FORMULATION AND EVALUATION OF SOLID LIPID NANOPARTICLES LADEN IN-SITU GEL OF QUERCETIN FOR OCULAR DISEASES

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In

Pharmaceutics

By

NOOR HASSAN SULAIMAN KHIL

(Enrollment No. 21122020048)

Guide

Prof. (Dr.) Shaweta Sharma

DEPARTMENT OF PHARMACY
SCHOOL OF MEDICAL AND ALLIED SCIENCES
GALGOTIAS UNIVERSITY, UTTAR PRADESH, INDIA

July, 2023

DECLARATION

I, hereby declare that the dissertation titled **"Formulation and Evaluation of Solid Lipid Nanoparticles laden in-situ Gel of Quercetin for Ocular Diseases"** submitted here in has been carried out by me in the School of Medical and Allied Sciences of Galgotias University, Uttar Pradesh. The work is original and has not been submitted earlier as a whole or in part for the award of any degree at this Galgotias University.

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CERTIFICATE

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Prof. (Dr.) Pramod Kumar Sharma
Dean
School of Medical & Allied Sciences

Prof. (Dr.) Shaweta Sharma
Guide
School of Medical & Allied Sciences

External Examiner

Approval Sheet

This thesis/dissertation/report entitled **"Formulation and Evaluation of Solid Lipid Nanoparticles laden in-situ Gel of Quercetin for Ocular Diseases"** by **Noor Hassan Sulaiman Khil** is approved for the degree of **Master of Pharmacy (Pharmaceutics)**

Examiners

____

____

Supervisor (s)

____

____

Chairman

____

Date:___________

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STATEMENT OF THESIS PREPARATION

1.	Thesis title	**Formulation And Evaluation of Solid Lipid Nanoparticles laden in-situ Gel of Quercetin for Ocular Diseases**
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6.	The thesis has been prepared without resorting to plagiarism.	Yes
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(Signature of the student)

Name: Noor Hassan Sulaiman Khil

Roll No: 21122020048

ABSTRACT

Conventional ocular drug delivery systems suffer from numerous limitations, including low bioavailability, rapid clearance, and poor residence time on the ocular surface. Synthetic dosage forms have also presented challenges due to their potential toxicity and limited biocompatibility. This research aims to overcome these drawbacks by formulating and evaluating solid lipid nanoparticles (SLNs) laden in-situ gel of Quercetin for ocular diseases. Drug excipient compatibility was assessed by FT-IR technique and it was found that the selected drug excipients were compatible to each other. The SLNs were prepared using Glyceryl monostearate as the lipid component and a combination of soya lecithin as polymer and poloxamer 407 as surfactant. The formulated SLNs were characterized using various techniques, Percentage yield, Entrapment efficiency, Particle size, Zeta potential, Scanning electron microscopy (SEM) and *In-vitro* drug release. The incorporation of Quercetin into the SLNs aimed to increase its residence time on the ocular surface by converting it into an in-situ gel formulation. The optimization of the SLNs formulation was achieved using a 3^2 full factorial design, with the independent factors being the amounts of Glyceryl monostearate and soya lecithin. The effects of these factors on the dependent variables were systematically evaluated.

A checkpoint batch, identified as the best batch, was obtained from the optimization study. This batch was then transformed into a nanoparticle formulation and subjected to comprehensive evaluation for various parameters. The outcomes of these evaluations, including physicochemical characterization, release kinetics, particle size, and morphology, confirmed the successful formulation of SLNs of Quercetin. The optimized formulation exhibited desirable attributes, such as controlled drug release, small particle size, and suitable surface charge. This batch was further converted into in-situ gel utilizing appropriate gelling agents. The prepared gel was also evaluated for Quality control parameters and satisfactory result were obtained.

The present work represented a significant advancement in ocular drug delivery systems, providing an innovative approach to enhance the therapeutic efficacy of Quercetin. By incorporating quercetin into SLNs and formulating them as in-situ gels,

the drawbacks associated with conventional and synthetic dosage forms can be overcome. The utilization of a factorial design allowed for the systematic optimization of the formulation, leading to the development of a promising ocular drug delivery system. The findings of this study have the potential to contribute to the development of effective treatments for ocular diseases and pave the way for future research in the field of ocular drug delivery.

Dedicated

To

My Parents

&

Guide

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Annexure 1

LIST OF MATERIAL

Ingredient	Manufacture/Supplier
Quercetin	HI Media Laboratories Pvt. Ltd. Mumbai, India
Glyceryl monostearate	CDH, New Delhi
Soya lecithin	HI Media Laboratories Pvt. Ltd. Mumbai, India
Poloxamer 407	HI Media Laboratories Pvt. Led. Mumbai, India
Carbopol 940	CDH, New Delhi
Hydroxy Propyl Methyl Cellulose (HPMC)	CDH, New Delhi

LIST OF EQUIPMENTS

Equipment's	Manufacturer/ Supplier
Digital weighing machine	B13082, KERRO
Rotatory Film Evaporator	Lab India
Prob Sonicator	Pro 650
High Speed Homogenizer	Ika T18D
pH meter	Pico pH
FT-IR	Alpha, Bruker
Dissolution	LABINDIA DS8000
Cooling Centrifuge	REMI cPR-30 Plus
UV-VIS 1800	Schimadzu 1800, Japan

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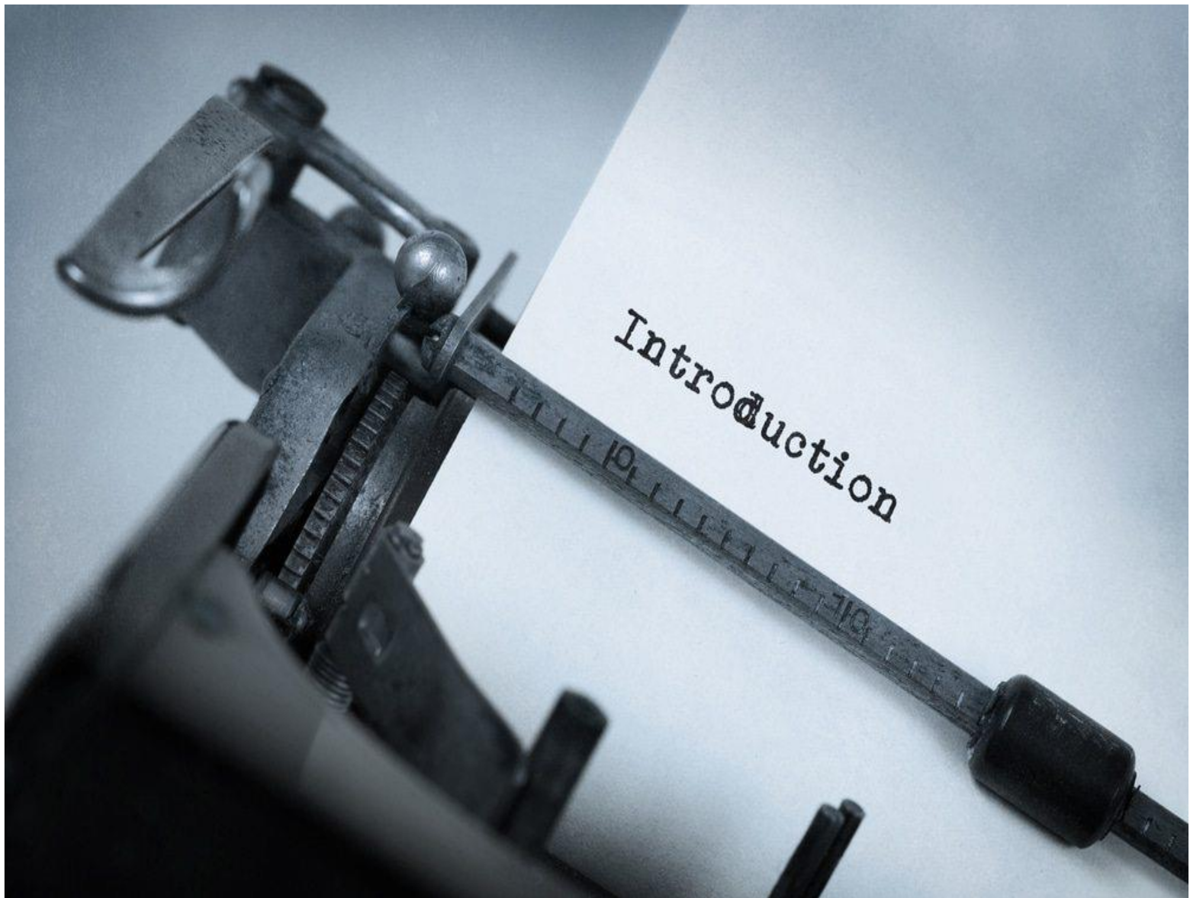
List of Abbreviations

S. No	Abbreviation	Full form
1	SLNs	Solid Lipid Nanoparticles
2	-	Absent
3	±	Standard deviation
4	Nm	Nanometers
6	RGCs	Retinal ganglion cells
7	WHO	World Health Organization
8.	IOP	Intraocular pressure
9	POAG	Primary open-angle glaucoma
10	AMD	Age related Macular degeneration
11	BOB	Blood Ocular Barrier
12	BRB	Blood retinal barrier
13	ADDE	Aqueous deficient dry eye
14	EDE	Evaporative dry eye
15	CNV	Choroidal neovascularization
16	VEGF	Vascular endothelial growth factor
17	DR	Diabetic retinopathy
18	NSAIDs	Nonsteroidal anti-inflammatory medications
19	ONS	New ocular nano systems
20	NLC	Nano lipid carriers
21	HSH	High shear homogenization
22	SNEDDS	Self-nanoemulsifying drug delivery system

23	**XRD**	X-ray diffraction
24	**Temp^o**	Temperature
25	**^{o}C**	Degree Celsius
26	**RBF**	Round Bottom Flask
27	**SEM**	Scanning Electron Microscopy
28	**EE%**	Encapsulation/Entrapment Efficiency
29	**HPLC**	High Performance Liquid Chromatography
30	**HLB**	Hydrophilic lipophilic balance
31	**v/v**	Volume by volume
32	**w/v**	Weight by volume
33	**Λmax**	Lambda maximum
34	**Mg**	Microgram
35	**Mg**	Mili garam
36	**Ml**	Mili litter

CHAPTER 1

INTRODUCTION



CHAPTER 1
INTRODUCTION

1.1 OCULAR DRUG DELIVERY SYSTEMS

Eye drops and other traditional formulations are among the most popular ocular medication delivery systems or dosage forms, and they need daily dosing. However, complicated implant systems require dosing just once every few years. Ocular inlays and/or implants, prepared gels, in-situ gels, liposomes, microparticles, nanotechnology-derived drug delivery systems such as nanoparticles, nano emulsions, and nano micelles; and the physical ways to promote drug delivery such iontophoresis and microneedles are examples Among the many methods and systems for ocular medication administration that have been studied in an effort to address unmet medical requirements and problems with current methods [1]. Application of solution, suspension, or ointment formulations into the conjunctival sac of the eye is the most preferred method for treating ocular conditions. Primarily, the topical route is used to administer medications to the anterior segment of the eye. Although the bioavailability of the drug administered through this route is constrained by formulation concerns such as aqueous solubility & stability, as well as permeability and delivery challenges including precorneal drainage, corneal ultrastructure, & drainage through the conjunctival vasculature or nasolacrimal duct [2-3]. Including subconjunctival, retrobulbar, subtenon, peribulbar, and posterior juxta scleral routes, periocular administration is the most efficacious and minimally invasive route for a posterior segment of the eye. Physical barriers associated with this pathway include the sclera, choroid-Bruch's membrane, and RPE. Another option is the systemic or oral administration of therapeutic agents to the ocular tissues. This route, however, is impeded by several physiological barriers (blood ocular barrier (BOB) and blood retinal barrier (BRB)) and results in excessive systemic drug exposure. Intravitreal administration, in which the medication is injected directly into the vitreous humor, is highly effective but intrusive.

The physicochemical properties of drug molecules play a crucial role in determining ocular bioavailability after topical administration. The relationship between the oil water partition coefficient & corneal bioavailability is generally parabolic [2]. In a series of tested steroids, compounds with log octanol–water partition coefficients (logP) between 2 and 3 exhibited the highest corneal permeability [4]. Therefore, for efficient ocular tissue permeation, compounds must be neither too hydrophilic nor too lipophilic [2]. Medication delivery to the eye has been difficult because of the many barriers present in the eye, making it more difficult than medication delivery to other regions of the body. Many of these obstacles are inherent & unique to ocular anatomy and physiology, making this a difficult challenge for scientists who study drug delivery. These obstacles vary based on the route of an administration, namely systemic, topical, and injectable. The majority of these are anatomical and a barrier that safeguard the eye from toxic substances. In addition, numerous reformulations and formulation factors must be taken into account when designing an ophthalmic formulation [5].

1.2 EYE

The eye is a visual organ that communicates with the visual centers of the brain via the optic nerve. There are ten principal types of composite optical systems with determining power, and 96% of animal species possess complex optical systems [6]. The eye is a remarkable organ with its own unique anatomy and physiology. The anterior segment and the posterior segment are the principal anatomical components of a eye (Figure 1). The anterior segment comprises approximately one-third of the eye, while the posterior section comprises the remainder. Including the cornea, conjunctiva, aqueous fluid, iris, ciliary body, and lens, the anterior segment of the eye comprises the most prominent structures. The posterior segment of the eye encompasses several anatomical structures, including the sclera, choroid, retinal pigment epithelium, optic nerve, neural retina, and vitreous humor. [7]. The shape of the lens is altered for near vision and ciliary muscle-restricted vision. Light-sensitive cells in the retina convert photons into electrical impulses, which are then transmitted to the brain via the optic nerve as vision and sight [8]. An anterior segment of the eye consists of the vitreous humor, cornea, iris, and ciliary body, as well as the lens, which

contains two fluid-filled chambers: I the posterior chamber between the iris, and (ii) The anterior chamber is located between a posterior surface of the cornea., the iris, and the front face of the vitreous. The anterior section is flooded with aqueous humor that nourishes the surrounding tissues. There exist three principal diseases that impact the anterior segment of the eye, namely hyphenate, anterior uveitis, and glaucoma [9]. The term "posterior segment" is used to describe the area near the rear of the eye. The vitreous humor, retina, choroid, and an optic nerve are all part of a posterior segment of an eye, together with the anterior hyaloids membrane [10]. The protein content of aqueous humor is lower than that of clear water fluid. Separation from the ciliary epithelium is possible, and its two chambers are located front and back. 3 Aqueous humors primarily enlarge the eyeball and helps keep intraocular pressure stable. Aqueous humor drainage must be maintained at a steady rate to counteract the aqueous humor drainage that is continually produced by the ciliary progressions. Intraocular pressure has a negligible effect on the amount of aqueous humor produced or lost. The initial portion of the posterior chamber is used for aqueous humor drainage, and then there is a small opening between the posterior iris and anterior lens that connects to the anterior chamber. Schlemm's canal receives the aqueous humor from the trabecular meshwork [11].

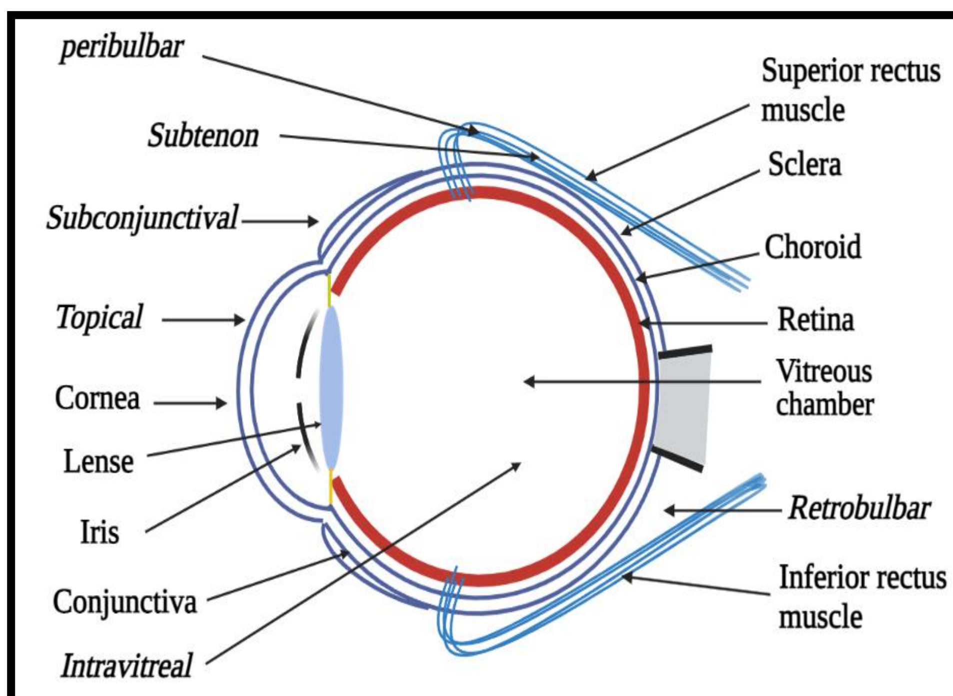


Figure 1.1: Routs of drug administration and Anatomy of eye

1.3 OCULAR DISEASES AND TREATMENT:

Several sight-threatening conditions, including independent eye disorders, may result in vision loss due to a variety of causes.

1.3.1 Dry Eye

A number of factors, such as tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities, can lead to dry eye, a disorder of the ocular surface marked by a loss of tear film equilibrium and accompanied by eye symptoms [12]. The two most prevalent types of dry eye are aqueous deficient dry eye (ADDE) & evaporative dry eye (EDE), but they are not mutually exclusive. Reduced tear secretion is a defining characteristic of dry eye disease. EDE is characterized by excessive tear fluid evaporation from an ocular surface [13]. Reduced inflammation and improved tear film function are the two primary approaches of treating DED [14]. Anti-inflammatory medications such as corticosteroids, lifitegrast and cyclosporin A, as well as artificial tears and autologous or allogenic serum, are common treatments for DED [15-16]. There is a possibility that sex hormones could assist with DED, but hormone replacement therapy is not recommended [17].

1.3.2 Glaucoma

Glaucoma is the primary cause of permanent blindness worldwide. Progressive optic neuropathy is a collection of conditions that result in progressive vision loss [18]. Degeneration of retinal ganglion cells (RGCs) and their axons causes gradual vision loss and an abnormal appearance of the optic disc, which are symptoms of age-related macular degeneration [19]. The typical IOP ranges between 12 and 22 mmHg. The most prevalent form of glaucoma is primary open-angle glaucoma (POAG), which is characterized by an open angle and elevated intraocular pressure (IOP). NTG is the subtype of glaucoma that is distinguished by a normal intraocular pressure (IOP) and an open angle. Both open-angle and angle-closure glaucoma are characterized by optic neuropathy and visual field abnormalities [20]. Without treatment, glaucoma could result in irreversible vision loss. Intraocular pressure (IOP) reduction is the only treatment for glaucoma that has shown any promise to date [21]. IOP-lowering

medications include prostaglandin derivatives, Agonists of beta-receptors, agonists of beta-2-receptors, inhibitors of carbonic anhydrase, and agonists of cholinergic receptors [22]. When medical treatment fails to reduce intraocular pressure (IOP) sufficiently with tolerable adverse effects, laser or incisional surgery is advised [21]. The current standard of care is trabeculectomy to reduce intraocular pressure [22].

1.3.3 Cataract

Cataracts are currently the leading worldwide cause of blindness. Seniors were disproportionately afflicted, indicating a connection between the two. Cataract is the prevalent name for the opacification of the crystalline lens of an eye. Cataracts may result in decreased visual acuity and contrast sensitivity, altered refractive & color vision, monocular diplopia or multi-view, glare, visual field defects, and other visual impairments. [23].

Currently, the most effective treatment for cataracts is surgery (intraocular lens implantation). Intracapsular extraction, extracapsular extraction, & phacoemulsification are the three distinct surgical techniques for removing a cataract lens. The intracapsular extraction method has been replaced by two alternatives [23]. However, drugs such as glutathione and N-acetylcysteine can only prevent so much lens oxidative injury [24].

1.3.4 Age-related macular degeneration

After the age of 50, AMD is leading cause of blindness in a Western world. By 2040, it is anticipated that 300 million persons will have age-related macular degeneration (AMD). An atrophic form of age-related macular degeneration inevitably results in the degeneration of the retinal pigment epithelium (RPE) and the photoreceptors. Choroidal neovascularization (CNV) complex, which may include fluid or retinal hemorrhage, retinal pigment epithelial detachments, rigid exudate, or a subretinal fibrous scar tissue, are characteristic lesions of exudative AMD. The medications used to treat AMD will be divided into three categories: those that help prevent the disease, those that treat exudative AMD, and those that treat atrophic AMD. High dietary consumption of lutein and zeaxanthin had the same effect, and studies indicate

that zinc and antioxidant supplements (vitamin C, vitamin E, beta-carotene, and copper) may reduce the likelihood of AMD progressing to advanced phases [25]. Hypoxia and other factors generate the angiogenesis-promoting vascular endothelial growth factor (VEGF). Anti-VEGF is the most prevalent treatment for exudative AMD at present, although it only treats the symptoms and not the underlying cause. Anti-VEGF medications may delay a progression of exudative AMD, but their effects are only temporary, and the disease frequently flares up again when treatment is discontinued. The pharmaceuticals eculizumab and lampalizumab, which target the complement system, are undergoing Phase 2 and 3 clinical trials, respectively [26].

1.3.5 Diabetic retinopathy

As a prevalent complication of diabetes mellitus, DR remains the leading cause of blindness worldwide [27]. Retinal neovascularization, microaneurysms, and vitreous protein exudates are typical DR symptoms, all of which contribute to a gradual decrease in retinal capillary density [28].

First, glycemic control is the cornerstone of treatment. Insulin has been found in clinical studies to reduce the prevalence and severity of DR [29]. For PDR, the most common therapy is pan-retinal laser photocoagulation [27]. Second, medications may be classified in the following ways based on their role in the pathophysiology of DR: medicine that prevents blood vessel growth, including anti-vascular endothelial growth factor medication, and medicine that reduces inflammation [28].

1.4 OCULAR INFLAMMATION

Ocular inflammation can arise from a diverse range of aetiologias, encompassing autoimmune disorders, infectious agents, allergic reactions, physical injury or trauma, surgical interventions, and, albeit infrequently, malignant neoplasms. Various ocular conditions can be influenced by inflammation, encompassing both the external eye and eyelids (blepharitis, scleritis), as well as the internal ocular structures, including the uvea (uveitis), and in more severe cases, the entire eyeball (endophthalmitis). This pathological condition leads to the disturbance of phospholipids within the cellular membrane and the synthesis of prostaglandin.

Prostaglandins induce vasodilation and heightened vascular permeability, leading to the manifestation of pain and sensitivity to light, elevated intraocular pressure (IOP), the development of posterior-capsule opacification, and impairment of vision [30]. Nonsteroidal anti-inflammatory medications (NSAIDs) and corticosteroids are two significant types of compounds that may be used to treat ocular inflammation. However, for these medications to be effective, they must go to the needed place in the right concentration and remain there for an adequate amount of time [31]. The administration of most of these medications typically involves the use of eye drops, ocular ointments, and gels. However, these formulations may not always provide sustained delivery of the medication at the eye site, necessitating frequent application. However, it should be noted that the current drug delivery methods for ocular administration are considered conventional and have certain limitations. These methods often do not provide sufficient contact time with the ocular surface and are highly susceptible to elimination through the nasolacrimal pathway and other elimination mechanisms such as tear dilution and turnover. Additionally, the differential lipophilicity of various ocular barriers, as well as the presence of lysozymes and carbonic anhydrase enzymes, can lead to biochemical degradation of the drug, ultimately reducing its effective bioavailability at the intended ocular site [32]. In point of fact, roughly 5% of the dosage that is supplied makes it to the anterior chamber of the eye through the topical route, and the remainder of the dose will be eliminated (within five minutes) by continual tear flow in reaction to the formulation being instilled [33].

1.5 NOVEL OCULAR DRUG DELIVERY SYSTEMS

The utilization of colloidal carriers has been extensively employed in the domain of drug delivery research. The approach offers enhanced specificity in targeting and sustained release of molecules at the intended location. The utilization of nanotechnology exhibits great potential in the management of a diverse range of ocular diseases, encompassing both the anterior & posterior segments of the eye, thereby generating considerable enthusiasm. A thorough understanding of the physiological and biochemical mechanisms that govern both normal and pathological

conditions is essential for the successful development of an effective ocular drug delivery system.

A desirable therapeutic approach entails the precise targeting of active agents to specific ailments such as choroidal neovascularization, diabetic retinopathy, and ocular solid tumors. The lymphatic system is absent in the retina, and it demonstrates angiogenesis that bears resemblance to solid tumors, such as the presence of enhanced permeability and retention (EPR) effects. The utilization of nanotechnology-based products for drug delivery serves three primary purposes: (i) augmentation of drug permeability, (ii) regulation of drug release, and (iii) targeted drug delivery. [34, 35]. Biodegradable carriers have the potential to serve as a substitute for nonbiodegradable polymer-based implants, which necessitate surgical removal after a specific duration. During the surgical procedure, various complications may arise, including astigmatism, vitreous hemorrhage, and patient noncompliance [36]. In order to address these concerns, researchers have been investigating nanotechnology-based new ocular nano systems (ONS) over the last two decades in the hopes of increasing bioavailability by prolonging the amount of time that a substance is in touch with the eye and so slowing down the eye's natural removal process [37]. This result was accomplished by inserting the medications into solid-lipid nanoparticles (SLNs), nanoemulsion, liposomes, cubosomes, niosomes, spanlastics, nanomicelles, and nanostructured lipid-carriers (NLC) (Figure 2) [38].

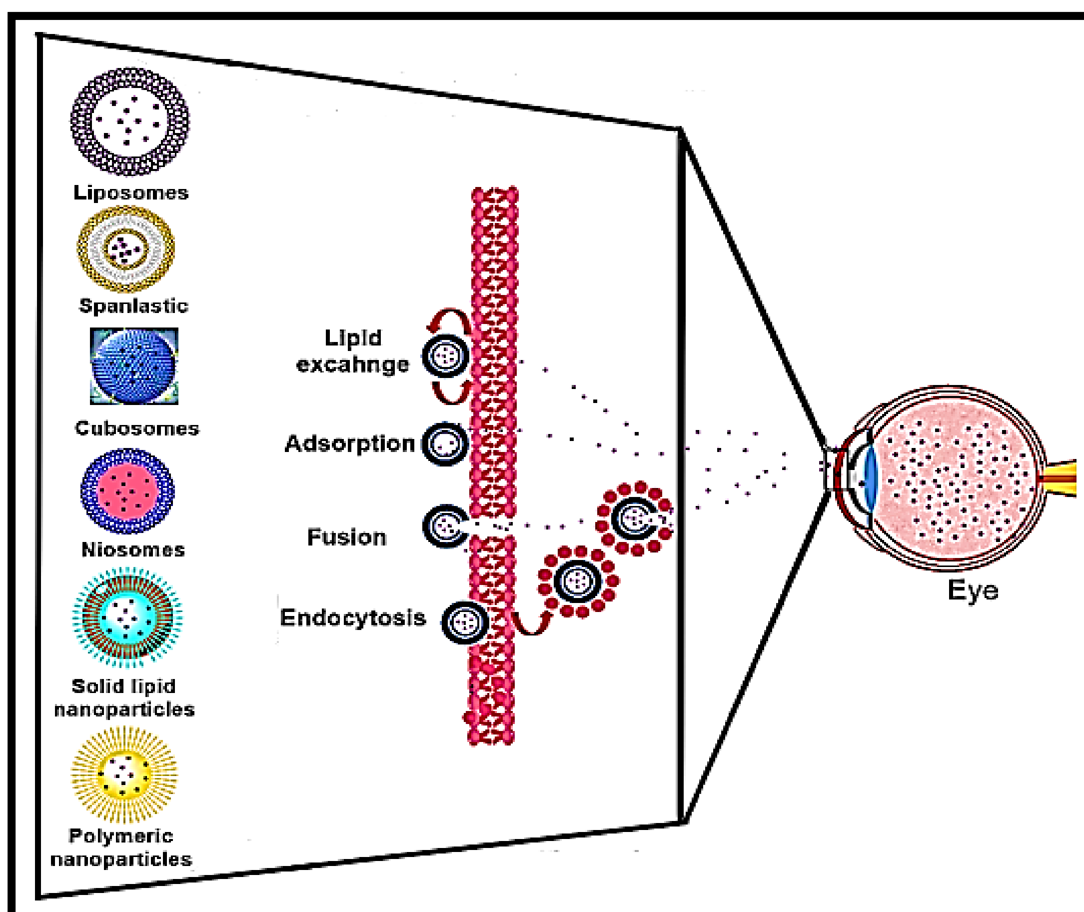


Figure 1.2: Ocular Nano system (ONS)

1.6 SOLID LIPID NANOPARTICLE (SLNs)

Solid lipid nanoparticles, formerly referred to as "lipospheres," are a potential nanocarrier for controlled drug delivery [39, 40]. Colloidal nanoparticles range in size from 10 to 1000 nanometers. These synthetic/natural lipids are suitable for optimizing drug distribution and reducing toxicity. They have emerged as a versatile alternative to liposomes as drug carriers over the years. The ability of nanoparticles to overcome numerous anatomical barriers, their extended content discharge, and their nanometer-scale stability all contribute to the efficient use of nanoparticles in drug delivery. Although the lack of secure polymers with regulatory approval and the high cost of nanoparticles have hampered their widespread use in clinical treatment [41]. SLNs are attractive due to their ability to improve the efficacy of pharmaceuticals as a result of their unique characteristics, such as their small size, extensive surface area, high drug loading, and phase interaction at the interface [42].

The materials' biocompatibility and biodegradability [43]. SLNs have several distinguishing characteristics and advantages, including lower toxicity, a larger surface area, a prolonged time before drug release, enhanced drug uptake by cells, and increased drug solubility and bioavailability (Figure 3) [44, 45]. Solid lipid nanoparticles (SLNs) combine the advantages of multiple carrier systems, including liposomes and niosomes, into one. Similar to other carrier systems, SLNs are composed of biocompatible, physiologically acceptable excipients with a similar nanoparticle structure to polymeric nanoparticles. In addition, it is hypothesized that the solid matrix of SLNs will shield the laden therapeutic molecules from the severe biological environment and other chemical degradations that have the greatest potential to alter the release patterns of the therapeutic molecules. Due to their numerous advantages, the SLNs constitute an exceptional transport network [46].

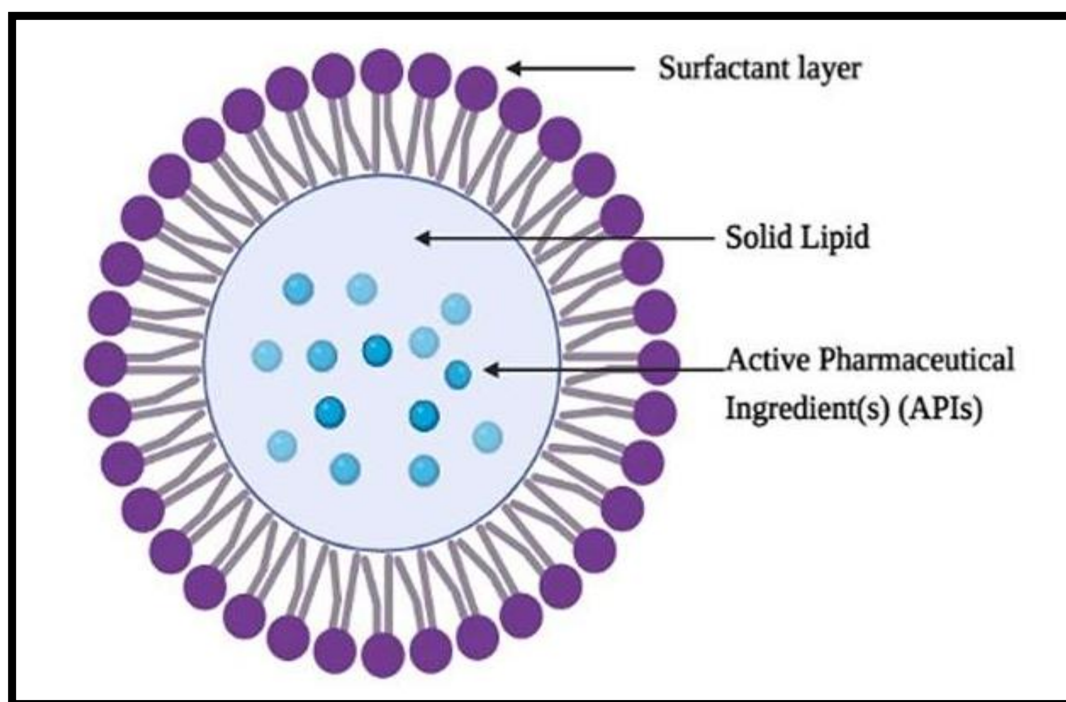


Figure 1.3: Structure of solid lipid nanoparticles

1.6.1 ADVANTAGES OF SOLID LIPID NANOPARTICLE (SLNs):

- Because of their nano size range, Reticuloendothelial system (RES) the cells are unable to absorb SLNs., allowing to circumvent spleen and liver filtration [47, 48].
- Scale up and simplify sterilization.
- Improve the bioavailability of compounds that are weakly water soluble [49].

- Outstanding biocompatibility [50].
- It is possible to provide dynamic medicine in a method of control over a lengthy period of time [51].
- The transporter lipids are biodegradable and hence safe. Natural solvents should be avoided. Enhanced bioavailability of atoms that are insufficiently water soluble [52].
- Bypassing the first pass of hepatic metabolism [53].
- Preventing negative medication effects [54].

1.6.2 METHOD OF PREPARATION OF SOLID LIPID NANOPARTICLE (SLNS):

1.6.2.1 HIGH PRESSURE HOMOGENIZATION TECHNIQUE

Cavitation is created by forcing a molten lipid solution at high pressure (100–200 bars) through a tiny opening (typically in the micron range) at high speed. The collision with the solid surface further agitates the mixture before it is discharged as a uniform substance. Particles are broken down to the submicron range due to cavitation and shear stress. Nano dispersion can be achieved with a lipid concentration as high as 40%, despite the fact that the typical lipid content is between 5% and 10% [55]. Hot- and cold-homogenization methods are the two primary routes for making SLNs using high pressure-homogenization[56].

1.6.2.1.1 HOT HIGH-PRESSURE HOMOGENIZATION

Here, the lipid phase is heated to 90 °C before being mixed with the surfactant-containing aqueous phase at the same temperature. Three cycles are performed on the pre-emulsion in a high-pressure homogenizer at 90 °C and 5×10^7 Pa. Finally, solid lipid nanoparticles or Nanostructured lipid carriers are solidified by cooling the obtained oil in the water emulsion to room temperature [57].

1.6.2.1.2 COLD HIGH-PRESSURE HOMOGENIZATION

SLNs Lipid microparticles are produce by cooling the molten lipid phase until it solidifies, and then grinding it. The obtained SLNs lipid microparticles are pre-suspended by dispersing them in a cool aqueous phase containing surfactants. After

that, a high-pressure homogenizer is used to cycle the pre-suspension five times at room temperature and a pressurne of 1.5×10^8 Pa. [58].

1.6.2.2 ULTRA SONICATION TECHNIQUE

The use of sound waves to reduce particle size is key to the success of this technique. Here, both high-pressure homogenization and ultrasonication are used to make SLNs that are between 80 and 800 nm in size (Figure 4). Other cutting-edge methods have also been implemented for SLNs formulation [59]. Since its inception, solid lipid nano dispersions have been produced using the dispersing technique known as ultrasonication [60]. Ultrasonication and high-speed homogenization methods are also used in the preparation of SLNs. Smaller particles can only be achieved through a combination of ultrasonication and high-velocity homogenization. It has the advantage of lowering shear stress but also a number of drawbacks, such as the risk of metal contamination and the physical instability introduced by particle growth during storage. This process makes use of a probe sonicator or a bath sonicator [61-62].

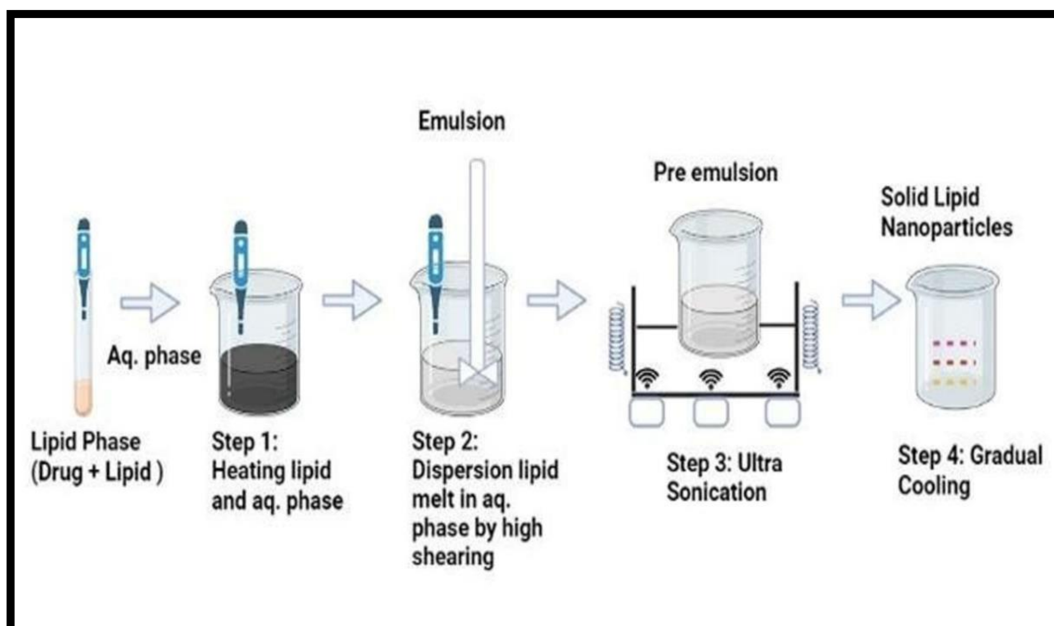


Figure 1.4: Ultrasonication technique for solid lipid nanoparticles.

