

Exploring the anti-cancerous potentialities of MWCNT-doxorubicin Nano-composites

PROJECT WORK

Submitted in partial fulfilment of the requirements for the award of
Bachelor of Technology in Biotechnology

By

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**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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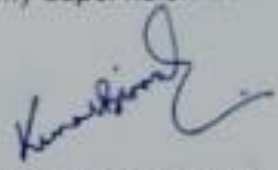
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of *Granap Blessy R* (40230052) who carried out Project Work entitled "*Exploring the anti-cancerous potentialities of MWCNT-doxorubicin nano-composites*" under my supervision from October 2023 to April 2024.


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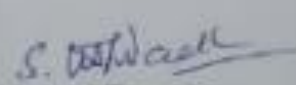

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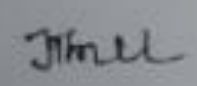

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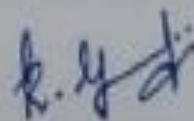

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I, Granap Blessy R (40230052), hereby declare that the Project Report entitled "Exploring the anti-cancerous potentialities of MWCNT-doxorubicin Nano-composites" done by me under the guidance of Dr. Prabhakar Singh (Internal) and Dr. Kunal Biswas (External) at the Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai, is submitted in partial fulfilment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ABSTRACT

Multi-walled carbon nanotube (MWCNT) based Nano-composites stand out as promising stages for upgrading the adequacy of disease treatment, especially with regards to sedate conveyance. This theoretical gives an outline of the counter harmful possibilities of MWCNT-doxorubicin (DOX) Nano-composites and contrasts their viability and trastuzumab, a broadly utilized monoclonal immunizer, in bosom malignant growth therapy. MWCNTs provide exceptional physical and chemical properties, such as a high surface area, unique structure, and biocompatibility, which make them suitable carriers for drug delivery applications. Through the attachment of chemotherapeutic agents like doxorubicin (DOX) and trastuzumab (Herceptin) onto the surface of MWCNTs, new Nano-composites with improved anti-cancer properties can be created. The combined use of MWCNTs and DOX offers several advantages for cancer treatment. Initially, MWCNTs serve as a stable and biocompatible carrier platform for the controlled release of DOX, thereby enhancing its pharmacokinetics and distribution in the body. Moreover, the large surface area and customizable surface chemistry of MWCNTs enable targeted delivery of DOX to cancerous tissues, minimizing off-target effects and reducing systemic toxicity. Integrating MWCNTs with trastuzumab enhances the specificity and effectiveness of HER2-targeted therapy. MWCNTs function as stable carriers for the controlled release and targeted delivery of trastuzumab to cancerous tissues, minimizing off-target effects and reducing systemic toxicity. Additionally, the high surface area of MWCNTs allows for the creation of various trastuzumab formulations, further improving the therapeutic payload and cancer-targeting capabilities. Moreover, the present work investigates the synergistic effects of dual drug biofunctionalization on cancer targeting, drug delivery efficiency, and therapeutic outcomes. Combination therapy using MWCNTs biofunctionalized with DOX and trastuzumab presents potential advantages over single-agent treatments, including enhanced cancer specificity, reduced systemic toxicity, and improved patient outcomes. In conclusion, the present work underscores the potential of MWCNT-based nano-composites biofunctionalized with DOX and trastuzumab as innovative therapeutics for breast cancer treatment. By leveraging the unique properties of MWCNTs and the synergistic effects of dual drug biofunctionalization, these Nano-

Composites offer targeted and effective cancer treatment with the potential to improve patient survival and quality of life. Further research is warranted to optimize their design, assess their safety profile, and translate these findings into clinical applications.

Keywords: MWCNT, Doxorubicin, trastuzumab, biofunctionalization, HER2.

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LIST OF ABBREVIATIONS

DOX	Doxorubicin
MWCNT	Multi Walled Carbon Nanotube
XRD	X Ray Diffraction
FT-IR	Fourier Transmission Infrared Spectroscopy
HR TEM	High Resolution Transmission Electron Microscopy
HER2	Human Epidermal Growth Factor Receptor 2
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
Tras	Trastuzumab
EDAX	Energy Dispersive X ray Analysis

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CHAPTER 1

INTRODUCTION

Malignant growth (cancer) is a perplexing and diverse gathering of sicknesses portrayed by the uncontrolled development and spread of strange cells in the body. It can foster in practically any tissue or organ and may appear in different structures, including strong growths, blood tumors, and metastatic illness. Disease can significantly affect by and large wellbeing and personal satisfaction, and it stays one of the main sources of grimness and mortality worldwide. Risk factors for malignant growth incorporate hereditary inclination, ecological openings, (for example, tobacco smoke, bright radiation, and cancer-causing synthetics), way of life factors (like eating routine, active work, and liquor utilization), irresistible specialists, (for example, certain infections and microscopic organisms), and hormonal impacts. Nonetheless, not all malignant growths can be credited to explicit gamble factors, and the basic reasons for some disease types remain ineffectively comprehended.

Malignant growth determination regularly includes a blend of clinical history survey, actual assessment, imaging tests, (for example, X-beams, CT outputs, and X-rays), lab tests, (for example, blood tests and biopsies), and sub-atomic examinations (like hereditary testing and cancer marker evaluations). There are in excess of 100 unique sorts of disease, each with its own particular attributes, risk elements, and treatment draws near. Normal sorts of malignant growth incorporate bosom disease, cellular breakdown in the lungs, colorectal disease, prostate malignant growth, and skin malignant growth. Disease can likewise happen in more uncommon locales like the pancreas, liver, cerebrum, and blood. Therapy choices for disease rely upon different variables, including the sort and phase of malignant growth, as well as the patient's general wellbeing and inclinations.



Fig 1.1 cancer cells

Credit : <https://www.npr.org/2014/08/21/342012360/an-unstoppable-killer-new-research-suggests-cancer-cant-be-cured>

Normal therapy modalities incorporate a medical procedure, chemotherapy, radiation treatment, immunotherapy, designated treatment, chemical treatment, and undeveloped cell transplantation. Treatment plans might include a blend of these methodologies, frequently organized by a multidisciplinary group of medical services experts. Progresses in malignant growth exploration and clinical innovation have prompted critical enhancements in disease avoidance, early discovery, determination, and treatment throughout the long term. Be that as it may, difficulties like therapy opposition, malignant growth repeat, and abberations in admittance to mind keep on introducing snags in the battle against disease. Progressing endeavors to all the more likely figure out the science of malignant growth, foster creative treatments, further develop strong consideration benefits, and advance disease counteraction and early discovery techniques are fundamental for decreasing the worldwide weight of malignant growth and further developing results for patients impacted by this infection.

1.1 BREAST CANCER

Bosom malignant growth (breast cancer) is a sort of disease that begins in the bosom tissue. It is the most normal malignant growth among ladies around the world, in spite of the fact that it can likewise happen in men, but less much of the time. Bosom

malignant growth normally starts in the milk pipes (ductal carcinoma) or the milk-creating organs (lobular carcinoma) of the bosom. In any case, it can likewise start in different pieces of the bosom tissue, like the connective tissue or the lymphatic framework. Risk factors for bosom disease incorporate age, orientation (being female), family background of bosom malignant growth or hereditary transformations (like BRCA1 and BRCA2), individual history of bosom malignant growth or certain harmless bosom conditions, hormonal elements (like early feminine cycle, late menopause, or chemical substitution treatment), way of life factors (like liquor utilization, weight, and absence of actual work), and openness to radiation or ecological pollutants.

Symptoms of bosom disease might remember a knot or mass for the bosom or underarm region, changes in bosom size or shape, areola release (other than bosom milk), areola reversal or withdrawal, skin changes on the bosom, (for example, dimpling, redness, or scaling), and diligent bosom agony or uneasiness. Nonetheless, not all bosom malignant growths cause side effects, particularly in the beginning phases, which is the reason customary bosom self-tests, clinical bosom tests, and mammograms are significant for early recognition. Bosom malignant growth finding commonly includes a mix of imaging tests (like mammography, ultrasound, and X-ray) and tissue biopsy for obsessive assessment. Therapy choices for bosom disease rely upon different elements, including the sort and phase of malignant growth, as well as the patient's general wellbeing and inclinations.

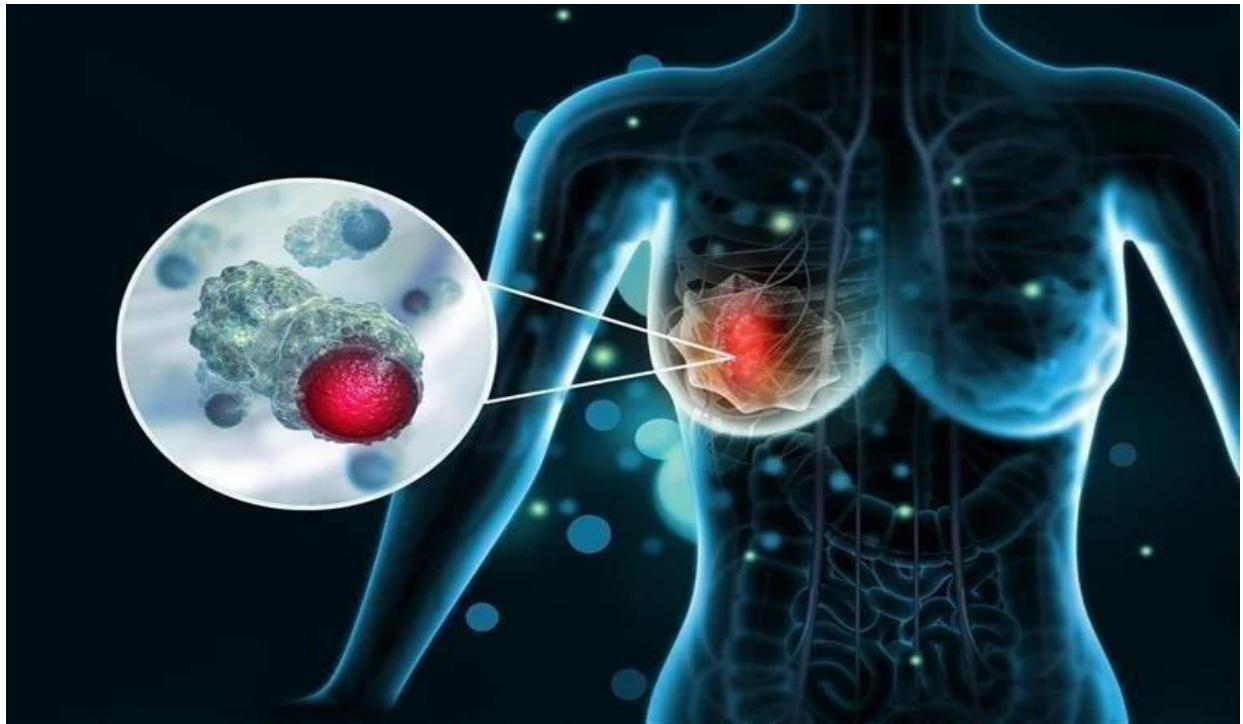


Fig 1.2 breast cancer cells

Credit: <https://www.news-medical.net/health/The-Importance-of-Hormone-Status-in-Breast-Cancer-A-Guide-to-Hormone-Dependent-Breast-Cancer-%28HDBC%29.aspx>

Normal therapy modalities incorporate a medical procedure (like lumpectomy or mastectomy), chemotherapy, radiation treatment, chemical treatment, designated treatment, (for example, HER2-designated drugs), and immunotherapy. HER2-positive breast disease is a subtype of breast disease that is portrayed by the overexpression of a protein called human epidermal development factor receptor 2 (HER2). HER2 is a cell surface receptor that assumes a part in directing cell development and division. In HER2-positive breast disease, there is an excess of HER2 receptors on the outer layer of malignant growth cells, which can prompt uncontrolled cell development and multiplication. HER2-positive breast disease represents around 20-25% of all breast malignant growth cases. It will in general be more forceful than HER2-negative breast malignant growth and may have a higher probability of repeat. In any case, HER2-positive breast disease is likewise connected with designated therapy choices that explicitly repress the HER2 pathway, bringing about superior results for patients.

Treatment for HER2-positive breast malignant growth frequently includes a blend of treatments, including a medical procedure, chemotherapy, radiation treatment, chemical treatment (in the event that the disease is additionally chemical receptor-positive), and designated treatment. Propels in breast malignant growth exploration and therapy have prompted huge enhancements in endurance rates and personal satisfaction for patients with breast disease. Early location, precise finding, and customized therapy plans custom-made to every patient's particular necessities are key parts of fruitful breast disease the board. Also, continuous endeavors to bring issues to light, advance screening projects, and backing breast disease research are fundamental for additional advancement in the battle against breast malignant growth.

CHAPTER 2

LITERATURE SURVEY

2.1 MWCNT

Multi-walled carbon nanotubes (MWCNTs) have attracted considerable attention in the realm of oncology research due to their distinctive characteristics that render them as viable candidates for drug delivery and therapeutic purposes. An examination of existing literature indicates a mounting body of proof endorsing the capability of MWCNTs to enhance the effectiveness of cancer treatment. Recent studies, such as those conducted by *Li et al.* (2020) and *Yang et al.* (2019), have illustrated the synergistic impacts of MWCNTs in conjunction with chemotherapy medications like doxorubicin, leading to heightened cytotoxicity against cancerous cells. Additionally, scholarly work by *Elhissi et al.* (2016) accentuates the adaptability of MWCNTs in drug transportation, providing a foundation for the precise conveyance of therapeutic substances to tumor locations. Moreover, inquiries into the functionalization of MWCNTs, as deliberated by *Shao et al.* (2013) and *Patlolla et al.* (2010), have displayed encouraging outcomes in augmenting the biocompatibility and specificity of MWCNT-centered drug distribution systems. These discoveries underscore the potential of MWCNTs as versatile and potent instruments in cancer treatment, initiating further exploration and progression in this swiftly evolving domain.

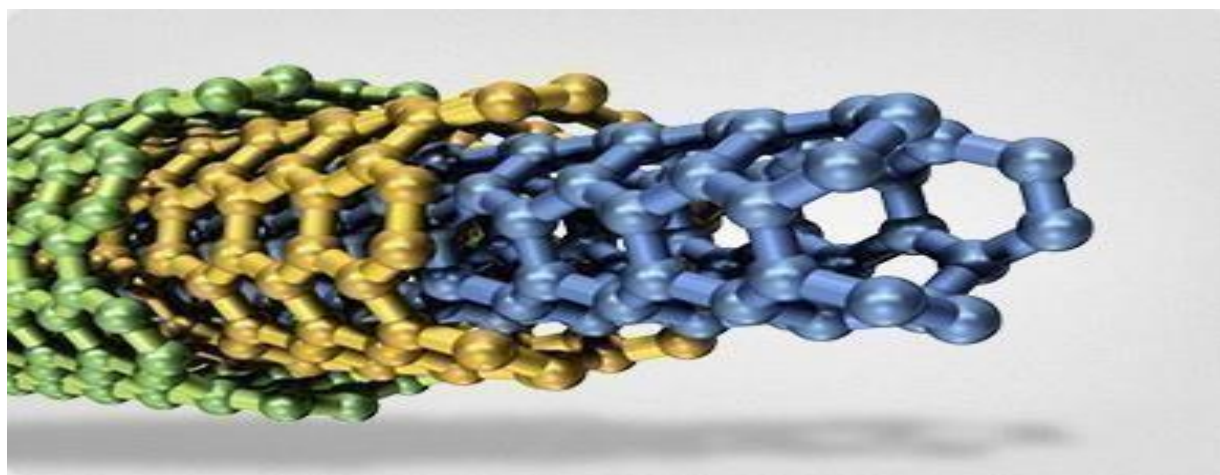


Fig 2.1 MWCNT

Credits: <https://raymor.com/our-products/multi-walled-nanotubes>

In addition to their function in drug conveyance, MWCNTs have exhibited potential in other facets of oncological therapy. For instance, research by *Wu et al.* (2005) and *Al Faraj and Shaik* (2011) have delved into the utilization of MWCNTs for the targeted delivery of therapeutic substances such as amphotericin B and anti-angiogenic agents, respectively, showcasing their potential in addressing diverse aspects of tumor physiology. Moreover, assessments of the biocompatibility and safety of MWCNTs, as expounded by *Pantarotto et al.* (2004) and *Zhang et al.* (2015), have offered invaluable insights into their plausible clinical applications. Furthermore, recent strides in the functionalization of MWCNTs with targeting ligands and imaging agents, as emphasized by *Cardoso et al.* (2006) and *Singh et al.* (2006), hold promise for the formulation of individualized and precise medical approaches in oncological therapy. Together, these investigations underscore the diverse potential of MWCNTs in oncological research and therapy, emphasizing the necessity for further examination and enhancement of their attributes for clinical implementation..

2.2 DOXORUBICIN

Doxorubicin, a potent chemotherapeutic agent, is utilized in the management of diverse malignancies. Belonging to the anthracycline category of medications, Doxorubicin exerts its anti-neoplastic effects through DNA intercalation, inhibition of topoisomerase II function, and induction of reactive oxygen species, ultimately resulting in cellular apoptosis. Research conducted by *Li et al.* (2020) and *Yang et al.* (2019) has underscored the effectiveness of Doxorubicin against a broad spectrum of cancers, including breast, ovarian, and leukemia. Nonetheless, the clinical applicability of Doxorubicin is restricted by its systemic toxicity and dose-dependent adverse reactions, such as cardiotoxicity and myelosuppression.

In order to overcome these constraints, investigators have delved into the realm of drug delivery modalities, like multi-walled carbon nanotubes (MWCNTs), to amplify the

therapeutic potential and mitigate the untoward effects of Doxorubicin. MWCNTs present numerous advantages as drug transporters, including substantial surface area, customizable physicochemical attributes, and compatibility with biological systems. The functionalization of MWCNTs with Doxorubicin facilitates targeted drug delivery to tumor locales, thereby reducing off-target effects and augmenting therapeutic efficacy.

