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DEVELOPMENT AND CHARACTERIZATION OF LIPOSOMAL FORMULATION OF TRILACICLIB

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ABSTRACT

Trilaciclib dihydrochloride, is a kinase inhibitor, chemically; he chemical name for trilaciclib is 2'-{[5-(4-methylpiperazin-1-yl)pyridin-2-yl]amino}-7',8'dihydro-6'H-spiro [cyclohexane-1,9'-pyrazino[1',2':1,5]pyrrolo[2,3-d]pyrimidin]-6'-one. Trilaciclib di hydrochloride is commercially available COSELA (trilaciclib) for injection, which is a lyophilized formulation available as 300 mg / vial and (trilaciclib) for injection is a yellow lyophilized cake supplied in a single-dose vial. Each vial contains one 300 mg strength single-dose vial. The current investigation was designed to alternative stable liposomal formulations. The inventors of the present invention have surprisingly found that it is possible to prepare a stable lipid Nano composition of *Trilaciclib.* Liposomal formulations are less toxic than drugs alone and have better pharmacological parameters. Although they seem to be the first choice for drug delivery systems for various diseases. The usage of natural products in pharmaceuticals has steadily seen improvements over the last decade, and this study focuses on the utilization of palm oil in formulating liposomal Trilaciclib. The liposomal form of Trilaciclib generally minimizes toxicity and enhances target delivery actions. Taking into account the antiproliferative and antioxidant properties of palm oil, the aim of this study is to design and characterize a new liposomal Trilaciclib by replacing phosphatidylcholine with 5% and 10% palm oil content. Liposomes were formed using the freeze thaw method, and Trilaciclib was loaded through pH gradient technique and characterized through in vitro and ex vivo terms. Based on TEM images, large lamellar vesicles (LUV) were formed, with sizes of 438 and 453 nm, having polydispersity index of 0.21 ± 0.8 and 0.22 ± 1.3 and zeta potentials of about −31 and −32 mV, respectively. In both formulations, the entrapment efficiency was about 99%, and whole Trilaciclib was released through 96 hours in PBS (pH = 7.4) at 37°C. Comparing cytotoxicity and cellular uptake of LUV with on MCF7 and MDA-MBA 231 breast cancer cell lines indicated suitable uptake and lower IC50 of the prepared liposomes.

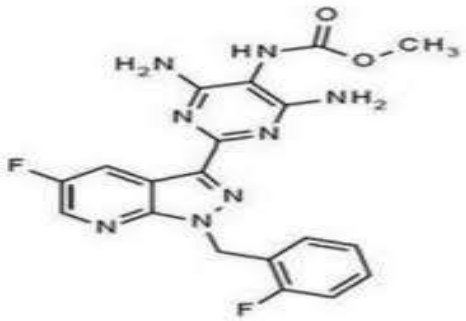
INTRODUCTION

Trilaciclib dihydrochloride is commercially available COSELA (trilaciclib) for injection, which is a lyophilized formulation available as 300 mg / vial and (trilaciclib) for injection is a yellow lyophilized cake supplied in a single-dose vial. Each vial contains one 300 mg strength single-dose vial. The current Investigation was designed to alternative stable liposomal formulations.

Drug Substance: Trilaciclib dihydrochloride is a water-soluble yellow solid.

Chemical Properties: COSELA for injection contains trilaciclib dihydrochloride, a kinase inhibitor. The chemical name for trilaciclib is 2'-{[5-(4-methylpiperazin-1-yl) pyridin- 2-yl]

amino} - 7',8'- dihydro- 6'H-spiro [cyclohexane - 1, 9' pyrazino[1',2':1,5]pyrrolo[2,3-d]pyrimidin]- 6'-one. Trilaciclib has the following structure:



Mechanism of Action: Trilaciclib is a transient inhibitor of CDK 4 and 6. Hematopoietic stem and progenitor cells (HSPCs) in the bone marrow give rise to circulating neutrophils, RBCs, and platelets. HSPC proliferation is dependent on CDK4/6 activity.

Materials: Trilaciclib, Hydrogenated palm oil (palm oil), cholesterol (CH), L-alpha-phosphatidylcholine (PC), polyethylene glycol (PEG), methanol, and chloroform were purchased from Sigma-Aldrich. Sodium hydroxide and potassium dihydrogen phosphate were purchased from Merck.

