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Formulation and Evaluation of Cisplatin Niosomal Drug Delivery System

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Abstract
The present study was focused on formulating and evaluating Cisplatin containing niosomes formulation for invitro studies. Niosomal formulations were prepared by using different ratio of surfactant (Tween 80 and Span 80) and cholesterol by thin film hydration method and were evaluated for *in vitro* characteristics, stability studies. Span 80 containing niosomal formulation displayed highest entrapment efficiency with desired particle size. SEM analyses showed that niosomal formulation was spherical in shape. Niosomes containing Span 80 displayed higher percentage of drug release after 8h as compared to other formulations. F-4 formulation was found to be stable at the end of the study on storage condition. The present study suggested that niosomal formulations provide sustained and prolonged delivery of drug with enhance bioavailability.

Keywords: Niosomes, Cisplatin, bioavailability, thin film hydration technique, *in vitro* drug release studies

Introduction
Niosomes are non-ionic surfactant-based vesicular carriers have emerged as a promising platform to address these limitations. Structurally similar to liposomes, niosomes are composed of non-ionic surfactants and cholesterol that self-assemble into bilayer vesicles capable of encapsulating both hydrophilic and hydrophobic drugs. [1]Compared with other colloidal carriers, niosomes offer advantages such as greater chemical stability, lower cost, ease of scale-up, and the ability to modify surface properties for passive or active targeting. [2] For anticancer drugs like cisplatin, niosomal encapsulation can potentially enhance plasma half-life, improve accumulation at tumor sites via the enhanced permeability and retention (EPR) effect, and provide a sustained release profile that lowers peak systemic concentrations and associated toxicities. [3] Cisplatin (cis-diamminedichloroplatinum II) is a cornerstone chemotherapeutic agent widely used for the treatment of a variety of solid tumors, including testicular, ovarian, bladder, lung, and head-and-neck cancers. [4, 5] This study aims to design, formulate, and evaluate cisplatin-loaded niosomes with the objective of improving drug delivery to tumor tissue and reducing systemic toxicity. [6] Formulating cisplatin into niosomal carriers represents a rational strategy to enhance its therapeutic index. [7] The present research addresses both formulation optimization and comprehensive evaluation, with the ultimate goal of producing a niosomal cisplatin system that offers controlled release, improved tumor targeting, and reduced systemic toxicity thereby contributing to safer and more effective platinum-based chemotherapy. [8]

Materials
Cisplatin procured from Hetero Labs, Hyderabad. Cholesterol, Span 80 and Tween 80 were obtained from Synpharma Research Labs, Hyderabad. Other chemicals and the reagents used were of analytical grade.

Methodology
Fourier Transform Infrared Spectroscopy (FTIR) study
FTIR is a useful technique to check and confirm any interaction that may occur between excipients and drug. The FTIR spectra of drug, excipients, briefly, solid sample (1 mg) along with 100 mg dried potassium bromide was compressed into a disc. The

sample were place onto NaCl or KBr aperture plate and sandwiched it under another aperture plate, such that no gas bubbles were trapped. The sample allowed formation of a thin liquid membrane between the two aperture plates. Thereafter, sample was scanned for absorbance over the range from 4000 to 400 (cm-1) wave numbers. The obtained spectrum was then compared with standard group frequencies of Cisplatin. [9]

Preparation of Niosomes

Cisplatin niosomes were prepared using thin film-hydration method. Accurately weighed quantities of the surfactant (Span 80 and Tween 80) and cholesterol in different Ratios in around- bottom flask. Afterwards, Cisplatin dissolved in 5ml of chloroform: methanol mixture (2:1) was added to the lipid solution. The organic solvents were removed under vacuum in a rotary evaporator at 40◦ C for 20min to form a thin film on the wall of the flask, and kept in a desiccator under vacuum for 2 h to ensure total removal of trace solvents. After removal of the last trace of organic solvents, hydration of the surfactant film was carried out using 10mL of distilled water at55◦C. The resulting niosomal suspension was mechanically shaken for 1 h using a horizontal mechanical shaking water bath at 55 ◦ C. Then, the vesicle suspension was sonicated in 3 cycles' of1min "on" and 1min "off" leading to the formation of multi lamellar niosomes. The niosomal suspension was left to mature overnight at 4 ◦ C and stored at refrigerator temperature for further studies.[10]

