DEVELOPMENT, CHARACTERIZATION AND EVALUATION OF CURCUMIN AND EGCG CO-ENCAPSULATED SELF NANO EMULSIFYING DRUG DELIVERY SYSTEM (SNEDDS) FOR EFFECTIVE MANAGEMENT OF DIABETES MELLITUS

Submitted by

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Declaration of Originality and Compliance of Academic Ethics

I hereby declare that this thesis contains literature survey and original research work performed by me (**Rinchen Palzong Bhutia**) as a part of my Master of Pharmacy studies. All the information in this document have been obtained and presented in accordance with academic rules and ethical conduct.

I also declare that, as required by these rules and conduct, I have cited and referenced the materials and results that are not original to this work.

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CERTIFICATE OF APPROVAL

This is to certify that ***Rinchen Palzong Bhutia*** (Exam Roll No. – M4PHP23011 Reg. No. 160244 of 2021-2023) has sincerely carried out the research work on the subject entitled "***Development, characterization and evaluation of curcumin and EGCG encapsulated Self Nano Emulsifying Drug Delivery System (SNEDDS) for effective management of diabetes mellitus***" under the supervision of **Dr. Manas Bhowmik,** Associate Professor, Department of Pharmaceutical Technology, Jadavpur University. He has incorporated his findings in this thesis submitted by him in partial fulfilment of the requirements for the Post Graduate Degree of Master of Pharmacy in the division of Pharmaceutics of Jadavpur University. He has carried out the research work independently and sincerely with proper care and attention to our entire satisfaction.

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Dedicated to
My Parents, My Brother,
Teachers, and
Friends

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CHAPTER: 1

AIMS & OBJECTIVES

1. Aim and Objectives

1.1 Aim of the present work

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels (hyperglycemia) either, due to failure of insulin secretion from the β-cells of pancreas or due to insulin resistance. Due to the increased blood glucose level, symptoms like polyuria, polydipsia and polyphagia are observed in patients suffering from DM. With the progression of disease, pathological changes like nephropathy, retinopathy and cardio-vascular complications occur leading to multiple organ failure eventually [1].

Oral route is the most common and preferred route for drug administration due to convenience and ease of administration [2]. However, for oral administration, low concentration gradient between the gut and blood vessel; due to poor solubility of the drug leads to a limited transport, consequently influencing its oral absorption. A drug with a low solubility and dissolution rate has limited absorption, resulting in inconsistent bioavailability, high inter- and intrasubject variability, and a lack of dosage proportionality [3]. Isolated bioactive compounds with many chemical entities have poor aqueous solubility and dissolution rate, influencing its bioavailability [4].

Bioactive compounds are naturally occurring substances found in plants, animals, and microorganisms that have the potential to positively influence human health and exhibit a range of therapeutic effects, including those against diabetes, cancer, inflammation etc [5,6]. Curcumin, a bioactive compound found in turmeric possess a wide range of medicinal properties. Curcumin has been widely studies and has been reported to possess anti-inflammatory, antioxidant, and potential anti-diabetic effects. It has also been studied for its potential in hepatoprotective, nephroprotective, neuroprotective properties, immunomodulatory and wound healing. However, its low oral bioavailability limits its therapeutic potential [7]. Epigallocatechin-3-gallate (EGCG), a polyphenolic compound, is a major catechin found in green tea. EGCG exhibits a variety of therapeutic effects and is highly effective in diabetes, exercise enhancement inflammatory bowel disease, skin disorders, hair loss, weight loss and iron overload. Regardless of promising medicinal activities of EGCG, it suffers from poor bioavailability due to low aqueous solubility and poor dissolution properties [8].

To address the issue of poor bioavailability, there is a need to develop a formulation for curcumin and EGCG with the aim of improving its dissolution properties which might further thereby improve its bioavailability. Novel drug delivery methods are effective in enhancing the dissolution properties of poorly water-soluble drugs. Various approaches have been investigated for the purpose of such drugs [9]. Furthermore, scalability and cost effectiveness make delivery a feasible method.

Currently, drug delivery systems utilizing lipids as carriers have gained an excellent reputation for enhancing the aqueous solubility and oral bioavailability of BCS class II drugs [10]. Among lipid-based drug delivery systems, the self-nanoemulsifying drug delivery system (SNEDDS) is a favorable option that has attracted academic and commercial interest [11].
SNEDDS is an isotropic mixture of oil, surfactant, cosurfactant, and a drug or bioactive. Under peristaltic movement upon contact with the aqueous medium in the gastrointestinal (GI) tract, they spontaneously form a fine oil-in-water (o/w) nanoemulsions with a globule ranging in size from a few nanometers to less than 200 nm [12]. The enhanced interfacial area of micronized globules will facilitate the dissolution of drug thereby improving the bioavailability and enhance permeability through biological membranes due to presence of lipid and surfactant [13].

1.2 Objectives of the present work

The aim of the present study was to design, develop and evaluate SNEDDS for better management of diabetes with the below specific objectives.

i. Improvisation in solubility of poorly water-soluble drug; curcumin and EGCG by encapsulating them in SNEDDS.
ii. In-vitro evaluations and characterization studies of optimized formulations.
iii. In-vivo study of best formulation developed for treatment of streptozotocin induced diabetes mellitus in mice model.

CHAPTER 2:

INTRODUCTION

2. Introduction

2.1 Diabetes Mellitus (DM)

Diabetes mellitus, commonly referred to as diabetes, is a chronic metabolic disorder characterized by elevated blood glucose levels (hyperglycemia) either, due to failure of insulin secretion from the β-cells of pancreas or due to insulin resistance [14]. Due to the increased blood glucose level, symptoms like polyuria, polydipsia and polyphagia are observed in patients suffering from DM. With the progression of disease, pathological changes like nephropathy, retinopathy and cardio-vascular complications occur leading to multiple organ failure eventually [15]. It is a global health concern with an increasing prevalence, imposing significant burdens on individuals, healthcare systems, and economies worldwide. According to IDF's report of 2017, about 425 million people were reported to be suffering from DM, and 90% of whom have type 2 diabetes. This number is expected to reach 628.6 million by 2045 [16].

India is facing a diabetes epidemic, with a substantial increase in the number of people affected by the disease. According to the International Diabetes Federation (IDF) Diabetes Atlas, India had an estimated 72.9 million adults living with diabetes, and this number is projected to increase to 134.3 million by 2045 [16]. The prevalence of diabetes in India is around 8-10% of the adult population, with a higher prevalence in urban areas compared to rural regions.

2.1.1 Types of DM

1) Type 1 diabetes mellitus (T1DM)

TIDM is a heterogeneous disorder characterized by autoimmune mediated destruction of pancreatic beta cells that culminates in absolute insulin deficiency. T1DM is most commonly diagnosed in children and adolescents, usually presents with symptomatic hyperglycaemia, and imparts the immediate need for exogenous insulin replacement [17]. In this form of diabetes, the rate of β-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycaemia that can rapidly change to severe hyperglycaemia and/or ketoacidosis in the presence of infection or other stress. Still others, particularly adults, may retain residual β-cell function sufficient to prevent ketoacidosis for many years; such

individuals eventually become dependent on insulin for survival and are at risk for ketoacidosis. At this latter stage of the disease, there is little or no insulin secretion, as manifested by low or undetectable levels of plasma C-peptide [18]. Approximately 10% of all diabetes cases are of T1DM. Since, the pancreas cells that produce insulin are destroyed, T1DM patient will have the disease for life and will need treatment in the form of insulin shots or an insulin pump [19].

2) Type 2 diabetes mellitus (T2DM)

Type 2 diabetes mellitus (T2DM) is a metabolic disorder that is characterized by insulin resistance and relative insulin insufficiency, both of which lead to elevated blood glucose levels. The classical symptoms of T2DM are frequent urination, increased thirst, fatigue, and weight loss. T2DM is due to a combination of lifestyle and genetic factors. In addition to any genetic component, environmental factors, particularly diet and obesity, have a significant role in the development of T2DM. Insulin resistance occurs when human tissues do not respond effectively to insulin. In contrast to T1DM, insulin resistance is often a problem with insulin receptors in cells that do not respond correctly to insulin rather than a problem with insulin synthesis [20]. Approximately 90% of all cases of diabetes worldwide are of this type. Normally, T2DM has adult-onset, however, it can affect people at any age, even children. But T2DM develops most often in middle-aged and older people [21].

3) Gestational Diabetes

This type of Diabetes affects females during pregnancy. Some women have very high levels of glucose in their blood, and their bodies are unable to produce enough insulin to transport all of the glucose into their cells, resulting in progressively rising levels of glucose [22]. Approximately 4% of pregnant women may develop gestational diabetes. Unlike Type 1 and Type 2 diabetes, gestational diabetes will disappear after the baby is born.

2.1.2 Diabetes Management

Diabetes can be managed by healthy eating habits, regular exercise, and medications to lower blood glucose levels. Many people with type 2 diabetes can control their blood glucose by following a healthy meal plan and a program of regular physical activity, losing excess weight, and taking medications [18]. Another important part of diabetes management is reducing cardiovascular disease risk factors including high blood pressure, hyper lipidaemia, and use of tobacco. Patient counselling and self-care practices are also important aspects of diabetes management that help people to cope with disease. Medications for each individual with diabetes will often change during the course of the disease depending upon individual need of the patient [23].

2.1.3 Treatment strategies in DM

2.1.3.1 Current treatments for Diabetes mellitus

The major components of the treatment of diabetes are shown below [24]:

1. Diet (combined with exercise if possible)
2. Oral therapy
3. Insulin treatment

Antidiabetic drugs can be classified into two categories:

A. Insulin injections: Mostly used in serious cases of diabetes.
B. Oral hypoglycemic agents: Suitable for most adult patients.

There are following common types of oral antidiabetic drugs:

1. Sulphonyl ureas – Sulfonyl ureas act by increasing insulin release from the beta cells of the pancreas. They are insulin secretagogues, triggering insulin release by inhibiting the K_{ATP} channel of the pancreatic beta cells. Glimepiride, a member of this class, appears to have a useful secondary action in increasing insulin sensitivity in peripheral cells.

E.g., First generation (Tolbutamide, Chlorpropamide); Second generation (Glibenglamide, Glipizide, Glimepiride).

2. Biguanides– Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose and increases peripheral glucose uptake and utilization. Metformin may be used as monotherapy, or in combination therapy with sulfonylurea or meglitinides.

E.g., Metformin

3.**Meglitinides** – The mechanism of action of the Meglitinides is to stimulate insulin production. Meglitinides help the pancreas to produce insulin and are often called "short acting secretagogues." They act on the same potassium channels as sulfonylureas, but at a different binding site. By closing the potassium channels of the pancreatic beta cells, they open the calcium channels, thereby enhancing insulin secretion. The meglitinides may be used alone, or in combination with metformin.

E.g., Nateglinide, Repaglinide

4. Thiazolidinediones –They act by both reducing glucose production in the liver and increasing insulin dependent glucose uptake in muscle cells. They do not increase insulin production. These drugs may be used in combination with metformin or a sulfonylurea.

E.g., Rosiglitazone, Pioglitazone

5. Alpha glucosidase inhibitors – Alpha-glucosidase inhibitors do not enhance insulin secretion. Rather, they inhibit the conversion of disaccharides and complex carbohydrates to glucose. This mechanism does not prevent conversion, but only delays it, reducing the peak blood glucose levels. Alpha-glucosidase inhibitors are useful for either monotherapy or in combination therapy with sulfonylureas or other hypoglycemic agents.

E.g., Acarbose, Miglitol

6. Dipeptidyl peptidase-4 (DPP-4) inhibitors –This drug, called a dipeptidyl peptidase 4 inhibitor, works by helping the pancreas release insulin.

E.g., Sitagliptin, Linagliptin

Limitations:

Drugs belonging to all these categories have associated side effects. GLP-1 analogues which are administered through subcutaneous route have shown clinical effectiveness in reducing glucose levels in clinical trials [25]. The most common adverse effect seen with sulfonylureas is hypoglycemia (most common with the use of chlorpropamide and glibenclamide) [26,27]. The other side effects that have been reported include cholestatic jaundice and cardiovascular mortality. The reports, nevertheless, are not very significant. Meglitinides are reported to cause a range of side effects, most commonly hypoglycemia, visual disturbances, abdominal pain, diarrhoea, constipation, nausea and vomiting. More rarely, hypersensitivity reactions as well as elevation of liver enzymes may also occur. Thiazolidienediones like rosiglitazone and pioglitazone may cause oedema, particularly in patients with hypertension, and risks of other cardiovascular diseases. α-glucosidase inhibitors like acarbose cause abdominal discomfort associated with flatulence and diarrhea [28]. Gliptins and GLP-1 analogues can cause pancreatitis while SGLT2 inhibitors can lead to urinary tract infections [29].

Hence, there is a need for safer and effective medicine for the treatment of DM, irrespective of the fact whether they belong to synthetic or natural/herbal origin.

2.1.3.2 Bioactive compounds for the treatment of diabetes

Herbal medicine has been used for the prevention and treatment of various health diseases from the distant past beyond memory. Phytoconstituents have been long investigated by the bio-pharmaceutical sciences because of the diversity seen in their chemical structure. Also, wide range of pharmacological activities and low side effects are added merits, so had gain popularity and tremendous growth of phytopharmaceutical usage [30]. Phytoconstituents play an important role in traditional medicine and are investigated for various pharmacological activities such as antineoplastic, antibacterial, and other [31]. Plant based medicines, pharmaceuticals, food supplements etc are increasing in demand in both rural and urban areas. Market of herbal supplements is estimated to reach $107 billion globally by 2017 [32]. Indian herbal industry is flourishing with a growth rate of 15 percent against pharmaceutical industry [33].

Herbal drugs could be the best alternative to conventional synthetic drugs for management of DM. Various bioactive compounds of the herbal plants e.g., catechins from green tea, curcumin

from turmeric, mangiferin from mango bark, etc. have been reported for their antidiabetic potential. However, these formulations suffer from certain restrictions. The bioactive molecules exhibit poor solubility and hence low oral bioavailability limits its therapeutic potential. Various approaches of novel drug delivery have been investigated to formulate the herbal drug with improved solubility and dissolution properties [34]. Other than these includes lack of targeting to site, presystemic metabolism, etc [35]. Versatile novel approaches have been utilized to improve the characteristics of various phytoconstituents.

2.1.3.3 Novel treatment strategies in DM

Antidiabetic drugs are either available in oral dosage form or in the form of injectables. As diabetes is a chronic disease requiring long term therapy, oral route is most suitable route due to its ease of administration and compliance. Most of the antidiabetic drugs which are available in oral dosage form are either tablets or capsules. These suffer from certain limitations in terms of side effects i.e., gastric irritation, diarrhoea, loss of appetite and lactic acidosis in people with abnormal kidney or liver function [36].

There are several approaches that are reported to improve the dissolution rate limited bioavailability of poorly soluble drugs [37]. These approaches include increasing the surface area [37], particle size reduction [38], formulation in a dissolved state [39], liquisolid compacts [40], preparation of inclusion complexes [41], solid dispersions [42], use of pro drugs [43], and generation of metastable polymorphs [44].

Various nano formulation strategies are being developed to address challenges associated with the absorption and availability of orally administered drugs. These innovative formulations have been employed not just to increase the solubility and availability of these bioactive compounds, but they have also demonstrated effectiveness in improving their stability against physical, chemical, biological, and light-induced degradation. Additionally, these formulations provide an extra benefit by allowing controlled and extended drug release, along with targeted delivery to specific sites. This targeted delivery prevents unintended exposure to non-target areas [45].

Numerous plant-derived components such as curcumin, flavonoids, and vitamins have been reported to exhibit more potent therapeutic effects at lower doses when incorporated into novel delivery systems compared to the conventional drug delivery systems [46,47,48,49].

In recent times, various advanced lipid-based nano delivery approaches such as liposomes, niosomes, transfersomes, ethosomes, phytosomes, nanoemulsions, solid lipid nanoparticles, and self-emulsifying delivery systems have emerged. These methodologies have been designed to significantly improve the solubility, bioavailability, and stability of compounds [50].

2.2 Self nano-emulsifying drug delivery system (SNEDDS)

Enhancing the oral bioavailability of poorly water-soluble medications can be achieved by if formulated with lipids [51]. One established method involves using lipid and surfactant-based systems for drug delivery [52]. Lipid based drug delivery consists of delivering a drug dissolved in a mixture of one or more excipients such as mono-, di-, and triglycerides, along with lipophilic and hydrophilic surfactants, and a co-surfactant [53,54]. The advantage of this approach is that the drug remains in a dissolved state within the formulation, eliminating the complexities associated with solid-state formulations during oral absorption. This eliminates the necessity for complete dissolution, leading to enhanced absorption of the drug. [55].

