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SYNTHESIS AND EVALUATION OF NANO PARTICLES FOR ANTICANCER APPLICATION

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Master's Thesis

Supervisor

Asst. Prof. Dr. Arkan Yashar BARBAR

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The thesis titled SYNTHESIS AND EVALUATION OF NANO PARTICLES FOR ANTICANCER APPLICATION, prepared by Shaden A. A. SHAHWAN and submitted on 09/12/ 2025, has been **accepted unanimously** for the degree of Master of Science in Biomedical Science.

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I hereby declare that all information/data presented in this graduation project has been obtained in full accordance with academic rules and ethical conduct. I also declare all unoriginal materials and conclusions have been cited in the text and all references mentioned in the Reference List have been cited in the text, and vice versa as required by the abovementioned rules and conduct.

Shaden A A SHAHWAN

Signature

DEDICATION

First and foremost, all praise and gratitude are due to God for His guidance, mercy, and strength that made the completion of this work possible. To the land of resilience, dignity, and unwavering spirit, my homeland Palestine, to the souls of all martyrs, who departed this world yet remain alive in memory and conscience. to my parents and my brother, whose unwavering support, encouragement, and belief in me have been my greatest strength throughout my academic journey. Their sacrifices and constant motivation made this achievement possible. to my friends, whose kindness, understanding, and sincere support lightened the weight of this journey. Their presence, words of encouragement, and belief in me made even the most challenging moments easier and more meaningful.

PREFACE

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The experimental analyses presented in this thesis were carried out in collaboration with Yıldız Teknik University. The FESEM, XRD, and MTT assays were performed at the research laboratories of Yıldız Technical University to support the characterization and biological evaluation of the synthesized nanoparticles. The institution's cooperation and technical assistance are sincerely acknowledged and greatly appreciated.

ABSTRACT

SYNTHESIS AND EVALUATION OF NANO PARTICLES FOR ANTICANCER APPLICATION

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Nanotechnology-based drug delivery systems have emerged as promising tools in cancer therapy, offering improved drug targeting, reduced systemic toxicity, and enhanced therapeutic efficiency. Among various nanomaterials, iron oxide nanoparticles (IONPs) are widely recognized for their biocompatibility, magnetic responsiveness, and surface modification potential. In this study, quercetin-loaded iron oxide nanoparticles (Q-IONPs) were synthesized and evaluated for their physicochemical properties and cytotoxicity against the MCF-7 breast cancer cell line. The nanoparticles were prepared using the co-precipitation method by mixing ferrous sulfate ($FeSO_4$) and ferric chloride ($FeCl_3$), followed by the addition of sodium hydroxide (NaOH) to form iron oxide nanoparticles. Quercetin was subsequently incorporated to enhance the formulation's therapeutic efficacy. Particle size analysis revealed an average hydrodynamic diameter of 394.2 ± 6.5 nm, confirming the formation of nanosized particles. Preliminary pH monitoring suggested good stability of the nanoparticles under near-physiological conditions. The cytotoxicity of Q-IONPs will be further assessed against MCF-7 cells using the MTT assay to evaluate their potential as anticancer agents. The outcome of this research is expected to provide valuable insights into the development of quercetin-based iron oxide nanoparticles as a promising nanocarrier system for targeted cancer therapy.

Keywords: Quercetin, Iron Oxide Nanoparticles, MCF-7, Nanocarrier, Cancer Therapy.

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ABBREVIATIONS

DLS	:	Dynamic Light Scattering
FESEM	:	Field Emission Scanning Electron Microscopy
FTIR	:	Fourier Transform Infrared
IONPs	:	Iron Oxide Nanoparticles
NPs	:	Nanoparticles
QIONPs	:	Quercetin Iron Oxide Nanoparticles
UV-Vis	:	Ultraviolet-visible light
XRD	:	X-Ray Diffraction

LIST OF SYMBOLS

ζ : Zeta Potential

λ : Wavelength

ω : Angular Frequency

1. INTRODUCTION

1.1 CANCER AS A GLOBAL HEALTH PROBLEM

1.1.1 Global Prevalence and Mortality Rates

Cancer remains a major global health problem, with approximately 20 million new diagnoses (including non-melanoma skin cancer) and approximately 9.7 million related deaths recorded in 2022. Current estimates suggest that approximately one in five people will be diagnosed with cancer in their lifetime, and approximately one in nine men and one in twelve women will die from the disease (Bray et al., 2024).

1.1.2 Economic and Social Burden of Cancer

The financial impact on households of most cancer sufferers and society is significant (Cheng et al., 2021), Between 2020 and 2050, the global economic burden of most cancers is projected to reach $25.2 trillion (in 2017 worldwide dollar price), representing an annual economic burden equal to 0.55% of the global gross domestic product (Zafar et al., 2025).

1.1.3 Major Types and Why Treatment Is Challenging

In 2022, lung cancers ranked as one of the most diagnosed cancers, with about 2.5 million new cases, or 12.4% of all cancers recognized globally, equivalent to 1 in 8 cases. Followed by breast cancer in women (11.6%), colon cancer (9.6%), prostate cancer (7.3%), and stomach cancer (4.9%). In terms of mortality, lung cancer remained the leading cause of cancer deaths, contributing to at least 1.8 million deaths (18.7%), followed by colorectal (9.3%), liver (7.8%), breast (6.9%), and stomach (6.8%) cancers (Chen et al., 2023). Although many treatment techniques along with surgical techniques, chemotherapy, radiotherapy, and hormonal treatments have advanced over the years, their effectiveness continues to be confined. Surgery frequently fails to ensure complete tumour resection because of tumour heterogeneity, chemotherapy suffers from toxicity and resistance, radiation therapy is challenged by a restricted focus on accuracy and availability, and hormone therapy routinely affects resistance and unfavourable physical outcomes (Zafar et al., 2025).

1.1.4 Current Cancer Therapies & Limitations

1.1.4.1 Conventional methods: surgery, chemotherapy, etc

Surgery is a well-known and important approach to treating solid malignant tumours. In diagnostics, a biopsy of malignant tissue is the best way to subtype and grade cancers. However, it is not very effective because the sample size is too small and it isn't easy to track regularly. Incision surgery is used to ensure the complete removal of tumour cells. Although surgical treatment may additionally seem effective, undetected tumour remnants can nonetheless be found in margin tissues or as small metastatic foci, whether stromal or hematogenous, thereby confirming the incidence of minimal residual disorder (MRD) (Zafar et al., 2025).

Since X-rays were discovered in 1896, RT has become a significant way to treat cancer, either by itself or with surgery and other medical treatments. RT's purpose is to deliver the target tumour volume with an optimal radiation dose while almost completely avoiding radiation to organs at risk (OARs). At low doses, radiation mainly initiates tumour development by altering DNA, while at higher doses, radiation relevant to RT is thought to promote tumour growth.

Chemotherapy is the main conventional approach to treating cancer. It uses drugs that eliminate cancer cells. Chemotherapeutic drugs work best on tumour cells because they target cells in the growing or dividing phase. There are various ways to use chemotherapy: It may serve as an initial and only treatment, such as for blood or lymphatic system cancers, or it might be utilized to reduce tumours by limiting cancer growth before further treatment or to get rid of any cancer cells that are still there after surgery or radiation therapy.

In addition to surgery, chemotherapy, radiation therapy, and targeted therapy, immunotherapy is the fifth foundational approach in most cancer management, profoundly reshaping cutting-edge oncological treatment. Immune cells from both the adaptive and innate immune systems can affect how a tumour grows, which is why they are the cellular basis of immunotherapy. Cancer immunotherapy uses the immune system to get rid of cancer cells by making the body's natural defences stronger (Naser et al., 2022).

1.1.5 Challenges

1.1.5.1 Lack of selectivity, damage to healthy cells.

Although many discoveries in tumour biology have been made, standard treatments like chemotherapy often remain the best options. However, these treatments have significant drawbacks; they are not very precise, which means healthy cells can also absorb the drugs, leading to serious side effects. Additionally, many antitumor medications struggle to dissolve effectively (Sanchez-Moreno et al., 2016).

1.1.5.2 Development of multidrug resistance in cancer

Multidrug resistance (MDR) remains one of the most crucial limitations to the success of most cancer treatments. Although chemotherapy is recognized as a key strategy for controlling tumour growth, its effectiveness is constrained—nearly 90% of patients experience treatment failure as cancer cells broaden resistance to anticancer agents. This adaptive resistance does not diminish treatment success but also contributes to a more rapid progression of the condition. In the oncology context, MDR describes the resilience of most cancer cells against multiple anticancer drugs, a phenomenon akin to antibiotic resistance. Cancer treatments are extensively labelled into local treatment (e.g., surgical and radiation therapy) and systemic treatment (e.g., chemotherapy, targeted therapy, and hormonal therapy) modalities, the latter being critical for metastatic or late-phase cancers. Growing evidence suggests that MDR is due to the overexpression of efflux transporters that pump drugs out of most cancer cells, reducing drug uptake. Furthermore, modifications in the tumour microenvironment, genetic variability, target mutations, and epigenetic changes are also implicated in resistance development (Emran et al., 2022).

1.1.5.3 Poor bioavailability of many anticancer drugs

Anticancer medications taken orally are an emerging alternative in the management of patients with cancer. Likewise, it can be challenging to develop oral anticancer drugs that do not dissolve well or don't cross cell membranes. Oral administration of anticancer drugs is commonly impeded by low bioavailability, which is associated with significant variability. Because most anticancer drugs have a limited therapeutic duration and are given at or near the maximum tolerated dose, a wide range of bioavailability can affect treatment outcomes. The degree of oral bioavailability is contingent upon various factors, including the drug's

release from the pharmaceutical dosage form, its stability within the gastrointestinal tract, factors influencing dissolution, the rate of translocation through the gut wall, and the pre-systemic metabolism occurring in the gut wall and liver (Stuurman et al., 2013).

1.1.5.4 Severe side effects

One factor that distinguishes anticancer drugs from other drugs is that they have significantly more severe side effects at therapeutic doses. A lot of solid tumours grow less than normal tissues, such as the normal bone marrow, gastrointestinal lining, reticuloendothelial system, and the gonads. These drugs have different effects on these tissues depending on the dose, and a patient's sensitivity is also significant. As a result, these tissues are more likely to be toxic. Development in chemotherapy has offered significant evidence supporting the principle that anticancer drugs may eliminate cancer, leading to their incorporation into treatment programmes alongside surgery and radiation therapy (Remesh, 2012).

1.1.6 Cancer Cell Lines

Cancer cell lines are beneficial for improving cancer research and treatment. They have been significant for learning more about cancer biology, developing novel methods to treat it, and identifying potential therapeutic targets. There are many cancer cell lines derived from various cancer types, and scientists use them widely in their research.

1.1.6.1 Breast cancer MCF-7

MCF-7 is a widely recognized cell line related to human breast cancer, where it is currently used in numerous cancer studies. It was named after the Michigan Cancer Foundation, which was the first to be established. An example of breast cancer that effectively assesses for oestrogen receptors (ER) is MCF-7. This cell line stimulates oestrogen receptors, making it energetic, which could be very beneficial for research on hormone-dependent breast cancer. MCF-7 cells exhibit many of the same trends as breast cancer cells, including the potential to form in vivo, shape tumours, and respond to hormones, particularly oestrogen. Researchers regularly make use of them to gain insights into the function of oestrogen and anti-oestrogen treatments within the development of breast cancer. MCF-7 is used in a lot of breast cancer studies, together with studying how cancer develops, trying out drugs, and researching how hormones affect cells. Researchers use MCF-7 cells to better understand

how breast cancer works and to evaluate potential treatments. The MCF-7 cellular line has significantly advanced understanding of breast cancer pathophysiology and has been instrumental in the development and evaluation of therapeutic interventions, particularly those targeting oestrogen signalling pathways. It has additionally been used to find possible biomarkers and targeted treatments for breast cancer. Researchers often use more than one cell line to account for the diversity of breast cancer. MCF-7 is a beneficial tool for breast cancer research. And its extensive use led to great learning about this type of cancer and how to treat it (Popov, n.d.).

1.1.6.2 Pancreatic cancer PANC-1

PANC-1 is a type of cancer that originates in the pancreas. Pancreatic cancer is aggressive and challenging to treat. PANC-1 cells commonly grow as a single layer and are adherent. They are used to study various aspects of pancreatic cancer biology, genetic mutations, and drug responses. Many studies on pancreatic cancer use PANC-1 cells. These studies investigate the genetic and molecular causes of the disorder, drug testing, and the development and evaluation of potential therapeutic agents. PANC-1 has helped researchers better understand how pancreatic cancers work. It has aided scientists in exploring treatment options for this tough-to-treat and often excessive form of cancer. In summary, PANC-1 is a crucial pancreatic cancer cell line that has advanced understanding of pancreatic cancer biology and supported the development of potential therapies for this challenging cancer (Popov, n.d.).

1.1.6.3 Melanoma A375

A375 is an extensively used human cancer cell line in oncological research. That stands for malignant melanoma, a type of cancer that begins in melanocytes, the cells that give skin its colour. Melanoma is known for being aggressive and easily spreading. In culture, A375 cells adhere to one another and grow as a single layer. Scientists use them to learn about many aspects of melanoma cancer biology, genetic mutations, and how the disorder reacts to treatments. A375 cells have been utilised in numerous research studies on melanoma, including investigations of the genetic and molecular mechanisms of the disease, drug testing, and the development and assessment of potential therapeutic agents. A375 has been very beneficial about how cancer works. It has helped scientists discover viable approaches to treat this competitive form of skin cancer. In short, A375 is a critical cancer cell line that

has helped examine more of how cancer works and how to make treatments for this severe form of skin cancer (Popov, n.d.).

