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FORMULATION AND EVALUATION OF CEFPODOXIME PROXETIL LOADED NIOSOMES USING TAMARIND SEED POLYSACCHARIDE

Rohit, Rajesh Manchanda and Rashmi Manchanda*

Department of Pharmaceutics, R.K.S.D. College of Pharmacy, Ambala Road, Kaithal, India.

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***Corresponding Author**
Rohit
Department of Pharmaceutics, R. K. S. D. College of Pharmacy, Ambala Road, Kaithal, India.

ABSTRACT

Non-ionic surfactant vesicles containing Cefpodoxime Proxetil were prepared using Tamarind Seed Polysaccharide, Span 60 and Cholesterol in the ratios (by weight) of 1:2:1, 1:4:1, 1:6:1, 1:8:1, and 1:10:1. The prepared vesicles were characterized for the shape, size, entrapment efficiency, and in-vitro drug release and stability studies. The particle size distributions were carried out by optical microscopic technique and spherical shape observed by TEM studies. The drug Entrapment Efficiencies varied from 76% to 86%. In vitro drug release studies were carried out by using PBS (pH: 7.4) as a dissolution medium for 24 hours. From the in vitro studies the N_6 was found to be more satisfactory which exhibit a retarded release of 98.16% for 24 hours. The stability of vesicles was assessed by storage at 4 ± 1ºC and at room temperature for three month. The results suggested that the Niosomes of Cefpodoxime Proxetil using Tamarind Seed Polysaccharide can be used for Sustained release for Sustained release drug delivery system.

KEYWORDS: *Cefpodoxime Proxetil, Non-ionic Surfactant, Tamarind Seed Polysaccharide, Entrapment efficiency, Particle size, In vitro release stability studies.*

1. INTRODUCTION

An ideal drug delivery system delivers drug at rate dictated by the need of the body over the period of treatment and it channels the active entity solely to the site of action. Drug targeting can be defined as the ability to direct a therapeutic agent specifically to desired site of action with little or no interaction with non target tissue.[1] Controlled release drug products are often formulated to permit the establishment and maintenance of drug concentration at target site for longer intervals of time.[2,3] Different novel approaches used for delivering these

drugs include Niosomes, Liposomes, Microspheres, Nanoparticles, Micro emulsions, Antibody-loaded drug delivery, Magnetic Microcapsules etc.[4] In 1909, A German physician and scientist (Paul Ehrlich), initiated the development of the targeted drug delivery system when he presumed a drug delivery mechanism that would target directly to diseased cell.[5] Rapid and significant progress in the use of nanotechnology in treatment and diagnosis of diseases has made a new field called nanomedicine and related subfields, such as pharmaceutical nanocarriers known as new branches of medical science. Nanostructures can be made from various materials including polymers, metals, metal oxides, nanogel, lipid-based carriers (Liposomes) and surfactant based carriers (Niosomes).[6] Polysaccharides are long chains of carbohydrate molecules, specifically polymeric carbohydrates composed of monosaccharide units bound together by glycoside linkages. This carbohydrate can react with water (hydrolysis) using amylase enzymes as catalyst, which produces constituent sugars (monosaccharide's or oligosaccharides). They range in structure from linear to highly branched.[7] Recent trends towards the use of natural polysaccharides as novel drug carriers primarily remain attractive because of their easy availability, cost effectiveness, capability of chemical modifications, excellent biodegradability and acceptable biocompatibility.[8,9] Drug release from Niosomes can be controlled with more efficacious manner by incorporating polysaccharides such as Fenugreek Galactomannan Polysaccharide, Black Gram Polysaccharide, Tamarind Seed Polysaccharide, Soybean Polysaccharides, Guar Gum, Chitosan, Locust Bean Gum etc. Tamarind Seed Polysaccharide (TSP) is cheap and naturally derived polysaccharide obtained from the seeds of Tamarindus indicia L., a common tree of India and South East Asia. TSP is composed of (1 → 4)-β-D-glucan back-bone substituted with side chains of α-D-xylopyranose and β-D-galactopyranosyl (1 → 2)-α-D-xylopyranose linked (1 → 6) to glucose residues. TSP is non-carcinogenic, biocompatible and extraordinarily stable even in the acid pH range.[10] It is used as binder, gelling, thickening, emulsifying, and suspending agent in different pharmaceutical formulations and acts as stabilizer in food and pharmaceutical industries.[11,12] Nanoparticulate drug delivery system has emerged to be an important area in the field of sustained drug delivery systems. Nanoparticles made from natural polysaccharides have been proved efficient in terms of better drug loading capacity, biocompatibility and better bioavailability.[13] Mucoadhesive nanoparticles made up of hydrophilic polysaccharides may also sustain the release of drug and hence improve the bioavailability. Nanotechnology is currently employed as a tool to fight more efficiently against human pathogens. Nanoparticles can be prepared from a variety of materials such as protein, biodegradable polymers and synthetic polymers. *Tamarindus*

