VOLUME 11 ISSUE 2 2025

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 11, No. 2, 921-930 (2025)

International Journal of Zoological Investigations

Contents available at Journals Home Page: www.ijzi.net

Editor-in-Chief: Prof. Ajai Kumar Srivastav

Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Formulation, Evaluation and Release Kinetics of Ofloxacin Nanoparticles

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Received: 18th February, 2025; **Accepted:** 22nd September, 2025; **Published online:** 17th November, 2025

https://doi.org/10.33745/ijzi.2025.v11i02.089

Abstract: The ofloxacin-loaded nanoparticles were formulated using egg albumin and chitosan. Cross-linking agents used for the nanoparticles were calcium chloride, glutaraldehyde, and dimethyl sulfoxide. The evaluation process of the nanoparticles was carried out by scanning electron microscopy (SEM), percentage of yield, drug entrapment study, *in vitro* dissolution study, and release kinetics parameters. All formulations show good entrapment efficiency, but 58.31% was the maximum entrapment, which was governed by F3, and the highest percentage of yield was 61.53%, which was acquired by F1. By analysing the particle size of the formulations, the smallest particle size was 453.33±29.31 nm.From these experiments, comparing the other two formulations, the formulation F1 with 1% calcium chloride exhibits the highest drug release, whereas the formulation F3 with 3% calcium chloride exhibits the lowest drug release. Drug entrapment efficiency % for F1 was least and for F3 was highest. Among the three formulations, F1 follows the Higuchi model, while F2 and F3 follow the Korsmeyer-Peppas model.

Keywords: Nanoparticles, Ofloxacin, Egg albumin, Chitosan, Calcium chloride, Dimethyl sulfoxide, Glutaraldehyde

Citation: Dey Rimi, Das Sudipta, Roy Madhusri, Jana Shubhadip and Bhowmick Souktik: Formulation, evaluation and release kinetics of ofloxacin nanoparticles. Intern. J. Zool. Invest. 11(2): 921-930, 2025.

https://doi.org/10.33745/ijzi.2025.v11i02.089

Introduction

Nanotechnology is used in various fields of physics, chemistry, biology, medical science, and engineering. Nanoparticles are nano-sized particles ranging from 1-100 nm, so they are incapable of being seen with the human eye. The word nanoparticle refers to a mixture of nano-spheres and nanocapsules (Nikam *et al.*, 2014; Prakash *et al.*, 2020). The foremost goal for developing nanoparticles is to control the discharge of the drug to the targeted organ at determined time intervals with a determined dosing frequency (Nikam *et al.*, 2014). Nanoparticles can be divided into two types: hard nanoparticles and soft nanoparticles. Nano-particles can also be obtained from different natural sources (Prakash *et al.*, 2020).

Nanoparticles can be of different shapes and sizes, like spheres, tubes, or cones, and sometimes they do not have any definite shape (Kumari and Sarkar, 2021).

There are different methods used for making nanoparticles-

(i) Solvent evaporation method: In this method, the polymer and the drug are dissolved in the organic solvent; this mixture forms an o/w emulsion by the emulsification process. The organic solvent is being evaporated by continuous stirring at reduced pressure and temperature which results in the formulation of nanoparticles.

(ii) Nano-precipitation: In this process, polymers are mixed with the solvent (acetone, methanol) along with surfactant in some specific cases. Then the mixture is scattered in polylactic acid solution (PLA), and due to the polarity range of PLA, it dissolves in the water-miscible solvent and forms nanoparticles (Prakash *et al.*, 2020).

Emulsification diffusion is an improved form of the solvent evaporation method. In this method, a water-miscible solvent is mixed with the water-immiscible organic solvent. Due to continuous diffusion, it causes turbulence, which helps in making small particles. Due to the high concentration of water-miscible solvent, the size of the particle decreases. The solvents are evaporated, and nanoparticle formation occurs (Nikam *et al.*, 2014). Dialysis, this method is also known as nano-precipitation. In this process, organic solvent is used as a polymer, which is kept in the dialysis tube. Polymers gather with each other to lose their solubility, and thus the formation of nanoparticles occurs. Supercritical fluid technology, this process is known for producing large-scale nanoparticles. This method has very few disadvantages. The fluid used in this technology is eco-friendly in nature. CO_2 is mostly used in this process because of its non-toxic and non-inflammatory nature (Prakash *et al.*, 2020).

