e-ISSN:2321-6204
p-ISSN:2347-2359

Research & Reviews: Journal of Food and Dairy Technology

Synthesis of Silver Nanoparticles through Chemical Reduction and its Antibacterial Effect

Asadi M[1], Khosravi-Darani K[2]*, Haj-Seyed Javadi N[2], Esmaeili S[2] and Azadnia E[2]

[1]Department of Food Science and Technology, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran

[2]Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, P.O. Box 19395-4741, Tehran, Iran

Research Article

Received Date: 04/11/2015
Accepted Date: 19/11/2015
Published Date: 30/11/2015

***For Correspondence**

Khosravi-Darani K, Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, P.O. Box 19395-4741, Tehran, Iran

E-mail: k.khosravi@sbmu.ac.ir

Keywords: Silver Nanoparticles, Chemical Reduction, Antimicrobial Activity, Process Variable

ABSTRACT

Traditionally, antimicrobial activities of silver have been known for human, but recently effects of silver nanoparticles and their antimicrobial effects have evaluated by researchers. In this study silver nanoparticles were produced by chemical reduction and then antimicrobial effects of these particles for two of the most threatening bacteria in food industries, *Staphylococcus aureus* and *Escherichia coli* as gram-positive and gram-negative bacteria examined by concentrations of 5, 10, 25, 50 mg L^{-1}. The Results indicated that the chemical reduction method is a good and suitable procedure for production of silver nanoparticles, but it needs high accuracy. The minimum inhibitory effect of silver nanoparticles for *Escherichia coli* and *Staphylococcus aureus* were 5 and l0 mg L^{-1} respectively, which *Escherichia coli* was more resistance. So, these particles could be considered as a suitable alternative to conventional antimicrobial agents.

INTRODUCTION

Reduction in size of particles is one of the effective and useful tools to improve particles' effectiveness; in fact nano technology assists to reduce the size and to eliminate restriction of sizes that had existed from the past [1,2]. Microorganisms like bacteria, yeast and molds exist in environment frequently infected people [3]. Increasing rate of infectious diseases which caused by pathogenic bacteria also increasing their resistance against antibiotics, pushed Physicians to use these new antimicrobial agents [4-6].

The antimicrobial activities of silver have been known from ancient time [7]. It is supposed that due to small size of silver nanoparticles, specific surface of atom and considerable amount of it has antibacterial activities in comparison with mass silver [3,8-15]. Various mechanisms are suggested for the impacts of silver's germicide but silver is different from other germicide materials and contrary to them, it seems that microorganisms have no resistance against silver in process of time and this apparently is because silver has more than one method to reveal the its antimicrobial impact on microorganisms [16].

In recent years to synthesis of metal nanoparticles a variety of chemical and physical methods have been developed, including chemical reduction, electrochemical, photochemical, sonochemical, thermal reduction, physical synthesis of steam, condensation neutral gases, chemical synthesis of steam, compression of steam atomic metal and etc. [17-23]. Physical methods are used rarely due to their need to high temperatures more than 1000°C, expensive equipment and complexity of how to control synthesis circumstances. Since facility of controlling conditions of reaction, inexpensive process and simple equipments and raw

e-ISSN:2321-6204
p-ISSN:2347-2359

material, chemical methods are so common instead and among these methods, chemical reduction utilize more than others because of simple equipment's and operations [24]. As yet different components have been used as reducer and stabilizer which are mostly toxic compounds.

Purposes of this research are synthesis of stable silver nanoparticles by using chemical reduction without using toxic solvents and evaluation of antibacterial effects of silver nanoparticles on *S. aureus* and *E. coli* as two harmful and problematic bacteria in food.

MATERIAL AND METHODS

Materials

Silver nitrate, Ethanol, medium of Eosin-Methylene blue, Nutrient Broth, Muller-Hinton agar, Mannitol-Salt-agar also peptone water provided from Germany Merck Company. Polyvinylpyrrolidone with 55000 molecular mass was purchased from Sigma-Aldrich Germany. Moreover, *S. aureus* PTCC No. 1112 (ATCC No: 6537) and *E. coli* PTCC No. 1330 (ATCC No: 8739) supplied from Iranian Research Organization for Science and Technology.