Recently, SNEDDS have gain more importance among lipid-surfactant based drug delivery systems due to their capability to create an emulsion with an extensive large surface area upon dispersion [56]. SNEDDS covers emulsions with a droplet size ranging from 100-250 nm. SNEDDS is an isotropic mixture of oil, surfactant, cosurfactant, and a drug or bioactive. Under peristaltic movement upon contact with the aqueous medium in the gastrointestinal (GI) tract, they spontaneously form a fine oil-in-water (o/w) nanoemulsions with a globule ranging in size from a few nanometres to less than 200 nm [10]. The enhanced interfacial area of micronized globules will facilitate the dissolution of drug thereby improving the bioavailability and enhance permeability through biological membranes due to presence of lipid and surfactant [11]. These conditions mimic the digestive motility in GI tract which is necessary to provide agitation required for in-vivo self-emulsification.

2.2.1 Mechanism of formation of SNEDDS

Self-emulsification is a phenomenon that occurs spontaneously during the formation of SNEDDS. It occurs when entropy change that favours dispersion is greater than energy required to increase the surface area of emulsion. Free energy of an emulsion is considered as a direct function of the energy required to create a new surface between any two immiscible phases. The two immiscible phases of an emulsion exhibit a tendency to separate so as to reduce

interfacial area to minimum and thus, to minimize free energy of system. These systems are stabilized by use of emulsifying agents that reduce the interfacial tension. Thus, for SNEDDS, such kind of emulsifiers and co-solvents need to be selected that will be able to reduce the interfacial tension. This, in turn, will lower the free energy required by SNEDDS so that when they come in contact with aqueous medium in GIT, the self-emulsification process sets in. **Fig 2.1** depicts the mechanism of SNEDDS formation.

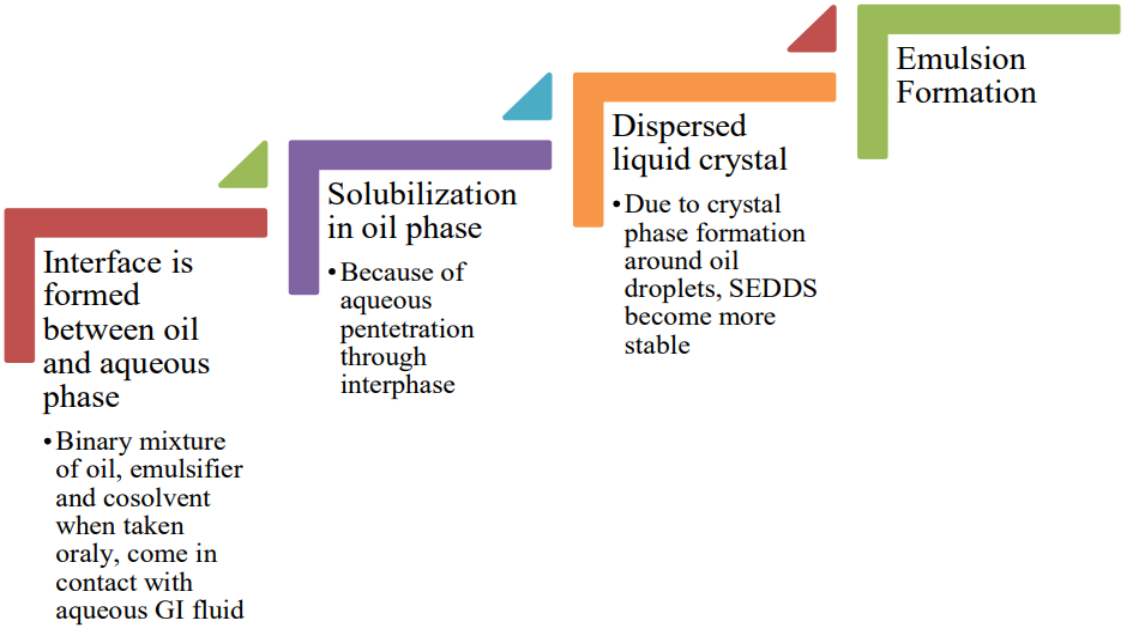


Fig.2.1 *Mechanism of SNEDDS formation*

2.2.2 Drug transport mechanism of SNEDDS

SNEDDS offer bioavailability of water insoluble drugs even through oral administration. Once they reach the GIT, they undergo three processes, i.e., digestion, absorption, and circulation. These three phases are depicted in **Fig 2.2**. During digestion, SNEDDS form a coarse emulsion, which undergoes enzymatic hydrolysis at oil water interphase and thereby gets ready for absorption phase. After formation of mixed micelles, due to interaction of fatty acids with bile, digestion process stops. The next phase of drug absorption then starts. These colloids are taken up by passive diffusion or active transport through enterocyte membrane. Some drugs may get absorbed via lymphatic circulation through chylomicrons. In circulatory phase, drug is released from chylomicrons and the residual lipid is used in the body.

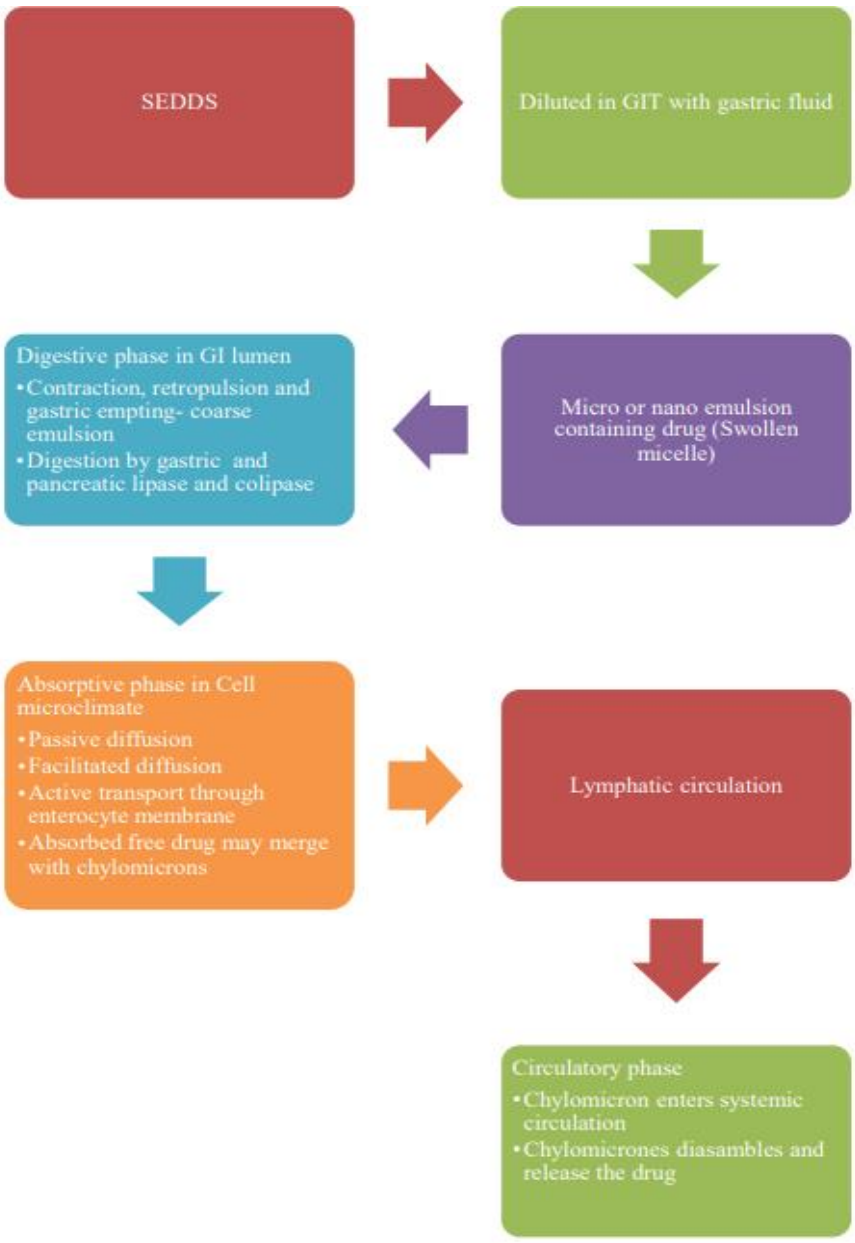


Fig.2.2. *Drug transport mechanisms of SNEDDS*

2.2.3 Advantages of SNEDDS

Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) offer several advantages in the field of pharmaceutical formulations. Some of these advantages are supported by references:

1. ***Enhanced Bioavailability***: SNEDDS can significantly improve the bioavailability of poorly water-soluble drugs by enhancing their solubility and dissolution rate. This leads to improved drug absorption and therapeutic efficacy [57].

2. ***Uniform Drug Dispersion***: SNEDDS spontaneously form nanoemulsions when in contact with gastrointestinal fluids. This leads to a more uniform distribution of the drug within the emulsion, promoting consistent drug delivery [58].

3. ***Reduced Variability***: The self-emulsifying nature of SNEDDS minimizes inter- and intra-subject variability in drug absorption, which can be a concern with conventional formulations. This ensures more predictable and reproducible pharmacokinetic profiles [59].
4. ***Ease of Manufacturing and Formulation***: SNEDDS are relatively simple to manufacture and can be easily incorporated into different dosage forms, including capsules and tablets. This makes them a practical choice for industrial production [60].

5. ***Stability Enhancement***: SNEDDS can improve the stability of sensitive drugs by protecting them from degradation, as the emulsified state can shield the drug molecule from environmental factors [61].

6. ***Reduced Food Effect***: SNEDDS formulations often exhibit reduced, or no food effects compared to conventional formulations, making them less susceptible to changes in bioavailability when taken with food [62].

Incorporating these advantages, SNEDDS have become a promising tool in pharmaceutical research for improving drug delivery and therapeutic outcomes.

2.2.4 Marketed Formulations

Various drugs had been formulated and the list of marketed preparations of SNEDDS is summarised in **Table 2.1.**

Table 2.1. Marketed formulations based on SNEDDS [63].

Drug (Indication)	Brand Name	Dose (mg)	Dosage form	Excipients
Cyclosporine A (Anticancer)	Sandimune® (Novartis)	10-100	SGC	Corn oil, polyoxyethylated linoleic glycerides (Labrafil M2125CS)
Lopinavir (Antiviral)	Kaletra® (Abbott)	130	SGC	Oleic acid, polyoxyl 35 castor oil (Cremophore EL
Fenofibrate (Antiplatelet)	Lipirex® (SanofiAventis)	200	HGC	Lauryl macrogol-glycerides (Gelucire 44/14), PEG 200
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

2.2.5 Formulation Approach

The phenomenon of self-emulsification is only specific to certain combinations of pharmaceutical excipients. Type of oil and surfactant pair, their ratios, the surfactant concentration, solubility of drug in lipid/surfactants blends, uniform droplet size distribution and the temperature at which self-emulsification occurs are some of the important parameters to be taken care of during the development of SNEDDS [64].

Selection of oil, surfactant and co-surfactant is done based on solubility of drug in oil, surfactant, and co-surfactant [65].

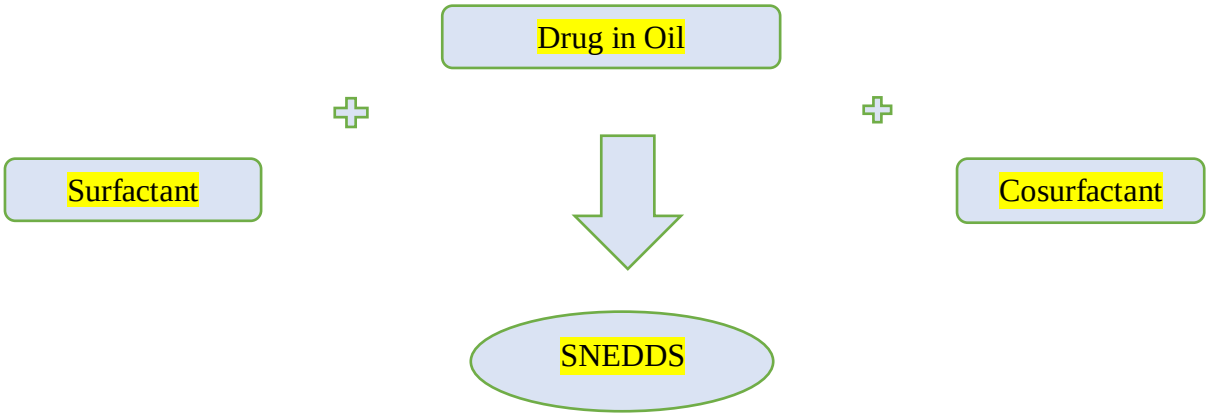


Fig 2.3. *Schematic representation of formulation of SNEDDS*

SNEDDS are composed of drug, oil, hydrophilic surfactant, and a cosurfactant. There are various lipids and surfactants available which can be utilized for the formulation of SNEDDS, but their toxicity and unclear mechanism of action limits their use. Therefore, an emphasis is made on the use of excipients that are generally regarded safe [66].

i) Drug

When dealing with drugs with larger doses, their solubility in the formulation of SNEDDS becomes a crucial factor. Ideally, the drug should readily dissolve in lipophilic phases. The effectiveness of SNEDDS in keeping the drug dissolved hinges largely on the drug's solubility in the lipid phase. If the drug's solubility relies on the surfactant or cosurfactant, the likelihood of drug precipitation increases since dilution of SNEDDS reduces the solvent-holding capability of surfactant and co-surfactant [67].

ii) Oils

The oil represents one of the most important excipients in the SEDDS formulation not only because it can solubilize the required dose of the lipophilic drug or facilitate self-emulsification but also and mainly because it can increase the fraction of lipophilic drug transported via the intestinal lymphatic system, thereby increasing absorption from the GI tract. The lipid part of SNEDDS forms the core of emulsion droplets. The selection of lipid should be done on the basis of potential of oil to assure maximum solubility of drug [68,69].

iii) Surfactants

Surfactants in SNEDDS reduce interfacial tension and adjust the spontaneous curvature of the interface so as to enable spontaneous emulsion formation [70]. Surfactants are amphiphilic in nature & they can dissolve or solubilize relatively high amount of hydrophobic drug compound. The high HLB and subsequent hydrophilicity of surfactants is necessary for the immediate formation of o/w droplets and rapid spreading of the formulation in the aqueous environment, providing a good dispersing/self-emulsifying performance. This ultimately helps in preventing precipitation of drug in GI lumen and prolongs the existence of drug molecules in soluble form which is vital for effective absorption.

iv) Cosurfactants

Role of the co-surfactant together with the surfactant is to lower the interfacial tension to a very small even transient negative value. Co-surfactants in SNEDDS help to facilitate dispersion process. Lipid mixtures with higher surfactant or co-surfactant ratios lead to the formation of SNEDDS. Cosurfactants affect the packing of surfactant molecules at the interface because of their short chain amphiphilic nature. Cosurfactants provides fluidity to the interfacial film by disrupting liquid crystalline phases. Cosurfactants can often improve the solubility of drug in SNEDDS preparation [71].

2.2.6 Drug Profile

2.2.6.1 Curcumin (Cur)

Cur (1, 7-bis [4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione) is a yellow-coloured compound isolated from Curcuma longa, family: Zingiberaceae. The solubility of cur is poor in water which leads to poor dissolution and bioavailability. Cur contains different functional moieties which are bonded to two phenol rings. The α- and β-unsaturated diketone moiety in the chemical framework of cur has a crucial role in the inhibition of nuclear factor-kB (NF-kB) and reactive oxygen species (ROS)-production (Nabavi et al., 2015). Commercial cur contains approximately 77% diferuloylmethane, 17% demethoxycurcumin, and 6% bisdemethoxycurcumin [72].