The benefits of nanoparticles are greater solubility, increased duration of drug release, the ability to be taken through different routes of administration, less harm due to the presence of a biodegradable polymer coating, and action at the targeted site (Prakash *et al.*, 2020). The drawbacks of nanoparticles are a complex procedure, which have a nominal size and more surface area; due to less size and more surface area, the particles get assembled (Nikam *et al.*, 2014; Prakash *et al.*, 2020). Nanoparticles are generally used for the distribution of proteins and peptides, drug and gene delivery, protein detection, and treatment processes of tumors and cancer and have antibacterial effects (Prakash *et al.*, 2020).

Ofloxacin is an anti-microbial drug belonging to the family fluoroquinolone. This drug got approval in 1990 by the FDA (Food and Drug Administration). It is generally used in the treatment of gram-positive and gram-negative bacterial infections (Graham and Tripp, 2024). Ofloxacin belongs to the class of broad-spectrum anti-microbial drugs that show bactericidal effects. It shows its activity by connecting to an obstructing bacterial topoisomerase II (DNA gyrase), an enzyme responsible for relaxing supercoiled DNA, as well as topoisomerase IV, an enzyme involved in separating linked daughter chromosomes following replication. Furthermore, fluoroquinolones are bactericidal and become concentrated intracellularly, resulting in rapid intracellular killing (Graham and Tripp, 2024). Some of the adverse effects are nausea, vomiting, diarrhoea, abdominal pain, dizziness, headache, and insomnia. (Halkin, 1988).Drugs that may interact with ofloxacin include- theophylline, sulphonyl urea hypoglycaemic drug (Davey, 1988).

In the present study, the ofloxacin-loaded nanoparticles were formulated using egg albumin and chitosan. Cross-linking agents used for the nanoparticles were calcium chloride, glutaraldehyde, and dimethyl sulfoxide. The evaluation process of the nanoparticles was carried out by scanning electron microscopy (SEM), percentage of yield, drug entrapment study, *in vitro* dissolution study, and release kinetics parameters.

Table 1: Formulations of Nanoparticles

FORMULATION	F1	F2	F3
Drug (mg)	50	50	50
Chitosan (mg)	300	300	300
Egg albumin (mg)	300	300	300
Acetic acid (ml)	20	20	20
GA (g)	1.5	1.5	1.5
DMSO (ml)	1	1	1
Water (ml)	50	50	50
CaCl2 (g)	0.5	1	1.5

Materials and Methods

Materials required

Ofloxacin (Yarrowchem Products, India), egg albumin (Loba Chemie Pvt. Ltd., India), chitosan (Yarrowchem Products, India), calcium chloride (Loba Chemie Pvt. Ltd., India), glutaraldehyde (Loba Chemie Pvt. Ltd., India), dimethyl sulfoxide (Loba Chemie Pvt. Ltd., India) were used in this research study. All chemicals and reagents used were of analytical grade.

Methodology

For the preparation of different formulations, about 300 mg of chitosan (Li *et al.*, 1996; Wang and Zhuang, 2022) and egg albumin (Aniesrani *et al.*, 2016), respectively, were added to 20 ml of acetic acid in a beaker along with 50 mg ofloxacin (Table 1). This drug-polymer solution was kept over the magnetic stirrer for continuous stirring until no residue is left. In another beaker, a cross-linking agent solution was prepared using 1.5 g GA (Sano *et al.*, 2005; Barbosa *et al.*, 2013) and 1 ml DMSO (Notman *et al.*, 2006; Sanmartín-Suárez *et al.*, 2011) along with different amounts of $CaCl_2$ (0.5 g, 1 g, and 1.5 g) in 50 ml distilled water (Vrana, 2001). This cross-linking agent solution was kept over the magnetic stirrer, and the drug-polymer solution was added drop-wise using a syringe, and then this solution was continuously stirred for about 1 h. Then the solution was stored in the refrigerator for about 12 h. After that, the solution was centrifuged for about 1 h at 1000 rpm. Then from the centrifuged solution, the settled sediment was separated and dried in a vacuum desiccator (Adlin *et al.*, 2009; Singh, 2011).

