10.48047/jocaaa.2024.33.06.101
ISSN: 1521-1398

DESIGN AND CHARACTERIZATION OF PALBOCICLIB LOADED NANO STRUCTURED LIPID CARRIERS FOR TREATMENT OF BREAST CANCER

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Abstract: Objective: To develop and characterize Palbociclib (PCB) loaded NLC. **Significance:** To overcome the specified limitations associated through oral PCB therapy, such as low aqueous solubility, suboptimal bioavailability, and systemic toxicity. **Methods:** In this study, Sterotex HM & Captex 300 were employed as solid & liquid lipid respectively. A blend of Tween 80 & Span 80 was used to stabilize the nano-emulsion system. The Box-behenken DOE was used for formulation development by microemulsion followed by ultra-sonication. **Results:** The optimized formulation exhibited a mean particle size 165 nm, a low PDI (0.3), zeta potential of approximately -34 mV, Entrapment efficiency & drug loading was observed above 98% & 9.9% respectively. Green approach to select blend of surfactants Span 80 and Tween 80 in 4.44:1 ratio offerings a highly promising drug delivery platform for Palbociclib. DSC and XRD studies confirmed the reduction in crystallinity of the lipid matrix and suggested successful incorporation of Palbociclib into the amorphous regions of the carrier. TEM images revealed that the NLCs possessed a spherical morphology with smooth surfaces and nanoscale size. In vitro drug release studies confirmed sustained drug release over 10 hours. In vitro cytotoxicity assessments on MCF-7 breast cancer cells showed significantly enhanced anti-cancer activity of the Palbociclib-loaded NLCs compared to the free drug, attributed to improved cellular uptake and retention. **Conclusion:** The study confirms that Sterotex HM and Captex 300-based NLCs stabilized by Span 80 and Tween 80 present a highly promising drug delivery for PCB, capable of overcoming key pharmacokinetic challenges.

Key words: Nano structured lipid carriers, NLCs, Palbociclib, Breast cancer, BCS II drug, required HLB.

1. INTRODUCTION

Palbociclib (PCB) is an oral cancer drug used mainly for certain breast cancers. As a Cyclin Dependent Kinase 4/6 (CDK4/6) inhibitor, it blocks proteins that drive cell division, slowing the growth and spread of malignant cells. PCB treats hormone receptor-positive (HR+), HER2-negative breast cancer that has progressed or metastasized, usually with hormone therapy like letrozole or fulvestrant. It prolongs progression-free survival by preventing the G1 phase from advancing to the S phase of the cell cycle, delaying disease progression and extending survival with manageable safety.[1,12]

However, as a BCS II drug, PCB faces formulation challenges due to low water solubility (0.5 mg/ml), which limits dissolution in the GI tract and reduces absorption efficiency. This poor solubility results in low bioavailability (~46%), meaning much of the dose is not fully utilized. Therefore, despite being an effective selective CDK4/6 inhibitor for ER-positive breast cancer, PCB's therapeutic potential can be limited by poor absorption.[2]

10.48047/jocaaa.2024.33.06.101
ISSN: 1521-1398

This issue can be addressed by loading PCB into Nanostructured Lipid Carriers (NLCs), a proven approach to enhance the solubility, absorption, and bioavailability of poorly soluble drugs. NLCs also help protect the drug from degradation, extend its residence time in target organs, and improve overall therapeutic effect while reducing side effects.[3]

NLCs are nano-sized colloidal carriers made of a core matrix of solid lipid (SL) and liquid lipids (LL).[4] As second-generation lipid nanocarriers, they overcome SLN limitations by using a less-ordered lipid blend stabilized by surfactants, forming 50–500 nm particles. This structure improves drug loading, stability, and controlled release. Their high loading capacity, sustained stability, and efficient drug trapping with minimal surfactant make NLCs especially useful.[5]

NLCs are a promising system for targeted delivery of highly lipophilic drugs. Targeting can be enhanced by selecting suitable solid and liquid lipids and attaching biomolecules to the carrier. Studies show NLCs can deliver drugs to the brain, lungs, liver, skin, and tumors. Strategies like PEGylation, folate or transferrin binding, and using ligands like anti-VEGFR-2, erythropoietin, hyaluronic acid, or biotin further improve targeted delivery.[6]

Although NLCs are constructed similarly to SLNs, they differ in three key ways based on how the drug is incorporated: Type I (imperfect crystal), Type II (multiple), and Type III (amorphous) (Fig. 1). This study focuses on improving the solubility of PCB, a BCS Class II drug, using NLCs. The objectives are to prepare and evaluate PCB-loaded NLCs, select suitable LL, SL, surfactant, and co-surfactant, and develop an effective, safe formulation to inhibit cancer cell growth.

2. MATERIAL AND METHOD

Materials: PCB were purchased from Sigma Aldrich, Sterotex HM and Captex 300 were obtained as gift sample from Abitech corporation, Tween 80 and span 80 (were purchased from Loba chemicals, Mumbai, Methanol, diethyl ether, Hydrochloric acid and other chemicals of analytical grades were procured from SD fine chemicals, Islampur, (MH). Double distilled water were obtained from, Symbiosis Pharmaceuticals Pvt. Ltd, Sangli.

Software: Design-Expert® (version 13.0, Stat-Ease Inc.), Ternaryplot.com, ACD/Checmsketch20212.0, Microsoft Excel 2024

Instruments: Electronic weighing balance (Wensar, India), UV-Visible spectrometer (Shimadzu (UV-1900), Japan), High pressure liquid chromatography (Thermo fisher scientific, USA) Fourier Transform Infrared Spectroscopy (FTIR) (JASCOFT/IR-410, Japan), Orbital shaker, Dissolution Apparatus, (Labindia DS8000, India), Probe sonicator (Johnson Plastosonic, India), Particle Size Analyzer (HORIBA, Japan), Differential Scanning Calorimetry (DSC) (SDT Q600 V20.9 Build 20), Magnetic stirrer, Heating mental, centrifuge & Ultra centrifuge (REMI, equipments Ltd. India), melting point apparatus etc

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Methods: RP-HPLC Analytical Method Development: High-performance liquid chromatographic analysis was carried out using an XTERRA MS C18 column (250 mm × 4.6 mm, 5 µm particle size) maintained at 25°C. The mobile phase consisted of ammonium acetate buffer and acetonitrile in the ratio of 30:70 (v/v), delivered at a flow rate of 1.0 mL/min. The detection was performed using a UV–Vis variable wavelength detector set at 266 nm, with an injection volume of 20 µL for conentrtion range 30-150µg/ml. The total run time was 20 minutes, which enabled proper separation and quantification of the analyte under the

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ISSN: 1521-1398

selected chromatographic conditions. The calibration curve was developed by plotting concentration (µg/ml) on X-axis and peak area (mAU)) on Y-axis.[9, 10]

Screening of lipids (Solid Lipid and Liquid Lipid): Selection of the lipids carried out on the basis of maximum solubility of PCB in lipids.