1.1.6.4 Leukaemia/Lymphoma K562

K562 is a famous human cell line derived from chronic myelogenous leukaemia (CML), a type of blood cancer that arises from rapidly proliferating white blood cells in the bone marrow. The Philadelphia chromosome is what makes CML special. It occurs during the copying of the genetic code along chromosomes 9 and 22. K562 cells are suspension cells that form clusters in culture that do not stick together. Researchers frequently utilise them to gain insights into various aspects of leukaemia biology, including genetic mutations, signalling pathways, and the disease's response to treatment. K562 cells are used in some studies on leukaemia, such as investigating the genetic and molecular causes of the disease, testing drugs, and developing and testing new drugs that could help. They are beneficial for analysing the efficacy of tyrosine kinase inhibitors in the treatment of CML. K562 has helped examine the mechanisms underlying leukaemia, particularly CML. It has aided researchers in exploring treatment options for this type of leukaemia, particularly those focused on the BCR-ABL fusion protein associated with the Philadelphia chromosome. In short, K562 is a very critical cellular line for studying leukaemia, specifically chronic myelogenous leukaemia. It has taught more about CML's biology and how to offer feasible remedies for it (Popov, n.d.).

1.1.6.5 Lung cancer A549

The A549 cell line is derived from human alveolar basal epithelial cells and is used in research. It is widely implemented in biomedical and cancer research. It serves as a famous experimental model for non-small mobile lung cancers (NSCLC), which account for almost all lung cancer cases. Owing to its well-characterised properties, A549 cells provide a reliable platform for studying cancer cell morphology, molecular signalling, and recovery responses. "A549 cells stick to surfaces" means they are "adherent." They grow in a single layer of cells. They preserve various characteristics of lung adenocarcinomas and are employed to study lung cancer biology, pharmacological assessment, and therapeutic progress. Researchers have used A549 cells in many lung cancer studies, including investigations into how cancer works, drug development, and testing new treatments for

NSCLC. A549 has been very helpful in lung cancer research by enabling scientists to understand NSCLC biology better and test different drugs to assess their efficacy.

The A549 cell line is an important tool in lung cancer research, especially for investigating non-small cell lung cancer (NSCLC). It has appreciably contributed to elucidating the molecular mechanisms underlying lung carcinogenesis and to improving and assessing advanced therapeutic techniques for NSCLC (Popov, n.d.).

1.1.7 Need for Novel, Targeted, and More Efficient Approaches

By manipulating materials at the nanoscale, scientists have developed nanocarriers that can deliver drugs to specific parts of the body. This makes the drugs work better and lowers the harmful effects they have on the whole body. Nanotechnology has emerged as a revolutionary breakthrough in cancer treatment, offering new ways to overcome the limitations of standard therapies such as chemotherapy and radiation. (Zhu et al., 2024).

By creating new approaches to supply capsules that make treatments work better and hurt much less. Researchers have improved the solubility, stability, and bioavailability of medicines using different types of nanoparticles, including liposomes, polymeric nanoparticles, and inorganic nanoparticles. Nanotechnology is crucial in medicine, as it enables to replace substances used to locate tumors and to provide patients with personalized treatment. Nanoparticle-based systems are created to improve the pharmacokinetic profile and efficacy of anticancer medications. This makes it simpler to present them and enables them to avoid becoming resistant (Venturini et al., 2025).

1.2 NANOTECHNOLOGY IN CANCER THERAPY

Nanotechnology is a brand-new way to improve the world by controlling things at the nanometre scale, which is a billion times smaller than a metre. Nanotechnology is any kind of technology that works on a tiny scale and can be used in lots of exclusive approaches in the real world (Nasrollahzadeh et al., 2019). Nanomaterials are substances that are between 1 and 100 nanometres in size and have unique optical, magnetic, and electrical properties. When the size of a substance changes from a large one to one less than 100 nm, its properties can change a lot (Cheng et al., 2021).

1.2.1 Unique Advantages

NPs serve as effective carriers for anticancer drugs, delivering them directly to tumours and improving tumour imaging. they can retain many drug molecules and are effective at addressing solubility, stability, and resistance issues. (Deivayanai et al., 2025).

1.2.1.1 Targeted delivery (passive via EPR effect, or active via ligands).

One key advantage of nanomaterial-based cancer therapy over free drugs is its ability to be specifically targeted. There has been a recent advancement in targeted delivery that uses nanomaterials. The concept of targeted delivery aims to deliver drugs to specific cancer cells, achieved through active or passive targeting methods. Passive targeting uses the enhanced permeability and retention (EPR) effect; active targeting uses antibodies, peptides, aptamers, and small molecules. Targeted delivery helps reduce toxicity in normal cells, prevents drug breakdown, extends their half-life, increases their loading capacity, and increases their solubility. (Cheng et al., 2021).

1.2.1.2 Enhanced drug solubility

Nanotechnology can alter materials at the molecular and atomic levels, opening new ways to improve drug solubility and delivery. The solubility of a drug is a key part of pharmacokinetics that directly affects its absorption, distribution, metabolism, and excretion (ADME), which, in turn, affects how well it works as a treatment. The solubility of a drug determines the degree to which it can dissolve in body fluids, which is necessary for it to cross biological membranes and enter the bloodstream. Consequently, understanding and improving drug solubility is essential for formulating efficacious therapeutic agents, especially considering biological variability that can complicate drug absorption and response (Zhuo et al., 2024).

1.2.1.3 Controlled drug release

Also, new nanodevices are currently being developed that can respond to specific physiological changes, such as temperature and pH. This enables regulation of drug release to enhance therapeutic outcomes.

1.2.1.4 Reduced toxicity to healthy cells

Nanodrugs can overcome the problems with traditional chemotherapy by being designed and modified in such a way that they are more specific and bioavailable, less harmful to healthy tissues, able to hold more drugs, have a longer half-life, and have regulated drug release properties (Cheng et al., 2021).

Nanocarriers can hold extensive quantities of drugs that would otherwise have poor pharmacokinetic properties or be highly toxic if consumed on their own. Because they are so small, the drugs included may easily enter and spread through tissues. Nanomaterials exhibit unique physicochemical properties, including size distribution, surface area-to-volume ratio, surface structure, chemical composition, and adhesion behaviour. These properties differ significantly from those of bulk materials. In the last ten years, nanotechnology has been used more widely in a broad spectrum of fields, such as cosmetics, medicine, food, and building substances (Hammami et al., 2021).

1.2.2 Targeting Mechanisms

Nanostructure targeting of cancer cells can be achieved using primary methods, including active and passive targeting. The latter depends on the enhanced permeability and retention (EPR) effect. This effect occurs when the leaky blood vessels that form during angiogenesis allow nanoscale structures to penetrate the tumour microenvironment passively. Also, these nanostructures can remain at the tumour site for a long time because lymphatic drainage is ineffective. Although this mechanism allows nanostructures to accumulate more in tumours, they are unable to distinguish between cancerous and healthy tissues. To improve their affinity for tumour cells, nanostructured surfaces were modified with targeting ligands that selectively bind receptors overexpressed on cancer cells—a strategy known as active targeting. Many different targeting ligands have been extensively studied for this objective, encompassing antibody fragments, antibodies, vitamins, peptides, nucleic acids, carbohydrates, and glycoproteins (Sabit et al., 2022).

1.2.3 Nanoparticles Classification

Nanomaterials are classified into five groups based on their size, origin, connectivity, pore size, and toxicity. Nanoparticles can be divided into four main groups depending on their

structure: inorganic, organic/dendrimers, composite, and carbon-based (Mekuye & Abera, 2023).

1.2.3.1 Liposome nanoparticles

Liposome nanoparticles are a new kind of nanostructure that can keep and release bioactive compounds. Liposomes can encapsulate a wide range of bioactive substances, such as drugs, cosmetics, and food ingredients. Liposomes have properties that make them beneficial in cosmetics, nanomedicine, and the food industry. These consist of being biocompatible and biodegradable (Panahi et al., 2017).

1.2.3.2 Gold nanoparticles (AuNPs)

AuNPs are the most important nanoparticles. They are used loads in medicine and other fields because they have characteristics that make them suitable for many things: non-harmful, biocompatible, and particularly no longer very poisonous (Hammami et al., 2021).

1.2.3.3 Polymeric nanoparticles

Polymeric nanoparticles are at the forefront of several advanced drug delivery systems, enabling researchers to control the size, morphology, architecture, charge, and surface properties of the drugs they deliver. Polymeric nanoparticles can also move distinctive organic boundaries to target precise regions of the body accurately, keep a wide range of healing substances, and release those materials quickly in response to signals from outside and inside the body (Beach et al., 2024).

1.2.3.4 Iron oxide nanoparticles

Iron-oxide nanoparticles (IONPs) are an important aspect of the current revolution in nanomedicine because they are inorganic nanomaterials. Their distinctive physical characteristics, extensive surface area to volume ratios and superparamagnetic characteristics, make them desirable for medical uses, including magnetic resonance imaging (MRI), drug and gene delivery, tissue engineering, and bio separation (Boyer et al., 2010).

Structure & Forms

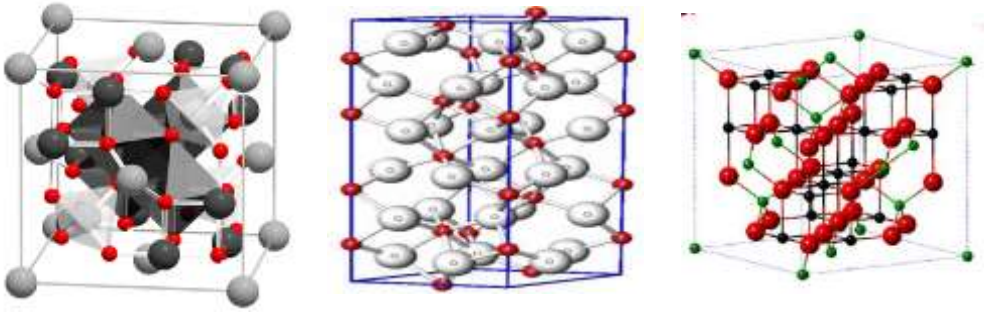

Figure 1.1: IONPs Structures and Forms.

The common and important iron oxide, magnetite (Fe_3O_4), has an equal amount of Fe2+ and Fe3+ in it. However, this ratio is often insufficient, leading to a layer low in Fe3+. Magnetite nanoparticles are extensively utilised in various medical and biological fields, whereas their use in optics is less prevalent. Nano magnetite suspensions, known as magnetic ferrofluids, have been used in optics in recent years because they have magneto-optical features (Baabu et al., 2023; Dudchenko et al., 2022).

Haematite (α-Fe2O3) is the second most important iron oxide. It is the most stable iron oxide at room temperature because it is thermodynamically stable. In recent years, haematite has attracted significant interest owing to its advantageous properties for various applications in electronics, optics, and photonics. There has been significant attention paid to undoped and doped haematite as materials for solar photoelectrochemical (PEC) cells, given the extensive research on that application. In addition to being economical, environmentally friendly, and highly productive, it also shows chemical stability throughout a wide range of pH levels, which makes it ideal for use in photocatalytic processes (Ahmad et al., 2016; Baabu et al., 2023).

Maghemite (γ-Fe_2O_3) is one of the most abundant forms of iron oxide, ranking 2nd in abundance after hematite (α-Fe_2O_3). In its superparamagnetic nanoparticle form, maghemite has a wide range of applications, including ferrofluids, magnetic data storage, high-frequency digital devices, permanent magnetic materials, magnetic refrigeration, MRI contrast enhancement, bio separation methods, most cancer hyperthermia therapy, and magnetic drug delivery. Overall, maghemite's primary importance lies in its good-sized use

in scientific diagnostics and statistical garage technologies. (Baabu et al., 2023; Shokrollahi, 2017).

Physicochemical Properties

IONPs have many advantageous physicochemical properties, such as being biodegradable, biocompatible, slightly toxic, highly magnetic, and superparamagnetic, with strong attraction, and a relatively low Curie temperature. These have enabled them to be successfully sold as new industrial and biomedical products.

The relatively low toxicity of IONPs for living cells has made them effective in biomedicine. The toxicity of substances in the biological environment depends on their physicochemical characteristics and the dosage administered. Nickel, zinc, cobalt, silver, and cadmium are among the metals that are harmful to organisms. Particles made of titanium and iron oxide are thought to be less harmful to cells.

Naked Fe_3O_4 nanoparticles (if not small enough) do not dissolve properly and tend to bind tightly together in physiological conditions. In commercially available ferrofluids, Fe_3O_4 nanoparticles are coated with surface molecules to improve their compatibility with living systems and facilitate their spread throughout the body. This also prevents the Fe_3O_4 core from oxidising, stabilises it, and prevents clumping. It also increases the Fe_3O_4 core's blood circulation half-life.

Iron-containing nanoparticles are biocompatible. When Fe_3O_4 breaks down in organs like the liver, the released iron binds to apoferritin and apo-transferrin, forming ferritin and transferrin. These are protein complexes that store and transport iron. After being filtered through the kidneys, the iron can either be taken up by red blood cells or released from the body. Several clinical trials have indicated slight adverse effects in patients who received the intravenous administration of clinically approved IONP-based fluids (Etemadi et al., 2021).

Synthesis Methods

Creating iron oxide nanoparticles remains difficult because their properties depend heavily on the method used in the trial. This is because the oxidation state, particle size, stoichiometry, or magnetic properties must be "traded off" against any additional property,

meaning one of the most important features is lost. Various techniques have been developed and refined to produce iron oxide nanoparticles that can do more than one thing, such as co-precipitation, thermal decomposition, hydrothermal synthesis, and green synthesis.

