Green synthesis of copper nanoparticles to investigate its properties
Group 1-18

ABSTRACT

Metal nanoparticles have been widely researched for their properties for their biological and pharmaceutical applications. In this study, we aimed to synthesize copper nanoparticles using green methods, via plant extracts. We found that Zingiber officinale and Syzygium aromaticum extracts were ineffective in reducing copper to form copper nanoparticles, and Senna leaf extract and Citrus limon fruit extracts were effective in reducing copper to form copper nanoparticles. We also found that copper nanoparticles cannot be used as a semiconductor due to its large band gap. Based on our results, we have also observed that the Cu NPs we synthesized have no antibacterial activity.

INTRODUCTION

Given their catalytic, optical, electrical and magnetic properties, many metal nanoparticles and nanomaterials are deemed very important for a range of biological and pharmaceutical applications (Mamunya et al. 2004). Copper nanoparticles, hence, have the potential to inhibit bacterial growth, especially in Gram Negative Bacteria such as Escherichia coli, whose bacterium cell only consists of a unique outer membrane layer and a single peptidoglycan layer (Fröhling & Schlüter, 2015).

Thus, our project decided to focus on investigating the properties of copper nanoparticles on their antibacterial properties, as well as investigate its band gap energy for use as a semiconductor. We plan to use green synthesis as our method of synthesizing copper nanoparticles as green synthesis has gained extensive attention as a reliable, biologically safe and eco-friendly alternative to traditional methods of synthesising nanoparticles.

Moreover, antibacterial activity is the most important characteristic of medical textiles, to provide adequate protection against microorganisms, biological fluids, and aerosols, as well as disease transmission (Cioffi et al. 2005).

As semiconductors are used in the fabrication of electronic devices (University of Washington, n.d.), semiconductors are a big part of our lives and by testing copper nanoparticles for band gaps, we can see its application as a semiconductor.

OBJECTIVES:

To investigate the methods of green synthesis of copper nanoparticles and the properties of copper nanoparticles, mainly its antibacterial properties, and band gap so as to find a cheaper and more environmentally friendly way to solve the problem of harmful bacterial growth. Originally, we wanted to investigate copper nanoparticles' ability to degrade organic dyes. However, due to time constraints, we decided to test the antibacterial properties of copper nanoparticles and its band gap energy for use as a semiconductor.

HYPOTHESES:

Copper nanoparticles can be synthesized with the use of plant extracts from zingiber officionale (ginger), Citrus Aurantium Dulcis (Citrus peels), Syzygium aromaticum (Clove) and Senna leaves.

Copper nanoparticles demonstrate antibacterial properties.

Copper nanoparticles have band gaps that allows for its usage in semiconductors

VARIABLES AND CHEMICALS

LIVING ORGANISMS:

Escherichia coli (E coli)

CHEMICALS:

Copper(II) sulfate, Copper(II) nitrate, Citrus limon fruits, Deionized and distilled water, Ginger (Zingiber Officinale), Clove (Syzygium aromaticum) and Senna leaves extracts,

Growth media nutrient broth (liquid nutrient growth medium), Aluminium stubs, Ascorbic acid

EQUIPMENT:

Knife, Whatman No. 40 and 41 filter paper , Refrigerator, Stirrer, Centrifuge, liquid chromatography-mass spectroscopy, Nutrient Agar Plates, Desiccators, UV-VIS spectrophotometer.

APPARATUS:

250ml Glass Beaker, Stoppered glass flask

EXPERIMENTAL PROCEDURES

<u>Green Methods</u>

Preparation of Cu NPs using Zingiber officinale (Ginger) extract

Zingiber Officinale rhizomes were obtained from the local market. They were crushed and grinded then 45gm was stirred vigorously in 150ml of water and boiled for 20 minutes. The solution was filtered with Whatman no. 40 filter paper. Filtrate was collected

The Zingiber Officinale extract was added dropwise to 1mM of copper (II) nitrate on a magnetic stirrer with constant stirring at room temperature. Supernatant was analyzed using a UV-VIS spectrophotometer.

Preparation of Cu NPs using Syzygium aromaticum (Clove) extract

1mM copper sulfate solution was prepared by dissolving 0.0622g of Copper (II) sulfate in 250 ml of double distilled water and then stored in a clean and dried reagent bottle.

10ml of leaves extract was added to 90ml of 1mM copper(II) sulfate solution, the colour of the solution changes from light brown to light green colour. The resulting solution must then be incubated for 24 hours at room temperature then centrifuged for 20 minutes at 10,000 rpm. The residue was dispersed in double distilled water to remove any unwanted material. The solution must then be filtered. Supernatant was analyzed using a UV-VIS spectrophotometer.

