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Development and Ev Aluation of Lns Loded Topical Ointment of Amoroifine by QBD Approach

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A B S T R A C T
This research work investigates the dissolvability of tetrahydrocannabinol in different excipients and its formulation into lipid nanoparticles (LNs) for enhanced drug delivery. THC showed the highest solubility in olive oil, but solid lipid nanoparticles (SLNs) were formulated using Tween 80 as the surfactant for improved solubilization and stabilization. A pseudoternary phase diagram optimized excipient concentrations, ensuring stable formulations. DLS demonstrated optimalsize of particle& PDI for effective skin penetration, while zeta potential analysis indicated stability through steric and reduced electrostatic stabilization. High-resolution transmission electron microscopy (HRTEM) and nanoparticle tracking analysis (NTA) confirmed uniform, consistent particle sizes. Spectroscopic analyses (DSC ,FTIR) &XRD validated the amorphous nature of THC within the formulations. Central Composite Design (CCD) optimization yielded a reliable model, showing strong potential for improving THC bioavailability in therapeutic applications.
Keywords: Tween 80, X-ray diffraction, Central Composite Design

A R T I C L E I N F O

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CONTENTS

1. Introduction

Solubility determination in solid lipids

Solubility of the drug in melted lipid indicates amount of the drug which gets incorporated into the lipid. Solubility estimation was carried out at elevated temperatures (at10°Chigherthelipid melting point) in Compritol® 888ATO, stearic acid, glyceryl monostearate (GMS) and cetylalcohol. Drug(30mg)was taken in a 10 ml beaker and lipid meltwas subsequently heated above its melting temperature. This lipid melt was added steadily in portions to the drug with stirring continuously. The end point was observed visually as indicated by formation of a clear solution of motlen lipid. The amount of lipid required for complete solubilisation of drug was then recorded.

Analysis Particle size &PDI

Size of Particle & PDI of THC-LNs & blank-LNs dispersion were quantify with specific dilutions with triple distilled H2O (20x) using Zetasizer Nano ZSP DLS, Malvern Panalytical, UK). Disposable polystyrene cells (10

mm diameter) were employed to measure hydrodynamic diameter (-average) and PDI of formulated nano-dispersions (Danaei et al., 2018). The lyophilized samples were also characterized for particle size, PDI and zeta potential. Reconstituted the lyophilized sample with 1 ml of water and sonicated using bath sonicator for 30 min. Then, the sample was used for ointment preparation.

2. Methodology

Potential of Zeta

It was identified by using Zetasizer Nano ZSP DLS, Malvern Panalytical, UK). The surface charge of THC-LNs and blank-LNs was analysed using a special zeta potential cell (DTS1060) obtained from Malvern, UK.

Nanoparticle tracking analysis (NTA)

Nanoparticle tracking analysis (NTA) is a technique that allows for the visualization and analysis of nanoparticles within a liquid which depends on the rate of Brownian movement related to the particle size.

TDC &EE

TDC is the total amount of drug present in the formulation. For estimation of TDC, 1 ml of the sample was added, followed by the sonication for 1 h. The later remaining volume was made up by the addition of THF and sonication was done for another 10 min. 1 ml of supernatant was diluted to 9 ml THF &sonicated for 10 min. Furthermore, 1 ml of supernatant was taken and diluted to 4 ml of mobile phase (THF:Citric acid) and, then injected into HPLC to determine the assay. The assay (expressed in mgg-1) of THC in the lipidic nanoparticles and ointment was calculated by using equation 19 as shown below.

$$\text{Assay (mgg-1)} = AT/AS \times Ws/100 \times 100/WT \times P/100$$

EE (%)Entrapment efficiency (EE%) is defined as the percentage of THC in lipidic nanoparticles, calculated by filtering un-entrapped THC using Amicon filters, centrifuging, and analyzing with HPLC.

$$EE\% = Wa\text{-}Ws/Wa \times 100$$

Fourier transform infrared spectrophotometer (FTIR)

Physicochemical interactions and drug entrapment within hybrid nanoparticles were analyzed by FTIR, comparing spectra of THC, THC-LNs, and other components in the range of 4000-400 cm^{-1}.

