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Development, In-Vitro Evaluation And In-Vivo Pharmacokinetic Study Of Buspirone Hcl Solid Lipid Nanoparticles

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Abstract:
Buspirone hydrochloride (BUS HCl) treats anxiety disorders and relieves symptoms, however its bioavailability is 4–10%. Solid lipid nanoparticles (SLNs) are commonly spherical with a 50 to 1000 nm diameter. The objective of the study to develop solid lipid nanoparticles and improve the oral bioavailability of buspirone HCl. Differential scanning calorimetry (DSC) studies showed no interactions between drugs and lipids. The SLNs were designed through a process of hot homogenization followed by ultrasonication. The prepared formulations were evaluated for size, zeta potential (ZP), Polydispersity Index (PDI), entrapment efficiency (EE), in-vitro drug release, and stability studies. Field emission scanning electron microscopy (FESEM) studies revealed that pure drug had irregular particles, whereas SLNs contained spherical-shaped and uniform-sized particles. Based upon the results, F3 formulation was optimized having size (163.1±5.2nm), PDI (0.163±0.007), and showed in-vitro release (76.44±1.4%) in 24hrs. The formulation (F3) significantly improved pharmacokinetic parameters such as Cmax, AUC0-t, and t1/2 and increased bioavailability by 2.3 folds that of coarse suspension.

Keywords: Solid lipid nanoparticles, lipids, bioavailability, and buspirone HCl

Introduction

Lipid-based drug delivery systems (LBBDS) are a promising strategy for improving bioavailability of drugs. Lipids have received much interest as drug carriers because of their high permeability nature. Encapsulating drugs with lipids increases their solubility and absorption, promoting bioavailability (Pouton and Porter., 2008). SLNs are commonly spherical, with a diameter range between 50 to 1000nm. The primary ingredients of SLNs include lipids, emulsifiers, solvents, and drug (Subroto et al., 2023).

BUS HCl is an anxiolytic agent. It reduces anxiety without producing significant sedative, hypnotic, or euphoric effects (Derakhshandeh M and Ghasemnejad M.B 2014). It is a BCS class-I drug with a limited oral bioavailability (5%), owing to substantial metabolism (Mahmood and Sahajwalla., 2012). The current study emphasizes the development and assessment of BUS HCl-SLNs.

Materials And Methods

MATERIALS

Buspirone HCl was a gift sample from Neutral Pharma, Surat, India. Compritol 888 ATO was procured from Gatteffose, Glycerol monostearate was obtained from choice Organochem LLP, Hyderabad), and Stearic acid, was getting from ennore chemicals, chennai. Lipoid E 80 was obtained from Lipoid, Germany. Kolliphor P188 from BASF, Mumbai. All other reagents used in the experiment were of analytical grade and purchased from their respective commercial sources.

METHODS

Preparation of BUS-SLNs:

The SLNs have been produced using a hot homogenization and ultrasonication process (Ganesan P. and Narayanasamy D., 2017). Drug, solid lipid, and lipoid E 80 were liquefied to make the oil phase in a 10 mL of solvent mixture with a ratio of 1:1. The lipid covering layer was melted using a heating system set at 5°C above

the melting point in which drug was encapsulated and the organic solvents were evaporated using a rota evaporator. Kolliphor®P188, a surfactant, was used to develop the aqueous phase. It was dissolved in double-distilled water and heated to the same temperature as the oil phase. The heated oil and aqueous phases were blended together for 5 min at 12,000 rpm in a homogenizer. Utilizing a probe sonicator, the resulting coarse oil in water (O/W) emulsion was sonicated for 20 min. SLNs formed when a heated nanoemulsion was allowed to cool to room temperature (Arjun and Kishan., 2013).

Table 1: Ingredients used in of SLNs formulations

Ingredients (mg)	**Formulations**					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
Organic phase						
Buspirone HCl	10	10	10	10	10	10
Compritol®888 ATO	100	150	-	-	-	-
Glycerol monostearate	-	-	100	150	-	-
Stearic acid	-	-		-	100	150
Lipoid E 80	100	100	100	100	100	100
Chloroform: methanol (1:1) (mL)	10	10	10	10	10	10
Aqueous phase						
Kolliphor ®P188	150	150	150	150	150	150
Double distilled water (mL)	10	10	10	10	10	10

Characterization studies of BUS-SLNs

Measurement of particle size (PS), polydispersity index (PDI), and zeta potential (ZP)

Malvern Zetasizer was employed to assess the PS, PDI, and ZP of the SLNs. The size of the formulated SLNs was measured at 90º angles after being diluted from 100 µL to 5 mL with double-distilled water (Manjunath and Venkateswarlu., 2005).

