EVALUATION OF THE ANTIDIABETIC POTENTIAL OF RUTIN AND EPIGALLOCATECHIN GALLATE-LOADED SOLID SELF-NANOEMULSIFYING FORMULATION AGAINST STREPTOZOTOCIN-INDUCED DIABETES IN MOUSE MODEL

Submitted by

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EXAMINATION ROLL NO.: **M4PHL24012**

REGISTRATION NO.: **131611** of **2015-2016**

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Declaration of Originality and Compliance with Academic Ethics

I, **Sagar Roy**, hereby declare that this thesis, submitted in partial fulfilment of the requirements for the degree of Master of Pharmacy, is a product of my original research and analysis. I affirm that all the information, data, and findings presented in this document have been obtained and reported in accordance with the highest standards of academic integrity and ethical conduct.

Furthermore, I confirm that any sources, materials, or results not originally generated by my work have been properly cited and referenced in compliance with academic regulations and ethical guidelines.

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CERTIFICATE OF APPROVAL

This is to certify that ***Sagar Roy***, bearing Exam Roll No: M4PHL24012 Registration No: 131611 of 2015-2016, has conducted the research work entitled ***"Evaluation of the antidiabetic potential of rutin and epigallocatechin gallate-loaded solid self-nanoemulsifying formulation against streptozotocin-induced diabetes in mouse model."***

This research was carried out independently with the necessary diligence and care under the supervision and guidance of ***Prof.(Dr.) Lakshmi Kanta Ghosh*** in the Pharmaceutics Research Laboratory, Department of Pharmaceutical Technology, Jadavpur University.

Sagar Roy has compiled his research findings into this thesis, which is being submitted in partial fulfilment of the requirements for the degree of Master of Pharmacy from Jadavpur University. I appreciate his dedicated efforts, and his work has met my full satisfaction.

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Dedicated to

My Parents, My Brother

& Teachers.

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LIST OF ABBREVIATION

1.	DM	Diabetes mellitus
2.	T1DM	Type1 diabetes mellitus
3.	T2DM	Type2 diabetes mellitus
4	HLA	Human leukocyte antigen
5	GAD	Glutamic acid decarboxylase
6	LADA	Latent autoimmune diabetes of adults
7	GLP 1	Glucagon-like peptide
8	GIP	Glucose dependent insulinotropic polypeptide
9	SGLT2	Sodium glucose transporter 2
10	SNEDDS	Self-nano emulsifying drug delivery system
11	HPMC	Hydroxypropyl methyl cellulose
12	PLGA	Poly lactic glycolic acid
13	BCS	Biological classification system
14	EGCG	Epigallocatechin Gallate
15	LDL	Low-density lipoprotein
16	BOD	Biological oxygen demand
17	SIF	Simulated intestinal fluid
18	SGF	Simulated gastric fluid
19	DHA	Docosahexaenoic acid
20	WHO	World health organization
21	NLCs	Nanostructured lipid carriers
22	SLNs	Lipid nanoparticles
23	HPLC	High performance liquid chromatography
24	FESEM	Field emission scanning electron microscopy
25	FTIR	Fourier transform infrared
26	STZ	Streptozotocin

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Chapter 1

Introduction

1.1 Diabetes mellitus

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic disorders marked by persistent hyperglycaemias (high blood sugar levels) resulting from defects in insulin secretion, insulin action, or both. The condition arises when the pancreas fails to produce sufficient insulin, or when the body's cells become resistant to the effects of insulin. This leads to impaired glucose regulation, which manifests as several characteristic symptoms, including excessive thirst (polydipsia), frequent urination (polyuria), unexplained weight loss, and blurred vision. Without proper management, diabetes can result in serious health complications affecting the cardiovascular system, kidneys (nephropathy), eyes (retinopathy), and nerves (neuropathy), among others [1].

Globally, diabetes is a major public health concern, contributing to approximately 4.2 million deaths annually. Of these, an estimated 1.5 million deaths are attributed to unmanaged or poorly managed diabetes. The prevalence of diabetes has seen a significant rise over the years. As of 2021, it was estimated that 537 million people worldwide were living with diabetes, accounting for 10.5% of the global adult population. The majority of these cases, around 90%, are classified as type 2 diabetes, which is closely linked to lifestyle factors such as obesity and physical inactivity [2,3].

Projections indicate that by 2045, the global diabetic population will rise to approximately 783 million adults, equating to 1 in 8 people and reflecting a 46% increase from current figures. This alarming growth is particularly pronounced in low- and middle-income countries, where rapid urbanization and lifestyle changes contribute to the surge in diabetes cases. Both men and women are equally affected by the disease, which ranks as the seventh leading cause of death worldwide [4].

The economic burden of diabetes is equally staggering, with global healthcare expenditure related to diabetes estimated at approximately US$760 billion annually. This includes the direct costs of treating diabetes and its associated complications, as well as the indirect costs such as loss of productivity. The increasing prevalence of diabetes underscores the urgent need for effective public health strategies focused on prevention, early detection, and comprehensive management to mitigate the impact of this chronic disease on individuals and society [5,6].

1.2. Types of diabetes

1.2.1. Type 1 diabetes mellitus (T1DM)

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune condition that accounts for approximately 5% to 10% of all diabetes cases. It is most frequently diagnosed in individuals under 20 years of age, though it can occur at any age, and the term "juvenile-onset diabetes" is no longer used. The disease is characterized by the destruction of insulin-producing beta cells in the pancreatic islets, leading to severe insulin deficiency. This destruction is primarily immune-mediated, involving a T-cell-mediated autoimmune attack against beta cells, resulting in the loss of insulin production. In some cases, T1DM may also occur without a known cause, in which case it is referred to as idiopathic T1DM [7,8].

Patients with T1DM often experience irregular and unpredictable blood glucose levels due to their critically low insulin levels and an impaired counter-regulatory response to hypoglycaemia. This makes management of the disease particularly challenging and necessitates lifelong insulin therapy to regulate blood sugar levels [9]

The development of T1DM is partly influenced by genetic factors. Multiple genes, including certain human leukocyte antigen (HLA) genotypes, have been identified as increasing the risk of developing the disease. However, genetic predisposition alone is not sufficient to cause T1DM. In genetically susceptible individuals, environmental factors, such as viral infections or dietary factors, are believed to trigger the onset of the disease. Although several viruses, including enteroviruses, have been implicated in the development of T1DM, conclusive evidence in humans remains elusive [10].

T1DM is not exclusive to children and adolescents; a significant number of cases are diagnosed in adulthood. In adults, a form of the disease known as latent autoimmune diabetes of adults (LADA) is recognized. LADA is characterized by a slower onset of beta cell destruction compared to the more rapid progression seen in children. Due to its gradual onset and occurrence in adults, LADA is often misdiagnosed as type 2 diabetes, which is more common in this age group. However, unlike type 2 diabetes, LADA is autoimmune in nature and eventually leads to insulin dependence, similar to T1DM. Some researchers and clinicians refer to LADA as "type 1.5 diabetes" due to its unique characteristics that straddle both type 1 and type 2 diabetes.[11].

1.2.2. Type 2 diabetes mellitus (T2DM)

Type 2 diabetes mellitus (T2DM) is the most prevalent form of diabetes, accounting for approximately 90% of all diabetes cases worldwide. Unlike Type 1 diabetes, T2DM is primarily characterized by insulin resistance—a condition in which the body's cells become less responsive to the effects of insulin. This resistance leads to an initial compensatory increase in insulin production by the pancreas. However, over time, the pancreas may become unable to produce sufficient insulin to maintain normal blood glucose levels, resulting in chronic hyperglycaemia [11,12].

T2DM typically develops in adults, particularly those over 45 years of age, although it is increasingly being diagnosed in younger populations, including children and adolescents, largely due to rising obesity rates. The condition is strongly associated with modifiable lifestyle factors such as physical inactivity, poor diet, and obesity. Additionally, genetic predisposition plays a significant role in the development of T2DM, with a family history of diabetes significantly increasing an individual's risk [13,14].

The pathophysiology of T2DM is complex and involves multiple interrelated mechanisms. Insulin resistance in peripheral tissues such as muscle, liver, and adipose tissue is a key feature. In the liver, insulin resistance leads to increased glucose production, contributing to elevated blood sugar levels. In the muscles, reduced glucose uptake further exacerbates hyperglycaemia. Over time, the pancreatic beta cells, which are responsible for insulin production, may become dysfunctional due to the chronic demands placed on them by insulin resistance, leading to decreased insulin secretion [15].

T2DM often develops gradually, and many individuals may remain asymptomatic for years before being diagnosed. Common symptoms, when present, include increased thirst (polydipsia), frequent urination (polyuria), fatigue, blurred vision, and slow healing of wounds. Because of its insidious onset, T2DM is frequently diagnosed through routine screening or during examinations for other health conditions [16].

Complications of T2DM can be severe and include both microvascular and macrovascular complications. Microvascular complications include diabetic retinopathy, which can lead to blindness; diabetic nephropathy, which can progress to kidney failure; and diabetic neuropathy, which can cause significant pain and disability. Macrovascular complications include an increased risk of cardiovascular diseases such as coronary artery disease, stroke, and peripheral artery disease. These complications are major contributors to the morbidity and mortality associated with T2DM.

Management of T2DM focuses on lifestyle modifications such as adopting a healthy diet, engaging in regular physical activity, and achieving and maintaining a healthy weight. Pharmacotherapy is also a cornerstone of treatment and may include oral medications that improve insulin sensitivity or increase insulin production, as well as injectable therapies like insulin. Early and aggressive management is essential to prevent complications and improve quality of life [17].

Given the rising global burden of T2DM, particularly in low- and middle-income countries, public health initiatives aimed at prevention and early detection are critical. These efforts include promoting healthy lifestyles, increasing awareness of the risks associated with obesity and sedentary behaviours, and implementing screening programs for at-risk populations.

1.2.3. Gestational diabetes mellitus (GDM)

Gestational diabetes mellitus (GDM) is a form of diabetes that arises during pregnancy and is characterized by a combination of insulin resistance and inadequate insulin secretion. It affects approximately 2% to 10% of pregnancies and typically emerges in the second or third trimester when the body's production of insulin-antagonist hormones, such as human placental lactogen and cortisol, increases. These hormones contribute to insulin resistance, making it more difficult for the body to regulate blood sugar levels [18].

Screening for GDM is recommended for all pregnant women between 24 and 28 weeks of gestation. Early detection is essential, as GDM is associated with potential health risks for both the mother and the fetus. Although gestational diabetes often resolves after childbirth, approximately 5% to 10% of women with GDM are later diagnosed with another form of diabetes, most commonly type 2 diabetes. Additionally, women who have experienced GDM are at a higher risk of developing type 2 diabetes in the future [19].

The management of GDM involves a combination of lifestyle changes and medical supervision to maintain healthy blood sugar levels. This includes a balanced diet, regular physical activity, and frequent monitoring of blood glucose levels. In some cases, insulin therapy or oral hypoglycaemic medications may be required to achieve optimal blood sugar control.

Even though GDM is often temporary, it can lead to serious complications if left untreated. For the fetus, uncontrolled gestational diabetes can result in macrosomia, or excessive birth weight, which increases the likelihood of complications during delivery, such as shoulder dystocia. Additionally, babies born to mothers with GDM face a higher risk of congenital abnormalities, particularly in the heart, central nervous system, and skeletal muscles [20].

Another critical concern associated with GDM is the potential for fetal hyperinsulinemia, where elevated insulin levels in the fetus can hinder the production of surfactant, a substance essential for lung development, leading to respiratory distress syndrome in newborns. High bilirubin levels, caused by the breakdown of red blood cells, can also lead to jaundice in the newborn [21].

In severe cases, GDM can lead to perinatal death, often due to compromised placental function resulting from vascular issues. To minimize these risks, labor may be induced if there are signs of declining placental function. If the fetus is particularly large or shows signs of distress, a caesarean section may be recommended to prevent delivery complications [22].

Early diagnosis and effective management of GDM are crucial in reducing the risk of complications and ensuring a healthy pregnancy. Postpartum follow-up is also important, as it provides an opportunity to monitor the mother's health and manage any ongoing risks related to diabetes.

1.3. Pathophysiology and complication of diabetes mellitus

1.3.1. Pathophysiology of DM

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia due to defects in insulin secretion, insulin action, or both. The underlying pathophysiology differs between the main types of diabetes, primarily Type 1 and Type 2, though certain mechanisms are common across both.

1.3.1.1. Pathophysiology of Type 1 DM

Type 1 diabetes is an autoimmune condition in which the immune system erroneously targets and destroys the insulin-producing beta cells in the pancreas. The onset of this destruction is often linked to a combination of genetic susceptibility and environmental triggers, such as viral infections.

i) **Autoimmune beta cell destruction**: The immune system generates an inappropriate response against beta cells in the islets of Langerhans, leading to their progressive loss.

ii) **Insulin deficiency**: As the beta cells are destroyed, the pancreas's ability to produce insulin diminishes, resulting in elevated blood glucose levels (hyperglycemia).

iii) **Shift to ketogenesis**: In the absence of sufficient insulin, the body resorts to breaking down fats for energy, producing ketones as a byproduct. This process can escalate to ketoacidosis if not properly managed.

1.3.1.2. Pathophysiology of Type 1 diabetes mellitus

Type 2 diabetes is primarily characterized by insulin resistance, where the body's cells do not respond effectively to insulin. This form of diabetes is often associated with a combination of genetic predisposition and lifestyle factors, such as poor diet, obesity, and physical inactivity.

i) **Insulin resistance**: Cells in target tissues like muscle, liver, and adipose tissue exhibit reduced responsiveness to insulin, impairing glucose uptake from the blood.

ii) **Compensatory hyperinsulinemia**: Initially, the pancreas compensates for insulin resistance by increasing insulin production. However, this compensatory mechanism may eventually become inadequate.

iii) **Beta Cell dysfunction**: Over time, the stress on pancreatic beta cells may lead to their dysfunction and a decline in insulin secretion, exacerbating hyperglycemia.

iv) **Chronic hyperglycemia**: Persistent elevated blood glucose levels can disrupt normal metabolic processes and contribute to further insulin resistance.

3. Common Mechanisms in diabetes mellitus

Several mechanisms are pivotal in the development and progression of both Type 1 and Type 2 diabetes:

i) **Glucotoxicity**: Prolonged exposure to high blood glucose levels can impair beta cell function and further decrease insulin secretion, creating a vicious cycle of worsening hyperglycemia.

ii) **Lipotoxicity**: Excess free fatty acids, particularly in non-adipose tissues like the liver and pancreas, can interfere with insulin signaling and beta cell function, contributing to insulin resistance and cell damage.

iii) **Inflammatory processes**: In Type 1 diabetes, autoimmune inflammation leads to beta cell destruction. In Type 2 diabetes, chronic low-grade inflammation is linked to the development of insulin resistance.

iv) **Oxidative stress**: The hyperglycemic state is associated with increased production of reactive oxygen species (ROS), which can cause oxidative damage to cells and tissues, further impairing metabolic functions.

1.3.2. Complications of diabetes mellitus

Diabetes mellitus is a chronic condition that can lead to a wide range of complications if not properly managed. These complications are generally divided into microvascular, macrovascular, and other associated complications.

1.3.2.1. Microvascular complications

i) **Diabetic retinopathy:** This condition involves damage to the small blood vessels in the retina, which can result in vision impairment and, in severe cases, blindness. It is one of the leading causes of vision loss among adults.

ii) **Diabetic nephropathy:** Prolonged elevated blood glucose levels can damage the kidneys, leading to chronic kidney disease (CKD) and potentially progressing to kidney failure.

iii) **Diabetic neuropathy:** High blood sugar over time can cause nerve damage, affecting various parts of the body. Symptoms include pain, tingling, numbness, and in advanced cases, loss of sensation or motor function.

1.3.2.2. Macrovascular complications

i) **Cardiovascular disease:** Individuals with diabetes are at increased risk for cardiovascular conditions, including coronary artery disease (which may lead to heart attacks), strokes, and peripheral artery disease.

ii) **Peripheral artery disease (PAD):** Diabetes can cause narrowing of the arteries, particularly those supplying blood to the legs and feet. This can result in pain and, in severe cases, ulcers or gangrene.

1.3.2.3. Other complications

i) **Foot problems:** Poor circulation and nerve damage in diabetes increase the risk of foot ulcers, infections, and, in extreme cases, amputations.

ii) **Skin disorders:** Diabetes can lead to various skin issues such as bacterial and fungal infections, as well as persistent itching.

iii) **Diabetic ketoacidosis (DKA):** This acute and potentially life-threatening condition is characterized by a dangerous accumulation of ketones in the blood due to very high blood sugar levels.

iv) **Hyperosmolar hyperglycemic state (HHS):** A severe complication where blood sugar levels become extremely high without significant ketone build-up, leading to severe dehydration and possibly coma.

v) **Gastrointestinal issues:** Diabetes can affect the digestive system, causing conditions such as gastroparesis, where stomach emptying is delayed, leading to symptoms like nausea, vomiting, and bloating.

vi) **Mental health challenges:** Diabetes is associated with an increased likelihood of developing mental health conditions such as depression, anxiety, and cognitive decline.

Effective management of diabetes, including medication adherence, lifestyle modifications, and regular health monitoring, is essential to reduce the risk or severity of these complications.

1.4. Diabetes management

Effective diabetes management is centred on maintaining blood glucose levels within a target range, aiming to keep them as close to normal as possible without causing hypoglycaemia (low blood sugar). This balance can typically be achieved through a combination of lifestyle modifications, such as adopting a healthy diet, engaging in regular physical activity, achieving and maintaining a healthy weight, and utilizing appropriate medications, which may include insulin and oral antidiabetic agents [23].

Education plays a crucial role in diabetes management. Individuals who are well-informed about their condition and actively participate in their treatment tend to experience fewer and less severe complications [24]. The primary goal of diabetes management is to achieve and maintain an haemoglobin A1C level below 7%, as this is associated with a reduced risk of long-term complications. The A1C test measures average blood glucose levels over the previous two to three months, providing a clear indicator of how well diabetes is being managed [25].

The A1C level, also known as haemoglobin A1C or HbA1C, is a blood test that provides information about a person's average blood glucose (sugar) levels over the past two to three months. It measures the percentage of haemoglobin (a protein in red blood cells) that is coated with glucose. The higher the blood glucose levels, the more glucose will attach to haemoglobin.

Key Points of HbA1C:

This are some of the key points of HbA1C

1. **Normal A1C levels:** For someone without diabetes, a normal A1C level is typically below 5.7%.
2. **Prediabetes:** A1C levels between 5.7% and 6.4% indicate prediabetes, which means an increased risk of developing diabetes in the future.
3. **Diabetes*:*** An A1C level of 6.5% or higher on two separate tests suggests that a person has diabetes.
4. **Diabetes management:** For many people with diabetes, the goal is to keep the A1C level below 7%. This target can vary depending on the individual, their age, and their overall health [24].

In addition to managing blood sugar levels, it is essential to address other health issues that can exacerbate the effects of diabetes. These include smoking, hypertension (high blood pressure), metabolic syndrome, obesity, and physical inactivity. Smoking cessation is particularly important, as smoking significantly increases the risk of cardiovascular disease, which is already elevated in people with diabetes. Similarly, controlling blood pressure and cholesterol levels is critical in reducing the risk of heart disease and stroke.

Weight management is another key component of diabetes care, especially for individuals with type 2 diabetes. Even modest weight loss can improve insulin sensitivity and glycaemic control. Regular physical activity not only helps with weight management but also improves cardiovascular health and insulin sensitivity, making it a cornerstone of diabetes management. Foot care is another vital aspect of diabetes management, given the risk of diabetic foot ulcers and other complications that can arise from poor circulation and nerve damage (neuropathy). Specialized footwear is often recommended to reduce pressure on the feet and prevent ulcers, which can lead to serious infections and even amputation if not properly managed. Regular foot examinations and prompt treatment of any foot issues are essential to prevent complications [26].

1.4.1. Current treatments for diabetes mellitus

The management of diabetes mellitus involves several key components, which include:

1. Antidiabetic drugs
2. Diet and Physical Activity

1.4.1.1. Classification of antidiabetic drugs

Antidiabetic medications can be categorized into two main types:

1.4.1.1.1. Insulin injections:

These are primarily utilized in more severe cases of diabetes, particularly for individuals with type 1 diabetes or those with advanced type 2 diabetes who require exogenous insulin to maintain glycaemic control.

Insulin, is a key hormone produced by the beta cells located in the islets of Langerhans within the pancreas. It is essential for regulating blood glucose levels by promoting the uptake of glucose into cells, where it is either used for energy or stored as glycogen in the liver and muscles. Additionally, insulin inhibits glucose production in the liver, helping to maintain balanced blood sugar levels.

