ZnCO3 nanoparticles synthesis, characterization, and cytotoxicity on the SK-OV-3 human ovarian cancer cells

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Abstract

Nanoparticles are effectively replacing anticancer medications in medicine (NPs). The co-precipitation approach (A) was used in this investigation to create ZnCO3-NPs, while green chemistry and turmeric extract were used in the latter (B). they were characterized by several techniques, such as FTIR, XRD, EDX, and SEM. ZnCO3-NPs (A) particles in the XRD had an average size of 21.10 nm, while ZnCO3-NPs (B) particles had an average size of 15.02 nm. The synthetic NPs have a high level of purity as determined by EDX. SK-OV-3 cells were tested against ZnCO3-NPs (A) at varying doses (25, 50, 100, 200, and 400 µg/ml). After 24 hours, the killing rate was, in descending order, 6.1%, 19.1%, 41.5%, 51.4%, and 66.34%. And the death rate of ZnCO3-NPs (B) was (4.02 percent, 15.8 %, 27.5 %, 45.13 %, and 65.59 percent) in that order. The approach of chemical precipitation is more effective at killing or inhibiting. ZnCO3-NPs have half-maximal inhibitory concentrations (IC50) of 183.8 and 128.5 µg/ml for A and B, respectively. As anticancer medications, ZnCO3-NPs have potential therapeutic benefits. At all concentrations, the substance was secure (not harmful).

Introduction

The "nano-world" proposed concepts have lately disclosed new scientific research areas. Over the past ten years, the number of articles, patents, initiatives, applications, and businesses devoted to nanotechnology has grown tremendously. Nanotechnology is a field that bridges the ideas of classical quantum physics and chemistry [1]. Compared to standard materials, studies made from nanoscale particles revealed unknown features or improved attributes. Alkaline carbonates have been consumed in large amounts, primarily for practical purposes. Metal carbonate is frequently utilized to make toothpaste, binding agents, and other products [2]. Metal carbonate is primarily utilized in the glass industry and as a water softening agent. Both compounds are practically insoluble in water, have a rhombic structure, and are crystalline. Various techniques have been used for nanoparticle production, including vapor transfer [3], homogeneous solution precipitation, and others [4]. Inorganic compound zinc carbonate is an odorless, white solid soluble in acidic and weak essential solutions but insoluble in water. It appears in nature as the mineral smithsonite [5]. ZnCO3 is essential in many industrial industries. Because of its ability to effectively remove harmful gases, it was employed in respirators. The production of ointments uses it primarily [6]. Compared to conventional cancer treatment techniques, inorganic nanoparticles have become more important in research and practical applications (surgery, chemotherapy, radiotherapy). Inorganic nanoparticles are perfect for cancer treatment because they have excellent stability, ease of installation, high biocompatibility, low toxicity, surface reactivity, and the capacity to carry medications to the affected and resistant areas [7]. Due to its late detection and recurrence, ovarian cancer is the most lethal gynecological malignancy [8,9]. The ovary and omentum are frequently affected by ovarian cancer, diffuse malignant ascites, and intraperitoneal metastases [10]. Less than 40% of stage 3 patients survive for five years, and around 75% of ovarian cancer patients initially have an intrabdominal diagnosis [11,12]. These variables contribute to treatment recurrence and resistance because, according to the available data, ovarian cancer cells are relatively resistant to standard chemotherapeutics [13]. In recent years, ovarian cancer has been labeled a gynecologically fatal malignancy [14]. Finding essential risk factors for ovarian cancer can have significant clinical and societal health effects because diagnostic techniques are often inadequate [15]. The phase or stage of ovarian cancer, which can be divided into grades I, II, III, and IV, performs an essential function during treatment [16]. In its early stages, ovarian cancer typically exhibits only a few symptoms. Patients' chances of survival are only % 10, and %30 when ovarian cancer is discovered in an advanced stage [17]. According to several preventative steps, the average mortality rate for ovarian cancer is expected to increase due to rising obesity rates and oral contraceptives [18]. This paper compares the efficacy of ZnCO3 nanoparticles as an anticancer agent prepared by two different methods on breast cancer cell lines.