XRD

X-ray diffraction (XRD) analyzed the crystallographic structure and chemical composition of THC-LNs, Compritol® 888ATO, THC, and THC-Amoro ointment using an XPERT-PRO diffractometer at 45 kV, 40 mA, and 25°C across 2Θ angles of 5-50°.

NMR:

1H-NMR measurements were conducted at 300 MHz and 25°C using Bruker Fourier 300, with THC and Compritol® 888ATO in CDCl3, tween 80 and phospholipon 90G in deuterated methanol, LNs dispersions in deuterated water, and spectra processed with MestReNova software.

SEM:

In order to have better conceptualization and visualization, surface characteristics of THC-LNs loaded amorolfine ointment, blank ointment, freeze dried THC-LNs and blank-LNs were examined by means of a scanning electron microscopy (JEOL, 6000 plus Tokyo, Japan). The formulations were coated with gold under vacuum using accelerating voltage of 5kV, following which the images were captured using software (ImageJ).

Determination of pH

A 2.5 g ointment sample was dissolved in 50 mL water, heated at 60-70°C for 10 min, cooled, centrifuged at 3000 rpm for 10 min, and the pH was measured using a pH meter.

Spreadability

Spreadability was determined using a modified parallel plate method, where a 500 mg sample was pressed with known weights at intervals, and the spread areas were measured and plotted to obtain spreading profiles and calculate the spreadability factor (Sf). Optimization of THC-LNs using statistical experimental design to optimize the concentrations of Tween 80 (A) and Compritol® 888 ATO (B), a CCD with 13 runs was selected, as detailed in the experimental run matrix for THC-LNs optimization.

Formulation of THC-LNs &its ointment with Amorolfine: THC-LNs were prepared using modified methods &keep at room temp, then refrigerated for further analysis. PC was added as a co-surfactant to enhance drug permeation in the skin, disrupting the stratum corneum lipid composition and increasing fluidity, which improved percutaneous penetration of the water-insoluble drug THC. Tween 80 was used with phospholipids to solubilize THC and enhance skin uptake of lipidic nanoparticles, adhering to FDA IIG limits.

Compritol® 888 ATO was used in the formulation, known for its polymorphic transition from less stable to more stable forms after crystallization. During nanoparticle formulation, surfactants help prevent this transition, reducing the risk of aggregation, particle size increase, and drug expulsion by stabilizing the lipid structure.

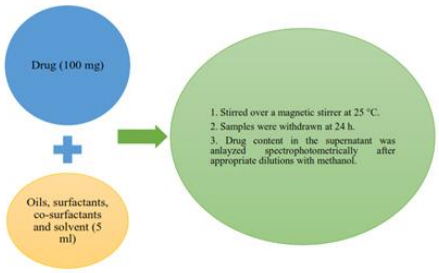

Figure 1: Procedure for solubility determination

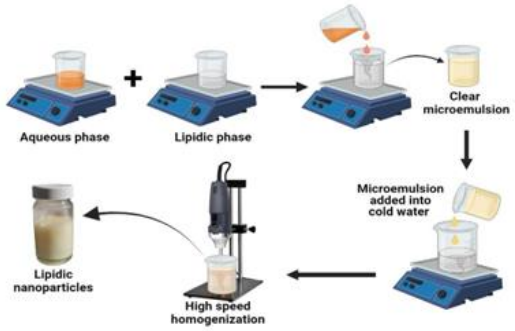

Figure 2: Preparation of THC-LNs by micro emulsification followed by high speed homogenization

Making of THC-LNs loaded Amorolfine ointment

THC-LNs loaded Amorolfine ointment was prepared by melting beeswax, paraffin, petrolatum, zinc oxide, and EDTA at 70°C, adding lyophilized THC-LNs and cooling to 40°C before adding Amorolfine and stirring to form a homogeneous ointment.

