



PAPER • OPEN ACCESS

Synthesis and study of silver nanoparticles for antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*

To cite this article: Xuan Hoa Vu *et al* 2018 *Adv. Nat. Sci. Nanosci. Nanotechnol.* **9** 025019

View the [article online](#) for updates and enhancements.

You may also like

- [Effect of biosynthesised silver nanoparticles as sterilant on physiological and biochemical characteristics in micropropagation of *Musa sapientum* L.](#)
Siriporn Phongtongpasuk, Thitikamon Liamnimit, Thanyaporn Buakaew et al.
- [Increasing the accumulation of aptamer AS1411 and verapamil conjugated silver nanoparticles in tumor cells to enhance the radiosensitivity of glioma](#)
Jing Zhao, Dongdong Li, Jun Ma et al.
- [Rapid microwave-assisted biosynthesis of chitooligosaccharide coated silver nanoparticles: assessments of antimicrobial activity for paediatric pulp therapy](#)
Subhathira Rajasekaran, Sneha S Rao, Pandurang Appana Dalavi et al.

Synthesis and study of silver nanoparticles for antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*

Xuan Hoa Vu¹, Thi Thanh Tra Duong^{1,2}, Thi Thu Ha Pham¹, Dinh Kha Trinh¹,
Xuan Huong Nguyen¹ and Van-Son Dang²

¹ Thainguyen University of Science, Tan Thinh, Thainguyen, Vietnam

² Hanoi University of Science (VNU), 334 Nguyen Trai, Thanh Xuan district, Hanoi, Vietnam

E-mail: hoavx@tnus.edu.vn and duongthanhtra803@mail.com

Received 22 September 2017

Accepted for publication 5 March 2018

Published 8 June 2018



Abstract

The colloidal silver solution was synthesized by reducing silver nitrate (AgNO_3) using sodium borohydride (NaBH_4) and starch as a stabilizer agent. The size and optical properties of synthesized AgNPs were characterized by UV-Vis spectroscopy, Fourier transform-infrared spectroscopy (FTIR) and transmission electron microscopy (TEM). The effects of several parameters on AgNPs were also investigated. The results have shown that the size of synthesized spherical silver nanoparticles was 8 ± 4 nm and disperse in water. The synthesized AgNPs of this study exhibited a strong antibacterial activity against Gram-negative bacteria *Escherichia coli* (*E. coli*) and Gram-positive *Staphylococcus aureus* (*S. aureus*). The average zones of inhibition of AgNPs were of 7.7 mm for bacteria *E. coli* and 7.0 mm for *S. aureus*. In this study, the zone of inhibition of AgNPs was also compared to the reference antibiotics drug.

Keywords: silver nanoparticle, antibacterial activity, surface plasmon, *Escherichia coli*, *Staphylococcus aureus*

Classification numbers: 2.04, 4.02

1. Introduction

Recently, silver nanoparticles (AgNPs) have attracted significant interest in the field of biomedical applications [1–3]. There are numerous researches on its biological labelling, drug delivery, cancer therapy, antibacterial activity and molecular imaging abilities [2, 4, 5]. Their unique optical properties related to the localized surface plasmon resonance (LSPR) have a great scientific interest [3]. When photons interact with the surface of AgNPs, the outer free electrons in the conduction band form localized plasmons. Surface plasmon resonance (SPR) is a collective excitation of the free electrons near the surface of the nanoparticles [6, 7]. Electrons

are limited to specific vibrations modes by the particle's size and shape, only specific wavelengths of light cause outer electrons to oscillate. When these resonances occur, the intensities of absorption and scattering are significantly higher than those of the same particles without plasmonic properties [8, 9]. The intrinsic properties of metal nanostructures can be tailored by controlling their size, shape, composition, crystallinity and structure depending on the experimental conditions. During the last decade, many synthesis methods have been reported for the preparation of silver nanoparticles (AgNPs). The metal nanoparticles can be prepared by two routes; the first one is a physical approach such as evaporation/condensation or laser ablation which requires the use of a tube furnace consuming a huge amount of energy [10]. The second one is a chemical approach in which the metal ions in solution are reduced. The chemical reduction approach is commonly used to prepare AgNPs because of time saving and cost-effective.



Original content from this work may be used under the terms of the [Creative Commons Attribution 3.0 licence](https://creativecommons.org/licenses/by/3.0/). Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.