Synthesis of silver nanoparticles

Chemical reduction was utilized to synthesis silver nanoparticles in this study. Some of the advantages of this method in comparison to other methods for production of silver nanoparticles are simplicity, inexpensive, ability to control the conditions of reaction .Silver salt, reducer, stabilizer and solvent, silver nitrate, ethanol, polyvinylpyrrolidone 55000 molecular mass and de-ionized water were required to synthesis of silver nanoparticles by this method. 0.1 g polyvinylpyrrolidone and 20 mL de-ionized water were added into a twin-neck flask. Then, exposed to nitrogen at 90°C for 10 min and stirred it strongly in order to solve completely beside de-oxygenation. Next, 0.01 g of silver nitrate with 1 mL ethanol added to the twin-neck flask, frequently color of solution bring to change. Which, after 20 min, the color changed from colorless into tawny which indicated synthesis of silver nanoparticles and end of the reaction. Examining UV-Vis (Ultraviolet-visible) spectrum of colloid solutions is a more accurate method to ensure of completing synthesis of silver nanoparticles. Appearance of a peak on 420 nm indicated the synthesis of silver nanoparticles was spheroid. Then, the volume of 1 mL of sample was reached to 10 mL with de-ionized water and read the UV-Vis spectrum of it with spectrophotometer. This procedure should be repeated every 5 min. At the beginning, advent of peak on 300 nm indicated existence of silver ions in the colorless solution. By time passed, peak moved to higher wavelengths which indicated reduction of silver ions to silver nanoparticles which in this step colorless solution changed into yellow. In the final step, appearance of peak on 420 nm and tawny color of solution presented production of silver nanoparticles and end of the reaction.

After the reaction ends, an image of silver nanoparticles was taken by Transmission electron microscopy (TEM); this is to ensure of synthesis of silver nanoparticles, moreover, determine mean of the particles' size.

After obtaining images by TEM, size and scattering of particles were calculated by Manual Microstructure Distance Measurement software which was a production of Nahamin Pardazane Asia Company. Biggest and smallest sizes of silver nanoparticles were 34 and 3 nm which most of them distributed in range of 8 to 15 nm. Then, colloidal solution of silver nanoparticles by concentrations of 5, 10, 25, 50 mg L^{-1} were prepared and applied to evaluate antimicrobial effects of those particles on *S. aureus* and *E. coli*.

Antimicrobial effects

First, McFarland Standard (0.5) for control treatment was prepared, then put 0.1 mL of *S. aureus* and *E. coli* in each test tubes containing Nutrient Broth medium (one type of bacteria per tube) and put tubes in incubator for 24 h on 37°C. After 24 h, the tubes removed from incubator which showed turbidity same to McFarland (0.5) turbidity with Light Absorption Coefficient (LAC) of 1. In accordance with McFarland (0.5) Standard it was accounted for 105 $\times 10^8$ CFU mL^{-1} of *S. aureus* in the solution.

Subsequently different dilutions of 10^3-10^7 of both mentioned bacteria were prepared and then all dilutions cultured with both bacteria in separate specific medium as Eosin-Methylene blue for *S. aureus*, Mannitol-Salt-agar for *E. coli* and Muller-Hinton agar applied to evaluate antimicrobial effects of both bacteria. Next, put each culture plate into incubator for 24 h on 37°C in order to those bacteria grow on foresaid culture medium. All plates which were cultured by this method consider as control plates.

After that, when the time passed for each one as 1, 12, 24 and 48 h, put 1 mL of all silver nanoparticles concentrations into each prepared bacteria concentrations on specific medium for each bacteria and also on Muller-Hinton agar for both bacteria; then placed in incubator for 24 h on 37°C to determine rate of inhibition effects silver nanoparticles on bacteria. All plates which were cultured by this method considered as test plates. Incubated control and test plates compared in order to understand silver nanoparticles impacts on percentage reduction of bacterial count.

RESULTS AND DISCUSSION

All plates including control and test plates have been evaluated after 24 h. Initial evaluation of those plates indicated that growth inhibition in 50 mg L^{-1} treatment of silver nanoparticles for all given times. **Figure 1** demonstrated inhibited growth of *S. aureus* and *E. coli* (10^7 CFU mL^{-1}) exposed to silver nanoparticles 50 mg L^{-1} after 1 h contact.

e-ISSN:2321-6204
p-ISSN:2347-2359

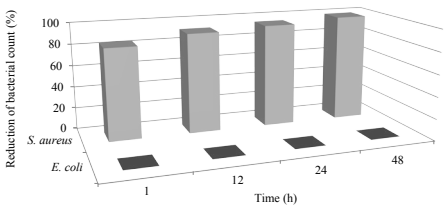

Figure 1. The interaction of time and type of bacteria on reduction of bacterial count in presence of silver nanoparticles at 25°C.

