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Original Research Article

Development and Optimization of Cubosomal Econazole Nitrate

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Abstract

Cubosomes are nanoparticle, more accurately nano structure particles of a liquid crystalline phase with cubic crystallographic symmetry formed by the self-assembly of amphiphilic or surfactants like molecule. This study is to investigate the potential of cubosomes as lipid nanocarrier to improve the controlled release of Econazole Nitrate used for treatment of dermatophytes. Econazole Nitrate cubosomes were prepared by Top-down approach employing GMO as lipid phase vehicle, Poloxamer 407 as stabilizer and distilled water as aqueous phase. The resultant cubosomes dispersion were characterized by encapsulation efficiency, in-vitro drug release, particle size, zeta potential and FTIR. Best formulation (F4) showed a maximum drug release of 93.96 % in 8 hours, particle size of 230.1 nm and zeta potential of -19.1 mV. The best formulation was chosen on the basis of Optimization by Design of Expert software which will be incorporated into the carbopol 934 gel for the proper and feasible application of the formulation on skin.

Keywords: Nanoparticles, Cubosomes, Top-down approach, Econazole Nitrate

INTRODUCTION

A vesicular drug delivery system is one that achieves targeted drug delivery by encapsulating the drug within a vesicular structure. Cubosomes are also found in the vesicular drug delivery system and the lipid-based colloidal system, which were discovered in 1980.[1-5]

Cubosomes are nano-structured, discrete, sub-micron particles of bicontinuous cubic liquid crystalline phase. Cubosomes have the same microstructure as the parent cubic phase but have a larger specific surface area and much lower viscosity than the bulk cubic phase.[6,7] The colloidal dispersion of bicontinuous cubic liquid crystalline structures in water using appropriate surfactants produces nanostructured systems known as 'cubosomes' with sizes ranging from 100 to 300 nm.8 It provides well-controlled delivery of a wide range of drug candidates, including anti-inflammatory compounds, local anaesthetics, anti-diabetics, antibiotics, and anticancer medications.[9]

Topical treatment of superficial fungal infections has several advantages over oral treatment, including the ability to target the site of infection, reduce systemic side effects, improve treatment efficacy, and increase patient compliance. Azone and dimethyl sulfoxide are two excipients that are frequently used in other formulations to improve drug permeation through the stratum corneum (SC). These excipients disrupt the SC layers, allowing antifungal drugs to penetrate deeper skin layers and, unfortunately, blood circulation, resulting in systemic side effects. As a result, in

order to overcome the challenges that formulation approaches have been investigated.[10]

Econazole nitrate is an imidazole antifungal that works by inhibiting fungus growth. Econazole nitrate is a Biopharmaceutical Classification System (BCS) Class II compound with high permeability and low solubility. This is a critical factor in fungal treatment success.[11] Econazole Nitrate is an imidazole antifungal that is used topically to treat superficial candidiasis, dermatophytosis, pityriasis versicolor, and skin infections. Because of its poor solubility, it is only partially absorbed after oral administration, and bioavailability varies between individuals.[12]

MATERIALS AND METHODS

Econazole Nitrate, Glyceryl Monooleate and Poloxamer were obtained from Yarrow chemical LRD., Mumbai. All other chemicals used were of analytical grade and obtained commercially.

Methods

Preformulation Studies: The organoleptic properties of drug were studied and compared with Pharmacopoeial specifications. Melting point of Econazole Nitrate was determined by capillary method. The solubility study of Econazole Nitrate was carried out in different solvents like Methanol, Phosphate buffer pH 7.4 and purified water.

