Development, characterization, and antidiabetic activity of curcumin and rutin-co-encapsulated self-nanoemulsifying drug delivery system (SNEDDS) in streptozotocin-induced diabetic mice

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Declaration of Originality and Compliance of Academic Ethics

As a requirement for earning my Master of Pharmacy, I, Mahesh Kumar, officially certify that this thesis includes both original research and a survey of past research. This document contains all of the information that was gathered and presented in compliance with ethical standards and academic regulations.

I further declare that I have cited and referenced any materials and results that are not original to my work, as required by these rules and conduct.

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CERTIFICATE OF APPROVAL

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This is to certify that Mr. Mahesh Kumar, B. Pharm. (2018-22), has carried out the research work on the subject entitled " **Development, characterization, and antidiabetic activity of curcumin and rutin-co-encapsulated self-nanoemulsifying drug delivery system (SNEDDS) in streptozotocin-induced diabetic mice.**" under my supervision of **Prof. Dr. L. K. Ghosh** in the Department of Pharmaceutical Technology of this university. He has incorporated his findings into this thesis of the same title, being submitted by him, in partial fulfilment of the requirements for the degree of Master of Pharmacy of Jadavpur University. He has carried out this research work independently and with proper care and attention to my satisfaction.

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Certificate

This is to certify that the project proposal no. **JU/IAEC-24/74** entitled **"Evaluation of antidiabetic activity of the combination of i) rutin & epigallocatechin gallate-loaded self nanoemulsifying drug delivery systems(SNEDDS) and ii) rutin & curcumin loaded self-nanoemulsifying drug delivery systems(SNEDDS)"** submitted by **Dr. Manas Bhowmik** has been approved/recommended by the IAEC of Department of Pharmaceutical Technology, Jadavpur University, in its meeting held on 09/04/2024 and 35 Swiss albino mice have been sanctioned under this proposal for a duration of next 12 months.

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LIST OF ABBREVIATION

1.	DM	Diabetes mellitus
2.	T1DM	Type1 diabetes mellitus
3.	HLA	Human leukocyte antigen
4	GAD	Glutamic acid decarboxylase
5	LADA	Latent autoimmune diabetes of adults
6	GLP 1	Glucagon-like peptide
7	GIP	Glucose dependent insulinotropic polypeptide
8	SGLT2	Sodium glucose transporter 2
9	SNEDDS	Self-nano emulsifying drug delivery system
10	HPMC	Hydroxypropyl methyl cellulose
11	PLGA	Poly lactic glycolic acid
12	BCS	Biological classification system
13	CUR	Curcumin
14	LDL	Low-density lipoprotein
15	BOD	Biological oxygen demand
16	SIF	Simulated intestinal fluid
17	SGF	Simulated gastric fluid
18	DHA	Docosahexaenoic acid
19	EPA	Eicosapentaenoic 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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INTRODUCTION

1. INTRODUCTION

1.1 Diabetes mellitus

Diabetes mellitus (DM), often known simply as diabetes, is a group of common endocrine diseases characterized by sustained high blood sugar levels. Diabetes is due to either the pancreas not producing enough insulin, or the cells of the body becoming unresponsive to the hormone's effects. Classic symptoms include thirst, polyuria, weight loss, and blurred vision. If left untreated, the disease can lead to various health complications, including disorders of the cardiovascular system, eye, kidney, and nerves. Diabetes accounts for approximately 4.2 million deaths every year, with an estimated 1.5 million caused by either untreated or poorly treated diabetes [1].

As of 2021, an estimated 537 million people had diabetes worldwide accounting for 10.5% of the adult population, with type 2 making up about 90% of all cases. It is estimated that by 2045, approximately 783 million adults, or 1 in 8, will be living with diabetes, representing a 46% increase from the current figures. The prevalence of the disease continues to increase, most dramatically in low- and middle-income nations. Rates are similar in women and men, with diabetes being the seventh leading cause of death globally. The global expenditure on diabetes-related healthcare is an estimated US $760 billion a year [2].

1.2 Types of diabetes

1.2.1. Type 1 diabetes mellites (T1DM)

T1DM accounts for 5 to 10% of diabetes cases and is the most common type diagnosed in patients under 20 years; however, the older term "juvenile-onset diabetes" is no longer used as onset in adulthood is not unusual. The disease is characterized by loss of the insulin-producing beta cells of the pancreatic islets, leading to severe insulin deficiency, and can be further classified as immune-mediated or idiopathic (without known cause). The majority of cases are immune-mediated, in which a T cell-mediated autoimmune attack causes loss of beta cells and thus insulin deficiency. Patients often have irregular and unpredictable blood sugar levels due to very low insulin and an impaired counter-response to hypoglycemia.

T1DM is partly inherited, with multiple genes, including certain HLA genotypes, known to influence the risk of diabetes. In genetically susceptible people, the onset of diabetes can be triggered by one or more environmental factors, such as a viral infection or diet. Several viruses have been implicated, but to date there is no stringent evidence to support this hypothesis in humans [3].

T1DM can occur at any age, and a significant proportion is diagnosed during adulthood. Latent autoimmune diabetes of adults (LADA) is the diagnostic term applied when type 1 diabetes develops in adults; it has a slower onset than the same condition in children. Given this difference, some use the unofficial term "type 1.5 diabetes" for this condition. Adults with LADA are frequently initially misdiagnosed as having type 2 diabetes, based on age rather than a cause. LADA leaves adults with higher levels of insulin production than type 1 diabetes, but not enough insulin production for healthy blood sugar levels [4].

1.2.2. Type 2 diabetes mellitus (T2DM)

T2DM is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. However, the specific defects are not known. Diabetes mellitus cases due to a known defect are classified separately. Type 2 diabetes is the most common type of diabetes mellitus accounting for 95% of diabetes. Many people with type 2 diabetes have evidence of prediabetes (impaired fasting glucose and/or impaired glucose tolerance) before meeting the criteria for type 2 diabetes. the progression of prediabetes to overt type 2 diabetes can be slowed or reversed by lifestyle changes or medications that improve insulin sensitivity or reduce the liver's glucose production.

Type 2 diabetes is primarily due to lifestyle factors and genetics. A number of lifestyle factors are known to be important to the development of type 2 diabetes, including obesity (defined by a body mass index of greater than 30), lack of physical activity, poor diet, stress, and urbanization. Excess body fat is associated with 30% of cases in people of Chinese and Japanese descent, 60–80% of cases in those of European and African descent, and 100% of Pima Indians and Pacific Islanders. Even those who are not obese may have a high waist–to–hip.

Dietary factors such as sugar-sweetened drinks are associated with an increased risk. The type of fats in the diet is also important, with saturated fat and trans fats increasing the risk and polyunsaturated and monounsaturated fat decreasing the risk. Eating white rice excessively may increase the risk of diabetes, especially in Chinese and Japanese people. Lack of physical activity may increase the risk of diabetes in some people [1,2].

1.2.3. Gestational diabetes

Gestational diabetes resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2–10% of all pregnancies and may improve or disappear after delivery. It is recommended that all pregnant women get tested starting around 24–28 weeks gestation. It is most often diagnosed in the second or third trimester because of the increase in insulin-antagonist hormone levels that occurs at this time. However, after pregnancy approximately 5–10% of women with gestational diabetes are found to have another form of diabetes, most commonly type 2 gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. Management may include dietary changes, blood glucose monitoring, and in some cases, insulin may be required [2]. Though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth, weight), congenital heart and central nervous system abnormalities, and skeletal muscle malformations. Increased levels of insulin in a fetus blood may inhibit fetal surfactant production and cause infant respiratory distress syndrome. A high blood bilirubin level may result from red blood cell destruction. In severe cases, perinatal death may occur, most commonly as a result of poor placental perfusion due to vascular impairment. Labor induction may be indicated with decreased placental function. A caesarean section may be performed if there is marked fetal distress or an increased risk of injury associated with macrosomia, such as shoulder dystocia [3].

1.3. Pathophysiology of diabetes mellitus

1.3.1. Pathophysiology type 1 diabetes mellitus

The hallmark of type 1 diabetes mellitus is the autoimmune destruction of insulin-producing cells in the pancreas caused by macrophages, CD4 and CD8, T lymphocytes, and islet infiltration.

Glutamic acid decarboxylase (GAD) in the pancreatic B cells is the target of most islet antibodies. Type 1 diabetes mellitus is associated with metabolic disruption due to insufficient insulin secretion resulting from the autoimmune death of pancreatic beta cells.

1.3.2. Pathophysiology type 2 diabetes mellitus

The pathophysiology of type 2 diabetes is primarily caused by reduced insulin secretion through malfunctioning pancreatic beta cells and impaired insulin action due to insulin resistance. These processes are broken down in type 2 diabetes. When insulin resistance predominates, the mass of beta cells has changed and is able to compensate for the excess insulin supply by increasing it. It also demonstrates the typical physiological effect of reduced insulin linked to low glucose concentration, which stimulates the secretion of glucagon by alpha cells. Based on the major events, it is thought that peripheral insulin resistance is primarily caused by a relative insulin shortfall, which is the result of an initial deficit in insulin secretion. Significant insulin resistance results in incompletely reduced hepatic glucose output, diminished fat's ability to absorb triglycerides, and decreased insulin-mediated glucose absorption in the peripheral tissues. A large increase in islet cells causes an increase in the amount of insulin secreted, which helps to overcome insulin resistance. Individuals diagnosed with type 2 diabetes exhibit either impaired fasting glucose or higher and accelerated endogenous glucose production.

1.4. Current antidiabetic drugs for treatment of diabetes mellitus

Antidiabetic drugs are classified into two categories:

1.4.1. Oral hypoglycemic agent

1.4.1.1. Sulphonylurea - Sulfonylureas were introduced in the 1950s and have been the main class of antidiabetic drugs used for Type II diabetes treatment. Sulfonylureas exhibit their hypoglycemic action by stimulating insulin secretion from β-cells. Treatment with sulfonylureas is only effective in patients who do not lack endogenous insulin. Insulin release is a result of the depolarization of β-cells due to enhanced calcium flux caused by the interaction between the sulfonylurea and cell membranes. Because of their long half-life and increased risk of hypoglycemia, which is difficult to reverse and usually requires hospitalisation [7].

E.g. first generation sulfonylureas - (tolbutamide, acetohexamide, tolazamide)

Second generation- (glyburide, glipizide and glimepiride)

1.4.1.2. Meglitinides- are known as non-sulfonylurea insulin secretagogues. Repaglinide was the first in this class to be introduced onto the market in 1998, followed by Nate glinide in 2001. These agents stimulate release of insulin from the β-cell by binding to a different receptor than sulfonylureas. Meglitinides promote insulin release by inhibiting potassium efflux via closure of ATP-regulated K and channels. This result in depolarization of the cell and opening of voltage-dependent Ca and channels, which increases influx of calcium into the β-cell and releases insulin [6,7].

E.g. Nateglinide, Repaglinide

1.4.1.3. Biguanide- The precise mechanism of action of biguanides is not completely understood However metformin shows hypoglycemic effects by decreasing hepatic gluconeogenesis and enhancing glucose uptake in peripheral tissue. The latter effect is related to improved insulin sensitivity in the muscles associated with reduction of circulating free fatty acid levels. Clinical trial reports showed that metformin therapy decreases the A1C value by 1.5 to 2%. Thus, metformin and sulfonylureas are equally effective in reducing A1C in Type II diabetes patients [2,3]

E.g. Metformin, phenformin

1.4.1.4. α- Glucosidase Inhibitors- Acarbose, an α-glucosidase inhibitor, was introduced in 1996. It is a pseudo tetrasaccharide with a maltose unit linked to an acarviosine unit in the active part of the molecule. The acarviosine moiety has a nitrogen linkage that is responsible for the high affinity towards the α-glucosidase. This α-glucosidase inhibitor competes with oligosaccharides for the catalytic enzymes. Acarbose inhibits the breakdown of oligosaccharides and disaccharides into monosaccharides [4,5].

E.g. Acarbose, Miglitol

1.4.1.5. Thiazolidinediones - they act by both reducing the glucose production in liver and Increasing insulin dependent glucose uptake into the muscle cells. They do not increase the Production of insulin. These drugs use with the combination of metformin or sulphonyl urea [8].
E.g. rosiglitazone, Pioglitazone

1.4.1.6 Dipeptidyl peptidase 4 (DPP-4) inhibitor - DPP-4 is a proline specific dipeptidyl. Aminopeptidase widely expressed in many tissues such as the liver, lung, kidney, intestinal brush border membranes, lymphocytes, and endothelial cells. This enzyme regulates the levels of several gastro intestinal hormones in the body including Glucagon-like peptide (GLP-1) and Glucose dependent insulinotropic polypeptide (GIP). GLP-1 has several beneficial effects for blood glucose control such as stimulation of insulin secretion, inhibition of glucagon secretion, and delaying gastric emptying [9,10].

E.g. sitagliptin, linagliptin

1.4.2. Insulin injection

Insulin's ability to restore health is so dramatic that its clinical pharmacology was initially described in biblical-like terms, such as "the raising of the dead." Beyond the miraculous pharmacology was the central role this hormone served in advancing our understanding of physiology, peptide chemistry, structural biology, immunoassays, cellular biochemistry, and rDNA-directed biosynthesis. These achievements constitute a phenomenal record that despite its age continues to grow.

1.4.2.1. Types of Insulin:

Fast-acting insulin (Insulin aspart, insulin lyspro)

Intermediate-acting insulin

Long-acting insulin analogs (Insulin glargine, Insulin detemir)

1.5. Limitation of oral hypoglycemic drug

Drug belonging to all of these categories have associate side effect. The most common problems with α-glucosidase function should be considered. Undigested oligosaccharides pass into the large bowl carbohydrates fermented by the flora in the large bowel cause flatulence, abdominal discomfort; and sometimes diarrhea [12]. The most common side effect of sulphonyl urea in recent decades, use of first-generation sulfonylureas has declined owing to a high incidence of adverse reactions. The prolonged action of chlorpropamide can produce severe hypoglycemia lasting for several days in patients with acute or chronic kidney disease. In addition, it can induce hyponatremia and water retention due to inappropriate secretion of antidiuretic hormone. Other side effects with first generation sulfonylureas are alcohol-induced flushing with tolbutamide and chlorpropamide [13]. The other side effect has been reported include cholestatic jaundice. The

most severe serious adverse event associated with metformin is lactic acidosis the occurrence is rare (about 0.03 cases per 1000 patient-years), but the mortality to Heigh. The meglitinide have diaphoresis, altered mental status, combative behavior, tremors, and confusion. These may be followed by increased CNS depression, seizures, and coma if the patient's blood glucose level continues to fall. Onset of symptoms is expected to be rapid (under 30 minutes) and of short durations (less than eight hours) [14]. α-glucosidase inhibitors will cause significant injury in acute overdose. Diarrhea and abdominal pain have been reported as adverse effects with therapeutic use and could be expected in the overdose situation. Similar to other α glucosidase inhibitors, miglitol appears to have no other clinically relevant extraintestinal effects. It is unlikely to produce hypoglycemia in overdose, but abdominal discomfort and diarrhoea may occur. The primary risk associated with acarbose appears to be hepatic injury from chronic therapy. Gliptin and glucose like peptide 1 (GLP1) analogues can cause pancreatitis while sodium-glucose transporter 2 (SGLT2) inhibitor can lead to urinary tract infection [15]. hence, there is a need of safer and more effective medicine for the treatment of diabetic mellites, weather it is synthetic or natural/ herbal origin.

