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Article

Preparation and Evaluation of Niosomal Formulation for Solubility Enhancement of Anti-Fungal Agent for the Treatment of Oral Candidiasis

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Abstract: Human fungal pathogen *Candida albicans* causes oral infectious diseases such as oral thrush in immunocompromised patients with hyposalivation, diabetes mellitus, prolonged use of anti-biotics or immunosuppressive medicines and poor oral hygiene. An anti-fungal medication classified as a BCS class II drug, Clotrimazole has high permeability and low solubility in water. It is commercially available as lozenges, which do not uniformly distribute the drug within saliva for local therapy. This results in higher dose frequency and lower patient compliance. Therefore, in this study we proposed a niosomal based subgingival film formulation of clotrimazole for enhancing drug efficacy by improving drug solubilization capacity and improving patient compliance by prolonging the release of drug at the targeted site and reducing the dose frequency to show effective anti-fungal activity. The prepared niosomal film showed good entrapment efficiency and anti-fungal activity. Niosomal film showed better release pattern than drug loaded film.

Keywords: *Candida albicans*; oral thrush; anti-fungal; subgingival film; niosomes

1. Introduction

Oral health is an important aspect of an individual's overall health status. Teeth and their supporting (periodontal) structures are of main importance to oral health. *Candida albicans*, a human fungal pathogen causes oral infectious diseases such as oral thrush which affect the tongue and supporting structures of the teeth such as gums [1]. Presence of *C. albicans* does not cause any harm as the beneficial bacteria controls the overgrowth of the *C. albicans* in the oral cavity. The risk of oral thrush increases in immunocompromised patients with hyposalivation, diabetes mellitus, prolonged use of anti-biotics or immunosuppressive medicines and poor oral hygiene. The white patches appearing as discrete lesions on the buccal mucosa, throat, tongue and gum linings that develop into confluent pseudo membranes resembling milk curds can be life-threatening as it may enter the bloodstream and affect the other organs and cause septic shock [2]. To treat oral thrush various formulations like gels, lozenges, creams, mouth rinses, and suspensions are used to deliver the drug locally into the oral cavity.

Administration of drug with less residence time or less solubilization capacity of drug decreases the efficiency of medication to treat oral thrush. So, local periodontal therapy was applied where anti-fungal agents can be directly administered to the periodontal pocket and thus inhibits the growth of human fungal pathogen. Subgingival film is a local sustained release delivery system for the delivery of an anti-microbial agent to achieve and maintain the therapeutic levels of the drug for the required period of time. This intrapocket delivery device is a dosage form that has several advantageous physical properties for intrapocket use. The dimensions and shape of the film can be easily controlled to correspond to the dimensions of the periodontal pocket to be treated. It is easily and rapidly inserted and remain submerged into the periodontal pocket with minimal discomfort to the patient while eating or practising oral hygiene habits.

Clotrimazole is a broad-spectrum antimycotic drug that is commonly used to treat *Candida albicans* and other fungal infections. Clotrimazole inhibits the biosynthesis of sterols, particularly

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ergosterol, an essential component of the fungal cell membrane, causing damage and altering the permeability of the cell membrane. This causes leakage and loss of essential intracellular compounds, ultimately leading to cell lysis. It is typically given as lozenges. Clotrimazole is a BCS Class II Drug. It shows low solubility and high permeability.

Niosomes are the best carriers to design the novel drug delivery system for poorly soluble drugs. Niosomes are self-assembled microscopic lamellar vesicles of size range between 20 nm to 50 μm formed on admixture of non-ionic surfactant and cholesterol with subsequent hydration in aqueous media and are capable of entrapping both hydrophobic and hydrophilic drug molecules [1]. Therefore, in this study we proposed a niosomal based subgingival film formulation of clotrimazole for enhancing drug efficacy by improving drug solubilization capacity and improving patient compliance by prolonging the release of drug at the targeted site and reducing the dose frequency to show effective anti-fungal activity.

2. Materials and Methods

2.1. Materials

Clotrimazole was procure as a gift sample from Cipla Ltd, Mumbai, India. Sorbitan Monostearate (span 60), Cholesterol, Polyethylene Glycol 400 (PEG 400), Chloroform and Methanol was purchased from Molychem, Mumbai, India. Polyvinyl alcohol was purchased from West Coast Laboratories, Mumbai, India and Dialysis Membrane-60 was acquired from HiMedia Laboratories Pvt., Ltd., Mumbai, India. The reagents and solvents used were all of analytical grade.

2.2. Optimization of Niosomes Encapsulated ClotrimazolE by Experimental Design

The Plackett Burman Factorial design was employed in this study for screening of the significant factors as shown in Table 1. Based on the results obtained from Plackett Burman design, a 2^3 Full Factorial design was employed to study the effect of independent variables i.e., speed of rotation, temperature and amount of solvent on dependent variable i.e., percentage entrapment efficiency. Design Expert software version 13, Stat Ease Inc., USA was used to correlate dependent and independent variables using the following polynomial equation:

$$Y = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_{12}X_1X_2 + A_{13}X_1X_3 + A_{23}X_2X_3 + A_{123}X_2X_3$$

where, Y is the response, A_0 the constant and A_1 to A_3 are the coefficients of the response values

Table 1. Different levels for variables in the Plackett Burman Factorial Design.

Factors	Lower Level (-)	Higher Level (+)
A) Speed of rotation	80	130
B) Cholesterol: Surfactant ratio	1:2	1:3
C) Hydration Time	30 mins	60 mins
D) Temperature	40°C	45°C
E) Amount of hydration solvent	10 ml	20 ml
F) Dummy	-	+
G) Dummy	-	+

2.3. Preparation of Niosomes Encapsulated Clotrimazole

Thin film Hydration method was employed to prepare niosomes. An optimized ratio 1:1:2.5 of drug, cholesterol and surfactant were dissolved in 10 ml of chloroform with continuous stirring. Then solvent was evaporated using rotatory evaporator (Trident Labortek) at 42. 9°C for 1 hour at 109 rpm.

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A thin film formed on the inner periphery of the round bottom flask was hydrated using 15.8 ml pH 6.8 phosphate buffer saline solution using rotary evaporator for 45 mins at 42.9°C. The prepared niosomes were kept overnight in refrigerator (4°C). The niosomal suspension was sonicated for 20 mins and were centrifuged at a speed of 10,000 rpm for 30 min using cooling centrifuge to separate out the supernatant and sediment.

2.4. Preparation of Niosomal Based SubgingivaL Film

The required amount of polyvinyl alcohol was dissolved in water by heating it on water bath with continuous stirring. This polymeric solution was kept on magnetic stirrer (Remi 2MLH) to avoid air entrapment. Then required amount of prepared niosomes and PEG 400 was added to this polymeric solution with continuous stirring and then it was poured at the center of the levelled rectangular glass slab and allowed to dry at room temperature.

2.5. Characterization of Niosomal Based Subgingival FiLm

2.5.1. Solubility Studies

The solubility study was studied by adding 1 gram of drug in different solvents like chloroform, methanol, ethanol, phosphate buffer pH 6.8, water and shaken on cyclomixer (Eltek VM 301) at room temperature and solubility of drug in solvent was recorded.

2.5.2. Scanning Electron Microscopy (SEM)

The morphology of drug was examined by SEM. Small amount of drug was mounted on the SEM sample stab using a double-sided sticking tape. The samples are coated with gold (200 A°) under reduced pressure (65 Pascal) for 2 min using an ion sputtering device. The gold-coated samples were observed under the SEM and photomicrographs of suitable magnifications were obtained by using Quanta 200 ESEM at Icon labs Pvt. Ltd., Sanpada, Navi Mumbai.

2.5.3. Spectral Characterization of drug by UV Spectroscopy

2.5.3. (a) Determination of Maximum Wavelength

Standard stock solution of 500 µg/ml was prepared by dissolving 50 mg of drug in 100 ml of methanol and diluted with methanol and phosphate buffer pH 6.8 to get respective UV spectra in the range of 200-400 nm using methanol and phosphate buffer pH 6.8 as blank. The absorbance maxima for the drug were determined using UV visible spectrophotometer 1800, Shimadzu.

2.5.3. (b) Calibration Curve of Drug

Standard stock solution of 500 µg/ml was used to prepare different dilutions with methanol and phosphate buffer pH 6.8 in concentration range of 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 µg/ml. The absorbance of these solutions was measured at 263 nm using methanol and phosphate buffer pH 6.8 as blank solution. The absorbance values were plotted against concentration to obtain standard graph.

2.5.4. Fourier Transform-Infrared Spectroscopy (FTIR)

The dry sample of drug and excipients in powdered form was analyzed for interference of excipients with the drug using potassium bromide pellet method. The prepared sample was scanned using Shimadzu IR Spirit.

2.5.5. Differential Scanning Calorimeter (DSC)

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The thermal analysis of pure drug and formulation was performed by keeping 1.5 mg of sample on aluminium pan at 10 to 350°C at a heating rate of 0.5°C/min using TA DSC 25 at SVKM's Shri C. B. Patel Research Centre, Vile Parle (W), Mumbai.

