CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.23863261

Available online at: http://www.iajps.com Research Article

DESIGN AND IN-VITRO ASSESSMENT OF CISPLATIN-LOADED SOLID LIPID NANO PARTICLES

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Abstract:

The present study was aimed at the formulation and evaluation of Cisplatin-loaded Solid Lipid Nanoparticles (SLNs) to improve drug encapsulation efficiency, provide sustained drug release, and enhance formulation stability. Four formulations (F1–F4) were prepared using varying concentrations of lipids and evaluated for their physicochemical properties, drug-excipient compatibility, particle size, zeta potential, entrapment efficiency, in vitro drug release, and stability.The particle size of the developed SLNs ranged from 116 to 151 nm, with formulation F4 exhibiting the smallest particle size (116 nm). Zeta potential values ranged from −23 mV to −27 mV, indicating acceptable physical stability of the formulations. Entrapment efficiency increased with increasing lipid concentration, and formulation F4 showed the highest entrapment efficiency of 83.50%.In vitro drug release studies demonstrated a sustained release profile over an 8-hour period, with the optimized formulation F4 exhibiting the highest cumulative drug release of 95.98%. Stability studies performed under different storage conditions (25°C/60% RH, 30°C/75% RH, and 40°C/75% RH) for three months indicated no significant changes in the physicochemical properties or drug release characteristics of the optimized formulation, confirming its stability.The findings of the present investigation suggest that Cisplatin-loaded Solid Lipid Nanoparticles represent a promising drug delivery system with improved encapsulation efficiency, sustained drug release, and enhanced stability. Among the formulations developed, F4 was identified as the optimized formulation based on its superior performance. Further in vivo studies are recommended to establish the therapeutic potential and clinical applicability of the developed SLN formulation.

Keywords: *Cisplatin, Solid Lipid Nanoparticles, SLNs, Entrapment Efficiency, Sustained Drug Release, Zeta Potential, Stability Studies.*

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Please cite this article in press ***P Sudharani*** *et al.,* ***Design And In-Vitro Assessment Of Cisplatin-Loaded Solid Lipid Nano Particles.,****. Indo Am. J. P. Sci, 2026; 13(10).*

INTRODUCTION:

Solid Lipid Nanoparticles (SLNs)

SLNs were designed in early 1990 as a substitute carrier system due to the drawback of other carrier system like emulsions, liposomes and polymeric nanoparticles. SLNs, the particle size lies between the ranges of 50–1000 nm have obtained massive interest in the field of controlled drug delivery as a carrier. SLNs show low cytotoxicity to mammalian cells because it is made up of mainly biocompatible and biodegradable solid lipid and demonstrating an acceptable tolerance to the body. An exceptional interest has been given to delivery systems based on those components which are really biocompatible and biodegradable components. Lipids and phospholipids are highly biocompatible and biodegradable materials. For these favourable characteristics of lipids and phospholipids multiple kind of nano drug delivery likes nanostructure lipid carriers and SLN have been studied and developed .

Generally biopharmaceutical classification system (BCS) describes class II and class IV as low soluble bioactive molecule. This kind of low soluble bioactive molecule is perfect for this colloidal carriers.SLN and NLC were formulating as surprisingly secure colloidal carriers for imperfectly soluble bioactive molecule delivery. Both SLN and NLC have unique characteristics that they can play a good role as excipients in various drug deliveries which are acceptable in human medication also. A matrix material is occur from SLNs for drug incorporation and introduce mono-,di- or triglycerides, fatty acids and complicated glyceride mixtures. They offer a big opportunity for composition with novel substance in compare to other exiting nanoparticulate technique. SLNs were demonstrated as a substitute bioactive delivery technique to conventional nanoparticle delivery with polymer basically for drug release mechanism, drug release in the delivery of drugs in controlled release system. SLNs are made up of lipid matrix which are originated from physiologically endured lipid ingredients and can lower the intensity for chronic and acute toxicity. It is a superiority of SLNs over polymeric nanoparticles. SLNs are good in physical stability, controlled release and tolerability and can also give a protection of introduced bioactive molecule from degradation. SLN can be produced conveniently on large industrial scale because of their physico-chemical stability and raw material and production costs are relatively low.

