

EVALUATING THE ANTI-CANCEROUS ACTIVITIES OF FUNCTIONALIZED AND PRISTINE REDUCED GRAPHENE OXIDE (rGO) NANOSHEETS

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Technology degree in Biotechnology

by

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**DEPARTMENT OF BIOTECHNOLOGY
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SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)
Accredited with Grade “A++” by NAAC**

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This is to certify that this Project Report is the bonafide work of **VYANKATESH PANTAM (40230056)** who carried out Project Phase-2 entitled “**Evaluating the anticancer activity of functionalized and pristine reduced graphene oxide (rGO) nanosheets**” under my supervision from January 2024 to April 2024.

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ABSTRACT

The graphene family has shown immense pertinence for sensing/imaging, gene/drug delivery, cancer therapy, regenerative medicine. Indeed, the large surface area, ease of functionalization, high drug loading capacity, and reactive oxygen species induction potentials have rendered graphene- (G-) and graphene oxide (GO)-based (nano)structures promising candidates for cancer therapy applications. In this work we report anti-angiogenesis and anti-proliferative activity with the help of using graphene materials from the graphene family. Functionalization of drug with a nanomaterial is effective against the cancer cell. As it hinders the growth of the cancer cell and stops the growth of tumor blood vessels. Colon cancer cell (HCT-116) which can be treated with drugs but as most drugs gets absorbed in the gastrointestinal tract so with the help of nanomaterial drugs can be easily transported to target sites. Even for hydrophilic drugs, high molecular weight drugs, also those which are substrates of efflux. Another advantage of the nano- drug delivery is the first-pass hepatic metabolism that can result in reduced drug bioavailability. Nanomaterial can also act on the receptor, so nano drugs can target the cancer cell responsible receptor for colon cancer cells there is Muscarinic receptors which leads to the M3R, MMP7, MMP which activates the MAPK pathway, which is responsible for cell proliferation, cell migration, invasion so alternate methods to target early cancer growth in this work.

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List of Abbreviations:

ABBREVIATIONS	FULL FORM
rGO	Reduced Graphene Oxide
GO	Graphene Oxide
SEM	Scanning Electron Microscopy
EHS	Environmental Health and Safety
GBM	Graphene-Based Materials
GFNs	Graphene Family of Nanoparticles
ZGNR	Zigzag graphene nanoribbon
ROS	Reactive oxygen species
FRET	Fluorescence Resonance Energy Transfer
ssDNA	Single-stranded DeoxyriboNucleic Acid
2D	Two Dimensional
ATP	Adenosine Triphosphate
HIV	Human Immuno Virus
PEG	Poly Ethylene Glycol
ZOI	Zone of inhibition
MRGO	Modified Reduced Graphene Oxide
OTC	Over the Counter drug

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1. INTRODUCTION

Carbon plays a unique role in nature. The formation of carbon in stars as a result of the combining of three α -particles is a crucial process that leads to the existence of all the relatively heavy elements in the universe. Carbon demonstrates unusually complicated behavior, forming several very different structures. the recent progress of graphene oxide-based multifunctional nanomaterials for cancer treatment. A comprehensive and in-depth depiction of the unique property of graphene oxide-based multifunctional nanomaterials is first interpreted, with descriptions about the suitability for applying in cancer therapy. Graphene oxide (GO) is the oxidized version of G, which is usually produced under harsh oxidation conditions. GO possesses numerous oxygen-bearing functionalities, such as hydroxyl, carboxylic, and epoxide groups on the carbon surface, rendering it more hydrophilic than (G. Zhu Y, *et al.*2010) Several investigations observed that rGO nanoparticles (NPs) exert selective toxicity toward tumor cells, causing fewer effects on normal cells. For example, earlier we observed that rGO NPs induce cytotoxicity in human cancer cells (*J. Nanomed,et, al.* 2012). Drug resistance and inability to distinguish between tumor and normal cells are important hurdles in cancer treatment. In recent times, nanoparticles (NPs) grabbed great attention to challenge this global issue because of its inherent potential of killing cancer cells without exerting much toxicity to normal cells. Graphene derivatives, especially reduced graphene oxide (rGO), has been considered a revolutionary nanomaterial and shows crucial application prospects in energy, environment, and biomedicine. Hence, NCs of rGO are expected to achieve enhanced anticancer performance. Moreover, anticancer potential of pure NPs and RGO NCs was investigated in two human cancer cell lines. Reduced graphene oxide (rGO) nanosheets that mimic single-layered graphene nanosheets have been generated by decreasing the polar groups on graphene oxide (GO). The planar structure of rGO serves a double-edged sword: it provides a high capacity for hydrophobic interactions among various functional molecules, but it leads to formation of aggregates with poor dispersion stability under

physiological conditions Zrazhevskiy P,et, al.2013). The major drawback of the current cancer therapy strategies is the inability to deliver specific drugs to the target, causing the drug to affect both healthy and cancerous cells alike.

Most drugs used in conventional therapeutic strategies have low solubility, high metabolism and are hydrophobic, making them biologically unavailable leading to systemic toxicity. (Zrazhevskiy P,et al .2013) The development of nanomedicine increases the possibility of specific targeted delivery, which overcomes tumor barriers. It is expected to better facilitate drug delivery and increase efficacy while minimizing the side effects of anticancer drugs (G., Kim, et al 2014). This study shows the enhance study against cancer cells with using graphene family and conjugate with the drug

Graphene is extremely conductive, and flexible, and has adjustable permittivity and hydrophilicity properties (Geim, 2009; K.S.Novoselov, 2011). Reduced graphene oxide (rGO) has a rough surface area, which disrupts the cell wall of the bacteria and fungus and elicits the apoptosis mechanism over them. The SEM image of reduced graphene oxide is depicted in *Figure 1.3*. These characteristics pave the way for the creation of multipurpose biomedical devices and antibiotics or drug carriers (Konstantin S Novoselov et al., 2012). Evaluation of binding affinities of graphene with components of the biological environment to ascertain the possibilities and constraints provided is a highly important and significant topic for such implementations. Graphene biological studies should be carried out using highly specialized, carefully constructed, and described materials under predetermined exposure. Nanomaterials linked to graphene have now been created in a variety of sizes, shapes, and surface modifications, giving them a range of different physical, chemical, and biological capabilities.

In-vitro, ecotoxicological investigations and thorough evaluations of the biocompatibility of graphene-related materials before in vivo application are essential to produce graphene-related biomedical products. The study of the biological effects of the graphene family of nanomaterials is rapidly growing, with new areas of inquiry focused mostly on medical implications as well as Environmental Health and Safety (EHS). Like any other nanomaterial, the possibility of toxicity raises questions in non

biomedical sectors where unanticipated occupational, consumer, and environmental exposures might arise (Kostarelos and Novoselov, 2014). Since 2010, several studies and analyses on the nanotoxicology and biocompatibility of materials connected to graphene have been published (Kiew *et al.*, 2016). The literature on the graphene-biological interface is evaluated in this study, with an emphasis on basic biological reactions and processes that are crucial for both practical applications and material safety. It is emphasized throughout the project that variations in the potential biological effects may lead to a variety of biological responses that, if understood, can be wisely used for function or security reasons (Sanchez *et al.*, 2015)

2. REVIEW OF LITERATURE

2.1 HISTORY OF GRAPHENE NANOSHEETS

Andre Geim and Konstantin Novoselov, two researchers at the University of Manchester in the United Kingdom, discovered graphene in 2004, making it a relatively new substance in the realm of materials science (Miller *et al.*, 2018). Yet, since the 1940s, the concept of a two-dimensional substance made exclusively of carbon atoms organized in a hexagonal lattice has been proposed. The discovery of graphene was made possible by the use of a simple procedure called mechanical exfoliation, in which a layer of graphite was repeatedly scraped away using sticky tape until just a single layer remained (Meyer and Hviid, 2013). Scanning tunneling microscopy was then used to study this single layer of graphene, confirming its special characteristics. Due to its remarkable mechanical, thermal, and electrical characteristics, graphene has subsequently become a hotly debated scientific topic. Being one of the most conductive materials and the strongest substance yet known, it is often regarded as both. Its special mix of attributes makes it a potentially valuable material for a variety of applications, including electronics and energy storage as well as biomedical devices and sensors. Geim and Novoselov received the 2010 Nobel Prize in Physics for their revolutionary work on graphene (Wolf, 2014). Since that time, graphene research has expanded, and new applications and prospective uses are consistently found.

2.1.1 *Reduced graphene*

Reduced graphene is a kind of graphene that has undergone a reduction procedure to remove part or all of the oxygen functional groups that are commonly present on the surface of graphene oxide (Thakur and Kandasubramanian, 2019). This technique results in a material that is more comparable in characteristics to pure graphene. A research team led by Rodney Ruoff at the University of Texas at Austin announced the first reduction of graphene oxide in 2006. They employed hydrazine as a reducing agent to remove the oxygen functional groups from graphene oxide, reduced graphene oxide (rGO). Since then, the manufacture of rGO has been a focus of

significant study, with numerous techniques for reducing graphene oxide and producing rGO with varying characteristics being discovered. Chemical reduction, thermal reduction, and electrochemical reduction are some of these approaches (Olabi *et al.*, 2021). Reduced graphene oxide possesses features comparable to graphenes, such as excellent electrical conductivity and mechanical strength, but with the additional benefit of being simpler to produce and process (Zhu *et al.*, 2010). It is a promising material for a variety of applications, including energy storage, catalysis, and sensing. Overall, the invention of reduced graphene oxide has created new opportunities for investigation and prospective applications in the area of materials science

2.1.2 Graphene oxide

A graphene derivative with surface-attached oxygen functional groups is called graphene oxide. By processing graphite with a solution of potassium chlorate and sulfuric acid, Benjamin Brodie created the first synthetic version of it in 1859 (Brisebois and Siaj, 2020). This substance was known as graphite oxide. Unfortunately, it took a long time for the genuine single-layer nature of graphene oxide to be understood. When Andre Geim and Konstantin Novoselov and their colleagues at the University of Manchester discovered the extraordinary characteristics of graphene in 2003, interest in graphite oxide and its derivatives was rekindled (Neto and Novoselov, 2011). The first effective mechanical exfoliation of graphene from graphite was announced in 2004 by a different team of researchers at the University of Manchester, headed by Geim and Novoselov (Tiwari *et al.*, 2018). They did, however, also point out that the exfoliation procedure often resulted in graphene oxide flakes, which they referred to as a "dirty version of graphene." Since then, several techniques have been developed to synthesis, analyze, and use graphene oxide, which has been a focus of significant study. It is often used as a starting material for the creation of reduced graphene oxide, a material with characteristics closer to those of pure graphene (Jiříčková *et al.*, 2016)

possible uses for graphene oxide exist, including healthcare, energy storage and electronics. It is a potential material for a variety of applications because of its distinctive qualities, including its large surface area, water dispersibility, and customizable functional groups (D. Chen *et al.*, 2012).

2.1.3 Reduced graphene oxide

Reduced graphene oxide (rGO) is a kind of graphene oxide that has had its surface oxygen functional groups partially or completely removed by a reduction process (Chowdhury *et al.*, 2015). A material with characteristics that are more akin to pure graphene is produced by this technique. Graphene oxide was successfully reduced for the first time in 2006, according to research done at the University of Texas at Austin under the direction of Rodney Ruoff (Warner *et al.*, 2012). They created rGO by stripping the oxygen functional groups from graphene oxide using the reducing chemical hydrazine. Since then, other approaches to reducing graphene oxide have been created, including chemical reduction, thermal reduction, and electrochemical reduction (Sharma *et al.*, 2017). Several techniques have been utilized to create rGO with different characteristics and levels of oxygen removal. High electrical conductivity and strong mechanical strength are two characteristics of reduced graphene oxide that are comparable to those of graphene, with the additional benefit of being simpler to produce and treat. It is a promising substance for several uses, such as energy storage, catalysis, and sensing. Overall, the creation of reduced graphene oxide has increased the possible uses of graphene-based materials in a variety of disciplines and created new prospects for the manufacturing of customized graphene-like materials (Jindal *et al.*, 2022).

2.2. SYNTHESIS OF GRAPHITE TO GRAPHENE

2.2.1. Physical method

There are several physical methods for the synthesis of graphene from graphite, including:

2.2.1.1 Mechanical exfoliation

With the help of scotch tape or another sticky tape, different layers of graphene is separated from bulk graphite in this process (Lin *et al.*, 2011). To remove a thin coating of graphite that may subsequently be further processed to produce graphene.

2.2.1.2 Chemical Vapor Deposition (CVD)

A carbon-containing gaseous precursor is introduced into a heated furnace during this procedure, where it breaks down and deposits a layer of graphene on a substrate. Different materials, such copper or nickel, may be used to construct the substrate, and the quality and thickness of the graphene can be controlled by adjusting the reaction conditions.

2.2.1.3 Plasma-enhanced chemical vapor deposition (PECVD)

A plasma-enhanced is usually employed in this CVD version to enhance up chemical graphene processes and increase the quality and uniformity of the graphene layer (Sun *et al.*, 2015)

2.2.2 Chemical method

Different types of methods are available for create graphene such as:

2.2.2.1 Hummer's method

This procedure, concentrated sulfuric acid, potassium permanganate, and sodium nitrate are used to oxidize graphite and form graphene oxide (GO). A reducing agent like hydrazine may be used to further reduce GO to produce reduced graphene oxide (rGO).

2.2.2.2 Chemical reduction of graphene oxide

By using then hummers method a reducing agent such as sodium borohydride, ascorbic acid, or hydroquinone, GO size reduced and generate rGO. Graphene is created when the sp^2 carbon-carbon bonds that were broken during the oxidation process are repaired during the reduction step.

2.2.2.3 Solvothermal method

With this technique, graphite is mixed with a solvent, such as N-methyl pyrrolidone (NMP), and then heated to high pressures. Exfoliation and reduction of graphite occur as a result of the process, of producing graphene (Zhang *et al.*, 2018).

2.2.2.4 Electrochemical exfoliation

In this technique, graphite is exposed to an electric field while submerged in an electrolyte solution. Graphene is created when graphite exfoliates due to an electrochemical process.

2.3 PHYSICO-CHEMICAL PROPERTIES OF 2D GRAPHENE SHEETS

2.3.1 Surface properties of graphene

Primary scattering mechanisms and associated transport features of graphene are strongly dependent on the extent of possible disturbance or breakdown of the underlying sublattice symmetries (Ritter *et al.*, 2009). Since all intravalley and intervalley scattering events between K and K' sites are permitted, short-range interactions in graphene may reveal a wide spectrum of physical phenomena. Chirality breaking, which can be brought about by short-range potentials produced by atomically sharp defects such as vacancies, Anderson disorder, or edge deformations, results in strong backscattering events and localization effects (Rosales *et al.*, 2009). Specifically, the increased reactivity of borders that may be susceptible to chemical passivation, irregularity, and structural restoration makes graphene nanoribbons intrinsically vulnerable to edge disorder (Cresti and Roche, 2009).

According to Bitounis *et al.* (2013), graphene oxide has significant physicochemical characteristics, such as a large surface area, ease of surface functionalization, and enhanced colloidal stability in aqueous environments. However, it's critical to carefully assess the effects graphene-based materials (GBMs) have on cells and tissues as more and more of them are manufactured and used. Understanding the interconnections of the immune system is vital (Farrera and Fadeel, 2015). The innate immune system's phagocytic cells, such as dendritic cells (DCs), macrophages, and neutrophils, will come into contact with an external substance that has entered the body. These cells eliminate dead cells, which is crucial for maintaining tissue homeostasis, in addition to acting as the body's first defense against external intruders.

2.3.1.1 Vibrational and Thermal properties

Acoustic phonons are primarily responsible for individual graphene thermal conductivity at room temperature. When the number of layers is reduced, umklapp processes are suppressed and the large value, which is related to long entail routes of phonons, is explained (Ghosh *et al.*, 2010). In two-dimensional crystals, the acoustic flexural mode ZA's quadratic dispersion is close to the Brillouin zone point and exhibits uniqueness in the density of states at zero energy. Because thermal fluctuations are expected to produce atomic displacements as large as the interatomic distance at certain temperatures, low-dimensional crystals should be unstable. On the other hand, it is demonstrated that macroscopic graphene samples remain stable and retain their crystal quality, It is thought to be caused by tiny crumpling that exists in the third dimension. In ballistic motion, graphene exhibits isotropic thermal conductivity. Below 20K, conduction is inhibited by the ZA mode. Above 20K, the LA and TA modes are also present, even though the ZA mode dominates in terms of thermal conduction. At higher temperatures, the primary heat transporter is likewise the ZA mode, according to the accurate solution of the phonon Boltzmann equation.

