ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# Solid Lipid Nanoparticles Of Hydroxypropyl β -Cyclodextrin Complexes Of Azithromycin For Enhancement Of In Vitro Drug Release.

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**ABSTRACT**

Azithromycin, a macrolide antibiotic with broad therapeutic potential suffers from poor aqueous solubility, acidic instability, and variable oral bioavailability (similar to 37%) leading to suboptimal therapeutic efficacy and increased resistance risk. To overcome these limitations, this study integrates two complementary formulation strategies such as β-cyclodextrin complexation and solid lipid nanoparticle encapsulation. Cyclodextrins from inclusion complexes with hydrophobic drugs, improving solubility and stability, while SLNs provide a lipid matrix for sustained release and protection from degradation. Cyclodextrin complexes of azithromycin were prepared by taking various ratios of drug to hydroxypropyl-β cyclodextrin (HP-βCD) by slurry method. Prepared complexes were converted into solid lipid nanoparticles (SLNs) by method of high-shear homogenization method. The SLNs were assessed for enhanced release rates. Among the prepared SLNs of CD complexes of azithromycin, formulation F2 showed optimum properties with particle size of 220nm, polydispersity index 0.197, high entrapment efficiency (92.5%), Zetapotential of -11.0mV, and sustained drug release of 88% over six hours. FTIR, DSC, XRD analysis and confirmed successful complexation indicating drug excipient compatibility and reduced crystallinity. Stability studies at storage (4°C) maintained formulation with minimal changes in size and drug content compared to room temperature. The optimized formulation is best in dissolution behavior. Combination effect of β-cyclodextrin complexation and SLN technology demonstrated significant enhancement in solubility and drug dissolution of azithromycin offering promising technology for efficient oral drug delivery for poorly water-soluble azithromycin.

**Keywords:** Solid Lipid Nanoparticlesb(SLNs), Azithromycin, High-shear homogenization, Entrapment Efficiency, Dissolution rate , stability studies.

https://doi.org/10.33887/rjpbcs/2026.17.3.3

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ISSN: 0975-8585

## INTRODUCTION

Azithromycin (AZT), a commonly prescribed macrolide antibiotic, exemplifies a drug with poor water solubility that requires innovative formulation methods to achieve optimal therapeutic performance. Its limited solubility and susceptibility to degradation in acidic gastric conditions hinder its absorption and bioavailability, emphasizing the need for improved drug delivery approaches. As a Biopharmaceutics Classification System (BCS) Class II drug, with oral bioavailability of 37% [1,2].

To address these barriers, scientists have developed advanced delivery systems such as solid dispersions, lipid-based formulations, and nanotechnology-derived carriers. Among these, solid lipid nanoparticles (SLNs) have gained significant importance because of their excellent properties, including improved solubility, stability, and bioavailability, along with their capacity for controlled and sustained drug release.

The present work is focused on the formulation and evaluation of azithromycin -loaded cyclodextrin complexes of solid lipid nanoparticles (CD-SLNs) to enhance the drugs solubility, and oral bioavailability.

An effective and innovative approach combines solid lipid nanoparticles (SLNs) with cyclodextrin complexation [3, 4]. Cyclodextrins play a key role in improving the water solubility of poorly soluble drugs and protecting them from chemical degradation, while SLNs ensure structural stability and controlled drug release. The fusion of these two systems works synergistically to enhance the overall therapeutic efficiency and performance of the drug.

The combined SLN–cyclodextrin formulation strategy shows great potential for azithromycin by enhancing its solubility, ensuring prolonged and controlled release, minimizing dosing frequency, and improving patient adherence[5,6]. This innovative nanotechnology-based approach represents a significant advancement in oral drug delivery, aimed at achieving superior bioavailability and enhanced therapeutic effectiveness.

The main objectives are preparation of cyclodextrin complexes of Azithromycin by taking various ratios of drug and HP-βCD by **slurry method and** conversion of all the complexes into SLNs to assess enhancement of dissolution.

## MATERIALS AND METHODS

### Materials

Azithromycin is a gift sample from Karnataka Antibiotics and Pharmaceuticals Ltd., Hydroxypropyl β-cyclodextrin, Glyceryl monostearate, Tween 80, Poloxamer 188 were obtained from Himedia, INdia Ethanol and distilled water were used as solvents, supplied by Central Drug House Pvt. Ltd. and a local source, respectively.

### Methods

Present research work is carried out in two steps including preparation of cyclodextrin complexes of azithromycin by taking various ratios of drug to hydroxypropyl- β cyclodextrin (HP-βCD) by slurry method and conversion of prepared complexes into solid lipid nanoparticles (SLNs).

