Procedure optimisation for green synthesis of silver nanoparticles by Taguchi method

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This study is dealing with optimisation of the green synthesised silver nanoparticles (AgNPs) using the Taguchi design. Orthogonal array of L_9 type was used as an experimental design to detect the optimum conditions for synthesis of AgNPs. AgNPs were synthesised using the extract of *Mentha longifolia* as a reducing agent. In addition to the colour changes, ultraviolet visible spectroscopy was used to attest the appearance of AgNPs. Meanwhile, yielded AgNPs were characterised by FT-IR, scanning electron microscopy and dynamic light scattering (DLS) technique. UV–visible spectrophotometer showed absorbance peak at 340 nm. DLS analysis showed that the synthesised AgNPs are 21.1 nm in optimum conditions. The optimised condition for the synthesis of AgNPs revealed that silver nitrate concentration was 10 mM, temperature was 60°C, pH was 9 and plant extract concentration was 3%. The results demonstrated that AgNPs can be synthesised by controlling silver nitrate and plant extract concentration, pH and temperature. The minimum diameter predicted by statistical model was 35 nm while the obtained diameter was 21.1 nm which is very close to the experimental designed measure. This indicates that the statistical predicated proposed by Taguchi was correct.

1. Introduction: During the last decade, metal nanoparticles have gained a great increasing attention on scientific research because of their specific properties on the nanoscale such as large surface area and structures as compared with bulky particles [1]. Nanotechnology is the field of science that involves the synthesis and development of different nanomaterials with size <100 nm [2].

By increasing surface area on the nanoscale, a corresponding increasing of the behaviour of the surface atoms can be observed which gives specific properties to the particles. Therefore it is extremely important to control size and morphology of nanomaterial [3]. Taguchi method was recommended by Dr. Genichi Taguchi in 1980. This method focuses on making the process or product insensitive to sources of variation [4].

Green synthesis techniques for the synthesis of AgNPs, have some valuable benefit over the chemical and physical nanoparticles synthesis methods [5–9] and between microbes such as bacteria and fungus [10], and a variety of plant (seed, leaf) extracts [11, 12] for green synthesis of metal nanoparticles, plant leaf extract has been gaining importance because of its cost-effectively, environmental compatibility and simplicity. Plants extract has secondary metabolites that can easily reduce silver nitrate to silver nanoparticles (AgNPs) [13].

Mentha longifolia L. (*M. longifolia*) that belongs to the Labiataes family, usually known as a wild mint named Puneh [14]. Different activities have been reported for various species of Mentha like antibacterial and antifungal properties [15].

Using of Taguchi method makes it possible to provide an acceptable formulation using minimum raw materials and save time [16, 17]. The main goal of Taguchi method is to analyse all factors simultaneously using a few experiments [18]. Software that implements this method can be utilised to optimise different formulations within a given set of independent variables [19–21].

In this Letter, Taguchi robust design was used as a mathematical and statistical techniques and the essential parameters were considered. After each synthesis, the nanoparticle size was measured by dynamic light scattering (DLS) to realise the effect of chosen experimental parameters on the diameter of silver particles and to investigate best experimental conditions for synthesis of AgNPs by the biological method.

For this purpose, the effect of various parameters including plant extract concentration, silver ion concentration, the temperature of reaction and pH was evaluated.

2. Materials and methods

2.1. Materials: Silver nitrate ($AgNO_3$) was purchased from Merck Company. Also, fresh leaves of *M. longifolia* were purchased from local market Tajrish, Iran and a botanist authenticated it.

2.2. Preparation of the extract: Healthy leaves were collected, rinsed thoroughly by distilled water and allowed to dry at room temperature. They were incised into fine pieces and the required amount of dried chopped leaves taken into a flask of a certain amount of distilled water. The beaker was boiled for about 15 min at 100°C. The aqueous extracts (3, 5 and 7%) were filtered using Whatman paper no.1 pore size 125 mm and then by 25 mm and were then collected in 250 mL Erlenmeyer flasks and stored in refrigerator for further use.

