Nat. Volatiles & Essent. Oils, 2020; 7(4): 54-65

NVEO
Natural Volatiles &
Essential Oils

Synthesis And Optimization Of Silver Nanoparticles By Bacterial Isolate E.Coli Spp

MS.RESMI NAIR[1] , DR. M.REJADHEESH[2]

[1]RESEARCH SCHOLA, LNCT UNIVERSITY, BHOPAL, INDIA.

[2]RESEARCH SUPERVISOR, DEPARTMENT OF MEDICAL MICROBIOLOGY, LNCT UNIVERSITY, BHOPAL, INDIA.

ABSTRACT

The use of microorganisms in the synthesis of nanoparticles emerges as an eco-friendly and exciting approach. In the present study, we observed the synthesis of silver nanoparticles and we reported the biosynthesis of silver nanoparticles employing the bacterium E.coli. The multi drug resistant pathogen E.coli was chosen due to its highest Ag NP synthesis. The higher level of nitrate reductase activity was measured at 731.24 U/ml in the 60th run with presence of (%) glucose: 0.1%, peptone: 1%, yeast extract: 0.4%, KNO3: 0.4%, pH: 7.5 at 25 ˚C and 3 days incubation period. AgNO3 showed more effective treated with gamma radiation of after mixing than before mixing with silver nitrate solution.

Keywords: Silver nitrate, nanoparticles, gamma radiation, bacterial isolates.

INTRODUCTION

The nanotechnology is the most dynamic research area in new materials science. The field of nanotechnology is one of the most active research areas in modern materials science. Nanoparticles reveal advanced properties based on determined characteristics like size, distribution and morphology. From the past years, the nanotechnology has majestic growth in various methods to synthesize the nanoparticles in specific size and shape for the particular requirements (Liau et al., 1997). Now a days the new applications are rapidly increased for the nanoparticles and nanomaterials.

The silver based compounds have been used mainly in the bactericidal applications (Gupta et al., 1998) because of their strong toxicity of silver at the levels of 116 microbes. Silver salts are applied as antimicrobial agents. The ionic silver strongly reacts with thiol groups of enzymes and inactivates it. The silver ions have the bactericidal activity on microorganisms.

Nanoparticles of silver have thus been studied as a medium for antibiotic delivery (Li et al., 2005), and to synthesize composites for use as disinfecting filters (Jain et al., 2005) (Jain et al.,2005) and coating

materials (Li et al., 2006). However, the nanoparticles bactericidal property of the nanoparticles depends on their growth medium stability and greater retention time for bacterium– nanoparticle interaction. The silver nanoparticles have stable to significantly restrict bacterial growth **(**Singh et al., 2008).

The silver nanoparticles synthesis participate in the number of chemical and physical methods and these methods employ toxic chemicals, intensive of energy and non-polar organic solutions and prevent their biomedical applications (Jain et al., 2010).The physical and chemical processes are mainly involved in the synthesis of nanoparticles. Nanoparticles synthesis acquired by 'top-down' and 'bottom up' methods. In involved in the bulk metals mechanical grinding subsequent stabilization that results in the formation of nanosized metal particles by the addition of scolloidal protecting agents (Gaffet et al., 1996 and Amulyavichus et al., 1998).

The nanoparticles synthesis in aqueous foams acts as template and involved in electrostatically complexing silver ions with anionic surfactant aerosol in highly stable liquid foam. After the foam is drained and reduced by sodium borohydride. The synthesized silver nanoparticles are stable and aerosol stabilizing it to give 5 to 40 nm in diameter of nanoparticles (Mandal et al., 2001). So in this study, synthesis of silver nanoparticles by microbes was determined using the bacterial strain Escherichia coli as a reducing agent.

MATERIALS AND METHODS

Materials

Silver nitrate was procured from Merck (Mumbai), India. The products used for bacterial study were EMB media, Nutrient agar, LB broth, PDA of Hi Media, India. The cultures were acquired from the Drug Radiation Research Department at National Center, Pune. The slope cultures were preserved at 4˚C in nutrient agar. The microbes were preserved in glycerol at -70˚C for long term storage. The antimicrobial activity of Silver nanoparticles was tested against Gram negative infectious multidrug resistant bacteria obtained from specimen at the National Cancer Institute, Pune.

