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Evaluation Of Self Nano Emulsfifying Drug Delivery System With Flavonoid For Anti Cancer Drug

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Abstract

Oral anticancer therapy faces several drawbacks: low aqueous solubility, poor and irregular absorption from gastrointestinal sites, high first-pass metabolism, food-influenced absorption, non-targeted delivery, severe systemic and local adverse effects, etc. Enhancement of oral bioavailability could reduce the drug load and associated adverse effects. Self-emulsifying drug delivery systems (SEDDS) can enhance in-vivo solubility and drug absorption from the gastrointestinal tract, bypass liver metabolism by lymphatic absorption and inhibit efflux transport. All these phenomena ultimately result in improved oral bioavailability. Anticancer drug delivery using the SEDDS has shown promising results for bioavailability and pharmacodynamic response. A handful of research studies have produced evidence of the successful loading of anticancer agents in SEDDS-based formulations. Various potent and established chemotherapeutic agents such as docetaxel, paclitaxel, etoposide, 5 Fluorouracil, doxorubicin etc., have been successfully formulated and evaluated. Improved bioavailability and reduction of dose might be possible by SEDDS. It could be effective for low-dose drugs. But, excessive surfactant- cosurfactant concentration, lacking predictive in-vitro models and adequate IVIVC, and unavailability of toxicity data are certain challenges for future researchers. No clinical trials have been recorded with anticancer drug-loaded SEDDS. Overcoming the challenges and further progression to clinical studies are required to avail the benefits of anticancer SEDDS.

Keywords: SEDDS; SNEDDS; Self-emulsifying; anticancer; bioavailability; oral delivery.

AIM & OBJECTIVE:

Aim: to enhance the poor aqueous solubility, oral bioavailability, and therapeutic efficacy of flavonoids and anti-cancer drugs

Objective:

Solubility & Bioavailability Enhancement: To screen and select appropriate oils, surfactants, and co-surfactants to maximize the solubility and absorption of both the flavonoid and the anti-cancer drug.

Formulation Optimization: To construct pseudo-ternary phase diagrams to identify the optimal nanoemulsion region and formulate a physically and thermodynamically stable SNEDDS.

Physicochemical Characterization: To evaluate the formulation for critical quality attributes, including droplet size, polydispersity index, zeta potential, and self-emulsification time.

Enhanced Drug Release: To perform in vitro dissolution and drug release studies, ensuring the system spontaneously forms nano-sized droplets in the gastrointestinal tract to improve systemic absorption.

Anti-Cancer Efficacy Evaluation: To assess the combination's cytotoxicity, apoptotic activity, and overall therapeutic potential using specific cancer cell lines in vitro or tumor-induced animal models.

Stability Testing: To conduct accelerated and long-term stability studies (as per ICH guidelines) to ensure the formulation maintains structural integrity and prevents drug precipitation over time.

1. Introduction

Both epidemiologic and experimental evidences revealed that modifications in lifestyle including diet, can have a major impact on the risk for various types of cancers . Based on this evidence, there has been an increasing interest in cancer chemoprevention via the use of dietary phytochemicals over the past 20 years. Among these compounds, phenolic compounds such as flavonoids , stilbenes coumarins , quinones and phenolic acids. have perhaps attracted the most attention. Phenolics are characterized by having at least one aromatic ring with hydroxyl group(s), which are widely distributed in fruits, vegetables, cereals, dry legumes, chocolate, wine, and beverages (e.g. tea, coffee) . Phenolic compounds are ubiquitous in the plant kingdom. More than 8,000 phenolic compounds have been isolated in a wide variety of forms . including compounds such as EGCG isolated from green tea, resveratrol derived from grape seed, and genistein from soybean. The chemistry, bioavailability, and benefit or toxicity on health of phenolics have been reviewed in several publications .[1]

Numerous experiments have successfully linked natural occurring dietary phenolics to anti-proliferative properties and thereby these compounds are regularly referred to as anticancer. For example, juices of prickly pears prevented growth of prostate and colon cells . phenolic-rich berry juice possessed antiproliferative activity against Caco-2 cells . and ginger-derived phenolics were shown to have chemopreventive and chemotherapeutic effects . Modern pharmacological research indicated that the mechanisms of action of phenolics include induction of apoptosis , inhibition of tumor angiogenesis . reduction of the expression of the proinflammatory gene cyclooxygenase-2 , down-regulation of the expression of pRb, cyclins, and CDKs , inhibition of AKR1C3, an target of hormeno-dependent cancer treatment [, and up regulation of MIC-1 gene expression . Perhaps due to these exciting findings, more

health conscious people are advocating the consumption of phenolic-rich food or dietary supplements.In contrast, some scientists questioned the ability of phenolics to exert clinical anticancer activities. Despite of the fact that experimental studies on cultured cell lines or animals models have established positive relationship between dietary phenolics and cancer, it is very difficult to extrapolate the results of these studies (often conduced in vitro or in rodents) to cancer prevention or therapy in humans. One of the reasons is that these studies have often been conducted at doses or concentrations far beyond those that can be achieved in humans for disease-prevention or therapy. For example, EGCG was shown to prevent melanoma tumor cell lines from growth with IC_{50} value from 11 to 89 μM . But in humans, the C_{max} of EGCG were 0.237-0.328 μg/mL (51.7 - 71.6 nM) after taking 1000 mg green tea extract or 250 g fresh grape plus EGCG riched-nutrient mixture .

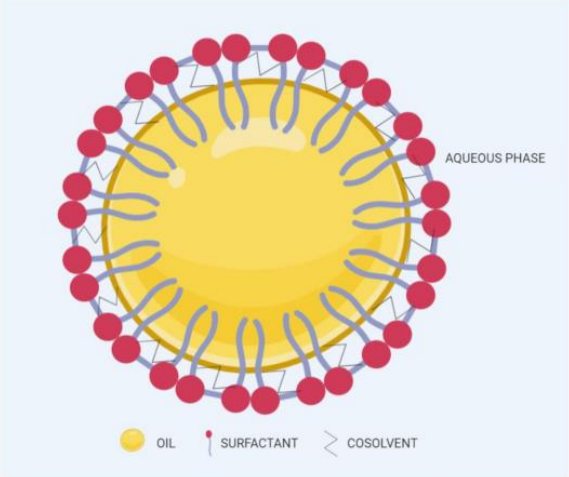


Fig.Self-Nanoemulsifying Drug Delivery System

Cancer is a diverse class of diseases characterized by uncontrolled cell growth with the potential to invade and spread to other parts of the body, and continues to be one of the leading causes of death worldwide . Previous reports have shown differences in cancer morbidity distribution between the developing and the developed world, and demonstrated that approximately 90-95% of all cancers are attributed to lifestyle factors, such as smoking, alcohol consumption, obesity, diet, physical inactivity, among other things, while the remaining 5-10% are due to inherited genes . Conventional cancer treatment modalities include antitumor drugs, surgical resection, locally targeted therapies such as radiation, radiofrequency ablation, and photodynamic therapy . [3]

Characterization of nanoemulsion :

Droplet Size & Polydispersity Index (PDI): Evaluated using Dynamic Light Scattering. The optimal droplet size determines optical clarity and permeation rate, while a PDI value under 0.3 generally indicates highly uniform, homogeneous droplet distribution.

Zeta Potential: Measured via Zeta Potential Analyzers to determine the electrostatic charge on droplet surfaces. Higher absolute values (typically \(\pm 20\) to \(\pm 30\) mV or greater) indicate strong repulsive forces, preventing droplet aggregation and ensuring long-term physical stability.

Morphology: Investigated using high-resolution imaging like Transmission Electron Microscopy to visualize droplet shape (typically spherical) and verify the size measured by light scattering techniques.

Optical Properties: The transparency or translucency of nanoemulsions is measured by percent transmittance using a UV-Visible Spectrophotometer. A transmittance close to 100% indicates an optimally small droplet size.

Rheology & Viscosity: Measured using a Viscometer or Rheometer. Nanoemulsions usually exhibit Newtonian or shear-thinning behavior, with specific viscosity targets depending on the intended route of administration (e.g., low viscosity for oral/ocular sprays or higher viscosity for topical gels).

Thermodynamic & Physical Stability: Tested through stress protocols like centrifugation, freeze-thaw cycling, and heating to check for phase separation, creaming, or cracking.

In Vitro Drug Release & Permeation: Evaluated using Franz Diffusion Cells to track how efficiently the therapeutic or active ingredient is released from the emulsion matrix and permeates through biological membranes (e.g., skin, cornea).[4]

Chemical Characterization: Includes testing for pH, Refractive Index (confirms isotropic nature), and drug encapsulation efficiency to measure the exact amount of active ingredient successfully loaded into the emulsion. [5]

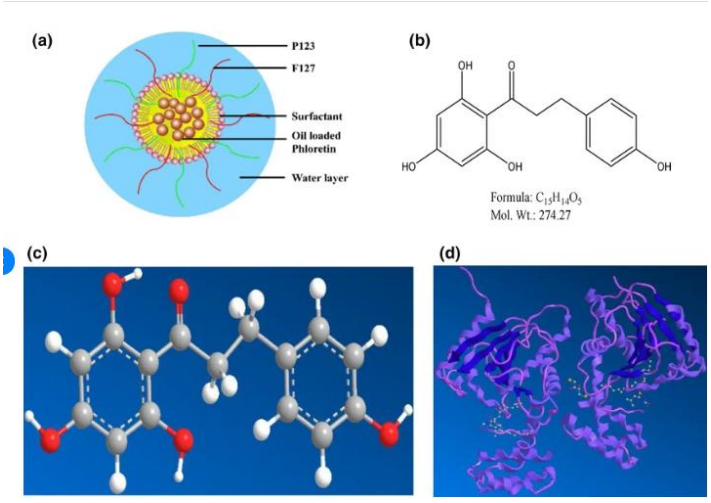
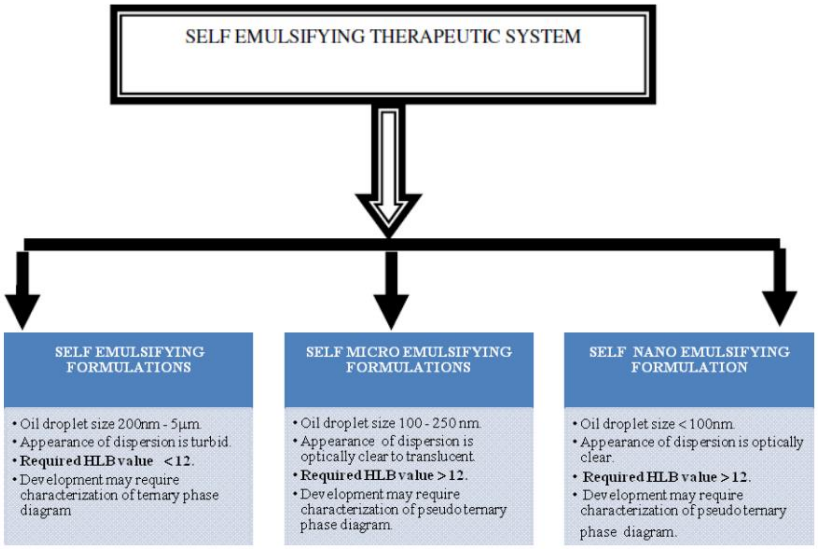