Method validation for Econazole Nitrate in pH 7.4 by UV Visible spectrophotometer:

100mg of the Econazole Nitrate was weighed accurately and transferred to 100ml of volumetric flask, dissolved in the sufficient quantity of buffer and the traditional topical formulations face, new volume was adjusted up to the mark (stock-A). 1 ml has to be taken from stock-A diluted with the buffer to get a solution containing about 10µm/ml. This solution was then scanned between 200-400nm in UV-Visible spectrophotometer to determine λmax. [13]

Preparation of Cubosomes

Glyceryl monooleate and Poloxamer 407 in various ratios were accurately weighed and melted in a water bath at 60°C. Econazole Nitrate was accurately weighed and mixed into the above mixture. The clear lipid solution was slowly added drop by drop to a suitable amount of preheated (60°C) distilled water while continuously stirring. After adding the lipid phase completely, it was set aside for one day to equilibrate. The formation of a two-phase system is disrupted by stirring. The entire system is taken and sonicated for 10 minutes at room temperature. The prepared dispersions were stored in closed containers at room temperature, away from direct sunlight, for later evaluation.

Experimental Design[14]

In the present work, a central composite design and response surface methodology was chosen to design Econazole Nitrate cubosomes using Design Expert Software (version 12.0.3 Stat-Ease, Inc., USA). Polymer range A GMO%(X1) and B P407% (X2) effects, as independent variables on one dependent variables, the particle size of the cubosomes (Y1) of all prepared possible combinations. Particle size (nm) were optimized using a Design Expert 12.0 at two levels: low (−1), and high (+1)

Table 1: Formulation chart of cubosomes

Formulation code	GMO(%)	Poloxamer 407(%)	Econazole Nitrate (%)	Water up to (ml)
F1	4.00	2.13	1	100
F2	4.80	0.20	1	100
F3	4.80	1.80	1	100

F4	5.13	1.00	1	100
F5	4.00	1.00	1	100
F6	2.87	1.00	1	100
F7	3.20	1.80	1	100
F8	3.20	0.20	1	100
F9	4.00	0.12	1	100

Characterisation of Cubosome containing Econazole Nitrate15-22

Entrapment efficiency

For the determination of entrapment efficiency, the cubosomes from the resulting dispersions were first separated by centrifugation. The separation of the free drug from the entrapped drug in the cubosome dispersion was achieved by centrifugation at 4000 rpm for 30 minutes. The resulting solution was then separated and supernatant liquid was collected. The collected supernatant was then diluted appropriately and estimated using UV visible spectrophotometer at 220 nm. The percent of encapsulation efficiency (%EE) was determined by the following equation:

% of EE = Ct−Cf/Ct× 100 Ct is equal to total drug concentration and Cf is equal to free drug concentration.

Average particle size

Average particle size (in nanometer) and of the cubosomes was measured using a Malvern nano zeta sizer instrument.

Zetapotential

Measurement of zetapotential of the cubosomes was done by using Malvern nano zeta sizer instrument. Measurements were performed on the samples prepared for size analysis. Zeta poetntial indicates the degree of repulsion between adjacent, similarly charged particles in dispersion system.

In vitro drug release from cubosomes

In vitro skin permeation studies were performed using bi chamber donor receiver compartment model (Franz diffusion cell). The formulation was taken in the donor compartment and phosphate buffer pH 7.4 was taken in the receptor compartment. The cellophane membrane, previously soaked overnight in the diffusion medium (phosphate buffer pH 7.4) was placed between the donor and receptor compartment. Cubosomal formulation was placed on the membrane, which is in contact with the receptor medium. The whole assembly was placed on the thermostatically controlled magnetic stirrer with continuous stirring and the temperature of the medium was maintained at 37±0.50 C. Samples were withdrawn from the receptor cell at specified time intervals of 1, 2, 3, 4, 5, 6 and 8 hours. Each time immediately after the removal of the sample, the medium was compensated with fresh Phosphate buffer (pH 7.4). The cumulative amount of drug from Cubosomes permeated through synthetic membrane was plotted against time.

