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A Niosomal Gel of Cefoperazone Sodium for Topical Application

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ABSTRACT

The present study endeavors to prepare a niosomal gel of Cefoperazone sodium (CFS), as a novel dermal delivery for the treatment of skin infections. CFS loaded niosomes were prepared using different molar ratio of Tween 80 and Cholesterol by ether injection method using experimental design. The optimized formula was evaluated for DSC, XRPD and AFM. A niosomal gel with the optimized formulation was prepared in Carbopol 934 and were evaluated for gelling properties, *in-vitro* release, *ex-vivo* permeation and skin irritation study on rats. Quality by design was successfully executed to get stable (Zeta potential -30mV), nano sized (365.3 nm) niosomal vesicles. The niosomal gel of CFS showed a pH around 5.5, and a viscosity of 84.13±0.25 cps, enhanced permeation and no skin irritation. Hence, the study depicts that a superior site-specific delivery of CFS can be achieved with a niosomal gel of the drug in the treatment of skin infections.

Keywords: Cefoperazone sodium, custom design, niosomes, niosomal gel, *ex-vivo* evaluation

INTRODUCTION

Niosomes are hydrated non-ionic vesicles of surfactant having the unique potential to entrap both hydrophilic and lipophilic drugs. They are unilamellar or multilamellar vesicles of surfactants with cholesterol or its derivatives, enclosed by an aqueous compartment[1]. The self-assembling properties of surfactants on hydration are responsible for formation of shapes like micelles or planar lamellar bilayer of microscopic and nanoscopic vesicles[2]. They are osmotically stable, non-immunogenic, biocompatible, biodegradable, and act as permeation en-

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hancers[3]. They can easily adsorb or fuse with stratum corneum and can pass through the intra epidermal channels, and diffuse deep into the skin to produce systemic effects. They can increase the fluidity of the skin membrane and results in enhancement in the permeability of drugs when applied topically[4].

Cefoperazone sodium, a semi synthetic broad-spectrum antibacterial drug used in the infections of skin caused by *Pseudomonas aeruginosa, Streptococcus pyogenes* and *Staphylococcus aureus* [5]. It is administered in divided doses of 2 to 4 gm intravenously per day depending on the severity. The drug has low serum half-life (2hours) and bound mostly in the plasma protein which is also reported to be dose dependent[6]. Therefore, an approach to develop a topical formulation of cefoperazone sodium could be beneficial in the treatment of skin infections considering localization of the drug at the site of action with improved patient compliance. As per the reported data, the drug has a low log P value[6], therefore, encapsulation of the drug in the niosomal vesicle could improve permeation of the drug through the skin. The drug can be encapsulated in the aqueous core of the nonionic vesicles. Cholesterol which is a common component in biological membrane, can influence the permeability and fusion of the vesicles through the stratum corneum, can be added to improvise the bilayer property of the nonionic surfactant vesicle[7]. Several studies have reported that the residence time of drug in stratum corneum was enhanced through niosomal delivery as it altered the horny layer properties[8] and enhance the stability of the entrapped drug[9].

Hence, the present study discusses on the development and evaluation of niosomal gel of cefoperazone sodium with targeting of the drug at the site of infection to provide better efficacy and patient compliance over the conventional dosage forms.

METHODOLOGY

Materials

Cefoperazone sodium (CFS) was gifted from Aurobindo Pharma Ltd, Hyderabad, India. Cholesterol was obtained from Loba Chemie, Mumbai, India. Carbopol-940 was purchased from SD Fine Chemicals Limited, Mumbai, India. Rest of the used chemicals and reagents were of analytical grade.

Methods

Employment of custom design in the methods of preparation of niosomes

The niosomes were prepared by ether injection method. The various process parameters like rate of injection, volume of injection and the property of the

materials can affect the encapsulation efficiency and the vesicular size of niosomes[10]. Presence of cholesterol plays an important role in bilayer stability of niosome and entrapment of drug[1]. The software JMP version 13 was availed to estimate the effect of surfactant and cholesterol ratio, and process parameters on the response on niosomes vesicle size, and entrapment efficiency with twelve experimental runs through custom design. A two level (low (-1) and high level (+1)) testing of each variable was done against the responses. From the initial screening study and review of reported literature the composition of surfactant: cholesterol molar ratio (X1) was varied from 6:4 to 8:6, and the process parameters, the rate of injection(X2) and the hydration volume(X3) were varied from 0.5 to 1.5 mL/min, and 10mL to 30mL respectively.

