Green synthesis and Characterization of Phyto -Metallic Quantum Dots

Report submitted in partial fulfillment of the requirement for the degree of

MASTER OF TECHNOLOGY

IN

BIOTECHNOLOGY

by

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MAY 2023

DECLARATION

I hereby declare that the work reported in the M. Tech. thesis entitled **"Green synthesis and Characterization of Phyto -Metallic Quantum Dots"** submitted at Jaypee University of Information Technology, Waknaghat, India, is an authentic record of my work carried out under the supervision of Dr. Garlapati Vijay Kumar, Dept. of Biotechnology and Bioinformatics, JUIT, Waknaghat, HP-173234, India. I have not submitted this work elsewhere for any other degree or diploma.

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SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the M. Tech. report entitled **"Green synthesis and Characterization of Phyto -Metallic Quantum Dots",** submitted by **Akshita Sharma (212551)** at Jaypee University of Information Technology, Waknaghat, India, is a bonafide record of the work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.

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ACKNOWLEDGMENTS

It is a genuine joy to express our profound feeling of thanks and gratitude to my mentor, philosopher and guide **Dr. Garlapati Vijay Kumar**, Department of Biotechnology &Bio informatics, Jaypee University of Information Technology, Waknaghat, and Himachal Pradesh. His commitment and distinct fascination over the entirety of his staggering disposition to help his understudies had been solely and generally liable for the most part answerable for finishing our work. His opportune counsel, careful examination, insightful admonishment and logical methodology have helped us to an exceptionally incredible degree to accomplish this assignment.

Thank you,

(**Akshita Sharma, 212551**)

ABSTRACT

The present work summarizes the state-of-art in synthesizing Phyto-based metallic quantum dots. We have taken copper as a metallic compound and *Solanum nigrum* as a phytochemical source as a prototype and outlined the simple production approach and characterization of copper quantum dots from plant leaf extracts. Green fabrication of quantum dots includes green chemistry, which provides mild reaction conditions that help reduce the metal copper into a nanoparticle of a specific size compulsorily equal to or below 10 nanometres. The plant extract of *Solanum nigrum* would provide phytochemicals that contribute to essential reduction, stabilization, and capping agents of copper Quantum dots. Further, the antimicrobial and antioxidant activities drew the efficacy of synthesized metallic quantum dots. The catalytic activity was measured with the methylene blue dye-based test. The antimicrobial activity would be tested against *E coli* and *S aureus*. The antioxidant activity of the fabricated metallic quantum dots was determined through the DPPH test. The following study shall provide a state-of-art approach to synthesizing and characterizing metallic quantum dots for a sustainable world.

Key words: *copper, Quantum dots, Solanum nigrum, antimicrobial, antioxidant, green synthesis.*

CHAPTER 1

INTRODUCTION & REVIEW OF LITERATURE

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1.1 Introduction

Diagnostics, imaging, therapy have always been important areas of science and medicine. These are the fundamental requirements for each field's study and progress. Science has advanced significantly over the years, from visualizing samples under a microscope to tracing genes with the aid of markers. With the advent of new approaches, new issues also surface. To make any new technology as secure as feasible, we must constantly work to enhance it in all respects (*Kubra et al. 2021*). It must be verified that there will be no harm to the environment or the procedure's target. A fluorophore is one of the most often employed substances in imaging, diagnostics, and prognostics, among other things.

A fluorophore is a molecule that, when excited, can emit light again. Fluorophores can be used as a dye, a tracer, a probe, and other things. They are mostly employed for staining tissues or cells when using spectroscopic or fluorescence imaging techniques. These substances and other organic dyes have been used for a very long period, but they have several drawbacks that could affect the outcomes. Some of the limitations are as follows:

- The general organic dyes show multiple peaks also called 'shoulders' which do not give accurate results or readings. Organic dyes do not have broad absorption spectrum. Organic dyes do not have high Quantum yield and are also extremely sensitive to light (*Wang et al. 2020*)
- Organic dyes undergo fast photobleaching hence the imaging has to be carried out immediately as these cannot be exposed to light for longer period of time (*Yan et al. 2020*).
- The fluorescence lifetime of these compounds is very low and they are not specific enough (*Zhou et al.* 2020)
- Use of dyes for so longer may cause toxicity in the subject.(*Gidwani et al.* 2021)
- These compounds are also not easily degraded and may even be biohazardous.

1.2 What are Quantum Dots?

In the year 1980, Alexei Ekimov and Louis E. Brus made the discovery of quantum dots. It is one of the most significant discoveries in nanotechnology. Nanoparticles called quantum dots are semiconducting and can transmit electrons. On the basis of shape and size of the particle, they produce light in a variety of colours. A certain colour of light can be adjusted into the quantum dot's emission. They could be synthesised using a wide range of materials, like silver, gold, copper, etc. (*Mariselvam et al. 2014*).

They are an excellent replacement of the organic dyes and fluorophores used in imaging. Quantum dots can be used as probes, markers, tracers, tags etc. They help to combat many obstacles due to nature of organic dyes such as, they are more photo stable, they have a broad absorption pattern, resistant to photo bleaching, have a long fluorescence life, has specific binding and shows specific peaks and no shoulder effect. Quantum dots produced by green synthesis or layered by ZnS have shown very less toxicity when compared to organic dyes which were used over long time periods. They display fluorescent properties because of quantum restriction. The Quantum captivation occurs when a substance is diminished to a dimension like the characteristic linear measure of the attribute being analyzed. For Quantum Dots, the aforementioned trademark linear measure is: exciton Bohr radius, accordingly QDson a similar dimension spectrum as their substance's trademark exciton Bohr range show quantum captivation. Quantum Dot fluorescence starts from the rejoining of an e-opening set, and the fluorescence is started via photo-excitation of an e- starting from quantum dot's valence band within its conduction band. The e- unwinds towards the most reduced energy state in the conduction band prior to amalgamate with the opening abandoned wherein the valence band. The distinction that is there in the energy allying the conduction and valence band stays preserved as a discharged photon, subsequently, the size of this hole directs frequency ofthe light produced out of the quantum dots. The QD bandgap is straight forwardly identified with its diameter, and thus QDs including a similar semiconductor substance can be attuned to radiate various frequencies by essentially converting their size.