1.6.2.3 SOLVENT EVAPORATION METHOD

Solvent evaporation is another method that can be used to make SLNs. In order to dissolve the lipophilic substance, an organic solvent that is incompatible with water

is used to create an aqueous emulsion (such as cyclohexane). As the solvent evaporates, nanoparticles of the typical size of 25 nm are precipitated from the lipid in the aqueous media. High-pressure homogenization was used to create an aqueous phase emulsion of the solution. The emulsion's organic solvent was evaporated at low pressure in order to purge it (40–60 mbar) [63].

1.6.2.4 SOLVENT EMULSIFICATION DIFFUSION METHOD

This strategy is useful for obtaining particles with diameters between 30 and 100 nm [61]. The biggest benefit of using this method is that moderate heat is required during the preparation process [64]. In this method, an acidic aqueous phase is combined with lipids dissolved in an organic phase in a water bath at 50 °C to modify the zeta potential and produce a coacervation of SLNs [65]. The production of the SLNs suspension was rapid. Once centrifuged, the system may be resuspended in clean water [66].

1.6.2.5 SUPER CRITICAL FLUID TECHNIQUE

This technique for making SLNs is innovative in its day. The thermophysical properties of the supercritical fluid are distinct and can be finely tuned by making small adjustments to the pressure. Processing without the use of solvents [67]. Using the ultrasonication method and supercritical carbon dioxide, which has a tendency to dissolve lipophilic drugs, SLNs can be prepared. Ultrasonication and supercritical carbon dioxide extraction have been used to create Xionggui-loaded SLNs. Xionggui loaded SLNs is a solid lipid nanoparticle loaded with a Xionggui powder-supercritical carbon dioxide fluid extract (Figure 5) [68].

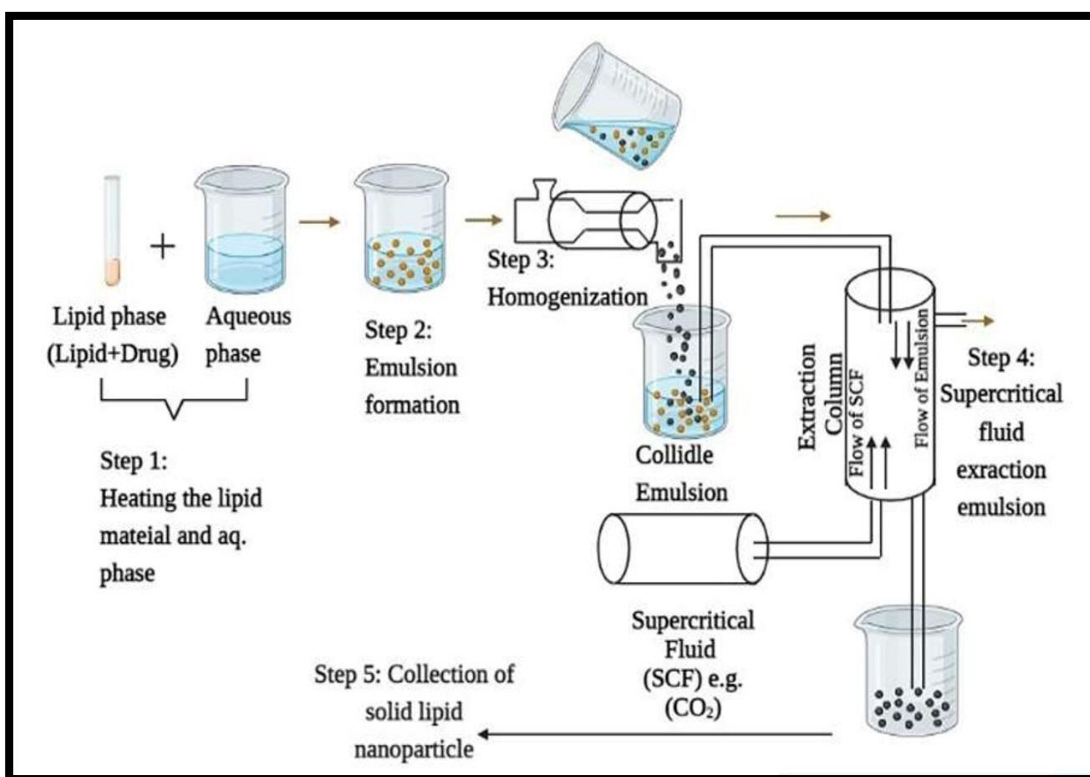


Figure 1.5: Super critical fluid technique

1.6.2.6 MEMBRANE CONTRACTOR TECHNIQUE

To facilitate industrial-scale production, this research looks into a novel method for preparing SLNs using a membrane contactor. For a visual representation of this procedure, see (Figure 6).

Lipid phase droplets develop when the lipid phase is heated over its melting point and is therefore pushed through the pores of the membrane. The aqueous phase moves around the membrane module itself, clearing the pore outlets of any droplets that may have formed. After the preparation is made, by bringing it down to room temperature, SLNs are created. The investigation focuses on the influence of process parameters, namely the size of the solid lipid nanoparticles (SLNs) and the flow rates of the lipid phase. Specifically, the temperature of the aqueous phase, a pressure of the lipid phase, the cross-flow velocity of the aqueous phase, and the pore size of a membrane are considered. Additionally, Solid lipid nanoparticle that have been loaded with vitamin E are made, and their stability is shown [69].

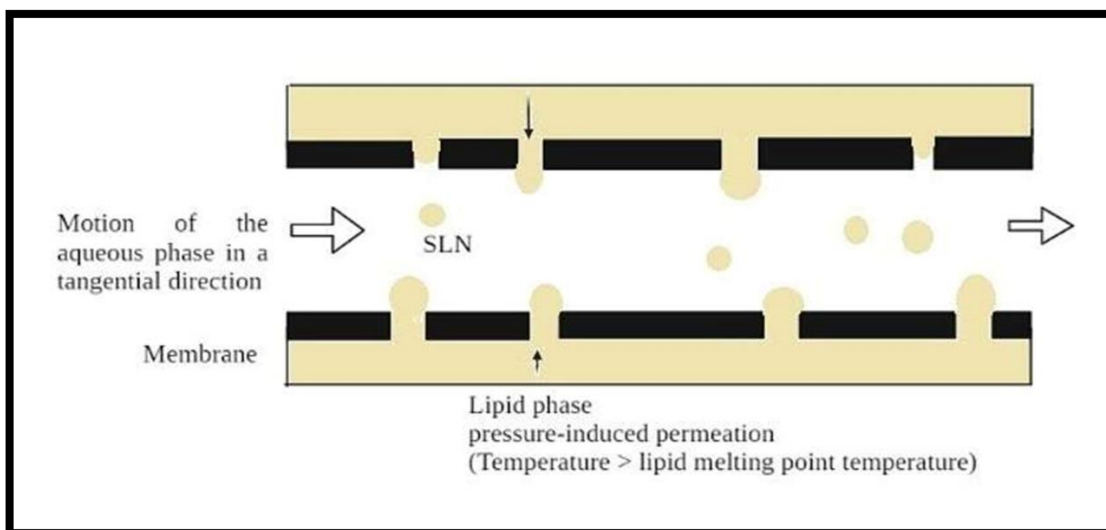


Figure 1.6: Diagrammatic representation of the Membrane Contractor used to make SLNs.

1.6.2.7 MICROEMULSION TECHNIQUE

Lipophilic phase (lipid) dispersions that consist of a surfactant, a co-surfactant, a lipid, and water that are transparent and thermodynamically stable are called microemulsions. The lipid phase of microemulsions precipitates out of solution when water is added. The Gasco research group used this method to make SLNs. We begin by dispersing the medicine throughout a molten lipid (fatty acid/glyceride). The lipid melting point is at least equal to the temperature at which heated water, surfactant, and co-surfactant. Transparent microemulsion is obtained by adding While swirling slowly, add this aqueous surfactant solution to the lipid melt.

The microemulsion is then mixed with water between 28- and 108-degrees Celsius while being gently stirred mechanically. The hot microemulsion is diluted with cold water at the volume ratio of between 1:25 and 1:50. As an oil droplet is dispersed in a cold aqueous medium, it rapidly recrystallizes, giving rise to SLNs. It's important to keep in mind that SLNs are formed not by a stirring process, but rather a precipitation process. After diafiltration, the resulting lipid nanoparticles suspension can be washed with water and lyophilized [60]. Lecithin, bile salts, taurodeoxycholate sodium, and butanol are some examples of the surfactants and co-surfactants used in this process [70].

1.6.2.8 SOLVENT INJECTION

SLNs formation works on a similar principle to the solvent diffusion technique. To make SLNs, however, solid lipids are dissolved in water-miscible solvents and injected into the water at high speed. Lipids in solid form can be dissolved in a solvent mixture that is miscible with water. Solvents like acetone, ethanol, isopropanol, and methanol are commonly used in this process. Particle sizes of 80-300 nm were obtained by Schubert and Muller-Goymann when they used this technique to prepare SLNs [71]. Diffusion appears to be the controlling force in the formation of SLNs, as approximately 96.5 percent of the used lipid was converted into SLNs [72].

1.6.2.9 DOUBLE EMULSION TECHNIQUE

A two-stage process, the double emulsion technique is used to create SLNs. The medication, typically one with hydrophilic properties, is solubilized within an aqueous solvent, referred to as the inner aqueous phase. This solution is subsequently dispersed within an emulsifier/stabilizer that contains lipids, resulting in the formation of a primary emulsion of the water-in-oil (w/o) type, such as lecithin. A double emulsion of the water-in-oil-in-water (w/o/w) type is formed through the introduction of an aqueous solution containing a hydrophilic emulsifier, such as poloxamer or polyvinyl alcohol (PVA), followed by agitation and subsequent filtration [73,74]. The double emulsion method may be used to create lipid nanoparticles that are loaded with proteins and peptides, which eliminates the need to melt the lipid, and allows for the possibility of satirical stabilizing the nanoparticles via the addition of a PEG-lipid derivative. Steric stabilization refers to the adsorption of bulky molecules on the surface of nanoparticles, which may include surfactants or polymers, to prevent aggregation. That these colloidal structures may withstand the fluids of the gastrointestinal tract was greatly enhanced by satirical stabilization [75,76]. With each iteration of emulsification, the droplet distribution becomes more and more heterogeneous, aiding in the creation of a double emulsion with a higher polydispersity. Therefore, those particles are double emulsion particle lack the structural and sizing precision necessary for use in applications requiring controlled release of active materials [77]. A colloidal system containing sodium cromoglycate and SLNs was produced, but its average particle size was in the micrometer range.

Sodium cholate phosphatidylcholine based mixed micelles was used in a novel reverse micelle-double emulsion technique to prepare insulin-loaded SLNs [78, 79].

1.6.2.10 A METHOD FOR DISPERSING MELT (HOT MELT ENCAPSULATION METHOD)

Instead of using a solution of lipids in an organic solvent, solid lipid can be liquefied and utilized with the melting dispersion method. After incorporating the medication into the lipid dissolve as a solid or solution using vigorous vortex mixing, the mixture is emulsified in a small volume of the aqueous phase heated above a melting point of the lipid. As a next step, SLNs are made by cooling the dispersion until it is at room temperature. In the melting dispersion method, there is no need to evaporate the organic solvent, making the final step identical to the solvent evaporation method. When compared to solvent emulsification-evaporation and ultrasonication, reproducibility is lower [80,81].

1.6.2.11 MICROCHANNEL/ MICROFLUIDIC TECHNIQUE

As can be seen in (Figure 7), it's a new kind of microchannel system in which the junction is in the shape of a cross and is formed by a primary microchannel and two branches [82]. The main channel carries in the form of a lipid solution (the lipid dissolved in a water-miscible organic solvent), while at side branches receive aqueous surfactant solutions. At the main channel's crossroads, the two liquids merged into one. Diffusion of the solvent from the flowing lipid solution into the surrounding aqueous phase caused local supersaturation of the lipid, this ultimately resulted in the establishment of SLNs [83,84]. The concentrations of lipid and surfactant, as well as the velocities of the lipid solution and aqueous phases, all have a role and were found to have an effect on the size of SLNs made using the microchannel method by Zhang S et al. SLNs mean size decreased as aqueous phase velocity increased, but lipid solution velocity increased [85].

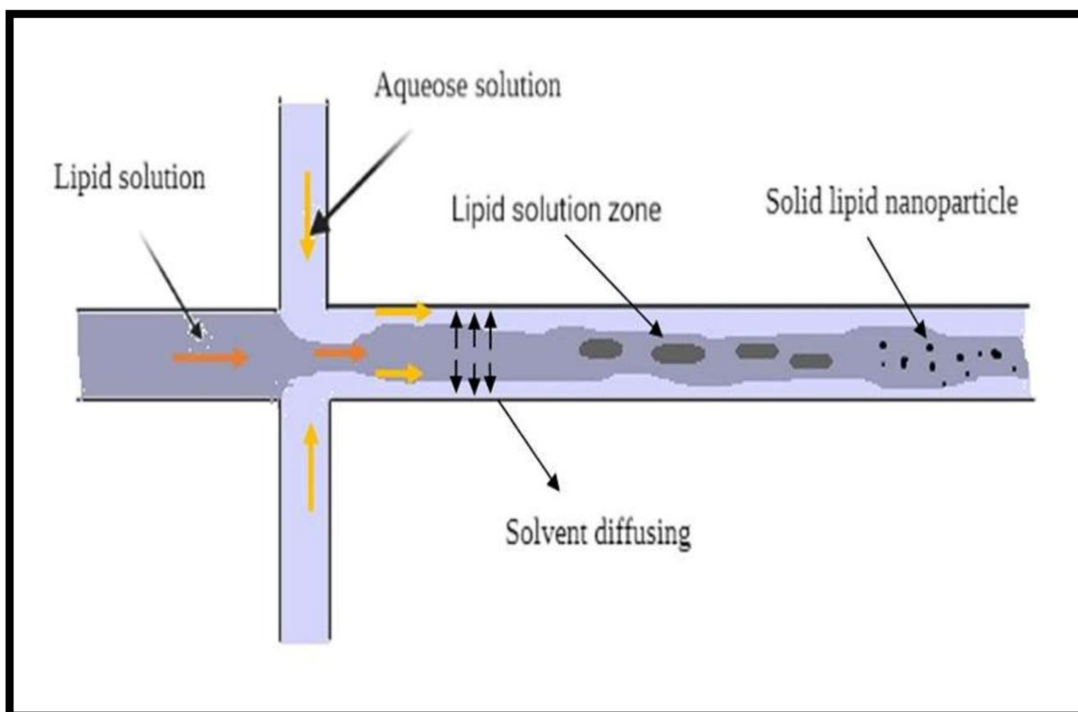


Figure 1.7: A simplified schematic depicting the formation of SLNs in a microchannel system

1.6.2.12 PRECIPITATION METHOD

An organic solvent (like chloroform) is used to dissolve the glycerides, and then the solution is emulsified in water. Nanoparticles of lipid will form after the organic solvent evaporates [86].

1.6.3 SECONDARY PRODUCTION STEPS:

1.6.3.1 SPRAY-DRYING

This is an alternative to a lyophilization for drying out SLNs that have been dispersed in water. The nature and amount of sugars used in the process, as well as the percentage of SLNs in the dispersion, all have an impact on the final product. Enhanced kinetic energy from high temperatures and shear forces causes particles to collide frequently during the spray-drying of SLNs. Lipid-based SLNs, which have a lower melting point than water, are vulnerable to melting during the spray drying process, which is a major drawback of this method. To avoid this issue, lipids with a higher melting point (like tribehenin 72^{0}C) should be used. Carbohydrates, which

surround the particles and stop lipid droplets from melting together, could mitigate the effect of heat [87,88].

1.6.3.2 LYOPHILIZATION

The chemical and physical stability can be increased through lyophilization, which could be useful for long-term storage [89]. Once, lyophilization was required to ensure the long-term stability of a product containing hydrolysable pharmaceuticals or a product suitable for oral administration [90]. By changing into a solid, the Oswald ripening and hydrolytic reactions are both avoided [91]. When the product is freeze-dried, there is an increase in the size and dispersion of the lipid nanoparticles produced by all of the lipid matrices. Freeze drying, by removing water, creates an environment favorable for SLNs to aggregate. While being frozen and dried, solid lipid nanoparticles can be shielded from aggregation with the help of a sufficient amount of cryoprotectant [92].

1.6.3.3 STERILIZATION

Autoclaving is the best way to sterilize nanoparticles before giving them through a vein. This can be done with formulations that contain heat-resistant drugs. Researchers looked into the impact of sterilization on particle size and found that it significantly increased particle size. Schwarz looked into how alternative sterilization processes (steam sterilization at 121°C (15 min) and 110°C (15 min), g-sterilization) affected SLNs properties. Results show that particles aggregation may develop after the treatment is applied. Both the sterilization temperature and the SLNs composition are crucial. In order to ensure the sample's physical stability at elevated temperatures, picking the right emulsifier is crucial. Each emulsifier's mobility and hydrophilicity will be affected differently by higher temperatures. When lipid particles are melted during steam sterilization, an o/w-emulsion is formed. Recrystallization results in the formation of solid particles. Due to the low increase in particle size during steam sterilization, Schwarz concluded that lecithin is an appropriate surfactant for the process. According to Freitas's experiments, preventing the particle increase and avoiding gelation can be achieved by lowering the lipid content (to 2%), as well as by modifying the surface of the glass vials. Moreover, Freitas noticed that nitrogen

purging provided some shielding during the sterilization process. Because of this finding, to some extent, particle destabilization might be due to chemical processes. For samples that are damaged by high temperatures, y-irradiation may be preferable to steam sterilization [93,94].

1.6.4 APPLICATION OF SOLID LIPID NANOPARTICLE (SLNs)

- Topical drug delivery system
- Controlled release of active substance
- Targeted carrier anticancer drug delivery to solid tumor
- Targeted brin drug delivery
- Cosmetics
- Antitubercular chemotherapy
- Antitubercular chemotherapy
- Carrier for peptides and protein drugs
- Gene vector carrier
- Anti-microbial drug delivery
- potential agriculture

1.7 IN-SITU GELLING SYSTEM

Drug delivery methods that transform into a gel once within the body are called in situ gel-forming systems. Once within the body, these pills transform into a gel and slowly release the medication in reaction to environmental conditions such as pH and temperature. In the early 1980s, the unique idea of generating in situ gel was initially proposed. The development of covalent bonds (chemical cross-linking) or non-covalent bonds (physical cross-linking) between polymer chains is what causes gelation. As a result of polymer conformational changes in response to the physiological environment, in-situ gel forming systems may be thought of as low viscosity solutions that undergo phase transition in the conjunctival cul-de-sac to create viscoelastic gels. Since the fluid mechanism of the eye produces a solution or weak gel between instillation and the creation of a strong gel, the pace at which the gel forms in situ are crucial. In situ gels may be made from a wide variety of polymers, including those derived from nature and the lab [95].

1.7.1 ADVANTAGES OF IN-SITU GELS [96]

- Vision blurring is reduced compared to using ointment.
- Reduced systemic side effects may occur as a result of decreased nasolacrimal drainage of the medicine, which may induce unpleasant side effects. The potential for precise and repeatable dosage, as opposed to previously gelled formulations, with the added benefit of enhancing precorneal retention.
- Sustained, long-term drug release and keeping a generally stable plasma profile.
- Reduced the number of times a patient had to use it. This made the patient more compliant and comfortable.
- In most cases, it's more pleasant than insoluble or soluble insertion.
- Increased precorneal residence duration and absorption has improved local bioavailability.
- Its production is simpler, which reduces the investment and manufacturing costs.

1.7.2. MECHANISM OF IN-SITU GELS

In situ gels operate through the following mechanisms: -

Based on the physical mechanism

- **Swelling**

  This method of in-situ gel formation involves the material absorbing water from its surroundings and expanding to occupy the desired space. For instance, polar lipid glycerol mono-oleate expands in water to form lyotropic liquid crystalline phase structures. It possesses some bio adhesive properties and is degradable in vivo via enzymatic action [97].

- **Diffusion**

  This procedure involves the diffusion of solvent from the polymer solution into the surrounding tissue, resulting in the precipitation or solidification of the polymer matrix. N-methyl pyrrolidone (NMP) has been demonstrated to be a useful solvent for this system [98].

- **Based on chemical reaction mechanism**

  In-situ gelation may result from chemical reactions involving the precipitation of inorganic substances from supersaturated ionic solutions, enzymatic processes, or photocatalyzed reactions [99].

1.7.3. TYPES OF STIMULI ACTIVITE IN-SITU GEL SYSTEM

- **Temperature triggered in-situ gel systems: -**

  The temperature-sensitive in-situ gel is the earliest, most investigated, and most prevalent form of stimuli-responsive gel. It can be introduced into the eye readily and precisely in liquid form without causing irritation or vision loss. The gel is formed at precorneal temperature (35°c) to withstand lachrymal fluid dilution without rapid precorneal drug elimination following administration [100]. It has been suggested that a good thermo-responsive ocular in-situ gel should have a gelation temperature above room temperature and undergo gel-sol transition at a precorneal temperature in order to avoid storing in a refrigerator prior to instillation, which can cause eye irritation due to its cold nature [101].

- **pH triggered in-situ gelling systems: -**

  This in-situ gelling system is comprised of pH-sensitive polymers that are poly electrolytes containing either an acidic (carboxylic or sulfonic) or a basic (ammonium salts) group that receive or release protons in response to a change in the pH of the ambient environment. At lower pH (pH 4.4), the formulation exists as a regular solution, but at pH 7.4, the pH of tear fluid, it undergoes gel formation [102].

- **Ion triggered in-situ gelling system: -**

  In ion triggered in situ gelling systems, the viscosity of the solution increases when exposed to the ionic concentration of tear fluids [103]. It is also known as osmosis-induced gelation. Ion-sensitive polymers are able to crosslink with cations (monovalent, divalent) present in lacrimal fluid on the ocular surface, thereby increasing the drug's retention time [104].

- **Multi-stimuli responsive in-situ gel: -**

  Recent advances in ocular in-situ gelling systems include the use of a combination of polymers with various gelling mechanisms, which have demonstrated enhanced therapeutic efficacy and greater patient compliance. Several studies involving the combination of thermos responsive polymers, pH-sensitive polymers, and ion-activated polymers in the same ophthalmic formulation have been published in recent years [105].

1.7.4. POLYMERS USED IN IN-SITU GEL [106]: -

- Temperature-triggered in-situ gel systems: -
  - Poloxamers
  - Xyloglucan
  - Chitosan
  - Hydroxy Propyl Methyl Cellulose (HPMC)
- pH triggered in-situ gelling systems: -
  - Carbopol
  - Polycarbophil
  - cellulose acetate phthalate (CAP)
- Ion-activated in-situ gel system: -
  - Gellan gum (Gelrite ®)
  - Hyaluronic acid
  - Sodium alginates.
- Multi-stimuli responsive in-situ gel: -
  - Sodium alginate and methylcellulose
  - Carboxymethyl chitosan (CMC)
  - poloxamer Chitosan with Gellan gum
  - Sodium alginate
  - chitosan
  - HPMC and Poloxamer.

CHAPTER 2
LITERATURE REVIEW

CHAPTER 2

LITERATURE REVIEW

2.1 LITERATURE REVIEW BASED ON TREATMENT: -

- **Alfredo G. Casanova et al. (2021)** Quercetin, an antioxidant flavonoid, has shown efficacy in mitigating nephrotoxicity in both animal models & clinical trials, making it a highly promising prophylactic agent under development. Due to its insolubility, Quercetin has limited clinical applications. This study aimed to determine a bioavailability & nephroprotective efficacy of the micellar formulation of Pluronic (F127) encapsulated Quercetin (P-Quercetin) with enhanced hydro solubility in rats. The intraperitoneal administration of P-Quercetin results in a higher plasma concentration and greater bioavailability of Quercetin compared to a administration of unmodified Quercetin at the same concentration. In addition, P-Quercetin exhibits nephroprotective properties and a modest improvement in certain renal function parameters compared to Quercetin, its natural counterpart. The study discovered that P-Quercetin significantly reduced the increase in plasma creatinine and urea levels, as well as the decrease in creatinine clearance, induced by the nephrotoxic chemotherapeutic drug cisplatin. In addition, P-Quercetin improved the histological evidence of tubular injury. The enhanced kinetic and biopharmaceutical properties of this novel formulation will facilitate further research into the potential of Quercetin as a nephroprotective agent, using reduced concentrations and administration routes that are more suitable for clinical application [107].
- **Jorge Raga-Cervera et al. (2021)** Glaucoma is a nerve disease that can cause people to lose their eyesight. It affects more than 100 million people around the world, with primary open angle glaucoma POAG) being the most common form. In ophthalmology, researchers have learned about the control of gene expression & gene networks, and many processes involved in the disease glaucoma. MicroRNAs (miRNAs or miRs) are small (18–22 nucleotides), single-stranded, noncoding RNA molecules that control the translation of genes. Analytical, observational, case-control study was done on 42 patients between the ages of 50 and 80. They were divided into two groups: (1) those with ocular hypertension OHT but no

glaucomatous neurodegeneration (ND), called the OHT group, and (2) those with POAG, called the POAG group. The volunteers were asked to get sociodemographic and personal/family information. They were also physically checked, and tissue samples were taken and frozen at 80 degrees Celsius before being processed for molecular-genetic tests. The RNA from the tissue was taken out, a collection was made, and next-generation sequencing was done. Different levels of the genes MiR-26b-5p, -miR-152-3-p, -miR-30e-5p, -miR-125b-2-5p-, -miR-224-5p, -miR-151a-3p, miR-1307-3p, and miR-27a-3p were found in the tears of OHT and POAG cases. Information about genes was taken from the databases DIANA-TarBase v7, DIANA-micrite-CDS, and Targets can v7.1. Only genes from at least four of the databases DisGeNet, Gene Distiller, MalaCards, OMIM PCAN, UniProt, & GO were used to make a network of metabolic pathways. We think that miRNAs & their target genes/signaling pathways could help us learn more about the molecular and genetic causes of glaucoma and, as a result, come up with the best ways to find HTO who are more likely to get glaucoma non-neovascular (ND). The function of these microRNAs as biomarkers for the diagnosis and/or prediction of OHT and prevention of glaucoma (ND) needs to be confirmed [108].

- **Nikolaos Katsinas et al. (2021)** Oxidative and inflammatory eye surface diseases are becoming a bigger problem in ophthalmology. Olive pomace "OP" is the most common pollutant in the olive oil business and could be harmful to the environment. However, it has beneficial phenolic substances like oleuropein (OL) and hydroxytyrosol (HT). On human corneal HCE & conjunctival epithelial (IM-ConjEpi) cells, an antioxidant & anti inflammatory effects of four OP extracts (CONV, OPT (1–3)), pure OL and HT, as well as their mixtures, were tested. CONV was recovered in the usual way, while OPT (1–3) were made by extracting liquids under pressure. Since CONV and OPT3 are high in HT and have more action, they were chosen for dose-dependent tests. Interleukin (IL)-1, IL-6, IL-8, and IL-17A, as well as interferon-induced protein [IP]-10 release and intracellular reactive oxygen species (ROS) generation, were measured in cells that had been activated with cancer necrosis factor or ultraviolet-B radiation. On HCE, both extracts and HT stopped the majority of measured ILs from being released, showing that they have a strong anti-inflammatory effect. In IM-ConjEpi, all materials stopped IP10 from

being released. Also, HT, OL, and both extracts had different amounts of antioxidant activity in both cell lines, depending on how much they were given. OPT3 was active at lower amounts than CONV, which shows that specific biomarkers are removed selectively using enhanced extraction methods. A high-value application as a potential eye surface treatment was suggested for OP valorization. [109].

- **Pooja Maharjan et al. (2019)** Ocular substance administration has been a well-established method for a treatment of ocular diseases. However, numerous anatomical & physiological barriers in an eye itself pose significant obstacles to achieving the required therapeutic efficacy and bioavailability in the eye. Although, recent advancements in nanoengineered strategies hold great promise for the development of improved ophthalmic drugs with enhanced ocular bioavailability via targeted drug delivery. Curcumin, a yellow-colored hydrophobic polyphenol, and its metabolic reduced product, tetrahydro curcumin (THC), are known for their beneficial pharmacological functions, such as anti-oxidant activities or anti-inflammatory at various tissue locations. However, these compounds' low aqueous solubility limits their bioavailability, thereby limiting their ubiquitous application. In the present study, we investigated how formation of inclusion complexes with various hydroxypropyl (HP)-cyclodextrins (CD) derivatives affects drug solubility. In order to enhance the storage stability of nanoengineered curcumin- or THC-containing formulations, the spray drying technique was employed. The formulations' physicochemical properties and cellular permeability were characterized [110].
- **Maria Letizia Manca et al. (2020)** Quercetin has been identified as a highly effective natural compound for mitigating inflammatory and oxidative mechanisms-associated skin disorders. The substance's limited bioavailability, which is primarily attributable to its poor water solubility, frequently limits its efficacy. The objective of the present study was to evaluate a potential of nanosizing Quercetin to improve its cutaneous bioavailability and saturation solubility. To accomplish this, a nanosuspension of Quercetin was created. Two different concentrations of Quercetin nanosuspensions (3% and 5%) were created by moist media milling with Tween 80 and Poloxamer 188 as stabilizing agents. In vitro studies utilizing keratinocytes have demonstrated the high biocompatibility of nanosuspensions as well as their ability

to reduce the oxidative effect of hydrogen peroxide on cells. These findings imply that nanosuspensions may have potential therapeutic applications for the treatment of cutaneous conditions [111].

- **Giuseppe Di Pede et al. (2020)** Quercetin, a flavanol found in foods, is known to have disease-preventing properties. However, its limited bioavailability may impede its efficacy. In order to enhance the stability and bioavailability of bioactive compounds, delivery systems have been developed. Using an in vitro model, the purpose of this study was to investigate the metabolic activity of Quercetin obtained from unformulated and phytosome-formulated sources in humans. Following an analysis of the two components' native (poly)phenolic composition, they were subjected to a 24-hour fermentation procedure using human faucal microbiota. Utilizing ultra-high performance liquid chromatography coupled with mass spectrometry (uHPLC-MSn) analyses, the concentrations of microbial metabolites were determined. During faucal incubation, the primary component of both products, native Quercetin, exhibited a lower susceptibility to microbial degradation when present in the phytosome-formulated variant compared to the unformulated variant. In the colonic microbiota, the bioavailability of Quercetin in both products was observed, leading to the production of derivatives of phenyl propanoic acid, phenylacetic acid, and benzoic acid. The level of Quercetin microbial metabolism was found to be higher in the unformulated constituent, with a time-dependent trend. This study provides novel insights into the examination of the effect of delivery systems on the modulation of flavanol microbial metabolism in the colonic milieu, which is a crucial stage in the anticipated bioactivity of flavanol consumption [112].
- **YAN-LING LI et al. (2017)** This study aimed to determine the effect of Quercetin on the cytotoxicity and cognitive decline caused by amyloid (A)-peptide in mice. The present study used the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method and a Y-maze assay to assess the radical quenching capacity and effect on working memory, respectively, of Quercetin treatment following a 4-day A administration. The study assessed the acute oral toxicity of Quercetin by determining the concentration necessary to elicit 50% neuronal cell death. A passive avoidance test was used to examine the effect of Quercetin on the A-induced decline in cognitive function. The results indicate that Quercetin suppressed the activity of DPPH radicals, resulting in

a 76.5% reduction in radical activity. Significantly protecting PC12 neuronal cells from A-induced mortality was Quercetin. Treatment of mice with 100 mg/kg of body weight per day of Quercetin for 30 days significantly improved A-induced learning and memory loss. The acute oral dose of Quercetin administered to rodents was calculated to be 575 mg/kg body weight. Thus, it has been determined that Quercetin has the potential to treat neurological disorders such as Alzheimer's. However, additional research is required to thoroughly investigate its effectiveness [113].

- **Marija Lesjak et al. (2018)** The bioavailability of Quercetin is limited and it undergoes significant metabolic transformation resulting in the formation of various metabolites. Despite limited knowledge regarding their biological functions, these metabolites play a pivotal role in elucidating the health advantages linked to the consumption of Quercetin through the diet. This study aimed to compare the antioxidant and anti-inflammatory properties of six Quercetin derivatives, namely Quercetin-3-O-glucuronide, isorhamnetin, tamarixetin, isorhamnetin-3-O-glucoside, Quercetin-3,4′-di-Oglucoside, and Quercetin-3,5,7,3′,4′-pentamethylether, with those of common onion extract, which is a primary source of dietary Quercetin, as well as with standards such as butylated hydroxytoluene and aspirin. The bioactivities of Quercetin derivatives were found to be significant, comparable to those of standard substances and onion. The antioxidant potency was observed to decrease upon derivatization of the hydroxyl groups of Quercetin. The potential of Quercetin to inhibit the production of inflammatory mediators was not found to be directly correlated with the number of its free hydroxyl groups. In conclusion, the Quercetin derivatives that are present in the systemic circulation subsequent to the consumption of Quercetin may exhibit robust antioxidant and anti-inflammatory properties, thereby potentially contributing to the overall biological efficacy of a diet that is rich in Quercetin [114].
- **Brigitte A. Graf et el. (2006)** The presence of Quercetin and Quercetin glycosides in food or dietary supplements results in the formation of glucoronate, sulphated, and methylated Quercetin conjugates in body tissues. This indicates that the bioactivity of Quercetin in vivo may be attributed to its metabolites. The present investigation aimed to analyze the metabolic processes of orally administered

Quercetin in both pre- and post-absorptive states. This was achieved by conducting a comparative analysis of the metabolite profile in the gastrointestinal (GI) tissues, contents, and internal tissues. A group of six F344 rats was subjected to a diet containing 0.45% Quercetin for a period of six weeks. The metabolite patterns in various tissues and contents such as stomach, small intestine, cecum, and colon, as well as in liver, kidney, and plasma, were determined using LCMS/MS. The gastrointestinal (GI) contents were found to have a high concentration of unmetabolized Quercetin, ranging from 94% to 100%. However, the Quercetin present in the GI tissues was observed to exist in the form of 11 different metabolites, which were sulfated, glucuronated, and methylated. The percentage of these metabolites was found to be 32% in the stomach, 88% in the small intestine, 27% in the cecum, and 46% in the colon. The metabolization of Quercetin occurred subsequent to its absorption, and it was discovered that the compound was present in the liver, kidney, and plasma in a predominantly sulphated methyl-Quercetin glucuronide form. The distinctive Quercetin metabolite profile observed in various gastrointestinal tissues of rats suggests that significant biotransformation occurs prior to absorption and distribution [115].

- **Randi L. Edwards et al. (2007)** The potential effectiveness of Quercetin supplementation in reducing blood pressure levels in individuals with hypertension has yet to be assessed. The present study aimed to investigate the hypothesis that the administration of Quercetin may lead to a reduction in blood pressure levels among individuals diagnosed with hypertension. Subsequently, an investigation was conducted to ascertain the potential correlation between the antihypertensive properties of Quercetin and the mitigation of systemic oxidant stress. A double-blind, randomized, placebo-controlled, crossover study was conducted to evaluate the effectiveness of 730 mg Quercetin/d for 28 days compared to placebo among a sample of 19 individuals with prehypertension and 22 individuals with stage 1 hypertension. Upon enrolment, prehypertensive individuals exhibited a blood pressure reading (measured in mm Hg and expressed as systolic/diastolic) of 137 6 2/86 6 1, while those diagnosed with stage 1 hypertension displayed a reading of 148 6 2/96 6 1. Quercetin supplementation did not result in any significant changes in the blood pressure of prehypertensive patients. Conversely, a statistically

significant decrease in systolic (27 ± 2 mm Hg), diastolic (25 ± 2 mm Hg), and mean arterial pressures (25 ± 2 mm Hg) was noted among patients with stage 1 hypertension following administration of Quercetin. Nevertheless, the indices pertaining to oxidant stress that were gauged in the plasma and urine exhibited no discernible impact from Quercetin. The present findings represent novel evidence indicating that Quercetin intake can effectively lower blood pressure levels among individuals with hypertension. In contrast to research conducted on animals, it was observed that there was no decrease in systemic indicators of oxidative stress in response to Quercetin [116].

