GREEN SYNTHESIS OF COPPEROXIDE NANOPARTICLES USING *ALIUM CEPA* AND ITS USAGE FOR LEAD DETECTION.

Project report submitted in partial fulfillment of the requirement for the degree of

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

By

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CERTIFICATE

This is to certify that the work reported in the B.Tech project report entitled **"Green synthesis of copperoxide nanoparticle using *Alium cepa* and its usage for lead detection. "**which is being submitted by Archita Johari in fulfillment for the award of Bachelor of Technology in Biotechnology Engineering by the Jaypee University of Information Technology, is the record of candidate's own work carried out by him/her under my supervision. This work is original and has not been submitted partially or fully anywhere else for any other degree or diploma.

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OBJECTIVE

The objectives of this project are:-

1. Green synthesis of metal nanoparticles from *AliumCepa.*
2. Characterization of nanoparticles (UV, TEM AND EDX)
3. Check of reactivity of copperoxide nanoparticles with different heavy metal salts.
4. Check the reactivity of CuO NP with lead in lake water.

ABSTRACT

Metal nanoparticles are mostly used in cases where we need to have small sized particles with more surface area. Therefore, it is good for being used as a biosensor. Copperoxide nanoparticles have already been used in sensing materials. Copperoxide nanoparticles have large surface area and the method of producing it using *Alium Cepa* is efficient, environment friendly, resourceful and reasonable. *Alium Cepa* used for producing copperoxide nanoparticles also works as reducing as well as stabilizing agent. Thus, no extra chemicals were required in producing it. UV – Vis, TEM and EDX characterization of thus produced copperoxide nanoparticles was done. UV-Vis characterization was done to check if nanoparticles were produced and of what type. TEM was done to find about size and morphology of the produced nanoparticles. And EDX was done to check the purity of the sample. The nanoparticles were checked for reaction with different heavy metal salts. And it was observed that copperoxide nanoparticles showed higher sensitivity and selectivity towards lead salt. UV-Vis, TEM and EDX characterization of reacted lead and copperoxide nanoparticles showed some positive results, confirming that green synthesized copperoxide naoparticles using *Alium Cepa* can be used as lead biosensor.

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LIST OF ACRONYMS

- **NPs** Nanoparticles
- **CuO** copper oxide
- **mM** Millimolar
- **nm** Nanometre
- **pM** picomolar
- **UV-Vis** Ultra-Violet Visible
- **EDX** Energy dispersive X-ray diffraction
- **SEM** Scanning Electron Microscope
- **JUIT** Jaypee University of Information Technology

CHAPTER 1

INTRODUCTION

INTRODUCTION

Lead is a non-degradable environment pollutant. It impacts human health severely by causing neurological [1], mental [2], anaemic [3], reproductive [4], hypertensive and cardiovascular [5] disorders. Lead sensors based on organic luminescent dyes [6], catalytic DNA [7] and quantum dots based fluorescent sensors [8] have been made but are disadvantageous due to photobleaching after repeated usage, fragility of nucleases and gradual weakening on addition of more lead respectively [9]. False signals are a possibility in G- quadruplex [10], DNAzyme [11] and fluorescent based sensors [12]. Fluorescent based biosensor's signal fluctuates due to environmental changes, probe concentration and instrumentational inefficiency and in DNAzyme based lead sensors, signals are affected due to RNA nuclease activity [13], intermolecular quenching and temperature sensitivity [14]. QCM- nanomagnetic beads biosensors require certain fixed environmental condition and complex processes to work [15]. FRET based biosensors [9] have poor selectivity and weak signal sensitivity [14]. Atomic absorption/ emission spectrometry [16], plasma mass spectrometry [17] and anodic voltametry methods [18] require a lot of time and needs highly sophisticated equipments. Therefore, here we have tried to make a very simple lead biosensor by green synthesizing copper oxide nanoparticles.

Green synthesis is a process of producing nanoparticles using biological entities like actinomycetes [19], bacteria [20], fungus [21], plants [22], viruses [23] and yeasts [24]. Also, plant approach is considered better than using microorganisms because it doesn't require complex procedure like isolation, culture preparation and maintenance, furthermore, it tends to be faster, cost effective and easy to scale up [25]. This method is clean, non-toxic and environment friendly. It also benefits in one step synthesis and eliminates the usage of reducing, stabilizing and capping agent[26].

Allium cepa a.k.a onion is an Angiosperm which has worldwide ease of access and is among the most explored plant group. *Allium* genus is high in Cs (S- alk(en)yl-L- Cysteine sulfoxides) [27]. Since, there is presence of surfactants in *Allium cepa,* it can stabilize the nanoparticles and prevent agglomeration. It also has various therapeutic uses, like, anti-diabetic, antibiotic, hypocholesterolaemic, fibrinolytic and various other biological uses. [28]

Copper oxide nanoparticles have been used as antimicrobial [29], antibiotic, antifungal, biocide, as gas sensor, magnetic resistance material, for solar energy transformation and organic/inorganic nanostructure composites [30]. It is a p-type semiconductor with good optical, electrical, physical and magnetic properties [31]. Copper oxide nanoparticles have already been made via electrochemical methods, sono-chemical methods, sol-gel technique, microemulsion system, precipitation, synthesis and microwave irradiation [32]. It has also been produced by green synthesis using many plants like, Convolvulus percicus L. [33], Gloriosa superba L. [30], Banana peel extract [34], Aloe vera leaf extract [35], Gum karaya [36], Azardirachta indica [37] and Abutilon indicum [38]. It is being produced using *Allium cepa* for the first time.

CHAPTER 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Nanotechnology is an interdisciplinary science which includes various fields like material sciences, physics, chemistry, biology, engineering and mathematics. One nanometer is one billionth of a meter or 10^{-9} m. Nanotechnology involves controlling atoms and molecules at nanoscale. Nanomaterials have enhanced properties like higher strength, lighter weight, increased control of light spectrum, and greater chemical reactivity than their macroscale counterparts [40]. Nanotechnology have been used in various fields like drug delivery targeting cancerous cells, fashioning water and stain resistant fabrics, to enhance reactivity of materials, making bullet proof jackets using carbon nanotubes, MEMS and NEMS [41]. A nanoparticle is any particle with at atleast one diamentionless than 100mM [42].

While, nanotechnology is known to have arrive very recently but actually its usage dates back to pre- Christian era. Ancient humans have knowing or unknowingly been using nanotechnology in many ways like they used to make glass using nanotechnology; Lycurgus cup which used to show different colors when light was thrown inside and outside [43], in middle age peopleused to paint glass of churches using nanotechnology, have been used in making Damask steel, etc [44].

Particle at nanoscale show varied properties like extraordinary strength, electrical conductivity, chemical reactivity, superparamagnetic behavior. These properties does not exist in the same element at macroscale. Size reduction has been known to one of the biggest reason for such novel properties. Many nanoparticle based material has been used commercially. These are cosmetics and personal care products, paints and coatings, household products, catalysts and lubricants, sports products and textiles, medical and healthcare products, food and nutritional ingredients, food and packaging, agrochemicals, veterinary medicine, construction materials, consumer electronic products, etc. [45]

Table 1.2. General classification and potential applications of NPs [14].

