JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

FORMULATION EVALUATION AND LYOPHILIZATION OF CISPLATIN LOADED LIPOSOMES

[1] NAGULA NANDINI, [2]SUSHMA DESAI, [3]CHANDRASEKHARA RAO BARU

[1]STUDENT, [2]RESEARCH SCHOLAR, [3]PROFESSOR &PRINCIPAL
[1]PHARMACEUTICS,
[1]CHILKUR BALAJI COLLEGE OF PHARMACY, HYDERABAD, INDIA

Abstract :
Cisplatin is an alkylating agent and an anticancer medication. The main objectives of this work were to prepare the Cisplatin Liposomes and assess their effectiveness. By creating pegylated liposomes, this formulation can lessen adverse effects and pinpoint the location of action with the stabilizer's effect on drug entrapment efficiency. Hydrogenated soy phosphatidyl choline and cholesterol, which are less hazardous, were used to create this liposomal formulation. The medication, carrier, ammonium sulphate, and stabilizer were combined in a rotary evaporator to create the liposomes using the dried thin film hydration process. The temperature, vacuum, and RPM were all kept constant in line with this. Following processing, the liposomes were frozen and sent for additional analysis. Liposomes from the formulations F1, F2, F3, F4, and F6 were examined, including their average size, shape, and zeta potential. The tested batches showed good physicochemical properties in the F6 formulation. The produced liposomes of F1 through F6 were assessed for assay and percentage of free drug. In comparison to other formulations, the F6 formulation had the highest percentage of free medicine.

Key words: *Formulation, Evaluation, Lyophilization, Cisplatin, Liposomes*

I. INTRODUCTION

Liposomes are tiny vesicles that range in diameter from 20 nm to 20 µm. They are made up of an aqueous membrane encircled by one or more concentric bilayers of phospholipids. When phospholipids are distributed in an aqueous media, the amphiphilic phospholipid molecules which contain both hydrophilic and hydrophobic regions—interact with water to form liposomes on their own. Because hydrophilic medications are trapped in the aqueous zone and hydrophobic materials are found in the hydrophobic sections, a variety of materials can be added. By negotiating exclusive distribution to pre-identified compartments, site-specific targeted medication delivery maximizes the intrinsic activity of pharmaceuticals while simultaneously reducing access to irrelevant non-target cells. Targeting characteristics include selective cell binding, controlled drug delivery rate and mode to the pharmacological receptor, and preservation of the drug's bioenvironment while it is being transported to the site of action. Each of the aforementioned events raises the drug's concentration at the site of action, which inevitably lowers the concentration in non-target tissue, where toxicity may occur. Drug liberation, efflux of free drug from the target site, and proportionate cellular absorption of the drug vehicle all contribute to the high concentration of the drug at the target site. Targeting is demonstrated when the active drug is present near the target site and the target compartment is isolated from the other compartments where toxicity may occur. Maintaining effective accessibility to the target site or sites while restricting the distribution of the parent drug to the non-target site or sites may optimize the benefits of targeted medicine administration.
Liposomes are mainly used as delivery systems for many substances which can easily be entrapped or act as anchor to various substituents. Liposomal delivery systems are well-known procedures used for encapsulation of anticancer and antimicrobial drugs, enzymes and antigens. The transport, distribution, controlled release, protection, and localization of encapsulated drugs are all aided by the use of liposomes in delivery systems. In order to improve the performance of the enclosed drug, it is useful to consider the basic reasons for applying liposome as drug carriers.

- Both in the lab and in the body, liposomes serve as medication transporters.
- As part of the targeted delivery system, liposomes carry anticancer drugs such as actinomycin- D and methotrexate.
- Liposome encapsulation has been used in enzyme purification.
- Liposomes containing growth hormone and insulin are administered orally. There have been reports of administration.
- Modifying the state of membrane lipid is the primary function of liposomes in cell biology.
- Key regulatory chemicals including CGMP, CAMP, and enzymatic cofactors can be inserted into cells via

liposomes.

- Liposome collagen gels have a clear benefit over multiple dosing regimens since they exhibit a gradual release of a range of bioactive medications into the circulation.
- Burns and wounds can also be treated topically with this technique.
- The addition of antimicrobials or cell growth agents to the liposomes can promote improved cell proliferation and infection avoidance.
- For research objectives, as a a modelling tool for membrane-level determinations of biological processes.
- In the field of membrane immunoassay, or microanalysis.
- Radiopharmaceuticals are transported by liposomes for diagnostic imaging.
- Employed in the manufacturing of novel chemicals and pharmaceuticals in the quickly evolving field of recombinant DNA technology.
- Cosmetic applications.

II. MATERIALS

Cisplatin was received as a kind gift from Nicholas and Piramal, Indore and Cipla Bangalore.
Hspc purchased from Lipoid Pvt. Ltd., Mumbai, Cholesterol purchased from Lipoid Pvt. Ltd., Mumbai.
Mpeg –dspe purchased from Sigma Aldrich, Mumbai.Ammonium sulphate purchased from Triveni chemicals, Mumbai. Sucrose purchased from Triveni chemicals, Mumbai.

III. METHODOLOGY

Cisplatin's standard calibration curve determination

UV Calibration Curve Cisplatin's standard calibration curve was computed at 301 nm in phosphate buffer pH 7.4 using a UV-Visible spectrophotometer. A phosphate buffer with a pH of 7.4 was used to make the necessary dilutions after a stock solution containing 1 mg/ml of the standard medication was prepared. Using phosphate buffer pH 7.4 as a makeup solution, successive dilutions were prepared from the stock solution to create rewrite solutions with concentrations ranging from 10 to 80 µg/ml. A UV-visible spectrophotometer was used to measure absorbance at 301 nm. To generate a standard calibration curve, absorbance measurements were plotted against the corresponding concentrations.

