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# Green Synthesis of Silver Nanoparticles Using Papaya Seed and Its Characterization

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Abstract: Inexpensive and environmentally friendly papaya seeds are used as a reducing agent in a cost-effective method for the synthesis of silver nanoparticles (AgNPs). The Response Surface Methodology (RSM) based on central composite design (CCD) was used to evaluate and optimize the effect of independent variables, temperature and concentration of reducing agent. Characterization of synthesized AgNPs were done by using nanoparticle size analyser, Fourier transform infrared spectroscopy (FTIR), Transmission electron microscopy (TEM), Energy Dispersive X-ray Spectroscopy (EDX) and X-ray diffraction technique (XRD). Synthesized spherical AgNPs were having particles range of 12 to 69 nm. This study established by the FT-IR that the papaya seeds were responsible for reduction and capping of the AgNPs.

Keywords: Central composite design, FTIR, nanoparticles, papaya seeds, silver.

## I. INTRODUCTION

Nanotechnology refers largely to a field of applied science and technology to control matter on the molecular level. There is incredible growth in nanotechnology and its application in engineering and materials science. It has broad collection of applications in areas such as mechanics, biomedical sciences, chemical industry, electronics, space industries, optics, energy science, catalysis, drug-gene delivery, [1-2], optoelectronic devices [3-4],nonlinear optical devices [5-6], and photo electrochemical applications [7].Silver is a health additive in traditional medicines and has been used since 1000B.C. It acts as an antibiotic as it is a non-selective toxic 'biocide'. Biocides are silver-based antimicrobials used for wood preservatives. In water usage, to control infectious agents, silver based disinfectants are used in hotels, hospitals and distribution systems. It was also used to sterilize recycled water in MRI space station and in the NASA space shuttle [8-9]. AgNPs possess antibacterial tendency due to its huge surface to volume ratio, high reactivity, by inhibiting bacterial growth in solid and aqueous media. Antibacterial property of silver containing materials can be used to remove microorganisms from textile fabrics [10] and also for water treatment [11].

AgNPs have been prepared in various ways with different morphologies as well as size distributions including  $NaBH_4$  reduction, use of plant extracts, polyol process, photo reduction, electrochemical, thermal decomposition of silver compounds, sono chemical, microwave assisted process, radiation assisted, chemical and photochemical reactions in reverse micelles and green chemistry way [12-16]. Amongst various pathways, green biological method shows a promising potential. These methods are found to be better due to slower kinetics, better manipulation and control over crystal growth and their stabilization [17].

The advantage of suing environmentally benign materials like bacteria, fungi and enzymes and plant extract for synthesis of AgNPs have gained importance for being eco-friendly and compatible with biomedical and pharmaceutical applications as well as no use of toxic chemicals [18]. Researchers have studied synthesized AgNPsby using extracts of variety of plant leaves such as Cardiospermumhelicacabum, Coriandrumsativum, Magnolia kobus, Diopyros kaki, Cinnamomumcamphora, Geranium, Gliricidiasepium, Acalyphaindica, Sorbusaucuparia, Rose, Camellia sinensis, and Neem [19-23].

Present research work is concerned with the investigation of the effect of temperature and concentration of reducing agent on synthesis of AgNPsby biological method using papaya seeds as a reducing agent by applying RSM.

## II. MATERIALS AND METHOD

## A. Materials

Silver nitrate and acetic acid were purchased from S. D. Fine-Chemicals Ltd (SDFCL), Mumbai, India. Papaya seeds were obtained from the canteen of the ICT hostel, Mumbai, India.

## B. Synthesis of AgNPs

Papaya seed were washed with distilled water and dried at 50°C in oven for 24 hours till it became brittle and was crushed to powder form.



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The experiments were performed using shaker bath of RossariLabtech, Mumbai, India. 0.01M silver nitrate solution was prepared using distilled water in 100 ml conical flask by adjusting the pH 3using acetic acid. Desired amount of papaya seeds were then added to this solution and were stirred continuously at 70 rpm to keep the papaya powder suspended. After 2 hour solution was withdrawn .The colloidal brown color obtained from colourless silver ion solution confirms the formation of AgNPs [24]. After complete reduction, this solution was filtered through nylon mesh and then centrifuged for 15minutes at 12000 rpm, the residues were further washed with distilled water and dried in vacuum oven at 80°C.