Table 1: Composition of Cisplatin Niosomes (F1 to F6)

S. No.	Ingredients (mg)	F1	F2	F3	F4	F5	F6
1	Cisplatin	20	20	20	20	20	20
2	Cholesterol	100	100	100	100	100	100
3	Tween 80	5	10	15	-	-	-
4	Span 80	-	-		5	10	15
5	Methanol	5	5	5	5	5	5
6	Chloroform	10	10	10	10	10	10

Evaluation of Niosomes

Particle size: Size and size distribution studies were done for niosomes prepared from Niosomes hydration. The Niosomes (100 mg) was hydrated in a small glass test tube using 10 ml of pH 7.4 phosphate buffer solution. The dispersion was observed under optical microscope at 40X magnification. Similarly, size was noted for niosomes formed spontaneously from Niosomes after hydration without agitation in a cavity slide.[11]

Zeta-potential: The sample was diluted with distilled water (1:100 (V/V)) and zeta potential was determined using Malvern zetasizer (Nano ZS, Malvern Instruments, United Kingdom). Measurement was based on the electrophoretic mobility of the particles, which was converted to the zeta potential by inbuilt software based on the Helmholtz-Smoluchowski equation. [12]

SEM Analysis : The shape, surface characteristics, and size of the niosomes were observed by scanning electron microscopy. Once again, 0.2 g of the Niosomes in a glass tube was diluted with 10 ml of pH 7.4 phosphate buffer. The niosomes were mounted on an aluminium stub using double-sided adhesive carbon tape. Then the vesicles were sputter-coated with gold palladium (Au/Pd) using a vacuum evaporator (Edwards) and examined using a scanning electron microscope (Hitachi 3700N, Germany) equipped with a digital camera, at 10 kV accelerating voltage. [13]

Entrapment efficiency: To 0.2 g of Niosomes, weighed in a glass tube, 10 ml phosphate buffer pH 7.4 were added. The aqueous suspension was then sonicated. Niosomes containing Cisplatin were separated from unentrapped drug by centrifugation at 9000rpm for 45 min at 4 ◦C. The supernatant was recovered and assayed spectrophotometrically using UV spectrophotometer (UV-1800 Shimadzu, Japan), at 250 nm [14]

The encapsulation percentage of drug (EP) was calculated by the following equation

$$EP = [(C_t - C_r)/ C_t] * 100$$

Where,

Ct, concentration of total Cisplatin, Cr, concentration of free Cisplatin.

In vitro drug* release Study: *in vitro release studies were carried out using unjacketed vertical franz diffusion cells with a diffusional surface area of 6.154 cm^2 and 20 mL of receptor cell volume. Prior to the study, the dialysis membrane was soaked in phosphate buffer pH 7.4 Formulation equivalent to 5mg of Cisplatin was placed in the donor compartment. The receptor compartment consisting of PB pH 7.4 was maintained at 37±2°C under constant stirring up to 8 hrs. The donor chamber and the sampling port were covered with lid to prevent evaporation during the study. Aliquots of 5 mL were withdrawn periodically at different time intervals and replaced with equal volume to maintain constant receptor phase volume. At the end of the study, the samples were suitably diluted and the amount of drug was determined spectrophotometrically at 250 nm. [15]

Stability Studies: To evaluate the physical and chemical stability of the optimized niosomal formulation over a period of 3 months under different storage conditions, in accordance with ICH guidelines, by monitoring changes in drug release studies. [16]

Results and Discussion

Drug - excipient compatibility studies (FT-IR)

Using the FTIR peak matching approach, the compatibility of the medicine with the chosen polymer and other excipients was assessed. The drug-Excipients mixture showed no peaks that appeared or vanished, indicating that there was no chemical interaction between the medication, lipids and other molecules.

