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Formulation and Evaluation of Titanium Dioxide Nanoparticles encapsulating Enzalutamide for Sustained drug delivery

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ABSTRACT

High surface area, chemical stability, low toxicity, and great photocatalytic activity are just a few of the exceptional physicochemical characteristics of titanium dioxide (TiO_2), an inorganic molecule that has been extensively researched. Because TiO_2 nanoparticles can encapsulate poorly soluble medicines and offer regulated or prolonged release, they have become attractive drug delivery vehicles in the field of nanomedicine This study reports the development and evaluation of titanium dioxide (TiO_2) nanoparticles as a carrier for enzalutamide to enhance therapeutic efficacy against prostate cancer. TiO_2 nanoparticles were synthesized and characterized for particle size, polydispersity index (PDI), zeta potential, drug loading capacity, and encapsulation efficiency (%EE). The optimized formulation exhibited a mean particle size of 95.1 ± 2.3 nm, PDI of 0.287, zeta potential of –11.2 mV, drug loading of 19.4 ± 0.6%, and %EE of 92.6 ± 1.2%. High-resolution transmission electron microscopy confirmed uniform, spherical morphology. In vitro drug release in phosphate-buffered saline (pH 7.4) demonstrated a biphasic profile with an initial burst followed by sustained release, reaching 78.5 ± 2.1% over 48 h. Cytotoxicity assays on DU-145 prostate cancer cells revealed significantly higher cell death with TiO_2–enzalutamide compared to free enzalutamide ($p < 0.05$), indicating enhanced intracellular delivery and antiproliferative activity. These findings suggest that TiO_2 nanoparticles are a promising platform for improving the solubility, stability, and bioavailability of enzalutamide in prostate cancer therapy.

Keywords: *Titanium dioxide, HPLC, FTIR, Enzalutamide.*

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1. INTRODUCTION

Prostate cancer is one of the most common cancers diagnosed and the leading cause of cancer-related death for men globally, particularly in older populations [1]. Because it inhibits androgen receptor signaling more effectively than previous treatments, enzalutamide, a potent second-generation nonsteroidal antiandrogen, has emerged as the main treatment for metastatic castration-resistant prostate cancer (mCRPC)[2]. Problems with enzalutamide therapy, such as low water solubility, decreased bioavailability, and off-target toxicity, can lead to significant systemic adverse effects and drug resistance [3,4].

The development of intelligent, targeted, and regulated drug delivery platforms has been made possible by nanotechnology-based drug delivery systems, revolutionizing cancer therapy [5]. Through passive (increased permeability and retention effect) or active targeted processes, these techniques aim to preferentially accumulate the drug in tumor tissues, improving therapeutic efficacy and lowering toxicity [6]. Due to its many benefits, such as high surface area, chemical stability, photocatalytic efficiency, biocompatibility, and ease of functionalization, titanium dioxide (TiO_2) nanoparticles have garnered a lot of attention [7]. The effectiveness of TiO_2 nanoparticles in precision oncology is further enhanced by the possibility of simultaneous therapeutic and diagnostic (theragnostic) applications [8].Higher drug loading, prolonged release, and improved intracellular absorption have been observed in recent studies that

have examined TiO_2-based nanocarriers for the administration of various anticancer drugs [9]. Due to their inert nature and ease of fabrication, they can be conjugated with a wide range of therapeutic molecules, which may increase intracellular uptake and cytotoxicity in cancer cells [10]. Research on TiO_2-based nanocarriers specifically for the treatment of prostate cancer is still scarce, nevertheless. Additionally, there is yet insufficient research on their application in the administration of hormone-targeting medications like enzalutamide.

In this work, a novel titanium oxide nanomaterial loaded with enzalutamide for targeted drug delivery is synthesized, characterized, and biologically evaluated. The formulation's drug release properties, encapsulation effectiveness, surface charge, and particle size were assessed. Its potential as a targeted treatment platform was also determined by evaluating its cytotoxicity properties in prostate cancer cell lines.

This study aims to advance nanomedicine in the treatment of prostate cancer by leveraging the unique properties of titanium oxide nanoparticles to get beyond the limitations of conventional enzalutamide treatment

2. MATERIAL AND METHODS

Materials

Titanium isopropoxide ($Ti[OCH(CH_3)_2]_4$) was employed as the precursor for the manufacture of titanium dioxide nanoparticles. Cadila Healthcare Pvt. Ltd. provided a free sample of enzalutamide (ENZ). Ethanol, distilled water, and analytical-grade chemicals were among the other solvents and reagents that were bought from conventional commercial sources and the hydrothermal synthesis process was carried out.