Preparation of Cu NPs using Citrus Aurantium Dulcis extract

Due to the failure of the past 2 plant extracts, we decided to use Citrus limon fruit.

Citrus limon fruit aqueous extract (100 ml) was mixed with 4 g of copper (II) sulfate pentahydrate under magnetic stirring at room temperature (27 o^C) for 4 h. The blue color of copper sulfate pentahydrate is expected to change to brown within 10 minutes, indicating the formation of CuNPs due to reduction of copper ions from Cu(II) ions to Cu metal. The samples will then be centrifuged at 3000 rpm for 10 min to get a clear supernatant at room temperature. Supernatant was analyzed using a UV-VIS spectrophotometer.

Preparation of Cu NPs using senna leaves extract

10 gm of leaves was boiled with 100 ml deionized water for 30 min. The extract was cooled down and filtered with Whatman filter paper no. 1 and extract was stored in a refrigerator at 4°C. The CuNPs were prepared by adding 10 ml of aqueous extract of plant material to 50 ml of 1 mM aqueous solution of cupric nitrate. The mixture was irradiated in the microwave oven for 2 minutes and allowed to cool at room temperature. Finally, the reaction mixture was centrifuged at 5000 rpm for 15 minutes and residue was dried at room temperature. Supernatant was analyzed using a UV-VIS spectrophotometer.

Calculation of band gap energy

Band gap energy can be calculated using the formula,

$$Band\ gap\ energy = \frac{h \times c}{\lambda}$$

$Where,$

$h = plancks\ constant$

$c = speed\ of\ light$

$\lambda = cut\ of f\ wave\ length$

Cut off wavelength is required to calculate the band gap energy. UV-VIS spectrophotometer will be used to determine the cut off wavelength of the copper nanoparticles.

Antibacterial/microbial Properties of Copper Nanoparticles

E coli and bacillus subtilis was inoculated in broth in an orbital shaker at 37 degrees at 150rpm for 24 hours. Bacteria samples were spread onto nutrient agar plates. Wells diffusion method was used to test the antibacterial properties of CuNP that were synthesized by senna leaves extract. Five samples were prepared for each type of bacteria.

RESULTS AND DISCUSSION

DATA ANALYSIS

UV-VIS Spectrophotometer was used to determine the peak and cut off wavelengths of the copper nanoparticles synthesised by various plant extracts.

The Citrus limon extract and Senna leaf extract both had a peak at 300 nm and cut off wavelength was around 450 nm, similar to copper nanoparticles, as shown in figure 6.

Hence our hypothesis that Cu NPs can be synthesized greenly using Citrus limon and Senna leaf is true.

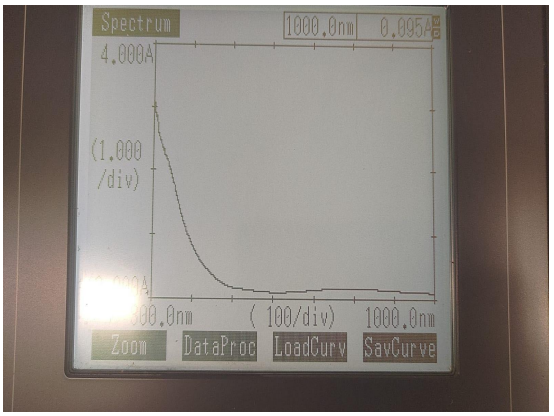

Figure 1. Senna leaves extract

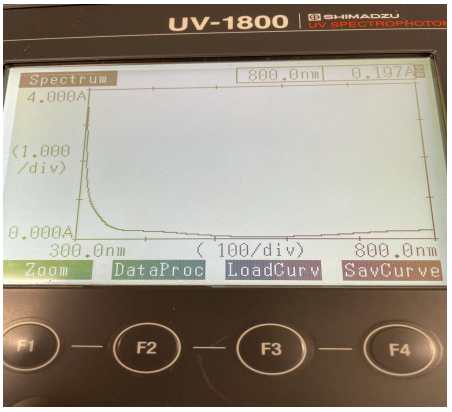

Figure 2. Citrus limon extract

The Ginger extract had a peak at 266 nm.
Hence our hypothesis that CuNPs can be synthesised using Ginger is false.