There are several reductants commonly being used for the synthesis of the AgNPs such as sodium borohydride, citrate, ascorbate and elemental hydrogen [11]. The choice of reductant might influence the size and shape of synthesized AgNPs, for example, the sodium borohydride reductant is desired to achieve monodispersed and stability AgNPs of smaller than 10 nm. It is also important to use a stabilizing agent or a protective agent to prevent the agglomeration of the dispersive nanoparticles during preparation. Polymers used as stabilizers are poly-vinylpyrrolidone (PVP), poly-ethylene glycol (PEG), poly-methacrylic acid (PMAA), starch and poly-methyl-methacrylate (PMMA). In this study, we used the chemical reduction method with sodium borohydride as the reductant and starch as the stabilizing agent. This simple method is cheap, fast and requires only basic equipment. The method allows the preparation of silver NPs with a different size and distribution. Several synthesis parameters were varied in order to find the optimum protocol allowing the formation of stable AgNPs with the meaning optical properties. The synthesized AgNPs were characterized by ultraviolet-visible (UV-Vis) absorption spectroscopy, transmission electron microscopy (TEM) and Fourier transform infrared spectroscopy (FTIR). The antibacterial effects of AgNPs on *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) were also investigated.

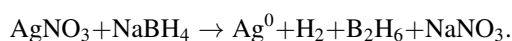
2. Experimental

2.1. Chemicals

All chemicals, supplied by Sigma-Aldrich, were of analytical grade and were used as received without further purification. Silver nitrate (AgNO_3 , 99.8%) was used as the precursor of Ag. Sodium borohydride (NaBH_4) and soluble starch (linear structure $(\text{C}_6\text{H}_{10}\text{O}_5)_n$) were used as the reductant and the stabilizer, respectively. The other solutions were freshly made for the experimental procedures in this work. Deionized water was used in the experiments, and all glassware was cleaned thoroughly by rinsing with ultrapure water prior to use.

2.2. Synthesis of AgNPs

The synthesis of colloidal silver nanoparticles (AgNPs) was performed using NaBH_4 as the reducing agent. First, a 100 ml of a 10^{-3} M solution of AgNO_3 were mixed with various amount of 0.2, 0.5, 1.0 and 2.0 gram of starch to generate 4 different starch solutions containing Ag^+ ions. Second, the solution was stirring vigorously on a magnetic stirrer at 70 °C to ensure that the mixture was homogeneous. While the solution was stable at 70 °C, 5 ml of 2×10^{-3} M sodium borohydrides solution was added gradually to the solution. There acted solutions then cooled to room temperature. The chemical reaction is the sodium borohydride reduction of silver nitrate [12]



The starch containing Ag^+ solutions turned to light yellow after the addition of the sodium borohydrides solution and to

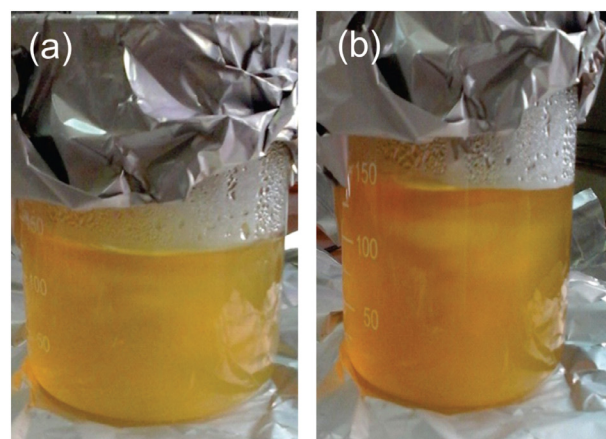


Figure 1. Color of the colloidal AgNPs after (a) 2 min and (b) 5 min of reaction.

brighter yellow when all of the sodium borohydride solution had been added (figure 1). The overall reaction process was carried out in the dark box to avoid unnecessary photochemical reactions [13]. Figures 1(a) and (b) describe the colloidal AgNPs after addition of the NaBH_4 solution within 2 min and 5 min, respectively.

To investigate the formation and property of the AgNPs, there are several reaction parameters were change including; the amount of starch, reaction time, the silver nitrate concentrations and pH environment.

2.2.1. Influence of the amount of starch on the stability of AgNPs. In order to investigate the stability of the AgNPs, a different amount of starch was use as the stabilizer as mentioned above. The samples were denoted as S0.2, S0.5, S1, and S2 for 0.2, 0.5, 1 and 2 g of starch using in the first solution, respectively. The reaction time was secured at 10 min and 70 °C. The samples were then analyzed by UV-Vis spectrophotometry, TEM image and FTIR spectra.

2.2.2. Effects of reaction time on synthesis of AgNPs. In this experiment, the sample prepared by 0.2 g of starch and the temperature of 70 °C was selected, then, the reaction time was change to 1, 3, 5, 10 and 20 min. The five reaction-time-dependence samples were analyzed by the UV-Vis spectrophotometry.

2.2.3. Effects of silver nitrate concentrations on synthesis of AgNPs. To investigate the dependence of the optical density of the colloidal AgNPs on the AgNO_3 concentrations, a series of 4 colloidal AgNPs samples were prepared by the reaction of different concentration of AgNO_3 of 10^{-3} , 2×10^{-3} , 3×10^{-3} and 6×10^{-3} M with the reducing agent solution. The reaction time of the samples was fixed at 10 min.

