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Preparation and Properties of Silver Nanoparticles by Heat-combined Electrochemical Method

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Abstract: Silver nanoparticles colloid has been prepared by heat-combined electrochemical method, which uses simple and low-cost equipment of easy installation and operation, is easily deployed in industrial scale. A silver plate was used as the cathode instead of silver salts to avoid unexpected ions from the salts. The cathode was made from a stainless steel plate. The chemical used as the electrolyte solution is TriSodium Citrate (TSC). Silver nanoparticles made by the above method are spherical, small-sized, its size distribution ranges from 3-12nm, stably dispersing in a non-toxic solution.

Bactericidal capacity of silver nanoparticles are tested on 4 types of common bacteria and fungi. The results showed that silver nanoparticles with a concentration of 10 ppm have a good bactericidal capacity against the said bacteria. This result shows that silver nanoparticles made by heat-combined electrochemical method can be used in antibacterial applications.

Keywords: Silvernanoparticle, electrolysis, TriSodium Citrate, antibacterial.

1. Introduction

Thanks to their good antibacterial properties, high electrical conductivity and special optical properties, silver nanoparticles are applied in many different areas such as biomedicine [1], textile [2], water and air treatment [3], food [4] and cosmetics [5]. At nanometer size, silver nanoparticles have large surface area, they are highly active materials. The bactericidal ability of silver nanoparticles is explained based on the high affinity of silver over sulfur and phosphorus. Silver nanoparticles link with sulfur atoms greatly available in cell membranes, altering the functions of the cell membranes [6]. There is also the theory that silver ions released from silver nanoparticles can interact with phosphorus in DNA and prevent the division of DNA or affect the sulfur atoms of protein, impeding the function of the enzyme [7].

Today, there are many methods to make silver nanoparticles with different sizes and shapes such as laser irradiation [8], gamma irradiation [9], redox [10], microwave exposure [11], hydrothermal

[12], electrochemical [13] and sonoelectrochemical methods [14]. Among these methods, the electrochemical method has a great advantage, it creates silver nanoparticles dispersed in a solution with high purity. Electrochemical method was first used by Reetz and Helbig to fabricate nanoparticles [15], in which a metal plate was used as the positive electrode and metal ions were reduced at the surface of the negative electrode to form metal nanoparticles. This method was then applied to produce silver nanoparticles [16] using the chemicals H_2SO_4, tetrabutylammoniumbromide (TBABr), acetate (TBAAcO) and silver electrodes. The electrochemical process is controlled with an electrochemical system named Autolab PGSTAT 20. Ultrasound can also be used in the electrochemical process to produce silver nanoparticles [14], in which ultrasound is used to dislodge silver nanoparticles from electrode surfaces. However, this method has a disadvantage that expensive silver salt must be used, making toxic ions such as NO_3^- or $S_2O_3^{2-}$ present in its products. For electrolysis using silver electrodes, a complex control systems must be used, it can manufacture at laboratory scale only.

This article presents a method to manufacture silver nanoparticles using electrochemical combined with heat, with simple and inexpensive equipment, easy to deploy at industrial scale. Its products are silver nanoparticles stably dispersed in a non-toxic solution, easy to control the size, with high bactericidal nature.

2. Experiment

Silver nanoparticles have been prepared by heat-combined electrochemical method, with positive electrode was made of silver, negative electrode was made of stainless steel, the surface area of both electrodes is 10x10mm^2. TriSodium Citrate (TSC) has a 99% purity purchased from Bio Basic Chemical Company. Electrochemical system diagram is shown in Figure 1. The distance between the two electrodes is 20 mm. Electrolyte solution contains 300 mg of TSC mixed with 100 ml of distilled water in a 200 ml beaker. The surface of negative electrode is continuously swept with a brush rotating at a speed of 60 rpm. Electrolysis duration is 60 minutes, at this time the solution is milky white (solution A). Current density is adjusted from 10 to 30 mA. The concentration of TSC is changed from 4 to 6 g/l. After that, the electrolyte solution was boiled for 10 minutes to obtain a solution with typical yellow color of silver nanoparticles solution (solution B).

