# FORMULATION AND EVALUATION OF CANNABIDIOL SOLID LIPID NANOPARTICLE

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**ABSTRACT**

In the present study Cannabidiol loaded solid lipid nanoparticles were prepared. Cannabidiol -loaded Solid lipid nanoparticles were prepared by employing a hot homogenization method. All the formulations were evaluated for drug content, entrapment efficiency and drug release studies, particle size and zeta potential. Out of all formulations, the best formulations were found to be F8 with drug content was found to be 83.1%, entrapment efficiency was 98.8% and drug release was 98% within 12h, particles size 286-386 nm and zeta potential -29.4 mv. Further trials were done for application of factorial design and optimization of formulation. 3 level 2 factor factorial design was applied for formulation optimization. Precirol ATO 5 and Soya lecithin was selected as independent variables. % Drug release at 1 hour, 4 hours and particle size were selected as dependent variables. All the batches C1-C9 found satisfactory with the results. The design was validated using DoE software. The Optimize batch was taken from the design and evaluated. Optimized formulation was charged in stability for 1 month at accelerated condition and found stable. Hence, O1 batch was considered as optimized batch.

**Key words: Cannabidiol, Precirol ATO 5 and Soya lecithin**

## 1. INTRODUCTION

### Introduction of Drug Delivery System[1]

### Introduction of Nanotechnology

Nanotechnology is the science of the small; the very small. It is the use and manipulation of matter on a tiny scale. At this size, atoms and molecules work differently, and supply a variety of surprising and interesting uses. Nanotechnology and Nanoscience studies have emerged rapidly during the past years in a broad range of products.

The poor solubility and slow dissolution rate of many drugs are major industrial problems, especially for pharmaceutical scientists involved in drug discovery and drug development. It has been reported that about 40% of the compounds being developed by the pharmaceutical industry are poorly water soluble or “insoluble” in water. Poor water solubility is the major hurdle that needs to be resolved in order to formulate a successful drug delivery system, which not only delivers the drug to systemic circulation but also produces enhanced therapeutic outcomes.

In recent years, there has been an exponential interest in the development of novel drug delivery systems using nanoparticles. Nanoparticles can offer significant advantages over the conventional drug delivery in terms of high stability, high specificity, high drug carrying capacity, ability for controlled release, possibility to use in different route of administration and the capability to deliver both hydrophilic and hydrophobic drug molecules.

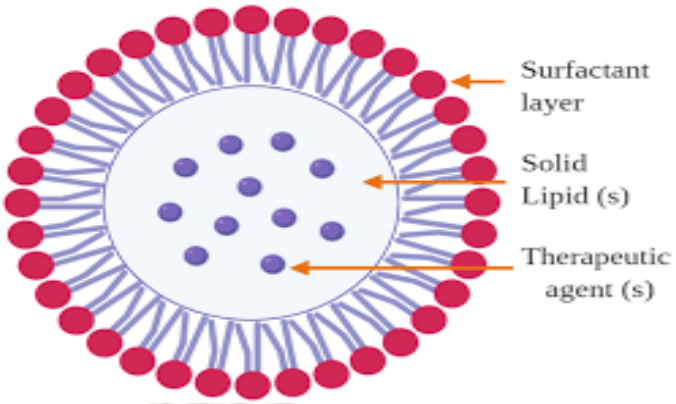


**Figure 1. structure of solid lipid nanoparticle**

Solid lipid nanoparticles (SLNs) are considered to be the most effective lipid based colloidal carriers, introduced in early nineties. This is the one of the most popular approaches to improve the oral bioavailability of the poorly water-soluble drugs. SLNs are in the submicron size range of 50-1000 nm and are composed of physiologically tolerated lipid components which are in solid state at room temperature.SLNs combine all the advantages of polymeric nanoparticles, fat emulsions and liposomes.

## ADVANTAGES OF SOLID LIPID NANO PARTICLE[1-2]

Increased efficacy and therapeutic index.

Increased stability via encapsulation.

Improved pharmacokinetic effect.

Producible with various sizes, compound surface.

Entrap both hydrophilic & lipophilic drug Protect entrapped drug from enzymatic degradation.

Large variety of drugs (antineoplastic, antibiotic) peptides or protein (including antibodies) &viruses &bacteria can be incorporated into nanoparticles.