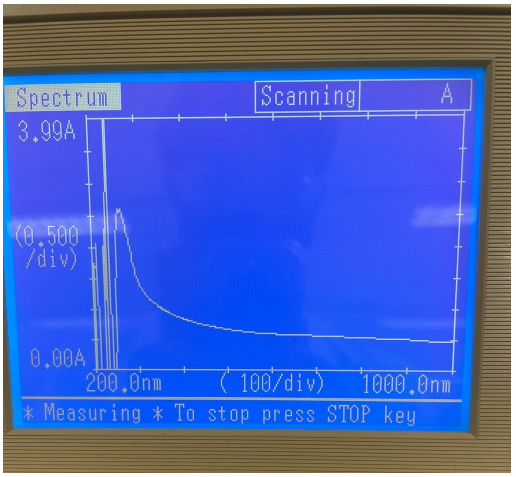

Figure 3.1 Ginger extract

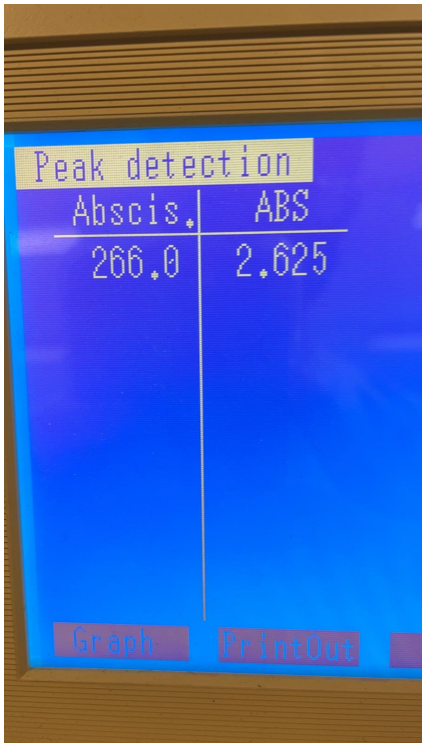

Figure 3.2 Ginger extract

The Clove extract had a peak at 382 nm.
Hence our hypothesis that Cu NPs can be synthesized using clove is false.

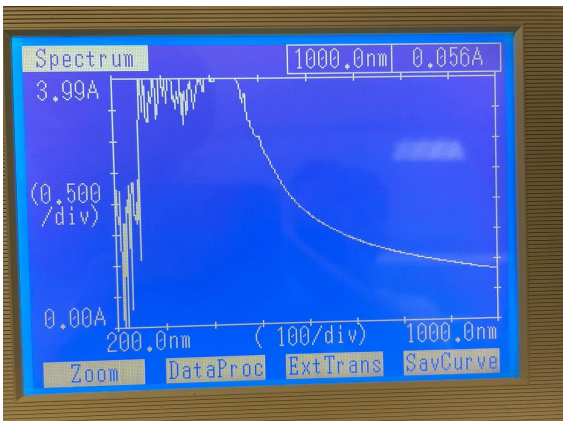

Figure 4.1. Clove extract

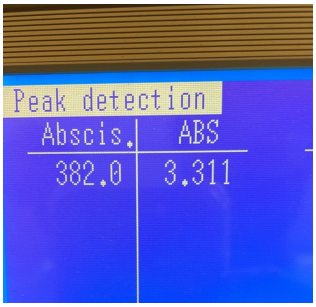

Figure 4.2. Clove extract

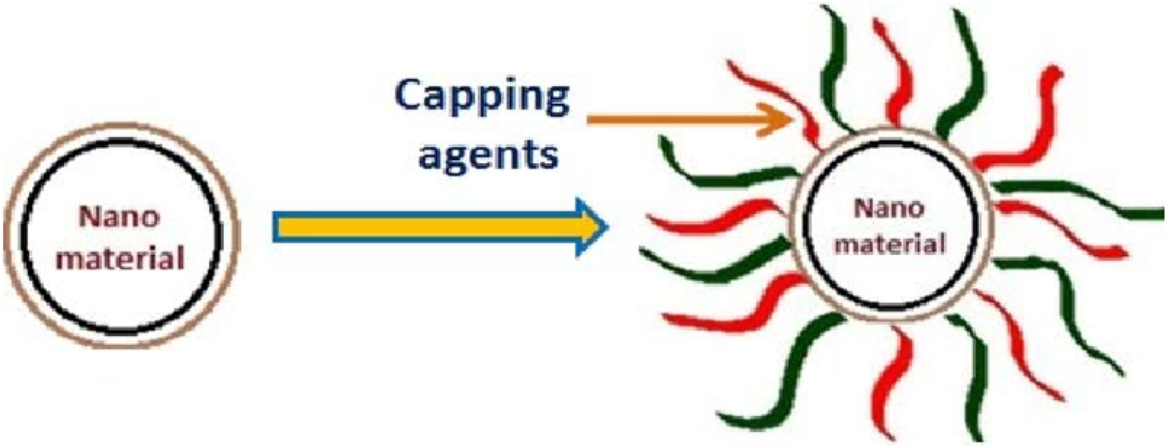

Figure 5. Reducing agent.

