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Research Article

Preparation and Characterization of Solid Lipid Nanoparticles Loaded with Cisplatin

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ABSTRACT

Cisplatin (Cis diaminedichloro platinum) is the first platinum drug to be used as an anticancer drug, and it is widely used in the treatment of testicular, head, neck, ovarian and lung cancer. The use of Cisplatin is limited due to its intrinsic and acquired resistance and severe side effects such as chronic neurotoxicity and nephrotoxicity. The colloidal carriers such as emulsion, liposomes, polymeric nanoparticles have been extensively studied to overcome above limitations. The solid lipid nanoparticles (SLNs), amongst other colloidal carriers, were found to be an ideal carrier for lipophilic drug for better stability and release retardation. Cisplatin loaded solid lipid nanoparticles was prepared by microemulsion technique. Stearic acid was used as lipid. The other excipients were used as DPPG, Soya lecithin and Poloxamer P407 and acidic buffer pH4. Probe sonication was used for 10 min at 79 Amplitude. Cisplatin SLNs Batch C13 showed particle size of 119.23 ± 1.52 nm, Zeta potential of -37.33 ± 2.47 mV, % Entrapment efficiency of 90.2 ± 2.1 %, % Drug loading capacity of 1.62 ± 1.34 %, The TEM study of optimized Cisplatin SLN illustrated the spherical shape of nanoparticles. Total release amount of Cisplatin was 82.62 ± 2.04 % after 48 hrs. The formulation performed kinetics study followed Peppas plot equation The SLNs of Cisplatin met all the requirements of a colloidal drug delivery system. They had particle size in nanosize; their size distribution was narrow and all the particles were in spherical shape and stable.

Keywords: Cisplatin, Solid Lipid nanoparticles, zeta potential, Particle size, Transmission electron Microscopy.

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INTRODUCTION

Cisplatin is a Antineoplastic agent used in the treatment of different malignancies. It is widely used in the treatment of testicular, head, neck, ovarian and lung cancer. The use of Cisplatin is limited due to its intrinsic and acquired resistance and severe side effects such as chronic neurotoxicity and nephrotoxicity.¹ Cisplatin has limited bioavailability due to its poor aqueous solubility and it also shows harmful effects on various organs. Furthermore, the intravenous administration of Cisplatin produces adverse effects like renal failure, ototoxicity, loss of hearing, cerebral blindness, tubular necrosis and papilloedema. All these effects limit the amount of drug given to the patients. The colloidal carriers such as emulsion, liposomes, and polymeric nanoparticles have been extensively studied to overcome above limitations. The solid lipid nanoparticles (SLNs), amongst other colloidal carriers, were found to be

an ideal carrier for lipophilic drug for better stability and release retardation. Further, the bioavailability of poorly soluble drug can be increased by incorporating them into SLNs.²

Nano particulate drug delivery system may offer plenty of advantages over conventional dosage forms which include improved bioavailability, reduced toxicity, enhanced bio distribution and improved patient compliance³. Solid Lipid Nanoparticles (SLNs) introduced in 1991 represent an alternative and better carrier system to traditional colloidal carriers such as emulsions, liposomes and polymeric micro and nanoparticles. Colloidal drug delivery systems offer a number of advantages over conventional dosage forms. Due to their small particle size, colloidal preparations lend themselves to parenteral administration and may be useful as sustained release injections for the delivery to a specific organ or target site.⁴

Targeting the drug to the desired site of action would not only improve the therapeutic efficiency but also enable a reduction of the amount of drug which must be administered to achieve a therapeutic response, thus minimizing unwanted toxic effects. Lipid-based particulate delivery systems, like liposomes, micelles, nanocapsules, and solid lipid nanoparticles have been developed especially to solubilize poorly water-soluble and lipophilic drugs. These lipid-based systems have the advantage that comprises bio-derived and/or biocompatible lipids that often result in lower toxicity.² This study is aimed to develop and characterize the Cisplatin loaded SLNs which are colloidal drug delivery in sustained manner with high degree of specificity.

