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# Gamma irradiation of cotton fabrics in $AgNO_3$ solution for preparation of antibacterial fabrics

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ABSTRACT

Silver nanoparticles (AgNPs) with diameter about 12 nm were immobilized on the surface of cotton fabrics by γ-irradiation of fabrics in the $AgNO_3$ chitosan solution. Effects of absorbed dose, concentration of $AgNO_3$ solution on immobilization of the AgNPs on fabrics were investigated. The optimal dose was selected to be 13.8 kGy and the suitable concentration of $AgNO_3$ was 1.5 mM in 1% chitosan solution. The content of AgNPs on fabrics was of 1696 ± 80 mg/kg for these conditions. The presence of AgNPs on fabrics was confirmed from scanning electron microscopy (SEM) images and X ray diffraction (XRD) patterns. The antibacterial activity of AgNPs/cotton fabrics after 40 washings against *Staphylococcus aureus* and *Escherichia coli* was found to be 99.99%. In addition, the AgNPs fabrics were innoxious to the skin ($k = 0$) after 1, 5, 10, 20, 30 and 40 washings by skin-irritation testing to animal (rabbit).



## 1. Introduction

The synthesis and functionalization of silver nanoparticles (AgNPs) have been extensively investigated in past decades due to their remarkable properties and potential applications. A number of reports are available on the synthesis of metal nanoparticles in solution by different methods (Hannan & Subbalaxmi, 2011; Sáez & Mason, 2009; Szczepanowics, Stefanska, Socha, & Warszynski, 2010). Bolge, Dhole, and Bhoraskar (2006) described the synthesis of dispersed nanoparticulate silver using gamma-irradiation. Chen, Song, Liu, and Fang (2007) also synthesized silver nanoparticles by γ-ray irradiation in acetic aqueous solution containing chitosan. The broad-spectrum of antimicrobial properties of AgNPs encourage its use in biomedicine, water and air purification, food production, cosmetics, numerous household products (Li et al., 2008; Mritunjai, Shinjini, Prasad, & Gambhir, 2008). Because of their effective antimicrobial properties and low toxicity toward mammalian cells, AgNPs have become one of the most commonly used nanomaterials in consumer products (Choi et al., 2008). Kokura et al. (2010) proved that silver nanoparticles were able to be used for preservation of cosmetics against mixed bacteria and mixed fungi. AgNPs can be immobilized on the fibers, bringing new properties to the final textile product, especially for antibacterial effect. The antibacterial fabrics can be used to make bandage, gauze, bed sheets, surgical clothes... (Dubas, Kimlangdudsana, & Potiyaraj, 2006; Gupta, Bajpai, & Bajpai, 2008). The AgNPs interact with the bacterial membrane and are able to penetrate inside the cell. The mechanisms of interaction involved AgNPs attached to bacterial cell membranes increase permeability and disturb respiration. The AgNPs may catalyze reactions with oxygen leading to reactive oxygen species (ROS) production which can cause DNA damage, protein and cell membrane breakdown. The catalytic silver can destroy bacteria's disulfide bonds to counteract the synthesis of bacterial cell. AgNPs are oxidized generating silver ions that can disrupt ATP production (adenosine triphosphate) to inhibit the adsorption of phosphate of protein (Jone & Hoek, 2010).

The AgNPs in the environment as wastewater treatment plant effluent were associated with reduced sulfur from organic thiols groups and inorganic sulfides to $Ag_2S$. Sulfide exhibits a much lower toxicity than other form of Ag (Kaegi et al., 2011).

In this study, AgNPs immobilized on cotton fabrics by *in situ* synthesis. Silver ions were reduced to silver atoms by γ-irradiation and simultaneously immobilized on the fabrics. The durability of AgNPs linked with cotton fabrics and antibacterial effects as well as skin irritation test were also investigated after repeated washings.