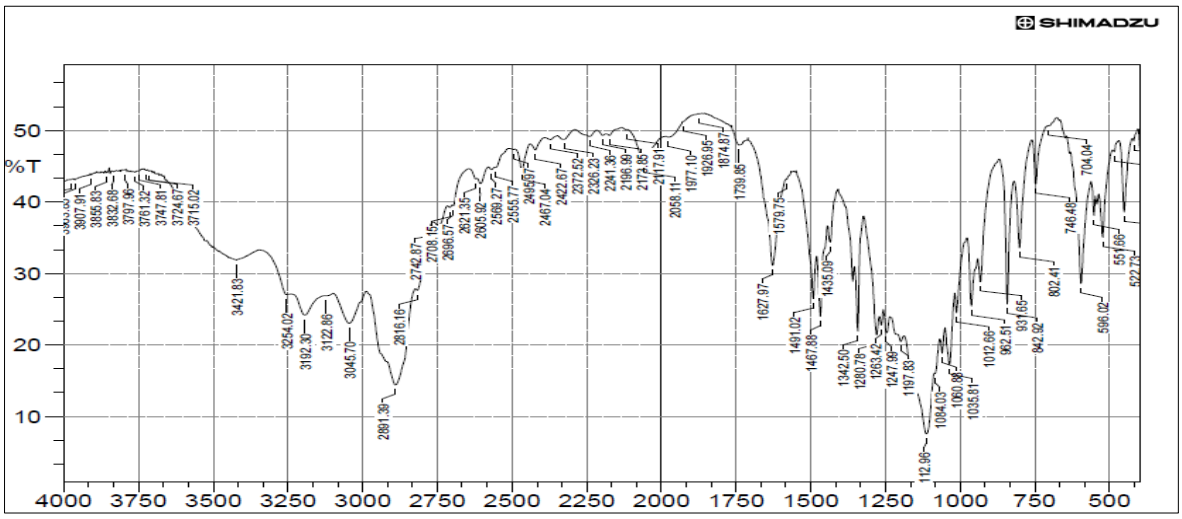

Fig 1: FT-IR Sample for Pure drug

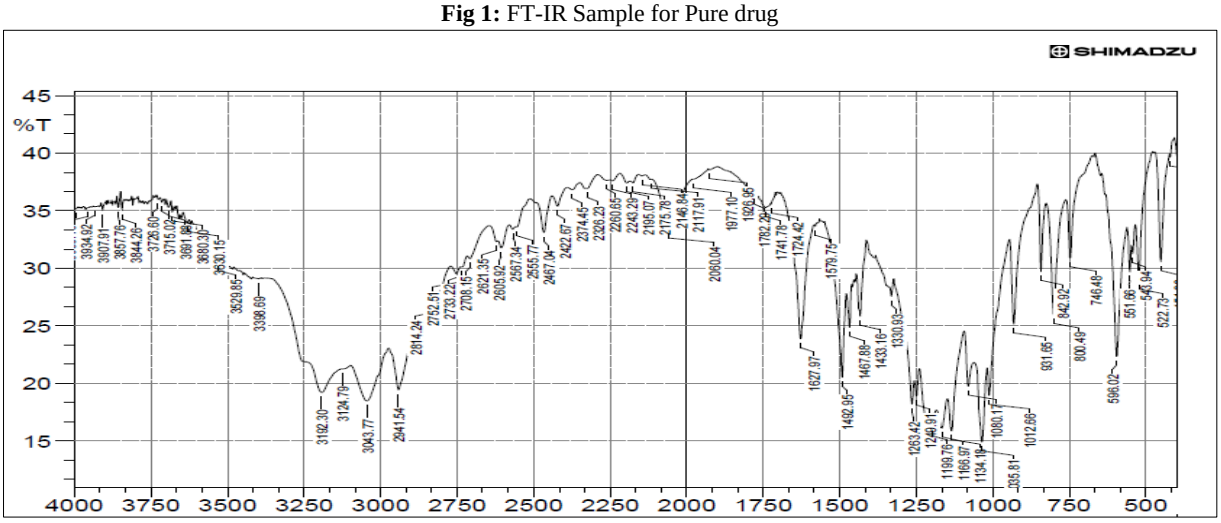

Fig 2: FT-IR Sample for Optimized formulation

Evaluation Parameters

Entrapment Efficiency

Table 2: Drug entrapment efficiency of all formulation

F.no	Drug entrapment efficiency
F1	79.68
F2	80.13
F3	76.35
F4	81.39
F5	77.81
F6	79.36

