Biosynthesis Of Copper Oxide and Its Optimization Utilizing Statistical method

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Abstract: Copper oxide nanoparticles were synthesized through novel eco-friendly biological method. Metabolits in Dalbergia sisso (Indian rose wood, shisham) leave extract played their role in capping and stabilizing of nanoparticles. Color of reaction mixture was found reddish to brownish black and UV- Vis spectrum found almost in ultra violets region. Parameters such as concentration (0.1mM-1mM), pH (7.5), time (12h-48h) and temperature (25˚C to 60˚C) are taken for statistical study and optimization of synthesis through Response Surface Method.

- Concentration, time and temperature were significant in ANOVA results. R^2, adjusted R^2, predicted R^2 were 99.63%, 98.96% and 94.85%.
- Optimum for maximum yield was found when 1mM precursor concentration, 48 hours reaction time and 25 degree Celsius temperature at pH 7.5 with 81 percent predictability. So that biological method was not only a eco-friendly but also effective method to control nanoparticles physical, chemical and electronic properties.
- UV- Vis spectrum shows considerable absorption in range 200-350 nm. Peak near 215 nm confirmed synthesis of copper oxide (CuO).

Keywords: *Dalbergia sissoo/*Indian rose wood/shisham, plant mediated nanoparticle synthesis, CuO nanoparticles, Response Surface Method, UV–Vis spectroscopy, Box–Behnken Design (BBD)

1. Introduction:

Nanotechnology word come from greek prefix which mean dwarf. Generally, in 10^{-9} is considered as nano but material that have dimension 1-100 nanometer(nm) is are taken as nanomaterial. The famous physicist dr. rechard feyanman introduced terms nanotechnology in his famous lecturer delivered at conference of American physical society 1959. This technique is based on changing material properties by reducing size in nanometer. Material in this size exhibits strange physical, chemical, biological, electrical, thermal behavior than their bulk counter parts. So that this field touched by almost every branch of science such as life science, chemistry, Physics, Engineering etc. (Benelmekki, 2015). Historically this technique is not new for our ancestors. Roman lycurgus cup (Wikipedia contributors, 2024b), medieval church-stained glass windows (*Early Nanomaterials*

- *International Institute for Nanotechnology*, 2021), Damascus sword (Yong, 2008) are some of examples found in history.

There are basically two approaches of synthesis bottom up and top down. Top down includes physical method such as mechanical milling, laser ablation, laser pyrolysis, physical vapor deposition, iron sputtering, inert gas condensation, lithography etc (Al-Harbi & Abd-Elrahman, 2024). Chemical method such as sol-gel, hydrothermal, spray drying, cryochemical are based on bottom-up approach. These methods expensive, toxic, required lots of energy, time consuming, environment hazardous.

Biological is novel cost effective, environment friendly, time saving method. Waste product of this method is biocompatible, has potential of large production of nanomaterials, well designed size and shapes, simple and fast, these are some of advantages of green or biological synthesis of nanomaterials. This method is getting popularity in modern days. In green method nanoparticles are synthesized through biological entities like bacteria, fungus, virus, plant, algae. Biological elements such as protein, metabolits, enzyme play role in reducing metal ions into nanoparticles. Diverse shapes of nanoparticles can be obtained like spherical, ring, rod, triangle, hexagonal etc. (Pandit et al., 2022).

Metal nanoparticles are known for their plasmonic, catalytic, magnetic, photocatalytic electric properties (Huynh et al., 2020). copper oxide can be use to waste water treatment by degrading antibiotics, (Nzilu et al., 2023), preventing fungicidal activity in foods (Gvozdenko et al., 2022), antibacterial (Černík & Padil, 2013), data storage devices, infrared photodetector (S. Wang et al., 2011). Copper nanoparticles in the form of cuprous oxide Cu_2O and cupric oxide CuO, copper hydroxide $Cu(OH)_2$ are synthesized with various methods. copper hydroxide $Cu(OH)_2$ is used as antifungal,

Dalbergia sisso plant also known as Indian rose wood belongs to legume family (Asif & Kumar, 2011). it is not only used is wood works, but it has medicinal properties. It is generally used throat infection, syphilis, gonoria, bronchitis, inflammations, infections, hernia, skin deseases, immunity disorders etc. (Mannan et al., 2017). This plant is generally found in sub–Himalayan Asian countries like India, Pakistan, Bangladesh, Bhutan and Ethiopia, Iran, Kenya, Nigeria, Sudan, Thailand etc. (Bhattacharya et al., 2014). This tree has some phytochemical that are responstible for nanoparticles synthiesis. They reduce the ions of metal atom and forms nanoparticles. Asif and Kumar (2011) and Al-Snafi (2017) report that this plant has various metabolites carbohydrates, proteins, amino acids, phenolic compounds, alkaloid s, saponin, phytosterols, steroids and tannins and flavanoids. But they are found in different parts of plants. Handayani et al. (2020) reports that pH value of extract and precursor salt solution affects size, shapes, stability of nanoparticles.