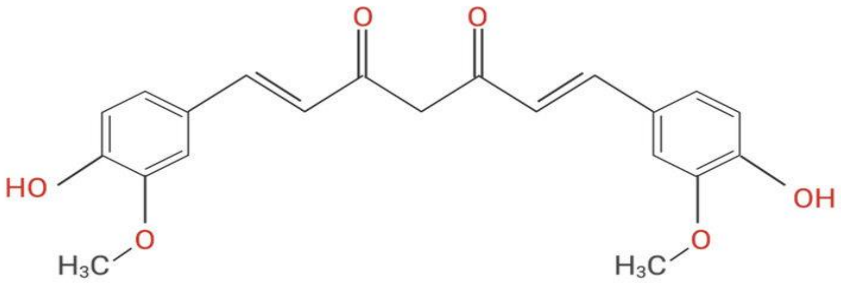


Fig.2.4*. Chemical structure of curcumin*

Fig. 2.5. *Powder curcumin extract*

2.2.6.1.1 Cur and its mechanism of action in DM

Cur is supposed to act as antidiabetic by various mechanisms as follows. It has the ability of induction of peroxisome proliferator-activated receptor gamma (PPAR-) activation. Cur helps to increase plasma insulin levels and lipoprotein lipase (LPL) activity. Activation of enzymes in liver, associated with glycolysis, gluconeogenic, and lipid metabolic process. Cur also induces secretion of GLP-1.

2.2.6.1.2 Traditional uses of curcumin in the treatment of DM

Curcumin is a bioactive compound found in turmeric, a spice widely used in traditional medicine and cooking. It has gained attention for its potential health benefits, including its use in the treatment of diabetes mellitus. Diabetes mellitus is a chronic metabolic disorder characterized by high blood sugar levels due to either insufficient insulin production or ineffective utilization of insulin.

There is a lot of ongoing research into the potential therapeutic effects of cur in DM. Here is an overview of some ways in which curcumin has been studied in relation to diabetes [73]:

1. ***Anti-Inflammatory Effects:*** Curcumin is known for its potent anti-inflammatory properties. Chronic inflammation is often associated with insulin resistance and the progression of type 2 diabetes. Some studies suggest that curcumin might help reduce inflammation in diabetes and improve insulin sensitivity.

2. ***Antioxidant Activity***: Curcumin possesses strong antioxidant properties, which can help counteract oxidative stress. Oxidative stress plays a role in the development of complications associated with diabetes, such as cardiovascular diseases and neuropathy.
3. ***Beta Cell Function:*** Beta cells in the pancreas are responsible for producing insulin. Some research suggests that curcumin might have a positive effect on beta cell function and survival, potentially aiding in insulin secretion.

4. ***Glucose Regulation:*** Curcumin has been studied for its potential to regulate blood sugar levels. It might influence glucose metabolism by improving insulin sensitivity and glucose uptake by cells.

5. ***Lipid Metabolism:*** Diabetes is often associated with dyslipidemia, an imbalance in lipid levels. Curcumin has been investigated for its ability to lower triglycerides and LDL cholesterol levels, which can be beneficial for individuals with diabetes.

6. ***Endothelial Function:*** Curcumin might have a positive impact on endothelial function, improving blood vessel health. This could be important in preventing diabetes-related complications such as cardiovascular diseases.

7. ***Neuropathy and Nephropathy:*** Some animal studies have suggested that curcumin might have protective effects against diabetic neuropathy (nerve damage) and nephropathy (kidney damage). These effects are likely linked to its anti-inflammatory and antioxidant properties.

2.2.6.2 Epigallocatechin gallate (EGCG)

Green tea derived from *Camellia sinensis* is the second most-consumed traditional drink in Asian countries and the extract of the leaves contain numerous antioxidants in the form of polyphenolic catechins namely (-)-epicatechin (EC), (−)-epigallocatechin (EGC), (−) -epicatechin3-gallate (ECG), (−)-epigallocatechin-3-gallate (EGCG). The most abundant catechin is EGCG (~60%), followed by EGC (~20%), then ECG (~14%), and EC (~6%) [74], and it is assumed that about 100mg of polyphenols are present in one bag of green tea [75].

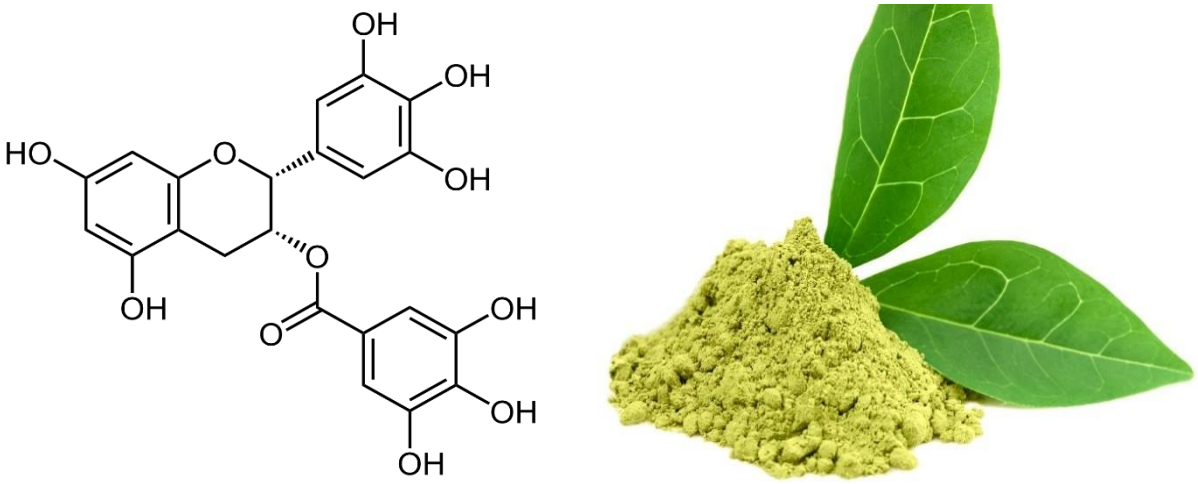


Fig.2.6. Chemical structure of EGCG and its extract

EGCG has been investigated in modern research for its effects on various health conditions, including diabetes. Here's a brief overview of EGCG:

1. ***Antioxidant Properties:*** EGCG is a potent antioxidant that helps neutralize harmful free radicals in the body. This antioxidant activity is believed to contribute to its

potential health benefits, including protecting cells from oxidative stress, which is often elevated in conditions like diabetes.

2. ***Anti-Inflammatory Effects:*** Chronic inflammation is linked to various health issues, including diabetes. EGCG has shown anti-inflammatory effects in studies, which could be relevant for managing inflammatory processes associated with diabetes and its complications.

3. ***Insulin Sensitivity:*** Some research suggests that EGCG might improve insulin sensitivity. Enhanced insulin sensitivity can help the body better regulate blood sugar levels, which is a key consideration in diabetes management.

4. ***Glucose Metabolism:*** EGCG has been studied for its potential to regulate glucose metabolism. It might influence the way cells take up glucose and use it for energy, which could be beneficial for people with diabetes.

5. ***Cardiovascular Health:*** Diabetes is often associated with an increased risk of cardiovascular complications. EGCG's antioxidant and anti-inflammatory effects might contribute to better cardiovascular health by improving factors like blood pressure and lipid profiles.

6. ***Potential Anti-Obesity Effects:*** Obesity is a major risk factor for type 2 diabetes. Some research suggests that EGCG might play a role in regulating metabolism and reducing body weight, which could indirectly benefit diabetes management.

2.2.7 Excipients Profile

i) Transcutol P

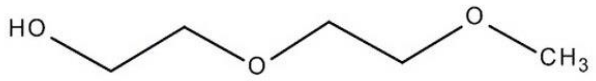


Fig.2.7 *Chemical structure of Transcutol P*

IUPAC name: 1-Methoxy-2-(2-methoxyethoxy) ethane

Chemical Name:	Diethylene glycol methyl ether
Molecular Formula:	$C_6H_{14}O_3$
Description:	Colourless and clear liquid with mild pleasant odour
Molecular Weight:	162 gm/mole
Boiling Point:	194˚C
Refractive Index:	1.425 at 25˚C
Solubility:	It is soluble in water and organic solvent like ethanol, acetone, ether, CHCl3 and benzene
Category:	Diluent, humectants, and solvent

ii) Tween 80

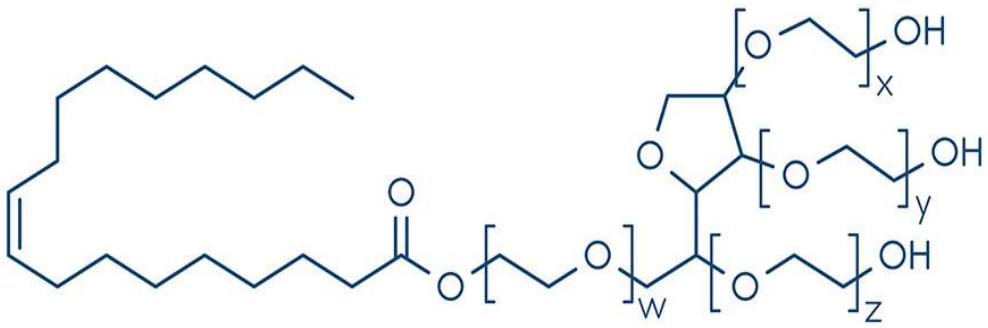


Fig.2.8 *Chemical structure of Tween 80*

IUPAC name: Polyoxyethylene (80) sorbitan monooleate

Chemical Name:	Polyoxyethylene sorbitan monooleate
Molecular Formula:	$C_{64}H_{124}O_{26}$
Solubility:	Soluble in water and ethanol and insoluble in mineral oil and vegetable oil

iii) Ascorbyl Palmitate

Ascorbyl palmitate is an ester formed from ascorbic acid and palmitic acid creating a fat-soluble form of vitamin C (Ascorbic acid, water soluble vitamin). In addition to its use as a source of vitamin C, it is also used as an antioxidant food additive (E number E304). It is approved for use as a food additive in the EU, the U.S, Canada, Australia, and New Zealand.

Ascorbyl palmitate is also marketed as "vitamin C ester". It is synthesized by acylation vitamin C using different acyl donors.

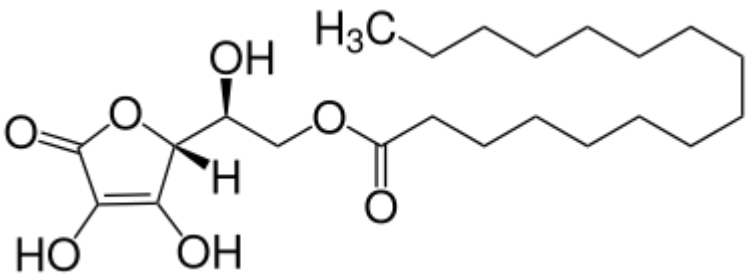


Fig.2.9 *Chemical structure of Ascorbyl palmitate*

Names:

Preferred IUPAC name**:** (2S)-2-[(2R)-3,4-Dihydroxy-5-oxo-2,5-dihydrofuran-2-yl]-2-hydroxyethyl hexadecanoate

Other names: Ascorbyl palmitate**,** L-Ascorbic acid 6-hexadecanoate**,** 6-O-Palmitoy-L-ascorbic acid.

iv) Aerosil 200

Aerosil 200 is a hydrophilic fumed silica with a specific surface area of 200 m^2/g. It has a shelf life of 2 years.

Chemical Name:	Silicon dioxide amorphous
Molecular Formula:	SiO_2
Description:	Transparent to grey, odorless powder.
Molecular Weight:	60.084 g/mol
Boiling Point:	4046 ºF at 760 mmHg
Melting Point:	3110 ºF
Solubility:	Poorly soluble in water

v) Isomalt

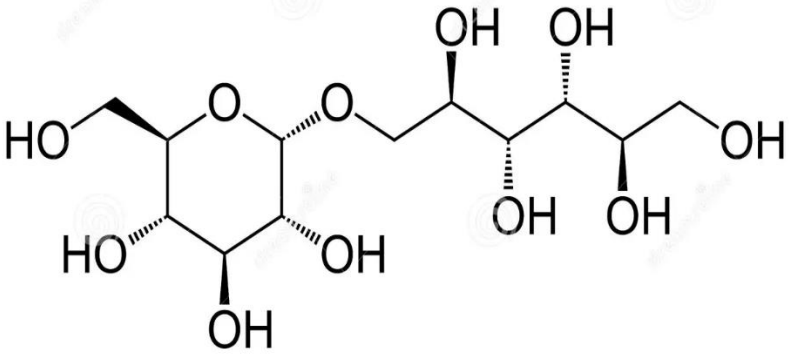


Fig.2.10 *Chemical structure of Isomalt*

IUPAC Name: (3R,4R,5R)-6-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl] oxyhexane-1,2,3,4,5-pentol

Isomalt is a noncariogenic excipient used in a variety of pharmaceutical preparations including tablets or capsules, coatings, sachets, suspensions, and in effervescent tablets. It can also be used in direct compression and wet granulation. In buccal applications such as chewable tablets it is commonly used because of its negligible negative heat of solution, mild sweetness and 'mouth feel'. It is also used widely in lozenges, sugar-free chewing gum, and hard-boiled candies, and as a sweetening agent in confectionery for diabetics.

Molecular Formula:	$C_{12}H_{24}O_{11}$
Molecular Weight:	344.31g/mol
Description:	Odourless, white, slightly hygroscopic, crystalline mass with pure sweet taste.
Solubility:	Soluble in water, very slightly soluble in ethanol.

vi) Fish Oil

Fish Oil is an oil derived from the tissues of oily fish. Fish oil is considered valuable due to its high content of omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid. Such fatty acids are not actually produced in fish but are accumulated from phytoplankton which produce omega-3 fatty acids.

Description:	Fish oil is a pale-yellow oily liquid with a fishy odour.
Solubility:	Insoluble in water

CHAPTER 3:

REVIEW OF LITERATURE

3. REVIEW OF LITERATURE

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

Tripathi et al.,2016 discusses the development of a self-nanoemulsifying drug delivery system (SNEDDS) loaded with quercetin, resveratrol, and genistein to improve their oral bioavailability and antioxidant potential. The optimized SNEDDS formulation showed a size of less than 200 nm and a polydispersity index (PDI) of less than 0.3. The antioxidant loaded SNEDDS demonstrated comparable antioxidant activity to the free antioxidants combination, as shown by the DPPH scavenging assay. The SNEDDS formulation showed rapid internalization within 1 hour of incubation by Caco-2 cells. Pharmacokinetic studies in rats showed that the SNEDDS formulation significantly increased the maximum concentration (Cmax) and area under the curve (AUC) of all three antioxidants compared to the free antioxidant suspension. The SNEDDS formulation exhibited a ~4.27 fold enhancement in the oral bioavailability of quercetin, ~1.5 fold for resveratrol, and ~2.8 fold for genistein compared to the free antioxidants suspension. The developed SNEDDS formulation demonstrated enhanced abeyance towards tumor growth in a DMBA-induced breast cancer model in rats compared to free antioxidants.

Jain et al.,2013 focuses on the rationalized development and characterization of a solidified self-nanoemulsifying drug delivery system (SNEDDS) for the oral delivery of a combinatorial therapeutic regimen. The authors used extreme vertices mixture design and 3 2 full factorial design for the optimization of the liquid SNEDDS and the concentration of the solid carrier in the lyophilization mixture. The developed formulation showed instantaneous emulsification

and maintained all quality attributes even after storage at accelerated stability conditions for 6 months. The formulation demonstrated significantly higher cellular uptake of tamoxifen and quercetin in comparison with free drug counterparts. The paper also discusses the polynomial equations for the analysis of QT loading, droplet size, and PDI of the resultant emulsion after dilution of the SNEDDS. The stability of the nanoemulsions after dilution was evaluated, and no phase separation or drug precipitation was observed. The selection of suitable oil was critical for the formulation of the liquid SNEDDS, and Capmul® MCM EP was found to solubilize both tamoxifen and quercetin effectively. The optimization of the composition of the SNEDDS was based on the desirability value, aiming to load maximum amount of quercetin while minimizing the concentration of surfactant.

Alfaro et al.,2022 developed a SNEDDS to improve the bioavailability of Betulinic acid (BA). SNEDDS-L and SNEDDS-C increased the bioavailability of BA 15.24-fold and 15.53 fold, respectively, compared to free BA. In an in vivo study using Wistar rats, SNEDDS demonstrated up to a 15-fold increase in BA bioavailability compared to free BA. The particle size of the SNEDDS nanoemulsions remained constant up to 105 minutes of simulated small intestine phase conditions. The addition of betulinic acid to the SNEDDS matrix maintained the particle sizes and verified caprylic acid as an oil phase for self-nanoemulsifying systems. FT-IR studies confirmed no interaction between the excipients of the SNEDDS and the encapsulated BA.

