Synthesis and characterization of surface-modified bismuth oxide nanoparticles with β-cyclodextrin as a therapeutic agent: Anticancer studies

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Abstract

Bismuth oxide (Bi_2O_3) NPs have received momentous attention in the field of biomedicine due to their exceptional properties such as high chemical stability and low toxicity. However, its hydrophobic nature and poor biocompatibility limit its application in biomedical fields. Bismuth oxide can be surface-modified to get around these drawbacks and improve its bioactivity and biocompatibility, making it a promising material for a range of biomedical uses. The surface modification of Bi_2O_3 NPs with β-cyclodextrin as a therapeutic agent is covered in the current study. Bi_2O_3 NPs were created using the sol-gel method and further functionalized with β-cyclodextrin. The surface-modified system was characterised using several techniques, including XRD, UV-Vis, FT-IR, SEM, and TEM. The MTT assay was used to assess this system's in vitro cytotoxicity.

Keywords: *Bi_2O_3 NPs, β-CD, surface modification, anticancer studies.*

1. Introduction

Concerns about human health have recently increased daily. Pharma firms and the scientific community have vowed to advance knowledge of and practical application of recently developed drugs and protocols. Although there are many well-established treatments available, there is still a critical need to create fresh, cutting-edge technologies that could aid in defining tumour margins, locating lingering tumour cells, and ultimately eliminating them.[1–3] Additionally, it has become crucial to develop new antimicrobial agents that can combat the rise in antibiotic resistance.[4] Nanoscience and material technology have thoroughly examined each of these problems. Despite their relative underutilization, bismuth-based materials have a lot of unrealized potential among the various promising nanosized or nanostructured materials.[5–7]

Bi_2O_3 is a p-type semiconductor with remarkable chemical and physical characteristics, including a significant energy bandgap, dielectric permittivity, a high refractive index, photoluminescence and photoconductivity.[8] Thus, it has potential uses in a variety of fields, including solid-state fuel cells,[9] photovoltaic cells,[10] high-temperature superconductors,[11] photocatalysts,[12] gas sensors,[13] optical coatings[14] and biomedical applications.[15] The five crystallographic polymorphs of Bi_2O_3 are α- Bi_2O_3 (monoclinic), β- Bi_2O_3 (tetragonal), γ- Bi_2O_3 (body-centred cubic), δ- Bi_2O_3 (cubic) and ε- Bi_2O_3 (triclinic). Two of these forms, α- Bi_2O_3 at ambient temperature and δ- Bi_2O_3 at high temperatures, are stable, whereas the remaining four are metastable phases.[16] The synthesis of Bi_2O_3 NPs can be done through various methods such as chemical vapour deposition (CVD),[17] precipitation method,[18] electrodeposition,[19] microwave-assisted synthesis,[20] microemulsion method,[21] sol-gel method,[22] and hydrothermal synthesis.[23] Surface modification of nanoparticles is the process of altering the surface chemistry of nanoparticles to improve their properties and enhance their performance for specific applications. Bismuth oxide nanoparticles can be highly reactive and prone to aggregation, leading to a loss of their unique properties. Surface modification can increase the stability of the nanoparticles and prevent aggregation. Bismuth oxide nanoparticles can be made more biocompatible by having their toxicity reduced and their ability to interact with living things improved through surface modification. As a result, they are better suited for use in biomedical applications like therapy, imaging, and drug delivery. The anticancer activity of spherical Bi_2O_3 NPs against malignant neoplasms was studied by Ovsyannikov, et al.[24] In an original in vitro study of the radiosensitizing ability of Bi_2O_3 NPs, Stewart and co-workers[25] used the highly radioresistant 9L gliosarcoma cells. Du et al.[26] investigated bifunctional Bi_2O_3 NPs for convincing CT imaging and radio-sensitization of tumours through hyaluronic acid (HA) functionalization. After coating with HA, the as-prepared HA- Bi_2O_3 NPs enabled the viability of precise targeting to cancer cells overexpressing CD44 receptors. Excellent biocompatibility and good water solubility were shown by the HA- Bi_2O_3 NPs, supporting a variety of chemical and biological effects. In mammalian cells, the toxicity of Bi_2O_3 NPs was investigated by Abudayyak and co-workers. They claimed that the oxidative damage caused by the Bi_2O_3 NPs in Caco-2, NRK-52E, and Hep G2 cells. Farahani et al.[27] used a polymer gel dosimetry technique to test bismuth nanoparticles in Kilovolt and Megavolt radiation therapy, demonstrating the strong energy dependence of dose enhancement.

