CeO_2/ZnO/GO HYBRID AS A THERANOSTIC NANOPLATFORM FOR CERVICAL CANCER DIAGNOSIS

PROJECT REPORT

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BONAFIDE CERTIFICATE

Certified that this project report "**CeO_2/ZnO/GO Hybrid as a Theranostic Nanoplatform for Cervical Cancer Diagnosis"** is the bonafide work of **"Saradha Preetha G (170801163), Saroja KK (170801165), Sherin S (170801171), Shivani KS (170801174)"** who carried out the project work under my supervision.

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ABSTRACT

As per the cancer statistics revealed by ICMR, India has over 17.3 lakh new cancer cases and over 8.8 lakh deaths due to breast, lung & cervix cancer in 2020. With an estimated 1 lakh new cases in 2016 and about 1.04 lakh cases in 2020, cervical cancer is the third most commonly occurring cancer. The most popular and widely used technique for screening for cervical cancer is traditional pap. But this technique suffers from a very high false negativity of about 40 percent. Although liquid based cytology has improved Pap screening sensitivity, it is found to be too costly for developing countries. In the present study, attempts have been made to clarify the biophysical signatures that can help diagnose the disease at a much earlier stage. Developed biosensor made from CeO_2/ ZnO/ GO nanocomposites are interacted with Cervical smear for an incubation period of 48 hours and its anti-cancer effects were recorded using standard MTT assay protocol. Also, the developed CeO_2/ ZnO/ GO nanocomposites are combined with cisplatin drug to evaluate its ability to act as drug delivery system. Here, Dual stain test & FACS study were performed to estimate the stage at which cancer cells were killed by the developed biosensor. Lastly, biosensor immersed in cervical lesion (as working electrode) is subject to CV study for recording its sensitivity and specificity of the biosensor that has been developed for cervical cancer screening.

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LIST OF SYMBOLS

mol/l	moles per litre
gm/mol	grams per mole
Degree C	Degree Celsius
n	Positive Integer
d	Particle Size
k	Shape Factor
lambda	Wavelength
beta	Full Width Half Maximum
Nm	Nanometer

LIST OF ABBREVIATIONS

ABBREVIATION	EXPANSION
NP	Nanoparticle
NC	Nanocomposite
GO	Graphene Oxide
CeO_2	Cerium di Oxide
ZnO	Zinc Oxide
CeO_2/ZnO/GO	Ternary Nanocomposite
XRD	X-Ray Diffraction
FTIR	Fourier Transform Infrared
SEM	Scanning Electron Microscope
AO/EB	AcridineOrange/EthidiumBromide
MTT	-3-(4,5-Dimethylthiazol -2-YI)- 2, 5Diphenyltetrazolium Bromide

CHAPTER 1
INTRODUCTION

Cancer is a disease with the ability to invade or spread to other parts of the body that involves irregular cell growth. There are more than 100 different identified human-affecting cancers. Due to the widespread prevalence of the disease, the high mortality rate, and recurrence following treatment, cancer detection is of great concern. From 2002 to 2006, the incidence rate (per 100,000 people) of cancer in white people was 470.6, 493.6 in black people, 311.1 in Asians, and 350.6 in Hispanics, according to the National Vital Statistics Reports, indicating that cancer is widespread among all races. According to the National Cancer Institute, lung cancer, breast cancer, and prostate cancer were the three leading causes of death in the US, claiming over 227,900 lives alone in 2007. Cancer is also greatly feared because of recurrences, as tumors can return after a period of time, even after chemotherapy, surgery, or radiotherapy, even if treated early. A cancer patient's survival is heavily dependent on early detection, and the development of technologies applicable to sensitive and specific cancer imaging techniques is really an inevitable task for cancer researchers. Existing cancer screening methods include the Papanicolau test for women to detect cervical cancer and mammography to detect breast cancer, prostate-specific antigen (PSA) level detection in blood sample for men to detect prostate cancer, occult blood detection for colon cancer, endoscopy, CT scans, X-ray, ultrasound imaging and MRI for various cancer detection. However, these traditional diagnostic methods, when it comes to cancer detection at very early stages, are not very powerful methods. Also, for several people, most of the screening techniques are quite expensive and not available. So, it is essential to develop technology that is specific, reliable and easily accessible to detect cancers at an early stage.

1.1 ROLE OF NANOTECHNOLOGY IN CANCER DIAGNOSTICS

Nanotechnology is a relatively new branch of science which studies 1 to 100 nm instruments and devices with different functions at the cellular, atomic and molecular levels. Nanotechnology, along with biology, applied physics, optics, computational analysis and modelling, as well as material science, has led to a remarkable convergence of diverse areas. Because of this, the application of analytical, computational, and synthetic nanoscale approaches to understanding and controlling complex biological structures creates incredible promise for advancement in cancer detection.

Properties that are not present in bulk materials of the same structure are also seen by artifacts in the nanometer scale regime. These new properties can be tuned in certain situations, which means that they can be changed by adjusting the form, size or composition of the nanoparticles. Nanotechnology's flexibility and broad applicability reflect the spectrum of composite materials (e.g., metals, semiconductors or polymers), geometries (e.g., spheres, prisms or rods) and structures (e.g., solids, core or shell or dendrimers) that have been developed to be used in various diagnostic and treatment procedures for cancer. In identifying cancer cells and how far the disease has spread across the body, nanotechnology has found several new ways. Nanoscience and nanotechnology have become a flexible and exciting medium for the production of novel materials with improved cancer detection properties and future applications. The use of nanoscale materials contributes to a "big revolution" in healthcare and medical therapies. Compared to large bio-molecules such as antibodies, receptors and enzymes, nanoparticles (NPs) are typically smaller than several hundred nanometers. Owing to their size (one hundred to ten thousand times smaller than human cells), NPs can undergo several interactions with biological molecules both on the surface of and within the cells, which can revolutionize cancer detection and treatment.

The biological function and bio-distribution of particles are therefore primarily affected by nanoparticle-protein complexes. In their ability to extravagate

from the bloodstream to enter the tumour tissue, nanoparticle size plays a significant role. The size of NPs should be large enough to prevent filtration by the kidney and small enough to avoid the capture by the liver and spleen. Nano-structures (<120nm) are capable of penetrating the extremely permeable capillaries of the blood that supply the tumours that develop rapidly. However, since the blood vessels are well developed and non-porous, this does not happen in normal tissue. As a consequence of inadequate lymphatic drainage, they accumulate and are stored in the tumour once within the capillaries. In addition, formulations with suitable nanoparticles may boost physicochemical properties, such as aqueous solubility, resulting in higher therapeutic performance.

1.2 EXISTING METHODS FOR CANCER DIAGNOSTICS

In general, the detection and diagnostic techniques used for cancer are classified into 3 categories: Non-invasive techniques, Invasive techniques, Analysis of Biopsy.

1.2.1 NON-INVASIVE METHODS

The most popular methods used in non-invasive techniques are

i. Often known as sonography, ultrasound scanning is an imaging method used to identify several different kinds of cancers. It utilizes sound waves and their echoes to visualize the internal structures of the body.

ii. A non-invasive means of looking at organs, tissues, bones, and other structures within the body is MRI, or magnetic resonance imaging. To produce images of the body, it utilizes large magnetic fields and radio waves.

iii. A Positron Emission Tomography (PET) scan is an imaging technique that creates a dynamic image of internal tissues and organs using radioactive molecules. PET scans create photographs that show living tissue activity. In contrast to methods that reveal structure but not activity, this is.

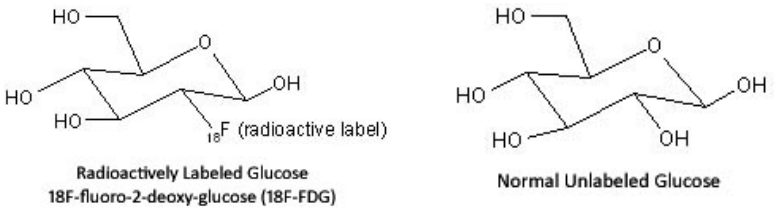

Fig.1.1 Difference in unlabelled and radioactively labelled Glucose

iv. A Computed Tomography (CT) scanner uses x-rays in the same manner as a conventional x-ray, but a CT scanner takes multiple images or slices instead of taking one image. To create a 3-dimensional image of the internal structures being examined, a computer gathers all the images and compiles them.

1.2.2 INVASIVE METHOD

Two invasive techniques are available,

i. Fine Needle Aspiration (FNA) is performed with a small needle of 20 to 27 gauges (same size or smaller than most needles used in ordinary blood test, a larger gauge corresponds to a smaller needle). To avoid infection, the area is sterilized with alcohol. Then the needle is inserted and directed at the lesion centre. A very small fragment is removed by suction as the needle hits the lesion. To ensure that a correct amount of available sample is collected, this is repeated.

ii. A fine needle aspiration (FNA) is similar to a core needle biopsy, except that a wider needle (11-18 gauge) is used and the pathology report is different. Local anaesthesia is used to numb the region before injection due to the needle being wider than in an FNA.

1.3 BIOPSY

Techniques used in biopsy research are,

i. The technique used to assess the existence and amount of particular cellular proteins is immunohistochemistry (IHC). Using specially labelled antibodies that can bind to the proteins of interest, IHC tests protein expression.

ii. Fluorescence in Situ Hybridization (FISH) is a technique using fluorescently labelled DNA to test gene amplification.

1.4 LIMITATIONS IN EXISTING METHODS

All these techniques are useful in cancer detection but has some limitations in terms of

- Accuracy
- Cannot detect all cancers
- Requires a greater number of infected cells to identify the tumour
- Only effective in detecting tumors that are close to the skin surface
- May identify a potential area of concern that is not malignant (high false results)
- Inherent technical limitations

1.5 NEED FOR NANOPARTICLES IN CANCER DETECTION

One of the key problems in the treatment of cancer is the early detection of the disease. Methods for the early detection of cancer are of utmost importance and are an active area of current research. Blood tests are a common and straightforward means of screening people for cancer in its early stages. If a chemical in the blood that signals the presence of even a small tumour can be detected, the cancer could be treated sooner and would be more likely to be cured. Often cancer is detected in its later stages, when it has comprised the function of one or more vital organ systems and is wide spread throughout the body. So, the use of nanoparticles to diagnose cancer now is becoming an emerging methodology. Nanoparticles also act as contrast agents in Magnetic Resonance Imaging for obtaining the clear image of cancer in the human body.

1.6 CERIUM OXIDE/ ZINC OXIDE/ GRAPHENE OXIDE NANOPARTICLES IN CANCER DIAGNOSTICS

Many researchers have been drawn to semiconducting nanomaterials because of their ideal flexible properties for a variety of applications.

ZnO nanoparticles have piqued the interest of researchers all over the world due to their low cost, biocompatibility, small band-gap, and ability to act as effective electron mediators in a variety of redox reactions. Because of their inherent properties, such as large band gap, binding energy, strong exciton, biocompatibility, nontoxicity, better electrochemical activities, photochemical stability, high electron communication features, and so on, zinc oxide (ZnO) nanoparticles are considered to be superior. Transition metal oxides with large band gaps can act as semiconductors or even insulators, which is one of ZnO's drawbacks. Due to pronounced volume expansion and contraction, these individual characteristics cause weak ion transport kinetics in electrode films. As a result, in order to increase their efficiency and preserve their properties throughout the fabrication process, they must be combined with other metal oxides, carbon-based materials, and polymers.

CeO_2 nanoparticles, like ZnO nanoparticles, have similar properties, but they also have excellent and fast electron transfer kinetics, allowing them to be sensitive to human health and the environment. The most significant feature of CeO_2 nanoparticles is that they have a high oxygen mobility on their surface, which makes it easier to convert between the valence states of Ce^{4+} and Ce^{3+} and keeps their crystallinity. This property aids in improving their sensitivity, catalytic activity, biological compatibility, structural stability, and high surface area, which will revolutionize numerous approaches to different applications.

Cerium oxide is a highly reactive rare-earth metal oxide used in a number of applications including catalysts, fuel cells, gas sensors, and biomedical devices. It has an oxygen vacancy that reduces and oxidizes at high temperatures, which adds to its technical importance. The proper optimization of nanostructured CeO_2-ZnO composites results in desirable properties such as high surface area, pore size accessibility, and adjustable surface chemistry, all of which greatly improve their suitability for biological and environmental sensors.

Due to its zero bandgap properties, which restrict its application to optoelectronics, graphene has received a lot of publicity. Graphene oxide (GO) is a graphene derivative with single-layered oxygen groups developed using Hummer's process. Since the functional groups at the edges of GO sheets collectively inhibit electron transfer, incorporating GO sheets as nanocomposite material is one way to take advantage of these properties for biomedical applications. Several attempts have been made to use GO to wage anti-cancer warfare. GO functionalized nanocarriers have recently been used for their drug loading and delivery capabilities. Pristine and fully functional because of its small scale, large surface area, low cost, and useful non-covalent interactions, GO has a high water dispersibility and colloidal stability, making it a potential candidate for biomedical applications. Efforts of several researchers have revealed that GO nanocarriers are very promising for biomedical applications.