## 2. Experimental

### 2.1. Materials

Cotton fabrics (100%) were provided by VICOTEX Company (Vietnam) with weighing 120 g/m$^2$. They were washed to remove

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glue then dried and cut into equal-sized square pieces measuring 0.5 m × 0.5 m, before using. Shrimp shell chitin was supplied by a factory in Vung Tau province, Vietnam. Chitin was treated in 3% sodium hydroxide (w/v) at 100 °C for 3 h for deproteinization and in 3% hydrochloric acid (v/v) for decalcification. Chitosan with a degree of deacetylation (DD%) about 80% was prepared by deacetylation of chitin in 50% sodium hydroxide at 100 °C for 1 h. This value was determined based on FT-IR spectra according to the following equation (Brugnerotto et al., 2001):

$$\frac{A_{1320}}{A_{1420}} = 0.3822 + 0.0313(100 - \mathrm{DD\%}) \quad (1)$$

where $A_{1320}$, $A_{1420}$ are absorbance of chitosan at 1320 and 1420 $cm^{-1}$, respectively.

The Mw of chitosan ($7.63 \times 10^4$) was measured by an Agilent 1100 gel permeation chromatography (GPC; Agilent Technologies, USA) with detector RI G1362A and the column ultrahydrogel model 250 from Waters (USA). The standards for calibration of the columns were pullulan (Mw 780–380 × $10^3$). All other chemicals, including silver nitrate ($AgNO_3$), (S) – lactic acid (90%), sodium hydroxide (NaOH) were of reagent grade. Distiled water was used in all experiments.

### 2.2. Preparation of AgNPs/cotton fabrics by γ-irradiation of cotton fabrics in $AgNO_3$ solution

Cotton fabrics about 60 g after washing were irradiated in 500 ml of 0.5 to 5.0 mM $AgNO_3$ solution using the stabilizer of 1.0% chitosan in the dose range from 5 to 20 kGy. The gamma-irradiation dose was determined by using the ethanol-chlorobenzene (ECB) dosimetry system from mean value of absorbed doses of three dosimeters at 30 °C. The AgNPs/cotton fabrics were washed according to a modified PN-EN ISO 105-C06: 2010 (AS1) (Marek Koz Elżbieta et al., 2013) in order to assess resistance to the washing process. The maximum number of washing was 40 cycles. An approximately 3 g weight sample was placed in 150 $cm^3$ of washing liquor and was washed at 40 °C for 30 min (one cycle). The concentration of the active surface agent was equal to 1 g/$dm^3$. Afterwards, samples were rinsed with water and dried at 40 °C for 40 min. A similar procedure was applied for 40 repeated washes. The content of AgNPs was evaluated by inductively coupled plasma atomic emission spectroscopy (ICP-AES), model Optima 5300DV (Perkin Elmer) after 1, 5, 10, 20, 30 and 40 washings.

All collected data were expressed as mean ± SE – standard error, in this study. The differences between sample values were assessed using two-tailed unpaired Student's *t*-tests. The standard error should be <±5% at a 95% confidence level, number of samples that were analyzed per condition to be three ($N = 3$).

### 2.3. Characterization of the AgNPs/cotton fabrics

The presence of AgNPs on fabrics was confirmed by SEM image, using a JSM-6480LV scanning electron microscopy was operated at 10 kV and used at 1,300 and 5,500 magnifications. X ray diffraction patterns were measured on a Shimadzu 5A diffractometer with CuKα radiation (35 kV and 25 mA), scanning at a speed of 0.5°/min from 5–80° ($2\theta$). The crystalline silver nanoparticle has been estimated by using Debye–Scherrer formula:

$$D = K \cdot \frac{\lambda}{\beta \cos\theta} \quad (2)$$

where $K = 0.89$, λ is the wave length of X-ray (1.5405 Å), $\beta$ is the full width at half maximum (FWHM), $\theta$ is the diffraction angle (Bragg) and $D$ is the particle diameter size (Baker, Pradhan, Pakstis, Pochan, & Shah, 2005)

The mechanical properties were determined using a Strograph V10-C (Toyoseiki Co., Japan) testing instrument at a constant crosshead speed of 50 mm/min. Five specimens with a dumbbells shape were prepared according to ASTM D 1882-L and were used for the measurement.