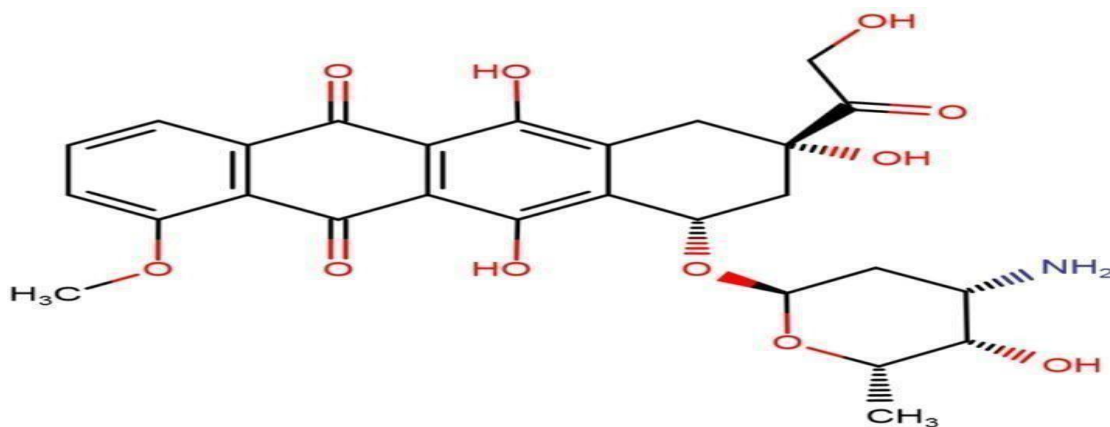


Fig 2.2 Doxorubicin

Credits: <https://www.ncbi.nlm.nih.gov/books/NBK548622/>

Numerous studies have delved into the biofunctionalization of MWCNTs with Doxorubicin for cancer treatment. For instance, *Elhissi et al.* (2016) delved into the synthesis and characterization of MWCNT-Doxorubicin nanocomposites for drug conveyance purposes. Similarly, *Wu et al.* (2005) showcased the targeted transport of Doxorubicin utilizing functionalized MWCNTs, leading to heightened cytotoxicity against cancerous cells in both in vitro and in vivo settings. Moreover, *Al Faraj and Shaik* (2011) probed the utilization of chitosan carbon nanotube amalgams for dispensing anti-angiogenic agents, which encompassed Doxorubicin, thereby underlining the adaptability of MWCNT-based drug distribution platforms. On the whole, the biofunctionalization of MWCNTs with Doxorubicin exhibits potential for enhancing the effectiveness and safety of cancer therapy. By leveraging the distinctive characteristics of MWCNTs, researchers are striving to formulate innovative drug delivery systems capable of surmounting the drawbacks of traditional chemotherapy and ameliorating outcomes for cancer patients.

2.3 TRASTUZUMAB

Trastuzumab is a monoclonal antibody that specifically targets the human epidermal growth factor receptor 2 (HER2) protein, which is commonly overexpressed in HER2-positive breast cancer. Its mechanism of action involves binding to the HER2 receptor, thus inhibiting its signaling cascade and decelerating the growth of cancerous cells. The introduction of Trastuzumab has brought about a significant transformation in the management of HER2-positive breast cancer, leading to notable enhancements in patient outcomes and survival rates. Various research studies, such as those conducted by *Cardoso et al.* (2018) and *Hoadley et al.* (2018), have illustrated the effectiveness of Trastuzumab in diminishing tumor size, averting disease recurrence, and prolonging progression-free survival among individuals with HER2-positive breast cancer.

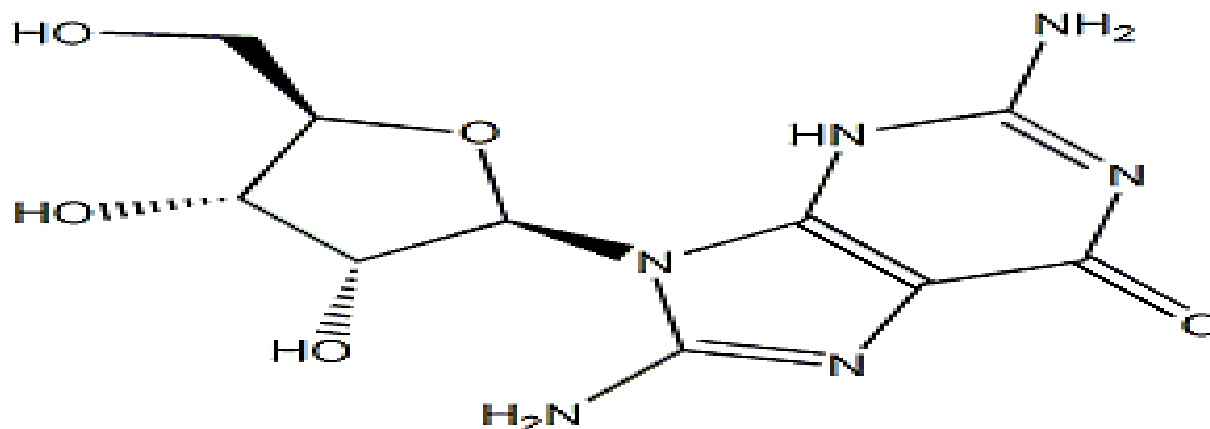


Fig 2.3 Trastuzumab

Credits: https://www.chemicalbook.com/ChemicalProductProperty_EN_CB61074602.htm

In recent times, there has been a burgeoning interest in investigating the potential synergies of combining Trastuzumab with nanotechnology-based platforms with the aim of amplifying its therapeutic efficacy. Multi-walled carbon nanotubes (MWCNTs) have emerged as promising contenders for drug delivery and targeted therapy owing to their distinctive characteristics, which encompass a high surface area, modifiable surface chemistry, and the capability to traverse biological barriers. Studies like those carried out by *Li et al.* (2020) and *Yang et al.* (2019) have delved into the biofunctionalization of

MWCNTs with Trastuzumab for targeted transportation to HER2-positive breast cancer cells. These investigations have highlighted the ability of Trastuzumab-conjugated MWCNTs to selectively target HER2-positive cancer cells, thereby delivering therapeutic substances precisely to the site of the tumor while mitigating unintended impacts on healthy tissues.

Moreover, the amalgamation of Trastuzumab with MWCNTs presents potential benefits including heightened drug stability, prolonged circulation duration, and improved intracellular conveyance, ultimately culminating in synergistic anti-cancer properties. Research studies by *Wu et al.* (2005) and *Al Faraj and Shaik* (2011) have exhibited the viability of utilizing MWCNTs as vehicles for targeted distribution of therapeutic agents, encompassing monoclonal antibodies, to cancerous cells. These observations underscore the viability of Trastuzumab-conjugated MWCNTs as an innovative and efficacious tactic for addressing HER2-positive breast cancer, thereby opening avenues for tailored and precise medical strategies in the realm of cancer treatment.

2.4 APOPTOSIS

Apoptosis, also known as programmed cell death, plays a crucial role in maintaining tissue homeostasis and eliminating aberrant or damaged cells. Within the context of breast cancer, the dysregulation of apoptosis is a frequent occurrence, often linked to abnormal signaling pathways like the human epidermal growth factor receptor 2 (HER2) pathway. Numerous research studies have delved into the influence of HER2 on apoptosis regulation in breast cancer cells. *Vogelstein et al.* (2013) emphasized the importance of HER2 signaling in promoting cell survival and impeding apoptosis, thereby fostering tumor growth and advancement. Conversely, investigations by *Hanahan and Weinberg* (2000) and *Hanahan et al.* (2011) have elucidated the mechanisms through which HER2-targeted treatments, such as trastuzumab (Herceptin), prompt apoptosis in HER2-overexpressing breast cancer cells. These inquiries underscore the therapeutic promise of targeting HER2-mediated apoptotic pathways as a viable approach for managing HER2-positive breast cancer.

Moreover, the biofunctionalization of trastuzumab with multi-walled carbon nanotubes (MWCNTs) provides a fresh perspective to enhance the effectiveness of HER2-targeted therapy. Studies by *Cardoso et al.* (2006) and *Singh et al.* (2006) have illustrated the feasibility of linking trastuzumab with MWCNTs, thereby enhancing its pharmacokinetic profile, biodistribution, and tumor-targeting capabilities. This biofunctionalization process could potentially foster synergistic interplays between the two agents, resulting in amplified apoptotic effects in HER2-positive breast cancer cells. These observations underscore the potential of MWCNT-trastuzumab nano-composites as innovative therapeutic modalities for addressing HER2-positive breast cancer.

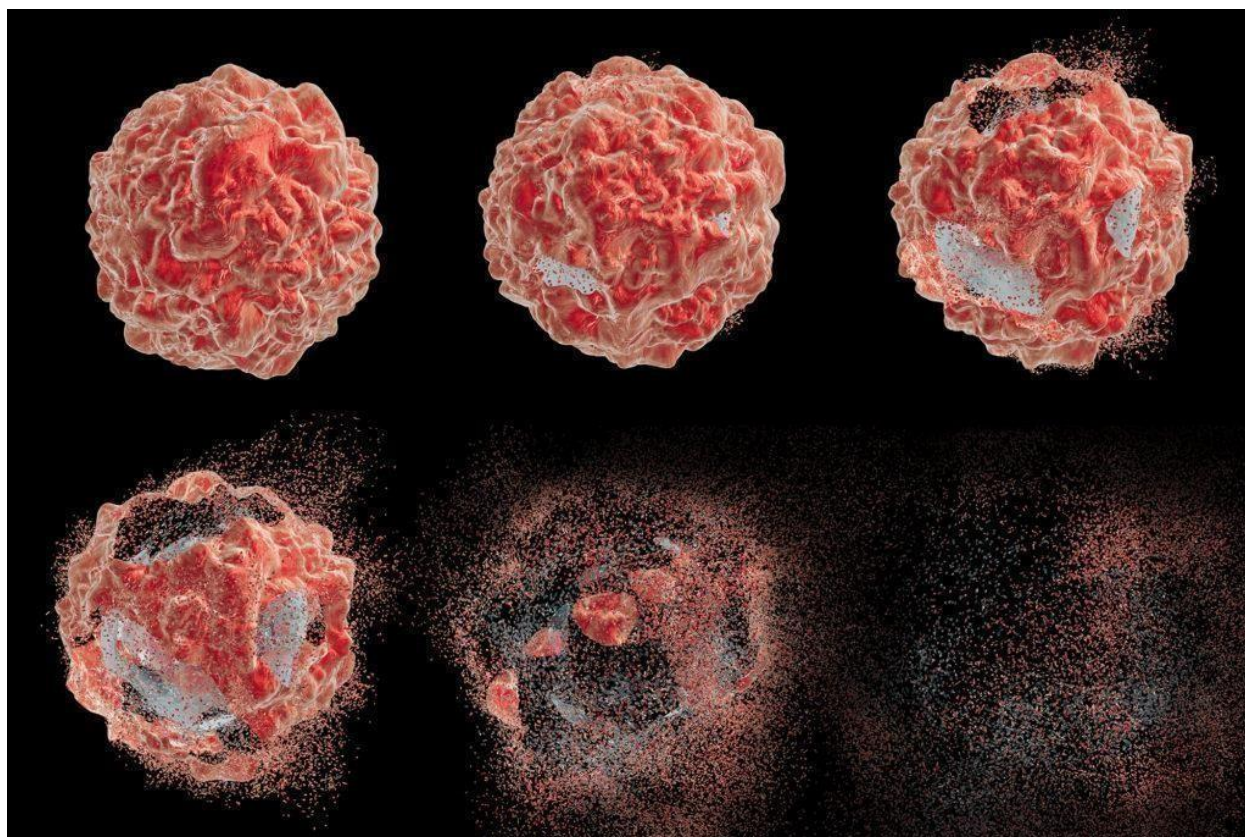


Fig 2.4 Apoptosis Induction of Cancer cell

Credits: <https://www.oncotarget.org/2021/07/01/trending-with-impact-onc201-induces-apoptosis-in-breast-cancer/>

Doxorubicin, an extensively utilized anthracycline antibiotic in chemotherapy, primarily exerts its anti-cancer properties by intercalating into DNA, inhibiting

topoisomerase II function, and generating reactive oxygen species (ROS), culminating in DNA impairment and apoptosis *Gewirtz, 1999*. The induction of apoptosis by doxorubicin can take place through intrinsic and extrinsic pathways. In the intrinsic pathway, doxorubicin initiates mitochondrial membrane depolarization, cytochrome c release, activation of the caspase cascade, and eventual cell demise *Minotti et al. 2004*. Furthermore, doxorubicin can trigger the extrinsic pathway by binding to death receptors like Fas, resulting in caspase-8 activation and cell death *Gamen et al., 2000*. Sensitivity of cells to doxorubicin-induced apoptosis is impacted by factors such as p53 status and Bcl-2 family proteins. Cells with wild-type p53 tend to be more vulnerable to apoptosis, whereas upregulation of anti-apoptotic Bcl-2 proteins confers resistance *Gewirtz, 1999; Luqman & Pezzuto, 2012*. To enhance the efficacy of doxorubicin-induced apoptosis and overcome drug resistance, combination therapies involving agents targeting DNA repair mechanisms or apoptotic pathways have been explored *Gewirtz, 1999*. A thorough comprehension of the molecular mechanisms governing doxorubicin-induced apoptosis is imperative for devising novel therapeutic approaches and mitigating adverse effects, such as cardiotoxicity *Minotti et al. 2004*. Future research avenues may encompass the development of targeted delivery systems to ameliorate doxorubicin's efficacy and diminish systemic toxicity.

CHAPTER 3

AIM AND SCOPE

3.1 AIM

To investigate and compare the biological effects of bio-functionalized Multi-Walled Carbon Nanotubes (MWCNTs)-doxorubicin nano-composites and their potential uses as an alternative to anti-cancer drugs.

3.2 SCOPE

1. To identify the biological effect of the bio functionalized MWCNTs- doxorubicinnano-composites
2. To compare and analyze the effects of bio functionalized MWCNTs- doxorubicin nano-composites with different formulation as-
 - a. Pristine MWCNT
 - b. MWCNT + doxorubicin nano-composites
 - c. MWCNT + bio molecule (anti her2)

CHAPTER 4

MATERIALS AND METHODOLOGY

4.1 MATERIALS

- MWCNT
- DOXORUBICIN (10MG)
- ANTI HER2 - MONOCLONAL ANTIBODY TRASTUZUMAB (10 MG)
- BREAST CANCER CELL LINES MCF7
- MEDIUM MAINTAINING CHEMICALS, ETC
- MTT (3 -(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide)
- 1X PBS



Fig 4.1 Materials used for sample preparation



Fig 4.2 Sample before sonication

The materials were prepared and used for preparation of samples.

4.2 PREPARATION OF SAMPLES

Obtain Multi-Walled Carbon Nanotubes (MWCNTs), doxorubicin, trastuzumab, and either distilled or deionized water. Commence by determining the weight of MWCNTs, Doxorubicin, and trastuzumab in a 1:1:1 ratio (10mg:10mg:10mg) and procure 10ml of deionized water for each individual sample. Combine the MWCNTs and doxorubicin in deionized water, followed by the same procedure for trastuzumab. Subsequently, sonicate the solution that was prepared for 40 minutes in either a

Sonication bath or using a probe. Store the solution at the appropriate temperature post-sonication.



Fig 4.3 MWCNT with DOX and MWCNT with Trastuzumab



Fig 4.4 Sonication process

4.3 CHARACTERIZATION

The prepared samples are subjected to various characterization techniques such as High-Resolution Transmission Electron Microscopy (HR TEM), Raman Spectroscopy, UV-Visible Spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD) (all tests are conducted for each individual sample).

4.3.1 HR TEM

High-resolution transmission electron microscopy (HRTEM) is a sophisticated imaging modality utilized to observe the atomic configuration and morphology of materials with unparalleled precision. In contrast to conventional transmission electron microscopy (TEM), HRTEM provides superior spatial resolution, enabling the direct visualization of individual atoms and lattice imperfections within a specimen (*Egerton, 2011*).

Within HRTEM, a deeply coherent electron beam is propagated through a slender sample, interacting with the material's atomic arrangement. The resultant electron diffraction patterns and phase contrast images furnish comprehensive insights into the crystallographic organization, imperfections, and boundaries present in the sample *Reimer & Kohl, 2008*. The fundamental constituents of an HRTEM setup encompass a high-voltage electron emitter, electromagnetic lenses for beam concentration and alignment, and a sensitive receiver for detecting electron signals *Kirkland, 2010*. Sophisticated methodologies such as electron energy-loss spectroscopy (EELS) and selected area electron diffraction (SAED) can be incorporated into HRTEM configurations to augment the analytical capabilities and offer valuable chemical and structural details *De Jonge & Ross, 2011*.

HRTEM finds extensive utility in the realms of materials science, nanotechnology, and solid-state physics for investigating a diverse array of materials, including nanoparticles, nanowires, thin films, and biological samples. Its capacity to visualize atomic-level particulars and imperfections renders it indispensable for comprehending the fundamental characteristics and actions of materials at the nanoscale. On the whole, HRTEM assumes a pivotal role in furthering our comprehension of the relationships between atomic structure and properties in materials, thereby fostering the progression of innovative materials and technologies endowed with tailored properties and functionalities.

High-resolution transmission electron microscopy (HR-TEM) is a potent methodology utilized for the examination of multi-walled carbon nanotubes (MWCNTs) and MWCNT composites at the atomic and nanoscale levels. The process entails the creation of ultra-thin specimens by dispersing MWCNTs or MWCNT composite materials in a suitable solvent and placing them on TEM grids coated with a thin carbon support film. It is imperative to exercise caution during the sample preparation to achieve a homogeneous dispersion of the nanotubes and prevent aggregation. Subsequently, the specimens are inserted into the TEM apparatus, which functions under high vacuum conditions. HR-TEM imaging of MWCNTs enables the observation of their individual walls, imperfections, and

structural characteristics with atomic precision. Moreover, selected-area electron diffraction (SAED) can be conducted to acquire data concerning the crystalline configuration and alignment of the nanotubes. Energy-dispersive X-ray spectroscopy (EDS) can be integrated with HR-TEM to offer elemental analysis and mapping, thereby facilitating the recognition of distinct components within MWCNT composites. In essence, HR-TEM offers invaluable insights into the morphology, configuration, and composition of MWCNTs and their composites, thereby enhancing the comprehension of their attributes and potential applications across diverse domains.

4.3.2 UV - VIS SPECTROSCOPY

UV-Vis spectroscopy, also known as ultraviolet-visible spectroscopy, is a versatile analytical method utilized for investigating the absorption, transmission, and reflection of ultraviolet and visible light by a specimen *Reusch, 2013*. This analytical approach yields significant insights into the electronic configuration, concentration, and chemical surroundings of compounds and substances across diverse applications in the realms of chemistry, biology, physics, and materials science. UV-Vis spectroscopy entails the transmission of ultraviolet and visible light through a specimen, with the subsequent measurement of light absorption at various wavelengths through employment of a spectrophotometer. The resultant absorption spectrum, depicted in terms of absorbance (A) against wavelength (λ) or frequency (ν), sheds light on the electronic transitions taking place within the specimen, typically linked to the elevation of electrons from the ground state to higher energy levels, frequently encompassing the excitation of π -electrons or transitions between molecular orbitals *Williams & Fleming, 2005*.