indicia Linn. (Tamarind) is one of the most important biodegradable polymer. Tamarind Seed Polysaccharide (TSP) is used for the synthesis of nanoparticulate formulation.[14] Cefpodoxime Proxetil is an oral, third-generation cephalosporin antibiotic. It is active against most Gram-positive and Gram-negative organisms. It is commonly used to treat acute otitis media, pharyngitis, sinusitis, and gonorrhea. It also finds use as oral continuation therapy when intravenous cephalosporins (such as ceftriaxone) are no longer necessary for continued treatment. Cefpodoxime Proxetil inhibits cell wall synthesis by inhibiting the final transpeptidation step of peptidoglycan synthesis in cell walls. It was patented in 1980 and approved for medical use in 1989.[15] The aim of the present study was to develop Cefpodoxime Proxetil loaded Niosomes using Tamarind Seed Polysaccharide to improve the physical stability of Niosomes. Cefpodoxime Proxetil loaded Niosomess were incorporated in Tamarind Seed Polysaccharide was investigated through the study of entrapment efficiency, physical stability study, Bioavailabilty and in vitro release study to evaluate the efficacy of this incorporation.

2. MATERIALS AND METHODS

2.1 Materials

Cefpodoxime Proxetil was purchased by Nectar Life sciences Ltd, (Chandigarh, India). Span-60 was purchased from Central Drug House (P) Ltd – CDH, (New Delhi, India). Cholesterol was purchased by Lobe Chemie Laboratories, (Mumbai, India). Tamarind Seed Polysaccharide was purchased from Local market (Kaithal, India). All the reagents were of analytical grade and used without further purification.

2.2 Preparation of cefpodoxime proxetil loaded niosome using tamarind seed polysaccharide[16]

Niosomal Suspensions were prepared by the Thin Film Hydration Method. Accurately weighed quantities of Drug, Non ionic Surfactant (Span-60), Polysaccharide and Cholesterol were dissolved in Chloroform in a Round Bottom Flask. The Chloroform was evaporated at 60°C under reduced pressure using a Rotary Flash Evaporator. After Chloroform evaporation, the flask was kept under vacuum overnight to remove residual solvent. The Thin film was hydrated with 10 ml of Phosphate Buffer of pH 7.4 and the flask was kept rotating at 60°C. Formulations were sonicated three times in a Bath-sonicator for 15 min with 5-min interval between successive times. The formed Niosomes were then filtered by vacuum filtration and

dried at room temperature and stored. An overview of the composition of the Niosomes is outlined in **Table 1**.

Table 1: Optimization of Cholesterol - Span60 - TSP Ratio and Its effect on Particle Size and Entrapment Efficiency.

Batch code	CHOL:Span60:TSP ratio wt (mg)		Drug (mg)	Particle Size (nm)	Entrapment Efficiency (%w/w)
F_1	1:2:1	50:100:50	50	538.42 ± 1.31	75.21 ± 1.26
F_2	1:4:1	50:200:50	50	377.58 ± 1.42	78.45 ± 0.42
F_3	1:6:1	50:300:50	50	456.25 ± 0.88	83.72 ± 0.79
F_4	1:8:1	50:400:50	50	478.12 ± 1.27	81.79 ± 1.43
F_5	1:10:1	50:500:50	50	873.36 ± 0.85	77.43 ± 0.67

***Each value is ± SD of three independent determinations**

2.3 Design of the experiment

A 3^2 full factorial design was used in this study. In this design 2 factors were evaluated, each at 3 levels, and experimental trials were performed at all 9 possible combinations. The Two independent variables were selected which were the CHOL: Span 60: Polysaccharide ratio (X_1) and the Hydration time (X_2) as given in **Table 2**.

Table 2: Test factors for optimization of process parameters.