2.5.6. Percent Drug Entrapment Efficiency (%EE)

Entrapment Efficiency of drug loaded niosomes was carried out by ultrafiltration method. The drug loaded niosomal suspension were centrifuged at a speed of 10000 rpm for 30 min using cooling centrifuge (Eltek Refrigerated Centrifuge RC 4010 F) and the sediment was assayed for entrapped drug concentration by diluting it with phosphate buffer pH 6.8 and determining the absorbance at 263 nm using UV spectrophotometer. The percent drug entrapment efficiency (%EE) was calculated using following formula:

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Entrapped Drug}}{\text{Total amount of drug}} \times 100$$

2.5.7. Particle Size, Polydispersity Index and Zeta Potential

The prepared niosomes was taken in small amount and diluted with 10 ml of double distilled water. This prepared sample was then sonicated for 20 min and placed in sample holder and scanned using HORIBA SZ-100 Nanoparticle Analyzer for determination of mean particle size, polydispersity index and zeta potential.

2.5.8. Transmission Electron Microscopy (TEM)

The characterization of vesicles was carried out by placing small amount of drug loaded niosomes on the carbon film covered copper grid and obtaining photomicrographs of suitable magnifications using FEI Tecnai 12 Transmission Electron Microscopes at Icon labs Pvt. Ltd., Sanpada, Navi Mumbai.

2.5.9. Antifungal Activity

A placebo subgingival film, drug loaded subgingival film and drug loaded niosomes based subgingival film was prepared and cut into 1×1 cm. These films were exposed to a fungi species, *Candida albicans* and incubated at 20-25°C for 2-5 days using disk diffusion method. These studies were carried out at Micro Bio Laboratory, Thane, Mumbai.

2.5.10. Drug Content

Drug loaded niosome based subgingival film was cut into 1×1 cm and dissolved in methanol and diluted upto 10 ml with pH 6.8 phosphate buffer solution. The absorbance of the solution was recorded at 263 nm using UV spectrophotometer by keeping pH 6.8 phosphate buffer solution as blank. The percent drug content was calculated by using following formula:

$$\text{Drug Content (\%)} = \frac{\text{Amount of drug in the formulation}}{\text{Total amount of drug}} \times 100$$

2.5.11. Percent Moisture Uptake

Drug loaded films were weighed individually and then it was kept in a desicator containing potassium chloride to maintain 84% RH for 24 hours. Then films were reweighed and percent moisture uptake was calculated using the following formula:

$$\text{Moisture Uptake (\%)} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Initial Weight}} \times 100$$

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2.5.12. Percent Moisture Content

Drug loaded films were weighed individually and then it was kept in a desicator containing calcium chloride at room temperature for 24 hours. After 24 hours it was taken out and reweighed and percent moisture content was calculated using the formula:

$$\text{Moisture Content (\%)} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Final Weight}} \times 100$$

2.5.13. Thickness Measurement

The niosomes based subgingival film were cut into 1×1 cm and thickness were determined by digital vernier caliper.

2.5.14. Weight Determination

The niosomes based subgingival film were cut into 1×1 cm and all the 20 films were measured individually on an electronic balance and average weight was calculated.

2.5.15. Tensile Strength

Tensile strength of the niosomes based subgingival film were determined with tensile strength tester by fixing the film between two load cell grips of the tester. When the force is gradually applied the upper movable load cell grip pulls the film till it breaks and shows the reading on the tester dial.

2.5.16. Folding Endurance

Folding endurance is determined by repeatedly folding the film till it breaks.

2.5.17. In-Vitro Diffusion and Drug Release

The *in-vitro* diffusion study was carried out using Franz Diffusion Cell. The receptor compartment was filled with 20 ml of Phosphate buffer pH 6.8 and sample was kept in donor compartment over a dialysis membrane that was soaked in phosphate buffer pH 6.8 solution for 24 hours. This assembly was kept on the magnetic stirrer at 50 rpm speed at room temperature. 2 ml of aliquot was withdrawn from the receptor compartment at the time interval of 1, 2, 3, 4, 5, 6, 24, 48, 72, 96, 120, 144, 168, 192 hours and 2 ml of medium i.e., phosphate buffer pH 6.8 was added back to the receptor compartment at the mentioned time interval and analyzed using UV spectrophotometer at 263 nm wavelength. Then percent cumulative drug release was determined and *in-vitro* drug release was studied using different release kinetic profiles.

2.5.18. Stability Studies

The stability of the optimized formulation was determined by wrapping the subgingival film in aluminium foil and storing the formulation at 5-8^{o}C and 25^{o}C for upto 3 months as per ICH guidelines and analyzing the stability by evaluating the appearance, percent entrapment efficiency and percent drug content.

3. Results and Discussions

3.1. Optimization of Niosomes

In this study, we employed Plackett Burman Factorial Design for screening of the significant factors which is depicted in Table 2. Based on these results 3 factors 2 level full factorial design was used to study the effect of independent variables i.e., speed of rotation, cholesterol: surfactant ratio, hydration time, temperature, amount of hydration solvent on dependent variable i.e., percent entrapment efficiency. The results of factorial design are depicted in Table 3. Based on these studies it was observed that when most significant factor i.e., temperature and speed of rotation increases

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the entrapment efficiency decreases as depicted in Figure 1. ANOVA statistical analysis study suggest that the selected model is significant for the responses as shown in Table 4. The model F-value of 53.57 implies the model is significant. There is only a 1.84% chance that an F-value this large could occur due to noise. p-values less than 0.05 indicate model terms are significant. In this case B, C, AB, ABC are significant model terms. Values greater than 0.1 indicate the model terms are not significant. If there are many insignificant model terms, model reduction may improve your model. The results of regression analysis suggest that the predicted R^2 of 0.8814 is in reasonable agreement with the adjusted R^2 of 0.9741; i.e., the difference is less than 0.2 (Table 5). The observed response was as per the predicted response which is indicating the validity of the optimization design (Table 6). So, the optimized formulation was prepared and used for further experiments.

The polynomial equation obtained from this study to correlate the independent and dependent variable was depicted as follow:

$$\mathbf{Y = 76.83 - 0.8475\,A - 1.44\,B - 2.96\,C - 1.39\,AB - 3.23\,ABC}$$

Table 2. Design of experiment using Plackett Burman Factorial Design for optimization of niosome.

Run	Speed of Rotation (rpm)	Cholesterol: Surfactant ratio	Hydration Time (mins)	Temperature (°C)	Amount of Hydration Solvent (ml)	Dummy	Dummy	Entrapment Efficiency (%)
1	130	1:1:2	30	45	10	+	+	85.14
2	130	1:1:3	30	40	20	-	+	69.92
3	130	1:1:3	60	40	10	+	-	78.40
4	80	1:1:3	60	45	10	-	+	88.46
5	130	1:1:2	60	45	20	-	-	83.64
6	80	1:1:3	30	45	20	+	-	85.00
7	80	1:1:2	60	40	20	+	+	81.64
8	80	1:1:2	30	40	10	-	-	84.88

Table 3. Design of experiment using 2^3 Factorial Design for optimization of niosomes.

Run	**Speed of Rotation (rpm)**	**Temperature (°C)**	**Amount of Hydration Solvent (ml)**	**Entrapment Efficiency (%)**
1	80	40	10	84.32
2	130	40	10	78.10
3	80	45	10	77.80
4	130	45	10	78.94
5	80	40	20	71.12
6	130	40	20	79.52
7	80	45	20	77.46
8	130	45	20	67.36

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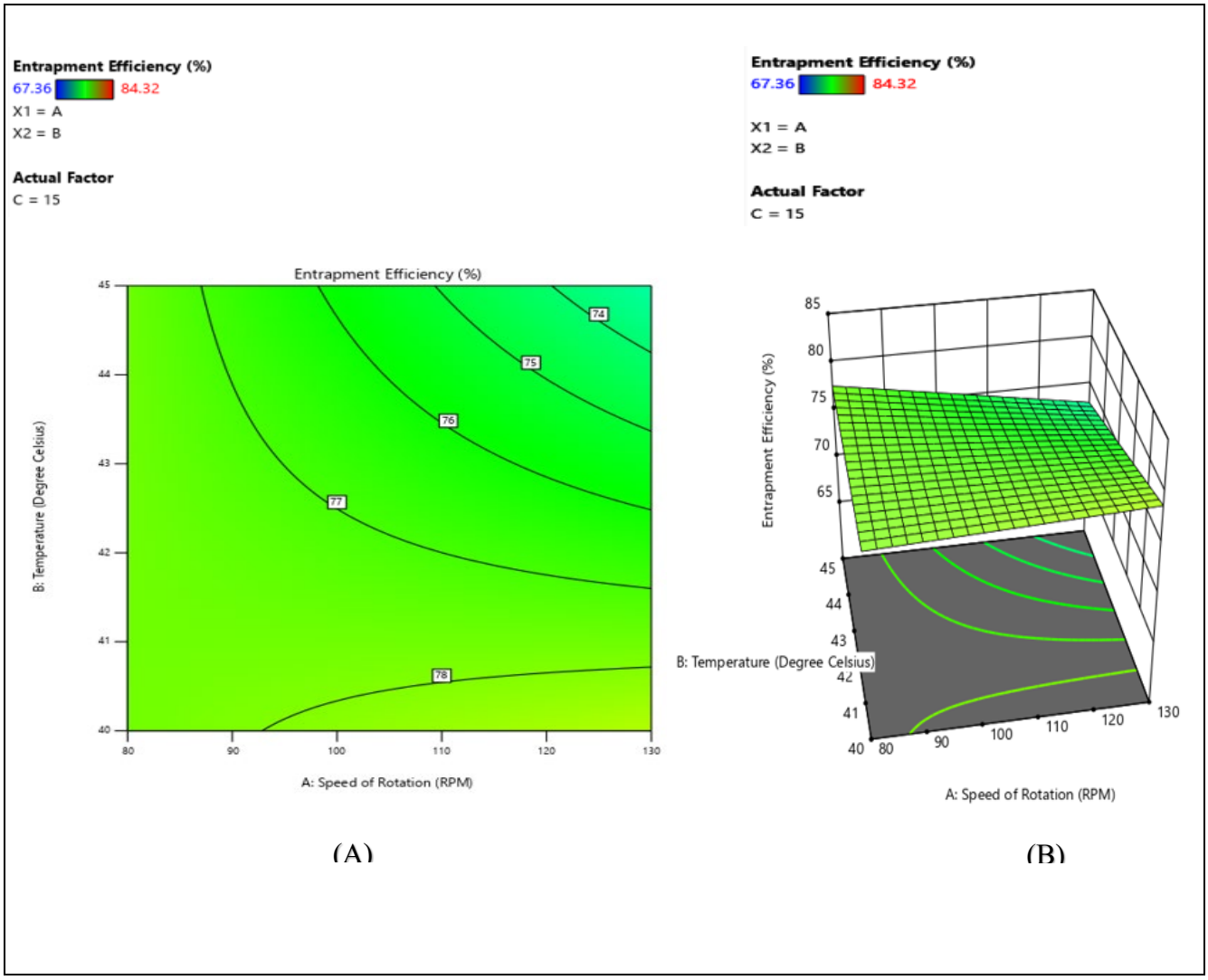

