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ORIGINAL

QBD Driven Approach Design and Optimization of Lipid-Based Nanoformulation of Cannabidiol for Enhanced Treatment of Non-Melanoma Skin Cancer

Diseño y optimización basados en el enfoque QBD de una nanoformulación lipídica de cannabidiol para mejorar el tratamiento del cáncer de piel no melanoma

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ABSTRACT

Introduction: the non-melanoma skin cancer (NMSC) is a very commonly detected malignancy globally, contributing significantly to patient morbidity and placing a substantial burden on healthcare systems.
Objective: this study aims to develop a stable cannabidiol (CBD)-incorporated nanostructured lipid carrier (NLC) for treating the non-melanoma skin cancer.
Method: in the current study, we report the design, development and characterization of a cannabidiol (CBD)-incorporated nanostructured lipid carrier (NLC) gel formulation (CBD loaded NLC gel), a novel topical therapeutic approach for treating the NMSC. The optimization of the NLC was effectively carried out using a central composite design (CCD), which enabled the systematic evaluation of formulation variables and their impact on key physicochemical parameters.
Results: the final optimized CBD-NLC formulation exhibited mean particle size of 154,7 nm and a zeta potential of +30,12 mV, indicating good stability and potential for effective skin interaction. The cumulative drug release and skin permeation studies using Wistar rat skin demonstrated enhanced and sustained delivery of CBD into the epidermal and dermal layers, facilitated by the lipid-based nanocarrier system. Further, the cytotoxic ability of the CBD Loaded NLC formulation was evaluated using A375 human melanoma cells. The MTT assay revealed a significantly lower IC_{50} value of 3,07 µg/mL for the CBD loaded NLC gel compared to conventional formulations, indicating improved anticancer activity.
Conclusion: cannabidiol (CBD)-incorporated nanostructured lipid carrier (NLC) gel formulation exhibited suitable release, skin permeation and cytotoxic potential for the treatment the non-melanoma skin cancer.

Keywords: Skin Cancer; Cannabidiol; Topical Route; NLC; A375 Human Skin Cancer Cell Lines.

RESUMEN

Introducción: el cáncer de piel no melanoma (CPNM) es una neoplasia maligna muy común a nivel mundial, que contribuye significativamente a la morbilidad de los pacientes y supone una carga considerable para los sistemas de salud.
Objetivo: este estudio busca desarrollar un transportador lipídico nanoestructurado (NLC) estable con cannabidiol (CBD) incorporado para el tratamiento del cáncer de piel no melanoma.
Método: en este estudio, informamos sobre el diseño, desarrollo y caracterización de una formulación en gel de transportador lipídico nanoestructurado (NLC) con cannabidiol (CBD) incorporado (gel NLC cargado con CBD), un novedoso enfoque terapéutico tópico para el tratamiento del CPNM. La optimización del NLC se llevó a cabo eficazmente mediante un diseño compuesto central (CCD), que permitió la evaluación sistemática de las variables de la formulación y su impacto en parámetros fisicoquímicos clave.

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Resultados: la formulación final optimizada de CBD-NLC presentó un tamaño medio de partícula de 154,7 nm y un potencial zeta de +30,12 mV, lo que indica una buena estabilidad y potencial para una interacción cutánea eficaz. Los estudios de liberación acumulada del fármaco y permeación cutánea con piel de rata Wistar demostraron una liberación mejorada y sostenida de CBD en las capas epidérmica y dérmica, facilitada por el sistema nanotransportador lipídico. Además, se evaluó la capacidad citotóxica de la formulación de NLC con CBD utilizando células de melanoma humano A375. El ensayo MTT reveló un valor de CI50 significativamente menor de 3,07 µg/mL para el gel de NLC con CBD en comparación con las formulaciones convencionales, lo que indica una mayor actividad anticancerígena.
Conclusión: la formulación de gel de portador lipídico nanoestructurado (NLC) con cannabidiol (CBD) incorporado mostró una liberación, permeación cutánea y potencial citotóxico adecuados para el tratamiento del cáncer de piel no melanoma.

Palabras clave: Cáncer de Piel; Cannabidiol; Vía Tópica; NLC; A375 Líneas Celulares de Cáncer de Piel Humana.

INTRODUCTION

The global incidence of skin cancers is on the rise due to chronic sun exposure, climate changes, and various individual and social factors.[1] Skin cancers are broadly categorized into cutaneous melanoma (CM) and non-melanoma skin cancer (NMSC), with the latter mainly comprising basal cell carcinoma (BCC) and squamous cell carcinoma (SCC).[2] Melanoma, a highly metastatic skin cancer whose frequency is still on the rise worldwide, is responsible for around 80 % of skin cancer-related deaths worldwide. A distinct subset within skin cancers is Merkel cell carcinoma (MCC), historically classified as a neuroendocrine tumor, though its aggressive nature and tendency to spread to lymph nodes closely resemble CM.[3] NMSC originates from epidermal cells and shares common epidemiological characteristics, such as increased prevalence in Caucasian populations. In contrast, MCC is believed to develop from Merkel cells and is more frequently observed in equatorial regions, particularly among individuals of white ethnicity.[4] The development of BCC, SCC, and MCC is influenced by multiple factors, with exposure to physical carcinogens being the most significant risk factor. Ultraviolet radiation (UVR) has a vital role in directly triggering the malignant transformation of progenitor cells.[5] Other factors also contribute to the risk of BCC development. In the United States, non-melanoma skin cancers (NMSC) are the most frequently diagnosed cancers, with between 900 000 and 1 200 000 new cases recorded each year.[6] The two main types, squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), represent a substantial public health burden despite having a negligible effect on mortality. Their incidence has risen sharply in recent decades. In New Hampshire, SCC cases increased by 235 % in males and 350 % in females, while BCC incidence rose by 80 % in both sexes over a 14-year period. Research indicates that individuals diagnosed with NMSC have a 20–60 % higher likelihood of developing a second primary malignancy, either before or after their initial diagnosis.[7]