β-cyclodextrin is a good biocompatible system for the surface modification of bismuth oxide nanoparticles. The structure of beta-cyclodextrin is cone-shaped with a hydrophobic inner cavity and a hydrophilic outer surface.[28] The hydrophobic cavity is formed by the glucose units, which are arranged in a toroidal shape with an internal diameter of approximately 0.78 nm and a height of approximately 1.1 nm. The hydrophilic outer surface of beta-cyclodextrin is composed of the hydroxyl groups of the glucose units, which can form hydrogen bonds with water molecules and other polar molecules. The hydrophobic inner cavity, on the other hand, can accommodate small hydrophobic molecules, such as lipids, steroids, and aromatic compounds. The cone-shaped structure of beta-cyclodextrin allows it to form inclusion complexes with guest molecules, in which the guest molecule is partially or completely enclosed within the hydrophobic cavity. This characteristic makes beta-cyclodextrin useful in a variety of applications, including the pharmaceutical delivery system, the food industry, and bioremediation. In the pharmaceutical industry, beta-cyclodextrin is used as a drug delivery system to improve the bioavailability and solubility of poorly soluble drugs.[29,30] It is also used as a stabilizer for drugs that are sensitive to moisture, light, or heat.

The current study focuses on the surface modification of Bi_2O_3 NPs with β-cyclodextrin. The developed surface modified system was characterized by XRD, UV-Vis, FT-IR, SEM and TEM. In vitro cytotoxicity of the surface modified Bi_2O_3 NPs was evaluated against three different cancer cells; Hep G2, MCF-7 and A549 using MTT assay.

2. Experimental

2.1 Materials and Methods

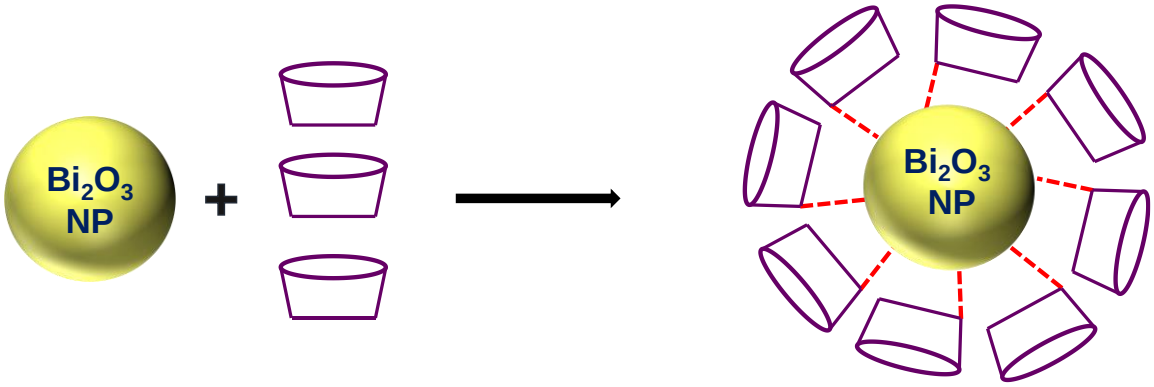
Bismuth nitrate alkaline $Bi_5O(OH)_9(NO_3)_4$ was used as a precursor. All of the chemicals and solvents used in the investigation were of analytical grade. Sodium-hypophosphite ($NaH_2PO_2.H_2O$), poly vinyl alcohol (PVA), cyclodextrin, nitric acid (HNO_3), sodium hydroxide (NaOH), DMSO, and ethanol are purchased from Nice chemicals. Throughout studies, deionized water was used, along with reagents and compounds that were employed without any additional purification.

2.2 Instrumentation

UV-Vis spectra were captured using a Shimadzu UV-visible NIR spectrophotometer operating between 190 and 1100 nm, and X-ray diffraction (XRD) measurements were performed using a Rigaku Miniflex-600 X-ray diffractometer with CuKα radiation in a θ-2θ configuration. The FT-IR spectra were captured using a Shimadzu-400 spectrophotometer with a scanning range of 4000-400 cm^{-1}. A HITACHI S-4200 scanning electron microscope operating at 20 kV and a JEOL JEM-2100 transmission electron microscope (TEM) were utilised to examine the morphology and size of the Bi_2O_3 NPs and surface-modified products.

2.3 Surface modification of Bi_2O_3 NPs with β-cyclodextrin

Bi_2O_3 NPs were synthesized via the sol-gel method. To obtain surface modified Bi_2O_3 NP with β-CD, about 1gm of β-CD and 0.25gm of Bi_2O_3 NP were dispersed in 10ml chloroform separately. Mix both solutions and stirred continuously for 3hrs. After the stirring, the solution was allowed to dry and the obtained powder is the surface modified Bi_2O_3 NPs. Scheme 1 schematically depicts the methods involved in creating surface-modified Bi_2O_3 nanoparticles using β-cyclodextrin.



Scheme 1. Graphical illustration of the surface-modified Bi_2O_3 nanoparticles with β-cyclodextrin.

