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A REVIEW: FORMULATION AND *IN-VITRO* EVALUATION OF SOLID LIPID NANOPARTICLES CONTAINING LEVOSULPIRIDE

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ABSTRACT

Objectives: The current study's objectives are to create Levosulpiride-loaded solid lipid nanoparticles (SLNs) to enhance the bioavailable portion of the medicine (often around 30% orally), decrease side effects, and minimize dosage and frequency. **Methods:** Preformulation investigations on levosulpiride included examinations of its physical characteristics, melting point, assay, calibration curve, FTIR analysis, and DSC analysis. The drug's calibration curve was created in phosphate buffer with a pH of 6.8. To make SLNs, the lipid phase of two lipids (stearic acid and palmitic acid) was employed. In order to create 16 formulations (8 for each lipid, SF1-SF8 and PF1-PF8), the factororial design (23) method was used. The solvent evaporation process was used to create levosulpiride SLNs, which were then homogenized. **Results:** Particle size analysis, zeta potential analysis, in vitro drug release, and drug release kinetics were used to describe the optimized formulations. By using FTIR, DSC, and TEM analyses, the drug-excipient interaction in the improved formulation was identified. ***Conclusion:*** The formulations SF1 (which contains stearic acid) and PF1 (which contains palimitic acid) were found to be better formulations among their groups with a regulated drug release after a period of 24 hours based on evaluation parameters.

KEYWORDS: Levosulpiride, Homogenization, Lipid, Nanoparticle, Assay, Calibration curve.

1. INTRODUCTION

Engineered adjustable devices with sizes in the billions of meters have been offered as an attractive tool that may be able to address the unmet need of improving medication transport

across the BBB in recent years with the emergence of nanomedicine.[1] The technology of nanoparticles (NPs) is growing quickly across a variety of devices. Structures having a size range of 1–100 nm in at least one dimension are referred to as nanostructures.[2] The use of science and technology in nanotechnology is to manipulate matter at the molecular level. The characteristics of matter differ dramatically from their macroscopic bulk properties at the nanoscale level.[3]

The ability to design, characterize, produce, and use structures, devices, and systems by regulating shape and size at the nanoscale scale is referred to as nanotechnology. Drugs are one industry where nanotechnology has the potential to have a big influence.[4] The introduction of various nanoscale drug delivery technologies into clinical settings has already had an impact, even if these systems' full potential is still being untapped. Small molecules that are hydrophilic or hydrophobic, peptides, protein-based medications, and nucleic acids are only a few examples of the therapeutic agents that can be contained in nanoscale drug delivery vehicles.[5]

Nanoparticles exhibit "enhanced permeability and retention effect" (EPR), which confirms their promise in precise targeting in order to maximize the therapeutic effects and minimize the unfavorable effects.[6] This is due to their distinct size range.

Solid lipid nanoparticles (SLNs), among other nanoparticles, were first developed in 1991 and offer an alternative to conventional colloidal carriers such emulsions, liposomes, and polymeric nanoparticles.[7] SLNs are tiny lipid nanoparticles made of solid lipids that are biocompatible and biodegradable. The physiological lipids that make up its matrix lessen the risk of both acute and long-term toxicity.[8]

Despite their modest size (10–1000nm), they have a considerable surface area, which increases their bioavailability and their ability to load drugs. SLNs are an intriguing drug delivery method because of these features.[9]

In the current investigation, levosulpiride (substituted benzamide: a levo-isomer of sulpiride) served as the model medication. According to Fig. (1)[10],

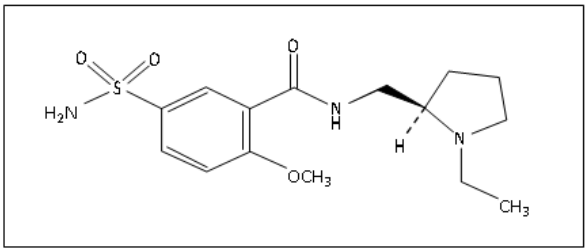

Fig. 1: Chemical structure of levosulpiride.

Levosulpiride is a chemical compound with the chemical formula N-[[(2S)-1-Ethylpyrrolidin-2-yl] methyl]-2-methoxy-5- sulfamoylbenzamide with a molecular weight of 341.43.

The presynaptic dopaminergic D2 receptors are blocked by levosulpiride. Levosulpiride exhibits antagonistic activity at D3 and D2 receptors that are present both pre- and postsynaptically, just like its parent drug. At low concentrations, the preferential binding of the presynaptic dopamine receptors reduces dopamine synthesis and release, whereas at greater levels, it results in antagonistic postsynaptic D2 receptors.[11] It is an atypical neuroleptic (anti-psychotic), and because it also has a prokinetic action that has been demonstrated, it can be used to treat a variety of GI diseases. Additionally, serotonergic receptors are said to be affected by it. It possesses extremely mild 5-HT3 antagonistic and moderate to partial 5-HT4 receptor agonist properties.[12]

Thereby serving as a helpful antiemetic. Unlike metoclopramide, levosulpiride rarely results in extrapyramidal side effects. At excessively high doses, extrapyramidal or sleep problems may be observed. It is a helpful medication for IBS, gastroesophageal reflux illness, nausea and vomiting, chemotherapy-induced emesis, and diabetic gastroparesis. The most frequent negative effects include drowsiness/sedation and endocrine consequences such amenorrhea, gynecomastia, galactorrea, and decreased libido.[13] It can also be taken parenterally.

Levosulpiride's bioavailability is less than 30%, which suggests a first pass impact. 3 hours make up Tmax. The substance has a 6 hour half life (t1/2), and it takes 3 hours to reach the maximum concentration (cmax). Over 40% of plasma proteins are bound. Distribution volume is 0.85 L/kg.[7] The liver is said to have an extremely sluggish rate of metabolism. The 25% share for renal excretion has not altered. Due to limited absorption, the medication is largely eliminated in the feces. Metabolic interactions with substrates associated to cytochrome P-450 are extremely rare due to the absence of hepatic metabolism.[14]

Levosulpiride is currently available in extended- and immediate-release tablet dosage forms for oral administration (e.g., Pirivo, Perfame, Levogut). The main problem with currently marketed formulations is that the medicine is only mildly present in the target tissue. The dosage form that is already on the market does not deliver the appropriate level of therapeutic impact due to the low bioavailable fraction of the drug that reaches the CNS of schizophrenia patients. The goal of the current study is to shed light on the state-of-the-art in schizophrenia treatment and develop Levosulpiride-loaded nanoparticles that could circumvent the bioavailability issue and cross the blood-brain barrier with sustained effect, reducing the frequency of dosing and boosting patient compliance.