### Step1: Preparation of Azithromycin- HP-βCD inclusion complexes:

Azithromycin- HP-βCD inclusion complexes were prepared by taking various ratios of azithromycin and HP-β- Cyclodextrin (CD)[7,8]. Eight compositions CD1 to CD8 of varied ratios of drug to CD were prepared and the composition is shown in **Table 1.** Each trial was prepared to contain 250mg azithromycin. Weighed quantity of HP-βCD was dissolved in 25 ml of water and stirred well. Then ethanolic solution azithromycin was added into HP-βCD dropwise with stirring at 600-800rpm. The mixture was dried at 40-50°C and the produced powder was pulverized and stored in screw capped vials for further work.

ISSN: 0975-8585

**Table 1: Composition of cyclodextrin complexes of azithromycin**

| S.no | Drug(mg) | HPβCD (mg) | Ratios |
|---|---|---|---|
| CD1. | 250 | 125 | 1:0.5 |
| CD2. | 250 | 250 | 1:1 |
| CD3. | 250 | 375 | 1:1.5 |
| CD4. | 250 | 500 | 1:2 |
| CD5. | 250 | 625 | 1:2.5 |
| CD6. | 250 | 750 | 1:3 |
| CD7. | 250 | 875 | 1:3.5 |
| CD8. | 250 | 1000 | 1:4 |

**Step 2: Preparation of Solid lipid nanoparticles (SLNs) of CD complexes of AZT :**

The prepared cyclodextrin complexes, CD1 to CD8 were converted as SLNs by high-shear homogenization method. The composition of SLNs were designated as F1-F8 and are shown in **Table 2.** In the preparation, glycerol monostearate (GMS) was used as lipid, tween 80 was used as surfactant and poloxamer 188 was a co-surfactant. During the preparation, weighed quantity of glycerol monostearate was melted at 5 to10°C above its melting point and typically between 70°C-75°C in a water bath. The pre-prepared AZ- HP-βCD complex equivalent of 250 mg of AZT was added into molten lipid while stirring to form a homogenous mixture. The aqueous phase was prepared separately by dissolving the surfactants (Tween 80 and poloxamer 188) according to the quantity in 60ml distilled water and heated to the same temperature. The volume was made up to 100ml quantity with distilled water. The hot aqueous phase was slowly added into the lipid phase under high-speed stirring for 10min to form a coarse emulsion. The obtained dispersion was cooled to 4°C. The emulsion was finally homogenized at 10,000 rpm for 15 minutes using a high-shear homogenizer. The solution was sonicated for 10 minutes to reduce the particle size further and achieve uniform dispersion [9,10].

**Table 2: Composition of CD-SLNs**

| INGREDIENTS | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
|---|---|---|---|---|---|---|---|---|
| **Cyclodextrin complexes of AZT** | **CD1.** | **CD2** | **CD3** | **CD4** | **CD5** | **CD6** | **CD7** | **CD8** |
| Glyceryl monostearate (mg) | 1500 | 1500 | 1500 | 1500 | 1500 | 1500 | 1500 | 1500 |
| Tween 80(ml) | 15ml | 10ml | 8ml | 20ml | 25ml | 0ml | 5ml | 10ml |
| Poloxamer 188 | 500mg | 400mg | 600mg | 750mg | 1000mg | 1200mg | 1000mg | 750mg |
| Distilled water | q.s to 100ml | q.s to 100ml | q.s to100ml | q.s to 100ml | q.s to 100ml | q.s to 100ml | q.s to 100m | q.s to 100ml |

**Evaluation of Solid Lipid Nanoparticles of Cyclodextrin Complexes of azithromycin:**

The Solid lipid nanoparticles of cyclodextrin complexes of azithromycin (F1 to F8) were evaluated for particle size determination, polydispersity index, zeta potential, entrapment efficiency, ***in-vitro*** drug release studies and stability studies.

**Particle Size and PDI determination of Solid Lipid Nanoparticles of Cyclodextrin complexes of azithromycin:**

To assess the size particle size and PDI to AZ- HPβCD of SLNs, the1ml sample was diluted into 10ml at room temperature and contents are gently stirred manually. The mean particle size and PDI of the resultant emulsion was determined by Zeta sizer (Horriba).

**Percentage Entrapment Efficiency:**

To assess percentage entrapment efficiency, an indirect technique centrifugation method was used. For this, the AZ- HPβCD of SLNs formulation were centrifuged with 4,500rpm for 30mins.Then 1ml of the supernatant was taken and dissolved in 10ml of water. The amount of azithromycin free/bound drug entrapped in the supernatant was measured with the help of UV spectrophotometry at 281nm wavelength by placing water as blank, the below formula was used to calculate the percentage of entrapment efficiency**.**

$$\textbf{\% Entrapment Efficiency: } \frac{\textit{Initial amount of drug} - \textit{amount of unbound drug}}{\textit{Initial amount of drug}} \times 100$$

Where $W_{total\ drug}$ is the drug amount initially added and $W_{free\ drug}$ the amount of drug in the aqueous phase after ultrafiltration- centrifugation.