1.6. Bioactive compound for the treatment of diabetes

Besides modern medication, traditional medicines have been used for a long time and play an important role as alternative medicines. According to WHO, a plant-based traditional system of medicine is still the chief support of about 75–80% of the world population mainly in developing countries having a diversity of plants. Traditional medicines are usually the first choice for primary healthcare of patients in developing countries because of better cultural acceptability, better compatibility with the human body and lesser side effects than modern therapies. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetics. More than 400 plant species having hypoglycemic activity have been available in the literature, however, investigating new antidiabetic drugs from natural plants has still been attractive because they contain phytoconstituents that demonstrate alternative and safe effects on the treatment of diabetes mellitus. Most plants contain bioactive components, such as phenolics, glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that have been improved as having antidiabetic activities [16]. Plant compounds have been shown to confer some protection

against the pathology of diabetes mellitus through the attenuation of inflammatory mediators. Therefore, this paper intends to review the most salient recent reports on the anti-inflammatory associated diabetes mellitus properties of phytochemicals and the molecular mechanisms underlying these properties [17]. Traditional medicine performed a good clinical practice and is showing a bright future in the therapy of diabetes mellitus. World Health Organization has pointed out this prevention of diabetes and its complications is not only a major challenge for the future, but essential if health for all is to be attained. Therefore, this paper briefly reviews active compounds, and pharmacological effects of some popular plants which have been widely used in diabetic treatment [18].

Herbal drug could be the best alternative for the conventical synthetic drug for the management of diabetic mellitus. various bioactive compound of the herbal plant e.g. Catechins from green tea curcumin from *Curcuma longa,* Mangiferine from *Mangifera indica, et*c. Have been reported for their antidiabetic activity. These formulations suffer from certain restriction. the bioactive compound has poor solubility hence low oral bioavailability limits the therapeutic activity. There is various approach of novel drug delivery have been investigated to formulate the herbal drug to improve their solubility and dissolution property. Various novel approach has been utilized to improve the characteristic of phytoconstituent.

1.7. Novel strategies for the treatment of diabetes mellitus

Antidiabetics are effortlessly accessible in the international market. Most of the oral hypoglycemics are available either in the form of tablets and capsules. However, these dosage forms offer various untoward effects/limitations like gastric irritation, diarrhea, loss of appetite, lactic acidosis in people with abnormal kidney or liver function due to gastrointestinal degradation, insolubility in water and do not comply with the safety and efficacy of patient [18].

Limitations associated with antidiabetics offered the opportunities for the investigators to come up with such delivery systems having improved therapeutic efficacy. Novel drug delivery carrier systems are developed to deliver antidiabetic drugs safely and more precisely to the specific site for scheduled period of time in a controlled manner for better therapeutic effectiveness and henceforth better control over DM [19].

Nano formulations not only improve solubility of the drug but also provide several other benefits such as reduced dose, rapid onset of action, sustained drug release, fewer side effects, targeted drug delivery, enhanced half-life of the drug, reduced patient variability along with improved bioavailability and may thus overcome many of the limitations of the current antidiabetics to cite a few examples; several studies have reported an improved area under curve (AUC) and higher bioavailability of sulfonylureas when delivered by nano formulations due to enhancement in their solubility. To overcome the permeability problems suffered by metformin, many sustained-release Nano formulations have also been reported [22].

Relatively higher intracellular uptake of nanoparticles (NPs) makes them the carriers of choice in delivering antidiabetic drugs. Moreover, they have a wide size range (1–1000 nm), covering a variety of structures, such as nanospheres, nanocapsules, nanodiamonds and nanofibers. They can be categorized as polymeric NPs, metal-based NPs, lipid-based NPs and biological NPs. Methods adopted to develop these nanostructures are emulsion solvent evaporation, solvent displacement, diffusion solvent evaporation, ionotropic poly-electrolyte pre-gelation, polyelectrolyte complexation, dispersion polymerization, interfacial polymerization of microemulsion, ionic gelation, free-radical precipitation/dis-pension, etc.

Various advanced lipid - based nano drug delivery approaches is liposome, niosomes, transferosomes, ethosomes, phytosomes, nano emulsion, solid lipid nanoparticle, and self-nano emulsifying drug delivery system have emerged. These methodologies have been designed to significantly improve the solubility, permeability and stability of compound.

1.8. Self-nanoemulsifying drug delivery system (SNEDDS)

In modern years, self-nanoemulsifying drug delivery systems (SNEDDS) are the most popular and commercially feasible lipid-based formulation approach for improving oral bioavailability of poorly water soluble and lipophilic drugs. SNEDDS are precisely defined as an isotropic multi-component drug delivery system composed of a synthetic or natural oil, surfactant, and co-surfactant that have a unique ability of forming fine oil in water micro- or nano-emulsion upon mild agitation followed by dilution in aqueous media such as gastro-intestinal fluid. As SNEDDS self-emulsifies in the stomach and presents the drug in minute droplets of oil (55 mm), it improves

drug dissolution through presenting a large interfacial area for partitioning of the drug between the oil and the git fluid. The other advantages include increased stability of drug molecules and ease of administering the final formulation as gelatin capsules (soft gelatin capsules in the case of liquid self-nanoemulsifying drug delivery system (L-SNEDDS) and hard gelatin capsules in the case of solid self-nanoemulsifying drug delivery system (S-SNEDDS) [23,24].

SNEDDS have been described as a blend of oils, surfactants, and cosurfactants or cosolvents. Following aqueous dispersion and mild agitation (such in GI tract), SNEDDS spontaneously form fine oil-in-water nanoemulsions with droplet size of 200 nm or below [25]. The spontaneous emulsification takes place when the entropy change favoring dispersion exceeds the energy required to increase the surface area of the dispersion. SNEDDSs have shown immense potential in overcoming limitations related to the oral administration of several compounds. Such limitations include low solubility in the GI tract, inconsistent dissolution, enzymatic degradation, and erratic intestinal absorption. Surfactants and lipid components used in SNEDDS can cooperate to enhance the GI absorption drugs. Furthermore, these components can be modified easily according to the need to make SNEDDS feasible for both hydrophilic and hydrophobic drugs. Recent studies have shown that SNEDDS could be effective oral drug carriers of peptides and proteins by preventing their GI degradation and improving their intestinal membrane permeability [26].

In comparison to other lipid nanocarriers such as nanostructured lipid carriers (NLCs), solid lipid nanoparticles (SLNs) or liposomes or solid dispersions, SNEDDS can be easily scaled up by mixing components with conventional equipment and then including the mixture in solid dosage form, i.e., capsule or tablet. Furthermore, drug-delivery-system-related issues such as a tendency to aggregate during the storage or to release the drug are not relevant to SNEDDS, as fine dispersion are directly produced in the GI tract. Therefore, SNEDDS display better pharmaceutical properties for enhancing solubility and oral bio-availability.

1.8.1 Advanced technology and trends of SNEDDS

1.8.1.1. Solid self-nano emulsifying drug delivery system (S-SNEDDS)

Conventional liquid SNEDDS (L-SNEDDS) are associated with some restrictions such as liquid drug-drug interactions, drug-excipient interactions and reactions between preconcentrate and

capsule shell, precipitation at lower temperature, high cost, palatability, manufacturing, handling and stability issues. Solidification of L-SNEDDS surpassed these limitations, joining the benefits of both traditional SNEDDS and the solid dosage forms. S-SNEDDS provide the advantages of improved solubility and bioavailability, control over manufacturing process, less overall cost, reproducibility, enhanced stability, robustness and scalability [27].

Adsorption of L-SNEDDS on some solid carrier is the most promising technique utilized for solidification. Aerosil, aeroperl, neusilin, coffee husk and avicel are frequently used solid carriers used to adsorb L-SNEDDS which can produce dry powder, granules and even tablets. Wet granulation, freeze drying, solvent evaporation, spray drying and extrusion spherization can be employed depending upon the physicochemical properties of excipients. Also, dry powders and small granules of S-SNEDDS can be filled in hard gelatin capsules [28].

1.8.1.2. Controlled release SNEDDS

SNEDDS can be employed as extended-release delivery systems for poorly water-soluble drugs. Various techniques used for controlled release SNEDDS include microencapsulation, polymer coating, sustained release pellets, and controlled release osmotic pump. Different polymers are used in the preparation of controlled release SNEDDS; such as, microcrystalline cellulose, hydroxypropyl methyl cellulose (HPMC), poly lactic glycolic acid (PLGA), and gelucire. From the study of Patil et al., an extended release was observed from felodipine SNEDDS developed using Aerosil 200 as the gelling agent, and the gelled SNEDDS were then entrapped in gelucire [30].

1.8.1.3. Targeted SNEDDS

SNEDDS have the ability to be a targeted drug delivery by surface functionalization. Nano emulsion droplets can be maintained in the circulation for a long duration of time. Cationic nano emulsions can also directly attach to the anionic membrane barrier. The lipid-based SNEDDS can be accepted by the liver and spleen, which is the simplest way to target these organs. Moreover, SNEDDS are preferentially useful for a drug targeting the lymphatic system and also have the opportunity for targeting macrophages. The surface tailor of the SNEDDS for stealth properties can be achieved by linking with hydrophilic polymers; such as pegylation by polyethylene glycol.

Active and passive targeting can be attained by attaching appropriate ligands; such as antibodies and peptides of the target receptors [31].

1.8.1.4. Self-double emulsifying drug delivery systems (SDEDDS)

Proteins and hydrophilic macromolecules are usually difficult to be administered orally in the form of SNEDDS. Therefore, SDEDDS are a promising technology that could resolve this problem. SDEDDS are w/o/w spontaneous emulsions that consist of hydrophilic surfactant and w/o emulsions where the w/o/w emulsions were spontaneously formed during dilution with water at mild agitation. SDEDDS are applicable for peptides, proteins, and other macromolecular drugs; such as, insulin and natto kinase. They can protect these macromolecules from enzymatic degradation in the gastrointestinal tract and improve drug efficacy.

1.8.1.5. Supersaturated SNEDDS

The reduction in the lipid content of the SNEDDS has resulted in the declining in vivo solubilizing capacity of the SNEDDS. Therefore, the drugs precipitate. Drugs which are more soluble in the surfactant than the lipid phase are at risk of precipitation because of a decrease in the solvent capacity of the surfactant upon dilution. For this reason, SNEDDS usually contain drug less than the equilibrium solubility. The supersaturated SNEDDS containing hydrophilic precipitation inhibitors have been developed to resolve this limitation. The hydrophilic precipitation inhibitor in supersaturated SNEDDS inhibits the nucleation process and subsequent drug precipitation in the gastrointestinal tract by achieving and sustaining a metastable supersaturated state. The polymeric precipitation inhibitors that are commonly incorporated in the SNEDDS include polyvinyl pyrrolidone, methyl cellulose, sodium carboxymethyl cellulose, and hydroxypropyl methyl cellulose, Super saturated SNEDDS could improve the stability, dissolution rate, and bioavailability of drugs in many studies; such as, simvastatin, silybin, paclitaxel, and hydrocortisone.

1.8.2. Mechanism of formation of SNEDDS

Self-emulsification occurs when the entropy change that favors dispersion is greater than the energy required to increase the surface area of the dispersion. The free energy of the conventional

emulsion is a direct function of the energy required to create a new surface between the oil and water phases. This can be described in Equation 1.

$$\Delta G = \Sigma N \pi r 2 \sigma \qquad \text{Eq. 1}$$

Where, ΔG is the free energy, N is the number of droplets of the radius r, and σ represents the interfacial energy. In case of the self-emulsifying systems, the energy required to form the emulsion is very low; therefore, the emulsification process can occur spontaneously. According to Wakerley et al. 24, the addition of a binary mixture (oil/nonionic surfactant) to water results in an interface formation between the oil and aqueous phases, followed by the solubilization of water within the oil phase owing to the aqueous penetration through the interface. This process would occur until the solubilization limit is close to the interface.

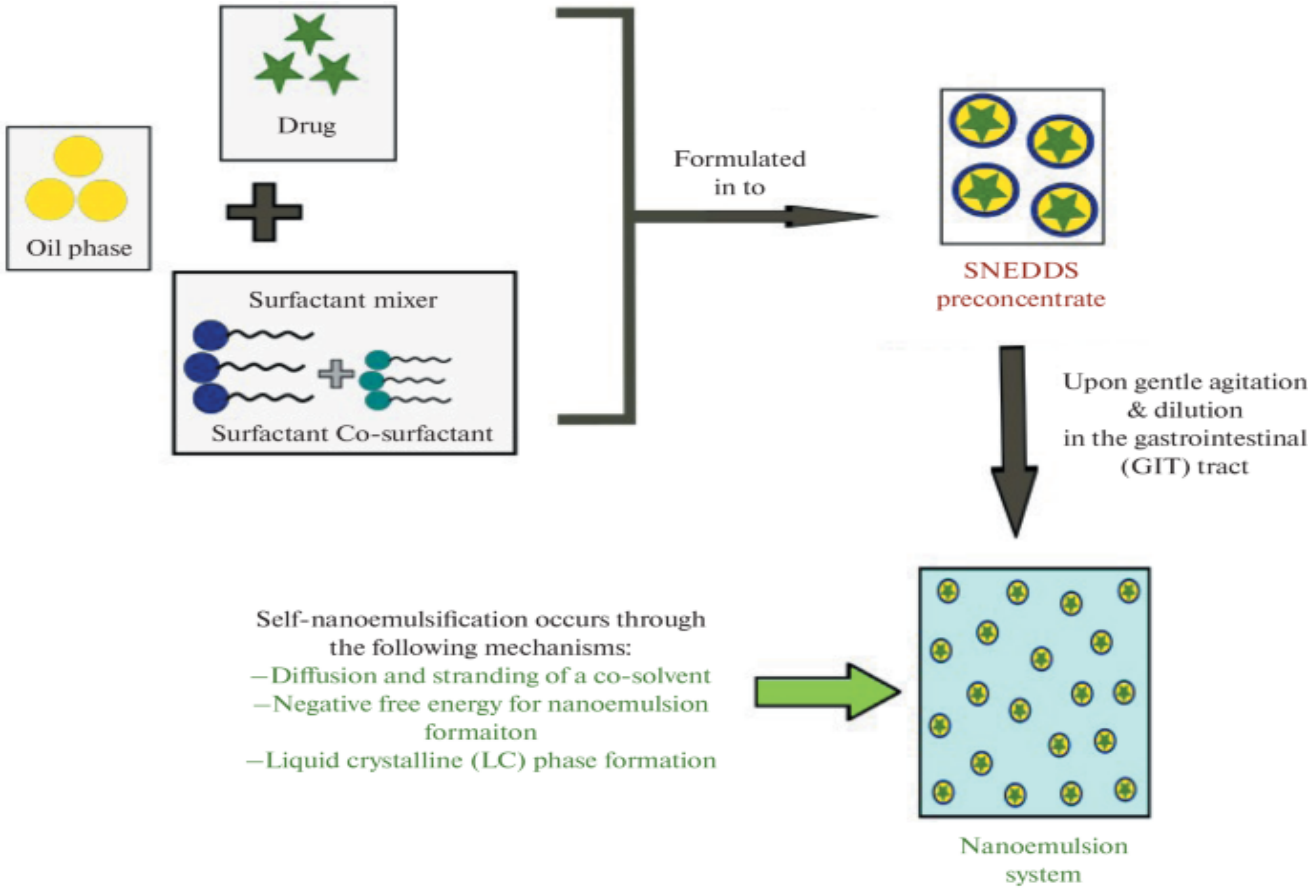


Fig. 1.1 Mechanism of formation of SNEDDS

1.8.3. Drug transport mechanism of SNEDDS

Even when taken orally, SNEDDS provide the bioavailability of water-insoluble medications. They go through three processes—digestion, absorption, and circulation—once they get to the git. Figure 1.2 shows these three stages. SNEDDS undergo enzymatic hydrolysis at the oil-water interphase during digestion, forming a coarse emulsion that prepares the absorption phase. The breakdown process comes to an end when mixed micelles are formed because of the interaction between fatty acids and bile. That's when the next stage of medication absorption begins. Through the enterocyte membrane, these colloids are either actively transported or passively diffused. Certain medications may be absorbed through chylomicrons in the lymphatic circulation. Drugs are liberated from chylomicrons during the circulatory phase, and the body uses the remaining lipid.