2.2.4. Effects of pH on the synthesis of AgNPs. To investigate the optical plasmon characteristic of the AgNPs prepared in different pH environment, the AgNPs were synthesis with the same conditions with the above experiments but at pH environment from 1.8 to 9.1 for 10 min reaction time. The pH

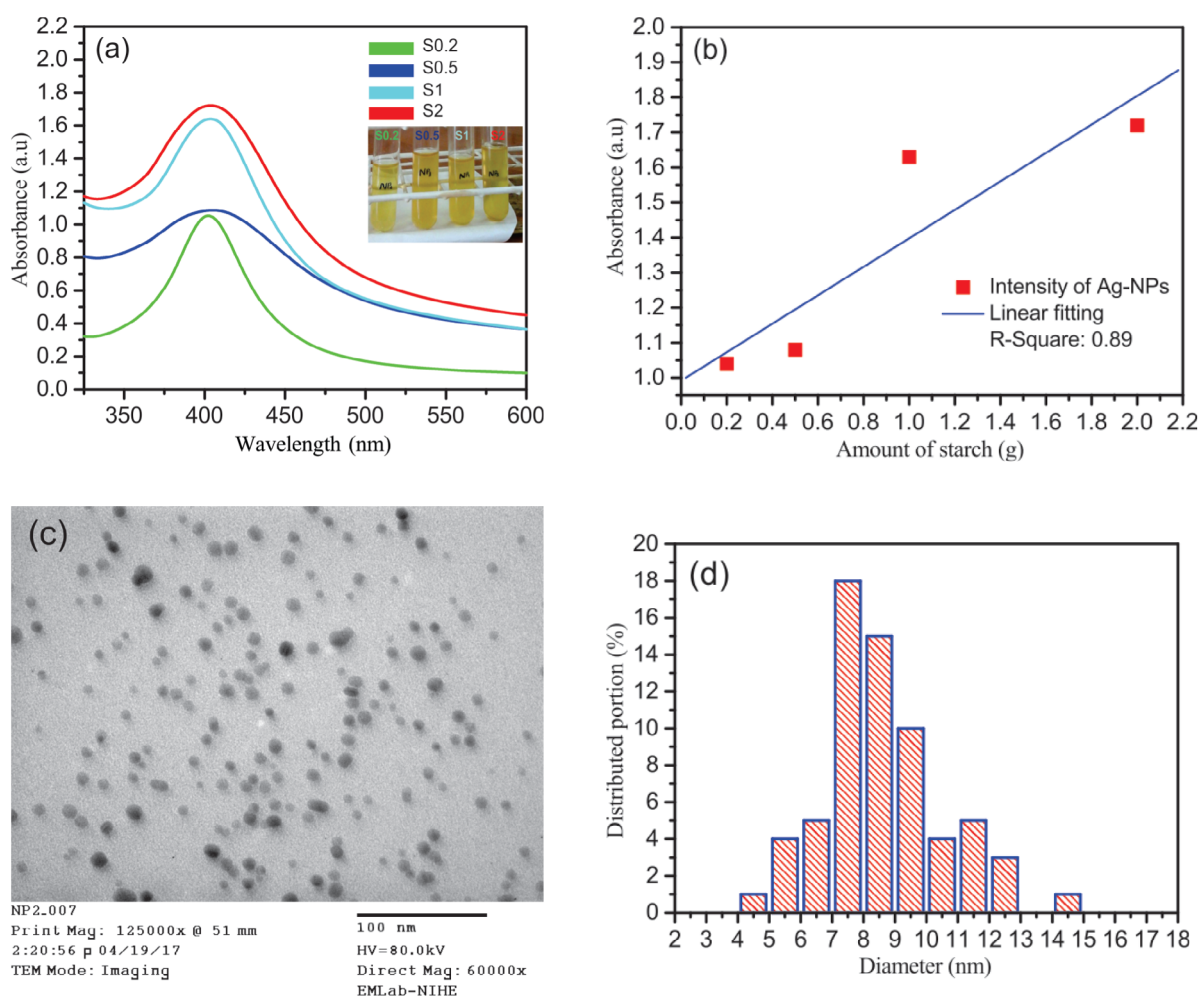


Figure 2. (a) The absorption spectra of the AgNPs solution at different amounts of starch (S0.2, S0.5, S1, and S2) and the inset picture are the images of the samples; (b) the plot of the absorption intensity of the AgNPs versus different amount of starch; (c) the TEM image of the S0.2 sample and (d) the distribution of the AgNPs size as measured from (c).

level was controlled by adding a small amount of acid or base to the Ag^+ in starch solution.