Selection of Liquid Lipid[11] :A small amount of PCB was added to 5 ml of various natural and semi-synthetic liquid lipids (oils). The mixtures were shaken on a digital orbital shaker for 24 hrs at 250 rpm. After 24 hrs, oils were checked for undissolved PCB. Oils where PCB fully dissolved were reloaded with more PCB and shaken for another 24 hrs to reach saturation. Tubes were then centrifuged for 5 min at 1000 rpm to separate any undissolved PCB. 1 ml of the supernatant was diluted in 10 ml methanol + diethyl ether (1:1), then further diluted (1 ml into 10 ml methanol + diethyl ether, 1:1). All oils were quantitatively analyzed for drug content.

Selection of Solid lipis: it screened on based on MP and highest solubility of drug by visual observation**.** Selected the number of SLs having meting point in between 40 to 60ºC. 1mg of drug were added in melted lipid and shaken the test tube in water bath having temperature just above the MP of the SL till drug get dissolved. The drug was gradually increased till un-dissolved drug particles observed. [11]

Lipids homogeneity test (Filter Paper Test)[12]: The SL should be miscible with the LL to allow for the formation of imperfections in the SL's crystal lattice structure, which will make API loading easier. This miscibility test gives ratio of SL LL at which both lipids homogenize with each other. Captex 300 and Sterotex HM were mixed in different ratios, heated to 70 °C, then cooled to room temperature. An aliquot was smeared on filter paper to visually assess miscibility; mixtures showing droplets were considered unstable, as visible droplets indicated poor miscibility. Solubility of drug further confirmed in selected ratio of solid-LL by DSC & XRD[13]

3. EVALUATION OF FORMULATION

Particle Size & zeta potential: The particle size study was carried out for all batches we are prepared and zeta potential study was carried out or checked for optimized batch.[16]

Polydispersity Index (PDI): It measures the uniformity of particle size distribution in a sample (like nanoparticles, polymers, lipid carriers). PDI ranges from 0 to 1 (< 0.1 very uniform (monodisperse), 0.1 - 0.3 acceptable uniformity, > 0.3 broad size distribution (polydisperse)[17]

Entrapment efficiency (EE%): To determine EE%, the PCB-loaded NLC dispersion is ultracentrifuged (15,000–25,000 rpm, 30–60 min, 4 °C) to separate free drug. The NLCs pellet while unentrapped PCB remains in the supernatant, which is quantified by HPLC, EE (%) = [(Total drug - Free drug) / Total drug] × 100. Here, the total drug refers to the initial amount of PCB used in the formulation, and the free drug is the amount of PCB detected in the supernatant.[18,30]

Drug loading (DL %): % DL were calculated based on the %EE results [19,], DL% = (Amount of drug entrapped / Total weight of nanoparticles) × 100

Statistical Optimization of trial batches: Using the statistical program Design-Expert® (version 13.0, Stat-Ease Inc.), a 3-level, 3-factor BBD was used to improve the NLCs formulation.[15,17]

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***In-vitro* Drug release study**: In vitro drug release of PCB-loaded NLCs was studied using the dialysis bag diffusion method. 5 mg of PCB-loaded NLC suspension was placed in a sealed dialysis bag and immersed in 900 ml PBS (pH 6.8) at 37 ± 0.5 °C with stirring at 50 rpm. At set intervals (5 min, 10, 15, 30, 45, 60 min, etc.), 5 ml samples were withdrawn and replaced with fresh pre-warmed PBS to maintain sink conditions. Samples were filtered through Whatman filter paper and analyzed by HPLC.[22,33]

Morphology Studies: Transmission electron microscopy (TEM) was used to examine the morphology of the optimized NLC**.**[23]

In vitro Anti-cancer activity: The National Center for Cell Science (NCCS), Pune, provided the MCF-7 human breast cancer cell line, maintained in MEM medium with 10% fetal bovine serum. Cells were incubated for 24 hours at 37 °C, 5% CO_2, at a density of 1×10^4 cells/ml. 70 µl of cells per well were seeded in 100 µl culture medium and 100 µl water. Samples (10–100 µg/ml) were added to tissue culture-grade 96-well plates. Control wells contained cell line and 0.2% DMSO in PBS. Each sample was tested in triplicate**.**[24] . Controls were included to determine cell viability. Cultures were incubated for 24 hours at 37 °C, 5% CO_2 in a CO_2 incubator (Thermo-Scientific BB150). After incubation, the medium was removed and 20 µl MTT reagent (5 mg/ml PBS) was added. Cells were then incubated for 4 hours at 37 °C in the CO_2 incubator. After removing the medium, 200 µl DMSO was added, kept for 10 min, and incubated at 37 °C wrapped in aluminum foil. Absorbance was measured at 570 nm using an ELISA microplate reader (Benesphera E21) for three samples.

4. RESULTS AND DISCUSSION

RP-HPLC Analytical method development: A stability-indicating RP-HPLC method was developed and validated in accordance with ICH Q2(R1) guidelines, demonstrating accuracy, precision, linearity, specificity, robustness, and sensitivity for reliable quantification of palbociclib. The method ensured reproducible retention time, sharp peak resolution, and compliance with regulatory acceptance criteria for analytical validation.

Table 1: Observation of RP-HPLC method validtion

Conc. µg/ml	**Peak area mAU**			**Average peak area**	**SD**	**LOD µg/ml**	**LOQ µg/ml**	**%RSD**
	1	2	3					
30	985.866	974.606	964.34	974.9373	±8.79	0.54	1.64	0.90
60	1932.517	1974.103	1945.868	1950.829	±17.33	1.07	3.25	0.88
90	3033.856	3051.149	3018.681	3034.562	±13.26	0.82	2.48	0.43
120	4070.611	4224.95	4117.901	4137.821	±64.56	3.99	12.10	1.56
150	5212.646	5204.875	5215.736	5211.086	±4.56	0.28	0.85	0.08

