2022-2024

ABU SUFYAN

SU92-MSCHW-F22-003

FOS

# Development of Nanoporous Drug Carriers for Ferroptosis Induced Cancer Therapy

SUPERIOR UNIVERSITY

**Thesis Submitted to**

**The Superior University Lahore**

**In Partial Fulfillment of the**

**Requirement for the Degree of**

**M.Phil. Chemistry**

**By**

**ABU SUFYAN**

**SU92-MSCHW-F22-003**

**Session: 2022-2024**

**Faculty of Sciences**

# Development of Nanoporous Drug Carriers for Ferroptosis Induced Cancer Therapy

SUPERIOR UNIVERSITY

**Thesis Submitted to**

**The Superior University Lahore**

**In Partial Fulfillment of the**

**Requirement for the Degree of**

**M.Phil. Chemistry**

**By**

**ABU SUFYAN**

**SU92-MSCHW-F22-003**

**Session: 2022-2024**

**Faculty of Sciences**

## Author's Declaration

I hereby state that my M.Phil. thesis titled **"Development of nanoporous drug carriers for ferroptosis induced cancer therapy"** is my work and has not been submitted previously by me for taking any degree from this University,

**The Superior University, Lahore**

or anywhere else in the country/world.

At any time if my statement is found to be incorrect even after my graduation, the university has the right to withdraw my M.Phil. degree.

Name of Student: Abu Sufyan

Date: _____

## Plagiarism Undertaking

I solemnly declare that research work presented in the thesis titled **"Development of nanoporous drug carriers for ferroptosis induced cancer therapy"** is solely my research work with no significant contribution from any other person. Small contribution/help wherever taken has been duly acknowledged and that complete thesis has been written by me.

I understand the zero-tolerance policy of the HEC and University,

**The Superior University, Lahore**

towards plagiarism. Therefore, I as author of the above-titled thesis declare that no portion of my thesis has been plagiarized and any material used as a reference is properly referred/cited. I undertake that if I am found guilty of any formal plagiarism in the above-titled thesis, even after awarding of M.Phil. degree, the University reserves the rights to withdraw/revoke my M.Phil. degree and that HEC and the University have the right to publish my name on the HEC/University website on which names of students are placed who submitted a plagiarized thesis.

Student/Author Signature: _____

Name: Abu Sufyan

## Research Completion Certificate

This is to certify that the thesis entitled **"Development of nanoporous drug carriers for ferroptosis induced cancer therapy"** submitted by **"Abu Sufyan"** has been accepted towards the partial fulfillment of the requirement for M.Phil. **"Chemistry"**. The quality of the work contained in this thesis is adequate for the award of degree.

Supervisor Name: Dr. Fizza Naseem

Designation:

Signature: _____

## Certificate of Approval

This is to certify that the research work presented in this thesis, titled **"Development of nanoporous drug carriers for ferroptosis induced cancer therapy"** was conducted by **"Abu Sufyan"** under the supervision of **"Dr. Fizza Naseem"**

No part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to the Faculty of Sciences, The Superior University, Lahore in partial fulfillment of the requirements for the degree of Master of Philosophy in the field of "**Chemistry"** in Faculty of Sciences at The Superior University, Lahore.

**Student Name: Abu Sufyan** Signature:_____

**Examination Committee:**

**Session Chair:** Dr. M. Mudassir Iqbal Signature:_____

a) External Examiner: Signature:_____

b) Internal Examiner: Signature:_____

c) Supervisor Name: Dr. Fizza Naseem Signature:_____

d) Name of HOD: Prof. Dr. Uqba Mehmood Signature:_____

e) Name of Dean: Prof. Dr. Muhammad Naveed Babur Signature:_____

f) Controller Examination: Dr. Muhammad Haris Signature:_____

## Dedication

To Rahmat-Ul-Lil Aalamin Hazrat Muhammad Sallallah-O-Alaih-E-Wasallam and to my beloved, teachers, parents whose prayers and affections are the source of strength of me in every step of life. Who devoted and sacrificed everything, at their disposal for the pursual of my studies. May god bless them with good health and long life to all those who blessed me with their affection and love i pay my special thanks to my father for their kind support and encouragement, which made me able to achieve this goal.

**Acknowledgments**

In the name of Allah, the Most Gracious and the Most Merciful, source of all wisdom and knowledge, and the sustainer of the Worlds.

I am thankful to Allah Almighty for granting us the ability to live, study, and thrive. Sincere thanks are sent to the Holy Prophet Muhammad (Peace be Upon Him), the greatest teacher in human history, who has led all people from darkness to light. Many people's advice and help were crucial to the project's progress and ultimate result, and I am very grateful to have had this support during the entire study process. My sincere gratitude and appreciation are extended to my supervisor **Dr. Fizza Naseem** and my co-supervisor **Dr. Quratulain Amjad** both Assistant Professors, Department of Chemistry, Superior University, Lahore for their guidance, cooperation and inspiration. This dissertation work would have not been in the current form without her insightful input and constructive criticism. I also pay my sincere regards to the Dean of Allied Health Sciences **Prof. Dr. Muhammad Naveed Babur**, the Head of the Department **Prof. Dr. Uqba Mehmood**, and the Program Leader **Dr. M. Mudassir Iqbal** for their necessary support and providing me with all the facilities.

I do acknowledge the role played by my Research fellows who not only helped me in research work but also provided me with that substantial culture to grow socially and morally. Special acknowledgement to my M.Phil fellow Sadia Younis who help me during my research work. I am grateful to lab assistants for their invaluable help, cooperation, and support during my research work.

**Abu Sufyan**

## Table of Contents

**Page**

Author's Declaration……II

Plagiarism Undertaking……..……III

Research Completion Certificate…….…...IV

Certificate of Approval……..…….V

DEDICATION…….....VI

ACKNOWLEDGEMENTS…….....VII

TABLE OF CONTENT….....VIII

LIST OF TABLES…….....XII

LIST OF FIGURES…….....XIII

LIST OF ABBREVATIONS…….....XIV

ABSTRACT…….....XV

CHAPTER 1…….....01

INTRODUCTION …….....01

1.1 Cancer……...01

1.2 History…….01

1.3 Cancer as a Chronic Disease…….....02

1.4 Liver Cancer……03

1.5 Risk Factor……04

1.6 Ferroptosis……06

1.7 Mechanism of Ferroptosis……06

1.8 Need for New Drug Delivery System……07

1.9 Nanoporous Drug Carriers……08

1.10 Function of Nanoporous Drug Carriers……...09

1.11 Magnetic Nanoparticles……..09

1.12 Graphene Oxide Nanoparticles……...09

1.13 Iron Oxide Nanoparticles……10

1.14 Tumor Targeting……10

1.15 Iron Doped Graphene Nanoparticles……...11

1.16 Iron Doped Graphene and Iron Doped Silica Nanoparticles……12

1.17 Treatment of Cancer……14

AIMS AND OBJECTIVES…….....15

CHAPTER 2 …….....16

LITERATURE REVIEW…….....16

CHAPTER 3 …….....24

METHODOLOGY…….....24

3.1 Chemicals…….....24

3.2 Cell Culture…….....24

3.3 Appratus…….....24

3.4 Synthesis of Graphene Oxide (GO) Nanoparticles.....24

3.5 Synthesis of Iron-Doped Graphene (FeGO) Nanoparticles……...25

3.6 Synthesis of Iron-Doped Silica (FeMS) Nanoparticles…….....26

3.7 Drug Loading on Nanocomposites……....26

3.8 Drug Loading Efficiency……...27

3.9 Drug Desorption Studies.....28

3.10 In-Vitro Cytotoxicity Studies.....28

3.11 Assessment of Cell Proliferation…….....28

3.12 MTT Assays…….....28

3.13 Real Time PCR……....28

3.14 Statistical Analysis……..29

CHAPTER 4…….....30

RESULTS …….....30

4.1 Characterization...……..30

4.1.1 FTIR of FeGO Nanocomposite...….....30

4.1.2 FTIR Spectra of FeGO and FeMS…….31

4.1.3 Uv-Visible of FeGO....……..32

4.1.4 XRD Analysis of FeGO and FeMS Nanocomposite.....33

4.1.5 TGA Analysis of FeGO and FeMS...……34

4.1.6 BET of FeGO and FeMS……...35

4.1.7 Scanning Electron Microscopy (SEM)….....36

4.2 Drug Loading…….....36

4.3 Release of Drug in Vitro…….……..37

4.4 Ferroptosis……38

4.5 Cytotoxicity of Cells (MTT Assay)……..38

4.6 Epithelial to Mesenchymal Transition……..39

4.6.1 E-Cadherin……40

4.6.2 CDC42……...40

4.6.3 N-Cadherin……40

4.6.4 Vimentin..……..40

4.6.5 Slit2……....41

4.6.6 Integrin..……41

CHAPTER 5 …….....43

DISCUSSION…….....43

CHAPTER 6…….....45

CONCLUSION …….....45

REFERENCES….....47

## List of Tables

**Description** **Page**

Table 3.1 Drug loading and release on FeGO and FeMS in percentage……...27

Table 4.1 Characterization parameters of different nanoparticles……...35

## List of Figures

**Description** **Page**

Figure 1.1 Chart of number of new cancer case and number of cancer death.....2
Figure 1.2 Chart of yearly cases of cancer in man and women…….....3
Figure 1.3 Flow chart of cancer risk factors…….....5
Figure 1.4 Nano-drug delivery system types…….....8
Figure 1.5 Tumor targeting strategies…….....11
Figure 3.1 Synthesis of graphene oxide nanoparticles…….....25
Figure 3.2 Synthesis of iron doped graphene oxide nanoparticles (FeGO)……..25
Figure 3.3 Synthesis of iron doped silica nanoparticles (FeMS)……26
Figure 4.1 FTIR of FeGO nanocomposite……...31
Figure 4.2 FTIR spectra of FeGO and FeMS……...32
Figure 4.3 Uv-Visible of FeGO……...33
Figure 4.4 XRD analysis of FeGO and FeMS nanocomposite……34
Figure 4.5 TGA analysis of FeGO and FeMS…….....35
Figure 4.6 Scanning electron microscopy (SEM) of GO, FeGO and FeMS……36
Figure 4.7 UV–Vis. of sorafenib (black line), FeGO- (red line), sorafenib loaded FeGO (blue line), FeMS (pink line) and sorafenib loaded FeMS (green line)……37
Figure 4.8 UV-Vis spectra of sorafenib loaded FeGO (a) FeMS (b) solutions at variable concentration and time……..37
Figure 4.9 Expression analysis of Ferroptosis inducing ACSL4 gene in liver cancer cells. Analyzed by RT-PCR.……...38
Figure 4.10 Effect of nanoparticles treatment on Liver cancer cell viability……..39
Figure 4.11 Effect of drug, drug loaded nanoparticles and nanoparticles on different genes (a) E-CAD, (b) CDC42, (c) N-CAD (d) Vimentin, (e) Slit2, (f) Integrin.....42

## List of Abbreviations

| | |
|---|---|
| CDT | Chemo-dynamic treatment |
| HCC | Hepatocellular carcinoma |
| ATCC | American Type Culture Collection |
| PL-OOH | Lipid bilayer, lipid peroxides |
| NDDS | Nanostructured drug delivery system |
| TDDS | Targeted drug delivery system |
| GO | Graphene Oxide |
| IONPs | Iron Oxide Nanoparticles |
| SIONPs | Superparamagnetic iron oxide nanoparticles, |
| FeGO | Iron doped Graphene |
| FeMS | Iron doped Mesoporous Silica |
| GQDs | Graphene quantum dots |
| TDM | Therapeutic drug monitoring |
| ROS | Reactive Oxygen specie |

# ABSTRACT

Hepatocellular carcinoma (HCC) ranks as one of the most therapeutically challenging cancer types, with sorafenib serving as first-line treatment. Its clinical challenges such as low solubility, systemic toxicity, and drug resistance underscore the need for next generation, nanocarriers to strengthen drug delivery capabilities. This research examined the nanoarchitecture of porous nanoparticles, with the aim of comparing iron-doped graphene (FeGO) and iron doped silica (FeMS) nanoparticles for targeted and controlled delivery of sorafenib, in terms of high-surface-area porous nanocarriers. Both, FeGO and FeMS nanocarriers, were synthesized and characterized systematically, using a combination of Brunauer–Emmett–Teller (BET) to evaluate surface area and porosity, thermogravimetric analysis (TGA) for thermal stability, scanning electron microscopy (SEM) to assess morphology, Fourier-transform infrared spectroscopy (FT-IR) for functional group evaluation, and ultraviolet-visible spectroscopy (UV-Vis) for optical properties. Results from BET indicated FeMS had a well-formed mesoporous framework beneficial for drug loading and sustained release, and Fe-G happened to have a greater surface area, but a lesser degree of controllability in release due to its flattened façade. The in vitro studies that were carried out on HepG2 liver cancer cells confirmed significant uptake and cytotoxicity of both types of nanocarriers. The $Fe_3O_4$ core silica nanoparticles could achieve not only magnetic targeting but also iron storage, which could result in ferroptosis a regulated cell death process involving iron-dependent lipid peroxidation and the accumulation of reactive oxygen species (ROS). The RT-PCR experiments indicated the activation of ferroptosis in HepG2 cells as evidenced by the up-`regulation of ACSL4 and the down-regulation of GPX4 that collectively confirm the apoptosis pathway through oxidative stress. This methodical evaluation illustrates the importance of porosity engineering to enhance drug delivery efficacy. Although FeGO nanoparticles provide a large surface area for adsorption of sorafenib, the characterization of FeMS nanoparticles developed increases controlled release, potential magnetic targeting, as well as induces ferroptosis. These nanoparticles are promising nanocarrier candidates for the next generation of liver cancer therapeutics. The findings of this study will assist in developing multifunctional high-performance nanocarriers in precision oncology and cancer therapies.

# CHAPTER 1

# INTRODUCTION

## 1.1 Cancer

The most common cause of death throughout human history, cancer's influence shifted as civilization became more industrialized and technologically sophisticated. The incidence of the most serious types of cancer has climbed this century, despite a sharp drop in the risk of a few types of the disease in affluent nations. In nations where risk factors including cigarette smoking, unhealthy eating habits, and exposure to hazardous chemicals at work or in surroundings are more prevalent, the incidence of lung, breast, prostate, colon, and rectal cancers has increased [1]. Cancer has always been considered of as a condition of proliferation, but new research indicates that it may also be a metabolic disease. In order to fulfil and even exceed the biosynthetic and bioenergetic requirements demands of ongoing cell development, growing tumors rearrange their metabolic pathways. Increased intake of glucose and glutamine, increased glycolysis, modifications in the utilization of metabolic enzyme isoforms, and The cancer cells' metabolic profile often exhibits elevated lactate generation [2]. It has also been found that oncogenes and tumor suppressors play a part in the metabolic alterations linked to cancer. It has been proposed that the metabolic profile of tumor cells reflects the rate of fast proliferation. Metabolic alterations linked to cancer may further highlight the significance of defense against ROS or the function of lactate released in the tumor microenvironment [3].

## 1.2 History

Cancer is an enigmatic and horrifying disease or collection of diseases. Cancer has afflicted multicellular species for almost 200 million years, and there is evidence that the ancestors of modern humans suffered from the disease more than a million years ago. Cancer is not primarily caused by an external factor, unlike parasites, infectious disorders, and a number of environmental conditions. In a way, human cells that have been recruited and partially transformed into pathogenic organisms have gotten away with it or tumor building blocks are its agents of destruction. Solving complex biological and medical problems is necessary to comprehend the causes of tumors,

how they spread, and particularly the elements that either facilitate or hinder their growth. These issues have received enormous resources, with varying degrees of success. Some of the achievements have been astounding. For instance, childhood leukaemia survival rates have risen from 10% to over 80% in the last 50 years. However, the cure (or prolonged remission) procedures are usually burdensome and risky, and they are frequently only accessible to people in wealthy nations. Medical experts still have a lot of work to perform because there is no such thing as a "cure" for cancer or malignancies [4].

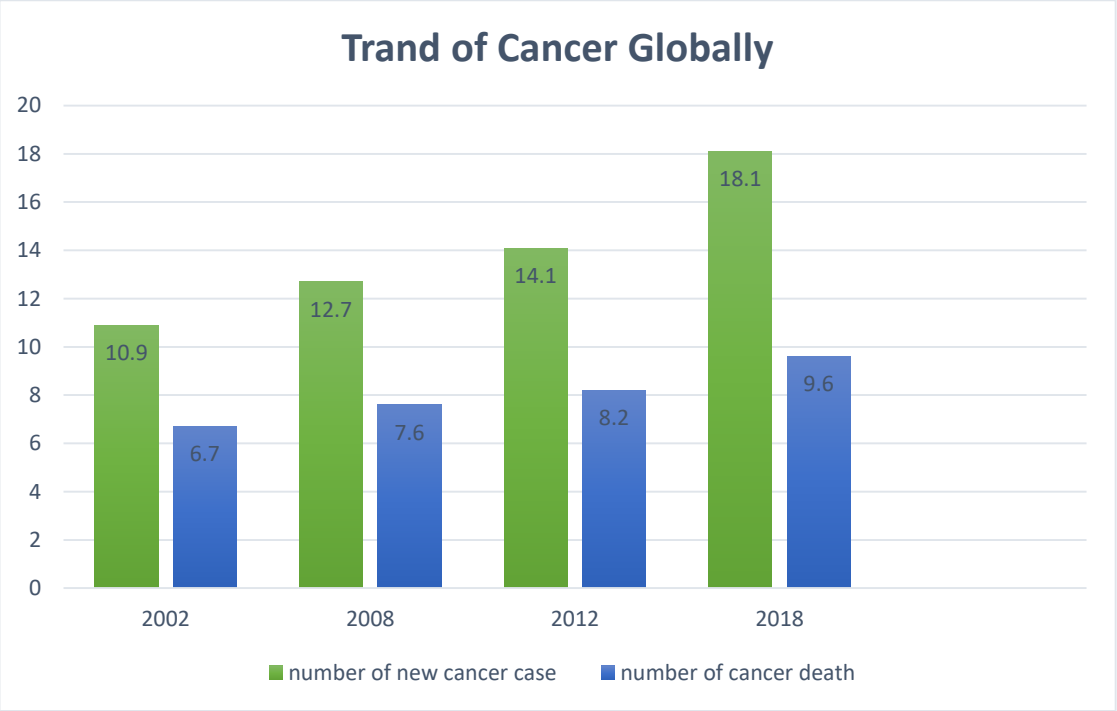



Figure 1.1 Chart of number of new cancer case and number of cancer death

### 1.3 Cancer as a Chronic Disease

In resource-rich countries like Australia, chronic diseases are the primary cause of illness, disability, and mortality, and they have an adverse effect on people's quality of life as well as that of their families and communities. In the healthcare system we currently have, which is frequently focused on managing acute illnesses, fails to emphasize the individual's function in self-management and offers sporadic disease-specific monitoring, and ignores the significance of secondary prevention, it can be difficult to provide cancer survivors with the support and care they need, just like it is for other chronic diseases [5]. A "disease that can develop gradually, persist for a long period of time (months to years), and be lethal", and be progressing and/or life-

limiting" is referred to as a chronic disease. Multiple sclerosis, amyotrophic lateral sclerosis, diabetes, dementia, heart failure, and HIV/AIDS, Chronic obstructive pulmonary disease, chronic renal disease, and arthritis, and some types of cancer are a few examples of these ailments. Over 40% of adults in Canada say they suffer from at least one of the seven prevalent chronic illnesses. Although there is no treatment for a chronic illness, symptoms like pain, exhaustion, and difficulty sleeping can be controlled. Physical restrictions, mental anguish, and the resulting low quality of life are other issues. Chronic illnesses have an impact on the health of the individual as well as their friends, family, neighbors, workplace, and careers [6].

### 1.4 Liver Cancer

According to estimates from 2000, Globally, liver cancer is the fifth most common cancer in men and the eighth most common in women. An estimated 564,000 new instances occur annually, with 166,000 occurring in women and 398,000 in males. In high-risk countries, liver cancer can develop before the age of 20, while it is rare in low-risk countries before the age of 50. In general, liver cancer is two to four times more common in males than in women. In many affluent nations, including the US, the prevalence of primary liver cancer is rising, and this trend is probably here to stay for a few decades [7].

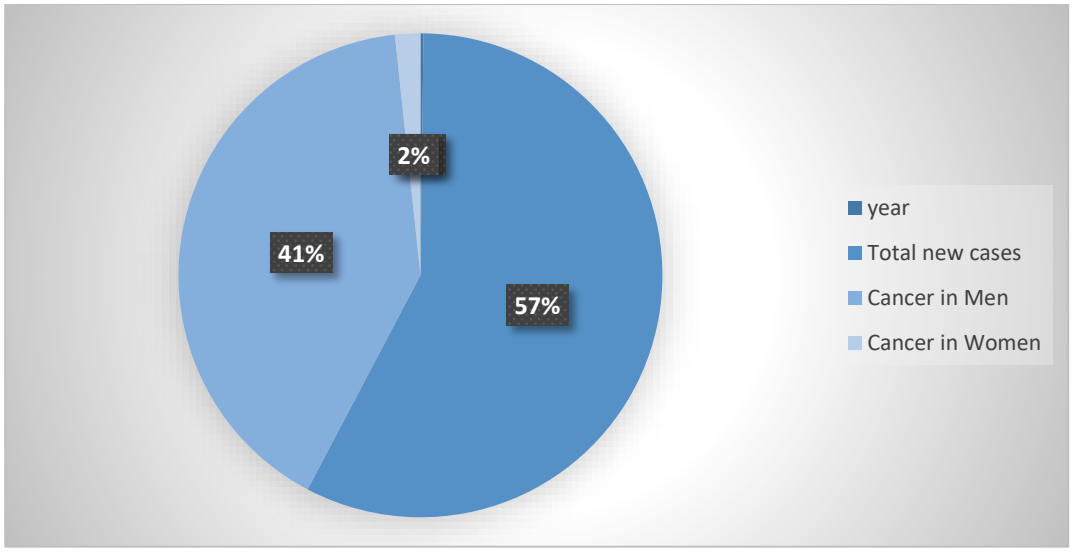



Figure 1.2 Chart of yearly cases of cancer in man and women

Although sorafenib is well tolerated, there are a number of toxicities that can occur, so it is strongly advised to monitor dosage in order to maximize the therapy's effectiveness and minimize its side effects, which include skin toxicity, hypertension,

diarrhea, and foot-hand syndrome. Therapeutic drug monitoring (TDM) can be used for this, therefore clinical labs require extremely sensitive and specific analytical techniques to measure sorafenib in various biological fluids [8]. Furthermore, the synergistic control of several medications on various metabolic pathways frequently overlooks their toxic side effects and multidrug resistance due to their nonspecific localization, which are significant factors contributing to chemotherapy failure. Therefore, the solution lies in maximizing the use of sorafenib-based treatment in conjunction with other therapeutic approaches. According to this study, the drug-loaded approach has a potent therapeutic effect and can successfully stop the hepatocellular carcinoma cells' ability to proliferate, migrate, and invade. Notably, the drug-loaded nanoparticles damaged mitochondrial homeostasis and sharply raised lipid peroxide and reactive oxygen species levels, which negatively impacted tumor cells' ability to produce GSH normally and finally resulted in ferroptotic cell death. Moreover, drug laden nanoparticles can efficiently exacerbate and focus on the tumor location effectively reduce HCC by inducing ferroptosis in situ, with no systemic negative effects in mice. Significantly, the development of the nanomedicine offers a fresh approach to chemotherapy for HCC as well as a fresh idea for creating a targeted, effective, stable, and Nanomedicine that is biocompatible that can find use in clinical settings [9].