2.3. Characterization of AgNPs

UV-Visible spectroscopy is one of the most widely used techniques for structural characterization of silver nanoparticles. It was monitored at room temperature using a double-beam UV-Vis spectrophotometer (HITACHI-U2900, Japan) at a wavelength of 300–600 nm. The deionized water was used as the blank. The morphological characteristics and the size of AgNPs were performed on JEM-1010 (JEOL) high-resolution transmission electron microscopy (TEM) operated at 80 kV. In order to detect the possible functional groups of the interaction of starch with the synthesized AgNPs, the samples were investigated by FTIR spectra recorded by Spectrum FTIR Affinity—1S (SHIMADZU).

2.4. Antibacterial activity

The antibacterial assays of the AgNPs were assessed by using the Kirby-Bauer method against human pathogenic [14]. The Gram-positive *Staphylococcus aureus* (*S. aureus*) and strains

of Gram-negative *Escherichia coli* (*E. coli*) bacteria were selected to evaluate the antibacterial activity of AgNPs. The microorganisms were provided from the culture collection of Biotechnology of Thai Nguyen University of Science in Vietnam. The identity of the bacterial strains was confirmed morphologically and through using of biochemical techniques. Agar plate punching method is used to evaluate the antimicrobial activity of the sensitized AgNPs. 10 ml of nutrient agar medium was poured into sterile Petridishes (as a basal layer), then 15 ml of seeded medium previously inoculated with bacterial suspension (100 of medium per 1 of 107 CFU) to attain 105 CFU/of the medium. The plates are perforated (10 mm in diameter) as a well loaded with of 100 μl of the nanoparticle solution. After incubator at 37 °C for 24 h, the zone of inhibition was recorded and measured.

3. Results and discussion

3.1. Influence of the amount of starch on the stability of AgNPs

In this study, the starch is used as the capping agent for AgNPs. Figure 2(a) shows the UV-Vis absorption spectra of

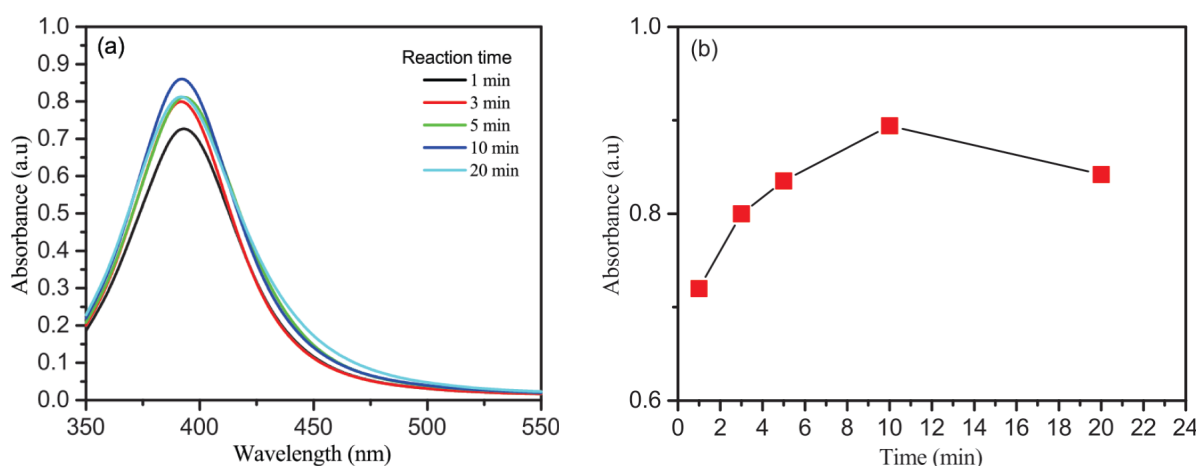


Figure 3. UV-Vis spectra of the AgNPs solutions in experiment recorded as a function of reaction time: (a) the absorption spectra corresponding to the different time of reaction; (b) the absorption intensity versus reaction time.

