# FORMULATION AND EVALUATION OF NIOSOMAL SUSPENSION OF CEFIXIME

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## ABSTRACT

**Objectives:** The present study was aimed to overcome the problems associated with the drug such as bioavailability, to reduce the dosage regimen, half–life, and to determine the appropriateness of niosomal formulation as a drug carrier.

**Methods:** The niosomal suspension was prepared by thin film technique, by varying ratios of span 60 and cholesterol and varying the concentration of span 60. The prepared four formulations were evaluated for various parameters.

**Results:** The optimized formulation had a vesicular size of 250-400 nm. Varying the concentration of span 60, the entrapment efficiency demonstrated that it had a considerable task. The highest entrapment efficiency was 95.3%. The kinetics study confirmed that the liberation of drug from the niosomal suspension is in a restricted manner. The statistical optimization showed that NS2 is the optimized formulation. The gastrointestinal enzymes showed no significant change in the release of drug from the formulation. The zone of inhibition showed that optimized formulation has a better activity than the marketed formulation. The MIC was found to be 0.05 mg, hence can be used as an efficient carrier for delivery of cefixime.

**Conclusion:** The present study concludes that the prepared niosomal suspension is a convenient and efficiency carrier for the delivery of antibacterial drug. Besides this, it provided controlled delivery of drug.

**Keywords:** Niosomes, Cholesterol, Thin film technique, Vesicular size, Controlled drug delivery.

## INTRODUCTION

Noisomes are non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media. These are lamellar structures that are microscopic in size. Structurally niosomes are bilayered in nature. On the basis of preparation methods used, niosomes may be unilayer or multilayer [1,2]. Depending on various factors such as stability and cost, the niosomes are considered ideal when compared with liposomes. For various routes such as topical, ophthalmic, and parenteral niosomes prove to be a potential and possible drug delivery mechanism [3]. It was prepared by the method of ether injection method, handshaking method (thin film hydration technique), sonication, microfluidization, multiple membrane extrusion method, reverse phase evaporation technique, transmembrane pH gradient (inside acidic) drug uptake process (remote loading), and the "Bubble" method [4-8].

Various factors affecting niosomal formulations are type of surfactant, drug and amount, charge and cholesterol content, osmotic stress due to resistance, and constitution of membrane [6,9-12].

The main aim of this project is to study about niosomal suspension which is emerging as a potential drug carrier, as a new drug delivery for the antibiotic drug cefixime. Therefore, the objective behind the study is to prolong the action by incorporating the drug into the vesicular carriers.

## MATERIALS AND METHODS

### Methodology

*Pre-formulation studies*

Pre-formulation investigations are to provide information on physicochemical and biopharmaceutical study properties of drug molecule, non-drug substance, and materials used for packaging as well as compressibility.

### Identification of the drug

*Melting point*

The purity of the sample is indicated by the melting point so obtained. The melting point values can get lowered because of the presence of unwanted substances.

*$\lambda_{max}$ of the drug*

Using phosphate buffer, the absorption maximum of cefixime was obtained. A range of solutions (2-10 μg/ml) was scanned using UV spectrophotometer.

*Solubility*

To obtain a desirable concentration of the drug in the blood, solubility plays a major role [13]. Varying solvents were taken including 0.1N HCL, methanol, water, ethanol, and certain buffers such as phosphate buffer (7.4) to determine the solubility.

### Partition coefficient of drug

Partition coefficient of cefixime in n-octanol-water was obtained.

### Physical drug excipient compatibility

At 75% relative humidity and 40°C, the formulations were kept sealed for 30 days. Then, it was compared with the initial state for any occurrence of interaction.

### Fourier transform infrared (FTIR) studies

With the help of infrared absorption spectral analysis (FTIR), the cefixime compressibility with individualized non-drug component and physical combination of the major formulation was produced. Spectral analysis using IR helped to identify any modification in the characters of the formulation when it was mixed with the non-drug substance.

## Formulation of niosomal suspension

*Method*

Niosomal suspension was prepared by thin film hydration method (Table I). The cholesterol and the surfactant were mixed and added in about 20 ml of chloroform into an evaporating flask of rotary evaporator and dipped in water bath at 300°C. It was then evaporated at very low pressure until it formed a dry thin film on the flask, which was then dissolved in 20 ml of phosphate buffer, and kept on a water bath at 450°C and 120 rpm rotation to hydrate the layers, later sonicated to obtain niosomal suspension. The prepared niosomal suspension was then stored in a tight container in refrigerator for further characterization [10].

## Characterization of niosomal suspension

*Vesicular size evaluation*

Microscopic evaluation

A little amount of niosomal suspension was dissolved with 10-12 ml of phosphate buffer (pH 7.2). The distribution of niosomal suspension was shaken in regular intervals of time. When an drop of dispersion was microscopically observed, rounded vesicular constituents were seen with uniform small size at ×100.