Water soluble drugs are trapped in aqueous compartment & lipophilic drugs without the need for chemical modification.

Nanoparticles encapsulated drugs are delivered intact to various tissue and cells and can be released when nanoparticles are destroyed, enabling site specific and targeted drug delivery

## DISADVANTAGES OF SOLID LIPID NANO PARTICLES[1-2]

Include their tendency to be taken up by cells of Retic endothelial system and the slow release of the drug when the liposomes are taken up by phagocytes through endocytosis, fusion, surface adsorption or lipid exchange.

Stabilizing the formulated liposomes is also difficult, but many approaches are now used for their stabilization.

## AIM OF SOLID LIPID NANONPARTICLES[8]

- ✓ More moderate (less costly than polymeric/surfactant-based transporters).
- ✓ Incorporation of lipophilic and hydrophilic medications attainable
- ✓ Avoidance of natural solvents.
- ✓ Problems concerning substantial scale generation and sanitization
- ✓ Increased sedate security.
- ✓ No biotoxicity of the transporter in light of the fact that, most lipids are biodegradable

## METHODS OF SOLID LIPID NANO PARTICLE[9]

A. High pressure homogenization

- Hot homogenization
- Cold homogenization

B. Ultrasonication/high speed homogenization

- Probe ultrasonication
- Bath ultrasonication
- Solvent evaporation method
- Solvent emulsification-diffusion method
- Supercritical fluid method
- Microemulsion based method
- Spray drying method
- Double emulsion method
- Precipitation technique
- Film-ultrasound dispersion

## 2. MATERIALS AND METHODS:

### 2.1 List of materials

| Sr. No. | Material | Role | Sources of Material |
|---|---|---|---|
| 1. | Cannabidiol | API | Ture Green, Jabalpur |
| 2. | Compritol | Lipid | ACS Chemicals, Ahmedabad. |
| 3 | Soya lecithin | Surfactant | ACS Chemicals, Ahmedabad. |
| 4 | Tween-80 | Surfactant | ACS Chemicals, Ahmedabad. |
| 5 | PEG-400 | Surfactant | ACS Chemicals, Ahmedabad. |

### 2.2 List of Equipments

| Sr. No. | Equipments | Manufacturers |
|---|---|---|
| 1. | Digital weighing balance | Reptech weighing balance ltd., Ahmadabad |
| 2. | Overhead Stirrer | Janki Impex Pvt. Ltd, Ahmedabad |
| 3. | Dissolution apparatus | Electro lab ltd, Mumbai |
| 4. | U.V. Visible spectrophotometer | Shimadzu-1601, Kroyoto, Japan. |
| 5. | FTIR | FTIR 8400S, Shimadzu, Kroyoto, Japan. |

### Pre-formulation studies

### Description

Check the description by visual observation of the API powder and record the observation.

### Solubility

Check the solubility of the API by adding the known quantity of the API powder in respective solvent. Calculate the mg/ml and record the results.

**Bulk Density:**

**a) Loose Bulk Density:** Weigh accurately 1 g of drug (M), which was previously passed through 20 # sieve and transferred in 25 ml graduated cylinder. Carefully level the powder without compacting, and read the unsettled apparent volume ($V_0$). Calculate the apparent bulk density in gm/ml by the following formula

**Bulk density = Weight of powder / Bulk volume …….. (1)**

**b) Tapped bulk density**: Weigh accurately 1 g of drug, which was previously passed through 20 # sieve and transfer in 25 ml graduated cylinder. Then mechanically tap the cylinder containing the sample by raising the cylinder and allowing it to drop under its own weight using mechanically tapped density tester that provides a fixed drop of 14± 2 mm at a nominal rate of 300 drops per minute. Tap the cylinder for 500 times initially and measure the tapped volume ($V_1$) to the nearest graduated units, repeat the tapping an additional 750 times and measure the tapped volume ($V_2$) to the nearest graduated units. If the difference between the two volumes is less than 2% then final the volume ($V_2$). Calculate the tapped bulk density in gm/ml by the following formula:

**Tapped Density = Weight of powder / Tapped volume …….. (2)**

**Carr's Index**

The Compressibility Index of the powder blend was determined by Carr's compressibility index. It is a simple test to evaluate the BD and TD of a powder and the rate at which it packed down. The formula for Carr's Index is as below:

**Carr's Index (%) = [(TD-BD) x100]/TD …….. (3)**

**Hausner's Ratio**

The Hausner's ratio is a number that is correlated to the flow ability of a powder or granular material.