The formulate niosomes formulation's entrapment efficiency was in the range of 76.35-81.39 %. The % EE of the prepared optimized formulation was found to be 81.39 %.

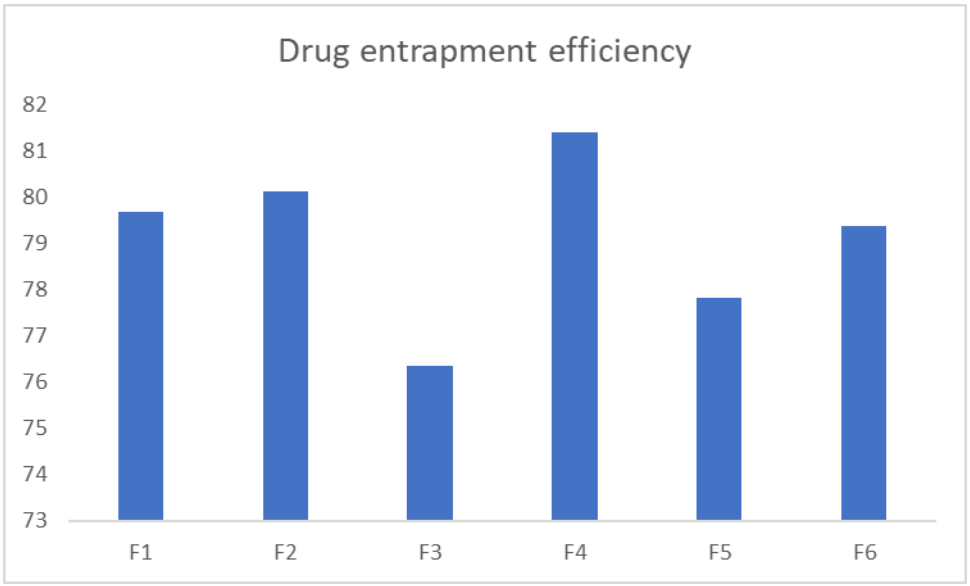

Fig 3: Drug entrapment efficiency of all formulation determination of Vesicle morphology and Size

The morphology of the prepared diverse types of nanoparticles was found to be virtually spherical in shape and have a rough surface, as illustrated in SEM photomicrographs of the nanoparticles.