### C. Experimental Design

Optimization studies were performed using RSM based on central composite design (CCD) for independent variables viz. temperature and concentration of reducing agent on absorbance of the solution as a response as is shown in Table I.

#### D. Characterization

UV-Visible spectrophotometer (UV-1800 ENG 240 V, Shimadzu, Japan) was used to confirm the formation of nanoparticles as a function of time in the range of 200-800 nm with a resolution of 1 nm. The particle size analysis was carried out using nano particle size analyser (SALD 7500 nano, Shimadzu, Japan). The morphology of the nanoparticles was studied by a Scanning transmission electron microscopy (Phillips TEM-200 Supertwin STEM, accelerating voltage-200kV, resolution-0.23 nm). The crystal structure of NPs was investigated by X-ray diffractometer (Shimadzu XRD-6100, Japan) with 20 scope of 10–80° using CuK $\alpha$  X-ray source. Fourier transforms infrared spectra (FTIR) were recorded at room temperature using FTIR (FTIR 8400S Shimadzu, Japan) in the spectral range of 750-4000 cm<sup>-1</sup> and elemental analysis was done in the Na-U channel using EDX (EDX-720, Shimadzu, Japan).

#### III. RESULTS AND DISCUSSION

#### A. Optimization analysis

A second-order polynomial model was considered to calculate the optimal levels of the temperature (A) and concentration of reducing agent (B) as summarized in Table I by determining the absorbance of the solutions.

	Temp. (°C)	Conc. of Reducing agent (gm/100 ml)	Absorbance at 431 nm		
Run			Experimental	Predicted	
1	75	7.5	0.1739	0.16021	
2	100	5	0.1474	0.139677	
3	75	2.5	0.0602	0.06891	
4	75	5	0.0993	0.106076	
5	50	2.5	0.0391	0.032128	
6	100	7.5	0.1752	0.184661	
7	75	5	0.0894	0.106076	
8	50	7.5	0.1375	0.141728	
9	75	5	0.1318	0.106076	
10	100	2.5	0.1134	0.111661	
11	75	5	0.0981	0.106076	
12	50	5	0.0757	0.078444	
13	75	5	0.1068	0.106076	

#### Table I- Ccd Experimental Run Of Trials For Synthesis Of Agnps

The results of ANOVA are shown in Table II. The P-values is a tool that assists to determine the significance of each coefficient which also indicates the interaction strength of each parameter. For the present experiment, the F-value (17.09) and P-values (p = 0.0008) testified the statistical significance of the obtained model. It was shown that the quadratic effects of input parameters were significant from the degree of significance level as they act as limiting factors and little variation in their value alter the production



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rate. Adjusted  $R^2(0.8701)$  depict that total variation of 87.01% of absorbance is recognized and only 12.99% cannot be explained by the model [25].

	5		, I		
	Sum of		Mean	F	p-value
Source	Squares	df	Square	Value	Prob> F
Model	0.018786	5	0.003757	17.09	0.0008
Pure error	0.001045	4	0.000261		
$\mathbb{R}^2$	0.9243				
Adj. R <sup>2</sup>	0.8701				

Table II- Analysis Of variance (Anova) For Oblimization Of Synthesis Of Agni	Table Ii-	Analysis O	f Variance	(Anova)	) For O	ptimization	Of Synthesis	Of Agnps
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In order to evaluate the interaction effects between response and independent variables, a second-order polynomial model (equation 1) was planned to calculate the optimal levels of these variables by determining the maximum absorbance. The results of experiments summarized are in Table I.

Absorbance =  $-0.071176+0.00124039A+0.015665B-0.000146400 \ AB+0.00000477517A^2+0.00135752 \ B^{2(1)}$ 



Fig. 1 Response surface plot of absorbance Vs temperature and concentration of reducing agent

Fig. 1 depicts that by increasing temperature and concentration of reducing agent, there was increase in absorbance of synthesized nanoparticles indicating increase in concentration of AgNPs. The quadratic model generated by RSM was used to calculate maximum absorbance achieved when the temperature and concentration of reducing agent were set at  $50^{\circ}$ C and 5.4 g/ 100mLrespectively which were the optimum conditions. The mean value of the absorbance obtained was 0.08736, which was in accord with the predicted value (0.0874).

## B. Characterization

## 1) UV- Visible spectral Analysis

UV-visible spectrophotometer was used to monitor the formation of AgNPs at different time intervals that is 15, 30, 45, 60 and 120 minutes as shown in fig. 2. The absorbance at 431 nm happens due to the surface Plasmon resonance phenomenon. [21, 26]. It was noted that the nanoparticle formation increases with time as more amount of reducing compounds present in the papaya seed leaches out.



