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# Development and Characterization of Polymeric Micelle for Ezetimibe

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**ABSTRACT**
Ezetimibe is the selective cholesterol absorption inhibitor. It is indicated for the treatment of primary hypercholesterolemia, which has poor aqueous solubility(0.00846gm/ml) and low solubility (35%). The current investigation was aimed to formulate polymeric micelles of ezetimibe to enhance solubility and dissolution rate which will leads to variability in absorption and it enhance oral bioavailability of the poorly soluble drug. The polymeric micelle was prepared using solvent evaporation technique. TPGS and Pluronic F68 were employed as lipid carrier and surfactant respectively. A 3 2 central composite design was applied to examine the combined effect of two formulation variables, each at 3 levels and the 9 possible combinations of EZM polymeric micelles. The concentration of EZM: TPGS (X1) and concentration of Pluronic (X2) were taken as independent variables. The particle size (Y1), and entrapment efficiency (Y2) were taken as dependent variables. The mean particle size and percentage entrapment efficiency co-loaded micelles were 142.34 nm and 39.21% respectively. The particle size of micelles in nanometre so we could expect better accumulation of micelles at site of action. Ezetimibe polymeric micelles evaluated by particle size analysis, %EE, differential scanning calorimetry (DSC), FTIR, In vitro drug release study and stability study. From prepared batches of polymeric micelle suggested that solubility of drug increases with increase in polymer concentration. The dissolution rate was substantially improved for ezetimibe from its polymeric micelle compared with pure drug. Combinations of polymer to drug shows enhanced drug loading capacity. The drug release experiments exhibited an initial rapid release followed by sustained release. From the results, it can be concluded that, polymeric micelles may serve as a pharmaceutical nano carrier with improved solubility and biological activity for ezetimibe.

**KEYWORDS:** Ezetimibe; Polymeric micelle; TPGS; Pluronic F68; Solvent evaporation method; 3 2 Central composite design.

## I. INTRODUCTION

**Hyperlipidaemia[1]** is a condition in which the blood contains an excessive amount of fatty molecules known as lipids, most notably cholesterol and triglycerides. These fatty molecules circulate in the bloodstream alongside proteins, it's also known as hyperlipoproteinemia. Different kinds of hyperlipidaemia exist based on whether lipid levels in the blood are elevated.Hyperlipidaemia is split into two subcategories in general. i. Hypercholesterolemia characterised by a high amount of cholesterol. ii. The most frequent kind of fat is hypertriglyceridemia, which is characterised by a high level of triglycerides

**Treatment for Hyperlipidaemia[2]**
Hyperlipidemia is treated using a number of different medication group

| Class | Example | Mechanism ofaction | Effectso nlipids |
|---|---|---|---|
| HMG CoA Reductaseinhib itors(statins) | Lovastatin,Simvastatin,Ator vastatin,Rosuvastatin | They act by inhibiting the ratelimitingenzyme(HMG-coenzymereductase)inthebiosynt hesisofcholesterolin the liver | ↓LDL ↓TG |
| Fibric acidde rivatives | Clofibrate,Fenofibrate,Gem fibrozil | Fibratesreduceplasmatriglycerid esbyinhibiting theirhepaticsynthesisand | ↓LDL ↓TG |

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| | | | |
|---|---|---|---|
| | | increasingtheir catabolism | |
| Bile acid sequestrants | Cholestyramine,colestipol | Bile acid sequestrants in theintestine form large insolublecomplexthatnotabsorbed andso exerted inthefaces | ↓LDL↑ HDL |
| Nicotinic acidderivative | Niacin | It inhibits hormone sensitivelipasewhichdecreasetriglycerideslipolysisthemainproducerofcirculating freefattyacids. | ↓TG ↑HDL |
| Cholesterol absorptioninhibitors | Ezetimibe | Inhibitabsorptionofbiliaryanddietarycholesterolfromsmall intestine | ↓LDL↑ HDL |
| Miscellaneousagents | Dextrothyroxine | Stimulateshepaticcatabolism andexcretion ofcholesterol | ↓LDL |

## CHOLESTEROL ABSORPTION INHIBITORS (EZETIMIBE)[3,4]

Ezetimibe (cholesterol absorption inhibitors) are a class of drugs used to lowers plasma cholesterol levels

↓

By inhibiting the absorption of cholesterol at the brush border of the small intestine

↓

Inhibition of Niemann- Pick C1 (NPC1L1) protein Which expressed in both the intestine and the liver

↓

Resulting in a reduction of cholesterol transport and selectively inhibits absorption of dietary and biliary cholesterol

↓

Decreased delivery of intestinal cholesterol to the liver

↓

Reduction of hepatic cholesterol stores and increased clearanceof cholesterol from the blood

**Fig. 1.MechanismofactionofEzetimibe**

## POLYMERIC MICELLES (PMS):

In comparison to other innovative drug delivery technologies, the preparation of polymeric micelle looks to be comparatively. High molecular weight, low CMC, increased stability, slower rate of dissociation, better retention of encapsulated drug and more site-specific accumulation of a drug at the target site are all characteristics of polymeric micelles. This is in contrast to other polymeric carriers such as drug-polymer conjugates, which have limited water solubility, precipitation and difficulty injecting into bloodstream due to most medications hydrophobic nature. These characteristics are attributed to architecture, which results in a wide range of desirable performance. PMs can easily be used to fill a wide range of medicines with low solubility resulting in increased bioavailability. Importantly, these can be utilized to successfully target specific areas of the body that are affected by disease. Because of the great drug loading capacity of the inner core and the unique disposition features in the body due to their size, PMs allow access to targeting. End-functionalization of block copolymers periphery with sugars and peptides results in a variety of micelles with changed biological properties that can be exploited for receptor-medicated targeted medication and gene delivery. Another method of targeting is Immunomicelles, which are made via covalent bonding monoclonal antibody molecules

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to a surfactant or PMs have great binding specificity and target ability, are another method of targeting. As a result, PMs as medication transporters have a bright future. PMs are aqueous solution-structured particles. hydrophobic domains are produced by the micelle core and can be exploited to solubilize hydrophobic molecules. To tackle the problem, solvency improvement usually results in hydrophobic drug higher oral bioavailability, and much of the poorly water-soluble medicine can be easily injected into the centre of PMs. Due to the strong drug loading capabilities of the inner core and PMs, they provide exposure to targeting. Due to the strong drug loading potential of the inner core and specific disposition properties in the body, PMs enable exposure to targeting.

Two methods are used to make PMs-

A.Solvent based direct dissolution of polymers accompanied by dialysis process

B. By adding a solvent, the precipitation of one block [6]

Drug loading in micelle is done by methodologies:

1. Direct dissolution process

- For moderately hydrophobic copolymers
- When copolymer + drug is combined in water over the critical micelle concentration (CMC), micellular production occurs.
- Technique is easy
- Efficiency of drug loading is poor

2. Indirect dissolution method:

- An amphiphile, which is not readily soluble in water. For drugs as well as copolymers, standard solvent is used and the solvent is extracted by any suitable process
- This method uses common organic solvent, e.g. Dimethyl sulfoxide DMSO,Acetone [7]

**ADVANTAGES OF PMS:**

- The solubility of the initial drug is improved by PMs, thus enhancing the biocompatibility
- Mechanical clearance is prevented by the hydrophilic shell and nanoscopic size
- Physical entanglement or chemical conjugation can be used to include different functional groups.
- High kinetic stability ensures that the integrity of the system is preserved.
- It has high inner core drug loading capacity ability
- It can be employed in a receptor- mediated drug delivery system

DISADVANTAGES OF PMS:

- Use only for lipophilic drug
- Low drug-loading capacity
- Dependency of critical micelles concentration [8]

PMs recyclable, biocompatible and easy to install, showing positive application to drug delivery systems. Polyme micelles are created by the self-aggregation of amphiphilic block polymers consist of hydrophobic core, and an outer hydrophilic shell, which stabilizes formulation in aqueous solution. So, ezetimibe (EZM) loaded PMs showed better therapeutic efficacy than free drug. This novel approach will justify for our category of drug. The purpose of this study will to develop PMs containing EZM lowers plasma cholesterol levels by inhibiting the absorption from intestine. [1]

In this present study, Ezetimibe polymeric micelle formula optimize by factorial design software the optimized formula is subjected to scale up process. Ezetimibe is low soluble and low bioavailable molecule. The bioavailability and therapeutic efficacy could be improved by sustained release formulations. In this study the Ezetimibe polymeric micelle formulation was optimized by using 2- factor, 3-level 3 2 full factorial design are one of the most efficient designs. The optimized formulation was evaluated in terms of parameters like particle size and entrapment efficiency.

## II. MATERIALS AND METHODS

Ezetimibe was a gifted from IPCA laboratories, Mumbai, India. D-α To copherol Polyethylene Glycol 1000 Succinate were purchased from Sigma Aldrich, Mumbai, India. Poloxamer F 68 were purchased from BASF, Mumbai. Ethanol, methanol were obtained from Fine Chemical LTD. ,Mumbai, India. Disodium Hydrogen and Phosphate Potassium Dihydrogen were purchased from Molychem, Mumbai. The distilled water used was prepared in our laboratory.

**PREPARATION OF FORMULATIONS**

**FORMULATION OF DRUG, AND PLURONICF 68-TPGS LOADED MICELLES**

The micelles of EZM were prepared by using Solvent evaporation.