Ether Injection Method for preparation of cefoperazone loaded niosomes (CFS-NIO)

A solution with appropriate molar ratio of cholesterol and surfactant (Tween 80) in ether was prepared. An aqueous solution of the drug (0.067%w/v) was prepared in phosphate citrate buffer at pH 3. The organic phase was slowly injected into the preheated aqueous solution of the drug maintained at 60 °C through a syringe pump. The vaporization of ether leads to the spontaneous formation of lamellar vesicles of the surfactants containing a drug[11]. The resultant drug-loaded niosome formulations (CFS-NIO) were equilibrated at room temperature and stored overnight at 4°C in the refrigerator prior taken for further evaluation.

Evaluation of niosomes

Particle size distribution

Horiba SZ-100 nanoparticle size analyzer was used to determine the particle size of the niosomes. After suitable dilution with double distilled water, the sample of niosomal dispersion was placed in disposable cuvettes for particle size measurements at a scattering angle of 90° at 25.2°C[12]. Three trials were done for determining the average particle size of each formulation.

Estimation of zeta potential

The zeta potential of all the CFS-NIOs was measured in Horiba SZ100. After dilution of the samples with doubled distilled water, three measurements were carried out for each sample at 25.2°C [12].

Estimation of drug entrapment efficiency

Entrapment efficiency of all the CFS-NIO formulations were determined by centrifugation method. Niosomal formulations were centrifugated at 14000 rpm

for 40 min at 4°C. The supernatant layer was separated to estimate the unentrapped drug (F_{drug}). A 0.22µm AST syringe filter was used to filter the supernatant layer. It was suitably diluted with phosphate buffer pH 5.5 and analyzed by UV-visible spectroscopy at λ max 286nm to estimate the free drug. The analytical method for assay was validated prior by establishing a linearity range between 5-25µg/mL with a regression coefficient value of (R^2) 0.9991. The method was found to be accurate, precise, and robust with relative standard deviation (RSD) of less than 2%. The total drug (T_{drug}) was estimated by lysing the equal volume of niosomal dispersion in methanol followed by centrifugation and analyzed spectrophotometrically at 286nm. A blank niosomal dispersion of each formulation was treated in the similar way and used as blank to nullify the effect of excipients in absorbance.

The entrapment efficiency was determined using the following formula [8,13]. Each result represents an average of three trials with standard deviation.

$$\%\ Drug\ entrapment\ efficiency = (Tdrug - Fdrug).100/Tdrug$$

Atomic force microscopy (AFM)

The surface morphology of optimized CFS-NIO was analyzed by atomic force microscopy (AFM). The sample was diluted with deionized water to make a nano dispersion. A drop of this dispersion was placed over a glass slide and covered with a coverslip. The sample was air dried to remove moisture from the sample. AFM was carried out by using Park systems NX-10 AFM at 80 kV . The images were recorded in 2D and 3D scales [14].

Freeze drying of optimized niosomes formulation

Lyophilization was done for the optimized drug loaded niosomal suspension. The lyoprotectants mannitol (15%w/w) and dextrose (5% w/w) were added to the niosomal suspension to yield a sugar: lipid ratio of 3:1. The suspension was subjected to freezing at -80'C for 24 h in a deep freezer (REMI ULT-90) followed by freeze drying in (LABCONCO freeze-dryer, Free-Zone 4.5, USA) at a preset condition of vacuum pressure maintained at a 54×10^{-3} bar and surface temperature held at -54'C for 8 h[15]. The freeze-dried samples were collected and stored in a tightly closed glass vials in a desiccator. The lyophilized sample (LYP-CFS-NIO) were further taken for thermal analysis, x-ray powder diffraction, and surface morphology study.