RESULTS AND DISCUSSION

Reformulation Study

Organoleptic characteristics like general description, colour was determined. It was found that Econazole Nitrate is White to pale cream-colored crystaline powder and whitish cream amorphous powder. Melting point was found to be 164˚C. Solubility was found to be insoluble in water (0.085mg/ml), soluble in phosphate buffer pH 7.4 (0.21gm/ml) and methanol (31.45mg/ml) was found to be within the standard specification limits

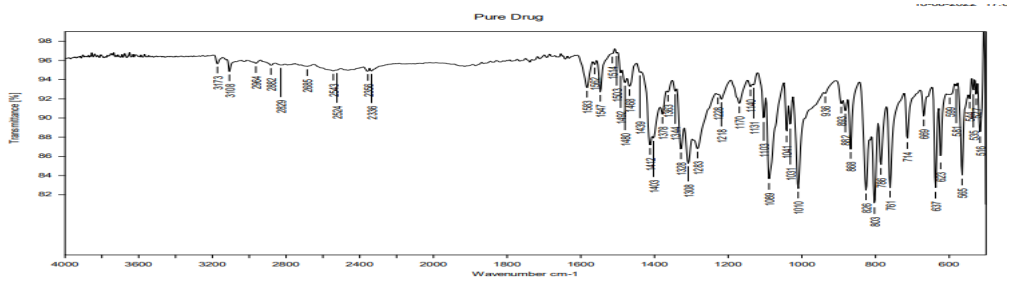


Figure 1 (a): FTIR of pure drug

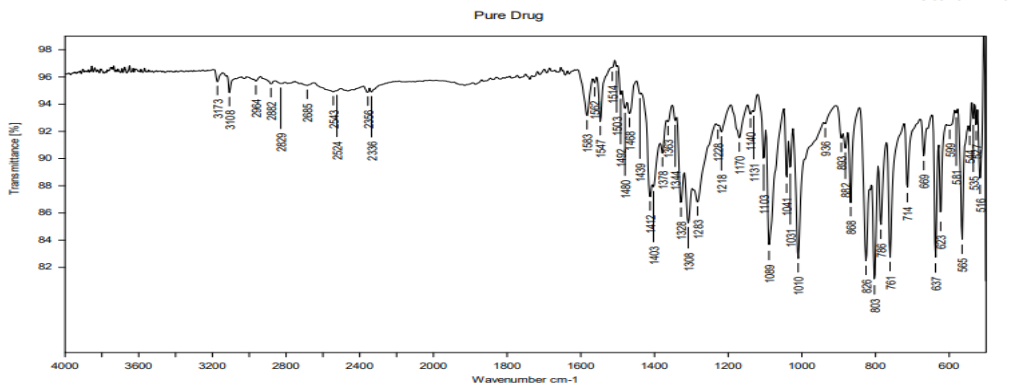


Figure 1(b): FTIR of Pure Drug + Polymer

The compatibility between the drug and excipients was carried out using the FTIR peak matching method. The characteristic peak associated with specific functional groups and bonds of the molecule and their presence / absence were noted.

All the characterestics of IR peaks related to pure drug Econazole Nitrate appeared in the FTIR spectrum of physical mixture . This result could infer that here there was no chemical incompatibility between the drug and excipients.

Analytical method validation of Econazole Nitrate in Phosphate Buffer 7.4

The absorption spectrum of pure Econazole Nitrate was scanned between 200-400nm. The λ_{max} of pure Econazole Nitrate was found to be 220 nm by using phosphate buffer pH 7.4. The curves obtained were shown in figure No 1.

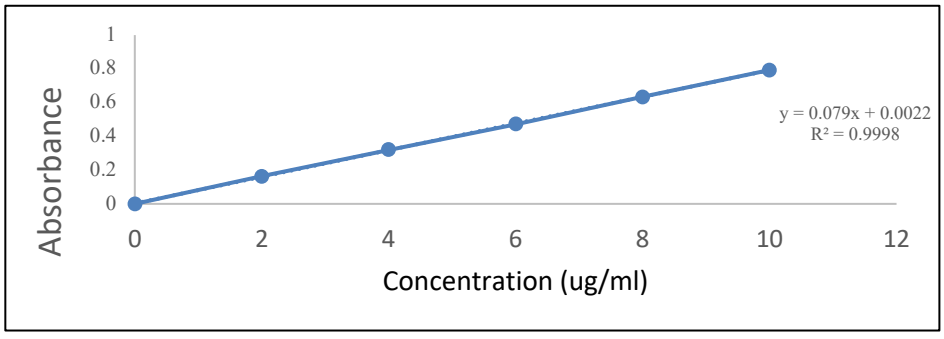


Figure 2: Calibration curve of Econazole Nitrate

Evaluation of cubosomes loaded with Econazole Nitrate

Entrapment efficiency(EE)