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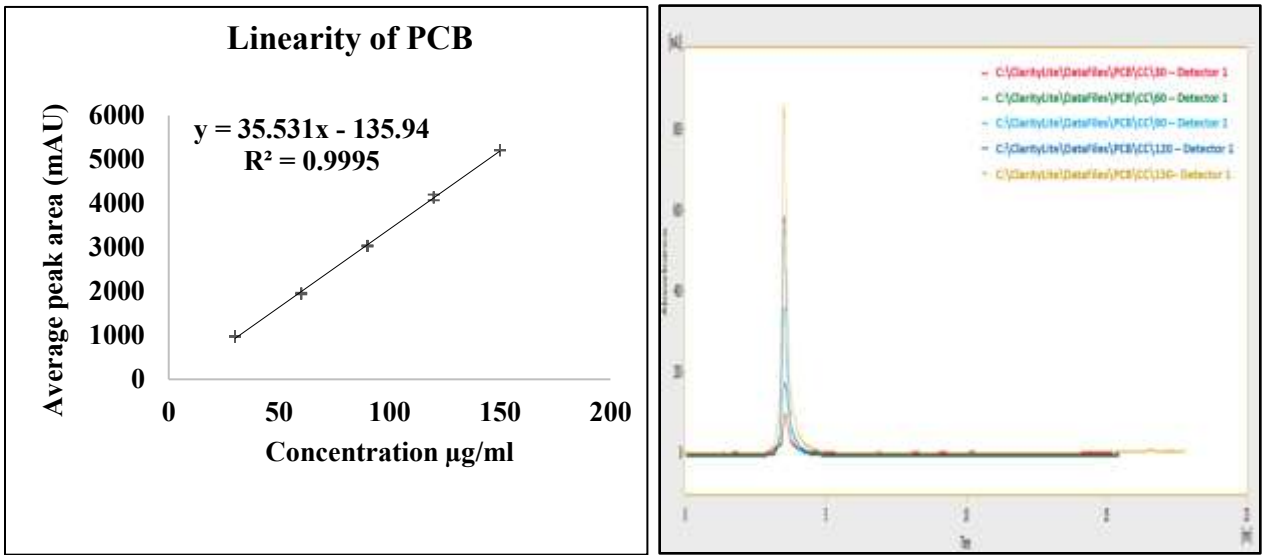
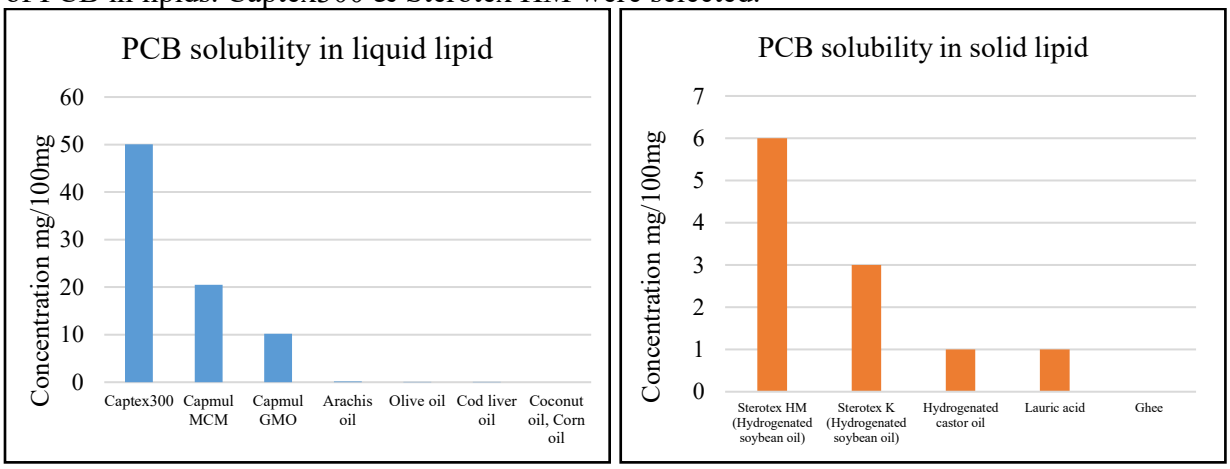


Figure 1: Calibration curve & overlapped chrpmtogrm of of PCB by RP-HPLC chromatogram

Screening of lipids (SL-LL): Selection of the lipids carried out on the basis of maximum solubility of PCB in lipids. Captex300 & Sterotex HM were selected.



(A) (B)

Figure 2: solubility of PCB in different (A) Liquid lipid (B) Solid & lipids

Lipid homogeneity test (Filter Paper Test): SS-LL homogeneity test is performed to ensure that the solid and LLs used in NLCs form a uniform mixture without phase separation or incompatibility. This test helps in selecting the appropriate lipid combination for suitable and effective lipid-based drug delivery system. 9:1 ratio was selected based on filter paper test. (Figure no. 3)

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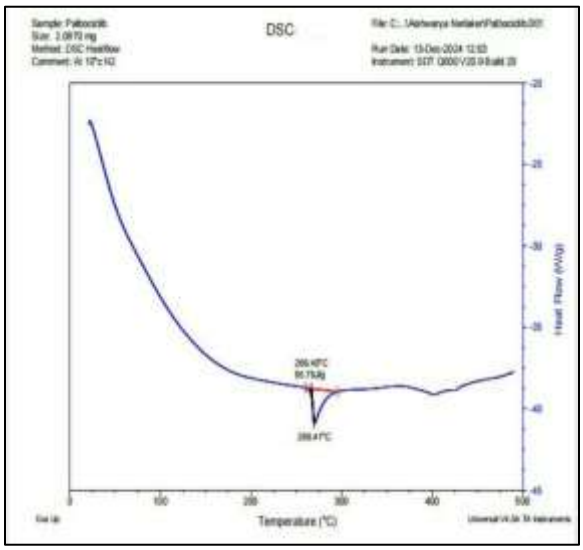
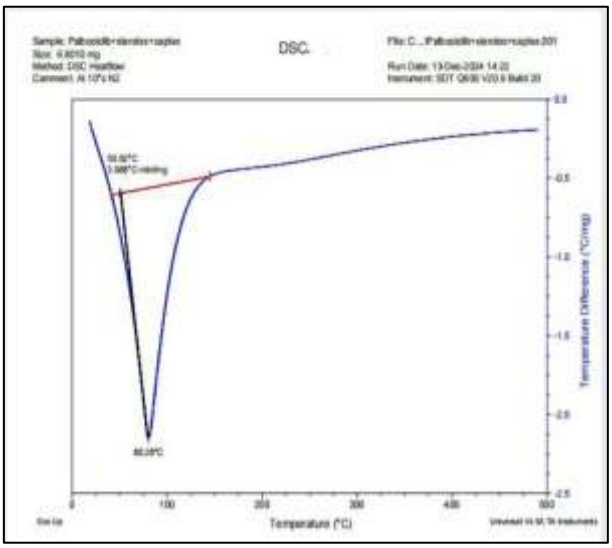
Liquid lipid : Solid lipid	Observation's
900:100	Oil spot observed
800:200	Oil spot observed
700:300	Oil spot observed
600:400	Oil spot observed
500:500	Oil spot observed
400:600	Oil spot observed
300:700	Oil spot observed
200:800	Oil spot observed
100:900	No oily spot observed on the filter paper

Figure 3: Observations of filter paper test





(A) **(B)**

Figure 4: DSC of PCB (B) DSC of physical mixture (Drug+SL+LL). Shifting of MP at lower side i.e depression of MP indicated PCB is soluble in both lipid

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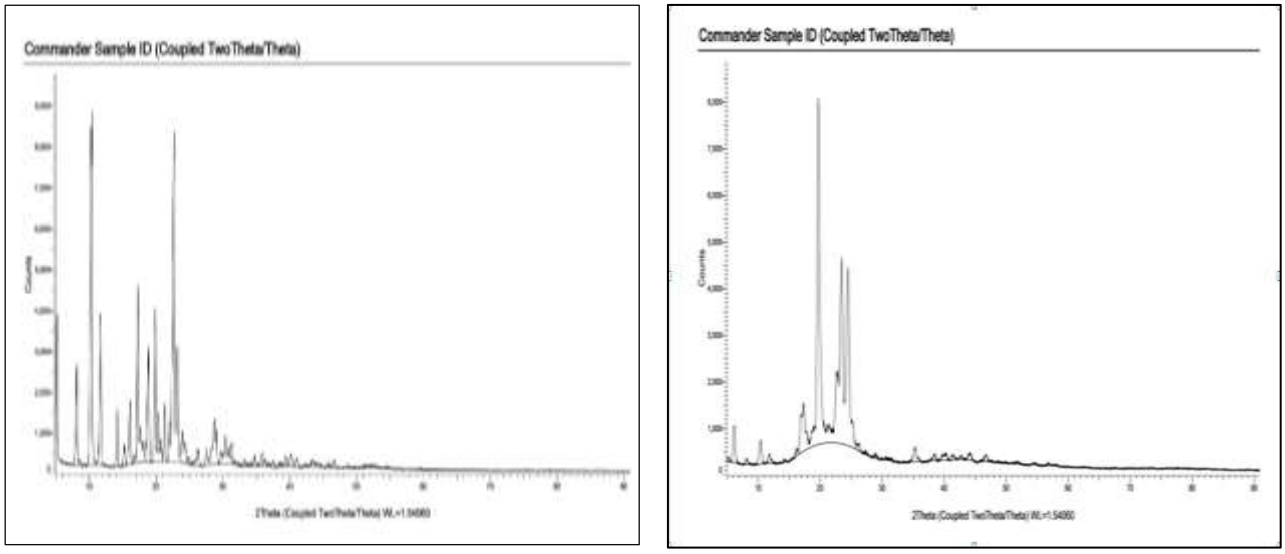

