $-OH$ with high affinity for Ag atoms [2] to stabilise Ag-NPs, such as PVA, PVP [3,4], gelatin and CMC [12], alginate [13], oligochitosan [14] 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 [15]. Owing to the interaction with $-NH_2$ groups of CTS chain (Figure 1), the Ag-NPs are enveloped by CTS fragments and so the nanoparticles could be kept from agglomerating during irradiation reduction process [11,15]. Using CTS as free radical scavenger and stabiliser 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 stabilising agent [11,14–16]. In addition, Ag-NPs stabilised by CTS are positive charge enrichment in surface so that antimicrobial property is significantly improved [17,18]. Therefore, preparation of Ag-NPs/CTS by γ-irradiation and antimicrobial effect on *Staphylococcus aureus* and *Corticium salmonicolor* were carried out in this work.

2. Experimental

2.1. *Materials*

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

2.2. *Methods*

CTS solution was prepared by dissolving 3 g CTS in 100 mL lactic acid 2% (v/v) solution (pH 3) and stored overnight. Then the pH of CTS solution was adjusted to about 6 by

NaOH 2 M solution. CTS solution after mixing with desired content of AgNO$_3$ was poured into glass tubes, which were deaerated by bubbling with N$_2$ for 15 min. The γ-irradiation was carried out on a Co^{60} irradiator 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 0.1 mM calculated as Ag$^+$ concentration were recorded on an UV-2401PC, Shimadzu, Japan. The size of Ag-NPs was characterised 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 Cu Kα radiation [8].

The antimicrobial activity of Ag-NPs was tested against *S. aureus* and *C. salmonicolor* by culture medium toxicity method [17–19]. 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 numbers 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 diameters of the colony of the control and studied samples, respectively.

3. Results

CTS has been used as a very effective reducing/stabilising agent for preparation of colloidal silver or gold nanoparticles by chemical method [5,15] and as a stabilising/ scavenging agent by ionising irradiation method [11,14]. So in all these experiments, the external agent to scavenge $^\bullet$OH free radical which arising from radiolysis of water is not employed. According to Chen et al. [11], stabilisation of CTS for Ag-NPs is due to their interaction with –NH$_2$ groups of CTS chain and the Ag-NPs are enveloped by CTS fragments. Concurrently, in aqueous solution the –NH$_2$ groups of CTS shell are protonated to $-$NH$_3^+$ ions and so the Ag-NPs could be kept from agglomerating through static repulsions between each other. However, the $^\bullet$OH radical can oxidise nascent metallic silver into silver ion that impacting on the formation of Ag-NPs. Fortunately, CTS scavenges $^\bullet$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. [14].

3.1. *Effect of pH*

The maximum absorption wavelength (λ_{max}) value of colloidal Ag-NPs depends on the size of Ag-NPs. As the size of Ag-NPs increases the λ_{max} will shift towards longer wavelengths [2,3,5]. The results in Table 1 showed that the λ_{max} of Ag-NPs was of 419.5 nm for pH 3 and 403.5 nm for pH 6 corresponding to the particle size of 15.0 nm and 7.3 nm. In addition, the size distribution of Ag-NPs prepared in pH 6 was narrower than that in pH 3 (Figure 2). The reason for that may be explained as follows, the reduction reaction of Ag$^+$ into Ag^0 could be unfavourable for the formation of small Ag-NPs in acidic medium with higher H$^+$ concentration. Moreover, Sun et al. [15] also concluded that CTS chains were broken in acidic aqueous solution that might partially reduce stabilising

Table 1. The characteristics of colloidal Ag-NPs from CTS (120kDa) 1%/ Ag^+ 5mM at different pH (dose 16kGy).