Synthesis of Titanium Dioxide Nanoparticles (TiO_2-NPs)

Titanium dioxide nanoparticles were synthesized via a hydrothermal method. Briefly, 5 mL of titanium isopropoxide (TTIP) was added dropwise to 100 mL of distilled water under continuous stirring. The reaction mixture was transferred into a Teflon-lined stainless-steel autoclave and heated at 200 °C for 24 hours [10,11]. After cooling to room temperature, the white precipitate obtained was centrifuged at 10,000 rpm for 10 minutes to remove unreacted precursors and by-products. The residue was washed thrice with distilled water and ethanol, then sonicated for 5 minutes to ensure proper dispersion. The obtained TiO_2 nanoparticles were dried at 60 °C and stored in a desiccator until further use [10]. The reaction mixture was transferred into a Teflon-lined stainless-steel autoclave and heated at 200 °C for 24 hours, following conventional hydrothermal protocols that enable control over crystal size and morphology by tuning temperature and time.

Preparation of Enz-TiO_2 Nanoparticles: Using an ultrasonic processor, 100 mg of TiNP-1 was dissolved in 25 mL of distilled water and sonicated. Increasing amounts of enzalutamide solution (5 mg/mL in ethanol) (6–14 mg) were applied. To guarantee that the medication was properly adsorbed onto the nanoparticles, the mixture was homogenized for 15 minutes at 13,500 rpm. Five formulations, Enz-TiO_2 1 through Enz-TiO_2 5, were created. After being lyophilized, the formulation was kept at 4 °C for additional analysis.

Homogenization process

After addition of the drug solution, the mixture was subjected to high-speed homogenization at 13,500 rpm for 15 minutes to promote interaction between Enzalutamide and TiO_2 nanoparticles.

Composition of API and TiNP-1 for formulating Nanoparticles

For all formulations, the nanoparticle substrate was 100 milligrams of TiNP-1. Five formulations were created by adding increasing volumes of Enzalutamide (ENZ) stock solution (5 mg/mL in ethanol). Table 1 displays each batch's precise makeup.

Table.no. 1 Composition of API and TiNP-1 for formulating Nanoparticles

Sr. No.	Name of Ingredients	Enz-TiO_2 1	Enz-TiO_2 2	Enz-TiO_2 3	Enz-TiO_2 4	Enz-TiO_2 5
1.	**TiNP-1**	100 mg	100 mg	100 mg	100 mg	100 mg
2.	Enzalutamide	6 mg	8 mg	10 mg	12 mg	14 mg
3.	Ethanol	1.2 ml	1.6 ml	2 ml	2.4 ml	2.8 ml
4.	Distilled Water	25 ml	25 ml	25 ml	25 ml	25 ml

Structural and morphological characterizations of Bare TiO_2 nanoparticles and drug loaded nanoparticles:

Evaluation of Titanium Dioxide Nanoparticles (TiO_2 NPs)

Using UV-VIS spectroscopy, FTIR, X-ray diffraction and SEM techniques, the morphologies and structures of drug-loaded TiO_2 nanocomposites and bare TiO_2 nanoparticles were calculated and verified. A double beam spectrophotometer was employed to examine the absorption spectra of nanoparticles suspended in deionized distilled water, using deionized distilled water as the blank from the Millipore system. The highest absorption wavelengths were analyzed to investigate bonding and spectral shifting interactions in materials. The capping formations on the surface of TiO_2 and the bonding contacts with drug were verified using IR analysis conducted with a Perkin Elmer series spectrometer, scanning the range of 500-4000 cm-1 using the KBr pellet technique. The morphology of these materials was validated by XRD spectra via the powder diffraction method [11-13].

The mean diameter of liposome particles was determined using photon correlation spectroscopy (PCS, Zetasizer 3000 HSA, Malvern Instruments Ltd., Worcestershire, UK). The zeta potential of liposome particles was assessed using a Malvern Zetasizer Nano ZS90. Each sample was diluted with distilled water to attain the requisite particle concentration, and each sample was tested in triplicate. All measurements were conducted in triplicate at 25 °C.

HPLC analysis

Enzalutamide, due to its poor water solubility, was first dissolved in ethanol to create a stock solution with a concentration of 1 mg/mL. Ethanol was chosen based on the drug's solubility profile and its compatibility with the aqueous dispersion medium. Quantitative analysis of Enzalutamide (ENZ) was performed using a validated high-performance liquid chromatography (HPLC) method. The separation was achieved on a Fortis C18 column (100 × 4.6 mm, 2.5 µm) with a mobile phase consisting of ammonium acetate buffer (pH 4.2) and acetonitrile in a 45:55 (v/v) ratio, delivered at a flow rate of 1.0 mL/min. Detection was carried out at 280 nm with an injection volume of 20 µL and a total run time of 15 minutes.

Drug Loading: Using high-performance liquid chromatography (HPLC), drug loading was ascertained. A 1:1, v/v mixture of ethanol and EDTA was used to dissolve one milligram of the nanoparticle formulation. To bring the final volume down to 10 mL, deionized water was added. A 0.45 µm membrane filter was used to filter the solution after it had been sonicated for five minutes. HPLC was used to examine the filtrate under the circumstances outlined in the HPLC analysis section.

