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# "Formulation, Development and In Vitro Evaluation of Nanoparticle Containing Fusidic Acid"

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**ABSTRACT**
The present study focuses on the formulation, development, and in vitro evaluation of fusidic acid-loaded nanoparticles aimed at enhancing the drug's solubility and bioavailability. Fusidic acid, a white and odorless solid with a melting point of 192°C and λmax of 229.5 nm, was formulated using the solvent evaporation method. Eudragit RS 100 and polyvinyl alcohol were employed as polymers for nanoparticle preparation. Characterization through Scanning Electron Microscopy (SEM) confirmed spherical nanoparticles with smooth and porous surfaces, while particle size analysis revealed nanometer-sized particles ranging from 146.5 to 663.7 nm. Zeta potential analysis indicated good stability of the formulation. The optimized batch (F1) showed high entrapment efficiency (81.13%) and sustained drug release over 16 hours. Overall, the study demonstrates that nanoparticle-based delivery effectively improves the solubility, stability, and controlled release of fusidic acid, suggesting its potential for enhanced therapeutic performance.

**Keywords:** Fusidic acid, Nanoparticles, Solvent evaporation, Eudragit RS 100, Polyvinyl alcohol, Entrapment efficiency, Drug release, Bioavailability

## I. INTRODUCTION

Nanotechnology offers a promising approach for overcoming such limitations through the development of nanoscale drug delivery systems. Nanoparticles, typically ranging in size from 1 to 1000 nm, provide unique advantages including an increased surface area-to-volume ratio, improved solubility of poorly soluble drugs, enhanced bioavailability, and controlled or sustained release profiles**(Alshoraet al., 2016).**These nanosystems can efficiently encapsulate both hydrophilic and hydrophobic drugs, protecting them from degradation and ensuring targeted delivery to specific sites. Moreover, nanoparticles can modulate drug release kinetics, thereby reducing dosing frequency and improving patient compliance**(Bai et al., 2022).**

Fusidic acid is a potent steroidal antibiotic primarily used for the treatment of infections caused by Staphylococcus aureus, including methicillin-resistant strains (MRSA). Despite its broad antibacterial spectrum and clinical relevance, the therapeutic application of fusidic acid is limited by its poor water solubility and low bioavailability**(Ahmedet al., 2020).** These physicochemical constraints often lead to suboptimal drug absorption, reduced efficacy, and the necessity for higher doses, which may increase the risk of side effects and resistance development. Therefore, the design of a novel delivery system that can enhance its solubility and controlled release has become an area of significant research interest**(Rai et al., 2016).** In this study, fusidic acid-loaded nanoparticles were formulated using the solvent evaporation method, a widely used and reliable technique for nanoparticle preparation. Polymers such as Eudragit RS 100 and polyvinyl alcohol (PVA) were selected due to their biocompatibility, stability, and ability to form uniform nanoparticles. The optimization of formulation parameters was conducted to achieve desirable particle size, surface morphology, entrapment efficiency, and drug release characteristics**(Saharan et al., 2019).**

Nanoparticle are small in size so they have special characteristics and the size of particle can be controlled by using surfactant and polymer and by controlling the size we can also controlled the absorption wavelength and emission wavelength **(Lucky et al., 2015).** Transparency in nanoformulation can be attained if particle are below the light critical wavelength of light. In metal nanoparticle the melting point of metal is decrease and also gives better paramagnetic properties. Nanoparticle has different chemical nature so it gives different affinity to electro and which give different properties of electron transport **(Mu et al., 2014).**

Characterization of the prepared nanoparticles was conducted to evaluate critical parameters such as particle size, surface morphology, zeta potential, entrapment efficiency, and in vitro drug release behaviour **(El-Naggar et al., 2015).**Scanning Electron Microscopy (SEM) was used to observe the surface structure and shape of nanoparticles, while a Malvern Zetasizer was employed to determine the particle size distribution and stability. The in vitro release profile was studied using the dialysis bag diffusion method to assess the sustained release behavior of the developed formulations **(Masoudipouret al., 2017).** Comprehensive characterization of the prepared nanoparticles was performed using techniques such as Scanning Electron Microscopy (SEM) to determine surface morphology, Malvern Zetasizer to measure particle size and zeta potential, and UV-visible spectroscopy for drug quantification. The in vitro drug release was assessed using the dialysis bag diffusion method to evaluate the sustained release behavior of the formulations **(Mahmood et al., 2017).**