In DM, there is a disruption in the body's ability to produce or effectively use insulin. In Type 1 diabetes, the immune system attacks and destroys beta cells, leading to an absolute lack of insulin. In Type 2, the body develops insulin resistance, where cells do not respond properly to insulin, often coupled with a gradual decrease in insulin production. Proper management of insulin through medications, lifestyle modifications, or insulin therapy is essential for controlling blood glucose levels and preventing diabetes-related complications [28].

1.4.1.1.2. Oral hypoglycaemic agents:

These medications are generally suitable for adult patients with type 2 diabetes and are designed to help manage blood sugar levels through various mechanisms.

Types of oral antidiabetic drugs:

i) Sulfonylureas: Introduced in the 1950s, sulfonylureas have been a cornerstone in the treatment of type 2 diabetes. They function by stimulating insulin secretion from the pancreatic β-cells. The effectiveness of sulfonylureas relies on the presence of endogenous insulin; hence, they are not suitable for patients with type 1 diabetes or those with significant β-cell dysfunction. The mechanism involves depolarization of the β-cell membrane and enhanced calcium influx, leading to increased insulin release. However, sulfonylureas have a prolonged half-life and can cause hypoglycaemia, which is sometimes severe enough to necessitate hospitalization.

E.g., include first-generation sulfonylureas such as tolbutamide, acetohexamide, and tolazamide, as well as second-generation agents like glyburide, glipizide, and glimepiride.

ii) Meglitinides: These drugs known as non-sulfonylurea insulin secretagogues, introduced in the late 1990s, meglitinides stimulate insulin release from β-cells by binding to distinct receptors compared to sulfonylureas. They achieve this by closing ATP-sensitive potassium channels, leading to cell depolarization and the opening of voltage-dependent calcium channels. This results in an increased influx of calcium into the β-cells, ultimately promoting insulin secretion. Meglitinides are often taken before meals to control postprandial blood glucose levels.

E.g., Repaglinide and Nateglinide.

iii) Biguanides: While the exact mechanism of action for biguanides remains partially understood, metformin, the most commonly prescribed drug in this class, exerts its hypoglycaemic effects by reducing hepatic gluconeogenesis and enhancing glucose uptake in peripheral tissues. This action is associated with improved insulin sensitivity in muscle tissues and a reduction in circulating free fatty acids. Clinical studies indicate that metformin therapy can lower A1C levels by 1.5% to 2%, making it as effective as sulfonylureas in managing blood sugar in type 2 diabetes patients.

E.g., Metformin, Phenformin

iv) α-Glucosidase inhibitors: Acarbose, a prominent α-glucosidase inhibitor, was introduced in 1996 and functions as a pseudo-tetra saccharide. Its structure features a maltose unit linked to an acarviosin unit, which is crucial for its mechanism of action. The acarviosin component contains a nitrogen linkage that confers a strong affinity for the enzyme α-glucosidase. This inhibitor works by competing with oligosaccharides for binding to the catalytic site of the enzyme, thereby preventing the breakdown of oligosaccharides and disaccharides into monosaccharides. This results in a reduction of glucose absorption in the intestine and helps control postprandial blood sugar levels.

E.g., α-glucosidase inhibitors include acarbose and miglitol.

v) Thiazolidinediones (TZDs): Thiazolidinediones are a class of antidiabetic agents that work by reducing hepatic glucose production and enhancing insulin-mediated glucose uptake in muscle cells. Unlike other antidiabetic medications, TZDs do not stimulate insulin production from the pancreas. Instead, they improve insulin sensitivity, making them particularly effective when used in combination with other agents, such as metformin or sulfonylureas.
E.g., Commonly prescribed thiazolidinediones include rosiglitazone and pioglitazone.

vi) Dipeptidyl peptidase-4 (DPP-4) inhibitors: DPP-4 inhibitors represent a class of medications that target the enzyme dipeptidyl peptidase-4, which is found in various tissues, including the liver, lungs, kidneys, and intestinal brush border. This enzyme plays a crucial role in regulating the levels of several gastrointestinal hormones, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). GLP-1 is beneficial for glycaemic control as it stimulates insulin secretion, inhibits glucagon release, and slows gastric emptying, thereby reducing postprandial glucose spikes.
E.g., DPP-4 inhibitors include sitagliptin and linagliptin.

1.4.2. Side effects and risks associated with antidiabetic medications

All drugs used in diabetes treatment are associated with side effects, and careful consideration must be given to their potential risks. For instance, α-glucosidase inhibitors, while effective in reducing postprandial blood glucose levels, often lead to gastrointestinal issues such as flatulence, abdominal discomfort, and diarrhoea. These symptoms result from undigested oligosaccharides reaching the large intestine, where they are fermented by gut bacteria, causing these adverse effects .
Sulfonylureas, particularly the first-generation drugs, have seen a decline in use due to their significant side effects. Chlorpropamide, for example, can cause prolonged and severe hypoglycaemia, especially in patients with renal impairment. It is also associated with hyponatremia and water retention due to inappropriate antidiuretic hormone secretion. Additionally, first-generation sulfonylureas like tolbutamide and chlorpropamide can cause alcohol-induced flushing, and rare but severe side effects such as cholestatic jaundice have also been reported [29,30].
Metformin, a widely used biguanide, is generally well-tolerated but carries a rare risk of lactic acidosis, a serious condition with a high mortality rate. Although the incidence of lactic acidosis is low (about 0.03 cases per 1,000 patient-years), its potential severity makes it a significant concern [38].

Meglitinides, another class of antidiabetic drugs, may cause symptoms such as diaphoresis, altered mental status, tremors, and confusion, particularly if blood glucose levels drop too low. These symptoms can escalate to seizures or coma if hypoglycaemia persists, although the onset of these side effects is usually rapid and of short duration.

Similarly, α-glucosidase inhibitors can cause gastrointestinal distress, with diarrhoea and abdominal pain being the most commonly reported adverse effects. While these drugs are less likely to cause hypoglycaemia, their chronic use, particularly with acarbose, has been linked to hepatic injury [39,40].

Newer classes of antidiabetic drugs, such as dipeptidyl peptidase-4 (DPP-4) inhibitors and glucagon-like peptide-1 (GLP-1) analogues, have been associated with an increased risk of pancreatitis. Sodium-glucose co-transporter 2 (SGLT2) inhibitors, on the other hand, have been linked to an elevated risk of urinary tract infections due to the increased glucose excretion in the urine.

1.5. Bioactive compounds for the treatment of diabetes

Bioactive compounds, particularly those derived from natural sources, have emerged as promising candidates in the management of diabetes mellitus. These compounds, often found in plants, offer a potential therapeutic advantage with fewer side effects compared to conventional antidiabetic medications. The following is a detailed exploration of several bioactive compounds that have shown significant potential in the treatment of diabetes.

i) Polyphenols

Polyphenols are a diverse group of naturally occurring compounds found in various plant sources, recognized for their antioxidant and antidiabetic properties. Epigallocatechin gallate (EGCG), a prominent polyphenol found in green tea, has been demonstrated to enhance insulin sensitivity and lower blood glucose levels. The mechanism of action for EGCG involves the activation of AMP-activated protein kinase (AMPK), which plays a crucial role in maintaining cellular energy balance. Additionally, EGCG has been shown to reduce oxidative stress, a major contributing factor in the development and progression of diabetes [31,32].

ii) Flavonoids

Flavonoids are another major class of polyphenolic compounds prevalent in fruits, vegetables, and medicinal plants, known for their therapeutic potential in diabetes management. Quercetin, a well-studied flavonoid, exhibits antidiabetic effects by inhibiting key enzymes such as α-glucosidase and aldose reductase, which are involved in glucose metabolism. By inhibiting these enzymes, quercetin effectively reduces postprandial blood glucose levels and may also offer protective effects against diabetic complications, including cataracts and neuropathy.

Rutin, a flavonoid glycoside, is another compound that has garnered attention for its antidiabetic properties. Found in citrus fruits, buckwheat, and certain vegetables, rutin has been shown to possess significant antioxidant activity, which helps in reducing oxidative stress—a key factor in the pathogenesis of diabetes. Rutin's hypoglycaemic effects are believed to be linked to its ability to enhance insulin secretion and improve glucose metabolism. Furthermore, rutin may inhibit advanced glycation end-product (AGE) formation, which is associated with diabetic complications [33].

iii) Curcuminoids

Curcumin, the active compound in turmeric, is noted for its potent anti-inflammatory and antioxidant effects, which are beneficial in the context of diabetes. Curcumin has been shown to improve insulin sensitivity, reduce blood glucose levels, and decrease the risk of diabetes-related complications, particularly cardiovascular diseases. The therapeutic effects of curcumin are primarily attributed to its ability to modulate inflammatory pathways and oxidative stress, both of which are pivotal in the pathogenesis of diabetes [34].

iv) Saponins

Saponins, naturally occurring glycosides found in various plants, have demonstrated hypoglycaemic activity in several studies. Ginsenosides, a class of saponins derived from ginseng, are particularly effective in improving insulin secretion and reducing insulin resistance. These effects are mediated through the modulation of signalling pathways that regulate glucose metabolism. Furthermore, saponins have been shown to enhance glucose uptake in peripheral tissues, contributing to improved glycaemic control [35].

v) Alkaloids

Berberine, an alkaloid obtained from plants such as *Berberis* sp., has been widely recognized for its antidiabetic properties. Berberine exerts its hypoglycaemic effects by increasing insulin receptor expression and enhancing insulin sensitivity. Additionally, berberine activates AMP-activated protein kinase (AMPK), a mechanism similar to that of metformin, a widely used antidiabetic drug. The modulation of gut microbiota by berberine is also believed to contribute to its overall hypoglycaemic effect, making it a multifaceted agent in diabetes management [46].

iv) Terpenoids

Terpenoids, including compounds like ginsenosides and limonoids, have shown potential in the treatment of diabetes. Ginsenosides not only improve insulin sensitivity but also protect pancreatic β-cells from oxidative stress-induced damage. Limonoids, found in citrus fruits, have been reported to reduce blood glucose levels and mitigate insulin resistance, potentially due to their antioxidant and anti-inflammatory properties [37].

1.6. Novel treatment strategies in diabetes mellitus (DM)

Antidiabetic medications are predominantly available either in oral dosage forms or as injectables. Since diabetes is a long-term condition requiring ongoing management, the oral route is generally preferred for its convenience and higher adherence among patients. However, oral antidiabetic drugs, often administered as tablets or capsules, have certain limitations. These include side effects like gastrointestinal disturbances, diarrhoea, reduced appetite, and in some instances, lactic acidosis, particularly in individuals with impaired kidney or liver function [38]. To address the challenge of bioavailability, especially in drugs with poor solubility, various strategies have been developed to enhance dissolution rates and improve bioavailability. These methods include increasing the drug's surface area, reducing particle size, formulating the drug in a dissolved state, creating liquisolid compacts, developing inclusion complexes, employing solid dispersions, utilizing prodrugs, and generating metastable polymorphs [38,39].

In recent years, novel nano-formulation strategies have been introduced to tackle the absorption and availability issues associated with oral drugs. These advanced formulations not only enhance the solubility and bioavailability of bioactive compounds but also protect them from degradation due to physical, chemical, biological, or light-induced factors. Additionally, these novel delivery systems enable controlled and sustained drug release, ensuring consistent therapeutic effects. Another significant advantage is the ability to deliver drugs directly to targeted sites, thereby minimizing exposure to non-target areas and reducing potential side effects [42].

Plant-derived bioactive compounds have shown promising results when incorporated into these advanced delivery systems. Compounds such as curcumin, flavonoids, and various vitamins have demonstrated greater therapeutic efficacy at lower doses when delivered through innovative formulations compared to traditional delivery methods. This enhanced effectiveness is primarily due to improved solubility, stability, and bioavailability.

Emerging lipid-based nano-delivery systems, such as liposomes, niosomes, transfersomes, ethosomes, phytosomes, nano-emulsions, solid lipid nanoparticles, and self-emulsifying drug delivery systems (SNEDDS), have been designed to significantly improve the solubility, bioavailability, and stability of therapeutic compounds. These cutting-edge approaches not only enhance the pharmacokinetic profiles of the drugs but also offer the potential for targeted and controlled release, optimizing therapeutic outcomes while minimizing adverse effects [40,41].

1.7. Self-nanoemulsifying drug delivery system (SNEDDS)

Enhancing the oral bioavailability of poorly water-soluble drugs remains a significant challenge in pharmaceutical development. One of the promising approaches to overcome this issue involves the use of lipid-based formulations. These formulations, particularly those based on self-nanoemulsifying drug delivery systems (SNEDDS), have gained substantial attention for their ability to improve the solubility and bioavailability of hydrophobic drugs.

SNEDDS are isotropic mixtures composed of oils, surfactants, co-surfactants, and the active pharmaceutical ingredient (API). Upon oral administration, when SNEDDS are exposed to the aqueous environment of the gastrointestinal (GI) tract, they spontaneously emulsify under gentle agitation provided by the digestive motility. This process forms a fine oil-in-water (o/w) nano emulsion, with droplet sizes typically ranging from a few nanometres(~5nm) to less than 200 nm. The small droplet size results in an increased interfacial surface area, which significantly enhances dissolution rate of the drug, thereby improving its bioavailability [43].

1.7.1. Composition and mechanism of SNEDDS

i) Lipid components:

The lipid component of SNEDDS plays a crucial role in solubilizing the poorly water-soluble drug. Typically, medium-chain triglycerides (MCTs) or long-chain triglycerides (LCTs) are used as the oil phase. These lipids not only aid in solubilization but also facilitate lymphatic absorption, which can bypass hepatic first-pass metabolism, further enhancing bioavailability [44, 45].

ii) Surfactants and co-surfactants:

Surfactants and co-surfactants are integral to the formation of a stable nano emulsion. Surfactants with high hydrophilic-lipophilic balance (HLB) values are typically used to reduce the interfacial tension between the oil and aqueous phases. Co-surfactants, often short-chain alcohols or glycols, are included to enhance the fluidity of the interfacial layer and reduce the size of the emulsion droplets . The synergistic action of surfactants and co-surfactants is critical for the spontaneous formation of nano-sized droplets upon contact with GI fluids [46]

1.7.2. Self-emulsification process

The self-emulsification process of SNEDDS is driven by the thermodynamic stability provided by the excipient mixture. When the formulation encounters the aqueous environment in the GI tract, the gentle agitation provided by peristalsis facilitates the formation of a nano emulsion. The nano emulsification process results in a significant reduction in droplet size, which increases the surface area available for drug dissolution, thereby enhancing absorption. The presence of lipids and surfactants in SNEDDS also enhances the permeability of the drug through the biological membranes, further improving its bioavailability [45].

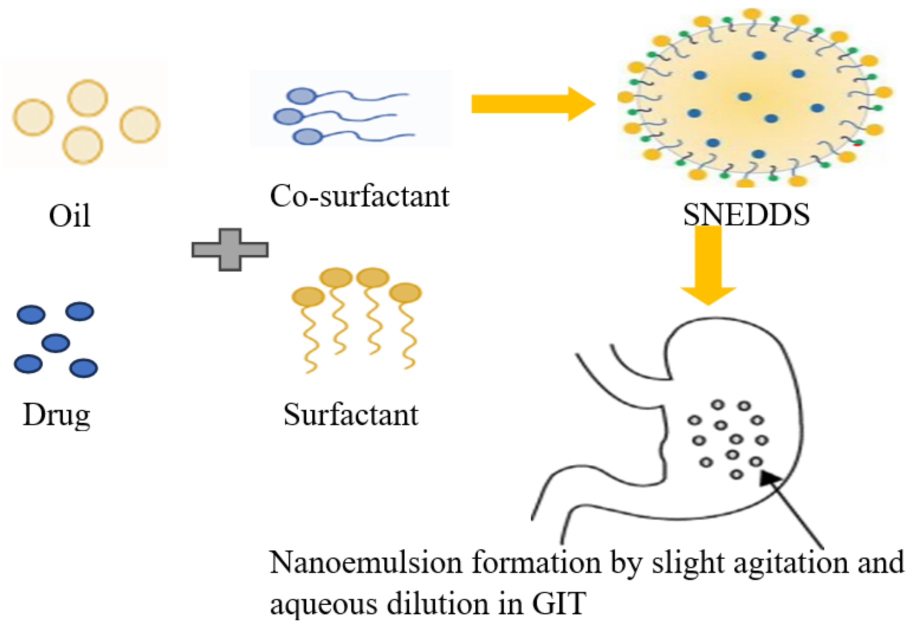


Fig. 1.1 Mechanism of formation of SNEDDS

1.7.3. Advantages of SNEDDS

i) **Improved bioavailability:**

The primary advantage of SNEDDS lies in their ability to improve the bioavailability of poorly soluble drugs. By maintaining the drug in a solubilized state within a nano-sized emulsion, SNEDDS overcome the limitations of solid-state formulations, leading to enhanced absorption and therapeutic efficacy.

ii) **Enhanced stability and protection:**

SNEDDS provide a protective environment for the drug, shielding it from degradation due to light, oxygen, and other environmental factors. This enhances the stability of the drug during storage and after administration.

iii) **Ease of manufacturing and scalability:**

The formulation and production of SNEDDS are relatively straightforward and do not require complex manufacturing processes. This ease of production makes SNEDDS a viable option for large-scale manufacturing .

iv) **Flexibility in dosage form:**

SNEDDS can be formulated in various dosage forms, including soft or hard gelatin capsules, tablets, and liquid formulations, providing flexibility in drug delivery.

v) **Reduced food effect:**

SNEDDS formulation often exhibit reduced, or no food effects compared to conventional formulations, making them more susceptible to changes in bioavailability when taken with food.

1.7.4. Marketed formulations of SNEDDS

SNEDDS have been effectively utilized in several commercial formulations to enhance the oral bioavailability of poorly water-soluble drugs. Below are some examples:

Table 1.1 Marketed formulation based on SNEDDS

Drug (Indication)	Brand Name	Dose (mg)	Dosage form	Excipients
Atorvastatin (Cholesterol)	Lipitor® (Pfizer)	20	TAB	Calcium carbonate, USP; candelilla wax, FCC, Tween 80
Cyclosporine A (Anticancer)	Sandimune® Neoral® (Novartis)	10-100	SGC	Corn oil, polyoxyethylated linoleic glycerides (Labrafil M2125CS)
Lopinavir (Antiviral)	Kaletra® (Abbott)	130	SGC	Oleic acid, polyoxyl 35 castor oil (Cremophore EL
Fenofibrate (Antiplatelet)	Lipirex® (SanofiAventis)	200	HGC	Lauryl macrogol-glycerides (Gelucire 44/14), PEG 200
Bexarotene (Anticancer)	Targetrin® (Ligand)	75	SGC	Tween 20, PEG 400
Indomethacin (NSAID)	Infree® (Eisai Co)	200	SGC	Polyoxoy 60 hydrogenated castor oil (Cremophor RH 60), hydrogenated oil, glyceryl mono-oleate

1.7.5. SNEDDS formulation approaches

The formulation of self-nanoemulsifying drug delivery systems (SNEDDS) involves several key components and approaches designed to improve the solubility, stability, and bioavailability of poorly water-soluble drugs. Below are the primary approaches and considerations in SNEDDS formulation:

1.7.5.1. Selection of oil phase

i) **Function:** The oil phase is critical in dissolving the lipophilic drug and forming the core of the nano emulsion droplets. It also affects the drug's release profile and the droplet size.

ii) **Common oils:** Frequently used oils include medium-chain triglycerides (MCTs) such as Capryol- 90, long-chain triglycerides (LCTs) like corn oil, and various synthetic oils. The choice of oil depends on the drug's solubility and the desired release characteristics.

1.7.5.2. Selection of surfactant

i) **Function:** Surfactants reduce the interfacial tension between the oil and aqueous phases, aiding in the formation of nano-sized emulsions. They also stabilize the emulsion and enhance drug absorption by improving wettability and permeability.

ii) **Common surfactants:** Non-ionic surfactants such as Tween 80, Cremophor EL, and Labrasol are commonly used. The surfactant's hydrophilic-lipophilic balance (HLB) value is a key factor in its emulsification efficiency.

1.7.5.3. Selection of co-surfactant

i) **Function:** Co-surfactants further reduce interfacial tension, enabling the formation of finer emulsions with smaller droplet sizes. They also help stabilize the emulsion and prevent droplet coalescence.

ii) **Common co-surfactants:** Common co-surfactants include Transcutol P, ethanol, and PEG 400. The selection is based on compatibility with the surfactant and drug.