**FT-IR spectroscopy:**

This study was performed to find out the compatibility between the drug and excipients using IR spectrometer (Bruker, Japan). Sample and KBr were taken in the ratio of 1:100 in a mortar, triturated, and compressed at 10 kg/cm$^2$ to form a transparent pellet using a hydraulic press. The pellet was kept in a sample holder and scanned between 4000cm-1 in an FT-IR spectrophotometer. The possible interaction between drug and excipients was assessed by comparing FT-IR spectra of the pure drug and selected formulation.

**Differential scanning calorimetry (DSC):**

Differential scanning calorimetry thermograms were recorded using differential calorimetry (DSC) (Mettle Toledo, Japan). Indian standards were used to calibrate the DSC temperature and enthalpy scale. Approximately 2-5 mg of each sample was heated in a pierced aluminium pan from 50ºC to 300°C at a heating rate of 10°C/min under a stream of nitrogen at a flow rate of 50ml/min. Thermal data analysis of the DSC thermogram was conducted using STARE software.

**X-Ray diffraction (XRD) studies:**

X-ray diffraction patterns of pure drug and AZY loaded CD-SLNs were obtained using X- ray diffractometer in which Cu-K line was used as a source of radiation by operating at the voltage 40 kV and the current applied was 25 mA over 10-80º range with a scanning rate of 3ºC / min and a step size of 0.02.

***In- vitro* drug release studies:**

Release studies from SLNs were carried out by a dialysis bag membrane method. Each sample 1ml was put into the semi-permeable dialysis bag, and then immersed 2h in 15ml of pH 1.6 stimulated gastric fluid (HCl 0.1N and Nacl 2.0g/l) and after 4h in 15ml of pH 6.8 stimulated intestinal fluid (6.8 g/l $KH_2PO_4$ and NaOH 0.89g/l) both maintained at 37±1°C and stirred at 300rpm. Samples are taken at given intervals of time up to 360min, and immediately replaced by an equal volume of fresh medium kept the same temperature. A correction was made for the cumulative dilution. The amount of release drug was assayed by UV- spectrophotometry at 281nm. Each test was repeated at least 2times.

## RESULTS AND DISCUSSION

**Solid lipid nanoparticles (SLNs) of cyclodextrin complexes of azithromycin:**

**Particle Size and PDI determination of Solid Lipid Nanoparticles of Cyclodextrin complexes of azithromycin:**

The results of mean particle size and polydispersity index of 8 formulations of cyclodextrin complexes of azithromycin SLNs, F1 to F8 are presented in **Table 3** and the data from Zeta sizer are shown in **Fig 2 and 3.** It is evident from the results that the metdos usd are successful in producing the SLNs red in nano

ISSN: 0975-8585

sizes ranging from 220nm to 539.0 nm. SLNs F2 are with lowest size of 220 nm and low polydispersity index 0.197 and zeta potential is -11.0mV.

**Table 3: particle size and polydispersity index of CD-AZY loaded SLNs**:

| S.no | Formulation | Particle size | Polydispersity index |
|---|---|---|---|
| 1 | F1 | 341nm | 0.418 |
| 2 | F2 | 220nm | 0.197 |
| 3 | F3 | 418nm | 0.514 |
| \4 | F4 | 534nm | 0.561 |
| 5 | F5 | 464nm | 0.574 |
| 6 | F6 | 507.4 | 0.432 |
| 7 | F7 | 455.5nm | 0.467 |
| 8 | F8 | 539.0 nm | 0.710 |