The two main chemotherapeutics used to treat non-melanoma are cisplatin and 5-fluorouracil (5FU); by disrupting DNA synthesis, these drugs specifically induce death in actively proliferating cells.[8] Antibodies that target T-lymphocyte associated protein (CTLA4) and selective inhibitors of v-Raf murine sarcoma viral oncogene homolog B (BRAF), such temozolomide and dacarbazine, have also been employed in contemporary melanoma treatments.[9]

The majority of data about the antimelanoma activity of phytocannabinoids is obtained from the experiments using cannabis/hemp extracts, hemp oils, CBD, or tetrahydrocannabinol (THC). Cannabinoids like THC and CBD can trigger apoptosis in cancer cells via: Activation of CB1 and CB2 receptors and increase in reactive oxygen species (ROS).[10]

The second generation of lipid-based nanoparticles are called nanostructured lipid carriers (NLCs),

which are made up of a mixture of liquid and solid lipids that create a less organized lipid core.[11] This unique structure allows for the integration of hydrophobic drugs within the lipid matrix, while hydrophilic drugs can be encapsulated in the aqueous compartments. The solid lipid matrix not only enhances the stability of the formulation but also protects the encapsulated drugs from degradation. NLCs offer several advantages, including sustained and controlled release of drug, targeted delivery to particular tissues or cells, and improved solubility and bioavailability of therapeutic agents.[12] Owing to their nanoscale size, NLCs can effectively penetrate the skin and localize drugs at the tumor site, thereby minimizing systemic side effects—an essential feature for the effective therapy of non-melanoma. This study aims to develop a stable cannabidiol (CBD)-incorporated nanostructured lipid carrier (NLC) for treating the non-melanoma skin cancer. The CBD-loaded NLC was formulated adopting a melt emulsification technique, sonication and optimization utilizing a central composite design (CCD). The formulations which were developed were characterized on the basis of particle size, PDI, zeta potential, DSC, TEM, FTIR, entrapment efficiency (% EE), % drug release, and cytotoxicity via

MTT assay. Furthermore, stability tests were performed in order to assess the formulation's robustness over time.[13]

METHOD

Materials

The drug cannabidiol was procured from SCY Chemical, USA. Compritol-888 ATO, stearic acid, Tefose 63, Lauroglycol and Transcutol P were sourced from Gattefossé, Saint-Priest, France. Poloxamer-407 and Tween 20 were acquired from Quiligens and CDH, New Delhi, India.

Screening of excipients

Several essential, synthetic, and herbal oils, including Clove oil, Coconut oil, Peceol, Rosemary oil, Lauroglycol, Lavender oil, Peppermint oil, and Labrafil M, were assessed for the solubility of the drug CBD. An excessive quantity of CBD was mixed in 2 ml of oil in a 5 ml vial to measure saturated solubility. The obtained mixture was subjected to vortex for 5 minutes, and maintained in an isothermal shaker at a temperature of 25°C for 72 hours. Once equilibrium was reached, the solution was centrifuged at 3000 rpm for a duration of 15 minutes.[14] The supernatant obtained was diluted with methanol in a 10 ml test tube. The analysis of the samples was performed in triplicate using a UV spectrophotometer (Shimadzu 1800, Japan) at 270 nm at 25°C.

The solubility of CBD was determined in various solid lipids, like stearic acid, Tefose 63, Sedefose, Compritol-188 ATO, and Precirol ATO. For this, 5 mg of the drug was mixed with 250 mg of solid lipid in a 5 mL glass vial. It was then melted at 85°C in a water bath with continuous stirring, and the solubilization of the drug was visually assessed.[15] The choice of surfactants to be used was dependent on their capacity to emulsify the specified solid and liquid lipid binary combination. After dissolving a 100 mg binary lipid mixture in 3 mL of methylene chloride, 10 mL of a 5 % surfactant solution (w/v) was added. The mixture so obtained was further mixed using a magnetic stirrer and then heated to a temperature of 40°C to remove the methylene chloride. The percentage transmittance (%T) of 1 mL of the solubilized mixture was measured at 270 nm using UV spectroscopy. Various surfactants, including Transcutol P, Poloxamer 188, Poloxamer 407, Tween 80, and a combination of Poloxamer 407 and Tween 20, were analyzed for their effectiveness.[16]

Development of NLC Formulation

The CBD-loaded NLC formulation was developed by the melt emulsification method followed by sonication. A 1,5 % binary lipid blend was melted at 75°C using a hot plate magnetic stirrer, and the appropriate amount of drug was incorporated. For the aqueous phase, equal proportions of Poloxamer-107 and Tween 20 were dissolved in water while constant stirring at 1000 rpm for a duration of 20 minutes. The molten lipid phase was slowly mixed into the aqueous phase dropwise, with constant stirring at 1000 rpm and maintaining the temperature at 75°C for 2 hours. The resultant emulsion was sonicated for 2 minutes to produce nanosized CBD-NLCs, and it was then kept at room temperature for further usage.[17]

Experimental Design for the optimization of CBD-loaded NLC

The CBD-loaded NLC was optimized by a central composite design (CCD) using the software, Design Expert® (version 13). Following a preliminary screening of excipients, a structured experimental design was set up for formulation development. A 20-run, 3-factor, 3-level CCD was selected to reduce the total number of experimental trials while effectively optimizing three or four formulation variables.[18] This method facilitated the construction of a quadratic response surface and refinement of second order polynomial equations by incorporating repeated center points and midpoints along the edges of a multidimensional cube. The design enabled detailed evaluation of critical formulation parameters and their interactions, focusing on the influence of a binary lipid mixture (X1) consisting of Tefose 63 and Compritol-888 ATO (solid lipid), Peceol (liquid lipid), Poloxamer 407 (surfactant, X2), and effect of sonication time (X3) on the mean particle size (PS, Y1), polydispersity index (PDI, Y2), and entrapment efficiency (% EE, Y3) (table 1). Statistical measures, including the multiple correlation coefficient, adjusted R-squared, and the predicted residual sum of squares (PRESS), were employed to validate the polynomial models that captured the main effects and interactions of the formulation components. The statistical significance of the developed models was confirmed through analysis of variance (ANOVA) using the Design Expert® platform.[19]