Making a pH 7.4 Phosphate Buffer

Using a tiny amount of distilled water, dissolve 1.564 grams of sodium hydroxide and 6.804 grams of potassium dihydrogen phosphate to create a volume of 1000 milliliters.
COMPATIBILITY STUDIES The resultant solution is phosphate buffer with a pH of 7.4.

Fourier Transform-Infrared Spectrophotometry

IR spectroscopy was used to study and forecast the physicochemical interactions between API and excipients in a formulation. Based on the results, appropriate and chemically compatible excipients were selected. Using infrared spectroscopy, the following substances were discovered: drug of mpeg-Dspg and drug of cholesterol, and hydroxy soy phosphatidyl choline rol-Dspe of Hspc. One part of the material and three parts potassium bromide were triturated in a mortar. A tiny portion of the triturated sample was compressed using a hydraulic press at a rate of 10 kg/cm utilizing a pellet maker2. The Bruker IR spectrophotometer was used to scan the pellet from 4000 cm to 400 cm while it was attached to the -1-1 sample holder. It was then contrasted with the initial spectrum. In the IR spectra, the functional peak shifts and the lack of a functional group were examined and compared. The spectra clearly show that the combinations and the medication have no interaction with the selected carriers. Therefore, it was discovered that the chosen carrier could entrap the chosen cisplatin with other carriers without causing any interactions.

Preparation of Cisplatin Liposome

Using a rotary evaporator and the dried thin film hydration process, liposomes were made with soy phosphatidylcholine (HSPC). Accurately weighed amounts of Hspc, cholesterol, and Mpeg-dspe are dissolved in methanol and chloroform, then rotated in a rota-evaparator at 25 degrees with a vacuum of about 025 mmHg until a thin film forms. After dissolving the necessary amounts of ammonium sulphate and sucrose (0.3%) in W.F.I., the mixture is added to the thin film above in the R.B. flask and spun until a milky white suspension appears. To make the liposomes smaller, the previously described solution is homogenized for 15 cycles. In order to eliminate free ammonia and sulphate from the lipid solution, the aforementioned solution is subjected to 25 dialysis cycles using a 10% sucrose solution.The necessary amounts of drug and tocoferol are combined in a W.F.I. to create the drug solution, which is then added to a lipid solution in an R.B. flask and rotated for one hour.

Table No: 1 The composition of Drug, Soy Hspc, Cholesterol, Mpeg-dspe, Tocoferol and Ammonium sulphate.

Formulation code	**Drug (cisplatin)**	**Hspc** (gm/100m)	**Cholesterol** (gm/100ml)	**Mpeg-dspe** (gm/100ml)	**Tocoferol** (ml/100ml)	**Ammonium Sulphate** (gm/100ml)
F1	40mg/ 15ml	3.5	1.75	1.5	0.1	3.96
F2		3.85	1.75	1.5	0.1	3.96
F3		4.0	2.0	1.85	0.1	3.96
F4		4.25	2.0	1.85	0.1	3.96

F5		4.25	2.25	2.0	0.1	3.96
F6		4.5	2.5	2.0	0.1	3.96

Liposome Characterization

Each liposomal formulation's physicochemical characteristics, including Particle size, zeta potential, and polydispersity analyses SEM examination.

Drug administration using subcutaneous means: stability studies

Determining the particle size distribution Determining the average liposome vesicle size was essential. This led to the use of the MALVERN INSTRUMENT. 5.3.2 Polydispersity Index: Using the formula Polydispersity = D (0.9) - D (0.1) / D (0.5), polydispersity was calculated. where the particle size just above 90% of the sample is represented by D (0.9). D (0.5) represents particle sizes that are just above 50% of the sample. The particle size just above 10% of the sample is represented by D (0.1).

Analysis of Zeta Potential

Any particle in suspension exhibits the physical characteristic known as zeta potential. It can be used to improve emulsion and suspension compositions. Long-term stability prediction is aided by it. The colloidal system's potential stability can be inferred from the zeta potential's magnitude. There won't be any inclination for the particles to clump together if they all have high negative or positive zeta potentials since they will tend to cancel each other out. However, if the particles' zeta potentials are low, there won't be any force to stop them from colliding and flocculating. 25 mV (positive or negative) is the arbitrary value that separates high-charged surfaces from low-charged surfaces. MALVERN ZETASIZER was used to assess the zeta potential.

Transmission Electron Microscopy

A technique for examining the microstructure of somewhat fragile systems, including micelles, liquid crystalline phases, vesicles, and emulsions, was TEM (Hitachi, H-7500, Germany). TEM analysis is used to determine the size and shape of the optimal liposomal composition.

Electron Microscopy Scanning

Cisplatin Liposomes' surface roundness, smoothness, and aggregate formation were assessed using scanning electron microscopy (SEM). The OSMANIA UNIVERSITY scanning electron microscope (SEM) was examined using the FIELD INSTRUMENT.

In Vitro Characterization

The Triton-X100 technique for quantifying free drug Fill a 10-milliliter volumetric flask with 1 milliliter of liposomal solution, top it off with 5% sucrose, and measure the absorbance. Since the absorbance for a 10 diluted sample is greater than 1, the absorbance of 1 milliliter of the dilution sample is measured using a UV- visible spectrophotometer. Calculate the concentration of the free medication in the liposomal solution based on the absorbance. Once more, place 1 ml of the liposomal sample, 1 ml of triton-x100, and a 5% sucrose solution in a 10 ml volumetric flask. The amount of free medication in the liposomal sample is calculated based on absorbance.

