Pluronic F-127 modified ZnO nanoparticles: Green synthesis, surface modification, characterization and anticancer properties

Nguyen Ngoc Son, Vu Minh Thanh, Nguyen Thi Huong*

Institute Chemistry and Materials, Academy of Military Science and Technology, Cau Giay, Hanoi, Vietnam.

*Email: nguyenhuong0916@gmail.com

Received 15 Oct. 2023; Revised 10 Dec. 2023; Accepted 10 Feb. 2024; Published 25 Feb. 2024.

DOI: https://doi.org/10.54939/1859-1043.j.mst.93.2024.91-98

ABSTRACT

In this study, we report a green synthesis method for ZnO NPs using guava leaf extract and surface modification of these nanoparticles with Pluronic. The structural, chemical characteristics, and morphological features of the synthesized ZnO NPs and their surface-modified counterparts were thoroughly described using appropriate techniques such as X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), energy-dispersive X-ray spectroscopy (EDX), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The anticancer activity of the Pluronic-coated ZnO NPs was also evaluated on cervical cancer cells (Hela). The results revealed that the synthesized nanomaterial exhibited excellent effectiveness in eradicating Hela cancer cells. The cell viability of Hela cells decreased significantly to 8.85% when the nanoparticle concentration used was 200 µg/mL. The half-maximal inhibitory concentration (IC50) was relatively low, around 28.07 µg/mL. These findings demonstrate the significant potential of using Pluronic F-127 surface-modified ZnO NPs in cancer treatment.

Keywords*:* Zinc oxide nanoparticles; Pluronic F127; Anticancer.

1. INTRODUCTION

Zinc oxide nanoparticles (ZnO NPs) have emerged as a promising and versatile nanomaterial for a wide range of biomedical applications, particularly in the fields of cancer therapeutics, diagnosis, and drug delivery. The synthesis of ZnO NPs can be achieved through different routes, including chemical, physical, and biological methods [1, 2]. While chemical methods, such as precipitation, sol-gel, and hydrothermal processes, have been commonly employed, greener alternatives, such as biological methods, have also been explored for eco-friendly synthesis [3, 4]. The unique properties of ZnO NPs position them as a promising candidate in the realm of cancer therapeutics and beyond, presenting potential breakthroughs in modern medicine and industry. Recently, ZnO NPs have found applications in pH-responsive drug delivery systems, specifically targeting tumor masses, and enabling drug release within cells due to their pH-sensitive solubility [5]. Several studies have demonstrated the ability of ZnO NPs to induce cancer cell apoptosis through the generation of reactive oxygen species (ROS), further affirming their potential as promising anticancer agents [3, 6].

Pluronic F-127 is a widely used triblock copolymer in the fields of medicine and pharmaceuticals. It consists of three different polymer segments: polyethylene oxide (PEO) at both ends and polypropylene oxide (PPO) in the middle [7]. This unique triblock structure allows Pluronic F-127 to be used for surface modification of nanoparticles [8, 9] and applied in drug delivery systems [10, 11] and various medical applications [12, 13]. Pluronic F-127 can self-assemble into stable micelles when present in an aqueous solution, making it suitable for transferring and delivering active drugs to the body. Its stability and cell-friendly properties improve drug solubility and enhance interactions with cells, thereby increasing treatment efficacy. Pluronic F-127, in particular, is commonly used to modify the surface of nanoparticles to enhance water solubility and stability. In scientific research, the combination of Pluronic F-127 with ZnO NPs has been reported to create effective drug delivery systems for cancer therapy and improve

treatment outcomes. A. Abuelsamen *et al*. [14] successfully synthesized F-127-coated ZnO nanoparticles to improve their stability and prevent nanoparticle agglomeration. The cytotoxicity of these nanoparticles was assessed using the MTT assay, revealing that the F-127-coated ZnO nanoparticles exhibited enhanced stability and maintained their crystalline structure. Furthermore, the cytotoxicity towards the healthy AE.hy296 cells was significantly reduced compared to uncoated ZnO nanoparticles. These results demonstrate the promising potential of F-127-coated ZnO nanoparticles for various biomedical applications.

