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Chrysin-Loaded Snedds: A Novel Approach To Boost Bioavailability Performance

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Abstract:
Chrysin, a natural flavonoid found in propolis, honey, and some plants, has potential uses in medicine because to its anti-inflammatory, neuroprotective, antioxidant, anti-cancer, and anxiolytic characteristics. However, its poor solubility and bioavailability pose issues that necessitate effective delivery strategies. Drug delivery systems (DDS) including liposomes, transdermal patches, and nanoparticles may boost bioavailability and limit unwanted effects. SNEDDS, a mixture of oils, surfactants, and co-surfactants, form nano-sized emulsions in the gastrointestinal system to increase medicine absorption. This research investigates the development of SNEDDS for chrysin, concentrating on formulation optimization, in vitro drug release assessment, and solubility augmentation. The best excipients were identified using pseudo-ternary phase diagrams, Tween 80, and PEG 400, and solubility tests were undertaken. Four formulations (F1–F4) were produced and tested using a 22-factorial design. Zeta potential studies proved the stability of the nanoemulsions, and Fourier Transform Infrared (FT-IR) analysis indicated no chemical interaction between the excipients and the chrysin. The revised chrysin SNEDDS formulation, F4, displayed better drug release, bioavailability, and stability, making it a feasible choice for future investigation and improvement in oral drug delivery systems. SNEDDS offer a realistic technique for overcoming chrysin's solubility and bioavailability difficulties, with F4 formulation showing the greatest potential for medicinal application.

Keywords: Chrysin, Development, Formulation, stability, bioavailability**,** SNEDDS

Introduction

Chrysin, a flavonoid compound derived primarily from plants, has garnered significant attention in recent years due to its diverse pharmacological properties. This natural compound, found in various botanical sources such as honey, propolis, and specific plant species, has exhibited potential therapeutic benefits in a range of conditions[1]. Chrysin's structural similarity to the hormone estrogen has led to investigations into its potential role in modulating estrogenic pathways[2]. Its antioxidant and anti-inflammatory activities have made it a subject of interest for researchers exploring its therapeutic applications in conditions associated with oxidative stress and inflammation[3]. This article provides the overview of chrysin, including its chemical structure, sources, pharmacological properties, and potential therapeutic applications and development Process of Self-Nanoemulsifying Drug Delivery Systems. SNEDDS are a potential method to improve the bioavailability of medicines that are not well soluble in water[4]. The systems consist of isotropic combinations of oil, surfactant, cosurfactant, and medicine. When diluted with aqueous medium, these systems spontaneously generate oil-in-water (O/W) nanoemulsions[5]. The generation of droplets at the nano-scale greatly enhances the pace at which hydrophobic medicines dissolve and are absorbed, therefore resulting in improved therapeutic effectiveness[6]. The constituents of SNEDDS are essential in determining their self-emulsification dynamics. Oils provide a hydrophobic milieu for the active ingredient, while surfactants and cosurfactants decrease the tension at the interface and promote the creation of stable nanoemulsions. Proper selection of suitable components is crucial to provide the best possible solubilisation and bioavailability of drugs[7]. Many studies have shown that Solid Nitrogen-Enhanced Drug Delivery Systems (SNEDDS) are successful in enhancing the oral absorption of many weakly water-soluble medications, such as anti-cancer medicines, anti-inflammatory pharmaceuticals, and lipid-soluble vitamins. Solid-state nanoemulsion drug delivery systems (SNEDDS) have also been investigated for additional uses, including topical and parenteral administration[8].

Biological difficulties to the administration of oral drug delivery systems: Given its simple administration, lack of discomfort, cheap cost, broad medication absorption/distribution, and high patient adherence, the oral route is the most often used method by patients. Nevertheless, the effectiveness of some oral medications is nevertheless constrained by several physiological challenges, leading to poor permeability and drug breakdown. Factors affecting the structure, biochemistry, and physiology of the gastrointestinal tract (GIT) summarise the constraints of oral medication administration[9].

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Anatomical factor: At anatomical level, the gastrointestinal tract (GIT) comprises the mouth cavity, oesophagus, stomach, small intestine, and colon. Each of these components has distinct characteristics that influence the administration of drugs[10]. Distinct anatomical features of the gastrointestinal tract (GIT) have various impacts on the absorption of drugs. The oral cavity is enveloped by oral mucosa, which provides a gentle microenvironment, convenient accessibility, uninterrupted circulation, excellent permeability, and efficient medication absorption[9,11]. medicines may be digested by the gut microbiota which impacts the release properties of pharmaceuticals. Targeting medications to the colon is of major relevance for treating bowel diseases with fewer side effects and lower drug dose. However, the intrinsic variability of the stomach emptying time and microbiota in different persons remains a fundamental barrier for colon targeting[12]
However, the restricted surface of oral cavity, saliva, and enzymatic makeup are the main obstacles to drug administration in mouth[9].

Due to the limited permeability and short residence duration of medicines, the esophagus is not a prominent target for drug delivery[13,14].

The stomach has a high acid environment with a pH range of 1.0–2.5, which can break down food, ectogenic infections[15], and acid-labile medications[16], which makes it the hardest barrier to drug absorption. In addition, the stomach has extrinsic epithelial cells[17] and a mucin–bicarbonate barrier[18]. The tight connections underneath the intrinsic barrier further impede the medication absorption. Moreover, pepsins in the stomach might contribute to the inactivation of protein medicines.
The small intestine has a vast surface area owing to the villi and microvilli in the intestinal lumen[19,20]. The small intestine is considered as a good location for oral drug administration owing to the vast surface and various transport channels. The gut mucosa may identify and convey ectogenic antigens to the immune system[21,22]. However, there are still certain obstacles of small intestine medication administration deriving from its special physiology. The harsh stomach chemical microenvironment, pancreatic enzymes, bile salts, and the mucosal layer limit the medication bioavailability. Drug delivery methods that can extend their retention duration at villi and microvilli, improve lipid solubility, and interact with particular receptor or carrier are able to boost their overall bioavailability.

ThecolonexhibitsahigherpHenvironmentandmuchlongerresidencetimecompared with the upper GIT, and the enzyme activity in colon is rather low[23,24].