2.3. Synthesis of AgNPs and optimisation experiments: To study the optimum parameters for AgNPs synthesis, Taguchi method was applied. Nine experiments were designed with three levels including aqueous solution (1, 5 and 10 mM as levels 1, 2 and 3) of silver nitrate, temperature (40, 60, 80°C as levels 1, 2 and 3), pH (5, 7, 9 as levels 1, 2 and 3) and aqueous extract (3, 5 and 7% as levels 1, 2 and 3). After conducting experiments, size of nanoparticles was considered as response. Minitab (version 16) software was used for statistical calculations.

Aqueous solution (1, 5 and 10 mM as levels 1, 2 and 3) of $AgNO_3$ was prepared for the synthesis of AgNPs. 0.5 mL of the $AgNO_3$ aqueous solution was drop wise added to 7 mL of aqueous extract. By de-ionised water the volume was adjusted to 10 mL and heated on the stirrer in dark condition at 150 rpm and required temperature (40, 60 and 80°C as levels 1, 2 and 3) for 2 h. The pH of the solution adjusted to determinate pH (5, 7 and 9 as levels 1, 2 and 3) using 0.1 N HCl or 0.1 N NaOH solutions). The colour change of solution from yellow to dark brown confirms the formation of AgNPs. Meanwhile, the formation of AgNPs was approved by spectrophotometric analysis. Obtained solution was centrifuged at 10,000 rpm for 10 min, subsequently washed three

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times with distilled water. The dilute colloidal solution was dried at room temperature and collected. Finally, the colloidal mixture was sealed and stored properly for future use.

2.4. Characterisation of AgNPs: The UV–vis spectrum analysis of the reaction mixture was done by using a double beam spectrophotometer (SHIMADZU Prestige-21 (Japan)) in a wavelength range between 200 and 800 nm. The synthesised AgNPs analysed by KYKY (EM3200) scanning electron microscopy (SEM) for morphology identification and DLS measurements were done by a Malvern Zeta size analyser (Zetasizer Nano Z) instrument (Malvern3600 Instruments Ltd., Malvern, UK) and finally the dried nanoparticles were analysed by FTIR-Nicolet IR100 spectrophotometer.

3. Results and discussion: In this study focused on detecting the effect of various parameters in size of AgNPs. Mixing of the $AgNO_3$ aqueous solution and aqueous extract to form AgNPs is a commonly used technology for green synthesis of AgNPs. Biosynthesis of AgNPs was exhibited by colour developed in solution during of reaction. The colour change from yellow to dark brown proved the reduction of silver ions from Ag^+ to Ag^0.

3.1. UV–visible results: Reduction of silver nitrate to silver particles was characterised by UV–vis spectrophotometer. The nanoparticle solution showed maximum absorbance at 340 nm. (Fig. 1). Velhal *et al.* [3] saw alike peak during their studied on size-controlled synthesis of AgNPs.

3.2. SEM and DLS results: The shape and arrangement of AgNPs was recognised using SEM. The images showed that synthesised AgNPs are spherical in shape (Fig. 2) [8]. DLS indicates the average particles size of the biosynthesis AgNPs present in solution. After conducting nine experiments designed by Taguchi method, size of nanoparticles was considered as response as shown in Table 1. Histogram of size distribution reported by intensity is indicated in Fig. 3.

3.3. FTIR results: FTIR analysis was used to detect the possible biomolecules responsible for the reduction of the silver ions into AgNPs. The FTIR spectrum indicated peak at 3388 cm^{-1} and other peaks were obtained at 2922, 1598 and 1404 cm^{-1}. The bands at 3388 and 2922 cm^{-1} were assigned to the stretching of primary and secondary amines, respectively, the peak at 1598 cm^{-1} may be due to C=C stretching and the band observed at 1404 cm^{-1} can be assigned to the C–N stretching vibrations of aromatic groups (Fig. 4) [11, 13].