The purpose of the current study was to create Levosulpiride-loaded SLNs using a solvent evaporation and homogenization technique, and to assess the physicochemical characteristics of the resulting Levosulpiride-loaded SLNs, including mean particle size, zeta potential, drug entrapment effectiveness, in vitro drug release, and drug release kinetics evaluation. In-depth research was done on the impacts of lipid material composition and surfactant combination on particle size, zeta potential, drug entrapment effectiveness, and in vitro drug release behavior. Analyses of the lipid and drug status were carried out using FTIR and DSC. TEM was used to determine the surface morphology and shape of the synthesized nanoparticles**.**

2. MATERIALS AND METHOD

Chemicals

A free sample of levosulpiride was obtained from Shagun Pharmaceuticals Pvt. Ltd. in Maharashtra, India. CDH (P) Ltd., New Delhi generously provided the stearic and palmitic acids. Qualikems Fine Chemicals Pvt. Ltd. sold us tween 80. Span 60, ethanol, and dialysis membrane-70 (MW cutoff, 8–12 kDa) were all obtained from Changshu Yangyuan Chemicals in China, Loba Chemie in Mumbai, India, and Hi-Media in Mumbai, India, respectively. The other substances, which were analytical reagent grade, were used exactly as they were given.

Preformulation Studies

Melting Point

A little amount of the medication was inserted in a capillary tube that was closed at one end and placed in a digital melting point equipment. The temperature at which the drug melted was then recorded.

Assay

The medication was tested using a UV spectrophotometric technique. Levosulpiride (10 mg) was dissolved in a few milliliters of phosphate buffer (pH 6.8) before the volume in the volumetric flask was increased to 100 milliliters. A 1 ml solution was taken from this stock solution and diluted up to 10 ml in a volumetric flask (10 g/ml). Using a UV spectrophotometer, the solution's absorbance was determined at the scanned wavelength (291.2 nm).[15]

Calibration Curve

Levosulpiride, accurately weighed at 10 mg, was put into a volumetric flask with a capacity of 10 ml. It was given a good shake after receiving a few mL of pH 6.8 phosphate buffer. To create a stock solution, the solution was diluted to the appropriate level with pH 6.8 phosphate buffer and sonicated for 1 minute in a bath sonicator. There were further dilutions made from this stock solution.

Preparation of Levosulpiride Loaded Solid Lipid Nanoparticles

To make the lipid phase, ethanol was mixed with levosulpiride, stearic acid/palmitic acid, and span 60. Tween 80 was combined with distilled water using a magnetic stirrer to create the aqueous phase. When a moist mass of drug-embedded lipid layer was remained after the ethanol from the lipid phase had evaporated, it was slowly added to an aqueous solution and homogenized at 10,000 rpm for 10 minutes.[16,17] For formulation batches (i.e., 8 formulations for each lipid), a full factorial design (23) was used in which 3 factors—lipid (stearic acid and palmitic acid), span 60, and tween 80—were examined at 2 levels of concentration, low and high.

The impact of various amounts of variables was assessed at the formulation's resultant particle size (i.e. response). The ingredients of the formulation batches for stearic acid and palmitic acid are listed in Tables 1 and 2, respectively.

Table 1: Composition of Stearic acid formulations.

Ingredients	Formulations							
	SF1	SF2	SF3	SF4	SF5	SF6	SF7	SF8
Levosulpiride (mg)	10	10	10	10	10	10	10	10
Stearic Acid (mg)	100	200	100	200	100	200	100	200
Span 60 (mg)	50	50	100	100	50	50	100	100
Tween 80 (mL)	0.50	0.50	0.50	0.50	0.75	0.75	0.75	0.75
Ethanol (mL)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Ingredients	Formulations							
	SF1	SF2	SF3	SF4	SF5	SF6	SF7	SF8
Dist. Water (mL)	25	25	25	25	25	25	25	25

Characterization of Formulated SLNs

Measurement of Particle Size and Zeta Potential

Using a Beckman Coulter Zeta sizer, the average particle size and zeta potential of the Levosulpiride-loaded SLN formulations were calculated. We calculated the average particle size, the number of particles in the size range, and the zeta potential.

Table 2: Composition of Palmitic acid formulations.

Ingredients	Formulations							
	PF1	PF2	PF3	PF4	PF5	PF6	PF7	PF8
Levosulpiride (mg)	10	10	10	10	10	10	10	10
Palmitic Acid (mg)	**100**	**200**	**100**	**200**	**100**	**200**	**100**	**200**
Span 60 (mg)	50	50	100	100	50	50	100	100
Tween 80 (mL)	0.50	0.50	0.50	0.50	0.75	0.75	0.75	0.75
Ethanol (mL)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Distilled Water (mL)	25	25	25	25	25	25	25	25

Drug Entrapment Efficiency

The percentage of entrapped Levosulpiride was determined spectrophotometrically at 291.2 nm. After centrifugation of the aqueous suspension, the amount of the free drug was detected in the supernatant and the amount of entrapped drug was determined as the result of the initial drug minus the free drug.[18] The entrapment efficiency can be calculated using the following formula:

$$\%EE = \{(\text{Total drug content} - \text{Free drug content})/\text{Total drug content}\} \times 100$$