MATERIAL AND METHOD

Material

Cisplatin (Khandelwal Laboratories Pvt. Ltd. Thane), DPPG (Lipoid Germany) Stearic Acid, Poloxamer P-407, Soya Lecithin, citric acid, Potassium dihydrogen phosphate,

Disodium hydrogen phosphate, sodium chloride (Loba chemie Pvt. Ltd, Mumbai)

Method of preparation of Solid Lipid Nanoparticles

Cisplatin loaded solid lipid nanoparticles were prepared by Microemulsion technique. In this technique Stearic acid melt at 80° C. and the DPPG was melted at 122° C and mixed with melted Stearic acid. To this mixture soya lecithin and Poloxamer P407 were added and stirred for 5 min. Cisplatin was dissolved in acidic buffer pH 4 and incubated at 37° C for 30 min. This solution was heated at 80 °C. This aqueous phase was added to melted lipid phase. This solution was kept on probe sonicator for a period of 10 min at 79 amplitude. Finally o/w emulsion was formed. This microemulsion was carefully added drop wise into 10 ml ice cold acidic buffer pH 6,5 and 4 present in beaker with a continuous stirring at 2000 rpm on magnetic stirrer. The SLN dispersion was stirred for 3hr after addition of microemulsion.^{5,6}

Table 1: Formulation of Cisplatin SLNs

Sr. No.	Ingredients	C11	C12	C13
1.	Cisplatin	10 mg	10 mg	10 mg
2.	Stearic acid	33.31 mg	33.31 mg	33.31 mg
3.	Soya lecithin	500 mg	500 mg	500 mg
4.	Poloxamer 407	28.50 mg	28.50 mg	28.50 mg
5.	DPPG	15 mg	15 mg	15 mg
6.	Acidic Buffer upto	20 ml (pH 6)	20 ml (pH 5)	20 ml (pH 4)

Characterization of Cisplatin Solid Lipid Nanoparticles:

1. Particle Size Analysis:

The particle size and polydispersity index (PDI) of prepared solid lipid nanoparticle were determined using a Malvern Zetasizer ZS90 (Nanoseries Malvern Instrument). Each sample was diluted 10 times with distilled water to avoid multiscattering phenomena and is placed in disposable sizing cuvette. The polydispersity index was studied to determine the narrowness of particle size distribution. The size analysis of sample consisted of 3 measurements and results were expressed as mean size ± Std. Deviation.⁵

2. Zeta Potential:

The zeta potential of prepared Cisplatin solid lipid nanoparticles was performed using Zeta meter. Zeta potential is a scientific term for electrokinetic potential in colloidal dispersion. Zeta potential is a key indicator of the stability of colloidal dispersions. The zeta potential of prepared Cisplatin solid lipid nanoparticles was determined using a Malvern Zetasizer ZS90 (Nanoseries Malvern Instrument). The pre concentrated Cisplatin SLNs 1ml sample was diluted 10 times with distilled water in 10 ml volumetric flask. From this solution 1 ml is placed in disposable zeta potential cuvette.⁷

3 Determination of % Entrapment efficiency:

Entrapment efficiency of Cisplatin loaded solid lipid nanoparticles was estimated by centrifugation method. In this method prepared solid lipid nanoparticles were placed in cooling centrifugation tube and centrifuged at 15000 rpm for 45 min at 6°C. The pellets were dissolved in 5 ml of

methanol and evaporated using heating Mantle. Then 1 ml of OPDA and 2 ml of Phosphate buffer pH 7.4 were added. The mixture was then heated for 10 min and volume was adjusted with Dimethyl formamide upto 10 ml. Entrapped Cisplatin was determined using UV spectrophotometer at 705 nm. The samples from supernatant were diluted before going for absorbance measurement. Encapsulation efficiency is also expressed as percent of drug trapped and was calculated using equation.⁸

%EE= Total amount of drug incorporated in SLN / initial weight of drug X 100.