The EE of all the formulation F1 – F9 is in the range of 55 – 86.10±0.76 as shown in table No.2. The highest entrapment efficiency was found in the batch F4, consisted of GMO and poloxamer 407 was found in the range 4.2 and 0.20 respectively. The result shows that the EE increased, as the amount of GMO increased. The increased GMO, which act as a solubilising agent of Econazole Nitrate and provide more space to accommodate excessive drugs. The effect

may be observed due to increased viscosity of the medium, because increasing the amount of lipid result in faster solidification of the cubosomal nanoparticles, which would prevent drug diffusion to the external phase of the medium. The Econazole Nitrate was incorporated in the surfactant layer at the surface of the cubosomes, leading to high entrapment efficiency.

Table 2: Entrapment Efficiency of F1-F9

Sl No	Formulation	EE %
1	F1	79.36 ±0.09 %
2	F2	82.51 ± 0.04 %
3	F3	80.31 ±0.07 %
4	F4	86.10 ±0.02 %
5	F5	75.81±0.01 %
6	F6	55.21 ±0.03%
7	F7	69.83±0.04 %
8	F8	66.51±0.06 %
9	F9	72.38±0.03 %

Average particle size

Particle size analysis of cubosomes was determined using Malvern zeta sizer instrument. The results showed that as the GMO & poloxamer content increases, the particle size decreases. The table No.3 shows that the particle size of formulation F1-F9 that is 230.1nm – 693.6 nm from which F4 was lesser than other formulation. It was found that the average particle size of F4 was approximately 230.1nm.

Zeta potential

Zeta potential of the Econazole Nitrate cubosomes was determined by Malvern nano zeta sizer instrument. It was found that zeta potential of all formulation was negative i.e -20 Mv Negative potential indicates that the particles have no charge as a whole system is stable.

In-vitro drug release from cubosomes

In-vitro drug dissolution profiles of cubosomes were studied with Franz diffusion cell. The results obtained for all formulations (F1 –F9) were shown fig.no 3. The cumulative percent drug release after 8 hours were found to be in the range 75.43-85.76 % respectively. Formulation F6 showed least percentage cumulative drug release value 75.43% at 8 hrs and formulation F4 showed highest percentage of drug release value 85.76 % at 8 hrs. As expected all formulations (F1 –F9) were shows controlled drug release for 8 hours.