**Hausner's Ratio = TD / BD ……. (4)**

**Table 1.1 Effect of Carr's Index and Hausner's Ratio on flow property**

| Carr's Index (%) | Flow Character | Hausner's Ratio |
|---|---|---|
| ≤ 10 | Excellent | 1.00–1.11 |
| 11–15 | Good | 1.12–1.18 |
| 16–20 | Fair | 1.19–1.25 |
| 21–25 | Passable | 1.26–1.34 |
| 26–31 | Poor | 1.35–1.45 |
| 32–37 | Very poor | 1.46–1.59 |
| >38 | Very, very poor | >1.60 |

**Angle of repose**

The angle of repose of API powder was determined by the funnel method. The accurately weight powder blend were taken in the funnel. The height of the funnel was adjusted in such a way the tip of the funnel just touched the apex of the powder blend. The powder blend was allowed to flow through the funnel freely on to the surface. The diameter of the powder cone was measured and angle of repose was calculated using the following equation.

$$\tan \theta = h/r \quad \text{.....(5)}$$

Where, h and r are the height and radius of the powder cone respectively.

**Table 1.2 Effect of Angle of repose (ϕ) on Flow property**

| Angle of Repose (Φ) | Type of Flow |
|---|---|
| < 20 | Excellent |
| 20-30 | Good |
| 30-34 | Passable |
| >35 | Very poor |

- **Calibration curve of Cannabidiol**

Standard stock solution of drug (100 µg/ml) was prepared by dissolving 10 mg of Cannabidiol in 100 ml using 0.1 N HCl to get a concentration of 100 µg/ml. The prepared solution is sonicated for 10 minutes and filtered through the Whatman No. 41 filter paper. Appropriate volumes of this solution were further diluted to obtain final concentrations in the range of 1 to 10 µg/ml. The spectrum of this solution was recorded from 200 nm to 400 nm using Shimadzu UV-VIS Spectrophotometer.

**5.2 Drug excipients compatibility study**

FTIR studies were carried out to determine the compatibility of excipients with the drug. Pure drug sample and physical mixture of excipients with drug compared by FTIR and check the compatibility.

**5.3 Preparation of Solid lipid Nanoparticle**

Cannabidiol loaded Solid lipid nanoparticles were prepared by hot homogenization followed by the sonication method.

- **Hot Homogenization Method:**
  - ✓ In the hot homogenization technique, the drug was dispersed in the lipid (Compritol) and Soya lecithin (hydrophobic surfactant) by melting them above 70 °C of their melting point.
  - ✓ This is considered as the lipid phase. The aqueous phase was prepared by adding hydrophilic surfactant (Tween 80 and PEG-400) in the distilled water and heated (70-80 °C) to the temperature of the lipid phase.

- ✓ The lipid phase was added to the aqueous phase slowly, drop by drop under continuous stirring at 2700 rpm for about 3 h.
- ✓ The formulation was further sonicated for half an hour and cooled to room temperature.
- ✓ At room temperature the lipid was recrystallized to the formation of Solid Lipid Nanoparticles.

**Table 1.3 Formulation Table**

| Name of Material | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
|---|---|---|---|---|---|---|---|---|
| **Cannabidiol (mg)** | 10 | 10 | 10 | 10 | 10 | 10 | 10 | **10** |
| **Compritol (g)** | 1 | 1 | 1 | 1 | - | - | - | - |
| **Precirol ATO 5** | - | - | - | - | 1 | 1 | 1 | **1** |
| **Soya lecithin (mg)** | 100 | 100 | 100 | 100 | 100 | 100 | 100 | **100** |
| **Tween-80 (mg)** | 50 | 100 | 150 | 200 | 50 | 100 | 150 | **200** |
| **Distilled water (ml)** | 20 | 20 | 20 | 20 | 20 | 20 | 20 | **20** |