Experimental

The chemicals used in this study are ZnCl2, NaOH, HCl, Na2CO3, and Deionized Water. It was from excellent international companies of high purity.

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Synthesis of ZnCO3 Nanoparticles by co-precipitation
ZnCl2 and Na2CO3 were combined in an amount of 0.5 M, and the mixture was stirred on a magnetic stirrer for six hours at 50 °C. A pH adjustment of 7 was made. The filtrate was then filtered and dried at 120 °C for four hours.

Synthesis of ZnCO3 Nanoparticles by green chemistry
A mixture of 100 g of turmeric was taken, and 1L deionized water was added and placed on the magnetic stirrer for hours at 50 °C. A pH adjustment of 7 was made. The filtrate was then filtered and dried at 120 °C for four hours.

Approaches for characterization

Numerous techniques, such as X-ray diffraction (XRD), Fourier-transform infrared (FTIR) spectroscopy, and scanning electron microscopy, were used to analyze the ZnCO3 nanoparticles (SEM). XRD was used to determine the crystallite size of the nanoparticles (Shimadzu, Kyoto, Japan). The samples' FTIR spectra were obtained using Shimadzu (Tokyo, Japan). SEM analysis was performed using a 200 kV Zeiss SEM (Germany).

ZnCO3 nanoparticle MTT assay
MTT dye (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) (10 mg/ml) was used for this experiment. Samples of ZnCO3 nanoparticles were dissolved in 0.2 % DMSO to obtain concentration gradients of 25, 50, 100, 200, and 400 µg/ml. sample of 200 µl suspended cells (1 × 104 cells/well) prepared in RPMI media was disseminated. The cells were cultured at 37 °C for 24 hours with 5% CO2. The cell cultures were then incubated for an additional 24 hours under the same circumstances after receiving 20 µl of ZnCO3-NPs treatment. Each sample was then given 10 µl of MTT reagent, which was then incubated for 5 hours at 37°C. At 570 nm, the absorbance was measured [20].

Hemolysis assay For ZnCO3 nanoparticle
To find toxic or non-toxic chemicals, the hemolysis assay was employed to screen for ZnCO3 at different doses (50, 200, and 400 µg/ml). After being taken from the lab and placed in an (EDTA) tube, the blood sample was analyzed on a slide and examined under a microscope at a magnification of (100). The blood cells and plasma were separated using an (EDTA) tube and placed in a centrifuge for 10 minutes. After removing the plasma layer from the cells, the cells were repeatedly washed with PBS, each time adding 1ML of PBS, and the centrifuge cycle was repeated for 10 minutes. After two minutes, the cells were withdrawn from the PBS. After the blood cells had been washed multiple times, the blood cell suspension was created by combining (1ML) with (9ML) PBS. The antagonist is added to each tube with a volume of (1200 µL) in varying concentrations, and (300 µL) of the cell suspension is added to the final volume (1.5 ml). Each tube is then incubated in the incubator for two hours before being spun apart at a rate of 1000 cycles per minute for five minutes. The Heh control parameters were then used to measure the difference in hemolysis (test tube containing blood and deionized water only, test tube containing blood and PBS). After centrifugation, the (+) option displays the compound's toxicity when mixed with blood components. The fact that the blood components were not combined after centrifugation, as shown by the (-) option, suggests that the drug was not harmful [21].

Results and discussion

Characterization of ZnCO3 nanoparticles by FTIR
ZnCO3 prepared by co-chemical precipitation A was diagnosed by FT-RT as shown in Figure 1. The appearance of a weak band at the frequency (462.92cm-1) due to the stretching of the (Zn-O) υ and a weak and sharp band at the site (1049.29cm-1) due to the frequency of the (C-O) υ, as we notice a band Long and sharp at a frequency (1512.19cm-1) due to the stretching of the (C = O) bond and a wide band at the frequency (13417.86cm-1) due to the stretching of the (O-H) bond. As for ZnCO3 prepared by green chemistry B, a weak band appears at the frequency (486.06) due to the stretching of the bond. (Zn-O) υ and a weak band in position (1041.56cm-1) that goes back to the frequency of the band (C-O) υ. We also notice a long and wide band at the frequency (1512.19cm-1) that goes back to the stretching of the band C=O) υ and a wide band at the frequency (3394.72cm-1) that goes back to the stretching of the υ(O-H) sphincter are consistent with the literature [22,23].

