Novel Anticancer Drug Development PEG-Oxaliplatin Liposomes

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Abstract: Background: The purpose of this study was to develop novel pegylated oxaliplatin (OXA)-loaded liposomes for targeting colorectal carcinoma. Methods: The liposomes were prepared by the rotary film evaporation method and were evaluated for in vitro and stability characteristics. Results: Rotary evaporation method was used for the preparation of encapsulated pegylated OXAliposomes consisting of Hydrogenated soyabean phosphatidylcholine (HSPC), Dipalmitoyl phosphatidylcholine (DPPC), Cholesterol (CH) and Polyethylene glycol-distearoyl phosphoethanol amine (PEG-DSPE) in the molar ratios of (45:20:25:10). In this study, pegylated liposomes containing oxaliplatin were prepared and their characteristics, such as particle size, zeta potential, encapsulation efficiency, stability study, in vitro drug release and in vitro cytotoxicity were investigated. The particle size and entrapment efficiency were found to be 182.3±8.6 nm and 34.2%±2.7%, respectively. The biophysical characterization of both pegylated liposomes were performed by transmission electron microscopy (TEM), HPLC and UV-visible spectrophotometry. The pegylated OXA liposomes showed a typical sustained and prolonged in vitro drug-release profile. In vitro cytotoxicity studies on HT-29 cell line was performed and the pegylated OXA liposomes exhibited higher potency than oxaliplatin. Conclusion: This liposomal formulation assisted sustained drug-release of oxaliplatin and also showed higher efficacy during in vitro cytotoxicity.

Keywords: oxaliplatin, pegylated liposomes, colorectal carcinoma, in vitro drug release, in vitro cytotoxicity.

I. INTRODUCTION

In cancer,cells divides in an uncontrolled manner and can invade other tissues; it encompasses a large and complex family of malignancies. The various treatment options are available like surgery, radiotherapy and chemotherapy. However traditional chemotherapy has several drawbacks such as requirement of high doses, intolerable cytotoxicity, and the development of multiple drug resistance. Therefore to enhance the therapeutic efficacy, there is a need to develop targeted drug delivery in order to overcome these drawbacks.[1]

Platinum anchored anticancer drugs have transformed cancer chemotherapy, and it is also clinically used for management of tumors of the ovary, testes, and the head and neck cancer.[2] However, the patient's quality of life has been severely affected due to various dose limiting toxicities related with platinum drug i.e., limited anti-tumor response, development of drug resistance, tumor relapse. These shortcomings have led to an extensive research effort towards development of new approaches for improving platinum therapy. Nano carrier-based (novel) delivery of platinum compounds is area of ongoing research that has provided good preclinical and clinical results as well as may help in the development of new generation of platinum compounds.[2-3]

Oxaliplatin[(1*R*,2*R*)-1,2-Cyclohexanediamine-*N*,*N*′]-[29xalate(2-)-*O*,*O*′]platinum provides a better therapeutic effect in colorectal carcinoma,. However, there is a need to reduce the toxic side effects of oxaliplatin. Liposomes as a novel type of drug carrier, exhibit good targeting properties, slow releasing potential, high stability and low toxicity. The active drug targeting is significant for altering the biological distribution of antitumor agents, reducing the multidrug resistance of tumor cells and improving the therapeutic efficacy of anticancer drugs. [3-4]

The new generation of liposomes are modified by the incorporation of polyethylene glycol (PEG) – derivatized with phospholipids into liposomal membrane. This results in sterically stabilized liposomes.[4] The presence of PEG on the surface of the liposomal carrier showed increase in blood-circulation time while reducing uptake by mononuclear phagocyte system (stealth liposomes).[4-5] These are mainly characterized by a decrease in their clearance.[5-6] Hence, the pharmacokinetics and bio-distribution of the encapsulated drugs are found to change. [7] Oxaliplatin belongs to third generation platinum antitumor drug, which can be encapsulated successfully to treat metastatic colorectal cancer.[4-7]

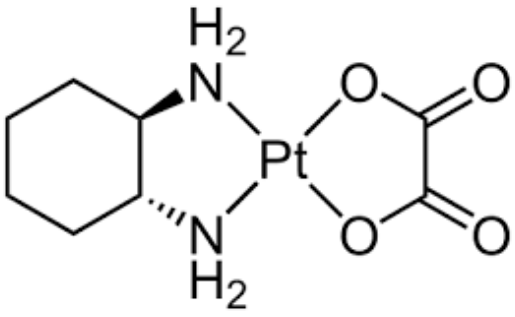


Figure 1 Chemical structure of oxaliplatin.

The objective of present study was to screen and develop novel pegylated oxaliplatin (OXA)-loaded liposomes. The liposomes were prepared by the rotary evaporation method to improve the entrapment efficiency and *in-vitro* release. To characterize the novel pegylated oxaliplatin (OXA)-loaded liposomes in terms of morphology, size, zeta potential, *in-vitro* release and stability. A *in-vitro* cytotoxicity study was carried out on Human colorectal cancer cell line (HT-29) cell lines using oxaliplatin drug, blank pegylated liposomes and pegylated oxaliplatin (OXA)-loaded liposomes.