In Vitro Drug Release Study

A 24-hour drug release investigation was conducted in phosphate buffer at pH 6.8. The procedure previously mentioned was used to prepare the buffer. A 50 mL pH 6.8 phosphate buffer maintained at 37°C with continuous stirring with a magnetic stirrer was incubated with 10 mL of formulation (put in a tiny cylinder fitted with an 8–12 kDa membrane at the bottom) to conduct an in vitro drug release investigation.[19] Periodically, the samples (2 mL each) were removed, and after each removal, an equal volume of medium was replenished. Using a UV-Visible double beam spectrophotometer to measure absorbance at 291.2 nm, the samples were then examined to determine the amount of medication released.[20]

Drug Release Kinetics

To describe the drug release process, the cumulative amounts of levosulpiride released from polymeric nanoparticles at various intervals were fitted with the zero-order kinetic model, first order kinetic model, Higuchi model, and Korsmeyer- Peppas model.[21,22]

Fourier Transform Infra-Red Spectroscopy (FTIR)

To investigate the functional groups contained in the various samples, the FTIR spectra of the natural medication Levosulpiride and the chosen formulations were both recorded. The medication and formulation samples were individually compressed to create the KBr sample discs. Using an FTIR spectrophotometer, the infrared spectra were captured in the wave number range of 4000-400 cm-1, and the distinctive bands were seen.

Differential Scanning Calorimetry

Using a Universal V4.5A TA DSC (Universal Instruments, USA), the thermal behavior of levosulpiride and its formulations was studied. 3.13 mg of precisely weighed samples were put in common aluminum pans and sealed with a perforated lid. The purge gas was dry nitrogen, flowing at a rate of 50 mL/min. The samples were heated at a rate of 10°C/min between 30°C and 300°C in order to produce the thermograms. We saw the melting point.

Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) was used to examine the morphology of the produced nanoparticles at a magnification of 2,70,000X. Drug-loaded SLNs were sonicated after being diluted with distilled water. A few drops of the diluted nanoparticles were dropped onto a Copper (Cu) grid, which was then put in a sample holder and put into the machine to take pictures of the particles.

1. RESULTS AND DISCUSSION

Preformulation Studies

Levosulpiride has a reported melting point of 177–181°C and appears as a white to cream-colored crystalline powder.

Melting Point

Along with the reported melting point range, the value of the observed melting point range is provided in Table 3 below.[23] The melting point value that was actually observed matched the reported value.

Table 3: Observed melting point of levosulpiride.

Parameter	Reference value	Experimental value
Melting point	177-181°C	180±0.6°C

*Mean ± S.D.

Assay

A UV spectrophotometric approach was used to test the medication, with a scanned wavelength of 291.2 nm. The drug content was discovered to be between 98.90 and 99.82%, which is acceptable.[15]

Calibration Curve

Each solution's absorbance was measured at 291.2 nm with a pH 6.8 phosphate buffer serving as the blank. Levosulpiride's correlation coefficient, regression equation, and calibration curve of absorbance vs. concentration were all calculated. In a concentration range of 10 to 100 g/mL, the medication was discovered to exhibit linearity.[23–24] In Fig. (2), the calibration curve is displayed.

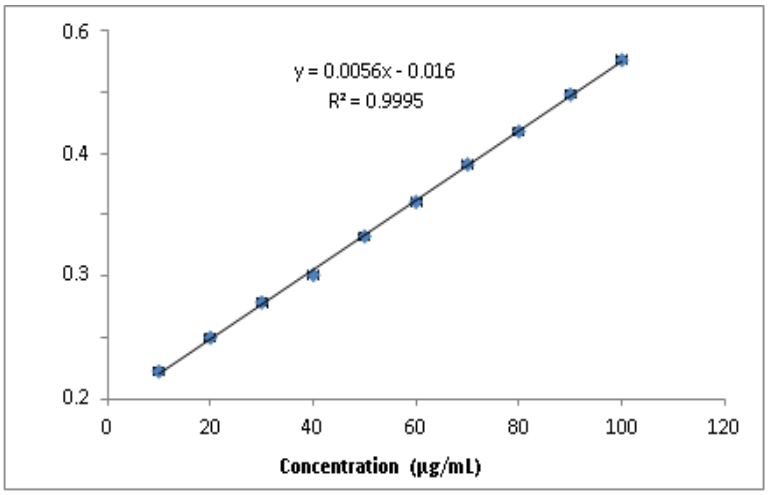

Fig. 2: Calibration curve of drug in pH 6.8 phosphate buffer. The error bars indicate the standard deviation of three tests.

Measurement of Particle Size and Zeta Potential

One of the initial goals was to determine the experimental parameters that control the particle size because our goal was to produce SLNs with particle sizes that were suitable for brain applications. For particle size analysis, the formulations were described. Six formulations (three of each lipid), SF1, SF2, SF6, and PF1, PF2, PF6 were found to have particle sizes within the required nanometer (nm) size range, while the remaining formulations were found

to have particle sizes greater than 250 nm. A total of 16 formulations (SF1-SF8 & PF1-PF8) were created using a 23 full factorial design.

Due to their smaller particle size and lower polydispersity index (an indicator of formulation stability and homogeneity of dispersion) than other formulations, the combination of tween 80 (an aqueous surfactant) and span 60 (a lipid surfactant) was found to be ideal in some formulations (Tables 4 and 5). This was most likely caused by increased drug solubilization in lipid and improved formulation stabilization. Zeta potential investigations (Table 6) validated these findings even further. The formulations' zeta potentials, which ranged from +15 to +25 for both the SF and PF batches, showed that the SLNs had a high degree of stability. We set out to comprehensively characterize the created SLNs for their stability after successfully improving the SLNs formulation and achieving particle within the administration range for drug delivery to brain. The stability of SLNs during synthesis and storage is important because unstable particles may congregate and expand. One of the metrics for the stability of SLNs is the particle's zeta potential. Further research on these formulations was taken into consideration.

Table 4: Particle size and PDI of Stearic acid formulations (SF Batches).

Sr. No.	Formulation	Particle Size (nm)	Polydispersity Index (PDI)
1.	SF1	124.4	0.262
2.	SF2	179.3	0.261
3.	SF3	281.9	0.278
4.	SF4	298.1	0.317
5.	SF5	298.0	0.330
6.	SF6	143.8	0.256
7.	SF7	269.8	0.650
8.	SF8	331.4	0.397

Table 5: Particle size and PDI of Palmitic acid formulations (PF Batches).