4. Determination of % Drug Loading capacity:

Loading capacity (LC %) can be calculated by dividing the amount of total entrapped drug by the total nanoparticle weight. In drug delivery, yield, given as a percent, is a reflection of the amount of drug delivered per amount encapsulated. Loading capacity helps you to deal with nanoparticles after their separation from the medium and to know their drug content. It is calculated using the following equation⁸

%LC = [Entrapped Drug/Amount of drug + Lipid+ Excipient] * 100

5. Surface Morphology:

TEM determines the particle size with or without staining. SEM uses electrons transmitted from the specimen surface, while TEM uses electrons transmitted through the specimen. TEM allows visualization of SLNs after freeze fracturing and freeze substitution. Transmission Electron Microscopy was used to visualize the morphology of solid lipid nanoparticle. By using TEM, various characteristics like particle size, shape and internal structure of

nanoparticulate carriers system can be efficiently evaluated.⁹

6. *In-Vitro* Drug Release:

In-vitro drug release of prepared cisplatin solid lipid nanoparticles was performed by using Dialysis bag. The release of Cisplatin from the solid lipid nanoparticles was measured in triplicate in Phosphate Buffer Saline pH 7.4. 2 mg equivalent cisplatin SLN were taken in a dialysis bag and both the end of dialysis bag were closed and placed in beaker containing 75ml PBS pH 7.4. This dialysis bag was arranged in such a way that it just touches the surface of buffer solution. The whole set up was placed on a magnetic stirrer which was rotated at 50rpm. Temperature of buffer was maintained at $37\pm 1^\circ\text{C}$. 2 ml aliquots of release medium were withdrawn at a time interval of 0.5, 1, 2, 4, 8, 12, 24 and 48 hrs. To maintain the sink condition, same volume of dissolution media was added. The aliquots were centrifuged at 3000rpm for 5 to 10 min. at room temperature. Supernatant was diluted with 2 ml phosphate buffer PH 7.4 and 1 ml OPDA solution. This solution was heated at 100°C for 15 min in order to get light green colored solution and volume was adjusted with Dimethyl Formamide to 10 ml. This was estimated by UV Visible spectrophotometer at 704 nm. The concentration of drug was calculated from straight line equation obtained from standard curve of Cisplatin.¹⁰

The data obtained from *In-vitro* release was subjected to kinetic study.¹⁰

7. Stability study of Optimized Cisplatin SLNs: The purpose of stability testing is to provide information on how the quality of drug product varies with time under the

influence of variety of environmental factors such as temperature, humidity and light and to establish a shelf life for drug product at recommended storage conditions.¹⁰

Procedure: From the different SLN batches, Formulation C13 was tested for stability studies. Formulation C13 was divided into 3 sample sets and stored at $4^\circ\pm 1^\circ\text{C}$, $25^\circ\pm 2^\circ\text{C}$ and $60\% \pm 5\% \text{RH}$, $40^\circ\pm 2^\circ\text{C}$ and $75\% \pm 5\% \text{RH}$.

After one month, particle size, entrapment efficiency of selected formulations was determined.

RESULT AND DISCUSSION

1. Particle size Analysis of Cisplatin SLNs:

The particle size is the most important property for *in vivo* integrity and biological fate of nanoparticles. Development of carriers with appropriate size plays an important role in the field of drug delivery. It is stated that nanoparticles in the range of 100-200nm have the highest potential to extend the circulation time in the blood stream, because they are small enough to avoid selective uptake in the liver. The vasculature of cancer cells is leaky unlike the normal cells. Small size of nanoparticles allows them to permeate through this leaky vasculature and to be retained in the tumour cells. This phenomenon improves the intracellular accumulation and localization of SLNs in tumor without affecting normal cells. All the batches produced nanoparticles in the size range between 100-200 nm. Amongst them the particle size of batch C13 was found to be 119 nm and was, therefore, optimized and selected for *In-vitro* drug release. Out of three graphs of particle size of each batch of Cisplatin SLNs one less particle size of graph showed in Figure 1,2,3.

Table 2: Characterization of Cisplatin SLNs

Sr. No	Batch	Average Particle size	PDI	Zeta Potential (mV)	% Entrapment efficiency	% Drug Loading Capacity
1.	C11	167.4 ± 1.15	0.411 ± 0.06	-30.26 ± 0.66	42.82 ± 3.15	0.85 ± 0.03
2.	C12	157.1 ± 3.25	0.321 ± 0.03	-33.53 ± 1.53	65.14 ± 0.12	1.38 ± 0.02
3.	C13	119.23 ± 1.52	0.416 ± 0.01	37.33 ± 2.47	90.2 ± 2.1	1.62 ± 1.34

Batch C11:

Results

Z-Average (d.nm): 166.3	Peak 1:	Size (d.nm): 342.6	% Intensity: 64.7	St Dev (d.n...): 179.0
Pdi: 0.289	Peak 2:	Size (d.nm): 96.93	% Intensity: 35.3	St Dev (d.n...): 28.20
Intercept: 0.857	Peak 3:	Size (d.nm): 0.000	% Intensity: 0.0	St Dev (d.n...): 0.000
Result quality : Good				

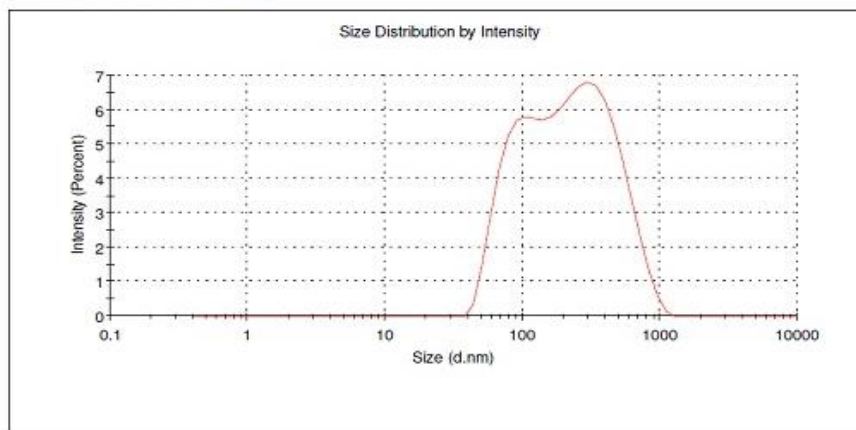


Figure 1: Particle Size and PDI of Batch C11 Cisplatin SLNs

Batch C12:

Results

Z-Average (d.nm): 153.9	Peak 1: 233.4	% Intensity: 100.0	St Dev (d.n...) 164.0
Pdi: 0.321	Peak 2: 0.000	0.0	0.000
Intercept: 0.879	Peak 3: 0.000	0.0	0.000

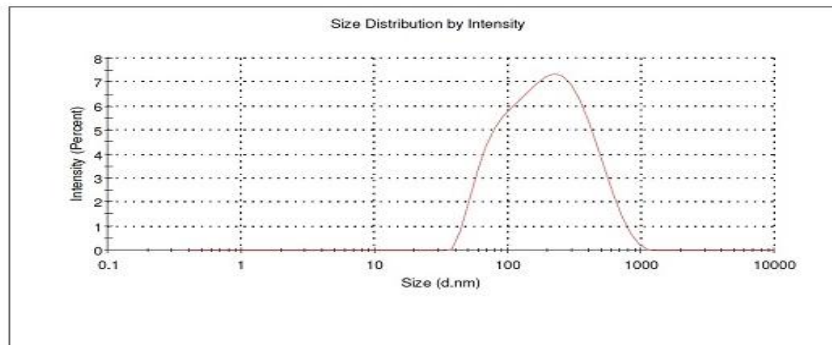


Figure 2: Particle Size and PDI of Batch C12 Cisplatin SLNs

Batch C13:

Results

Z-Average (d.nm): 117.9	Peak 1: 261.6	% Intensity: 74.8	St Dev (d.n...) 157.6
Pdi: 0.429	Peak 2: 54.10	25.2	15.97
Intercept: 0.880	Peak 3: 0.000	0.0	0.000