3.4. Taguchi's experimental designs: In this Letter, L_9 (3^3) orthogonal array design applied for the four variables with three different levels and their effect on synthesis of AgNPs were investigated (Tables 1 and 2) [18].

3.5. Main effect plot: In this study, the effect of silver nitrate concentration, temperature, pH and plant extract concentration on the particle size of silver at three different levels (1, 2 and 3) was investigated. Main effect plot of the nanoparticles size is shown in Fig. 5. The main effect plot was used to show the relationship between the variables and their response in the form of the size of the silver particle. It was found that by increasing pH from level 1 (pH = 5) to level 3 (pH = 9), the particle size decreases. The results show that changing this factor is effective on the size of particles. Also, temperature is a significant parameter for the control of the size of AgNPs. As temperatures rise from level 1 (40°C) to level 3 (80°C), increase of the nanoparticles is observed. Our finding showed that the size of AgNPs increases as it reaches to medium level in the amount of silver nitrate

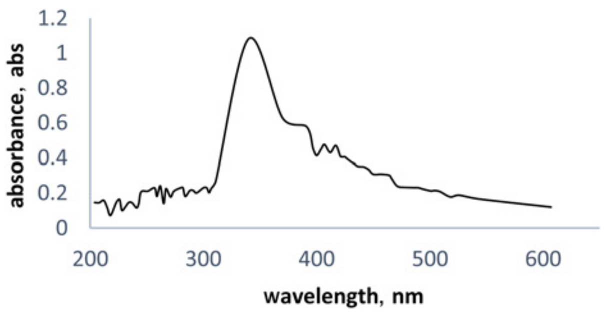

Fig. 1 *Enlarge image of UV–vis absorption spectrum of AgNPs*

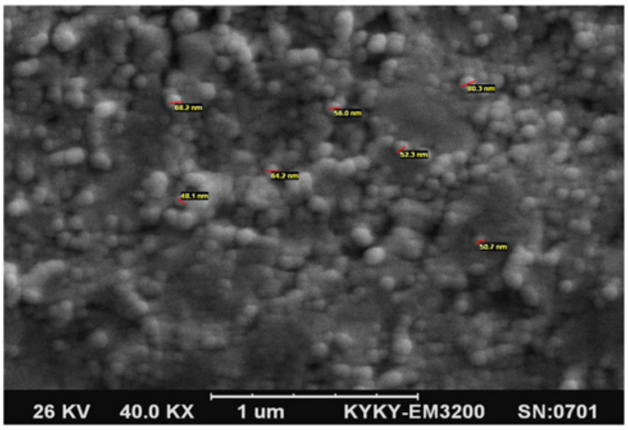

Fig. 2 *SEM image of spherical nanoparticles synthesised by aqueous extract of M. longifolia*

Table 1 Taguchi orthogonal array design. Each row represents a test, numbers show the levels, A factor represents the first variable (pH), factor B represents the second variable (temperature), C factor represents the third variable (plant material), D factor represents the fourth variable (silver nitrate concentration)

Trial number	A	B	C	D	Diameter NP, nm	PDI
1	1	1	1	1	120	0.342
2	1	2	2	2	260	0.387
3	1	3	3	3	301	0.702
4	2	1	2	3	41.7	0.346
5	2	2	3	1	84.2	0.959
6	2	3	1	2	228	0.464
7	3	1	3	2	126	0.408
8	3	2	1	3	21.1	0.493
9	3	3	2	1	68.1	0.738