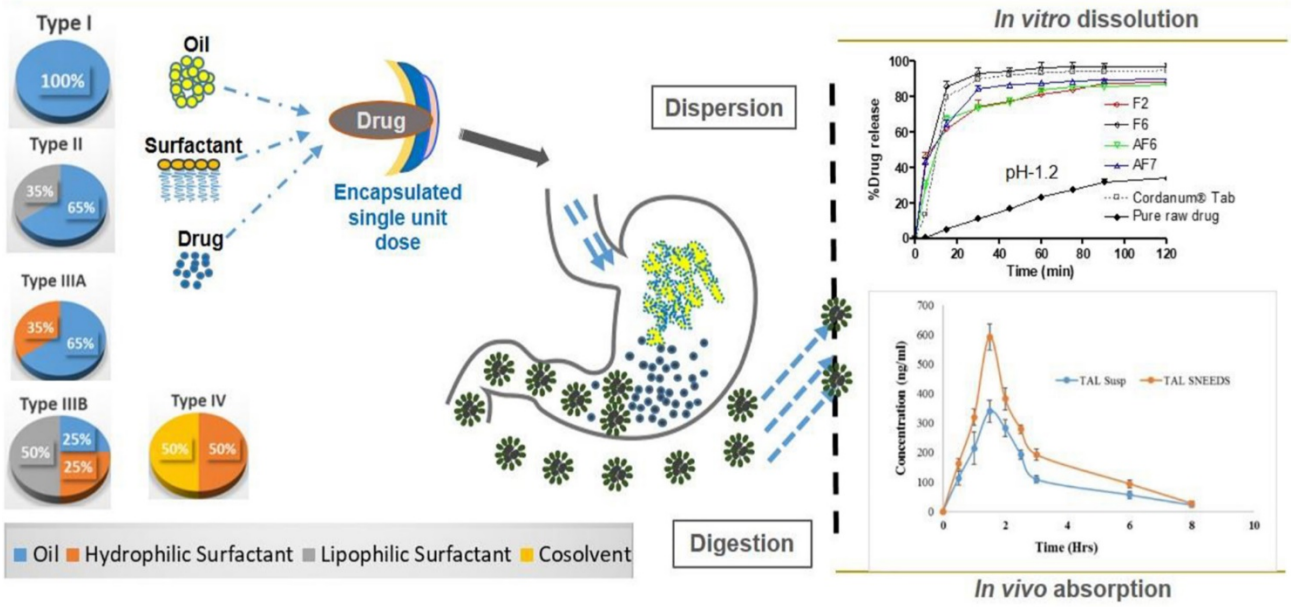


Fig 1.2 Transport mechanism of SNEDDS

1.8.4. Advantages of SNEDDS

Self-nano emulsifying drug delivery system (SNEDDS) have several advantages in pharmaceutical field some of them given below with supportive reference.

1.8.4.1. Improve the dissolution rate-limited absorption

Therapeutic agents with poor solubility in water; such as, drugs in the BCS classes II and IV usually have limited absorption in the gastro-intestinal tract according to their poor dissolution rate. The compositions used in the preparation of the SNEDDS providing solubilization potential for various hydrophobic drugs. Thus, a drug solubilized in the SNEDDS should produce a higher dissolution rate as compared to a pure drug. Furthermore, SNEDDS usually provide very fine nano emulsions after being presented in the gastrointestinal fluid. Have a high surface area for absorption the quick absorption and the improve oral bioavailability [33].

1.8.4.2. Improve the permeability of poorly permeable drugs

Therapeutic agents in the BCS class III have limited oral bioavailability according to their poor permeability property. Various compositions in the SNEDDS including oily phases, surfactants, and co-surfactants are known to help in the enhancing of the membrane permeability, subsequently providing the ability of the SNEDDS to improve the permeability and oral bioavailability of drugs [34].

1.8.4.3. Reduce the first pass metabolism or presystemic metabolism of the drugs

Various compositions of the SNEDDS have the potential to inhibit the activity of cytochrome P450 and gut metabolism enzymes. These compositions include gelucire (lauryl macrogol glycerides) and labrasol (caprylo-caproyl macrogol glycerides) Additionally, some lipid composition including long-chain tri- and mono- glycerides, i.e., glyceryl monooleate have the potential to stimulate the intestinal lymphatic transport of hydrophobic drugs. These two mechanisms are consequently associated with the reduction in the first pass metabolism of the drugs leading to an increase in oral bioavailability [33,34].

1.8.4.4. Reduce the first-pass metabolism or presystemic metabolism of the drugs

Various compositions of the SNEDDS have the potential to inhibit the activity of cytochrome P450 and gut metabolism enzymes. These compositions include gelucire (lauryl macrogol glycerides) and labrasol (caprylo-caproyl macrogol glycerides). Additionally, some lipid compositions including long-chain tri- and mono-glycerides, i.e., glyceryl monooleate have the potential to stimulate the intestinal lymphatic transport of hydrophobic drugs. These two mechanisms are

consequently associated with the reduction in the first-pass metabolism of the drugs leading to an increase in oral bioavailability [35].

1.8.4.5. Inhibit P-glycoprotein efflux

Some compositions of the SNEDDS including lipids; such as, Peceol (glyceryl monooleate), imwitor 742 (monoglyceride of caprylic acid), and akoline MCM (diglyceride of caprylic acid), and surfactants; such as, gelucire 44/14, labrasol, cremophor EL, and polysorbate 80 have the ability to inhibit the activity of a P-glycoprotein efflux pump, which is the intestinal efflux transporter. The entry of drugs from the gastrointestinal tract to the systemic circulation would be increased; thus, the bioavailability of drugs would be improved [33,35].

1.8.4.6. Reduction in food effects

The fed and fasted states dissolution media do not affect the droplet size of the SNEDDS. Therefore, the SNEDDS formulation could reduce the bioavailability ratio between the fed and fasted states and the reproducibility of the plasma profile of the drugs in fed and fasted conditions could be obtained. the bioavailability of probucol in minipigs showed that the probucol SNEDDS were not affected by the fed and fasted states. Alternatively, the variation in the fed and fasted states bioavailability was significantly observed in the probucol powder formulation [37].

1.8.4.7. Enhance mucus permeation

SNEEDS are the promising technology for mucus permeation. The interaction between self-emulsified nano emulsions and the mucus is low because of their hydrophobic surface that can cross the mucus layer without being entrapped. The very small vesicle size and shape deforming potential of self-emulsified nano emulsions help the SNEDDS to permeate through the mucus layer. The modification of the SNEDDS surface with positively or negatively charged surfactants could also result in the enhancement of mucus permeability [38].

1.8.4.8. Change in the pharmacokinetic parameters.

The improvement in oral absorption of the SNEEDS would result in the rapid onset of the action of drugs, which would be very beneficial for many drugs requiring quick action. In the study of Nepal et al. 56, the pharmacokinetic parameters of coenzyme Q10 SNEDDS were compared with a conventional formulation. The results showed that the tmax of the SNEDDS formulation was 6

h which was reduced by 2-fold as compared to the conventional formulation (12 h) indicating the quick onset of action of the SNEDDS formulation. Furthermore, the ability of the SNEDDS improved the Cmax from 86.6 ng/mL in a powder formulation to 480 ng/mL in a SNEDDS formulation and increased the oral bioavailability (AUC) from 1,110 ngh/mL in a powder formulation to 5,070 ngh/mL in a SNEDDS formulation. The results were related to the improvement in the therapeutic effects. An increment in the bioavailability of a drug might produce a reduction in the drug dosage and dose-related side effects of some drugs [37 38].

1.8.5. Commercially available products of SNEDDS

Table 1.1: Commercially available products of SNEDDS

Drug	Brand Name	Dose (mg)	Dosage form	Excipients
(Indication) Cyclosporine A (Anticancer)	SandimuneR (Novartis)	10-100	SGC	Corn oil, polyoxyethy lated 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
Hexamtrene (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

1.8.5. Formulation approach

SNEDDSs are multicomponent, homogeneous, anhydrous liquids that, after oral administration, under the mild agitation of digestive motility, spontaneously form translucent emulsions upon contact with gastrointestinal fluids. They consist of an oil or a lipid that is combined with a

surfactant or a blend of surfactants, a co-solvent, if required, and can incorporate a lipophilic drug. The variety of components that can be used for SNEDDS reveals the complexity of these systems and the almost endless number of possible combinations with each other. On the one hand, this innumerable combination variety tremendously increases the possibilities to develop a functioning SNEDDS formulation.

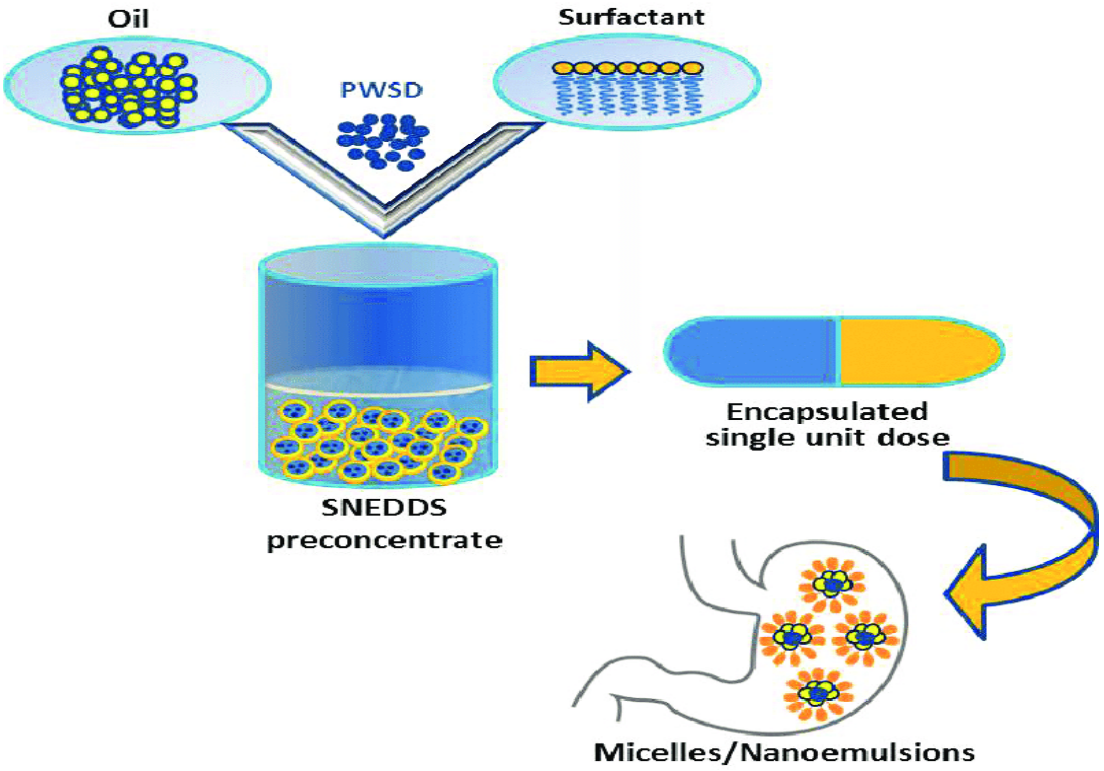


Fig 1.3 Formulation approach of SNEDDS

1.8.6. Component of SNEDDS

1.8.6.1. Oil phase

The oil phase is one of the most important compositions in the SNEDDS formulation. It has the function to solubilize the lipophilic drug, facilitate self-emulsification, and increase the fraction of lipophilic drug transport through the intestinal lymphatic system. Physicochemical properties of oil including the molecular volume, polarity, and viscosity potentially govern the spontaneity of the nano emulsification process, droplet size of the nano emulsions, drug solubility, and biological fate of the nano emulsions and drug. To achieve the maximum drug loading, the selected oil phase should have the maximum drug solubilizing potential [37].

1.8.6.2. DRUG

SNEDDS offer the potential to improve the oral bioavailability of drugs, preferentially those in BCS classes II and IV. The drug candidate should be a poorly water-soluble drug with an intermediate partition coefficient (log P between 2-4). The physicochemical properties; such as, log P, pKa, molecular weight, ionization group, and also the amount of drugs have an influence on the performance of the prepared SNEDDS including the phase behaviour and final droplet size. It has been further accepted that the drug candidate with a low melting point and low dose would be generally suitable for the preparation of the SNEDDS.

1.8.6.3. Aqueous phase

The properties of the aqueous phase where the SNEDDS are administered would affect the droplet size and stability of the nano emulsions. Therefore, the pH and ionic content of the aqueous phase should be taken into consideration during the preparation of the SNEDDS. The self-nano emulsification property and characteristic of the obtained nano emulsions of the SNEDDS in different pH and electrolyte concentrations of the aqueous phases should be evaluated in accordance with their route of administration.

1.8.6.4. Surfactant

The properties of the surfactants including the hydrophilic-lipophilic balance (HLB), viscosity, affinity for the oily phase, and concentration of the surfactants influence the self-nano emulsification process and droplet size of the nano emulsions. The surfactants are usually classified as anionic, cationic, and non-ionic. The non-ionic surfactants with high HLB values are commonly used in the preparation of the SNEDDS according to their less toxic properties compared with ionic surfactants. Many non-ionic surfactants have the ability to enhance the permeability and uptake of drugs via P-glycoprotein mediated efflux; such as; Cremophor EL3. Although, surfactants may cause irritation to the gastrointestinal mucosa, the association of the surfactant with the oil phase like the emulsion form could reduce the unfavorable adverse effect of the surfactant.

1.8.6.5. Co - surfactant and solubilizing agent

Co-surfactants and solubilizers are incorporated in the SNEDDS to increase the drug loading to the SNEDDS, improving the self-emulsification time, and modulate the droplet size of the nanoemulsions. The use of co-surfactants and solubilizers in the SNEDDS could result in an expanded self-nano emulsification region in the phase diagram. Co-surfactants and solubilizers

that are mostly employed in the preparation of the SNEDDS include propylene glycol, polyethylene glycol, polyoxymethylene, lauro glycol, transcutol.

1.8.7. Drug profile

1.8.7.1 Curcumin (Cur)

Table 1.2. Biological source of curcumin

Kingdom:	Tracheobionta
Subkingdom:	Spermatophyta
Sub division:	Magnoliophyta
Division:	Zingiber
Order:	Zingiberl
Family:	Curcuma
Genus:	C. longa
Species:	Cur*cuma longa*

1.8.7.1.1. Chemical structure of curcumin

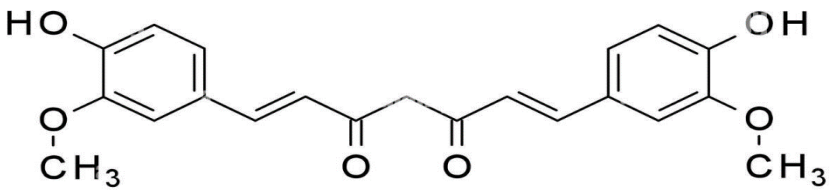


Fig. 1.4 Chemical structure of curcumin

Turmeric consists of several significant constituents isolated from the rhizome - structurally-related curcuminoids, including curcumin as the most important and the main active compound. The curcuminoids, besides including the yellow pigment ingredient curcumin (diferuloylmethane; 1,7-bis(4-hydroxy-3-methoxy-phenyl) hepta-1,6-diene-3,5-dione) also include desmethoxycurcumin (DMCur), bisdemethoxycurcumin (BDMCur) and the recently discovered cyclo curcumin (CCur). The main curcuminoids in commercial turmeric extracts are: Cur (~ 75%, molecular weight 368.37, melting point 183 °C), DMCur (~ 20%, molecular weight 338) and BDMCur (~ 5%, molecular weight 308).

Curcumin is a bis-*α, β*-unsaturated *β*-diketone and this form exists in equilibrium with its enol tautomer. The keto form predominates in acidic/neutral aqueous solutions and in cell membranes. On the contrary, the enol form of the heptadienone chain preponderates in alkaline medium.