Immune cells and other biomolecules in the biological environment experienced a dynamic modification in their fundamental properties upon exposure to GBNs, potentially leading to biotransformation or destruction. Thus, for applications in the future, in-situ evaluations are crucial (Jiménez and Tongeren, 2017). Recent studies have looked at the toxicity of GBNs in a number of taxa, including bacteria, plants, invertebrates, and vertebrates, as well as their principal target organs, the immune system and the cardiovascular system. After an initial invasion, there is evidence that GBNs can penetrate physiological barriers and reach distant secondary organs (Fadeel *et al.*, 2018).

2.3.1.2 Mechanical properties

Using an atomic force microscope, we were the first to directly evaluate the mechanical characteristics of monolayer graphene AFM by suspending nano-indenter-suspended monolayer graphene membranes. With the confirmation of its long-lasting breaking strengths with an intrinsic mechanical strain and Young's modulus, graphene was dubbed the "strongest material ever" (Lee *et al.*, 2008). It is also possible to adjust the material's mechanical thickness, as demonstrated by machine-driven pressure experiments performed on graphene sheets subjected to deflections caused by the application of various insulating capping layers (Ni *et al.*, 2008). A variety of theoretical investigations have been conducted to confirm the experimental results regarding the fundamental mechanical properties of graphene. Many methods, including the ab initio method (F. Liu *et al.*, 2007),

Precise measurements of graphene's Young's modulus and other intrinsic mechanical properties have been obtained by tight binding (Pereira *et al.*, 2009), molecular dynamics simulations, and semiempirical models (Scarpa *et al.*, 2009). Graphene's remarkable mechanical properties have also attracted the interest of electronic applications because these thin, robust, and versatile materials might be used to create structural components for nanoelectromechanical systems (NEMS). A perfect match between the machine-driven and electric responses of the conductive channel is necessary for the development of low-cost NEMS devices. According to Q. Lu and Huang (2010), the feasibility of conducting band gap engineering with the aid of external mechanical forces determines the effectiveness of a graphene-based NEMS.

2.3.1.3 Magnetic properties

Several theoretical research based on density functional theory or the mean-field Hubbard model predicts that the edge states of graphene would become spin-polarized

to a magnetic instability generated by the corresponding spike in the density of states only at Fermi energy (EF) (Yazyev, 2010). The atoms are antiferromagnetically arranged across opposing edges in the electromagnetic ground state when an energy gap at EF is formed and ferromagnetically directed along one edge. This antiferromagnetic ground state is in agreement with Lieb's theorem for a bi-particle lattice in a Hubbard model description. Edge magnetism is a phenomenon that is thought to occur in all graphene systems with zigzag edge segments, not just perfect ZGNRs (Cantele *et al.*, 2009). Building a magneto resistor exploiting the edge magnetism phenomena is an area of potential application (Muñoz-Rojas *et al.*, 2009). However, there hasn't been any experimental proof of graphene edge magnetism to date (Sepioni *et al.*, 2010). Only an indirect observation of magnetic edges using local probe microscopy has been made (Joly *et al.*, 2010). Theoretical works contend that the ideal zigzag-edged appearance is not very likely to occur, which supports the lack of magnetic edge states observed. Some nonmagnetic edge topologies have substantially better thermodynamics (Kunstmann *et al.*, 2011). The antiferromagnetic ground state was also shown to be only stable in perfect ZGNRs at freezing temperatures. Increasing amounts of evidence thus support the lack of edge magnetism as a true occurrence at ambient temperature.

2.3.1.3 Elastic properties

The indentation force-displacement link was thought to be caused by graphene's nonlinear elastic properties, which include a 2D Young's modulus of 340 nm and a second-order elastic toughness of -690 nm in the non-linear domain. Wei and Kysar (Wei and Kysar, 2012) carried out a more comprehensive investigation of the nanoindentation experiment using the finite element technique (FEM) and an anisotropic, singly perturbed elastic model for graphene as anticipated by density functional theory (DFT) simulations. AFM indentation studies were then used to examine the mechanical characteristics of polycrystalline graphene films produced by chemical vapor deposition (CVD) with different grain sizes. Additional research showed that the presence of out-of-plane ripples would significantly diminish the stiffness of graphene in a plane, Visual representations are provided of the small scale or nonlocal impacts on the non-dimensional buckling loads in the presence of aspect ratio and buckling modes (Tourki Samaei and Hosseini Hashemi, 2012).

2.3.1.4 Non-Elastic properties

The existence of faults and their evolution during redirection significantly affect the mechanical characteristics of 2D materials, even in the presence of elasticity. Endurance and robustness are two physically distinct traits that account for the start of failure in terms of stress and energy, respectively. According to basic mechanics, hardness is determined by a material's energy per unit volume, or J/m^3 . The unit J/m^2 , which is more accurately defined by fracture mechanics, is used to indicate the energy content of an area per unit of crack propagation. The energy/length of crack propagation may be used to represent the fracture toughness of 2D materials when utilizing a unit of J/m or eV/nm rather than the 2D Young's modulus (unit: N/m) (S. Singh and Patel, 2014).

2.3.1.5 Strength and Toughness

With an enduring endurance of 130 GPa, graphene has been determined to be the strongest material now in use as a conventional 2D material. The experiments cited have not even demonstrated a qualitative consistency, despite attempts to explain the link between roughness and particle size in polycrystalline graphene by atomic-level calculations (Mohan *et al.*, 2018). From an experimental point of view, the commonly used AFM nano-indentation method does not necessarily yield information on the weakest triple junction or grain boundary that governs a sample's failure strength. Molecular Dynamics (MD) simulations and theoretical models have demonstrated that the failure strength for polycrystalline graphene with randomly dispersed grains displays substantial statistical fluctuations following a Weibull distribution.

2.4 Electrical and electronic properties of graphene

A single layer of carbon atoms makes up the hexagonal lattice known as graphene. A single carbon atom in its ground state has four valence electrons and the electronic shell configuration [He] $2S^2 2p^2$. The carbon-carbon covalent bond in graphene is produced by hybridized orbitals, which are produced when 2s are overlapped with 2px and 2py orbitals (Lizhao Liu et al., 2016). The three nearby carbon atom bonds formed by the planar orbitals, which are responsible for the majority of the binding energy and the elastic characteristics of the graphene sheet, are effectively contained and stabilized by the hexagonal arrangement. The electrical characteristics of graphene are significantly influenced by the junction of these orbital states between nearby atoms. The remaining independent $2p_z$ orbitals have Π symmetry orientation (Yuan et al., 2015). As a result of its electronic states is easily represented by a linear combination of $2p_z$ orbitals, graphene's electrical structure may be accurately approximated by an orthogonal proximity tight-binding approximation. The energy dispersion relation of the Π (bonding) and π^* (antibonding) bands may be obtained by solving the Schrödinger equation, which is reduced to a matrix secular expression (Dubois et al., 2009). The extraordinary electrical qualities of graphene have propelled researchers to investigate their fundamental physical characteristics with the added benefit of converting them into useful applications. Nevertheless, its use in digital devices is severely constrained by the absence of an energy-band gap. Therefore, scientists are seeking fresh methods to make graphene develop a band gap. The electrical state of the graphene surface is altered by an antibody-antigen immunological response, which may be demonstrated, for instance, by a shift in the current passing through a graphene chip (Huang et al., 2020). The final sensitivity of such a device depends on the characteristics of graphene. (Beitollahi et al., 2019)

2.4.1 Opto-electronic properties of graphene sheets and applications

Graphene has extraordinary optical qualities, in addition to the burgeoning interest in its electrical properties. One of graphene's most well-known optical features is its ability to operate as a powerful quencher for a variety of luminous dyes and nanoparticles (Chang and Baek, 2016). Due to two opposing mechanisms—

photoinduced electron transfer and intramolecular energy transfer facilitated by a through-bond mechanism because of the luminophore's covalent attachment—this is the case (Terrones *et al.*, 2011). The wide spectrum luminescence and high optical transmittance of graphene, on the other hand, are outstanding optical qualities. (Figure 2.1)

2.4.2 Tribological properties of graphene sheets

Tribological behavior of the materials has a major impact on the performance of metal matrix composites, boundary lubrication, and ultra-precise mechanical systems such as micro-electromechanical systems (MEMS) and nano-electromechanical systems (NEMS). According to Kumar and Wani (2017), friction and wear in moving parts are frequently the cause of these mechanical failures. Because NEMS and MEMS components are small and have tight tolerances, their resistive conductance greatly affects the efficiency of parts like gears, switches, and actuators, while their wear and tear resistance determines the component's mechanical and commercial viability (Hae Jin Kim *et al.*, 2012).

However, because of surface tension, conventional methods of lubricating fluids to reduce friction cannot be used to microsystems. Improving the tribological characteristics of the parts/components under dry sliding circumstances is essential to the construction of dependable MEMS and NEMS devices. Organic materials such self-assembled monolayers (H. J. Kim *et al.*, 2009), diamond-like carbon (DLC) films (Penkov *et al.*, 2012), bi-layer and multi-layer hard coatings (Penkov *et al.*, 2013), and nanostructured coatings are used to achieve this. Graphene is one such innovative material that has demonstrated a great deal of promise in terms of tribological properties. (Figure 2.2)

Over the last ten years, the world of materials has been more interested in graphene due to its distinct properties, such as strength, hardness, and fracture toughness. Numerous unique physical, mechanical, and electrical features of graphene have been extensively studied (Peng *et al.*, 2008). Additionally, graphitic carbon exhibits a flawless crystallographic sheet. For improved lubricity and wear resistance, graphene

platelets have been used as oil additives (Lin *et al.*, 2011). More significantly, graphene works well as a corrosion protective layer on refined metals like copper and nickel when utilized in a reactive atmosphere for mechanical purposes (Prasai *et al.*, 2012). Prior studies on graphite flakes in tribology have shown that graphite scrolls form at the tribological contact. These scrolls work wonders for reducing surface energy and friction at sliding surfaces. Graphene sheets distinguish themselves from other materials used in tribological applications due to their remarkable chemical, mechanical, thermal, and electrical capabilities as well as their relatively extraordinarily cheap manufacturing costs (Figure 2.2). Consequently, graphene is considered a promising material for future usage in MEMS, NEMS, and other tribological applications. Because of its increased strength, graphene also holds tremendous potential for usage as a self-lubricating material and an ultra-thin protective coating for a range of precision components that are exposed to contact stress.

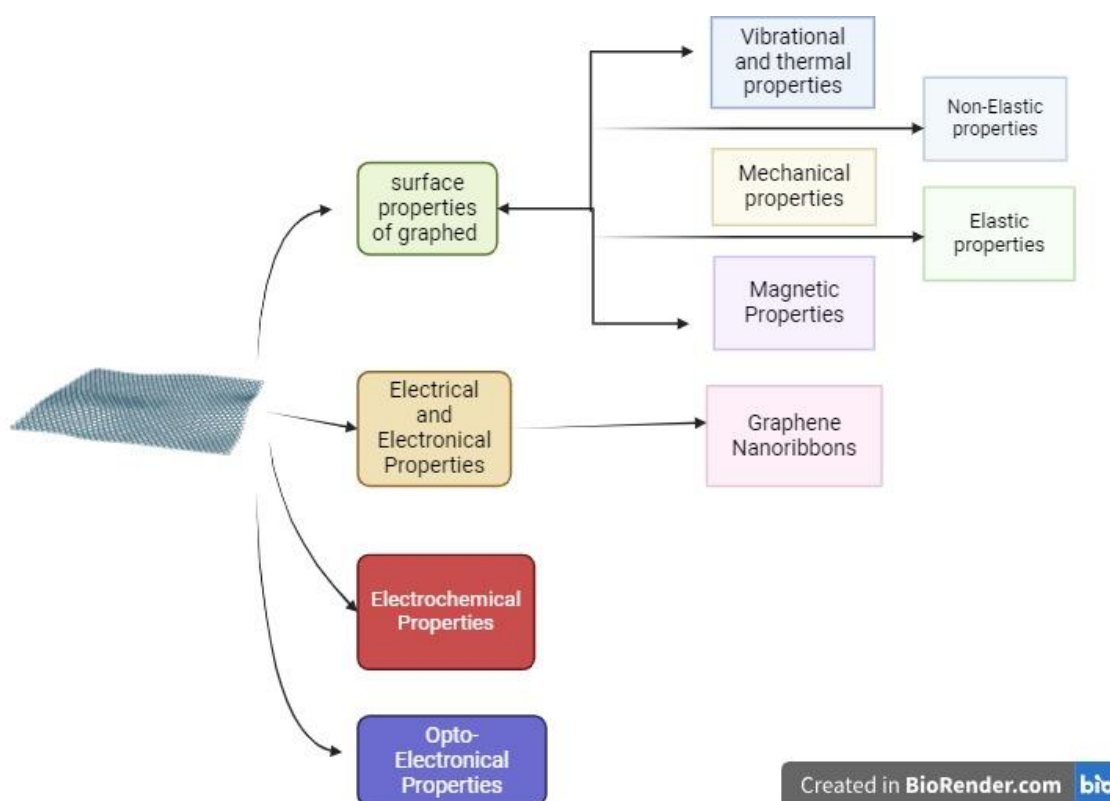


Figure 2.1: Physical and chemical characteristics of 2D graphene sheets



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Figure 2.2: Basic Tribological properties of graphene sheets

2.5 TOXICOLOGICAL STUDIES OF 2D GRAPHENE SHEETS IN BIOLOGICAL SYSTEM

2.5.1 Activity of graphene against cells

Compared to other materials like minerals, metals, and polymers, very little research has examined how cells interact with graphene. As graphene has an unusual surface which is in particular because of its high aspect ratio physical dimension, it is possible to design surfaces with specific qualities that might prevent or encourage bacterial adhesion as well as perhaps trigger cell death. The capacity to tailor the interaction with cells is made possible by various preparation techniques that create single-layer and multi-layer graphene sheets (Jastrzębska *et al.*, 2012). While some research has shown that these surfaces are anti-bacterial, other research has demonstrated that they promote cell adherence. In 2010, (Akhavan and Ghaderi, 2010)

The research by Ruiz (Ruiz *et al.*, 2011) was contradictory to the studies above, they demonstrated that cells are not adversely affected and noted that cells growth rates were normal.

2.5.1.1 Mechanism of interaction

In addition to deterring cell attachment and drug combination, they used PEG400 solution for mix and binding each other and attack on the cancer cells. (Hegab *et al.*, 2016; Zou *et al.*, 2016)

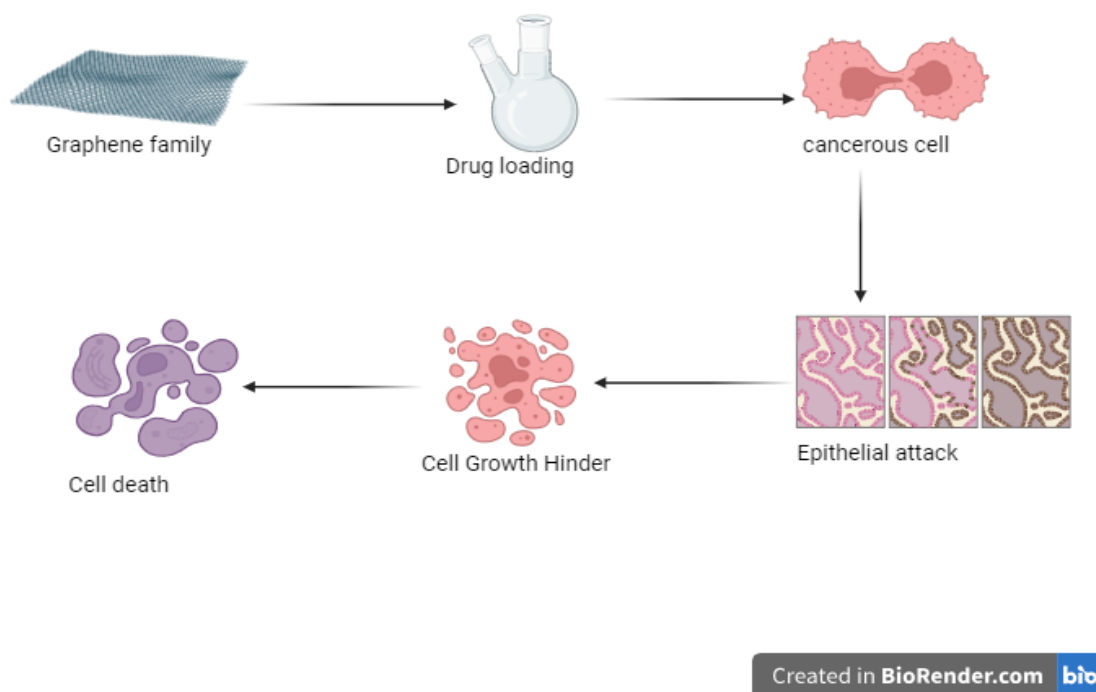


fig. 2.3. Nanomaterial-Drug action on cancer cell basic outer diagram

2.5.1.2 Graphene's action against cancer cell line

Recently, a remarkable use of graphene in biological research has been proposed, to manage plant infections. It is believed that graphene, namely GO, has unique optical, electrical, mechanical, and thermal properties that contribute to its target drug delivery activity. These properties include ease of surface modification, high mechanical strength, great water dispersibility, and photoluminescence (V. Singh *et al.*, 2011). This broadened the application of graphene, which can be used for the management of agricultural diseases. Around the globe, crop diseases brought on by a diverse range of may lower a cultivar's yield by 20–40%. Multidrug-resistant infections have been brought about by the formation of strong, unbreakable resistance in cancer cell as a consequence of continuous and extensive exposure to numerous (Rezzonico *et al.*, 2009). The question of whether graphene oxide has a wide range of anti-cancerous against cancer cell is one worry with employing it as an target site agent. (Figure 2.3)