UV-VIS and PL (i.e., photoluminescence spectroscopy) give a fast, non-destructive, and contactless method for optical portrayal of quantum dots. Hazdra and colleagues used an uncommon technique called photo modulated reflectance spectroscopy to examine deposited QD structures. This method provides the same energy resolution as photoluminescence at reduced ferocity and experiments a wider range of critical focal points, including the low energy state or ground state and additionally numerous high demand inter-band optical advancements through which the band structure, particularly of the dampcoating, can be thoroughly considered. The ocular characteristics (or the incandescent emanation) of quantum dots can be adapted by thequantum dots' dimension i.e. is a key specification that will decides the spectral location as well as the immaculateness of PL. Quantum Dots' dimension is commonly determined utilizing traditional procedures like SEM technique ,TEM, and DLS. For screening the dimension of the epitaxially arranged QDs, a few portrayal strategies are generally utilized, for instance transmission e- microscopy, AFM: atomic force microscopy / STM.

Moreover, quantum dots bio-compatible nature is fundamental to their natural and bioscience significance. All in all, bio-compatible quantum dots can therefore be acquired through 3 unique highways:

- Bio-mimetic synthesis: either via the utilization of artificial cellular construction/bio-molecules for example nucleic acids, peptides, proteins, and catalysts as layouts;
- Bio-synthesis: utilizing alive life forms in bio-reactors;
- Changing the outside of quantum dots from their synthetic merger. The bio-synthetic methodology gives a green route to getting ready bio-compatible quantum dots free from producing harmful items or alternatively hostile response state, while the exterior alteration proposition can construct an inflated QY to an excessive extent.

Table 1. Classification of QD

QD	Group no	Examples
Single core structure	II-VI	CdS, CdSe, ZnSe & CdTe
	III-V	GaN, InP & InAs
	IV-VI	PbSe & PbS
	IV	Ge & Si
Core/Shell structure	Type 1	CdSe/ZnTe, CdTe/ CdSe, ZnSe/CdS
	Type 2	CdSe/ZnS, InP/ZnS, CdSe/CdS/ZnS, CdSe/CdS/Cd0.5Zn0.5S/ZnS
Alloy		CuXZn1-XS, HgXCd1-XTe, ZnXCd1-XSe

Table 1: QDs classification according to the elements used.

1.3 Applications

- They are used in LEDs, solar cells, lasers etc (*Molaei et al., 2020*).
- They are also used albeit particular markers for cellular construction and particles, discovering cell parentage, keeping a track of physiological incidents in animate cells, calculating cell movability as well as trailing cells in vivo (*Pandey et al. 2020*).
- If we compare QDs with organic dyes, they are photostable, with longer fluorescence life, broad absorption patterns, and give clear peaks for accurate visualization.
- By tuning the structure we can make it emit light of the colour of our choice.
- Bioimaging
- Photovoltaic devices
- Light emitting devices

1.4 QD *Vs* NANO

Nanoparticle representation is frequently linked to the localising function of light in nanoscale structures or networks, which typically exhibit a massive field magnification on their exteriors. Common models include tiny solitary photonic components like complex nano radar, nanogaps, and nanoresonators. As a result, compact confinement is extraordinarily important, for example, for high-resolution surveys, regional substance transformation, big-field localization, strength enhancement, and an increase in the productivity of computational cycles like Raman scattering and harmonic production.

Nano-specks of semiconductor substances put together in bandgaps are referred as quantum dots. Quantum dots (QD) are the nanocrystals that are made out of the semiconductor substances which are adequately little to display quantum mechanical characteristics. Quantum dots are useful in making photovoltaic cells, light emitting diodes, and distinction specialists as diagnostics (*Nirala et al., 2020*)

1.5 Elements used for the preparation of Quantum Dots

Some of the examples of the elements used for the preparation of QDs are: cadmium selenide, cadmium telluride, zinc cadmium selenide, zinc sulfide, and lead selenide, to better their optical characteristic, moreover a cap to permit upgraded aqueous dissolvability for bio associated applications.

1.6 QD'S as Biomarkers

If a set of molecular probes can be measured and scientifically manipulated in the centre of cancerous and healthy cells, biomarker probes might be helpful for the identification and filtering of malignancy. There are usually disease indicators in very crowded assemblies; hence procedures that are effective enough to have a low detection threshold are required. QDs are emanating auto fluorescence on excitation with a light source. They consist of exceptional optical characteristics including high brightness, obstruction to photo-bleaching process and tunable frequency. Now a day's advancement in exterior alteration of quantum dots allows them to potentially be utilized in cancer diagnostics. Quantum Dots having near-infrared emission could be utilized for the process of sentinel lymph-node mapping to assist biopsied and operations (*Zhang et al.* 2020). Union of qds with bio-particles, plus antibodies as well as peptides, could be utilized for targeting tumors *in vivo* (*Freitas et al. 2020*). A great amount of research is going on in the advancement of QDs for cancer diagnostics and therapy as of scientific view and for further bettering of QD mechanisms to visualize and target metastatic malignant cell growth, and to be able to quantify the measure of explicit target points on malignant growth by delivering bio-dynamic probes for target hindrance.

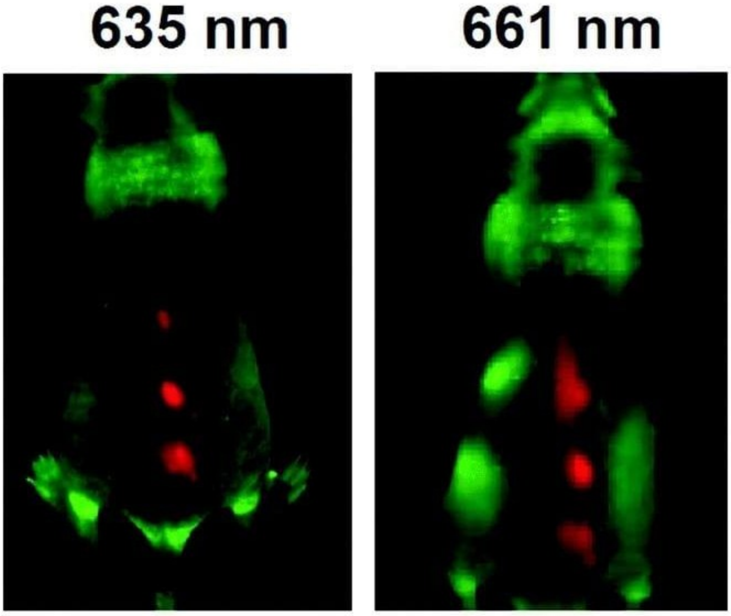

Fig 1.1: QDs as biomarkers

1.7 QD's instead of Natural Dyes

Quantum dots display the attractive distinctive absorption property that gradually increases towards smaller frequencies, in contrast to natural colours (below 1s texcitonic absorption band) and a constrained symmetrical emission band. Particle size affects the locations of the absorption and emission spectra (i.e., quantum size effect.) The transmission of the QD's size is primarily responsible for controlling the breadth of the emission spectrum. The wide absorption permits unbound choice when it comes to the excitation frequency and in this manner direct division of excitation and emanation (*Al-Zahrani et al. 2020*). The molar absorption (which is size dependent) quantities at the very 1stabsorption band of quantum dots are mostly huge when contrasted with natural dyes (*Jang et al., 2020*).