II. MATERIALS AND METHODS

Oxaliplatin (purity >98%) was obtained as gift sample from CIPLA Ltd (Mumbai central, Mumbai). Hydrogenated soyabean phosphatidylcholine (HSPC, LIPOID S PC-3), Dipalmitoyl phosphocholine (DPPC, LIPOID PC) and Polyethylene glycol-distearoyl phospho ethanolamine (PEG-DSPE, LIPOID PE) were donated by Lipoid GMBH (Ludwigshafen, Germany). Cholesterol (CH) was obtained from Fischer Scientific, India. Double-distilled water was obtained in-house by a Quartz double Distillation Water System, (Lab Sil Instruments, Mumbai). HPLC grade acetonitrile (ACN) was purchased from Sigma Aldrich, USA. All other chemicals used in the study were of analytical grade.

III. ANALYTICAL METHOD DEVELOPMENT

Determination of drug content for stability

A reverse phase HPLC method was modified to detect oxaliplatin content in liposomes throughout stability study. The HPLC system consisted of a JASCO-HPLC PU 2080 plus with MD-2018 PDA detector and chromatograms were processed using the ChromNAV software. The method consisted of mobile phase composition of acetonitrile: water adjusted with phosphoric acid (pH 3.0) in ratio of 95:5, stationary phase octadecylsilane (C18, 250 mm x 4.6 mm, 5µm particle size, Phenomenex) and detection wavelength was 252 nm. The chromatograms were obtained at a flow rate was 0.8 ml/min and injection volume was 50 µL.

Determination of entrapment efficiency

An UV (JASCO V-630) method was developed for oxaliplatin in phosphate buffer saline 7.4 for the quantification of drug from the formulation during entrapment efficiency and in vitro release testing. The drug exhibited absorption maxima at wavelength 260nm. Ideally, λmax selected for the analysis of drug formulation should not show any interference due to solvent and excipients present in formulation.

OPTIMIZATION OF PEG-OXA LIPOSOMES

The process parameters:

Liposomes were prepared by thin film hydration and reverse phase evaporation (REV) method. The final nano range liposomes were prepared with the aid of sonication. Generally two types of sonication methods were used bath and probe sonication. Probe sonication was used to obtain liposomes with particle size below 200 nm.

The formulation parameters:

The solvent system composition should be such that it prevents precipitation of formulation excipient during solvent stripping process. The organic solvent system of chloroform: diethylether in various proportions was used for dissolving the formulation components like HSPC, DPPC, CHOL, mPEG-2000-DSPE for preparation of liposomes. Increase in the lipid proportion relative to drug led to an increase in the drug entrapment, also entrapment improved by use of different lipid ratios. With increase in quantity of lipids, more number of liposomes per ml of the liposomal dispersion was formed, resulting into increased drug entrapment. Entrapment did not show significant increase Beyond 1: 5 drug to lipid ratio. Various combination in molar ratios of HSPC, DPPC, Cholesterol and PEG-DSPE (45/20/25/10, 55/10/30/5, 50/10/35/5, 40/45/10/5, 60/30/5/5 and 45/25/20/5) were tried and the optimum ratios selected on the basis of the entrapment efficiency of drug in liposomal formulation. Different lipid concentrations (36–152mg) were tried in order to observe the effect of varying concentrations on entrapment efficiency, particle size, zeta potential and stability of formulation.

Preparation of optimized PEG - oxaliplatin liposomes by reverse phase evaporation method

Liposomes modified with m-PEG2000-DSPE were composed of HSPC/ DPPC/ CHOL/ mPEG2000-DSPE (45/20/25/10 molar ratio). Briefly, lipids (50mmol) were dissolved in chloroform/ diethyl ether (1:2 v/v) and then oxaliplatin solution (2 mg/ml) in 10 % (w/v) sucrose was added to the lipid mixture to form a w/o emulsion. For preparation of empty PEG-coated liposomes, 10% sucrose solution was added instead of oxaliplatin solution. The volume ratio of the aqueous to the organic phase was maintained at 1:3.The emulsion was sonicated for 10 min and then the organic phase was removed to form liposomes by evaporation in a rotary evaporator at 40 °C under reduced pressure at 273 hPa for 1h. The resultant liposome solution was subjected to probe sonication for four minutes to obtain particle size below than 200 nm.