Assay for Cisplatin

Both a sample and a standard solution were made. 20 microliters of the standard and sample solutions should be injected separately under chromatographic conditions. The chromatogram should be recorded. Use the following formula to determine the amount of medication in a liposomal injection per milliliter. A/B/W/200/5/50/C/100/100-D/100/5/100/5 is the assay. where A is the cisplatin-containing region of the sample. The section of the working standard that represents cisplatin is B. C is the percentage of cisplatin purity in the working standard. The percentage of working standard water is denoted by D.

W is the working standard's milligram weight. Conditions for chromatography: Column: 250 x 4.6 mm C18 BDS

Mobile phase: 47 ml, 48 ml, and 5 ml of buffer, acetonitrile, and methanol

The buffer is composed of 2.8% w/w sodium lauryl sulfate and 2.3% w/v. Phosphoric Acid. The wavelength of the wave is 301 nm. Rate of flow: 1.7 ml/min. Acidified IPA (90 ml IPA + 0.68 ml Hcl+) is the solvent. Fill up to 100 milliliters with water.

Cisplatin liposome in vitro diffusion studies

All of the liposomal suspension's in vitro release experiments were conducted using exhaustive dialysis. Two open-ended glass tubes were removed, and a semi-permeable membrane was used to shut one side. Two milliliters of the liposomal suspension were moved from the donor compartment—the built tube—to the receptor compartment, which held 200 milliliters of phosphate buffer (PBS, pH 7.4) containing acetonitrile. A magnetic stirrer was used to agitate the odialysis, which was performed at 50 rpm and at C. Aliquots (4 ml) of the release medium were taken out at different periods to maintain the sink condition volume, and the same volume was then replenished with brand-new phosphate buffer (pH 7.4). After that, the samples were examined using UV-visible spectrophotometry up to 301 nm in wavelength.

IV. RESULTS AN DISCUSSION

CALIBRATION CURVE OF CISPLATIN

Table No 2: Standard Readings of Cisplatin in phosphate buffer pH 7.4

S. No.	Concentration	Absorbance at 301nm
1.	0	0
2.	5	0.101
3.	10	0.224
4.	20	0.311
5.	30	0.425
6.	40	0.540
7.	50	0.645
8.	70	0.830

The UV absorbance of the standard solution of cisplatin in the range of 10–80 g/ml medication showed linearity at a maximum of 301 nm in phosphate buffer pH 7.4. Plotting the linearity of absorbance versus concentration yielded a value of 0.9989 and the slope equation Y = 0.0106x + 0.0015.

PREFORMULATION STUDIES OF CISPLATIN: FT-IR STUDIES

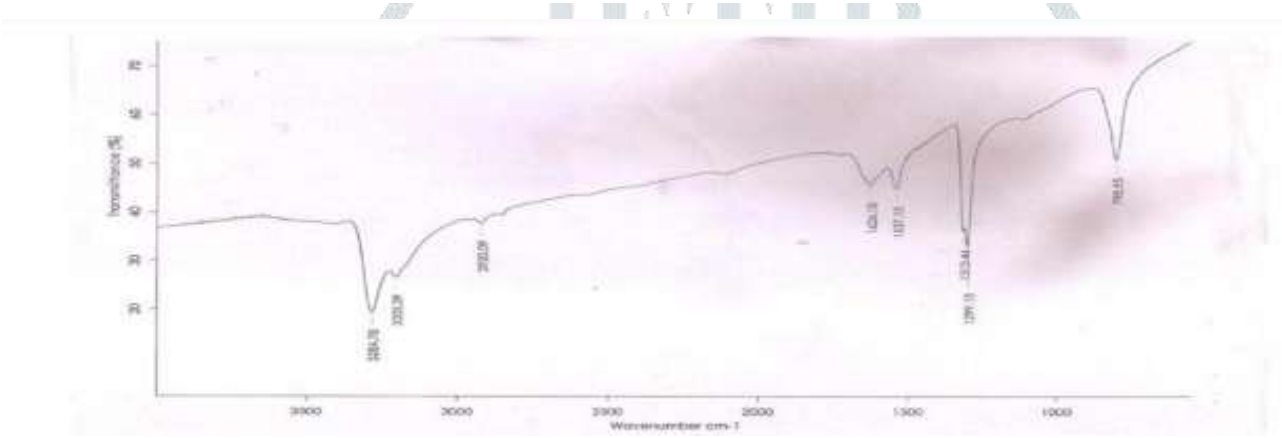


Figure No: 1 FTIR of cisplatin drug

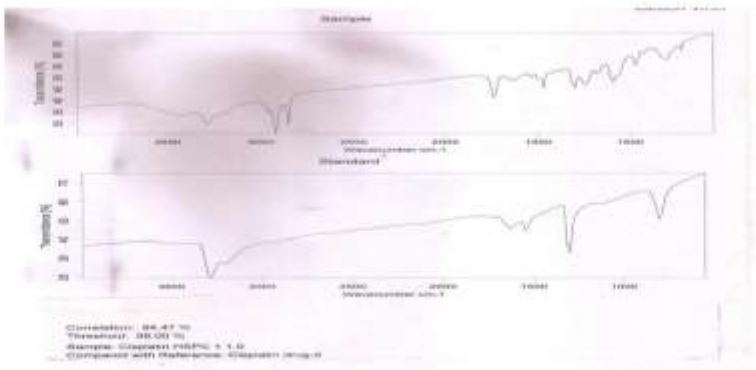


Figure No: 2 FTIR of drug and Hspc.(1:1)