Differential scanning colorimetry (DSC)

DSC was used for thermal analysis of pure drug (CFS) and LYP-CFS-NIO formulation. The instrument used was DSC-60, Shimadzu, Japan. Each sample was placed in an aluminum pan, sealed with pierced lids, and heated at 5 °C/min over a temperature range of 30–300 °C under a nitrogen purging of 40 mL/min[16].

X-ray powder diffraction (XRPD)

XRPD patterns of the pure drug (CFS) and LYP-CFS-NIO formulation were obtained using an X-ray diffractometer (X' Pert3 powder-Malvern Panalytical). A Cu-Ka radiation was used to analyze the sample between 4° and 100° 2θ with a scan rate of 4°/min. Voltage and current were maintained at 40 kV and 30 mA, respectively[16].

Preparation of niosomal gel CFS-NIO-gel

The LYP-CFS-NIO equivalent to 0.01%w/w drug was incorporated into a gel base of Carbopol 934(2%w/w). The required quantity of Carbopol 934 in a small amount was dispersed in distilled water and hydrated for 4 h. Propylene glycol (7%w/w) was added to the hydrated base. Triethanolamine solution (1%w/v) was used to adjust the pH of the base to 5.5. Finally, distilled water was added to adjust the gel weight to 10g[17]. A gel containing pure drug of the equivalent quantity was prepared in the same manner for comparative evaluation.

Evaluation of gel

pH of gel

Digital pH meter (Digisun Electronics System) was used to determine pH of **CFS**-NIO-gel. It was calibrated before its use. The pH measurement was made in triplicates[18].

Viscosity of gel

The viscosity of CFS-NIO-gel was determined at 25°C by using brook field viscometer. The niosomal gel (20g) was rotated at 10 rpm with spindle 2. Three trials were made for the estimation of viscosity[18].

Drug content for drug-loaded niosomal gel

A known quantity of the CFS-NIO-gel was taken in the Eppendorf tube and diluted with methanol and kept for vortex mixing for 10minutes. An aliquot was withdrawn, filtered with syringe filter, diluted suitably with phosphate buffer pH 5.5, and estimated for drug content spectrophotometrically at 286nm[19,20]. Three trials were run to confirm the estimation.

Spreadability

Spreadability was determined based on slide and drag method. An excess of the CFS-NIO-gel was placed on a glass slide. Another slide was placed over it. To spread the gel uniformly on slide, a weight of 500g was placed on the top of the slides for few minutes. Spreadability was determined by measuring the time to drag a fixed distance after placing a weight of 100 g on the slides[21,22]. Spreadability was calculated by

$$Spreadability = \frac{Mass\ on\ the\ slide\ X\ length\ of\ slide}{Time\ taken\ for\ complete\ separation\ of\ slides}.$$

In-vitro drug release and kinetic study

The *in-vitro* drug release of niosomal gel was carried out in Franz diffusion cell apparatus using 0.22µm dialysis membrane from Himedia. The dialysis membrane was soaked in phosphate buffer pH 7.4 overnight prior use. The receptor compartment was filled with 45mL of phosphate buffer of pH 7.4. A quantity of 1 g gel was placed in the donor compartment. The whole assembly was kept over magnetic stirring and the temperature of the assembly was maintained at 37°0.5°C. An aliquot of 1mL was withdrawn at a suitable time interval and replenished with equal volume of fresh media to maintain sink conditions. The study was carried out for 8h. The aliquots after suitable dilution were analyzed spectrophotometrically at 286nm. The % cumulative drug release was calculated[23]. The release kinetics data were analyzed for zero order, first order, Higuchi, Korsmeyer-Peppas model through linear regression analysis

Ex-vivo diffusion study

The *ex-vivo* studies were executed using the abdominal skin of albino Wistar rats weighing between 250-300gms. To conduct the study an approval from the institutional ethical committee vides the approval number KCP/IAEC/PCEU/39/2019 was procured in advance. The rats were euthanized using excess of carbon dioxide, the abdominal skin was depilated, and rinsed thoroughly with phosphate buffer of pH 7.4. A section of the skin was cut and tied to donor compartment of the Franz diffusion cell such that the dorsal side of the skin projecting the donor compartment. The receptor compartment was filled with 45mL phosphate buffer of pH 5.5. The LYP-CFS-NIO-gel and gel of pure drug containing equivalent drug were taken for permeation study. The receptor compartment was under magnetic stirring. The temperature of the assembly was maintained at 32°0.5°C. The samples were withdrawn at constant interval of time for 8h, the same volume of fresh solution was replaced to maintain sink condition. The withdrawn samples were suitably diluted and analyzed spectro-

photometrically at 286nm[24]. From the data the permeation constant (Kp) and the steady state permeation flux (Jss) were determined.