(A) **(B)**

Figure 5: (A) XRD of PCB (B) XRD of physical mixture (PCB+SL+LL). Noticeable reduction in the intensity and number of sharp peaks & broader peaks in (B) compared (A) with indicating a significant loss of crystallinity

Surfactants has been selected by calculating required HLB of formulation.

Surfactant selection was done based upon calculation of HLB of surfactant on the basis of required HLB of lipid phase in formulation. In order to prepare stable emulsion an emulsifier mixture having HLB equal to HLB of oil phase in the formulation should be used.

Based on solubility of drug in both lipids and ratio at which both lipid found miscible (9:1) the probable formula of the PCB loaded NLC was predicted as follows. (Table 2)

Table 2: Formula for PCB loaded NLC

Drug	PCB	10 mg
SL	Sterotex HM	90 mg
LL	Captex300	10 mg
Surfactant	Tween 80+Span 80	1gm
Water	Solvent	QS

Required HLB of the formulation were calculated in order to find the ratio of surfactant –co- surfactant having equal HLB.

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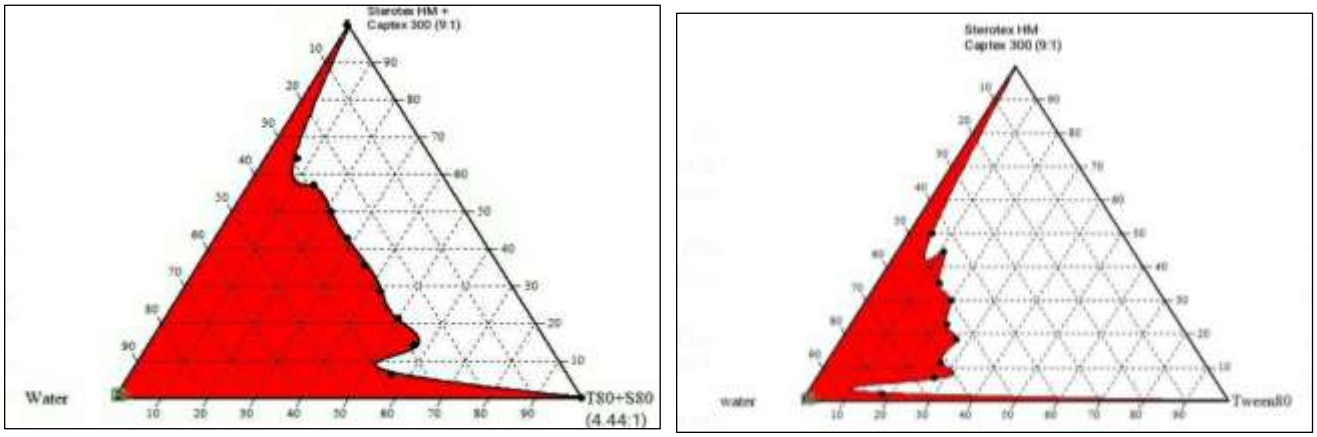

(A) **(B)**

Figure 6: Pseudo ternary phase diagram using (A) Tween80: Span80 4.44:1 (B) only Tween80

Based on pseudo-ternary phase diagram Tween 80 and Span 80 ratio (4.44:1) showing more micro-emulsion area as compare with only Tween 80 as a surfactant. The concentration 0.3%, 0.4%, 0.5% were selected to prepare PCB NLCs.(Figure 6)

Formulation development:
Using the statistical program Design-Expert® (version 13.0, Stat-Ease Inc.), a 3-level 3-factor BBD was utilized to improve the NLCs formulation. The BBD is highly suitable for optimizing the formulation of NLCs of PCB due to its ability to efficiently evaluate the influence of multiple formulation and process variables with a reduced number of experimental runs.

Micro-emulsion formation followed by probe sonication method: Formulation of NLCs:
Trial batches of PCB loaded NLCs were formulated using the micro-emulsion technique followed by probe sonication. This high-energy process helped in breaking down the lipid droplets into nano- sized carriers, ensuring a narrow size distribution and improved stability of the final formulation. The prepared NLCs appeared as uniform, milky-white dispersions with a slightly translucent to opaque appearance, indicating successful nano-encapsulation and good physical stability. (Figure No. 7)

Figure 7: Trial batches of PCB NLCs formulations

Evaluation of formulation:
The formulation were primarily evaluated for Particle size, PDI, % Entrapment efficiency:

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Table 3: Results for average size, PDI, and entrapment efficiency.

Run No.	Lipid mix (mg)	S-mix (%)	Sonication Time (min)	Particle size (nm)	PDI	EE (%)
1	200	0.4	3	137.6	0.3	90.17
2	200	0.4	7	87.3	0.349	71.37
3	250	0.3	3	146.1	0.296	91
4	250	0.4	5	129.8	0.301	84.65
5	300	0.4	3	155.6	0.3	95.65
6	250	0.3	7	158.4	0.359	95.84
7	200	0.5	5	102.2	0.355	80.27
8	250	0.4	5	135	0.31	82.31
9	250	0.4	5	150	0.317	95.21
10	250	0.5	3	148.4	0.373	91.56
11	300	0.5	5	93.6	0.307	74.33
12	300	0.4	7	169	0.309	99.84
13	250	0.4	5	128.6	0.313	95.21
14	250	0.5	7	96	0.353	79
15	300	0.3	5	164.9	0.33	98.99
16	250	0.4	5	129.8	0.301	84.16
17	200	0.3	5	136.7	0.326	86.36

Statistical optimization of formulation:

a) Effect of Variables on Particle size:

Multiple linear regression analysis results show that the X1, X2, and X3 have a significant impact on the particle size. The following polynomial equation can be used to illustrate the fitted equation for the whole model that links the particle size to specific factors:

Particle size = +134.64+14.91* X1 20.36*X2 -9.25 * C-9.20 * X1 * X2+15.93* X1 * X3 -16.93* X2 * X3-4.70 *$X1^2$ -5.60 * $X2^2$ +7.43 * $X3^2$ +7.43