Size and size distribution

Dynamic light scattering method was used to evaluate vesicular size distribution. The niosomal suspension was diluted with 10-12 ml distilled water with physical shaken. The temperature was fixed at 20°C, refractive index at 1.333 viscosity, and 0.01 poise.

Scanning electron microscopy

Niosomal suspension was diluted with distilled water and was attached with the help of a glued double-faced carbon tape on an aluminum stub. With the help of a vacuum evaporator, the vesicular components covered with gold and were examined.

Entrapment efficiency

The niosomal suspension was sonicated after loading the drug along with the phosphate buffer and was centrifuged and separated. Assay was done at 288 nm to quantify cefixime.

Zeta potential determination

The ideal formulation's zeta potential was obtained by Zetasizer.

### *In vitro* drug release

It carried out for the four niosomal suspension formulations.

### Activation of cellophane membrane

The cellophane membrane was activated first by dipping in hot water for 1 hr, and then membrane was immersed in ethanol for 30 minutes. The ethanol-immersed membrane was then transferred to acetate buffer of pH 4 and kept for overnight.

### *In vitro* drug release studies in gastrointestinal pH

Three milliliters of niosomal suspension containing known amount of drug was taken in a cellophane membrane tied to one of the ends of an open-ended cylinder. The entire apparatus was immersed in 30 ml 0.1 N HCL pH 1.2 and stirred at 370°C. Samples of definite amount were withdrawn at specific intervals of time (5, 10, 15, 30, 45, 60, 75, 90, 105, and 120 minutes) and were analyzed by UV spectrophotometry at 288 nm [11,12].

### *In vitro* drug release studies in phosphate buffer pH 7.2

Using spectrophotometry, the percentage of drug released was obtained.

### Statistical design of experiment

The software was used to perform the procedure for statistical optimization. The statistical optimization procedure was performed with the help of optimization software such as Statgraphics Centurion 16.

### ANOVA study by one-way method

When the F value is higher than critical F value, the null hypothesis is rejected.

### Study on the effect of gastrointestinal enzymes on the release of drug

The effect of the enzymes such as trypsin and pepsin was studied on the release of optimized formulation. 2 ml of niosomal suspension containing known amount of drug was taken in a cellophane membrane tied to one of the ends of an open-ended cylinder. The entire apparatus was immersed in 30 ml 0.1 N HCL pH 1.2 containing 0.25% pepsin and stirred at 370°C. Samples of definite amount were withdrawn at specific intervals of time (5, 10, 15, 30, 45, 60, 75, 90, 105, and 120 minutes) and were analyzed by UV spectrophotometry at 288 nm [13,14].

### *In vitro* antibacterial activity

The *in vitro* antibacterial activity was performed using agar diffusion assay method. The microorganism employed was *Escherichia coli* and Klebsiella. The activity was performed on optimized formulation, marketed formulation (cefixime - oral suspension), and plain niosomal suspension.

### Media and Chemicals

Media and chemicals were distilled water, 05% sodium chloride, 1.5% agar, 0.5% peptone, 0.3% beef extract/yeast extract, and pH adjusted to neutral (6.8) at 25°C.

### Additional materials required

Additional materials required were standard microbiological supplies and apparatus such as biochemical reagents, incubators, incinerator/autoclave, microscope, staining suppliers, slides, saline blank, and loops.

### Comparison study of antibacterial activity of optimized formulation with marketed formulation

The antibacterial activity of the ideal formulation was studied against commercially available formulation of the drug. 0.1-0.2 ml of the test microorganism was inoculated into the freshly prepared sterile nutrient agar plates. Wells were arranged, and a positive control was also added to another well. The plates were incubated for 18-48 hrs at room temperature. After incubation, they were examined for the presence or absence of the growth [15,16].

### Determination of minimum inhibitory concentration (MIC)

The bacterial activity was inhibited by the increase in the concentration of the sample. The MIC was taken at the lowest concentration that prevented the growth of the microorganisms [17].

### Stability studies

The ideal formulation of NS2 was preserved for 45 days at two different temperatures 30±2°C and 4±2°C to evaluate chemical and physical stabilities. For 7, 15, 30, and 45 days, the formulation was evaluated for entrapment efficiency [18-20].

### RESULT AND DISCUSSION

### Melting point of the drug

In accordance with the monograph, the melting point of the drug was found (218-225°C).

### $\lambda_{max}$ of the drug

The $\lambda_{max}$ of the drug was found to be 288 nm, and it was in agreement with the official standard as in Fig. 1.

The above parameters such as melting point, $\lambda_{max}$, and FTIR showed that it was in accordance with the reference and hence pure sample of cefixime I.P.

### Solubility of the drug

The pure drug solubility was compared with the standard sample, and it reveals that the pure drug is highly soluble in methanol (95%) and practically insoluble in water.

**Partition coefficient of the drug**

The partition coefficient of the drug was found to be 3.2. This indicates that cefixime is practically insoluble in water and having high lipid solubility, so this can be incorporated into niosomes for the efficient transport.