Factor	Name	Units	Low Level (-)	Medium Level (0)	High level (+)
A (X_1)	CHOL:Span 60: Polysaccharide ratio	----	1:4.5:1	1:6:1	1:7.5:1
B (X_2)	Hydration Time	Min.	20	40	60

2.4 Optimization of various parameters by 3^2 full factorial design

The results obtained after implementing 3^2 Full Factorial Design are summarized in **Table 3**.

Table 3: Effect of various parameters on characteristics of niosomes.

Batch Code	A: CHOL: Span60: TSP	B: Hydration Time (min.)
N_1	1:5:1(-)	20(-)
N_2	1:6:1(0)	20(-)
N_3	1:7:1(+)	20(-)
N_4	1:5:1 (-)	40(0)
N_5	1:6:1 (0)	40(0)
N_6	1:7:1 (+)	40(0)
N_7	1:5:1 (-)	60(+)
N_8	1:6:1 (0)	60(+)
N_9	1:7:1 (+)	60(+)

3. Characterization of prepared niosomes

3.1 Surface and Shape analysis by scanning electron microscopy

The shape and surface characteristics of Niosomes were analyzed by scanning electron microscopy (Meta-litesizer) operating at 10 kV. The samples were mounted on an aluminum stub with adhesive tape and excess samples were removed and coated with gold for 20 seconds. Then the metal stub was placed in E-1010 Ion sputter for 20 minutes under vacuum. After 20 minutes samples were analyzed under scanning electron microscope.

3.2 Particle Size and Zeta potential

The particle size and zeta potential of optimize Niosomes formulation F_6 were measured by (Malvern Instruments) after suitable dilution with distilled water. Zeta potential is a measure of surface charge of dispersed particles in relation to dispersion medium. The experiment was performed using clear disposable zeta cell, water as dispersant which having refractive index (RI) -1.330 and viscosity (cPs) -0.88 and the temperature was kept constant at 25°C.

3.3 Drug entrapment efficiency

The Entrapment Efficiency (EE%) is defined by the concentration of the incorporated material (such as active ingredients, drugs etc.) detected in the formulation over the initial concentration used to make the formulation. The entrapment efficiency of the Niosomes was determined spectrophotometrically. A sample of F_6 Cefpodoxime Proxetil Niosomes (10 mg) was dissolved in 10 ml of Methanol and kept it for overnight. 1 ml of the supernatant was taken and diluted to 10 ml with a solution containing Phosphate Buffer of pH 7.4 and was analyzed at 218 nm using UV-Visible spectrophotometer.

$\% EE = (W_{total} - W_{free})/ W_{total} \times 100$, Where W_{total} is the total amount of drug in used in preparing formulation, W_{free} is the amount of the drug in supernatant.

3.4 *In vitro* drug release studies[17]

In-vitro drug release study was done on the release pattern of the drug from the niosomal formulations prepared by Thin Film Hydration method. After separating the un-entrapped drug, the niosomal suspension containing drug equivalent to drug content was pipette into the dialysis bag which was previously soaked and washed several times with distilled water. This was placed in 100 ml of phosphate buffer saline (pH 7.4) and kept with constant agitation on a magnetic stirrer maintaining a temperature of 37ºC. Each periodical time the whole sample were withdrawn and same volume of fresh sample was replaced. Then the samples were assayed spectrophotometrically at 218nm using medium as blank.

3.5 Stability study

Freeze-dried optimized Niosomes formulation N_6 was subjected to stability studies as per ICH guidelines. The samples were placed in vials and kept at 25±2°C/60 ±5% RH and 4±2°C C/75 ± 5% RH atmospheric conditions using stability chamber over period of three months. The samples were analyzed physical appearance at specified time intervals (0, 15, 30, 60, 90 days of storage). Cumulative drug release study was also carried out at the end of stability study for both storage conditions.

4. RESULT AND DISCUSSION

4.1 Surface and Shape analysis by transmission electron microscopy

Surface morphology of the Niosomes was examined by TEM as shown in **Fig.1**. TEM analysis revealed nanosized, almost spherical particles with numerous pores on the surface. The pores characteristically tunnelled inwards that were probably the impressions of diffusion of solvent (dichloromethane) from the surface of Niosomes. Any residual crystals of the drug could not be seen on the surface of the Niosomes indicative of the matrix being constructed from drug, polysaccharide and non-ionic surfactant.