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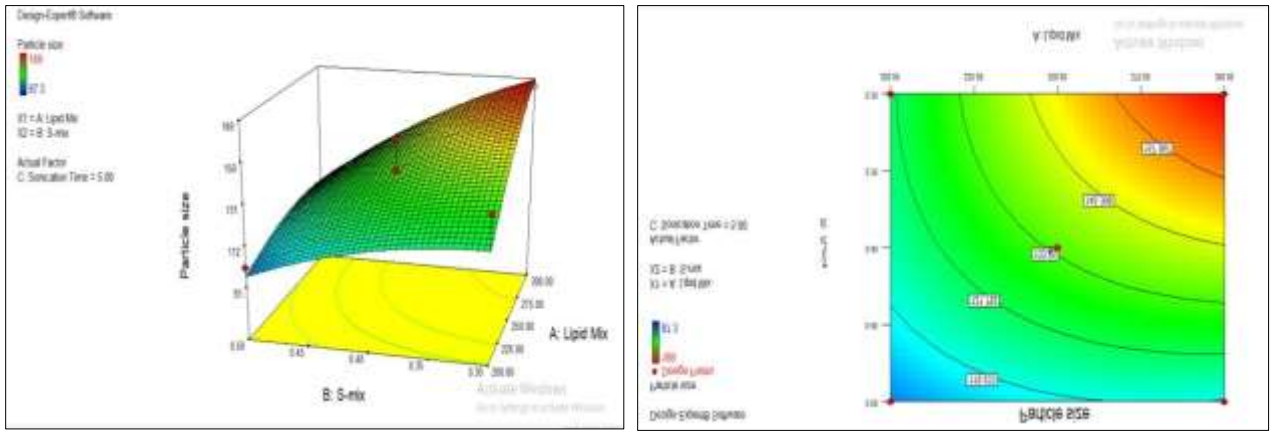


(A) **(B)**

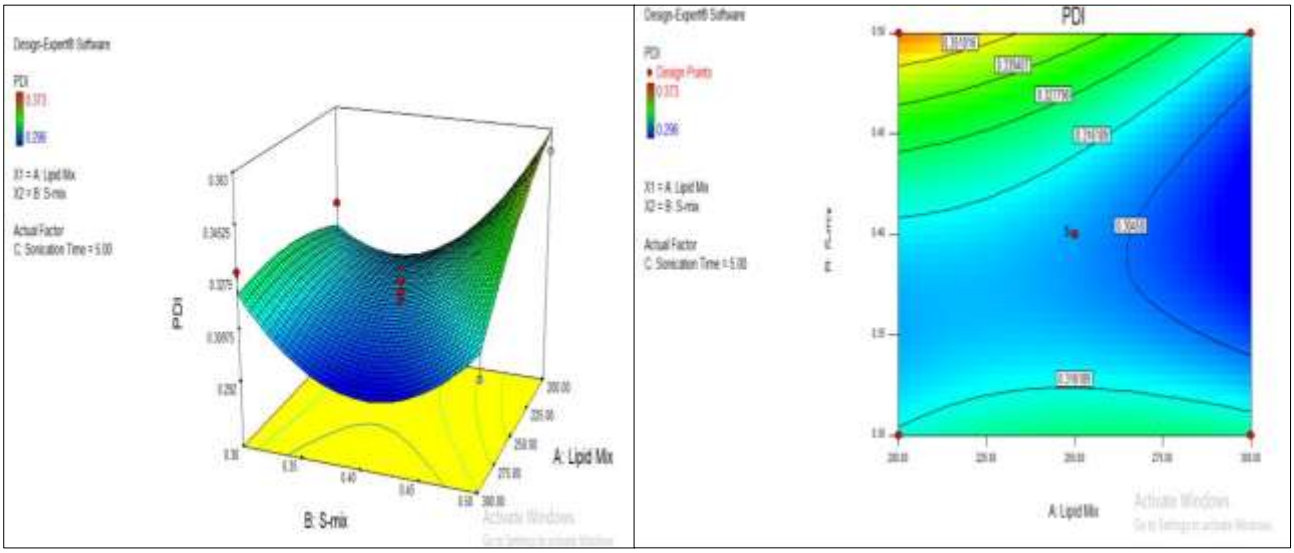
Figure 8: (A) Surface response curve (B) Contour plot for Particle Size.

The 3D surface plot & contour plot (Figure No.8) illustrates the combined effect of lipid mix and surfactant mix on the particle size of PCB-loaded NLCs. It was observed that increasing the lipid concentration led to a corresponding increase in particle size, likely due to the formation of larger droplets during emulsification and potential aggregation upon solidification. In contrast, increasing the concentration of the surfactant mix resulted in a significant reduction in particle size, as the surfactants effectively lowered interfacial tension and stabilized the lipid dispersion. The smallest particle sizes were achieved at low lipid and high surfactant levels, indicating an optimal balance for producing stable, nanoscale carriers.[7] These findings highlight the critical role of lipid-to-surfactant ratio in determining the physical characteristics of NLCs and optimizing them for improved solubility and delivery of poorly water-soluble drugs like PCB.

Effect of Variables on PDI:

It is evident from the data that Y2 (PDI) is highly dependent on the factors X1, X2, and X3.

PDI= +0.31-0.011 * X1+9.625E-003* X2+0.013 * X3-0.013* X1 * X2-0.010* X1 * X3-0.021* X2* X3-4.825E-00*$X1^2$+0.026*$X2^2$+0.011$X3^2$.



(A) **B)**

Figure 9: (A) Surface response curve (B) Contour plot for PDI

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The surface response plot & contour plot (Figure No.9) for PDI demonstrate the combined effect of lipid mix and surfactant mix concentrations on the uniformity of PCB-loaded NLCs. A lower PDI value, indicative of a more homogeneous particle size distribution, was achieved when both lipid and surfactant levels were moderately high. This reflects efficient emulsification and stabilization during formulation. Conversely, higher PDI values were observed at elevated lipid concentrations with insufficient surfactant, likely due to inadequate coverage and stabilization of the lipid droplets, resulting in particle aggregation and size variability. These results emphasize the critical balance between lipid and surfactant content in achieving a uniform and stable NLC formulation, which is essential for consistent drug delivery performance.

b) Effect of Variables on % EE:

The data clearly indicated that values of % EE intensely dependent on the designated independent variables i.e Amount of lipid mix, S-mix concentration, sonication time. The fitted equation (for full model) relating the response % EE to the transformed factor is:

$$EE = +88.31 + 5.08 * X1 - 5.88 * X2 - 2.79 * X3 - 4.64 * X1 * X2 + 5.75 * X1 * X3 - 4.35 * X2 * X3 - 1.71 * X1^2 - 1.61 * X2^2 + 2.66 * X3^2$$

The regression model for encapsulation efficiency (EE) shows that increasing lipid amount (X1) enhances EE, while higher surfactant concentration (X2) and longer sonication time (X3) reduce it, likely due to drug leakage or matrix disruption. Interaction effects reveal that combinations of high lipid with surfactant, or surfactant with sonication, further lower EE, whereas lipid with sonication shows a positive effect. Quadratic terms suggest excessive lipid and surfactant reduce EE, while moderate sonication may improve it before a decline. Thus, optimizing lipid and carefully balancing surfactant and sonication time is essential for maximizing EE.

