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Taguchi Design for Parameter Optimization of Size-Controlled Synthesis of Silver Nanoparticles

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***Abstract**: In this work, we focus on synthesis of silver ultrafine nanoparticles via a simple, fast and eco- friendly biological method using the Taguchi design. Orthogonal array of L_9 type was applied as an experimental design to analyze the results and to determine optimum conditions for synthesis of silver nanoparticles. Silver nanoparticles are synthesized by biological method using Fungus Aspergillus terreus (Thom) MTCC 6324. Addition of silver ions to the biomass of fungi and temperature of reaction are investigated. The effect of these factors on the particle size of synthesized silver nanoparticles was quantitatively studied by the analysis of variance (ANOVA). The results showed that silver nanoparticles can be synthesized by controlling silver concentration, biomass of fungi and temperature. The synthesized silver nanoparticles are characterized using X-Ray diffraction, and UV- visible spectrophotometer. Shape of synthesized silver nanoparticles was studied using Scanning Electron Microscopy and elemental composition of nanoparticles was determined using electron diffraction spectrum. The result showed that silver nanoparticles can be synthesized by biological method using Taguchi Design which was of spherical in shaped and average particle diameter was 2.18nm. The diameter predicated by statistical model was 2.23nm while the obtained diameter was 2.18nm which is very close to the predicated value. This indicates that the experimental design proposed by Taguchi was correct. Hence optimization of parameters for is achieved during this study and this method may be applicable further for large scale production of silver nanoparticles of smaller width.*

***Key Words**: Silver Nanoparticles, Biosynthesis, Taguchi Design, size.*

I. Introduction

Metal nanoparticles have attracted a great interest for scientific research and industrial application under the interdisciplinary field of nanotechnology. On the nanoscale, materials often have unique physical and chemical properties. By increasing surface area with respect to the volume of nanoparticles; a corresponding increasing of importance of the behavior of the surface atoms can be observed which gives specific properties to the particles[1]. Therefore it is extremely important to control size and morphology of nanomaterials.

Taguchi method is introduced by Dr. Genichi Taguchi in 1980[2]. Early traditional designs of fractional factorial experiments used for optimization was focus to determine and control the source of variation. While Taguchi experimental design is emphasis on to make the process or product insensitive to those sources of variation. This approach is called "robust design" and it is a central theme of Taguchi Method[3, 4]. Therefore, Taguchi methodology is used to achieve a predictive knowledge of a complex, multi-variables process with the fewest possible trials and optimization of the experimental process itself. Here Taguchi structured approach was used to determining the best combination of inputs to produce a product based on a design of experiments (DOE) methodology for determining parameter levels such as silver, biomass and temperature. Taguchi method is applied to analyze the mean response for each run in the inner array and to analyze the variation using as appropriately chosen S/N ratio, which can be calculated by the following equation (1) [5,6].

$$S/N = \frac{-10\log\left(Y_1^2+Y_2^2+\cdots+Y_n^2\right)}{n} \qquad (1)$$

Where, Y is the size of nanoparticles and n is the replication number of the experiment. The S/N ratios are different according to the type of the characteristics. Experimental data were processed with the "small the better" quality characteristics (a) to determine the optimum conditions for the silver nanoparticles synthesis, (ii) to identify significance of individual parameters, and (iii) to determine the significant parameters in the process by applying ANOVA[6].

In this paper, we focus on synthesis of silver ultrafine particles via a simple, fast and eco- friendly biological method. The synthesis of silver nanoparticles has been reported previously using microorganisms like bacteria, fungi and actinomycetes, yeast, etc[8-13]. But very less report on the development of protocol to obtained specific size of silver nanoparticles. On the other side low- dimension nanostructures have wide applications in various fields of science like in medicine and technological aspects[14, 15].

The aim of this study is to investigate the effect of chosen experimental parameters on the diameter of silver particles and to find best experimental conditions for synthesis of ultrafine particles of silver by biological method. Therefore, experimental is design to investigate the effect of parameters like silver ion, biomass of fungi and temperature on size of silver nanoparticles in this study.