Figure 1. (A) Contour plot showing the impact of factors on the response **(B)** Three-dimensional response surface plot indicating the impact of factors on the response.

Table 4. ANOVA Statistical Analysis.

Source	F-value	p-value	
Model	53.57	0.0184	significant
A- Speed of rotation	8.03	0.1052	
B- Temperature	23.11	0.0406	
C- Amount of Hydration Solvent	98.16	0.0100	
AB	21.69	0.0431	
ABC	116.87	0.0084	

Table 5. Results of regression analysis for response.

Responses	R^2	Adjusted R^2	Predicted R^2	Adeq Precision
Entrapment Efficiency (%)	0.9926	0.9741	0.8814	23.1561

Table 6. The optimized response predicted by design of experiments and the experimental data for the response under the optimum conditions, n = 3.

Response	Predicted by DOE	Experimental Data
Entrapment Efficiency (%)	75.85	74.66 ± 3.12

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3.2. Characterization of Niosomal Based Subgingival Film

3.2.1. Solubility Studies

The solubility of clotrimazole was observed in chloroform and methanol, slight solubility in phosphate buffer pH 6.8, insolubility in water and it was sparingly soluble in ethanol. So, analytical grade of chloroform and methanol was used as a solvent for this experimental study.

3.2.2. Scanning Electron Microscopy (SEM)

The morphology of clotrimazole was examined by SEM analysis. As per Figure 2, the pure form of drug was in crystal form.

3.2.3. Spectral Characterization of Drug by UV Spectroscopy

The absorbance maxima for the clotrimazole were observed at 263 nm in both methanol and phosphate buffer pH 6.8 (Figure 3 and 4). We observed a linear calibration curve for clotrimazole in both methanol and phosphate buffer pH 6.8 as depicted in Figure 5 and 6 respectively.

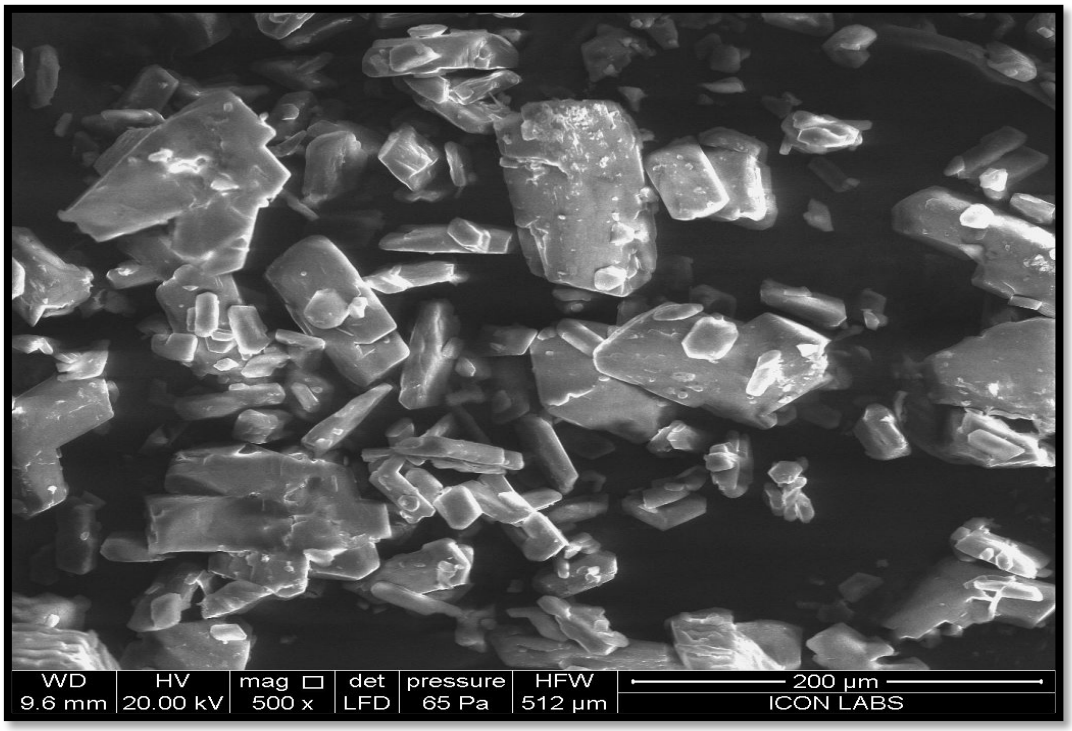

Figure 2: Morphology of Drug in 500x magnification.

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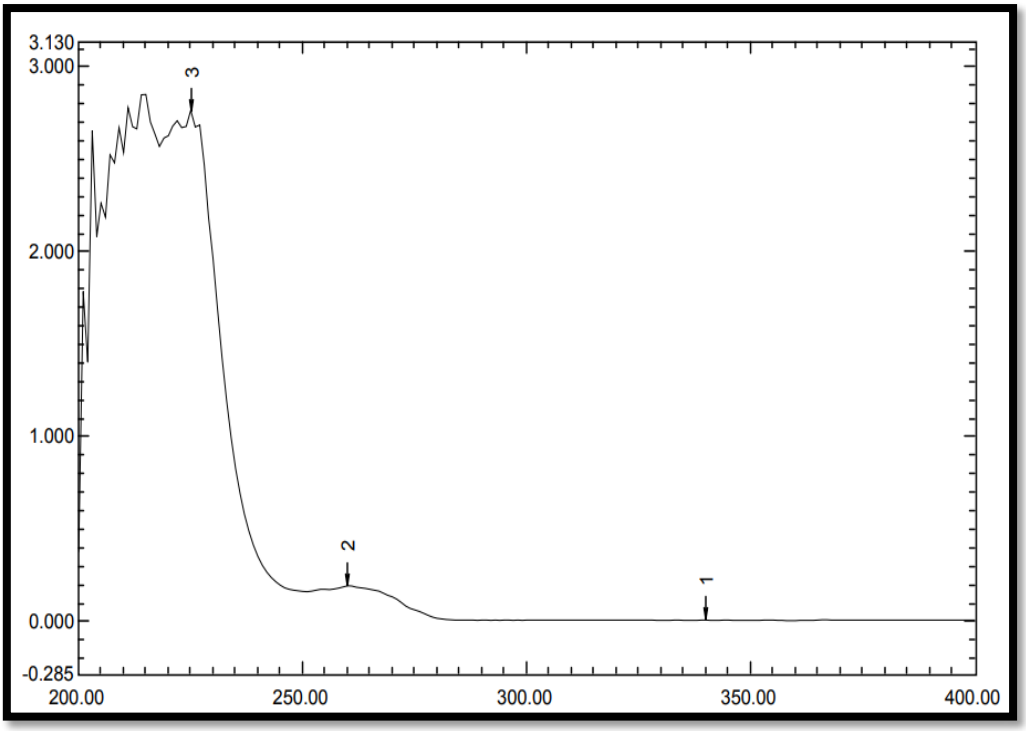

Figure 3: UV Spectra of clotrimazole in Methanol.