To understand the mechanism of interaction between GO, a detailed investigation into the use of graphene oxide (GO) against cancer cell lines (HCT-116) was conducted. This study postulated a cooperative mechanism in which a variety of aggregated graphene oxide sheets interact with the cidal activity of both drug (Trans-resveratrol) and nanoparticles to damage local cell membranes, reduce bacterial membrane potential, and cause electrolyte leakage from fungal spores (J. Chen *et al.*, 2014). Cell lysis may be one of GO's primary cytotoxic effects on phytopathogens. This is likely how GO interacts with the pathogens by physically encircling, damaging the pathogens locally, and eventually causing cell lysis

2.5.1.3 Mechanism of interaction (between cell and rGO)

Reduced graphene oxide (rGO) and cell interact in a complicated manner via several processes. Physical and chemical interactions between fungi and rGO may occur, and these interactions can be impacted by a variety of variables, including the size and form of the rGO,. Adsorption is one of the main ways that cell and rGO interact with one another. Many oxygen-containing functional groups, including carboxyl and hydroxyl groups, are present on the surface of rGO and may interact with the cell wall (Yu *et al.*, 2015). The growth, development, and metabolism of the cells may vary as a result of the adsorption of rGO onto the cancer cell wall due to changes in the shape, structure, and permeability of the cell wall. Transferring electrons is another method by which fungus and rGO interact. rGO can move electrons to or from the cells thanks to its strong electron conductivity. The redox balance of the cancer cells and subsequent effects on metabolism, growth, and survival may result from this electron transfer. Reactive oxygen species may also be produced as a result of the interaction between cancer cell and rGO (ROS). ROS may be produced as a result of rGO adhering to the cell wall, which can cause oxidative stress (Reshma *et al.*, 2017).

Overall, adsorption, electron transfer, ROS formation are some of the mechanisms that may be involved in the complicated mechanism of interaction between cell and rGO. Depending on the particular cancer cell line and the characteristics of the rGO used the exact nature of the interaction may change (Radhi *et al.*, 2021)

2.5.1.4 Interaction of graphene with Drug

Most studies have focused on how toxicity might accumulate in drugs with nanoparticles, but little is known about how it can spread to vascular tissues or the risks associated with modified graphene materials. In this work, we used a ^{13}C -stable isotope skeleton- labeling technique to investigate the translocation of graphene in cancer cell line (*HCT-116*.) (L. Chen *et al.*, 2019). Chemical reduction of graphene oxide (GO) produced reduced graphene oxide (rGO), These results emphasize the importance of chemical reduction in the translocation of graphene compounds with drug as well as the risks associated with such materials interfering with critical metabolic processes in the carbon cycle. On the other hand, non-reduced GO was restricted to plant roots and did not affect photosynthesis. This study underlines the importance of the chemical structure of rGO nanosheets and its safe interaction with higher drug concentration (J. Singh and Sengar, 2020).

2.5.1.5 Graphene as a Target drug delivery

At the drug delivery combined with nanoparticles to the cancer cell line, As a drug compound completely dissolves in the graphene and forms a liquid state, GO was tested on the cancer cell lines. The nanomaterial avoids the drug with digestion and absorption in the gastrointestinal tract. The hydrophilic character of GO and its ability to carry water aided in to the target side. The reason for this was that the hydrophobic sp^2 domains of GO were used to transport water molecules to the target from the oxygen- containing functional groups of GO (Y. He *et al.*, 2017). The combination of the sp^2 and sp^3 structures of GO gives it special abilities to function as a potent water transporter. Given the number of oxygen-containing functional groups and the size of its particular surface, GO has great potential as a target drug delivery to cancer cell lines(Zhao *et al.*, 2011).

2.5 *IN-SILICO* ANALYSIS OF REDUCED GRAPHENE OXIDE

With the advent of computer simulations and modeling tools for materials science research, *in-silico* investigation of reduced graphene oxide (rGO) has only recently become a reality. Graphene research, on the other hand, has a long history that dates back to the 1940s. P.R. Wallace predicted the presence of a two-dimensional structure composed of carbon atoms organized in a hexagonal lattice, which led to the discovery of graphene in 1947 (Tiwari *et al.*, 2018). Yet it was not until the 2000s that graphene was isolated and scientifically defined, sparking a boom in research interest in this substance. A derivative of graphene called reduced graphene oxide (rGO) is created by reducing graphene oxide (GO), which is created by oxidizing graphite (Ray, 2015). The majority of the oxygenated functional groups are removed during the reduction of GO to rGO, which also improves the material's mechanical and electrical conductivity by restoring some of the sp^2 carbon-carbon bonds.

Using simulations and computational techniques to analyze the characteristics and behavior of the material is referred to as an *in-silico* study of rGO. Studying the electrical structure, mechanical characteristics, and interactions with other materials are a few examples of this (Piras *et al.*, 2021). Understanding the characteristics of rGO and its prospective uses in a variety of industries, including electronics, energy storage, and sensors, requires the use of *in-silico* analysis. Recent years have seen a significant increase in the use of *in-silico* analysis for rGO research, with many studies concentrating on streamlining the reduction process, enhancing the characteristics of rGO, and creating new uses for this material. *In-silico* research is anticipated to become an increasingly crucial tool for the study of rGO and other graphene derivatives as computational power and simulation methods develop (Wang *et al.*, 2017).

Reduced graphene oxide (rGO) *in-silico* analysis has several potential uses in numerous industries. Here are a few illustrations:

2.5.1 Electronic devices

Based on rGO, electronic devices such as field-effect transistors, sensors, and energy storage devices may be designed and optimized via *in-silico* analysis (Ahmed and Yi, 2017). This includes researching rGO's electrical structure, transport characteristics, interactions with other materials, and more.

2.5.2 Energy storage

rGO is a potential material for energy storage applications because of its large surface area, electrical conductivity, and mechanical robustness. The performance of rGO-based supercapacitors and batteries may be studied, and the material parameters can be optimized for improved performance, using *in-silico* research (Liao *et al.*, 2021).

2.5.3 Catalysis

It has been shown that rGO possesses catalytic activity for several chemical processes, including oxygen reduction and hydrogen evolution. The catalytic capabilities of rGO may be investigated, and its composition and structure can be tailored for certain reactions using *in-silico* studies.

2.5.4 Water treatment

rGO is successful in purifying water of impurities such as organic pollutants and heavy metals. The interactions between rGO and water molecules may be studied using *in-silico* research, and the material characteristics can be improved for better water treatment performance (Pérez-Ramírez *et al.*, 2016).

2.5.5 Biomedical applications

rGO may be used in tissue engineering, bioimaging, and medication delivery. As well as optimizing the material's characteristics for particular biomedical applications, the *in-silico* analysis may be utilized to examine the interactions between rGO and biological molecules.

Overall, rGO *in-silico* analysis has the potential to speed up the creation of novel applications for this substance and to direct experimental research for a deeper comprehension of its characteristics and behavior.

A computer technique called "*in-silico* docking" is used to forecast how tiny compounds (or "ligands") will interact with proteins or macromolecular complexes (receptors). Predicting the binding affinity and position of the ligand inside the receptor's active region requires modeling the interaction between the ligand and receptor.

2.5.6 Trans-resveratrol

Trans-resveratrol is a naturally occurring polyphenolic compound found in various plants, including grapes, berries, and peanuts. It is a type of stilbenoid, characterized by its stilbene structure, which consists of two phenyl rings linked by an ethylene bridge. The trans- form is the biologically active isomer, while the cis- form is less common and less stable and reverse drug resistance in tumor cells by making them more sensitive to chemotherapeutic agents. Trans-resveratrol helps inhibiting STAT3 activation, modulating13K pathway in cancer cell also lead apoptosis. (Pérez-Ramírez *et al.*, 2016).

3. AIM AND SCOPE

- To analyze the physical characteristics of reduced graphene oxide synthesized.
- To recognize which surface property of graphene family nanomaterials elicits the toxicity phenomena over cancer cell lines.
- To formulate the accurate concentration of reduced graphene oxide for target drug delivery over the cancer cell line.
- To check the toxicity pathway of the reduced graphene oxide over the *cancer cell line* and combination of drug (*Trans-resveratrol*)
- To compare the concentrations of Reduced graphene oxide (rGO) with Drug against cancer cell line (*HCT-116*)
- To dock rGO nanosheets with various prokaryotic membranous proteins.
- To perform in-silico analysis of various anticancer and over the counter drug to check the ability of rGO nanosheets to act as drug carrier for targeted drug delivery.

3. MATERIALS AND METHODS

3.1 SYNTHESIS OF GRAPHENE NANOSHEETS AS rGO

Hummer's modified method was used to create rGO from graphite flakes (Deemer *et al.*, 2017). The solution of concentrated sulphuric acid and phosphoric acid, graphite flakes, and the necessary quantity of potassium permanganate (KMnO_4) along with sodium nitrate (NaNO_3) as an oxidizing agent was used in the improved technique (9:1). When the mixture had been properly diluted with ice-cold distilled water and was kept at a temperature below 35°C for 24 hours with steady stirring, 30% hydrogen peroxide was added. The brown GO was then slowly reduced to rGO by refluxing it at 100°C with hydrazine hydrate. The product was then centrifuged, cleaned at least four times with distilled water to remove any remaining contaminants, and dried under a vacuum for a whole night at 80°C . The modified procedure employed by Hummers included KMnO_4 as well as an equal quantity of (Sahudin *et al.*, 2021). Figure 4.1)

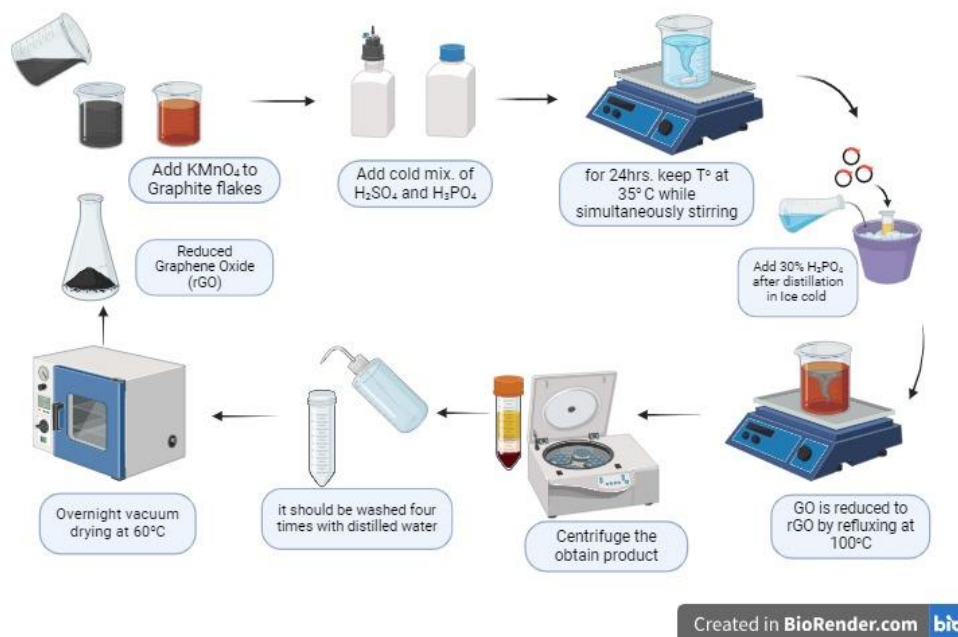


Figure 4.1: Schematic flowchart of synthesis of rGO nanosheets by Modified Hummer's method (Created using Biorender)

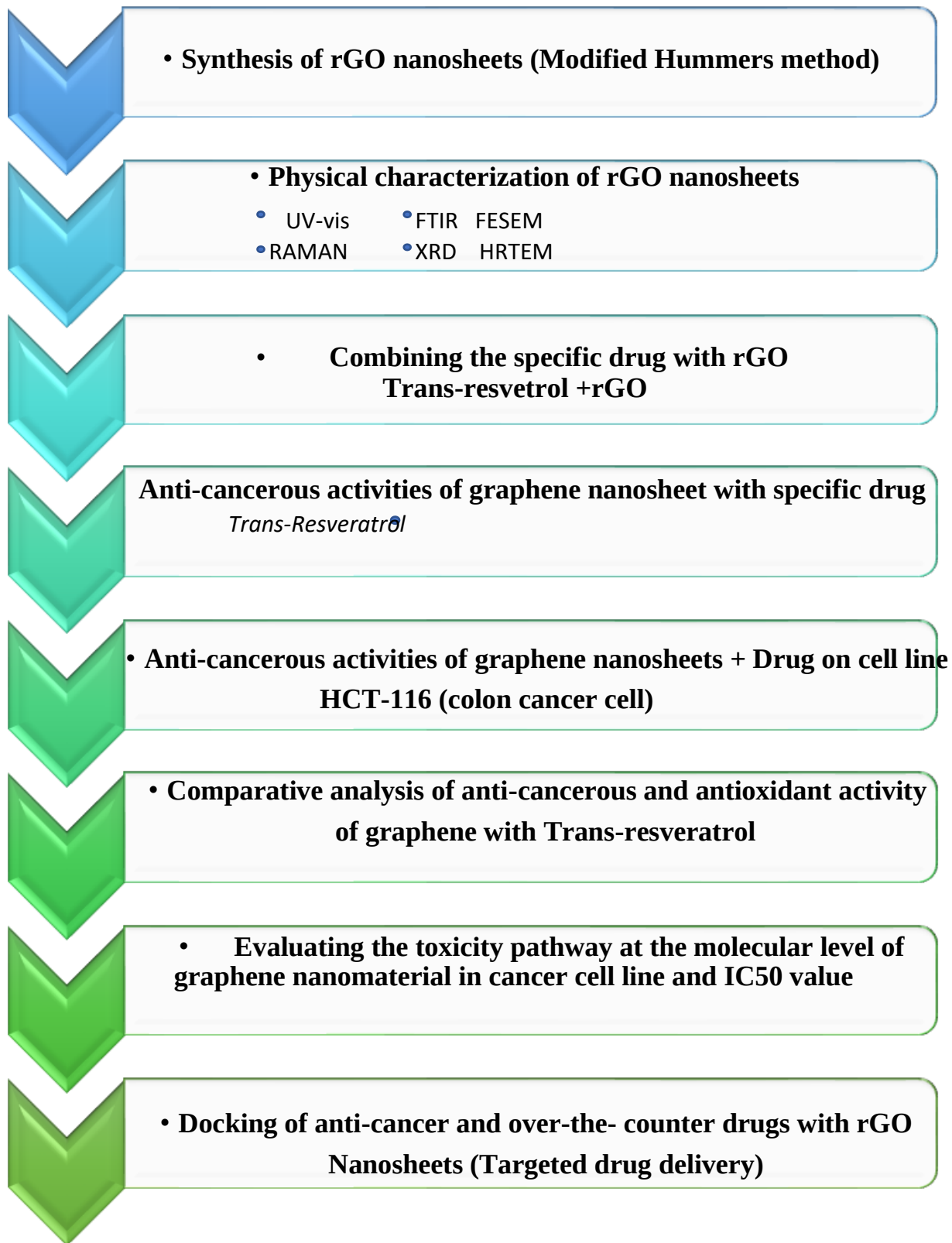


Figure 4.2: Flowchart of Methodology

4.2 PHYSICAL CHARACTERIZATION OF GRAPHENE NANOSHEETS

4.2.1 UV-Visible Spectrometry

An analytical technique suitable for a large class of organic chemicals as well as certain inorganic species, UV-vis spectroscopy is affordable, straight forward, adaptable, and non-destructive (Pons *et al.*, 2004). The absorbance or transmittance of light flowing through a material as a function of wavelength is measured using UV-vis spectrophotometers. UV-vis detectors are integrated into high-performance liquid chromatography and ultra-high-performance liquid chromatography to detect and measure the concentration of chemicals in liquid streams (Rocha *et al.*, 2018). All species identification is facilitated by combining these methods with mass spectrometry. A variation that has improved scattering capabilities for measuring the characteristics of solids and powders is UV-vis diffuse reflectance spectroscopy. The reduced graphene oxide (rGO) that we created using a modified Hummers technique was quantified for this experiment using a Shimadzu UV-1800 purchased from Japan (Piovesan *et al.*, 2018).

