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Biosynthesis and Characterization of Copper Oxide Nanoparticles †

Alina Matei *, Gabriel Craciun, Cosmin Romanitan, Cristina Pachiu and Vasilica Tucureanu *

National Institute for Research and Development in Microtechnologies—IMT Bucharest, 126A Erou Iancu Nicolae, 077190 Bucharest, Romania; email1@email.com (G.C.); email2@email.com (C.R.); email3@email.com (C.P.)

* Correspondence: alina.matei@imt.ro (A.M.); vasilica.tucureanu@imt.ro (V.T.)

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Abstract: In this paper, we report the use of the natural extract of Echinacea leaves [*Echinacea purples]* for the biosynthesis of copper oxide nanoparticles, and copper nitrate [$Cu(NO_3)_2$ $3H_2O$] as a metal precursor salt. The synthesized CuO was investigated using FTIR and Raman spectroscopy, X-ray diffraction, FESEM microscopy, and EDX analysis. The FTIR spectra confirm the presence of the Cu-O bond by the appearance of the characteristic peak at 402 cm^{-1}, but also the presence of the functional groups characteristic of the biomolecules presents in the plant extracts used. The Raman spectra indicate peaks at wavelength of 272 and 610 cm^{-1}, which are characteristic bands for CuO. The XRD diffractogram indicates the formation of a monoclinic crystalline structure by the appearance of distinctive peaks corresponding to (110), (002), and (111), planes, with an average crystallite size of 15 nm. The SEM images reveal the formation of spherical particles with dimensions below 40 nm. The EDX spectrum confirms the presence of the peaks attributed to (C) and (O) atoms, without other impurities. Due to the small size, morphology and precise elemental composition of CuO NPs, this approach allows the synthesis of biomaterials with applicability in the development of antibacterial agents and biosensors.

Keywords: copper oxide; nanoparticles; biosynthesis; natural extract; characterization

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1. Introduction

Ecological approaches regarding the synthesis of metal oxide nanoparticles have attracted special attention, due to their ability to prevent environmental contamination, but also to improve the quality of life and human well-being. Among metal oxide nanoparticles, CuO NPs have attracted particular interest, due to their distinctive physical and chemical properties, as well as the antibacterial, antioxidant, and antifungal characteristics, based on which it has expanded its applicability in many biomedical, industrial, agricultural, electronic, and environmental applications, etc. [1,2]

Until now, many researchers have reported different synthesis approaches, namely the methods: sol-gel, coprecipitation, hydrothermal, solid-state reaction, thermal decomposition of precursors, microemulsion, sonochemical, photochemical reduction, through which pure and well-defined CuO NPs are obtained, but that releases waste harmful to the environment and health. In this context, the biogenic method was considered an alternative to the known physical and chemical methods. This process is emerging as an economic and sustainable approach, that has gained significant attention due to the use of less harmful substances and biological systems (e.g., plants, fungi, yeast, algae, bacteria etc.), with low adverse effects and high biocompatibility. Among these, the suitable parts (e.g., leaves, flowers, fruit, root etc.) are the most common components in the synthesis of nanoparticles, due to their non-toxicity, easy handling and low pur-

chase price. The applicability of natural extracts is given by the phytochemical compounds, such as phenols, flavonoids, carboxylic acids, terpenoids, tannins, etc., which act as both reducing and capping agents for the formation of CuO NPs, with effect on the physico-chemical characteristics, stability, and toxicity. The morphology of the oxide nanoparticles is influenced by the reducing potential of the bioactive compounds determined by the type and amount of extract, the concentration, and ratio of the main raw materials, the pH of the solution, the process temperature, etc. [3–6]

From the literature survey, it is observed that exists studies on the biosynthesis of CuO by using different types of plants and substances derived from them, such as *Aloe vera, Psidium guajava, Ruellia tuberosa, Tribulus terrestris, Pterospermum acerifolium, Galeopsidis herba, Gloriosa superb, Chamomile, Ocimum sanctum,* etc. through which nanostructures of different shapes (nanoparticles, nanorods, nanoflowers, nanosphere or clusters) were synthesized, with dimensions in the nanometric range. CuO NPs obtained by biosynthesis methods present an efficiency against ethylene blue (MB) dye, excellent antibacterial, antioxidant and antifungal properties, and can be considered a candidate for photocatalytic, as well as antimicrobial activity [7,8].