Skin irritation test

The skin irritation study of LYP-CFS-NIO-gel was performed on six wistar albino rats. The dorsal and ventral side of the rats were shaved to remove the hairs. Marking was done on both sides. Ventral and the dorsal side served as control and test, respectively. Gel was applied once a day for three consecutive days. The skin irritation was recorded by observation for any skin sensitivity reactions like swelling, redness, and skin rash[25,26].

RESULTS and DISCUSSION

Optimization of the custom design through evaluation of particle size, zeta potential and % entrapment efficiency of CFS-NIO

The custom design constitutes a radical approach to find the possibility of investigating a high number of variables at different levels with minimal experimentation. The use of center points in the design increased the confidence level and helped to minimize the errors on experimentation. The experimental runs of the twelve formulations resulted the responses %entrapment efficiency (%EE), and particle size (PS) as shown in table 1.

Table 1: Factors and observed responses for the custom design

Formulation code	Surfactant: cholesterol (Molar ratio) (X1)	Rate of injection (mL/min) (X2)	Hydration volume (mL) (X3)	Particles size(nm) (PS)	Drug entrapment efficiency (%) (EE)
F1	1	1	1	317.4± 5.09	98.93±0.001
F2	-1	-1	1	416.5± 4.04	98.93±0.003
F3	0	0	0	499± 0.05	98.89±0.009
F4	1	-1	-1	339.8± 3.08	98.56±0.052
F5	-1	1	-1	433.5± 6.08	98.76±0.001
F6	-1	1	1	453.9±7.03	99.11±0.025
F7	1	-1	-1	361.3± 9.05	98.41±0.01
F8	1	-1	1	644.3± 507	98.81±0.085
F9	0	0	0	412.1±9.44	98.92±0.096
F10	1	1	-1	290.4±8.06	98.98±0.034
F11	1	1	1	249±6.01	98.99±0.047
F12	-1	-1	-1	378.5±8.08	98.94±0.011

Regression analysis was done to generate a relationship between factors and responses using JMP V13 software. The response surface diagrams depicted the significant effect of composition, hydration volume and rate of injection on entrapment efficiency. The particle size of the niosomes were greatly affected with rate of injection and hydration volume. The three-dimensional (3D) response surface graphs represented the most statistically significant variables on the evaluated responses as shown in figure 1.