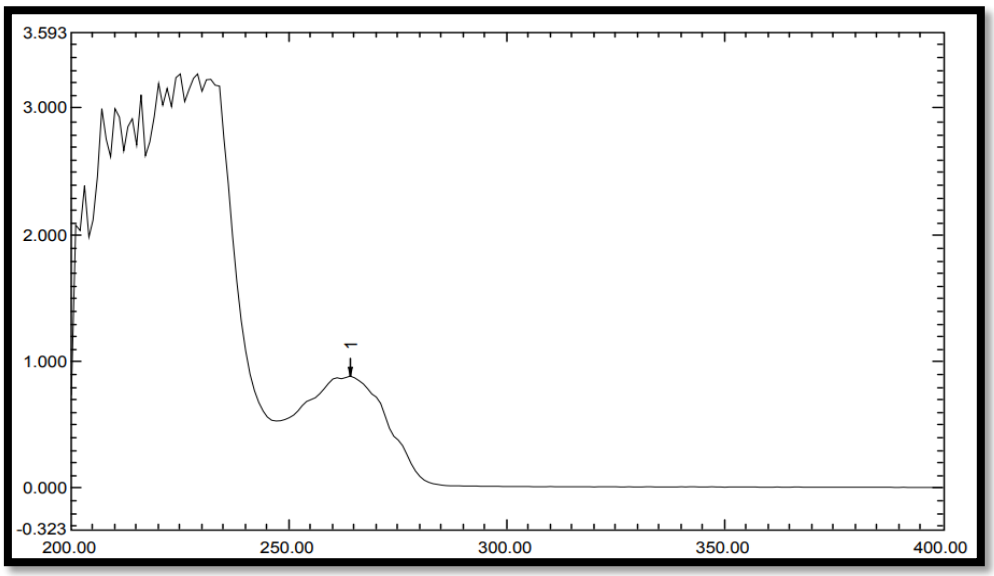

Figure 4: UV Spectra of clotrimazole in phosphate buffer pH 6.8.

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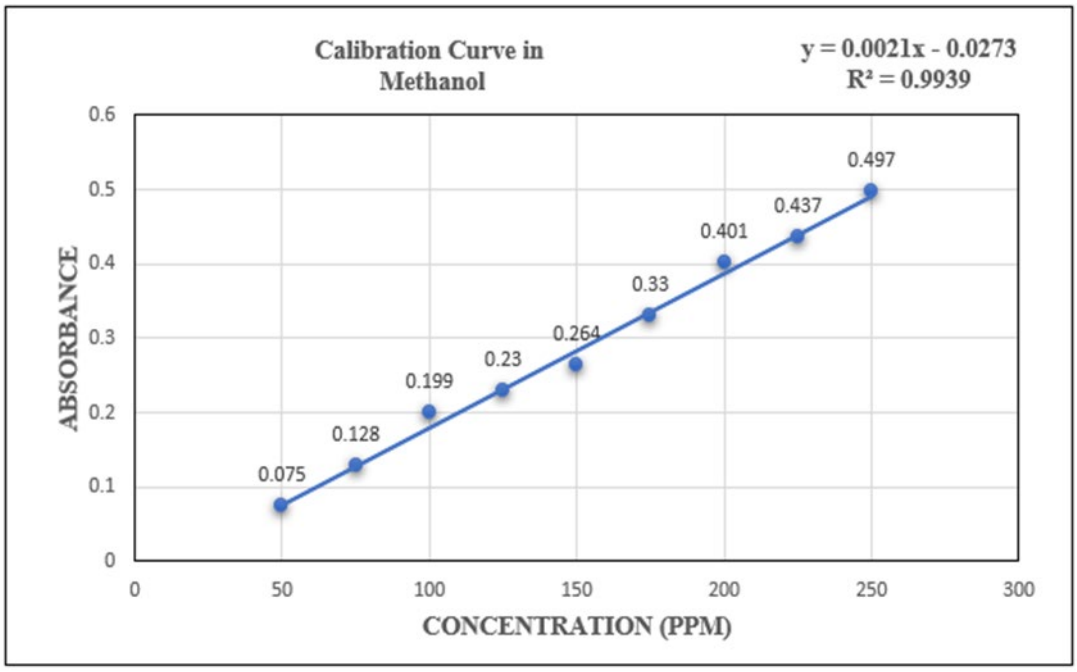

Figure 5. Calibration curve of clotrimazole in methanol.

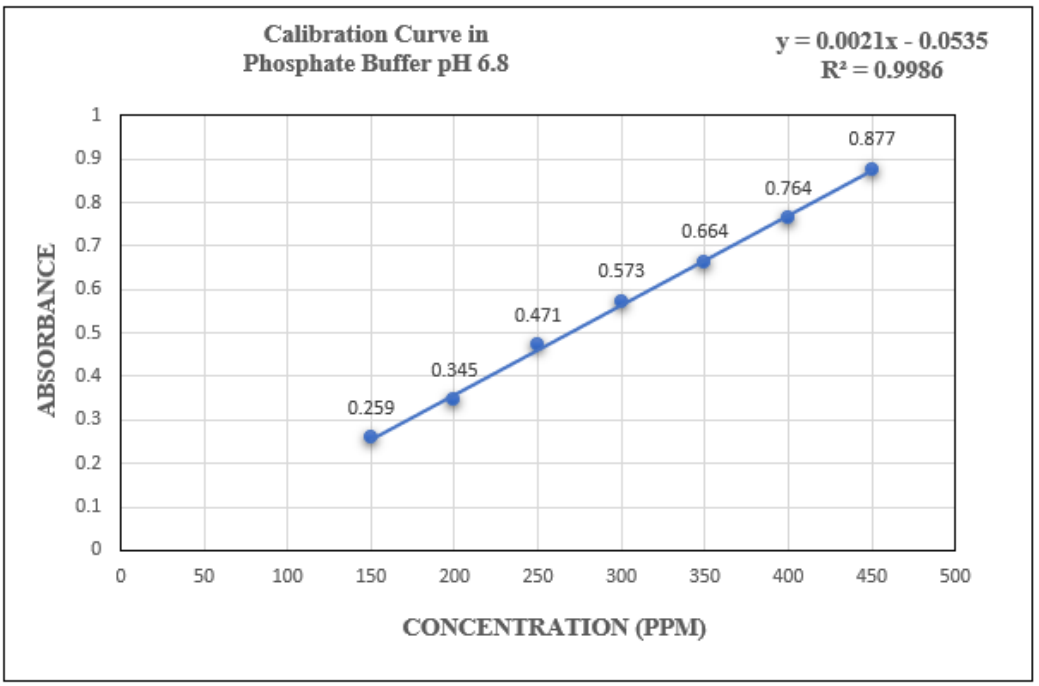

Figure 6: Calibration curve of clotrimazole in phosphate buffer pH 6.8.

3.2.4. Fourier Transform-Infrared Spectroscopy (FTIR)

The FTIR spectra of clotrimazole were compared with the FTIR spectra of physical mixture of drug and excipients. The comparative study showed that there was no significant shift in the positions of the wavenumbers, so there was no interaction between the drug and excipients (Table 7, Figure 7 and 8).

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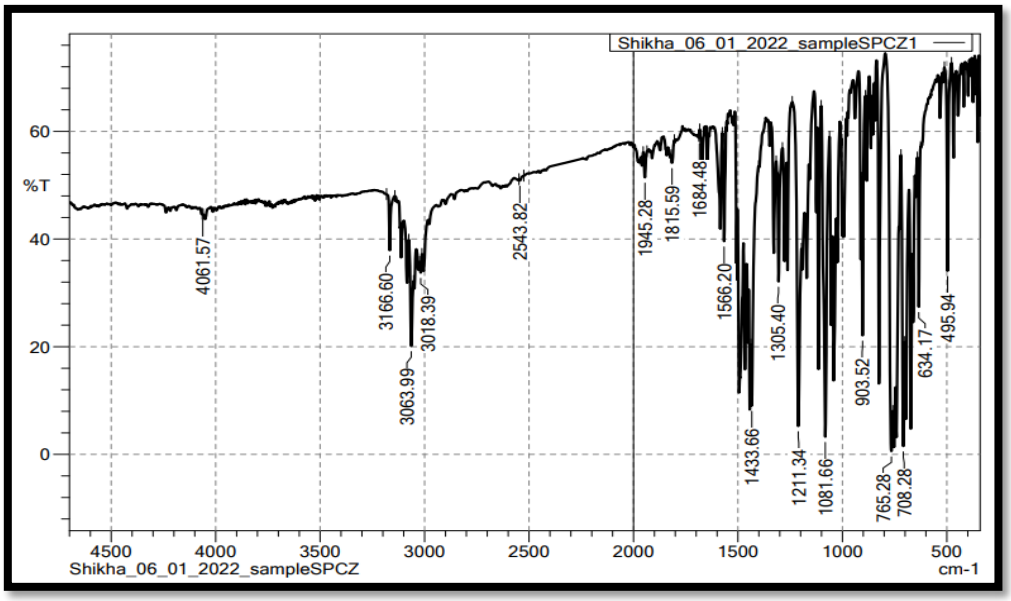

Figure 7. FTIR Spectra of clotrimazole.