1.7 SCOPE OF THE WORK

The objective of this project is to characterize GO, CeO_2 and ZnO nanoparticles prepared by co-precipitation method using XRD and SEM techniques. The main motivation was to synthesis the GO, CeO_2 and ZnO nanoparticles for cancer detection. The overall process involves the following steps:

- To develop a biosensor made of CeO_2 / ZnO/ GO Ternary Nanocomposite and subject the same to CV study for evaluation of its sensitivity on HeLa cell line.
- To perform CV study on Cisplatin induced CeO_2/ ZnO/ GO biosensor interacted with HeLa cell line.
- Compare the effectiveness of developed drug- free CeO_2/ ZnO/ GO biosensor and Cisplatin induced biosensor to estimate its application as drug delivery system in cervical cancer screening.

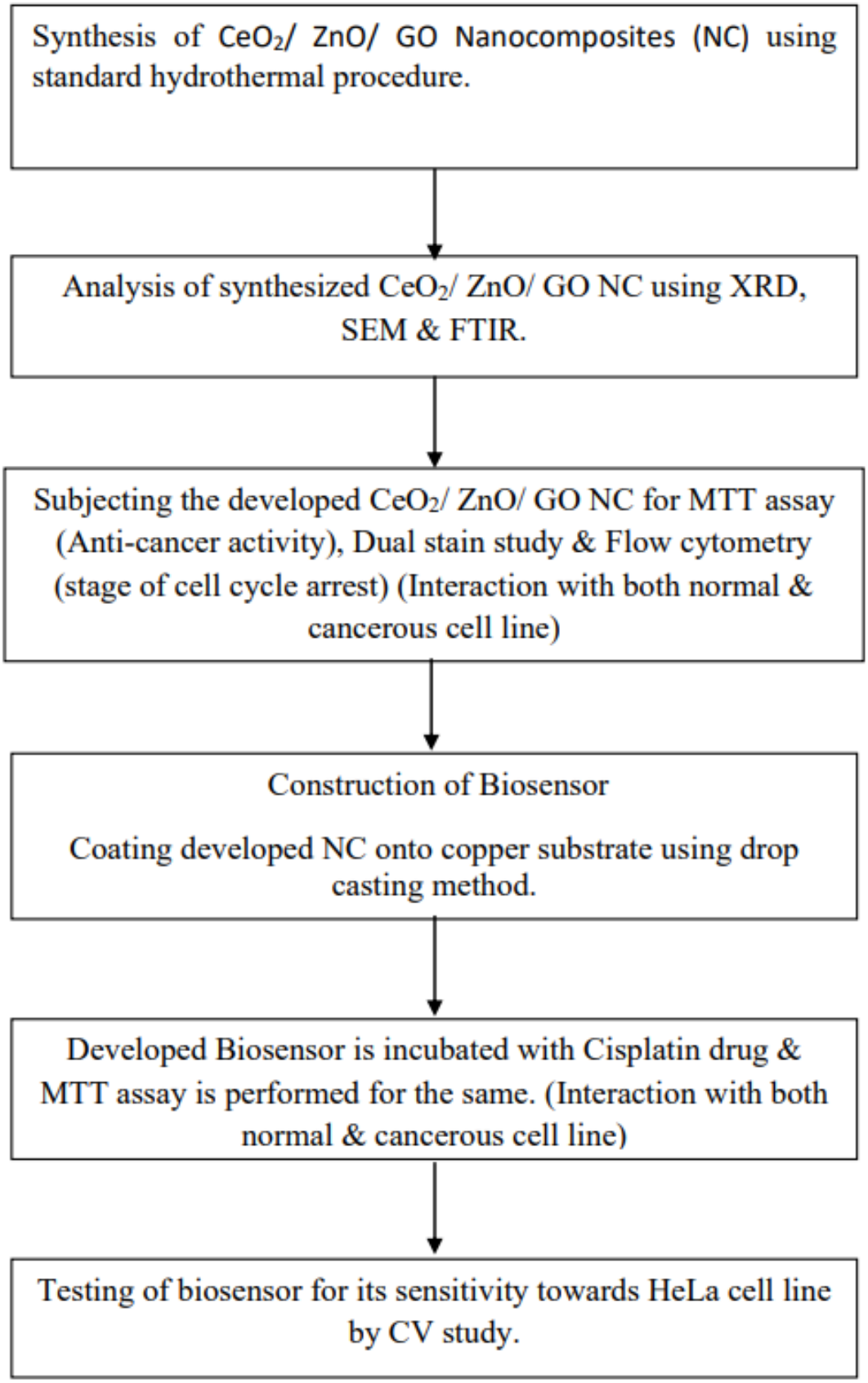

Fig.1.2 Methodology involved in our project

CHAPTER 2
LITERATURE SURVEY

J. Saranya, B. S. Sreeja, G. Padmalaya, S. Radha, M. Arivanandan (2020) studied the cyto-compatibility characteristics using anti-proliferative which revealed the toxicity behavior against HeLa cells and normal cells. All these results depicted that the nano system made of CeO_2/ ZnO is superior to the ZnO NPs.

Saranya J, Sherin S, Saroja K K and Samuel Jude S (2020) reviewed that when a customized nanomaterial has large surface to volume ratio, then it is very easy to hold more drugs onto it and release the same at various target sites. Also, it can capture more antigens on its surface, which makes the diagnosis easier at early stage.

A.Król, Pomasowski, B.Buszewsk, K. Rafińska and V. Railea-Plugaru (2017) reported that characteristics of ZnO nano-particles, such as their inherent toxicity against cancerous cells, their ability to induce intracellular ROS generation leading to death make them an appealing candidate for biomedical applications.

Jinzhao Liua, Jia Donga, Ting Zhangb and Qiang Peng (2018) reported that Graphene-based nanomaterials have great potentials in cancer therapy, either as drug delivery systems or active agents, due to their large surface area which promises a high drug loading capacity.

Kuang- Kai Liu, Wen-Wei Zheng, Chi- Ching Wang, Yu-Chung Chiu, Chia-Liang Cheng, Yu-Shiu Lo, Chinpiao Chen Jui-I Chao (2010) reported that linkage of ND and paclitaxel, is a potential nano material as a drug carrier for cancer drug delivery and therapy. 70 times more efficient for drug delivery than traditional systems.

Esmail Nour-mohammadi, Hoda Sarkarizi, Reza Nedaeinia, Hamid Sadeghni, Leila Hasan, Majid Darroudi and Reza oskuee (2018) observed that in MTT assay method, in WEHI164 cells, toxicity effects were observed from concentration ≥ 15.63. Protects normal cells against the drug.

CHAPTER 3

SYNTHESIS OF GRAPHENE OXIDE/ CERIUM OXIDE/ ZINC OXIDE NANOPARTICLES

3.1 MATERIALS AND METHODS

All chemicals and solvents were of analytical grade and are used without further purification. Cerous Nitrate hexahydrate ($CeN_3O_9.6H_2O$) and sodium hydroxide (NaOH) were purchased from Fischer Scientific, India. Other reagents used were of analytical grade. 0.1 M of phosphate buffer solution (PBS 7.4) and distilled water was used in all the experimentation works. X-Ray diffraction (XRD) spectrum of CeO_2/ZnO/GO hybrid nano system was recorded using RIGAKU mini flux 2C, Japan. Surface Morphology was obtained using SEM model SUPRA 55, Carl Zeiss NTS GMBH, Germany.

3.2 SYNHESIS OF NANOPARTICLES

3.2.1 PROCEDURE TO PREPARE GO NANOPARTICLES

Graphene oxide (GO) was prepared by the hummer mechanism. Here a mixture of 1g of sodium nitrate and 1g of graphite flakes is applied to 100 ml of sulphuric acid and put in an ice bath at a temperature below 5 °C. This mixture is then stirred for two hours on a continuous basis. 8g of Potassium permanganate is added to it after intense stirring. The ice bath is removed and the mixture is mixed for two days before a brownish paste turns into the solution. The paste is then combined with Hydrogen Peroxide and diluted with deionized water. Till it turns yellow, the solution is stirred. To extract impurities from the solution, it is washed several times with diluted hydrochloric acid and deionized water. For the particles to settle down, the solution is centrifugated. The particle is then dried for the GO powder to be obtained.

Fig.3.1 Graphene Oxide Synthesis

3.2.2 PROCEDURE TO PREPARE CeO_2 NANOPARTICLES

Cerium oxide nanoparticles (CeO_2 NPs) have been pre-pared by using a simple wet chemical technique at room temperature. Firstly, 0.1 M of cerous nitrate hexahydrate ($CeN_3O_9 \cdot 6H_2O$) was dissolved in 100 ml of distilled water. 50 ml of 0.5 M sodium hydroxide (NaOH) solution was added drop-wise to 100 ml cerous nitrate hexahydrate solution under constant stirring. The solution continued to be in a stirring condition for 2 h and a brown color was observed Upon continuous stirring, a yellowish color was noticed indicating the formation of CeO_2 NPs. This yellowish solution was kept aside for the CeO_2 NPs to settle down and further it was washed thrice using distilled water. Finally, it was dried under 150 °C and annealed at 350 °C. The powder thus obtained was used for further analytical purposes.

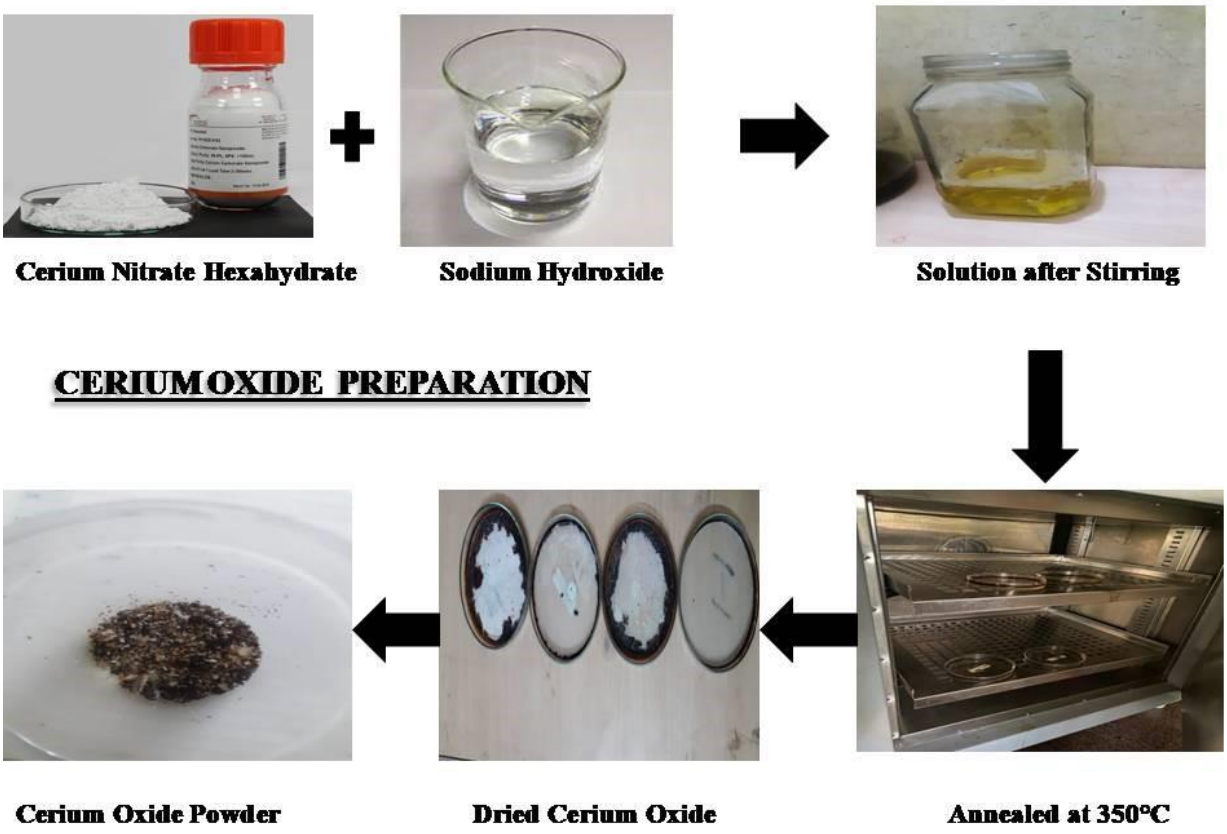

Fig.3.2 Cerium Oxide Synthesis

3.2.3 PROCEDURE TO PREPARE ZnO NANOPARTICLES

Zinc oxide nano-particles were prepared by wet chemical method.500ml 1%starch solution was obtained by mixing 5gm starch solution with 500ml distilled water and boiling the mixture until transparent.14.87 gm Zinc nitrate Hexahydrate was added to the above solution to obtain a 0.1M Zinc Nitrate solution.100ml 0.2M Sodium Hydroxide solution was prepared by dissolving 0.8gm NaOH crystals in distilled water. 25ml of the 0.2M NaOH solution was added drop wise along the walls of the vessel to the 0.1M Zinc Nitrate solution while continuously stirring with magnetic stirrer. The mixture was continuously stirred for 2hours by the magnetic stirrer and then kept undisturbed overnight to allow the precipitation of zinc Hydroxide. Supernatant was discarded carefully and the remaining solution was centrifuged in a cooling centrifuge at 11000 rpm for 10min to separate the residue from the excess supernatant in the form of a pellet. The pellet was washed

for 2-3 times with distilled water to remove starch from zinc hydroxide pellet hence obtained. The paste was kept in oven at 80C to allow dehydration. Zinc oxide formed is suspended.