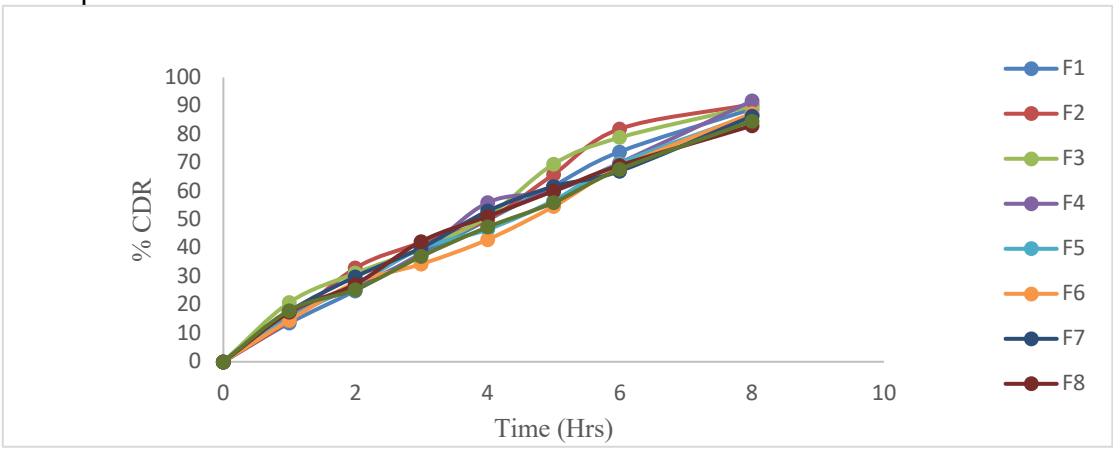


Figure 3: Invitro Diffusion studies F1-F9

Optimization of Prepared Cubosomes Sequential Model Sum of Squares

Table 4: Sequential Model sum of squares

Source	Sum of Squares	DF	Mean Square	F-value	p-value	
Mean vs Total	1.217E+06	1	1.217E+06			
Linear vs Mean	17524.51	2	8762.25	0.2991	0.7520	
2FI vs Linear	912.04	1	912.04	0.0261	0.8780	
Quadratic vs 2FI	**1.672E+05**	**2**	**83580.64**	**32.49**	**0.0093**	**Suggested**
Cubic vs Quadratic	7656.79	2	3828.40	63.28	0.0885	Aliased
Residual	60.50	1	60.50			
Total	1.410E+06	9	1.567E+05			

Model summary statistics

Table 5: Model summary statistics

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	171.17	0.0907	-0.2125	-0.5773	3.049E+05	
2FI	187.02	0.0954	-0.4474	-0.7724	3.426E+05	
Quadratic	50.72	0.9601	0.8935		*	Suggested
Cubic	7.78	0.9997	0.9975		*	Aliased

Evaluation of model selected by analysis of variance (ANOVA)

Quadratic polynomial equations that represents the 2FI and quadratic interactions for each response were generated based on the obtained experimental data and the significance of each regression coefficient was statistically evaluated by analysis of variance (ANOVA).

ANOVA for response surface 2FI Model

Table 6: ANOVA for Response surface 2FI Model

Source	Sum of Squares	DF	Mean Square	F-value	p-value	
Model	1.856E+05	5	37119.57	14.43	0.0261	significant
A-GMO	12274.92	1	12274.92	4.77	0.1169	
B-P 407	5249.58	1	5249.58	2.04	0.2485	
AB	912.04	1	912.04	0.3545	0.5935	
A^2	1.646E+05	1	1.646E+05	64.00	0.0041	
B^2	48185.28	1	48185.28	18.73	0.0227	
Residual	7717.29	3	2572.43			
Cor Total	1.933E+05	8				

Final Equation in Terms of Coded Factor

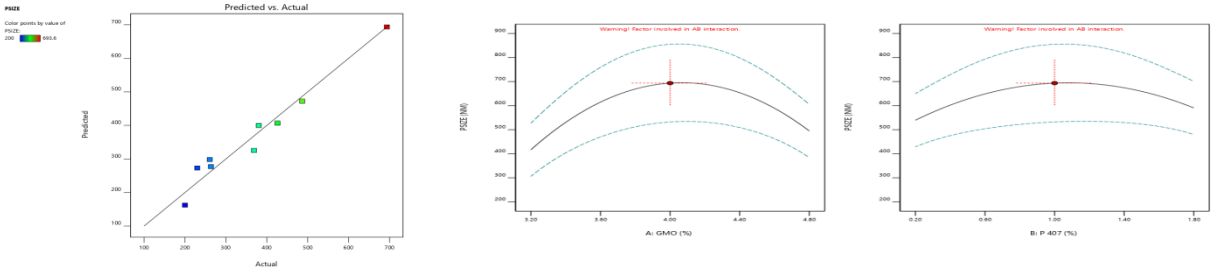
693.60 + 39.17 A + 25.62 B + 15.10 AB – 237.90 A2 – 128.70 B2

Final Equation in Terms of Actual Factors

2999.1197 A + 339.83 B + 23.593 AB – 371.71 A2 – 201.037 B2

Model Graphs

Design Expert software has provided various graphs to help interpret the model selected. They were generated after fitting a model. Special forms of response plots such as Effect of variables on particle size and predicted vs actual graphs were used to interpret the model selected and were given in figure No 4.