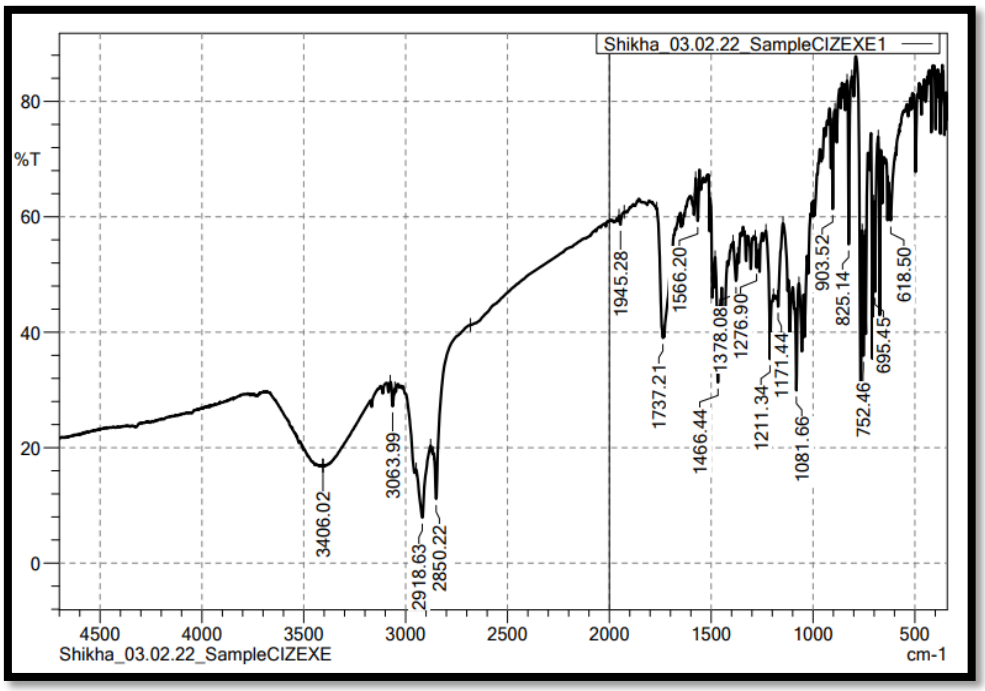

Figure 8: FTIR Spectra of physical mixture of drug and excipients.

Table 7. Peaks of FTIR Spectra of drug and physical mixture of drug and excipients.

Expected Peak Range (cm^{-1})	Observed Peak Range of drug (cm^{-1})	Observed Peak Range of physical mixture (cm^{-1})	Functional Group
3100-3000	3063.99	3063.99	=C-H stretch
1600-1400	1433.66	1466.44	Aromatic C=C stretch
1350-1200	1305.40	1276.90	C-N stretch
860-680	765.28	752.46	Aromatic C-H bending
1700-1500	1566.20	1566.20	Aromatic C=C bending
800-600	708.28	695.45	C-Cl stretch

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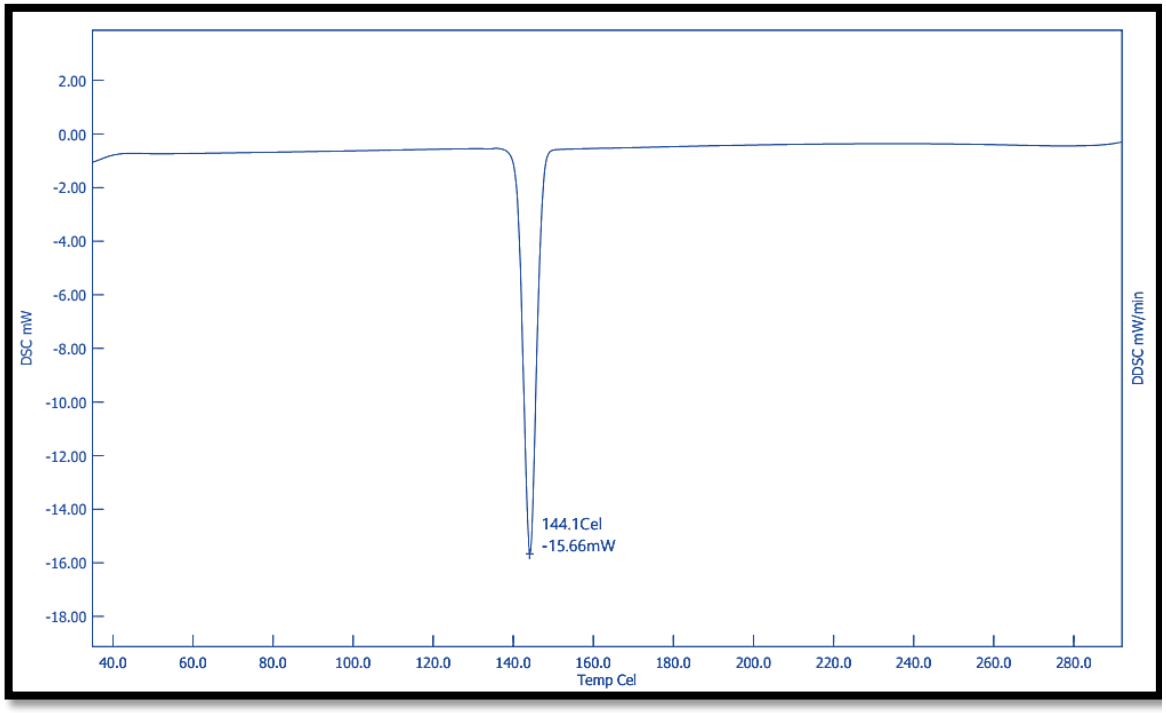
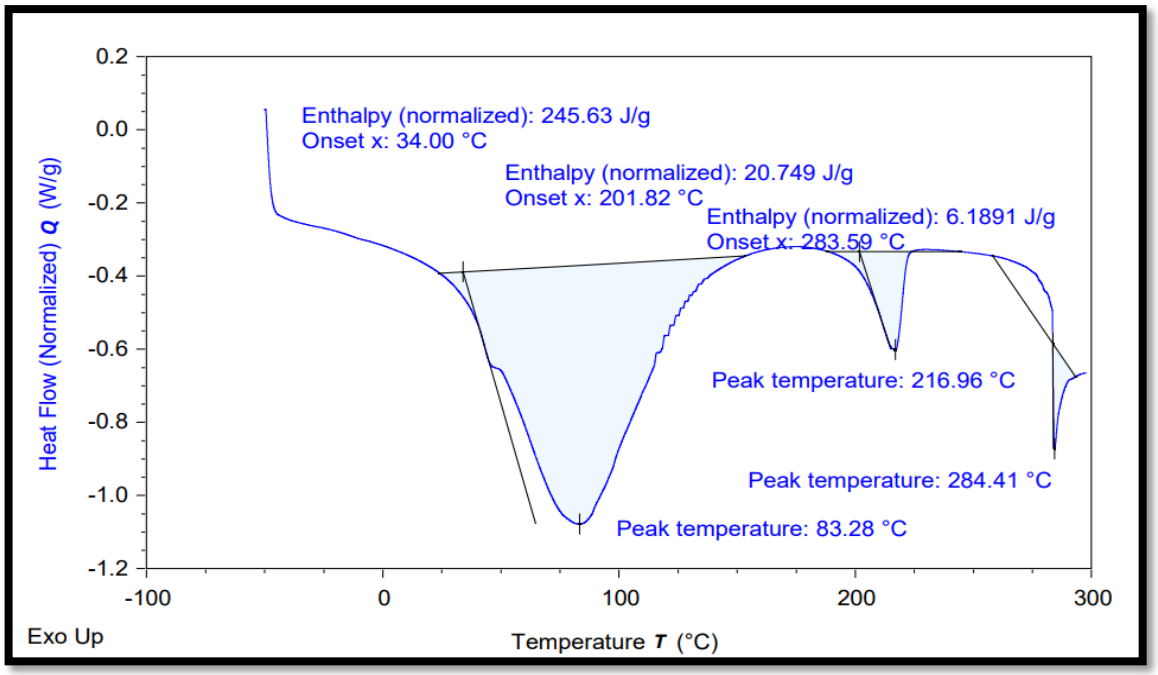
3.2.5. Differential Scanning Calorimeter (DSC)

The thermal analysis of clotrimazole and formulation of drug loaded niosomes based subgingival film shows sharp endothermic peak at 144.1°C and less intense endothermic peak at 83.28°C. Based on this study we can conclude that drug was entrapped in niosomes and hence, solubility of the drug will increase (Figure 9).



(**A**)



(**B**)

Figure 9. Thermogram of (A) Clotrimazole (B) Formulation.