**Table 1.4 Factorial Batches formulation table**

| Ingredients (mg) | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | V9 |
|---|---|---|---|---|---|---|---|---|---|
| **Cannabidiol (mg)** | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| **Soya lecithin** | 80 | 100 | 120 | 80 | 100 | 120 | 80 | 100 | 120 |
| **Poloxamer 188** | 0.5 | 0.5 | 0.5 | 1.0 | 1.0 | 1.0 | 1.5 | 1.5 | 1.5 |
| **Tween-80 (mg)** | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 |
| **Distilled water (ml)** | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |

## 1. RESULTS & DISCUSSION

### Characterization of Cannabidiol

Physico-chemical characterization of API was done and the results were recorded in below table. Flow properties, description, solubility and melting point were checked and results recorded in table 6.1.

**Table 1.1 Characterization of Drug Cannabidiol**

| Sr. No. | Characteristic Properties | | Observation/Result |
|---|---|---|---|
| 1 | **Organoleptic Characteristics** | *Colour* | White to off-white |
| | | *Odour* | Odorless |
| 2 | **Description** | | White to off white crystalline powder |
| 3 | **Flow Properties** | *Bulk density (g/ml)* | 0.21 ± 0.03 |
| | | *Tapped density (g/ml)* | 0.37 ± 0.05 |
| | | *Carr's index (%)* | 43.2 ± 0.04 |
| | | *Hausner's ratio* | 1.76 ± 0.02 |
| | | *Angle of repose (θ°)* | 51.2 ± 0.8 |
| 4 | **Melting Point** | | 68 ° C |
| 5 | **Solubility** | | Insoluble in water |

As per above results, it found that the API is white colored crystalline powder. The flow properties found poor flow in nature. Further, drug was found in-soluble in water as it belongs to BCS class-II drug. Melting point by capillary melting method was found and found satisfactory.

### 6.2 Standard Calibration curve of Cannabidiol

Identification of drug was done by UV spectroscopic method and the $\lambda_{max}$ was found 244 nm. The UV identification curve was taken over the range of 200-400 nm and it is shown in below figure.

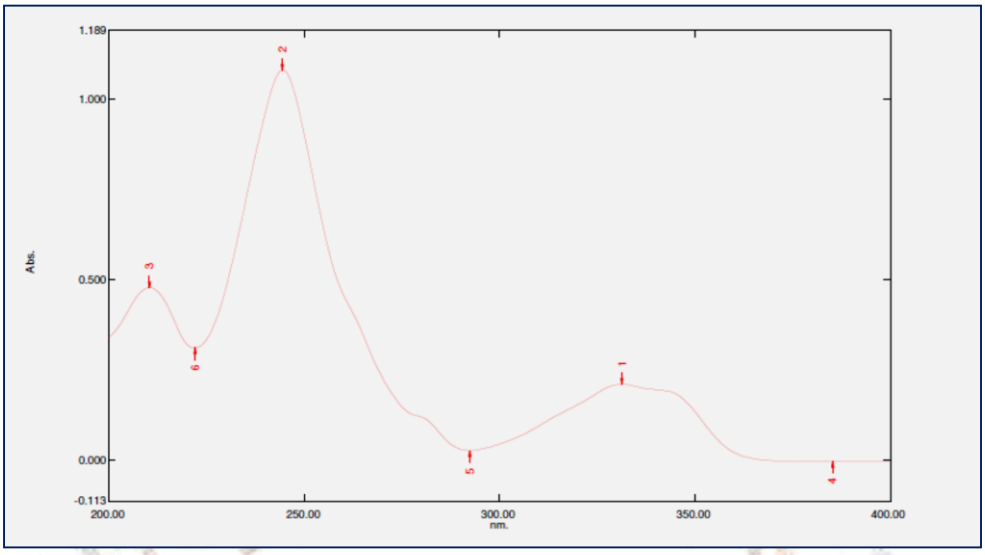


**Figure 1.1 UV Identification curve of Cannabidiol**

The standard calibration curve was prepared using 0.1 N HCl and 6.8 phosphate buffer as solvent. A linearity curve was prepared over the range of 10-50 µg/ml and found linear.