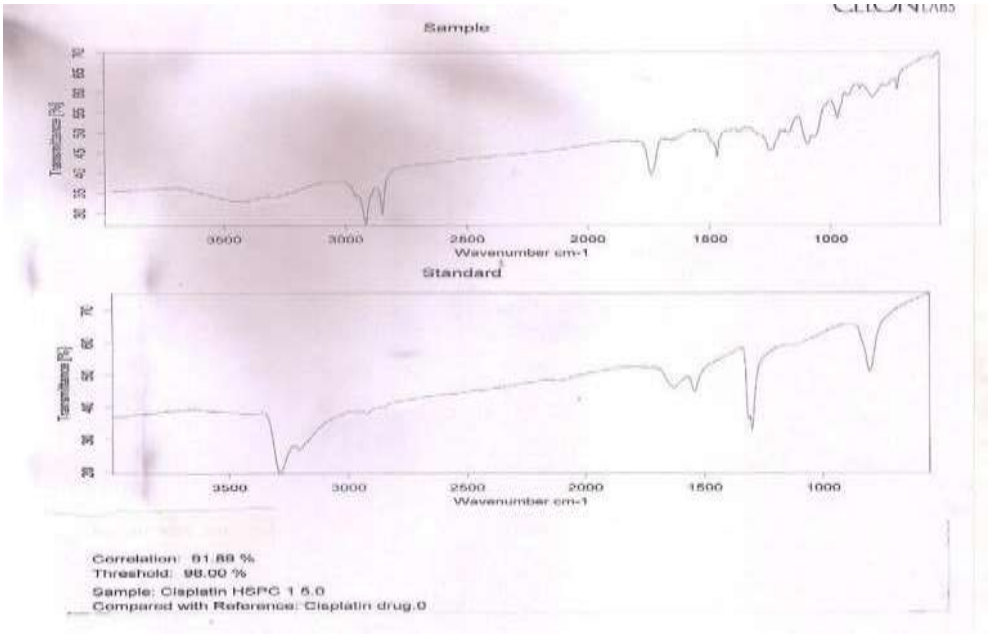


Figure No: 3 FTIR of drug and Hspc.(1:5).

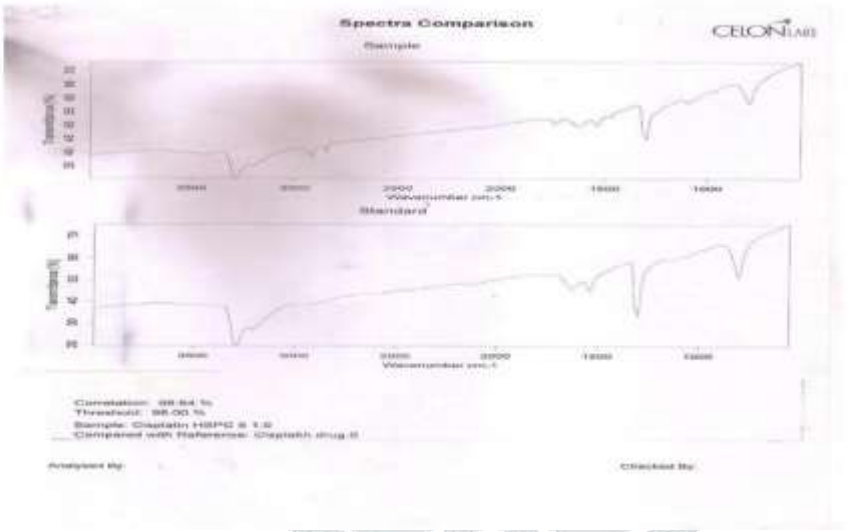


Figure No: 4 FTIR of Cisplatin and Hspc (5:1)

Figure No: 5 FTIR of Cisplatin and Hspc (1:1)

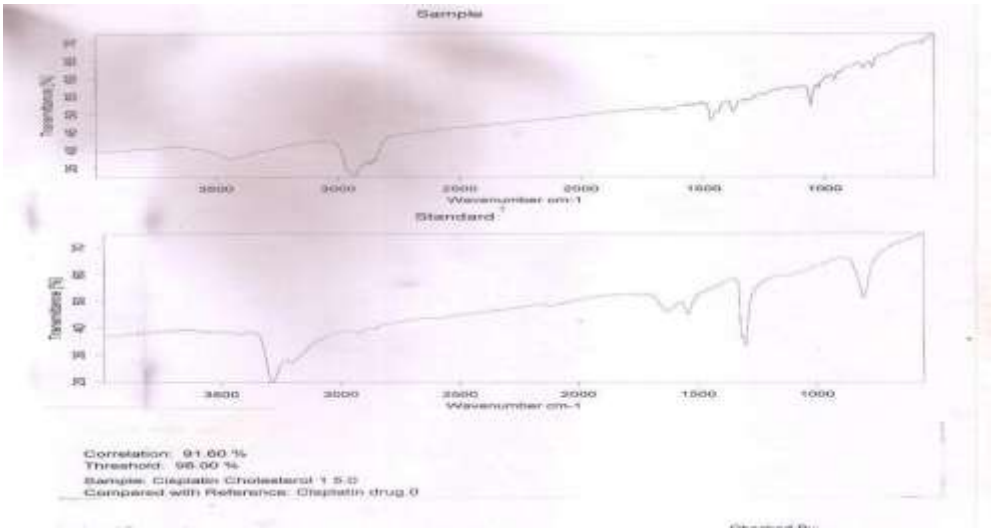


Figure No: 6 FTIR of Cisplatin and cholesterol (1:5)

