*ejpmr, 2025, 12(7), 123-132*

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

# FORMULATION, DEVELOPMENT AND EVALUATION OF DORZOLAMIDE HYDROCHLORIDE NANOPARTICLES

***Varanasi Alekhya, M. Pallavi, Dr. Avinash Dundigalla and Dr. A. Yasodha**

Department of Pharmaceutics, Dhanvanthri College of Pharmaceutical Sciences Tirumala Hills Appannapally Mahabubnagar Telangana India 509001.

***Corresponding Author: Varanasi Alekhya**

Department of Pharmaceutics, Dhanvanthri College of Pharmaceutical Sciences Tirumala Hills Appannapally Mahabubnagar Telangana India 509001.

**Article Received on 01/05/2025** **Article Revised on 21/05/2025** **Article Accepted on 11/06/2025**

**ABSTRCT**

The goal of this study was to assess the efficacy of a method based on the creation of polymeric nanoparticles as an innovative formulation of Dorzolamide hydrochloride with enhanced therapeutic efficacy. Dorzolamide hydrochloride has low solubility and permeability, which result in limited and variable bioavailability; its low stability makes it difficult to develop stable aqueous liquid formulations. The Dorzolamide hydrochloride Polymeric nanoparticles were created using the solvent evaporation process. The numerous formulations with varied drug-polymer and surfactant ratios were analyzed and improved. Dorzolamide hydrochloride Polymeric nanoparticles containing PLGA were created using the solvent evaporation method, then the particle size was decreased by sonication. Particle size, surface morphology by SEM, drug excipient compatibility by FTIR, and in-vitro drug release experiments were used to characterize the produced nanoparticles. The formulation with the best encapsulation efficiency was (F-3) A drug encapsulation effectiveness of up to 92.85 % has been attained in this study. It was discovered that the efficiency of encapsulation improved along with the polymer content. According to the results of the current investigation, the manufacture of Dorzolamide hydrochloride Polymeric nanoparticles can be done using a solvent evaporation process followed by sonication.

**KEYWORDS:** Dorzolamide hydrochloride drug, Polymeric Nano Particles, Solvent Evaporation, lipid, FTIR, invitro drug release.

## INTRODUCTION

Nanotechnology Nanotechnologies can be defined as the design, characterization, fabrication and application of structures by controlling morphology and dimension at a nanometer scale. Potential benefits of nanomaterials are well documented in the literature and nanotechnology promises to far exceed the impact of the Industrial Revolution, extrapolative to become a $1 trillion market by 2015.[1] Nanotechnology offers unique approaches to revolutionary impact on biology and medicine because of size dependent physical and chemical properties. Among the approaches for exploiting nanotechnology in diagnostics and therapeutics, nanoparticles offer some unique advantages as sensing, image improvement, and antimicrobial agents. Therefore nanoparticles (NPs) used for parenteral, oral, ocular and transdermal application and sustained released formulations.[2]

Nanotechnology has gained huge attention over time. The fundamental component of nanotechnology is the nanoparticles. Nanoparticles are particles between 1 and 100 nanometers in size and are made up of carbon, metal, metal oxides or organic matter. The nanoparticles exhibit a unique physical, chemical and biological properties at nanoscale compared to their respective particles at higher scales.[3]

## METHODOLOGY

### Preformulation study

**a) Determination of melting point**

The capillary method was used to determine the melting point of Dorzolamide Hydrochloride.

**b) Solubility**

Dorzolamide Hydrochloride's solubility was assessed in DMSO, dimethyl formamide, and 7.4 pH buffer.

**c) Compatibility study (IR spectroscopy)**

The drug-polymer compatibility was ascertained by subjecting the drug and homogenates of drug and polymer to Infrared spectrophotometric study.

Preparation of standard curve of Dorzolamide Hydrochloride.[29]

Utilizing a solution made in a 7.4 pH buffer and a UV/visible spectrophotometer set to 240 nm, Dorzolamide Hydrochloride was examined.

**Preparation of 7.4 -pH**

2.48 gm of Disodium hydrogen phosphate and 0.19 gm of potassium dihydrogen phosphate and 8 gm of sodium chloride in 1000 ml of water.

**Preparation of standard curve of Dorzolamide Hydrochloride in 7.4 pH**

Dorzolamide Hydrochloride 10 mg was precisely measured and diluted in 10 ml of 7.4 phosphate buffer for the standard graph. Using 7.4 phosphate buffer, several concentrations of Dorzolamide Hydrochloride — 10, 20, 30, 40, and 50 mcg/ml— were produced from the stock solution (1 mg/ml).