- **Jaisinghani, R.N. et al. (2017)** This study investigated the antibacterial properties of the substance against various bacterial strains, including Staphylococcus aureus, Shigella flexneri, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, and Lactobacillus casei var Shirota, using the broth dilution method. At a concentration of 20 mcg/mL, Quercetin demonstrated inhibition of S. aureus and P. aeruginosa, whereas P. vulgaris and E. coli were inhibited at concentrations of 300 mcg/mL and 400 mcg/mL, respectively. At a concentration of 500 mcg/mL, there was complete indifference observed between Shigella flexneri and Lactobacillus casei var Shirota [117].
- **Chao Huang et al. (2020)** According to the available evidence, brachial plexus avulsion (BPA) is associated with oxidative stress and inflammation. Anti-inflammatory, anti-oxidant, neuroprotective and anti-apoptotic, are all characteristics of Quercetin. Using a temperature-sensitive poly (D, L-lactide-co-glycolide)-poly(ethylene-glycol)-poly (D, L-lactide-co-glycolide) (PLGA-PEG-PLGA) material, the present study evaluated the therapeutic efficacy of a hydrogel sustained-release system of Quercetin in bisphenol A (BPA). Hydrogel injections containing varying concentrations of Quercetin were administered directly to a rat model of bisphenol A (BPA) in the study. The study observed significant reductions in reactive oxygen species and interleukin-6 levels 24 hours after surgery, as well as an increase in the number of motor and functional neurons in the anterior horn of the spinal cord 6 weeks after surgery. utilizing a temperature-sensitive hydrogel system composed of PLGA-PEG-PLGA infused with Quercetin has been shown to reduce oxidative damage and inflammation within the spinal cord. Additionally, it

has been demonstrated that this system improves neuron survival rates, facilitates nerve regeneration, and promotes the recovery of motor function in rodents with early BPA exposure. The results indicate that the drug-containing hydrogel has promising potential for use in the clinical treatment of BPA [118].

- **Nancy Tripathi et al. (2022)** Sustained and extended exposure to sunlight, particularly the ultraviolet (UV) radiation it contains, poses a potential threat not only to the development of skin cancer, but also to premature skin ageing, photo dermatoses, and actinic keratoses. Flavonoids, which encompass Flavone, Flavanone, Flavone, Flavanol, Isoflavone, Neo flavone, among others, exhibit robust antioxidant properties and are employed as topical agents to safeguard against skin damage induced by UV radiation, as well as for dermatological purposes. Quercetin (Flavanol) is the most frequently utilized flavonoid and can be found in a variety of fruits, vegetables, and herbs. Our objective is to conduct a comprehensive analysis of the scholarly literature pertaining to the advancement of various innovative formulations for the treatment of skin ailments induced by exposure to UV radiation. The present review encompasses an analysis of diverse Quercetin formulations, with a focus on their solubility, stability, and efficacy. The outcomes of these formulations have been compiled and compared to provide a comprehensive understanding of their respective applications. Based on the comparative analysis conducted, it has been determined that three formulations, nanostructured lipid carriers, specifically glycerosomes and deformable liposomes, exhibit promising potential for future applications in the topical delivery of Quercetin. The aforementioned formulations exhibited improved stability, heightened Quercetin accumulation across various skin layers, facilitated skin permeation of the drug, and sustained drug release [119].

2.2 LITERATURE REVIEW BASED ON FORMULATION TECHNOLOGY: -

- **Terreni E et al. (2021)** The present study aimed to investigate the potential of utilizing a combination of in-situ gelling system and a drug-loaded self assembling nano micellar carrier as an innovative Ocular Drug Delivery System (ODDS) for the poorly water-soluble drug Cyclosporine-A (CyA). In this study, two non-ionic

surfactants, namely d--tocopherol polyethylene glycol succinate (VitE-TPGS) & polyoxyl 40 hydrogenated castor oil (RH-40), were employed for the synthesis of nano micelles. The nano micelles were subjected to physical-chemical characterization to assess their CyA entrapment efficiency (EE%) and loading efficiency (LE%), cloud-point (CP), regeneration-time (RT), size & polydispersity index (PI). This analysis enabled us to identify the optimal surfactant mixture that demonstrated satisfactory stability, high CyA-EE (99.07%), uniform and minimal dimensions, and facilitated the solubilization of a comparable quantity of CyA (0.144% w/w). The nano micellar formulation was characterized from technological and biopharmaceutical perspectives, considering parameters such as osmolality, pH, gelling capacity, rheological behaviour, wettability, transmission electron microscopy (TEM), and storage stability at 4 and 20°C. The utilization of this novel integrated approach facilitated the acquisition of aqueous dispersions that possessed transparency, injectability, and the ability to form a gelatinous substance when exposed to tear fluid. Consequently, this enhanced the extent to which CyA could be absorbed and utilized within the ocular environment. Furthermore, when compared to Ikervis®, this novel ocular drug delivery system (ODDS) effectively inhibited the trans corneal permeation of CyA, exhibited minimal cytotoxic effects, and prolonged the residence time of CyA in the precorneal area [120].

- **Badr MY et al (2021)** The administration of Tacrolimus (TAC) suspension is indicated for the treatment of moderate-to-severe cases of atopic keratoconjunctivitis (AKC) and vernal -keratoconjunctivitis (VKC), respectively. The objective of this study was to develop an aqueous eye drop formulation containing the hydrophobic compound TAC (TAC) and examine its distribution within the eye after being applied topically to a healthy rabbit model. The ultimate goal was to utilize this formulation for the treatment of allergic keratoconjunctivitis (AKC) and vernal keratoconjunctivitis (VKC). The technique of thin-film hydration was employed to encapsulate TAC (triacetin) within the chitosan-based amphiphile known as N-palmitoyl-N-monomethyl-N, N-dimethylN, N, N-trimethyl-6-O-glycolchitosan. This encapsulation process is referred to as Molecular Envelope Technology (MET). The formulation was subjected to characterization, and its stability was evaluated over a period of one month under three different storage

conditions. The present study aimed to examine the ocular distribution of the formulation in rabbits with normal health conditions. This was achieved through the utilization of LC-MS/MS analysis, which involved the examination of ocular tissues as well as whole blood samples. The formulation of the MET-TAC nanoparticle, with a size of 200 nanometers, exhibited properties such as viscosity, osmolarity, and pH that fell within the acceptable range for ocular comfort. Moreover, the stability of this formulation was observed to be maintained for a period of one month when stored under refrigeration conditions. The concentrations of TAC in the cornea and conjunctiva of rabbits were measured to be 4452.2289 and 516.180 ng/g of tissue, respectively, one hour following the application of the substance topically. The objective of this study is to develop a topical ocular aqueous formulation of TAC eye drops that can effectively deliver an adequate amount of the drug to the relevant tissues on the ocular surface [121].

- **Terreni E et al. (2020)** The eye's physiological resistance mechanisms have the effect of decreasing the bioavailability of drugs that are administered topically, particularly those that have a high molecular weight and/or lipophilic properties. An example of such a drug is Cyclosporine A (CyA). Given the recent approval of a limited range of commercial products containing CyA, the utilization of a combined approach involving nano micelles and mucoadhesive polymer shows potential. The biopharmaceutical effectiveness of Assembling Surfactants-Mucoadhesive Polymer Nano micelles (ASMP-Nano) was assessed using a binary system comprising two surfactants in conjunction with hyaluronic acid. The selection of the Nano1HAB-CyA formulation was determined through an optimization process that considered the number of surfactants, CyA-loading, and size of the formulation. The chosen formulation consists of 0.105% w/w CyA-loaded nano micelles, which exhibit a size of 14.41 nm and demonstrate clarity and stability. The nanostructured system exhibited a protective effect on epithelial corneal cells, resulting in a cell viability rate exceeding 80%. The distribution of a fluorescent probe was used to demonstrate that the compound interacted with cellular barriers in reconstituted corneal epithelial tissue, inhibiting the permeation of CyA. This interaction facilitated the uptake and accumulation of CyA within cells. The inclusion of the mucoadhesive polymer hyaluronic acid (HA) in the nano micellar carrier in a pharmacokinetics study

involving rabbits resulted in an extended retention time of CyA in the precorneal area [122].

- **Suriyaamporn P et al. (2021)** Dissolving microneedles (DMNs) utilize minimally invasive micron-scale technology to facilitate the delivery of medication to the ocular region. The objective of this study was to develop and fabricate highly efficient drug-loaded micro/nanoparticles (DMNs) with the potential to serve as an ocular drug delivery system. The utilization of the central composite design-response surface methodology (CCD-RSM) computational design strategy was employed in order to design and determine the most optimal formulation. The sodium fluorescein (FS), which is a hydrophilic compound, was introduced into DMNs. The production of DMNs involved the combination of Gantrez® S-97 (GAN) and hyaluronic acid (HA) polymer in different proportions. The study encompassed the evaluation of various parameters including physical and mechanical properties, ocular permeation, retention of the drug in ocular tissue, insertion force, insertion depth, dissolution time, and ex vivo ocular drug delivery. The study found that the most effective combination of DMNs consisted of 20.06% GAN, 5% HA, and 1% FS. The DMNs were arranged in 11 MN arrays, with each array measuring approximately 570.83 ± 14.78 m in height, 300.23 ± 3.30 m in width, and 600.10 ± 2.10 m in interspacing across a patch area of 25 mm2. The DMNs that exhibited optimal performance demonstrated the following characteristics: a reduction in height of 25.42 ± 0.72%, a permeation rate of FS of 52.15 ± 20.59% within a 24-hour period, and a retention rate of FS in ocular tissue of 2.42 ± 0.21%. The DMNs underwent complete dissolution in the sclera tissue within a time frame of two minutes. The specified force for insertion was 0.08 Newtons per needle, while the depth of insertion varied between 200 and 400 meters. In the ex vivo investigation, it was observed that the optimal drug-matrix nanocomposites (DMNs) exhibited a significantly greater permeation of FS over a 24-hour period compared to the control patch. This finding suggests that the optimal DMNs possess the capability to traverse the ocular barrier and effectively transport FS into the eye. In summary, the optimal drug-loaded microneedles demonstrated features that are minimally invasive and suitable for an efficient ocular drug delivery system [123].

- **Guangya Xu et al. (2018)** Extensive research has been conducted on ovarian cancer as a significant threat to women's health. The well-established knowledge regarding the potential effects of Quercetin, a naturally occurring bioflavonoid, has led to its recognition as a valuable compound for anticancer purposes. Nevertheless, one of a major challenge faced by Quercetin is its limited solubility in water, instability in physiological environments, and consequently, its insufficient bioavailability. Hence, it is imperative to optimize the most effective drug delivery strategies in order to maximize the therapeutic advantages of Quercetin. The present study involved the development of a thermosensitive injectable hydrogel-system, referred to as Qu-M-hydrogel composites, which incorporated nanotechnology and loaded Quercetin. Prior to incorporation into the thermosensitive hydrogel-Pluronic F-127, the compound Quercetin was encapsulated in MPEG-PCL. This encapsulation process involved a high drug concentration of 7% and resulted in small particles with a size of 32 nm. The Qu-M–hydrogel composites demonstrated a notable delay in release when compared to Qu-M in an in vivo setting. The cytotoxicity, induction of apoptosis, and antitumor effects of Qu-M hydrogel composites were also investigated in vivo using mouse models of abdominal SKOV-3 ovarian cancer. The Qu-M–hydrogel composites exhibited enhanced apoptotic induction and inhibition of cell growth in comparison to other experimental groups. Furthermore, in vivo experiments revealed a higher level of anti-tumor efficacy in the Qu-M–hydrogel composite group when compared to other groups, while maintaining an equivalent dosage of the drug. In summary, the utilization of Qu-M-hydrogel composites resulted in enhanced antitumor efficacy through the localized delivery of Quercetin at a concentrated level, sustained and consistent release of the drug, prolonged retention of the drug within the tumor, and minimal toxicity towards healthy tissues. The potential clinical applicability of Qu-M-hydrogel composites in the context of ovarian cancer activity is considerable [124].
- **Carmelo Puglia et al. (2020)** The objective of this study was to develop a nanostructured-lipid-carrier (NLC) platform for the targeted delivery of N-palmoylethanolamide (PEA) to the posterior segment of the eye. PEA is a naturally occurring compound that possesses inherent anti_inflammatory & neuroprotective characteristics. Nanoencapsulation of PEA within drug delivery systems is

necessary due to its inherent limitations, namely poor solubility and high instability. In order to develop NLC, a comparative analysis was conducted between the high shear homogenization technique (HSH) and a procedure that combines the HSH technique with ultrasonication (HSH/US). The mean size, homogeneity, physical stability, and surface charge, of the nanoparticles were assessed utilizing Turbiscan technology. The distribution of palmitoylethanolamide (PEA) in the retina of a rat's eye was determined after a single administration of PEA-loaded nanostructured lipid carriers (PEA-NLC) ophthalmic formulation. The novel formulation exhibited exceptional efficacy in delivering high concentrations of PEA to the retina. The application of PEA-NLC ophthalmic formulation through topical administration resulted in a significant reduction in retinal tumor necrosis factor- (TNF-) levels in rats with streptozotocin-induced diabetes. Present research indicates that the newly developed ophthalmic formulation exhibits potential efficacy in addressing retinal disorders, specifically diabetic retinopathy. At present, ongoing clinical research endeavors are being undertaken to assess the feasibility of this proposition [125].

- **Swarupa Ghosh et al. (2017)** Ischemia–reperfusion of the brain is a classic illustration of a transient brain lesion mediated by reactive oxygen species (ROS). Mitochondrial damage is caused by the post-ischemic reperfusion-induced production of 11 oxygen free radicals in brain cells. Antioxidants such as Quercetin (Qc) may manage pathophysiology related to oxidative stress. Poor oral bioavailability and low cell membrane permeability limit its therapeutic efficacy, however. To overcome these obstacles, thirteen mitochondria-specific Qc nano capsules were designed to combat cerebral ischemia–reperfusion–induced cell death and fourteen neurodegenerations in young and aged rats. Using 15 triphenyl phosphonium cation as one of a matrix component, the mitochondria-specificity of the orally-administrable Quercetin-loaded polymeric nano capsules (N1QC) was enhanced. After 16 cerebral ischemia–reperfusion procedures, N1QC demonstrated remarkable mitochondrial localization and enhanced brain uptake. This novel mitochondrial delivery of Quercetin reduced histopathological severity by preserving mitochondrial 17 structural and functional integrity through ROS sequestration, thereby modulating mitochondrial ROS-mediated apoptotic cell death in young and aged rats [126].

- **A. J. Sousa-Batista et al. (2017)** There is a pressing need for the development of novel oral therapies to effectively treat all forms of leishmaniasis. The objective of this study was to assess the improved oral effectiveness of Quercetin (Qc) and its penta-acetylated derivative (PQc) in the treatment of cutaneous leishmaniasis. This was achieved by encapsulating the compounds in lipid-core nano capsules (LNCs) composed of poly(-caprolactone). BALB/c mice infected with Leishmania amanuensis were administered a total of 51 doses of either placebo drugs (at a dosage of 16 mg kg1) or drugs loaded with LNC (at a dosage of 0.04 mg kg1) on a daily basis. The results of the study indicate that treatment with free Qc resulted in a reduction of lesion sizes by 38% and parasite loads by 71%. In contrast, treatment with LNC-Qc led to a more significant decrease in lesion sizes by 64% and parasite loads by 91%. The antileishmanial efficacy of PQc was enhanced to a lesser degree compared to Qc through the process of encapsulation. None of the treatments resulted in elevated serum levels of aspartate aminotransferase, alanine aminotransferase, or creatinine. The results suggest that the use of Qc and, to a lesser extent, PQc, within LNC can effectively enhance the antileishmanial effect at a significantly lower dosage, indicating potential for the development of a novel oral medication for cutaneous leishmaniasis [127].
- **Man-Yi Wong et al. (2011)** The treatment options for hormone and trastuzumab-resistant breast cancer are currently ineffective and limited in their efficacy. The objective of this study was to develop a liposome formulation that combines vincristine and Quercetin in order to treat a specific subtype of breast cancer. The formulation aimed to achieve prolonged drug circulation and synchronized drug release in vivo, with the goal of enhancing treatment efficacy. A notable synergistic effect was observed when vincristine and Quercetin were combined in JIMT-1 cells that were resistant to hormones and trastuzumab, with a molar ratio of 2:1. The co-encapsulation of the two medications within liposomes resulted in an extended duration of plasma circulation and ensured the maintenance of the optimal drug ratio in vivo. In addition, the liposome formulation that co-encapsulated various compounds demonstrated a greater ability to suppress the growth of tumors in the JIMT-1 human breast tumour xenograft model compared to the control group treated with only the vehicle, as well as groups treated with free Quercetin, free vincristine,

and combinations of free vincristine and Quercetin. The liposome formulation that co-encapsulated the drug vincristine demonstrated noteworthy antitumor efficacy, even when administered at a dosage equivalent to two-thirds of the maximum tolerated dose. Importantly, this formulation did not induce substantial weight loss in the animal subjects [128].

- **Lei Gao et al. (2010)** The ability of an evaporative precipitation into aqueous solution (EPAS) & high homogenization press (HPH) processes to produce a chemically stable nanosuspension of Quercetin was evaluated in this study. The Zeta potential particle size of the EPAS nanosuspension were identical to those of a HPH nanosuspension. The results of differential scanning calorimeter and X-ray measurements for the two processes were distinct. The profile of EPAS desiccated powder demonstrated the transition from crystalline to amorphous phase. In contrast, the fundamental crystalline state of the substance was maintained throughout the HPH process. According to the results of dissolution experiments, the EPAS technique improved the solubility and dissolution rate of the drug more than the HPH method. A (HPLC) analysis demonstrated the superiority of both nanosuspensions over the QCT solution formulation in terms of chemical and photostability. Using the EPAS and HPH methodologies, it was possible to produce a chemically stable QCT nanosuspension with a significantly increased dissolution rate [129].
- **Ankit Kumar S. Jain et al. (2013)** The present study reports the successful fabrication of novel lipid nanocarriers, namely Gelu Pearl (GP), which consist of Precirol ATO 5 lipid nanoparticles and (GPNLC) or without oil (GPSLN), loaded with Quercetin (QR). A objective of this study was to enhance the therapeutic efficacy of the nanocarriers. The QR loaded GP nanoparticles was optimized to achieve satisfactory colloidal stability, with a mean particle size falling within the range of 350-380 nm, and an entrapment efficiency exceeding 90%. The morphological evaluation of GPSLN and GPNLC was conducted through cryo-TEM, surface charge analysis, assessment of protection provided to QR against alkali-mediated degradation, & fluorescence studies to determine QR-lipid interaction. Differential scanning calorimetry (DSC) analysis was conducted to gain understanding of the physical state of quantum dots (QR) loaded within nano

systems. The results of the in vitro release studies indicate that the gelatin particles loaded with QR possess the ability to sustain the release of the drug. The solubilization of QR can be enhanced by lipidic nanocarriers, as confirmed by in vitro lipolysis studies. The study found that the administration of QR loaded GP nano systems resulted in a statistically significant reduction (P < 005) in the volumes of flank tumors in C57BL/6 mice over a period of 22 days, as compared to the administration of QR suspension. The administration of GPSLN resulted in a significant reduction in lung colonization and an increase in antimetastatic activity (P < 005) of the drug when tested against B16F10 melanoma cells in C57BL/6 mice, as compared to QR suspension. The lyophilization of QR loaded GPSLN and GPNLC was found to be successful with minimal alterations in particle size and drug content. This was achieved through the use of 15% w/v mannitol as a cryoprotectant [130].

- **Amit K. Jain et al. (2014)** This study centers on the examination of the anticancer properties of a self-nanoemulsifying drug delivery system (SNEDDS) containing Quercetin (QT). The SNEDDS is comprised of Capmul MCM, Tween 20, and ethanol. The developed formulation was found to have potential cell cytotoxicity based on in vitro cell culture studies, as evidenced by its ability to induce DNA damage & apoptosis in MCF-7 cells. The study found that administering QT-SNEDDS at a dosage of 50 mg/kg resulted in a higher latency to tumor growth and demonstrated antioxidant activity when measured in terms of prophylactic antitumor efficacy against DMBA-induced breast tumors, as compared to administering free QT. The aforementioned recognition was reinforced by standardized levels of markers for tumor angiogenesis, namely MMP-2, MMP-9, TNF-α, and IL-6. The administration of higher doses (100 mg/kg) resulted in the observation of pro-oxidant activity. This exhibited a significantly greater therapeutic anticancer efficacy, with approximately 65% tumor suppression, in comparison to the efficacy of free QT which was approximately 20%. These results were obtained in the same model. Ultimately, the safety profile of the formulated product was established through the evaluation of several markers of hepatotoxicity [131].
- **Guangya Xu Colorectal et al. (2015)** Colorectal cancer is a malignancy originating from the epithelial cells that line the colon and/or rectum. It is the third most

common type of cancer and is a major cause of cancer-related deaths in the United States. Quercetin (Qu) is a bioflavonoid that exhibits significant anticancer properties, making it a promising candidate for chemotherapy in various types of cancer. Despite its potential therapeutic benefits in cancer treatment, the clinical use of Quercetin is limited by its hydrophobic nature, which results in poor hydrophilicity. Hence, there is a need for a plan of action to enhance the solubility of Quercetin in a water and/or augment its bioavailability. The process of encapsulating hydrophobic agents that have poor water solubility within polymer micelles has the potential to enhance drug dissolution in aqueous solutions. In our study, we employed nanotechnology to integrate the incorporation of Quercetin into biodegradable nanosized amphiphilic block copolymers composed of monomethoxy poly (ethylene glycol)–poly (ε-caprolactone) (MPEG–PCL). The primary aim of our research was to encapsulate Quercetin within these copolymers. The objective of this study was to present corroborative evidence of the effectiveness of the polymeric micelles drug delivery system. The Quercetin-loaded MPEG-PCL nano micelles, known as Qu-M, demonstrate a notable drug loading capacity of 6.85% and possess a diminutive particle size of 34.8 nm. The micelles possess the ability to achieve thorough dispersion in aqueous solutions and exhibit sustained release of Quercetin in both laboratory settings and living organisms. Concurrently, in an in vitro setting, the compound Qu-M exhibited heightened efficacy in inducing apoptosis and inhibiting cell proliferation in CT26 cells, surpassing the effects observed with free Quercetin. Furthermore, the researchers employed a subcutaneous CT26 colon cancer model in mice to thoroughly evaluate the therapeutic effectiveness of Qu-M. The results demonstrated an augmented anti-colon cancer effect in vivo, with Qu-M exhibiting greater efficacy in inhibiting colon tumor growth compared to free Quercetin. Furthermore, the immunofluorescence analysis revealed enhanced efficacy of Qu-M in promoting cellular apoptosis, impeding tumor angiogenesis, and suppressing cellular proliferation. The findings of our study suggest that Qu-M represents a newly discovered nano agent of Quercetin that exhibits superior antitumor properties. This agent holds great potential as a viable candidate for chemotherapy in the treatment of colon cancer [132].

- **Emilli Antônio et al. (2016)** Quercetin, a flavonoid, has been documented to possess anti-inflammatory, anti-allergic, anti-microbial, antiplatelet, antioxidant, anti-neurodegenerative, and antitumoral properties. The limited effectiveness of the substance is attributed to its low solubility in water. The utilization of nanotechnology has the potential to serve as a significant mechanism for enhancing the properties of Quercetin and augmenting its bioavailability. The present investigation involved the development of bovine serum albumin (BSA) nanoparticles incorporating Quercetin through desolation technique. The nanoparticles underwent characterization in terms of mean particle size, polydispersity, encapsulation efficiency, zeta potential, physical state of the drug within the nanoparticles, drug release-profile, and antioxidant activity. The impact of varying glutaraldehyde concentrations on the properties of nanoparticles was assessed, and it was determined that such alterations did not significantly affect the parameters of the nanoparticles. The nanoparticles exhibited a mean size of approximately 130 nm and an encapsulation efficiency of approximately 85%. The X-ray diffractometry findings indicated that the drug's crystal underwent a conversion to an amorphous state while in a polymeric matrix. The release profile of Quercetin exhibited a biphasic pattern, with an approximate release of 18% of the drug after 96 hours. The release of Quercetin was found to conform to a second-order model and was controlled by Fickian diffusion, as evidenced by the kinetic models. Following a 96-hour period, it was observed that nanoparticles containing Quercetin exhibited greater efficacy in scavenging radical ABTS·+ and hypochlorous acid in comparison to free Quercetin. Nanoparticles of BSA have the potential to serve as carriers for enhancing the properties of Quercetin [133].

2.3 LITERATURE REVIEW BASED ON FORMULATION OF SOLID LIPID NANPARTICLES (SLNs)

- **Li K, Pi C et al. (2022)** The present investigation aimed to examine the impact of structural alteration of curcumin (CU) in conjunction with the solid lipid nanoparticles (SLN) drug delivery mechanism on the in vitro anti-tumor efficacy. The phenolic hydroxyl group of curcumin was modified to synthesis a novel derivative, denoted as CU1. The synthesis of cu1 was accomplished with success.

At a temperature of 45°c or under constant light conditions, CU1 exhibited twice the stability as compared to cu. A SLN comprising of cu1 (CU1-SLN) was formulated and characterized for its particle size, polydispersity index, drug loading, entrapment efficiency, and zeta potential. The results showed that the particle size of CU1-SLN was (104.1±2.43) nm, with a polydispersity index of 0.22±0.008. The entrapment efficiency and drug loading were found to be (95.1±0.38) % and (4.28±0.02) %, respectively. The zeta potential of CU1-SLN was (28.3±1.60) mv. The amorphous nature of CU1 in SLN was confirmed through the utilization of x-ray diffraction (XRD) and differential scanning calorimetry (DSC). The drug cu1-sln exhibited a sustained release profile over a period of 48 hours, whereas the drugs cu and CU1 demonstrated a rapid release profile within 8 hours. CU1 demonstrated a greater degree of cytotoxicity compared to cu against a549 and smmc-7721 cells, with a 1.5-fold increase in inhibition. Additionally, CU1-SLN exhibited a 2-fold increase in inhibition compared to CU1. The toxicity observed in normal hepatocytes was found to be significantly reduced upon treatment with both CU1 and CU1-SLN, exhibiting a respective 2.6-fold and 12.9-fold decrease in toxicity as compared to CU. The experimental results indicate that cu1-sln exhibited a noteworthy apoptotic effect, with statistical significance at a p-value of less than 0.05. To summarise, CU1 maintained the inhibitory impact of cu on cancerous cells, while enhancing its stability and safety. Moreover, the CU1-SLN exhibits a potential approach for managing liver and lung carcinoma [134].

- **Nilesh R. Rarokar et al al. (2022)** The objective of the current study was to formulate a hydrogel containing solid lipid nanoparticles (SLNs) loaded with terbinafine hydrochloride (TH) in order to enhance its antifungal activity. The present study involved the production of SLNs loaded with TH, which were prepared using glyceryl monostearate as the lipid and Pluronic® F68 as the surfactant through high-pressure homogenization. The optimization of the drug-to-lipid ratio was conducted, taking into account various factors such as the desired particle size and the maximum percentage of encapsulation efficiency. The lyophilized solid lipid nanoparticles (SLNs) were subsequently integrated into a hydrogel composed of Carbopol 934P at concentrations ranging from 0.2% to 1.0% w/v, and their rheological properties were further assessed. The study yielded

measurements of 241.3 nm for the z-average, -15.2 mV for the zeta potential, and 0.415 for the polydispersity index. The results indicate that the SLNs exhibit a significantly elevated entrapment efficiency of approximately 98.36%, while displaying a drug loading range of 2.12 to 6.3602%. The successful preparation of solid lipid nanoparticles (SLNs) through the process of freeze drying is evidenced by the obtained SEM images, XRD data, and results from DSC and FTIR analyses. The hydrogel containing TH-loaded SLNs exhibited a protracted release of the drug, with a rate of 95.47 ± 1.45%, sustained for a duration of 24 hours. The findings presented in this research demonstrate a noteworthy impact on the zone of inhibition in Candida albicans cultures, as compared to both the marketed formulation and pure drug. Furthermore, the tested substance exhibited superior physical stability at lower temperatures. The ex vivo study demonstrated that it facilitated skin deposition and both in vitro & in vivo experiments indicated an improvement in antifungal activity [135].

- **Hamdan N. Alajami et al. (2022**) The objective of this study were to optimize a solid lipid nanoparticle (SLN) system loaded with celecoxib (CXB) for targeted delivery to the colon, with the aim of improving its anticancer activity. The present study utilized an ultrasonic melt-emulsification technique to fabricate solid lipid nanoparticles (SLNs). The physical characteristics of the particles were analyzed with respect to their sizes, charges, morphology, and entrapment efficiency (%EE), as well as through DSC and FTIR analyses. The study assessed the drug release profiles in vitro and analyzed the anticancer efficacy through an MTT assay across three cancer cell lines, namely HT29 colon cancer, Daoy medulloblastoma, and HepG2 hepatocellular carcinoma cells. The SLNs formulations that were prepared exhibited particle sizes within the nanoscale range, with measurements ranging from 238 nm to 757 nm. Reported were high zeta-potential values (in millivolts) falling within the range of negative 30 to negative 1 second millivolts. The percentage of EE (exactitude error) fell within the interval of 86.76% to 96.6%. The confirmation of the amorphous state of CXB entrapped in SLNs was ascertained through the analysis of SLNs DSC thermograms. The in vitro release kinetics exhibited a consistent and gradual release pattern without any initial rapid release, a phenomenon atypical of solid lipid nanoparticles. The F9 and F14 exhibited a near-

total CXB release within a 24-hour period, with a mere 25% of the release occurring in the initial 5-hour timeframe. After 24 hours of incubation, F9 induced a noteworthy percentage of cell death in the three cancer cell lines that were tested. This effect was sustained for a period of 72 hours. The solid lipid nanoparticles (SLNs) loaded with CXB demonstrated distinctive characteristics, including a sustained release profile without any initial burst effect and a high percentage of encapsulation efficiency (%EE). The observed formulation exhibited a noteworthy level of anticancer activity across all tested cancer cell lines and incubation periods, indicating a high degree of promise [136].

- **Abdul Shakur Khan et al. (2022)** The objective of this study was to optimize a solid lipid nanoparticle (SLNs) system loaded with celecoxib (CXB) for targeted delivery to the colon, with the aim of improving its anticancer activity. The present study utilized an ultrasonic melt-emulsification technique to fabricate solid lipid nanoparticles (SLNs). The physical characteristics of the particles were analyzed with respect to their sizes, charges, morphology, and entrapment efficiency (%EE), as well as through DSC and FTIR analyses. The study assessed the drug release profiles in vitro and analyzed the anticancer efficacy through an MTT assay across three cancer cell lines, namely HT29 colon cancer, Daoy medulloblastoma, and HepG2 hepatocellular carcinoma cells. The SLNs formulations that were prepared exhibited particle sizes within the nanoscale range, with measurements ranging from 238 nm to 757 nm. Reported were high zeta-potential values (in millivolts) falling within the range of negative 30 to negative 1 second millivolts. The percentage of EE (exactitude error) fell within the interval of 86.76% to 96.6%. The confirmation of the amorphous state of CXB entrapped in SLNs was ascertained through the analysis of SLN DSC thermograms. The in vitro release kinetics exhibited a consistent and gradual release pattern without any initial rapid release, a phenomenon atypical of solid lipid nanoparticles. The F9 and F14 exhibited a near-total CXB release within a 24-hour period, with a mere 25% of the release occurring in the initial 5-hour timeframe. After 24 hours of incubation, F9 induced a noteworthy percentage of cell death in the three cancer cell lines that were tested. This effect was sustained for a period of 72 hours. The solid lipid nanoparticles (SLNs) loaded with CXB demonstrated distinctive characteristics, including a

sustained release profile without any initial burst effect and a high percentage of encapsulation efficiency (%EE). The observed formulation exhibited a noteworthy level of anticancer activity across all tested cancer cell lines and incubation periods, indicating a high degree of promise [137].

- **Davida Mirra et al. (2022)** A chronic inflammatory condition accompanied by variable airway hyperresponsiveness (AHR) is the defining feature of asthma. Prior research has established the involvement of Nociceptin/Orphanin FQ (N/OFQ) and its corresponding receptor, N/OFQ peptide (NOP), in the development of airway hyperresponsiveness (AHR). The objective of this investigation was to enhance the bioavailability of N/OFQ through the formulation of solid lipid nanoparticles (SLNs). The Quasi Emulsion Solvent Diffusion (QESD) technique was utilized to prepare SLNs that were loaded with N/OFQ, followed by their characterization. The study involved the sensitization of Brown Norway rats to ovalbumin (OVA) followed by an intratracheal administration of either saline solution or N/OFQ-SLNs. Subsequent to the final challenge, a series of functional, histological, and molecular assessments were conducted after a 24-hour interval. The SLNs exhibited an average diameter of 233 ± 0.03 nm, a PDI value of approximately 0.28 ± 0.02, and a drug release rate of 84.3%. The release of Nociceptin/Orphanin FQ (N/OFQ) from solid lipid nanoparticles (SLNs) was observed in vitro, indicating that the peptide began to be released within two hours of incubation. The administration of N/OFQ-SLNs to animals resulted in a significant reduction in allergen-induced airway hyperresponsiveness (AHR) when compared to the control group. The findings of this study indicate that N/OFQ-SLNs have a favorable impact on the inflammatory process and mechanical characteristics of the airways. This suggests that the employment of nanotechnology may have therapeutic advantages for individuals suffering from asthma [138].
- **Rahul Nair et al. (2012)** Chitosan (CT) is a biopolymer that displays a variety of fascinating features, including regulated drug delivery, and the current study intends to construct an aqueous solution of Solid lipid Nanoparticles incorporating CT. Lipophilic medication carbamazepine (CBZ) demonstrates antiepileptic action via blocking sodium channels. Using the solvent injection technique, ethanol was used to create Chitosan-CBZ solid lipid Nanoparticles (SLNs). The formulated SLNs

showed excellent encapsulation efficiency and excellent physical stability. SLNs containing the medication have shown to have regulated release profiles over extended time periods. Surface morphology, entrapment efficiency, zeta potential, FTIR, DSC, and In-vitro diffusion investigations were performed on the produced SLNs. 168.7 1.8 nm was the hydrodynamic mean diameter and 28.9 2.0 mV was the zeta potential for SLN-chitosan-CBZ. As a result, chitosan-SLNs may be an attractive option for encapsulating CBZ in order to boost the drug's therapeutic effectiveness in the management of Epilepsy [139].

- **Roohi Kesharwani et al. (2016**) The goal of this study were to develop and test a topical gel formulation of the NSAID etoricoxib for a treatment of arthritis that would reduce the gastrointestinal side effects that come from taking the drug orally. SLNs were made by combining stearic acid and tween 80 into a melt emulsion, which was then solidified at a low temperature. Particle size, particle size distribution, scanning electron microscopy, zeta potential crystallinity research by DSC, and in-vitro release tests were performed on each and every formulation. The formulation with the highest concentration of lipids has been shown to entrap the most particles. Stability of the formulation is shown by the zeta potential of -25.6 and the entrapment rate of 70.766% observed in the SLN- dispersion. Using a modified Franz diffusion cell with a dialysis membrane and a phosphate buffer at pH 7.4 as the receptor media, the drug release rate of the gel was determined in vitro. Carbopol gel and hydroxypropyl methylcellulose (HPMC) gel were used in the in-vitro release study. When comparing the SLN-F3C (Carbopol) and SLN-F3HPMC (hydroxypropyl methylcellulose) formulations, the steady-state flux (Jss) was found to be substantially higher in the former. It was determined that the topical administration of an SLN-based gel formulation comprising Carbopol and Etoricoxib had much superior results in terms of anti-inflammatory action [140].
- **Kushwaha AK et al. (2013)** Raloxifene hydrochloride (RL-HCl) is classified as an orally selective estrogen receptor modulator (SERM) that exhibits limited bioavailability of approximately 2% due to its inadequate aqueous solubility and significant first pass metabolism. The present study reports the development of (SLNs) loaded with raloxifene, which aim to enhance its oral bioavailability. Compritol-888 ATO was employed as the lipid carrier, while Pluronic F68 was used

as the surfactant. The preparation of Raloxifene loaded solid lipid nanoparticles (SLNs) was carried out using the solvent emulsification/evaporation method. The optimization process involved the manipulation of process variables such as surfactant concentration and homogenization speed at varying levels. The SLNs were subjected to characterization with regards to various parameters such as zeta potential, particle size-entrapment efficiency- surface morphology, & crystallinity of both the lipid and drug components. The study conducted drug release experiments in vitro, utilizing the dialysis bag diffusion technique, in a phosphate buffer with a pH of 6.8. The particle sizes of all formulations fell within the range of 250 to 1406 nm, while the entrapment efficiency exhibited a range of 55 to 66%. The results of FTIR and DSC analyses suggest that there is no discernible interaction between the drug and lipid components. Additionally, the XRD spectrum indicates that RL-HCl exists in an amorphous state within the formulation. The release profiles observed in vitro exhibited biphasic characteristics and conformed to the Higuchi model of release kinetics. The present study investigated the pharmacokinetics of solid lipid nanoparticles loaded with raloxifene following oral administration to Wistar rats. The bioavailability of solid lipid nanoparticles (SLNs) loaded with RL-HCl was found to be approximately five times greater than that of pure RL-HCl [141].

