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SYNTHESIS OF COPPER DOPED ZINC OXIDE NANOPARTICLES AND INVESTIGATION OF THEIR ACTIVITY AS SKIN ANTICANCER AGENTS

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Abstract: Skin cancer is a common illness in many regions of the planet, and indeed a danger to human existence, so we tried to assess the effectiveness of the drug which is copper doped zinc oxide nanoparticles and which might be a beneficial future medication. Copper doped zinc oxide (Cu-ZnO) nanoparticles were prepared using a solvothermal method at 180°C for 12 hours to create a selective drug material for the treatment of skin cancer cells. The crystallite size is 38.081 nm (based on XRD measurements), and the three-dimensional images of FE-SEM revealed that the prepared nanostructures have high regularity, with an average length of 233.3 nm and a diameter of 68.14 nm. The presence of copper, zinc, and oxygen was detected using EDS, indicating that the prepared form (Cu-ZnO) is pure. The IC50 has been determined for skin cancer cells (A375) 520.33 $\mu g \cdot mL^{-1}$ and normal cells (HdFn) 539.3 $\mu g \cdot mL^{-1}$. Weak Cu-ZnO selectivity due to crystal size was expected, as the shape and crystal size, in addition to the percentage and type of doping, play an important role in increasing Cu-ZnO selectivity, so we recommend Cu-ZnO nanoparticles. Smaller sizes to increase

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surface area in scientific research to find an anti-skin cancer drug, although its toxicity is low on normal cells due to the weak response to this type of cell, while We believe that we can enhance of response and selectivity can be greatly via changing the geometry of the nanocrystals of the same material.

Keywords: Nano zinc oxide; copper-doped zinc oxide; nanostructures; Skin cancer; Nanopharmaceuticals and a human melanoma.

Introduction

Nanoscience has affected numerous disciplines of physics, chemistry, and engineering during the last three decades, resulting in many basic discoveries as well as applications and products.[1] As a result of the unique electro-optical and magnetic properties of materials on the Nanoscale, as well as very high efficiency due to the enormous surface area. Nanoscience is considered one of the sciences that has brought about a great scientific revolution in various fields, and this is why their investment in the medical fields has yielded great results at the level of nanoscience and nanotechnology.[2] There are many characteristics that distinguish a nanostructure from a bulk material like this improves the capacity to distribute poorly water-soluble medicines and to decrease drug build up in healthy tissue. This also aids in the retention of the medicine in the body for successful therapy.[3,4] While the prolongation of medication bioactivity by biological environmental protection drugs can now be transported past epithelial and endothelial barriers as well as incorporate therapeutic and diagnostic capabilities into a single agent.[5] Nanoparticles are becoming increasingly important in current cancer treatment due to their specific targeting capabilities and higher effectiveness, and they are beginning to obliterate traditional cancer therapies such as radiation, chemotherapy, and surgery.[6,7] ZnO NPs. may be a viable anticancer drug due to their unique qualities as in biocompatibility and superior selectivity, improved cytotoxicity, and ease of manufacturing. Zinc being a key component of the human body in addition a cofactor for greater than 300 mammalian enzymes, is essential for cellular activities such as DNA replication,

oxidative stress, cell cycle progression, DNA repair, and apoptosis.[8] Cancer therapy with ZnO nanoparticles has been demonstrated to be promising, including the destruction of cancer cells. Skin cancer is the most frequent kind of cancer among fair-skinned people all over the world. Skin cancer incidence, morbidity, and death rates are growing, posing a substantial public health problem. Ultraviolet radiation (UVR) is the most important etiologic agent in the expansion of skin malignancies.[9] UVR damages DNA with creates genetic mutations, which contribute to skin cancer. A better knowledge of Ultraviolet radiation is critical for skin cancer prevention. Ozone depletion, latitude, UV light elevation, meteorological and altitude conditions all have an impact on the quantity of UVR reaching the earth's surface.[10-13] The solvothermal technique, which is distinguished by convenience of use and the ability to regulate the dimensions of the nanoparticles of the created materials by manipulating pressure, temperature, and time, is one of the methods for creating zinc oxide nanoparticles.[14,15]

Results and Discussion

UV-vis Spectrum

Energy gap value is one of the most important characteristics that control the photoelectric distinctive of semiconductors, especially the ZnO nanostructures. The energy gap was calculated using Tauc plot methods based on absorption and wavelength $(\alpha hv)^n = K(hv-E_g)$, where K is constant, hv is photon energy, and Eg is band gap energy. The exponent (n) represents the nature of a transition (Direct Transition). The value of the energy gap (Eg = 3.28 eV) was less than the normal value for ZnO nanostructures, and this reduction is due to the presence of copper doping as in the figure 1.