**Method of preparation of Dorzolamide Hydrochloride loaded nanoparticles: Formulation development**

**Table-1: Composition of the Nanoparticles.**

| Ingredients | Batch no | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
| Dorzolamide Hydrochloride | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| PLGA | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 |
| Poloxamer 407 | 0.5 | 1 | 1.5 | 0.5 | 1 | 1.5 | 0.5 | 1 |
| SLS | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

Loaded nanoparticles were prepared using PLGA as a polymer, poloxamer 407, and sodium lauryl sulfate (SLS) as a stabilizer utilizing the solvent evaporation method. PLGA concentration was kept constant (10 mg), while poloxamer 407, SLS, and drug were used in varying concentrations. The developed nano formulations were characterized for their physicochemical properties, drug loading, % entrapment efficiency, and stability. The optimized Nano formulations were then decorated with Dorzolamide Hydrochloride.[30]

**Evaluation of Dorzolamide Hydrochloride loaded nanoparticles**

**Particle size:** All of the generated batches of nanoparticles were observed under a microscope to establish their sizes. The average size of the nanoparticles was determined by measuring the size of each batch's nanoparticles in a small drop of nanoparticle dispersion on a slide.[31]

**SEM analysis**

The morphology of nanoparticles was examined using the scanning electron microscope (SEM, Hitachi, Tokyo, Japan). After being properly diluted (1:100) in double-distilled water, Dorzolamide Hydrochloride -freeze-dried SLNs were added to a drop of the nanoparticle formulation and left to air dry. The sample was then observed under various magnifications and a 15,000-volt accelerating voltage. The imaging was performed in a high vacuum.[32]

**Drug encapsulation efficiency**

A set volume of the nanoparticle's dispersion (10 ml) was poured into a centrifuge tube at room temperature, and it was spun at 18,000 rpm for 20 minutes (Remi Instruments Pvt. Ltd, India). The drug's absorbance in the supernatant was measured spectrophotometrically at a maximum wavelength of 310 nm after the lipid component was removed (Shimadzu 1800, Japan).[33]

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount entrapped}}{\text{Total drug loaded}} \times 100$$

**In-vitro drug release studies**

Utilizing the dialysis bag approach, in vitro release tests were carried out. Prior to the release trials, the dialysis membrane (molecular weight cutoff between 12,000 and 14,000) was immersed in double distilled water for an overnight period. As releasing media, phosphate buffer pH 7.4 were also employed. A donor compartment and a receptor compartment make up the experimental unit. A boiling tube that was cut open at one end and tied with a dialysis membrane at the other end serves as the donor compartment, into which 3 ml of polymeric dispersion was injected for the release research. The receptor compartment is made up of a 250 ml beaker that contains 100 ml of release media and was kept at a temperature of 37 0.5 °C. Every 3 ml sample was taken out of the receiver compartment and replaced with the same amount of release medium at the 1, 2, 3, 4, 5, 6, 7 and 8h time periods. The collected samples were appropriately diluted before being examined at 310 nm with a UV-visible spectrophotometer.[34]

Percentage of drug release was determined using the following formula.

$$\text{sPerentage drug release} = \frac{Da}{Dt} \times 100$$

Where, Dt = Total amount of the drug Da = The amount of drug released

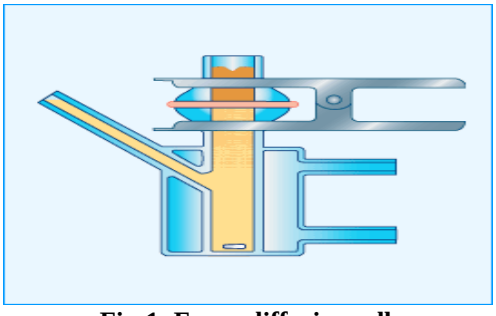

**Fig-1: Franz diffusion cell.**

**Kinetics of drug release**[35]

To study kinetices data obtained from invitro relesase were plotted in various kinetic models.

- **Zero-order Equation**

% R = Kt

This model represents an ideal release profile in order to achieve the pharmacological prolonged action. This is applicable to dosage forms like transdermal systems, coated forms, osmotic systems, as well as matrix tablets with low soluble drugs.

- **First order equation**

Log % unreleased = Kt / 2.303

This model is applicable to study hydrolysis kinetics and to study the release profiles of pharmaceutical dosage forms such as those containing water soluble drugs in porous matrices.

- **Higuchi equation**

$\% R=Kt^{0.5}$

- **Korsmeyer-Peppas equation**

$\%R=Kt^{n}$

This model is widely used, when the release phenomenon could be involved. The end value could be used to characterize different release mechanisms as.

| N | Mechanism |
|---|---|
| 0.5 | Fickiandiffusion (Higuchi matrix) |
| 0.5<n<1 | Anomalous transport |
| 1 | Case- II transport (zero order release) |
| n>1 | Super case- II transports |

**Stability studies**[36]

Over the course of 90 days, the stability of Dorzolamide Hydrochloride nanoparticle dispersion in screw-capped glass vials was assessed. Four samples were split into two groups and kept at 4°C and 25°C, respectively. At the end of the 90 days, the amount of drug leaking from nanoparticles and the average particle size of the samples were calculated.

## RESULTS AND DISCUSSION

**Preformulation studies**

**a) Organoleptic evaluation**

**Table-2: Organoleptic properties of Dorzolamide Hydrochloride.**

| Properties | Results |
|---|---|
| Description | Powder |
| Taste | Tasteless |
| Odor | Odorless |
| Color | white powder |

**b) Determination of melting point**

Dorzolamide Hydrochloride melting point was discovered to be in the region of 264 $^{0}$c, which met the requirement and demonstrated the medication sample's purity.

**c) Solubility**

Dorzolamide hydrochloride is soluble in water. It is also described as slightly soluble in methanol and ethanol.

**Preparation of standard curve of Dorzolamide Hydrochloride**

The Dorzolamide Hydrochloride standard curve was created by graphing absorbance versus concentration at 310 nm. use a pH 7.4 solution at 310 nm. It also adheres to Beer's law. The R 2 value is 0.998.