Samples	OD	λ_{max} (nm)	d (nm)
pH 3	0.97	419.5	15.0 ± 5.4
pH 6	1.06	403.5	7.3 ± 1.4
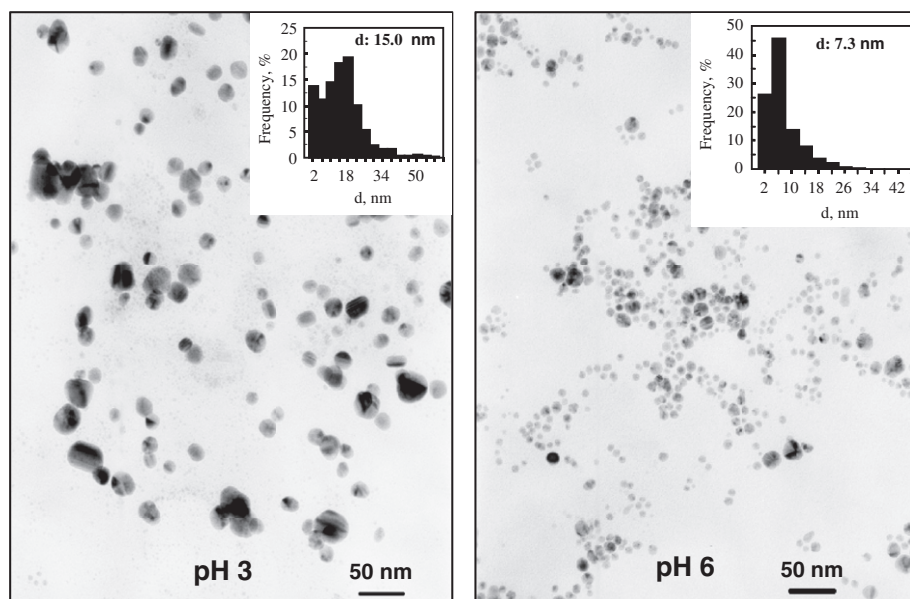
Figure 2. TEM images and histograms of size distribution of Ag-NPs from Ag^+ 5mM/CTS 1% with different pH.

activity of CTS for metallic nanoparticles. Recently, several studies on preparation of Ag-NPs by γ-irradiation in CTS solution were performed [11,14,20], but the effect of pH has not yet been investigated. However, the effect of pH for other stabilisers has been carried out. For instance, Huang et al. reported that pH 12.4 was an ideal condition for preparation of Ag-NPs in carboxyl methyl CTS solution [21]. The results of Ramnani et al. [2] indicated that neutral and acid media (pH 2–4) were desired for the synthesis of Ag clusters on SiO_2. Thus, the effect of pH plays an important role in the formation of small size of Ag-NPs and optimal pH values may be varied upon stabiliser agents. Based on our results for CTS, it inferred that the nearly neutral medium (pH 6) is suitable for preparation of Ag-NPs with small size.

3.2. *Influence of Ag^+ concentration*

As known from the Mie theory for the optical absorption bands of small metal particles, the size and amount of nanoparticles effect both the absorption wavelength and the
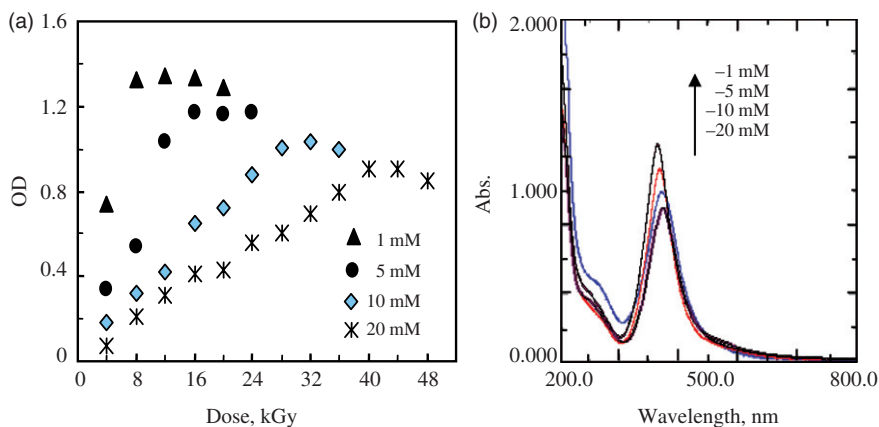
Figure 3. The relationship of OD and dose (a) and UV-Vis spectra of colloidal Ag-NPs at conversion dose (b) from CTS (120 kDa) 1%/Ag$^+$ 1–20 mM.

Table 2. The characteristics of colloidal Ag-NPs from CTS (120 kDa) 1%/Ag$^+$ 1–20 mM.

