Preparation and Optimization of Cisplatin Polymeric Nanoparticles

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ABSTRACT:
The goal of this study was to assess the efficacy of a method based on the creation of polymeric nanoparticles as an innovative formulation of Cisplatin with enhanced therapeutic efficacy. Cisplatin has low solubility and permeability, which result in limited and variable bioavailability; its low stability makes it difficult to develop stable aqueous liquid formulations. The Cisplatin Polymeric nanoparticles were created using the precipitation method.The numerous formulations with varied drug-polymerwere analyzed and improved. Particle size, surface morphology by SEM, drug excipient compatibility by FTIR, and in-vitro drug release experiments were used to characterize the produced nanoparticles. The formulation with the best encapsulation efficiency was (F-8). A drug encapsulation effectiveness of up to 80.12% has been attained in this study. It was discovered that the efficiency of encapsulation improved along with the polymer content. According to the results of the current investigation, the manufacture of Cisplatin Polymeric nanoparticles can be done using a precipitation process followed by sonication.
Key words: Cisplatin drug, Polymers, FTIR, Precipitation method, Polymeric Nano Particles, In-vitro drug release.

I.INTRODUCTION

Nanotechnology is a term used to define areas of science and engineering in which phenomena occurring at nanoscale dimensions are used in the design, characterization, manufacture, and applications of materials, structures, devices, and systems. The nano drugs employed have demonstrated that bioavailability is enhanced, side effects are eliminated, and therapeutic medicine is absorbed more effectively.[1] Polymeric nanoparticles (NPs) have attracted considerable interest over recent years due to their properties resulting from their small size. Advantages of polymeric NPs as drug carriers include their potential use for controlled release, the ability to protect drug and other molecules with biological activity against the environment, improve their bioavailability and therapeutic index.[2] The term "nanoparticle" comprises both Nano capsules and nanospheres, which differ with respect to their morphology.The properties of PNPs include particle size and shape, hydrophobicity, surface charges and coating material that influence the targeted cells or tissues. It also influences the penetration of particles into the cell membrane and passages by the biological barrier. [3]The genotoxic agent cisplatin, used alone or in combination with radiation and/or other chemotherapeutic agents, is an important first-line chemotherapy for a broad range of cancers.[4] The main aim of the research study was formulate and evaluate of cisplatin polymeric nanoparticles used in the treatment of cancer.

II. EXPERIMENTAL WORK

MATERIALS

Cisplatin procured from Hetero Labs, HYD. Eudragit RS 100 and sodium alginate was obtained from Synpharma Research Labs, Hyderabad. Other chemicalsand the reagents used were of analytical grade.

METHODOLOGY

Compatibility study (IR spectroscopy)

FTIR analysis was performed in order to study the compatibility of ingredients used in the preparation of nanoparticles, using a Shimadzu FTIR spectrophotometer (Prestige21, Shimadzu Corporation, Kyoto, Japan). Cisplatin and Excipients their mixture with ratio (1:1) was evaluated using FTIR spectrophotometer using potassium bromide disc technique where 1mg of the sample is mixed with 100 mg of dry powdered KBr; the mixture is pressed into a transparent disc and was inserted in the apparatus for IR scan.[5]

Method of preparation of Cisplatin loaded nanoparticles:

Table-1: Composition of the Nanoparticles

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Cisplatin	50	50	50	50	50	50	50	50
Sodium alginate	100	200	300	400	-	-	-	-
Eudragit RS 100	-	-	-	-	100	200	300	400

Ethanol	10	10	10	10	10	10	10	10
PVA	1%	1%	1%	1%	1%	1%	1%	1%

Precipitation method

1. Prepare Organic Phase

Dissolve the polymers in 10 mL ethanol.Add 50 mg Mix thoroughly until fully dissolved **(Organic Phase).** Prepare 50 mL of 1% PVA solution in distilled water (as stabilizer). **(Aqueous Phase)** Stir to ensure complete dissolution.Under moderate magnetic stirring (400–800 rpm), add the organic phase dropwise into the aqueous phase.A turbid colloidal suspension will form as nano particles precipitate. Stir the suspension for 2–4 hours at room temperature to allow full solvent diffusion and evaporation. Remove residual ethanol by Continued stirring in a fume hood by using a rotary evaporator. Centrifuge the suspension at 15,000 rpm for 30 min. Discard the supernatant and resuspend the pellet in distilled water. Repeat 2–3 times to remove unencapsulated drug and PVA.[6]