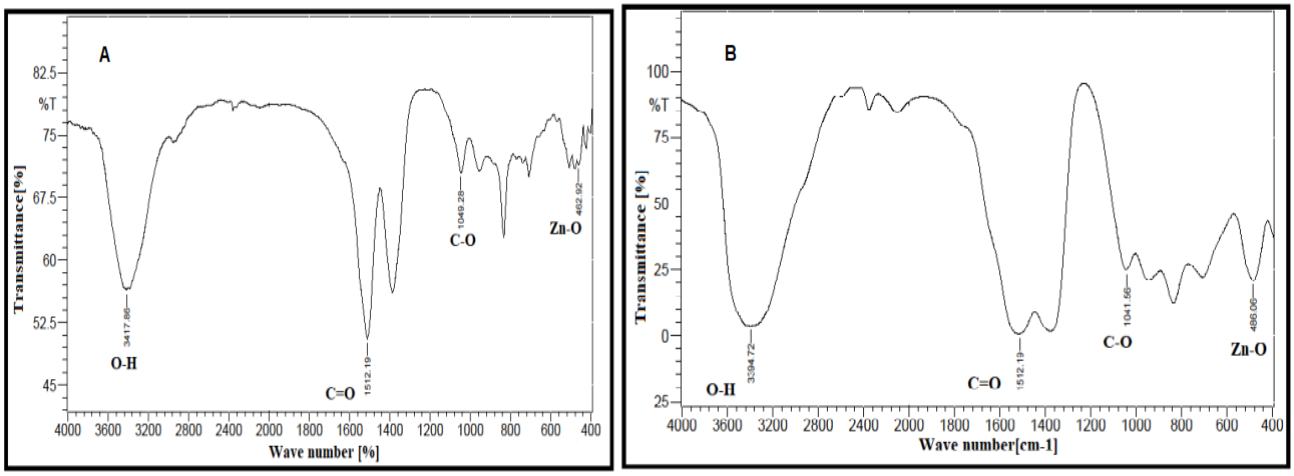

Fig. 1: Characterization of ZnCO3 nanoparticles by FTIR

Characterization of ZnCO3 nanoparticles by X-ray diffraction

Using the International Center for XDR Database (ICDD), the ZnCO3 X-ray spectra were compared to the ZnCO3 standard spectrum in Figure 2. Card number 01-083-1765 ZnCO3 nanoparticles produced using the chemical

precipitation method had an average crystal size of (21.10 chemistry B had an average crystal size of 15.02 nm. nm). A. ZnCO3 nanoparticles made using green