Yin et al.,2017 developed a nanoemulsions (NEs) consisting of hemp oil and less surfactants were used to improve the oral bioavailability of BCL. BCL-NEs were prepared using the high-pressure homogenization technique to reduce the amount of surfactants. BCL-NEs showed significantly enhanced oral bioavailability of BCL compared to suspensions and conventional emulsions. BCL-NEs exhibited excellent intestinal permeability and transcellular transport ability. The cytotoxicity of BCL-NEs was found to be low and acceptable for oral use. The particle size of BCL-NEs was around 90 nm with a high entrapment efficiency of 99.31%. BCL-NEs were characterized using particle size analysis and transmission electron microscopy. The pharmacokinetic parameters of BCL were determined using ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC-qTOFMS) analysis. Caco-2 cells were used to investigate the cell uptake and internalization of BCL-NEs.

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 50-66.57nm 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. The solubility of sitagliptin and dapagliflozin in anhydrous SNEDDS was evaluated, and the formulation with black seed oil and mixed glycerides at a ratio of 3:7 with cremophor EL as surfactant exhibited the highest drug solubility and better aqueous dispersibility. The solubility studies aimed to increase the loading capacity of the drugs in the SNEDDS formulation to improve oral bioavailability. 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.

Zang et al.,2020 developed a supersaturable self-nanoemulsifying drug delivery system (S-SNEDDS) to enhance the solubility and oral bioavailability of luteolin. The formulation for

SNEDDS consisted of caprylic/capric triglyceride, polyoxyl 35 hydrogenated castor oil, and polyethylene glycol 400. Hydroxypropyl methylcellulose (HPMC) K4M was determined to be the optimal precipitation inhibitor for luteolin-loaded SNEDDS. Luteolin S-SNEDDS formed a clarified nanoemulsion with a particle size of 25.60 nm and a zeta potential of −10.2 mV. S-SNEDDS achieved an excellent in vitro dissolution of 99% in phosphate buffer at pH 6.8 with 0.5% Tween 80. In vivo pharmacokinetics study showed a significant increase (2.2-fold) in the oral bioavailability of luteolin in rats compared to conventional SNEDDS. SNEDDS can enhance drug absorption by lymphatic transport and GI drug absorption, improve drug absorption through P-gp inhibitory effect and the permeability of the intestinal barrier, and induce a high supersaturation concentration of LUT in the GI tract. The interaction between LUT and HPMC K4M was determined through FT-IR spectroscopy and 1H NMR spectroscopy. The optimized SNEDDS formulation consisted of Crodamol GTCC, Kolliphor EL, and PEG 400 at a ratio of 20.1:48.2:31.7 wt. Luteolin has poor aqueous solubility and undergoes extensive first-pass metabolism, limiting its clinical applications.

Corrie et al.,2022 investigated the role of isomalt (GIQ9) as a pharmaceutical carrier for solid self-nanoemulsifying drug delivery systems (S-SNEDDSs) and improve the oral bioavailability of curcumin (CUN). The suitability of GIQ9 as a carrier was assessed by calculating the loading factor, flow, and micromeritic properties. The S-SNEDDSs were prepared using a surface adsorption technique, and the formulation variables were optimized using central composite design (CCD). The optimized S-SNEDDS showed a significant enhancement in dissolution rate and oral bioavailability compared to the naïve curcumin. The entrapment efficiency of the CUN-S-SNEDDS was calculated using a formula, and the droplet size, zeta potential, and PDI were measured before and after solidification. DSC, FTIR, XRD, and microscopic studies were conducted to analyze the physical and chemical properties of the optimized S-SNEDDS. In vivo studies were performed on Wistar rats to evaluate the pharmacokinetics of the optimized formulation.

Agrawal et al., 2015 formulated S-SEDDS of glipizide, an antidiabetic with erratic absorption due to its poor water solubility. They transformed the selected microemulsion system utilizing phase diagram into solid SEDDS (S-SEDDS) using Syloid 244 FP as adsorbing agent. Slight increase in the globule size was observed in the S-SEDDS from the Liquid SEDDS. Dissolution studies revealed improvement in dissolution properties of glipizide with more than 85% of drug

released from SSEDDS in 20 min as compared to 2.68% of pure drug in simulated gastric fluid. In vivo studies in rats demonstrated efficiency of S-SEDDS to control blood glucose level as compared to pure drug**.**

Patel et al., 2014 worked on development and optimization of S-SNEDDS of poorly water-soluble drug, nelfinavir mesylate with an aim to improve its dissolution rate and oral bioavailability. Scheffe's mixture design was utilized to optimize the amount of components in L-SNEDDS. Globule size, drug loading and percentage transmittance were taken as dependent variables. Optimized L-SNEDDS containing drug with average globule size of 12.80 nm was then adsorbed on Neusilin US2 to form SSNEDDS. In-vitro drug release study of prepared formulations exhibited remarkable improvement in dissolution properties of nelfinavir mesylate with more than 90% of drug release in 20 min. In-vivo studies in rabbits showed improvement in bioavailability of drug in L-SNEDDS and S-SNEDDS as compared to pure drug suspension. Stability studies for 3 months showed insignificant differences in the observed values.

Kim et al., 2013 reported improved oral bioavailability of fenofibrate by S-SMEDDS. Optimized L-SMEDDS was converted into S-SMEDDS using dextran as inert carrier material. Average diameter of optimized batch was found to be 240 nm. They reported no significant difference in mean droplet size and size distribution of the emulsions formed from L-SMEDDS and S-SMEDDS. In-vitro drug release studies exhibited higher dissolution rates than pure drug powder. Solid state characterization studies exhibited molecular dispersion of drug in the formulation. Furthermore, in-vivo studies in rats demonstrated 2 fold increase in AUC values S-SMEDDS than that of pure drug.

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

Akhter et al., 2014 investigated SNEDDS of coenzyme CoQ10, a fat soluble vitamin like compound with an aim to improve its dissolution properties and thereby oral bioavailability.

Optimized formulation was then converted into solid form (SSNEDDS) by spray drying L-SNEDDS onto Aerosil 300. Dissolution studies exhibited faster drug release from the prepared formulations. Drug release of 97.5 % was achieved in 1 h from S-SNEDDS as compared to only 0.36 % drug release from CoQ10 powder. In-vivo studies suggested enhancement in drug absorption with 3.4and 5 fold increase in Cmax and AUC values respectively, for CoQ10 in S-SNEDDS as compared to CoQ10 powder. DSC and XRD studies suggested amorphous nature of CoQ10 in S-SNEDDS.

Yeom et al., 2015 formulated and optimized a self-nanoemulsifying drug delivery system of poorly water-soluble drug, atorvastatin by formulating SMEDDS using a Doptimal mixture design. The optimized formulation containing 7.16% Capmul MCM (Oil), 48.25% Tween 20 (surfactant) and 44.59% Tetraglyol (cosurfactant) showed a significant enhancement in dissolution properties of atorvastatin. Optimized formulation showed 12.3-fold increase in dissolution rate of drug in simulated gastric fluid. Pharmacokinetic studies carried out in rats suggested 3.4- and 4.3-fold increase in AUC and tmax of atorvastatin from SMEDDS when compared with pure drug suspension.

Seo et al., 2015 worked on self-nanoemulsifying drug delivery system of tacrolimus, a water insoluble immunosuppressant with an aim of improving its dissolution properties and thereby bioavailability. Liquid SNEDDS were then converted into solid-SNEDDS by spray drying onto colloidal silica. Optimized formulations comprising of Capryol PGMC, Transcutol HP and Labrasol (10:15:75 % v/v/v) showed highest dissolution rate of tacrolimus from SNEDDS. In-vivo bioavailability studies in rats showed faster absorption with a 2-fold increase in AUC value of solid SNEDDS as compared to commercial product.

El-Badry et al., 2014 studied solubility and dissolution enhancement of tadalafil, a poorly water-soluble drug by formulating SNEDDS using Capryol 90 as oil, Triton X100 as surfactant and Transcutol HP as cosurfactant. The lowest droplet size observed was to be 64.7 nm. Dissolution studies showed significant improvement in dissolution properties of tadalafil with 96.6 % of drug release from the optimized batch of SNEDDS whereas only 12.4 % of drug release was observed from the pure drug suspension after 24 h study. They reported a 1434-fold increase in solubility of tadalafil from the optimized batch.

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 reported dissolution enhancement of poorly water-soluble drug, cilostazol by formulating SNEDDS. A 23 full factorial design was employed to study the influence of independent variables viz. Amount of oil (Capmul MCM), amount of surfactant (Tween 80) and amount of cosurfactant (Transcutol HP) on dependent variables. Liquid SNEDDS were solidified by adsorbing onto Neusilin US2. Optimized formulation showed globule size of 215.2 nm. Results of solubility and dissolution studies suggested remarkable improvement in respective properties. Reported values of solubility and dissolution efficiency at 30 min were 9.82 mg/mL and 83.3 % respectively.

Yoo et al., 2010 worked on SNEDDS formulations of lutein, a poorly water soluble herbal active ingredient. SNEDDS containing Phosal 53 MCT as oil, Labrasol as surfactant and Transcutol HP as cosurfactant were prepared with an aim of improving solubility and dissolution of lutein. They reported optimized formulation of SNEDDS with concentration of 25 % of oil, 60 % of surfactant and 15 % of cosurfactant. Liquid SNEDDS were converted into Solid SNEDDS by adsorbing it onto Aerosil 200. Globule size of 93 nm was observed with the optimized formulation. Results of dissolution studies suggested remarkable improvement in dissolution properties of lutein from the formulation.

Villar et al., 2012 formulated and optimized SNEDDS of gemfibrozil, a water insoluble antihyperlipidemic drug. They employed Box-Behnken experimental design to optimize the formulation factors viz. Amount of oil (lemon oil), amount of surfactant (Cremophor EL) and amount of cosurfactant (Capmul MCM-C8). The mean droplet size observed for optimized formulation was 56.5 nm. DSC studies suggested conversion of drug into amorphous form. Results of in-vitro release studies suggested significant improvement in dissolution properties of gemfibrozil. Drug release followed Weibull mathematical model release.

Mohd et al., 2015 developed SNEDDS of poorly aqueous soluble drug, glimepiride with an objective of improving its oral delivery. They reported its therapeutic efficacy in albino rabbits. SNEDDS were formulated using Miglyol 812 (oil), Tween 80 (surfactant) and PEG 400 (cosurfactant). The average globule size of best formulation was found to be 152 nm. Liquid SNEDDS were converted into S-SNEDDS by adsorbing onto solid carrier, Aerosol 200. They

reported more than 85 % drug release within 15 min. In-vivo studies revealed significant enhancement in therapeutic efficacy with AUC value of 234.64 for optimized S-SNEDDS. Solid state characterization studies exhibited the amorphous nature of drug in the prepared formulation.

CHAPTER 4:

MATERIALS AND METHODS

4. MATERIALS AND METHODS

4.1. Materials

The chemicals and instruments used for the development and evaluation of Cur and EGCG loaded SNEDDS are given in **Table 4.1** and **Table 4.2** respectively.

4.1.1. List of chemicals used instruments used for the development and evaluation of Cur and EGCG loaded SNEDDS.

Table 4.1: *List of chemicals*

S. No	Name of the chemicals	Name of the suppliers
1.	Curcumin	Lobachemie Pvt. Ltd., Mumbai, India
2.	EGCG	Tokyo Chemical Industries, Tokyo, Japan
3.	Streptozotocin	Sigma-Aldrich Chemicals Pvt. Ltd., Bengaluru
4.	Glibenclamide	
5.	Tween 80	
6.	Transcutol P	
7.	Ascorbyl Palmitate	
8.	Fish oil	Indiamart, Noida
9.	Aerosil - 200	Sisco Research Laboratories Pvt. Ltd., Maharashtra
10.	Isomalt	Tokyo Chemical Industries, Tokyo, Japan
11.	Methanol (HPLC grade)	Sigma-Aldrich Chemicals Pvt. Ltd., Bengaluru
12.	Acetonitrile (HPLC grade)	

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

Table 4.2 *List of instruments:*

S. No	Name of the Instruments	Model and name of the manufacturer
1.	Electronic balance	Sartorius AG, Germany
2.	Hot air oven	VO-INC-13 Incon Engineers ltd, Hyderabad, India
3.	Vacuum pump	DC-52 Torrlits Engineers ltd, Kolkata, India
4.	Centrifuge	R4R-V /FM Plasto Crafts Industries Pvt. Ltd., Mumbai, India
5.	Magnetic stirrer	Tarsons digital spinot
6.	Vortex shaker	
7.	B.O.D Incubator shaker	BOD-INC-1S Incon Engineers ltd, Hyderabad, India
8.	HPLC	Prominence AD 30, Shimadzu, Japan
9.	Transmission electron microscopy	Philips EM430 TEM, USA
10.	Particle size analyzer and Zetasizer	MALVERN Instruments Model: - ZEN 1600
11.	Field emission scanning electron microscopy	FEI Model: Quanta 250 FEG
12.	X-ray diffractometer	Panalytical Model: X'Pert Pro
13.	Deep freezer	Samsung
14.	Refrigerator	

4.1.3. Animals

Male Swiss Albino mice with an average weight of 25-30gm age about 2-3 months (on the day of study) were used for the in vivo study of the optimized formulation. The animals were procured from West Bengal Livestock Breeding Centre, Kalyani, India. Mice were housed in polypropylene cages lined with husk and were acclimatized at a temperature of 24 ± 2 ˚C and relative humidity of 45 ± 15 %, with a 12 h light/dark cycle over a period of 2 weeks prior to dose administration. Animals were kept under observation during the acclimatization period. Animals with good health were included in the study. All mice were maintained at standard laboratory diet and domestic mains tap water was provided ad libitum. The animals were kept fasted for 12 h prior to the experiment. 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-22/51** Dated **15.06.2023** before beginning the experiment.

4.2. Methods

4.2.1. Preparation of SNEDDS

(FORMULATION 1 – 230222)

A formulation was prepared to check the solubility of cur in tween 80 and transcutol. Oil was not used in this formulation.

S. No	Materials	Quantity
1.	Oil	Not used
2.	Drug (Curcumin)	100mg
3.	Surfactant (Tween 80)	3ml
4.	Co-surfactant (Transcutol)	3ml
5.	Aerosil- 200	615mg
6.	Double distilled water	40ml

Ratio of Surfactant and Co-surfactant = 1:1

Procedure:

i) Tween 80 and transcutol were added in a beaker in 1:1 ratio and stirred with the help of a magnetic stirrer at 500 rpm at a temperature of 45ºC for 5 minutes.

ii) Curcumin was added to the above mixture and stirred for about 10-15 minutes or until transparent.

iii) 40 ml DDW was added to the above mixture and stirred for another 5 minutes.

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

v) The slurry was transferred to a petri dish and stored at -20ºC overnight and kept in the vacuum oven the next day for the formulation to be dried.

vi) The formulation was weighed after drying and the decrease in weight was noted until the weight was constant.

(FORMULATION 2 – 230227)

Fish oil was used in this formulation.

S. No	Materials	Quantity
1.	Fish oil	3ml
2.	Drug (Curcumin)	150mg
3.	Surfactant (Tween 80)	1.5ml
4.	Co-surfactant (Transcutol)	1.5ml
5.	Aerosil- 200	1.2g
6.	Double distilled water	40ml

Procedure:

i) 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.

ii) Curcumin was added to the above isotropic mixture and stirred for about 10-15 minutes or until transparent.

iii) 40 ml DDW was added to the above mixture and stirred for another 5 minutes.

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

v) The slurry was transferred to a petri dish and stored at -20ºC overnight and kept in the vacuum oven the next day for the formulation to be dried.

vi) The formulation was weighed after drying and the decrease in weight was noted until the weight was constant.

(FORM ULATION 3 – 230228)

Glyceryl trioleate oil was used in this formulation.

S. No.	Materials	Quantity
1.	Glyceryl trioleate	3 ml
2.	Drug (Curcumin)	150mg
3.	Surfactant (Tween 80)	1.5ml
4.	Co-surfactant (Transcutol)	1.5ml
5.	Aerosil- 200	1.2g
6.	Double distilled water	40ml

Procedure:

Same as in formulation 2

(FORMULATION 4 – 230303)

Isomalt was introduced in this formulation for solidification of the L-SNEDDS. The quantity of transcutol was increased as compared to tween80. The quantity of the fish oil was also reduced.