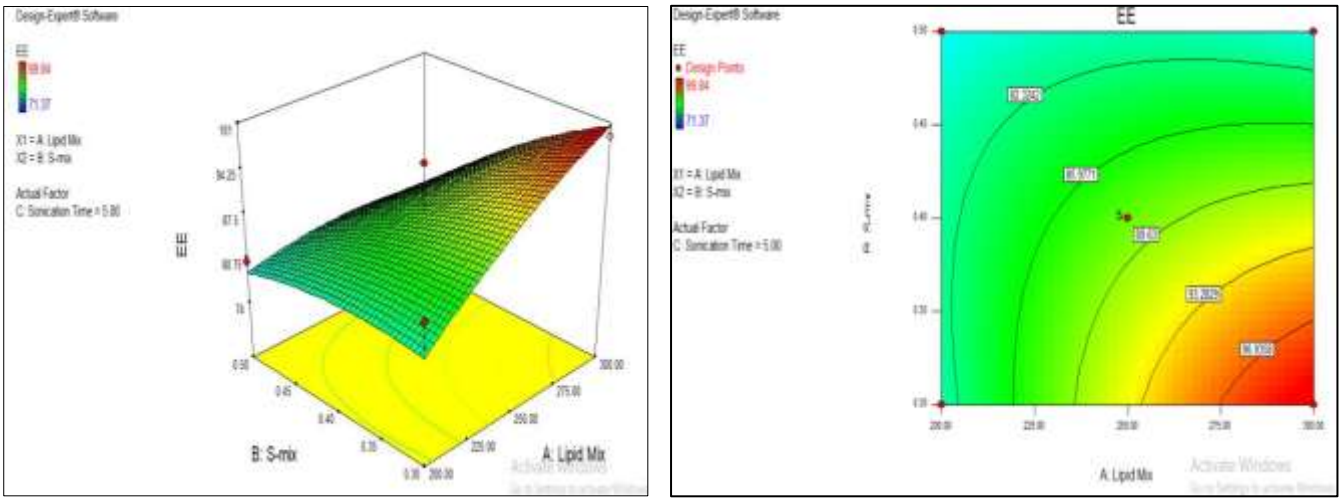

Figure 10: (A) Surface response curve (B) Contour plot for %EE

Due to spatial incompatibility, the mixture of the SL and LL matrix in NLCs results in a significant number of defects that aid in the ability to accommodate larger drug quantities. When Captex300 and Sterotex HM are present, imperfect crystals form, giving lipophilic medications additional room and affinity for integration. A high concentration of surfactant slows down the rate at which the medication diffuses by making the aqueous phase more viscous, which raises the percentage of EE. (Figure No.10)

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Determination of optimized of batch: by selecting the following criteria F15 was found to be optimized batch with desirability 1.00

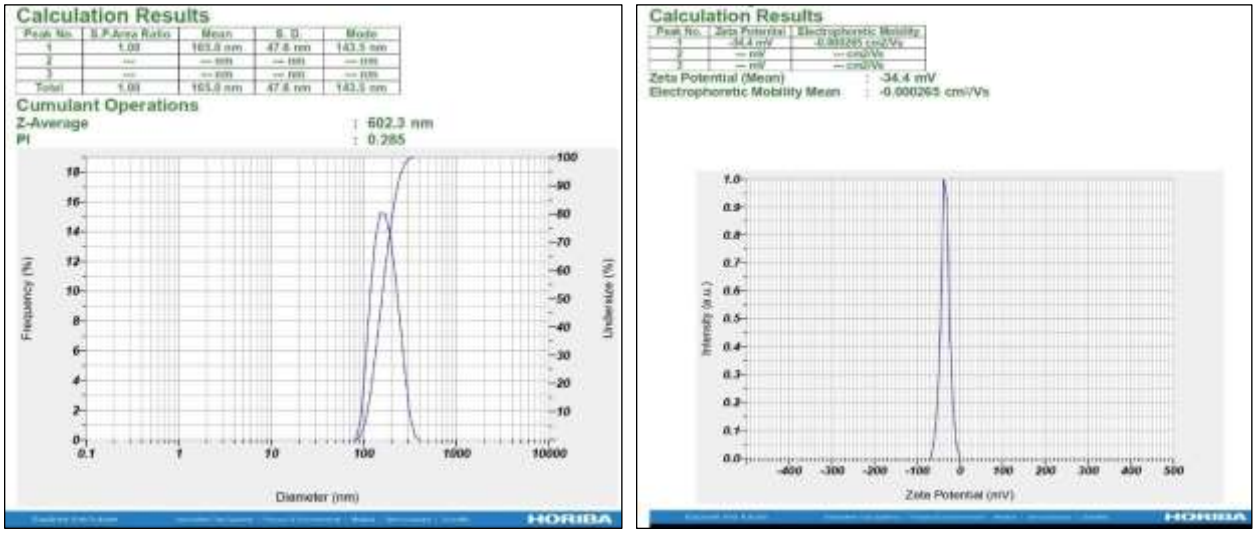
Table 4: Selection criteria for optimization of trial batch

Criteria	Amount of lipid (mg)	S-mix (%)	Sonication time (min)	Particle size (nm)	PDI	EE (%)
Goal	maximize	in range	in range	in range	in range	maximize

Table 5: Optimized batch F15

Batch no.	**Lipid mix (mg)**	**S-mix (%)**	**Sonication Time (min)**	**Particle size (nm)**	**PDI**	**EE (%)**
F15	300	0.3	5	164.9	0.33	98.99

With an average particle size of 165 nm and zeta potential of –34 mV (Figure 11), PCB-loaded NLCs demonstrate effective drug delivery. The negative zeta potential suggests strong electrostatic repulsion, reducing aggregation and enhancing stability. The nanosize supports better cellular uptake and bioavailability. These features align with literature-reported successful NLCs, indicating formulation F15 meets desired criteria for stability and therapeutic efficacy.



(A) **(B)**

Figure 11: (A) Particle size (nm) & (B) zeta potential of optimized batch

Drug Loading (%DL): Calculated on the basis of results of %EE (98.99%) of optimized batch F15
DL% = (Amount of drug entrapped ÷ Total weight of nanoparticles) × 100 = (29.70mg ÷ 300mg) × 100 = 9.9%

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The drug loading was determined to be 9.9% based on the overall weight of the nanoparticles (300 mg) and the amount of drug entrapped.