Media

Ready Made Media

According to the manufacturer's instruction, the Ready Made Media were prepared and included.

Nutrient agar

4 gm/l of Yeast extract, 5 gm/l of tryptone, 50 gm/l of glucose, 0.55 gm/l of potassium dihydrogen phosphate, 0.425 gm/l of potassium chloride, 0.125 gm/l of calcium chloride, 0.125 gm/l of magnesium sulphate, 0.0025 gm/l of ferric chloride, 0.0025 gm/l of manganese sulphate, 0.022 gm/l of bromocresol green and agar at 15 gm/l (oxoid).

Muller–Hinton Agar

300gm/l of Beef, 17.5gm/l of casein hydrolysate, 1.5gm/l of starch and 17gm/l of agar.

Luria bertani broth

10gm/l of Tryptone, 5gm/l of yeast extract and 2gm/l of sodium chloride.

Nitrate media

1.5 gm/l of glucose, 15 gm/l of peptone, 3.5 g m/l of yeast extract and 3.5 gm/l of KNO_3, after mixing and autoclaving of these components at 121°c for 15 min and then cooled and inoculated the pathogen (Table-1).

Table-1: The different variables and their levels used for optimization of nitrate reductase productivity.

Levels	Glucose g/l	Peptone g/l	Yeast exract g/l	Potassium nitrate g/l	pH	Temp ˚C	Incubation period Day
1	2	20	4.2	4.2	7.6	36	3
0	1.6	16	3.4	3.4	7	31	2
-1	1	11	3	3	6.7	24	1

Optimized media

The mixing of the components such asglucose 1 gm/l, peptone 10 gm/l, yeast extract 4 gm/l and KNO3 4 gm/l for the production of the Ag NPs from E.coli then mixed the components and autoclaved at 121°c for 15 min then cooled and inoculated with the pathogen.

Production of cell supernatant from E.coli

For the synthesis of silver nano paricles, the cell supernatants from bacterial isolates were grown aerobically in the medium of nitrate. The bacterial cultured flasks were inoculated using shaker and agitated at 120 rpm. After 24 hours the collected cell supernatants were centrifuged by using centrifugation at 6,000 rpm for 10 minutes at 6˚C.

Synthesis of silver nanoparticles

The method of Synthesis of silver nanoparticles was described by (Kalishwaralal et al., 2008) with minor changes. For the synthesis of silver nanoparticles, the cell supernatant prepared by the above method was placed in the Erlenmeyer flasks containing AgNO3 at a concentration of 1 mM were incubated for 5 min. By using UV–visible spectrophotometer, the absorption spectrum of the sample was recorded at a resolution of 1 nm. The cell supernatant was irradiated one time at before and after mix with the 1 Mm of silver nitrate and it was exposed to room temperature at 0.25 to 3kGy Gamma-rays. After mixing , the silver nitrate filtrate was irradiated then we used different ranges of silver nitrate.

Screening of the most potent bacterial isolate for microbiological synthesis of silver nanoparticles

For synthesis of silver nanoparticles the cell supernatant of E.coli species was growned in nitrate medium. The different cell supernatant was measured by optical density of Ag NPs synthesized.

Estimation of nitrate reductase activity

The modified procedure of Nitrate reductase activity was estimated by Vaidyanathan et al, 2010.For the crude enzyme ,the cell free supernatant obtained was used. Mixing of 25 mM of potassium phosphate buffer with 30 of mM potassium nitrate and 0.05 of mM ethylene diamine tetra acetic acid acid at pH 7.3 was prepared and used as a enzyme substrate solution.

An aliquont of 100 µl enzyme was mixed with 1.8 ml substrate solution and supported by 100 µl of 2.5 mM β-nicotinamide adenine dinucleotide reduced to form the solution (β-NADH). This solution was incubated at 30°C for 5min and terminated promptly by adding and agitating with 1ml of 58 mM sulphanilamide solution with 3M of HCl. After that 1ml 0.77mM N-(1-naphthyl) ethylenediamine dihydrochloride solution (NED) was added and the color developed was noted after 5 min incubation. The absorbance was measured at 540 nm and defined as micromoles at optical density at nitrite produced per min.