Product areas with end-products containing NPs	Sectors where nanotechnology is expected to have a considerable impact
• Cosmetics and personal care products	• Medical and pharmaceutical sector
• Paints and coatings	• Bio-nanotechnology, bio-sensors
• Household products	• Energy sector, including fuel cells, batteries and photovoltaics
• Catalysts and lubricants	• Environment sector including water remediation
• Sports products and textiles	• Automotive sector
• Medical and healthcare products	• Aeronautics sector
• Food and nutritional ingredients	• Construction sector, including reinforcement of materials
• Food packaging and agrochemicals	• Composite materials
• Veterinary medicines	• Electronics and optoelectronics, photonics
• Construction materials	
• Consumer electronics	

Figure 2.1: Various commercial usage of nanoparticles

Nanoparticles have superior photo stability, narrow range of emission, broad excitation wavelength, multiple possibilities of modification therefore they have been used efficiently for imaging. They have used quantum dots and magnetic nanoparticles for imaging purposes. Various multifunctional nanoparticles have been used therapeutically, like nanoparticle used not only for imaging but also for drug delivery. These type of Nanoparticles have been used in treating Hyperthermia. They have also been used in photodynamic therapy.

High surface area to volume ratio makes nanoparticles a better catalyst and therefore they can bind with biological entities efficiently and therefore can be used to detect toxicity outside as well as inside of the cells. They have also been used to study cell cytotoxicity. [46]

Nanoparticles have been produced using physio-chemical methods but these are expensive, produce toxic solvents, hazardous and mostly gives imperfect surface structure. Chemical methods can be more reactive and toxic. But in green synthesis, a bottom up approach, expensive and toxic chemicals are replaced by natural extract of either plants or microbes. Giving us a method which is less expensive, eco friendly and better quality of nanoparticles are produced free of chemical contamination. Furthermore, they help in recycle of expensive metal salts. Biological materials such as proteins, enzymes, sugars and even whole cells gives us a more stably produced nanoparticles. Infact it is known that biologically produced nanoparticles are more efficient than chemically produced one in terms of biomedical applications.

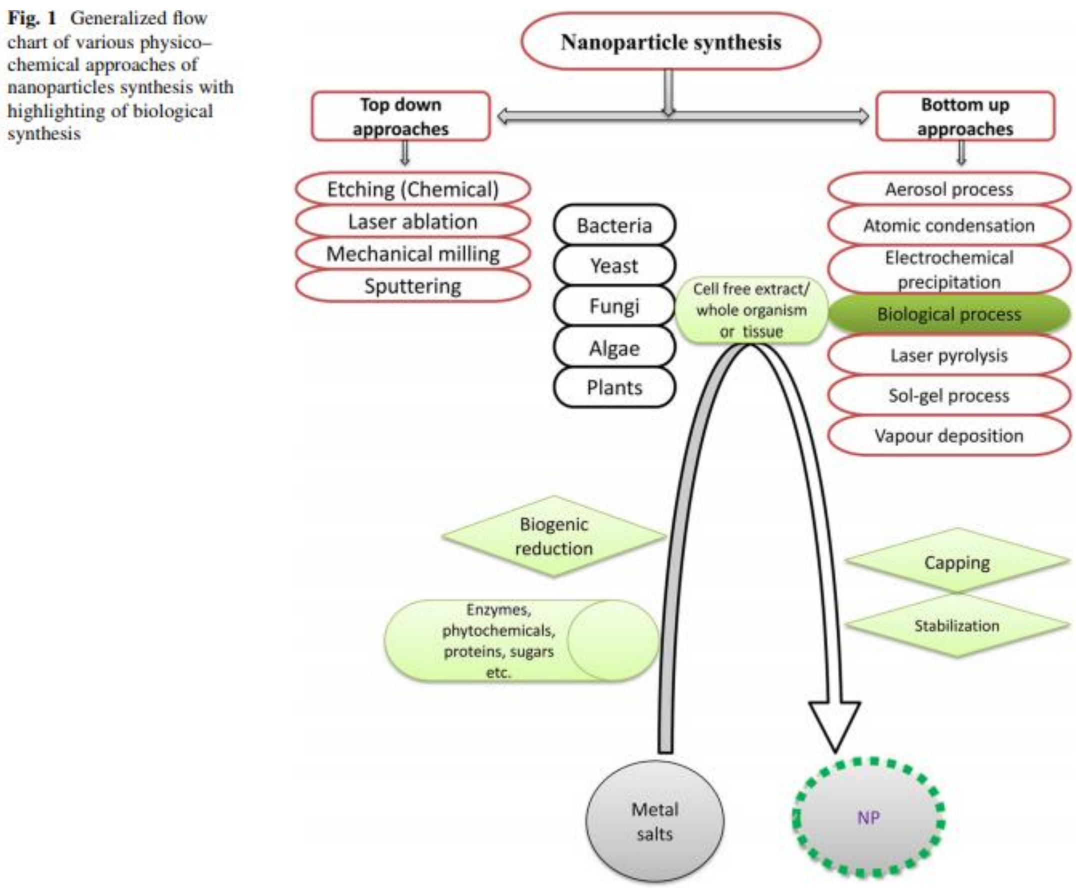


Figure 2.2: Synthesis of nanoparticles.

Natural resources based naoparticles are thus sustainable, eco-friendly, inexpensive and chemical free. It can be used for large scale production of nanoparticles. Capping and stabilizing agents present in biological entities enhances stability and inhibits agglomeration process. [47]

Plant mediated green synthesis is considered the best because it is more complex system and easy to handle. Microbial systems requires more maintenance and are prokaryotic, thusnot more suitable green synthesizing nanoparticles.

Alium cepa L. is a very commonly used plant and cultivated all over the world, it has been used in food and medicine since forever. Onions are rich in sulfur compounds , it is therefore rich in aroma and flavor. Onion also posses various medicinal properties like antibiotic, antidiabetic, anticancerous and antiatherogenic.

Green synthesis using onion is therefore very common. Silver nanoparticles have been produced using *Alium cepa* L., which has been used as a biosensor for ascorbic acid [48]. It has also been produced in another study similarly but for studying the antimicrobial activities of thus produced silver nanoparticles [49]. Silver nano-coated fabric have been made and its anti microbial activity was observed in another study using onion [50]. Gold nanoparticles have been made using onion extract [51]. Copper nanoparticles using onion was made which was used a plant growth precursor [52].

Copperoxide is a metal nanoparticle, it has high surface to volume ratio and therefore has excellent catalytic properties and can be used as a biosensor. Copperoxide nanoparticles have been made using precipitation method, via various precursors in which nanoparticles of different shapes, size and morphology was made[53]. Copperoxide nanoparticle was made using banana peel and its photocatalytic activities were checked [54]. *Gloriosa superb* L. based copperoxide had excellent antibacterial activities. [55]. Copperoxide nanoparticle made from *Abulition indicum* leaf extract showed various biomedical applications like antioxidant, antimicrobial and photochemical dye degradation [56]. Different size range of copperoxide nanopaticle (49nm, 89nm, 120nm, 324nm) were produced using *Azardica Indica* [57].