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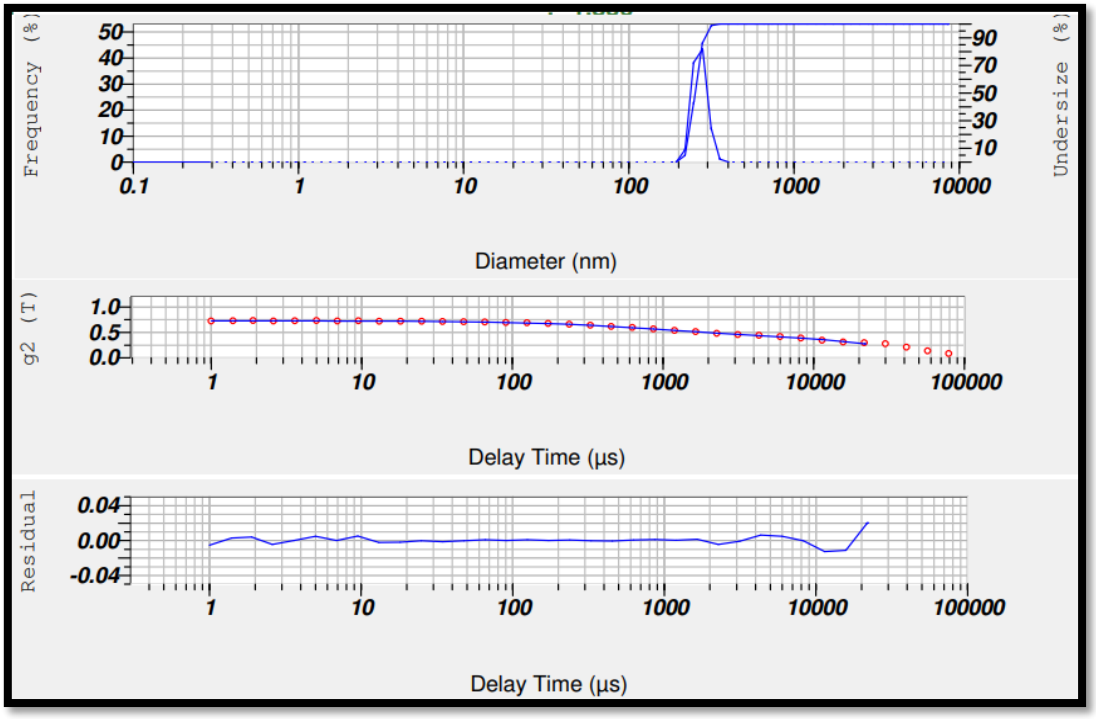
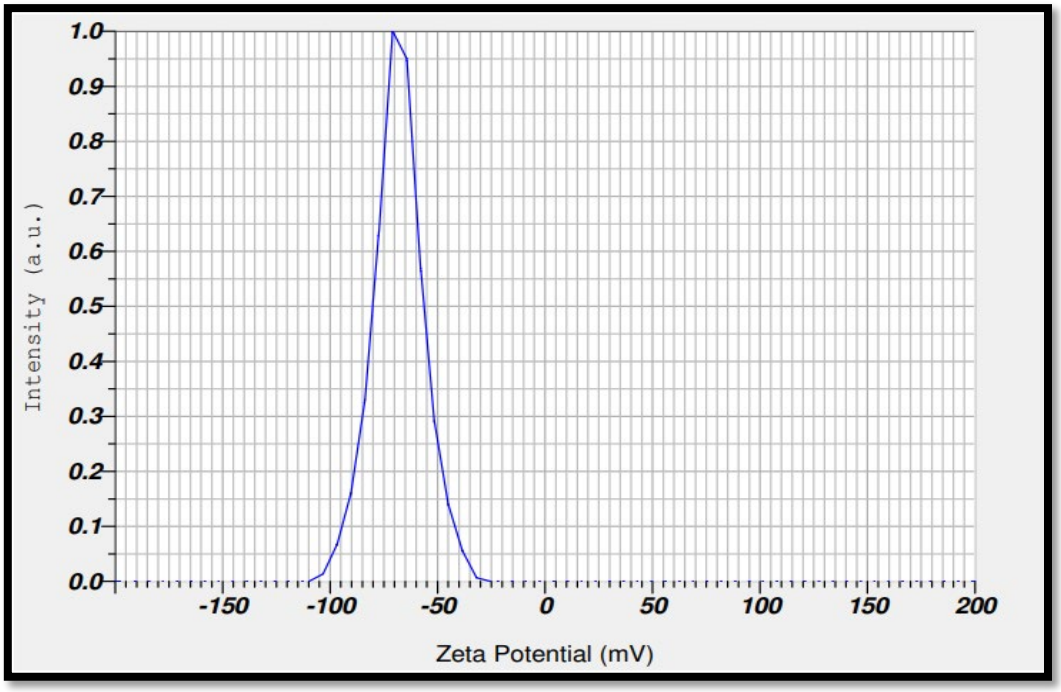
3.2.6. Particle Size, Polydispersity Index and Zeta Potential

The observed particle size of optimized batch of niosomes was 2930.4 nm which lies within the range of 20 nm-50 µm. The polydispersity index was found to be 1.836 and zeta potential of optimized niosomes was found to be -68.2 mV which is depicted in Figure 10.



(A)



(B)

Figure 10. Graphical representation of (A) Particle size (B) Zeta Potential.

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3.2.7. Transmission Electron Microscopy (TEM)

The morphology of prepared niosomes were studied through TEM analysis. Figure 11 illustrates the internal structure of drug loaded niosomes. The study validates that the prepared niosomes are spherical in shape with rigid boundaries.

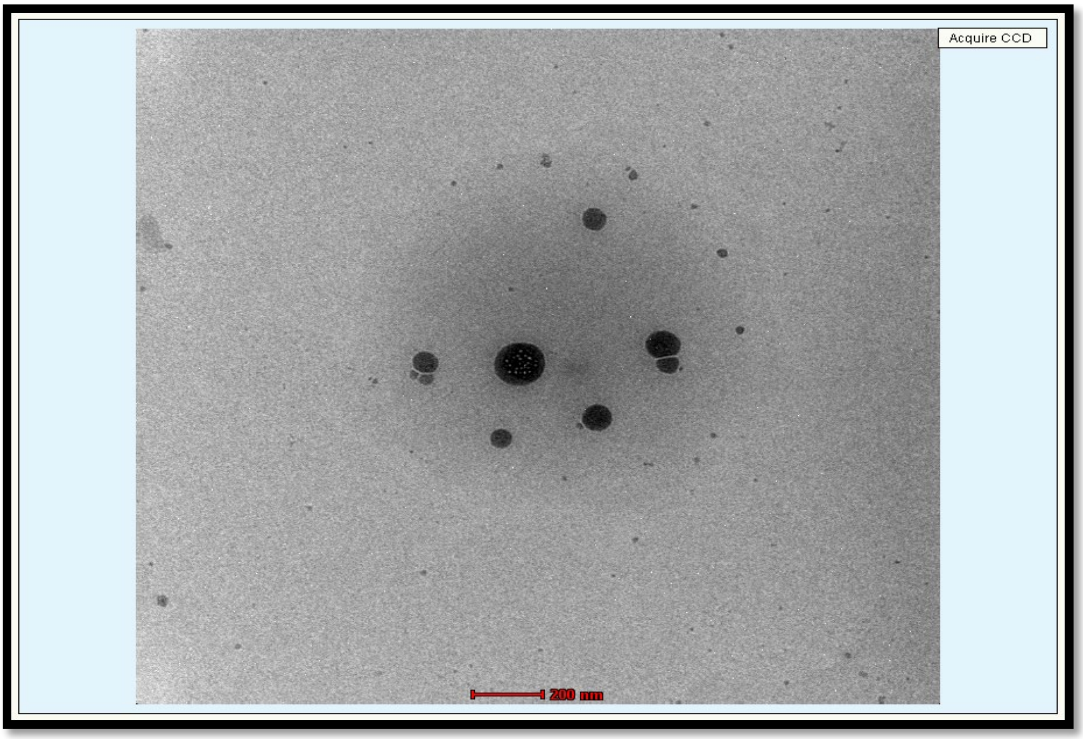

Figure 11. TEM images of prepared niosomes.

3.2.8. Anti-FUNGAL Activity

The anti-fungal study illustrates that the clotrimazole loaded film and optimized clotrimazole loaded niosomal film shows anti-fungal activity but optimized clotrimazole loaded niosomal film shows better zone of inhibition against *C. albicans* than clotrimazole loaded film and no zone of inhibition was observed in placebo film sample and hence, we concluded that excipients does not show antifungal activity. The clotrimazole loaded film showed 32.68 mm zone of inhibition and optimized clotrimazole loaded niosomal film showed 36.07 mm zone of inhibition against *C. albicans* which is depicted in Figure 12.

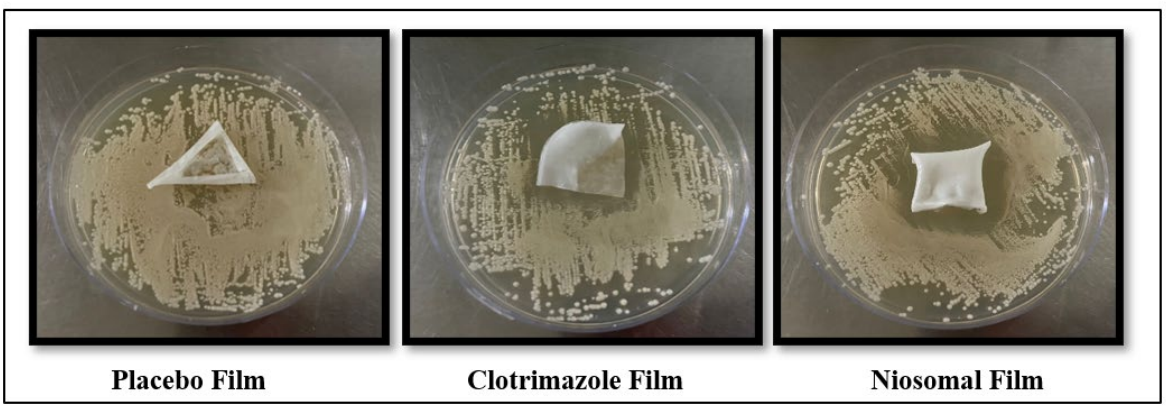

Figure 12. Images depicting anti-fungal activity.

