Formulation and *In vitro* characterization of Quercetin Loaded Solid Lipid Nanoparticles

by

Rubayet Rafsan-22146078

Md. Mohiyminul Hassan-22146084

Jafrin Akter Mim-22146028

Md Sabbir Hosen Sohan-22146007

A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements of the degree of

Bachelor of Pharmacy (Hons.)

School of Pharmacy

BRAC University

April 2026

© 2026. BRAC University

All rights reserved.

Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at BRAC University.

2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.

4. I/We have acknowledged all main sources of help.

Student's Full Name & Signature:

_____ _____

Rubayet Rafsan
22146078

Jafrin Akter Mim
22146028

_____ _____

Md. Mohiyminul Hassan
22146084

Md Sabbir Hosen Sohan
22146007

Approval

The thesis/project titled "Formulation and *In vitro* characterization of Quercetin Loaded Solid Lipid Nanoparticles" submitted by Rubayet Rafsan-22146078, Md. Mohiyminul Hassan-22146084, Jafrin Akter Mim-22146028 & Md Sabbir Hosen Sohan-22146007 of Spring 2026 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

Supervised By: _____

Dr. Zara Sheikh, PhD
Assistant Professor, School of Pharmacy
BRAC University

Approved By:

Chairperson _____

Professor Sharmind Neelotpol, PhD
Chairperson, School of Pharmacy
BRAC University

Ethics Statement

This project does not involve any kind of animal and human trial.

Abstract

Quercetin is a common bioflavonoid with various potent therapeutic effects such as antioxidant anti-inflammatory and anticancer activity. However, the use of quercetin is limited owing to its low water solubility, limiting its oral bioavailability and in vivo beneficial functions. To enhance the bioavailability of quercetin, solid lipid nanoparticles of the quercetin were developed using the ultra-speed homogenization technique. Stearic acid has been used in the formulation as a solid-lipid matrix and Tween-80 as a surfactant, comprising the aqueous part of the formulation. 16 different types of formulations (labelled as F1-F16) were prepared and optimized by varying the amount of stearic acid, Tween 80 and rotational speeds of the homogenizer. Among the 16 formulations, F5 formulation (composition: 25 mg of quercetin drug, 0.5 gm of stearic acid, 2% of Tween 80) and was found to be the optimum formulation with the highest encapsulation efficiency of 80% and further displaying a sustained release profile following a 2-hour dissolution study. The present study suggests that high-speed homogenization technique is a suitable method to develop solid-lipid nanoparticles of Quercetin with high entrapment efficiency. Further work is required to determine the efficacy of the formulation in terms of particle size, zeta potential, release profile, morphology using scanning electron microscopy, in vitro antioxidant study, anti-inflammatory study, and bioequivalence study in animal models.

Keywords : Quercetin, Solid lipid nanoparticles, Encapsulation efficiency, Sustained release, homogenization, ultrasonication, dissolution study

Dedication

Dedicated to my Supreme Creator, beloved parents, and my siblings who constantly support me on this journey. I also extend my sincere gratitude to a special person and to my dear friends for their constant encouragement, motivation, and support during the completion of this work.

Acknowledgement

I am sincerely grateful to my respected supervisor, Dr. Zara Sheikh, PhD, Assistant Professor at the School of Pharmacy, BRAC University, for her invaluable guidance, constructive feedback, and unwavering support throughout this project. Her patience, encouragement, and insightful mentorship greatly helped me overcome challenges and successfully complete this thesis. I would like to express my heartfelt gratitude to Professor Arshad Mahmud Chowdhury, PhD, Dean and Professor Sharmind Neelotpol, PhD Chairperson, for their encouragement and leadership, as well as all faculty members.

Table of Contents

List of Tables

List of Figures

List of Acronyms

ADME(T) — Absorption, Distribution, Metabolism, Excretion and Toxicology

API / APIs — Active Pharmaceutical Ingredient(s)

COX — Cyclooxygenase

CT26 — Colon Tumor Cell Line 26

DL — Drug Loading

Dox — Doxorubicin

EE — Entrapment Efficiency

EMT — Epithelial–Mesenchymal Transition

H_2O_2 — Hydrogen Peroxide

IL-1α — Interleukin-1 alpha

IL-8 — Interleukin-8

LPS — Lipopolysaccharide

LOX — Lipoxygenase

MAPK — Mitogen-Activated Protein Kinase

MC38 — Mouse Colon Cancer Cell Line 38

MCF-7 — Michigan Cancer Foundation-7

mRNA — Messenger Ribonucleic Acid

NAD(P)H — Nicotinamide Adenine Dinucleotide Phosphate

NQO1 — NAD(P)H Quinone Oxidoreductase 1

NPs — Nanoparticles

PLGA — Poly(lactic-co-glycolic acid)

QC — Quercetin

QLSN / QSLN / QSLNs — Quercetin-loaded Solid Lipid Nanoparticles

ROS — Reactive Oxygen Species

RPM — Revolutions Per Minute

SA — Stearic Acid

SH-SY5Y — Human Neuroblastoma Cell Line

SLN / SLNs — Solid Lipid Nanoparticle(s)

SLN-Qt — Solid Lipid Nanoparticles loaded with Quercetin

TIMPs — Tissue Inhibitors of Metalloproteinases

TNF-α — Tumor Necrosis Factor Alpha

USP — United States Pharmacopeia

UV-Vis — Ultraviolet–Visible (Spectrophotometry)

Chapter 1

Introduction

Chapter 1. Introduction

1.1 Introduction

In recent times, there has been a significant increase in research centered around the utilization of natural bioactives for the management of chronic ailments owing to their low toxicity and environmentally friendly properties. A diverse range of chemical compounds and phytoconstituents with potential pharmacological and biological activities have been extracted and discovered from medicinal herbs in recent years [1]. Quercetin (3,5,7,3′,4′-pentahydroxyflavone) is a natural flavonoid, widely present in plants in nature including apples, berries, vegetables, capers, grapes, onions, spring onions, tea and tomatoes and is also present in many seeds, nuts, flowers, barks and in leaves has been the subject of extensive research owing to its diverse pharmacological properties including antioxidant, antiviral, immune-modulatory, and anticancer activity with a low toxicity profile . Chemically, quercetin is composed of three benzene rings and five hydroxyl groups and the most abundant source of quercetin is shown in Figure 1. Although there are a number of reports demonstrating the therapeutic efficacy of quercetin, however, due to lack of clinical data and an exact understanding of its mechanism of action, quercetin has not yet received the status of an established molecule in the pharmaceutical industry [2].

Figure 1. Main Sources of quercetin

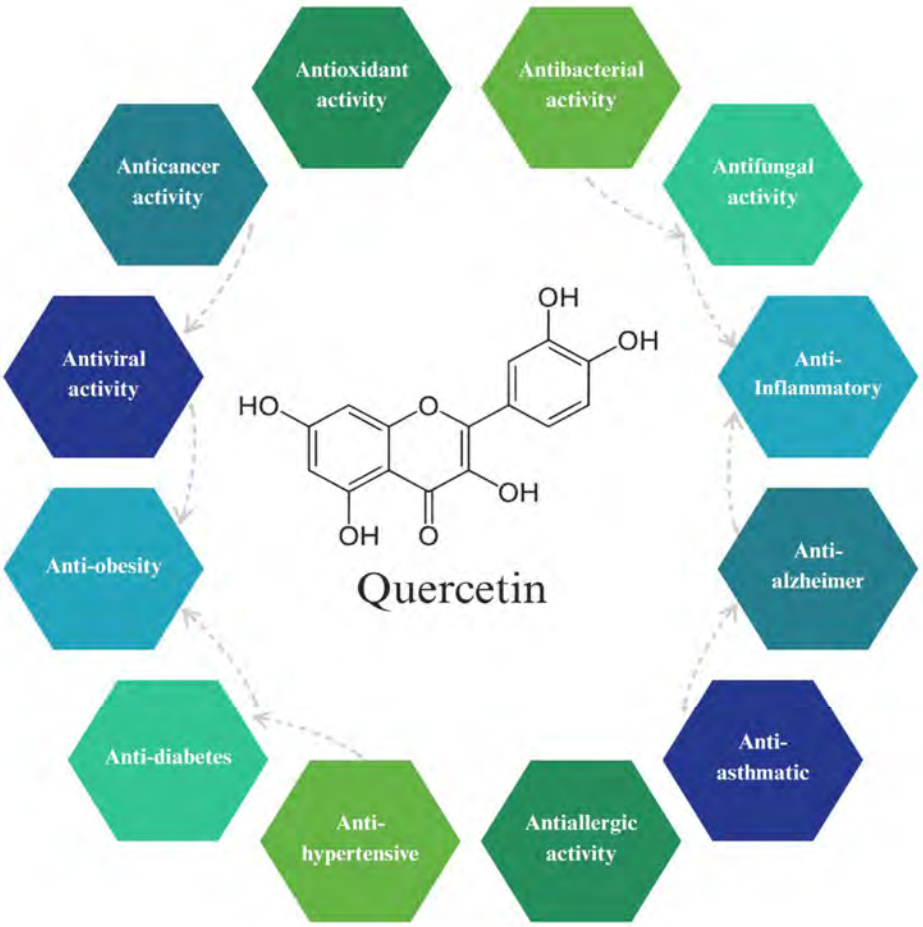

Figure 2. Quercetin Structure and Potential Therapeutic Applications

1.2 Sources of Quercetin

Quercetin is widely distributed in various plant-based foods and main plant sources of quercetin which contain the highest amount of quercetin include capers, rocket, dill, coriander, fennel, juniper berries, corn poppy, bee pollen and okra [3]. Plant sources of quercetin include plant species, including *Santalum album*, *Mangifera indica*, *Emblica ofcinalis*, *Curcuma domestica valenton*, *Withania somnifera*, *Foeniculum vulgare*, and *Cuscuta refexa* [4]. Quercetin is frequently utilized as a dietary supplement in capsule and powder form due to its extensive range of pharmacological effect [5]. Depending on consumption of vegetables, tea, and fruits quercetin intake varies from 50 to 800 mg per day; other nations reported varying dietary intakes. Among the investigated foods, it has been discovered that onions contain the most quercetin (approximately 300 mg/kg) [6].

Quercetin and kaempferol were also present in other plants, such as broccoli and kale, albeit in considerably lesser amounts. However, tea has a high catechin content but a low quercetin flavonoid content. As red grapes, cherries, and blueberries have a substantial number of different anthocyanins, the color of fruits and vegetables reflects the amounts of flavonoids present. Additionally, most of these fruits include flavonoids, particularly quercetin. According to reports, the average daily consumption of quercetin in nations like the USA, Spain, Japan, and China is 9.75, 18.48, 16.2, and 18 mg [7].