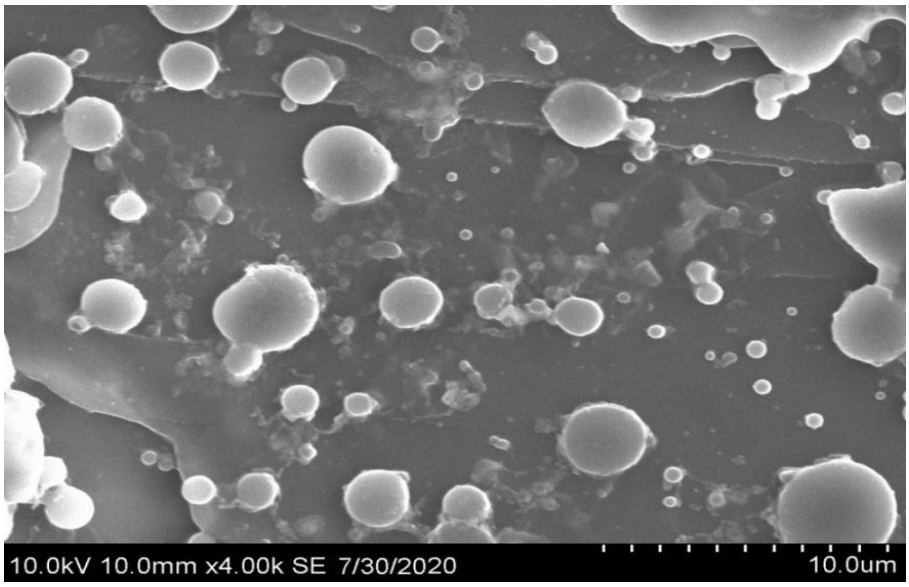

Fig. 1: TEM photograph of cefpodoxime proxetil loaded niosomes.

4.2 Particle Size and Zeta potential

The particle size of the optimized Niosomes formulation N_6 showed considerably mean size of 376.5 nm as shown in **Fig. 2**. Zeta potential is necessary for analyzing stability of colloidal dispersion during storage. The zeta potential of optimized formulation was found to be -48.6 mV as shown in **Fig. 3**, which imparts good stability of Niosomes dispersion.

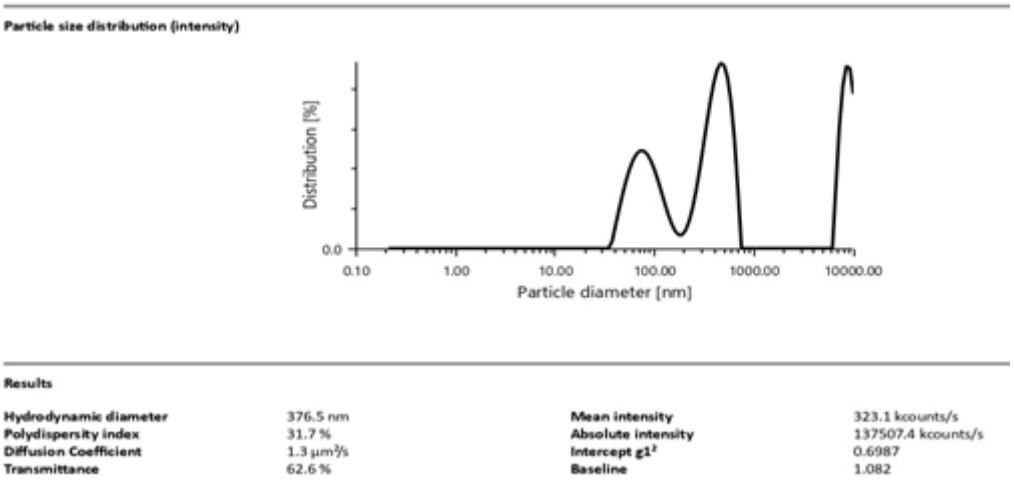

Fig. 2: Particle size analysis of cefpodoxime proxetil loaded niosomes coated with tamarind seed polysaccharide.