Methods: Liposomes were prepared using the freeze-thaw method and pH gradient technique, carried out in order to maximize the loading of Trilaciclib, within liposome. Two formulations were designed; both consisted of 45 mg CH and 5 mg PEG with different percentages of PC and palm oil. The first formula (Fa) contains 5% palm oil and 45% of PC, while the second formula (Fb) contains 10% palm oil and 40% PC in their respective formulations. Then, all of the lipid components and PEG were dissolved in a chloroform: methanol mixture of (2: 1, v/v) in a round-bottom flask. The solvent was removed under vacuum using a rotary evaporator (Rotavapour R-124, BÜCHI) at 40°C and 50 rpm. After a thin lipid film was formed in the interior of the flask, the system was purged with nitrogen to remove organic solvent entirely. The lipid film layer was hydrated with 10 mL Citrate buffered solution (pH = 4) and then sonicated for 30 minutes in a bath type sonicator (Sonicor). The freeze-thaw cycle was carried out five times via freezing under −80°C and then heated mixture in water bath at 65°C with the intention of decreasing the size, further entrapping the acidic buffer inside the liposome. Bicarbonate buffer (pH = 0.5) was added dropwise to the mixture (for the reason of adjusting outer liposomes space into a physiological pH) until its pH reaches 7. Afterwards, 10 mL of Trilaciclib medium in distilled water (2000 μg/mL) was added to the mixture and shaken at room temperature for 30 minutes at 60 rmp. Liposomes are specialized delivery vehicles that serve multiple roles in enhancing the capabilities of active pharmaceutical ingredients (APIs). These lipid bilayers form in the shape of hollow spheres, encapsulating cargo of interest within an aqueous interior or lipid bilayer.

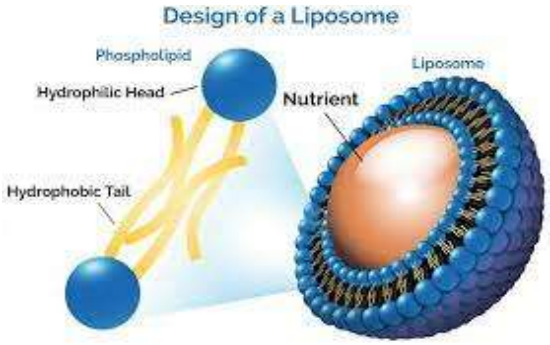


Figure 1.0: Design of Liposomes

Liposomes are a novel drug delivery system (NDDS), they are vesicular structures consisting of bilayers which form spontaneously when phospholipids are dispersed in water. They are microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid bilayers. Liposomes are microscopic fat-soluble vesicles derived from lipids. In liposomal delivery, liposomes are used to encapsulate and transport active ingredients in drugs and nutritional supplements to locations in the body where they are most efficiently absorbed. A liposome is a spherical vesicle having at least one lipid bilayer. The liposome can be used as a drug delivery vehicle for administration of nutrients and pharmaceutical drugs, such as lipid nanoparticles in mRNA vaccines, and DNA vaccines. Traditional liposomes include one or more lipid bilayer rings surrounding an aqueous pocket, but not all lipid nanoparticles

have a continuous bilayer that would qualify them as lipid vesicles or liposomes. Some lipid nanoparticles are micellar-like structures, encapsulating drug molecules in a non-aqueous core.

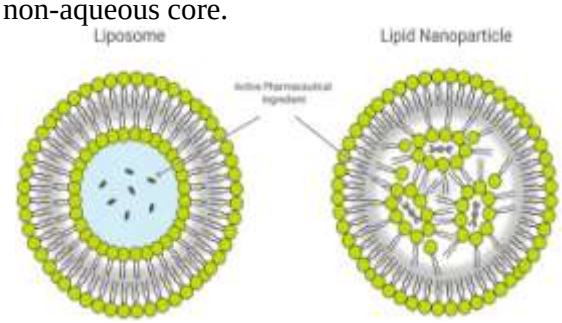


Figure 1.1 Liposomes and Lipid Nanoparticles

The application of liposomes to assist drug delivery has already had a major impact on many biomedical areas. They have been shown to be beneficial for stabilizing therapeutic compounds, overcoming obstacles to cellular and tissue uptake, and improving bio distribution of compounds to target sites *in vivo.*

Liposome preparation: The Trilaciclib Liposome Injection, is a sterile filtered product and the manufacturing process includes Compounding(Ammonium Sulfate & Lipid Phase Preparation, Lipid Phase Injection & Hydration, Particle Size Reduction/Extrusion, Tangential Flow Filtration, Filtration and Drug loading), Pre-filtration & Sterile filtration, Aseptic filling followed by Stoppering and Sealing.