Assessment of entrapment efficiency (EE)

The produced SLNs formulations' EE was calculated through an estimation of the amount of free drug (unentrapped) present in an aqueous medium that was separated by ultrafiltration using Centrisart tubes, which were made of a filter membrane (M.Wt. 20 KDa) at the bottom sample collecting compartment. A sample collecting unit was placed over the collected material with approximately 5 mL of an SLNs preparation in the outside compartment, and the sample was centrifuged at 3500 rpm for 30 minutes. After passing through the filter membrane and the sample collecting compartment, the aqueous medium retained the SLNs and drug encapsulated in the outer unit. HPLC was used to determine the amount of drug in the aqueous medium (Bhalker et al., 2017).

In-vitro drug release studies

These studies were carried out using the dialysis bag diffusion technique. A dialysis membrane (M.Wt 12,000–14,000Da) with an appropriate pore size of 2.4 nm, which had been soaked in double-distilled water for 12 hours before use, was used for the study. The developed SLN formulations were tested for release utilizing an open tube technique at pH 1.2 simulated gastric fluid (SGF) for 2 hours and pH 6.8 simulated intestinal fluid (SIF) for 22 hours. The dialysis membrane was connected to an open tube (SLN dispersion) as the donor compartment and a buffer (100 mL) containing a 200 mL beaker as the receptor compartment while maintaining the requisite temperature of 37.5°C. At various time intervals up to 24 hours (0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 hrs), 2 mL of the sample was removed from the receiver compartment and replaced with a new medium. A UV-visible spectrophotometer assessed drug release in samples at 236 nm (Takahashet al., 2016).

Stability studies
The changes in the PS, ZP, PDI, and EE of SLNs were used to measure stability. The optimized SLNs formulation (F3) was stored in a room (25°C/60% RH) and refrigerated (4°C) at temperatures for 90 days. The physical stability of the sample was evaluated on the first, 30^{th}, 60^{th}, and 90^{th} days (n=3) (El-say and Hosny., 2018).

Lyophilisation
Lyophilization (freeze-drying) increased the stability of SLNs. In a deep freezer, SLNs with cryoprotectant (10% w/v trehalose) were prepared and stored overnight at -40oC. The frozen sample was then placed in a freeze-dryer (U-Tech). The vacuum was then supplied, exposing the sample to multiple drying methods over 48 hours to achieve a stable powdered lyophilized product (Battaglia et al., 2023). The free-flowing powder was used in additional studies, such as solid-state characterization with DSC and surface morphology by FESEM (Choi, M.J et al., 2012).

Drug and excipient compatibility studies by DSC
The DSC was used to assess the drug-excipient compatibility and crystalline behavior of the drug and lipids. Thermograms of the drug, lipids, and physical mixture (PM) in a 1:1 ratio and lyophilized formulation were carried out using a Perkin Elmer (DSC 4000, USA) thermometer. The thermograms were taken between 20 and 200°C at a heating rate of 20°C/min (Hongbin Liu et al., 2021).

Surface morphology studies of SLNs by FESEM
The pure drug and SLN morphology has been examined using FESEM (JEOL, Japan, 0.1kV to 30kV). The measured samples were placed on the aluminium stubs consecutively. As a reflective layer, a thin layer of gold-palladium (5-10 nm) was sputter deposited on the surface of the samples at various magnifications (Mahmood et al., 2017).

Pharmacokinetic studies
The studies were conducted on healthy male Wistar rats weighing 200±30 gm. The animals fasted overnight and had adequate water access. The Institutional Animal Ethical Committee (IAEC) reviewed and approved the research protocol at UCPSc, Kakatiya University, Warangal, India (IAEC/02/UCPSc/KU/2022).

Study protocol
The animals were separated into two groups (n=6); BUS coarse suspension (Group I) and optimized SLNs (F3) formulation (Group II) at 10 mg/kg body weight and given orally using a rat oral feeding tube.