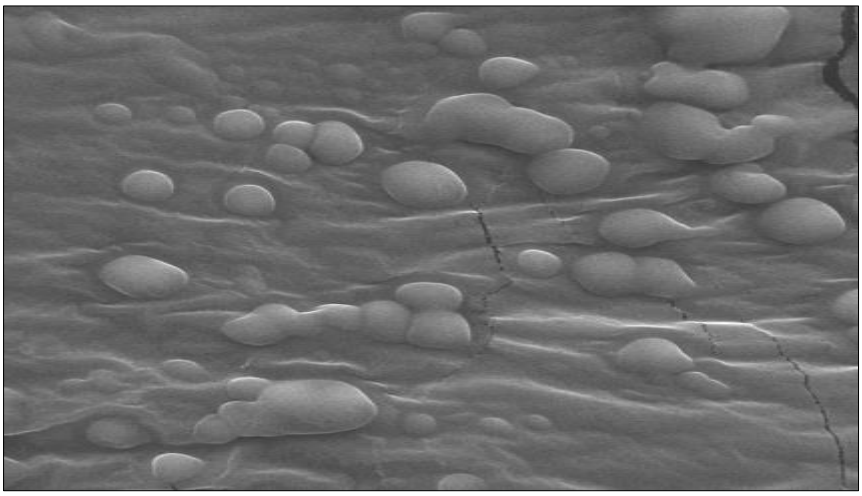

Fig 4: SEM analysis of Optimized niosomes

Evaluation Studies of particle size and Zeta potential Niosomes Particle size

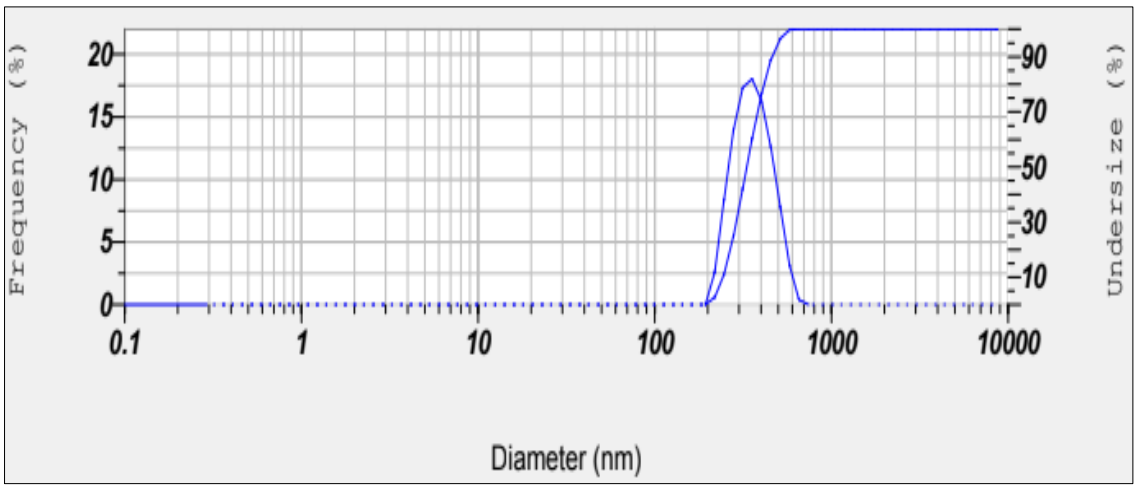

Fig 5: Particle size analysis of Optimized Niosomes Zeta potential

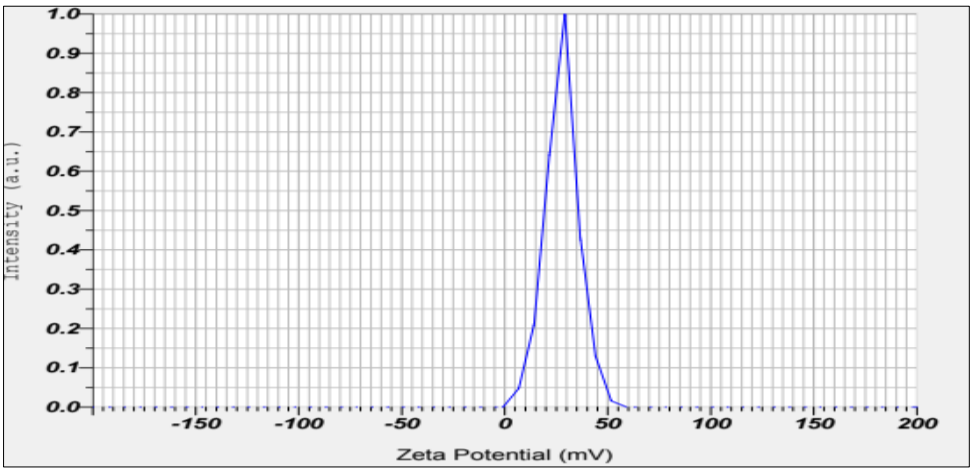

Fig 6: Zeta potential analysis of Optimized niosomes

The ZP or change in the surface of colloidal particles in niosomes was studied to determine the charge on the particles to avoid agglomeration. Figure indicates the ZP of the optimized formulation as -25mV.