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

intensity of the plasmon absorption band [21,22]. Generally, colloidal metal nanoparticles solution with small sizes and high content of particles has a high intensity at the maximum absorption band and λ_{max} shifts to a shorter wavelength. The results in Figure 3(a) 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 [3,9]. The influence of Ag$^+$ concentration on OD, λ_{max} and size of Ag-NPs is manifested in Table 2. 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. The Ag-NPs obtained were mainly quasi-spherical in shape and Gaussian type in size distribution (Figure 4). The XRD pattern of CTS and Ag-NPs/CTS shown in Figure 5 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-centred cubic (fcc) lattice of silver [11,13,14]. 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 stabiliser [3], the particles size of 5–11 nm (Table 2) obtained in this work was slightly smaller. The reason may be explained by the contribution of electrostatic repulsion of protonated amine
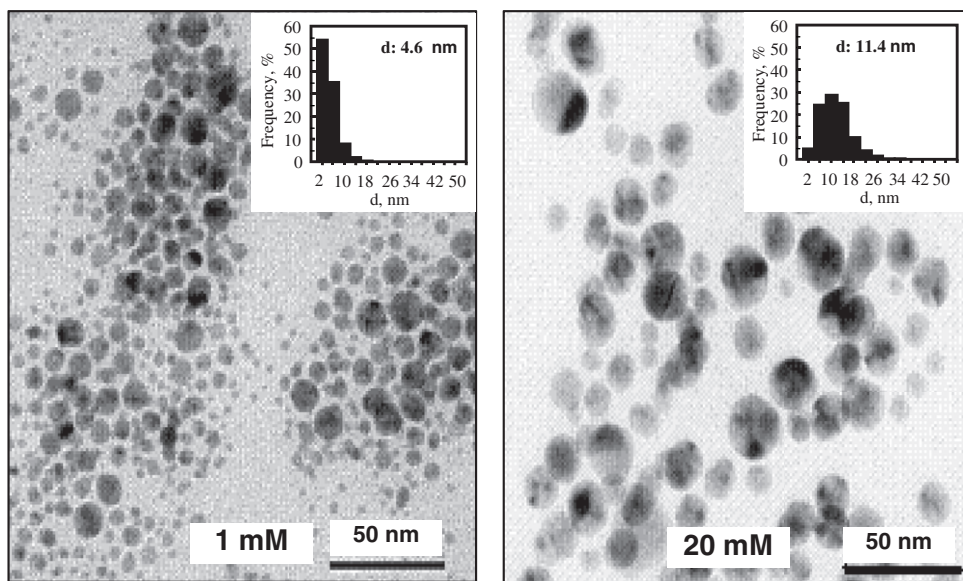
Figure 4. Typical TEM images and histograms of size distribution of Ag-NPs from CTS (120 kDa) 1%/Ag$^+$ 1 and 20 mM.
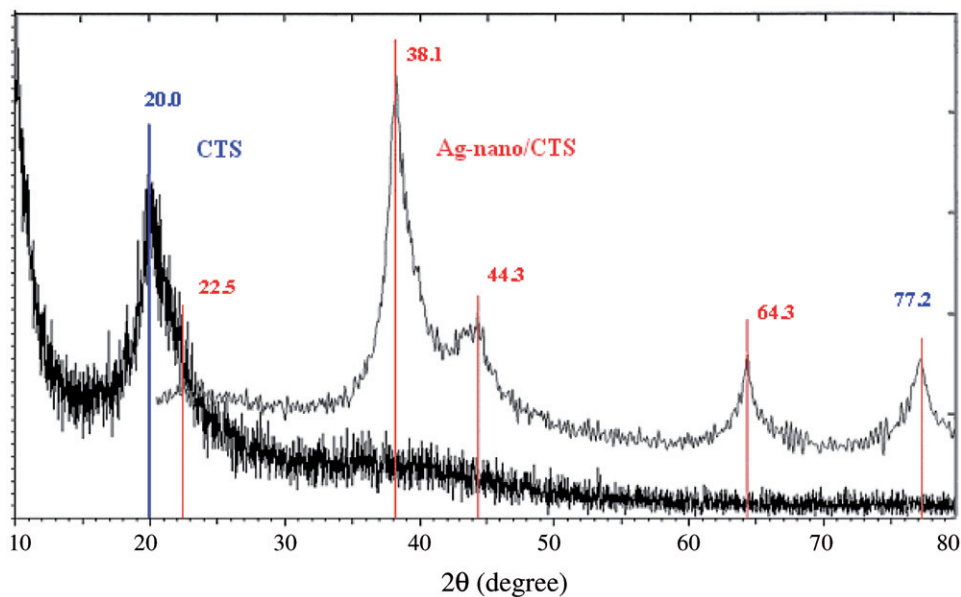

Figure 5. XRD patterns of CTS (lower line) and Ag-NPs/CTS (upper line).