1.3 Pharmacological Properties of Quercetin

1.3.1 Antioxidant Properties

Quercetin is well-known for its antioxidant properties according to several published reports [8], [9]. Hydrogen peroxide is a key reactive oxygen species in many neurological disorders and the

presence of quercetin showed reduction in lactate dehydrogenase and H_2O_2-mediated cytotoxicity in human neuronal SH-SY5Y cells [10].Additionally, quercetin significantly increased the amount of the antiapoptotic gene Bcl-2 and reduced the level of the pro-apoptotic gene Bax in neural cells, suggesting that quercetin could prevent neurological diseases resulting from oxidative stress and apoptosis [11]. Quercetin is known to prevent progression of different types of cancer including brain, liver, lung, colon, prostrate, breast and cervical cancer by controlling antioxidant enzymes and oxidative stress factors [12], [13], [14], [15]. Quercetin has been demonstrated to have antioxidant and hepatoprotective effects against acute liver damage induced by tertiary butyl hydrogen peroxide in *in vivo* investigations. Quercetin has demonstrated to efficiently shield cells against radiation-induced damage and genetic toxicity by removing free radicals and elevating the amounts of endogenous antioxidants [16]. Table 1 shows a summary of the antioxidant properties of Quercetin.

Table 1. Antioxidant Properties of Quercetin

Antioxidant Agent	**Activity Carried Out**	**Key Findings**	**Study Models**
Quercetin	Aging ovary-oxidative stress	Related genes are more prevalent	Animal models/in vivo
Quercetin	H_2O_2	Quercetin has neuroprotective properties in addition to reducing the cytotoxicity of H_2O_2	Clinical trial/in vitro

Onion extract plus six different types of quercetin	DPPH	There was an increase in the antioxidant activity of quercetin-3-O-glucuronide, isorhamnetin, tamarixetin, and quercetin	In vitro
Quercetin with Hesperidin	Hydrogen peroxide radical, DPPH, hydroxyl radical, nitric oxide, reducing power assay, and superoxide	Enhanced effectiveness of both substances against DPPH	In vitro
Quercetin and its glucosides	Hydroxyl groups' function in DFT	The antioxidative exercises are predominantly OH, groups in the C-ring and B-ring	In vitro
Quercetin	Oxidative stress and BP in factors in urine and blood	No effects on oxidative stress; BP reduction	Clinical trial/ex vivo
Quercetin	Anti-reactive oxygen species (ROS)	GSH level regulated; decreases MDA levels while increasing SOD activity	Animal model/in vivo
Quercetin	Anti-free radical	Scavenging DNA from the free radicals	In vitro
Quercetin	Anti-aging	Reduced the severity of mouse senescence and lengthened the life of *C. elegans* by 15%	Animal model/in vivo
Quercetin with Na^+, K^+-ATPase	Anti-aging in humans	Influences the activity of ATP-dependent protein transporter	Clinical trial/in vitro

Quercetin	Cancer treatment	The antioxidant activity of quercetin caused inhibition of tumor growth	Milad
Quercetin	Anti-aging	Prevention of developing eye diseases such as macular degeneration and cataracts	Clinical trial/in vitro
Quercetin	Antidepressant activity	Increased 5-HT levels and prevented MAO-A levels in brain	Animal model
Quercetin	Antidepressant activity	Quercetin treatment with a concentration of 50 mg/kg for 8 weeks restored the levels of serum elements	Animal model
Quercetin	Antidepressant activity	Lipid hydroperoxide levels in the hippocampus rose after olfactory bulbectomy, proving improved depression in rats	Animal model
Quercetin	Anti-non-alcoholic liver	Liver protection from NASH by decreasing the levels of CYP2E1	Animal model
Quercetin	Anti-free radical	Maintains homeostasis in the human body by removing free radicals	In vivo
Quercetin	Fatty liver treatment in non-alcoholics	Prevention of NAFLD and liver steatosis	In vivo

Dihydroquercetin	Renal protection	Regulating the nuclear factor 2 (Nrf2) signaling pathways connected to erythroid 2 (Nrf2)	Animal model/in vivo and in vitro
Quercetin	Quercetin treatment in kidney and tumor tissues	Regulated mitogen-activated protein kinases (MAP kinases)	Animal model/in vivo
Quercetin	Kidney disorder	Negatively regulated the phosphatidylinositol 3-kinase (PI3K/Akt)	Animal model/in vitro
Quercetin	Kidney fibrosis treatment	Activated B-cell nuclear factor kappa light-chain enhancer hedgehog	Animal model/in vivo and in vitro
Quercetin	Anti-inflammatory	Participated in the overall biological activity of a quercetin-rich diet	Ex vivo

1.3.2 Anticancer activity

Several studies have shown quercetin to have anti-cancer properties by inducing apoptosis and cell cycle arrest in different cancer cell lines such as those of breast, prostate, lung, and colon cancers. In a published study by Minei et al, 2016, it has been demonstrated that administration of nano-quercetin to MCF-7 breast cancer cells augments mRNA expression and apoptosis [17]. Additionally, it was shown that quercetin decreased the expression of genes multidrug resistance

protein 1 and NAD(P)H quinone oxidoreductase 1 and sensitized MCF-7 cells to the chemotherapy medication doxorubicin (Dox) [18]. According to a study, quercetin inhibited cell viability in colon 38 (MC38) and colon 26 (CT26) cells, induced apoptosis in CT26 cells through the MAPK pathway, inhibited cell viability through the MAPK pathway, inhibited cell viability through the MAPK pathway in CT26 cells, and regulated the expression of EMT markers by nontoxic doses of quercetin. Inhibiting CT26 cells' migration and invasion abilities by inhibiting their expression of tissue inhibitors of metalloproteinases (TIMPs) inhibits their invasion and migration abilities [19]. Studies demonstrating anti-cancer properties of Quercetin is summarized in Table 2.

Table 2. Anticancer properties of Quercetin

Cancer Types	**Mechanisms**
Lung cancer	BCL2/BAX-mediated necrosis, apoptosis, and mitotic catastrophe are all triggered Inhibits the ability of A549 cells to migrate;
Lung cancer	Increases miR-21 expression and inhibits [Cr(VI)]
Lung cancer	In nanoparticle form, improved antitumor effect
Gastric cancer cell lines	Combination treatment results in improved anticancer effects
13 HCC liver cancer cell line	Both substances' combined anticancer effects
Ovarian cancer mouse model	Hydrogel of quercetin demonstrates improved apoptosis

EMT6 breast cancer cell line	In combined form, synergistic antitumor effects
Breast cancer	Slows cell cycle progression and increases cell apoptosis; p51, p21, and GADD45 signaling activity are upregulated, as is FasL mRNA expression
Colon cancer	ERK activation triggers G2 phase arrest and autophagic cell death
Kidney cancer	Increases the cytotoxic effects of DOX and guards against nephrotoxicity caused by DOX; reduces the expression of TNF, IL1B, iNOS, and caspase-3 in the kidneys
Thyroid cancer	Reduces chymotrypsin-like proteasome activity; raises the rate of apoptosis and decreases the rate of cell proliferation via activating caspases;reduces the concentration of Hsp90
Eye cancer	Reduces the secretion of VEGF, RPE cell proliferation, and migration dose-dependently; VEGF production generated by CoCl2's hypoxia is prevented
Brain cancer	Hsp27 inhibition reduces COX2 expression and serves as both a COX2 and Hsp27 inhibitor
Neck and head cancer	HSC3 cell colony growth is reduced; retards MMP2 and MMP9 levels
Gastric cancer	EBNA1 and LMP2 protein expression from EBV is inhibited
Prostate cancer	Prevents vimentin and N-cadherin expression that is stimulated by TGF-β; reduces Twist, Snail, and Slug expression when TGF-β is present in the prostate cancer 3 cell line
Pancreatic cancer	Cellular FLICE-like inhibitory protein expression is decreased

Skin cancer	Blocks the NF-kB activation and COX-2 up-regulation caused by UVB light in the Hacat cell line
Bone cancer	Reduces the expression of cyclin D1 in U2OSPt and SKOV3 cells
Liver cancer	Induces apoptosis
Breast cancer	Increases the amounts of cleaved caspases 8 and 3; inhibits the manufacture of phosphorylated JAK1 and STAT3; -reduces the activity of the STAT3-dependent luciferase reporter gene in BT474 cells.

1.3.3 Anti-inflammatory and Immunosuppressive effects

Quercetin has proved to be a long-standing anti-inflammatory agent both in animal and human models in different cell types [20]. In addition to a wide range of biochemical and pharmacological activities, quercetin has been repeatedly shown to exert anti-inflammatory effects on endothelial and monocyte/macrophage systems *in vitro* [21]. Li et al. [22]conducted experiments in different animal models and found that quercetin inhibited the production of tumour necrosis factor alpha (TNF-α) induced by lipopolysaccharide (LPS) in macrophages [23] and lung A549 cells LPS-induced IL-8 production [24] Furthermore, it has even been shown in glia cells that quercetin can suppress LPS-induced mRNA levels of TNF-α and interleukin- (IL-)1α: neuronal cell death is also reduced [25]. Quercetin can inhibit the enzymes that produce inflammation (cyclooxygenase (COX) and lipoxygenase (LOX) [26]

1.3.4 Anti-Alzheimer activity

Quercetin has also shown potential health benefits against Alzheimer's disease due to inhibition of acetylcholinesterase [27]. One key mechanism is the reduction of oxidative stress in the brain, which is caused by an imbalance between reactive oxygen species (ROS) and antioxidant defense mechanisms. Quercetin acts as a potent antioxidant by scavenging ROS, inhibiting lipid peroxidation, and enhancing the activity of antioxidant enzymes that enables to counteract oxidative stress and protect against neurodegenerative processes that contribute to Alzheimer's disease. Another known mechanism is the modulation of neuroinflammation, which is a hallmark of Alzheimer's disease. Quercetin inhibits the activation of microglial cells and reduces the production of pro-inflammatory cytokines, thereby mitigating the detrimental effects of chronic inflammation in the brain. Overall, quercetin may exert its anti-Alzheimer effects by reducing oxidative stress and modulating neuroinflammation [28], [29].

1.4 Rationale and Aim of the Research

Quercetin has been widely tested against diseases like tumor, diabetes, obesity, neurological and cardiovascular diseases because of its vast therapeutic potential including antioxidant and anti-inflammatory properties [30]. But poor solubility and lower bioavailability of quercetin has limited its clinical application [31]. As a result different nanosystems (Fig. 3) were targeted to improve the bioavailability and efficacy of quercetin [31]. Most of these systems are biocompatible and appropriate for delivery with controlled release enhancing the Absorption, Distribution, Metabolism, Excretion and Toxicology (ADMET) profile of encapsulated active pharmaceutical ingredients (APIs).