Optimization of nanoparticle synthesis is often done through statistical method. Researcher used area of UV-Vis absorbance graph, absorbance peak, size of nanoparticles as response or outcome in statistical method.

RSM is powerful tool to establish relation between input variable and output of experiment through regression analysis. This method relies on mathematical and statistical technique and use to predict new observation, optimize the process, effect of single or multiple input variable on output of experiment. Basically, this method is popular among engineering, research and manufacture. (Lamidi et al., 2022)

2. Materials and Methodology

2.1 Materials

Analytic grade copper sulfate petahydrate ($CuSO_4.5H_2O$), double distil water, whatmann no.1 filter, magnetic stirrer with hot plate (REMI -2 MLH), for pH measuring machine LABTRONICS digital pH meter (Model LT-11), a digital weighing balance for precise weight measurement, laboratory thermometer (0˚C -100˚C), refrigerator, and Philips grinder were used to desired synthesis condition. UV-Vis spectrum was taken from JASCO UV–Vis spectrophotometer (Model V-750).

2.2 Leaf Extract

Healthy leaves of Dalbergia Sisso are collected from botanical garden of Dr. Bhimrao Ambedkar College Sriganganagar Rajsthan. Plant and leaves are made authenticated from Prof. Rajendra Kadwasra department of botany of Dr. Bhimrao Ambedkar College Sriganganagar. Leaves were washed initially with normal water to remove dust. After it washed with distil water various times. Then leaves were shadow dried for two weeks at room temperature. Then dried leaves were ground to powder with domestic grinder and collected in bottle.

10 gm of obtained leave powder were taken and mixed in 100 ml distil water in 400 ml capacity beaker. Mixture heated at 60-70 ˚C for 1 hour. This process produced extract of leaves. This extracts contained various phytochemicals which helped in reducing copper ions in copper oxide nanoparticles. Leaf extract was viscous so that solution was first filtered with normal strainer and after it centrifuged various time at 8000 rpm. After multiple times of centrifugation , confirmation that no impurities was obtaining during filtration process, extracts was collected in sterile bottle and placed in refrigerator at 4 ˚C for further experimental use.

2.3 Sample Preparation

Different molarity (0.1 Mm to 1 Mm) solutions of copper sulphate pentahydrate ($CuSO_4.5H_2O$) was prepared by taking required amount in distill water. Keeping ratio of plant extract to precursor salt solution 1:4, 27 samples were prepared according to table 1.1 for Box-Bahken Design. pH of solution was controlled by 1N sodium hydroxide (NaOH) solution. Yield of each experiment was obtained through area of UV –Vis extinction. Table 1.1 and table 1.2 shown selected parameters, their ranges and coded values

Table 1.1 included factors for optimization process:

Parameters/factors	Experimental value	Coded value
Precursor Concentration (A)	0.10 mM	-1
	0.55 mM	0
	1.00 mM	1
Temperature (B)	25.0˚C	-1
	42.5 ˚C	0
	60.0 ˚C	1
Incubation time (C)	12 h	-1
	30 h	0
	48 h	1

Zayyoun et al. (2016) in his work found that at increasing pH from 6 to 12, cuprous oxide (bandwidth 2.04 eV) was produced initially and after it passed through some transition state convert into cupric oxide (4.08 eV). Color of reaction mixture was varying in each test tube. Color was ranging from brown to brownish black. Samples were collected directly from test tubes after 12 hours, 30 hours and 48 hours. Samples of plant extracts and precursor solution in same dilution as experimental design were also prepared. These samples were then analyzed through UV- Vis spectroscopy. Outcome experiments were analyzed through statistical software.

