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PREPARATION, CHARACTERISATION AND EVALUATION OF CISPLATIN LOADED NANO PARTICLES BY EMULSION POLYMERISATION TECHNIQUE WITH IMPROVED INVITRO ANTI-CANCER ACTIVITY

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Abstract: The present research work is based on the formulation, optimization and characterization of Nano particulate drug delivery system. The main rationale of the work is to formulate a targeted drug delivery system with enhanced drug entrapment efficiency. Here the drug of interest is Cisplatin hydrochloride trihydrate, an antineoplastic agent while the polymer used is poly lactide -o - glcolic acid (PLGA), a biodegradable polymer. The analytical method development is carried out using acetonitrile and phosphate buffer saline. The formulation optimization is also carried out optimizing its various process and formulation parameters. Different organic solvents were tried and various surfactants were used to optimize the Nano particulate formulation. The size range and zeta potential was measured using Malvern zeta sizer. The lyofilization was carried out using two different cry protectants.

Keywords: Characterization, Evaluation, Cisplatin, Polymerization

Introduction: Targeted cancer chemotherapy aims to direct adequate concentration of the chosen agent to tumor cells while affecting as few healthy cells as possible. In principle, this can be achieved by passive or active targeting. Passive targeting exploits the enhanced permeability and retention (EPR) characteristics of tumor vessels. Rapidly growing tumors develop extensive vasculatures to meet their requirement for nutrient supply and waste disposal, but the blood vessels are abnormally hyper-permeable, with defective architecture and impaired lymphatic drainage. Circulating macromolecular drugs or particulate delivery systems that have difficulty permeating normal blood vessels can extravasate through such tumor blood vessels, and they become entrapped due to the impaired lymphatic drainage in tumortissues.

Mechanism of Action: Cisplatin inhibits the action of topoisomerase I. Cisplatin prevents relegation of the DNA strand by binding to topoisomerase I-DNA complex. The formation of this ternary complex interferes with the moving replication fork, which induces replication arrest and lethal double-stranded breaks in DNA. As a result, DNA damage is not efficiently repaired and apoptosis (programmed cell death) occurs.Indication: For the treatment of metastatic colorectal cancer (first-line therapy when administered with 5-fluorouracil and leucovorin). Also used in combination with cisplatin for the treatment of extensive small cell lung cancer. Cisplatin is currently under investigation for the treatment of metastatic or recurrent cervical cancer.Method of Preparation: Preparation of nanoparticles is frequently based on the use of dispersed systems in which solid or liquid phases are dispersed in fluid media to constitute embryos of

the final particles. Single and multiple emulsion systems have been used to encapsulate drugs into polymeric particles. Normally, an organic solvent is required to dissolve the polymers in the emulsion step. To decrease droplet size and avoid droplet coalescence, surfactants are usually required.

CALIBRATION CURVE OF CISPLATIN IN ACETONITRILE:

A stock solution of Cisplatin in ACN was prepared by dissolving 5 mg of the drug in 50 ml of ACN. A serial dilution of stock solution was done to prepare various strength of CIS solution and the absorbance was measured using UV Visible spectrophotometer (UV-1700, Pharma spec, Shimadzu, Japan) at wavelength of 256 nm. A calibration plot of absorbance vs. concentration was plotted.

RESULTS AND DISCUSSION

The calibration curve of cisplatin hydrochloride trihydrate was carried out in acetonitrile and phosphate buffer saline.

Table 1: Calibration curve of CIS in CAN

Concentration	Mean Absorbance
0	0
10	0.708
20	1.455
30	2.105
40	2.731
50	3.221

POWDER X-RAY DIFFRACTION

Cisplatin hydrochloride was characterized by its powder X-ray diffraction pattern, comprising 20 angle values of about 7.60; 8.30; 9.55; 11.00; and 12.40. The Relative Intensity (%) of the mentioned characteristics reflection peaks of Form b at the 20 angle values are reported in table.

Table 2: X-Ray diffraction pattern

Angle 2□	Relative intensity %
7.60	47.9
8.30	33.4
9.55	36.9
11.00	97.3
12.40	88.1

FORMULATION OF CISNANOPARTICLES

Nanoparticlepreparation:

Nanoparticles were prepared by using solvent evaporation method. Polymer and drug was firstly dissolved in organicphase. Then a weighed quantity of surfactant was dissolved inaqueous phase. Then the organic phase was added to aqueous phase in a dropwise manner. The suspension was kept on mechanical stirring until the complete evaporation ofsolvent. The residual quantity of solvent was removed by rotatory vacuum evaporator for 1hour. Then this dispersion was passed through the Sephadex G-25 column (size exclusion chromatography) for the separation of free drug and entrappeddrug. Preparation of organic phase: The required amount of the organic solvent was measured and was taken in a 10 ml beaker. Then drug and polymer was separately weighed and was dissolved in organic phase.