Quercetin-loaded Solid Lipid Nanoparticles (QSLN) offer significant advantages over traditional polymeric delivery systems (such as PLGA or chitosan nanoparticles) primarily due to

their superior biocompatibility, enhanced stability, and high loading capacity for lipophilic compounds. SLNs use solid lipids (like stearic acid, glyceryl monostearate) that mimic natural body lipids, providing better safety profiles and improved bioavailability for poorly soluble compounds like quercetin [32]

Key Advantages of Quercetin-Loaded SLNs over Polymeric Systems:

- **Higher Biocompatibility and Reduced Toxicity:** Unlike many synthetic polymers (e.g., PLGA), SLNs are synthesized using biodegradable, physiologically well-tolerated solid lipids. This reduces the risks of chronic toxicity and immune reactions.
- **Superior Encapsulation and Loading of Quercetin:** Quercetin is a hydrophobic (lipophilic) drug. SLNs provide a lipid core where quercetin is highly soluble, resulting in higher drug loading capacity (e.g., up to 12.4%–13.2%) and entrapment efficiency (>90%) compared to many polymeric systems, preventing drug leakage.
- **Enhanced Stability and Protection against Degradation:** SLNs create a rigid solid matrix that protects labile, easily oxidized molecules like quercetin from environmental degradation (light, heat, pH changes) better than polymeric matrix systems.
- **Improved Bioavailability and Oral Absorption:** SLN-Qt formulations can enhance quercetin's oral bioavailability, with some studies showing up to 571% (5.7-fold) higher bioavailability compared to raw quercetin suspensions. They prevent rapid metabolism by intestinal bacteria and enzymes.
- **Avoidance of Organic Solvents:** The fabrication of SLNs often uses high-pressure homogenization or similar methods that can be done without organic solvents, making them safer and more eco-friendly compared to solvent-evaporation techniques used for polymeric NPs.

- **Sustained Release Characteristics:** QSLN particles provide a sustained drug release profile, often showing an initial controlled release followed by prolonged release over several days (e.g., up to 142 hours) in simulated intestinal fluid.
- **Scalability and Cost-Effectiveness:** The production methods (hot homogenization, ultrasonication) are widely used and easily scaled up, making them more economical than complex polymeric nanoparticle synthesis.

1.4.1 Aim of the research

The aim of the research was to prepare novel solid-lipid nanoparticle formulations of Quercetin for enhanced drug delivery. In this study, stearic acid (SA) was used as the lipid core. Subsequently, Tween 80 was used as an emulsifiers to prepare Quercetin-loaded solid lipid nanoparticles (QLSN) and the formulations were formulated using the micro-emulsion technique. To achieve uniform distribution and lower particle size, a combination of ultrasonication and high-speed homogenization has been employed and subsequently characterized.

Chapter 2

Materials and Methods

Chapter 2: Materials and Methods

2.1 Materials

Quercetin drug was purchased from Sisco Research Laboratories Pvt. Ltd. (Maharashtra, India). Stearic Acid and Tween 80 (Polysorbate 80) surfactant was purchased from Research-Lab Fine Chem Industries (Mumbai, India). Ethanol was also purchased from Research-Lab Fine Chem Industries (Mumbai, India). All solvents used in this research were of analytical grade.

2.2 Standard curve of Quercetin

10 mg Quercetin Drug was weighed on wax paper. A volumetric flask (10 ml) was taken and the 10 mg of drug (Quercetin) was transferred to it. Then 10 ml ethanol was added to the volumetric flask containing the drug. The concentration of the stock solution was found to be 1000 µg/ml (1mg/ml). This stock solution was labelled as Stock-1. 1ml of solution was taken from it and added to another 10 ml volumetric flask. It was dissolved with 9 ml ethanol to make it up to the mark. The concentration of the solution was found to be 100 µg/ml and the solution was labelled as Stock-2. From Stock-2, serial dilution was done to obtain different concentrations (1, 2, 4, 6, 8, 10 µg/ml) for preparing the standard curve. An UV-Vis Spectrophotometer (Agilent, USA) was used to measure the absorbance at 373 nm [33]. The experiment was done in triplicates and data is represented as mean ± standard deviation.

2.3 Formulation of Quercetin-loaded Solid Lipid Nanoparticles (QSLNs)

A combined method of homogenization and ultrasonification was used to prepare Quercetin-loaded Solid Lipid Nanoparticles (QSLNs) [34]. Stearic acid was used as a solid lipid for the preparation of Quercetin Solid Lipid Nanoparticles (QSLNs) [35]. Four formulations varying the amounts of stearic acid (0.5, 0.75, 1, 1.5 gm) in a 25ml beaker and Quercetin drug (25mg) was

added to each beaker and the formulations were named were F1, F2, F3, F4 respectively as shown in Table 3. 10 ml of Ethanol was then added to the mixture to dissolve the solution. This part represents the lipid phase of the formulation.

The mixture was kept in a water bath at 70°C (it is necessary to make sure it does not reach Ethanol's boiling point: 78.37°C) and the solution was stirred continuously for the stearic acid to mix in the ethanol properly. Aqueous surfactant solutions were prepared by adding 2 gm of Tween 80 in 50 mL distilled water in a separate beaker and stirred at 70°C by keeping the solution in a water bath. When both the beakers (aqueous phase and lipid phase) reached the same temperature (70°C), the 2% Tween 80 solution (aqueous surfactant solution) was placed in the homogenizer (Model: SH-HZD, Brand: SH Scientific, Country of Origin: Korea) and the speed was set at 3000 rpm. Simultaneously, the lipid phase containing the drug (Quercetin drug in stearic acid and ethanol solution) was added dropwise to the aqueous solution by using a syringe (Company: JMI Group, Country: Bangladesh) and thoroughly mixed under the homogenizer (Model: SH-HZD, Brand: SH Scientific, Country of Origin: Korea) at a rotational speed of 3000 rpm. The mixture was homogenized for 30 minutes. Subsequently, the emulsion was sonicated for 10 minutes using an ultrasonicator (Model: KSU-500, Country of Origin: China). Lastly, QSLN mixture was obtained by quickly cooling the nano emulsion at room temperature and later dried at 60°C in a Vacuum Drying Oven (Model: LVO-2030, Brand: LabTech, Country of Origin: South Korea) for 6-7 hours until it was completely dried.

Following the same procedure, another 4 formulations were prepared by varying the amounts of Tween 80 (0.5%, 1%, 1.5%, 2%) and the formulations were labelled as F5, F6, F7, F8 respectively as shown in Table 4. Additionally 4 more formulations were prepared by varying the amount of

Quercetin drug (15 mg, 25 mg, 35 mg, 45 mg) and were labelled as F9, F10, F11, F12 respectively as shown in Table 5, and furthermore, another set of 4 formulations were prepared by changing the rotation speed of homogenizer and labelled as F13, F14, F15, F16 as shown in Table 6.

Table 3. Quercetin-loaded nanoparticles formulation composition with varying amounts of Stearic Acid

Composition	Amount			
	Formulation (F1)	Formulation (F2)	Formulation (F3)	Formulation (F4)
Quercetin	25 mg	25 mg	25 mg	25 mg
Tween 80	2%	2%	2%	2%
Stearic Acid	0.5 gm	0.75 gm	1 gm	1.5 gm
Ethanol	10 ml	10 ml	10 ml	10 ml

Table 4. Quercetin-loaded nanoparticles formulation composition with varying amounts of Tween 80

Composition	Amount			
	Formulation (F5)	Formulation (F6)	Formulation (F7)	Formulation (F8)
Quercetin	25 mg	25 mg	25 mg	25 mg
Tween 80	0.5%	1%	1.5%	2%
Stearic Acid	1 gm	1 gm	1 gm	1 gm
Ethanol	10 ml	10 ml	10 ml	10 ml

Table 5. Quercetin-loaded nanoparticles formulation composition with varying amounts of Quercetin Drug

Composition	Amount			
	Formulation (F9)	Formulation (F10)	Formulation (F11)	Formulation (F12)
Quercetin	15 mg	25 mg	35 mg	45 mg
Tween 80	2%	2%	2%	2%
Stearic Acid	1 gm	1 gm	1 gm	1 gm
Ethanol	10 ml	10 ml	10 ml	10 ml

Table 6. Quercetin-loaded nanoparticles formulation composition with varying rotation speed

Composition	Amount			
	Formulation (F13)	Formulation (F14)	Formulation (F15)	Formulation (F16)
Quercetin	25 mg	25 mg	25 mg	25 mg
Tween 80	2%	2%	2%	2%
Stearic Acid	0.5 gm	0.75 gm	1 gm	1.5 gm
Ethanol	10 ml	10 ml	10 ml	10 ml
Rotation Speed	3000 rpm	2500 rpm	2000 rpm	1500 rpm

2.4 Entrapment efficiency (% EE)

1 ml of the nanoparticle suspension was taken in an eppendorf and centrifuged at 7000-10,000 rpm using a microcentrifuge (Model: D1008, Brand: DLAB, Country of Origin: USA). 400µl of supernatant was collected from the eppendorf using a micropipette and transferred to a test tube. 3.6 ml ethanol was added to it to dilute it 10 times. The quantity of entrapment Quercetin (% EE) at 373 nm was determined by an UV- Vis Spectrophotometer (Agilent Cary 60, USA). The concentration was calculated and multiplied by 10 to obtain the free drug concentration in the supernatant. To ascertain the drug percentage entrapment efficiency (% EE) in the solid lipid nanoparticles (SLNs), the following equation was utilized:

$$\% \text{ EE} = \frac{Total\ Drug\ Concentration - Free\ Drug\ Concentration}{Total\ Drug\ Concentration} \times 100$$

2.5 Percentage Drug Loading (% DL)

The percentage drug loading (DL) of the Quercetin-Loaded Solid lipid Nanoparticles (QSLNs) was determined by determining the amount of Quercetin present in the SLNs relative to the total weight of the SLN formulation. The centrifugation of an aliquot of the SLN dispersion was performed at 12,000 rpm 30 min to separate the nanoparticles with the unbound drug [36]. Agilent Cary 60 UV-vis spectrophotometer was therefore used to measure unencapsulated Quercetin in the supernatant at 373 nm.

By utilizing the following equation, the %DL was calculated:

$$\% \text{ DL} = \frac{Amount\ of\ Quercetin\ in\ SLNs}{Total\ amount\ of\ SLNs} \times 100$$

2.6 Dissolution Study

An *in vitro* dissolution test was done for Quercetin-Loaded Solid Lipid Nanoparticles (QSLNs) formulation in distilled water of pH 5.5 for a time period of 120 minutes by the help of a magnetic stirrer and a Hot Plate (Model: MS-H380-pro DLAB, USA) at an optimum speed of 300 rpm and a temperature of 37 ± 0.05°C [36]. A 50 ml beaker containing 50 ml of distilled water was used to conduct the test to enhance the aqueous solubility of Quercetin. Distilled water was selected because it is an easy and reproducible dissolution medium. Since it had low volume and a small sample size, a magnetic stirrer was selected over the traditional USP dissolution apparatus as a cost-effective and time-efficient method [36].