Sr. No.	Formulation	Particle Size (nm)	Polydispersity Index (PDI)
1.	PF1	137.1	0.269
2.	PF2	189.7	0.240
3.	PF3	231.7	0.217
4.	PF4	246.3	0.339
5.	PF5	284.5	0.219
6.	PF6	154.1	0.241
7.	PF7	285.8	0.362
8.	PF8	318.1	0.415

Table 6: Zeta potential of selected formulations from SF and PF Batches.

Sr. No.	Formulation	Zeta Potential (mV)
1.	SF1	+15.74
2.	SF2	+25.89
3.	SF6	+25.44
4.	PF1	+21.53
5.	PF2	+19.69
6.	PF6	+15.11

Drug Entrapment Efficiency

The UV-Visible spectrophotometer was used to measure the entrapment effectiveness of the prepared SLNs. The maximum absorbance at 291.2 nm was identified, and following calculations, the entrapment efficiency was calculated and is shown in the table below. The strong affinity of the lipophilic drug for the lipid material and the presence of span 60 are likely to be the causes of the beneficial effect that lipids have on entrapment efficiency. The chosen formulations could entrap 90% or more of the medication. The outcomes are listed in Table 7 below.

Table 7: Entrapment Efficiency of selected formulations.

Sr. No.	Formulation	Entrapment Efficiency (%)
1.	SF1	90.79
2.	SF2	88.57
3.	SF6	90.29
4.	PF1	92.04
5.	PF2	89.62
6.	PF6	91.16

***In Vitro* Drug Release Study**

To determine the release profile, the cumulative amount of medication release was plotted versus time. A rapid initial release was noted, which was followed by a slower release rate. The slower release in the later stage was related to the fact that solubilized drug can only be released slowly from the lipid matrices due to dissolution and diffusion. The first burst rate may be caused by the drug desorption linked with the surface of nanoparticles. In phosphate buffer at pH 6.8, all 6 formulations were successful in releasing the medication over the course of up to 24 hours. Figures (3 and 4) depict the release pattern seen in formulations including stearic acid and palmitic acid, respectively. Dissolution data is provided below (Table 8).

% Cummulative Drug Release

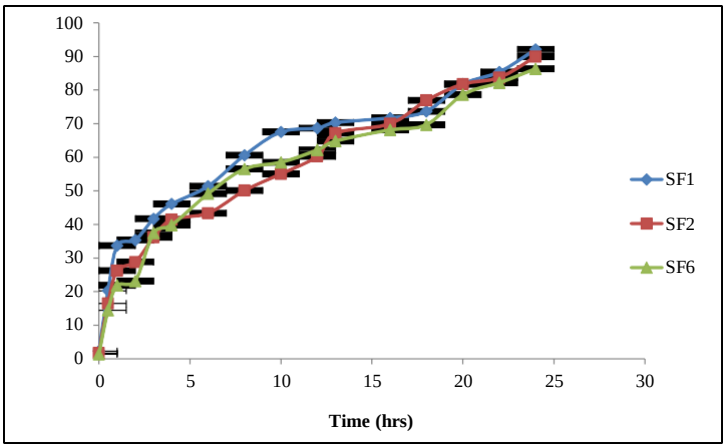

Fig. (3): % Cumulative drug release of Levosulpiride loaded SLNs from Stearic acid batch (selected formulations). The error bars indicate the standard deviation of three tests.

% Cummulative Drug Release

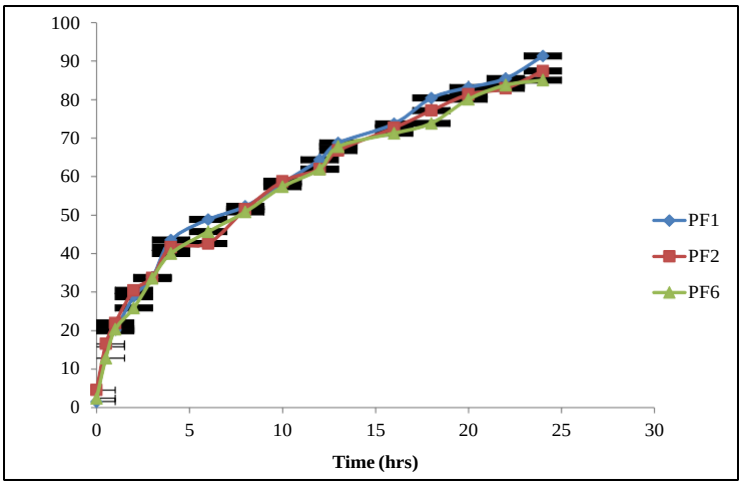

Fig. 4: % Cumulative drug release of Levosulpiride loaded SLNs from Palmitic acid batch (selected formulations). The error barsindicate the standard deviation of 3 tests.

Table 8: % cumulative drug release of Levosulpiride loaded SLNs.

Time	% Cummulative Drug Released ± SD (n=3)					
	SF1	SF2	SF6	PF1	PF2	PF6
0 min	2.33±0.35	1.72±0.20	1.38±0.08	1.55±0.07	4.55±0.28	2.44±0.67
30 min	20.27±0.34	16.49±0.13	14.46±1.29	15.83±0.13	16.55±0.15	12.83±0.13
1 hr	33.71±0.47	26.27±0.20	21.88±0.28	19.99±0.23	21.99±0.13	20.33±0.75
2 hr	35.38±0.79	28.83±0.20	23.16±0.13	28.83±0.26	30.49±0.23	25.88±0.08
3 hr	41.72±0.20	36.16±0.23	37.49±0.13	33.83±0.26	33.72±0.20	33.55±0.20
4 hr	46.11±0.54	41.49±0.36	39.83±0.13	43.55±0.20	41.66±0.23	40.05±0.54
6 hr	51.38±0.47	43.33±0.13	49.16±0.13	48.88±0.90	42.61±0.28	45.71±0.47
8 hr	60.61±0.43	50.10±0.34	56.55±0.20	52.27±1.02	51.49±0.13	50.83±0.13