3. Results and Discussion

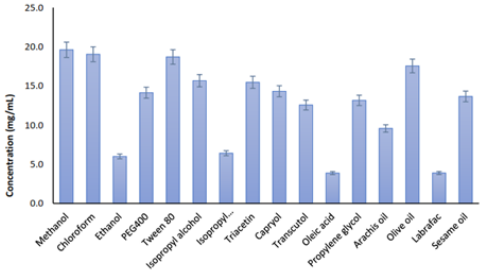

Figure 3: Solubility studies in different liquid lipids, surfactants and solvents.

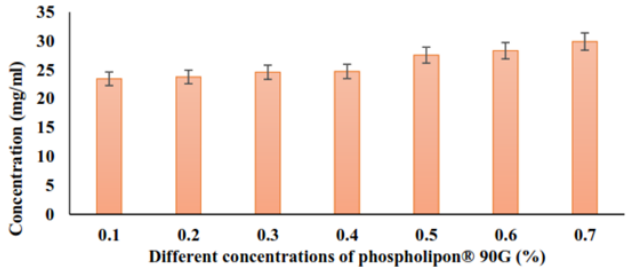

Figure 4: Solubility studies indifferent concentrations of Phospholipon®90 G.

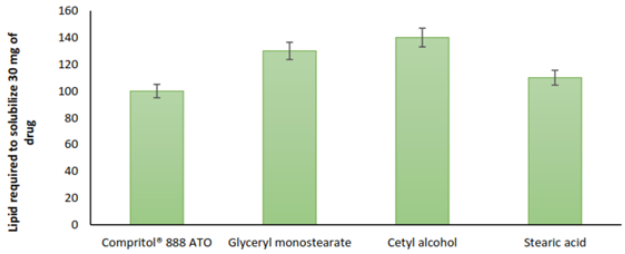

Figure 5: Solubility solid lipids studies in different solidlipid

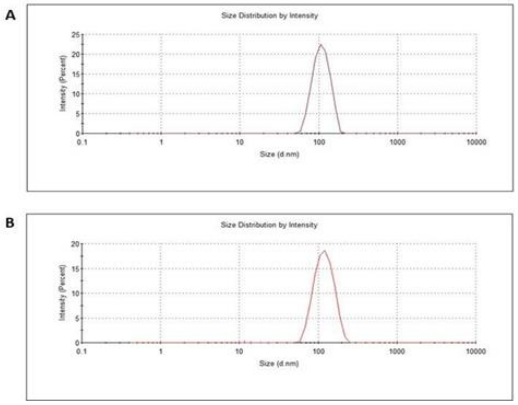

Figure 6: Particle size distribution of THC-LNs and blank-LNs.

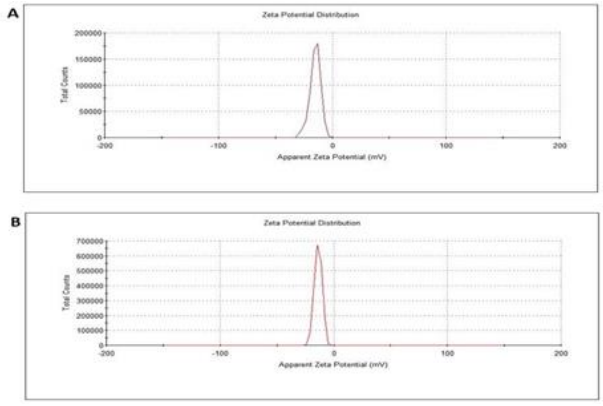

Figure 7: Zetapotential of THC-LNs and blank-LNs.