Evaluation of Cisplatin loaded polymeric nanoparticles:

Particle Size and Zeta Potential

The particle size of the formulation was determined by photo correlation spectroscopy with a zeta master (Malvern Instruments, UK) equipped with the Malvern PCS software. Every sample was diluted with distilled water. The surface charge (Zeta potential) was determined by measuring the electrophoretic mobility of the nanoparticles using a Malvern zeta sizer (Malvern Instruments, UK). Samples were prepared by diluting with distilled water.[7]

SEM analysis

Scanning and transmission electron microscopy (SEM) have been widely used to obtain information regarding the shape and size of polymeric NPs. These are usually combined with cryofracture techniques to perform the NPs morphology analysis.[8]

Drug entrapment efficiency

For determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation was determined (w) by UV spectrophotometer at 252 nm. A standard calibration curve of drug was plotted for this purpose. The amount of drug in supernatant was then subtracted from the total amount of drug added during the preparation (W). [9] Effectively, (W-w) will give the amount of drug entrapped in the particles. Then percentage entrapment of a drug was calculated according to Equation 2

$$\%\text{Drug Entrapment} = (W\text{-}w/W) \times 100$$

In-vitro drug release studies:

In vitro dissolution of drug was carried out by the method with Dialysis bag. Dialysis membrane was used for the release study. Dialysis membrane was soaked in distilled water 24 h before the release studies. 2 mg equivalent weight of the Cisplatin loaded polymeric nanoparticles was incorporated into the dialysis that is tied at the two ends. 50 ml of phosphate buffer pH 6.8 was added to a beaker and the dialysis membrane was fixed in it, where the solution was stirred using magnetic stirrer at 50 rpm and the temperature was maintained at 37±0.5 °C, at the time intervals of 1, 2, 3, 4, 5, 6, 7, 8 h the sample of 1 ml was taken and same volume was added to the beaker to maintain the sync condition. Concentration of drug release from the Cisplatin loaded polymeric nanoparticles was calculated using UV spectrophotometer at 252 nm.[10]

Kinetic analysis of Cisplatin from nanoparticles [11]

The diffusion of the Cisplatin from the nanoparticles formulation was evaluated by fitting the experimental data to different kinetic equations. The data were analyzed using the linear regression according to:

Zero order kinetics:

$Ct=Co-kot$ …

First order kinetics:

$Ct=Coe-kt$ …

According to the simplified

Higuchi diffusion model: $Ct=kh\sqrt{t}$ …

Where,

Ct is the total amount of drug dissolved (%) after time t,

Co is the initial amount of the drug (%),

Ko is the zero order rate constant (% min-1),

K is the first order rate constant (min-1),

Kh is the rate constant obtained according to Higuchi equation (% min-1/2).

Korsmeyer–Peppas Model

F = Kptn, where F represents the fraction of drug released in time t, Kp is the Koresmeyer–Peppas release rate constant, and n is the diffusion exponent

The order of the drug dissolution from each was nanoparticle determined by calculating the coefficient of determination (r2) in each case. The highest (r2) value represents the order of drug dissolution from the nanoparticles.

Stability studies:

Over the course of 90 days, the stability of Cisplatin nanoparticle dispersion in screw-capped glass vials was assessed. Four samples were split into two groups and kept at 4°C and 25°C, respectively. At the end of the 90 days, the amount of drug leaking from nanoparticles and the average particle size of the samples were calculated.[12]

III. 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-polymer mixture showed no peaks that appeared or vanished, indicating that there was no chemical interaction between the medications, polymer andother molecules.