II. Materials and Methods

1) **Fungal strain and growth conditions**

Fungus *Asperillus terrerus* (Thom) MTCC 6324 was obtained from Microbial type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh. The fungal culture was grown on medium containing g/100ml: Glucose 1.5, Peptone 1.0, Yeast extract 0.35 and KNO_3 0.35 at 28^0C for 72h in incubator shaker at 150 rpm speed. After incubation, the biomass was separated by filtration and wash thrice with deionized water to remove medium impurities from biomass. The washed biomass was dried at 50^0C and mill in to fine powder. The powder fungal biomass was further used for study.

2) **Synthesis of silver nanoparticles and optimization experiments:**

Analytical- grade silver nitrate was used as received from Fisher Scientific, Mumbai. The silver particles were synthesized by adding Ag^+ solution at various concentration and fungal biomass of at various temperatures (Table 2). The experiments were performed in triplicate and all flasks were kept at 150rpm for 24h in incubator shaker (Steelmet).

To optimize experimental parameters for the synthesis of silver nanoparticles, an experimental design approach was applied. The level of variables (silver concentration, amount of biomass and temperature) used during study were shown in Table1. To design the experiments following steps were used:

1. Problem statement and objective of the experiment
2. Identification of factors and interactions
3. Choice of factor levels
4. Selection of orthogonal array
5. Assignment of factors
6. Experimental setup
7. Statistical data analysis-
   i. ANOVA to determine the significant parameters in the process
   ii. Draw main effect plot, S/N ratio plot and analyze the optimal level of control variables
8. Interpretation and experimental conclusion.

The factors used during study were namely, concentration of silver (1 to 10mM/ml), amount of dried biomass (0.1 to 1.0gm/100ml) and temperature of the synthesis process (20 to 45 ^{0}C). The experimental was carried out as indicated in Table 2 where each row represents one experimental run. After conducting experiments, size of nanoparticles was observed as response. For statistical calculations and modeling Minitab (version 16) software was used.

3) **Characterization of silver nanoparticles:**

All synthesized samples were characterized by Jas. Co V670 UV- visible spectrophotometer where spectrum of dried powder was record at wavelength 200 to 800nm. The scanning electron micrographs with EDS were recorded using JSM- 8360A JEOL. The size of the silver nanoparticles was determined by X-ray diffraction (XRD) studies using Burker 9XS, D8 advance model. The average particles size was obtained by using well known Scherrer's equation (2) from full width at half maximum (FWHM).

$$t = \frac{K\,\lambda}{\beta\ cos\theta_B} \qquad (2)$$

Where, t is thickness of crystallite, K is constant dependent on crystallite shape (0.89), λ is x- ray wavelength, β is FWHM or integral breadth, and θ_B is Bragg angle.

III. Results and discussion:

A. Synthesis and characterization of silver nanoparticles

The purpose of this study was to determine the effect of various parameters in size of silver nanoparticles. During synthesis, mixing of fungal biomass and silver ions in aqueous solution to form silver nanoparticles is a commonly used technology for biological synthesis of silver nanoparticles[16, 17]. The interaction between silver ions and fungal biomass is a complex process where fungal mycelium play important role[18]. The factors included in this study were silver concentration, amount of biomass, and the temperature of the solution. Factors and level tested are reported in Table 1 and 2. Also, data obtained by results of the experiments are given in Table 2.

In fungal mediated reduction of silver ions, silver was reduced from Ag^+ to Ag^0 in presence of various functional groups from fungal cell wall. This reduction was also responsible for change in color of biomass from pale white to brown. This color change is may be due to the excitation of surface plasmon vibrations of silver nanoparticles[19]. Zhang *et al* (2005) reported the process of entrapment of silver nanoparticles upon microbial cell wall[20]. This study indicate that the presence of carboxyl group of amino acid residues and the amide of peptide chains along with reducing groups like aldehyde and ketones were responsible for bio-reduction of silver to $[Ag(NH_3)_2]^+$ group[21]. Along with chemical groups some enzymes like Nitrate Reductase has been also reported by Anil Kumar, et al. which play important role in silver nanoparticles synthesis[22]. In fact it has been shown that presence of hydrogenanse and nitrate reductase is the essential element for metal reduction. Hence cell wall medicated silver nanoparticles synthesis can be possible while presence of carboxyl group of amino acid enhance the entrapment of particles intracellularly.