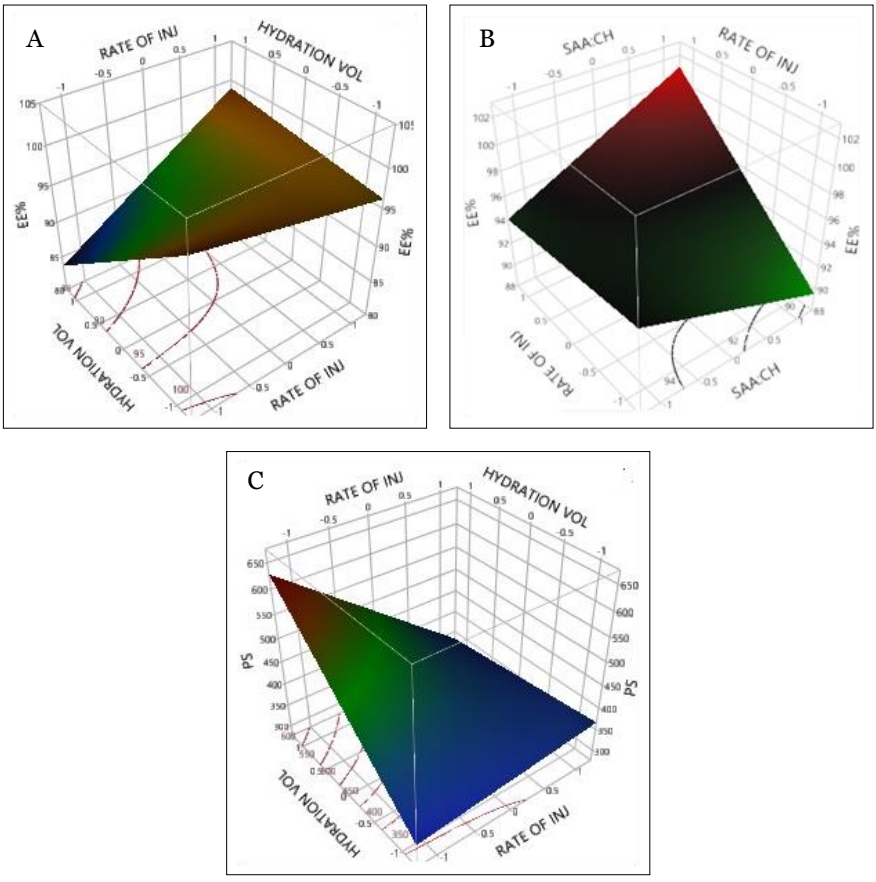

Figure 1: Response surface diagram of variables on the three responses A: %EE, B: %EE C:PS

The response surface diagrams revealed that higher the hydration volume the higher the EE. The particle size was significantly affected by the rate of injection. The effect of the various factors on the responses were estimated through parameter sensitivity analysis and listed in table 2.

Table 2: Parameter sensitivity analysis

Factors	Prob> t	
	% Entrapment Efficiency (EE)	Particle Size (PS)
SAA:CH	0.042*	0.088
Rate of injection	0.036*	0.013*
Hydration	0.010*	0.011*

*indicates significance

The best-fitted model for the design was found to be the quadratic model when the validation was carried out at a significance level of P<0.05. The ANOVA study showed the model was significant for estimation of the effects of compositions and process parameters on entrapment efficiency and particle size as shown in figure 2 in the predicted vs observed graph. The regression coefficient value of more than 0.9 proved further the significance of the model with respect to all the responses.

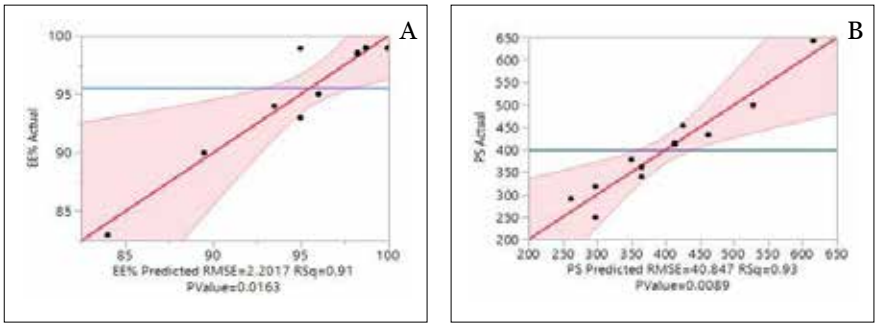

Figure 2: Actual vs predicted plot of variables on the two responses A: %EE, B: PS.

The model optimization was carried out at desirability of 0.78 and an optimized condition was predicted at a SAA/CH molar ratio of 7:5, rate of injection 1 mL/min and a hydration volume of 20 mL to yield a drug loaded niosome with high EE and small PS. The optimized formulation was prepared with the predicted parameters and evaluated for the responses. The listed experimental values of the optimized product prepared are shown in table 3. The responses of the optimized product were close to the predicted values with low percentage bias, suggesting the rationality and reliability of the model.

Table 3: Comparison of the predicted and experimental values of the optimal condition

Responses	Predicted	Experimental	%Bias
%Entrapment efficiency	96.65	94±0.66	2
Particle size (nm)	374	365.3±0.82	2.94

The zeta potential of the optimized formulations was found to be -30mV. The particle size and the zeta potential of the optimized formulations are shown in figure 3.