Fig. 1.5. Powder curcumin extract

1.8.7.1.2. Curcumin and its mechanism of action in DM

Curcuminoids have been shown to improve insulin resistance, decrease glucose and insulin levels, increase adiponectin release, and reduce the levels of leptin, resistin, interleukin (IL)-6 IL-1β, and tumor necrosis factor-α in patients with T2DM. These findings suggest that these compounds can

affect glucose homeostasis and diabetic complications, and the vascular risk of patients with T2DM.

1.8.7.1.3 Traditional uses of curcumin in treatment DM

1.8.7.1.3.1. Antioxidant Effects - A study of ischemia in the feline heart demonstrated that curcumin pretreatment decreased ischemia-induced changes in the heart. An in vitro study measuring the effect of curcumin on endothelial heme oxygenase-1, an inducible stress protein, was conducted utilizing bovine aortic endothelial cells. Incubation (18 hours) with curcumin resulted in enhanced cellular resistance to oxidative damage.

1.8.7.1.3.2. Hepatoprotective Effects - Turmeric's hepatoprotective effect is mainly a result of its antioxidant properties, as well as its ability to decrease the formation of pro-inflammatory cytokines. In rats with CCl4-induced acute and subacute liver injury, curcumin administration significantly decreased liver injury in test animals compared to controls.

1.8.7.1.3.3. Anti-inflammatory Effects - The volatile oils and curcumin of Curcuma longa exhibit potent anti-inflammatory effects. Oral administration of curcumin in instances of acute inflammation was found to be as effective as cortisone or phenylbutazone, and one-half as effective in cases of chronic inflammation.

1.8.7.1.3.4. Anticarcinogenic Effects - Animal studies involving rats and mice, as well as in vitro studies utilizing human cell lines, have demonstrated curcumin's ability to inhibit carcinogenesis at three stages: tumor promotion, angiogenesis, and tumor growth. In two studies of colon and prostate cancer, curcumin inhibited cell proliferation and tumor growth.

1.8.7.1.3.5. Antimicrobial growth - Turmeric extract and the essential oil of Curcuma longa inhibit the growth of a variety of bacteria, parasites, and pathogenic fungi. A study of chicks infected with the caecal parasite *Eimera maxima* demonstrated that diets supplemented with 1-percent turmeric resulted in a reduction in small intestinal lesion scores and improved weight gain.

1.8.7.1.3.6. Cardiovascular Effects - Turmeric's protective effects on the cardiovascular system include lowering cholesterol and triglyceride levels, decreasing susceptibility of low-density

lipoprotein (LDL) to lipid peroxidation, and inhibiting platelet aggregation. These effects have been noted even with low doses of turmeric.

1.8.7.2. Rutin

Table 1.3 Biological source of rutin

Buckwheat:	This grain is one of the best natural sources of rutin.
Citrus fruits:	Especially in the skin, citrus fruits contain rutin.
Grapes:	These juicy fruits also provide rutin
Red wine	Enjoyed in moderation, red wine contains this beneficial compound.

1.8.7.2.1. Chemical structure of rutin

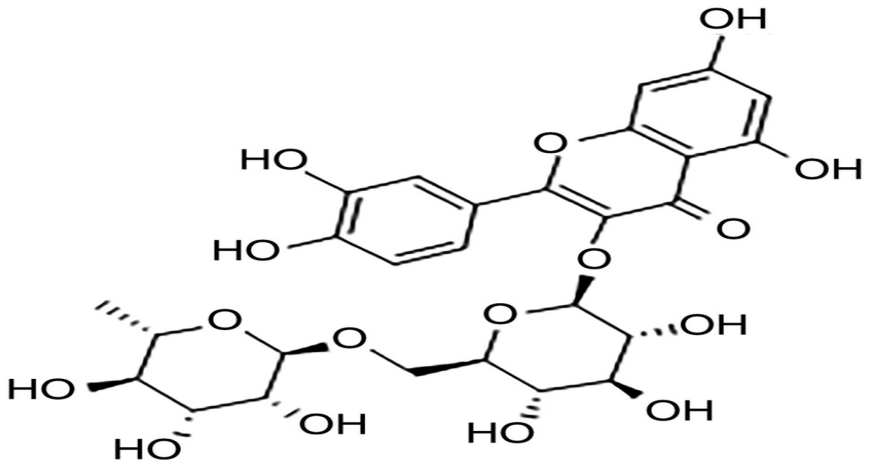


Fig. 1.6. Chemical structure of rutin

Rutin (3, 3′, 4′, 5, 7-pentahydroxyflavone-3- rhamnoglucoside) (Figure 2) also called as Ruto side, quercetin-3-rutinoside or sophorin is a flavanol category of flavonoid. Chemically, rutin is a glycoside combining the flavonol quercetin with the disaccharide rutinose (rhamnose and glucose). The name 'rutin' has been drawn from the plant Ruta graveolens (common Rue) (Figure 1.7) whose aerial parts contain rutin. Rutin is a kind of flavonoid glycoside, also known as vitamin P or purple quercitrin, present in more than seventy plants species and food products of plant origin especially

buckwheat seeds, apricots, cherries, grapes, grapefruit, onion, plums, and oranges. The large intestine metabolizes rutin to a variety of compounds that include quercetin and phenol derivatives such as 3, 4-dihydroxyphenylacetic acid, 3-hydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenylacetic acid (Hom vanillic acid) and 3, 4-dihydroxytoluene and they are devoid of significant antioxidant anti diabetic activity [44].

Fig 1.7 A- Plants of Ruta graveolens B- its flowers, C- its Ruta graveolens leaves

Table 1.4 The physicochemical properties of rutin

Chemical formula	C27H30O16
Molar mass	610.52 mol−1
Form and colour	Powder yellow to green
Melting point	242 °C
Solubility in water	12.5 mg in 100 ml
Site of absorption	Intestine

1.8.7.2.2. Antidiabetic property of rutin

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin resulting in hyperglycemia, or raised blood sugar level which may lead to serious damage to many of the body's systems. World Health Organization (WHO) in its fact sheets on diabetes 2018, indicated that the global prevalence of diabetes is rising every year and estimated that diabetes was the seventh leading cause of death in 2016. Flavonoids present in vegetables and medicinal plants have beneficial effects on diabetes by improving glycemic control, lipid profile, and antioxidant status. The probable reason and mechanisms for the anti-hyperglycemic effect of rutin include a decrease of carbohydrates absorption in the small intestine, inhibition of tissue gluconeogenesis, an increase of tissue glucose uptake, stimulation of insulin secretion from pancreatic beta cells, and protecting and exhibited significant Langerhans islet against degeneration. Rutin treatment improved the histo-architecture of beta islets and reversed hypertrophy of hepatocyte antidiabetic activity by inhibiting inflammatory cytokines, and improving antioxidant and plasma lipid profiles. The antihyperglycemic property of rutin and its protective effects against the development of diabetic complications have been associated with a rutin-mediated decrease in the formation of sorbitol, reactive oxygen species, advanced glycation end-product precursors, and inflammatory cytokines. The antidiabetic effect of rutin has been suggested to involve enhanced peripheral glucose utilization either by direct stimulation of glucose uptake or via the mediation of enhanced insulin secretion and by inhibiting the glucose transporter activity [44, 41].

1.8.8. Excipient profile

1.8.8.1. Transcutol P

Fig 1.8 Chemical structure of transcutol P

Table 1.5 Chemical properties of transcutol P

IUPAC Name:	2-(2-Ethoxyethoxy) ethanol
Chemical Name:	Diethylene glycol monomethyl ether
Molecular formula:	C6H10O3

Transcutol P is a clear, colorless, hygroscopic liquid with a mild odor. It is a versatile pharmaceutical excipient known for its solubilizing, emulsifying, and enhancing properties in drug delivery systems. Transcutol P is miscible with water and most organic solvents. Transcutol P is used in (curcumin and rutin) co-encapsulated SNEDDS, acts as a co-surfactant, aiding in the solubilization of rutin and cur in the oil phase of the formulation. This improves the dispersibility and absorption of drug in the gastrointestinal tract, thereby enhancing its therapeutic effectiveness in pharmaceutical applications.

Table 1.6 Physiochemical property of transcutol P

Density	0.989 g/cm³ at 20°C
Molar Mass	134.175 g/mol
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.

1.8.8.2. Tween 80

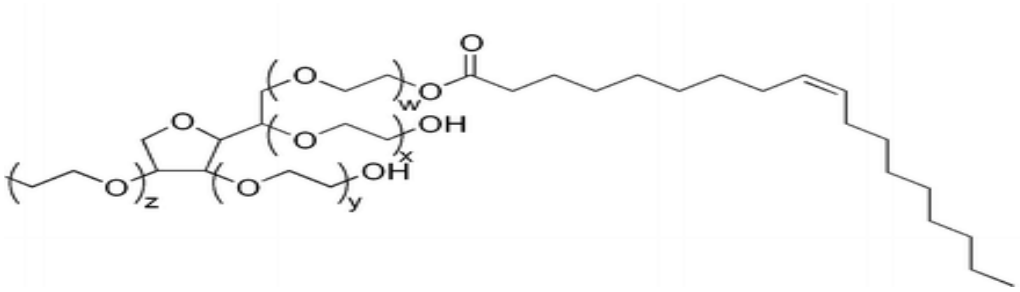


Fig. 1.9 Chemical structure of tween 80

Table 1.7. Physiochemical property of tween 80

IUPAC Name	Polyoxyethylene (80) sorbitan monooleate
Chemical Name	Polyoxyethylene sorbitan monooleate
Molecular Formula	C24 H44 O6
Melting Point	-25 °C
Boiling Point	>100 °C
Density	1.08 g/mL at 20 °C
Solubility	soluble in water and ethanol and mineral oil Vegetable oil

1.8.8.2. Ascorbyl palmitate

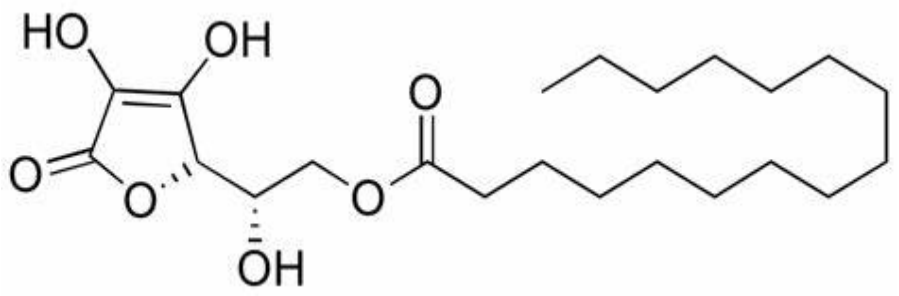


fig 1.10 Chemical structure of ascorbyl palmitate

Table 1.8. Physiochemical property of ascorbyl palmitate

IUPAC name	(2R)-2-[(2S)-2-[(2R)-2-Hydroxy-3,4-dihydro-2H-pyran-2-yl]-2-hydroxyethoxy]-2-methylbutyl hexadecanoate
Molecular formula	$C_{22}H_{38}O_7$
Chemical Name	L-Ascorbic acid 6-hexadecanoate

Ascorbyl palmitate ($C_{22}H_{38}O_7$) is a fat-soluble form of vitamin C used in some pharmaceutical products, such as suppositories. It is synthesized through the esterification of ascorbic acid (derived from the fermentation of corn) with palmitic acid (extracted from palm oil).

1.8.8.3. Aerosil 200

Aerosil 200 is a hydrophilic fumed silica with a specific 200 m^2/g surface area. It serves various purposes in different applications.

Table 1.9. Physiochemical property of aerosil 200

Chemical name	silicon dioxide amorphous
Molecular Formula	SiO2
Melting Point	3110°F
Boiling Point	4046 °F at 760 mmHg
Molecular weight	60.084 g/mol

1.8.8.4. Isomalt

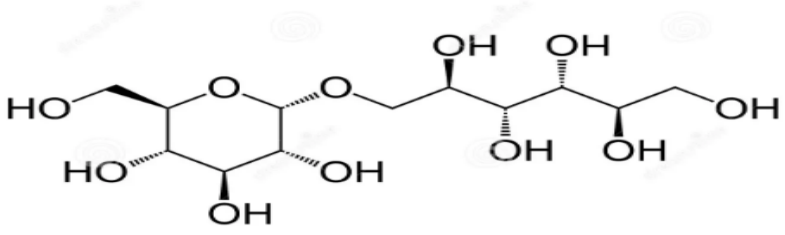


Fig 1.9 Chemical structure of isomalt

Isomalt is a sugar substitute composed of two disaccharide alcohols: 1,6-GPS and 1,1-GPM. It is primarily used for its sugar-like physical properties. Notably, isomalt has minimal impact on blood sugar levels, does not stimulate insulin release, and is considered tooth-friendly as it does not promote tooth decay. In pharmaceutical applications, isomalt serves as a coating agent, granulation aid, sweetening agent, and tablet and capsule diluent.

Table 1.10. Physiochemical property of isomalt

IUPAC Name:	1-O-(6-Deoxy-1,6-Dimethyl-D-glucitol)
Chemical Name:	Isomaltitol
Molecular Formula	$C_{12}H_{24}O_{11}$
Molecular weight	344.31 g/mol
Solubility	soluble in water poorly soluble in ethanol
Description	Odourless, white, slightly hygroscopic crystalline

1.8.8.5. Fish oil

Fish oil is a valuable dietary source of omega-3 fatty acids. These essential nutrients play crucial roles in various bodily functions. Here are the primary sources: Fatty Fish: Salmon, mackerel, and trout are rich in omega-3 fatty acids. These fish contain two key types: docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Shellfish, Mussels, oysters, and crabs also provide omega-3 fatty acids.

Table 1.11. Physiochemical properties of fish oil

Solubility:	Insoluble in water
Description:	fish oil is a pale-yellow oily liquid with a fishy odor

1.8.8.6. Dimethyl sulphoxide

The formula for dimethyl sulfoxide (DMSO), an organosulfur chemical, is (CH3)2SO. The most often used sulfoxide in commerce is this colourless liquid. This significant polar aprotic solvent is miscible in a variety of organic solvents including water, and it dissolves both polar and nonpolar substances. It boils at a rather high temperature. After DMSO is absorbed by the skin, it is metabolized into substances that taste like garlic. Drug rutin is highly soluble in DMSO.

Chapter: 2

LITERATURE REVIEW

2. LITERATURE REVIEW

Tripathi et al.,2016 explains the creation of a quercetin, resveratrol, and genistein-co-encapsulated self-nanoemulsifying drug delivery system (SNEDDS) to increase the compounds' 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-co-encapsulated 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 and area under the curve of all three antioxidants. The oral bioavailability of quercetin was ~4.27 times higher in the SNEDDS formulation, ~1.5 times higher.

Yoo et al., 2010 To increase lutein solubility and dissolution, DS containing Phosal 53 MCT as oil, Labrasol as surfactant, and Transcutol HP as cosurfactant was created. They reported on an optimized SNEDDS formulation including 25% oil, 60% surfactant, and 15% cosurfactant by concentration. By adsorbing it onto Aerosil 200, liquid SNEDDS were transformed into solid SNEDDS. A 93 nm globule size was reported using the improved formulation. The solubility qualities of lutein from the formulation showed a notable improvement, according to the results of the dissolution studies.

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. Results of in-vitro release tests revealed significant improvement in dissolving characteristics of gemfibrozil. 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 SNEDDS of the poorly soluble in water medication glimepiride with the aim 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. In less than 15 minutes, they reported more than 85% medication release. Research conducted in vivo shown a noteworthy improvement in therapeutic efficacy, with an AUC value of 234.64 for optimized S-SNEDDS.

El-Badry et al., 2014 Tadalafil is a weakly water-soluble medication. El-Badry et al. (2014) investigated the solubility and dissolution enhancement of this medication by preparing SNEDDS with Capryol 90 as the oil, Triton X100 as the surfactant, and Transcutol HP as the 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.