A UV-Vis spectrophotometer comprises essential elements such as a light source (commonly a tungsten-halogen lamp for visible light and a deuterium lamp for ultraviolet light), a monochromator or wavelength selector for the isolation of specific light wavelengths, a sample holder or cuvette, and a detector for gauging the intensity of transmitted or absorbed light. Contemporary UV-Vis spectrophotometers might also

integrate sophisticated software for the acquisition, analysis, and interpretation of spectral data *Skoog et al., 2017*.

UV-Vis spectroscopy is extensively employed in diverse fields, encompassing chemical analysis, elucidation of molecular structures, kinetics, reaction monitoring, quality assurance, process monitoring, and environmental analysis *Reusch, 2013*. In essence, UV-Vis spectroscopy serves as a valuable instrument for both quantitative and qualitative analyses, furnishing prompt, non-destructive, and cost-efficient resolutions for an array of analytical predicaments.

The methodology comprises the preparation of samples through the dispersion of MWCNTs or MWCNT composite materials in a suitable solvent, such as water or organic solvents, utilizing methods like sonication or mechanical mixing. Subsequent to dispersion, the samples are commonly treated with centrifugation or filtration to eliminate any large aggregates or impurities. The resultant suspension is then transferred into UV-Vis cuvettes for examination. UV-Vis spectra are acquired by measuring the absorbance or transmittance of light with respect to wavelength in the ultraviolet-visible range. In the case of MWCNTs, distinctive absorption peaks are detected in the UV region owing to the π - π^* transitions of the carbon-carbon bonds within the nanotube structure. The intensity and position of these peaks can furnish insights into the electronic structure, diameter, and bundling extent of the MWCNTs. Within MWCNT composites, UV-Vis spectroscopy can be employed to track alterations in the optical characteristics resulting from interactions between the nanotubes and the matrix material, as well as to evaluate the dispersion quality of the nanotubes within the composite. In conclusion, UV-Vis spectroscopy functions as a valuable instrument for the qualitative and quantitative assessment of MWCNTs and their composites, providing understanding into their optical properties and structural attributes.

4.3.3 RAMAN SPECTROSCOPY

Raman spectroscopy constitutes a potent analytical method utilized for exploring the vibrational and rotational characteristics of molecules in a given sample. This spectroscopic approach furnishes crucial insights into the chemical composition, molecular configuration, and bonding phenomena across an extensive array of materials, encompassing solids, liquids, and gases. Within Raman spectroscopy, a coherent laser beam is directed towards the sample, inducing molecular oscillations that lead to the inelastic scattering of photons. The resultant scattered light, altered in frequency due to energy transfer with the oscillating molecules, is amassed and scrutinized to construct a Raman spectrum, featuring peaks corresponding to distinct vibrational modes *Smith & Dent, 2005*.

A Raman spectrometer's essential elements encompass a laser light source, a monochromator or wavelength selector for isolating specific light wavelengths, a sample container or cuvette, and a detector for gauging scattered light intensity. Various types of Raman spectroscopy techniques, such as confocal Raman spectroscopy, surface-enhanced Raman spectroscopy (SERS), and resonance Raman spectroscopy, can be deployed contingent on the particular application, each offering distinct advantages for examining diverse samples and materials *Matousek & Stone, 2013*.

The utility of Raman spectroscopy spans across multiple domains, including chemistry, materials science, pharmaceuticals, biology, and forensics. It serves purposes like qualitative and quantitative analysis, identification of unknown substances, portrayal of molecular configuration and structural alterations, monitoring of chemical reactions and processes, and visualization of biological specimens *Smith & Dent, 2005*. Overall, Raman spectroscopy emerges as a valuable instrument for researchers and analysts aiming to unravel the molecular attributes and conduct of materials with a high degree of sensitivity and specificity.

Raman spectroscopy is extensively applied in the assessment of multi-walled carbon nanotubes (MWCNTs) and MWCNT composites due to its sensitivity towards vibrational modes within carbon-based materials. The procedure commonly entails employing a Raman spectrometer equipped with a laser light source, often operating at wavelengths like 532 nm or 785 nm. MWCNT samples or composites are typically prepared by dispersing them in a suitable solvent and depositing a droplet onto a pristine substrate. Alternatively, the specimens can be directly placed on a Raman-compatible substrate such as silicon or quartz. The acquisition of Raman spectra involves focusing the laser beam on the sample and capturing the scattered light, which undergoes Raman scattering owing to interactions with the vibrational modes of carbon atoms in the nanotube structure. The Raman spectrum typically exhibits peaks corresponding to the radial breathing mode (RBM) and the tangential (G-band) and disorder-induced (D-band) modes of the MWCNTs. The ratio of intensity between the D-band and the G-band (I_D/I_G) offers insights into the level of disorder and imperfections present in the nanotube structure, while the RBM peak position can be utilized to estimate the nanotubes' diameter. Additionally, Raman spectroscopy enables the characterization of MWCNT composites, facilitating the recognition of interactions between the nanotubes and the matrix material, as well as the evaluation of nanotube dispersion and alignment within the composite.

4.3.4 XRD

X-ray diffraction (XRD) is a potent analytical method utilized for the examination of the crystallographic configuration and characteristics of substances. Grounded on Bragg's law principles, XRD encompasses the projection of a monochromatic X-ray beam onto a crystalline specimen, inducing the constructive interference of X-rays diffused by the crystal lattice. The resultant diffraction pattern furnishes crucial insights into the atomic or molecular arrangement within the sample, encompassing crystal phase, lattice parameters, crystallite dimensions, and orientation *Cullity & Stock, 2001*.

The essential elements of an XRD apparatus comprise an X-ray generator, like a sealed X-ray tube or synchrotron radiation, a sample holder, a diffractometer for

managing the angle of incidence and measuring the intensity of diffracted X-rays, and a detector for capturing the diffraction pattern. Various XRD methodologies include powder X-ray diffraction (XRPD), single-crystal X-ray diffraction (SCXRD), and thin-film X-ray diffraction, each presenting distinct perspectives on the structure and characteristics of materials *Dinnebier & Billinge, 2008*.

XRD is extensively applied across diverse domains such as materials science, geology, pharmaceuticals, and metallurgy. It is utilized for phase recognition, quantitative phase assessment, determination of crystallinity, structural profiling, and exploration of phase alterations and imperfections in materials *Cullity & Stock, 2001*. XRD is notably beneficial for scrutinizing crystalline materials like minerals, polymers, ceramics, metals, and pharmaceutical compounds, delivering comprehensive insights into their atomic layout and characteristics. Overall, X-ray diffraction emerges as a foundational tool for researchers and engineers endeavoring to comprehend the structure-property correlations of materials, facilitating advancements in materials design, synthesis, and analysis.

X-ray diffraction (XRD) is a robust technique employed in the evaluation of multi-walled carbon nanotubes (MWCNTs) and MWCNT composites to scrutinize their crystalline structure and phase constitution. The methodology typically encompasses the preparation of samples by dispersing MWCNTs or MWCNT composite materials onto a flat substrate or incorporating them into a matrix material. Subsequently, the samples are exposed to X-ray radiation generated by an X-ray source, such as a copper (Cu) or cobalt (Co) target. The incident X-rays interact with the atoms in the sample, prompting them to diffract at specific angles as per Bragg's law, contingent on the interatomic spacing within the crystal lattice. The diffracted X-rays are captured by a detector, and the resulting diffraction pattern, termed the XRD spectrum, is scrutinized to recognize the crystallographic phases present in the sample and ascertain their relative abundance and orientation. In the scenario of MWCNTs, the XRD spectrum typically displays distinct peaks corresponding to the graphite (002) reflection, indicating the existence of organized carbon layers within the nanotube structure. The location and intensity of these peaks

can offer insights into the degree of crystallinity, interlayer spacing, and alignment of the MWCNTs. In MWCNT composites, XRD can be leveraged to identify alterations in the crystalline structure and phase composition arising from interactions between the nanotubes and the matrix material. Overall, X-ray diffraction functions as a valuable tool for the qualitative and quantitative exploration of MWCNTs and their composites, presenting perspectives on their structural attributes and phase behavior.

4.3.5 FT-IR

Fourier-transform infrared spectroscopy (FT-IR) is a widely utilized analytical method for examining the chemical composition, molecular structure, and bonding interactions of substances. Through the absorption of infrared radiation by molecules, FT-IR spectroscopy yields significant insights into the functional groups, molecular vibrations, and chemical bonds within a specimen. The process involves transmitting infrared radiation through a sample and measuring the absorption spectrum by assessing the intensity of transmitted or reflected light in relation to the wavelength or frequency of the infrared radiation *Griffiths & de Haseth, 2007*. An FT-IR spectrometer comprises essential elements such as an infrared light source, an interferometer for modulating the infrared beam, a sample holder or cell, and a detector to gauge the infrared radiation intensity. FT-IR spectra are conventionally collected across a broad spectrum of wavelengths (e.g., mid-infrared, near-infrared) and can be examined to recognize functional groups, ascertain molecular structure, quantify components in mixtures, and track chemical reactions *Griffiths & de Haseth, 2007*.

The utility of FT-IR spectroscopy spans diverse domains including chemistry, pharmaceuticals, materials science, biology, and environmental science. It serves purposes like qualitative and quantitative analysis, polymer characterization, identification of unknown substances, biomolecule analysis, pollutant detection, and chemical process monitoring *Griffiths & de Haseth, 2007; Smith, 1999*. All in all, FT-IR spectroscopy emerges as a versatile and potent instrument for researchers and analysts aiming to comprehend the molecular attributes and behavior of substances.

FTIR spectroscopy is commonly utilized in the assessment of multi-walled carbon nanotubes (MWCNTs) and MWCNT composites to scrutinize their chemical composition and functional groups. The procedure involves sample preparation by dispersing MWCNTs or MWCNT composite materials in a suitable solvent or embedding them in a matrix. Subsequently, the samples are typically dried, ground into a fine powder, and placed on an FTIR-compatible substrate like a potassium bromide (KBr) pellet or an attenuated total reflectance (ATR) crystal. FTIR spectra are captured by exposing the sample to infrared radiation across various frequencies and measuring the absorbance or transmittance of light as it interacts with the molecular vibrations. In the instance of MWCNTs, characteristic peaks in the FTIR spectrum might originate from carbon-carbon bond vibrations in the nanotube structure and functional groups on the nanotube surface like hydroxyl (-OH), carbonyl (C=O), and carboxyl (-COOH) groups. The presence and intensity of these peaks offer insights into the chemical composition, surface functionalization, and oxidation level of the MWCNTs. In MWCNT composites, FTIR spectroscopy can identify interactions between the nanotubes and the matrix material, as well as evaluate changes in functional groups and chemical structure within the composite. In essence, FTIR spectroscopy stands out as a valuable instrument for both qualitative and quantitative analysis of MWCNTs and their composites, providing valuable information about their chemical attributes and surface properties.

4.4 BIOLOGICAL ASSAY

After all the characterization are done the sample is then cultured with MCF7 cell lines and MTT assay is done to check the cytotoxicity.

The MTT assay, utilizing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, is a frequently employed colorimetric technique for evaluating cell viability and proliferation in cell culture studies. This method hinges on the transformation of the yellow tetrazolium salt MTT into purple formazan crystals by mitochondrial enzymes in metabolically active cells, with the quantity of formazan generated being directly correlated with the viable cell count in the culture. Upon a specified incubation duration with MTT, the formazan crystals are dissolved utilizing a solvent like dimethyl sulfoxide

(DMSO), and the absorbance of the resultant solution is assessed via spectrophotometry at a wavelength typically near 570 nm. Elevated absorbance readings signify enhanced cell viability and proliferation according to *Mosmann (1983)*.

The MTT assay holds widespread application in fields such as cell biology, pharmacology, and toxicology, serving to evaluate drug-induced cytotoxicity, determine cell proliferation rates, ascertain cell viability post-treatment, and screen compounds for potential therapeutic utility *Mosmann, 1983; van Meerloo et al., 2011*. Despite its extensive utility, the MTT assay is subject to certain constraints, including its endpoint nature, susceptibility to interference from colored or insoluble substances, and reliance on mitochondrial function, which can exhibit variability across cell types and experimental setups. Nonetheless, when accompanied by appropriate controls and optimization strategies, the MTT assay endures as a valuable and economical tool for scrutinizing cell viability and proliferation in vitro.

Fibroblast cells were cultured in 96-well plates, followed by the application of drugs onto the cellular monolayer. Subsequently, the cells underwent a 24-hour observation period to assess cellular morphology. Post this evaluation, the cells underwent two washes with 1XPBS and were subjected to a three-hour incubation with 0.2 mg/ml of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The resultant purple-colored product was dissolved using isopropyl alcohol, and the optical density was gauged at 620nm using an ELISA Reader.