S. No.	Materials	Quantity
1.	Fish oil	1.2 ml
2.	Drug (Curcumin)	250mg
3.	Surfactant (Tween 80)	2.5ml
4.	Co-surfactant (Transcutol)	7.5ml

5.	Aerosil- 200	1.5g
6.	Isomalt	6g
7.	Double distilled water	40ml

Procedure:

Same as in formulation 2

(FORMULATION 5 – 230315)

S. No.	Materials	Quantity
1.	Fish oil	1.2 ml
2.	Drug (Curcumin)	250mg
3.	Surfactant (Tween 80)	2.5ml
4.	Co-surfactant (Transcutol)	7.5ml
5.	Aerosil- 200	2g
6.	Isomalt	5g
7.	Double distilled water	40ml

Procedure:

Same as in formulation 2

FORMULATION 6

i) 230330 A

S. No.	Materials	Quantity
1.	Fish oil	1.2 ml
2.	Drug (Curcumin)	350 mg
3.	Surfactant (Tween 80)	2.5 ml
4.	Co-surfactant (Transcutol)	4 ml

5.	Aerosil- 200	2 g
6.	Isomalt	4 g
7.	Double distilled water	40 ml

ii) 230330 B

S. No.	**Materials**	**Quantity**
1.	Fish oil	1.2 ml
2.	Drug (Curcumin)	350 mg
3.	Surfactant (Tween 80)	2.5 ml
4.	Co-surfactant (Transcutol)	2.5 ml
5.	Aerosil- 200	2 g
6.	Isomalt	5 g
7.	Double distilled water	40 ml

iii) 230330 C

S. No.	**Materials**	**Quantity**
1.	Fish oil	1.2 ml
2.	Drug (Curcumin)	350 mg
3.	Surfactant (Tween 80)	2.5 ml
4.	Co-surfactant (Transcutol)	2.5 ml
5.	Aerosil- 200	3.5 g
6.	Isomalt	3.5 g
7.	Double distilled water	40 ml

(FORMULATION 8 – 230410)

Formulation contains both Cur and EGCG. Ascorbyl Palmitate was also added to this formulation.

S. No.	Materials	Quantity
1.	Fish oil	1.2 ml
2.	Drug (Curcumin)	175 mg
3.	Drug (EGCG)	175 mg
4.	Surfactant (Tween 80)	2.5 ml
5.	Co-surfactant (Transcutol)	2.5 ml
6.	Aerosil- 200	3.5 g
7.	Isomalt	3.5 g
8.	Ascorbyl Palmitate	100 mg
9.	Double distilled water	40 ml

4.2.1.2 Dispersion test:

1mg powdered SNEDDS from each of the formulations was dissolved in 1ml DDW in an effendurf tube and vortexed for 2-3 minutes. The precipitation was noted after different intervals of time.

4.2.1.3 High Performance Liquid Chromatography (HPLC):

In high-pressure liquid chromatography (HPLC), UV detectors and diode array detectors (DAD) were used for drug loading and in vitro drug release. The HPLC system consisted of a quaternary pump (Model: Agilent 1260 series) with an autosampler, UV-Visible detector and thermostat column compartment (TCC). Mobile phase used was Buffer (30 mm KH_2PO_4) and Acetonitrile in the ratio of 50:50. Flow rate was 1.0ml/min. Wavelength used for the detection of absorbance was 258nm. Ez chrome software was used for data collection and analysis. The stationary phase generally used C18 column of Phenomenex Luna octadecylsilane, having dimension of 250 mm × 4.6 mm, particle size 5µm. For sample preparation, an ultrasonic bath was used. The weighing of the Samples and standards were usually carried out on the analytical balance.

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

The EE of Cur & EGCG loaded SNEDDS was determined by centrifugation at 12,000 rpm for 60 minutes. After the supernatant was decanted, it was analyzed by High-Performance Liquid Chromatography (HPLC). As for the drug loading, the Cur & EGCG loaded SNEDDS was analyzed by HPLC, with acetonitrile and buffer (KH2POH) as the mobile phase. The entrapment efficiency (% EE) and drug loading (% DL) were calculated using the following equations:

$$\% EE = (A - B)/A \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$\%DL = (C/A+D) \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

A = Total amount of drug in SNEDDS

B = Total amount of drug present in the supernant

C = Total amount of drug added

D = Total amount of excipients added

4.2.1.3.2 In-vitro dissolution studies

The in-vitro drug release of Cur & EGCG loaded SNEDDS 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. An equivalent amount of drug encapsulated SNEDDS powder was weighed and suspended in equivalent amount of DDW and vortexed for 15-20 minutes in a magnetic stirrer at a temp of 45ºC-50ºC. 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. The temperature of the B.O.D incubator shaker was maintained at 37 ± 0.5ºC, at a stirring speed of 100 rpm. At predetermined intervals (, 10, 15, 20, 30, 45, 60 and 90 min), 2 ml samples were withdrawn and filtered using membrane filter (0.45 µm). 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.

4.2.1.4 Characterization of Cur & EGCG loaded SNEDDS.

i) Particle Size:

Particle size and size distribution of all the batches of Cur & EGCG loaded SNEDDS were analyzed after dilution (1000 times) with double distilled water in a volumetric flask. All samples were vortexed in a magnetic stirrer for 15-20 minutes at a temp of 45ºC - 50ºC in order to minimize any aggregation if present. The samples were analyzed by particle size analyzer at 25 ˚C with an angle of 90˚. All studies were done in triplicates and average results were reported.

ii) Zeta Potential (ζ)

For all the formulations of Cur & EGCG loaded SNEDDS, the zeta potential (ζ) values were evaluated by determining the particle electrophoretic mobility using particle size analyzer. The method employed for the sample preparation was similar to that of particle size determination study. The analysis was performed in purified water adjusted to a standard conductivity of 50 µS cm with sodium chloride solution (0.9 % w/v) in order to avoid changes in zeta potential values due to day-to-day variations occurring in the conductivity of water.

iii) X- Ray Diffraction (XRD)

To observe crystallographic pattern of pure drug, optimized SNEDDS and blank SNEDDS were carried out using Powder X-ray diffractometer using a voltage of 45 kV, generator current 40 mA, scan step time 9 sec-1 and scan step size of 0.008° (2θ). The scanning rate employed was maintained over the interval of 10 to 80°C (2θ).

ELECTRON MICROSCOPY

Electron microscopy is a versatile method for two and three dimensional characterizations of materials. High resolution of SEM (Scanning electron microscopy) and TEM (Transmission electron microscopy) is usually sought after because of its non-destructive materials characterization technique. SEM and TEM find its application in evaluating critical microstructures of materials.

iv) Transmission Electron Microscope (TEM)

TEM studies were performed in order to detect the droplet morphology of the selected SNEDDS formulation. Briefly, a drop of diluted (100 times) SNEDDS was placed on a copper grid and was left for air drying overnight. The copper grid was stained with 1% w/v phosphotungistic acid solution for 5 min at room temperature. The image was taken with transmission electron microscope at an accelerated voltage of 100 kV.

v) Field emission electron microscopy (FE-SEM)

The surface morphology of the Cur & EGCG loaded SNEDDS were visualized through FE-SEM. Briefly, a drop of diluted (100 times) SNEDDS was placed on a glass slide (substrate) and was left to be air dried overnight. The slide was fixed by aluminium stub and was coated with gold under high vacuum for 4-5 minutes. The scanning was carried out at a voltage of 10 kV.

4.2.1.5 In *vivo* studies

4.2.1.5.1 Experimental animal model

Streptozotocin-induced mice model was selected to evaluate the anti-diabetic activity of curcumin and EGCG 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. **JU/IAEC-22/51** Dated **15.06.2023** before beginning the experiment.

4.2.1.5.2 Study design

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

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

Group II: Disease control group, normal diet + STZ single intraperitoneal injection (100 mg/kg body weight).

Group III: Standard control group, normal diet + STZ + Standard drug (Glibenclamide) (administered by oral gavage at the dose of 2mg/kg body weight) for 60 days.

Group IV: SNEDDS group, Normal diet + STZ + SNEDDS (administered by oral gavage at the dose of 120 mg/kg body weight) for 60 days.

Group V: Free drug group, Normal diet + STZ + Free drugs (administered by oral gavage at the dose of 240 mg/kg body weight) for 60 days.

Table.4.3 *Groups of animals*

Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Total no. of animals**
Normal	Disease Control	Standard control	SNEDDS (Cur + EGCG)	Free drug treated	
3	3	3	3	3	15

4.2.1.5.3 Experimental induction of diabetes

After overnight fasting, diabetes was induced by intraperitoneal (i.p.) injection of streptozotocin (STZ) which was dissolved in 0.1 M cold sodium citrate buffer (pH 4.5), at a dose of 100 mg/kg body weight. Blood samples were collected before the administration of STZ and after seven days of its administration. Animals with fasting blood glucose above 250 mg/dL were considered diabetic and were included in the study.

4.2.1.5.4 Regular monitoring of body weight and blood glucose level

The body weight and blood glucose level were monitored every weekend for 7 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.

4.2.1.5.5 Drug administration

After 2 weeks of STZ induction, the formulations were administered orally through oral gavage. Dose calculation was done according to the body weight of the animals and fresh dose were prepared for each day administration of the formulations once a day. Overnight fasted animals were analyzed weekly for their body weights and blood glucose level. After completion of the experimental duration, the animals were fasted overnight, terminal sacrifice was carried out by overdosing on diethyl ether and pancreas was dissected out. The dissected

pancreas was immediately rinsed in ice cold saline and paraffin sections of pancreatic, tissues were prepared. These were stained with hematoxylin and eosin to observe histopathological changes.

4.2.1.5.6 Histopathological studies

Paraffin sections of pancreas tissues were made and stained with hematoxylin and eosin to observe histopathological changes.

4.2.1.5.7 Statistical analysis

All the data are expressed as mean ± SD. The statistical analysis was performed using one-way ANOVA and was followed by Dunnett's multiple comparison test. $P < 0.05$ was considered as significant. GraphPad Prism software was used.

CHAPTER 5:

RESULTS AND DISCUSSION

5. Results and Discussion

5.1 Preparation of Curcumin loaded SNEDDS.

(Formulation 1 – 230222)

A formulation was prepared to check the solubility of cur in tween 80 and transcutol. Oil was not used in this formulation. Aerosil – 200 was used as a solidifying agent.

Fig.5.1. *Formulation 230222*

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

(Formulation 2 – 230227)

Fish oil was used to prepare the mixture. Compared to formulation 1, the amounts of surfactant and co-surfactant were cut in half. The amount of aerosil-200 dosage was increased compared to formulation 1.

Fig.5.2. *Formulation 230227*

The product after drying for more than 7 days appeared to be agglomerated and not a free flowing powder as shown in **Fig.5.2**. Hence, the formulation was discarded.

(FORMULATION 3 – 230228)

Glyceryl trioleate oil was used in this formulation.

Fig.5.3. *Formulation 230228*

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

(FORMULATION 4 – 230303) & (FORMULATION 5 – 230315)

As the product was not drying properly, isomalt was introduced to the formulation alongside aerosil-200 for the solidification of SNEDDS. Compared to tween80, transcutol was used in greater amounts. Additionally, less fish oil was used.

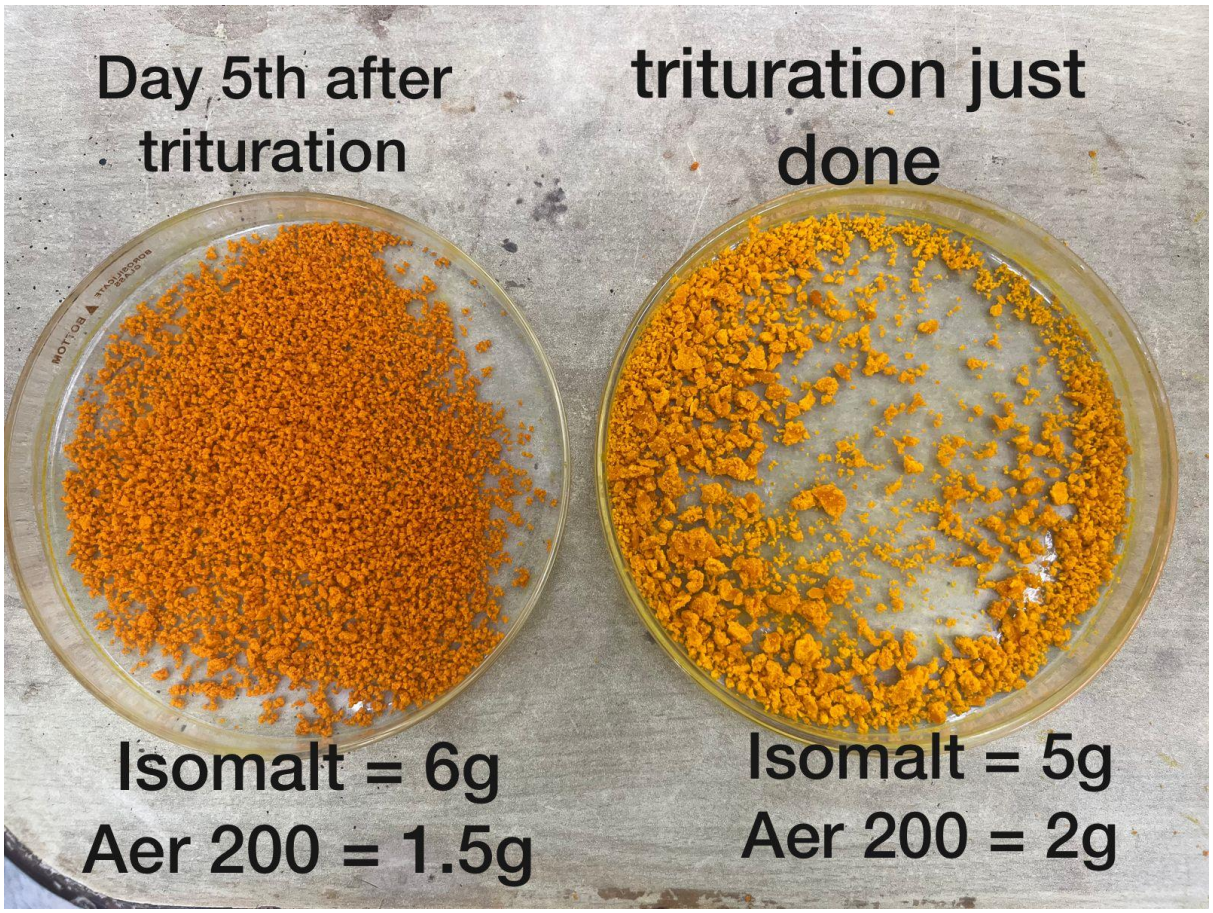


Fig.5.4. *Formulation 230303 & Formulation 230315*

Formulation 4 & and formulation 5 were prepared with isomalt and aerosil – 200 being the solidifying agent. The product showed stickiness and agglomerates. The formulations were discarded.