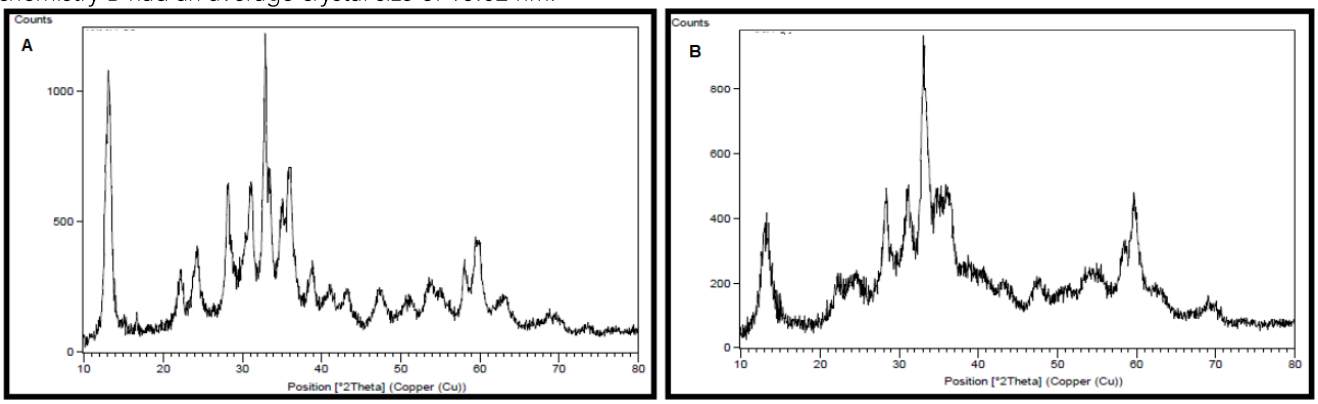

Fig. 2: X-ray diffraction spectrum of ZnCO3nanoparticles

Characterization of ZnCO3 nanoparticles by energy-dispersive X-rays

The elements found in ZnCO3NPs were identified using energy-dispersive X-ray, as depicted in Figure 3. The results showed that zinc 67.5%, oxygen 21.1%, and carbon11.3% were present in sample A, demonstrating the high degree of purity of the zinc carbonate nanoparticles. The results from experiment B showed high levels of purity in the zinc carbonate nanoparticles, with a zinc content of 67.9%, oxygen at 20.3%, and carbon at 11.9%.

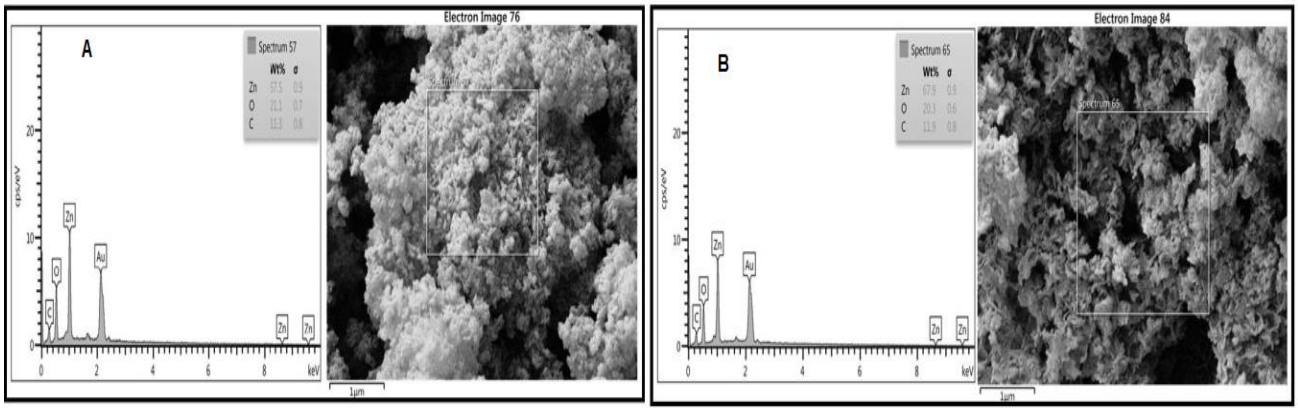

Fig. 3: Energy-dispersive X-rays of ZnCO3nanoparticles

Characterization of ZnCO3nanoparticles by SEM

The morphological and structural makeup of ZnCO3NPs was studied using an SEM scanning electron microscope. The nanoparticles were produced at the nanoscale level, and experiment A data from Figure 4 demonstrate that their average diameter is 52.81 nm. The diameter of the results from experiment B is 64.51 nm on average.