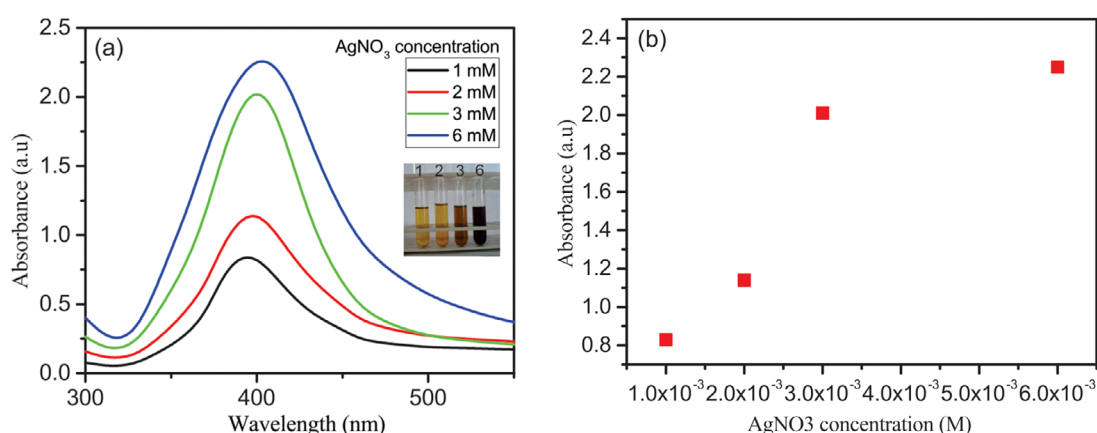


Figure 4. (a) The UV-Vis spectra of the AgNPs fabricated by different AgNO_3 concentrations; (b) the plot of the absorption intensity versus AgNO_3 concentration.

the AgNPs solutions which were capped with various amount of starch of 0.2, 0.5, 1 and 2 g defined as S0.2, S0.5, S1, and S2, respectively. The spectrum revealed characteristics of silver Surface plasmon resonance (SPR) located in between 405–413 nm and the spherical AgNPs. The absorption spectrum indicates that these AgNPs have a small size and regular distribution. The absorption peaks increase with increasing the starch concentration and slightly blue shift (S0.2 at 413, S0.5 at 410, S1 at 408 and S2 at 405 nm) as shown in figure 2(b). This result could be explained that an increase of starch concentration leads to an increase in the number of the silver nanoparticles to be capped. Figure 2(c) describes TEM image of the S0.2 sample, the TEM image reveals a spherical and uniform AgNPs. The image shows clearly monodisperse AgNPs with the diameter distributed around 8 ± 4 nm as seen in figure 2(d).

3.2. Effects of reaction time on synthesis of the AgNPs

Figure 3(a) presents the UV-VIS spectra of the colloidal AgNPs solutions in which the samples were synthesized at different reaction time from 1 to 20 min and capped by 0.2 g starch. The Surface Plasmon resonance intensity which is at

392.8 nm increases slightly for the reaction time from 1 to 10 min and stays quite stable at 5 min of the reaction time (green line) with the value of the absorbance intensity of about 0.8 (a.u). But when the reaction time increases to 20 min, the intensity of the absorption peak decreases. The dependence of the reaction time and the intensity of absorption peaks of the AgNPs solution is plotted in figure 3(b). It might reveal that the reaction time to synthesize the stable AgNPs was around 10 min.

3.3. Effects of silver nitrate concentrations on the synthesis of AgNPs

The UV-Vis absorption spectra of the samples synthesized by various AgNO_3 concentration are shown in figure 4. The absorption intensity increases quasi linearly with an increasing of AgNO_3 concentration during the reaction (figure 4(a)). All the solutions exhibited characteristic of the AgNPs surface Plasmon resonance (SPR) typically located in between 393–403 nm. This is characteristic of silver nanoparticles [14]. The intensity of SPR absorption peak increase with the increasing of the AgNO_3 concentration before reacting and slightly red shift. The concentration of the AgNO_3 solution was 1, 2, 3,

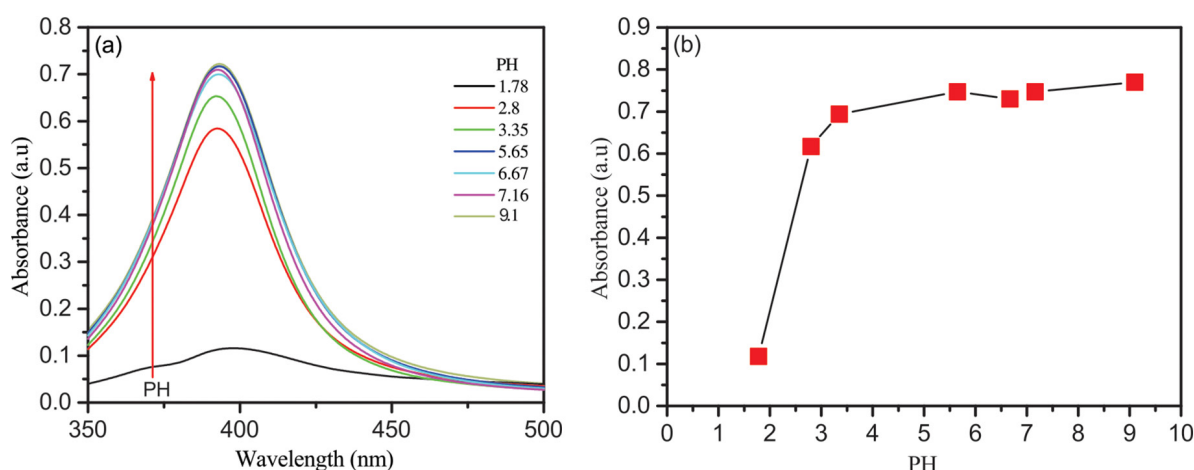


Figure 5. (a) UV-Vis absorbance spectra of silver nanoparticles for the samples prepared in different pH-values; (b) UV-Vis absorbance intensities at the plasmon peak (maximum absorbance) of silver nanoparticles for the samples prepared in different pH-values.

