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Formulation and Evaluation of Simuvastatin Loaded self nano-emulsifyng Drug Delivery System

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ABSTRACT

The chronic neurological disease known as Alzheimer's disease (AD) is characterised by cognitive decline, impaired memory, and aberrant behaviour. Simvastatin and other potential neuroprotective medications have low absorption and solubility, which restricts their application in the treatment of AD. Simvastatin is a lipophilic HMG-CoA reductase inhibitor with anti-inflammatory, anti-amyloid, and antioxidant properties. However, due to its poor aqueous solubility and rapid first-pass metabolism, it has limited clinical value. By creating and evaluating a self-nanoemulsifying drug delivery system (SNEDDS), the current study aimed to increase the oral bioavailability and solubility of simvastatin. Pre-formulation tests assessed the physicochemical properties of simvastatin and its compatibility with excipients. The solubility investigation led to the selection of Transcutol P as the oil, Tween 80 as the surfactant, and Capmul MCM as the co-surfactant. The formulation was optimised through the application of a Box-Behnken design, and pseudo-ternary phase diagrams were produced. The optimised formulation displayed thermodynamic stability, nano-sized globules (less than 100 nm), and rapid emulsification. Evaluation metrics, including droplet size, viscosity, zeta potential, and in vitro diffusion, confirmed the improved solubility and dissolution rate compared to pure simvastatin. The results of the study show that simvastatin-loaded SNEDDS effectively increase solubility, dissolution, and maybe oral bioavailability, suggesting that it has potential as a state-of-the-art nanocarrier solution for Alzheimer's disease treatment. It is possible to overcome the pharmacokinetic limitations of simvastatin and improve its therapeutic efficacy in neurodegenerative illnesses by employing this formulation approach.

1. INTRODUCTION:

Among older adults, Dementia is most commonly caused by Alzheimer's disease observed clinically. This debilitating neurologic disorder is defined by a variety of behavioural and mental symptoms, cognitive decline, trouble carrying out daily activities, and memory issues. Although many symptoms of AD are common to all people, every individual has a unique experience with the illness. At the beginning, short-term memory loss is common. As the disease worsens in the brain, the ability to perform complex tasks and basic daily tasks declines. Persistent memory loss, mood swings, social isolation, animosity, and confusion are signs of the advanced stage. Studies have connected AD is caused by abnormal build-up of β-

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amyloid plaques together with neurofibrillary tangles. Neuritic plaques are amyloid-β protein aggregates caused by the disease. When APP is improperly cleaved by enzymes like gamma secretase, a 42-amino acid peptide known as Aβ is produced. Neurofibrillary tangles and apoptotic cell death result from the accumulation and deposition of Aβ in glutamatergic neurones, which also causes oxidative stress, inflammation, and increased excitotoxicity. Neurofibrillary tangles, which are required for healthy cells' axons to grow and mature, are primarily composed of tau protein. Neuronal cell death results from tangles that form inside nerve cells of the hippocampus and medial temporal lobe due to excessive tau protein phosphorylation. As a result, neurotransmitter systems like serotonin, acetylcholine (ACh), and norepinephrine diminish. According to a study, AD patients may have neurotoxic consequences and memory impairment due to cholinergic system deficiency.[i-iii]

The factor associated with Disease Risk is **a**ge because **t**he strongest non-modifiable risk factor for AD; prevalence rises sharply after 65 years and reaches ~50% in those over 85, with aging also increasing cardiovascular risks that contribute to AD.[iv,v] **Genetics,** ApoE4 increases AD risk, while ApoE2 is protective; familial AD is rare (<10%) and linked to mutations in chromosomes 1, 14, and 21. **Education,** Lower education is associated with higher AD risk due to reduced cognitive reserve and fewer synaptic connections.[vi-viii] Early symptoms include memory loss and mild cognitive impairment (MCI), with later involvement of language, vision, judgment, and motor functions. AD is diagnosed clinically using medical history, cognitive testing, daily functioning assessment, and behavioral changes, with biomarkers under investigation for early detection.[ix-xiii]

Various Stages of AD:

Table -1.1- Various Stages of AD.

Stages	Description
Preclinical AD	Singles show signatures of AD pathology without any noticeable cognitive or systemic deterioration. The duration of this stage mainly spans from VI to X years, and the development of MCI caused by AD depends on characteristics such as age, sex, and ApoE status.
MCI due to AD	Characterized by modest cognitive impairments, such as deficiencies in short-term memory and challenges in language, executive function, and visuospatial ability. Patients have the potential to maintain a certain level of autonomy, but there is a chance that they may develop AD dementia.
AD Dementia	Severe cognitive impairments hinder everyday functioning, necessitating help with basic daily tasks. The behavioural symptoms progressively intensify, resulting in a substantial impact on both patients and carers.

Mechanisms of AD-

Alzheimer's disease (AD) has a complex and heterogeneous pathophysiology, with no single unifying theory explaining its development. AD occurs as rare familial forms (1–5%), caused by autosomal dominant mutations in APP, PS1, and PS2 genes, and common sporadic forms (>95%) influenced by genetics, environment, and comorbidities. Genome-wide association studies link AD to immune response, lipid metabolism, amyloid-β plaques, neurofibrillary tangles, and endocytosis, though many risk genes remain unidentified. Non-genetic factors such as lifestyle, psychosocial stressors, and environmental exposures further increase disease risk. AD shows wide clinical and pathological variability, including neuroinflammation, synaptic loss, neuronal degeneration, Aβ plaques, and NFTs. High clinical trial failure rates reflect this heterogeneity and limitations of conventional drug delivery systems. Modified-release drug formulations are being explored to improve therapeutic efficacy and patient outcomes.[xiv-xvi]

Innovative drug delivery systems for Alzheimer's-

The blood-brain barrier makes it difficult for traditional medication delivery techniques to transfer therapeutic substances to the brain. The goal of innovative medicine delivery systems is to overcome these obstacles. Bypassing the blood-brain barrier, Medication can be administered delivered to the brain by way of the olfactory route via techniques like nasal sprays. Focused ultrasound can induce a temporary dissolve the blood-brain barrier when used in conjunction with microbubbles, increasing the efficiency of drug delivery to the brain. By ensuring that higher dosages of medicinal medications get to the right parts of the brain, these cutting-edge delivery techniques may improve the effectiveness of Alzheimer's treatments.[xvii]