- **Kalam MA et al. (2010)** The present study outlines the formulation and analysis of (SLNs) utilizing stearic acid (SLN-A) and a combination of stearic acid and Compritol (SLN-B) as the lipid matrix, poloxamer-188 as the surfactant, and a co-surfactant mixture of sodium taurocholate and ethanol. The objective of this research is to investigate the potential of the SLNs for use in topical ocular drug delivery. The o/w microemulsion technique was utilized to prepare SLNs. SLNs were subjected to a variety of characterization techniques, including time-resolved zeta ()-potential, particle size analysis, polydispersity index, differential scanning calorimetry (DSC), infrared spectroscopy, and wide-angle X-ray diffractometry (WAXD). The outcomes derived from these investigations were juxtaposed with those of the SLNs formulated solely with stearic acid. The results of IR, WAXD, and DSC analyses indicated that the SLNs had low crystallinity and exhibited favorable ζ-potential values following a storage period of three months. The findings of the study revealed

that the utilization of a mixed lipid-matrix led to the production of solid lipid nanoparticles (SLNs) with reduced crystallinity and smaller particle sizes, as well as increased drug entrapment in comparison to SLNs that were prepared solely with stearic acid. As a result, it can be concluded that SLN-B would be a viable option for the formulation of nanosuspension. The nanosuspensions underwent rheological and physicochemical assessments, as well as in vitro drug release and ex vivo corneal permeation investigations. Additionally, their impact on corneal hydration levels was analyzed. The combination of stearic acid and compritol in SLNs exhibits favorable characteristics for ocular drug delivery, such as smaller particle size, particle size stability, and biocompatible components [142].

- **Mohanty B et al. (2014)** The goal of this research was to find out whether the water-insoluble medicine itraconazole could be delivered topically to the eyes by being encapsulated in solid lipid nanoparticles (SLNs). Different amounts of polyvinyl alcohol were used as an emulsifier in the creation of the drug-loaded SLNs from stearic acid and palmitic acid. We created SLNs by sonicating a melt emulsion at a low temperature, then characterized them for particle size, zeta potential, drug loading, and entrapment effectiveness. Stearic acid-prepared SLNs exhibited a mean particle size of 139–199 nm, whereas palmitic acid–prepared SLNs varied from 126–160 nm. The SLNs took on a spherical form. Drug entrapment was shown to be greater in stearic acid-SLNs than in palmitic acid-SLNs. Measurements using X-ray diffraction and differential scanning calorimetry (DSC) revealed that the SLNs formulations had lower drug crystallinity. Drug corneal permeability testing was performed using a modified Franz-diffusion cell and newly removed goat corneas. Itraconazole permeation was better from stearic acid-SLNs than palmitic acid-SLNs. Antimicrobial activity of the SLNs formulations was shown by a distinct zone of inhibition against Aspergillus flavus [143].

CHAPTER 3
AIM AND OBJECTIVE

CHAPTER 3
AIM AND OBJECTIVE

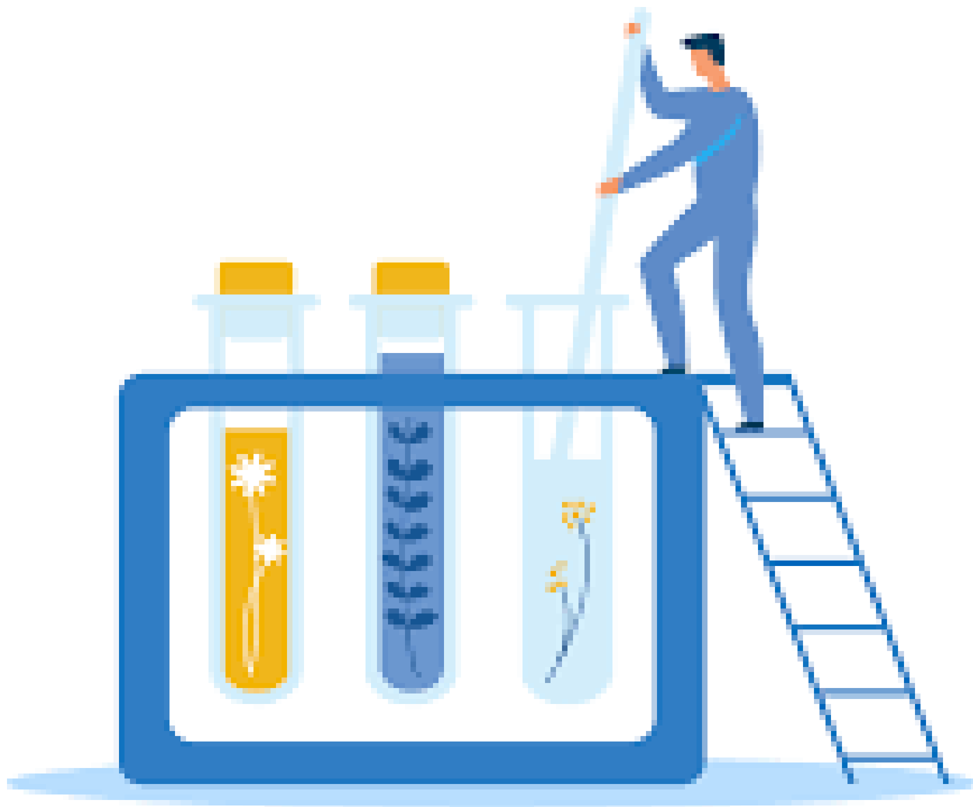
AIM: Formulation and evaluation of solid lipid nanoparticles laden in-situ gel of Quercetin for Ocular diseases.

❖ **OBJECTIVE:**

The main objective of the study was to develop a Nanoparticulate In-situ gel of Quercetin:

- Development of Solid Lipid Nanoparticles of Quercetin
- Evaluation of prepared Solid Lipid Nanoparticles of Quercetin
- Development of Solid Lipid Nanoparticles laden in-situ gel of Quercetin
- Evaluation of Solid Lipid Nanoparticles laden in-situ gel of Quercetin
- To increase the bioavailability of drug
- To increase the residence time on ocular surface
- To increase the patient compliance

CHAPTER 4
DRUG&EXCIPIE TPROFILE



CHAPTER 4
DRUG AND EXCIPIENT PROFILE

4.1 DRUG PROFILE:

4.1.1 Quercetin

4.1.1.1 IUPAC Name: 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one

4.1.1.2 Molecular weight: 302.236 g/mol

4.1.1.3 Molecular formula: $C_{15}H_{10}O_7$

4.1.1.4 Physical state: Quercetin appears as yellow needles or yellow powder

4.1.1.5 Solubility: Soluble in ethanol, DMSO and DMF, purged with an inert gas. Solubility in ethanol is 2 mg/ml and solubility in DMSO and DMF is 30 mg/ml.

4.1.1.6 Boiling Point: 643.0° C at 760 mmHg

4.1.1.7 Melting point: >275° C

4.1.1.8 Drug Description:

Quercetin is a flavanol antioxidant that is often found in the form of the Quercetin glycoside, As shown in (Figure 1) [144,145]. Glucose, xylose, and retinues may all conjugate with the hydroxyl group of the Quercetin aglycone to produce different Quercetin glycosides [146]. Quercetin-3-O-glycoside is mostly used as a pigment in plants like fruits and vegetables [147]. Rather of being present as aglycones, nutritional Quercetin is found predominantly in the form of glycosides [148]. When compared to other phytochemicals, its bioavailability is rather high [149]. Grapes, berries, cherries, apples, citrus fruits, onions, buckwheat, kale, tomatoes, red wine, and black tea are all good sources of polyphenols [150,151,152]. However, the amount of Quercetin present varies from plant to plant and even within the same plant at various locations [153-154]. Quercetin, a nutritional flavonoid, is a more potent antioxidant than vitamins C and E [155]. compounds with a 3-OH and 3′,4′-catechol in the formula have been shown to be 10 times more effective than ebselen in neutralizing peroxynitrite [156]. Onion-derived Quercetin (including Quercetin glucoside) was more bioavailable than apple-derived Quercetin in a variety of food preparations (containing Quercetin rhamnoside and Quercetin galactosidase) [157]. Quercetin's antioxidant and anti-inflammatory properties are important to its ability

to control oxidative stress, kinases, and the cell cycle, and to ensure the survival of neurons. Quercetin's anticancer potential lies on its ability to induce apoptosis [158].

Chemical Structure:

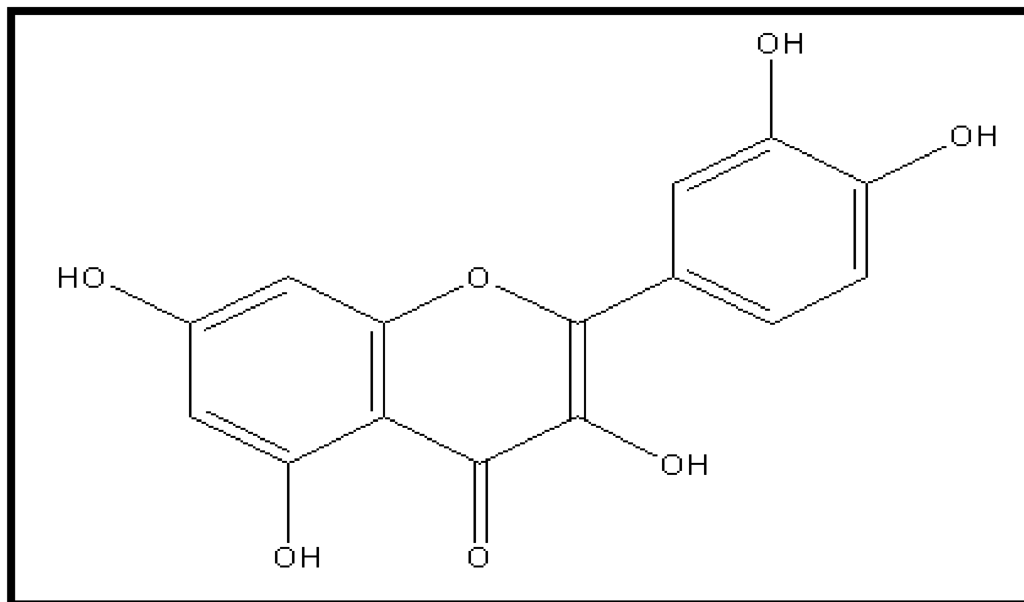


Figure 4.1: Chemical structure of Quercetin

4.2 EXCIPIENT PROFILE

4.2.1 GLYCERYL MONOSTEARATE [159]

4.2.1.1 IUPAC Name: 2,3-dihydroxypropyl octadecenoate

4.2.1.2 Molecular weight: 358.6 g/mol

4.2.1.3 Molecular formula: $C_{21}H_{42}O_4$

4.2.1.4 Physical state: Powder, pure-white or cream-colored, wax-like solid

4.2.1.5 Solubility: insoluble in water, soluble in hot oils and organic solvents

4.2.1.6 Boiling Point: 410.96° mmHg

4.2.1.7 Melting point: 78-81°C

4.2.1.8 Pharmaceutical Applications

Many different kinds of glyceryl monostearate are used in food, medicine, and beauty products as nonionic emulsifiers, stabilizers, emollients, and plasticizers. It functions as a proficient stabilizer, specifically as a solvent that is capable of dissolving both polar and nonpolar substances, thereby facilitating the formation of emulsions characterized by either water-in-oil or oil-in-water compositions. Additionally, these

characteristics render it advantageous in its application as a dispersant for pigments in oil or as a medium for dissolving solids in fats. It can also serve as a solvent for phospholipids, such as lecithin. Glyceryl monostearate has been employed in a novel fluidized hot-melt granulation technique for the purpose of manufacturing granules and tablets. Glyceryl monostearate serves as a binding agent in the formulation of tablets. Additionally, it has the potential to be utilized in the production of sustained-release media for solid dosage forms. Sustained-release applications encompass the development of pellet formulations for tablets or suppositories, as well as the production of veterinary boluses. GMS has been employed as a constituent matrix in the development of a biodegradable, implantable dosage form designed for controlled release of pharmaceutical substances. When incorporating glyceryl monostearate into a formulation, it is important to take into account the potential occurrence of polymorph formation. The form in question possesses the characteristic of being dispersible and foamy, rendering it advantageous for its applications as an emulsifying agent or preservative. The b-form, which is denser and more stable, is considered to be appropriate for wax matrices. The aforementioned application has been employed for the purpose of concealing the taste of clarithromycin in a formulation intended for pediatric use.

Chemical structure:

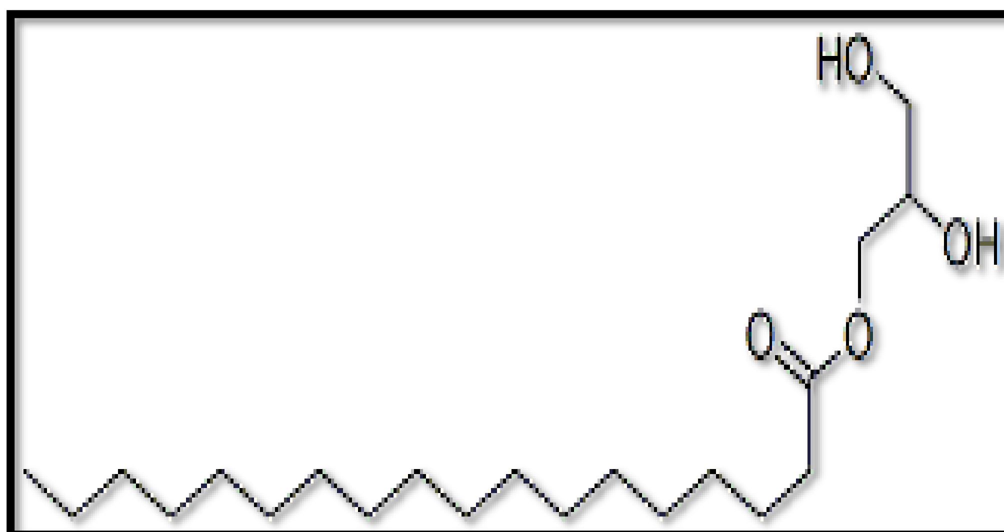


Figure 4.2: Chemical structure of glyceryl monostearate

4.2.2 Soya lecithin [160]

4.2.2.1 IUPAC Name: (2-nonanoyloxy-3-octadeca-9,12-dienoyloxypropoxy)-[2-(trimethylazaniumyl)ethyl phosphonate

4.2.2.2. Molecular weight: 643.9 g/mol

4.2.2.3. Molecular formula: $C_{42}H_{80}NO_8P$

4.2.2.4. Physical state: Soya lecithin is commonly found in a semi-solid or liquid state, depending on the temperature and the specific form it is in. Its physical state can vary based on factors such as processing, composition, and any additives present. Liquid: Soya lecithin is often available in liquid form, which is commonly used in various industries. The liquid form may vary in viscosity and consistency, ranging from relatively thin to more viscous, depending on the specific product and processing methods. Semi-Solid: Soya lecithin can also be found in a semi-solid state. This form is typically more solid or waxy in texture and may have a higher viscosity compared to the liquid form. The semi-solid state is often encountered in products like solidified granules, flakes, or pastes.

It's important to note that the physical state of Soya lecithin can be influenced by temperature. At higher temperatures, it tends to be more liquid-like, while lower temperatures can cause it to solidify or thicken. Additionally, the addition of other ingredients or processing techniques can also impact its physical properties.

4.2.2.5 Solubility: Soya lecithin is partially soluble in water, Soya lecithin is soluble in organic solvents such as ethanol, chloroform, and hexane, which are nonpolar or lipophilic in nature. In these solvents, Soya lecithin can dissolve readily due to the presence of its lipid components.

4.2.2.6 Applications: In pharmaceuticals, Soya lecithin serves as an excipient, contributing to the stability, solubility, and bioavailability of active pharmaceutical ingredients (APIs). It is used in the various drug delivery systems, including liposomes, micelles, and nano emulsions, to improve the solubility and targeted

delivery of drugs. Soya lecithin is also employed in topical formulations such as creams and ointments for its emulsifying and stabilizing properties.

4.2.2.7 Description:

Soya lecithin is commonly used as the excipient in pharmaceutical formulations. It serves as a versatile ingredient that can enhance the stability, solubility, and bioavailability of active pharmaceutical ingredients (APIs). As an excipient, Soya lecithin helps in the dispersion of APIs and aids in their absorption and delivery within the body. Due to its emulsifying properties, Soya lecithin is used as an emulsifier and stabilizer in various pharmaceutical formulations. It helps in mixing and dispersing oil and water-based components, thereby ensuring uniformity and stability in suspensions, creams, ointments, and other pharmaceutical products.

Lipid-Based Drug Delivery Systems: Soya lecithin is a key ingredient in lipid-based drug delivery systems, such as liposomes, micelles, and nano emulsions. These systems enable the encapsulation of drugs within lipid structures, improving their solubility, stability, and targeted delivery. Soya lecithin acts as a building block for these lipid-based systems, providing structural integrity and facilitating the encapsulation and release of drugs. Soya lecithin has been investigated for its potential to enhance drug permeation across biological barriers. By incorporating Soya lecithin into drug formulations, researchers aim to improve the absorption and bioavailability of poorly soluble drugs, thereby optimizing their therapeutic effects. Soya lecithin is utilized in the encapsulation of nutritional supplements and vitamins. It helps to improve the stability, bioavailability, and taste masking of these ingredients, ensuring their effective delivery and consumption [161,162].

Chemical structure

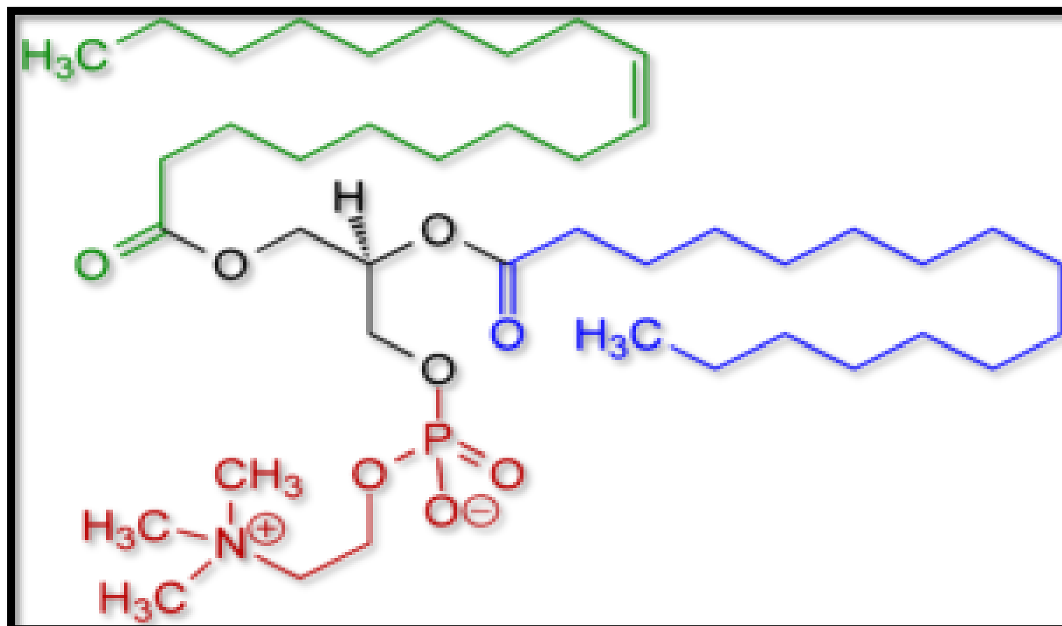


Figure 4.3: Chemical structure of Soy lacertine

4.2.3 POLOXAMER 407 [163]

4.2.3.1 IUPAC Name: Oxirane, methyl-, polymer with oxirane

4.2.3.2 Molecular weight: 12,600g/mol

4.2.3.3 Molecular formula: $C_{572}H_{1146}O_{259}$

4.2.3.4 Physical state: Poloxamer 407 is a triblock copolymer composed of a hydrophobic polypropylene glycol block in the center and two hydrophilic polyethylene glycol blocks on each side (PEG).

4.2.3.5 Solubility: Poloxamer 407, also known as Pluronic® F127, is a non-ionic, water-soluble triblock copolymer made up of a hydrophobic poly oxy propylene (POP) residue sandwiched between two hydrophilic polyoxymethylene units (POE)

4.2.3.6 Boiling Point: 53 °C

4.2.3.7 Melting point: 53–57 °C

4.2.3.8 Applications: The bulk of the common uses for poloxamer 407 are related to its surfactant properties. It's often used in cosmetics, for example, to dissolve oily components in water. It's utilized in multi-purpose contact lens cleaning solutions to help remove lipid deposits from the lenses.

4.2.3.9 Description: Poloxamer 407 is a triblock copolymer composed of a hydrophobic polypropylene glycol block in the center and two hydrophilic polyethylene glycol blocks on each side (PEG). The two PEG blocks have around 101 repetition units each, whereas the propylene glycol block has about 56 repeat units.

Chemical Structure

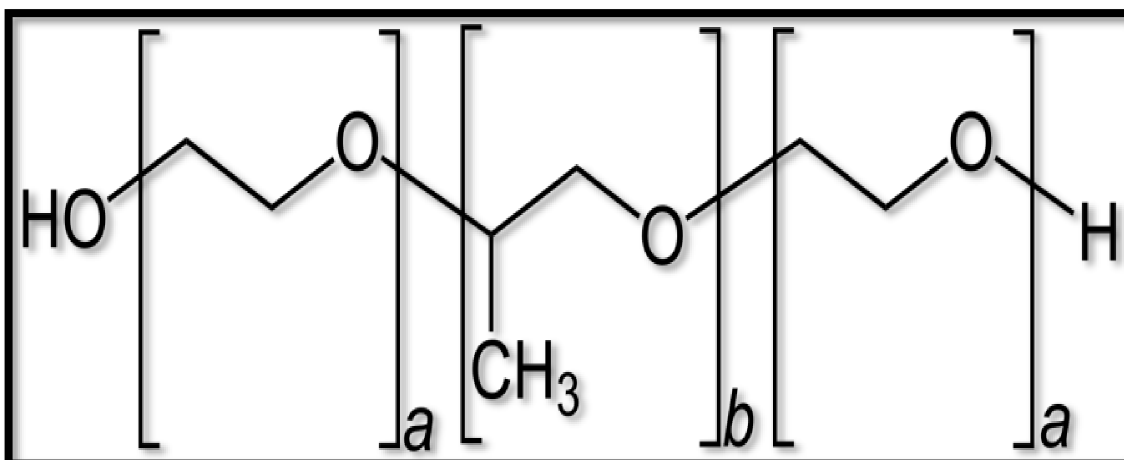


Figure 4.4: Chemical structure of Poloxamer 407

4.2.4 HYDROXY PROPYL METHYL CELLULOSE [164]

4.2.4.1 IUPAC Name: Cellulose, 2-Hydroxypropylmethyl Ether: Hypromellose

4.2.4.2 Molecular weight: 1261.4

4.2.4.3 Boiling Point: 1,102 °C

4.2.4.4 Melting point: 1.39

4.2.4.5 Molecular formula: $C_{56}H_{108}O_{30}$

4.2.4.6 Physical state: An HPMC polymer may be used in a broad variety of industries, including medicine, cosmetic, adhesives, coatings and textiles. HPMC is a biodegradable and biocompatible polymer.

4.2.4.7 Solubility: Non-aqueous solvents may be employed with HPMC since it is soluble in organic polar solvents. In both hot and cold organic solvents, it possesses a unique solubility profile that permits it to dissolve. HPMC has more organ solubility and thermos plasticity than other methyl cellulose counterparts. When heated to a gelation temperature of 75–90°C, it forms a gel.

4.2.4.8 Applications: HPMC is used as a thickening, binder, and film forming agent in agrochemicals, coatings, ceramics, adhesives, inks, and a range of other applications. Heat causes HPMC aqueous solutions to reversibly gel, allowing for a controlled increase in the green strength of green ceramic bodies.

4.2.4.9 Description: HPMC is a cellulose ether in which one or both of the cellulose ring's three hydroxyl groups have indeed been replaced by any one of the cellulose ring's three hydroxyl groups. An HPMC polymer may be used in a broad variety of industries, including medicine, cosmetics, adhesives, coatings and agriculture. Soluble in organic solvents, HPMC may be used in either aqueous or nonaqueous solutions. Solubility in hot and cold chemical methods is possible because to its unique solubility profile. HPMC has a higher degree of organ solubility and thermos plasticity than some other methyl celluloses. At 75–90ºC, the gelation temperature, it forms a gel.

Chemical Structure

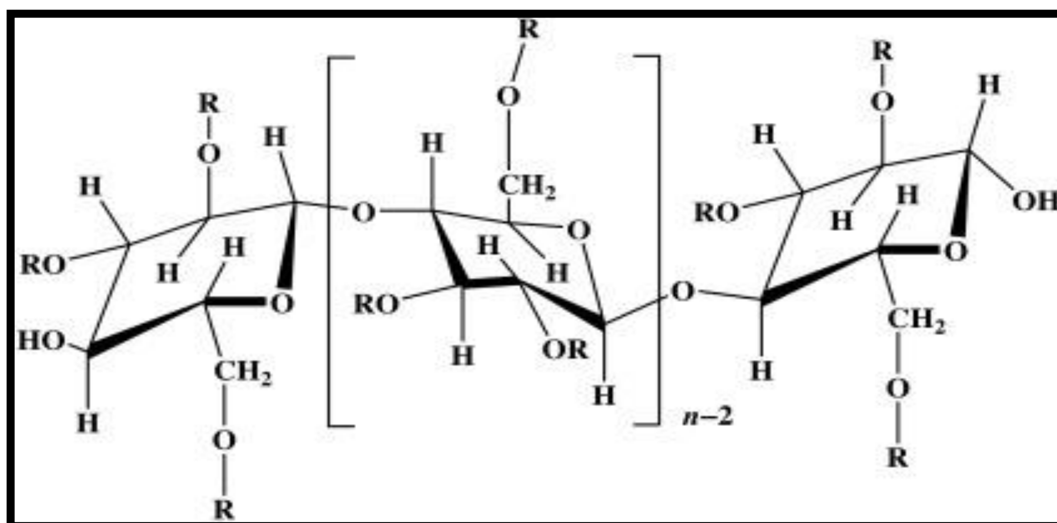


Figure 4.5: Chemical structure of HPMC

4.2.5 CARBOPOL 940 [165-166]

4.2.5.1 Molecular Formula: C3H4O2 (isomer)

4.2.5.2 Density: 1.2 g/mL at 25 °C

4.2.5.3 Melting Point: 12.5° C

4.2.5.4 Boiling Point: 116 °C

4.2.5.5 Description: Carbopol 940 polymer is a type of crosslinked polyacrylic acid polymer that comes in a white powder form. It's a very effective rheology modifier that creates dazzling clear gels & hydro-alcoholic gels & creams with high viscosity. Its non-drip characteristics and short flow make it excellent for clear gels, hydroalcoholic gels, and creams.

4.2.5.6 Solubility: The substance exhibits the property of swelling in both water and glycerin, and upon neutralization, it also demonstrates swelling in ethanol (95%). Carbomers exhibit limited solubility and primarily undergo significant swelling due to their three-dimensional crosslinked microgel structure.

4.2.5.7 Usage: Binder, film-former, and emulsion stabilizer, carboxy poly methylene It can also aid in increasing the viscosity of a product.

Chemical Structure

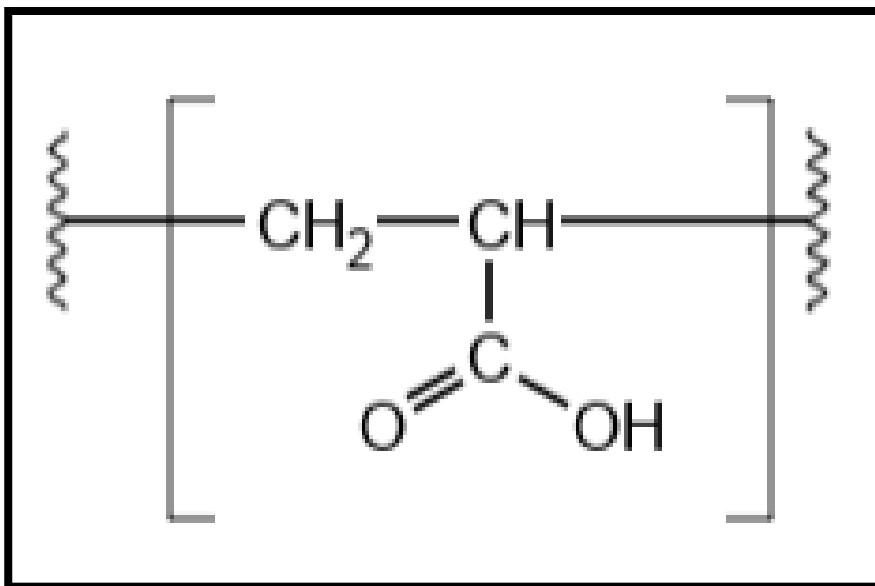


Figure 4.6: Structure of Carbomer isomer

CHAPTER 5
ADOPTED METHODOLOGY

CHAPTER 5
ADOPTED METHODOLOGY

5.1 EXPERIMENTAL WORK

5.1.1 Determination of absorbance maxima (λmax) of Quercetin

The absorbance maxima (λmax) of Quercetin was determined using a U.V visible spectrophotometer (Shimadzu UV-1800 spectrophotometer). A 10µg/ml solution of Quercetin was scanned between 200 to 400 nm.

5.1.2 Preparation of calibration curve of Quercetin in phosphate buffer pH 7.4

5.1.2.1 Preparation of the phosphate buffer pH 7.4

About 27.218 gm of potassium dihydrogen ortho phosphate was dissolved in distilled water and dilute with water to 1000 mL to make 0.2M solution. Also, sodium hydroxide was dissolved in water to produce 40 to 60 percent w/v solution and allowed to stand. To avoid absorption of carbon dioxide, the clear supernatant liquid was siphoned off and diluted with carbon dioxide-free water to a suitable volume of the liquid to contain 8.0 gm of NaOH in 1000 mL to make 0.2M NaOH solution. A 50.0 mL of 0.2M potassium dihydrogen phosphate was placed in a 200 mL volumetric flask, and the specified volume of 0.2M sodium hydroxide was added and then water was added to the volume [167].

5.1.2.2 Preparation of Calibration curve of Quercetin in phosphate buffer pH 7.4

For the preparation of calibration curve of Quercetin in phosphate buffer pH 7.4, 10 mg of Quercetin was weighed accurately and dissolved in 100 ml of a phosphate buffer pH 7.4 in a 100 ml of volumetric flask and volume was made up to 100 ml with phosphate buffer pH 7.4. An amount of 1 ml of solution was withdrawn and transferred to 10 ml volumetric flask and diluted up to 10 ml with pH 7.4 phosphate buffer to obtain a stock solution of 10µg/ml. From this stock solution, an amount of 1ml, 2ml, 3ml, 4 ml and 5 ml were transferred to the 10 ml volumetric flasks and volume was made up to a 10 ml phosphate buffer pH 7.4. To make 10µg/ml - 50

µg/ml solution of Quercetin, the absorbances of these solutions were measured at 266 nm against blank phosphate buffer pH 7.4. The calibration curve was obtained by plotting the concentration of Quercetin versus the absorbance [168].

5.1.3 Fourier transforms infrared spectroscopy (FT-IR)

IR Spectroscopy is often used as a tool for the determination of identity of pharmaceutical compounds. FT-IR spectroscopy helps in confirming the formation of the complex between the polymer and drug by comparing it with the individual spectrum of polymer and drug with the spectrum of polymer-drug complex [169]. Compatibility of selected drug with chosen excipients was determined by FT-IR analysis. The drug alone and in combination with selected excipients was analyzed by FT-IR using KBr pellet.

5.1.4 Formulation development

5.1.4.1 Preparation of Solid Lipid Nanoparticles of Quercetin

Quercetin solid lipid nanoparticles were prepared by hot homogenization followed by ultrasonication. Quercetin, Glyceryl monostearate, and Soya lecithin were dissolved in 10 mL of a 1:1 mixture of chloroform and methanol. Organic solvents were completely removed by using a Rota evaporator. The drug-embedded lipid layer was molten by heating to 78°C above melting point of the lipid. Aqueous phase was prepared by dissolving Poloxamer 407 in double distilled water and heated to same temperature (based on lipid melting point) of lipid phase. Hot aqueous phase was added to the lipid phase, and homogenization was carried out (at 12 000 rpm) for 5 minutes. The coarse hot lipid in water emulsion so obtained was ultrasonicated using a probe sonicator for 20 min. Quercetin solid lipid nanoparticles were obtained by allowing hot nano emulsion to cool up to room temperature [170]. The composition of various formulations were shown in Table 5.1.

5.1.4.2 Formulation Development and Optimization of Solid lipid Nanoparticles

A 3^2 factorial design was selected for optimization study. For this study, two independent variables i.e., Glyceryl monostearate and Soya lecithin were selected and their effect on dependent variables such as particle size (PS), and percentage encapsulation efficiency (%EE) was studied.

Table 5.1: Formulation design of Quercetin loaded solid lipid nanoparticles

Ingredients	Batch No								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Quercetin (mg)	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Glyceryl monostearate (mg)	200	350	350	350	50	50	50	200	200
Soya lecithin (mg)	125	100	150	125	100	150	125	150	100
Chloroform & Methanol (1:1) (mL)	10	10	10	10	10	10	10	10	10
Poloxamer-407 (mg)	150	150	150	150	150	150	150	150	150
Double distilled water (mL)	10	10	10	10	10	10	10	10	10

5.1.5 Evaluation of solid lipid nanoparticles (SLNs) of Quercetin

5.1.5.1 Percentage yield

Percent yield refers to the percent ratio of actual yield to the theoretical yield. Percentage yield of prepared nanoparticles was determined by following formula.

$$\text{Percentage Yield} = \frac{\text{Actual Yield}}{\text{Theoreticla Yield}} \times 100$$

5.1.5.2 Encapsulation Efficiency (EE) of Solid lipid nanoparticles (SLNs)

Separation of unentrapped drug from the nano lipid formulation was done by the ultracentrifugation method. Centrifugation of nano lipid dispersion was carried out at 13000 rpm for 90 min. The clear supernatant from the resulting solution was diluted properly using pH 7.4 phosphate buffer and analyzed by the U.V visible spectrophotometer at the 266nm. entrapment efficiency was calculated by using the following formula [171]: -

$$\text{EE} = \frac{\text{Total quantity of the drug} - \text{quantity in supernatent}}{\text{Total quantity of the drug}} \times 100$$

EE=Encapsulation Efficiency

5.1.5.3 Particle size

Particle size of prepared nanoparticles was measured by Malvern zetasizer (Malvern P analytical Ltd).

5.1.5.4 Zeta potential (ζ)

Zeta potential (ζ) measurements were performed on solid lipid nanoparticles using a Zetasizer 4 (Malvern Instruments Ltd., Malvern, UK). The zeta potential was measured using an aqueous dip cell in an automated mode. Samples were diluted with ultra purified water and put in a capillary measuring cell, with the cell position adjusted.

5.1.5.5 Scanning electron microscope (SEM)

A scanning electron microscopic technique was used to analyze the shape and size of the prepared nanoparticles.

5.1.5.6 *In-vitro* drug release of solid lipid nanoparticles of Quercetin

The dialysis bag approach was used to analyze all 9 batches of Quercetin solid lipid nanoparticles (F1 - F9). The solid lipid nanoparticles equivalent to dose of Quercetin (6.5mg) was introduced to in a USP dissolution test equipment equipped with a basket-style stirring element. The dialysis bag was used in the basket. A phosphate buffer solution (pH 7.4) was used as a dissolving medium and was kept at 37 °C. The basket was rotating at a rate of 50 RPM. 5.0mL of medium was withdrawn using a 5.0mL syringe at 60, 120, 180, 240, 300, 360, ,420, 480, 540,600 660and 720 minutes interval and replaced with 5.0mL of fresh phosphate buffer solution (pH 7.4). The drug content was determined using a UV visible spectrophotometer at 266 nm [172].

5.1.5.7 Optimization study

Central composite design was used to optimize the formula required for preparation of nanoparticles with desired shape and with good entrapment efficiency. A check point batch was obtained after conducting ANOVA study which was further formulated and evaluated for said parameters.

5.1.5.8 Formulation and evaluation of optimized batch (Fopt)

A check point batch was obtained which was further formulated and evaluated. The composition of optimized batch was given in the table 5.2.

Table 5.2: Composition of optimized batch (Fopt)

Formulation batch	Composition			
	Drug (mg)	**Lipid**	**Polymers (mg)**	**Solvents (ml)**
F-optimized	Quercetin, (equivalent to dose)	Glyceryl monostearate 200 (mg)	Soya lecithin (150)	Chloroform: Methanol (1:1) (10mL)

5.1.5.8.1 Stability study of Optimized batch (Fopt)

The optimized batch thus prepared was evaluated for stability study also. The stability investigations was conducted in accordance with the ICH standards. The optimized batch was tested for stability by keeping and sealing it in tightly sealed transparent containers at ambient temperature (25°C ± 2°C and 60 ± 5% RH) for 90 Days. The *in-vitro* drug release study profile on the first day of storage as well as at the end of the 30 days, 60 days, and 90 days storage period was obtained.