Table 3. The characteristics of colloidal Ag-NPs from Ag^+ 5 mM/ CTS (120 kDa) 0.5–3.0%.

Samples (in %)	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

groups on CTS chains that cannot be attained in the case of PVP. Long et al. [14] studied to prepare Ag-NPs by gamma irradiation using oligochitosan as stabiliser, the particles size was 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 (17 kDa) in their experiments. In addition, the concentration of oligochitosan was of 0.03% compared to 1% CTS in our work.

3.3. *Effect of CTS concentration*

The particle size of Ag-NPs usually varies with both precursor ion and stabiliser concentration [2,3,13]. The change in particles sizes on CTS concentration in the range 0.5–3.0% was studied. Results in Table 3 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. [20]. 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. Results in Table 3 indicated no obvious changes in particles size ($\sim$7 nm) for the CTS concentration from 1% to 3%. It was also found in our previous work that PVA concentration of 2–3% was a critical range for Ag^+ concentration of 20 mM in order to obtain the smallest Ag-NPs ($\sim$10 nm) [23]. Thus, we speculated that there will be an optimal stabiliser 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.

3.4. *Effect of CTS molecular weight*

The influence of molecular weight of CTS on characteristics of colloidal Ag-NPs is shown in Table 4. The λ_{max} values of colloidal Ag-NPs appeared in the range of 399–410 nm, that is the specific surface plasmon resonance band of Ag-NPs [20,21]. It was also obvious in Table 4 that the higher the M_w of CTS, the shorter the λ_{max} and the smaller the size of Ag-NPs. The reason for that may be due to the cumbersomeness of high M_w CTS which could antiagglomeration among Ag clusters to form small Ag-NPs. Similar results were

Table 4. The characteristics of colloidal Ag-NPs from Ag^+ 5 mM/ CTS 1% with different $M_{w.}$

Samples (in kDa)	OD	λ_{max} (nm)	d (nm)
CTS 3.5	0.82	410.5	15.5 ± 1.6
CTS 60	1.03	409.5	8.4 ± 1.3
CTS 120	1.06	403.5	7.3 ± 1.4
CTS 460	1.20	399.5	5.0 ± 1.7
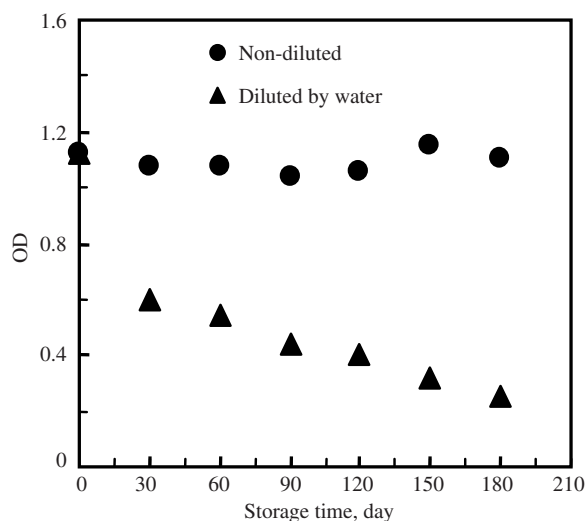
Figure 6. The change in OD of colloidal Ag-NPs from CTS 1%/Ag^+ 5 mM with storage time.

reported by Du et al. [3] for PVP K90 (1100 kDa) and PVP K30 (50 kDa) in the synthesis of Ag-NPs by γ-irradiation. Yin et al. [6] also concluded that PVP with a short polyvinyl chain was unfavourable for the electrochemical synthesis of Ag-NPs. Temgire and Joshi [22] prepared Ag-NPs by γ-irradiation using PVA as stabiliser, the particles sizes obtained were 18.6, 19.4 and 21.4 nm for PVA 125 kDa, PVA 30 kDa and PVA 14 kDa, respectively. In addition, results of Huang et al. [21] confirmed that the diameter of Ag-NPs prepared by UV irradiation in carboxyl methyl CTS (0.8 kDa) was larger than that in carboxyl methyl CTS (31 kDa).