### 1.5 Risk Factors

Iron overload, Diabetes, smoking, cirrhosis from alcohol, fatty liver disease, and obesity, hepatitis B and hepatitis C viruses, and other dietary exposures are risk factors. For liver cancer, the prognosis is not good. Merely 5% to 15% of patients qualify for surgical excision, which is only appropriate for people in the early stages and because of a reduced capacity for hepatic regeneration, usually in the absence of cirrhosis; the risk of complications following a right hepatectomy is higher than that of a left hepatectomy [10].

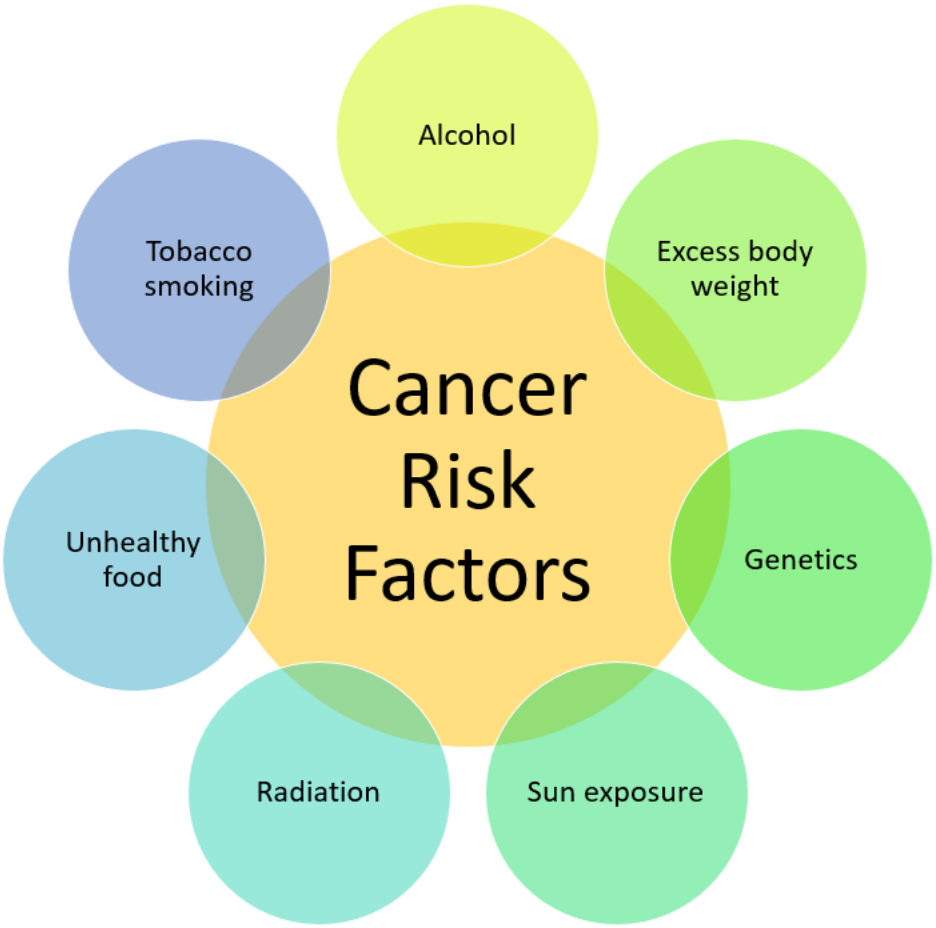



Figure 1.3 Flow chart of cancer risk factors

Although there are currently around HepaRG, Huh7, SK-Hep-1, Hep3B, and HepG2 are the most commonly used among 40 distinct hepatic cancer cell lines that have been obtained from various malignancies. The HepG2 cell line is the most well-known of the aforementioned cell cultures because of its numerous uses in scientific research. Hepatocellular carcinoma, often known as hepatoma or HCC, was the name given to this line when it was identified in 1975In 1980, Wistar Institute researchers submitted a patent application for the HepG2 cell line, "a human hepatoma-derived cell line." Since then, HepG2 cells, "extracted from a white guy 15-year-old's liver tissue that had a well-differentiated hepatocellular carcinoma," have been recorded as a human cell line (HB 8065) within the American Type Culture Collection (ATCC), located at Rockville, Maryland, USA repository [10-12]. Cancer of the liver is among the two most frequent cause of cancer-related death (HepG2). Regretfully, there isn't yet a cure that works completely. By controlling intracellular lipid peroxidation, glutathione

(GSH) levels, and other associated chemicals, ferroptosis may have an impact on the development of HepG2 [13].

### 1.6 Ferroptosis

A research screening recently discovered ferroptosis, a non-apoptotic planned death of cells process. Ferroptosis differs from other types of several methods of programmed cell killing, with its two main characteristics being the accumulation of lipid peroxides and iron reliance [14]. Recent data suggests that ferroptosis may be induced for cancer treatment, especially to eradicate aggressive cancers that don't respond to conventional treatments [15]. A lot of work has recently gone into creating anticancer medications that use ferroptosis induction. As nanotechnology has advanced in recent years, so too has the application of nanomedicine in cancer treatment. Nanomaterials can effectively kill cancer cells because of their distinct physicochemical characteristics, as well as some unique properties (For instance, an electrochemical property, the magnetism property, the photothermal effect, etc.). Additionally, it has been discovered that nanomaterials can cause ferroptosis [16].

### 1.7 Mechanism of Ferroptosis

The ferroptotic cascade is initiated predominantly by lipid peroxidation, and due to their capacity to harm the lipid bilayer of the membrane, lipid peroxides (PL-OOH), particularly lipid hydroperoxides, are considered to be the primary mediators of ferroptosis. Lipid peroxides may also impart additional toxicity through their degradation products by altering protein structure and function as well as nucleic acids, such as aldehydes and Michael acceptors. In order to act as ferroptotic signals, free polyunsaturated fatty acids (PUFAs) have to be first esterified into membrane phospholipids (like phosphatidylethanolamines, or PEs), which reactive oxygen species (ROS) have to oxidise into lipid peroxides. Therefore, ferroptosis induced by RSL3 and erastin is dictated to a considerable extent by acyl-CoA synthetase long-chain family member 4 (ACSL4), a fatty acid metabolism enzyme (to acylate AA), and lysophosphatidylcholine acyltransferase 3 (LPCAT3), an enzyme required to reacylate the lysophospholipid of the membrane. Additionally, when used in conjunction In combination with other drugs like cisplatin, temozolomide, cytarabine/ara-C, and adriamycin, erastin can be used to treat a number of cancer types [17].

## 1.8 Need for New Drug Delivery System

Cancer treatment has advanced significantly during the last few decades. Since chemotherapy is so effective when compared to other therapies, it has become a common strategy for managing cancer. Chemotherapy relies on the high cytotoxicity of chemotherapeutic drugs against cancer cells. Regretfully, traditional chemotherapeutic drugs always have intrinsic drawbacks, including poor solubility in physiological settings, quick blood clearance, limited absorption, and nonspecific distribution. Fast-growing cells are the primary target of chemotherapeutics since cancer cells grow more quickly than healthy ones. However, because certain healthy cells grow quickly as well, the medications used in chemotherapy also target these healthy cells. Because of this unwelcome attack, traditional chemotherapy fails. Due to the drawbacks of traditional chemotherapy, intelligent nanocarrier-based drug delivery devices have been developed [18]. The technical capacity to analyze the molecular mechanisms of tumor biology, surface modification techniques, nanotechnology, especially the creation of biocompatible nanoparticles, microelectronics, and material sciences has improved. Consequently, a large variety of nanostructured carriers that are highly effective at delivering medications to particular malignant areas have been created. The nanostructured drug delivery system (NDDS) is a specific technique that allows the entrance of a therapeutic nanostructured substance into the body by regulating the rate, timing, and location [19]. One obvious and popular treatment approach for treating malignant disorders is to cause cell death in cancer. Apoptosis, the most well-known type of controlled cell death, is one process that causes cancer cells to die when exposed to chemotherapeutics like cisplatin [20].

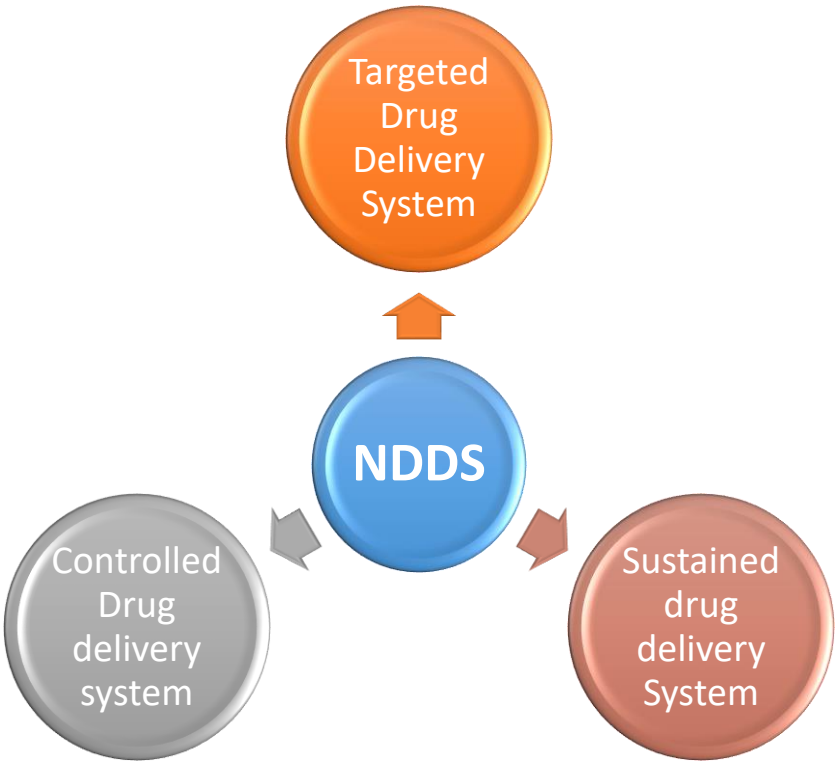



Figure 1.4 Nano-drug delivery system types

### 1.9 Nanoporous Drug Carriers

The development of porous carriers as controlled drug delivery matrices has received a lot of attention lately due to their many characteristics, which include a stable, homogeneous porous structure, well-defined surface properties, a wide surface area, and tunable pore size. Because of its many beneficial qualities, porous carriers have been used in pharmaceuticals for a variety of applications, such as the creation of sustained and floating drug delivery systems [17, 21]. Nanocarriers are carriers that are nanoscale in size and are based on the targeted drug delivery system (TDDS) idea. Its benefits of controlled release, targeting, high efficiency, minimal toxicity, and great stability have made it a current research hotspot. With the advancement of nanotechnology, anti-tumor drugs that use nanoparticles as carriers can use specific materials and surface modification to accomplish targeted drug delivery and controlled release. Additionally, it can overcome the drawbacks of conventional anti-tumor medications, which have been used extensively, and increase the stability and bioavailability of these medications [22]. Chemotherapy and targeted therapy can only marginally increase a patient's chances of survival and enhance their frequently inadequate quality of life [23]. Since the pores of nonporous materials are nanoscale,

a significant portion of their structure is made up of empty space. These materials' excellent selectivity, high permeability, high surface-to-volume ratio, and superior resistance to heat and sound are important characteristics. Ion exchangers, pollutant separation and removal, catalysts, sensors (in biological applications), membranes, and insulating materials are just a few of the delicate and varied applications that use these materials because of their highly desirable qualities. Nonporous silica, carbon, and zeolite are the most widely used and prevalent nonporous materials [24].

### 1.10 Function of Nanoporous Drug Carriers

The development of nanostructures prompted numerous attempts to examine the unique qualities and capabilities of this new material as well as other related ones for use in various contexts and expectations. In fact, the nanostructures provide a suitable surface for interacting with different kinds of substances to create the intended nano-based sensing or adsorbing capabilities and applications. As a result, the interaction of various species with nanostructures has been studied up to this point, and the development of nano-based drug delivery methods has been influenced by the particular interactions of pharmacological compounds. As previously said, the concept of targeted drug delivery is crucial to developing a more effective pharmaceutical regimen, particularly for complex illnesses like cancer [25].

### 1.11 Magnetic Nanoparticles

The ability of magnetic nanoparticles to be attracted to and concentrated in specific locations by an external magnetic field makes them a promising technique for in-vivo medication administration. In the radiofrequency spectrum, alternating and extremely strong magnetic fields have recently been used to effectively absorb energy for medicinal purposes. Nevertheless, there is no information on photothermal treatment employing magnetically localized magnetic carbon nanoparticles for photo absorption [26].

### 1.12 Graphene Oxide Nanoparticles

A two-dimensional substance made up of carbon and oxygen atoms, graphene oxide (GO) belongs to the graphene nanomaterial family. High mechanical strength, flexibility, and chemical stability are some of its special qualities. It is also naturally hydrophilic, which makes it easier to dissolve in water and gives the solution a

brownish hue. It is usually created by modifying graphite chemically, which alters its later characteristics, particularly the quantity and kind of oxygen-containing groups. This material's remarkable properties and ease of production have made it a desirable choice for a number of sectors, including electronics, materials science, healthcare, and energy applications [27].

### 1.13 Iron Oxide Nanoparticles

Iron oxide nanoparticles (IONPs), one of the more promising nanomaterials, have a large surface area for conjugating theranostic moieties, exceptional biocompatibility, and distinct and adjustable magnetic characteristics. IONPs are used for antimicrobial agents, drug delivery, magnetic resonance imaging (MRI) contrast dyes, environmental pollutant separation, illness detection, and pathogen diagnosis. It's interesting to note that IONPs become more sensitive to bacteria and aid in the fight against drug resistance when antibiotics or other antimicrobial compounds are conjugated with them [28]. Among the most attractive options are superparamagnetic iron oxide nanoparticles, or SPIONs. SPIONs can be used as an MRI contrast agent for the diagnosis of disease and monitoring of therapy due to their super paramagnetism. Due to the biodegradability of iron oxide, iron from degraded SPIONs can become deposited in the natural iron stores of the body, such as red blood cell hemoglobin [29].

### 1.14 Tumor Targeting

The term "tumor targeting" describes the targeted accumulation and absorption of treatment within the tumor. The EPR effect can be used passively to target tumor cells, or a targeting drug can be used actively. Active targeting offers improved uptake and distribution inside the tumor, whereas passive tumor targeting greatly increases the uptake of nanosized treatments. A ligand that recognizes particular receptors that are highly expressed selectively on cancer cells is typically attached to create a targeted SPION. It is also possible to target cancer cells by attaching substances like folic acid or glucose to their enhanced metabolic activity [30]. Significant progress has recently been made in the discipline of magnetism nanoparticle-based nonviral drug delivery methods thanks to the surface modification of SPIONs. Such systems can concentrate in the tumor site because to the superparamagnetic SPION capabilities under an external magnetic field (active) or the enhanced permeability and retention (EPR)

effect (passive). These characteristics, however, differ greatly between tumor types and within tumors, and they are not always adequate for precise targeting [31]. There are three strategies to treat tumors with targeted IO nanoparticles. First, the IO nanoparticles can be coupled with particular antibodies to specifically bind to relevant receptors and stop the growth of tumors. Second, hyperthermia for tumor therapy can be achieved by targeted IO nanoparticles. Thirdly, for targeted therapy, medications can be placed onto the IO nanoparticles. A growing body of research indicates that delivering therapeutic drugs selectively into a tumor mass may reduce damage to healthy tissues and increase the absorption of cytotoxic chemicals. Drug incorporation into targeted IO nanoparticles can be accomplished in a number of ways. Drugs may be embedded in the IO nanoparticles themselves, attached to the carrier coating, or deposited on the surface layer. Diffusion, vehicle rupture, formulation dissolution, or endocystosis can all release them [32].


Figure 1.5 Tumor targeting strategies

### 1.15 Iron Doped Graphene Nanoparticles

Because of their high water dispensability and strong biocompatibility, graphene-based carbon nanoparticles, including reduced forms of graphene oxide (rGO) and graphene oxide (GO), make excellent candidates for the administration of

chemotherapeutic medicines. Their application in cancer nanomedicine has been strengthened by their high surface area for loading large amounts of hydrophobic anticancer medicines via π–π interaction and their adjustable surface functionalization capabilities. Following intracellular uptake, their pH-responsive drug release behaviour also makes it easier for drugs to be released in the low pH environment seen in endosomes. However, graphene-based nanomaterials also work effectively as photothermal agents to treat cancer. Despite the fact that GO is a promising nanomaterial with high drug loading and good photothermal conversion efficiency for chemo-photothermal applications [33].

### 1.16 Iron Doped Graphene and Iron Doped Silica Nanoparticles

Iron-doped graphene oxide nanoparticles (Fe-doped GO) and iron-doped mesoporous silica nanoparticles (Fe-doped MSNs) have demonstrated potential in cancer treatment. A multifunctional nanoregulator for chemodynamic treatment (CDT) has been developed using Fe-doped MSNs. An aberrant and uncontrollably growing cell population is a sign of cancer [33]. Due to its effective antitumor efficaciousness, the integration of various therapeutic modalities, such as chemo-photodynamic, chemo-chemo, and chemo-thermal therapies, has garnered significant interest in recent years [34]. Chemo-photodynamic therapy in particular can surpass the limitations of utilizing mono chemotherapy and combine the advantages of the two approaches, greatly boosting the effectiveness of treatment [35]. As a result, in the past few years, study on cancer therapy has focused on intelligent drug delivery systems (DDS), which precisely deliver cargo to tumor areas to improve payload durability and minimize hazardous side effects. Numerous nanostructured matter drug carriers, including liposomes, nano gels, nano micelles, nanoparticles, and so on, have been created thus far. Mesoporous silica nanoparticles (MSNs), which are biocompatible, were thought to be a viable vector among them because of their simple functionalization and adjustable pore size and volume [36].

The encapsulated medications can be released under controlled conditions thanks to the mesoporous nature of MSNPs. The drug release rate might be handled through modifications MSNP pores' size or surface characteristics. Because of its controlled release feature, the medication is delivered continuously, extending its therapeutic benefits and perhaps reducing the number of times a dosage is administered.

Additionally, MSNPs can be programmed to react to particular stimuli, like variations in temperature or pH [37]. In biomedicine, porous materials are becoming more and more important. Porosity is a crucial component used in tissue scaffolds and medical implants. Adsorbents made of porous materials have already been used to treat drug overdoses and remove pollutants [38]. Because iron can improve the biodegradation behavior and therapeutic efficacy of silica-based nanoplatforms, it is utilized to dope mesoporous silica. Mesoporous silica can be doped with iron ions to provide the nanoparticles with a special coordination-responsive biodegradability and on-demand drug-releasing behavior.

Ferromagnetic or ferrimagnetic behavior are examples of magnetic qualities that can be provided by the iron oxide's presence in silica materials and are beneficial in a variety of contexts [39]. Furthermore, Materials based on iron, such particles of iron oxide are frequently employed as oxygen scavengers and can have a high oxygen adsorption capacity when supported on mesoporous silica nanospheres [40]. The confirmation of iron doping in mesoporous silica can also be achieved using methods like Mossbauer spectroscopy, which can identify the arrangement of iron-containing groups in the silica matrix [41]. All things considered, mesoporous silica with iron doping provides a flexible way to improve these nanoparticles' characteristics and functionality for a range of uses. Graphene nanoparticles doped with iron have demonstrated promise in the treatment of cancer. These nanoparticles can be employed in combination with photothermal therapy and chemotherapy to deliver drugs for a variety of cancer treatments, including brain cancer [42].

Furthermore, because nanoparticles of superparamagnetic iron oxide can be employed for both simultaneous cancer diagnosis and therapy, they have attracted interest in the field of cancer theranostics. Specifically, magnetite ($Fe_2O_4$)/maghemite ($\gamma$-$Fe_3O_4$) are non-toxic, biocompatible, and chemically stable SPIONs. They can be functionalized for multifunctional purposes by using organic coatings and chemotherapeutic medicines, as they can easily penetrate tumor tissues [43]. Iron-doped graphene nanoparticles coated in an Au layer have also been produced as a radiopharmaceutical for anticancer therapy, a magnetic agent, and an anticancer immunological medication. These nanoparticles have the possibility of applying magnetic hyperthermia in vivo and immunotherapy against cancer tissues. They also show cytotoxicity towards cancer cells that express HER2 receptors [44].