5.1.6 Formulation of solid lipid nanoparticles laden in-situ gel of Quercetin

5.1.6.1 Preparation of solid lipid nanoparticles laden in-situ gel Formulation

The exact amount of hydroxy propyl methyl cellulose (HPMC) was dispersed in warm water with continuous stirring to form a gel. To prepare the Carbopol 940 gel, it was dispersed in distilled water and kept in the dark place overnight to allow for complete swelling [173]. Both polymers were taken in the ratio of 1:1, and nanoparticle optimized formulation was added to the above suspension was gently stirred (magnetic stirrer, 150rpm) till the gel swelled. Then triethanolamine was added to neutralize the acidic nature of the Carbopol 940 molecule which led to gelatinization followed by slow stirring until a homogeneous gel was formed [174, 175]. An appropriate quantity of preservative was added to the formulation, the composition of nanoparticles laden in-situ gel of Quercetin showed in table 5.3.

Table 5.3: Composition of nanoparticles laden in-situ gel of Quercetin

Ingredients	Batch No		
	B1	B2	B3
Quercetin	Equivalent to dose	Equivalent to dose	Equivalent to dose
Carbopol 940	250mg	500mg	750mg
HPMC	250mg	500mg	750mg
Benzalkonium chloride (BAK)	qs	qs	Qs
Distilled water	100 ml	100 ml	100 ml

5.1.7 Evaluation 0f solid lipid nanoparticles laden in-situ gel of Quercetin

Physical parameters

The formulated in situ gel solution was tested for clarity, pH, gelling capacity, and drug content.

5.1.7.1 Gelling capacity

The gelling capacity of the formulation was evaluated by adding a drop of the generated formulation to a 2 ml vial of freshly prepared simulated tear fluid and observed it visually. The duration of its gelatinization was recorded [176].

5.1.7.2 Measurement of pH:

The pH of Solid lipid nanoparticles laden in-situ gel of Quercetin formulation was measured using pH meter. The pH meter was calibrated every time using standard pH buffers 4.01, 7.00 and 10.01.

5.1.7.3 Drug content estimation

The drug concentration was calculated by diluting the generated formulation in 100 ml of distilled water and analyzing it at 266 nm using a UV-visible spectrophotometer (Shimadzu UV-1700 PC, Shimadzu Corporation, Japan).

5.1.7.4 *In-vitro* diffusion study

The produced gel was tested for in-vitro diffusion in a Franz diffusion cell setup. In a Franz diffusion cell, 1g of gel was evenly placed across a cellophane membrane that had been soaked in phosphate buffer pH 7.4 for 24 hours and was sandwiched between the donor and receptor compartments. As the receptor compartment, 30 mL of phosphate buffer was employed. The temperature shall be kept at 37±0.5°C. The whole system was mounted on a magnetic stirrer, and the solution in the receptor compartment was constantly stirred at 50 rpm with a magnetic bead. At hourly intervals, a 1 mL sample was taken and replaced with 1 mL of the fresh buffer. The drug concentration in the receptor fluid was measured U.V visible spectrophotometrically at 266 nm against a blank. The percentage drug release was calculated using regression equation [177-179].

CHAPTER 6
RESULTS AND DISCUSSION

CHAPTER 6
RESULT AND DISCUSSION

6.1 EXPERIMENT WORK

6.1.1 Determination of Absorption maxima of Quercetin:

The λmax of (10µg/ml) solution of Quercetin in phosphate buffer pH 7.4 was found to be 266 nm as shown in figure 6.1.

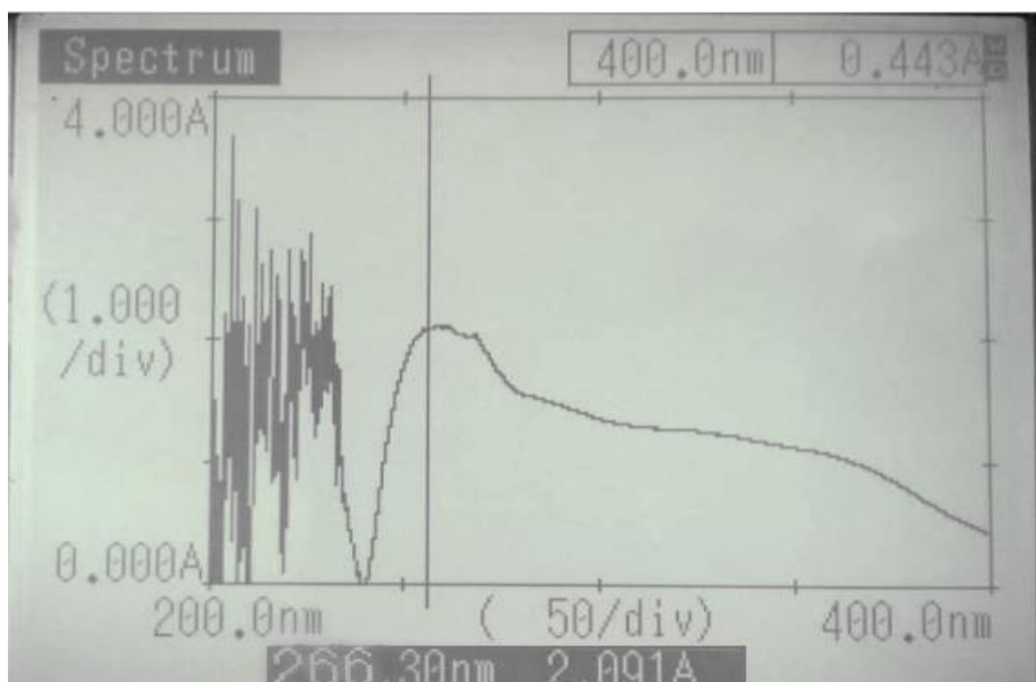


Figure 6.1: λmax of Quercetin in phosphate buffer pH 7.4

6.1.2 Preparations of Standard calibration curve of Quercetin in phosphate buffer pH 7.4:

The serial dilutions of Quercetin in the concentration rang of 10-50 µg/ml was prepared and absorbance was taken on U.V visible spectrophotometer at 266nm. The standard calibration curve of Quercetin was constructed in between concentration and absorbance as shown in table 6.1 and figure 6.2. It was observed that Quercetin showed good linearity ($y = 0.0232x - 0.0248$ and $r^2 = 0.9993$) over the range of 10-50 µg/ml.

Table 6.1: Standard calibration curve of Quercetin in phosphate buffer pH 7.4

S.NO.	Conc. (µg/ml)	Absorbance
1	0	0
2	10	0.02
3	20	0.044
4	30	0.069
5	40	0.091
6	50	0.115

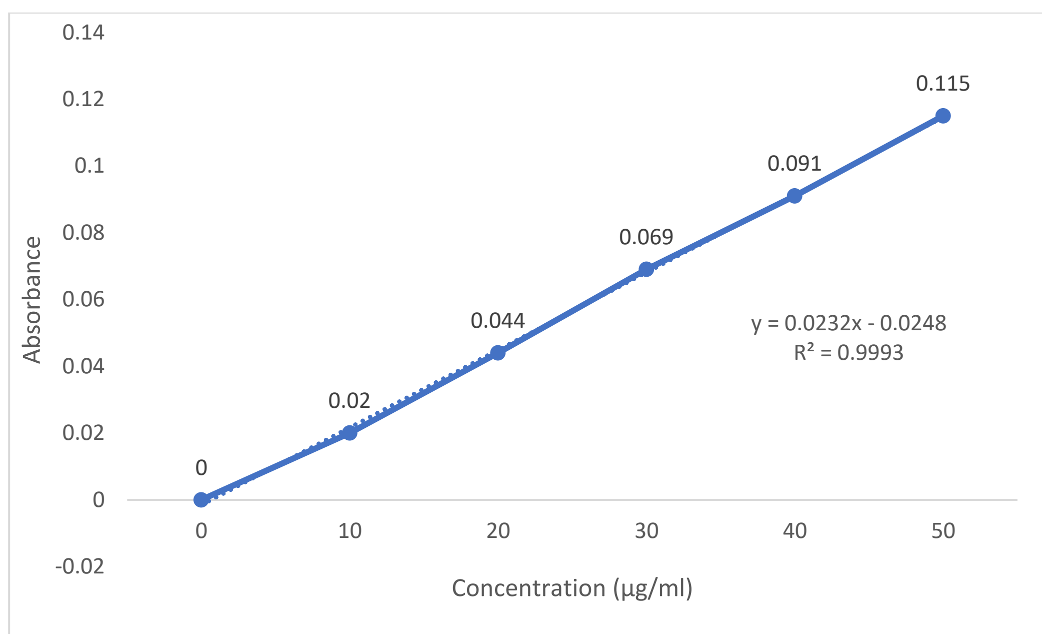


Figure.6.2: Standard calibration curve of Quercetin

6.1.3 Compatibility study

FT-IR Spectroscopy is often used as a tool for the determination of the identity of pharmaceutical compounds. FT-IR spectroscopy helps in confirming the formation of the complex between the polymer and drug by comparing it with the individual spectrum of the polymer and drug with the spectrum of the polymer-drug complex.

6.1.3.1 FT-IR

The FTIR spectra of the pure drug Quercetin was obtained and shown in figure 6.3 and interpretation was represented in table 6.2.

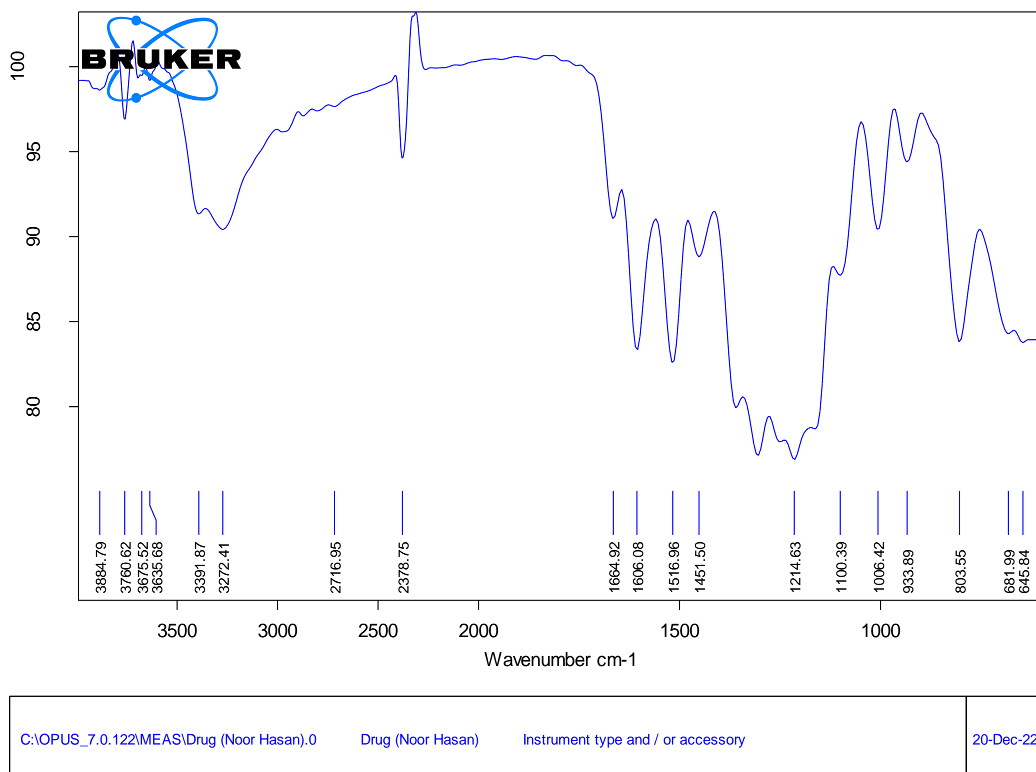


Figure 6.3: FT-IR spectra of Quercetin (pure drug)

Table 6.2: FT-IR Interpretation of Quercetin (pure drug)

Peak assignment	Actual Peak (cm-1)	Corresponding peak (cm-1)
(O-H) alcohol, phenol	3635 – 3848	3500-4000
(N-H) (O-H) Amines, Amides, and carboxy acids	3391	3300-3500
(N-H) (O-H) Amines, Amides, and carboxy acids	3271	3300-3500
(C=O) Carbonyl compounds	1664	1600-1800
(C=O) Carbonyl compounds	1606	1600-1800
C=CH of the aromatic compounds	645-1006	650-1000
(C-O) ethers	1100	1050-1100

Similarly, FT-IR spectra of selected excipients were obtained and represented in figure 6.4-6.8 their interpretation was presented in table 6.3-6.7.

6.1.3.2 FT-IR spectra of Glyceryl monostearate

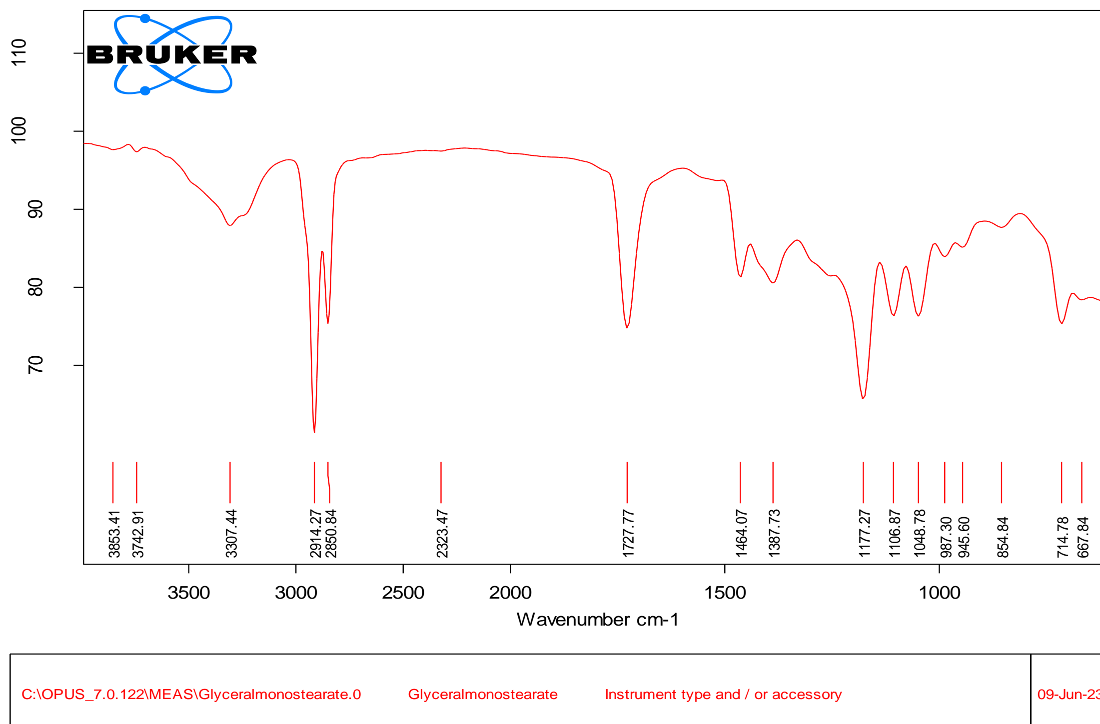


Figure 6.4: FT-IR spectra of Glyceryl monostearate

Table 6.3: FT-IR Interpretation Glyceryl monostearate

Peak assignment	Actual Peak (cm-1)	Corresponding peak (cm-1)
(O-H) alcohol, phenol	3853	3500-4000
(N-H) (O-H) Amines, Amides, and carboxy acids	3307	3200-3500
(C=O) Carbonyl compounds	1727	1600-1800
(CH2) methylene group	1464	1465
(C-O) ethers	1048-1177	1000-1300
(C-H) Alkanes	987	900-1000
(C-H) Alkanes	945	900-1000
(C=C) Trans-disubstituted alkenes	854	800-900

6.1.3.3 FT-IR spectra of Soya lecithin

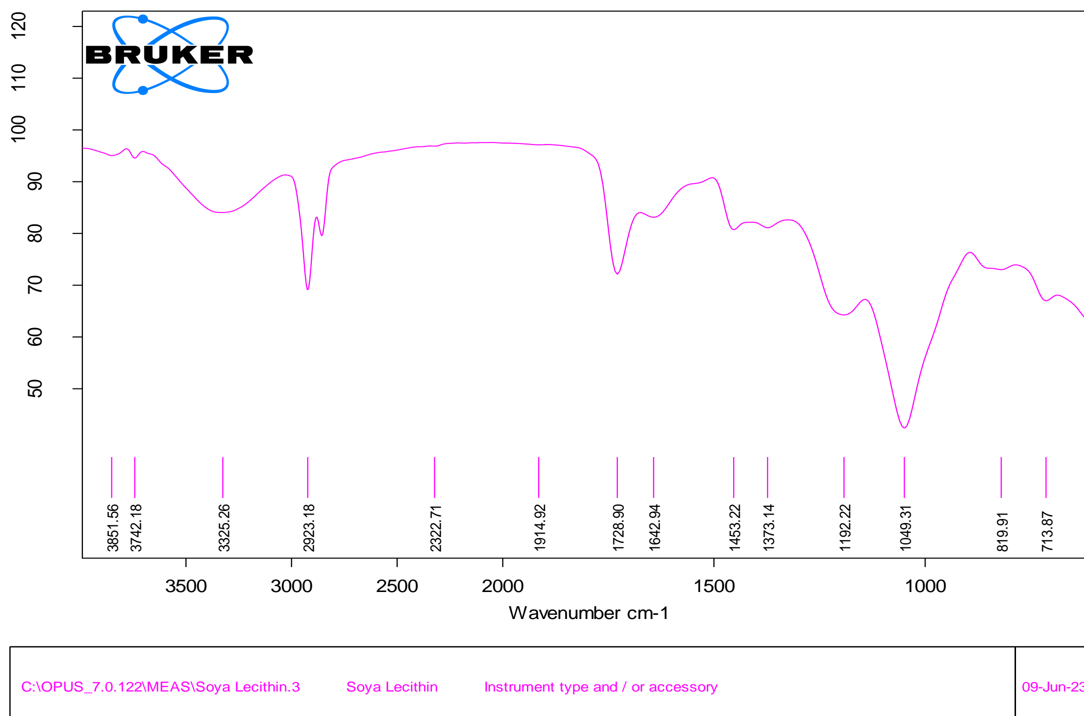


Figure 6.5: FT-IR spectra of Soya lecithin

Table 6.4: FT-IR Interpretation of Soya lecithin

Peak assignment	Actual Peak (cm-1)	Corresponding peak (cm-1)
(O-H) alcohol, phenol	3851	3500-4000
(N-H) (O-H) Amines, Amides, and carboxy acids	3325	3200-3500
(C-H) Alkane	2923	2850-2960
(C=O) Carbonyl compounds	1728	1600-1800
(C=O) Carbonyl compounds	1642	1600-1800
(CH2) Methylene group	1453	1465
(C≡H) Alkynes	1373	1300-1400
(C-O) Ethers	1192	1000-1300
(C-O) Ethers	1049	1000-1300
(C=C) Trans-disubstituted alkenes	819	800-900

6.1.3.4 FT-IR spectra of Poloxamer 407

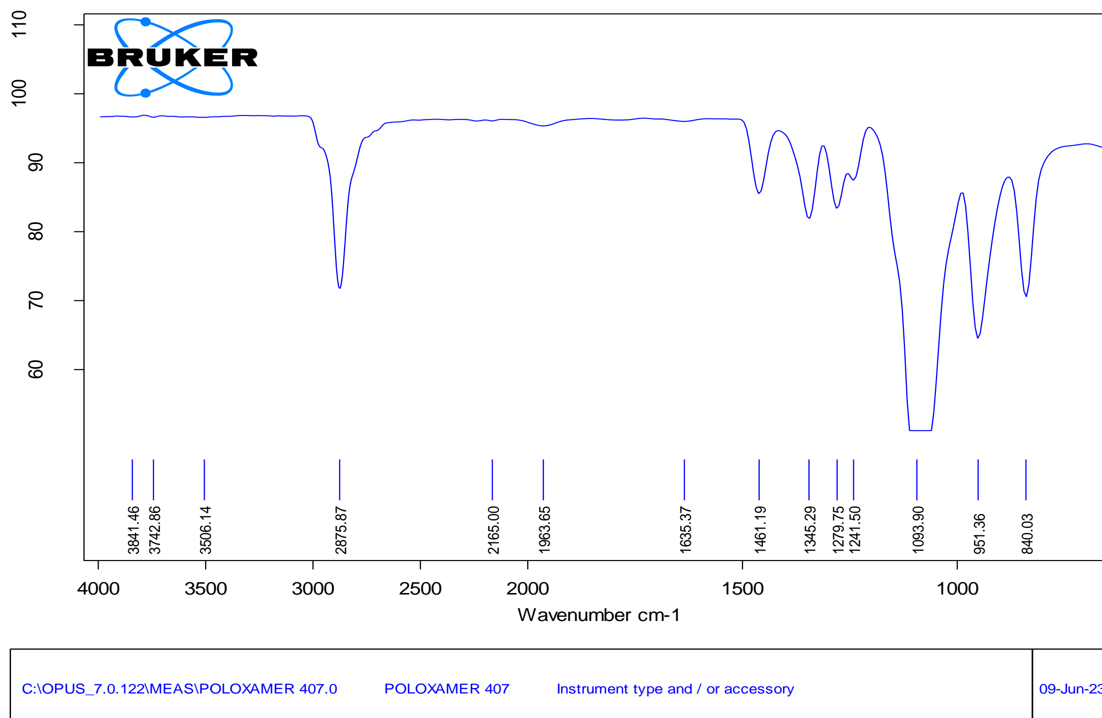


Figure 6.6: FT-IR spectra of Poloxamer 407

Table 6.5: FT-IR Interpretation of Poloxamer 407

Peak assignment	Actual Peak (cm-1)	Corresponding peak (cm-1)
(O-H) alcohol, phenol	3841	3500-4000
(O-H) alcohol, phenol	3742	3500-4000
(C-H) Alkane	2875	2850-2960
(C=O) Carbonyl compounds	1635	1600-1800
(CH2) Methylene group	1461	1465
(C≡H) Alkynes	1345	1300-1400
(C-O) Ethers	1279	1000-1300
(C-O) Ethers	1039	1000-1300
(C=C) Trans-disubstituted alkenes	851	800-900
(C=C) Trans-disubstituted alkenes	841	800-900

6.1.3.5 FT-IR spectra of Hydroxypropyl methylcellulose (HPMC)

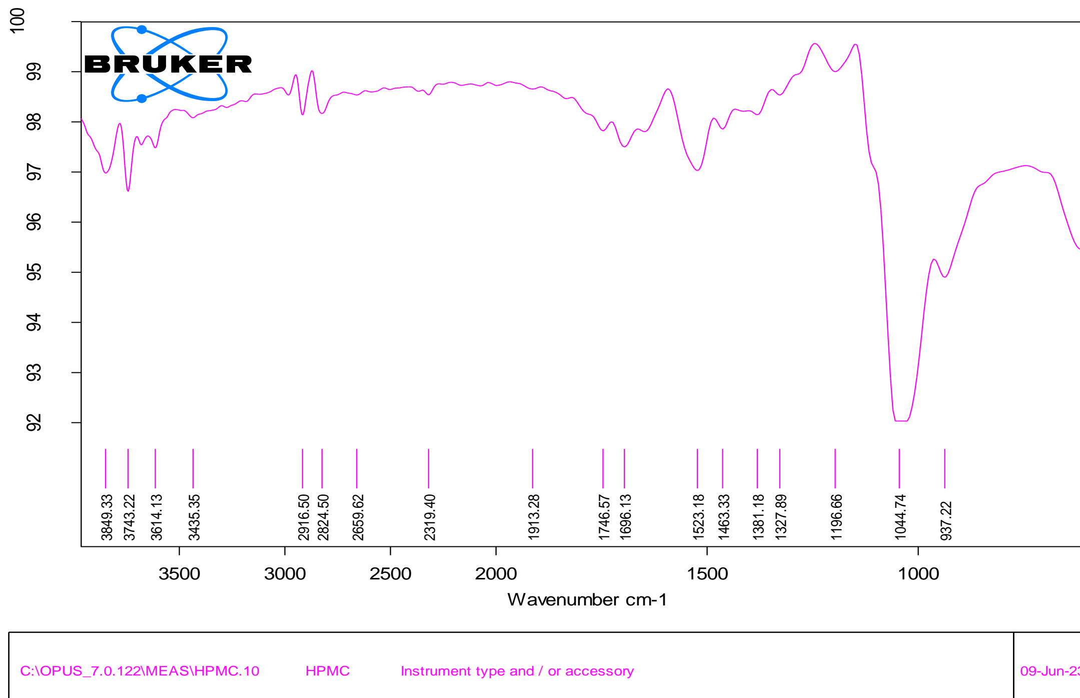


Figure 6.7: FT-IR spectra of Hydroxypropyl methylcellulose (HPMC)

Table 6.6: FT-IR interpretation of Hydroxypropyl methylcellulose (HPMC)

Peak assignment	Actual Peak (cm-1)	Corresponding peak (cm-1)
(O-H) alcohol, phenol	3614-3849	3500-4000
(N-H) (O-H) Amines, Amides, and carboxy acids	3435	3200-3500
(C-H) Alkane	2824	2850-2960
(C=O) Carbonyl compounds	1746	1600-1800
(C=O) Carbonyl compounds	1696	1600-1800
(CH2) Methylene group	1463	1465
(C≡H) Alkynes	1381	1300-1400
(C-O) Ethers	1196	1000-1300
(C-O) Ethers	1044	1000-1300
(C-H) Alkanes	937	900-1000

6.1.3.6 FT-IR spectra of Carbopol 940

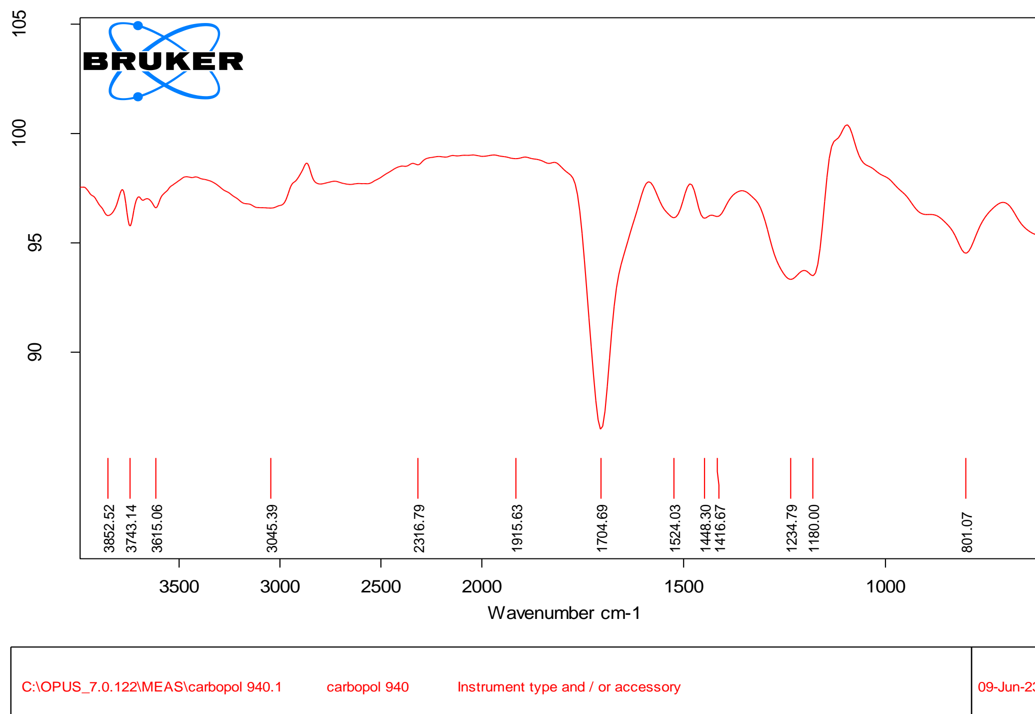


Figure 6.8: FT-IR spectra of Carbopol 940

Table 6.7: FT-IR Interpretation of Carbopol

Peak assignment	Actual Peak (cm-1)	Corresponding peak (cm-1)
(O-H) alcohol, phenol	3615-3852	3500-4000
(C-H) Alkanes, Alkenes, and Aromatics compounds	3045	3045
(C=O) Carbonyl compounds	1704	1600-1700
(CH2) Methylene group	1448	1465
(C-O) Ethers	1234	1000-1300
(C-O) Ethers	1180	1000-1300
(C=C) Trans-disubstituted alkenes	801	800-900

The FT-IR spectra of drug with selected excipients was also obtained and result were presented in figure 6.9 table 6.8.

6.1.3.7 FT-IR spectra of Quercetin with their excipients

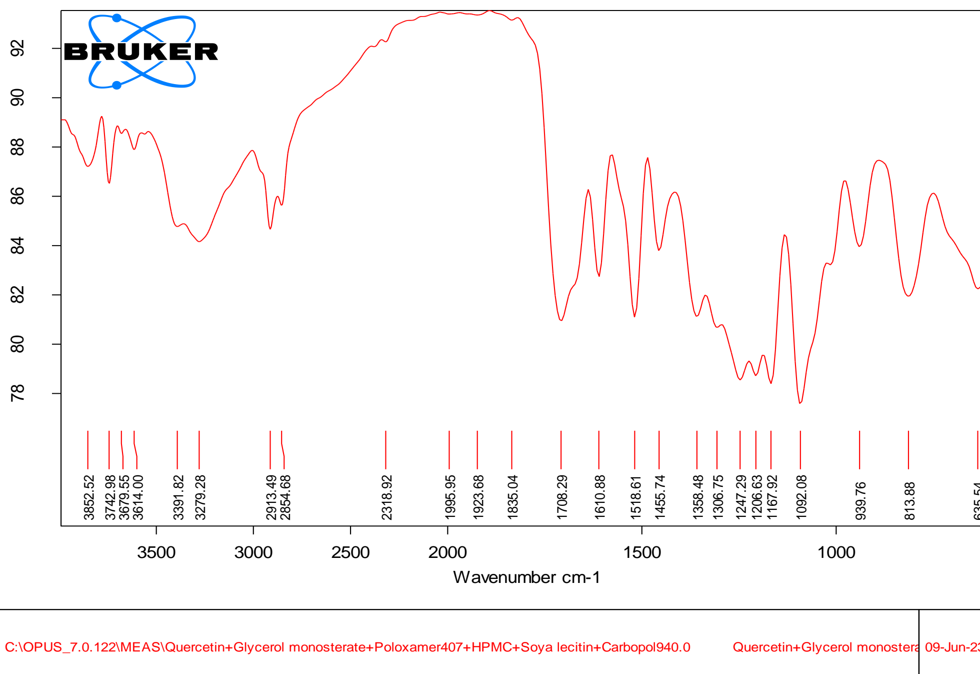


Figure 6.9: FT-IR spectra of Quercetin with selected excipients

Table 6.8: FT-IR Interpretation of Quercetin with selected excipients

Peak assignment	Actual Peak (cm-1)	Corresponding peak (cm-1)
(O-H) alcohol, phenol	3614-3852	3500-4000
(N-H) (O-H) Amines, Amides, and carboxy acids	3391	3200-3500
(N-H) (O-H) Amines, Amides, and carboxy acids	3213	3200-3500
(C-H) Alkane	2913	2850-2960
(C-H) Alkane	2854	2850-2960
(C≡H) Alkynes	1923	1923
(C=O) Carbonyl compounds	1835	1835
(C=O) Carbonyl compounds	1708	1600-1800
(C=O) Carbonyl compounds	1610	1600-1800
(CH2) Methylene group	1455	1465
(C≡H) Alkynes	1356	1300-1400
(C-O) Ethers	1092	1000-1300
(C-H) Alkanes	939	900-1000
(C=C) Trans-disubstituted alkenes	813	800-900

From the result of compatibility studies, it was observed that the characteristic peak of pure drug were retained at respective place, thus confirmed the compatibility of selected drug with the chosen excipients.

6.1.4 Evaluation of solid lipid nanoparticles of Quercetin

The solid lipid nanoparticles of Quercetin were prepared and evaluated for following parameters.

6.1.4.1 Percentage yield

The amount of Quercetin in each formulation was determined. The percentage yield of formulations F1-F9 was found respectively. The formulation F8 showed the maximum % yield. The percentage yield for all batches of solid lipid nanoparticles was depicted in table 6.9 and figure 6.10.

Table 6.9: Percentage yield of solid lipid nanoparticles of Quercetin (Formulation batches F1-F9)

Batches of Nanoparticle	Percentage yield (%)
F1	64.87
F2	78.05
F3	72.12
F4	69.65
F5	65.21
F6	75.91
F7	69.57
F8	86.33
F9	58.55

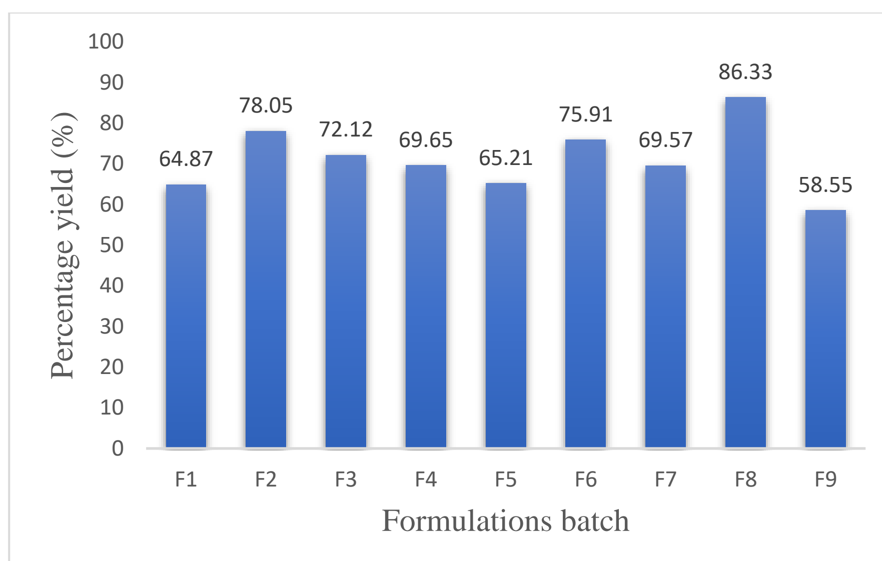


Figure 6.10: Percentage yield of Quercetin solid lipid nanoparticles (Formulation batches F1-F9)

6.1.4.2 Encapsulation Efficiency

The amount of active constituent in the supernatant was determined using UV spectrophotometer at 266 nm and the absorbance readings were used to calculate the amount of free drug which further determined the %EE. The %EE of all the batches was calculated & mentioned in table 6.10 & figure 6.11. The %EE ranged between 64.59% to 83.85%.

Table 6.10: %EE of solid lipid nanoparticles of Quercetin (Formulation batches F1-F9)

Batch	Encapsulation Efficiency (EE%)
F1	79.31
F2	71.93
F3	73.82
F4	75.67
F5	64.59
F6	69.15
F7	67.78
F8	83.85
F9	77.9

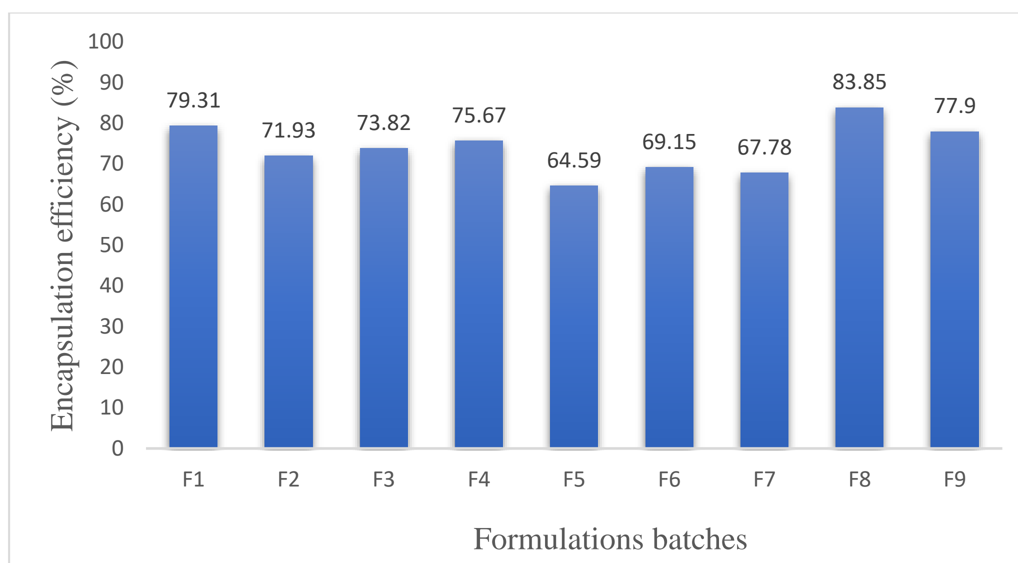


Figure 6.11: EE% of solid lipid nanoparticles of Quercetin (Formulation batch F1-F9)

6.1.4.3 Particle size

The particle size of the Nanoparticles was determined by using a Zetasizer 4 (Malvern Instruments Ltd., Malvern, UK). The particle size and size distribution are the most important characteristics of Nanoparticles system. The particle size of formulations F1-F9 was found in the range of 279-520nm respectively. The particles size of solid lipid nanoparticles was shown in table 6.11 and figure 6.12.