I II III

Figure 4: I) Predicted vs Actual II) Effect of variable A and B on particle size

Contour plot

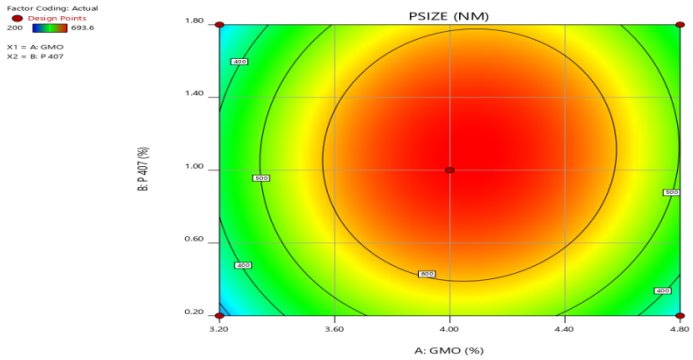


Figure 5: Contour plot of Y1: Particle size (nm) against A: GMO % and P407 %

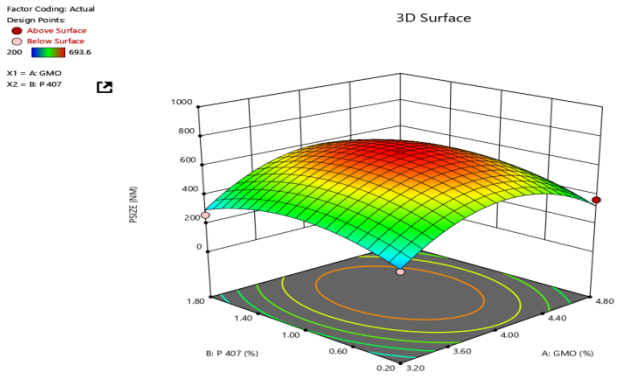


Figure 6: 3D Surface plot of Y1: Particle size (nm) against A: GMO and P407

Preparation of the optimized formulation Cubosomes were prepared by the method previously mentioned in methodology.

Table 7: Comparision of Predicted and actual experimental values

Optimized formulation		OF1	OF2	OF3
Particle size (nm)	Predicted	298.04	296.35	293.26
	Actual	298.76	295.54	293.86
	% Error	0.72	0.81	0.6

Model summary statistics: In the model summary statistics (Table no.4) Focused on the model maximizing the Adjusted R and the Predicted R^2. The software suggested the 2F1 (2-factor interaction) model to be the best fitting model. PRESS is a measure of the fit of the model to data points in the design. The smaller value of PRESS statistic for a 2F1 model indicated that the model fits to the data. points.

ANOVA: The selected 2F1 model was evaluated by analysis of variance Table no.6. shows ANOVA for Response Surface 2F1 Model. The Model F-value of 14.43 implied the model was significant. There was only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicated model terms were significant. In this case A, B. AB were significant model terms.

Model graphs: The model graphs for the response (particle size) is given in the Fig. 4, Figure no (4.(I)) presents predicted vis actual. here higher degree of linearity was observed in all the runs.Fig. 4(II) shows the effects of GMO (A) on particle size (YI). An antagonistic impact on response Y can be observed in the case of A as particle size was found to increase when the GMO was increased. Fig. 4(II) shows the effect of P407 (B) on particle size (YT), A positive impact on response Y can be observed in the case of B as particle size was found to decrease when the P407 was increased.

Contour plot: The contour plot (Fig.no.5) is a two-dimensional (2D) representation of the response (Y1) plotted against GMO% w/v (A) and P407 (B). The plot revealed that low concentration of GMO and P407 could lead to an optimum formulation with smallest particle size.

The 3D Surface plot: It is a projection of the contour plot giving shape in addition to the colour and contour. The plot clearly shows the variable's effects and the interaction effect of variables on particle size. The plot also revealed that optimum formulation with a small particle size can be obtained at a low concentration of GMO and a P40.

CONCLUSION

Glyceryl monooleate and Poloxamer 407 in various ratios were used for formulation of cubosomes by Top-down techniques. In vitro drug release study revealed that cubosomal formulation have prolonged release, good entrapment efficiency, and good stability. It can be concluded that cubosomes are promising vehicle for delivery of Econazole Nitrate.

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