Fig of Characterization of nanoemulsion

SELF EMULSIFYING THERAPEUTIC SYSTEM :



Self-nano emulsifying drug delivery system(SNEDDS) are isotropic mixtures of oil, surfactant, co-surfactant and drug that form fine oil-in-water nanoemulsion when introduced into aqueous phases under gentle agitation. SNEDDS spread readily in the gastrointestinal tract, and the digestive motility of the stomach and the intestine provide the agitation necessary for self-emulsification.[6]Mechanism of self emulsification According to Reiss, Self-emulsification occurs when the entropy change that favours dispersion is greater than the energy required to increase the surface area of the dispersion. The free energy of the conventional emulsion is a direct function of the energy required to create a new surface between the oil and water phases and can be described by the DG= S N p r 2s …… (Equation 1) Where, DG = free energy associated with the process, N = number of droplets, r = radius of droplets, s = interfacial energy. The two phases of emulsion tend to separate with time to reduce the interfacial area and subsequently, the emulsion is stabilized by emulsifying agents, which form a monolayer of emulsion droplets and hence reduce the interfacial energy as well as providing a barrier to prevent coalescence. The specificity of surfactant combination required to allow spontaneous emulsification may be associated with a minimization of the phase inversion temperature, thereby increasing the ease of emulsion.

Advantages of SNEDDS :

Enhanced Bioavailability: By keeping poorly water-soluble drugs (typically BCS Class II and IV) in a dissolved, nanosized state, SNEDDS maximize the interfacial surface area available for absorption.

Bypass of First-Pass Metabolism: The lipid components promote lymphatic transport, allowing the drug to enter systemic circulation directly through the thoracic duct, avoiding liver metabolism.

Reduced Fed/Fasted Variability: Unlike conventional oily solutions, SNEDDS disperse independently of bile salts and stomach enzymes, resulting in more consistent and reproducible plasma concentration levels.

Faster Onset of Action: Because the drug is already in a solution within nanosized droplets, it empties rapidly from the stomach, facilitating faster absorption and quicker therapeutic effects.
Protection of Labile Drugs: The system shields sensitive drug molecules from enzymatic degradation in the gastrointestinal tract.
Improved Patient Compliance: Liquid SNEDDS can be easily solidified and filled into conventional hard or soft gelatin capsules, minimizing palatability issues, preventing drug leakage, and offering excellent commercial viability.[7]

Disadvantages of SNEDDS :
Drug Precipitation: Dilution of the formulation in the gastrointestinal (GI) tract can sometimes reduce the solvent capacity of the mixture, leading to the precipitation of the active drug.

High Surfactant Toxicity: SNEDDS require high concentrations of surfactants and co-surfactants (typically 30–60%), which can cause GI irritation, nausea, or localized toxicity.

Capsule Incompatibility: Liquid SNEDDS can interact with capsule shells, leading to capsule softening, hardening, or drug leakage during long-term storage.

Production and Scale-Up Costs: Formulations require highly specialized equipment and precise oil-surfactant balancing, significantly increasing manufacturing and overall product costs.

Poor Dosing Capacity: They are not suitable for drugs that require high therapeutic doses, as the oil and surfactant phases have a limited solubilization capacity.

Evaluation Difficulties: Traditional dissolution testing methods are often ineffective for SNEDDS because the formulation may depend on the body's digestive processes prior to drug release. There are also no well-established, standardized in vitro models to accurately predict in vivo performance. **[8]**

Factors affecting SNEDDS :
Drugs which are administered at very high dose are not suitable for SNEDDS, unless they exhibit extremely good solubility in at least one of the components of SNEDDS, preferably lipophillic phase. The drugs exhibit limited solubility in water and lipids are most difficult to deliver by SNEDDS. The ability of SNEDDS to maintain the drug in solubilized form is greatly influenced by the solubility of the drug in oily phase. If the surfactant or co-surfactant is contributing to a greater extent for drug solubilization, then there could be a risk of precipitation, as dilution of SNEDDS will lead to lowering of solvent capacity of surfactant or co-surfactant.[9]

Characterization of solid SNEDDS :
As the final dosage form of the solid SNEDDS is a tablet or a capsule, the powder properties of the solid emulsion particles are important. The nature and the quantity of liquid SNEDDS adsorbed on the surface of a particular excipient would influence the properties of the obtained solid particles. The ratio of liquid:adsorbent quantity is important. Powder properties, such as density, angle of repose, flow, compressibility index and particle size distribution, are important for processing into dosage form. The

globule size of spontaneously formed nanoemulsion would govern its performance in vivo. The desorption of SNEDDS from the surface of the solid particles and its conversion into nanoemulsion is the rate-limiting step for the dissolution and absorption of the drug. In our study, an increase in the globule size of the nanoemulsions was observed when the solid nanoemulsifying particles were dispersed in water. Increase in size was not only related to the carrier used but also to the composition of SNEDDS and properties of the drug. It is necessary to carry out physical characterization of the solid SNEDDS using x-ray diffraction spectroscopy, differential scanning calorimetry and scanning electron microscopy to ensure there is no drug precipitation during preparation of solid SNEDDS. The absence of characteristic drug melting endotherm in differential scanning calorimetry suggests that the drug is in a solubilized state in solid SNEDDS. X-ray diffraction is a useful technique employed in the characterization of crystalline materials. The formation of a diffuse diffraction pattern and the disappearance of characteristic drug peaks indicate that the drug is in a solubilized state in solid SNEDDS. [10]

Formulation considerations and potential components :

Successful formulation of SNEDDS depends on the through understanding of the spontaneous nano emulsification process and also on the physicochemical and biological properties of the components used for the fabrication of SNEDDS. The factors influencing the phenomenon of selfnano emulsification are: The physicochemical nature and concentration of oily phase, surfactant and co-emulsifier or co surfactant or solubilizer (if included); The ratio of the components, especially oil-tosurfactant ratio; The temperature and pH of the aqueous phase where nanoemulsification would occur; Physicochemical properties of the drug, such as hydrophilicity/lipophilicity, pKa and polarity. These factors should receive attention while formulating SNEDDS. In addition, the acceptability of the SNEDDS components for the desired route of administration is also very important while formulating SNEDDS.[11]

Cancer preventive activities of the flavonoids :

Apoptosis is an essential part of the maintenance of tissue homeostasis, and is a tightly regulated process under the control of several signaling pathways . Generally, apoptosis induction occurs through multiple pathways, and thus various stimuli activate different pathways . So far, two principal signal pathways of apoptosis have been identified. The intrinsic mechanism of apoptosis involves a mitochondrial pathway. Apoptosis stimuli destruct mitochondrial membrane structure under the control of Bcl-2 (B-cell

leukemia/lymphoma) family, resulting in the release of mitochondrial proteins including cytochrome c. Once cytochrome c is released it activates caspase-9 through interaction with Apaf-1 and dATP, and ultimately leads to caspase-3 and -7 activation . On the other hand, the extrinsic pathway induced by death receptors, such as tumor necrosis factor receptor (TNFR) and Fas, which is responsible for the activation of caspase-8 and -10 accompanied by the activation of caspase-3 and -7 .Caspase-3 and -7 are the final mediators in the two principal signal pathways that cleave substrates and lead to cell death.[12]

Many diseases have been associated with aberrantly regulated apoptotic cell death, ultimately leads to inhibition of apoptosis and propagation of diseases such as cancer. Most of the anticancer therapies trigger apoptosis induction and related cell death networks to eliminate malignant cells .On the other hand, cancer cells are often found to overexpress many of the proteins that play important roles in resisting the activation of apoptotic cascade, ultimately escape from apoptosis and lead to tumor development, progression and treatment resistance . Bcl-2, a well-known antiapoptotic factor, functions through hetero-dimerization with proapoptotic members of the BH3 family to prevent mitochondrial pore formation and prevent cytochrome c release and initiation of apoptosis . In addition, Bcl-2 has been suggested to play an oncogenic role through survival pathways other than its functions at the mitochondrial membrane .

Anti-angiogenic and anti-metastatic properties of flavonoids

Angiogenesis is the process which forms new blood vessels and occurs in many physiological and pathological processes such as reproduce in adults, wound healing, tumor development and some inflammatory diseases . It is a process that is tightly controlled by a wide range of angiogenic inducers such as VEGF and adhesion molecules as well as various endogenous angiogenesis inhibitors including angiostatin and thrombospondin. It can also be stimulated by many inflammatory factors, which contribute to the pathology of inflammation and cancer, indicating angiogenesis, inflammation and cancer are closely related . Uncontrolled angiogenesis is considered as a key step in cancer growth, invasion and metastasis, a great deal of attention has thus been paid to develop potent inhibitors of angiogenesis. In fact, a number of anti-angiogenesis drugs have been approved by FDA and are being used in cancer treatment . Despite this, researchers never stop exploring novel candidates of angiogenesis inhibitors due to side effects of these drugs . It has been demonstrated that wogonin inhibits LPS-induced tumor angiogenesis via suppressing PI3K/Akt/NF-κB signaling in breast cancer cell lines in vitro and in vivo . [14]

Bioavailability of Flavonoids:

Flavonoids can interact with other nutrients they can decrease glucose absorption due
to suppression of carbohydrate-hydrolyzing enzymes (alpha-amylase and alpha-glucosidase) .and glucose transporter in the brush border . Fat intake improves flavonoid bioavailability andincreases their intestinal absorption via augmented secretion of bile salts which increase micellarincorporation of flavonoids .However, protein intake can decrease flavonoid bioavailability .affecting both antioxidant efficacy and protein digestibility . The gut microbiome is very importantfor the absorption and metabolism of flavonoids. After consumption, prior to absorption intestinal or colon microflora are able

to hydrolyze glycosylated flavonoids such as flavones, isoflavones,flavonols and anthocyanins into their respective aglycones . Aglycones are lipophilic, and therefore passive diffusion is responsible for their pathway to the intestinal epithelial cells while the uptake of glycosides into the intestinal epithelial cells is regulated by the epithelial transporters .After absorption, flavonoids undergo metabolic transformations first in the small intestine, liver andkidney .Methylation, sulfation, or glucuronidation of flavonoids before they reach the circulation and, afterwards, the tissues, could influence their biological activities. Unabsorbed flavonoids remaining in the proximal intestine are further digested in the colon by microbes able to split their heterocyclic oxygen containing ring and the hydroxylated phenyl carboxylic acids formed could be absorbed . [16]

Anticancer Effect of Flavonoids :

The ability of flavonoids to scavenge free radicals, regulate cellular metabolism, and prevent oxidative stress–related diseases have been demonstrated in numerous studies . There is accumulating evidence that many flavonoids exert anticancer activity, however, the molecular mechanisms responsible for this effect have not been fully elucidated yet.