In the coprecipitation method, magnetic iron oxide nanoparticles are synthesized via a wet-chemical process, which is considered more effective and easier than other available synthesis techniques. In this procedure, ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions in an aqueous solution react with an alkaline base, main to the formation of magnetite, maghemite, and ferrous hydroxide phases upon incubation. This technique is especially favoured because of its ease of operation, fee efficiency, and potential to supply nanoparticles and scale up the yield (Baabu et al., 2023).

In hydrothermal synthesis, autoclaves are used to maintain high temperatures and high pressure, conditions vital for nanoparticle formation. Within this closed-response vessel, iron-based aqueous precursors undergo a two-step process hydrolysis and oxidation of blended metallic hydroxides resulting in the formation of ferrites. This technique offers considerable versatility, because the morphology, length, and crystallinity of the produced nanoparticles can be managed with the aid of manipulating key experimental parameters, which include response time, temperature, stress, and the chemical composition of the solution, inclusive of reactant recognition, solvent kind, and complexing agent homes (Baabu et al., 2023).

The inexperienced synthesis technique has attracted significant interest among materials scientists for the fabrication of iron oxide nanoparticles due to its green and non-toxic nature. Unlike conventional chemical or physical strategies, this approach employs biological entities such as vegetation, fungi, microorganisms, viruses, and algae as natural assets for nanoparticle synthesis, which function to reduce, cap, and stabilize nanoparticles. The synthesis way commonly involves two primary steps: bio-reduction, wherein metallic ions are converted into strong nanoparticles, and biosorption, which allows their attachment to organic matrices to form sturdy complexes (Baabu et al., 2023).

Biomedical Applications

Superparamagnetic iron oxide nanoparticles (IONPs) have become very useful in biomedical applications because they combine multiple properties into a single nanostructure. IONPs

serve as efficient contrast agents in magnetic resonance imaging (MRI) and magnetic particle imaging (MPI) by creating targeted dipolar magnetic fields. This allows for non-invasive visualisation of inflammatory reactions and tracking of therapeutic or stem cells in cell-based therapies.

When an external magnetic field gradient is applied to IONPs, they experience a magnetic force that drives drug vectors, including IONPs or IONP-labeled cells, through magnetophoresis. Magnetic alteration has been proposed for targeted drug delivery to specific body locations, to enhance cell therapy by maintaining therapeutic cells at the site of regeneration, and for tissue engineering to replicate the native cellular structure of tissues.

Finally, IONPs can create mechanical or thermal energy when exposed to an alternating magnetic field. This provides many opportunities to burn tumors (thermal destruction), alter the tumor microenvironment's temperature, trigger specific and controlled cellular responses, or modulate neuronal activity or gene expression.

Researchers are looking into using remote stimulation of IONPs as a new way to treat brain disorders, cancer, and regenerative medicine (Faivre, 2016).

1.2.4 Quercetin As an Active Ingredient

Recently, there has been a significant increase in the use of natural biological agents to treat chronic diseases, as they tend to be less harmful and safer for the environment. Quercetin, a bioactive molecule, has received extensive investigation due to its multifaceted pharmacological properties, including antioxidant, antiviral, immunomodulatory, and anticancer effects, alongside an acceptable toxicity profile.

While several studies indicate that quercetin is an effective therapeutic agent, it has not yet been approved as a pharmaceutical because there is insufficient clinical data or an accurate understanding of how it works. Also, a wide range of phytoconstituents and chemical compounds with extensive biological and pharmacological effects has been identified in medicinal herbs. Quercetin (3,5,7,3′,4′ pentahydroxyflavone) is found in many places, including stems, roots, wine, flowers, bark, vegetables, tea, fruits like lovage, onions, apples, dill, berries, cilantro, and capers (Aghababaei & Hadidi, 2023).

1.2.4.1 Chemical structure & properties

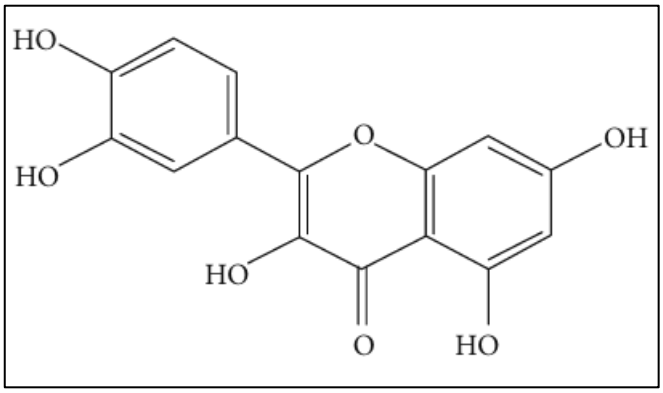

Figure 1.2: Structural Formula of Quercetin (Yang et al., 2020)**.**

Quercetin is a well-known flavonoid that appears as a yellow crystalline compound, showing poor solubility in water but dissolving readily in alcohols and lipids, with only moderate solubility in warm water and a pleasant sour taste. Structurally, quercetin is an aglycone, meaning it lacks carbohydrate mobility, and it contributes to the vibrant pigmentation observed in many plants. This compound possesses super antioxidant activity, which is frequently attributed to the hydroxyl groups at the 3, 5, 7, 3′, and 4 ′ positions of the A and B rings, the C2–C3 double bond, and the carbonyl group at C4. These structural capabilities allow quercetin to neutralize unpaired radicals by donating electrons, thereby interrupting oxidative chain reactions that might otherwise damage living cells. Owing to its presence in those houses, quercetin plays an essential role in protecting natural structures from oxidative stress and contributes to various therapeutic applications. It additionally provides a lot of interesting uses for various medical conditions, such as dealing with cancer, allergies, diabetes, obesity, high uric acid levels, and gouty arthritis. The most significant aspect about quercetin is that it can stop the progression of some cancers, like breast, cervical, lung, colon, prostate, and liver cancers (Aghababaei & Hadidi, 2023).

1.2.4.2 Pharmacological activities

Antioxidant

The body generates free radicals during metabolism, and they are among the factors that cause many diseases. They can hurt cell membranes and change genes, speed up the body's ageing process, and cause several diseases, including liver damage, diabetes, and heart disease. Quercetin is the most effective free radical scavenger in the flavonoid family. By

looking into the chemical nature of quercetin, it was discovered that there are four hydroxyl groups on the benzodihydropyran ring of the polyphenol, so quercetin has a high antioxidant capacity that can reduce free radicals formed in the body, and can aid the body in staying in a steady state. Quercetin's antioxidant activities in vitro primarily involve direct elimination of free radicals, chelation of metal ions, and inhibition of lipid peroxidation (Yang et al., 2020).

Antimicrobial Properties

Quercetin has developed a wide variety of antibacterial and antifungal drugs that successfully inhibit the growth of large numbers of pathogenic microorganisms. Studies have shown that quercetin inhibits the proliferation of microorganisms, which include Pseudomonas aeruginosa, Salmonella Enteritidis, Staphylococcus aureus, Escherichia coli, Proteus, and the fungus Aspergillus flavus. The antimicrobial mechanism of the compound is usually related to disruption of the bacterial mobile department and modifications in membrane permeability, which together impair essential mobile talents. These disturbances disrupt protein synthesis and expression, reduce enzyme affinity, and inhibit nucleic acid synthesis, ultimately leading to cell death. In addition, quercetin is reported to inhibit bacterial adhesion to surfaces, disrupt quorum-sensing pathways, damage or alter the plasma membrane, and inhibit the efflux pump system, thereby enhancing its antimicrobial activity. Through these multifaceted mechanisms, quercetin shows sturdy capability as an antimicrobial herbal remedy with fitness relevance (Yang et al., 2020).

Anti-Obesity

Quercetin is a naturally occurring flavonoid found in numerous plant-based foods. Many studies have examined its potential anti-obesity effects. Numerous in vitro and in vivo studies demonstrate that quercetin exerts anti-obesity effects via multiple mechanisms. It has been demonstrated for impeding adipogenesis and adipocyte differentiation, which are essential processes in the pathogenesis of obesity. It is also believed to accelerate lipolysis, the breakdown of stored fat, and thermogenesis, the process by which brown adipose tissue produces heat. This means that more energy is used. Quercetin also has anti-inflammatory effects because it prevents the production of pro-inflammatory cytokines, which have been shown to be involved in inflammation associated with obesity (Aghababaei & Hadidi, 2023).

1.2.4.3 Therapeutic applications of quercetin

Quercetin in Diabetes

Many research investigations have demonstrated that Quercetin is a promising pharmacological target for diabetes management. Several mechanisms have been suggested for Quercetin's antihyperglycemic effects, including enhanced insulin sensitivity, stimulation of glycogen synthesis, and reduced insulin resistance. Quercetin enhances insulin sensitivity by stimulating pancreatic β-cell proliferation, thereby improving glucose metabolism and insulin secretion. Researchers have also confirmed that quercetin prevents α-glucosidase and α-amylase from working. Quercetin reduced blood glucose ranges in streptozotocin (STZ)-induced diabetes models and increased plasma insulin levels by way of maintaining the mass and function of β-cells, thereby enhancing the impact of serum insulin (Salehi et al., 2020).

Quercetin in Neurodegenerative Disorders

Quercetin has been utilised in the development of anti-Alzheimer's disease (AD) drugs because of its neuroprotective effects against oxidative stress and excitotoxicity, mediated by modulation of apoptotic pathways.

Several mechanisms had been advised for the neuroprotective outcomes of Quercetin, in conjunction with the bargain of amyloid-β (Aβ) synthesis, the prevention of intracellular neurofibrillary tangles (NFTs) improvement, the suppression of amyloid precursor protein (APP), the blockade of cleaving enzyme (BACE1), the inhibition of acetylcholinesterase (AChE), and similarly pathways that alleviate oxidative stress in Alzheimer's sickness (AD). Quercetin and its bioactive substances promote neurogenesis and neuronal durability via modulating signalling pathways, which include P13 kinase, AKT/PKB tyrosine kinase, and protein kinase C in Alzheimer's sickness (Salehi et al., 2020).

Quercetin in Cancer Therapy

Quercetin stops the growth of most cancer cells and tumors by altering the PI3K/Akt/mTOR, Wnt/β-catenin, and MAPK/ERK1/2 pathways. Quercetin causes most cancer cells to die by inducing β-catenin and HIF-1α, activating caspase-3, and preventing the phosphorylation of Akt, mTOR, and ERK. Quercetin also prevents metastasis by reducing VEGF and MMP levels. QUE has an impact on cancer metabolism by blocking key enzymes in glycolysis and

glucose uptake. It does this by interacting with the PI3K/Akt/mTOR pathways. Quercetin also targets mitochondria in cancer, reducing bioenergetics and triggering intrinsic apoptosis. Quercetin also targets mitochondria in cancer cells, disrupting bioenergetics and triggering natural apoptosis. The impact of QUE on glucose metabolism and cellular energy production contributes to its ability to decrease cell viability, prevent metastasis, and induce apoptosis in cancer cells (Reyes-Farias & Carrasco-Pozo, 2019).

The molecular mechanism and main strategies are as follows:

a. Inhibition of the PI3K/Akt/mTOR pathway

The PI3K/Akt/mTOR pathway is vital for preserving cells alive, growing, dividing, and moving. In many forms of cancer, like breast, lung, and prostate cancer, it really works too hard. Quercetin inhibits the PI3K/Akt/mTOR pathway by reducing the likelihood that Akt is phosphorylated. This inhibits mTOR, slowing cell growth and hastening the death of cancer cells. MTOR is responsible for essential processes such as protein synthesis and the formation of new blood vessels, both of which are required for tumors to grow. Quercetin blocks mTOR activity, slowing tumor growth. In breast cancer models, Quercetin inhibited phosphorylation in this pathway, promoting apoptosis in cancer cells while exerting minimal consequences on healthy cells. Also, by blocking this pathway, Quercetin makes most cancer cells more likely to respond to traditional chemotherapies, thereby reducing drug resistance (Silva-Pinto et al., 2025).

b. Modulation of oxidative stress and antioxidant systems

Quercetin could be essential for preserving oxidative stress in the test. This is because oxidative stress damages DNA, proteins, and mobile membranes, which is a major cause of most cancer development. It can also directly neutralize ROS by donating hydrogen atoms or electrons, thereby lowering oxidative stress in cells. This interest prevents cells from suffering oxidative damage that could cause cancer. Quercetin not only removes ROS directly but also supports the body's antioxidant systems by restoring levels of vitamins C and E, which are endogenous antioxidants. These antioxidants work together to keep cells healthy by stabilizing their membranes. Quercetin's ability to bind transition metals that initiate ROS reactions makes it even more effective at clearing free radicals. This stops the tumor microenvironment from making more ROS. This wide range of antioxidant effects

not only prevents tumors from growing and forming but also protects healthy cells from oxidative damage during treatment (Silva-Pinto et al., 2025).

c. Apoptosis induction and anti-inflammatory activity

Quercetin possesses strong anticancer effects, as it causes tumor cells to undergo apoptosis and exerts anti-inflammatory effects that help regulate the tumor microenvironment. Quercetin promotes programmed cell death in cancer cells through multiple mechanisms. This reduces the risk of damage to healthy cells. Death receptors like FAS and TNFR1 need to be activated, and caspases, a group of proteases essential for apoptosis, must be activated. Research indicates that Quercetin enhances the expression of pro-apoptotic proteins, such as Bax, while suppressing anti-apoptotic proteins, such as Bcl-2, thereby promoting cell death in cancer cells. Quercetin additionally induces apoptosis and alters inflammatory pathways. It does this mainly by inhibiting transcription factors such as NF-κB and COX-2, which are important for preventing chronic inflammation in the tumor microenvironment. By reducing the expression of these inflammatory mediators, Quercetin lowers the production of pro-inflammatory cytokines, thereby limiting cancer cells' ability to evade the immune system and spread to other parts of the body (Silva-Pinto et al., 2025).