Crystalline Quercetin, SLN formulations, and a standard market product were all carefully weighed to keep the amount of Quercetin in the 50 ml beaker at 200 mg. A 0.3 ml sample was taken with a micropipette and replaced with an equal volume of distilled water at the following timepoints (5, 15, 30, 45, 60, 75, 90 and 120 minutes). Each sample was centrifuged at 15,000 g for 30 minutes and diluted with ethanol (1:3). The dissolved Quercetin concentrations was measured using a UV spectrophotometer at 373 nm . All experiments were performed in triplicates.

Chapter 3

Results and Discussion

Chapter 3: Results and Discussion

3.1 Standard curve of Quercetin

The standard curve of Quercetin was constructed by preparing a series of Quercetin solutions with different concentrations (2, 4, 6, 8, 10 µg/mL) and absorbance was measured at 373 nm. Figure 1 shows the standard curve of Quercetin (with absorbance values on y axis and concentrations in x axis (µg/mL)). The regression coefficient R^2 value was found to be 0.998 that is close to 1, overall indicating good linearity and a close fit and this method was used every time to prepare standards for subsequent experiments.

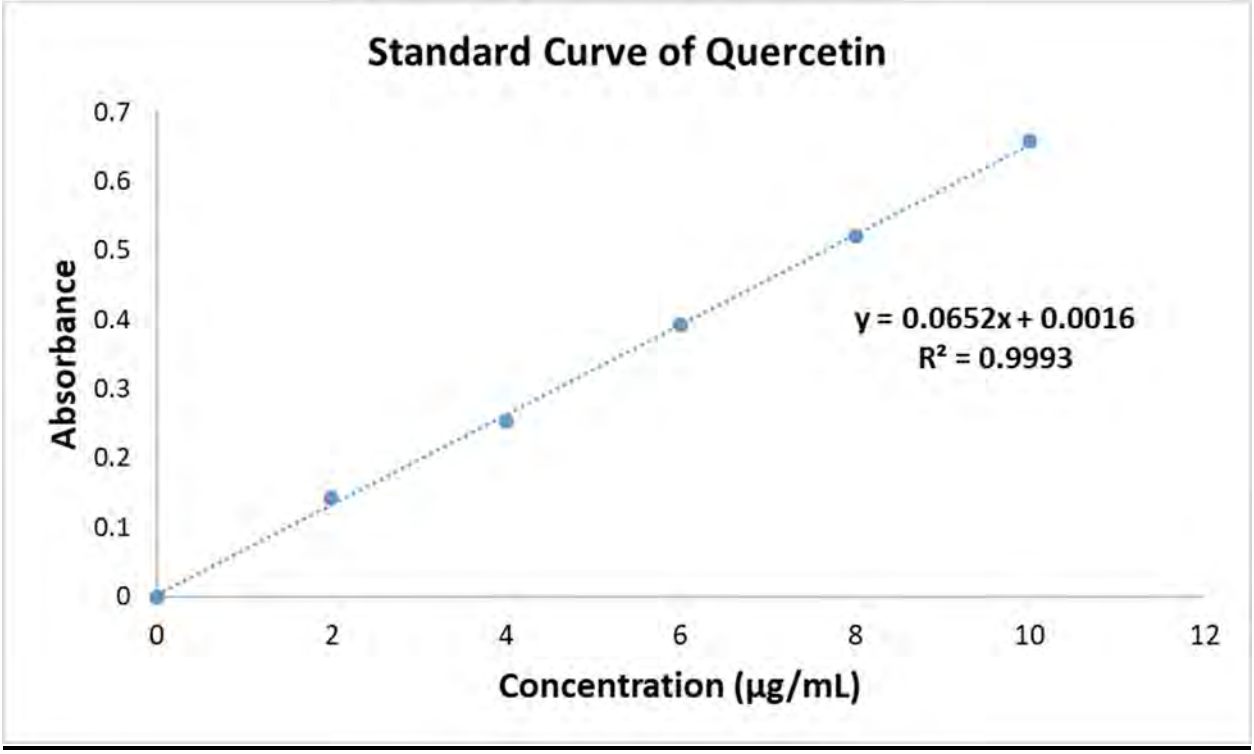

Figure 3. Standard Curve of Quercetin

3.2 Entrapment Efficiency and Drug Loading

Entrapment Efficiency (EE) is defined as the percentage of drugs entrapped into the nanocarrier matrix in reference to the total drug input [37]. It is a crucial parameter for the evaluation of nanocarriers and the accurate measurement of EE requires clear separation of nanocarriers from free drugs [37]. Measuring entrapment efficiency is important to ensure that the maximum amount of drug is entrapped in nanoparticles, reflecting formulation effectiveness, minimizing dosage requirements and reducing drug loss. Drug Loading (DL) is a promising strategy to produce highly stable drugs with improved dissolution rate, solubility and bioavailability [38]. DL is essential for ensuring therapeutic efficacy and to ensure that an adequate amount of drug is present in each nanoparticle for effective drug delivery [36].

In total 16 formulations were prepared and named as F1 to F16. The composition of F1 to F4 had varying amounts of stearic acid (0.5 gm, 0.75 gm, 1 gm & 1.25 gm) keeping the other parameters fixed such as Amount of Drug (25 mg), Tween 80 (2%) and rotation speed (3000 rpm). Similarly, F5 to F8 had varying amounts of Tween 80 (0.5%, 1%, 1.5% and 2%) keeping the other parameters fixed such as amount of Drug (25 mg), stearic acid (1 gm) and rotation speed (3000 rpm). Formulations F9 to F12 had different amounts of drug (15 mg, 25 mg, 35 mg and 45 mg) while F13 to F16 had different rotation speed (1500 rpm, 2000 rpm, 2500 rpm and 3000 rpm) keeping the other parameters constant.

The % EE of QSLNs ranged from 4.35% (lowest for F14) to 81.46% (highest for F5) from among the 16 formulations that were developed. F5 formulation showed the highest entrapment efficiency (Table 2) and this high % EE indicates the successful encapsulation of Quercetin within the SLNs, minimizing drug loss during preparation and storage. From this finding, we can state that F5 is the

optimum formulation which includes the following parameters (0.5% Tween 80, 1gm Stearic Acid, 25mg drug/Quercetin formulated at a rotation speed of 3000 rpm).

Table 7: Percentage of Entrapment Efficiency with varying amounts of Stearic Acid

Percentage of Entrapment Efficiency with varying amounts of Stearic Acid				
Formulations	F1 (0.5 gm of stearic acid)	F2 (0.75 gm of stearic acid)	F3 (1 gm of stearic acid)	F4 (1.25 gm of stearic acid)
Entrapment Efficiency (%)	52.66	70.44	58.65	65.27
	39.63	68.16	58.45	68.37
	46.46	69.20	58.65	66.51
Average	46.25	69.27	58.58	66.72
Std	6.52	1.14	0.12	1.56

Table 8: Percentage of Entrapment Efficiency with varying amounts of Tween 80 and using 0.5 gm of stearic acid

Percentage of Entrapment Efficiency with varying amounts of Tween 80				
Formulations	F5 (0.5%)	F6 (1%)	F7 (1.5%)	F8 (2%)
Entrapment Efficiency (%)	81.6	56.99	59.27	71.06
	81.39	56.99	58.65	70.85
	81.39	56.79	58.45	70.85
Average	81.46	56.92	58.79	70.92
Std	0.12	0.12	0.43	0.12

Table 9: Percentage of Entrapment Efficiency with varying amounts of Quercetin

Percentage of Entrapment Efficiency with varying amounts of Quercetin				
Formulations	F9 (15 mg of drug)	F10 (25 mg of drug)	F11 (35 mg of drug)	F12 (45 mg of drug)
Entrapment Efficiency (%)	14.83	71.06	21.63	16.67
	14.14	70.85	21.78	16.78
	14.48	70.85	21.63	16.67
Average	14.48	70.92	21.68	16.71
Std	0.35	0.12	0.09	0.06

Table 10: Percentage of Entrapment Efficiency with varying rotation speed

Percentage of Entrapment Efficiency with varying rotation speed				
Formulations	F13 (3000 rpm)	F14 (2500 rpm)	F15 (2000 rpm)	F16 (1500 rpm)
Entrapment Efficiency (%)	71.06	4.28	34.88	80.98
	70.85	4.49	35.29	81.19
	70.85	4.28	35.09	80.98
Average	70.92	4.35	35.09	81.05
Std	0.12	0.12	0.21	0.12

3.3 *In-vitro* dissolution studies

Distilled water was chosen as the dissolution medium for its simplicity and reproducibility, especially when comparing formulations in a standardized environment without the influence of surfactants or complex biological fluids [36]. While more complex media like simulated gastric or intestinal fluids could be used in later stages, the goal at this stage was to highlight the solubility-enhancing potential of the SLN system in a baseline aqueous environment. Additionally, using a magnetic stirrer instead of a conventional USP dissolution apparatus was driven by the small volume and limited sample size, which allowed us to work more efficiently and reduce material use [36].

Quercetin's low solubility/dissolution behavior is also one of the most important issues that needs to be addressed for it to be used for therapeutic applications [39]. *In vitro* dissolution assessments evaluate the drug release profile within the physiological environment and determine drug release behavior from various dosage forms. To determine whether various QSLNs formulations can improve the dissolution behavior of Quercetin compared to commercially available reference products, dissolution studies were conducted on all QSLNs formulations in distilled water and the results are represented in Figures 2-17. Among all the formulations, F2 showed the highest drug release percentage compared to other formulations (Figure 6). It demonstrated a 3-fold increase in dissolution behavior compared to other formulations at 120 min.