MATERIALS:

Cisplatin was procured from Aurobindo ltd, Hyd, Glyceryl Monostearate, Tween 80, Chloroform, and Methanol were obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

METHODOLOGY:

Method of preparation of Cisplatin loaded nanoparticles:

Table -1: Composition of Cisplatin for preparation of solid lipid nanoparticles

Ingredients (mg)	F1	F2	F3	F4
Cisplatin (mg)	10	10	10	10
Glyceryl Monostearate (mg)	100	200	300	400
Tween 80(mg)	50	50	50	50
Methanol(ml)	10	10	10	10
Chloroform(ml)	20	20	20	20

Preparation Method (Modified Hot Emulsification – Ultrasonication Method)

Step 1: Lipid phase preparation

- Accurately weigh the required quantity of Glyceryl Monostearate (GMS).
- Dissolve GMS in a mixture of chloroform and methanol (2:1).
- Dissolve Cisplatin in a minimum quantity of suitable aqueous medium (PBS pH 7.4) and disperse it into the lipid phase under continuous stirring.
- Heat the lipid phase to 5–10°C above the melting point of GMS (approximately 70–75°C).

Step 2: Aqueous phase preparation

- Prepare an aqueous solution containing Tween 80 as a surfactant.
- Heat the aqueous phase to the same temperature as the lipid phase (70–75°C) to avoid premature lipid solidification.

Step 3: Emulsification

- Add the aqueous solution slowly to the molten lipid phase under high-speed homogenization (10,000–15,000 rpm for 5–10 min).
- Alternatively, use ultrasonication (probe sonicator) for 3–5 min in pulses to reduce particle size.

Step 4: Solidification of nanoparticles

- Cool the emulsion to room temperature or in an ice bath with gentle stirring.
- Lipid solidifies, forming SLNs encapsulating Cisplatin.

Step 5: Purification

- Remove unencapsulated drug by centrifugation
- Wash nanoparticles with cold water to remove excess surfactant.

Evaluation of Cisplatin loaded nanoparticles:

Particle size:

The particle size of Cisplatin -loaded solid lipid nanoparticles (SLNs) can be determined using Dynamic Light Scattering (DLS). First, the nanoparticle suspension is appropriately diluted with deionized water or a suitable buffer to prevent multiple scattering, ensuring the sample is free of aggregates by gentle vortexing or sonication. If necessary, the dispersion can be filtered through a 0.22 µm filter to remove dust or large particles.

Zeta potential

The zeta potential of nanoparticles is an important parameter that indicates the surface charge and stability of the colloidal system. For Cisplatin -loaded solid lipid nanoparticles (SLNs), the zeta potential can be measured using a Zeta Potential Analyzer or a Dynamic Light Scattering (DLS) instrument equipped with zeta potential measurement.

SEM analysis:

Scanning Electron Microscopy (SEM) analysis is commonly used to study the surface morphology, shape, and size of nanoparticles, including Cisplatin -loaded solid lipid nanoparticles (SLNs). In this technique, a small amount of the lyophilized or dried nanoparticle sample is carefully mounted on a metallic stub using double-sided conductive tape. To prevent charging and enhance image quality, the sample is usually coated with a thin layer of a conductive material, such as gold or platinum, using a sputter coater.

Drug encapsulation efficiency:

The encapsulation efficiency of Cisplatin in solid lipid nanoparticles is typically determined by separating the free, unentrapped drug from the nanoparticles and quantifying it. First, a known volume of the nanoparticle suspension is subjected to centrifugation at high speed (e.g., 15,000–20,000 rpm for 30–60 minutes) or ultrafiltration using a suitable membrane to separate the nanoparticles from the supernatant containing unencapsulated drug. The concentration of free Cisplatin in the collected supernatant is then measured using an appropriate analytical method, such as UV-Vis spectroscopy [58]

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount entrapped}}{\text{Total drug loaded}} \times 100$$

In-vitro drug release studies:

The in-vitro drug release of Cisplatin from solid lipid nanoparticles is typically performed using the dialysis bag diffusion method. A known quantity of SLNs is placed inside a pre-soaked dialysis membrane with a suitable molecular weight cut-off and sealed at both ends. The dialysis bag is then immersed in a release medium such as phosphate-buffered saline (PBS, pH 7.4) to simulate physiological conditions, and maintained at 37 ± 0.5°C under continuous stirring at 100–150 rpm to ensure uniform dispersion. At predetermined time intervals Percentage of drug release was determined using the following formula.[59]

$$\text{Perentage drug release} = \frac{Da}{Dt} \times 100$$

Where, Dt = Total amount of the drug

Da = The amount of drug released

Stability studies:

The stability of Cisplatin -loaded solid lipid nanoparticles is evaluated by storing the formulated nanoparticles under various controlled conditions and assessing their physical and chemical properties over time. The SLNs are typically divided into samples and stored at refrigerated (2–8°C), room temperature (25 ± 2°C), and accelerated conditions (40 ± 2°C with 75% relative humidity) for a predefined period, such as 1, 2 and 3 months.