Bactericidal capacity of silver nanoparticles is tested on the bacterium *Salmonella typhimutium* ATCC 14028 causing food poisoning, the streptococcus *Enterococcus faecalis* ATCC 29212 thriving on burns, the fungus Ca*ndida albicans* ATCC 26790 causing gynaecological infection in humans and the fungus *Aspergillus niger* ATCC 16888 causing the syndrome of opportunistic infection in humans.

The strains of the bacteria Salmonella typhimutium and E. faecalis are tested at respective concentrations of $3.9x10^5$ CFU/ml and $2.4x10^5$ CFU/ml, the fungus Candida albicans at a concentration of $5.8x10^5$ CFU/ml and the fungus Aspergillus niger at a concentration of $4.2x10^4$ CFU/ml. These bacterial strains are preserved, proliferative in culture medium for 24 hours and then diluted to achieve the concentration of 10^6 CFU/ml (using the Mc Farland method [17]). The silver nanoparticles solution is diluted to a concentration of 10 ppm. Add 1 ml of bacteria with a concentration of 10^6 CFU/ml to the test tube containing 9 ml of silver nanoparticles solution with a

concentration of 10 ppm, with exposure time of 24 hours. The negative control sample uses physiological brine of 0.9% concentration. After 24 hours of exposure, check the number of alive bacteria in the sample exposed with the culture method spreading on the surface. Suck 0.25 ml of silver nanoparticles solution which has been exposed to micro-organisms in each sample spreading on sterilized plastic petri dishes, of 90 mm diameter, containing 20 ml of culture medium *(Salmonella typhi* using Hektoen agar medium; *E.faecalis* using BEA (Bile Esculine Agar); *Candida albicans* using DRBC (Dichloran Rose Bengal Chlortetracycline); *Asperfillus niger* using DRBC (Dichloran Rose Bengal Chlortetracycline), carry out the culture for each type of bacteria in one plate. Repeat the above steps with silver nanoparticles solution samples exposed to microorganisms and diluted for 10 times and 100 times to find the solution concentration for the density of colonies that can be counted with a counter. Similarly perform with the control sample.

Incubate the plates at 37 °C ± 1 °C for 24-48 hours for the bacteria and 72 hours for the fungi. The number of bacteria and fungi on the surface of agar plates after incubation is determined by a Interscience Automatic Colony Counter (France), determining the number of alive bacteria in the sample A and alive bacteria in the control sample B then calculating the percentage of killed bacteria by the formula (B-A)*100/B (%).

The structure of materials was examined by X-ray diffractometer (XRD) D5005, Bruker, using Cu Ka radiation. Transmission electron microscope (TEM) measurements were carried out using a JEM-1200EX TEM instrument working at an accelerating voltage of 80 kV. UV-vis spectra of the samples were acquired in a UV-Vis Specord 200 spectrophotometer (Analytik Jena, Germany) between 200 and 900 nm in a quartz cell of 5 mm path length. Centrifugation was carried out by a Hettich Universal 320, 9000 rpm, 20 min. The concentration of silver in the solution was determined by a Shimadu AA-6800F atomic absorption spectroscopy (AAS). Zeta potential was recorded by using a Zeta phoremeter IV-CAD Instrumentation at 40 °C.

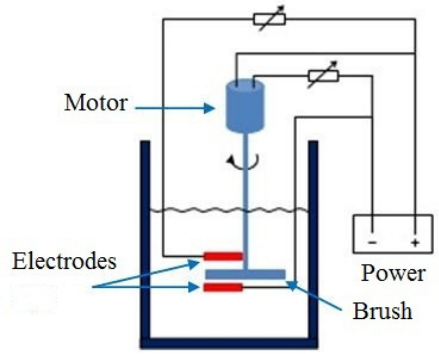

Fig. 1. Diagram of electrochemical system.

3. Results and discussion

X-ray diffraction spectra of silver nanoparticles (I = 30 mA, c = 6 g/l) is shown in Fig. 2. The diagram presents three diffraction peaks at the angle 2θ is 38.11°; 44.29° and 64.41°, which match

well with the diffraction from the (111), (200) and (220) planes, respectively. The results show that the diffraction peaks coincide with the positions of the standard peaks of the silver particles (JCPDS standard card No. 04-0783) with face-centered cubic structure.