Lead is a hazardous heavy metal which needs to be eliminated from the environment in order to prevent human health. Lead pollution is one of the main concern in medical sciences, therefore its detection and removal is important. Colorimetric based lead sensor using DZAzyme mrthode have been made which can detect up to 100nM of lead. [58] QCM – nanomagnetic bead biosensor for lead detection was made which can detect up to 0.3pM of lead concentration. [59]

However, these are mostly physio-chemical systems, so more green synthesis based systems are required for detecting lead.

CHAPTER 3

MATERIALS AND METHOD

MATERIALS AND METHOD

3.1. MATERIAL USED

CHEMICALS

1. Cupric sulfate
2. Lead acetate
3. Mercury (II) chloride
4. Manganese (II) chloride
5. Sodium molybdate
6. Barium chloride
7. Zinc chloride

OTHER MATERIALS

1. *Alium cepa* without its covering
2. Distilled water

APPARATUS

1. Gloves
2. Cotton
3. Beaker
4. Conical flask
5. Eppendorffs
6. Magnetic stirrer
7. Magnetic bead
8. Glass rod
9. Hot plate
10. Micropipette
11. Tips
12. Refrigerator
13. Weighing machine

14. What man filter paper
15. Parafilm
16. Spectrophotometer
17. Cuvette
18. Tissue paper
19. Falcon tubes
20. Muslin cloth

3.2 **METHOD**

3.2.1 GREEN SYNTHESIS OF COPPEROXIDE NANOPARTICLES

Following steps are included in preparation of copperoxide nanoparticle preparation:-

1. Preparation of onion extract.
2. Preparation of copper solution in different concentration.
3. Preparation of nanoparticles.

3.2.1.1.PREPARATION OF EXTRACT

1. 1 kg Fresh onions (*Alium cepa)* were bought from the local market.
2. Its cover was peeled out and the peeled onions were washed.
3. The onion was cut into small pieces and was blended.
4. From the blended onions extract was taken out using a muslin cloth.
5. The extract was the filtered twice using whattman filter paper so that no impurities were left.
6. It was then stored at 4°C in refrigerator.

3.2.1.2. PREPARATION OF COPPEROXIDE SOLUTION OF DIFFERENT CONCENTRATION

1. Cupric sulfate ($CuSO_4.5H_2O$) salt weight was measured according to the concentration of the solution to be prepared (0.05M).
2. The salt was mixed with distilled water according to the ratio (10ml).
3. The solution was kept aside for further reactions.

3.2.1.3.<u>PREPARATION OF NANOPARTICLES</u>

1. The onion extract and cupric sulfate solution were mixed in different ratios (5:10, 7:10, 1:1 and 12.5:10)
2. The solution mixture was kept on the hot plate magnetic stirrer at temperature of 55°C for 40 mins.
3. The water was completely evaporated from the solution and a brown coloured layer was at the bottom.
4. 10 ml of water was added to it.
5. It was further kept for characterization.

Figure 3.1: Brown layer to which water id added to make NP solution.

3.2.2. <u>REACTIVITY WITH HEAVY METAL SALTS</u>

Following steps are involved in checking the reactivity of copperoxide nanoparticles towards heavy metals:

- Preparation of 100mM salt solution of Lead acetate, Mercury (II) chloride, Manganese (II) chloride, Sodium molybdate, Barium chloride and Zinc chloride.
- Preparation of 120 times diluted nanoparticle solution enough for reacting with each salt.
- Mixed 500µL of salt with 1000µL of nanoparticle solution.
- Repeated step 3 for each of the salts.
- Observed the spectra under UV – Vis spectrophotometer.

3.2.3. REACTIVITY WITH LEAD SALT

- 100mM Lead acetate salt solution was prepared.
- Aliquots were prepared of this salt solution.
- 120 times diluted nanoparticle solution and water was added to it accordingly.
- All the solutions were observed under UV – Vis spectrophotometer individually.

Table 3.1: Aliquots of Lead Acetate salt solution with NPs.

Nanoparticle solution µl	Water µl	Lead acetate solution µl
1000	500	0
1000	450	50
1000	400	100
1000	350	150
1000	300	200
1000	250	250
1000	200	300
1000	150	350
1000	100	400
1000	50	450
1000	0	500
1000	0	600
1000	0	700

3.2.4. REACTIVITY OF MERCURY CHLORIDE

1. 100mM Mercury chloride salt solution was prepared.
2. Aliquots were prepared for the salt solution
3. 120 times diluted nanoparticle solution and water was accordingly.
4. Each solution sample was observed under UV- Vis spectrophotometer individually.

Table 3.2: Aliquots of Mercury chloride salt solution with NPs.

Nanoparticle solution µl	Water µl	Mercury chloride solution µl
1000	500	0
1000	450	50
1000	400	100
1000	350	150
1000	300	200
1000	250	250
1000	200	300
1000	150	350
1000	100	400
1000	50	450
1000	0	500

3.2.5. REACTION WITH LEAD ACETATE IN LAKE WATER

1. 100mM of Lead acetate salt solution was prepared in “Gambar kad” water, taken from near the university.
2. Aliquots of this solution were prepared.
3. 120 times diluted nanoparticles solution was made using “Gambar kad” water.
4. Nanoparticle solution and “Gambar kad” water was mixed accordingly.
5. Solutions were observed under UV – Vis spectrophotometer.

Table 3.3: Aliquots of Lead Acetate salt solution with NPs in “Gambar kad” water.

Nanoparticle solution µl	Gambar kad Water µl	Lead acetate solution µl
1000	500	0
1000	450	50
1000	400	100
1000	350	150

1000	300	200
1000	250	250
1000	200	300
1000	150	350
1000	100	400
1000	50	450
1000	0	500

CHAPTER 4

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

4.1 Nanoparticle synthesis

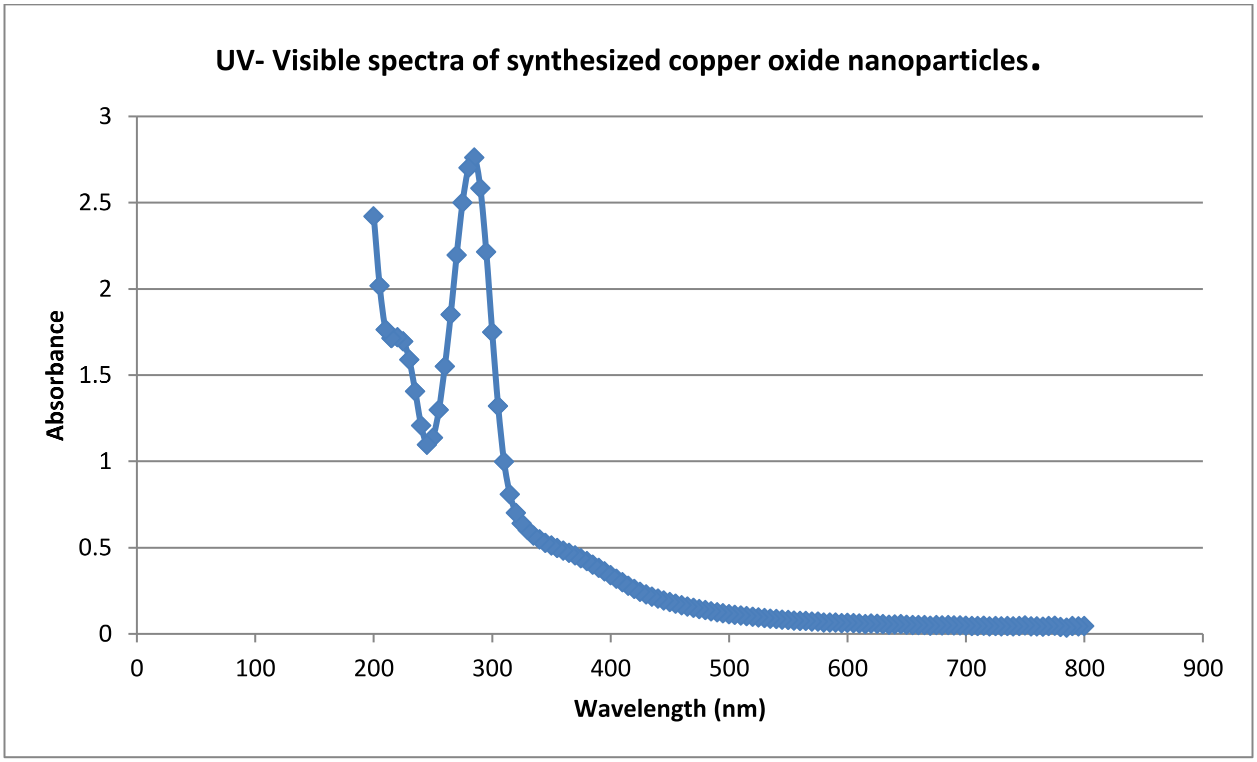
It was observed that nanoparticles were produced when the whole water in solution was evaporated and a brown colored layer was left at the bottom to which water was added to make a solution.