Table 1 Components of formulation system

Hydrogenated soyabean phosphatidylcholine
Dipalmitoyl phosphocholine
Polyethylene glycol-distearoyl phospho ethanolamine
Cholesterol
Chloroform
Diethylether
Oxaliplatin solution
10% sucrose solution

PHYSIOCHEMICAL CHARACTERIZATION

Particle size

The particle size and zeta potential of the liposomes were measured using a Zetasizer Nano ZS analyzer (Malvern Instruments, Malvern, UK). The prepared liposomes were diluted 10 times with ultrapure water and shaken prior to measurement at 25°C. Dynamic light scattering data were collected using a helium laser as the light source and mean results were provided by photon correlation spectroscopy.

Entrapment efficiency

Entrapment efficiency was determined by equilibrium dialysis method. PEG-oxaliplatin liposomes equivalent to 4 mg of oxaliplatin were filled in a pre-soaked dialysis membrane (Himedia dialysis bag with a MW cutoff of 10-12 K), followed by dialysis in phosphate buffer pH 7.4 for a total duration of 2 h to remove the un-entrapped oxaliplatin. After attaining equilibrium, the un-entrapped drug was analyzed by UV spectroscopy. The % entrapment efficiency was calculated using the formula shown below: [8]

$$\%\text{Entrapment efficiency} = \frac{\text{Total drug} - \text{un-entrapped drug}}{\text{Total drug}} \times 100$$

Transmission electron microscopy

A transmission electron microscope (JEM-2100, JEOI Institute of chemical technology Mumbai, India) was to characterize the morphology of the PEG-oxali liposomes (2 mg/mL). A drop of the diluted drug-l liposomes was placed on a copper grid and stained phosphotungstic acid (2%) for observation. Upon dry room temperature, a thin film was formed and observ transmission electron microscopy.[9]

In vitro drug release study of liposomes

Freeze dried liposomes were reconstituted with equivalent amount of filtered distilled water. The reconstituted formulation so obtained was kept for equilibrium dialysis for a duration of 2h. This was done to separate the un-entrapped drug present in formulation. After completion of dialysis, un-entrapped drug was removed and was calculated for the un-entrapped content of oxaliplatin. Then the release of oxaliplatin from total entrapped drug containing formulation and drug solutions was evaluated by comparing *in vitro* drug release profiles using 50 ml of PBS. Briefly, 4 ml of reconstituted liposomal oxaliplatin sample was placed into the dialysis bag and then suspended in a flask containing 50 ml of phosphate-buffered saline (PBS, pH 7.4) with temperature maintained on a magnetic stirrer at 37 ± 2 ^{0}C with at 50rpm. After predetermined time intervals, 1ml aliquots were removed with replacement of same medium. Samples were collected and analyzed by UV at 260 nm.

Lyophilization assay

Formulations were lyophilized following three different strategies: without cryoprotectant, with lactose (4:1, w/w sugar:lipid) and lactose (8:1, w/w sugar:lipid). The optimized batch was selected after final characterization by particle size, polydispersity index (PDI) and Zeta potential.

Detection of solvent residue

The level of residual organic solvent (chloroform) in freeze dried PEG-oxaliplatin liposomes was determined by Head-space analysis. (gas chromatography-Varian CP-3800-GC Chromatograph), with set of standard chloroform solutions. The analysis was performed by the static head space using Varian µ software.

Stabilities studies:

The freeze dried PEG-oxaliplatin liposomes were kept in glass vials with rubber closure and aluminium cap. These glass vials were kept in stability chamber maintained at 40 ± 2^{0}C and 75±5 % RH for a period of 3 months. The PEG-oxaliplatin liposomes were evaluated for drug content after 1, 2 and at the end of third month by the developed HPLC method. Freeze dried PEG-oxaliplatin liposomes (10 mg); were accurately weighed and dispersed in 2 mL of methanol and sonicated for 5 min on a bath sonicator followed by centrifugation at 14000 rpm (Remi, India) for 15 minutes. The supernatant was analysed for drug content by validated HPLC method after suitable dilution with mobile phase.

In vitro cytotoxicity study of PEG-oxaliplatin liposomes

The human colorectal cell lines HT-29 were grown in RPMI 1640 medium containing 10 % fetal bovine serum

and 2 mM L-glutamine. Cells were inoculated into 96 well plates (100 µL), depending on the doubling time of individual cell line. After cell inoculation, the96 well plates were incubated at 37 ^{0}C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. Experimental drugs (blank PEG-liposomes, PEG-oxa liposomes and oxaliplatin solutions) were initially solubilized in dimethyl sulfoxide at 100 mg/ml and diluted 1 mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate (1 mg/ml) was thawed and diluted to 100, 200, 400, 800 µg/ml with complete medium containing test article. Aliquot of 10 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentration i.e.10, 20, 40, 80 µg/ml. After compound addition, plates were incubated at standard conditions for 48 h and assay was terminated by the addition of cold TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4^{0}C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye washing was done five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

IV. RESULT AND DISCUSSION

ANALYTICAL METHOD DEVELOPMENT

Determination of content for stability

Quantification of drug content for stability of PEG oxaliplatin liposomes was performed on Reverse phase HPLC method. The retention time of oxaliplatin was found to be 7.4 min. Linearity (R^2 = 0.999) was observed from 2.5 to 100 µg/mL with limit of detection at 250 ng/mL and limit of quantification at 500 ng/mL for RP-HPLC.