Preparation of aqueous phase: the required amount of selected surfactant was dissolved in water.

Characterization of CIS nanoparticles

Percent drug entrapment

An aliquot of CIS NP dispersion was added to CAN and sonicated well to dissolve nanoparticles completely.

The absorbance of the solution was measured at □max of 256nm using U.V. visible spectrophotometer (UV-1700, Pharma spec, Shimadzu, Japan).

The percent drug entrapment was calculated using following formula.

$$\text{Drug entrapment (\%, w/w)} = \frac{\text{Amount of drug in nanoparticles}}{\text{Total amount of the drug used}} \times 100$$

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Table 3: IN VITRO RELEASE PROFILE OF CIS NP:

Time	Cumulative Percent drug release
15 min	0
30 min	0.4
1 hours	1.3
2 hours	4.75
4 hours	20.59
6 hours	31.85
8 hours	48.32
24 hours	87.3

In vitro profile of CIS NP:

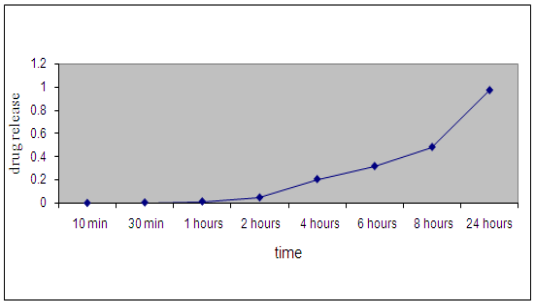

Figure 1: plot showing in vitro release pattern of CIS NP

SUMMARY AND CONCLUSION:

Summary:

As the field of targeted drug delivery continues to move forward, it will be increasingly important to design nanoscale systems with tailorable properties for efficient delivery and improved therapeutic efficacy. Important design considerations will include the physicochemical properties that govern targeting, bio distribution, and clearance as well as the system's effectiveness in carrying, protecting, and even releasing active therapeutic and diagnostic agents. The formulation, optimization and characterization of cisplatin hydrochloride trihydrate nanoparticles was successfully carried out.

Conclusion:

The analytical method developed for CIS in ACN and PBS pH 7.4 showed regression coefficient near to 1 and followed Beer Lambert's law.

Cisplatin PLGA nanoparticles were prepared using solvent evaporation method.

Formulation and optimization of CIS NP was carried out using various formulation and process parameters.

Solvent selection: Acetonitrile was selected as the solvent of choice

Surfactant selection: Poloxomer 188 was selected as surfactant.

Drug to polymer ratio: The drug to polymer ratio was optimized to be 1:10

Organic phase to aqueous phase ratio: The organic phase to aqueous phase ratio was optimized as 1:2.

Poloxomer 188 concentration: The poloxomer188 concentration was selected as 2%.

RPM of the magnetic stirrer: The speed of rotation of the magnetic stirrer was kept 600 rpm

Rate of addition of organic phase to aqueous phase: The rate of addition of organic solvent to the aqueous solvent was 0.5 ml/min.

Zeta potential: Zeta potential is the overall charge acquired by particles in a particular medium and its value gives the indication of potential physical stability of nanoparticles dispersion. The zeta potential obtained was –13.3 mV.

Percent drug entrapment: The maximum percent drug entrapment was found out to be 37.2% of the batch CIS F009.**In vitro drug release**: The in vitro drug release of CIS NP was also found out using dialysis method in phosphate buffer saline pH 7.4. The in vitro drug release showed sustained release of drug over 24 hours.

Lyofilization: Lyophilized CIS NP with different cry protectant at Svarious ratios of solid drug to cry protectants was used. Compared to sucrose, trehalose served as better cry protectant and maintained the original particle size.Hence the CIS loaded PLGA nanoparticles have potential as a drug delivery system. Furthermore, they may have utility for site-specific drug delivery since the small size of the particles may allow their delivery to extra vascular target sites through the leaky endothelia of inflamed and cancerous areas.

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