Drug Used in AD

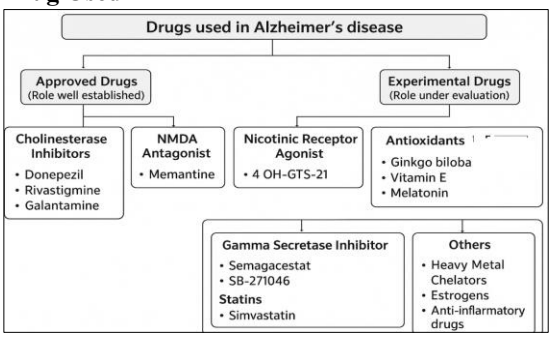

Figure-1- Drug Used in AD

Simvastatin:

Compared to hydrophilic statins, simvastatin, a lipophilic HMG-CoA reductase inhibitor, can more

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easily cross the BBB and subsequently alter central nervous system processes. Simvastatin is a statin class medicine that severely inhibits HMG-CoA reductase, a critical regulator of cholesterol production. The lipophilic statin that has the easiest affinity for the brain and spinal cord and the (BBB) is simvastatin. Beyond its lipid-lowering action, simvastatin has pleiotropic properties that include antioxidant, anti-inflammatory, and endothelium protective actions. These properties may aid in neuroprotection in AD.[xviii] Preclinical research demonstrates that simvastatin improves learning and memory in AD animal models inhibit tau hyperphosphorylation, and reduce Aβ accumulation. Epidemiological studies have also shown that long-term statin users have a lower risk of AD.[xx-xxi] However, contradictory findings from clinical trials indicate that additional study is required to identify the optimal dosage, treatment plan, and illness stage at which simvastatin may be most effective.[xix-xxv]

Pharmaceutical Challenges of SIM:
SIM is quite lipophilic and prone to stomach disintegration. Because of the substantial enzymatic breakdown and first-pass metabolism in the gastrointestinal tract, this non-biodegradable substance becomes intractable in the gastrointestinal medium following oral therapy. Consequently, oral bioavailability and overall therapeutic efficacy are reduced. SNEDDS can increase drug solubility and safeguard the gastrointestinal tract from toxicity is well known. It has been extensively studied if SNEDDS can enhance the bioavailability *regarding drugs which are not* sufficiently soluble in watery medium. In thermodynamic terms, it is stable. The therapeutic compounds' absorption is improved by the nano globules' larger surface area.[xxiii]

Nanoformulations are a method of treating AD:
Nanoformulations are the most effective way to boost the bioavailability and therapeutic efficacy of pharmaceutical compounds. Reduced particle size, enhanced systemic circulation, and the ability to lower dosage requirements are characteristics that set nano-formulations apart. Many scientists have created nanoparticles of various pharmaceutical substances and are presently examining how they might enhance the effectiveness of AD treatment. This is because these particles have special properties.[xxiv]

Pathogenesis:
An extensively researched system that is thermodynamically stable, SNEDDS maximises the bioavailability of lipophilic pharmaceuticals. The medicinal chemical's improved bioavailability is a result of the nanoscale size's enhanced surface area. For fifteen minutes, the isotropic solutions were vortexed after the amounts including he selected oil, surfactant, and co-surfactant were combined. With oral delivery, SNEDDS progresses along the digestive tract, absorptive, and circulatory phases. When the SNEDDS were digesting, they created a gritty emulsion. Enzymatic hydrolysis of the prepared coarse emulsion is currently taking place along the oil-water boundary. As a result of the interaction between fatty acids and bile, mixed micelles are formed. By passive or active diffusion across the enterocyte membrane and lymphatic circulation, colloidal micelles are absorbed as chylomicrons. After absorption, the circulatory phase begins, during which time the body releases the drugs from the surplus lipids and chylomicrons it has used. Deterioration of neurons in many areas of the brain is a hallmark of AD, a chronic illness. There are several factors linked to AD. The pathogenic elements of AD(AD) can be efficiently linked to SIM; it provides symptom relief in AD by reducing oxidative stress, neuroinflammation, accumulating amyloid β, and obesity.[xxv]

Self-nanoemulsifying Drug Delivery System (SNEDDS):
SNEDDS (Self-Nanoemulsifying Drug Delivery Systems) are lipid-based formulations composed of oils, surfactants, and co-surfactants that spontaneously form stable oil-in-water nanoemulsions in aqueous media with mild agitation. They enhance the solubility, dissolution rate, and absorption of poorly water-soluble drugs by providing a large interfacial surface area. SNEDDS improve oral bioavailability and ensure more consistent plasma drug levels, particularly for hydrophobic drugs.[xxvi-xxx] These systems are thermodynamically stable, transparent or translucent, and undergo digestive, absorptive, and circulatory phases after oral administration. A coarse emulsion was produced by the SNEDDS during the digestion stage. Bile and fatty acids interacted to produce the mixed micelles, which were subsequently hydrolysed by enzymes at the oil–water contact surface. This procedure was now applied to the created coarse emulsion.[xxxi-xxxviii] Presently, both active and passive diffusion are used to absorb the produced colloidal micelles as chylomicrons through the lymphatic system and enterocyte membrane. After absorption, the circulatory phase begins, during which the body uses chylomicrons and releases drugs from excess lipid.[xxxix-Li]

HYPOTHESIS:
The management of AD, a complex neurological disorder, is a prime illustration of the deficiencies in the medical, pharmaceutical, and healthcare sectors. AD ranks sixth among the leading causes

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of death, with approximately 55 million individuals currently experiencing the condition. Neurological diseases make up 6.3% of the worldwide burden of disease,with 30% of AD patients pass away before the age of 80. The solubility of SIM has been inadequately addressed through the investigation of a variety of formulation methods like cyclodextrin complexes, solid dispersions, nanosuspensions, bio nanocomposites, co-solvent assisted solubilization, and micro. SNEDDS, A stable isotropic formulation of oil, surfactant, co-surfactant, and drug that spontaneously produces an oil-in-water nanoemulsion with droplet sizes ranging from 20 to 200 nm upon dilution, introduction to an aqueous medium with gentle agitation. Self-nano emulsification is a spontaneous process that is facilitated by the minimal utilization of free energy. This method enhances the dissolution of medications and their bioavailability and permeability across biological membranes by increasing the interfacial area of micronized globules owing to the inclusion of lipids and surfactants. SNEDDS can provide multiple advantages, including blocking P-glycoprotein (P-gp)-mediated drug transport and enhancement of lymphatic drug transport. improved chemical and enzymatic stability, and the solubilization of drugs within the gastrointestinal lumen, thereby increasing the interfacial area for drug absorption. This investigation establishes and assesses SIM's SNEDDS.