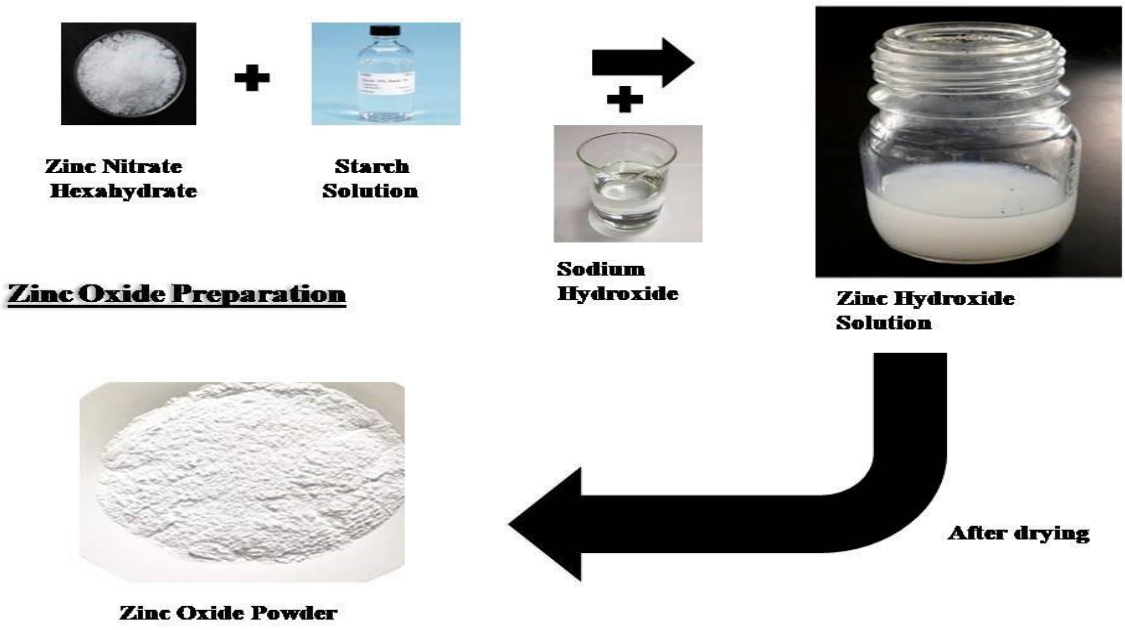

Fig.3.3 Zinc Oxide Synthesis

CHAPTER 4

CHARACTERIZATION TECHNIQUES

The findings and discussion of the approaches described in the previous chapters are discussed in this chapter. We use X-ray Diffraction Spectroscopy, Scanning Electron Microscopy and Fourier Transform Infrared Spectroscopy (FTIR) to classify nanoparticles for our research. The nanoparticles are then subjected to biomarker characterization to determine their viability for cancer detection based on the results obtained using the techniques.

Due to their special and often beneficial properties, nanoparticles and nanoparticle-based devices are of interest in a wide range of industrial applications. Many size-dependent phenomena, such as chemical, electrical, magnetic, and mechanical properties, are introduced by nanoparticles' high surface-to-volume ratio combined with size effects (quantum effects). Particle sizing is an important task in the property characterization of nanoparticles because particle size plays such an important role in their properties.

Several commercially available instruments can be used to assess the particle size and size distribution of nanoparticles. Dry powders and powders spread in suspension can also be examined with these instruments. There are two simple methods for determining particle size in general. The first approach involves physically inspecting the particles and taking measurements of their proportions. Particle pictures, for example, can be used to quantify a variety of dimensional parameters using microscopic techniques. The second approach makes use of the relationship between particle size and behaviour. Furthermore, by analysing the findings of various instruments, additional knowledge about the sample can be gleaned.

4.1 XRAY DIFFRACTION

4.1.1 INTRODUCTION TO X-RAY DIFFRACTION

About 95 percent of all solid materials are crystalline in nature, and X-ray powder diffraction (XRD) is a fast analytical method mainly used for phase identification of crystalline materials and can provide information on unit cell dimensions. A diffraction pattern is produced when X-rays are passed over a crystalline substance. A pure substance's X-ray diffraction pattern acts as a fingerprint. As a result, powder diffraction is an excellent method for identifying and characterizing polycrystalline phases. Powder diffraction is primarily used in a search/match process to identify components in a sample.

XRD has been successfully used to investigate the structure of materials in which atoms are only ordered over short distances. When these materials are irradiated with x-rays, they act as very imperfect gratings, resulting in highly diffuse XRD patterns.

4.1.2 THEORY AND METHODOLOGY:

Basic principle:

When electromagnetic radiation impinges on periodic structures with geometrical variations on the length scale of the wavelength of the radiation, diffraction effects are observed. In crystals and molecules, interatomic distances range from 0.15 to 0.4 nm, which corresponds to the wavelength of x-rays with photon energies ranging from 3 to 8 keV in the electromagnetic spectrum. When crystalline and molecular structures are exposed to x-rays, phenomena such as constructive and destructive interference maybe visible.

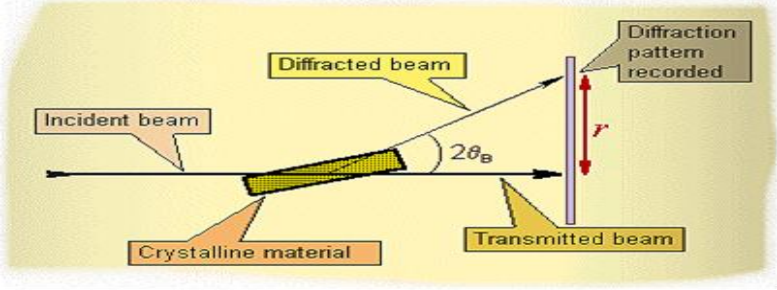

Fig.4.1 Experimental setup of X-ray powder diffraction

4.1.3 BRAGG'S LAW:

The detector can only give the peak corresponding to the radiation diffracted by the sample along the direction if Bragg's law is satisfied. It's important to remember two geometrical facts:

(1) The incident beam, the normal to the reflecting plane, and the diffracted beam are all always coplanar.

(2) There is always a 2° angle between the diffracted and transmitted beams. This is known as the diffracted angle, and it is this angle that is normally measured experimentally rather than.

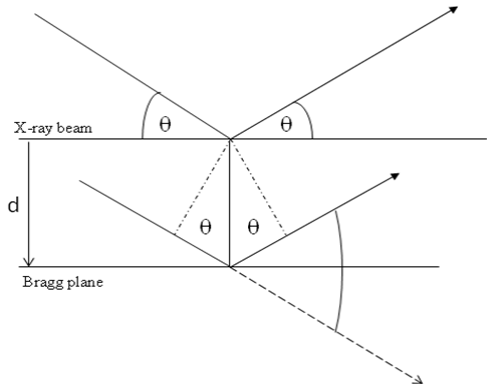

Fig.4.2 Bragg's Law

A crystal could not diffract possible ultraviolet radiation. On the other hand, if λ is very small, the Bragg law may be written in the form:

$$n\lambda = 2d\sin\theta$$

where n is integer.

This is the Bragg law of diffraction. Thus, the diffracted beam makes an angle θ with the Bragg plane and the angle between the incident beam and the diffracted beam is 2θ.

4.1.4 X-RAY SPECTROSCOPY

The Bragg law can be used in two ways in experiments. Structure analysis is the process of determining the spacing d of different planes in a crystal by using x-rays of known wavelength λ and measuring. Alternatively, we can use a crystal

with known spacing d to measure and thus calculate the wavelength of the radiation being used, which is known as x-ray spectroscopy.

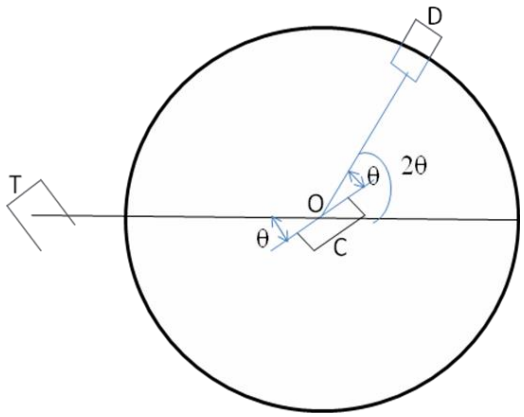

Fig.4.3 X-Ray Spectrometer

T – X-ray source C – crystallite sample D – Detector

The following are the basic characteristics of an x-ray spectrometer:

(1) X-rays from the tube T strike a crystal C, which can be rotated around an axis through O, the spectrometer's center, to any desired angle to the incident beam. D is an ionization chamber or some other kind of counter that measures the intensity of diffracted x-rays; it can also be rotated around O and set at any angle.

(2) The crystal is positioned so that its reflecting planes form a specific angle with the incident beam, and D is set to that angle. The wavelength of the diffracted beam is calculated using the Bragg law after the intensity of the diffracted beam is measured.

4.1.5 DIFFRACTED DIRECTIONS

The order of reflections can be used to determine possible angles 2θ in which the incident planes can be obtained. The crystal system to which the crystal belongs and its lattice parameters determine when a beam of a given wavelength is diffracted by a given set of lattice planes. Diffraction directions are strictly determined by the unit cell's shape and size. The positions of the atoms within the unit cell determine the intensities of diffracted beams.

4.1.6 DIFFRACTED METHODS

Diffraction can occur whenever the Bragg law, $\lambda=2d\sin\theta$ is satisfied. An

arbitrary setting of a single crystal in an x-ray beam will not produce any diffracted beams in monochromatic radiation. The Bragg law must be satisfied in some way, and this can be obtained by constantly varying either λ or θ during the experiment.

4.1.7 RECIPROCAL LATTICE

The most useful method for describing diffraction phenomena is known as "reciprocal lattice". It constructs a geometrical construction using the reciprocal of d_{hkl}. When arranging an x-ray source, specimen, and detector in order to predict the motions of specific diffracting effects. The reciprocal vector is defined as:

$$*\mathbf{d}_{\mathbf{hkl}} = \frac{1}{d_{hkl}}$$

Where, hkl - Miller Indices

Because the units are in reciprocal angstroms, the space is reciprocal as well. A reciprocal lattice is a space lattice defined by periodic intervals. a*, b*, and c* are the repeating translation vectors. There are several key properties of the reciprocal lattice:

• The numerator's cross-product indicates that b1 is perpendicular to a2 and a3. The vectors of a three-dimensional lattice are defined by the vectors a, b, and c.

• The plane in direct space whose miller indices are hkl is perpendicular to a vector Hhkl drawn from the origin of reciprocal space to any point in reciprocal space with coordinates h, k, and l. The length Hhkl of the reciprocal lattice vector Hhkl = hb1 + kb2 + lb3 equals the reciprocal of the periodicity of (*hkl*), i.e., Hhkl = 1/dhkl.

In non-cubic structures, the direct space vector [hkl] will not be perpendicular to (hkl). Direct measurement, therefore, shows that the reciprocal lattice vectors' lengths are equal to the inverse of the spacing between corresponding planes. As a result, the infinite series of physical direct space planes

can be represented by a single lattice point in reciprocal space, defined by vector H_{hkl}.

4.1.8 APPLICATIONS OF XRD

XRD has a wide range of applications in geology, material science, environmental science, chemistry, forensic science, and the pharmaceutical industry.

4.1.8.1 Identification

The most used method for identifying unknown crystalline materials is X-Ray diffraction. This may include phase identification (search/match), high/low temperature phase investigation, solid solutions, and unit cell parameter determinations for new materials.

Current applications of line broadening for the prediction of material property gradients such as yield strength in machined and shot peened surfaces, and hardness in steels are discussed. With the introduction of x-ray diffractometers and the development of the plane-stress residual stress model, hardened steels could be successfully studied.

4.1.8.2 Texture analysis

Texture analysis is the process of determining the preferred crystallite orientation in polycrystalline aggregates, and the term texture refers to the preferred crystallographic orientation of a polycrystalline material, usually a single phase. Pole figures are often used to describe the preferred orientation.

4.1.8.3 Polymer crystallinity

Partially crystalline and partially amorphous polymers exist. The crystalline domains act as a reinforcing grid, similar to the iron framework in concrete, and increase performance across a broad temperature range. Brittleness is caused by too much crystallinity. The crystallinity components produce narrow, sharp diffraction

peaks, while the amorphous component produces a wide peak (halo). The amount of crystallinity in the material can be calculated using the ratio between these intensities.

4.2 SCANNING ELECTRON MICROSCOPY (SEM)

4.2.1 INTRODUCTION TO SEM

The scanning electron microscope (SEM) is used to examine the surfaces of specimens. Secondary electrons are emitted from the specimen surface when it is irradiated with a fine electron beam (called an electron probe). The topography of the surface can be seen by scanning the electron probe in two dimensions over the surface and capturing an image from the detected secondary electrons.

4.2.2 BASIC PRINCIPLE AND METHODOLOGY

1. A metallic filament at the top of the microscope generates electrons, which is referred to as the electron gun. The filament looks a lot like the filament inside a light bulb.

2. The emitted electrons are then accelerated down the column toward the specimen in the form of a beam.

3. As the beam moves down the column, electromagnetic lenses focus and direct it even more.

4. Electrons are kicked loose from the surface of the specimen when the beam reaches it. Secondary electrons are the electrons that make up the nucleus.

5. A detector detects these electrons, amplifies the signal, and sends it to a monitor.

6. The electron beam scans back and forth across the sample, generating an image based on the number of electrons emitted from each spot.