Figure 4.1: Water added to brown colored layer at the bottom, giving us the nanoparticle solution.

After the addition of water to this brown colored residue, we could see a copper layer formed at the bottom of the vessel. This proved that the nanoparticle produced is of copper.

Figure 4.2: Copper layer formed at the bottom of vessel.

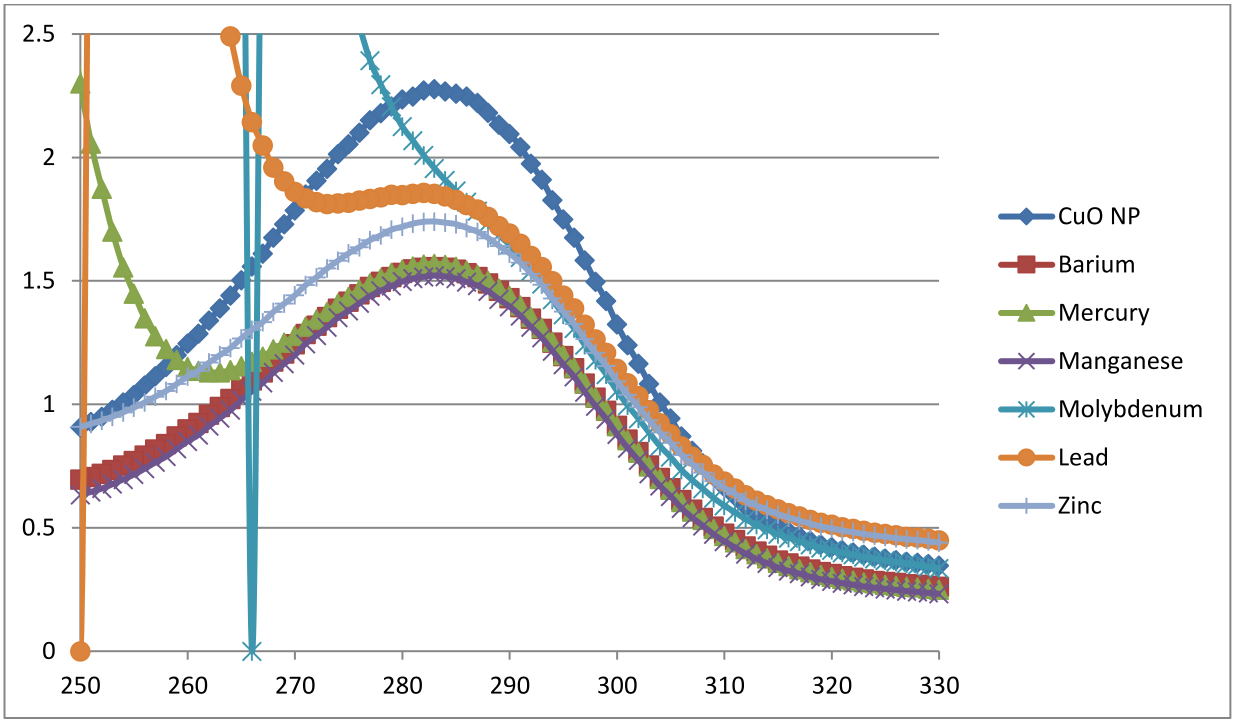
UV – Vis spectroscopy was performed for this solution, showing us a peak at 285nm, ranging from 25nm to 330nm. Confirming the synthesis of copperoxide nanoparticles {ref.}.



Graph 4.1: UV Spectra of synthesized nanoparticles.

3.3 **Reaction with different heavy metal salts**

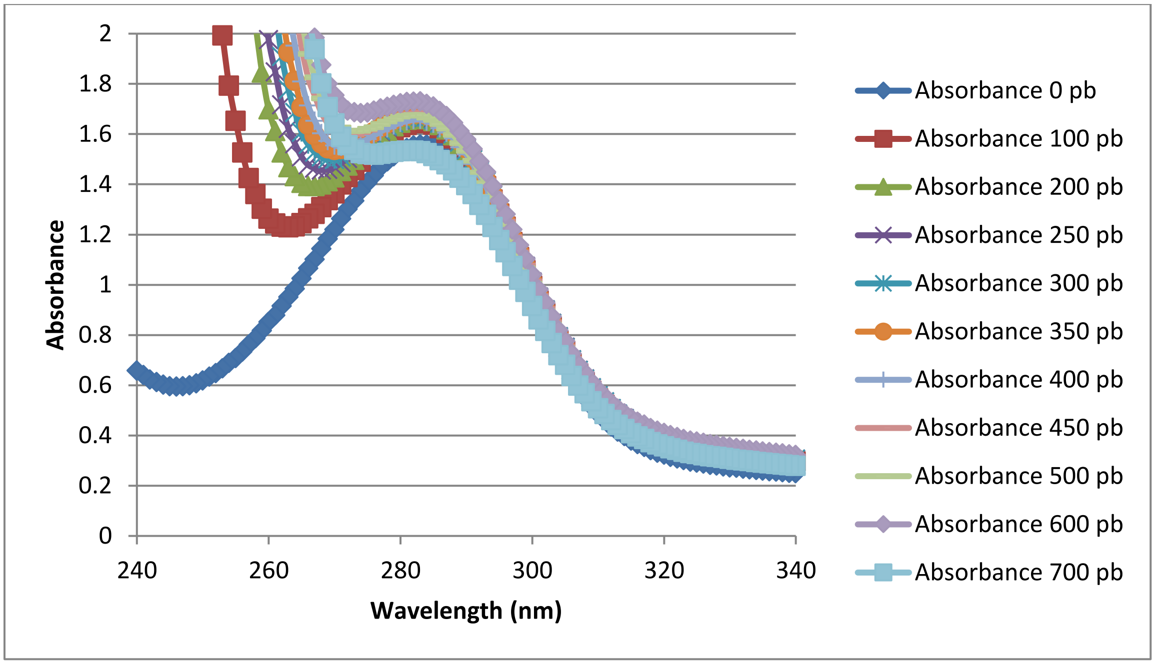
The reaction with different heavy metal salts i.e., Lead acetate, Mercury (II) chloride, Manganese (II) chloride, Sodium molybdate, Barium chloride and Zinc chloride, showed that copperoxide nanoparticles best reacts with Lead salt.