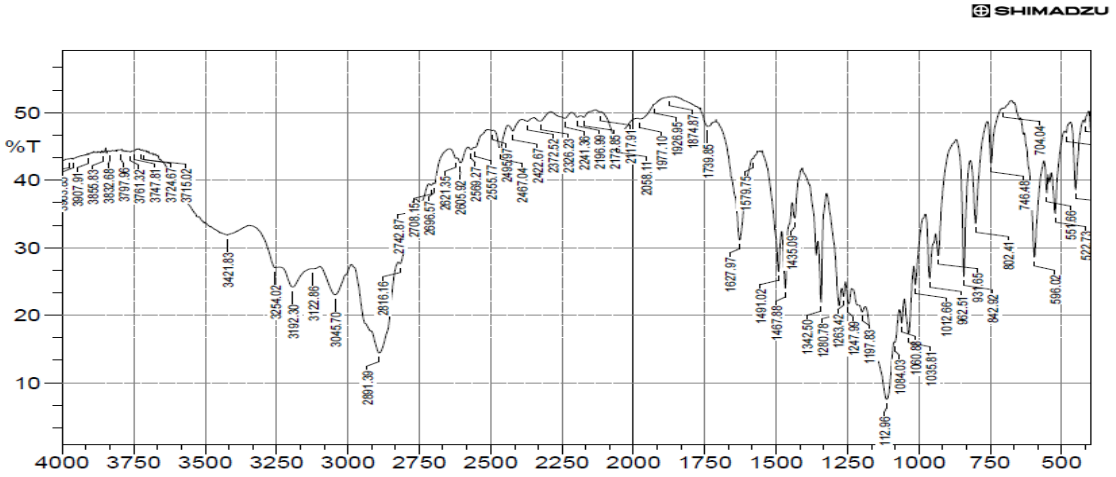

Fig-1: FT-IR Sample for Pure drug

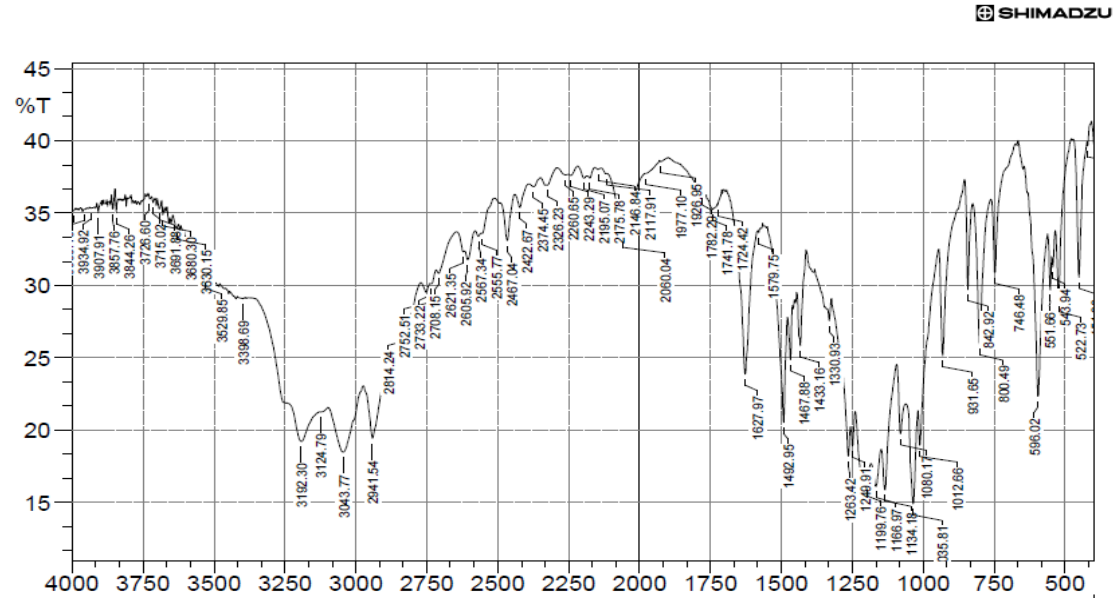

Fig-2: FT-IR Sample forOptimized formulation

EVALUATION PARAMETERS

Particle size:

With an increase in lipid concentration, the particle size increased. Based on entrapment effectiveness and particle size distribution.