Cancer is a heterogeneous disease characterized by uncontrolled proliferation and impaired cell cycle leading to the growth of abnormal cells that invade and metastasize to other parts of the body . Oxidative stress, hypoxia, genetic mutations and lack of apoptotic function are the main internal causes of cancer, whereas the external causes are related to increased exposure to stress, pollution, smoking, radiation and ultraviolet rays.Altered metabolism, impaired cell cycles, frequent mutations, resistance to immune response, chronic inflammation, formation of metastasis, and induction of angiogenesis are the main characteristics of the cancer cells . There isemerging evidence that cancer is a metabolic disease determined by various degrees of mitochondrial dysfunctions and metabolic alterations . Mitochondria play essential roles in cellular energysupply, regulation of metabolism, cell death signaling and reactive oxygen species (ROS) generation.[17]

The main metabolic alterations of the tumor cells involve increased aerobic glycolysis deregulated pH impaired lipid metabolism increased generation of ROS and compromised enzyme activities (Figure 8). As a direct consequence, the extracellular environment becomes acidic andmore favorable to inflammation glutamine-driven lipid biosynthesis increases and upregulatesthe pathways involved in tumorigenesis initiation and metastasis cardiolipin levels decrease inmembranes causing impaired enzyme activities mitochondria are hyperpolarised . andthis effect correlates with the malignancy and invasiveness of cancer cells .

Flavonoids exert a wide variety of anticancer effects: they modulate ROS-scavenging enzyme activities, participate in arresting the cell cycle, induce apoptosis, autophagy, and suppress cancer cell proliferation and invasiveness .

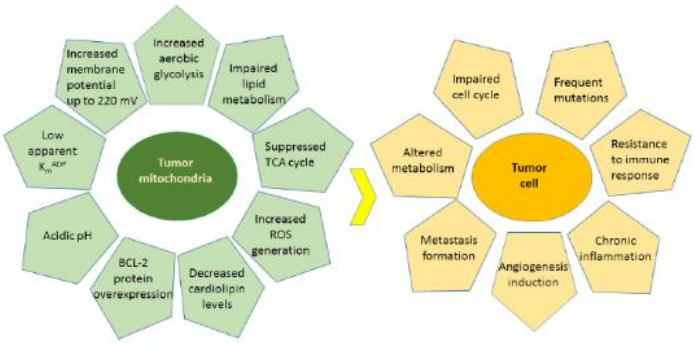
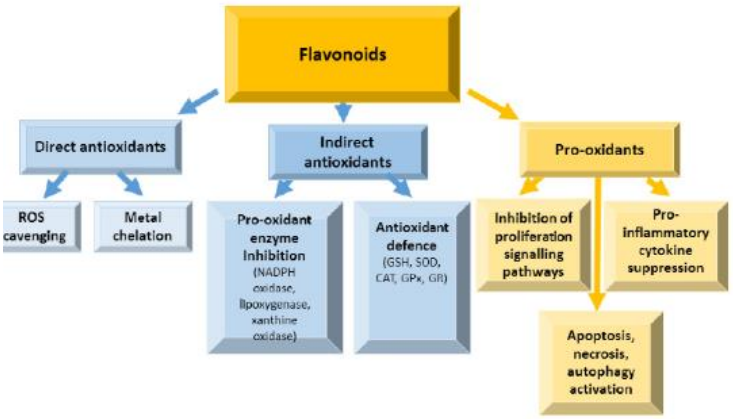


Figure 8. The main characteristics of tumor mitochondria and tumor cells.

Flavonoids in Oxidative Stress

When the cellular homeostasis between the pro-oxidant activities and antioxidant defense is impaired, the production of ROS increases, and free radicals accumulate . ROS are mainly generated in the electron transport chain in mitochondria as the byproducts of oxidative phosphorylation in the cell . The amount of ROS produced causes oxidative stress which is involved in the development of inflammation processes leading to many degenerative diseases and cancer. Flavonoids have dual action regarding ROS homeostasis—they act as antioxidants under normal conditions and are potent pro-oxidants in cancer cells triggering the apoptotic pathways.[18]

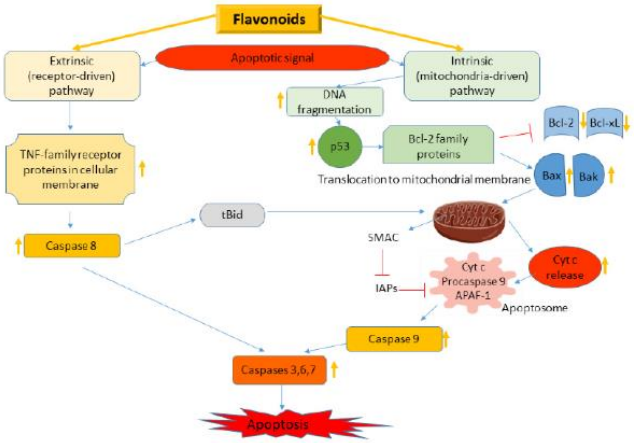


Flavonoids can directly scavenge ROS, and chelate metal ions due to their ability stabilize the free radicals due to the presence of phenolic hydroxyl groups . Indirect flavonoid antioxidant effects are related to activation of antioxidant enzymes, suppression of pro-oxidant enzymes, and stimulating production of antioxidant enzymes and phase II detoxification enzymes . Both antioxidant andpro-oxidant activities are involved in flavonoid anticancer effects .

Isoflavone genistein promoted breast cancer cell arrest at G2/M phase and subsequent ROS dependent apoptosis . Daidzein promoted apoptosis in breast cancer MCF-7 cells due to the ROSgeneration . Flavanone hesperetin induced apoptosis of gall bladder carcinoma, esophagealcancer, hepatocellular carcinoma and human breast carcinoma MCF-7 cells via activatingthe mitochondrial apoptotic pathway by increasing the ROS production. Flavanone naringenin exerted anti-cancer effects on choriocarcinoma JAR and JEG 3 cell lines by inducing the generation of ROS and activation of signaling pathways . [19]

Flavonoids in Apoptosis

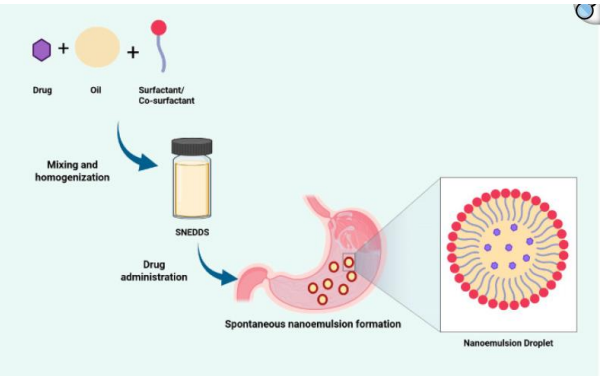
Cancer cells are resistant to apoptosis—a programmed cell death, usually induced by a series of signal transduction pathways and pro-apoptotic proteins—caspases and Bcl-2 family proteins .There are two main signaling cascades of apoptosis—extrinsic, related to tumor necrosis factor (TNF)superfamily with main signaling protein—caspase 8; and intrinsic—mitochondrial pathway, where Bcl-2 family proteins launch the activation of caspases 9, 3 and 7 . There is an overexpression of oncogenic genes (e.g., c-Myc), leading to cellular proliferation and p53 suppression, and activated anti-apoptotic proteins of Bcl-2 family in cancer cells whereas pro-apoptotic proteins and caspases could be downregulated .



Flavonoids acting as pro-oxidants could suppress proliferation of cancer cells by inhibition of epidermal growth factor receptor/mitogen activated protein kinase (EGFR/MAPK), phosphatidylinositide 3-kinases (PI3K), protein kinase B (Akt) as well as nuclear factor
kappa-light-chain-enhancer of activated B cells (NF-κB) .]. Isoflavonoid genistein could regulate estrogen receptor-α expression and change Bax/Bcl-2 ratio downregulating roliferation, differentiation, and activating apoptosis in MCF-7 and 3T3-L1 cells .Moreover, genistein suppressed Bcl-2, Bcl-xL, c-inhibitor of apoptosis protein 1 (c-IAP1), survivin, and NF-κB in 200 and A2780 cells . increased caspase-3 activity in HT-29 colon cancer cells and
activated intrinsic apoptotic signaling pathway in HCT-116 and LoVo cells . Isoflavonoid daidzein also acted as phytoestrogen . It promoted cytochrome c release from mitochondria, leading to caspase 7 and 9 activation and also altered Bax/Bcl-2 ratio in MCF-7 cells . Daidzein induced apoptosis in the HCCSK-HEP-1 cell line via Bak upregulation and ownregulation of anti-apoptotic proteins, resulting in cytochrome c release from mitochondria and activating subsequent apoptotic pathway involving caspases 3 and 9 . Flavanone esperetin induced cytochrome c release, activation of caspases-3 and -9, and reduced Bax to Bcl-2 ratio in gastric cancer cells in the Eca109 cellline as well as in the HT-29, MCF-7, and MDA-MB-231 cell lines . In H522 cells, hesperetin induced extrinsic apoptotic athway due to overexpression of TNF-protein superfamily members,caspase-9 activation, and decrease in p53 level .Furthermore, hesperetin inhibited the NF-κBsignaling pathway and reduced Bcl-2 transcription and translation in PC-3 cells . Flavanone naringenin

Basic Principles of SNEDDS Formulation :

SNEDDS is a drug delivery system of oil, surfactants, and co-surfactants that spontaneously forms a nanoemulsion in water with gentle agitation . Unlike nanoemulsions or microemulsions, SNEDDS exists as an anhydrous preconcentrate and only forms a nanoemulsion in situ upon contact with gastrointestinal fluids. This unique characteristic contributes to enhanced physical stability during storage, as there is no aqueous phase present that could lead to phase separation or degradation before administration. Spontaneous emulsification of SNEDDS occurs because the free energy (ΔG) approaches zero, achieved by the flexible interface of the surfactant and co-surfactant molecules, which reduces surface tension . Compared to other lipid-based systems like nanostructured lipid carriers (NLCs), solid lipid nanoparticles (SLNs), and liposomes, SNEDDS offers the advantages of ease of production and avoids aggregation risks as it is a preconcentrated nanoemulsion that only forms a nanosystem in the gastrointestinal tract.[21]