Echinacea purpurea is a medicinal herb, belonging to the *Asteraceae family*, which contains chicoric acid as the dominant component (among the phenylpropanoids), along with phenolic compounds such as flavonoids, tannins, alkaloids, starch, furochromones, and glycosides. It can be used as reducing agents and stabilizing agents in the synthesis of different types of nanoparticles, due to their antibacterial, antiproliferative, inhibitory, and antioxidant properties. This plant gained its reputation due to its phytochemical compounds and special properties, being used in different practical applications for oral consumption, skin treatment, intestinal pain, etc. However, the literature presents scarce studies on the use of Echinacea extract in the biosynthesis of nanoparticles. It was found that by using *Echinacea purpurea* extract as reducing and capping agents, nanoparticles of ZnO, TiO_2, Ag and Au, with sizes below 100 nm, were synthesized, with antibacterial susceptibility against both *Gram positive* (*S. aureus*) and *Gram negative* (*E. coli*) bacteria, enhanced antioxidant effects, antimicrobial properties (against *Escherichia coli* and *Candida albicans*) [9–11].

In this paper, we have reported an eco-friendly and simple approach for the synthesis of CuO NPs using a natural extract of Echinacea leaves [*Echinacea purples*] as a reducing and capping agent. The synthesized particles were investigated using FTIR and Raman spectroscopy, X-ray diffraction, FESEM microscopy, and EDX analysis. The results of this study offer a good understanding of the process parameters and the types of extracts (in our case Echinacea) that can be used to synthesize CuO NPs, and the extension of their applicability in various fields depending on the obtained properties (e.g., shape, size, composition, structure etc.).

2. Experimental Detail

2.1. Synthesis of CuO Nanoparticles

For the biosynthesis of CuO nanoparticles, we used the natural extract of Echinacea leaves [*Echinacea purples*], copper nitrate [$Cu(NO_3)_2$ $3H_2O$], sodium hydroxide [NaOH], deionized water [H_2O]. All chemicals were purchased from Sigma -Aldrich, without any further purification and the required solutions were prepared in deionized water. The dried Echinacea leaves were washed to remove dust particles, after which a quantity of 10 g was added to 100 mL DIW and heated at 100 °C for 2 h. The mixture was then cooled to room temperature and the extract was filtered using a Whatman filter paper No: 1. To synthesize CuO NPs, Echinacea extract was added dropwise over the aqueous solution of $Cu(NO_3)_2$ $3H_2O$ [0.1M], under continuous stirring using a magnetic stirrer, at a temperature of ~80 °C, for 2 h, the solution becomes dark green. The ratio between the main components, $Cu(NO_3)_2$: Echinacea extract = 2:1. The NaOH solution [0.1 M] was added drop by drop to the obtained solution, until a pH = 12, and this reaction was left

for 3 h, under continuous stirring, at a temperature of ~80 °C. After the end of the reaction and the formation of a brown precipitate, it was left to mature for 24 h. The obtained precipitate was centrifuged at 9000 rpm for 20 min. and washed 3 times with DIW. The powder was dried at 100 °C, for 4 h, followed by thermal treatment at 450 °C, for 3 h, with an oven heating speed of 5 °C/min., to obtain a brownish-*black* powder.

2.2. Characterization

FTIR spectra for CuO sample were recorded using Tensor 27 FTIR spectrometer (Bruker Optics, Germany). The spectral range was 4000–370 cm^{-1}, with a resolution of 4 cm^{-1}, at room temperature, and averaging 64 scans, using an ATR Platinum holder. Raman spectra was collected at room temperature using a Witec Raman spectrometer (Alpha-SNOM 300 S, WiTec. GmbH, Germany) using 532 nm as an excitation, and processing of collected datawas performed by a dedicated computer using WiTec Project Five software. Diffraction pattern for CuO sample was collected using a Rigaku Smartlab diffractometer using monochromatic Cu-Kα radiation, operating at 7a current of 5 mA and a voltage of 40 kV. The intensity data were recorded in the range between 20° and 95°. To assess morphology and particle size of the CuO sample a Field Emission Scanning electron microscope (FEI Company, Hillsboro, OR, USA) was used. The CuO NPs were dispersed in an organic solvent, deposited on silicon substrate and allowed to dry overnight. Elemental analysis on synthesized particles was carried out using Energy Dispersive X-Ray Analyzer (EDX) (Smart Insight AMETEK) attached to SEM equipment.