2.4 In vitro cytotoxicity studies- MTT assay

For cytotoxicity studies of chemicals and drug screening, *in vitro* cell viability and cytotoxicity studies with cultivated cells are often performed. Currently, these tests are utilized in cancer research to evaluate the cytotoxicity and growth inhibition of possible cancer treatment candidates. MTT (3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide) assay is the most widely used colorimetric method for assessing cytotoxicity or cell viability. The MTT method is widely used because it is simple to use, safe, and well-reproducible. In the present work, in vitro cytotoxicity of the surface modified Bi_2O_3 NPs with β-CD was assessed against cancer cell lines; A549 (Human lung cancer), MCF-7 (Human breast cancer), and Hep G2 (Human liver cancer). The study was conducted at the Center for Research on Molecular and Applied Sciences *Pvt Ltd*, Biogenix, DNRA 41, Thiruvananthapuram, Kerala, India. The viability of cells was evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method. At a wavelength of 540 nm, absorbance values were measured using a microplate reader. Using the formula, the percentage of growth inhibition was computed:

$$\%\ of\ viability = \frac{Mean\ OD\ samples}{Mean\ OD\ of\ control\ groups} \times 100$$

2.5 Statistical analysis

All studies were conducted in triplicate, with results expressed as Mean ± SE. A one-way analysis of variance (ANOVA) and Dunnets test were used to analyse the results. $p < 0.001$ in comparison to the control group was statistically significant.

3. Results and discussion

3.1 Characterization of surface-modified Bi_2O_3 NPs with β-cyclodextrin

Figure 1 represent the XRD pattern of the surface-modified Bi_2O_3 NPs using β-CD. The peaks are in support of the monoclinic crystals phase of Bi_2O_3 based on COD No. 96-152-6459 at the 2θ value of 23.91°, 27.27°, 30.22°, 32.90°, 38.07°, 39.74°, 48.82°, 56.16° and 64.63° which are associated with the planes (102), (120), (012), (121), (131), (122), (113), (222), and (104). The crystallite size of Bi_2O_3 NPs is calculated by the Debye Scherrer equation and obtained in the range of 12-16 nm. The low-intensity peaks which are obtained below 30º are due to the β–CD in the final surface-modified system.

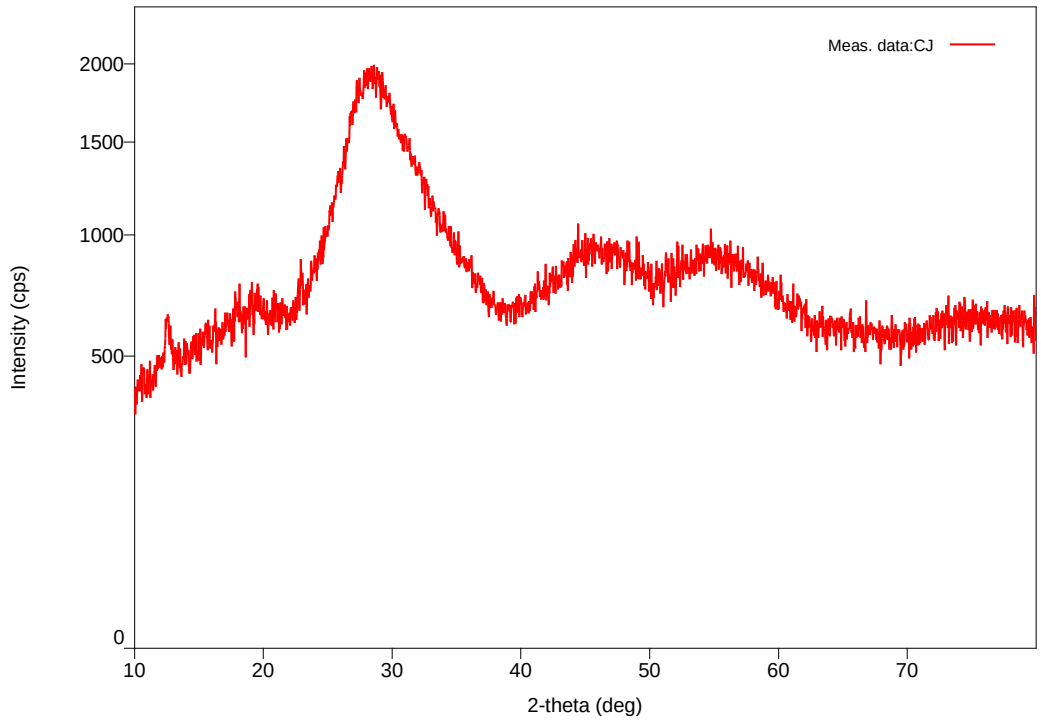


Figure 1. XRD pattern of the surface-modified Bi_2O_3 NPs using β-CD.

The FT-IR spectrum of the surface-modified Bi_2O_3 NPs using β-CD showed (**Figure 2**) a broad peak in the region of 3500–3200 cm^{-1} due to the characteristic peak of O–H groups and at 1649 cm^{-1} is due to H–O–H bond of beta-cyclodextrin. Peaks at 1154 cm^{-1}, 940 cm^{-1} and 838 cm^{-1} appear due to the C–O, C–O–C (glucose units) and C–O–C bond present in β-CD attached over the surface of Bi_2O_3 NPs. In the region of 570-400 cm^{-1} significant peak corresponds to the Bi–O bond. In the FT-IR spectrum of the surface-modified Bi_2O_3 NPs using β-CD, the appearance and slight shift of β-CD peaks confirm the successful surface coating of β-CD over Bi_2O_3 NPs.