Nearly 0.2 mL of blood was taken from the retro-orbital plexus into eppendorf tubes and spun at 10,000 rpm for 10 minutes to separate serum at regular predefined intervals of 0, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours after oral administration. It was kept at -20ºC until the HPLC analysis was done (Weiping et al., 2020). The Kinetica tool (version 5.0) calculates C_{max}, t_{max}, AUC_{0-t}, $t_{1/2}$, and MRT. Graph pad prism software (version 6.4) was used to compare data from two samples statistically, and $p<0.01$ was considered statistically significant (Narendar, D. and Kishan., 2016)

Results And Discussion

DSC analysis
A sharp endothermic peak with a high enthalpy was visible on the pure drug's DSC thermogram at 204.24ºC, which was also its melting point. It was pure since the peak was observed at the drug's reported melting point (204ºC). The endothermic peak temperatures of the bulk stearic acid, GMS, and Compritol ATO 888 lipids were 69.65°C, 68.37°C, and 68.52°C, respectively. The drug peak was found in a physical mixture of optimized formulation at 204ºC, indicating no interaction between the drug and excipients (Montenegro, L et al., 2011). The DSC studies help to assess the interaction of different components of the formulation.

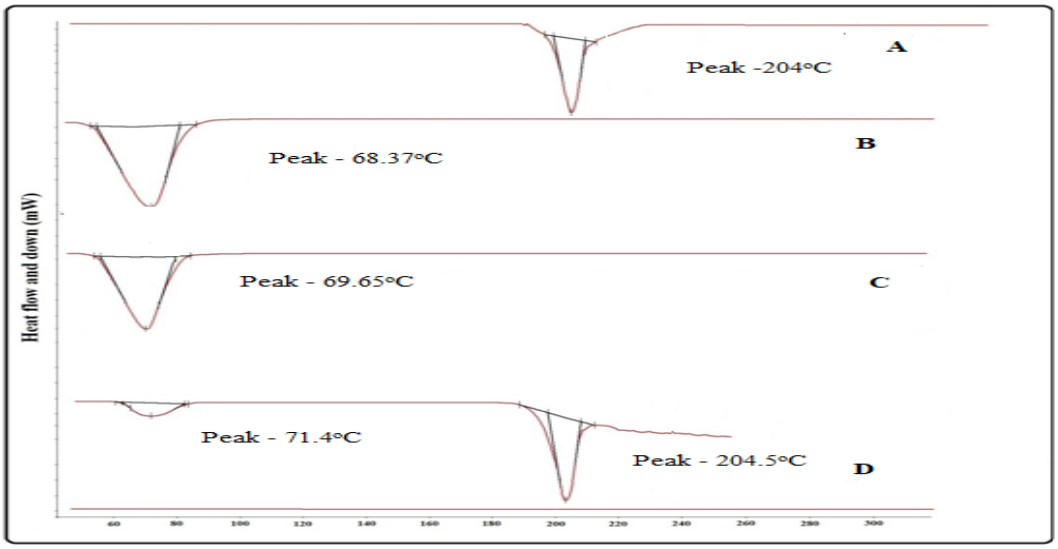

Fig.1: DSC thermograms A) Pure drug B) Compritol 888 C) GMS D) Optimized formulation

Determination of PS, PDI, ZP, assay and EE of BUS-SLNs

The ZP, PS distribution, EE and assay of the BUS-SLNs were determined. The polydispersity indices were within the permissible range (below 0.3) for developed formulations and the findigs are tabulated in Table 2. The average particle size of BUS-SLNs was found to be 163-262nm.

Table 2: Results of PS, PDI, ZP, assay and EE

Formulation	PS (nm)	PDI	ZP (mV)	Assay (%)	EE (%)
F1	249.1 ± 10.5	0.329 ± 0.03	-27.84 ± 2.79	9.42 ± 0.51	63.63 ± 0.59
F2	262.4 ± 12.5	0.314 ± 0.06	-30.25 ± 3.16	9.87 ± 0.39	67.35 ± 0.72
F3	163.8 ± 4.89	0.240 ± 0.02	-22.68 ± 2.63	10.24 ± 0.72	71.29 ± 1.12
F4	171.5 ± 5.21	0.263 ± 0.05	-26.41 ± 2.82	10.01 ± 0.27	68.54 ± 1.24
F5	214.9 ± 6.92	0.297 ± 0.04	-25.37 ± 2.52	10.11 ± 0.35	62.13 ± 0.68
F6	228.3 ± 7.84	0.312 ± 0.07	-28.59 ± 2.85	9.65 ± 0.43	66.59 ± 0.81