Entrapment Efficiency: A Remi cooling centrifuge was utilized to ascertain the entrapment efficiency. To extract the free medication from the nanoparticles, a formulation of nanoparticles (10 µg/mL) was centrifuged at 4000 rpm for 18 minutes at 4 °C. After collecting the supernatant, it was centrifuged once again for 30 minutes at 4 °C and 15,000 rpm. To release the medication, the resultant pellet of nanoparticles was resuspended in ethanol and EDTA (final volume 100 mL). HPLC was used to measure the quantity of enzalutamide present in the sample. The following formula was used to determine the entrapment efficiency (%):

$$\textbf{Percentage Entrapment Efficiency} = \frac{\textbf{Wc}}{\textbf{Wt}} \times \textbf{100}$$

Where amount of drug content (entrapped) in the NP is denoted as Wc [18]and total amount of drug in the dispersion is denoted as Wt.

In vitro drug release: The dialysis membrane method was used to study drug release in vitro. Either free enzalutamide dispersion or the Enz-TiO_2-3 formulation equivalent to 5 mg of enzalutamide was employed in a dialysis bag (12,000 Da cut-off, Sigma). A magnetic stirrer was used to swirl the dialysis bags at 100 rpm while they were submerged in 100 mL of phosphate-buffered saline (pH 7.4) that was kept at 37 ± 0.5 °C. At specified intervals (0, 1, 2, 4, 6, 8, 12, 24, and 48 hours), samples (5 mL) were taken out and replaced with an equivalent volume of brand-new buffer. After passing through a 0.45 µm membrane, the extracted samples were examined at 234 nm using the verified HPLC technique [19]. The standard calibration curve was used to calculate drug release.

MTT Assay

PC-3 human prostate cancer cells (passage no. 41) were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco) under humidified conditions at 37 °C in a carbon dioxide incubator (Eppendorf CellXpert) until confluency was achieved. Cells were harvested and approximately 1×10^4 cells were seeded into each well of a 96-well plate (Tarsons). Control groups included untreated wells (cells with medium only) and vehicle control wells (cells with solvent treatment alone) to assess the effect of the solvent on cell viability. After cell attachment, test sample TENZ was added at graded concentrations, and plates were incubated for 24 h.

Following treatment, cell viability was assessed by MTT assay. Briefly, MTT reagent was prepared in PBS at a concentration of 5 mg/mL, and 50 µL was added to each well after 24 h of incubation. Plates were further incubated for 3–4 h in the dark until purple formazan crystals were observed microscopically. The crystals were solubilized in 100 µL of dimethyl sulfoxide (DMSO; HiMedia), and absorbance was recorded at 570 nm using an Epoch multiplate reader (BioTek). The entire assay was performed under light-protected conditions by wrapping plates in aluminum foil. Cell viability was calculated relative to untreated control wells.

3. RESULTS

Formulation of TiO_2 Nanoparticles

TiO_2 nanoparticles were successfully synthesized in three concentrations by varying the amount of titanium isopropoxide (10 mL, 15 mL, and 20 mL in 100 mL distilled water), designated as TiNP-1, TiNP-2, and TiNP-3. The obtained nanoparticles appeared as fine white powders after hydrothermal treatment and drying [10,11].

Morphological and Physicochemical Characterization

UV-Vis spectra

The UV–Vis spectrum of pure ENZ exhibited a maximum absorption peak at ~236 nm. The spectrum of Enz–TiO_2-3 displayed a similar peak with a slight bathochromic shift[14], indicating possible interaction between ENZ and TiO_2