**Physical drug excipient compatibility**

There were no incompatibilities or interaction between drug and excipients.

**FTIR spectroscopy**

From the spectra, it is evident that all the peaks in the FTIR of the drug are shown (Fig. 2) in the FTIR peak of the formulation. This finding confirms that cefixime does not interact with any of the ingredients of the niosomal suspension.

**Analytical method**

Calibration curve (Fig. 2) in phosphate buffer pH 7.2 shows the absorption reading of standard drug solution containing 2-10 µg/ml of drug in phosphate buffer (7.2) at a wavelength of 288 nm.

On the basis of this standard curve (Fig. 3), the calculations of drug contents and *in vitro* drug release are studied.

**Characterization of niosomal suspension**
- Vesicular size evaluation
- Microscopic evaluation

Dissolved niosomes from niosomal suspension were observed microscopically and found to be having similarity and uniformity in vesicle size.

**Size and size distribution**

When evaluated for vesicular size and size distribution, it showed that ionic concentration rise can increase the mean vesicular size which ranged between 215 and 400 nm. The niosomal suspensions formed by hydration were of uniform size as suggested by the low polydispersity index as shown in Fig. 4.

**Scanning electron microscopy**

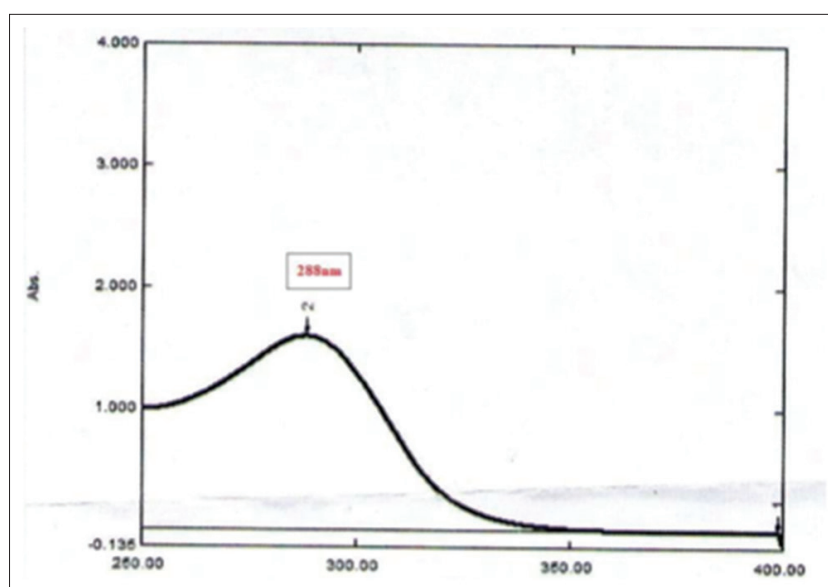Fig. 5 shows that the optimized formulation has a particle size between to be 250 and 400 nm.



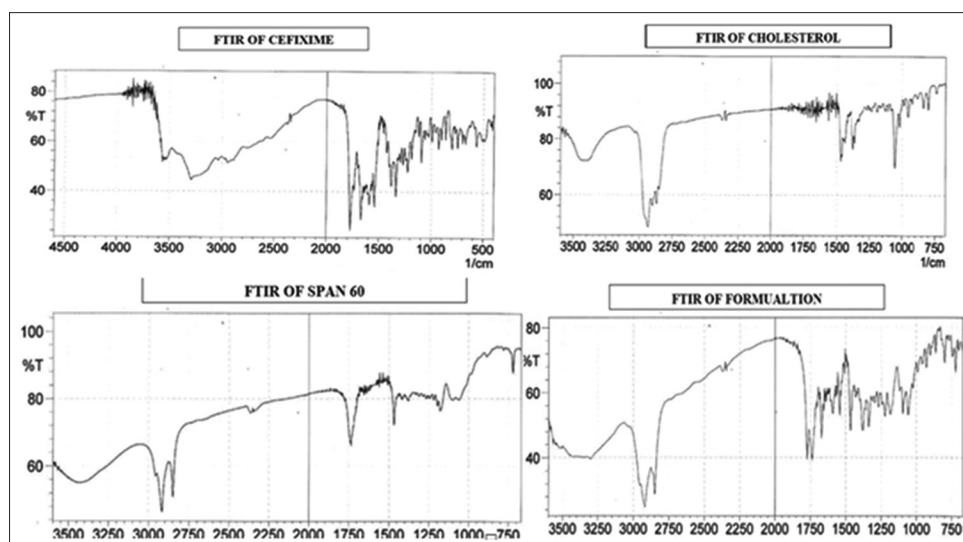Fig. 1: Absorption maxima of cefixime in phosphate buffer in phosphate buffer (pH = 7.2)



Fig. 2: Fourier transform infrared of cefixime, cholesterol, span 60, formulation

**Entrapment efficiency**

Entrapment efficiency of these formulations was found considerable at $p < 0.05$ (Fig. 6). Depicts the entrapment efficiency of the different formulations. NS2 has the highest entrapment efficiency.