REDUCING AGENT

Refer to figure 5, reducing agents form biological and chemical compounds around the nanomaterial, preventing the nano material from forming aggregate. Most reducing agents in plant extracts contain flavonoid. Low flavonoid content in the Ginger and Clove extracts means that the mixture could form aggregate and hence nanoparticles cannot be formed.

We can determine the total flavonoid content of the ginger and clove extracts if the experiment was repeated.

DETERMINATION OF FLAVONOID CONTENT

Add 400 mL methanol to separate portions of 100mL of the plant extract in a test tube and shake the mixture. Add 100mL of Aluminium Chloride to the mixture and shake the mixtures. Add 100mL of 1M Sodium Acetate and shake the mixtures. Incubate the samples at room temperature for 45 minutes in the dark for further analysis using UV-VIS spectrophotometer. Plot a calibration curve, and the content of flavonoid compounds can be calculated using the formula,

$$C = \frac{(c \times v)}{m}$$

$Where,$

$$C = total\ content\ of\ flavonoid\ compounds, \frac{mg}{g}\ plant\ extract$$

$$c = concentration\ of\ flavonoid\ established\ from\ the\ calibration\ curve\ , \frac{mg}{ml}$$

$$V = volume\ of\ extract,\ ml$$

$$m = the\ weight\ of\ pure\ plant\ extract,\ gm$$

CALCULATION OF BAND GAP ENERGY

The copper nanoparticles synthesized via senna leaf and Citrus limon extract have band gap energy of 276 eV, larger than common semiconductor materials.

$$Band\ gap\ energy = \frac{h \times c}{\lambda}$$

$Where,$

$$h = plancks\ constant$$

$$c = speed\ of\ light$$

$$\lambda = cut\ off\ wave\ length$$

$$\frac{6.62607004 \times 10^{-34}\, m^2 kgs^{-1} \times 2.99792458 \times 10^8 ms^{-1}}{4.50 \times 10^{-9} m} = 4.41 \times 10^{-7} J = 276\, eV$$

A small band gap allows for the solid to have a strong enough flow of electrons from the valence to conduction bands in order to have some conductivity. With a large band gap, copper nanoparticles have poor conductivity and cannot be used as an effective semiconductor.

	Peak wavelength (nm)	Cut off wavelength (nm)
Citrus limon	300	450
Senna leave	300	450
Ginger	266	Undetermined (> 1000)
Clove	382	Undetermined (> 1000)
Copper nanoparticles	300	450

Figure 6. Table of wavelength against plant extracts

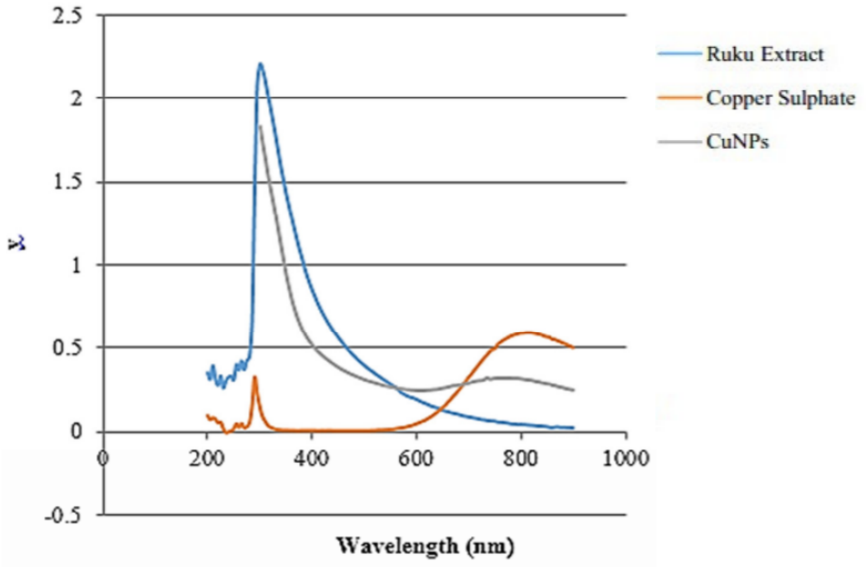

Figure 7. UV-VIS spectra of copper nanoparticles. Ramadhan, 2019.