3. Results and Discussions

3.1. FTIR Analysis

Figure 1 shows the ATR-FTIR spectra for both the Echinacea extract (a) and for CuO NPs obtained from the extract (b) to establish the possible phytochemical compounds responsible for the bioreduction, capping, and stabilization of the CuO. The characteristic spectrum of the extract (Figure 1a) indicates the presence of metabolites (e.g., flavonoids, alkaloids, terpenes, etc.) which are responsible for the process of reduction and stabilization of these nanoparticles. Table 1 shows the possible assignments of the spectral bands both for the extract and for the oxide nanoparticles obtained. The FTIR spectrum of the CuO sample (Figure 1b) confirms the occurrence of the characteristic peak at 402 cm^{-1}, which can be attributed to the vibrations of Cu-O, but also the presence of the functional groups characteristic of the biomolecules present in the extract, that indicate the presence of C-O stretching alcohols, carboxylic acids, esters, and ethers. In Figure 1b, a peak of high intensity centered at around 402 cm^{-1} can be observed, which can be associated with the vibration mode of the Cu-O bonds, but also peaks of reduced intensity and shifted towards higher wave numbers, compared to the extract, due to the anchoring of the molecules from the extract on the surface of the oxide nanoparticles.

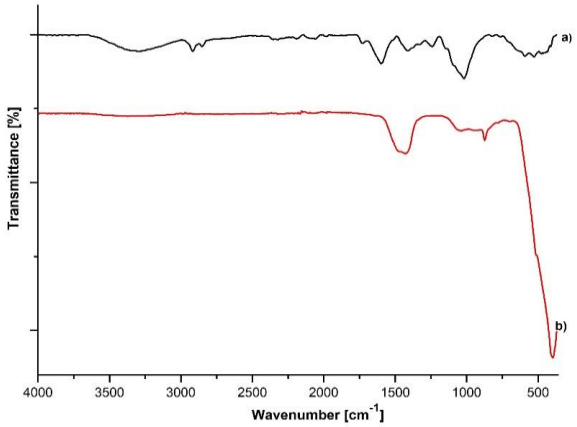

Figure 1. FTIR spectra of (**a**) Echinacea extract, (**b**) CuO NPs synthesized from Echinacea extract.

Table 1. Possible assignments of the FTIR spectral bands of Echinacea extract and the CuO NPs [12,13].

Possible Assignments	From…	Wavenumber [cm^{-1}]	
		Echinaceea	CuO
O-H	Stretching vibration from phenols hydroxyl groups	3300	-
-C-H	Aliphatic stretching vibration	2926	-
		2855	-
C=O	Stretching vibration band of esterified carbonyl groups overlap with carboxylic group	1730	-
C=O+ C=C, ν	C=O conjugated to the aromatic ring	1598	
C=O	Stretching vibrations of carboxylic group	1414	1425
S=O	Stretching vibration from sulfate ester	1330	-
C-O	Stretching vibrations	1246	-
S=O	Stretching vibration from sulfate ester	1151	-
C–O	Stretching vibration band	1022	1037
Cu-O	CuO	-	402

3.2. RAMAN Analysis

The Raman spectrum of CuO nanoparticles is presented in Figure 2, being obtained in the range 100–1000 cm^{-1}, at room temperature. The recorded spectrum shows the presence of three scattering bands with peaks located at 272, 333, and 610 cm^{-1}. The peak centered at 272 cm^{-1} is assigned to the A_g mode, and the other two peaks at 333 and 610 cm^{-1} are assigned to the B_g modes. No active modes related to other phases or any impurities have been detected, which indicates the formation of monoclinic phase CuO.

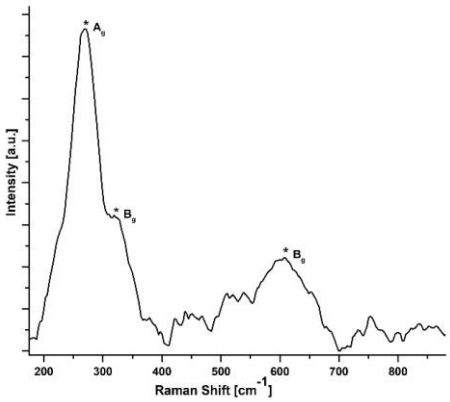

Figure 2. RAMAN spectrum of CuO NPs synthesized from Echinacea extract.

3.3. XRD Analysis

The XRD pattern of the CuO synthesized using the Echinacea extract is shown in Figure 3. The XRD pattern indicates the formation of a monoclinic crystalline structure by the appearance of well-defined diffraction peaks observed at the 2θ = 32.47°, 35.52° and 38.63°. These distinctive peaks can be assigned to (110), (002) and (111) crystallographic planes of the monoclinic structure of CuO with lattice parameters: a = 4.6936 Å, b = 3.42834 Å, c = 5.1377 Å, according to the International Center for Diffraction Data (ICDD), card no. 080-1916 and other data that exist in the literature [5,14].