**Table-3: Calibration curve of Dorzolamide Hydrochloride in 7.4 phosphate buffer.**

| S. no | Concentration (µg/ml) | Absorbance |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 10 | 0.124 |
| 3 | 20 | 0.234 |
| 4 | 30 | 0.334 |
| 5 | 40 | 0.467 |
| 6 | 50 | 0.565 |

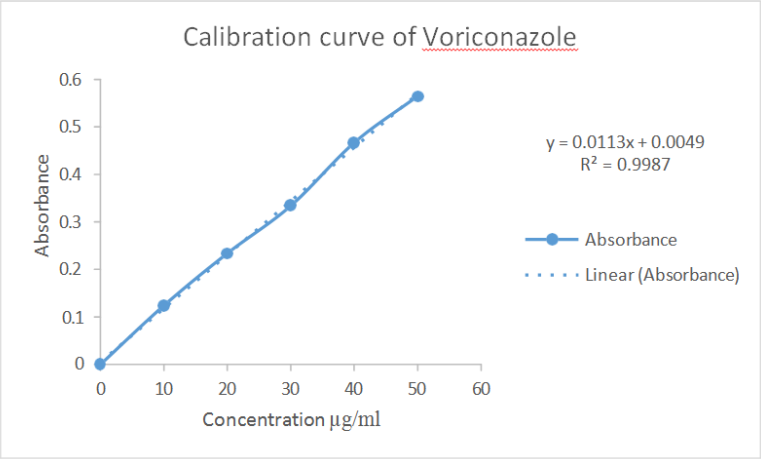


**Fig-2: Calibration curve of Dorzolamide Hydrochloride.**

**Drug - excipient compatibility studies (FT-IR)**

Using the FTIR peak matching approach, the compatibility of the medicine with the chosen polymer and other excipients was assessed. The drug-polymer mixture showed no peaks that appeared or vanished, indicating that there was no chemical interaction between the medication, polymer and other molecules.

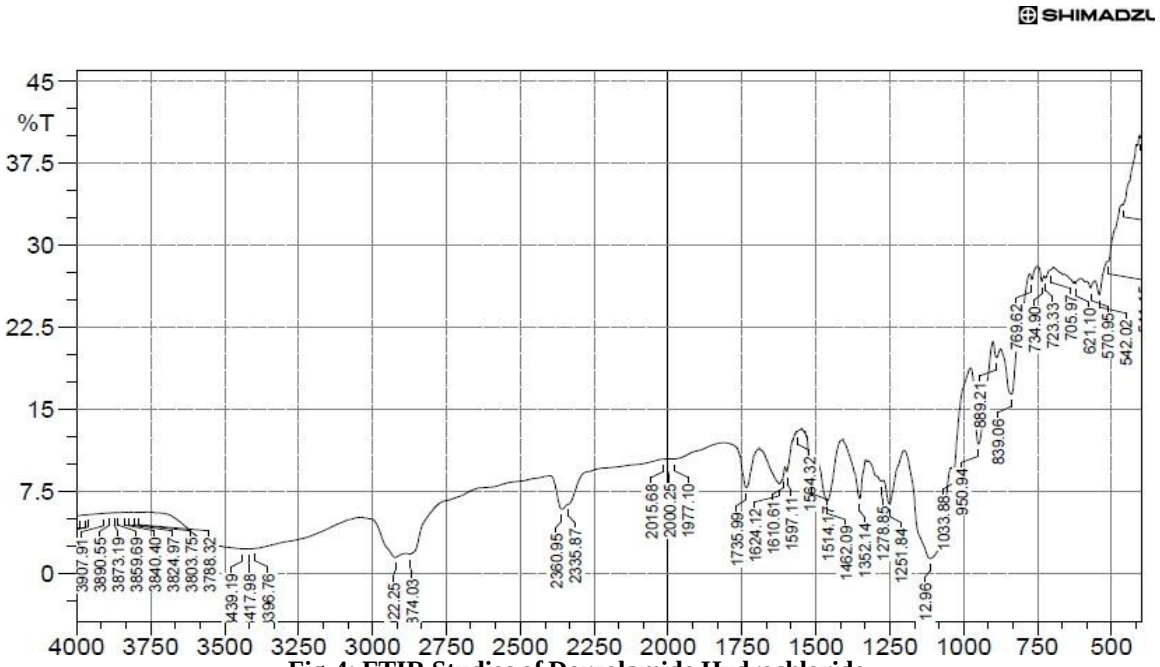


**Fig-4: FTIR Studies of Dorzolamide Hydrochloride.**

**Table-4: Characteristic Peaks for Dorzolamide Hydrochloride.**

| S.No. | Characteristic Peaks | Frequency range (cm-1) | Frequency (cm-1) |
|---|---|---|---|
| 1 | OH stretching | 4000-3500 | 3890.55 |
| 2 | OH Bending | 3500-3000 | 3417.96 |
| 3 | C-H stretching | 2750-2250 | 2335.87 |
| 4 | C=O stretching | 1500-1000 | 1278.85 |