The overall objective of this research was to enhance the solubility, bioavailability, and therapeutic performance of fusidic acid by developing an efficient nanoparticle-based drug delivery system. The optimized formulation aimed to provide controlled drug release, minimize dose-related side effects, and improve treatment efficacy. The study underscores the potential of nanotechnology in reformulating conventional drugs like fusidic acid into advanced, effective, and patient-friendly delivery systems.

## II. MATERIAL AND METHODS

### 2.1 Chemicals

Ethanol and Methanol were obtained from Molychem, a reputable supplier of analytical reagents. Loba provided the Light liquid paraffin, and Sigma-Aldrich provided the Ethyl cellulose, Chloroform and Eudragit RS-100. Fine chem provided the DMSO and Acetone.

### 2.2 Pre-formulation studies

#### 2.2.1 Organoleptic Properties

The organoleptic studies of Fusidic acid like general appearance like color, odor, state, etc. were performed**(Ahmed et al., 2020).**

#### 2.2.2 Solubility study

Qualitative solubility of Fusidic acid in different solvents was determined according to USP NF, 2007. Approximately 1 mg of Fusidic acid was weighed and transferred into a 10 ml test tube and dissolved in the respective solvents (1 ml each of methanol, ethanol, acetonitrile, and water) **(Jain and Verma 2020).**

#### 2.2.3 Melting Point

Melting point was analyzed by open Capillary method using Thiele's tube. The temperature at which the sample starts to melt was taken as the melting point of the sample **(Chowk, M. I. 2020).**

#### 2.2.4 Determination of Lambda max and calibration curve

- **Lambda (λ) max**

A stock standard solution containing 1 mg/mL of Fusidic acid was prepared in 80% methanol. Working standard solution equivalent to 100 µg/mL of Fusidic acid was prepared by appropriate dilution of stock solution with the same solvent. The solution was scanned in the range of 200 – 400 nm UV spectrum using shimadzu 1700 double beam spectrophotometer (**Kumbhar and Salunkhe 2013)**.

- **Standard calibration curve**

100 mg of Fusidic acid was accurately weighted into 100 ml volumetric flask, dissolved in 80% Methanol and volume was made up with same solvent. Pipette 1ml of this solution into another 10 ml volumetric flask and the volume was made with Methanol and marked as Stock. The resultant solution is scanned in the range of (200-400 nm) by UV Spectrophotometer to get absorption maximum (λ max).

#### 2.2.5 Preparation of calibration curve

The prepared stock solution was further diluted with solvent to get working standard solution of 5, 10, 15, 20, 25 and 30 µg/ml of Fusidic acid to construct Beer's law plot for the pure drug, the absorbance was measured, against solvent as blank. The standard graph was plotted by taking concentration of drug on X-axis and absorbance on Y-axis in the concentration range of 5-30µg/ml (**Behera et al., 2012).**

#### 2.2.6 Fourier transmission Infra-Red Spectroscopy

FT-IR spectrum of Drug and drug + excipients was recorded over the range of 4000 to 400 cm−1 by KBr pellet method using a FT-IR spectrophotometer. The KBr disc was prepared using 1 mg of each Drug and drug + excipients in

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100 mg of spectroscopic grade KBr which has been dried using IR lamp. Both KBr and drug was mixed and subjected to hydraulic pressure to form disc. This disc was placed in FT-IR chamber. Infrared spectrum was recorded in the 4000 - 400 cm-1 region **(Silva et al., 2024).**

**2.3 Formulation of nanoparticle**

Solvent evaporation method was used for the preparation of Eudragit RS-100 nanoparticles. Firstly, the emulsification of polymeric solution was done in an aqueous solution containing a surfactant. Then the evaporation of polymeric solution was done by precipitation of the polymer. In the solution of methanol drug was dissolved. With constant stirring using a magnetic stirrer, the organic solution was added into an aqueous phase containing polyvinyl alcohol (PVA). The emulsion was sonicated using sonicator for nanosize of the emulsion. The organic solvent was then evaporated using constant stirring on a magnetic stirrer for about 4-5 hrs. After centrifugation (30 min, 10000 rpm), the nanoparticles were collected. The prepared emulsion was then kept for lyophilization for 48 hrs **(Saharan et al., 2019).**