**Table 1.2 Standard Calibration curve of Cannabidiol**

| Sr. No | Concentration (µg/ml) | Absorbance ± SD (n=3) | |
|---|---|---|---|
| | | 0.1 N HCl | 6.8 Phosphate Buffer |
| 1 | 10 | 0.192 ± 0.003 | 0.186 ± 0.002 |
| 2 | 20 | 0.389 ± 0.001 | 0.369 ± 0.005 |
| 3 | 30 | 0.573 ± 0.006 | 0.551 ± 0.004 |
| 4 | 40 | 0.772 ± 0.004 | 0.723 ± 0.007 |
| 5 | 50 | 0.961 ± 0.002 | 0.903 ± 0.004 |

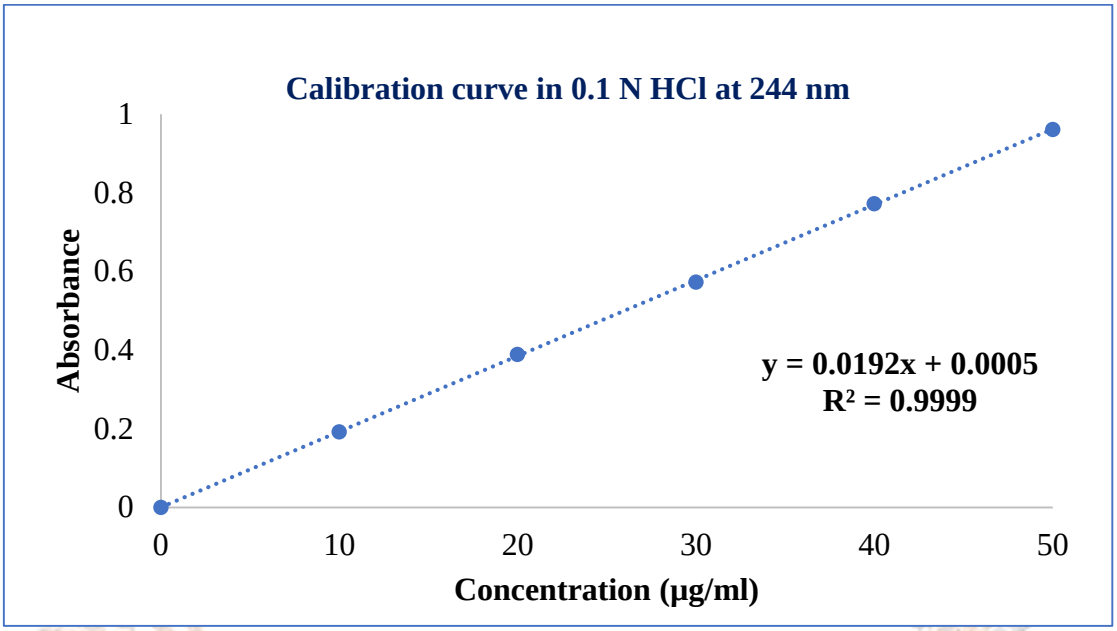


**Figure 1.2 Calibration curve of Cannabidiol in 0.1 N HCl**

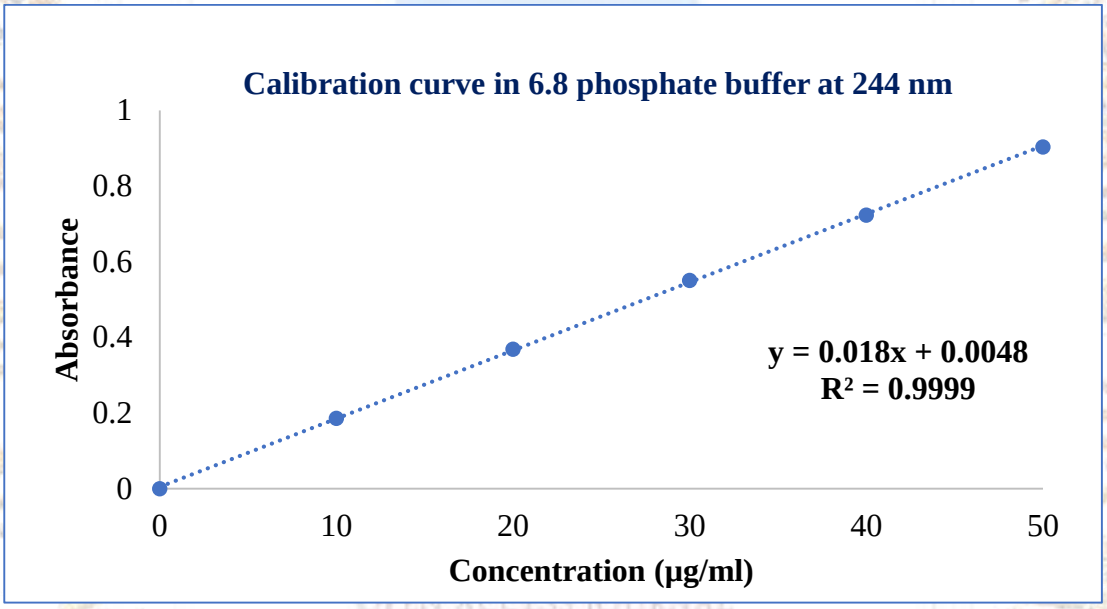


**Figure 1.3 Calibration curve of Cannabidiol in 6.8 phosphate buffer**

### 6.3 Drug Excipient Compatibility Study

To check the possible drug interaction with the excipients, drug excipient compatibility study was performed. Pure drug sample and final formulation mixture were scanned under IR region and the FTIR graph was reported below. All characteristic peaks of API were remained same in Final formulation. Hence, the selected excipients were found compatible with the Drug substance.