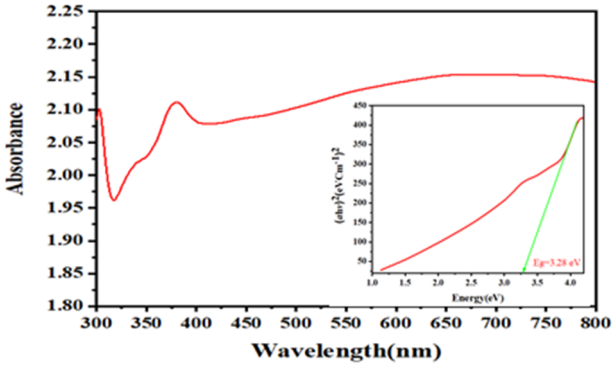


Figure 1. UV spectrum at the region of 300- 800 nm with absorption peak at λ max = 378 nm and Tauc plot (inset) for Cu-ZnO nanostructures at 180°C.

X-ray diffraction (XRD Crystallography)

XRD Crystallography revealed that the crystal form belongs to (hexagonal wurtzite) as shown in Figure 2, as well as match with zinc oxide (JCPDS 01-075-1526). Three new peaks in the Cu-ZnO diffraction pattern have been discovered at 43.46, 50.62 and 74.30° (marked with a black star) that match with copper (JCPDS 00-001-1241) as shown in Figure 3, although the measurement of the crystal size for Cu-ZnO is 38.081 nm (according to the Scherrer equation).

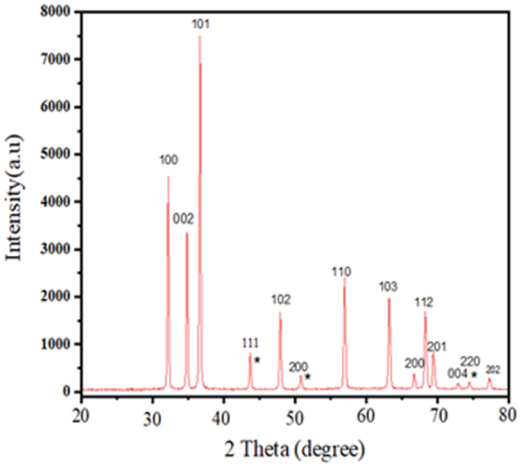


Figure 2. XRD crystallography for Cu-ZnO nanostructures at temperatures 180 °C.

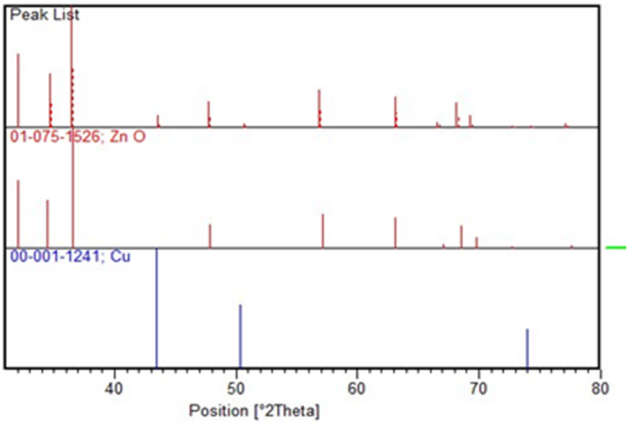


Figure 3. Comparison of XRD crystallography for Cu-ZnO nanostructures at temperatures 180 ℃ (top), ZnO (midst) and Copper (bottom).

Through comparison, as in the figure 3, it becomes clear to us that the locations of the peaks correspond to what is found in the source of Zinc oxide (JCPDS 01-075-1526) and Copper (JCPDS 00-001-1241), and this means that the crystalline faces of the manufactured sample match with these two materials. This confirms that Copper actually entered the crystalline structure of Zinc oxide and that the shape is hexagonal wurtzite.