**Zeta potential determination**

The zeta potential determination confirmed that the ideal formulation (NS2) illustrates that the sample is stable suspension.

***In vitro* release studies**

Both *in vivo* and delivery system functioning inside the body can be predicted by the release profile of a drug. All the four formulations underwent *in vitro* release studies in stomach pH and intestinal pH. The release data represented in the graph in Figs. 7-9.

The *in vitro* release pattern of the four formulations in the Stomach pH was studied, and it was concluded that there is no significant release of drug in the stomach pH as the release is only 0.4%. The release profiles suggest that the release of drug occurs slowly and later immediate release. Therefore, the release first occurs slowly and then immediately due to penetration enhancement of nonionic surfactant.
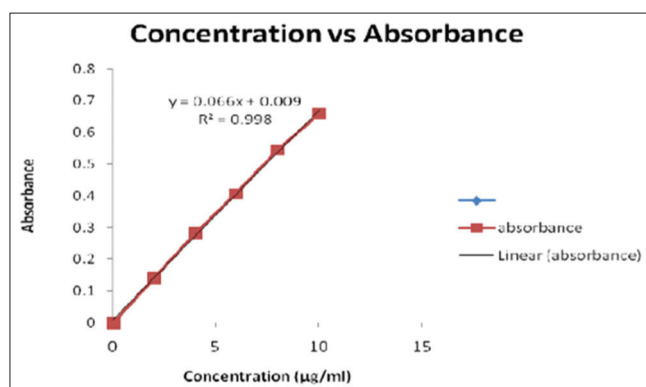


**Fig. 3: Calibration in phosphate buffer pH 7.2**

**Kinetic modeling of *in vitro* drug release**

The *in vitro* drug liberation of NS2 was fitted into various kinetic equations, and the linearity of each plot was determined by the closeness of regression coefficient to unity. The kinetic study of the release data showed that NS2 showed Higuchi model as the best fit model. The linearity of Higuchi plots indicates that as the distance of the diffusion increases the release of drug will be at a slower pace. This concludes that the drug is being released from the inner side of niosomal suspension in a restricted method. Kinetic study of the drug release profile optimum formulations NS2 is shown, and the graphical representations are shown in Fig. 9.

**Optimization of formulation by one-way ANOVA method**

Analysis of variance was used to optimize the formulation; it was carried out at 95% confidence interval. The report is represented in Figa. 10 and 11.

**ANOVA analysis report**

The ANOVA of entrapment efficiency indicates that both surfactant and cholesterol are significant terms as the F values are above the critical F values and thus making the $p < 0.05$ (threshold level).

There is a statistically significant difference between the means of the two variables at the 95.0% confidence level, represented in Fig. 12.

**Study on the effect of gastrointestinal enzymes on the release of drug**

*In vitro antibacterial activity*

a. Comparison study of antibacterial activity of the optimized formulation and marketed formulation by zone of inhibition.
   The optimized formulation NS2 was found to have better activity in both *E. coli* and Klebsiella than the marketed formulation of the drug (Table 2).
b. Determination of MIC of cefixime loaded niosomal suspension (Fig. 13).
   The MIC of the optimized formulation was obtained.
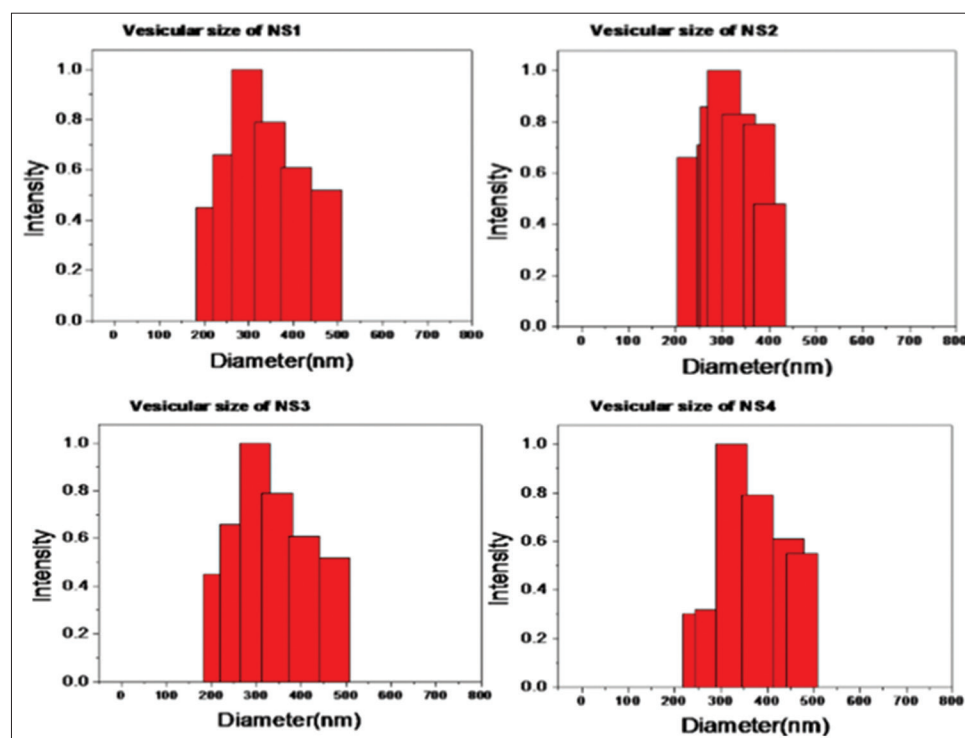
Furthermore, the value is tabulated in Table 3.