**Table 1: Ingredients used in nanoparticle formulation**

| Ingredients | Formulation 1 | Formulation 2 | Formulation 3 | Formulation 4 | Formulation 5 |
|---|---|---|---|---|---|
| Drug (mg) | 75 | 75 | 75 | 75 | 75 |
| Eudragit RS 100 (mg) | 55 | 105 | 155 | 205 | 255 |
| PVA (%) | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Sonication Time (Min.) | 10 | 15 | 20 | 25 | 30 |
| Methanol (ml)(Solvent) | 10 | 10 | 10 | 10 | 10 |
| Water | q.s | q.s | q.s | q.s | q.s |

**2.4 Evaluation parameter of Nanoparticle formulation**

**2.4.1 Particle size**

The size of nanoparticle was measured using Malvern Zeta sizer (Malvern Instruments) **(Sharma et al., 2011)**

**2.4.2 Zeta potential**

The zeta potential was measured for the determination of the movement velocity of the particles in an electric field, particle charge and analyzed by Zetasizer Malvern instruments **(Kumar et al., 2011; Penjuri et al., 2016).**

**2.4.3 Entrapment efficiency**

% Entrapment efficiency was determined by indirect estimation. Drug -loaded nanoparticles were centrifuged at 15,000 rpm for 30 min using REMI Ultra Centrifuge. The non-entrapped drug (free drug) was determined in the supernatant solution using UV spectrophotometer. The peak area was determined and amount of free drug is determined by extrapolating the calibration curve. And drug entrapment calculated by using below equation **(Balla et al., 2020).**

**2.4.4 Scanning Electron Microscopic (SEM)**

The electron beam from a scanning electron microscope was used to attain the morphological features of the optimized drugs loaded Fusidic acid were coated with a thin layer (2–20 nm) of metal (s) such as gold, palladium, or platinum using a sputter coater under vacuum. The pre-treated specimen was then bombarded with an electron beam and the interaction resulted in the formation of secondary electrons called auger electrons. From this interaction between the electron beam and the specimen's atoms, only the electrons scattered at 90° were selected and further processed based on Rutherford and Kramer's Law for acquiring the images of surface topography**(Anweret al., 2021).**

**2.4.5 In vitro drug release study (kinetics model)**

The release kinetics study of the optimized fusidic acid nanoparticle batch (F5) was conducted to determine the drug release mechanism. The dissolution data were fitted into various kinetic models, including Zero-order ($Qt = Q0 + K0t$), First-order ($\ln Qt = \ln Q0 + K1t$), Higuchi ($Qt = Kt^{1/2}$), and Korsmeyer-Peppas ($Mt/M\infty = Kt^n$) models using linear regression analysis. Zero-order kinetics indicates a concentration-independent release, while first-order follows a concentration-

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dependent pattern. The Higuchi model describes drug diffusion through a porous matrix, showing a square root time-dependent release, and the Korsmeyer-Peppas model helps identify the release mechanism based on the release exponent (n). The model that best fits the data indicates the predominant drug release mechanism of the optimized formulation**(Paarakhet al.,2018).**

### 2.5 Stability studies

The drug loaded Nanoparticle formulation was packed and were placed in the stability test chamber and subjected to stability studies at accelerated testing ($25^0$C±$2^0$C and 60 ± 5% RH) and ($40^0$C±$2^0$C and 70 ±5% RH) for 3 months. The formulation were checked for evaluation parameter particle size, entrapment efficiency and in-vitro drug release studies at the interval of 30, 45, 60, 90 days (3 month) months. The formulation was tested for stability under accelerated storage condition for 3 months in accordance to International Conference on Harmonization (ICH) guidelines. Formulation was analyzed for the change in evaluation parameter particle size, entrapment efficiency and in-vitro drug release studies.