d. Modulation of the JAK/STAT pathway

Quercetin has been proven to target the JAK/STAT pathway, which is a crucial part of the immune reaction and is frequently too active in certain kinds of cancer, particularly breast and blood cancers. Quercetin may stop alerts that help most cancer cells survive, grow, and evade the immune system by reducing key components of this pathway. Quercetin inhibits the phosphorylation of STAT proteins, preventing their activation and nuclear entry. This prevents the expression of genes crucial for mobile growth and immune suppression, slowing tumor growth. The effect of Quercetin on the JAK/STAT pathway is profoundly enormous in immuno-oncology, as this pathway is typically exploited by cancer cells to evade immune detection. Quercetin helps restore immune surveillance by blocking JAK/STAT signaling. This allows T cells to find and kill cancer cells (Silva-Pinto et al., 2025).

e. Inhibition of the Wnt/β-catenin pathway

The Wnt/β-catenin pathway plays a huge role in controlling development, differentiation, and apoptosis. When it is not operating properly, it's frequently related to different varieties of cancer, particularly colorectal cancer. When this pathway is overly lively, β-catenin can increase inside the cytoplasm and then circulate to the nucleus, where the transcription of oncogenes like c-Myc and Cyclin D1 begins. These motives cause cells to grow out of control and form tumors. Quercetin has been tested for its capacity to block this pathway by reducing nuclear β-catenin accumulation, thereby inhibiting the expression of genes involved in cellular proliferation. Studies show that Quercetin works nicely to save you elements of the Wnt device, like β-catenin and Disheveled (DVL) proteins, which can be essential for sending signals within the Wnt pathway. This inhibition reduces mobile proliferation and induces apoptosis in most cancer cells, suggesting Quercetin's potential in cancers associated with Wnt/β-catenin dysregulation (Silva-Pinto et al., 2025).

f. Inhibition of the MAPK/ERK pathway

The MAPK/ERK (Mitogen-Activated Protein Kinase/Extracellular Signal-Regulated Kinase) pathway is a vital signaling pathway that controls how cells grow, survive, and trade. If this pathway is activated incorrectly, it could cause cells to grow out of control and become resistant to apoptosis. This takes place in cancers like breast cancer, lung cancer, and melanoma. Quercetin has been shown to inhibit the MAPK/ERK pathway by blocking ERK phosphorylation, which is essential for its activation, after which it shifts to the nucleus. When ERK is activated and enters the nucleus, it allows the expression of genes crucial for cell division and survival. Quercetin stops the transcription of these cancer-causing targets by way of decreasing ERK activation. This slows the increase of cancer cells and quickens their death. Studies on lung cancer cell lines suggest that Quercetin treatment markedly reduces ERK phosphorylation, thereby inhibiting cell cycle progression and promoting apoptotic cell death. In addition, Quercetin's potential to inhibit the MAPK/ERK pathway may enhance the effectiveness of other cancer treatments. Cancer cells frequently rely upon MAPK/ERK signaling for his or her survival, specifically under the strain of chemotherapy and radiation. Quercetin makes tumor cells more sensitive to those well-known treatments by blocking this pathway. This could result in better remedy results. The twin movement of inhibiting tumors without delay and making them more sensitive to various therapies

indicates how useful Quercetin will be in combination treatment plans for cancers that depend on MAPK/ERK (Silva-Pinto et al., 2025).

g. Modulation of the NF-κB pathway

The NF-κB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) pathway is a key regulator of infection, cellular survival, and immune function. In cancer, NF-κB is frequently overactivated, leading to the transcription of genes that help cells survive, grow, and avoid apoptosis. This pathway's consistent activation also leads to the release of pro-inflammatory cytokines that create a microenvironment that supports tumor growth and spread in most cancers. Quercetin has been shown to downregulate the NF-κB pathway, which prevents most cancer cells from expressing genes that block apoptosis and promote infection. The essential way this happens is by preventing IκB kinase (IKK), which prevents the phosphorylation and degradation of IκB, the NF-κB inhibitor. NF-κB stays inside the cytoplasm due to this. This prevents it from binding on the nucleus and prevents the transcription of gene targets important for cell survival and inflammation. Research demonstrates that Quercetin treatment reduces NF-κB activity in some types of cancer, including breast and prostate cancers, thereby improving tumor cell sensitivity to apoptosis and inhibiting inflammation-prompted tumor proliferation. Furthermore, with the aid of modifying NF-κB signaling, Quercetin now not only promotes apoptosis but also establishes a less conducive microenvironment for tumor growth (Silva-Pinto et al., 2025) .

Cardiovascular Protection

Quercetin has positive effects on cardiovascular conditions like high blood pressure, atherosclerosis, ischemia-reperfusion injury, and cardiotoxicity. These effects are closely linked to quercetin's anti-inflammatory and antioxidant properties. The protective effect of quercetin on the cardiovascular system involves lowering systolic, diastolic, and mean arterial blood pressure. The levels of ST segment, plasma lipid peroxidation, free fatty acids, phospholipids, total cholesterol, and triglycerides in serum were reduced, which can help blood vessels regenerate and lower blood sugar. It can help make the aortic wall thinner (Yang et al., 2020).

1.2.4.4 Limitations of free quercetin

Quercetin has many advantages, but it can't be used as a medicine because it doesn't dissolve well in water, doesn't pass through membranes well, is unstable in physiological media (like the stomach and intestines), has a short biological half-life, and goes through a lot of first-pass metabolism in the liver before it gets into the bloodstream. This means that it doesn't work well when taken orally. However, recent advances in drug delivery have enabled the development of new drug-delivery systems that address Quercetin's poor bioavailability and improve therapeutic outcomes, specifically designed to boost its biological activity. The most effective delivery systems have to protect Quercetin in the upper gastrointestinal tract, maintain its stability, and release it in the colon with longer-lasting diffusion and higher bioavailability (Salehi et al., 2020). Pharmacokinetic studies of quercetin have indicated that inadequate absorption and quick metabolism are the primary factors contributing to its low bioavailability.

1.2.5 Quercetin Iron Oxide Nanoparticle

1.2.5.1 Rationale for loading quercetin into IONPs

The restricted solubility of this compound in water can be improved through several tactics. Increasing the particle’s floor area, reducing its size to the nanoscale, and reducing crystallinity are among the best techniques. Alternatively, generating an extremely strong amorphous form with a higher solubility than the crystalline form, or using an embedding compound such as cyclodextrin. Liposomes, phospholipid complexes, mesylates, and similar molecules are promising new drug transport strategies that can offer better solubility, better permeability, and increased resistance to quercetin metabolism (Vijayasri et al., n.d.).

1.2.5.2 Methods of loading quercetin into IONPs

The three basic strategies for loading potent therapeutic drugs into a nanocarrier system are covalent bonding, conjugation, encapsulation, and electrostatic interaction.

covalent bonding conjugation

Both drug loading and release are efficient thanks to the presence of functional groups on their surfaces. The three main ways therapeutic drugs can be loaded into the nanocarrier system are via covalent bonds, encapsulation, and electrostatic interactions. The nanocarrier-

drug combination slowly diffuses into the cell membrane, allowing the drug to be released exactly where it needs to be and stay there. This covalent bond makes it possible to create a stable nanocarrier technology for delivering certain drugs (Sabit et al., 2022).

Encapsulation

Another way to put drugs into nanocarrier systems is to encapsulate them. Nanocarriers have a concave surface that lets them hold therapeutic drugs. Polymeric nanocarriers, nanocapsules, dendritic plants, and other nanocarriers have hollow interiors that make it easy to load drugs into them. The nanocarrier's inner chambers are hydrophobic, allowing more hydrophobic drugs to be loaded via hydrophobic interactions or hydrogen bonding. Encapsulation can also result from physical reactions. Liposomes hold drugs that can be used on the skin in either an active or passive way. To release the drug, pH-sensitive neutralisation, hydrolysis, tocolysis, and thermal decomposition methods are used (Sabit et al., 2022).

Electrostatic Interactions

The incorporation of functionalized nanocarriers has demonstrated effectiveness in enhancing the solubility of hydrophobic capsules. The presence of amine and carboxyl functionalities on those vendors affords charged websites that interact electrostatically with drug molecules. Such interactions promote stable incorporation of nonsteroidal anti-inflammatory marketers along with ibuprofen, diflunisal, ciprofloxacin, and indomethacin inside the nanocarrier system, ultimately enhancing their aqueous solubility and bioavailability (Sabit et al., 2022).

1.2.5.3 Advantages over free quercetin

Quercetin is an evidently occurring bioflavonoid recognized for its large pharmacological activities, in conjunction with antioxidant, anti-inflammatory, anticancer, antiviral, and anti-ischemic effects. However, its therapeutic potential is severely constrained by its poor solubility and low bioavailability, posing a significant challenge for development. To address these boundaries, nanotechnology-based systems, particularly superparamagnetic iron oxide nanoparticles (IONPs), have emerged as promising delivery systems. These nanoparticles exhibit unique properties, including a high surface-to-mass ratio, colloidal stability, and cellular uptake, making them effective for delivering drugs, proteins, and

biosensors. When quercetin is coated onto IONPs, its solubility and bioavailability are markedly superior, leading to more pharmacologically common performance. Moreover, coating IONPs with biocompatible polymers along with beta-cyclodextrin and polyethylene glycol not only minimizes nanoparticle toxicity but also increases stability and drug-loading efficiency. Consequently, Quercetin-loaded iron oxide nanoparticles represent a complex and effective strategy for achieving controlled release, enhanced therapeutic efficacy, and targeted drug delivery (Vijayasri et al., n.d.).

2. MATERIALS AND METHOD

2.1 CHEMICALS

a. Ferric chloride ($FeCl_3$•$6H_2O$) - Sigma Aldrich (USA)

b. Ferrous sulphate ($FeSO_4$•$7H_2O$) - Sigma Aldrich (USA)

c. Sodium hydroxide (NaOH) - Sigma Aldrich (USA)

d. Quercetin (Qu) - Sigma Aldrich (USA)

e. Ethanol - Sigma Aldrich (USA)

f. Deionized water - Sigma Aldrich (USA)

2.2 INSTRUMENTS

a. Ultrasonicator - SK7210HP, KUDOS, China

b. Magnetic Stirrer - Benchmark, United States

c. pH meter - Table top pH meter, ISOLAB, Germany

d. Zeta sizer – Horiba, Kyoto, Japan

e. Centrifuge - ROTOFIX 32 A Hettich, Germany

f. Analytical balance, Sartorius, Germany

g. Micropipette - Sartorius, Germany

h. Ultraviolet and visible spectrometer - UV-1800, SHIMADZU, Japan

i. Infrared spectroscopy using Fourier transform - IRAffinity-1S, Shimadzu, Japan

2.3 PREPARATION

2.3.1 Synthesis of Iron Oxide Nanoparticles (IONPs)

The preparation of the Iron Oxide Nanoparticles was conducted in the previous study (Ba-Abbad et al., 2022).

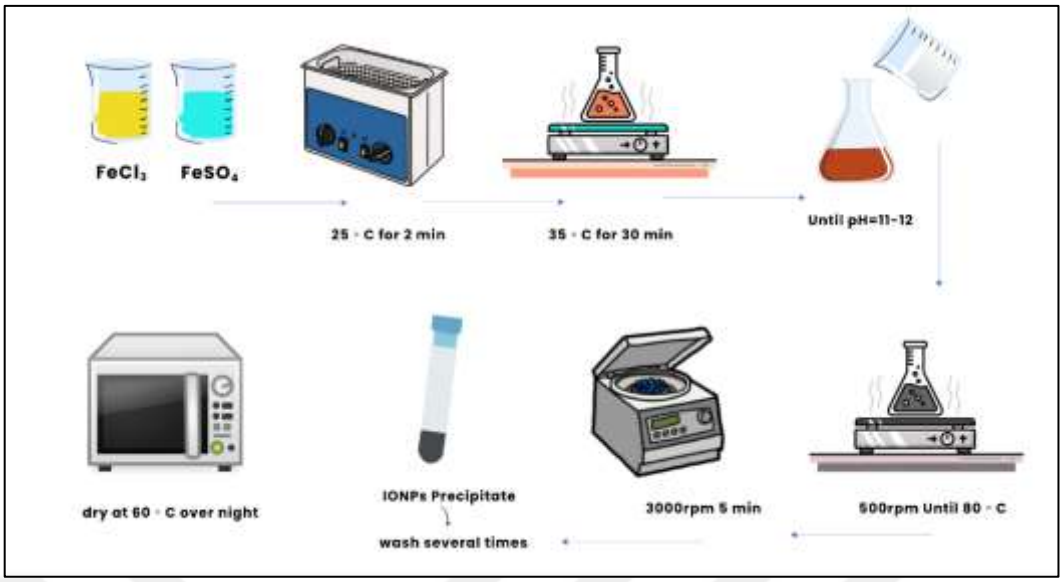

Figure 2.1: Synthesis of Iron Oxide Nanoparticles (IONPs).

Step 1: Dissolve iron salts

a. Prepare 100 mL of deionized water.

b. Dissolve $FeCl_3.6H_2O$ (0.4 M) and $FeSO_4.7H_2O$ (0.2 M) under stirring.

c. Maintain the solution at 70-80°C

Step 2: Precipitation with NaOH

a. Slowly add NaOH dropwise until the pH reaches 11-12.

b. A black precipitate of IONPs will form.