**Fig. 4: Vesicular size of niosomal suspension of NS1, NS2, NS3, and NS4**

**Stability studies**

The optimized formulation at two temperatures, i.e. 30±2°C and 4±2°C were done for 45 days; it does not show any variation when studied against the newly prepared formulation. There is no major variation in the entrapment efficiency while kept for 45 days in both of these conditions represented in Fig. 14.

**CONCLUSION**

The current article was intended to build up an appropriate transporter for the delivery of cefixime, third-generation cephalosporin antibiotic drug to conquer the troubles associated with it, like the poor solubility, low bioavailability, and half-life of the drug, thereby increasing the duration of action and thereby increasing the half-life. The pre-formulation studies were carried out; from the study, it was clear that it satisfy the entire characteristic for oral drug delivery. The partition coefficient of the drug confirmed that the drug is lipid soluble and hence confirmed that niosomal suspension may be a proficient for the delivery of drug. The niosomal suspension was prepared by thin film hydration technique by varying the concentration of surfactant, while keeping the concentration of cholesterol same. The characterization of the niosomal suspension was conducted and had a particle size of 350-450 nm. *In vitro* drug release was done in both stomach pH and intestinal pH. The release in stomach pH was only 0.4%, while the release in intestinal pH was 57.72%. The effect of gastrointestinal tract enzymes such as pepsin and trypsin on the *in vitro* liberation of drug from the formulation was also carried out. It was found that there is no much significant effect of the enzymes on the release of the drug from the formulation. The kinetics study of the formulation follows Higuchi model which states that the distance of the diffusion increases the release of drug will be at a slower pace. This concludes that the drug is being released from the inner side of niosomal suspension in a controlled manner. The optimization was based on the entrapment efficiency and *in vitro* release studies. ANOVA showed that NS2 (Span 60-300 mg and cholesterol - 200 mg) is the optimized formulation. *In vitro* antibacterial study against *E. coli* and Klebsiella revealed that the zone of inhibition higher than the marketed formulation. The MIC confirmed that the inhibitory concentration was much less 0.05 mg; hence, it can be used as a proficient transporter for the delivery of cefixime. The formulation was stable for about 45 days at two different temperatures. From the present study, it is clear that the surfactant at the medium level showed highest entrapment efficiency and release. The *in vitro* antibacterial activity showed that the activity is more than the marketed formulation and the MIC was much lower than the
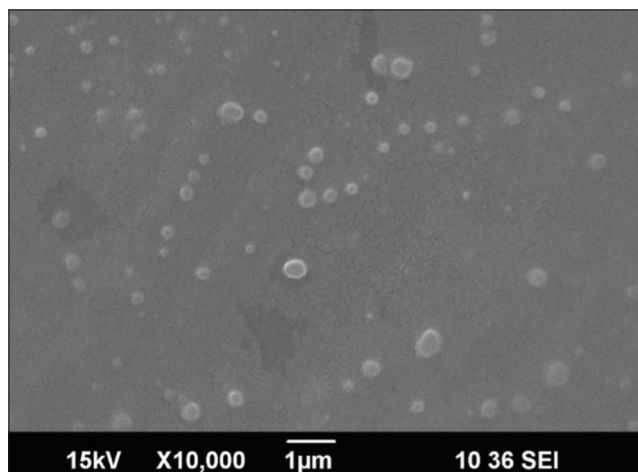


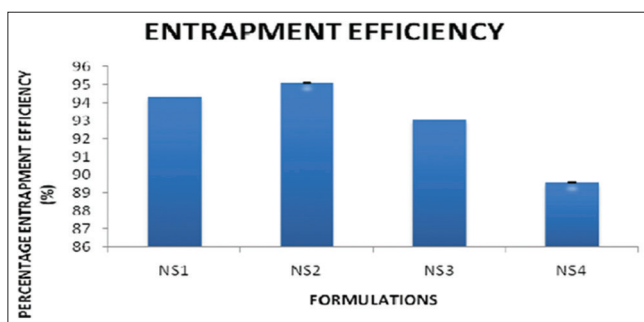**Fig. 5: Scanning electron microscopy of optimized formulation**