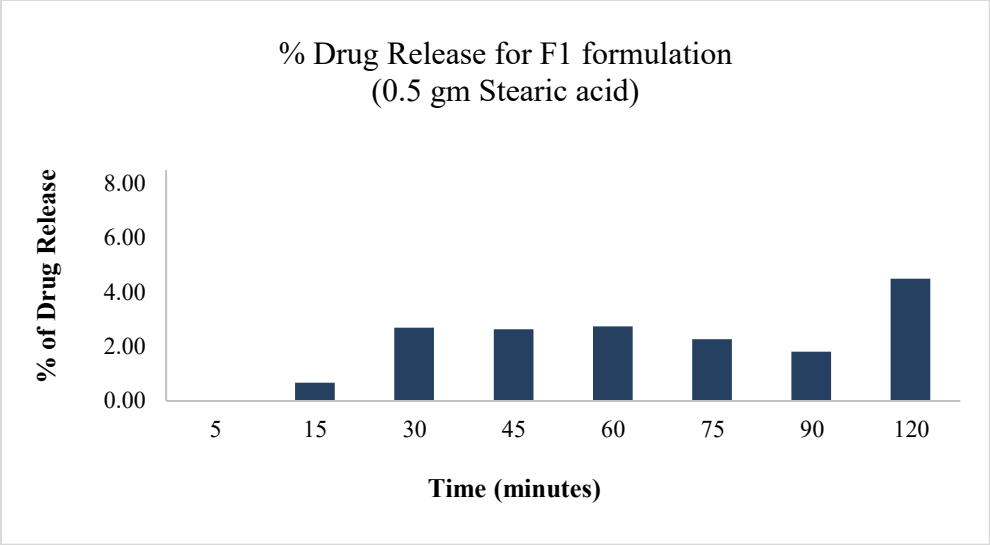

Figure 4. Percentage Drug Release of F1 formulation

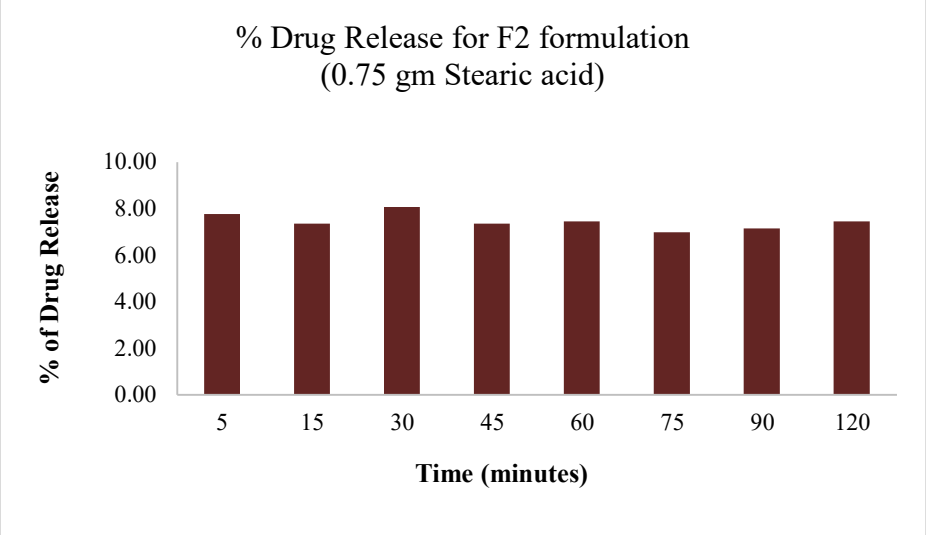

Figure 5. Percentage Drug Release of F2 formulation

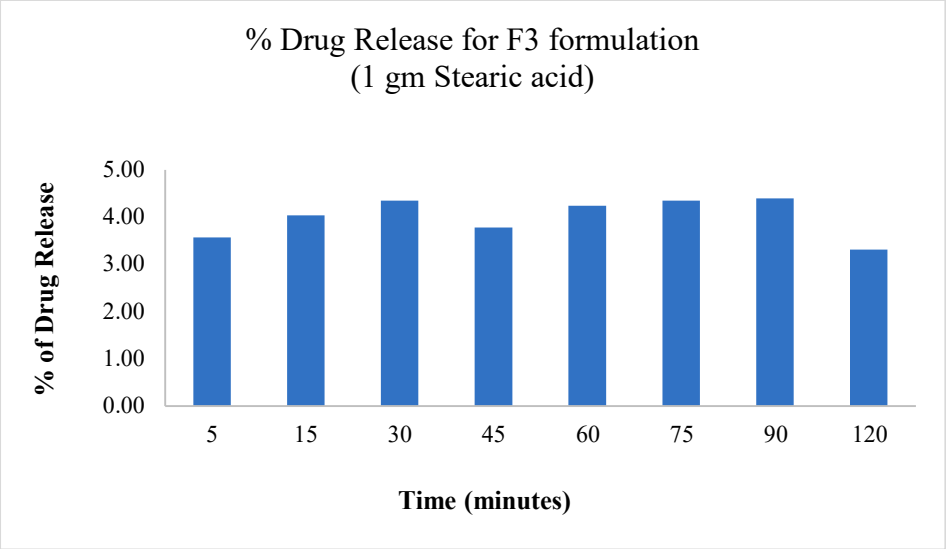

Figure 6. Percentage Drug Release of F3 formulation

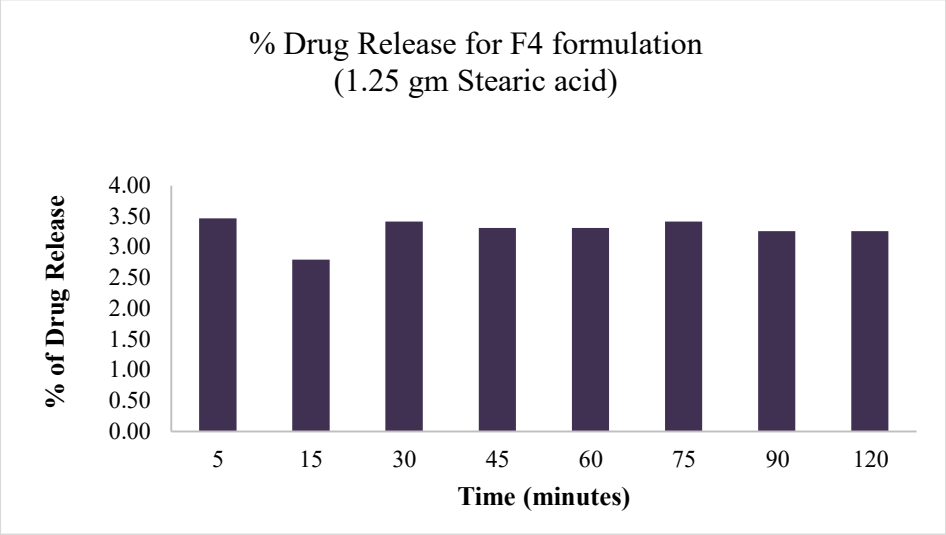

Figure 7. Percentage Drug Release of F4 formulation

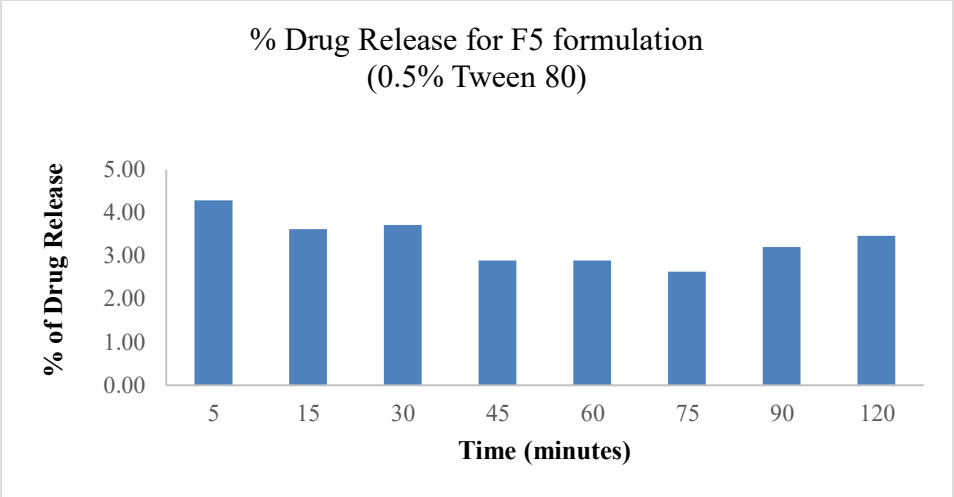

Figure 8. Percentage Drug Release of F5 formulation

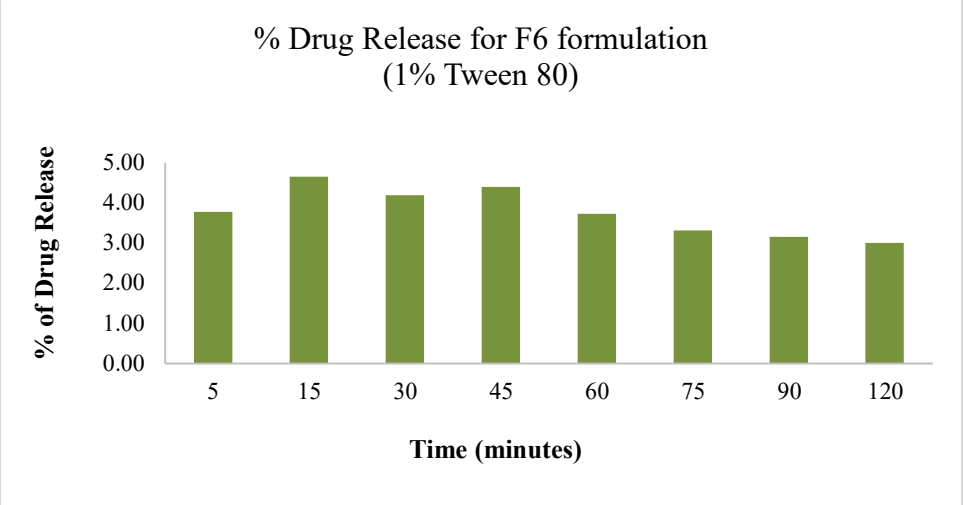

Figure 9. Percentage Drug Release of F6 formulation

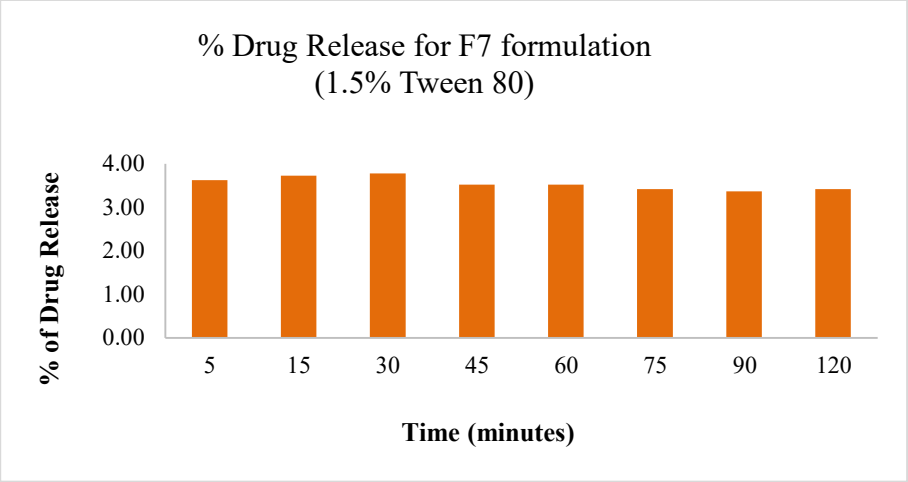

Figure 10. Percentage Drug Release of F7 formulation

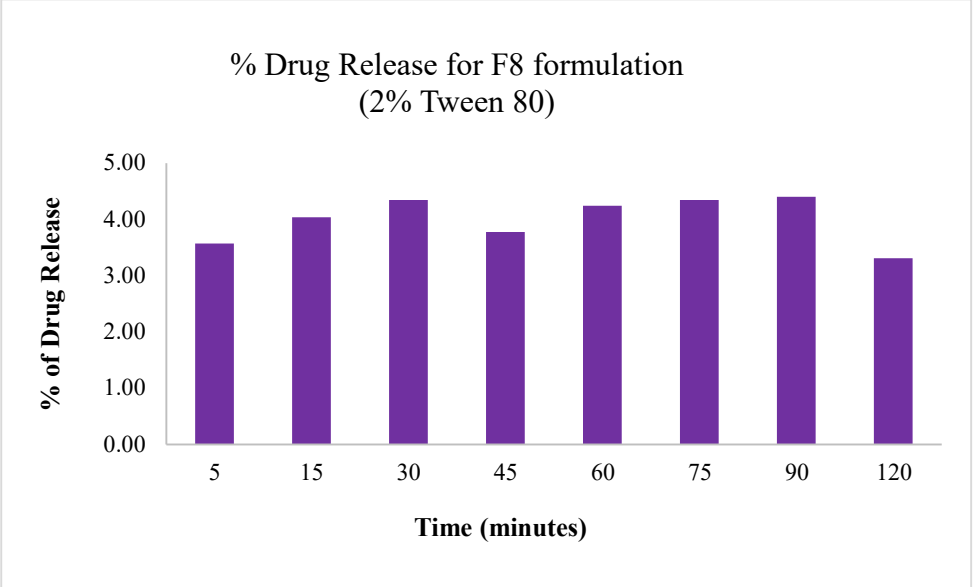

Figure 11. Percentage Drug Release of F8 formulation

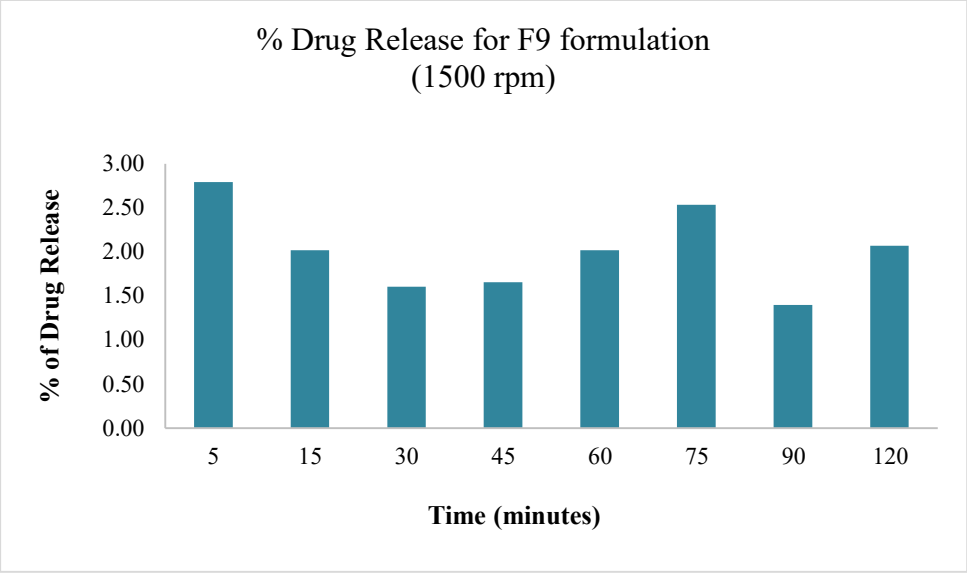

Figure 12. Percentage Drug Release of F9 formulation

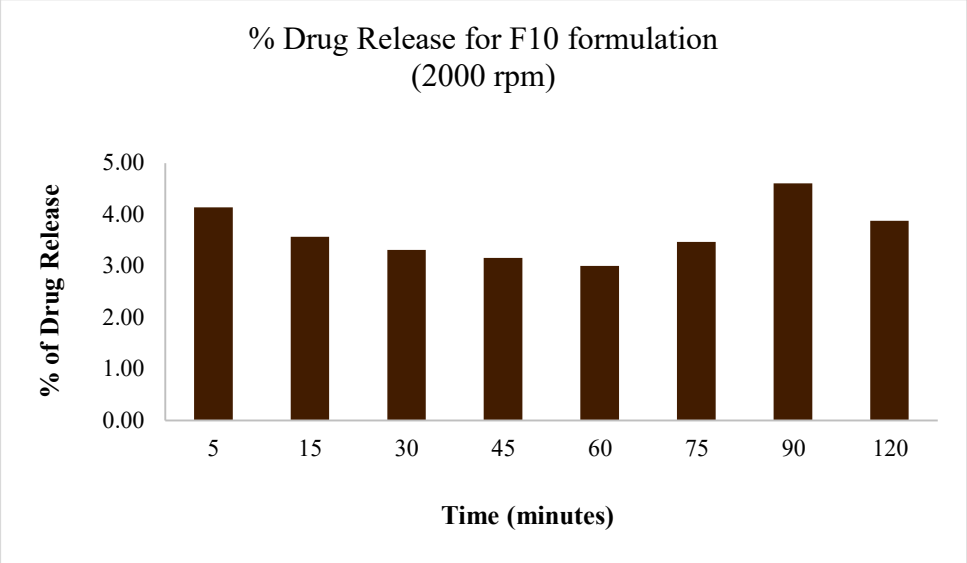

Figure 13. Percentage Drug Release of F10 formulation

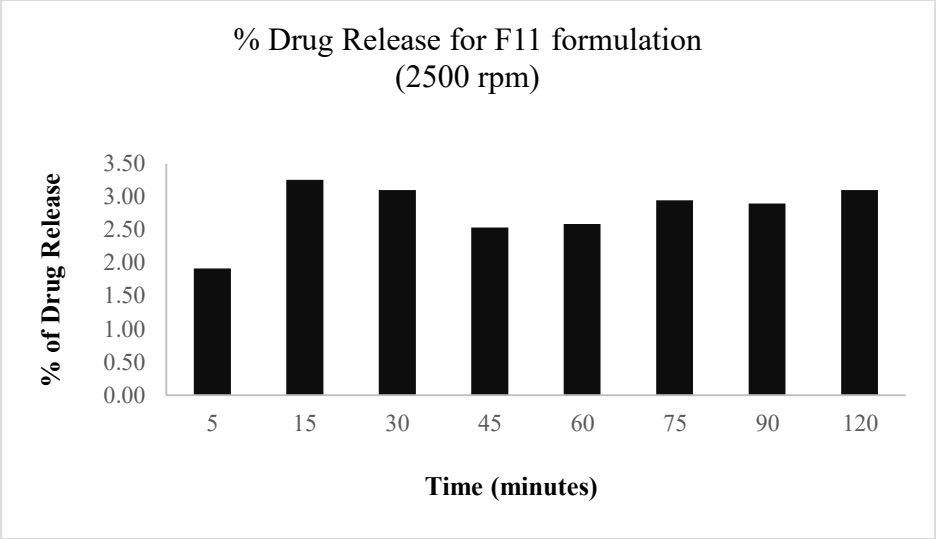

Figure 14. Percentage Drug Release of F11 formulation

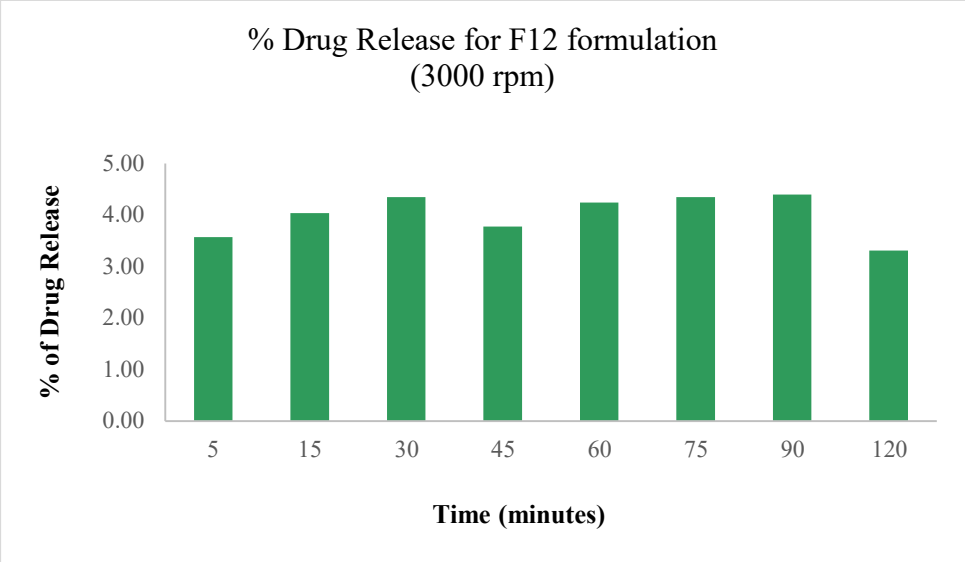

Figure 15. Percentage Drug Release of F12 formulation

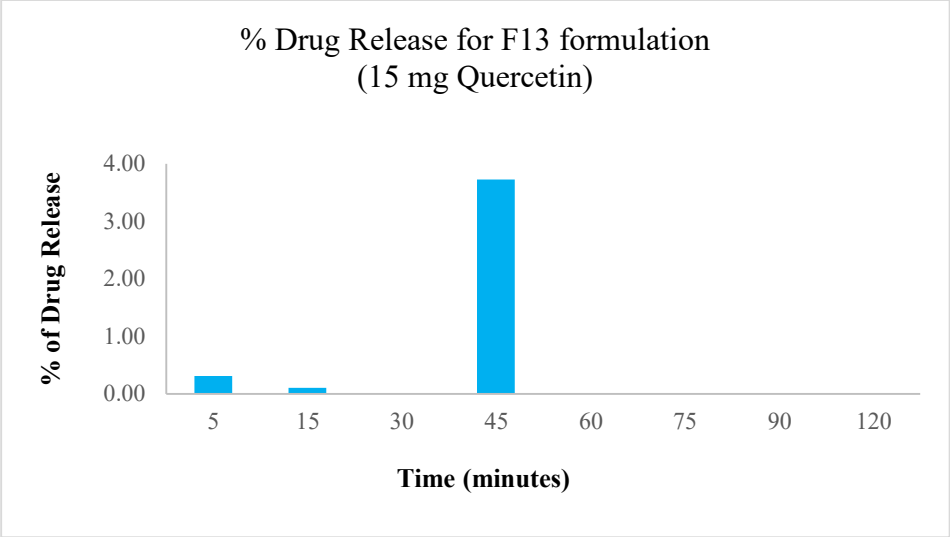

Figure 16. Percentage Drug Release of F13 formulation

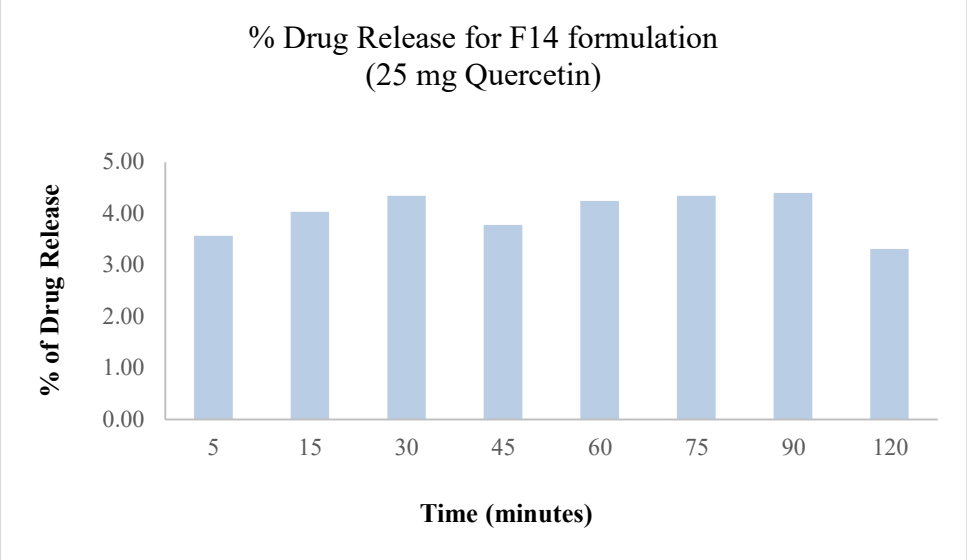

Figure 17. Percentage Drug Release of F14 formulation

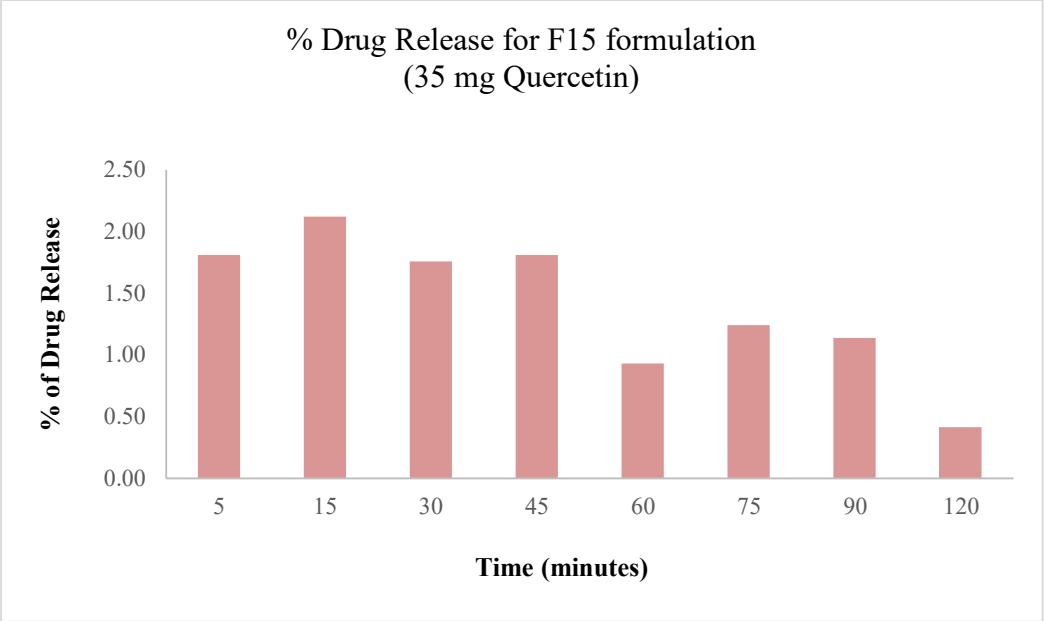

Figure 18. Percentage Drug Release of F15 formulation

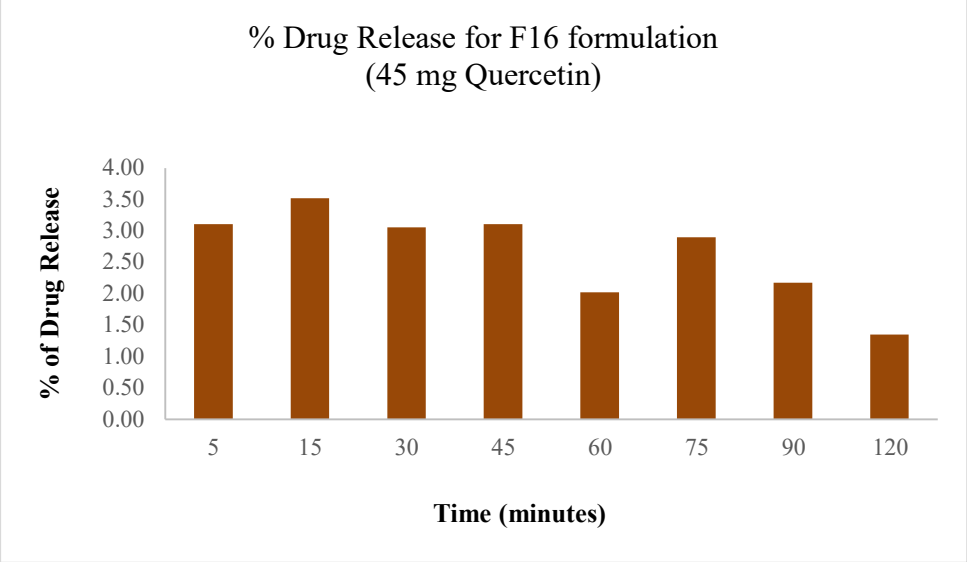

Figure 19. Percentage Drug Release of F16 formulation

F1 formulation showed a slow release of drug from starting time point and after 120 minutes, approximately 4.5% of drug was released. Comparatively F2 showed a significantly higher drug release post 120 minutes (7.5 %) (Figure 3). Formulations F3 – F12 showed similar dissolution profiles with a total release of approximately 4% after 120 minutes dissolution study. All these formulations displayed a sustained release profile owing to the incorporation of stearic acid that has a retarding effect and has been employed in this formulation as a solid-lipid