Optimization of medium components for nitrate reductase production

The influence of reductase activity of different factors was estimated by RSM with 97 runs totally. Glucose, peptone, yeast extract, KNO3, pH, temp and incubation period are the independent variables against the dependent variable nitrate reductase activity.

RESULT AND DISCUSSION

In modern years, the silver nanoparticles have been explored by numerous researchers nationwide and universally. It is mostly due to the dormant utilization of nanomaterials in biologically, clinically, optically and electronically. Silver nanoparticles have power with other decent nanoparticles (e.g., gold and copper) since the surface Plasmon resonance energy of silver is located away from the interband transition energy.

Screening of the most potent local Bacillus strains for microbiological synthesis of silver Nanoparticles

The E.coli strains were evaluated for silver nanoparticles synthesis and used for the following study. Silver has long-lasting to manifest a powerful virulence to a broad range of 116 micro-pathogens (Liau et al., 1997) because of these silver-based components have been used comprehensively in most of the applications of bactericidal organisms (Gupta et al., 1998 and Nomiya et al., 2004). The focus of this research was to perform the Ag NPs synthesis by multi drug resistant bacterial isolate, the nitrate reductase production by those bacteria using the optimization of medium components and to evaluate the antibacterial activity for synthesized Ag NPs

From this study, the local bacterial isolate of E.coli spp. Which was predominantly established as positive by observing a color change from pale yellow to brown ,which denoted the synthesis of silver nanoparticles (Ag+ to Ag0) (Ranganath et al., 2012). The treated AgNO3 flask exhibited the changes that

have been assigned to the surface Plasmon resonance (SPR) which represented the Ag NPs formation (Kalishwaralal et al., 2008).

Synthesis of silver nanoparticles

The cell free supernatant of E.coli was added to the aqueous Ag+ within 10 hr after incubation the Ag NPs (*sentence to check)were reduced before optimization of media. At the time incubation, the whitish yellow to brown colour was observed and the control has no colour change. The SPR band for Ag NPs is obtained at ƛ 431 nm was measured and denotes the appearance of spherical or roughly spherical Ag nanoparticles.

Optimization of medium components for nitrate reductase production

In this study, nitrate reductase activity was observed highly at the range of 630.20U/ml during 60th run with 0.2% of glucose, 1% of peptone, 0.4% of east extract, 0.4% of KNO3 at pH of 7.5 with 25 ˚C for the duration of 3 days (Table-2). The reduction of Ag+ to Ag0 together with nitrate reductase and other proteins formation by reductase enzymes. The reduction of silver nitrate to silver nanoparticles was observed in the solution through capping medium of proteins by using the released enzymes (Vaidyanathan et al ., 2010).

Table-2: Combination of variables and Response in RSM design

No. of run	Glucose g/l	Peptone g/l	Yeast extract g/l	Pot. nitrate g/l	pH	Temp ˚C	Incubation period day	Enzyme activity U/ml	OD at ƛ 430 nm of silver nano paricles
1.	1.5	15	3.5	3.5	7	30	2	295.6	1.5
2.	2	20	3	3	6.5	25	1	566.21	2
3.	2	10	3	4	7.5	35	1	176.6	1.9
4.	1.5	15	3.5	3.5	7	30	2	295.6	1.5
5.	1	10	4	4	6.5	25	1	126.35	1.9
6.	1	20	3	4	7.5	35	1	172.3	1.4
7.	2	20	4	3	7.5	25	1	493.42	1.9
8.	1.5	15	3.5	3.5	7	30	2	295.6	1.8
9.	1.5	15	3.5	3.5	8.41	30	2	601.6	1.4