The particle size of SLNs compositions containing compritol ATO 888 (F1 & F2) ranged from 249.1 ±10.5 to 262.4 ±12.5 nm, the PDI went from 0.329 ±0.03 to 0.314 ±0.06, and the ZP ranged from -27.84 ±2.79 to -30.25 ±3.16 mV. GMS composed of formulations (F3 & F4) have a reduced particle size ranging from 163.8 ±4.89 to 171.5 ±5.21 nm, a lower PDI ranging from 0.240 ±0.02 to 0.263± 0.05, and a lower ZP ranging from -22.68± 2.63 to -26.41± 2.82 mV. The average particle size of stearic acid formulations (F5 & F6) was 214.9± 6.92 to 228.3± 7.84 nm, with a PDI ranging from 0.297± 0.04 to 0.312± 0.07 and a ZP ranging from -25.37 ±2.52 to -28.59± 2.85 mV.

The particle size of BUS-SLNs was shown to be dependent on the extent of the alkyl chain in the lipids; the greater the length of the alkyl chain, the larger the particle size. The stability of colloidal dispersion is maintained by the external surface charge, which plays a vital part in this process. When it comes to SLNs, particles with smaller particle sizes have a higher degree of stability at their disposal. In the formulation, the surfactant poloxamer 188 was used. It is a non-ionic surfactant, and it was responsible for reducing the electrostatic repulsion between the particles after the nanoparticles were sterically stabilised. This was accomplished by generating a coat over the surface of the nanoparticles, which helped to ensure that the stability of the SLNs was maintained.

***In vitro* drug release**

The *in-vitro* drug release of SLNs was done using a dialysis approach in pH 1.2 for 2 hours and pH 6.8 for the remaining 22 hours. The lipids, compritol ATO 888, GMS, and stearic acid formulations of SLNs showed drug release rates of 65.52% (F1), 69.53 1.12% (F2), 76.44 % (F3), 72.32% (F4), 70.16 % (F5), and 66.24 % (F6). Table 3 and Figure 2 suitably represent the release data and profiles. The results are presented in Fig 2.

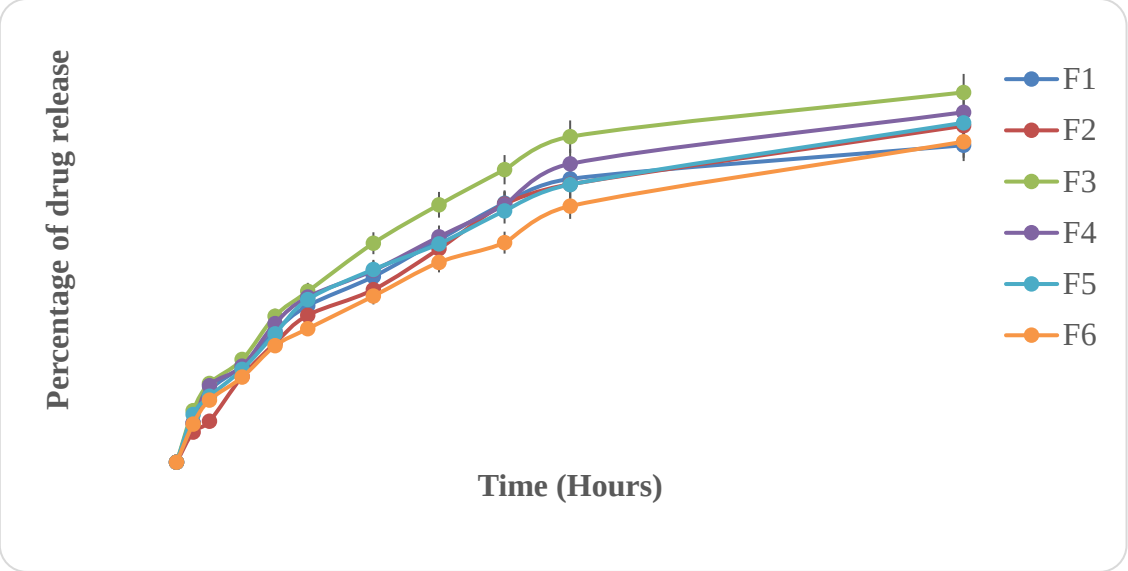

Fig 2: *In-vitro* release profiles of BUS-SLNs formulations

The *in vitro* release patterns of all BUS-SLN formulations that were developed followed a typical biphasic structure, consisting of an initial rapid phase followed by an exepidited phase release pattern observed in phosphate buffer (Takka S et al., 2003). The primary rapid phase is facilitated by the drug's bursting release. A realisable assertion is a small diffusion pathway generated by drug encapsulation in the periphery of solid-state nanoparticles (Venkateswarlu V, Manjunath K 2004).