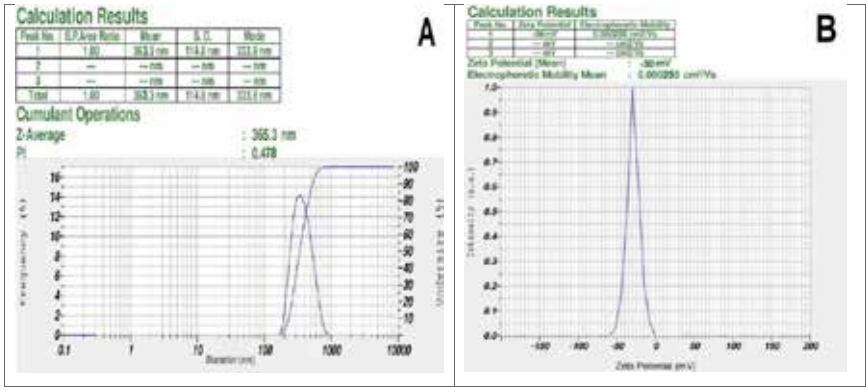

Figure 3: Particle size (A) and zeta potential (B) of optimized formulation

A stable vesicular system was predicted from the study of surface charge[27]. Therefore, the proposed design was capable to produce CFS loaded stable nano sized niosomes.

Atomic force microscopy (AFM)

The surface morphology of the niosomes were shown in figure 4, which revealed the formation of spherical and smooth surface niosomes. The AFM images in different scales further revealed the formation of spherical nano sized particles of similar size range as predicted by the design.

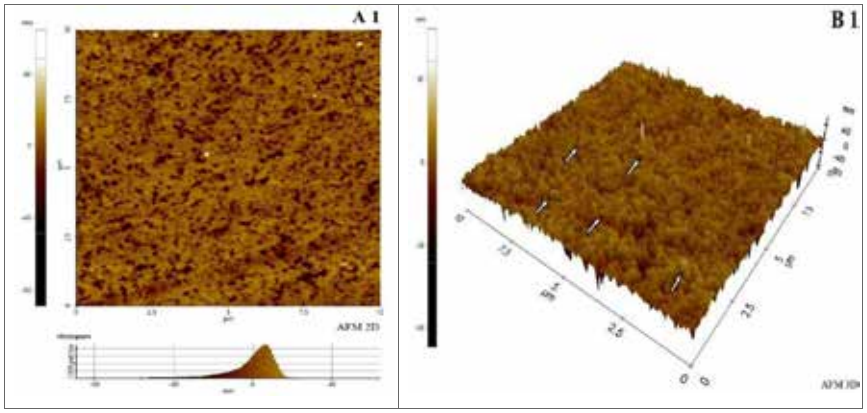

Figure 4: AFM images of optimized drug-loaded niosomes formulation (A1, 2D image B1, 3D Image)

Differential scanning colorimetry (DSC)

The DSC thermogram (figure 5) of the pure drug showed an endothermic peak corresponds to its melting point 180°C, whereas the freeze-dried formulation (LYP-CFS-NIO) revealed a shift in the peak to 149 °C, showing a decrease of phase transition temperature and heat (ΔH), which was an indication of the localization of drug inside the bilayer of lipids and surfactant. This agrees with the reported studies that the presence of cholesterol affects the gel liquid transition temperature of the vesicles[28]. Presence of long alkyl chain and hydrophilic moiety in tweens showed greater entrapment efficiency in niosomes while presence of cholesterol ensures greater bilayer stability[29].

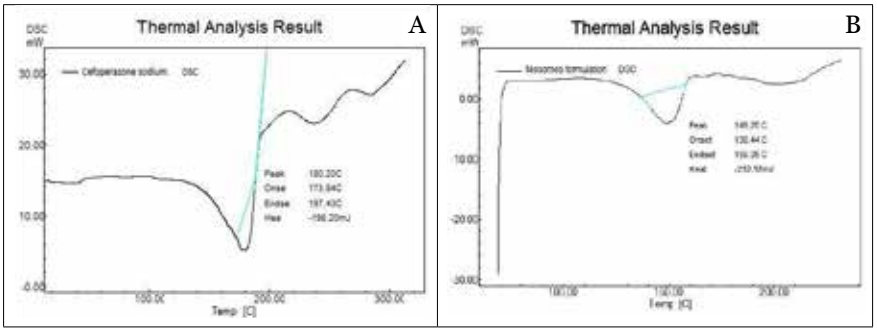

Figure 5: DSC thermograms of a pure drug(A) and Formulation (B).