6 mM and the absorption peaks of the samples synthesized by those solution were 393, 397, 400, 403 nm, respectively. It means that the size of AgNPs made of from those solutions was increasing and those solutions color changes from brighter yellow to red-orange. These results show that the absorption of the AgNPs solutions is directly proportional to the AgNO_3 concentration in the range of optical density < 2.5 (see figure 4(b)).

3.4. Effects of pH on the synthesis of AgNPs

We also investigated the influence of pH environment on the AgNPs solution which was prepared in experimental section 2.2.4. Because the pH of the solution is a critical parameter influencing the properties of the synthesized Ag NPs [15–17]. Figure 5(a) shows the absorption spectra at 393 nm of the AgNPs solutions prepared in different pH value from 1.78 to 7.16 during the reaction time. Figure 5(b) shows the evolution of the plasmon peak intensities as a function of pH-values of which the samples were synthesized. A very low Plasmon peak intensity for the samples prepared in the pH level of 1.78 is observed. Then, the absorbance intensity increases rapidly with the increase of the pH value and reaches a plateau for the pH value which is greater than 3. The disappearance of the surface plasmon absorption peak from the spectra of the sample prepared the pH level of 1.78 could correspond to an aggregation of AgNPs [18]. We might conclude that an almost instantaneous aggregation of silver NPs appears for the sample prepared in the pH value of around 2. The colors of the samples could also give information about the AgNPs properties. AgNPs are not stable at very low pH and it is seen that the colloidal silver solutions prepared by this method are stable in the base environment.

3.5. Fourier transforms infrared (FTIR) spectroscopy

FTIR spectroscopy is used to analyze the chemical structure and determine the function group of the colloidal silver nanoparticles. The FTIR spectra of pure starch and AgNPs (sample S0.2) are shown in figure 6. From this FTIR spectrum

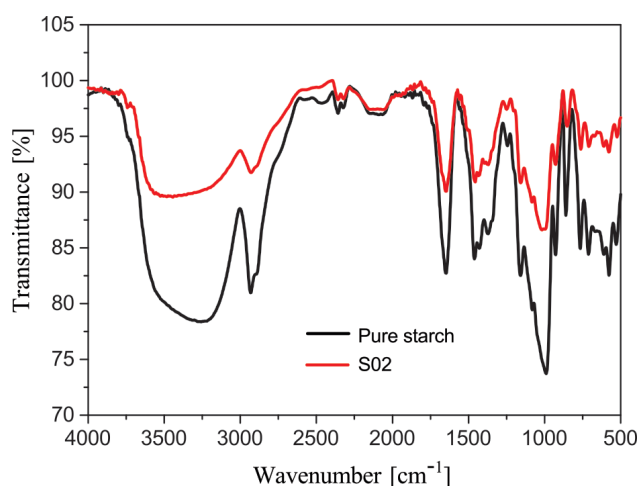


Figure 6. FTIR spectra of pure starch and ST0.2 sample.

of starch, the wavenumber at 3221 cm^{-1} is typical absorption bands of OH stretching. The different wavenumber and their identification are listed as: 1647 cm^{-1} (OH bending of water), 1159 cm^{-1} (C-O stretching), 1082 cm^{-1} (C-O-C symmetrical stretching), 989 cm^{-1} (COH bending) [19, 20]. From the FTIR spectrum of S0.2, we can see that it exhibits a similar pattern to that of pure starch. However, a shift in frequencies is detected for the signals associated with the OH functional group, a noticeable wavenumber at 3506 cm^{-1} (OH stretching) and 997 cm^{-1} (COH bending) and 1157 cm^{-1} (C-O stretching) evidently indicate the interaction of OH groups with AgNPs [21].