10.	1	10	4	3	7.5	25	1	435.77	1.9
11.	2	10	4	4	6.5	35	1	316.77	1.3
12.	1.5	15	3.5	3.5	7	30	2	295.6	1.8
13.	2	10	3	3	7.5	35	3	102.16	1.8
14.	2	10	3	3	7.5	25	1	52.67	1.4
15.	2	10	4	3	7.5	35	1	399.5	2
16.	2.91	15	3.5	3.5	7	30	2	533.3	1.9
17.	1	20	4	4	6.5	35	1	523.73	1.2
18.	1.5	15	3.5	3.5	7	30	2	295.6	1.6
19.	1.5	15	3.5	3.5	7	30	4.83	2281.1	1.4
20.	1.5	15	3.5	3.5	7	30	2	295.6	1.9
21.	1.5	15	3.5	3.5	7	30	2	295.6	1.7
22.	2	20	3	3	6.5	35	3	227.7	2
23.	1.5	15	3.5	4.91	7	30	2	435.8	1.1
24.	1	10	4	3	6.5	25	3	571.677	2
25.	2	10	3	4	6.5	35	3	259.2	2
26.	1.5	15	3.5	3.5	7	30	2	295.6	1.8
27.	1	20	4	3	6.5	35	3	36-.6	1.6
28.	2	10	3	3	6.5	35	1	420.33	1.7
29.	1	20	4	3	6.5	25	1	142.06	1.1
30.	1	20	3	4	6.5	25	1	142.06	1.1
31.	1.5	15	3.5	3.5	7	30	2	295.6	1.2
32.	2	20	4	4	7.5	35	1	244.7	1.3
33.	1	20	4	4	7.5	35	3	370.012	1.9

34.	2	20	3	4	7.5	35	3	271.66	1.7
35.	2	20	4	4	6.5	35	3	167.98	1.5
36.	2	10	4	3	7.5	25	3	542.86	2
37.	1	10	4	3	6.5	35	1	215.8	1.5
38.	1.5	15	3.5	3.5	7	30	2	295.6	1.9
39.	2	20	4	3	6.5	35	1	186.5	1.7
40.	1	10	3	4	7.5	25	1	169.63	1.5
41.	2	20	4	4	7.5	25	3	525.22	1.4
42.	2	20	3	3	7.5	25	3	555.28	1.2
43.	1	20	3	3	6.5	25	3	427.33	1.6
44.	1.5	15	3.5	3.5	7	30	2	295.6	1.3
45.	2	20	4	4	6.5	25	1	524.22	1.7
46.	1.5	15	3.5	3.5	7	30	2	295.6	1.5
47.	1	10	4	4	6.5	35	3	325.79	2
48.	1	10	3	4	6.5	25	3	529.69	1.1
49.	1	20	3	4	6.5	35	3	165.4	1.6
50.	1	10	4	3	7.5	35	3	219.8	1.9
51.	2	20	3	4	6.5	35	1	263.5	1.3
52.	1	20	3	3	6.5	35	1	426.9	1.8
53.	2	10	3	3	6.5	25	3	441.5	1.4
54.	1.5	15	3.5	3.5	7	30	2	259.6	1.7
55.	1	10	3	3	7.5	35	1	340.8	1.4
56.	2	20	3	4	7.5	25	1	102.36	1.2
57.	1	10	3	3	7.5	25	3	307.16	2

58.	2	10	3	4	6.5	25	1	56.65	1.1
59.	1	20	4	4	7.5	25	1	231.76	1.9
60.	1	10	4	4	7.5	25	3	731.24	2.2
61.	2	10	4	4	7.5	35	3	120.45	1.7
62.	1.6	14	3.4	3.4	6	29	2	289.6	1.2
63.	2	11	3	3	7.8	24	1	715.9	1.6
64.	1.4	14	3.4	3.4	6	31	2	268.3	1.3
65.	1	19	4	3	7.8	23	2	345.77	1.5
66.	1	19	4	3	7.8	23	2	298.0	1.6
67.	1	11	4	4	6.4	34	3	206.4	1.6
68.	2	19	3	4	6.2	23	2	710	2
69.	1.4	16	3.6	3.6	6	29	2	298.6	1
70.	2	21	2	2	7.1	34	1	216.0	1.2
71.	2	21	2	2	6.4	24	2	389.5	1.5
72.	1	19	3	4	7.4	24	2	687.5	1.3
73.	2	11	3	3	6.9	24	4	694.6	1.5
74.	1.4	16	3.4	3.4	6	20	2	300.4	1.5
75.	1.5	16	3.4	3.4	6	29	2	300.4	1.6
76.	1	11	3	3	6.7	23	1	63.9	1.6
77.	1	10	3	3	7.1	34	1	325.7	1.8
78.	1.5	16	3.4	3.4	6	31	2	296.8	1.6
79.	1.4	16	4.50	3.4	6	31	2	350	3
80.	0.054	14	3.4	3.4	6	29	2	295.0	1.7
81.	1.6	0.546	3.4	3.4	6	29	2	356.3	1.5