Biochemical factor: Different pH environments and digestive enzymes were regarded as the main bio chemical barriers for oral drug delivery systems. The pH varies distinctly in different parts of the GIT, it rises gradually from the stomach to the colon in the range from 1 to 8 [25,26]. The variation from acidic to alkaline environment affects the drugs' activities and bioavailability. pH variation not only affects drug delivery, but also a route for targeting the design of oral drugs. The existence of various enzymes will critically influence the bioavailability of drugs in the GIT, especially for protein drugs. There are over 400 different species of aerobic and anaerobic microorganisms in the colon; they can produce hydrolytic and reductive metabolizing enzymes, which can catalyze the metabolism of xenobiotics and other biomolecules. Polysaccharides can only be metabolized in the colon by anaerobic bacteria and be stable in the stomach and intestine, making it possible for colon-targeted drug delivery. Since drugs are also susceptible to colonic enzymes and generate biotransformation, the "prodrug" approach is often used for the colon-specific drug delivery[23].

Physiology Factors: The GIT exerts a low permeability to the bloodstream and extraneous substances, which restricts the bioavailability and absorption of drugs. The physiological barriers mainly consist of epithelium cellular barrier and the mucus barrier. The gastrointestinal epithelium is a phospholipid bilayer membrane, which allows the penetration and absorption of lipophilic macromolecules[27], while it is a primary absorp tion barrier for hydrophilicity and macromolecules[28]. The existence of tight junctions between adjacent cells also limits the paracellular pathway for hydrophilic drug[29]. Mucus is a dynamic semipermeable barrier, which restricts the direct interaction of drugs with epithelial cells[16]. Mucus is a viscous gel formed by mucins and glycoproteins; it can serve as a lubricant for ingested food and also a strong barrier to entrap foreign particles and eliminate potentially harmful compounds and bacteria[30-34]. Secreted mucins are linked together through disulfide bonds to form highly glycosylated macromolecules, which makes the mucin complex more stable and protects them from enzymatic degradation[18]. The mucus structure and intermolecular interactions dictate the permeation of peptides, large molecules, and microorganisms through the mucus layer[35,36].

Application of oral Drug delivery system: Oral drug delivery systems have profoundly revolutionised the pharmaceutical sector, giving various benefits over conventional drug administration approaches. These devices offer regulated delivery of drugs, targeting particular locations inside the gastrointestinal tract, and boosting patient compliance. They are especially advantageous for medications with limited bioavailability, small therapeutic windows, or those prone to degradation in the hostile gut environment[37].

One of the primary benefits of oral drug delivery systems is their capacity to offer continuous release of drugs, lowering dose frequency and boosting patient comfort. By releasing medications gradually over time, these systems maintain therapeutic drug levels for prolonged durations, boosting effectiveness and minimising the need for frequent administration. Additionally, oral medication delivery systems may be tailored to target particular parts of the gastrointestinal tract, such as the colon or small intestine, by the introduction of targeting agents[38]. This tailored distribution enhances medicine effectiveness and decreases negative effects.

Moreover, these systems may shield active substances from degradation by stomach acid or enzymes, preserving their stability and bioavailability. By protecting medications from the hostile environment of the stomach, oral drug delivery devices boost their effectiveness and minimise the need for greater dosages. Furthermore, accessible dose forms, such as once-daily or weekly formulations, might promote patient adherence to treatment regimens, leading to improved results[39]. Oral drug delivery systems may be utilised to administer a broad variety of bioactive chemicals, including proteins, peptides, and nucleic acids, for diverse therapeutic uses. This adaptability makes them essential instruments for tackling a varied variety of medical issues[40].

Methods and Material:

Drug & Excipient used: Chrysin dosage recommendations vary depending on a number of variables, including age, health, and other illnesses. According to FDA Pharmacokinetics research, 0.5 to 3 g of chrysin taken daily is deemed safe.

Research on pharmaceuticals revealed that chrysin is significantly bio transformed in the body, resulting in the formation of conjugated metabolites chrysin-7-glucuronide and chrysin-7-sulfate, and that its oral bioavailability is very low (<1%) due to its limited water solubility. Urine and faeces quickly contain the unaltered chrysin.Capryol 90 functions as an oil phase component and solubilizing agent. It is purchased from Central Drug House in New Delhi. Cremophor EL operates as a surfactant and is also offered by Central Drug House, New Delhi. Transcutol P functions as a co-solvent and co-surfactant, and is bought from Central Drug House. Labrasol is a surfactant and co-emulsifier, produced by Central Drug House. Miglyol 812 is an oil phase component and is acquired from Sigma-Aldrich. Tween 80 is a surfactant and is also offered by Sigma-Aldrich. PEG 400 serves as a co-solvent and co-surfactant and is purchased from Merck. Oleic Acid functions as an oil phase component and penetration enhancer, provided by Sigma-Aldrich.

Sefsol 218 is an oil phase component and is acquired from Central Drug House. Lecithin is an emulsifier and is also offered by Central Drug House.

4.3.1. Solubility studies: The most essential criteria for the analysis of components when it comes to microemulsion is the solubility of weak medications in oils, creams and co-solvents. The solubility of Chrysin in various ointments was evaluated by adding the quantity of the medication in 2 ml of the recommended ointments and creams and supplements in 5 ml ampoules and combined using a vortex mixer. The vials holding the samples were held at a temperature of 25±10°C for 48 hours in an ultrasonic device to attain equilibrium. The prepared samples were removed from the shaker and chilled at 5000 rpm for 15 minutes. The supernatant was collected and filtered using a 0.45 µm membrane filter. The content of Chrysin in the samples was evaluated using an ultraviolet (UV) spectrophotometer by measuring the absorbance of the samples at a wavelength of 313 nm[41].

Building of pseudo-ternary phase diagrams: Using the water titration technique, a pseudo-ternary phase diagram was produced to calculate the concentration range of components for the present range of microemulsions. Using oil, surfactant, and co-surfactant with different surfactant proportions—co-surfactant, or S/Co (1:4, 1:3, 1:2 1:1, and 4:1 w/w)—ternary plots were constructed. In a pre-weighed test container, S-mix and oil were blended in the following ratios: 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. Water was added to the oil and surfactant and co-surfactant solutions at appropriate weight ratios while whirling them gently. The mixtures were visually examined and categorised as coarse or microemulsions after they had stabilised. Using Triplot V4.1 software, the gathered data was utilised to construct ternary charts (Todd Thompson)[42].