REASON FOR SELECTION OF DRUG SIMVASTATIN:

Simvastatin was selected for the formulation about SNEDDS designed for Alzheimer's disease due to its potential neuroprotective, anti-inflammatory, and cholesterol-lowering effects that may help in reducing amyloid-β accumulation and neuronal damage. However, its therapeutic its use is restricted due to poor water solubility and reduced oral bioavailability and extensive first-pass metabolism. Being highly lipophilic, simvastatin can readily penetrate biological membranes and potentially cross the blood–brain barrier, but its dissolution in gastrointestinal fluids remains a major challenge. Formulating simvastatin into an SNEDDS can overcome these limitations by enhancing solubilization, improving absorption facilitated via lymphatic uptake, leading to lower first-pass metabolism, and achieving higher and more consistent plasma concentrations. Therefore, SNEDDS serves as an idealtransport system to enhance the drug exposure and therapeutic benefit of simvastatin in Alzheimer's disease management.[Lii-Lv]

REASON FOR SELECTION OF EXCIPIENT CAPMUL MCM:

Capmul MCM was picked for the SNEDDS formulation on account of its excellent solubilizing capacity for lipophilic drugs like simvastatin and its ability to form stable formulations with nanosized droplets. It is a medium-chain mono- and diglyceride that enhances drug loading, promotes rapid emulsification, and facilitates efficient absorption through the gastrointestinal tract. Capmul MCM also supports lymphatic transport, which helps circumvent hepatic first-pass effect and improves the oral uptake of water-insoluble drugs. Additionally, it is biocompatible, pharmaceutically accepted, and safe for oral use, making it a suitable lipid phase for SNEDDS formulations. Its good miscibility with using surfactants like Tween 80 and co-surfactants including Transcutol P ensures formation of a stable, homogeneous nanoemulsion system with improved drug solubilization and absorption efficiency.[Liv]

REASON FOR SELECTION OF EXCIPIENT TWEEN 80:

Tween 80 (Polysorbate 80) was selected for the SNEDDS formulation because it is a non-ionic surfactant with excellent emulsification and solubilization properties for lipophilic drugs like simvastatin. It has an increased hydrophilic-lipophilic ratio (HLB ≈ 15), which renders it effective in forming stable oil-in-water nanoemulsions with fine droplet sizes, thereby enhancing the surface exposed for drug absorption. Tween 80 is biocompatible, non-toxic, and pharmaceutically approved, making it safe for oral formulations. Moreover, it enhances drug permeability across biological membranes and may facilitate lymphatic uptake, augmenting pharmacokinetic availability of poorly soluble drugs. Its good miscibility with oils such as Capmul MCM and co-surfactants like Transcutol P ensures rapid self-emulsification and physical stability of the SNEDDS, resulting in consistent and efficient drug delivery.[Lv]

Simvastatin- Simvastatin is chemically known as "(1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl] ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate with molecular formula $C_{25}H_{38}O_5$. It has melting point134-138°C with 3 hours half-life.[Liv]

Capmul MCM- it is chemically known as 2,3-dihydroxypropyl octanoate. Its synonyms are Monocaprylin, Monoctanoin and molecular formula is $C_{11}H_{22}O_4$ with melting 60-65 °C and boiling point176-177 °C is Emulsifier and Stabilizer agent.

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Tween 80- It is a Non-ionic surfactant, Emulsifying agent, Solubilizer with molecular formula $C_{64}H_{124}O_{26}$ and Synonyms are Polysorbate 80, Polyoxyethylene (20) sorbitan monooleate. It is highly hydrophilic and Yellow to amber, oily liquid with a characteristic odour.[Li]

METHODS:

Pre-formulation Studies-

The first and most important stage of drug development is pre-formulation studies, which are carried out to assess the drug substance's physicochemical characteristics and possible interactions with different excipients prior to formulation design. The scientific basis for creating a stable, secure, and efficient dosage form with the best possible bioavailability and therapeutic efficacy is provided by these studies. Pre-formulation's primary goal is to produce data that will help determine the drug's compatibility with excipients and provide insight into its solubility, stability, partition coefficient, polymorphic behaviour, pKa, hygroscopicity, and dissolving properties. Such information is essential for choosing the best manufacturing procedures, dosage forms, and formulation strategies. Potential formulation issues that can be resolved early in the development phase, such as poor solubility, instability, or incompatibility, are also identified with the aid of pre-formulation. In doing so, it guarantees consistent product quality, improves formulation resilience, and shortens the time needed for product development.

Drug Characterization

The physical constitution, melting point, and λmax of SIM were determined through characterization.

Physical description- A glass slide was coated with approximately 1 gram of the substance. Personal inspection was employed to determine the drug's colour.

Determination of Melting Point- Thermal analysis by DSC and capillary fusion methods were employed to determine the drug's melting point.

Determination of ultraviolet absorption maxima (λ_{max})- A UV spectrophotometer was employed to determine the absorption maxima within the 200-800 nm range of a drug sample (SIM) in methanol at a dosage of 10µg/mL, with methanol serving as the baseline.

Linearity and range- To develop the calibration curve, the average peak area of six samples was graphed against SIM concentrations, and the regression line was derived.

Precision- The average response was reported after the preparation and injection of all three quality control standards (QCS) solutions, namely LQC, MQC, and HQC, six times. Equation 1 (143) is used to determine the percentage of accuracy.

Accuracy- This was determined by the established procedure's intermediate precision and repeatability. Six injections were administered on the same day and under identical chromatographic conditions to assess the standard concentration's repeatability. Three distinct analysts (inter-analyst) executed the identical technique within the same experimental environment to detect the intermediate procedure. The percentage %RSD was calculated from the averaged data.

%Relative standard deviation = Standard deviation of peak area ∗ 100 - Eq.2
Peak area on average

Robustness- The impact of minor modifications in chromatographic conditions on the established method was evaluated by varying the HPLC flow rates and mobile phase proportions (0.8-1.2 mL/min), (88:12, 90:10, 92:08), and λ max (236 nm, 238 nm, 240 nm) for six consecutive injections of MQC samples into the HPLC. The medication's peak area, retention duration, and percentage recovery were recorded (146).

Solubility Investigation- Solubility screening was done to select suitable **oil, surfactant, and co-surfactant** for designing the **SIM-SNEDDS**. Simvastatin solubility was evaluated in various excipients to achieve optimal drug loading and effective self-nano-emulsification. The study assessed different **oils** (castor oil, olive oil, eucalyptus oil, almond oil, cottonseed oil, Lauro-glycol FCC, and C-MCM), **surfactants** (Tween 20, Tween 80, Polyoxamer 407, Polyoxamer 188, and Labrafil M 1944 CS), and **co-surfactants** (TP). In a 5 mL glass vial, SIM (10 milligrams) was combined with one millilitre each of the oil, surfactant, and co-surfactant, which was mixed thoroughly and vortexed for 2-3 minutes. Furthermore, all vials were sealed and kept in a shaking water bath at 37 ± 0.2°C and 50 R.P.M. for 48 hours. The supernatant was dissolved with methanol as the solvent, and the drug level was determined by introducing the samples into an RP-HPLC system. The response was measured at 238 nm.