7. The entire procedure is carried out in a vacuum. The electron beam interacts with the sample rather than the air because of the vacuum.

8. Samples to be viewed with the SEM must be conductive and able to resist a vacuum.

9. A procedure known as sputter coating can be used to coat non-conductive samples with a thin layer of conductive material.

Fig.4.4 SEM Setup

4.2.3 APPLICATIONS OF SEM

4.2.3.1 Medical Science

SEM is currently used to definitively characterize a small but significant proportion of clinical presentations (3-8%), particularly cancer and non-neoplastic renal disease. These figures likely understate SEM's potential contribution, as it is constrained not only by its lack of usefulness in many clinical situations, but also by its expense, time to produce results, and low output compared to histological techniques. The SEM technique, we believe, has the potential to address the latter three issues because it is especially well suited to fast, simple, and cost-effective tissue specimen preparation and imaging.

4.2.3.2 Crohn's Disease

Crohn's disease is a particular type of inflammatory bowel disease (IBD). Traditional SEM has been used to characterize biopsied tissues for early pathological changes that are not visible macroscopically.

4.3 FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

Fourier Transform Infrared Spectroscopy, also known as FTIR Analysis or FTIR Spectroscopy, is an analytical technique used to identify organic, polymeric, and in some cases, inorganic materials. The FTIR analysis method uses infrared light to scan test samples and observe chemical properties.

4.3.1 VALUABLE ANALYSIS WITH FTIR SPECTROSCOPY

- Assessing purity
- Identifying:
    - Base polymer composition
    - Additives
    - Organic contaminants
- General type of material being analyzed when there are unknowns

The infrared spectrum of absorption or emission of a solid, liquid, or gas is obtained using the Fourier transform infrared spectroscopy (FTIR) technique. An FTIR spectrometer gathers high spectral resolution data over a wide spectral range at the same time. This gives it a big advantage over a dispersive spectrometer, which only measures intensity over a small range of wavelengths at a time.

Fig.4.5 An example of an FTIR spectrometer with an attenuated total reflectance (ATR) attachment

The goal of any absorption spectroscopy technique (FTIR, UV-Vis, etc.) is to determine how well a sample absorbs light at different wavelengths.

The simplest method, known as "dispersive spectroscopy," involves shining a monochromatic light beam at a sample, measuring how much of the light is absorbed, and repeating the process for each different wavelength. (Some UV-Vis spectrometers, operate in this manner.)

The use of Fourier transform spectroscopy to obtain the same information is less intuitive. Rather than shining a monochromatic beam of light at the sample, this technique shines a multi-frequency beam at the sample and measures how much of it is absorbed. The beam is then changed to contain a different frequency combination, yielding a second data point. This procedure is repeated a number of times. After that, a computer uses all of this information to infer absorption at each wavelength by working backwards.

Starting with a broadband light source—one that contains the entire spectrum of wavelengths to be measured—the beam described above is created. The light shines through a Michelson interferometer, which is a set of mirrors with one of them being moved by a motor. Due to wave interference, the interferometer regularly blocks, transmits, blocks, transmits each wavelength of light in the beam as it moves. Different wavelengths are modulated at different rates, resulting in a different spectrum for each beam exiting the interferometer.

As previously stated, computer processing is required to convert raw data (light absorption for each mirror position) into the desired outcome (light absorption for each wavelength). The necessary processing is revealed to be the Fourier transform, which is a widely used algorithm. An "interferogram" is a term used to describe the raw data.

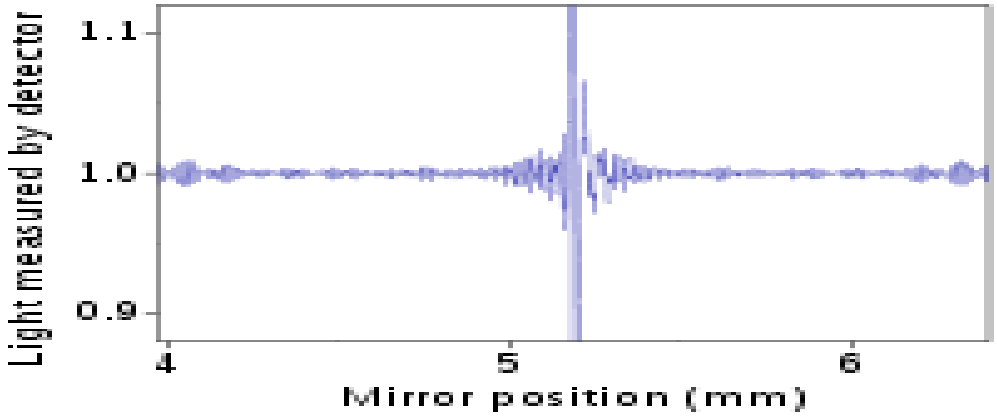

Fig.4.6 Interferogram

4.3.2 ADVANTAGES OF FOURIER TRANSFORM SPECTROSCOPY

When compared to a scanning (dispersive) spectrometer, an FT spectrometer has three major advantages.

1. The multiplex or Fellgett's advantage. This arises from the fact that information from all wavelengths is collected simultaneously. It results in a higher Signal-to-noise ratio for a given scan-time for observations limited by a fixed detector noise contribution (typically in the thermal infrared spectral region where a photo detector is limited by generation-recombination noise). For a spectrum with m resolution elements, this increase is equal to the square root of m. alternatively; it allows a shorter scan-time for a given resolution. In practice multiple scans are often averaged, increasing the signal-to-noise ratio by the square root of the number of scans.

2. The Jacquinot advantage, or throughput. This is due to the fact that the monochromator in a dispersive instrument has entrance and exit slits that limit the amount of light that passes through it. Only the diameter of the collimated beam coming from the source determines the interferometer throughput. FTIR spectrometers need an aperture to limit the convergence of the collimated beam in the interferometer, even though no slits are required. Because the path difference varies, convergent rays are modulated at different frequencies. A Jacquinot stop is the name for such an aperture. For a given resolution and wavelength this circular aperture allows more light through than a slit, resulting in a higher signal-to-noise ratio.
3. The advantage of Connes, or wavelength precision. A laser beam of known wavelength passes through the interferometer, calibrating the wavelength scale. This is much more stable and precise than dispersive instruments, which rely on the mechanical movement of diffraction gratings to determine scale. In practice, the accuracy of the interferometer is limited by the divergence of the beam, which is dependent on the resolution.

Another minor benefit is reduced sensitivity to stray light, which is light of one wavelength that appears at a different wavelength in the spectrum. This is caused by flaws in the diffraction gratings and accidental reflections in dispersive instruments. The apparent wavelength in FT instruments is determined by the modulation frequency in the interferometer, so there is no direct equivalent.

4.3.3 COMPONENTS

4.3.3.1 IR sources

The mid and near IR regions are where FTIR spectrometers are most commonly used. The most common source for the mid-IR region, 225 m (5000–400 cm−1), is a silicon carbide element heated to about 1200 K. The output is comparable to that of a blackbody. Shorter near-IR wavelengths, like 12.5 m (10000–4000 cm−1), necessitate a higher temperature source, like a tungsten-halogen lamp. Because of the absorption of the quartz envelope, the long wavelength output of these is limited to about 5 m (2000 cm−1). A mercury discharge lamp produces more output in the far-IR than a thermal source, particularly at wavelengths beyond 50 m (200 cm−1).

4.3.3.2 Detectors

Pyroelectric detectors, which respond to changes in temperature as the intensity of IR radiation falling on them varies, are widely used in mid-IR spectrometers. Deuterated triglycine sulphate (DTGS) or lithium tantalate are the sensitive components in these detectors (LiTaO3). These detectors work at room temperature and have enough sensitivity for most everyday applications. A scan usually takes a few seconds to achieve the best sensitivity.

For circumstances requiring higher sensitivity or faster response, cooled photoelectric detectors are used. In the mid-IR, liquid nitrogen-cooled mercury cadmium telluride (MCT) detectors are the most common. An interferogram can be measured in as little as 10 milliseconds using these detectors. Near-IR systems typically use uncooled indium gallium arsenide photodiodes or DTGS. In the far-IR, where both sources and beam splitters are inefficient, very sensitive liquid-helium-cooled silicon or germanium bolometers are used.

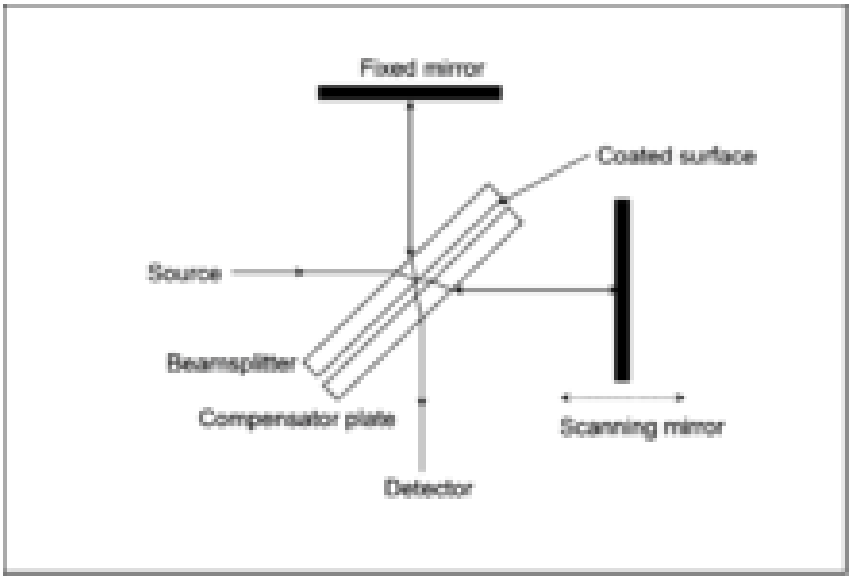

Fig.4.7 Simple interferometer with a beam-splitter and compensator plate

In an ideal beam splitter, half of the incident radiation is transmitted and half is reflected. Due to the limited range of optical transmittance of any material, several beam-splitters can be used interchangeably to cover a wide spectral range. The beam splitter for the mid-IR area is normally made of KBr with a semi-reflective germanium-based coating. Because KBr absorbs strongly beyond 25 m (400 cm−1), CsI is sometimes used to extend the wavelength range to around 50 m (200 cm−1). Where moisture vapor is a concern, ZnSe is an option that is limited to about 20m (500 cm−1). CaF2 is the usual material for the near-IR, being both harder and less sensitive to moisture than KBr but cannot be used beyond about 8 μm (1200 cm^{-1}).

One beam passes through the beam splitter twice in a plain Michelson interferometer, while the other passes through only once. An additional compensator plate of equal thickness is included to compensate for this. The majority of far-IR beam splitters are made of polymer films and only cover a narrow wavelength range.

4.3.3.3 Attenuated total reflectance (ATR)

ATR is an FTIR spectrophotometer accessory that is used to measure surface properties of solid or thin film samples rather than bulk properties. Depending on your sample conditions, ATR has a penetration depth of around 1 or 2 micrometres.

4.3.3.4 Fourier transform

In practise, an interferogram is a set of intensities measured for discrete retardation values. The distinction between consecutive values of retardation is always the same. As a result, the discrete Fourier transform is required. The algorithm used is the fast Fourier transform (FFT).

4.3.3.5 Far Infrared FTIR

For the far-infrared range, the first FTIR spectrometers were developed. This is due to the mechanical tolerance required for good optical performance, which is proportional to the wavelength of the light being used. Tolerances of 10 m are sufficient for the relatively long wavelengths of the far infrared, but tolerances of more than 1 m are required for the rock-salt region. The cube interferometer, designed at the NPL and sold by Grubb Parsons, was an example of a typical instrument

4.3.3.6 Mid – Infrared FTIR

With the introduction of low-cost microcomputers, it became possible to have a computer dedicated to operating the spectrometer, gathering data, performing the Fourier transform, and displaying the spectrum. The development of FTIR spectrometers for the rock-salt region was sparked by this. It was necessary to overcome the challenges of producing ultra-high precision optical and mechanical components. The constant mirror velocity is no longer strictly needed in modern FTIR systems, as long as the laser fringes and the original interferogram are recorded simultaneously at a higher sampling rate and then re-interpolated on a constant grid, as pioneered by James W. Brault.

4.3.3.7 Near – Infrared FTIR

Between the rock-salt region and the start of the visible region at about 750 nm, the near-infrared region covers the wavelength range. In this area, fundamental vibrations' overtones can be seen. It's mostly used in industrial settings like process control and chemical imaging.

4.3.4 APPLICATIONS OF FTIR

FTIR can be used in any application that previously required the use of a dispersive spectrometer. Furthermore, the increased sensitivity and speed have opened up new application possibilities. Spectra can be measured even when only a small amount of energy enters the detector, and scan rates can reach 50 spectra per second. In geology, chemistry, materials science, and biology, Fourier transform infrared spectroscopy is used for study.

4.3.4.1 Microscopy and imaging

Samples as small as 5 microns across can be detected and spectra measured using an infrared microscope. Combining a microscope with linear or 2-D array detectors will produce images. With tens of thousands of pixels, the spatial resolution can approach 5 microns. Each pixel in the image has its own spectrum, which can be interpreted as a map of intensity at any wavelength or combination of wavelengths. This makes it possible to see the distribution of various chemical species within the sample. Typical studies include investigating the homogeneity of pharmaceutical tablets and analysing tissue sections as an alternative to traditional histopathology.