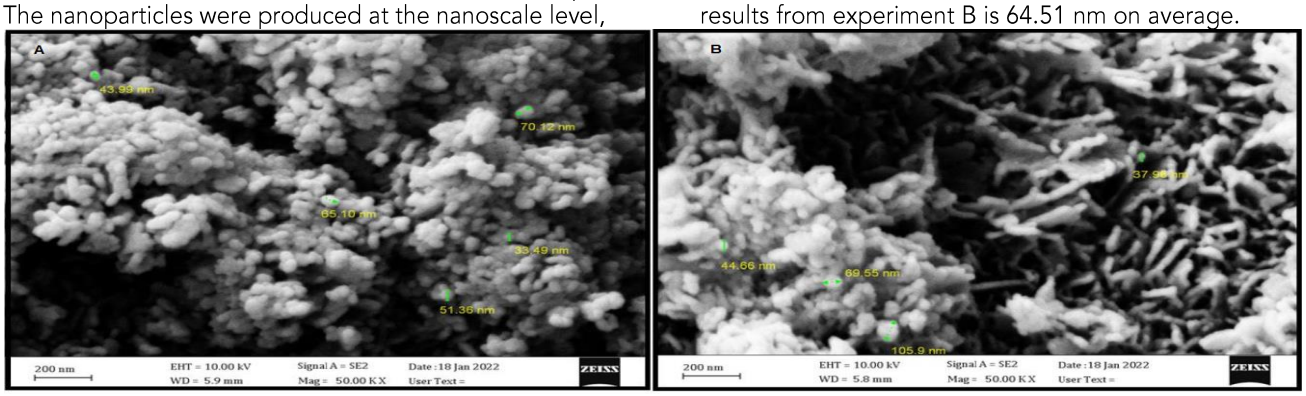

Fig. 4: SEM of ZnCO3nanoparticles

Inhibition of ZnCO3 nanoparticles for SK-OV-3 ovarian cancer cells

The results in Figure 5 showed the survival rate of SK-OV-3 cells after adding the mixed ZnCO3 by chemistry precipitation method A at different concentrations (25-400 µg/ml) compared to Blank for 24 hours of concentration of 100, the killing rate was 41.5%, at the concentration of 200, the killing rate was 51.4%, which indicates a relationship between the increase in the concentration and the percentage of inhibition or killing. At a concentration of 400, the killing rate is 66.3%As for the ZnCO3 prepared using the green chemistry method B at a concentration of 25, the killing rate was 4.02 percent, and at a concentration incubation. At a concentration of 25, the killing rate was 6%, and at a concentration of 50, the killing rate was 19%. While at the of 50, the killing rate was 15.8%. While at the concentration of 100, the killing rate was 27.5%, at the concentration of 200, the killing rate was 45.13%. At the concentration of 400, the killing rate was 65.59%, which indicates that the ZnCO3 prepared by the chemical precipitation method is more effective in inhibiting or killing.

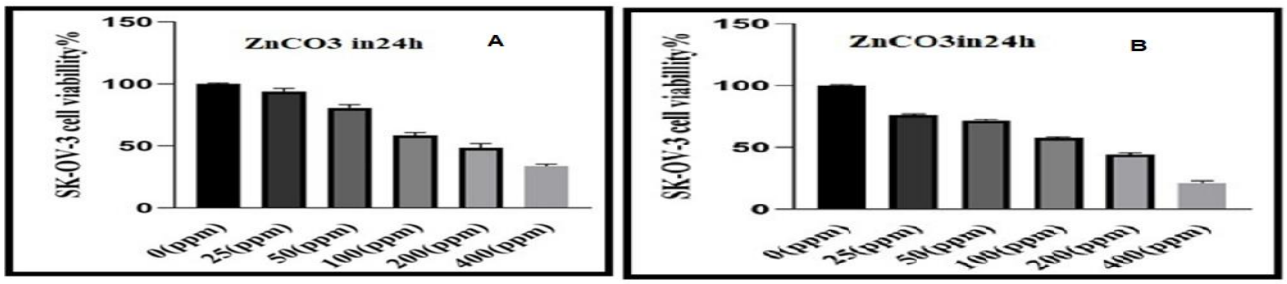

Fig. 5: Inhibition of ZnCO3 nanoparticles for SK-OV-3in 24 h

After 24 hours of incubation with SK-OV-3 ovarian cancer cells, the half-maximal inhibitory concentration (IC50) of ZnCO3 nanoparticles via chemical precipitation technique A was assessed using a normalized response. Fig. 6 depicts the IC50 value as 183.8 µg/ml. Using method B of green chemistry, ZnCO3 was created. A relatively low IC50 value was 128.5 µg/ml.