82.	1.4	16	3.4	3.4	6.6 1	29	2	356.2	1.8
83.	2	9	4	2	6.1	29	1	450.4	1.4
84.	1	19	4	4	7.2	32	3	158.9	1.5
85.	1.4	16	3.4	3.4	6	29	2	279.5	1.3
86.	1	19	3	3	6.3	24	2	655.3	1.1
87.	1.3	17	2.00	3.6	6	29	2	490	1.1
88.	1.4	30.12	3.2	3.2	6	29	2	500.6	1.2
89.	1	9	2	3	6.3	32	1	516	1
90.	1.5	16	3.4	3.4	6	29	2	300.3	1.3
91.	1.6	16	3.4	3.4	6	29	2	289.6	1.5
92.	2	11	4	3	7.3	30	1	217	1.7
93.	1	19	4	3	7.3	34	1	234.6	1.5
94.	1	9	4	3	7.3	34	3	470.2	2
95.	2	21	3	4	7.3	34	3	175.23	1.5
96.	2	9	3	4	6.4	34	2	123.9	1.5
97.	1.5	15	3.5	2.93	6	31	2	170.00	1.2

Thenitrate reductase activity was constructed by dependent variable, (independent variables, ANOVA and mean significance. The analysis of variance was tested by Fisher's statistical test showed the range of 2.1300 higher than the F tabulated 0.0001. The model determination coefficient R2 (0.416500) recommended that the fitted model could explain 45.9% of the total variation. The gamma rays are exposed to aqueous solutions by the radiolytic method formed solvated electrons that reduced the metal ions equally to form accumululation. The synthesis of Ag NPs by radiolytic reactions and stabilization to form the capping by prevention of aggregates. The high level of irradiation and low level of SPR band intensity was 0.75 kGy might be due to the free radicals present in the gamma radiation of extract bioactive compounds (Marignier et al., 1985).

The determination coefficient R2 (0.678058) suggested that the fitted model could explain 67.8% of the total variation**.** The t-value and p-value statistical parameters were measured by dividing each

coefficient by its standard error. The p-value is the chance of getting higher t value by getting alone. The higher magnitude of the t-value and smaller the p-value is more significant than the corresponding coefficient. The nitrate reductase activity was remarkably affected by yeast extract, temperature and period of incubation (Table-3). The yeast extract showed high level of nitrate reductase activity at 0.5, increased incubation period (3 days) at the low level of temperature of 25°C (fig-1). And also the peptone and potassium nitrate, glucose and temp, yeast extract and temperature was observed and showed in fig-2.

Table-3: Regression analysis of the RSM design

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	35	1632348.1	46638.5	3.6707
Error	61	775040.2	12705.6	Prob > F
C. Total	96	2407388.3	-	<0.0001* (significant)

DF: degree of freedom, *: Significant , F: Fisher F- Test

Fig-1: Effect of different doses of gamma radiation on nitrate reductase enzyme activity