10 hr	67.55±0.34	55.05±0.31	58.49±0.27	58.38±0.82	58.77±0.16	57.38±2.39
12 hr	68.66±0.36	60.27±0.16	62.16±0.13	64.38±1.80	61.94±0.90	61.94±1.01
14 hr	70.33±0.49	67.16±0.36	64.82±0.23	68.77±1.02	66.77±1.14	67.83±1.29
16 hr	71.72±0.28	69.94±0.28	68.16±0.13	73.71±0.75	72.66±1.20	71.27±0.88
18 hr	73.66±0.27	76.94±0.15	69.66±0.13	80.44±1.41	77.16±3.08	73.83±0.35
20 hr	81.77±0.41	81.77±0.39	78.66±0.23	83.16±1.77	81.33±0.81	80.10±0.034
22 hr	85.38±0.69	83.71±0.47	82.16±0.36	85.49±1.53	82.94±0.34	83.77±0.56
24 hr	92.11±0.43	89.94±0.28	86.33±0.35	91.33±0.13	87.44±0.20	85.05±0.34

Drug Release Kinetics

The drug release kinetics and release mechanism were tested on the produced SLNs. The formulations were examined by fitting the drug release time profile with several equations, including the Higuchi and Korsmeyer pappas, the first order, zero order, and first order. For the drug release mechanism, all of the formulations (SF1, SF2, SF6, PF1, PF2 and PF6) were examined.

The data showed that Fickian diffusion for SF batch formulations and time-dependent drug release provided a superior fit to the Higuchi diffusion model with n value smaller than 0.43. Contrarily, Higuchi anomalous diffusion (non-Fickian) for PF batch formulations was discovered due to n value > 0.431, which may be explained by the fact that the diffusion relates to a mixture of diffusion and erosion regulated rate release.[25]

Tables (9 and 10) and Figures (5 and 6) following present the findings:

Table 9: Drug release kinetics for Stearic acid formulations.

Formulations	Zero Order		First Order		Higuchi		Korsmeyer Peppas	
	$K(h^{-1})$	R^2	$K\ (h^{-1})^1$	R^2	$K\ (h^{-1/2})^H$	R^2	N	R^2
SF1	3.003	0.906	0.038	0.963	16.71	0.984	0.256	0.968
SF2	3.062	0.937	0.034	0.966	16.82	0.991	0.321	0.975
SF6	2.991	0.902	0.030	0.976	16.72	0.988	0.405	0.973

Table 10: Drug release kinetics for Palmitic acid formulations.

Formulations	Zero Order		First Order		Higuchi		Korsmeyer Peppas	
	$K\ (h^{-1})$	R^2	$K\ (h^{-1})$ 1	R^2	$K\ (h^{-1/2})$ H	R^2	N	R^2
PF1	3.223	0.928	3.223	0.928	17.84	0.996	0.464	0.982
PF2	3.046	0.937	3.046	0.937	16.77	0.996	0.496	0.989
PF6	3.109	0.929	3.109	0.929	17.21	0.997	0.464	0.984

FTIR Studies

FTIR spectrum and DSC thermogram analyses were used in the compatibility studies. The

results for the individual FTIR spectra of levosulpiride and lipids (stearic acid/phosphoric acid) and the final formulation suggested that the characteristic bands seen in levosulpiride pure samples were largely identical, and the peaks in the final formulation of levosulpiride and lipids adhered within their ranges without changes in the functionalities, indicating its compatibility (Fig. (7)).

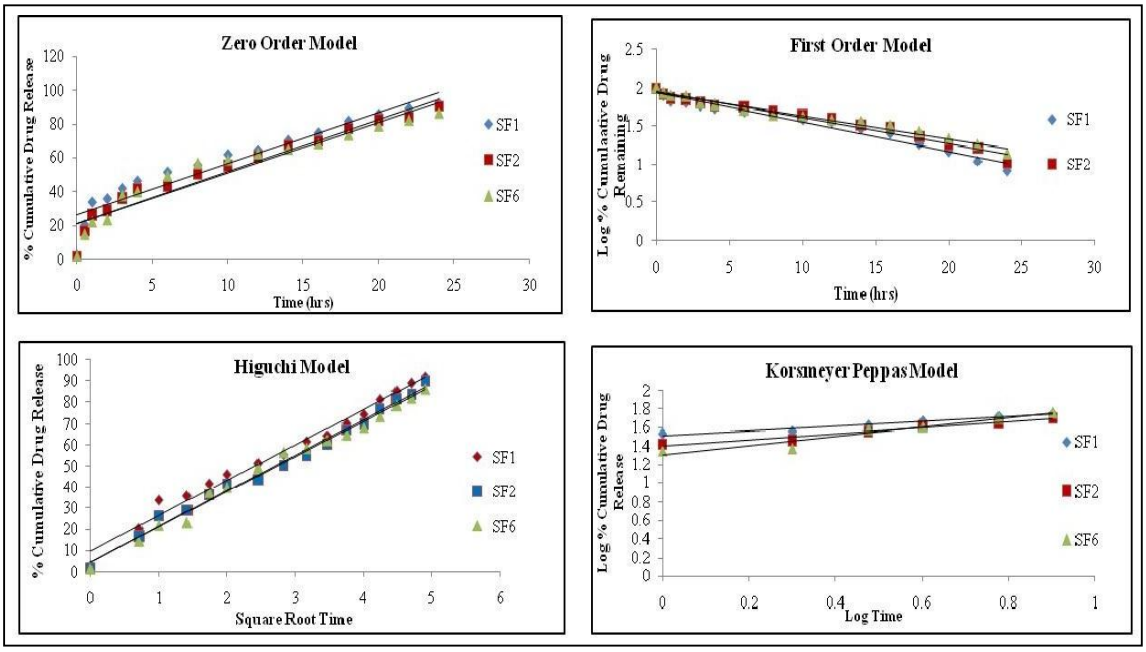

Fig. 5: Kinetics of drug release for Stearic acid formulations- a). Zero order model; b). First order model; c). Higuchi model; d).