X-ray powder diffraction (XRPD)

The peak intensities of pure drug and the optimized freeze-dried formulations at various diffraction angles are represented in figure 6. The pure drug showed high intensity peaks whereas the peak intensities of the same peak were reduced in the formulation graph. The high-intensity peaks of the pure drug represented its crystalline nature, while the optimized formulation showed the appearance of same peaks with low intensities. The low intensities of the peak area were attributed to the localization of the drug in the lipid and surfactant matrix and was an indication of high entrapment Which was in confirmation with DSC thermograms[16].

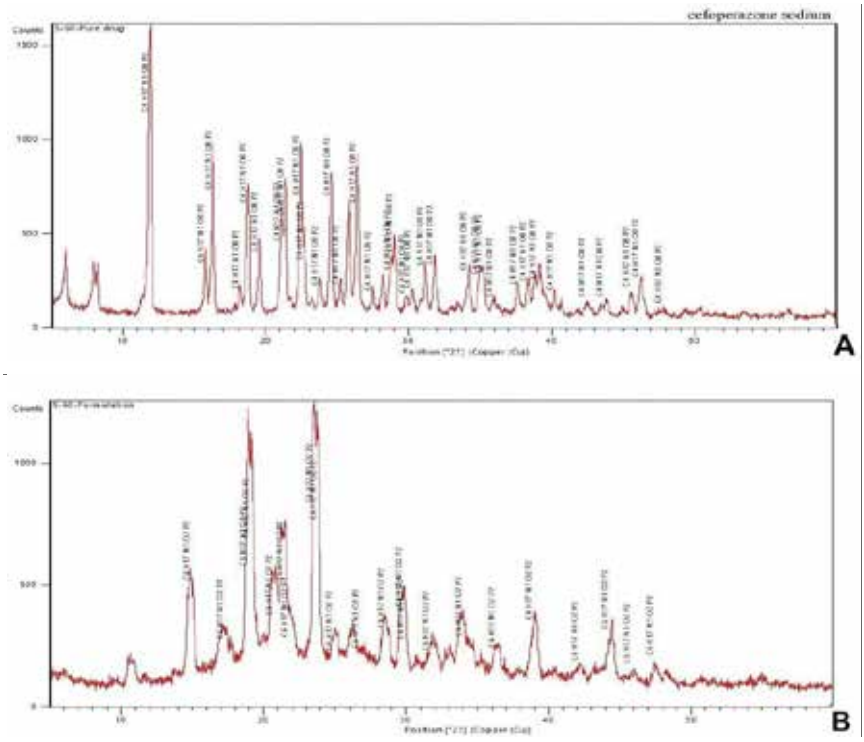

Figure 6: PXRD patterns of a pure drug (A) and formulation (B)

Evaluation of gel

The LYP-CFS-NIO-gel showed a pH around 5.5 optimal to the skin conditions, a viscosity, drug content and spreadability of 84.13±0.25 cps, 95±0.52%, and 7.63 ± 0.125gm.cm/sec respectively. Therefore, the gel was found to be suitable for dermal application based on its pH, mechanical and rheological properties.

In-vitro release and kinetics study

The *in-vitro* release of the LYP-CFS-NIO-gel revealed a slow release of drug upto 86% in 8 hours as presented in figure 7. The release kinetics followed Higuchi model as per the highest regression coefficient value(R^2). The Korsmeyer-Peppas modelling yielded a release component "n" value of 0.57, indicated that the drug release followed quasi-Fickian diffusion model with a matrix swelling and diffusion of the drug from the formulation.