## III. RESULT AND DICUSSION

### 3.1 Organoleptic properties

**Table 2: Organoleptic properties of Fusidic acid**

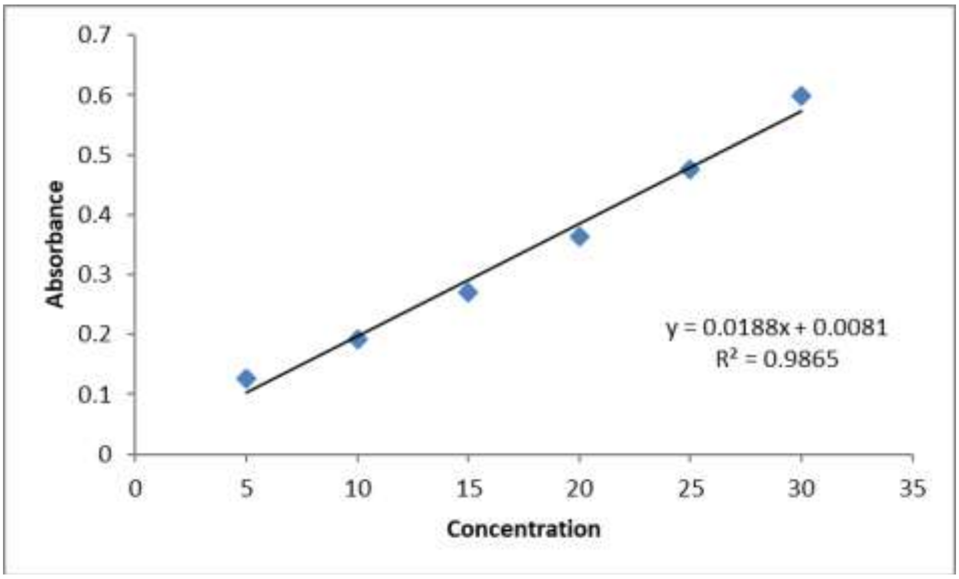
| Drug | Organoleptic properties | Observation |
|---|---|---|
| **Fusidic acid** | Color | White |
| | Odor | Odour less |
| | Appearance | Solid powder |
| | State | Solid |

### 3.2 Melting point study

**Table 3: Melting point study of Fusidic acid**

| Drugs | Observed | Reference |
|---|---|---|
| Fusidic acid | 192°C | 192°C-193°C |

### 3.3 Calibration curve




**Graph 1: Calibration curve of Fusidic acid**

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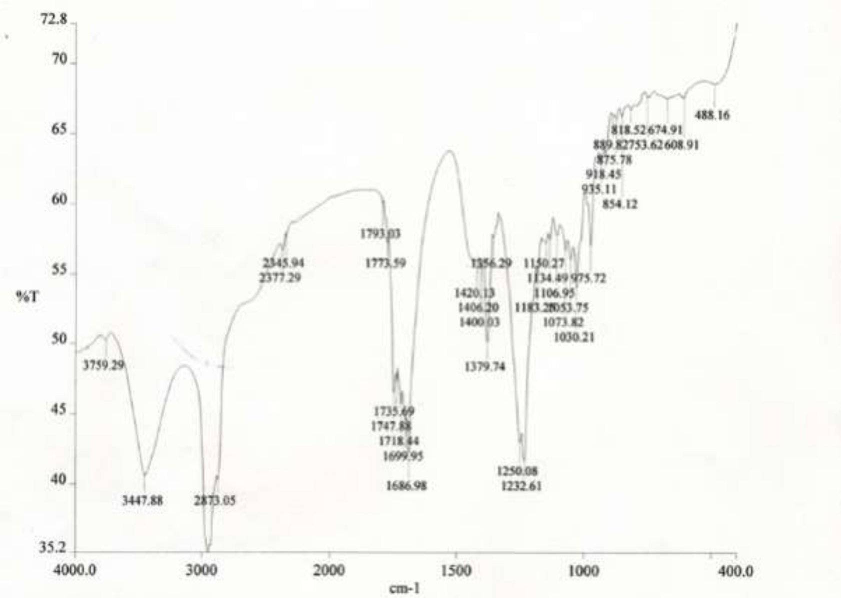
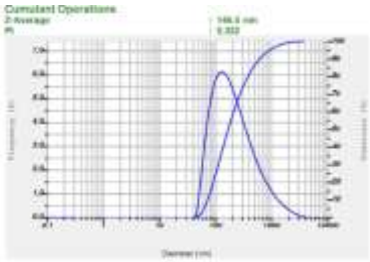
### 3.4 Functional group identified by FTIR