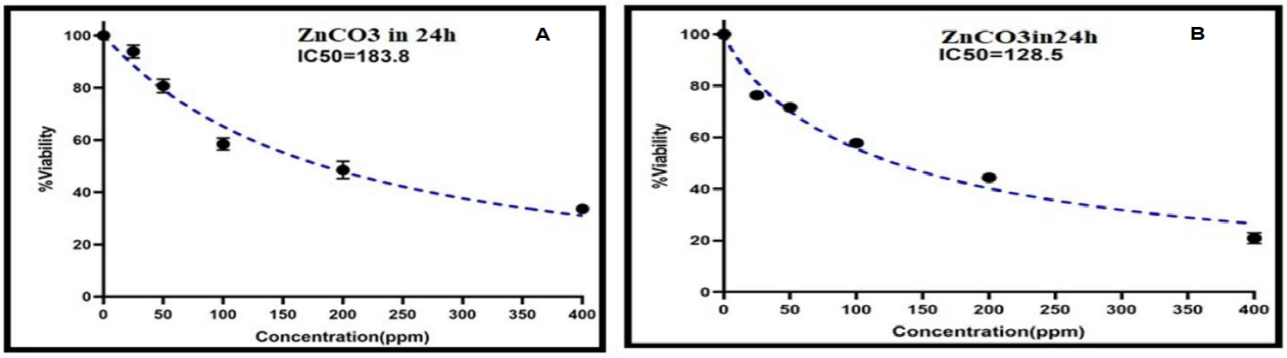

Fig. 6: IC50 of ZnCO3 nanoparticles for SK-OV-3 ovarian cancer cells

Fig. 7. Shown cytotoxicity of the compound of ZnCO3 nanoparticles via chemical precipitation technique A was investigated, and the results showed that the compound was safe (non-toxic) at all concentrations. Also, for method B of green chemistry