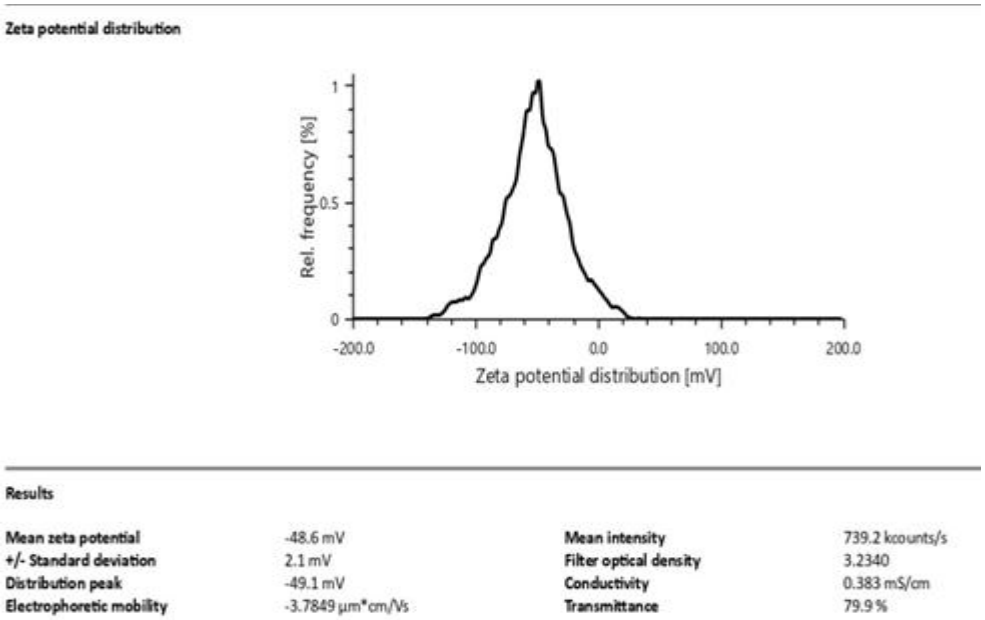

Fig. 3: Zeta potential of cefpodoxime proxetil loaded niosomes coated with tamarind seed polysaccharide.

4.3 Drug entrapment efficiency

The Cefpodoxime Proxetil loaded Niosomes using Tamarind Seed Polysaccharide were formulated using by the Thin Film Hydration technique. Out of Nine formulations (N_6) seems to exhibit good physical stability indicated by high entrapment efficiency value as shown in the **Table 4.**

Table 4: Entrapment efficiency of niosomes.

Batch Code	Entrapment Efficiency (%)
N_1	76.2 ± 0.19
N_2	78.3 ± 0.62
N_3	80.7 ± 1.31
sN_4	79.5 ± 1.41
N_5	83.2 ± 0.86
N_6	**86.3 ± 0.62**
N_7	78.8 ± 1.14
N_8	77.4 ± 0.68
N_9	79.6 ± 0.91

***Each value is ± SD of three independent determinations**

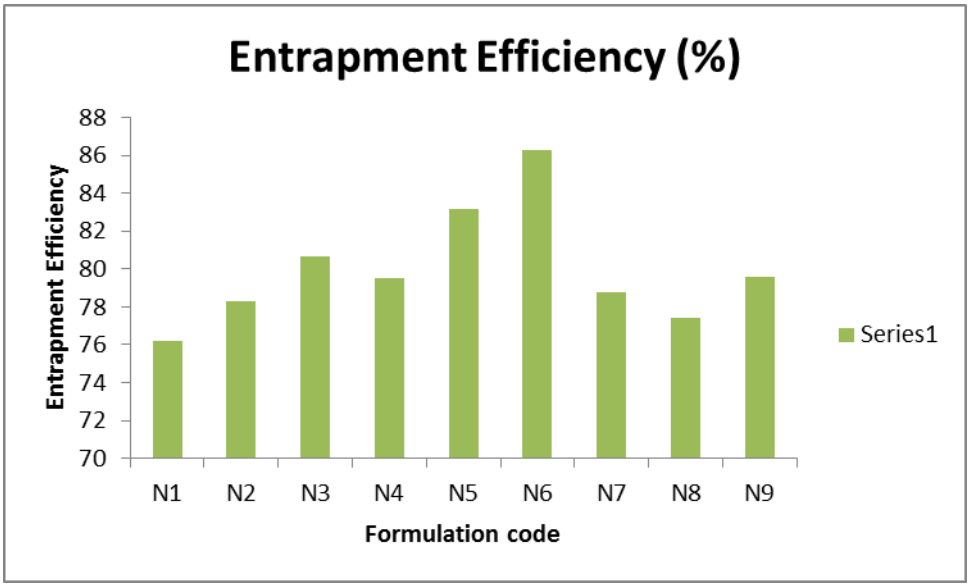

Fig. 5: Entrapment efficiency of niosomes coated with TSP.