### 2.4. Antibacterial efficacy and skin irritation test of the AgNPs/cotton fabrics

Antibacterial properties of resultant fabrics were verified according to AATCC Test Method 100–2004 against *Staphylococcus aureus* No. 6538, a Gram-positive organism and *Escherichia coli* No. 10229, a Gram-negative organism (AATCC Test method: 100–2004). The inoculum concentration of bacteria in a germ containing nutrient solution was $1 \times 10^7$ to $2 \times 10^7$ CFU/ml. Test specimens were cut in 4.8 ± 0.1 cm diameters and absorbed 1 ml of inoculum in sterile Petri plates. Then, specimens were placed in the jar contained 100 ml neutralizing solution. Jars were shaken vigorously for 1 min, serial dilutions were made. From each of three suitable dilutions, 0.1 ml liquid was drawn and transferred to nutrient agar, then incubated all plates for 48 h at 37 °C. Percent reduction of bacteria was calculated from the number of bacteria recovered in the jar immediately after inoculation and the jar incubated over desired period.

Skin irritation test was carried out by Institute of Drug Testing following ISO 10993-10 (2002). This test was carried out on grown and healthy rabbits weighing at least 2 kg. The rabbits using for the irritation test were reared at 25 °C, with humidity of 30–70%. The AgNPs/cotton fabrics were applied to shaved skin about 10 cm × 15 cm in the back of rabbit. The skin situation of the rabbits was observed after 12, 24, 48 and 72 h.

## 3. Results and discussion

### 3.1. Preparation of AgNPs/cotton fabrics

The AgNPs/cotton fabrics were prepared by γ-irradiation of cotton fabrics in silver nitrate solution. The influential factors such as the initial $Ag^+$ concentration and absorbed dose on the immobilization of AgNPs onto fabrics were investigated by ICP-AES. During γ-irradiation of cotton fabrics in the $AgNO_3$ solution, the hydrated electron ($e_{aq}^-$) and radical of H• that were generated by water radiolysis can reduce $Ag^+$ to Ag as shown in Eqs. (3)–(5) (Long, Wu, & Chen, 2007). Continuous reduction of the $Ag^+$ solution causes the aggregation of clusters into AgNPs. In chitosan solution, the •OH groups can react with chitosan to abstract hydrogen and form macromolecule free radicals as Eq. (6). These radicals can reduce the cluster of $Ag^+$ ions forming the cluster of Ag atoms on fabric surface (Eq. (7)). The binding of silver clusters by chitosan is achieved through the Ag—O bond (Chen, Wu, & Zeng, 2005). The reactions can be proposed as follows:

$$H_2O(\gamma\text{-rays}) \rightarrow {}^{\bullet}OH + e^-_{aq} + {}^{\bullet}H + H_2O_2 + H_2 + \ldots \quad (3)$$

$$Ag^+ + e^-_{aq} \rightarrow Ag^0 \quad (4)$$

$$Ag^+ + H^{\bullet} \rightarrow Ag^0 + H^+ \quad (5)$$

$$[C_6H_{11}O_4N]_n(\text{chitosan}) + {}^{\bullet}OH \rightarrow [C_6H_{11}O_4N]_{n-1}[C_6H_{10}O_4N]^{\bullet} + H_2O \quad (6)$$

$$Ag^+ + [C_6H_{11}O_4N]_{n-1}[C_6H_{10}O_4N]^{\bullet} \rightarrow Ag^0\text{-}[C_6H_{11}O_4N]_{n-1}[C_6H_9O_4N] + H^+ \quad (7)$$