RESULTS AND DISCUSSION:

Scanning Electron Microscopy:

The surface characteristic of prepared crystal was studied by SEM (ZEISS Electron Microscope, EVO MA 15). Powder samples was mounted onto aluminum stub using double sided adhesive tape and sputter coated with a thin layer of gold at 10 Torr vacuum before examination. The specimens were scanned with an electron beam of acceleration potential of 20 kV and the images were collected as secondary electron mode.

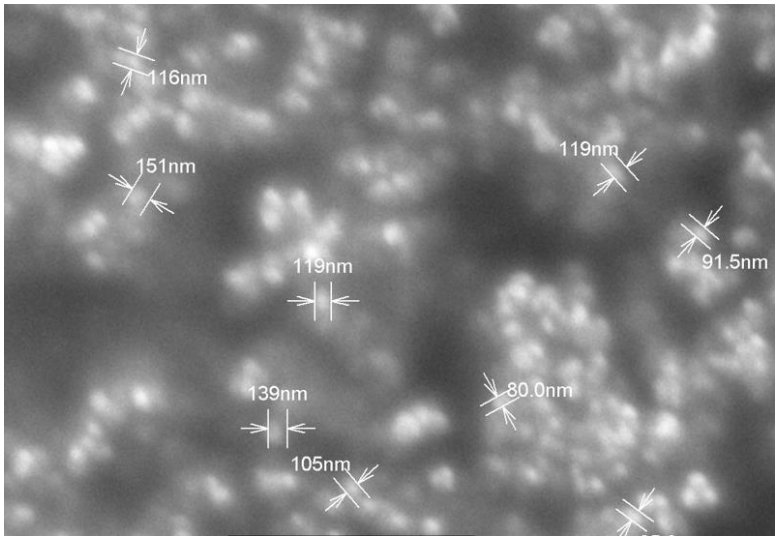


Fig-1: SEM Analysis of nanoparticles

Determination of Zeta potential:

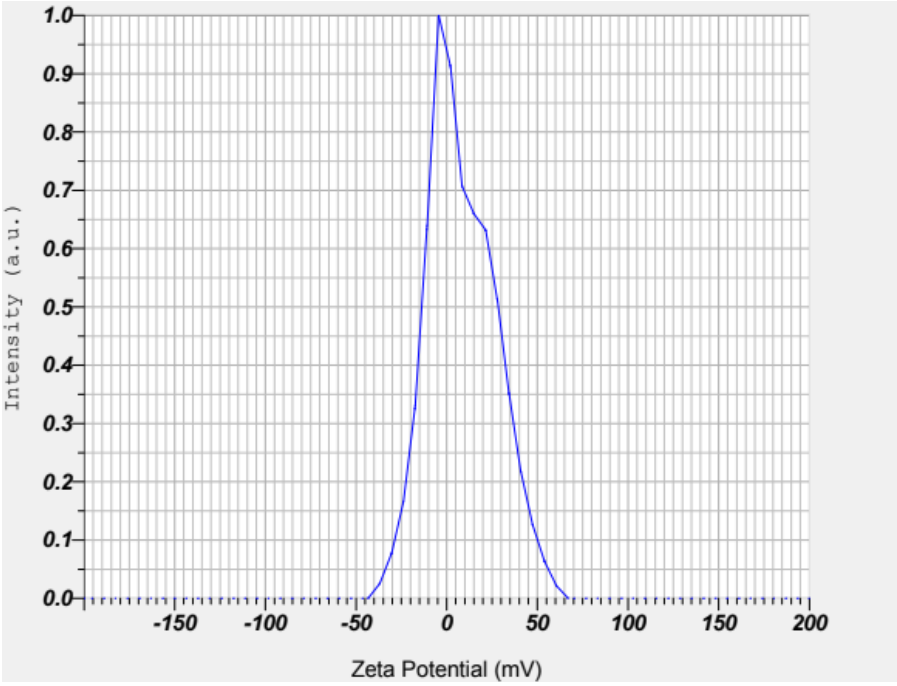


Fig-2: Zeta potential of Optimized formulation

Particle size

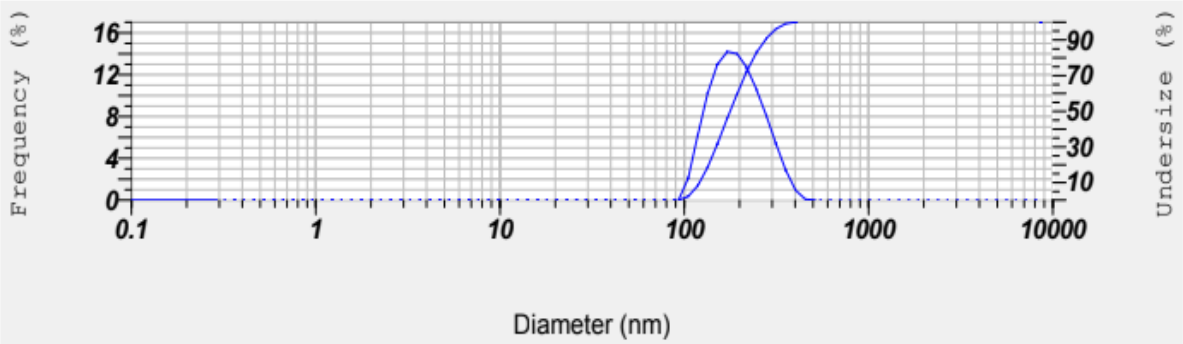


Fig-3: Particle size of optimized formulations

In general, particle size was with a diameter of 116 nm. The surfaces of the nanoparticles were smooth.

Characterization of Nanoparticles of Cisplatin

Table-2: Evaluation Studies of solid lipid nanoparticles

F. no	Particle size (nm)	Zeta Potential(mV)
F1	119	-25
F2	151	-27
F3	139	-26
F4	116	-23

Entrapment efficiency

F. no	Entrapment Efficiency (%)
F1	76.93
F2	79.81
F3	81.23
F4	83.50

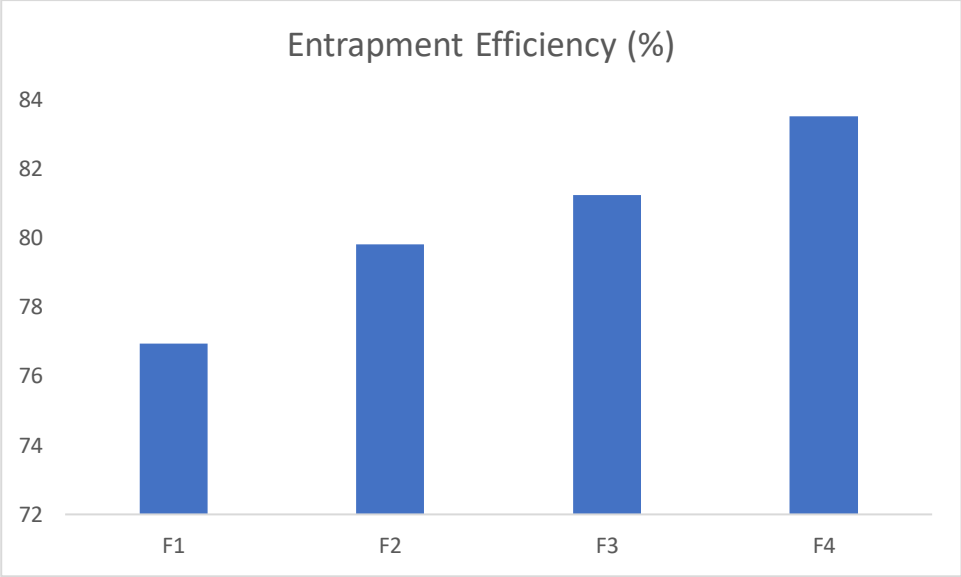


Fig-4: In vitro drug entrapment efficiency studies

In-vitro Dissolution Study

Table-3: In-vitro release data of Cisplatin-loaded SLNs (F1–F4)

Time (hrs.)	F_1	F_2	F_3	F_4
0	0	0	0	0
1	22.42	26.37	19.86	25.49
2	35.39	32.25	33.50	36.45
3	41.25	37.53	42.35	45.10
4	53.58	48.20	49.86	55.55
5	62.38	59.92	62.33	63.37
6	74.50	73.50	72.20	73.25
7	81.28	81.85	85.21	83.52
8	92.35	92.35	94.36	95.98