After observing the plates, the results must be compared statistically to evaluate independent and interaction effects between factors on percentage reduction count of *S. aureus* and *E. coli*. So, it would be cleared whether they are significant or not. **Table 1** indicated the results obtained from analysis of variance (ANOVA) by full-factorial model.

Table 1. Analysis of variance for variables impacts on percentage reduction count of bacteria by full-factorial model.

Source	SOS**	Degree of freedom	mean squares	F value	P-value
Model Significant	2.027E+005	31	6538.43	2003.43	<0.0001
A-Time(h)	312.32	3	104.11	31.90	<0.0001
B-Type of Bacteria	28316.58	1	28316.58	8676.42	<0.0001
C-Concentration of nano	95974.95	3	31991.65	9802.49	<0.0001
AB	29.13	3	9.71	2.97	0.0334
AC	345.00	9	38.33	11.75	<0.0001
BC	77520.25	3	25840.08	7917.61	<0.0001
ABC	193.01	9	21.45	6.57	<0.0001
Pure Error	522.18	160	3.26	-	-
Cor Total	2.032E+005	191	-	-	-

*Std. Dev. 1.81, R-Squared 0.9974, Mean 85.53, Adj R-Squared 0.9969, C.V. %2.11, Pred R-Squared 0.9963, PRESS 751.94, Adeq Precision 135.589
** Sum of square

The results demonstrated that effects of independent factors like time, type of bacteria, silver nanoparticles concentration and also interaction effects between time and silver nanoparticles concentration, type of bacteria and silver nanoparticles concentration and time, type of bacteria, silver nanoparticles concentration were significant in level of 1% and effect of time and type of bacteria were significant in level of 5%. The outputs presented a linear relationship among three given factors on percentage reduction count of bacteria. Desired fitting of data could be evaluated by information such as R^2, R^2_{adj}, C.V or coefficient of variation that those amounts were equal to R-Squared= 0.9974, Adj R-Squared= 0.9969 and C.V= 2.11%.

Figure 2 indicated the comparisons effects of different time growth on bacterial reduction count in presence of silver nanoparticles with concentration of 5 mg L^{-1} and at the temperature of 25°C. Also, The interaction of time and type of bacteria on reduction of bacterial count in presence of silver nanoparticles at the temperature of 25°C has been shown in **Figure 3** which indicated that the effect of time on reduction of *E. coli* was not significant but by increasing time from 1 to 48 h, the number of survived *S. aureus* bacterial cell was reduced.

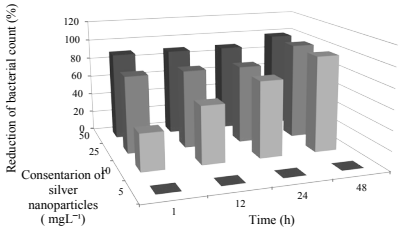

Figure 2. The interaction effects of concentration of silver nanoparticles and time on reduction of bacterial count at 25°C.

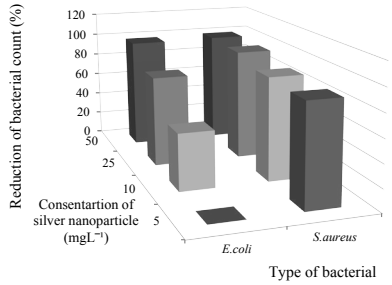

Figure 3. Interaction effect of silver nanoparticles and type of bacteria on reduction of bacterial count at 25°C.

According to Figure 2, which showed the interaction effects of concentration of silver nanoparticles and type of bacteria on reduction of bacterial cells at the temperature of 25°C, the concentration of 5 mg L^1 silver nanoparticles reduced the number of *S.*

aureus bacterial cells but this concentration hadn't any effects on *E. coli* bacterial cells. The inhibitory effect of silver nanoparticles on *E.* coli was obscured in concentration of 5 mg L^1. So, the concentrations of silver nanoparticles which can inhibit the growth of *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) were respectively 5 and 10 mg L^{-1}. Moreover, concentration of 50 mg L^{-1} silver nanoparticles inhibited the growth of both bacteria completely. Structure of membrane Gram-positive and Gram-negative bacteria is different from each other and also peptidoglycan layer may be in different thicknesses, which were reasons that *E. coli* had more resistance than *S. aureus*. Gram-positive bacteria as *S. aureus* have thick and multi-layer peptidoglycan. However, Gram-negative bacteria as *E. coli* have thinner layer and its outer membrane containing lipopolysaccharide which protected the bacteria from antibiotic and antimicrobial factors.