Graph 4.2: Graph showing reaction of copperoxide NPs with different heavy metal salts.

4.2 Reaction with Lead acetate

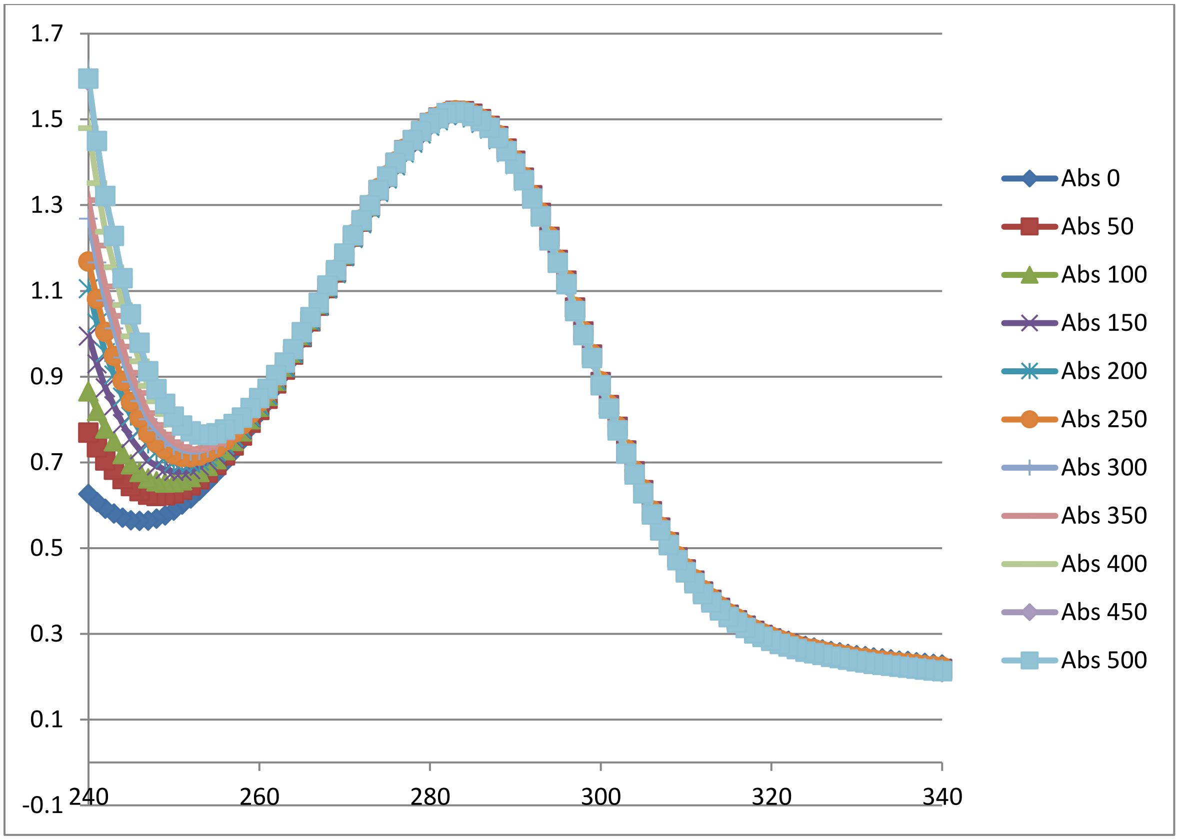
The reaction of copperoxide nanoparticles showed that its reaction with different concentration of Lead acetate, gives us the miniconcentration of its detection i.e., 500mM.



Graph 4.3: Shows various concentration of Lead acetate reaction with NP solution.

4.3 Reaction of nanoparticles with Mercury Chloride.

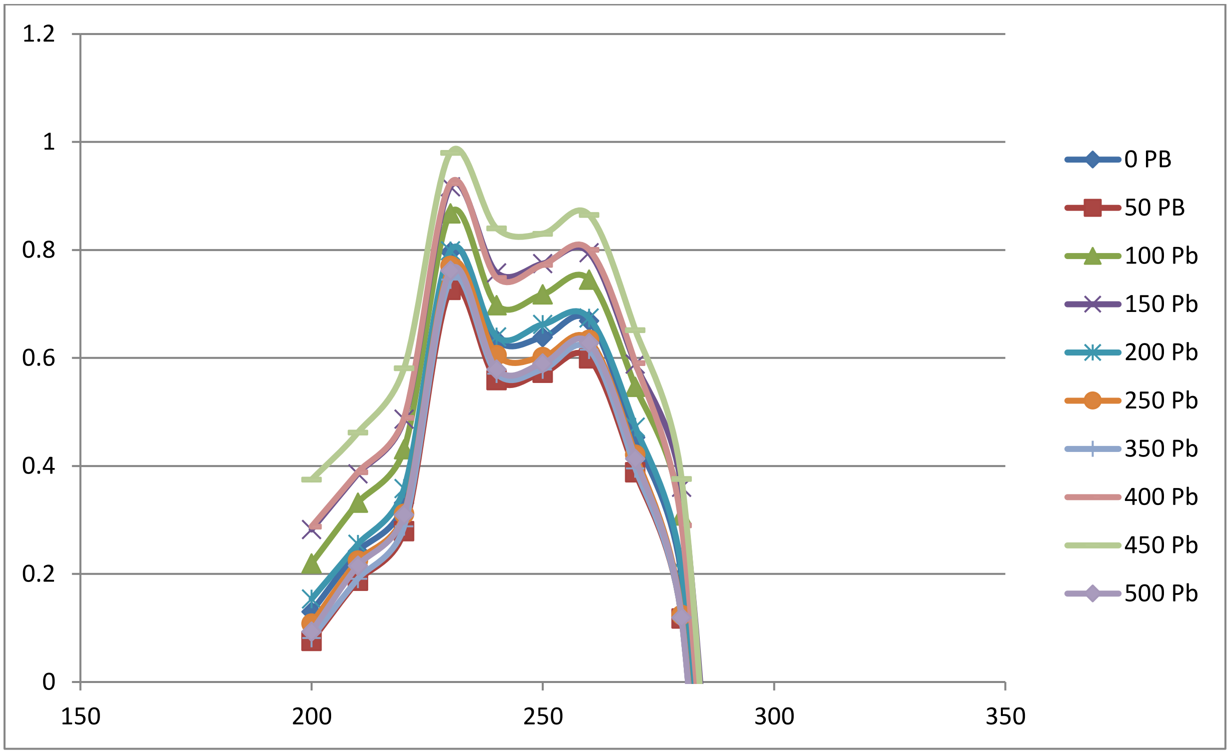
The reaction of copperoxide nanoparticles with different concentration of Mercury chloride shows no visible reaction with it, proving that copperoxide nanoparticles are very specific for Lead salt.