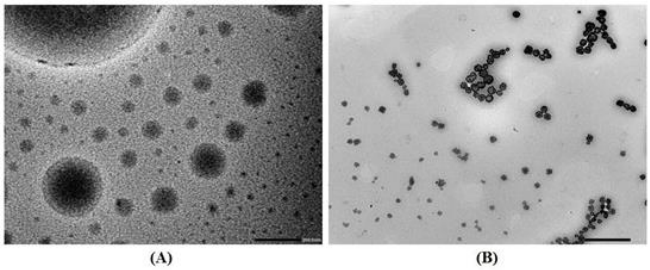

Figure 8: HRTEM images of prepared (A) THC-LNs (B) THC-Amoro ointment

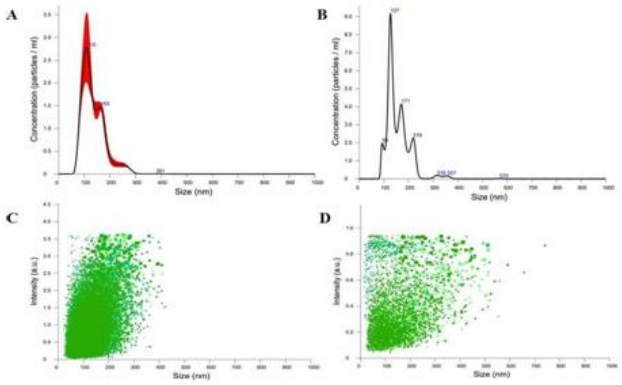

Figure 9: Nanoparticletrackinganalysis (NTA)ofTHC-LNs (A)andblank-LNs (B) the averaged Concentration vs. Size plot and THC-LNs (C) and blank-LNs (D) Intensity vs. Size graph

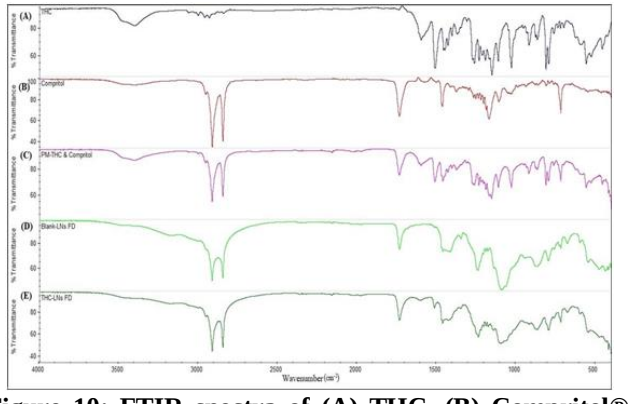

Figure 10: FTIR spectra of (A) THC, (B) Compritol® 888 ATO, (C) physical mixture (THC and Compritol® 888 ATO), (D) blank-LNs FD and (E) THC-LNs FD.

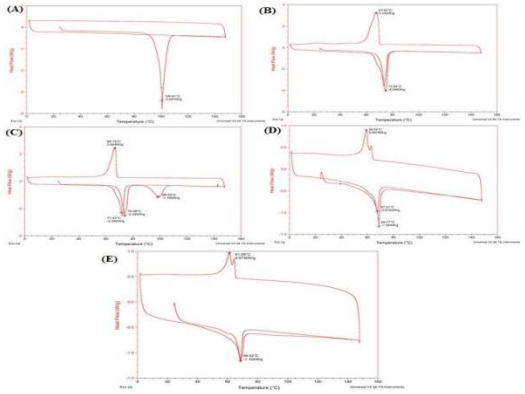

Figure 11: DSC thermogram of (A) THC (B) Compritol® 888 ATO (C) physical mixture of THC and Compritol® 888 ATO (D) blank-LNs (E) THC- LNs