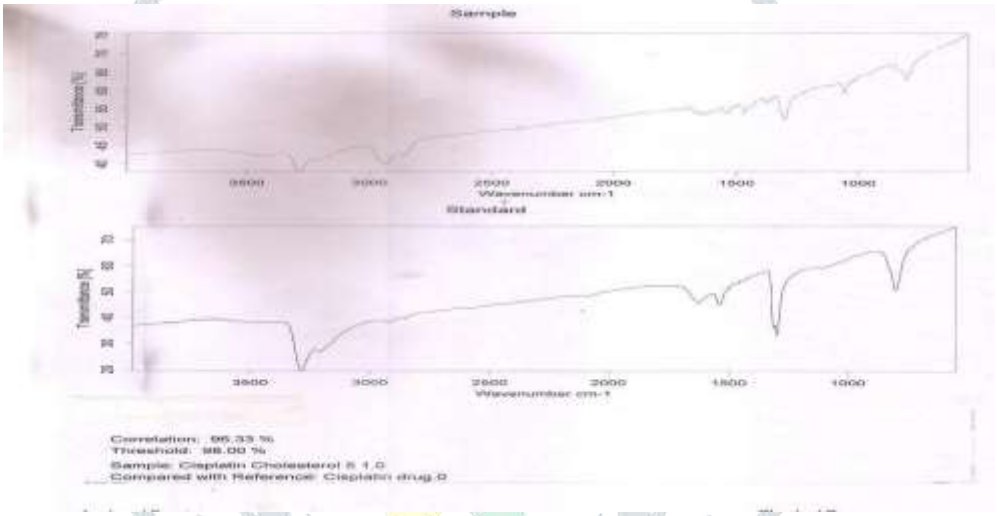


Figure No: 7 FTIR of Cisplatin and mpeg-dspe (5:1)

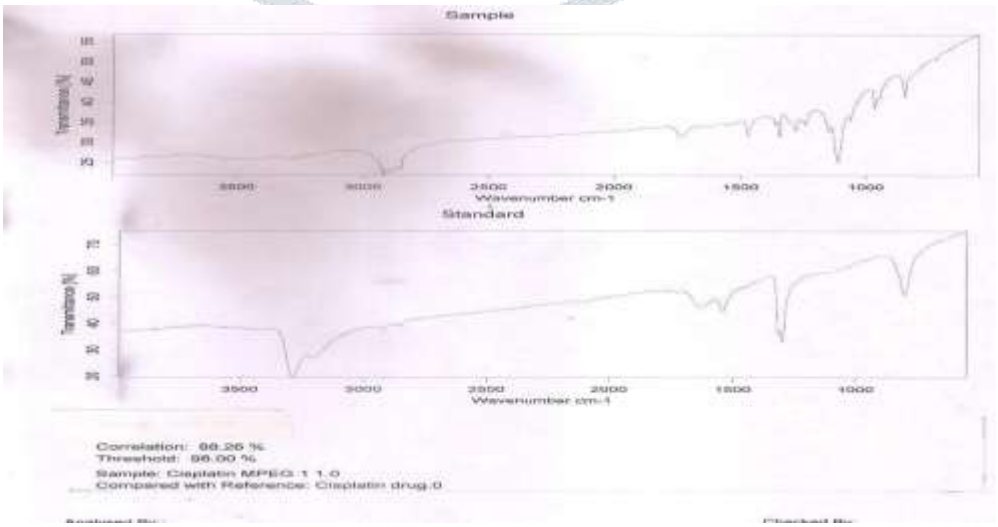


Figure No: 8 FTIR of Cisplatin and mpeg-dspe (1:1)

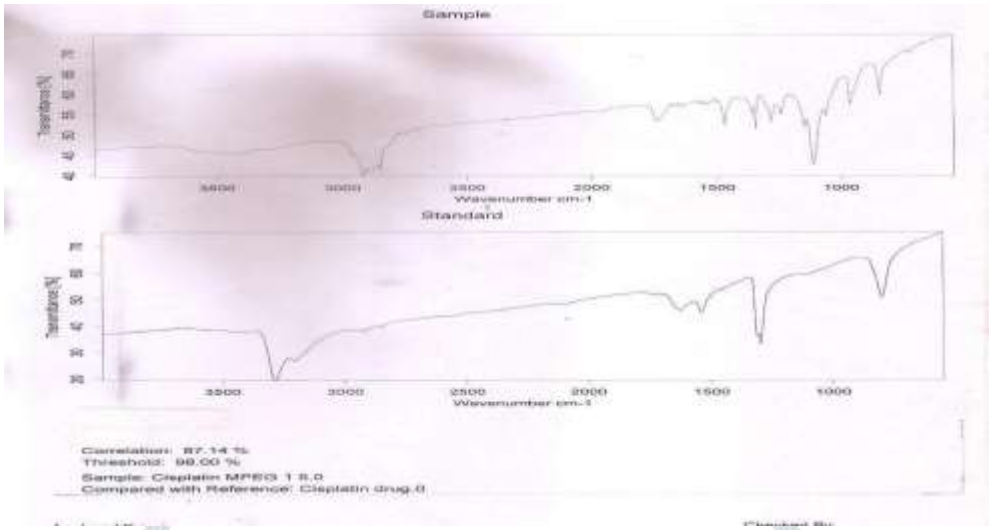


Figure No: 9 FTIR of Cisplatin and mpeg-dspe (1:5)