It's interesting to note that natural dyes also exhibit significant fluorescence quantum yields as of visible light spectrum; these values are, at best, averaged throughout the NIR frequency spectra. Utilization of natural dyes for NIR-frequency fluorescence imaging is currently hindered with the negative effects of reduced quantum yield at NIR frequencies as well as the limited photostability of certain NIR-frequency dyes. When compared to natural dyes, another dependent, bi- or multiex ponential QD decay behaviour makes it very difficult to identify species from time-established fluorescence estimates. Unfortunately, this is an inherent flaw with these materials.

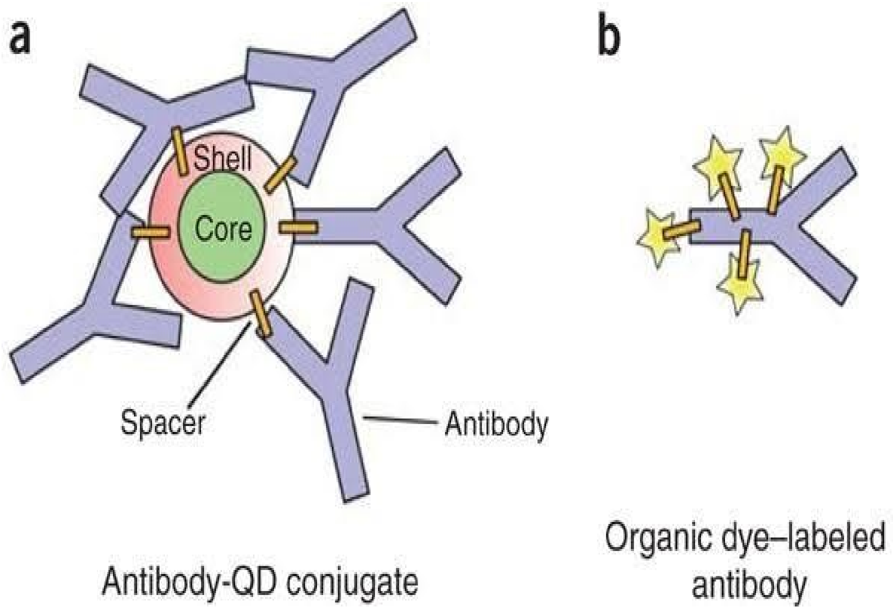

Fig 2 : QDs *Vs* Organic dyes

1.8 Benefits of QDs over traditional Fluorophores

Compared to natural fluorophores, which are often used for in vivo labelling, quantum dots provide a few advantages. Rhodamine and fluorescein are two examples of natural fluorophores that do not have the broad range of optical characteristics that quantum dots can. (*Emam and Ahmed, 2020*) Fluorophores that are natural or organic have a narrow range of absorption, which results in a narrow range of outflow. Additionally, the symmetric outflow top of the natural dyes is not sharp.

It has a red-tail and is further extended. On the other hand, compared to natural fluorophores, which are typically used for in vivo naming, quantum dots have a few advantages. Unlike natural fluorophores, quantum dots can have a wide range of optical characteristics, for example, rhodamine 6g and fluorescein. Natural fluorophores have a tight absorption range which brings about a thin scope of discharge. Likewise, the natural dyes don't have a sharp symmetric emanation peak which is additionally expanded by a red-tail. Conversely, quantum dots have a more extensive excitation range and a tight more forcefully characterized outflow peak. Because of these properties, a solitary light source can be utilizedto energize multicolor quantum dots all the while without signal cover st, quantum dots have a more extensive excitation range and a narrow all the more pointedly characterized emission top. Because of these properties, a solitary light source can be utilized to energize multicolor quantum dots all the while without any overlaps (*Li et al. 2020*).

1.9 QD's distinguishing characteristics

It is a semiconductor nanocrystal that has gained recognition for having large bandgap energy, or the power required to move an e- from one electronic band to another at a significantly higher energy level. An e-opening pair eventually comes to be known as an exciton due to the excitationconspire. The exciton releases energy as a fluorescence photon after returning to its ground state. The molecule now develops distinctive electrical and optical properties as the semiconducting nanocrystal measure approaches the mass of the Bohr exciton span, a hallmark dimension falling within the range of 2–10 nm.

1.9.1 Photostability

Photostability of QDs generally relies upon surface passivation which is dictated by the QD covering atoms. Photostability of semiconductor QDs is allegedly higher than that of natural dyes, however QDs may likewise be influenced by the exposureof light. The result of such exposure may rely upon numerous trial factors, can prompt either an increment or decrease in the photoluminescent proficiency of QDs and is hard to anticipate (*Kulakovich et al. 2020*).

1.9.2 Absorption

By and large, the optical retention peaks of QDs in the UV-region is typically assessed as π-π* change of sp2 formed carbon and n-π* change of hybridization with hetero atom and so forth. The absorption characteristic can be controlled through surface passivation or alteration measure. Jiang et al. fostered a simple aqueous technique to integrate red, green and blue radiant CQDs by utilizing three isomers of phenylenediamines. The UV-visible absorption spectra of the as-acquired CQDs visualised undifferentiated from design. Curiously, the absorption changes of these three CQDs were red-moved, showing the electronic bandgaps of the CQDs were more compact than their relating antecedents (*McBride et al. 2020*).

1.9.3 Photoluminescence

The most fascinating aspects of QDs might be their photoluminescence, both in terms of their theoretical research and their usefulness. Overall, the specific dependence of the outflow frequency and intensity is one uniform component of the PL for QDs. This extraordinary feat may have been made possible by the surface-level optical detection of nanoparticles of varied sizes or QDs with various emissive trap. The broad and excitation-subordinate PL emission range can indicate the variability of molecule size and PL outflow (*Nie et al. 2020*)

1.9.4 Electroluminescence

Since semiconductor nanocrystals are notable to show electroluminescence (ECL), there ought to be nothing unexpected that QDs have enlivened different interests for ECL contemplates which can well be utilized in electrochemical. One example is, *Zhang et al. (2013)* revealed a CQDs-based light- emanating diodes (LED) gadget, in which the outflow tone can be constrained by the driving current. Shading switchable ECL from a similar CQDs going from blue to white was seen under various working voltages (*Shu et al. 2020*).