In this study, green-synthesized zinc oxide nanoparticles (ZnO NPs) were surface-modified with pluronic F-127 after being synthesized using the leaf extract of guava (Psidium guajava). The resulting material, ZnO@F-127, was then characterized, and its anticancer activity was evaluated on several cell lines.

2. MATERIALS AND METHODS

2.1. Materials

Guava leaves Guava leaves are harvested at the farm (geographic coordinates: 21.03746, 105.71918) in the summer, zinc acetate dihydrate ($Zn(CH_3COO)_2.2H_2O$, Merk), Pluronic F127 (12400 Da, Sigma-Aldrich). Ethanol absolute (EtOH, Emsure), Deionized water (DI, TDS $\leq$ 5). Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12), along with penicillin, streptomycin, and trypsin/EDTA, were procured from Thermo Fisher Scientific, USA. Fetal bovine serum (FBS) was acquired from Wisent, Saint-Bruno, Canada. The human breast cancer cell lines HeLa were sourced from the American Type Culture Collection (ATCC), based in Manassas, Virginia, USA. These cell lines were nurtured in DMEM/F12 medium supplemented with 10% FBS and 2% penicillin/streptomycin solution (10,000 U/mL penicillin and 10 mg/mL streptomycin) at 37 °C within a humidified incubator enriched with 5% CO_2.

2.2. Methods

2.2.1. Preparation and characterization of ZnO NPs using guava leaf extract

Guava leaves were collected, dried, and then finely ground. 100 grams of the ground Guava leaves were then boiled with a 50:50 mixture of ethanol and deionized water (v/v) for 2 hours. The resulting extract (GL-ext) was recovered after filtration and centrifugation to remove any solid residues. Meanwhile, 10 grams of zinc acetate dihydrate were completely dissolved in 200 mL of deionized water. This solution was then added dropwise (at a rate of 1.0 - 1.5 mL per minute) into a reaction mixture consisting of 200 mL of GL-ext and 200 mL of ethanol (EtOH) under mechanical stirring at 380 rpm. The reaction was conducted at 60 °C for 3.5 hours.

After the reaction, the reaction mixture was centrifuged, and the solid residue obtained was dried at 50 °C for 8 hours. Subsequently, the dried product was calcined at 600 °C for 3 hours. The resulting product was a white powder of ZnO nanoparticles (ZnO NPs).

2.2.2. Preparation of Pluronic F127-coated ZnO NPs

In a 250 mL Erlenmeyer flask, 0.5 grams of ZnO nanoparticles (synthesized as described in section 2.2.1) were completely dispersed in a mixture of 25 mL of deionized water (DI) and 25 mL of ethanol (EtOH). This flask was then placed inside an ultrasonic bath and subjected to ultrasonication for 30 minutes. Subsequently, 20 mL of a 4 mM Pluronic F-127 solution was added to the reaction flask. Ultrasonication was continued at various time intervals from 10 to 30 minutes.

After the reaction, the resulting product was subjected to centrifugation at 10,000 rpm for 5 minutes to obtain a solid material with a pale ivory to light yellow color. The solid material was transferred onto a Petri dish and dried at 60 °C for 24 hours to obtain Pluronic F-127-coated ZnO.

2.2.3. Characterization

The as-prepared nanoparticles were evaluated using various analytical techniques, including

Fourier-transform infrared spectroscopy (FT-IR, Bruker), X-ray diffraction (XRD, PANalytical), scanning electron microscopy (SEM) and field emission scanning electron microscopy (FESEM, Jeol-JMS 6490), and dynamic light scattering (DLS) for hydrodynamic size analysis.

2.2.4. MTT assay for evaluating the anticancer activity of Pluronic F-127 coated ZnO.

The anticancer activity of Pluronic F-127 coated ZnO was tested on several cancer cell lines using the MTT assay. The cell viability can be determined using the following formula:

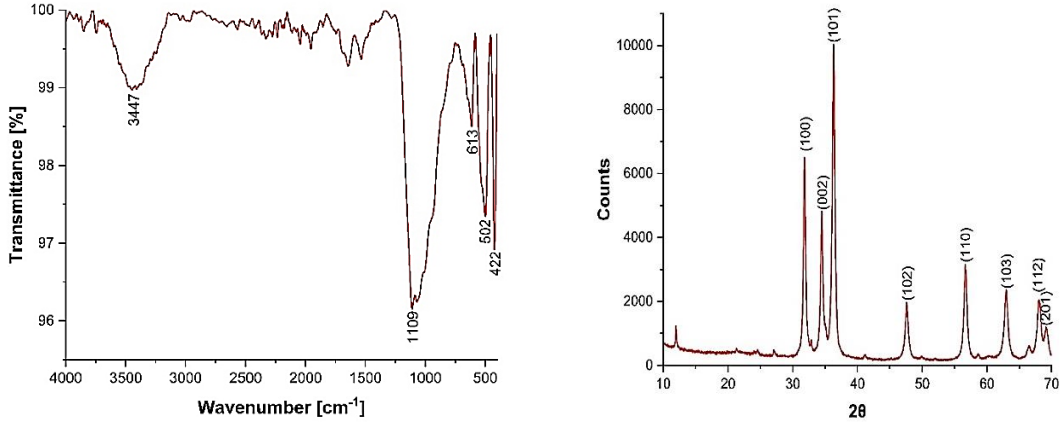
$$\text{Cell viability (\%)} = \frac{\text{OD}_{570}(\text{sample})}{\text{OD}_{570}(control)} \times 100\%$$

where OD_{570}(sample) is the optical density of cells treated with various concentrations of ZnO NPs and OD_{570}(control) is the optical density of cells incubated with medium only.

3. RESULTS AND DISCUSSION

3.1. Synthesis and characterization of ZnO NPs

Zinc oxide (ZnO) was synthesized using guava leaf extract and its characteristics were analyzed using Fourier-transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD), as shown in figure 1. The FTIR spectrum (figure 1*a*) exhibits absorption peaks at 3447 cm^{-1}, which are characteristic of hydroxyl groups, and a peak at 1109 cm^{-1}, which corresponds to C-O stretching on polysaccharide skeleton [15]. Its presence can be attributed to the source originating from the guava leaf extract. Polysaccharides are a class of carbohydrates composed of long chains of sugar molecules, and they are commonly found in plants, including guava leaves. When the guava leaf extract is used in the synthesis process, these polysaccharides from the plant source become part of the resulting zinc oxide nanoparticles. This observation further supports the eco-friendly and bio-inspired synthesis method using the guava leaf extract as a green and sustainable source of stabilizing and functionalizing agents in the preparation of zinc oxide nanoparticles. The characteristic absorption peaks of zinc oxide appear in the range of 400-615 cm^{-1} [16, 17], with three sharp and intense peaks at 422 cm^{-1}, 502 cm^{-1} and 613 cm^{-1} representing the Zn-O bonding in the zinc oxide compound. The XRD pattern of the synthesized ZnO nanoparticles (ZnO NPs) is presented in figure 1b. The main X-ray diffraction peaks appear in the range of 20° to 70° of the 2-theta angles. The major diffraction peaks (2θ) are observed at 31.82°, 34.50°, 35.00°, 36.30°, 47.59°, 56.65°, 62.97°, 68.02°, and 69.09°, corresponding to the crystallographic planes (100), (002), (101), (102), (110), (103), (112), and (201) of a hexagonal crystal structure of ZnO (Library patterns of hexagonal zincite ZnO (PDF 01-071-6424)). The FTIR and XRD analyses confirm the successful synthesis of zinc oxide using the guava leaf extract.



(a) *(b)*

Figure 1. *FTIR spectra (a) and XRD pattern (b) of as-prepared ZnO NPs.*

The morphological characteristics of the synthesized nano-sized particles were assessed using SEM and TEM methods, as illustrated in figure 2. These observations reveal that the as-prepared ZnO NPs exhibit uniformity in both size and shape. The particles have a spherical shape, with a consistent size ranging from approximately 20 to 25 nanometers. The SEM images clearly show well-defined boundaries between individual particles, indicating minimal agglomeration or clustering of the nanoparticles. This observation demonstrates the excellent efficiency of the guava leaf extract as a stabilizing agent, preventing the aggregation of nanoparticles during the formation process. The uniform and well-separated spherical nanoparticles obtained through this synthesis method highlight the effectiveness of the guava leaf extract as a capping and stabilizing agent. Successful control over the size and morphology of the ZnO nanoparticles is vital for tailoring their properties and enhancing their performance in biomedicine applications.