**GENERAL SCHEMATIC PRESENTATION OF FORMULATION DEVELOPMENT**

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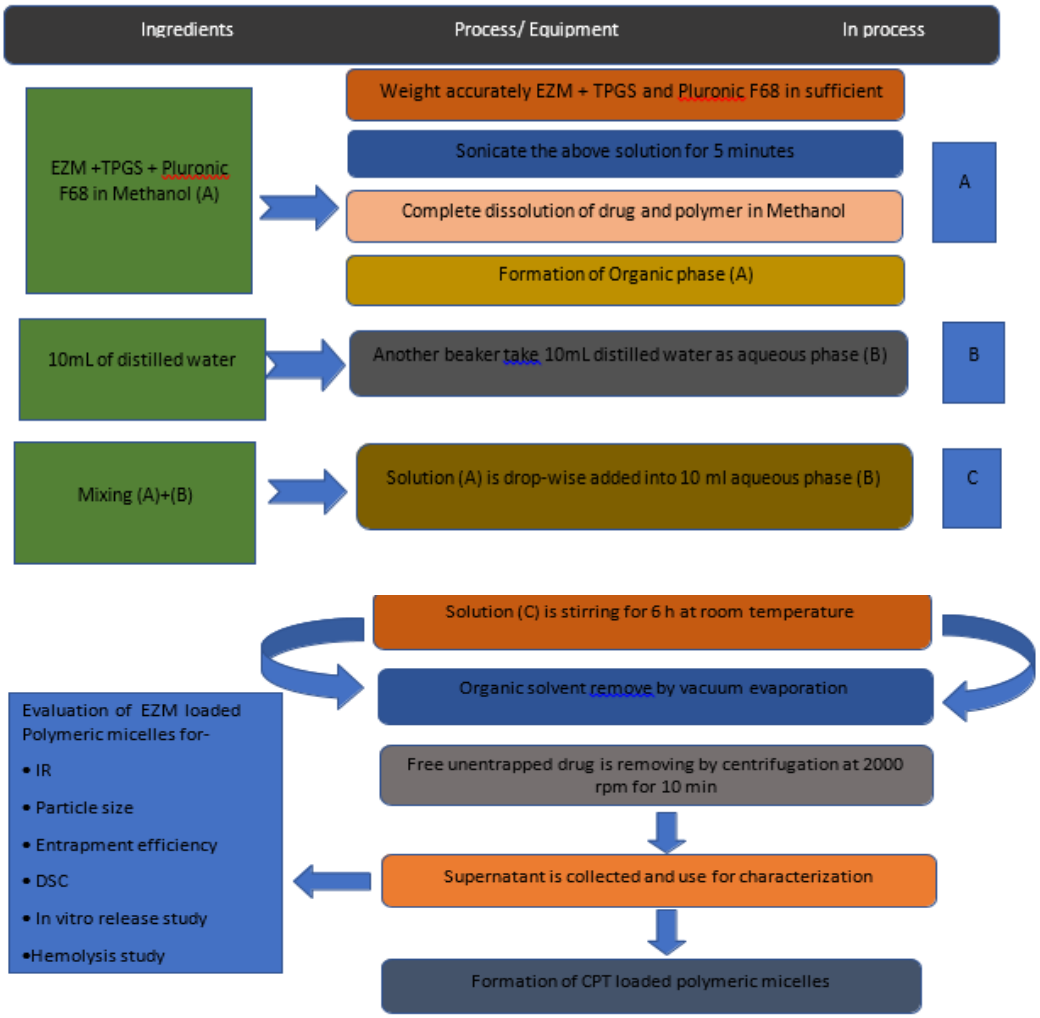


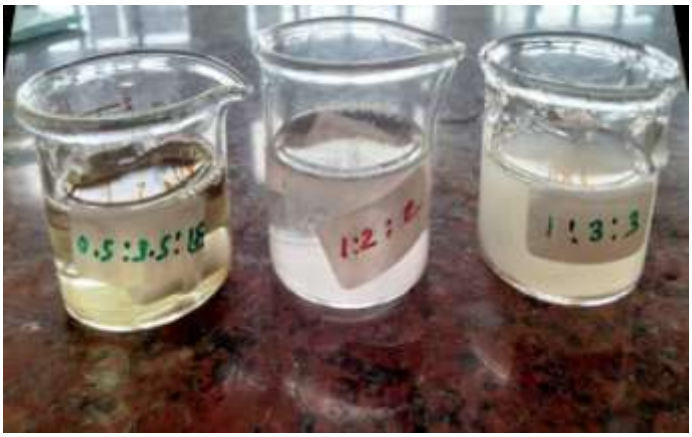


**Fig.2 :Polymeric micelle batches(A,B,C)**

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**Tableno.1:Formulation design for solvent evaporation batches(w/w)**

| Batch | EZM | PluronicF68 | TPGS |
|---|---|---|---|
| A | 0.5 | 3.5 | 1.5 |
| B | 1 | 2 | 2 |
| C | 1 | 7 | 3 |

**32 CENTRAL COMPOSITE DESIGN (CCD):**

The design of $3^2$ plaques was applied to study the co-effect of the formulation variants, eachon 3 levels and 9 possible groups of PMs. The combination of Drug: TPGS (X1) and PluronicF68 (X2) concentrations were exercised as objective variables. Particle sizes (Y1), %EE (Y2) had used as secondary variables.

**Tableno.2: Factor and levels for 32central composite design**

| Independentvariables | Levels | | |
|---|---|---|---|
| | Low(-1) | Medium(0) | High(+1) |
| Concentration ofEZM:TPGS(X1) | 1% | 2% | 3% |
| ConcentrationPluronic F68(X2) | 1% | 2% | 3% |

**BATCHES FORM DESIGN-EXPERT**

The full design of the factors with the help of the design expert provides the number of batches needed to improve the various standards. The design expert has a number of designs included, such as Fractional, Central Composite, Placket Burman, Box-Behnken and so on. The central composite of the design was picked from all of the above, since it is used for stardesign and central point variables.

**Table No.3:Batches for Design Expertinactual terms**

| | | **Factor1** | **Factor2** | **Response 1** | **Response2** |
|---|---|---|---|---|---|
| **Std** | **Run** | **A:EZM -TPGS** | **B:Pluronic F68** | **Particlesize** | **%EE** |
| | | **M.mol** | **M.mol** | **nm** | **%** |
| 5 | 1 | 1.58579 | 5.25 | 142.29 | 25.21 |
| 8 | 2 | 3 | 7.72487 | 133.7 | 37.3 |
| 2 | 3 | 4 | 3.5 | 104.4 | 16.06 |
| **6** | **4** | **4.41421** | **5.25** | **145.1** | **32.44** |
| 4 | 5 | 4 | 7 | 140.3 | 35.54 |
| 1 | 6 | 2 | 3.5 | 110.1 | 21.23 |
| 9 | 7 | 3 | 5.25 | 142.34 | 39.21 |
| 3 | 8 | 2 | 7 | 139.27 | 30.01 |
| 7 | 9 | 3 | 2.77513 | 101.01 | 19.22 |

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**Characterization of prepared micelles**

**PERCENTAGE ENTRAPMENT OF EZM LOADED MICELLES (SOLVENT EVAPORATION METHOD)**

0.1 ml of batch of drug loaded micelle formulations formulated by solvent evaporation technique were diluted up to 10 ml by methanol (StockA). Then withdrawn 0.5ml from stock A diluted up to 10ml by methanol. These samples were scanned and read for absorbance on Single beam UV- Visible spectrophotometer (Agilent 1800) and matched quartz cells of 1 cm in width. The readings obtained at 234nm were used for determining the % EE and % DL of formulations.

$$\%\text{Encapsulation Efficiency}=\frac{\text{Weig ht of drugmicelles}}{\text{weig htof drug taken initially}}\text{X } 100$$

$$\%\text{Drug Loading Efficiency } =\frac{\text{Weig ht of drug micelles}}{\text{Weig ht of drugfed initially } +\text{Weig ht of polymers}}\text{X } 100$$

**PARTICLESIZEANDZETAPOTENTIALANALYSIS**

The particle size and Zeta potential of EZM and Pluronic F68- TPGS loaded micelles were analyzed using Malvern zeta size analyzer. The sample was placed in cuvette and it was then kept in DLS analyzer, zeta potential and particle sizes were recorded.

**DIFFERENTIAL SCANNING COLORIMETRIC STUDY (DSC):**

DSC has shown to be an efficient tool for the pharmaceutical business in disseminating critical information on the physiochemical properties of pharmaceuticals and excipient compounds, such as purity, drug stability, and formulation compatibility, among other things. This process necessitates a minimum purity of around 97 percent. As a result, the DSC method is rarely employed to ensure purity.

**FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)**

Infrared spectra of polymeric micelle were obtained using FTIR spectrometer in the range of 4000-400cm -1 . This was done to study about the compatibility between drug and polymer in the polymeric micelle In-vitro drug release study.

**PREPARATION OF DISSOLUTION MEDIUM PBS PH**

6.8 28.20 gm. of disodium hydrogen phosphate, 11.45 gm. of potassium dihydrogen phosphate was dissolved in 1000 ml of distilled water. Adjust the pH if necessary.

**PREPARATION OF 2% SODIUM BICARBONATE**

2. gm. of sodium bicarbonate was dissolved in 100 ml of distilled water PREPARATION OF 1MM EDTA

74.44 mg EDTA was dissolved in 200ml of distilled water.

**ACTIVATION OF DIALYSIS BAG**

Dialysis bag (Hi-media) was placed for approximately 30 min in distilled water to remove storage solutions (glycerol). Then it was rinsed with distilled water. The rinsed dialysis bag was soaked for 3 to 4 hrs. in 2% sodium bicarbonate solution containing 1mM EDTA in order to remove metal traces. The bag was then rinsed with distilled water. It was then placed in Phosphate buffer saline solution for overnight before the study.