Table 3: Evaluation Studies of particle size and Zeta potential Niosomes

F. No	Particle size (nm)	Zeta potential
F1	153	-26
F2	149	-28
F3	168	-29
F4	160	-25
F5	155	-27
F6	173	-22

The mean particle size of optimized niosomes was found to be 160 nm.

In vitro drug release studies

Table 4: ***in vitro*** drug release profiles of Cisplatin niosomes (F1-F6)

Time (hr)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	16.98	17.63	18.67	19.68	17.54	15.96
2	28.13	27.46	28.64	29.64	25.19	27.14
3	35.17	36.89	38.98	40.25	39.83	34.67
4	47.89	48.24	49.82	51.37	50.10	49.14
5	58.10	59.68	60.16	61.64	62.14	60.20
6	69.76	70.25	72.34	73.35	71.25	72.36
7	81.53	82.34	83.15	85.19	83.15	83.15
8	93.67	94.57	95.68	98.10	97.63	95.84

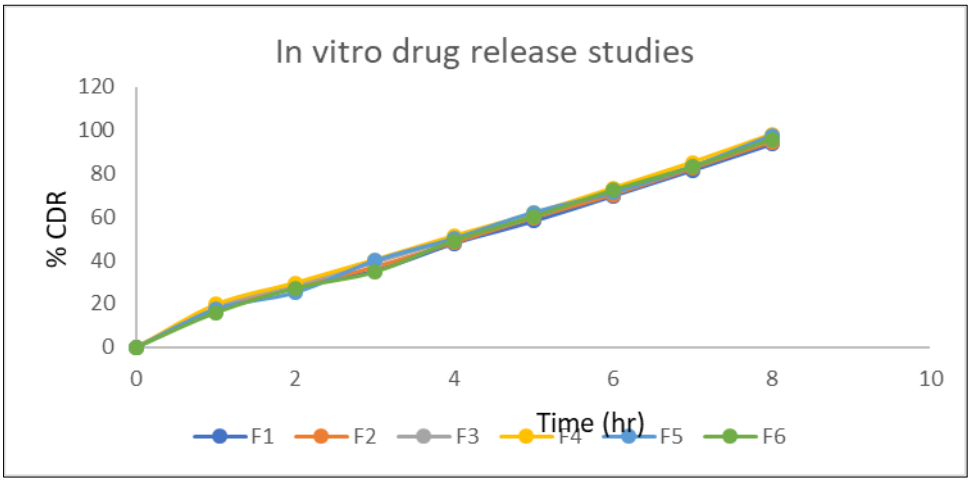

Fig 7: Drug release of (F1-F6) formulations

The drug release studies of all formulations of Cisplatin were conducted by means of Franz diffusion cell apparatus for a time period of 8 hrs. From the drug release studies as depicted in Figure, the results showed that 4th formulation showed maximum drug release rate of 98.10% within 8 hrs.

Stability studies

Optimized formulations F4 was selected for accelerated stability studies as per ICH guidelines. The Niosomes were observed for drug release for a period of three months.

Table 5: Stability studies of optimized formulations at 4 ± 2 °C, 25 ± 2 °C and 40 ± 2 ^{0}C and 75 ± 5% RH for 3 months

Formulation Code	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-4	98.10	97.46	96.37	95.80	Not less than 85 %
F-4	98.10	97.35	96.23	95.67	Not less than 85 %
F-4	98.10	97.19	96.10	95.55	Not less than 85 %

Conclusion

The goal of the current formulation study on Cisplatin is to create a niosomal drug delivery system and assess how well it functions *in vitro*. Different proportions of cholesterol and surfactant were used to create the formulations. High entrapment efficiency is regarded as the ideal or best niosome formulation. This study discovered that the ratio of cholesterol to surfactant affected entrapment efficiency. Formulations were discovered to guarantee the drug's good oral bioavailability. The niosomes were seen to be smooth-surfaced, spherical vesicles. The highest entrapment efficiency was demonstrated by Formulation F4. These facts lead to the conclusion that niosomes may be a promising method for increasing Cisplatin bioavailability.

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