Description of the Manufacturing Process

Manufacturing process of Trilaciclib Liposome Injection includes following stages:

- ❖ Ethanol Injection and Hydration – MLV's Formation.
- ❖ Extrusion Process (Particle size reduction) –SUV's Formation.
- ❖ Tangential Flow Filtration (TFF) – External (NH4)2SO4Removal
- ❖ Drug Loading & Potency adjustment –Trilaciclib entrapment. Filtration & Filling
- ❖ Following critical studies of manufacturing process were studied: Ammonium sulfate phase preparation – Mixing speed, time & Temperature
- ❖ Lipid Phase preparation - Mixing speed, time & Temperature
- ❖ Lipid Phase Injection – Injection Pressure
- ❖ Hydration Time and Temperature optimization
- ❖ Extrusion temperature & Pressure optimization

- Compatibility study with TFF Sample
  - SS316L compatibility study (Hold Time study) with TFF sample
  - Filter compatibility study with TFF sample
  - Tubing compatibility study with TFF sample
- Compatibility study with Liposomal Bulk Dispersion
  - SS 316L Compatibility study with Liposomal Bulk Dispersion (Hold time study)
  - Filter compatibility study with Liposomal Bulk Dispersion. Tubing compatibility study with Liposomal Bulk Dispersion. Gasket compatibility study with Liposomal Bulk Dispersion

Tangential Flow Filtration Parameter optimization (No of DV,TMP & Shear rate) Drug Loading process optimization – Heating (temperature & time) & Cooling (temperature & time) Sterilization selection method

Formation and morphology: The formation of liposomes was observed with a transmission electron microscope (TEM). Samples were prepared by applying a drop of the mixture to a carbon-coated copper grid and left for a minute to allow some of the particles to adhere onto the carbon substrate. After removing the excess dispersion with a piece of filter paper, a drop of 1% phosphotungstic acid solution was applied for one minute and then left to be air-dried. The samples were viewed with a TEM.

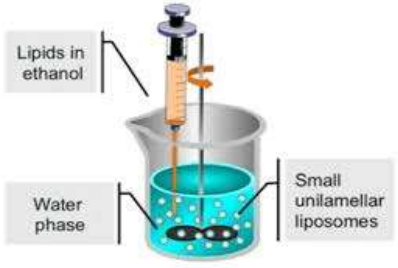


Figure 1.3: Ethanol Injection and Hydration –MLV's Formation.

Particle Size Distribution, Polydispersity Index (PDI), and Zeta Potential (ZP) Measurement: To evaluate the size distribution, PDI, and value ZP of each sample, 50 mg of liposome was weighted and dispersed in 20 mL distilled water and then those parameters were measured by the zetasizer (ZetasizerNanoseries, Malbern Instrument). This test was repeated thrice.

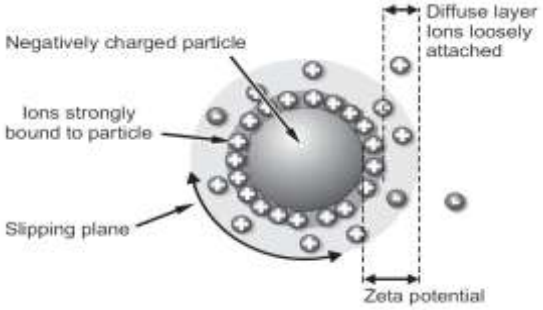


Figure 1.4: Zeta potential

Liposome Particle size distribution (PSD) : Dynamic Light Scattering (also known as Photon Correlation Spectroscopy or Quasi-Elastic Light Scattering) is a technique for measuring the size of particles typically in the sub-micron region. Dynamic Light Scattering (DLS) measures Brownian motion and relates this to the size (Hydrodynamic diameter) of the particles. The relationship between the size of a particle and its speed due to Brownian motion is defined by Stokes-Einstein equation. Hydrodynamic Diameter: The size of a particle is calculated from the translational diffusion coefficient by using the Stokes-Einstein equation;

$$D(H) = kT / 3\pi\eta D$$

Where

D (H) = hydrodynamic diameter, D = translational diffusion coefficient.

k = Boltzmann's constant, T = absolute temperature, η = viscosity

It does this by illuminating the particles with a laser and analysing the intensity fluctuations in the scattered light. Brownian motion is the random movement of particles due to the bombardment by the solvent molecules that surround them.