**DRUG RELEASE STUDY**

Release of EZM from micelle was studied using the dialysis method at 37+2°C room temperature and was compared with the pure drug solution. The drug Solution micelles equivalent to 2 mg of EZM were placed in dialysis tubes (MWCO 12000) and tightly sealed. Then, the tubes were immersed in 50 ml of release medium, i.e., PBS (pH 6.8) c. While stirring the release medium using the magnetic stirrer at 150 rpm/min, the samples (1 ml) were withdrawn at predetermined time intervals (30 min, 1, 2, 4, 3, 4, 5,6, and 24 hrs.) from the release medium and the same volume was replaced with fresh medium. The 1 mL of sample was centrifuged at 150 rpm for 5 min and the supernatant was bath sonicated for 5 min and analyzed using by UV-visible spectrophotometer

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(Agilent) at 234nm.

**Fig.3:Drug release study by using dialysisbag**

**STABILITY STUDY OF MICELLES:**

The micelle formulations were stored at 2-8°C and at room temperature for one month. After a period of 15 days & 30 days the formulations were analyzed for % EE and % DLE using UV- visible spectrophotometry as an analytical method. 1 mi of formulation was centrifuged and the supernatant was diluted up to 10 ml with methanol, sonicated for 10 and analyzed for EZM remaining.

## III. RESULTS AND DISCUSSION

The trend toward Quality by Design was beneficial to the enhancement of ezetimibe polymeric micelles. Organoleptic characteristics, melting point determination, solubility analysis, UV- spectroscopy investigation, FTIR study, and DSC analysis were all part of the ezetimibe preformulating study. The choice of polymer in the formulation of polymeric micelles, such as TPGS and pluronic F68, has an impact on the loading effectiveness of polymeric micelles.

**PREFORMULATION STUDY**

**ORGANOLEPTIC TESTS**

The results obtained from organoleptic tests are mentioned in table

| Sr.No. | Physicalproperties | Methods | Description |
|---|---|---|---|
| 1. | PhysicalState | Visualobservation | Solid amorphouspowder |
| 2. | Color | Visualobservation | White |
| 3. | Odour | Smellingby nose | Noodour |

**Table No.4:Organoleptic tests**

**IDENTIFICATIONTEST**

The identification tests for ezetimibe were performed by the different respective methods. The results are mentioned in table and values observed are within range. From the results it is clear that the ezetimibe is in pure form.

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| Sr.No. | Physicalproperties | Methods | Description |
|---|---|---|---|
| 1. | Solubility | Visualobservation | Insoluble in water andverysolubleinmethanol andethanol |
| 2. | Melting Point | Capillarymethod | 164 to166$^0$C |
| 3. | UVSpectra | UVVisiblespectrometer | UVspectrawereobtainedatstandardcondition and it showsλmaxof234nm inmethanol |
| 4. | Infra-redspectra | IRspectroscopy | Theinfra-redspectrumisaccordantwithreference spectrum. |
| 5. | DifferentialScanning Calorimetry (DSC)study | Colorimeter | Melting point ofezetimibeis 164$^0$C |

**Table No.5 :Identification tests of ezetimibe**

**FTIR ANALYSIS**

The FTIR spectrum of Ezetimibe was recorded using FTIR (cary-630 Agilent technology). The spectrum was recorded over the range of wave no. 4000 to 400 cm -1 The spectra observed is shown in figure.4. The values of major peaks in FTIR spectrum of Ezetimibe are mentioned in table 6. From the observed peak it is clear that the sample is Ezetimibe

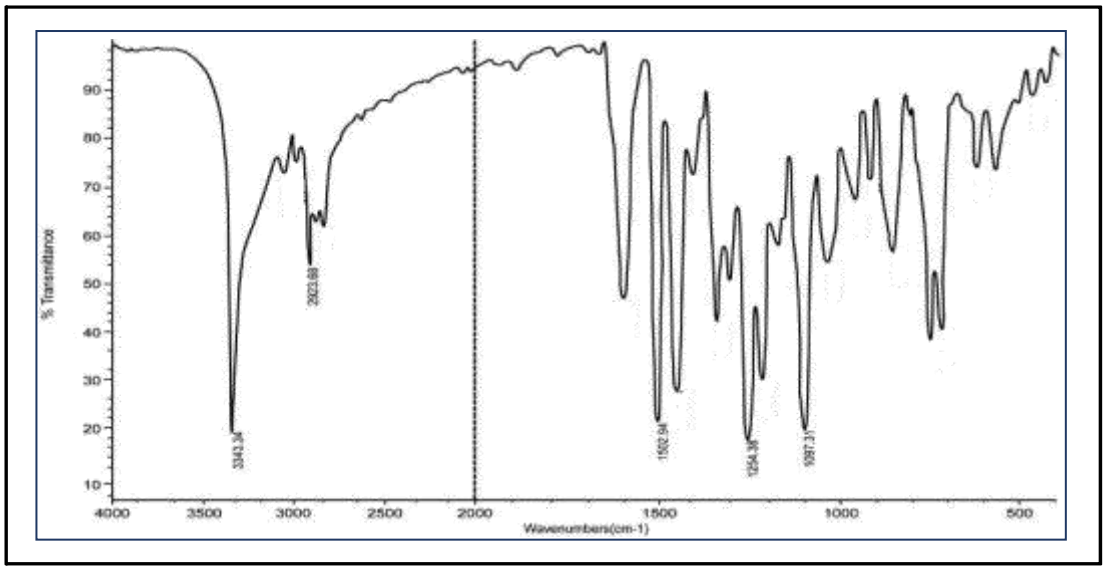


**Fig.4: FTIR spectra of Ezetimibe pure drug**

| Sr. No. | Functionalgroup | Peak(Wavenumber)cm$^{-1}$ (Observedvalue) | Peak (Wavenumber)cm$^{-1}$ (Reportedvalue) |
|---|---|---|---|
| 1. | O-H$_{Ar}$stretch | 3315.63 | 3600-3400 |
| 2. | O-H$_{Ali}$stretch | 3343.34 | 3600-3400 |

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| | | | |
|---|---|---|---|
| 3. | $C=O_{Amide}$ | 1684.28 | 1700 |
| 4. | $C=C_{Ar}$ | 1588.33 | 1600 |
| 5. | C-N | 1271.09 | 1400 |
| 6. | C-F | 1097.31 | 1100 |

**Table. 6:Functional groups of Ezetimibe pure drug**

**DSC OF THE PURE DRUG EZETIMIBE**

The DSC study of pure ezetimibe shows endothermic peak indicating the melting of ezetimibe at 164 0 C which marches with reported melting point of drug and confirms the drug under study was ezetimibe and highly pure.

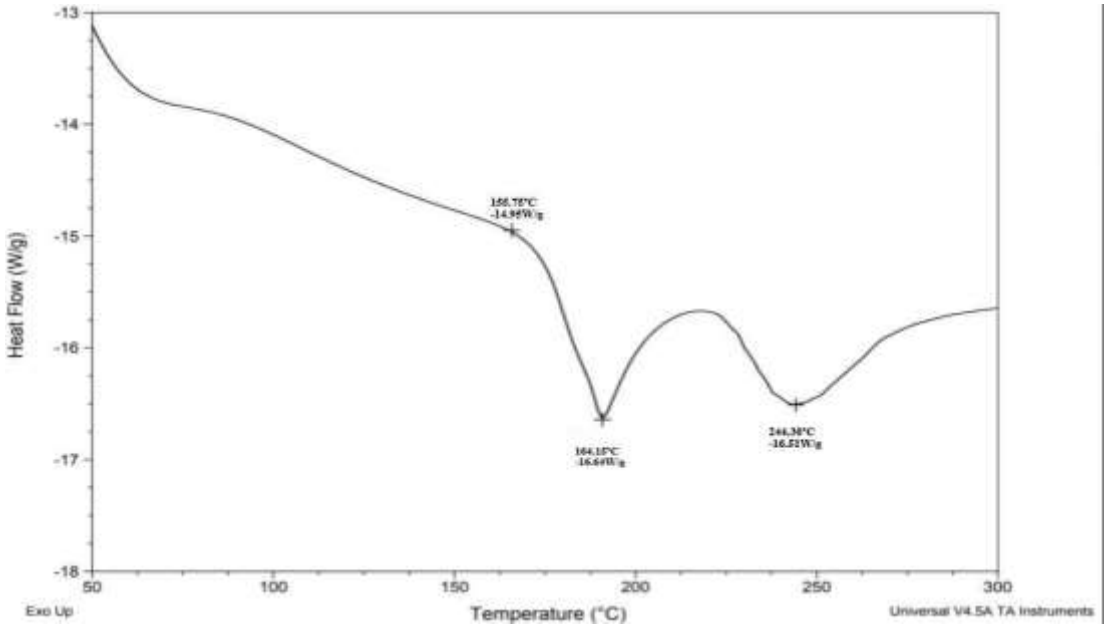


**Fig.5. DSC of the pure drug EZM**

**UV-SPECTROSCOPIC ANALYSIS**
**DETERMINATION OF Λ MAX**

λ max of Ezetimibe by UV-spectrophotometer in methanol was found to be 234 nm and it is very close to standard λ max the UV- Visible spectrum of Ezetimibe is shown in Figure 6. and the value observed is shown in table 7.