Stability aspects of BUS-SLNs

BUS-SLNs were preserved at room and refrigerated temperature for the period of 90 days and characteristic size of particles, PDI, ZP and EE were studied and results are summarized in Table 3.

Table 3: Stability studies of BUS-SLNs (F3) (mean ± SD, n=3)

Day	At room temperature (25ºC)				At refrigerated temperature (4°C)			
	Size (nm)	PDI	ZP (mV)	EE (%)	Size (nm)	PDI	ZP (mV)	EE (%)
Initial	163.8±3.89	0.240±0.02	-26.48±2.63	71.29±0.28	164.5±2.61	0.241±0.10	-26.51±1.17	71.46±0.35
30	169.4±4.12	0.243±0.03	-25.19±2.72	70.76±0.31	171.6±4.37	0.244±0.02	-26.75±2.06	70.89±0.29
60	174.9±4.35	0.249±0.05	-29.85±2.69	68.95±0.39	175.8±3.82	0.251±0.04	-30.11±2.59	67.91±0.31
90	180.6±4.89	0.254±0.04	-28.79±2.95	66.28±0.42	181.0±5.14	0.256±0.12	-29.50±2.72	67.07±0.35

There was no significant variation in the parameters of the optimised formulation (F3) after 90 days of storage at ambient temperature (25°C) and refrigerated (4°C).

FESEM studies of BUS-SLNs

The BUS-SLN particles were round with a small size distribution and flat, uniform surfaces, as shown by their outward morphology result. The samples were looked at in a SEM with a 20kV high-vacuum setting. In the

sample, no drug crystals or groups of solid lipid nanoparticles lived coexisting together. This picture shows the shape of a particle or how particles are grouped together and pure drug exist as irregular short form of rods and the results presented in Fig 3.

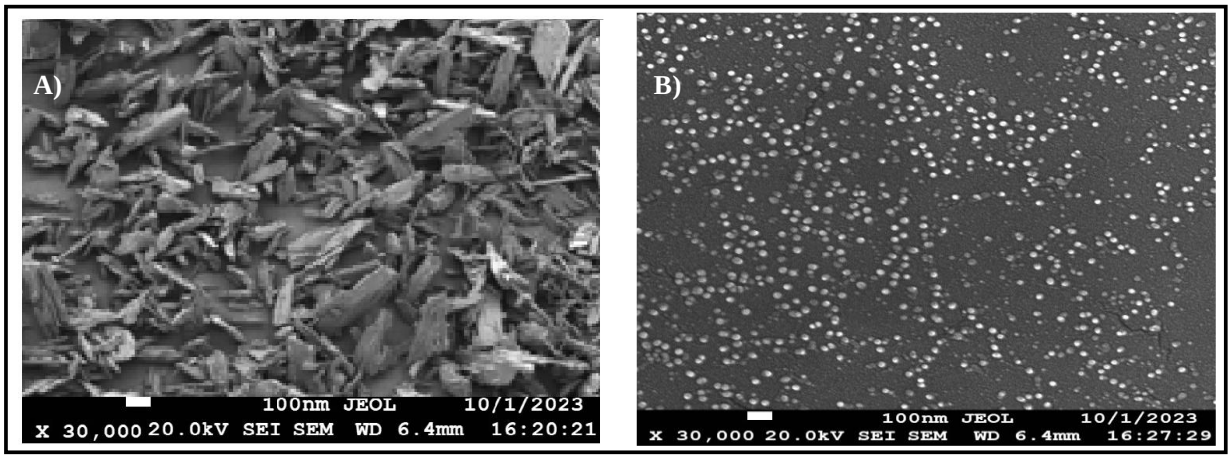

Fig.3: FESEM studies of pure drug & optimized formulation (F3)

Pharmacokinetic study of BUS-SLNs

The optimised formulation of BUS-SLNs (F3) achieved acceptable pharmacokinetic characteristics such as AUC, Cmax, Tmax, MRT, and t ½, which were then compared to the coarse suspension of the drug. The findings of the various pharmacokinetic parameters are computed and shown in Table 4 and Fig 4.