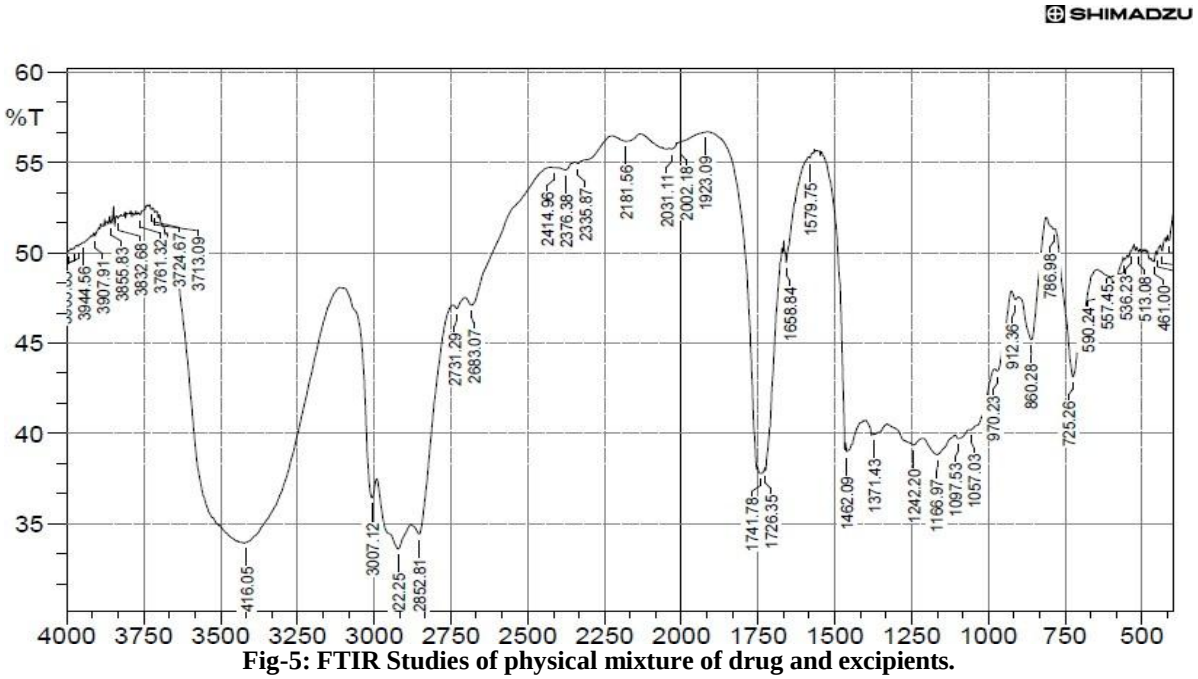


**Fig-5: FTIR Studies of physical mixture of drug and excipients.**

**Table-6: Characteristic Peaks for drug and excipients.**

| S.No. | Characteristic Peaks | Frequency range (cm-1) | Frequency (cm- 1) |
|---|---|---|---|
| 1 | OH stretching | 4000-3500 | 3907.91 |
| 2 | OH Bending | 3500-3000 | 3416.05 |
| 3 | C=O stretching | 1500-1000 | 1242.20 |

**EVALUATION PARAMETERS EVALUATION PARAMETERS**

**Particle size**

With an increase in polymer concentration, the particle size increased. Based on entrapment effectiveness and particle size distribution.

**Drug entrapment efficiency**

Optimizing the polymer concentration to be used in the creation of polymeric nanoparticles was the first step of the work plan. Based on the particle size and entrapment effectiveness of the discovered polymeric nanoparticles, the polymer content was optimized.

**Determination of Zeta potential**

Zeta potential is a measure of charge present on the vesicle surface. It was determined by using phase analysis light scattering with Malvern zetasizer at field strength of 20V/cm in distilled water and based on electrophoretic mobility of charged particles present in the nanocarrier system. Charged particles were attracted to the electrode with the opposite charge when an electric field is applied.

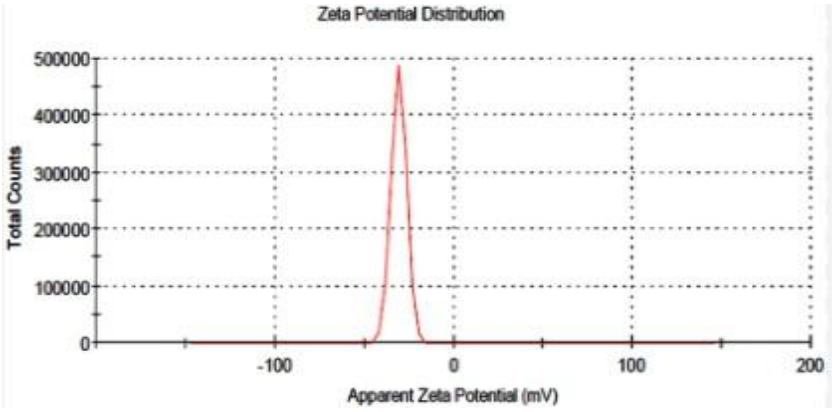


**Fig-6: Zeta potential of Optimized formulation.**

The addition of membrane additives affects zeta potential value depending on the type of membrane additives. Zeta potential of optimized nanoparticles formulation was measured and found to 24- mv. The obtained result of the zeta potential of the prepared formulation indicates particles in the formulation remains suspended and so were found to be stable.