Evaluation

Percentage of yield: The yields of ofloxacin-loaded nanoparticles were computed by measuring the initial weight of drug-polymers used in the preparation and weight of the finally prepared nanoparticles. The percentage yield of nanoparticle was determined by using the following formula:

% yield = (Practical yield / Theoretical yield) X 100

Scanning electronmicroscopy: The particle size of different formulations has been measured using Scanning Electron Microscopy (SEM) (JEOL, Japan). (Adlin *et al.*, 2009; Singh, 2011).SEM photographs were taken at 5.0 kV and their morphology was examined (Das *et al.*, 2024).

Drug entrapment efficiency: Firstly, three formulations were taken into three different test tubes, each comprising 10 mg nanoparticle formulation in 100 ml water, respectively. Then, the absorbance is being measured at 288 nm against phosphate buffer pH 7.4 as a blank in a UV spectrophotometer. The drug content was determined from the standard curve(Saha *et al.*, 2010; Kharia *et al.*, 2012).

Drug entrapment efficiency = (Estimated drug content/Theoretical drug content) X 100

In vitro dissolution study: At first, three formulations were taken and then measured about

Table 2: % Yield of three formulations

Formulation Number	% Yield
F1	61.53
F2	58.46
F3	52.30

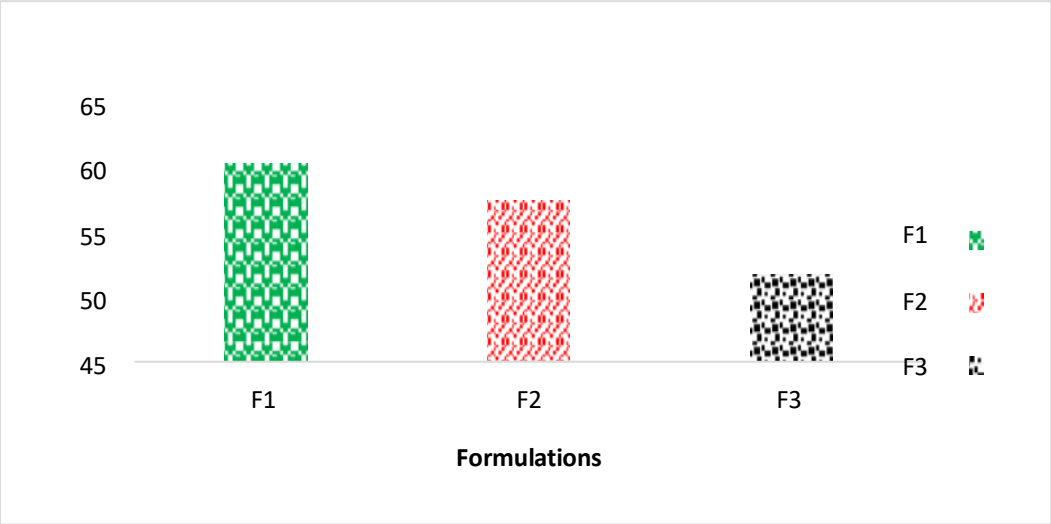


Fig. 1: Percentage yield of Ofloxacin nanoparticles.

306.17 mg, 427.91 mg, and 222.98 mg. Now all these three formulations were given into three individual test tubes covered with semi-permeable membranes. 100 ml of water was taken as dissolution medium in three individual beakers, a test tube holder, stirrer, and pipette were used in this study. All three test tubes were perpendicularly touching the water surface. After that, the dissolution rate was taken after each 30 min of time up to 240 min. 8 individual test tubes were needed for each three formulations to collect the samples from the beakers which were undergoing through UV spectrometer for determining the drug dissolution rates (Ekambaram and Abdul Hasan, 2011; Thakur *et al.*, 2012; Lokhande *et al.*, 2013).