Graph 4.4: Reaction of various concentration of Mercury Chloride with CuO NPs.

4.4 REACTION OF NANOPARTICLE WITH LAKE WATER

Nanoparticles were mixed with Lead acetate salt solution made in lake water taken from "Gambar kad". This did not show any reaction, meaning that the produced nanoparticles are yet inefficient to be used as waste water sample Lead biosensor.



Graph 4.5: Graph showing reaction of Lead with CuO NPs in "Gambar kad" water.

4.5 SCANNING ELECTRON MICROSCOPE(SEM) (COPPEROXIDE NANOPARTICLES).

The results of scanning electron microscope (Quanta 450 FEG) carried out in IITR Lucknow, showed that the average size of copperoxide nanoparticles is 19.5 nm.

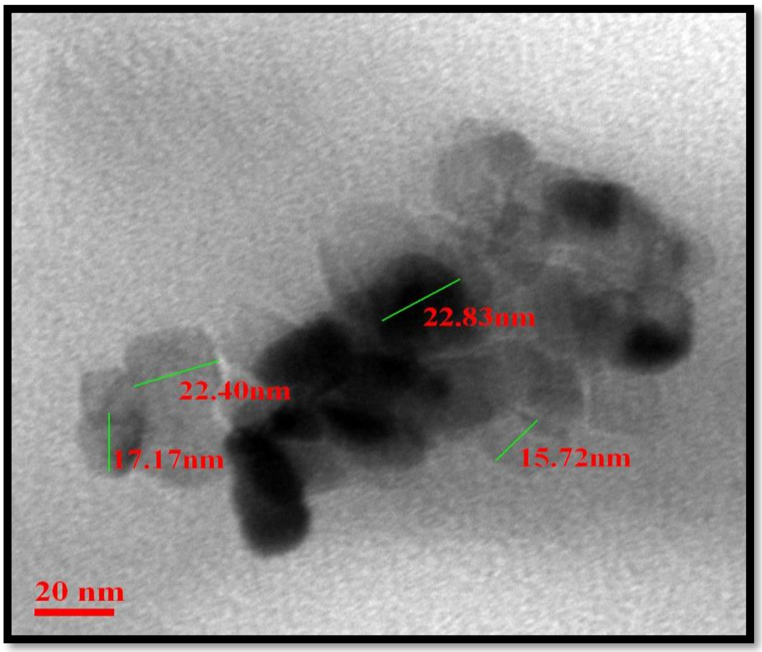


Figure 4.3: SEM image of copperoxide nanoparticles.

4.6 SEM (SCANNING ELECTRON MICROSCOPE) OF COPPEROXIDE NANOPARTICLES REACTED WITH LEAD ACETATE

The SEM (Quanta 450 FEG) image of copperoxide nanoparticle shows both change in morphology of nanoparticles as well as reduction in aggregation of copperoxide nanoparticles, showing that Lead acetate salt is reacting with the functional component present on the surface of copperoxide nanaoparticles. Thus, confirming the biosensor activity of copperoxide nanopaticle for detecting Lead. The average size of nanoparticles gets reduced to an average of 10.9 nm.

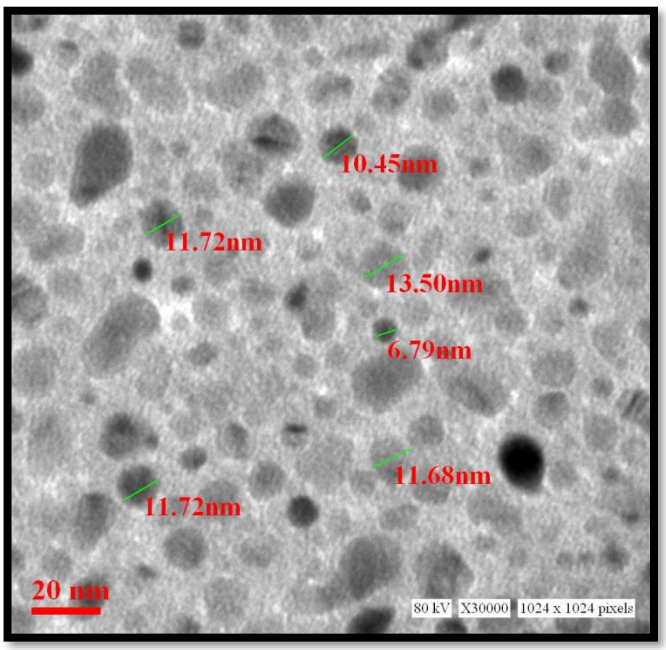


Figure 4.4: SEM image of copperoxide nanoparticles after reaction with Lead.

4.7 EDX (ENERGY DISPERSIVE X-RAY) OF COPPEROXIDE NANOPARTICLES

The EDX (Quanta 450 FEG) results of copperoxide nanoparticles shows the presence of Kα , Kβ and L electronic shells, confirming the presence of copper nanoparticles.

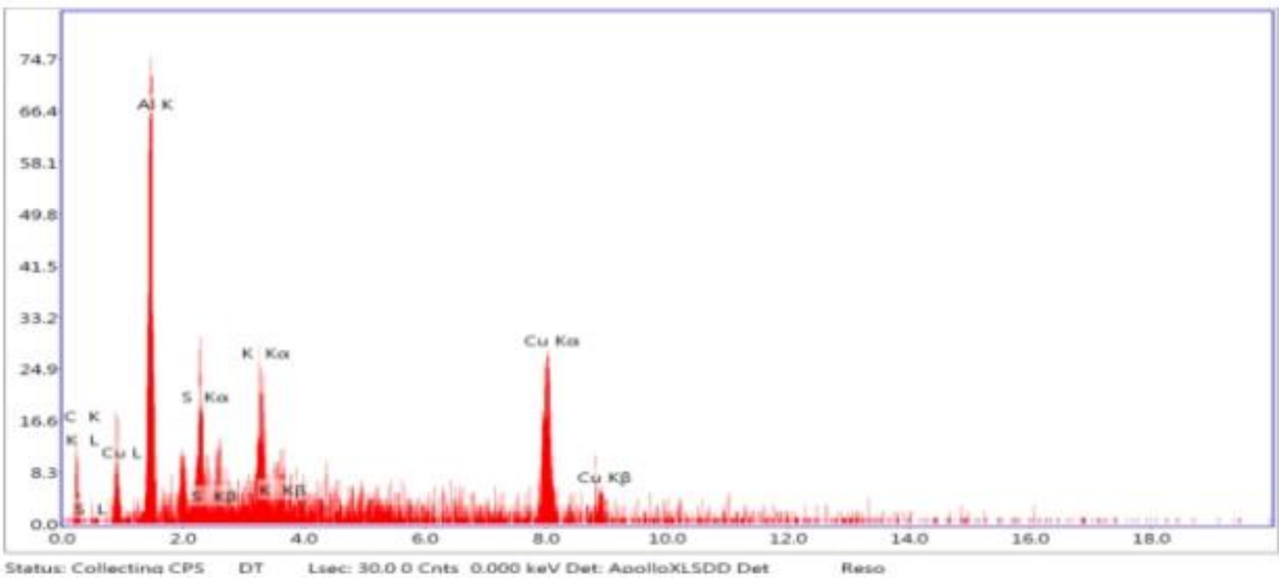


Figure 4.5: EDX results of copperoxide nanoparticles.