Preparation of SNEDDS

CMCM, T-80, and TP were optimized as a lubricant, assigned as the surfactant and co-surfactant, respectively in the preparation of

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SNEDDS, following the analysis of solvent compatibility data. The concentrations of oil and Smix (surfactants and co-surfactants) were adjusted to produce a total of 27 prototype formulations. By altering the ratio between 1:1, 1:2, and 2:1, Smix can be achieved. Table 1 illustrates the composition of the SNEDDS prototypes. The SNEDDS pre-concentrate (1 mL) for all volumes was developed by vortexing the oil, surfactants, and co-surfactants for 15 minutes using a Cyclo Mixer (REMI, India). Additionally, SIM (10 mg) was introduced to each pre-concentrate that had been prepared and vortexed for 15 minutes. By diluting the pre-concentrate with 500 mL of distilled water, it was transformed into an emulsion. The system was maintained at 37 ± 0.2 °C for 5–7 min while the mixture was agitated at 500 rpm.

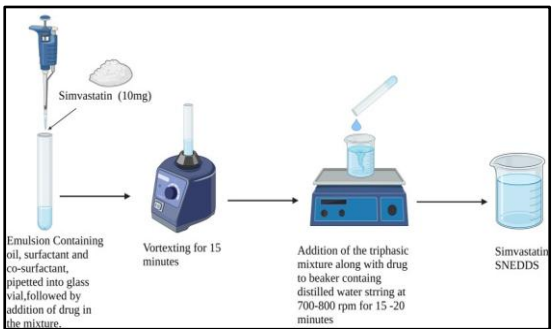

Figure-4- illustrates the method of preparing SIM-SNEDDS.

The development of a pseudo-ternary phase diagram (TPD)

The solubility data evaluation yielded the following: CMCM, T-80, and TP were identified as the used as oil, surfactant, and co-surfactant, correspondingly for the preparation of SNEDDS. The levels of oil and Smix were adjusted toto develop a total of twenty-seven SNEDDS formulations. The ratios of Smix compositions are 1:1, 1:2, and 2:1. SNEDDS were the classification for all stable emulsions that exhibited a clear and transparent appearance, while SMEDDS were the designation for stable but translucent emulsions. A simulated pseudo TPD was produced using the Triplot program version 4.1.2. The vertices of triangles are used to represent the formulation components, which include oil, surfactant, and co-surfactant.

Table:4.1. shows the composition of SNEDDS for the ternary phase investigation. Formulation Smix (1:1)

S. No.	Formulation Code	Capmul MCM (µL)	Tween 80 (µL)	Transcutol P (µL)
1.	F1	100	450	450
2.	F2	200	400	400
3.	F3	300	350	350
4.	F4	400	300	300
5.	F5	500	250	250
6.	F6	600	200	200
7.	F7	700	150	150
8.	F8	800	100	100
9.	F9	900	50	50

Table:4.2. shows the composition of SNEDDS for the ternary phase investigation. Formulation Smix (2:1)

S. No.	Formulation Code	Capmul MCM (µL)	Tween 80 (µL)	Transcutol P (µL)
1.	F10	100	600	300
2.	F11	200	530	270
3.	F12	300	470	230
4.	F13	400	400	200
5.	F14	500	330	170
6.	F15	600	270	130
7.	F16	700	200	100
8.	F17	800	130	70
9.	F18	900	70	30

Table:4.3. shows the composition of SNEDDS for the ternary phase investigation. Formulation Smix (1:2) Capmul MCM, Tween 80, and Transcutol P

S. No.	Formulation Code	Capmul MCM (µL)	Tween 80 (µL)	Transcutol P (µL)
1.	F19	100	300	600
2.	F20	200	270	530
3.	F21	300	230	470
4.	F22	400	200	400
5.	F23	500	170	330
6.	F24	600	130	270
7.	F25	700	100	200
8.	F26	800	70	130
9.	F27	900	30	70

Optimization of the formulation through the design of experiments (DOE):

A Box Behnken design was employed to optimize the SNEDDS, using oil, surfactant, and co-surfactant evaluated investigated at three coded levels: high (+1), medium (0), and low (–1), which were designated as independent variables. PDI, ZP (mA), and GS (nm) were assessed as dependent variables. The degree of relevance was evaluated after the model was calibrated to align with the design model. The impact of varying concentrations of independent factors (Factors) on the SNEDDS formulations was evaluated using the perturbation plots and 3D surface responses that were generated. The ideal region of the complete model was delineated by the resultant overlay plot, which was accomplished through graphical optimization. The polynomial equation that was identified served to examine the effect of independent variables on the responses.

The table displays the compositions of SNEDDS that are calculated using BBD.

Table:4.4. Optimization of the formulation through the design of experiments (DOE).

	Factor 1	Factor 2	Factor 3
Run	A: Oil	B: Surfactant	C: Co-Surfactant
	(µL)	(µL)	(µL)
1	150	530	300
2	100	565	270
3	200	530	285

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4	200	600	285
5	150	565	285
6	200	565	270
7	100	600	285
8	150	600	270
9	100	565	300
10	150	600	300
11	200	565	300
12	100	530	285
13	150	530	270

Assessment of optimized SNEDDS-Thermodynamics, centrifugation, and cloud point determination

The enhanced SIM-SNEDDS were tested under stress conditions through freeze-thaw (-21°C and +25°C) cycles and heating-cooling (4°C and 40°C) cycles in order to evaluate thermodynamic stability. The cloud point temperature was assessed by placing 10 mL of diluted formulations in a heated water bath while continuously recording the temperature. The onset of cloudiness was then documented. The cloud point is the temperature at which a clear and translucent SNEDDS became turbid became turbid was recorded. The centrifugation of 1 mL of the attenuated formulation at 10,000 rpm for 20 minutes resulted in the visible detection of phase separation.

pH and dilution effects

The study examined the effects of pH and dilute volume changes on the mean droplet size, drug precipitation, and phase separation of SIM-SNEDDS formulations. The effects of dilution and pH variation were revealed by the solubility of SIM-SNEDDS in varying quantities of water (250, 500, and 900 mL) and pH levels (1.2, 6.8, and 7.4).

Drug Incorporation of Simvastatin in SNEDDS

1 mL of the optimized SNEDDS formulation was used to vortex the SIM-infused SNEDDS for 15 minutes. Using a magnetic agitator, 500 mL of double-distilled water was combined with this at 500 r.p.m. The water bath was maintained at a temperature of 37±2ºC. The undissolved SIM was extracted from the 5 mL sample following centrifugation at 11,200 g for 15 min. Distilled water was utilized to produce the necessary dilutions, which were defined by the area of the diluted solution.