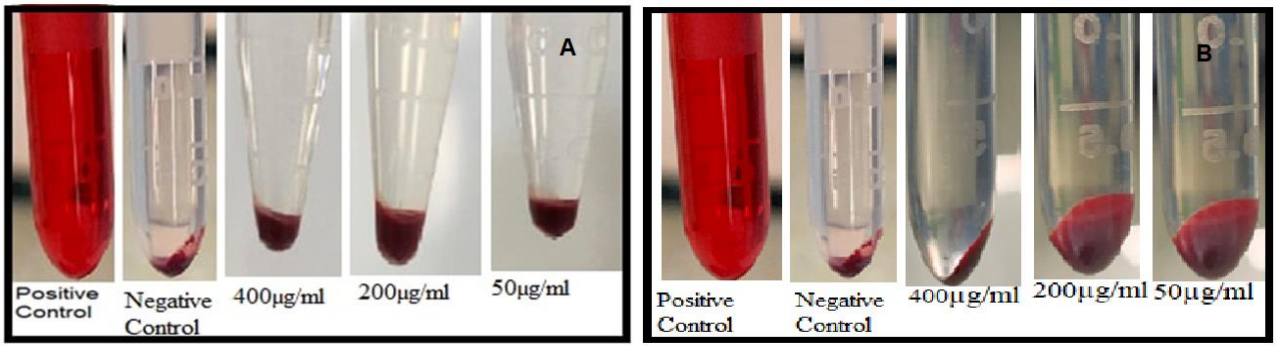

Fig. 7: Hemolysis test for ZnCO3 nanoparticles

Recent years have seen a lot of interest in using nanoparticles to treat ovarian cancer. To create a useful nanotechnology method, metal nanoparticle creation and modification depend on the form, size, and target accumulation. ZnCO3 nanoparticles are among the nanoparticles that are showing promise in modern nanobiotechnology for a variety of uses, including antioxidant, antibiofilm, antibacterial, and anticancer effects [24,25]. By raising the intracellular amount of ROS, disrupting the mitochondrial membrane, and inducing programmed cell death against human metastatic ovarian cancer cell lines, the chemical approach showed a potent in vitro cytotoxic effect (SK-OV-3 cells). According to earlier research, the NPs have reportedly demonstrated sound anticancer effects against various cancer cells, including colon, cervical, leukemia, breast, and neuroblastoma [26,27].

Conclusions

The current study discussed the green chemistry of employing turmeric extract in co-precipitation to synthesize ZnCO3 nanoparticles. FTIR, XRD, and SEM were used to analyze the structural characteristics of ZnCO3 nanoparticles. ZnCO3 nanoparticles offer potential therapeutic benefits as anticancer agents, and studies have shown that they can reduce the spread of ovarian cancer ZnCO3 nanoparticles can prevent breast cancer stem cells' ability to migrate and invade by increasing the quantity of ROS inside cells, rupturing the mitochondrial membrane, and causing programmed cell death against them.

References

1. Karagiozov, C., & Momchilova, D. (2005). Synthesis of nano-sized particles from metal carbonates by the method of reversed mycelles. Chemical Engineering and Processing: Process Intensification, 44(1), 115-119.
2. Dagaonkar, M. V. (2001). Effect of a microphase on gas-liquid mass transfer. Rijksuniversiteit te Groningen.
3. Dong, X. L., Choi, C. J., & Kim, B. K. (2002). Chemical synthesis of Co nanoparticles by chemical vapor condensation. Scripta Materialia, 47(12), 857-861.
4. Du, Y., Fang, J., Zhang, M., Hong, J., Yin, Z., & Zhang, Q. (2002). Phase character and structural anomaly of Bi4Ti3O12 nanoparticles prepared by chemical