Energy-dispersive X-ray spectroscopy (EDS)

The EDS analysis shows that the prepared sample is completely pure and there are no other materials other than Zinc, Oxygen, and Copper as in the figure 4, this explains the high orderliness in the crystal structure. The absence of any undesirable impurities enhances the chemical and physical properties of the crystal structure and makes Cu-ZnO nanoparticles possess more distinct and unique properties.

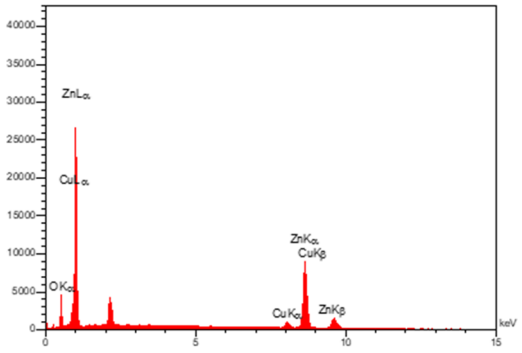


Figure 4. EDS spectra of Cu-ZnO nanoparticles synthesized via solvothermal technique at 180 °C with 0.07 wt% copper acetate Cu $(CH_3COO)_2.H_2O$ as precursor and incubation time of 12 hours.

Field Emission -Scanning Electron Microscopy (FE-SEM)

The scanning electron microscope gives us a three-dimensional image of the prepared nanoparticles through the scanning electron microscope images and the three shown images in figures (5,6,7). The hexagonal wurtzite shape of the nanoparticles has high crystal regularity, which is due to several main factors, including temperature, pressure, type of impurity, and time. There are other factors that affect the growth of nanostructures, including the percentage of doping as well as the type of solvent. The average length of the nanostructures at a temperature of 180 °C, after 12 hours of preparation, is 233.3 nm, and their average diameter is 68.14 nm. Zinc oxide can be invested in to improve the quality of some products or apply it to other products to make them more efficient, and this is due to the optical properties that it has acquired. The growth of nanostructures results from the alignment of nanocrystals according to Ostwald Ripening (Small crystals or colloidal solution molecules dissolve, re-deposit on bigger crystals or colloidal solution molecules, and Ostwald ripening is a phenomenon observed in solid solutions or liquids that depicts a change in a heterogeneous structure over time).[16,17]

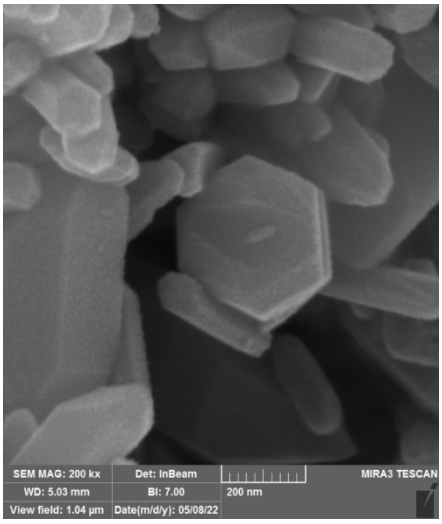


Figure 5. FE-SEM images for Cu-ZnO NPs. synthesized at 180 ℃ by a solvothermal method. Images are shown in 200nm scale for Cu-ZnO NPs.

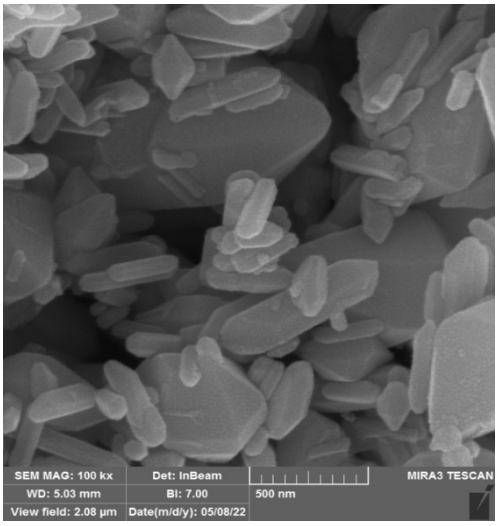


Figure 6. FE-SEM images for Cu-ZnO NPs. synthesized at 180 ºC via solvothermal method. Images are shown in 500nm scale for Cu-ZnO NPs.

MTT assay

MTT assay measures cell viability by converting MTT into purple formazan in living cells. It can also determine the IC50 (half-maximal inhibitory concentration), which indicates the drug dose required to inhibit 50% of cell growth. The results are quantified by measuring absorbance.