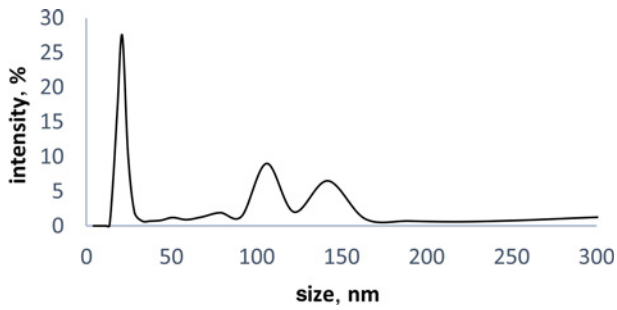

Fig. 3 *Histogram of size distribution report by intensity*

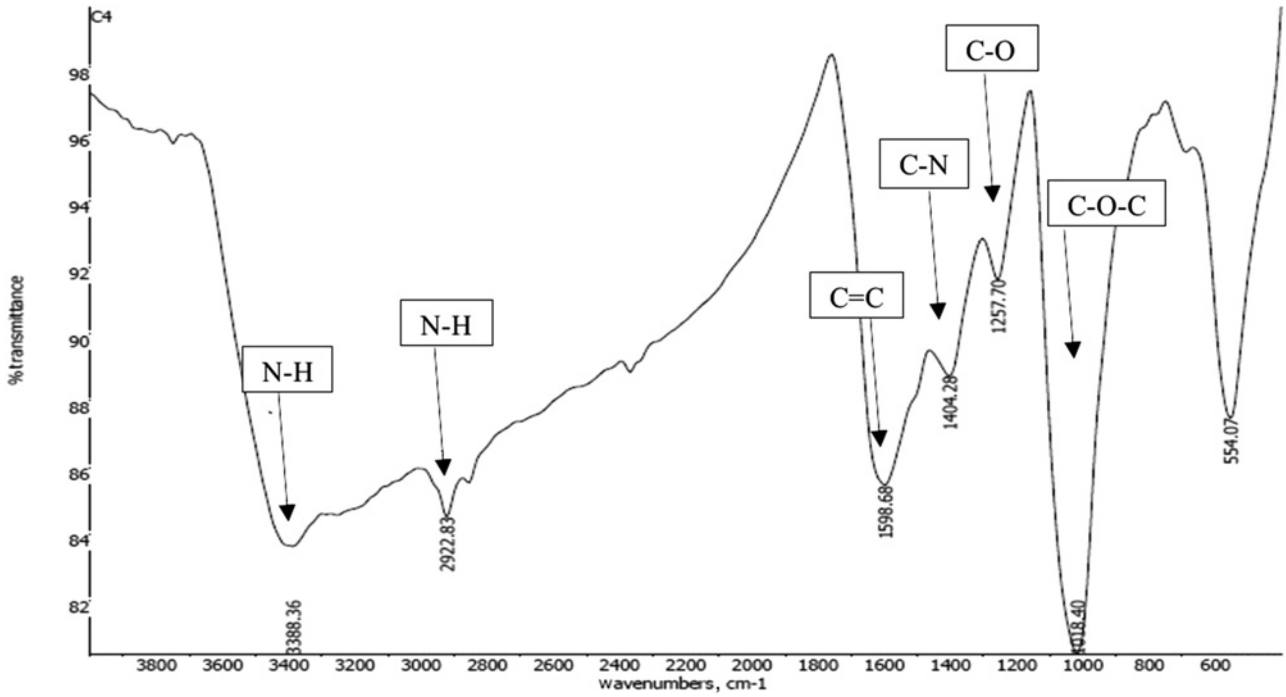

Fig. 4 *FT-IR spectra for AgNPs prepared at optimum conditions which showed the possible biomolecules responsible for the reduction of the silver ions into AgNPs*

Table 2 Factors and their level of variation used during study

	Factor	Level 1	Level 2	Level 3
A	pH	5	7	9
B	temperature, °C	40	60	80
C	plant material, %	3	5	7
D	silver nitrate concentration, mM	1	5	10