Table 1. The independent variables including Binary mixture concentration and surfactant and dependent variables including Particle size, PDI, % Entrapment efficiency

Factors 7		Coded Levels	
Independent Variable	**Low (-1)**	**Medium (0)**	**High(+1)**
X1= Lipid (Binary Mixture) (%)	0,1	5	1
X2= Surfactant (%)	2	3	4

X3=Sonication time (min)	2	5	10
Dependent variables		Constraints	
Y1= PS (nm)		(<200)	
Y2= PDI		Minimum	
Y3= Average EE (%)		Maximum	

Characterization of NLC

Particle size, Polydispersity Index, and Zeta potential

The size, polydispersity index (PDI), and zeta potential was measured using the dynamic light scattering (DLS) at a temperature of 25 ± 1°C with a Malvern Instruments system (UK). Prior to measurement, the nanostructured lipid carrier (NLC) was diluted using Milli-Q water at a 1:100 ratio with to achieve uniform dispersion. The mean values for PS, ZP, and PDI were obtained by averaging results from three batches of the CBD Loaded NLC formulation.[20]

Transmission Electron Microscopy (TEM)

The final optimized CBD-loaded NLCs were diluted ten times with Milli-Q water, a tiny drop of the diluted formulation was applied onto a copper grid, then left at room temperature for drying. The sample which was dried was then stained with 1 % phosphotungstic acid to improve contrast. Transmission electron microscopy (TEM) images were acquired using a Tecnai G4 S-Thrin microscope (SEF, Netherlands) operating at 150 kV, with a 500 nm scale and capable of PTP resolution.[21]

Differential Scanning Calorimetry analysis

To evaluate the compatibility between drug and excipients, exactly 2 mg of CBD, and CBD- Loaded NLC samples was precisely weighed and taken into hermetically sealed aluminum pans for DSC analysis. The pans were sealed using a hydraulic press and heated at a rate of 10°C per minute across the temperature varying from 40 to 400°C. The analysis was conducted using a differential scanning calorimeter (DSC) manufactured by PerkinElmer Inc., USA.[22]

Encapsulation Efficiency

The % encapsulation efficiency of CBD in the CBD-loaded NLC was detected by the ultracentrifugation technique. A 30 mL portion of the formulation was placed into a 50 mL Eppendorf tube and subjected to centrifugation at a speed of 20 000 rpm at 4°C for a duration of 30 minutes. After centrifugation, the sample was vortexed, and the supernatant was taken to quantify the unencapsulated drug. The absorbance of the collected supernatant was detected at 270 nm (λmax) using UV spectroscopy (Shimadzu, Japan) for further analysis.[23]

$$\%\ \text{Entapment efficiency(EE)} = \frac{\text{Total drug} - \text{Free drug}}{\text{Total amount of drug}} \text{x } 100 \qquad \text{1 Equation}$$

$$\text{Loading Capacity (LC)} = \frac{\text{Total drug} - \text{free drug}}{\text{Nanoparticles weight}} \text{x } 100 \qquad \text{2 Equation}$$

In-vitro release

The cumulative release of drug from the final CBD-loaded NLC formulation was evaluated over a 24-hour period under sink conditions. For this study, 5 mL of the optimized formulation was taken into a dialysis bag (the molecular weight cut-off was12 kDa) which was pre-activated, with both ends securely sealed. Then it was immersed in a beaker with 900 mL of phosphate buffer (pH 7,4), at a temperature of 37 ± 0,5 °C and subjected to constant stirring at 100 rpm on a magnetic stirrer. The samples (5 mL) were withdrawn at fixed time intervals, and analyzed using the UV spectroscopy at 270 nm to detect the concentration of CBD. The same procedure was performed for the CBD suspension as a control.[24]

Ex-Vivo skin permeation

The study was carried out using the Franz diffusion apparatus, with Wistar rat skin positioned between the donor and receptor compartments. Fixed amount of phosphate buffer (pH 5,5) i.e. 10 mL was filled in the receptor compartment, while 1 g of CBD-loaded NLC gel was taken in the donor compartment above the skin. The setup was kept at a temperature of 37 ±1 **°C. Samples of** 0,5 mL were withdrawn at regular intervals ranging from 15 minutes to 8 hours, like the in-vitro study. After each sample collection, an equal volume i.e. 0,5 mL of fresh buffer was replenished in the receptor compartment. The collected samples were then

examined using UV spectroscopy at 270 nm.[25]

In vitro % Cytotoxicity assay (MTT assay)

The cytotoxic activity of CBD Loaded NLC was analyzed using the MTT assay on A375 Human skin cancer cells. The cells were seeded in a 96-well flat-bottom microtiter plate at a density of 1050 cells per well and then incubated for 36 hours to facilitate cell growth. After incubation, varying concentrations of CBD (1–5 µg/mL) were added, followed by a 72-hour incubation. Following the replacement of the medium with new medium, 20 µL of MTT solution i.e. 5 mg/mL in PBS was taken in each well. Further, the cells were incubated at 37°C for 4 hours, allowing viable cells to convert MTT into formazan crystals through the activity of mitochondrial dehydrogenase. After dissolving these crystals in DMSO, a Mark microplate reader (X Mark Bio-Rad) was used to measure their absorbance at 540 nm.[26]

RESULTS

Solubility study for selection of excipients

Various liquid and solid lipids were taken based on solubility and miscibility studies to understand the optimal solubility mixture of CBD. The solubility in solid lipids was as follows: Tefose 63 Compritol-188 ATO, Sedefose, Stearic acid, Precirol ATO, while in liquid lipids, it was Frankincense oil, Peceol, Rosemary oil. The final formulation components included Compritol-888 ATO combined with Tefose 63 (solid lipid) and Poloxamer-407 & Tween-20 (surfactant), as they exhibited the highest solubility for CBD. The binary mixture of liquid and solid lipids maintained a stable formulation at a 2:7 ratio without phase separation, allowing further development of the formulation.[27]

Preparation of CBD NLC by CCD approach

Polynomial models were constructed for the formulation optimization using 20 experimental runs and three level of central composite design, as indicated in table 2.