Shakeel et al., 2013 developed ultra fine super SNEDDS of indomethacin a poorly water-soluble drug with an aim to improve its solubility and dissolution properties. They employed Labrafil as oil, Tween-80 as surfactant and Transcutol-HP as cosurfactant. They reported globule size of formulations in range of 8.7 nm to 23.8 nm. Remarkable improvement in solubility and dissolution properties were observed. In-vitro drug release revealed 98.4% drug release from the optimized formulation. Results of solubility studies indicated 4573-fold enhancement in solubility of drug from the prepared formulations as compared to pure drug.

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. According to reports, the solubility and dissolving efficiency at 30 minutes were 83.3% and 9.82 mg/mL, respectively.

Yeom et al., 2015 developed and refined a self-nanoemulsifying drug delivery system (SNEDDS) employing an Optimal 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.

Gursoy, R.N., Benita, S et al., 2004 The goal of this review article is to improve the oral bioavailability of lipophilic medications by highlighting the latest developments in SNEDDS technology. The authors go over a number of cutting-edge methods for improving the effectiveness of medication distribution, including the use of novel excipients and sophisticated methodologies. They give a summary of the physicochemical characteristics of SNEDDS, taking into account variables such as surface charge, droplet size, and thermodynamic stability. The performance of SNEDDS in vivo is also examined in the paper, along with information on how they affect medication absorption and bioavailability. Understanding the advancements in the area and the potential of SNEDDS in drug delivery applications is made easier with the help of this overview.

Jain et al.,2013 emphasizes the logical design and description of a solidified self-nanoemulsifying drug delivery system (SNEDDS) intended for oral administration of a combination therapy regimen. 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. The persistence of the nano emulsions following.

Yin et al.,2017 created hemp oil-based nano emulsions (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. The excellent entrapment effectiveness of 99.31% was accompanied with a particle size of around 90 nm in BCL-NEs. 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 was studied using Caco-2 cells.

Kazi et al.,2020 developed and optimized a combined oral dosage form of sitagliptin and dapagliflozin using self-nanoemulsifying drug delivery systems (SNEDDS) for the treatment of type 2 diabetes mellitus. The SNEDDS were developed using bioactive medium-chain/long-chain triglycerides oil, mixed glycerides, and nonionic surfactants, and showed excellent self-emulsification performance with nanodroplets of around 50-66.57mm in size. The SNEDDS exhibited high drug loading capacity without causing any precipitation in the gastrointestinal tract and provided higher antioxidant activity compared to the pure drugs. In vivo pharmacokinetic studies in rats showed significant increase in drug absorption and bioavailability compared to the

commercial product. Anti-diabetic studies in mice demonstrated the significant inhibition of glucose levels with the combined dose of sitagliptin-dapagliflozin using SNEDDS. Visual observation was used as a primary means of assessment to differentiate good and poor formulations. The study aimed to prepare an effective combined oral dosage form of sitagliptin and dapagliflozin encapsulated in liquid SNEDDS with improved anti-diabetic effects. The formulations showed a fine dispersing appearance when diluted with water, indicating improved solubility and stable nanodroplets without precipitation. The study also mentioned the use of absorbance values to measure antioxidant activity. The mixing ratio of oil to surfactant played an important role in SNEDDS formulation development. Zeta potential measurement was used to determine the potential stability of the SNEDDS in an aqueous system. The study found that the SNEDDS formulations had zeta potential values that indicated good electrical properties and dispersion stability. The presence of cremophor EL as a surfactant contributed to the stability of the SNEDDS system by forming a protective layer around the droplets. Dynamic dispersion tests were conducted to examine the release performance and absence of drug precipitation upon dispersion of the SNEDDS formulation. The results showed that the SNEDDS formulations maintained a high percentage of drug in solution without precipitation for up to 24 hours in simulated intestinal fluid. This indicated the potential of the SNEDDS formulation to avoid in vivo drug precipitation and improve drug solubilization.

Kazi 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.

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.

Chapter: 3

AIM AND OBJECTIVES

Objectives of the present work

The present study aimed to design, develop and evaluate (curcumin and rutin) co-encapsulated SNEDDS for better management of diabetes with the specific objectives stated below.

I. Curcumin and EGCG, two medications with low water solubility, are made more soluble by encasing them in SNEDDS.
II. Characterization investigations and in vitro assessments of optimized formulations.
III. An in-vivo investigation was conducted to determine the optimal formulation for treating mice model of streptozotocin-induced diabetes mellitus.

Chapter: 4

MATERIALS AND METHODS

4. Materials and methods

4.1. Materials

The materials and tools used to create and analyze the (curcumin and rutin) co-encapsulated SNEDDS are listed in Table 4.1 and Table 4.2 respectively.

4.1.1. List of substances and equipment used in the manufacture and testing of (curcumin and rutin) co-encapsulated 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.	Ethanol	
09.	Fish oil	Glenham® 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 (curcumin and rutin) co-encapsulated 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 Itd, Hyderabad, India
08.	HPLC	Prominence AD 30, Shimadzu, Japan
09.	Transmission electron microscopy	Philips EM430 TEM, USA
10.	Particle size analyzer and Zeta Sizer	MALVERN Instruments Model: - ZEN 1600
11.	Field emission scanning electron andmicroscopy (FESEM)	FEI Model: Quanta 250 FEG
12.	X-ray diffractometer	Panalytical, Model: X'Pert Pro
13.	Deep freezer	Godrej EON
14.	Refrigerator	Godrej EON
15.	FTIR	PERKINELMER FRONTIER

4.1.3 Animals

For the in vivo study of the optimized formulation, male *Swiss albino* mice weighing an average of 25–30 grammes and approximately 2-3 months of age (on the day of the study) were obtained from the West Bengal Livestock Breeding Centre, Kalyani, India. The mice were kept under observation in polypropylene cages lined with husk and were acclimatized at 24 ± 2 °C and relative humidity of 45 # 15% over a period of 2 weeks prior to dose administration. All animals in the study were kept under observation during the acclimatization period. The mice were kept on a standard laboratory diet and were given unlimited access to domestic mains tap water. The animals were kept fasted for the duration of the study. The experimental protocol was subjected to the scrutiny and ethical clearance was obtained from Institutional Animal Ethics Committee of Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India Project proposal no. JU/IAEC-24/74 Dated 04.09.2024 before beginning the experiment.

4.2. Methods

4.2.1. Preparation of SNEDDS

FORMULATION 1 - 2308015

A formulation was prepared to evaluate curcumin'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 (curcumin)	100mg
03.	Surfactant	3ml
04.	Co-surfactant (Transcutol)	3ml
05.	Aerosil- 200	615mg
06.	Double distilled water	40ml

Ratio of surfactant and co-surfactant = 1:1

Procedure:

Tween 80 and transcutol were added in a beaker in 1:1 ratio and stirred with the help of magnetic stirrer at 500 rpm at a temperature of 45°C for 5 minutes until its transparent. 40 ml DDW was added to the above mixture and stirred for another 5 minutes. The above liquid SNEDDS was then transferred to a mortar and Aerosil-200 was added and triturated with pestle until it formed a slurry. the slurry was transferred to a petri dish and stored at -20°C overnight and kept in the vacuum oven the next day for the formulation to be dried. The formulation was weighed after drying and the decrease in weight was noted until the weight was constant.

FORMULATION 2 - 230816

Glyceryl trioleate was used in the formulation.

S. No.	Materials	Quantity
01.	Glyceryl trioleate	1.5ml
02.	Drug (curcumin)	150mg
03.	Surfactant (Tween 80)	1.5ml
04.	Co-surfactant (Transcutol)	1.5ml
05.	Aerosil- 200	1.2g
06.	Double distilled water	40ml

Procedure;

The mentioned amount of fish oil, transcutol and tween 80 was added in a beaker and mixed homogenously to form an isotropic mixture in a magnetic stirrer.Curcumin was added to the above isotropic mixture and stirred for about 10-15 minutes or until transparent.40 ml DDW was added to the above mixture and stirred for another 5 minutes.The above liquid SNEDDS was then transferred to a mortar and Aerosil 200 was added and triturated with pestle until it formed a slurry.The slurry was transferred to a petri dish and stored at -20°C overnight and kept in the vacuum oven the next day for the formulation to be dried. The formulation was weighed after drying and the decrease in weight was noted until the weight was constant.

FORMULATION 3 - 230818

Fish oil was used in the formulation.

S. No.	Materials	Quantity
01.	Fish Oil	1.5ml
02.	Drug (curcumin)	150 mg
03.	Surfactant (Tween 80)	1.5ml
04.	Co-surfactant (Transcutol)	1.5ml
05.	Aerosil- 200	1.2g
06.	Double distilled water	40ml

Procedure:

Same as in formulation 2

FORMULATION 4 - 230820

Isomalt was added to the formulation to solidify the L-SNEDDS. The amount of Transcutol and Tween 80 were in same ratio while the quantity of fish oil was decreased.

S. No.	Materials	Quantity
01.	Fish oil	3ml
02.	Drug (curcumin)	375mg
03.	Surfactant (Tween 80)	2.5ml
04.	Co-surfactant (Transcutol)	2.5ml
05.	Aerosil- 200	1.5g
06.	Isomalt	5g
07.	Double distilled water	40ml

Procedure:

Same as in formulation 2.

FORMULATION 5 - 230902

Quantity of Aerosil-200 increased. The amount of Transcutol and Tween 80 were in same ratio

S. No.	Materials	Quantity
01.	Fish oil	3ml
02.	Drug (curcumin)	375 mg
03.	Surfactant (Tween 80)	2.5ml
04.	Co-surfactant (Transcutol)	2.5ml
05.	Aerosil- 200	3g
06.	Isomalt	5g
07.	Double distilled water	40ml

Procedure:

Same as formulation 2.

FORMULATION 6- 230908

The formulation includes both curcumin and rutin. Additionally, Ascorbyl Palmitate was incorporated into the formulation.

S. No.	Materials	Quantity
01.	Ethanol	1.5ml
02.	Fish oil	1.5 ml
03.	Drug (Rutin)	175mg
04.	Drug (curcumin)	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

Procedure

Curcumin was mixed with fish oil in a 25 ml beaker, and the solution was stirred until the drug dissolved into the oil. Similarly, rutin was dissolved in ethanol. Both solutions were then mixed, and the mixture was stirred for 1 to 2 minutes using a magnetic stirrer at a temperature of 40 to 50°C. Afterward, Tween 80 and Transcutol were added to the solution, followed by ascorbyl palmitate, and the mixture was stirred for a short period. Water was then added to the solution and stirred for 1 minute, resulting in the formation of liquid SNEDDS. The solidification of the curcumin and rutin liquid SNEDDS was carried out by first crushing isomalt into a powder using a mortar and pestle, followed by adding Aerosil 200. The liquid SNEDDS was mixed with the

Aerosil and isomalt mixture and triturated for 10 to 15 minutes. The mixture was then stored at -20°C overnight, followed by vacuum drying at 40 to 50°C until the final weight was obtained. The final SNEDDS was stored in a well-closed container and kept at 4°C for further analysi

Formulation no7 – 230912

This formulation did not contain any drug all the ingredient same as formulation no 6

S. No.	Materials	Quantity
01.	Ethanol	1.2ml
02.	Fish oil	1.2 ml
03.	Surfactant (Tween 80)	2.5ml
04.	Co-surfactant (Transcutol)	2.5ml
05.	Aerosil- 200	3.5g
06.	Isomalt	3.5g
07.	Ascorbyl Palmitate	100mg
08.	Double distilled water	40ml

Procedure; same as formulation no 6

4.3. Dispersion test:

In an effendurf tube, 2 mg of powdered of (curcumin and rutin) co-encapsulated SNEDDS curcumin free drug each formulation was dissolved in 2 mL of DDW and vortexed for two to three minutes. The precipitation was observed at various time intervals.

4.4. High Performance Liquid Chromatography (HPLC):

UV detectors and diode array detectors (DAD) were utilized in high-pressure liquid chromatography (HPLC) for both drug loading and in vitro drug release. The HPLC system was comprised of a thermostat column compartment (TCC), UV-visible detector, autosampler, and

quaternary pump (Model: Agilent 1260 series). Acetonitrile and buffer (30 mm KH2P04) were employed as the mobile phase in a 50:50 ratio. There was 1.0 ml/min of flow. The absorbance was detected at a wavelength of 265 nm. Software called Ez Chrome was utilized to gather and examine the data. The C18 column of Phenomenex Luna octadecylsilane, with dimensions of 250 mm x 4.6 mm and a particle size of 5um, is typically employed in the stationary phase. The sample was prepared using an ultrasonic bath. Typically, the samples and standards were weighed using the analytical balance.

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

Centrifugation was used for 10 minutes at 12,000 pm to determine the EE of the SNEDDS co-encapsulated with curcumin and rutin. Following the decantation process, High-Performance Liquid Chromatography (HPLC) was used to evaluate the supernatant. Regarding drug loading, acetonitrile and buffer (KH2POH) were used as the mobile phase in an HPLC analysis of the Cur & rutin co-encapsulated SNEDS. The following formulas were used to determine the drug loading (% DL) and entrapment efficiency (% EE):

% EE = (A - B)/A × 100% .....(1)

%DL = (C/A and D) × 100% .....(2)

A = Total amount of drug in SNEDDS

B = Total amount of drug present in the supernatant

C = Total amount of drug added

D = Total amount of excipients added

4.6. In-vitro dissolution studies

The in-vitro drug release of (curcumin and rutin) co-encapsulated SNEDDS free drug was done in B.O.D incubator shaker. Employing 200 ml of simulated gastric fluid (SGF) (pH 1.2) and 200 ml of simulated intestinal fluid (SIF) (pH 6.8) respectively in a 250 ml conical flask. Two dialysis bags of around 10 cm were separately immersed into the flask containing SGF and SIF overnight. An equivalent amount of (100mg) drug encapsulated SNEDDS powder and free drug was weighed and suspended in equivalent amount of (10ml) DDW and vortexed for 15-20 minutes in a magnetic stirrer at a temp of 45°C-50°C. SNEDDS suspended in DDW was filled in a dialysis bag

(membrane) and tied from both the ends. The membranes were placed inside the conical flasks respectively. Each conical flask contains (200 ml) SIF AND SGF. The temperature of the B.O.D incubator shaker was maintained at 37±0.5°C, stirring at speed of 100 rpm. at predetermined intervals (1, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144, 168 hours), 2 ml samples were withdrawn and filtered using syringe filter (0.45 um). The sink condition was maintained by replacing the sample with an equal volume of fresh dissolution medium. Filtered samples after appropriate dilutions were then subjected to HPLC for the determination of in-vitro drug release. The study was repeated in triplicates and the average values were utilized to construct the dissolution profiles.

4.7. Characterization of curcumin and rutin-loaded SNEDDS.

4.7.1. Particle size:

In a flask, distilled water was used to dilute the SNEDDS formulation, which included 100 times dilution of SNEDDS sample. (curcumin & rutin) co-encapsulated SNEDDS were vortexed in a magnetic stirrer for 15-20 minutes at a temp of 45°C - 50°C in order to minimize any aggregation if present. Photon correlation spectroscopy was used to determine the nano emulsion's droplet size. Zetasizer 1000 HS (Malvern Instruments, Worcestershire, UK) was used for the measurement. At 25 °C, light scattering was observed from a 90° angle [44].

The larger droplet size was not detected the droplet size using particle size analyzer because the equipment only covers the droplet size less than 1000nm. Nano emulsion size has a droplet size of less than 200nm. Oil phase in high concentration will produce a large droplet size. Meanwhile, a large number of cases indicate that increasing surfactant concentration will decrease droplet size because surfactant molecule localization on the interface of oil-water will stabilize oil droplets. High surfactant concentration will improve the penetration of water into oil droplets that lead to the breakdown of oil droplets and formation of larger droplets [45].