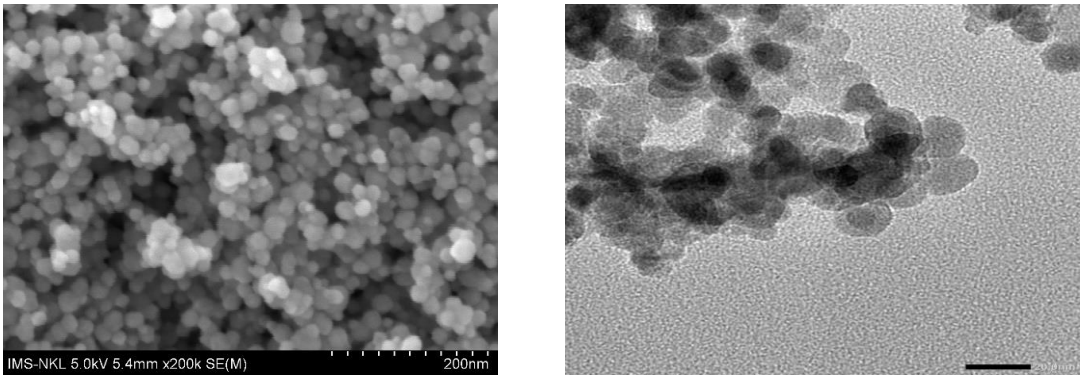


Figure 2. *Image of SEM (a) and TEM (b) of the synthesized ZnO NPs using guava leaf extract.*

The EDX spectrum of ZnO nanoparticles (ZnO NPs) is presented in figure 3. In the EDX spectrum, characteristic peaks corresponding to the elements present in the ZnO NPs will be observed. The prominent peaks are expected to represent zinc (Zn) and oxygen (O) elements, as these are the constituents of ZnO. The EDX analysis provides valuable confirmation of the elemental composition of the synthesized ZnO nanoparticles, further supporting the successful synthesis process. The presence of distinct peaks for zinc and oxygen in the spectrum validates the formation of pure ZnO nanoparticles.

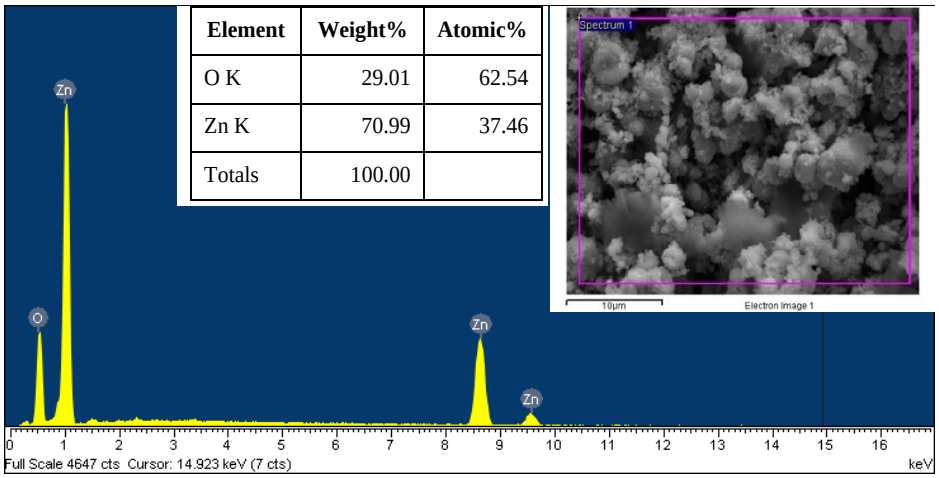
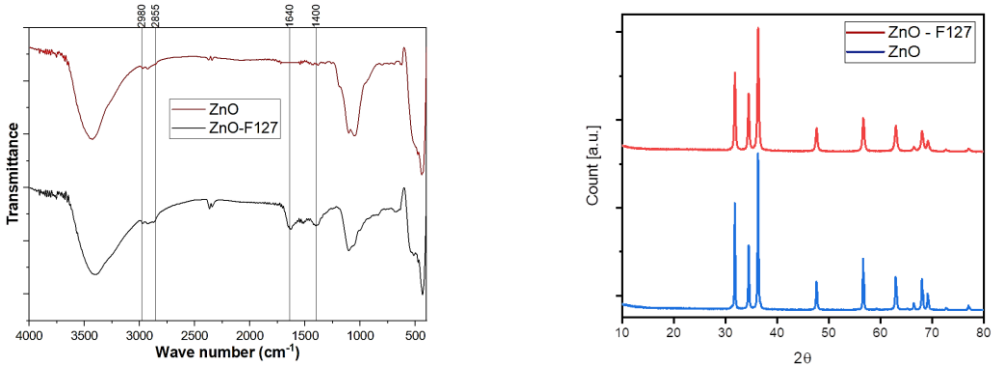