The scientists developed two theories based on the edge impact caused by another surface imperfection and the band gap emission of the generated p domain in order to more clearly understand the iridescence component of CQDs (defect). The quantum confinement effect (QCE) of the p-formed electrons in the sp2 nuclear structure is the source of the PL properties of the fluorescence emanation of QDs from the produced p space, and these qualities may be altered by the size, edge arrangement, and shape of the electrons. The sp2 and sp3 hybridised carbon and other surface flaws of QDs cause the fluorescence discharge of CQDs, and even the fluorescence power and pinnacle position are associated with this deformity.

1.10 *Solanum nigrum*

The Solanaceae family of plants includes the therapeutic herb *Solanum nigrum*, also known as black nightshade. The family includes numerous genera and is famous for their healing capacities. Along with *S. nigrum*, members of this family generally involve ornamental plants like petunia, vegetables and fruits like potato (*Solanum tuberosum*), tomato, and peppers, as well as herbs like *Atropa belladonna L.* (deadly nightshade), Datura ,*Hyoscyamus niger L*. (black henbane) and *Stramonium L.* (Jimson weed).

- *S. nigrum*, also famous as Makoi or black nightshade, invasive species in the damp places having different soil samples. In tropical and subtropical agro - climatic condition places id developed by planting the seeds in between April - May in fertilised raised bed form.

- The plant shows admirably important pharmacological attributes. Traditional medicine has made considerable use of *S. nigrum* in treating many of conditions, including pain, fever, and inflammation.
- Anticarcinogenic, antioxidants, nonsteroidal anti-inflammatory, diuretic, and alexipyretic substance, *S.nigrum* is widely utilized in Oriental systems of medicine. Numerous substances have indeed been found to be the cause of a variety of actions.
- *S. nigrum* is broadly applied in many traditional medical systems across the globe for a variety of diseases, although it has not gained waves for use in contemporary therapeutics. Glycoalkaloids, glycoproteins, and polysaccharides are the main active substances found in *S. nigrum* and are each accountable for a variety of actions. The polyphenolic substances, gallic acid, caffeic acid, are also found in it Solamargine, Solasonine, and Solanine are examples of the tropane group of chemicals' glycoalkaloids. Several studies have been conducted on the action and functionality of solanine. It is generally present in all parts of the plant and accounts for 95% of the total alkaloid concentration. Given that it is toxic even in little amounts, it serves to be one of the main natural defences of the plant. Because of the ability to modulate the immune system, polysaccharides extracted from aqueous extracts of *S. nigrum* have been demonstrated to exhibit antiproliferative action (*Jain and Sharma et al. 2011*)

Fig 3: ***Solanum nigrum***

It hasleafy and branched stems. Leaves can be observed as: large,alternate, higher mostleaves are a bit smaller looking, 3-fid or entire, lanceolate. Flowers can be observed as small and they appear extensively in clumps. Spicate/ suberect/ parallelpanicled. The color seen is brownish yellow. Flowering tops as well as the leaves are: bitter, astringent and aromatic. Fruits, on the other hand, berry like. The oil percentage and oil yield differs as theplant distribution, moreover, it also depends ondifferent the phases of growth (*Ijaz et al. 2020*).

The plant shows admirably important pharmacological attributes. Traditionally its use is seen as antioxidants, anti-inflammatory, hepatoprotective, diuretic, and antipyretic substance, *S. nigrum* is widely utilised in Oriental systems of medicine. Over the years, a large number of actions of aerial parts are examined and studied carefully and it was observed that they possess important properties like:anti- microbial, anti-fungal, antibacterial, and anti- oxidant properties. Also used in the treatment of bacterial infections, cough and indigestion. This plant has also been investigated for antiproliferative, antioxidant activities. The Oil possesses anti- microbial, anti-fungal, insecticidal properties. All of these evidences just confirm that the whole plant is of great therapeutic value (*Odey and et al. 2012*).

1.10.1 Why only copper?

Copper is a low-cost metal that is readily available everywhere. Moreover, CuQDs can be efficiently exploited as catalysts, antioxidant and antimicrobial agents.

1.10.2 How it would be economical?

There's a need for a new, innovative and efficient as well as cost effective method for the breakdown of natural dyes and to be able to keep a check on pathogenic microbial growth in wastelands of various industries.

1.10.3 Why *Solanum nigrum* Fruit?

Various *Solanum nigrum* fruit extracts have been shown to have antitumor capability. *Solanum Nigrum* is a herbaceous plant which has small size, spherical berry fruits that has been utilised in traditional medicine for the therapy of various illnesses, which includes cancer (*Muthu et al. 2006*).

Additionally utilized for treating cough, indigestion, and infections caused by bacteria. Antiproliferative, antioxidant, and antiviral properties of this plant have also been studied.

1.11 OBJECTIVES

- Preparation of *S. nigrum* leaf extract.
- Optimization of Reaction Conditions for Cu QD's of *S.nigrum*.
- Evaluation of antioxidant, and antibacterial of Cu'QDs of *S. nigrum.*

1.12 Characterization Techniques for QD's

Definite nuclear scale portrayal of Quantum Dots is subsequently important to enhance their optoelectronic properties. Distinctive portrayal procedures which can be utilized for measure of the dimension, shape and anatomy of the QDs are depicted in this part. Every portrayal procedure has its own benefits and constraints in giving primary and creation data of the QDs.

1.12.1 Atomic Force Microscopy

It comprises of a cantilever tip connected to a piezo-actuator to examine across the surface. The nuclear powers between the tip and the surface cause a quantifiable avoidance of the cantilever which is utilized to repeat the surface geology. By and large an optical switch strategy is utilized to identify the cantilever redirections, where a laser shaft is centered

around the rear of the cantilever and the reflected bar is gathered by a photodiode. The laser bar diversion framework is associated with a criticism circle to control the power and the situation of the tip. AFM can be worked in 3 unique modes: (i) Contact mode: - where the head is consistently touching the surface; (ii) Non-contact mode: - where the head wavers over the surface and also the forces were estimated; (iii) tapping mode: - where the head contacts the surface intermittently swaying near the reverberation recurrence. At brief good ways from surface the van der Waals forces are available which draw in the tip toward the surface. At the point when the distance of the tip from the surface is additionally diminished, the horrendous forces because of the collaboration between electronic clouds of tip and the surface become prevailing (*Khan et al. 2020*).