1.7.5.4. Drug solubilization

i) **Approach:** The drug is solubilized in the oil phase, often alongside surfactants and co-surfactants. This ensures the drug remains in a dissolved state, which is essential for enhanced absorption.

ii) **Considerations:** The drug's physicochemical properties, such as lipophilicity, pKa, and molecular weight, are considered to optimize its solubilization within the SNEDDS.

1.7.5.5. Optimization of formulation components

i) **Pseudo-ternary phase diagrams:** These diagrams help identify the optimal ratios of oil, surfactant, and co-surfactant to achieve a stable nano emulsion. They are used to visualize the emulsification region and determine the best proportions for self-emulsification.

ii) **Screening and testing:** The components and their ratios are screened through solubility studies, emulsification efficiency tests, and stability assessments.

1.7.5.6. Preparation method

i) **Spontaneous emulsification:** Upon dilution with gastrointestinal fluids, the isotropic mixture of oil, surfactant, and co-surfactant spontaneously forms a nano emulsion. This process occurs without the need for external energy, such as heat or mechanical agitation.

ii) **Solid SNEDDS:** SNEDDS can also be converted into solid dosage forms through adsorption onto solid carriers, spray drying, or freeze-drying, improving stability and ease of administration.

1.7.5.7. Evaluation of SNEDDS

i) **Droplet size analysis:** The average droplet size of the nano emulsion is measured using techniques such as dynamic light scattering (DLS) or laser diffraction. Smaller droplet sizes, typically less than 200 nm, are preferred for enhanced bioavailability.

ii) **Zeta potential:** The surface charge (zeta potential) of the nano emulsion droplets is measured to assess formulation stability. Higher absolute zeta potential values usually indicate better stability.

iii) **In vitro dissolution and release studies:** These studies evaluate the rate and extent of drug release from the SNEDDS, providing insights into the drug's bioavailability and therapeutic potential.

1.7.5.8. Stability testing

i) **Long-term stability:** SNEDDS formulations are subjected to stability studies under various conditions (e.g., temperature, humidity) to ensure the nano emulsion retains its properties over time.

ii) **Physical stability:** The formulation is monitored for phase separation, drug precipitation, and changes in droplet size over time.

1.8. Drug profile

1.8.1 Rutin

Rutin is a bioflavonoid glycoside commonly found in plants such as buckwheat and citrus fruits. It is recognized for its antioxidant, anti-inflammatory, and antidiabetic effects. Rutin aids in enhancing insulin sensitivity, reducing oxidative stress, and promoting cardiovascular health by strengthening blood vessels and minimizing capillary fragility.

1.8.1.1 Chemical structure and basic information

- **Name:** Rutin
- **Alternative name:** RUTIN rutoside Quercetin 3-rutinoside
- **IUPAC name:** (2S)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-(4-hydroxy-3-methoxy-phenyl)-4H-chromen-4-one 3-rhamnosyl-(1→6)-glucoside.
- **Molecular formula:** $C_{27}H_{30}O_{16}$
- **Molecular weight:** 610.521 g/mol
- **Chemical structure :** [Fig. 2.3]

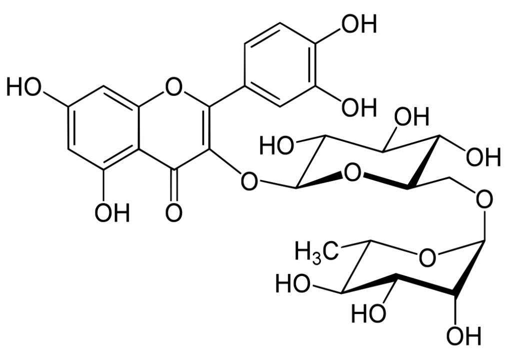


Fig. 1.2. Chemical structure of rutin **Fig. 1.3. Powder rutin extract**

1.8.1.2. Traditional uses of rutin in the treatment of DM

Rutin has been traditionally used in various cultures for its health benefits, particularly in managing diabetes and related conditions. Its historical applications include:

i) **Blood sugar control:**
Rutin has been used in traditional medicine to help regulate blood sugar levels. Found in foods like buckwheat and citrus fruits, rutin is believed to support insulin secretion and enhance the uptake of glucose by cells, helping to maintain stable blood sugar levels.

ii) **Anti-inflammatory and antioxidant effects:**
Rutin is known for its anti-inflammatory and antioxidant properties, which are important in managing diabetes. These properties help reduce oxidative stress and inflammation, both of which can lead to insulin resistance and damage to pancreatic beta cells. By mitigating these factors, rutin aids in improving insulin sensitivity.

iii) **Vascular health support:**
Traditional practices have used rutin to promote vascular health, especially in people with diabetes. Rutin helps strengthen blood vessels and improve circulation, which is crucial in preventing complications like diabetic retinopathy and neuropathy.

iv) **Herbal formulations:**

In various traditional medicine systems, rutin is commonly included in herbal blends designed to manage diabetes. It is often used alongside other natural compounds to enhance its effectiveness in controlling blood sugar and preventing diabetes complications.

v) **Support for insulin function:**

Rutin is traditionally believed to aid in the function of insulin by improving insulin signaling pathways, which helps the body use glucose more effectively. This is important in preventing the progression of diabetes and minimizing reliance on pharmaceutical treatments.

1.8.2. Epigallocatechin -3- gallate (EGCG)

Green tea, derived from the leaves of *Camellia sinensis*, is the second most widely consumed traditional beverage in many Asian countries. The leaves of this plant are rich in various antioxidants known as polyphenolic catechins, which contribute to the tea's health benefits. Among these catechins, epigallocatechin gallate (EGCG) is the most abundant, comprising approximately 60% of the total catechin content. Other catechins include epicatechin (EC), epigallocatechin (EGC), and epicatechin gallate (ECG), which represent about 6%, 20%, and 14%, respectively.

Typically, a single tea bag of green tea contains around 100 mg of these beneficial polyphenols, making it a significant source of antioxidants. EGCG, in particular, is noted for its potent antioxidant properties, which help neutralize harmful free radicals and reduce oxidative stress in the body. This catechin has gained attention for its potential health benefits, including anti-inflammatory, anti-cancer, and cardioprotective effects.

Research suggests that EGCG may play a role in managing various health conditions, such as diabetes, due to its ability to enhance insulin sensitivity and regulate blood sugar levels. Its efficacy in promoting weight loss and supporting metabolic health is also being studied. The consumption of green tea, therefore, not only provides hydration but also delivers a myriad of health benefits attributed to its high catechin content, especially EGCG.

1.8.2.1. Chemical structure and basic information

- **Name:** (-)-Epigallocatechin gallate (EGCG)
- **Alternative name:** Epigallocatechin -3- Gallate
- **IUPAC name:** [(2R,3R)-5,7-Dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-3-yl] 3,4,5-trihydroxybenzoate
- **Molecular formula:** $C_{22}H_{18}O_{11}$
- **Molecular weight:** 458.372 g/mol
- **Chemical structure :** [*Fig. 2.5*]
- **Appearance:** Yellow to white crystalline powder.
- **Stability:** Light, heat and oxygen sensitive, can degrade its quality.
- **Source:** Green tea *(Camellia sinensis)*

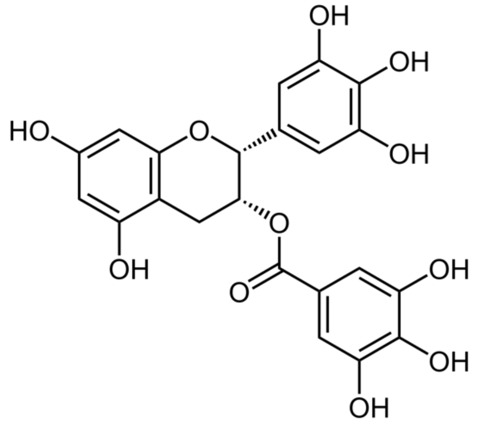


Fig. 1.4. Chemical structure of EGCG **Fig. 1.5. Powder EGCG extract**

1.8.2.2. Traditional Uses of EGCG in managing diabetes mellitus

Epigallocatechin gallate (EGCG), a prominent compound found in green tea, has been historically utilized in various cultures for its health benefits, particularly in the management of diabetes mellitus (DM). Green tea's use dates back centuries in Asian societies, where it has been recognized for its medicinal qualities.

i) **Blood sugar control:**
Traditionally, green tea has been consumed to help regulate blood glucose levels. The polyphenols in green tea, particularly EGCG, have demonstrated the ability to enhance insulin sensitivity, which is essential for individuals with diabetes. Regular consumption of green tea is associated with reduced fasting glucose levels and improved glycaemic control.

ii) **Weight management:**
In traditional practices, green tea is acknowledged for its potential role in weight management. Maintaining a healthy weight is crucial for controlling type 2 diabetes, and EGCG is believed to promote fat oxidation and increase metabolic rates, thereby aiding weight loss. This is especially helpful for those at risk of developing diabetes or those already managing the disease.

iii) Antioxidant properties:

Traditional uses of green tea also emphasize its antioxidant capabilities. EGCG can help alleviate oxidative stress, a contributor to the progression of diabetes and its complications. By neutralizing free radicals, EGCG may protect pancreatic cells, supporting their ability to produce insulin.

iv) Anti-inflammatory effects:

Chronic inflammation is linked to insulin resistance and the onset of diabetes. Traditional practices often involve drinking green tea for its anti-inflammatory properties, which can help reduce inflammation associated with metabolic disorders. EGCG has been shown to inhibit inflammatory cytokines, contributing to its beneficial effects.

v) Cultural relevance:

Across different cultures, green tea has been valued not only for its taste but also as a preventive measure against diabetes. This cultural practice reflects the historical recognition of the health benefits of EGCG, leading to its incorporation into diets aimed at promoting overall health and managing chronic conditions like diabetes.

1.9. Excipients profile

1.9.1. Transcutol P

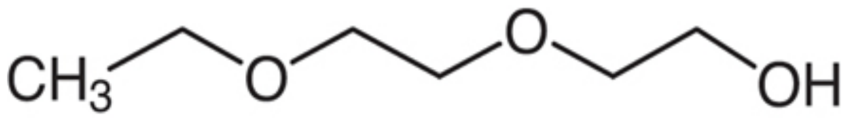


Fig 1.6. Chemical structure of Transcutol P

Transcutol P is a clear, colorless, hygroscopic liquid with a mild fragrance. It is a multifunctional pharmaceutical excipient recognized for its ability to solubilize, emulsify, and enhance drug delivery systems. In the formulation of EGCG-SNEDDS, Transcutol P serves as a co-surfactant, facilitating the solubilization of EGCG within the oil phase of the formulation. This process improves the dispersibility and absorption of EGCG in the gastrointestinal tract, thereby increasing its therapeutic efficacy in pharmaceutical applications.

Table. 1.2 Basic information of Transcutol P

Molecular formula	$C_6H_{14}O_3$
IUPAC name	2-(2-Ethoxyethoxy) ethanol
Chemical name	Diethylene glycol monomethyl ether
Molar mass	134.175 g/mol
Density	0.989 g/cm³ at 20°C
Viscosity	4.8 mPa·s at 20°C
Log P (Octanol: Water)	-0.43
Melting point	-76°C
Boiling point	196-200°C
Vapor pressure	16 Pa
Solubility	It is soluble in water and organic solvent like ethanol, acetone, benzene.
Category	Diluent, humectants and solvent.

1.9.2. Tween-80

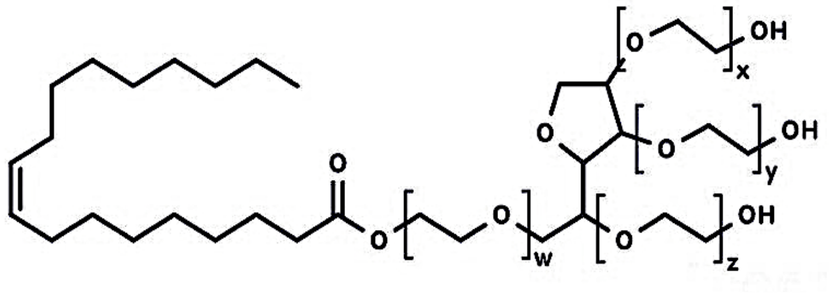


Fig. 1.7. Chemical structure of Tween-80

Tween 80, also known as Polysorbate 80, is a widely utilized non-ionic surfactant and emulsifier in both pharmaceuticals and food applications. Its IUPAC designation is Poly(oxyethylene) (20) sorbitan monooleate. Tween 80 is effective in enhancing solubility and lowering surface tension, which makes it suitable for use in emulsions and lipid-based drug delivery systems.

Table. 1.3 Basic information of Tween-80

Molecular formula	$C_{64}H_{124}O_{26}$
IUPAC name	Polyoxyethylene (20) sorbitan monooleate
Chemical name	Polyoxyethylene sorbitan monooleate
Other names	Kolliphor PS 80, Polysorbate 80, PS 80
Melting point	-25 °C
Boiling point	>100 °C
Density	1.08 g/mL at 20 °C
Solubility	Soluble in water, ethanol, mineral oil and vegetable oil.
Category	Non-ionic surfactant and emulsifier.

1.9.3. Ascorbyl palmitate

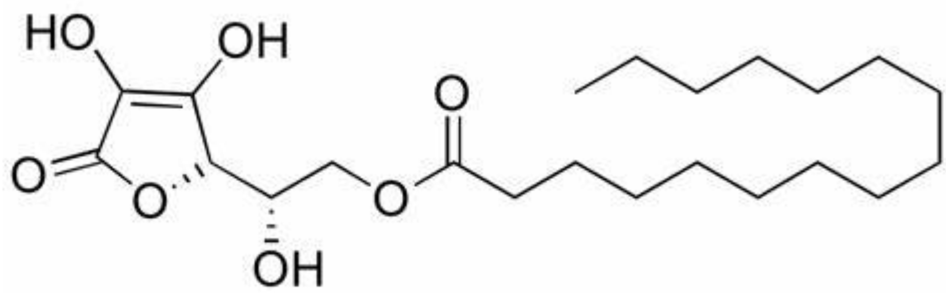


Fig. 1.8. Chemical structure of Ascorbyl palmitate

Ascorbyl palmitate is a fat-soluble form of vitamin C (fermentation of corn) combined with palmitic acid (extracted from palm oil), frequently utilized as an antioxidant in food, cosmetics, and pharmaceutical products. It serves to stabilize and safeguard other components against oxidation, thereby prolonging their shelf life. Furthermore, it may contribute to skin health and promote collagen production.

Table. 1.4. Basic information of ascorbyl palmitate

Molecular formula	$C_{22}H_{38}O_7$
IUPAC name	[(2S)-2-[(2R)-4,5-Dihydroxy-3-oxo-2-furyl]-2-hydroxy-ethyl] hexadecanoate
Chemical name	L-Ascorbic acid 6-hexadecanoate
Other names	Vitamin- C ester
Molar mass	414.533 g/mol
Melting point	116 to 117 °C
Boiling point	178.1 °C
Density	1.150±0.06 g/cm3 (Predicted)
Solubility	Ethanol
Category	Chemical preservative food additive

1.9.4. Aerosil 200

Aerosil 200 is a hydrophilic fumed silica with a specific surface area of 200 m²/g. Aerosil 200 serves as an important excipient due to its properties as a fumed silica. It enhances the viscosity of the formulation, helping to stabilize the nano emulsion and prevent phase separation.

Table. 1.5. Basic information of aerosil 200

Chemical name	Silicon di oxide amorphous
Molecular formula	SiO2
Melting point	1710 °C
Boiling point	2230 °C at 760 mmHg
Molecular weight	60.084 g/mol
Solubility	Poorly soluble in water

1.9.5. Isomalt

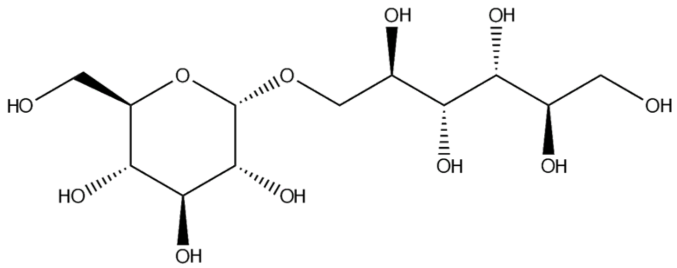


Fig 1.9. Chemical structure of isomalt

Isomalt is a sugar substitute classified as a sugar alcohol (polyol) and is derived from sucrose. It is often utilized as a low-calorie sweetener in various food products, especially in sugar-free and reduced-calorie options. With about 50% of the sweetness of regular sugar, isomalt is valued for its stability at high temperatures, making it ideal for cooking and baking. Isomalt has a negligible effect on blood sugar levels and does not trigger insulin release, making it a suitable option for those monitoring their sugar intake. Additionally, it is considered tooth-friendly since it does not contribute to tooth decay. In pharmaceutical settings, isomalt is utilized as a coating agent, granulation aid, sweetening agent, and diluent for tablets and capsules.

Table. 1.6. Basic information of isomalt

Molecular formula	**$C_{12}H_{24}O_{11}$**
IUPAC name	(2R,3R,4R,5R)-6- [[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)-2-tetrahydropyranyl]oxy]hexane-1,2,3,4,5-pentol
Chemical name	L-Ascorbic acid 6-hexadecanoate
Melting point	145°C -150°C
Molecular weight	344.31 g/mol
Solubility	soluble in water poorly soluble in ethanol
Description	odorless, white, slightly hygroscopic crystalline mass, sweet in taste.
Category	Sugar Alcohol

1.9.6. Sunflower oil

Sunflower Oil is a non-volatile oil extracted from the seeds of the sunflower plant (*Helianthus annuus*). It is primarily composed of triglycerides, with a high content of polyunsaturated fatty acids, especially linoleic acid (omega-6 fatty acid) and oleic acid (omega-9 fatty acid). In the pharmaceutical industry, sunflower oil is used for various purposes like Excipient, solvent, Emollient, Stabilizer and Nutritional Supplement.

Table. 1.7. Basic information of sunflower Oil

Molecular weight	Approximately 886.5 g/mol
Appearance	Clear, light yellow to amber liquid
Density	Approximately 0.92 g/cm³
Viscosity	Varies, typically around 60–70 mPa·s at 25°C
Smoke point (refined)	232 °C
Smoke point (unrefined)	107 °C

1.9.7. Dimethyl sulfoxide (DMSO)

Dimethyl sulfoxide (DMSO) is an organic solvent with the chemical formula $(CH_3)_2SO$. Known for its high polarity and ability to dissolve both polar and nonpolar compounds, DMSO is widely used in pharmaceutical formulations and laboratory research. It serves as a vehicle for drug delivery due to its ability to enhance the permeability of drugs through biological membranes.

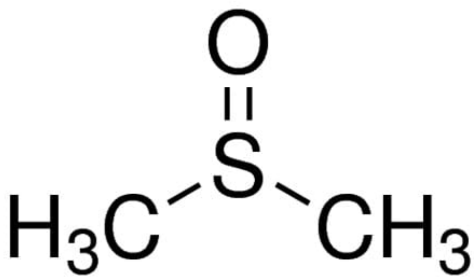


Fig 1.10. Chemical structure of DMSO

Additionally, DMSO has anti-inflammatory and analgesic properties, which make it useful in topical applications. Its unique solubilizing characteristics and relatively low toxicity make DMSO a valuable solvent in the development of various drug formulations.

Table. 1.8. Basic information of DMSO

Property	**Details**
Molecular formula	$(CH_3)_2SO$
Chemical name	Dimethyl sulfoxide
Other names	Methyl sulfoxide, Dimethyl sulphoxide
Molar mass	78.13 g/mol
Melting point	18.45°C (65.21°F)
Boiling point	189°C (372.2°F)
Density	1.1004 g/cm^3

1.9.8. Ethyl alcohol (Ethanol)

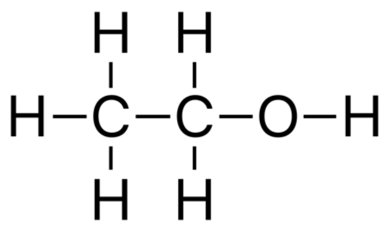


Fig 1.11. Chemical structure of ethanol

Ethanol, also known as ethyl alcohol or grain alcohol, is a clear, volatile liquid with a distinctive odour and taste. It is widely used in the pharmaceutical industry as a solvent for formulating both oral and topical medications. Its antimicrobial properties make it an effective antiseptic in hand sanitizers and disinfectants. Ethanol also serves as a preservative in pharmaceutical formulations, aids in extracting active pharmaceutical ingredients from plants, and enhances drug solubility in various dosage forms like syrups and tinctures.