1) **SEM- EDS results**

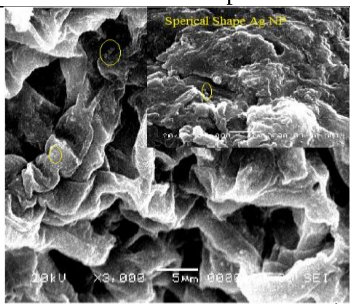
The shape and arrangement of silver nanoparticles was studied using scanning electron micrograph (SEM). The SEM image (Fig.1:A) shows that silver nanoparticles was present intracellularly and morphology of the synthesized nanoparticles was spherical in shape. Studies suggested that dried biomasses of microorganism are responsible for reduction of silver ions through interaction between silver ions and groups of microbial cell wall[23, 24]. The elemental composition of silver particle was studied using electron diffraction studies (EDS) indicted in Fig 1:B. The amount of silver present was 0.11 atomic weight % while due to presence of fungal biomass, carbon (65.48%), oxygen (34.15%), phosphorous (0.19%), sulphur (0.06%) were also identified in EDS analysis.

2) **UV- Vis spectrophotometery**

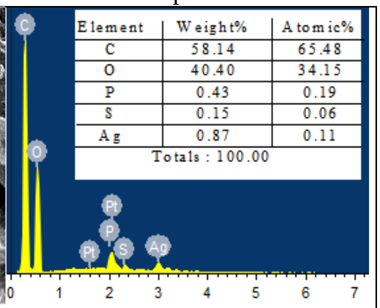
These bio- reduced silver particles were characterized by UV-Vis spectrophotometer and illustrated in Fig 1:C. The spectrum of samples was obtained at 200- 800nm and absorbance peak was observed at 290 nm. Similar peak was also observed by Yen San *et al* during their studied on fungal mediated biosynthesis of silver nanoparticles in intra as well as extracellular type[25].

3) **X-Ray Diffraction study of silver nanoparticles**

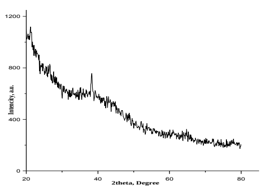
X-ray diffraction tool is known to calculate the approximate particle size of synthesized nanoparticles. During first phase of design of experiments, silver nanoparticles of particle size range 4.30 to 13.80 nm was obtained. As we know, properties of nanoparticles are highly size and shape dependent. On other hand smaller size particle were rapidly aggregate in absence of stabilizer or capping agent. But in biosynthesis of nanoparticles, various cell mediated bonds like –C-O-C-, -C-O- and –C=C= derived from heterocyclic compounds and the amide I bond derived from proteins were responsible for capping ligands of the nanoparticles[26]. This will help in synthesis and stabilization of smaller size of nanoparticles. Therefore, particle size 2.35nm was obtained was obtained in second phase of design of experiment. This indicates that biological synthesis of silver nanoparticles is relatively produce smaller size silver nanoparticles than other known process.



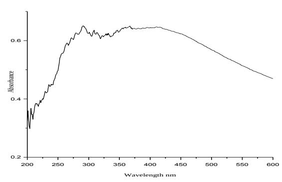
A. SEM image of silver nanoparticles



B.EDS of silver nanoparticles



C.XRD pattern of silver nanoparticles



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D.UV-Vis spectrophotomatery of silver nanoparticles

Fig 1. Shows- A) SEM image of silver nanoparticles embedded in cell wall of fungal biomass, B) elemental composition of synthesized silver nanoparticles was estimated by electron diffraction studies C) XRD graph of dried silver nanoparticles embedded fungal biomass, and D) synthesized nanoparticles was analyzed by UV- Vis spectrophotometer.

B. Taguchi's Experimental Designs

In this study, L_9 (3^3) orthogonal array design was used for the three variables with three different levels for which each pair of the columns had all possible combination of levels and their effect on synthesis of silver nanoparticles were studied.

Table 1. Factors and their level of variation used during study

Factor		Levels		
		1st	2nd	3rd
A	$AgNO_3$ (mM/ml)	6.0	8.0	10.0
B	Biomass (g100ml/)	0.3	0.5	0.7
sC	Temperature (^{0}C)	35	40	45

Table 2. Taguschi's L_9 (3^3) Orthogonal Array Design experimental setup

Sr.No.	Factors			Mean of NP size
	A	B	C	
1.	1	1	1	6.4
2.	1	2	2	3.95
3.	1	3	3	2.23
4.	2	1	2	12.7
5.	2	2	3	5.65
6.	2	3	1	5.15
7.	3	1	3	4.35
8.	3	2	1	6.1
9.	3	3	2	6.65

1**) Main effect plot:**

In this study, the effect of silver and biomass concentration on the particle size of silver at three different levels (1. 2, and 3) was investigated. Main effect plot for the nanoparticles size reduction using intracellular biosynthesis is shown in Fig 2. The main effect plot was used to visualize the relationship between the variables and their response in the form of size of silver particle[2, 6, 25, 27]. It was found that the concentration of silver ions is a significant parameter for the control of size of silver nanoparticles. The effect of initial amount of silver is indicated by factor 'A' which shows that size of nanoparticles decreases with decrease in amount of silver.

