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Lercanidipine hydrochloride loaded self-nanoemulsifying: A novel approach drug delivery for hypertension treatment as single dose liquid oral ampoule

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ABSTRACT: Lercanidipine Hydrochloride (LRCH) is a third-generation dihydropyridine calcium channel blocker. It is a safe antihypertensive drug with a protective effect on the kidneys in hypertensive patients who have diabetes or chronic renal disease. It is practically insoluble in water and belongs to the Biopharmaceutical Classification System (BCS) Class II. It has a high lipophilic nature due to its Log P value of 6.42, distinguishing it from older dihydropyridines. Its oral bioavailability is only 10%, and erratic absorption is observed due to its extensive first-pass metabolism. However, when taken with food, especially high-fat foods, its bioavailability can increase (30-40%). In this study, based on the pharmacokinetic characteristics of LRCH, it is suggested that this drug is an excellent candidate for formulating a unique self-nano emulsifying drug delivery system (SNEDDS) oral dosage form to overcome the challenges associated with this drug using peppermint oil as an oil phase, Tween 20 as a surfactant, and propylene glycol as a co-surfactant. Pseudo-ternary phase diagrams generated by varying ratios of surfactant and co-surfactant 1:1, 2:1, 3:1, as well as 4:1. The results showed that we successfully loaded LRCH in SNEDDS liquid formulation with different Smix ratios in excellent properties, ranging from (9 - 24) seconds emulsification time, (9.415 - 32.14) nm droplet size, (0.11 - 0.699) PDI, (98.265 - 99.889) % drug content, and higher dispersion rate (over 97% during 60 min). The Fourier transform infrared spectroscopy (FTIR) data show that the excipients are compatible with the drug, and the globules are indeed nano-sized and spherical, as confirmed by atomic force microscopy (AFM).

KEYWORDS: Lercanidipine HCL; self-nano emulsifying; Tween20; pseudo ternary phase diagram; oral ampule.

1. INTRODUCTION

Hypertension is a significant risk factor for adverse cardiovascular complications affecting 30% of the global population and over double that percentage in the elderly [1]. Major complications such as heart failure, stroke, cerebral hemorrhage, thrombotic stroke, vascular dementia, chronic kidney failure, and myocardial infarction can result from uncontrolled hypertension [2]. Cardiovascular disease was the primary cause of 64% of deaths in 2019 alone, these illnesses cause deaths every 32 seconds throughout the world [3]. It is important to promote non-pharmacological methods for preventing and treating the underlying causes of a condition. According to a WHO report in 2023, effectively treating hypertension could save 76 million lives, 120 million strokes, 79 million heart attacks, and 17 million episodes of heart failure between now and 2050 [4]. Improving treatment coverage for hypertension is a global priority. Many conventional drugs are available for treating high blood pressure, and most are administered orally. This method of drug delivery is preferred because it is safe, easy for patients to comply with, and allows for self-administration. Unfortunately, several rugs suffer from restricted oral bioavailability due to physicochemical characteristics such as poor solubility, low permeability, and rapid metabolism; thus, their efficacy may be impacted [1,5]. Lercanidipine hydrochloride (LRCH) is a novel third-generation dihydropyridine calcium channel blocker 2-[(3,3diphenylpropyl) methylamine]-1,1-dimethylethylmethyl 1, 4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5 pyridinedicarboxylic ester [6], Since 1996, Recordati of Italy has been granted the first world marketing LRCH under the trade mark Zanidip® 10 and 20 mg film-coated tablets in several European countries for the treatment of hypertension [7].

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LRCH has a safe antihypertensive and anti-ischemic effect by blocking high-voltage dependent L-type calcium channels in the smooth muscle cells of blood vessels. This helps decrease peripheral vascular resistance, relaxing arterial smooth muscles [8]. It has been proven through studies that lercanidipine has a protective effect on the kidneys in hypertensive patients who suffer from diabetes or chronic renal disease. One of the most significant characteristics of LRCH is its high lipophilicity (Log P 6.42) compared to older DHPs. It exhibits a strong affinity for lipid membranes and a prolonged interaction with the L-type calcium channel, resulting in a gradual onset of action and long-lasting antihypertensive effect with the advantage of avoiding reflex tachycardia and causing significantly less peripheral edema compared to older DHPs [9].

According to the Biopharmaceutics Classification System, it is classified as a BCS class II drug due to its poor water solubility and high lipophilic nature [10]. It has low oral bioavailability; only 10% and erratic absorption was observed due to its extensive first-pass metabolism. It is soluble in ethanol, very soluble in methanol and chloroform, sparingly soluble in acetone, and practically insoluble in water. Its dissociation constant (pKa) is 6.83 at 37°C [6].

Developing an effective formulation of such drugs is a significant challenge. Although various solubilization technologies such as solid dispersions [10], cyclodextrin complexes [11], and micronization have been attempted to improve the solubility of LRCH, they have had limited success. These methods have also not been effective in reducing hepatic first-pass metabolism and enhancing the drug's permeability through the oral route. As a result, we have developed an alternative therapeutic system to deliver the drug with improved bioavailability and antihypertensive activity.