Step 3: Centrifugation to precipitate IONPs.

a. Collect IONPs via centrifugation (20,000 rpm, 15 min).

Step 4: Washing & Collection

a. Wash the precipitate several times with deionized water and ethanol until pH becomes 7.

b. Dry at 60°C in a vacuum oven.

2.3.2 Quercetin Iron Oxide Nanoparticles Preparation

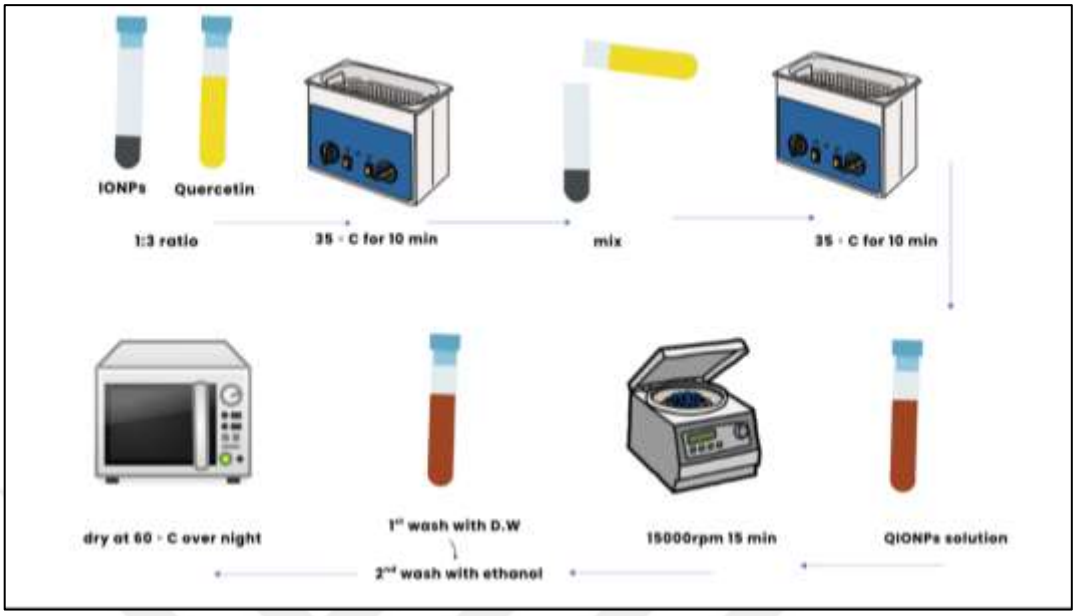

Figure 2.2: Quercetin Iron Oxide Nanoparticles Preparation.

Functionalization with Quercetin

Step 1: Quercetin Solution Preparation

a. Dissolve quercetin in water under stirring.

Step 2: Nanoparticle Coating

a. Add Quercetin to the IONPs solution.
b. Sonicate for 60 min to enhance adsorption.
c. Stir at room temperature to allow functionalization.

Step 3: Separation & Purification

a. Collect QIONPs via centrifugation (15,000 rpm, 15 min).
b. First wash with water to remove unbound quercetin, and second wash with ethanol.
c. Dry under vacuum at 60°C.
d. Store the obtained QIONPs in an amber glass vial and seal tightly at room temperature away from direct light, heat, and moisture for characterization.

2.3.3 Preparation of Different Concentrations of QIONPs For Evaluation Test

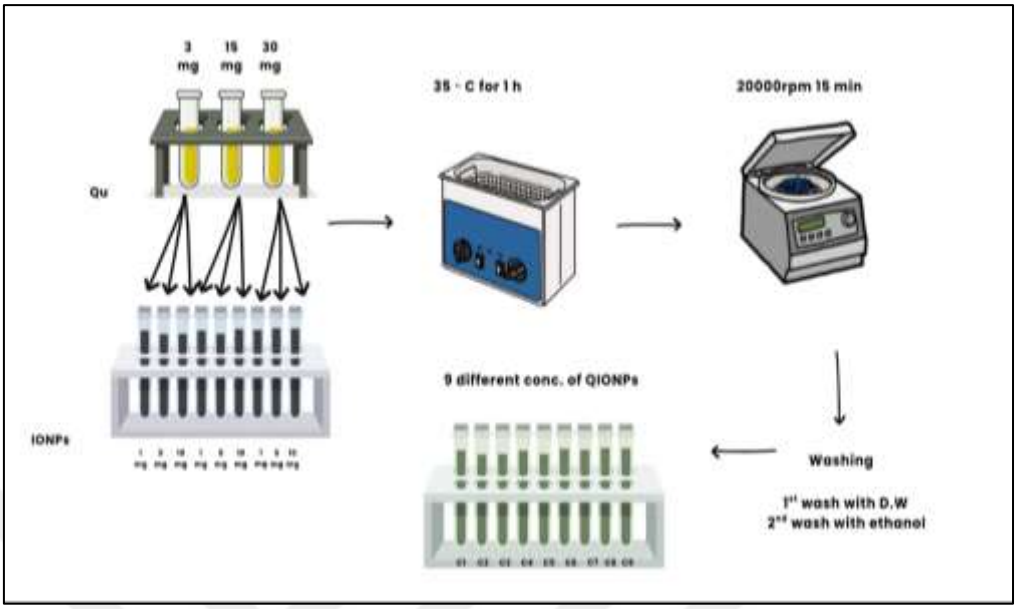

Figure 2.3: Preparation of Different Concentrations of QIONPs.

For the evaluation test on MCF7 cell line

Step 1: Solution Preparation

a. Prepare three different concentrations: 0.3 mg/mL,1.5 mg/mL, and 3.0 mg/mL of Quercetin solution.

b. Prepare three different concentrations: 0.1 mg/mL,0.5 mg/mL, and 1.0 mg/mL of IONPs solution.

Step 2: Nanoparticle Coating

a. Add each conc. Of Quercetin to each conc. of the IONPs solutions as shown in Figure 5.

b. Sonicate for 60 min at 35°C to enhance adsorption.

Step 3: Separation & Purification

a. Collect QIONPs via centrifugation (20,000 rpm, 15 min).

b. First wash with water to remove unbound quercetin, and second wash with ethanol.

2.4 CHARACTERIZATION

To confirm the synthesis and functionalization, different characterization tests were performed

2.4.1 Ultraviolet–Visible Spectrometer

2.4.1.1 Quercetin ultraviolet–visible spectrometer study

Sample solutions and the blank were placed in a quartz cuvette, and the baseline was corrected using ethanol as the blank. Quercetin spectral curves were obtained covering the wavelength between 200-700 nm (Rosli et al., 2018).

2.4.1.2 IONPs ultraviolet–visible spectrometer study

Sample solutions and the blank were placed in a quartz cuvette, and the baseline was corrected using ethanol as the blank. IONPs spectral curves were obtained covering the wavelength between 200-700 nm (Rosli et al., 2018).

2.4.1.3 QIONPs ultraviolet–visible spectrometer study

Sample solutions and the blank were placed in a quartz cuvette, and the baseline was corrected using ethanol as the blank. QIONPs spectral curves were obtained covering the wavelength between 200-700 nm (Rosli et al., 2018).

2.4.2 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR measurements were employed to identify the functional groups and demonstrate the interaction between Quercetin molecules and the iron oxide nanoparticle surface. Spectra were recorded on a spectrometer in the region 4000–400 cm^{-1}, recorded at 4 cm^{-1} resolution with 64 scans (Wierzbinski et al., 2018).

2.4.3 Zeta Potential Analysis

Zeta potential analysis of QIONPs-coated nanoparticles was measured with the HORIBA Zetasizer system to determine the surface charge and colloidal stability of QIONPs, in a capillary cell. The measurements were taken 3 times, with no fewer than 20 counts per measurement, at 25 °C. (Wierzbinski et al., 2018).

2.4.4 Size Measurement Using Dynamic Light Scattering (DLS)

The size of quercetin-loaded iron oxide nanoparticles was measured using a Horiba particle size analyser to determine the hydrodynamic particle size and size distribution. The nanoparticles were diluted with distilled water and analysed at least three times in open, double-sided quartz cuvettes. The average values were used to calculate the response surfaces (Vijayasri et al., n.d.) .

2.4.5 pH Analysis

A digital pH meter is first calibrated with three pH standard solutions at 4, 7, and 10. Later, it is used to determine the pH of the prepared IONPs formulations. Each system's pH readings are repeated three times (Naga Sravan Kumar Varma et al., 2014).

2.4.6 Morphological Structure

The morphological structure of the synthesized QIONPs nanoparticles was measured using a field emission scanning electron microscope (FESEM) S-3700N. FESEM is a testing method in which a sample is examined with an electron beam to create an expanded image that can be studied. in FESEM analysis, the signals generate a two-dimensional image displaying data regarding the sample's external morphology (Vijayasri et al., n.d.).

2.4.7 X-ray Diffraction Analysis (XRD)

X-ray diffractograms of the QIONP were collected on an X-ray diffractometer (XRD) at 25 °C using Cu Kα (1.54060 Å) radiation. With a voltage of 45 kV, an electric current intensity of 40 mA. The X-ray scanning was carried out at diffraction angles (2θ) between 5° and 90°, with increments of 0.02° and a pace of 2° per minute.

2.4.8 Stability Studies

The stability of QINPs was studied by monitoring their time-dependent pH, UV-Vis spectra, and physical appearance for up to 2 weeks, as-synthesized, in a 50:50 ethanol: water solution. Synthesized QINPs (at a concentration of 50mg/mL) underwent 10s of sonication. On day 1, day 7, and day 14, QIONPs spectrum was collected (Prestianni et al., 2023). Drug Release Analysis

2.4.9 Drug Release

Studies were conducted using vertical Franz diffusion cells, with a regenerated cellulose dialysis membrane with a molecular weight cut-off of approximately 12 kDa. The membranes were hydrated in distilled water prior to mounting in the cell. The receptor compartment was filled with an 80:20 (v/v) mixture of water and ethanol to maintain sink conditions and enhance drug solubility, and was maintained at 37 °C throughout the experiment. At predetermined time intervals (e.g., 0, 30, 60, 120, 180, 240 min), aliquots were withdrawn from the receptor compartment and replaced with fresh media to maintain constant volume. The concentration of diffused drug in each sample was quantified using UV-visible spectrophotometry at 370 nm (Salamanca et al., 2018).

2.4.10 Anticancer Activity Assay of QIONPs

The results of the MTT assay performed using the MCF-7 cell line are presented in the appendix. The experiment was conducted in five replicates. An MCF-7 cell suspension was seeded in each well of a 96-well plate at a density of 1×10^5 cells/mL. The plates were incubated for 24 hours at 37°C in a 5% CO_2 incubator to ensure cell adherence.

At the end of the incubation period, sample solutions at different concentrations were added to the cells, and the plates were incubated again. Following the 24-hour treatment period, the MTT viability assay was performed. For this purpose, MTT solution was added to each well, and absorbance was measured at the end of the incubation period.

DMSO solution was used as a positive control, and cell culture medium was used as a negative control. Photometric measurements were performed at 570 nm, and the negative control group was assigned a 100% viability value; cell viability was calculated for the samples.

3. RESULTS

3.1 PREPARATION RESULTS

Quercetin iron nanoparticles were obtained, kept in a vial, in a dry space, at room temperature, away from direct light, as shown in the figure below.

Figure 3.1: Pure Quercetin Solution (Left), IONPs Dispersed in Water (Middle), and Quercetin IONPs (Right).

3.2 CHARACTERIZATION RESULTS

3.2.1 Ultraviolet–Visible Spectrometer

3.2.1.1 Quercetin UV-Vis spectroscopy study.

A UV-Vis Spectrophotometer was used to confirm the formation of QIONPs. The Spectro absorbance curve is shown in the figure below; the maximum absorbance was observed at 312.20 nm, with an absorbance of 1.647 A.

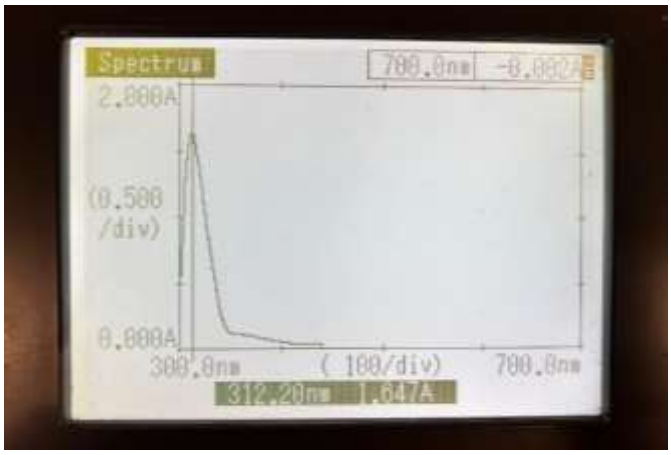

Figure 3.2: Quercetin UV-Vis Spectroscopy.

3.2.1.2 IONPs ultraviolet–visible spectrometer study

Ultraviolet–Visible Spectrometer was used to confirm the formation of QIONPs. The Spectro absorbance curve is shown in the figure below, and the maximum absorbance was observed at 352.60 nm, and the absorbance was found to be 0.837 A.