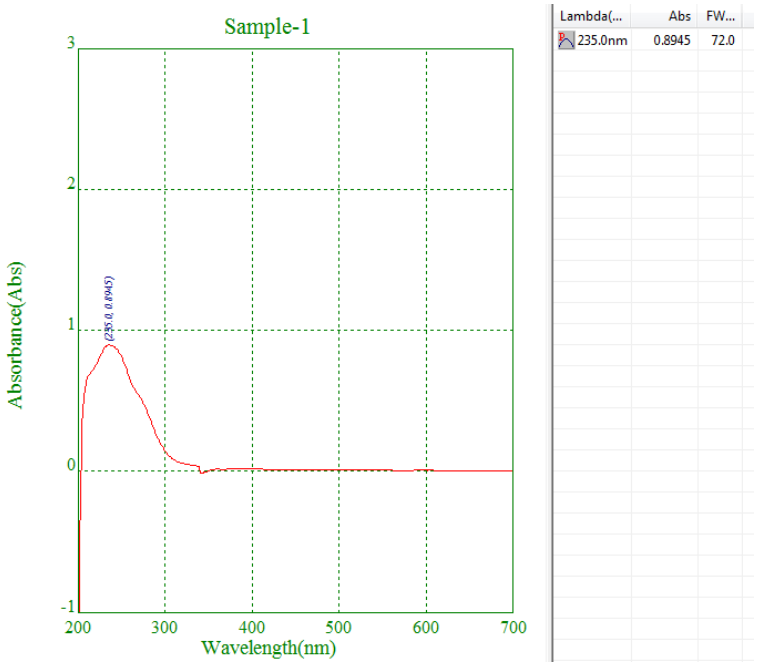

Fig. no 1 . UV Spectra of API

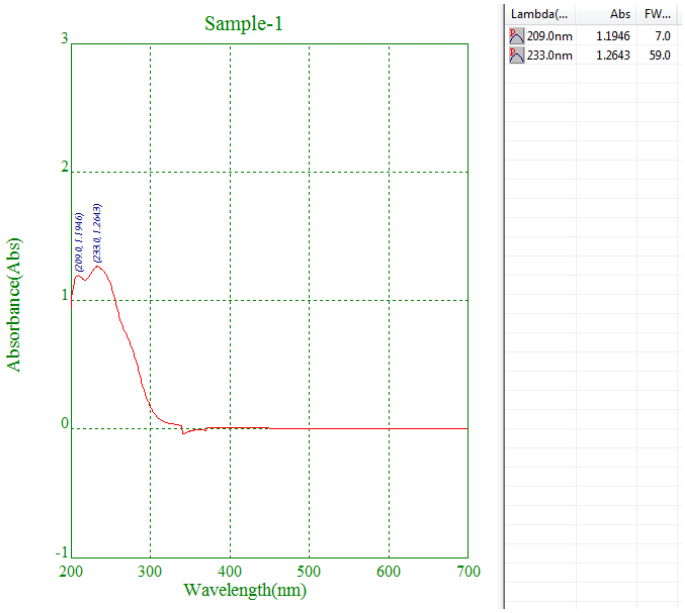

Fig. no 2. UV Spectra of Formulation

FTIR Characterizations of TiO_2 nanoparticles and drug-encapsulated TiO_2 nanoparticles

FTIR spectra of Enz–TiO_2-3 nanoparticles exhibited characteristic absorption bands at 2850–2970 cm^{-1} (C–H stretching), 1045–1300 cm^{-1} (C–O stretching), and 598–607 cm^{-1} (C–H bending), confirming the presence of functional groups and purity of the metal oxide along with the drug absorption band[12].

The FTIR spectrum of Enz–TiO_2-3 displayed peaks corresponding to both TiO_2 and ENZ, confirming drug encapsulation within the nanoparticles.

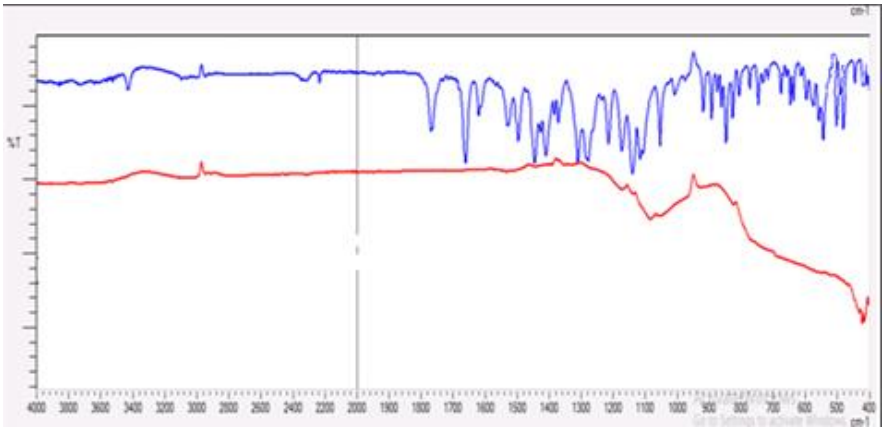

Fig. no 3. FTIR Spectra of Enz–TiO_2-3 and TiNp-3

X-Ray Diffraction (XRD) analysis

The distinctive TiO_2 peaks in the XRD patterns of TiNP-3 as well as in Enz-TiNP-3 matched reference data, confirming the nanoparticles crystalline structure [13]. The XRD pattern shows that the drug as well encapsulated in TiNp-3 nanoparticle without disturbing the crystalline structure.