Table. 1.9. Basic information of ethanol

Molecular formula	C_2H_5OH
Chemical name	Ethyl alcohol
Other names	Grain alcohol, alcohol
Molar mass	46.068 g/mol
Melting point	-114.1 °C (-173.4 °F)
Boiling point	78.37 °C (173.07 °F)
Density	0.789 g/cm³ at 20 °C
Solubility	Miscible with water, soluble in ether, acetone, benzene
Category	Solvent, antiseptic, pharmaceutical excipient.

CHAPTER 2

REVIEW OF LITERATURE

Kazi, M., et al., 2019 comprehensive review provides detailed insights into the formulation, applications, and advancements of SNEDDS. The authors explain the fundamental principles behind SNEDDS, highlighting their role in enhancing the bioavailability of poorly water-soluble drugs. Key formulation strategies, such as the selection of oils, surfactants, and co-surfactants, are discussed in depth. Additionally, the review covers various characterization techniques used to evaluate SNEDDS, including droplet size analysis, zeta potential measurement, and stability studies. The applications of SNEDDS in improving the pharmacokinetics and therapeutic efficacy of drugs are illustrated with numerous examples from recent research. This article is a valuable resource for understanding the current state and future prospects of SNEDDS technology.

Pouton, C.W. et al., 2000 In this seminal paper, Pouton provides a foundational overview of the formulation and characterization of SNEDDS, specifically focusing on lipophilic drugs. The review delves into the mechanics of self-emulsification, emphasizing the importance of the oil phase and the role of surfactants and co-surfactants in achieving nano-sized emulsions. It outlines various methods for preparing SNEDDS and highlights the critical parameters that influence the stability and performance of these systems. The paper also discusses the advantages of SNEDDS in enhancing the oral bioavailability of lipophilic drugs, making it a cornerstone reference for researchers and formulators working with SNEDDS.

Khursheed et al.,2022 discusses the preparation of a self-nanoemulsifying drug delivery system (SNEDDS) containing curcumin and quercetin for the treatment of type 2 diabetes mellitus in rats. The SNEDDS formulation was prepared by dissolving curcumin and quercetin in an isotropic mixture of Labrafil M1944CS®, Capmul MCM®, Tween-80®, and Transcutol P®. It was then solidified using Ganoderma lucidum extract, probiotics, and Aerosil-200® through spray drying. The mean droplet size and zeta potential of the SNEDDS were measured to be 63.46 ‡ 2.12 nm and -14.8 3.11 mV, respectively. The dissolution rate and permeability of curcumin and quercetin were significantly enhanced when loaded into SNEDDS pellets. The developed SNEDDS formulation showed improved bioavailability and was able to normalize blood glucose levels, lipid profiles, antioxidant biomarkers, and tissue architecture in streptozotocin-induced diabetic rats. The study also highlighted the role of prebiotics and probiotics in reducing blood glucose levels and managing dyslipidemia in diabetic rats.

Tripathi et al.,2016 he talks about how quercetin, resveratrol, and genistein were added to a self-nanoemulsifying drug delivery system (SNEDDS) to increase their oral bioavailability and antioxidant potential. With a dimension of less than 200 nm and a polydispersity index (PDI) of less than 0.3, the optimized SNEDDS formulation was demonstrated. The DPPH scavenging assay revealed that the antioxidant-loaded SNEDDS exhibited similar antioxidant activity to the mixture of free antioxidants. Caco-2 cells quickly internalized the SNEDDS formulation after one hour of incubation. Rat pharmacokinetic analyses revealed that, in contrast to the free antioxidant suspension, the SNEDDS formulation dramatically raised the maximum concentration (Cmax) and area under the curve (AUC) of all three antioxidants. When compared to the free antioxidants suspension, the SNEDDS formulation showed an increase in the oral bioavailability of ~4.27 fold for quercetin, ~1.5 fold for resveratrol, and ~2.8 fold for genistein. When compared to free antioxidants, the new SNEDDS formulation showed improved resistance to tumor growth in a rat model of breast cancer generated by DMBA.

Gursoy, R.N., Benita, S et al., 2004 This review article highlights the recent advancements in SNEDDS technology aimed at enhancing the oral bioavailability of lipophilic drugs. The authors discuss various novel formulation approaches, such as the use of new excipients and advanced techniques to improve the efficiency of drug delivery. They provide an overview of the physicochemical characterization of SNEDDS, including parameters like droplet size, surface charge, and thermodynamic stability. The paper also examines the in vivo performance of SNEDDS, presenting data on their impact on drug absorption and bioavailability. This review is particularly useful for understanding the progress made in the field and the potential of SNEDDS in drug delivery applications.

Singh, B., et al. 2009 Singh and colleagues provide an in-depth review of Self-Emulsifying Drug Delivery Systems (SEDDS), a broader category that includes SNEDDS. The article covers the theoretical and practical aspects of SEDDS, discussing the principles of self-emulsification and the factors influencing the formation of stable emulsions. The authors review various formulation strategies, highlighting the selection of suitable excipients and the optimization of formulation parameters. They also discuss the characterization techniques used to evaluate SEDDS and the potential benefits of these systems in enhancing drug solubility and bioavailability. The review includes numerous case studies and examples of SEDDS formulations, providing a comprehensive overview of their applications in pharmaceutical technology.

Jain et al.,2013 The study conducted by Jain et al. (2013) centers on the methodical creation and description of a solidified self-nanoemulsifying drug delivery system (SNEDDS) intended for the oral administration of a combination therapy plan. The liquid SNEDDS and the solid carrier concentration in the lyophilization mixture were optimized by the authors using 3 2 full factorial design and extreme vertices mixture design. The created mixture exhibited immediate emulsification. It kept all of its quality characteristics even after six months of storage under accelerated stability conditions. When compared to free drug competitors, the formulation showed noticeably higher cellular absorption of quercetin and tamoxifen.

The polynomial equations for the analysis of the droplet size, PDI, and QT loading of the resulting emulsion following SNEDDS dilution are also covered in the publication. After dilution, the stability of the nano emulsions was assessed, and neither phase separation nor drug precipitation were seen. The formulation of the liquid SNEDDS required careful consideration of the oil to be used, and it was discovered that Capmul® MCM EP efficiently dissolved both quercetin and tamoxifen. With the goal of loading the most quercetin possible while lowering the surfactant concentration, the composition of the SNEDDS was optimized based on the attractiveness value.

Alfaro et al.,2022 created a SNEDDS to increase betulinic acid's (BA) bioavailability. When compared to free BA, SNEDDS-L and SNEDDS-C enhanced the bioavailability of BA by 15.24 and 15.53-fold, respectively. When compared to free BA, SNEDDS showed up to a 15-fold improvement in BA bioavailability in an in vivo investigation with Wistar rats. For 105 minutes, the SNEDDS nanoemulsions' particle size did not change under the small intestine phase circumstances simulation. Betulinic acid was added to the SNEDDS matrix in order to preserve the particle sizes and confirm the use of caprylic acid as an oil phase in self-nanoemulsifying systems. No interaction between the encapsulated BA and the SNEDDS excipients was confirmed by FT-IR tests.

Yin et al.,2017 created hemp oil-based nanoemulsions (NEs) with fewer surfactants to increase BCL's oral bioavailability. The high-pressure homogenization method was used to create BCL-NEs in order to lower the surfactant content. When BCL-NEs were evaluated against suspensions and traditional emulsions, they demonstrated a markedly improved oral bioavailability of BCL. Excellent intestinal permeability and transcellular transport capacity were demonstrated by BCL-NEs. BCL-NEs were discovered to have a low level of cytotoxicity, making them suitable for oral usage. BCL-NEs had a high entrapment effectiveness of 99.31% and a particle size of about 90 nm. Transmission electron microscopy and particle size analysis were used to characterize BCL-NEs. Using ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC-910FMS) analysis, the pharmacokinetic characteristics of BCL were ascertained. The cell uptake and internalization of BCL-NEs were examined using Caco-2 cells.

Kazi et al.,2020 developed and improved a self-nanoemulsifying drug delivery system (SNEDDS)-based combination oral dosage form for dapagliflozin and sitagliptin to treat type

2 diabetes mellitus. Using mixed glycerides, bioactive medium-chain/long-chain triglycerides oil, and non-ionic surfactants, the SNEDDS demonstrated outstanding self-emulsification capability with nanodroplets ranging in size from 50 to 66.57 mm. In comparison to the pure medicines, the SNEDDS demonstrated a greater capability for drug loading without precipitating in the gastrointestinal system and had increased antioxidant action. When compared to the commercial product, in vivo pharmacokinetic studies in rats shown a considerable improvement in drug absorption and bioavailability. Studies on mice with anti-diabetes showed that the combination dosage of sitagliptin and dapagliflozin utilizing SNEDDS significantly inhibited glucose levels.

The main method of evaluation employed to distinguish between excellent and subpar formulations was visual inspection. The goal of the trial was to create a liquid SNEDDS-encapsulated oral dosage form of sitagliptin and dapagliflozin that would have better anti-diabetic effects. When diluted with water, the formulations had a fine dispersion appearance, suggesting enhanced solubility and stable nanodroplets free of precipitation. The study also discussed measuring antioxidant activity using absorbance values. The development of the SNEDDS formulation was significantly influenced by the oil to surfactant mixing ratio. The formulation including black seed oil and mixed glycerides at a ratio of 3:7 with cremophor EL as a surfactant had the best drug solubility and superior aqueous dispersibility when sitagliptin and dapagliflozin solubility in anhydrous SNEDDS was assessed. The goal of the solubility tests was to boost the medications' loading capacity in the SNEDDS formulation in order to enhance oral bioavailability. The potential stability of the SNEDDS in an aqueous system was ascertained by means of zeta potential measurement. Zeta potential values for the SNEDDS formulations were found to be indicative of good electrical characteristics and dispersion stability. Cremophor EL, a surfactant, formed a protective coating over the droplets, which helped to stabilize the SNEDDS system. To investigate the release performance and lack of drug precipitation following dispersion of the SNEDDS formulation, dynamic dispersion tests were carried out. The outcomes demonstrated that, in simulated intestinal fluid, the SNEDDS formulations were able to maintain a high percentage of medication in solution for up to 24 hours without precipitating.

Zang et al.,2020 developed a super saturable self-nanoemulsifying drug delivery system (S-SNEDDS) to enhance the solubility and oral bioavailability of luteolin. The formulation for SNEDDS consisted of carylic/capric triglyceride, polyoxyl 35 hydrogenated castor oil, and polyethylene glycol 400. Hydroxyropyl methylcellulose (HPMC) K4 was determined to be the optimal precipitation inhibitor for luteolin-loaded SNEDDS. Luteolin S-SNEDDS formed a clarified nanoemulsion with a particle size of 25.60 m and a zeta potential of - 10.2 mV. S-

SNEDDS achieved an excellent in vitro dissolution of 99% in phosphate buffer at pH 6.8 with 0.5% Tween 80. In vivo pharmacokinetics study showed a significant increase (2.2-fold) in the oral bioavailability of luteolin in rats compared to conventional SNEDDS. SNEDDS can enhance drug absorption by lymphatic transport and GI drug absorption, improve drug absorption through P-gp inhibitory effect and the permeability of the intestinal barrier, and induce a high supersaturation concentration of LUT in the Gi tract. The interaction between LUT and HPMC K4M was determined through FT-IR spectroscopy and IH MR spectroscopy. The optimized SNEDDS formulation consisted of Crodamol GTCC, Kolliphor EL, and PEG 400 at a ratio of 20.1:48.2:31.7 wt. Luteolin has poor aqueous solubility and undergoes extensive first-pass metabolism, limiting its clinical applications.

Corrie et al., 2022 examined how to increase the oral bioavailability of curcumin (CUN) and explore the role of isomalt (GIQ9) as a pharmaceutical carrier for solid self-nanoemulsifying drug delivery systems (S-SNEDDSs). Loading factor, flow, and micromeritic property calculations were used to evaluate GIQ9's suitability as a carrier. Central composite design (CCD) was utilized to improve the formulation variables and create the S-SNEDDSs using surface adsorption approach. When compared to the naïve curcumin, the optimized S-SNEDDS demonstrated a notable improvement in oral bioavailability and dissolving rate. A formula was used to determine the CUN-S-SNEDDS's entrapment efficiency, and measurements of the droplet size, zeta potential, and PDI were made both before and after solidification. Microscopic, FTIR, XRD, and DSC analyses were performed to examine the chemical and physical characteristics of the optimized S-SNEDDS. Wistar rats were used in in vivo experiments to assess the pharmacokinetics of the improved formulation.

Agrawal et al., 2015 created lipizide S-SEDDS, an antidiabetic with inconsistent absorption because of its low water solubility. Using Syloid 244 FP as an adsorbing agent, they converted the chosen microemulsion system into solid SEDDS (S-SEDDS) by employing a phase diagram. The S-SEDDS from the Liquid SEDDS showed a little increase in globule size. Glipizide's dissolving qualities improved, according to dissolution studies, with over 85% of the medication released from SSEDDS in 20 minutes as opposed to 2.68% of pure drug in simulated stomach contents.Rats used in in vivo experiments showed that S-SEDDS was more effective than pure medication at controlling blood glucose levels.

Kim et al., 2013 observed increased fenofibrate oral bioavailability with S-SMEDDS. S-SMEDDS was created by converting optimized L-SMEDDS with dextran serving as an inert carrier substance. The optimized batch's average diameter was discovered to be 240 nm. They found that there was no discernible difference between the emulsions made from L-SMEDDS and S-SMEDDS in terms of the size distribution or mean droplet size. Pure drug powder showed lower dissolving rates than in vitro drug release trials. Studies on the characterization of solid states revealed the drug's molecular dispersion in the formulation.

Cerpnjak et al., 2015 reported a comparative study of L-SNEDDS and S-SNEDDS as a tool for solubility and dissolution properties enhancement of poorly water-soluble drug, naproxen. L-SNEDDS was transformed to S-SNEDDS by spray drying on maltodextrin used as a carrier. Results demonstrated remarkable improvement in solubility and dissolution properties of naproxen by preparing SNEDDS as compared to pure drug. About 99% of drug release was achieved in SNEDDS preparation in about 45 min which was remarkably greater than drug release of pure naproxen i.e., around 35% in 45 min. Solid state characterization studies of optimized formulations demonstrated conversion of crystalline drug to amorphous form.

Akhter et al., 2014 investigated SNEDDS of coenzyme CoQ10, a fat-soluble vitamin like compound with an aim to improve its dissolution properties and thereby oral bioavailability. Optimized formulation was then converted into solid form (SSNEDDS) by spray drying L-SNEDDS onto Aerosil 300. Dissolution studies exhibited faster drug release from the prepared formulations. Drug release of 97.5% was achieved in 1 h from S-SNEDDS as compared to only 0.36% drug release from CoQ10 powder. In-vivo studies suggested enhancement in drug absorption with 3.4and 5 fold increase in Cmax and AUC values respectively, for CoQ10 in S-SNEDDS as compared to CoQ10 powder. DSC and XRD studies suggested amorphous nature of CoQ10 in S-SNEDDS.

Patel et al., 2014 worked on development and optimization of S-SNEDDS of poorly water-soluble drug, nelfinavir mesylate with an aim to improve its dissolution rate and oral bioavailability. Scheffe's mixture design was utilized to optimize the amount of components in L-SNEDDS. Globule size, drug loading and percentage transmittance were taken as dependent variables. Optimized L-SNEDDS containing drug with average globule size of 12.80 mm was then adsorbed on Neusilin US to form SSNEDDS. In-vitro drug release study of prepared formulations exhibited remarkable improvement in dissolution properties of nelfinavir mesylate with more than 90% of drug release in 20 min. In-vivo studies in rabbits showed improvement in bioavailability of drug in L-SNEDDS and S-SNEDDS as compared to pure drug suspension. Stability studies for 3 months showed insignificant differences in the observed values.

Yeom et al., 2015 developed and refined a self-nanoemulsifying drug delivery system (SMEDDS) employing a Doptimal mixture design for the weakly water-soluble medication atorvastatin. The optimal combination of 7.16% Capmul MCM (Oil), 48.25% Tween 20 (surfactant), and 44.59% Tetraglyol (cosurfactant) significantly improved the atorvastatin's dissolving characteristics. In simulated stomach fluid, the optimized formulation demonstrated a 12.3-fold improvement in medication dissolving rate. Rat pharmacokinetic studies revealed that atorvastatin from SMEDDS had a rise in AUC and tmax of 3.4 and 4.3 times, respectively, in comparison to pure drug suspension.

El-Badry et al., 2014 Tadalafil is a weakly water-soluble medication. To improve its solubility and dissolution, researchers created SNEDDS by combining Capryol 90 as an oil, Triton X100 as a surfactant, and Transcutol HP as a cosurfactant. 64.7 nm was the smallest droplet size that was found. Dissolution investigations revealed a notable improvement in the tadalafil's dissolving characteristics, with 96.6% of the drug released from the optimized batch of SNEDDS and just 12.4% from the pure drug suspension during a 24-hour examination. They reported that the improved batch of tadalafil had a 1434-fold improvement in solubility.

Shah, S., et al., 2016 investigates the development and enhancement of SNEDDS for the oral delivery of curcumin, a drug having significant medical promise but low water solubility and restricted bioavailability. The writers go into great detail about the selection of oils, surfactants, and co-surfactants, as well as how to optimize each of these ingredients to create an effective SNEDDS formulation. The review also includes a range of characterization techniques, such as droplet size analysis, zeta potential, and in vitro dissolution studies. It also examines the in vivo performance of the optimized SNEDDS in enhancing the oral bioavailability and therapeutic efficacy of curcumin, demonstrating the potential of SNEDDS to mitigate bioavailability problems.

Shakeel et al., 2013 created ultra fine super SNEDDS of the poorly water-soluble medication indomethacin in an effort to enhance the drug's solubility and dissolving characteristics. They used Tween-80 as a surfactant, Transcutol-HP as a cosurfactant, and Labrafil as an oil. They stated that the formulations' globule sizes ranged from 8.7 nm to 23.8 nm. Significant improvements in the characteristics of solubility and dissolution were noted. 98.4% of the drug released from the improved formulation was seen in in vitro drug release. The developed formulations' solubility of the medication was 4573 times more than that of the pure drug, according to the results of solubility studies.

Pund et al., 2014 revealed that creating SNEDDS improved the dissolution of the medication cilostazol, which is weakly soluble in water. To investigate the effects of independent factors, such as the amount of oil (Capmul MCM), surfactant (Tween 80), and cosurfactant (Transcutol HP), on dependent variables, a 23 complete factorial design was used. SNEDDS in liquid form were stabilized through adsorption onto Neusilin US2. The globule size of the optimized formulation was 215.2 nm. The findings of investigations on solubility and dissolution indicated a notable improvement in the corresponding qualities. The solubility and dissolving efficiency at 30 minutes were reported to be 9.82 mg/mL and 83.3%, respectively.

Yoo et al., 2010 worked on lutein SNEDDS formulations; lutein is an active botanical ingredient that has a low water solubility. SNEDDS containing Phosal 53 MCT as an oil, Labrasol as a surfactant, and Transcutol HP as a cosurfactant were created to improve lutein solubility and dissolution. An optimized SNEDDS formulation including 25% oil, 60% surfactant, and 15% cosurfactant by concentration was presented by them. Liquid SNEDDS were converted to solid SNEDDS by adsorbing it onto Aerosil 200. A globule size of 93 nm was recorded with the enhanced formulation. The results of dissolve studies indicated a significant improvement in the formulation's lutein dissolving properties.

Villar et al., 2012 developed and enhanced SNEDDS of the water-insoluble antihyperlipidemic medication gemfibrozil. To optimize the formulation factors—the amount of oil (lemon oil), the amount of surfactant (Cremophor EL), and the amount of cosurfactant (Capmul MCM-C8)—they used the Box-Behnken experimental design. With the optimized formulation, the average droplet size was 56.5 nm. DSC research indicated that the medication might become amorphous. The findings of in vitro release investigations indicated a notable enhancement in gemfibrozil's dissolving characteristics. Weibull mathematical model release was followed by drug release.

Seo et al., 2015 attempted to improve the solubility and bioavailability of tacrolimus, a water-insoluble immunosuppressant, by developing a self-nanoemulsifying drug delivery system. Then, by spray drying onto colloidal silica, liquid SNEDDS were transformed into solid SNEDDS. Tacrolimus dissolved from SNEDDS most quickly in optimized formulations containing Capryol PGMC, Transcutol HP, and Labrasol (10:15:75% v/v/v). Rats used in in-vivo bioavailability experiments demonstrated quicker absorption of solid SNEDDS, with a two-fold increase in AUC® value when compared to commercial products.