1.12.2 Transmission Electron Microscopy

TE is a high goal imaging strategy in which an electron beam is communicated through a meager sample. The association of transmitted electron beam with the sample delivers the picture. The electron source and the identifier are situated on inverse sides as for the example. The e-beam communicates through the sample and afterward is caught and prepared by the detector(s). The connection volume of the e-beam is little and comparable to the de Broglie frequency of the electrons. On a fundamental level, TEM can distinguish the situation of cross section planes in a crystalline solid and can deliver the lattice diffraction design also. The QDs can be recognized by the dark pyramid like highlights in the splendid network. In a unique report electron microscopy has been utilized to make a 3D tomographic picture of a QD (*Marin et al. 2020*).

In any case, test readiness and acknowledgment of appropriate diminishing for TEM examination is profoundly mind boggling and tedious. During the readiness of meager lamellae of the sample by utilizing miniature and nano-machining procedures or utilizing focused ion beam (FIB), there is a high likelihood to change or harm the construction of the example. The local data is restricted and the acquired outcomes are found the middle value ofover the entire lamellae where likewise nearby strain fields can influence the picture contrast. Extra strategies are expected to determine the local changes in the morphology and organization at the atomic scale.

CHAPTER 2

MATERIALS AND METHODS

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2.1 Materials

Less costly samples were taken for the conduction of the experiment. *Solanum nigrum* leaf extract was obtained from the institute laboratory; Copper sulphate was easily available in the institute. For the formation of copper quantum dots green synthesis method was used in which plant source and copper sulphate solution was used. Other materials like DPPH, Copper sulphate, sodium hydroxide etc were collected for the experiment. All the glassware's used for the biofabrication of Quantum Dots were first washed with the help of distilled water and then dried in the oven.

2.2 Methods

Nanoparticles can be produced by various methodologies such as physical or mechanical, chemical or green synthesis. Other than green methods the other two generally include toxic pre cursors and may lead to production of by products or waste products that could be hazardous to environment. Sustainable building is of utmost importance and hence, this is where green synthesis comes into play (Matussin et al., 2020).

Green synthesis is the utilization of environmentally congruent materials that can be microbessuch as fungus or plants for the synthesis of nanoparticles or in this case Quantum dots (*Chand et al. 2020*). It eliminates the need of toxic precursors and uses sensitive andmild reaction conditions (*Olaokun et al. 2020*). It also minimizes waste production, is environment friendly and the whole process is clean and non toxic hence it is the most effectively sustainable way of synthesizing nanoparticles are Quantum dots.

Plant extract was prepared from different ways-

2.2.1 Preparation of plant extract:

Leaf extracts can be prepared by various methods the most prevalent of which are hot water extraction and ethanol extraction (*Abubakar et al. 2016*).

Fig 4: *Solanum nigrum* leaves (Sangija et al., 2021)

2.2.2 Making of Hot water extract

To make plant extract by this process we need to first drive the leaves completely and thenmake fine powder out of it. Then the leaf powder is put in the hot water to soak and isboiled for 30 minutes and put aside to cool for 24 hours (*Chand et al. 2020*).

2.2.3 Ethanol extract preparation

In ethanol extraction the Powder of the dried plant it is taken and soaked in ethanol to completely submerge it. This mixture is left inside and ultra low temperature freezer for about 24 hours. In these 24 hours all the soluble components will be separated by the ethanol and thus this way the plant extract would be formed and can be further used for the preparation of quantum dots (*Andleeb et al. 2020*).

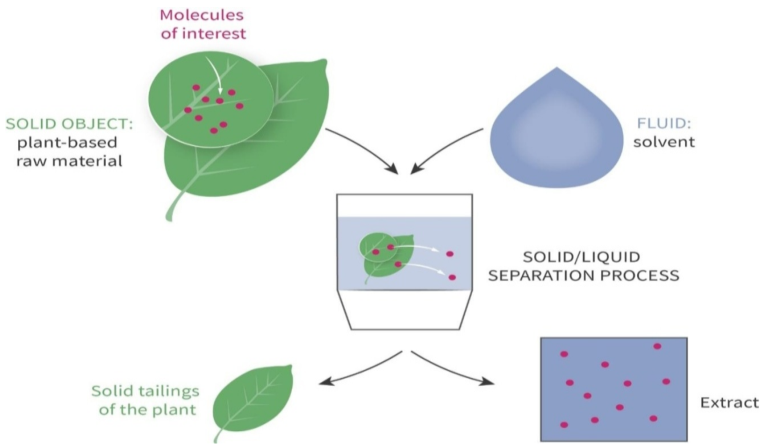

Fig 5: Ethanol extraction process.

2.2.4 *Solanum nigrum* leaf extracts preparation-

The fine powder of leaves was made by grinding them in mixer grinder. The aqueous extract was made by mixing 10g of leaf powder with 200ml of distilled water in a flask, and then vigorous shaking with the help of stirrer for about 1 hour was done. After roughly 15 minutes of continuous agitation, the resulting mixture had been set over the steam from water that was boiling and then filtration was done. Prepared *S.nigrum* extract was then kept at 4°C and consumed within two weeks (*Rawani et al. 2013*).

Fig 6: Filtration of leaf extract.

2.2.5 **Final Fabrication step of Quantum Dots**

After the extract has been successfully prepared it is now used for bio fabrication of copper Quantum dots. The first step in the preparation is to make a fresh batch of $CuSO_4$ (*Romanik et al. 2007*). The filtered plant extract it is then mixed with this copper sulphate solution continuously with magnetic stirring at RT. This reaction slowly turns to yellowish and then brown colour in about 10 minutes. This confirms successful fabrication of the copper Quantum dots (*Rani et al. 2020*).