Table 1.2 Box-Bahken experiment design and obtained yield

Run	Concentration (mM)	Time (hours)	Temperature (˚C)	Yield
1	0.1	12	40	66.311
2	1.0	12	40	132.162
3	0.1	48	40	115.264
4	1.0	48	40	137.959
5	0.1	30	25	35.848

6	1.0	30	25	138.703
7	0.1	30	60	106.975
8	1.0	30	60	95.250
9	0.5	12	25	54.265
10	0.5	48	25	139.606
11	0.5	12	60	133.163
12	0.5	48	60	74.562
13	0.5	30	40	126.350
14	0.5	30	40	124.517
15	0.5	30	40	121.634

3. Result and Discussion

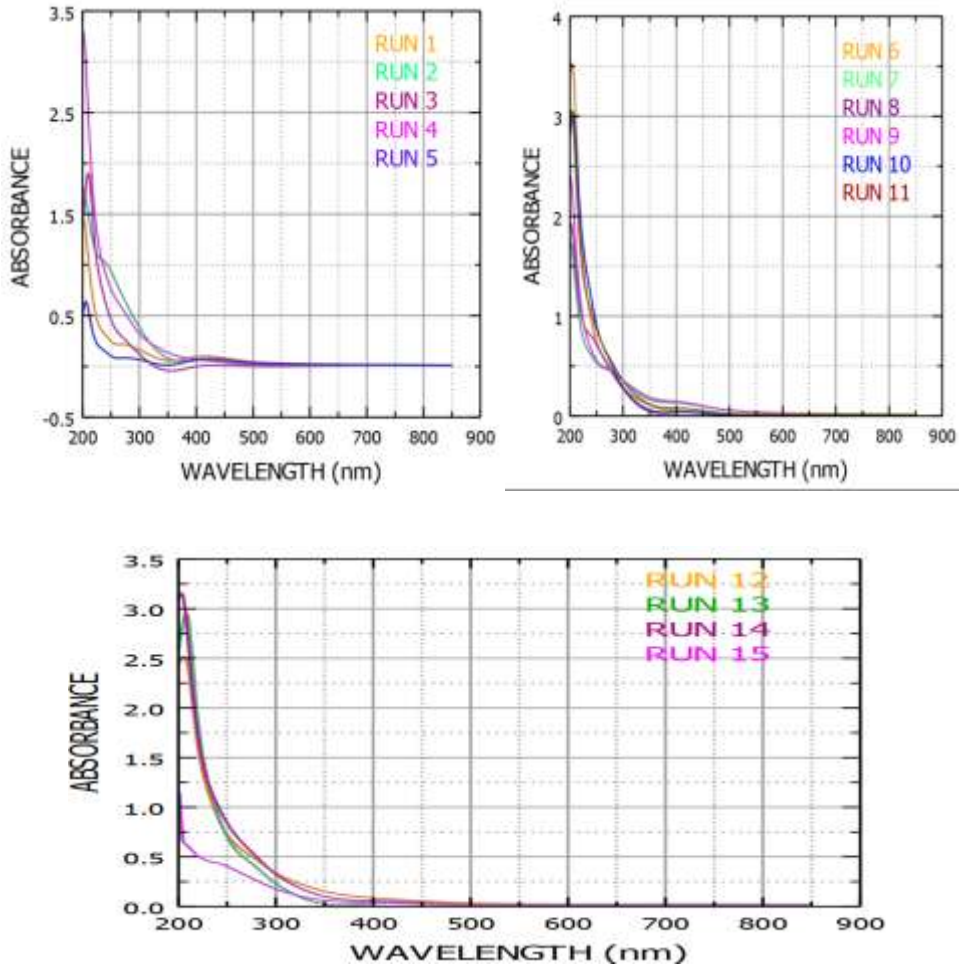


Figure 1.1: Absorbance graphs

Optimization of synthesis process was based on Response Surface Method (RSM). This is a power full method comprises of statistical and mathematical structure. Figure 1.1 shows UV- Vis graphs of various experimental designs.

UV-Vis spectrum shows that there were two peaks. One found in between 200-210 nm and another between 382-413 nm. A high absorbance peak found in ultra violet region and small speak found in visible region. Nzilu et al. (2023) in his study reported that formed nanoparticles shown absorbance in range 250nm to 450nm and SPR peak was on 340 nm. Sukumar et al. (2020) synthesized copper oxide nanoparticles from green route and found strong absorbance peak at 250 nm and broad width of absorbance between 320-600 nm. Similar research was done by Barati et al. (2024) and got SPR peak at 194 nm with absorbance ranges from 190 to 350 nm. Alishah et al. (2016) reported absorbance intensity peak at 215 nm and absorbance region lies mostly in ultra violet region. So that results were matching with previous studies. Minitab® software (v15.1.30.0) software was used to analyze outcomes of experiments.