matrix. Interestingly, F13 showed very low drug release, probably as only 15 mg of the drug was used to prepare the formulation. However, F14, F15 and F16 showed release profiles similar to F3 – F12. Overall, the developed QLSN formulations showed a sustained release effect over time. Further studies are required to understand the dissolution behavior of the optimized formulation of F5 that exhibits the highest encapsulation efficiency (approximately 80%).

Chapter 4

Conclusion and

Future Perspectives

4. Conclusions and Future Perspectives

Quercetin is a common bioflavonoid with low water solubility, which limits its oral bioavailability and *in vivo* beneficial functions. However, the poor solubility of Quercetin in water (0.17–7 μg/mL), artificial gastric juice (5.5 μg/mL), and artificial intestinal juice (28.9 μg/mL) greatly limits its bioavailability after oral administration [40]. Bioavailability studies of QC in humans revealed that the mean plasma values ranged between 15 and 24 μg/L in subjects consuming their habitual diets [41] and was 42 μg/L after a diet containing 815 g/d of vegetables, fruits, and berries [42]. Therefore, to address these limitations, a novel formulation of Quercetin Solid Lipid Nanoparticles was developed using stearic acid as a solid lipid matrix and Tween 80 as a surfactant using a high-speed homogenization technique. 16 formulations were prepared varying the amounts of stearic acid (lipid phase), Tween 80 (surfactant), Quercetin drug and the rotation speed. F5 formulation was found to be the optimum formulation with the highest encapsulation efficiency of 80% displaying a sustained release profile following a 2-hour dissolution study. The present study suggests that high-speed homogenization technique is a suitable method to develop solid-lipid nanoparticles of Quercetin with high entrapment efficiency. Further work is required to determine the efficacy of the formulation in terms of particle size, zeta potential, release profile, morphology using scanning electron microscopy, *in vitro* antioxidant study, anti-inflammatory study, bioequivalence study in animal models etc.