New advancements in oral medication consumption involve the use of lipid-based nanomedicine. Incorporating lipids in the medication can enhance their dissolution and permeability in the intestines and speed up the dispersion process by reducing gastrointestinal transit time. Drugs integrated with lipids can bypass first-pass metabolism and directly enter the bloodstream through lymphatic transport, increasing their bioavailability [12]. Various lipid nanosystems are available, such as solid lipid nanoparticles, nanostructured lipid carriers, liposomes, and related structures, including ethosomes, transfersomes, niosomes, and cubosomes. However, many of these nanocarriers have significant drawbacks, such as complex and time-consuming preparation procedures, limited physical stability, poor encapsulation efficiency, and lack of biocompatibility. Using self-emulsifying drug delivery with the right formula can reduce or even eliminate these drawbacks. These nanometric emulsions have excellent stability, dispersion capacity, and easy preparation methods [13,14]. Self-nano-emulsifying drug-delivery systems (SNEDDSs) have become increasingly popular in recent years as a method of improving the oral bioavailability of medications with poor water solubility [15]. A Self-Nanoemulsifying Pharmaceutical Delivery System (SNEDDS) is a mixture of anhydrous nanoemulsions (NEs) (isotropic mixture) that contain oils (both natural and synthetic), surfactants, and co-surfactants. When diluted with body fluids and mildly agitated in the stomach, these NEs form globules smaller than 200 nm [16]. When LRCH is taken with food, especially high-fat foods, its absorption rate increases three to four times due to its high lipophilicity. However, this may lead to variation in bioavailability among individuals. SNEDDS can be used as a promising solution to overcome this issue. SNEDDS not only enhances oral bioavailability but also helps to reduce the variability caused by consuming food alongside the medication [17,18]. In this study, based on the pharmacokinetic properties, LRCH is a promising option for creating unique SNEDDS formulations. These formulations can improve the dispersion rate of the drug, enhance drug stability, and increase oral bioavailability by being absorbed through the lymphatic system rather than undergoing first-pass metabolism.

2. RESULTS and DISCUSSION

2.1. LRCH saturated solubility

Selecting the right components is a crucial step in developing stable SNEDDS formulations. These components include oil, surfactant, and co-surfactant, and they must have high solubilizing ability for LRCH and be highly miscible with each other. The results of the solubility tests conducted on LRCH in different oils and surfactants are listed in Tables 1 and 2. Peppermint oil exhibited the highest drug solubility when tested in an oil phase and Tween 20, propylene glycol as surfactant and co-surfactant, respectively.

Table 1. LRCH saturation solubility values in various oil types and dissolution medium

Oils	Solubility (mg/mL) mean ± SD*	Oils	Solubility (mg/mL) mean ± SD*
Peppermint oil	76.870 ± 0.014	Capryol 90 oil	11.885 ± 0.011
Clove oil	71.105 ± 0.389	Black seed oil	7.312 ± 0.016
Lavender oil	70.425 ± 0.193	Castor oil	4.831 ± 0.002
Ginger oil	47.074 ± 0.053	Triacetin oil	3.797 ± 0.070
Oleic acid oil	23.806 ± 0.126	Dissolution media 1.2 HCl&0.5% Brij35	0.380 ±0.006

*Standard Deviation from Mean, n=3

Table 2. LRCH saturation solubility values in various surfactants and co-surfactants

Surfactants	Solubility (mg/mL) mean ± SD*	Co-surfactants	Solubility (mg/mL) mean ± SD*
Tween 20	25.642 ± 0.003	Propylene glycol	27.066 ± 0.025
Tween 80	23.554 ± 0.099	PEG 200	19.596 ± 0.063
Cremophor EL	16.763 ± 0.141	PEG 400	8.094 ± 0.066
Span 20	6.926 ± 0.006		
Tween 40	5.465 ± 0.076		

*Standard Deviation from Mean, n=3

2.2. Construction of pseudo-ternary phase diagrams

The change when body fluids are mixed with SNEDDS (Self-Nanoemulsifying Drug Delivery System) must be carefully considered. The decrease in solvent capacity can lead to the precipitation of drugs. A pseudo-ternary phase diagram must be developed to generate the SNEDDS formulation. The self-nanoemulsifying area and component concentrations can be found in this diagram. The Smix ratio is one of the most critical parameters affecting the development of the nanoemulsion category. Figure 1 depicts the pseudo-ternary phase diagram for various Smix ratios (Tween 20: propylene glycol 1:1, 2:1, 3:1, and 4:1).

The phase diagrams show the formation of nanoemulsion systems in the purple area, while the unshaded region displays turbid liquid with multiphase systems. Fully dilutable nanoemulsions, with an oil: Smix ratio of 1:9 across all Smix ratios, maintain their miscible, transparent nano-emulsion properties, even more so after an infinite cycle of titration or water diluting. The feasibility of the process is attributed to two factors. Firstly, the Tween 20/PG mixture in the nanoemulsion droplets lowers the interfacial free energy. This leads to the dispersion occurring spontaneously. Secondly, increasing the Smix ratio also causes the nanoemulsion zone to broaden. The surfactant lowers interfacial tension and ensures optimal intermolecular interaction between the oil phase, Smix, and deionized water [19].

2.3. The preparation of lercanidipine HCl SNEDDS liquid

Upon visual inspection, all liquid LRCH-SNEDD formulations exhibited a homogeneous, yellowish mixture devoid of drug precipitation or phase separation. As depicted in Figure 2.

2.4. Evaluation of the prepared LRCH-SNEDDS liquid formulations

2.4.1. Thermodynamic stability testing

All the LRCH-SNEDDS formulations showed excellent stability in the thermodynamic stability tests, which included alternative temperature cycles (-4°C and 45°C), freeze-thaw cycles (-21°C and +25°C), and centrifugation at 3500 rpm, without showing any signs of drug precipitation or phase separation consistency at varying degrees of temperature and centrifugal force.

2.4.2. Droplet size measurement and PDI

Table 3 shows the results of droplet size determination for LRCH formulations with the same lower percentage (10% w/w) of oil but different Smix ratios. The size of droplets is directly correlated to the concentration of surfactant. As a result, a higher surfactant concentration generally produces smaller droplets [13]. During this test, it was determined that the globules of LRCH-SNEDDS were in the nanometer range between (9.415 and 32.140) nm. The PDI is a dimensionless measurement used to determine the width of the size distribution. LRCH-SNEDDS PDI values range from 0.11 to 0.699, where values below 0.3 indicate a monodisperse system with a narrow droplet size distribution. In contrast, a higher value indicates that the

system is heterogeneous and has a wider size distribution range [20]. The PDI of less than 0.3 in F3 and F4 formulations indicated stable and homogenous globular size during long-term storage.