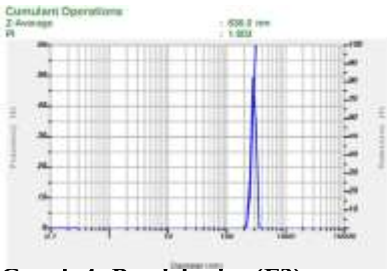

Graph 2: FTIR study

### 3.5 Evaluation parameter of drug loaded Nanoparticle

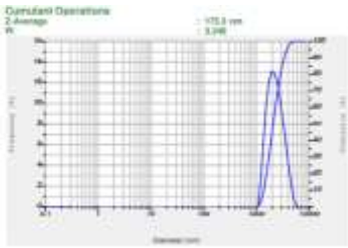
### 3.5.1 Particle size determination



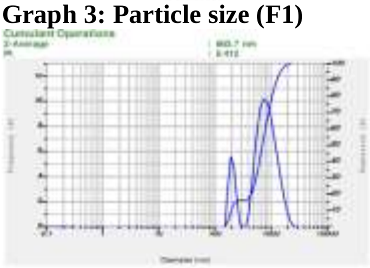
Graph 3: Particle size (F1)



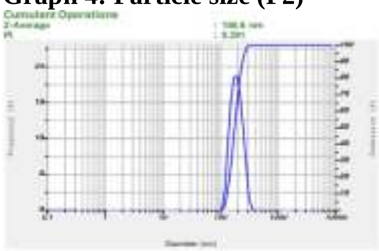
Graph 4: Particle size (F2)



Graph 5: Particle size (F3)



Graph 6: Particle size (F4)



Graph 7: Particle size (F5)

**Table 4: Result of Particle size of all formulations**

| Formulations | Particle size (nm) | PDI Value |
|---|---|---|
| **Nanoparticle (F1)** | **146.5 nm** | **0.332** |
| Nanoparticle (F2) | 636.0 nm | 1.603 |
| Nanoparticle (F3) | 175.3 nm | 3.248 |
| Nanoparticle (F4) | 663.7 nm | 0.412 |
| Nanoparticle (F5) | 156.6 nm | 0.291 |

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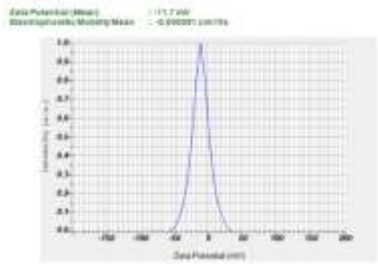
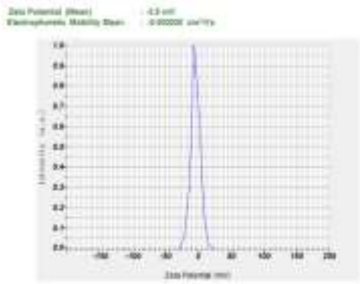
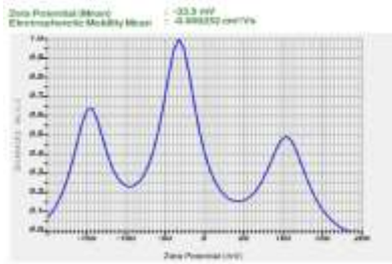
### 3.5.2 Zeta potential