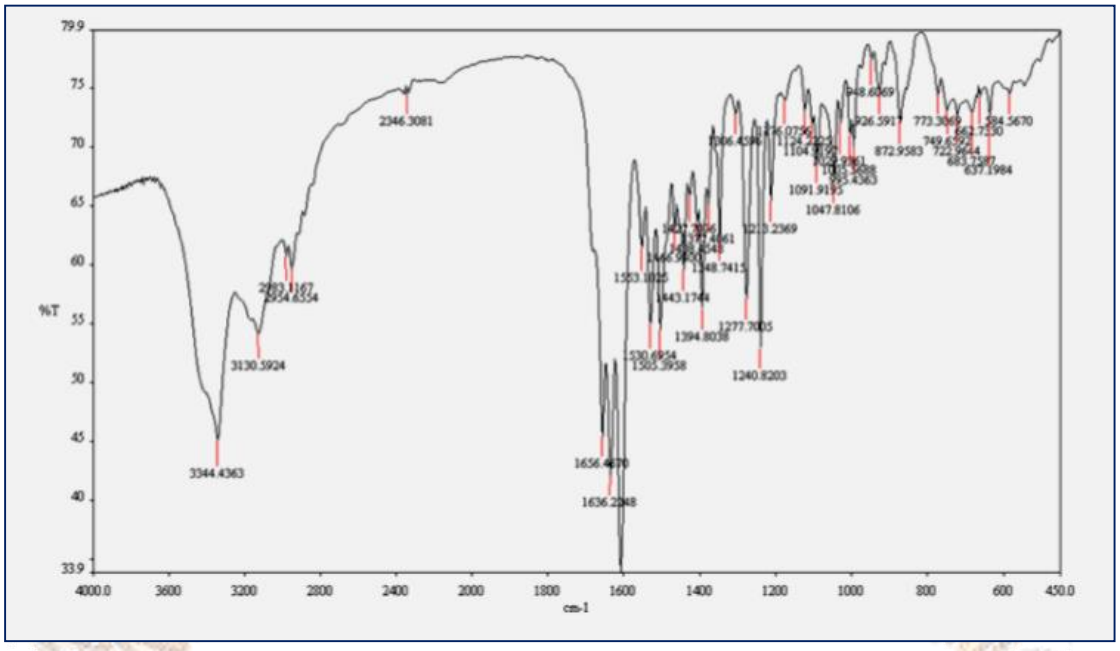


**Figure 1.4 FTIR graph of Cannabidiol**

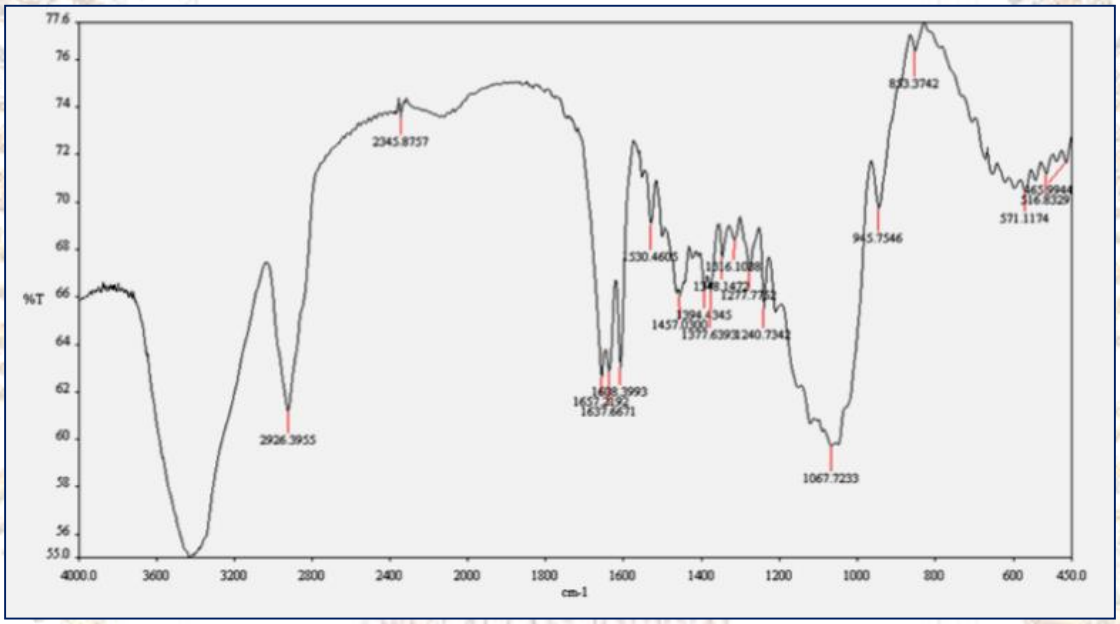


**Figure 1.5 FTIR graph of physical mixture**

**Table 1.3 FTIR Data Interpretation**

| Stretching | Pure Drug Peak ($cm^{-1}$) | Formulation Mixture Peak ($cm^{-1}$) |
|---|---|---|
| C=O | 1213 | 1277 |
| C-H | 2954 | 2926 |
| C-N | 1307 | 1348 |

### 6.4 Evaluation of Preliminary Trial Batches

Initial trials were taken with different concentration of surfactant with constant amount of lipid barrier. Trial batch F1-F8 were taken to check the important factors of the formulation. Evaluation was done for all F1-F8 batches and discussed below;

The drug content of the trial batches F1-F8 were checked and reported in below table. It was found that the drug content was found satisfactory. The comparative chart also prepared for all data and given below. Batch F8 have 83% drug content which is higher among all the formulations.

Further, the Entrapment Efficiency for F1-F8 batch was found in range of 46 – 98%. Batch F8 have 898% Entrapment Efficiency which is higher among all the formulations.