## 1.17 Treatment of Cancer

The targeted administration of sorafenib was accomplished using an aptamer-conjugated iron doped GO nanocarrier. $Fe_3O_4$ attachment to the GO layer and subsequent aptamer joining as a targeting moiety resulted in the formation of iron-doped nanocarriers. Additionally, the anticancer medication sorafenib was loaded onto the nanocarrier in a manner that demonstrated both in vitro release findings and great loading, with a 95.75% entrapment efficiency and strong pH-responsive release. Iron-doped GO nanocarriers were shown to be biocompatible with a cell death rate of less than 20% for fibroblast L-929 cells, according to a cellular toxicity assay. However, at varying medication dosages, sorafenib and sorafenib-loaded iron doped GO were found to cause significant cell death in HepG2 tumour cells [45]. Although sorafenib is well tolerated, there are a number of toxicities that can occur, so it is strongly advised to monitor dosage in order to maximize the therapy's effectiveness and minimize its side effects, which include skin toxicity, hypertension, diarrhoea, and foot-hand syndrome. Therapeutic drug monitoring (TDM) can be used for this, therefore clinical labs require extremely sensitive and specific analytical techniques to measure sorafenib in various biological fluids [8]. Furthermore, the synergistic control of several medications on various metabolic pathways frequently overlooks their toxic side effects and multidrug resistance due to their nonspecific localization, which are significant factors contributing to chemotherapy failure. Therefore, the solution lies in maximizing the use of sorafenib-based treatment in conjunction with other therapeutic approaches. According to this study, the drug-loaded approach has a potent therapeutic effect and can successfully stop the hepatocellular carcinoma cells' ability to proliferate, migrate, and invade. Notably, the drug-loaded nanoparticles damaged mitochondrial homeostasis and sharply raised lipid peroxide and reactive oxygen species levels, which negatively impacted tumor cells' ability to produce GSH normally and finally resulted in ferroptotic cell death. Moreover, drug laden nanoparticles can efficiently exacerbate and focus on the tumor location alongside minimal detrimental systemic implications in vivo, and effectively decrease HCC via inducing in real time iron metabolism. Significantly, the development for nan-medication offers a fresh approach to chemotherapy for HCC as well as a fresh idea for creating a intended, effective, consistent and safe nano-medication that can find use in clinical settings [9].

# AIMS AND OBJECTIVES

Objectives of this study are:

- To synthesize iron-doped mesoporous silica nanoparticles
- To synthesize iron oxide doped reduced graphene
- To characterize the as synthesized nanoparticles
- To investigate the effect of nanoparticles on liver cancer metastasis.

# CHAPTER 2

## LITERATURE REVIEW

Uncontrolled cell growth, or cancer, is one of the main killers worldwide. It killed nearly 7,900,000 individuals worldwide in 2007 and was responsible for approximately 13% of all deaths. Cancer is the second most common cause of mortality in the United States after cardiovascular disease. Although cancer therapy has evolved dramatically over the past 50 years, it still constitutes a serious medical problem, so considerable efforts have been made to discover new therapeutic approaches (Donna.S Shewach, et al.2009) [46]. Almost all human cancers can develop in people who are genetically susceptible. As demonstrated for colon cancer in individuals with familial adenomatous polyposis, Mendelian dominant inheritance is the most pronounced type of genetic vulnerability, resulting in high penetrance and an earlier than typical age at which cancer appears. Knudson, Alfred G. et al. (1993) [47]. Fear of recurrence or advancement of cancer has been defined by a group of scientists, legislators, learners, and patient advocates as "fear, be concerned, or alarm relating to the potential of a cancer may recur or develop." Persistent worry, hypervigilance to ailments, intense apprehensive negative expectancies (e.g., intrusive thoughts that are difficult to control), intense preoccupation (e.g., repetitive thinking or worries), and a repeated focus of emotion and attention on potential cancer-related symptoms that could indicate a recurrence or progression of the illness are all included in this broad concept. As a result, daily activities are disrupted. Bergerot, Cristiane Decat et al. (2022) [48].

The majority of people with hepatocellular carcinoma in the US and other industrialized nations have liver cirrhosis. Hepatocellular carcinoma has likely increased as a result of the increasing prevalence of cirrhosis, which has likely been caused by a number of reasons. In spite of the recent dramatic drop in hepatitis C virus frequency, an estimated In the US, nearly four million individuals have a persistent viral infection, and up to one-third of them may develop cirrhosis. Once a hepatitis C virus infection, cirrhosis takes an average of 20 years to develop. The annual incidence of cancer of the liver ranges from one percent to 4 percent. Once cirrhosis is

established. (Hashem B. El-Serag and others, 2000) [49]. Two pathways have been identified as responsible for the high occurrence of hepatic metastases. First, the possibility of liver metastases is increased by the entryway and circulation throughout the body provide the liver with two sources of blood. Second, metastatic cells can more easily enter the liver parenchyma thanks to fenestrations in the hepatic sinusoidal epithelium (Ashwin Ananthakrishnan, M.D., M.P.H. et al 2006) [50].

With a high tumor cell density, HCC has a solid, trabecular, and pseudoglandular appearance. HCC can be divided into three categories: clearly separated, mildly distinctive, and weakly defined. roughly two centimetres across, well-differentiated HCCs are frequently made up of cells with a greater nuclear to cytoplasmic ratio, organised in a thin trabecular pattern, and occasionally containing pseudoglandular features. Larger tumors (greater than 3 cm) with polygonal tumour cells arranged in frequently occurring pseudoglandular patterns with a thin trabecula shape are typically classified as moderately distinct HCCs. Poorly integrated HCCs are composed of versatile cancer cells that grow in a hard or dense form. (Mei Feng and others, 202) [51]. Between 40 and 70 percent of patients with colorectal cancer (CRC) have liver metastases. The main course of treatment is surgical resection. However, because of low hepatic reserve at diagnosis or the presence of multifocal tumours, resection is only possible in a small percentage of patients. (Wáng YX and others, 2015) [52]. Most patients with liver cancer (HCC) are only initially identified with the disease. when the disease has progressed. Transarterial chemoembolization (TACE) is the conventional treatment for advanced, non-metastatic stages. Systemic sorafenib treatment is the recommended treatment for metastatic illness. In this trial, we assessed the advantages of giving patients with advanced metastatic illness an extra local liver therapy.(Laura Schmidt and colleagues, 2014) [53].

Drug delivery vehicles at the nanoscale may be made of inorganic, organic, or a combination of these materials. Numerous papers have illustrated the benefits of nano carriers, including their adjustable dimensions and form, extensive capability as well as adaptability on the exterior alterations in effective medicine administration during lung tumours [54, 55]. The relevance of nanoscale drug delivery for cancer treatments is demonstrated by a brief review of the last five years of research in this area [55]. Regardless of the wide availability of porous in nature tiny size drug delivery drivers for drug/gene/peptide delivery through systemic injection in lung cancer, research on

porous small particles for drug delivery in the lungs continues to receive a lot of traction, including the use of these porous tiny particles in breathing route of oversight [55, 56]. Because of its extensive surface area, huge porous size, lack of density, and adjustable size, pore nanomaterials provide significant benefits over their non-porous counterparts [57]. One of the biggest problems in the delivery of cancer treatments can be resolved by using pathways of permeable substances that are nano-porous to capture and deliver a lot of poorly soluble medications. Additionally, the large exterior promotes a high level of uptake of drugs, which may lower the frequency of patient administrations and the medication dose cycle. However, medication atoms of different dimensions and forms, ranging huge polypeptide molecules from tiny peptide, can be accommodated by adjusting The size of the pores in materials that are nanoporous. For control the medicine release, the nano-porous fragments' surfaces may additionally be. altered to attach a number of components that respond to stimulation, including temperature, redox, pH as well as magnetism variables. Furthermore, these materials can affect Particle deposit caused by dispersion and damage containing the therapeutic substances because of the extra porosity characteristic [58, 59].

With its large surface area and pore size, mesoporous silica is a kind of silica material that is ideal for several uses, including chromatography, drug delivery, separations, and catalysis. It can be made with a variety of techniques and starting materials. Mesoporous silica nanoparticles were created by Rudzani Sigwadi and Touhami Mokrani using the sol-gel process. Their huge specific exterior as well as pore size Create something perfect for use for fuel cells as well as super capacitors [35, 40]. Mesoporous silica microspheres were created by Yang Liu et al. and given antibacterial modifications, which allowed them to be employed as materials for support and separation [60]. Mesoporous silica mechanical characteristics and chemical stability were examined by Andriayani et al. They discovered that the material can stabilize in acidic environments, which makes it appropriate for adsorption applications [61]. Utilizing sterile as a source of silica, Langston Tillman et al. demonstrated the synthesis of mesoporous silica, offering a potentially more affordable and environmentally friendly method of producing porous silica products [62]. Using hexane as an addition to improve crystal size and Na2SiO3 as a recursor, Syamsi Aini et al. synthesized mesoporous silica, demonstrating its potential use in

chromatography, adsorption, medicine, and catalyst synthesis [63]. Mesoporous silica nanoparticles doped with iron have been created as a cancer therapeutic agent. Numerous research have yielded encouraging outcomes using these nanoparticles. Fe-doped hollow mesoporous silica nanoparticles are combined with other substances in Wang and Zhang's multifunctional nanoregulator to improve chemodynamic treatment (CDT) to destroy tumor cells [41]. Sagir et al. synthesized mesoporous silica-coated magnetic nanoparticles with a polyamidoamine dendrimer functional tailored at the folate receptor for photodynamic therapy medication delivery [64]. Superparamagnetic iron oxide-cored mesoporous silica nanoparticles were produced by Vaz-Ramos et al. for cancer theranostics, and they demonstrated promise as T2 contrast agents and drug carriers [65]. For cancer theranostics, Vaz-Ramos et al. created superparamagnetic iron oxide core-centered mesoporous silica nanoparticles, which demonstrated promise as T2 contrast agents and medication carriers [66]. Todea and colleagues synthesized core-shell silica microspheres featuring iron oxide shells to improve magnetic resonance imaging contrast and facilitate localized hyperthermic cancer treatment [67]. These findings highlight iron-doped mesoporous silica nanoparticles' potential for photodynamic therapy, medication delivery, and cancer treatment-related hyperthermia.

Cancer research has demonstrated the promise of iron-doped graphene. For imaging, targeting, and cancer phototherapy, Lin et al. created a multipurpose nanoparticle platform made of GO [68]. $Fe_3O_4$-RGO nanohybrid of superparamagnetic iron oxide and reduced graphene oxide was created by Gupta et al., and it effectively showed chemo-thermotherapeutic actions on human cervical cancer cells [69]. Tade and Patil discussed the application of graphene quantum dots (GQDs) in photodynamic therapy, hyperthermia therapy, and photothermal therapy for the treatment of breast cancer [70]. According to Zhou et al., pristine Oxides of graphene and graphene at low concentrations can prevent breast cancer cells from migrating and invading, which may offer fresh perspectives on how to treat the disease [71]. These investigations demonstrate the promise of iron-doped graphene and its derivatives for imaging and cancer treatment. Ferroptosis is a controlled mechanism of cellular death that requires iron and has garnered a lot of interest lately. It is essential for the treatment of several illnesses, including cancer. As of October 1, 2021, the number of unique publications published on ferroptosis has expanded significantly, totaling 1690 [72]. Research on

ferroptosis in the field of cancer has grown rapidly; from 2012 to 2021, 1833 publications were found [73]. Ferroptosis research is primarily conducted in China and the US, Biochemical and Biophysical Research Communications and Cell, respectively, are the top publications in terms of output and co-citation frequency [74]. Another well-regarded field of study is ferroptosis in the brain, with 656 papers found between 2012 and 2021 [75].

VEGFR, PDGFR, and the Raf kinase family are among the tyrosine protein kinases that are inhibited by the small chemical sorafenib (SO) (Wilhelm et al., 2006). It enhances the death of a variety of tumors and suppresses angiogenesis and tumor cell proliferation (Chang et al., 2007, Wilhelm et al., 2004). It can be used to treat advanced thyroid cancer that is resistant to radioactive iodine, liver cancer, and advanced renal cell carcinoma. According to several recent studies, SO may impede the Xc-transport system, which would inhibit GSH synthesis in a manner similar to that of erastin (Fig. 1D) (Chang et al., 2007, Lachaier et al., 2014). Later, additional studies verified that SO might cause cancer cells to undergo ferroptosis (Lachaier et al., 2014, Louandre et al., 2015,) [13]. Although sorafenib is well tolerated, there are a number of toxicities that can occur, so it is strongly advised to monitor dosage in order to maximise the therapy's effectiveness and minimise its side effects, which include skin toxicity, hypertension, diarrhoea, and foot-hand syndrome. Therapeutic drug monitoring (TDM) can be used for this, therefore clinical labs require extremely sensitive and specific analytical techniques to measure sorafenib in various biological fluids [8].

Exact inserting of medications on tiny particles is a crucial component of nanomedicine. The way polymeric materials and medicinal particles combine used frequently determines drug loading. Since compounds with a high molecular mass produce heavy loading of drugs as well as regulated mechanics of delivery, the molecular weight of molecules frequently taken into account. Since amorphous polymers are better at loading pharmaceuticals than crystalline polymers, the polymers' crystallinity is also taken into consideration [76]. The mechanism of addition can be used to categorise drug loading into nanoparticles. These include drug conjugation, inclusion, and adsorption/absorption. After both the medication mixture and the tiny particles are prepared, the medication mixture gets absorbed into the particles' core on adsorption/absorption process. When the nanoparticles are being prepared, the drug solution is introduced during integration. This technique frequently

results in a higher medication loading. Although this relies on how the medications interact with the polymer in the nanoparticles, drug conjugation occurs when the medication and the material in small particles are attached. Drug loading into nanocarriers can be divided into three categories based on the loading duration [77].

The process of pre-loading involves first creating the drug nanoparticles, after which the medicine is covered in covers for protection to create medication's barrier of protection or interior. One of the many benefits of this approach is that it can deliver a tunable release, which is a controlled or sustained release of the medication. The medication core may potentially receive a protective covering from the shell. Using microfluidic techniques, immunoprecipitation and freeze-drying (emulsion templated) can be used to create the drug core nanoparticles. This method typically yields formulations with controlled release. Kheradmandnia et al. (2010) produced ketoprofen nanoparticles with a 97% encapsulation [78].

Co-loading involves putting the medication into the nanoparticles as they are being formulated. There are the fewest stages in this strategy. Covalent bonding could be used to load all into small particles from the medicinal molecules. Through non-covalent interactions, such as hydrophobic, pi-pi, and electrostatic interactions, certain drug conjugates have the ability to self-assemble. Because of The method of reversal self-assembling, these self-assembled linked frequently exhibit instability [79].

After the nanoparticles are created, the medicine is loaded into them in a process known as post-loading. These nanocarriers are combined with drug solutions by electrostatic, trapping, and adsorption processes. Carbon, Iron and silica nanoparticles are frequently used [80].

The physical and chemical characteristics of the medicine and structure, as well as interactions within drug, structure, and surroundings, determine how well the drug loads onto nanoparticles. Covalent bonds, hydrogen bonds, dipole interactions, precipitation, and surface adsorption are among ways that drugs might be integrated into nanoparticles. The kind of nanoparticle and the method of production also affect drug loading [77, 81]. Drug loading is also influenced by the incubation period. To obtain appropriate loading, the incubation period must be long enough. Because drug loading and entrapment efficacy improve with increasing interaction, while release rate decreases, the drug-carrier system interaction is a crucial aspect. Instead of

interacting with the surrounding medium, the medicine needs to be preferred engage through the vehicle system [82]. The nominal drug loading is also one of the elements that influence drug loading. Drug loading is improved by raising this component. The medication loading, the process for nanoparticle manufacturing, the method of nanoparticle alteration (e.g., changing pH), and the kind of drug are additional considerations [81]. In conclusion, the medication which ought to be included in the system of small carriers, the target, the planned administrative path, and a description of the delivery (burst release or sustained release) all affect drug loading; a nanocarrier and loading method can be chosen based on these considerations [82].

The proportion between the drug content of the particle and the total quantity of drug employed in the composition is known as drug loading efficiency [76]. The following formula provides an additional expression for this.

$$\text{Drug loading efficiency \%} = \frac{Wp}{Wi} \times 100$$

From an economic perspective, drug loading efficiency is crucial. High drug-loading capacity is more difficult to achieve than high effectiveness of medication packing. The effectiveness regarding the design process to integrate a medication into an assembly of transmitters is further referred to as effectiveness of medication load. How medicine was used in supply apparatus as it is being prepared is indicated by the drug loading efficiency. The weight of the medications in the feeds, the drug loading mechanism, and additional experimental configurations all affect the effectiveness of medication carrying. Medications whose dosage effectiveness are produced by electrostatic adsorption, whereas high drug loading efficiency nanoparticles are produced by crystallization, covalent bonding, and coordinate bonds.

As nanotechnology has advanced, SF delivery methods based on nanocarriers have been extensively researched. Its subpar pharmacokinetic characteristics have somewhat improved. Because of their greater therapeutic advantages over traditional monotherapies, synergistic medication combinations also show promise for the treatment of cancer. Anti-tumor drug bioavailability and treatment efficacy are enhanced by creative nanocarrier structure optimisation (particle size modulation, increased area of surface particular to a particle, altered surface with many uses, etc.). The synergistic effects of combination medications are significantly influenced by the chemical and physiological characteristics of nanocarriers. Nonetheless, certain

obstacles persist, including inadequate tissue penetration, unpredictable drug release, poor drug loading, loss of targeting capacity, biosafety, etc. Specifically, to improve the SF anti-tumor efficacy, nanocarriers that have been surface or ligand changed are used. Certain agonists, including vitamin C, aptamers and peptides, or antibodies, were added to the outer layer of tiny carriers to accomplish active targeting by recognizing biological substrates and receptors (antibodies and antigens), in accordance with the sensors that are overexpressed at above of tumor cells. However, because of tumor heterogeneity of pertinent sensors on the tumor surface is insufficient to provide good selectivity. Furthermore, complicated purification, manufacturing, and preparing/modification of tiny carriers are frequently needed for the development of nano formulations. Steering and durability difficulties, consistency of blood from unit to unit repeatability, and scale-up problems are some of the drawbacks. Another issue is batch-to-batch repeatability. The majority of SF-based nanodrugs are investigated in animals and cells. When it comes to clinical translation, there are several physiological hurdles [83].

# CHAPTER 3

# METHODOLOGY

## 3.1 Chemicals

Graphite powder, de-ionized water, ethanol ($C_2H_4OH$), sodium nitrate ($NaNO_3$), sulphuric acid ($H_2SO_4$), acetic acid ($C_2H_4O_2$), hydrochloric acid (Hcl), hydrogen peroxides ($H_2O_2$), sodium hydroxide (NaOH), potassium permanganate ($KmNO_4$), iron (iii) chloride hexahydrate ($FeCl_3.6H_2O$), and tetraethylorthosilicate (TEOS).

## 3.2 Cell Culture

Human liver cancer cells (HepG2) were cultivated using 10% foetal bovine serum added to Dulbecco's modified Eagle's medium (DMEM). (Invitrogen), 100 µg/mL streptomycin and 100 U/mL penicillin (Invitrogen), and maintained at 37 ˚C with 5% $CO_2$ supply. The media was changed regularly and cells were sub-cultured when 80% confluent.

## 3.3 Apparatus

Hot plate, ice bath, round bottom flask, magnetic stirrer, thermometer, iron and tripod stand, centrifuge machine, autoclave or hydrothermal reactor, heating furnace and other usual laboratory glassware's

## 3.4 Synthesis of Graphene Oxide (GO) Nanoparticles

Hummers method was used to synthesize the graphene oxide (GO). Graphite powder (2g) and $NaNO_3$ (2g) were added to 150 mL of $H_2SO_4$ with continuously stirring. Mixture was kept in an ice bath and stirred for two hours. To oxidize graphite, 6g of $KMnO_4$, is added pinch by pinch in one hour. Kept the solution on stirring for 20 to 24 hours. After that, mixture was diluted by using 100 ml DI water drop-wise, 20 ml $H_2O_2$ added drop-wise. The final product was centrifuged at 4000 rpm. Graphene was washed with 10% HCl solution and deionized water, the synthesized graphene was placed for 3 days to dry in oven at 70$^o$C. The final product obtained was greyish-black [84].

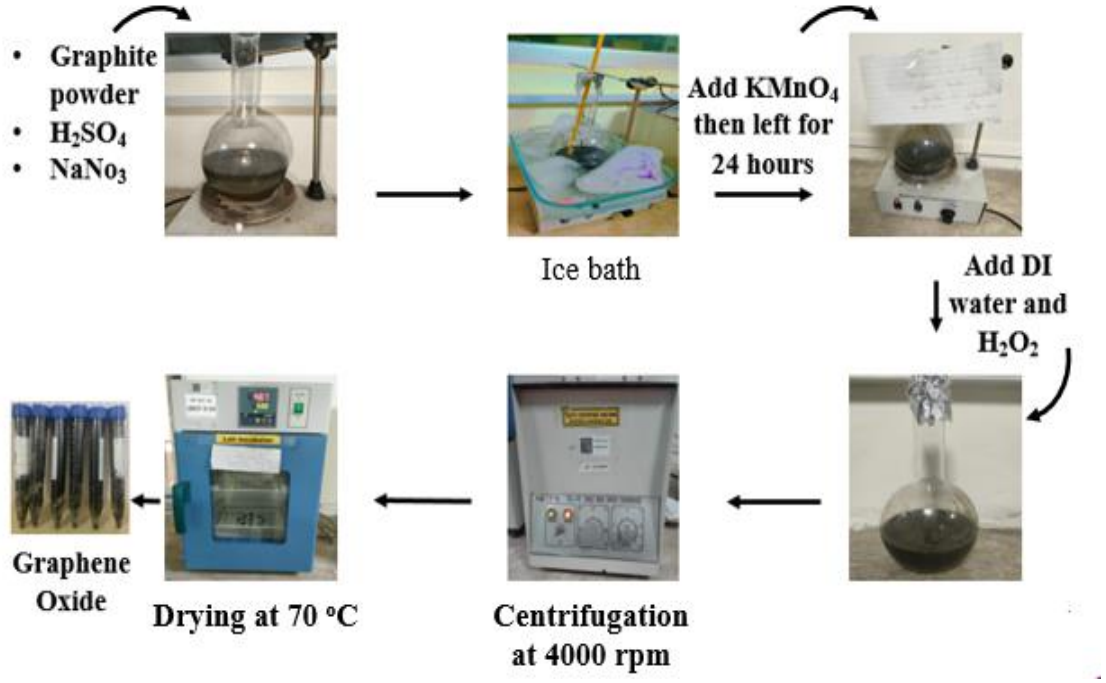



Figure 3.1 Synthesis of graphene oxide nanoparticles

### 3.5 Synthesis of Iron-Doped Graphene (FeGO) Nanoparticles

To synthesize a homogenous solution of FeGO, 0.15 g GO was ultrasonically dissolved in 200 mL of deionized water (DI). The GO suspension was injected with an aqueous solution of $FeCl_3 \cdot 6H_2O$ (0.33 g) in DI $H_2O$ (5 mL) and reaction mixture was heated at 80°C temp during continuous stirring. After bringing the mixture's pH-10 via adding 10 mL of 30% sodium hydroxide solution, the mixture was agitated and maintained at 80°C for 30 minutes. Finally, the solution was heated to 95°C and 1.0 g of trisodium citrate was added to generate a suspension with a black hue. The mixture was centrifuged and rinsed with DI water and dried the product at 60°C. Synthesized hybrid materials of iron oxide and GO were collected and dried at 80°C [85].