The size of the globules in a nanoemulsion impacts the stability, absorption rate, and rate of drug release. Hence, measuring the globule size is essential for optimizing the nanoemulsion formulation and assessing its performance [21].

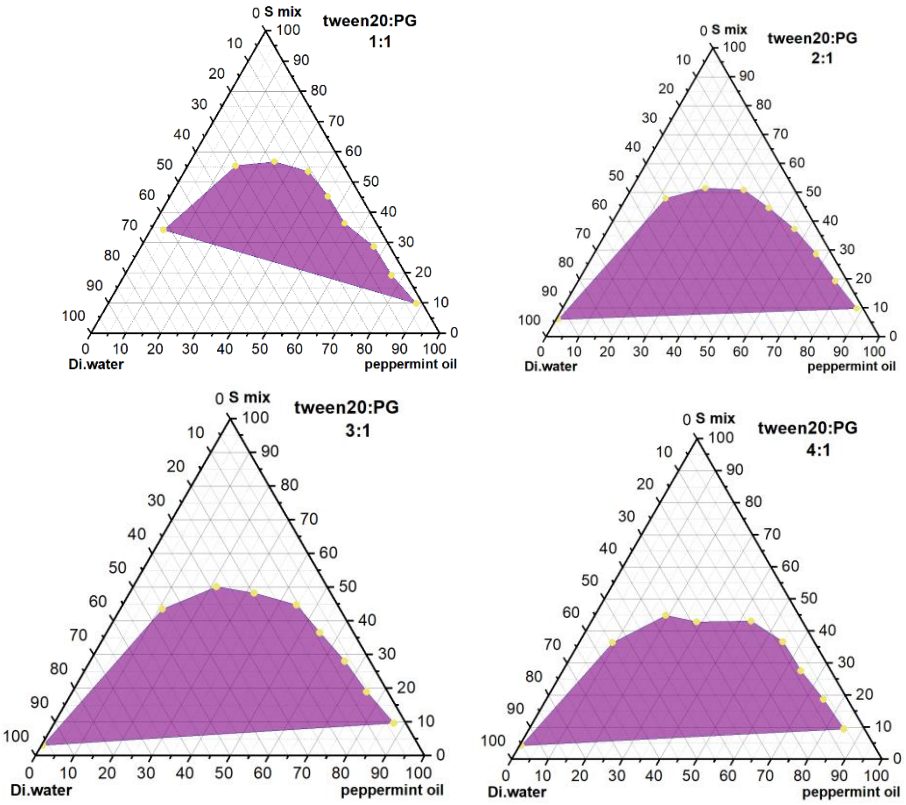

Figure 1. The pseudo ternary phase diagram for various Smix ratios (Tween 20: propylene glycol 1:1, 2:1, 3:1, and 4:1) purple domain indicates the self-emulsification region.

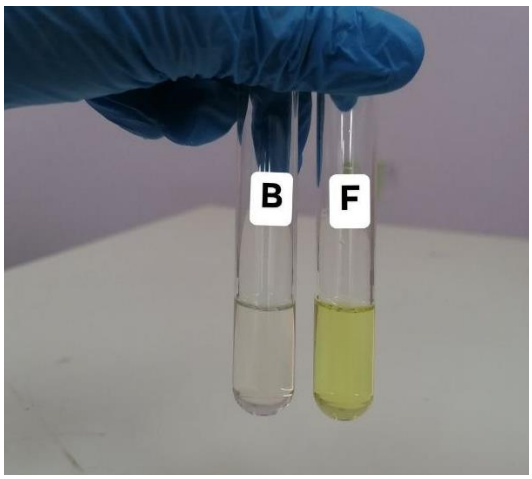

Figure 2. (B) blank formulas without drug, (F) LRCH loaded SNEDDS formulation

Table 3. displays the droplet size and PDI measurements of the liquid LRCH- SNEDDS samples.

F	Mean droplet size (nm) ± SD	PDI ± SD
SNEDDS-1	32.140 ± 1.581	0.378 ± 0.048
SNEDDS-2	19.546 ± 0.161	0.699 ± 0.007
SNEDDS-3	9.721 ± 0.115	0.110 ± 0.029
SNEDDS-4	9.415 ± 0.029	0.113 ± 0.024

*Standard Deviation from Mean, n=3

2.4.3. Robustness to dilution

SNEDDS need to be diluted without phase separation or drug precipitation to use them as drug delivery vehicles; this is important to ensure their effectiveness. The resulting nanoemulsions remained stable, clear, and transparent when all LRCH SNEDDS formulations were at infinite dilution with distilled water (D.W) and 0.1N hydrochloric acid (HCl). They did not show any phase separation even after 24 hours of storage at room temperature. This indicates that these systems are suitable for oral administration [22].

2.4.4. Dispersibility tests and self-nano emulsification time

Since emulsification is considered the rate-limiting step for drug absorption, efficient self-emulsification is essential for SNEDDS. According to visual observations, all the created LRCH-SNEDDS formulations successfully produced a nanoemulsion with grade A in under 30 seconds. The findings of the self-emulsification time (Table 4) show that the emulsification process became more spontaneous, and the self-emulsification time declined as the surfactant concentration increased.