The particle size was checked by microscope and the results were recorded. The particle size of batch F8 was found 286 to 386 nm. The best formulation of F8 showed the zeta potential value of -29.4 mV. All parameters were recorded in below table and the comparative graph also drawn for side by side comparison and listed.

**Evaluation of Solid Lipid Nano-particles factorial batches**

| Batch | % Yield | Drug content (%) | Entrapment Efficiency |
|---|---|---|---|
| **C1** | 72.4 | 97.5 | 85.4 |
| **C2** | 74.6 | 96.9 | 86.9 |
| **C3** | 73.9 | 95.2 | 87.5 |
| **C4** | 78.6 | 97.8 | 89.6 |
| **C5** | 79.1 | 96.4 | 95.4 |
| **C6** | 77.6 | 97.3 | 95.9 |
| **C7** | 75.9 | 96.2 | 96.7 |
| **C8** | 81.4 | 95.3 | 97.1 |
| **C9** | 82.3 | 96.7 | 98.6 |

**Evaluation of solid lipid nano particles**

| Batch | Particle Size (nm) | Zeta Potential (mV) |
|---|---|---|
| **C1** | 283 | -21.6 |
| **C2** | 296 | -22.3 |
| **C3** | 302 | -23.5 |
| **C4** | 325 | -24.1 |
| **C5** | 336 | -24.9 |
| **C6** | 342 | -25.2 |
| **C7** | 357 | -26.9 |
| **C8** | 369 | -27.8 |
| **C9** | 378 | -28.6 |

**Drug release studies**

% Drug release study of trials batches C1-C9 performed and results recorded in Table 6.9. based on data it has been observed that the amount of lipid increase, retard the drug release and vice-versa.