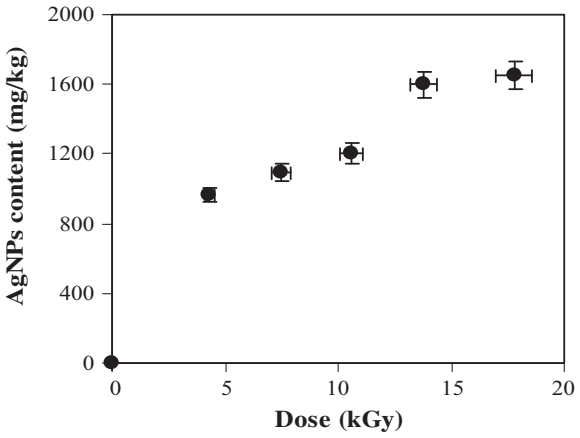


**Fig. 1.** Relationship between the content of AgNPs on the cotton fabrics and irradiation dose.

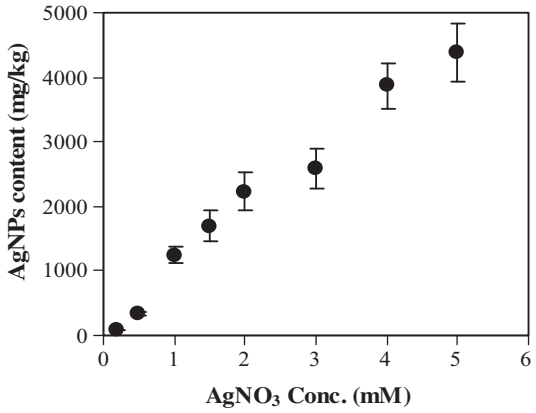


**Fig. 2.** Effect of $AgNO_3$ concentration on the content of AgNPs on the cotton fabrics.

$$nAg^0 \rightarrow Ag_2 \rightarrow \ldots Ag_n \quad (8)$$

The absorbed dose plays an important role in formation and growth of AgNPs by reduction of $Ag^+$ to $Ag^0$ atom by γ-irradiation. At a low dose, nanoparticles have not appeared yet, while nanoparticles will be formed at a higher dose. In this study, the preparation of the AgNPs/cotton fabrics by *in situ* synthesis in which $Ag^+$ ions were reduced to Ag atoms and simultaneously deposited on cotton fabrics. The interaction between fibers and metallic AgNPs which were stabilized by chitosan results from formation of chemical bond between silver and alcoholic groups of cotton and physical adsorption of AgNPs on the fabric surface (Perelshtein, Applerot, & Perkas, 2008). Fig. 1 clearly indicates that the content of AgNPs on the cotton fabrics has changed insignificantly in the range of low doses from 4 to 10 kGy, but increased at higher doses. A maximal value of the AgNPs content on fabrics was found to be 1696 mg/kg for the cotton fabrics in 1.5 mM $AgNO_3$ solution irradiated at the dose of 13.8 ± 0.6 kGy.

The effect of $Ag^+$ concentration on the content of the AgNPs when irradiation of the cotton fabrics in $AgNO_3$ solution was also investigated. In Fig. 2, the content of AgNPs on the fabrics increased as using the initial $Ag^+$ concentrations from 0.5 to 5 mM at the dose of 13.8 kGy. In order to control the release of silver nanoparticles into environment but keeping the antibacterial purpose after washings, the cotton fabrics which were immobilized with AgNPs at the content of 1696 ± 80 mg/kg corresponding to $Ag^+$ salt concentration of 1.5 mM was selected for further investigation.