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Fig. 2 UV-Vis absorption spectrum of AgNPssolutions at different time intervals

The visual change in the colour of the silver nitrate solution is shown in fig. 3. The reduction of silver nitrate into AgNPs converts the colourless solution into colloidal brown with increase in reaction time [27].



Fig. 3 Images of AgNPs solutions taken out at different time intervals

2) Particle size analysis : The determination of the particle size of the AgNP was analyzed in the nanoparticle size analyser, TEM and XRD (Fig. 4 a,b and c) and is given in Table III.From TEM image we can note that the particles were predominantly spherical in shape (Fig. 4b). Synthesized NPs ranged in size from 8 nm to 25 nm with an average diameter of 12 nm shape (Fig. 4a).



Fig. 4b TEM image of silver nanoparticles



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The peaks observed in fig. 4c at  $31.9039^{\circ}$ ,  $37.7965^{\circ}$ ,  $45.8762^{\circ}$  and  $64.94^{\circ}$  were for (101), (111), (200) and (220) Miller indices respectively which corresponds to the AgNPs.

The intense X-ray diffraction pattern clearly showed the crystalline nature of the nano particles (JCPDS card no 04-0783, 1991).[28] The crystallinity percentage was 37.59% which is evident from the (101) peak.

The average particle size of the nano particle was estimated from the Debey–Scherrer's formulas per equation. (2)

$$D = \frac{0.94\lambda}{\beta cos\theta} \tag{2}$$

Where,  $\lambda$  is X-ray wavelength (0.15406 nm),  $\beta$  is full width at half maximum (FWHM) of the diffraction peak in radians,  $\Theta$  is Bragg's diffraction angle respectively. [29] The particle size obtained was 11.96 nm. The results obtained by the XRD and TEM are in good agreement. However in case of nano particle size analyzer the particle size is little bit higher. This could be because of the different methodology involved in the measurement [30].

Table Iii- Particle Size Of Agnps Calculated By Different Methods

Crystallite size (nm)				
Debey-Scherrer formula	DLS	TEM		
11.96	69	12		

*3) Energy Dispersive X-Ray analysis:* The energy dispersive X-ray shown in fig. 5 was carried out to identify the presence of elemental silver due to the signals of the silver nanoparticles. The optical absorption bandpeak located in the range of 3 to 4 keV is typical for the absorption of metallic silver nanocrystallites. [31]



Fig.5EDX pattern of the synthesized silver nanoparticles

## C. FTIR Analysis

FTIR analysis was used to identify the chemical nature of the biomolecules responsible for capping and efficient stabilization of the metal NPs synthesized by papaya seed (fig. 6). The broad band at 3200cm<sup>-1</sup> can be due to the O-H stretching vibrations from different fatty acids and alcohols. We can see the clear change in the IR spectra around 2850cm<sup>-1</sup>, the decrease in the intensities of the C-H streaching bands might be due to the possible breakdown of the aliphatic chain while formation of nanoparticles. The intense sharp peaks at  $1741 \text{ cm}^{-1}$  are attributed to the carbonyl group (C=O) of carboxylic acid or ester which has become weaker



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for AgNPs [32]. The sharp peak around 2400cm<sup>-1</sup> was due to the nitrile groups from benzylisothiocyanate (BITC) present in the extract [33]. Thus we can postulate that different functional groups detected in FTIR spectrum are due to the presence of organic matter from the extract which are reducing in interacting with Ag ions either by binding or capping it [34].



Fig. 6 FTIR spectrum of papaya seed and AgNPs

## IV. CONCLUSION

The present research showed that AgNPs were synthesized by using papaya seed as a reducing agent. Papaya seeds performed the dual function of formation and stabilization of AgNPs due to the presence of polyols such as terpenoids and flavones naturally present in it. The nano particles were spherical in shape and crystallinity % was 37.59%. The optimal conditions were achieved at  $50^{\circ}$ C using 5.4 gm/100mL concentration of papaya seeds as per CCD for maximum absorbance of 0.0874. The particle size of the nano particles synthesized were in the range of 12 nm to 70 nm.

## V. ACKNOWLEDGMENTS

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## VI. COMPETING INTERESTS SECTION

The authors declare that they have no competing interests

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