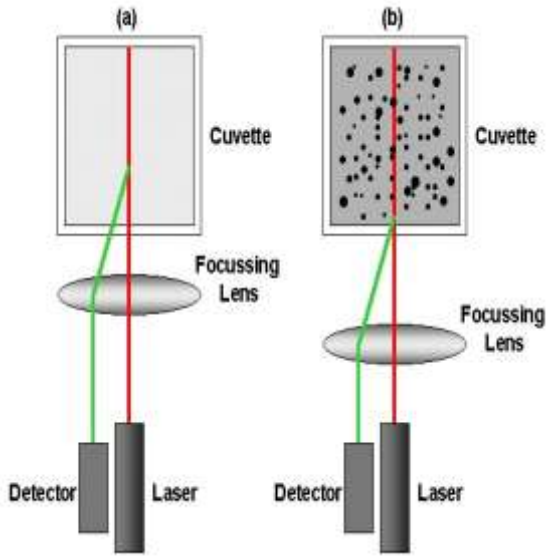


Figure 1.5: Working Principle of Malvern Zeta-Sizer

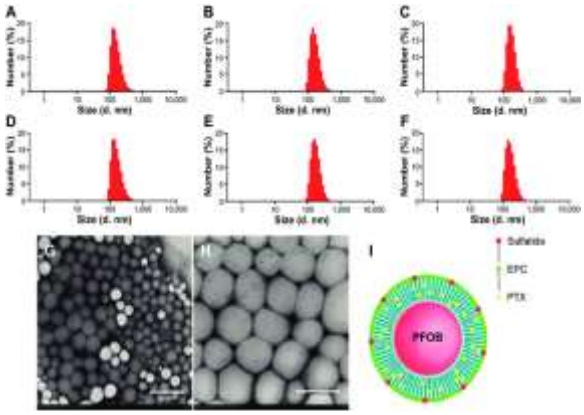


Figure 1.6: Malvern Zeta-Sizer

The correlator compares the scattering intensity at successive time intervals to derive the rate at which the intensity is varying. This correlator information is then passed to a computer, where the software will analyze the data and derive size information. To overcome this, an attenuator is used to reduce the intensity of the laser source and hence reduce the intensity of scattering. The scattering intensity signal from the detector is passed to a digital processing board called a correlator. The correlator compares the scattering intensity at successive time intervals to derive the rate at which the intensity is varying. This correlator information is then passed to a computer (6), where the software will analyze the data and derive size information. The fundamental particle size distribution obtained from a dynamic light scattering measurement is based upon the intensity of light scattered by

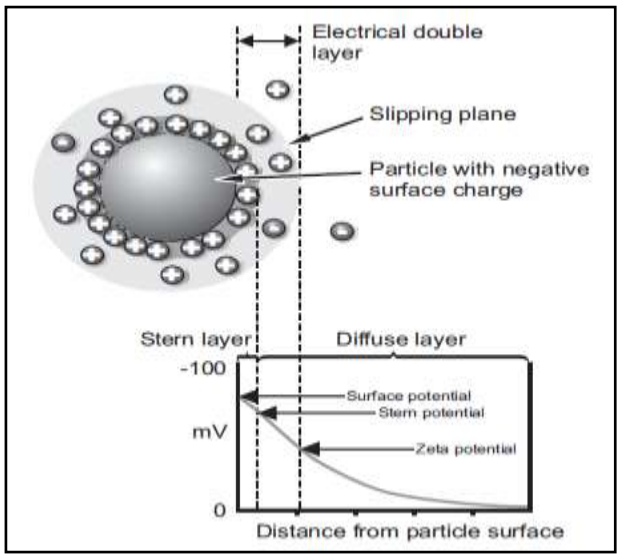
the particles being measured. The mean particle size obtained is the intensity based mean size which is called as "Z Average". The particle size distribution of Trilaciclib liposome injection lies between approx. 30 nm to 300 nm. So the formulation can be analyzed using Zeta-Sizer instrument with detector positioned either at 173° (Nano S & ZS range: 0.6nm to 6µm) or 90° (Nano S90 &ZS90 range: 2nm to 3µm).

Procedure: Approx 1mL Sample was put into disposable Polystyrene cuvette and particle size distribution was analysed using Malvern Zetasizer Nano ZS using Particle absorbance 0.010 and Refractive index 1.35.