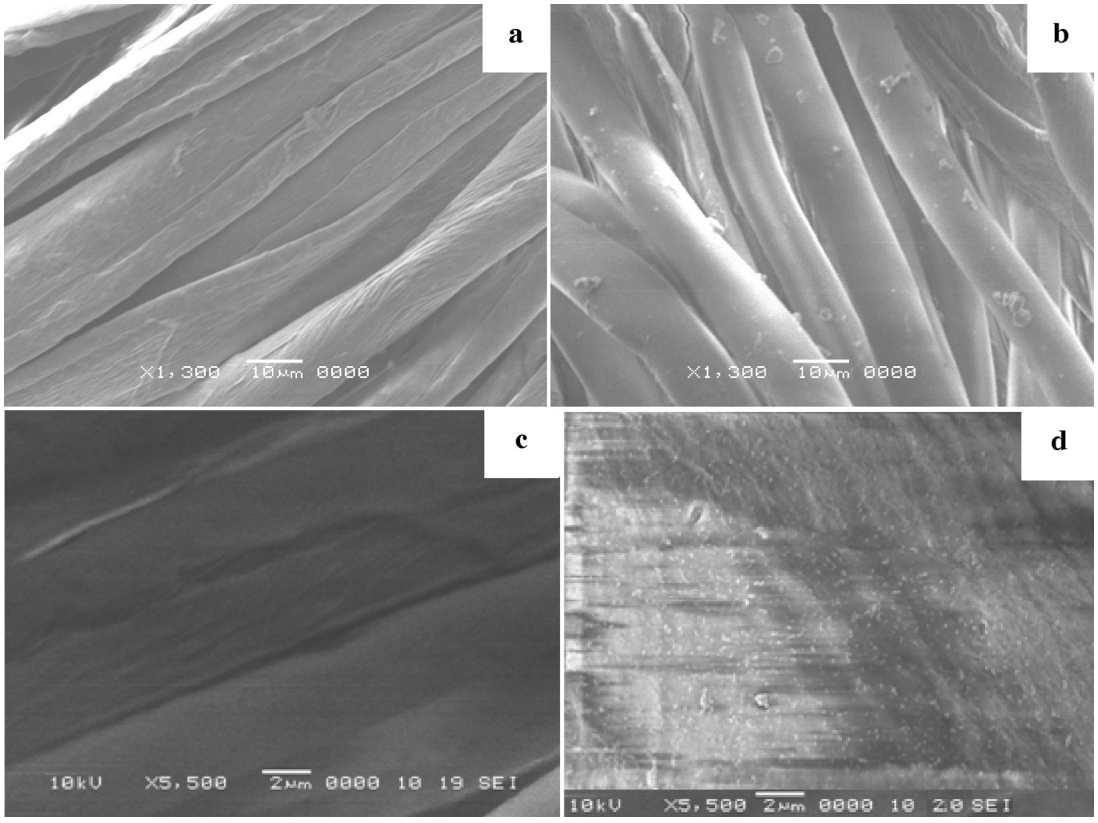


**Fig. 3.** SEM images of: original cotton fabrics (a) ×1300 and (c) ×5500; AgNPs cotton fabrics (b) ×1300 and (d) ×5500.

**Table 1**
The tensile strength and elongation at break of cotton fibers against irradiation doses.

| Dose (kGy) | Tensile strength (kf/mm$^2$) | Elongation (%) |
|---|---|---|
| 0 | 31.0 ± 1.6 | 50 ± 2.3 |
| 4.3 ± 0.2 | 30.5 ± 1.5 | 46 ± 2.0 |
| 7.5 ± 0.4 | 29.5 ± 1.5 | 44 ± 2.0 |
| 10.6 ± 0.5 | 27.0 ± 1.3 | 38 ± 1.5 |
| 13.8 ± 0.6 | 26.5 ± 1.3 | 36 ± 1.5 |
| 17.8 ± 0.8 | 21.3 ± 1.1 | 27 ± 1.3 |
| 20.3 ± 0.9 | 20.0 ± 1.0 | 22 ± 1.0 |

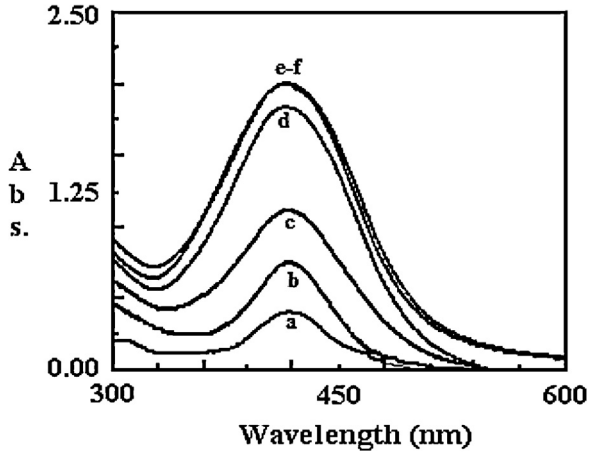


**Fig. 4.** UV–vis spectra of colloidal AgNPs from 1.5 mM $AgNO_3$ solution corresponding to irradiation doses of: (a) 4.3 kGy, (b) 7.5 kGy, (c) 10.6 kGy, (d) 13.8 kGy, (e) 17.0 kGy, and (f) 20.3 kGy.