Determination of entrapment efficiency

An UV (JASCO V-630) method was developed for determination of oxaliplatin entrapped in PEG-oxaliplatin liposomes and *in-vitro* release study. The drug exhibited absorption maxima at wavelength 260 nm. Linearity (R^2 = 0.999) was observed from 30 to 150 µg/mL with limit of detection at 0.58µg/mL and limit of quantification at 1.71 µg/mL for UV Visible spectroscopy. The sandell's sensitivity was found to be 0.00167µg/cm^3/AU[10].

OPTIMIZATION OF PEG-OXA LIPOSOMES

i. The process parameters:

The optimized formulation was prepared by reverse phase evaporation It showed higher entrapment efficiency than thin film hydration. Sonication for 2 minutes was not able to convert liposomes into nano size range; while bath sonication for 6 to 8 minutes resulted in lesser entrapment possibly due to drug leakage from the liposomes. So optimum time considered for sonication was 4 min (60 seconds x 4 times).

ii. The formulation parameters:

The organic solvent system of chloroform: diethyl ether (1:2) was used for dissolving the formulation components like HSPC, DPPC, CHOL, DPPA and drug.

Table 2 Different process and formulation parameter affected on formulation.

Parameter	Sonication time(min)	Inference
Sonication	2	Submicron range liposomes
	5	Nano-range liposomes with less EE%
	6	Nano-range liposomes with less EE%
	4	Nano-range liposomes with good EE%
Solvent system selected	Ratios	Inference
Chloroform: diethyl ether	1:1	Lipids not completely soluble
	1:3	Improper emulsion formed
	2:1	Incomplete removal of $CHCl_3$
	1:2	Suitable removal of solvent, proper emulsion formed
Ratio of drug to lipid	Drug to lipid ratios	EE%
Oxaliplatin to lipids	1:5	34±2
	1:10	7±6
	1:3	18±5
	1:4	21±3

Table 3 Effect of various combination of lipids ratios on entrapment efficiency, particle size and zeta potential.

Combination of lipid	Entrapment efficiency%	Particle size (nm)	Zeta potential (mV)
30/60/10/-	18±0.85	1351	-11.6
45/20/20/5	19±1.26	238	-32
50/15/20/5	30±1.8	196	-34.4
45/20/20/5	22±2	228	-34
45/20/25/10	36±0.45	182	-43.6

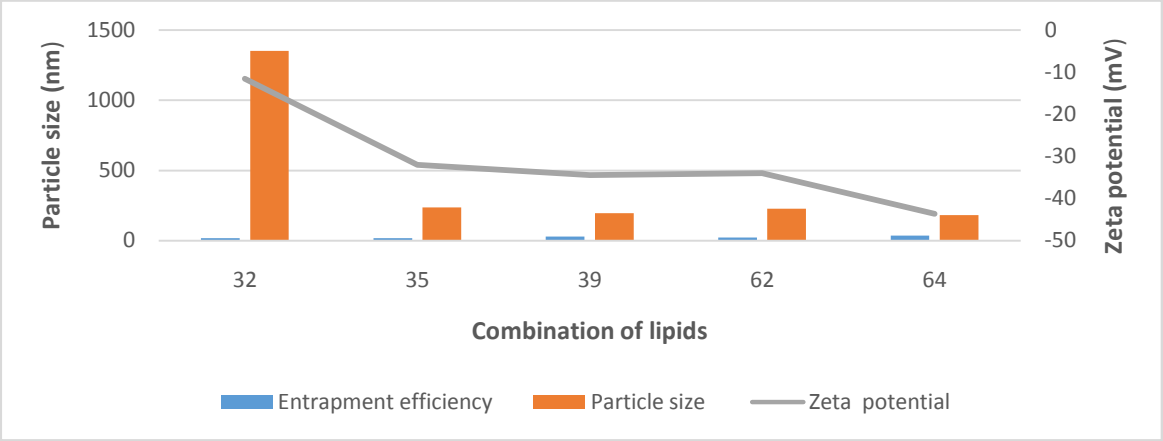


Fig 2 Effect of various combination of lipids ratios on entrapment efficiency, particle size and zeta potential.

Table 4Effect of total lipid content concentration on entrapment efficiency, particle size and zeta potential.