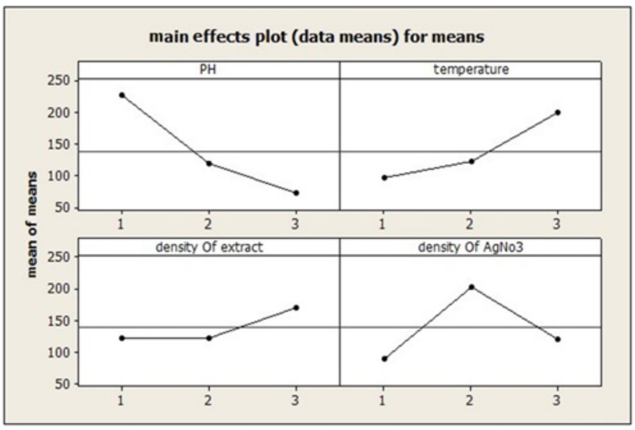

Fig. 5 *Main effect plot of AgNPs synthesised by aqueous extract of M. longifolia. The relationship between the variables and the answers received shows the size of the nanoparticles formed*

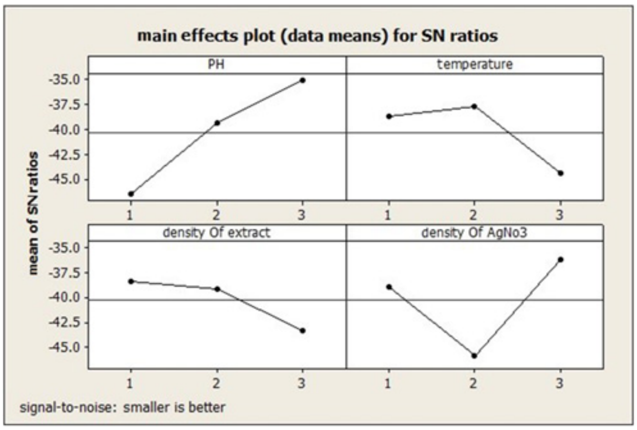

Fig. 6 *S/N ratio in the synthesis of AgNPs synthesised by aqueous extract of M. longifolia*

concentration and changing plant extract concentration from levels 1 to 2 had no effect on particle size. While high concentrations of extracts (level 3) caused particle size increase [20].

3.6. Main effect plot for signal-to-noise (S/N) ratio: Taguchi method was applied to detect the optimal conditions and influencing parameters on minimising the size of AgNPs. For this purpose, the obtained experimental data were analysed with the ‘small the better’ quality characteristics. In this study, smaller S/N ratio is better for the size of AgNPs synthesis. Fig. 6 shows the S/N ratio of the size of AgNPs. The S/N ratio was detected by equation (1) and the results show that this plot is opposite to main effect plot. It was found that at higher S/N ratio smaller width particle size was collected. So optimal conditions according to this plot were level 3 for plant extract concentration, level 2 for pH, level 1 for temperature and level 3 for $AgNO_3$ concentration [21]

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

3.7. Regression analysis: In this study, only machining parameters such as pH, temperature, plant material and silver nitrate concentration are analysed so least square linear regression was

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used. The regression equation was calculated by the mean values of different operating condition.

The equation is as follows:

$$\text{result} = 183 - 64.0\,\text{pH} - 17.2\,\text{temperature} + 33.3\,\text{density of extract} + 26.4\,\text{density of AgNO}_3$$

4. Conclusion: The plant *M. longifolia* belonging to the family Labiatae was selected and used in this report on the synthesis of AgNPs from its leaf extracts and to realise the optimised conditions for AgNP synthesis in this plant. Optimisation of the procedure variables is essential to achieve the optimum conditions of AgNPs formation. The operating factors involved in this process were the concentration of silver nitrate, *M. longifolia* leaf extract, pH, and temperature. Absorption spectra of AgNPs have absorbance peak at 350 nm and broadening of peak showed that the particles are poly dispersed. Optimum conditions involved in this study were: temperature = 60°C, concentration of silver nitrate = 10 mM, pH = 9 and concentration of leaf extract = 3%.

AgNPs were synthesised at optimum conditions 21.1 nm as shown in DLS. This study exhibits the potential of *M. longifolia* aqueous extract for the simple and green synthesis of AgNPs which is rapid, cost effective and environmentally safe. This method can be used in different biotechnological applications.

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