Formulation and Optimization of Chrysin SNEDDS Using a 2^2 Full Factorial Design Approach: It is ideal to create a pharmaceutical formulation that is acceptable in the shortest amount of time while using the fewest man-hours and raw resources. Pharmaceutical formulations are typically generated by adjusting one variable at a time. The process is labour-intensive and demands a great deal of creativity. Furthermore, since the combined impacts of independent variables are not taken into account, it might be challenging to create the perfect formulation using this traditional method. Therefore, it is crucial to use well-established statistical methods like factorial design to comprehend the complexity of pharmaceutical formulations. The factorial design methodology, in addition to the art of formulation, is a useful way to show the relative importance of many factors and their interactions.

Formulations for SNEDDS were created by taking into account the microemulsion regions and the maximum amount of medication that may dissolve in a given ratio of surfactant to co-surfactant with oil that fulfils the parameters for microemulsion creation after dispersion in aqueous medium. chrysin and the chosen excipients that were found employing solubility research screening and pseudo-ternary phase diagram plotting made up the developed formulation. The phase diagrams allowed for the determination of the appropriate oil and S/CoS ratios. chrysin was dissolved in S/CoS mixes, gently vortexed, heated to ≤90°C, and oil was added to produce SNEDDS formulations. Different batches containing chrysin and varied quantities of oil and S/CoS were created using 2^2 factorial designs in order to investigate the impact of the formulation variables. Formulations were kept for future research at room temperature in a desiccator. The chrysin was dissolved in a solution of surfactant and co-surfactant at 50°C in a water bath to create the formulation. After that, oil was added. Using a cyclomixer, this mixture was combined until a translucent preparation was achieved. Hard gelatine capsules were filled with the produced chrysin SNEDDS[43].

Characterization of SNEDDS: Substance level of drugs 100 mg of chrysin of a self-emulsifying drug delivery system formulation were taken and dissolved in a tiny quantity of methanol. 0.1 N HCl (1 mg/ml) was used to get the volume up to 100 ml. 0.2 ml (200 µg/ml) of the solution above was collected, and it was diluted with 20 µg/ml of methanol to yield 10 ml. A UV-visible spectrophotometer was used to measure the absorbance at 313 nm after the samples were produced in triplicate. 0.1 N HCl served as the standard [44].

Compatibility study of pure drug and with excipients: A suitable design and formulation of the dosage form involves considerations of the physical, chemical and biological aspects of both medication and excipients utilised in the creation of the product. Compatibility must be developed between the active component and other excipients to provide a stable, effective, attractive and safe product. If the excipients(s,) are new and if no previous literature addressing the employment of those exact excipients with an active component is available, then compatibility studies are of significant value. Infrared (IR) is associated to covalent bonding, the spectra supplied considerable information about molecule structure. Hence, before designing the genuine formulation, compatibility of chrysin with different polymers and other excipients were examined using the Fourier transform infrared (FT-IR) spectroscopy technique.

Fourier transforms infrared spectroscopy is a significant analytical approach employed to examine the chemical interaction between medication and other excipients contained in the formulations. Drug and the intended excipients interaction were evaluated using FT-IR. The appropriate samples were crushed and tightly combined with dry powdered potassium bromide. The powdered mixture was collected in a diffuse reflectance sampler, and the spectra was produced by scanning in the wavelength region of 4000-400 cm−1 in FT-IR spectrophotometer[45].

Determination of droplet size and zeta potential: Zeta potential measurement was used to ascertain the charge of the droplets. Zeta potential aids in forecasting the stability and flocculation impact in emulsion systems. At some point, the zeta potential will drop below which the colloidal will coalesce because of attraction forces. A Zeta-sizer ZS 90 (Malvern Instruments, UK) was used to measure the droplet size and zeta potential of the resulting emulsion. At a 90° angle, light scattering was seen at 25°C[46].

In vitro diffusion study: Using the dialysis method, an in vitro diffusion investigation of the chrysin SNEDDS was carried out. We utilised 0.1N HCl as the dialysis medium. The experimental formulation sample was inserted in the dialysis tubing (Dialysis membrane 70, MWCO 12,000-14,000; pore size: 2.4 nm) after one end of the tubing was clamped. A magnetic stirrer (Remi Instrument Ltd., Mumbai, India) was used to stir the 900 ml of dialysing media at 37°C while the other end of the tube was fastened with dialysis closure clips. Five millilitre aliquots were taken out at 30-minute intervals and further diluted as needed. Every time, a new dialysing media was added to the aliquot volume. These samples were examined using a UV-visible spectrophotometer set at 313 nm to determine if chrysin was present in the dialysing media at the appropriate time[47].

Measurement of self-emulsification duration: The self-emulsification time of SNEDDS was estimated according to USP XXIII, dissolving apparatus type II. Each formulation added drop-wise to 900 ml of 0.1N HCl at 37°C. Gentle agitation was delivered by a standard stainless steel dissolving paddle at fifty rotations per minute. Emulsification time was determined visually[48].

Rheological characteristics determination: The SNEDDS systems were encapsulated in firm gelatin capsules in the current investigation. So, it may be readily pourable into capsules, and such systems should not be too thick. Viscosity studies are important for SNEDDS to characterise the system physically and to manage its stability. The rheological properties (viscosity, flow) of the microemulsion are determined by use of Brookfield viscometer (Japan) DV-E use of spindle RV-6 at 100 rpm at 25°C ± 0.5°C. This viscosities decision indicates whether the system is w/o or o/w. If the system has low viscosity, then, it is o/w type of the system and if a high viscosity, then it is w/o type of the system[49].

Thermodynamic stability studies: The physical stability of the formulation is critical to its efficacy since drug precipitation in an excipient matrix might have a negative impact. Phase separation of the excipients due to inadequate physical stability of the formulation may impact both the medicinal effectiveness and bioavailability. Moreover, the formulation's incompatibilities with the capsule's gelatin shell may result in brittleness, softness, delayed drug decomposition, or partial release. For these investigations, the cycles listed below are completed[50].

Cycle of heating and cooling: There are six cooling and heating cycles, with exposure times of no less than 48 hours at both refrigerated (4°C) and high (45°C) temperatures. The centrifugation test is thereafter used to those formulations that show stability.

Centrifugation: Formulations that successfully complete the heating-cooling cycle are spun for 30 minutes at 3500 rpm. For the freeze-thaw stress test, formulations without any phase separation are used.
Stress cycle of freeze-thaw: Three cycles of chilling and defrost between 21°C and 25°C, with storage at each temperature for a minimum of 48 hours. The formulations that pass this test indicate great stability, displaying no evidence of flaking, creaming, or phase separation. The formulations that pass this test are subsequently put to a dispersibility test to assess how effectively they self-emulsify.