***In-vitro* drug release study:**

The significant enhancement in PCB release from PCB-NLCs compared to the pure drug can be attributed to several formulation-related factors. In this study, PCB-NLCs achieved approximately 28 % cumulative drug release in 0.1 N HCl and 98 % in pH 6.8 phosphate buffer over 10 hours, whereas pure PCB exhibited only about 36% release in the same timeframe.(Figure no. 22)

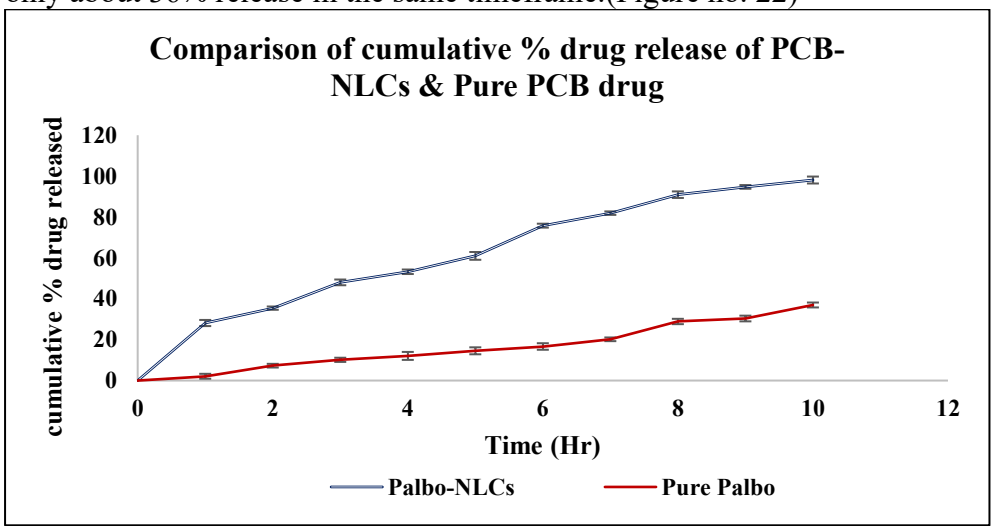

Figure 12: Comparison of cumulative % drug release of PCB-NLCs & Pure PCB drug

Drug diffusion kinetics study:

Table 6: Correlation coefficient (R^2 values) of Optimized batch

Model	Higuchi	Korsmeyer Peppas	Zero order	First order	Hixson Crowell
R^2	0.9796	0.9154	0.9441	0.9555	0.9658

The in-vitro drug release study of PCB-loaded NLCs was performed using the dialysis bag diffusion method in phosphate buffer (pH 7.4) at 37 ± 0.5°C. The cumulative percentage of drug released was determined at predetermined time intervals and plotted against the square root of time to assess the drug release kinetics.(Figure no. 23 (A)) Optimized batch F15 primarily followed Higuchi drug release kinetic model.

Morphology study:

The TEM images shows particle size measurements, ranging from 100 nm to 169 nm, (Figure no. 24 (B)) clearly confirm the nanoscale nature of the formulation. The image also reveals a moderate dispersion of particles across the field, indicating that the formulation exhibits good colloidal stability with minimal aggregation. (Figure no. 24 (A)

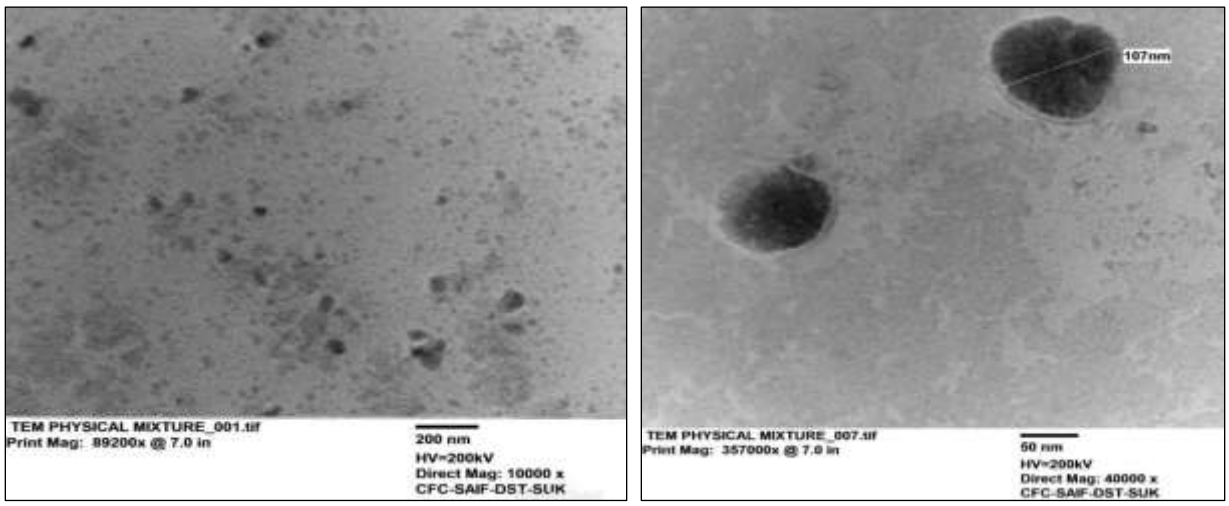

Figure 13: (A) TEM image of PCB-NLCs (A) Magnified at 10000X showing moderate dispersion of NLCs (B) magnifies at 40000X showing nano sized particle

In-vitro anti-cancer activity:

Sample F1 was pure drug (PCB) and F2 coded for PCB loaded NLCs for the MTT test. The cytotoxic effects of samples F1 and F2 were assessed against the MCF-7. A dose-dependent decrease in cell viability was seen in samples F1 and F2, indicating that higher concentrations resulted in more cytotoxic effects. The pure drug sample F1 exhibited considerable cytotoxicity, as evidenced by a drop in cell viability from 93.60% to 58.51%. In comparison to sample F1 (Pure drug), sample F2 (PCB NLC) showed more pronounced cytotoxic effects, with cell viability dropping from 87.34%. (Figure 14 & 15)

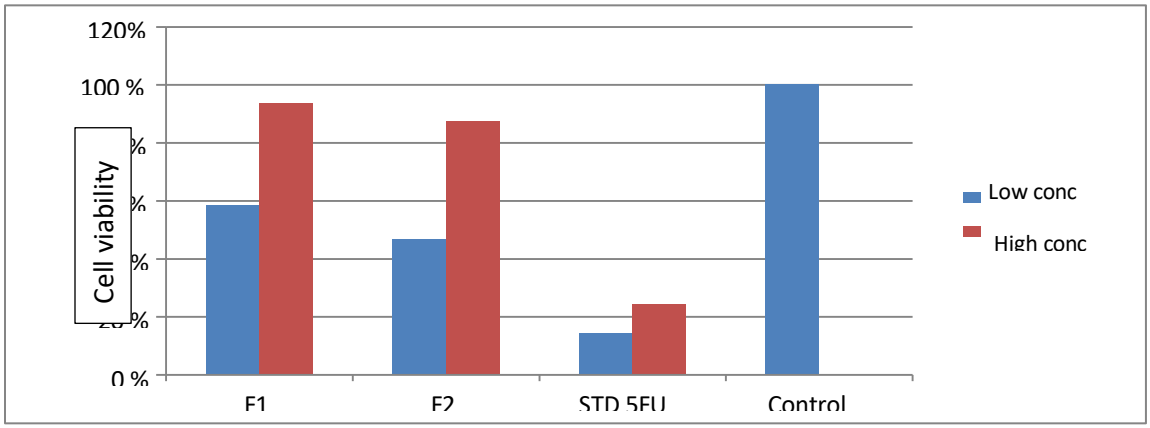

Figure 14: Comparison of % Cell Viability of MCF-7 Cells F1:Pure Drug vs F2:PCB-NLCs)