Mohd et al., 2015 created SNEDDS of glimepiride, a medication that is poorly soluble in water, with the intention of enhancing its oral administration. They discussed how well it worked as a treatment on albino rabbits. Miglyol 812 (oil), Tween 80 (surfactant), and PEG 400 (cosurfactant) were used in the formulation of SNEDDS. It was discovered that the optimal formulation's average globule size was 152 nm. SNEDDS in liquid form were adsorbed onto Aerosol 200, a solid carrier, to create S-SNEDDS. Within 15 minutes, they reported that over 85% of the drugs were released. Research conducted in vivo shown a noteworthy improvement in therapeutic efficacy, with an AUC value of 234.64 for optimized S-SNEDDS. Studies on the characterisation of solid states revealed that the medication in the produced formulation was amorphous.

Yu, M., et al., 2017 explains the creation of a SNEDDS for berberine, a bioactive substance with low oral bioavailability and antidiabetic qualities. The authors give a thorough explanation of the formulation and optimization procedure, covering the choice of suitable excipients and the application of design of experiments (DoE) for formulation parameter optimization. A thorough description of the SNEDDS characterisation is given, including information on droplet size, stability, and zeta potential. The study also includes the findings of in vivo investigations showing that berberine administered by SNEDDS has better bioavailability and increased antidiabetic efficacy. The potential of SNEDDS in the creation of powerful oral formulations for bioactive substances is highlighted by this work.

Ali, J., et al., 2010 The authors concentrate on the development and assessment of SNEDDS for glibenclamide, a popular anti-diabetic medication with low water solubility. In order to produce a stable and efficient SNEDDS, the formulation process—which includes the choice and optimization of oils, surfactants, and co-surfactants—is fully explained. There is a thorough discussion of the characterization methods used, including droplet size analysis, zeta potential testing, and in vitro dissolving tests. Additionally, the review includes in vivo studies that show a notable increase in oral bioavailability and assess the pharmacokinetic properties of glibenclamide administered via SNEDDS. This paper offers insightful information about how SNEDDS may improve the effectiveness and delivery of poorly soluble medications.

Chapter 3

Aim and objectives

Aim and objectives

3.1. Aim of work:

The primary aim of this study was to design, develop, and evaluate a self-nanoemulsifying drug delivery system (SNEDDS) for improving the management of diabetes.

3.2. Objectives of the present work

The specific objectives were:

i) **Enhancement of solubility and bioavailability**

Improve the solubility and bioavailability of poorly water-soluble bioactive compounds, Rutin and EGCG, by encapsulating them in SNEDDS.

ii) **In-vitro evaluation and characterization**

Conduct in-vitro evaluations and characterization of the optimized SNEDDS formulations, including droplet size, stability, and interaction studies.

iii) **In-vivo efficacy study**

Evaluate the in-vivo efficacy of the best-developed SNEDDS formulation in a streptozotocin (STZ)-induced diabetic mouse model, focusing on blood glucose levels, body weight, and pancreatic histopathology.

Chapter 4

Materials and methods

4.1. Materials

The materials and tools used to create and analyse the rutin and EGCG loaded SNEDDS are listed in Table 4.1 and Table 4.2 respectively.

4.1.1. List of chemicals and equipment used in the creation and assessment of rutin and EGCG loaded SNEDDS.

Table 4.1: List of chemicals

S.No	Name of chemicals	Name of the suppliers
01.	Rutin	Simson Pharma Ltd, Mumbai
02.	EGCG	BLD Pharmtech Ltd, China
03.	Streptozotocin	Sigma-Aldrich Chemicals Pvt. Ltd., Bengaluru
04.	Glibenclamide	
05.	Tween 80	
06.	Transcutol P	
07.	Ascorbyl palmitate	
08.	Dimethylsulfoxide (DMSO)	
09.	Sunflower oil	Glentham® Life Sciences Ltd, United Kingdom
10.	Isomalt	Tokyo Chemical Industries, Tokyo, Japan
11.	Aerosil-200	Sisco Research Laboratories Pvt. Ltd, Mumbai
12.	Methano1 (HPLC grade)	Sigma-Aldrich Chemicals Pvt. Ltd., Bengaluru
13.	Acetonitrile (HPLC grade)	

4.1.2. List of instruments used for the development and evaluation of Rutin and EGCG loaded SNEDDS.

Table 4.2 List of instruments

S. No	Name of the instruments	Model and name of the manufacturer
01.	Electronic balance	Sartorius AG, Germany
02.	Hot air oven	VO-INC-13 Incon Engineers Ltd, Hyderabad, India
03.	Vacuum pump	DC-52 Torrlits Engineers Ltd, Kolkata, India
04.	Centrifuge	R4R-V /FM Plasto Crafts Industries Pvt. Ltd., Mumbai, India
05.	Magnetic stirrer	Tarsons digital spinot
06.	Vortex shaker	Tarsons digital spinot
07.	B.O.D Incubator shaker	BOD-INC-1S Incon Engineers ltd, Hyderabad, India
08.	HPLC	Prominence AD 30, Shimadzu, Japan
09.	Transmission electron microscopy	Philips EM430 TEM, USA
10.	Particle size analyzer and Zetasizer	MALVERN Instruments Model: - ZEN 1600
11.	Field emission scanning electron microscopy (FESEM)	FEI Model: Quanta 250 FEG
12.	X-ray diffractometer	Panalytical, Model: X'Pert Pro
13.	FTIR	PerkinElmer
13.	Deep freezer	Godrej EON
14.	Refrigerator	Godrej EON

4.1.3 Animals

Male Swiss Albino mice, aged 2-3 months and weighing between 25-30 grams on the day of the study, were utilized for the in vivo evaluation of the optimized formulation. These animals were procured from the West Bengal Livestock Breeding Centre in Kalyani, India. The mice were housed in polypropylene cages lined with husk and acclimatized to laboratory conditions for two weeks prior to dose administration. During this period, they were maintained at a temperature of 24 ± 2°C and a relative humidity of 45 ± 15%, with a 12-hour light/dark cycle. Only animals in good health were included in the study. They were fed a standard laboratory diet and provided with domestic mains tap water ad libitum. The mice were fasted for 12 hours before the experiment commenced. The experimental protocol received ethical clearance from the Institutional Animal Ethics Committee of the Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India [Project Proposal No.: **JU/IAEC-24/74 dated 09/04/2024**.]

4.2. Methods

4.2.1. Preparation of SNEDDS

FORMULATION 1 – 230822

A formulation was prepared to evaluate EGCG's solubility in transcutol and tween 80. In this formulation, there was no oil used.

S. No	Materials	Quantity
01.	Oil	Not used
02.	Drug (EGCG)	100mg
03.	Surfactant (Tween 80)	3ml
04.	Co-surfactant (Transcutol)	3ml
05.	Aerosil- 200	615 mg
06.	Double distilled water	40ml

Ratio of surfactant and co-surfactant = 1:1

Procedure:

i) Tween 80 and Transcutol were combined in a 1:1 ratio in a beaker and stirred using a magnetic stirrer at 1000 rpm at 45°C for 5 minutes.

ii) EGCG was then added to this mixture and stirred for 10-15 minutes or until the mixture became transparent.

iii) To the above mixture, 40 ml of double-distilled water (DDW) was added and stirred for an additional 5 minutes.

iv) The resulting liquid SNEDDS was transferred to a mortar, and Aerosil-200 was added. The mixture was triturated with a pestle until it formed a slurry.

v) This slurry was then transferred to a petri dish and stored at -20°C overnight. The following day, it was placed in a vacuum oven to dry the formulation.

vi) After drying, the formulation was weighed, and the decrease in weight was recorded until the weight remained constant.

FORMULATION 2 – 230825

Glyceryl trioleate Oil was used in the formulation., surfactant and co-surfactant ratio was 1:1.

S. No	Materials	Quantity
01.	Glyceryl trioleate oil	3ml
02.	Drug (EGCG)	150mg
03.	Surfactant (Tween 80)	1.5ml
04.	Co-surfactant (Transcutol)	1.5ml
05.	Aerosil- 200	3 g
06.	Double distilled water	40 ml

Procedure:

i) The specified quantities of oil, Transcutol, and Tween 80 were combined in a beaker and mixed thoroughly to form a homogeneous isotropic mixture using a magnetic stirrer.

ii) EGCG was added to the isotropic mixture and stirred for approximately 10-15 minutes or until the mixture became transparent.

iii) Next, 40 ml of double-distilled water (DDW) was added to the mixture, which was then stirred for an additional 5 minutes.

iv) The liquid SNEDDS was transferred to a mortar, and Aerosil-200 was incorporated. The mixture was triturated with a pestle until it formed a slurry.

v) This slurry was transferred to a petri dish and stored at -20°C overnight. The following day, it was placed in a vacuum oven to dry.

vi) After drying, the formulation was weighed, and the reduction in weight was recorded until it remained constant.

FORMULATION 3 – 230829

Sunflower Oil was used in the formulation, surfactant and co-surfactant ratio was 1:1.

Procedure:

Same as in **FORMULATION 2**

S. No	Materials	Quantity
01.	Sunflower Oil	3ml
02.	Drug (EGCG)	150mg
03.	Surfactant (Tween 80)	1.5ml
04.	Co-surfactant (Transcutol)	1.5ml
05.	Aerosil- 200	3 g
06.	Double distilled water	40ml

FORMULATION 4 – 230911

Isomalt was added to the formulation to solidify the L-SNEDDS. Other excipients and drug ratios similar to formulation 3

S. No	Materials	Quantity
01.	Sunflower Oil	3 ml
02.	Drug (EGCG)	350 mg
03.	Surfactant (Tween 80)	2.5 ml
04.	Co-surfactant (Transcutol)	2.5 ml
05.	Aerosil- 200	3 g
06.	Isomalt	6 g
07.	Double distilled water	40ml

Procedure:

Same as in **FORMULATION 2**

FORMULATION 5 – 230915

Quantity of Aerosil-200 increased, isomalt was decreased and aerosol-200 was increased.

S. No	Materials	Quantity
01.	Sunflower Oil	1.2ml
02.	Drug (EGCG)	250mg
03.	Surfactant (Tween 80)	2.5ml
04.	Co-surfactant (Transcutol)	7.5ml
05.	Aerosil- 200	6 g
06.	Isomalt	3 g
07.	Double distilled water	40ml

Procedure:

Same as in **FORMULATION 2**

FORMULATION 6 – 230922

Isomalt and aerosol- 200 were same ratio. Surfactant and co-surfactant were used as 1:1. Drug and oil amount were increased.

Procedure:

S. No	Materials	Quantity
01.	Sunflower Oil	3 ml
02.	Drug (EGCG)	350 mg
03.	Surfactant (Tween 80)	2.5 ml
04.	Co-surfactant (Transcutol)	2.5 ml
05.	Aerosil- 200	3.5 g
06.	Isomalt	3.5 g
07.	Double distilled water	40 ml

Same as in **FORMULATION 2**

FORMULATION 7- 231003

The formulation includes both rutin and epigallocatechin gallate (EGCG) in equal ratio. Additionally, Ascorbyl palmitate was incorporated into the formulation, dimethyl sulfoxide (DMSO) was added in same ratio as sunflower oil.

S. No	Materials	Quantity
01.	DMSO	1.2ml
02.	Sunflower oil	1.2ml
03.	Drug (Rutin)	175mg
04.	Drug (EGCG)	175mg
05.	Surfactant (Tween 80)	2.5ml
06.	Co-surfactant (Transcutol)	2.5ml
07.	Aerosil- 200	3.5g
08.	Isomalt	3.5g
09.	Ascorbyl palmitate	100mg
10.	Double distilled water	40ml

4.2.2. Dispersion test

To evaluate the dispersion characteristics of the self-nanoemulsifying drug delivery systems (SNEDDS), a dispersion test was performed. Initially, 1 mg of powdered SNEDDS from each formulation was accurately weighed and transferred into an Effendurf tube. The SNEDDS powder was then dissolved in 1 ml of distilled deionized water (DDW). The mixture was subjected to vortexing for 2-3 minutes to ensure complete dissolution and uniform dispersion. Following the vortexing process, the dispersion was allowed to stand, and observations were made at specific time intervals to monitor the formation of any precipitates. This step was crucial for assessing the stability and the potential for phase separation in the dispersion over time. The results from these observations provide insight into the behavior of the SNEDDS formulations in aqueous environments, which is essential for understanding their performance and stability in practical applications.

4.2.1.3 High-performance liquid chromatography (HPLC) analysis of SNEDDS

High-performance liquid chromatography (HPLC) is a widely used analytical technique for measuring, identifying, and purifying chemical substances. In this study, HPLC was employed to assess drug loading and monitor in vitro drug release. The HPLC system used was an Agilent 1260 series, equipped with a quaternary pump, autosampler, UV-Visible detector, and a thermostatted column compartment (TCC), ensuring precise and consistent analytical results.
Both UV detectors and diode array detectors (DAD) were utilized for analyte detection. The DAD offered detailed spectral data, which was helpful in confirming peak purity and identifying compounds. The mobile phase comprised a 50:50 mixture of Buffer (30 mM KH_2PO_4) and Acetonitrile, chosen for its ability to produce optimal separation and peak shape for the analytes. The flow rate of the mobile phase was consistently set at 1.0 mL/min, balancing resolution and analysis duration. The detection wavelength was 260 nm, selected based on the drug's maximum absorbance (λ max) to ensure accurate and sensitive quantification. EZ Chrom software was used for data collection and analysis, valued for its robust data management and user-friendly interface, facilitating efficient chromatographic data processing and interpretation.
The stationary phase was a C18 column, specifically a Phenomenex luna octadecylsilane column with dimensions of 250 mm x 4.6 mm and a particle size of 5 µm. The C18 column was chosen for its non-polar nature, making it ideal for separating hydrophobic compounds, such as the drug and its metabolites. Sample preparation involved the use of an ultrasonic bath

to ensure complete dissolution of the samples and standards, which enhanced the accuracy and reproducibility of the analysis. The samples and standards were precisely weighed using an analytical balance, which was essential for preparing accurate calibration curves and conducting the analysis. The HPLC method underwent rigorous validation to confirm its suitability for the intended purpose, with evaluations covering linearity, precision, accuracy, and robustness—critical parameters in validating analytical methods.

4.2.1.3.1 Estimation of drug loading (DL) and entrapment efficiency (EE)

The estimation of entrapment efficiency (EE) and drug loading (DL) is crucial for evaluating the performance of rutin and epigallocatechin gallate (EGCG) loaded self-nanoemulsifying drug delivery systems (SNEDDS). The entrapment efficiency was assessed by subjecting the SNEDDS formulation to centrifugation at 12,000 rpm for 10 minutes. This process facilitated the separation of the encapsulated drug from the free drug. The supernatant, containing the unentrapped drug, was carefully removed and subsequently analyzed using high-performance liquid chromatography (HPLC). For the drug loading assessment, the Rutin and EGCG loaded SNEDDS were directly analyzed by HPLC. The mobile phase used in the HPLC consisted of a mixture of acetonitrile and buffer (KH_2PO_4), selected for its compatibility and effectiveness in separating the analytes.

The entrapment efficiency (% EE) and drug loading (% DL) were calculated using the following equations:

$$\% \text{ EE} = \left[\frac{A-B}{A}\right] \times 100 \text{ - - - - - - } (1)$$

$$\% \text{ DL} = \left[\frac{C}{A+D}\right] \times 100 \text{ - - - - - - } (2)$$

Where:

- A represents the total amount of drug initially present in the SNEDDS formulation.
- B denotes the amount of drug that remains unentrapped in the supernatant after centrifugation.
- C is the total amount of drug added to the formulation.
- D signifies the total amount of excipients included in the formulation.

The entrapment efficiency (% EE) provides a measure of the proportion of the drug that is successfully encapsulated within the SNEDDS, while the drug loading (% DL) indicates the ratio of the drug to the total weight of the formulation, including excipients. These metrics are essential for determining the effectiveness of the drug delivery system and optimizing the formulation parameters to achieve the desired therapeutic outcomes.

4.2.1.3.2 In-vitro dissolution studies

In-vitro dissolution studies are essential for evaluating the release behavior of drugs from the formulated self-nanoemulsifying drug delivery systems (SNEDDS). For this study, the in-vitro drug release of the active compounds loaded in SNEDDS was conducted using a B.O.D. incubator shaker. The experiments were carried out in 250 mL conical flasks containing 200 mL of simulated gastric fluid (SGF) at pH 1.2 and 200 mL of simulated intestinal fluid (SIF) at pH 6.8, replicating the physiological conditions of the stomach and small intestine, respectively.

The samples were as follows- rutin and EGCG-loaded SNEDDS, pure EGCG and pure rutin. Precise amount of the mentioned samples was separately weighed and suspended in an appropriate volume of double-distilled water (DDW). The sample mixtures were then vortexed for 15-20 minutes using a magnetic stirrer, maintained at a temperature of 45°C-50°C, to ensure thorough mixing and uniform suspension. The resulting suspensions in DDW were carefully transferred into 10 cm dialysis bag (membrane) and securely tied at both ends to prevent leakage. These dialysis bags were then immersed in the respective conical flasks containing the SGF and SIF. The flasks were placed in the B.O.D. incubator shaker, which was set to maintain a constant temperature of 37 ± 0.5°C, closely mimicking human body temperature. The shaker was operated at a controlled stirring speed of 100 rpm to simulate the dynamic conditions of the gastrointestinal tract. At specific predetermined time intervals (01, 02, 04, 06, 08, 10, 12, 24, 48, 72, 96, 120, 144, 168 hours), 2 mL aliquots of the dissolution medium were carefully withdrawn from the conical flasks. These samples were immediately filtered through a 0.45 µm syringe filter to remove any undissolved particles or excipients. To maintain sink conditions and ensure consistent dissolution throughout the experiment, the withdrawn volume was replaced with an equal amount of fresh dissolution medium. The filtered samples, after appropriate dilution, were analyzed using HPLC to quantify the amount of drug released at each time point.

4.2.1.4 Characterization of rutin and EGCG-loaded SNEDDS

4.2.1.4.1 Dynamic light scattering (DLS) analysis

Dynamic Light Scattering (DLS) is a technique used to measure the size distribution of nanoparticles in suspension by analyzing how light scatters as particles move. It is commonly used to assess particle size and aggregation in nanoparticle formulations.

i) Particle size analysis

The particle size and size distribution of the self-nanoemulsifying drug delivery systems (SNEDDS) loaded with active compounds were thoroughly analyzed. To prepare the samples, each batch of SNEDDS was diluted 100 times with double-distilled water in a volumetric flask. The samples were then vortexed on a magnetic stirrer for 15-20 minutes at a temperature range of 45°C to 50°C. This process was essential to minimize any potential aggregation that might occur during the preparation.

Following preparation, the samples were subjected to particle size analysis using a particle size analyzer. The measurements were conducted at a temperature of 25°C, with the detection angle set at 90°. This method allows for precise determination of the particle size and size distribution within the SNEDDS formulation. Each experiment was performed in triplicate to ensure the accuracy and reproducibility of the results, and the average values were reported.

ii) Zeta potential measurement

The zeta potential, which reflects the surface charge and stability of the SNEDDS particles, was evaluated for all the formulations. The zeta potential values were determined by measuring the electrophoretic mobility of the particles using the same particle size analyzer employed in the particle size analysis. The sample preparation method was identical to that used in the particle size determination, ensuring consistency in the analysis.

To maintain the accuracy of the zeta potential measurements, the analysis was performed in purified water, which was adjusted to a standard conductivity of 50 µS/cm. This was achieved by adding a sodium chloride solution (0.9% w/v) to avoid fluctuations in zeta potential values due to variations in the conductivity of the water from day to day.

4.2.1.4.2 X-Ray diffraction (XRD) analysis

X-Ray diffraction (XRD) analysis was conducted to investigate the crystallographic patterns of the pure drugs (rutin and EGCG), the optimized SNEDDS formulation, and the blank SNEDDS. This technique is crucial for understanding the physical state of the drug within the formulation and any changes that might occur during the encapsulation process. The XRD analysis was carried out using a powder X-ray diffractometer under specific conditions. The instrument was set to a voltage of 45 kV and a generator current of 40 mA. The scanning parameters included a scan step time of 9 seconds per step and a scan step size of 0.008° (2θ). The scanning rate was maintained over a temperature range of 10°C to 80°C (2θ), providing a comprehensive analysis of the crystallographic properties.

This characterization allowed for the identification of any changes in the crystallinity of the active compounds when incorporated into the SNEDDS, as well as comparisons with the pure drug and blank formulations. The results from these analyses provide valuable insights into the structural integrity and stability of the SNEDDS formulations.