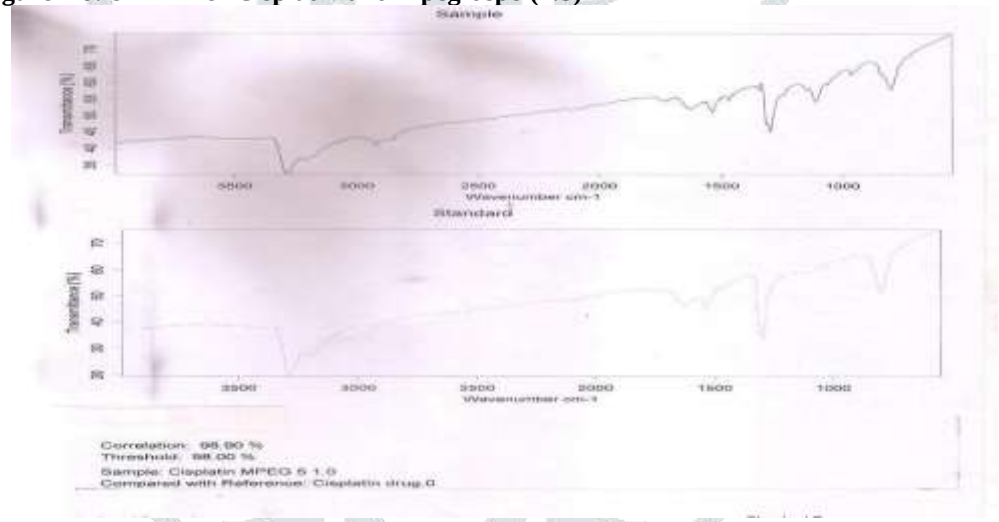


Figure No: 10 FTIR of Cisplatin and mpeg-dspe (1:5)

DIFFERENTIAL SCANNING CALORIMETRY (DSC) RESULTS

Figure No: 11 D.S.C of Cisplatin drug

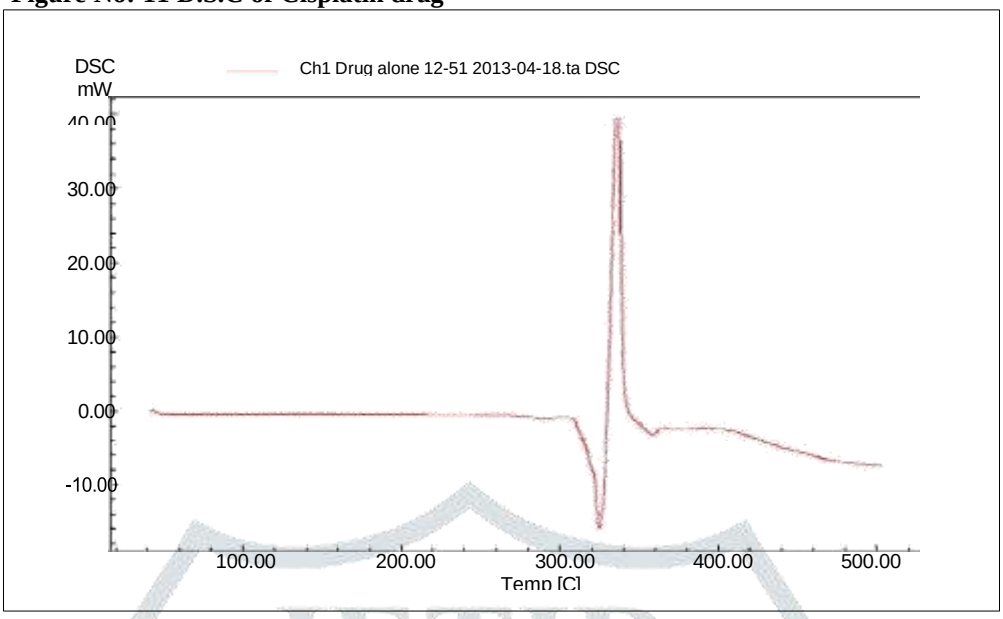


Table no 3: Correlation percentages between drug and lipid mixtures

S.no	Formulation code	Percentage compatibility ratio of drug and excipients
1	Drug and Hspc.(1:1)	94.47
2	Drug and Hspc.(1:5)	91.89
3	Drug and Hspc.(5:1)	98.64
4	Drug and cholesterol (1:1)	93.08
5	Drug and Cholesterol (1:5)	91.60
6	Drug and cholesterol (5:1)	96.33
7	Drug and mpeg-dspe (1:1)	88.26
8	Drug and mpeg-dspe (1:5)	87.14
9	Drug and mpeg-dspe (5:1)	98.50

The drug's compatibility with the selected lipid and other excipients was evaluated using the FTIR peak matching technique. The drug-lipid combination showed no peaks or disappearances based on the percentage correlations examined, suggesting that the drug, lipid, and other chemicals did not interact chemically.