ANTIBACTERIAL TEST

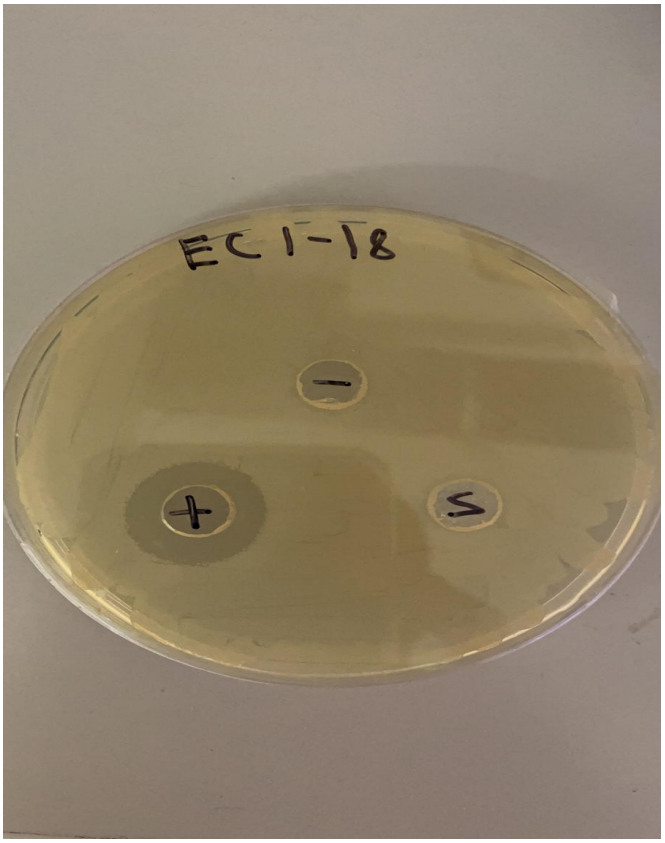

Figure 8. E coli agar plate after 24 hours incubation

Figure 9. Bacillus subtilis agar plate after 24 hours incubation

There was a zone of inhibition for the positive control (bleach), and no zone of inhibition for the negative control (distilled water). All 5 samples for escherichia coli showed no zone of inhibition, similar to that of the negative control. All 5 samples for bacillus subtilis showed no

zone of inhibition, similar to that of the negative control. Hence, our hypothesis that CuNP has antibacterial properties is false.

The CuNP synthesized using Senna leaves was not used immediately for the antibacterial study so the nanoparticles might have formed an aggregate and hence did not exhibit antibacterial properties.

CONCLUSION

Copper nanoparticles cannot be synthesized using Ginger and Clove extract.
Copper nanoparticles can be synthesized using Senna leaves and Citrus limon extract.
Copper nanoparticles synthesized using Senna leaves and Citrus limon extract cannot be used as semiconductors.
Copper nanoparticles synthesized using Senna leaves do not exhibit antibacterial properties.

CHALLENGES

Synthesis of nanoparticles:
Refer to figure 5, Clove and Ginger extracts are not good reducing agents, possibly due to lower flavonoid content and cannot reduce copper to form copper nanoparticles

Antibacterial activity:
Low concentration of nanoparticles could be a possible factor in the nanoparticles not exhibiting antibacterial properties. Another factor could be lack of hydrogen peroxide as some nanoparticles exhibit antibacterial properties in hydrogen peroxide.

Time constraints:
Experiments could not be conducted during the June Holidays, hence experiments had to be conducted in the first half of Term 3 so opportunities to visit the laboratory were reduced.
Some procedures required waiting times of around 3 hours, so we could only conduct these procedures during SPACE periods.

FUTURE WORK

More effective plants with reducing agents should be considered for the formation of copper nanoparticles. Other metal nanoparticles can be researched further like gold and silver due to their common commercial use. They are also more easily synthesized. Copper nanoparticles cannot be used as semiconductors due to their band gap energy. Semiconductors play a big role in the fabrication of electronic devices but the large band gap of copper nanoparticles results in poor conductivity hence it cannot be used as semiconductor. Antibacterial tests should have been conducted immediately after the synthesis of copper nanoparticles, to prevent the formation of aggregate.

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