The following parameters are considered important during the Particle size analysis:

- Average Particle Size Z_{avg}
- Polydispersity Index (PDI)
- D10, D50 and D90 values
- SPAN (D90-D10)/D50

Zeta Potential/Electrical Surface Potential: Surface charge/zeta potential on liposomes can affect the clearance, tissue distribution, and cellular uptake. The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. If all the particles in suspension have a large negative or positive zeta potential then they will tend to repel each other and there is no tendency to flocculate. If the particles have low zeta potential values then there is no force to prevent the particles coming together and flocculating. The general dividing line between stable and unstable suspensions is generally taken at either +30mV or-30mV. Particles with zeta potentials more positive than +30mV or more negative than -30mV are normally considered stable. The Zeta potential analyzer calculates the zeta potential by determining the Electrophoretic Mobility and then applying the Henry equation. The development of a net charge at the particle surface affects the distribution of ions in the surrounding interfacial region, resulting in an increased concentration of counter ions (ions of opposite charge to that of the particle) close to the surface. Thus an electrical double layer exists around each particle. The liquid layer surrounding the particle exists as two parts; an inner region, called the Stern layer, where the ions are strongly bound and an outer, diffuse, region where they are less firmly attached. Within the diffuse layer there is a notional boundary inside which the ions and particles form a stable entity. When a particle moves (e.g. due to gravity), ions within the boundary move with it, but any ions beyond the boundary do not travel with the particle. This boundary is called the surface of hydrodynamic shear or slipping plane. The potential that exists at this boundary is known as the Zeta potential.



Concept of Zeta potential and electric double layer

The **Henry equation** is:

Z: Zeta potential.
$\mathbf{U_E}$: Electrophoretic mobility.
ε: Dielectric constant.
η: Viscosity.
***f* (Ka):Henrys** function.

Two values are generally used as approximations for the *f*(Ka) determination either 1.5 or 1.0. Electrophoretic determinations of zeta potential are most commonly made in aqueous media and moderate electrolyte concentration. *f*(Ka) in this case is 1.5, and is referred to as the Smoluchowski approximation. For small particles in low dielectric constant media *f*(Ka) becomes 1.0 and allows an equally simple calculation. This is referred to as the Huckel approximation. Non-aqueous

measurements generally use the Huckelapproximation.

Liposome morphology: Liposome morphology and degree of lamellarity governs the drug loading, drug retention, and the rate of drug release from the liposomes.

Construction of standard curve: Dilutions of Trilaciclib were in the range of 400, 200, 100, 50, 25, and 12.5 ng/mL, prepared and detected by HPLC with a fluorescence detector Mixture of Acetonitrile. Heptanesulfonic acid (0.2%, pH 4) by a ratio of 25 : 75 was applied as mobile phase with the flow rate of 1 mL/min. Trilaciclib has an excitation wavelength of 480 nm and an emission wavelength of 560 nm.

Evaluation of Entrapment Efficiency and In Vitro Release: The mixture was centrifuged (Universal 32) for 70 minutes at 14000 rpm, the supernatant containing free Trilaciclib was obtained, and the absorbance was measured using HPLC [15]. The entrapment efficiency of liposomes was determined by the following formula: where EE is the concentration of entrapped drug (ng/mL), is the initial concentration of drug used in formulating the liposomes (ng/mL), is the concentration of drug in the supernatant (ng/mL), and EE (%) is the percentage of the drug's entrapment. To estimate the in vitro drug release of liposomal Trilaciclib, a dialysis bag was used. After separating free drug, 100 mg of liposome was weighted and then placed directly into the dialysis bag (Mw12000). The dialysis bag was sealed at both ends and located in a 1000 mL fresh PBS buffer medium (pH 7.4) at 37°C, at 90 rpm under perfect sink conditions. At predetermined time intervals, 1 mL of the medium was sampled for further analysis by HPLC. The concentrations of Trilaciclib throughout the releasing time were calibrated using the calibration equation. The results recorded are the mean value of the three runs carried out for each liposome concentration. The percentage of released Trilaciclib at certain time was plotted using Microsoft Excel and was defined by the following formula: where is the concentration of drug released (ng/mL) at time and is the initial drug concentration (ng/mL).