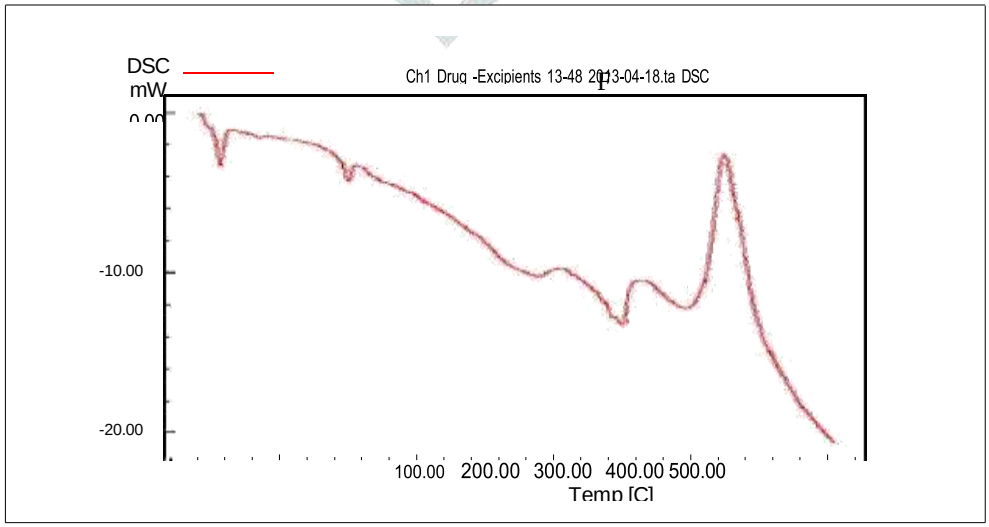


Figure No: 12 D.S.C: Cisplatin, Hspc, Mpeg-dspe and Cholesterol

The DSC curve of pure drug Cisplatin exhibits a sharp heat-absorbing peak at 320.1°C. The thermogram of drug-lipid mixture displayed endothermic peaks at 32.1°C, 120.0°C,400.5°C. DSC measurements showed that drug-lipid mixture was having less ordered arrangement of crystals, and this was favorable for increasing the drug loading capacity.

ZETA POTENTIAL ANALYSIS

TableNo:4 Zeta potential values of Cisplatin Liposomes for Optimized Batch.

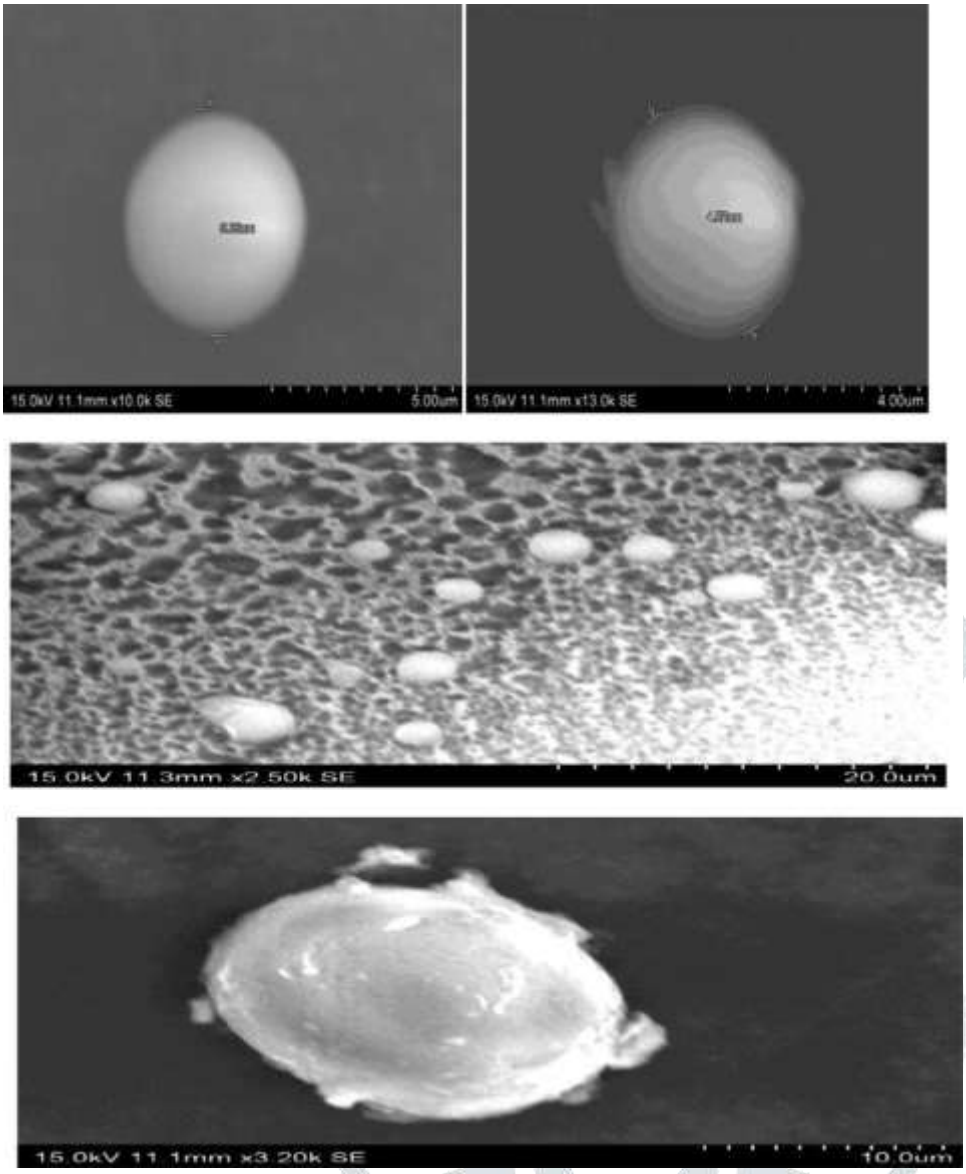
S.No	Formulation Code	Zeta Potential(mV)
1	F2	-17.0
2	F6	-20.0

It was discovered that the improved formulation (F6) had a zeta potential of -20mV.One crucial factor affecting stability is the zeta potential. While repulsion between particles with equivalent electric charges prevents the particles from aggregating and hence enables easy redispersion, extremely positive or negative zeta potential values result in higher repulsive forces. A minimum zeta potential of 20 mV is desirable for electrostatic and steric stabilization together.