The effect of factor 'B' as concentration of fugal biomass on the width of silver particles was investigated. Our finding showed that the size of silver nanoparticles decreases as it reaches to medium level in amount of biomass. Also effect of the temperature factor 'C' on the size of particles was studied. Three different temperatures (35, 40 and 45^{0}C) were investigated. The results of this study showed that the size of silver nanoparticles decrease as it reaches to maximum level of temperature.

2) **Signal to noise (S/N) ratio: Main effect for S/N ratio and optimization of S/N ratio**

Taguchi method was used to identify the optimal conditions and influencing parameters on size of silver nanoparticles. For this purpose the obtained experimental data were processed with the "small the better" quality characteristics. In this experimental setup, we used smaller S/N ratio is better for size of silver nanoparticles synthesis. Fig. 3 shows the S/N ratio of size of silver nanoparticles reduction using *Asperillus terrerus* (Thom) MTCC 6324 mediated biosynthesis.

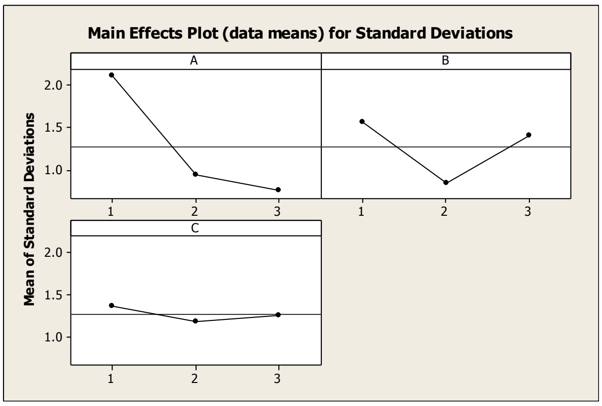

Fig.2. Taguchi design generated main effect plot of each variables

The S/N ratio was determined using equation no.1 and found that the interpretation of plot of S/N ratios is opposite to main effect plot. The results of S/N ratio study showed that at higher S/N ratio smaller width particle size was obtained. In other words, higher level of factors (A, B, C) gives us smaller size of silver nanoparticles.

The factors involved in synthesis process were optimized based on S/N ratio and a result obtained was showed in Fig 3. It has been found that the optimum performance of each factor were based on larger the S/N ratio smaller the particle size. While Fig 3. Shows that the optimal operating condition of experimental parameters are A(1), B (3), and C (3).

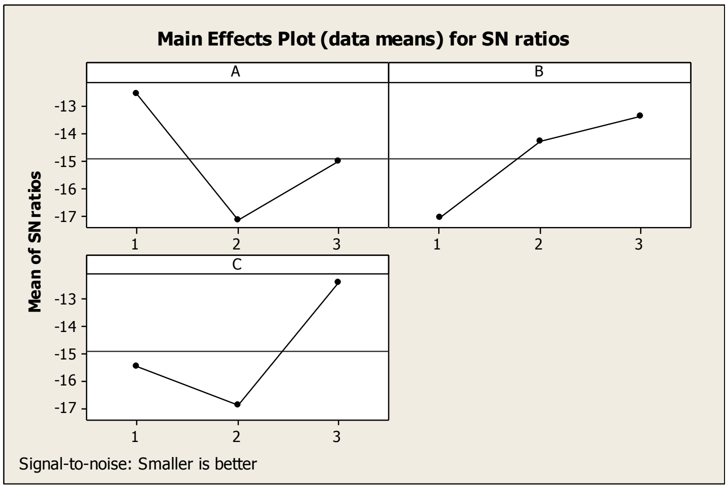

Fig 3.Main effect for S/N ratio

3) **Analysis of variance (ANOVA):**

Analysis of variance (ANOVA) of these experiments was studied for statistical significance using p- value tool. The significant contribution of each parameter on determining width of silver nanoparticles was studied at 0.5% level of significance. At this level none of the factors (silver and biomass concentration, temperature) have contributed significantly. As showed in Table 3.