Korsmeyer-Peppas model.

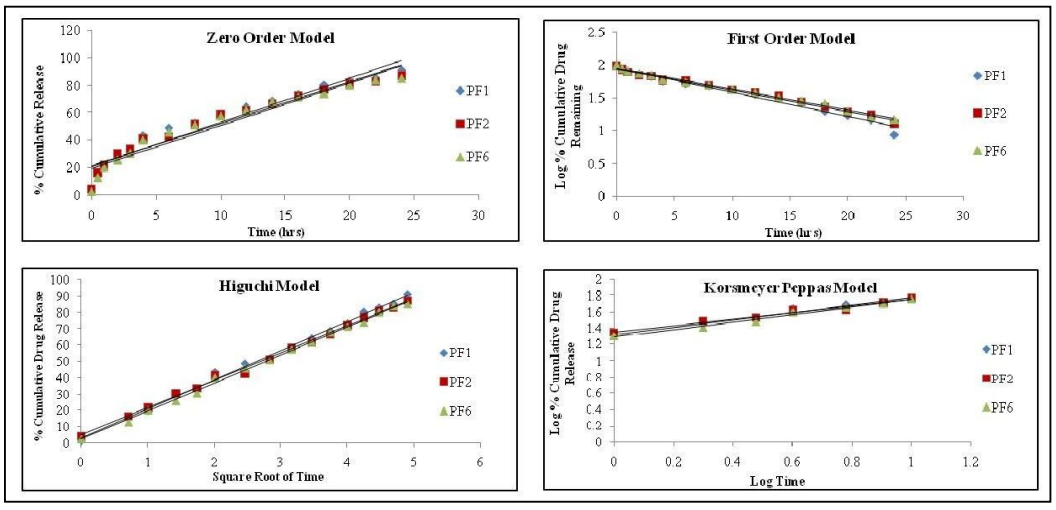

Fig. 6: Kinetics of drug release for Palmitic acid formulations- a). Zero order model; b). First order model; c). Higuchi model; d).

Korsmeyer-Peppas model.

DSC Studies

Because there are no melting events of Levosulpiride on the SLN formulations in DSC themograms (Fig. 8), it is obvious that the medication was solubilized on the lipid. In addition, a little reduction in the lipid's onset and melting temperature when it is drug-formulated was noted. Peak width values make this more clear: the decline was greater as drug dosage increased.

Transmission Electron Microscopy (TEM)

The Hitachi H7500 was used to take the TEM pictures for the formulations SF1 and PF1 (Figs. 9 and 10, respectively). According to the photos, the Levosulpiride-loaded solid lipid nanoparticles were below 250 nm in size and had an uneven, rough-surfaced sphere form.

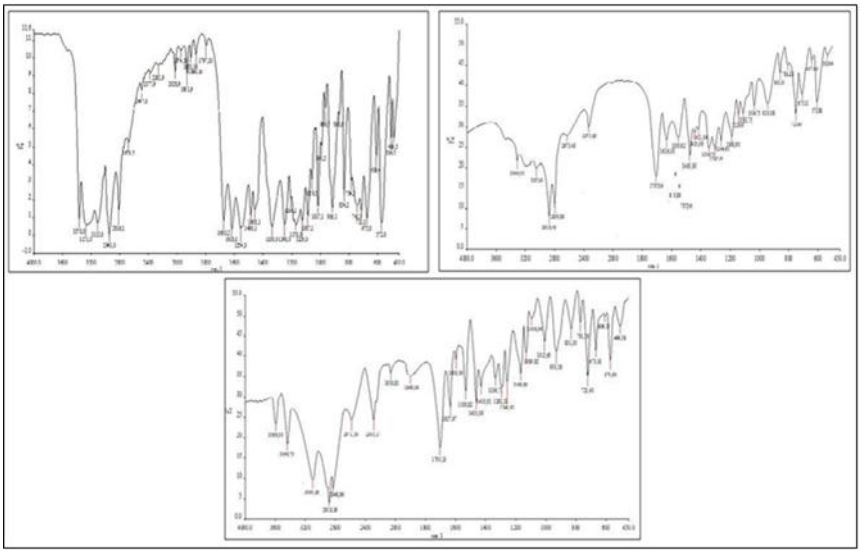

Fig. 7: FTIR spectra of- a). Levosulpiride; b). Formulation SF1; c). Formulation PF1.

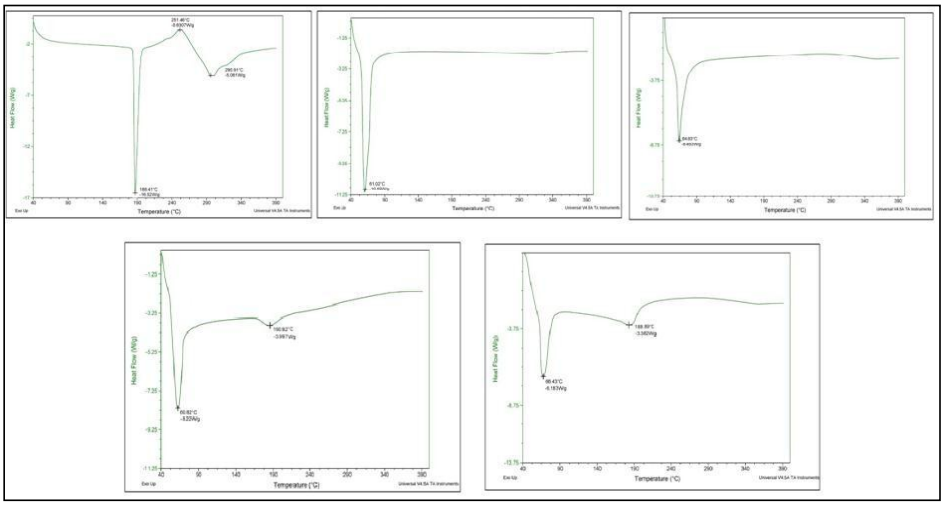

Fig. 8: DSC Thermograms of- a). Levosulpiride (Pure Drug); b). Stearic acid; c).