Surface morphology:

According to scanning electron microscopy (SEM), the polymeric nanoparticles were round, smooth, and free of any aggregation.

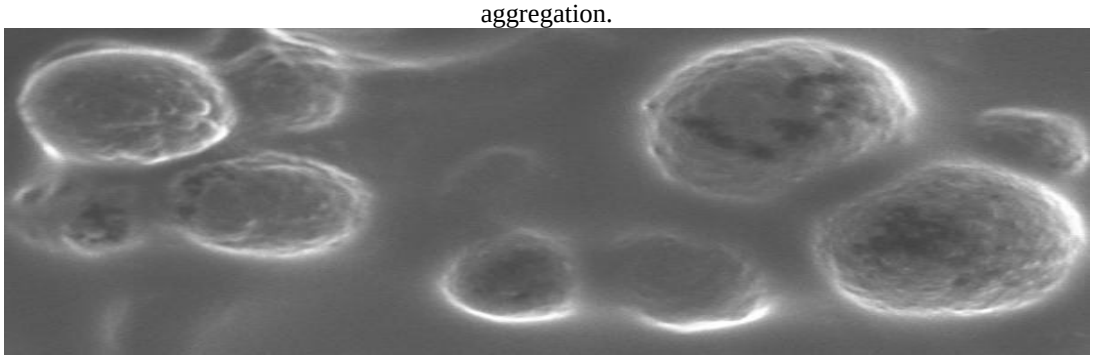

Fig-3: SEM analysis of Optimized polymeric nanoparticle

Particle size

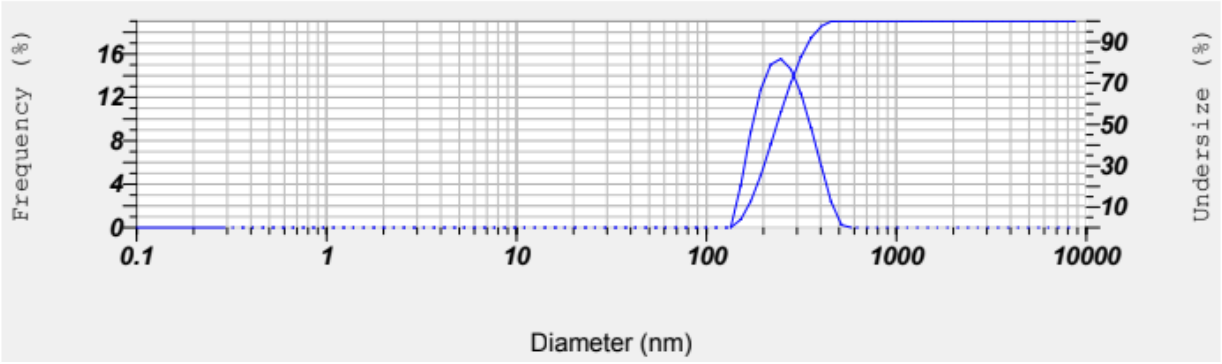

Fig-4: Particle size of Polymeric nanoparticles

The mean particle size of optimized Polymeric nanoparticles was found to be 247 nm

Determination of Zeta potential:

Zeta potential is a measure of charge present on the vesicle surface. It was determined by using phase analysis light scattering with Malvern zetasizer at field strength of 20V/cm in distilled water and based on electrophoretic mobility of charged particles present in the nanocrystal system. Charged particles were attracted to the electrode with the opposite charge when an electric field is applied.