**Particle size**

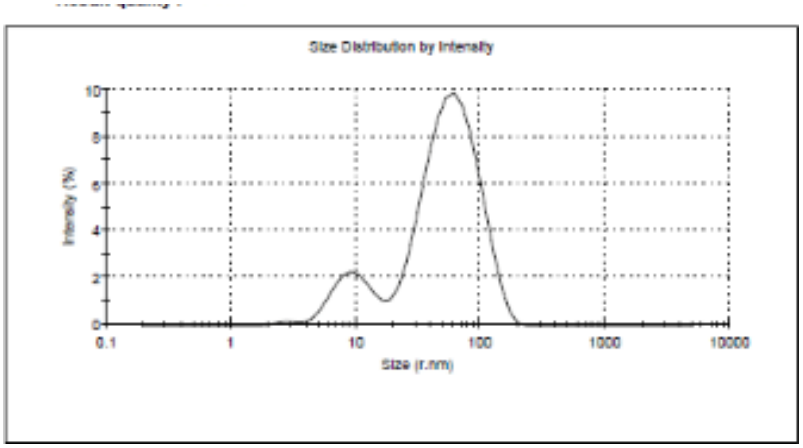


**Fig-7: Particle size of optimized formulations.**

The surfaces of the nanoparticles were smooth.

**Surface morphology**

According to scanning electron microscopy (SEM), the polymeric nanoparticles were round, smooth, and free of any aggregation.

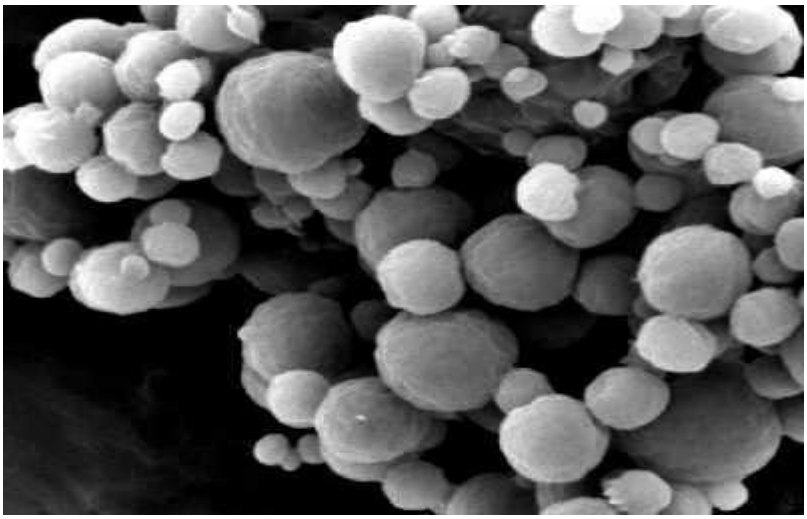

**Fig-8: SEM analysis of Optimized polymeric nanoparticle Drug entrapment efficiency.**

Optimizing the polymer concentration to be used in the creation of polymeric nanoparticles was the first step of the work plan. Based on the particle size and entrapment effectiveness of the discovered polymeric nanoparticles, the polymer content was optimized.

**Table-8: Evaluation Studies of Prepared polymeric nanoparticles: Entrapment Efficiency and Particle size and zeta potential.**

| Batch No | Particle size (nm) | Entrapment Efficiency (%) | zeta potential |
|---|---|---|---|
| F1 | 243 | 75.86 | -30 |
| F2 | 241 | 78.12 | -28 |
| F3 | 270 | 73.92 | -17 |
| F4 | 256 | 75.20 | -19 |
| F5 | 274 | 77.14 | -20 |
| F6 | 290 | 79.85 | -24 |
| F7 | 276 | 76.82 | -26 |
| F8 | 268 | 71.25 | -29 |

**In vitro drug release studies**

Using a dialysis membrane and a pH 7.4 buffer, the in vitro diffusion investigations were carried out for eight hours. The initial release of the medication from all three batches was discovered to be between 25 and 30 percent in 8 hours. This resulted from the drug's release from the surface of the nanoparticles. Later, for 8 hours, a consistent and gradual medication release was seen. The polymer ratio in the F6 formulation was shown to be the most effective one.

**Table-9: *In vitro* drug release profiles of Dorzolamide Hydrochloride polymeric nanoparticles (F1-F8).**

| Time | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 15.98 | 14.71 | 14.89 | 15.81 | 16.82 | 17.10 | 15.93 | 16.10 |
| 2 | 28.90 | 27.82 | 27.56 | 27.57 | 28.93 | 24.69 | 29.63 | 28.51 |
| 3 | 37.46 | 35.60 | 34.68 | 32.54 | 33.25 | 35.10 | 38.40 | 37.19 |
| 4 | 48.19 | 47.92 | 48.25 | 47.90 | 46.80 | 45.89 | 48.81 | 45.66 |
| 5 | 53.64 | 52.38 | 55.74 | 52.20 | 53.69 | 55.50 | 56.93 | 50.35 |
| 6 | 68.90 | 67.91 | 69.86 | 65.94 | 64.77 | 69.98 | 65.89 | 66.98 |
| 7 | 78.17 | 80.40 | 78.90 | 77.51 | 80.13 | 81.25 | 83.25 | 80.19 |
| 8 | 91.68 | 92.86 | 93.58 | 94.18 | 95.82 | 97.10 | 95.32 | 94.56 |