According to Figure 3 which demonstrated interaction effects of concentration of silver nanoparticles and type of bacteria on percentage reduction count of bacterial cells at the temperature of 25°C, the concentration of 5 mg L^{-1} silver nanoparticles hadn't any effect on reduction of number of bacterial cells in any time. But, the concentration of 10 mg L^{-1} silver nanoparticles by increasing the time can reduce more number of bacterial cells. Moreover, the concentration of 25 and 50 mg L^{-1} silver nanoparticles were important. The results obviously showed that after 1 h exposure of bacteria to these concentrations of nanoparticles causes high antibacterial effects on *E. coli* and *S. aureus*.

Table 2 demonstrated comparison of mean between effects of silver nanoparticles in different concentration on percentage reduction count of bacteria. As they cleared, there was a significant difference between silver nanoparticles by concentration of 5, 10, 25 or 5, 10, 50 mg L^{-1}; but there was no significant difference among silver nanoparticles by concentration of 25 and 50 mg L^{-1}. Silver nanoparticles with concentrations of 25 and 50 mg L^{-1} had the most also 5 and 10 mg L^{-1} had the least effectiveness on bacteria. The effects of type of bacteria on bacterial reduction count in presence of silver nanoparticles with concentration of 5 mg L^{-1} and temperature 25°C were compared in **Table 3.** Obviously there was a significant difference between types of bacteria which the most reduction occurred in *S. aureus*. These results were like to those had been obtained by Cho et al., who also found that inhibitory concentration were at least respectively 5 and 10 mg L^{-1} for *S. aureus* and *E. coli*. However, they described 50 mg L^{-1} could be reasonable concentration for *S. aureus* and subsequently for *E. coli* would be 100 mg L^{-1}. Ruparelia also reported same outputs that *E. coli* is more resistance than *S. aureus*. They evaluated effects of silver nanoparticles which were produced through reduction on *S. aureus* and *E. coli* and determined *E. coli* is more resistance than *S. aureus* [25]

Table 2. Effects of different concentration of silver nanoparticles on reduction count of bacteria at temperature 25°C.

Concentration of Nanoparticles (mg L^{-1})	Decrease of Bacterial Count (%)	Duncan Grouping
5	46.9	C
10	90.0	B
25	99.7	A
50	100.0	A

Data reported are average of 6 replication, Different Latin letters shows significant difference between data reported in each row ($P<0.05$) *

Table 3. Effects of type of bacteria on bacterial count in presence of silver nanoparticles with concentration of 5 mg L^{-1} and temperature 25°C.

Type of Bacteria	Decrease of Bacterial Count (%)	Duncan Grouping
E. coli	73.38	B
S. aureus	97.67	A

Data reported are average of 6 replication, Different Latin letters shows significant difference between data reported in each row ($P<0.05$) *

Silver nanoparticles are caused destruction of bacteria by impact on bacteria cell-wall [26-28]. There are several reports about mechanism of silver nanoparticles' impacts on bacteria. Aggregation of silver nanoparticles on bacteria cell-wall and penetration to cell has been explained as bacteria destruction [29]. Also similar studies have been demonstrated that size and shape of silver nanoparticles effect on germicide activities; as smaller silver nanoparticles with spherical shape have the most germicide effects. Cho [30] reported that surface of *E. coli* cell-wall strongly damage in contact with silver nanoparticles. Lack of *S. aureus* and *E. coli* growth on test plates consisting silver nanoparticles with concentration of 50 mg L^{-1} presented high inhibitory effectiveness which is a result of destructing cell-wall from both bacteria. Our results support the theory that production of silver nanoparticles could provide a simple and inexpensive method in order to inhibit bacterial growth.

CONCLUSIONS

Generally, the results of study demonstrated that chemical reduction method is a good and suitable procedure for production of silver nanoparticles, but it needs high accuracy and the most important step is preventing the aggregation of particles by use of appropriate stabilizer.

Moreover, according to the results, silver nanoparticles can destroy *S. aureus* and *E. coli* in low concentrations which are both harmful bacteria in food industries. This can considerably assist to food industry in all over the world; including food packing and antiseptic of assembly line by silver nanoparticles.

The minimum inhibitory concentration of silver nanoparticles for *S. aureus* and *E. coli* were 5 and 10 mg L^{-1} respectively.

Moreover, both bacteria were killed in concentration of 50 mgL^{-1} of silver nanoparticles. Also, the results showed that there was no resistance to silver nanoparticles in bacteria by passing the time. So, these particles can be considered as a suitable alternative to conventional antimicrobial agents.

ACKNOWLEDGEMENTS

This study was partially supported by National Nutrition and Food Technology Research Institute (NNFTRI) Master of Science project, Iran. Hereby, this support is appreciated.