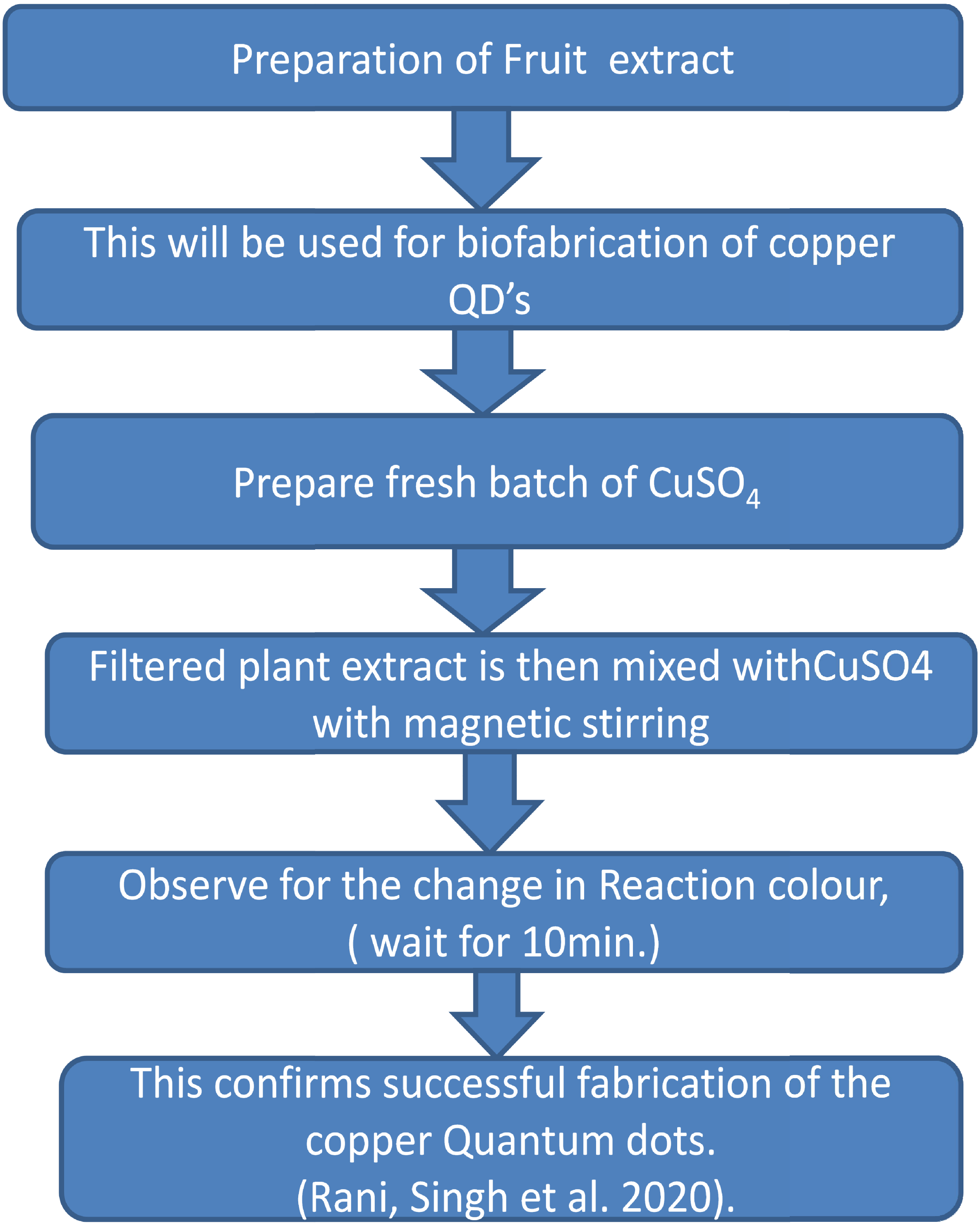

Fig 7: Phytometallic Quantum Dots Preparation.

However, for the optimization of the experiment many parameters had to be observed at different values.

- Firstly, the quantity of the extract to be used was determined by making batches from 1 ml to 4 ml separately and efficiency was observed.
- Next the effect of quantity of copper sulphate solution was observed. The concentration was varied from 1 to about 5 mM.
- The temperature ranges were also observed from 30 degree Celsius to 60°C with equal intervals.
- The pH was also determined by using a wide range starting from 6 to 12. 0.01M of HCL and NaOH were used to regulate it.
- The optimal incubation time was also determined.

Fig 8: Colour shift from yellow to Brown indicating formation of CuQD's.

CHAPTER 3

RESULTS AND DISCUSSION

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3.1 Biofabrication of Quantum Dots.

The QD's were prepared, as plant extract was made first and then the filtered plant extract was used for the biofabrication of Cu QD's fresh batch of copper sulphate was prepared and then both the plant extract and copper sulphate solution were mixed and magnetic stirred at RT. The reaction slowly turns to yellowish and then brown colour in about 10 minutes. This confirms successful fabrication of the copper Quantum dots. The concentration of copper was varied from 1mm to 5mm and its efficiency was compared and the quantity of plant extract used was also varied from 1ml to 5ml.

The Copper Quantum Dots showed peak at wavelength range between 250-280 nanometer.

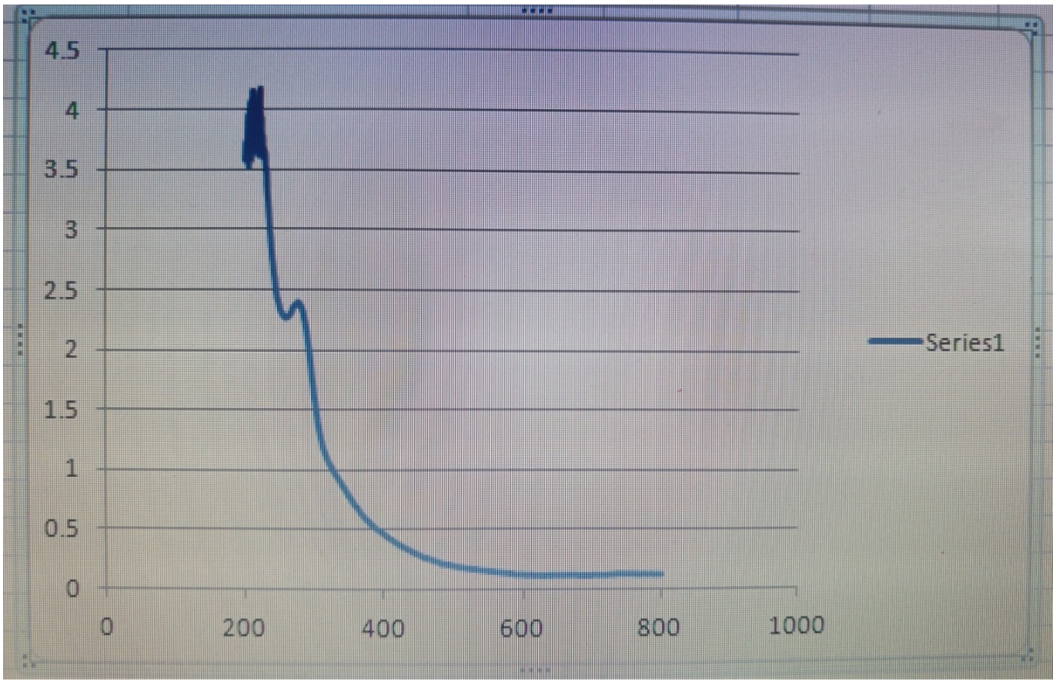

Fig 9 : UV-VIS Absorption spectra of Cu QDs of *S. nigrum* leaf extract.