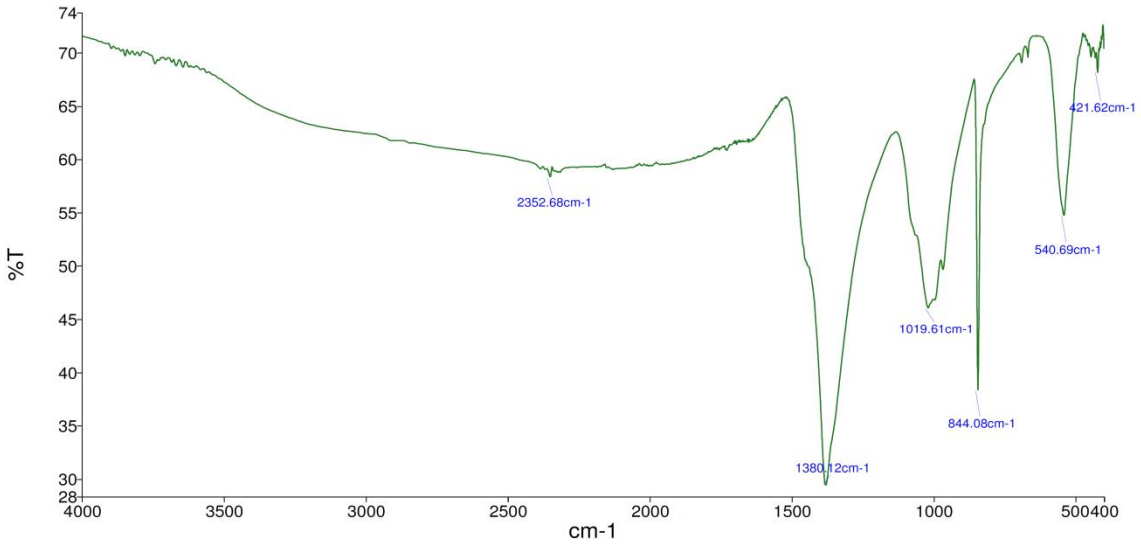


Figure 2. FT-IR spectrum of the surface modified Bi_2O_3 NPs using β-CD.

The UV-Vis DRS spectrum of the surface modified Bi_2O_3 NPs using β-CD (**Figure 3**) also confirms the formation of Bi_2O_3 NPs by exhibiting absorption peaks at 283 nm and 268 nm.

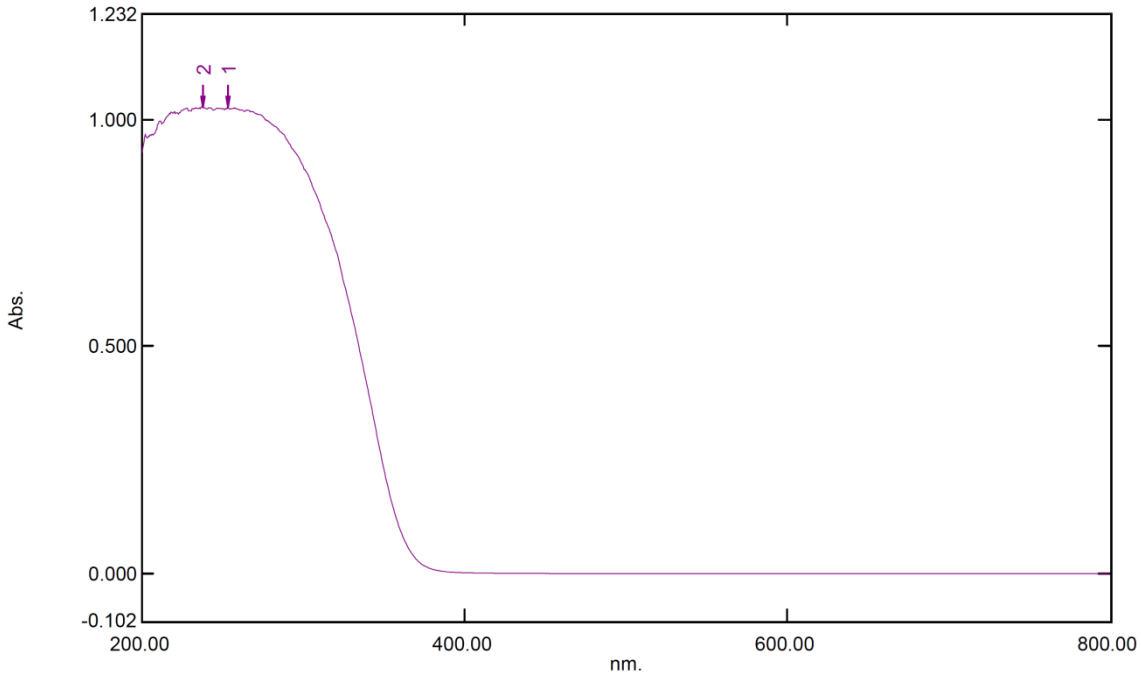


Figure 3. UV-Vis spectrum of the surface-modified Bi_2O_3 NPs using β-CD.