3.6. Antibacterial activity of AgNPs

Figure 7 presents the synthesized AgNPs in experiment 2.4. The samples exhibit an effective antibacterial activity against *E. coli* and *S. aureus*. The diameter of the inhibition zones is shown in comparison with silver ion ($100 \mu\text{l}$ of 10^{-3} M of silver nitrate solution) in figure 7 and table 1. The inhibition zones of 9 and 10 mm in *E. coli* were larger for samples S0.2 and S1, respectively, than 7 and 5 mm for samples S0.5 and S2, respectively. And in *S. aureus* the inhibition zones of 12, 11, 3 and 2 mm for the samples S0.5, S0.2, S2 and

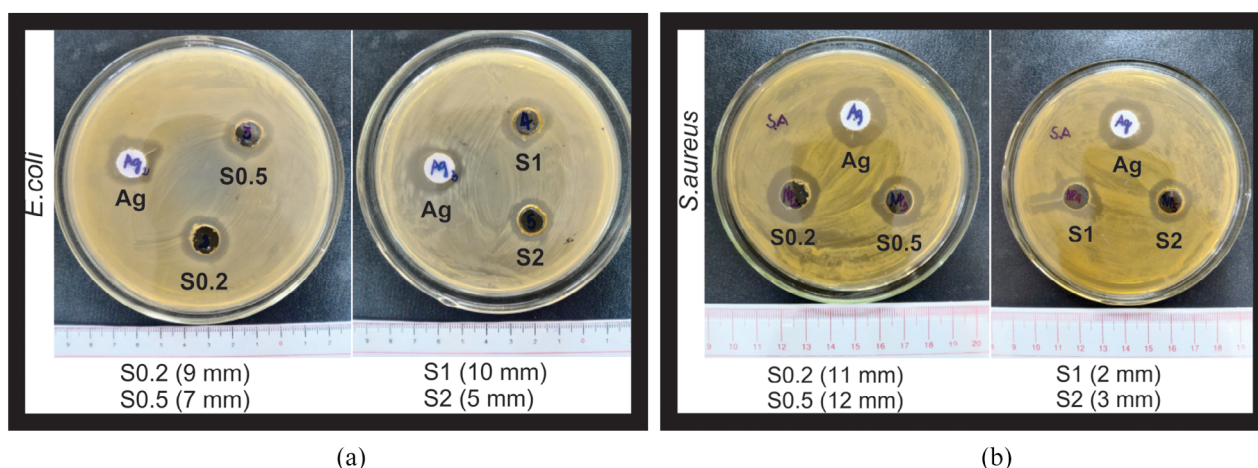


Figure 7. Antibacterial activity of AgNPs against *E. coli* (a) and *S. aureus* (b).

Table 1. Antibacterial activity of AgNPs against *E. coli* and *S. aureus*.

Sample	Zone of inhibition (mm)			
	S0.2	S0.5	S1	S2
<i>E. coli</i>	9	7	10	5
<i>S. aureus</i>	11	12	2	3

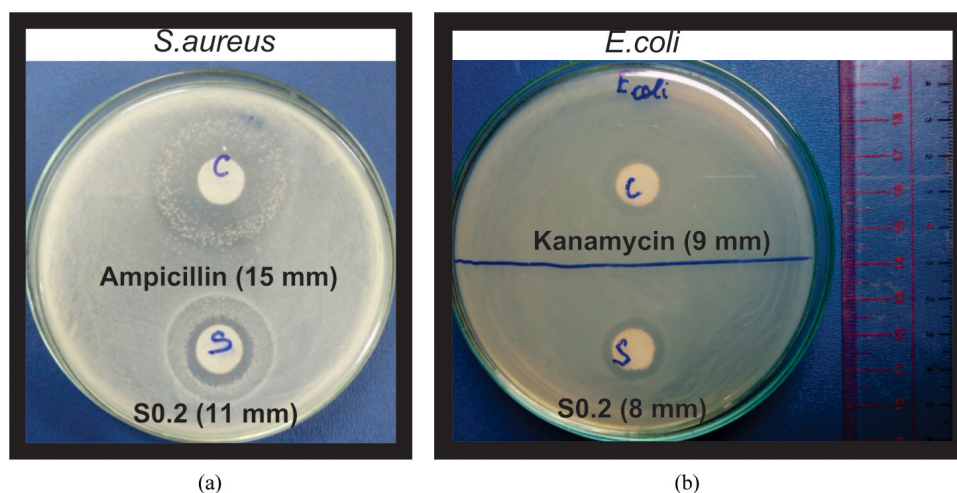


Figure 8. Zone of inhibition of AgNPs was compared against the reference antibiotics drug: ampicillin for bacterial *S. aureus* (a) and kanamycin for bacterial *E. coli* (b).

S1, respectively are listed in table 1. It was clearly that the inhibition zones of silver ion were qualitatively similar to the inhibition zones of AgNPs in *E. coli* bacteria and larger than that of the inhibition zones of AgNPs in *S. aureus* case. This means that it is difficult to fully explain the observed toxicity of the AgNPs suspension and initial silver ions. Kim et al suggested that the toxicity of AgNPs is mainly due to oxidative stress and independent of silver ions [22]. However, it is not clear to which degree the toxicity of AgNPs results from released silver ions and how much toxicity is related to the AgNPs per second. The mechanism of the growth inhibition of microorganisms by the AgNPs has been suggested that AgNPs penetrate the cell walls of the bacteria, causing

deterioration in the plasma membrane, which leads to bacterial cell death [23].