The SNEDDS consists of three main components: oil, surfactant, and co-surfactant . Oil serves as the primary carrier for drugs, enhancing the solubility of the active ingredients in the lipid phase. The oil used can be natural or synthetic and can be selected based on its ability to dissolve the active ingredient.Natural oils are commonly chosen based on their safety and biocompatibility considerations . Some studies have used natural oils with pharmacological activities that align with the active ingredient to form a bioactive SNEDDS formulation .Many SNEDDS formulations utilize synthetic oils primarily because of their consistent composition, higher solubilization capacity, and improved stability compared to natural oils . Surfactants reduce interfacial tension, facilitating the formation of uniformly sized nanoemulsion globules .Non-ionic surfactants are commonly used because of their low toxicity and ability to stabilize emulsions across a wide pH range . Beyond emulsification, non-ionic surfactants like Tween 80 and Cremophor EL/RH40 enhance membrane fluidity and inhibit efflux transporters, improving drug bioavailability . Co-surfactants enhance nanoemulsion stability and homogeneity by improving interfacial fluidity while simultaneously reducing the required concentration of surfactants, which helps minimize potential toxicity risks. Common co-surfactants include short-chain alcohols and glycols, such as SNEDDS development generally involves several stages. Each stage is crucial for selecting proper excipients and achieving efficient self-nanoemulsification.[22]

Solubility Testing

The first stage is solubility testing, which aims to determine the solubility of the active pharmaceutical

ingredient (API) in various oils, surfactants, and co-surfactants. Because SNEDDS relies on dissolving the drug in lipid-based excipients, selecting components with a high solubilizing capacity is essential. Oils improve drug absorption, whereas surfactants and co-surfactants facilitate emulsification . This step ensures that the final formulation can carry an adequate drug load without precipitation by identifying excipients that can dissolve the maximum amount of API .

Emulsification Efficiency

Emulsification efficiency, also referred to as emulsification capability, evaluates the ability of a given combination of oil, surfactant, and co-surfactant to spontaneously form a nanoemulsion upon contact with an aqueous medium . Because the oil phase is generally selected based on the solubility results from the previous stage, this test was primarily conducted to determine the most effective combination of surfactant and co-surfactant. Rapid and spontaneous emulsification is a key characteristic of SNEDDS, allowing the drug to be efficiently dispersed in the gastrointestinal tract. This procedure involved introducing a small volume of the preconcentrate (SNEDDS formulation) into an aqueous medium under mild stirring to simulate the gastrointestinal environment. The emulsification efficiency was assessed visually, and the globule size and/or % transmittance were measured using an appropriate instrument .

Construction Diagram Pseudo-Ternary

Once suitable excipients are selected, a pseudo-ternary phase diagram defines the range of oil, surfactant, and co-surfactant concentrations that lead to stable nanoemulsion formation. The optimum ratio of surfactant to co-surfactant can also be determined by observing the area of the nanoemulsion formation within the phase diagram. Because SNEDDS formulations require a careful balance between these components, mapping the phase diagram helps to effectively visualise the regions where nanoemulsification occurs . Oil, surfactant, and co-surfactant mixtures were prepared in various ratios and titrated with water while stirring. Each system was observed, and the results were plotted on a ternary diagram to define the nanoemulsion region . Evaluation of self-emulsification and turbidity after dilution is an alternative to titration methods for assessing the effectiveness of SNEDDS formation .[23]

Optimization of SNEDDS Formulation

After obtaining the SNEDDS formation area through the analysis of the pseudo-ternary phase diagram, the next step was to optimize the SNEDDS formulation. This optimization process can be performed by creating several formula variations. Variations are generally applied to the oil-to-Smix ratio and surfactant-to-cosurfactant ratio to select the formulation that produces the optimum SNEDDS characteristics . SNEDDS was prepared by mixing oil, surfactant, and co-surfactant in specific proportions, followed by homogenization using magnetic stirring or ultrasonication methods until a stable mixture was formed. Several approaches can be used to determine an optimal formulation. Beyond these conventional approaches, SNEDDS optimization can be further enhanced by implementing a QbD strategy, enabling a more structured and risk-based development process.

Application of QbD in SNEDDS Development :

The conventional approach to developing SNEDDS commonly only allows the observation of One Factor at a Time (OFAT), making it less efficient in understanding the interactions between formulation variables. This limitation has led to the implementation of a more systematic and scientific approach, known as QbD. Through QbD, SNEDDS development not only focuses on formulation optimization but also enables a deeper understanding of the influence of each factor on product quality (CQAs). Consequently, the formulated product exhibits a better controlled quality and consistency than the conventional empirical approach . Several studies have developed SNEDDS formulations based on the QbD approach following the general stages outlined . The process began by determining the QTPP, CQAs, CMAs, and CPPs, followed by a risk assessment to evaluate the impact of CMAs and CPPs on CQAs. Finally, a design space was established. However, the number of studies implementing a comprehensive QbD approach is relatively limited. Most studies have focused on determining the CQAs, CPPs, and CMAs and designing experiments to optimize the formulation. In many cases, QTPP and risk assessment have not yet been clearly defined .

Define of QTPP in SNEDDS Development

The QTPP is the expected final quality specification of a product. In pharmaceutical dosage forms, such as SNEDDS, QTPP serves as a guideline to ensure that the formulation meets the optimal quality, safety, and efficacy criteria. The QTPP, one of the key aspects of QbD, serves as the basis for identifying CQAs . As shown in , several elements of the QTPP in the SNEDDS dosage form include clinical target, dosage form, dosage type, dosage strength, route of administration, packaging/container closure system, pharmacokinetic parameters, stability, and alternative administration methods. Specific targets were established for each QTPP element, along with justifications explaining the rationale behind their selection. These targets ensure that the formulation satisfies the required quality, efficacy, and safety standards . shows the common QTPP elements, their targets, and justifications for SNEDDS formulation.[24]

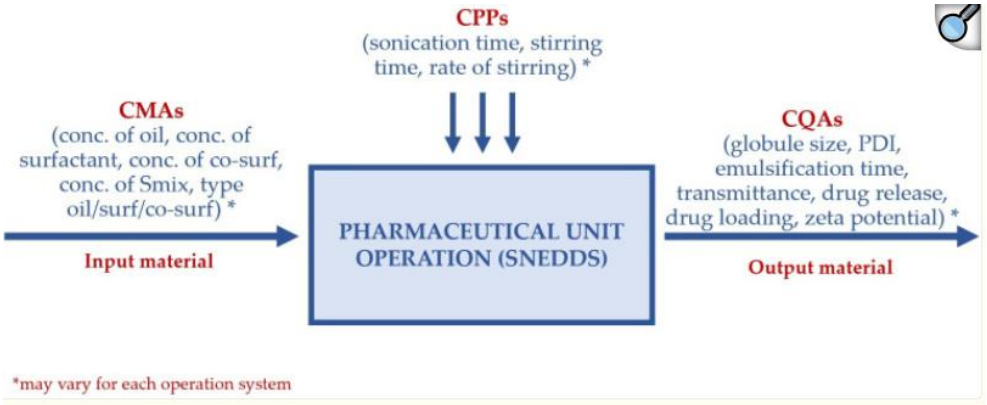
Identify CQAs in SNEDDS Development:

The process begins by defining the quality attributes of the product and collectively contributing to meeting the predefined QTPP. Once these quality attributes are identified, each attribute is further analyzed to assess its potential impact on product performance, allowing for the identification of Critical Quality Attributes. An attribute is critical if it significantly affects the safety, efficacy, or stability of a product. This is critical when variations substantially change the quality of the final product . In the context of SNEDDS, several common attributes that may be categorized as CQAs include the globule size, emulsification time, % transmittance, drug release/dissolution, zeta potential, and polydispersity index . These attributes directly affect the performance of SNEDDS, particularly in enhancing the bioavailability of poorly soluble drugs. Similar to establishing the QTPP, strong justification must also support the identification of the CQA. This justification should include a scientific rationale and experimental data to validate the selection of specific CQA. Generally, not all CQAs are implemented; only those most suitable for particular research conditions are selected.[25]

Risk Assessment in SNEDDS Development

In the QbD approach, risk assessment is used to identify factors that may affect the quality of pharmaceutical products. After the CQAs were established in the previous stage, a risk assessment was conducted to evaluate factors that may cause variations in product quality, including CMAs and CPPs. CMAs refer to the inherent characteristics and attributes of raw materials, whereas CPPs refer to manufacturing process parameters that influence the quality of the final product. CMAs and CPPs have a significant impact on CQAs and are controlled through risk assessments to ensure quality consistency and optimal product performance. Risk assessment typically begins with identifying potential risks using tools such as the Ishikawa diagram, followed by assessing the impact of each identified risk using appropriate methods. According to ICH Q9's quality risk management section, the risk management process can be conducted using various approaches such as Failure Mode and Effects Analysis (FMEA) and Risk Ranking and Filtering (REM). [26]

The selection of an appropriate method depends on the level of risk, process complexity, and data availability. REM is commonly applied for initial screening, followed by FMEA when a more detailed analysis is required, particularly for high-risk processes. Although REM does not necessarily have to precede FMEA, combining both methods is often considered the best practice to achieve a comprehensive risk assessment In SNEDDS development, nearly all researchers used the Ishikawa diagram for risk identification . The Ishikawa diagram, also known as the fish-bone diagram, is used to systematically identify and analyze the factors contributing to a problem or risk within a process. It is widely applied in quality control and risk management, including in pharmaceutical formulations, based on the QbD approach. The diagram resembles a fish skeleton, where the main issue (effect) is positioned at the head of the fish, while the potential causes are categorized into branches along the fishbone. To construct an Ishikawa diagram, the first step is to define the main problem, identify the primary cause categories, add specific contributing factors to each category, and then conduct an in-depth analysis to determine the factors with the most significant impact. These causes are typically grouped into six categories: man (human), machines (equipment), materials (raw materials), methods (processes), measurements (evaluations), and the environment (external factors) Several studies on SNEDDS development using the QbD approach have demonstrated various strategies for conducting risk assessments. Some articles only described the construction of the Ishikawa fish-bone diagram without further elaborating on the quantitative risk assessment methods. In contrast, others describe subsequent stages using Risk Ranking and Filtering (REM), a Risk Assessment Matrix (RAM), or FMEA to classify and prioritize risks systematically. REM ranks risks by assigning scores to predefined criteria, typically severity and probability, and then sorts risks from highest to lowest priority. [27]