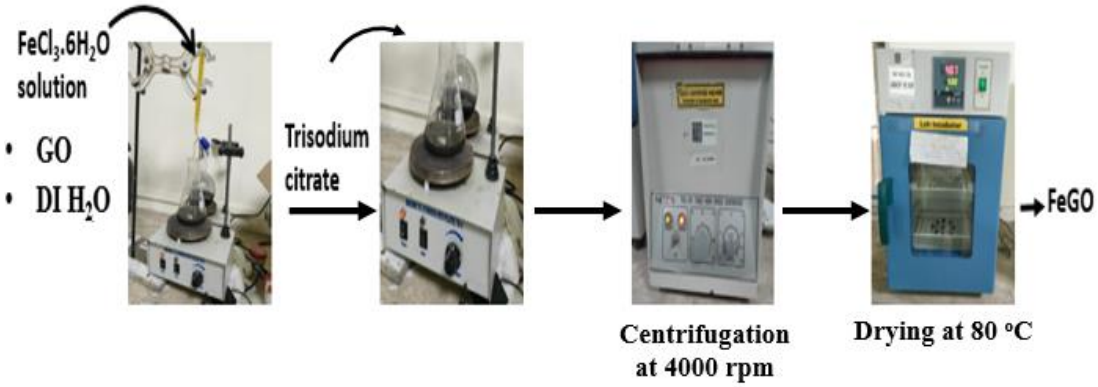



Figure 3.2 Synthesis of iron doped graphene oxide nanoparticles (FeGO)

### 3.6 Synthesis of Iron-Doped Silica (FeMS) Nanoparticles

The iron-doped silica nanoparticles were synthesized by using one-pot synthesis with some modifications in previously reported method. Surfactant CTAB (0.03g, 1mmol) was dissolved in 30 ml of deionized water and 3.33 ml of TEOS, then 10 ml of 1M NaOH solution was added while stirring at room temperature. Mixture was treated with iron chloride (0.433 g; 40.04 mmol) for 24 hours at pH 11 and 1 M hydrochloric acid was added to the suspension to bring its pH down to 7. Centrifugation was used to gather the yellowish-white precipitates, which were then cleaned twice with ethanol and deionized water before being dried for a whole night at 60°C in a vacuum desiccator. The solid materials were calcined at 600°C for five hours with airflow (100 mL $min^{-1}$) in an electric furnace to eliminate the surfactant. Sample code FeMS was assigned to this sample [86].

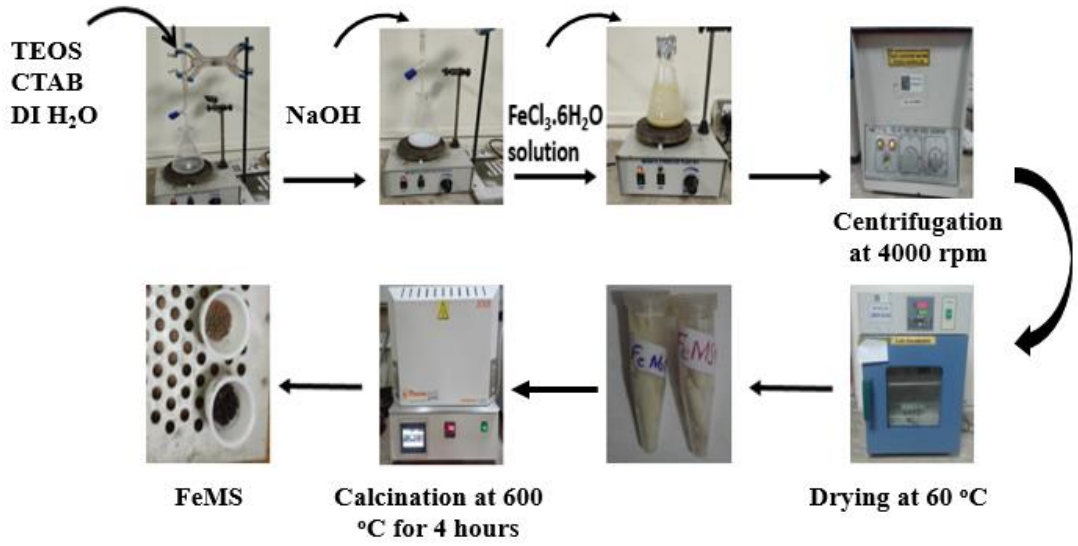



Figure 3.3 Synthesis of iron doped silica nanoparticles (FeMS)

### 3.7 Drug Loading on Nanocomposites

The SO-encapsulate FeGO and FeMS used in this study were prepared with minor modifications from previous studies. Each of 40 mg FeMS and FeGO were added in separate solution of 40 mg sorafenib in 20mL (PBS). The drug-encapsulated nanoparticles were centrifuged at 4,000 rpm for 5 minutes take out the supernatant to check the absorbance of drug loaded nanoparticles at 240 nm. Sorafenib absorption spectrum within the nanoparticles exhibits a profile that is very comparable to that of the medication in phosphate-buffered saline (PBS), with an absorption maximum at

240 nm. As was previously established, sorafenib has very poor water solubility, and precipitation happens at very low concentrations (>10–5 M). This indicates that more sorafenib is transported in water by the NPs than is soluble in it, and that the drug precipitates when released, resulting in a drop in absorbance and an increase in scattering [87-89].

### 3.8 Drug Loading Efficiency

Through solvent-assisted SO extraction and subsequent quantification tests, the drug loading efficiency (DLE) can be ascertained. The calculation of the DLE is estimated by the following equations:

$$\text{Drug loading efficiency \%} = \frac{Wp}{Wi} \times 100$$

Here, Wi is the starting weight of the drug feed, and Wp is the weight of the drug in the nanoparticles. Drug-loading nanoparticles weighing 5 mg were carefully weighed and re-dispersed in acetate buffer (pH 5.4 and 7.4) for 20 minutes in order to estimate the loaded SO. These procedures led to the comprehensive detachment of the FeGO@SO and FeMS@SO nano-complex and hence a 95% release of SO. Following a series of centrifugations, the concentration of SO at an absorbance of 285 nm was measured using ultraviolet-visible spectroscopy (UV-Vis). The SO released was examined by quantifying the supernatant of FeGO@SO and FeMS@SO derivative nanocomposites over a range of time periods. Each nanoparticle's supernatant was collected using a centrifuge set to 13,000 rpm for 10 minutes [76, 87, 90, 91].

Table 3.1 Drug loading and release on FeGO and FeMS in percentage

| Sample name | Drug loading | Sample | Drug release time (minutes) | Drug release % |
|---|---|---|---|---|
| FeGO | 85% | FeGO-SO | 15 | 70% |
| | | | 30 | 10% |
| | | | 60 | 7% |
| | | | 120 | 5% |
| FeMS | 96 % | FeMS-SO | 240 | 4% |
| | | | 1440 | 3% |
| | | | 2880 | 1% |

### 3.9 Drug Desorption Studies

To check the release profile of SO was studied at different times like 15, 30, 60, 120, 1440, and 2880 minutes with the help of UV spectrometry. 20 mg of FeGO@SO and FeMS@SO were added to 20 mL of buffers at various pH values and agitated at 37°C for the drug desorption tests. The absorbance measured at 285 nm was used to calculate the SO concentrations using ultraviolet light.[92].

### 3.10 In-Vitro Cytotoxicity Studies

Using an MTT-based colorimetric test, the cytotoxic potentials of drugs, nanoparticles, and drug-loaded nanoparticles against HepG2 cells ($1 \times 10^4$ cells/well) were determined at two distinct time periods (24 and 48 h) [93].

### 3.11 Assessment of Cell Proliferation

The cells were cultivated at a density of $1x10^4$ cells/well in 96-well plates. Following induction by iron doped graphene and iron-doped silica nanoparticles, various assays were performed to assess alterations in the viability, proliferation, and metabolism of cells.

### 3.12 MTT Assays

Cell metabolic activity was estimated using the MTT test. For that, cells were treated with 0.1 mg/ml MTT salt (Thermo Fisher Scientific-M6494) in PBS and allowed to incubate for three hours. The BioTek ELx808 absorbance reader was used to measure the absorbance at 570 nm following the addition of the de-staining solution [37].

### 3.13 Real Time PCR

Liver cancer cells' whole RNA was extracted using phenol: chloroform extraction method. After DNAase treatment 1 µg of total RNA was subjected to reverse transcription with cDNA synthesis kit (Invitrogen Life Technologies, Carlsbad, CA, USA). RT-PCR was conducted in 6 well plates using an Applied Biosystems 7900HT Fast Real-Time PCR System (Applied Biosystems Life Technologies, Foster City, CA, USA) using SYBR Green I PCR Master Mix kit (Thermofisher Scientific), according to manufacturer's recommendations, in a volume of 5 µl using 5µM primers each (Table 3.2). Analysis of genes involved in adhesion and ECM remodeling were

analyzed using ΔCT values. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control for normalization [94].

### 3.14 Statistical Analysis

Three sets of quantitative data were computed and presented as mean standards and associated standard errors. The one-way ANOVA test was used to calculate the differences between the obtained results and the control groups; $P < 0.05$ and $P < 0.01$ are regarded as statistically significant. The Origin 2018R program was used to display the data and do statistical analysis on it. The SANS and SAXS raw data were reduced using in-house software, and Sas View version 5.0.3 was used to plot the data [95].

# CHAPTER 4

# RESULTS

## 4.1 Characterization

### 4.1.1 FTIR of FeGO Nanocomposite

FTIR spectra of nanocomposites (Figure 4.1) were recorded under transmission mode to determine the functional groups of compounds exhibited in FeGO composite. GO spectrum established the occurrence of the alkoxy C–O stretching vibrations (1112.435 $cm^{-1}$), epoxy C–O–C stretching modes (1224.316 $cm^{-1}$), and aromatic C=C skeleton vibrations (1615.248 $cm^{-1}$). The C=O stretching band, which appeared at the edge of GO sheets, was emerged at 2333.852 $cm^{-1}$. All these functional groups may be observed in FeGO spectrum, but the bond positions are red shifted and the peak sharpness is altered showing the alteration in coordination environment of different functional groups in FeGO. The band at 3339.945 $cm^{-1}$ is due to O–H stretching vibration of C–OH group, where this broad absorption is reduced in FeGO spectrum because the GO is reduced during heat treatment and the conjugated aromatic system is formed. Besides that after coating with $Fe_2O_3$, the C=O stretching band at 2300.902 $cm^{-1}$ became weaker than GO due to formation of –COO–. In contrast to GO, Fe–O stretching mode at the absorption bands 784.645 $cm^{-1}$ is attributed to the presence of $Fe_2O_3$ chemical compounds bonded to the –COO on the GO edge nano sheets. The second indication for the monodentate and bidentate ligand formation in a complex between Fe and the carboxyl group is the presence of the extra vibrational band at 1224. 316 $cm^{-1}$, which indicates the covalent bond formation between hematite and GO.

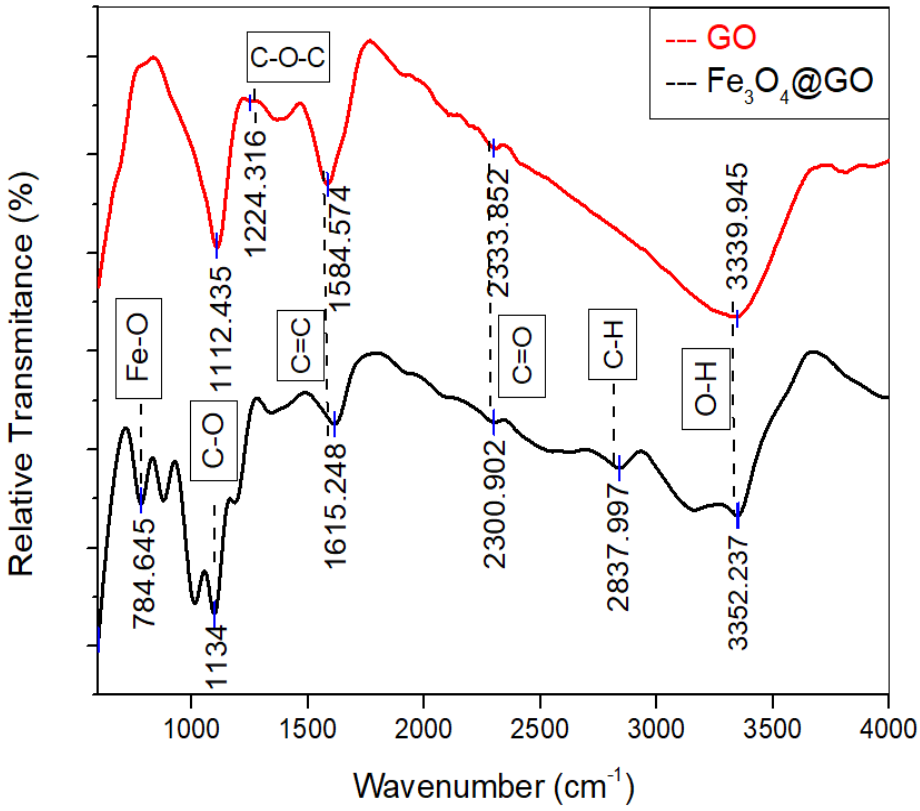



Figure 4.1 FTIR of FeGO nanocomposite

### 4.1.2 FTIR Spectra of FeGO and FeMS

Figure 4.2 shows the FTIR spectra of FeGO and FeMS. The band located at 3127.52 $cm^{-1}$ is assigned to the O–H stretching vibration of C–OH groups. Absorption peaks were found at 777.51$cm^{-1}$, which was attributed to the Fe-O vibration. The Si-O-Si asymmetric stretching vibration is represented by a strong broadband at 1070.60 $cm^{-1}$ [96], whereas the Fe-Si-O vibration, which reflects the iron coating on silica, is assigned a weak band at 803.95$cm^{-1}$ [97, 98].

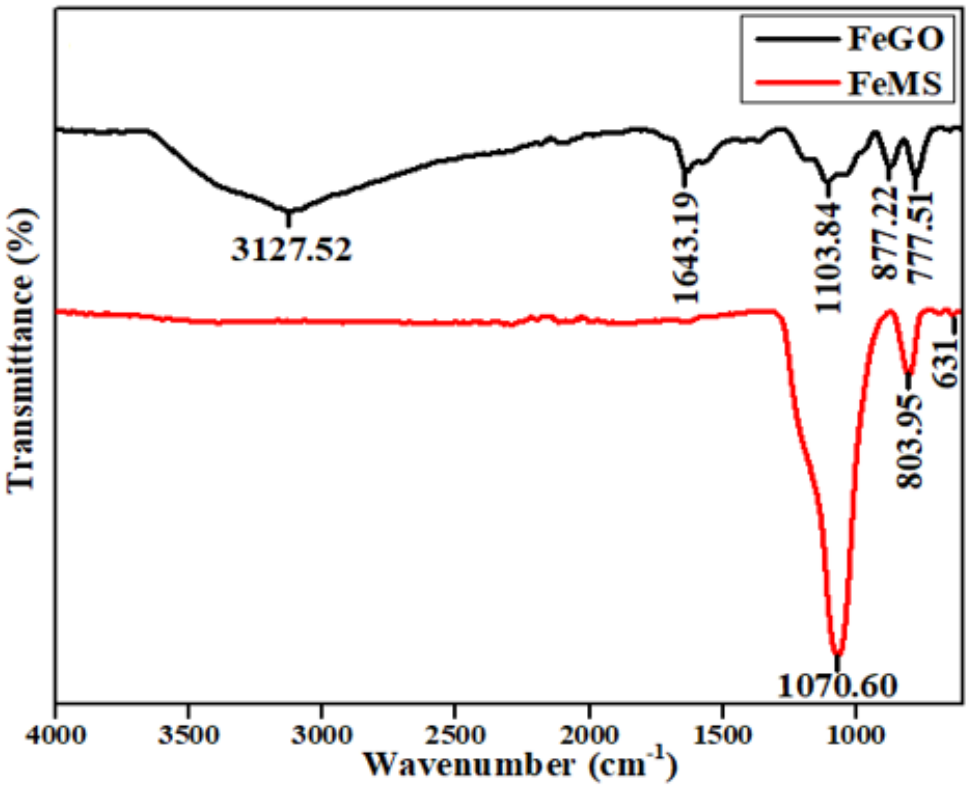



Figure 4.2 FTIR spectra of FeGO and FeMS

### 4.1.3 UV-Visible of FeGO

The optical properties of the GO and FeGO using UV-Vis analysis are shown in Figure 4.3(c). It is evident that GO generates an absorbance peak at 235 nm due to the π–π* transition of the C = C [99] aromatic bond and the C = O bond, which are both at 270–300 nm. The presence of $Fe_3O_4$ causes the peaks for FeGO to move to higher wavelengths at 235 nm, resulting in the restoration of electronic conjugation for the graphene sheet's carbon bond.

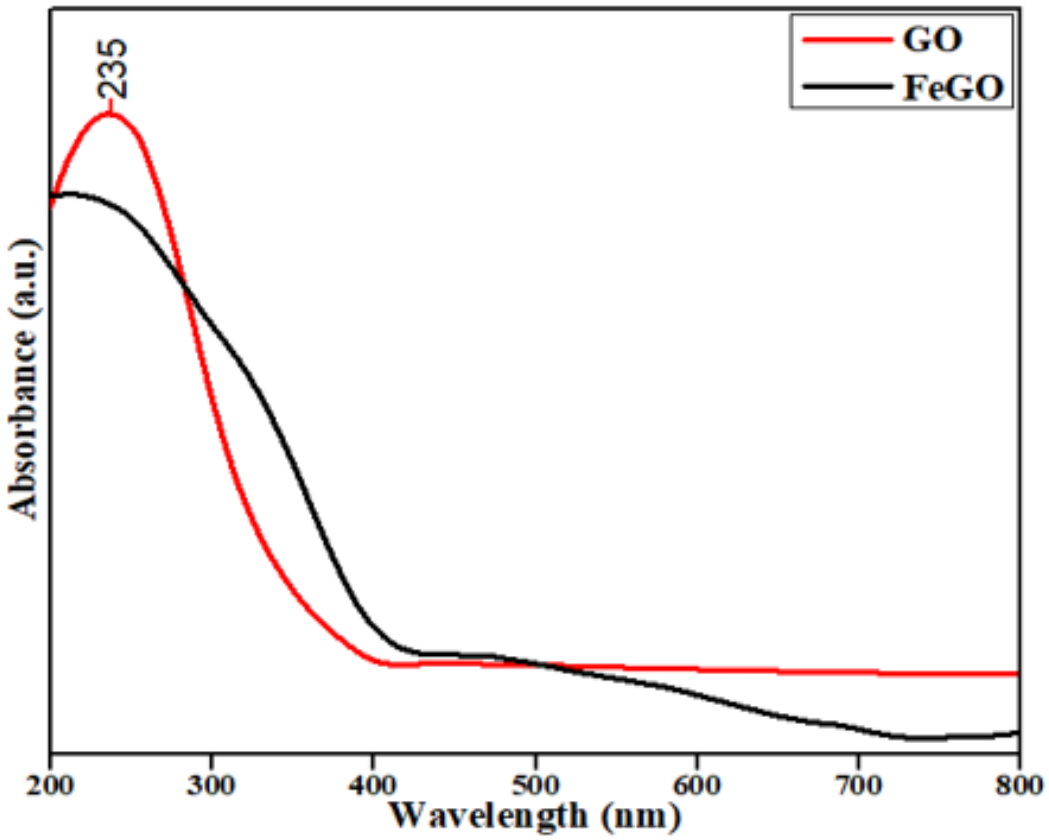



Figure 4.3 UV-Visible of FeGO

### 4.1.4 XRD Analysis of FeGO and FeMS Nanocomposite

The crystalline structure of FeGO and FeMS nanocomposite were studied by powder X-ray diffraction (XRD), as shown in Figure 4.4(a). The XRD spectra of FeGO shown a distinct diffraction peak at 2θ of 38.32, 46.14, and 64.54, corresponding to crystal planes of (110), (202), and (1010) This symbolizes the crystal structure of $Fe_3O_4$ face-centered cubic lattice (fcc). Figure 4.4(a) illustrates that each peak can be identified as the magnetite phase of $Fe_3O_4$ (JCPDS 96-154-8825) [100, 101]. And XRD spectra of FeMS shown a distinct diffraction peak at 2θ of 23.81, 37.45, 39.34, 43.21**,** 45.52, 65.62, 78.93, and 82.49 corresponding to crystal planes of (121), (202), (051), (004), (213), (420),(084) and (345) which represent the face-centered cubic lattice (fcc) crystal structure of $Fe_3O_4$ (JCPDS 96-900-1920) [102]. There were no distinctive $SiO_2$ peaks found, suggesting that the material was amorphous [96, 103, 104].