Figure 3. *EDX spectrum of as-prepared ZnO NPs.*

3.2. Surface modification and characterization of ZnO@F-127

The FTIR spectrum of Pluronic F-127 modified ZnO is presented in figure 4*a*. For both coated and bare ZnO, characteristic absorption peaks of ZnO appear in the regions of 400 - 500 cm^{-1}, with a peak

at 3400 cm^{-1} representing the hydroxyl group. Additionally, for the coated ZnO, there are additional peaks observed in the regions of 2855-2980 cm^{-1} and 1400 and 1640 cm^{-1}, which are characteristic of the CH_2 and CH_3 group vibrations. This indicates the presence of F-127 in the ZnO NPs.



(a) *(b)*

Figure 4. *Infrared spectrum (a) and X-ray diffraction (b) of zinc surface modified with Pluronic F-127.*

The XRD results in figure 4*b*, after the modification of nano ZnO with Pluronic F127, show that the characteristic structural features of the material remain relatively unchanged. The positions of the characteristic peak locations are preserved, with only slight shifts observed. At the same time, the intensity of the characteristic peaks decreases to some extent, which may be attributed to the effect of the F127 modification. This observation is consistent with the findings reported by Smilja M. Marković and colleagues [18], suggesting that the introduction of Pluronic F127 as a modifying agent leads to minor alterations in the XRD pattern of the ZnO nanoparticles. The overall crystal structure of the material remains intact, but the presence of F127 could cause some subtle changes in the XRD peaks. The XRD data confirms the successful modification of nano ZnO with Pluronic F127 and provides important insights into the structural properties of the resulting material.

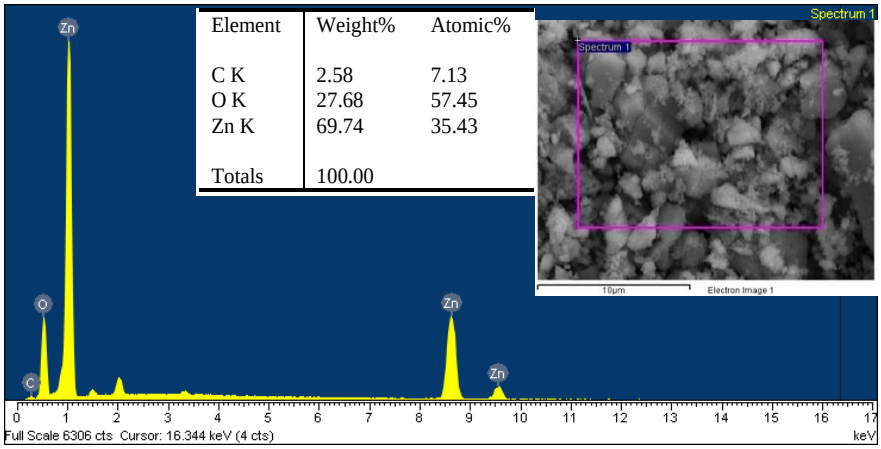
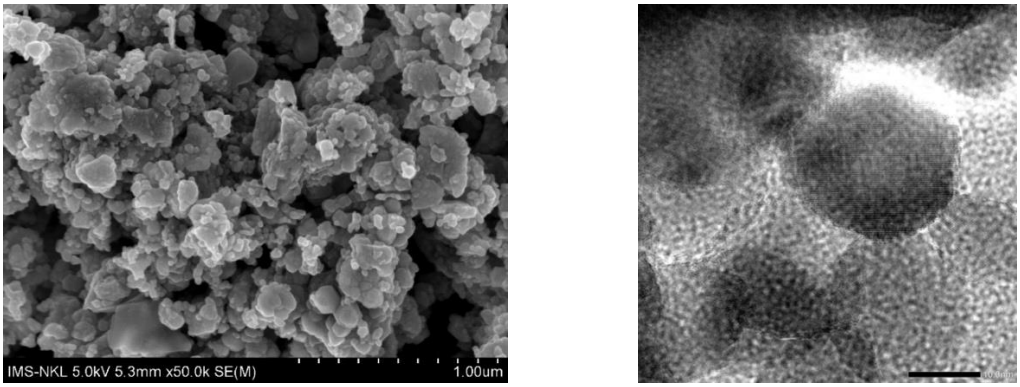