Figure 4 illustrates the surface morphology of the surface-modified Bi_2O_3 NPs using β-CD under SEM. The Bi_2O_3 NP has a spherical shape in morphology with an average diameter of 12-16 nm, and the agglomeration behavior was much reduced due to the presence of the β-CD.

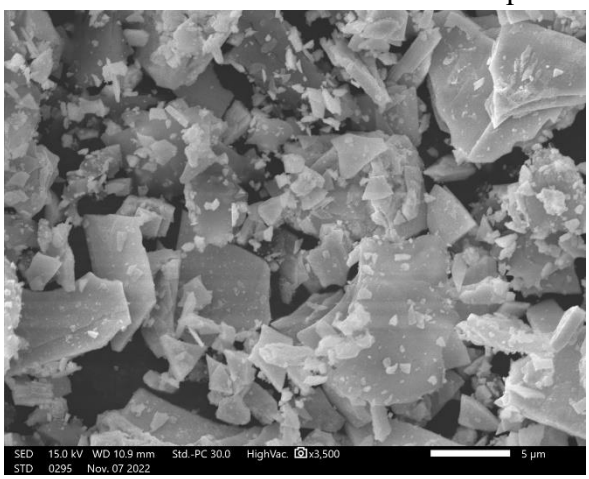


Figure 4. SEM image of the surface-modified Bi_2O_3 NPs using β-CD.

The amount of each particle's non-organic elemental composition was evaluated using EDS (**Figure 5**). In order to characterize the elemental composition of the studied volume, the EDS technique measures the x-rays that are emitted by the sample during irradiation by the electron beam. Results of EDS analysis are given in Table 1.

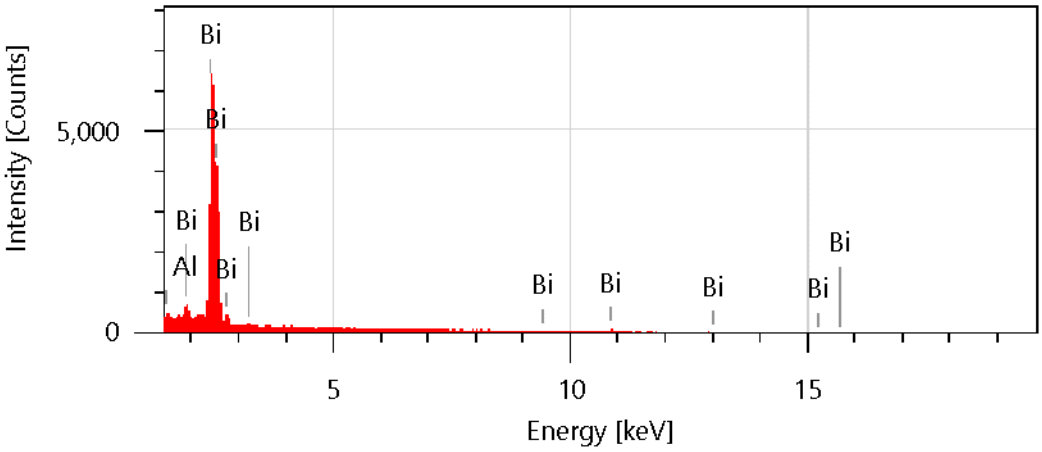


Figure 5. EDS of the surface-modified Bi_2O_3 NPs using β-CD.

Table 1. Results of EDS analysis.

Elemer	Lir	Mass%	Atom%
C	K	2.40±0.0	13.42±0.1
O	K	14.05±0.1	58.89±0.4
Al	K	0.41±0.0	1.02±0.0
Bi	M	83.13±0.3	26.67±0.1
Total		100.00	100.00

Figure 6 depicts the TEM image of Bi_2O_3 NPs, and it is clear that the synthesized monoclinic Bi_2O_3 NPs possess a spherical shape having a crystalline diameter in the range of 12-16 nm.

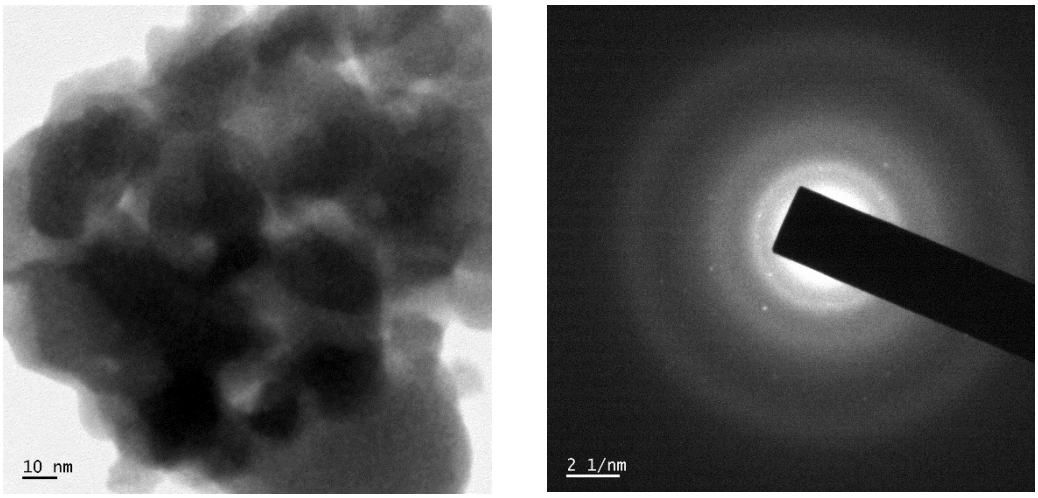


Figure 6. TEM image of the surface-modified Bi_2O_3 NPs using β-CD.