RP-HPLC was employed to analyze the samples at a wavelength of 238 nm. The percentage of medication intake was quantified using the formula from Equation 5. Samples were analyzed using RP-HPLC at a wavelength of 238 nm. The percentage of medication loading was quantified using the formula.

%Drug Loading = concentration of the formulation concentration of the standard ∗ 100 – Eq. Five

The optimized formulation's zeta potential (ZP) and globule size (GS) are determined.

The Malvern ZetaSizer Nano ZS-90 was employed to evaluate the zeta potential (ZP) and global stability (GS) of SNEDDS. Samples in polystyrene cuvettes were exposed to 50 mV laser light at a 90° angle, after being passed through a 0.2 µm syringe filter was used to filter the distilled water (100 mL) that was diluted with the optimum formulation combination (0.1 mL). Extracted from the attenuated sample, one milliliter was examined in the sample cell. The operation was performed at a temperature of 25° C (63° F).

DSC analysis

The Perkin Elmer DSC apparatus was employed to evaluate the Differential Scanning Calorimetry (DSC) of unadulterated SIM, C-MCM, T-80, TP, and SIM-SNEDDS. A pulverized sample (2-3 mg) was placed in an Al3+ pan and heated from 30 to 4400°C at a rate of 100°C/min, while a dry nitrogen flush was applied at a flow rate of 19.8 mL/min.

Scanning electron microscopy (SEM)

In order Examine morphological, optimized SNEDDS, Scanning Electron Microscopy (SEM) was implemented. The material undergoes electron scanning in a zigzag pattern. This is accomplished by applying a thin layer of gold palladium (1.5 - 3.0 nanometer). The FE-SEM is utilized to detect extremely fine topographical details on the surfaces of entire or aliquoted materials.

High-Resolution Transmission Electron Microscope (HR-TEM)

SNEDDS formulation's surface architecture was evaluated with HR-TEM. 1 mL of the pre-concentrate SNEDDS was combined with water until the preferred droplet size was achieved in the nano-emulsion. The created nano-emulsion was applied in a single drop to a metal plate, and surplus formulation was blotted off with filter paper. The HR-TEM picture was taken after the squares had dried.

In-vitro dissolution investigations

A quantity equivalent to 10 mg of SIM was employed for the analysis, and the dissolution apparatus (USP type II) was employed. A subsequent naïve and optimized batch of SIM-SNEDDS was incorporated into this medium and subjected to dissolution testing. While the mixture was stirred at 50 rpm, with the dissolution medium kept at 37 ± 0.5 °C. As per the conventional method, 0.1N HCl (pH 1.2) and 900 mL of 6.8 phosphate buffer were also utilised. The solution was subjected to filtration through a 0.2 µm membrane before analysis. five millilitre aliquots

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that were collected at 5, 10, 15, 20, 30, 45, and 60 minutes. The release characteristics of SIM (101) were evaluated by analyzing the supernatant using HPLC at 238 nm after the filtered solution was centrifuged for 15 minutes at 9864g.

Stability analysis

Over the course of three months, the SIM-SNEDDS were housed in a stability testing chamber at three different temperatures: Stability studies were conducted at three storage conditions: 5 ± 3 °C, 25 ± 0.2 °C/65 ± 5% RH, and 40 ± 0.2 °C/75 ± 5% RH. At several time points, samples were analysed for droplet/particle size, polydispersity index, and assay, and compared to newly generated samples. We examined the dissolution of freshly made SIM-SNEDDS with samples aged three months at 40±0.2°C and 75±5% relative humidity.

RESULTS AND DISCUSSIONS:

API Characterization

The physical description of SIM- The Physical properties of powdered Simvastatin were observed. The test substance's sensory qualities are consistent with the provided standard data.

Table 5.1. represents the various physical properties of the Drug.

S. No.	Physical Properties	Observation
1.	Appearance	Crystaline Powder
2.	Colour	Off White
3.	Odour	Odourless
4.	Taste	Bitter

Determination of the melting point-

Capillary fusion method- Table 5.2 displays the M.P. value that was determined using capillary fusion methods.

Table 5.2 illustrates the M.P. of SIM as determined by the capillary method. Drug Reported value Observed value Reference.

Table: 5.2. Capillary fusion method

S. No.	Observed Melting Point	Average Melting Point	Standard Melting Point
1.	147°C	143°C	138-141°C
2.	143°C		
3.	141°C		

Investigation of Solubility-

The sol, SIM in a variety of lubricants, surfactants. & co-surfactants were determined and reported. The drug exhibited a maximum solubility of 42.78±0.05% in CMCM oil, 43.96±0.15% in TP co-surfactant, and 42.63±0.25% in T-80 surfactant as shown in figure-5.1 & 5.2, table- 5.3 & 5.4.

Table: 5.3. Oil solubility

S. No.	Solvents	Percentages of solubility of each ingredient
1.	Capmul MCM	42.78±0.05
2.	Lauroglycol FCC	39.04±0.15
3.	Cotton Seed	19.26±0.36
4.	Eucalyptus oil	18.56±0.55
5.	Olive oil	17.52±0.42
6.	Labrafac PG	16.35±0.36
7.	Captex 300	15.45±0.19
8.	Castor oil	14.95±0.21
9.	Peanut oil	12.85±0.29

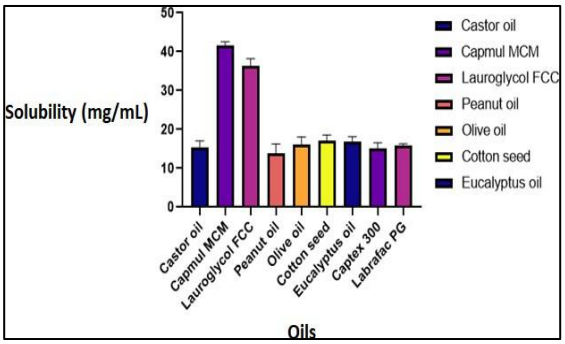

Figure- 5.1-Oil solubility

Table- 5.4- Solubility in surfactant &co-surfactant

S. No.	Solvents	Percentages of solubility of each ingredient
1.	Transcutol P	43.96±0.15
2.	Tween 80	41.26±0.18
3.	Labrasol	41.26±0.18
4.	Tween 20	37.65±0.05
5.	Polyoxamer 407	19.65±0.09
6.	Polyoxamer 188	18.56±0.18
7.	Labrafil M	16.35±0.21

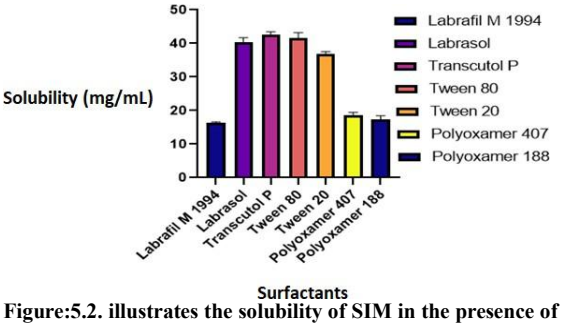

Figure:5.2. illustrates the solubility of SIM in the presence of various surfactants and co-surfactants.