Cellular Uptake: To observe the cellular uptake, two breast cancer cell lines, received from Pasture Institute, were utilized separately. MCF-7 cells were cultured in RPMI 1640, and 10% FBS were then seeded in 24-well plates with a density of 1 × 105 cells/well and incubated in 37°C with 5% CO2 for 24 h. 50 µLTrilaciclib liposome (2000 µg/mL) was added into each well and incubated for 24 h, and then the cells were washed thrice with BPS, respectively. Afterward, image analyses of cells were performed with confocal microscopy (IX71, Olympus, Japan), and the same procedure was carried out for MDA-MBA, 231 cells as well.

Cytotoxicity Assay: MTT assay was performed to observe the cytotoxic activities of designed liposome, IC50 of formulation assessed in cell culture media and compared with (pegylated liposomal Trilaciclib). The human breast cell lines MCF 7 and MDA-MBA 231 were seeded in 96-well plates with a density of 7 × 103 cells/well, using RPMI 1640 and 10% FBS added and then incubated in 37°C with 5% CO2 for 24 h. The cells were then treated with various concentrations of (2000 µg/mL), Fa, and Fb (liposome containing 5% and 10% of palm oil loaded with 2000 µg/mL Trilaciclib which is in the same concentration of), respectively, and then incubated for 48 h. Afterwards, the media were removed, and 10 µL MTT was added to each well, incubated for a further four hours. Finally, the MTT was removed, and 100 µL DMSO was added to each well, and the absorbance was measured with an ELISA.

Statistical Analysis: All of the results were remarked with the mean ± SD, and the one-way analysis of variance (ANOVA) was employed for statistical analysis of the data.

Results: TEM images in Figure 1 demonstrate the formation of vesicles. Considering the TEM images, one layer liposome with large inside capacity confirms the fine formation and well shape of the LUV in both formulations.

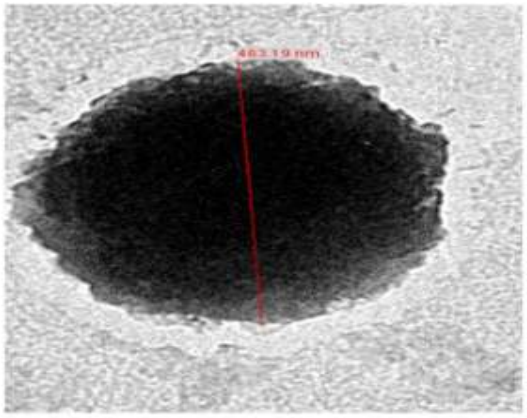


Figure 1 (Fa)

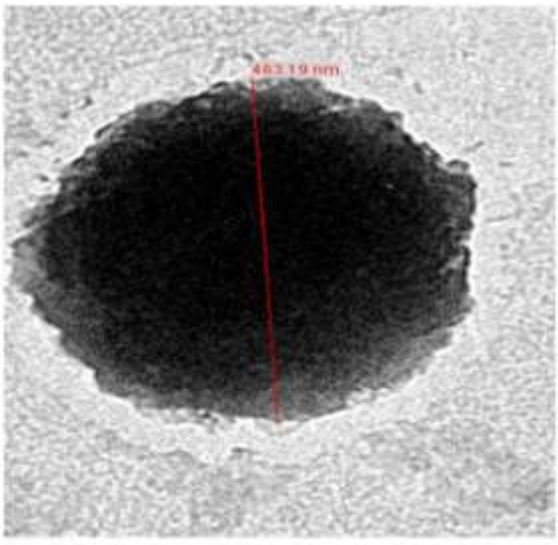


Figure 1 (Fb)
TEM images of Trilaciclib liposome with magnification 8000x, (a) (Fa), (b) (Fb).

Particle Size Distribution and Zeta Potential Measurement: Particle size determinations are mostly performed to confirm that the desired liposome size range has been obtained during preparation because suitable size of particles is important for their interaction with the biological situation; for instance, through intravenous administration of loaded particles, their ability to pass or leave the vascular capillaries effectively is dependent on the sizing. Referring to Table 1, Fa has a size of 438 nm, while Fb has a size of 453 nm; the nanosize of LUVs would result in advance drug delivery. The polydispersity index value is a measure of the heterogeneity of particle sizes in a compound. Liposomes with PDI value between 0.1 and 0.25 display more uniformity and physical stability. Further PDI value more than 0.5 indicates the poor uniformity of mixture. Looking at Table 1, the PDI values of liposomes are 0.22 and 0.21 which confirm the uniformity and homogeneity of LUVs in the mixture as well.

Construction of Calibration Curve: The following equation from the HPLC results was obtained: Y = 21998 X + 8938, where Y is the area under the curve and X is the concentration of Trilaciclib; the regression line of R2 = 0.999 was obtained as well.