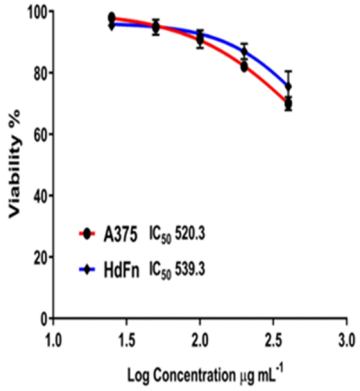


Figure 7. Results of MTT assay performed for 400, 200, 100, 50 and 25 µg·mL^{-1} concentrations of Cu-ZnO.

From the figure (7) that the IC50 of skin cancer cells is 520.3 μg, while for normal cells it is 539.3 μg according to the six concentrations used, 400, 200, 100, 50, and 25 $\mu g \cdot mL^{-1}$. This means that Cu-ZnO show Fairly good selectivity for skin cancer cells, and its performance can be improved by controlling the nanoscale dimensions of Cu-ZnO to increase the surface area and thus become more active, such as in the other types of cancer such as breast cancer (MCF7) and uterine cancer (HeLa)[18] as shown in the Table1.

Table 1. MTT cytotoxicity assay results (mean± sd) for A375 and HdFn cell samples.

NO.	Concentration	skin cancer cells (A375)	normal cells (HdFn)
		Mean ± Sd	Mean ± Sd
1.	400.00	69.98 ± 2.18	75.50 ± 4.98
2.	200.00	82.02 ± 0.64	86.96 ± 2.5
3.	100.00	90.97 ± 2.91	92.21 ± 0.47
4.	50.00	94.83 ± 2.43	95.72 ± 0.64
5.	25.00	97.96 ± 1.39	95.41 ± 1.14

Table 1 explain the impact of varying concentrations of copper doped zinc oxide nanoparticles (Cu-ZnO NPs) on skin cancer cells (A375) and normal skin cells (HdFn) was assessed using a viability assay (MTT assay) to calculate cell survival rates (%) with standard deviation (±SD).

Our investigation into the cytotoxic effects of Cu-ZnO nanoparticles (NPs) revealed a pronounced concentration-dependent relationship against A375 melanoma cells. Specifically, cell viability remained high (~98 %) at a lower concentration of 25 μg/mL, indicating minimal toxicity. However, as the concentration escalated to 400 μg/mL, a dramatic reduction in cancer cell survival to approximately 70 % was observed, demonstrating significant cytotoxic efficacy.

Furthermore, a critical finding of this study is the evident selective toxicity of Cu-ZnO NPs towards cancer cells compared to normal human dermal fibroblasts (HdFn). Although both cell lines experienced a decline in viability at higher concentrations, normal cells exhibited notably greater resilience, maintaining a survival rate above 75 % even at the maximum tested concentration of 400 μg/mL. This compelling disparity in cytotoxicity is underscored by the widening gap in survival rates between the two cell lines with increasing concentration; the difference grew from approximately 4.94 % at 200 μg/mL to 5.5 % at 400 μg/mL. This trend suggests that while absolute toxicity increases for all cells at high doses, the therapeutic window for selectively targeting malignant cells is indeed present.

This selective toxicity can be attributed to the fundamental mechanism of action of metal oxide nanoparticles. Cu-ZnO NPs are believed to induce cell death primarily through the generation of reactive oxygen species (ROS), which causes oxidative stress. It is well-established that cancer cells, due to their altered metabolic state, often operate under higher basal levels of oxidative stress and possess less efficient antioxidant defenses compared to their normal counterparts. Consequently, they are inherently more vulnerable to further ROS-induced damage, which triggers pathways leading to apoptosis and inhibited cell division. The modest yet consistent differential toxicity observed here provides a foundational rationale for further optimization of these nanoparticles – for instance, through surface functionalization – to enhance their specificity and therapeutic index.

Finally, these findings are in strong agreement with the existing body of literature, which confirms that the cytotoxic activity of Cu-ZnO

NPs is not only selective but also critically dependent on their physicochemical properties, such as size, crystalline structure, and synthesis methodology. Therefore, this study corroborates the potential of Cu-ZnO NPs as a promising agent in cancer therapeutics while highlighting the necessity for precise nano-engineering to fully exploit their selective anti-cancer properties.[18,19]

Table 2. Comparative cytotoxicity of Cu-ZnO and ZnO NPs across cancer cell lines: IC_{50} profiling.