The discussion on QTPP, CQA, CMA, and CPP has predominantly focused on the development of SNEDDS for oral administration. This is mainly due to the extensive research and well-established applications of SNEDDS in enhancing the solubility and bioavailability of poorly water-soluble drugs via the oral route. However, some studies have also investigated the use of SNEDDS for non-oral routes such as ocular, intranasal, and transdermal administration, although the number of such studies remains relatively limited . For these alternative routes, adjustments in QTPP, CQA, CMA, and CPP are necessary to accommodate the distinct physiological conditions, absorption mechanisms, and therapeutic objectives, despite many aspects being shared across different routes. For example, in one ocular SNEDDS development study using DoE, the CMAs included variations in oil, surfactant, and co-surfactant concentrations, as well as surfactant type. The CQAs were defined based on parameters such as globule size, emulsification time, and % transmittance after dilution. Although CMAs and CQAs were similar to those considered in oral SNEDDS development, key differences appeared in the process parameters and testing conditions. For instance, dilution was conducted using simulated tear fluid (STF, pH 7.4) at a 1:10 volume ratio under gentle stirring (30 rpm) at 35 °C to closely mimic the ocular environment, including corneal surface conditions and eyelid movement.[28]

Application of DoE in SNEDDS Development :

In QbD, the Design of Experiments (DoE) is a systematic approach for understanding the relationship between CMAs, CPPs, and CQAs. Compared to other optimization methods, such as OFAT, DoE has the advantages of studying multiple factors simultaneously, providing more accurate information and identifying interactions between factors affecting product quality. This approach is expected to reduce development costs and time while improving the reproducibility of the results DoE in pharmaceutical product development can be carried out using various types of design, including mixture design (simplex lattice design, extreme vertices design, and optimal mixture design), response surface methodology (Box–Behnken design and Central Composite Design), and factorial design. Various experimental design methods can be applied as optimization tools in the development of SNEDDS formulations, each offering distinct advantages .

Mixture Design

Mixture design has been widely applied in the development of SNEDDS formulations . Mixture design is a specialized approach within the DoE framework utilized to optimize pharmaceutical formulations.

This method is particularly relevant when the response of the system is determined by the relative proportions of the multiple components in a formulation. A fundamental characteristic of mixture design is that the total sum of all elements in the formulation remains constant (100% or one) . Composition adjustments were made by modifying the relative ratios of the components, without altering the overall quantity of the mixture. This makes the mixture design well-suited for developing SNEDDS formulations, mainly when the defined factors are the oil, surfactant, and co-surfactant concentrations. The data in show that the mixture design can yield a final SNEDDS formulation with a 100% proportion of oil, surfactant, and co-surfactant. However, the table also highlights that applying mixture design is limited to evaluating CMAs because it does not consider CPPs as contributing factors in the formulation process. If the process parameters are not considered critical under certain conditions, mixture design is an appropriate choice for optimizing SNEDDS formulations.[29]

Response Surface Methodology

Response surface methodology (RSM) is another design widely applied in SNEDDS development RSM is a statistical technique widely used for optimizing processes by evaluating the relationships between multiple independent and response variables. This approach is beneficial in experimental design as it reduces the required trials while providing a comprehensive understanding of factor interactions. RSM employs mathematical models to describe the response surface and predict the optimal conditions The Box–Behnken Design (BBD) and Central Composite Design (CCD) are the most commonly used RSM designs. BBD is a three-level design that efficiently models factor interactions while avoiding extreme (corner) points in the experimental space. By contrast, CCD consists of factorial, axial, and central points, making it a more flexible design that explores a broader experimental space, mainly due to the inclusion of axial points Unlike the mixture design, the total proportion of the mixture components (oil, surfactant, and co-surfactant) cannot be directly fixed at 100% in the RSM, as shown in . This is because selecting experimental points in the RSM focuses more on exploring the effects of individual variables than on maintaining a fixed total composition Therefore, further calculations are required to determine the optimal percentage formulation. Several studies of SNEDDS using RSM have employed volume or weight units for component factors as alternatives to percentage-based compositions Apart from enabling efficient point selection and experimental design, this method allows one to assess the influence of the manufacturing process (CPPs). This aspect is not feasible in mixture design, as observed in studies of SNEDDS for Bedaquiline Docetaxel and Venetoclax Additionally, the CMAs analyzed in the RSM were not limited to the mixture proportions. They can also include other variables, such as the surfactant/co-surfactant ratio, dosage, and type of surfactant used . The RSM allows for including numerical and categorical variables, whereas the mixture design is restricted to numerical variables

Factorial Design

Several studies have employed factorial design in addition to mixture design and RSM in the development of SNEDDS formulations . Factorial design is a statistical approach used to evaluate the effects of multiple independent factors and their interactions on the responses. It includes a Full Factorial Design, which examines all possible combinations of factor levels, and a fractional factorial design, which reduces the number of runs by selecting a subset of combinations. The factorial design incorporates both numerical and categorical factors . The selection of experimental points represents a key distinction between factorial design and RSM. In a factorial design, experimental points are

typically placed at low and high levels, with an additional intermediate level in three-level designs. This approach efficiently identifies main effects and interactions but is less suitable for capturing nonlinear responses. In contrast, RSM enables the modelling of response surface curvature and higher-order relationships. In SNEDDS development, factorial design is commonly used for screening critical formulation and process parameters (CMAs and CPPs), while RSM is preferred for optimization .[30]

Optimization stages in SNEDDS Development Based on DoE :

DoE is applied in SNEDDS optimization to achieve two main objectives: (1) analyze the influence of CMAs and CPPs on CQAs and (2) obtain an optimal formulation that meets the desired CQA criteria. Several systematic steps must be followed in order to achieve these goals.

Determination of Factors and Responses

In DoE, the terms, factors and responses, play a crucial role. A factor or independent variable refers to a variable that can influence the outcome of an experiment and is classified as a CMA or CPP 1 By contrast, a response or dependent variable represents the measured outcome of the experiment, corresponding to the CQA. As explained in the QbD section, determining CQAs is the first step and must be supported by proper justification 1 Based on 1 the responses of the SNEDDS formulation included the globule size, emulsification time, polydispersity index (PDI), % transmittance, % drug release, zeta potential, and drug loading. However, researchers are not required to designate all these parameters as responses. Instead, they should be selected with proper justification, focusing on the quality attributes that are most relevant to the desired product performance. The most commonly established responses are the globule size, emulsification time, and drug release. Globule diameter is a critical parameter to ensure that SNEDDS spontaneously forms a nanoemulsion upon contact with aqueous fluids, typically measuring less than 200 nm. The emulsification time reflects the ability of the system to create a nanoemulsion spontaneously upon contact with an aqueous fluid. Meanwhile, the percentage of drug release is significant when the primary objective of SNEDDS development is to enhance the dissolution of poorly water-soluble drugs 1

Selecting Experimental Design

Selecting the most appropriate DoE approach involved basing it on study objectives and characteristics. However, the available publications generally do not explain the reasons for choosing a particular DoE type in detail. As previously discussed, three main DoE approaches can be applied in SNEDDS optimization: mixture design, response surface methodology, and factorial design. Factorial design is commonly used to screen for significant factors, helping identify key variables that influence SNEDDS performance. Response surface methodology and mixture design are preferred for optimization, mainly when dealing with nonlinear relationships. When the optimization focuses solely on the composition of the SNEDDS components, oil, surfactant, and co-surfactant, mixture design (e.g., optimal mixture design) is ideal. This method ensures that the proportion of these three components is 100%, thereby providing an optimal formulation based on their relative ratios. However, if optimization involves additional factors beyond composition, such as numerical or categorical variables and Critical Process Parameters (CPPs), RSM becomes more suitable. RSM optimizes the formulation composition (CMAs)

and process parameters (CPPs) while effectively capturing the interaction effects and nonlinear relationships that influence the SNEDDS characteristics .

Establishment of Experimental Points

The experimental points can be established after defining the factors/responses and selecting the DoE type. This process ensured the experimental design was efficiently structured, allowing systematic data collection and optimization. The system generates experimental runs based on the chosen DoE model, considering factors such as the number of variables, their levels, and the requirements for replication. The design can focus on the most relevant formulation space by setting appropriate upper and lower limits, particularly for numerical factors, such as oil, surfactant, and co-surfactant concentrations. The pseudo-ternary phase diagram often serves as a reference for defining these concentration ranges, ensuring that only the stable nanoemulsion regions are explored. Software such as Design-Expert version 9/10/11/12/13, Systat version 13, Statgraphics® centurion XV version 15.2.05, and MODDE software version 2.1 can assist in generating experimental runs . Referring to . the number of runs used in the experimental designs varied widely, ranging from 8 to 32. However, the most commonly used run is between 13 and 17. The sertraline SNEDDS formulation was developed using a 2^3 factorial design with eight experimental runs. Oil, surfactant, and co-surfactant concentrations were evaluated at two levels (low and high) without replication. This design choice ensures the efficient screening of the selected factors while maintaining a minimal number of runs .However, this approach is limited in capturing nonlinear relationships and is not ideal for formulation optimization .Venetoclax SNEDDS was optimized using 32 experimental runs with a Central Composite Design. Five factors were considered: the oil concentration, surfactant concentration, co-surfactant concentration, stirring rate, and stirring time. Four response parameters were measured: the globule size, PDI, emulsification time, and % transmittance. Many factors directly contributed to the higher number of experimental runs. Although this approach requires more effort, time, and resources, it allows a more comprehensive understanding of the formulation. This design provides valuable insights into CMAs and CPPs, leading to more precise optimization of the SNEDDS formulation . Selecting an appropriate number of runs is crucial for balancing experimental efficiency and data accuracy, ensuring that only the most relevant factors are considered for analysis and optimization.

Preparation and Characterization of SNEDDS

This stage involved preparing SNEDDS formulations according to designated experimental points. API is typically incorporated alongside oil, surfactant, and co-surfactant, ensuring the required dosage to achieve the desired pharmacological effect. The formulation process involved mixing the components via vortexing, stirring, or ultrasonication. Additionally, the solubility of APIs in the oil phase or mixture must be ensured to achieve optimal dissolution. Once all formulations have been prepared, they are characterized using appropriate and validated methods based on predefined response parameters.

Data Analysis and Polynomial Modelling

DoE provides a systematic framework for analyzing the relationship between factors and responses within a given system. The process begins with data analysis, where experimental responses are statistically processed to identify trends, interactions, and significant factors. Statistical tools, such as ANOVA, assess factor significance and interactions, ensuring that the selected model accurately

represents the system. Polynomial modelling establishes a mathematical relationship between factors and responses, choosing an appropriate polynomial model crucial for accurately describing the system .