**Fig. 6: Representing entrapment efficiency of formulations**
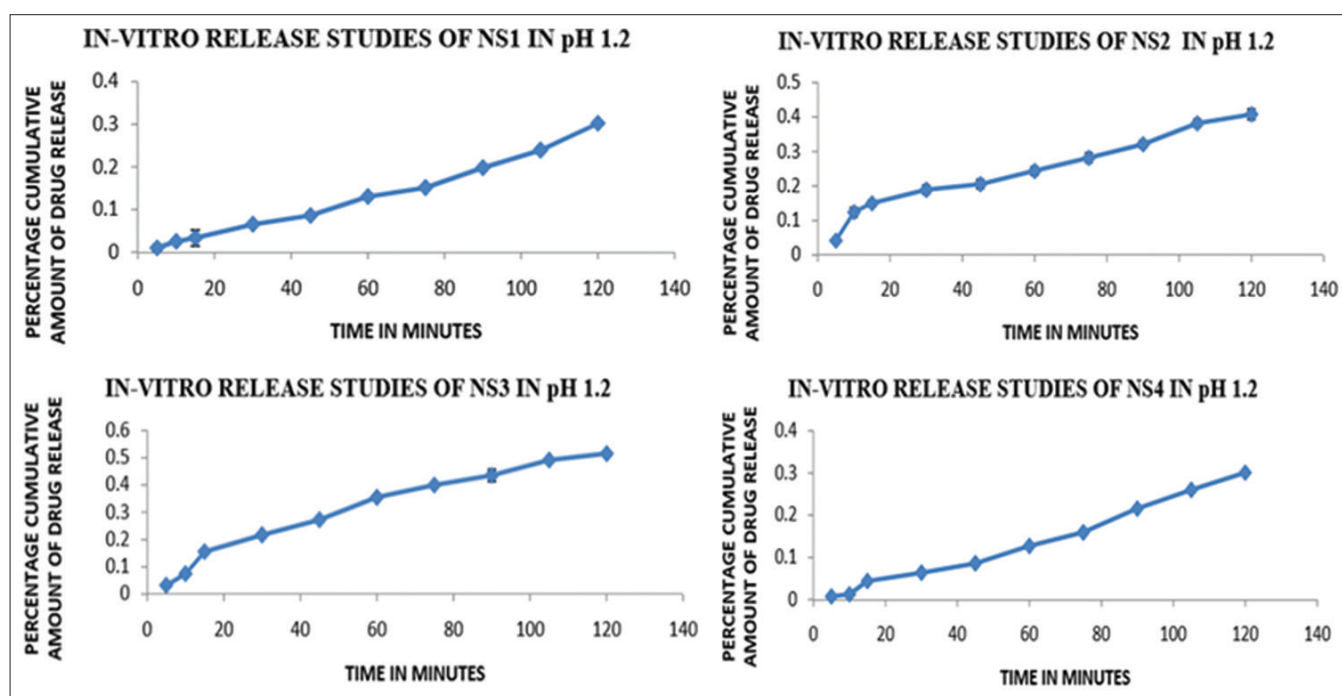


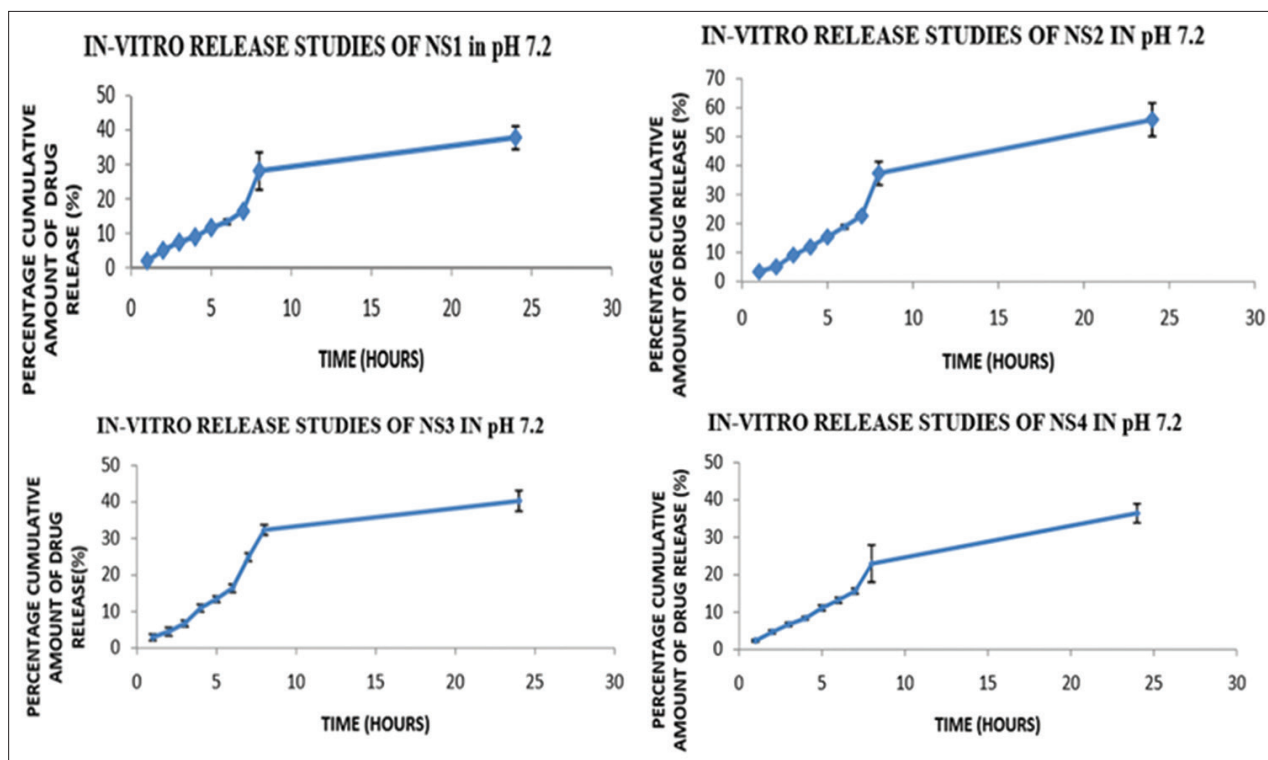**Fig. 7: *In vitro* release studies of NS1, NS2, NS3, and NS4 in pH 1.2**