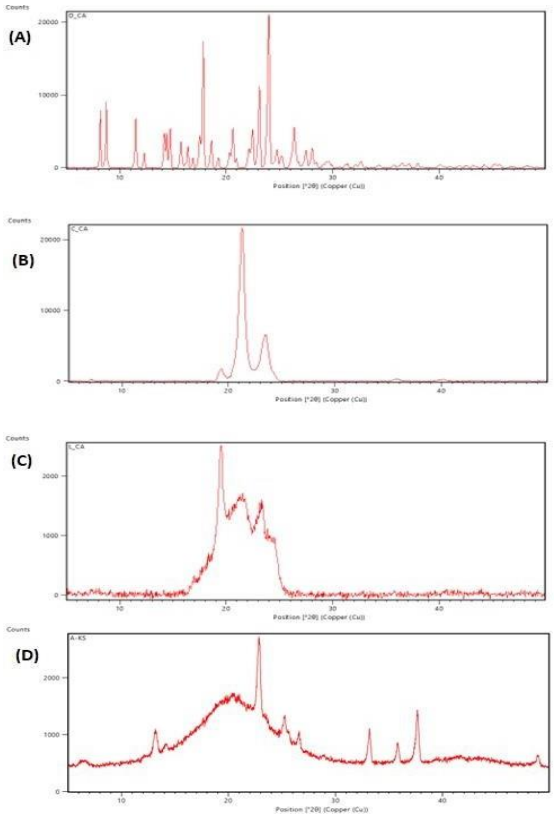

Figure 12: Diffractograms of (A) Pure THC (B) Compritol® 888 ATO (C) THC- LNs (D) TTO

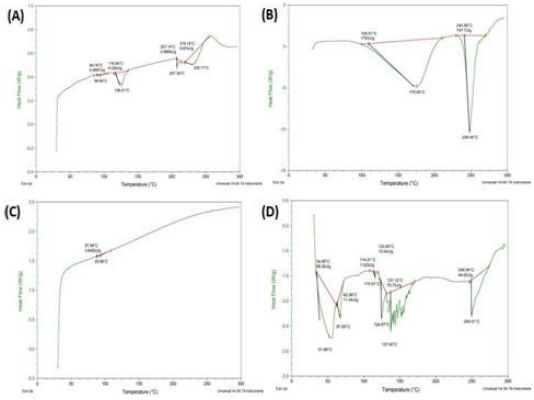

Figure 13: DSC thermogram of (A) amorolfine (B) EDTA (C) ZnO (D) THC- Amoro ointment.

SEM: SEM analysis uses a focused beam of high-energy electrons to study solid and semi-solid specimens, revealing details about surface morphology, chemical composition, and crystalline structure. The SEM images that THC-LNs loaded amorolfine ointment had a porous, flaky texture with some compact points (Figure 33A), while the blank ointment had a smooth, irregular surface (Figure 33B). Both THC-LNs and blank-LNs, lyophilized with trehaloseas a cryoprotectant, displayed similar spherical structures embedded in trehalose (Figure 33C and D). This observation aligns with previous studies on the effects of cryoprotectants in nanoparticle formulations.

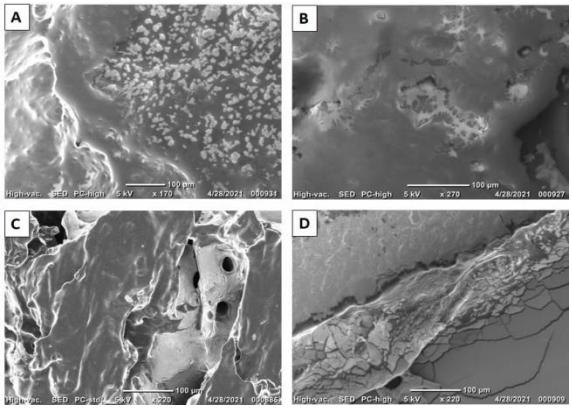

Figure 14: SEM

Spreadability

Spreadability measures a semisolid formulation's ability to be applied over the skin, influenced by its structural and viscoelastic properties. THC-Amoro ointment had slightly lower spreadability compared to blank-LNs ointment due to the concentrated form of lyophilized THC, which increased stiffness. Spreadability is categorized as low, moderate, or high and is inversely related to the ointment base concentration—the thicker the base, the lower the spreadability. The THC-Amoro ointment had a spread diameter of 1.8 cm, compared to 2 cm for the blank-LNs ointment. While the blank-LNs formulation spread more easily, the reduced spreadability of THC-Amoro ointment can be beneficial for targeting specific affected areas.