Table 4. Self-Emulsification Formulation times for LRCH-SNEDDS

F	Self-Emulsification Time * (s)	Gade
SNEDDS-1	24 ± 0.370	A
SNEDDS-2	15± 0.110	A
SNEDDS-3	9 ± 0.170	A
SNEDDS-4	8 ± 0.110	A

*Standard Deviation from Mean, n=3

2.4.5. Drug content

All the created LRCH-SNEDDS had a drug content percentage higher than 97%, which is within the acceptable range of 90-110% according to the British Pharmacopoeia, as depicted in Table 5. There is a significant difference between F3 and F1, F2 ($P \leq 0.05$), except with F4

Table 5. The percent of drug content for liquid LRCH SNEDDS

F	%Drug content
F1	98.265 ± 0.251
F2	98.656 ± 0.389
F3	99.897 ± 0.102
F4	99.685 ± 0.162

*Standard Deviation from Mean, n=3

2.4.6. In-vitro dissolution test

The drug release study of LRCH SNEDDS and LRCH pure powder used the USP dissolution test apparatus type II. The experiment had a 500 mL (1.2) pH of 0.1 N HCl. 0.5% Brij35 was added to the solution to maintain the optimal sink condition. Dialysis membranes were used due to their small pores and low probability of blockage [23]. For this experiment, diluting liquid LRCH SNEDDS with distilled water kept the formulation from adhering to the dialysis membrane, allowing for easy dissolution and permeation of dissolved molecules from the dialysis bag [24].

In this *in-vitro* dissolution, we used four different Smix concentrations. We found, that with increased Smix ratios, all LRCH SNEDDS formulations show significant improvement in dissolution rate of more than 97% after 60 minutes.

As follows, formulas ordered according to the faster dissolution:

F3 > F4 > F2 > F1 > pure drug.

There could be several reasons for this faster release rate. Firstly, the drug was dissolved in the SNEDDS system due to the solubilization effects of peppermint oil and tween 20; secondly, the excellent nanoglobule

size allowed for a larger surface area of nanoemulsion droplets, thus increasing the dissolution rate. Thirdly, a higher Smix ratio (tween20/PG) played a role in this faster release, especially when well balanced with an oil ratio [25,26].

The effect of the Smix ratio upon drug release was depicted in Figure 3; all SNEDDS formulations exhibited a better dissolution profile, approximately seven times more than the LRCH powder. This improvement can ultimately lead to a better oral absorption of LRCH.

A comparison between the dissolution profile of pure LRCH and SNEDDS formulation was made using the similarity factor. $f2$ is a parameter used in pharmaceutical sciences to evaluate the likeness between two dissolution profiles. As per the FDA guidelines, the two profiles are deemed similar when the $f2$ value exceeds 50, ranging from 50 to 100. In this case, the resulting value of the similarity factor is less than 50, indicating a significant difference in the dispersion behavior between LRCH-SNEDDS formulations and pure LRCH powder, as shown in Table 6 [27].

Table 6. Similarity factor tests of F1, F2, F3, and F4 compared versus pure lercanidipine.

Formula no. compared with pure drug	$f2$ Similarity value
F1	18.43
F2	17.86
F3	10.39
F4	12.60

Figure (4) shows the Faster dispersion rate of LRCH ampule as compared to zandip® due to the nanodroplet size of the prepared formulations because of increased surface area The release profiles of the two dosage forms are also different ($f2 < 50$).

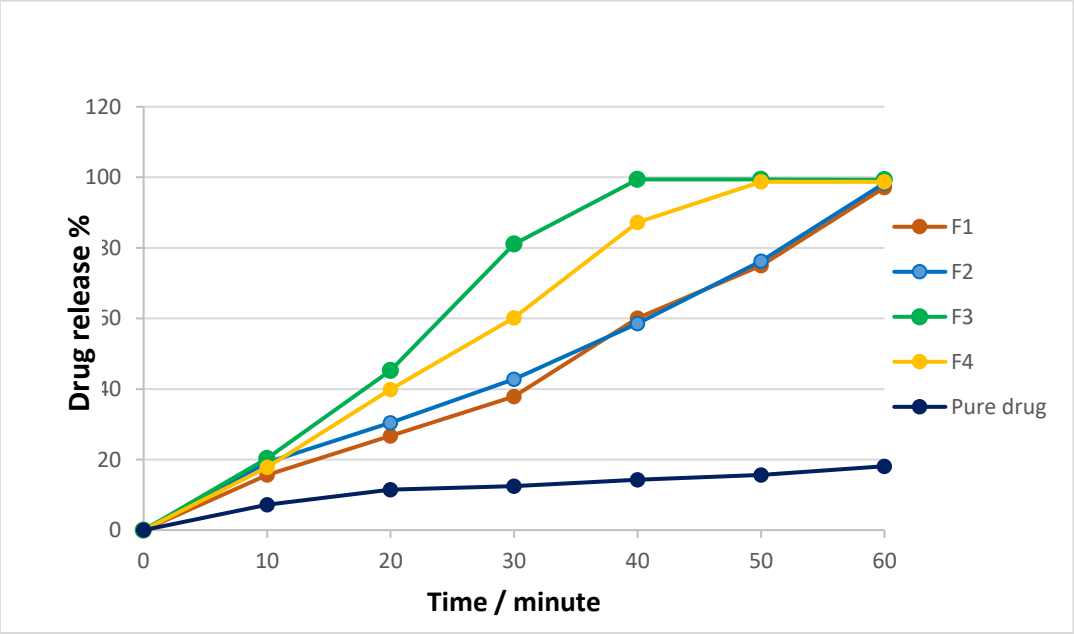

Figure 3. In-vitro release of the pure LRCH, F1, F2, F3, and F4 in 0.1N HCl with 0.5% Brij35 at 100 rpm and 37°C.