4.3.4.2 FTIR as detector in chromatography

FTIR can obtain spectra from compounds as they are separated by a gas chromatograph. However, in comparison to the more sensitive GC-MS (gas chromatography-mass spectrometry), this method is seldom used. The GC-IR method is especially useful for detecting isomers, which have identical masses by definition. Because of the solvent, liquid chromatography fractions are more difficult. One noteworthy exception is the use of chlorinated solvents with no absorption in the region in question to measure chain branching as a function of molecular size in polyethylene using gel permeation chromatography.

4.3.4.3 TG-IR (thermogravimetric analysis-infrared spectrometry)

Measuring the gas produced as a material is heated allows for qualitative species identification in addition to the purely quantitative information provided by weight loss measurements.

CHAPTER 5

FABRICATION OF NANO SYSTEM USING DROP CASTING TECHNIQUE

Biosensors are analytical devices in which specific recognition of the chemical substances is performed by biological material. The biological material that serves as recognition element is used in combination with a transducer. The transducer transforms concentration of substrate or product to electrical signal that is amplified and further processed. The biosensors may utilize enzymes, antibodies, nucleic acids, organelles, plant and animal tissue, whole organism, or organs. Biosensors containing biological catalysts (enzymes) are called catalytical biosensors. Biosensors of this type are the most abundant, and they found the largest application in medicine, ecology, and environmental monitoring.

5.1 FABRICATION TECHNIQUE

Techniques for the deposition of thin films, many of which have been developed for the fabrication of magnetic and semiconductor devices and optical coatings, can also be applied to grow thick films (1-5 μm) for the fabrication of optical waveguides. The deposition of materials on a substrate can be done with techniques such as Drop Casting, Spin coating, Dip coating, etc.

5.1.1 DROP CASTING TECHNIQUE

The simple, easy and rapid technique of “drop casting” is widely used to prepare the surface of chemically modified electrodes in which the modifying layer is composed of particles such as nanotubes or nanoparticles and used for electro-catalysis notably for chemical sensing as well as materials evaluation.

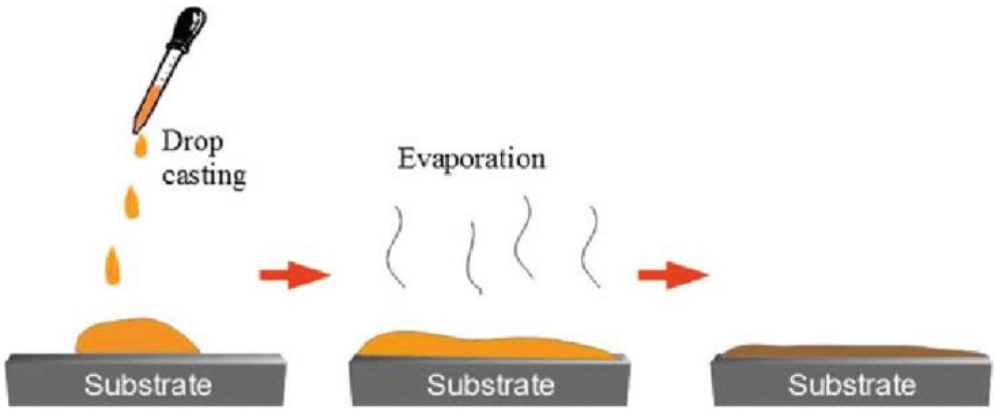

Fig.5.1 Steps for Drop Casting Method

The nanocomposite material is mixed with DMSO solvent and drop casted onto a glass film. This film is then placed in a hot air oven for evaporation process to take place, at 80C for 15 minutes. The final product is then utilized for cyclic voltammetry study.

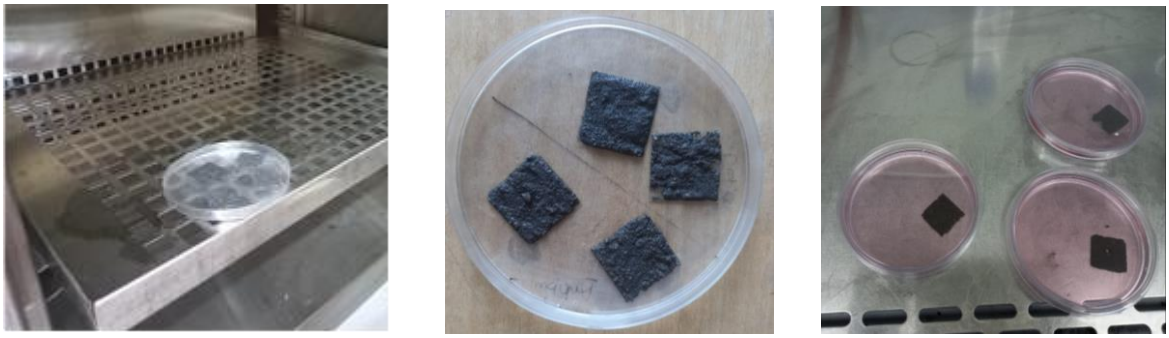

Fig.5.2 Fabrication through Drop Casting Technique

The advantage is, the method is very simple. Mix the material in a suitable solvent (the material should not change its form) and cast by dropping on the target substrate.

The disadvantage is, not easy to get a uniform coating and not well controlled to obtain a thickness.

CHAPTER 6

IMAGING MODALITIES FOR CERVICAL CANCER DIAGNOSIS UNDER IN VITRO CONDITIONS

6.1 INTRODUCTION TO CELL LINES

Cancer cell lines have made a substantial contribution to cancer translational research and biomedical discovery. The ready availability and widespread dissemination of the cell lines to investigators worldwide have resulted in more than 9000 citations, including multiple examples of important biomedical discoveries. The laboratory technique of maintaining live cell lines separated from their original tissue source became more robust in the middle 20th century. The cancer genome is amazingly complex, as manifested by a detailed analysis of cancer cell lines. Cell lines have played crucial roles in the identification and characterization of driver mutations. Every single important driver mutation present in cancer tumours is also represented in the large bank of cancer cell lines that are available for investigation, providing crucial, and in some cases essential, resources for the study of cancer pathogenesis.

6.2 IN-VITRO STUDIES OF NANOCOMPOSITE MATERIAL

The nanocomposite material is tested on cancer cells to check how well it can reduce the number of cancer cells. It is also tested on normal cells to check if there is any damaging effect. The non –cancerous cells are referred as *vero* cells. The cervical cancer cells are called *HeLa* cells.

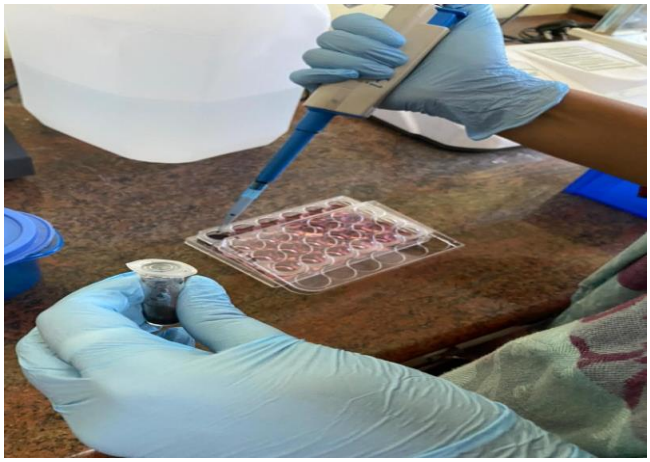

Fig.6.1 Addition of sample dilution to the HeLa cell-line

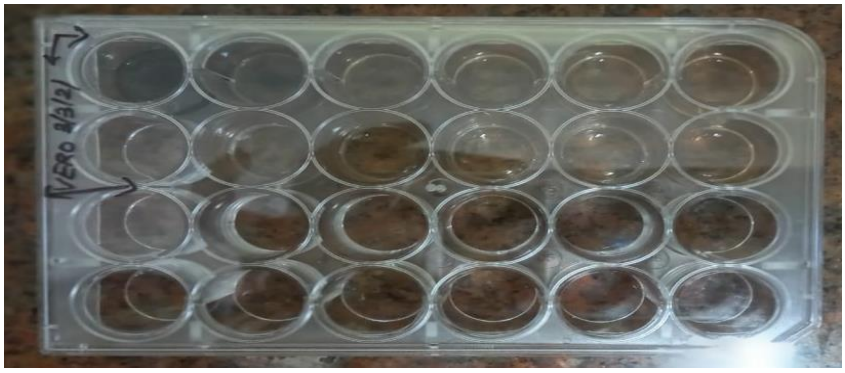

Fig.6.2 VERO cell-lines in incubation with sample

6.2.1 MTT ASSAY

a. Cell Line & Culture

VERO cell lines were obtained from The King Institute Guindy, Chennai and HeLa cell lines were purchased from Saveetha Dental College, Velappanchavadi, Chennai. The cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO2 at 37 °C.

b. Reagents:

MEM was purchased from Hi Media Laboratories Fetal Bovine Serum (FBS) was purchased from Cistron laboratories Trypsin, methylthiazolyldiphenyl-tetrazolium bromide (MTT) and Dimethyl sulfoxide (DMSO) were purchased from

(Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

c. In Vitro assay for Cytotoxicity/Anti-Cancer activity (MTT assay) (Mosmann, 1983):

Cells (1 × 105/well) were plated in 24-well plates and incubated in 370C with 5% CO2 condition. After the cell reaches the confluence, the sample was added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate- buffered saline (pH 7.4) or MEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4,5- dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically. The % cell viability was calculated using the following formula:

% cell viability = A570 of treated cells / A570 of control cells × 100

6.2.2 DUAL STAINING (ACRIDINE ORANGE AND ETHIDIUM BROMIDE STAINING)

Principle:

Acridine orange is a vital dye and will stain both live and dead cells. Ethidium bromide will stain only cells that have lost membrane integrity. Live cells will appear uniformly green. Early apoptotic cells will stain green and contain bright green dots in the nuclei as a consequence of chromatin condensation and nuclear fragmentation. Late apoptotic cells will also incorporate ethidium bromide and therefore stain orange, but, in contrast to necrotic cells, the late apoptotic cells will show condensed and often fragmented nuclei.

Procedure:

- HeLa cells were seeded (one lakh cells per ml) on a cover slip placed in a 6 well plate and incubated for 24 hours of time.
- After incubation, the mono layer of cells was treated with sample (Con:1000µg/ml Con:500µg/ml and Con:250µg/ml) and incubated for 24 hours of time.
- The treated cells were washed with sterile PBS. 70% ethanol was used to fix the cells.
- Incubate 25 µl of cell suspension (0.5×10^6 to 2.0×10^6 cells/ml) with 1 µl of AO/EB solution.
- Each sample should be mixed just prior to microscopy and quantification.
- Samples must be evaluated immediately.

Place 10 µl of cell suspension onto a microscopic slide, cover with a glass cover slip, and examine at least 300 cells in a fluorescence microscope using a fluorescein filter.

6.2.3 FACS

Principle:

The basic working principle of Flow Cytometry is – this analytical technique requires fluorescent labelling of cells, followed by the passage of these cells suspended in a fluid sheet as a single file to a laser beam. The scattered and fluorescent wavelengths are detected and analysed or used for the sorting of cells. The preparation protocol differs based on the source, target, and purpose. In case some tissue is used as a source of cells then it should be first disintegrated into single cells, but when blood is the source then no need for disintegration. Further, it is important to tag a fluorescent probe mostly an antibody that can detect cell-specific protein. In many cases, commercially available antibodies are used. Sometimes unknown antibodies can be developed and used. But for both prior to flow cytometry microscopic evaluation of specific and non-specific staining or any

abnormal staining is important. Once the staining is optimized using microscopy, it can be used for sample preparation in flow cytometry.

Procedure:

- HeLa cells (1 x 106) were cultured in a tissue culture dish and kept to mature all-night.
- The cells were treated for 72 hrs and cells without CeO_2/ZnO/GO nanocomposite were kept as control.
- After attaining 75% confluence, the cells were trypsinized and collected in appropriate centrifugal tubes.
- The above were centrifuged at 2500 rpm for 5 min at room temperature. Then re-suspended in 300 mL of phosphate buffer solution – Ethylenediaminetetra acetic acid to which 700 mL of chilled 70% ethanol was added drop-wise with slow mixing.
- The solution was added to ensure absolute combination of ethanol, and the samples were stored at 0 ºC overnight. The solution was added to ensure absolute combination of ethanol, and the samples were stored at 0 ºC overnight.
- Subsequently, 100 µl RNase A was added to the cells suspension and the mixture was incubated at 37 ºC for 1 hr.
- 400 µl of propidium iodide was added to the stain and incubated for 10 – 20 min at room temperature in darkness.
- The stained cells were analysed for cell phase distribution, using flow cytometry.

6.2.4 INTRODUCTION TO CV STUDY

Principle:

Cyclic voltammetry (CV) is a type of potentiodynamic electrochemical measurement. In a cyclic voltammetry experiment, the working electrode potential is ramped linearly versus time. Unlike in linear sweep voltammetry, after the set potential is reached in a CV experiment, the working electrode's potential is ramped in the opposite direction to return to the initial potential.

These cycles of ramps in potential may be repeated as many times as needed. The current at the working electrode is plotted versus the applied voltage (that is, the working electrode's potential) to give the cyclic voltammogram trace. Cyclic voltammetry is generally used to study the electrochemical properties of an analyte in solution or of a molecule that is adsorbed onto the electrode.