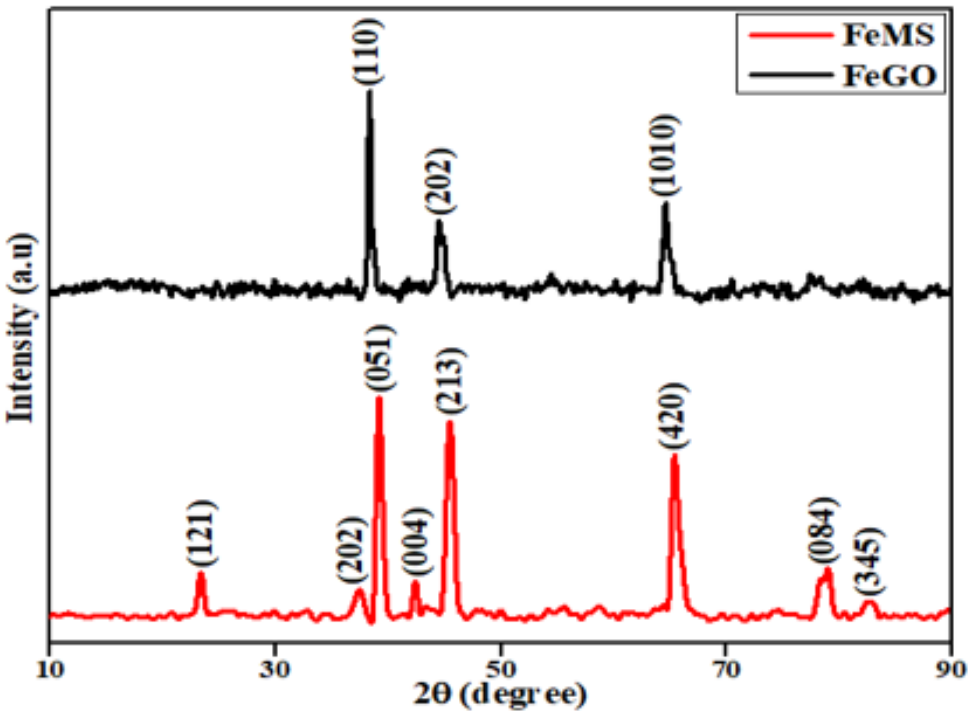



Figure 4.4 XRD analysis of FeGO and FeMS nanocomposites

### 4.1.5 TGA Analysis of FeGO and FeMS

Thermal stability of FeGO, and FeMS samples investigated via TGA technique at heating rate 5 °C/min as shown in Figure 4.5. The FeGO showed three main stages of weight losses. First weight loss at 143 $^{o}$C (5.81 wt%) has been attributed to evaporation of adsorbed water, second decomposition of labile oxygen deriving from functional groups such as hydroxyl at 143-496 $^{o}$C (14.56 wt%), and third to the removal of more stable oxygen functionalities deriving from phenol and carbonyl at 496-743 $^{o}$C (14.3 wt%) [105]. The TGA curve of FeMS has two major weight losses. The first weight loss below 295 °C (7.81%) is due to the vaporization of the physically adsorbed water. While the second minor weight loss (5.42%) between 500 and 600 °C was resulting from the physisorption molecules [106].

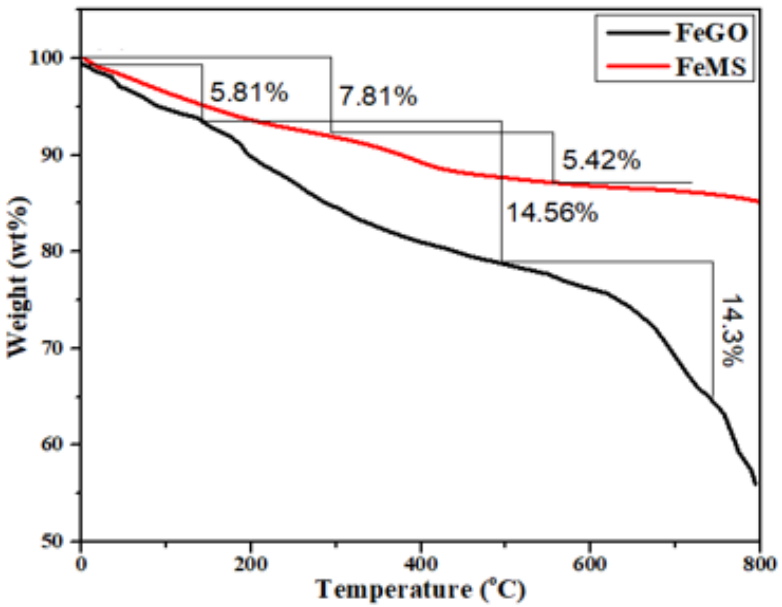



Figure 4.5 TGA analysis of FeGO and FeMS

### 4.1.6 BET of FeGO and FeMS

According to Table 2, the BET surface areas of FeGO and FeMS were 79.45 $m^2/g$ and 98.92 $m^2/g$, respectively. Additionally, FeMS nanocomposites' high BET surface area was attributed to their highly porous structure and plentiful supply of active sites on their surface [107, 108]. Table 2 shows the BET surface areas for FeGO and FeMS, which were 79.45 $m^2/g$ and 98.92 $m^2/g$, respectively. Additionally, the high BET surface area of FeMS nanocomposites was attributed to their high porosity and a large number of active sites on their surface [107, 108].

Table 4.1 Characterization parameters of different nanoparticles

| Sample | BJH* | | | BET ($m^2/g$) | Langmuir |
|---|---|---|---|---|---|
| | Surface area ($m^2/g$) | Pore size (nm) | Pore volume ($cm^3/g$) | Surface area ($m^2/g$) | Surface area ($m^2/g$) |
| FeGO | - | - | - | 79.45 | - |
| FeMS | 87.35 | 206.43 | 0.51 | 98.92 | 102.20 |

### 4.1.7 Scanning Electron Microscopy (SEM)

The morphology was characterised using scanning electron microscopy (SEM). of FeGO and FeMS nanocomposites. Figure 4.6 (a) and (b) demonstrate the morphology of FeGO, displaying smooth and homogeneous 2D GO sheets and particle aggregation as a result of Fe doping on GO sheets, respectively [109]. The resulting particles are shown in the image (c) and (d) to be hollow with a continuous wall devoid of any obvious holes, fissures, or other deformations [110].

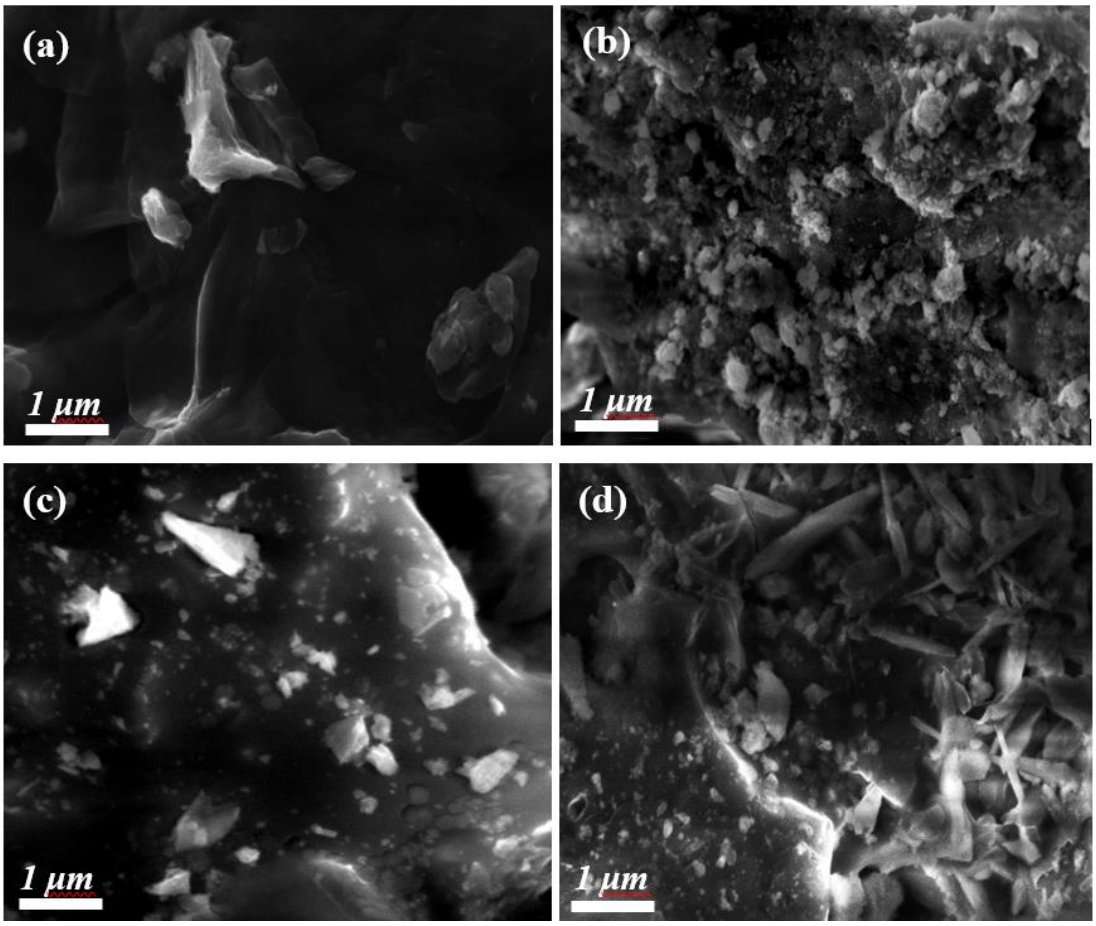



Figure 4.6 Scanning electron microscopy (SEM) analysis of GO (a), FeGO (b), FeMS (c) and (d).

## 4.2 Drug Loading

Figure 4.7 shows the ultraviolet-visible absorption spectra of SO, FeGO, FeGO@SO and FeMS, FeMS@SO composites. The interactions between SO and nanoparticles

were confirmed by the absorption characteristic of SO at 285 nm and the absorption peak indicating the noncovalent loading of SO on nanoparticles [111, 112].

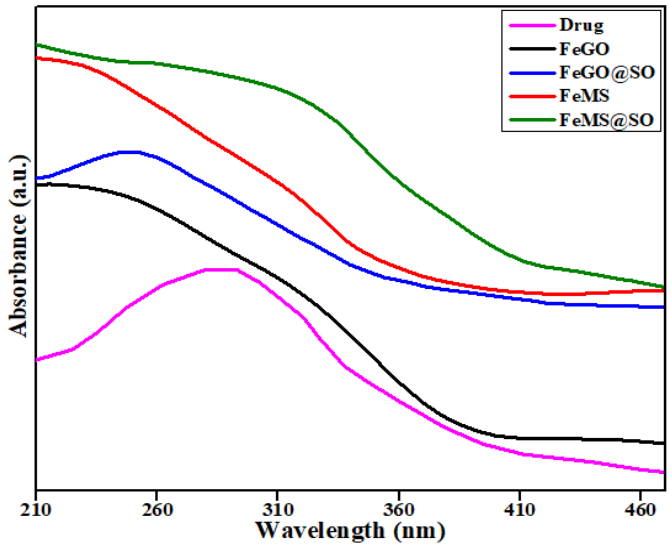



Figure 4.7 UV–Vis. of sorafenib (black line), FeGO- (red line), sorafenib loaded FeGO (blue line), FeMS (pink line) and sorafenib loaded FeMS (green line)

### 4.3 Release of Drug in Vitro

To check the release profile of SO was studied at different times like 15, 30, 60, 120, 1440, and 2880 minutes with the help of UV spectrometry. In the drug desorption experiments, 20 mg of FeGO@SO and FeMS@SO was added into 20 mL of buffers at a series of pH values and stirred at 37°C. The concentrations of SO was determined by Uv with the absorbance detected at 285nm [113].

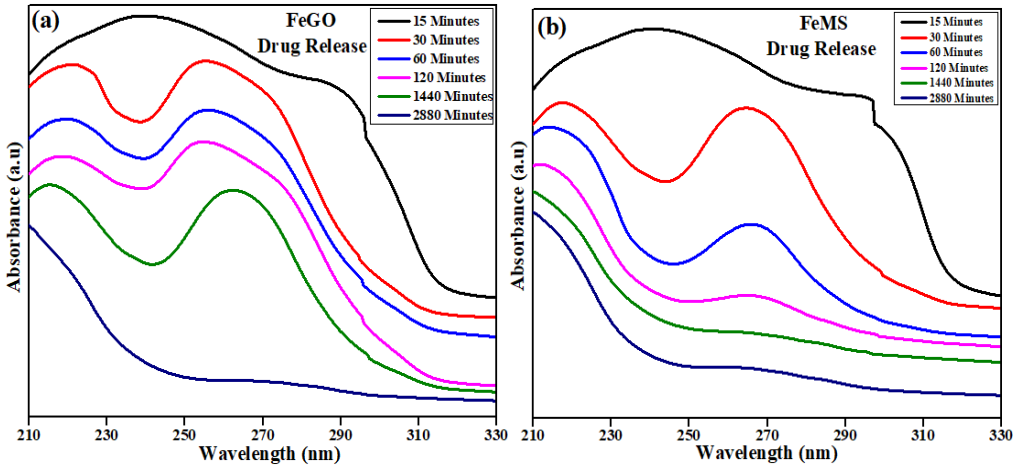



Figure 4.8 UV-Vis spectra of sorafenib loaded FeGO (a) FeMS (b) solutions at variable concentration and time.

### 4.4 Ferroptosis

Ferroptosis was successfully triggered in HepG2 cells by ACSL4-promoted lipid peroxidation upon exposure to sorafenib-loaded nanocarriers. Both sorafenib-loaded FeGO and FeMS systems possessed the capability for ferroptotic cell death induction. Nevertheless, the drug delivery system based on FeGO possessed a more significant effect. Sorafenib-loaded FeGO showed greater enhanced ACSL4 expression as compared with FeMS. These observations indicate that FeGO is a more effective vehicle for the delivery of sorafenib to induce ferroptosis in liver cancer cells, possibly because it is more efficiently loaded with the drug and has better cellular uptake.

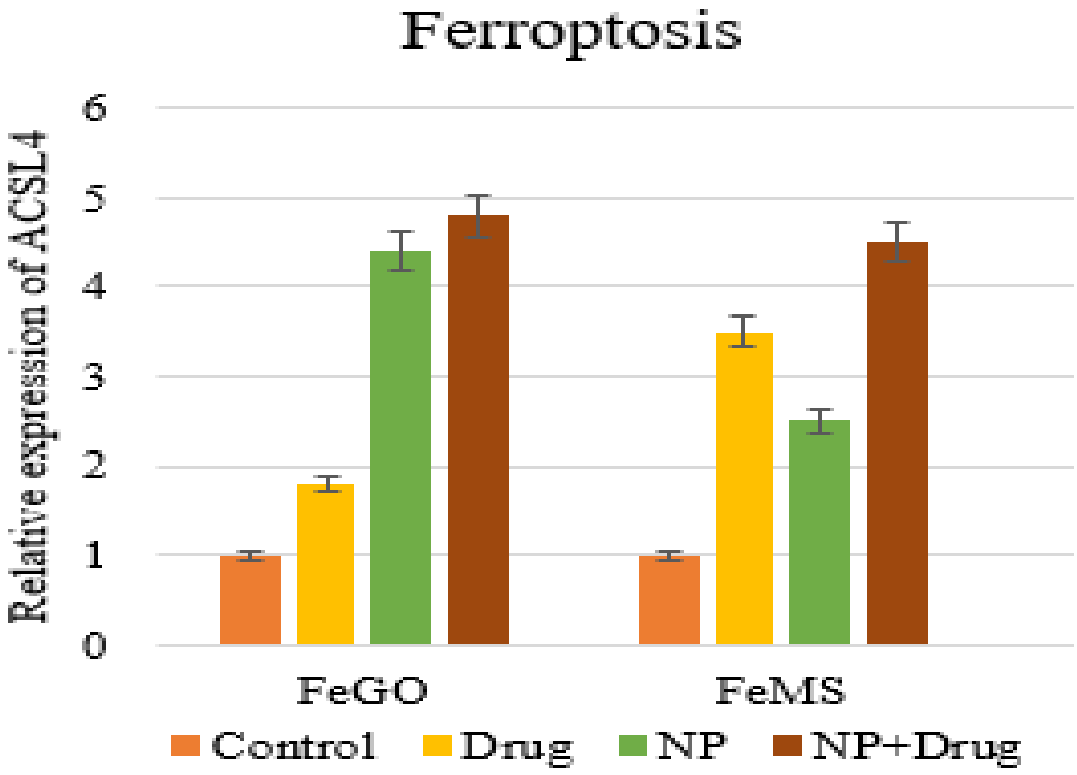



Figure 4.9 Expression analysis of Ferroptosis inducing ACSL4 gene in liver cancer cells. Analyzed by RT-PCR.

### 4.5 Cytotoxicity of Cells (MTT assay)

The anticancer activity of samples on HepG2 cells was determined by the MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay, that was used to

assess the cytotoxicity [114]. A decrease in cell viability was observed when the SO was introduced into the cells either bare or loaded in nanocarriers. But the number of viable cancer cells was lesser when SO was administered along with nanocarriers. These results also indicated that drug loaded FeGO is more effective than drug Loaded FeMS, as the decrease in cell viability was more pronounced when SO was loaded on FeGO [115].

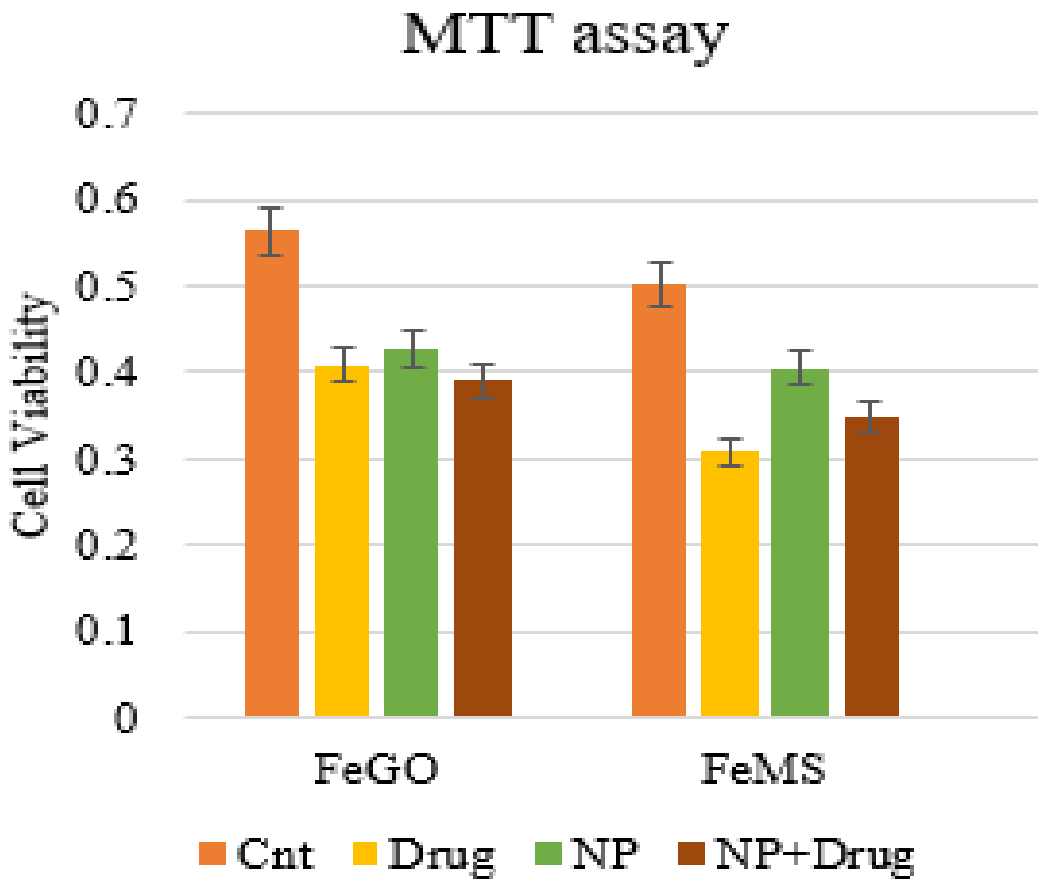



Figure 4.10 Effect of nanoparticles treatment on Liver cancer cell viability

### 4.6 Epithelial to Mesenchymal Transition

The expression of multiple epithelial to mesenchymal marker genes was analyzed by RT-PCR. The expression of Slit2 (that is reported to have a tumor suppressive role in Liver Cancer according to various reports) was also analyzed after exposure to multiple nanocarriers. Expression of CDC42 (a cytoskeletal modulator) was also analyzed.

### 4.6.1 E-Cadherin

The expression of E-Cadherin (epithelial marker gene) gene was checked in liver cancer cells administered with sorafenib and sorafenib loaded nanoparticles (FeMS, and FeGO). The expression of E-Cad in the presence of SO was equal as compared to treated with the sorafenib loaded FeMS and SO loaded FeGO. The expression of E-Cad gene is lowest in SO loaded FeMS as compared to the SO loaded FeGO.

### 4.6.2 CDC42

The expression of CDC42 gene was checked in liver cancer cells with sorafenib and sorafenib loaded nanoparticles (FeMS, and FeGO). The expression of CDC42 in the presence of SO was downregulated as compared to treated with the sorafenib loaded FeMS, and SO loaded FeGO. The expression of gene in SO loaded FeMS is higher to the SO loaded FeGO.

### 4.6.3 N-Cadherin

The expression of N-Cadherin (A mesenchymal marker) gene was checked in sorafenib and sorafenib loaded nanoparticles (FeMS, and FeGO). The expression of N-Cad in the presence of SO was equal as compared to treated with the sorafenib loaded FeMS. The gene expression in SO is more as compared to the SO loaded FeGO. The expression of N-Cad gene is highest in SO loaded FeMS as compared to the SO loaded FeGO.