Graphene nanosheets may be examined using UV-Visible (UV-Vis) spectroscopy. For this, the following methods may be applied:

4.2.1.1 Sample preparation

An appropriate technique, such as the chemical reduction of graphene oxide, may be used to create graphene nanosheets. To create a stable suspension, the manufactured graphene nanosheets should be mixed with a suitable solvent, such as water or ethanol.

4.2.1.2 Instrument setup

Setting up the UV-Vis spectrophotometer involves modifying variables including baseline correction, slit width, and wavelength range.

4.2.1.3 Measurement

The produced suspension of graphene nanosheets is put into the sample holder of a UV-Vis spectrophotometer, and an appropriate wavelength range is selected for recording the absorbance spectra. The graphene nanosheets' electrical structure and optical characteristics are revealed through the absorbance spectrum.

4.2.1.4 Analysis

The resultant absorbance spectrum may be examined to ascertain the bandgap, number of layers, and degree of oxidation of the graphene nanosheets, among other optical characteristics. The analysis may be carried out using software techniques like peak deconvolution or Gaussian fitting, or it can be done by comparing the resulting spectrum with reference spectra.

It is vital to remember that the stability and quality of the suspension of graphene nanosheets as well as the settings for the instrument may have a big impact on how accurate and repeatable the UV-Vis readings are. To get accurate findings, careful sample preparation and calibration of the equipment are required (Zare *et al.*, 2022).

4.2.2 X-Ray Diffraction

The method of X-ray diffraction (XRD) is often used to describe the composition and characteristics of nanoparticles (da Silva *et al.*, 2011). While the XRD process for nanoparticles is similar to that for bulk materials because, to the nanoparticles' tiny size, several unique considerations must be made. The following are the general stages involved in the XRD analysis of nanoparticles:

4.2.2.1 Sample preparation

The first step is to create a sample of nanoparticles that is evenly distributed. This may be accomplished by utilizing sonication or other techniques to disperse the nanoparticles in a suitable solvent, such as water or ethanol.

4.2.2.2 Instrumentation

A high-intensity X-ray beam with a tiny spot size should be able to be produced by the XRD apparatus used for nanoparticle investigation. For the X-rays to be diffracted from the nanoparticles and not the substrate or any other contaminants in the sample, this is important.

4.2.2.3 X-ray diffraction

A detector then picks up the dispersed X-rays after the sample has been subjected to a high-intensity X-ray beam. The nanoparticles' crystal structure may be recognized by the X-ray diffraction pattern that was produced.

4.2.2.4 Data Analysis

The crystal structure of the nanoparticles is then determined by analyzing the XRD pattern. This entails comparing the pattern's peaks to those in a database of crystal structures that are already known to exist. With the use of several analytical techniques, such as the Scherrer equation, the size, and form of the nanoparticles may also be ascertained from the XRD pattern.

4.2.2.5 Interpretation of results

The XRD data may provide details on the nanoparticles' size, shape, and orientation as well as their crystal structure. The production of nanoparticles may be made more efficient using this knowledge, and their characteristics and behavior can be better understood (Manik *et al.*, 2020).

To establish the crystal structure, size, and form of the nanoparticles, an XRD examination of nanoparticles entails the preparation of a well-dispersed sample exposure to a high-intensity X-ray beam, analysis of the resultant diffraction pattern, and interpretation of the finding

4.2.3 Raman Spectrometry

An effective technique for characterizing nanoparticles is Raman spectroscopy. The technique of Raman spectroscopy for powdered nanoparticles is similar to that used for bulk materials, but owing to the tiny size of the particles, certain special considerations need to be made (Li Bassi *et al.*, 2005). The following are the general stages involved in the examination of powdered nanoparticles using Raman spectrometry:

4.2.3.1 Sample preparation

The first step is to make a sample of nanoparticles that is powdered. This may be accomplished by using a ball mill or a mortar and pestle to ground the nanoparticles to a tiny powder. Any impurities or pollutants that might taint the Raman findings should not be present in the sample.

4.2.3.2 Instrumentation

A high-intensity laser beam with a tiny spot size should be able to be produced by the Raman spectrometer utilized for nanoparticle investigation. Making sure the laser only interacts with the nanoparticles and not the substrate or any other contaminants in the sample, is essential. To achieve a smooth surface, the powdered sample is typically put onto a flat sample holder and leveled off (Slavov *et al.*, 2010).

4.2.3.3 Laser excitation

After then, the sample is subjected to a powerful laser beam, usually in the visible or near-infrared spectrum. The nanoparticles shake as a result of the laser activation, creating a Raman scattering signal.

4.2.3.4 Raman Spectroscopy

A spectrometer is used to gather and examine the dispersed light. The nanoparticles' vibrational modes are characterized by the Raman spectrum that was acquired.

4.2.3.5 Data Analysis

The content and structure of the nanoparticles are then determined by examining the Raman spectra. To do this, the peaks in the spectrum are compared to those in a database of well-known Raman spectra.

4.2.3.6 Interpretation of results

The Raman results may provide details about the nanoparticles' chemical and physical makeup, size, shape, and content. The production of nanoparticles may be made more efficient using this knowledge, and their characteristics and behavior can be better understood.

In conclusion, the Raman spectrometry analysis of powdered nanoparticles entails preparation of a fine powder sample, exposure to a high-intensity laser beam, analysis of the resulting Raman spectrum, and interpretation of the results to ascertain the composition, size, and shape of the nanoparticles, as well as their chemical and physical properties (Li *et al.*, 2018).

4.2.3 Zeta potential

For the determination of the zeta potential of suspended nanoparticles, the Horiba Nanopartica Zetasizer is a commonly used tool. For powdered nanoparticles, the following stages are used in the process for measuring zeta potential using the Horiba Nanopartica Zetasizer machine:

3.2.3.1 Sample preparation

The powdered nanoparticles must first be dispersed in a suitable solvent to create a stable suspension. Sonication or mechanical stirring may be used to accomplish this. Based on the characteristics of the nanoparticles and their planned usage for them, the solvent should be selected.

4.2.3.2 Instrumentation

A measuring cell that is intended for zeta potential measurements is part of the Horiba Nanopartica Zetasizer machine. The device measures the zeta potential and size of the suspended nanoparticles using dynamic light scattering (DLS) (Jain *et al.*, 2020).

4.2.3.3 Setting up the machine

The measuring cell is cleaned before use, and the device is calibrated. After loading the sample into the measuring cell, the apparatus is prepared for zeta potential measurement.

4.2.3.4 Measurement of zeta potential

The device exposes the sample to an electric field and gauges how the particles move as a consequence. Usually, it takes a few minutes to finish the measurement. The Smoluchowski equation or other appropriate equations are then used to compute the zeta potential using the observed electrophoretic mobility.

4.2.3.5 Interpretation of results

Information on the stability and colloidal behavior of the nanoparticles in the suspension may be gleaned from the zeta potential measurements. When the zeta potential is high, the nanoparticles are evenly distributed and stable, but when it is low, the nanoparticles are more likely to aggregate or settle. The outcomes of the zeta potential analysis may also be utilized to improve nanoparticle manufacturing and formulation for a variety of applications. In conclusion, the Horiba Nanopartica Zetasizer machine can measure the zeta potential of powdered nanoparticles by dispersing them in a suitable solvent, loading the sample into the measurement cell, applying an electric field, measuring the zeta potential using dynamic light scattering, and interpreting the results to determine the stability and colloidal behavior of the nanoparticles in suspension (Brandhonneur *et al.*, 2018).

4.2.4 FTIR

The measurement of a sample's infrared radiation transmission or absorption using FTIR spectroscopy is a method often used to characterize nanoparticles in terms of their chemical makeup and functional groups (Petit and Puskar, 2018). An expert tool, such as an FTIR spectrophotometer, is needed to conduct FTIR spectroscopy on nanoparticle samples.

The procedures listed below may be used to conduct FTIR spectroscopy on samples of powdered nanoparticles using an FTIR spectrophotometer (S. Yang *et al.*, 2021):

4.2.4.1 Prepare the sample

The sample of powdered nanoparticles has to be combined with an appropriate infrared-transparent matrix, such KBr or Nujol. A hydraulic press is then used to shape the mixture into a thin pellet.

4.2.4.2 Place the pellet in the sample holder

The Shimadzu UV-1800 spectrophotometer's sample container is filled with the pellet, and the device is then mounted with the sample holder inside.

4.2.4.3 Collect the spectrum

The FTIR spectrophotometer is set up to scan the sample across several wavelengths to obtain the spectrum. In transmission mode, when infrared light passes through the sample and is detected on the opposite side, the spectrum is normally gathered.

4.2.4.4 Analyze the spectrum

The functional groups present in the sample and the nanoparticles' chemical makeup are both determined by analyzing the acquired FTIR spectrum

4.2.5 HR-TEM

A method for seeing the atomic-scale structure of materials, including nanoparticles, is High-Resolution Transmission Electron Microscopy (HRTEM) (Hrnjic *et al.*, 2021). The approach that may be utilized to conduct HRTEM analysis on samples of powdered nanoparticles is as follows:

4.2.5.1 Sample preparation

A drop of the solution is applied to a thin copper grid that has been coated with carbon after the powdered rGO nanoparticle sample has been dissolved in a solvent such as ethanol or isopropanol. On the grid, a small layer of nanoparticles is left behind when the solvent evaporates.

4.2.5.2 Instrument setup

The HR-TEM apparatus, which is normally a highly specialized and sensitive microscope, is then filled with the carbon-coated copper grid. Next, by altering the magnification, beam intensity, and other settings, the equipment is prepared for imaging.

4.2.5.3 Imaging

A beam of electrons is then used to photograph the nanoparticle sample by passing through it and landing on a detector on the opposite side. The atoms in the sample scatter the electrons as they go through it, giving rise to a picture of the nanoparticles' atomic structure.

4.2.5.4 Image analysis

Using specialist software, it is possible to identify the size, shape, and crystal structure of the nanoparticles from the HR-TEM pictures. It is crucial to remember that HR-TEM is a highly specialized method that calls for specialist tools and knowledge to execute successfully. To guarantee that high-quality pictures are acquired, it is crucial to carefully manage the sample preparation, instrument setup, and imaging.

4.2.6 FESEM

With the help of the field emission scanning electron microscope (FE-SEM), it is possible to get high-resolution images of the surfaces of many materials, including nanoparticles (Dalglish *et al.*, 2004). The approach listed below may be used to conduct FESEM analysis on samples of powdered nanoparticles:

4.2.6.1 Sample preparation

A drop of the suspension is applied to an appropriate substrate, such as a silicon wafer, after the powdered rGO nanoparticle sample has been dissolved in a solvent like ethanol or isopropanol. A tiny layer of nanoparticles is left on the substrate after the solvent has been allowed to evaporate.

4.2.6.2 Instrument setup

The rGO nanoparticle sample substrate is then put into the FE-SEM device. Next, by altering the magnification, beam energy, and other settings, the equipment is prepared for imaging.

4.2.6.3 Imaging

A beam of electrons is then focused onto the sample's surface to scan the rGO nanoparticle sample. A detector in the FE-SEM equipment picks up secondary electrons that are released as a result of the electrons' interactions with the sample's atoms. The generated photos provide high-resolution details on the shape and surface structure of the nanoparticles.

4.2.6.4 Image analysis

Using specialist software, it is possible to examine the FESEM pictures and identify the size, shape, and surface characteristics of the nanoparticles.

4.3 BIOLOGICAL ASSAYS ON CANCER CELL WITH rGO AND TRANS-RESVETROL

4.3.1 MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay

To quantify changes in cellular metabolic activity, NAD(P)H-dependent enzymatic reduction of the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; MP Biomedicals, Solon OH) to insoluble formazan precipitate was measured. Stock MTT solution was prepared in PBS at 5 mg/mL. 10 µL add to each well and incubated during the 2 h 15 min of each treatment condition. Cells were washed three times with PBS and 100 µL dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan, the plates were covered with aluminum foil, and gently agitated on a nutating shaker for 15 min, and optical densities (OD) at 540 nm were measured on Spectramax i3x spectrophotometer (Molecular Devices, Sunnyvale, CA). Background OD measurements were subtracted out using DMSO from atop cells without MTT incubation, and changes in metabolic activity were calculated relative to respective vehicle control-treated cells never exposed to microplastics. The MTT assay results for each condition were normalized to their respective number of cells per well.

4.3.2 Acridine orange/Ethidium bromide (AO/EB) staining

Acridine orange (AO) is a vital dye that stains the nuclei of both living and dead cells green. Ethidium bromide (EB) exclusively stains cells that have lost membrane integrity red. Live cells are uniformly green, but early apoptotic cells exhibit constricted or fragmented nuclei with vivid green hue. Late-apoptotic cells will have constricted and broken orange chromatin.

As resveratrol concentrations grew, orange and red staining increased and green staining decreased, indicating cell damage and death. By counting the amount of red, orange, and green cells using different concentrations of drug find the drug concentration of apoptosis and IC50 value.

4.3.3 DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay

DPPH is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule so that the molecules do not dimerize, like most other free radicals. The delocalization also gives rise to the deep violet color, with an absorption in ethanol solution at around 520 nm. Mixing DPPH solution with a substance that can donate a hydrogen atom, it gives rise to the reduced form with the loss of violet color. Representing the DPPH radical by $Z^{\bullet}$ and the donor molecule by AH, the primary reaction is $Z^{\bullet} + AH = ZH + A^{\bullet}$. DPPH is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole so that the molecules do not dimerise, like most other free radicals. The delocalisation also gives rise to the deep violet colour, with an absorption in ethanol solution at around 520 nm. On mixing DPPH solution with a substance that can donate a hydrogen atom, it gives rise to the reduced form with the loss of violet colour. Representing the DPPH radical by $Z^{\bullet}$ and the donor molecule by AH, the primary reaction is: While DPPH can accept an electron or hydrogen radical to become a stable, diamagnetic molecule, it can be oxidized only with difficulty, and then irreversibly. DPPH shows a strong absorption band at 517 nm due to its odd electron and solution appears a deep violet colour, the absorption vanishes as the electron pairs off. The resulting decolorization is stoichiometric with respect to the number of electrons taken up. The alcoholic solutions of 0.5 mM are densely colored and at this concentration, the Lambert-Beer law is obeyed over the useful range of absorption. With different concentration of drug Trans-resveratrol 5 μ M, 10 μ M, 20 μ M, 40 μ M, 50 μ M added and as standard antioxidant drug ascorbic acid added to the wells

4.4 IN-SILICO ANALYSIS

Reduced graphene oxide nanosheets were created using Chem Draw software, where the nanoparticle can be used as a ligand while docking with specific proteins and protein while docking it with specific anti-cancer and over-the-counter drugs for performing targeted drug delivery in higher organisms.

The stages followed for the docking process:

4.4 Preparation of the ligand and receptor structures

In molecular docking studies, the creation of the ligand and receptor structures is crucial. The molecule that is docking into the receptor, which is often a protein or nucleic acid molecule, is referred to as the ligand. Predicting the binding manner and affinities of the ligand to the receptor is the aim of molecular docking. Getting the molecules' three-dimensional coordinates is the initial step in creating the ligand and receptor structures. Experimental techniques like X-ray crystallography, NMR spectroscopy, or electron microscopy may be used to do this. Other methods for obtaining the structures include computational molecular modeling methods like ChemDraw software (Guedes *et al.*, 2014).

4.4.1 Grid generation

Grid generation, which entails building a three-dimensional grid of dots to depict the receptor molecule's possible binding site, is an essential stage in the molecular docking process. The grid determines the search space where the ligand will dock and aids in determining the best mode of binding and receptor affinity for the ligand. The ligand molecule is docked into the grid's predetermined search space once the grid has been constructed. The grid enables the effective computation of the receptor-ligand interaction energies, which are used to foretell the best binding mode and affinity of the ligand to the receptor (Elokely and Doerksen, 2013).

4.4.2 Ligand docking

A crucial phase in the molecular docking process is ligand docking, in which a ligand molecule is positioned in a receptor molecule's binding site and the interaction between the two molecules is anticipated. The purpose of ligand docking is to discover prospective therapeutic candidates for further experimental validation and to predict the binding mechanism and affinity of the ligand to the receptor. The ability to anticipate the binding mechanism and affinity of ligand molecules to receptor molecules makes ligand docking a potent computational tool that may be utilized for drug development as well as other molecular biology and biochemistry applications.