The selection of a polynomial model depends on how well it represents experimental data. A first-order polynomial assumes a simple linear relationship between the factors and the responses, which is suitable when the initial data show no curvature. A higher-order model may be required if the residual analysis or statistical fit tests indicate poor model adequacy. A second-order polynomial (quadratic model) is applied when nonlinearity is detected, with significant quadratic terms (X^2) indicating nonlinear factor–response interactions. It is preferable that this quadratic model significantly improves the lack-of-fit test and increases R^2 without overfitting. A third-order polynomial (cubic model) may be required for more complex response patterns to capture higher-order interactions when the quadratic model is insufficient .

Immunomodulatory effects of flavonoids :

It has been known that flavonoids such as quercetin, fisetin, luteolin and kaempferol have specific immunomodulatory effects that are might be linked to their anti-allergic activities and beneficial effects against autoimmune diseases .Previous studies on the effects of quercetin on the immune system showed its inhibitory effects on cytotoxic lymphocyte function, and clarified that quercetin can affect the balance of Th1/Th2 in a murine model of asthma . Fisetin also significantly inhibited Th1 and Th2 cytokine production, cell cycle and the ratio of T $CD4^+/CD8^+$ cells in vitro through the suppression of NF-κB and nuclear factor of activated T cells (NFAT) signaling pathway . Furthermore, kaempferol has been recently reported to enhance the function of $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells by inhibiting FOXP3 phosphorylation . In fact, Treg cells have received considerable attention due to their immunosuppressive properties in vitro and in vivo .These previous findings thus support the immunosuppressive effects of flavonoids. On the other hand, immunosuppression has been widely recognized in cancer patients due to immune tolerance induced by malignant tumor cells and/or undesirable side-effects of many types of chemotherapeutic drugs . Accumulating evidence has shown an increased number and function of Treg cells in patients with solid tumors and hematologic malignancies, suggesting its critical role in limiting antitumor immune response and promoting immunological ignorance of cancer cells .

Effects of flavonoids on drug transporters and metabolic enzymes :

Since drug action usually requires uptake of the drug, it was considered that intracellular drug concentrations might determine the efficacy of respective drug. Cancer cells usually express a high protein level of ATP binding cassette (ABC) transporters that can attenuate the efficacy of treatment by actively pumping drugs out of the cells, leading to the multidrug resistance phenotype . It has been established that multidrug resistance-associated protein 1 (MRP1)/MRP2, P-glycoprotein (P-gp), multidrug resistance 1 (MDR1) and breast cancer resistance protein (BCRP), which belong to the ABC transporter superfamily, play a prominent role in the chemoresistance to various anticancer drugs . Fortunately, flavonoids have been demonstrated to serve as modulators of drug transporters and metabolic enzymes, consequently exhibit their cancer chemopreventive activity .Recently, novel flavone derivatives have been demonstrated to serve as selective and dual inhibitors of the transporters P-gp and

BCRP .Nobiletin, a major flavonoid compound from oranges (Citrus sinensis) peel, has been shown to inhibit MRP1, resulting in the accumulation of intracellular adriamycin (ADR) in A549 human non-small-cell lung cancer (NSCLC) cells, ultimately enhance chemosensitivity to ADR .Flavonoids including genistein, quercetin, wogonin were found to downregulate MRP1 in resistant human tumor cell lines, such as pancreatic adenocarcinoma cells (Panc-1) and chronic myelogenous leukemia (CML) cells (K562/A02), suggesting their MDR reversal potential . Previous reports further demonstrated that flavonoids might inhibit MRP1 by binding to certain regions of the transporter (substrate-binding site, nucleotide-binding domains) or depleting intracellular glutathione . Structure-activity relationships seem to be linked with the inhibition activity against MRP1, suggesting that the degrees of hydroxylation and methoxylation, as well as 2,3-double bonds, play important roles in MRP1 inhibition . Regarding P-gp, Mohana et al. recently reported that flavonoids such as quercetin, rutin, epicatechin 3

Differentiation-inducing activity of flavonoids in cancer cells :

The aim of differentiation therapy is to induce the differentiation of malignant cells, consequently cause them to cease proliferation, ultimately control their tumorigenic and malignant potential . Differentiation therapy arises from the fact that leukemic cells have lost their ability to differentiate and eventually become malignant . Use of all-trans retinoic acid (ATRA) and/or arsenic trioxide in the treatment of APL has acquired a therapeutic niche, represented as one of most successful model of differentiation therapy .Differentiation therapy possesses the obvious characteristics of relatively low toxicity compared with conventional chemotherapy . Therefore, there is an urgent need to develop novel agents with potent differentiation-inducing activity and less toxicity for differentiation therapy due to poor cellular differentiation of cancer cells and their acquired resistance to differentiation agents.

Bioavailability of flavonoids :

Low bioavailability of flavonoids has been a concern as it can limit or even hinder their health effects . Flavonoids are present in food products mostly in the form of glycosides that are generally hydrolyzed, consequently converted to their respective aglycones by intestinal or colon microflora prior to absorption in the gastrointestinal tract, followed by biotransformation to various metabolites which enter bloodstream .Previous pharmacokinetic data indicate that the absorption of anthocyanins into the bloodstream of rodents and humans is minimal, suggesting that they may have little efficacy in tissues other than the gastrointestinal tract and skin, where they can be absorbed locally . Therefore, a number of formulation strategies including liposomes, nanoparticles, nanoemulsions and mucoadhesive buccal films have been developed in recent years in order to maximize the bioavailability of flavonoids . More recently, Deepika and colleagues developed a novel therapeutic polymeric complex of rutin (a hydrophobic polyphenolic flavonoid phytochemical) and fucoidan (a well-known sulfated polysaccharide of brown seaweed), which aimed to overcoming the limitations of bioavailability of rutin, and showed that the rutin-fucoidan complex induced G_0/G_1 and S phase cell cycle arrest, and has the ability to induce apoptosis via reactive oxygen species generation and mitochondrial potential loss in cervical cancer cell, but was biocompatible on normal cells .Of note, Chen et al. have recently expressed concerns about the potential toxic effect of some polyphenol including apigenin against non-transformed cells when used at high concentrations, markedly higher than that assumed with diet, suggesting higher

concentrations of flavonoids could be toxic and therefore more toxicological studies should be warranted.

Anticancer activity of combination treatment of flavonoids and conventional chemotherapeutic drugs :

Combination treatments, which aims to improve overall clinical efficacy, are widely accepted as safe and effective approach in cancer therapy . Due to multidrug resistance and tumor recurrence, the development of new strategies aimed at improving chemotherapy sensitivity and minimizing the adverse side effects is still urgently needed. In this regard, flavonoids have been considered to be one of most promising candidates by virtue of its diverse biological properties such as anticancer activity . We have previously provided evidence for the potential combination of arsenite and natural product including delphinidin, one of anthocyanin compounds, against human APL cells NB4 and HL-60, in which delphinidin sensitized leukemia cells to arsenite by strengthening intrinsic/extrinsic pathway-mediated apoptosis induction, modulating the amount of intracellular glutathione and NF-κB binding activity .

We further demonstrated that the combination treatment strongly preferred to selectively enhance the cytotoxicity of arsenite against cancer cells rather than human peripheral blood mononuclear cells . In agreement with our findings, a comprehensive review paper recently summarized the detailed chemomodulating effects of flavonoids in human leukemia cells, and further demonstrated that the secondary metabolites of flavonoids can also sensitize malignant cells to conventional chemotherapeutic drugs and could be considered as potential adjunctive agents in cancer treatment . Despite a wide clinical application of ATRA and its successful clinical efficacy in the treatment of APL patient, continuous effects have been made to explore novel promising candidate aiming to improve the effectiveness of ATRA and overcome clinical problems such as resistance. In this regard, He and colleagues recently demonstrated that dihydromyricetin (DMY), one of flavonoid bioactive compound extracted from Ampelopsis grossedentata, exhibited a strong synergy with ATRA to promote NB4 cells differentiation . They further clarified that DMY sensitized the NB4 cells to ATRA-induced cell growth inhibition, CD11b expression, nitrobluetetrazolium (NBT) reduction and myeloid regulator expression, all of which seemed to be dependent on the activation of p38-STAT1 signaling pathway, providing new opportunities for the combination of DMY and ATRA as a promising approach for future differentiation therapy .

The beneficial effects of combination treatment are also observed in various types of solid tumor cancer cells. Quercetin has been demonstrated to sensitize human glioblastoma U87 and U251 cells to temozolomide, an oral alkylating chemotherapeutic agent, in vitro via inhibition of heat-shock protein 27 . In fact, flavonoids including quercetin have been clarified to be able to enter the brain to influence brain function by modulating the activity of gamma-aminobutyric acid A ($GABA_A$)-receptor and monoamine oxidase A/B . Similarly, the anticancer potential of combination of isoflavone biochanin A and temozolomide against glioblastoma U87 and T98G cells was reported to be linked to enhanced expression of p-p53, and inhibition of cell viability, expression of cell survival proteins EGFR, p-ERK, p-Akt, c-myc and membrane-type-MMP1 . The combination treatment also induced G_1 arrest, and a shift

in the metabolic phenotype from glycolytic to oxidative phosphorylation in cancer cells . Casticin has been demonstrated to potentiate TNF-related apoptosis-inducing ligand-induced apoptosis in colon cancer cells through downregulation of survival proteins such as Bcl-2, Bcl-xL, survivin, X-linked inhibitor of apoptosis protein (XIAP) and cellular FLICE-like inhibitory protein (cFLIP) and upregulation of death receptor 5 . Palko-Łabuz et al. recently demonstrated that combined use of statins, inhibitors of the key enzyme of mevalonate pathway 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, and flavonoids such as baicalein strengthened cell growth inhibition and apoptosis induction as compared to the application of statins alone to a human colorectal adenocarcinoma cell line LoVo . They further demonstrated that in doxorubicin-resistant cell line LoVo/Dx, a stronger decrease of resistance to doxorubicin was observed in the presence of statins in combination with flavones in comparison with the effect observed in the presence of statins alone .EGCG was recently suggested to serve as a novel chemo-sensitizer to enhance the sensitivity of cancer cells to 5-fluorouracil (5-FU) by inhibiting glucose-regulated protein 78 (GRP78)/NF-κB/miR-155-5p/MDR1 pathway, and the IC_{50} values of 5-FU in the presence of EGCG were approximately 8-fold and 10-fold lower in comparison to the IC_{50} values for 5-FU alone in human colon carcinoma cell line-HCT-116 and DLD1, respectively .