4.2.1.4.3 Electron microscopy

Electron microscopy is a powerful technique that enables both two-dimensional and three-dimensional characterization of materials at the nanoscale. The high resolution offered by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) makes these methods invaluable for non-destructive analysis of materials, allowing researchers to examine critical microstructural features with precision.

i) Transmission electron microscopy (TEM)

To investigate the droplet morphology of the selected self-nanoemulsifying drug delivery system (SNEDDS) formulation, TEM studies were conducted. A diluted sample of the SNEDDS (diluted 100 times) was placed onto a copper grid and allowed to air dry overnight to facilitate proper morphology observation. Subsequently, the copper grid was treated with a 1% w/v solution of phosphotungstic acid for 5 minutes at room temperature, which served to enhance the contrast of the imaging. The morphological characteristics were then captured using a transmission electron microscope operating at an accelerated voltage of 100 kV. This approach allows for detailed observation of the internal structure and droplet size of the SNEDDS, which is critical for understanding its behavior and efficacy in drug delivery.

ii) Field emission scanning electron microscopy (FESEM)

Field Emission Scanning Electron Microscopy (FESEM) is a crucial technique for analyzing the surface characteristics and particle size of powder SNEDDS. The process begins with the preparation of the SNEDDS powder, which is placed on a carbon tape mounted on an aluminum stub. To prevent charging under the electron beam, the sample may be coated with a thin layer of conductive material, such as gold or platinum.

In the FESEM chamber, the sample is subjected to a high-energy electron beam. This interaction causes the emission of secondary electrons, which are captured to generate high-resolution images. These images reveal detailed information about the surface morphology, including particle shape, size, and distribution.

FESEM analysis is vital for ensuring the consistency and quality of SNEDDS formulations. By examining the particle size and surface structure, we can optimize the formulation to enhance drug solubility and bioavailability, ultimately improving therapeutic efficacy.

By utilizing both TEM and FE-SEM, a comprehensive understanding of the morphology and surface characteristics of the SNEDDS formulations can be achieved, providing valuable insights for further development and optimization in pharmaceutical applications.

4.2.1.4.4 Spectroscopic analysis

4.2.1.4.4.1 Fourier-transform infrared spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was utilized to investigate the chemical structure and potential interactions between the bioactive compound and excipients in the SNEDDS (Self-Nanoemulsifying Drug Delivery System) formulation. For this analysis, a small portion of the SNEDDS formulation was placed on a potassium bromide (KBr) pellet, which served as the medium for the infrared analysis. The sample was then exposed to infrared radiation across a spectrum range of 4000 to 400 cm^{-1}, and the resulting FTIR spectra were captured to identify characteristic peaks that correspond to various functional groups within the formulation.

The FTIR analysis was primarily focused on identifying any shifts or alterations in peak positions that might suggest interactions between the bioactive compound and the lipid components of the SNEDDS. Such interactions are critical to the stability and effectiveness of the drug delivery system, as they can influence the drug's release profile, bioavailability, and overall therapeutic performance.

Specifically, the analysis targeted peaks related to functional groups such as hydroxyl (O-H), carbonyl (C=O), and amine (N-H) to determine if any significant interactions or chemical changes had occurred during the formulation process. If the FTIR spectra showed no new peaks or significant shifts, it would indicate that the bioactive compound remained stable and unchanged within the SNEDDS matrix, thereby confirming the compatibility of the formulation components.

4.2.1.5 In vivo studies

4.2.1.5.1 Experimental animal model

In this study, a streptozotocin-induced diabetic mice model was employed to evaluate the anti-diabetic efficacy of the encapsulated bioactive compound within the Self-Nanoemulsifying Drug Delivery System (SNEDDS). The streptozotocin (STZ) model was chosen due to its well-documented ability to induce a diabetic condition in mice, making it a reliable model for studying diabetes and potential therapeutic interventions.

Prior to initiating the experimental procedures, the study protocol received approval from the institutional animal ethics committee (IAEC) of the Department of Pharmaceutical Technology at Jadavpur University, Kolkata, India. The approval was granted under project proposal number **JU/IAEC-24/74, dated April 09, 2024**. This ethical clearance ensured that all experimental work adhered to the guidelines for the care and use of laboratory animals, maintaining high standards of animal welfare and research ethics.

This ethical approval was crucial in ensuring that the research was conducted with responsibility and integrity, providing a strong foundation for the study's exploration of the anti-diabetic potential of the encapsulated bioactive compound within the SNEDDS formulation.

4.2.1.5.2 Study design

The animals were divided into 5 different groups, each receiving different treatments:

Group I: Control group

- Normal diet
- Normal saline

Group II: Disease control group

- Normal diet
- Single intraperitoneal injection of STZ (60 mg/kg body weight)

Group III: Standard control group

- Normal diet
- STZ injection
- Standard drug (Glibenclamide) administered orally (2 mg/kg body weight) for 28 days [61].

Group IV: SNEDDS group

- Normal diet
- STZ injection
- SNEDDS administered orally (20 mg/kg body weight) for 28 days

Group V: Free drug group

- Normal diet
- STZ injection
- Free drug administered orally (100 mg/kg body weight) for 28 days

Table. 4.3. Groups of animals

Group 1	Group 2	Group 3	Group 4	Group 5	Total no. of animals
Normal	Disease Control	Standard control	SNEDDS (Rutin + EGCG)	Free drug treated	
05	05	05	05	05	25

4.2.1.5.3. Experimental induction of diabetes

Diabetes was experimentally induced in the mice following an overnight fast to ensure stable baseline glucose levels. The induction was performed using an intraperitoneal (i.p.) injection of streptozotocin (STZ), a compound known for its selective toxicity to insulin-producing beta cells in the pancreas. STZ was freshly prepared by dissolving it in a 0.1 M ice cold sodium citrate buffer, adjusted to a pH of 4.5, to maintain its stability and efficacy. Each mouse received a dose of 60 mg/kg body weight, administered under controlled conditions to ensure accurate dosing. Blood samples were collected from the animals both before the administration of STZ and seven days post-injection to monitor the induction of diabetes. The fasting blood glucose levels were measured to confirm the onset of diabetes. Mice with fasting blood glucose levels exceeding 250 mg/dL were considered successfully diabetic and were subsequently included in the study for further evaluation of the experimental treatments. This threshold ensured that only animals with significant hyperglycaemia, indicative of a diabetic state, were selected for the study.

4.2.1.5.4. Regular monitoring of body weight and blood glucose levels

Throughout the study, the body weight and blood glucose levels of the experimental animals were systematically monitored to assess the progression of diabetes and the effects of the treatment. These parameters were recorded weekly over a period of 04 weeks, providing a continuous assessment of the animals' health and metabolic status.

To measure blood glucose levels, blood samples were collected by carefully pricking the tail vein with a syringe needle. This minimally invasive method ensured that the animals experienced minimal discomfort during the sampling process. The blood glucose levels were then measured using the Accu-Chek Active blood glucose monitoring system, a reliable tool for obtaining accurate readings. This regular monitoring allowed for the early detection of any significant changes in the animals' metabolic condition, facilitating timely adjustments to the experimental protocol if necessary. By tracking both body weight and blood glucose levels, the study aimed to provide a comprehensive understanding of the effects of the treatment on the diabetic condition over the study period.

4.2.1.5.5. Drug administration

Two weeks following the induction of diabetes with streptozotocin (STZ), the experimental formulations were administered orally using an oral gavage technique. The dosage for each animal was carefully calculated based on their body weight to ensure accurate and consistent dosing. Fresh doses of the formulation were prepared daily, and the administration was performed once a day to maintain the efficacy of the treatment.

The animals, which were fasted overnight prior to the weekly monitoring, had their body weight and blood glucose levels recorded throughout the study.

4.2.1.5.6. Statistical analysis

All experimental data were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test to evaluate the significance of the differences between the groups. A p-value of less than 0.05 ($P < 0.05$) was considered statistically significant, indicating that the observed effects were not due to random variation. The data analysis was performed using GraphPad Prism software, which provided robust tools for statistical evaluation and graphical representation of the results.

Chapter 5

Results and Discussion

5. Results and Discussion

5.1. Preparation of EGCG Loaded SNEDDS

Formulation 1- 230822

A formulation was prepared to check the solubility of EGCG in tween 80 and Transcutol-P. Oil was not used in this formulation. Aerosil - 200 was used as a solidifying agent.

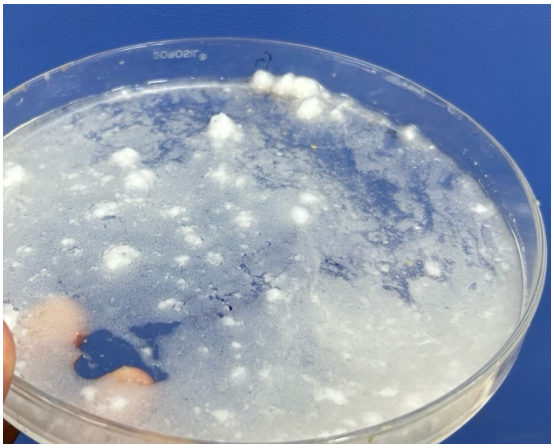


Fig. 5.1. Formulation 1- 230822

The formulation after preparation, storing in -20°C overnight and drying in vacuum oven for 3 days, it formed a sticky jelly like substance as shown in **Fig.5.1**.
The formulation was discarded.

Formulation 2 – 230825

Glyceryl trioleate oil was used in the formulation. The amount of aerosil-200 and EGCG was increased compared to formulation 1. Surfactant and co-surfactant ratio was 1:1.

Fig. 5.2. Formulation 2 – 230825

After drying for more than 7 days, the product exhibited agglomeration and did not maintain a free-flowing powder form, as illustrated in *Fig. 5.2*. Consequently, the formulation was discarded.

Formulation 3 – 230829

Dimethyl sulfoxide (DMSO) was incorporated into the formulation, with the surfactant and co-surfactant used in a 1:1 ratio.

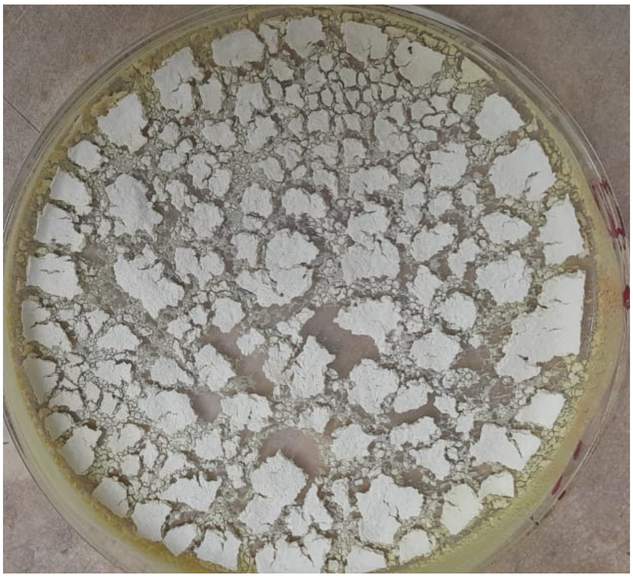


Fig. 5.3. Formulation 3 – 230829

The formulation closely resembled that of formulation 2. However, insufficient drying led to the formation of sticky agglomerates, solid cake, making the product unsuitable for further use. As a result, the formulation was deemed unsatisfactory and subsequently discarded.

Formulation 4 – 230911

Isomalt was incorporated into the formulation to aid in solidifying the L-SNEDDS (Liquid Self-Nanoemulsifying Drug Delivery System). The remaining excipients, as well as the drug-to-excipient ratios, were kept consistent with those used in formulation 3 to ensure uniformity in the formulation process.

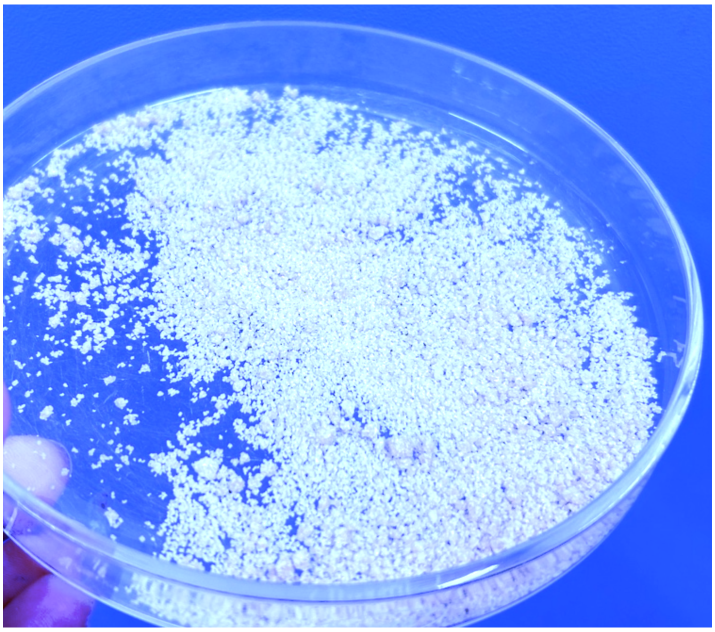


Fig. 5.4. Formulation 4 – 230911

The product showed stickiness and agglomerates. The formulation was deemed unsatisfactory and subsequently discarded.

Formulation 5 – 230915

The amount of Aerosil-200 was increased in the formulation, while the quantity of isomalt was reduced. This adjustment was made to enhance the formulation's flow properties and improve the overall stability. By increasing Aerosil-200, a commonly used glidant and anti-caking agent, the formulation aimed to achieve better consistency and prevent issues such as agglomeration or clumping.

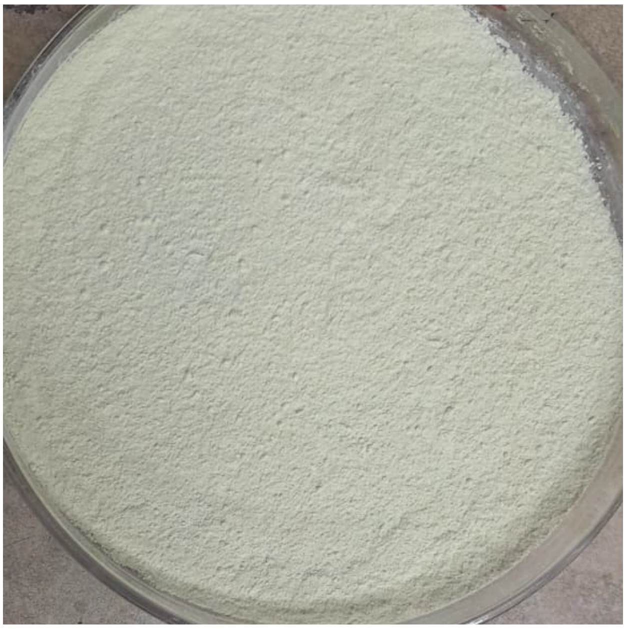


Fig. 5.5. Formulation 5 – 230915B

After drying more than 7 days Formulation 5 appeared to be free flowing powder.

Formulation 6 – 230922

The formulation maintained an equal ratio of isomalt and Aerosil-200, ensuring a balanced mix of excipients. The surfactant and co-surfactant were utilized in a 1:1 ratio to promote efficient emulsification. Additionally, the amounts of both the drug and the oil were increased to potentially enhance the drug loading capacity and improve the formulation's overall performance.

Fig. 5.6. Formulation 6 – 230815C

After more than 7 days of drying, Formulation 6 achieved a free-flowing powder consistency. Due to its improved flow properties, especially when compared to Formulation 5, this formulation was selected for further characterization studies.

5.1.2. Characterization of EGCG - SNEDDS

5.1.2.1. Particle size and polydispersity index (PDI)

The formulation labelled as 230922 underwent analysis for particle size and polydispersity index (PDI). The droplet size within the emulsion is a critical parameter influencing the self-emulsification process, as it directly impacts the rate and extent of drug release and subsequent absorption. The analysis revealed that formulation 230922, which incorporated Aerosil-200 and isomalt in a 1:1 ratio, exhibited an average particle size of 169.0 nm. Details of the particle size distribution, zeta potential, and PDI are presented in Table 5.1.

Table: 5.1: Particle size distribution, zeta potential and PDI for formulation 230922

Formulation	Avg. Particle Size(nm)	Zeta Potential	Polydispersity index
230922	169.0	-34.32	0.3121

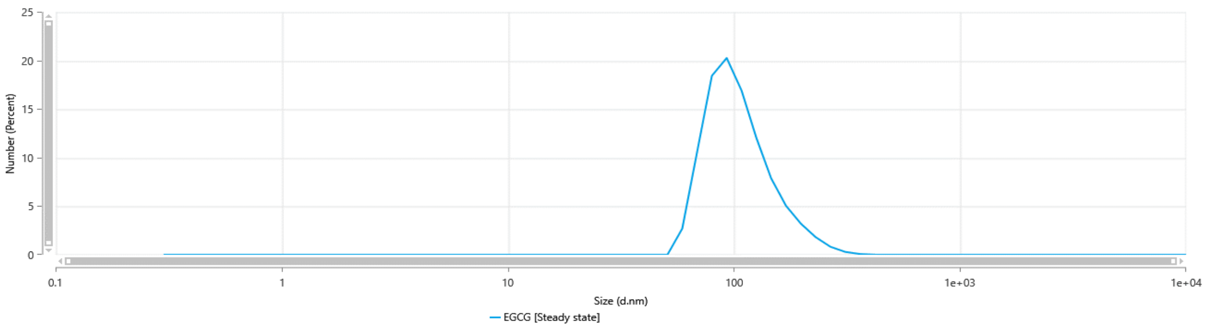


Fig. 5.7. Particle size graph of formulation 230922

Table 5.2 Particle size and PDI of formulation 230922

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Z-Average (nm)	169	-	-	169	169
Polydispersity Index (PI)	0.3121	-	-	0.3121	0.3121
Peak 1 Mean by Intensity ordered by area (nm)	176.2	-	-	176.2	176.2
Peak 1 Area by Intensity ordered by area (%)	100	-	-	100	100

5.1.2.2. Zeta Potential

Zeta potential measurements are essential for determining the surface charge of oil globules within the formulation, which plays a key role in assessing stability. A zeta potential value ranging from ±25 mV to ±30 mV generally indicates a stable formulation. In the case of Formulation 230922, which is an EGCG-loaded SNEDDS, the zeta potential was measured at -34.32 ± 0.5 mV, suggesting good stability. The corresponding zeta potential graph is illustrated in *Fig. 5.8*.

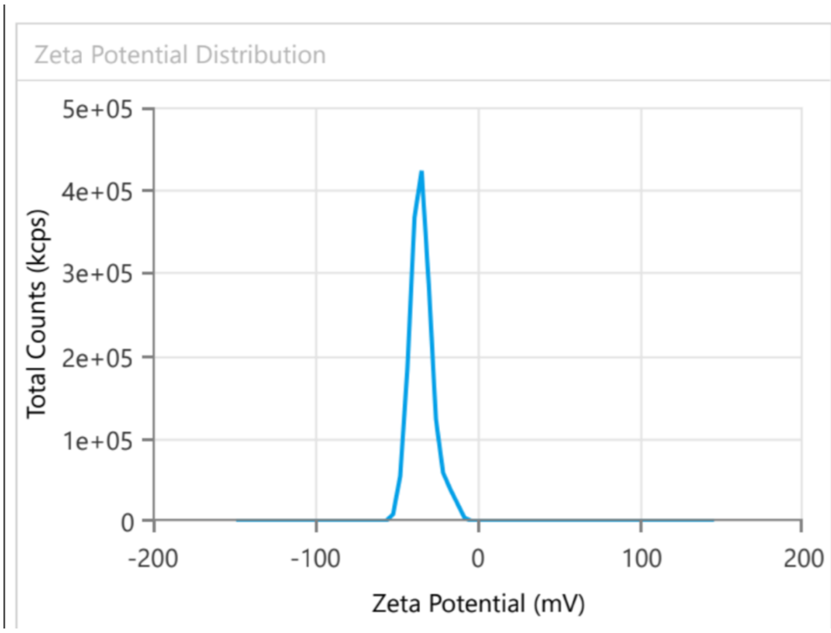


Fig. 5.8. Zeta potential graph of formulation 230922

Formulation 7 - 231003

This formulation contains rutin and epigallocatechin gallate (EGCG) in equal proportions. Ascorbyl Palmitate was also integrated into the formulation. Furthermore, Dimethyl sulfoxide (DMSO) was included in the same ratio as sunflower oil.