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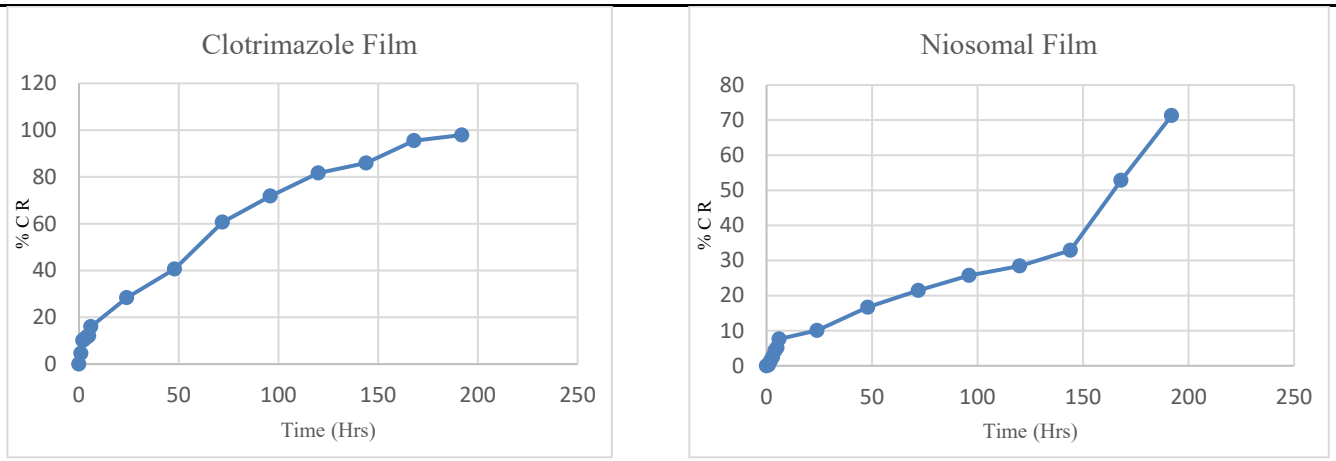
3.2.9. In-Vitro Diffusion and Drug Release

A comparative study was performed between clotrimazole loaded film and clotrimazole loaded niosomal film for drug release profile. *In-vitro* diffusion study, as depicted in Figure 13 shows that 97% of drug was release in 192 hours from clotrimazole loaded film and 71% of drug was release in 192 hours from clotrimazole loaded niosomal film. Based on these drug release data various kinetic models was plotted to study the mechanism of drug release which is depicted in Figures 14 and 15 and Table 8. The zero-order kinetic model for clotrimazole loaded niosomal film shows the regression coefficient near to one. So, based on this model a constant amount of drug was release from the film at per unit time.



(**A**) (**B**)

Figure 13. *In-vitro* diffusion study of (A) Clotrimazole loaded film (B) Clotrimazole loaded niosomal film.

Table 8. Regression Coefficient value obtained from kinetic release models.

Release Model	Equation	Clotrimazole Film (R^2)	Niosomal Film (R^2)
Zero-order	$C_t = C_0 + K_0t$	0.876	0.961
First-order	$\log C = \log C_0 + Kt/2.303$	0.885	0.957
Korsmeyer-Peppas	$Kt^n = M_t/M_\infty$	0.867	0.900
Higuchi	$Q = KH\sqrt{t}$	0.950	0.785
Hixson- Crowell	$K_t = C_0^{1/3} - C_t^{1/3}$	0.882	0.959

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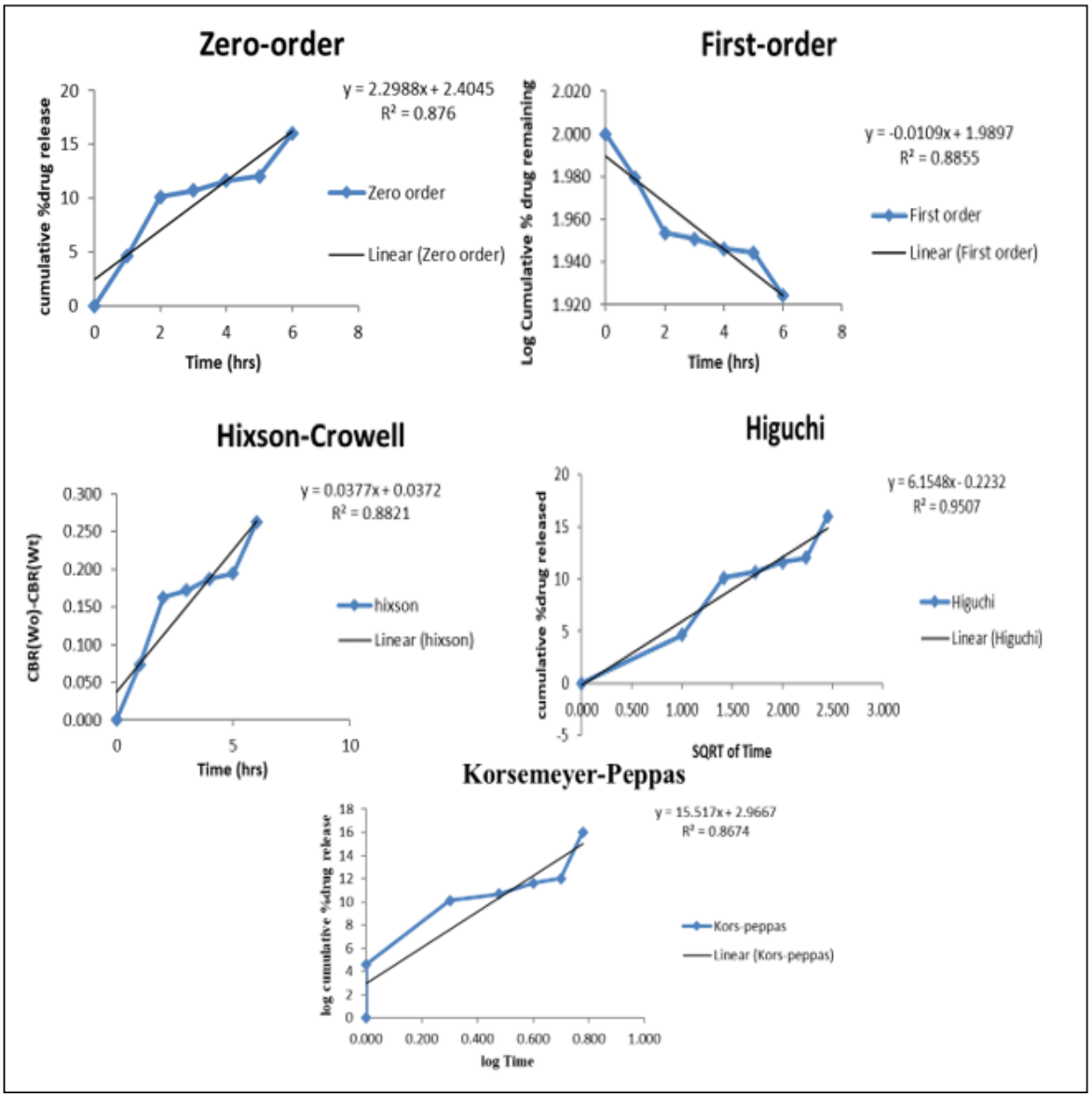

Figure 14. Kinetic Models for Clotrimazole Loaded Film.

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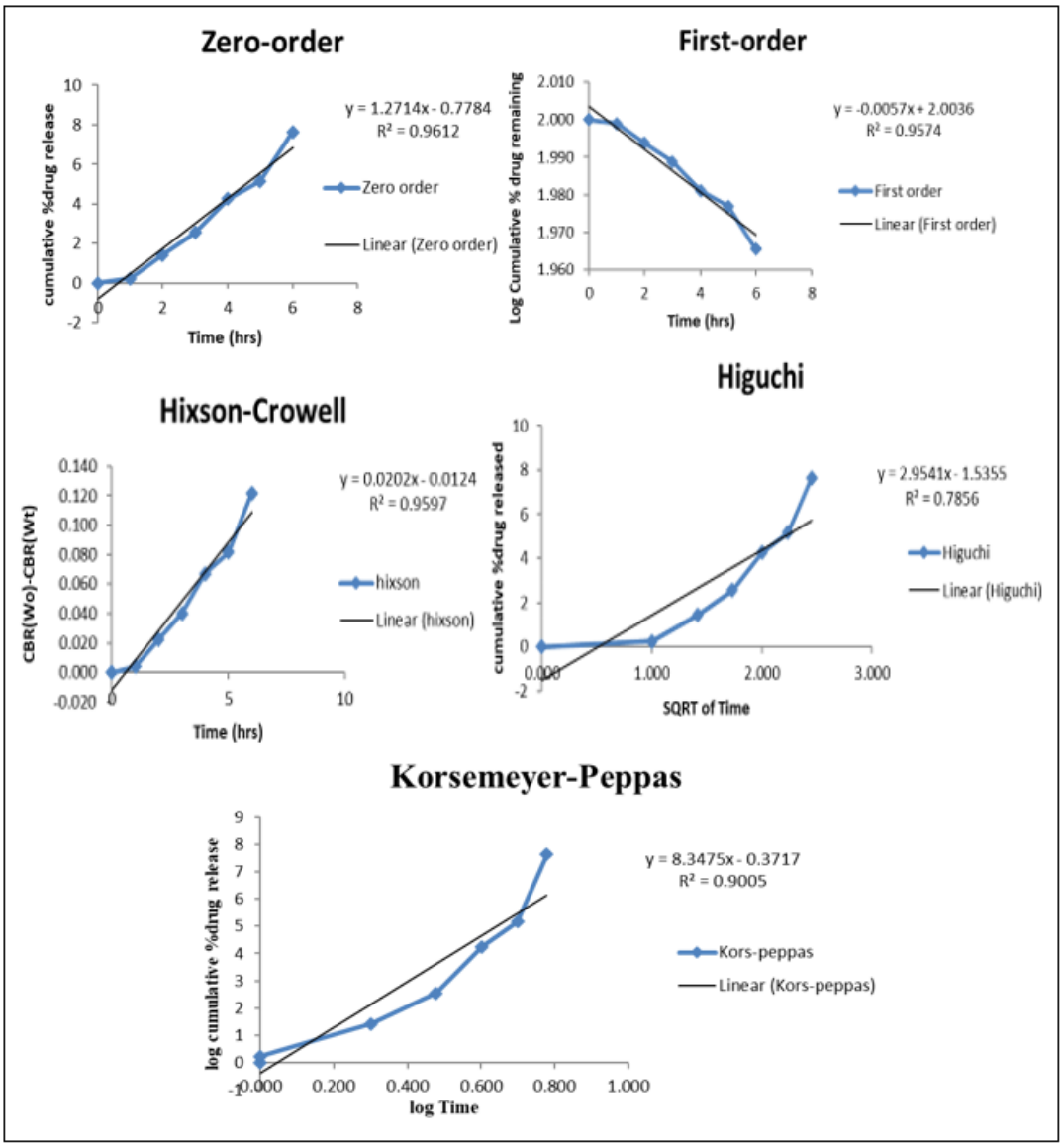