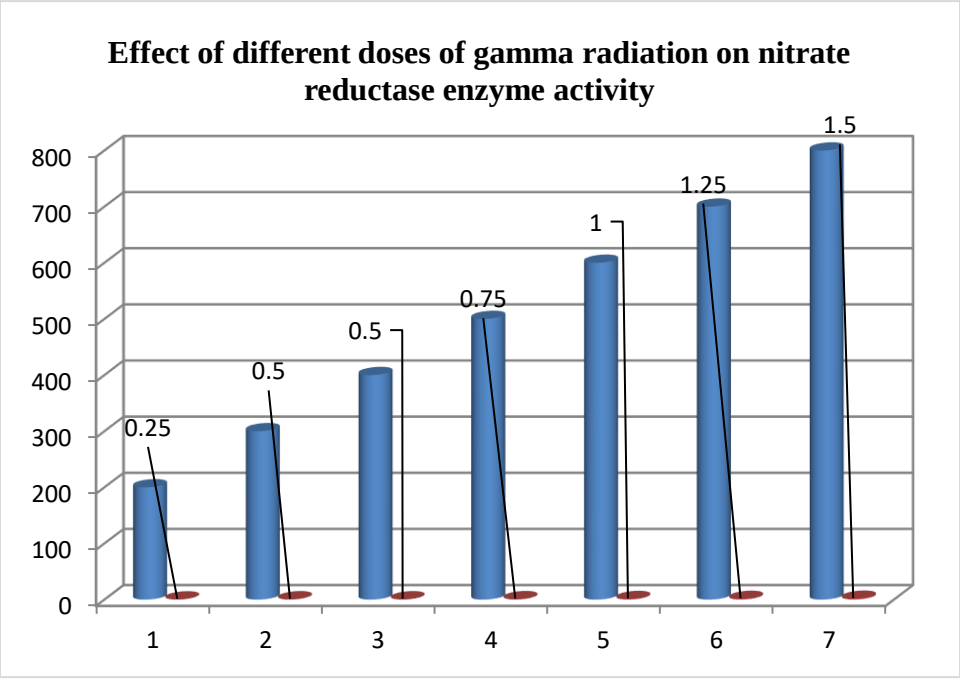
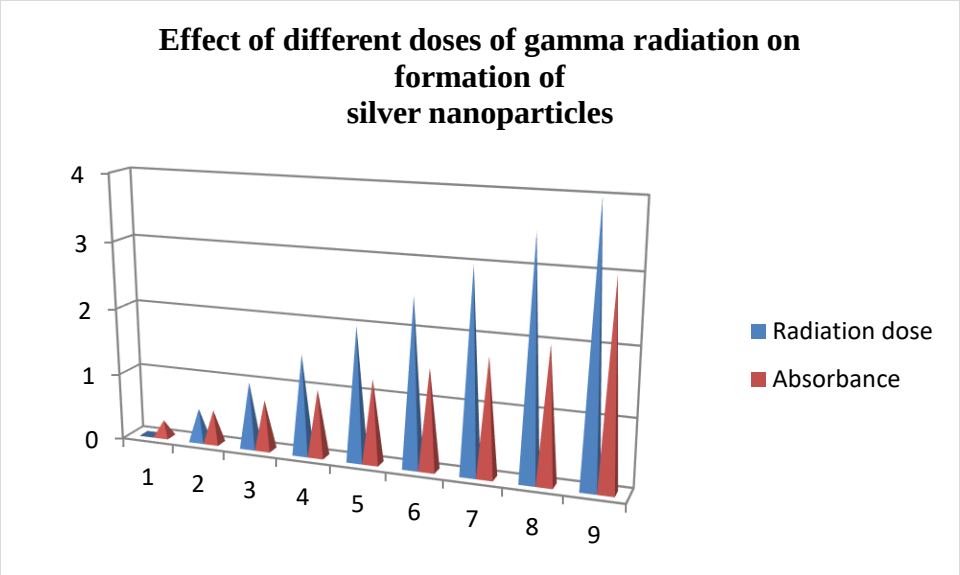


Fig-2: Effect of different doses of gamma radiation on formation of silver nanoparticles



The surface methodology of the nitrate reductase activity was remarkably over done by extract of yeast and incubation time. The high level of yeast extract (0.4) and incubation period (3 days) have increasing enzymatic activity. The various physiochemical parameters on the nitrate reductase affected the various physiochemical parameters against independent variable at their central levels (Silveira et al., 2008).