Table 2. Composition of 3 variables, 3 levels CCD for the CBD Loaded NLC formulation development

Run	A: Binary Mixture %(w/w)	B: Surfactant %(w/w)	C: Sonication Time(min)	Response 1 Particle Size	Response 2 PDI	Response 3 %EE
1	0,55	4,68179	6	163	0,456	69
2	1	2	10	298	0,578	74
3	0,1	4	2	165	0,561	61
4	0,55	3	-0,727171	278	0,356	80
5	0,55	1,31821	6	186	0,778	76
6	0,55	3	6	154,7	0,219	87
7	0,55	3	6	154,7	0,219	87
8	0,1	2	2	139	0,745	88
9	0,55	3	12,7272	294	0,671	76
10	1	4	2	338	0,456	63
11	0,1	4	10	146	0,378	75
12	1	4	10	289	0,478	89
13	0,55	3	6	154,7	0,219	87
14	-0,206807	3	6	180	0,49	68
15	1	2	2	278	0,445	58
16	0,55	3	6	154,7	0,219	87
17	0,55	3	6	154,7	0,219	87
18	0,55	3	6	154,7	0,219	87
19	1,30681	3	6	295	0,578	61
20	0,1	2	10	185	0,771	65

Effect of variables on the particle size (Y1)

Based on the experimental findings, the particle size varied between 150 and 300, as presented in table 2. The concentration of the binary mixture (Tefose 63 + Compritol-888 ATO) and Peceol had a major influence on particle size. The relationship between these factors and particle size can be explained using the equation given as follows: Particle Size (Y1) +154,95= 55,75A-0,0499B+1,82C+8,00AB-7,00AC-16,75BC+27,66A^2 +5,39B^2

+44,81C^2

Y1, Y2, and Y3 represent the primary outcomes observed when each variable is individually increased from its lower to upper level. Positive coefficients indicate a favorable impact on particle size, whereas negative coefficients suggest a reducing effect. figure 1(a) presents the evaluation of these coefficients within the previously described second-order polynomial model. Additionally, the Model-F value for the full quadratic model analyzing nanoparticle size was 17,59, signifying that both the linear response surface and the quadratic model were statistically significant, as detailed in table 3. The response surface analysis, as illustrated in figure 1(a), shows that the enhancement in the concentration of Poloxamer-188 results in a reduction of particle size.[2]

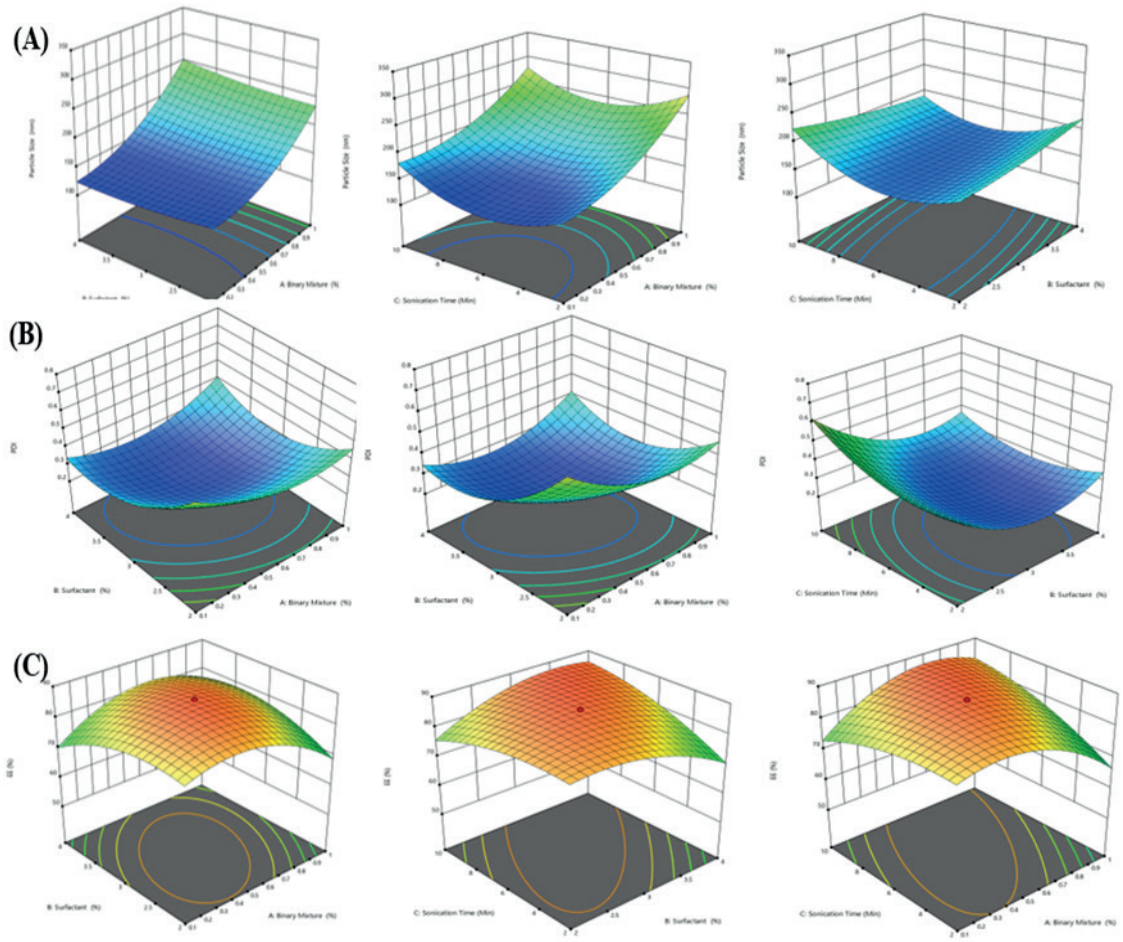

Figure 1. Response surface plots of (A) Particle Size, (B) Polydispersity Index, and (C) Encapsulation Efficiency

Effect of the variable on Poly Dispersity Index (Y2)