Results and Discussion

Percentage of yield

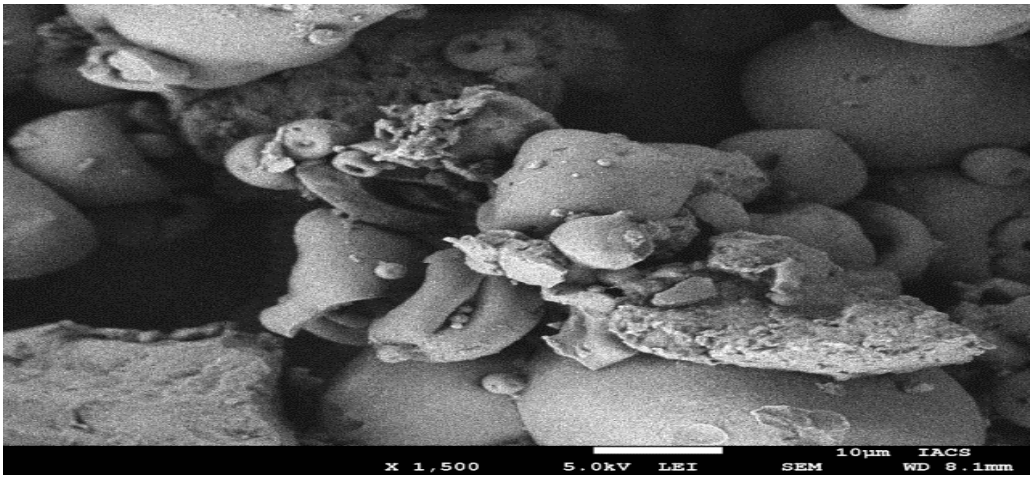
In this study, ofloxacin-loaded nanoparticles were formulated using chitosan and egg albumin with GA and $CaCl_2$ as cross-linking agents. Ofloxacin-loaded nanoparticles containing 1%$CaCl_2$ (F1) exhibited comparatively better percent yields (61.53%) than the ofloxacin-loaded nanoparticles containing 2% $CaCl_2$ (F2) and 3% $CaCl_2$ (F3) i.e. 58.46% and 52.30%, respectively (Table 2, Fig. 1). The percent yield was found to be comparatively lower in case of higher concentration of $CaCl_2$ while the concentration of GA remained same in all the three formulations.

Scanning electron microscopy (SEM)

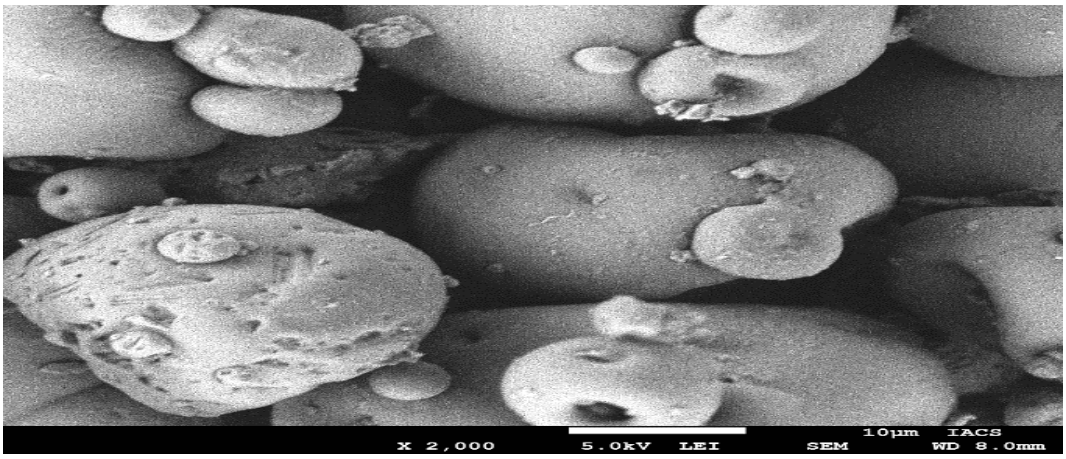
SEM photographs presenting surface morphology of ofloxacin-loaded nanoparticles are presented in Figure 2. A number of derbies and drug crystals were found onto the surface of nanoparticles. The obtained data of particle size of the formulations was reported as mean ± SD, which was analyzed by Microsoft Excel (Tables 3, 4).