Fig. 8: *In vitro* release studies of NS1, NS2, NS3, and NS4 in pH 7.2
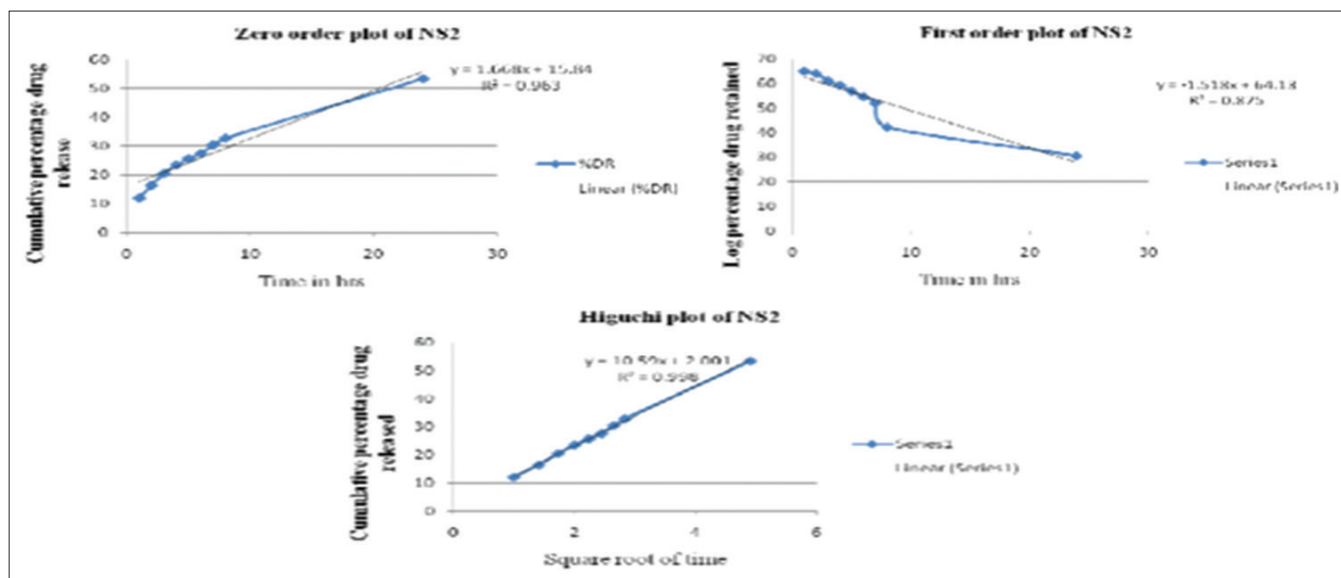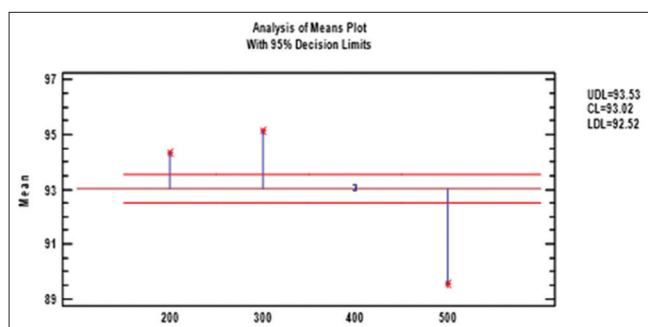


Fig. 9: Zero order, first order, and Higuchi plot of NS2



Fig. 10: ANOVA analysis of entrapment efficiency

Table 1: Composition of formulation

| Ingredients | Formulation code | | | |
|---|---|---|---|---|
| | NS1 | NS2 | NS3 | NS4 |
| Surfactant (mg) | 200 | 300 | 400 | 500 |
| Cholesterol (mg) | 200 | 200 | 200 | 200 |
| Chloroform (ml) | 20 | 20 | 20 | 20 |
| Phosphate buffer (7.2) (ml) | 20 | 20 | 20 | 20 |
| Drug (mg) | 20 | 20 | 20 | 20 |

standard used. Thus, niosomal suspension is a promising carrier for the delivery of cefixime.