4.8 EDX(ENERGY DISPERSIVE X-RAY) OF COPPEROXIDE NANOPARTICLES AFTER REACTION WITH LEAD ACETATE.

The EDX (Quanta 450 FEG) shows the results of reaction of copperoxide nanoparticles with Lead salt. The image shows Lα, Lβ and M electronic shells of Lead present in the sample.

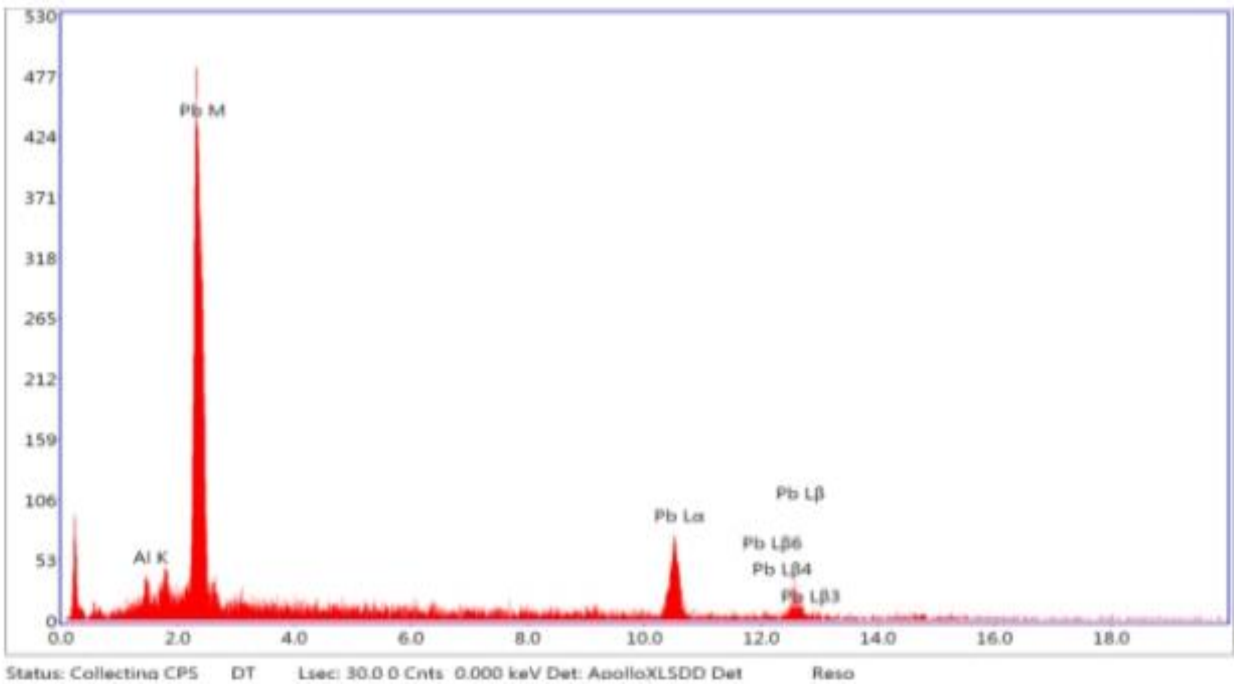


Figure 4.6: EDX results of copperoxide nanoparticles after reaction with Lead.

CHAPTER 5

SIGNIFICANCE OF THE PROJECT

SIGNIFICANCE OF THE PROJECT

Since, the discovery of nanoparticles there have been a huge impact of this technology in many areas of day to day life. Therefore, there is a huge demand of producing the nanoparticles cheaply, safely and efficiently. Physical and chemical methods of producing nanoparticles have various drawbacks like time consumption, health hazard and varied results. With the evolution of green synthesis for production nanoparticles there have been many advantages like less time consumption, environment and health safety and due to the presence of various organic substances like secondary metabolites, better quality of nanoparticles have been produced. Plant is one of the best system to produce nanoparticles because it is easy to handle as compared to microbial systems. Onion (*Alium cepa)* is one of the most common plant we use in our day to day life which is known to have various medicinal properties since the ancient times like treating digestive disorders, bone strength, blood related problems, etc. Copper is a very abundant and therefore is safe and cheap metal. Hence, producing it will be cost effective, cheap and easy.

Metal nanoparticles have been known to have more surface area with less volume, therefore it will be very effective as a biosensor. And as we can see from scanning electron microscope (SEM) results, the average size of nanoparticles have been reduced from 19.5nm to 10.9nm plus, aggregation was reduced , therefore we can say that Lead had a significant reaction with copperoxide nanoparticles. So, these nanoparticles produced from *Alium cepa* shows sensitivity towards lead. The nanoparticles are also very selective in nature for lead as mentioned in the report. Also, we did not need any extra chemical to functionalize or stabilize nanoparticle, since onion is used which is very rich in sulfur content. Therefore, I have produced copperoxide nanoparticles to be used as a biosensor for detectionof lead with a minimum concentration of 100mM.

REFERENCES

2. Sanders, T., Liu, Y., Buchner, V. and Tchounwou, P.B. (2009). Rev Environ Health.; 24(1): 15–45.
3. Bouchard, M., Bellinger, D.C., Weuve, J., Bellinger, J.M., Gilman, S.E., Wright, R.O., Schwartz, J. and Weisskopf, M.G. (2009) Arch Gen Psychiatry.; 66(12): 1313–1319.
4. Waldron, H.A. (1966) Br J Ind Med. ; 23(2): 83–100.
5. Xuezhi J, Youxin L, Yilan W (1992) Biomed Environ Sci.;5(3):266-75.
6. Vaziri N.D. (2008) Am J Physiol Heart Circ Physiol. ;295(2):H454-65.
7. Chen C.T., and Huang W.P, (2002) *J. Am. Chem. Soc.*, 2002, *124* (22), pp 6246–6247
8. Li, J. and Lu Y. (2000) *J. Am. Chem. Soc.*, *122* (42), pp 10466–10467.
9. Meshik, X. ,Xu, K., Dutta, M. and Stroscio, M.A. (2014) IEEE TRANSACTIONS ON NANOBIOSCIENCE, VOL. 13, NO. 2.
10. Xu S, Xu S, Zhu Y, Xu W, Zhou P, Zhou C, Dong B, Song H. (2014) Nanoscale ;6(21):12573-9.
11. Kotch F.W., Fettinger J.C. and Davis J.T. (2000) *Org. Lett.*, *2* (21), pp 3277–3280.
12. Kuang H. ,Yin H., Xing C., and Xu C. (2013) Materials (Basel).; 6(11): 5038–5046.
13. Zhan S.,Wu Y.,Luo Y.,Liu L.,He L.,Xing H.,Zhou P. (2014) Analytical Biochemistry, Volume 462, Pages 19-25.
14. Liang L, Lan F, Ge S, Yu J, Ren N and Yan M (2017) Anal Chem.;89(6):3597-3605.
15. Nadarajan S.P., Ravikumar Y., Deepankumar K., Lee C.S. and Yun H. (2014) *Chem. Commun.*,50, 15979-15982
16. Zhang Q , Cui H , Xiong X, Chen J , Wang Y , Shen J , Luo Y , Chen L (2018) Analyst.;143(2):549-554.
17. Tan Q. , Wu P., Wu L. and Hou X. (2006) Microchimica Acta, 155:441
18. Hall E.S. and Murphy E. (1993) Journal of Radioanalytical and Nuclear Chemistry; Volume 175, Issue 2, pp 129–138.
19. Zhu L. , Tian C. , Yang R. and Zhai J. (2008) Electroanalysis ; Volume20, Issue5; Pages 527-533.
20. Sowani H., Mohite P., Munot H., Shouche Y., Bapat T., Kumar A. R. , Kulkarni M. and Zinjarde S. (2016) Process Biochemistry Volume 51, Issue 3, Pages 374-383.