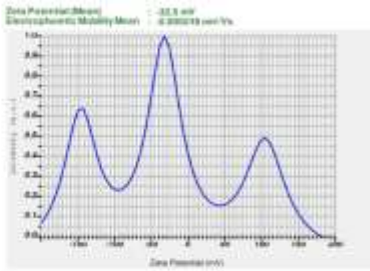
**Graph 8: Zeta potential (F1)**



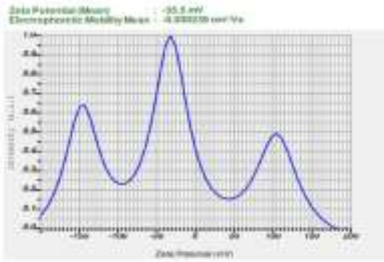
**Graph 9: Zeta potential (F2)**



**Graph 10: Zeta potential (F3)**



**Graph 11: Zeta potential (F4)**



**Graph 12: Zeta potential (F5)**

**Table 5: Result of Zeta potential and Entrapment efficacy of all formulations**

| Formulation | Zeta potential | Entrapment efficacy (%) |
|---|---|---|
| **Nanoparticle (F1)** | **-11.7 mV** | **81.13** |
| Nanoparticle (F2) | -3.9 mV | 76.13 |
| Nanoparticle (F3) | -33.5 mV | 74.65 |
| Nanoparticle (F4) | -32.5 mV | 79.32 |
| Nanoparticle (F5) | -35.5 mV | 73.53 |

### 3.5.3 SEM analysis

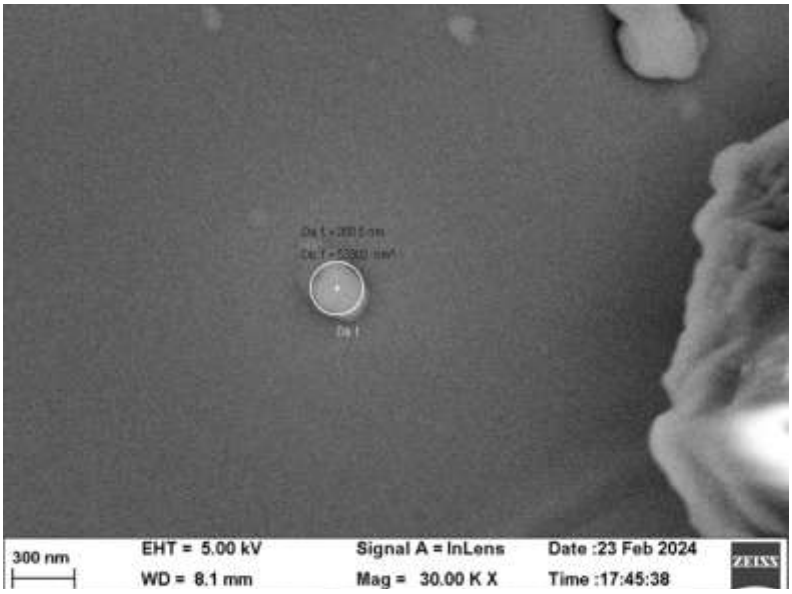



**Figure 1: SEM**

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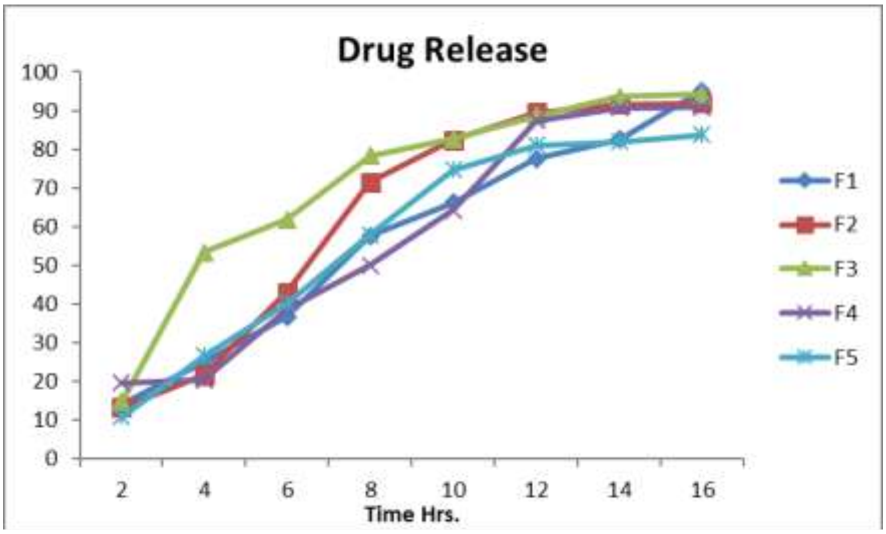
**3.5.4In- vitro drug release kinetics study of F1 to F5 formulation**