(FORMULATION 6 – 230330)

Formulation 6 was divided into:

i) 230330 A
ii) 230330 B
iii) 230330 C

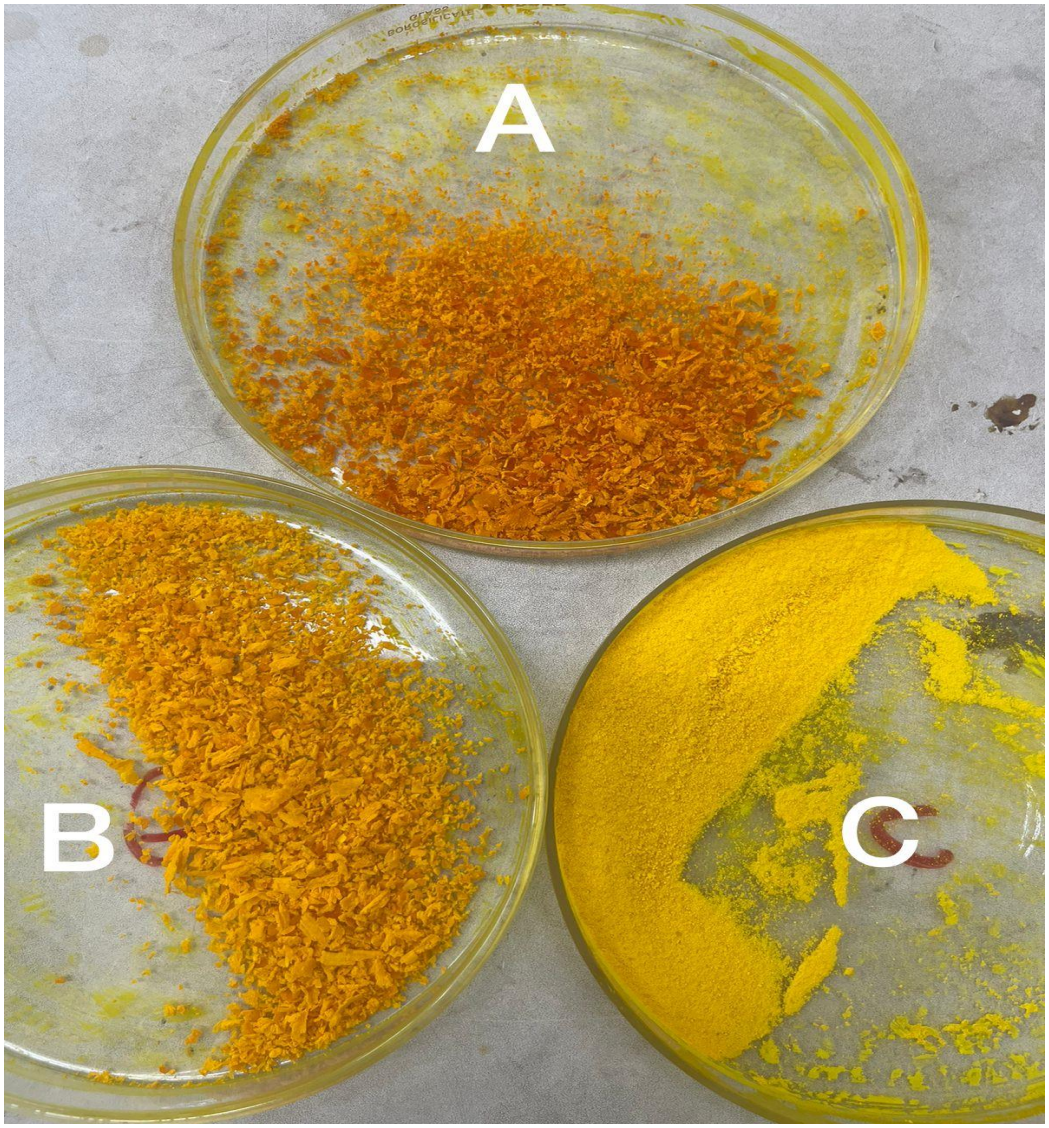


Fig.5.5. *Formulation 230330 (A, B, C)*

After drying for more than 7 days, formulation 230330 B & formulation 230330 C appeared to be free-flowing powders. Hence, it was considered final optimized formulation for Cur SNEDDS. The Cur-SNEDDS was further evaluated with particle size, polydispersity index and zeta potential.

5.1.2. Characterization of Cur – SNEDDS

5.1.2.1 Particle size and polydispersity index (PDI)

The formulations 230330 B & 230330 C were subjected to particle size and polydispersity index analysis. The droplet size of the emulsion is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release, as well as absorption [76,77]. It was observed that formulation 230330 C containing aerosil-200 & isomalt in the ratio of 1:1 revealed droplet size having an average particle size of 125.4 ± nm and formulation 230330 B revealed an average particle size of 130.2 ± nm as shown in **Fig.5.6** and **Fig 5.7**. It was also observed that there was not much change in the average particle size among the formulations.

Similar to mean particle size, size distribution is a crucial metric in nanotechnology. The poly dispersibility index (PDI) quantifies the uniformity of globule size. The more homogenous the particles are, the closer the value is to zero. For experimental batches of SNEDDS, the poly dispersibility index (PDI) was evaluated to estimate the globule size distribution. [78,79]. The poly dispersibility index of the optimized batch of cur loaded SNEDDS formulation 230330 B & formulation 230330 C was observed to be 0.386 and 0.285 respectively exemplifying narrow size distribution of system [80,81]. Comparison of particle size distribution, zeta potential and PDI is shown in **Table 5.1**.

Table 5.1: *Comparison of particle size distribution, zeta potential and PDI for formulation 230330 B and 230330 C.*

S.No.	Formulation:	Particle size(nm)	Zeta potential	Polydispersity Index
1.	230330 B	130.2 ±	-16.16	0.386
2.	230330 C	125.4 ±	-12.2	0.285

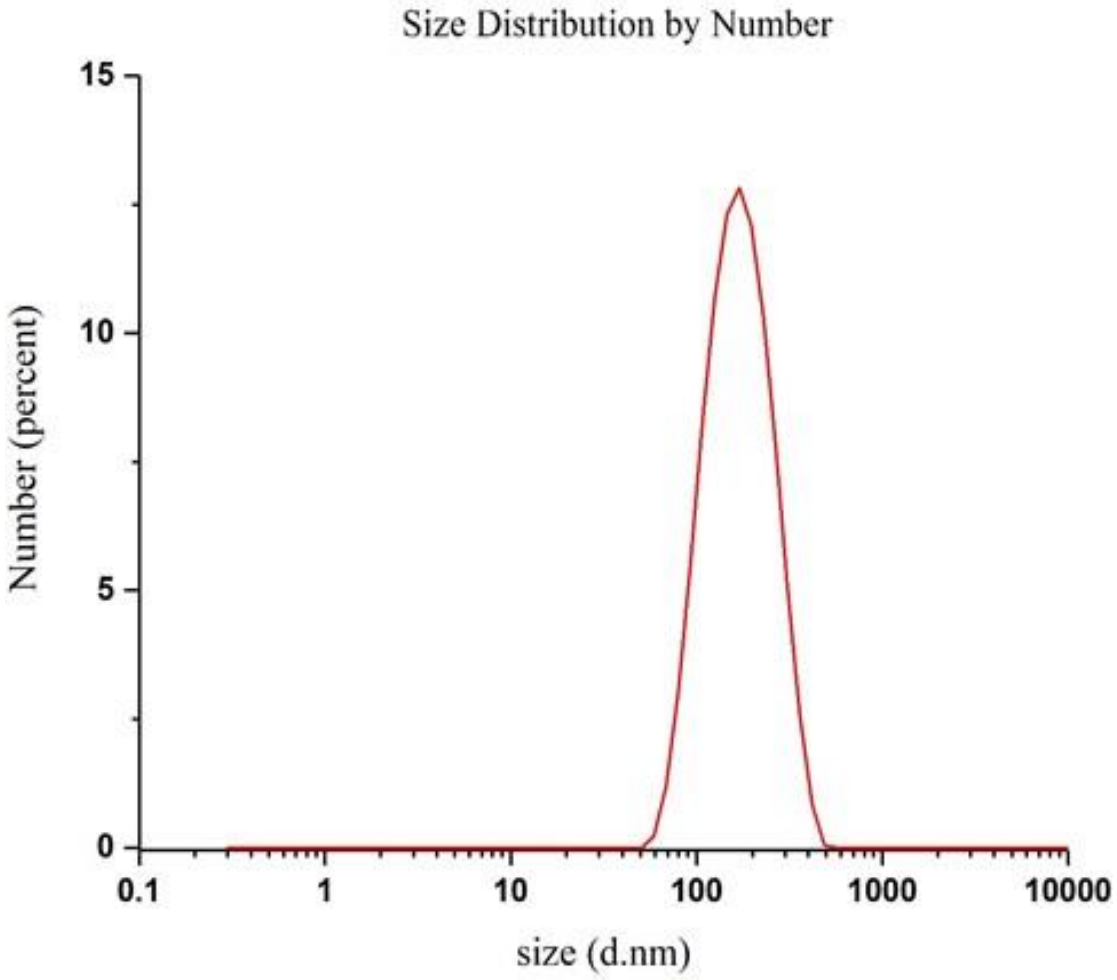


Fig.5.6 *Particle size graph of formulation 230330 B*

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Z-Average (nm)	130.2	-	-	130.2	130.2
Polydispersity Index (PI)	0.3866	-	-	0.3866	0.3866
Peak 1 Mean by Intensity ordered by area (nm)	180.6	-	-	180.6	180.6
Peak 1 Area by Intensity ordered by area (%)	92.78	-	-	92.78	92.78
Peak 2 Mean by Intensity ordered by area (nm)	23.08	-	-	23.08	23.08
Peak 2 Area by Intensity ordered by area (%)	7.222	-	-	7.222	7.222

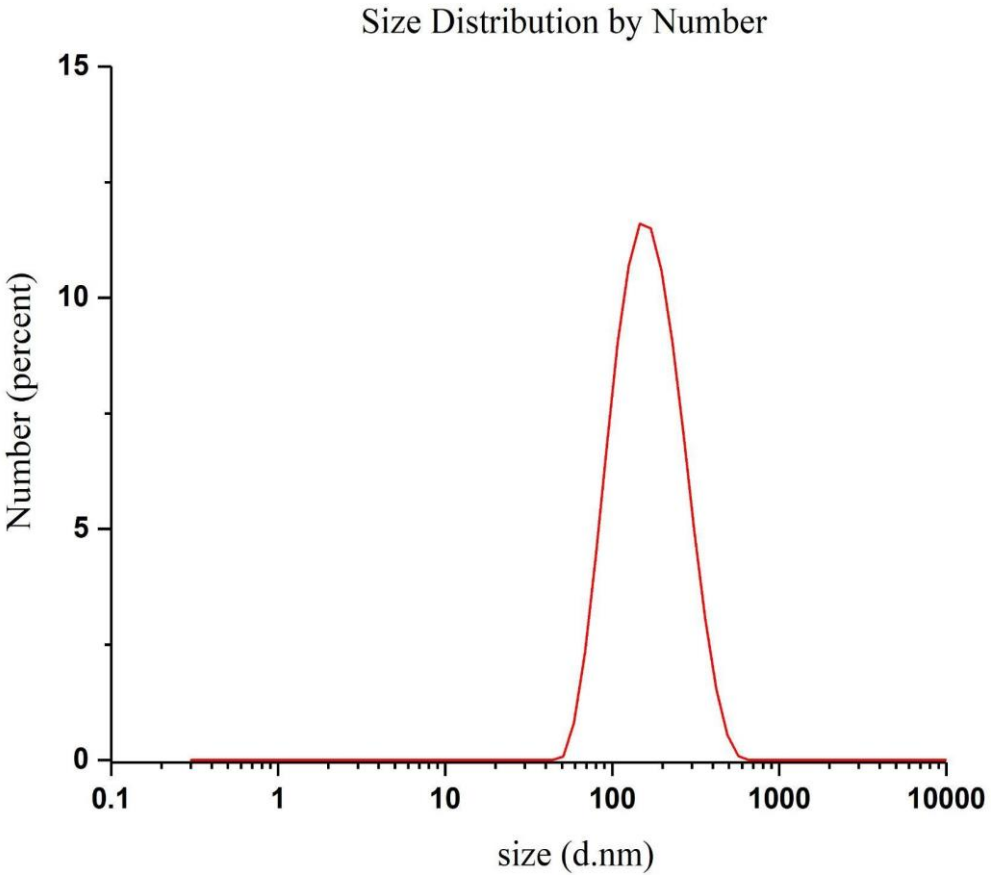


Fig.5.7 *Particle size graph of formulation 230330 C*

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Z-Average (nm)	125.4	-	-	125.4	125.4
Polydispersity Index (PI)	0.2851	-	-	0.2851	0.2851
Peak 1 Mean by Intensity ordered by area (nm)	178.7	-	-	178.7	178.7
Peak 1 Area by Intensity ordered by area (%)	94.1	-	-	94.1	94.1
Peak 2 Mean by Intensity ordered by area (nm)	21.17	-	-	21.17	21.17
Peak 2 Area by Intensity ordered by area (%)	5.905	-	-	5.905	5.905

5.1.2.2 Zeta *Potential (ζ)*

Zeta potential measurement is necessary to identify the charge of oil globules. Zeta potential values in the range of 25 mV to 30 mV in either charge signifies a stable formulation. Formulation 230330 B & formulation 230330 C cur loaded SNEDDS depicted zeta potential value of -16.16 ± 0.5 mV and -12.2 ± 0.5 mV respectively. The graph for zeta potential is shown in **Fig.5.8** & **Fig.5.9**.

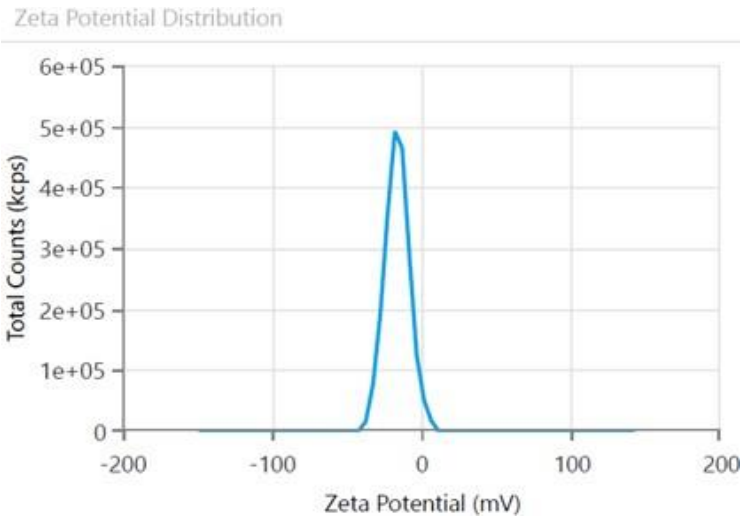


Fig.5.8 *Zeta potential graph of formulation (230330 B)*

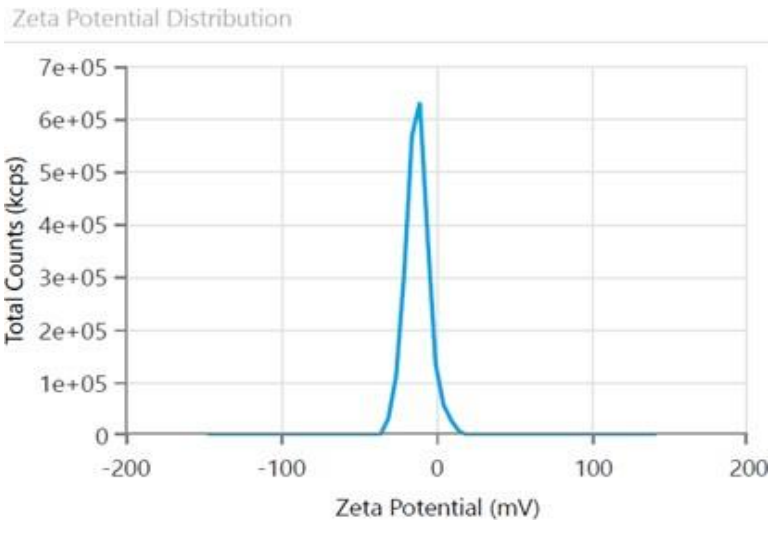


Fig.5.9 *Zeta potential graph of formulation (230330 C)*

5.2 Formulation of Cur-EGCG loaded SNEDDS.

(FORMULATION 7 – 230410)

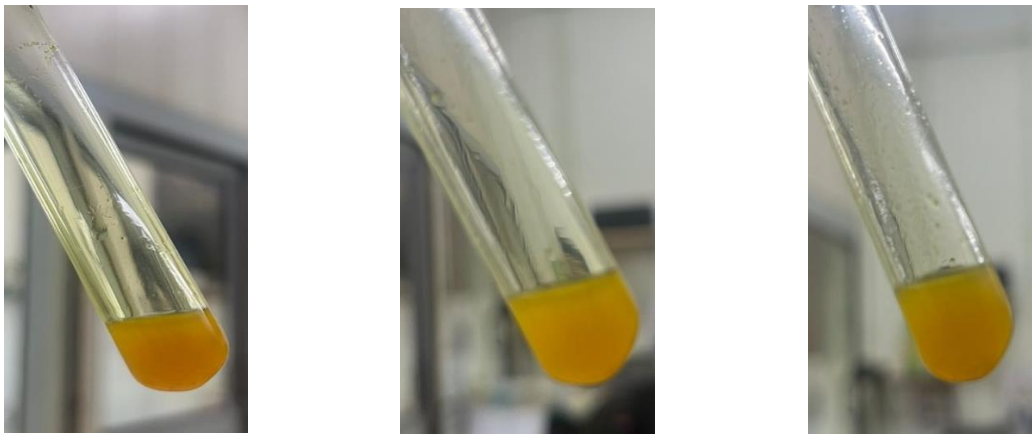
Cur and EGCG loaded SNEDDS was prepared with the same amount of excipients used in preparing Cur loaded SNEDDS.