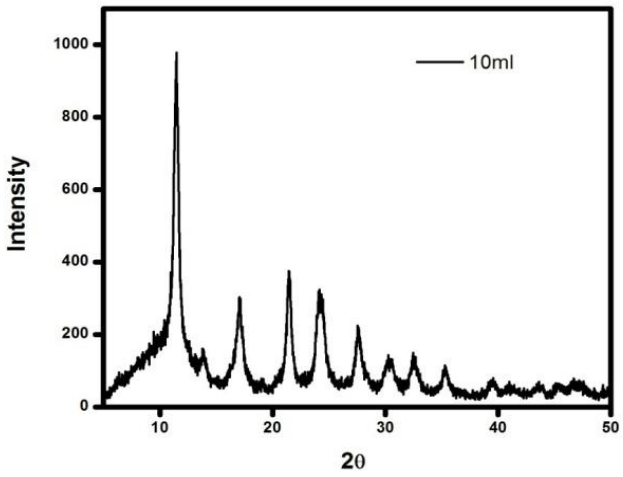

Fig. No.4. XRD pattern for TiNP-3

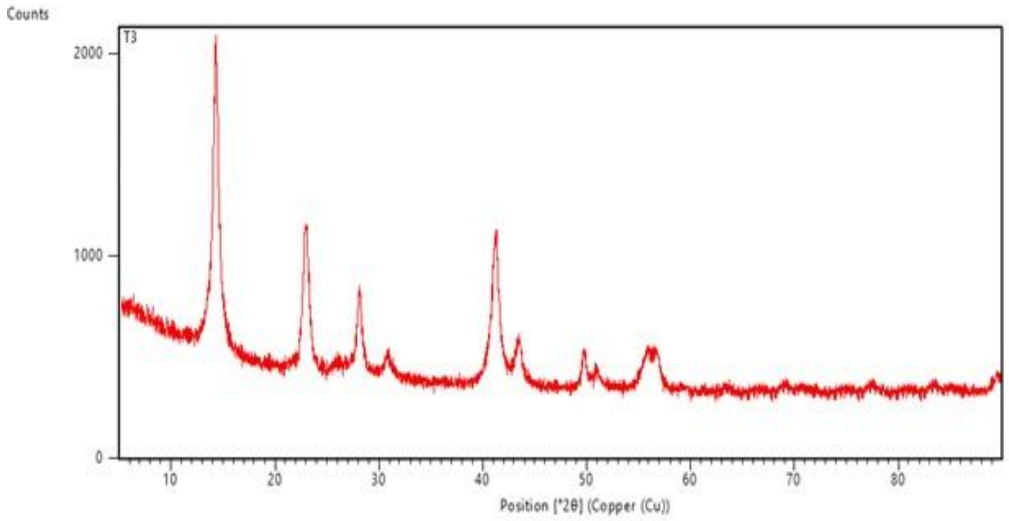

Fig. No.5. XRD pattern for EN-TiNP-3

Particle size and Zeta potential analysis

Titanium dioxide nanoparticles (TiNP-3) synthesized via hydrothermal method exhibited a spherical morphology with an average particle size of approximately 30 nm as confirmed by scanning electron microscopy (SEM) (Fig. 6&7). Upon loading with enzalutamide (Enz), the particle size increased to about 69.4 nm for the optimized formulation Enz-TiO_2 3 (Fig. 8). This size increase is attributed to the adsorption and encapsulation of drug molecules on the nanoparticle surface, possibly inducing slight aggregation. In general, nanoparticles could form a stable dispersion when the absolute value of zeta potential was above 30 mV due to the electric repulsion between particles [18]. The polydispersity index (PDI) of 0.287 and zeta potential of –11.2 mV suggest moderate particle uniformity and colloidal stability, respectively.