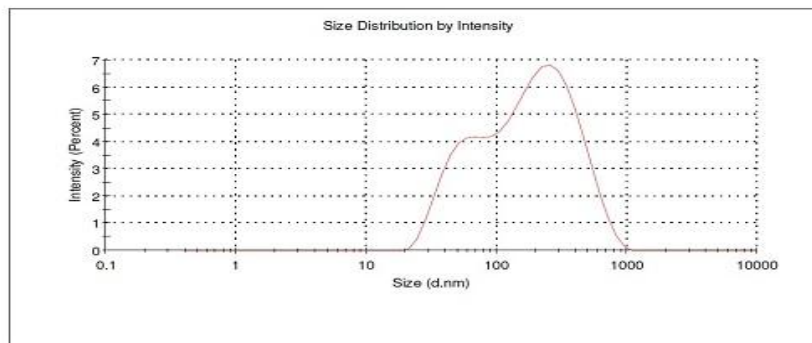


Figure 3: Particle Size and PDI of Batch C13 Cisplatin SLNs

2. Determination of Zeta potential:

The zeta potentials of all the batches were greater than 30mV with negative charge indicating the stability of the formulations. The highest stability as measured by the zeta potential was shown by batch C13 which was formulated at pH 4. Moreover, the anionic lipid DPPG might have added to the negative charge thereby improving the stability of formulation. Out of the three graphs of Zeta Potential of Each batch of Cisplatin SLNs, one graph which is high zeta potential value showed in Figure 4,5and 6.

Batch C11:

Results

Zeta Potential (mV): -30.6	Mean (mV)	Area (%)	St Dev (mV)
Zeta Deviation (mV): 11.9	Peak 1: -21.8	50.2	6.73
Conductivity (mS/cm): 0.223	Peak 2: -39.5	49.8	7.86
	Peak 3: 0.00	0.0	0.00