**Table 1.4 Cumulative % drug release of batches C1 to C9**

| Time (hrs) | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 38.1 | 35.9 | 34.1 | 32.5 | 30.1 | 27.1 | 29.4 | 25.8 | 23.1 |
| 2 | 45.3 | 42.6 | 40.3 | 40.2 | 37.2 | 34.3 | 35.6 | 32.7 | 29.4 |
| 3 | 53.6 | 50.1 | 48.3 | 48.1 | 45.1 | 42.1 | 43.6 | 40.5 | 38.4 |
| 4 | 61.3 | 58.9 | 55.6 | 55.6 | 53.2 | 50.3 | 47.9 | 45.9 | 42.5 |
| 6 | 78.2 | 74.3 | 71.2 | 72.3 | 69.4 | 65.8 | 65.4 | 61.5 | 59.4 |
| 8 | 90.1 | 86.4 | 84.3 | 85.3 | 82.1 | 79.2 | 78.5 | 74.6 | 71.2 |
| 10 | 99.9 | 99.2 | 98.5 | 96.9 | 95.1 | 93.2 | 93.5 | 88.8 | 85.8 |
| 12 | 99.9 | 99.9 | 99.5 | 99.1 | 99.2 | 98.2 | 99.8 | 99.4 | 97.1 |

**Table 1.5 Table for Optimized Batch**

| Sr. No. | Ingredients (mg) | O1 |
|---|---|---|
| 1 | Cannabidiol (mg) | 10 |
| 2 | Precirol ATO 5 | 1.07 |
| 3 | Soya Lecithin | 83.98 |
| 4 | Tween-80 (mg) | 200 |
| 5 | Distilled water (ml) | 20 |

**Table 1.6 Evaluation of Optimized Batch**

| Ingredients (mg) | C1 |
|---|---|
| **% Yield** | 86.9 ± 2.6 |
| **Drug content (%)** | 98.5 ± 2.1 |
| **Entrapment Efficiency** | 92.5 ± 1.3 |
| **Particle Size (nm)** | 335 ± 9 |
| **Drug release at 12 hours.** | 98.9 ± 1.2 |

- **Stability Study**

Stability study of final optimized batch O1 performed for 1 month at 40°C and 75% RH. Initial results and after 1-month results compared and found satisfactory. The batch was found stable during stability. The results were recorded in below table.

**Table 1.7 Stability study of O1 batch**

| Evaluation Parameters | Initial Results | After 1 Month Results |
|---|---|---|
| **Appearance** | No Change | No Change |
| **Assay (%)** | 98.5 ± 2.1 | 98.4 ± 1.6 |
| **% Drug Release after 12 hrs** | 98.9 ± 1.2 | 98.3 ± 1.8 |

## 3. CONCLUSION

In the present study Cannabidiol loaded solid lipid nanoparticles were prepared. Cannabidiol -loaded Solid lipid nanoparticles were prepared by employing a hot homogenization method. All the formulations were evaluated for drug content, entrapment efficiency and drug release studies, particle size and zeta potential. Out of all formulations, the best formulations were found to be F8 with drug content was found to be 83.1%, entrapment efficiency was 98.8% and drug release was 98% within 12h, particles size 286-386 nm and zeta potential -29.4 mv. Further trials were done for application of factorial design and optimization of formulation. 3 level 2 factor factorial design was applied for formulation optimization. Precirol ATO 5 and Soya lecithin was selected as independent variables. % Drug release at 1 hour, 4 hours and particle size were selected as dependent variables. All the batches C1-C9 found satisfactory with the results. The design was validated using DoE software. The Optimize batch was taken from the design and evaluated. Optimized formulation was charged in stability for 1 month at accelerated condition and found stable. Hence, O1 batch was considered as optimized batch

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