The experimental results indicated that particle size over a Poly Dispersity Index (PDI) range of 0,219 to 0,778, as shown in table 2. A lower PDI value was generally preferred, because it denotes a more uniform size distribution. The PDI was affected by the quantity of the Binary Mixture and the sonication time. The equation illustrates the association between these factors and the PDI.[28]

Poly Dispersity Index (Y2) +0,1740= -0,0950A+0,0945B-0,0054C+0,1340AB-0,2330AC-0,1125BC+0,0375A^2 +0,1784B^2 +0,1075C^2

The "Model F-value" of 12,69 indicates that the model is significant, as shown in table 3. The probability of obtaining a "Model F-value" this large by chance is only 0,0001 %. Figure 1(b) demonstrates how various variables impact the PDI. Increasing the amount of Compritol-888 ATO and sonication time lead to an increase in polydispersity. Conversely, the content of Poloxamer-188 had a notable effect in reducing the PDI.[29]

Table 3. ANOVA Quadratic model results

Response	F-value	P-value	Mean square	Adjusted R^2	Predicted R^2	Remarks
Particle size (Y1)	17,59	<0,0001	9160,03	0,8871	0,8421	Significant
PDI(Y2)	12,69	<0,0001	0,0744	0,8470	0,8284	Significant
EE (Y3)	10,48	<0,0001	224,61	0,8189	0,8234	Significant

The Effect on Encapsulation Efficiency (Y3)

The encapsulation efficiency will depict the preparation efficiency of the Compritol-888 ATO, Tefose 63, and Peceol formulation. As shown in table 3, the p-values for X1 (Binary Mixture), X2 (Poloxamer-188), and X3 (Sonication duration) were all below 0,0001 %, indicating that these factors had a notable effect on the encapsulation efficiency. An enhancement in the Binary Mixture concentration and a reduction in Poloxamer-188 concentration were found to reduce encapsulation efficiency, as represented in the following equation.[30]

Encapsulation Efficiency (Y3) +86,97= -1,23A-0,6424B+1,92C+4,62AB+6,37AC+5,87BC-7,77A^2 -4,94B^2 -2,99C^2

The "Model F-value" of 10,48 depicts that the model is significant, with a 0,2 % probability that such a large "Model F-value" could arise because of random noise. Figure 1(c) illustrates the impact of various variables on entrapment efficiency. Increase in the quantity of Binary Mixture and sonication time led to a reduction in entrapment efficiency. However, the surfactant content had a significant impact in enhancing the encapsulation efficiency.[31]

Particle size and Polydispersity Index

All the formulations ranged in size from 154 to 338 nm and PDI from 0,219 to 0,778 as depicted in table 2.[32] This optimized CBD Loaded NLC formulation showed particle size in a range of 154,7 nm with PDI of 0,219, as shown in figure 2 (A) and Zeta Potential is most important physical parameter for formulation stability more the zeta potential more will be the stability (-30 to +30) but due to nonionic charge of lipid zeta potential was found to be 30,12 mv as shown in figure 2B.[33]

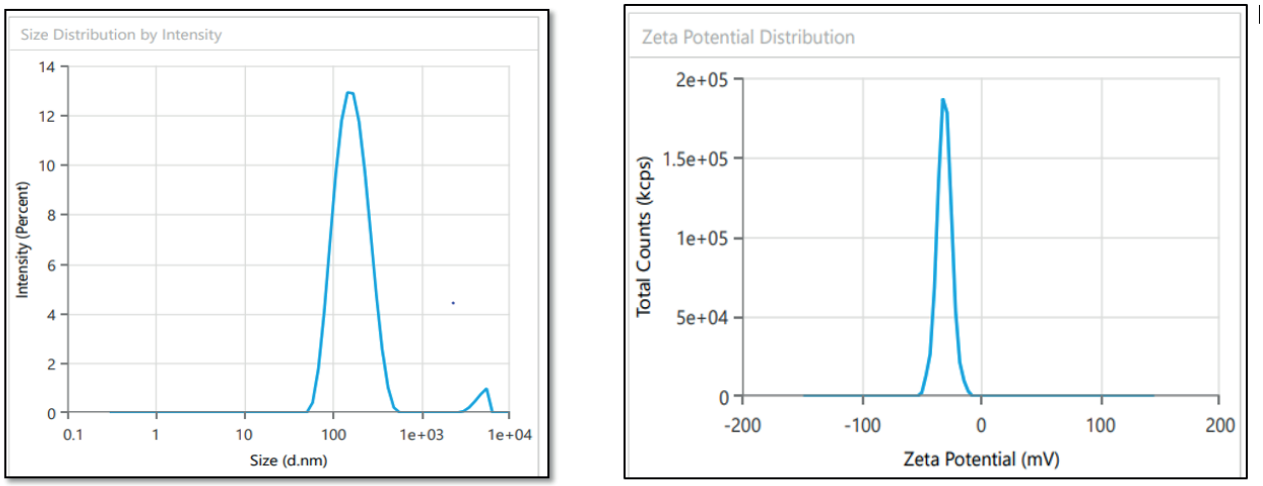

Figure 2. (A) Particle Size and (B) Zeta Potential of the optimized CBD Loaded NLC

Morphology study (TEM)

The technique used to study the morphology of CBD loaded NLC was TEM, revealing a nearly spherical shape with a diameter of approximately 150 nm, as shown in figure 3. The TEM findings were consistent with the results of the particle size analysis and zeta potential measurements.[34]