Total lipid content	Entrapment efficiency (%)	Particle size (nm)	Zeta potential (mV)
32	18	1351	-11.6
35	19	238	-32
39	30	196	-34.4
62	22	228	-34
64	36	182	-43.6

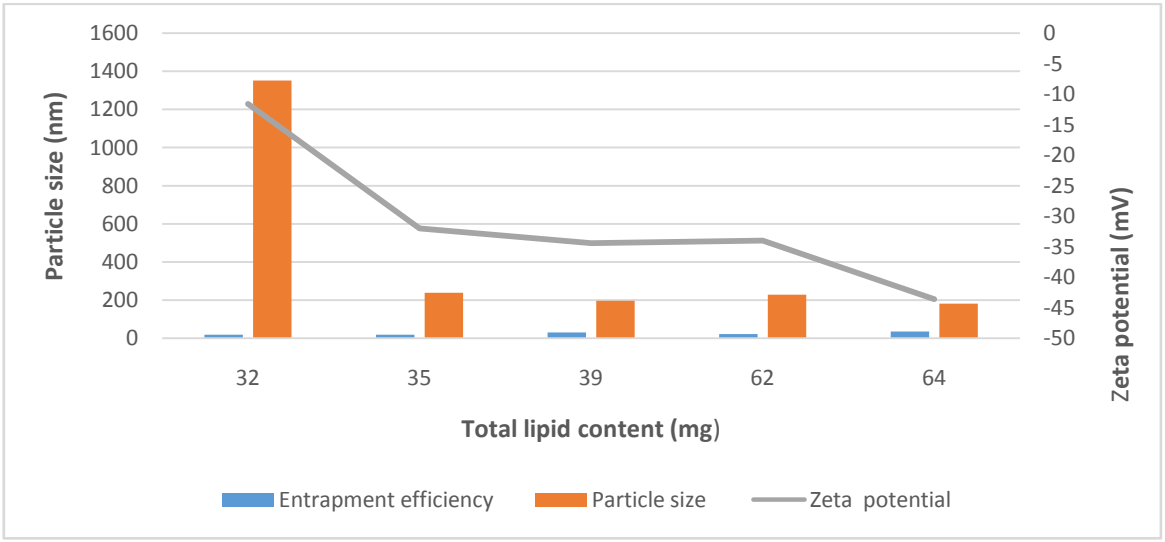


Fig 3 Effect of total lipid content concentration on entrapment efficiency, particle size and zeta potential.

In optimization studies, it was observed that particle size, entrapment efficiency and stability of liposomes were better at 20 molar ratio of cholesterol. This ensured improved integrity of liposomal membrane and stability of liposomes. DPPC and HSPC at 25 and 45 molar ratio respectively were also found to be appropriate for the liposomes. Final optimized batch was prepared using reverse phase evaporation with 64 mg of lipid content, drug: lipid ratio of 1:5 was selected and probe sonication of 4 minutes (particle size reduction from 500 to less than 200 nm). Lipid combinations selected were HSPC: DPPC: Cholesterol: PEG- DSPE (45/20/25/10) with lactose as cryoprotectant. The organic solvent system of chloroform: diethyl ether (1:2) was used for dissolving the formulation components like HSPC, DPPC, CHOL, mPEG-2000-DSPE for preparation of liposomes and aqueous to organic phase ratio was also optimized to 1:3.

PHYSIOCHEMICAL CHARACTERIZATION:

Particle size

The REV method rendered nanosized liposomes with particle size less than 200 nm and narrow (PDI). Particle size obtained by dynamic light scattering was in accordance with size determined from transmission electron micro photographs of the liposomal systems.

Zeta potential

Zeta potential of all the liposomal dispersion was negative (PUT AVG VALUE FOR ZETA). PEG-oxaliplatin liposome exhibited a zeta potential of - 33 mV and had an apparently higher negative zeta potential as compared to other batches.

Entrapment efficiency

The % entrapment efficiency of PEG-oxaliplatin liposome was more than 35%. The final drug to lipid ratio was at 1:5.

Transmission electron microscopy

Transmission electron microscopy revealed that the drug-loaded liposomes had a homogeneous size and spherical in shape. In the present study the TEM photomicrograph of oxaliplatin loaded liposomes were shown as in (Figure 3) that shows liposomes had spherical shape and multilamellar structure. Liposomal membrane was showed the inner aqueous compartments as slightly darker than the surrounding perimeters and also pegylation of liposomes or coating with PEG was also observed in the TEM photomicrogram.