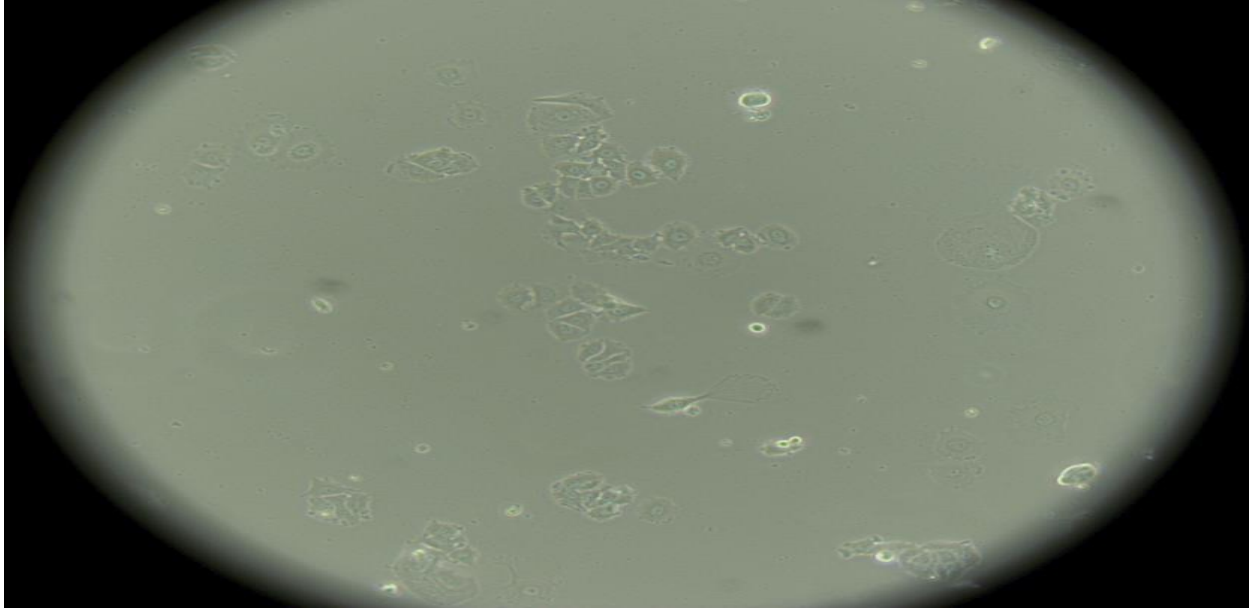


Fig 4.5 a

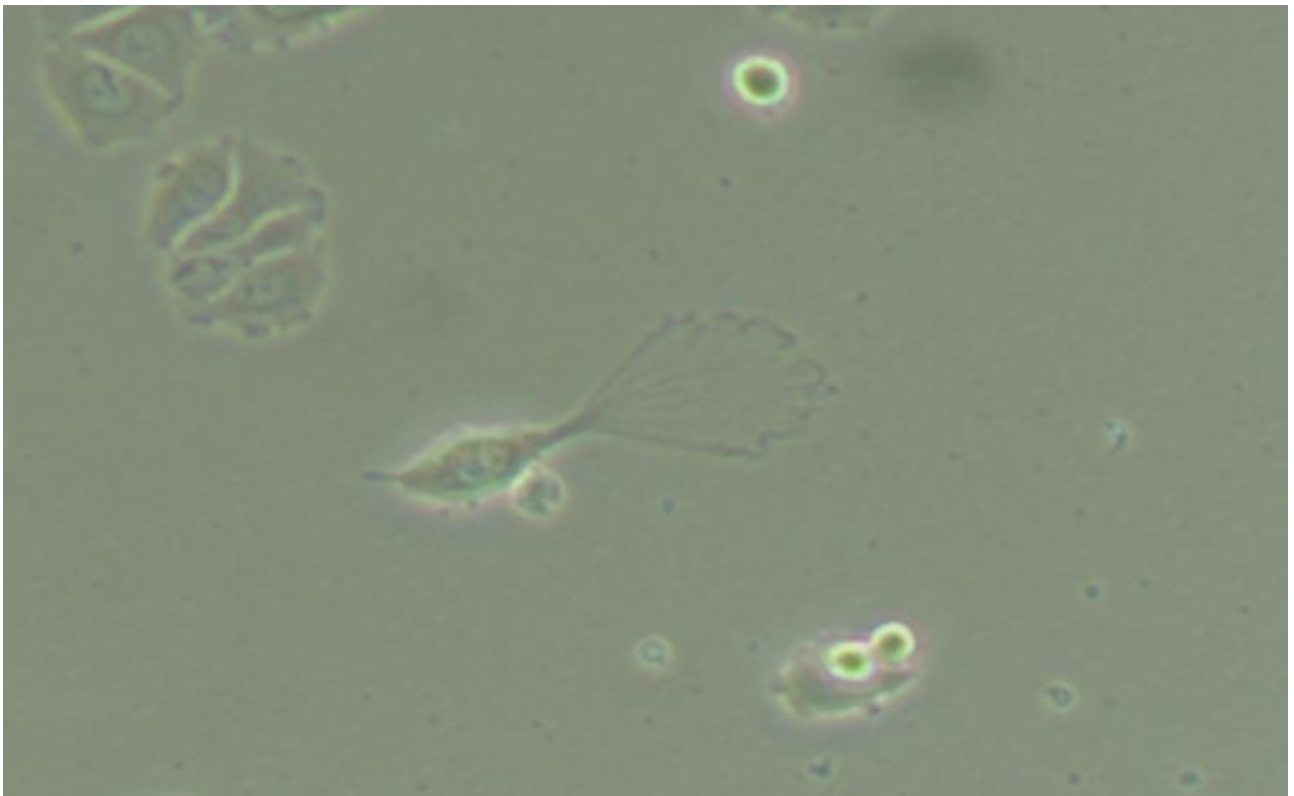


Fig 4.5 b

Both figures 4.5 a and 4.5 b are MCF7 cell lines in microscopic images

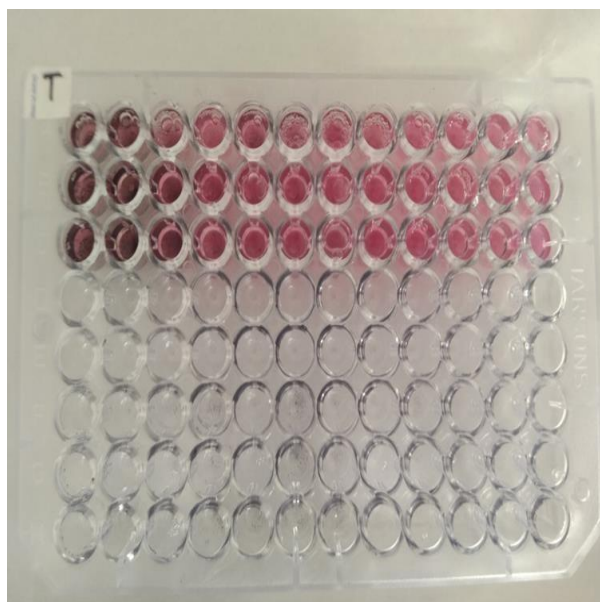


Fig 4.6 a Before MTT assay - tras



Fig 4.6 b After MTT assay - tras

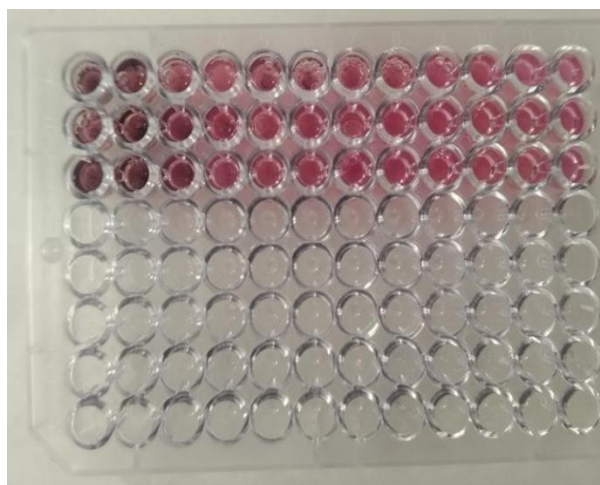


Fig 4.7 a Before MTT assay - Dox

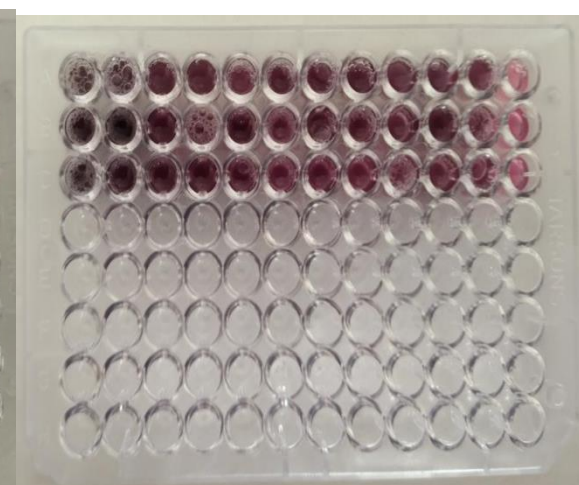


Fig 4.7 b After MTT assay - Dox

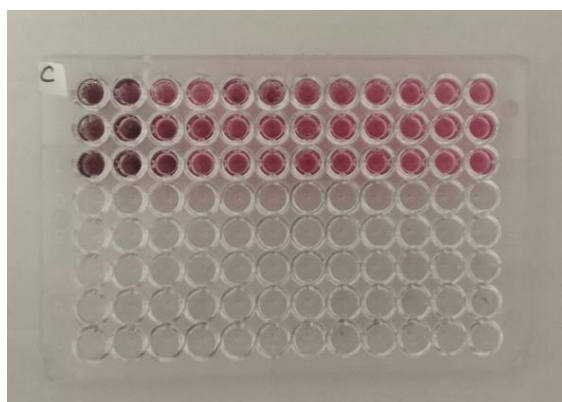


Fig 4.8 a Before MTT assay – MWCNT

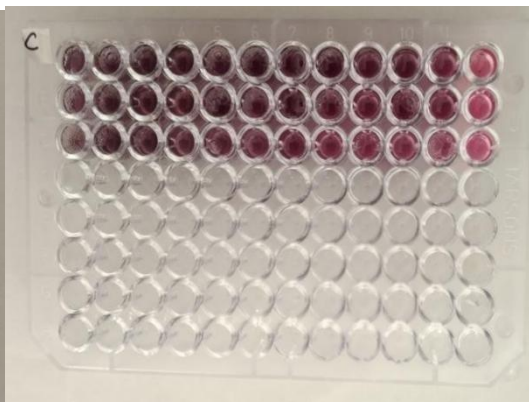


Fig 4.8 b After MTT assay – MWCNT

CHAPTER 5

RESULTS AND DISCUSSION

5.1 MATERIAL ANALYSIS

The sonication was successful and lyophilization was done to obtain powdered samples for characterization.



Fig 5.1 Samples after sonication

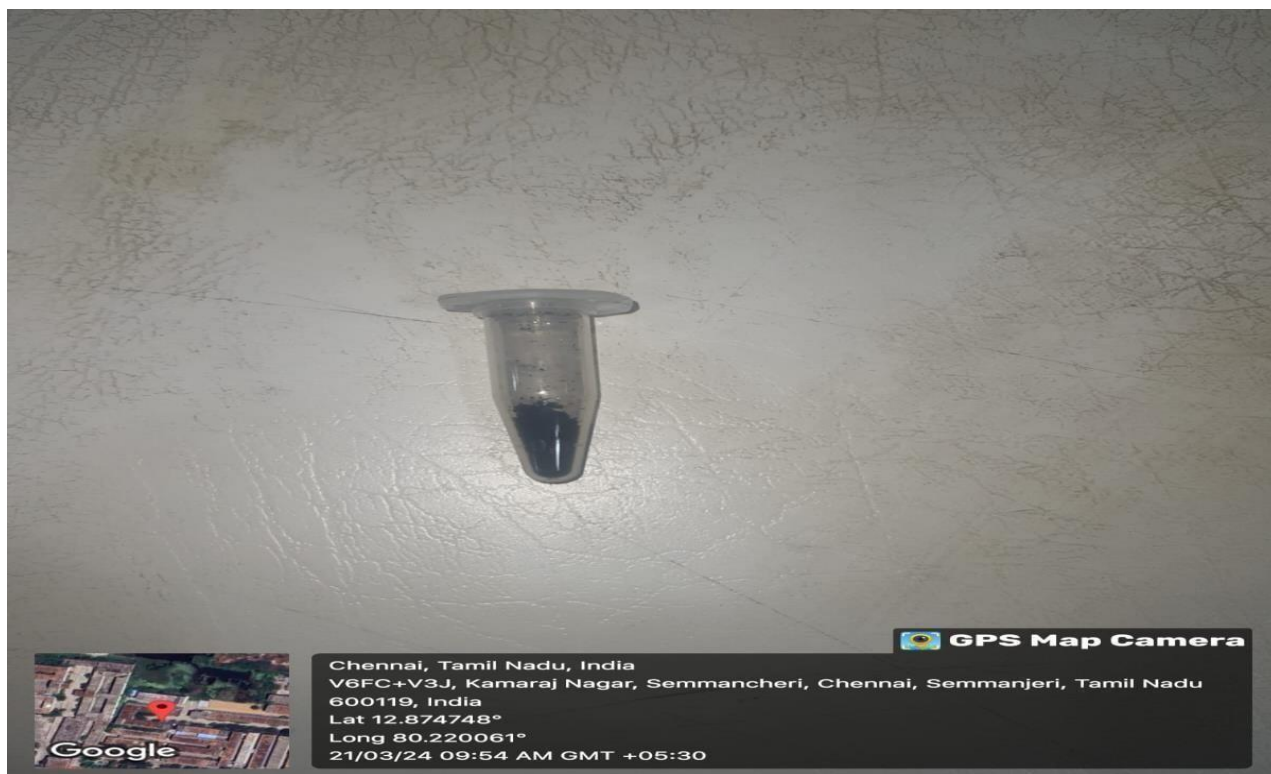


Fig 5.2 Sample after lyophilization

5.2 CHARACTERIZATION ANALYSIS

Each characterization tests gave results for all 3 samples that includes: mwcnt, mwcnt with doxorubicin and mwcnt with trastuzumab.

5.2.1 HR TEM ANALYSIS

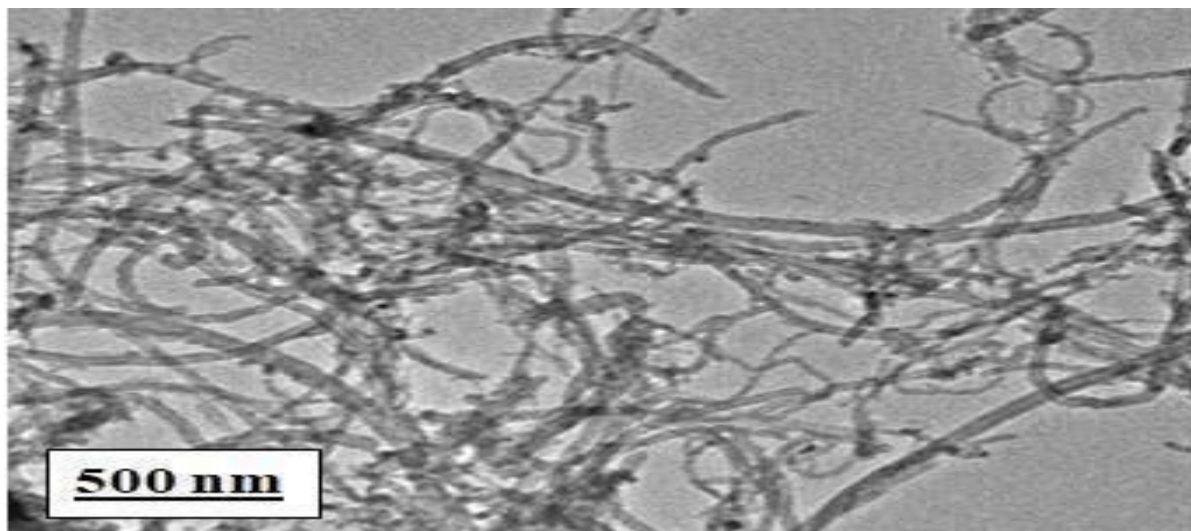


Fig 5.3 HR TEM image of MWCNT

Figure 5.3 reveals the HR TEM result for MWCNT. High-resolution imaging techniques, operating at an extremely fine scale down to the atomic level, have unveiled the presence of elongated rod-shaped formations exhibiting a measured diameter of around 500 nanometers, a dimension that aligns well with the distinctive structural morphology commonly associated with multi-walled carbon nanotubes (MWCNTs).

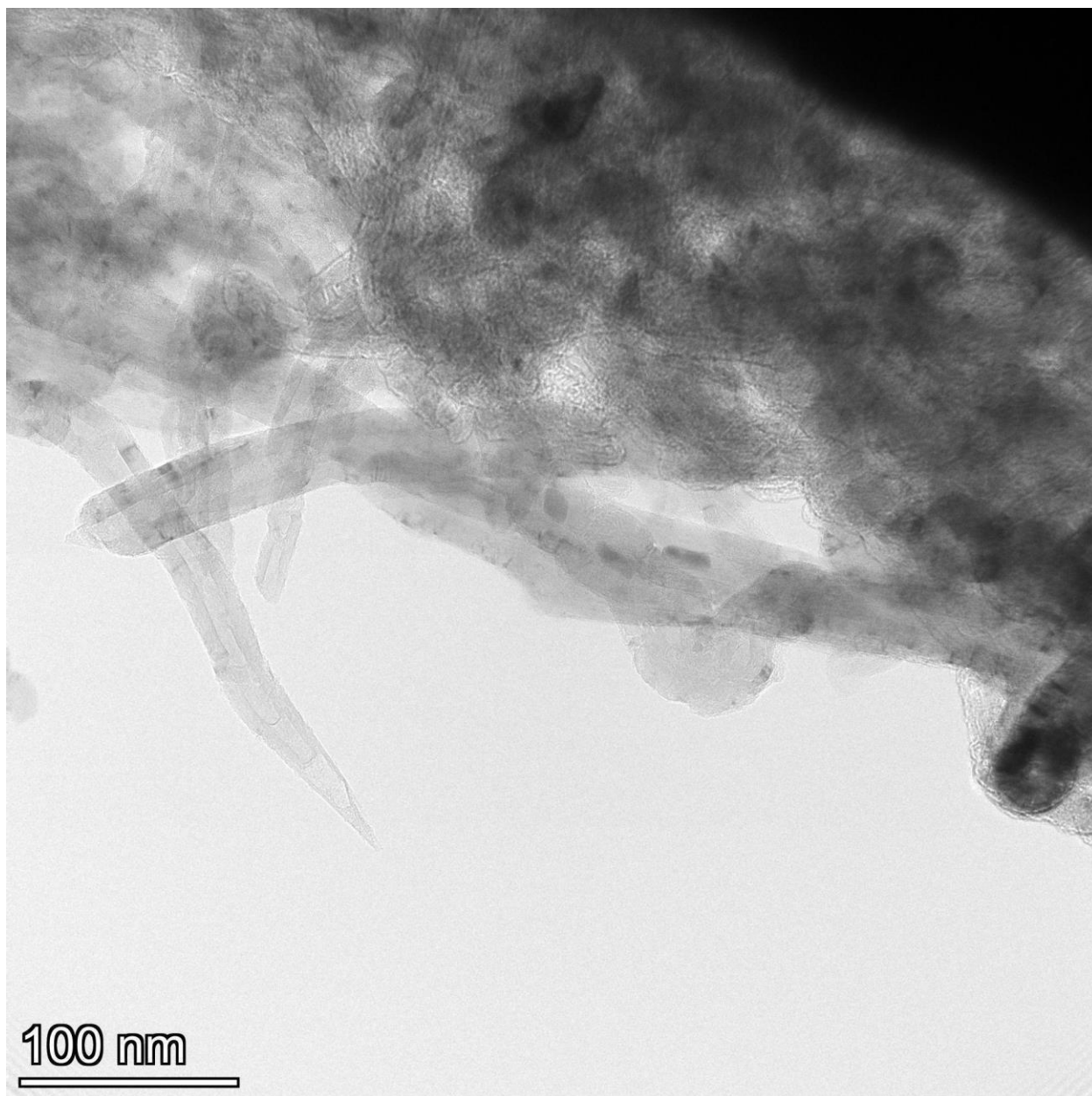


Fig 5.4 HR TEM image of MWCNT with Trastuzumab

The molecular interactions taking place between the trastuzumab compound and the MWCNT architecture is clearly visible at the nanoscale (100 nm) as shown in Figure 5.4. The functionalization by the trastuzumab compound over MWCNT is understood from the cloudy images surrounding the native MWCNT Nano-structures. The interactions between the two compounds at the nanoscale is further validated in the EDAX analysis as shown in Figure 5.6 where the two interacting compounds of the bio and nano-scaled

materials interacted, proving a successful functionalisation of the compound (trastuzumab) over the MWCNT structures.

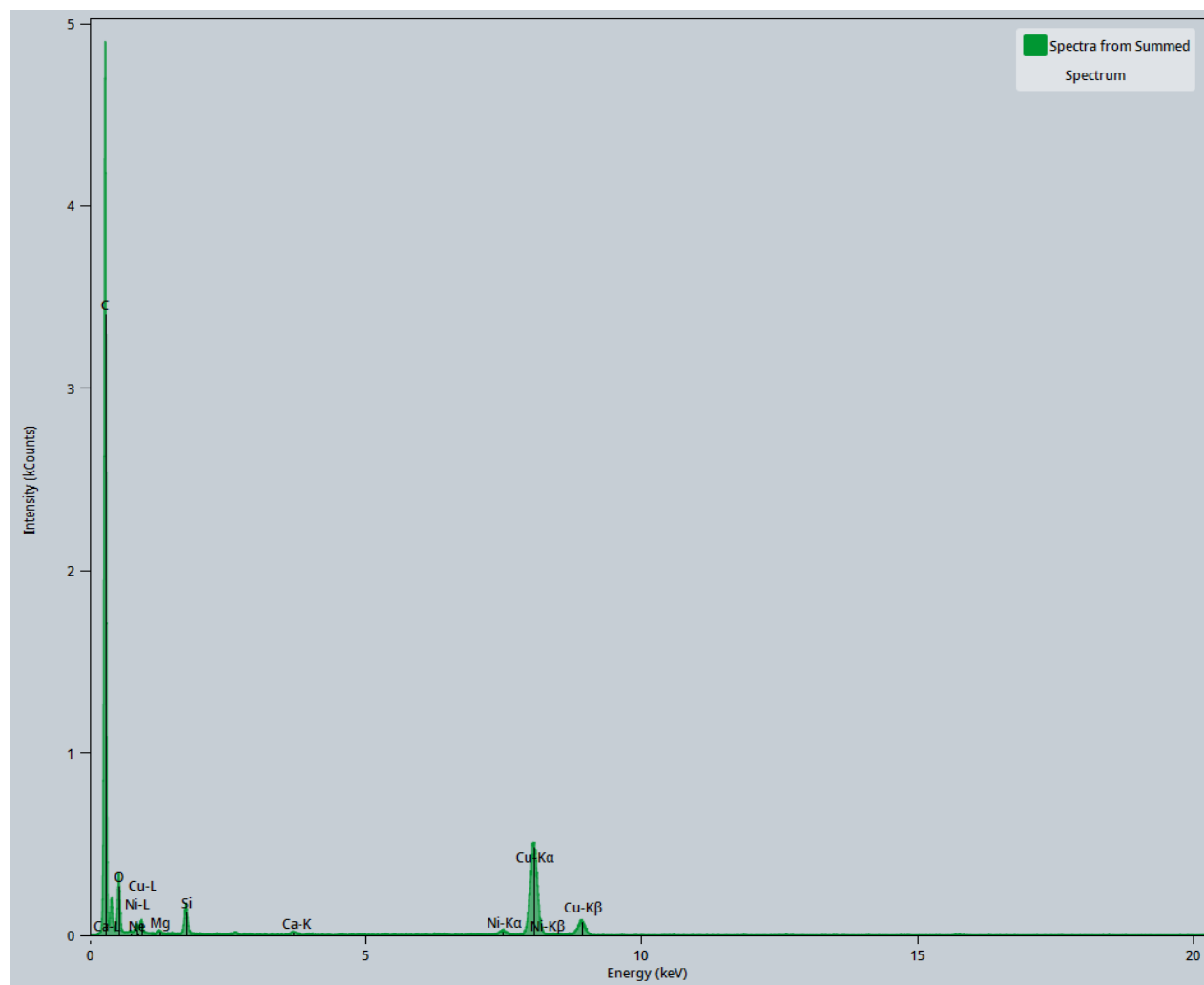


Fig 5.5 EDAX of MWCNT with Trastuzumab

It could be seen from the EDAX elemental profiling Figure 5.5, that Oxygen groups were augmented in the case of '*Trastuzumab-functionalised MWCNTs*' as compared to the lower proportions of Oxygen group presence in case of '*Doxorubicin-functionalised MWCNTs*'. On the other hand, the proportion of Copper (Cu) is suppressed in percentage in case of '*Trastuzumab-functionalised MWCNTs*' as when compared with heightened Cu group proportions in case of '*Doxorubicin- functionalised MWCNTs*'.