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Figure 15. Kinetic Models for Clotrimazole Loaded Niosomal Film.

3.2.10. Physico-Chemical Properties of Niosomal film

The prepared clotrimazole loaded niosomes based subgingival film was white in color with smooth appearance. This formulation was evaluated for various physico-chemical properties which is depicted in Table 9.

3.2.11. Stability Studies

The stability studies of the optimized formulation were carried out as per ICH guidelines at 5-8 ± 2°C and 25 ± 2°C for upto 3 months which is depicted in Table 10 and Figure 16.

Table 9. Physico-chemical Properties.

Parameters	Results
Thickness	0.52 ± 0.03 mm
Drug Content	90.82 ± 0.67 %
Weight Variation	0.64 ± 0.21 gm
Tensile Strength	1.62 ± 0.02 kg/cm^2
Folding Endurance	250 ± 12 times

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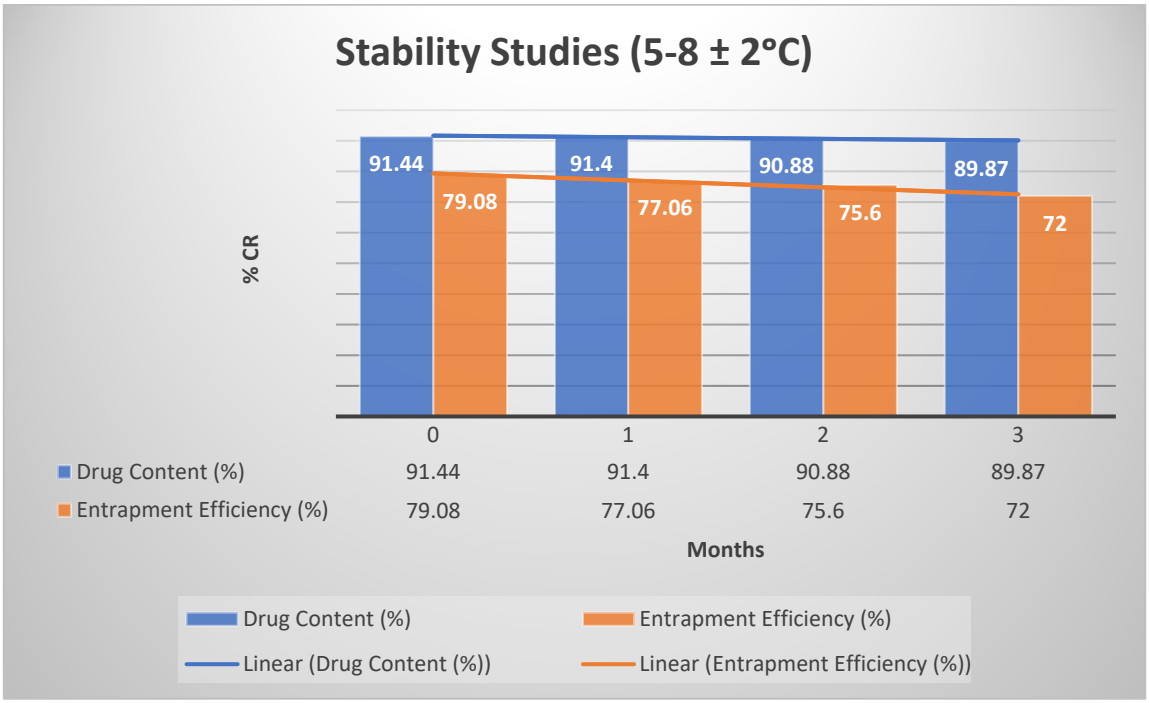
Percent Moisture Uptake	20.47 ± 0.66 %
Percent Moisture Content	40.25 ± 7.85 %

Table 10. Stability Studies.

Parameters (Months)	Appearance	Drug Content %	Entrapment Efficiency %
		5-8 ± 2°C	
0	White and Smooth	91.44	79.08
1	White and Smooth	91.40	77.06
2	White and Smooth	90.88	75.60
3	White and Smooth	89.87	72.00
		25 ± 2°C	
0	White and Smooth	91.44	79.08
1	White and Slightly Shrink	91.25	78.40
2	White and Slightly Shrink	90.00	75.03
3	White and Slightly Shrink	89.02	72.03



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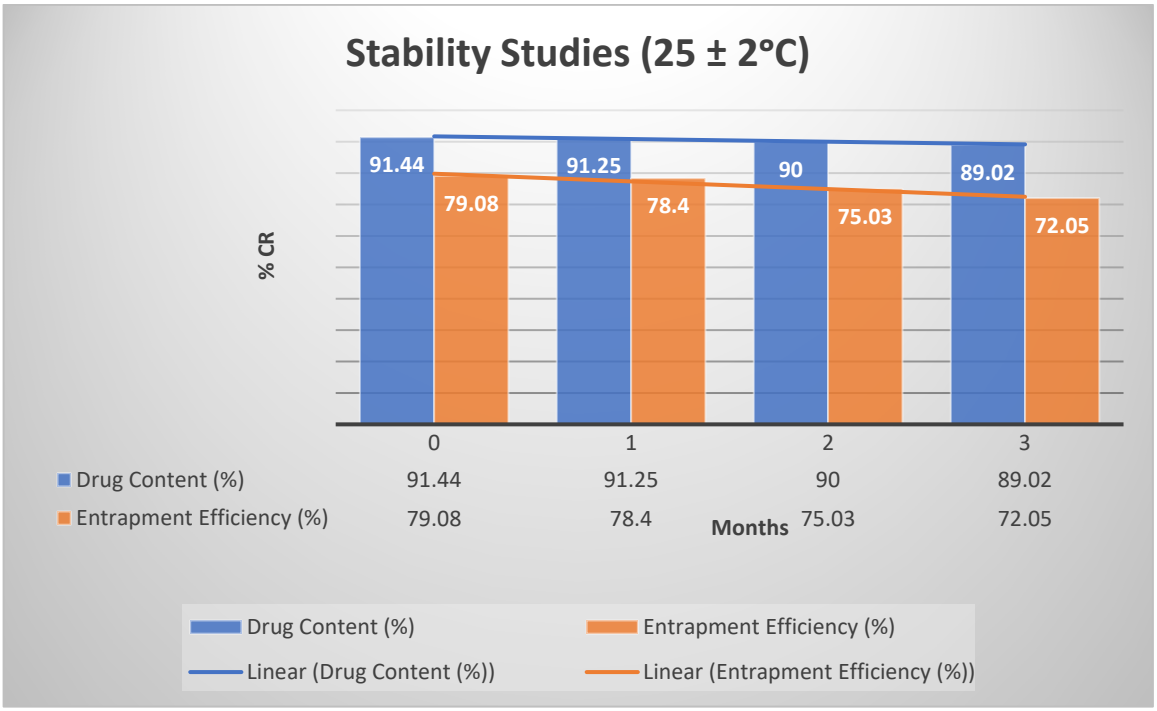

Figure 16. Stability Studies.

4. Conclusion

As per the literature survey it was found that marketed preparation of niosomal subgingival film was not available for oral thrush. So, in this study, an optimized clotrimazole loaded niosomes were prepared using thin film hydration method and these niosomes were incorporated in a film by solvent casting method and evaluated for its physico-chemical properties. *In-vitro* characterization studies revealed that Clotrimazole can be incorporated in a slow-release device for the treatment of oral thrush. Niosomal subgingival film containing clotrimazole can be a promising alternative nano-carrier system for local drug delivery and enhanced anti-fungal activity against *C. albicans*.

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