coprecipitation. Materials Letters, 57(4), 802-806.
5. Gao, J., Zhu, F., Lai, X. J., Huang, R., Qin, S., Chen, D. L., ... & Wu, X. (2014). Compressibility of a natural smithsonite ZnCO3 up to 50 GPa. High Pressure Research, 34(1), 89-99.
6. Alhawi, T., Rehan, M., York, D., & Lai, X. (2015). Synthesis of zinc carbonate hydroxide nanoparticles using microemulsion process. Procedia engineering, 102, 346-355.
7. Chen, T., & Liu, Y. (2016). Semiconductor Nanocrystals and Metal Nanoparticles: Physical Properties and Device Applications. CRC Press.
8. Rojas, V., Hirshfield, K. M., Ganesan, S., & Rodriguez-Rodriguez, L. (2016). Molecular characterization of epithelial ovarian cancer: implications for diagnosis and treatment. International journal of molecular sciences, 17(12), 2113.
9. Bhatt, P., Vhora, I., Patil, S., Amrutiya, J., Bhattacharya, C., Misra, A., & Mashru, R. (2016). Role of antibodies in diagnosis and treatment of ovarian cancer: Basic approach and clinical status. Journal of controlled release, 226, 148-167.
10. Chen, S. N., Chang, R., Lin, L. T., Chern, C. U., Tsai, H. W., Wen, Z. H., ... & Tsui, K. H. (2019). MicroRNA in ovarian cancer: biology, pathogenesis, and therapeutic opportunities. International journal of environmental research and public health, 16(9), 1510.
11. Stewart, C., Ralyea, C., & Lockwood, S. (2019, April). Ovarian cancer: an integrated review. In Seminars in oncology nursing (Vol. 35, No. 2, pp. 151-156). WB Saunders.
12. Giampaolino, P., Della Corte, L., Foreste, V., Vitale, S. G., Chiofalo, B., Cianci, S., ... & Bifulco, G. (2019). Unraveling a difficult diagnosis: the tricks for early recognition of ovarian cancer. Minerva Medica, 110(4), 279-291.
13. Tarhriz, V., Bandehpour, M., Dastmalchi, S., Ouladsahebmadarek, E., Zarredar, H., & Eyvazi, S. (2019). Overview of CD24 as a new molecular marker in ovarian cancer. Journal of cellular physiology, 234(3), 2134-2142.
14. Boitano, T. K., Barrington, D. A., Batra, S., McGwin Jr, G., Turner, T. B., Farmer, M. B., ... & Leath III, C. A. (2019). Differences in referral patterns based on race for women at high-risk for ovarian cancer in the southeast: Results from a Gynecologic Cancer Risk Assessment Clinic. Gynecologic oncology, 154(2), 379-382.
15. Casey, M. J., & Salzman, T. A. (2018). Reducing the risk of gynecologic cancer in hereditary breast ovarian cancer syndrome mutation carriers: moral dilemmas and the principle of double effect. The Linacre Quarterly, 85(3), 225-240.
16. Narod, S. (2016). Can advanced-stage ovarian cancer be cured? Nature reviews Clinical oncology, 13(4), 255-261.
17. Guo, J., Yang, W. L., Pak, D., Celestino, J., Lu, K. H., Ning, J., ... & Bast Jr, R. C. (2019). Osteopontin, macrophage migration inhibitory factor and anti-interleukin-8 autoantibodies complement CA125 for detection of early-stage ovarian cancer. Cancers, 11(5), 596.
18. Jochem, C., Schlecht, I., & Leitzmann, M. (2018). Epidemiologic relationship between obesity and ovarian cancer. In focus on gynecologic malignancies (pp. 21-30). Springer, Cham.
19. Shin, S. W., Song, I. H., & Um, S. H. (2015). Role of physicochemical properties in nanoparticle toxicity. Nanomaterials, 5(3), 1351-1365.
20. Salah, M., Hammadi, M., Hummadi, E.H.(2021). Anticancer activity and cytotoxicity of ZnS nanoparticles on MCF-7 human breast cancer cells. ***Biochemical and Cellular Archives***, 21(1), pp. 95–99
21. Wayne, P. A. (2011). Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing.
22. Mohamed, N. H., Hamed Esam, A., Gomaa, S. G., & Sanad, A. (2014). Thermodynamics of solvation for nano zinc carbonate in mixed DMF-H2O solvents at different temperatures. Int. J. Eng. Innov. Tech. (IJEIT), 4, 203-207.
23. Shamsipur, M., Pourmortazavi, S. M., Hajimirsadeghi, S. S., Zahedi, M. M., & Rahimi-Nasrabadi, M. (2013). Facile synthesis of zinc carbonate and zinc oxide nanoparticles via direct carbonation and thermal decomposition. Ceramics International, 39(1), 819-827.
24. Arakha, M., Pal, S., Samantarrai, D., Panigrahi, T. K., Mallick, B. C., Pramanik, K., ... & Jha, S. (2015). Antimicrobial activity of iron oxide nanoparticle upon modulation of nanoparticle-bacteria interface. Scientific reports, 5(1), 1-12.
25. Khatami, M., Alijani, H. Q., Fakheri, B., Mobasseri, M. M., Heydarpour, M., Farahani, Z. K., & Khan, A. U. (2019). Super-paramagnetic iron oxide nanoparticles (SPIONs): Greener synthesis using Stevia plant and evaluation of its antioxidant properties. Journal of Cleaner Production, 208, 1171-1177.
26. Taghavi, F., Saljooghi, A. S., Gholizadeh, M., & Ramezani, M. (2016). Deferasirox-coated iron oxide nanoparticles as a potential cytotoxic agent. MedChemComm, 7(12), 2290-2298.
27. Zhi, D., Yang, T., Yang, J., Fu, S., & Zhang, S. (2020). Targeting strategies for superparamagnetic iron oxide nanoparticles in cancer therapy. Acta biomaterialia, 102, 13-34.