Drug entrapment efficiency

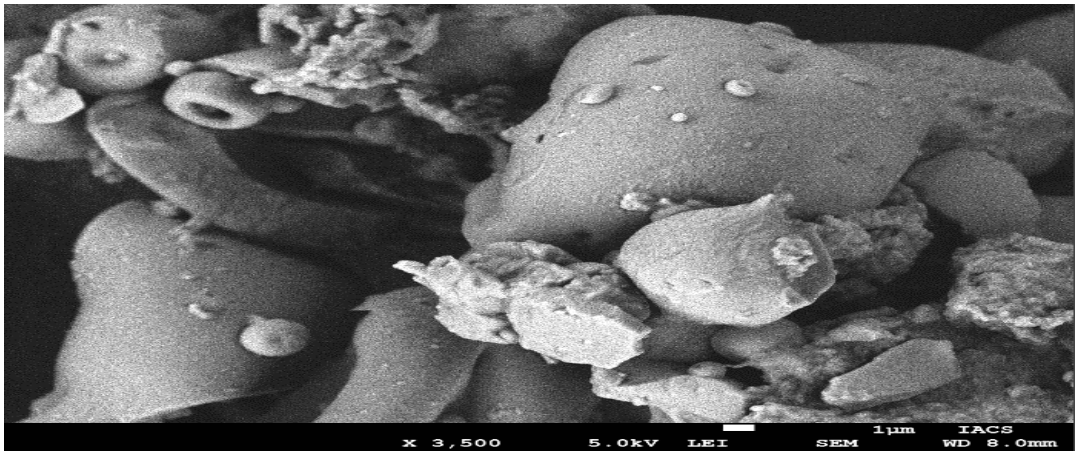
Ofloxacin-loaded nanoparticles containing 1% $CaCl_2$ (F1) exhibited comparatively lower drug entrapment efficiency (30.38%) than the ofloxacin-loaded nanoparticles containing 2% $CaCl_2$ (F2) and 3% $CaCl_2$ (F3). The drug entrapment efficiency of F2 and F3 was 42.46%



(a) SEM of F1



(b) SEM of F2



(c) SEM of F3

Fig. 2: SEM of 3 different formulations. (a) magnification = X1500, (b) magnification = X2000 and (c) magnification = X3500. Instrument: 7500F, Acc. Voltage: 10.0 kV, Probe Current: 0.69400 nA, PHA mode: T4, Real Time: 51.03 sec.

Table 3: Particle size analysis of Ofloxacin nanoparticles

S. No.	Particle size (nm)		
	F1	F2	F3
1	450	510	429
2	489	412	640
3	535	438	483

Table 4: Particle size range of different formulations

Formulation Number	Particle size (nm)
F1	491.333±24.565
F2	453.333±29.310
F3	517.333±63.283