LITERATURE REVIEW :

1. **Box and Wilson (1951)**

George E. P. Box and K. B. Wilson first introduced Response Surface Methodology for process optimization. Their work established the foundation for statistical optimization techniques used in industrial and pharmaceutical research.

2. **E Shukla, DD Kara, T Katikala… - Drug Development and …, 2023 - Taylor & Francis**

Objective A significant problem faced by the health care industry today is that though there are numerous drugs available to tackle diseases like cancer, their intrinsic properties make it …

3. **Priya Batra and Anil K. Sharma2013**

Reported that flavonoids possess strong anticancer activity through apoptosis induction, inhibition of cell proliferation, antioxidant activity, and suppression of angiogenesis in cancer cells.

4. **Irina Cherniakov, Abraham J. Domb and Amnon Hoffman2015**

Explained that SNEDDS improve oral absorption and bioavailability of lipophilic drugs by enhancing dissolution and intestinal permeability.

5. **Maria Heleno et al.2015**

Studied phenolic antioxidants and concluded that flavonoids exhibit antiproliferative and cytotoxic effects against various cancer cell lines.

6. **Preethi G. Anantharaju et al.2016**

Reported that dietary phenolic compounds including flavonoids inhibit tumor growth by inducing apoptosis and blocking oncogenic signaling pathways.

7. **Neeraj Chouhan et al.2015**

Concluded that SEDDS and SNEDDS are promising carriers for phytoconstituents because they enhance solubility and oral bioavailability of herbal drugs.

8. **Eugenia D. Teodor et al.2020**

Reviewed flavonoids and tannins from medicinal plants and confirmed their anticancer and antitumor

potential through selective cytotoxicity against abnormal cells.

9. Dalia M. Kopustinskiene et al.2020

Explained that flavonoids act as anticancer agents by modulating ROS activity, inducing apoptosis, suppressing proliferation, and arresting cell cycle progression.

10. Aristote B. Buya, Ana Beloqui and Véronique Préat 2020

Discussed formulation development, characterization, and industrial applications of SNEDDS for poorly soluble oral drugs.

11. Bhupinder Kapoor et al.2021

Reported that plant flavonoids show anticancer effects through anti-oxidant activity, inhibition of angiogenesis, and reversal of multidrug resistance.

12. Alena Liskova et al.2021

Explained that flavonoids can sensitize cancer cells toward chemotherapy and improve therapeutic outcomes through nano-technological approaches.

13. Abhishek Singh and Fahad Khan 2021

Reviewed hesperidin flavonoid and reported significant chemotherapeutic potential against different cancer types.

14. Mohd Farhan et al.2023

Reported that flavonoids demonstrate antioxidant, anti-inflammatory, and anticancer activities with improved effectiveness when delivered using nanocarrier systems.

15. Chandramouli Manojmouli et al.2023

Studied flavonoid derivatives and found that they exhibit strong anticancer effects with fewer adverse effects compared to conventional chemotherapy.

16. Eesha Shukla et al.2023

Concluded that SNEDDS significantly improve the bioavailability and therapeutic performance of poorly soluble anticancer drugs.

17. Pratibha Pandey et al.2024

Reported that flavonoids inhibit NF-κB signaling pathways and suppress cancer progression, metastasis, and drug resistance.

18. Irina Cherniakov, Abraham J Domb and Amnon Hoffman2015

Reported that SNEDDS significantly improve oral bioavailability of lipophilic and poorly soluble drugs by enhancing dissolution and intestinal absorption.

19. Neeraj Chouhan et al.2015

Explained that SEDDS are highly useful for phytoconstituents and flavonoids because they increase aqueous solubility and oral bioavailability of plant actives.

20. Aristote B. Buya, Ana Beloqui and Véronique Préat 2020

Described formulation components, optimization methods, characterization, and industrial applications of SNEDDS for oral drug delivery.

21. Akiladevi D et al.2020

Reported that SNEDDS enhance in-vitro drug release, stability, and clinical efficacy of poorly soluble drugs after oral administration.

22. Muthadi Radhika Reddy and Kumar Shiva Gubbiyappa 2021

Reviewed supersaturable SNEDDS and concluded that they improve drug solubility, absorption, and bioavailability of anticancer drugs with poor aqueous solubility.

23. Lakshmi Devi Gottemukkula and Sunitha Sampathi 2022

Discussed SNEDDS as lipid-based nanocarriers capable of improving permeability and oral absorption of hydrophobic drugs.

24. Eesha Shukla, Divya Dhatri Kara and Mahalaxmi Rathnanand2023

Concluded that SNEDDS are promising nanoplatforms for oral delivery of anticancer drugs and significantly enhance solubility and bioavailability of BCS class II and IV drugs.

25. Rahmi Annisa et al.2023

Explained mechanisms of self-nanoemulsification and characterization methods including droplet size, zeta potential, and drug release studies.

MATERIALS AND METHODS :

- **Material**

Chemicals used in the study were purchased from Sigma-Aldrich (Munich, Germany). The solvents used were of HPLC/analytical grade purchased from Merck (Kenilworth, NJ, USA). Chlorpromazine was received from Global Pharmaceutical Pvt. Ltd. Islamabad, Pakistan.

MethodsDetermining Drug Solubility

The solubility of chlorpromazine was determined in all components used for the synthesis of SNEDDS, which include oils (captex, triacetin, linseed oil, and olive oil), surfactants (tween 85), and ethanol. The drug was taken in a stoppered glass vial of 5 mL capacity and mixed for 10 min with each component by using a vortex mixer (China). The vials were kept in an isothermal shaker (GFL1092, Burgwedel, Germany) at 50 ± 1.0 °C for 72 h until homogeneity is achieved. The homogenate was then centrifuged at 3,000 rpm for 10 min to remove the insoluble drug. The supernatants were filtered with 0.45 µ syringe filter and drug concentration was determined through HPLC (Agilent Technologies, Inc., Santa Clara, CA, USA) method reported earlier with following conditions . Separation of the drug was carried out through C_8 column (ZORBAX Eclipse XDB Agilent Technologies, Santa Clara, CA, USA) by injecting 20 µL sample, using mobile phase acetonitrile and methanol (10:90, v/v) with a flow rate of 1.0 mL/min at 35 °C. Run time was fixed at 6 min and absorbance was measured at 308 nm.

Synthesis of SNEDDS

Each formulation with a total weight of 1 g was prepared by taking 20 mg of drug in Teflon lined screw-capped glass vial and then adding various proportions of glycerides and surfactants. The drug was dissolved in components by gentle stirring and heating at 50 °C in a water bath. The mixture was cooled down to room temperature followed by addition of ethanol and stirring to achieve uniformity. Formulations were kept at ambient temperature for 48 h to achieve the equilibrium and observed for any phase separation and turbidity prior to emulsification and particle size determination. Formulations with no phase separation were selected for stability testing and further characterization.

Dispersibility and Stability Investigations

The efficiency and dispersibility of self-emulsification were determined through USP dissolution apparatus 2. Briefly, 1 mL of each formulation was added dropwise into 200 mL of simulated intestinal

fluid (pH 6.8, without enzymes), maintained at 37 °C, with gentle stirring using stainless steel paddles rotated at 60 rpm. Each formulation was assessed visually for the rate of emulsification, dispersibility, apparent physical stability, and appearance. The precipitation of the drug was evaluated after 24 h. The formulations were further categorized as stable (no precipitation), milky, dull white, whitish, or unstable (showing precipitation). The stable formulation with small particles size that passed the dispersibility test was selected for further characterization.

Drug Content Determination

The selected formulations were evaluated for drug entrapment. Extraction of the drug from SNEDDS was carried out by taking one part of each formulation and diluting it with nine parts of 100% methanol (v/v) and centrifuged at 10,000 rpm for 30 min. The supernatant obtained was then diluted with methanol (2.5 times) and drug content was determined through HPLC using the earlier reported conditions.

Thermodynamic Stability Profile

Thermodynamic stability studies were carried out for the selected formulations. Nanoemulsions were subjected to centrifugation at 18,000 rpm at 4 °C for 30 min. The stable formulations with no phase separation were further subjected to 6 heating and cooling cycles by incubating them for 48 h at 45 °C and 4 °C, respectively. The formulations that remained stable at former conditions were proceeded to 3 freeze–thaw cycles between −21 °C and 25 °C, and monitored for the time-dependent physical changes like drug precipitation. HPLC analysis for chlorpromazine was carried out to check the chemical stability of drug within SNEDDS. Moreover, selected formulations were kept for three months at 37 ± 2 °C and refrigerator (2–8 °C) to check their stability upon storage and shelf-life. The stability was measured in terms of the change in particle size, dispersibility, and transmittance.

Percentage Transmittance

The percentage transmittance of the SNEDDS gave an idea about the formulation features including uniformity and size of the droplets. Percentage transmittance was measured by taking 1 mL of each formulation and diluting it 10 times with distilled water. A UV spectrophotometer was used to measure the percentage transmittance at 308 nm by taking distilled water as a blank.

Particle Size and Zeta Potential Analysis

The particles size and zeta potential of the chlorpromazine nanoemulsions were measured through PSS NicompTM 380 DLS/ZLS devise. Furthermore, transmission electron microscopy (TEM) using (FEI Nova NanoSEM 450) was done to examine the surface morphology and particle size of selected SNEDDS formulations.

Ex Vivo Transport Studies

Ex vivo transport profile of entrapped chlorpromazine from SNEDDS was conducted in simulated gastrointestinal fluid (SIF, pH 6.8) using everted sac method [18]. The study protocol was approved by the Institutional Ethics Committee of Riphah International University, Lahore (REC/RIPS-LHR/2018-

018, dated 9 January 2019). Briefly, rats (weighing 250–300 g) were anesthetized using chloroform and abdomen was opened with the middle incision. The small intestine was detached by cutting each end. Middle region of small intestine was taken from the proximal-distal part. The entire length of small intestine was cleaned with saline solution to eradicate blood and debris. The intestine was everted by carefully passing a narrow glass rod from one end of the intestine and then gently rolling it on a glass rod. Ligatures were fixed over the condensed part of the glass rod and exert the sac by softly pushing the rod through the whole length of intestine. The rod was then detached, and the intestine was placed in SIF at room temperature. A 4 cm long piece was tied off with thread and slice an open sac from the main length. Second ligature was positioned loosely around the open end of the sac and a blunt needle was inserted attached with a syringe. The loose ligature was fastened over the needle and 2 mL of chlorpromazine formulation was injected into the sac. The intestine was then placed in 150 mL of SIF (pH 6.8) in a shaking incubator. The samples were collected from the surrounding medium at pre-defined time intervals that was replaced with the same amount of fresh solution. Samples were analyzed using HPLC and percent transport was calculated using the following equation.