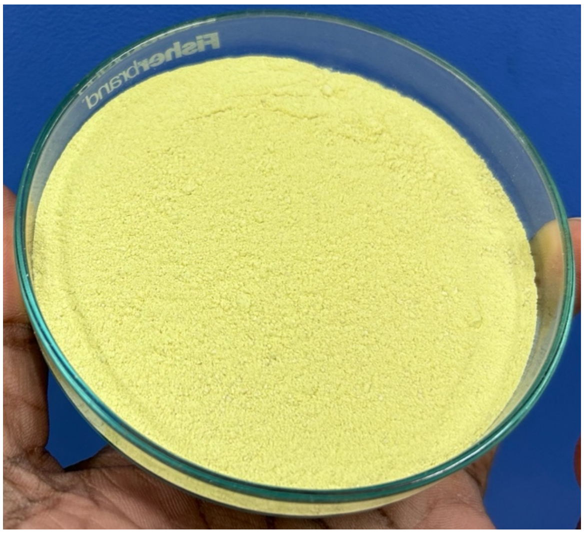
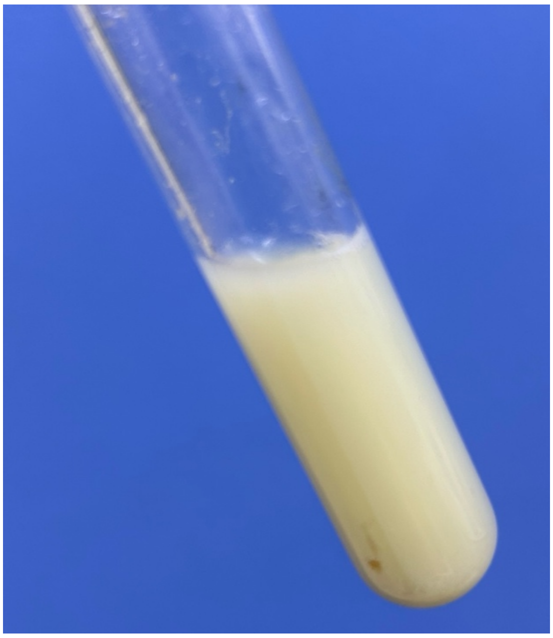


Fig. 5.9. Formulation 7 – 231003

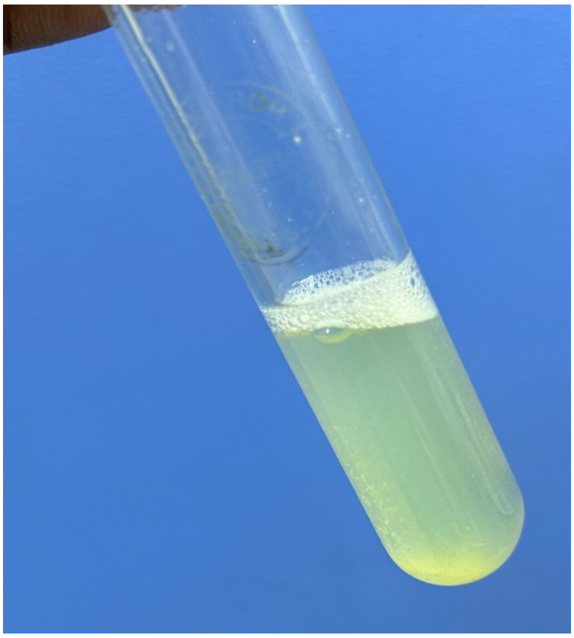
The final formulation of the Rutin-EGCG loaded SNEDDS was prepared, and after drying for 7-8 days, a constant weight was achieved. The resulting product was a free-flowing powder, making it suitable for further characterization studies.

5.1.2.3. Dispersion test

To assess the dispersion characteristics of the formulation 230922 and 231003, 1 mg of each powdered formulation was dissolved in 1 ml of distilled deionized water (DDW) in an Eppendorf tube. The mixture was vortexed for 2-3 minutes to ensure complete dissolution and uniform dispersion. The dispersion was then observed at specific intervals for precipitate formation, providing insights into the stability and behaviour of the SNEDDS formulations in an aqueous environment.



A



B

Fig. 5.10. Dispersion test of A) Pure EGCG and B) Rutin-EGCG SNEDDS.

It was observed that the subjected formulations exhibited minimal to no precipitation.

5.1.2.4. Estimation of drug loading (DL) & entrapment efficiency (EE)

5.1.2.4.1. EGCG encapsulated SNEDDS

The optimized EGCG-loaded SNEDDS demonstrated a significant enhancement in drug incorporation, achieving an entrapment efficiency of 87.31% and a drug loading capacity of 8.58%.

Table. 5.3. Entrapment efficiency of EGCG-loaded SNEDDS

Drug Loading	Entrapment Efficiency
8.58%	87.31%

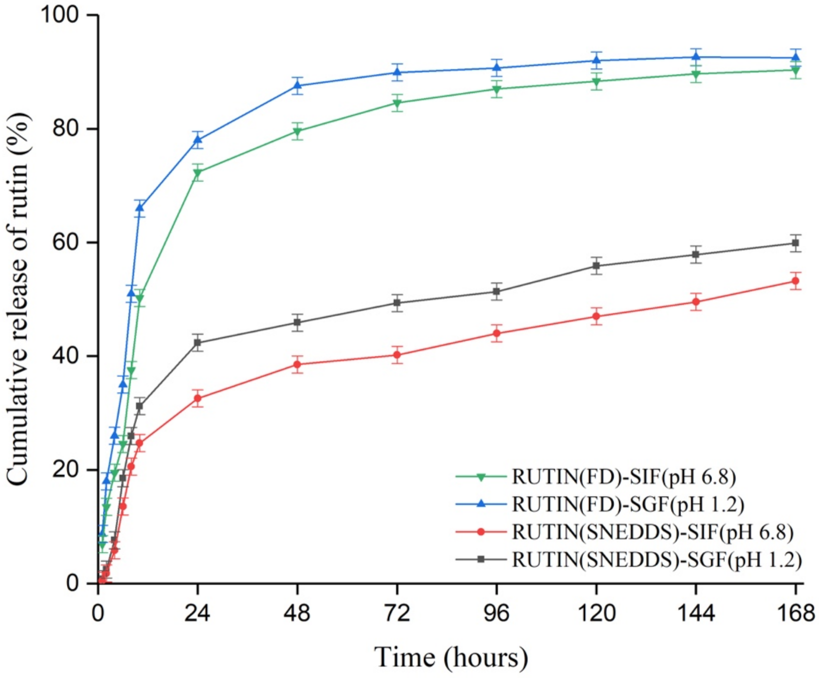
5.1.2.4.2. Rutin - EGCG encapsulated SNEDDS

The optimized Rutin-EGCG loaded SNEDDS achieved an entrapment efficiency of 86.39% for Rutin and 85.76% for EGCG. The drug loading capacities were 12.14% for Rutin and 11.53% for EGCG.

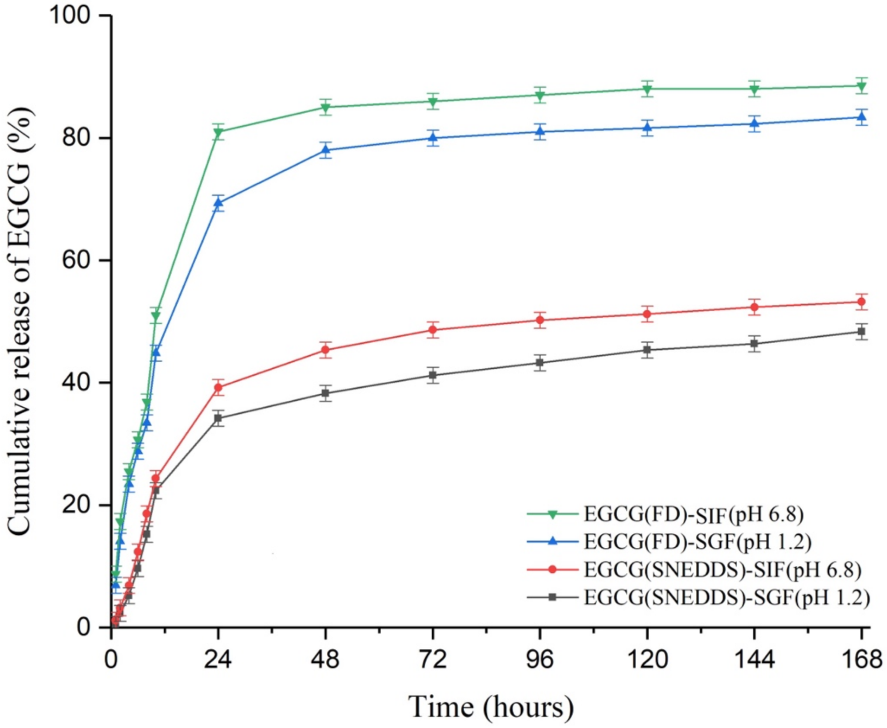
Table. 5.4. Entrapment efficiency of rutin-EGCG-loaded SNEDDS

Drug	Drug loading	Entrapment efficiency
Rutin	12.14 %	86.39%
EGCG	11.53 %	85.76%

5.1.2.5. In vitro dissolution studies



(A)



(B)

Fig 5.11. In vitro dissolution studies of rutin-EGCG co-encapsulated SNEDDS & rutin-EGCG free drug as denoted as A & B

5.1.2.6. Characterization of rutin and EGCG co-encapsulated SNEDDS

5.1.2.6.1. Particle size and polydispersity index (PDI)

Dynamic light scattering (DLS) is a widely employed technique for determining the hydrodynamic diameter of nanoparticles within colloidal systems, particularly in the submicron and nano ranges. Smaller particles offer a larger surface area, which enhances drug release through mechanisms like surface erosion and diffusion. Additionally, these smaller particles improve the ability of drug-encapsulated nanoparticles to penetrate and cross physiological barriers.

In the case of the prepared rutin-EGCG co-encapsulated SNEDDS, the mean hydrodynamic diameter was measured at 146.2 ± 3.1 nm. The polydispersity index (PDI) of the rutin-EGCG co-encapsulated SNEDDS was found to be 0.25. A PDI value below 0.3 indicates a narrow size distribution, whereas values above 0.3 suggest a broader range of particle sizes.

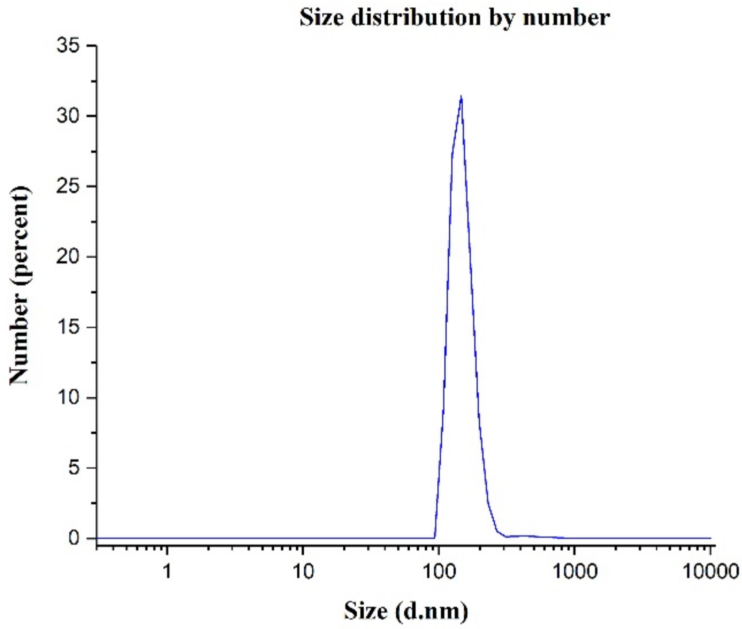
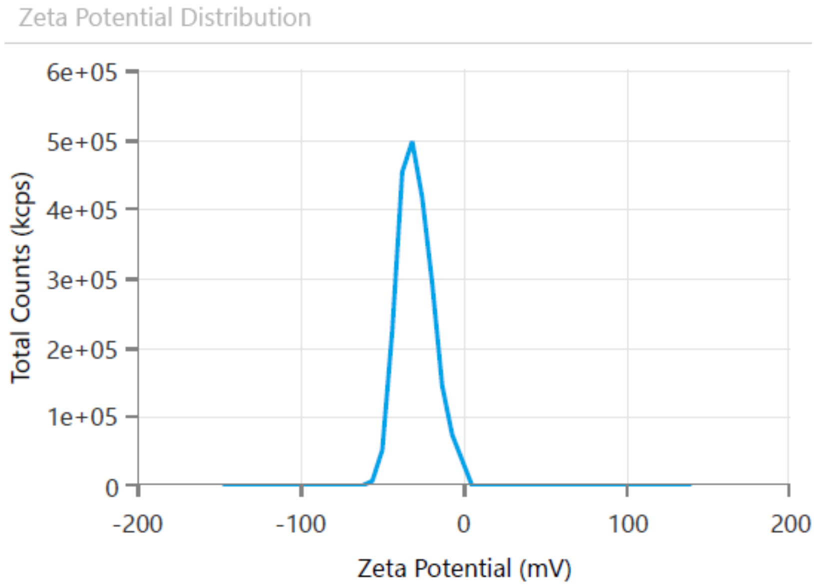


Fig 5.12. Particle size distribution rutin-EGCG co-encapsulated SNEDDS

5.1.2.6.2. Zeta Potential

The zeta potential of the rutin-EGCG co-encapsulated SNEDDS formulation was recorded at -29.06 mV. Zeta potential is an essential factor in evaluating the stability of colloidal systems, as it indicates the surface charge of nanoparticles. A zeta potential value around ±30 mV generally implies that the formulation is stable, as the electrostatic repulsion between particles minimizes the risk of aggregation. The measured zeta potential of -29.06 mV suggests adequate stability in this formulation, which helps to prevent particle agglomeration and ensures a consistent dispersion of the SNEDDS. This stability is vital for maintaining the bioavailability and effectiveness of the encapsulated rutin and EGCG, as well as for the formulation's longevity and practical use.



Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-29.06	-	-	-29.06	-29.06
Zeta Peak 1 Mean (mV)	-29.06	-	-	-29.06	-29.06
Conductivity (mS/cm)	0.0441	-	-	0.0441	0.0441
Wall Zeta Potential (mV)	0	-	-	0	0
Zeta Deviation (mV)	10.6	-	-	10.6	10.6
Derived Mean Count Rate (kcps)	1770	-	-	1770	1770
Reference Beam Count Rate (kcps)	1814	-	-	1814	1814
Quality Factor	1.65	-	-	1.65	1.65

Fig. 5.13. Zeta potential value of rutin-EGCG loaded SNEDDS.

5.1.2.7. X-ray diffraction (XRD)

The X-ray diffraction (XRD) pattern of rutin displayed several prominent, sharp crystalline peaks at various diffraction angles (2θ): 10.5°, 15.5°, 21°, 22°, 23.5°, 29°, 32°, and 33°, indicating its crystalline nature. Similarly, the XRD pattern of pure EGCG exhibited several distinct and intense peaks, highlighting its high crystallinity, with the most notable peak at 17.25°, and other significant peaks at 12.27°, 14.53°, 15.83°, 18.15°, 21.13°, 23.28°, and 24.69°.

In contrast, the XRD pattern of the EGCG and rutin co-encapsulated SNEDDS showed the absence of the characteristic peaks of both EGCG and rutin. This suggests a reduction in crystallinity or a transformation of the drugs into an amorphous phase within the nanoparticles. The loss of these peaks in the co-encapsulated SNEDDS indicates that both drugs have been successfully incorporated into the formulation. The XRD pattern of the physical mixture revealed intense but less pronounced peaks compared to the pure drugs, indicating that the crystalline structure of the drugs remained somewhat intact in the mixture. Conversely, the blank mixture showed no distinct peaks, reflecting an amorphous nature.

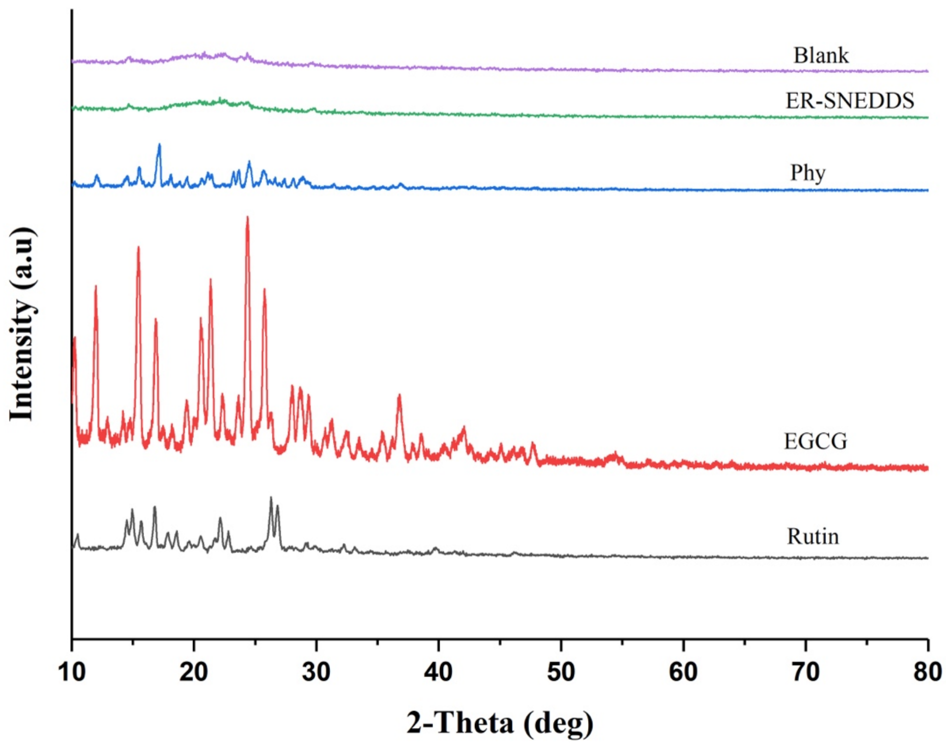


Fig. 5.14. XRD spectra of rutin-EGCG loaded SNEDDS, blank, physical mixture of the excipients, pure rutin and pure EGCG

5.1.2.8. FTIR analysis

5.1.2.8.1. The FTIR data for rutin reveals the following key functional groups:

i) **3419.35 cm⁻¹ (-OH stretching):** Indicates the presence of hydroxyl groups, common in rutin's polyphenolic structure, with broadness suggesting hydrogen bonding.

ii) **1649.36 cm⁻¹ (C=O stretching in ester):** Corresponds to the ester carbonyl groups in rutin, confirming its esterified structure.

iii) **1204.77 cm⁻¹ (C-O stretching):** Linked to ester or fatty acid groups within rutin, particularly in its glycosidic bonds [63].

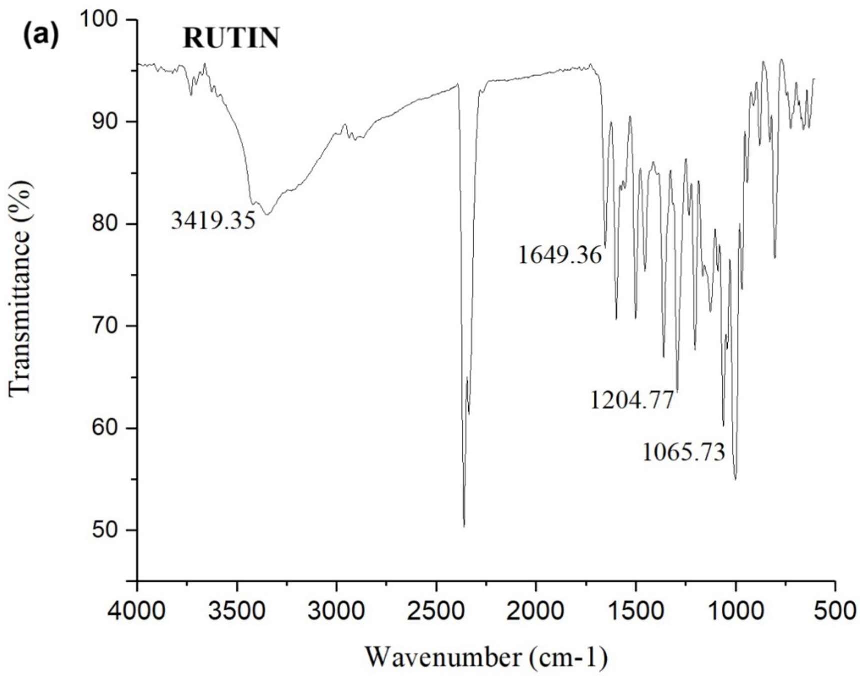


Fig. 5.15. FT-IR analysis of rutin.

These peaks confirm the presence of hydroxyl, carbonyl, and ester groups in rutin.

5.1.2.8.2. The FTIR spectrum of EGCG reveals the following key peaks:

i) **3350.72 cm⁻¹ (O-H stretching):** This peak corresponds to the hydroxyl (O-H) group attached to the aromatic ring in EGCG.

ii) **1688.20 cm⁻¹ (C=O stretching):** This strong peak is associated with the carbonyl (C=O) group that connects the trihydroxybenzoate and chroman groups.

iii) **1540.22 cm⁻¹ (C-C stretching):** This peak indicates the C-C stretching vibrations within the aromatic ring of EGCG [63].