4.7.2. Zeta potential

Zeta potential of the optimum formulations was determined by dynamic light scattering using particle size analyzer Zetasizer 1000 HS (Malvern Instruments, Worcestershire, UK) was used for the measurement. CUR & rutin co-encapsulated SNEDDS were diluted with a ratio of 1:100 (v/v) with distilled water and repeated in triplicate [45]. A high value of zeta potential has better stability since there is resistance to aggregation of the formulation. Zeta potential of less than -30mV and more than and30mV is generally appropriate for the stability of a system. A negative value of zeta

potential indicates the existence of free fatty acid, surfactant, and co-surfactant in the formulation. It also shows the considerable force of repulsion between droplets to prevent aggregation [46].

4.7.3. FOURIER TRANSFORMED INFRARED SPECTROSCOPY (FTIR)

To ascertain the interplay among excipients and the active drug, the most effective method for examining potential chemical interactions between pharmaceuticals and formulation excipients is Fourier-transform infrared spectroscopy (FTIR). CUR & rutin co-encapsulated SNEDDS, pure curcumin, pure rutin, blank SNEDDS and physical mixer FTIR spectra were scanned. The samples were scanned using an FTIR spectrophotometer Model RZX (Perkin Elmer UK) over the scanning range of 4000–650 cm−1[48].

4.7.4. X-ray diffraction (XRD)

X-ray diffraction (XRD) patterns of pure curcumin, pure rutin, (curcumin & rutin) co-encapsulated SNEDDS, blank SNEDDS and physical mixer were recorded by using an X-ray diffractometer (X'Pert; Malvern PANalytical Ltd, Eindhoven, Netherlands). An appropriate amount of sample powder was taken and equipped with an intended wavelength K-Alpha1 (1.54060 Å) operating a generator power of 45 kV and current of 40 mA. The samples were filled in the sample holder and patterns were recorded over angular range intervals of 2 to 80° (2θ) at a scanning speed of 67 s per step [48, 49].

4.7.5. Electron microscopy

A flexible technique for characterizing materials in two and three dimensions is electron microscopy. Due to its non-destructive method of characterizing materials, high resolution of TEM (Transmission electron microscopy) and SEM (Scanning electron microscopy) is typically sought after. SEM and TEM are used in the assessment of important material microstructures.

4.7.5.1. Scanning electron microscopy (SEM)

Using a scanning electron microscope (Carl Zeiss EVO LS10; Cambridge, UK), the (curcumin and rutin) co-encapsulated SNEDDS, were analyzed to assess the impact of solidification on the adsorbent particle form and identify any indications of inadequate solidification. Samples were secured on stubs with double-sided carbon tape before being coated in gold in an argon atmosphere (20 mA) vacuum for 60 seconds using a Q150R sputter coater equipment (Quorum Technologies Ltd., East Sussex, UK).

4.7.5.2. Transmission Electron Microscope (TEM)

To identify the droplet morphology of the chosen (curcumin & rutin) co-encapsulated SNEDDS formulation, TEM investigations were carried out. In short, a copper grid was covered with a drop of diluted (100 times) SNEDDS, which was then allowed to air dry for the entire night. Using a transmission electron microscope, the picture was captured at a 100 kV accelerated voltage.

4.2.6 In vivo studies

4.2.6.1 Experimental animal model

Streptozotocin-induced mice model was selected to evaluate the anti-diabetic activity of (curcumin and rutin) co-encapsulated SNEDDS. The study has been approved by the Institutional Animal Ethics Committee of Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India Project proposal no. JUAAEC-24/74 Dated 09.04.2024 before beginning the experiment.

4.2.6.2. Study design

The animals were divided into 5 different groups, they are as follow:

Group I: Control group, normal diet and normal saline.

Group II: Disease control group, normal diet and STZ single intraperitoneal injection (65mg/kg body weight).

Group III: Standard control group, normal diet and STZ and standard drug (Glibenclamide) (administered by oral gavage at the dose of 2 mg/kg body weight) for 30 days [51].

Group IV: Free drug group, normal diet and STZ and free drugs (administered by oral gavage at the dose of 100 mg/kg body weight) for 30 days.

Group V: SNEDDS group, normal diet and STZ and SNEDDS (administered by oral gavage at the dose of 20 mg/kg body weight) for 30 days.

Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Total no. of animal**
Normal	Disease Control	Standard control	Free drug treated	SNEDDS (curcumin and rutin)	Animals
3	3	3	3	3	15

4.2.6.3. Experimental induction of diabetes

Intraperitoneal (IP) injection of streptozotocin (STZ) dissolved in 0.1 M cold sodium citrate buffer (pH 4.5) at a dose of 65 mg/kg body weight was used to induce diabetes following an overnight fast. Prior to and seven days following the delivery of STZ, blood samples were taken. Animals that tested positive for diabetes at a fasting blood glucose level of more than 250 mg/dL were included in the study [60,61].

4.2.6.4. Regular monitoring of body weight and blood glucose level

The body weight and blood glucose level were monitored every weekend for 4 weeks. The blood was collected by pricking the tail vein using a syringe needle and the blood glucose level was determined using Accu-Check Active blood glucose monitoring system.

4.2.6.5. Drug administration

Oral gavage was used to deliver the formulations after two weeks of STZ induction. The animal's body weight was taken into account while calculating the dosage, and new doses were made daily for the formulations that were administered once a day. Every week, the blood glucose level and body weight of mice that had fasted overnight were measured.

4.2.7. Statistical analysis

The mean ± SD is used to express all the data. One-way ANOVA was used for the statistical analysis, and Dunnett's multiple comparison test came next. P less than 0.05 was regarded as significant. The program GraphPad Prism was utilized.

Chapter; 5

RESULTS AND DISCUSSION

5. Result and Discussion

5.1 Preparation of curcumin co-encapsulated SNEDDS

(Formulation 1- 230815)

A formulation was prepared to check the solubility of the rutin in tween 80 and transcutol oil is not used aerosil 200 was used as a solidifying agent.

Fig 5.1 Formulation 230815

After preparing the formulation, it was stored at –20°C overnight and dried in a vacuum oven for 4 days. It appeared as a solid mass, which was distributed in a Petri plate, as shown in Figure 5.1. The formulation was then discarded

(Formulation 2- 230816)

The combination was prepared using fish oil. Half the amount of surfactant and cosurfactant is reduced when compared to the formulation. Aerosil 200 is more prevalent than in the formulation one.

Fig 5.2 Formulation – 230818

Figure 5.2 illustrates that the product appeared agglomerated and was not free-flowing after drying for up to seven days. Therefore, the formulation will be eliminated.

(FORMULATION 3 - 230818)

Glyceryl trioleate oil was used in place of fish oil.

Fig .5.3 Formulation no – 230818

The formulation seemed to be very similar to the mixture to be 2nd formulation. Inadequate drying of the product resulted as sticky agglomerates. The formulation was discarded.

FORMULATION 4 - 230820

Since the product does not dry completely, isomalt was added to the formulation together with aerosil 200 to help solidify it. Compared to tween80, transcutol was utilized in larger quantities. Furthermore, less fish oil was utilized.

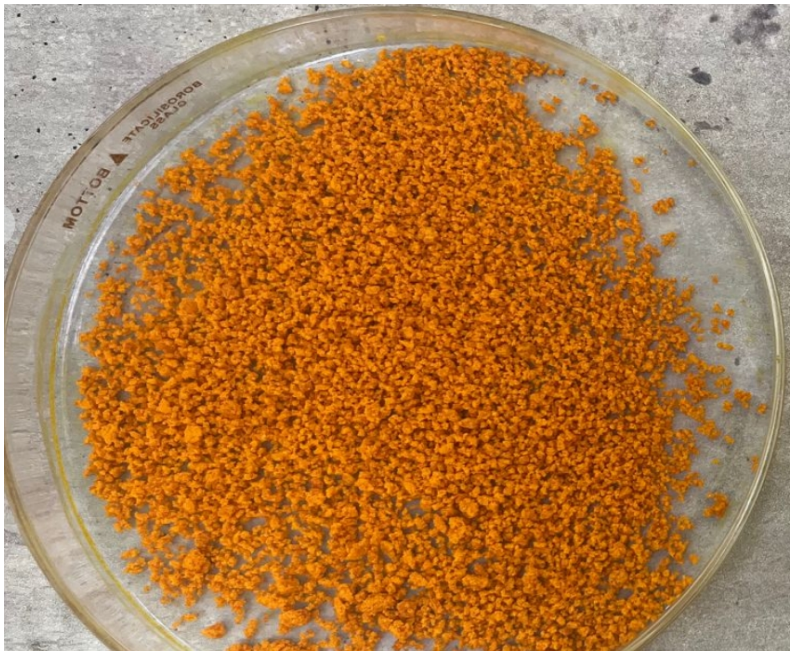


fig 5.4 Formulation – 230820

formulation 4 was created by combining aerosil and isomalt as solidifying agents. The resultant product was sticky and clumped together, therefore it was thrown out.

FORMULATION 5 – 230902

In this formulation, the quantity of Aerosil was increased, while the amounts of isomalt and Transcutol were slightly reduced.

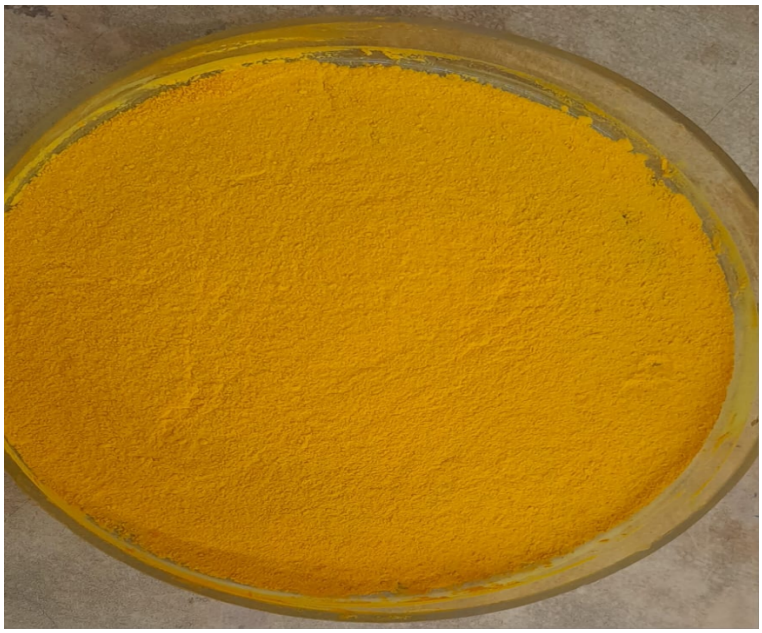


Fig 5.5 Formulation no. 5

Formulation no 5 used isomalt and aerosil, both are used as solidifying agent. The flow property of the formulation increases.

Formulation no 6 – 230908

(Curcumin and rutin) co-encapsulated SNEDDS was prepared where quantity of surfactant and co-surfactant same quantity and aerosil and isomalt were also same.

Fig 5.6 Formulation no. 6 (curcumin and rutin) co-encapsulated SNEDDS

After drying for 7 to 8 days we found the final formulation. The formulation appears to be free-flowing and subjected to further characterization.

Formulation no 7 – 230912

Blank SNEDDS this formulation did not contain any drug. All the ingredient will same as the formulation no 6.

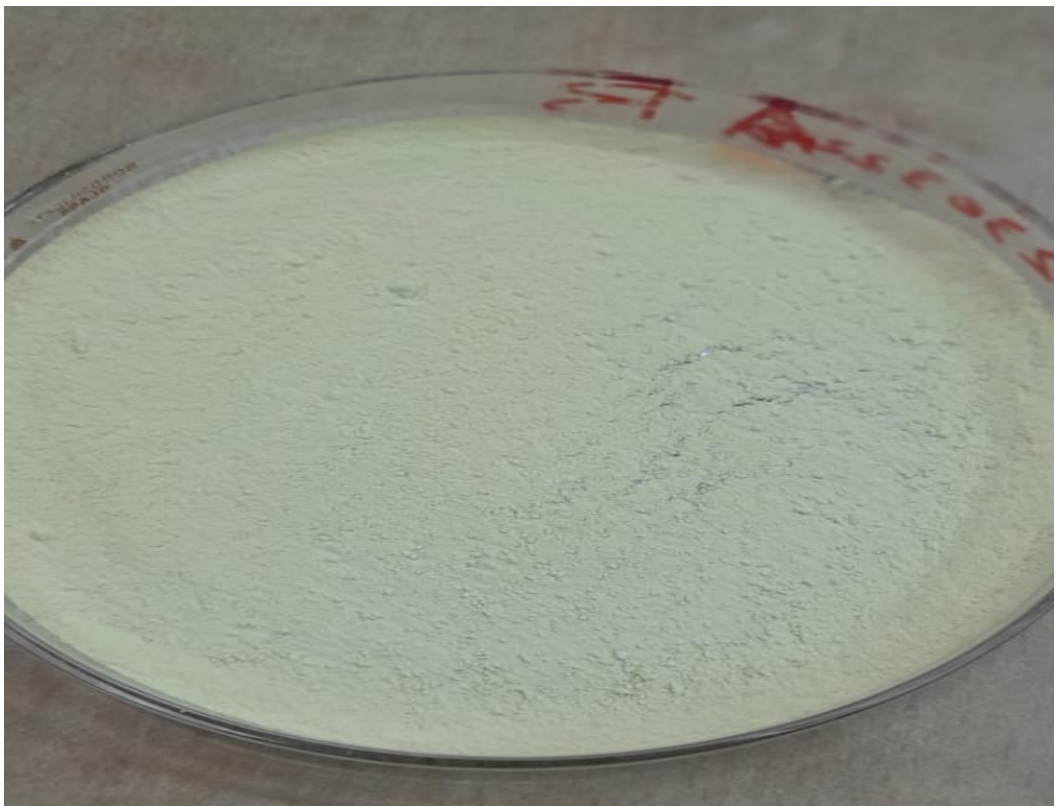
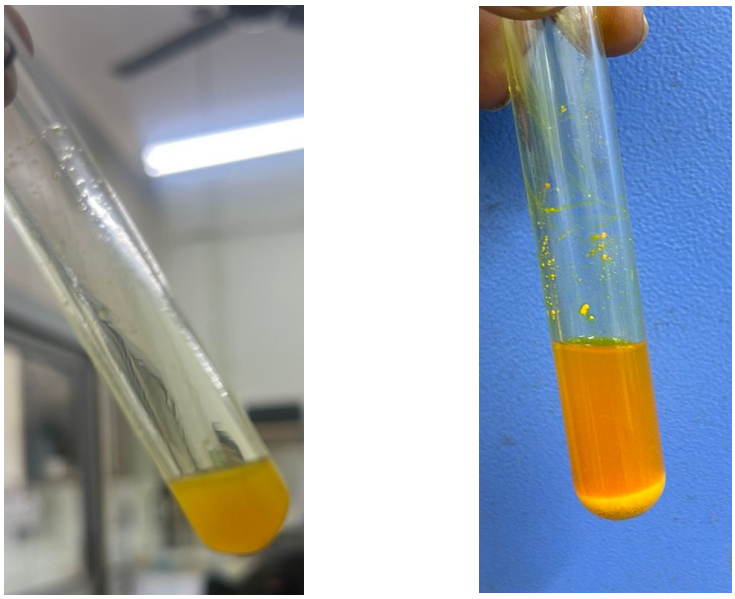


Fig 5.7 Formulation no 7 blank SNEDDS

After drying the formulation 7 to 8 days the formulation it stores at 4 °C for further characterization.

5.2. Dispersion test

The dispersion test was conducted on two formulations of (curcumin & rutin) co-encapsulated SNEDDS and curcumin free drug.



(a) (b)

Fig 5.8 (a) dispersion test of (cur and rutin) co-encapsulated SNEDDS; (b) dispersion test of free curcumin drug

For two to three minutes, 1 mg of each formulation was dissolved in 1 ml of DDW in an effendurf tube and centrifuged. It was observed that the formulation showed very little to no precipitation but in formulation b (free curcumin drug) show more precipitation.

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

The entrapment efficiency of the optimized formulation of (curcumin and rutin) co-encapsulated SNEDDS was 82.36 % for cur and 84.76% rutin. The drug loading was 9.26 and 8.16 for curcumin and rutin respectively.