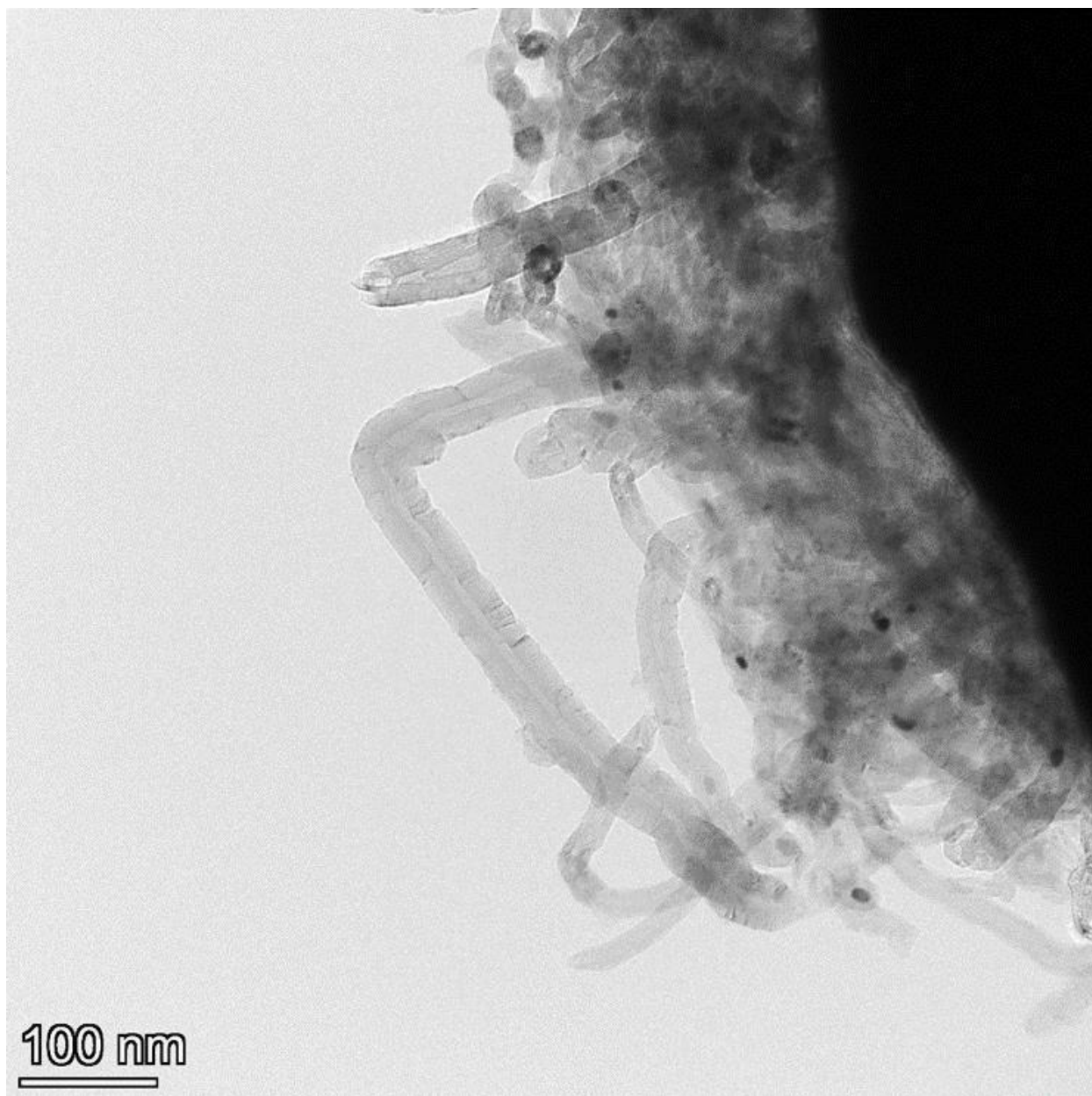


Fig 5.6 HR TEM image of MWCNT with Doxorubicin

The entanglement of the doxorubicin compound over the MWCNT structure at the nanoscale (100 nm) is clearly visible from Figure 5.6 from the darker agglomerating compounds images over the native nano-MWCNT architectures. Such functionalisation is further corroborated using the EDAX elemental analysis as shown in Figure 5.7, here different interacting entities of doxorubicin and MWCNT are intermingled and is providing respective elemental peak profiling, indicating successful functionalisation of the

doxorubicin compound over the MWCNT structure.

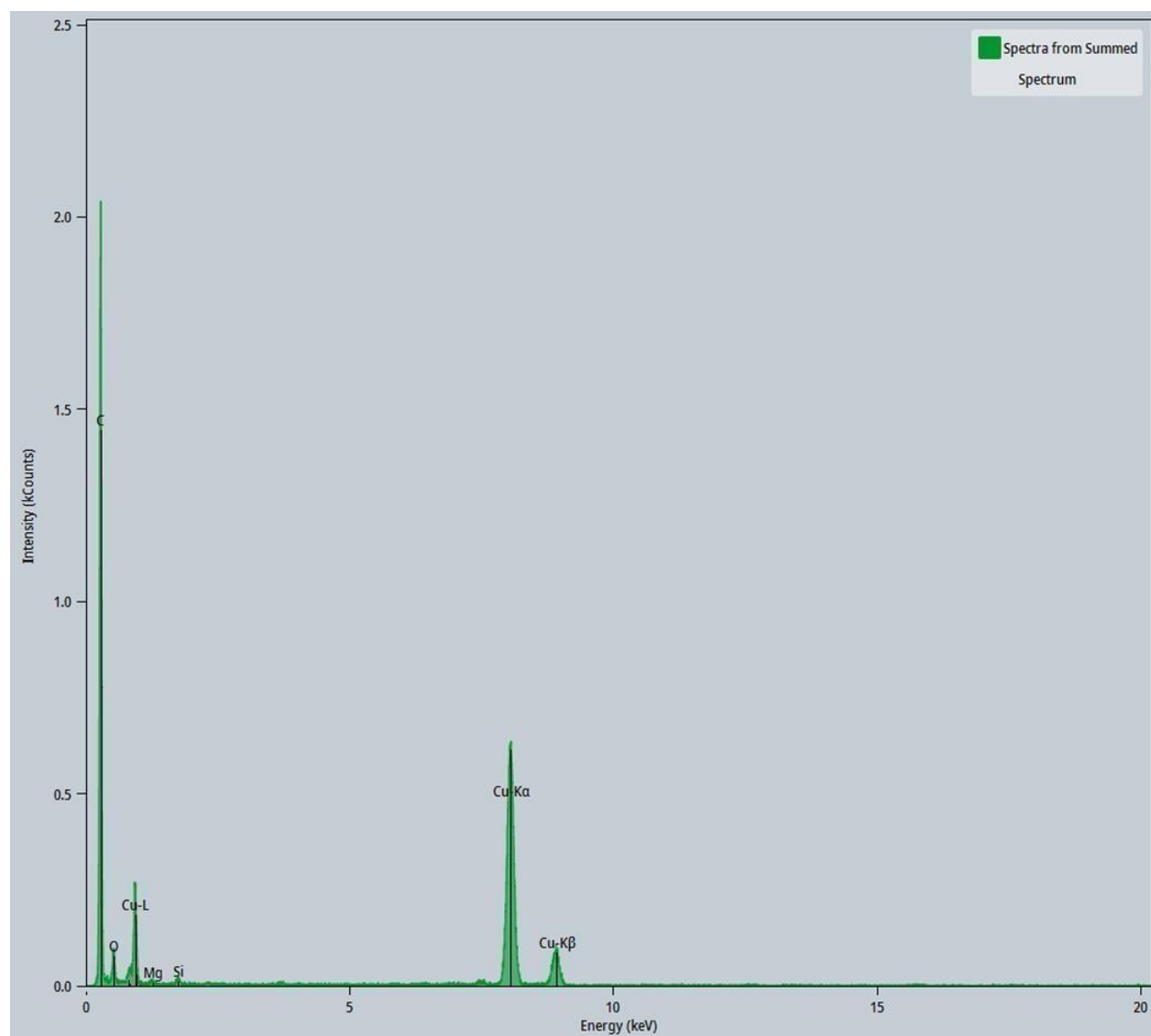


Fig 5.7 EDAX of MWCNT with Doxorubicin

It could be seen from Figure 5.7, that Oxygen groups were suppressed in case of '*Doxorubicin-functionalised MWCNTs*' as compared to the presence of 'Oxygen' group in case of '*Trastuzumab-functionalised MWCNTs*'. On the other hand, the proportion of Copper (Cu) is augmented in percentage in case of '*Doxorubicin-functionalised MWCNTs*' as when compared with Cu group in case of '*Trastuzumab-functionalised MWCNTs*'. Such differential proportion of 'Cu' and 'O' groups evident from the HR-TEM results of MWCNT functionalised trastuzumab and doxorubicin clearly demarcates the

differential anti-cancerous responses achieved using both the functionalised cases.

5.2.2 UV VIS SPECTROSCOPY ANALYSIS

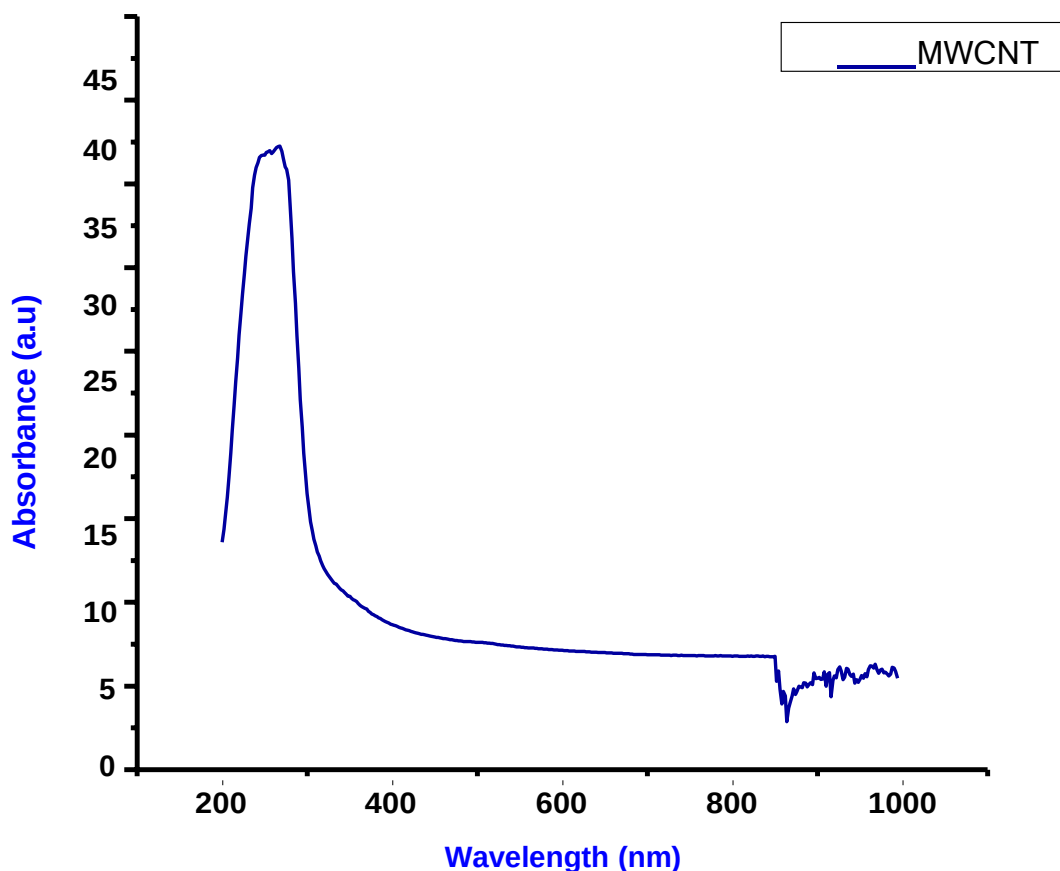


Fig 5.8 UV spectroscopy graphical image for MWCNT

It could be seen from Figure 5.8 that UV-Vis absorbance peaks is clearly noticed at ~300 nm wavelength. It signifies the absorbance peak for the inherent structure of MWCNT in the aqueous solution of distil water. The C and O groups present in the pristine structure of MWCNT as evident from the UV –Vis plot is signified in terms of the absorbance peaks as noted in the specific wavelength of light.

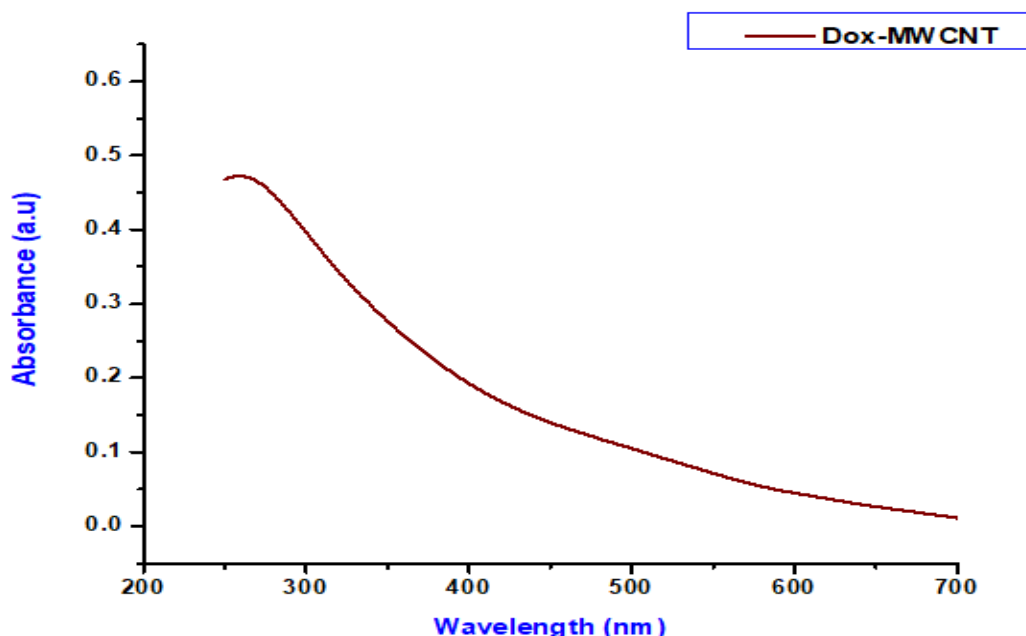


Fig 5.9 UV spectroscopy graphical image for MWCNT with Doxorubicin

Figure 5.9 shows the UV result for MWCNT with doxorubicin sample. It could be clearly seen from Figure that upon functionalisation in the aqueous phase for the MWCNT with the Doxorubicin molecule, due to the charge transfer and redox state reactions taking place between the MWCNT and dox molecule, there is a gradual shift in the UV-vis absorbance peaks in the functionalised case. It is understood that due to surface functionalisation of dox molecule over the pristine structure of MWCNT, there is a change in the pristine surface properties of MWCNTs, leading towards an altered absorbance plot. Such changes in the complex of DOX-MWCNT aids in understanding the fact that upon dox functionalisation, there is a probability in the change in the biocompatibility and solubility aspects in the native structure of MWCNT, helping thereby in achieving a robust anti-cancerous activity in chosen cancer cell lines.

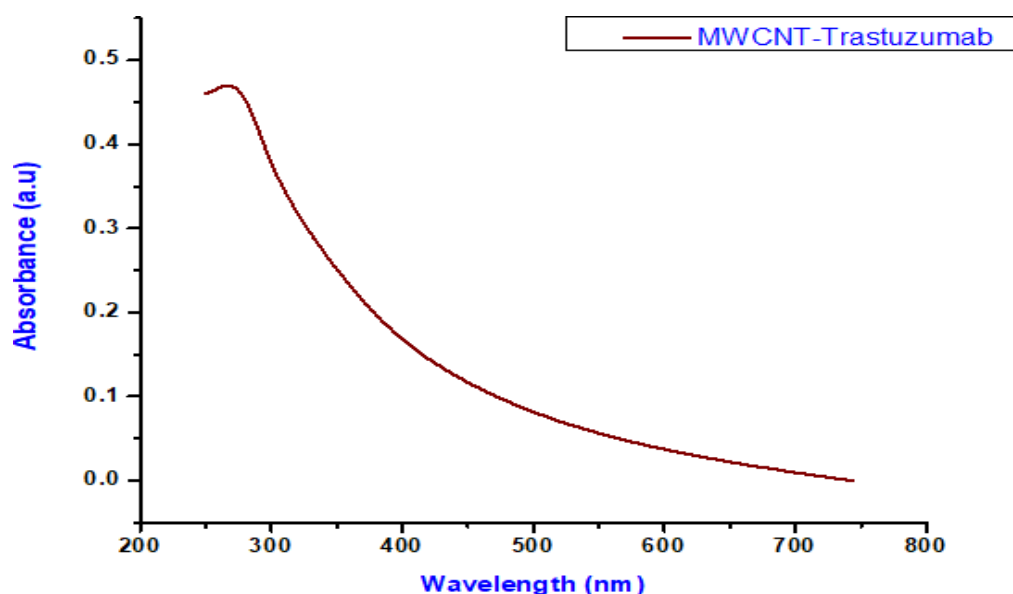


Fig 5.10 UV spectroscopy graphical image for MWCNT with Trastuzumab

It could be clearly seen from Figure 5.10, that upon functionalisation with the Trastuzumab molecule over the pristine MWCNT structure, there is an alteration in the structure of MWCNT surface and at the intersites, leading thereby an altered absorbance plot as noticed in the Figure 5.10. Such alteration in the structure of MWCNT, leads towards the differing in the shifting of the absorbance plots as compared to the native structure of the MWCNT. The alterations in the structural and physico-chemical differences of the complex structure, would result into the utilisation of the trastuzumab-MWCNT complexes in the anti-cancerous studies in a bio-compatible and safer manner.