CONCLUSION

This study concluded that the silver nitrate was added to supernatant of E.coli synthesized the Silver nanoparticles in the range of 14±4 nm. The Response surface methodology estimates the main factors and explored the interaction among different factors. This cheap and easy method can be used as others to chemical, physical, and microbial mediated methods used for synthesis of silver nanoparticles. The environment friendly synthesized silver nanoparticles by the endophytic bacteria offers vast scope for their application in the biomedicine field.

REFERENCE

1. Liau, S. Y.; Read, D. C.; Pugh, W. J.; Furr, J. R. and Russell, A. D. (1997): Interaction of silver nitrate with readily identifiable groups: relationship to the antibacterial action of silver ions. Letters in Applied Microbiology, 25: 279.
2. Gupta, A. and Silver, S. (1998): Molecular Genetics Silver as a biocide: Will resistance become a problem. Nature Biotechnology, 16: 888.
3. Li, Y.; Leung, P.; Yao, L.; Song, Q. W. and Newton, E. (2006): Antimicrobial effect of surgical masks coated with nanoparticles. Journal of Hospital Infection, 62: 58.
4. Singh, M.; Singh, S.; Prasad, S. and Gambhir, I. S. (2008): Nanotechnology in Medicine and Antibacterial Effect of Silver Nanoparticles. Digest Journal of Nanomaterials and Biostructures. 3(3): 115 – 122.

5. Jain, N.; Bhargava, A.; Majumdar, S.; Tarafdar, J. C. and Panwar, J., (2010): Extracellular biosynthesis and characterization of silver nanoparticles using Aspergillus flavus NJP08: A mechanism perspective. The Royal Society of Chemistry Nanoscale; 3: 635–641.
6. Gaffet, E.; Tachikart, M.; El Kedim, O. and Rahouadj, R. (1996): Nano structural materials formation by mechanical alloying: morphologic analysis based on transmission and scanning electron microscopic observations. Materials Characterization, 36, 185–190.
7. Amulyavichus, A.; Daugvila, A.; Davidonis, R. andSipavichus, C. (1998): Study of chemical composition of nano structural materials prepared by laser cutting of metals. Fizika Metallov I Metallovedenie, 85, 111–117
8. Mandal, S.; Arumugam, S.; Pasricha, R.; Sastry, M. (2001): Silver nanoparticles of variable morphology synthesized in aqueous foams as novel templates. Bulletin of Materials Science, 28, 503–510.
9. Kalishwaralal, K.; Deepak, V.; Ramkumar pandian, S.; Nellaiah, H. and Sangiliyandi, G. (2008): Extracellular biosynthesis of silver nanoparticles by the culture supernatant of Bacillus licheniformis. Materials Letters, 62: 4411–4413.
10. Nomiya, K.; Yoshizawa, A.; Tsukagoshi, K.; Kasuga, N. C.; Hirakawa, S. and Watanabe, J. (2004): Synthesis and structural characterization of silver (I), aluminium (III) and cobalt(II) complexes with 4-isopropyltropolone (hinokitiol) showing noteworthy biological activities. Action of silver (I)- oxygen bonding complexes on the antimicrobial activities. Journal of Inorganic Biochemistry, 98: 46.
11. Ranganath, E; Rathod, V. and Banu, A. (2012): Screening of Lactobacillus spp, for mediating the biosynthesis of silver nanoparticles from silver nitrate. IOSR Journal of Pharmacy; 2(2): 237-241.
12. Vaidyanathan, R.; Gopalram, S.; Kalishwaralal, K.; Deepak; V.; Pandian, S. R. K. and Gurunathan, S. (2010): Enhanced silver nanoparticle synthesis by optimization of nitrate reductase activity. Colloids and Surfaces B: Biointerfaces. 75: 335–341.
13. Marignier, J.L.; Belloni, J.; Delcourt, M.O. and Chevalier, J.P. (1985): Microaggregates of non-noble metals and bimetallic alloys prepared by radiation-induced reduction. Nature, 317:344-345.
14. Silveira, S. T.; Daroit, D. J. and Brandelli, A. (2008): Pigment production by Monascus purpureus in grape waste using factorial design. LWT-Food Science and Technology, 41:170–174.