### 3.2. Characterization of AgNPs/cotton fabrics

The presence of silver nanoparticles onto fabrics can be seen by SEM images (Fig. 3). The images in Fig. 3a and c show the smooth structure of the original cotton fabrics before coating with silver nanoparticles. After gamma-irradiation of cotton fabrics in $AgNO_3$ solution, the nanoparticles that were dispersed on the surface of the AgNPs/fabrics demonstrated the immobilization of silver nanoparticles on the fabrics (Fig. 3b). The homogeneous deposition is observed in a higher magnification image (Fig. 3d). UV–vis spectra were recorded for the colloidal AgNPs from 1.5 mM $AgNO_3$ in 1.0% chitosan solution irradiated by gamma-irradiation with cotton fabrics, in the dose range from 4 to 20 kGy (Fig. 4). The chitosan concentration was maintained at 1% solution as this provided sufficient chitosan for stabilization of the AgNPs and also allowed for an acceptable solution viscosity at 30 °C. The UV–vis absorbance spectra of AgNPs depict peak maxima in the range from 416–420 nm with respective increase in optical density (OD) in gamma dose from 4 to 13.8 kGy. These results demonstrate a proportional yield increase of AgNPs with gamma-irradiation dose below 13.8 kGy. Above 13.8 kGy (17.0 and 20.3 kGy), the optical density changed insignificantly. Therefore, the dose of 13.8 kGy is optimal for conversion of the 1.5 mM $AgNO_3$ to AgNPs in 1% chitosan solution at 30 °C.

Mechanical properties of cotton fabrics were determined in the dose range from 0 to 20 kGy. The results showed that tensile strength ($\sigma$) and elongation at break ($\varepsilon$) of cotton fabrics slightly lower at the doses below 13.8 kGy in comparison with non-irradiated cotton fabrics (Table 1). However, these values were much lower than at the higher doses, so that the irradiation dose was selected to be 13.8 kGy for irradiation of cotton fabrics.

The XRD patterns in Fig. 5a shows peaks at 12° and 21°, which can be attributed to the crystalline structure of cellulose. Four peaks at 38.25°, 43.28°, 62.46° and 76.32° in Fig. 5b assigned to diffractions from the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes of face-centered cubic silver, respectively. This result is in agreement with reports from other authors who used polysaccharides for stabilizing AgNPs (Phu et al., 2010; Staszewski, Kopyto, Becker Wrona, Dworak, & Kwarcinski, 2008). The full width at half-maximum of diffraction peaks (1 1 1) in Fig. 6 is employed to determine the average crystalline size using Debye–Scherrer as formula (2). The crystalline size of AgNPs was calculated to be about 12 nm.

### 3.3. Antibacterial effect and skin irritation test

The durability of antibacterial substance linked with fabrics is also an important factor for using. The AgNPs may be immobilized on fabrics by the bonds of the atomic silver with alcoholic groups in cellulose structure and chitosan capped AgNPs. According to the result in Table 2, the AgNPs content on fabrics decreased after the first washing cycle but changed insignificantly for next washings. The signs of $Ag/CF_0$, $Ag/CF_1$, $Ag/CF_5$, $Ag/CF_{10}$, $Ag/CF_{20}$, $Ag/CF_{30}$ and $Ag/CF_{40}$ were the abbreviations of the AgNPs/cotton fabrics before washing and washing 1, 5, 10, 20, 30 and 40 cycles, respectively. The layer-by-layer links of the stabilizer molecules coating outside were formed from electrostatics force, so that they were broken easily by physical impact or detergent. However, the bonds from the reactive center of cellulose to AgNPs capping by chitosan may be durable.

Results in Table 2 also demonstrated that antibacterial activity was influenced by the content of AgNPs on the fabrics. After 40 washings, an amount of AgNPs was remained on the fabrics of about 858 mg/kg, which inhibited the growth of *S. aureus* and *E. coli* to be 99.95 and 99.98%, respectively. Gupta et al. (2008) also reported that the AgNPs content is a key factor in controlling the antibacterial activity. In addition, the AgNPs diameter is also an important role for antibacterial effect. Lee and Jeong (2005) proved that the AgNPs size about 2–3 nm has a better antibacterial effect than 30 nm one. In this study, the AgNPs diameter about 12 nm was also effective for antibacterial activity.