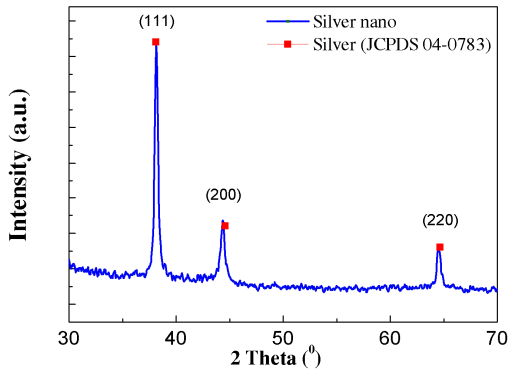

Fig. 2. X-ray diffraction spectra of silver nanoparticles.

Fig. 3 presents the TEM images and size distribution of silver nanoparticles (I=15 mA) as a function of TSC concentration. Figures 3a, 3b, 3c show the TEM image of silver nanoparticles made with TSC concentration of 4 g/l, 5 g/l and 6 g/l, respectively. TEM image of silver nanoparticles shows that silver nanoparticles are spherical and the increase of TSC concentration leads to the increase of the density and size of silver nanoparticles in the solution. Figure 3d shows the size distribution diagram of the silver nanoparticles samples with TEM image shown in Figures 3a, 3b and 3c. The results show that the size of these particles mainly rises from 3 to 5 nm when the TSC concentration rises from 4 to 6 g/l.

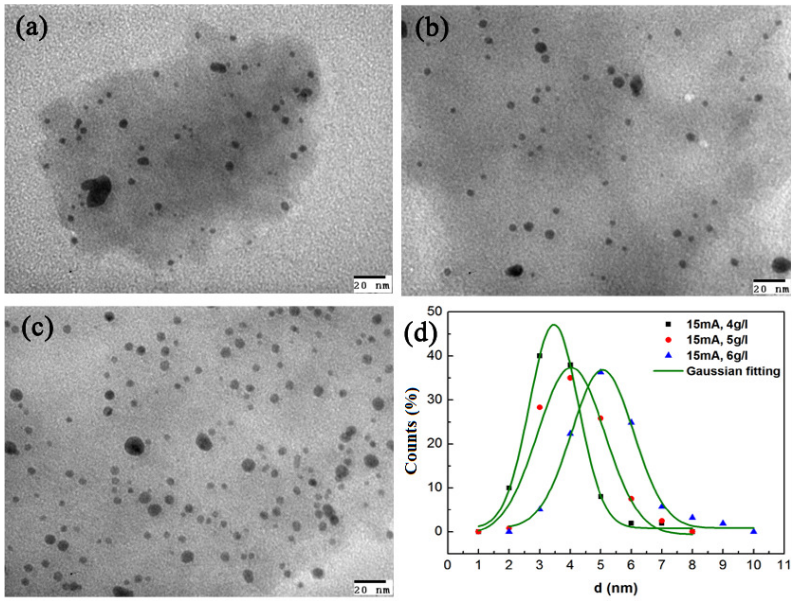

Fig. 3. TEM images of silver nanoparticles (I = 15 mA): c = 4 g/l (a); c = 5 g/l (b); c = 6 g/l (c) and size distribution diagram of silver nanoparticles (d).

Silver nanoparticles are formed in the following mechanism: During the process of electrolysis, TSC serves as a conductor in the solution, the silver ions are released from the positive electrodes by the equation $Ag^{\circ} - e^{-} = Ag^{+}$. These silver ions move toward the negative electrode.

Only a few of these ions are reduced to zero-valent Ag atoms on the cathode by the equation $Ag^{+} + e^{-} \rightarrow Ag^{\circ}$; silver nanoparticles are formed via nucleation and growth due to attractive van der Waals forces between Ag atoms [18]. Then, synthesised silver nanoparticles are separated from the cathode by the brush. Fig. 4 presents the TEM images of electrolyte solution before boiling. Fig. 4 shows that there are only some silver nanoparticles exist in the electrolyte solution before boiling.