4.4.3 Scoring

In molecular docking, scoring entails determining the anticipated binding affinity of a ligand to a receptor. The objective of scoring is to rank the ligands according to their expected binding affinities and to locate the ligand-receptor complex with the highest advantageous interaction energy (Krovat *et al.*, 2005).

4.4.4 Analysis

To understand the interactions between ligands and receptors and to find prospective therapeutic candidates, analysis is a crucial stage in the molecular docking process. It is essential to analyze the docking data to comprehend how ligands and receptors interact as well as to find promising medication candidates. This information can be used to inform the design and optimization of ligand molecules for drug discovery and other uses in molecular biology and biochemistry. It can also give insight into the binding mode, binding affinity, selectivity, and other important characteristics of the ligand-receptor complex (Morrone Xavier *et al.*, 2016).

In-silico docking is utilized in drug discovery and design because it enables scientists to forecast how tiny compounds will attach to certain proteins and to refine the structures of those molecules to increase their binding affinity and selectivity.

Reduced graphene oxide (rGO) and proteins may interact, and one computer technique to anticipate this interaction is called *in-silico* docking. The procedure entails modeling the interaction between the protein and the rGO surface and making predictions about the protein's affinity for binding and its orientation within the surface (Rao and Srinivas, 2011). To forecast the interactions between them, reduced graphene oxide (rGO) may be docked with both ligands and proteins individually.

When an rGO and a ligand are docked, the interaction between the two is simulated, and the binding affinity and orientation of the ligand inside the rGO surface are predicted. Since it enables scientists to foresee the ideal ligands for functionalizing the surface of rGO and improves their targeting and therapeutic effectiveness, this kind of docking is often utilized in drug discovery and design.

The method of docking rGO with a protein, on the other hand, entails modeling the interaction between the protein and the rGO surface and predicting the binding affinity and orientation of the protein inside the rGO surface. Due to its ability to aid in the creation of innovative graphene- based materials for biomedical applications including biosensors, drug transport, and tissue engineering, this kind of docking is often utilized in the fields of nanobiotechnology and drug discovery. In general, *in-silico* docking of rGO with either ligands or proteins may provide insightful information on the nature of interactions between rGO and biomolecules and aid in the development of novel materials and applications.

5. RESULTS AND DISCUSSION

5.1 SYNTHESIS OF REDUCED GRAPHENE OXIDE

Using the modified hummers method reduced graphene oxide nanosheets were successfully synthesized in powdered form.

5.1 PHYSICAL CHARACTERIZATION OF GRAPHENE NANOSHEETS

5.1.1 UV-Visible Spectrometer (Shimadzu UV-1800, Japan)

For the confirmation of the synthesized nanoparticle, the rGO powder was subjected to UV-visible spectroscopy. There was a peak identified at 300 to 400 nm which is depicted in *Figure 5.1*. This result confirms that the particle synthesized is reduced to graphene oxide nanosheets.

5.1.2 X-Ray Diffraction (Rigaku MiniFlex--II Desktop X-ray diffractometer)

XRD has been employed for studying the diffraction angle at which characteristic crystalline peaks are formed for MRGO nanosheets. *Figure 5.2* describes the XRD patterns of MRGO. During the process of XRD, exfoliation had taken place from graphite to GO, and then further removal of oxygen groups from Intercalating layers of GO occurred. This resulted in the formation of (002) crystalline peaks at 24.3 Å of X-ray imparted. The additional appearance of a small diffraction peak of (111) at 42.8 Å reveals the short-range order in stacked graphene layers.

5.1.3 Raman Spectroscopy (Renishaw inVia Raman Microscope)

This particular physical characterization has been carried out to study the crystallinity, disorder, and defect in rGO nanosheets. The results of this analysis are depicted in *Figure 5.3*. G peak at 1600 cm⁻¹.

this is attributed due to the re-graphitization of the carbon network resulting in the creation of a large number of sp^2 carbon species in the graphene network and D peak is at 1400 cm^{-1} . Using the relative intensity ratio, that is I_D/I_G , the strength of defects present in the crystal lattice of a material can be evaluated. The I_D/I_G for MRGO is 1.375 ($I_D/I_G = 110000/8000 = 1.375$). This shows that the concentration of defects is higher than normal, which can result in the formation of many internal benzene-derived sp^2 domains in a distinctive graphene structure.

5.1.4 Zeta potential (Horiba Nanopartica Zetasizer)

The zeta potential of reduced graphene oxide nanosheets has been studied in an aqueous medium where an rGO powdered sample was dissolved with distilled water by sonication, to inspect the colloidal stability of nanomaterials in the dispersion state. The zeta potential mean value is -47 mV as shown in *Figure 5.4*, which is greatly above the acceptable limit of colloidal stability in the dispersion state.

5.1.5 FTIR (Agilent Cary630 FTIR)

To understand the major functional groups, present in the rGO nanosheet synthesized, FTIR studies have been carried out, the result is depicted in *Figure 5.5*. The broad and intense peak of wavenumbers at $\sim 3000\text{ cm}^{-1}$ arising from ($-OH$) stretching vibration, a peak at $\sim 1715\text{ cm}^{-1}$ due to ($-C=O$) stretching vibrations of carboxylic groups and sharp $C=C$ band within the range of 1500 cm^{-1} .

5.1.6 HR-TEM ((S)TEM Microscope, (TALOS F200S G2 TEM)

The morphology of rGO nanosheets synthesized is characterized by HR-TEM analysis. The results depicted in *Figure 5.6* indicate the presence of wrinkles and folded regions on the surface of rGO. The images were collected at 10 nm, 50 nm, and 100 nm to find out the intensity of folds present on the surface of rGO present on the surface.

5.1.7 FE-SEM (Carl Zeiss NTS GMBH, SUPRA 55)

FE-SEM images (Figure: 5.7) of rGO synthesized by the modified hummers method depict the sharp edges, which proves its toxicity over lower organisms such as bacteria and fungal phytopathogens, as this saw-like surface can cut open the peptidoglycan layer of bacteria and a chitinous layer of fungus easily, which in turn will stimulate reactive oxygen species and result in the death of the organisms.

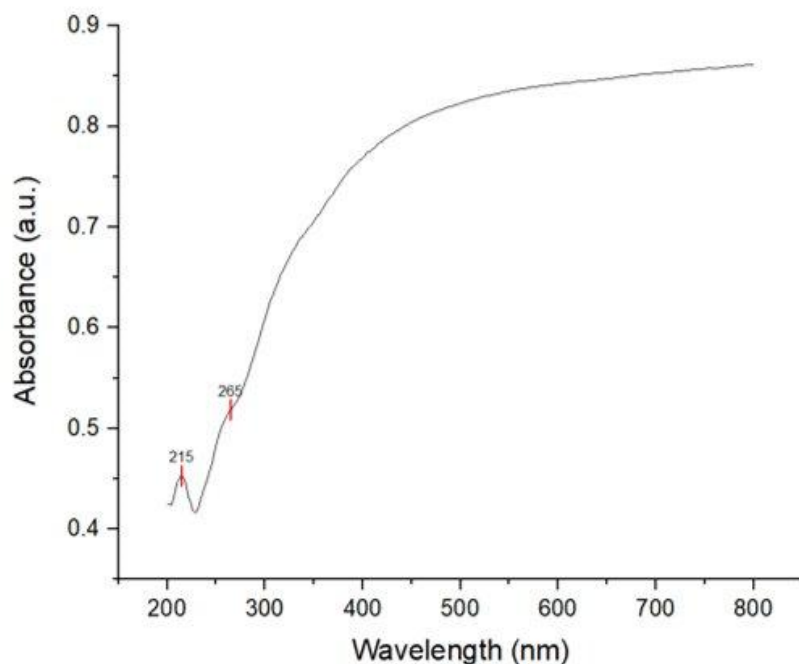


Figure 5.1: UV- Visible spectroscopy results of rGO

-it indicates the two peaks over the absorbance at 1) 215 nm and 2) 265 nm wavelength