Figure 5. *EDX analysis of Pluronic F-127 modified ZnO.*

The results of elemental composition analysis using EDX technique are presented in figure 5. As expected, only characteristic peaks of Zn, O, and C elements are observed, with a carbon content of up to 7.13% atomic fraction (representing the Pluronic component). This once again confirms the successful surface modification of zinc oxide nanoparticles with Pluronic F-127.

The morphological characteristics of Pluronic F-127 modified ZnO NPs are illustrated in figure 6. The SEM image (figure 6*a*) reveals that the nano-sized particles no longer exhibit a uniform

size distribution as observed for the original ZnO NPs (figure 2). Instead, the nano-sized particles start to agglomerate, forming clusters. However, this might be merely a loose physical agglomeration, and under suitable conditions, they still retain their very small nano size. This is demonstrated in the TEM image (figure 6*b*), where the nano-sized particles maintain a size of around 20 nm. Additionally, the surface of the nano-sized particles (the boundaries between particles) shows some irregular layers, with a different contrast from the nanoparticles. This could be attributed to the formation of an organic Pluronic layer on the surface of ZnO NPs.



(a) *(b)*

Figure 6. *SEM and TEM Images of Pluronic F-127 Modified ZnO NPs.*

3.3. Anticancer activity of Pluronic F-127 modified Zinc Oxide

The anticancer activity of Pluronic F-127 modified ZnO NPs has been tested on cervical cancer cells (Hela). The results demonstrate a strong dependence of the cancer cell-killing efficacy of these nano-sized particles on their concentration, following an exponential function. With test concentrations of nano-sized particles ranging from 10 to 200 µg/mL, the cell survival rate decreased from 69.96% to 8.85%. Therefore, with a sufficiently high concentration of nano-sized particles, almost all cancer cells were effectively eliminated. These promising findings suggest that Pluronic F-127 modified ZnO nanoparticles hold significant potential as a potent anticancer agent. The observed concentration-dependent cell-killing effect further supports the idea that these nanoparticles could serve as a targeted and efficient therapy for cervical cancer.

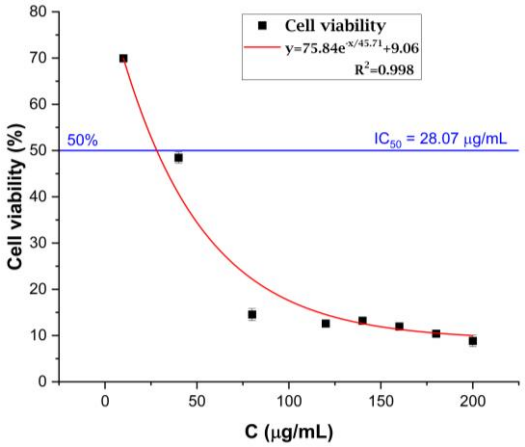


Figure 7. *Effect of Pluronic F-127 coated ZnO NPs concentration on the cytotoxicity against Hela cells.*

Moreover, through graph interpolation, the half-maximal inhibitory concentration (IC50) value was estimated to be around 28.07 µg/mL. This value provides a crucial insight into the effective

concentration required to achieve a 50% reduction in cancer cell viability, making it an essential parameter for potential future therapeutic applications. The enhanced cancer cell-killing efficacy of ZnO nanoparticles after being coated with Pluronic F-127 can be attributed to the unique properties of this agent. Pluronic F-127 acts as a stabilizing and targeting agent, enabling the nanoparticles to selectively interact with cancer cells while sparing healthy cells. This selective targeting minimizes the potential side effects and cytotoxicity on normal cells, making the treatment more promising for cancer therapy.