Palmitic acid; d). Formulation SF1; e).

Formulation PF1.

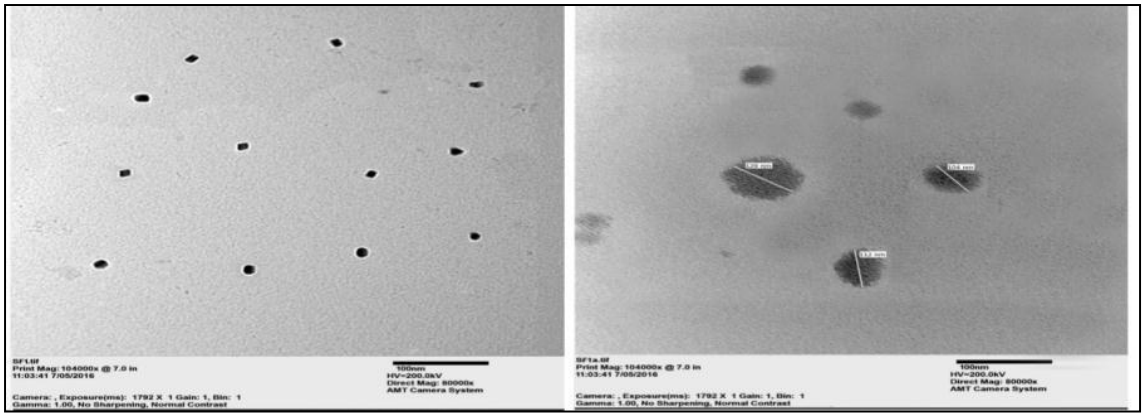

Fig. 9: TEM images of formulation SF1.

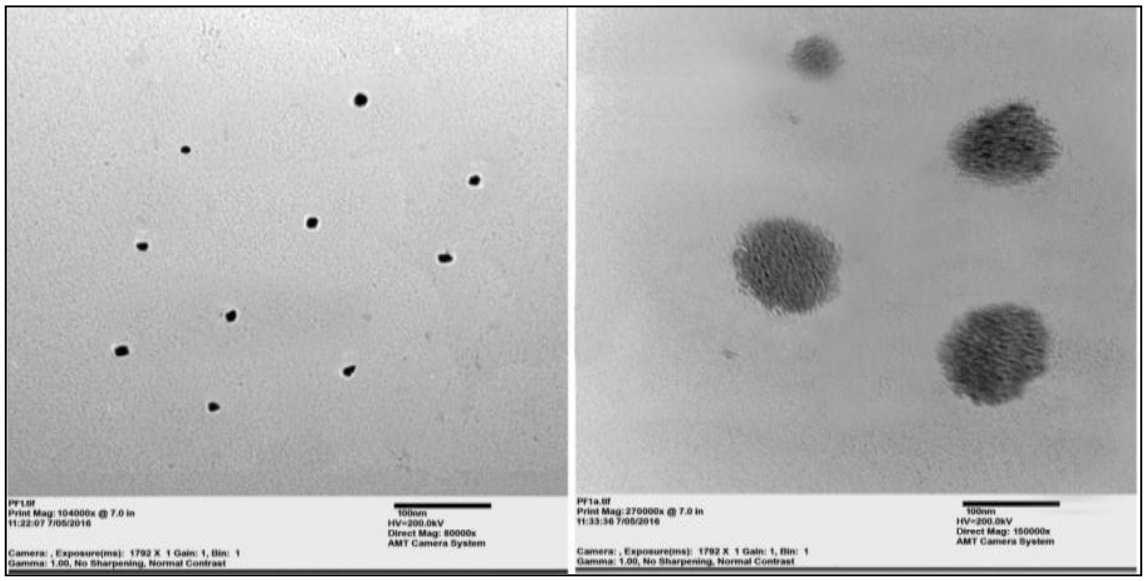

Fig. 10: TEM images of formulation PF1.

CONCLUSION

A 23 complete factorial design was used to create a total of 16 formulations, 8 for each lipid (stearic acid and palmitic acid; SF1-SF8 and PF1-PF8 respectively). Particle size analysis, zeta potential, drug entrapment effectiveness, and an in vitro drug release research were used to describe optimized formulations. Using Zetasizer, the particle sizes of SF1, SF2, SF6, PF1, PF2, and PF6 were determined to be, respectively, 124.4 nm, 179.3 nm, 143.8 nm, 137.1, 189.7, and 154.1 nm, which was within the intended range. Zeta potentials of +15 to +30 mV were found to be appropriate for the stability of SLN formulations.

And for some formulations, drug entrapment efficiency was found to be around 90%. The cumulative drug release after 24 hours from SF1, SF2, SF6, PF1, PF2 and PF6 was calculated from an in vitro drug release research to be 92.1143%, 89.940.28%, 86.330.35%, 91.3313%, 87.440.20% and 85.050.34%, respectively. The Higuchi diffusion model was determined to be the mechanism of drug release, with non-Fickian diffusion for PF batch formulations and Fickian diffusion for SF batch formulations. From each batch, the formulation with the highest cumulative% drug release (SF1 and PF1) was chosen for additional testing. The reduction in particle size and the drug's transformation from crystalline to amorphous nature were both confirmed by a drop in the enthalpy and onset temperature for the melting points of lipids (stearic acid and palmitic acid) in the DSC thermograms. No physical or chemical changes were discovered using FTIR analysis. Using TEM images, morphological investigations revealed round to oval particles with well-defined peripheries. The center of the SLNs looked to be less thick, and the shell was clearly delineated. Levosulpiride has been successfully included into SLNs, which expands the scope of research into the delivery system with regard to sustained and targeted drug delivery. To confirm the potential of compounded SLNs, in vivo research must still be conducted.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

REFERENCES

1. Malewar N, Avachat M, Pokharkar V, Kulkarni S. Controlled release of ropinirole hydrochloride from a multiple barrier layer tablet dosage form: effect of polymer type on pharmacokinetics and IVIVC. AAPS PharmSciTech., 2013; 14(3): 1178-89.