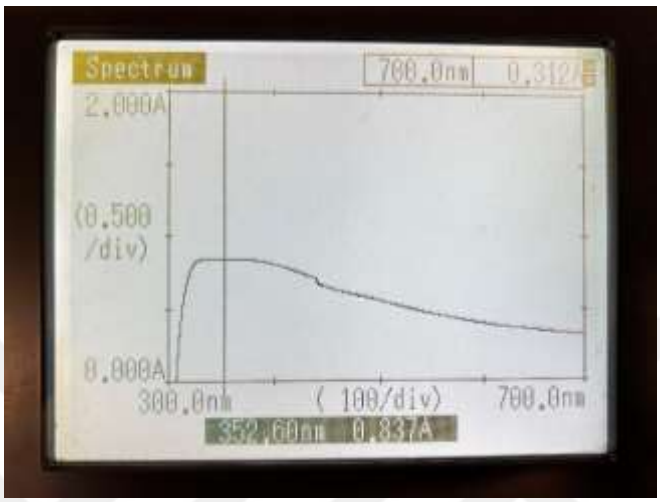

Figure 3.3: IONPs UV-Vis Spectroscopy study.

3.2.1.3 QIONPs ultraviolet–visible spectrometer study

Ultraviolet–Visible Spectrometer was used to confirm the formation of QIONPs. The Spectro absorbance curve is shown in the figure below, and the maximum absorbance was observed at 311.40 nm, and the absorbance was found to be 1.253 A.

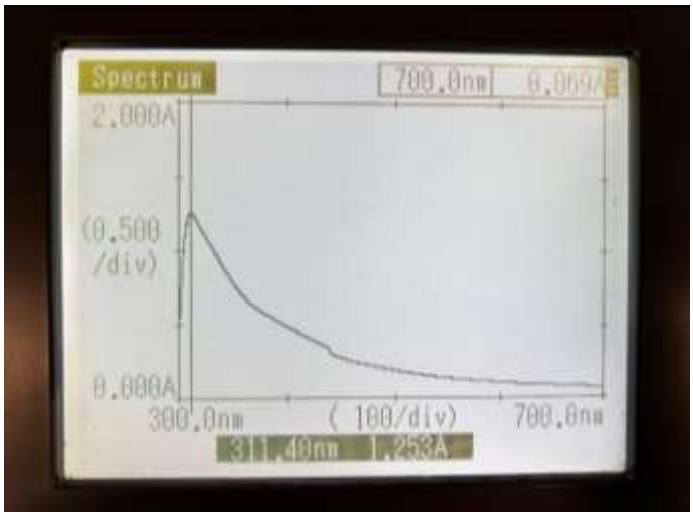

Figure 3.4: QIONPs UV-Vis Spectroscopy Study**.**

3.2.2 Fourier Transform Infrared (FTIR) Spectroscopy

This Fourier transform infrared spectroscopy displays three overlaid spectra labelled as: Iron nanoparticles (green), QIONPs (red), Quercetin (blue). FTIR was applied to identify essential groups and confirm the complex formations between components. In the figure below, the x-axis represents the wavenumber (cm^{-1}), ranging from 4000–400 cm^{-1}, while the y-axis shows % transmittance (%T).

Observations:

i. Broad band around 3400–3200 cm^{-1}

a. Seen in all spectra, but most intense in QIONPs (red) and Quercetin (blue).

b. This corresponds to the O–H stretching vibration, typical of phenolic –OH groups and possibly absorbed moisture.

c. The Iron nanoparticles (green) show a less intense and slightly shifted O–H band, suggesting metal–ligand coordination that reduces hydrogen bonding.

ii. Region around 2900 cm^{-1}

a. Represents C–H stretching vibrations of aliphatic or aromatic groups.

b. All samples show similar weak features, indicating hydrocarbon components are present in each.

iii. Region around 1650–1600 cm^{-1}

a. Prominent peaks for IONPs and Quercetin comp indicate C=O stretching (carbonyl group) and C=C aromatic ring vibrations.

b. In Iron nanoparticles, this band appears slightly shifted and less intense, which supports the formation of a metal–complex (Fe–O or Fe–C=O interaction).

iv. Region 1300–1000 cm^{-1}

a. Strong peaks observed in all spectra.

b. Corresponds to C–O stretching and C–O–C vibrations, characteristic of flavonoid structures (like quercetin).

c. The shifts and intensity changes in the iron nanoparticles spectrum suggest coordination between Fe ions and the oxygen atoms of hydroxyl/carbonyl groups.

v. Region below 800 cm^{-1}

a. Vibrations here (metal–oxygen bonds) are most visible in iron nanoparticles, confirming Fe–O bond formation, a signature of metal–ligand complexation .

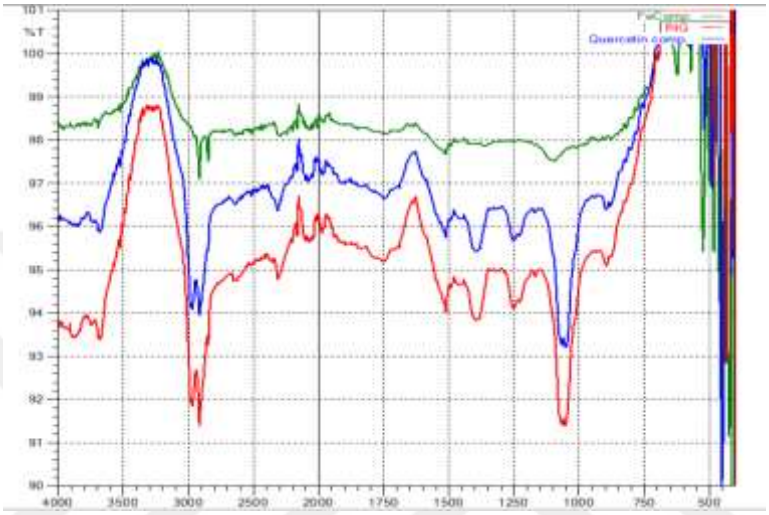

Figure 3.5: Fourier Transform Infrared (FTIR) Spectroscopy.

3.2.3 Size Measurement Using Dynamic Light Scattering (DLS)

DLS analysis of the iron-oxide–quercetin complex revealed a single, intensity-weighted population with an average hydrodynamic diameter of approximately 394.2 ± 6.5 nm.

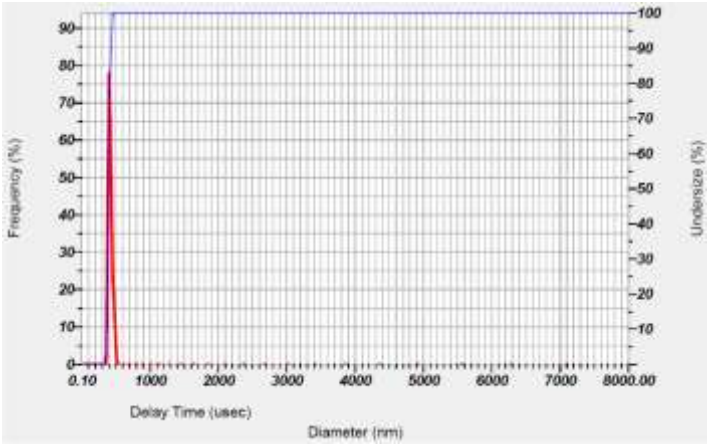

Figure 3.6: Dynamic Light Scattering.

3.2.4 Zeta Potential

The electrophoretic mobility distribution is monomodal and sharply peaked at −56,8 ± 6,7 mV.

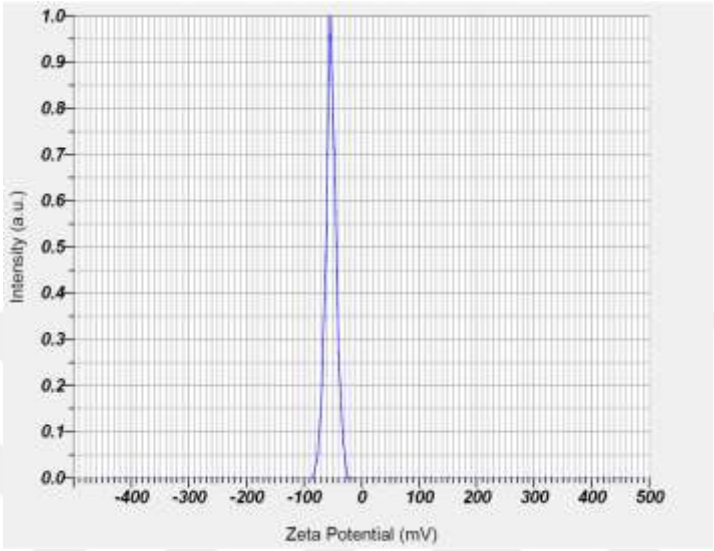

Figure 3.7: Zeta Potential.

3.2.5 pH Analysis Result

It was found that the pH of all formulations was 7.0, as shown in the figure below.

Figure 3.8: pH Analysis.

3.2.6 X-ray Diffraction Analysis (XRD)

3.2.6.1 X-ray diffraction analysis for IONPs

X-ray diffraction (XRD) analysis confirmed the crystalline structure of the synthesized iron oxide nanoparticles. The diffraction pattern exhibited characteristic peaks at approximately $2\theta = 30°, 35°, 43°, 53°, 57°$, and $63°$, corresponding to the (220), (311), (400), (422), (511), and (440) planes of magnetite (Fe_3O_4), in agreement with the standard JCPDS card no. 19-0629. The broadness of the peaks indicates the formation of nanoparticles with small crystallite size. No additional diffraction peaks related to hematite or maghemite phases were observed, suggesting high purity and successful synthesis of crystalline Fe_3O_4 nanoparticles.

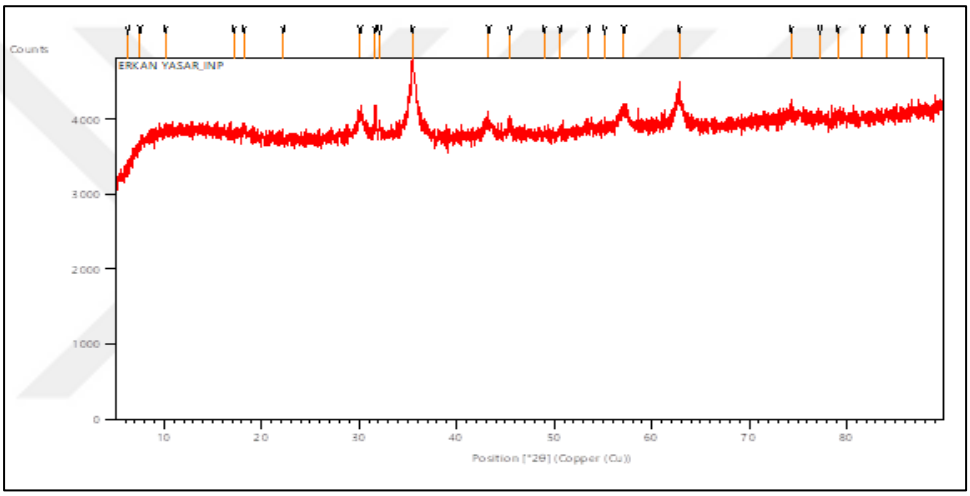

Figure 3.9: X-ray Diffraction Analysis for IONPs (XRD).

3.2.6.2 X-ray diffraction analysis for QIONPs

The XRD pattern of the quercetin-loaded iron oxide nanoparticles (Q-IONPs) shows characteristic diffraction peaks at $2\theta \approx 30°, 35°, 43°, 53°, 57°$, and $63°$, corresponding to the (220), (311), (400), (422), (511), and (440) planes of magnetite (Fe_3O_4) (JCPDS 19-0629). The presence of these peaks confirms that the crystalline structure of the iron oxide core was retained following quercetin loading. Compared with the pure IONPs, the Q-IONP peaks exhibited reduced intensity and increased broadening, which indicates successful surface coating by quercetin and a partial decrease in crystallinity due to the amorphous nature of the quercetin layer. The disappearance of characteristic crystalline quercetin peaks further supports that quercetin was successfully loaded onto the nanoparticle surface in an

amorphous form. No additional peaks related to hematite or other impurities were detected, confirming the purity and structural stability of the synthesized Q-IONPs.

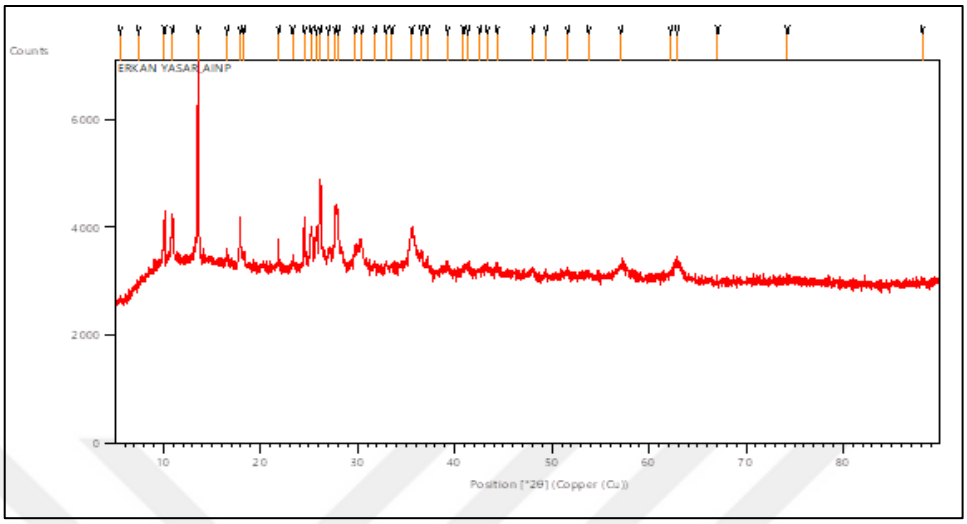

Figure 3.10: X-ray Diffraction Analysis for QIONPs (XRD).