REFERENCES

1. Gade AK, et al. J. Biol. Mater. Bioener. 2008; 2: 1.
2. Lin YE, et al. Inactivation of Mycobacterium avium by copper and silver ions, Water Res. 1998; 32: 1997-2000.
3. Chen X, et al. Toxicol. Lett. 2008; 178:1.
4. Feng QL, et al. J. Biomed. Mater. 2000; 52: 662.
5. Gurunathan S, et al. Biosynthesis, purification and characterization of silver nanoparticles using Escherichia coli, Biointerfaces. 2009; 74:328-335.
6. Mirkin CA and Taton TA. Semiconductors meet biology, Nat. 2000; 405: 626-627.
7. Kapoor S, et al. Reduction and Aggregation of Silver Ions in Aqueous Gelatin Solutions, Langmuir 1994; 10:3018-3022.
8. Dragieva I, et al. Extracellular synthesis of silver nanoparticles using dried leaves of *pongamia pinnata* (L) pierre Nanostruct. Mater 1999; 12: 267-270.
9. Hidalgo E and Domíngue C. Study of cytotoxicity mechanisms of silver nitrate in human dermal fibroblasts, Toxicol. Lett. 1998; 15: 169-179.
10. Hugo WB and Russell AD. Types of antimicrobial agents. In: Principles and practice of disinfection, preservation and sterilization, Blackwell Scientific Publications, Oxford, UK, 2004; 106.
11. Klasen HJ. Historical review of the use of silver in the treatment of burns. I. Early uses, Burns. 2003; 26: 117-130.
12. Lin YE, et al. Individual and combined effects of copper and silver ions on inactivation of *Legionella pneumophila* Water. Res. 1996; 30: 1905-1913.
13. Sarkar S, et al. Facile synthesis of silver nano particles with highly efficient anti-microbial property Polyhedron. 2007; 26:4419-4426.
14. Shahverdi AR, et al. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*, Nanomed. Nanotechnol. Biol. Med. 2007; 3:168-171.
15. Shiraishi Y and Toshima N. J. Mol. Catal. A. 1999; 141:187-192.
16. Williams DN, et al. Evaluation of the microbial growth response to inorganic nanoparticles, J. Nanobiotechnol. 2006; 4.
17. Nersisyan HH, et al. New and Effective chemical reduction method for preparation of silver powder and colloid al dispersion, Mater. Res. Bull. 2003; 38 949-956.
18. Khademhosseini A and Langer R. Chem. Eng. Prog. 2006; 102:38.
19. Henglein A. Physicochemical properties of small metal particles in solution: "microelectrode" reactions, chemisorption, composite metal particles, and the atom-to-metal transition, J. Phys. Chem. 1993; 97: 5457-5471.
20. Wright GD. Bacterial resistance to antibiotics: enzymatic degradation and modification, .Adv. Drug. Deliv. Rev. 2005; 57:1451-1470.
21. Sondi I and Salopek-Sondi BJ. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria, Colloid Interf. Sci. 2004;275: 177-182.
22. Shrivastava S, et al. Characterization of enhanced antibacterial effects of novel silver nanoparticles, Nanotechnol. 2007; 18:103.
23. Panacek A, et al. Silver Colloid Nanoparticles: Synthesis, Characterization, and Their Antibacterial Activity, J. Phys. Chem. 2006; 110: 16248-16253.
24. Souza GIH, et al. Utilization of Fusarium oxysporum in the biosynthesis of silver nanoparticles and its antibacterial activities, in Xth National Meeting of Environmental Microbiology, Curtiba, Brazil, 2004 25.
25. Ruparelia JP, et al. Strain specificity in antimicrobial activity of silver and copper nanoparticles, Acta Biomater. 2008;4: 707-716.

26. An J, et al. Physical, chemical and microbiological changes in stored green asparagus spears as affected by coating of silver nanoparticles-PVP, LWT – Food Sci. Technol. 2008;41:1100-1107.

27. Anil Kumar S, et al. Nitrate reductase-mediated synthesis of silver nanoparticles from AgNO3, Biotechnol. Lett. 2007; 29:439-445.

28. Blanc DS, et al. Water disinfection with ozone, copper and silver ions, and temperature increase to control Legionella: seven years of experience in a university teaching hospital, J. Hosp. Infect 2005; 60:69-72.

29. Sondi I and Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria, J. Colloid Interf. Sci. 2004; 275:177-182.

30. Cho KH, et al. The study of antimicrobial activity and preservative effects of nanosilver ingredient, Electrochim. Acta. 2005; 51:956-960.