Apparent Permeabilty ($[\mu g/cm]^2$) = Concentration × Volume/Mucosal area

Mucosal surface area was calculated by assuming intestine a cylinder and using the formula:Mucosal surface area cm^2 = Circumference (πr^2) × Length

In Vivo Oral Bioavailability Study

Oral bioavailability of the optimized formulations was investigated on male Sprague–Dawley rats weighing 200–250 g. In vivo studies were carried out as per guidelines of approved protocol by Institutional Ethics Committee of Riphah International University, Lahore (REC/RIPS-LHR/2018-018, dated 9 January 2019). Animals were divided into 4 groups (n = 6) and housed one day before starting experiment with free access to food and water. The nanoformulations small chain triglyceride (SCT_{15}), medium chain triglyceride (MCT_6) long chain triglyceride (LCT_{14}) and chlorpromazine suspension were given orally through gavage at a concentration of 2 mg/kg of body weight of chlorpromazine. The blood samples (200 µL) were collected from the tail vein at predetermined time points of 1, 2, 4, 8, 12, and 24 h in heparinized syringes and centrifuged at 5,000 rpm for 10 min. The plasma was separated, transferred to separate Eppendorf and stored in a freezer at −20 °C until further analysis. The drug was extracted from plasma samples through liquid-liquid extraction by adding 200 µL of chilled acetonitrile and 150 µL of methanol followed by vortex for 5 min and centrifugation at 3,000 rpm for 10 min. The supernatant of each sample was then transferred to labeled HPLC vials and run one by one on HPLC using method described above. Peak areas according to concentration were recorded and a graph between area against time was drawn to calculate plasma drug concentration for all formulations .

Pharmacokinetic Parameters and Statistical Analysis

Pharmacokinetic parameters of orally administered chlorpromazine were obtained by using a non-compartments pharmacokinetic analysis of plasma concentration-time data. PK Solver (a free Microsoft Excel Add-in) was used to calculate the area under the curve from concentration versus time curve to last measured time ($AUC_{0–24}$) and other pharmacokinetic parameters. Absolute bioavailability was calculated from absolute dose and areas under curves (AUC) for oral against intravenous administration.

Statistical Analysis

Statistical data analysis was performed using Student's t-test with $p < 0.05$ as the minimal level of significance. All values were expressed as mean ± SD. Finally, the results were compared with control and literature.

Evaluation parameters of SNEDDS :

Thermodynamic stability studies

The physical stability of a lipid –based formulation is also crucial to its performance, which can be adversely affected by precipitation of the drug in the excipient matrix. In addition, poor formulation physical stability can lead to phase separation of the excipient, affecting not only formulation performance, but visual appearance as well. In addition, incompatibilities between the formulation and the gelatin capsules shell can lead to brittleness or deformation, delayed disintegration, or incomplete release of drug.

i. **Heating cooling cycle:** Six cycles between refrigerator temperature (4°C) and 45°C with storage at each temperature of not less than 48 h is studied. Those formulations, which are stable at these temperatures, are subjected to centrifugation test.

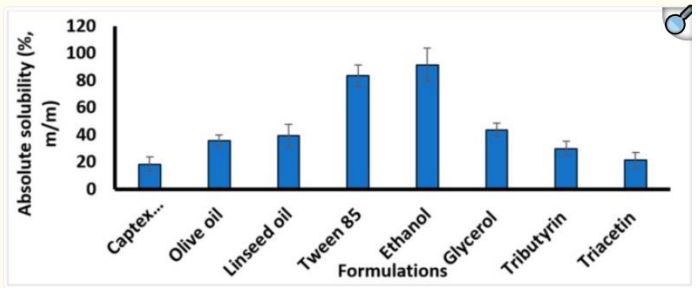
ii. **ii. Centrifugation:** Passed formulations are centrifuged thaw cycles between 21°C and +25°C with storage at each temperature for not less than 48 h is done at 3500 rpm for 30 min. Those formulations that does not show any phase separation are taken for the freeze thaw stress test.

iii. **iii. Freeze thaw cycle:** Three freeze for the formulations. Those formulations passed this test showed good stability with no phase separation, creaming, or cracking.

Result:

Drug Solubility in Nanoemulsion ComponentsThe solubility of chlorpromazine was tested in all formulation components individually. Chlorpromazine was found to be more soluble in ethanol (91%) compared to other vehicles as shown in The choice of formulation components was driven by the fact that chosen excipients must have definite regulatory status. Three different types of triglycerides, i.e., long chain, (C18 from linseed and olive oil), medium chain (mono and di-glycerides), and short chain (triacetin, a trimester of glycerol and acetic acid) were chosen. The emulsion is stabilized by the presence of surfactants and co-surfactants, so Tween 85 was added to stabilize the SNEDDS.

Figure 2.



Characterization and Evaluation of SNEDDS Formulations

Lipid-based formulations were prepared by using short chain triglycerides (SCT), medium chain triglycerides (MCT) or long-chain triglycerides (LCT). Triacetin was selected as SCT, Captex 355 was used as medium chain triglyceride and olive oil and linseed oil were used as long chain triglycerides. Fifteen different formulations of each of SCT, MCT, and LCT were prepared as SNEDDS by using different ratios of triglycerides, surfactants and co-surfactants as presented in respectively. These formulations were tested and evaluated to find optimized formulations for further characterization.

Dispersibility Test

In the formulation of SCT, MCT, and LCT SNEDDS, different concentrations of the oil phase, surfactants and co-surfactants were used, and dispersion time was found to be dependent on composition as shown in An increase in surfactant to co-surfactant ratio produced smaller particle size and reduced dispersion time. Formulation SCT_{15} (has the lowest dispersion time of 35 ± 2 s, which is due to the decreased particle size and greater emulsification ability produced by the highest proportion of surfactant (tween 85). Similar trends were observed in the dispersion time with an increase in the oil phase and surfactant/co surfactants ratio in case of MCT-SNEDDS. However, in comparison to SCT, MCT requires a greater concentration of surfactant (50% tween 85) as presented in . In the case of LCT formulation combination of linseed oil and olive oil was used. Formulations with higher contents of linseed oil provided greater solubility but the rate of emulsification was compromised due to a proportional decrease in concentration of olive oil. LCT provided optimum emulsification and dispersion time at surfactant (tween 85) concentration of 40% without the use of co-surfactant. However, a further increase in surfactant ratio resulted in precipitation as is evident from results

Stability Tests

Only those formulations that proved their thermodynamic, chemical and physical stability were selected for further studies. The results indicated that formulations SCT_{15}, MCT_6, and LCT_{14} were the most stable formulations from each group of SNEDDS. These formulations were stored for three months at 37 °C ± 2 and refrigerator for stability studies upon storage. Formulations were observed to be more stable in the refrigerator as there was no significant change observed in particle size, poly dispersity, drug loading, percentage transmittance, and dispersibility. Thus, LCT_{14}, among the three formulations, was considered to be the most stable nanoemulsion as it showed no absorbance (highest transmittance).

Zeta potential Analysis

The zeta potential analysis was carried out on the selected formulation SCT_{15}, MCT_6, and LCT_{14} and respective values are presented in The higher the zeta potential, the greater the stability because increased surface charge opposed the aggregation of particles. Zeta potential of SCT_{15}, MCT_6, and

LCT_{14} was found to be −17.1, −14.2, and −21.4, respectively.

Particle Size, Poly Disersity, and Surface Morphology

The selected formulation SCT_{15}, MCT_6, and LCT_{14} were subjected to particle size analysis and the particle size was found to be 159 ± 15, 186 ± 20, and 178 ± 16, respectively The uniformity of the synthesis of SNEDDS was displayed through the polydispersity index (PDI) value. The PDI was found to be 0.27 ± 0.43, 0.13 ± 0.67, and 0.31 ± 0.17 for SCT_{15}, MCT_6, and LCT_{14}, respectively. Transmission electron microscopy (TEM) revealed that particles were spherical shaped in case of LCT_{14} SENDDS as compared to SCT_{15} and MCT

Drug Content Determination

Selected formulations SCT_{15}, MCT_6, and LCT_{14} were evaluated by HPLC for estimation of drug content in individual formulations. The aim of this test was to evaluate the formulations for drug loading efficiency. The linearity curve of the chlorpromazine over a range of 0.0312–0.5 µg/mL is shown ina. The value of R^2 was found to be 0.998 with equation Y = 59460 x − 410.61. The chromatogram shown in b, presents the chromatogram of chlorpromazine detection in formulation with retention time at 2.296 min. Whereas, shows the chromatogram of pure chlorpromazine with retention time at 2.926 min. A decrease in drug content was observed with an increase in chain length of triglyceride, like 92.3% for SCT_{15}, 82.7% for MCT_6, and 85.5% for LCT_{14} . Thus, shorter chain triglycerides showed better encapsulation of drug as compared to LCT.

Discussion :

In the past decade, much attention has been directed to lipid-based formulations with emphasis on improving and enhancing the solubility and oral bioavailability of poorly water-soluble BCS class II drugs. Ideally, SNEDDS transport a hydrophobic drug in solubilized form and retain satisfactory solubilization through gastrointestinal passage. Moreover, SNEDDS protect drugs against enzymatic degradation, foster super saturation, surfactant-provoked membrane fluidity and permeability enhancement that is often sufficient for drug absorption .

SNEDDS composed of surfactants, co-surfactants, oil, and drug should turn into a monophasic, clear dispersion once added to the aqueous phase, at room temperature. Upon mild agitation in aqueous media SNEDDS were converted to very fine oil/water emulsion. Surfactants used in the formulation are responsible for the conversion of oil phase into the very fine particles by reducing the surface tension at the oil and water interface. The finer the droplet size of oil phase, the lesser the dispersion time of SNEDDS . As the total weight of the SNEDDS formulation was kept constant to 1 g, an increase in the concentration of triacetin produced a proportional increase in the dispersion time due to increased particle size and a simultaneous decrease in the surfactant/co-surfactant ratio .

Conclusion :

In this study, SNEDDS based on small chain triglycerides (SCT), medium chain triglycerides (MCT), and long-chain triglycerides (LCT) for oral delivery of highly lipophilic drug chlorpromazine, were successfully designed with the significantly superior features based on different component ratios. Among these, LCT_{14} showed greater potential in term of reduced particle size (178 ± 16), high drug loading (85.5%), and increased oral bioavailability. The formulation was stable over a 3-month storage

period at 25 °C and 4 °C in terms of particle size, physical appearance, and drug loading. Hence, the present approach demonstrated the substantial increase in oral bioavailability of highly lipophilic drugs through the use of SNEDDS that adopts intestinal lymphatic route along with para- and trans-cellular route.

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