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Research



Formulation and optimization of cisplatin liposomal drug delivery system

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	Abstract
Published on: 22 Jun 2024	<p>In the present work an attempt is being made to provide for stable drug delivery system with or having improved therapeutic index for Cisplatin in form of lyophilized liposomes. By varying the composition of lipids six formulations of Liposomes were prepared and loaded by Cisplatin as a model drug using physical dispersion method for targeted delivery of this drug. Various evaluation parameters were studied for the prepared liposomes which includes encapsulation efficiency, particle size analysis, <i>in-vitro</i> drug release, stability studies etc. Because F5 has higher encapsulation effectiveness and a smaller particle size than the other formulations, it was determined to be the best choice. The phosphatidylcholine and cholesterol-containing formulation F5 satisfies the liposomal formulation criterion. More than 95.73% of the drug was released in 8 hours of in vitro testing, with 80.25% drug encapsulation efficiency.</p>
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<p>Keywords: Cisplatin, Cholesterol, phosphatidylcholine, physical dispersion method, <i>In-vitro</i> drug release studies.</p>	

INTRODUCTION

Liposomes are biocompatible as well as biodegradable bilayer vesicles comprising of a hydrophilic aqueous core and are made of phospholipids. From the last several years the liposomal vesicles have been extensively considered as carrier of preference for the delivery of various potential drug candidates that are lipophilic as well as hydrophilic.¹ Generally, the liposomes used in clinical practice have the size with diameter in the range of 50 to 300 nm. The membranes of liposomal vesicle are linked with the plasma membrane, and are consisting of bilayers made up of phospholipids. Instinctively, vesicles formation occurs upon hydration by means of aqueous media from phospholipids as a consequence of tails of hydrophobic fatty acid, and a head group of hydrophilic phosphatidyl that exist in the typical amphiphilic molecular structure.^{2,3} In the liposomal membrane cholesterol (CH) can be simply incorporated just as plasma membrane in the matching style which stabilizes the membrane and adjust the release of the drug.^{4,5} The drug release rate is reliant on the components present in the membrane of liposome, like types of phospholipid's fatty acid acyl chain, degree of unsaturation, charge on lipid and inclusion of CH.^{6,7}

MATERIALS AND METHODS

Cisplatin drug was collected from Carbanio and other chemicals like Phosphatidylcholine, Cholesterol and Chloroform were collected from Synpharma Research Labs, HYD.

Table 1: Instruments used for the research work

S. No	Name	Supplier
1	Double Beam UV/Vis Spectrophotometer	Lab India
2	Franz diffusion cell apparatus	AR Chemicals
3	FT-IR	Shimdzu, India .
4	Scanning Electron Microscopy	Hitachi, Japan.
5	Electronic Balance	Shimdzu, Japan.
6	Magnetic Stirrer	Genuine Equipment Manufactures, Coimbatore
7	pH Meter	Systronics, Chennai

Method

Melting Point Determination

Melting point of drug was observed by placing a specified quantity of drug (nearly 2-5 mg) in a capillary tube which was sealed at one side. The tube was kept in a melting point apparatus (Thiele's apparatus, capillary method). The observed melting point of the drug was noted in triplicate.

Solubility Determination

100 mg of API was added into 20ml volumetric flask. Qualitative solubility in individual solvents was noted after proper mixing by vortexing /sonication. The solvents evaluated were Water, Dehydrated Alcohol, Methanol, Chloroform, Acetone and Hexane.

Preparation of standard calibration curve of Cisplatin

The concentration range of 10–50 µg/ml of Cisplatin was used to plot the calibration curve. Since the maximum absorbance was obtained at 270 nm, appropriate dilutions were made and absorbance was measured for each solution at this wavelength. The absorbance vs. concentration graph was plotted.

IR Spectroscopic Analysis

For IR spectroscopy KBr powder was dried out at 60°C for one hour. The dried KBr powder was uniformly mixed with drug and IR spectra was taken for this mixture using ATR-FTIR 4000 (Jasco, Japan) IR Spectrophotometer.