3.2 Determination of antioxidant activity

1 Approach 1:

The antioxidant activity of Cu quantum dots was assessed with little adjustment using DPPH: 1,1-diphenyl-2-picrylhydrazyl was used like free radicle model. DPPH provides a method that is speedy for evaluating the antioxidant property. Here, in the examination, different makeup - (300, 600, 900 and 1200 μL) of *S. nigrum* leaf extricate or/ Cu QDs were mixed to a 2 ml of 0.1 mM DPPH arrangement with the presence of pure methanol with option of DW in order makeup the last volume upto 3000 μL. Resultant reaction blend was then shaken energetically, trailed by incubating for 30 minutes at room Temperature in dark area. Control already was set up by addition of 1ml of distilled H_2O to 2ml of DPPH arrangement (*Jovanović et al. 2020*). The color change can be observed as follows: violet → yellow; it was a recognized attributable to its anti-oxidant potential. Moreover, reaction blends absorbance was resolved at 517 nm.

The formula used for determining the free radicalscavenging activity was:

INHIBITION → (%) = ¼ {[1- Absorbance of sample/Absorbance of control] x 100}

2 Approach 2:

Antioxidant properties are researched utilizing two unique kinds of free radicals, to be specific, superoxide and hydroxyl. Superoxide radical elimination productivity was resolved utilizing the autoxidation of pyrogallol technique, while OH- ones were controlled byFenton reaction. For determining the capacity of free radical scavenging (denoted as S), the conditions that were utilized are as follows:

$$S_S \rightarrow \% = \{[(A_S - A_0)/A_0] \times 100\%\}$$

Where:

S_S: scavenging activity to remove superoxide radicals (%)

A_0: - standard absorbance, (-)

A_S: - sample absorbance, (-)

$$S_{OH} \rightarrow (\%) = \{[(A_S - A_0)/(A - A_0)] \times 100\%\}$$ Where:

S_{OH}: - Scavenging activity to remove OH- radicals, (%)

A_0: - Std absorbance

A_S: - Sample absorbance

A: - Solution without absorbance

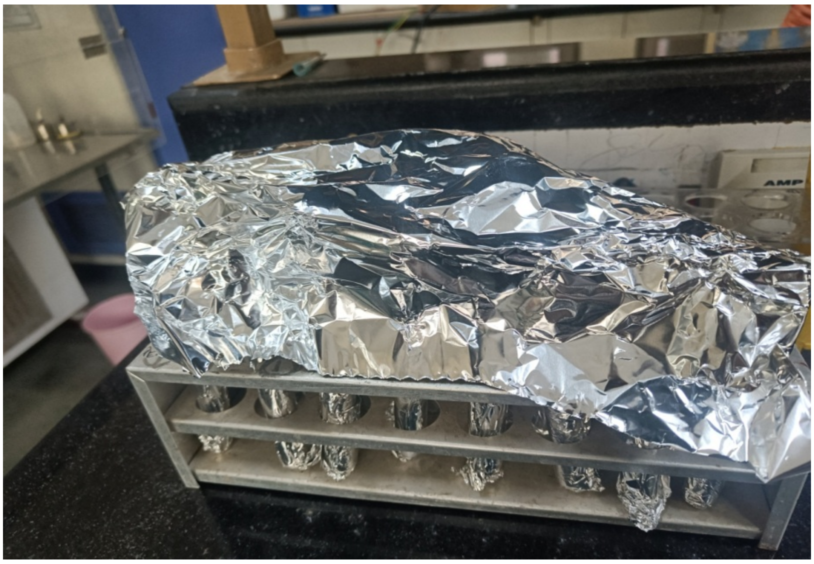

Fig 10: Determination of Antioxidant activity.

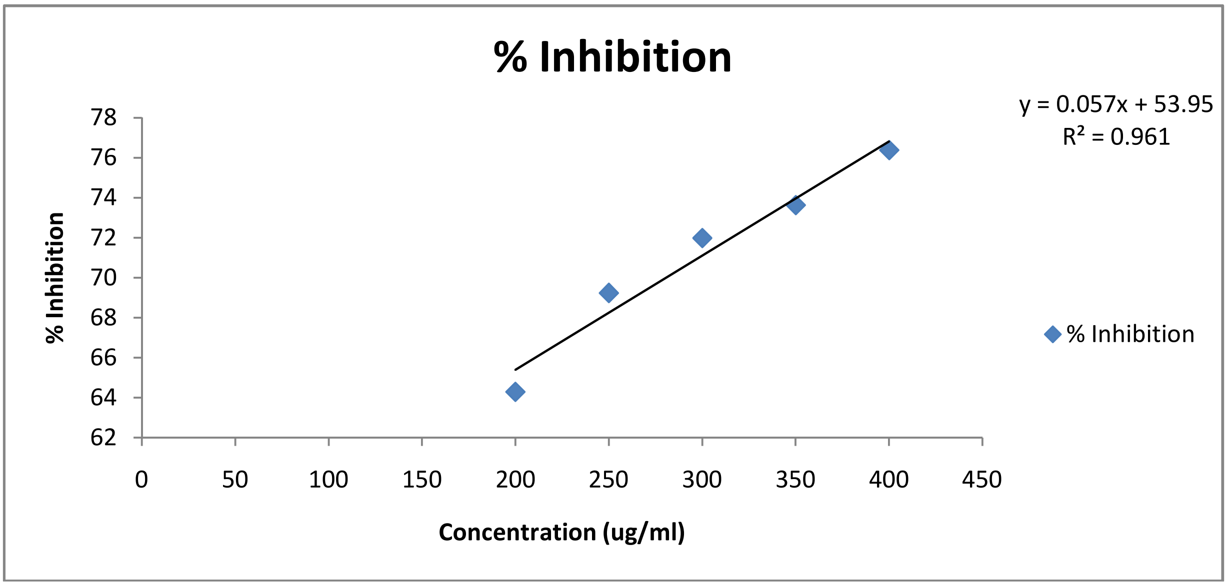

Fig 11: Antioxidant activity of Cu QD's

3.3 Determination of antibacterial activity

Firstly, the antibacterial evaluation of Copper Quantum Dots was done and it was then analyzed against the strains of *E. coli* ATCC25922 and *S. aureus* MTCC3760, respectively. Shortly, followed by the technique of agar well diffusion. Agar slants of supplement media are formed and then are utilized for keeping up the cultures of *E. coli* and *S. aureus* prepared in stock about 4C. Prior to tests, the unadulterated pure cultures are then sub-refined onto nutrient agar inclinations which are afterwards put into incubation for the time being at 37°C (*Jose et al. 2020*). Afterward, the nutrient agar plates are then arranged and then inoculation is done with one or the other strains of *E. coli/ S. aureus* and then followed by incubating for 24 h. It brought about even development (as seen all over the supplement nutrient agar plates). Wells are then punch and loaded with 50µL of Cu QDs, growth inhibition zone could be seen after keeping them for 17 hrs in incubator. Additionally, the activities of *S.nigrum* fruit extricate just as a mass of Cu_2SO_4 composite were examined (*Alavi et al. 2021*).