In vitro dissolution technique: Using a USP type II dissolving apparatus and 900 cc of pH 1.2 buffer solution of phosphate at 100 rpm while maintaining a 37°C ± 0.5°C temperature, quantitative in vitro dissolution studies are done to measure drug release from oil phase into aqueous phase. At regular intervals, 5 ml aliquots of the samples were taken out, and the volume taken out was replaced with fresh medium. After that, samples were analysed using a UV spectrophotometer calibrated to 313 nm[51].

Results

Screening of oils and surfactants: The Tables 1 revealed the solubility results of chrysin inappropriate vehicles. Oleic acid, tween 80, and PEG 400 were selected as the oil, surfactant, and co-surfactant, respectively, based on the solubility data.

Table 1 Screening of Surfactants and oils

Co-Surfactant	Solubility mg/ml (X±SD)
Coconut oil	0.71±0.03
Arachis oil	1.81±0.05
Castor oil	5.41±0.06
Olive oil	0.61±0.07
oleic acid	15.81±0.08
Tween 20	5.33±0.01
Tween 80	9.63±0.04
PEG 200	2.07±1.11
PEG 400	5.98±1.92
PG	5.547±0.04

Plot of pseudo ternary phase diagrams: To determine the existence of a microemulsion zone, phase diagrams of the systems with oleic acid as the oil phase, Tween 80 as the surfactant, and PEG 400 as the co-surfactant were constructed at the surfactant/co-surfactant (S/CoS) ratio of 1:4, 1:3, 1:2, 1:1, and 4:1 (w/w), respectively. These diagrams are given in Figures. Comparing the ensuing microemulsion zones at S/CoS ratios of 1:1 [Figure 1] to all other ternary plots, the phase research indicated that they were low. The potential to create microemulsion is controlled by the co-surfactant, as demonstrated by the continuous growth in microemulsion areas at S/CoS ratios of 1:2, 1:3, and 1:4, which increase as co-surfactant concentration rises. When compared to the other ternary plots, the ratio 4:1 of S/CoS displayed the biggest microemulsion area, demonstrating that the highest microemulsion regions are formed by increasing surfactant concentration. It implies that the surfactant concentration greatly impacts SNEDDS's potential to create microemulsion areas.

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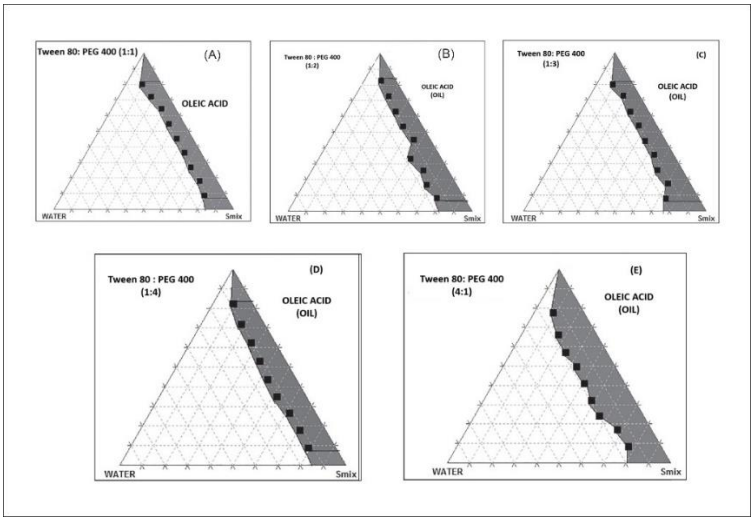

Figure 1 Pseudo ternary phase diagrams

When compared to all other ratios, the surfactant: co-surfactant ratio of 4:1 displayed the biggest microemulsion area, according to the testing results. Therefore, based on the potential to generate microemulsion zones, a S/Co-S ratio of 4:1 was selected for the formation of SNEDDS.

Formulation and optimization of chrysin SNEDDS by using 2^2 full factorial design: From the examination of pseudo ternary phase diagrams, it was noticed that the Surfactant: Co-surfactant ratio of 4:1 was exposing vast micro emulsion zones. And it was selected for formulation of SNEDDS. In the 4:1 (S:Cs), we have 9 unique ratios of Smix : Oil, that is, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9. In the aforementioned ratios, the top two greatest water consumption ratios are picked and are employed for the formulation and optimization of chrysin SNEDDS by employing 22 factorial design. The outcomes of Formulation and Optimization of chrysin SNEDDS by using 22 full factorial design were provided in Table 2

Table 2 Composition of formulation

Component	F1	F2	F3	F4
Chrysin (mg)	100	100	100	100
S_{mix}	47.2	52	47.2	52
Oleic acid	24	32.3	32.3	24

Characterization of self-emulsifying drug delivery systems: UV-visible spectrophotometer was used for chrysin-SNEDDS analysis. On 313 nm, a linear calibration curve was obtained with a calibration coefficient (R2) of 0.999 in the 2-10 μg/mL range. The results of the analysis are shown in table 3

Table 3 Characterization of self-emulsifying

Component	Solubility mg/ml (X±SD)
F1	96.3±1.73
F2	97.1±1.24
F3	97.9±1.91
F4	98.7±0.08

Fourier transform infrared studies: To evaluate the drug interacts with other excipients in the formulation, we are running competitive research on FT-IR analysis using pure drug and the formulation SNEDDS. The figures show the spectra for the pure medication and SNEDDS preparation. The vibration measured at 758.787 cm−1 is indicative of the C–H bending of the aromatic group. Alcohols show C=O stretching, which is defined by the vibration created at 1289 cm−1. The vibration detected at 1461 cm−1 is indicative of the C=C stretching of the aromatic group. The peaks detected at 1585 and 1643 cm−1 are suggestive of N=N and C=N stretching, respectively. The signal found at 3184 cm−1 is suggestive of the C–H alkene group of the molecule. On comparing the SNEDDS optimum formulation spectrum to the pure drug spectrum, no interaction was identified between the excipients and chrysin.