Experimental Setup:

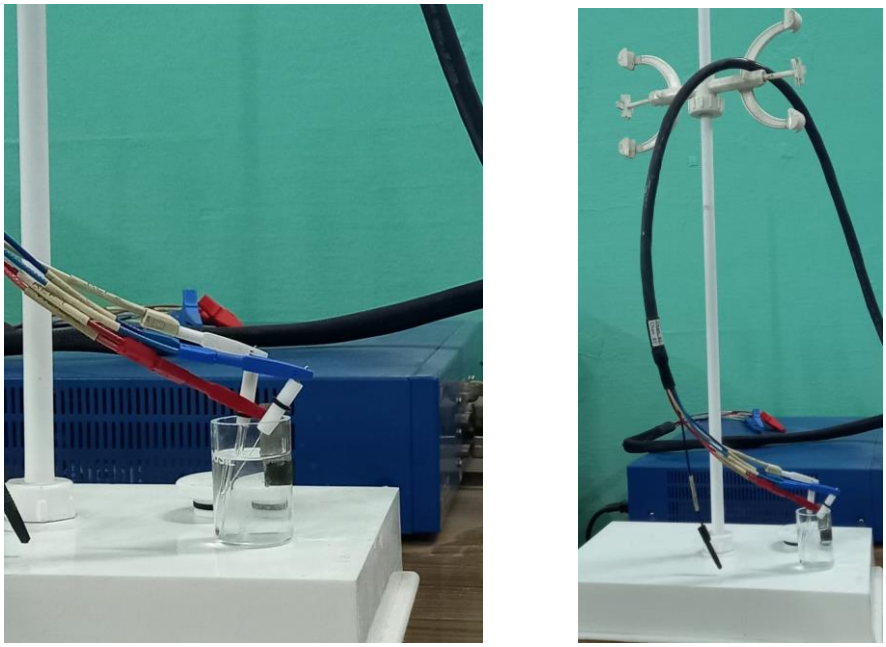

Fig.6.3 Experimental Setup for CV Study

In cyclic voltammetry, the electrode potential ramps linearly versus time in cyclical phases. The rate of voltage change over time during each of these phases is known as the experiment's scan rate (V/s).

The most common arrangement is the electrochemical cell with three different electrodes as shown Fig.6.3.

• Working electrode (WE) - Working electrodes intended for general purpose work are usually made from a metal that is electrochemically inert over a wide range of potentials. The most widely used metals are mercury, platinum, gold, and various forms of carbon.

• Reference electrode (RE) - The potential of a working electrode in a voltammetry experiment is always controlled with respect to some standard, and that standard is the reference electrode.

• Counter electrode (CE) - This auxiliary (or counter) electrode provides an alternate route for the current to follow, so that only a very small current flows through the reference electrode.

The potential is measured between the working electrode and the reference electrode, while the current is measured between the working electrode and the counter electrode. These data are plotted as current (*i*) versus applied potential (*E*, often referred to as just 'potential').

At some point after the reduction potential of the analyte is reached, the cathodic current will decrease as the concentration of reducible analyte is depleted.

If the redox couple is reversible then during the reverse scan (from t1 to t2) the reduced analyte will start to be re-oxidized, giving rise to a current of reverse polarity (anodic current) to before. The more reversible the redox couple is, the more similar the oxidation peak will be in shape to the reduction peak. Hence, CV data can provide information about redox potentials and electrochemical reaction rates.

For instance, if the electron transfer at the working electrode surface is fast and the current is limited by the diffusion of analyte species to the electrode surface, then the peak current will be proportional to the square root of the scan

rate. This relationship is described by the Randles–Sevcik equation. In this situation, the CV experiment only samples a small portion of the solution, i.e., the diffusion layer at the electrode surface.

A standard CV experiment employs a cell fitted with three electrodes: reference electrode, working electrode, and counter electrode. This combination is sometimes referred to as a three-electrode setup. Electrolyte is usually added to the sample solution to ensure sufficient conductivity.

The electrolyte ensures good electrical conductivity and minimizes iR drop such that the recorded potentials correspond to actual potentials. For aqueous solutions, many electrolytes are available, but typical ones are alkali metal salts of perchlorate and nitrate. In nonaqueous solvents, the range of electrolytes is more limited.

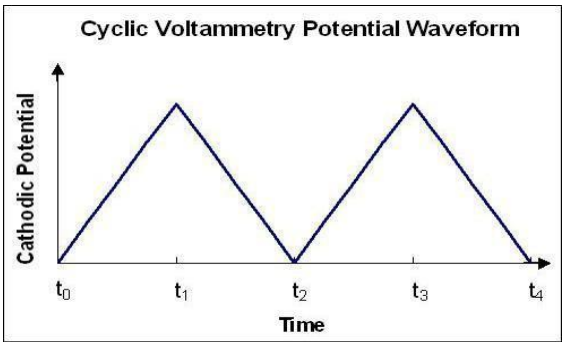

Fig.6.4 Cyclic Voltammetry Potential Waveform

CHAPTER 7

RESULTS AND DISCUSSION

7.1 SYNTHESIS OF NANOPARTICLES

The figures represent Cerium Oxide/Zinc Oxide/Graphene Oxide the nanoparticles.

Fig.7.1(a) CeO_2

Fig.7.1(b) ZnO

Fig.7.1(c) GO

7.2 XRD RESULTS OF CeO_2/ZnO/GO

The sharp peaks from diffraction patterns show the crystalline nature of the samples. The diffraction peaks obtained for crystal plane confirms the cubic inverse spinel structure. The Crystallite size of XRD Patterns is obtained by using Debye Scherrer formula.

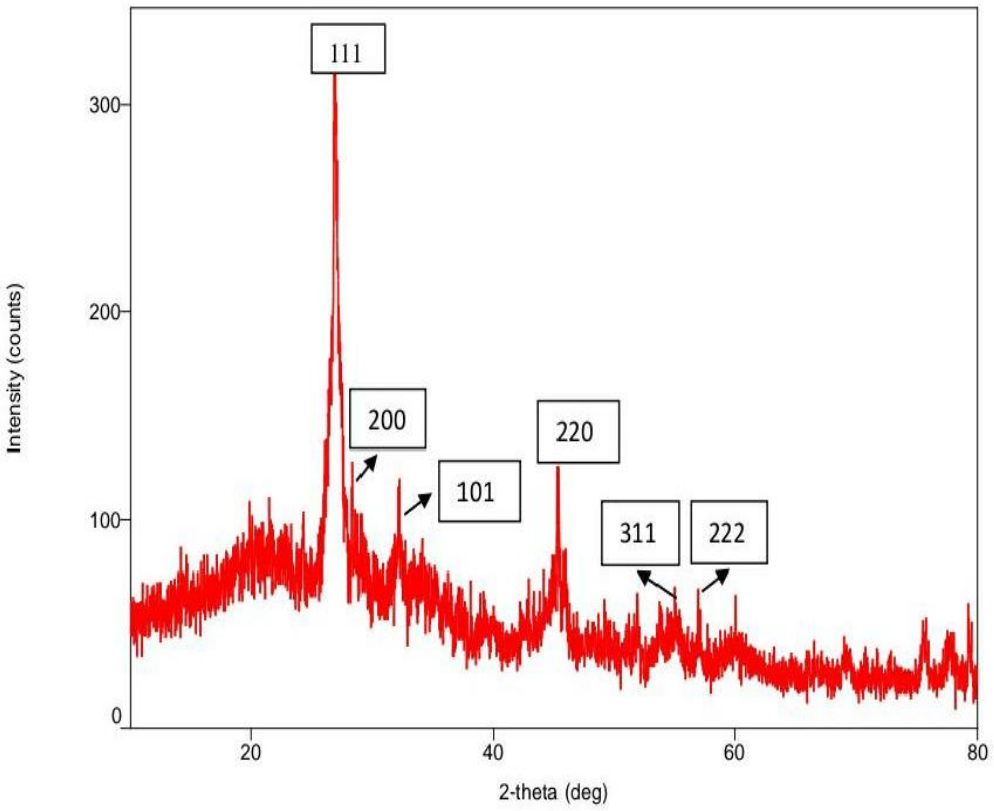

Fig.7.2 XRD Results of CeO_2/ZnO/GO

Fig.7.3 shows the peaks at 111, 200, 101, 220,311 & 222 and this confirms the formation of CeO_2/ZnO/GO NP. The sharp XRD peaks indicate that the particles are polycrystalline, and the nanostructure grew with a random orientation. After confirming, the crystallite size can be calculated from the following formula:

$$d=k\lambda/\beta\cos\theta$$

7.3 SEM RESULTS OF CeO_2/ZnO/GO

The figure describes the size of the nanoparticles. Image shows the intracellular distribution of nanoparticles which together with cellular morphology can give important information on the biocompatibility and demonstrate the potential of nanoparticle utilization in medicine.

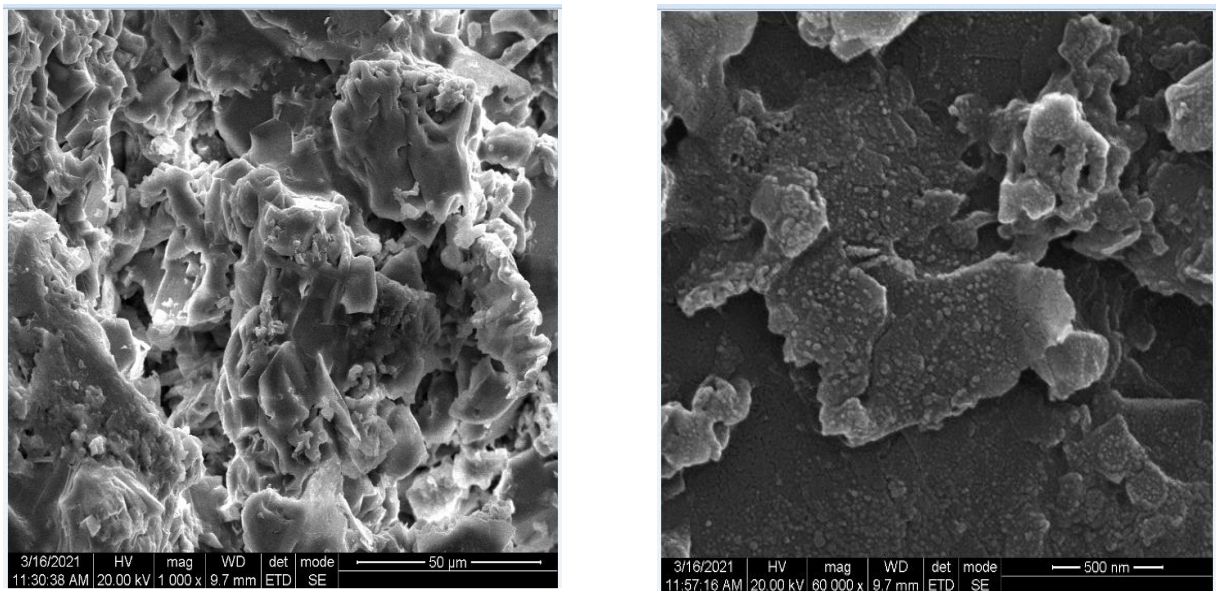

Fig.7.3 SEM images of Graphene sheets decorated with ZnO & CeO_2 NPs

Here GO is obtained for a dimension of 50 µm and ZnO and CeO_2 NPs were deposited onto GO sheets which is at the dimension of 500nm.

7.4 FTIR CHARACTERISTICS OF CeO_2/ZnO/GO

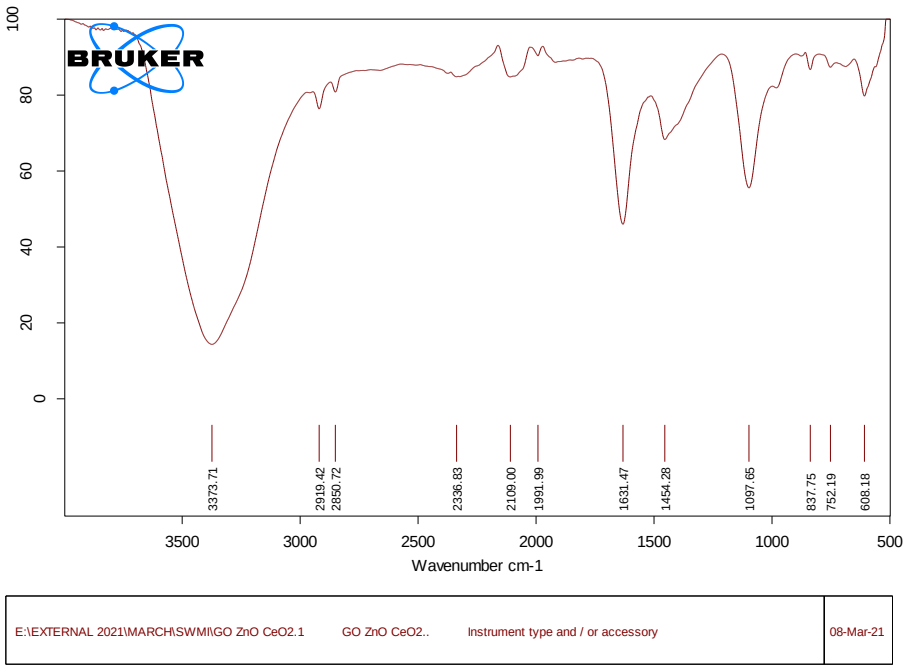

Fig.7.4 FTIR Spectrum of CeO_2/ZnO/GO

FTIR is used to study about the absorption and emission characteristics of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high resolution data over wide spectral range.