TRANSMISSION ELECTRON MICROSCOPY (TEM)

The syllable structure of optimized formulation (F6) produced was assessed by Transmission electron microscopy (TEM) and it is shown in Fig.No.13 confirming the spherical shaped particles in nanometric range.

Figure no 13: TEM images of liposomes



SCANNING ELECTRON MICROSCOPY (SEM)

Figure no 14: SEM images of Cisplatin liposomes

SEM was used to analyze the liposomes' morphology and surface appearance. The smooth surface of the particles was demonstrated by the SEM images of the F2 and F6 formulations. Figure 14 displayed the SEM images.

DETERMINATION OF PERCENTAGE FREE DRUG

Table No: 5 Cisplatin liposomal solution's percentage free drug for Formulations F1, F2, F3, F4, F5, and F6

S. No.	Code for formulation	Percentage of free drug
1.	F0	45.23
2.	F1	39.3
3.	F2	30.20
4.	F3	25.31

5.	F4	17.21
6.	F5	8.72

The percentage of free drug in each formulation from F1 to F6 has been calculated.
The F6 formulation had the highest percentage of free drug, which was under the 10% restriction.

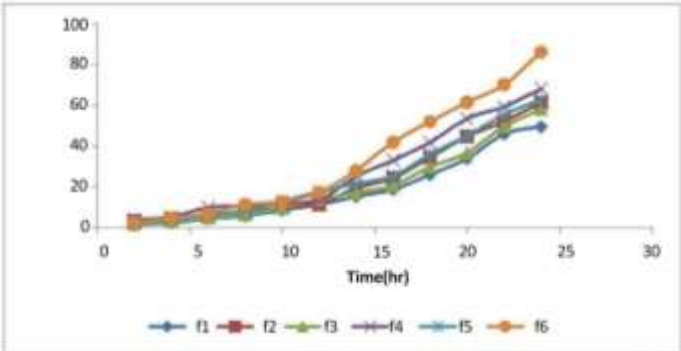


Figure No: 15 In-vitro drug release of F1 to F6 Formulations

The membrane diffusion method was used to determine the produced formulations' in-vitro diffusion profile. For a total of 24 hours, the diffusion took place in phosphate buffer with a pH of 7.4. Tables 11 to 16 show the cumulative percent release of the F1 to F6 formulations at different time periods. A graph showing the cumulative percentage of medication release over time for all formulations is shown in Figure 26. The F6 formulation, which has the highest amount of drug entrapment, had the highest percentage of drug release.

CONCLUSION

In conclusion, cisplatin's bioavailability could be improved via liposomal administration as an alternative to the traditional dosage form. According to the conducted experimental results, the stabilizer, along with hydrogenated soy phosphatidylcholine
and cholesterol, were appropriate carriers for the creation of Cisplatin Liposomes. It is also established that liposomes would maintain the drug release.

V. REFERENCES

1.Zahednezhad F, Zakeri-Milani P, Shahbazi Mojarrad J, Valizadeh H. The latest advances of cisplatin liposomal formulations: essentials for preparation and analysis. Expert opinion on drug delivery. 2020 Apr 2;17(4):523-41.
2.Stathopoulos GP. Liposomal cisplatin: a new cisplatin formulation. Anti-cancer drugs. 2010 Sep 1;21(8):732-6.
3.Peleg-Shulman T, Gibson D, Cohen R, Abra R, Barenholz Y. Characterization of sterically stabilized cisplatin liposomes by nuclear magnetic resonance. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2001 Feb 9;1510(1-2):278-91.
4.Carvalho Júnior AD, Vieira FP, De Melo VJ, Lopes MT, Silveira JN, Ramaldes GA, Garnier-Suillerot A, Pereira-Maia EC, De Oliveira MC. Preparation and cytotoxicity of cisplatin-containing liposomes. Brazilian Journal of Medical and Biological Research. 2007;40:1149-57.
5.Krieger ML, Eckstein N, Schneider V, Koch M, Royer HD, Jaehde U, Bendas G. Overcoming cisplatin resistance of ovarian cancer cells by targeted liposomes in vitro. International journal of pharmaceutics. 2010 Apr 15;389(1-2):10-7.
6.Boulikas T. Clinical overview on Lipoplatin™: a successful liposomal formulation of cisplatin. Expert opinion on investigational drugs. 2009 Aug 1;18(8):1197-218.
7.Schroeder A, Honen R, Turjeman K, Gabizon A, Kost J, Barenholz Y. Ultrasound triggered release of cisplatin from liposomes in murine tumors. Journal of controlled release. 2009 Jul 1;137(1):63-8.
8.Toro-Cordova A, Flores-Cruz M, Santoyo-Salazar J, Carrillo-Nava E, Jurado R, Figueroa-Rodriguez PA, Lopez-Sanchez P, Medina LA, Garcia-Lopez P. Liposomes loaded with cisplatin and magnetic nanoparticles: physicochemical characterization, pharmacokinetics, and in-vitro efficacy. Molecules. 2018 Sep 6;23(9):2272.