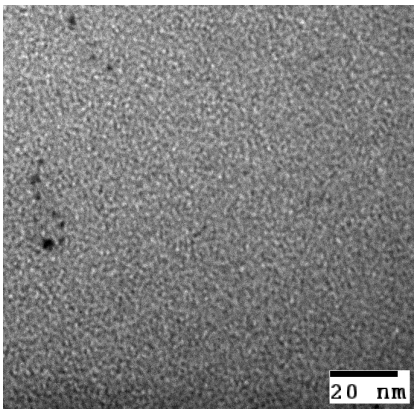

Fig. 4. TEM images of electrolyte solution before boiling.

Most of these silver ions are dispersed by the brush into the milky white solution A. When boiling this solution, TSC reduces silver ions Ag^{+} to silver atoms Ag°. In this process, the reaction can be expressed as follows [19]:

$$4Ag^{+} + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^{\circ} + C_6H_5O_7H_3 + 3Na^{+} + H^{+} + O_2$$

The produced Ag atoms then acted as nucleation centres and grow to become silver nanoparticles stably dispersed in the solution B. In this process, TSC acts as surfactant agent that can modify the surface of silver particles and prevents the growth of the particles and inhibit the aggregation of silver nanoparticles. The higher the TSC concentration is, the more Ag^{+} ions are reduced to become Ag° atoms. The greater the concentration of Ag° in solution is, the more easily Ag° atoms combine together, leading to the greater size and greater density of the particles.

The effect of pH on the zeta potential of silver nanoparticles was also investigated (Figure 5). At natural conditions (pH = 9.1), the zeta potential was equal to -63.8 mV. In the colloid solution, there exist citrate ions adsorbed on the surface of Ag nanoparticles with the result that the surface charge of the Ag nanoparticles will be negative. The magnitude of the zeta potential is predictive of the colloidal stability. Nanoparticles with Zeta Potential values greater than +30 mV or less than -30 mV typically have high degrees of stability. Dispersions with a low zeta potential value will eventually aggregate due to van der Waal inter-particle attractions. We can conclude that the silver nanoparticles got a negative zeta potential and the isoelectric point is above pH = 2. At pH > 4, particles are fairly stable due to the electrostatic repulsion. On the other hand, in acidic solutions (pH < 4), low negative values of zeta potential clearly indicate instability of the aggregates.

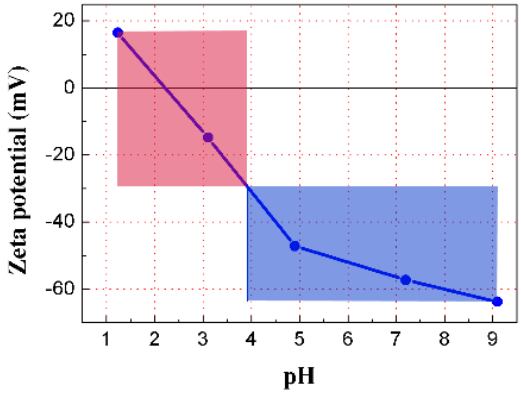

Fig. 5. Zeta potential of silver nanoparticles at different pH values.

The dependence of the wavelength of absorption peak (λ_{max}) of silver nanoparticles solution on the parameters of the electrolysis process is shown in Figure 6. Figure 6a describes the dependence of λ_{max} of silver nanoparticles solution on TSC concentration of the electrolyte solution. Figure 6a shows that λ_{max} tend to be shifted toward the long wavelength as TSC concentration rises from 4 to 6 g/l. The dependence of λ_{max} on the particle size is described by Mie theory [20], in which the λ_{max} shifts towards the long wavelength as the particle size rises. Comparing the obtained results with Mie theory, we see that the particle size rises as TSC concentration rises, which is completely consistent with the above result of TEM image analysis.

The particle size can also be controlled by changing the current density. Figure 6b shows the dependence of λ_{max} on the current density of the electrolysis process. The result shown in Figure 6b shows that as the current density gradually increases, λ_{max} shifts towards the long wavelength, i. e. the particle size rises. This can be explained that with the same electrolysis time, as the current density increases, the quantity of Ag^+ ion in the solution also rises, boiling the solution, TSC reduces more Ag^+ ions to become Ag° atoms, thus the particles develop faster, increasing the particle size.