The results of ANOVA indicated that enhancing concentration of silver solution from 0.6mM to 10mM/ml increased the size of silver nanoparticles increased. The biomass concentration also has influence on size of silver nanoparticles and it has been found that higher the concentration of biomass better was the nanoparticles size. On the other hand 45^0C temperature was the optimum temperature for synthesis of smaller size silver nanoparticles.

Recall that for silver nanoparticles with smaller diameter, the study of the main factor shows that the optimum conditions proposed according to the results of ANOVA are: 0.6mM/ml concentration of silver solution, 0.7g/100ml concentration of biomass and 45^0C of temperature. Hence the optimum condition for smaller size silver nanoparticles synthesis was obtained at run 3 of Table 2 and has particle size 2.23nm. Also it has been proved by our obtained data that biological mediated synthesis can produce smaller size nanoparticles.

Table 3. Analysis of variance for S/N ratios

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
A	2	31.79	31.79	15.895	2.00	0.334
B	2	22.47	22.47	11.234	1.41	0.415
C	2	31.78	31.78	15.890	2.00	0.334
Residual Error	2	15.92	15.92	7.959		
Total	8	101.96				

Table 4: response for S/N ratios smaller is better

Level	**A**	**B**	**C**
1	-12.55	-17.07	-15.44
2	-17.15	-14.29	-16.89
3	-15.01	-13.35	-12.38
Delta	4.60	3.72	4.51
Rank	1	3	2

The S/N ratio study also used to find individual factorial importance in synthesis of smaller size silver nanoparticles and the results obtained was showed in Table 4. The result described as ranking of each parameter as per their importance in biosynthesis of silver nanoparticles as: concentration of $AgNO_3$ is important and first essential component than temperature as second essential factor while biomass is present in third place. The contributions of all the factors were shown in Fig. 5 which illustrated as, 37% each is contributed by silver concentration and temperature while 26% contributed is by biomass. Hence the optimal operating condition of the parameters is shown in Table 5.

Table 5. Comparison between predicted and obtained value for confirmation of Experiment

Optimal set of parameters	**Predicated optimal value**		**Actual value**
A_1, B_3, C_3	Mean	S/N ratio	2.185
	2.23	-9.2787	

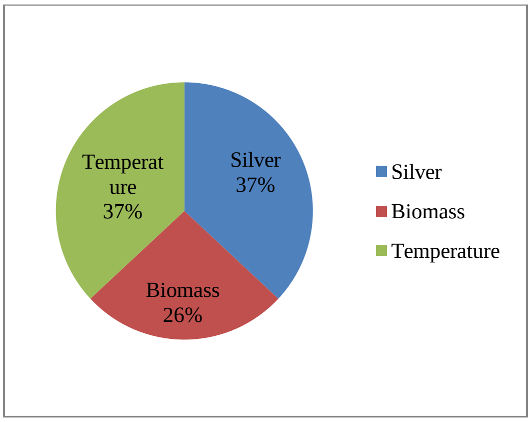

Fig 4. Percent Distribution of each variable in synthesis of silver nanoparticles

IV. Conclusion

In summary, we have studied a eco-friendly, rapid and efficient method for the synthesis of silver nanoparticles synthesis using fungus *Asperillus terrerus* (Thom) MTCC 6324. Silver nanoparticles have lot of applications in various fields like antimicrobials, preservatives, paints, biosensors and cosmetics. Due to these vast applications, synthesis of sliver nanoparticles with well-defined particle size were focused area.The Taguchi design of experiment was applied for the optimization of reaction conditions. Experimental parameter such as silver ion concentrations, amount of biomass and temperature were fund play significant roles in controlling size of silver nanoparticles. The experiments proved that by using biological mediated synthesis, silver nanoparticles could be synthesized at optimum conditions. The data obtained by various analysis like XRD, UV- Vis spectrophotometer and SEM also proven that silver nanoparticles were synthesized intracellularly and has approx. diameter of 2.18nm. This research requires further research on the effect of different parameters like pH of the reaction, using wet biomass, and time taken to complete the reaction.

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