References

[1] P. Hagde *et al.*, "Therapeutic potential of quercetin in diabetic foot ulcer: Mechanistic insight, challenges, nanotechnology driven strategies and future prospects," *J. Drug Deliv. Sci. Technol.*, vol. 74, p. 103575, Aug. 2022, doi: 10.1016/j.jddst.2022.103575.

[2] F. Aghababaei and M. Hadidi, "Recent Advances in Potential Health Benefits of Quercetin," Jul. 01, 2023, *Multidisciplinary Digital Publishing Institute (MDPI)*. doi: 10.3390/ph16071020.

[3] N. Suganthy, K. P. Devi, S. F. Nabavi, N. Braidy, and S. M. Nabavi, "Bioactive effects of quercetin in the central nervous system: Focusing on the mechanisms of actions," *Biomedicine & Pharmacotherapy*, vol. 84, pp. 892–908, Dec. 2016, doi: 10.1016/j.biopha.2016.10.011.

[4] H. Yi *et al.*, "The Therapeutic Effects and Mechanisms of Quercetin on Metabolic Diseases: Pharmacological Data and Clinical Evidence," *Oxid. Med. Cell. Longev.*, vol. 2021, no. 1, Jan. 2021, doi: 10.1155/2021/6678662.

[5] M. Azeem, M. Hanif, K. Mahmood, N. Ameer, F. R. S. Chughtai, and U. Abid, "An insight into anticancer, antioxidant, antimicrobial, antidiabetic and anti-inflammatory effects of quercetin: a review," *Polymer Bulletin*, vol. 80, no. 1, pp. 241–262, Jan. 2023, doi: 10.1007/s00289-022-04091-8.

[6] S. Ozgen, O. K. Kilinc, and Z. Selamoglu, "Antioxidant Activity of Quercetin: A Mechanistic Review," 2016. [Online]. Available: www.agrifoodscience.com,

[7] Y. Li *et al.*, "Quercetin, Inflammation and Immunity," *Nutrients*, vol. 8, no. 3, p. 167, Mar. 2016, doi: 10.3390/nu8030167.

[8] M. Hadidi, J. C. Orellana-Palacios, F. Aghababaei, D. J. Gonzalez-Serrano, A. Moreno, and J. M. Lorenzo, "Plant by-product antioxidants: Control of protein-lipid oxidation in meat and meat products," *LWT*, vol. 169, p. 114003, Nov. 2022, doi: 10.1016/j.lwt.2022.114003.

[9] A. W. Boots, G. R. M. M. Haenen, and A. Bast, "Health effects of quercetin: From antioxidant to nutraceutical," *Eur. J. Pharmacol.*, vol. 585, no. 2–3, pp. 325–337, May 2008, doi: 10.1016/j.ejphar.2008.03.008.