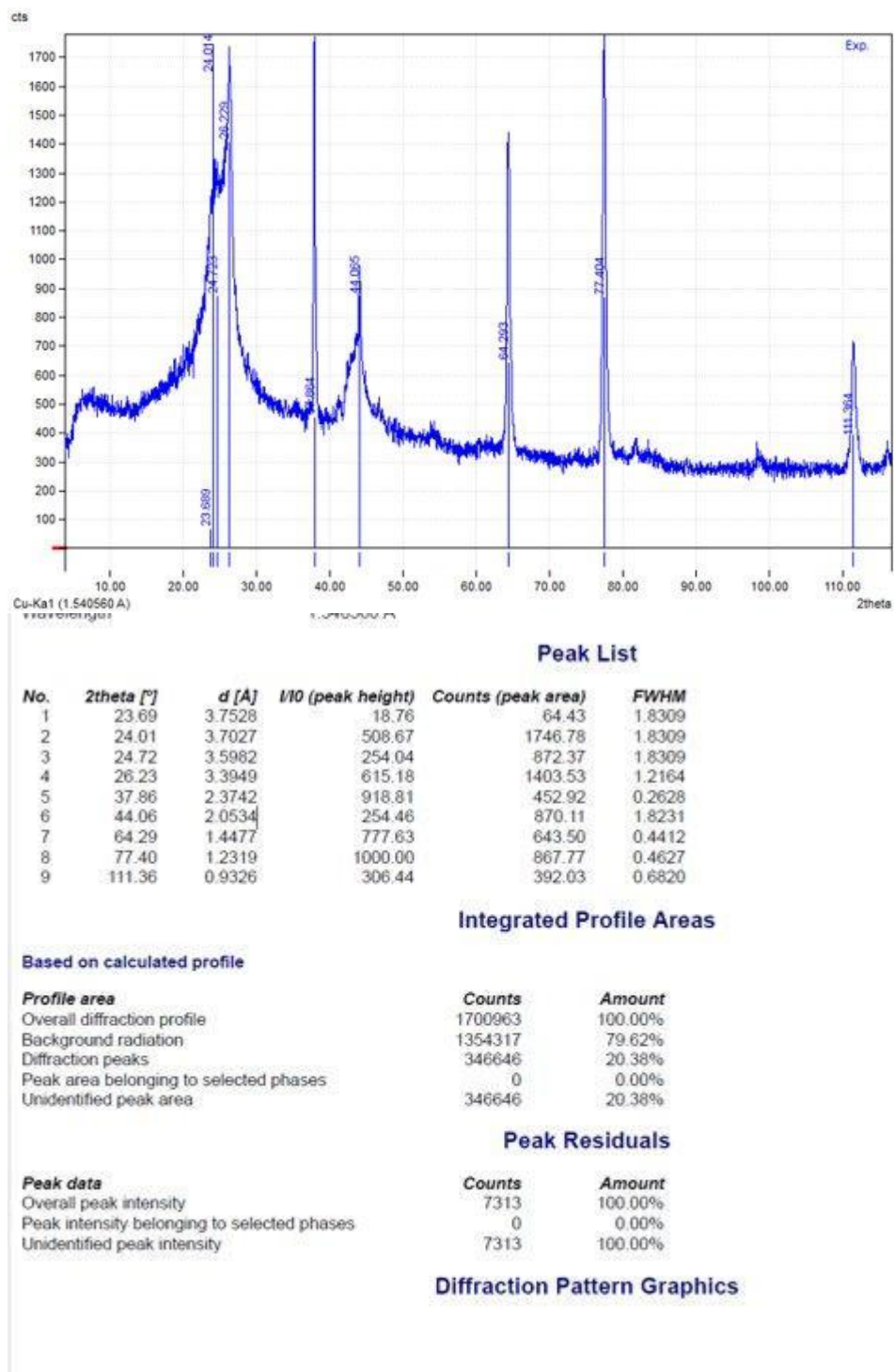


Figure 5.2: XRD peaks of rGO

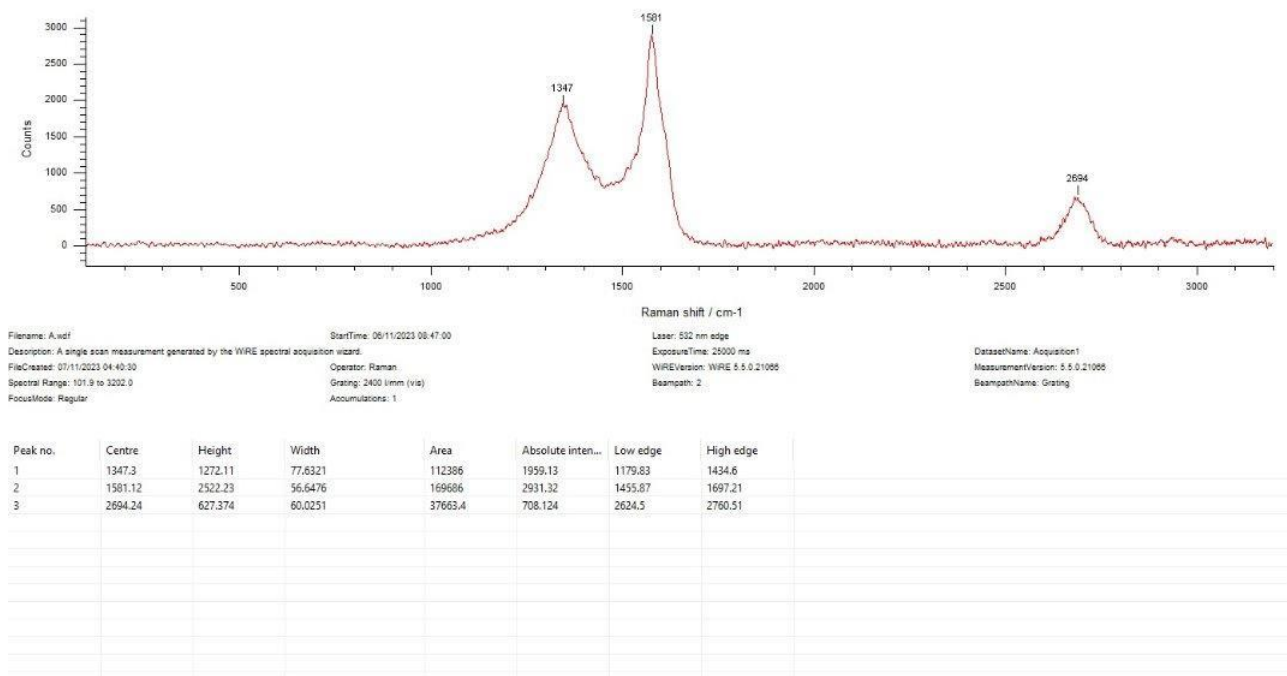


Figure 5.3: Raman spectroscopy results of rGO

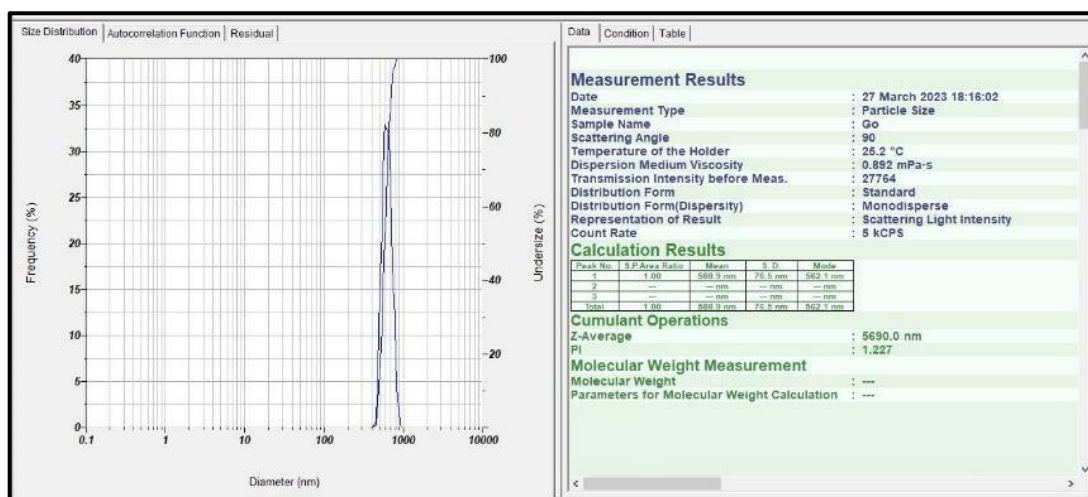


Figure 5.4: Zeta potential (particle size analysis) results of rGO

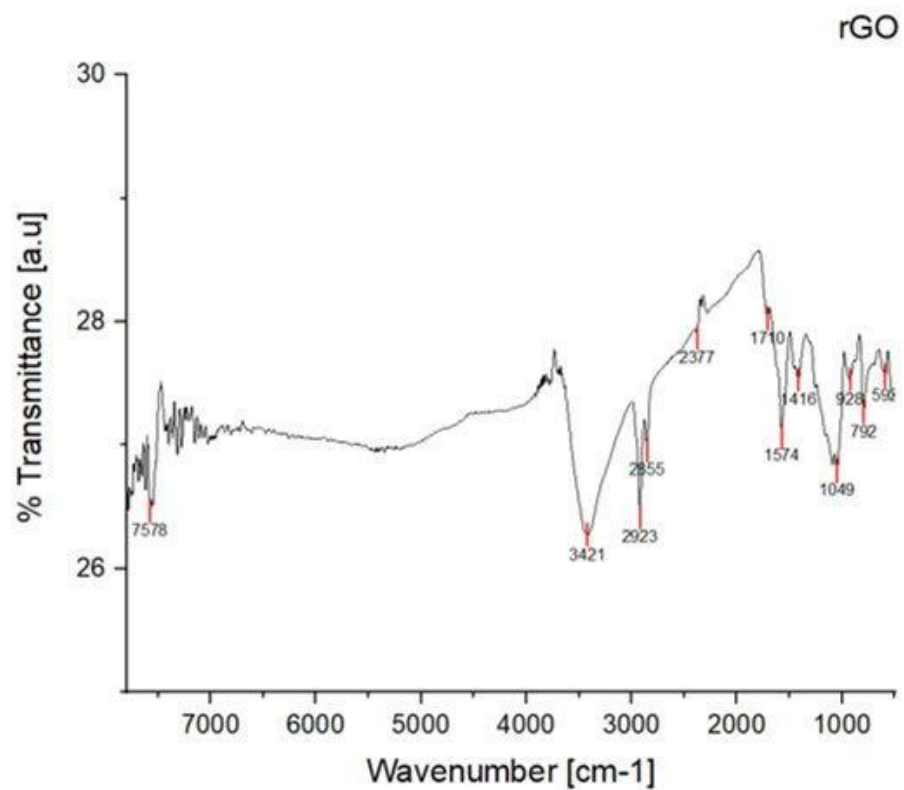
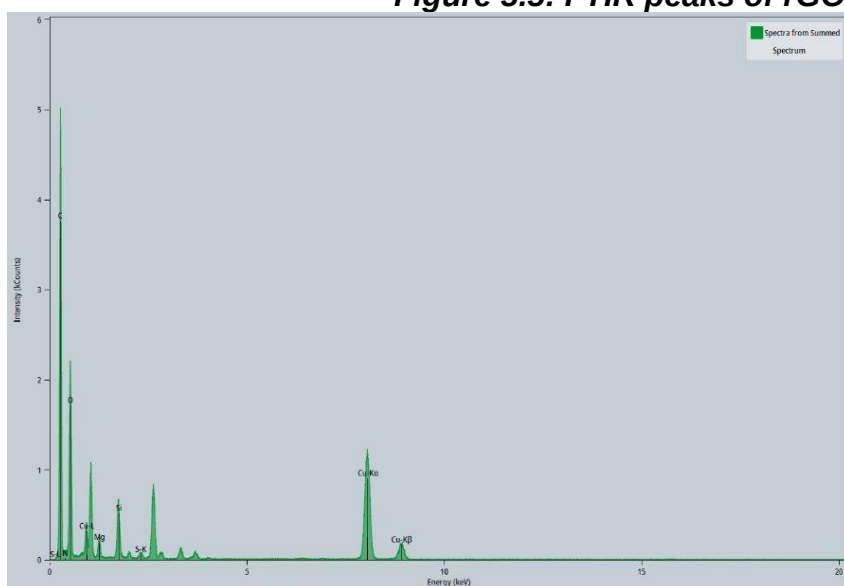


Figure 5.5: FTIR peaks of rGO



(a) Graph of rGO peaks in HR-TEM

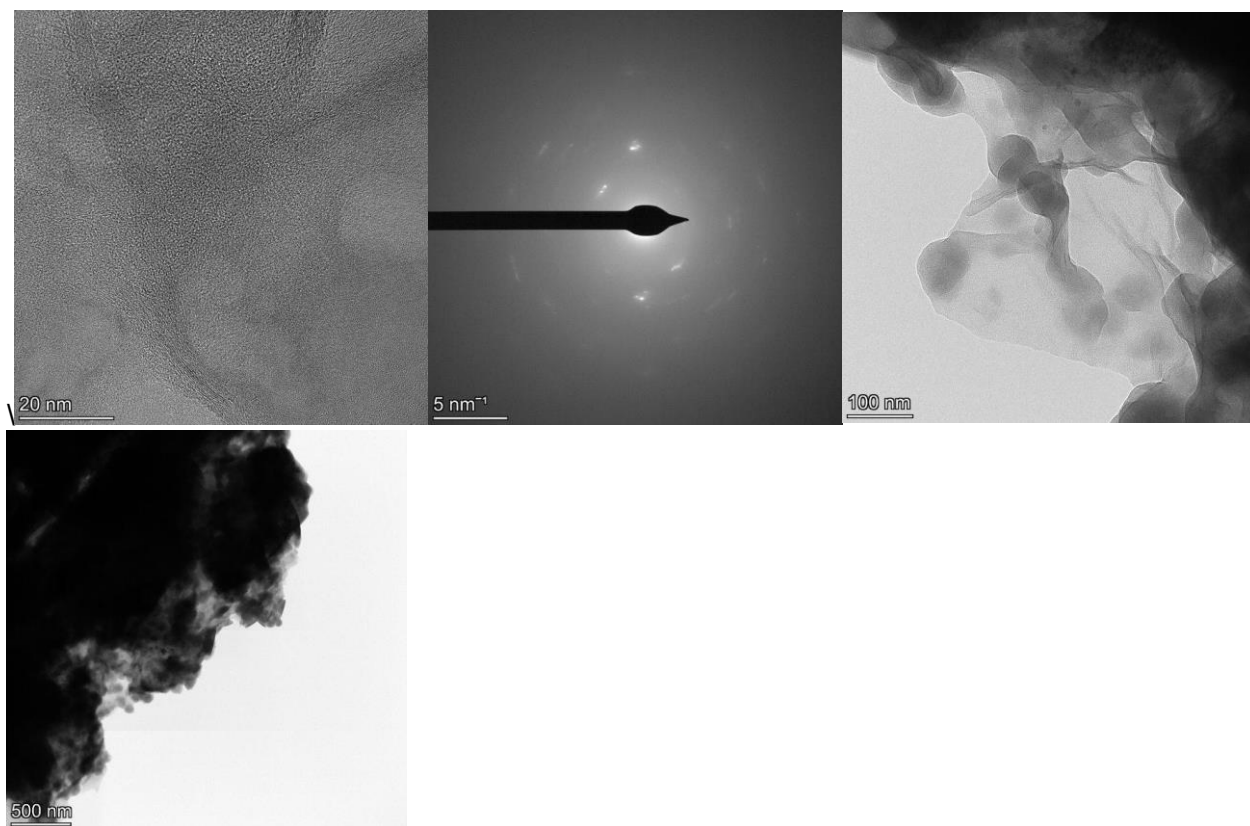


Figure 5.6: HR-TEM images of rGO

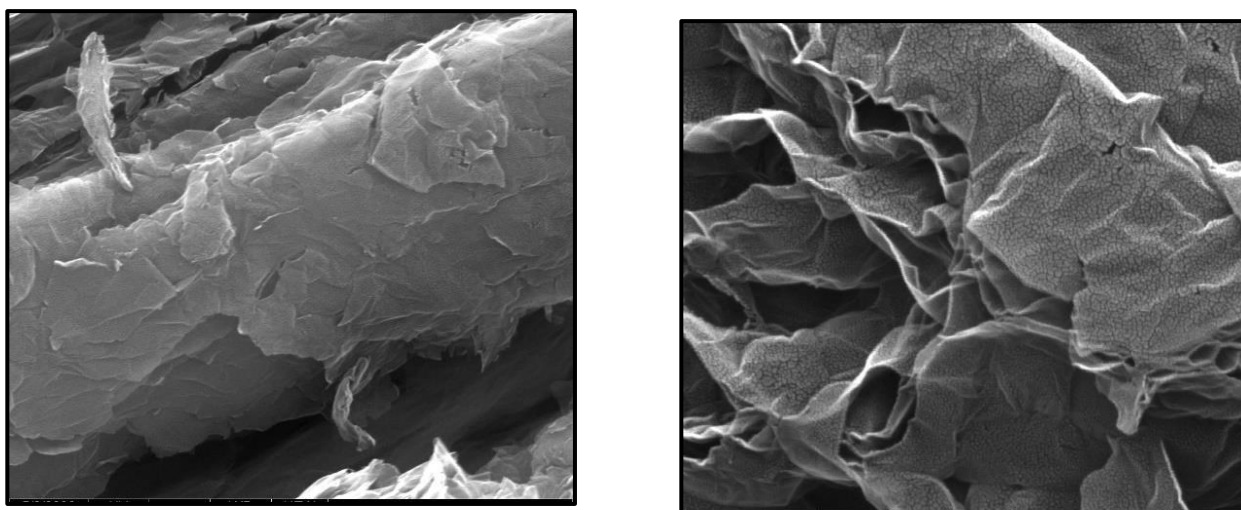
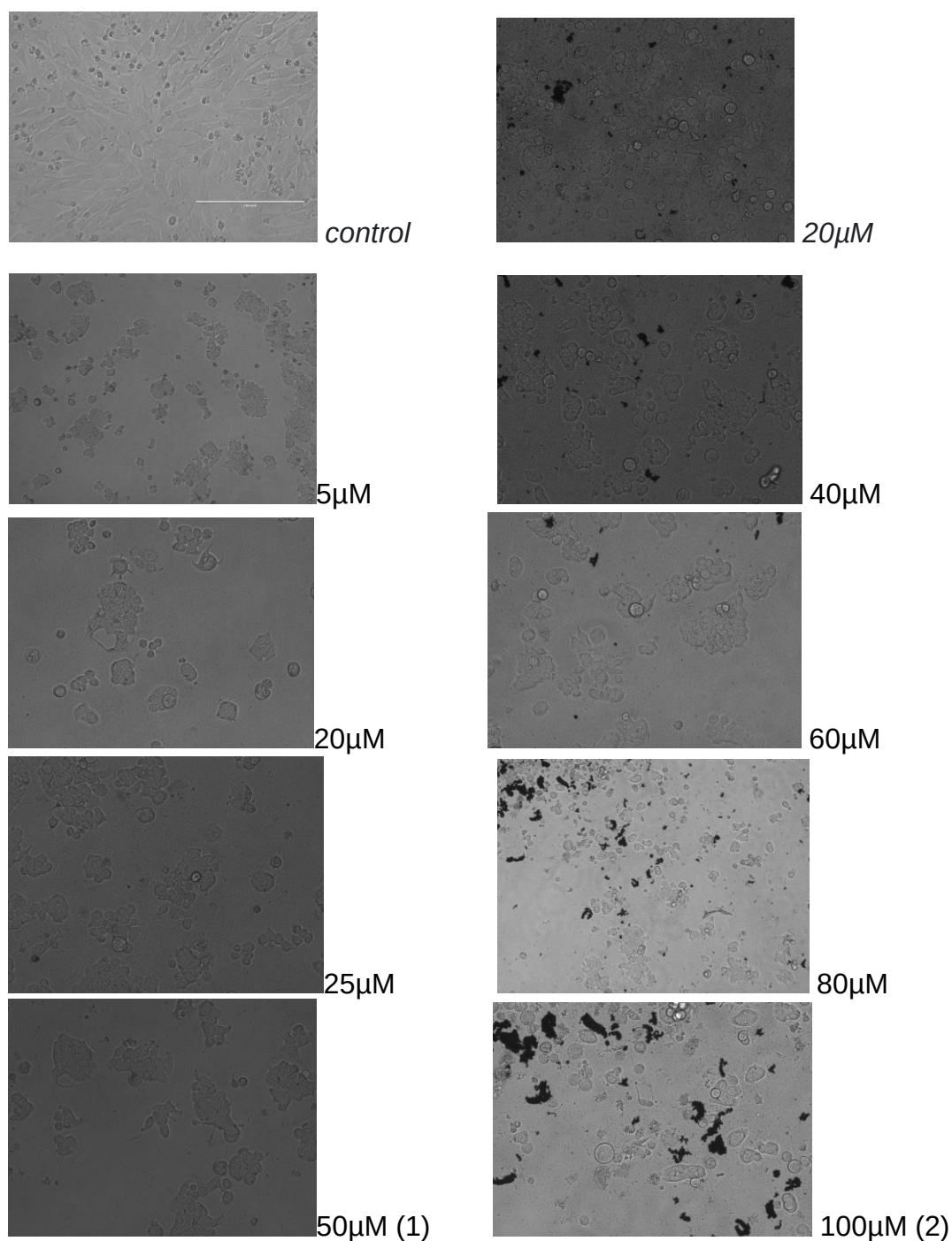
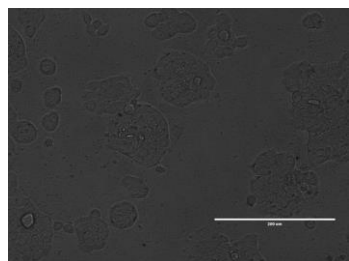


Figure 5.7: FE-SEM images of rGO

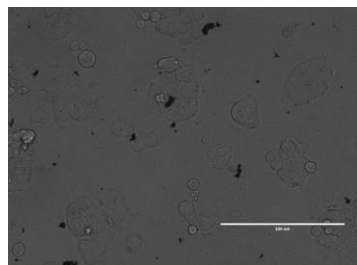
5.3 MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay

The concentration of the drug for the Trans-Resveratrol is 5 μ M, 10 μ M, 20 μ M, 25 μ M, 50 μ M (Figure 5.8 (1)) and for separate rGO concentration is 20 μ M, 40 μ M, 60 μ M, 80 μ M, 100 μ M (Figure 5.8 (2)) and for the combination of the rGO and Trans-Resveratrol 5 μ M+ 20 μ M, 10 μ M+ 40 μ M, 20 μ M+ 60 μ M, 25 μ M+ 100 μ M (Figure 5.8(3)) for graph of these concentration cytotoxicity given in (Figure 5.9)

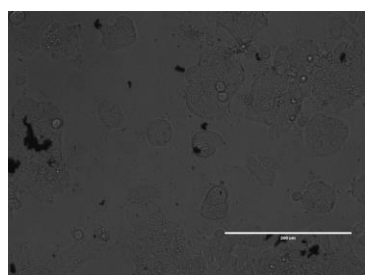




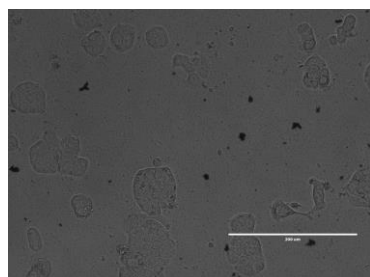
20μM rGO+5μM Trans-Resveratrol



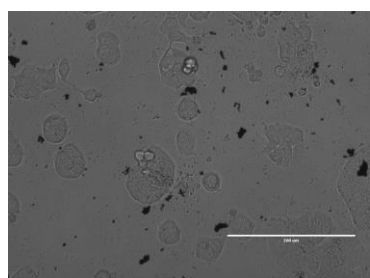
40μM rGO+ 10μM Trans-Resveratrol



60μM rGO+ 20μM Trans-Resveratrol



80μM rGO+ 25μM Trans-Resveratrol



100μM rGO+ 50μM Trans-Resveratrol

(3) Trans-resveratrol and rGO combined

Fig.5.8 : MTT assay results (1) trans-resveratrol (2) rGO (3) Trans-resveratrol and rGO combined

MTT assay was done to check the cell viability and cytotoxicity. 1×10^4 cells/100 μ l were seeded in each well of 96-well plate and incubated for 24 hrs in CO₂ incubator.