Testing skin-irritation was carried out for the fabric samples, which were synthesized *in situ* by γ-irradiation of fabrics in the 1.5 mM $AgNO_3$ solution at the dose of 13.8 kGy. The fabrics imbedded AgNPs were washed from 1, 5, 10, 20,

**Table 2**
Bacterial reduction of AgNPs cotton fabrics and numerical grades for testing skin irritation on the rabbit skin.

| Samples | Washings (times) | AgNPs content on the fabrics (mg/kg) | Bacterial reduction (%) | | Numerical grades | | Remark |
|---|---|---|---|---|---|---|---|
| | | | *S. aureus* | *E. coli* | *Erythema* | *Edema* | |
| $Ag/CF_0$ | 0 | 1696 ± 80 | 100 | 100 | 0 | 0 | Skin-innoxious ($k = 0$) |
| $Ag/CF_1$ | 1 | 1034 ± 50 | 99.94 | 100 | 0 | 0 | Skin-innoxious ($k = 0$) |
| $Ag/CF_5$ | 5 | 1024 ± 45 | 99.99 | 100 | 0 | 0 | Skin-innoxious ($k = 0$) |
| $Ag/CF_{10}$ | 10 | 910 ± 45 | 99.94 | 100 | 0 | 0 | Skin-innoxious ($k = 0$) |
| $Ag/CF_{20}$ | 20 | 880 ± 40 | 99.99 | 100 | 0 | 0 | Skin-innoxious ($k = 0$) |
| $Ag/CF_{30}$ | 30 | 860 ± 40 | 99.99 | 100 | 0 | 0 | Skin-innoxious ($k = 0$) |
| $Ag/CF_{40}$ | 40 | 858 ± 40 | 99.95 | 99.98 | 0 | 0 | Skin-innoxious ($k = 0$) |