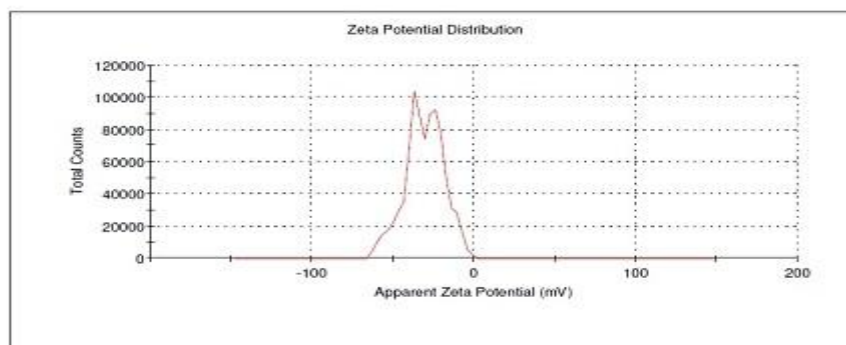


Figure 4: Zeta Potential of Batch C11 Cisplatin SLN

Batch C12

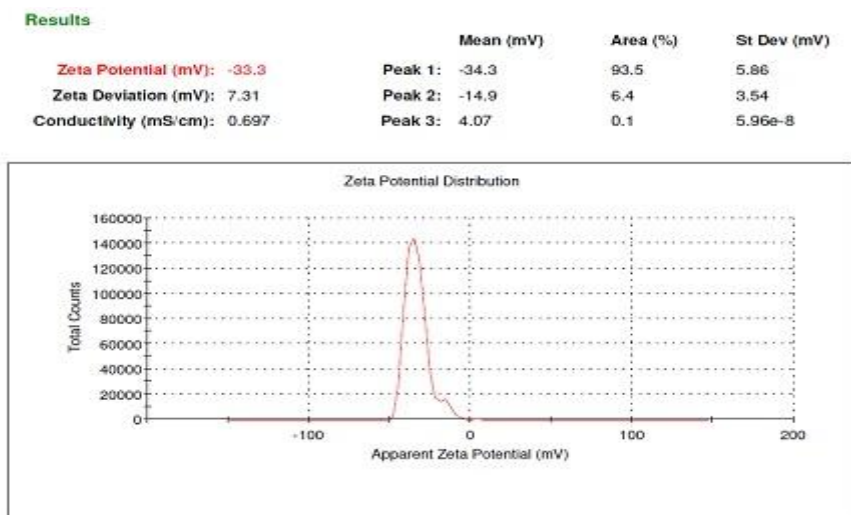


Figure 5: Zeta Potential of Batch C12 Cisplatin SLN

Batch C13

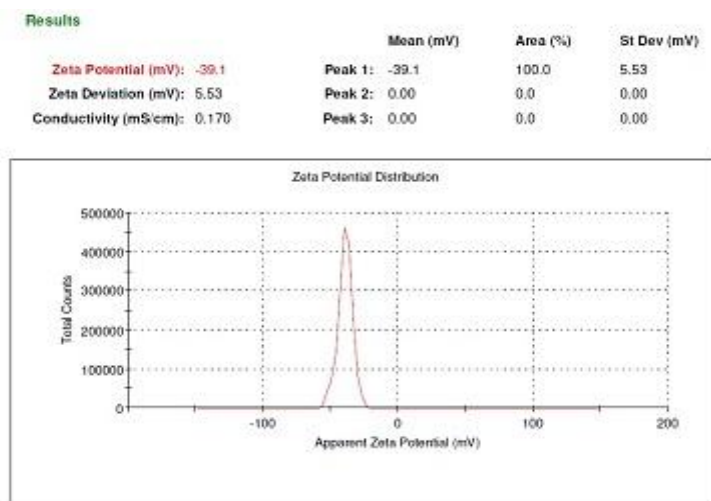


Figure 6: Zeta Potential of Batch C13 Cisplatin SLN

3. % Entrapment Efficiency:

Drug entrapment efficiency is an important property in drug loaded nanocarriers and directly affects the therapeutic effect of system. The entrapment efficiencies have been shown in table no. 2. The results show that the amount of drug entrapped was more due to the DPPG, soya lecithin and three times Stearic acid increased acidic buffer pH 4. The entrapment efficiency of pH 5 and 6 batches showed less entrapment than pH 4 batch C13. The effect of acidic pH buffer on the % Entrapment efficiency. The effect on entrapment was observed in batch C13 i.e. 90.2 ± 2.1 %. The higher the encapsulation capacity of nanoparticles the larger the amount of drug released at the tumor site.

4. % Drug Loading Capacity:

The % Drug loading capacity of Cisplatin C13 was found to be 1.62 ± 1.34 %. It shows a good result.

5. Surface Morphology:

The TEM study of optimized Cisplatin SLN illustrated the spherical shape of nanoparticles. The TEM image of the nanoparticles is depicted in Figure No.7. It is evident from

the image that the particles were spherical in shape with narrow size distribution and no aggregation. Particles of optimized Cisplatin SLN formulation were of nanosize. The surfaces of particles are smooth moreover their integrity was found to be intact.

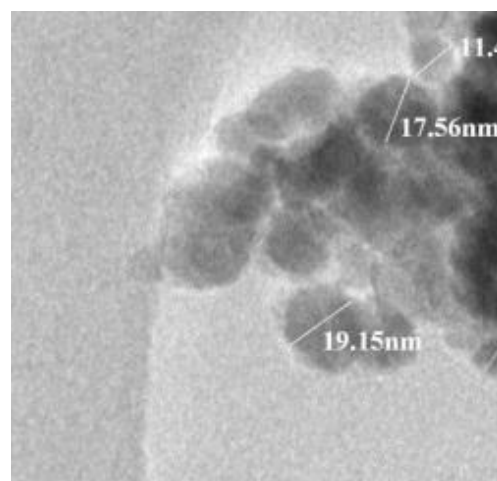


Figure 7: TEM of Cisplatin SLN

6. *In-vitro* drug release of Cisplatin Solid lipid Nanoparticles:

Table 3: *In-vitro* release profile of Cisplatin SLN (Formulation C13)

Sr. No	Time hr	% Cumulative Drug Release			% Cumulative Average \pm S.D	SEM
		I	II	III		
1.	0	0	0	0	0	0
2.	0.5	5.06	7.07	4.05	5.39 \pm 1.53	0.88
3.	1	8.81	7.20	11.09	9.03 \pm 1.95	1.12
4.	2	13.03	15.24	18.26	15.51 \pm 2.62	1.51
5.	4	24.43	26.37	30.12	26.97 \pm 2.89	1.66
6.	8	42.86	40.31	45.74	42.97 \pm 2.71	1.56
7.	12	60.95	64.17	66.99	64.03 \pm 3.02	1.74
8.	24	75.03	77.04	73.12	75.06 \pm 1.96	1.13
9.	48	84.41	83.07	80.39	82.62 \pm 2.04	1.17