3.5. *Stability of Ag-NPs colloid*

The stabilisation of colloidal Ag-NPs as-prepared was studied through the change in OD value with storage time at ambient temperature. Results in Figure 6 revealed that the OD of colloidal Ag-NPs solution without dilution was stable for more than 6 months. However, when it was diluted by water with ratio 1/50 (v/v), OD decreased from 1.12 to 0.44 after 3 months and to 0.25 after 6 months. Furthermore, it was also observed that the λ_{max} of the diluted solution shifted from 408.5 nm to 423 nm after 6 months. The decrease
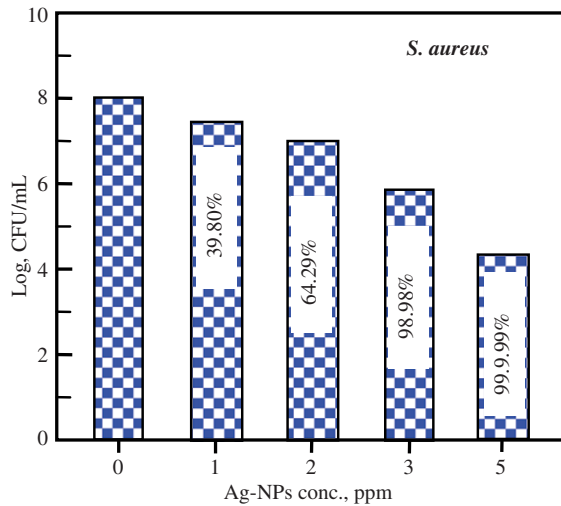
Figure 7. The antibacterial effect of Ag-NPs/CTS on *S. aureus*.

in OD and the increase in λ_{max} mean that aggregation occurred to form larger particles [4,14]. In addition, it was interesting to note that OD of colloidal Ag-NPs diluted by 0.5% CTS solution with ratio of 1:50 (v/v) was almost unchanged after 6 months of storage (data not shown).

3.6. *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 4logCFU/mL at 5 ppm Ag-NPs. Sanpui et al. [18] 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. [17] 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 fact that 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 in 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 (Figure 7).

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 Figure 8, ED_{50} (effect dose for 50% inhibition) of Ag-NPs on *C. salmonicolor* was found to be 27.2 ppm. 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 plants was also investigated by Park et al. [10]. Their results revealed that the inhibitory effect on bacteria (*B. subtilis*, *P. syringae*, ...) was
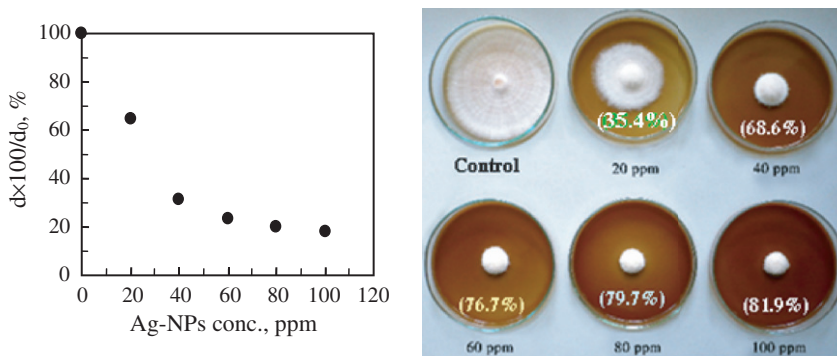
Figure 8. The antifungal effect of Ag-NPs/CTS on *C. salmonicolor*.

also higher in comparison to that on fungi (*M. grisea*, *B. cinerea*, ...). This may be because of differences in organisation, structure and function of their cells [24,25].

4. Conclusions

Colloidal Ag-NPs were prepared by γ-irradiation using CTS as a stabiliser and free radical scavenger. The particles size was in the range 4.6–11.4 nm for Ag^+, concentration from 1 to 20 mM and feasibly controllable by adjusting the pH, as well as selecting concentration and molecular weight of CTS. The colloidal Ag-NPs solution can be storable for at least 6 months with good stability. Ag-NPs of ~7 nm showed strongly inhibition effects against *S. aureus* bacteria with η of 99.99% at 5 ppm and fungal *C. salmonicolor* with ED_{50} 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.

Acknowledgement

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