**Table-10: Drug release kinetics of optimized formulation.**

| TIME | %CDR | SQARE T | LOG T | LOG%CDR | ARA | LOG%ARA |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 17.1 | 1 | 0 | 1.23299611 | 82.9 | 1.91855453 |
| 2 | 24.69 | 1.41421356 | 0.30103 | 1.39252109 | 75.31 | 1.87685265 |
| 3 | 35.1 | 1.73205081 | 0.47712 | 1.54530712 | 64.9 | 1.8122447 |
| 4 | 45.89 | 2 | 0.60206 | 1.66171806 | 54.11 | 1.73327753 |
| 5 | 55.5 | 2.23606798 | 0.69897 | 1.74429298 | 44.5 | 1.64836001 |
| 6 | 69.98 | 2.44948974 | 0.77815 | 1.84497394 | 30.02 | 1.47741069 |
| 7 | 81.25 | 2.64575131 | 0.8451 | 1.90982337 | 18.75 | 1.27300127 |
| 8 | 97.1 | 2.82842712 | 0.90309 | 1.98721923 | 2.9 | 0.462398 |

**Zero orer kinetics**

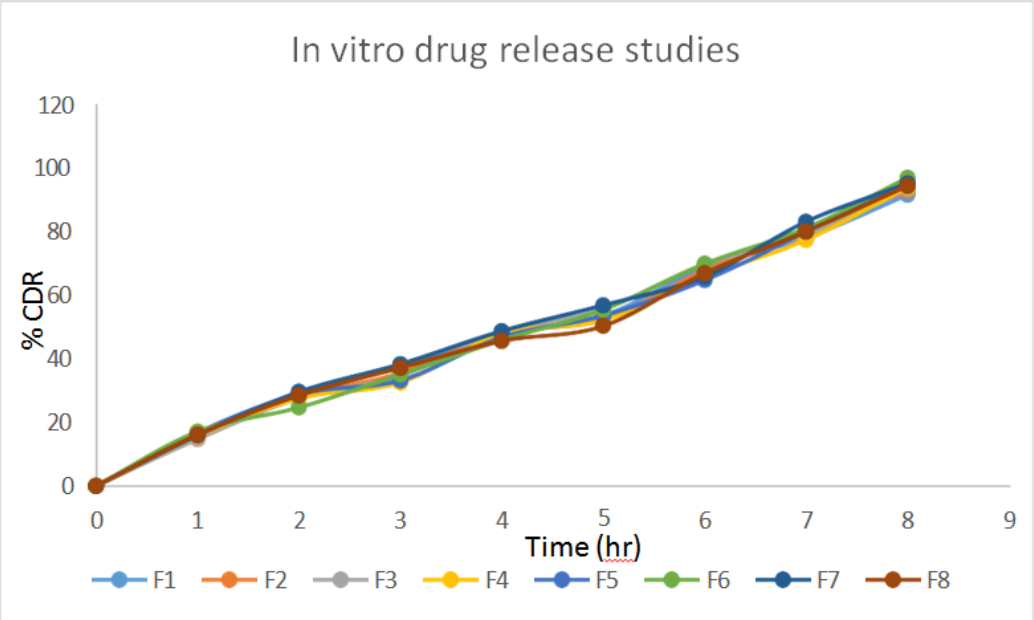


**Fig-9: Drug release for all formulations Drug release kinetics.**

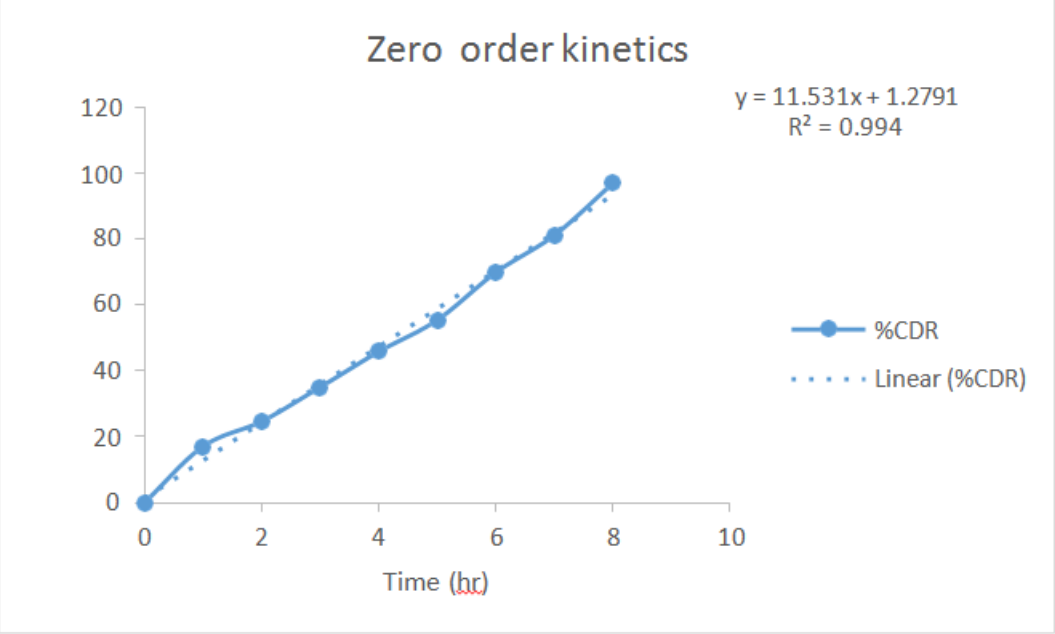


**Fig-10: Zero order kinetics of optimized formulation First order kinetics.**

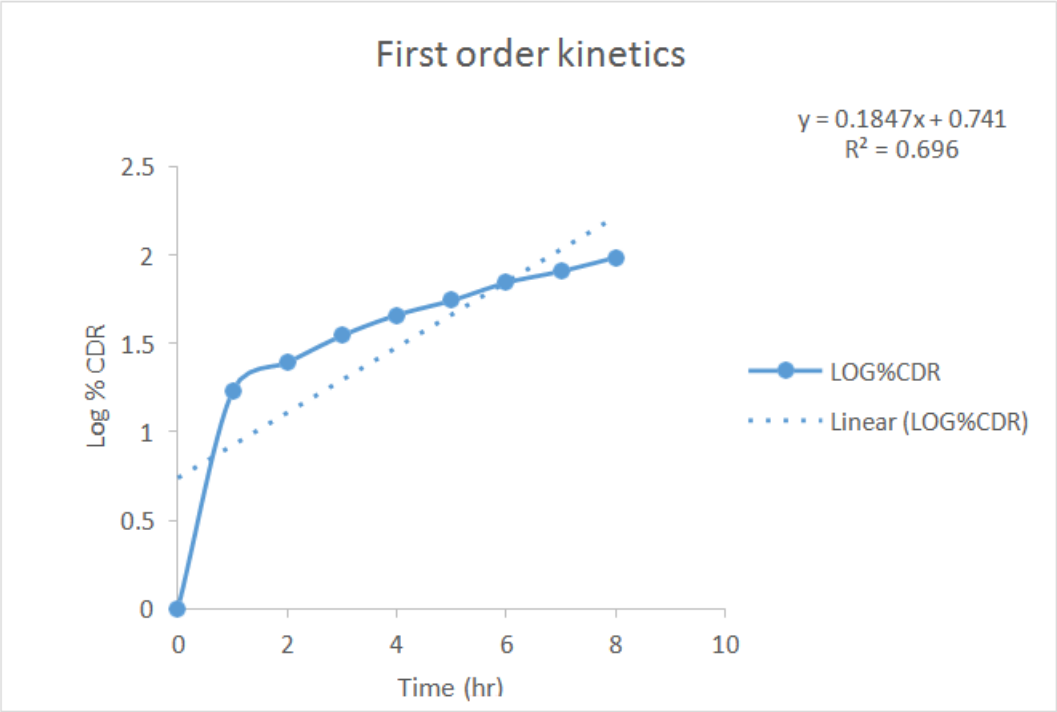


**Fig-11: Zero order kinetics of optimized formulation.**

**Higuchi model**

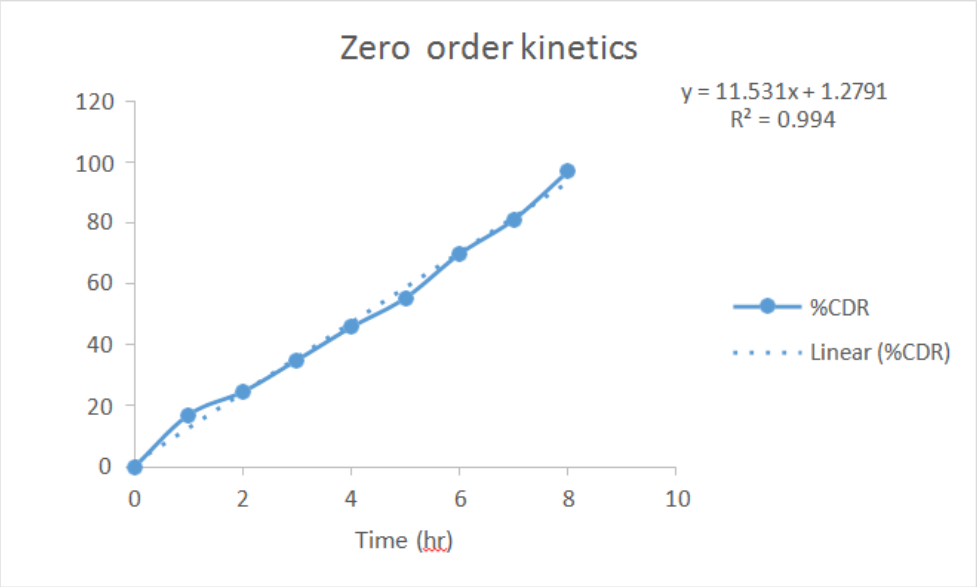


**Fig-12: Higuchi model of optimized formulation Korsmeyer poppas.**

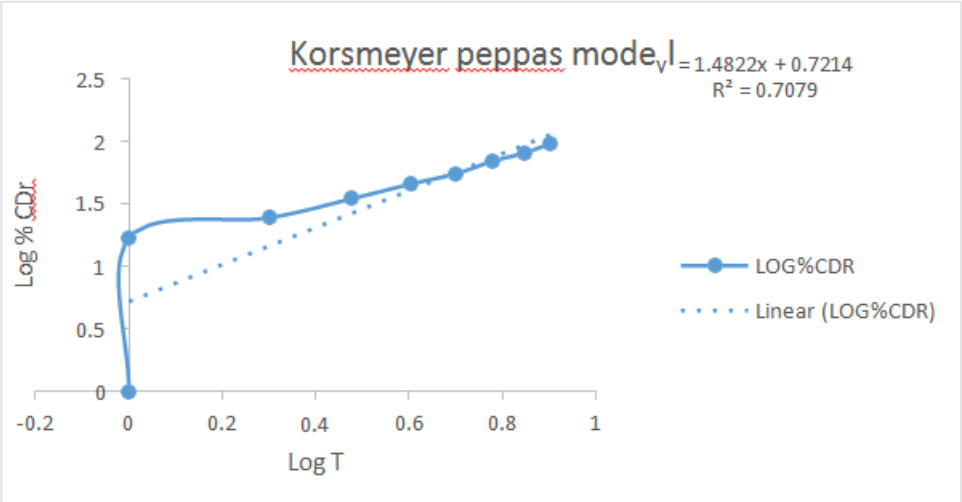


**Fig-13: Korsmeyerpeppas of optimized formulation.**

The release kinetics for all the prepared nanoparticles was evaluated to determine the release behavior of Dorzolamide Hydrochloride from the prepared nanoparticles. The release data were analyzed with zero-order kinetic, first-order kinetic, and Korsmeyer–Peppas kinetic models, as well as the Higuchi kinetic model. It was revealed that the release data from nanoparticles s fit to Higuchi kinetic model with the highest (r) value, while for free Dorzolamide nanoparticles, the release data fit the zero-order kinetic model.