3.2 In vitro cytotoxicity studies- MTT assay

In vitro cytotoxicity of three different cancer cell lines; A549, MCF-7, and Hep G2 were evaluated using MTT assay and the results were illustrated as a graphical plot in **Figure 7** and Table 2. For this purpose, the cancer cell lines were exposed to the surface modified Bi_2O_3 NPs with β-CD at various concentrations (6.25, 12.5, 25, 50 and 100 µg/mL) for 24 h. In all three cases, the cell viability declined with a rise in the concentration of the surface modified Bi_2O_3 NPs with β-CD. The IC_{50} value of surface-modified Bi_2O_3 NPs with biotinylated β-cyclodextrin

reported against A549, MCF-7, and Hep G2 cell lines are 101.086, 90.269, 119.578 and µg /mL respectively and it was determined using ED50 PLUS V1.0 Software.

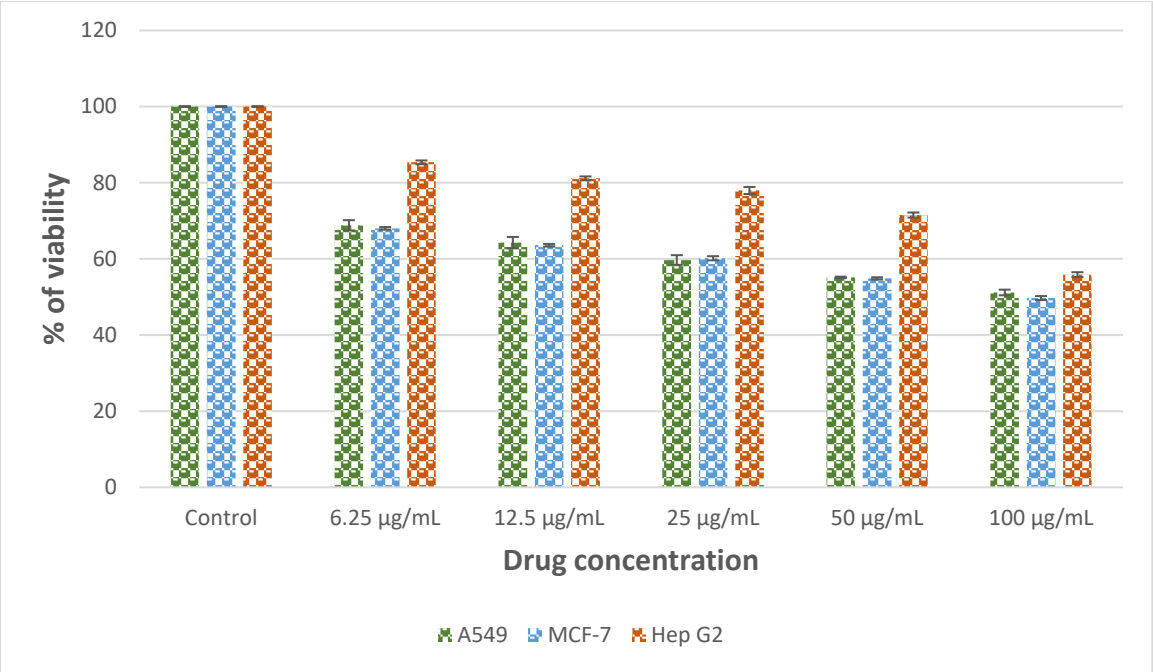


Figure 7. Graphical plot of *in vitro* cytotoxicity of surface-modified Bi_2O_3 NPs using β-CD against A549, MCF-7,and Hep G2.

Table 2. *In vitro* cytotoxicity of the surface-modified Bi_2O_3 NPs using β-CD against A549, MCF-7,and Hep G2.