Preparation of liposomes

Different lipid ratios were used in the physical dispersion method and thin-film hydration approach to create liposomes. The lipids were dissolved in chloroform using this approach. This lipid solution in chloroform has been applied to a conical flask with a flat bottom. After that, the solution was allowed to evaporate at room temperature without being disturbed. The aqueous medium phosphate buffer (pH 7.4) was used to hydrate the lipid film form. In order to achieve this, the flask was tilted to one side, and then a drug-containing aqueous medium was added down the side of the flask before it was gradually rotated back to its upright position. The lipid layer was gently covered with the fluid, and the flask was let to stand at 37°C for two hours to allow for full expansion. Vesicles are gathered by twirling the flask's contents to produce a milky white solution once they have swelled. Centrifugation was then applied to the formulations. Various liposome batches were made in attempt to find the best possible formulation. Every batch of liposomes was made using the above-described standard procedure.

Table 2: Composition of lipids for preparation of liposomes

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Phosphatidylcholine	100	100	100	100	100	100	100	100
Cholesterol	100	200	300	400	500	600	700	800
Solvent(Chloroform)	10	10	10	10	10	10	10	10
Drug	20	20	20	20	20	20	20	20
Phosphate buffer pH 7.4	10	10	10	10	10	10	10	10

Evaluations of liposomes**Encapsulation efficiency determination⁸⁻¹⁰**

The entrapment efficiency (EE) of liposomal preparations was evaluated by ultra-centrifugation technique. Briefly, ultra-centrifugation devices from Amicon (Amocon ultra, 0.5ML 10K, Merck, USA) were used to process 500µL sample of cisplatin liposomes. After centrifugation for 20 min at 10,000 rpm in a TarsonMC-1, Spin win micro centrifuge, the ultrafiltrate was collected and injected into HPLC device for free drug determination. The entrapment efficiency of the liposomes was estimated using the following equation.

$$\text{Entrapment efficiency (\%)} = [(TD - FD)/TD] \times 100 \quad (1)$$

Where

FD is the quantity of drug estimated in the filtrate and

TD is the theoretical amount of drug that is present in liposomal preparation.

Particle size analysis

To examine the size of each generated batch of liposomes, a microscope was used. By placing a little drop of liposomal dispersion on each batch's liposomal vesicle at a different point on the slide, the average size of the liposomal vesicles was calculated.

SEM analysis

A scanning electron microscope was used to examine the liposomes' morphology. With order to do this, the sample was lyophilized, mounted on aluminium stubs, and then a sputter coater was used to coat the surface with gold particles. Scanning electron microscopy (SEM) (XL30, Philips, the Netherlands) at 15 kV and 750 mA was used to determine the liposomes' appearance.

In-Vitro Drug release study

An 8 ml receptor compartment in a Franz diffusion cell was used for *in-vitro* drug release experiments. The drug from the produced liposomes was determined using a cellulose acetate membrane. The diffusion cell's donor and receptor compartments were separated by a 0.45 µ pore-size cellulose acetate membrane. After the manufactured liposomes were put on the cellulose acetate membrane, aluminium foil was used to cover them. Phosphate buffer, pH 7.4, was put within the diffusion cell's receptor compartment. The entire assembly was mounted on a hot plate magnetic stirrer, and since human skin normally has a temperature of 32°C, the fluid in the receptor compartment was continually and continuously swirled using magnetic beads. The samples were taken out at various intervals and subjected to spectrophotometric analysis to determine the drug concentration. After every sample removal, the receptor phase was refilled with the same volume of phosphate buffer.¹¹

Stability studies

The stability of optimized liposomes formulation was assessed for a period of 3 Months by storing the liposomes at 3 specified temperature conditions, i.e. 25±2°C /60% RH (Room temperature; RT), 40±2°C/75% RH (Accelerated conditions) and 2-8°C (Refrigerator; control). The liposomal formulations were kept in sealed laminated aluminium tubes (10 ml capacity) which were previously flushed with nitrogen. Samples were periodically taken at specified time period of 0, 1, 2 and 3 months. These were tested for drug release, in the manner described.

RESULTS & DISCUSSION**Determination of melting point**

Melting point of Cisplatin was found in the range of 270-272°C, which complied with the standard, indicating purity of the drug sample.

Solubility

Soluble in dimethyl formamide (16mg/ml) or DMSO (10mg/ml); sparingly soluble in water; insoluble in 100% ethanol.