[http://dx.doi.org/10.1208/s12249-013-0009-3] [PMID: 23897037]

2. Costa P, Sousa Lobo JM. Modeling and comparison of dissolution profiles. Eur J Pharm Sci., 2001; 13(2): 123-33. [http://dx.doi.org/10.1016/S0928-0987(01)00095-1] [PMID: 11297896]
3. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol Pharm., 2010; 67(3): 217-23.
4. [PMID: 20524422]
5. Nazir I, Abbas J, Asad M, Bashir S, Rasul A, Ahmad N. Formulation and *in vitro* Evaluation of Orodispersible tablet of Levosulpiride. J Pharm Cosm Sci., 2013; 1: 53-6.
6. Kumar R, Singh S, Kamal SS, Kaur D, Singh M, Katual MK. Development and Validation of Spectrophotometric Method for Estimation of Levosulpiride in Bulk and Tablet Dosage Form. Euras J Anal Chem., 2017; 12: 265-73.
7. Kushwaha AK, Vuddanda PR, Karunanidhi P, Singh SK, Singh S. Development and Evaluation of Solid Lipid Nanoparticles of Raloxifene Hydrochloride for Enhanced Bioavailability. BioMed Res Int., 2013; 2013: 584549. [http://dx.doi.org/10.1155/2013/584549]
8. Kim BD, Na K, Choi HK. Preparation and characterization of solid lipid nanoparticles (SLN) made of cacao butter and curdlan. Eur J Pharm Sci., 2005; 24(2-3): 199-205. [http://dx.doi.org/10.1016/j.ejps.2004.10.008] [PMID: 15661491]
9. Ji P, Yu T, Liu Y, *et al.* Naringenin-loaded solid lipid nanoparticles: preparation, controlled delivery, cellular uptake, and pulmonary pharmacokinetics. Drug Des Devel Ther., 2016; 10: 911-25. [PMID: 27041995]
10. Tonini M, Cipollina L, Poluzzi E, Crema F, Corazza GR, De Ponti F. Review article: clinical implications of enteric and central D2 receptor blockade by antidopaminergic gastrointestinal prokinetics. Aliment Pharmacol Ther., 2004; 19(4): 379-90. [http://dx.doi.org/10.1111/j.1365-2036.2004.01867.x] [PMID: 14871277]
11. Corazza GR, Tonni M. Levosulpiride for Dyspepsia and Emesis: A review of its Pharmacology, Efficacy and Tolerability. Clin Drug Investig, 2000; 19: 151-62. [http://dx.doi.org/10.2165/00044011-200019020-00008]
12. Pharmacokinetics. Pantocid.com. Available at: http://pantocid.com/pharmacokinetics.html
13. Yoganand BD, Jigar AP, Moreshwar PM, Sanjay DS. UV spectrophotometric method for simultaneous estimation of rabeprazole sodium and levosulpiride in bulk and tablet dosage form. Pharma Chem., 2013; 5: 163-8.
14. Mehnert W, Mäder K. Solid lipid nanoparticles: Production, characterization and

applications. Adv Drug Deliv Rev., 2001; 47(2-3): 165-96. [http://dx.doi.org/10.1016/S0169-409X(01)00105-3] [PMID: 11311991]

15. Müller RH, Mäder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery - a review of the state of the art. Eur J Pharm Biopharm, 2000; 50(1): 161-77. [http://dx.doi.org/10.1016/S0939-6411(00)00087-4] [PMID: 10840199]
16. Mehnert W, Mäder K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev., 2001; 47(2-3): 165-96. [http://dx.doi.org/10.1016/S0169-409X(01)00105-3] [PMID: 11311991]
17. Severino P, Andreani T, Macedo AS, Fangueiro JF, Santana MH, Silva AM, *et al.* Current state-of-art and new trends on lipid nanoparticles (SLN and NLC) for oral drug delivery. J Drug Del., 2012; 2012: 750891.
18. Martindale: The complete drug reference. Published by Pharmaceutical Press, Lambeta high street, Londan SEI 75M, UK. 2006.
19. Song CW, Chun HJ, Kim CD, Ryu HS, Choe JG, Hyun JH. Effects of levosulpiride in patients with functional dyspepsia accompanied by delayed gastric emptying. Korean J Intern Med., 1998; 13(1): 15-21. [http://dx.doi.org/10.3904/kjim.1998.13.1.15] [PMID: 9538626]
20. Re F, Gregori M, Masserini M. Nanotechnology for neurodegenerative disorders. Maturitas, 2012; 73(1): 45-51. [http://dx.doi.org/10.1016/j.maturitas.2011.12.015] [PMID: 22261367]
21. Youns M, Hoheisel JD, Efferth T. Therapeutic and diagnostic applications of nanoparticles. Curr Drug Targets., 2011; 12(3): 357-65. [http://dx.doi.org/10.2174/138945011794815257] [PMID: 20955146]
22. Uchegbu IF. Pharmaceutical nanotechnology: polymeric vesicles for drug and gene delivery. Expert Opin Drug Deliv, 2006; 3(5): 629-40. [http://dx.doi.org/10.1517/17425247.3.5.629] [PMID: 16948558]
23. Farokhzad OC. Nanotechnology for drug delivery: The perfect partnership. Expert Opin Drug Deliv., 2008; 5(9): 927-9. [http://dx.doi.org/10.1517/17425247.5.9.927] [PMID: 18754745]
24. Sun Y, Kang C, Wang M, Zhu J, Jin L, Cheng X. Nanosized camptothecin conjugates for single and combined drug delivery. Eur J BioMed Res., 2016; 2: 8-16.
25. [http://dx.doi.org/10.18088/ejbmr.2.1.2016. 8-14]
26. Ritger PL, Peppas NA. A simple equation for description of solute release I. Fickian and non-fickian release from nonswellable devices in the form of slabs, spheres, cylinders or

discs. J Control Release., 1987; 5: 23-36.

27. [http://dx.doi.org/10.1016/0168-3659(87)90034-4]