4.4 *In vitro* drug release studies

The *in vitro* drug release study was performed using dialysis technique in Phosphate Buffer pH 7.4 as shown in the **Table 5**. *In vitro* release profile of Cefpodoxime Proxetil loaded Niosomes formulations portrayed in **Fig.6** and showed burst drug release for initial 0.5h followed by slow and sustained release up to 24 h.

Table 5: *In-vitro* drug release study niosomes containing cefpodoxime proxetil.

Batch code	% Drug release in 24 Hours
N_1	90.39 ± 0.56
N_2	92.47 ± 1.54
N_3	94.23 ± 0.65
N_4	93.17 ± 0.63
N_5	95.45 ± 0.86
N_6	**98.16 ± 1.62**

N_7	92.52 ± 0.83
N_8	91.57 ± 1.28
N_9	94.12 ± 0.65

***Each value is ± SD of three independent determinations**

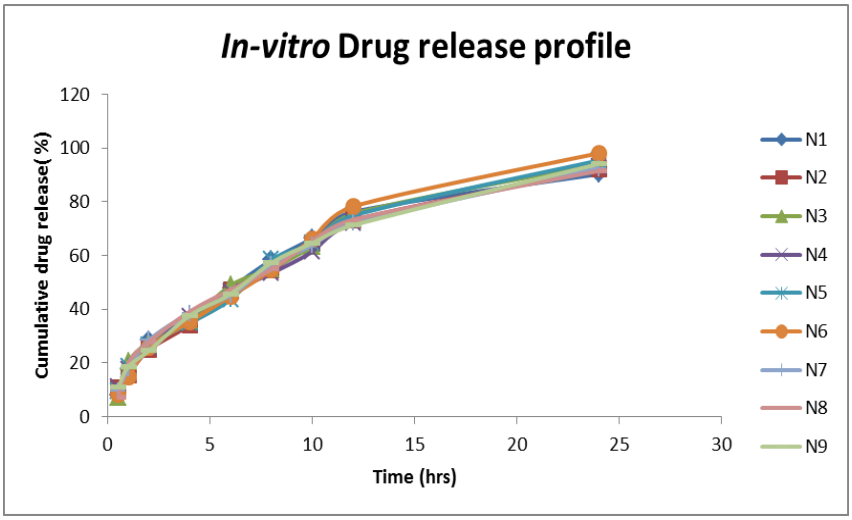

Fig. 6: *In-vitro* drug release profile of niosomes coated with TSP.

4.5 Drug release kinetics of drug release

In order to investigate the release mechanism of present drug delivery system, the data obtained from *in-vitro* release of final optimized batch were fitted into equations for the zero-order, first-order, Higuchi release model and Korsmeyer-Peppas equation **Table 6.** Enlists the values of regression coefficient obtained from various kinetics models.

Table 6: Regression coefficient (R^2) obtained from various kinetics models.

Batch Code	Zero Order Kinetics	Higuchi Kinetics	Korsmeyer-Peppas Kinetics	n	First Order Kinetics
N_1	0.844	0.979	0.969	0.608	0.624
N_2	0.883	0.989	0.994	0.580	0.704
N_3	0.874	0.986	0.950	0.620	0.617
N_4	0.892	0.995	0.997	0.552	0.712
N_5	0.888	0.987	0.984	0.581	0.691
N_6	0.895	0.984	0.989	0.639	0.682
N_7	0.875	0.992	0.983	0.575	0.663
N_8	0.860	0.986	0.953	0.606	0.609
N_9	0.887	0.994	0.994	0.558	0.706

The interpretation of data was based on the values of the resulting regression coefficients. The *in vitro* drug release showed the regression coefficient values of optimized formulation (N_6) for Zero order (R^2= 0.895), Higuchi's model (R^2= 0.984), Peppas model (R^2= 0.989) and with a value of n = 0.639 and First order (R^2 = 0.682). On the basis of best fit with the highest

correlation (R^2) value it is concluded that the optimized formulation of Niosomes follows the Korsmeyer-Peppas model with release exponent value n = 0.639. The magnitude of the release exponent **n** indicates the release mechanism is N-fickian diffusion.