A549 cancer cell lines					
Drug Concentratio	6.25	12.5	25	50	100
Percentage of cell viability	68.82 ± 1.35	64.28 ± 1.4	59.70 ± 1.2	55.13 ± 0.2	51.17 ± 0.74
MCF-7 cancer cell lines					
Drug Concentratio	6.25	12.5	25	50	100
Percentage of cell viability	68.02 ± 0.33	63.58 ± 0.3	60.16 ± 0.5	54.87 ± 0.3	49.71 ± 0.52

Hep G2 cancer cell lines					
Drug Concentratio	6.25	12.5	25	50	100
Percentage of cell viability	85.36 ± 0.49	81.16 ± 0.5	77.96 ± 0.9	71.52 ± 0.6	55.85 ± 0.64

4. Conclusion

Surface modified nanoparticles have been an active area of research and development for several years, and their potential applications are vast. The modification of nanoparticle surfaces allows for the optimization of their properties and the tailoring of their behavior to suit specific applications. This work describes the surface modification of Bi_2O_3 NPs with β-CD which exhibited greater biocompatibility and lower toxicity. The modified system was characterized using various techniques such as XRD, FT-IR, UV-Vis, SEM and TEM analysis. The in vitro cytotoxicity of the surface-modified Bi_2O_3 NPs using β-CD was evaluated by the MTT assay, against A549, MCF-7, and Hep G2 cell lines. The future outlook for surface modified nanoparticles is bright, as they offer a broad spectrum of potential applications and continue to be an area of active research and development.

References

[1] S.N. Gardner, Modeling multi-drug chemotherapy: Tailoring treatment to individuals, J. Theor. Biol. 214 (2002) 181–207. https://doi.org/10.1006/jtbi.2001.2459.

[2] K.J. Helzlsouer, T.W. Kensler, Cancer Chemoprotection by Oltipraz: Experimental and Clinical Considerations, Prev. Med. (Baltim). 22 (1993) 783–795. https://doi.org/10.1006/pmed.1993.1072.

[3] G. Girish, K. Finlay, D. Fessell, D. Pai, Q. Dong, D. Jamadar, Imaging review of skeletal tumors of the pelvis malignant tumors and tumor mimics, Sci. World J. 2012 (2012). https://doi.org/10.1100/2012/240281.

[4] S. Bin Zaman, M.A. Hussain, R. Nye, V. Mehta, K.T. Mamun, N. Hossain, A Review on Antibiotic Resistance: Alarm Bells are Ringing, Cureus. 9 (2017). https://doi.org/10.7759/CUREUS.1403.

[5] M.A. Shahbazi, L. Faghfouri, M.P.A. Ferreira, P. Figueiredo, H. Maleki, F. Sefat, J. Hirvonen, H.A. Santos, The versatile biomedical applications of bismuth-based nanoparticles and composites: therapeutic, diagnostic, biosensing, and regenerative properties, Chem. Soc. Rev. 49 (2020) 1253–1321. https://doi.org/10.1039/C9CS00283A.

[6] M. Bartoli, P. Jagdale, A. Tagliaferro, A Short Review on Biomedical Applications of Nanostructured Bismuth Oxide and Related Nanomaterials, Materials (Basel). 13 (2020) 5234. https://doi.org/10.3390/MA13225234.

[7] R. Hernandez-Delgadillo, D. Velasco-Arias, J.J. Martinez-Sanmiguel, D. Diaz, I. Zumeta-Dube, K. Arevalo-Niño, C. Cabral-Romero, Bismuth oxide aqueous colloidal nanoparticles inhibit Candida albicans growth and biofilm formation, Int. J. Nanomedicine. 8 (2013) 1645–