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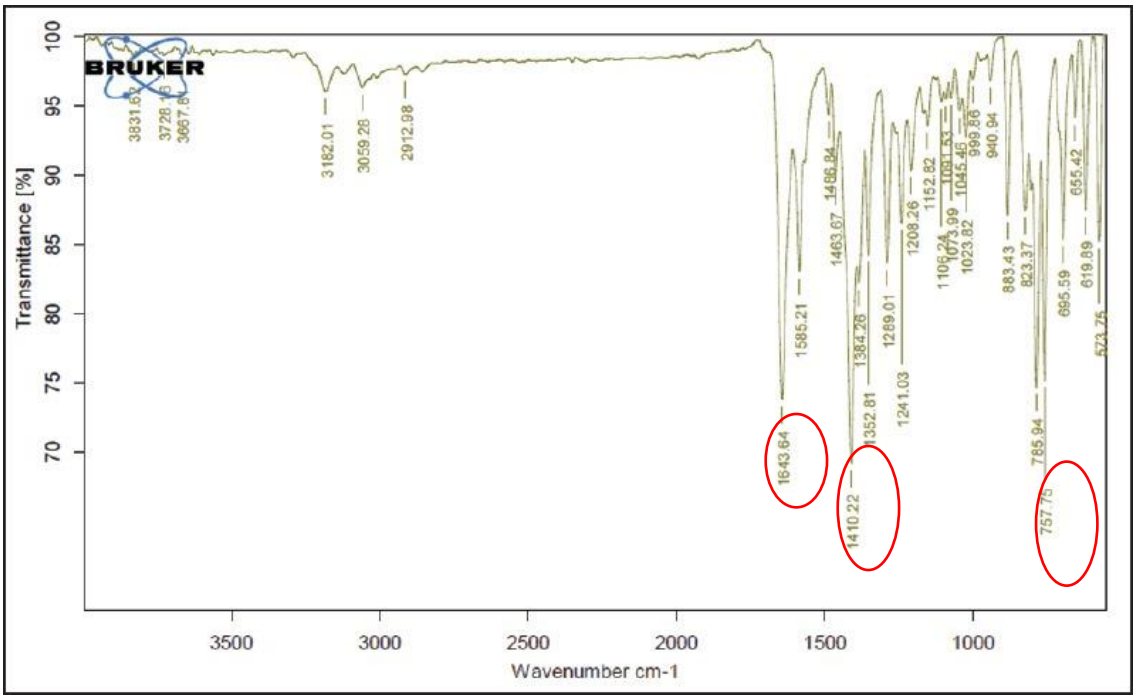

Figure 2 Fourier transform infrared graph of Chrysin pure form

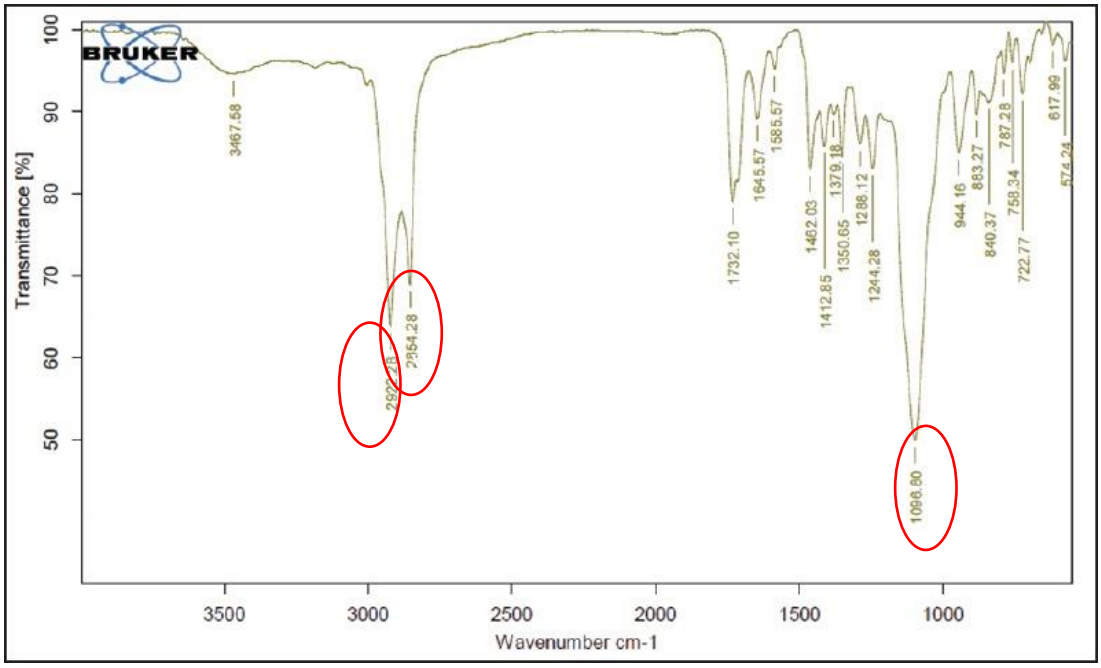

Figure 2 FTIR graph of chrysin SNEDDS

Zeta potential and droplet size determination: The measurement was utilised to establish the charge of the droplets. Using a Zeta-sizer ZS 90 (Malvern Instruments, UK), the droplet size and zeta potential of the resultant emulsion were measured. At 25°C, light scattering was noticed at a 90° angle. Table 4 revealed the SNEDDS formulation results. Based on the information acquired from Table 4, it was found that the F4 formulation outperformed all other formulations, demonstrating a droplet size of 220 nm and a zeta potential of -78.2 mV.

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Table 4 Zeta potential of SNEDDS

Component	Droplet Size (nm)	Polydispersibility Index	Zeta potential
F1	1578	1.00	-39.5
F2	1012	0.87	-50.4
F3	546	0.59	-65.4
F4	220	0.38	-78.2

In vitro Diffusion investigation

The table presents the release characteristics of four formulations (F1, F2, F3, F4) during a period of 6.5 hours, shown by the proportion of drug released at various time intervals. At the start of the experiment, there was no observable release of the drug in any of the formulations. F2 had the largest drug release (49.42%) during the first hour, followed by F4, F1, and F3. During the time period of 1 to 3 hours, the release rates of all formulations showed a consistent rise. Among them, F2 had the highest release rate, while F1 and F3 had slower release rates compared to both F2 and F4. At the 3-hour point, F2 had the greatest release rate (70.60%), whereas F4 and F1 displayed comparable patterns but with significantly lower percentages. After a duration of 4.5 hours, formulation F4 outperformed all other formulations by achieving a release rate of 88.25%, while formulas F2 and F1 exhibited somewhat lower release rates. After 6.5 hours, all formulations achieved their maximal release, with F4 exhibiting the greatest release percentage (96.89%), followed by F1, F3, and F2. Overall, F2 and F4 exhibited faster release patterns, with F4 achieving the greatest release % at the conclusion of the research. F1 and F3 had a more progressive pattern of release, with F1 reaching similar levels of release as F4 after 6 hours. All formulations exhibited a consistent pattern of escalating release over time, although with different rates of release.