We also investigate the zone of inhibition of AgNPs was compared against the reference antibiotics drug (see figure 8). The present study clearly indicates that the stabilized AgNPs has excellent antimicrobial activity against gram positive organism of *S. aureus* and gram negative organism of *E. coli*. In the case of *E. coli*, the result shows that the antibacterial activity of the 20 μ l (M) of AgNPs is equal to approximated 89% of the antibacterial activity of the 5 mg of kanamycin antibiotics. For a case of *S. aureus*, ampicillin antibiotics used to compare and the result shows an equal of 73.3% of the antibacterial activity of the 2 mg of ampicillin.

4. Conclusion

In conclusion, we have demonstrated that the method of reduction Ag^+ to Ag^0 by sodium borohydride as the reaction agent and the starch as stability agent was used for the synthesis of spherical shape and diameters average of 8 nm AgNPs. Our method showed that the synthesized AgNPs exhibited a good antibacterial activity against Gram-positive pathogenic bacteria (*S. aureus*) and Gram-negative bacteria *E. coli*. So, this AgNPs can be applied as antibacterial coatings for many foods production.

Acknowledgment

Author Van Son Dang would like to acknowledge National Foundation for Science and Technology Development (NAFOSTED) for financial support under the grant number: 103.99-2015.81.

References

- [1] Tran H N *et al* 2015 *Adv. Nat. Sci.* **6** 023002
- [2] Kumari J, Mamta B and Ajeet S 2016 *J. Radiat. Res. Appl. Sci.* **9** 217–27
- [3] Haes A J and Van Duyne R P 2004 *Expert Rev. Mol. Diagn.* **4** 527–37
- [4] Gownolla M R, Tippabattini J, Kokkarachedu V, Rotimi S, Sinha Ray S and Konduru M R 2013 *Carbohydrate Polym.* **93** 553–60
- [5] Mlalila N G, Hulda S S, Askwar H and Dattatreya M K 2017 *Nanotechnol. Sci. Appl.* **10** 1–9
- [6] Anambiga V, Suganthan V and Arunai Nambi Raj N 2014 *Int. J. Sci. Eng. Res.* **5** 3
- [7] Jawaad R S, Sultan K F and Al-Hamadani A H 2014 *ARPN J. Eng. Appl. Sci.* **9** 4
- [8] Fan X, Zheng W and Singh D J 2014 *Light* **3** e179
- [9] Pris M 2014 Influence of different parameters on wet synthesis of silver nanoparticles *Bachelor Thesis* University of Twente
- [10] Abou El-Nour K M M, Eftaiha A, Al-Warthan A and Ammar R A A 2010 *Arab. J. Chem.* **3** 135–40
- [11] Khodashenas B and Ghorbani H R 2015 *Arab. J. Chem.* **48** 385002
- [12] Solomon S D, Bahadory M, Jeyarajasingam A V, Rutkowsky S A and Boritz C 2007 *J. Chem. Educ.* **84** 322–5
- [13] Le Ouay B and Stellacci F 2015 *Nano Today* **10** 339–54
- [14] Cormican M, Wilke G, Barrett W, Pfaller M S and Jones M A 1996 *Diagn. Microbiol. Infect. Dis.* **25** 107e112
- [15] Alqadi M K, Abo Noqtah O A, Alzoubi A Y, Alzoubi J and Aljarrah K 2014 *Mater. Sci.* **32** 1
- [16] Seitz F, Rosenfeldt R R, Storm K, Metreveli G, Schaumann G E, Schulz R and Bundschuh M 2015 *Ecotoxicol. Environ. Saf.* **111** 263–70
- [17] Singha M, Sinha I and Mandal R K 2009 *Mater. Lett.* **63** 425–7
- [18] Sivera M *et al* 2014 *Plos One* **9** 8
- [19] Subramanian S B, Francis A P and Devasena T 2014 *Carbohydr. Polym.* **114** 170–8
- [20] Mano J F, Koniarova D and Reis R L 2003 *J. Mater. Sci., Mater. Med.* **14** 127–35
- [21] Kumar B, Smita K, Cumbal L, Debut A and Pathak R N 2014 *Bioinorg. Chem. Appl.* **2014** 784268
- [22] Kim S, Choi J E, Choi J, Chung K H, Park K, Yi J and Ryu D Y 2009 *Toxicol. In Vitro* **23** 1076–84
- [23] Raghavendra G M, Jayaramudu T, Varaprasad K, Mohan Reddy G S and Raju K M 2015 *RSC Adv.* **5** 14351–8