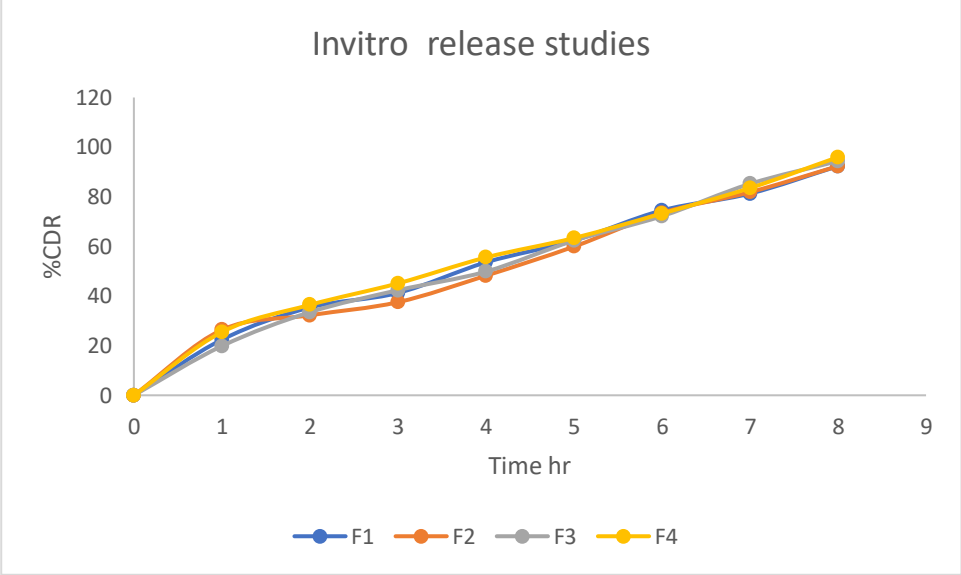


Fig-5: In vitro drug release studies of F1-F4 Formulation

Stability studies

There was no significant change in physical and chemical properties of the SLN of formulation F-4 after 3 months. Parameters quantified at various time intervals were shown;

Table-4: Results of stability studies of optimized formulation F4

Formulation Code	Parameters	Initial	1st Month	2nd	3rd	Limits as per Specifications
F-4	**25^{0}C/60%RH**	95.98	94.52	93.48	92.66	**Not less than**
F-4	**30^{0}C/75% RH**	95.98	94.24	93.25	92.72	**Not less than**
F-4	**40^{0}C/75% RH**	95.98	94.33	93.2	92.23	**Not less than**

SUMMARY AND CONCLUSION:

Summary

The present study was carried out to formulate and evaluate Cisplatin-loaded Solid Lipid Nanoparticles (SLNs) using varying concentrations of lipids. Four formulations (F1–F4) were successfully prepared and evaluated for their physicochemical characteristics, drug-excipient compatibility, particle size, zeta potential, entrapment efficiency, in vitro drug release, and stability.FTIR compatibility studies indicated that there was no significant interaction between Cisplatin and the selected excipients, as evidenced by the retention of characteristic peaks in both the pure drug and optimized formulation spectra. SEM analysis confirmed the formation of nanoparticles with smooth surface morphology.The particle size of the developed formulations ranged from 116 to 151 nm, indicating successful preparation of nanoparticles within the desired size range. Among all formulations, F4 exhibited the smallest particle size (116 nm). The zeta potential values ranged from −23 mV to −27 mV, suggesting acceptable physical stability of the SLN formulations.Entrapment efficiency increased with increasing lipid concentration, with F4 showing the highest entrapment efficiency of 83.50%, indicating effective encapsulation of Cisplatin within the lipid matrix.In vitro drug release studies demonstrated a sustained release pattern for all formulations over an 8-hour period. The optimized formulation, F4, exhibited the highest cumulative drug release of 95.98%, indicating its ability to provide prolonged drug release.Stability studies conducted under different storage conditions (25°C/60% RH, 30°C/75% RH, and 40°C/75% RH) for a period of three months showed no significant changes in drug release characteristics. The optimized formulation retained more than 90% drug release throughout the study period, confirming its stability.

CONCLUSION:

The present investigation successfully developed and evaluated Cisplatin-loaded Solid Lipid Nanoparticles using a suitable nanoparticulate delivery approach. The results demonstrated that the developed SLNs possessed desirable physicochemical characteristics, good drug entrapment ability, nanosized particle distribution, and sustained drug release properties.Among the four formulations prepared, formulation F4 was identified as the optimized formulation based on its superior performance, including small particle size (116 nm), high entrapment efficiency (83.50%), satisfactory zeta potential (−23 mV), maximum cumulative drug release (95.98%), and excellent stability profile.The findings of this study suggest that Solid Lipid Nanoparticles are a promising carrier system for the delivery of Cisplatin, offering advantages such as improved drug encapsulation, controlled release behavior, enhanced formulation stability, and the potential to reduce the frequency of administration and systemic adverse effects associated with conventional Cisplatin therapy.

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