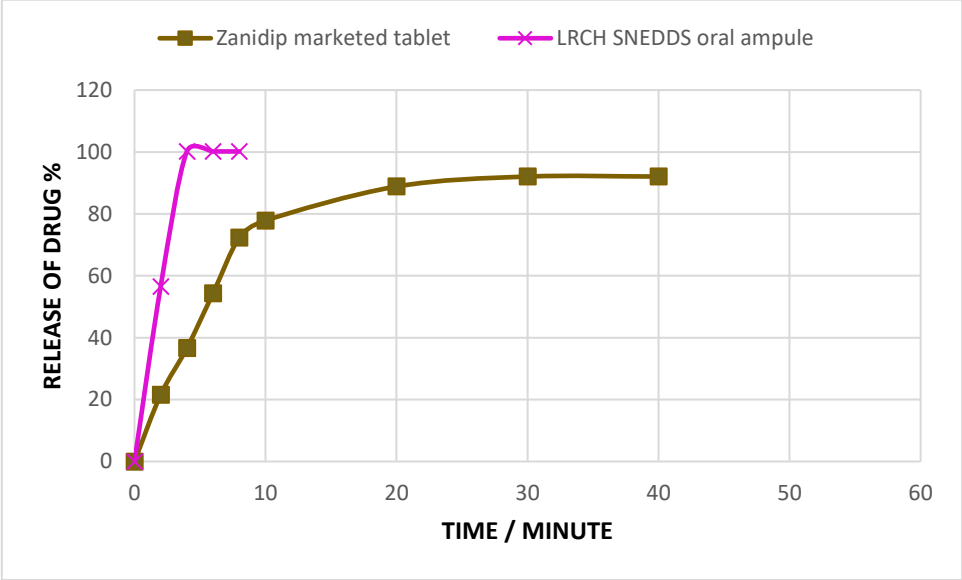

Figure 4. The dissolution profile of the final prepared dosage form LRCH SNEDDS ampule and marketed tablet Zanidip® in 0.1N HCl with 0.5% Brij35 at 37°C according to the USP monograph.

2.4.7. Fourier transform infrared spectroscopy (FTIR).

The LRCH-SNEDDS formulations and the pure LRCH powder were analyzed using FTIR. The LER spectrum displayed strong and characteristic peaks at various wavelengths, including 3267 cm^{-1} (N–H stretching), 3186 and 3078 cm^{-1} (aromatic C–H stretching), 2947 and 2773 cm^{-1} (aliphatic C–H stretching), 1674 cm^{-1} (ester C=O stretching), 1523 cm^{-1} (aromatic C=C stretching), 1489 cm^{-1} (aromatic NO2 stretching), 1400 cm^{-1} (N–H bending), 1346 cm^{-1} (C–H bending from CH2, CH3), 1234 cm^{-1} (C–N stretching), 1195 and 1126 cm^{-1} (ester C–O stretching), 1091 cm^{-1} and 1014 cm^{-1} (in-plane bending from C–H aromatic ring) and 902 cm^{-1} (out-of-plane bending from the C–H aromatic ring) [28].

The results of the FTIR study, as shown in Figures 5 and 6, indicate that the LRCH spectrum's unique peaks are still evident in the SNEDDS formulation, although with less intensity than the pure drugs. the LRCH and excipients in the formulation were compatible, as no chemical interactions were observed.

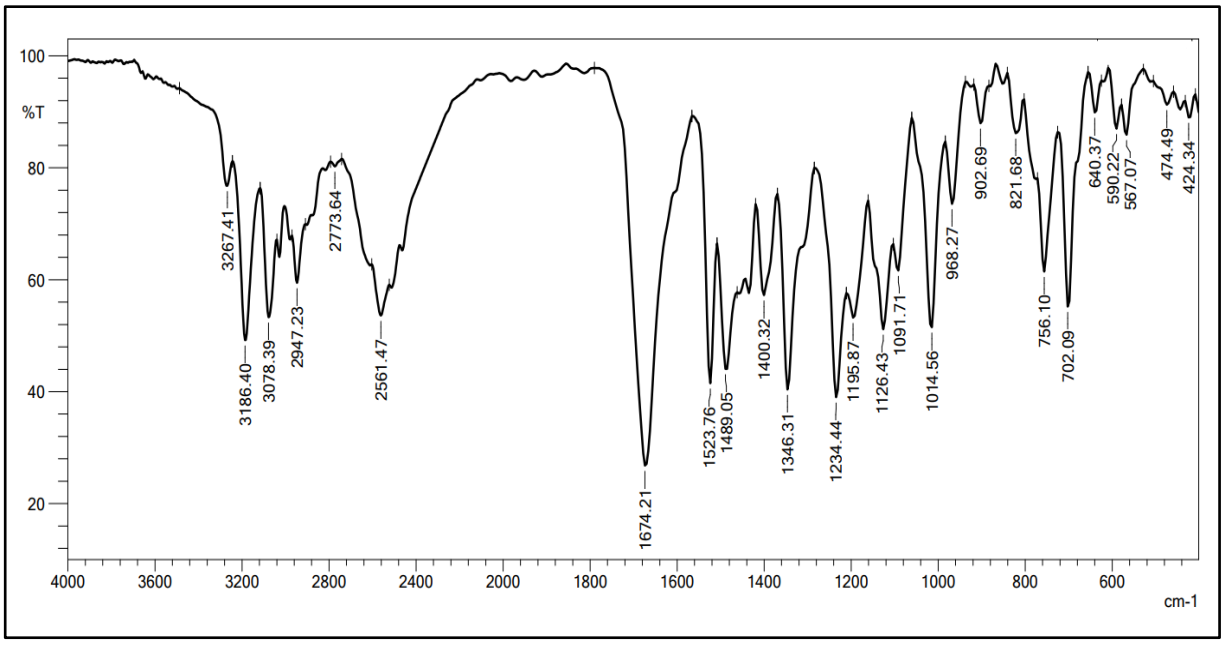

Figure 5. FTIR specrtoscopy of pure LRCH.