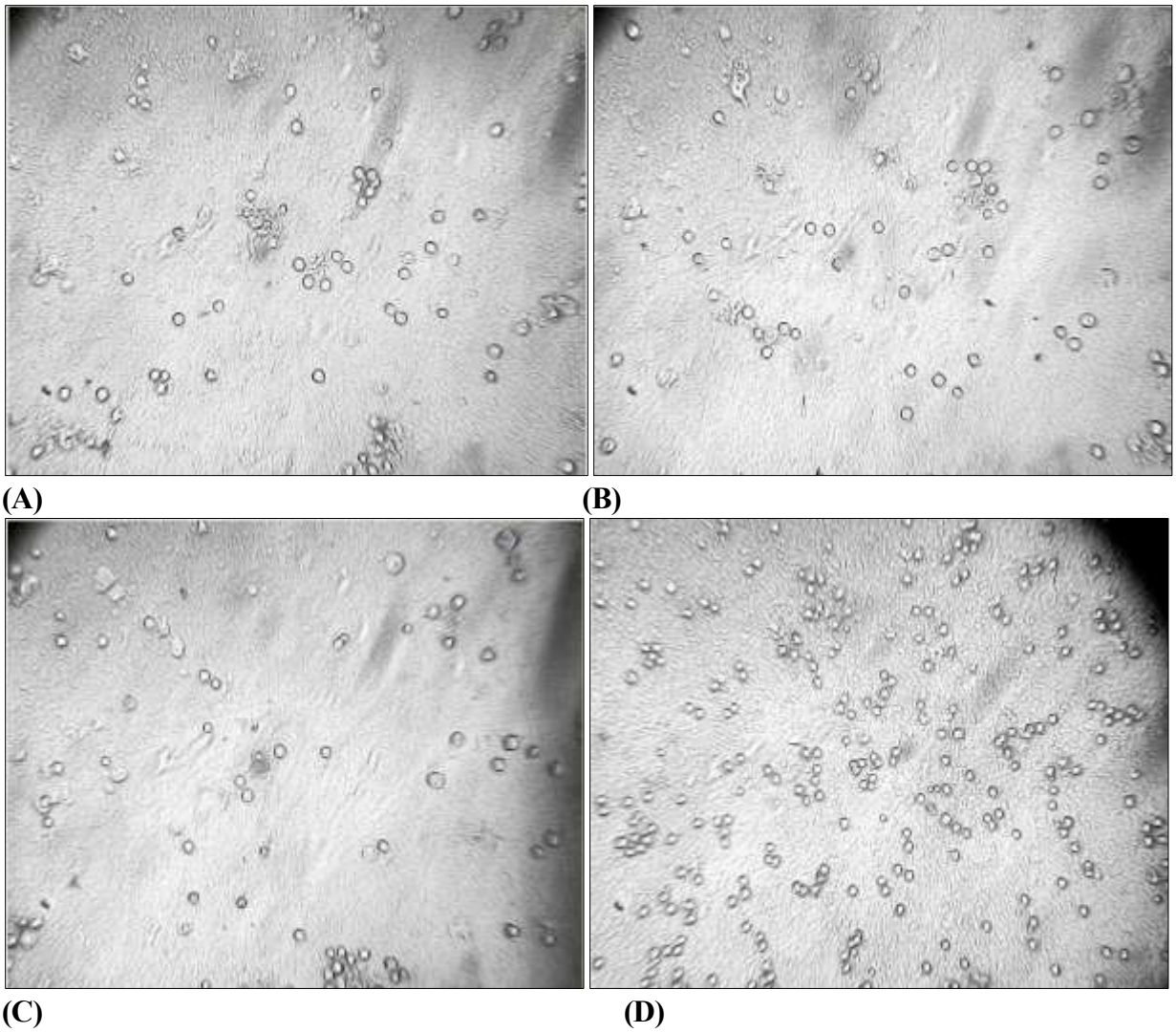

Figure 15: MTT assay results of A) Sample F1 B) Sample F2 C) STD 5-FU D) Control.

Discussion:

PCB, a CDK4/6 inhibitor for hormone receptor–positive breast cancer, has poor aqueous solubility (log P ≈ 3.9, pKa ~7.4), limiting its oral bioavailability (BCS Class II). These properties make PCB suitable for NLC encapsulation to enhance bioavailability and efficacy. An RP-HPLC method validated for PCB–NLCs showed excellent linearity (30–150 µg/mL, R^2 = 0.999), precision (%RSD < 2%), and sensitivity, confirming suitability for routine analysis.

PCB showed high solubility in Sterotex HM (solid lipid, MP 55–66 °C) and Captex 300 (liquid lipid), which were miscible at a 9:1 ratio.[26] Their triglyceride nature and fatty acid compatibility supported stable hydrophobic interactions. DSC and XRD confirmed PCB solubilization and reduced crystallinity, indicating amorphization and improved dissolution [25,27].

The required HLB of 13 was achieved with Tween 80:Span 80 at 4.44:1, producing a larger microemulsion region than arbitrary ratios. This rational surfactant selection enhanced stability

and emulsification efficiency, aligning with green chemistry. NLCs were prepared by microemulsion followed by probe sonication using Sterotex HM/Captex 300 (9:1) and T80/S80 (4.44:1).

Optimized batch F15 showed particle size 164 nm, zeta potential –34 mV, drug loading 9%, and entrapment efficiency 98%, with a 36-fold increase in solubility compared to pure drug. In vitro release reached 98% over 10 h, compared to rapid release from pure PCB. Sustained release was attributed to nanosize, amorphization, and surfactant-enhanced dispersion. Data fitted the Higuchi model ($R^2 = 0.9796$), and the Korsmeyer–Peppas n value of 1.16 indicated Super

Case II transport involving diffusion, erosion, and lipid matrix reorganization [29]. MTT assay confirmed dose-dependent cytotoxicity against MCF-7 cells. Pure PCB (F1) reduced viability from 93.6% to 58.5%, while NLC-loaded PCB (F2) showed greater cytotoxicity, reducing viability from 87.3%. Enhanced activity of F2 resulted from improved solubility, sustained release, and nanoscale uptake, leading to higher intracellular drug levels and stronger CDK4/6 inhibition [1,2].

Conclusion

This study aimed to develop and characterize PCB-loaded NLCs to improve delivery and therapeutic efficacy for breast cancer treatment. Given PCB's poor water solubility, low bioavailability, and systemic toxicity, NLCs offer a promising alternative. Sterotex HM (SL), Captex 300 (LL), and a Span 80–Tween 80 blend were used to stabilize the system. The formulation, prepared by high shear micro emultion followed by ultrasonication, produced stable NLCs with desirable properties. Overall, Sterotex HM–Captex 300 NLCs stabilized with Span 80 and Tween 80 effectively improve PCB's bioavailability, stability, and anti-cancer efficacy, making them a promising delivery system for breast cancer therapy. Future prospects: Although this study successfully formulated and characterized PCB-loaded NLCs, several gaps remain. Lipid selection relied on conventional solubility tests; future work could use AI-based solubility prediction for better efficiency and stability. The NLCs were not converted into a final dosage form, so developing patient-friendly systems like capsules or injections is needed. In vivo pharmacokinetic and biodistribution studies are crucial to confirm bioavailability, efficacy, and safety. Targeted delivery strategies, long-term stability, scalable manufacturing, and co-encapsulation with synergistic drugs should also be explored. Finally, patient-centric design will help improve compliance and therapeutic outcomes, advancing clinical translation of PCB-NLCs.

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