**Fig 1: Particle size of F1**

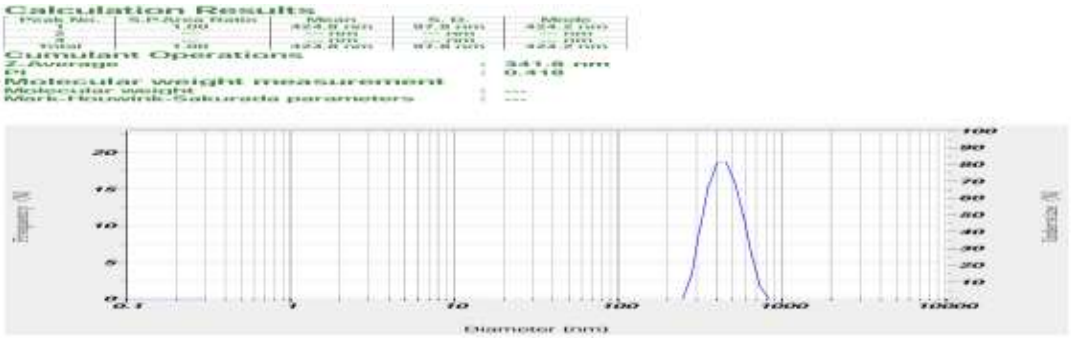


**Fig 2: particle size of F2**

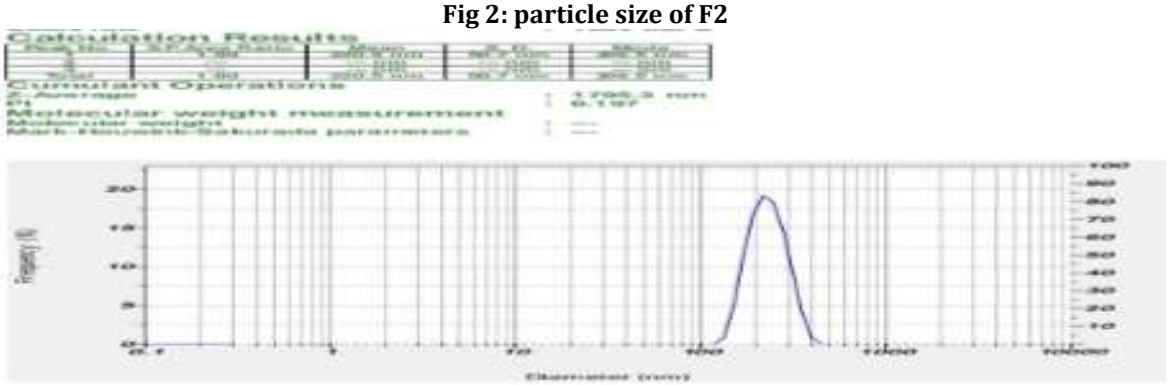


**Fig 3: Particle size of F3**

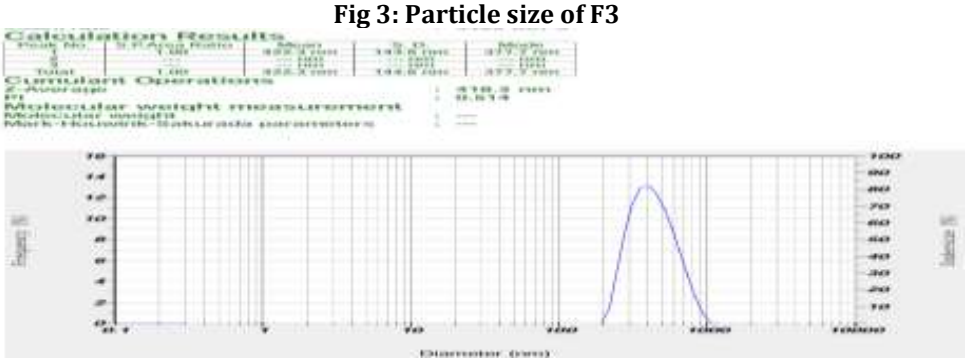

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Fig 4: particle size of F4

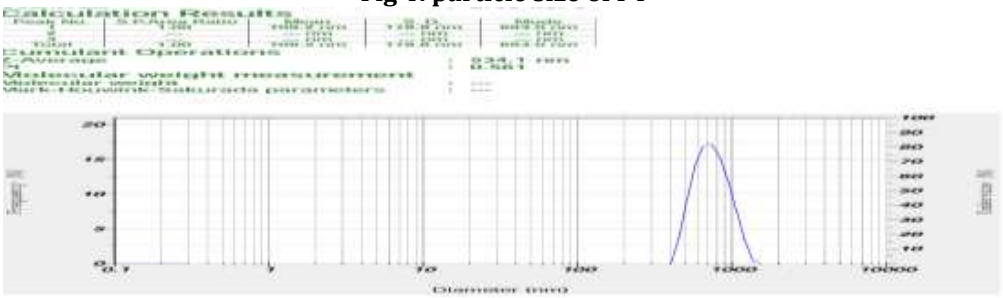


Fig 5: particle size of F6

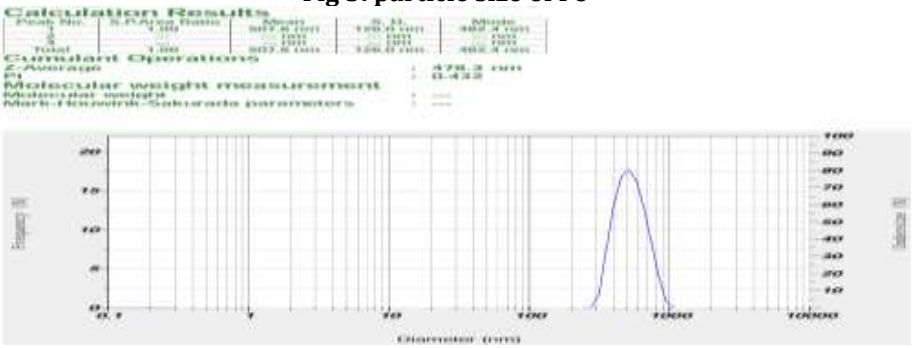


Fig 6 : particle size of F7

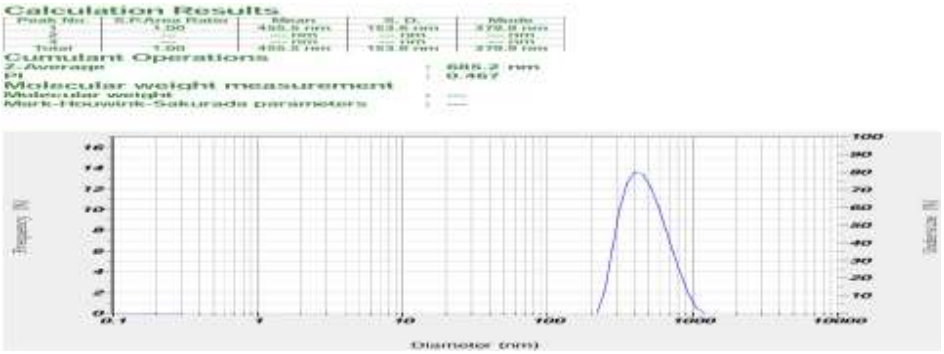


Fig7: particle size of F7

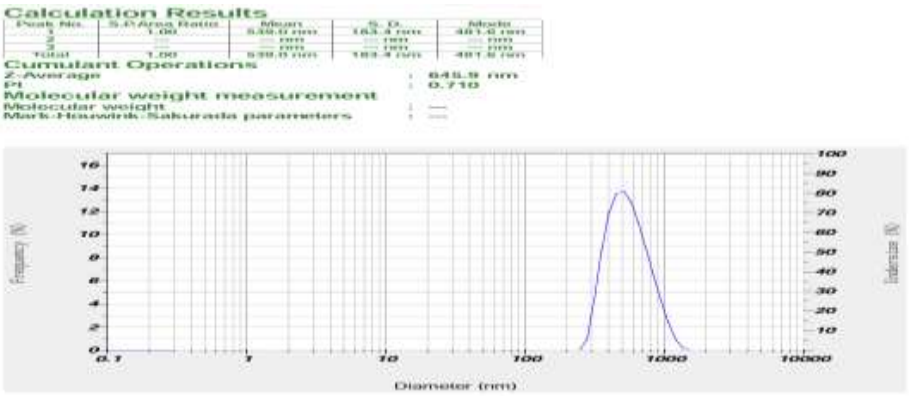


Fig8: particle size of F8