**Stability studies**

After three months, the physical and chemical characteristics of the nanoparticles of formulation F-6 had not significantly changed. The parameters quantified at various times were displayed.

**Table-11: Results of stability studies of optimized formulation F-6.**

| Formulation Code | Parameters | Initial | 1st Month | 2nd Month | 3rd Month | Limits as per Specifications |
|---|---|---|---|---|---|---|
| **F-6** | **$25^0$C/60%RH % Release** | 97.10 | 96.85 | 95.81 | 94.86 | **Not less than 85 %** |
| **F-6** | **$30^0$C/75% RH % Release** | 97.10 | 96.21 | 95.25 | 94.10 | **Not less than 85 %** |
| **F-6** | **$40^0$C/75% RH % Release** | 97.10 | 96.08 | 95.46 | 94.02 | **Not less than 85 %** |

**CONCLUSION**

The current study suggested a unique Gemcitabine polymeric nanoparticle formulation for regulated release. Investigation into the polymeric nanoparticles' production, characterization, and in-vitro release was done. The numerous formulations with varied drug-polymer and surfactant ratios were analyzed and improved. A drug encapsulation effectiveness of up to 84.50% has been attained in this study. Gemcitabine polymeric nanoparticles containing polymers were created using the solvent evaporation method, then the particle size was decreased by sonication. Formulations using polymeric nanoparticles performed well in terms of medication content and encapsulation effectiveness. This shows that the formulation procedure was suitable and reproducible in nature, and it provided a good yield. The formulation with the best encapsulation efficiency was (F-6) It was discovered that