Entrapment Efficiency and In Vitro Drug Release: As seen from Table 1, in both formulations, liposomes contained maximum entrapment efficiency, nearly 100%, using the pH gradient technique .Figure 2 shows the in vitro release of Trilaciclib during 96 hours where both formulations demonstrate a constant and continuous release profile. Since Fa and Fb liposomes include same ingredient with only difference in amount of PC and palm oil, they also have comparable releasing pattern with small variation. Within the first 6 hours, Fa demonstrated a faster release rate compared to Fb. During 6–24 hours, Fa and Fb liposomes showed almost similar release whereas, after 36 hours, Fa release goes slower than Fb; however no significant difference is observed.

Cellular Uptake and Cytotoxicity

Figure 3 demonstrates the cellular fluorescence images and cellular uptake of the Trilaciclib after the cells were incubated with liposome for 24 h. As Trilaciclib emits red fluorescence, the presence of Trilaciclib liposome can be clearly observed in MCF-7 and MDA-MBA 231 cells. After incubation of cell lines with Trilaciclib liposomes, they would cross the cell's membrane and the viable cells appear to have a red basis, while the apoptosis cells exhibited brighter reds, respectively

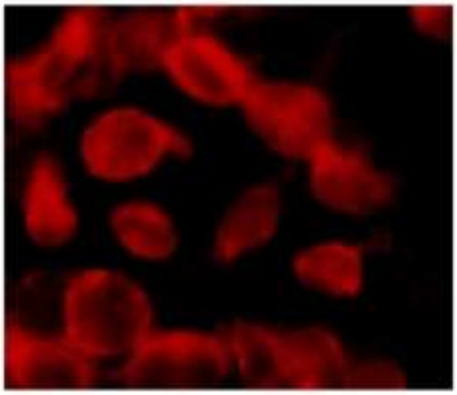


Figure 1(a)

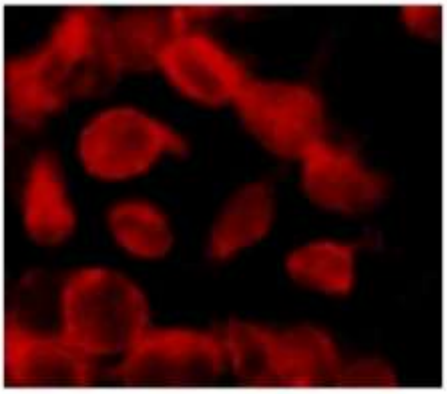


Figure 2(b)

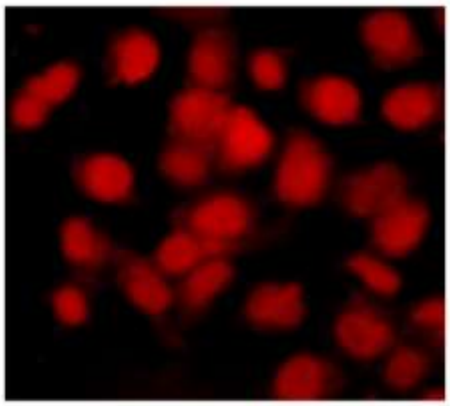


Figure 3(c)

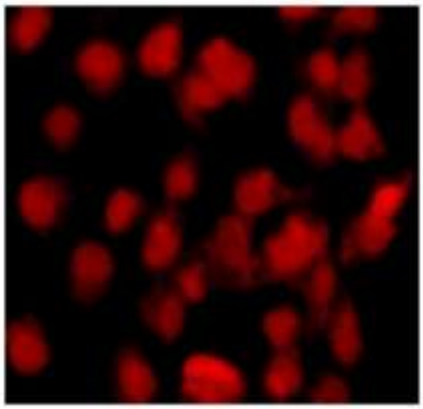


Figure 4 (d)

Figure 3 -Cellular uptake of Trilaciclib liposome: (a) Fa liposome in MCF 7 cells, (b) Fb liposome in MCF 7 cells, (c) Fa liposome in MDA-MBA 231 cells, and (d) Fb liposome in MDA-MBA 231 cell

Table 1: Particle size, zeta potential, and entrapment efficiency of the liposomes.