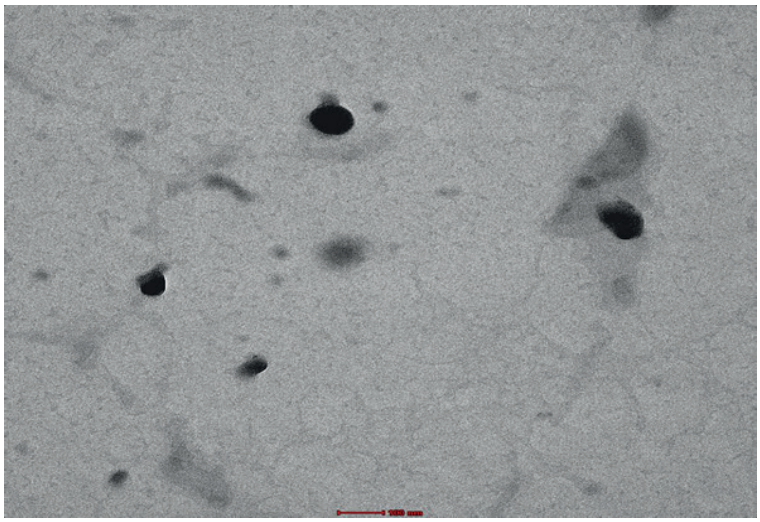

Figure 3. (TEM) CBD-NLC Formulation

Differential Scanning calorimetry analysis

The DSC thermogram of the formulations is shown in figure 4. A distinct endothermic peak for CBD was observed at 66 °C. The results suggest that there were no incompatibilities or drug-excipients interaction that could impact the formulation's stability or performance. However, in the NLC nanoformulation, no peaks corresponding to the excipients were detected, except for mannitol, which showed a peak at 166,108 °C. This indicates the disappearance of the CBD peak in the DSC thermogram of the optimized formulation.[35]

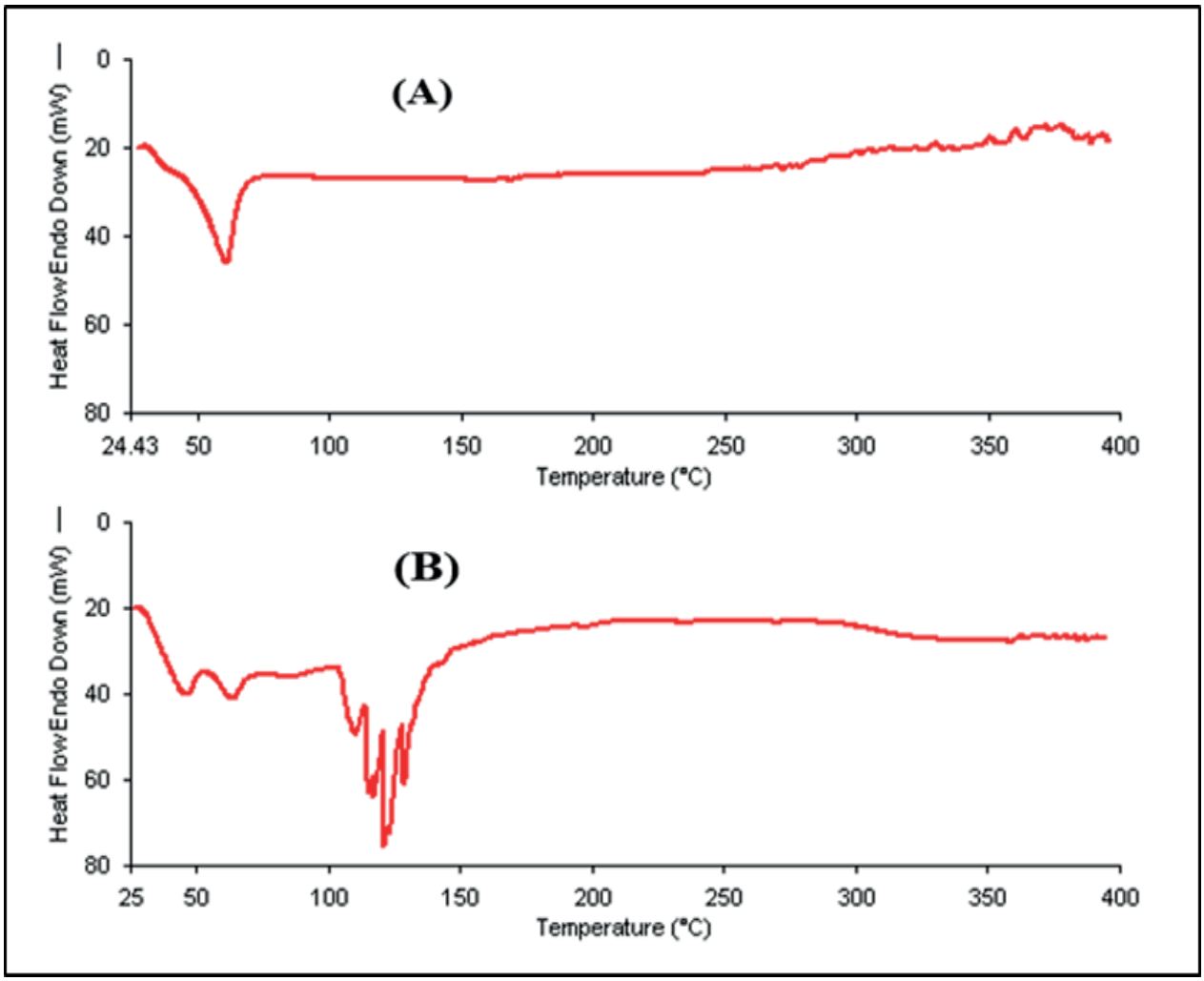

Figure 4. DSC Analysis CBD (A) Physical mixture of CBD and Excipients (B)

Drug loading and encapsulation

The loading of drug and percentage encapsulation efficiency (%EE) of CBD in the CBD-NLC formulation were determined to be 11 ± 0,67 % and 87 ± 0,67 %, respectively. These values indicate the amount of CBD effectively incorporated into the nanostructured lipid carrier (NLC) and the efficiency with which CBD was encapsulated within the formulation. The results suggest a high degree of CBD entrapment in the NLC, reflecting the capacity of the formulation to efficiently encapsulate the drug.[36]

In-vitro drug release

The drug release profile of the optimized CBD loaded NLC formulation, and its suspension is represented in (figure 5). The results demonstrated that, in the first 2 hours, 36,78 % of CBD was released from the CBD NLC, in contrast to only 12,20 % released from the suspension. By the end of 24 hours, 49,57 % of CBD was released from the CBD-loaded NLC, while 12,45 % CBD was released from the suspension. The drug was encapsulated within the lipid core is confirmed by an initial burst release of pharmaceuticals adsorbed on the surface of the CBD-loaded NLC, followed by a continuous release. This data was studied using different kinetic models: zero-order (R^2 = 0,7178), first-order (R^2 = 0,578), Higuchi square root (R^2 = 0,9823), and Korsmeyer–Peppas (R^2 = 0,8166). The Higuchi model, which showed the maximum R^2 value, provided the most optimum fit for the drug release kinetics of the optimized CBD-loaded NLC formulation.[37]