**Zeta potential:**

Zeta potential indicates the stability of the formed nano emulsion. The charge of the SLNs is another property that should be assessed for increased absorption. The charge of oil droplets in SLNs was

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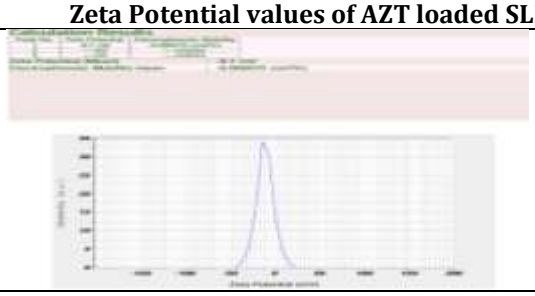
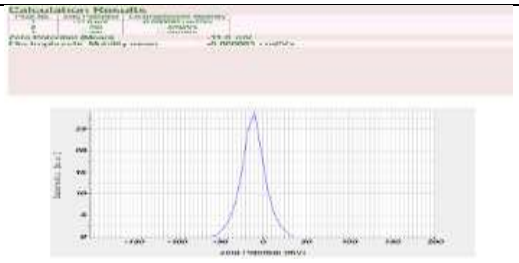
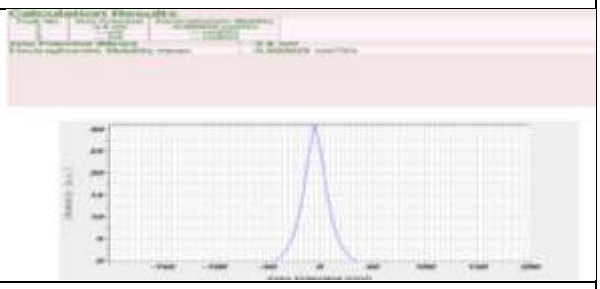
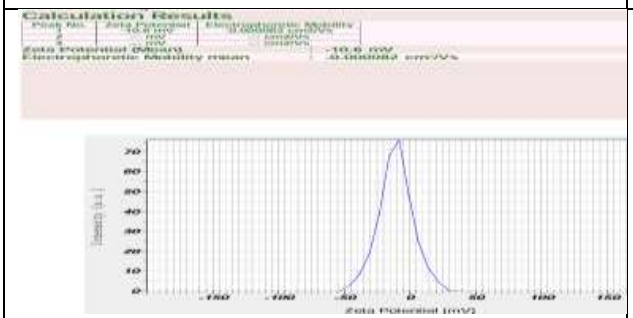
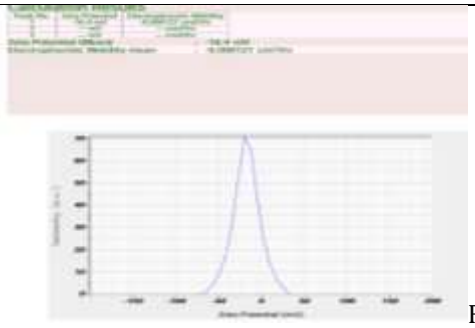
negative due to the presence of free fatty acids which shows relatively negative charge. The observed zeta potential for all prepared formulations ranged in between -3.8 to -20.3mV was reported in **table 4 and fig19 to fig26** indicated stability of prepared trial SLNs.