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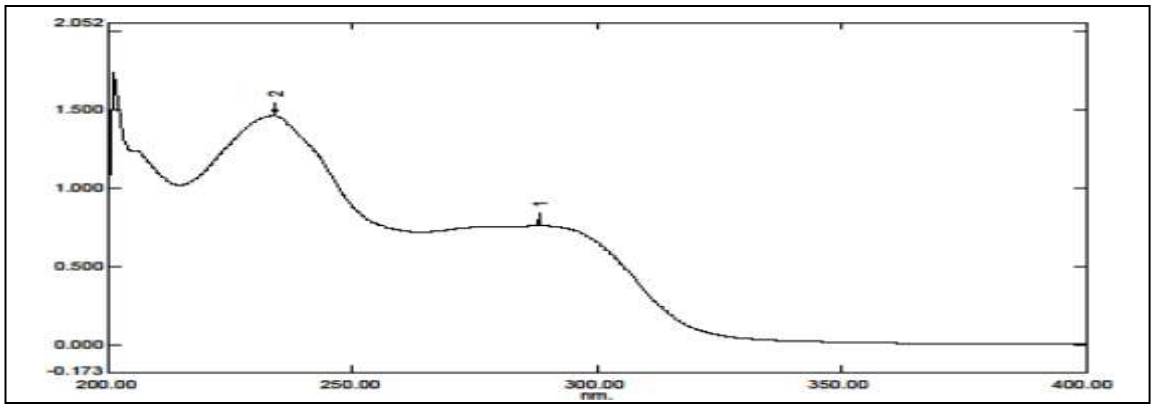


**Fig.6:UV-Visible adsorption spectrum of ezetimibe**

| Sr.No | Solvent | ƛ max(nm) |
|---|---|---|
| 1. | Methanol | 234 |

**Table.7:Wave length of maximum absorbance(ƛ max)**

**DEVELOPMENT OF CALIBRATION CURVE OF EZETIMIBE USING UV-VISIBLE SPECTROPHOTOMETRY**

The graph of absorbance vs. concentration for pure Ezetimibe was found to be linear in concentration range 0.5-3.5 ppm at 234nm. The drug obeys Beer-lamberts law in the range0.5- 3.5 ppm. Standard calibration curve values of ezetimibe in methanol are shown in Table.8. and calibration curve shown in fig.7. The R 2 value was found to be 0.9993

| Sr.No. | Concentrationµg/ml | Absorbance |
|---|---|---|
| 1 | 0.5 | 0.228 |
| 2 | 1 | 0.408 |
| 3 | 1.5 | 0.598 |
| 4 | 2 | 0.782 |
| 5 | 2.5 | 0.978 |
| 6 | 3 | 1.190 |
| 7 | 3.5 | 1.40 |

**Table.8: Calibration curve value of ezetimibe in methanol by UV-Visible spectrophotometry**

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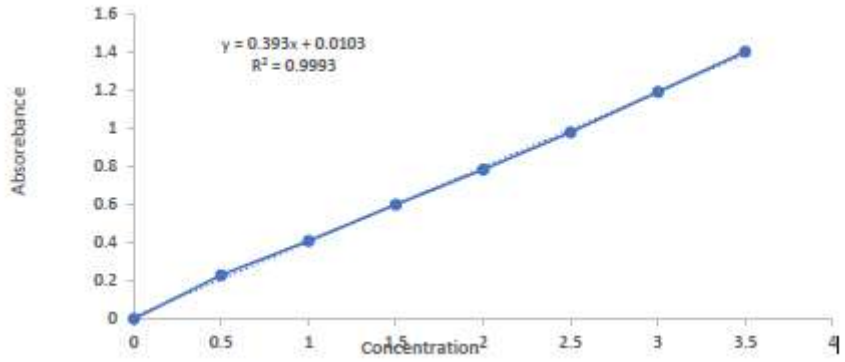


**Fig 7:Calibration curve of ezetimibe in methanol UV-Visible spectrophotometry**

**DEVELOPMENT OF CALIBRATION CURVE OF EZETIMIBE IN PHOSPHATE BUFFER PH 6.8 USING UV-VISIBLE SPECTROPHOTOMETRY**

The graph of absorbance vs. concentration for pure Ezetimibe was found to be linear in concentration range0.5-3.5 ppm at 234nm. The drug obeys Beer-lamberts law in the range 0.5-3.5 ppm µg/ml. Standard calibration curve values of ezetimibe in phosphate buffer pH 6.8 are shown in Table 9. and calibration curve shown in fig 8. The R 2 value was found to be 0.9995.

| Sr.No | Concentrationµg/ml | Absorbance |
|---|---|---|
| 1 | 0.5 | 0.162 |
| 2 | 1 | 0.312 |
| 3 | 1.5 | 0.477 |
| 4 | 2 | 0.602 |
| 5 | 2.5 | 0.768 |
| 6 | 3 | 0.914 |
| 7 | 3.5 | 1.08 |

**Table.9: Calibration curve value of ezetimibe in phosphate buffer pH 6.8 by UV-Visible spectrophotometry**

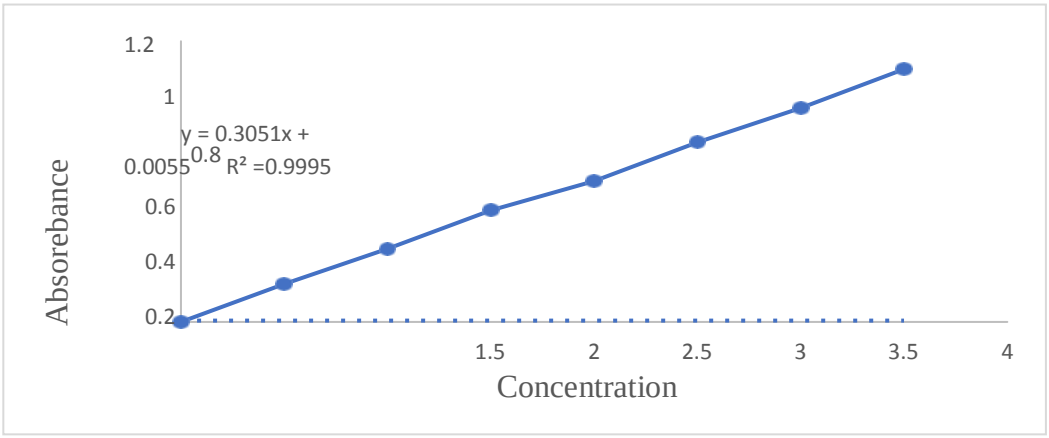


**Fig.8: Calibration curve of ezetimibe in phosphate buffer pH 6.8 UV-Visible spectrophotometry**

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**DEVELOPMENT OF CALIBRATION CURVE OF EZETIMIBE IN ACETATE BUFFER PH 4.5 USING UV-VISIBLESPECTROPHOTOMETRY**

The graph of absorbance vs. concentration for pureAcetate buffer pH4.5was found to belinear in concentration range 0.5-3.5 ppm at 234nm. The drug obeys Beer-lamberts law in therange 0.5-3.5ppm. Standard calibration curve values of ezetimibe in phosphate buffer pH6.8are shown in Table 10. and calibration curve shown in fig.9. The $R^2$ value was found tobe0.9977.

| Sr.No | Concentrationµg/ml | Absorbance |
|---|---|---|
| 1 | 0.5 | 0.172 |
| 2 | 1 | 0.312 |
| 3 | 1.5 | 0.477 |
| 4 | 2 | 0.654 |
| 5 | 2.5 | 0.808 |
| 6 | 3 | 0.941 |
| 7 | 3.5 | 1.104 |

**Table.10:Calibration curve value of ezetimibe in Acetate buffer pH 4.5 by UV-Visible spectrophotometry**

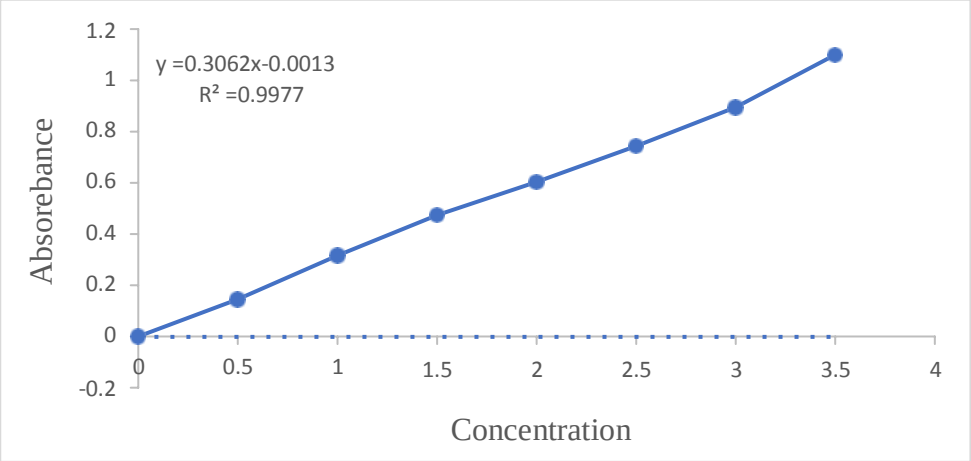


**Fig.9: Calibration curve of ezetimibe in Acetate buffer pH 4.5 UV-Visible spectrophotometry**

**DESIGN OF EXPERIMENT FORMULATION OPTIMIZATION 32 CENTRAL COMPOSITE DESIGN (CCD):**

A 32 Central Composite Design (CCD) was used in the study. The data obtained held combined into the software Design expert 10. It was functional to observe the united consequence of two formulation variables, each at 3 levels and the possible 9 combinations of ezetimibe PMs. The attentiveness of Ezetimibe: TPGS (X1), Pluronic F68 (X2) was taken as independent variables. The Particle size (Y1) and the EE% (Y2) did take as dependent variables. They prepared and tested nine formulations for the response. The data obtained held combined into the software. Variance Analysis was used to verify architecture. After considering both the positive or negative scale of the coefficients and a statistical symbol it carries, the

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polynomial equations may be utilized for inference.

| Batch No. | Factor1 | Factor2 | Response1 | Response2 |
|---|---|---|---|---|
| | A:EZM-TPGS (X1) | B:PluronicF68 (X2) | Particlesize (Y1) | %EE (Y2) |
| | M.mol | M.mol | nm | % |
| B1 | 1.58579 | 5.25 | 142.29 | 25.21 |
| B2 | 3 | 7.72487 | 133.7 | 37.3 |
| B3 | 4 | 3.5 | 104.4 | 16.06 |
| B4 | 4.41421 | 5.25 | 145.1 | 32.44 |
| B5 | 4 | 7 | 140.3 | 35.54 |
| B6 | 2 | 3.5 | 110.1 | 21.23 |
| **B7** | **3** | **5.25** | **142.34** | **39.21** |
| B8 | 2 | 7 | 139.27 | 30.01 |
| B9 | 3 | 2.77513 | 101.01 | 19.22 |

**Table.11:Summary of experimental design**

**OPTIMIZATION USING 22 CENTRAL COMPOSITE DESIGNS:**

**MATHEMATICAL MODELING AND STATISTICAL ANALYSIS USING ANOVA:**

Mathematical model was mechanized to understand effect of amount of EZM, concentration of TPGS, and concentration of Pluronic F68 on the responses.