Each value is represented as a mean ± SEM, n=3

Table 5: Drug entrapment efficiency % of three formulations

Formulation Number	Drug entrapment efficiency (%)
F1	30.38
F2	42.46
F3	58.31

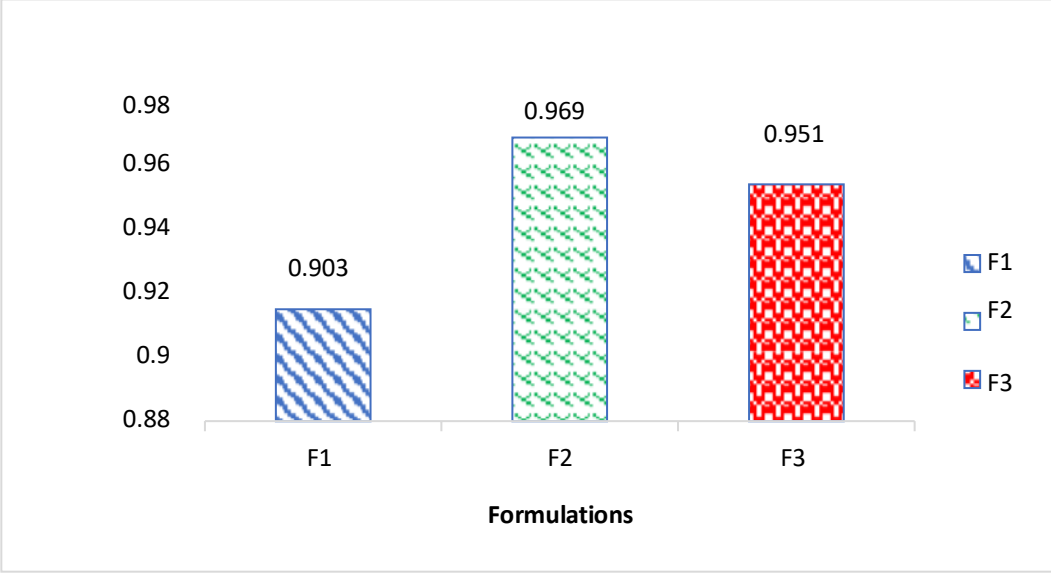


Fig. 3: % of drug entrapment efficiency of Ofloxacin nanoparticles.

and 58.31%, respectively Table 5, Fig. 3). The concentration of glutaraldehyde was kept equal in all the three formulations. Then it was the concentration of $CaCl_2$ that affected the entrapment efficiency. The drug entrapment efficiency was found to be comparatively higher in case of higher amount of $CaCl_2$.

In vitro dissolution study

The drug release study demonstrated that all the ofloxacin-loaded nanoparticles showed a sustained release pattern over 240 min, *in vitro*. Ofloxacin-loaded nanoparticles containing 1% $CaCl_2$ (F1) exhibited the highest drug release i.e. 56.32%, whereas the formulation F2 with 2%

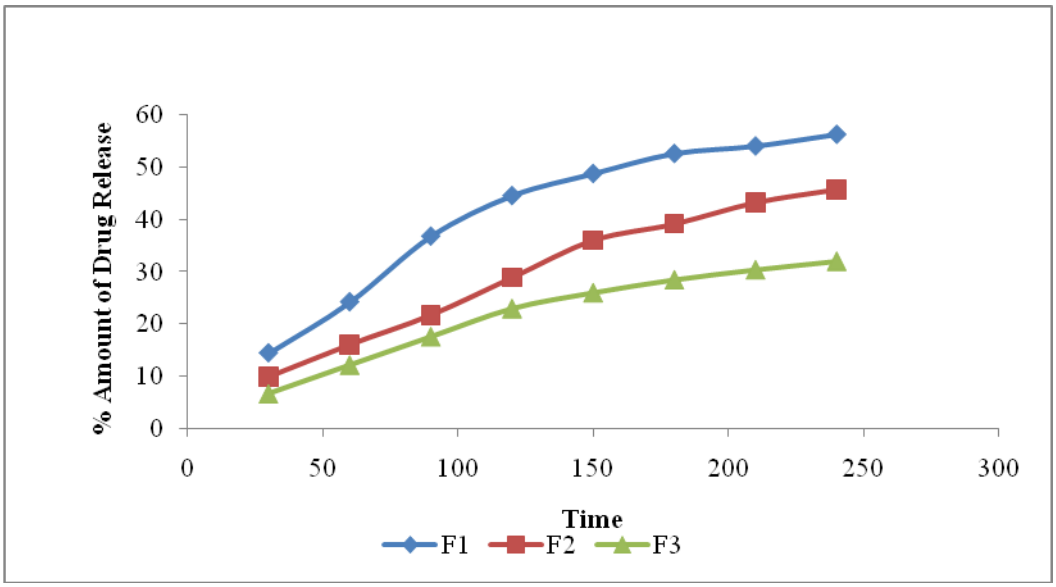


Fig. 4: *In vitro* dissolution study of ofloxacin nanoparticles.

Table 6: R^2 (Phosphate Buffer)

Formulations	Zero Order	First Order	Hixon-Crowel	Higuchi	Korsmeyer-Peppas	Follows
F1	0.903	0.950	0.906	0.974	0.965	Higuchi Model
F2	0.969	0.991	0.982	0.966	0.994	Korsmeyer-Peppas
F3	0.951	0.968	0.963	0.972	0.983	Korsmeyer-Peppas
Average	0.941	0.969	0.950	0.970	0.980	-

calcium chloride and F3 with 3% calcium chloride exhibited the lower drug release i.e. 45.74% and 32.03%, respectively (Fig. 4). This fact could be occurred due to the greater cross-linking degree for higher concentration of $CaCl_2$ in F2 and F3. Among the three formulations, F1 followed Higuchi model while F2 and F3 followed Korsmeyer-Peppas model.