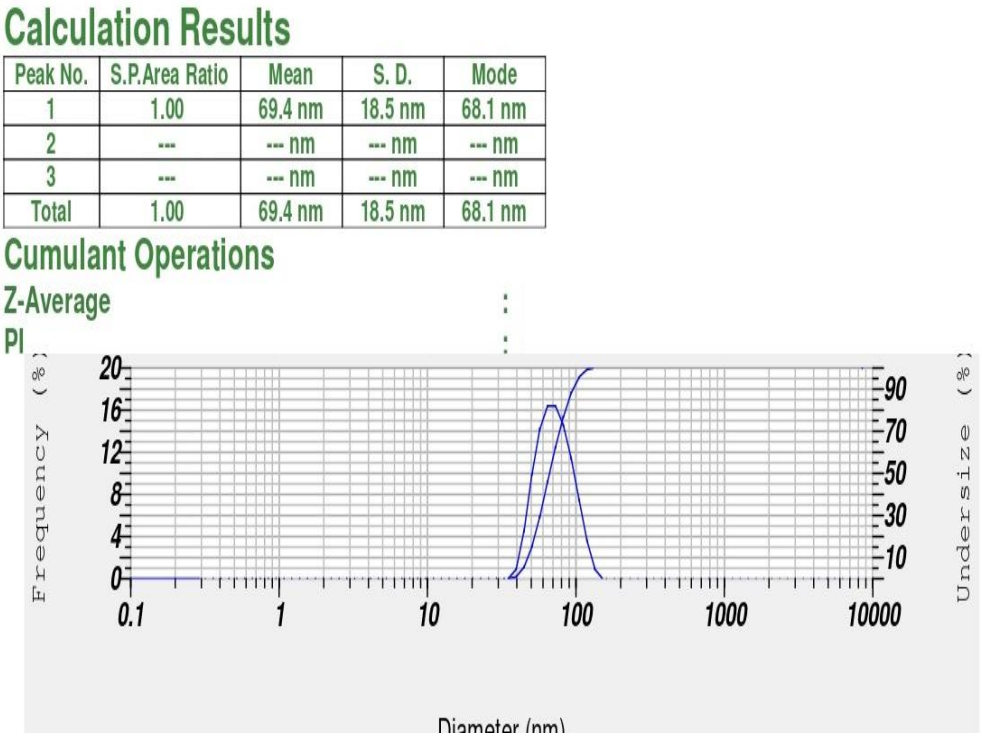

Fig. no.6 Particle size images of TiNP-3

SEM Analysis

SEM micrographs revealed spherical nanoparticles with smooth surfaces and uniform morphology. The average particle size of TiO_2 nanoparticles was approximately 30 nm (Fig. no.7 &8)

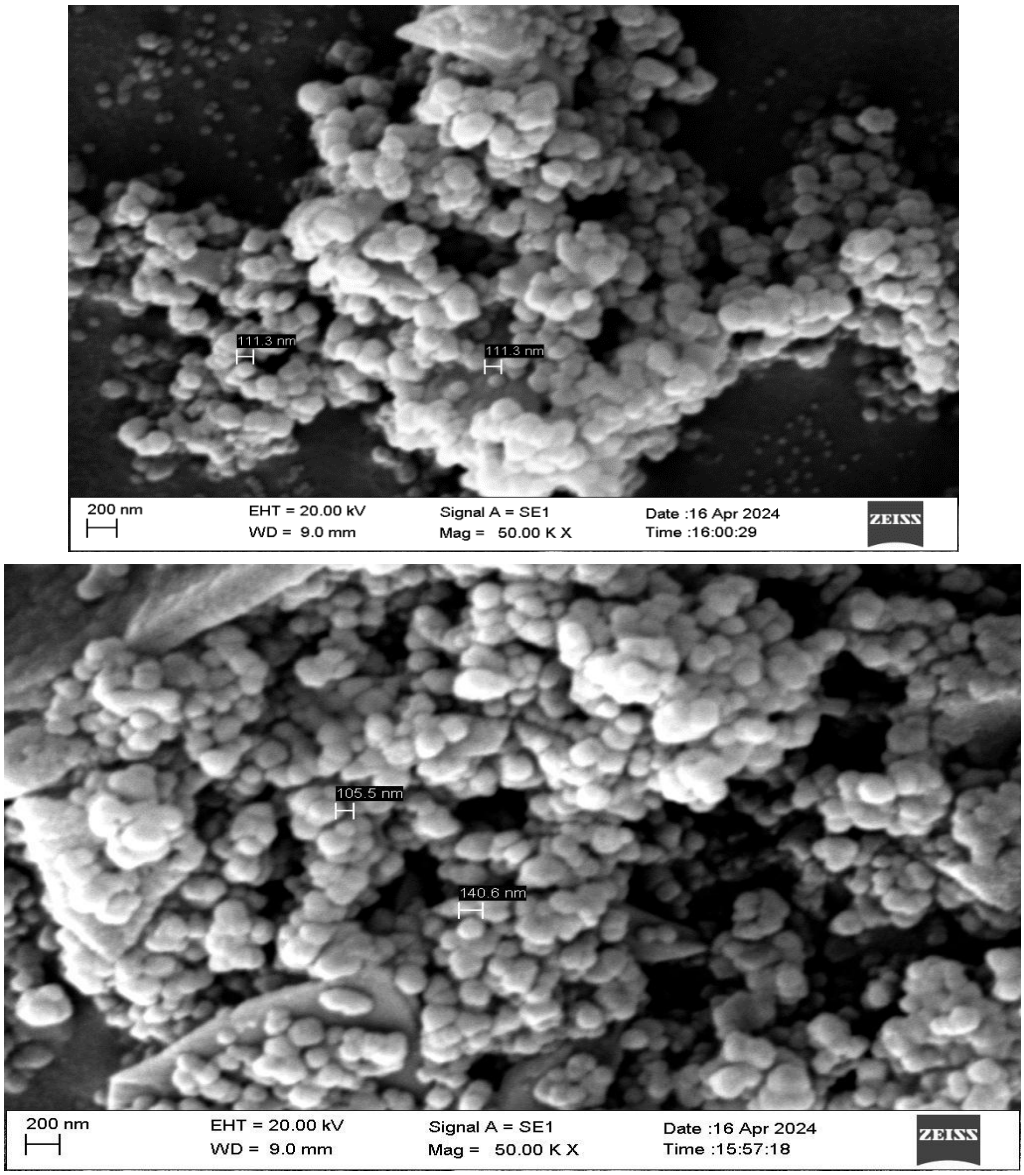

Fig. no.7 &8 SEM images of TiNP-3 and En- TiNP-3 showed that both are spherical in shape with a nano-metric size range

Drug loading and Entrapment efficiency

The drug loading was found to be in the range of 45 to 76.96% and entrapment efficiency (EE) values for the formulations ranged from 51.10% to 69.71% (Table 2). En–TiNp-3 exhibited the highest drug loading of (76.96 ± 1.36%), and EE (69.71 ± 1.00%).