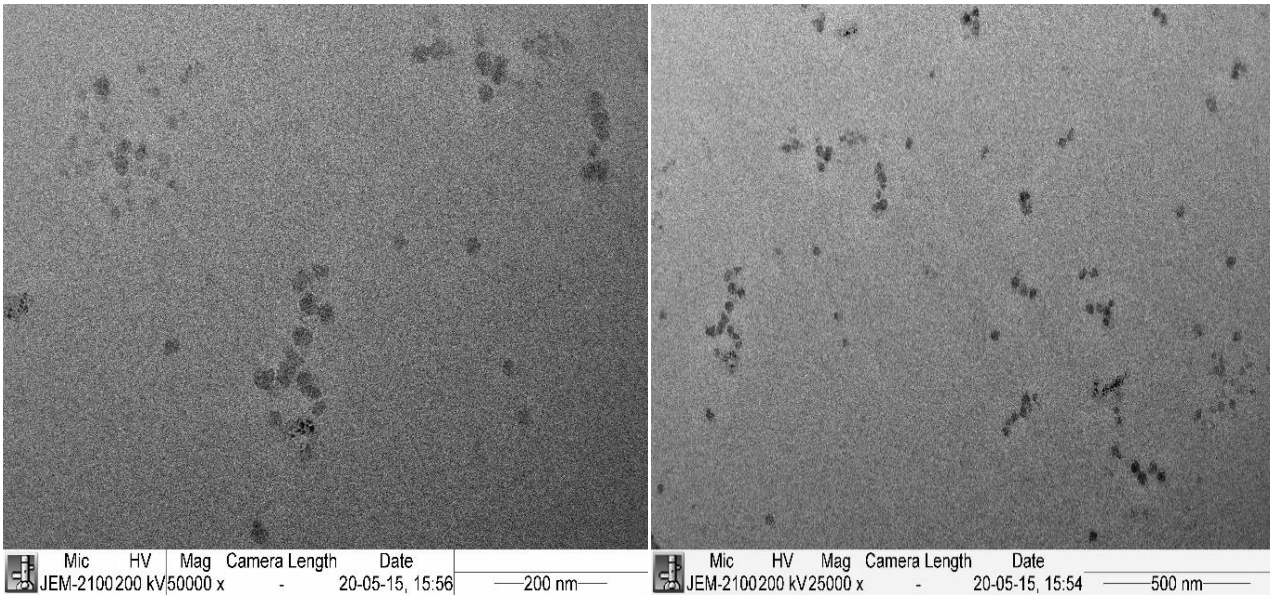


Figure 4 Transmission electron micrograph of the pegylated oxaliplatin liposomes. The scale bar represents 250nm and 500nm. (If u have ZOOM image please put that)

In vitro release study

The pegylated oxaliplatin liposomes showed sustain release profile as evident from the graph as shown in (Figure 4), while oxaliplatin did not exhibit sustain release profile compared to liposomal formulation. The pegylated oxaliplatin liposomes was found to have 80-90% drug release in 24h while oxaliplatin standard had release of 90% in 9h. This could be attributed to encapsulation of oxaliplatin in liposomal vesicle, thus not available for fast release. At the end of 24 h, release from liposomes was found to be 92 ± 1.91%. This indicated that oxaliplatin loaded liposomes can provide sustain

release effect and also surface modification with pegylation can improve drug circulation time.

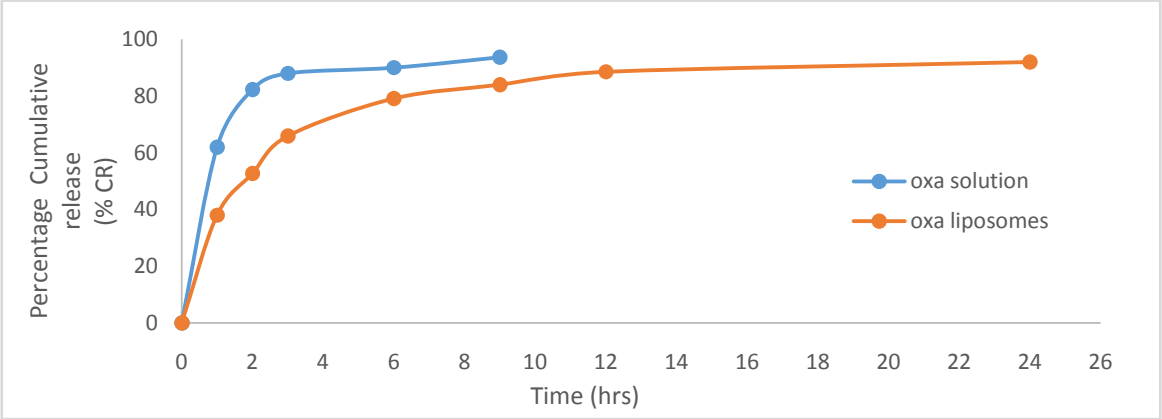


Figure 5 Drug release profiles for pegylated oxaliplatin liposomes and oxaliplatin in phosphate-buffered saline (pH 7.4) for 24 hours (n=6).

Abbreviation: OXA, oxaliplatin.

Lyophilization assay

Lyophilization carried out with and without cryoprotectant showed difference in primary characterization. Entrapment efficiency, particle size and zeta potential of the liposomal formulation with (4:1) cryoprotectant were found to be better than liposomal formulation without cryoprotectant and 1:8 lipid to lactose ratio.

Table 5 Lipid to cryoprotectant ratio with particle size, zeta potential and entrapment efficiency.