Table 4: Pharmacokineticss of BUS coarse suspension and BUS-SLNs in rats

PK parameters	**Drug coarse suspension** (n=6)	**SLNs (F3)** (n=6)
C_{max} (µg/mL)	1.332 ± 0.021	3.341 ± 0.158**
T_{max} (h)	2	2
AUC_{0-t} (µg/mL.h)	6.897 ± 0.089	15.863 ± 0.824***
t1/2 ($^{h-1}$)	2.325 ± 0.166	4.324 ± 0.733**
MRT (h)	5.418 ± 0.134	8.981 ± 0.593**

***p<0.001 and **p<0.01
Statistically significant at p<0.01 when compared with suspension.

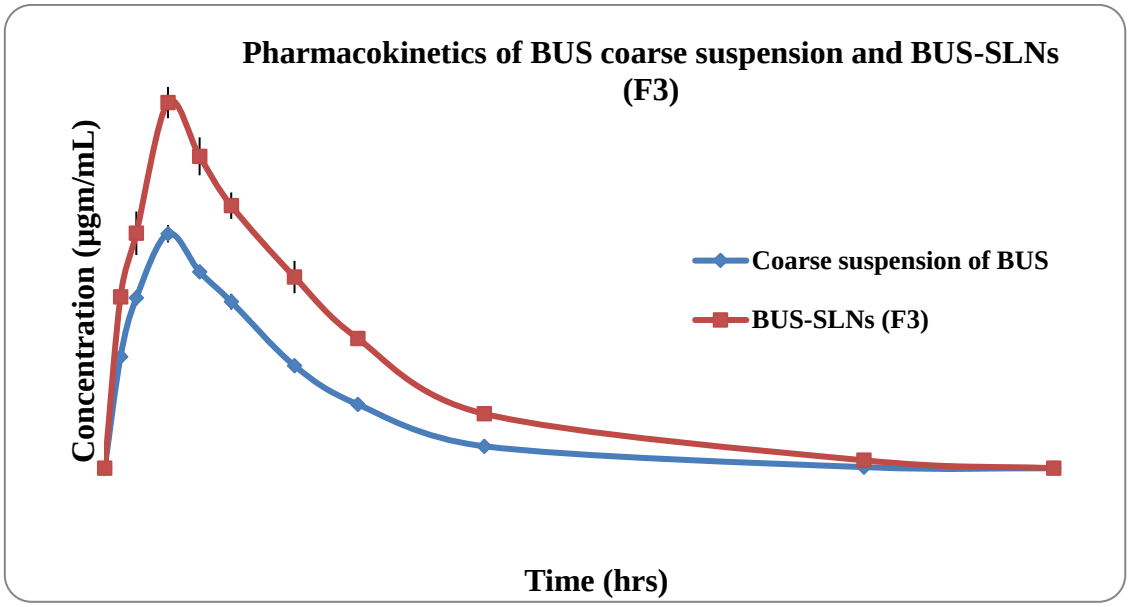

Fig 4: Pharmacokinetic profile of BUS coarse suspension & SLNs (F3)

According to the present findings, the PK study was conducted for drug suspension and SLNs (F3). The optimized formulation SLNs (F3) showed a significant improvement in PK parameters such as C_{max}, AUC_{0-t}, $t_{1/2}$, and MRT, but Tmax remains unchanged. SLNs (F3) increased bioavailability by 2.3 folds that of coarse suspension. This could be due to the smaller size and the fact that bioavailability has improved significantly (Nazief *et al.*, 2020).

Conclusion

The developed process for producing buspirone SLNs, based on hot homogenization followed by ultrasonication, was discovered to be easy and suited for manufacturing highly water-soluble drug SLNs. Remarkably, our attempt to make buspirone SLN using an easy conventional procedure indicated that employing SLN as a carrier boosted the drug's bioavailability and was found to be 2.3-fold superior to its suspension. According to the current research, SLN carriers may aid in improving buspirone bioavailability. However, more research is needed to uncover SLN lymphatic absorption mechanisms.

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