Table.no. 2 Drug Loading (%) and variability of Enz-TiO_2 Nanoparticle formulations

Formulation	Average % Drug loaded	SD
Enz-TiO_2 1	64.10	1.88
Enz-TiO_2 2	58.57	1.46
Enz-TiO_2 3	76.96	1.36
Enz-TiO_2 4	68.30	1.80

Enz-TiO_2 5	45.97	1.16

Table. No. 3 Average Entrapment Efficiency and Variability of Enz-TiO_2 Formulations

Entrapment Calculation		
Formulation	**Average %EE**	**SD**
Enz-TiO_2 1	51.10	1.58
Enz-TiO_2 2	67.57	1.39
Enz-TiO_2 3	69.71	1.00
Enz-TiO_2 4	57.28	1.82
Enz-TiO_2 5	55.86	1.50

In vitro drug release

The in vitro release profile (pH 7.4) showed that Enz–TiNp-3 exhibited a sustained drug release over 48 h, with an initial burst release in the first hour approximately three times higher than free ENZ. The free drug displayed significantly faster release and lower cumulative release over the same period.(Fig No. 9)

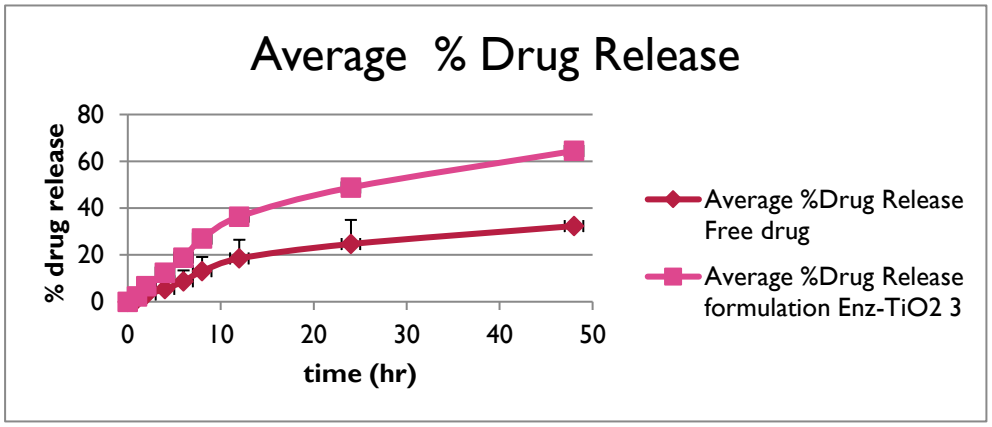

Fig. no.9 Cumulative in-vitro release profile of enzalutamide from Enz-TiO_2 3 compared to free enzalutamide over 24 hours

***In vitro* anticancer study by MTT assay**

Cytotoxicity study was done on PC-3 cells by MTT assay to express the influence of the new form of EnZ (loaded in TiO_2 nanoparticles) on induction of cell death. Data reveals that 42.26 % and 27.59% of cell viability of PC-3 cells was inhibited by 100 µM equivalent Ti-Np-3 and En-Ti-Np3 as compared to 71.74% of free drug. (Fig. No. 10 &11) These findings showed that En-Ti-Np3 significantly exhibits increase in apoptotic cells when compared with free drug.

En-Ti-Np3 exhibit IC_{50} of 49.28 µg/ml whereas TiNp3 and free drug etoposide exhibit IC_{50} of 72.27 and 169.67 µg/ml respectively. The finding concluded that titanium nanoparticles loaded drug demonstrated increased its bioavailability at lower doses, thereby increasing its therapeutic efficiency compared with free drug.