Capmul MCM (42.78%), Lauroglycol FCC (39.04%), Transcutol P (43.96%), and Tween 80 (42.63%) were found to have the highest solubility of SIM.

The construction of the TPD-

"A total of 27 prototype formulation were developed by adjusting the concentrations of oil and Smix (surfactants and co-surfactants)." Vary the ratios of 1:1, 1:2, and 2:1 to adjust the mixture. Figure 5.3 illustrates the ternary phase of these formulations.

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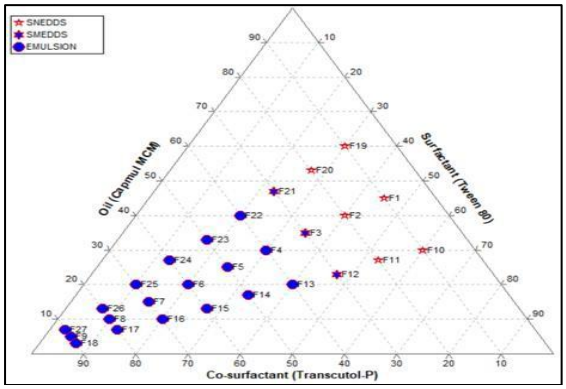

Figure-5.3- illustrates a ternary phase diagram.

The transparency of the formulation was reduced as a result of increasing the oil ratio, as indicated by the TPD. Increasing in particulate size. The emulsion's lipid content was elevated result of a larger oil phase, but because of an increase in interfacial tension at the oil/water contact, the surfactant's concentration was not high enough to guarantee a uniform dispersion of nano-sized particles. In contrast, the oil/water interface's interfacial tension dropped when the surfactant conc. Increased oil conc. Reduced. This suggests that the nanoparticles are uniformly dispersed, which leads to transparency and clarity. Figure (designated as SNEDDS) illustrates that "F1 to F20" were transparent and clear emulsions, as indicated by the results. The 27 SNEDDS formulations were subjected to an additional evaluation.

Drug compatibility study-

The figure displays the infrared spectrum of a pure Simvastatin sample, Capmul MCM, and Tween 80 obtained using an FTIR spectrometer. This spectrum was compared to the typical Simvastatin sample. Capmul MCM, Tween 80 functional group frequencies are displayed in the table.

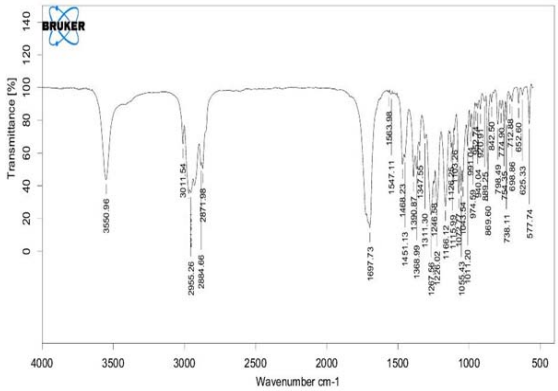

Figure-5.4. FTIR Spectra of Simvastatin

Table-5.5. Represents the FTIR Spectra of Simvastatin

S. No.	Functional Group	Range (cm^{-1})	Observed (cm^{-1})
1	O–H (alcohol)	3200–3600	3550.96
2	C–H (alkane)	2850–2960	2955.26, 2924.86
3	C=O (lactone/ester)	1735–1750	1697.73
4	C=C (aromatic)	1450–1600	1515.13, 1457.11
5	C–O (ester/ether)	1000–1300	1279.82, 1238.20, 1172.52
6	C–H bending (CH_2/CH_3)	1340–1470	1366.79, 1374.26
7	C–H wagging (alkane/aromatic)	650–900	879.60, 825.33, 677.14

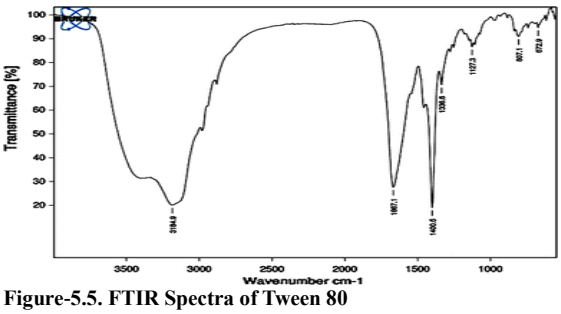

Figure-5.5. FTIR Spectra of Tween 80

Table-5.6. Represents the FTIR Spectra of Tween 80

S. No.	Functional Group	Range (cm^{-1})	Observed (cm^{-1})
1	O–H (alcohol, hydrogen bonding)	3200–3600	~3419
2	C–H (alkane, sp^3)	2850–2960	~2924
3	C=O (ester carbonyl)	1735–1750	~1697
4	C–O (ester, ether)	1000–1300	1238, 1172, 1107
5	C=C (aromatic)	1450–1600	1460
6	C–H bending (alkane)	1350–1470	1382
7	C–H wagging (out-of-plane)	650–900	672

Thermostability investigation-

SIM-SNEDDS exhibited commendable stability. No phase separation was observed during centrifugation, the cycle of heating and cooling, or freeze-thaw cycle testing, according results. Particle diameters under 100 nm a transmittance percentage of 100%, and a PDI of 0.20-0.40 were all indicated by SIM-SNEDDS results. Among the 20 SNEDDS prototypes that were evaluated (centrifugation), Formulation F11 demonstrated the lowest GS and optimal stability against thermodynamic and kinetic stress.

Optimization of SNEDDS-

A total of thirteen experiments were conducted to assess the effect of ingredients on the SIM-SNEDDS. The responses from all BBD trials are

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summarized in detailed. Evaluate the design's appropriateness (i.e., p>0.05), analysis of variance was implemented. GS, ZP, and PDI were substantially influenced by each variable, as indicated by the derived polynomial equation for the design. A linear equation was constructed for PDI and GS.

