PHARMACEUTICALLY RELEVANT CuNP, PbNP AND LiNP FROM ASPERGILLUS SPECIES

Mirza Farhana Ishaque, Nabila Anwar Patrawala and Dr. D. R. Majumder*

Abeda Inamdar Senior College 2390-B, K.B Hidayatullah Road, Azam Campus, Pune - 411001.

ABSTRACT

The microorganisms showed the unique potential in the production and accumulation of nano metals with different shapes and sizes by ‘bioleaching’. The biological system – Aspergillus species a fungus, isolated from soil was capable of synthesizing nanoparticles such as lithium, lead, and copper using LiCl₃ (0.025M), Pb(NO₃)₂ (0.0002M), and CuSO₄(0.025M) as a precursor metal salt. The morphology and the size of the synthesized nano metals as per Scanning Electron Microscopy micrograph confirmed the formation of LiNPs with 85 nm – 100 nm size range, CuNPs with 80 nm – 86 nm in range and PbNPs with 70 nm – 95 nm in range and all three were almost spherical in shape. These nano Metals were further characterized by UV Visible Spectrophotometry, X-ray differraction analysis, Fourier Transform Infrared analysis, Cyclic Voltammetry. The ability of these nanoparticles in the field of electronics for the use in batteries were also tested. This green synthesis at room temperature was the most ecofriendly way to produce nano metals.

KEYWORDS: Bioleaching, Biosynthesis of Nano Metals, LiNP, CuNP, PbNP, Aspergillus species.

1. INTRODUCTION

The most consistent ecofriendly method for synthesis of nanometals biologically is of utmost importance in the field of nanoscience and nanotechnology. The biosynthesis of nanoparticles have gained an increasing attention due to the great need to develop safe, cost-effective & ecofriendly technologies for nano-materials synthesis.[1] This mainly involves the use of microorganisms. Nanotechnology is mainly the synergy of mechanical, material sciences,
microelectronics, electrical, chemical and biological screening. The extraction of metals from their ores by using the living organisms is called bioleaching. This is much cleaner than the traditional heap leaching using cyanide.

Nanoparticles (NPs) are mainly the atoms in the size range of 1–100 nm. It is well known that the properties of a metal NPs are determined by its size, shape, composition, and structure.[2] As compared to the traditional synthetic methods, biological systems provides a novel idea for the production of nano-materials.[3] A variety of microorganisms are capable of synthesizing inorganic components either intra- or extracellularly to produce metal nano particles. Thus microorganisms are potential eco-friendly nano factories. As compared to bacteria, fungi have been known to secrete much higher amounts of bioactive substances, which made fungi more suitable for large-scale production.[4] Furthermore, the extracellular biosynthesis using fungi could also make downstream processing much easier than bacteria. With respect to mono dispersity, using fungi, nanoparticles of well-defined dimensions can be obtained. Compared to bacteria, fungi could be used as a source for the production of large amount of nanoparticles. This is because of the fact that fungi secrete more amounts of proteins which directly translate to higher productivity of nanoparticle formation.[5]

Nano materials have been synthesized by an array of physical, chemical and biological methods.[6] Different methodologies have been formulated to synthesize these noble metal nano particles of particular shape and size. These methods are costly and involve the procedure where hazardous chemicals are used. Biosynthesis of gold, silver, silica, platinum, magnetite gold–silver alloy, selenium, and uranite nanoparticles by fungi, bacteria, actinomycetes, yeasts and viruses have been already reported extensively.[7-14] Recently metal nano particles are being applied in a wide range in industries as they possess excellent optical, magnetic, catalytic and electronic properties. The potential applications of lithium nanoparticles are in lithium ion batteries.[15] Copper nano particles has application as an antibiotic, anti-microbial, and anti-fungal agent when added to plastics, coatings and textiles.[16-21] As magnetic nanoparticles lead nano metal is used for magnetic data storage and magnetic resonance imaging (MRI).

2. MATERIALS AND PROCEDURES
2.1 Isolation and identification of Fungi
Soil sample was collected from Abeda Inamdar College Campus and plated on Glucose Peptone Yeast Extract Agar (GPYA) plates. Further, the culture was enriched in Glucose
Peptone Yeast Extract Broth (GPYB) and incubated on shaker for 20 days at 120rpm-37ºC. Wet mount of biomass was performed with Lacto phenol Cotton blue stain.

2.2 Bioynthesis of LiNPs, CuNPs and PbNPs using microorganism
The biomass from the incubated flask was separated by centrifugation at 3000 rpm for 15minutes and inoculated in the flasks containing 50ml sterile solutions of LiCl₃, CuSO₄, Pb(NO₃)₂, further incubated at room temperature for 96 hrs in dark to check the reaction. Control (Without biomass) were also run along with reaction flasks.

2.3 UV-Visible spectroscopy analysis
The cell free filtrate from all the 3 flasks were analyzed using UV-Visible spectrophotometer (wavelength ranging 200nm-800nm) at room temperature for different periods of time.