Fig.5.10. (*Formulation 7 – 230410) Cur-EGCG loaded SNEDDS.*

The final formulation was prepared for Cur - EGCG loaded SNEDDS and after drying for 7-8 days, we got constant weight. The formulations appeared to be a free flowing powder and was subjected to further characterization.

Dispersion test:

Cur loaded SNEDDS, formulations 6 B & 6 C and Cur-EGCG loaded SNEDDS, formulation 7 was subjected for dispersion test.



(a) (b) (c)

Fig.5.11. *Formulations 6 B, 6 C and formulation 230410 marked as (a), (b) and (c) respectively.*

1mg powdered SNEDDS from each of the formulations was dissolved in 1ml DDW in an effendurf tube and vortexed for 2-3 minutes. It was noted that there was very little, or no precipitation seen in the subjected formulations.

5.2.1 *Estimation of drug loading (DL) & entrapment efficiency (EE)*

5.2.1.1 Curcumin encapsulated SNEDDS

The optimized Cur loaded SNEDDS significantly improved the drug incorporation, with an entrapment efficiency of 88.21%. The drug loading was 8.51%.

Drug Loading	**Entrapment Efficiency**
8.51%	88.21%

5.2.1.2 Cur – EGCG encapsulated SNEDDS

The optimized Cur-EGCG loaded SNEDDS was 85.36% for cur and 84.67% for EGCG. The drug loadings were 4.32% and 4.21% for cur and EGCG respectively.

Drug	**Drug loading**	**Entrapment Efficiency**
Curcumin	4.32%	85.36%
EGCG	4.21%	84.67%

5.2.2 In-vitro dissolution studies

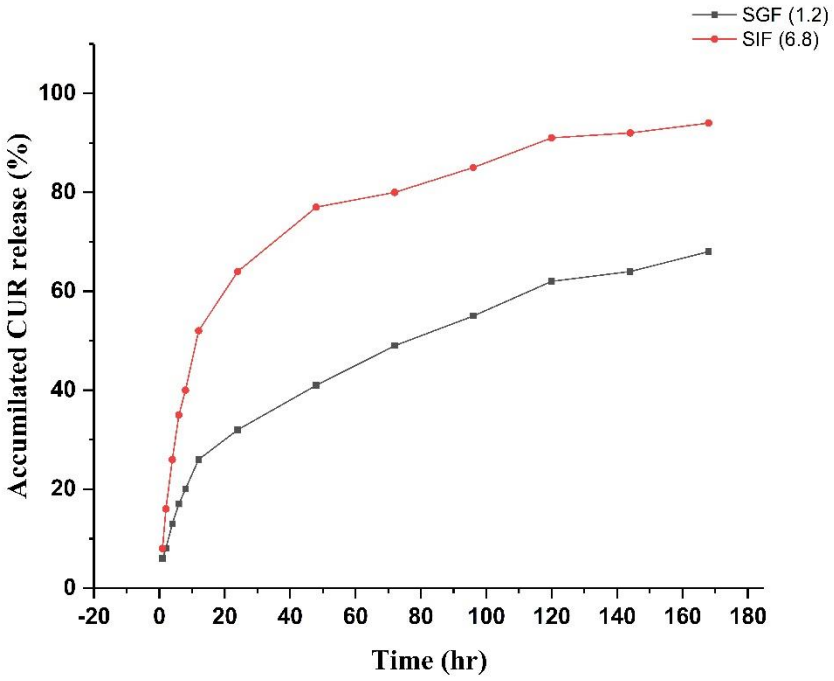


Fig.5.12. *In-vitro dissolution studies of Curcumin in SGF (1.2) & SIF (6.8)*

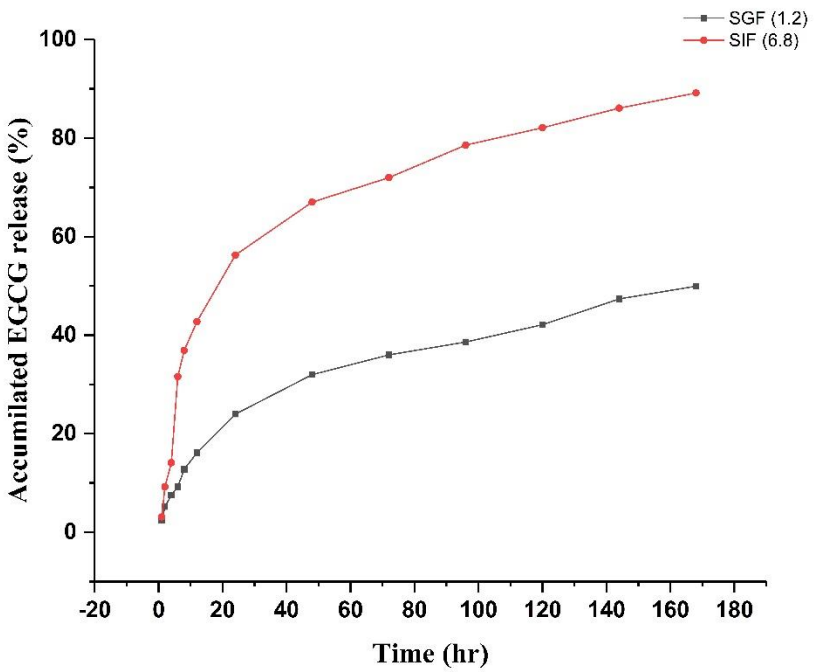


Fig.5.13. *In - vitro dissolution studies of EGCG in SGF (1.2) & SIF (6.8)*

Fig.5.12 and **Fig.5.13** represent the drug release profiles of Cur and EGCG respectively in simulated gastric fluid pH 1.2 and simulated intestinal fluid pH 6.8. Both the drugs showed greater release in SIF pH 6.8.

5.2.3 Characterization of Cur-EGCG SNEDDS

5.2.3.1. *Particle size and polydispersity index (PDI)*

The final formulation Cur-EGCG loaded SNEDDS was subjected to particle size and polydispersity index analysis. The droplet size of the emulsion is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release, as well as absorption. It was observed that formulation revealed droplet size having an average particle size of 151.3 ± nm as shown in **Fig.5.14.**

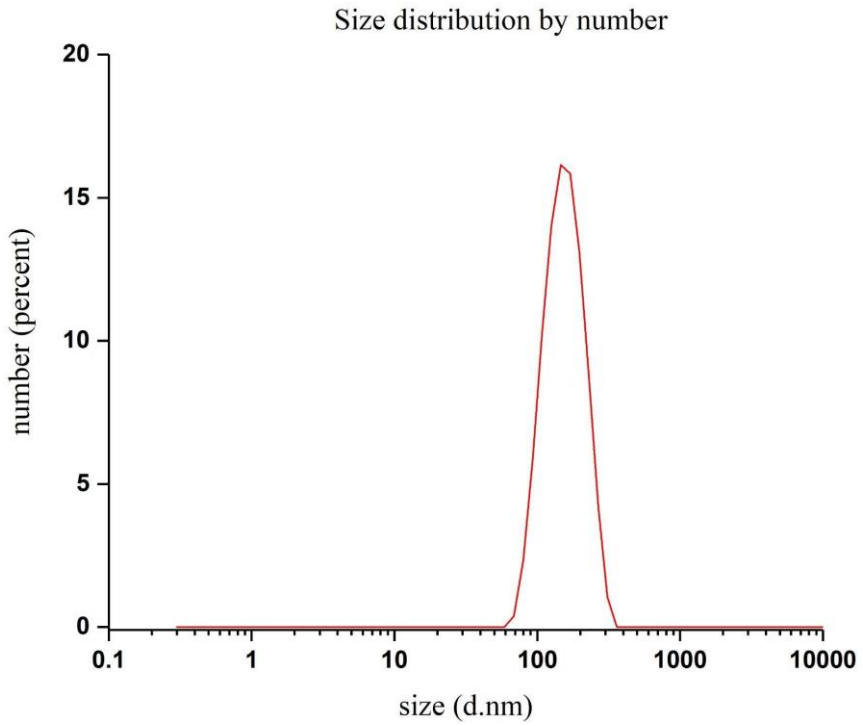


Fig.5.14. *Particle size graph of Cur-EGCG SNEDDS*

Similar to mean particle size, size distribution is a crucial metric in nanotechnology. The poly dispersibility index (PI) quantifies the uniformity of globule size. The more homogenous the particles are, the closer the value is to zero. For experimental batches of SNEDDS, the poly dispersibility index (PI) was evaluated to estimate the globule size distribution. The poly dispersibility index of the optimized batch of cur-ECGC loaded SNEDDS formulation was observed to be -11.62 mV exemplifying narrow size distribution of system.

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Z-Average (nm)	151.3	-	-	151.3	151.3
Polydispersity Index (PI)	0.3387	-	-	0.3387	0.3387
Peak 1 Mean by Intensity ordered by area (nm)	160.1	-	-	160.1	160.1
Peak 1 Area by Intensity ordered by area (%)	92.06	-	-	92.06	92.06
Peak 2 Mean by Intensity ordered by area (nm)	23.14	-	-	23.14	23.14
Peak 2 Area by Intensity ordered by area (%)	7.939	-	-	7.939	7.939

5.2.3.2 Zeta ***Potential (ζ)***

Zeta potential measurement is necessary to identify the charge of oil globules. Zeta potential values in the range of 25 mV to 30 mV in either charge signifies a stable formulation. Cur-EGCG loaded SNEDDS depicted zeta potential value of -11.62 mV.

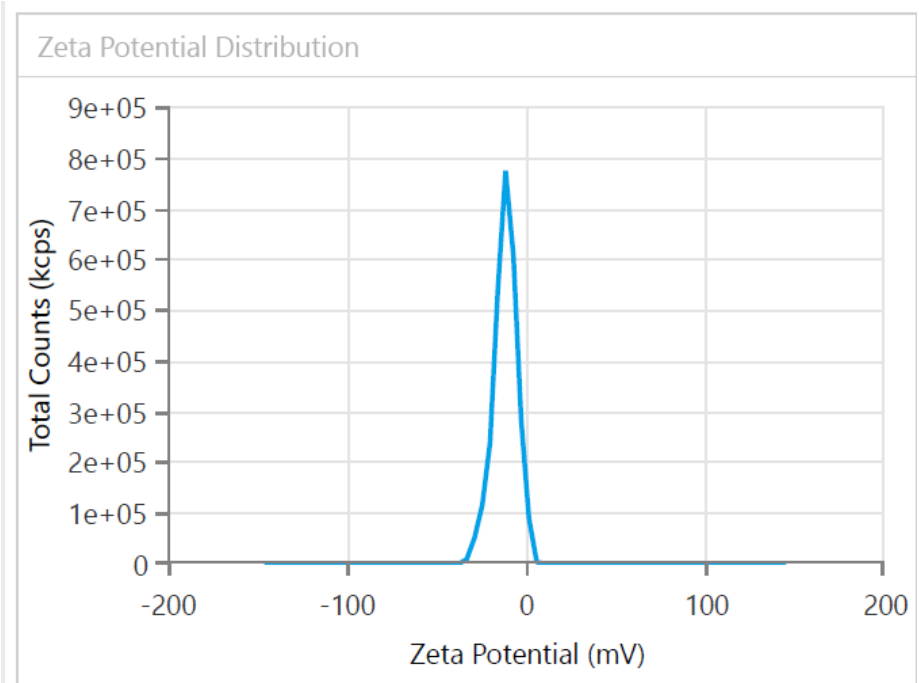


Fig.5.15. *Zeta potential value of Cur-EGCG loaded SNEDDS.*

5.2.3.3 X- *Ray Diffraction (XRD)*

The XRD graph of Cur & EGCG in **Fig.5.16** showed sharp peaks demonstrating its crystalline nature whereas spectra of optimized S-SNEDDS showed disappearance of sharp peaks of Cur & EGCG indicating conversion into amorphous form [82]. Amorphous halo peaks were observed in the diffractogram of blank S-SNEDDS. The result confirmed the formation of drug solution into liquisolid system which again supports the DSC results.

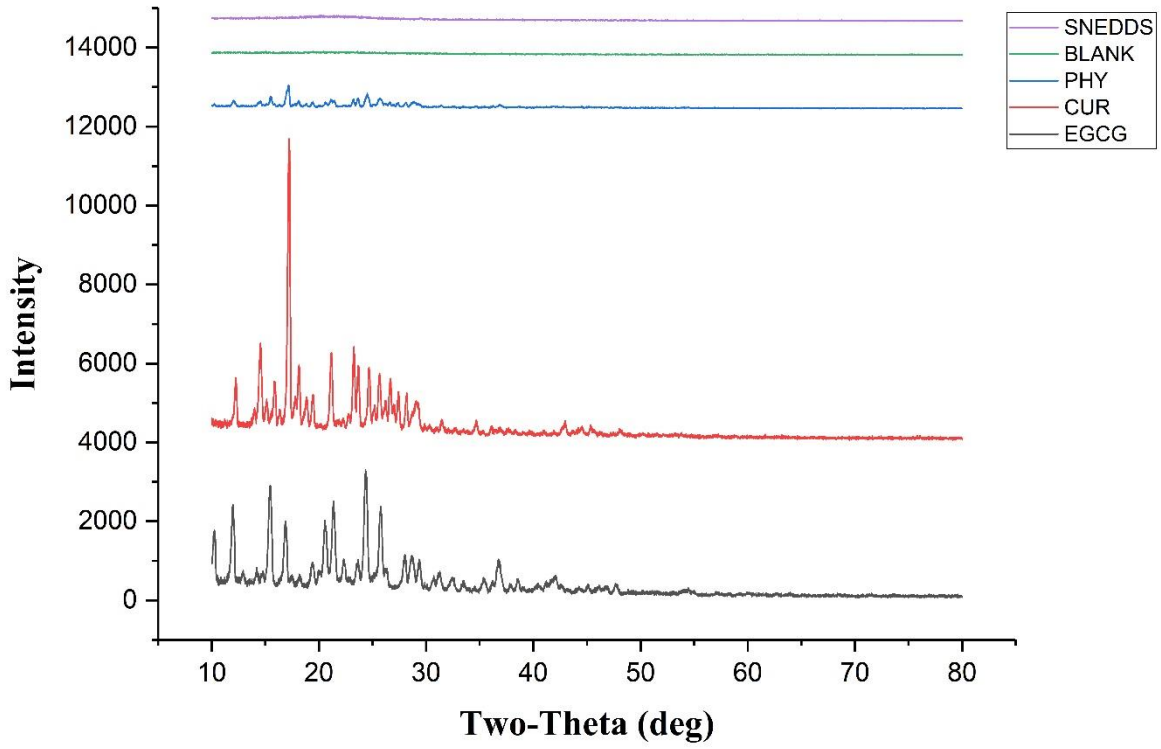


Fig.5.16. *XRD spectra of (a) Cur-EGCG loaded SNEDDS (b) Blank (c) Physical mixture of the excipients (d) Pure cur (e) Pure EGCG*

5.2.3.4 Transmission Electron Microscope (TEM)

The TEM images of Cur-EGCG encapsulated SNEDDS clearly indicate spherical droplets of reconstituted S-SNEDDS at a scale of 100 nm. These images confirmed that the droplets were unagglomerated, distinct, spherical, and nano size.

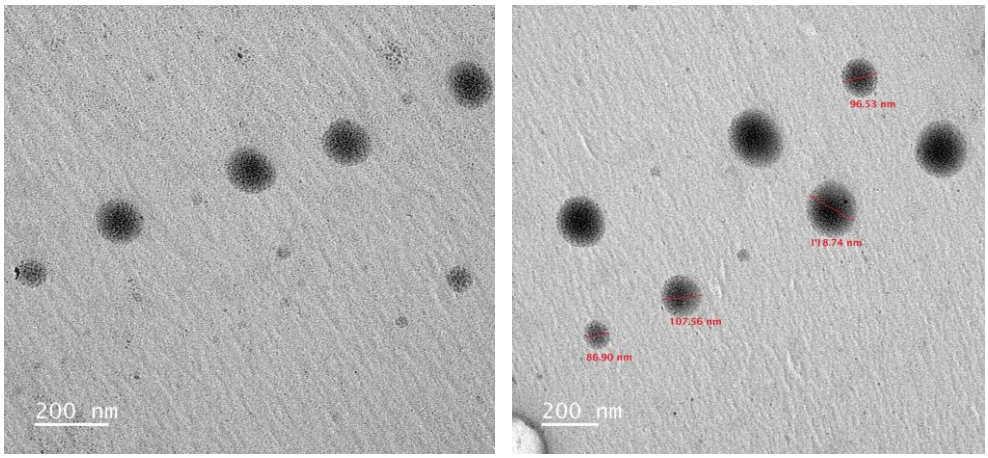


Fig.5.17 *TEM images of Cur-EGCG loaded SNEDDS.*

5.2.3.5 *Field emission electron microscopy (FE-SEM)*

SEM studies were carried out to study the morphological characteristics of the optimized formulation. The SEM images of Cur-EGCG SNEDDS are shown in **Fig.5.18**. The formulation appeared as rough-surfaced particles with porous and irregular aperture which might be attributed to absorption of Liquid SNEDDS [83].