7.5 ANTI-CANCER EFFECTS STUDY THROUGH MTT ASSAY

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell Viability(%)	
				24hrs.	72hrs
1	1000	Neat	0.23	40.35	18.18
2	500	1:1	0.27	47.36	27.27
3	250	1:2	0.34	59.64	35.78
4	125	1:4	0.39	68.42	45.45
5	62.5	1:8	0.42	73.68	55.84
6	31.2	1:16	0.45	78.94	61.03
7	15.6	1:32	0.47	82.45	71.42
8	7.8	1:64	0.54	94.73	83.11
9	Cell control	-	0.57	100	100

Table.7.1 Anti-Cancer activity of sample against HeLa cell-line

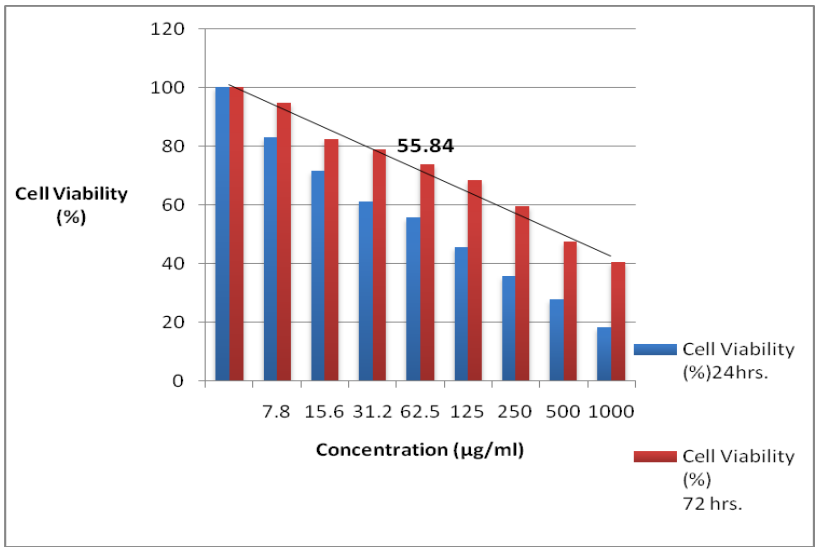

Fig.7.5 Anti-Cancer activity of sample against HeLa cell-line

Anticancer effect of Sample + Cisplatin on *HeLa* Cell line

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.05	4.53
2	500	1:1	0.09	9.89
3	250	1:2	0.13	14.28
4	125	1:4	0.19	20.87
5	62.5	1:8	0.23	25.27
6	31.2	1:16	0.29	31.86
7	15.6	1:32	0.32	35.16
8	7.8	1:64	0.37	40.65
9	Cell control	-	0.91	100

Table.7.2 Anti-Cancer activity of sample+cisplatin against HeLa cell-line

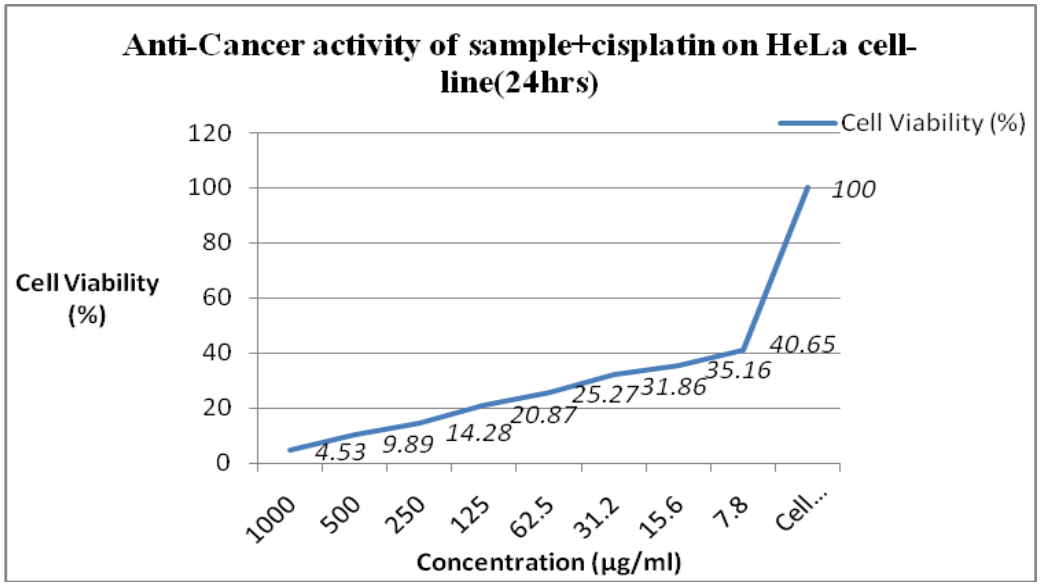

Fig.7.6 Anti-Cancer activity of sample+cisplatin against HeLa cell-line

Cytotoxicity effect of Sample on *VERO* Cell line

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell Viability (%)
1	1000	Neat	0.42	67.74
2	500	1:1	0.44	70.96
3	250	1:2	0.47	75.80
4	125	1:4	0.50	80.64
5	62.5	1:8	0.53	85.48
6	31.2	1:16	0.54	91.93
7	15.6	1:32	0.58	93.54
8	7.8	1:64	0.60	96.77
9	Cell control	-	0.62	100

Table.7.3 Anti-Cancer activity of sample against VERO cell-line

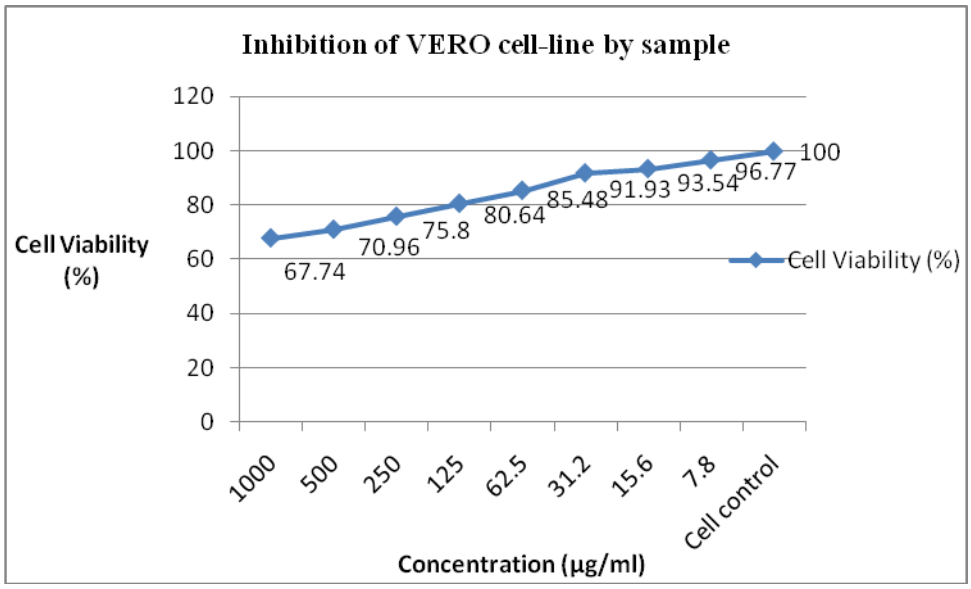

Fig.7.7 Anti-Cancer activity of sample against VERO cell-line

7.6 DUAL STAINING STUDY OF CeO_2/ZnO/GO

Upon subjecting AO/EB dye onto cell culture medium that holds 1000 µg/ml test concentration of hybrid nanocomposite, the live cells absorb AO and emits green fluorescence and dead cells absorb EB and emits red fluorescence as shown in Fig.7.8. Fig.7.9 shows the dual staining test performed at cell culture medium which holds 500 µg/ml test concentration of the hybrid nanocomposite. Fig.7.9 reveals that, the quantity of live cells was drastically reduced (green dots is found less) and the projection of dead cells alone (red dots) is available by emitting a red fluorescence. On observing the Fig.(7.9 – 7.11), there was no indication of the morphological changes in the cell boundaries but there was the existence of necrosis. This evidenced that a significant number of dead cells was caused by the hybrid nanocomposite which further confirms the existence of the anticancer property of our developed CeO_2/ZnO/GO hybrid against HeLa cells. The quantitative apoptotic activity of the CeO_2/ZnO/GO nanocomposite was further evaluated using flow cytometry study.

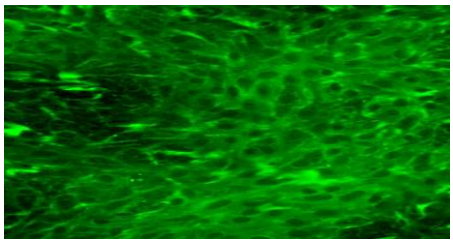

Fig.7.8 Normal HeLa Cell Line

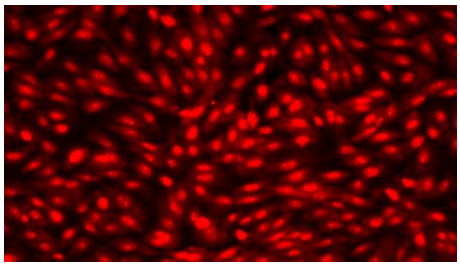

Fig.7.9 Con:1000µg/ml

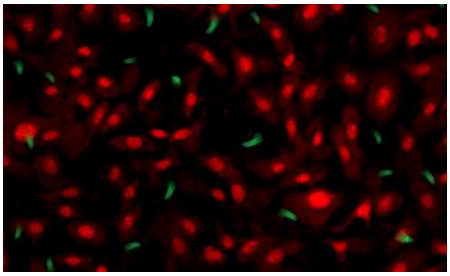

Fig.7.10 Con:500µg/ml

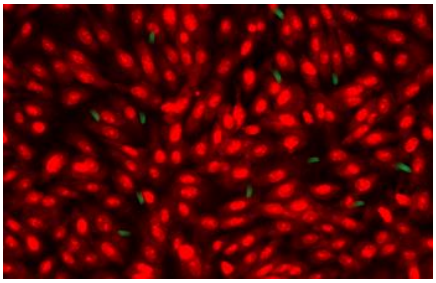

Fig.7.11 Con:250µg/ml

7.7 FACS STUDY OF CeO_2/ZnO/GO

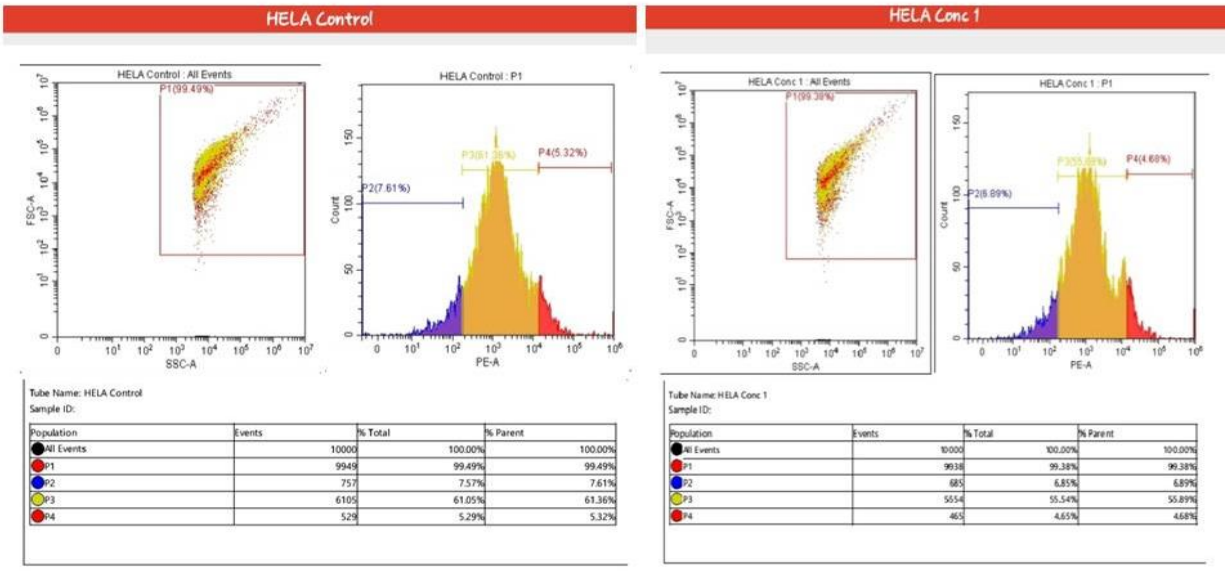

Fig.7.12 With comparison to the control unit, in conc. 1 at cycle P2, it is observed that the alive cells are only 6.85%