21. Divya K., Kurian L.C., Vijayan S. and Shaikmoideen J.M. (2016) JWENT, Article 9, Volume 1, Issue 1, Page 63-74.

22. Molnár Z., Bódai V., Szakacs G., Erdélyi B., Fogarassy Z., Sáfrán G., Varga T., Kónya Z., Tóth-Szeles E., Szűcs R. and Lagzi I. (2018) *Scientific Reports* volume 8, Article number: 3943
23. Siavash Iravani (2011) *Green Chem.*, 13, 2638-2650.
24. Velusamy P., Kumar G.V., Jeyanthi V, Das J, and Pachaiappan R. (2016) Toxicol Res.; 32(2): 95–102.
25. Niknejad F., Nabili M., Ghazvini R.D., and Moazeni M. (2015) Curr Med Mycol.; 1(3): 17–24.
26. Shah M., Fawcett D., Sharma S., Tripathy S.K., and Poinern G.E.J. (2015) Materials (Basel); 8(11): 7278–7308.
27. Patil M.P., Palma J., Simeon N.C., Jin X., Liu X., Ngabire D., Kim N, Tarte N.H. and Kim G. (2017) *New J. Chem.*,41, 1363-1371.
28. Rose P, Whiteman M, Moore PK and Zhu YZ. (2005) Nat Prod Rep.;22(3):351-68
29. Augusti KT (1996) Indian J Exp Biol.;34(7):634-40.
30. Amiri M. , Etemadifar Z., Daneshkazemi A., and Nateghi M. J (2017) Dent Biomater; 4(1): 347–352.
31. Naika H.R. , Lingaraju K., Manjunath K., \Kumar D., Nagaraju G., Suresh D., Nagabhushana H. (2015) Journal of Taibah University for Science 9 ; 7–12.
32. Phiwdang K., Suphankij S. , Mekprasart W. and Pecharapa W. (2013) Energy ProcediaVolume 34, Pages 740-745.
33. Grigore M.E., Biscu E. R., Holban A.M., Gestal M.C., and Grumezescu A.M. (2016) Pharmaceuticals (Basel); 9(4): 75.
34. Hosseinzadeh R., Mohadjerani M. and Mesgar S. (2017) IET Nanobiotechnology (Volume: 11, Issue: 6,) Page(s): 725 - 730
35. Aminuzzaman M., Kei L.M., and Liang W.H. (2017) AIP Conference Proceedings **1828**, 020016.
36. Kumar V., Shameem U., Kollu P., Kalyani R.L. and Pammi S.V.N. (2015) BioNanoScience 5(3)
37. Padil V.V.T. and Černík M. (2013) Int J Nanomedicine.; 8: 889–898.
38. Ansilin S., Nair1 K.J., Aswathy C., Rama V., Peter J.and Persis J.J. (2016) Journal of Nanoscience and Technology 2(5); 221–223
39. Ijaz F., Shahid S., Khan S.A., Ahmad W. and Zaman S. (2017) Tropical Journal of Pharmaceutical Research; 16 (4): 743-753.

40. https://www.nano.gov/nanotech-101/what/definition

41. http://www.understandingnano.com/introduction.html

42. https://www.sciencedaily.com/terms/nanoparticle.html

43. TheLycurgus Cup www.britishmuseum.org/explore/highlights/highlight_objects/pe_mla/t/the_lycurgus_cup.aspx
44. NANOSCIENCE AND NANOTECHNOLOGIES - History Of Nanotechnology - N.K. Tolochko
45. IOP Concise Physics, Designing Hybrid Nanoparticles, Maria Benelmekki, Chapter 1, An introduction to nanoparticles and nanotechnology.
46. Biomedical engineering, Chapter 13, Nanoparticles in Biomedical Applications and Their Safety Concerns, Jonghoon Choi and Nam Sun Wang
47. Biotechnol Lett (2016) 38:545–560; Green synthesis of nanoparticles and its potential application; Imtiyaz Hussain
48. Mohammad A.Khalilzadeh; Green synthesis of silver nanoparticles using onion extract and their application for the preparation of a modified electrode for determination of ascorbic acid; Journal of Food and Drug Analysis; Volume 24, Issue 4, October 2016, Pages 796-803
49. Geetika Sahni; Controlled green synthesis of silver nanoparticles by *Allium cepa* and *Musa acuminata* with strong antimicrobial activity; International Nano Letters; June 2015, Volume 5, Issue 2, pp 93–100
50. Prashansa Sharma; Green synthesis and characterization of silver nanoparticles by *Allium cepa L.* to produce silver nano-coated fabric and their antimicrobial evaluation; AOC; Volume32, Issue3**;** March 2018; Pages e4146
51. Umesh Kumar Parida; Green Synthesis and Characterization of Gold Nanoparticles Using Onion (*Allium cepa*) Extract; WJNSE; vol.1, no. 4, 2011
52. Bhanushali Mansi P; Copper Nanoparticles using Onion (Allium cepa) Extract and their Application in Plant Growth ; Int. J. of Life Sciences, 2017, Vol. 5 (4): 661-666
53. KankanitPhiwdan; Synthesis of CuO Nanoparticles by Precipitation Method Using Different Precursors;
54. Mohammod Aminuzzaman; Green synthesis of copper oxide (CuO) nanoparticles using banana peel extract and their photocatalytic activities; AIP Conference Proceedings 1828, 020016 (2017)

55. H. RajaNaika; Green synthesis of CuO nanoparticles using *Gloriosa superba* L. extract and their antibacterial activity; JTUSCI; Volume 9, Issue 1, January 2015, Pages 7-12
56. Faheem Ijaz; Green synthesis of copper oxide nanoparticles using Abutilon indicum leaf extract: Antimicrobial, antioxidant and photocatalytic dye degradation activities; Tropical Journal of Pharmaceutical Research April 2017; 16 (4): 743-753.
57. S. Ansilin; Green Synthesis and Characterisation of Copper Oxide Nanoparticles using Azadirachta indica (Neem) Leaf Aqueous Extract; Journal of Nanoscience and Technology 2(5) (2016) 221–223.
58. Juewen Liu; A Colorimetric Lead Biosensor Using DNAzyme-Directed Assembly of Gold Nanoparticles; *J. Am. Chem. Soc.* 125, 22, 6642-6643.
59. Qingli Zhang, QCM-nanomagnetic beads biosensor for lead ion detection; *Analyst*, 2018,143, 549-554.