Quadratic equation for ZP. The Design-Expert software has identified the definitive mathematical model regarding encoded factors, which is represented by Equations 13, 14, and 15.

Particle size = +68.04 + 47.16 * A + 4.81 * B + 15.38 * C – Eq. (13)

PDI = +0.4069 + 0.0125 * A – 0.1388 * B + 0.0013 * C – Eq. (14)

Zetapotential = -13.40-1.05*A+0.7625*B-1.79*C+0.6750*AB-1.97*AC-3.50*BC-0.3250*A2-5.10*B2+0.0500*C2 –Eq.(15)

The aforementioned equation indicates a synergistic effect when a positive sign is present, negative sign indicates antagonistic responses of components on the specified response. The GS of SNEDDS increased in proportion to the increases in factors A, B, and C, as indicated by Equation 13. An increase in oil concentration (factor A) and co-surfactant concentration (factor C) led to a higher PDI of the SNEDDS, as indicated by Equation 14. Conversely, an increase in surfactant concentration (factor B) had the opposite effect. Factors affecting the zeta potential of SNEDDS included the oil and co-surfactant concentrations, which had an antagonistic effect, and by the concentration of surfactant, which exhibited a synergistic effect (Eq. 15). Multiple contour graphs were generated for various independent variables using these equations, which illustrated that each variable had a substantial impact on the development of SNEDDS.

Table-5.5- illustrates the optimisation of SNEDDS through the use of BBD.

Table 24: Optimization of SNEDDS using BBD

"Run	"Factor 1 A: Oil (µL)	Factor 2 B: Surfactant (µL)	Factor 3 C: Co-Surfactant (µL)	Response 1 Size (nm)	Response 2 PDI	Response 3" ZETA (mAU)"
1	150	530	300	76.60	0.54	-17.20
2	100	565	270	14.48	0.22	-12.10
3	200	530	285	129.00	0.41	-20.90
4	200	600	285	176.50	0.24	-18.20
5	150	565	285	54.03	0.4	-13.40
6	200	565	270	78.58	0.54	-10.90
7	100	600	285	12.81	0.29	-18.10
8	150	600	270	53.09	0.26	-12.70
9	100	565	300	40.99	0.53	-12.50
10	150	600	300	72.19	0.24	-22.50
11	200	565	300	105.70	0.43	-19.20
12	100	530	285	44.26	0.48	-18.10
13	150	530	270	26.26	0.71	-21.40"

Table -5.7- Provides a summary of the ANOVA results for the BBD samples.

Independent variables	Regression coefficient	P value	
R^2		Fvalue	
Globules Size	0.7502	9.01	0.0045
PDI	0.5661	3.91	0.0484
Zeta Potential	0.9886	28.99	0.0092

Factors A, B, and C significantly affected GS, as shown by the perturbation plot and 3D plot (Figure 5.6A) for GS. On the other hand, the 3D plot and perturbation plot showed that independent factor B had a greater impact than factors A and B. As the surfactant concentration rose, this suggested that the formulation's factor D dropped. Both the 3Daimention graphs and the perturbation plot showed that Factor B had a dominant effect on the ZP of the formulation. This demonstrated that ZP shifted more toward the positive side as the concentration of surfactant (factor B) increased.

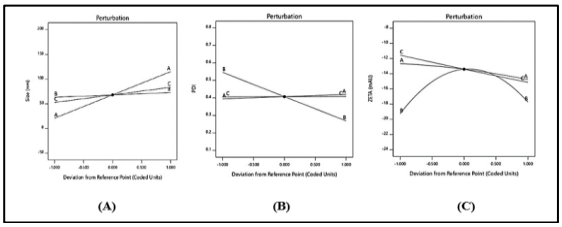

Figure-5.6- illustrates the perturbation plots for GS (A), PDI (B), and ZP (C)

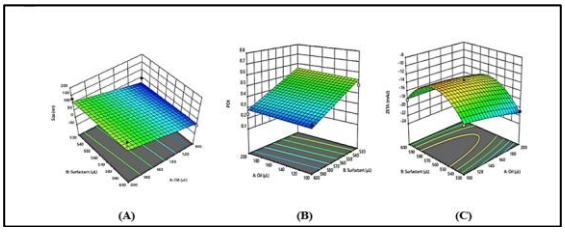

Figure-5.7- illustrates the 3D plots for PDI (B), ZP (C), and GS (A).

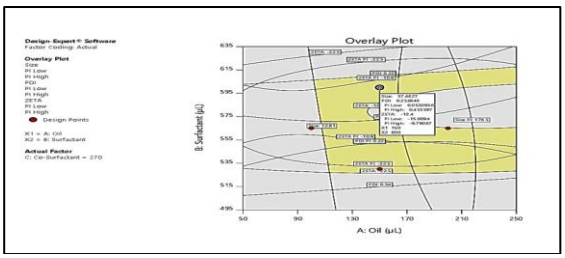

Figure-5.8- illustrates the optimized composition of SNEDDS through an overlay plot.

Characterization and preparation of optimized SNEDDS-

The DoE study's results indicate that the optimal formulation for SNEDDS is Capmul MCM (150µL), Tweens 80 (600µL), and Transcutol P (270µL). The mixture was sufficiently vortexed, and SIM (10 mg) was incorporated to produce liquid SNEDDS of SIM. These SNEDDS were subsequently evaluated for a variety of parameters.

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Stability of centrifugation and thermodynamics-
The optimized formulation was analyzed following centrifugation, and no precipitation or phase separation was observed during the thermodynamic stability testing (heating, chilling, and freeze-thaw cycles) when the temperature was adjusted. The enhanced formulation's cloud point was determined to be 95 ± 0.36 °C. After seven days, the diluted SNEDDS showed no indications that drugs had precipitated.

Investigation of pH change and dilution effect-
Mild agitation during dilution creates a fine oil-in-water emulsion, as liquid SNEDDS are pre-concentrated mixtures. L-SNEDDS undergo infinite dilution in the gastrointestinal tract and transition from an acidic to a basic pH environment upon oral administration. The GS must maintain a degree of stability in the face of fluctuations in the pH or volume of the GIT medium. Additionally, they must be stable within the organism. To assess the effects of dilution and pH, optimised SNEDDS were diluted in a variety of buffers and water at pH levels of 1.2, 6.8, and 7.4. Throughout a variety of pH levels and volumes, during the dilution process, neither phase separation nor drug precipitation was seen. The size of droplets (Figure **5.9**) varied in accordance with the pH and dilution volume. Eighty

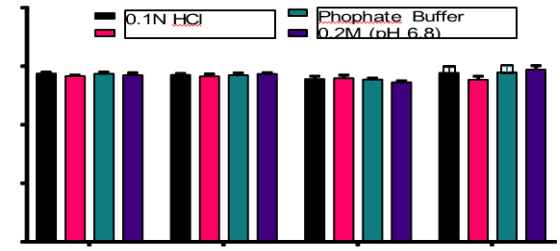

Figure-5.9- Analyze the pH change and dilution effect (n = 3)

Zeta potential & size of emulsion globules
Optimized formulation's mean GS, PDI, and ZP were determined to be 57.46± 2.65 nm, 0.253± 0.005 and -13.6 ± 4.1 mV, respectively (Figure 5.10).