Table 5.1 Drug loading and entrapment efficiency

Drug	**Drug loading**	**Entrapment efficiency**
Curcumin	9.26 %	82.36 %
Rutin	8.16 %	84.76 %

5.3. In vitro dissolution studies

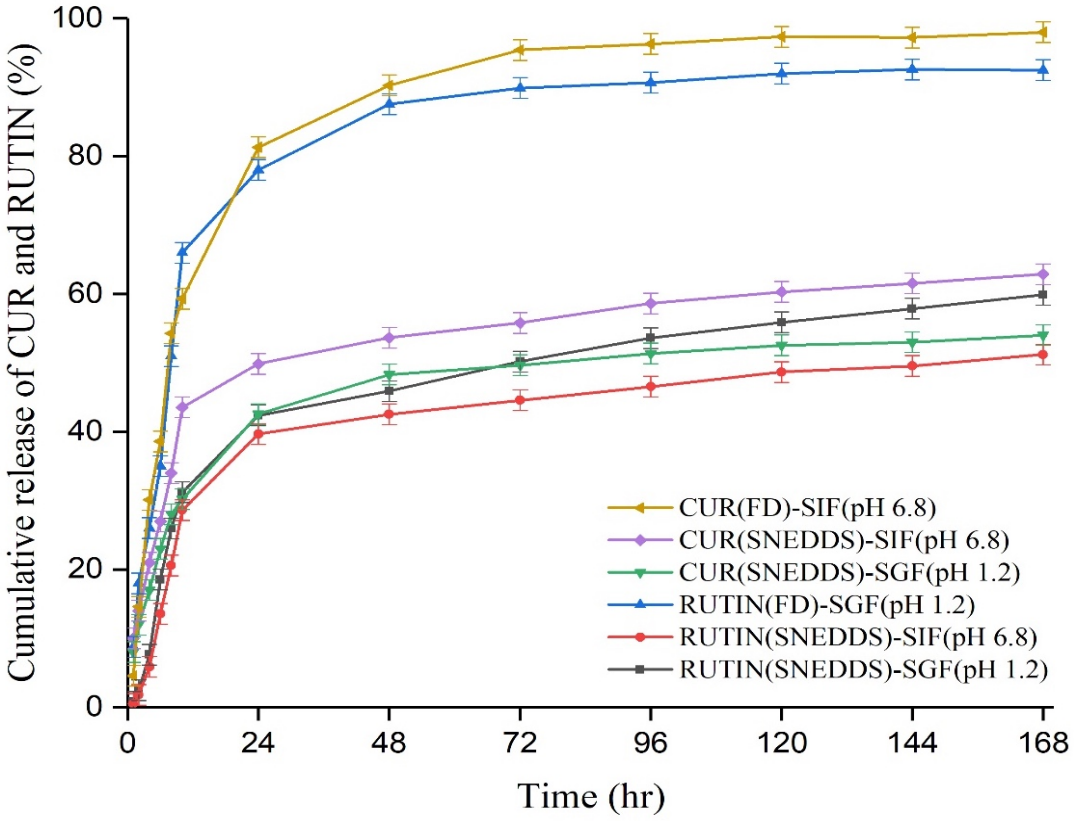


Fig 5.9 In vitro dissolution studies (curcumin and rutin) co-encapsulated SNEDDS 7 & curcumin and rutin free drug

Figure 5.9 shows the release profiles of curcumin and rutin from their free forms and (curcumin & rutin) co-encapsulated SNEDDS at pH 1.2 and 7.4. A 12-h burst release was followed by a 7-day prolonged drug release, leading to a biphasic release profile. Encapsulated drugs were released from the polymer matrix at a sustained rate for 7 days. The weakly bound drug molecules on the surface of the polymer matrix may be responsible for the burst release of drugs from nanoparticles.

According to the in vitro drug release tests, curcumin and rutin were released faster in SIF pH 7.4 compared to SGF pH 1.2 [50]. This slower release profile indicates that the (curcumin & rutin) co-encapsulated SNEDDS formulation can control and extend the release of curcumin and rutin which may improve its stability and absorption in the body. The controlled release from (curcumin and rutin) co-encapsulated SNEDDS could potentially enhance the therapeutic benefits by maintaining a sustained level of the compound in the system, thus maximizing its bioavailability and efficacy.

5.4. Characterization of (curcumin and rutin) co-encapsulated SNEDDS

5.4.1. Particle size and polydispersity index (PDI)

One method that is widely used to measure the hydrodynamic diameter of nanoparticles in colloidal systems in the submicron and nano ranges is dynamic light scattering (DLS). Because smaller particles have a bigger surface area, surface erosion and diffusion can facilitate drug release from nanoparticles. Furthermore, it makes it possible for drug-encapsulated nanoparticles to penetrate and cross physiological drug barriers [49]. The prepared (curcumin and rutin) co-encapsulated SNEDDS's mean hydrodynamic diameter was discovered to be 168.1 [49,50]. It was discovered that the PDI observations in (curcumin and rutin co-encapsulated SNEDDS) was 0.25 When the PDI score is less than 0.3, the sample is deemed to have a narrow size distribution. On the other hand, PDI values >0.3 are associated with a greater diversity of particle sizes [51].

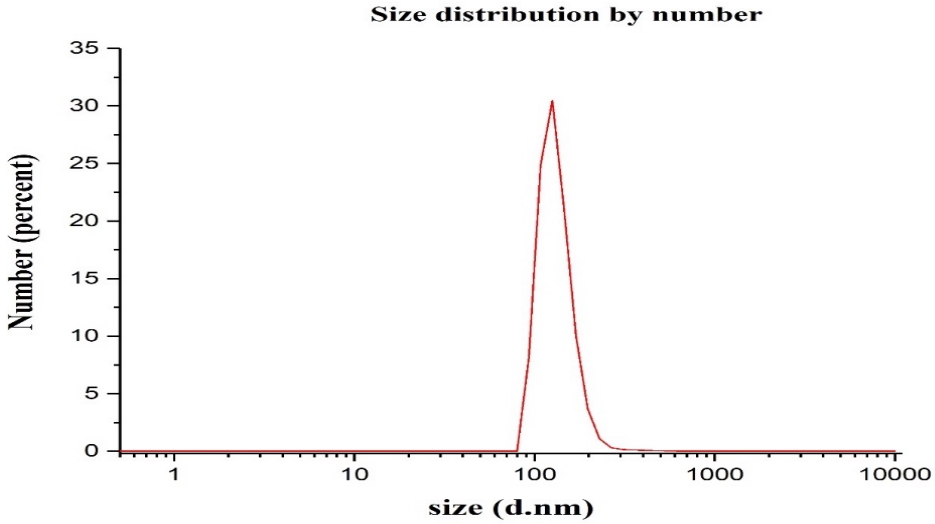
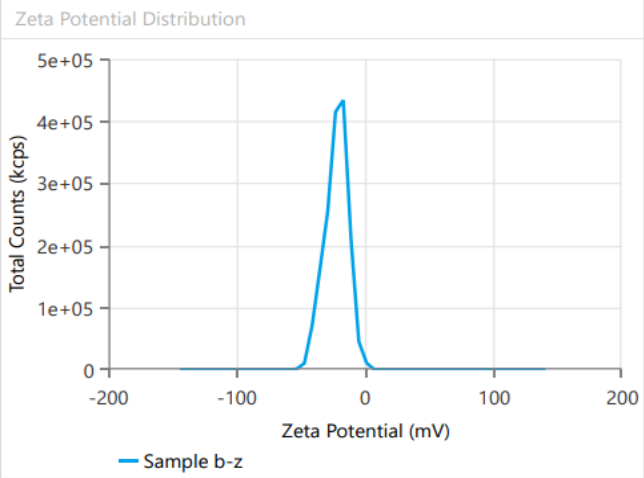
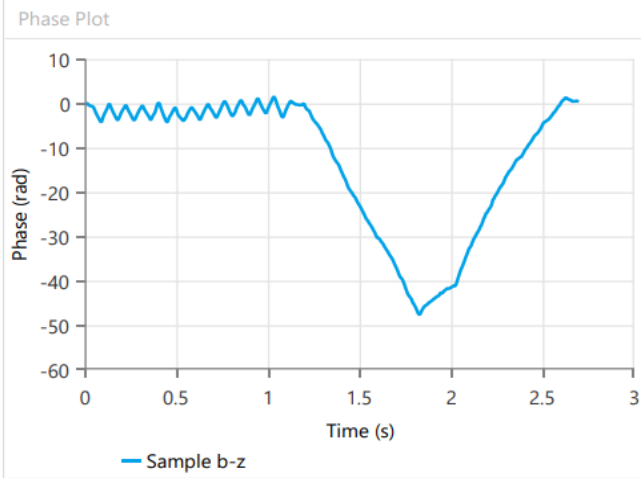


fig5.10 Particle size distribution (curcumin and rutin) co-encapsulated SNEDDS.

5.4.2 ZETA POTENTIAL

The zeta potential of curcumin and rutin co-encapsulated SNEDDS of the formulation was found to be (22.28 ± 1.6). ±20 is good) zeta potential of the sample heigh that indicate the more repulsion force in between the particle it prevents the particle to be agglomerates. That help to improve the stability of the curcumin and rutin co-encapsulated SNEDDS.





Statistics Table

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-22.28	-	-	-22.28	-22.28
Zeta Peak 1 Mean (mV)	-22.28	-	-	-22.28	-22.28
Conductivity (mS/cm)	0.04636	-	-	0.04636	0.04636
Wall Zeta Potential (mV)	0	-	-	0	0
Zeta Deviation (mV)	8.991	-	-	8.991	8.991
Derived Mean Count Rate (kcps)	9147	-	-	9147	9147
Reference Beam Count Rate (kcps)	1715	-	-	1715	1715
Quality Factor	1.698	-	-	1.698	1.698

fig 5.11 Zeta potential of (curcumin and rutin) co-encapsulated SN

5.4.3. FTIR analysis

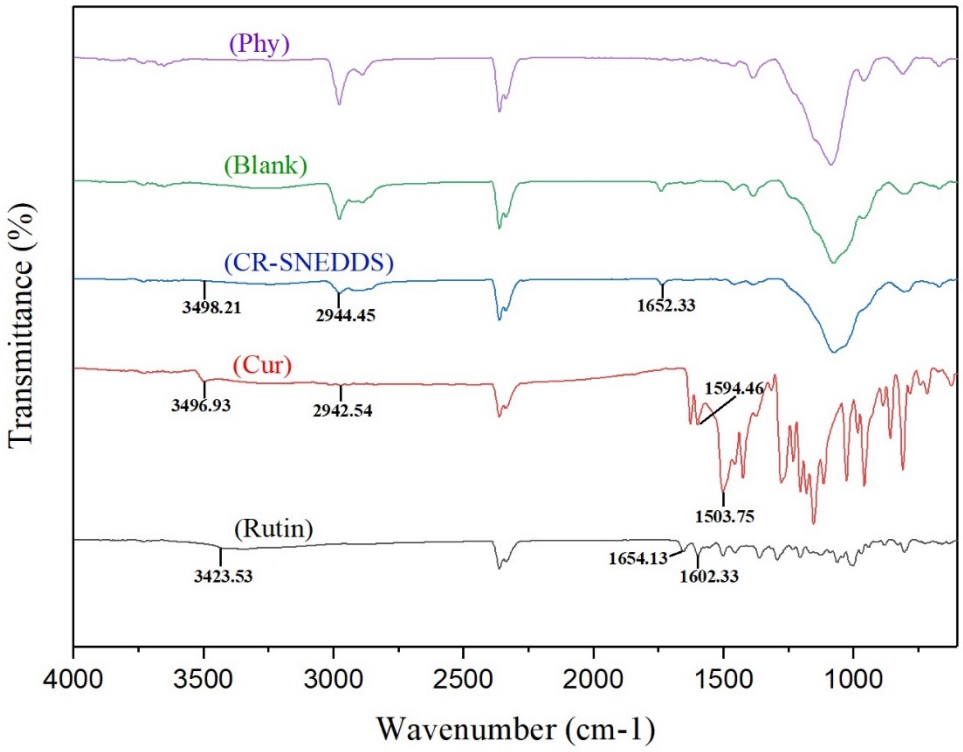


Fig 5.12 FT-IR analysis (curcumin and rutin) co-encapsulated SNEDDS

In fig 5.12 principal peaks of curcumin were observed at 3496.93 cm-1 and 2942.54 cm−1 which correspond to the -OH stretch and vibration of C-H bonds, respectively. An aromatic(C=C) stretching of curcumin was observed at the peaks 1594.46 cm-1, 1503.75 cm-1, and 1416.62 cm-1[52]. The spectra for rutin alone showed peaks at; 3410 cm -1 (-OH alcohol or phenol stretching). Chemical shift at a wavelength of 1654.13 cm−1 corresponds to C=O group in ester and the wavelength of 1602.33 cm−1 of the C=O group in the fatty acid [53]. The FTIR spectra of (curcumin and rutin) co-encapsulated SNEDDS NP exhibited the disappearance of characteristic absorption peaks of both curcumin and rutin and new peaks confirming the bond interaction of curcumin and rutin with the excipients in (curcumin and rutin) co-encapsulated SNEDDS.

5.4.4. XRD analysis

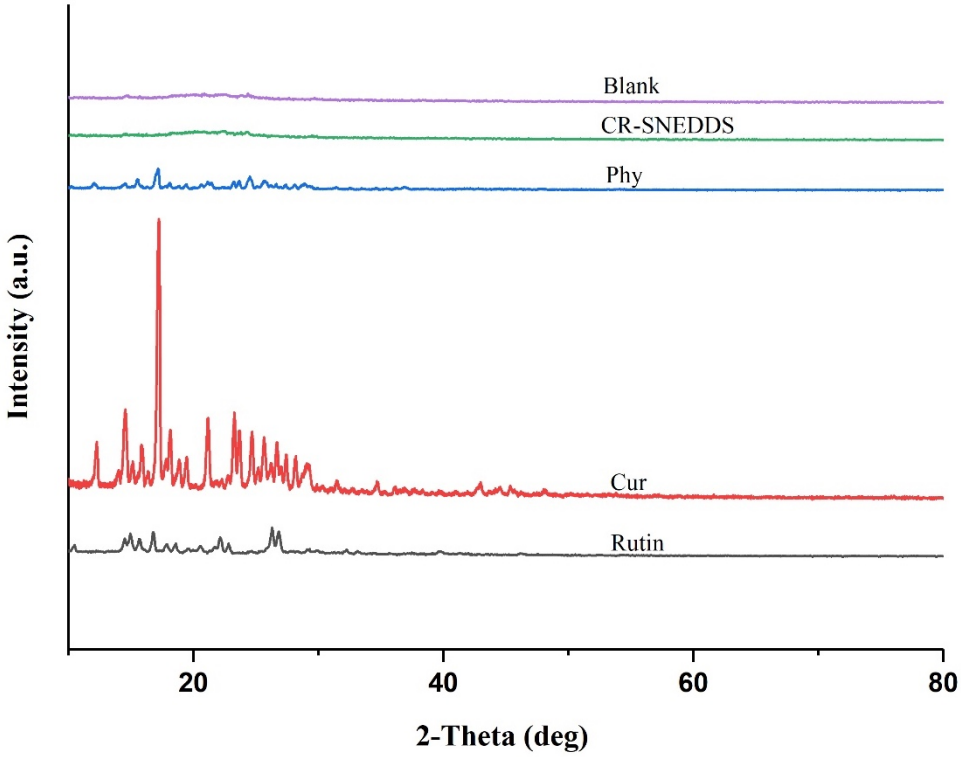


Fig 5.12 XRD pattern of cur, rutin and (curcumin and rutin) co-encapsulated SNEDDS

In Fig 5.12 rutin's XRD pattern showed multiple high-intensity, crisp crystalline peaks at different diffraction angles (2θ): 7.5°, 20.5°, 21°, 22°, 23.5°, 29°, 32°, and 33°, in that order. Similarly, pure curcumin's XRD pattern showed several distinct, strong peaks, suggesting that it is a highly crystalline material. The central peak, located at 17.25◦, stood out the most among these peaks. Other notable peaks at 2θ angles were 12.27◦, 14.53◦, 15.83◦, 18.15◦, 21.13◦, 23.28◦, and 24.69◦. In contrast, the XRD pattern of (curcumin and rutin) co-encapsulated SNEDDS revealed the disappearance of characteristic peaks of both curcumin and rutin, suggesting a decrease in crystallinity or transformation of the drugs into an amorphous phase. The disappearance of curcumin and rutin peaks in (curcumin and rutin) co-encapsulated SNEDDS confirms the successful loading of these drugs into the nanoparticles. The XRD pattern of the physical mixture displayed intense but diminished peaks compared to pure drugs, indicating that the crystalline

structure of the drugs was still present in the mixture. In contrast, the blank mixture exhibited no peaks, indicating an amorphous nature.