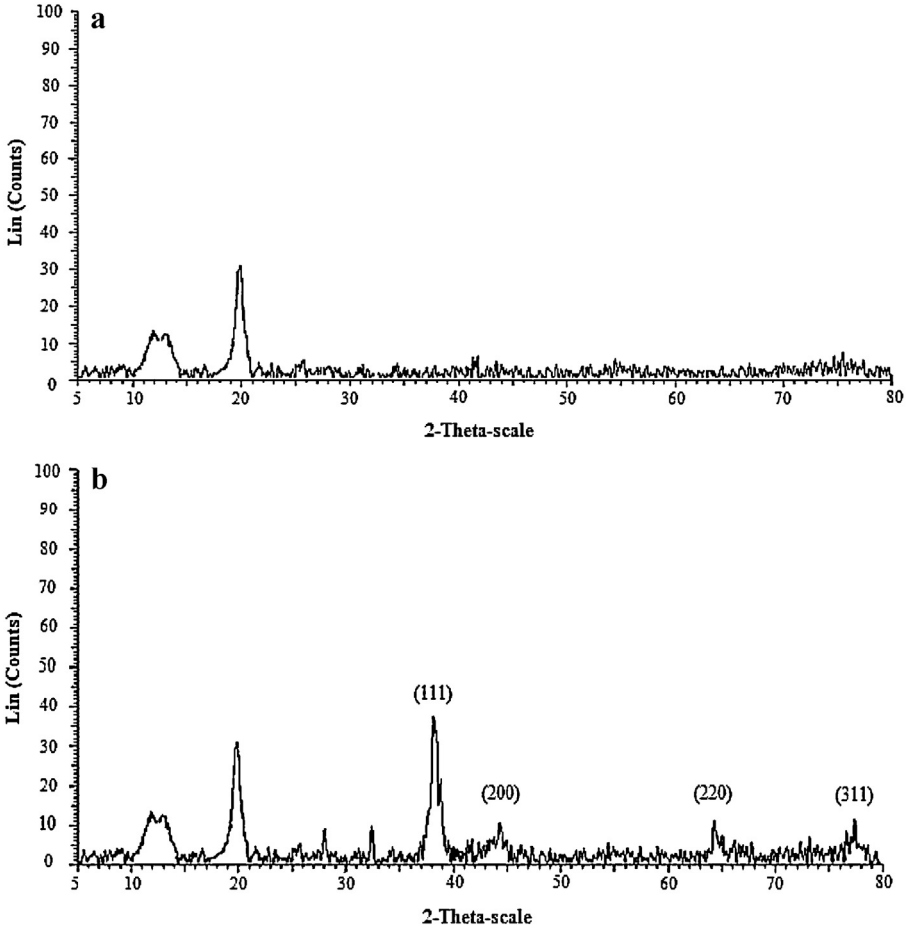


**Fig. 5.** XRD patterns of: (a) The cotton fabrics, and (b) AgNPs cotton fabrics.

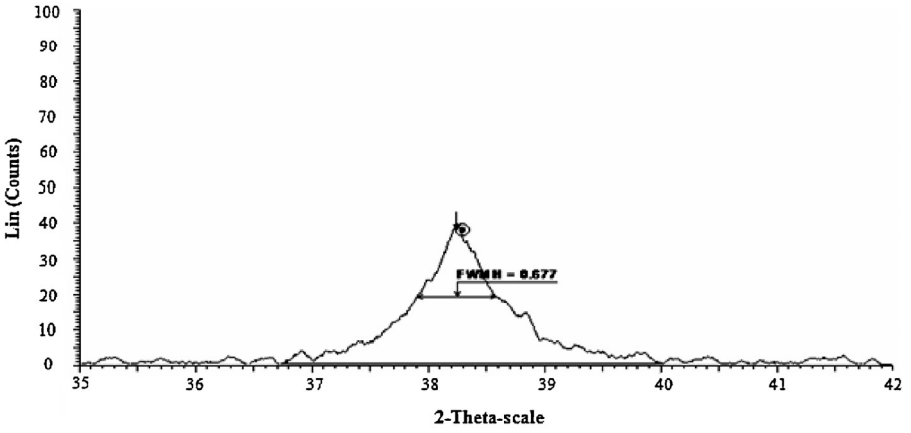


**Fig. 6.** Diffraction angle and the value FWHM at $2\theta = 38.25^{o}$.

30 and 40 cycles then were applied on the rabbit skin. The noxiousness on skin was determined from observing the skin situation after contact times for 12, 24, 48 and 72 h at 25 °C. The erythema and edema on the rabbit skin were recorded at the numerical grades to be 0 during coating AgNPs fabrics. These results confirmed that AgNPs/cotton fabrics are innoxious to skin with coefficient of $k = 0$ (Table 2). Lee and Jeong (2005) also reported that the AgNPs of 2–3 nm in diameter have the numerical grading to be 0 for erythema and edema. They included AgNPs with 2–3 nm size was skin-innoxious.

## 4. Conclusions

The silver ions were reduced effectively by gamma irradiation and immobilized on the cotton fabrics by *in situ* synthesis. The AgNPs content deposited on the fabrics was of 1696 mg/kg, when the fabric sample was irradiated in 1.5 mM $AgNO_3$ and 1.0% chitosan solution at the dose of 13.8 kGy at 30 °C. The AgNPs were confirmed by UV–vis spectra and SEM images. The average diameter of AgNPs was determined by XRD pattern to be about 12 nm. The presence of AgNPs on the fabrics was depicted by SEM images.

Antibacterial efficacy of the AgNPs fabrics after washing 1, 5, 10, 20, 30 and 40 cycles was about 99.99% for *S. aureus* and *E. coli*. The AgNPs/cotton fabrics washing from 1 to 40 cycles were innoxious to skin ($k = 0$). These results demonstrate that gamma irradiation of cotton fabrics in the presence of aqueous solution of $AgNO_3$ and chitosan is a promising approach for preparation of stable, safe and efficacious antibacterial fabrics.

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