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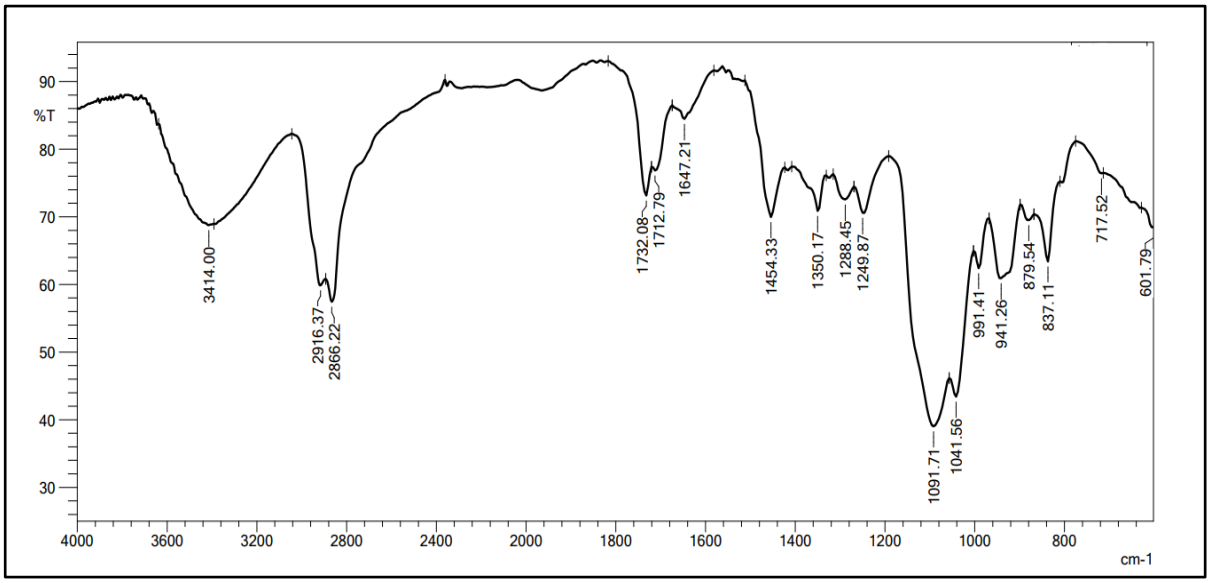

Figure 6. FTIR spectroscopy of liquid LRCH SNEDDS F3 Formula.

2.4.8. AFM

The AFM investigation of the selected LRCH SNEDDS formulation F3 demonstrates the creation of a stable and uniform nanoemulsion with consistent droplet size distribution and surface topography. The average droplet size was 46 nm, as depicted in Figures 7 and 8.

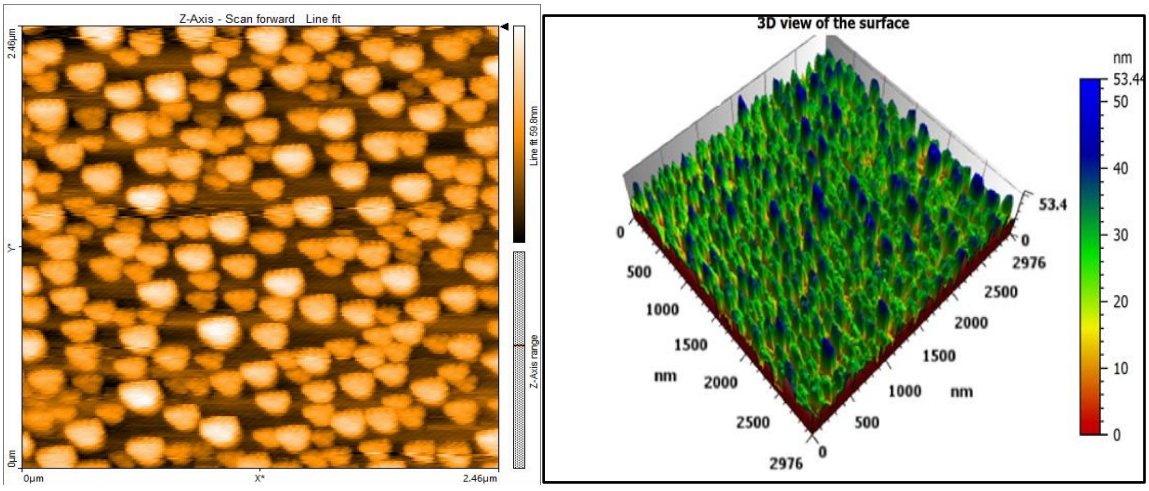

Figure 7. Chosen formula: AFM-imaged surface 3D model of lercanidipine HCl SNEDDS

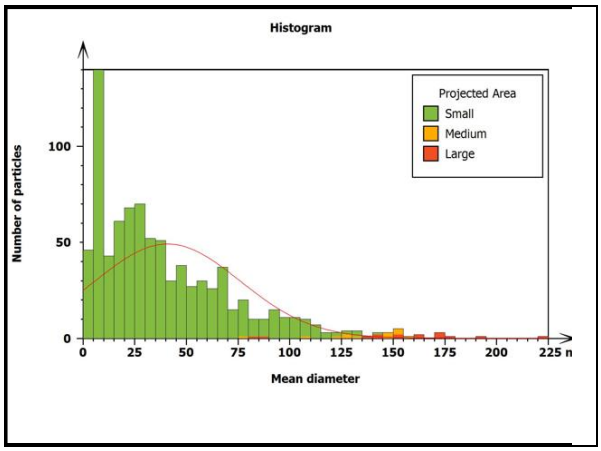

Figure 8. Histogram of LRCH-SNEDDS chosen formula globule size analysis using AFM

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2.5. Future aspect

We hope that this study receives attention from the pharmaceutical industry as it provides an easy and suitable way for the elderly or those who have difficulty swallowing to take their medication. Taking chronic medicines can have a psychological impact, so manufacturing them in this distinctive way provides a departure from the monotonous routine and improves the mood of patients. As depicted in Figure 9, a single ampule of the (LRCH-SNEEDDS) oral liquid contains 10 mg of lercanidipine HCl daily.

Mix it with some water, then drink all the contents without waiting.