Standard graph of Cisplatin

Standard solution of Cisplatin Prepared with phosphate buffer saline of pH 7.4

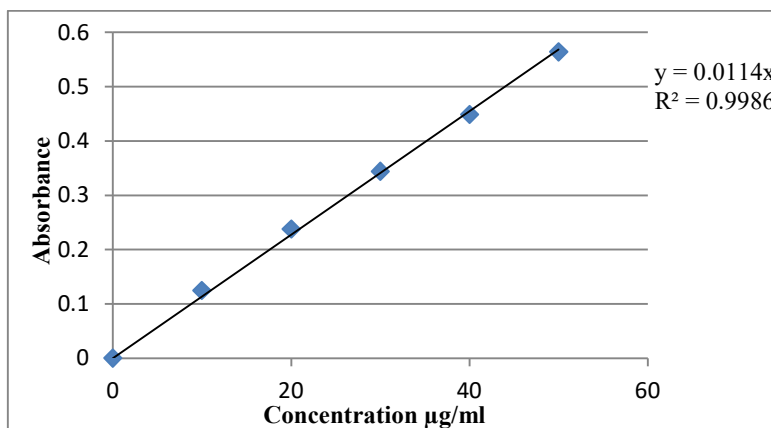


Fig 1: Standard graph and regression value of the standard solution with pH 7.4

Drug-excipient compatibility studies (FT-IR)¹²⁻¹⁴

The compatibility between the pure drug and the optimized formulation (F5) was evaluated using FTIR peak matching method. There were no significance changes in the peaks between the drug and F5 formulation, which confirmed the compatibility between the Drug, lipid and other excipients.

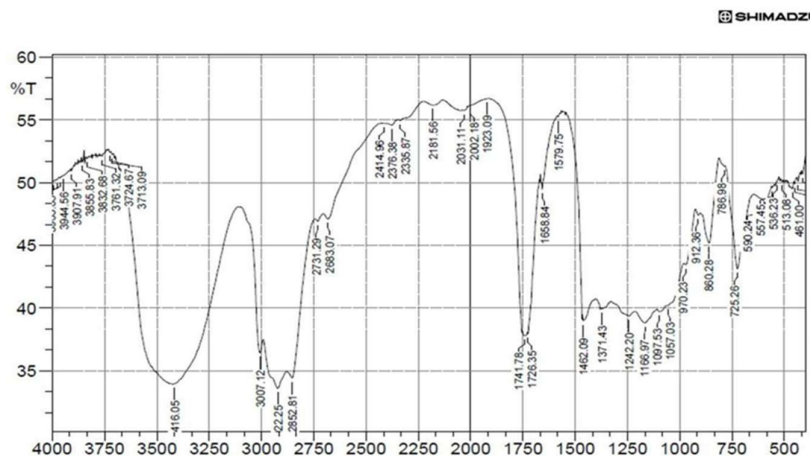


Fig 2: FTIR Studies of Cisplatin

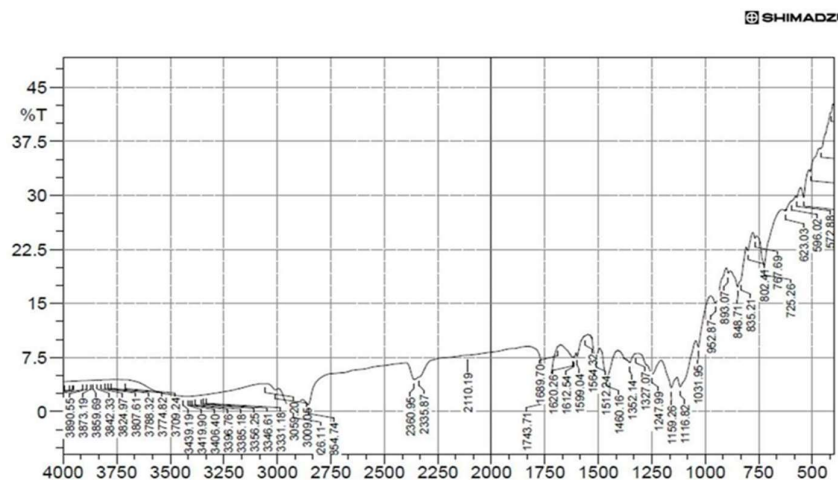


Fig 3: FTIR Studies of optimized formulation (F5)

Drug entrapment efficiency**Table 3: Drug entrapment efficiency of all formulations**