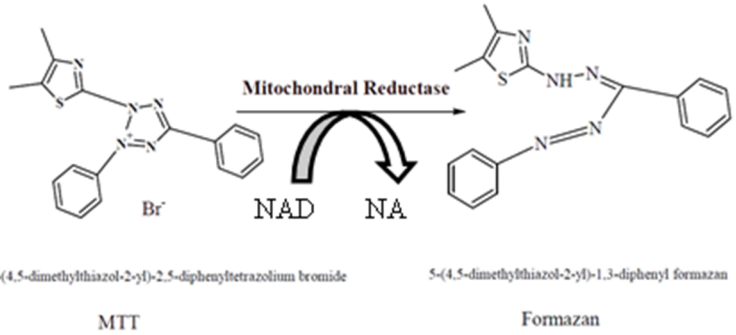
Nanomaterial	Cell line	IC_{50} µg/mL	IC_{50} (µg/mL) Normal cell	Reference
Cu-ZnO NPs	A375	520.3	539.3	Our current research
Cu-ZnO NPs	MCF7	219.56	> 300	Rishikesan et al. (2020)[18].
	HeLa	137.27		
ZnO/Cu NPs	G-292	77.7	54.5	Ghaznavi et al (2025)[19].
ZnO NPs	G-292	147.96	129.71	

Table 2 provides significant data on the cytotoxic properties of Cu-ZnO NPs in various cell lines while stressing perception of their selective cytotoxicity, the most meaningful and important enhancement over previous research, which primarily provides data on efficacy without adequate consideration of safety. The findings demonstrate that these Cu-ZnO NPs exhibit selectivity against breast cancer cells (MCF7) while studies on Cu-ZnO NPs have shown unwanted toxicity to normal cell lines. To note, the efficacy of copper-doped zinc oxide nanoparticles (Cu-ZnO NPs) can be improved through crystal engineering methods by controlling crystal dimension and crystal morphology. This has the potential to successfully improve their anticancer properties and maintain selectivity. This research is the first to assess these materials for cytotoxicity against melanoma cells

(A375), filling an important gap in the literature. Our findings demonstrate an opportunity to further the design of safer and effective nano-materials for targeted cancer therapy. Our comparison further highlights the innovative contribution of this work in balancing the potential for therapeutic benefit and biosafety recommendations, while crystal structure manipulation could emerge as a viable option for improved performance.

Experimental

The zinc oxide nanoparticles impregnated with copper were prepared by the solvothermal method by: 10 mL solution 1M of zinc acetate ($(CH_3COO)_2Zn{\cdot}2H_2O$ 98%, MW=219.49 g/mol, THOMAS BAKER), 0.07 % (weight percent), copper acetate ($(CH_3COO)_2Cu{\cdot}H_2O$ 99 %, MW=199.65 g/mol, THOMAS BAKER), 40 mL of 5M sodium hydroxide (NaOH 98 %, MW=40 g/mol, THOMAS BAKER) and MeOH as solvent, at 180 °C for 12 hours. The obtained nanoparticles were characterized using (UV-vis, EDS, FE-SEM, XRD) techniques. Then the sample was examined by the MTT technique, against HdFn normal skin cells and skin cancer cells (A375) at the concentration of Cu-ZnO of 400, 200, 100, 50, and 25 $\mu g{\cdot}mL^{-1}$. The color of active cells changed from yellow tetrazolium salt (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals as in Scheme 1.



Scheme 1. MTT is metabolized to formazan salt via viability cells.

Conclusions

The solvothermal approach is a simple and promising way of controlling the form and size of nanomaterials. Temperature, pressure, solvent type, and time are all important elements in influencing the size, physical and chemical characteristics of ZnO NPs. The kind and degree of doping have a significant impact on the photoelectric characteristics of ZnO NPs. Copper reduces the ZnO energy gap value (from 3.37 to 3.28 eV). A decrease in crystal size and an increase in surface area, in addition to the percentage and type of doping, are critical factors in determining the selectivity of Cu-ZnO against cancer cells. Cu-ZnO can be an effective treatment for many forms of cancer such as skin cancer cells due to its high selectivity and cancer cell permeability; however, selectivity and mechanism of action should be further enhanced. Potential exists as an adjunct to cancer therapy.

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