5.2.3 RAMAN SPECTROSCOPY ANALYSIS

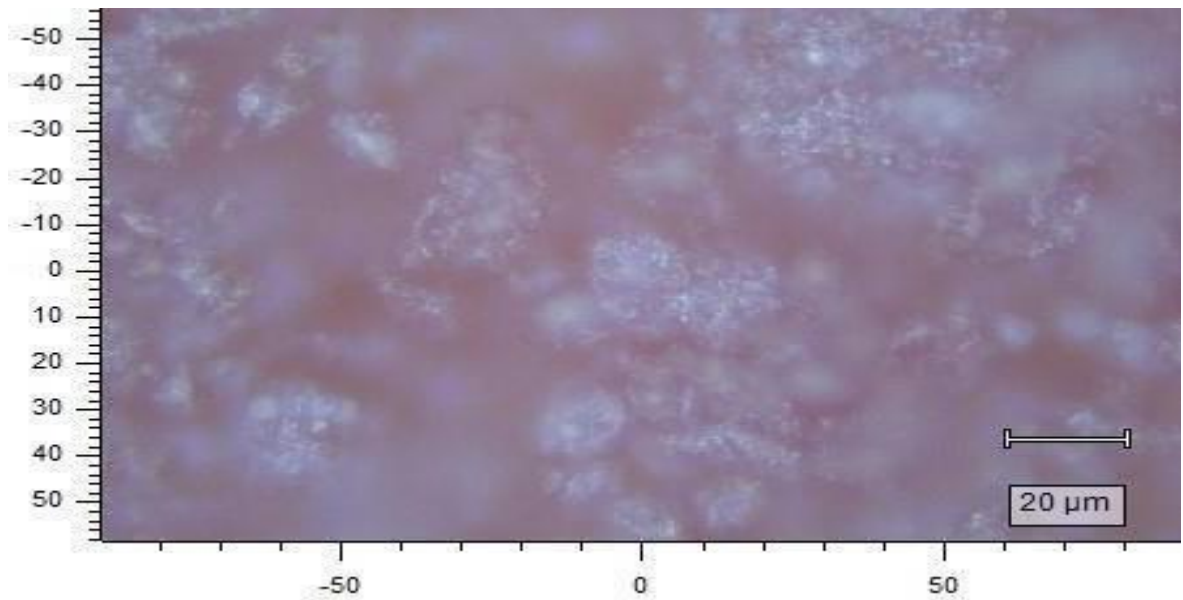


Fig 5.11 Raman spectroscopy image for MWCNT at 20 micrometer.

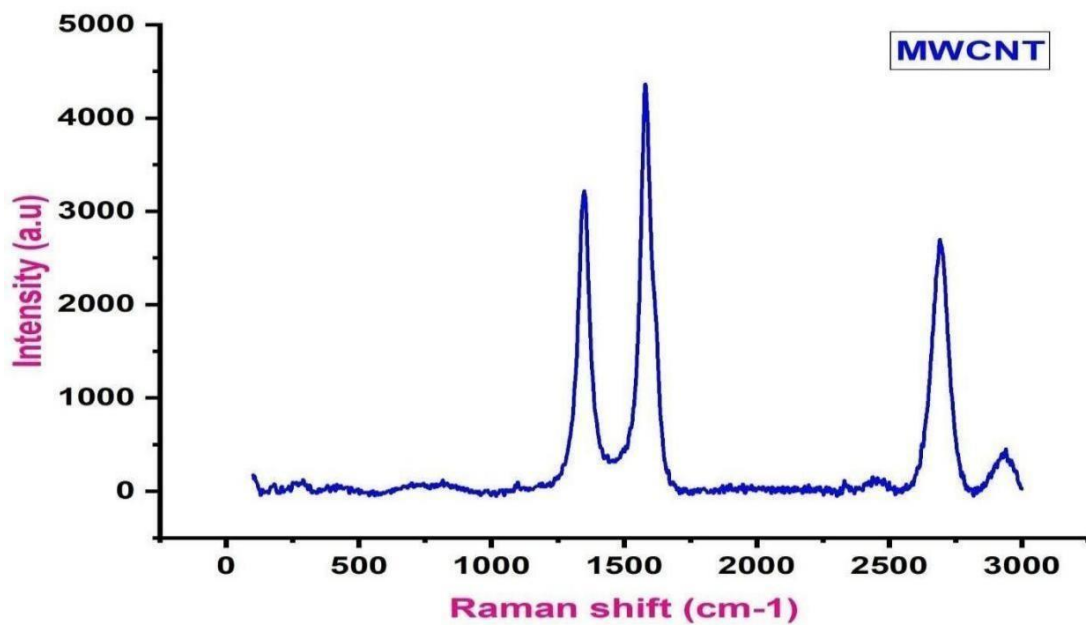


Fig 5.12 Graphical representation of Raman for MWCNT

The Raman spectrum provided exhibits consistency with multi-walled carbon nanotubes (MWCNTs), which are categorized as a form of nanomaterial comprising numerous layers of graphene sheets arranged in a cylindrical configuration. A material's Raman spectrum serves as a distinctive indicator of its vibrational modes, enabling material identification and property determination. The G peak, situated at approximately 1580 cm^{-1} , arises from the stretching mode of sp^2 -bonded carbon atoms within the graphene sheets. The D peak, positioned around 1350 cm^{-1} , originates from defects present in the graphene sheets. The comparison of the D and G peak intensities offers insight into the quality assessment of MWCNTs, with a higher D/G ratio generally indicating an increased prevalence of defects in the MWCNT structure. The intensity of the Raman spectrum also facilitates the estimation of MWCNT quantity in a given sample, with higher intensities correlating to elevated MWCNT concentrations. A comprehensive interpretation of Raman spectra necessitates consideration of all peaks within the spectrum, alongside variables like laser excitation wavelength and sample preparation procedures. Furthermore, when analyzing Raman spectra of MWCNTs, factors such as the nanotubes' diameter influencing the G and D peak characteristics, the chirality dictating the Raman spectrum based on graphene sheet rolling, and the potential impact of impurities or contaminants in the MWCNT sample on the Raman spectrum, should all be taken into account.

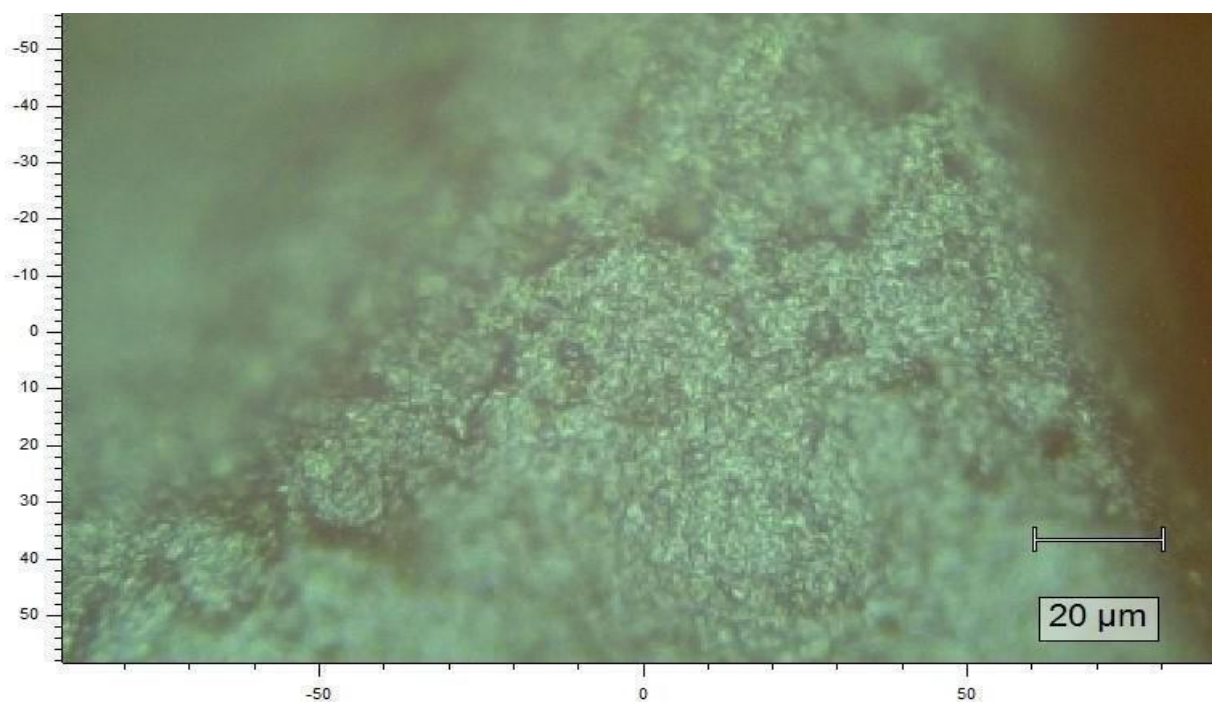


Fig 5.13 Raman spectroscopy image for MWCNT with Trastuzumab at 20 micrometer.

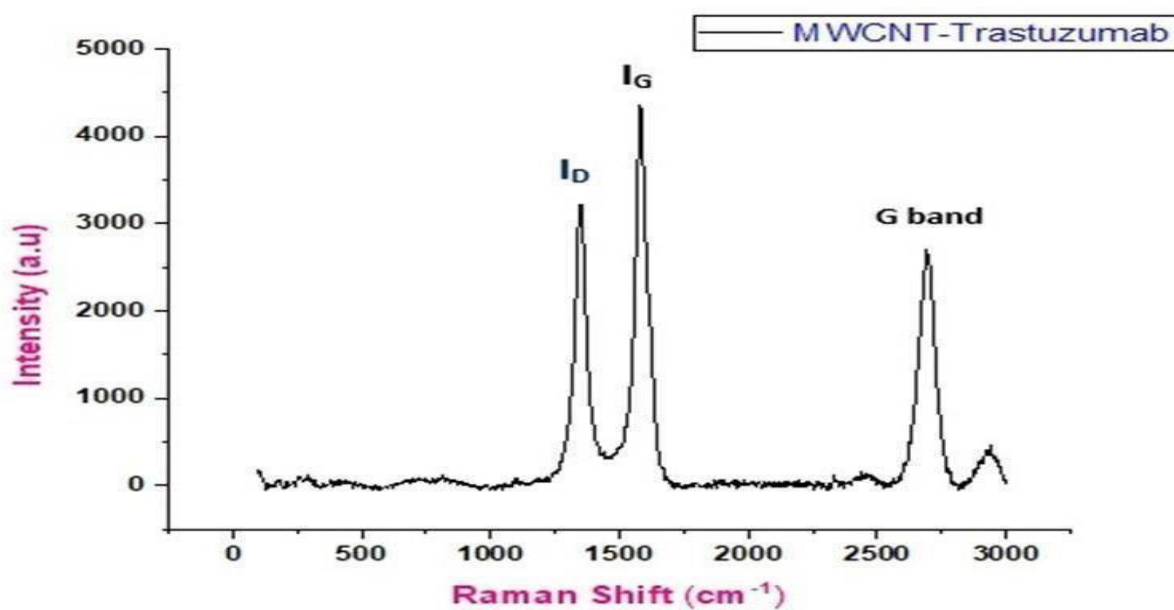


Fig 5.14 Graphical representation of Raman for MWCNT with Trastuzumab

It could be seen from the Figure 5.14 that upon conjugation of the 'MWCNT-trastuzumab', the defect proportions are altered in terms of the intensity graphs of I_D/I_G ratio. Also, the 'G- band' seems to be shifted upon the conjugation with the trastuzumab compound upon the MWCNT structure. The vibrations and its shifts in the structure of the composite indicates the successful conjugation of the trastuzimab compound over the native structure of MWCNT. The proportions of the defect states in the structure of MWCNT seems to be entangled with the compound of trastuzumab molecule.

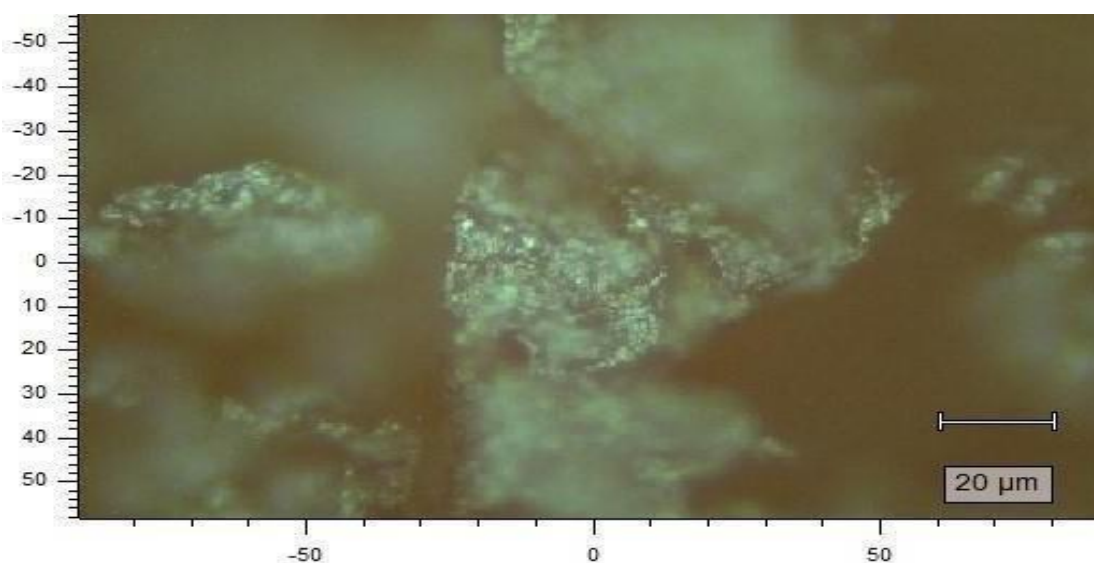


Fig 5.15 Raman spectroscopy image for MWCNT with Doxorubicin at 20 micrometer.

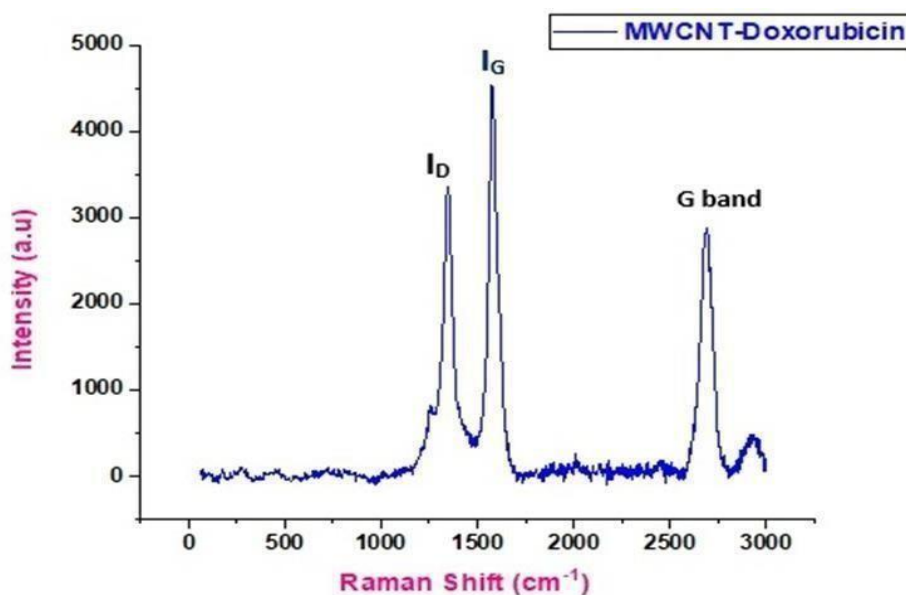


Fig 5.16 Graphical representation of Raman for MWCNT with Doxorubicin

It could be seen from the Figure 5.16 that upon conjugation of 'MWCNT with doxorubicin' compound, as compared to trastuzumab conjugation, similar defect intensity alterations could be noticed in the present composite form. The 'G bands' also seems to be altered in comparison to the native raman vibrational spectra. Such observations clearly indicate the possible inter-entanglement of the 'MWCNT- doxorubicin' compound mediated by the interface redox state interactions between the material and biological context. Such composites when introduced into the biological systems like cancer cells and allied would harbour the potentiality of eliciting the robust anti-cancerous activities which when compared to native un-conjugated anti-cancerous agents.

5.2.4 XRD ANALYSIS

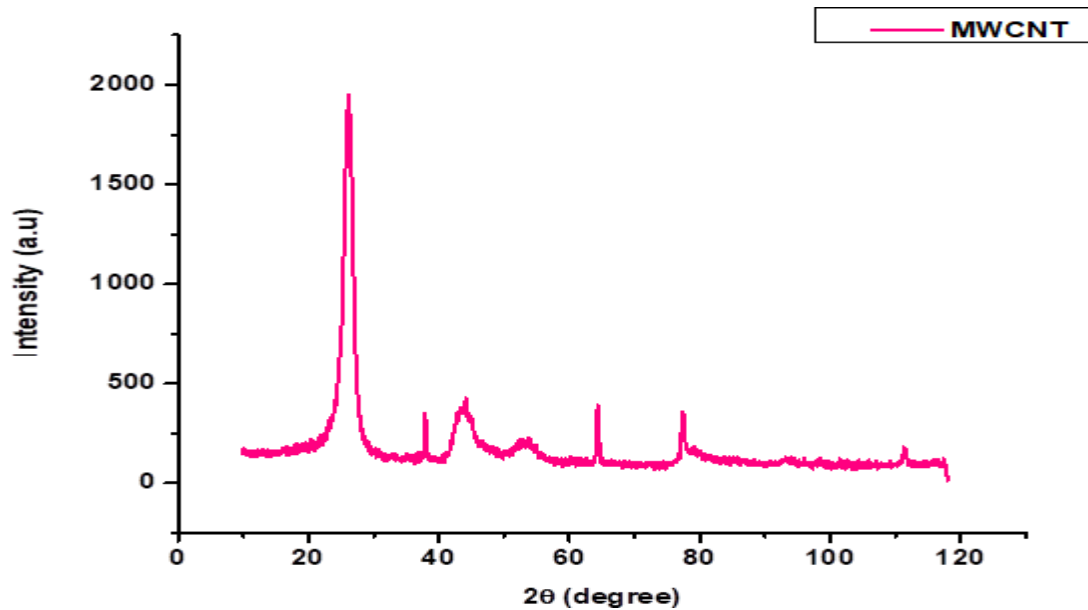


Fig 5.17 XRD FOR PRISTINE MWCNT

The powder samples were subjected to XRD instrument (provide name and make of company) and were analysed using X-Ray Diffraction (XRD) measurements for analysing the underlying samples diffraction planes and braggs diffraction angles. The nature of diffraction peaks would indicate the type of sample's crystallinity or amorphosity in the examining sample. It could be seen from Figure that Xrd diffraction planes at (002) corresponding to diffraction angle $\sim 26^\circ$, which corresponds to the typical graphitic peaks arising from the tubular structure of the carbon atoms in the sample. The diffraction planes at (100) and (004) corresponding to $\sim 45^\circ$ diffraction and 55° angle is noticed for MWCNT samples, which are in agreement with the previously published data. The type of peaks indicates that the sample is crystalline in nature as sharp prominent peaks were observed in the X-Ray diffractogram.