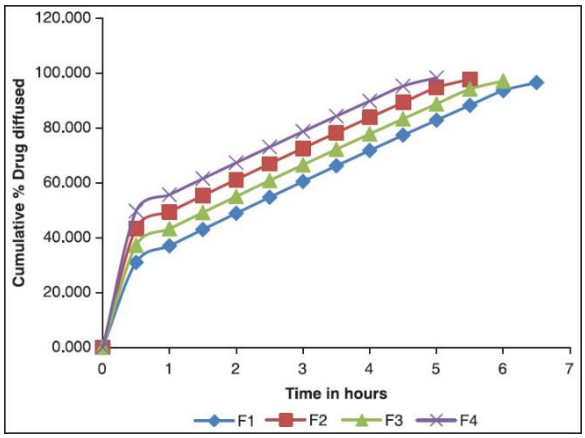

Figure 3 Comparative in vitro diffusion formulations in 0.1N HCl

Table 5 Comparative in vitro diffusion formulations in 0.1N HCl

Time (h)	F1	F2	F3	F4
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.5	31.95±0.23	42.45±0.87	29.25±1.56	31.87±1.45
1	37.99±0.12	49.42±0.47	34.69±0.79	38.43±0.56
1.5	42.96±0.25	55.32±0.65	40.13±0.23	45.99±0.39
2	48.87±0.05	61.15±0.72	45.57±0.19	51.55±0.41
2.5	54.71±0.27	66.92±0.28	51.01±0.03	58.61±0.37
3	60.43±0.33	70.60±0.54	56.45±0.13	65.27±0.34
3.5	66.15±0.59	72.15±0.60	61.89±0.12	71.93±0.31
4	70.77±0.30	77.77±0.31	67.33±0.25	75.59±0.28
4.5	74.15±0.60	82.33±0.22	72.77±0.38	88.25±0.25
5	78.77±0.31	84.81±0.53	78.21±0.51	90.91±0.22

5.5	83.15±0.61	85.23±0.24	83.65±0.63	93.57±0.19
6	87.77±0.32	89.57±0.03	89.09±0.76	94.23±0.16
6.5	96.15±0.62	95.52±0.39	94.53±0.89	96.89±0.13

The correlation (R2) values of first order release kinetics were found to be greater than those of zero order release kinetics in Table 5, indicating that all formulations followed first order kinetics. The slope of the first order linear plot seen in was used to get the first order rate constant (K).

Table 6 In Vitro diffusion kinetics of chrysin SNEDDS formulations in 0.1N HCl

Dissolution Medium	Formulation	Correlation co-efficient		K (min^{-1})	T50 (min)	T90 (min)
		Zero order	First order			
0.1N HCL	F1	0.7419	0.9267	0.0419	10.45	39.34
	F2	0.7056	0.9123	0.1119	11.45	40.56
	F3	0.8693	0.9493	0.0293	13.61	45.53
	F4	0.6673	0.8633	0.0593	9.77	37.56

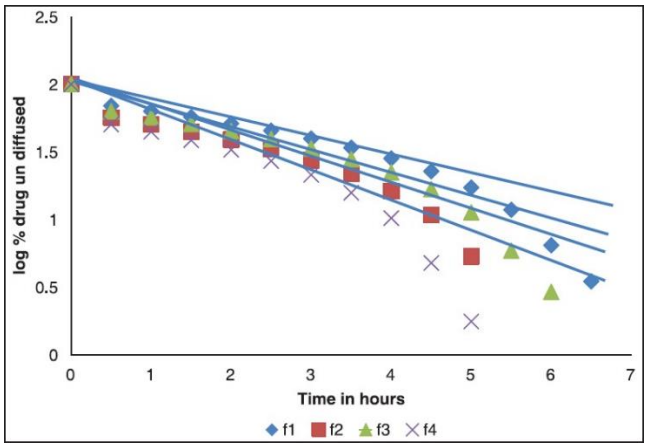

Figure 4 Zero and First order plot of diffusion data of all formulations

Important information on the kinetic behaviour of the four distinct formulations (F1–F4) was obtained from their dissolving research in a 0.1N HCl medium. The rate constant (K), time for 50% drug dissolution (T50), time for 90% drug dissolution (T90), and correlation coefficients for both zero-order and first-order kinetics were assessed. Formulation F1, which has a rate constant of 0.0419 min^{-1}, a T50 of 10.45 min, and a T90 of 39.34 min, correlated better with first-order kinetics (0.9267) than zero-order (0.7419). Similarly, Formulation F2, with a rate constant of 0.1119 min^{-1}, a T50 of 11.45 min, and a T90 of 40.56 min, showed a larger first-order correlation (0.9123) than zero-order (0.7056). Formulation F3, with a rate constant of 0.0293 min^{-1}, a longer T50 o13.61 min, and a T90 of 45.53 min, showed the greatest correlation values for both zero-order (0.8693) and first-order (0.9493).

With a rate constant of 0.0593 min^{-1}, a T50 of 9.77 min, and a T90 of 37.56 min, Formulation F4 exhibited the lowest correlation coefficients for both kinetics (0.6673 for zero-order and 0.8633 for first-order). All formulations follow first-order kinetics more closely than zero-order kinetics, according to the data overall, with Formulation F3 exhibiting the slowest dissolving time of the group.

As a result, it was discovered that the drug release from the chrysin SNEDDS F4 formulation was noticeably faster and more concentrated than that of the other SNEDDS formulations. One theory is that the SNEDDS F4 formulation caused a tiny droplet size microemulsion to spontaneously develop, allowing for a higher rate of drug release into the aqueous phase. Therefore, stronger and faster absorption as well as oral bioavailability may result from the SNEDDS F4 formulation's increased availability of dissolved chrysin.

Determination of self-emulsification time

An essential metric for evaluating the effectiveness of emulsion formation is the emulsification time. When SNEDDS are diluted in aqueous solution with little disturbance, they should spread out quickly and fully. Table 7 provided the emulsification time for each formulation.