Batch	Particle size (nm)	Zeta potential (mV)	Entrapment efficiency (%)
Freeze dried Liposomes without lactose	238±1.34	-33±1.8	28±2
Freeze dried liposomes Lipids: lactose (1:4)	202±6	-30±1.9	32±2
Freeze dried liposomes Lipids: lactose (1:8)	208±4	-31±2	26±4

Differential Scanning Calorimetry (DSC):

A sharp melting endotherm of oxaliplatin was seen at 182.38 ^{0}C. Broadening of peak in pegylated oxaliplatin liposomes could be attributed to the change in physical form of drug from crystalline to amorphous or a less ordered structure. A shift in endotherm was seen from 182.38^{0}C to 219.38^{0}C.Thus, it can be concluded that oxaliplatin was compatible with liposomal components.

Stabilities studies:

Table 6 Stability of pegylated oxaplatin liposome at zero month, one month and after two month.

oxaliplatin content found in formulation	INITIAL zero month (%)	One month (%)	Two month (%)
	98.59	97.02	96.68

After completion of stability study it may be inferred that degradation of oxaliplatin loaded liposomes had not occurred in the formulations. The method is suitable for routine analysis of oxaliplatin in liposomal formulation. The stability indicating assay method developed for HPLC was found to be simple, sensitive, accurate and precise. The method was proved to be reproducible and selective for analysis of oxaliplatin from formulation without any interference from the

excipients. The method can be used in quality control laboratories to determine the purity of the drug and for identification purposes and, it can be further extended to determine degradation kinetics of oxaliplatin.

In vitro cytotoxicity study of PEG-oxaliplatin liposomes

The HT-29 cell line was sensitive for oxaliplatin with GI50 values 23.8 and 18.8(from result table) for the free drug and equivalent loaded drug. Oxaliplatin-loaded liposomes showed a comparable cytotoxicity for the incubation period of 24 h. This effect was observed for the four types of lipids used in the oxaliplatin formulation. Table 7 showed the lists of GI50 values found for all treatments in HT-29 cell lines. The cytotoxic effect were observed to be more significant in HT-29 cell line for free drug than for the pegylated liposomes. However, the blank liposomal formulation did not showed any anti proliferative activity compared to drug loaded liposomes.

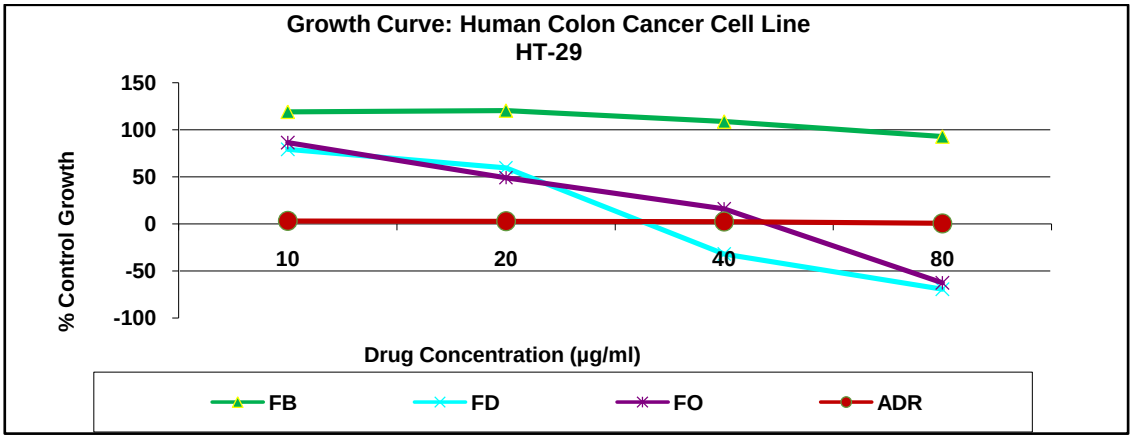


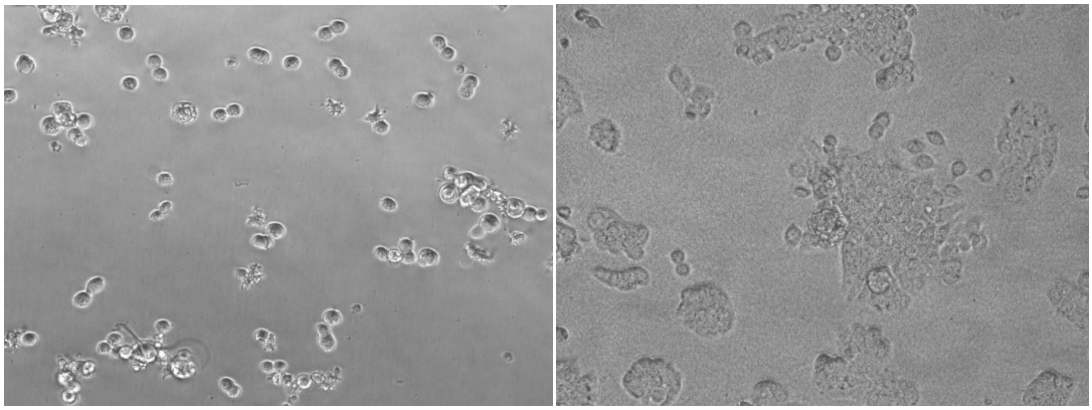
Figure 6 % control growth on human colon cancer HT-29 cell line.