3.2.7 Morphological Structure

3.2.7.1 Morphological analysis for IONP FESEM

The surface morphology of the synthesized iron oxide nanoparticles was examined using scanning electron microscopy (SEM) at a magnification of 500,000×. The SEM micrograph reveals a densely packed, aggregated structure composed of nearly spherical nanoparticles with uniform distribution. The particles exhibit a distinct granular appearance, indicating successful formation of nanosized iron oxide clusters. The average particle size appears to be in the nanometre range, with individual particles measuring approximately 20–40 nm in diameter. The aggregation observed in the micrograph is likely due to the high surface energy and magnetic dipole–dipole interactions among the nanoparticles, which tend to promote clustering. Despite this aggregation, the particles maintain a relatively homogeneous morphology, suggesting controlled nucleation and growth during synthesis. The absence of irregular or elongated structures indicates that the employed synthesis route produced isotropic nanoparticles.

The porous-like network visible between the clusters may contribute to a high surface area, which is advantageous for applications in catalysis, drug delivery, and magnetic resonance imaging. Overall, the SEM analysis confirms the successful synthesis of iron oxide

nanoparticles with nanoscale dimensions, spherical morphology, and uniform distribution, consistent with previously reported features of magnetite and maghemite nanoparticles synthesized via wet chemical or co-precipitation routes.

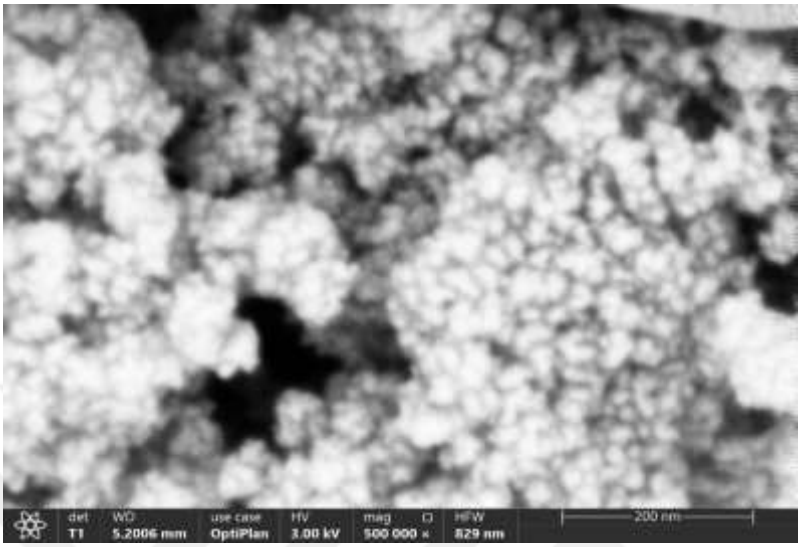

Figure 3.11: Morphological Analysis for IONP FESEM.

3.2.7.2 Morphological analysis for QIONP FESEM

The surface morphology of the synthesized quercetin–iron oxide nanoparticles (QINO) was examined using scanning electron microscopy (FESEM), as presented in Figure below. The micrograph reveals that the nanoparticles predominantly exhibit a different shape. The particles tend to form dense aggregates, which is a common occurrence in nanoscale materials due to magnetic dipole–dipole interactions and van der Waals forces. This aggregation may have been further enhanced by the centrifugation and drying steps during sample preparation, which promote particle–particle contact.

Closer inspection of the surface features shows that the nanoparticles possess a rough and irregular texture, which can be attributed to the surface coating of quercetin molecules on the iron oxide core. The presence of this organic layer is significant, as it not only stabilizes the nanoparticles but also enhances their potential biocompatibility and antioxidant functionality. The quercetin coating likely serves as a capping and reducing agent during synthesis, resulting in the formation of a heterogeneous surface morphology.

In certain regions, small crystalline structures can be observed adhering to or surrounding the nanoparticle clusters. These are indicative of unbound or recrystallized quercetin molecules, confirming partial crystallization during the drying process. The coexistence of these crystalline regions with the iron oxide nanoparticles suggests a successful conjugation between the inorganic and organic components, forming a composite structure. The distinct boundary between the smooth iron oxide cores and the irregular quercetin crystals further supports this observation.

Overall, the FESEM analysis confirms the successful formation of quercetin–iron oxide nanocomposites with distinct morphological features characteristic of hybrid organic–inorganic nanoparticles. The spherical geometry, surface roughness, and visible quercetin crystals collectively verify the integration of quercetin into the nanoparticle matrix and the stabilization of iron oxide cores through surface modification.

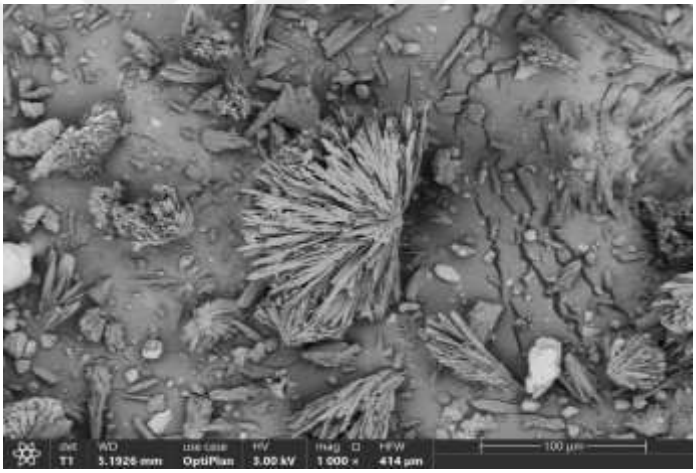

Figure 3.12: Morphological Analysis for QIONP FESEM.

3.2.8 Stability Studies Result

According to the stability studies, the QINPs were found stable during the course of the one-month testing period. Table below presents the outcomes (Prestianni et al., 2023).

Table 3.1: Stability Assay Results.

Days	pH results	Uv vis spectra	Physical appearance
1	7	374.5 nm_ Absorbance 2.531	yellow brown liquid
7	6.7	375 nm _ Absorbance 2.537	yellow brown liquid
14	6.98	375.5 nm _ Absorbance 2.522	yellow brown liquid

3.2.9 Drug Release Analysis

The result was found as shown in the figures below. The calibration curve for the drug was constructed by plotting UV-Vis absorbance at 370 nm against known concentrations of the standard solutions. A linear relationship was observed over the tested range, and linear regression analysis yielded the equation $y = 0.0018x + 0.1227$ with a high coefficient of determination ($R^2 = 0.9804$), indicating excellent linearity for quantification.

The cumulative percentage release of the drug over time was plotted, and the release profile showed a progressive increase in released drug up to ~75% by 270 min. Linear regression of the main release phase gave the equation $y = 0.3318x – 3.3281$ with $R^2 = 0.9817$, suggesting a strong linear correlation over the measured interval. Error bars indicate standard deviation from triplicate measurements.

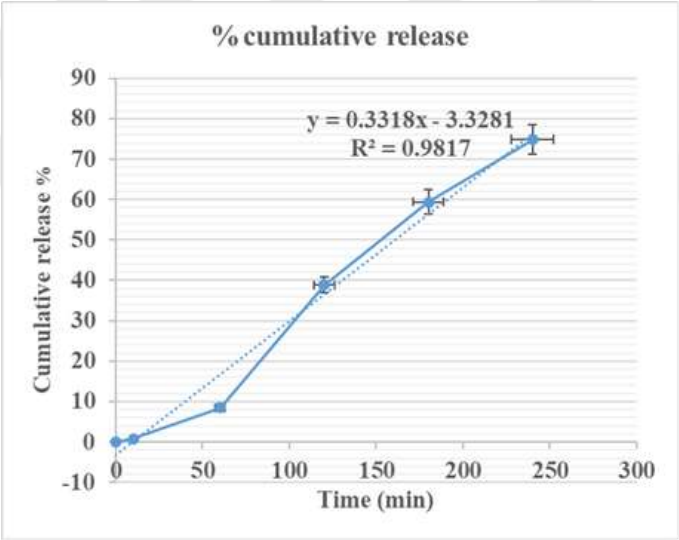

Figure 3.13: Drug Release Analysis (Cumulative Release).

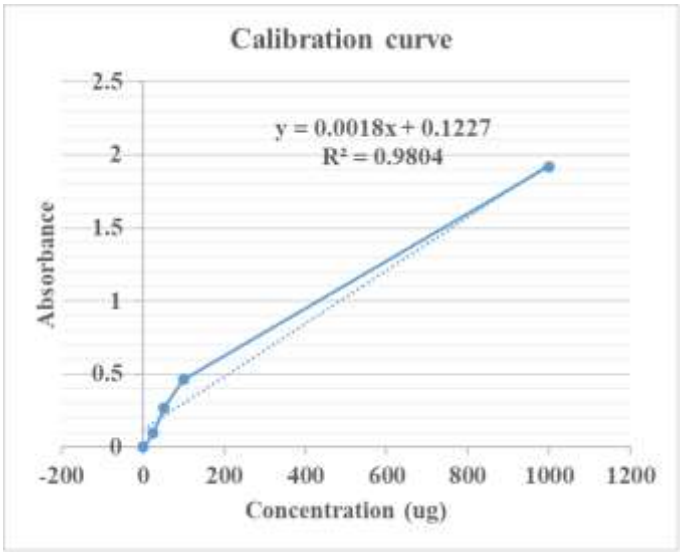

Figure 3.14: Calibration Curve for Drug Release Analysis.

3.2.10 Anticancer Activity Assay Result

The cytotoxicity of 9 distinct concentrations of quercetin-loaded iron oxide nanoparticles (Q-IONPs) was evaluated using the MCF-7 breast cancer cell line. The effects were compared with those of iron oxide nanoparticles (IONPs) and are shown in the Figure below.

Across all tested concentrations, Q-IONPs exhibited the best cytotoxic activity toward MCF-7 cells, followed by quercetin, whereas naked IONPs confirmed minimum cytotoxicity. This confirms that the anticancer effect observed is commonly attributed to the presence of quercetin and is considerably greater when introduced in nanoparticle form.

At the bottom concentrations (1–3), Q-IONPs brought about 18–36% cytotoxicity, while free quercetin resulted in the most effective 3 –7% cellular death, and bare IONPs produced less than 5% cytotoxicity. These findings suggest that even at low doses, Q-IONPs exhibit superior anticancer activity, likely due to improved solubility, stability, and cell uptake compared to free quercetin.

The maximal cytotoxic response was observed at attention 4, where Q-IONPs produced almost 40% cell death. At this factor, quercetin additionally confirmed a slight increase (~15%), whereas IONPs remained low (~10%). This concentration appears to represent an excellent level for nanoparticle–mobile phase interactions, which are crucial for efficient quercetin release within cells.

At intermediate concentrations (5–6), a slight decline in Q-IONP pastime led to cytotoxicity (approximately 26–32% cytotoxicity), suggesting saturation of cellular uptake mechanisms or altered release kinetics at these concentrations. Free quercetin maintained a slight cytotoxic effect (~15%), and IONPs remained at low cytotoxic levels (5–10%).

A high-quality cytotoxicity peak was observed again at awareness 7, where Q-IONPs induced ~38% cell death, and unfastened quercetin reached its maximum impact (~30%). Bare IONPs remained minimally cytotoxic. At higher concentrations (8–9), both Q-IONPs and free quercetin showed a stabilization of cytotoxicity around 28–30%, indicating a plateau segment in which, in addition, increases in attention did not produce proportional increases in mobile death.

Overall, Q-IONPs showed greater cytotoxicity than quercetin at almost all examined concentrations, confirming that nanoparticle encapsulation significantly enhances the anticancer activity of quercetin against MCF-7 cells. IONPs displayed limited cytotoxicity throughout, supporting their biocompatibility and confirming that the observed cytotoxicity is mainly mediated by quercetin. These findings together highlight Q-IONPs as a promising transport platform for improving the healing capacity of quercetin in breast cancer treatment.

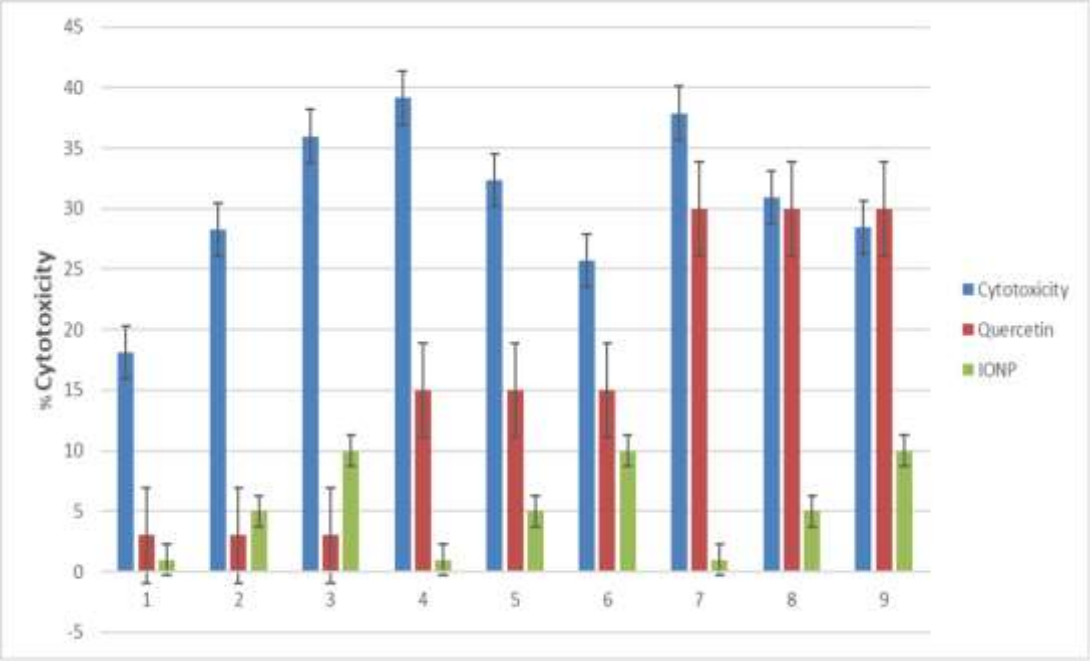

Figure 3.15: Anticancer Activity Assay Result.