**COUNTER PLOT:**

Two dimensional counter plots are really helpful for studying the part's interface effect on the answers. These kinds of graphs are helpful for simultaneously observing the impact of two variables on the reaction.

**COUNTER PLOT OF PARTICLE SIZE (Y1) WITH VARIABLES X1 AND X2:**

It was determined from counter plot fig. No.10 that the PS of EZM PMs was decreased with of increase in concentration of TPGS upto certain concentration followed by increase in the particle size. Similarly, in the external stage, the high surfactant concentration increases the drug solubility and can increase the partitioning of the drug from the internal process. The particle size increases with increase in polymeric concentration and produce larger particles.

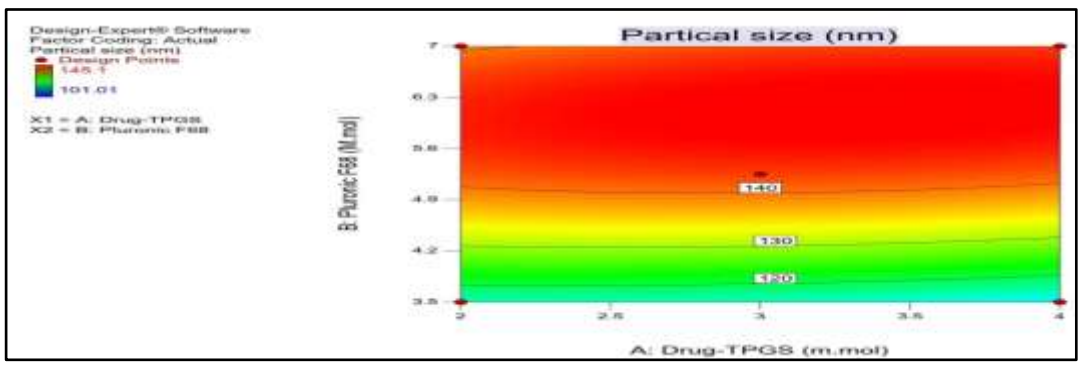


**Fig.No.10.Counter plot of Particle size (Y1) with variables X1 and X2**

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**COUNTER PLOT OF %EE (Y2) WITH VARIABLES X1 AND X2:**

It was determined from counter plot fig. No.11. that the %EE of EZM PMs was increased with increase in concentration of TPGS. [36] Therefore, entrapment efficiency increases with enhance in the amount of the TPGS and Pluronic F68 helped the drug to remain within the particles and/ or the surface of the particles. As expected, EE significantly increased with decreasing the particle size.

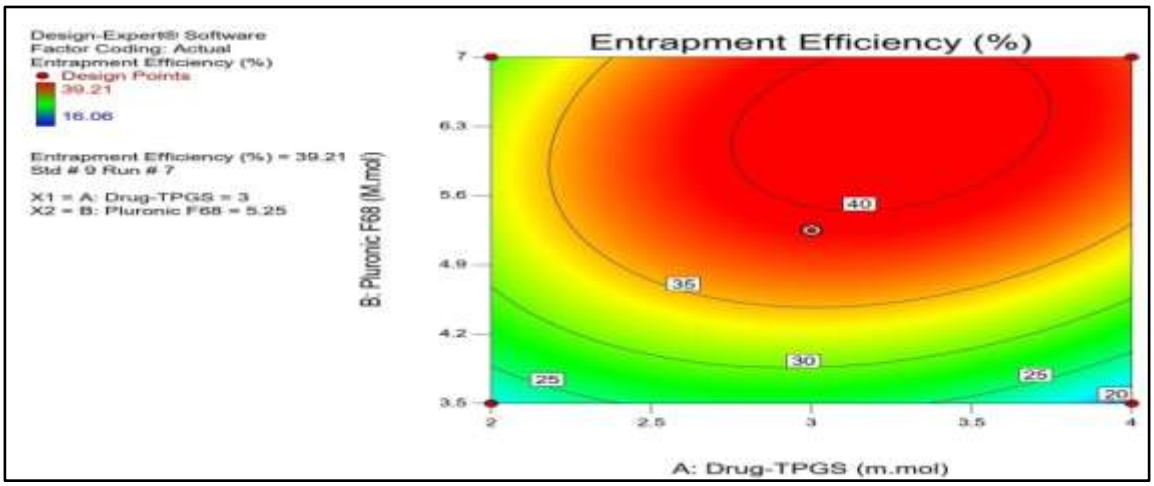


**Fig.11. Counter plot of % EE (Y2) with variables X1 and X2**

**3DRESPONSE PLOT OF PARTICLE SIZE:**

It was determined from 3D plot fig. No.12. that the PS of EZM PMs was decreased with increase in concentration of TPGS upto certain concentration followed by increase in the particle size. The particle size increases with enhance in polymeric concentration and produce larger particles.

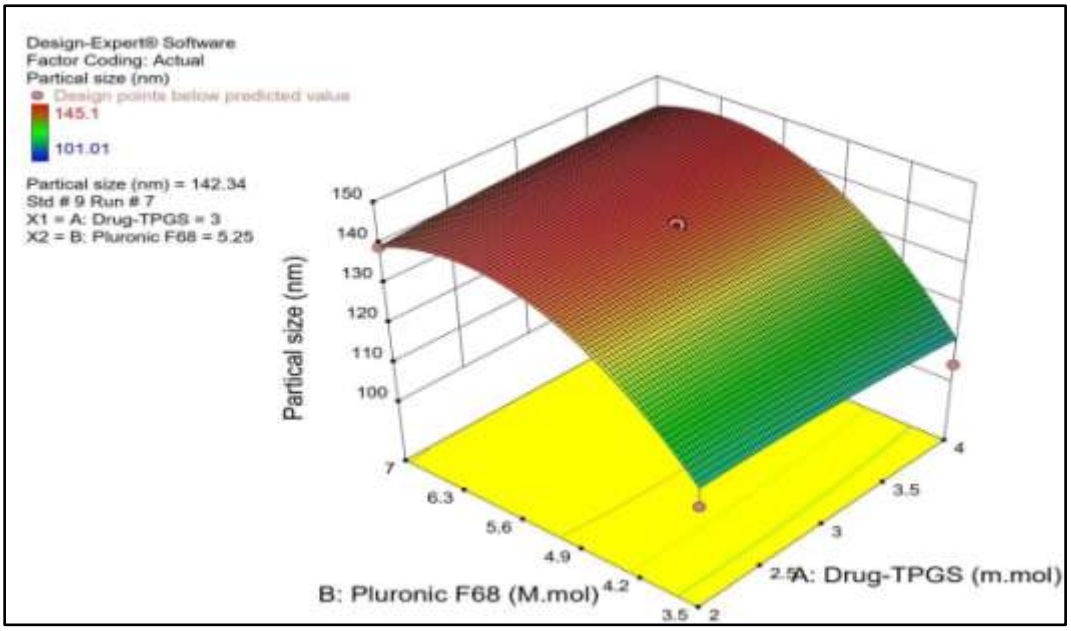


**Fig.No.123D Response plot of Particle size**

**3DRESPONSEPLOTOF%EE**

It was determined from 3D plot fig.No.13. that the %EE of EZM PMs was increased with increase in concentration of TPGS. Similarly, in the

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external stage, the high concentration of surfactant increases the drug solubility and can increase the partitioning of the drug from the internal process. Therefore entrapment efficiency increases with enhance in the amount of the TPGS and Pluronic F68 helped the drug to remain within the particles and/ or the surface of the particles. As expected, EE significantly increased with increasing the polymeric concentration.