Table 6.11: Particle size of solid lipid nanoparticles of Quercetin (Formulation batch F1-F9)

Batch	Particle size (nm)
F1	299
F2	412
F3	401
F4	365
F5	520
F6	425
F7	486
F8	279
F9	322

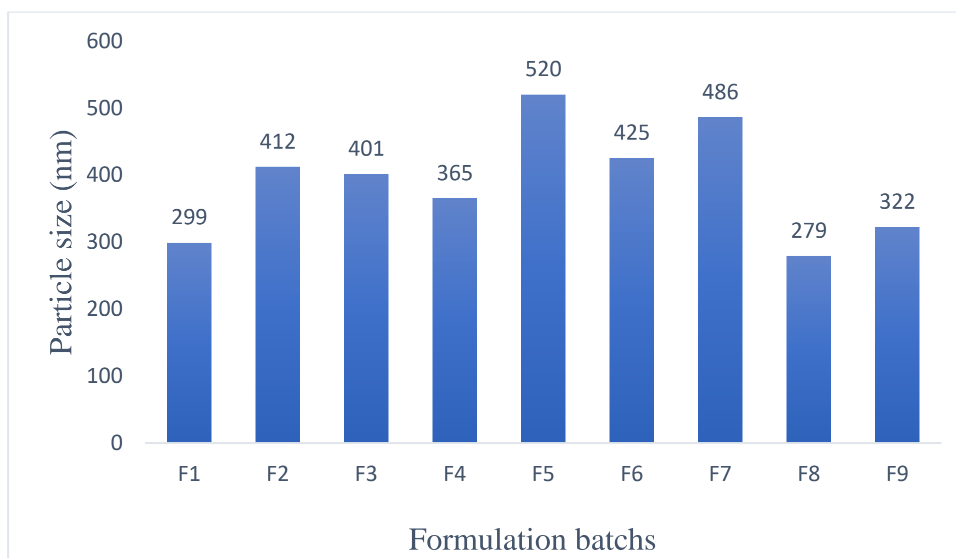


Figure 6.12: Particle size of solid lipid nanoparticles of Quercetin (Formulation batch F1-F9)

6.1.4.4 Zeta potential

Zeta potential of drug loaded SLNs was determined and sound to be in the range of -6.55 to -15.73mV. Results were shown in table 6.12.

Table 6.12: Zeta potential of solid lipid nanoparticles of Quercetin (Formulation batch F1-F9)

Batch	Zeta potential (mV)
F1	-13.66
F2	-14.37
F3	-12.01
F4	-11.43
F5	-6.55
F6	-10.83
F7	-10.12
F8	-15.73
F9	-11.89

6.1.4.5 Scanning electron microscope (SEM)

The surface morphology study of prepared batches of Quercetin solid lipid nanoparticles was conducted and it was found that nanoparticles were spherical in shape with rough surface.

6.1.4.6 *In-vitro* drug release

The dissolution profile of all the batches of solid lipid nanoparticles of Quercetin was obtained in phosphate buffer pH 7.4. The *in-vitro* dissolution testing was performed for12 hr. (720 minutes). The in-vitro drug release from solid lipid nanoparticles of Quercetin ranged from 71.98% to 92.13%. The maximum *in-vitro* drug release was found to be 92.13% from F8 at the end of 720 minutes as shown in the table 6.13 and figure 6.13.

Table 6.13: *In-vitro* drug release profile of solid lipid nanoparticles of Quercetin (Formulation batches F1-F9)

Time (minute)	F1	F2	F3	F4	F5	F6	F7	F 8	F9
0.00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
60	6.14	7.43	7.27	6.93	6.12	8.12	6.76	8.51	5.85
120	14.19	13.21	16.76	16.19	13.27	13.36	12.84	17.31	11.77
180	22.72	26.35	21.71	19.11	20.93	19.95	18.13	27.48	17.02
240	29.01	33.93	28.31	24.32	24.78	27.85	23.78	36.53	22.12
300	34.56	36.64	32.87	29.32	30.91	37.78	29.31	41.9	31.51
360	39.41	41.21	38.59	34.76	33.21	46.57	34.87	48.91	35.91
420	45.89	47.12	44.85	39.34	38.45	53.11	40.36	56.11	41.65
480	51.87	52.87	53.12	43.67	42.06	59.91	47.56	62.23	45.76
540	57.12	59.65	61.91	48.21	49.68	66.45	54.16	69.91	51.09
600	63.78	68.32	69.23	54.89	54.15	72.78	61.98	76.56	55.43
660	69.19	79.54	74.17	64.43	62.11	81.08	69.12	84.21	64.32
720	74.96	86.11	84.93	73.12	71.98	88.32	78.03	92.13	73.87

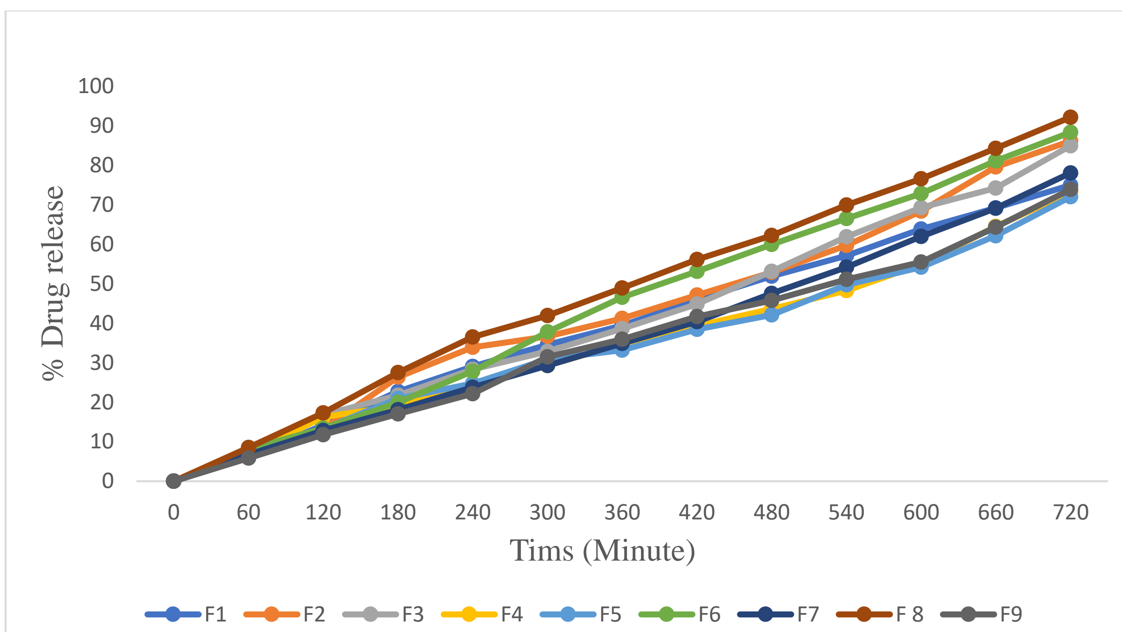


Figure 6.13: Percentage drug Release of solid lipid nanoparticles of Quercetin (Formulation batches F1-F9)

Based on the result obtained from evaluation study, it was observed that the batch F8 showed better results when compared with other batches.

6.1.5 Optimization of Solid lipid nanoparticles of Quercetin

For the selection of an optimized formulation, solid lipid nanoparticles of Quercetin were undergone optimization study by using 3^2 full factorial design. Two independent parameters i.e., Glyceryl monostearate and Soya lecithin were chosen and their effect on dependent variables i.e., particle size and encapsulation efficiency (%EE) was determined.

6.1.5.1 Effect of Glyceryl monostearate and Soya lecithin concentration on particle size and Entrapment efficiency

Solid lipid nanoparticles were prepared by the hot homogenization followed by ultrasonication method. The different concentrations of Glycerol monoetherate and Soya lecithin were used to prepare nanoparticles. Optimization study was conducted to see the effect of lipid and polymer concentration on particle size, and entrapment efficiency.

6.1.5.2 Response 1-particle size

Table 6.14: Showing the effect of independent variable on Particle size

Run	Factor 1	Factor 2	Response 1
	A: Glyceryl monostearate	B: Soya lecithin	Particle size. Nm
F1	200	125	299
F2	350	100	412
F3	350	150	401
F4	350	125	365
F5	50	100	520
F6	50	150	425
F7	50	125	486
F8	200	150	279
F9	200	100	322

ANOVA for Reduced Cubic model

Table 6.15: Response 1: Particle size

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	53727.44	7	7675.35	110.53	0.0731	not significant
A-Glyceryl monostearate	7320.50	1	7320.50	105.42	0.0618	
B-Soya lecithin	924.50	1	924.50	13.31	0.1703	
AB	1764.00	1	1764.00	25.40	0.1247	
A^2	36360.06	1	36360.06	523.58	0.0278	
B^2	193.39	1	193.39	2.78	0.3437	
A^2B	33.33	1	33.33	0.4800	0.6143	
AB^2	1008.33	1	1008.33	14.52	0.1634	
Residual	69.44	1	69.44			
Cor Total	53796.89	8				

Factor coding is **Coded**.
Sum of squares is **Type III – Partial**
The **Model F-value** of 110.53 implies there is a 7.31% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are a significant. In this case, A^2 is a significant model term. Values exceedingly more than 0.1000 indicate model terms are not significant. If there are many insignificant model terms (not counting those required to support the hierarchy), model reduction may improve your model

The data obtained for the particle size (Y1) was fitted into various polynomial models. It was observed that the response Y1was fitted in Cubic model response surface model. The equation is given below (Equation 1).

$$Y1 = 293.44 - 60.50 * A - 21.50 * B + 21.0 * AB + 134.83 * A^2 + 9.83 * B^2 - 5.00 * A^2B + 27.50 * AB^2 \quad \textbf{Eq--1}$$

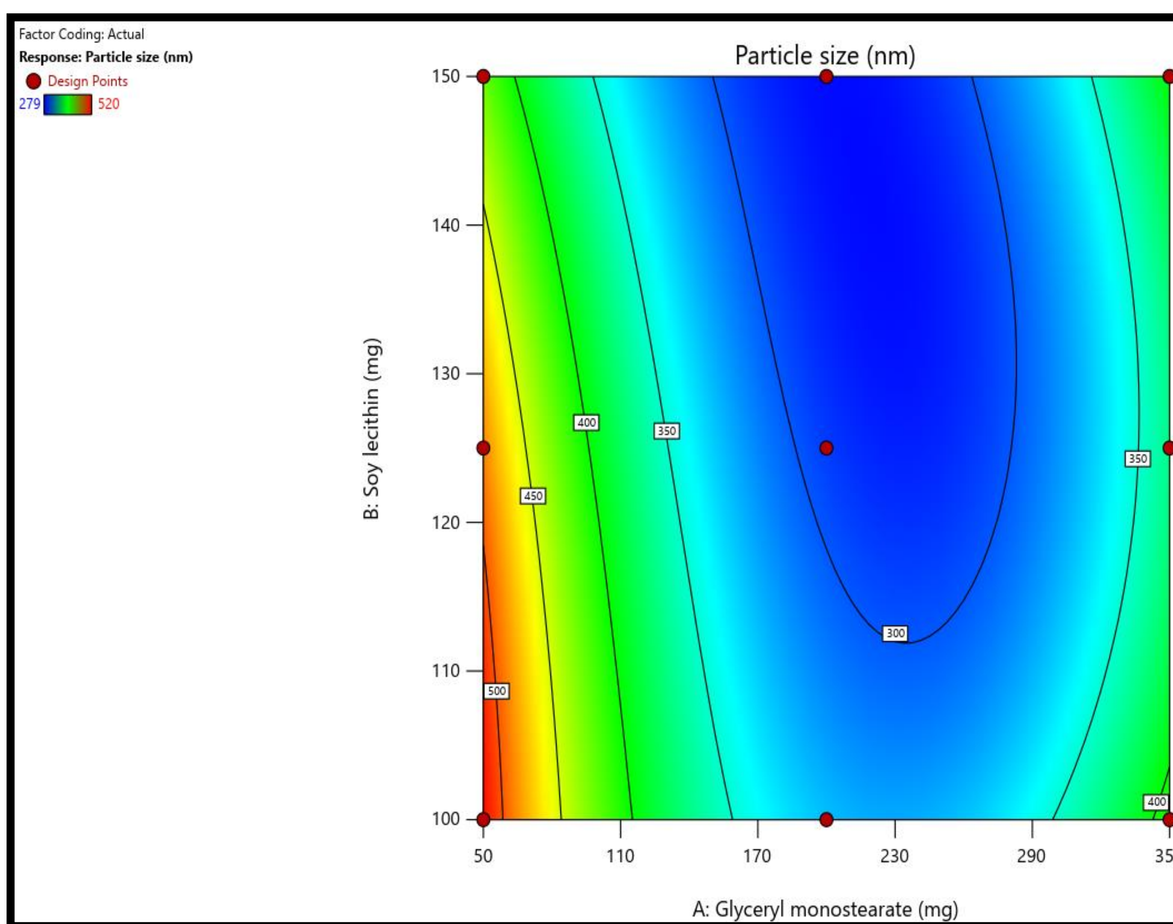


Figure 6.14: Model graph showing effect of glyceryl monostearate and soya lecithin on particle size

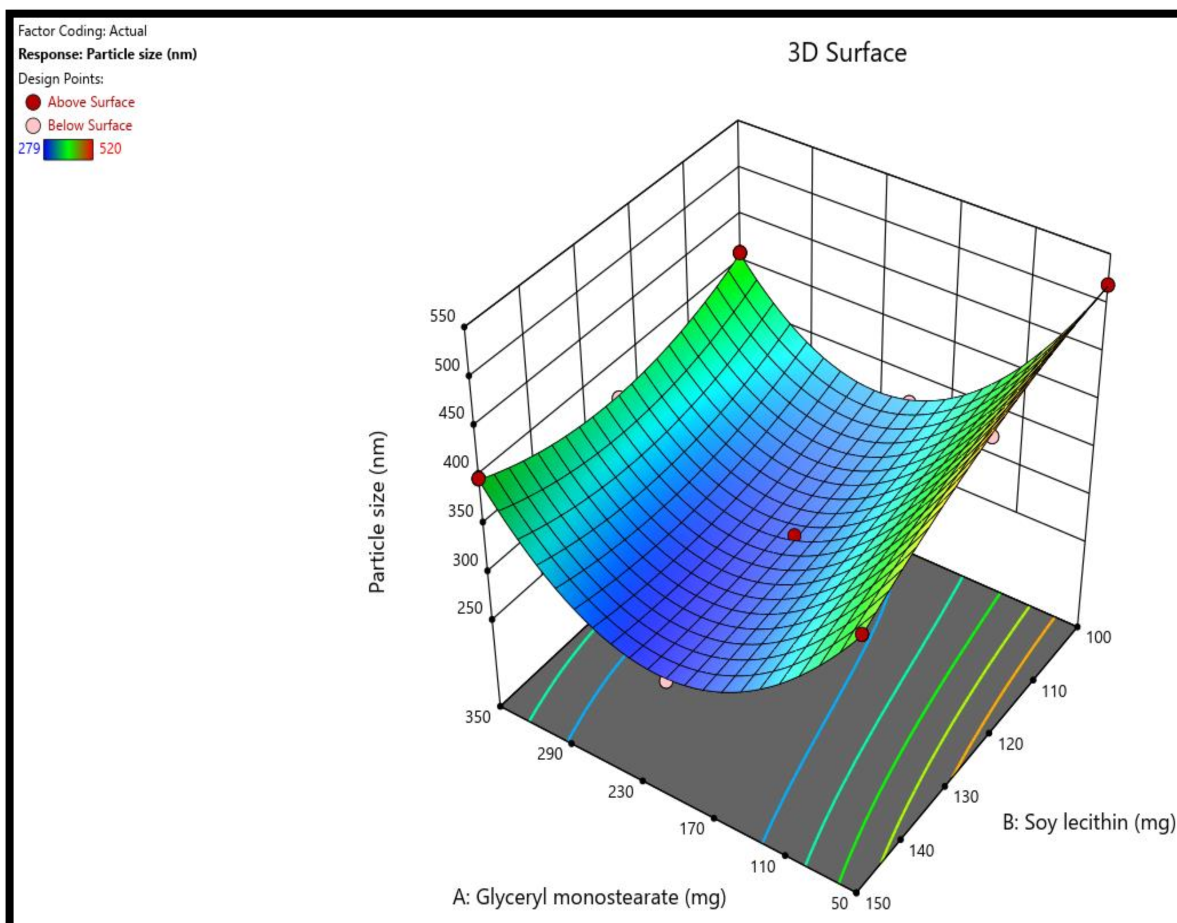


Figure 6.15: 3D graph showing effect of Glyceryl monostearate and Soya lecithin on Particle size

6.1.5.3 Response 2-Entrapment efficiency

ANOVA for Reduced Cubic model

Table 6.16: Response 2: Entrapment efficiency

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	292.66	7	41.81	8.05	0.2651	not significant
A-Glyceryl monostearate	31.13	1	31.13	6.00	0.2468	
B-Soya lecithin	17.70	1	17.70	3.41	0.3160	
AB	1.78	1	1.78	0.3433	0.6626	
A^2	194.57	1	194.57	37.48	0.1031	
B^2	1.02	1	1.02	0.1961	0.7346	
A^2B	2.48	1	2.48	0.4768	0.6153	
AB^2	1.18	1	1.18	0.2282	0.7163	
Residual	5.19	1	5.19			
Cor Total	297.85	8				

Factor coding **Coded**.

sum of squares is **Type III – Partial**

The **model F-value** of 8.05 implies that the model is not significant relative to noise. There is a 26.51% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate that model terms are significant. In this case, there are no important model terms. Values above 0.1000 indicate model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The data obtained for the % EE (Y2) was fitted in which cubic response surface model as shown in figure 6.18. The equation for the same is given below (Equation 2).

$Y2 = 80.83 – 3.94 * A\ 2.97 *B – 0.6675 * AB – 9.89 *A^2 – 0.7133 * B^2 – 1.36 * A^2B – 0.9425 * AB^2$ **Eq-2**

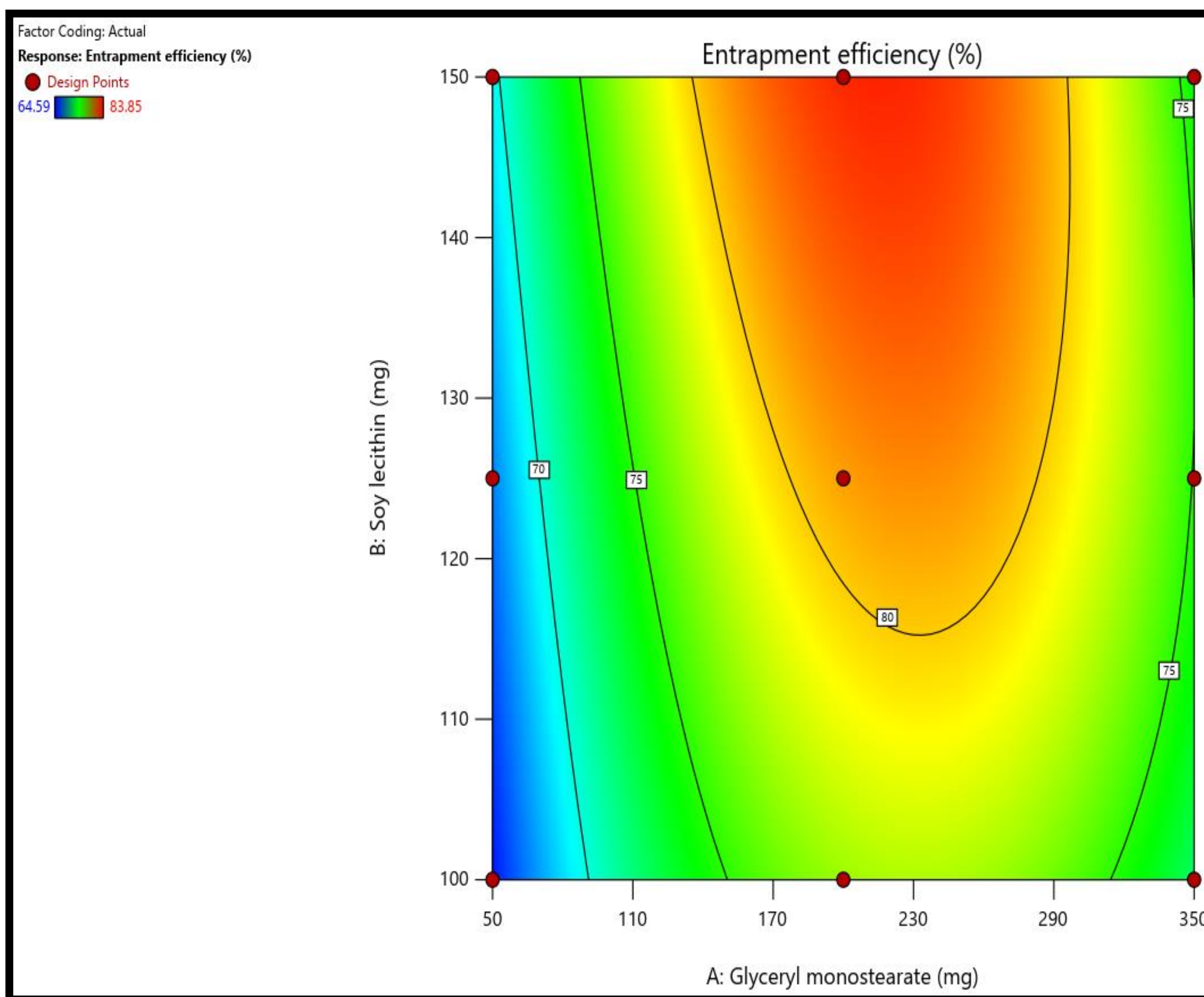


Figure 6.16: Model graph showing effect of Glyceryl monostearate and Soya lecithin on %EE

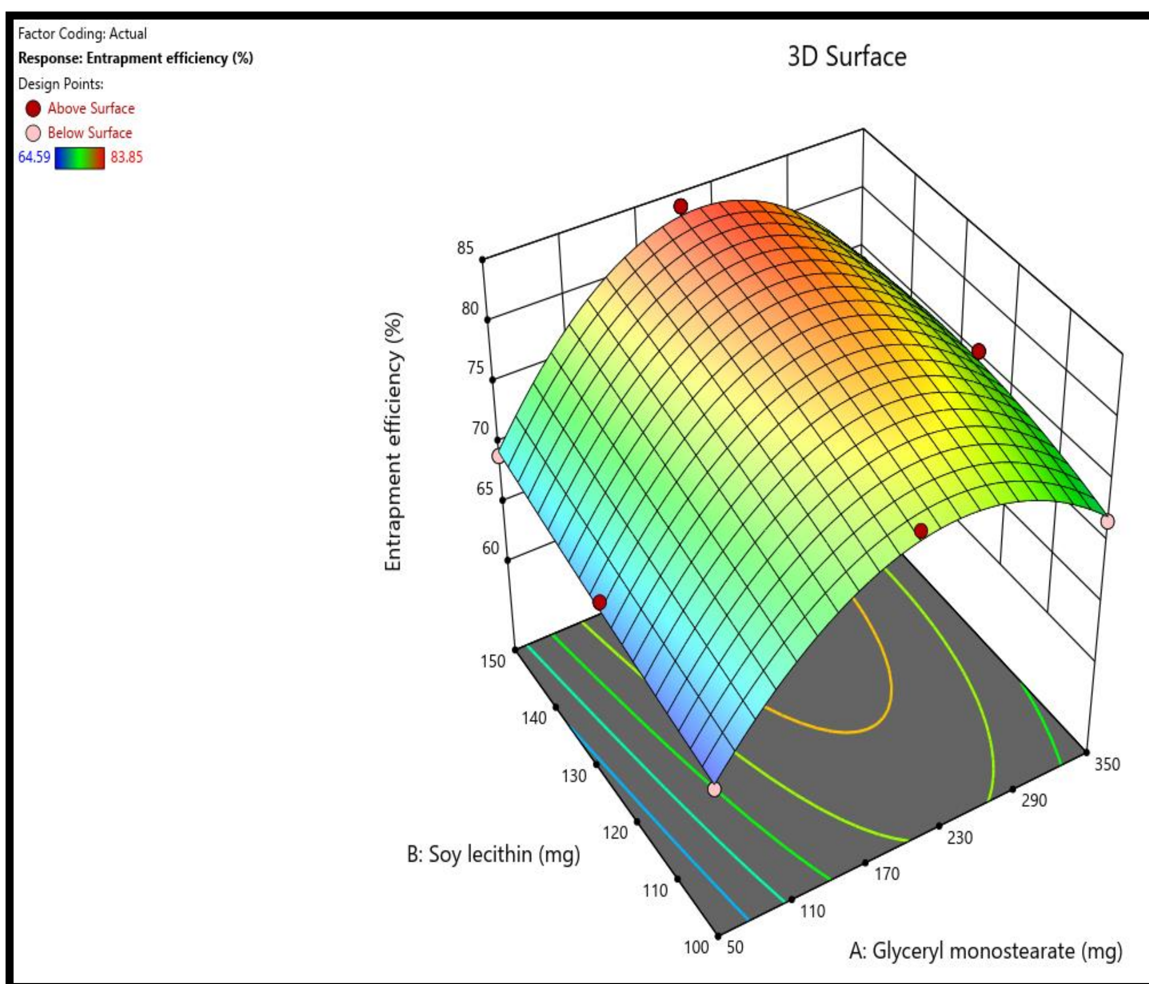


Figure 6.17: 3D graph showing effect of Glycerol monosteraate and Soya lecithin on %EE

Based on the evaluation of solid lipid nanoparticles batches F1-F9 by factorial design, a check point batch was obtained and it was further formulated as per formula given by factorial design.

Y2 was fitted into reduced cubic response surface model (Equation 2). The relationship between independent variables and dependent variables was depicted by constructing three-dimensional (3D) surface response plots.

After conducting ANOVA study on prepared batches, an optimized formula was obtained (Table 6.17 And figure 6.18) and used for future study.

Table 6.17: Check point batch

Number	Glyceryl monostearate	Soya lecithin	Particle size	Entrapment efficiency	Desirability	
1	211.487	150.000	281.620	83.204	0.978	Selected

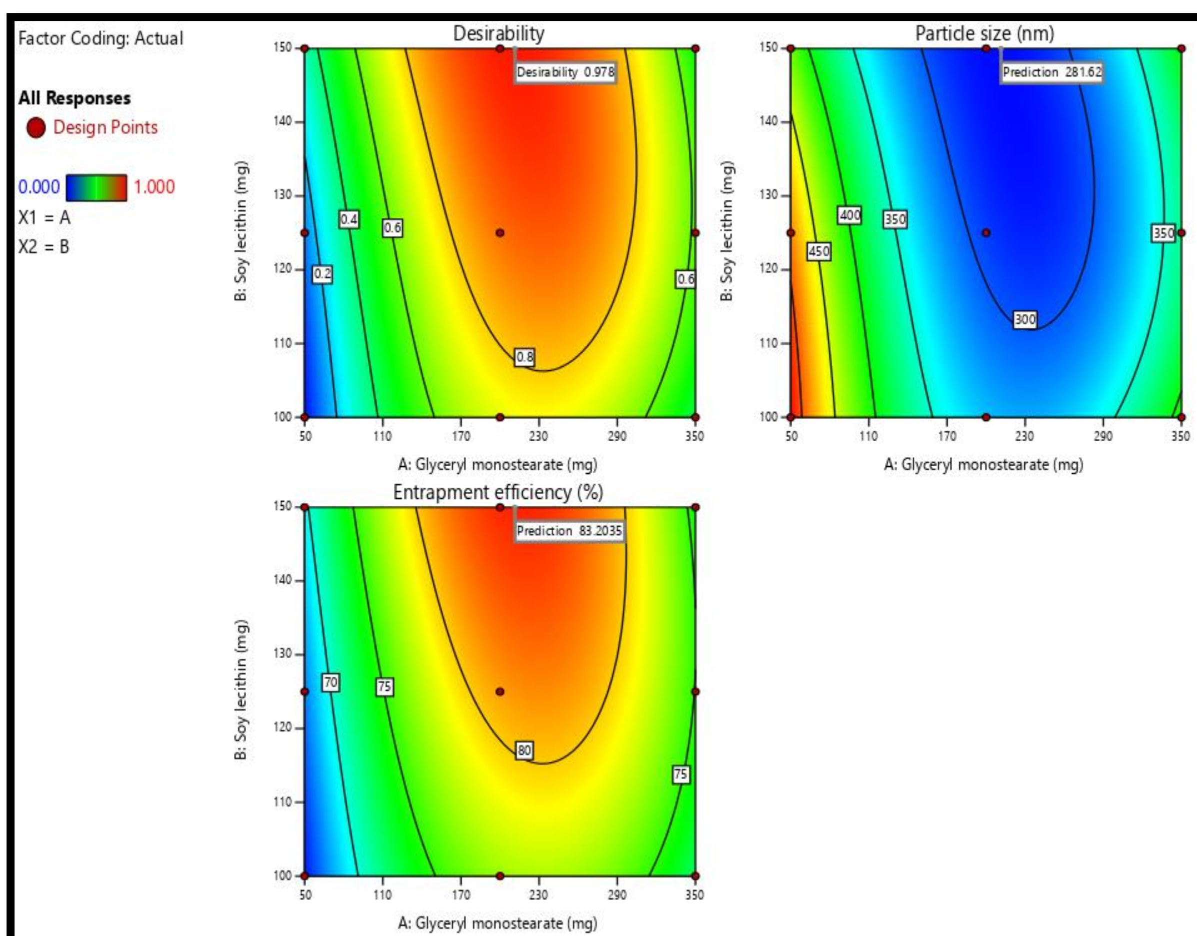


Figure 6.18: Desirability Graph showing optimized formula

6.1.5.4 Evaluation of optimized batch

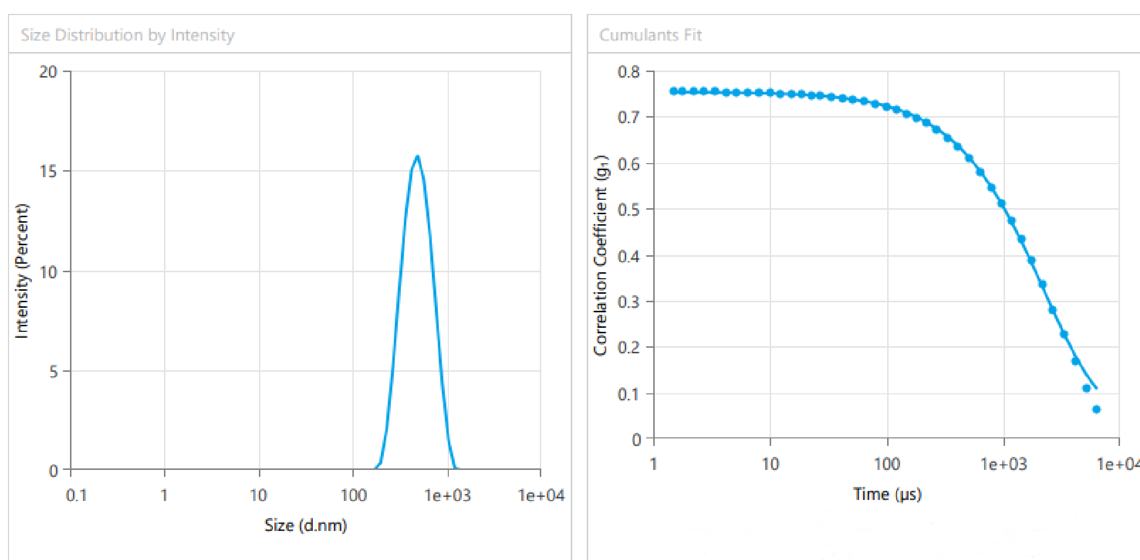
Optimized formula thus obtained was converted into a formulation and evaluated for various parameters as discussed earlier and results were represented in table 6.18 and figure 6.19-6.20.

Table 6.18: Evaluation parameters for optimize batch (Fopt)

Formulation batch	Evaluation Parameters			
	Particle size (nm)	**Zeta potential (mv)**	**Encapsulation efficiency (% EE)**	**Percentage yield (%)**
Fopt	279	-15.73	83.85	86.33

6.1.5.4.1 Particle size

Particle size value of optimized batch was represented figure 6.19.

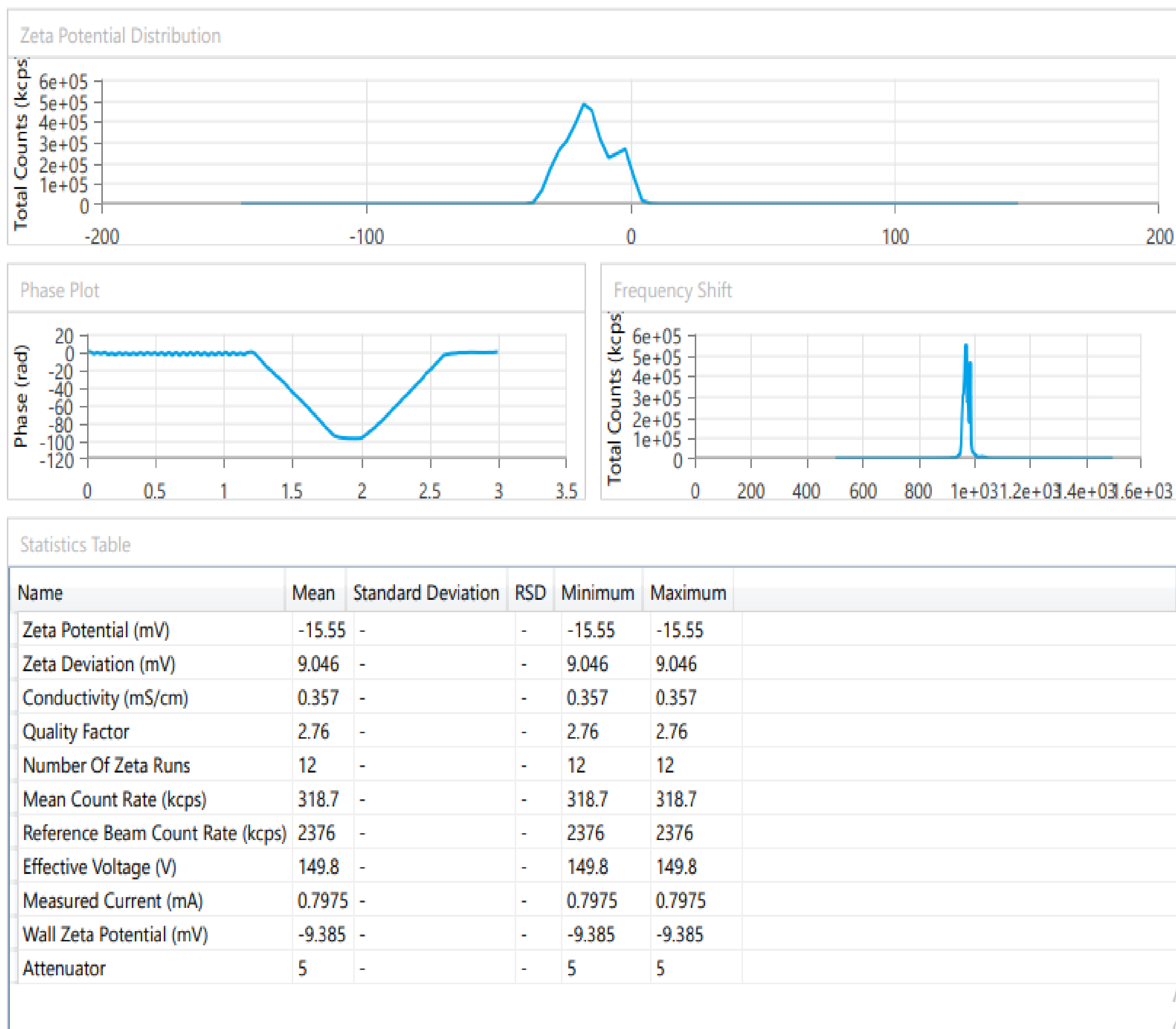


Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Z-Average (nm)	279	-	-	279	279
Polydispersity Index (PI)	0.4249	-	-	0.4249	0.4249
Intercept	0.9629	-	-	0.9629	0.9629
Derived Mean Count Rate (kcps)	1.815E+04	-	-	1.815E+04	1.815E+04
Cuvette Position (mm)	4.64	-	-	4.64	4.64
Number Of Size Runs	30	-	-	30	30
Run Retention (%)	100	-	-	100	100
In Range (%)	93.26	-	-	93.26	93.26
Fit Error	0.004959	-	-	0.004959	0.004959
Detector Angle (°)	90	-	-	90	90

Figure 6.19: Particle size of Optimized batch (Fopt) of solid lipid nanoparticles of Quercetin

6.1.5.4.2 Zeta potential

Zeta potential value of optimized batch was represented figure 6.20



Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-15.55	-	-	-15.55	-15.55
Zeta Deviation (mV)	9.046	-	-	9.046	9.046
Conductivity (mS/cm)	0.357	-	-	0.357	0.357
Quality Factor	2.76	-	-	2.76	2.76
Number Of Zeta Runs	12	-	-	12	12
Mean Count Rate (kcps)	318.7	-	-	318.7	318.7
Reference Beam Count Rate (kcps)	2376	-	-	2376	2376
Effective Voltage (V)	149.8	-	-	149.8	149.8
Measured Current (mA)	0.7975	-	-	0.7975	0.7975
Wall Zeta Potential (mV)	-9.385	-	-	-9.385	-9.385
Attenuator	5	-	-	5	5

Figure 6.20: Zeta potential of Optimized batch (Fopt) of solid lipid nanoparticles of Quercetin

6.1.5.4.3 Surface morphology

The optimized batch of Quercetin nanoparticles was checked for surface morphology.