[10] M. C. Christodoulou *et al.*, "Spectrophotometric Methods for Measurement of Antioxidant Activity in Food and Pharmaceuticals," *Antioxidants*, vol. 11, no. 11, p. 2213, Nov. 2022, doi: 10.3390/antiox11112213.

[11] N. Suematsu, M. Hosoda, and K. Fujimori, "Protective effects of quercetin against hydrogen peroxide-induced apoptosis in human neuronal SH-SY5Y cells," *Neurosci. Lett.*, vol. 504, no. 3, pp. 223–227, Oct. 2011, doi: 10.1016/j.neulet.2011.09.028.

[12] A. Kale *et al.*, “Neuroprotective effects of Quercetin on radiation-induced brain injury in rats,” *J. Radiat. Res.*, vol. 59, no. 4, pp. 404–410, Jul. 2018, doi: 10.1093/jrr/rry032.

[13] H. Kalantari, H. Foruozandeh, M. J. Khodayar, A. Siahpoosh, N. Saki, and P. Kheradmand, “Antioxidant and hepatoprotective effects of Capparis spinosa L. fractions and Quercetin on tert-butyl hydroperoxide- induced acute liver damage in mice,” *J. Tradit. Complement. Med.*, vol. 8, no. 1, pp. 120–127, Jan. 2018, doi: 10.1016/j.jtcme.2017.04.010.

[14] G. Sharmila *et al.*, “Chemopreventive Effect of Quercetin in MNU and Testosterone Induced Prostate Cancer of Sprague-Dawley Rats,” *Nutr. Cancer*, vol. 66, no. 1, pp. 38–46, Jan. 2014, doi: 10.1080/01635581.2014.847967.

[15] G. Sharmila *et al.*, “Chemopreventive effect of quercetin, a natural dietary flavonoid on prostate cancer in in vivo model,” *Clinical Nutrition*, vol. 33, no. 4, pp. 718–726, Aug. 2014, doi: 10.1016/j.clnu.2013.08.011.

[16] H. Kalantari, H. Foruozandeh, M. J. Khodayar, A. Siahpoosh, N. Saki, and P. Kheradmand, “Antioxidant and hepatoprotective effects of Capparis spinosa L. fractions and Quercetin on tert-butyl hydroperoxide- induced acute liver damage in mice,” *J. Tradit. Complement. Med.*, vol. 8, no. 1, pp. 120–127, Jan. 2018, doi: 10.1016/j.jtcme.2017.04.010.

[17] A. Minaei, M. Sabzichi, F. Ramezani, H. Hamishehkar, and N. Samadi, “Co-delivery with nano-quercetin enhances doxorubicin-mediated cytotoxicity against MCF-7 cells,” *Mol. Biol. Rep.*, vol. 43, no. 2, pp. 99–105, Feb. 2016, doi: 10.1007/s11033-016-3942-x.

[18] J. Suksiriworapong, K. Phoca, S. Ngamsom, K. Sripha, P. Moongkarndi, and V. B. Junyaprasert, “Comparison of poly(ε-caprolactone) chain lengths of poly(ε-caprolactone)-co-d-α-tocopheryl-poly(ethylene glycol) 1000 succinate nanoparticles for enhancement of quercetin delivery to SKBR3 breast cancer cells,” *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 101, pp. 15–24, Apr. 2016, doi: 10.1016/j.ejpb.2016.01.008.

[19] J.-Y. Kee *et al.*, “Inhibitory effect of quercetin on colorectal lung metastasis through inducing apoptosis, and suppression of metastatic ability,” *Phytomedicine*, vol. 23, no. 13, pp. 1680–1690, Dec. 2016, doi: 10.1016/j.phymed.2016.09.011.

[20] D. Aggarwal *et al.*, “Anti-inflammatory potential of quercetin: From chemistry and mechanistic insight to nanoformulations,” *Current Research in Pharmacology and Drug Discovery*, vol. 8, p. 100217, 2025, doi: 10.1016/j.crphar.2025.100217.

[21] S. R. Alizadeh and M. A. Ebrahimzadeh, “<scp> *O* -Glycoside </scp> quercetin derivatives: Biological activities, mechanisms of action, and structure–activity relationship for drug design, a review,” *Phytotherapy Research*, vol. 36, no. 2, pp. 778–807, Feb. 2022, doi: 10.1002/ptr.7352.

[22] Y. Li *et al.*, “Quercetin, Inflammation and Immunity,” *Nutrients*, vol. 8, no. 3, p. 167, Mar. 2016, doi: 10.3390/nu8030167.

[23] D. Yang, T. Wang, M. Long, and P. Li, “Quercetin: Its Main Pharmacological Activity and Potential Application in Clinical Medicine,” *Oxid. Med. Cell. Longev.*, vol. 2020, pp. 1–13, Dec. 2020, doi: 10.1155/2020/8825387.

[24] L. Geraets, H. J. J. Moonen, K. Brauers, E. F. M. Wouters, A. Bast, and G. J. Hageman, “Dietary Flavones and Flavonoles Are Inhibitors of Poly(ADP-ribose)polymerase-1 in Pulmonary Epithelial Cells ,” *J. Nutr.*, vol. 137, no. 10, pp. 2190–2195, Oct. 2007, doi: 10.1093/jn/137.10.2190.

[25] G. Bureau, F. Longpré, and M. -G. Martinoli, “Resveratrol and quercetin, two natural polyphenols, reduce apoptotic neuronal cell death induced by neuroinflammation,” *J. Neurosci. Res.*, vol. 86, no. 2, pp. 403–410, Feb. 2008, doi: 10.1002/jnr.21503.

[26] D. C. NIEMAN *et al.*, “Effects of Quercetin and EGCG on Mitochondrial Biogenesis and Immunity,” *Med. Sci. Sports Exerc.*, vol. 41, no. 7, pp. 1467–1475, Jul. 2009, doi: 10.1249/MSS.0b013e318199491f.

[27] X.-W. Zhang, J.-Y. Chen, D. Ouyang, and J.-H. Lu, “Quercetin in Animal Models of Alzheimer’s Disease: A Systematic Review of Preclinical Studies,” *Int. J. Mol. Sci.*, vol. 21, no. 2, p. 493, Jan. 2020, doi: 10.3390/ijms21020493.

[28] D. Xu, M.-J. Hu, Y.-Q. Wang, and Y.-L. Cui, “Antioxidant Activities of Quercetin and Its Complexes for Medicinal Application,” *Molecules*, vol. 24, no. 6, p. 1123, Mar. 2019, doi: 10.3390/molecules24061123.

[29] P. Poprac, K. Jomova, M. Simunkova, V. Kollar, C. J. Rhodes, and M. Valko, “Targeting Free Radicals in Oxidative Stress-Related Human Diseases,” *Trends Pharmacol. Sci.*, vol. 38, no. 7, pp. 592–607, Jul. 2017, doi: 10.1016/j.tips.2017.04.005.

[30] D. Aggarwal *et al.*, “Anti-inflammatory potential of quercetin: From chemistry and mechanistic insight to nanoformulations,” *Current Research in Pharmacology and Drug Discovery*, vol. 8, p. 100217, 2025, doi: 10.1016/j.crphar.2025.100217.

[31] D. Aggarwal *et al.*, “Anti-inflammatory potential of quercetin: From chemistry and mechanistic insight to nanoformulations,” *Current Research in Pharmacology and Drug Discovery*, vol. 8, p. 100217, 2025, doi: 10.1016/j.crphar.2025.100217.

[32] J. M. M. Mohamed *et al.*, “Optimization and characterization of quercetin-loaded solid lipid nanoparticles for biomedical application in colorectal cancer,” *Cancer Nanotechnol.*, vol. 15, no. 1, p. 16, Dec. 2024, doi: 10.1186/s12645-024-00249-3.

[33] M. Sharma and S. Chauhan, “Determination of Quercetin by UV spectroscopy in feed premix,” *Pharma Innov.*, vol. 14, no. 3, pp. 43–46, Jan. 2025, doi: 10.22271/tpi.2025.v14.i3a.26063.

[34] W. Zhou, Shuyu Xie, Luyan Zhu, Zhao Dong, Y. Wang, and Xiaofang Wang, “Preparation and evaluation of ofloxacin-loaded palmitic acid solid lipid nanoparticles,” *Int. J. Nanomedicine*, p. 547, Mar. 2011, doi: 10.2147/IJN.S17083.

[35] S. Sharma, R. Thukral, L. Das Singla, N. Singla, and D. Choudhury, “Quercetin-loaded solid lipid nanoparticles for enhanced anti-helminthic activity,” *Int. J. Pharm.*, vol. 672, p. 125308, Mar. 2025, doi: 10.1016/j.ijpharm.2025.125308.

[36] S. Halder, F. Nasrin, M. Chandra Shill, M. L. Shuma, M. Z. Sultan, and M. S. Reza, “Improved Biopharmaceutical Performance of Coenzyme Q10 Through Solid Lipid Nanoparticles for Enhanced Brain Delivery,” *Scientifica (Cairo).*, vol. 2025, no. 1, 2025, doi: 10.1155/sci5/9034181.

[37] Y. Lv *et al.*, “Visual validation of the measurement of entrapment efficiency of drug nanocarriers,” *Int. J. Pharm.*, vol. 547, no. 1–2, pp. 395–403, Aug. 2018, doi: 10.1016/j.ijpharm.2018.06.025.

[38] A. H. Khalbas, T. M. Albayati, N. S. Ali, and I. K. Salih, “Drug loading methods and kinetic release models using of mesoporous silica nanoparticles as a drug delivery system: A review,” *S. Afr. J. Chem. Eng.*, vol. 50, pp. 261–280, Oct. 2024, doi: 10.1016/j.sajce.2024.08.013.

[39] M. Lu, C.-T. Ho, and Q. Huang, “Improving quercetin dissolution and bioaccessibility with reduced crystallite sizes through media milling technique,” *J. Funct. Foods*, vol. 37, pp. 138–146, Oct. 2017, doi: 10.1016/j.jff.2017.07.047.

[40] Y. Gao *et al.*, “Formulation optimization and in situ absorption in rat intestinal tract of quercetin-loaded microemulsion,” *Colloids Surf. B Biointerfaces*, vol. 71, no. 2, pp. 306–314, Jul. 2009, doi: 10.1016/j.colsurfb.2009.03.005.

[41] I. Erlund, M. Silaste, G. Alfthan, M. Rantala, Y. Kesäniemi, and A. Aro, “Plasma concentrations of the flavonoids hesperetin, naringenin and quercetin in human subjects following their habitual diets, and diets high or low in fruit and vegetables,” *Eur. J. Clin. Nutr.*, vol. 56, no. 9, pp. 891–898, Sep. 2002, doi: 10.1038/sj.ejcn.1601409.

[42] I. Erlund, “Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology,” ., vol. 24, no. 10, pp. 851–874, Oct. 2004, doi: 10.1016/j.nutres.2004.07.005.