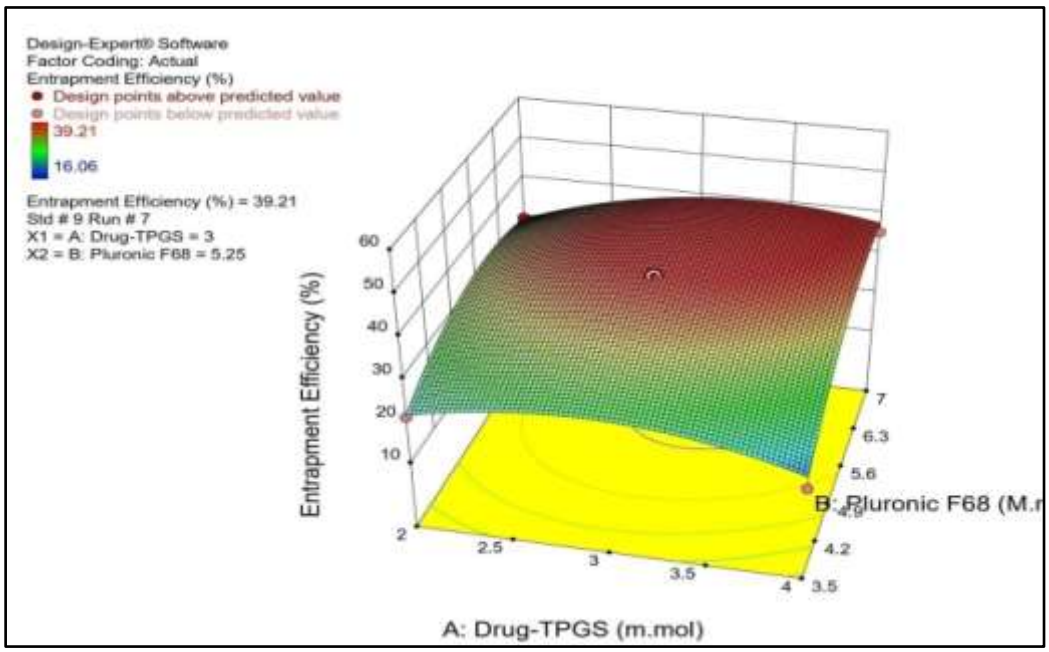


**Fig.No.13 3DResponse plot of % EE**

**PROBABILITY PLOTS:**

Figures 14-15 showed the usual likelihood graph of particle size and percent EE: describe whether the residual follows a normal distribution, where that situation the objects would follow a linear line. Also with data points, expect any dispersion. Look for only particular trends, such as the S- shaped curve, which means that a better interpretation can be given by a transformation of the answer. We can infer from this that the usual wave equation of the blue spot implies that the variable distributed along the straight line has no significant effect.

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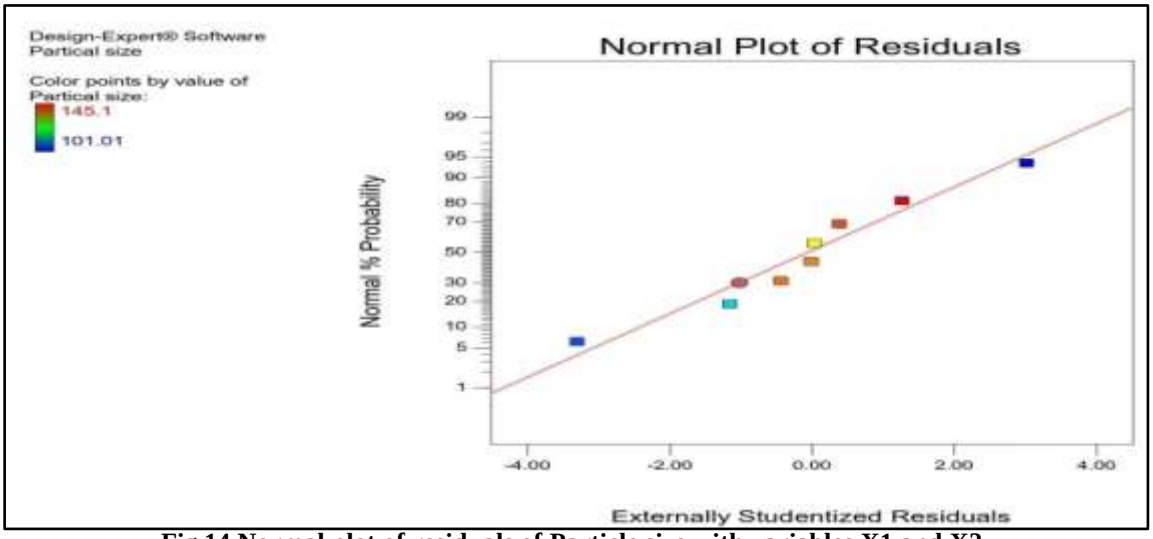


**Fig.14 Normal plot of residuals of Particle size with variables X1 and X2**

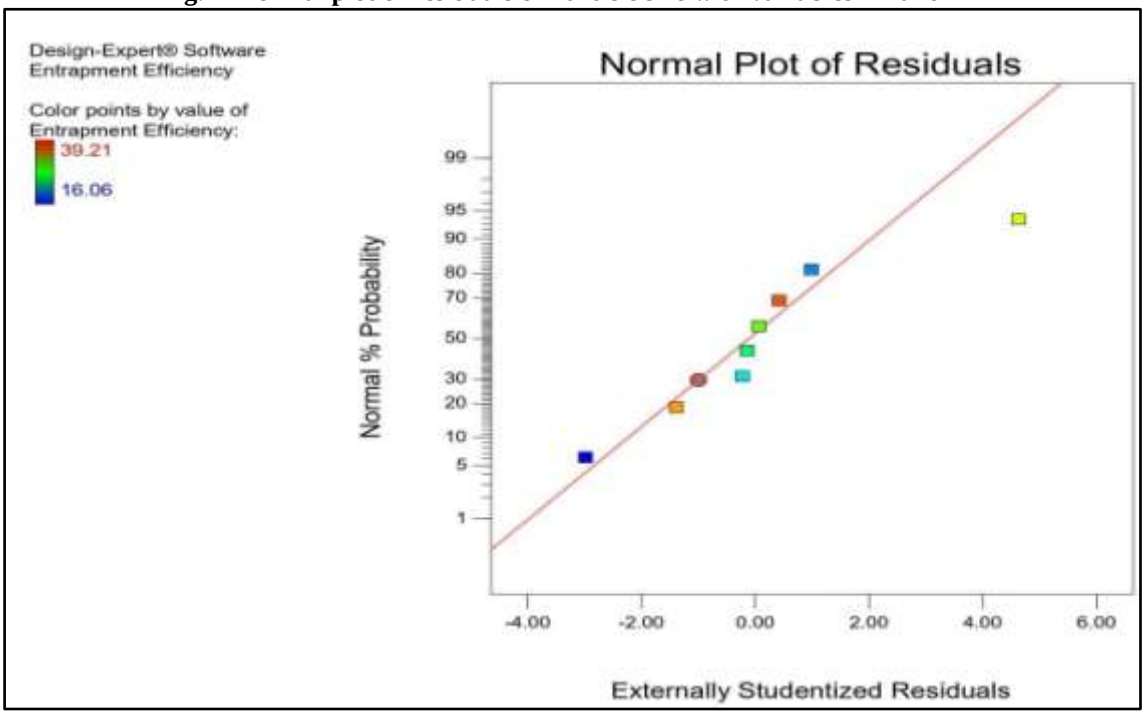


**Fig.15 Normal plot of residuals of Entrapment Efficiency with variables X1 andX2**

**MULTIPLE REGRESSION ANALYSIS:**

The central composite design was done using the Design Expert. Answer surface methodology graphs were used to evaluate the interaction factor for the variable considered. The importance of linear models implies that model terms are important. As an independent variable, the concentrations of EZM - TPGS and Pluronic F68 were picked. In order to analyse the answer, a numerical form comprising dynamical and polynomial terms was used. The equation for the two Y2 (% EE) and Y1 (PS) responses in terms of coded parameter is provided in the table. For line a rmodels,

$$Y1=\beta0+\beta2X1+\beta2X2+\beta11X1Y1$$

**ANALYSIS OF VARIANCE OF PARTICLE SIZE:**

The Model F-value of 9.72 implies the

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model is significant.Thereisonlya4.51% chance that an F-value this large could occur due to noise. Values of "Prob > F" less than 0.0500indicate model terms are significant. In this case B, $B^2$ are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Particle size=+142.34 -0.087*A +13.91*B+1.68*AB- 1.07*A2-14.24*B2

The equation in terms of coded factors can be used to make predictions about the response forgiven levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels of the factors are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

| Source | Sumof Squares | df | Mean Square | FValue | p-value Prob> F |
|---|---|---|---|---|---|
| Model | 2462.25 | 5 | 492.45 | 9.72 | 0.0451 |
| A-DRUG-TPGS | 0.061 | 1 | 0.061 | 1.196E-003 | 0.9746 |
| B-Pluronic F68 | 1548.48 | 1 | 1548.48 | 13.58 | 0.0117 |
| AB | 11.32 | 1 | 11.32 | 0.22 | 0.6686 |
| $A^2$ | 3.36 | 1 | 3.36 | 0.066 | 0.8134 |
| $B^2$ | 590.26 | 1 | 9590.26 | 11.66 | 0.0420 |
| Residual | 151.92 | 3 | 50.64 | | |
| Cor Total | 2614.16 | 8 | | | |
| Std.deviation | 7.12 | R-Squared | 0.9419 | | |
| Mean | 128.72 | AdjR-Squared | 0.8450 | | |
| C.V.% | 5.53 | PredR-Square | N/A | | |
| PRESS | N/A | AdeqPrecisio | 8.322 | | |

**Table.12Analysisof variance table (Partial sum of squares-type III) of particle size**

**ANALYSISOF VARIANCEOF EE%**

The Model F-value of 11.02 implies the model is significant. There is only a 3.80% chance that an F-value this large could occur due to noise. Values of "Prob > F" less than 0.0500indicatemodeltermsare significant. In this caseB, $A^2$,$B^2$ are significant model terms.
Values greater than 0.1000 indicate the model terms are not significant. If there are many in significant model terms (not counting those required to support hierarchy), model reduction may improve your model.
Entrapment Efficiency=+39.21+1.32*A+6.73*B+2.67*AB - 5.90*$A^2$-6.18*$B^2$

The equation in terms of coded factors can be used to make predictions about the response forgiven levels of each factor. By default, the high levels of the factors are coded as +1 and thelow levels of the factors are coded as -1. The coded equation is useful for identifying therelativeimpact

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of thefactors by comparing the factor coefficients.