Formulation	Mean particle size (nm, ±SD)	Mean zeta potential (mV, ±SD)	Mean polydispersity index (PDI)	Mean entrapment efficiency (%, ±SD)
Fa	438.74 ± 1.9	-31.1 ± 2.6	0.22 ± 1.3	99.98 ±3.18
Fb	453.71 ± 1.1	-32.2 ± 4.1	0.21 ± 0.8	99.99 ± 5.22

The value of zeta potential (ZP) proves the stability of the particulate systems. It is a measurement of the repulsive forces between the particles. Particles having a ZP of less than −30 mV or more than +30 mV are usually regarded as stable. Considering the ZP values were higher than −30 mV (Table 2), which confirms the acceptable stability of LUVs as well as their uniformity and size homogeneity suspension.

Table 2: IC50 of Fa, Fb after 48 hours of treatment.

Formulation	IC50 MCF7(µg/mL,n=3)	IC50MDA-MBA 231(µg/mL,n=3)
Fa	376.45 ± 9.20	726.40 ± 7.58
Fb	387.22 ±6.93	755.73 ± 6.81

Table 3: Stability study results of TrilaciclibInjection 10 mg/mL

Sr. No.	Tests	Specification	Trilaciclib injection 10 mg/mL			
			Stability condition (5±3°C Inverted)			
		Stage	Initial	6M	9M	12M
1	Description	Clear color yellow solution	*	*	*	*
2	Assay (%)	Between 90-110%	98.50	94.2	94.20	93.41
3	Lipid content (%)					
	Palm oil content	Between 80-120%	96.90	92.73	95.33	94.14
	L-alpha-phosphatidylcholine	Between 80-120%	95.30	92.31	88.52	90.11
	Cholesterol	Between 80-120%	97.20	93.79	96.25	96.81
4	Free Drug (%)	NMT 5% of assay	2.50	1.59	2.57	2.14
5	Entrapped Drug (%)	NLT 92% of assay	98.50	98.77	96.10	97.96
6	Zeta Potential (mV)	-5mV to -15mV	-9.38	-9.81	-9.93	-9.02
7	Osmolality (mOsm/kg)	250-400mOsm/kg	324	327	336	327
8	pH	Between 6.0 to 7.0	6.49	6.4	6.65	6.62
9	**Particle Size Distribution**					
	Zavg (nm)	95 ± 15	91.7	91.2	93.69	92.28
	D10 (nm)	45 to 80	64	63.1	65.20	63.90
	D50 (nm)	80 to 120	95.4	94.97	97.50	96.20
	D90 (nm)	120 to 190	143	143	147	145
10	**Related substances (%)**					
	Impurity -I	NMT 1.0 %	0.03	0.12	0.10	0.11
	Impurity -II	NMT 0.2 %	0.01	0.05	0.08	0.05
	Impurity -III	NMT 0.2 %	0.09	0.03	0.01	0.06
	Any unspecified impurity	NMT 0.2 %	0.02	0.13	0.07	0.14
	Total Impurities	NMT 3.0%	0.09	0.17	0.46	0.41
11	**Lipid Degradation Products**					
	Lyso–PC content (%)	NMT 6.0%	2.19	NA	2.62	3.27
	Lyso PE-PEG2000 (%)	NMT 10.0%	NA		4.33	3.52
	Stearic acid	NMT 2.0%			1.1	1.26
	Palmitic acid	NMT 0.5%			0.1	0.05
12	**In-Vitro Release (%) {Dissolution media pH 6.4; Temperature 52±0.5°C}**					
	After 0.5 Hrs.	1.5 Hrs - NLT 15%	14	2	NA	2
	After 1.5 Hrs.		32	24		19
	After 3.0 Hrs.	3.0 Hrs - NLT 35%	57	54		50
	After 5.0 Hrs.	7.0 Hrs - NLT 80%	81	78		70
	After 7.0 Hrs.		98	94		88

CONCLUSION

In order to take advantage of the therapeutic effects of palm oil, liposomal Trilaciclib formulations were prepared by replacing PC with different ratios of palm oil. Liposomal formulations containing 5% and 10% of palm oil were made through the freeze-thaw method, and then the TEM images revealed satisfactory morphology and formation of LUVs, respectively. Liposomal size distribution, zeta potential, and stability remain in the acceptable range. The HPLC results confirm the optimal drug loading through pH gradient technique and sophisticated in vitro release profile as well. The finest cellular uptake was observed on MCF-7 and MDA-MBA 231 cell lines through 24 hr furthermore, cytotoxicity assay confirms the more effectiveness of the liposomal Trilaciclib containing palm oil.

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