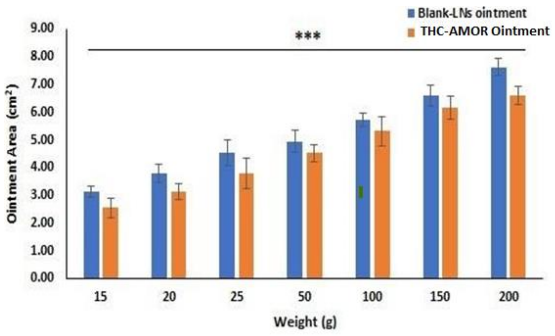

Figure 15: Spreadability

Optimization data analysis & validation

Thirteen experimental runs analyzed the effects of two formulation variables on particle size, %EE, and total drug content, showing variations from 174-201 nm, 72-88%, and 61-101%, respectively.

The optimal conc. of lipid & surfactant for the formulation of lipidic nanoparticles were determined based on minimizing particle size, maximizing drug content, and achieving the highest entrapment efficiency (%EE). Compritol® 888 ATO was selected for its superior drug entrapment ability due to its complex structure and interchain intercalation capacity, though its high melting point could affect particle size and PDI. Tween 80 was optimized for its role in stabilizing THC-LNs by forming a protective layer around nanoparticles, preventing aggregation, and maintaining integrity during storage.

Model equations were generated to analyze the effects of Tween 80 (A) and Compritol® 888 ATO (B) on particle size (Y1), %EE (Y2), and total drug content (Y3). Significant findings included the observation that Tween 80 concentration significantly impacts particle size, while Compritol® 888 ATO influences %EE. Increasing Tween 80 concentration initially increases particle size and %EE, but excessive concentrations lead to larger particles and reduced %EE due to micelle formation and distorted drug partitioning.

The optimized THC-loaded LNs formulation, containing 16% Tween 80 and 7.5% Compritol® 888 ATO, exhibited a particle size of 172.9 nm, %EE of 86%, and total drug content of 95%. The formulation showed high desirability based on set criteria.

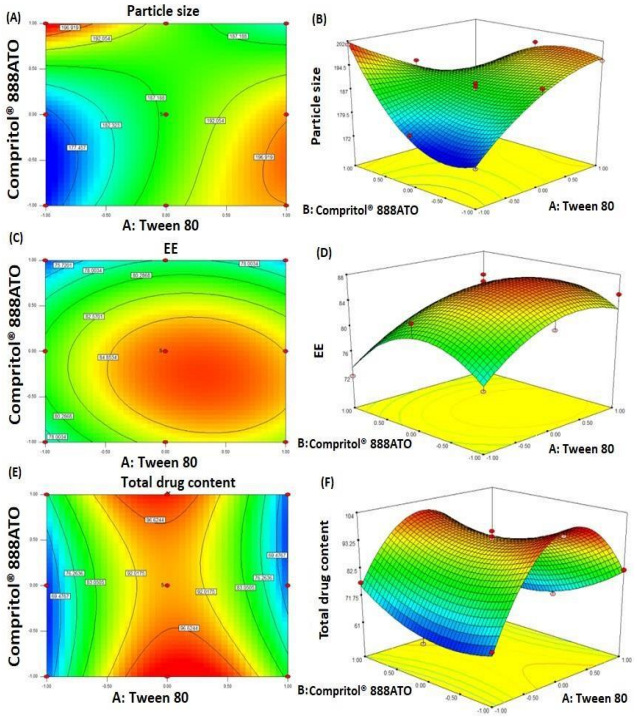

Figure 16. 2D contour plots and 3D response surface plots portraying the effect of tween 80 content and Compritol® 888 ATO on (A-B) particle size, (C-D) %EE and (E-F) total drug content.

Table : 1 Design matrix summarizing the levels of 13runs of CCD for optimization of THC loaded LNs.