5.4.5. *TEM analysis*

The TEM analysis was used to examine the morphology of the synthesized (curcumin and rutin) co-encapsulated SNEDDS. It was identified as spherical, with sizes ranging from 80 to 100 nm (Fig 5.13). The scale bar represents 100 nm [53].

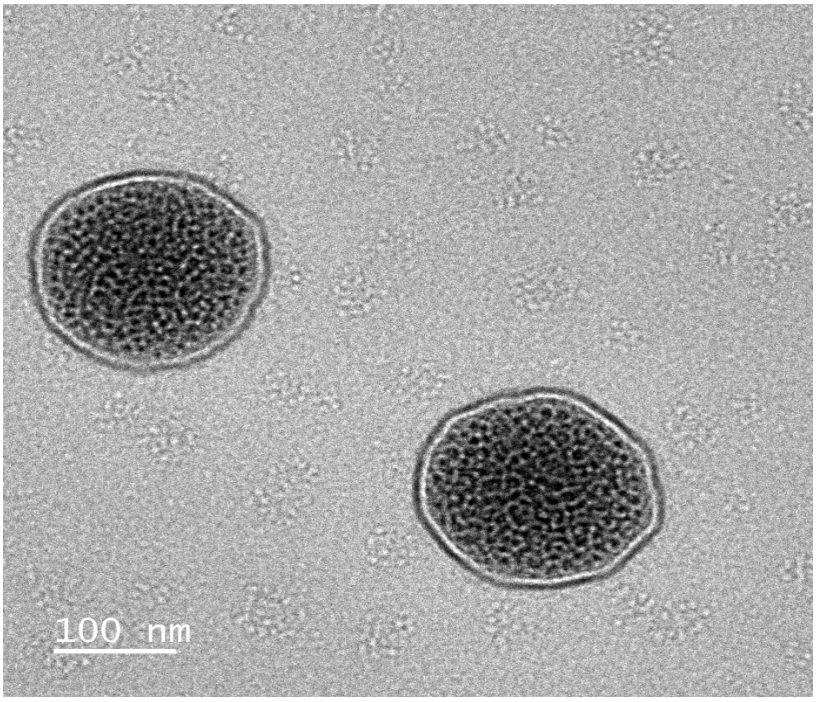


Fig 5.13 TEM analysis of (curcumin and rutin) co-encapsulated SNEDDS

5.2.4.6. *FESEM analysis*

The FESEM analysis confirmed the size and morphology of the (curcumin and rutin) co-encapsulated SNEDDS as observed in the DLS and TEM studies. The prepared nanoparticles were observed to have a spherical morphology under FESEM.

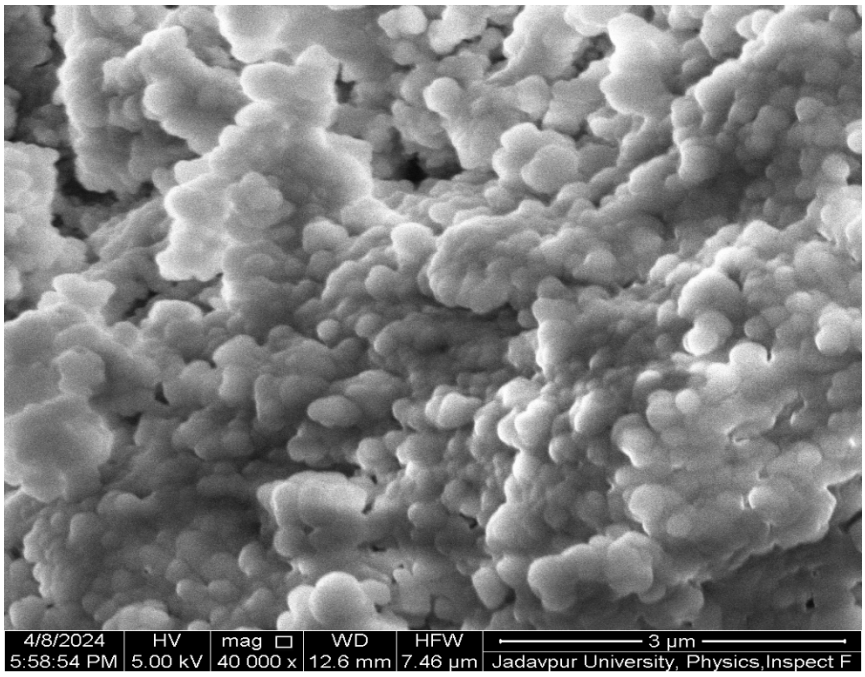


Fig 5.14 FESEM analysis of (curcumin and rutin) co-encapsulated SNEDD

5.2.4.7. ***In vivo antidiabetic study***

5.2.4.7.1. Effect of glibenclamide (standard drug), free drug (curcumin and rutin) and (curcumin and rutin) co-encapsulated SNEDDS on blood glucose levels

In the present investigation, the treatment with (curcumin and rutin) co-encapsulated SNEDDS demonstrated an improvement in protection against the rise in blood glucose levels until the end of the study. It has been observed that the optimized curcumin and rutin-co-encapsulated SNEDDS have been shown to decrease blood glucose levels compared to the STZ group. The free drug (curcumin and rutin) was not able to significantly lower blood glucose levels compared to the optimized (curcumin and rutin) co-encapsulated SNEDDS. This might be due to their low solubility and dissolution. In the third week, the blood glucose levels of the optimized SNEDDS groups are nearly comparable to those of the standard drug group. In the 4th week of the study, the optimized SNEDDS formulation demonstrated effective results by lowering blood glucose levels, comparable to the normal group and the standard drug. All the experimental data were analyzed using ANOVA followed by Dunnett's multiple comparison test in GraphPad Prism 5 software at a 95% confidence level. The comparison of normal control data with others revealed that there is a highly significant difference with STZ. It confirms the successful induction of diabetes. A

significant difference was also observed in the group treated with the free drug. The comparison of the STZ group with other groups confirms that the reduction in blood glucose levels for the STD drug group and the SNEDDS (curcumin and rutin) group showed a highly significant difference, indicating similar blood glucose-lowering effects as the normal control group. Whereas the free drug group showed no significant difference in blood glucose-lowering effects compared to the STZ group. It was concluded that the free drugs were not able to lower blood glucose levels compared to the formulated SNEDDS. So, Curcumin and EGCG should be delivered in the form of SNEDDS instead of their free form. The data for blood glucose level shown in table 5.2.

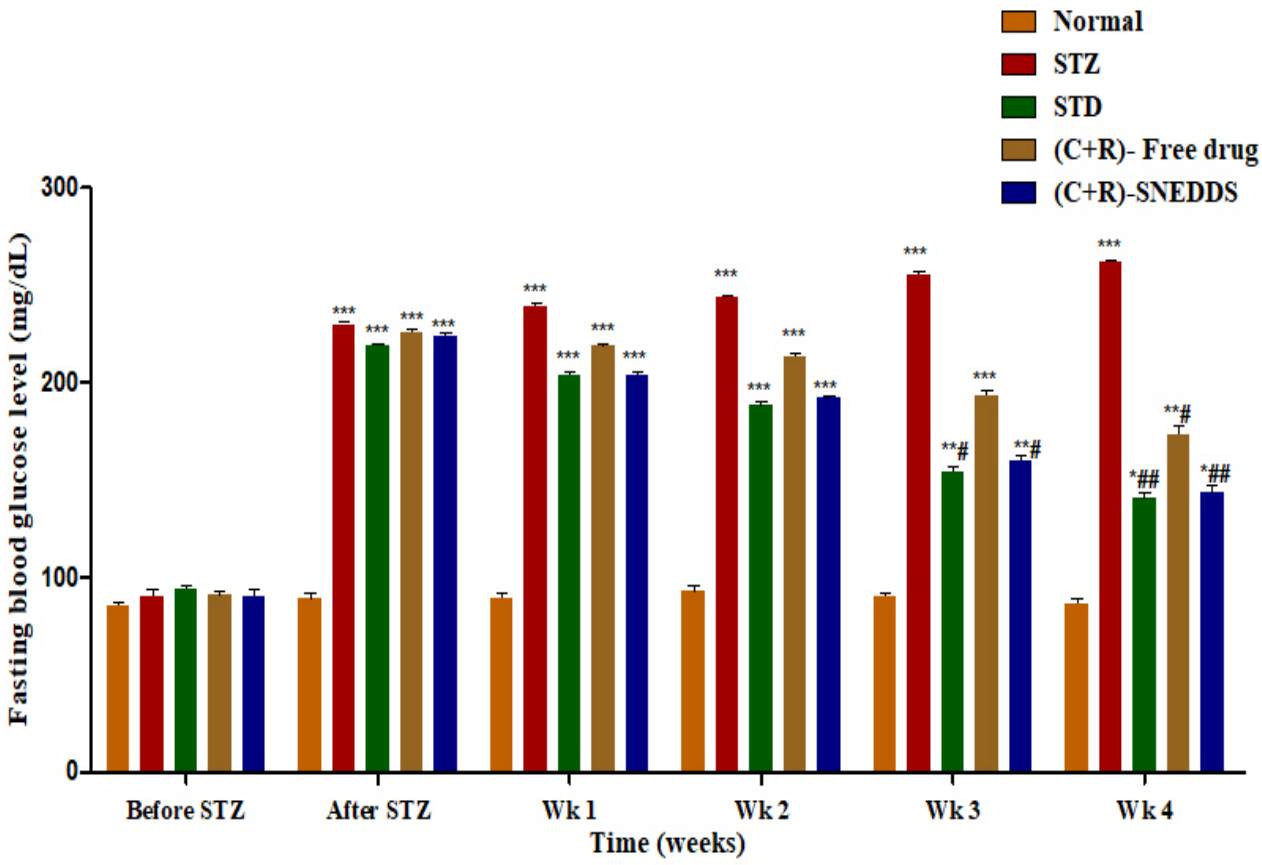


Fig 5.14 In vivo antidiabetic study of glibenclamide, free drug (curcumin and rutin) and (curcumin and rutin) co-encapsulated SNEDDS.

Table 5.2 Blood glucose levels of mice

	Normal (average)	STZ (average)	STD (average)	(Curcumin and Rutin) Free drug (average)	(Curcumin and Rutin) SNEDDS (average)
Before STZ	85 ± 1.2	90 ± 33	94 ± 1.43	90 ± 1.3	90 ± 1.0
After STZ	89 ± 1.3	229 ± 1.1	219 ± 1.45	225 ± 1.44	223 ± 1.67
Week 1	88 ± 1.25	238 ± 1.3	225 ± 1.22	218 ± 1.55	203 ± 1.6
Week 2	92 ± 1.5	243 ±1.5	188 ± 1.21	213 ± 1.23	191 ± 1.42
Week 3	90 ± 2.0	255 ± 1.34	154 ± 1.33	190 ± 1.34	159 ± 1.2
Week 4	86 ± 1.33	261 ± 1.44	140 ± 1.1	160 ± 1.44	143 ± 1.3

Chapter 6:

CONCLUSION

6. Conclusion

Fish oil, Tween 80, Transcutol P, and ascorbyl palmitate were used as excipients in successfully developing (curcumin and rutin) co-encapsulated SNEDDS. A 1:1 ratio of isomalt to Aerosil 200 was employed as the solidifying agent. A total of eight batches were prepared and tested for dissolution and flow properties of the powdered SNEDDS. The particle size of the optimized (curcumin and rutin) co-encapsulated SNEDDS was found to be 155 ± 0.5 nm, with a zeta potential of –22 mV, indicating a high repulsive force between particles. In vitro dissolution studies showed that the intestinal fluid, with its larger surface area for drug absorption, exhibited higher drug release for both curcumin and rutin. The (curcumin and rutin) co-encapsulated SNEDDS demonstrated sustained release compared to the free drugs. FTIR analysis revealed the disappearance of characteristic absorption peaks of both curcumin and rutin, confirming their interaction with the excipients in the SNEDDS formulation. XRD analysis further indicated that the originally crystalline curcumin and rutin were present in the SNEDDS in an amorphous state. These findings suggest that the drugs were successfully encapsulated in the delivery system while meeting optimal specifications. The study also concluded that a self-nanoemulsifying drug delivery system could improve the dissolution properties of bioactive compounds and enhance their bioavailability. After four weeks of antidiabetic testing on STZ-induced diabetic mice, statistical analysis showed that the (curcumin and rutin) co-encapsulated SNEDDS exhibited significantly greater antidiabetic potential than the free drugs. In summary, this development may lead to a more effective treatment strategy for diabetic patients, as (curcumin and rutin) co-encapsulated SNEDDS can be used for extended periods without adverse effects. However, the full conclusion will depend on the results of clinical testing.

Chapter:7

SUMMARY

7. Summary

Hyperglycemia, or high blood sugar, is a hallmark of diabetes mellitus, a long-term metabolic disease caused by either insulin resistance or insufficient insulin release from the pancreatic B-cells. Patients with diabetes mellitus experience symptoms such as polyuria, polydipsia, and polyphagia as a result of their elevated blood glucose levels. Pathological alterations such as nephropathy, retinopathy, and cardio-vascular problems arise as the disease progresses, ultimately resulting in multiple organ failure.

Because it is convenient and simple to administer, the oral route is the most popular and recommended method for drug delivery. On the other hand, low concentration gradient between the blood vessel and the stomach after oral delivery results from the drug's poor solubility, which limits transport and affects oral absorption. Low solubility and dissolution rate drugs have poor absorption, leading to substantial inter- and intrasubject variability, non-proportional dosage administration, and uneven bioavailability. Poor water solubility and dissolution rate of isolated bioactive substances with many chemical entities affects their bioavailability.

Natural molecules called bioactive compounds can be found in plants, animals, and microorganisms. They have the potential to improve human health and have a variety of therapeutic effects, such as reducing inflammation, diabetes, and cancer. Turmeric contains a bioactive substance called curcumin, which has numerous therapeutic uses. Many studies have been conducted on curcumin, which has been shown to have anti-inflammatory, antioxidant, and maybe anti-diabetic properties. Its potential for hepatoprotective, nephroprotective, immunomodulatory, and wound-healing qualities has also been investigated. But its therapeutic potential is limited by its low oral bioavailability. One of the main catechins in green tea is a polyphenolic molecule called epigallocatechin-3-gallate (EGCG). EGCG is very beneficial in treating inflammatory bowel disease, and diabetes, and improving exercise performance.

It is necessary to create a formulation for curcumin and EGCG to improve its dissolving properties, which may further increase its bioavailability and address the problem of low bioavailability. Drugs that dissolve poorly in water can be effectively made to dissolve better with the use of

innovative drug delivery techniques. Numerous methods have been looked into to produce such medications. Furthermore, delivery is a practical approach because of its scalability and cost-effectiveness.

Nowadays, lipid-based drug delivery methods have established a stellar reputation for improving the oral bioavailability and water solubility of BCS class II medications. The self-nanoemulsifying drug delivery system (SNEDDS) is a promising lipid-based drug delivery method that has drawn interest from academia and industry.

Chapter:8

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