| Source | Sumof Squares | df | Mean Square | FValue | p-value Prob> F |
|---|---|---|---|---|---|
| Model | 534.93 | 5 | 106.99 | 11.02 | 0.0380 |
| A-DRUG-TPGS | 14.00 | 1 | 14.00 | 1.44 | 0.3158 |
| B-POLAXOMAR | 362.19 | 1 | 362.19 | 37.32 | 0.0088 |
| AB | 28.62 | 1 | 28.62 | 2.95 | 0.1844 |
| $A^2$ | 101.29 | 1 | 101.29 | 10.44 | 0.0482 |
| $B^2$ | 111.22 | 1 | 111.22 | 11.46 | 0.0429 |
| Residual | 29.12 | 3 | 9.71 | | |
| Cor Total | 564.05 | 8 | | | |
| Std.deviation | 3.12 | R-Squared | 0.9484 | | |
| Mean | 28.47 | AdjR-Squared | 0.8624 | | |
| C.V.% | 10.94 | PredR-Squa | N/A | | |
| PRESS | N/A | Adeq Precision | 8.603 | | |

**Table 13.Analysis of variance Analysis of variance table (Partial sum of squares type III) of % EE**

**DRUG RELEASE**

At room temp, the in vitro DR of EZM loaded Polymeric micelles was studied and the results are presented in Table.14 and Figure. 16 after 24 hours, the percentage of CDR from the EZM loaded PM solution was set to be 30-40%. It's really evident at the above reports thatthe EZM loaded PMs demonstrate very slow release of EZM into the blood vessels, thus fulfil the component of an effective system of delivery systems.

| BatchNo. | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 |
|---|---|---|---|---|---|---|---|---|---|
| %CDRat8hrs. | 25.21 | 37.3 | 16.06 | 32.44 | 35.54 | 21.23 | 39.21 | 30.01 | 19.22 |

**Table. 14Cumulative Percentage Drug Release of Design Expert Batches**

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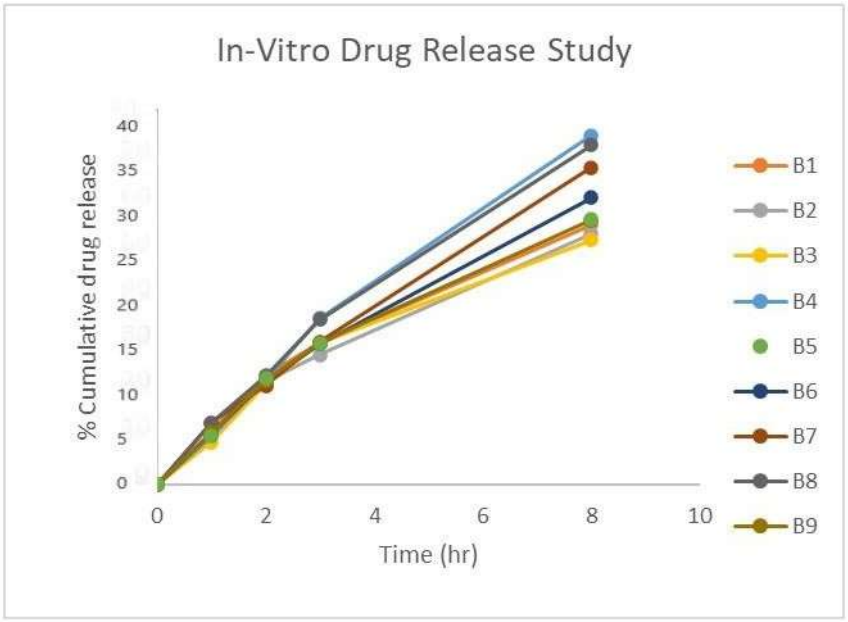


**Fig.No.16In-Vitro Drug Release Study**

**FTIR STUDY:**

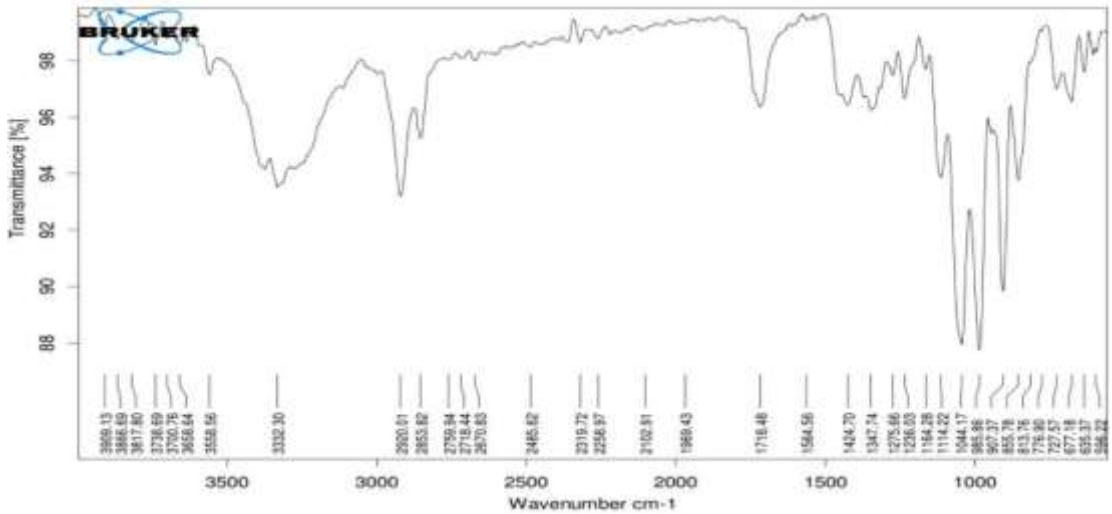


**Figure No17.FTIR analysis of EZMPMs of optimized batch B7**

The FTIR spectra of Ezetimibe and Physical mixture (EZM + TPGS + Pluronic F68) were recorded. The FTIR (Bruker) spectra were recorded over the range of wave no 4000 - 400cm$^{-1}$.Theobserved spectra and results are mentioned below.

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| Sr.No. | Functionalgroup | Observed peak(cm-1)ofplain EZM | Observedpeak($cm^{1}$)ofBatch F7 | Standard peak(cm-1) |
|---|---|---|---|---|
| 1 | O-$H_{Ar}$stretch | 3315.63 | 3000.56 | 3600-3400 |
| 2 | O-$H_{Ali}$stretch | 3343.34 | 2850.22 | 3600-3400 |
| 3 | C=O Amide | 1684.28 | 1718.48 | 1700 |
| 4 | C=$C_{Ar}$ | 1588.33 | 1465.28 | 1600 |
| 5 | C-N | 1271.09 | 1350.28 | 1400 |
| 6 | C-F | 1097.31 | 1114.48 | 1100 |

**Table.15 FTIR analysis of EZMPMs of optimized batch F7**

**DSC ANALYSIS:**

- The DSC (SDT Q600 V20.9 Build 20) graph of measuring melting point temperature of Ezetimibe and Ezetimibe PMs were recorded using.
- The Ezetimibe DSC thermogram showed a high endothermic peak at 164 0 C that leading to the melting of drugs and EZM PMs shows 157 0 C.
- The DSC thermogram of EZM PMs shows the melting endotherm of the drug was decrease which indicates that EZM was completely solubilized inside the matrix of the PMs.
- The absence of a EZM melting endotherm indicated drug transition from crystalline to the amorphous nature.
- These findings indicate substantial controlled drug with the bilayer structure and may explain for improved absorption of EZM into formulations of micelles and sustained release of drugs.

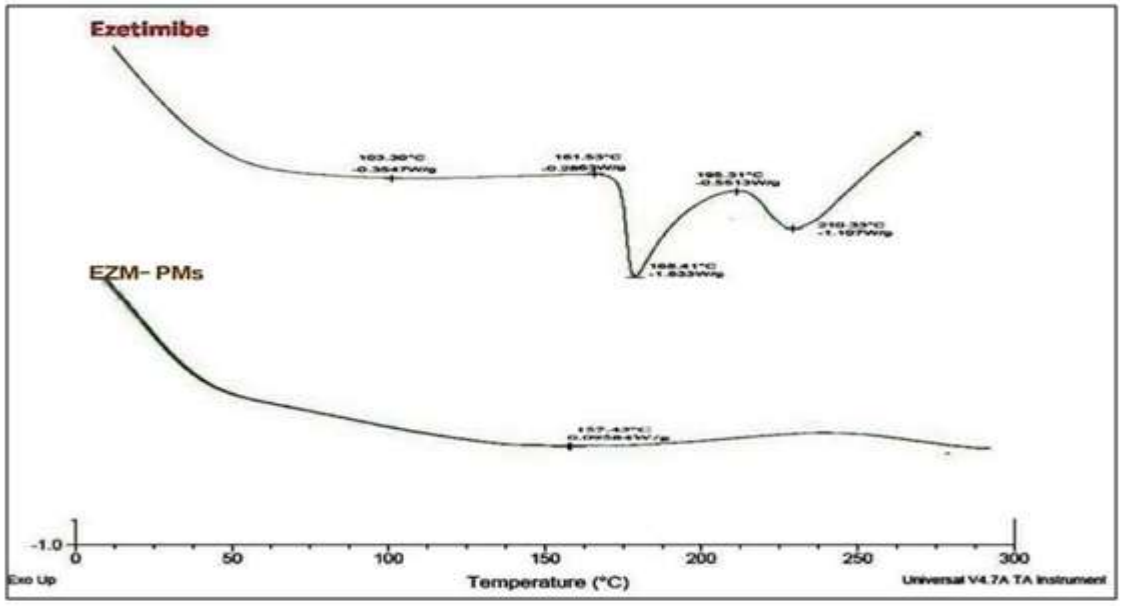


**Figure No.18 DSC thermo graph of EZM**

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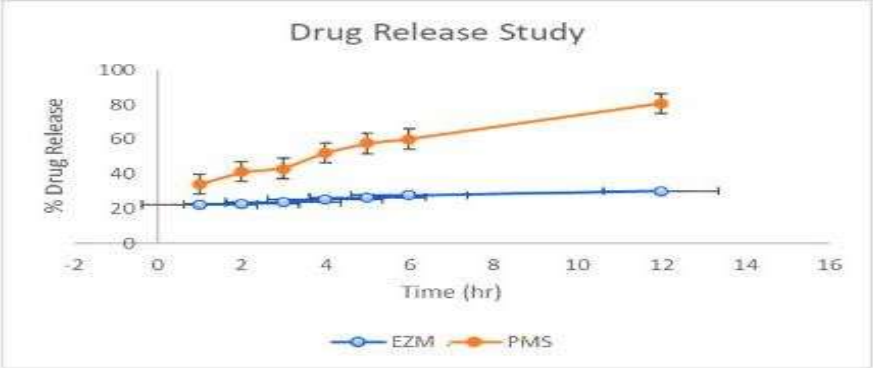
**DISSOLUTION STUDY**