### 4.6.4 Vimentin

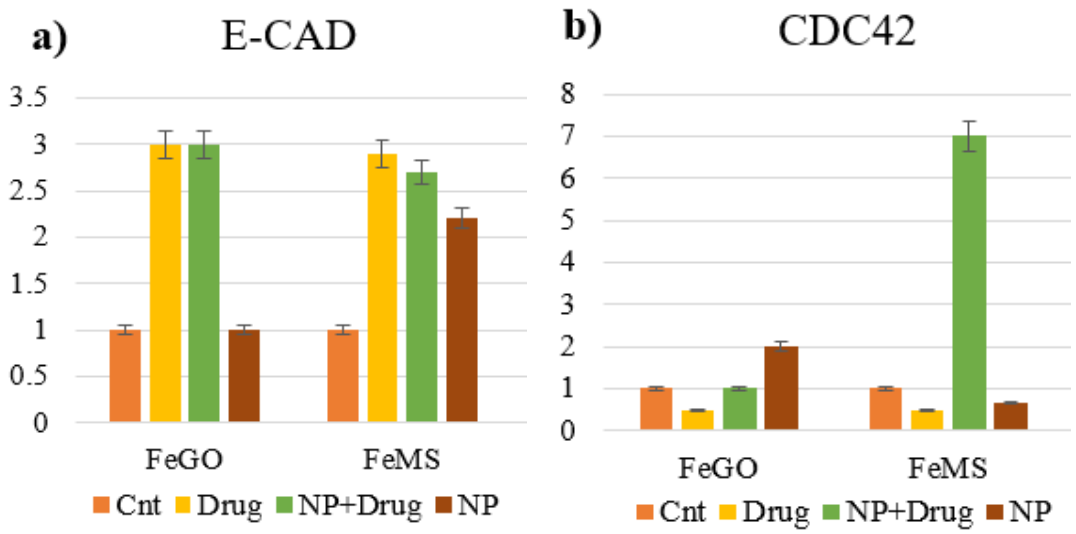
The expression of vimentin gene was also analyzed with sorafenib and sorafenib loaded nanoparticles (FeMS, and FeGO). The expression of vimentin in the presence of drug was less as compared to treated with the sorafenib loaded FeMS. The gene expression in SO is more as compared to the SO loaded FeGO. The expression of vimentin gene is highest in SO loaded FeMS as compared to the SO loaded FeGO.

### 4.6.5 Slit2

The expression of Slit2 was checked in sorafenib and sorafenib loaded nanoparticles (FeMS, FeGO). Expression of Slit2 in the presence of SO was less as compared to treated with the sorafenib loaded FeMS, and SO loaded FeGO. The expression of Slit2 gene is highest in SO loaded FeMS as compared to the SO loaded FeGO.

### 4.6.6 Integrin

The expression of integrin gene was checked in the sorafenib and sorafenib loaded nanoparticles (FeMS, and FeGO). The expression of integrin in the presence of SO was more as compared to when treated with the sorafenib loaded FeMS. The SO expression is less as compared to the SO loaded FeGO. The expression of integrin gene is highest in SO loaded FeGO as compared to the SO loaded FeMS.



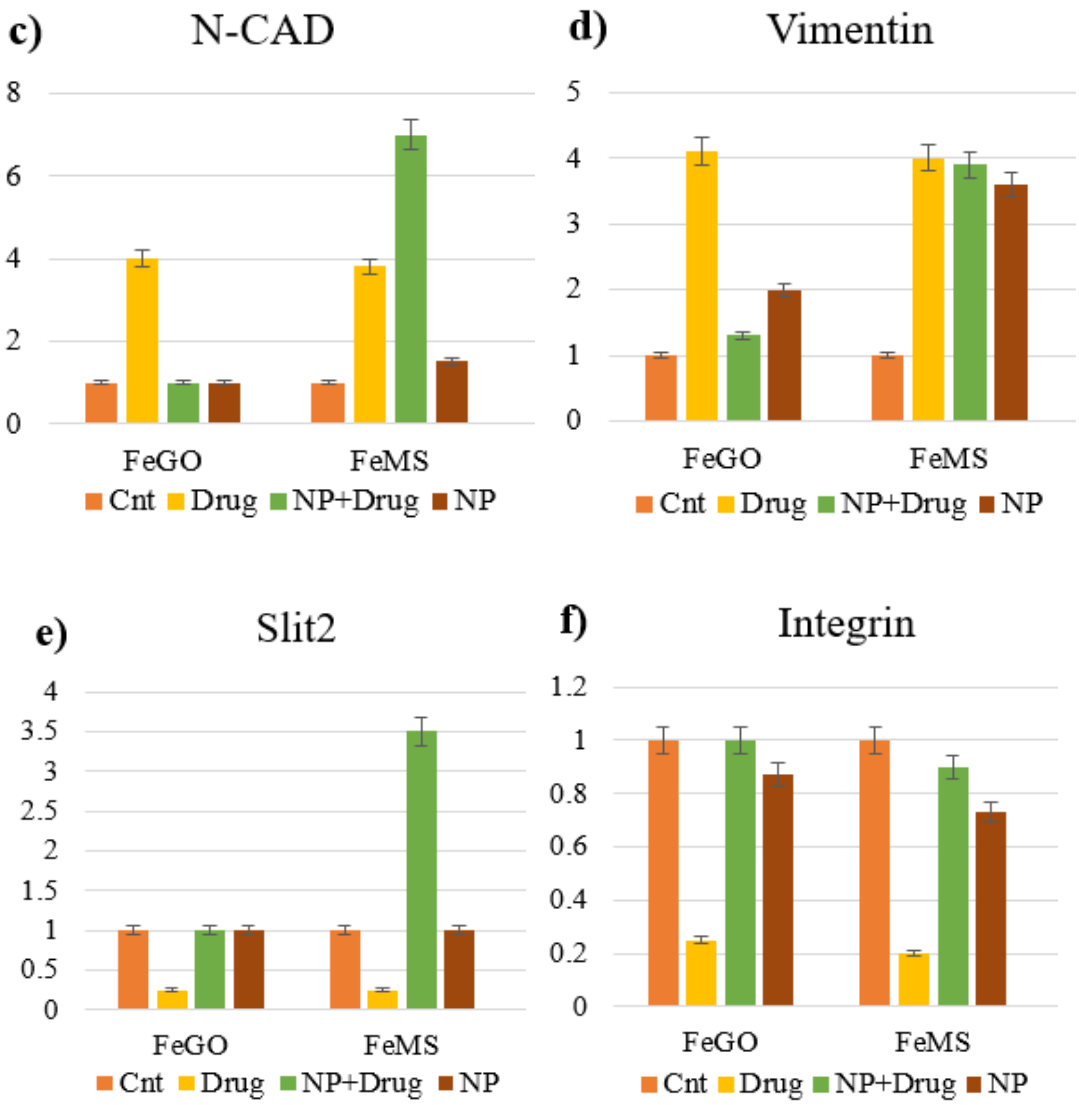



Figure 4.9 Effect of drug, drug loaded nanoparticles and nanoparticles on different genes (a) E-CAD, (b) CDC42, (c) N-CAD (d) Vimentin, (e) Slit2, (f) Integrin

# CHAPTER 5

# DISCUSSION

HepG2 liver cancer is a malignant tumor that has a high rate of morbidity and death. Combining sorafenib with FeGO and FeMS nanoparticles can increase the effectiveness of targeted therapy against liver cancer cell lines. This seems to be partially caused by sorafenib's capacity to prevent tumor angiogenesis. Sorafenib's poor water solubility and non-specific medication distribution are two of its main drawbacks. In order to mitigate these drawbacks, we created FeGO and FeMS nanoparticles and added sorafenib to them. Compared to FeGO, the FeMS exhibited a higher DL% and sustained drug release across three pH values, with a greater release in the tumor microenvironment's acidic circumstances. When sorafenib is encapsulated on FeGO and FeMS, its sustained release may result in a longer half-life at tumor sites than when the drug is administered freely. The nanoparticles were about 2 μm in size, which could help lessen the negative effects of the medication by demonstrating good EPR effects to target tumors.

For loading a variety of medications, post-loading is simple and ubiquitous, particularly for mesoporous silica and graphene nanomaterials. However, because of the unspecific binding or adsorption of pharmaceuticals on the particle surface, these nanoparticles have problems such as limited loading efficiency and undesired burst release. Although applying a thin layer of additional material as the gatekeeper is a popular and successful strategy to reduce the first burst release, it has drawbacks, including additional coating and purification procedures that may reduce drug loading and cause unintended drug degradation, among other things. Since pharmaceuticals are loaded during the nanoparticle production process, the co-loading approach frequently involves the fewest stages for creating high drug loading nanoparticles. However, because of their reversible self-assembly and dis-assembly process, drug-conjugate self-assembled nanoparticles are often unstable. Additionally, it is challenging to create a uniform method for all medications, even though drug-drug conjugates might achieve a maximum of 100% drug loading. According to the

experimental findings, sorafenib's anticancer activity is completely maintained when it is encapsulated in hydrophobic micelle cores, and a magnetic field improves FeGO and FeMS cell internalization without compromising cell viability.

In this in vitro experiment, ferroptosis was effectively achieved in HepG2 liver cancer cells by ACSL4-catalyzed lipid peroxidation after treatment with sorafenib-loaded nanocarriers. Between the two tested systems—FeGO (iron-loaded graphene oxide) and FeMS (iron-loaded mesoporous silica) FeGO displayed better efficacy in enhancing ferroptotic cell death. This was evidenced by elevated ACSL4 expression and higher cell viability reduction, as validated by the MTT assay. The improved performance of FeGO is due to its higher drug loading capacity and better cellular uptake than FeMS. Additionally, RT-PCR analysis of epithelial to mesenchymal transition (EMT) markers disclosed that FeGO retained more epithelial features as evidenced by enhanced E-Cadherin expression, but downregulate mesenchymal features, including decreased N-Cadherin, Vimentin, and CDC42 expression, than FeMS. Whereas Slit2, a tumor suppressor gene, expressed slightly greater in FeMS, it was induced even by FeGO compared to free sorafenib. Integrin expression was also maximum in the FeGO-treated group, indicating improved interaction with cellular membranes and therefore possibly aiding greater drug internalization. Overall, these in vitro results strongly suggest that FeGO is a superior and efficient nanocarrier for sorafenib delivery and triggering ferroptosis in liver cancer cells and affecting the major pathways linked with metastasis inhibition.

# CONCLUSIONS

Creating efficient drug delivery systems based on nanoparticles, the current work aimed to increase the anticancer activity of sorafenib (SO) against HepG2 liver cancer cells. Two types of iron-containing nonporous carriers were created: iron-doped graphene oxide (FeGO) and iron-doped mesoporous silica (FeMS). The emulsion solvent evaporation process was used to create the nanocarriers, which offer a nanoscale delivery platform with an excellent drug loading capacity and a desired release behavior. Comparing the two, FeMS nanoparticles showed a higher drug loading capacity of about 11% compared to FeGO. It was attributed to the existence of hollow mesoporous, which offer improved trapping capabilities and controlled drug release. The two nanocarriers' drug release is pH dependent, meaning that sorafenib releases more quickly in acidic environments. This type of targeted release pattern enhances the medications' site-specific targeting while lowering the risk of off-site effects. Both FeMS and FeGO were shown to be effective in delivering sorafenib and to have significant cytotoxic effects in HepG2 cells in vitro. Additionally, preliminary findings suggest a process of cell death driven by ferroptosis, which would be a crucial strategy for overcoming resistance brought on by conventional chemotherapy. The well-thought-out nanoparticles exhibited high drug entrapment, stable physicochemical characteristics, and therapeutic potential in a regulated environment. By showing that altering the size, surface chemistry, and porosity of nanoparticles can significantly improve drug delivery effectiveness and therapeutic benefit in liver cancer models, the study has been successful in achieving its goals. However, the investigation was carried out in vitro. More in-depth mechanistic analyses of ferroptosis-related gene and protein expression as well as assessments of the stability and long-term biocompatibility of these nanoparticles should be part of future studies. To further improve tumor-specific concentration, more optimization is suggested, such as ligand conjugation to enable targeted delivery. All things considered, this work highlights the potential of FeMS and FeGO as enhanced nanocarriers for anticancer medication delivery in the future and encourages greater research into more effective, individualized, and selective anticancer therapies.

This in vitro study proves that FeGO is a more effective and powerful nanocarrier than FeMS for sorafenib delivery in liver cancer cells. Its upregulated Ferroptosis induction, EMT-related gene regulation, and cellular uptake place it at the forefront as a hopeful agent for targeted liver cancer therapy. These results lay a firm groundwork for subsequent in vivo studies and justify the continued development of FeGO-based drug delivery systems for treating hepatocellular carcinoma.

# REFERENCES


1. Trichopoulos D, Li FP, Hunter DJ. What causes cancer? Scientific American. 1996;275(3):80-7.
2. Seyfried TN, Shelton LM. Cancer as a metabolic disease. Nutrition & metabolism. 2010;7:1-22.
3. Coller HA. Is cancer a metabolic disease? The American journal of pathology. 2014;184(1):4-17.
4. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat M-L, Gabbiani G. Biological Perspectives. The American Journal of Pathology. 2007;170(6).
5. Phillips JL, Currow DC. Cancer as a chronic disease. Collegian. 2010;17(2):47-50.
6. Harris C. Exploring why people decline cancer treatment: perspectives from patients and psychologists: Canterbury Christ Church University (United Kingdom); 2023.
7. Bosch FX, Ribes J, Díaz M, Cléries R. Primary liver cancer: worldwide incidence and trends. Gastroenterology. 2004;127(5):S5-S16.
8. El-Zahry MR, Ali MF. A novel polymer integrated Vertically-Oriented reduced graphene oxide sheets supported over palladium nanoparticles based sensor for Real-Time monitoring of Sorafenib; a Multi-kinase inhibitor in complex biological samples. Microchemical Journal. 2022;180:107549.
9. Wang L, Tong L, Xiong Z, Chen Y, Zhang P, Gao Y, et al. Ferroptosis-inducing nanomedicine and targeted short peptide for synergistic treatment of hepatocellular carcinoma. Journal of Nanobiotechnology. 2024;22(1):533.
10. Arzumanian VA, Kiseleva OI, Poverennaya EV. The curious case of the HepG2 cell line: 40 years of expertise. International journal of molecular sciences. 2021;22(23):13135.
11. Pechlivani L, Ntemou N, Pantazi D, Alivertis D, Skobridis K, Tselepis AD. Synthesis of novel nilotinib analogues and biological evaluation of their antiplatelet activity and functionality towards cancer cell proliferation in vitro. Pharmaceuticals. 2024;17(3):349.
12. Glebavičiūtė G, Vijaya AK, Preta G. Effect of Statin Lipophilicity on the Proliferation of Hepatocellular Carcinoma Cells. Biology. 2024;13(6):455.
13. Tang H, Chen D, Li C, Zheng C, Wu X, Zhang Y, et al. Dual GSH-exhausting sorafenib loaded manganese-silica nanodrugs for inducing the ferroptosis of hepatocellular carcinoma cells. International journal of pharmaceutics. 2019;572:118782.
14. Xu T, Ding W, Ji X, Ao X, Liu Y, Yu W, et al. Molecular mechanisms of ferroptosis and its role in cancer therapy. Journal of cellular and molecular medicine. 2019;23(8):4900-12.
15. Liang C, Zhang X, Yang M, Dong X. Recent progress in ferroptosis inducers for cancer therapy. Advanced materials. 2019;31(51):1904197.
16. Nie Q, Hu Y, Yu X, Li X, Fang X. Induction and application of ferroptosis in cancer therapy. Cancer cell international. 2022;22(1):12.
17. Wang Y, Wei Z, Pan K, Li J, Chen Q. The function and mechanism of ferroptosis in cancer. Apoptosis. 2020;25(11):786-98.

18. Qin S-Y, Zhang A-Q, Cheng S-X, Rong L, Zhang X-Z. Drug self-delivery systems for cancer therapy. Biomaterials. 2017;112:234-47.
19. Hossen S, Hossain MK, Basher M, Mia M, Rahman M, Uddin MJ. Smart nanocarrier-based drug delivery systems for cancer therapy and toxicity studies: A review. Journal of advanced research. 2019;15:1-18.
20. Khan MI, Hossain MI, Hossain MK, Rubel M, Hossain K, Mahfuz A, et al. Recent progress in nanostructured smart drug delivery systems for cancer therapy: a review. ACS applied bio materials. 2022;5(3):971-1012.
21. Ahuja G, Pathak K. Porous carriers for controlled/modulated drug delivery. Indian journal of pharmaceutical sciences. 2009;71(6):599.
22. Wu H, Wang M-D, Zhu J-Q, Li Z-L, Wang W-Y, Gu L-H, et al. Mesoporous nanoparticles for diagnosis and treatment of liver cancer in the era of precise medicine. Pharmaceutics. 2022;14(9):1760.
23. Kianfar E, Sayadi H. Recent advances in properties and applications of nanoporous materials and porous carbons. Carbon Letters. 2022;32(7):1645-69.
24. Saadh M, Mirzaei M, Dhiaa S, Hosseini LR, Kushakova G, Da'i M, et al. Density functional theory assessments of an iron-doped graphene platform towards the hydrea anticancer drug delivery. Diamond and Related Materials. 2024;141:110683.
25. Gu L, Vardarajan V, Koymen AR, Mohanty SK. Magnetic-field-assisted photothermal therapy of cancer cells using Fe-doped carbon nanoparticles. Journal of Biomedical Optics. 2012;17(1):018003-.
26. Kofroňová J, Jiříčková A, Jankovský O, Luxa J, Melliti A, Vurm R. Toxicity of iron-doped graphene Oxide: Towards eco-friendly carbon-based nanomaterials. FlatChem. 2024;47:100737.
27. Tasnim NT, Ferdous N, Rumon MMH, Shakil MS. The promise of metal-doped iron oxide nanoparticles as antimicrobial agent. Acs Omega. 2023;9(1):16-32.
28. Kievit FM, Zhang M. Surface engineering of iron oxide nanoparticles for targeted cancer therapy. Accounts of chemical research. 2011;44(10):853-62.
29. Gupta AK, Gupta M. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. biomaterials. 2005;26(18):3995-4021.
30. Zhi D, Yang T, Yang J, Fu S, Zhang S. Targeting strategies for superparamagnetic iron oxide nanoparticles in cancer therapy. Acta biomaterialia. 2020;102:13-34.
31. Peng X-H, Qian X, Mao H, Wang AY, Chen Z, Nie S, et al. Targeted magnetic iron oxide nanoparticles for tumor imaging and therapy. International journal of nanomedicine. 2008;3(3):311-21.
32. Dash BS, Lu Y-J, Chen H-A, Chuang C-C, Chen J-P. Magnetic and GRPR-targeted reduced graphene oxide/doxorubicin nanocomposite for dual-targeted chemo-photothermal cancer therapy. Materials Science and Engineering: C. 2021;128:112311.
33. Nagai H, Kim YH. Cancer prevention from the perspective of global cancer burden patterns. Journal of thoracic disease. 2017;9(3):448.
34. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA: a cancer journal for clinicians. 2019;69(1):7-34.

35. Abbasi M, Ghoran SH, Niakan MH, Jamali K, Moeini Z, Jangjou A, et al. Mesoporous silica nanoparticle: Heralding a brighter future in cancer nanomedicine. Microporous and Mesoporous Materials. 2021;319:110967.
36. Yang M, Ding H, Zhu Y, Ge Y, Li L. Co-delivery of paclitaxel and doxorubicin using mixed micelles based on the redox sensitive prodrugs. Colloids and Surfaces B: Biointerfaces. 2019;175:126-35.
37. Chen K, Chang C, Liu Z, Zhou Y, Xu Q, Li C, et al. Hyaluronic acid targeted and pH-responsive nanocarriers based on hollow mesoporous silica nanoparticles for chemo-photodynamic combination therapy. Colloids and Surfaces B: Biointerfaces. 2020;194:111166.
38. Zhang Q, Wang X, Li PZ, Nguyen KT, Wang XJ, Luo Z, et al. Biocompatible, uniform, and redispersible mesoporous silica nanoparticles for cancer-targeted drug delivery in vivo. Advanced Functional Materials. 2014;24(17):2450-61.
39. Thapa R, Ali H, Afzal O, Bhat AA, Almalki WH, Alzarea SI, et al. Unlocking the potential of mesoporous silica nanoparticles in breast cancer treatment. Journal of Nanoparticle Research. 2023;25(8):169.
40. Day GS, Drake HF, Zhou H-C, Ryder MR. Evolution of porous materials from ancient remedies to modern frameworks. Communications Chemistry. 2021;4(1):114.
41. Ling J, Wang BB, Li XN, Zhang R, Pan LF, Zhang H, et al. Multifunctional iron-doped hollow mesoporous silica nanoregulator for enhanced tumor chemodynamic therapy. Particle & Particle Systems Characterization. 2023;40(9):2300054.
42. Lérida-Viso A, Estepa-Fernández A, García-Fernández A, Martí-Centelles V, Martínez-Máñez R. Biosafety of mesoporous silica nanoparticles; towards clinical translation. Advanced Drug Delivery Reviews. 2023;201:115049.
43. He Y, Khaleed A, Lo PS, Ahmad I, Ching Ng AM, Djurišić AB. Iron-Based Oxygen Scavengers on Mesoporous Silica Nanospheres. ACS omega. 2023;8(24):21689-95.
44. Shariati M. The cancer therapy materialization by theranostic nanoparticles based on gold doped iron oxide under electromagnetic field amplification. Nanomedicine: Nanotechnology, Biology and Medicine. 2021;35:102406.
45. Hoseini-Ghahfarokhi M, Mirkiani S, Mozaffari N, Abdolahi Sadatlu MA, Ghasemi A, Abbaspour S, et al. Applications of graphene and graphene oxide in smart drug/gene delivery: is the world still flat? International Journal of Nanomedicine. 2020:9469-96.
46. Shewach DS, Kuchta RD. Introduction to cancer chemotherapeutics. ACS Publications; 2009. p. 2859-61.
47. Knudson AG. Antioncogenes and human cancer. Proceedings of the National Academy of Sciences. 1993;90(23):10914-21.
48. Bergerot CD, Philip EJ, Bergerot PG, Siddiq N, Tinianov S, Lustberg M, editors. Fear of cancer recurrence or progression: what is it and what can we do about it? American Society of Clinical Oncology educational book American Society of Clinical Oncology Annual Meeting; 2022.
49. Tanne JH. US drug regulator issues its first ever non-compliance notice for not posting trial results. BMJ: British Medical Journal (Online). 2021;373.