- Trans-Resveratrol and nanomaterial as a combination with concentrations (20nm,40nm,60 nm,80 nm,100 nm) were added.
- Trans-Resveratrol alone with concentrations (20nm,40nm,60 nm,80nm100nm) and rGO alone with concentrations (20nm,40nm,60 nm,80nm100nm) were added.
- The absorbance was measured at 570 nm using a multi well microplate reader.
- The IC₅₀ concentration 40nm drug in combination and as an individual for HCT-116 cell lines were calculated plotting concentration vs inhibition.
- The Two-way ANOVA is explained In row factor and the column factor have p-values less then 0.0001, so we can reject the null hypothesis for both. (Figure 5.10)



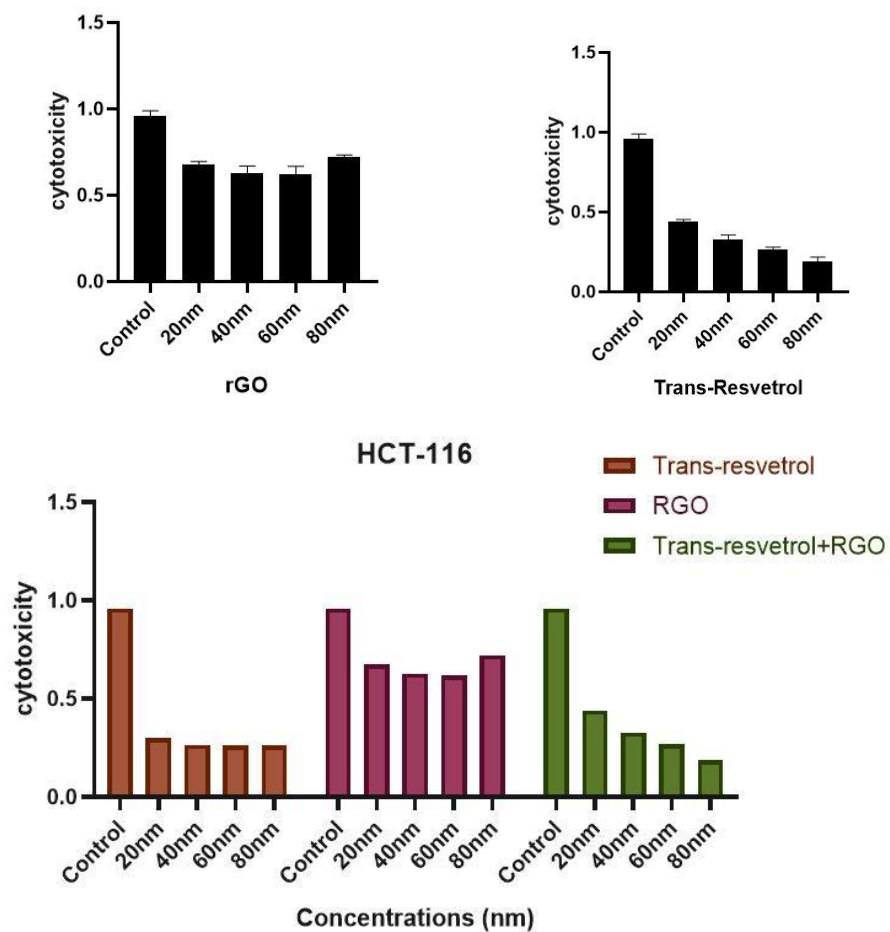


Fig. 5.9 MTT graph for (1) Trans-resveratrol (2) rGO (3) Trans-resveratrol and rGO combined

Two-way ANOVA analysis

Table Analyzed	Data 1				
Two-way ANOVA	Ordinary				
Alpha	0.05				
Source of Variation	% of total variation	P value	P value summary	Significant?	
Interaction	8.876	<0.0001	****	Yes	
Row Factor	64.28	<0.0001	****	Yes	
Column Factor	26.07	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.3026	8	0.03783	F (8, 30) = 43.07	P<0.0001
Row Factor	2.191	4	0.5479	F (4, 30) = 623.8	P<0.0001
Column Factor	0.8889	2	0.4445	F (2, 30) = 506.1	P<0.0001
Residual	0.02635	30	0.0008782		

Fig.5.10: Two way ANOVA analysis for MTT values

- Both the row factor and the column factor have p-values less than 0.0001, so we can reject the null hypothesis for both. This means that there is a statistically significant main effect for both the row factor and the column factor.

5.4 acridine orange/ethidium bromide (AO/EB) staining

For the Trans-Resveratrol drug concentration is $5\mu\text{M}$, $10\mu\text{M}$, $20\mu\text{M}$, $25\mu\text{M}$, $50\mu\text{M}$. This concentration added to the HCT-116 each well 30000 cells were added in the 24 well plate. (Figure 5.11) cells observed at 12 hrs, 48hrs, 72hrs with different concentration under epoch inverted microscope.

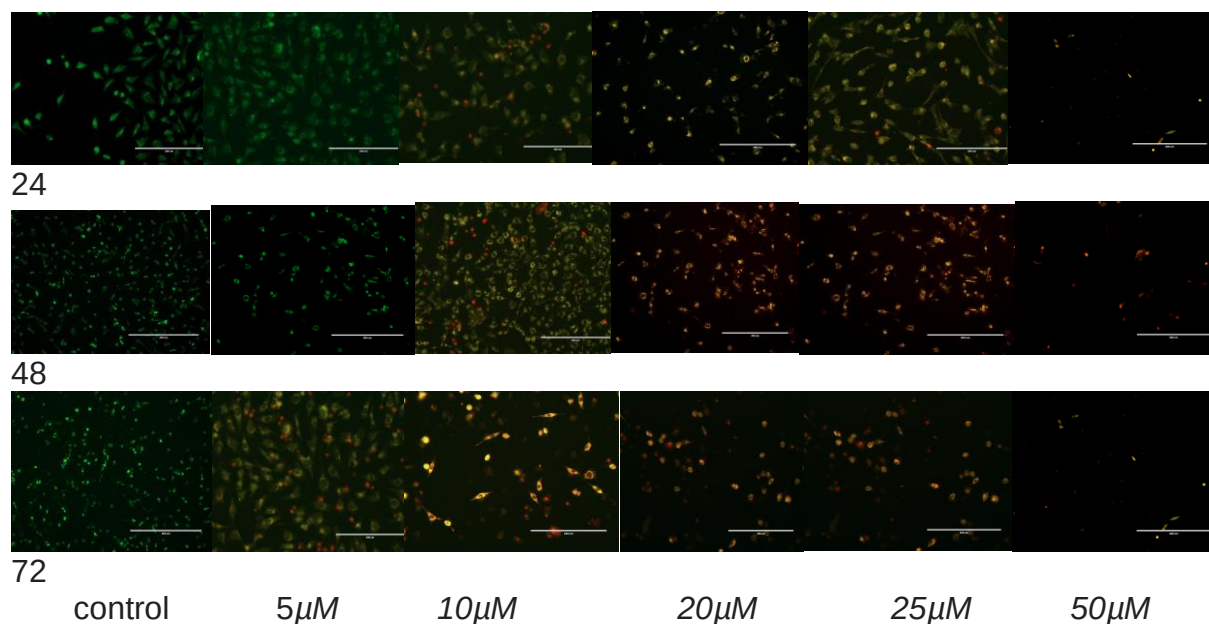


Fig. 5.11: AO/EB assay control, $5\mu\text{M}$, $10\mu\text{M}$, $20\mu\text{M}$, $25\mu\text{M}$, $50\mu\text{M}$ for 24 hrs, 48 hrs, 72 hrs interval

5.5 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

the concentration of trans-resveratrol is added to the 9 well plate and DPPH solution added to the each well by making it up to 1000 μ l and absorbed epoch multiplate reader with 517 nm where it shows that trans-resveratrol has antioxidant effect in (Figure 5.12)

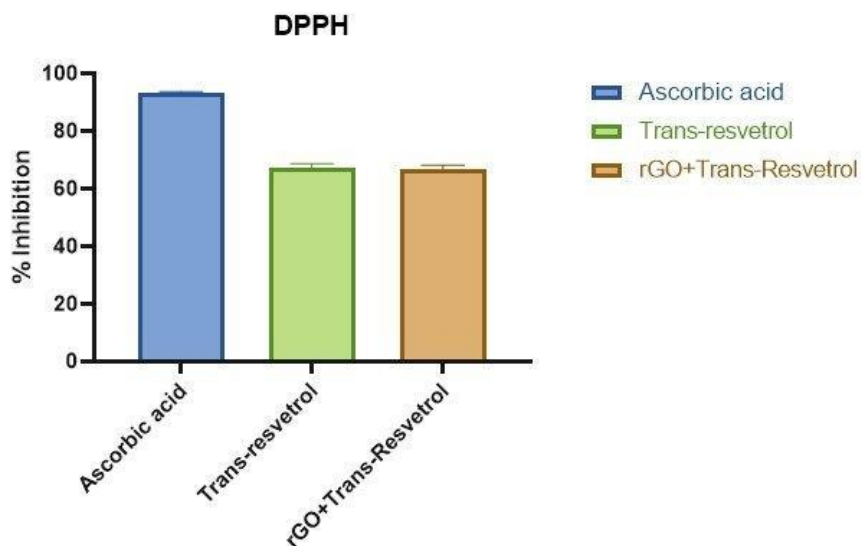
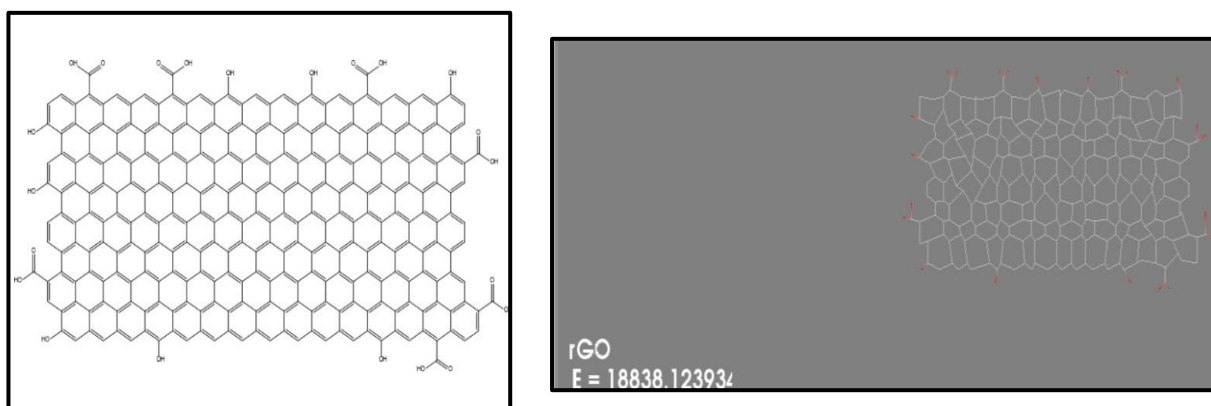


Fig. 5.12: DPPH assay graph

5.4 IN-SILICO ANALYSIS

rGO nanosheet was created using Chemdraw software and it was used as both ligand and macromolecule to dock with specific proteins and drugs respectively for finding the toxicity pathway and the scope for rGO to act as drug carrier for targeted drug delivery binding affinity of the same. The results are tabulated in Table 5.1 and with the pictorial depiction in *Figure 5.13*



(a)

(b)

Figure 5.13: Preparing rGO nanosheets for in-silico docking analysis

(a) **Construction of rGO nanosheets using chemdraw software**

(b) **Energy minimization of rGO**

5.4.1 In-silico docking of membrane proteins and rGO

rGO nanosheets were docked with selected membrane proteins present in to identify which protein has the highest binding affinity and to bring out the toxicity pathway by comparing these results with wet-lab analysis ones. (Figure 5.14)

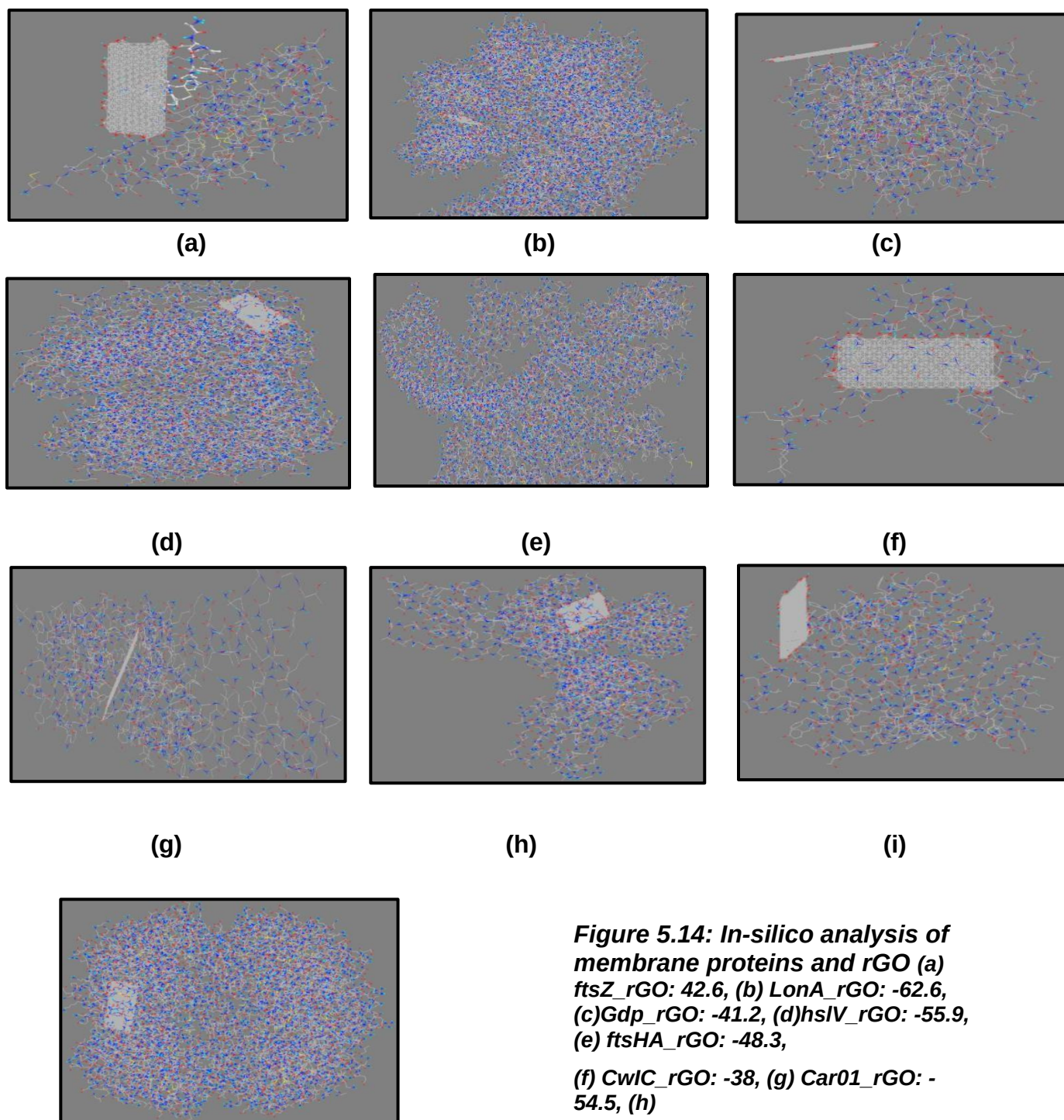


Figure 5.14: In-silico analysis of membrane proteins and rGO (a) *ftsZ* rGO: 42.6, (b) *LonA* rGO: -62.6, (c) *Gdp* rGO: -41.2, (d) *hslV* rGO: -55.9, (e) *ftsHA* rGO: -48.3, (f) *CwIC* rGO: -38, (g) *Car01* rGO: -54.5, (h) *Transpeptidase* rGO: -53.4, (i) *VDAC* rGO: -46.1, (j) *ClpP* rGO: -54.4

5.4.2 In-silico docking of DNA binding proteins and rGO

rGO nanosheets were docked with DNA binding membrane proteins present in Homo sapiens to check if the synthesized nanoparticle can interfere with the central dogma. The results are depicted below.in (Figure 5.15)

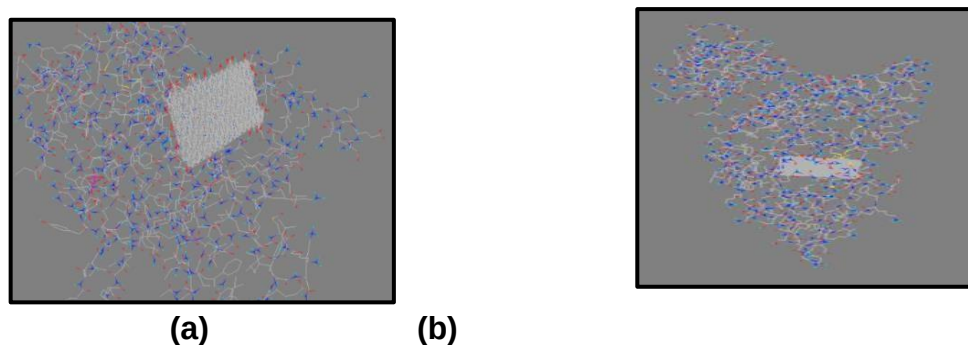


Figure 5.15: In-silico analysis of DNA binding proteins and rGO

(a) RecA_rGO: -45.9, (b) LexA_rGO: -47.1

5.4.2.1 In-silico docking of cancer related proteins and rGO

rGO nanosheets were docked with selected proteins to detect its anticancer activity.(Figure 5.16)