**Table 6: In- vitro drug release kinetics study**

| Time | Formulation 1 | Formulation 2 | Formulation 3 | Formulation 4 | Formulation 5 |
|---|---|---|---|---|---|
| 2 | 14.18 | 13.22 | 14.78 | 19.57 | 10.78 |
| 4 | 25.09 | 21.53 | 53.26 | 20.33 | 26.54 |
| 6 | 36.63 | 43.01 | 61.73 | 38.71 | 40.17 |
| 8 | 57.6 | 71.53 | 78.25 | 50.00 | 57.89 |
| 10 | 66.13 | 82.33 | 82.63 | 64.23 | 74.58 |
| 12 | 77.56 | 89.45 | 88.63 | 87.39 | 80.96 |
| 14 | 82.45 | 91.53 | 93.56 | 90.75 | 81.86 |
| 16 | 95.01 | 91.89 | 94.11 | 90.8 | 83.69 |




**Graph 13: Drug release study of All Formulation F1 to F5**

**Table 7: Kinetics study of F1 formulation**

| Time (Hr) | cumulative % drug released | % drug remaining | Square root time | log Cumu % drug remaining | log time | log Cumu % drug released |
|---|---|---|---|---|---|---|
| 0 | 0 | 100 | 0.000 | 2.000 | 0.000 | 0.000 |
| 2 | 14.18 | 85.82 | 1.414 | 1.934 | 0.301 | 1.152 |
| 4 | 25.09 | 74.91 | 2.000 | 1.875 | 0.602 | 1.400 |
| 6 | 36.63 | 63.37 | 2.449 | 1.802 | 0.778 | 1.564 |
| 8 | 57.6 | 42.4 | 2.828 | 1.627 | 0.903 | 1.760 |
| 10 | 66.13 | 33.87 | 3.162 | 1.530 | 1.000 | 1.820 |
| 12 | 77.56 | 22.44 | 3.464 | 1.351 | 1.079 | 1.890 |
| 14 | 82.45 | 17.55 | 3.742 | 1.244 | 1.146 | 1.916 |
| 16 | 95.01 | 4.99 | 4.000 | 0.698 | 1.204 | 1.978 |

**Table 8: Correlation value ($R^2$ value)**

| Formulation | Model | Kinetic parameter values |
|---|---|---|
| Nanoparticle | Zero Order | $R^2 = 0.987$ |
| | First Order | $R^2 = 0.890$ |
| | Higuchi | $R^2 = 0.932$ |
| | Korsmeyerpeppas | $R^2 = 0.895$ |

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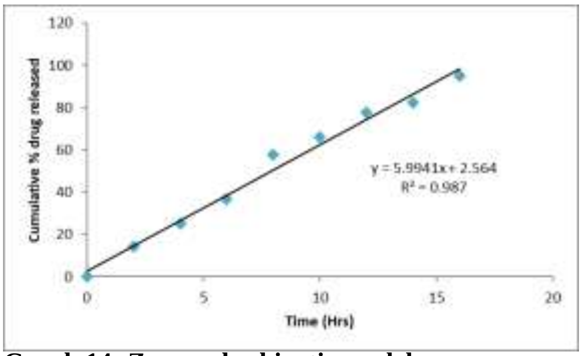
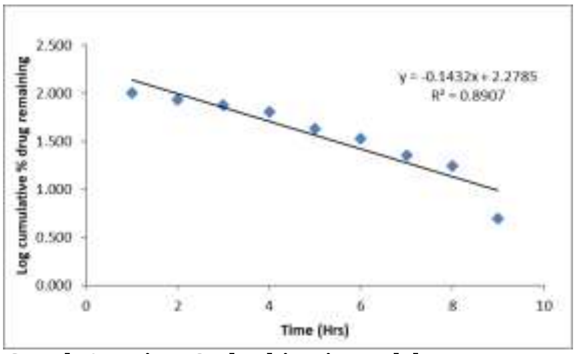
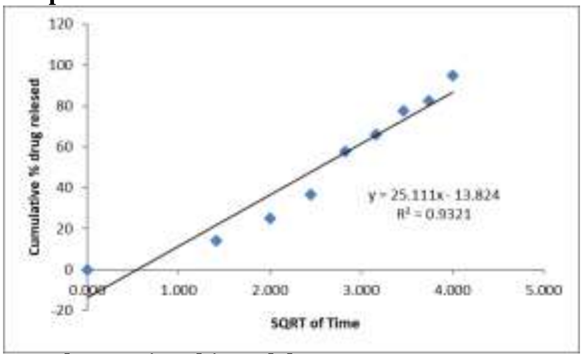