2.4 Scanning Electron Microscopy studies
The cell free filtrate were evaporated at 100ºC for 3hrs and characterized by Scanning Electron Microscopy (JEOL JSM- 6360A (The Department of Physics, University Of Pune) for their morphological studies.

2.5 X-ray Diffraction Analysis (XRD)
X-ray Diffraction pattern, using Cu Kα radiation with( λ = 0.15148 nm) were recorded on X-ray Diffractometer BRUKER axs,D8 advance, (Department of Physics, University Of Pune) were used to determine the formation and quality of nanoparticles in the samples.

2.6 Electronical analysis
Samples were analyzed to check their capacity as conductor by using LiNPs, PbNPs, CuNPs as electrolyte solutions where cathode and anode were dipped into it. Further volt was recorded by voltammeter (Department of Electronics, Abeda Inamdar Senior College).

2.7 Fourier Transform Infrared studies (FTIR): The FTIR spectra were conducted on Jasco FT-IR-4100 (Department of Chemistry, Abeda Inamdar Senior College) to determine the presence of covalent bond and functional groups in the molecules.

2.8 Cyclic Voltammogram: In Cyclic Voltammetry, three electrode system is used, platinum disc as the working electrode, Platinum wire as the auxiliary electrode and Lithium, Copper, Lead as reference electrode respectively. This analysis were carried on BAS Epsilon Electrochemical Instrument (Department of Chemistry, Abeda Inamdar Senior College).
3. RESULTS

3.1 Fungal morphology: The wet mount of the growth from plate with Lacto phenol Cotton blue stain was performed. Microscopically at high power (450X) the fungal mycelial morphology matched with *Aspergillus sp.* (Fig 1).

![Figure 1: Aspergillus sp.](image1)

3.2 Synthesis and characterization of LiNPs, CuNPs and PbNPs using *Aspergillus* species: In this study, it was generally recognized that there was color change in the solutions (Fig. 2a, Fig. 2b and Fig. 2c) due to incubation for 96 hrs. Thus the color change in the solution was indicative of the formation of LiNPs, CuNPs and PbNPs.

![Figure 2(a): LiCl$_3$ with *Aspergillus* (B) and LiCl$_3$ without *Aspergillus* (C).](image2)
Clear solution    Brownish

Figure. 2(b): Pb(NO$_3$)$_2$ without Aspergillus (C) and Pb(NO$_3$)$_2$ with Aspergillus (B).

Light Blue    Dark Blue

Figure. 2(c): CuSO$_4$ without Aspergillus (C) and CuSO$_4$ with Aspergillus (B)

3.3 UV-Visible Spectroscopic Studies

The reactions between biomass and metal solution and the stability of the metal nano particles formed in the solutions were monitored using UV-Visible Spectroscopic analysis. The UV-Vis spectra of the cell filtrate with LiCl$_3$, CuSO$_4$ and Pb(NO$_3$)$_2$ showed a broad peak at 200nm, 300nm and 200nm respectively. When the spectral analysis were performed after 24hrs, 72hrs and 120hrs periods of incubation, the absorbance maxima was observed to be at the same wavelength (Fig. 3a, Fig. 3b and Fig. 3c) with concentration of nano metals varying. The maximum concentration of LiNP and PbNP was at 24 hours and for CuNP it was 120 hours.
Figure 3(a): UV-Visible spectrum of Lithium nano particles formed in reaction mixture.

Figure 3(b): UV-Visible spectrum of Copper nano particles formed in the reaction mixture.

Figure 3(c): UV-Visible spectrum of Lead nano particles formed in the reaction mixture.
3.4 Scanning Electron Microscopy

The morphology and the size details of the synthesized metal NPs were determined using Scanning Electron Microscopy. SEM micrograph confirmed the formation of LiNPs with 85 nm – 100 nm size range, CuNPs with 80 nm – 86 nm in range and PbNPs with 70 nm – 95 nm in range (Fig. 4) and are almost spherical in shape.

Figure 4a: SEM images of the synthesized LiNPs.

Figure 4b: SEM images of the synthesized PbNPs.

Figure 4c: SEM images of the synthesized CuNPs.
3.5 X-Ray Diffraction Analysis: For further study, the metal NPs were analysed by XRD. The confirmation of the LiNPs, CuNPs and PbNPs by the characteristic peaks observed in XRD pattern are shown in Fig. 5. The particle size of the metal NPs were determined by a equation called Debye-Scherrer equation.\[22\]

\[
D_p = \frac{0.94 \lambda}{\beta_{1/2} \cos \theta}
\]

Where,

\(D_p (\text{nm})\) = average Crystallite size

\(K\) = Scherrer constant=0.94

\(\lambda\) (nm) = wavelength of X-Ray Diffraction

\(\theta\) (degrees) = diffraction angle

\(\beta\) (radian) = full width of half maxima of highest peak.