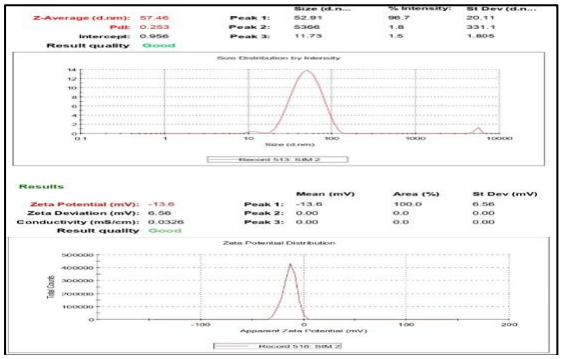

Figure- 5.10- illustrates the GS, PDI, and ZP of SIM-SNEDDS that have been optimised.

Field Emission Scanning Electron Microscopy (FESEM)-
The SIM-SNEDDS were round in form, as demonstrated by the FESEM image. The shape of individual particles with irregular surfaces was plainly revealed in the FESEM image, Figure **5.11**

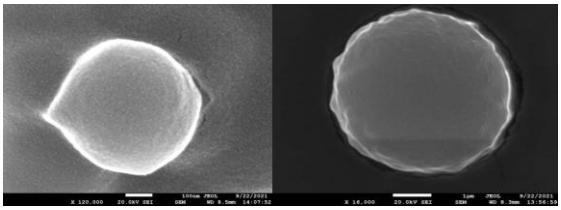

Figure -5.11- illustrates an SEM image of SIM-SNEDDS.

TEM analysis
Nanometer-sized spherical and un-agglomerated globules were noticed TEM picture. The diameter globule was assessed in this image. The globules were uniformly sized and spherical, as illustrated in Figure 5.12 No aggregation of globules was observed. Additionally, the GS obtained by TEM indicated that the GS was less than 100 nm. This was the result of a dynamic scattering experiment. Indicating that SNEDDS that were developed were genuinely nanometer-sized.

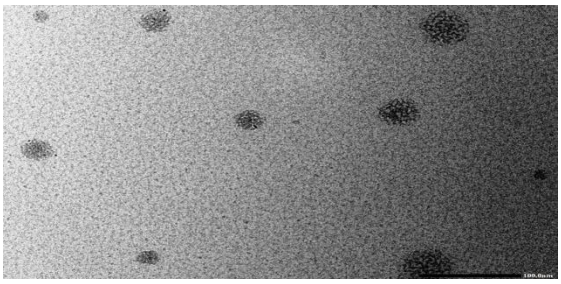

Figure 5.12 Presents the TEM micrograph of SIM-SNEDDS nanoparticles

DSC analysis
The crystallinity of pure SIM was indicated by the endothermic DSC peak at 138°C. The halo pattern of the remaining excipients, which included CMCM, T-80, and TP, demonstrated that they did not exhibit any distinct crystalline peaks.

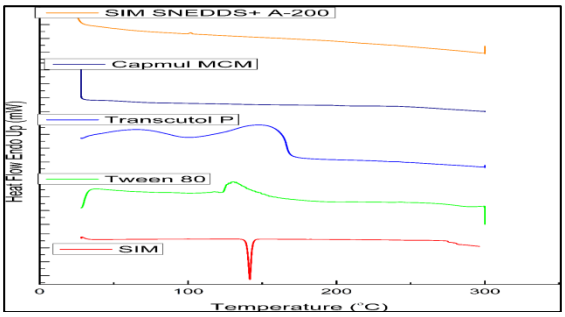

Figure: 5.13. illustrates the DSC thermograms of TP, T-80, Pure SIM, and SIM-SNEDDS.

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This demonstrates the excipients' amorphous properties. The final formulation did not exhibit any abrupt peaks or peaks at the medication's melting point, which indicates the absence of precipitation and demonstrates outstanding drug loading, as illustrated in Figure 5.13.

Stability Study of SIM-SNEDDS-

Table: 5.8. Stability Study of SIM-SNEDDS

S. No.	Storage Condition	Time Point	Droplet Size (nm)	PDI	Drug Assay (%)
1	5°C ± 3°C	0 month	68.4 ± 1.8	0.192	99.5 ± 0.4
		1 month	68.7 ± 2.0	0.194	99.1 ± 0.6
		3 months	69.2 ± 2.1	0.196	98.9 ± 0.5
2	25°C ± 0.2°C / 65% ± 5% RH	0 month	68.4 ± 1.8	0.192	99.5 ± 0.4
		1 month	69.3 ± 1.9	0.198	98.7 ± 0.7
		3 months	70.1 ± 2.0	0.201	98.1 ± 0.8
3	40°C ± 0.2°C / 75% ± 5% RH (stress)	0 month	68.4 ± 1.8	0.192	99.5 ± 0.4
		1 month	71.5 ± 2.4	0.215	97.8 ± 0.9
		3 months	74.8 ± 2.7	0.229	96.2 ± 1.1
10	Freshly prepared (comparison)	0 month	68.4 ± 1.8	0.192	99.5 ± 0.4

CONCLUSION:

Alzheimer's disease (AD) is a severe neurological disorder, affecting about 55 million people globally and causing significant mortality and disease burden. Current AD treatments highlight major shortcomings in medical and pharmaceutical research. Drug repurposing offers a low-risk, cost-effective strategy to identify new therapeutic uses for existing drugs. Several drugs have been successfully repurposed, demonstrating the feasibility of this approach. Simvastatin (SIM), originally used to treat hyperlipidemia, shows potential for repurposing in AD therapy. SIM inhibits HMG-CoA reductase, reducing cholesterol levels and influencing neuroprotective pathways. Its clinical utility is limited by poor solubility, rapid metabolism, and low oral bioavailability. Self-nanoemulsifying drug delivery systems (SNEDDS) enhance solubility, absorption, and bioavailability of lipophilic drugs like SIM. Optimized SIM-SNEDDS showed high drug loading, nanoscale droplet size, and favorable stability characteristics. SIM-SNEDDS demonstrated anti-inflammatory, antioxidant, and neuroprotective effects, suggesting promise for slowing AD progression and improving cognition.

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