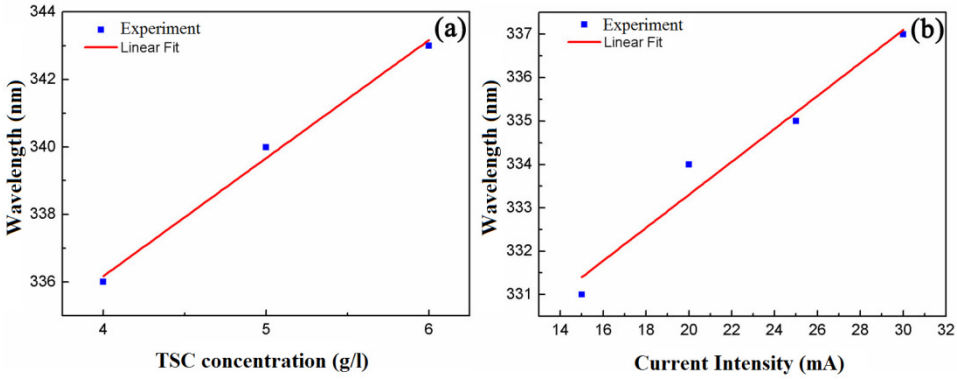

Fig. 6. Dependence of the *wavelength* of *maximum absorbance* of silver nanoparticles solution on TSC concentration (a) and current density (b).

The wavelength of absorption peak (λ_{max}) and the intensity of absorption peak (I_{max}) of silver nanoparticles solution also depends on the sample boiling time. This dependence is shown in Figure 7. Figure 7a describes the change of λ_{max} by the sample boiling time. As the sample boiling time is increased, λ_{max} also rises and reaches a stable value of 358 nm from the 8th minute onwards. Figure 7b shows the dependence of I_{max} by the boiling time. I_{max} also rises as the boiling time rises and reaches a stable value after the 8thminute. This is because when the boiling time is increased, TSC continues reducing Ag^+ ions to Ag° atoms. Silver nanoparticles continue to grow in size and quantity, leads to rise of λ_{max} and I_{max}. After 8 minutes, all Ag^+ ions in the solution are reduced to Ag° atoms, the solution runs out of material for silver nanoparticles to develop or form new particles, so λ_{max} and I_{max} reach stable values.

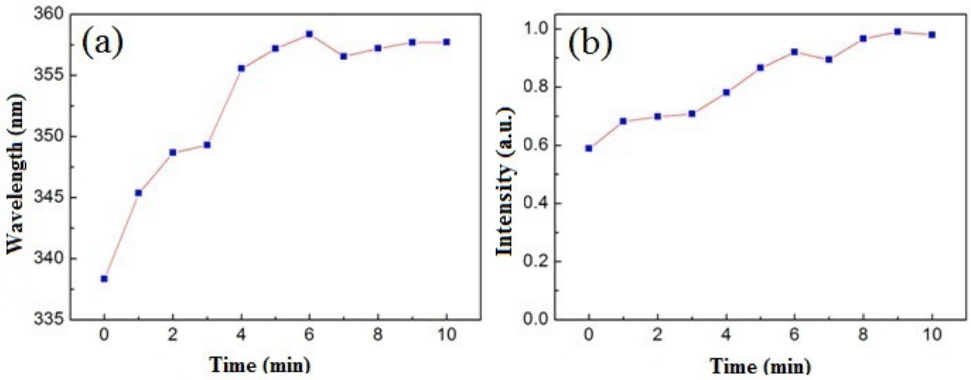

Fig. 7. Dependence of the wavelengths and intensities of absorption peaks of silver nanoparticles solution on the sample boiling time: a) the wavelengths; b) the intensities.