Ezetimibe loaded PMs were tested at room temperature with in Vitro drug release and findings are described in the figure 8.16. The percent cumulative drug release from the plain EZM solution was establish to be 80.56 % after 12 hours and 30.12 % after 24 hours were found to be EZM loaded PMs. In the case of EZM PMs, we observed slightly less and continued release of EZM after 24 h of the sample (only 30 ± 2.6 percent). It is very clear from the above findings that micelles loaded with EZM will display a very slow release of EZM in the bloodstream and therefore meet the criteria for an efficient drug delivery system.




**FigureNo.19. %Drug release study of EZM and PMs**

**STABILITYSTUDY**

The CPT PMs were tested for stability on the terms of the drug content. The findings showed no major improvements in any of the parameters analyzed.

| SamplingTime | Appearance | DrugContent% |
|---|---|---|
| 0 week | StableSolution | 39 |
| 1 week | StableSolution | 32 |
| 2weeks | StableSolution | 25 |
| 3weeks | StableSolution | 21 |

**Table.16 stability study**

## IV. SUMMARY AND CONCLUSION

- The characterization of ezetimibe was done with melting point by capillary method and FTIR spectroscopy.
- The analytical method of UV- Visible spectrophotometry was used for construction of calibration curve of ezetimibe in methanol.
- Excipients were selected on the basis of non-irritating and non-toxic properties.
- The ezetimibe loaded Pluronic F68-TPGS micelles formulations by using different drug to polymer ratios of ezetimibe and Pluronic F68- TPGS were prepared by solvent evaporation technique.
- The prepared micelles were analysed for %EE, %DLE by UV-Visible spectroscopy, particle size, zeta potential, in-vitro drug release ( dialysis method) and stability studies by UV-Visible spectrophotometer (Agilent 1800).
- QbD was applied to the development of EZM loaded polymeric micelle.
- The %EE is 39.21% results showed maximum encapsulation of ezetimibe and Pluronic F68-TPGS loaded micelles at drug to polymer ratio, indicating that increase in drug to lipid concentration increase the ezetimibe loading, however it is also well known that, increase in lipid concentration increase risk of lipid toxicity. To overcome this drawback and to improve encapsulation efficiency of micelles

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by using minimum concentration of lipid, micelles can be prepared.

- All micelles formulations shows size below 142.34 nm which indicates that they can easily permit the cell membrane.
- The in vitro drug release study results showed that complete release of ezetimibe from standard drug solution micelle .While ezetimibe loaded micelle, ezetimibe and Pluronic F68-TPGS loaded micelles would show very slow release of ezetimibe in the blood circulation and therefore meets the requirements for an effective drug delivery system.
- The results of 3 2 full factorial design results revealed that the concentration of TPGS and amount of Poloxamer 188 has significant effect on dependent variables like the particle size (Y1), entrapment efficiency (Y2) and drug release (Y4). B7 batch was selected as optimized batch based on design space and particles size Y1 (142.34nm), entrapment efficiency Y2
- Hence, it is concluded that quality by design approach was versatile method as it gives the cost- effective production method, low risk when the formulation was carried out and all the steps in the QbD were done systematically and within limit which gives best and reliable product to the patient. Thus, polymeric micelles are promising approach for the solubility enhancement of poorly aqueous soluble drug.

## REFERENCES:

[1]. Mathur M., Kusum Devi V., 2016. Potential of novel drug delivery strategies for thetreatmentofhyperlipidemia.JDrugTarget.10,916-926.doi:10.3109/1061186X.2016.1172586.

[2]. LibbyP.,BuringJE.,BadimonL.,HanssonGK., DeanfieldJ.,BittencourtMS.,Tokgözoğlu L., Lewis EF., 2019. Atherosclerosis. Nat Rev Dis Primers 1, 56. doi:10.1038/s41572-019-0106-z.

[3]. M.H.Shukr.,S.Ismail.,S.M.Ahmed.,2019.Developmentandoptimizationofezetimibe nanoparticles with improved antihyperlipidemic activity. J drug deliverysciencreand Technalogy.doi:https://doi.org/10.1016/j.jddst.2018.12.001.

[4]. Phan BA., Dayspring TD., Toth PP., 2012. Ezetimibe therapy: mechanism of actionandclinicalupdate.VascHealthRiskManag.8,415-27.doi:10.2147/VHRM.S33664.

[5]. Wilczewska.,A.Z.,Niemirowicz.,K.,Markiewicz.,K.H.,Car,H.,2012.Nanoparticles as drug delivery systems. Institute of pharmacology policy academy ofscience.64,1020–1037.https://doi.org/10.1016/s1734-1140(12)70901-5.

[6]. Mourya, V., Inamdar, N., Nawale, R., Kulte, S. S., 2011. PMs: General considerationandtheirapplications.IndianJ.Pharm.Educ.Res.45,128–138.https://doi.org/10.3390/cancers10070238.

[7]. Wakaskar, R.R., 2017. PMs and their properties. J. Nanomed. Nanotechnol. 8, 433.https://doi.org/10.4172/2157-7439.1000433.

[8]. Deshmukh,A.S.,Chauhan,P.N.,Noolvi,M.N., Chaturvedi,K.,Ganguly,K.,Shukla, S. S., Nadagouda, M. N., Aminabhavi, T. M., 2017. PMs: Basic research toclinical practice. Int J. Pharm. 532, 249–268.https://doi.org/10.1016/j.ijpharm.2017.09.005.

[9]. Tran PH., Tran TT., Vo TV., 2014. Polymer conjugate-based nanomaterials for drugdelivery.J Nanosci Nanotechnol. 14,815-27.doi: 10.1166/jnn.2014.8901.

[10]. KedarU.,PhutaneP.,ShidhayeS.,KadamV.,2010.Advancesinpolymericmicellesfordrugdeliveryandtumortargeting.Nanomedicine.6,714-29.doi:10.1016/j.nano.2010.05.005.

[11]. Kore G., Kolate A., Nej A., Misra A., 2014. Polymeric micelle as multifunctionalpharmaceuticalcarriers.JNanosciNanotechnol.1,288-307.doi:10.1166/jnn.2014.9021.

[12]. Isoglu IA., Ozsoy Y., Isoglu SD., 2017.Advances in Micelle-based Drug Delivery:Cross-linkedSystems.CurrTopMedChem.13,1469-1489.doi:10.2174/1568026616666161222110600.

[13]. Reddy BP., Yadav HK., Nagesha DK., Raizaday A., Karim A., 2015. PolymericMicellesasNovelCarriersforPoorlySolubleDrugs--AReview.JNanosciNanotechnol.6,4009-18.doi: 10.1166/jnn.2015.9713.

[14]. SezginZ.,YükselN.,BaykaraT.,2006.Preparationandcharacterizationofpolymeric micelles for solubilization of poorly soluble anticancer drugs. Eur J PharmBiopharm.3,261-8. doi: 10.1016/j.ejpb.2006.06.003.

[15]. TorchilinVP.,2004.Targetedpolymericmicell esfordeliveryofpoorlysolubledrugs.Cell Mol LifeSci. 19-20, 2549-59. doi: 10.1007/s00018-004-4153-5.
[16]. Kesharwani,P.,Gorain,B.,Low,S.Y.,Tan,S.A. ,Ling,E.C.S.,Lim,Y.K.,Chin,
[17]. M.,Lee,P.Y.,Lee,C.M.,Ooi,C.H.,Choudhury, H.,Pandey,M.,2018.Nanotechnologybasedap proachesforanti-diabeticdrugsdelivery.DiabetesRes.Clin.Prac t.136,52–77.https://doi.org/10.1016/j.diabres .2017.11.018.
[18]. Salimi,A.,SharifMakhmalZadeh,B.,Kazemi, M.,2019.Preparationandoptimization of PMs as an oral drug delivery system for deferoxamine mesylate: invitro and ex vivo studies. Res. Pharm. Sci. 14, 293–307. https://doi.org/10.4103/1735-5362.263554.
[19]. Peng CL., Lai PS., Lin FH., Wu SY., Shieh Mj., 2009. Dual chemotherapy andphotodyanamic therapy in an HT- 29 human colon cancer xenografts model using SN-38-loadedchlorine-corestarblockcopolymer micelles.Biomaterials. 30,3614-3625.
[20]. AllenC.,YuY.,EisenbergA.,1999.Maysinger D.CelllularinternalizationofPCL(20)-b-PEO(44)blockcopolymer micelles.BiochimBiophys Acta.8,1421-32.
[21]. Xu W., Ling P., ZhangT., 2013. Polymeric micelles, a promising drug deliverysystemtoenhancebioavailabilityofpo orlywater-solubledrugs.JDrugDeliv.340315.doi: 10.1155/2013/340315.