Figure 9. Final dosage form and method of its administration

3. CONCLUSION

This study's findings support that SNEDDS is a useful dose form for lipophilic oral medications. Using a simple mixing method and spontaneous emulsification technique, lercanidipine hydrochloride was readily and successfully encapsulated in a liquid SNEDDS system with peppermint oil, Tween 20, and propylene glycol as oil phase, surfactant, and co-surfactant respectively. All formulations show significant improvement in drug release with an increased Smix ratio. The drug's release rate is approximately now seven times faster than in its pure powder form, which is a vast improvement. At the nanoscale, atomic force microscopy (AFM) demonstrated uniformly sized, spherically shaped, and surface-free particles. The outcomes have the required and unique characteristics of the oral dose form, concluding the investigation. The LRCH-SNEDDS oral liquid dosage form has great promise for the treatment of hypertension, particularly in patients with renal failure. However, an in-*vivo* bioavailability study is required to alter the dosage.

4. MATERIALS AND METHODS

4.1. Material

Lercanidipine HCl (Hyperchem, China), Tween 20,40 and 80 (Chemical Point, Germany), propylene glycol (Barcelona, Spain), peppermint oil and methanol (Thomas Baker, India), Clove and Ginger oil (Alpha Chemika, CO., India), Lavender oil (Shaanxi Guanjie Technology CO, LTD, China), Oleic acid oil (Central Drug House(P)LTD, India), Capryol 90 oil(Gattefosse Sas, France), Black seed and castor oil (NOW® CO., USA), Triacetin oil, Cremophor EL and Span 20 (Hyperchem, China), PEG 200 and PEG 400 (Sigma Aldrich, USA), Zandip®10 mg film-coated tablet (Recordati, Italy) and Brij35 (HIMEDIA, chemicals India) and hydrochloric acid (ReAgent Chemicals, UK).

4.2. Methods

4.2.1. Solubility Study

To determine the best solvents for dissolving LRCH, a saturation solubility test was conducted in several oils such as Peppermint oil, lavender oil, Ginger oil, oleic acid, Capryol 90 oil, Black seed oil, Castor oil, and triacetin oil. Additionally, surfactants like tween 20, tween 80, Cremophor EL, span 20, and tween 40, co-surfactants such as Propylene glycol, PEG 200, PEG 400, and dissolution media consisting of 0.1 N HCl containing 0.5% Brij35 were used to ensure sink condition.

The excess LRCH powder was added to 2ml of each selected individual oil, surfactant, and co-surfactant in stoppered vials. After sealing the mixture, it was sonicated for five minutes. Afterward, the oils, surfactants, and co-surfactants were shaken for 48 hours at 25 ± 0.5°C, while the dissolving media were shaken at 37±0.5°C to achieve a saturated solution. Once the mixture was retracted, it was centrifuged at 3000 rpm for 20 minutes. The supernatant was removed, and the solution was filtered through a 0.45µm millipore syringe filter. The next step was to dissolve it in methanol by diluting it [29]. LRCH concentration in diluted samples was measured spectrophotometrically at methanolic λ max 237 nm [30]. The mean ± SD of the solubility data was computed.

4.2.2. Constructing pseudo-ternary phase diagrams

A pseudo-ternary phase diagram was created using a low-energy aqueous titration approach to determine the component ratios in SNEDDS. The components of blank formulation were developed based on solubility, miscibility, and stability studies [31]. Peppermint oil (oil phase), tween 20 (surfactant), and propylene glycol as co-surfactants, while the aqueous phase deionized water. The ratios of surfactant and co-surfactant were varied, ranging from 1:1 to 4:1. Each phase diagram was made by carefully mixing oil and Smix in several glass vials at ratios ranging from 1:9 to 9:1. Mild magnetic stirring was used to gradually dilute the Smix: oil mixture with DI.W until a stable and transparent system was achieved. The ternary plot was generated using Origin 2018 64Bit software.

4.2.3. Preparing Lercanidipine HCl liquid SNEDDS

Several liquid SNEDDS formulations were created following the pseudo-ternary phase diagram. The formulations consisted of peppermint oil, tween 20 surfactants, and propylene glycol co-surfactant in different ratios, including 1:1, 2:1, 3:1, and 4:1. The oil to Smix ratio was constant at 1:9, as shown in Table 7.

Firstly, blank formulas were prepared by mixing peppermint oil, tween 20, and propylene glycol in a vortex mixer before adding the drug for about 3 min in a screw-capped glass tube to ensure homogeneity and obtain excellent miscibility; then, accurately weighted LRCH was added to the(oil, surfactant, and co-surfactant) homogenous mixture, then mixing by a vortex mixer for 5 minutes until all drug dispersed and dissolve in oil/Smix mixture, then sonicated for 10 min until a clear yellow liquid obtained [19] Finally, the formulations were left at room temperature and checked visually for at least 48 hours before further evaluation. The presence of turbidity, phase separation, or precipitation was examined.

Table 7. Lercanidipine HCl liquid SNEDDS composition (% w/w).

Formulation	Smix Ratios	Oil: Smix Ratios	Peppermint oil % w/w	Tween 20 %w/w	Propylene Glycol % w/w	LRCH % w/w
F1	1:1	1:9	10	45	45	10
F2	2:1	1:9	10	60	30	10
F3	3:1	1:9	10	67.5	22.5	10
F4	4:1	1:9	10	72	18	10

4.2.4. Evaluation of prepared Lercanidipine HCl liquid SNEDDS

I. Thermodynamic stability studies

All liquid SEDDS formulations that were prepared were subjected to thermodynamic stability studies (centrifugation, heating-cooling cycle, and freeze-thaw cycle) to evaluate the effect of temperature variation and phase separation on SEDDS formulations to overcome selecting a metastable formulation [32].

A- Centrifugation test

All formulations were centrifuged at 3500 rpm for 30 minutes and checked for instability, such as creaming, cracking, phase separation, and precipitation. Stable formulations that passed this test and maintained a monophasic state were chosen for the heating/cooling test cycle [32].