The bactericidal capacity experiment results of silver nanoparticles are shown in Table 1. The experiment results show that after being exposed for 24 hours, silver nanoparticles can completely destroy the bacterial strains *S. typhi* and *E. faecalis*. For the two types of fungi *Candida albicans* and *Aspergillus niger*, the bactericidal rates are 99.8% and 93.8%, respectively. Silver nanoparticles kill bacteria in many ways: silver ions released will interact with SH functional group of systeine by replacing the positions of H atoms to form AGS links, changing the function of enzymes and inhibiting the growth of bacteria [21]; silver nanoparticles can penetrate through cell membranes, including positive-gram and negative-gram bacteria, preventing the respiration of cells and destroying the structure of the cell membranes [22]. A similar mechanism occurs for the fungicide, silver nanoparticles destroy the structure of cell membranes and prevent the branch development of fungi [23]. The results show that the antibacterial ratio of silver nanoparticles solution against fungi is lower than that against bacteria. This is because the antibacterial mechanisms of silver particles for bacteria and fungi are similar, but the fungi have spores, they are more difficult to be acted than the bacteria.

The bactericidal capacity of silver nanoparticles is also related to the particle size: the smaller the particle size is, the greater the ratio of surface area over particle volume becomes, increasing the number of surface atoms, releasing more silver ions, increasing the bactericidal capacity of the silver particles. Moreover, the smaller the particle size is, the more easily the silver particles penetrate into

the cells [24]. Silver nanoparticles made with the above method have a very small size, so they show strong bactericidal capacity only at a concentration of 10 ppm.

Table 1. Bactericidal capacity experiment results of silver nanoparticles on microbial samples

Testedmicrobes		After 24 hours of exposure	
		Alive bacteria (CFU/ml)	Bactericidal rate (%)
S. typhi	$3.9x10^5$ CFU/ml	<1	99.9
E. faecalis	$2.4x10^5$ CFU/ml	<1	99.9
C. albicans	$5.8x10^5$ CFU/ml	1100	99.8
A. niger	$4.2x10^4$ CFU/ml	2600	93.8

4. Conclusion

Silver nanoparticles solution is made with by heat-combined electrochemical method using simple and inexpensive equipment, easy to deploy at industrial scale. The obtained products are spherical silver nanoparticles with sizes ranging from 2 nm to 12 nm, a concentration of 90 ppm, stably dispersed in non-toxic and environmentally friendly solution, showing a high bactericidal nature against four types of common bacteria and fungi.

References

[1] L. Karthik, G. Kumar, A. V. Kirthi, A. Rahuman, and K. B. Rao, Streptomyces sp. LK3 mediated synthesis of silver nanoparticles and its biomedical application. Bioprocess and biosystems engineering, 2014, 37(2), 261-267.

[2] L. Budama, B. A. Çakır, Ö. Topel, and N. Hoda, A new strategy for producing antibacterial textile surfaces using silver nanoparticles. Chemical Engineering Journal, 2013, 228, 489-495.

[3] D. Ren and J. A. Smith, Retention and Transport of Silver Nanoparticles in a Ceramic Porous Medium Used for Point-of-Use Water Treatment. Environmental science & technology, 2013, 47(8), 3825-3832.

[4] P. Kanmani and S. T. Lim, Synthesis and structural characterization of silver nanoparticles using bacterial exopolysaccharide and its antimicrobial activity against food and multidrug resistant pathogens. Process Biochemistry, 2013, 48(7), 1099-1106.

[5] S. Kokura, O. Handa, T. Takagi, T. Ishikawa, Y. Naito, and T. Yoshikawa, Silver nanoparticles as a safe preservative for use in cosmetics. Nanomedicine: Nanotechnology, Biology and Medicine, 2010, 6(4), 570-574.

[6] S. Pal, Y. K. Tak, and J. M. Song, Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia coli. Applied and environmental microbiology, 2007, 73(6), 1712-1720.

[7] V. K. Sharma, R. A. Yngard, and Y. Lin, Silver nanoparticles: green synthesis and their antimicrobial activities. Advances in colloid and interface science, 2009, 145(1), 83-96.

[8] G. M. Herrera, A. C. Padilla, and S. P. Hernandez-Rivera, Surface Enhanced Raman Scattering (SERS) Studies of Gold and Silver Nanoparticles Prepared by Laser Ablation. Nanomaterials, 2013, 3(1), 158-172.