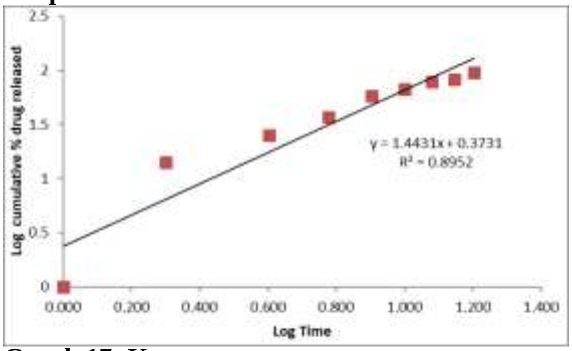

**Graph 14: Zero order kinetic model**




**Graph 15: First Order kinetic model**




**Graph 16: Higuchi model**




**Graph 17: Korsmeyerpeppas**

**3.6 Stability study**

**Table 9: Stability Study of nanoparticle formulation**

| Time (Days) | $25^0C\pm2\ ^0C$ and 60 ± 5% RH | | | $40^0C\pm2\ ^0C$ and 70 ±5% RH | | |
|---|---|---|---|---|---|---|
| | Appearance | Particle size nm | Zeta potential mV | Appearance | Particle size nm | Zeta potential mV |
| 0 | Solid Powder | 146.5 nm | -11.7mV | Solid Powder | 146.5 nm | -11.7 mV |
| 30 | Solid Powder | 146.4 nm | -11.9mV | Solid Powder | 146.1 nm | -12.2 mV |
| 45 | Solid Powder | 145.9 nm | -12.1mV | Solid Powder | 146.6 nm | -12.5 mV |
| 60 | Solid Powder | 146.0 nm | -12.4mV | Solid Powder | 146.4 nm | -12.10 mV |
| 90 | Solid Powder | 146.3 nm | -12.8mV | Solid Powder | 146.7 nm | -12.13 mV |

## IV. DISCUSSION

The evaluation of fusidic acid confirmed that its organoleptic properties—appearance, color, odor, and state complied with I.P. standards, exhibiting a white, odorless solid form. The drug was found to be freely soluble in dimethyl sulfoxide, ethanol, and methanol, but insoluble in water. Its melting point (192°C) and λmax (229.5 nm) were within specification limits. Calibration studies showed excellent linearity in the 5–30 µg/mL range with a correlation coefficient ($R^2$) of 0.986, confirming the method's accuracy.

Nanoparticles prepared using the solvent evaporation method showed particle sizes ranging from 146.5 to 663.7 nm, with F1 having the smallest size. Zeta potential values between –3.9 and –35.5 mV indicated good colloidal stability, while entrapment efficiency ranged from 73.53% to 81.13%, the highest being for F1. SEM analysis revealed spherical nanoparticles with smooth, uniform surfaces.

Drug release kinetics studies demonstrated that F1 followed zero-order release with an $R^2$ value of 0.987, indicating a concentration-independent and sustained release profile. In vitro release was further supported by fitting to Higuchi and Korsmeyer-Peppas models, confirming diffusion-controlled release. Stability studies conducted under accelerated conditions for three months showed no significant change in physical or chemical parameters, confirming the formulation's robustness and long-term stability.

## V. CONCLUSION

The study successfully developed fusidic acid-loaded nanoparticles using the solvent evaporation method, achieving high entrapment efficiency and stable particle size in the nanometer range. Among the formulations, F1 exhibited optimal performance in terms of drug release and stability. The nanoparticle system enhanced the solubility and bioavailability of fusidic acid, addressing limitations associated with poor solubility. Overall, this nanotechnology-based formulation demonstrates significant potential for improving the therapeutic efficacy of fusidic acid.

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