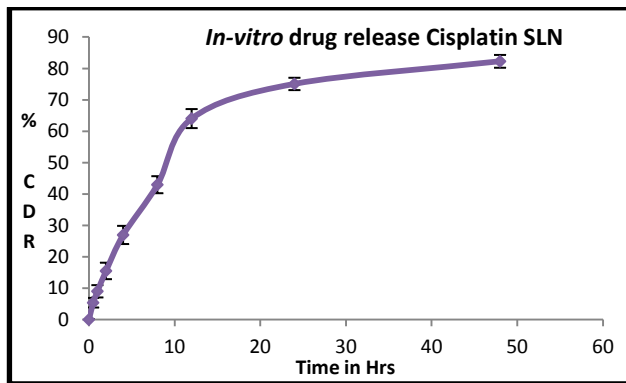


Figure 8: *In-Vitro* Release Profile of Cisplatin SLNs Batch C13

In-vitro drug release profile of Cisplatin solid lipid nanoparticles was performed in order to evaluate the stability and release behavior of Cisplatin SLN. The

prepared SLN showed a long term stable drug release profile up to 48hrs. Total release amount of Cisplatin was 82.62 \pm 2.04 % after 48 hrs Thus, Cisplatin SLN may serve as stable nanoparticles for a longer period thereby increasing drug accumulation into tumor site.

Treatment of Kinetic release study:

Table 4: Cisplatin SLNs Model Fitting Profile

Cisplatin SLN	Zero Order	First Order	Peppas	Higuchi
R ²	0.7517	0.8338	0.9663	0.9298

The results of *In-vitro* release study were treated by mathematical model. Cumulative percentage drug release of C3 batch after 48 hrs was shown 82.62%. The formulation followed Peppas plot equation. This indicates that the Cisplatin nanoparticle followed more than one type of release phenomenon and it appeared to be by the Non Fickian diffusion control mechanism.

Stability Study:

Table 5: Stability Study of Selected formulation C13

Formulation C13	4 ⁰ \pm 1 ⁰ C	25 ⁰ \pm 2 ⁰ C and 60% \pm 5% RH	40 ⁰ \pm 2 ⁰ C and 75% \pm 5 % RH
% Entrapment efficiency	89.04 \pm 1.26	87.32 \pm 1.58	86.25 \pm 2.22
Particle Size	120.2 \pm 2.24	122.5 \pm 1.65	123.4 \pm 3.02

Stability study of the prepared Cisplatin solid lipid nanoparticles C13 were carried out, by storing the formulation C13 at 4⁰ \pm 1⁰C, 25⁰ \pm 2⁰C and 60% \pm 5% RH, and 40⁰ \pm 2 ⁰C and 75% \pm 5 % RH in humidity control oven for month. The %Entrapment efficiency and Particle size

were carried out. The results % Entrapment efficiency and Particle size at different storage condition are shown in Table 5.The comparing this data with the previous data of C13 it was observed that there is slight increase in Particle size and little Decrease in the % Entrapment efficiency.

Results

Z-Average (d.nm): 118.9
 Pdl: 0.410
 Intercept: 0.881

Size (d.nm):
 Peak 1: 201.7
 Peak 2: 0.000
 Peak 3: 0.000

% Intensity:
 100.0
 0.0
 0.0

St Dev (d.n...
 147.1
 0.000
 0.000

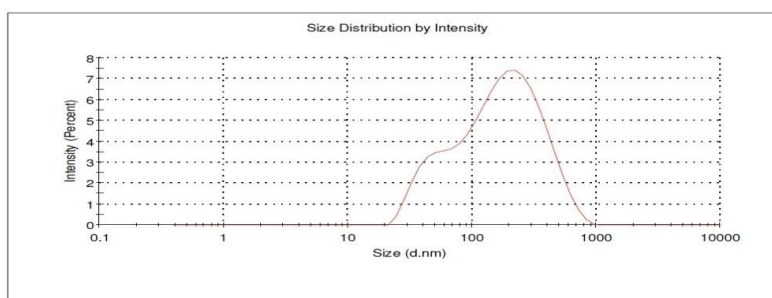


Figure 9: Particle size of Cisplatin SLNs of batch C13 after stability

CONCLUSION

The Cisplatin loaded solid lipid nanoparticles met all the requirements of a colloidal drug delivery system. They had particle size in nanosize; their size distribution was narrow and all the particles were in spherical shape. The percent entrapment efficiency and percent drug loading capacities were in the acceptable limits. Moreover, the *In-vitro* release pattern revealed that they served the purpose of being a sustained drug delivery system

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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