**Table 4: Zeta potential values**

| S. No | Formulation | Zeta potential |
|---|---|---|
| 1 | F1 | -9.7 mv |
| 2 | F2 | -11.0 mv |
| 3 | F3 | -10.6 mv |
| 4 | F4 | -5.1 mv |
| 5 | F5 | -20.3 mv |
| 6 | F6 | -3.8 mv |
| 7 | F7 | -16.4 mv |
| 8 | F8 | -8.8 mv |

**Table 4: Zeta potential of AZY loaded CD-SLNs**

| Zeta Potential values of AZT loaded SLNs of cyclodextrin complexes of azithromycin | |
|---|---|
|  |  |
| **F1** | **F5** |
|  |  |
| **F2** | **F6** |
|  |  F7 |
| **F3** | |

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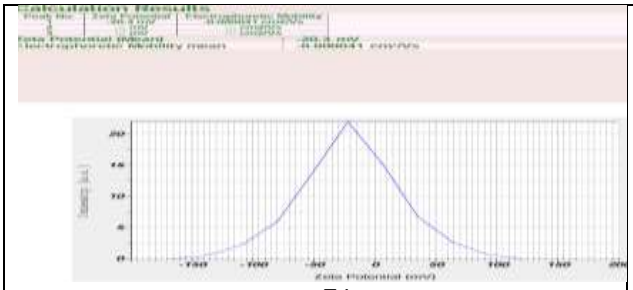
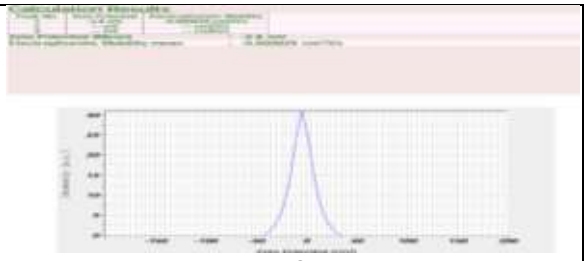


F4



F8

**Drug Entrapment efficiency of CD-SLNs:**

The results of % drug Entrapment efficiency values Azithromycin loaded CD-SLNs formulations F1 to F8 are given in **Table 5** and they are in the range of 61% to 92%and. The formulation, F2 has the highest value of 92%±0.05 indicates its highest drug entrapment efficiency. Due to this property and particle size of 220nm this formulation F2 was considered as more promising and assessed for further studies.

**Table 5: %drug entrapment efficiency of Azithromycin loaded CD-SLNs**

| Formulation | %Entrapment efficiency |
|---|---|
| F1 | 81.7±0.03 |
| F2 | 92.5±0.05 |
| F3 | 74.4±0.03 |
| F4 | 85.8±0.07 |
| F5 | 80.6±0.06 |
| F6 | 77.0±0.04 |
| F7 | 61.7±0.06 |
| F8 | 87.2±0.01 |

***In-vitro* Drug Release Studies:**

The results of ***in-vitro*** drug release studies are presented in **Table 6.** The results indicate good release characteristics from all SLNs and the formulation F2 exhibited highest drug release of 88%±23.72 . Moreover due to the optimum particle size of 220nm and high entrapment efficiency of formulation F2, it was considered as promising formulation and further investigated for drug excipient interaction study, XRD analysis.