Overall, these results demonstrate the high potential of Pluronic F-127 modified ZnO nanoparticles as a powerful anticancer agent, opening new avenues for further research and development in the field of nanomedicine and targeted cancer therapies.

4. CONCLUSIONS

Zinc oxide nanoparticles with 20 - 25 nm in size were successfully synthesized using a green, simple, and environmentally friendly method. A straightforward and eco-friendly process was employed to surface modify the zinc oxide nanoparticles with Pluronic F-127. The crystalline structure of ZnO remained unchanged after surface modification. The results of the anticancer cell tests for the Pluronic F-127 coated ZnO NPs were highly intriguing. The effectiveness of the Hela cell-killing exhibited an exponential dependence on the nanoparticle concentration, following an exponential decay pattern. The half-maximal inhibitory concentration (IC_{50}) for the Pluronic F-127 coated ZnO NPs was determined to be 28.07 µg/mL. These findings demonstrate the significant potential of surface-modified ZnO NPs with Pluronic F-127 as a promising candidate for cervical cancer treatment. The results highlight the effectiveness of this modified nanomaterial in targeting and eliminating Hela cancer cells, indicating its potential for further development as a targeted therapeutic agent for cervical cancer.

Acknowledgments: *This study was funded by the 562 Program (Basic Science Development in the fields of life, earth, and marine science in the period of 2017 - 2025, orienting to 2030), under grant number ĐTĐL.CN-53/21.*

REFERENCES

[1]. Bandeira, M., et al., "*Green synthesis of zinc oxide nanoparticles: A review of the synthesis methodology and mechanism of formation. Sustainable Chemistry and Pharmacy*". **Vol**. **15**, No. (2020), pp. 100223.

[2]. Chung, I.-M., et al., "*An Investigation of the Cytotoxicity and Caspase-Mediated Apoptotic Effect of Green Synthesized Zinc Oxide Nanoparticles Using Eclipta prostrata on Human Liver Carcinoma Cells. Nanomaterials*". **Vol**. **5**, No. 3, pp. 1317-1330, (2015).

[3]. Abbasi, B.A., et al., "*Bioactivities of Geranium wallichianum Leaf Extracts Conjugated with Zinc Oxide Nanoparticles. Biomolecules*". **Vol**. **10**, No. 1 (2019), pp. 38.

[4]. Agarwal, H., S. Venkat Kumar, and S. Rajeshkumar, "*A review on green synthesis of zinc oxide nanoparticles – An eco-friendly approach. Resource-Efficient Technologies*". **Vol**. **3**, No. 4, pp. 406-413, (2017).

[5]. Muhammad, F., et al., "*pH-Triggered Controlled Drug Release from Mesoporous Silica Nanoparticles via Intracelluar Dissolution of ZnO Nanolids. Journal of the American Chemical Society*". **Vol**. **133**, No. 23, pp. 8778-8781, (2011).

[6]. Xia, T., et al., "*Comparison of the Mechanism of Toxicity of Zinc Oxide and Cerium Oxide Nanoparticles Based on Dissolution and Oxidative Stress Properties. ACS Nano*". **Vol**. **2**, No. 10, pp. 2121-2134, (2008).

[7]. Yu, J., et al., "*Polymeric Drug Delivery System Based on Pluronics for Cancer Treatment. Molecules*". **Vol**. **26**, No. 12, (2021).

[8]. Vu-Quang, H., et al., "*Pluronic F127-Folate Coated Super Paramagenic Iron Oxide Nanoparticles as Contrast Agent for Cancer Diagnosis in Magnetic Resonance Imaging. Polymers*". **Vol**. **11**, No. 4 (2019).