ANOVA analysis was shown linear quadratic and interaction terms were significant. Lack of fits test had $p=0.226$ ($p>>0.05$) indicated non-significance of test. This implied that data adequately fitted in regression model without any significant over fitting and under fitting. It was also clear that overall model is significant $F=148.83$ and $p<0.05$. This suggested that mostly all variations in outcome of experiments were explained by model.

Table 1.3: ANOVA result

Sr.	Source	F-value	P-value
1.	Regression	148.83	0.00
2.	Linear	148.71	0.00
3.	Square	52.59	0.00
4.	Interaction	245.20	0.00
5.	Lack Of Fit	4.73	0.226

Table 1.4: Model summary

Term	Coefficient	T	P
Constant	124.715	62.275	0.000
A	22.460	18.314	0.000
B	11.274	9.193	0.000
C	6.279	5.120	0.004
A2	-9.132	-5.029	0.004
B2	-2.659	-1.473	0.201
C2	-21.389	-11.849	0.000
AB	-10.789	-6.221	0.002
AC	-28.645	-16.516	0.000
BC	-35.717	-20.594	0.000

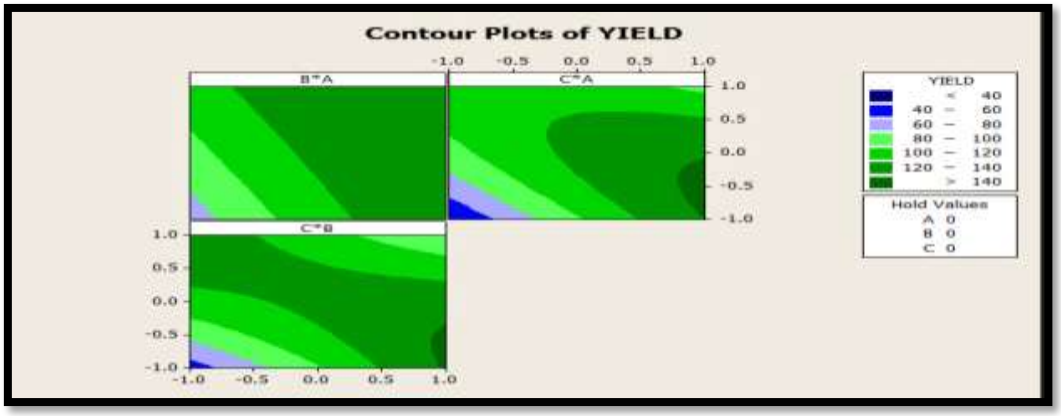
S = 3.46866 PRESS = 832.517

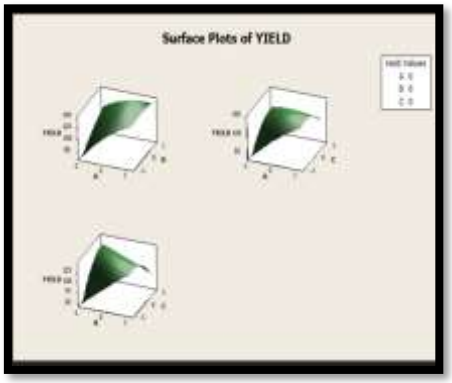
R^2 = 99.63% R^2 (pred) = 94.85% R^2(adj) = 98.96%

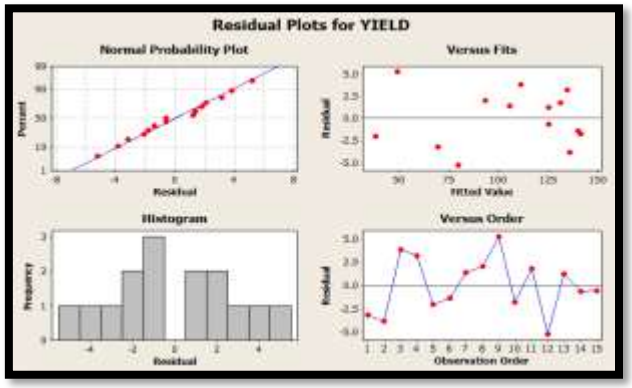
Table 1.4 has shown information about regression coefficients. All parameters and interaction terms were statistical significant expect B^2 term. R2 value in 99.93 % and R2 adj is 98.96 %. R2 predicated was 94.85%. These values defined the ability of predication and optimization. Regression equation on the basis of these coefficients were

$$Yield=124.715+22.460A+11.274B+6.279C-9.132A^2-2.659B^2-21.389C^2-10.789AB-28.645AC-35.717BC$$