**Table 6: Drug release data of studies Azithromycin loaded CD-SLNs**

| S. No | Formulation | % Drug Release In 60min |
|---|---|---|
| 1 | F1 | 61.5±18.39 |
| 2 | F2 | 98±23.72 |
| 3 | F3 | 38.5±10.95 |
| 4 | F4 | 70.1±20.62 |
| 5 | F5 | 28.1± 8.52 |
| 6 | F6 | 67±18.23 |
| 7 | F7 | 44.7±12.79 |
| 8 | F8 | 61.5±17.74 |

**FT-IR Analysis pure(AZY) and AZY loaded Cyclodextrin complexes of SLNs:**

The FT-IR spectrum of AZY loaded Cyclodextrin complexation of SLNs shows the characteristic AZY bands, a broad O–H / N–H stretch around ~3386 $cm^{-1}$, aliphatic C–H stretches at ~2917 and ~2850 $cm^{-1}$, a strong carbonyl (C=O) band at ~1733 $cm^{-1}$ and a C–O band near ~1030 $cm^{-1}$. In the AZY-loaded CD-SLNs

these bands are still visible but generally display decreased intensity and small shifts particularly the broad O–H/N–H band and the C=O and C–O vibrations were reported in table12 .

Comparative assessment of FT-IR spectrum of pure AZY from AZY loaded CD-SLNs shows some same peaks in **Fig. 9 and 10.**

The aliphatic C–H peaks remain largely unchanged, indicating the alkyl backbone of AZY is preserved. Overall, the spectral changes support successful loading/interaction of AZY with the CD-SLN matrix rather than chemical degradation. Hence, it was concluded that there was no interaction between drug and excipients used to develop the CD-SLNs.

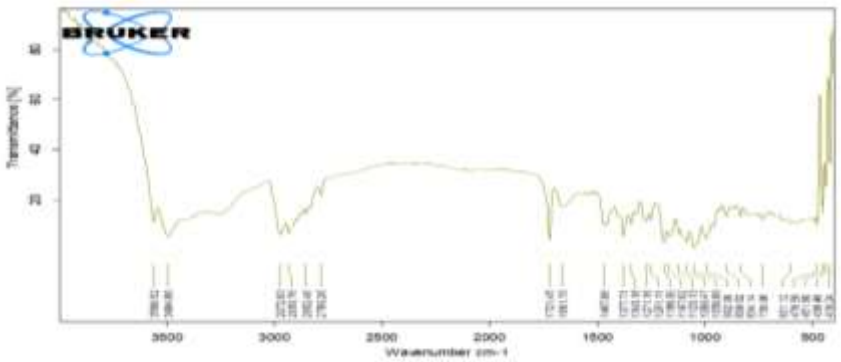



**Fig 9: FTIR Spectrum of AZY**

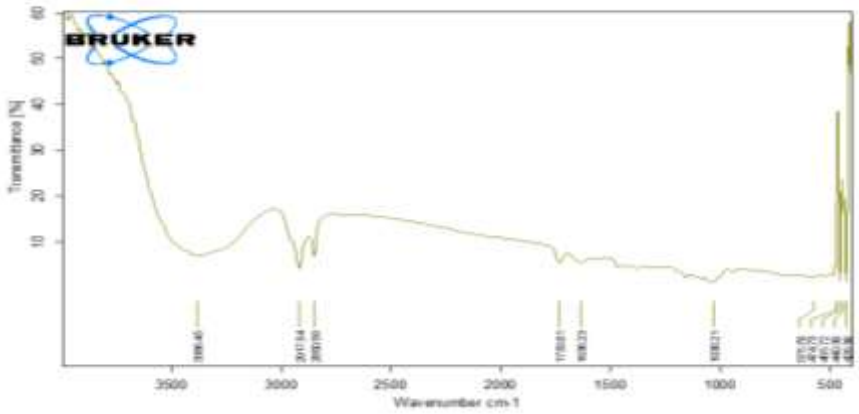



**Fig 10: FTIR Spectrum of AZY loaded CD-SLNs optimized formulation**

| S.no | Functional groups | Absorption peaks of AZY pure Wavenumber ($cm^{-1}$) | Absorption peaks of AZY loaded CD-SLNs |
|---|---|---|---|
| 1 | O–H (Alcohol/phenol) or N–H stretch (Amine) | 3500–3400 | 3386 $cm^{-1}$ |
| 2 | C–H stretch (Aromatic/Alkane) | 2950–2850 | 2917 $cm^{-1}$ |
| 3 | C=O stretch (carbonyl /ester) | 1735–1720 | 1733 $cm^{-1}$ |
| 4 | C=C / C=N stretch (Alkene/aromatic) | 1650–1600 | 1638$cm^{-1}$ |
| 5 | C–H bending | 1450–1375 | 2850 $cm^{-1}$ |
| 6 | C–O–C stretch | 1260–1050 | 1030 $cm^{-1}$ |
| 7 | C–H bending (aromatic or out-of-plane) | 900–700 | 577,472,458,443,428 $cm^{-1}$ |