[9]. Elamin, N.Y., T. Indumathi, and E. Ranjith Kumar, "*Pluronic f127 encapsulated titanium dioxide*

nanoparticles: Evaluation of physiochemical properties for biological applications. Journal of Molecular Liquids". **Vol**. **379**, pp. 121655, (2023).
[10]. Shriky, B., et al., "*Pluronic F127 thermosensitive injectable smart hydrogels for controlled drug delivery system development. Journal of Colloid and Interface Science"*. **Vol**. **565**, pp. 119-130, (2020).
[11]. Ma, W.-D., et al., "*Pluronic F127-g-poly(acrylic acid) copolymers as in situ gelling vehicle for ophthalmic drug delivery system. International Journal of Pharmaceutics"*. **Vol**. **350**, No. 1, pp. 247-256, (2008).
[12]. Melbiah, J.S.B., D. Nithya, and D. Mohan, "*Surface modification of polyacrylonitrile ultrafiltration membranes using amphiphilic Pluronic F127/CaCO3 nanoparticles for oil/water emulsion separation. Colloids and Surfaces A: Physicochemical and Engineering Aspects"*. **Vol**. **516**, pp. 147-160, (2017).
[13]. Lu, H., et al., "*Pluronic F127–folic acid encapsulated nanoparticles with aggregation-induced emission characteristics for targeted cellular imaging. RSC Advances"*. **Vol**. **4**, No. 35, pp. 18460-18466, (2014).
[14]. Abuelsamen, A., et al., "*Novel Pluronic F-127-coated ZnO nanoparticles: Synthesis, characterization, and their in-vitro cytotoxicity evaluation. Polymers Advanced Technologies"*. **Vol**. **32**, No. 6, pp. 2541-2551, (2021).
[15]. Taleb, M.F.A., H.L.A. El-Mohdy, and H.A.A. El-Rehim, "*Radiation preparation of PVA/CMC copolymers and their application in removal of dyes. Journal of Hazardous Materials"*. **Vol**. **168**, No. 1, pp. 68-75, (2009).
[16]. Handore, K., et al., "*Novel Green Route of Synthesis of ZnO Nanoparticles by Using Natural Biodegradable Polymer and Its Application as a Catalyst for Oxidation of Aldehydes. Journal of Macromolecular Science, Part A"*. **Vol**. **51**, No. 12, pp. 941-947, (2014).
[17]. Teixeira, L.T., et al., "*Sustainable Cellulose Nanofibers-Mediated Synthesis of Uniform Spinel Zn-Ferrites Nanocorals for High Performances in Supercapacitors."* **Vol**. **24**, No. 11, pp. 9169, (2023).
[18]. Rajić, V.B., et al., "*Synthesis and optical properties of ZnO and ZnO/PEO nanonsructured powders. Tehnika"*. **Vol**. **71**, No. 1, pp. 9-15, (2016).

TÓM TẮT

Tổng hợp xanh và hoạt tính chống ung thư của hạt nano kẽm oxit biến tính bề mặt với Pluronic F-127

Trong nghiên cứu này, nano ZnO được tổng hợp bằng phương pháp xanh sử dụng dịch chiết lá ổi và biến tính bề mặt hạt nano này bằng tác nhân Pluronic. Cấu trúc, đặc trưng hoá học, hình thái học của nano ZnO và nano ZnO sau biến tính được đánh giá bằng các phương pháp phân tích công cụ hiện đại: XRD, FTIR, EDX, SEM, TEM. Hoạt tính chống ung thư của ZnO NPs sau khi biến tính bề mặt bằng Pluronic cũng đã được thử nghiệm đối với tế bào ung thư cổ tử cung (Hela). Kết quả cho thấy loại vật liệu nano được tổng hợp thể hiện hiệu quả trong tiêu diệt tế bào ung thư Hela. Khả năng sống của tế bào Hela giảm xuống còn 8.85% khi hàm lượng hạt nano sử dụng là 200 µg/mL. Sự phụ thuộc của tỷ lệ này vào lượng hạt nano sử dụng tuân theo quy luật của hàm số mũ. Giá trị ức chế một nửa (IC_{50}) đạt khá thấp, khoảng 28.07 µg/mL. Các kết quả này cho thấy tiềm năng lớn sử dụng ZnO NPs biến tính Pluronic F-127 trong điều trị ung thư.

Từ khóa: Hạt nano kẽm oxit; Pluronic F-127; Chống ung thư.