Table 7 All formulations self-emulsification times

Formulation	Self-emulsification time
F1	131.87±0.56
F2	79.45±0.38
F3	97.03±0.67
F4	52.27±0.29

Four different formulations' self-emulsification times were noted as follows: At 131.87 ± 0.56 seconds, Formulation F1 had the longest self-emulsification time. The self-emulsification time of Formulation F2 was much quicker, at 79.45 ± 0.38 seconds. Formulation F3 emulsified in 97.03 ± 0.67 seconds, which was a little bit longer than formulation F2. At 52.27 ± 0.29 seconds, Formulation F4 finally showed the fastest self-emulsification time of all the formulations. Table 7 observation revealed that the F4 formulation creates microemulsion in the shortest amount of time compared to the other formulations, suggesting that the F4 was the best prepared formulation.

Rheological properties determination

Hard gelatine capsules were used to contain the SNEDDS systems. Therefore, SNEDDS was simple to pour into capsules, and systems of this kind shouldn't be very thick. Table 8 provides information on the microemulsion's rheological characteristics (viscosity, flow).

Table 8 Rheological properties for different formulations

Formulation	Type of flow	Viscosity
F1	Plastic flow	1231
F2	Plastic flow	884
F3	Plastic flow	1498
F4	Plastic flow	734

PF: Plastic flow

The table highlights the flow type and viscosity of four distinct formulations. All formulations (F1, F2, F3, and F4) demonstrate plastic flow characteristics. Among these, formulation F3 had the greatest viscosity, measuring 1498 units, followed by F1 with a viscosity of 1231 units. F2 has a lower viscosity of 884 units, while F4 has the lowest viscosity, reported at 734 units. From Table 8, it was discovered that F4 formulation was displaying low viscosity and plastic flow, which shows stability and pourability of formulation F4 was best among all other formulations.

Thermodynamic stability studies

The physical stability of the formulation is especially critical for its performance as it can be severely influenced by precipitation of the medication in an excipient matrix. Poor physical stability of the formulation could lead to phase separation of excipients that compromises bioavailability, as well as therapeutic efficacy. Furthermore, the mismatch between formulation and gelatin shell created brittleness, softness and delayed the disintegration or partial release of medication. The following cycles were carried out for these tests, and the results were provided in the Table 9.

Table 9 Thermodynamic stability studies

Formulation	Heating coil Cycle	Centrifugation	Freeze thaw stress cycle
F1	NPS	NPS	NPS
F2	NPS	NPS	NPS
F3	NPS	NPS	NPS
F4	NPS	NPS	NPS

NPS: No Phase Separation

The formulations were determined to be thermodynamically stable based on Table 10 observation that there were no discernible changes made to the formulations during stability tests.

In vitro dissolution study

To compare the drug release from the manufactured chrysin SNEDDS formulations and pure drug, an in vitro dissolution investigation was undertaken. Using a USP type II dissolving apparatus, quantitative in vitro dissolution studies are done to determine drug release from the oil phase into the aqueous phase. The results of the in vitro dissolution investigations were shown in Table 10 and the first order plot in Figures 20 and 21. Upon reviewing the data, it was discovered that the

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chrysin SNEDDS F4 formulation released approximately 96.15%, 96.99%, 97.73%, and 99.65%. Over the course of 65 minutes, the dissolution characteristics of the four formulations (F1–F4) as well as the pure drug were observed. None of the tests showed any drug release at 0 minutes. The pure drug released 5.67% after 5 minutes, but the discharges from F1 through F4 were much greater, ranging from 30.95% to 35.89%. The pure drug release rose to 8.11% after ten minutes, whereas the discharges of F1 through F4 ranged from 36.99% to 41.67%. The pure drug demonstrated a progressive rise in release over time, reaching 40.55% after 65 minutes. By the 65-minute point, however, F1, F2, F3, and F4 showed much greater drug releases, with values of 96.15%, 96.99%, 97.73%, and 99.65%, respectively. The formulations continuously showed better release patterns than the pure drug across the whole time period, with F4 demonstrating the maximum release at each interval.

Table 10 dissolution studies of SNEDDS formulations & pure drug

Time (min)	Pure Drug	F1	F2	F3	F4
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
5	5.67±0.56	30.95±0.23	31.76±0.88	33.29±0.49	35.89±0.12
10	8.11±0.38	36.99±0.12	37.51±0.31	37.51±0.56	41.67±0.73
15	10.55±0.67	42.96±0.25	43.26±0.26	41.73±0.17	47.45±0.34
20	13.99±0.29	48.87±0.05	48.01±0.83	47.95±0.44	52.23±0.95
25	17.43±0.34	54.71±0.27	52.76±0.42	52.17±0.28	57.01±0.56
30	19.97±0.29	60.43±0.33	58.41±0.97	56.99±0.22	62.69±0.17
35	22.91±0.24	66.15±0.59	63.66±0.54	63.81±0.72	67.97±0.78
40	25.85±0.18	70.77±0.30	68.91±0.11	70.63±0.18	73.25±0.39
45	28.79±0.13	74.15±0.60	74.16±0.68	76.45±0.64	78.53±0.51
50	31.73±0.85	78.77±0.31	80.41±0.25	81.27±0.11	83.81±0.61
55	34.67±0.33	83.15±0.61	85.66±0.82	86.09±0.44	89.09±0.22
60	37.61±0.18	87.77±0.32	89.16±0.39	90.91±0.98	94.37±0.83
65	40.55±0.71	96.15±0.62	96.99±0.96	97.73±0.52	99.65±0.44

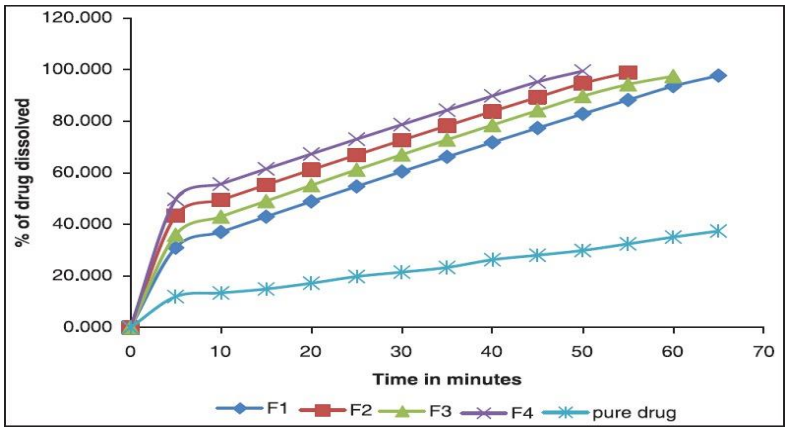

Figure 5 Comparative in vitro dissolution of different formulations & pure drug in 0.1N HCl