50. Ananthakrishnan A, Gogineni V, Saeian K, editors. Epidemiology of primary and secondary liver cancers. Seminars in interventional radiology; 2006: Copyright© 2006 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New ….
51. Feng M, Pan Y, Kong R, Shu S. Therapy of primary liver cancer. The Innovation. 2020;1(2).
52. Wáng Y-XJ, De Baere T, Idée J-M, Ballet S. Transcatheter embolization therapy in liver cancer: an update of clinical evidences. Chinese Journal of Cancer Research. 2015;27(2):96.
53. Schmidt L, Fischer K, Straub G, Rauch B, Paprottka PM, Göke B, et al. Addition of local hepatic therapy to sorafenib in patients with advanced hepatocellular carcinoma (stage BCLC C). Digestion. 2014;90(4):219-28.
54. Cho EC, Au L, Zhang Q, Xia Y. The effects of size, shape, and surface functional group of gold nanostructures on their adsorption and internalization by cells. Small. 2010;6(4):517-22.
55. Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. Engineering precision nanoparticles for drug delivery. Nature reviews drug discovery. 2021;20(2):101-24.
56. Edwards DA, Hanes J, Caponetti G, Hrkach J, Ben-Jebria A, Eskew ML, et al. Large porous particles for pulmonary drug delivery. Science. 1997;276(5320):1868-72.
57. Abdelaziz HM, Gaber M, Abd-Elwakil MM, Mabrouk MT, Elgohary MM, Kamel NM, et al. Inhalable particulate drug delivery systems for lung cancer therapy: Nanoparticles, microparticles, nanocomposites and nanoaggregates. Journal of Controlled Release. 2018;269:374-92.
58. Bayne L, Ulijn RV, Halling PJ. Effect of pore size on the performance of immobilised enzymes. Chemical Society Reviews. 2013;42(23):9000-10.
59. Rahimi-Gorji M, Van de Sande L, Debbaut C, Ghorbaniasl G, Braet H, Cosyns S, et al. Intraperitoneal aerosolized drug delivery: technology, recent developments, and future outlook. Advanced drug delivery reviews. 2020;160:105-14.
60. Liu Y, Zhang R, Tian Y-C, Cong H-L, Shen Y-Q, Yu B. Preparation and application of novel poly-tetrahydropyrimidine modified-mesoporous silica microspheres. Ferroelectrics. 2023;607(1):73-8.
61. Muis Y, Pakpahan J, Daulay A. Stability of mesoporous silica using ricinoleic methyl ester as a template with the addition of HCl and application of Cd 2+ adsorption optimized by Box–Behnken design. RSC advances. 2023;13(11):7329-38.
62. Tillman L, Voskanyan A, Navrotsky A. Synthesis of mesoporous silica using a mineral silica source. Journal of the American Ceramic Society. 2023;106(3):1993-9.
63. Meilita P, Aini S, Azra F, Zainul R. Effect of Hexane Addition on Mesoporous Silica Crystal Size Using Na2SiO3 from Chert Stone. Periodic. 2022;11(2):36-9.
64. Sagir T, Huysal M, Senel M, Isık S, Burgucu N, Tabakoglu O, et al. Folic acid conjugated PAMAM-modified mesoporous silica-coated superparamagnetic iron oxide nanoparticles for potential cancer therapy. Journal of Colloid and Interface Science. 2022;625:711-21.
65. Vaz-Ramos J, Cordeiro R, Castro MMC, Geraldes CF, Costa BF, Faneca H, et al. Supercritically dried superparamagnetic mesoporous silica nanoparticles for cancer theranostics. Materials Science and Engineering: C. 2020;115:111124.

66. Reczyńska K, Marszałek M, Zarzycki A, Reczyński W, Kornaus K, Pamuła E, et al. Superparamagnetic iron oxide nanoparticles modified with silica layers as potential agents for lung cancer treatment. Nanomaterials. 2020;10(6):1076.
67. Todea M, Simon V, Muresan-Pop M, Vulpoi A, Rusu M, Simion A, et al. Silica-based microspheres with aluminum-iron oxide shell for diagnosis and cancer treatment. Journal of Molecular Structure. 2021;1246:131149.
68. Lin C-H, Chen Y-C, Huang P-I. Preparation of multifunctional dopamine-coated zerovalent iron/reduced graphene oxide for targeted phototheragnosis in breast cancer. Nanomaterials. 2020;10(10):1957.
69. Gupta J, Prakash A, Jaiswal MK, Agarrwal A, Bahadur D. Superparamagnetic iron oxide-reduced graphene oxide nanohybrid-a vehicle for targeted drug delivery and hyperthermia treatment of cancer. Journal of Magnetism and Magnetic Materials. 2018;448:332-8.
70. Yadav N, Kannan D, Patil S, Singh S, Lochab B. Amplified activity of artesunate mediated by iron oxide nanoparticles loaded on a graphene oxide carrier for cancer therapeutics. ACS Applied Bio Materials. 2020;3(10):6722-36.
71. Tade RS, Patil PO. Theranostic prospects of graphene quantum dots in breast cancer. ACS Biomaterials Science & Engineering. 2020;6(11):5987-6008.
72. Dong X, Tan Y, Zhuang D, Hu T, Zhao M. Global characteristics and trends in research on ferroptosis: a data-driven bibliometric study. Oxidative medicine and cellular longevity. 2022;2022(1):8661864.
73. Zhou Q, Wu F, Zhao M, Yang M. Bibliometric Evaluation of 2012–2020 Publications on ferroptosis in cancer treatment. Frontiers in Cell and Developmental Biology. 2022;9:793347.
74. Miao M, Han Y, Wang Y, Yang Y, Zhu R, Sun M, et al. The research landscape of ferroptosis in the brain: a bibliometric analysis. Frontiers in Pharmacology. 2022;13:1014550.
75. Chen Y, Fan Z, Hu S, Lu C, Xiang Y, Liao S. Ferroptosis: A new strategy for cancer therapy. Frontiers in Oncology. 2022;12:830561.
76. Liu Y, Yang G, Jin S, Xu L, Zhao CX. Development of high-drug-loading nanoparticles. ChemPlusChem. 2020;85(9):2143-57.
77. Singh R, Lillard Jr JW. Nanoparticle-based targeted drug delivery. Experimental and molecular pathology. 2009;86(3):215-23.
78. Kheradmandnia S, Vasheghani-Farahani E, Nosrati M, Atyabi F. Preparation and characterization of ketoprofen-loaded solid lipid nanoparticles made from beeswax and carnauba wax. Nanomedicine: Nanotechnology, Biology and Medicine. 2010;6(6):753-9.
79. Huang P, Wang D, Su Y, Huang W, Zhou Y, Cui D, et al. Combination of small molecule prodrug and nanodrug delivery: amphiphilic drug–drug conjugate for cancer therapy. Journal of the American Chemical Society. 2014;136(33):11748-56.
80. Wu M, Wang Q, Liu X, Liu J. Highly efficient loading of doxorubicin in Prussian Blue nanocages for combined photothermal/chemotherapy against hepatocellular carcinoma. Rsc Advances. 2015;5(39):30970-80.
81. Shen S, Wu Y, Liu Y, Wu D. High drug-loading nanomedicines: progress, current status, and prospects. International journal of nanomedicine. 2017:4085-109.
82. Judefeind A, de Villiers MM. Drug loading into and in vitro release from nanosized drug delivery systems. Nanotechnology in drug delivery. 2009:129-62.

83. Caputo TM, Cusano AM, Principe S, Cicatiello P, Celetti G, Aliberti A, et al. Sorafenib-loaded PLGA carriers for enhanced drug delivery and cellular uptake in liver cancer cells. International Journal of Nanomedicine. 2023:4121-42.
84. Yasir AT, Benamor A, Ba-Abbad M, Hawari AH. Performance of polysulfone ultrafiltration membranes incorporating graphene oxide nanoparticles functionalized with different generations of poly (amido amine). Journal of Water Process Engineering. 2024;60:105095.
85. Pak WS, Ko KH, Ri JH, Ro JH. Magnetic Fe3O4/GO nanocomposite-modified graphite-epoxy composite electrode for high-performance glucose sensing. International Journal of Electrochemical Science. 2025;20(1):100918.
86. Coelho JV, Guedes MS, Prado RG, Tronto J, Ardisson JD, Pereira MC, et al. Effect of iron precursor on the Fenton-like activity of Fe2O3/mesoporous silica catalysts prepared under mild conditions. Applied Catalysis B: Environmental. 2014;144:792-9.
87. Mdlovu NV, Lin K-S, Mavuso FA, Weng M-T. Preparation, characterization, and in-vitro studies of doxorubicin-encapsulated silica coated iron oxide nanocomposites on liver cancer cells. Journal of the Taiwan Institute of Chemical Engineers. 2020;117:190-7.
88. Oh JY, Yang G, Choi E, Ryu J-H. Mesoporous silica nanoparticle-supported nanocarriers with enhanced drug loading, encapsulation stability, and targeting efficiency. Biomaterials Science. 2022;10(6):1448-55.
89. Han S, Teng F, Wang Y, Su L, Leng Q, Jiang H. Drug-loaded dual targeting graphene oxide-based molecularly imprinted composite and recognition of carcino-embryonic antigen. RSC advances. 2020;10(19):10980-8.
90. Gholami A, Habibi B, Hosseini SA, Matin AA, Samadi N. Controlled release of anticancer drugs via the magnetic magnesium iron nanoparticles modified by graphene oxide and polyvinyl alcohol: Paclitaxel and docetaxel. Nanomed J. 2021;8:200-10.
91. Tang S, Li Y. Sorafenib-loaded ligand-functionalized polymer-lipid hybrid nanoparticles for enhanced therapeutic effect against liver cancer. Journal of Nanoscience and Nanotechnology. 2019;19(11):6866-71.
92. Ganguly S, Dewanjee S, Sen R, Chattopadhyay D, Ganguly S, Gaonkar R, et al. Apigenin-loaded galactose tailored PLGA nanoparticles: A possible strategy for liver targeting to treat hepatocellular carcinoma. Colloids and Surfaces B: Biointerfaces. 2021;204:111778.
93. Kumar CS, Raja M, Sundar DS, Antoniraj MG, Ruckmani K. Hyaluronic acid co-functionalized gold nanoparticle complex for the targeted delivery of metformin in the treatment of liver cancer (HepG2 cells). Carbohydr Polym. 2015;128:63-74.
94. Barraud L, Merle P, Soma E, Lefrançois L, Guerret S, Chevallier M, et al. Increase of doxorubicin sensitivity by doxorubicin-loading into nanoparticles for hepatocellular carcinoma cells in vitro and in vivo. Journal of hepatology. 2005;42(5):736-43.
95. Fuchsova B, Juliá AA, Rizavi HS, Frasch AC, Pandey GN. Expression of p21-activated kinases 1 and 3 is altered in the brain of subjects with depression. Neuroscience. 2016;333:331-44.

96. Shang H, Xu A, Yan H, Xu D, Zhang J, Fang X. PIEZO2 promotes cell proliferation and metastasis in colon carcinoma through the SLIT2/ROBO1/VEGFC pathway. Advances in Clinical and Experimental Medicine. 2023;32(7):763-76.
97. Mdlovu NV, Lin K-S, Weng M-T, Hsieh C-C, Lin Y-S, Espinoza MJC. In vitro intracellular studies of pH and thermo-triggered doxorubicin conjugated magnetic SBA-15 mesoporous nanocarriers for anticancer activity against hepatocellular carcinoma. Journal of Industrial and Engineering Chemistry. 2021;102:1-16.
98. Kurnaz Yetim N, Kurşun Baysak F, Koç MM, Nartop D. Characterization of magnetic Fe3O4@ SiO2 nanoparticles with fluorescent properties for potential multipurpose imaging and theranostic applications. Journal of Materials Science: Materials in Electronics. 2020;31(20):18278-88.
99. Yang S, Zeng T, Li Y, Liu J, Chen Q, Zhou J, et al. Preparation of graphene oxide decorated Fe3O4@ SiO2 nanocomposites with superior adsorption capacity and SERS detection for organic dyes. Journal of Nanomaterials. 2015;2015(1):817924.
100. Arshad M, Abbas M, Ehtisham-ul-Haque S, Farrukh MA, Ali A, Rizvi H, et al. Synthesis and characterization of SiO2 doped Fe2O3 nanoparticles: Photocatalytic and antimicrobial activity evaluation. Journal of Molecular Structure. 2019;1180:244-50.
101. Damodaran SP. Novel Nanohybrid Containing Magnetite Nanocluster-Decorated Reduced Graphene Oxide Nanosheets for Heat Transfer Applications. ChemistrySelect. 2021;6(26):6698-706.
102. Donga C, Mishra SB, Ndlovu LN, Abd-El-Aziz AS, Kuvarega AT, Mishra AK. Magnetic magnetite-graphene oxide (Fe3O4-GO) nanocomposites for removal of dyes from aqueous solution. Journal of Inorganic and Organometallic Polymers and Materials. 2024;34(9):4192-202.
103. Rehman GU, Ismail A, Goh P, Arzhandi MR-D, Ismail N. Aptes and teos modified binary recyclable hybrid Fe3O4@ GO nanocomposite for photocatalytic dye removal. Jurnal Teknologi (Sciences & Engineering). 2018;80(4).
104. Geng J, Men Y, Liu C, Ge X, Yuan C. Preparation of rGO@ Fe 3 O 4 nanocomposite and its application to enhance the thermal conductivity of epoxy resin. RSC advances. 2021;11(27):16592-9.
105. Yaseen M, Khan A, Humayun M, Farooq S, Shah N, Bibi S, et al. Facile synthesis of Fe3O4− SiO2 nanocomposites for wastewater treatment. Macromolecular Materials and Engineering. 2023;308(7):2200695.
106. Kurnaz Yetim N, Hasanoğlu Özkan E. Synthesis of Au-doped magnetic nanocomposites: structural, magnetic, and catalytic properties. Journal of Materials Science: Materials in Electronics. 2021;32(20):24766-74.
107. da Costa Júnior AE, Mota JPF, Pontes SMA, Maia FJN, Clemente CdS, Fechine PBA, et al. A self-assembly of graphene oxide@ Fe 3 O 4/metallo-phthalocyanine nanohybrid materials: Synthesis, characterization, dielectric and thermal properties. Journal of Materials Science. 2017;52:9546-57.
108. Gemeay AH, Keshta BE, El-Sharkawy RG, Zaki AB. Chemical insight into the adsorption of reactive wool dyes onto amine-functionalized magnetite/silica core-shell from industrial wastewaters. Environmental Science and Pollution Research. 2020;27:32341-58.

109. Li Y, Lu H, Wang Y, Zhao Y, Li X. Efficient removal of methyl blue from aqueous solution by using poly (4-vinylpyridine)–graphene oxide–Fe3O4 magnetic nanocomposites. Journal of Materials Science. 2019;54(10):7603-16.
110. Han JS, An GS. Preparation of dual-layered core–shell Fe3O4@ SiO2 nanoparticles and their properties of plasmid DNA purification. Nanomaterials. 2021;11(12):3422.
111. Alosaimi EH, Azeem N, Tahir N, Jilani A, Zahid M, Alharthi SS, et al. Investigation of Fe-Doped Graphitic Carbon Nitride-Silver Tungstate as a Ternary Visible Light Active Photocatalyst. Journal of Chemistry. 2021;2021(1):4660423.
112. Mendez N, Liberman A, Corbeil J, Barback C, Viveros R, Wang J, et al. Assessment of in vivo systemic toxicity and biodistribution of iron-doped silica nanoshells. Nanomedicine: Nanotechnology, Biology and Medicine. 2017;13(3):933-42.
113. Chen J, Liu J, Hu Y, Tian Z, Zhu Y. Metal-organic framework-coated magnetite nanoparticles for synergistic magnetic hyperthermia and chemotherapy with pH-triggered drug release. Science and Technology of Advanced Materials. 2019;20(1):1043-54.
114. Huang Y, Hemmer E, Rosei F, Vetrone F. Multifunctional liposome nanocarriers combining upconverting nanoparticles and anticancer drugs. The Journal of Physical Chemistry B. 2016;120(22):4992-5001.
115. Zhang H, Zhang F-M, Yan S-J. Preparation, in vitro release, and pharmacokinetics in rabbits of lyophilized injection of sorafenib solid lipid nanoparticles. International journal of nanomedicine. 2012:2901-10.

1. Trichopoulos D, Li FP, Hunter DJ. What causes cancer? Scientific American. 1996;275(3):80-7.
2. Seyfried TN, Shelton LM. Cancer as a metabolic disease. Nutrition & metabolism. 2010;7:1-22.
3. Coller HA. Is cancer a metabolic disease? The American journal of pathology. 2014;184(1):4-17.
4. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat M-L, Gabbiani G. Biological Perspectives. The American Journal of Pathology. 2007;170(6).
5. Phillips JL, Currow DC. Cancer as a chronic disease. Collegian. 2010;17(2):47-50.
6. Harris C. Exploring why people decline cancer treatment: perspectives from patients and psychologists: Canterbury Christ Church University (United Kingdom); 2023.
7. Bosch FX, Ribes J, Díaz M, Cléries R. Primary liver cancer: worldwide incidence and trends. Gastroenterology. 2004;127(5):S5-S16.
8. El-Zahry MR, Ali MF. A novel polymer integrated Vertically-Oriented reduced graphene oxide sheets supported over palladium nanoparticles based sensor for Real-Time monitoring of Sorafenib; a Multi-kinase inhibitor in complex biological samples. Microchemical Journal. 2022;180:107549.
9. Wang L, Tong L, Xiong Z, Chen Y, Zhang P, Gao Y, et al. Ferroptosis-inducing nanomedicine and targeted short peptide for synergistic treatment of hepatocellular carcinoma. Journal of Nanobiotechnology. 2024;22(1):533.
10. Arzumanian VA, Kiseleva OI, Poverennaya EV. The curious case of the HepG2 cell line: 40 years of expertise. International journal of molecular sciences. 2021;22(23):13135.

11. Pechlivani L, Ntemou N, Pantazi D, Alivertis D, Skobridis K, Tselepis AD. Synthesis of novel nilotinib analogues and biological evaluation of their antiplatelet activity and functionality towards cancer cell proliferation in vitro. Pharmaceuticals. 2024;17(3):349.
12. Glebavičiūtė G, Vijaya AK, Preta G. Effect of Statin Lipophilicity on the Proliferation of Hepatocellular Carcinoma Cells. Biology. 2024;13(6):455.
13. Tang H, Chen D, Li C, Zheng C, Wu X, Zhang Y, et al. Dual GSH-exhausting sorafenib loaded manganese-silica nanodrugs for inducing the ferroptosis of hepatocellular carcinoma cells. International journal of pharmaceutics. 2019;572:118782.
14. Xu T, Ding W, Ji X, Ao X, Liu Y, Yu W, et al. Molecular mechanisms of ferroptosis and its role in cancer therapy. Journal of cellular and molecular medicine. 2019;23(8):4900-12.
15. Liang C, Zhang X, Yang M, Dong X. Recent progress in ferroptosis inducers for cancer therapy. Advanced materials. 2019;31(51):1904197.
16. Nie Q, Hu Y, Yu X, Li X, Fang X. Induction and application of ferroptosis in cancer therapy. Cancer cell international. 2022;22(1):12.
17. Wang Y, Wei Z, Pan K, Li J, Chen Q. The function and mechanism of ferroptosis in cancer. Apoptosis. 2020;25(11):786-98.
18. Qin S-Y, Zhang A-Q, Cheng S-X, Rong L, Zhang X-Z. Drug self-delivery systems for cancer therapy. Biomaterials. 2017;112:234-47.
19. Hossen S, Hossain MK, Basher M, Mia M, Rahman M, Uddin MJ. Smart nanocarrier-based drug delivery systems for cancer therapy and toxicity studies: A review. Journal of advanced research. 2019;15:1-18.
20. Khan MI, Hossain MI, Hossain MK, Rubel M, Hossain K, Mahfuz A, et al. Recent progress in nanostructured smart drug delivery systems for cancer therapy: a review. ACS applied bio materials. 2022;5(3):971-1012.
21. Ahuja G, Pathak K. Porous carriers for controlled/modulated drug delivery. Indian journal of pharmaceutical sciences. 2009;71(6):599.
22. Wu H, Wang M-D, Zhu J-Q, Li Z-L, Wang W-Y, Gu L-H, et al. Mesoporous nanoparticles for diagnosis and treatment of liver cancer in the era of precise medicine. Pharmaceutics. 2022;14(9):1760.
23. Kianfar E, Sayadi H. Recent advances in properties and applications of nanoporous materials and porous carbons. Carbon Letters. 2022;32(7):1645-69.
24. Saadh M, Mirzaei M, Dhiaa S, Hosseini LR, Kushakova G, Da'i M, et al. Density functional theory assessments of an iron-doped graphene platform towards the hydrea anticancer drug delivery. Diamond and Related Materials. 2024;141:110683.
25. Gu L, Vardarajan V, Koymen AR, Mohanty SK. Magnetic-field-assisted photothermal therapy of cancer cells using Fe-doped carbon nanoparticles. Journal of Biomedical Optics. 2012;17(1):018003-.
26. Kofroňová J, Jiříčková A, Jankovský O, Luxa J, Melliti A, Vurm R. Toxicity of iron-doped graphene Oxide: Towards eco-friendly carbon-based nanomaterials. FlatChem. 2024;47:100737.
27. Tasnim NT, Ferdous N, Rumon MMH, Shakil MS. The promise of metal-doped iron oxide nanoparticles as antimicrobial agent. Acs Omega. 2023;9(1):16-32.
28. Kievit FM, Zhang M. Surface engineering of iron oxide nanoparticles for targeted cancer therapy. Accounts of chemical research. 2011;44(10):853-62.
29. Gupta AK, Gupta M. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. biomaterials. 2005;26(18):3995-4021.
30. Zhi D, Yang T, Yang J, Fu S, Zhang S. Targeting strategies for superparamagnetic iron oxide nanoparticles in cancer therapy. Acta biomaterialia. 2020;102:13-34.