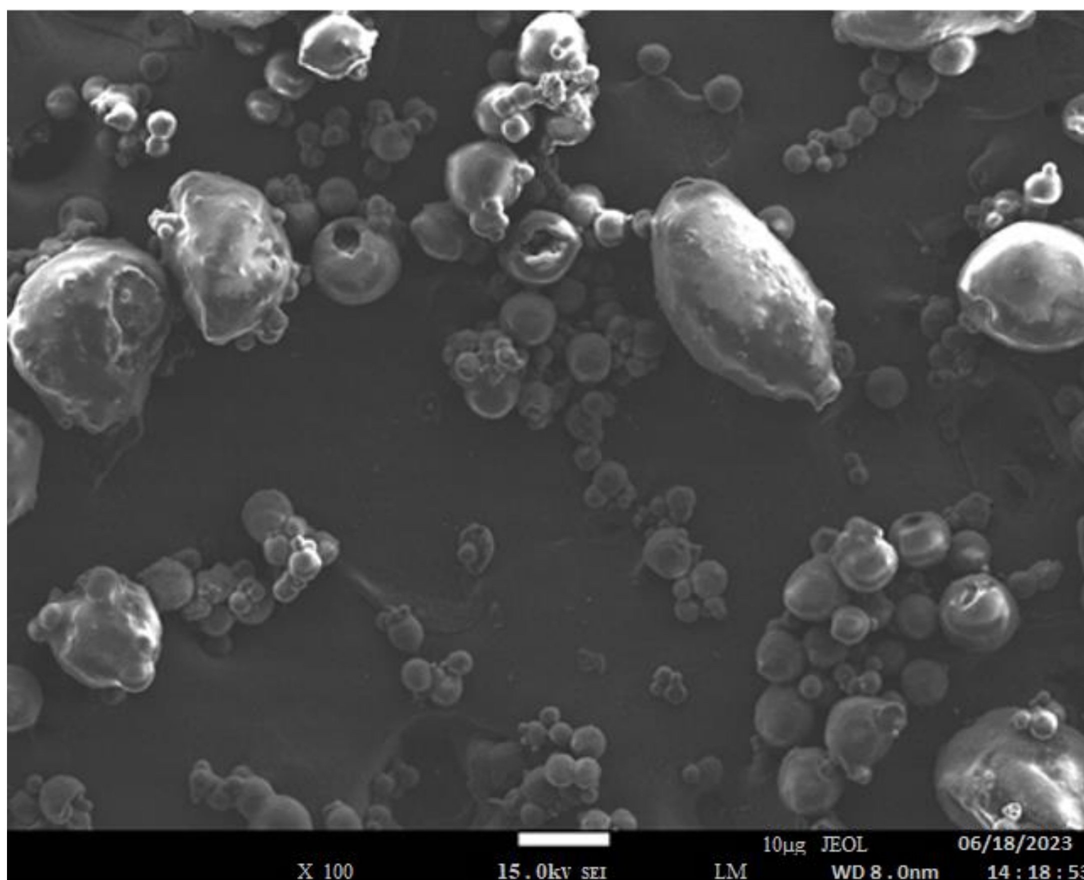


Figure 6.21: SEM image of optimize batch (Fopt)

The surface morphology study of optimized batch (Fopt) of Quercetin solid lipid nanoparticles have shown that nanoparticles were spherical in shape with rough surface (Figure 6.21).

6.1.5.4.1 *In-vitro* drug release study of optimized batch

The optimized batch was also undergone for *in-vitro* drug release study. Results have been shown in table 6.19 and figure 6.22 and it was observed around that 92.13% drug was released from optimized batch in 12 hr.

Table 6.19: *In-vitro* drug release from optimized (Fopt) batch of Quercetin solid lipid nanoparticles

Time (minute)	% drug release from Fopt
0.00	0.0
60	8.51
120	17.31
180	27.48
240	36.53
300	41.9
360	48.91
420	56.11
480	62.23
540	69.91
600	76.56
660	84.21
720	92.13

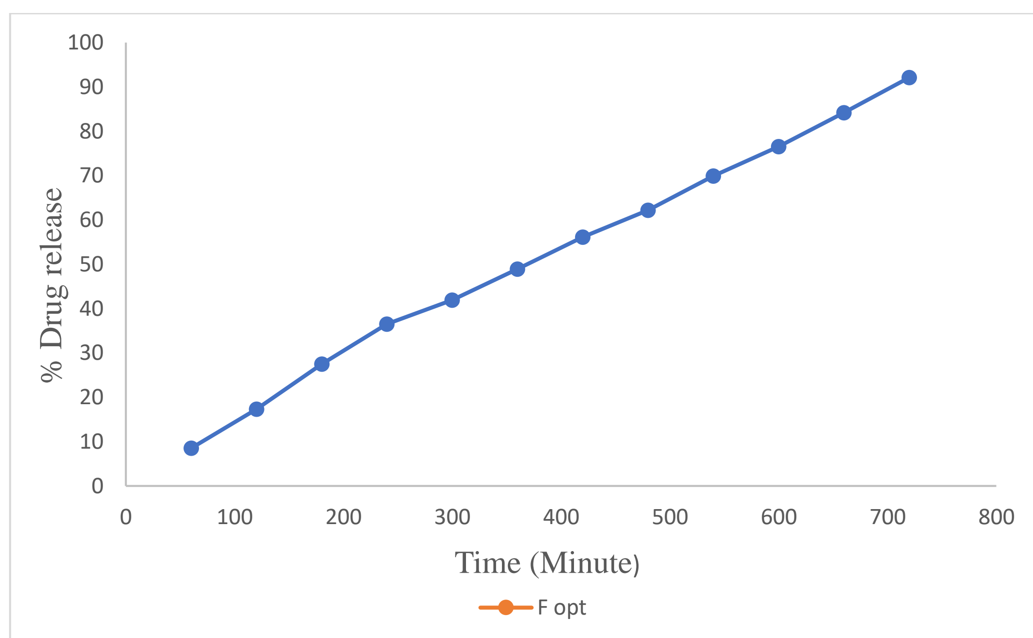


Figure 6.22: *In-vitro* drug release profile of optimized (Fopt) batch of Quercetin (SLNs)

6.1.5.4.2 Stability Studies

The optimized batch was kept on accelerated conditions to check the stability of optimized batch. The physical appearance *in-vitro* drug release studied were observed for stability testing. The results of appearance, and in-vitro drug release studies, on 0 day and after 30 days, 60 days and 90 days of storage are shown in table 6.20 and figure 6.23

.

Table 6.20: Effect of stability conditions on different parameters of optimized batch formulation (Fopt)

S.NO.	Parameters	Results			
		Day 0	Day 30	Day 60	Day 90
1	Appearance	Transparent	Transparent	Transparent	Transparent
2	*In-vitro* Drug release at 12 hr	92.13%	90.89	89.56	89.21

Table 6.21: Effect of stability condition on the release of drug from optimized batch of Quercetin SLNs (Fopt)

Time (minute)	Day 0	Day 30	Day 60	Day 90
0	0	0	0	0
60	8.51	7.91	8.9	6.23
120	17.31	15.23	14.87	13.96
180	27.48	26.11	26.5	24.91
240	36.53	34.98	33.18	31.02
300	41.9	42.06	41.32	39.65
360	48.91	47.14	45.67	44.98
420	56.11	57.32	56.23	54.87
480	62.23	60.43	61.08	59.92
540	69.91	68.12	66.77	65.59
600	76.56	75.23	74.83	72.98
660	84.21	82.98	82.14	80.76
720	92.13	90.89	89.56	89.21

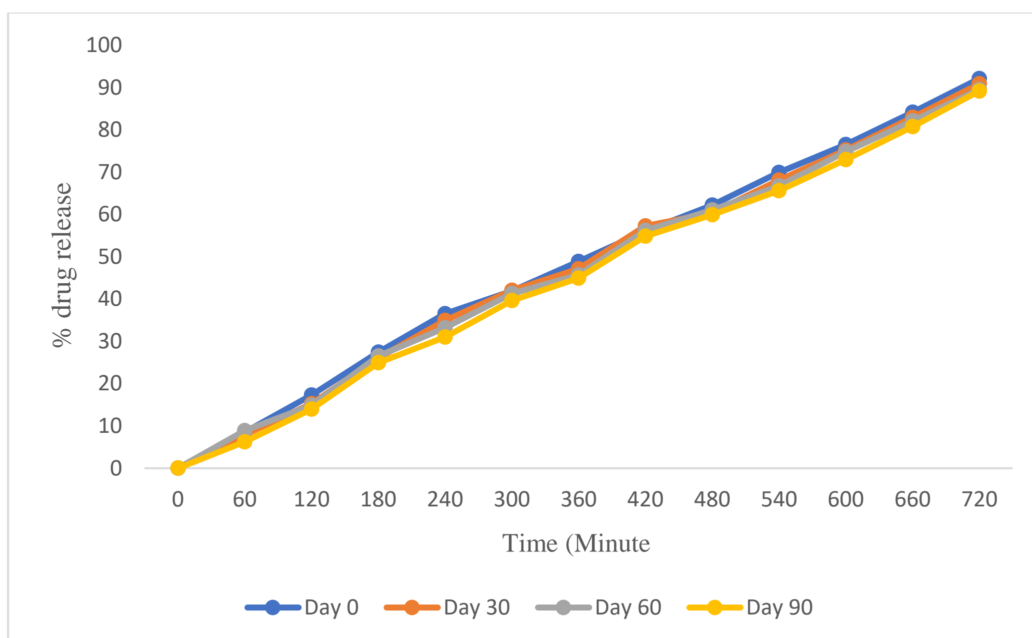


Figure 6.23: Effect of stability conditions on the release of drug from optimized batch of Quercetin SLNs (Fopt)

6.1.6 Evaluation of solid lipid nanoparticles laden in-situ gel of Quercetin

The optimized batch (Fopt) of Solid lipid nanoparticles was converted into in-situ gel by utilizing appropriate gelling agents. The prepared gels were evaluated for following parameters.

6.1.6.1 Physical parameters

The Quercetin solid lipid nanoparticle in-situ gel was tested for clarity, pH, gelling capacity, drug content and *in-vitro* drug release.

6.1.6.2 pH of Solid lipid nanoparticles laden in-situ gel of Quercetin

The Solid lipid nanoparticulate in-situ gel of Quercetin formulation was checked for pH. The pH of in-situ gel of Quercetin SLNs was found to be in the range 5.98-6.12. All measurement of pH was given in table 6.22 and figure 6.24.

Table 6.22: Evaluation data of solid lipid nanoparticles laden in situ gel of Quercetin formulation batches (B1-B3)

Formulations	Clarity	Visual appearance	pH	Drug content (%)	Gelling capacity
B1	Clear	Transparent	6.12	78.64	++
B2	Clear	Transparent	6.04	88.57	+++
B5	Clear	Transparent	5.98	82.31	++

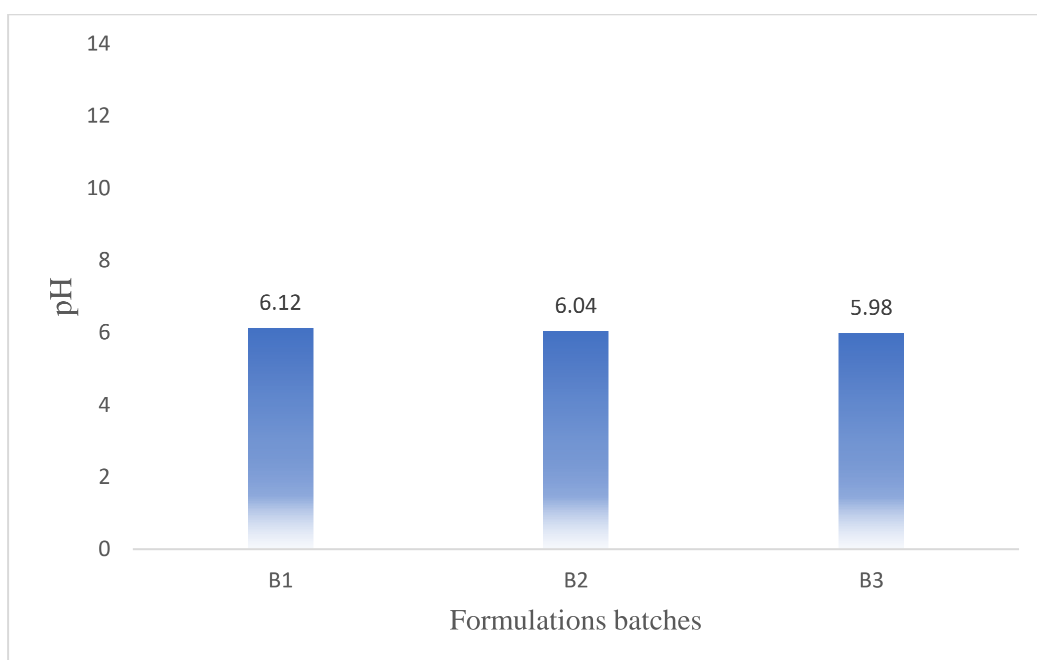


Fig 6.24: pH of Quercetin SLNs in-situ gel formulations (B1-B3)

6.1.6.3 Drug content

The amount of Quercetin in the prepared in-situ gel of Quercetin SLNs was determined by U.V visible spectrophotometer (UV-1800, Shimadzu). The UV absorbance of the sample was determined at a wavelength of 266nm. The drug content of formulations B1-B3 was found in between 78.64-88.57% respectively. The formulation B2 showed the-maximum drug content. The drug content for all batches of in-situ gel was depicted in table 6.22 and figure 6.25.

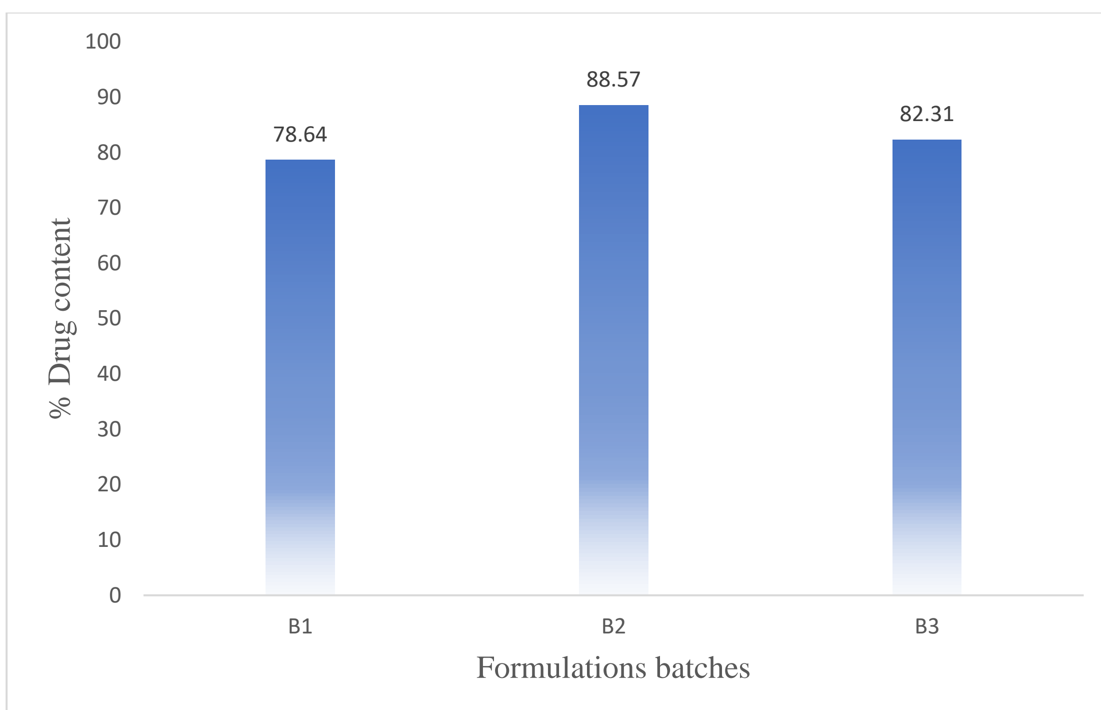


Figure 6.25: %Drug content of Quercetin in-situ gel formulations (B1-B3)

6.1.6.4 *In-vitro* diffusion study

The amount of drug release is an important parameter for controlled release formulation. The prepared batches of in-situ gel of Quercetin nanoparticles were evaluated for in-vitro drug release parameters. The drug release of formulation B1-B3 was found to be in between 81.26-90.05 % respectively. The batch B2 showed the maximum release. The drug release data of all the formulations was depicted in table 6.23 figure 6.26.

Table 6.23: *In-vitro* drug release data of Solid lipid nanoparticles laden in-situ gel of Quercetin (B1-B3)

Time (minute)	B1	B2	B3
0.00	0.0	0.0	0.0
60	6.24	6.91	5.91
120	13.78	13.54	14.11
240	28.11	29.12	30.98
360	41.94	46.07	44.08
480	58.09	64.23	62.37
600	67.45	78.11	73.12
720	81.26	90.05	87.55

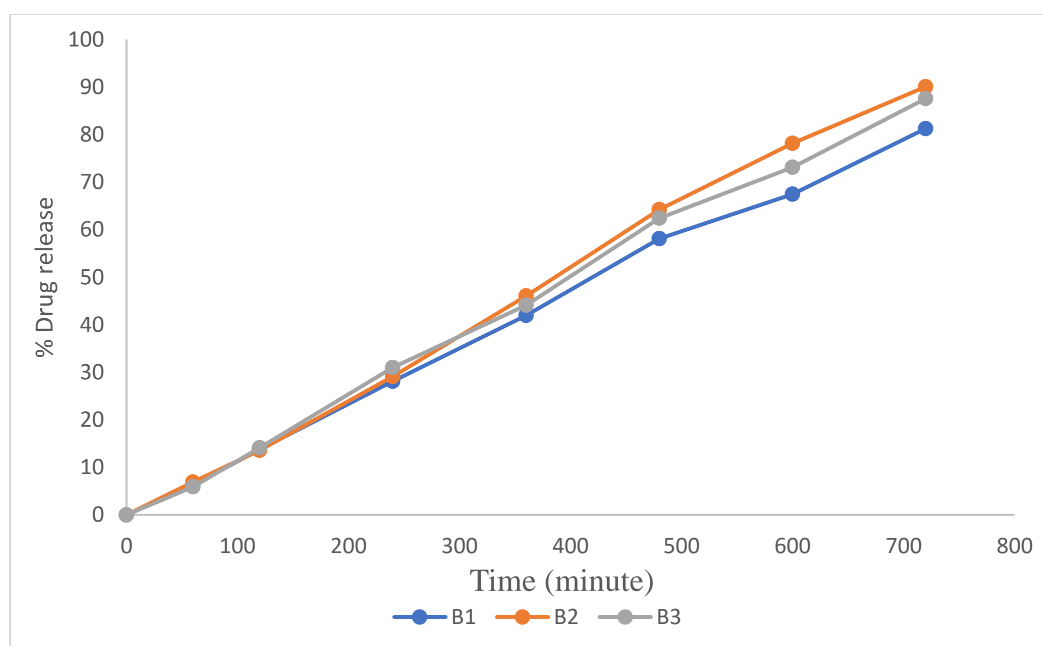


Figure 6.26: %Drug Release of Solid lipid nanoparticles laden in-situ gel of Quercetin (B1-B3).

From the Results it was observed that drug release from in-situ gel was in sustained manners for a duration of 12 hr.

CHAPTER 7
SUMMARY & CONCLUSION

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Conventional ocular drug delivery systems suffer from low bioavailability, rapid clearance, poor penetration, patient non-compliance, invasive procedures, lack of sustained release, and limited drug selection. Majority of dosage concentrations undergo mere wastage when applied as conventional dosage forms. The efficacy of many drugs is often limited by their potential to reach the site of therapeutic action. In most cases (conventional dosage forms), only a small amount of administered dose reaches the target site while the majority portion is distributed throughout the body in accordance with its physicochemical and biochemical properties. The present work consisting of formulation & evaluation of solid lipid nanoparticle laden In-situ gel of Quercetin. Quercetin was formulated as SLNs and converted to in-situ gel, and conceptualized as controlled release drug delivery formulation. Nanoparticles of the selected drug were formed individually with various ingredients by employing nanotechnology. Glyceryl monostearate, soya lecithin, Polyxamer-407 and Quercetin were nanosized by adopting hot homogenization followed by ultra sonication, then converted into in-situ gel by mixing HPMC and Carbapol-940. The drug was run on UV visible spectrophotometer in predetermined concentrations to reckon and confirm the absorbance maxima (λmax) and individual standard calibration curves were prepared by plotting concentration vs absorbance graph. The above determination revealed λmax for Quercetin (266nm). The compatibility studies were carried out, in each case, by adopting FT-IR Spectrometry alone as well as in combination with chosen ingredient/polymer(s). The study revealed that all of them were found compatible with the selected ingredients/polymer(s).

The nanoparticles produced with the drug i.e., Quercetin were evaluated for Percentage yield, Entrapment efficiency, determination of Particle size & Zeta potential, Surface morphology and *In-vitro* drug release.

Such an evaluation of produced nanoparticles revealed Percentage yield (58.55-86.33), Encapsulation entrapment (64.59-83.85%), Particle size (279-520nm), Zeta potential (-6.55-15.73mv), and *In-vitro* drug release (71.98-92.13%). Surface morphology showed that nanoparticles were found to be spherical in shape with rough surfaces. A 3^2 full factorial design was used in the present study. In this design, 2 factors were evaluated, at 3 levels (-1,0,1) and experimental trials were performed on all 9 possible combinations. Glyceryl monostearate and soya lecithin were chosen as independent variables and their effect on dependent variable was evaluated. One checkpoint batch was obtained from optimization study which was further formulated and evaluated. The optimized batch was shown Particle size 279nm and Encapsulation efficiency 83.85% respectively. The surface morphology of Optimized batch of Quercetin SLNs was shown that prepared nanoparticles were spherical in shape. In-vitro drug release study conducted on optimized batch of Quercetin SLNs showed that about 92.13% drug was released at the end of 12 hr. The optimized batch was also tested for stability by keeping and sealing it in tightly sealed transparent containers at ambient temperature (25°C ± 2°C and 60 ± 5% RH) for 90 Days. The *in-vitro* drug release study profile on the first day of storage as well as at the end of the 30 days, 60 days, and 90 days storage period was obtained. Results were complied with official requirements. The optimized batch was further transformed into in-situ gel and prepared in-situ gel was also evaluated for various evaluation parameters. Results revealed that the gel was transparent in color having pH from 5.98-6.12 approx. which was similar to pH of lacrimal fluid. In-vitro drug release study showed that about 88.57 % drug was released from the in-situ gel at the end of 720 min. The prepared in-situ gels were found to be clear as no particulate matter was seen in the preparation. All the results were in compliance with the given standards.

BIBLIOGRAPHY

BIBLIOGRAPHY

[1]. Yavuz B, Kompella UB. Ocular drug delivery. InPharmacologic Therapy of Ocular Disease 2016 (pp. 57-93). Springer, Cham.

[2]. Sreeraj M et al. Ophthalmic Drug Delivery Systems 2. New York: Marcel Dekker, Inc., 2003.

[3]. Maurice D, Mishima S. Ocular pharmacokinetics in: Sears ML, ed. Handbook of Experimental Pharmacology: Pharmacology of the Eye. Berlin: Springer, 1986: 19–116.

[4]. Schoenwald RD, Ward RL. Relationship between steroid permeability across excised rabbit cornea and octanol-water partition coefficients. J Pharm Sci 1978; 67: 786–788

[5]. Gaudana R, Ananthula HK, Parenky A, Mitra AK. Ocular drug delivery. The AAPS journal. 2010 Sep;12(3):348-60.

[6]. Land, MF & Fernald, RD, 1992, 'The Evaluation of Eyes', Annual Review of Neuroscience vol. 15, pp. 1-29.

[7]. Bourlais CL, Acar L, Zia H, Sado PA, Needham T, Leverge R. Ophthalmic drug delivery systems-- recent advances. Prog Retin Eye Res. 1998; 17:33–58.10.1016/S1350-9462(97)00002-5

[8]. Csillag, A 2005, 'The organ of vision. In: Atlas of the Sensory Organs: Functional and Clinical Anatomy', New York, NY: Springer-Verlag, LLC; pp. 85-164.

[9]. Cassin, B & Solomon, S 1990, 'Dictionary of eye terminology. Gainesville', Fla: Triad Pub. Co. ISBN 978-0-937404-33-1.

[10]. Patel, A, Cholkar, K, Agrahari, V & Mitra, AK 2013, 'Ocular drug delivery systems: An overview', World Journal of Pharmacology, vol. 2, no. 2, pp. 47-64.

[11]. Weinstein, BI, Kandalaft, N, Ritch, R, Camras, CB, Morris, DJ, Latif, SA, Vecsei, P, Vittek, J, Gordon, GG & Southren, AL 1991, '5 alpha-dihydrocortisol in human aqueous humour and metabolism of cortisol by human lenses in vitro', Investigative Ophthalmology & Visual Science. vol. 32, no. 7, pp. 2130-35.

[12]. Craig JP, Nichols KK, Akpek EK, Caffery B, Dua HS, Joo CK, et al. TFOS DEWS II definition and classification report. Ocul Surf. 2017;15(3):276–83.

[13]. Messmer EM. The pathophysiology, diagnosis, and treatment of dry eye disease. Dtsch Arztebl Int. 2015;112(5):71–82.

[14]. Thulasi P, Djalilian AR. Update in current diagnostics and therapeutics of dry eye disease. Ophthalmology [Internet]. 2017;124(11): S27–33.

[15]. Novack GD, Asbell P, Barabino S, Bergamini MVW, Ciolino JB, Foulks GN, Goldstein M, Lemp MA, Schrader S, Woods C, Stapleton F TFOS DEWS II clinical trial design report. Ocul Surf [Internet] 2017;15(3):629–649.

[16]. Kuo Y, Lin I, Chien L, Lin T, How Y. Dry eye disease: a review of epidemiology in Taiwan, and its clinical treatment and merits. J Clin Med Rev. 2019;8(1227):1–25.

[17]. Clayton JA. Dry eye. N Engl J Med. 2018;378(23):2212–23.

[18]. van Oterendorp C, Sgouris S, Bach M, Martin G, Biermann J, Jordan J, et al. Quantification of retrograde axonal transport in the rat optic nerve by Fluorogold spectrometry. PLoS One. 2012;7: e38820.

[19]. Weinreb R, Khaw P. Primary open-angle glaucoma. Lancet. 2004;363(9422):1711–20.

[20]. Zhang L, Zhang YQ, Xu L, Yang H, Wu XS. Is normal-tension glaucoma different from primary open-angle glaucoma. Chinese J Ophthalmol. 2011;47(2):105–8.

[21]. Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma: a review. JAMA - J Am Med Assoc. 2014;311(18):1901–11

[22]. Lusthaus J, Goldberg I. Current management of glaucoma. Med J Aust. 2019;210(4):180–7.

[23]. Liu YC, Wilkins M, Kim T, Malyugin B, Mehta JS. Cataracts. Lancet. 2017;390(10094):600–12.

[24]. Lim JC, Morton JD, Bunt CR. Drug delivery to the lens for the management of cataracts. Adv Drug Deliv Rev [Internet]. 2018; 126:185–194.

[25]. Mitchell P, Liew G, Gopinath B, Wong TY. Age-related macular degeneration. Lancet [Internet] 2018;392(10153):1147–1159

[26]. Wang S, Wang X, Cheng Y, Ouyang W, Sang X, Liu J, et al. Autophagy dysfunction, cellular senescence, and abnormal immune-inflammatory

responses in AMD: from mechanisms to therapeutic potential. Oxidative Med Cell Longev. 2019;2019

[27]. Stitt AW, Curtis TM, Chen M, Medina RJ, McKay GJ, Jenkins A, et al. The progress in understanding and treatment of diabetic retinopathy. Prog Retin Eye Res [Internet] 2016; 51:156–186.

[28]. Wang W, Lo ACY. Diabetic retinopathy: pathophysiology and treatments. Int J Mol Sci. 2018;19(6).

[29]. Whitehead M, Wickremasinghe S, Osborne A, Van Wijngaarden P, Martin KR. Diabetic retinopathy: a complex pathophysiology requiring novel therapeutic strategies. Expert Opin Biol Ther [Internet]. 2018;18(12):1257–1270

[30]. E. Donnenfeld, Current use of non-steroidal anti-inflammatory drugs in the treatment of ocular inflammation related to cataract surgery, Eur. Ophthalmic 48 Rev. (2012) 173–177.

[31]. E.B. Rodrigues, M.E. Farah, J.M. Bottós, F.B. Aggio, Nonsteroidal anti-inflammatory drugs in the treatment of retinal diseases, in: Retinal Pharmacotherapeutics, Karger Publishers, 2016, pp. 212-220.

[32]. T. Yorio, A. Clark, M.B. Wax, Ocular therapeutics: eye on new discoveries, academic press, 2011.

[33]. K. Adibkia, M.R. Siahi Shadbad, A. Nokhodchi, A. Javadzedeh, M. Barzegar-Jalali, J. Barar, G. Mohammadi, Y. Omidi, Piroxicam nanoparticles for ocular delivery: physicochemical characterization and implementation in endotoxin-induced uveitis, J Drug Target, 15 (2007) 407-416.

[34]. T. Yasukawa, Y. Ogura, Y. Tabata, H. Kimura, P. Wiedemann, and Y. Honda. Drug delivery systems for vitreoretinal diseases. Prog. Retin. Eye Res. 23:253–281 (2004) doi:10.1016/j. preteyeres.2004.02.003.

[35]. S. K. Sahoo, F. Dilnawaz, S. Krishnakumar. Nanotechnology in ocular drug delivery. Drug Discov Today. 13:144–151 (2008).

[36]. Lalu L, Tambe V, Pradhan D, Nayak K, Bagchi S, Maheshwari R, Kalia K, Tekade RK. Novel nanosystems for the treatment of ocular inflammation: Current paradigms and future research directions. Journal of Controlled Release. 2017 Dec 28; 268:19-39.

[37]. M.A. Kamaleddin, Nano-ophthalmology: Applications and considerations, Nanomedicine: Nanotechnology, Biology and Medicine, (2017).

[38]. Lalu L, Tambe V, Pradhan D, Nayak K, Bagchi S, Maheshwari R, Kalia K, Tekade RK. Novel nanosystems for the treatment of ocular inflammation: Current paradigms and future research directions. Journal of Controlled Release. 2017 Dec 28;268:19-39.

[39]. Paliwal R, Rai S, Vaidya B, Khatri K, Goyal AK, Mishra N, Mehta A, Vyas SP. Effect of lipid core material on characteristics of solid lipid nanoparticles designed for oral lymphatic delivery. Nanomedicine: Nanotechnology, Biology and Medicine. 2009 Jun 1;5(2):184-91.

[40]. Cortesi R, Esposito E, Luca G, Nastruzzi C. Production of lipospheres as carriers for bioactive compounds. Biomaterials. 2002 Jun 1;23(11):2283-94.

[41]. Scheffel U, Rhodes BA, Natarajan TK, Wagner HN. Albumin microspheres for study of the reticuloendothelial system. Journal of Nuclear Medicine. 1972 Jul 1;13(7):498-503.

[42]. Hanumanaik M, Patel SK, Sree KR. Solid lipid nanoparticles: a review. Int J Pharm Sci Res. 2013;4(3):928-40.

[43]. Silva AC, González-Mira E, García ML, Egea MA, Fonseca J, Silva R, Santos D, Souto EB, Ferreira D. Preparation, characterization and biocompatibility studies on risperidone-loaded solid lipid nanoparticles (SLN): high pressure homogenization versus ultrasound. Colloids and Surfaces B: Biointerfaces. 2011 Aug 1;86(1):158-65.

[44]. Manjunath K, Reddy JS, Venkateswarlu V. Solid lipid nanoparticles as drug delivery systems. Methods Find Exp Clin Pharmacol. 2005 Mar 1;27(2):127-44.

[45]. Sathali AH, Ekambaram P, Priyanka K. Solid lipid nanoparticles: a review. Sci Rev Chem Commun. 2012;2(1):80-102.

[46]. Das S, Chaudhury A. Recent advances in lipid nanoparticle formulations with solid matrix for oral drug delivery. Aaps Pharmscitech. 2011 Mar;12(1):62-76.

[47]. Parhi R, Suresh P. Preparation and characterization of solid lipid nanoparticles-a review. Current drug discovery technologies. 2012 Mar 1;9(1):2-16.

[48]. Souto EB, Wissing SA, Barbosa CM, Müller RH. Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. International journal of pharmaceutics. 2004 Jun 18;278(1):71-7.

[49]. Pardeike J, Hommoss A, Müller RH. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. International journal of pharmaceutics. 2009 Jan 21;366(1-2):170-84.

[50]. Naguib YW, Rodriguez BL, Li X, Hursting SD, Williams III RO, Cui Z. Solid lipid nanoparticle formulations of docetaxel prepared with high melting point triglycerides: in vitro and in vivo evaluation. Molecular pharmaceutics. 2014 Apr 7;11(4):1239-49.

[51]. Gasco MR, inventor; Gasco Maria R, assignee. Method for producing solid lipid microspheres having a narrow size distribution. United States patent US 5,250,236. 1993 Oct 5.

[52]. Sathali AH, Ekambaram P, Priyanka K. Solid lipid nanoparticles: a review. Sci Rev Chem Commun. 2012;2(1):80-102.

[53]. Venkateswarlu V, Manjunath K. Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. Journal of controlled release. 2004 Mar 24;95(3):627-38.

[54]. Sathali AH, Ekambaram P, Priyanka K. Solid lipid nanoparticles: a review. Sci Rev Chem Commun. 2012;2(1):80-102.

[55]. Mukherjee S, Ray S, Thakur RS. Solid lipid nanoparticles: a modern formulation approach in drug delivery system. Indian journal of pharmaceutical sciences. 2009 Jul;71(4):349.

[56]. Parhi R, Suresh P. Preparation and characterization of solid lipid nanoparticles-a review. Current drug discovery technologies. 2012 Mar 1;9(1):2-16.

[57]. Souto EB, Wissing SA, Barbosa CM, Müller RH. Development of a controlled release formulation based on SLN and NLC for topical clotrimazole delivery. International journal of pharmaceutics. 2004 Jun 18;278(1):71-7.

[58]. Pardeike J, Hommoss A, Müller RH. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. International journal of pharmaceutics. 2009 Jan 21;366(1-2):170-84.

[59]. Naguib YW, Rodriguez BL, Li X, Hursting SD, Williams III RO, Cui Z. Solid lipid nanoparticle formulations of docetaxel prepared with high melting point triglycerides: in vitro and in vivo evaluation. Molecular pharmaceutics. 2014 Apr 7;11(4):1239-49.

[60]. Gasco MR, inventor; Gasco Maria R, assignee. Method for producing solid lipid microspheres having a narrow size distribution. United States patent US 5,250,236. 1993 Oct 5.

[61]. Sathali AH, Ekambaram P, Priyanka K. Solid lipid nanoparticles: a review. Sci Rev Chem Commun. 2012;2(1):80-102.

[62]. Venkateswarlu V, Manjunath K. Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. Journal of controlled release. 2004 Mar 24;95(3):627-38.

[63]. Sathali AH, Ekambaram P, Priyanka K. Solid lipid nanoparticles: a review. Sci Rev Chem Commun. 2012;2(1):80-102.

[64]. Hu FQ, Yuan H, Zhang HH, Fang M. Preparation of solid lipid nanoparticles with clobetasol propionate by a novel solvent diffusion method in aqueous system and physicochemical characterization. International journal of pharmaceutics. 2002 Jun 4;239(1-2):121-8.

[65]. Trotta M, Debernardi F, Caputo O. Preparation of solid lipid nanoparticles by a solvent emulsification–diffusion technique. International journal of pharmaceutics. 2003 May 12;257(1-2):153-60.

[66]. Yuan H, Huang LF, Du YZ, Ying XY, You J, Hu FQ, Zeng S. Solid lipid nanoparticles prepared by solvent diffusion method in a nanoreactor system. Colloids and Surfaces B: Biointerfaces. 2008 Feb 15;61(2):132-7.

[67]. Kaiser CS, Rompp H. schmidt PC. Pharmaceutical application of supercritical carbon dioxide. pharmazie. 2001; 56:907-26.

[68]. Svilenov H, Tzachev C. Solid lipid nanoparticles—a promising drug delivery system. Nanomedicine. 2014; 2:187-237.

[69]. Charcosset C, El-Harati A, Fessi H. Preparation of solid lipid nanoparticles using a membrane contactor. Journal of controlled release. 2005 Nov 2;108(1):112-20.