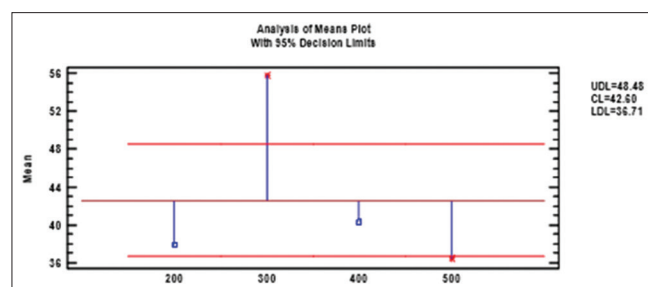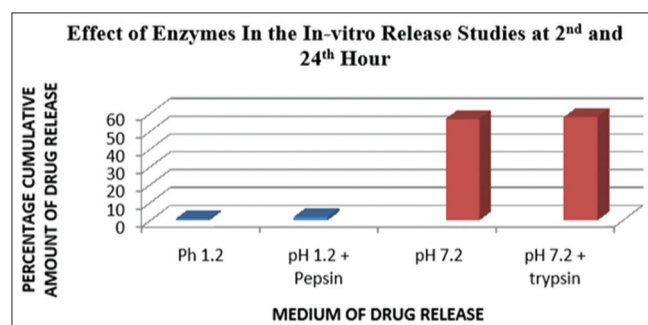**Table 2: Evaluation of *in vitro* antibacterial activity by zone of inhibition**

| Microorganism | Concentration (μl/well) | Zone of inhibition (mm) | | |
| --- | --- | --- | --- | --- |
| | | **Marketed formulation** | **Optimized formulation** | **Plain niosomes** |
| *E. coil* | 2 | 0 | 0 | 0 |
| | 5 | 11 | 13 | 0 |
| | 10 | 14 | 18 | 0 |
| | 15 | 16 | 2 | 0 |
| Klebsiella | 2 | 0 | 0 | 0 |
| | 5 | 1 | 14 | 0 |
| | 10 | 12 | 16 | 0 |
| | 15 | 14 | 19 | 0 |

*E. coli: Escherichia coli*

**Table 3: Minimum inhibitory concentration of cefixime loaded niosomal suspension**

| Microorganisms | Minimum inhibitory concentration (mg/ml) |
| --- | --- |
| | **Optimized formulation** |
| *E. coli* | 0.05 mg |
| Klebsiella | 0.05 mg |

*E. coli: Escherichia coli*



**Fig. 11: ANOVA analysis of release study *in vitro***
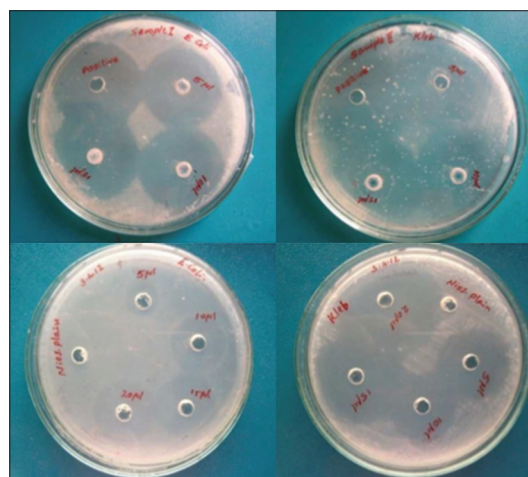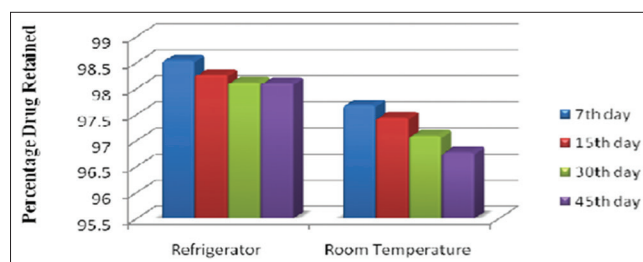


**Fig. 13: Photograph of *in vitro* antibacterial activity of optimized formulation and photograph of *in vitro* antibacterial activity of optimized formulation**



**Fig. 12: Effect of enzymes in the *in vitro* release of optimized formulation**



**Fig. 14: Stability study of optimized formulation NS2**

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