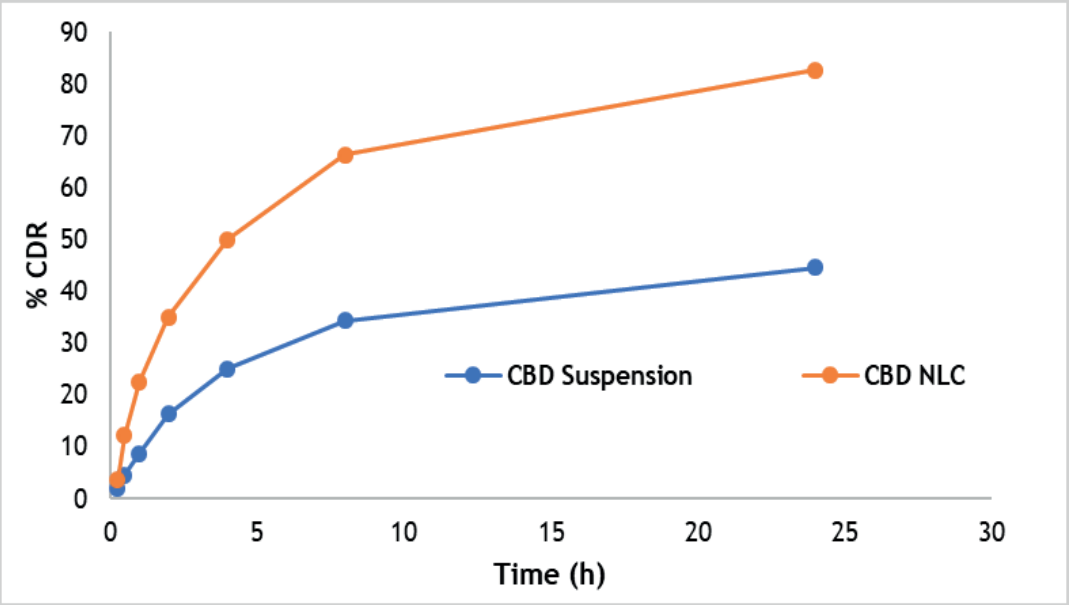

Figure 5. Drug release profile of CBD-Suspension and CBD- NLC formulations

Ex-vivo permeation study

The skin permeation study of the CBD-loaded nanostructured lipid carrier (NLC) gel demonstrated a significantly enhanced drug penetration profile in contrast to the CBD suspension. Specifically, the CBD-loaded NLC formulation exhibited a cumulative drug permeation of approximately 96 % over the study period, whereas the conventional CBD suspension showed only about 47 % permeation under the same conditions. This indicates that the CBD-loaded NLC achieved nearly a twofold increase in skin permeation efficiency.[38] The enhanced permeation can be due to the lipidic nature of the NLCs, which enhances skin penetration by interacting with the stratum corneum, as well as their occlusive properties that increase skin hydration and facilitate deeper drug absorption. These findings clearly highlight the superiority of the NLC-based formulation in promoting topical drug delivery of CBD (figure 6).[39]

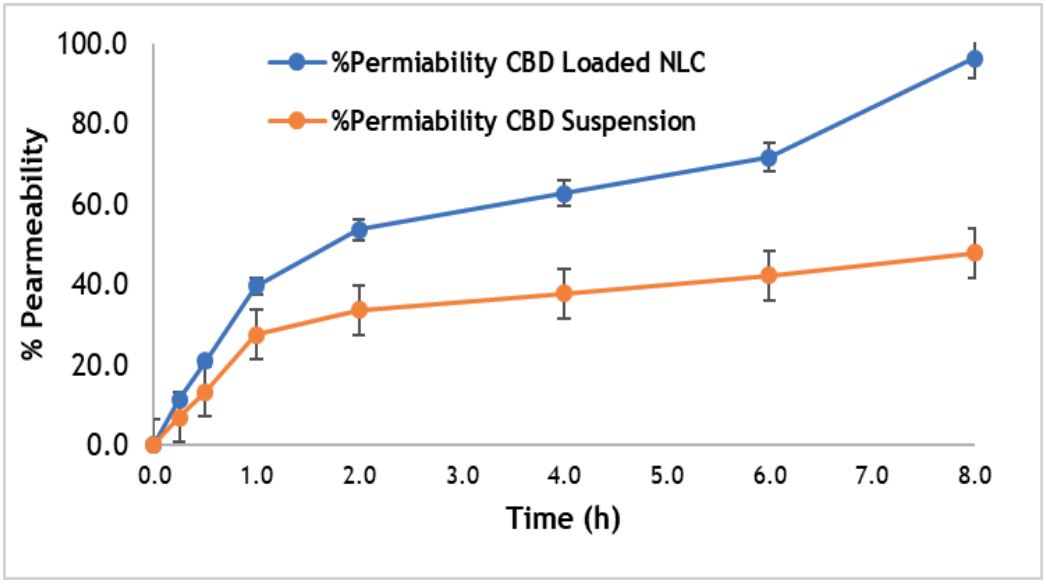

Figure 6. Ex-vivo permeation of CBD-loaded NLC gel

In vitro % cytotoxicity assay (MTT assay)

The results shown in figure 7 illustrate the investigation of cytotoxicity for CBD, and CBD Loaded NLC in A375 cells. The percentage of cytotoxicity was assessed using the standard MTT assay across various concentrations over 72 hours, with IC50 values also calculated. After 72 hours of incubation with A375 cells, the cell inhibition effect of CBD loaded NLC was significantly higher than that of CBD shown in figure. Specifically, the IC50 values for CBD loaded NLC, and were determined to be 6,12 µg/mL, 3,07 µg/mL, respectively, toward A375 cells.[40]