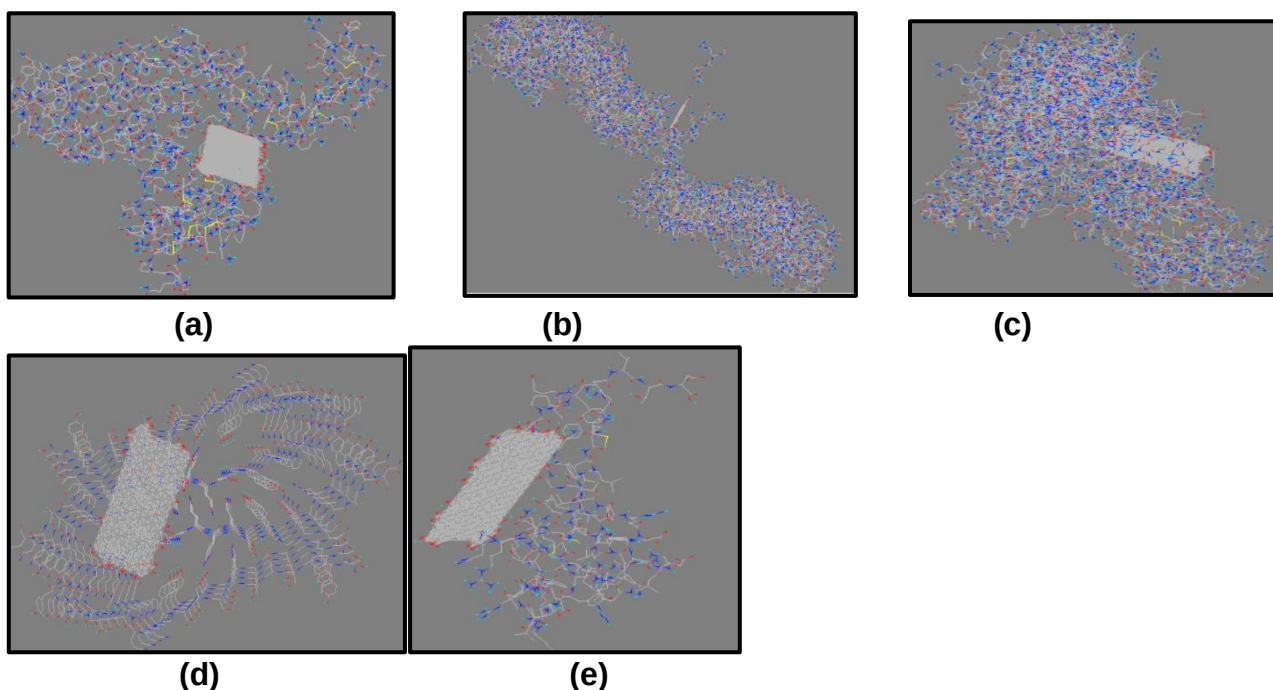
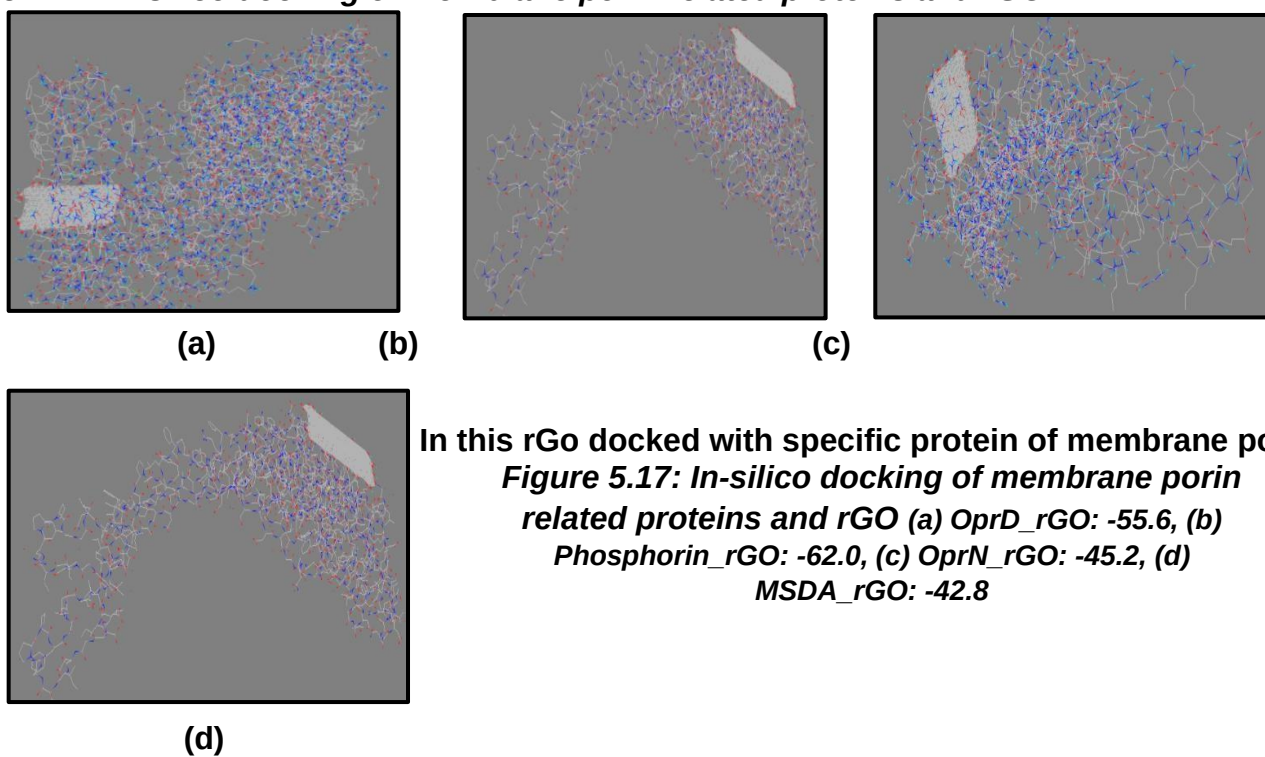


Figure 5.16: In-silico docking of cancer related proteins and rGO (a) TNF_rGO:

-47.4, (b) Menin_rGO: -46.7, (c) TP53_rGO: -50.6, (d) Lc_rGO: -56.8, (e) Caspase 7_rGO: -37.8

5.4.2.2 In-silico docking of membrane porin related proteins and rGO



5.4.2.3 In-silico docking of transport proteins and rGO

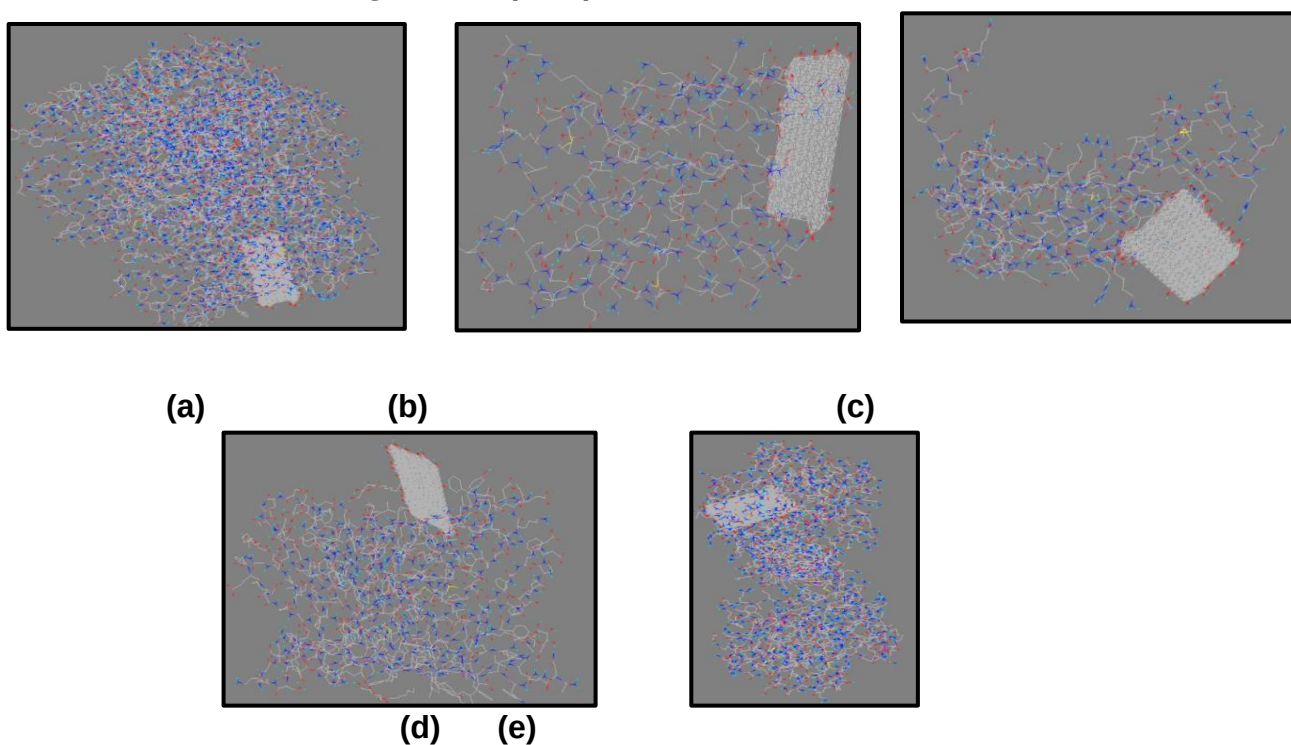


Figure 5.18: In-silico docking of transport proteins and rGO (a) OmpC_rGO: -49.9, (b) OmpATb_rGO: -40.2, (c) OmpA_rGO: -41.1, (d) OmpG_rGO: -57.7, (e)

Table 5.1: Results of *In-silico* analysis of rGO nanosheet with specific proteins

S. No	Proteins	Classification	Role	Binding affinity(KD)	PDB number	Organism
1	BclW	Cancer related protein	Anti-apoptotic protein, inhibition of this is helpful for cancer patients	-38	1MK3	<i>Homo sapiens</i>
2	Cas	Cancer related protein	Sequence specific RNA cleavage, control of transcription	-37.8	5O2M	<i>Homo sapiens</i>
3	Menin	Cancer related protein	Regulates gene transcription	-46.7	3U84	<i>Homo sapiens</i>
4	TNF	Cancer related protein	Inflammation-associated carcinogenesis	-47.4	1TNR	<i>Homo sapiens</i>
5	TP53	Cancer related protein	Tumor suppressor (regulates cell division)	-50.6	1XNI	<i>Homo sapiens</i>
6	VDAC	Membrane protein, Apoptosis	Regulates the mitochondrial porins	-46.1	2K4T	<i>Homo sapiens</i>
7	Atg	Transport protein	Autophagy-related	-50.8	6Y09	<i>Homo sapiens</i>

6. SUMMARY AND CONCLUSION

The synergistic potential of reduced graphene oxide (rGO) and trans-resveratrol presents a promising avenue for cancer therapy. Through this exploration, we have observed the multifaceted mechanisms by which these two agents can target cancer cells, inhibit tumor growth, and potentiate the efficacy of conventional treatments. The combination of rGO and trans-resveratrol offers several advantages, including enhanced bioavailability, improved cellular uptake, and targeted delivery to cancerous tissues. Additionally, their ability to modulate multiple signaling pathways involved in cancer progression, such as apoptosis, angiogenesis, and inflammation, highlights their potential as versatile anticancer agents.

Furthermore, the biocompatibility and low cytotoxicity of rGO, when functionalized appropriately, complement the therapeutic properties of trans-resveratrol, mitigating concerns regarding systemic toxicity and off-target effects. Despite these promising findings, challenges such as optimization of dosage, elucidation of optimal treatment regimens, and comprehensive evaluation of long-term safety profiles remain to be addressed. Additionally, further research is warranted to explore the specific mechanisms underlying the synergistic interaction between rGO and trans-resveratrol and their potential application across various cancer types.

Graphene belongs to the family of nanomaterials because of its surface chemistry, lateral dimension, and several layers. These changes are likely to have an impact on biological response. This thesis examines the electrical and structural characteristics of graphene and discusses current findings in graphene-based biocompatibility. GO and rGO exhibit various mechanical, electrical, and chemical capabilities because of their disparate chemical and structural compositions. rGO nanosheets display the target drug delivery for HCT-116 cell line by drug Trans-resveratrol in MTT assay it rGO has shown his effect against the cancer cell line. Also Trans-resveratrol has shown the antioxidant effect in the DPPH assay where oxygen groups bind to the nanomaterial with more affinity.

In conclusion, the integration of rGO and trans-resveratrol holds great promise as a novel approach in cancer therapy, offering a targeted, efficient, and potentially less toxic alternative to conventional treatments. Continued interdisciplinary research and clinical trials are imperative to fully harness the therapeutic potential of this innovative combination for the benefit of cancer patients worldwide.

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ANNEXURE

Poster published in the International conference



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उम्मीदवार का नाम (Candidate's Name)	Vysankesh	कनिष्ठकारी की तारीख तिथि (Candidate's Date of Birth)
पिता/परिवारिक का नाम (Father/Guardian's Name)	Ganesh	माता/परिवारिक का नाम (Mother/Guardian's Name)
राष्ट्रियता (Nationality)	INDY OTHER VALID GOVT. IDENTITY WITH PHOTOGRAPH (EXCEPT ADAAHAR NUMBER)	लिंग (Gender)
व्यक्ति का प्रकार (Identity Type)	SC	व्यक्ति संख्या (Identification Number)
तैयारी का तरीका (Mode of Preparation)	Self Study	देश का राज्यकोड प्राप्त करें (State/U.T of Residence)
आपके बारे में सूचना दें (Are you Disabled?)	NO	देश का स्थान (Place of Residence)
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अन्य जानकारी (Additional Details)		
अन्य चयनित (Aadhaar Selected)	YES	
अन्य चयनित विकल्प (Aadhar Selected Option)	मे पसंद है अलग आधार नहीं माफ़ा करने के लिए सहायक और सुझाव देना है कि इसका उपयोग पुष्करिकाओं के अध्यापक के प्रत्यक्ष में व्यक्तिगत रूप से किया जाना चाहिए। यदि आप इसे अपूर्ण रूप से भरेंगे तो आपको अपने आवेदन पर निरस्त कर दिया जाएगा। (It will be used To Establish my Identity for Graduate Aptitude Test - Biotechnology And Biotechnology Eligibility Test (GAT-B & BET) Examination By Matching My Personal Identification Details Available In Aadhar Records Of UIDAI.)	
अन्य संख्याएं(Aadhar No)	XXXX XXXX XXXX	
परीक्षा केंद्र और विकल्प (Exam Center And Details)		
के लिए अधिकृत (Authorized Exam Center)	GAT-B & BET-2024	प्रश्न पत्र माध्यम (Question Paper Medium)
पिता/परिवारिक की योग्यता (Father/Guardian Qualification)	Graduate	पिता/परिवारिक का व्यवसाय (Father/Guardian Occupation)
माता/परिवारिक की योग्यता (Mother/Guardian Qualification)	Graduate	माता/परिवारिक का व्यवसाय (Mother/Guardian Occupation)
क.स. (S.No.)	प्रोग्राम के लिए लागू कार्यक्रम (Programme Applied For GAT-B)	चयनित कार्यक्रम (Programme Selected)
1	Programme applied for GAT-B (1 st Choice)	M.Sc. Microbiology

2	Programme applied for GAT-B (1 st Choice)	M.Sc. Molecular Biology and Biotechnology with specialization in Animal Biotechnology
3	Programme applied for GAT-B (1 st Choice)	M.Sc. Molecular Biology and Biotechnology
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श्री नाम (S.No.) श्री नाम का पंज नाम (Choice For Exam Center)		श्री नाम का शहर (State का नाम) (City Name (State Name)) Chennai (Tamil Nadu) Coimbatore (Tamil Nadu) Madurai (Tamil Nadu) Tiruchirappalli (Tamil Nadu)
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श्री नाम का विभाग का नाम (College/Institute/Department Name) Sathyabama Institute Of Science And Technology		श्री नाम का नाम (University Name) SATYABAMA INSTITUTE OF SCIENCE & TECHNOLOGY, CHENNAI
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श्री नाम की विधि (Exam Status) Appearing/Result awaited		श्री नाम का शहर का पंज नाम (Selected Name Of The Qualifying Programme) B. TECH
श्री नाम का विभाग का नाम (College/Institute/Department Name) Sathyabama Institute Of Science And Technology		श्री नाम का नाम (University Name) SATYABAMA INSTITUTE OF SCIENCE & TECHNOLOGY, CHENNAI
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