4.5 Stability study

The stability studies indicate no physical change in appearance and colour, indicating that the optimized formulation **N_6** was physically stable. On chemical evaluation, it was observed that the percent residual drug content of the optimized batch at the end of 3 months was found to be 97.34 ± 0.16% at 4 ± 1ºC and 84.31 ± 0.15% at room temperature as shown in **Table 7.**

Table 7: Effect of aging on residual content at 4 ± 1°C and room temperature.

Sr. No.	Days	Physical Change	Mean Percent Residual Drug Content (at 4 ± 1°C)	Mean Percent Residual Drug Content (at room temp.)
1	0	No Change	100	100
2	15	No Change	99.61 ± 0.08	98.19 ± 0.06
3	30	No Change	99.03 ± 0.07	95.24 ± 0.05
4	45	No Change	98.48 ± 0.21	92.64 ± 0.10
5	60	No Change	97.98 ± 0.06	89.13 ± 0.12
6	90	No Change	97.34 ± 0.16	84.31 ± 0.15

***Each value is ± SD of three independent determinations**

The stability studies indicate no physical change in appearance and colour, indicating that the optimized formulation **N_6** was physically stable at the accelerated conditions. On chemical evaluation, it was observed that the percent residual drug content of the optimized batch at the end of 3 months was found to be 97.34 ± 0.16% at 4 ± 1^o C and 84.31 ± 0.15% at room temperature as shown in **Fig. 7**.

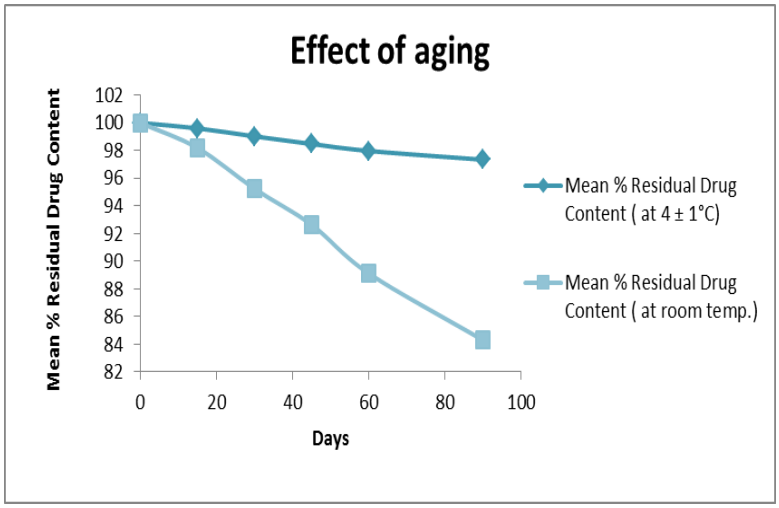

Fig. 7: Effect of aging on % residual drug content at 4 ± 1°C and room temperature.

5. CONCLUSION

Niosomes, a non-ionic surfactant vesicular system, is a novel and efficient approach to drug delivery. With the incorporation of appropriate non-ionic surfactant and cholesterol in the vesicular membrane, a wide range of drugs can be encapsulated in Niosomes. The use of natural polysaccharides in Niosomes for pharmaceutical applications is attractive because they are economical, readily available, non-toxic, and capable of chemical modifications, potentially biodegradable. In this study, Cefpodoxime Proxetil loaded Niosomes using Tamarind seed polysaccharide has been developed and characterized which exhibited many features such as low dosing frequency and sustained release of drug. Cefpodoxime Proxetil loaded Niosomes using Tamarind Seed Polysaccharide have been prepared using Thin Film Hydration Method by the use of Span 60 as non ionic surfactant, Tamarind Seed Polysaccharide as natural polysaccharide and Cholesterol as stabilizers.

Morphological investigations showed that all Niosomes were spherical in shape, having mean diameter of 376.5 nm. Selection of the appropriate experimental conditions resulted in the production of Cefpodoxime Proxetil loaded Niosomes and **N_6** batch was found to be the optimized formulation having high entrapment efficiency of 86.3%(w/w) and high percent cumulative drug release of 98.16% (w/w) at 24th hr which showed that Cefpodoxime Proxetil Niosomes have the potential for prolonged drug release. Over three months of investigation on stability at 4 ± 1˚C and room temperature, formulation show faster degradation at higher temperature. The results indicate that the ideal storage temperature for the Niosomes is a cold place.

Hence, it can be concluded that it is possible to design Cefpodoxime Proxetil loaded Niosomes for the treatment of Respiratory tract bacterial infections where efficacy and patient compliance are of prime importance.

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