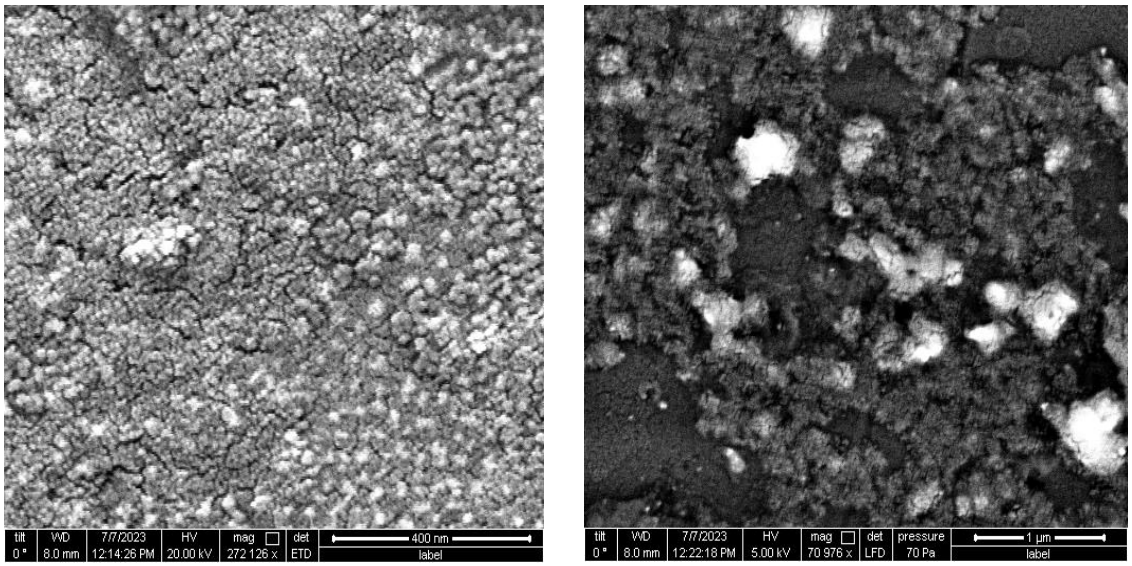


Fig.5.18. *SEM images Cur-EGCG loaded SNEDDS.*

5.3 In vivo evaluation of optimized formulation

STZ is commonly used for experimental induction of DM as it causes selective cytotoxicity to pancreatic islet β-cell. This damage is mediated through the release of nitric oxide (NO) which brings rapid reduction in pancreatic islet pyridine nucleotide concentration and necrosis of β-cell subsequently. STZ also produces SOD anions in mitochondria, which results in diabetic complications [84]. Hence, in the present study, such perspectives were undertaken to evaluate the anti-hyperglycaemic activity in STZ-induced diabetic mice.

5.3.1 Effect of Glibenclamide (standard drug), free drug (Cur-EGCG) and Cur-EGCG loaded SNEDDS on blood glucose levels:

In the present investigation, the treatment with Cur-EGCG loaded SNEDDS showed enhancement in protection towards increase in blood glucose till the end of study. It has been observed that the optimized Cur-EGCG loaded SNEDDS have been shown to decrease the blood glucose levels as compared to the STZ group. Free drug (Cur-EGCG) was not able to significantly reduce the blood glucose levels as compared to the optimized Cur-EGCG SNEDDS. This might be due to their poor solubility and dissolution. On 6^{th} week, the blood glucose level of optimized SNEDDS groups is almost comparable to standard drug group. On 8^{th} week of the study, the optimized SNEDDS formulation has shown an effective result by decreasing the blood glucose level and is similar to normal group and standard drug. All the experimental data were analysed by ANOVA followed by Dunnett's multiple comparison test in GraphPad Prism 5 software at 95% confidence limit. The comparison of normal control data with others revealed that there is highly significant difference with STZ. It confirms successful induction of diabetes. Significant difference was also seen in the free drug treated group. The comparison of STZ group with other groups confirm that the reduction of blood glucose levels for STD drug group and SNEDDS (Cur-EGCG) group showed high significant difference that indicates the similar blood glucose lowering effects as the normal control group. Whereas free drug group showed no significant difference in blood glucose lowering effects as compared to STZ group. It was concluded that the free drugs not capable to lower blood glucose level as compared to the formulated SNEDDS. So, Cur and EGCG should be delivered in the form of SNEDDS instead of free form. The data for blood glucose level is shown in **Table 5.1.**

Table 5.1 *Fasting Blood Glucose level (mg/dl)*

S. No.	Treatment	Fasting Blood Glucose level (mg/dl)					
		Before STZ	**After STZ**	**2nd Week**	**4th Week**	**6th Week**	**8th Week**
1.	Normal	98.6±3.055	101±6.24	98±2.00	100±9.16	100.33±4.1	97±2.00
2.	STZ	98.33±2.08	266±4.00***	295.6±5.68***	325±9.84***	344±13.74***	346.33±14.46***
3.	STD	83.33±3.21	275.66±5.85***	233±10.81$^{**\#}$	185.3±14.01$^{*\#\#}$	128.66±4.16$^{*\#\#}$	102.66±5.03$^{\#\#\#}$
4.	SNEDDS	87.33±2.082	272.33±5.50***	227.33±6.50$^{**\#}$	183.33±8.14$^{*\#\#}$	134.66±8.08$^{*\#\#}$	112±3.606$^{\#\#\#}$
5.	Free Drug	90±3.00	253±8.88***	240.33±8.02$^{**\#}$	231.33±10.78$^{**\#}$	215.66±6.11$^{**\#}$	187.66±5.13$^{**\#}$

All values are expressed as Mean ± SD, n=3. The statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test, comparing the groups with normal(*) and STZ($^{\#}$) group, $p < 0.05$ considered to be significant at 95% confidence limit.

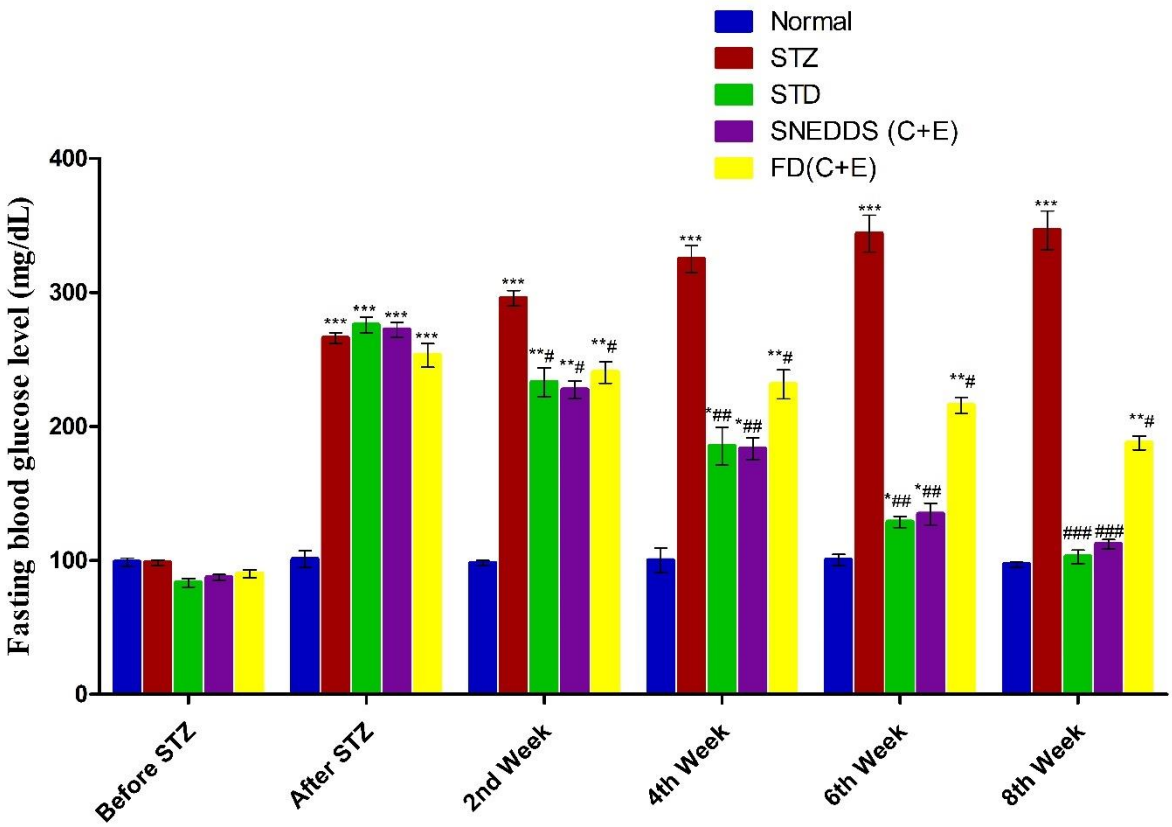
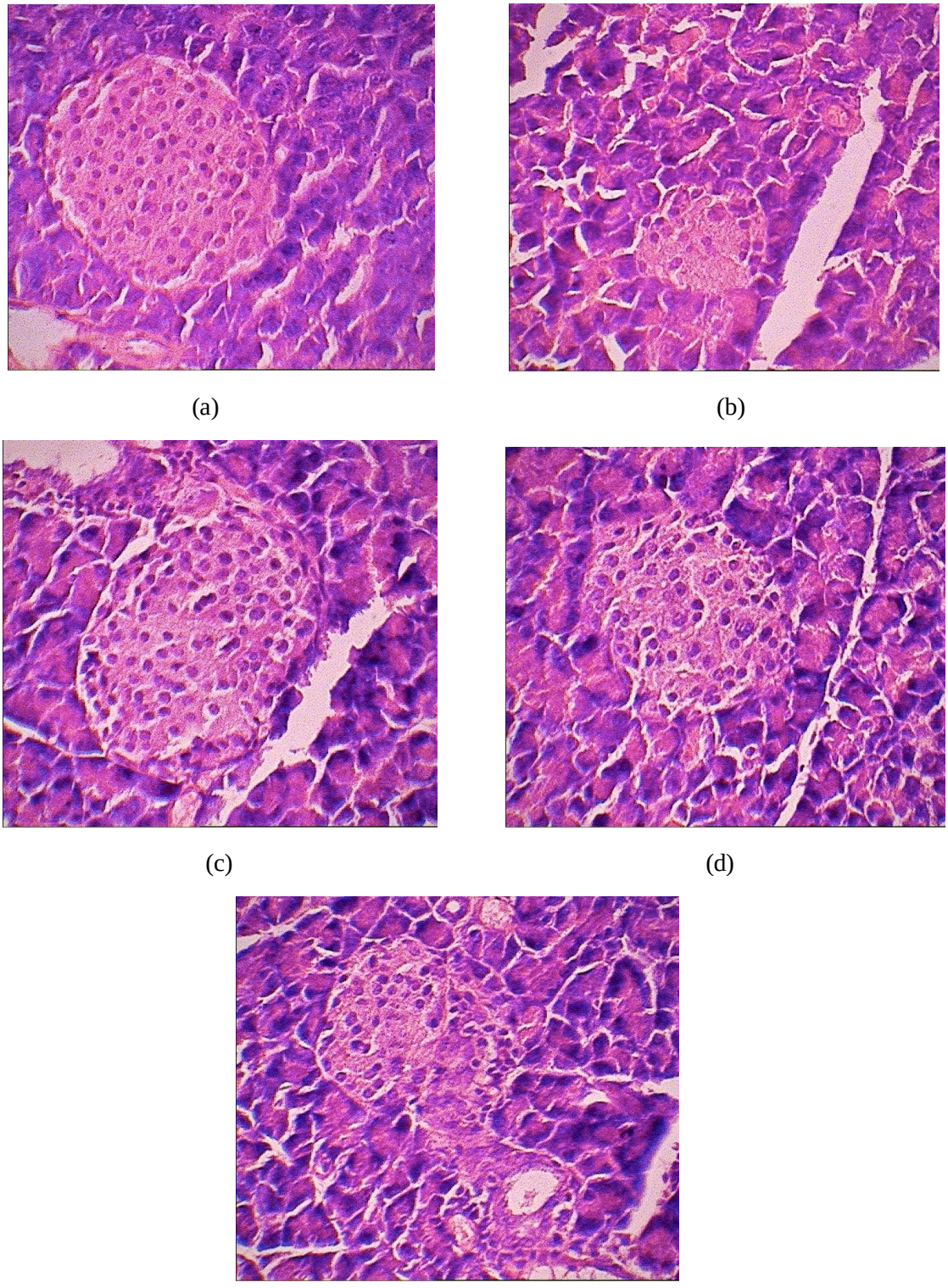


Fig.5.19. *Effect of glibenclamide (std), free drug (Cur+EGCG) and Cur-EGCG loaded SNEDDS on fasting blood glucose levels of mice. Data expressed as mean ± SD (n=3) ***p<0.001 vs normal group, **p<0.01 vs normal group, *p<0.05 vs normal group and ###p<0.001 vs STZ group, ##p<0.01 vs STZ group and #p<0.05 vs STZ group.*

5.3.2 Histopathological studies

The histopathological data revealed that the pancreatic damage occurred due to diabetic mellitus induced by STZ was found to be improved in mice receiving the optimized Cur-EGCG loaded SNEDDS and glibenclamide treatment. However, improvement was found to be little less in case of mice treated with free drug (Cur+EGCG). This clearly provided an indication towards rejuvenation of pancreas. These results indicate that Cur-EGCG loaded SNEDDS may prove to be a workable treatment strategy for diabetes. The group treated with free drug did not show any improvement in the cellular structure of pancreas. This could be attributed to the degradation of pure cur and EGCG in the GI environment as well as their poor aqueous solubility, leading to poor availability of both the drugs in the systemic circulation. The histopathology images of the pancreas in shown in **Fig.5.20.**



(a) (b)

(c) (d)

(e)

Fig.5.20. *Histopathological images of the pancreas (a) Normal group (b) STZ group (c) Glibenclaclamide group (d) Cur-EGCG loaded SNEDDS (e) Free drug (Cur+EGCG)*

CHAPTER: 6
CONCLUSION

CONCLUSION

Cur-EGCG encapsulated SNEDDS was successfully developed with fish oil, tween 80, transcutol p, ascorbyl palmitate as the excipients. Aerosil – 200 and isomalt at the ratio of 1:1 was used as a solidifying agent. Total 8 batches were developed and tested for its solubility and flowability of the powdered SNEDDS. The optimized formulation of Cur-EGCG loaded SNEDDS revealed a particle size of 125 ± 0.5 nm. In vitro dissolution study revealed that the drug release was more in intestinal fluid for both Cur and EGCG which is a greater area for drug absorption. PXRD revealed the crystalline drugs (Cur, EGCG) were present in the form of amorphous state in the SNEDDS formulation. The finding of the current study, therefore, proves that the drugs was properly encapsulated in the delivery system following its optimized parameters. The study also concluded that self-nanoemulsifying drug delivery system, could be employed for improving the dissolution characteristics of bioactive compounds, thereby increasing its bioavailability.

The statistical comparison of parameters and histopathological results of 8th Week antidiabetic activity performed on STZ caused mice demonstrated that Cur-EGCG loaded SNEDDS had significantly greater antidiabetic potential than their free medication. In a nutshell, this advancement is predicted to lead to a better treatment plan for diabetic patients, as Cur-EGCG loaded SNEDDS can be used for a longer period of time without generating any negative side effects. However, the entire conclusion would be established only after the formulation's clinical testing.

CHAPTER:7
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