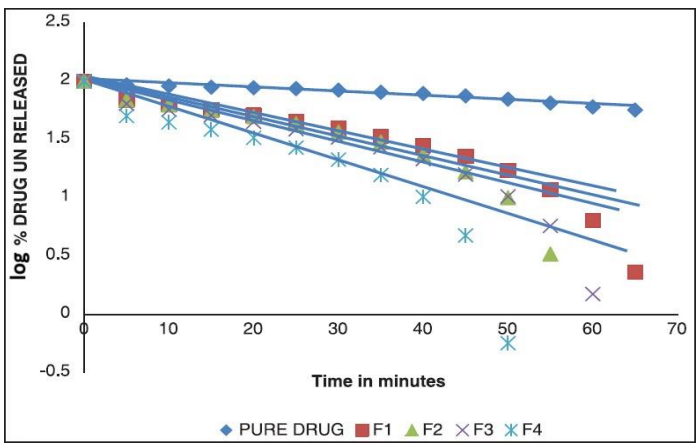

Figure 6 Zero order and First order plot of dissolution data of different formulations and pure drug

The correlation (R2) values of first order release kinetics were found to be greater than those of zero order release kinetics, indicating that all formulations followed first order kinetics. The slope of the first order linear plot seen in Figure 11 was used to get the first order rate constant (K). For all SNEDDS formulations and pure drug, Table 11 shows the in vitro dissolution characteristics, such as T50 (time required to dissolve 50% of drug), T90 (time required to dissolve 90% of drug), DE45 (Dissolution Efficiency), and correlation co-efficient values (0 & 1st order).

Table 11 In vitro dissolution of Chrysin SNEDDS formulations and pure drug in 0.1N HCl

Dissolution Medium	Formulation	Correlation co-efficient		K (min-1)	T50 (min)	T90 (min)	% of Dissolution Efficiency (DE_{45})
		Zero order	First order				
0.1N HCL	**Pure Drug**	0.9411	0.9197	0.8983	92.69	305.55	18.41
	F1	0.8421	0.9069	0.0119	15.45	59.34	51.34
	F2	0.7001	0.9241	0.1119	11.45	40.56	61.05
	F3	0.7693	0.9356	0.0293	13.61	48.31	49.53
	F4	0.6173	0.8633	0.0593	9.77	33.06	54.16

It seems from looking at every outcome that there was greater drug release from every SNEDDS formulation than there was from pure drug. It suggests that improving chrysin's solubility was made possible by the SNEDDS formulations.

The F4 formulation had the fastest rate of chrysin release out of the four SNEDDS and pure drug formulations. Compared to other formulations utilised in this examination, the formulation F4, which was made with surfactant Tween 80, co-surfactant PEG 400 and 24% oil (oleic acid), gave a comparatively quick release of chrysin. For this reason, it was chosen for more research.

Conclusion:

The Present work focuses on the invention and enhancement of chrysin self-emulsifying drug delivery systems (SNEDDS), especially when employing the F4 formulation. It has been found that oleic acid, Tween 80, and PEG 400 were appropriate components for solubilizing chrysin. Oleic acid exhibited the greatest solubility for chrysin at 15.81 mg/ml, topping other oils. Tween 80 and PEG 400 were chosen as surfactants and co-surfactants because to their high solubility levels. Pseudoternary phase diagrams demonstrated a considerable sensitivity of microemulsion zone size on the surfactant/co-surfactant (S/CoS) ratio. When the S/CoS ratio went from 1:1 to 4:1, the microemulsion area expanded dramatically. The highest microemulsion area was obtained at a S/CoS ratio of 4:1, suggesting that larger surfactant concentrations are more effective in forming bigger microemulsion areas. Thus, the S/CoS ratio of 4:1 was determined to be the most suited for the creation of self-nanoemulsifying drug delivery systems (SNEDDS). The formulation method was developed utilising a 22-factorial design, which permitted systematic optimization of the SNEDDS formulations. The UV-visible spectrophotometer was employed for the study of chrysin-SNEDDS, providing a linear calibration curve at 313 nm with a calibration coefficient (R^2) of 0.999 across the 2-10 µg/mL range. Competitive research employing FT-IR analysis was done using the pure medication and the SNEDDS formulation. The FT-IR spectra for both the pure medicine and the SNEDDS formulation demonstrated no interaction between the excipients and chrysin. Figures 16 and 17 illustrate the Fourier transform infrared graphs of chrysin in its pure form and within the SNEDDS formulation, respectively. Zeta potential studies offered vital insights into the characteristics of self-nanoemulsifying drug delivery systems (SNEDDS) formulations. F4 displayed greater performance with the smallest droplet size and largest zeta potential, demonstrating its benefits for stability and dispersion. The four formulations (F1, F2, F3, F4) were examined, and F4 demonstrated the maximum drug release throughout a 6.5-hour period, peaking at 96.89%. F4's increased release may be attributed to its capacity to form a microemulsion with smaller droplet sizes, which promotes higher drug release and may boost oral bioavailability. Emulsification times for the four formulations exhibited large differences in their self-emulsification effectiveness. Formulation F4 demonstrated the shortest self-emulsification time, followed by Formulation F2 and Formulation F3. F4 was the most effective in promptly forming a microemulsion, making it the recommended alternative among the assessed formulations. The use of firm gelatine capsules for containing the SNEDDS systems proved useful, since they are simple to pour owing to their non-thick structure. Thermodynamic stability experiments demonstrated that all formulations, including F1, F2, F3, and F4, exhibited no phase separation across numerous stress cycles, assuring their physical stability without notable modifications. The absence of phase separation and the lack of adverse effects on

bioavailability and therapeutic effectiveness further illustrate the suitability of these formulations for their intended uses. In vitro dissolving research demonstrated that all SNEDDS formulations of chrysin exhibited markedly enhanced drug release compared to the pure drug. Formulation F4 displayed greater dissolving rates at each time period, with F4 having the largest release percentages across the board. The dissolving efficiency and first-order kinetics further underscore the effectiveness of the SNEDDS in enhancing chrysin's solubility and release profile. Among the formulations investigated, Formulation F4, comprising Tween 80, PEG 400, and oleic acid, produced the fastest and most complete release of chrysin, making it the most feasible alternative for further investigation and development. In conclusion, the optimised chrysin SNEDDS (F4) demonstrated enormous promise for enhanced drug release, bioavailability, and stability, making it a suitable option for future investigation and development in oral drug delivery systems.

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