[9] S. Wang, Y. Zhang, H. -L. Ma, Q. Zhang, W. Xu, J. Peng, J. Li, Z. -Z. Yu, and M. Zhai, Ionic-liquid-assisted facile synthesis of silver nanoparticle-reduced graphene oxide hybrids by gamma irradiation. Carbon, 2013, 55, 245-252.

[10] Z. Jia, H. Sun, and Q. Gu, Preparation of Ag nanoparticles with triethanolamine as reducing agent and their antibacterial property. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2013, 419, 174-179.

[11] X. Zhao, Y. Xia, Q. Li, X. Ma, F. Quan, C. Geng, and Z. Han, Microwave-assisted synthesis of silver nanoparticles using sodium alginate and their antibacterial activity. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2014, 444, 180-188.

[12] V. N. Phan, H. Lan, N. T. Thuy, T. M. Hien, T. Q. Huy, N. Van Quy, H. D. Chinh, L. M. Tung, P. A. Tuan, and V. D. Lam, Characterization and antimicrobial activity of silver nanoparticles prepared by a thermal decomposition technique. Applied Physics A, 2013, 113(3), 613-621.

[13] M. Roldán, N. Pellegri, and O. de Sanctis, Electrochemical Method for Ag-PEG Nanoparticles Synthesis. Journal of Nanoparticles, 2013, 2013.

[14] T. Q. Tuan, N. V. Son, H. T. K. Dung, N. H. Luong, B. T. Thuy, N. T. V. Anh, N. D. Hoa, and N. H. Hai, Preparation and properties of silver nanoparticles loaded in activated carbon for biological and environmental applications. Journal of hazardous materials, 2011, 192(3), 1321-1329.

[15] M. T. Reetz and W. Helbig, Size-selective synthesis of nanostructured transition metal clusters. Journal of the American Chemical Society, 1994, 116(16), 7401-7402.

[16] L. Rodriguez-Sanchez, M. Blanco, and M. Lopez-Quintela, Electrochemical synthesis of silver nanoparticles. The Journal of Physical Chemistry B, 2000, 104(41), 9683-9688.

[17] J. McFarland, The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. Journal of the American Medical Association, 1907, 49(14), 1176-1178.

[18] Khaydarov R., R. Khaydarov, O. Gapurova, Y. Estrin, and T. Scheper (2009), Electrochemical method for the synthesis of silver nanoparticles. Journal of Nanoparticle Research. 11(5), pp.1193-1200.

[19] Šileikaitė A., I. Prosyčevas, J. Puišo, A. Juraitis, and A. Guobienė (2006), Analysis of silver nanoparticles produced by chemical reduction of silver salt solution. Mater. Sci.-Medzg. 12, pp.287-291.

[20] S. Link and M. A. El-Sayed, Shape and size dependence of radiative, non-radiative and photothermal properties of gold nanocrystals. International Reviews in Physical Chemistry, 2000, 19(3), 409-453.

[21] J. Y. Kim, C. Lee, M. Cho, and J. Yoon, Enhanced inactivation of< i> E. coli</i> and MS-2 phage by silver ions combined with UV-A and visible light irradiation. Water research, 2008, 42(1), 356-362.

[22] W. -R. Li, X. -B. Xie, Q. -S. Shi, H. -Y. Zeng, O. -Y. You-Sheng, and Y. -B. Chen, Antibacterial activity and mechanism of silver nanoparticles on Escherichia coli. Applied microbiology and biotechnology, 2010, 85(4), 1115-1122.

[23] K. -J. Kim, W. S. Sung, B. K. Suh, S. -K. Moon, J. -S. Choi, J. G. Kim, and D. G. Lee, Antifungal activity and mode of action of silver nano-particles on Candida albicans. Biometals, 2009, 22(2), 235-242.

[24] G. Martinez-Castanon, N. Nino-Martinez, F. Martinez-Gutierrez, J. Martinez-Mendoza, and F. Ruiz, Synthesis and antibacterial activity of silver nanoparticles with different sizes. Journal of Nanoparticle Research, 2008, 10(8), 1343-1348.