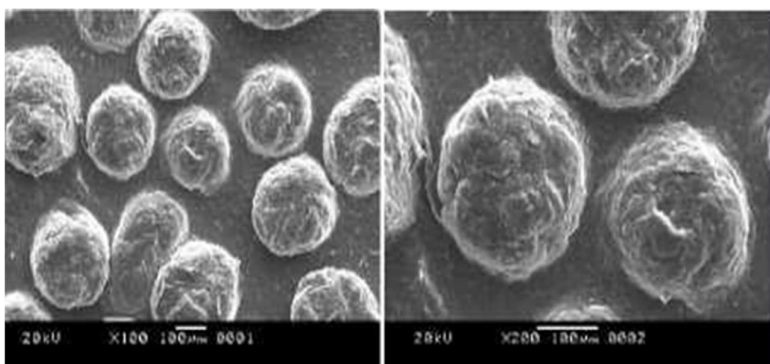
Formulation code	DEE
F1	78.95
F2	75.68
F3	74.57
F4	79.62
F5	80.25
F6	76.93
F7	77.82
F8	73.58

Particle size analysis

The synthesised formulation's vesicle shape was determined to be spherical using scanning electron microscopy investigation at 15.00 kV.

Table 4: Mean particle size (mps) of different formulation of liposomes

Formulation code	Particle size
F1	180
F2	163
F3	172
F4	175
F5	182
F6	178
F7	181
F8	177

SEM Analysis**Fig 4: SEM Analysis of Cisplatin liposomes****In-Vitro Drug release study****Table 5: Cumulative percentage drug release from various formulations of liposomes**

Time (Hrs)	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	17.65	15.68	15.87	14.89	15.99	14.96	13.69	16.58
2	28.74	24.67	25.93	26.65	27.83	25.90	27.55	25.97
3	32.46	31.49	32.67	34.52	35.95	34.69	33.57	35.64
4	47.89	45.74	44.25	46.98	45.72	44.76	42.30	46.52
5	56.30	55.60	56.98	58.49	55.64	53.61	52.11	53.67
6	65.79	64.22	63.54	65.55	67.98	65.90	64.98	65.88

7	78.51	75.74	78.85	79.88	80.02	75.93	78.82	77.56
8	91.23	90.55	91.64	92.20	95.73	93.60	94.55	93.34

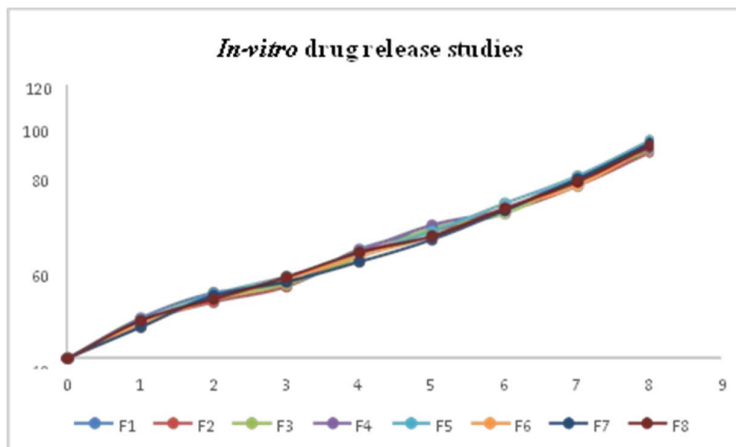


Fig 5: *In-vitro* drug release of (F1-F8) formulations

Formulation code F5 were found to release the drug in 8 hrs and considered as best formulation in terms of *In-vitro* drug release. The cumulative percentage release was found to be 95.73 %.

Stability studies

After three months, the formulation F-5's chemical and physical characteristics did not significantly alter. Displayed were parameters measured at different time periods.

Table 6: Results of stability studies of optimized formulation F-5

Formulation code	Parameters	Initial	1 st Month	2 nd Month	3 rd Month	Limits as per specifications
F5	25°C/60%RH % Release	95.73	94.89	93.69	92.78	Not less than 85%
F5	30°C/75%RH % Release	95.73	94.75	93.58	92.46	Not less than 85%
F5	40°C/75%RH % Release	95.73	94.58	93.47	92.74	Not less than 85%

CONCLUSION

A novel method of medicine delivery is liposomes. They are an excellent means of administering medications with less toxicity and increased effectiveness. They do, however, have certain drawbacks, and sterically stabilised liposomes appear to be the preferred method of drug administration. Cisplatin possesses all requisite qualities required for liposomal drug delivery. The combination F5 was determined to be the most appropriate among the several formulations due to its high encapsulation effectiveness and reduced particle size. The phosphatidylcholine and cholesterol-containing formulation F5 satisfies the liposomal formulation criterion. Up to eight hours of *in-vitro* drug release, 95.73% of the medication was released. It has a 182 nm particle size and an encapsulation effectiveness of 80.25%. Thus, liposomes may be a more effective drug delivery method for cytotoxic medications like cisplatin.

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