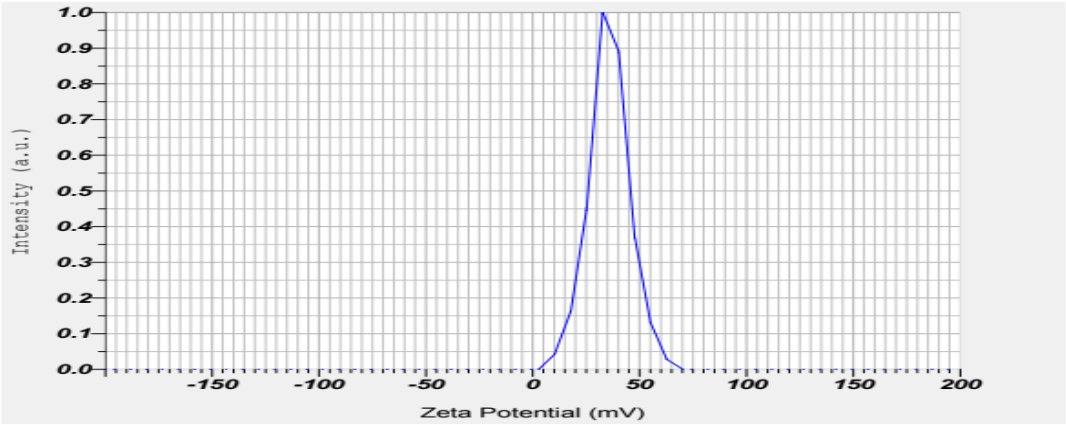

Fig-5: Zeta potential of Polymeric nanoparticles

Drug entrapment efficiency:

Optimizing the polymer concentration to be used in the creation of polymeric nanoparticles was the first step of the work plan. Based on the particle size and entrapment effectiveness of the discovered polymeric nanoparticles, the polymer content was optimized.

Table-2: Evaluation Studies of Prepared polymeric nanoparticles: Entrapment Efficiency and Particle size

Batch No	Particle size (nm)	Zeta potential	Entrapment Efficiency (%)
F1	205	-26	63.59
F2	220	-29	70.15
F3	219	-28	69.82
F4	225	-30	75.50
F5	238	-28	73.91
F6	199	-25	68.17
F7	218	-30	76.39
F8	247	-24	80.12

In vitro drug release studies

Using a dialysis membrane and a pH 7.4 buffer, the in vitro diffusion investigations were carried out for eight hours. This resulted from the drug's release from the surface of the nanoparticles. Later, for 8 hours, a consistent and gradual medication release was seen. The polymer ratio in the F8 formulation was shown to be the most effective one.

Table-3: *In vitro* drug release profiles of Cisplatin polymeric nanoparticles (F1-F8)

Time	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	14.56	15.75	16.98	17.10	18.12	17.26	18.21	19.82
2	29.81	28.91	27.69	29.32	27.84	28.15	30.56	32.25
3	34.56	35.69	36.56	38.56	37.96	38.95	40.25	42.58
4	45.78	46.38	47.20	48.51	47.59	48.96	53.67	55.50
5	58.17	59.83	60.15	62.34	65.81	66.10	67.89	68.33
6	68.25	70.12	71.25	73.59	72.32	75.58	76.51	77.15
7	79.86	81.20	80.19	82.50	83.52	85.22	86.95	87.90
8	91.25	92.65	93.65	95.68	94.55	96.39	97.52	98.21