![Figure 5a](image_url)

**Figure 5a:** Representative X-ray diffraction pattern of LiNPs.

![Figure 5b](image_url)

**Figure 5b:** Representative X-ray diffraction pattern of CuNPs.
Using XRD spectrum analysis, diffraction peaks were obtained at 2θ values for Lithium, Copper and Lead nanoparticles. The crystalline size of NPs were LiNp-4.22nm, CuNP-2.96nm and PbNP-3.38nm.

3.6 Electricity Generation
By using CuNPs, LiNPs, PbNPs, DC electricity can be generated which derives load like Light Emitting Diode (LED) which requires 1.58V, 1.56V and 1.48V respectively to glow. As lead nanoparticle was a weak electrolyte, more cells need to be connected in series to glow the LED. (Fig 6)
3.7 FT-IR Analysis: The FTIR spectra determined the presence of covalent bond and functional groups in the molecules. The FT-IR measurements were carried out to identify the possible biomolecules responsible for capping and efficient stabilization of metal nanoparticles synthesized by *Aspergillus sp*. Fourier Transform Infrared peaks were ranging from 1000-4000 cm\(^{-1}\) which confirmed the presence of polyphenols with aromatic ring and bound amide region, required for the synthesis and stabilization of copper, lead and lithium nanoparticles. The peaks near 3012.27 cm\(^{-1}\), 3015.16 cm\(^{-1}\), 3009.37 cm\(^{-1}\) for a) CuNPs, b) LiNPs and c) PbNPs respectively correspond to N-H stretching bond. 1739.48 cm\(^{-1}\), 1740.44 cm\(^{-1}\), 1737.55 cm\(^{-1}\) for a) CuNPs, b) LiNPs and c) PbNPs correspond to C-H stretching bond. The weaker band at 1368.25 cm\(^{-1}\) and 1367.28 cm\(^{-1}\) for a) CuNPs, b) LiNPs and c) PbNPs correspond to amide arising due to carbonyl stretch in proteins. The peak at 1220.72 cm\(^{-1}\) corresponds to C-O stretching vibration of the carboxy group in a) CuNPs, b) LiNPs and c) PbNPsO.
Figure 7 a): FT-IR spectra of CuNPs.

Figure 7 b): FT-IR spectra of LiNPs.

Figure 7 c): FT-IR spectra of PbNPs.
3.8 Cyclic Voltamogramm: Cyclic Voltammetry is used to study the redox property of the chemical species. The CV of copper NPs showed only one oxidation peak which can be accounted for conversion of Cu$^0$ to Cu$^{+1}$. Further, no redox peak was observed for Cu$^{+1}$ to Cu$^0$ as the reaction is not feasible at given potential. Similarly, there is no peak observed for Li and Pb NPs as the redox reaction is not feasible at the given potential range. (Fig 8)

![Cyclic Voltammogram of synthesized CuNPs.](image)

Figure. 8: Cyclic Voltammograph of synthesized CuNPs.

4. DISCUSSION

In recent years Fungi is being exploited in a big way for eco-friendly synthesis of metal nanoparticles. In our work we found Aspergillus sp. Capable of producing all three metal nanoparticles viz. CuNP, LiNP and PbNP.$^5$ Fungi has successfully produced CuNP previously. As per SEM studies Fusarium oxysporum leached out copper in the range of 93-115 nm$^{18}$ while in our present study Aspergillus sp. produced CuNP in the range of 80 nm – 86 nm. Extracellular PbNP measuring 1.77–5.8 μm was produced by Aspergillus sp.$^6$ as stated in the reference,$^{23}$ while our Aspergillus sp. produced extracellular PbNP measuring in the range of 70 nm – 95 nm. Lithium nanoparticles measuring 85 nm – 100 nm were produced by Aspergillus sp.$^7$ for the first time. In UV-Vis Spectroscopy, CuNP showed maximum absorbance at 300nm which is consistent with the present reference which states the maximum absorbance of CuNP between 250-350$^{24}$, PbNP showed maximum absorbance at 200nm which matches with 239nm from the reference$^{25}$ and for LiNP our study showed maximum absorbance at 200nm while reference$^{26}$ says 263nm which is comparable. The crystalline size of NPs were LiNP-4.22nm, CuNP-2.96nm and PbNP-3.38nm, are comparable to the references$^{25}$ for CuNP and PbNP while$^{26}$ for LiNP. Capping agents in myco synthesis of nanoparticles are mainly biomolecules like polysaccharides and aminoacids which is
evident from the FTIR results.\textsuperscript{[28]} Electrochemical behavior of CuNP as per Cyclic Voltammogram has been studied.\textsuperscript{[18]}

5. CONCLUSION
In this study, LiNPs, CuNPs and PbNPs were synthesized by \textit{Aspergillus sp} at room temperature and the Lithium nanoparticles were produced biologically for the first time as there were only physical and chemical methods practiced previously. Also, this study gave the outline for the first time that these nanoparticles can be used as diode and by increasing their voltage they can be used in working of batteries by connecting the cells in series.

ACKNOWLEDGEMENT
I would like to thank Abeda Inamdar Senior College for providing the necessary infrastructure conducive for research.

REFERENCE