**Table 12: Absorption peaks of FT-IR spectrum of pure and optimized formulation.**

ISSN: 0975-8585

**XRD Analysis:**

The X-ray diffractogram of pure AZT and CD–SLN formulation F2 are presented in Fig. 11 and 12. The X-ray diffraction (XRD) pattern of pure AZY exhibited numerous sharp and intense peaks at diffraction angles (2θ) around 7.8°, 9.1°, 13.8°, 14.4°, 18.8°, 19.8°, 20.6°, 23.3°, 26.1°, and27.8°, indicating the highly crystalline nature of the drug. The presence of multiple intense peaks confirms that AZY exists in a well-defined crystalline form with an ordered lattice structure. The XRD graph of the CD–SLN formulation shows a few distinct peaks with reduced intensity and increased broadness compared to the pure AZY drug pattern. Indicating conversion of crystalline form of drug to amorphous. This may the reason for enhanced dissolution characterises of SLNs of cyclodextrin complex of AZT.

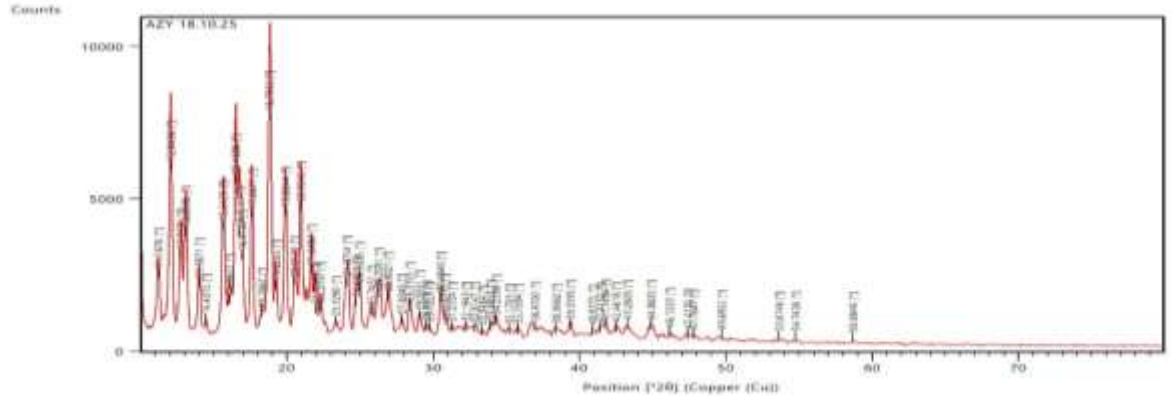



**Fig11 : XRD graph of pure drug (Azithromycin)**

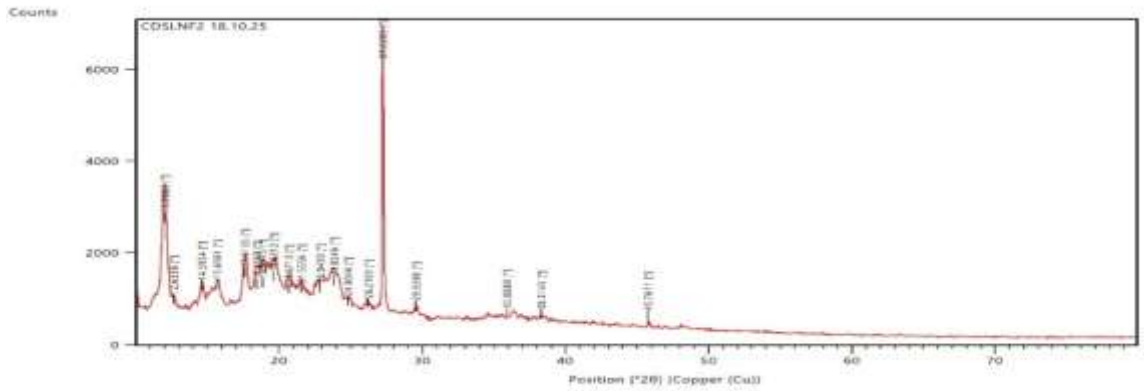



**Fig. 12: XRD graph of optimized formulation**

**Microscopic study:**

The shape and morphology of the optimized formulation F2 was examined by using phase contrast microscope. The microscopic image of the SLNs is shown in **Fig 153.** All the particles appear with good spherical shape with smooth surface which is an identical feature of SLNs.

**Fig. 13: Phase Contrast image of the optimized formulation**

## CONCLUSION

Glyceryl monostearate solid lipid nanoparticles of azithromycin produced from cyclodextrin complexes of azithromycin prepared in the ratio of 1:1 are promising to exhibit fast rate of dissolution of 98% which is an ideal character for azithromycin formulations since it is a a poorly soluble drug.

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