3.3. 1 Antimicrobial Suceptibility Test (AST)

For performing AST the cultures were revived, and the petri plates were made by pouring of MHA (Muller Hinton Agar) , then spreading of culture was done on the plate, then after that the wells were made on the plate , the wells were properly labelled according to what has to be loaded in each well. In this in each plate six wells were punched in first two wells $cuso_4$ was loaded in the next two wells CuQD's were loaded and in the last two wells Plant extract was loaded to see their activities against the strain used. This experiment was done in duplicates. After this the plates were kept in the incubator at 37^oC for about 17-24 hours.

The results were analysed after a proper incubation time and zones were seen only in case of CuQD's that is known as zone of inhibition. It states that the CuQD's has shown antibacterial activity. The plant extract and Copper sulphate alone loaded did'nt showed some significant results. From here it is clear that biofabricated CuQD's have been stated as good antibacterial agents. The zone of inhibition diameter in case of *E.coli* was 18.5mm, for *S. aureus* it was 15.5mm. Showing that both *E.coli* and *S. aureus* are good antibacterial agents. The results are shown below in the figure.

Fig 12: Antibacterial activity of Cu QD's against (1) *E. coli* ATCC25922 (-) and (2) S. aureus MTCC3760 (+) (a) $CuSO_4$ (b) Cu QD's (c) *S. nigrum* extract.

3.3.2 MIC (Minimal Inhibitory Concentration)

The minimum concentration of an antimicrobial substance which entirely prevents a bacteria from growing, whether in tube or microdilution wells, is known as the minimum inhibitory concentration, or MIC.

Microtiter Plate was taken and the proper amount of sample was taken with media, further dilutions of sample were done and the amount of culture in each well was taken same, so as to see which minimum sample is completely inhibiting the growth of the organism. The positive and negative controls were also taken, the whole setup was incubated for 24hrs and then the results were analysed, visually we can see the well where there is no turbidity, and then after that the turbidity started appearing in the next wells it means the well which was clear without any turbidity is showing MIC (Minimal Inhibitory Concentration), as turbidity in the wells indicate that the amount of antimicrobial agent is not sufficient to inhibit the growth of organisms. The MIC values for CuQD's was analysed and calculated. The MIC value of CuQD's was 0.25mM

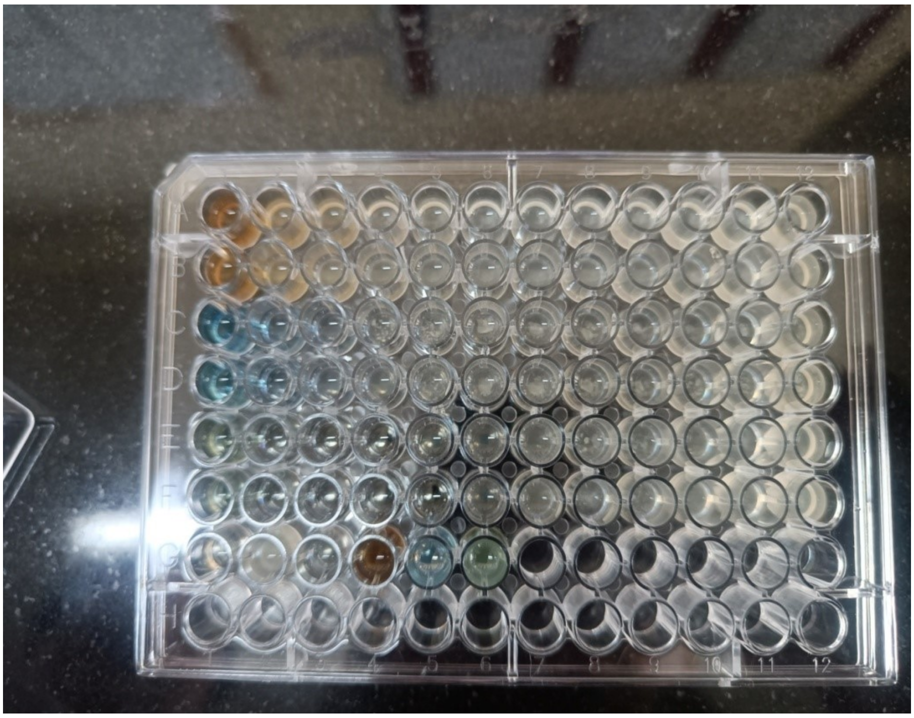

Fig 13: Determination of MIC (Minimun Inhibitory Concentration)

CHAPTER 4

CONCLUSIONS AND FUTURE PERSPECTIVES

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4.1 Conclusions

- The present state-of-art provides the basic, conservative, fast and green engineered fabrication of Cu QDs utilizing *S. nigrum* leaf extract.
- The investigation exhibited the as synthesized copper quantum dots can be potentially used like an effective antioxidant and antimicrobial and catalytical specialists.
- The copper quantum dots so-delivered may be viable as the antimicrobial specialist against the *E. coli/S. aureus* strains yet additionally portray the fast decrease of Methylene Blue, recommending that copper quantum dots may be productively utilized in industrial wastewaters both as antimicrobial agents (*Singh et al., 2020*).
- The synthesis of Copper QDs, which serve like reducing or capping agents, was predominantly attributed to the polyphenols in *S. nigrum* leaf extract.
- According to the results of this study, such QDs might be utilized as anti- inflammatory, copper-based dressings and antimicrobial coatings for wounds, as well as a substitute substance to fight illnesses associated to bioterrorism and multiple drug-resistant pathogenic bacteria. (*Rani et al. 2020*).

4.2 Future directions

- Additional research is necessary to fully characterise the sensor characteristics, cytotoxicity, and mechanisms of action associated with both the antioxidant and antibacterial activity of Copper QDs (*Alavi et al. 2021*).
- Future work aims to find less harmful QDs, such as Green sythesized QDs with distinctive optical properties, and it covers broad range of uses, like solar cells, photocatalysis, other bioapplications (biosensing and bioimaging) (*Zhang et al. 2021*).

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