Determinants of release kinetics parameters

The *in vitro* release data obtained in phosphate buffer were analyzed using various kinetic models i.e., Zero-order, First-order, Higuchi, Hixson–Crowell, and Korsmeyer–Peppas—to check the mechanism of drug release from the formulations. The linear regression coefficients (R^2) for each model were calculated for all the 3 formulations, and the model showing the highest R^2 value was considered to best represent the release pattern (Table 6). As shown in Figure 5, formulation F1 showed maximum linearity with the Higuchi model (R^2 = 0.974), representing diffusion-controlled release. In contrast, formulations F2 and F3 were better described by the Korsmeyer–Peppas model, having R^2 values of 0.994 and 0.983, respectively, involving both drug diffusion as well as polymer chain relaxation (Fig. 6). These results recommend that the release behaviour was strongly influenced by the polymer composition along with degree of cross-linking within the matrix.

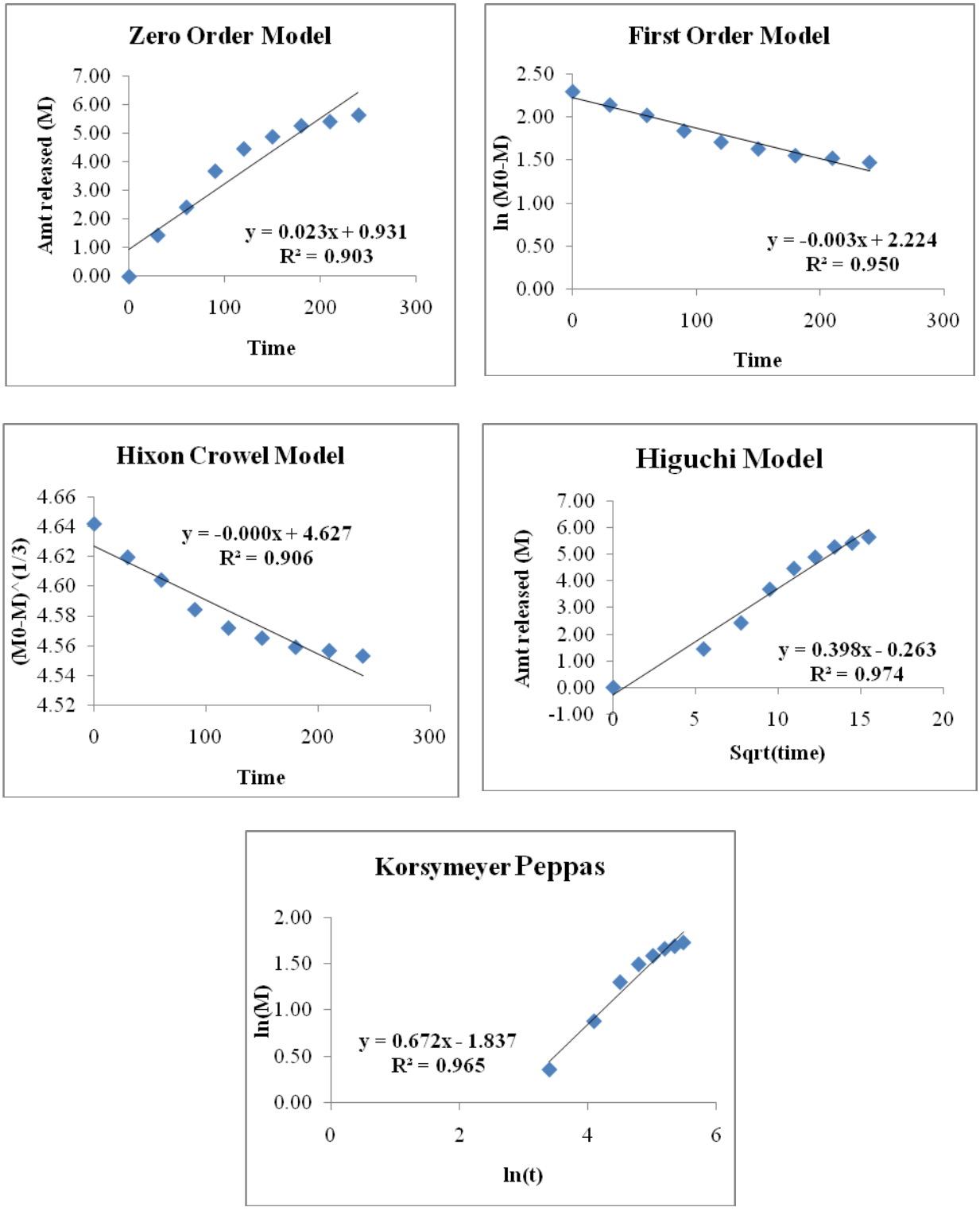


Fig. 5: Curves of release kinetics parameters of F1 (Zero order, First order, Higuchi, Hixon-Crowell, Korsmeyer-Peppas).

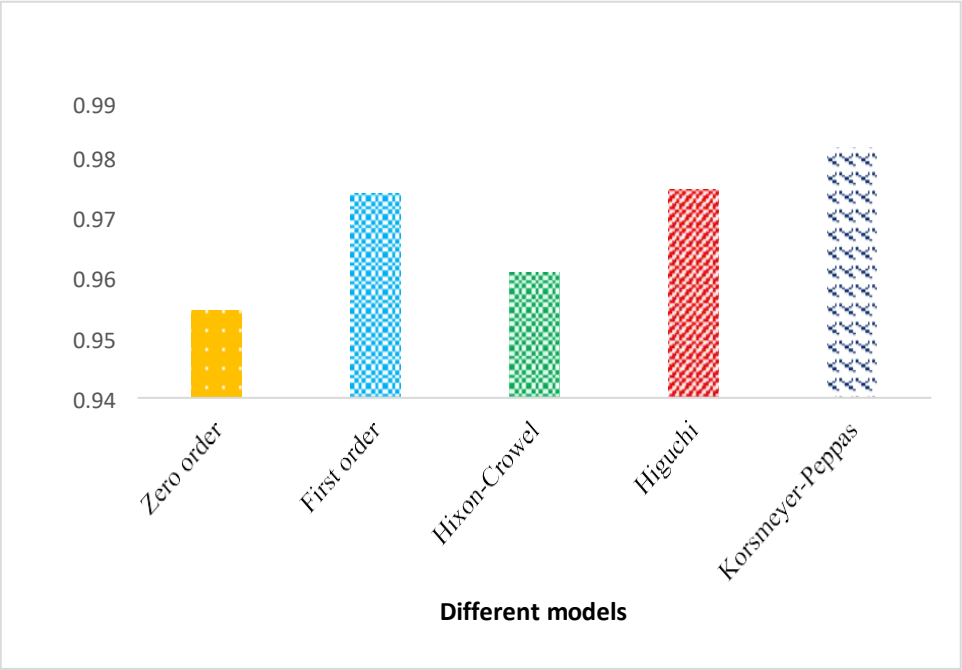


Fig. 6: Average of R^2.

Conclusion

From the present study it can be inferred that the three nanoparticle formulations (F1, F2 and F3) have particle size 491.333±24.565 nm, 453.333±29.310 nm, and 517.333±63.283 nm, respectively. Compared to the other two formulations, the formulation F1 with 1% calcium chloride exhibits the highest drug release i.e. 56.32%, whereas the formulation F3 with 3% calcium chloride exhibits the lowest drug release i.e. 29.55%. The three formulations F1, F2, F3 have percentage yield 61.53%, 58.46%, 52.30%, respectively. Drug entrapment efficiency % for F1 was least i.e. 30.38% and for F3 was high i.e. 58.31%. Among the three formulations, F1 follows Higuchi model while F2 and F3 follow Korsmeyer-Peppas model.

Ethical Statement

No animal or microbes have been used or sacrificed for this study, hence Ethical Approval not required.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

The authors extend thank to the Principal and Authority of Netaji Subhas Chandra Bose Institute of Pharmacy, Chakdaha, Nadia, West Bengal, India, for providing the necessary facilities to perform the present study.

Conflict of Interest

The authors declare no conflicts of interest.

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