[70]. Manjunath K, Reddy JS, Venkateswarlu V. Solid lipid nanoparticles as drug delivery systems. Methods Find Exp Clin Pharmacol. 2005 Mar 1;27(2):127-44.

[71]. Schubert MA, Müller-Goymann CC. Solvent injection as a new approach for manufacturing lipid nanoparticles–evaluation of the method and process parameters. European journal of pharmaceutics and biopharmaceutics. 2003 Jan 1;55(1):125-31.

[72]. Manjunath K, Reddy JS, Venkateswarlu V. Solid lipid nanoparticles as drug delivery systems. Methods Find Exp Clin Pharmacol. 2005 Mar 1;27(2):127-44.

[73]. Luo Y, Chen D, Ren L, Zhao X, Qin J. Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability. Journal of controlled release. 2006 Aug 10;114(1):53-9.

[74]. Cortesi R, Esposito E, Luca G, Nastruzzi C. Production of lipospheres as carriers for bioactive compounds. Biomaterials. 2002 Jun 1;23(11):2283-94.

[75]. Garcıa-Fuentes M, Torres D, Alonso MJ. Design of lipid nanoparticles for the oral delivery of hydrophilic macromolecules. Colloids and Surfaces B: Biointerfaces. 2003 Feb 1;27(2-3):159-68.

[76]. Utada AS, Lorenceau E, Link DR, Kaplan PD, Stone HA, Weitz DA. Monodisperse double emulsions generated from a microcapillary device. Science. 2005 Apr 22;308(5721):537-41.

[77]. Liu J, Gong T, Wang C, Zhong Z, Zhang Z. Solid lipid nanoparticles loaded with insulin by sodium cholate-phosphatidylcholine-based mixed micelles: preparation and characterization. International journal of pharmaceutics. 2007 Aug 1;340(1-2):153-62.

[78]. Liu J, Gong T, Fu H, Wang C, Wang X, Chen Q, Zhang Q, He Q, Zhang Z. Solid lipid nanoparticles for pulmonary delivery of insulin. International journal of pharmaceutics. 2008 May 22;356(1-2):333-44.

[79]. Reithmeier H, Herrmann J, Göpferich A. Development and characterization of lipid microparticles as a drug carrier for somatostatin. International journal of pharmaceutics. 2001 May 7;218(1-2):133-43.

[80]. Mehnert W, Mäder K. Solid lipid nanoparticles: production, characterization and applications. Advanced drug delivery reviews. 2012 Dec 1; 64:83-101.

[81]. Gasco MR, inventor; Gasco Maria R, assignee. Method for producing solid lipid microspheres having a narrow size distribution. United States patent US 5,250,236. 1993 Oct 5.

[82]. Zhang S, Yun J, Shen S, Chen Z, Yao K, Chen J, Chen B. Formation of solid lipid nanoparticles in a microchannel system with a cross-shaped junction. Chemical Engineering Science. 2008 Dec 1;63(23):5600-5.

[83]. Zhang SH, Shen SC, Chen Z, Yun JX, Yao KJ, Chen BB, Chen JZ. Preparation of solid lipid nanoparticles in co-flowing microchannels. Chemical Engineering Journal. 2008 Oct 15;144(2):324-8.

[84]. Zhang S, Yun J, Shen S, Chen Z, Yao K, Chen J, Chen B. Formation of solid lipid nanoparticles in a microchannel system with a cross-shaped junction. Chemical Engineering Science. 2008 Dec 1;63(23):5600-5.

[85]. Manjunath K, Reddy JS, Venkateswarlu V. Solid lipid nanoparticles as drug delivery systems. Methods Find Exp Clin Pharmacol. 2005 Mar 1;27(2):127-44.

[86]. Müller RH, Mehnert W, Lucks JS, Schwarz C, Zur Mühlen A, Meyhers H. Solid lipid nanoparticles (SLN): an alternative colloidal carrier system for controlled drug delivery. European journal of pharmaceutics and biopharmaceutics. 1995;41(1):62-9.

[87]. Freitas C, Müller RH. Spray-drying of solid lipid nanoparticles (SLNTM). European Journal of Pharmaceutics and Biopharmaceutics. 1998 Sep 1;46(2):145-51.

[88]. Sharma G, Ballauff M. Cationic spherical polyelectrolyte brushes as nanoreactors for the generation of gold particles. Macromolecular rapid communications. 2004 Feb;25(4):547-52.

[89]. Mehnert W, Mäder K. Solid lipid nanoparticles: production, characterization and applications. Advanced drug delivery reviews. 2012 Dec 1; 64:83-101.

[90]. Ohshima H, Miyagishima A, Kurita T, Makino Y, Iwao Y, Sonobe T, Itai S. Freeze-dried nifedipine-lipid nanoparticles with long-term nano-dispersion stability after reconstitution. International journal of pharmaceutics. 2009 Jul 30;377(1-2):180-4.

[91]. Subedi RK, Kang KW, Choi HK. Preparation and characterization of solid lipid nanoparticles loaded with doxorubicin. European journal of pharmaceutical sciences. 2009 Jun 28;37(3-4):508-13.

[92]. Dong X, Mattingly CA, Tseng M, Cho M, Adams VR, Mumper RJ. Development of new lipid-based paclitaxel nanoparticles using sequential simplex

optimization. European journal of pharmaceutics and biopharmaceutics. 2009 May 1;72(1):9-17.

[93]. Sinha VR, Srivastava S, Goel H, Jindal V. Solid lipid nanoparticles (SLN'S)-trends and implications in drug targeting. International Journal of Advances in Pharmaceutical Sciences. 2010 Jul 1;1(3).

[94]. Mehnert W, Mäder K. Solid lipid nanoparticles: production, characterization and applications. Advanced drug delivery reviews. 2012 Dec 1; 64:83-101.

[95]. Patil RN,Kumar RS. In-situ gelling system: Novel approach for ophthalmic drug delivery. World Journal of Pharmacy and Pharmaceutical Sciences 2014; 3(7):423- 440.

[96]. Patil Rajeshwari N, Kumar Rachana S. In-situ gelling system: Novel approach for ophthalmic drug delivery.World Journal of Pharmacy and Pharmaceutical Sciences 2014; 3(7):423-440.

[97]. Parekh HB, Rishad J, Jivani NP, Patel LD, Makwana A, Sameja K. Novel In situ polymeric drug delivery system: A Review. J. Drug Deliv. & Therap. 2012; 2(5):136-145.

[98]. Nirmal HB, Bakliwal SR, Pawar SP. In Situ Gel: New trends in controlled and sustained drug delivery system. Int. J. Pharm.Tech. Res. 2010; 2(2):1398-1408.

[99]. Nerkar TS, Gujarathi NA, Bhushan R, Bakliwal SR, Pawar SP. In situ Gel: Novel Approach in Sustained and Controlled DrugDelivery System, Pharma. Sci. monitor 2013; 4(4):1-18.

[100]. Wei G ,Xu H ,Ding PT ,Li SM ,Zheng JM . Thermosetting gels with modulated gelation temperature for ophthalmic use: the rheological and gamma scintigraphic studies. J Control Release 2002;83(1):65–74.

[101]. Patel N ,Nakrani H ,Raval M ,Navin S . Development of loteprednol etabonate-loaded cationic nanoemulsified in-situ ophthalmic gel for sustained delivery and enhanced ocular bioavailability. Drug Deliv 2016;23(9):3712–23.

[102]. Klouda L . Thermoresponsive hydrogels in biomedical applications: a seven-year update. Eur J Pharm Biopharm 2015;97(Pt B):338–49.

[103]. 28. Ma WD, Xu H, Wang C, Nie SF, Pan WS. Pluronic F127-g-poly (acrylic acid) copolymers as in situ gelling vehicle for ophthalmic drug delivery system. Int J Pharm. 2008; 350(1-2):247-256.

[104]. Kumar D, Jain N, Gulati N, Nagaich U. Nanoparticles laden in situ gelling system for ocular drug targeting. J Adv Pharm Technol Res. 2013; 4(1):9-17.

[105]. Almeida H, Amaral MH ,Lobao P . Temperature and pH stimuli-responsive polymers and their applications in controlled and selfregulated drug delivery. J App Pharm Sci 2012;2(6):1–10.

[106]. Majeed A, Khan NA. Ocular in situ gel: An overview. Journal of Drug Delivery and Therapeutics. 2019 Jan 15;9(1):337-47.

[107]. Casanova AG, Prieto M, Colino CI, Gutiérrez-Millán C, Ruszkowska-Ciastek B, de Paz E, Martín Á, Morales AI, López-Hernández FJ. A micellar formulation of quercetin prevents cisplatin nephrotoxicity. International Journal of Molecular Sciences. 2021 Jan 13;22(2):729.

[108]. Raga-Cervera J, Bolarin JM, Millan JM, Garcia-Medina JJ, Pedrola L, Abellán-Abenza J, Valero-Vello M, Sanz-González SM, O'Connor JE, Galarreta-Mira D, Bendala-Tufanisco E. miRNAs and genes involved in the interplay between ocular hypertension and primary open-angle glaucoma. Oxidative stress, inflammation, and apoptosis networks. Journal of Clinical Medicine. 2021 May 21;10(11):2227.

[109]. Katsinas N, Rodríguez-Rojo S, Enríquez-de-Salamanca A. Olive pomace phenolic compounds and extracts can inhibit inflammatory-and oxidative-related diseases of human ocular surface epithelium. Antioxidants. 2021 Jul 20;10(7):1150.

[110]. Maharjan P, Jin M, Kim D, Yang J, Maharjan A, Shin MC, Cho KH, Kim MS, Min KA. Evaluation of epithelial transport and oxidative stress protection of nanoengineered curcumin derivative-cyclodextrin formulation for ocular delivery. Archives of pharmacal research. 2019 Oct; 42:909-25.

[111]. Manca ML, Lai F, Pireddu R, Valenti D, Schlich M, Pini E, Ailuno G, Fadda AM, Sinico C. Impact of nanosizing on dermal delivery and antioxidant activity of quercetin nanocrystals. Journal of drug delivery science and technology. 2020 Feb 1; 55:101482.

[112]. Di Pede G, Bresciani L, Calani L, Petrangolini G, Riva A, Allegrini P, Del Rio D, Mena P. The human microbial metabolism of quercetin in different formulations: An in vitro evaluation. Foods. 2020 Aug 14;9(8):1121.

[113]. Li YL, Guo H, Zhao YQ, Li AF, Ren YQ, Zhang JW. Quercetin protects neuronal cells from oxidative stress and cognitive degradation induced by amyloid β-peptide treatment. Molecular Medicine Reports. 2017 Aug 1;16(2):1573-7.

[114]. Lesjak M, Beara I, Simin N, Pintać D, Majkić T, Bekvalac K, Orčić D, Mimica-Dukić N. Antioxidant and anti-inflammatory activities of quercetin and its derivatives. Journal of Functional Foods. 2018 Jan 1; 40:68-75.

[115]. Graf BA, Ameho C, Dolnikowski GG, Milbury PE, Chen CY, Blumberg JB. Rat gastrointestinal tissues metabolize quercetin. The Journal of nutrition. 2006 Jan 1;136(1):39-44.

[116]. Edwards RL, Lyon T, Litwin SE, Rabovsky A, Symons JD, Jalili T. Quercetin reduces blood pressure in hypertensive subjects. The Journal of nutrition. 2007 Nov 1;137(11):2405-11.

[117]. Jaisinghani RN. Antibacterial properties of quercetin. Microbiology research. 2017 May 2;8(1):6877.

[118]. Huang C, Fu C, Qi ZP, Guo WL, You D, Li R, Zhu Z. Localised delivery of quercetin by thermo-sensitive PLGA-PEG-PLGA hydrogels for the treatment of brachial plexus avulsion. Artificial Cells, Nanomedicine, and Biotechnology. 2020 Jan 1;48(1):1010-21.

[119]. Tripathi N, Verma S, Vyas M, Yadav NS, Gain S, Khatik GL. Nano formulations of quercetin: a potential phytochemical for the treatment of UV radiation induced skin damages. Brazilian Journal of Pharmaceutical Sciences. 2022 Apr 22;58.

[120]. Terreni E, Zucchetti E, Tampucci S, Burgalassi S, Monti D, Chetoni P. Combination of nanomicellar technology and in situ gelling polymer as ocular drug delivery system (ODDS) for cyclosporine-A. Pharmaceutics. 2021 Feb 1;13(2):192.

[121]. Badr MY, Abdulrahman NS, Schatzlein AG, Uchegbu IF. A polymeric aqueous tacrolimus formulation for topical ocular delivery. International Journal of Pharmaceutics. 2021 Apr 15; 599:120364.

[122]. Badr MY, Abdulrahman NS, Schatzlein AG, Uchegbu IF. A polymeric aqueous tacrolimus formulation for topical ocular delivery. International Journal of Pharmaceutics. 2021 Apr 15; 599:120364.

[123]. Suriyaamporn P, Opanasopit P, Ngawhirunpat T, Rangsimawong W. Computer-aided rational design for optimally Gantrez® S-97 and hyaluronic acid-based dissolving microneedles as a potential ocular delivery system. Journal of Drug Delivery Science and Technology. 2021 Feb 1; 61:102319.

[124]. Xu G, Li B, Wang T, Wan J, Zhang Y, Huang J, Shen Y. Enhancing the anti-ovarian cancer activity of quercetin using a self-assembling micelle and thermosensitive hydrogel drug delivery system. RSC advances. 2018;8(38):21229-42.

[125]. Puglia C, Santonocito D, Ostacolo C, Maria Sommella E, Campiglia P, Carbone C, Drago F, Pignatello R, Bucolo C. Ocular formulation based on palmitoylethanolamide-loaded nanostructured lipid carriers: Technological and pharmacological profile. Nanomaterials. 2020 Feb 8;10(2):287.

[126]. Ghosh S, Sarkar S, Choudhury ST, Ghosh T, Das N. Triphenyl phosphonium coated nano-quercetin for oral delivery: neuroprotective effects in attenuating age related global moderate cerebral ischemia reperfusion injury in rats. Nanomedicine: Nanotechnology, Biology and Medicine. 2017 Nov 1;13(8):2439-50.

[127]. Sousa-Batista AJ, Poletto FS, Philipon CI, Guterres SS, Pohlmann AR, Rossi-Bergmann B. Lipid-core nanocapsules increase the oral efficacy of quercetin in cutaneous leishmaniasis. Parasitology. 2017 Nov;144(13):1769-74.

[128]. Wong MY, Chiu GN. Liposome formulation of co-encapsulated vincristine and quercetin enhanced antitumor activity in a trastuzumab-insensitive breast tumor xenograft model. Nanomedicine: Nanotechnology, Biology and Medicine. 2011 Dec 1;7(6):834-40.

[129]. Gao L, Liu G, Wang X, Liu F, Xu Y, Ma J. Preparation of a chemically stable quercetin formulation using nanosuspension technology. International Journal of Pharmaceutics. 2011 Feb 14;404(1-2):231-7.

[130]. Jain AS, Shah SM, Nagarsenker MS, Nikam Y, Gude RP, Steiniger F, Thamm J, Fahr A. Lipid colloidal carriers for improvement of anticancer activity of

orally delivered quercetin: formulation, characterization and establishing in vitro–in vivo advantage. Journal of biomedical nanotechnology. 2013 Jul 1;9(7):1230-40.

[131]. Jain AK, Thanki K, Jain S. Novel self-nanoemulsifying formulation of quercetin: Implications of pro-oxidant activity on the anticancer efficacy. Nanomedicine: Nanotechnology, Biology and Medicine. 2014 Jul 1;10(5): e959-69.

[132]. Xu G, Shi H, Ren L, Gou H, Gong D, Gao X, Huang N. Enhancing the anti-colon cancer activity of quercetin by self-assembled micelles. International Journal of Nanomedicine. 2015; 10:2051.

[133]. Antçnio E, Khalil NM, Mainardes RM. Bovine serum albumin nanoparticles containing quercetin: characterization and antioxidant activity. Journal of nanoscience and nanotechnology. 2016 Feb 1;16(2):1346-53.

[134]. Li K, Pi C, Wen J, He Y, Yuan J, Shen H, Zhao W, Zeng M, Song X, Lee RJ, Wei Y. Formulation of the novel structure curcumin derivative–loaded solid lipid nanoparticles: synthesis, optimization, characterization and anti-tumor activity screening in vitro. Drug Delivery. 2022 Dec 31;29(1):2044-57.

[135]. Rarokar NR, Menghani SS, Kerzare DR, Khedekar PB, Bharne AP, Alamri AS, Alsanie WF, Alhomrani M, Sreeharsha N, Asdaq SM. Preparation of Terbinafin-Encapsulated Solid Lipid Nanoparticles Containing Antifungal Carbopol® Hydrogel with Improved Efficacy: In Vitro, Ex Vivo and In Vivo Study. Pharmaceutics. 2022 Jun 30;14(7):1393.

[136]. Alajami HN, Fouad EA, Ashour AE, Kumar A, Yassin AE. Celecoxib-loaded solid lipid nanoparticles for colon delivery: formulation optimization and in vitro assessment of anti-cancer activity. Pharmaceutics. 2022 Jan 5;14(1):131.

[137]. Khan AS, Shah KU, Mohaini MA, Alsalman AJ, Hawaj MA, Alhashem YN, Ghazanfar S, Khan KA, Niazi ZR, Farid A. Tacrolimus-loaded solid lipid nanoparticle gel: Formulation development and in vitro assessment for topical applications. Gels. 2022 Feb 18;8(2):129.

[138]. Mirra D, Spaziano G, Esposito R, Santonocito D, Filosa R, Roviezzo F, Malgieri G, D'Abrosca G, Iovino P, Gallelli L, Fattorusso R. Formulation of Solid Lipid Nanoparticles Loaded with Nociceptin/Orphanin FQ (N/OFQ) and

Characterization in a Murine Model of Airway Hyperresponsiveness. Pharmaceuticals. 2022 Sep 29;15(10):1210.

[139]. Nair R, Kumar AC, Priya VK, Yadav CM, Raju PY. Formulation and evaluation of chitosan solid lipid nanoparticles of carbamazepine. Lipids in health and disease. 2012 Dec;11(1):1-8.

[140]. Kesharwani R, Sachan A, Singh S, Patel D. Formulation and evaluation of solid lipid nanoparticle (SLN) based topical gel of etoricoxib. Journal of Applied Pharmaceutical Science. 2016 Oct 29;6(10):124-31.

[141]. Kushwaha AK, Vuddanda PR, Karunanidhi P, Singh SK, Singh S. Development and evaluation of solid lipid nanoparticles of raloxifene hydrochloride for enhanced bioavailability. BioMed research international. 2013 Oct 20;2013.

[142]. Kalam MA, Sultana Y, Ali A, Aqil M, Mishra AK, Chuttani K. Preparation, characterization, and evaluation of gatifloxacin loaded solid lipid nanoparticles as colloidal ocular drug delivery system. Journal of drug targeting. 2010 Apr 1;18(3):191-204.

[143]. Mohanty B, Majumdar DK, Mishra SK, Panda AK, Patnaik S. Development and characterization of itraconazole-loaded solid lipid nanoparticles for ocular delivery. Pharmaceutical development and technology. 2015 May 19;20(4):458-64.

[144]. Spencer JP, Vauzour D, Rendeiro C. Flavonoids and cognition: the molecular mechanisms underlying their behavioural effects. Archives of biochemistry and biophysics. 2009 Dec 1;492(1-2):1-9.

[145]. David AV, Arulmoli R, Parasuraman S. Overviews of biological importance of quercetin: A bioactive flavonoid. Pharmacognosy reviews. 2016 Jul;10(20):84.

[146]. Sytar O, Kosyan A, Taran N, Smetanska I. Anthocyanin's as marker for selection of buckwheat plants with high rutin content. Gesunde Pflanzen. 2014 Dec;66(4):165-9.

[147]. Miles SL, McFarland M, Niles RM. Molecular and physiological actions of quercetin: need for clinical trials to assess its benefits in human disease. Nutrition reviews. 2014 Nov 1;72(11):720-34.

[148]. Guo Y, Bruno RS. Endogenous and exogenous mediators of quercetin bioavailability. The Journal of nutritional biochemistry. 2015 Mar 1;26(3):201-10.

[149]. Russo M, Spagnuolo C, Tedesco I, Bilotto S, Russo GL. The flavonoid quercetin in disease prevention and therapy: facts and fancies. Biochemical pharmacology. 2012 Jan 1;83(1):6-15.

[150]. Sytar O, Kosyan A, Taran N, Smetanska I. Anthocyanin's as marker for selection of buckwheat plants with high rutin content. Gesunde Pflanzen. 2014 Dec;66(4):165-9.

[151]. Sultana B, Anwar F. Flavonols (kaempeferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants. Food chemistry. 2008 Jun 1;108(3):879-84.

[152]. Pérez-Jiménez J, Neveu V, Vos F, Scalbert A. Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database. European journal of clinical nutrition. 2010 Nov;64(3): S112-20.

[153]. Dinelli G, Bonetti A, Minelli M, Marotti I, Catizone P, Mazzanti A. Content of flavonols in Italian bean (Phaseolus vulgaris L.) ecotypes. Food chemistry. 2006 Jan 1;99(1):105-14.

[154]. Sytar O, Bruckova K, Hunkova E, Zivcak M, Konate K, Brestic M. The application of multiplex fluorimetric sensor for the analysis of flavonoids content in the medicinal herb's family Asteraceae, Lamiaceae, Rosaceae. Biological research. 2015 Dec;48(1):1-9.

[155]. Sokół-Łętowska A, Oszmiański J, Wojdyło A. Antioxidant activity of the phenolic compounds of hawthorn, pine and skullcap. Food chemistry. 2007 Jan 1;103(3):853-9.

[156]. Haenen GR, Paquay JB, Korthouwer RE, Bast A. Peroxynitrite scavenging by flavonoids. Biochemical and biophysical research communications. 1997 Jul 30;236(3):591-3.

[157]. Kaşıkcı MB, Bağdatlıoğlu N. Bioavailability of quercetin. Current research in nutrition and food science journal. 2016 Oct 25;4(Special Issue Nutrition in Conference October 2016):146-51.

[158]. Kaşıkcı MB, Bağdatlıoğlu N. Bioavailability of quercetin. Current research in nutrition and food science journal. 2016 Oct 25;4(Special Issue Nutrition in Conference October 2016):146-51.

[159]. Yu CC, Lee YS, Cheon BS, Lee SH. Synthesis of glycerol monostearate with high purity. Bulletin-Korean Chemical Society. 2003 Aug 20;24(8):1229-31.

[160]. Mehmood T, Ahmed A, Ahmed Z, Ahmad MS. Optimization of soya lecithin and Tween 80 based novel vitamin D nanoemulsions prepared by ultrasonication using response surface methodology. Food chemistry. 2019 Aug 15; 289:664-70.

[161]. Valenta C, Wanka M, Heidlas J. Evaluation of novel soya-lecithin formulations for dermal use containing ketoprofen as a model drug. Journal of controlled release. 2000 Jan 3;63(1-2):165-73.

[162]. Wang X, Luo Z, Xiao Z. Preparation, characterization, and thermal stability of β-cyclodextrin/soybean lecithin inclusion complex. Carbohydrate polymers. 2014 Jan 30; 101:1027-32.

[163]. Fakhari A, Corcoran M, Schwarz A. Thermogelling properties of purified poloxamer 407. Heliyon. 2017 Aug 1;3(8): e00390.

[164]. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Bampidis V, Azimonti G, de Lourdes Bastos M, Christensen H, Dusemund B, Kos Durjava M, Kouba M, López-Alonso M, López Puente S, Marcon F. Safety and efficacy of hydroxypropyl methyl cellulose for all animal species. EFSA Journal. 2020 Jul;18(7): e06214.

[165]. Carbopol and its Pharmaceutical Significance: A Review [Internet].

[166]. Niharika, Verma N. Adhesive polymers in fabrication of transdermal drug delivery. Res J Pharm Technol. 2016 Jul 1;9(7):945–56.

[167]. Indian Pharmacopoeia Commission. Indian Pharmacopoeia 2007, volume I, pp. 241-242

[168]. Patel mk, shah sk, tyagi ck, usman mr. Method development and validation for estimation of quercetin using uv and rp-hplc in bulk and formulation. Plant archives. 2020;20(2):4343-7.

[169]. Chandra S, Khan S, Avula B, Lata H, Yang MH, Elsohly MA, et al. Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of

aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. Evidence-based Complement Altern Med. 2014;2014.

[170]. Manjunath K, Venkateswarlu V. Pharmacokinetics, tissue distribution and bioavailability of clozapine solid lipid nanoparticles after intravenous and intraduodenal administration. Journal of Controlled Release. 2005 Oct 3;107(2):215-28.

[171]. Pandurangan DK, Bodagala P, Palanirajan VK, Govindaraj S. Formulation and evaluation of voriconazole ophthalmic solid lipid nanoparticles in situ gel. International journal of pharmaceutical investigation. 2016 Jan;6(1):56.

[172]. S. Ramesh, R. Ranganayakulu, S. P. Reddy, and E. Tejaswi, "Formulation and in vitro evaluation of nanoparticles containing ciprofloxacin hydrochloride," Journal of Innovative Trends in Pharmaceutical Sciences, vol. 1, no. 2, pp. 79–85, 2010.

[173]. Al-Suwayeh SA, Taha EI, Al-Qahtani FM, Ahmed MO, Badran MM. Evaluation of skin permeation and analgesic activity effects of carbopol lornoxicam topical gels containing penetration enhancer. The Scientific World Journal. 2014 Jan 1;2014.

[174]. Firmansyah F, Vajrika SA, Muhtadi WK. Effect of Combination of Carbopol-940 Base and HPMC Gel Extract of Aloe Vera Flesh on Physical Properties and Antibacterial Activity of Propionibacterium acnes. Malahayati Nursing Journal. 2022 Dec 1;4(12):3347-57.

[175]. Bairagi U, Mittal P, Singh J, Mishra B. Preparation, characterization, and in vivo evaluation of nanoformulations of ferulic acid in diabetic wound healing. Drug Development and Industrial Pharmacy. 2018 Nov 2;44(11):1783-96

[176]. Varshosaz J, Tabbakhian M, Salmani Z. Designing of a thermosensitive chitosan/poloxamer in situ gel for ocular delivery of ciprofloxacin. The Open Drug Delivery Journal. 2008 Aug 21;2(1).

[177]. Singh B, Kaur T, Singh S. Correction of raw dissolution data for loss of drug and volume during sampling. Indian journal of pharmaceutical sciences. 1997;59(4):196.

[178]. Chatterjee A, Bhowmik BB, Thakur YS. Formulation, in vitro and in vivo pharmacokinetics of anti-HIV vaginal bioadhesive gel. Journal of Young Pharmacists. 2011 Apr 1;3(2):83-9.

[179]. Khullar R, Kumar D, Seth N, Saini S. Formulation and evaluation of mefenamic acid emulgel for topical delivery. Saudi pharmaceutical journal. 2012 Jan 1;20(1):63-7.

PUBLICATIONS

PUBLICATIONS

S.NO.	Title	Review/ Research article	Journal Name	Current Status
1.	Serval applications of solid lipid nanoparticles in drug delivery	Review article	Current Molecular Medicine, Bentham Science Publishers **Impact factor: 2.616**	Provisionally accepted
2.	Neoteric role of quercetin in visual disorders	Review article	Current Drug Research Review, Bentham Science Publishers, Scopus	Provisionally accepted
3.	Development and optimization of Quercetin Solid lipid nanoparticles for management of Ocular disorders	Research article		Communicated
4.	Formulation and Characterization of pH sensitive in-situ Gels Containing Quercetin for Ocular Drug Delivery: A Combination of Carbopol®/ HPMC Poly mer	Research article		Communicated

ACHIEVEMENTS

S. No.	Title	Conference / E-Conference / Workshop / Webinar / Training	Place	Date
1.	My Story Motivational Session	Webinar	IIC, Galgotias University, Greater Noida	27 November 2021
2.	Problem Solving and Ideathon	Workshop	IIC, Galgotias University, Greater Noida	29 November 2021
3.	IPR Workshop	Workshop	IIC, Galgotias University, Greater Noida	23rd to 27th October 2021
4.	International conference on covid-19; Recent Development, Challenges and opportunities in pharmaceutical science.	International conference	IIMT, College of Pharmacy	11 December 2021
5.	National Energy Conservation Day	Webinar	IIC, Galgotias University, Greater Noida	14 December 2021
6.	Two days International E-Conference on Recent Advancements in Pharmaceutical and Health Science Domains	International E Conference	SMAS, Galgotias University, Greater Noida	25th to 26th March 2022
7.	National Intellectual property awareness mission (NIPAM)	Training	Galgotias University, Greater Noida	05th April 2022
8.	Pharmacovigilance and pharma Regulatory-current challenges and way forward	Webinar	Accurate college of pharmacy	14th May 2022

9.	World Entrepreneurs' Day-2022	Webinar	Galgotias University, Greater Noida	21 August 2022
10.	Expert talk Series-1 on Innovation and Entrepreneurship	Webinar	IIC (MoE), Galgotias University, Greater Noida	15th October 2022
11.	Workshop on Design Thinking, Critical Thinking and Innovation Thinking	Webinar	IIC, Galgotias University, Greater Noida	28th January 2023
12.	HealthHack 4.0 – Ideathon workshop	Workshop	DPSRU New Delhi	20th February 2023
13.	National Intellectual property awareness mission (NIPAM)	Training	Galgotias University Greater Noida	24th February 2023
14.	Online Workshop on Manufacturing of Phytopharmaceutical Drugs (Herbal Drug Manufacturing)	Workshop	Galgotias University, Greater Noida, IIIM-Technology Business Incubator & Skill Development Initiative of CSIR-IIIM, Jammu	27th March to 29th March 2023
15.	National Conference on Recent Advances in Pharmaceutical Sciences	Conference & poster presentation (Abstract published in Souvenir)	IIMT College of Pharmacy, Greater Noida	27th April 2023

Thesis

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Current Molecular Medicine

Date: 2023-07-17

Attn: Dr. Noor Hassan Sulaiman Khil, Dr. Shaweta Sharma and Dr. Pramod Kumar Sharma

Dear Authors,

Thank you for your contribution entitled "**ENORMOUS APPLICATIONS OF SOLID LIPID NANOPARTICLES IN DRUG DELIVERY**" by *Noor Hassan Sulaiman Khil, Shaweta Sharma and Pramod Kumar Sharma* to the journal **Current Molecular Medicine** being published by Bentham Science.

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Dr. Shaweta Sharma <shaweta.sharma@galgotiasuniversity.edu.in> Fri, Jul 21, 2023 at 9:39 AM
To: Noorhassan Sulaimankhil <hassan.sulaimankhil@gmail.com>

Thanks & Regards,
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Dear Dr. Dr.Shaweta Sharma,

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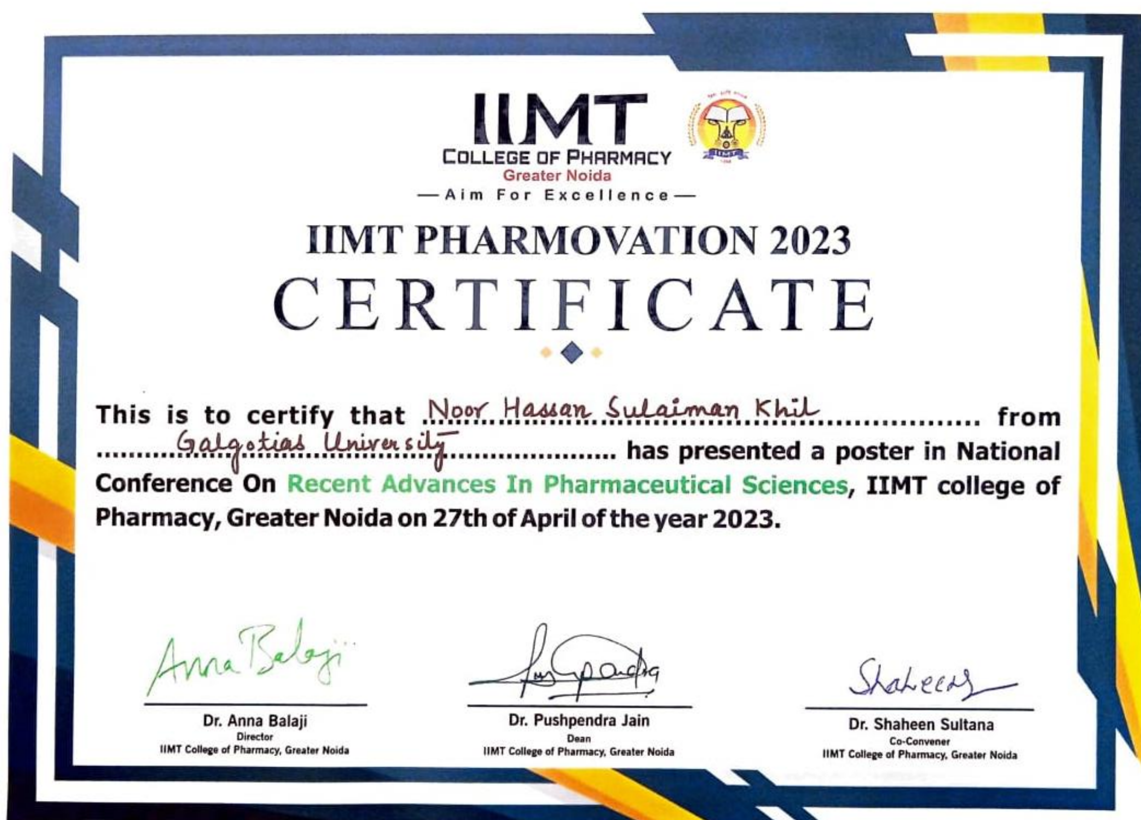
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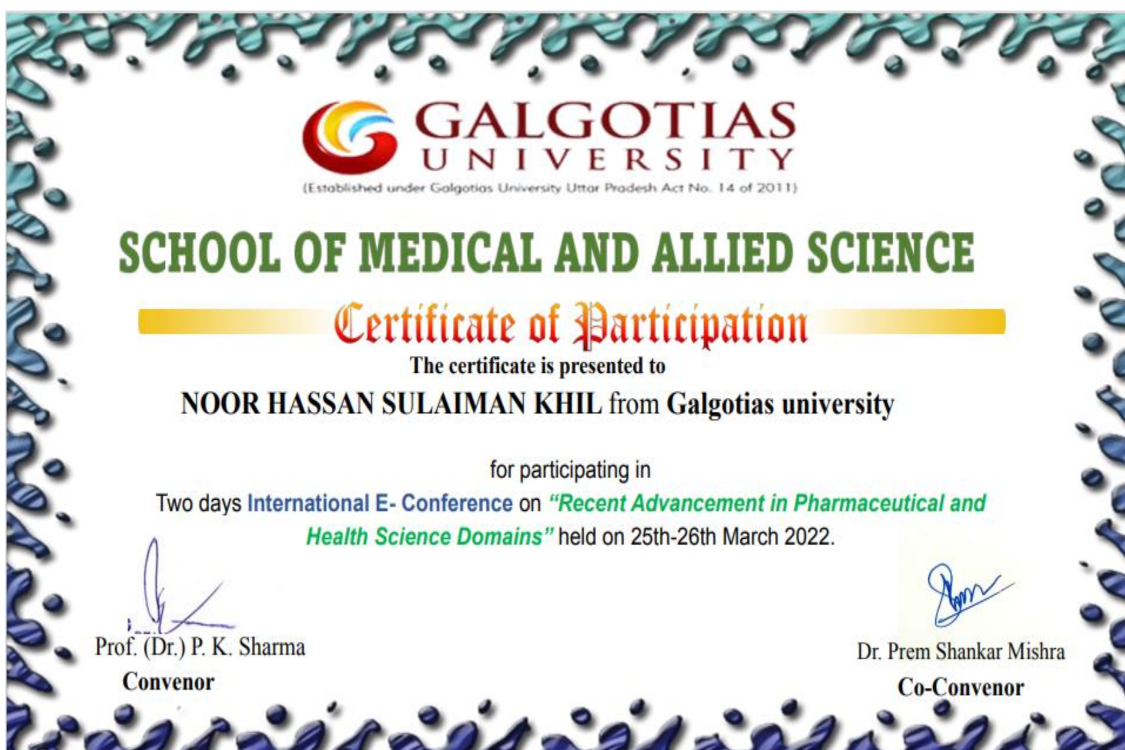
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Er. Ankush Varma
Coordinator, IIIM-Technology Business Incubator, Jammu

Dr. Saurabh Saran
Principal Scientist, CSIR-IIIM
& Principal Investigator, IIIM-TBI

Er. Abdul Rahim
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NOORHASSAN SULAIMANKHIL

has actively participated in the Ministry of Education Innovation Cell (MIC) driven activity "Workshop on Problem Solving and Ideation" organized by Institute Innovation Council (IIC), Galgotias University, Greater Noida, Uttar Pradesh.

NOVEMBER 29, 2021

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IIC In Charge, GU

Ms. Vinny Sharma
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21 August 2022

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IIC In Charge, GU

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15 October, 2022

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PROF. ANAMIKA PANDEY
Dean, SoB, Galgotias University

DR. GAURAV KUMAR
IIC In Charge, GU

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