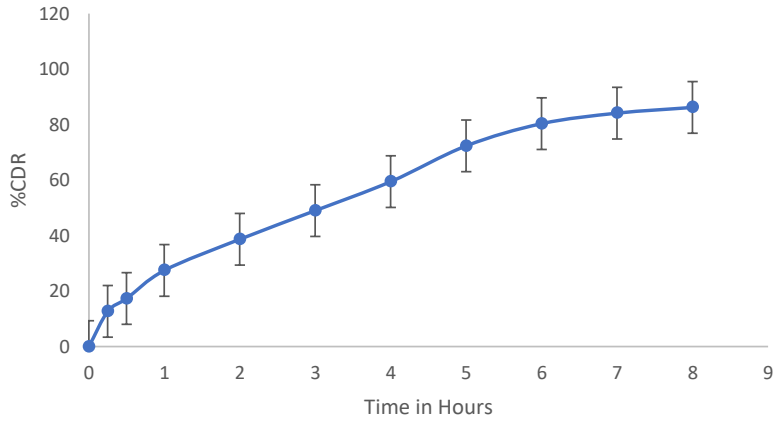

Figure 7: *In-vitro* release study of niosomal gel of CFS.

Ex-vivo permeation study

The *ex-vivo* permeation study unveiled that the niosomal gel showed higher penetration than the non niosomal gel of pure drug as shown in figure 8. It was further proved by the calculation of permeation parameters as mentioned in table 4. The niosomal gel had a 3.28 times higher flux value than the non-niosomal gel of cefoperazone sodium. This observations attributes to the formulation characteristics, as niosomes can be effectively used as permeation enhancer.

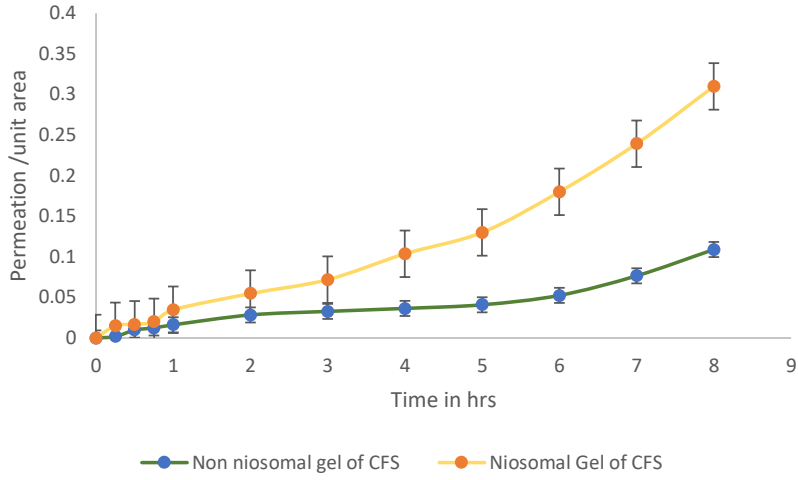

Figure 8: *Ex-vivo* permeation study of niosomal gel and non niosomal gel of CFS.

Table 4: Permeation parameters

Formulation	Steady state flux(mg/cm^2/hr)	Permeation constant (Kp) cm/hr	Drug permeation at 8 hours (mg/cm$^{2)}$
LYP-CFS-NIO-gel	0.032	0.016	0.309
Pure drug gel	0.009	0.005	0.108

Skin irritation study

The results of the skin irritation study revealed that following three days application of CFS-NIO- gel, there was no signs of skin irritation, no evidence on patchy or severe erythema associated with edema. Therefore, the gel was found to be suitable for dermal application.

The present study was designed to develop a novel delivery of cefoperazone sodium in niosomal gel for the treatment of skin infections. A highly permeable gel was prepared with niosomal cefoperazone sodium in Carbopol base, by screening of factors with custom design using JMP V13 software. The CFS-NIO were characterized by their particles size, drug entrapment efficiency and surface charge. The optimized formulation was evaluated for AFM, DSC, XRPD studies and revealed the formation of nanosized stable vesicles with high encapsulation of drug. The CFS-NIO loaded Carbopol 934 gel showed its good mechanical and rheological property. The *in-vitro* release study showed a quasi Fickian release of the drug. The *ex-vivo* permeation and skin irritation study proved the improved penetrability and suitability of the gel for dermal appli-

cation, respectively. Therefore, an effective transdermal delivery of CFS can be made with the prepared niosomal gel of CFS. These findings can create a paradigm for future studies for superior delivery of cefoperazone sodium in the treatment of skin infections.

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