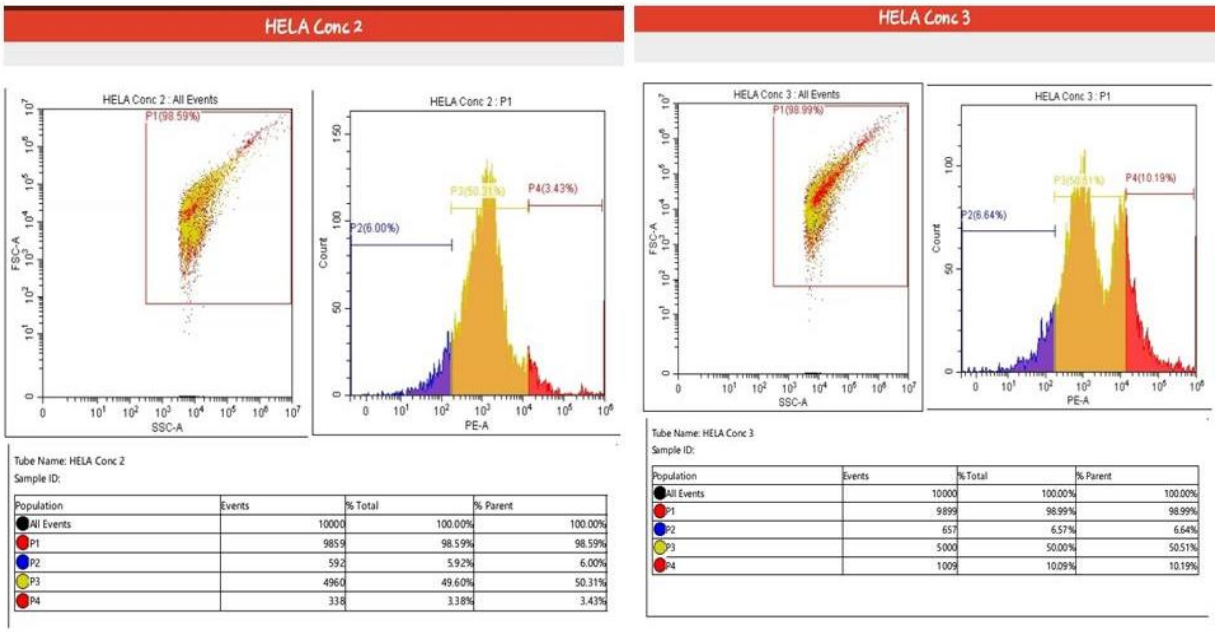

Fig.7.13 The above images depict that at conc. 2, the alive cells at P2 are only 5.92% and at conc. 3, the alive cells at P2 are only 6.57%

To evaluate the molecular mechanisms of the cell cycle distribution, the significant inhibitory effects of the CeO_2/ ZnO /GO nanostructured hybrid nano system was analysed by different cell cycle phases, using flow cytometry as shown in Fig.7.12 and Fig.7.13. The measurements were based on the quantitative number of cells (i.e., nuclear DNA cells content) present with different dosages. The human cervical cancer cells were treated with different concentrations (dose 1 and dose 2) for 24 h. The percentage of the death cell rate (55.84%) was increased during the R1 (G0/G1) cycle when HeLa cells were treated with 62.5 µg/ml of the CeO_2/ZnO/GO hybrid nano system (Fig.7.13).

7.9 CYCLIC VOLTAMMETRY

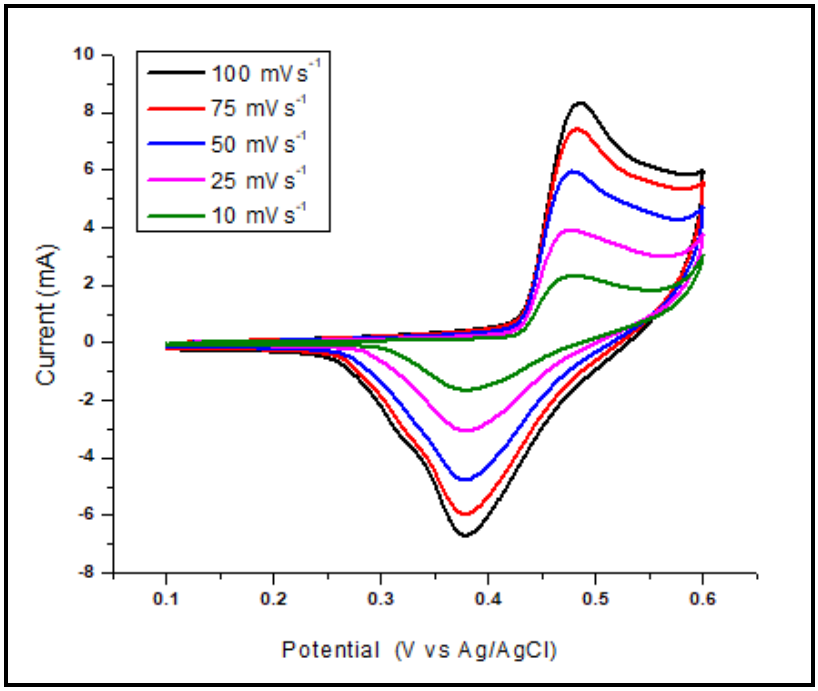

Fig.7.14 Differential pulse voltammetry for varying scan rate in 2 molar concentration of KOH electrolyte at pH 7 medium

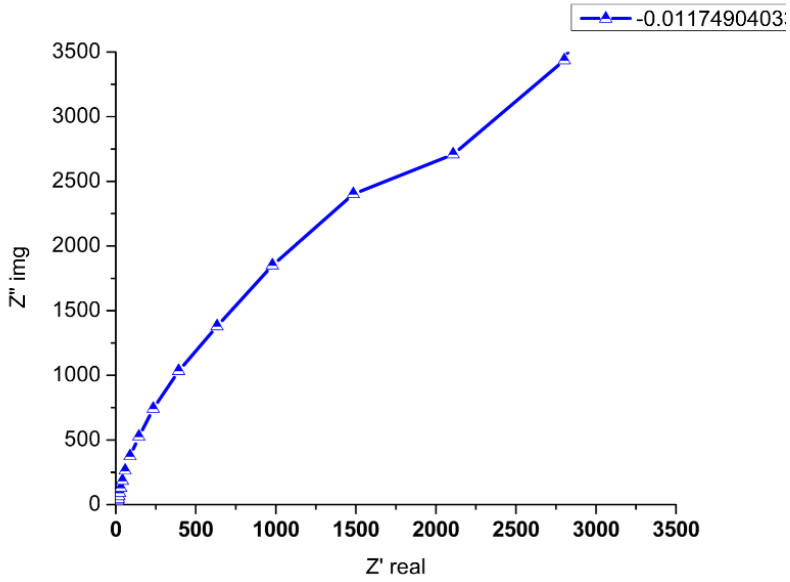

Fig.7.15 Electrical charge transfer based on the resistance of the sensing material

Fig.7.14 illustrates rising and decaying potentials (i.e.) Oxidation & reduction potential with respect to rise in scan rate of CeO_2/ZnO/GO using Ag/AgCl as reference electrode and platinum as counter electrode. These results affirm the sensing behavior of developed nano system towards HeLa cell line.

A Nyquist Plot for the developed working electrode is shown in Fig.7.15. R was assumed to be 0.9 Ω. Here the impedance decreases linearly and thus affirms the sensing behavior of proposed CeO_2/ZnO/GO electrode.

CHAPTER 8

CONCLUSION AND FUTURE SCOPE

8.1 CONCLUSION

A novel Cerium Oxide/ Zinc Oxide/ Graphene Oxide - based thin film device will be developed. The developed platform will be made to interact with HeLa Cancerous cells and is also combined with Cisplatin drug under in vitro conditions. The interaction will intoxicate the HeLa cancerous cells in an efficient manner when it is combined with Graphene Oxide. This process ensures the destruction of cancerous cells. Lastly our present study will be compared with the existing system to know the benefits and extension of the same to clinical trials.

8.2 FUTURE SCOPE

The developed bio-sensor can be used against different cancerous cell lines from breast, lung, prostate tissues and the % cell viability can be compared to determine its killing effects. Further the developed nano composite can be subject to in-vivo studies to estimate its toxicity levels & biocompatibility.

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APPENDIX 1

International Conference

Presented the oral presentation titled **CeO_2/ZnO/GO Hybrid as a Theranostic Nanoplatform for Cervical Cancer Diagnosis** in the **World Nano Congress on Advanced Science and Technology (WNCST-2021)** organized by the Centre for Nanotechnology and Research, Vellore Institute of Technology, Vellore, India.

CeO_2/ZnO/GO Hybrid as a Theranostic Nanoplatform for Cervical Cancer Diagnosis

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ABSTRACT:

In this work, Cerium Oxide/Zinc Oxide/ Graphene Oxide (CeO_2/ZnO/GO) based nano system is fabricated under microwave assistance using hydrothermal technique. Their physical and chemical properties were analysed using X-Ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The presence of functional groups was affirmed using FTIR analysis. The morphological features of the hybrid were verified using SEM. SEM images reveals that, pure CeO_2 nanoparticles are highly agglomerated and CeO_2/GO hybrid possess nano-rectangular morphology. The aim of this study is to analyse their anti-cancer properties against HeLa cells for screening of cervical cancer. The developed structural CeO_2/ZnO/GO composite have been evaluated for anti-cancer efficacy against HeLa (cervical cancerous cell line) and normal (VERO-non cancerous) cell lines using MTT assay. Their results reveal that, CeO_2/ZnO/GO - based nano system have higher cytotoxic behaviour than CeO_2/ZnO and CeO_2/ GO-based nano system against HeLa cancer cells. Hence the proposed CeO_2/ZnO/GO nanoplatform can be used for cervical cancer diagnosis.

Keywords*: XRD, SEM, Hybrid, HeLa, FTIR, MTT, Vero.*

RAJALAKSHMI ENGINEERING COLLEGE

DEPARTMENT OF ECE

PROGRAM OUTCOMES (POs)

Engineering Graduates will be able to:

PO1 Engineering knowledge: Apply the knowledge of mathematics, science, engineering fundamentals, and an engineering specialization to the solution of complex engineering problems.

PO2 Problem analysis: Identify, formulate, review research literature, and analyze complex engineering problems reaching substantiated conclusions using first principles of mathematics, natural sciences, and engineering sciences.

PO3 Design/development of solutions: Design solutions for complex engineering problems and design system components or processes that meet the specified needs with appropriate consideration for the public health and safety, and the cultural, societal, and environmental considerations.

PO4 Conduct investigations of complex problems: Use research-based knowledge and research methods including design of experiments, analysis and interpretation of data, and synthesis of the information to provide valid conclusions.

PO5 Modern tool usage: Create, select, and apply appropriate techniques, resources, and modern engineering and IT tools including prediction and modeling to complex engineering activities with an understanding of the limitations.

PO6 The engineer and society: Apply reasoning informed by the contextual knowledge to assess societal, health, safety, legal and cultural issues and the consequent responsibilities relevant to the professional engineering practice.

PO7 Environment and sustainability: Understand the impact of the professional engineering solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.

PO8 Ethics: Apply ethical principles and commit to professional ethics and responsibilities and norms of the engineering practice.

PO9 Individual and team work: Function effectively as an individual, and as a member or leader in diverse teams, and in multidisciplinary settings.

PO10 Communication: Communicate effectively on complex engineering activities with the engineering community and with society at large, such as, being able to comprehend

and write effective reports and design documentation, make effective presentations, and give and receive clear instructions.

PO11 Project management and finance: Demonstrate knowledge and understanding of the engineering and management principles and apply these to one's own work, as a member and leader in a team, to manage projects and in multidisciplinary environments.

PO12 Life-long learning: Recognize the need for, and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change.

PROGRAM SPECIFIC OUTCOMES (PSOs)

PSO1: An ability to carry out research in different areas of Electronics and Communication Engineering fields resulting in journal publications and product development.

PSO2: To design and formulate solutions for industrial requirements using Electronics and Communication engineering.

PSO3: To understand and develop solutions required in multidisciplinary engineering fields.

COURSE OUTCOMES (CO)

CO1	To conceive an idea and develop confidence in designing, analyzing and executing the project in the emerging fields of Electronics and Communication and multidisciplinary research areas.
CO2	Identification of modern tools for the implementation of project through simulation and prototype.
CO3	Develop products that meets the specified needs in industrial applications with appropriate consideration for the public health and safety, societal, environmental and ethical considerations.

EC17811 –PROJECT WORK

Project Title:

CeO_2/ZnO/GO Hybrid as a Theranostic Nanoplatform for Cervical Cancer Diagnosis

Batch Members:

1. SARADHA PREETHA G (170801163)
2. SAROJA K K (170801165)
3. SHERIN S (170801173)
4. SHIVANI K S (170801174)

Name of the Supervisor:

J SARANYA, M. Tech.,(Ph.D.)
ASSITANT PROFESSOR
DEPARTMENT OF ELECTRONICS AND COMMUNICATION ENGINEERING

CO - PO – PSO matrices of course

PO/PSO CO	PO1	PO2	PO3	PO4	PO5	PO6	PO7	PO8	PO9	PO10	PO11	PO12	PSO1	PSO2	PSO3
CO1	2	3	3	3	3	2	2	3	3	3	3	2	3	2	3
CO2	2	1	2	1	2	3	3	2	3	3	2	3	3	2	2
CO3	3	3	3	3	3	3	2	3	3	3	2	3	2	3	3
Average	2.3	2.3	2.6	2.3	2.6	2.6	2.3	2.6	3	3	2.3	2.6	2.6	2.3	2.6

Note: Enter correlation levels 1, 2 or 3 as defined below:

1: Slight (Low) 2: Moderate (Medium) 3: Substantial (High), If there is no correlation, put - “

Signature of the Supervisor