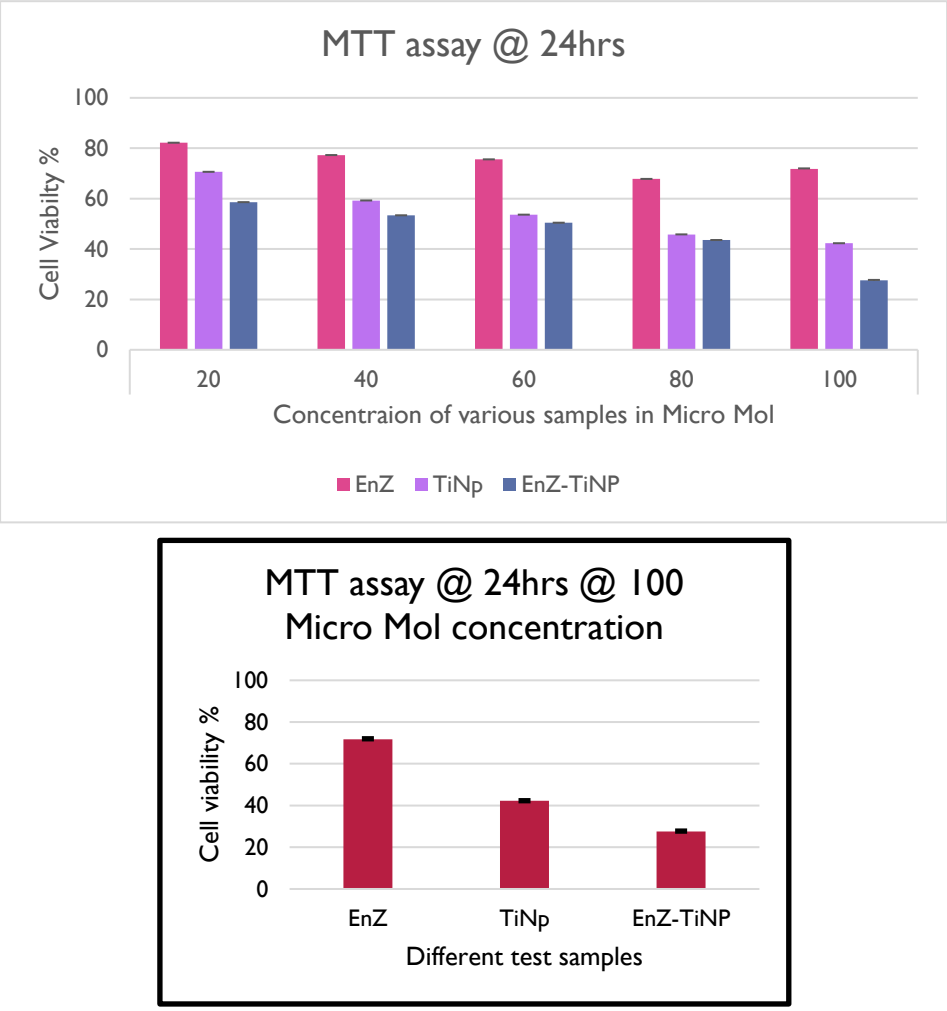

Fig. no.10 & 11 MTT Assay of EnZ, TiNP's & Enz-TiO_2 3 at 24 hours

4. DISCUSSION

In order to address the drug's low solubility and bioavailability problems, which are frequently encountered in prostate cancer therapy, the current study effectively produced and evaluated titanium dioxide (TiO_2) nanoparticles as carriers for enzalutamide. The spherical morphology and nanoscale size (~30 nm) of the TiO_2 nanoparticles produced by the hydrothermal technique are in line with other research showing the stability and biocompatibility of TiO_2 in drug-delivery platforms [7, 8, 16]. Upon drug loading, the observed increase in particle size to around 69.4 nm indicated efficient adsorption of enzalutamide molecules onto the nanoparticle surface. Given that it frequently corresponds with improved drug payload capacity and sustained-release features, this size growth is both anticipated and advantageous [10, 17]. In line with results from optimized nanoparticulate formulations, the comparatively narrow polydispersity index (PDI) suggests a homogeneous size distribution, which is essential for predictable pharmacokinetic behavior [18]. After loading, XRD examination verified that TiO_2's crystalline structure was retained, preserving its physicochemical integrity, which is essential for long-term stability and cell contact [9, 19]. The existence of enzalutamide in the formulation without any chemical change was further supported by FTIR spectra, indicating effective encapsulation as opposed to merely adsorption. Formulations differed in their drug loading and entrapment efficiency; Enz-TiO_2 3 had the greatest values (~77% drug loading; ~70% EE). This is consistent with earlier results showing enhanced loading efficiency in comparable nanocarrier systems [20] and represents the ideal drug–carrier ratio balance. For cancer treatment techniques that aim to deliver immediate therapeutic dosages followed by prolonged maintenance, the in vitro release profile showed a desired biphasic pattern, with an early burst release followed by sustained release over 48 hours. The improved intracellular delivery and therapeutic potential of this nanocarrier system are highlighted by the enhanced cytotoxic effects seen in prostate cancer cells treated with Enz-TiO_2 nanoparticles. These findings are consistent with evidence from nanomedicine approaches that improve cellular uptake, overcome drug resistance, and lessen off-target toxicity.

In conclusion, TiO_2 nanoparticles loaded with enzalutamide demonstrate promising physicochemical properties and biological efficacy, making them a potential platform for targeted and sustained delivery in prostate cancer therapy. Further

in vivo studies and surface modifications for active targeting could enhance their clinical applicability.

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