the percentage of encapsulation efficiency along with the polymer concentration. According to the method described, permeation studies with dialysis membrane were conducted. The in vitro drug release profiles of all the formulations indicated an initial burst effect, followed by a gradual drug release. The formulations demonstrated good drug release from the polymer. These polymeric nanoparticles contained more Gemcitabine and released it more quickly.

**REFERENCES**

1. S. Mukherjee, S. Ray and R. S. Thakur, Ind. J. Pharm. Sci., 2009; 349-358.
2. M. R. Mozafari, 2006; 41-50.
3. Rainer H. Muller, KarstenMader and Sven Gohla, Eur. J. Pharm. Biopharm., 2000; 50(1): 161- 177.
4. Wolfgang Mehnart and KarstenMader, Adv. Drug. Deliv. Rev., 2001; 47: 165-196.
5. Houli Li, Xiaobin Zhao, Yukun Ma and Guangxi Zhai, Ling Bing Li and Hong Xiang, Lou. J. Cont. Release, 2009; 133: 238-244.
6. MelikeUner, GulgunYener, Int. J. Nanomedicine, 2007; 2(3): 289-300.
7. Annette ZurMehlen, Cora Schwarz and Wolfgang Mehnart, Eur. J. Pharm. Biopharm., 1998; 45: 149-155.

8. Elena Ugazia, Roberta Cavalli and M. R. Gasco, Int. J. Pharm., 2002; 241: 341-344.
9. Indu Pal Kaur, Rohit Bhandari, Swati Bhandari and Kakkur. J. Cont. Rel., 2008; 127: 97-109.
10. GhadaAbdelbary and Rania H. Fahmy, AAPS Pharm. Sci. Tech., 10(1): (2009).
11. Ramu, B., Manasa, M.S. (2015). Formulation and Evaluation of Colon Specific Drug Delivery of Press Coated Lansoprazole Tablets. Indo American Journal of Pharmaceutical Research, 5(4): 1522.
12. Ramu B. Formulation of Lamotrigine Orodispersible Tablets By Using New Generation Superdisintegrants Generation Superdisintegrants World Journal Of Pharmacy And Pharmaceutical Sciences, 2015; 4: 631- 43.