4. DISCUSSION

Cancer is the second leading cause of death worldwide, and the conventional therapies as chemotherapy, surgery, and radiation, have many undesirable side effects and many restrictions, such as MDR, poor bioavailability, and lack of selectivity. To overcome these limitations, nanotechnology is a promising approach for cancer therapy.

This research synthesizes iron oxide nanoparticles via the co-precipitation method, using the reaction between $FeCl_3$ and $FeSO_4$, adding NaOH to the mixture to obtain black IONPs, and then prepares a Quercetin: Iron oxide Nanoparticle in the ratio of 3:1 to evaluate the nanoparticles' physicochemical properties. Three different weights of Quercetin powder (3, 15, 30 mg) were added to each of three different samples of IONPs (1, 5, 10 mg) to obtain a total of nine samples of quercetin-iron oxide Nanoparticles. and examine the effects of nine QIONP concentrations on the MCF7 breast cancer cell line using the MTT assay.

The synthesis of Iron oxide nanoparticles gives a solution with a black participate, which indicates the formation of nanoparticles as (Kumar et al., 2021) approved. A solution of quercetin led to a clear yellow solution. The quercetin iron oxide nanoparticle led to the smooth dispersion of the liquid. When quercetin was added to iron oxide, the colour changed from yellow to brown, indicating that interactions had occurred.

UV–Visible spectroscopy was performed to make sure that quercetin was attached to the iron oxide nanoparticles and to confirm the long-term stability of the Nanoparticle dispersion. The spectrum of pure quercetin showed a strong absorption peak at about 312 nm (Ravichandran et al., 2014), This spectral change indicates the establishment of coordination bonds between iron ions and the hydroxyl groups of quercetin, aligning with prior research on flavonoid–metal oxide complexes. Consequently, UV–Vis analysis offers compelling evidence for the effective synthesis of quercetin-coated iron oxide nanoparticles.

The Fourier transform infrared results clearly show the disappearance or shifting of the characteristic quercetin peaks in iron coordination. The appearance of new bands below 800 cm^1 is consistent with an iron-oxygen expansion. A reduction in O–H band intensity, indicating that the phenolic groups are involved in metal binding. Hence, the data confirm the formation of an Fe–Quercetin complex, in which Fe likely coordinates to the hydroxyl and carbonyl oxygen atoms of quercetin. Such complexation can enhance stability, modify

antioxidant activity, and influence biological properties. Quercetin (blue) shows the standard spectrum of a flavonoid compound with characteristic –OH, C=O, and C–O bands. INQ (red) shows a similar pattern, likely representing a quercetin intermediate or precursor. Iron nanoparticles (green) display notable shifts and intensity changes at the O–H, C=O, and C–O regions, indicating successful complexation between Fe and quercetin.

The measured size likely reflects the hydrodynamic radius of clustered nanoparticles (core + ligand Shell composed of quercetin flavonoid) rather than the size of individual crystalline cores. The narrow peak indicates the monodispersity of the nanoparticles. The small size of the nanoparticles indicates successful formation.

For the zeta potential assay, a very large negative value was found. In practical colloid science, thresholds are often quoted as: $|\zeta| \geq 30$ mV good stability, $|\zeta| \geq 60$ mV excellent electrostatic stability. A value near $-56{,}8 \pm 6{,}7$ mV indicates good stability and strong electrostatic repulsion between particles, suggesting the suspension should be highly stable against aggregation under the measurement conditions. A single sharp peak means the sample is dominated by a single population with uniform surface charge. Although zeta potential measurements indicate a strong negative surface charge predictive of electrostatic stabilization the relatively large DLS size suggests the presence of stable aggregates or quercetin-mediated bridging between particles.

The pH measurement was performed to assess the chemical environment and stability of the nanoparticle suspensions. The iron oxide nanoparticles had a high pH (about 11), as expected from the alkaline conditions during mixing with NaOH. The pure quercetin solution had a pH that was close to neutral, while the quercetin–iron oxide nanoparticle suspension had a pH of 7, which is slightly basic. This drop in pH after conjugation indicates that some surface hydroxyl groups have been neutralized, as quercetin's phenolic hydroxyls interact with Fe ions. The slightly basic pH also indicates a stable colloidal dispersion that can be used for biological testing later.

X-ray diffraction (XRD) analysis was performed to investigate the crystalline nature and phase purity of the synthesized nanoparticles. The XRD pattern of the iron oxide nanoparticles showed distinct diffraction peaks at 2θ values of 30.1°, 35.5°, 43.1°, 53.4°, 57.0°, and 62.6°, corresponding to the (220), (311), (400), (422), (511), and (440) planes of

the cubic spinel structure of magnetite (Fe_3O_4). These results confirm the successful synthesis of crystalline magnetite nanoparticles. After conjugation with quercetin, the main diffraction peaks remained visible, although their intensity and amplitude were slightly reduced, indicating that the crystalline phase was preserved while the quercetin coating introduced a thin amorphous layer onto the nanoparticle surface. These results are consistent with previous reports on quercetin-functionalized Fe_3O_4 nanoparticles, indicating a successful coating without compromising structural integrity.

FESEM is a powerful imaging technique used to study the surface morphology and structural properties of various materials. Field Emission Scanning Electron Microscopy (FESEM) was used to examine the surface morphology and particle size distribution of the nanoparticles we prepared. The FESEM images of iron oxide nanoparticles should show particles that are almost spherical and tend to group together due to magnetic interactions. The quercetin–iron oxide nanoparticles are expected to have a similar shape, but the surface will be smoother, and the particles will stick together less because of the quercetin coating. This change to the surface probably stabilizes the particles by making it harder for them to attract each other directly with magnets. The measured particle sizes are expected to fall within the nanoscale range, consistent with the findings from the DLS analysis. Previous research on quercetin-functionalized magnetite nanoparticles has documented similar morphological traits, affirming that the organic layer does not substantially alter the core shape but does improve dispersibility and stability in aqueous environments. In general, FESEM characterization provides direct visual evidence that nanoparticles have formed and that their surfaces have been functionalized, corroborating findings from UV–Vis, FTIR, and XRD analyses.

The stability of the quercetin–iron oxide nanoparticles was assessed over several weeks by monitoring pH, UV–Visible absorbance, and physical appearance. The UV–Vis spectra taken at 374.5 nm and 375 nm over three different measurements showed very little change in the characteristic absorption peak of quercetin. This means there was no significant aggregation or degradation during storage. This steady absorption profile indicates that the nanoparticles' optical properties have been preserved and suggests that the quercetin coating effectively protected the iron oxide core from oxidation and sedimentation.

The pH of the suspension remained fairly stable during the testing period, changing only slightly from 7.00 to 6.68 and 6.98. These minor variations are acceptable for colloidal dispersions and show that there have been no significant chemical changes or the release of acidic degradation products. The samples' physical appearance remained unchanged, with no sedimentation, color change, or precipitation observed during the study.

The overall stability indicators indicate that the QIONPs remain stable over time under normal conditions. The preservation of optical, chemical, and physical properties over time indicates that quercetin serves not only as a bioactive molecule but also as an efficient capping agent, improving the long-term stability of the nanoparticle suspension. These results corroborate earlier studies that emphasize the function of flavonoid coatings in enhancing the dispersion and chemical stability of metal oxide nanoparticles

The drug release profile demonstrated sustained increases in drug diffusion through the dialysis membrane over the duration of the experiment. The release kinetics exhibited a predominantly linear phase after an initial lag, as shown by the high R^2 value of the regression line (*0.9817*), suggesting a diffusion-controlled process. The dialysis membrane with a 12 kDa MWCO provided an effective barrier, limiting the passage of free drug molecules and ensuring the observed release reflected diffusion rather than bulk flow. The calibration curve exhibited a strong linear relationship ($R^2 = 0.9804$), validating the UV-Vis method at 370 nm for quantitative analysis of samples. These findings are consistent with the literature, which reports that Franz diffusion cells and UV-Vis quantification are successfully used to characterize drug permeation and release profiles under physiological conditions, confirming the reliability and reproducibility of the experimental setup.

The 3-(4,5-dimethiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test: Measuring cell viability and proliferation forms the basis of many laboratory tests that assess a cell population's response to external factors. The MTT cell proliferation test measures the rate of cell division, and conversely, when metabolic events lead to apoptosis or necrosis, the rate of cell viability decreases. The MTT test is a colorimetric assay that measures the decrease in yellow MTT by the enzyme mitochondrial succinate dehydrogenase.

The present study demonstrates that quercetin-loaded iron oxide nanoparticles exhibit a significantly more potent cytotoxic effect against MCF-7 breast cancer cells than

unencapsulated quercetin or naked iron oxide nanoparticles. This enhancement was consistently observed across all nine examined concentrations, confirming the effectiveness of nanoparticle-mediated delivery in improving quercetin's anticancer activity.

Quercetin is known for its anticancer properties; however, its healing utility is constrained by its poor aqueous solubility, chemical instability, and low cell permeability. The markedly low cytotoxicity observed with unfastened quercetin at most concentrations, particularly at lower levels, aligns with these recognised limitations. In contrast, encapsulation within iron oxide nanoparticles significantly enhanced quercetin's bioavailability and cellular uptake, resulting in a 2- to 6-fold increase in cytotoxicity at the highest concentrations. This finding is consistent with previous research showing that nanoparticle-based formulations enhance the internalization of hydrophobic flavonoids and facilitate sustained intracellular release, thereby increasing their cytotoxicity against cancer cells. The maximum cytotoxicity was observed at awareness 4, where Q-IONPs caused almost 40% cell death. This attention likely represents the greatest balance between nanoparticle uptake and intracellular quercetin release. Interestingly, a slight decrease in cytotoxicity was observed at concentrations 5 and 6, as evidenced by a rise at awareness 7 and a subsequent plateau at higher concentrations. Such fluctuations may also replicate differences in nanoparticle aggregation, cell uptake saturation, or variations in quercetin launch kinetics at certain concentrations. The plateau observed at the highest concentrations indicates that, after an important intracellular threshold of quercetin is reached, increases in dose do not proportionally increase cytotoxicity because cells have already reached their maximum sensitivity.

The minimum cytotoxicity of bare IONPs across all concentrations indicates that the iron oxide middle is inherently biocompatible and does not induce significant toxicity on its own. This supports their suitability as a safe nanocarrier and confirms that the anticancer effect observed is primarily due to quercetin rather than the nanoparticle matrix. These findings also align with previous reviews describing the low cytotoxicity of magnetite nanoparticles at comparable concentrations.

Taken collectively, the results strongly suggest that conjugating quercetin to iron oxide nanoparticles successfully overcomes the pharmacological limitations of unencapsulated quercetin by enhancing its solubility, stability, and cellular uptake in MCF-7 cells. The stepped forward cytotoxicity of Q-IONPs highlights their capability as a promising healing

platform for breast cancer treatment. Future paintings ought to encompass additional mechanistic research to examine apoptosis induction, reactive oxygen species generation, and cell cycle arrest, as well as in vivo evaluation to further validate the therapeutic potential of this Nano formulation.

5. CONCLUSION

Modern researchers have sought to synthesize and evaluate quercetin-loaded iron oxide nanoparticles (Q-IONPs) for potential anticancer applications. The synthesis is efficiently performed with the aid of co-precipitating ferrous ($FeSO_4$) and ferric ($FeCl_3$) salts, using sodium hydroxide as a precipitating agent, and is eventually accompanied by functionalization with quercetin. The formation of a solid quercetin–iron oxide complex was evident from distinct color changes and confirmed by spectral and physicochemical analyses. The UV–Vis spectroscopy evaluation showed a clear absorption peak at 311.40 nm, which indicated that quercetin had been effectively loaded onto the iron oxide surface. The pH stability indicates that the substance was very stable in everyday situations. The nanoparticle suspension's physical appearance remained unchanged, further indicating that it had become stable. The X-ray diffraction (XRD) sample confirmed that the iron oxide middle transformed into a crystalline state, and the presence of quercetin did not alter the crystal lattice, indicating that the surface change occurred rather than breaking the structure. Field Emission Scanning Electron Microscopy (FESEM) analysis revealed nearly spherical nanoparticles with a uniform size distribution, confirming successful synthesis and morphological consistency. The MTT assay performed on MCF7 breast cancer cells showed that the quercetin-loaded nanoparticles displayed a dose-dependent cytotoxic effect, markedly reducing cell viability compared to controls. These effects show that combining quercetin with iron oxide nanoparticles makes it even more powerful against most cancers, likely because it allows cells to take up the active compound more effectively and release it in a controlled manner. In the end, this study shows that iron oxide nanoparticles loaded with quercetin possess the desired physicochemical properties, are stable, and exhibit promising anticancer activity. This indicates that they will be useful nanocarriers for most cancer treatments. More research is needed to investigate ways to improve the scale and floor functionalization of nanoparticles to enhance their loading in tablets and their targeting of specific cells. Comprehensive in vitro and in vivo studies are cautioned to examine cell internalization mechanisms, pharmacokinetics, and biodistribution. Additionally, assessing the therapeutic efficacy of these nanoparticles against diverse cancer cell strains and integrating targeted ligands may facilitate their development toward clinical applications in nanomedicine.

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