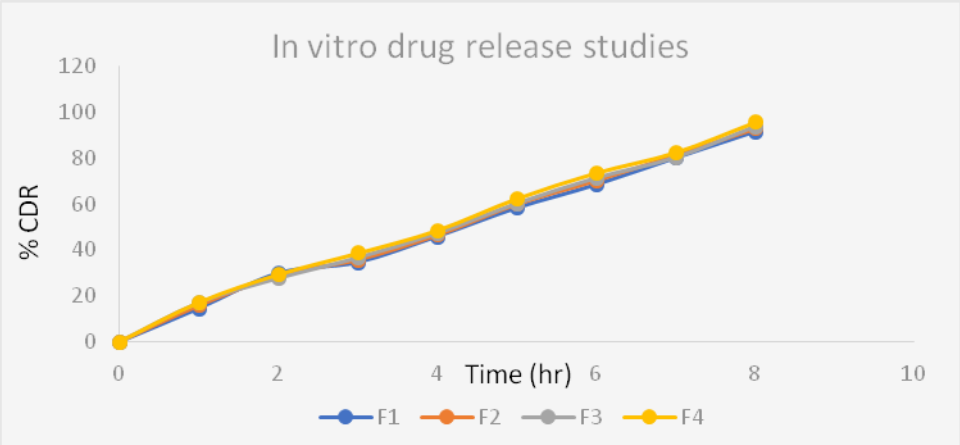

Fig-6: Drug release for (F1-F4) formulations

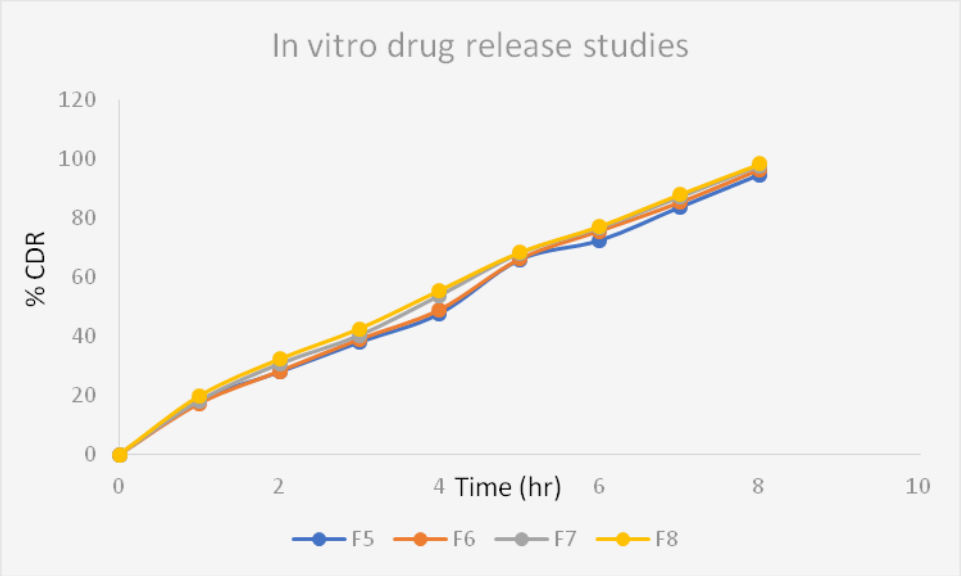

Fig-7: Drug release for (F5-F8) formulations

Stability studies:

After three months, the physical and chemical characteristics of the nanoparticles of formulation F-8 had not significantly changed. The parameters quantified at various times were displayed.

Table-4: Results of stability studies of optimized formulation F-8

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-8	**25^0C/60%RH**	98.21	97.86	96.58	95.87	**Not less than**
F-8	**30^0C/75% RH**	98.21	97.80	96.45	95.50	**Not less than**
F-8	**40^0C/75% RH**	98.21	97.16	96.23	95.18	**Not less than**

CONCLUSION

The current study suggested a unique Cisplatin polymeric nanoparticle formulation for regulated release. Investigation into the polymeric nanoparticles' production, characterization, and in-vitro release was done. The numerous formulations with varied drug-polymer and surfactant ratios were analyzed and improved. A drug encapsulation effectiveness of up to 80.12 % has been attained in this study. Cisplatin polymeric nanoparticles containing polymers were created using the precipitation method, then the particle size was decreased by sonication.Formulations using polymeric nanoparticles performed well in terms of medication content and encapsulation effectiveness. This shows that the formulation procedure was suitable and reproducible in nature, and it provided a good yield. The formulation with the best encapsulation efficiency was (F-8) It was discovered that the percentage of encapsulation efficiency along with the polymer concentration. According to the method described, permeation studies with dialysis membrane were conducted. The in vitro drug release profiles of all the formulations indicated an initial burst effect, followed by a gradual drug release. The formulations demonstrated good drug release from the polymer. These polymeric nanoparticles contained more Cisplatin and released it more quickly.

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