31. Peng X-H, Qian X, Mao H, Wang AY, Chen Z, Nie S, et al. Targeted magnetic iron oxide nanoparticles for tumor imaging and therapy. International journal of nanomedicine. 2008;3(3):311-21.
32. Dash BS, Lu Y-J, Chen H-A, Chuang C-C, Chen J-P. Magnetic and GRPR-targeted reduced graphene oxide/doxorubicin nanocomposite for dual-targeted chemo-photothermal cancer therapy. Materials Science and Engineering: C. 2021;128:112311.
33. Nagai H, Kim YH. Cancer prevention from the perspective of global cancer burden patterns. Journal of thoracic disease. 2017;9(3):448.
34. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA: a cancer journal for clinicians. 2019;69(1):7-34.
35. Abbasi M, Ghoran SH, Niakan MH, Jamali K, Moeini Z, Jangjou A, et al. Mesoporous silica nanoparticle: Heralding a brighter future in cancer nanomedicine. Microporous and Mesoporous Materials. 2021;319:110967.
36. Yang M, Ding H, Zhu Y, Ge Y, Li L. Co-delivery of paclitaxel and doxorubicin using mixed micelles based on the redox sensitive prodrugs. Colloids and Surfaces B: Biointerfaces. 2019;175:126-35.
37. Chen K, Chang C, Liu Z, Zhou Y, Xu Q, Li C, et al. Hyaluronic acid targeted and pH-responsive nanocarriers based on hollow mesoporous silica nanoparticles for chemo-photodynamic combination therapy. Colloids and Surfaces B: Biointerfaces. 2020;194:111166.
38. Zhang Q, Wang X, Li PZ, Nguyen KT, Wang XJ, Luo Z, et al. Biocompatible, uniform, and redispersible mesoporous silica nanoparticles for cancer-targeted drug delivery in vivo. Advanced Functional Materials. 2014;24(17):2450-61.
39. Thapa R, Ali H, Afzal O, Bhat AA, Almalki WH, Alzarea SI, et al. Unlocking the potential of mesoporous silica nanoparticles in breast cancer treatment. Journal of Nanoparticle Research. 2023;25(8):169.
40. Day GS, Drake HF, Zhou H-C, Ryder MR. Evolution of porous materials from ancient remedies to modern frameworks. Communications Chemistry. 2021;4(1):114.
41. Ling J, Wang BB, Li XN, Zhang R, Pan LF, Zhang H, et al. Multifunctional iron-doped hollow mesoporous silica nanoregulator for enhanced tumor chemodynamic therapy. Particle & Particle Systems Characterization. 2023;40(9):2300054.
42. Lérida-Viso A, Estepa-Fernández A, García-Fernández A, Martí-Centelles V, Martínez-Máñez R. Biosafety of mesoporous silica nanoparticles; towards clinical translation. Advanced Drug Delivery Reviews. 2023;201:115049.
43. He Y, Khaleed A, Lo PS, Ahmad I, Ching Ng AM, Djurišić AB. Iron-Based Oxygen Scavengers on Mesoporous Silica Nanospheres. ACS omega. 2023;8(24):21689-95.
44. Shariati M. The cancer therapy materialization by theranostic nanoparticles based on gold doped iron oxide under electromagnetic field amplification. Nanomedicine: Nanotechnology, Biology and Medicine. 2021;35:102406.
45. Hoseini-Ghahfarokhi M, Mirkiani S, Mozaffari N, Abdolahi Sadatlu MA, Ghasemi A, Abbaspour S, et al. Applications of graphene and graphene oxide in smart drug/gene delivery: is the world still flat? International Journal of Nanomedicine. 2020:9469-96.
46. Shewach DS, Kuchta RD. Introduction to cancer chemotherapeutics. ACS Publications; 2009. p. 2859-61.
47. Knudson AG. Antioncogenes and human cancer. Proceedings of the National Academy of Sciences. 1993;90(23):10914-21.
48. Bergerot CD, Philip EJ, Bergerot PG, Siddiq N, Tinianov S, Lustberg M, editors. Fear of cancer recurrence or progression: what is it and what can we do about it? American

Society of Clinical Oncology educational book American Society of Clinical Oncology Annual Meeting; 2022.
49. Tanne JH. US drug regulator issues its first ever non-compliance notice for not posting trial results. BMJ: British Medical Journal (Online). 2021;373.
50. Ananthakrishnan A, Gogineni V, Saeian K, editors. Epidemiology of primary and secondary liver cancers. Seminars in interventional radiology; 2006: Copyright© 2006 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New ….
51. Feng M, Pan Y, Kong R, Shu S. Therapy of primary liver cancer. The Innovation. 2020;1(2).
52. Wáng Y-XJ, De Baere T, Idée J-M, Ballet S. Transcatheter embolization therapy in liver cancer: an update of clinical evidences. Chinese Journal of Cancer Research. 2015;27(2):96.
53. Schmidt L, Fischer K, Straub G, Rauch B, Paprottka PM, Göke B, et al. Addition of local hepatic therapy to sorafenib in patients with advanced hepatocellular carcinoma (stage BCLC C). Digestion. 2014;90(4):219-28.
54. Cho EC, Au L, Zhang Q, Xia Y. The effects of size, shape, and surface functional group of gold nanostructures on their adsorption and internalization by cells. Small. 2010;6(4):517-22.
55. Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. Engineering precision nanoparticles for drug delivery. Nature reviews drug discovery. 2021;20(2):101-24.
56. Edwards DA, Hanes J, Caponetti G, Hrkach J, Ben-Jebria A, Eskew ML, et al. Large porous particles for pulmonary drug delivery. Science. 1997;276(5320):1868-72.
57. Abdelaziz HM, Gaber M, Abd-Elwakil MM, Mabrouk MT, Elgohary MM, Kamel NM, et al. Inhalable particulate drug delivery systems for lung cancer therapy: Nanoparticles, microparticles, nanocomposites and nanoaggregates. Journal of Controlled Release. 2018;269:374-92.
58. Bayne L, Ulijn RV, Halling PJ. Effect of pore size on the performance of immobilised enzymes. Chemical Society Reviews. 2013;42(23):9000-10.
59. Rahimi-Gorji M, Van de Sande L, Debbaut C, Ghorbaniasl G, Braet H, Cosyns S, et al. Intraperitoneal aerosolized drug delivery: technology, recent developments, and future outlook. Advanced drug delivery reviews. 2020;160:105-14.
60. Liu Y, Zhang R, Tian Y-C, Cong H-L, Shen Y-Q, Yu B. Preparation and application of novel poly-tetrahydropyrimidine modified-mesoporous silica microspheres. Ferroelectrics. 2023;607(1):73-8.
61. Muis Y, Pakpahan J, Daulay A. Stability of mesoporous silica using ricinoleic methyl ester as a template with the addition of HCl and application of Cd 2+ adsorption optimized by Box–Behnken design. RSC advances. 2023;13(11):7329-38.
62. Tillman L, Voskanyan A, Navrotsky A. Synthesis of mesoporous silica using a mineral silica source. Journal of the American Ceramic Society. 2023;106(3):1993-9.
63. Meilita P, Aini S, Azra F, Zainul R. Effect of Hexane Addition on Mesoporous Silica Crystal Size Using Na2SiO3 from Chert Stone. Periodic. 2022;11(2):36-9.
64. Sagir T, Huysal M, Senel M, Isık S, Burgucu N, Tabakoglu O, et al. Folic acid conjugated PAMAM-modified mesoporous silica-coated superparamagnetic iron oxide nanoparticles for potential cancer therapy. Journal of Colloid and Interface Science. 2022;625:711-21.
65. Vaz-Ramos J, Cordeiro R, Castro MMC, Geraldes CF, Costa BF, Faneca H, et al. Supercritically dried superparamagnetic mesoporous silica nanoparticles for cancer theranostics. Materials Science and Engineering: C. 2020;115:111124.

66. Reczyńska K, Marszałek M, Zarzycki A, Reczyński W, Kornaus K, Pamuła E, et al. Superparamagnetic iron oxide nanoparticles modified with silica layers as potential agents for lung cancer treatment. Nanomaterials. 2020;10(6):1076.
67. Todea M, Simon V, Muresan-Pop M, Vulpoi A, Rusu M, Simion A, et al. Silica-based microspheres with aluminum-iron oxide shell for diagnosis and cancer treatment. Journal of Molecular Structure. 2021;1246:131149.
68. Lin C-H, Chen Y-C, Huang P-I. Preparation of multifunctional dopamine-coated zerovalent iron/reduced graphene oxide for targeted photothera gnosis in breast cancer. Nanomaterials. 2020;10(10):1957.
69. Gupta J, Prakash A, Jaiswal MK, Agarrwal A, Bahadur D. Superparamagnetic iron oxide-reduced graphene oxide nanohybrid-a vehicle for targeted drug delivery and hyperthermia treatment of cancer. Journal of Magnetism and Magnetic Materials. 2018;448:332-8.
70. Yadav N, Kannan D, Patil S, Singh S, Lochab B. Amplified activity of artesunate mediated by iron oxide nanoparticles loaded on a graphene oxide carrier for cancer therapeutics. ACS Applied Bio Materials. 2020;3(10):6722-36.
71. Tade RS, Patil PO. Theranostic prospects of graphene quantum dots in breast cancer. ACS Biomaterials Science & Engineering. 2020;6(11):5987-6008.
72. Dong X, Tan Y, Zhuang D, Hu T, Zhao M. Global characteristics and trends in research on ferroptosis: a data-driven bibliometric study. Oxidative medicine and cellular longevity. 2022;2022(1):8661864.
73. Zhou Q, Wu F, Zhao M, Yang M. Bibliometric Evaluation of 2012–2020 Publications on ferroptosis in cancer treatment. Frontiers in Cell and Developmental Biology. 2022;9:793347.
74. Miao M, Han Y, Wang Y, Yang Y, Zhu R, Sun M, et al. The research landscape of ferroptosis in the brain: a bibliometric analysis. Frontiers in Pharmacology. 2022;13:1014550.
75. Chen Y, Fan Z, Hu S, Lu C, Xiang Y, Liao S. Ferroptosis: A new strategy for cancer therapy. Frontiers in Oncology. 2022;12:830561.
76. Liu Y, Yang G, Jin S, Xu L, Zhao CX. Development of high-drug-loading nanoparticles. ChemPlusChem. 2020;85(9):2143-57.
77. Singh R, Lillard Jr JW. Nanoparticle-based targeted drug delivery. Experimental and molecular pathology. 2009;86(3):215-23.
78. Kheradmandnia S, Vasheghani-Farahani E, Nosrati M, Atyabi F. Preparation and characterization of ketoprofen-loaded solid lipid nanoparticles made from beeswax and carnauba wax. Nanomedicine: Nanotechnology, Biology and Medicine. 2010;6(6):753-9.
79. Huang P, Wang D, Su Y, Huang W, Zhou Y, Cui D, et al. Combination of small molecule prodrug and nanodrug delivery: amphiphilic drug–drug conjugate for cancer therapy. Journal of the American Chemical Society. 2014;136(33):11748-56.
80. Wu M, Wang Q, Liu X, Liu J. Highly efficient loading of doxorubicin in Prussian Blue nanocages for combined photothermal/chemotherapy against hepatocellular carcinoma. Rsc Advances. 2015;5(39):30970-80.
81. Shen S, Wu Y, Liu Y, Wu D. High drug-loading nanomedicines: progress, current status, and prospects. International journal of nanomedicine. 2017:4085-109.
82. Judefeind A, de Villiers MM. Drug loading into and in vitro release from nanosized drug delivery systems. Nanotechnology in drug delivery. 2009:129-62.
83. Caputo TM, Cusano AM, Principe S, Cicatiello P, Celetti G, Aliberti A, et al. Sorafenib-loaded PLGA carriers for enhanced drug delivery and cellular uptake in liver cancer cells. International Journal of Nanomedicine. 2023:4121-42.

84. Yasir AT, Benamor A, Ba-Abbad M, Hawari AH. Performance of polysulfone ultrafiltration membranes incorporating graphene oxide nanoparticles functionalized with different generations of poly (amido amine). Journal of Water Process Engineering. 2024;60:105095.
85. Pak WS, Ko KH, Ri JH, Ro JH. Magnetic Fe3O4/GO nanocomposite-modified graphite-epoxy composite electrode for high-performance glucose sensing. International Journal of Electrochemical Science. 2025;20(1):100918.
86. Coelho JV, Guedes MS, Prado RG, Tronto J, Ardisson JD, Pereira MC, et al. Effect of iron precursor on the Fenton-like activity of Fe2O3/mesoporous silica catalysts prepared under mild conditions. Applied Catalysis B: Environmental. 2014;144:792-9.
87. Mdlovu NV, Lin K-S, Mavuso FA, Weng M-T. Preparation, characterization, and in-vitro studies of doxorubicin-encapsulated silica coated iron oxide nanocomposites on liver cancer cells. Journal of the Taiwan Institute of Chemical Engineers. 2020;117:190-7.
88. Oh JY, Yang G, Choi E, Ryu J-H. Mesoporous silica nanoparticle-supported nanocarriers with enhanced drug loading, encapsulation stability, and targeting efficiency. Biomaterials Science. 2022;10(6):1448-55.
89. Han S, Teng F, Wang Y, Su L, Leng Q, Jiang H. Drug-loaded dual targeting graphene oxide-based molecularly imprinted composite and recognition of carcino-embryonic antigen. RSC advances. 2020;10(19):10980-8.
90. Gholami A, Habibi B, Hosseini SA, Matin AA, Samadi N. Controlled release of anticancer drugs via the magnetic magnesium iron nanoparticles modified by graphene oxide and polyvinyl alcohol: Paclitaxel and docetaxel. Nanomed J. 2021;8:200-10.
91. Tang S, Li Y. Sorafenib-loaded ligand-functionalized polymer-lipid hybrid nanoparticles for enhanced therapeutic effect against liver cancer. Journal of Nanoscience and Nanotechnology. 2019;19(11):6866-71.
92. Ganguly S, Dewanjee S, Sen R, Chattopadhyay D, Ganguly S, Gaonkar R, et al. Apigenin-loaded galactose tailored PLGA nanoparticles: A possible strategy for liver targeting to treat hepatocellular carcinoma. Colloids and Surfaces B: Biointerfaces. 2021;204:111778.
93. Kumar CS, Raja M, Sundar DS, Antoniraj MG, Ruckmani K. Hyaluronic acid co-functionalized gold nanoparticle complex for the targeted delivery of metformin in the treatment of liver cancer (HepG2 cells). Carbohydr Polym. 2015;128:63-74.
94. Barraud L, Merle P, Soma E, Lefrançois L, Guerret S, Chevallier M, et al. Increase of doxorubicin sensitivity by doxorubicin-loading into nanoparticles for hepatocellular carcinoma cells in vitro and in vivo. Journal of hepatology. 2005;42(5):736-43.
95. Mdlovu NV, Lin K-S, Weng M-T, Hsieh C-C, Lin Y-S, Espinoza MJC. In vitro intracellular studies of pH and thermo-triggered doxorubicin conjugated magnetic SBA-15 mesoporous nanocarriers for anticancer activity against hepatocellular carcinoma. Journal of Industrial and Engineering Chemistry. 2021;102:1-16.
96. Kurnaz Yetim N, Kurşun Baysak F, Koç MM, Nartop D. Characterization of magnetic Fe3O4@ SiO2 nanoparticles with fluorescent properties for potential multipurpose imaging and theranostic applications. Journal of Materials Science: Materials in Electronics. 2020;31(20):18278-88.
97. Yang S, Zeng T, Li Y, Liu J, Chen Q, Zhou J, et al. Preparation of graphene oxide decorated Fe3O4@ SiO2 nanocomposites with superior adsorption capacity and SERS detection for organic dyes. Journal of Nanomaterials. 2015;2015(1):817924.
98. Arshad M, Abbas M, Ehtisham-ul-Haque S, Farrukh MA, Ali A, Rizvi H, et al. Synthesis and characterization of SiO2 doped Fe2O3 nanoparticles: Photocatalytic and antimicrobial activity evaluation. Journal of Molecular Structure. 2019;1180:244-50.

99. Damodaran SP. Novel Nanohybrid Containing Magnetite Nanocluster-Decorated Reduced Graphene Oxide Nanosheets for Heat Transfer Applications. ChemistrySelect. 2021;6(26):6698-706.
100. Donga C, Mishra SB, Ndlovu LN, Abd-El-Aziz AS, Kuvarega AT, Mishra AK. Magnetic magnetite-graphene oxide (Fe3O4-GO) nanocomposites for removal of dyes from aqueous solution. Journal of Inorganic and Organometallic Polymers and Materials. 2024;34(9):4192-202.
101. Rehman GU, Ismail A, Goh P, Arzhandi MR-D, Ismail N. Aptes and teos modified binary recyclable hybrid Fe3O4@ GO nanocomposite for photocatalytic dye removal. Jurnal Teknologi (Sciences & Engineering). 2018;80(4).
102. Geng J, Men Y, Liu C, Ge X, Yuan C. Preparation of rGO@ Fe 3 O 4 nanocomposite and its application to enhance the thermal conductivity of epoxy resin. RSC advances. 2021;11(27):16592-9.
103. Yaseen M, Khan A, Humayun M, Farooq S, Shah N, Bibi S, et al. Facile synthesis of Fe3O4− SiO2 nanocomposites for wastewater treatment. Macromolecular Materials and Engineering. 2023;308(7):2200695.
104. Kurnaz Yetim N, Hasanoğlu Özkan E. Synthesis of Au-doped magnetic nanocomposites: structural, magnetic, and catalytic properties. Journal of Materials Science: Materials in Electronics. 2021;32(20):24766-74.
105. da Costa Júnior AE, Mota JPF, Pontes SMA, Maia FJN, Clemente CdS, Fechine PBA, et al. A self-assembly of graphene oxide@ Fe 3 O 4/metallo-phthalocyanine nanohybrid materials: Synthesis, characterization, dielectric and thermal properties. Journal of Materials Science. 2017;52:9546-57.
106. Gemeay AH, Keshta BE, El-Sharkawy RG, Zaki AB. Chemical insight into the adsorption of reactive wool dyes onto amine-functionalized magnetite/silica core-shell from industrial wastewaters. Environmental Science and Pollution Research. 2020;27:32341-58.
107. Li Y, Lu H, Wang Y, Zhao Y, Li X. Efficient removal of methyl blue from aqueous solution by using poly (4-vinylpyridine)–graphene oxide–Fe3O4 magnetic nanocomposites. Journal of Materials Science. 2019;54(10):7603-16.
108. Han JS, An GS. Preparation of dual-layered core–shell Fe3O4@ SiO2 nanoparticles and their properties of plasmid DNA purification. Nanomaterials. 2021;11(12):3422.
109. Alosaimi EH, Azeem N, Tahir N, Jilani A, Zahid M, Alharthi SS, et al. Investigation of Fe-Doped Graphitic Carbon Nitride-Silver Tungstate as a Ternary Visible Light Active Photocatalyst. Journal of Chemistry. 2021;2021(1):4660423.
110. Mendez N, Liberman A, Corbeil J, Barback C, Viveros R, Wang J, et al. Assessment of in vivo systemic toxicity and biodistribution of iron-doped silica nanoshells. Nanomedicine: Nanotechnology, Biology and Medicine. 2017;13(3):933-42.
111. Chen J, Liu J, Hu Y, Tian Z, Zhu Y. Metal-organic framework-coated magnetite nanoparticles for synergistic magnetic hyperthermia and chemotherapy with pH-triggered drug release. Science and Technology of Advanced Materials. 2019;20(1):1043-54.
112. Huang Y, Hemmer E, Rosei F, Vetrone F. Multifunctional liposome nanocarriers combining upconverting nanoparticles and anticancer drugs. The Journal of Physical Chemistry B. 2016;120(22):4992-5001.
113. Zhang H, Zhang F-M, Yan S-J. Preparation, in vitro release, and pharmacokinetics in rabbits of lyophilized injection of sorafenib solid lipid nanoparticles. International journal of nanomedicine. 2012:2901-10.

114. Senthilraja P, Kathiresan K. In vitro cytotoxicity MTT assay in Vero, HepG2 and MCF-7 cell lines study of Marine Yeast. Journal of applied pharmaceutical science. 2015;5(3):080-4.
115. Mdlovu NB, Lin K-S, Weng M-T, Mdlovu NV. Formulation and in-vitro evaluations of doxorubicin loaded polymerized magnetic nanocarriers for liver cancer cells. Journal of the Taiwan Institute of Chemical Engineers. 2021;126:278-87.

# Report:Development Of Nanoporous Drug Carriers For Ferroptosis Induced Cancer Therapy (Abu Sufyan)

ORIGINALITY REPORT

| 9% | 6% | 6% | 2% |
|---|---|---|---|
| SIMILARITY INDEX | INTERNET SOURCES | PUBLICATIONS | STUDENT PAPERS |

PRIMARY SOURCES

| | | |
|---|---|---|
| 1 | ro.ecu.edu.au<br>Internet Source | 1% |
| 2 | link.springer.com<br>Internet Source | 1% |
| 3 | researcherslinks.com<br>Internet Source | 1% |
| 4 | Ndumiso Vukile Mdlovu, Kuen-Song Lin, Fikile Agath Mavuso, Meng-Tzu Weng. "Preparation, characterization, and in-vitro studies of doxorubicin-encapsulated silica coated iron oxide nanocomposites on liver cancer cells", Journal of the Taiwan Institute of Chemical Engineers, 2020<br>Publication | 1% |
| 5 | Shuang Han, Fu Teng, Yuan Wang, Liqiang Su, Qiuxue Leng, Haiyan Jiang. "Drug-loaded dual targeting graphene oxide-based molecularly imprinted composite and recognition of carcino-embryonic antigen", RSC Advances, 2020<br>Publication | <1% |
| 6 | Yun Liu, Guangze Yang, Song Jin, Letao Xu, Chun-Xia Zhao. "Development of High-Drug-Loading Nanoparticles", ChemPlusChem, 2020<br>Publication | <1% |