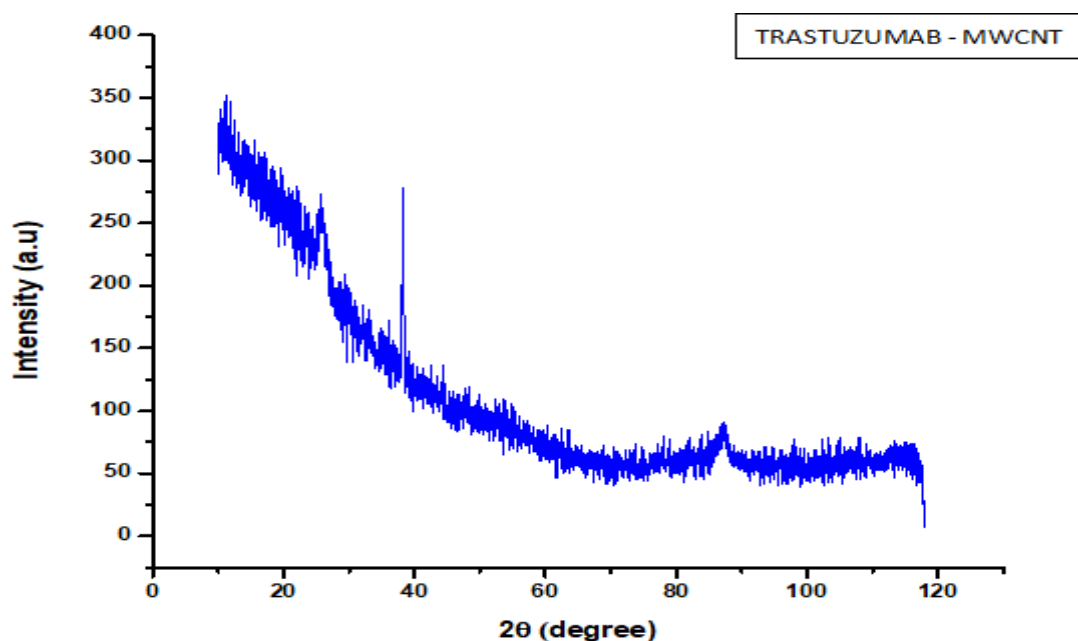


Fig 5.18 XRD FOR MWCNT with Trastuzumab

It could be seen from Figure 5.18 , that upon functionalisation with trastuzumab over the pristine structure of MWCNT, there is an occurrence in the suppression of the crystal lattice peaks at diffraction angle $\sim 26^\circ$. Further suppression and reduction in the lattice peaks could be seen in the shoulder peaks $\sim 45^\circ$ & $\sim 55^\circ$ indicating the effect of trastuzumab molecule over the pristine structure of MWCNT. Such alterations in the crystal lattice planes in the pristine structure of MWCNT and its functionalised counterpart, indicates the induction of charge transfer mechanism taken place at the interface of trastuzumab and the MWCNT interface leading to the more bio-functionalised and robust solubility aspects in the MWCNT structures aiding thereby the anti-cancerous activities in selected cancer cell lines

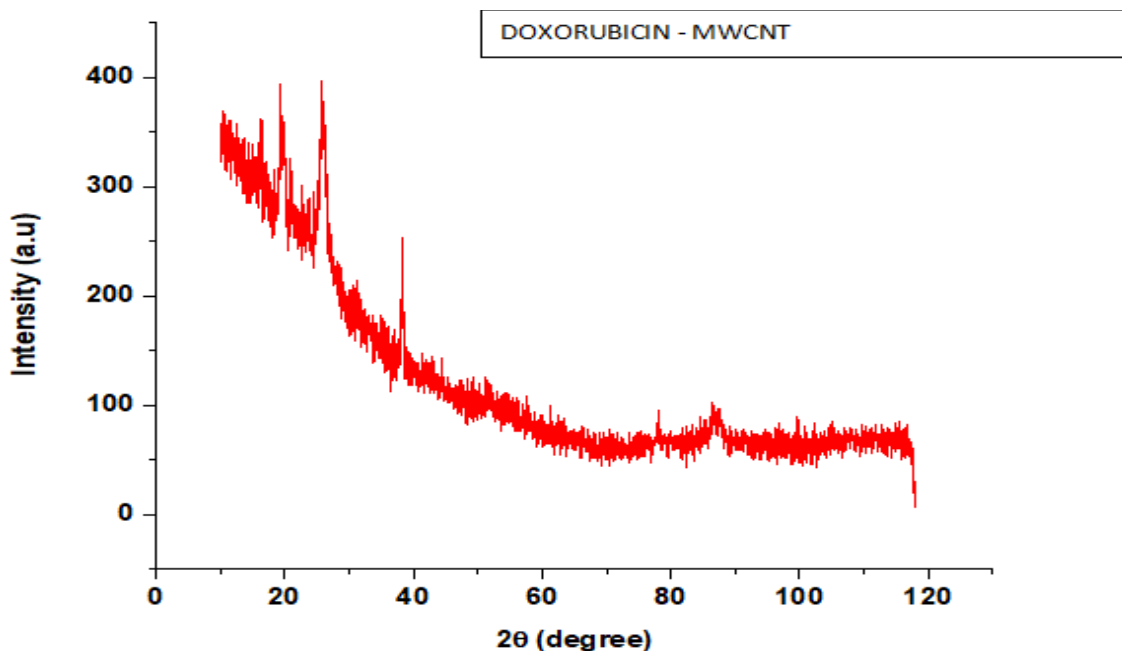


Fig 5.19 XRD FOR MWCNT with Doxorubicin

It could be seen from Figure 5.19, the studies involving the dox-MWCNT composites, the molecule of doxorubicin induces the structural alterations in the inherent pristine structure of MWCNTs. The alterations in the pristine structure could be evident from the generation of the two shoulder peaks ~ 25 and ~ 30 respectively. Such alterations in the pristine MWCNT lattice planes signifies the effect of trastuzumab impact over the native structure of the MWCNTs. The effect of such anti-cancerous drugs functionalisation over the pristine structure of MWCNT makes the study of MWCNT in aqueous solution more soluble and bio-functionalised in the approach towards embarking anti-cancerous activities.

5.2.5 FT-IR ANALYSIS

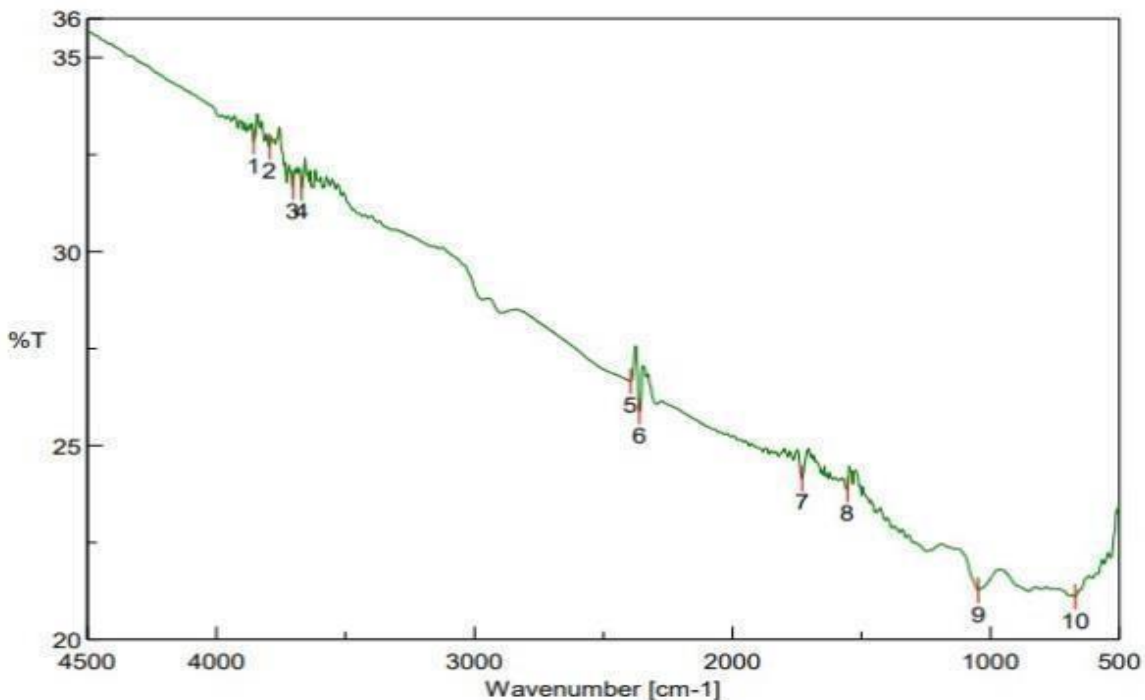


Fig 5.20 FT-IR graphical image for MWCNT

It is seen in Figure 5.20 the native structure of MWCNT shows the predominant **inverse-wavenumbers** around 3500 cm⁻¹, 2500 cm⁻¹, 1500 cm⁻¹ & around 1000 cm⁻¹, respectively. Such inverse peaks are attributed due to **C-H** stretching, **C-O** stretching, **C=O** and **C-C** stretching vibrations, respectively. Such characteristic vibrations in the molecules of MWCNT indicates the predominant proportions of Carbon (C) & Oxygen (O), respectively.

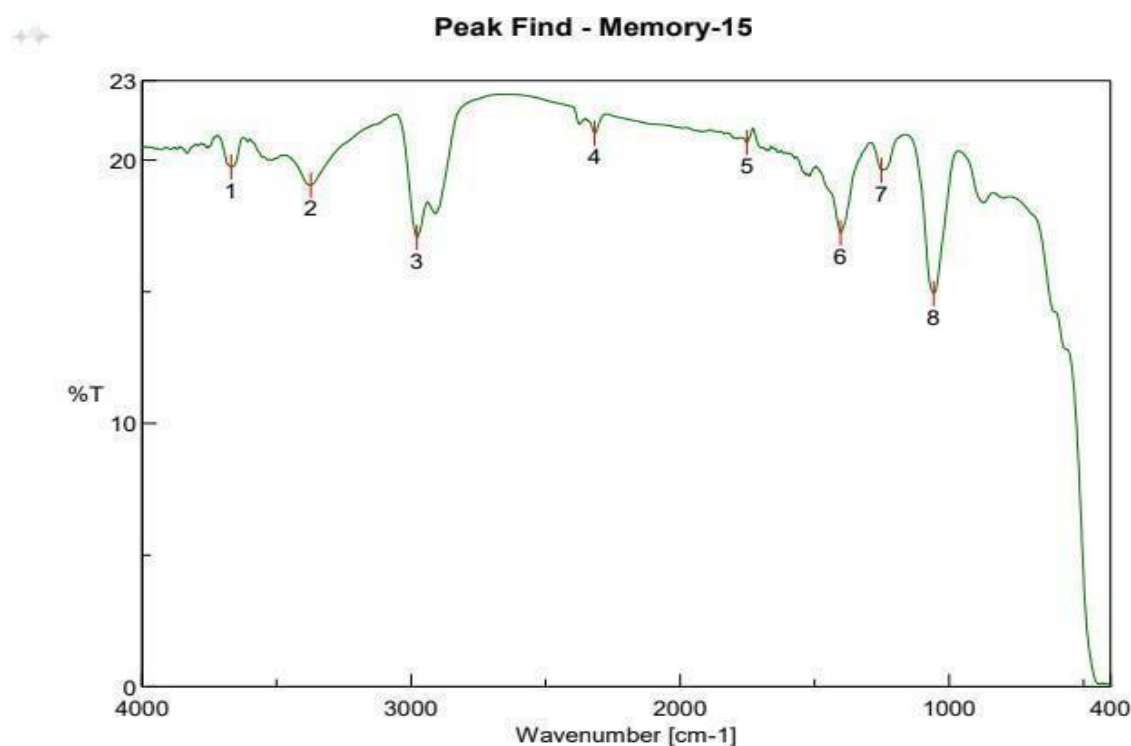


Fig 5.21 FT-IR graphical image for MWCNT with Trastuzumab

It is seen in Figure 5.21 that upon conjugation with the trastuzumab compound, the basic architectural inverse peaks at around 3500 cm^{-1} , 2500 cm^{-1} , 1500 cm^{-1} & around 1000 cm^{-1} seems to be altered and more dipping in peaks is observed in the conjugated compounds. The characteristic inverse dipping in the conjugated version signifies the native structural deformations in the basic inherent structures of MWCNT, which was noticed in the un-conjugated case. Due to the incorporation of the trastuzumab compound over and inside by both physisorption and chemisorption mechanisms over the MWCNT structure, the stretching vibrations of **carbon** and **Oxygen** seems to be perturbed, which further justifies the successful conjugation of the MWCNT-trastuzumab compound. Such composites would be introduced into the biological milieu for understanding the complex interplay of the nano-biological interactions.

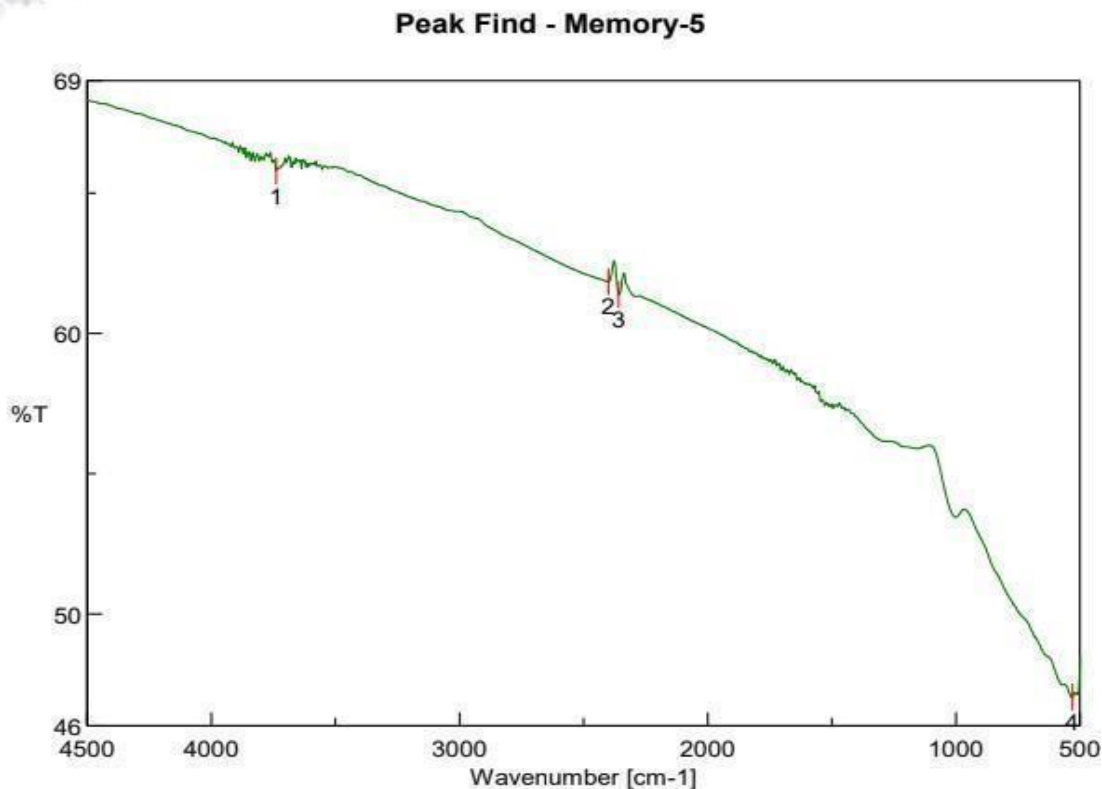


Fig 5.22 FT-IR graphical image for MWCNT with Doxorubicin

It could be seen from the figure 5.22 that upon conjugation of the **MWCNT with the doxorubicin** structure, there is a net significant interactions taking place at the surface of the MWCNT structure. The native vibrations around 3500 cm^{-1} , 2500 cm^{-1} , 1500 cm^{-1} & 1000 cm^{-1} indicates the suppressive mode of inverse dipping taking place at the **MWCNT-doxorubicin** composite. The inherent re-arrangements of the MWCNT-doxorubicin composites are taking negligibly at the surface of the MWCNT compound upon introduction with the doxorubicin molecule. It is evident from the FT-IR analysis of the composite that, only surface passivation and physisorption mechanism has been taken place at the surface of the MWCNT structure with doxorubicin introduction. Such composites are sufficient enough to elicit potential biological responses, when introduced into the biological systems.

5.3 BIOLOGICAL ASSAY ANALYSIS

MTT assay of each sample to check cytotoxicity

MWCNT WITH TRASTUZUMAB:

Table 5.1 MTT assay – sample concentration and cell viability for MWCNT with Trastuzumab

Sample concentration	% of cells
Stock (1mg/ml)	15.6
Dil 1 (100ug/ml)	49.7
Dil 2 (10ug/ml)	101.6
Dil 3 (1ug/ml)	107
Dil 4(100ng/ml)	110
Dil 5(10ng/ml)	120.3
Dil 6(1ng/ml)	123
Dil 7(100pg/ml)	124.6
Dil 8(10pg/ml)	109.3
VC	105.9
NC	79.7
Control	100

Calculation proceeds as per standard protocol of reference paper.

Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods 1983, 65, 55-63.