Std	Runs	Factor A	Factor B
8	1	0.00	1.00
7	2	0.00	-1.00
10	3	0.00	0.00
12	4	0.00	0.00
2	5	1.00	-1.00
13	6	0.00	0.00
5	7	-1.00	0.00
1	8	-1.00	-1.00
9	9	0.00	0.00
4	10	1.00	1.00
6	11	1.00	0.00
3	12	-1.00	1.00
11	13	0.00	0.00

Table : 2 Differential scanning calorimetry (DSC) data

Composition	Temperature(°C)	Heat
THC	100.61	-3.407W/g
Compritol®888ATO	67.63	3.245W/g
	74.84	-4.046W/g
Physical mixture	66.75	2.448W/g
	71.42	-2.292W/g
	98.59	-2.535W/g
	-	-1.160W/g

Blank-LNs	59.04	0.8978J/g
	67.51	-0.9763J/g
	68.77	-1.324J/g
THC-LNs	61.06	0.9730J/g
	68.52	-1.164J/g
ZnO	93.86	-
	87.99	0.8482J/g
EDTA	109.51	1793J/g
	175.83	-
	240.88	747.7W/g
	248.45	-
AMOROLFINE	89.76	0.4897J/g
	94.84	-
	116.94	6.226J/g
	126.01	-
	207.14	0.9889J/g
	207.36	-
	219.18	8.874J/g
	235.11	-
THC-Amoroointment	34.45	98.36J/g
	51.96	-
	62.99	11.44J/g
	67.28	-
	114.21	1.529J/g
	115.91	-
	123.85	15.64J/g
	124.67	-
	137.12	75.70J/g
	137.40	-
	248.94	44.63J/g
	249.51	-

Table : 3 Experimental runs as per CCD for optimization of THC loaded LNs.

Std	Runs	A:Tween 80 (%w/w)	B: Compritol® 888ATO (%w/w)	Y1: Particle size(nm)	Y2:%EE	Y3:Total drug content(%)
8	1	16	10	192	78	98
7	2	16	5	192	82	101
10	3	16	7.50	187	86	95
12	4	16	7.50	185	87	95
2	5	20	5	196	85	82
13	6	16	7.50	188	86	97
5	7	12	7.50	178	83	61
1	8	12	5	174	76	67
9	9	16	7.50	187	87	94
4	10	20	10	182	77	66
6	11	20	7.50	198	81	65
3	12	12	10	201	72	77
11	13	16	7.50	189	88	94

Table 4: Optimum solutions obtained using numerical optimization with different values of desirability function

Formulations	Factor A (Tween 80)	Factor B (Compritol® 888 ATO)	Particle size	%EE	Total drug content	Desirability function value
F1	16	7.50	172.9	86	95	0.833
F2	17.5	6.90	179.5	89.5	94	0.901
F3	17	7.20	166.4	90	96.6	0.998

4. Conclusion

The study developed and characterized THC-loaded lipid nanoparticles (THC-LNs) and blank lipid nanoparticles (blank-LNs), focusing on particle size, PDI ,& other physicochemical properties. Dynamic Light Scattering (DLS) revealed that THC-LNs had a particle size of 166.4 nm with a PDI of 0.247, while blank-LNs were 143.2 nm with a PDI of 0.294, attributed to the specific choice of lipid and surfactant for enhanced skin uptake. Zeta potential measurements showed negative charges of -15.0 mV for THC-LNs and -13.7 mV for blank-LNs, indicating stability through steric stabilization. Nanoparticle Tracking Analysis (NTA) confirmed the poly disperse nature of the nanoparticles, with modal sizes of 109.4 nm for THC-LNs and 126.5 nm for blank-LNs. THC-LNs exhibited a high total drug content (96.6%) and entrapment efficiency (90.0%), highlighting the effectiveness of the microemulsion method used. FTIR and DSC analyses provided insights into the functional groups, molecular conjugation, and thermal behavior of the materials. X-Ray Diffraction (XRD) showed the disappearance of crystalline peaks after incorporation into lipid nanoparticles, suggesting an amorphous structure. Scanning Electron Microscopy (SEM) revealed the surface morphology differences between THC-LNs and blank-LNs ointments. The pH of the THC-Amoro ointment was 6.58, safe for skin application, with lower spreadability compared to blank-LNs, beneficial for targeted application. The study optimized formulation variables, achieving a particle size of 172.9 nm, 86% entrapment efficiency, and 95% total drug content. The model was validated, showing close alignment between observed and predicted outcomes.

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