B- Heating-cooling cycle (H/C cycle)

The SNEDDS formulations underwent six heating-cooling cycles, ranging from 4°C to 45°C. Each formulation was stored at each temperature for at least 48 hours. The formulations that were stabilized at these temperatures were frozen and thawed [33].

C- Freezing/ thawing cycles

Three freeze-thawing cycles at temperatures between (-21°C and +25°C) with storage at each temperature for at least 48 hours. Formulations that remain clear and not separate were selected for further studies [34].

II. Globule size measurement and polydispersity index (PDI)

Dynamic light scattering (DLS) was used to evaluate all the formulations. The samples were stirred gently with a magnetic stirrer for 1 minute after being diluted with distilled water at a ratio of 1:100 (v/v). The formulation was checked to be adequately disseminated in water before the measurement. Light scattering monitoring at 25°C and a 90° angle was used to measure the droplet size and PDI. The particle size analyzer was the Malvern Zetasizer, manufactured by Spectris Company in the United Kingdom [35,36].

III. Robustness to dilution

In two separate volumetric glass containers, distilled water, and 0.1N HCl buffer (pH 1.2) were used as dissolution media to dilute all SNEDDS formulations to concentrations of 50, 100, 1000, and 3000 times, respectively. The resulting nanoemulsion solutions were combined with a magnetic stirrer at 37°C, close to the human core temperature, to mimic the mixing process inside the body. After 24 hours, they were examined visually for indications of drug precipitation, droplet coalescence, or phase separation [37].

IV. *Self-nano emulsification time and dispersibility test*

The self-emulsification time was used to assess the SNEDDS self-emulsifying efficiency and the time required for a formulation to create a homogeneous nanoemulsion when reconstituted with deionized water. The USP dissolution apparatus II was used for the in vitro evaluation. Add 10 mg of LRCH- SNEDDS formulation dropwise to 500 mL of distilled water at 37°C±0.5°C. Emulsification time was manually recorded while a paddle operating at 50 rpm gently agitated the mixture [38]. An easily identifiable indicator of *in vitro* efficiency is forming a clear, uniform system. In minutes, it is possible to calculate the time required to achieve full nano-emulsification by utilizing a grading system, as demonstrated in Table 8 [37].

Table 8. Categorization of the SNEDDS Formulation Based on Comparative Grades.

Grade	Self-nanoemulsification time	Visual appearance
NE A	Quickly creating a nanoemulsion (in under a minute)	Displaying a transparent or blue light
NE B	Quickly Forming in under two minutes	Emulsion that looks blue-white but is relatively less translucent
NE C	Within 2min	Bright white emulsion
NE D	It takes more than three minutes to emulsify.	A slightly oily emulsion that appears white or greyish-dull.
NE E	Slowly emulsify for more than 3 min	Displaying surface oil globules and poor emulsification.

V. Determination of content of drug

The LRCH-SNEDDS formulation dissolved in 100 mL of methanol and the mixture was subjected to sonication for around 15 minutes. The correctly filtered extracted solution was obtained using a 0.45 µm filter syringe. After additional dilution, a UV/Vis spectrophotometer measured the drug content at 237nm [39].

VI. In-vitro dissolution study

This study utilized the USP Dissolution Apparatus-II (paddle type) to investigate the in vitro dissolution profiles of both stable LRCH-SNEDDS and the pure drug. As a dissolution medium, 500 mL of 0.1N hydrochloric acid with 0.5% Brij35 at 37±0.5°C [40]. In the dialysis bag technique (8000- 14000), Da was soaked in a solution of 1.2 (0.1N) HCl for 24 hours at room temperature [23]. The SNEDDS formulations were diluted with distilled water at a specific ratio for each sample, filled in a dialysis bag, then tightly sealed at both ends, and placed in the using medium [24]. The speed at which the paddle was rotating was 100 RPM. Fresh media was added to maintain constant sink conditions after removing 5 ml of the dissolving medium at 10, 20, 30,

40, 50, and 60-minute intervals. A 0.45 µm pore size membrane filter was used to filter the samples [38]. The amount of drug dissolved was quantified at its maximum absorption wavelength using a UV-Spectrophotometer. The DDSolver used a similarity factor to compare dissolution profiles.

VII. ***The FTIR spectroscopy technique***

Successful LRCH SNEDDS formulation depends on the compatibility and stability of its components. FTIR (IRAffinity-1 designed by Shimadzu in Japan) was used to analyze the functional groups present in the components, assess any chemical interactions or changes that may occur with excipients during formulation, and ensure the stability of the final SNEDDS. FTIR study was performed by making spectrums from 4000-400 cm^{-1}. Pure powdered Lercanidipine HCl was analyzed using a KBR disc, while liquid LRCH -SNEDDS samples were analyzed using a specially developed cuvette [41].

VIII. Atomic force microscope (AFM) analysis

The morphology and size of nanoemulsions may now be determined using the AFM technique, a complementing analytical approach. Many benefits make it superior to traditional methods of characterizing high-resolution interfaces, such as TEM (Transmission Electron Microscopy) and SEM (Scanning Electron Microscopy). In addition to being easy to use, it allows us to study the droplet interface in a setting that mimics actual nanoemulsion systems. Tapping AFM combines the two types of modes, contact and non-contact. This mode enables the AFM tip to briefly touch the sample surface by oscillating at or near its natural resonance frequency across the surface. When tapping a sample, very few shear forces are exerted on its surface. As a result, tapping mode AFM has become the gold standard for high-resolution imaging of water-based soft materials such as nanoemulsions [42]. An AFM system called CoreAFM 2023, manufactured by Nanosurf AG in Switzerland, was used for the experiment.

4.3. Statistical analysis

An average of three samples with a standard deviation was used to present the research outcomes. The data were examined by one-way analysis of variance (ANOVA) at a significance threshold of ($P = 0.05$) to detect statistical significance ($P < 0.05$) or non-significant ($P > 0.05$) in the changes in the applied parameters [43, 44].

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