No other peaks characteristic of secondary phases such as Cu_2O or other impurities were detected, which confirmed the high purity of the sample. The average crystallite size of CuO NPs was calculated using the Debye equation, D = kλ/βcosθ, where D is the

average crystalline size (Å), k is the shape factor: 0.93, λ is the wavelength of X-ray radiation, and β is the full width at half maximum (FWHM) of the peaks at the diffracting angle θ, and was found to be 15 nm.

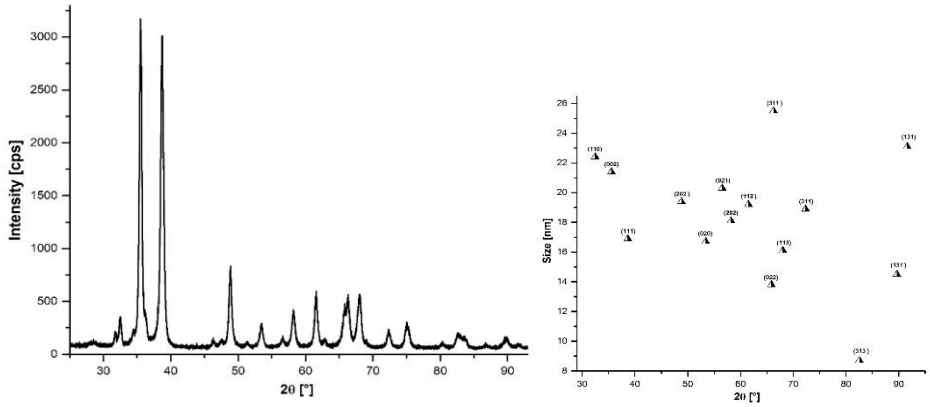

Figure 3. XRD diffraction patterns of CuO NPs synthesized from Echinacea extract.

3.4. SEM Analysis

Figure 4 shows the morphology of the CuO sample obtained by using Echinacea extract. From the examination of the SEM image, the formation of spherical particles was found, and most of them are found in the agglomerated form. The synthesized nanoparticles have sizes varying between 15–40 nm.

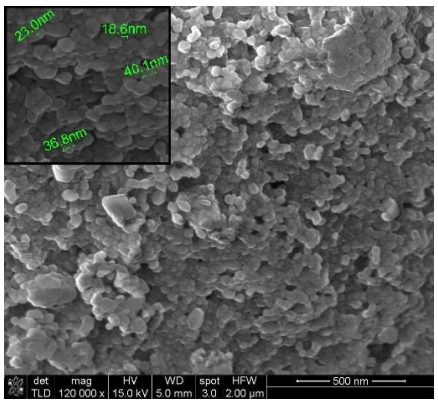

Figure 4. SEM images of CuO NPs synthesized from Echinacea extract**.**

3.5. EDX Analysis

The elemental composition of the CuO sample is presented in Figure 5. In the EDX spectrum of the analyzed sample, only the peaks attributed to Cu and O elements are presented, confirming the purity of the synthesized material. The weight and atomic compositions of the main elements (Cu and O) are presented in the inset table of the EDX spectrum.

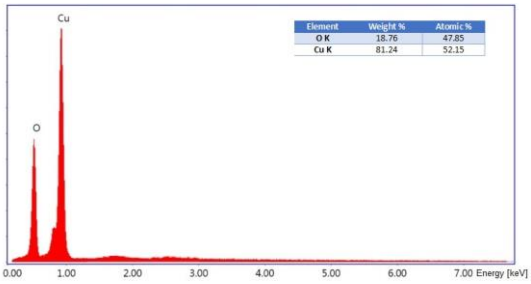

Figure 5. EDX spectrum of CuO NPs synthesized from Echinacea extract.

4. Conclusions

CuO nanoparticles were obtained, using a convenient and eco-friendly method, the aqueous extract of Echinacea as a biological reducing and stabilization agent. The FTIR and Raman spectra, for the oxide sample, confirm the existence of Cu-O bonds, but also the existence of molecules from the extract on the surface of the nanostructured oxide. The XRD patterns suggest the nature of crystallinity in the formation of the CuO NPs accompanied by the crystallite size in the range of 8–25 nm. A spherical morphology with the agglomerated form of nanoparticles, and with a size range from 15 to 45 nm, was observed from SEM analysis. The obtained results provide information about the performances of these nanoparticles, which allow the expansion of the field of applicability in the food industry, agriculture sector, and biomedical science.

Author Contributions: A.M. conceived, planned, carried out the experiments for the synthesis of CuO nanoparticles; FTIR characterization, V.T.; XRD characterization, C.R.; SEM and EDX characterization, G.C.; RAMAN characterization, C.P.; writing—original draft preparation, A.M.; writing—review and editing, A.M., V.T., C.R., G.C., C.P. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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