FB, blank formulation; FD, pegylated oxaliplatin liposomes; FO, oxaliplatin solution; ADR, adriamycin.

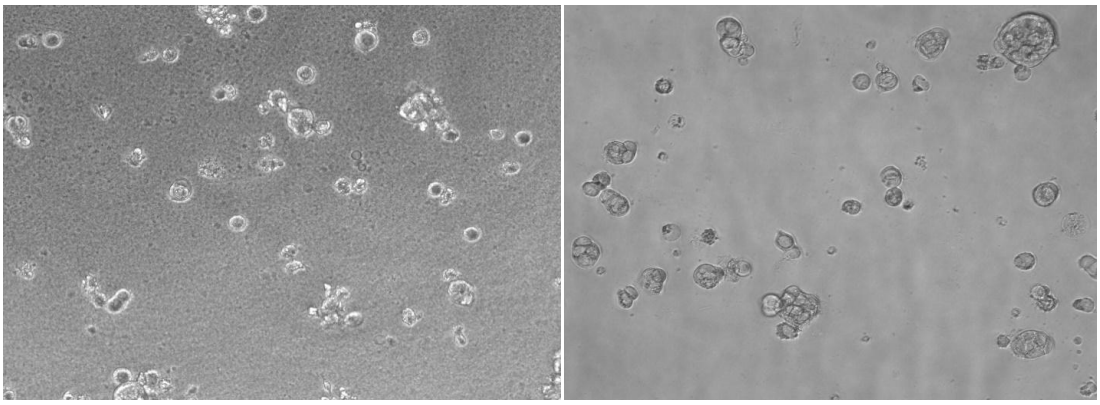
Table 7 Results of LC50, TGI, and GI50 on HT-29 (put values only for which graph is there).

HT-29	LC50	TGI	GI50*
FB	>80	>80	>80
FD	64.8	41.8	18.8
FO	73.0	48.4	23.8
ADR	>80	34.7	10

LC50,Concentration of drug causing 50% kill; TGI, concentration of drug causing total inhibition of cell; GI50;concentration of drug causing 50% inhibition cell growth.



HT-29 FB HT-29 FD



HT-29 FO HT-29 Positive control

Figure 7 Effects of FB, FO, FD and ADR on human colorectal cell line (HT-29).

FB, blank formulation; FD, pegylated oxaliplatin liposomes; FO, oxaliplatin solution; ADR, adriamycin.

V. CONCLUSION

In preformulation studies, it was observed that particle size, entrapment efficiency were better at 20 molar ratio of cholesterol. This ensured improved integrity of liposomal membrane and stability of liposomes. DPPC and HSPC at 25 and 45 molar ratio respectively were also found to be appropriate for the liposomes. Final optimized batch was prepared using reverse phase evaporation with 64mg of lipid content, drug: lipid ratio of 1:5 was selected and probe sonicated for 4 minutes. Lipid combinations selected were HSPC: DPPC: Cholesterol: PEG- DSPE (45/20/25/10) with 252mg of lactose as cryoprotectant. The organic solvent system of chloroform: diethyl ether (1:2) was used for dissolving the formulation components like HSPC, DPPC, CHOL, mPEG-2000-DSPE for preparation of liposomes and aqueous to organic phase ratio was also optimizes to 1:3. The reverse phase evaporation method rendered nanosized liposomes with particle size less than 200nm and narrow polydispersity index. Blank liposomes and oxaliplatin liposome exhibited a zeta potential of -28mV and -33mV, which showed good stability. The percentage of entrapment efficiency was observed to be 34 ± 2% of oxaliplatin optimized formulation. The optimized formulation showed sustained release profile as compared to oxaliplatin drug solution since, 80-90% drug release was obtained within 2h for oxaliplatin as compared to the same amount in 24h of optimized formulation. Entrapment efficiency, particle size and zeta potential of the liposomal formulation with (4:1) cryoprotectant were found to be better than liposomal formulation without cryoprotectant.

Encapsulation of oxaliplatin in nanocarriers like pegylated liposomes resulted in improving the *in-vivo* circulation time of drug. Pegylated Oxa-liposomes could be effectively used to target colorectal and other carcinomas resulting in improved therapeutic efficacy. Encapsulation of the drug in liposomes also ensures comparable cellular cytotoxicity. Moreover, the formulation ensures sustained release of the drug, thus, avoiding the need for frequent dosing. Thus oxaliplatin encapsulated liposomes can be a promising drug delivery system for treating colorectal carcinoma.

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