1652. https://doi.org/10.2147/IJN.S38708.
[8] L. Leontie, M. Caraman, M. Alexe, C. Harnagea, Structural and optical characteristics of bismuth oxide thin films, Surf. Sci. 507–510 (2002) 480–485. https://doi.org/10.1016/S0039-6028(02)01289-X.
[9] A.M. Azad, S. Larose, S.A. Akbar, Bismuth oxide-based solid electrolytes for fuel cells, J. Mater. Sci. 29 (1994) 4135–4151. https://doi.org/10.1007/BF00414192.
[10] W.E. Mahmoud, A.A. Al-Ghamdi, Synthesis and properties of bismuth oxide nanoshell coated polyaniline nanoparticles for promising photovoltaic properties, Polym. Adv. Technol. 22 (2011) 877–881. https://doi.org/10.1002/pat.1591.
[11] A. Sergeev, I. Golev, High-Temperature Superconducting Materials Based on Bismuth with a Low Critical Current, Mater. Today Proc. 11 (2019) 489–493. https://doi.org/10.1016/J.MATPR.2019.01.019.
[12] A.P. Reverberi, P.S. Varbanov, M. Vocciante, B. Fabiano, Bismuth oxide-related photocatalysts in green nanotechnology: A critical analysis, Front. Chem. Sci. Eng. 12 (2018) 878–892. https://doi.org/10.1007/S11705-018-1744-5.
[13] S.S. Bhande, R.S. Mane, A. V Ghule, S.-H. Han, A bismuth oxide nanoplate-based carbon dioxide gas sensor, (2011). https://doi.org/10.1016/j.scriptamat.2011.09.022.
[14] V. Fruth, M. Popa, D. Berger, R. Ramer, M. Gartner, A. Ciulei, M. Zaharescu, Deposition and characterisation of bismuth oxide thin films, J. Eur. Ceram. Soc. 25 (2005) 2171–2174. https://doi.org/10.1016/j.jeurceramsoc.2005.03.025.
[15] M. Bartoli, P. Jagdale, A. Tagliaferro, A Short Review on Biomedical Applications of Nanostructured Bismuth Oxide and Related Nanomaterials, (n.d.). https://doi.org/10.3390/ma13225234.
[16] M. Kumar Trivedi, R. Mohan Tallapragada, A. Branton, D. Trivedi, G. Nayak, O. Latiyal, S. Jana, Evaluation of Atomic, Physical, and Thermal Properties of Bismuth Oxide Powder: An Impact of Biofield Energy Treatment, Am. J. Nano Res. Appl. 2015 (2015) 94–98. https://doi.org/10.11648/j.nano.20150306.11.
[17] H.W. Kim, J.H. Myung, S.H. Shim, One-dimensional structures of Bi2O3 synthesized via metalorganic chemical vapor deposition process, Solid State Commun. 137 (2006) 196–198. https://doi.org/10.1016/J.SSC.2005.11.012.
[18] R. Irmawati, M.N.N. Nasriah, Y.H. Taufiq-Yap, S.B.A. Hamid, Characterization of bismuth oxide catalysts prepared from bismuth trinitrate pentahydrate: Influence of bismuth concentration, Catal. Today. 93–95 (2004) 701–709. https://doi.org/10.1016/J.CATTOD.2004.06.065.
[19] Z. Hamid, Synthesis of Bismuth oxide Nano powders viaelectrolysis method and study the effect of change voltage on the size for it, Aust. J. Basic Appl. Sci. 11 (2017) 97–101. www.ajbasweb.com (accessed January 20, 2023).
[20] P. V Shinde, B.G. Ghule, S.F. Shaikh, N.M. Shinde, S.S. Sangale, V. V Jadhav, S.-Y. Yoon, K.H. Kim, R.S. Mane, Microwave-assisted hierarchical bismuth oxide worm-like nanostructured films as room-temperature hydrogen gas sensors, (2019). https://doi.org/10.1016/j.jallcom.2019.06.182.
[21] W. Dong, C. Zhu, Optical properties of surface-modified Bi2O3 nanoparticles, J. Phys. Chem. Solids. 64 (2003) 265–271. https://doi.org/10.1016/S0022-3697(02)00291-3.
[22] A. Shokuhfar, K. Nasir, A. Esmaeilirad, V. Mazinani, M. Mallahi, A. Shokuhfar, M.R. Vaezi,

A. Esmaeilirad, V. Mazinani, Synthesis and characterization of Bismuth oxide nanoparticles via sol-gel method, Am. J. Eng. Res. 03 (2014) 162–165. www.ajer.org (accessed January 20, 2023).

[23] Q. Yang, Y. Li, Q. Yin, P. Wang, Y.B. Cheng, Hydrothermal synthesis of bismuth oxide needles, Mater. Lett. 55 (2002) 46–49. https://doi.org/10.1016/S0167-577X(01)00617-6.

[24] V.A. Ovsyannikova, M. V. Zamoryanskayaa, A. V. Semenchab, K.A. Lychevab, T.S. Kol'tsovab, O. V. Tolochkob, L.N. Blinov, Development of Bismuth OxideBased Nanopreparation for the Destruction of Malignant Neoplasms: Theoretical Prerequisites, Challenges, and Practical Approaches, Glas. Phys. Chem. 41 (2015) 533–536. https://doi.org/10.1134/S1087659615050119.

[25] C. Stewart, K. Konstantinov, S. McKinnon, S. Guatelli, M. Lerch, A. Rosenfeld, M. Tehei, S. Corde, First proof of bismuth oxide nanoparticles as efficient radiosensitisers on highly radioresistant cancer cells, Phys. Medica. 32 (2016) 1444–1452.

[26] F. Du, J. Lou, R. Jiang, Z. Fang, X. Zhao, Y. Niu, S. Zou, M. Zhang, C. Wu, Hyaluronic acid-functionalized bismuth oxide nanoparticles for computed tomography imaging-guided radiotherapy of tumor, Int. J. Nanomedicine. 12 (2017) 5973–5992. https://doi.org/10.2147/IJN.S130455.

[27] S. Farahani, N. Riyahi Alam, S. Haghgoo, A. Shirazi, G. Geraily, E. Gorji, N. Kavousi, The effect of bismuth nanoparticles in kilovoltage and megavoltage radiation therapy using magnetic resonance imaging polymer gel dosimetry, Radiat. Phys. Chem. 170 (2020) 108573. https://doi.org/10.1016/J.RADPHYSCHEM.2019.108573.

[28] V.J. Stella, R.A. Rajewski, Cyclodextrins: Their future in drug formulation and delivery, Pharm. Res. 14 (1997) 556–567. https://doi.org/10.1023/A:1012136608249.

[29] R. Challa, A. Ahuja, J. Ali, R.K. Khar, Cyclodextrins in drug delivery: An updated review, AAPS PharmSciTech. 6 (2005) 329–357. https://doi.org/10.1208/pt060243.

[30] A.L. Laza-Knoerr, R. Gref, P. Couvreur, Cyclodextrins for drug delivery, J. Drug Target. 18 (2010) 645–656. https://doi.org/10.3109/10611861003622552.