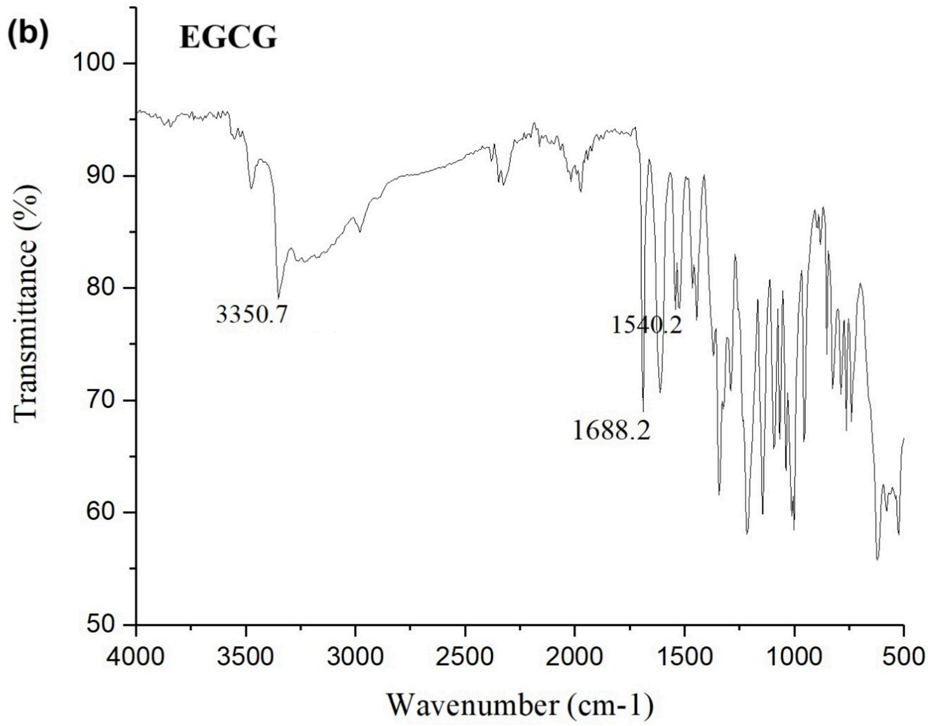


Fig. 5.16. FT-IR analysis of EGCG

5.1.2.8.2. The FTIR data for rutin and EGCG loaded SNEDDS:

The FTIR data for rutin-EGCG-loaded SNEDDS reveals several important peaks:

i) **3419.35 cm⁻¹ (O-H stretching):** This peak indicates the presence of hydroxyl groups from both rutin and EGCG. The broad nature of the peak suggests strong hydrogen bonding interactions within the SNEDDS formulation.

ii) **1649.36 cm⁻¹ (C=O stretching):** This peak is associated with the carbonyl (C=O) groups, likely from the ester linkages present in both rutin and EGCG. The specific wavenumber reflects the intact ester groups within the SNEDDS.

iii) **1065.73 cm⁻¹ (C-O stretching):** The peak at 1065.73 cm⁻¹ corresponds to the C-O stretching vibrations, which may be related to the ester bonds or glycosidic linkages in rutin and EGCG. This confirms the presence of these functional groups in the SNEDDS formulation [64].

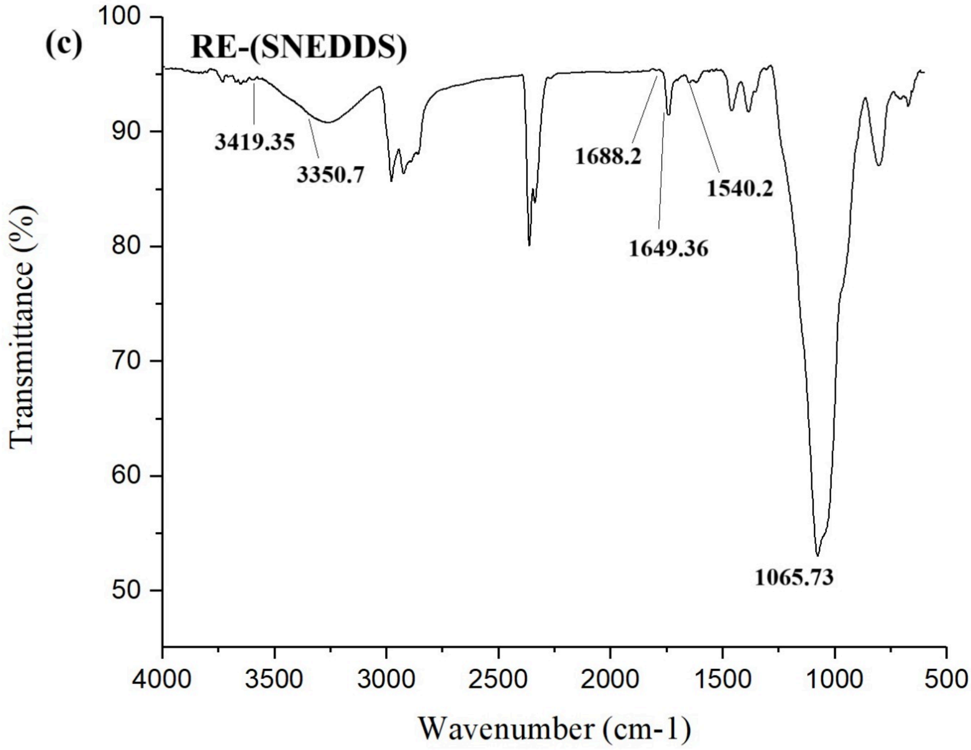


Fig. 5.17. FT-IR analysis rutin-EGCG co-encapsulated SNEDDS

These peaks confirm that both rutin and EGCG maintain their structural integrity within the SNEDDS, retaining key functional groups like hydroxyl, carbonyl, and ester bonds.

5.1.2.9. Transmission electron microscopy (TEM

The morphology of the synthesized rutin and EGCG co-encapsulated SNEDDS was analyzed using transmission electron microscopy (TEM). The particles were found to be spherical, with diameters ranging between 50 and 100 nm (see Fig. 5.15). The scale bar in the image represents 100 nm.

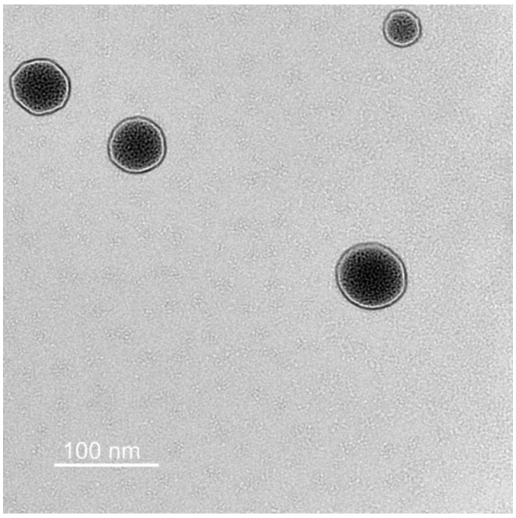


Fig. 5.18. TEM image of rutin-EGCG loaded SNEDDS

5.1.2.10. Field emission scanning electron microscopy (FESEM)

SEM studies were performed to examine the morphological characteristics of the optimized formulation. The SEM images of the rutin-EGCG loaded SNEDDS, presented in *Fig. 5.16*, show that the particles have a rough surface with porous and irregular apertures. These morphological features are likely due to the absorption of the Liquid SNEDDS during the formulation process.

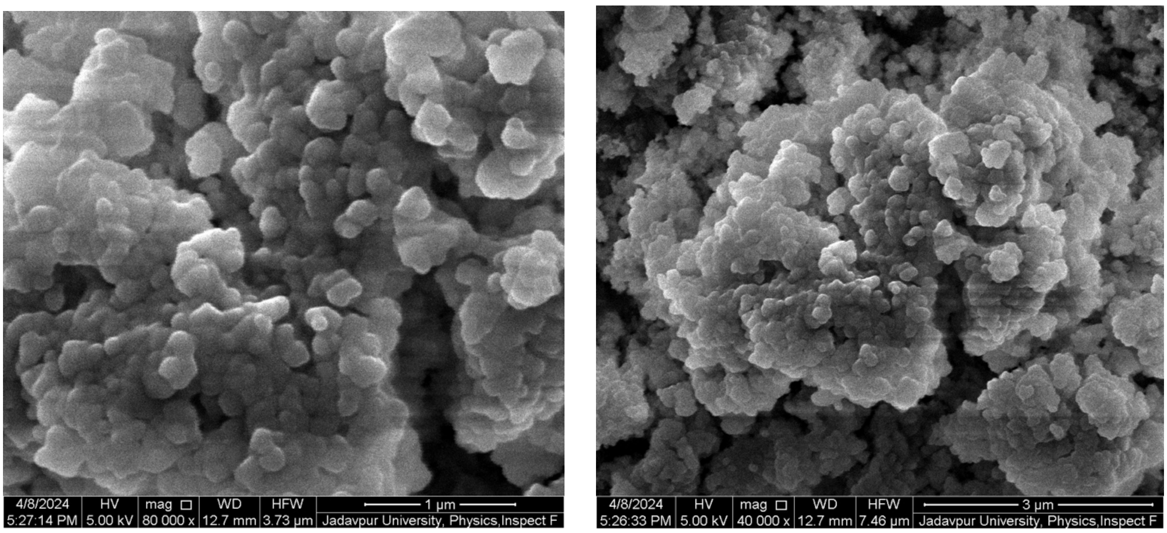


Fig 5.19. FESEM image of rutin-EGCG loaded SNEDDS

5.1.2.11. In vivo antidiabetic study

5.1.2.11.1. Impact of Glibenclamide (standard drug), free EGCG, free rutin, and rutin-EGCG co-encapsulated SNEDDS on blood glucose levels

The investigation revealed that EGCG and rutin co-encapsulated in SNEDDS significantly improved the management of blood glucose levels throughout the study. The optimized SNEDDS formulation notably reduced blood glucose levels compared to the STZ group. Conversely, the free EGCG and rutin did not show a significant reduction in blood glucose levels when compared to the optimized co-encapsulated SNEDDS, likely due to their limited solubility and dissolution.

By the third week, blood glucose levels in the optimized SNEDDS group were nearly equivalent to those observed in the standard drug group. By the fourth week, the optimized SNEDDS formulation effectively lowered blood glucose levels to a level comparable to both the normal control group and the standard drug group. Statistical analysis, performed using ANOVA followed by Dunnett's multiple comparison test in GraphPad Prism 5 software with a 95% confidence level, confirmed a significant difference between the normal control and the STZ group, verifying successful diabetes induction. Significant differences were also noted in the free drug group compared to the STZ group. Comparisons between the STZ group and both the standard drug and SNEDDS (EGCG and rutin) groups showed highly significant reductions in blood glucose levels, similar to those in the normal control group. In contrast, the free drug group did not exhibit significant differences in glucose-lowering effects compared to the STZ group. This suggests that EGCG and rutin should be delivered in the SNEDDS formulation rather than as free drugs. Blood glucose level data are detailed in ‘Table 5.2’.

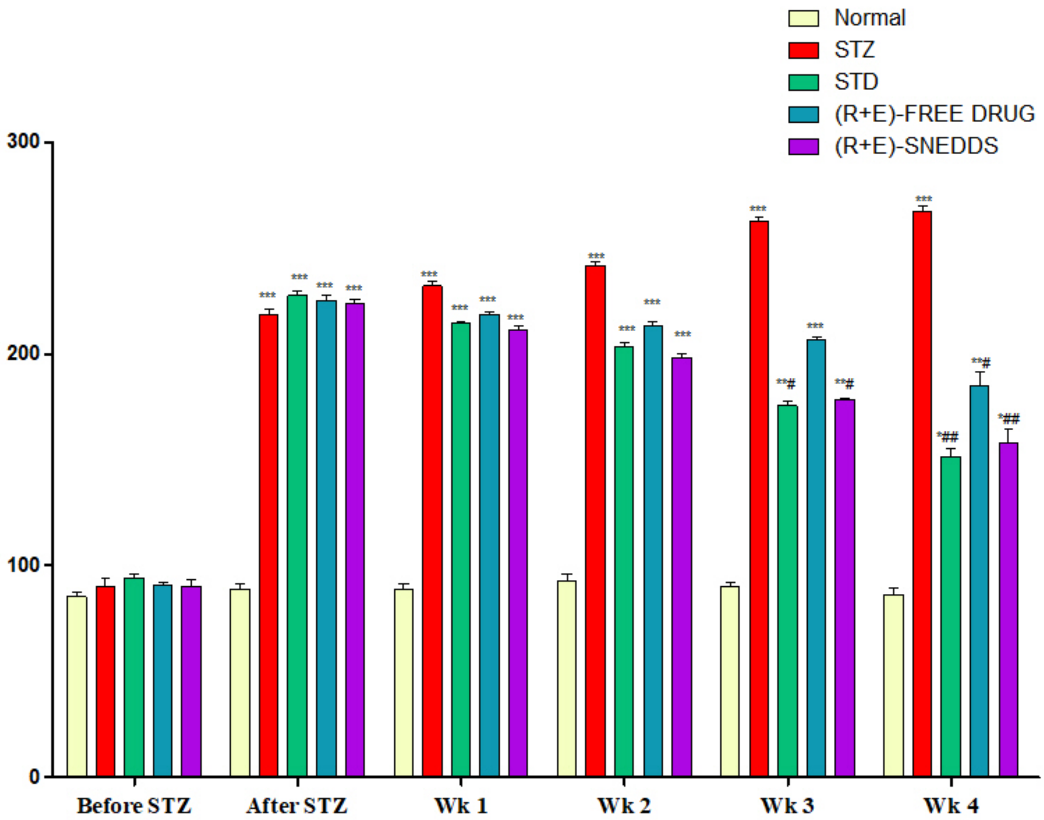


Fig 5.20. In vivo effects of Glibenclamide (standard drug), free EGCG, free rutin, and rutin-EGCG co-encapsulated SNEDDS on blood glucose levels.

Table 5.5. Blood glucose levels of mice

	Normal (mg/dL)	**STZ (mg/dL)**	**STD (mg/dL)**	**(Rutin and EGCG) Free drug (mg/dL)**	**(Rutin-EGCG) SNEDDS (mg/dL)**
Before STZ	85 ± 1.30	90 ± 2.55	94 ± 1.45	90 ± 1.45	90 ± 2.45
After STZ	89 ± 1.96	218 ± 1.88	227 ± 1.61	225 ± 1.45	223 ± 2.35
Week 1	88 ± 2.67	232 ± 1.30	214 ± 1.75	218 ± 2.34	211 ± 1.58
Week 2	92 ± 1.30	241 ± 3.48	203 ± 1.34	213 ± 1.48	198 ± 1.45
Week 3	90 ± 1.69	262 ± 1.66	175 ± 2.25	206 ± 1.30	178 ± 2.46
Week 4	86 ± 1.45	267 ± 1.30	151 ± 2.45	184 ± 1.80	158 ± 3.23

Chapter 6

Conclusion

Conclusion

The development of an EGCG and rutin co-encapsulated SNEDDS using sunflower oil, DMSO, Tween 80, Transcutol P, and Ascorbyl palmitate as excipients was successful. The use of a 1:1 ratio of isomalt to Aerosil 200 as the solidifying agent was crucial in optimizing the formulation, resulting in eight batches that were systematically evaluated for their dissolution and flow properties. The optimized SNEDDS exhibited a particle size of 146.2 ± 3.1 nm and a zeta potential of –29.06 mV, indicating strong particle stability due to high repulsive forces.

In vitro dissolution studies highlighted the enhanced drug release of both EGCG and rutin in the intestinal fluid, owing to the increased surface area available for absorption. The co-encapsulated SNEDDS demonstrated sustained drug release profiles, surpassing the performance of free drugs. The FTIR analysis confirmed the successful interaction of EGCG and rutin with the excipients, as evidenced by the disappearance of their characteristic absorption peaks. Additionally, XRD analysis revealed that the originally crystalline forms of EGCG and rutin transitioned into an amorphous state within the SNEDDS, further supporting their effective encapsulation.

These findings suggest that the SNEDDS formulation not only improves the dissolution characteristics of the bioactive compounds but also significantly enhances their bioavailability. Furthermore, the antidiabetic potential of the EGCG and rutin co-encapsulated SNEDDS was validated through four weeks of testing on STZ-induced diabetic mice, demonstrating superior efficacy compared to the free drugs. The study concludes that this novel drug delivery system holds promise for developing more effective and sustained treatment strategies for diabetes, with the potential for extended use without adverse effects. Future clinical trials will be essential to fully confirm these benefits in human patients.

Chapter 7

Summary

Summary

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycaemia, which arises either from inadequate insulin secretion by the pancreatic β-cells or from resistance to insulin action. The elevated blood glucose levels associated with DM lead to classic symptoms such as polyuria (excessive urination), polydipsia (excessive thirst), and polyphagia (excessive hunger). As the disease progresses, it can result in severe complications, including nephropathy, retinopathy, and cardiovascular diseases, ultimately leading to multi-organ failure.

The oral route remains the most commonly utilized method for drug administration due to its convenience and ease of use . However, oral administration of drugs often faces significant challenges, particularly for compounds with poor solubility. The low concentration gradient between the gastrointestinal tract and the bloodstream, combined with poor drug solubility, limits the drug's transport across the gut, thereby reducing its absorption. Drugs with low aqueous solubility and dissolution rates suffer from inconsistent bioavailability, high variability between and within subjects, and challenges in achieving dosage proportionality.

Many bioactive compounds, which are naturally occurring substances in plants, animals, and microorganisms, have been identified to possess therapeutic potential across a range of diseases, including diabetes, cancer, and inflammatory conditions . Despite their potential, the clinical application of these compounds is often hampered by their poor water solubility and low oral bioavailability.

Rutin, a flavonoid glycoside, is one such bioactive compound that has been extensively studied for its medicinal properties. Rutin exhibits a range of pharmacological effects, including anti-inflammatory, antioxidant, and potential anti-diabetic activities. It has also shown promise in hepatoprotective, nephroprotective, neuroprotective, immunomodulatory, and wound healing applications. However, the therapeutic potential of rutin is significantly limited by its poor oral bioavailability.

Similarly, epigallocatechin-3-gallate (EGCG), a major catechin found in green tea, is another bioactive compound with diverse therapeutic effects. EGCG has been shown to be effective in managing diabetes, enhancing exercise performance, treating inflammatory bowel disease, addressing skin disorders, preventing hair loss, promoting weight loss, and reducing iron overload. Despite these promising medicinal properties, EGCG's clinical use is constrained by its low bioavailability, which is primarily due to its poor aqueous solubility and limited dissolution rate.

Addressing the challenge of poor bioavailability, particularly with bioactive compounds like rutin[56] and epigallocatechin-3-gallate (EGCG), necessitates the development of advanced formulations designed to enhance their dissolution properties and, consequently, their bioavailability. Improving the dissolution rate of poorly water-soluble compounds is crucial for ensuring consistent and effective absorption, ultimately leading to better therapeutic outcomes. Novel drug delivery systems have demonstrated significant potential in overcoming the solubility limitations of such compounds, providing viable solutions for enhancing bioavailability. Various strategies have been explored, with scalability and cost-effectiveness being essential considerations to ensure the practicality of these solutions for broader application.

Lipid-based drug delivery systems have garnered substantial attention for their ability to enhance the solubility and oral bioavailability of drugs, particularly those classified under BCS Class II, which are known for their poor water solubility. Among these systems, the self-nanoemulsifying drug delivery system (SNEDDS) has emerged as a particularly promising approach. SNEDDS formulations have gained interest in both academic research and commercial applications due to their efficacy in improving drug delivery.

SNEDDS is an isotropic mixture composed of oil, surfactant, co-surfactant, and the bioactive compound or drug. Upon encountering the aqueous environment of the gastrointestinal (GI) tract and under the influence of peristaltic movement, SNEDDS spontaneously forms fine oil-in-water (o/w) nano emulsions. The droplet size of these nano emulsions typically ranges from a few nanometres(~5nm) to less than 200 nanometres. This substantial reduction in particle size increases the interfacial surface area, which in turn enhances the dissolution of the encapsulated compound, thereby improving its bioavailability. Moreover, the presence of lipids and surfactants in the SNEDDS formulation further enhances permeability across biological membranes, contributing to the improved absorption of the bioactive compound.

By leveraging SNEDDS and other lipid-based delivery systems, this research aims to overcome the challenges associated with the poor bioavailability of rutin and EGCG, ultimately enhancing their therapeutic potential in various clinical applications.

Chapter 8

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