T test indicated that factor A had strongest effect on response variable. Looking at B, C it was cleared that they have positive significant of outcome or response of experiments. Significance of quadratic term indicated a quadratic effect and optimization would be present in RSM. A, B and B, C had high t test value indicated strongest interaction between time, temperature and concentration.







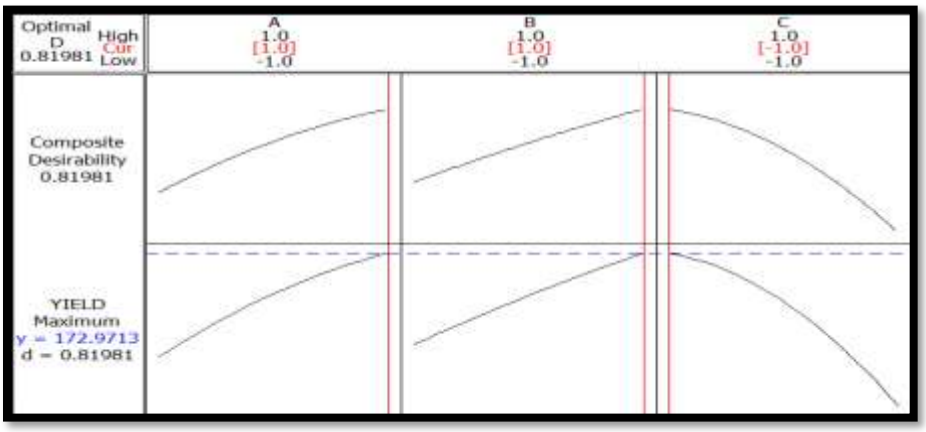


Figure 1.2: Surface, contour and residual plots

Keeping temperature at middle value increasing concentration and reaction time enhanced yield of copper oxide. If time kept at middle value then higher temperature and lower concentration made yield higher. Contour plot analyses revealed significant antagonistic interactions impacting yield. An interaction between Factor A and Factor C (AC = -28.645) showed that while factor A independently boosts yield, increasing factor C at high levels of A reduces it. Optimal yields can be achieved with high value of factor A and low factor C, whereas low levels of both result in the lowest yields.

Similarly, a strong antagonistic interaction exists between Factor B and Factor C (BC = –35.717). Factor B alone positively influenced yield, but this effect diminished or reversed when factor C is present at high concentrations. The best yield occured with high factor B and low factor C, while low levels of both lead to the lowest yields.

Collectively, these findings indicated that factor C consistently acting as an antagonist, negating the positive effects of both factor A and Factor B at elevated levels. Surface again shown fact again that yield would be higher if A and B kept higher and C at lower level. Residual plots shown normality existence as most of points are perfectly lies near or on straight line. Residual vs fitted value shown constant variance and unbiased nature of predicted value as point were scattered randomly around horizontal line and no specific pattern in residual point were observed. From histogram of residuals it was cleared that residual follow normal probability distribution as residuals were making almost bell shaped envelop. Residual versus order indicated that the observations were independent and there were no time-related biases or external factors systematically influencing the residuals over the course of the experiment.

Based on this comprehensive optimization analysis, the most favorable conditions for maximizing YIELD are achieved when Factor A is set at its high level (1.0), Factor B at its high level (1.0), and Factor C at its low level (-1.0). Under these optimized conditions, the model predicts a maximum YIELD with predictability 81.98%.

4. Conclusion

Copper oxide nanoparticles in not only successfully synthesized but also optimized. Confirmation of nanoparticle was done through visual inspection changing of color of mixture solution from brown to brownish black. At strong absorbance peak found in ultraviolet region wavelength ranging from between 200-212 nm. Residuals plots confirmed satisfactory statistical result and almost no biasness. ANOVA test revealed that regression model not only statistically significant but also had good predication ability with R^2 value in 99.93 % ,R^2 (adj) is 98.96 % . R2 predicated was 94.85%. Optimum condition with 81.98 pre found when concentration and time at its maximum while temperature remain its minimum level.

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