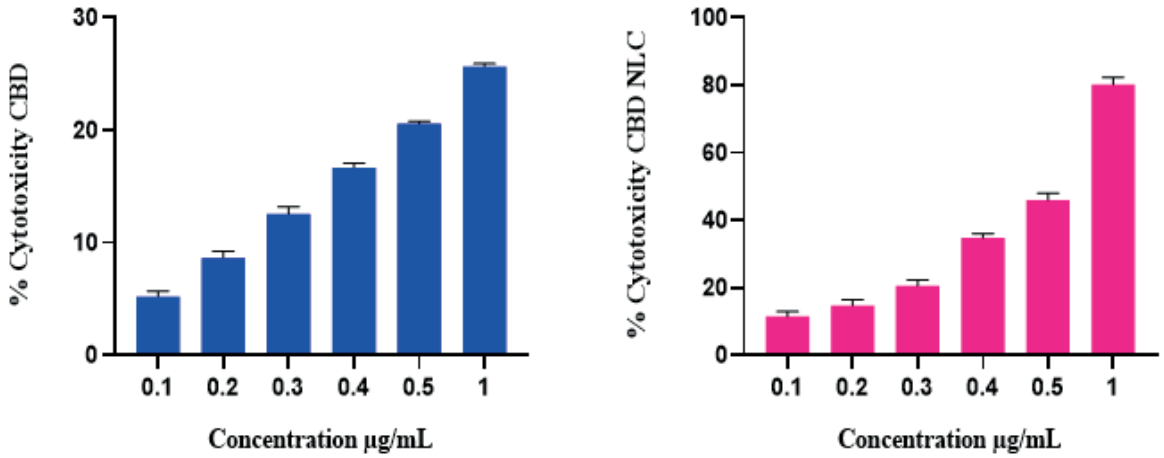

Figure 7. In vitro cytotoxicity of CBD & CBD Loaded NLC formulation

DISCUSSION

The current study intended to develop and optimize a CBD-loaded nanostructured lipid carrier (NLC) system utilizing a quality-by-design (QbD) strategy to improve solubility, stability, and therapeutic performance. The early solubility screening allowed for the reasonable selection of excipients by determining the lipid combinations that maximized CBD solubility and miscibility. Among the solid and liquid lipids evaluated, the binary combination of Compritol-888 ATO and Tefose 63, along with surfactants Poloxamer-407 and Tween-20, displayed excellent solubilization capacity and formulation stability, resulting in a homogenous lipid matrix appropriate for NLC production. The use of a Central Composite Design (CCD) allowed for systematic optimization by investigating the interacting effects of formulation factors on important quality parameters. The results showed that the concentration of the binary lipid mixture and Peceol had a strong influence on particle size, while Poloxamer-188 had a considerable size-reducing effect. The polynomial model developed for particle size was statistically significant, showing the effectiveness of the optimization technique. Similarly, PDI results showed that uniform particle distribution was mostly determined by the binary lipid content and sonication time, with higher surfactant concentrations enhancing homogeneity. In terms of encapsulation efficiency, the model demonstrated that high binary lipid content lowered entrapment due to structural packing restrictions, whereas surfactant content increased drug inclusion. The optimized NLCs had a particle size of 154,7 nm and a PDI of 0,219, indicating homogeneous nanoscale dispersion. Although the zeta potential was 30,12 mV, the non-ionic character of the chosen lipids contributed to steric rather than electrostatic stabilization, which was adequate to maintain colloidal stability. TEM investigation validated the nano-spherical form, which is consistent with the dynamic light scattering data. DSC analysis demonstrated the elimination of CBD's distinctive melting peak in the formulation, indicating effective amorphous dispersion inside the lipid core and the absence of excipient-drug incompatibility. The modified lipid matrix effectively entrapped CBD, with encapsulation efficiency of 87 ± 0,67 % and drug loading of 11 ± 0,67 %. The in-vitro release research revealed a biphasic profile with an initial burst followed by persistent release, which best fits the Higuchi kinetic model and confirms diffusion-controlled release. The ex-vivo permeation investigation revealed considerably improved cutaneous diffusion of CBD from the NLC gel when compared to the solution, which was attributable to lipid-skin affinity and increased occlusivity. Finally, MTT assay results showed that CBD-NLC had significantly higher cytotoxic activity against A375 cells than pure CBD, indicating improved cellular uptake and therapeutic efficacy.

Overall, the data demonstrate that the optimized CBD-NLC formulation has greater solubility, controlled release, enhanced penetration, and anticancer potential, indicating its suitability for effective topical drug delivery.

CONCLUSIONS

This study demonstrates the successful development, optimization, and comprehensive assessment of cannabidiol (CBD)-incorporated nanostructured lipid carriers (NLCs) intended for the topical therapy of non-melanoma skin cancer. Through a structured formulation and optimization process, the effect of the formulation parameters on the critical quality attributes of the CBD-loaded NLCs—namely particle size, polydispersity index (PDI), and % entrapment efficiency was thoroughly explored. The optimized CBD-NLC formulation indicated desirable physicochemical properties, including an appropriate nanoscale particle size, narrow PDI, and high drug entrapment efficiency, all of which are essential for effective skin delivery. The drug release studies depicted that the optimized CBD-NLC formulation provided a sustained release of CBD over a duration of 24-hours, indicating its potential for prolonged therapeutic action. Moreover, skin penetration studies performed using

Franz diffusion apparatus across excised Wistar rat skin confirmed a two-fold enhancement in CBD penetration from the optimized NLC formulation when compared to a conventional CBD suspension. This suggests improved bioavailability and skin retention of the active compound. Cytotoxicity studies performed on A375 human melanoma cells demonstrated that the optimized CBD-loaded NLCs retained comparable anticancer activity to pure CBD, indicating that the formulation process did not compromise the therapeutic efficacy of the drug. Furthermore, stability studies showed that the optimized NLC formulation remained physically and chemically stable for a duration of at least three months when stored at room temperature, highlighting its potential for practical application and commercial viability.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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