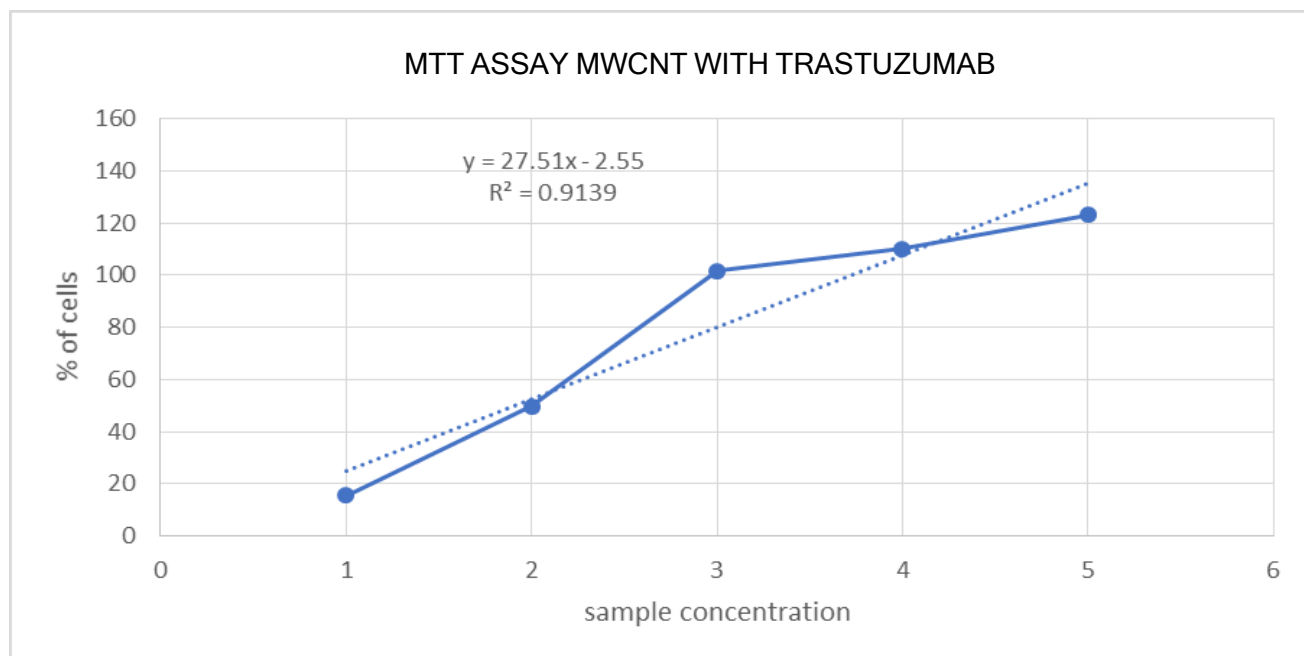


Fig 5.23 Graphical representation of mtt assay for MWCNT with Trastuzumab

IC50 VALUE CALCULATION:

$$y = mx + c$$

$$y = 50 \text{ (ic50)}$$

$$m = 27.51$$

$$c = -2.55$$

$$x = (y - c) / m$$

$$\text{IC50} = 1.7248$$

The IC50 value for MWCNT with trastuzumab shows that the effectiveness of the compound is at 1.7248 concentration which when compared to other samples is relatively less therefore this is more suitable for cancer therapeutics.

Table 5.2 MTT assay – sample concentration and cell viability for MWCNT with Doxorubicin

Sample concentration	% of cells MCF7
Stock (1mg/ml)	10.1
Dil 1 (100ug/ml)	32.5
Dil 2 (10ug/ml)	62.5
Dil 3 (1ug/ml)	76.5
Dil 4(100ng/ml)	83
Dil 5(10ng/ml)	83
Dil 6(1ng/ml)	65
Dil 7(100pg/ml)	72
Dil 8(10pg/ml)	68
VC	92
NC	78
Control	100

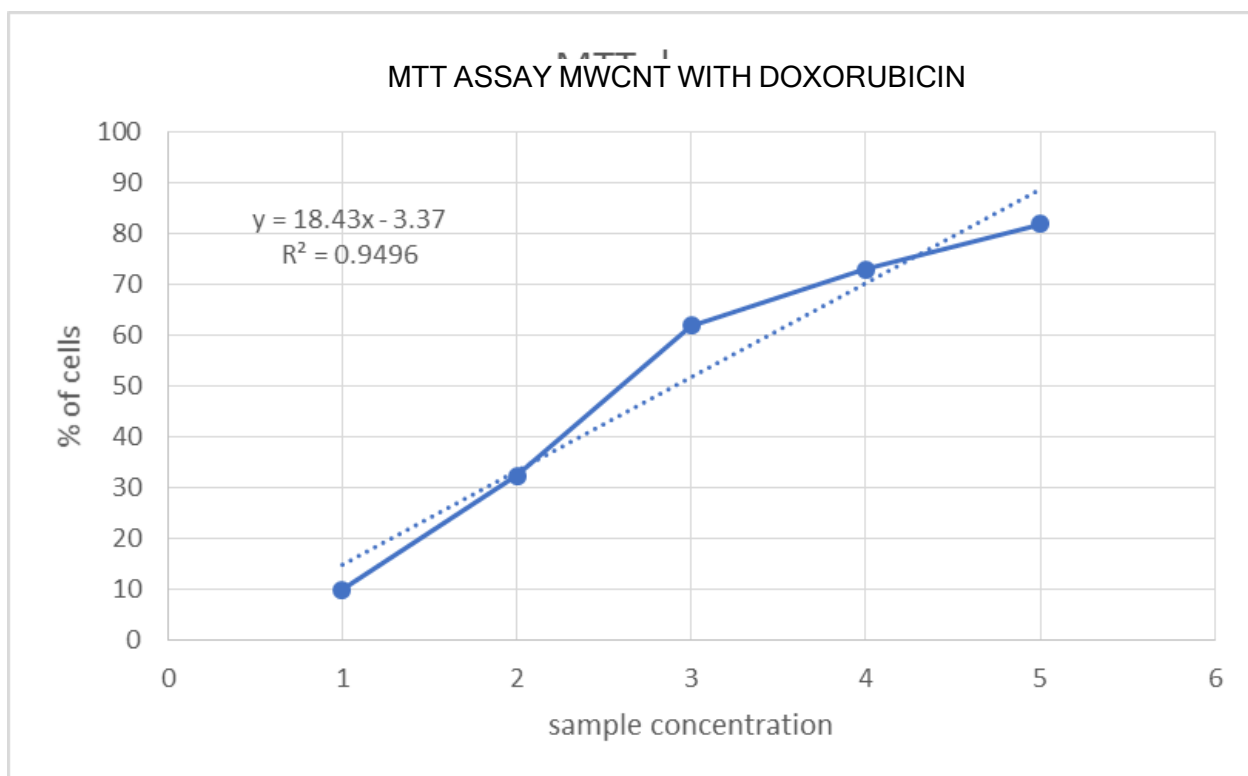


Fig 5.24 Graphical representation of mtt assay for MWCNT with Doxorubicin

IC50 VALUE CALCULATION:

$$y = mx + c$$

$$y = 50 \text{ (ic50)}$$

$$m = 18.43$$

$$c = 3.37$$

$$x = (y - c) / m$$

$$\text{IC50} = 2.5301$$

The IC50 value for doxorubicin is relatively higher compared to MWCNT with trastuzumab though it provides good efficiency it is comparatively lower than trastuzumab

Table 5.3 MTT assay – sample concentration and cell viability for MWCNT (CONTROL)

Sample concentration	% of cells MCF7
Stock (1mg/ml)	60
Dil 1 (100ug/ml)	75.5
Dil 2 (10ug/ml)	85
Dil 3 (1ug/ml)	92
Dil 4(100ng/ml)	105
Dil 5(10ng/ml)	104
Dil 6(1ng/ml)	110
Dil 7(100pg/ml)	118
Dil 8(10pg/ml)	98
VC	101
NC	79.7
Control	100

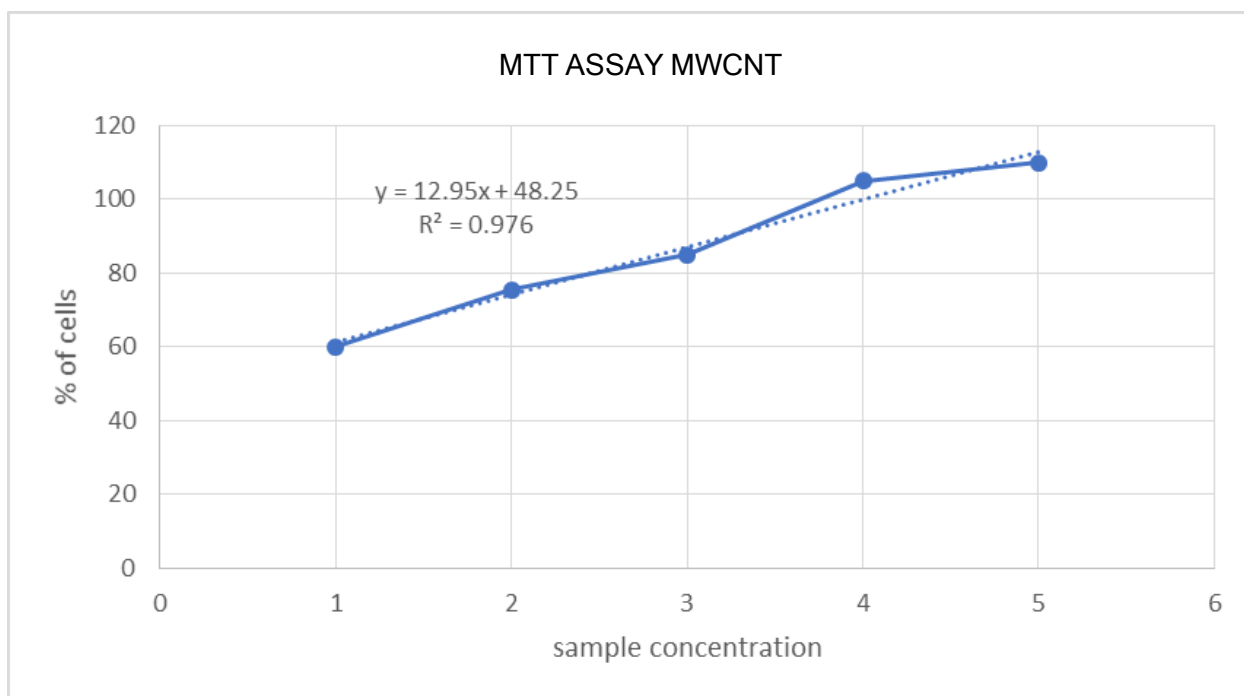


Fig 5.25 Graphical representation of mtt assay for MWCNT (control)

IC50 VALUE CALCULATION:

$$Y = MX + C$$

$$Y = 50$$

$$m = 12.95$$

$$c = 48.25$$

$$x = (y - c) / m$$

$$IC_{50} = 0.13513$$

The IC₅₀ value for mwcnt is used as a control and generally proves that this value alone can also decrease the cell viability of the cancer cells.

The amalgamation of multi-walled carbon nanotubes (MWCNTs) with chemotherapeutic agents like doxorubicin (DOX) and targeted therapy agents such as trastuzumab holds substantial potential in the realm of cancer treatment. MWCNTs possess distinctive characteristics like a high surface area, thermal conductivity, and mechanical robustness, rendering them appealing candidates for drug conveyance

systems.

The integration of DOX into MWCNTs has the capacity to amplify drug delivery effectiveness and mitigate adverse effects. Conversely, trastuzumab targets HER2- positive breast cancer cells, positioning it as a prime contender for combined therapeutic interventions.

The characterization of MWCNT-Doxorubicin nanocomposites plays a pivotal role in comprehending their configuration and attributes. Methodologies such as transmission electron microscopy (TEM) can be utilized to visualize the structure of the nanocomposites, validating the successful incorporation of DOX onto MWCNTs. Moreover, Fourier-transform infrared spectroscopy (FTIR) can be deployed to ascertain the chemical linkages between DOX and MWCNTs, furnishing insights into the mechanism of drug loading. In the research conducted by Li et al., the investigators delved into the utilization of functionalized carbon nanotubes for the purpose of delivering cancer drugs. Their analysis underscored the capacity of these nanotubes to improve the transportation of chemotherapeutic substances to cancerous cells, akin to the methodology expounded in the present article. Nevertheless, in contrast to the current investigation, Li et al. did not concentrate specifically on the amalgamation of MWCNT-Doxorubicin nanocomposites with targeted therapeutic agents such as trastuzumab. The anti-cancer potentials of MWCNT-Doxorubicin nanocomposites emanate from their efficacy in delivering DOX proficiently to cancerous cells. MWCNTs can infiltrate cell membranes and discharge DOX intracellularly, intensifying its cytotoxic impacts. Furthermore, the synergy between DOX and MWCNTs may surmount drug resistance in cancer cells, culminating in enhanced therapeutic consequences. In a separate investigation conducted by Wang et al., the utilization of MWCNTs for conveying paclitaxel, a different chemotherapeutic substance, was explored. The researchers determined that MWCNTs exhibited the capability to effectively transport paclitaxel to malignant cells, thereby augmenting its anti-neoplastic properties. Although this particular research did not directly juxtapose MWCNT-Doxorubicin nanocomposites against alternative drug conveyance mechanisms, it offers supplementary substantiation regarding the prospective application of MWCNTs in the realm of cancer treatment.

The amalgamation of MWCNT-Doxorubicin nanocomposites with trastuzumab can bolster their anti-cancer efficacies, particularly in the context of HER2-positive breast cancer. Trastuzumab precisely targets HER2 receptors, which are amplified in specific breast cancer instances, whereas MWCNT-Doxorubicin nanocomposites transport DOX to cancerous cells. This dual-targeting strategy has the potential to ameliorate the efficacy of chemotherapy and curtail systemic toxicity. To conclude, the examination of MWCNT-Doxorubicin nanocomposites in conjunction with MWCNT and trastuzumab for cancer therapy exhibits substantial potential.

The characterization of these nanocomposites is imperative for understanding their structure and functionality. The anti-cancer capabilities of these nanocomposites, particularly when combined with targeted therapeutic agents like trastuzumab, present a fresh approach to enhancing cancer treatment outcomes. Further investigation in this domain is warranted to fully harness the therapeutic advantages of MWCNT-based drug delivery systems in oncology.

The alignment with Engineering Responsibilities and SDG3 can be observed in the development and utilization of MWCNT-Doxorubicin nanocomposites. This alignment encompasses various engineering responsibilities, particularly PSO1, which involves the conceptualization and implementation of solutions for technical challenges within the healthcare sector and the environment. The utilization of these nanocomposites introduces a new strategy for drug delivery that has the potential to tackle obstacles in cancer treatment, showcasing the engineer's role in contributing to the health and well-being of society (PO6). Moreover, the research on MWCNT-Doxorubicin nanocomposites directly contributes to the achievement of Sustainable Development Goal 3 (SDG3) - aiming to ensure healthy lives and promote well-being for individuals of all ages. The focus on developing innovative cancer therapies through nanotechnology signifies a dedication to enhancing healthcare outcomes on a global scale.

CHAPTER 6

SUMMARY AND CONCLUSION

In conclusion, the investigation into the potential anti-cancer properties of multi-walled carbon nanotube (MWCNT)-doxorubicin nano-composites, in conjunction with MWCNT-trastuzumab, emerges as a highly promising path within the realm of cancer therapy research. By amalgamating MWCNTs with chemotherapeutic agents such as doxorubicin and targeted antibodies like trastuzumab, researchers have achieved significant progress in enhancing both the effectiveness and specificity of cancer treatment modalities. The distinctive characteristics exhibited by MWCNTs, which encompass a substantial surface area, biocompatibility, and the capability to traverse cellular membranes, render them as exceptionally suitable candidates for drug conveyance mechanisms. Furthermore, the precision in delivery facilitated by trastuzumab serves to further heighten the specificity of treatment towards cancerous cells that exhibit an overexpression of the HER2 receptor. Through the utilization of these nanocomposites, the primary objective of researchers revolves around the enhancement of drug delivery efficiency, mitigation of systemic toxicity, overcoming of drug resistance, and amplification of therapeutic outcomes in individuals afflicted by cancer. The ongoing dedication to research endeavors that concentrate on refining the synthesis, delineation, and biological assessment of MWCNT-centered nano-composites harbors immense potential for the origination of groundbreaking and efficacious anti-cancer interventions.

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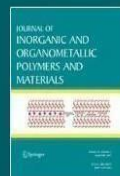
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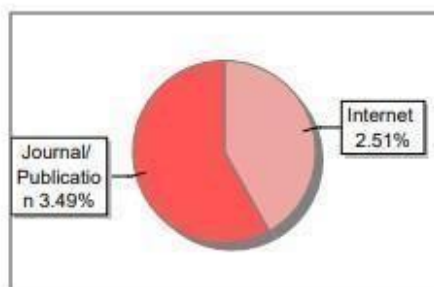
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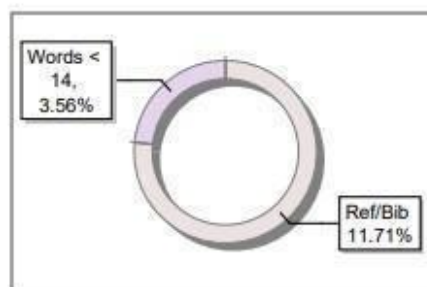
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PSO1	Exploring the anti-cancer potentialities of MWNT-Doxorubicin nanocomposites aligns with PSO1 by enabling engineers to conceptualize and implement innovative solutions for technical problems in the healthcare domain. By leveraging nanotechnology to enhance cancer treatment, engineers contribute to addressing healthcare challenges, integrating technology into medicine, and fostering interdisciplinary collaboration to improve patient care and outcomes.
P06	Exploring the anti-cancer potentialities of MWNT-Doxorubicin nanocomposites aligns with PO6 - The engineer and society by enabling engineers to contribute positively to society's well-being, prioritizing health and safety considerations, addressing legal and cultural issues, recognizing their global responsibilities, and upholding their professional duties in engineering practice.
SDG3	Exploring the anti-cancer potentialities of MWNT-Doxorubicin nanocomposites aligns with SDG 3 by contributing to the improvement of cancer treatment, enhancing access to affordable medicines, advancing research and innovation in healthcare, promoting health and well-being, and fostering international collaboration to address global health challenges.
PEO2	By exploring the anti-cancer potentialities of MWNT-Doxorubicin nanocomposites, technologists not only contribute to the advancement of cancer research but also embody the traits outlined in PEO 2. They demonstrate commitment, research aptitude, lifelong learning, investigative approach, and multidisciplinary thinking, which are essential for making meaningful contributions to science and technology.



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