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FORMULATION AND CHARACTERIZATION OF CUBOSOMES OF ISOCONAZOLE FOR TOPICAL DISEASE

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ABSTRACT

This study focuses on the formulation and characterization of Cubosomes loaded with Isoconazole for enhanced topical drug delivery. Cubosomal dispersions were prepared using Glyceryl monooleate (GMO) and Poloxamer 407 via a top-down technique. Isoconazole was incorporated into the dispersion, and formulations were optimized to maximize drug entrapment efficiency and minimize particle size. The optimal formulation was then integrated into a gel matrix using Carbomer, Triethanolamine (TEA), and propylene glycol to achieve a stable Cubosomal gel. Characterization studies included evaluation of particle size, entrapment efficiency, drug release kinetics, and physical properties of the gel formulations. In vitro drug release studies demonstrated sustained release profiles, and regression analysis indicated the Korsmeyer-Peppas model best described the release kinetics. The findings highlight the potential of Cubosomal technology for improving Isoconazole delivery in dermatological applications.

Keywords: Cubosomes, Isoconazole, topical drug delivery, Glyceryl monooleate, Poloxamer 407, Carbomer, sustained release.

INTRODUCTION

Cubosomes are a novel class of drug delivery systems composed of self-assembled cubic liquid crystalline phases, characterized by their unique cubic structure. These nanocarriers are particularly promising for topical applications due to their ability to encapsulate hydrophilic and lipophilic drugs, enhance skin penetration, and provide sustained release (Bansal *et al.,* 2019). Isoconazole, an imidazole derivative, is widely used as an antifungal agent for the treatment of skin infections, including dermatophytosis and candidiasis. However, its poor solubility and skin permeability limit its therapeutic efficacy (Kumar *et al.,* 2020).

The formulation of isoconazole-loaded cubosomes aims to improve drug solubility and enhance its delivery to the targeted site of action. The cubic phase of cubosomes facilitates the formation of a highly ordered structure that can encapsulate the drug effectively while providing controlled release properties. This is especially beneficial for topical treatments, where prolonged drug action is desirable (Wang *et al.,* 2018).

Moreover, the incorporation of surfactants, such as Poloxamer and glyceryl monooleate, has been shown to stabilize the cubosomal structure and improve the drug loading efficiency (Bhardwaj *et al.,* 2021).

This study investigates the formulation and characterization of isoconazole-loaded cubosomes, focusing on their physicochemical properties, drug entrapment efficiency, and in vitro release profiles, aiming to develop an effective topical delivery system for isoconazole.

MATERIALS AND METHODS

Formulation of Cubosomes

Cubosomal dispersions of Isoconazole were prepared by top-down technique. Accurately weighted quantity of Glyceryl monooleate (GMO) and poloxamer 407 polymer mixed and melted in a water bath at 60°C, to this mixture add Isoconazole drug and stir until completely dissolved, then to this solution add drop by drop preheated (up to 70°C) distilled water of suitable quantity by continuous stirring for 2 hours, This whole system is taken into subjected for homogenization at 1500 rpm for 1 minute under at room temperature (Bhosale *et al.,* 2013). Thus, formed liquid dispersion of cubosomes was kept at a room temperature, avoids direct sunlight and which will used for further study.

Table 1: Formulation ingredient for preparation of Cubosomes

F. Code	Drug (mg)	Poloxamer 407 (mg)	Glyceryl monooleate (mg)	Water (ml)
F1	100	300	250	50
F2	100	300	200	50
F3	100	300	150	50
F4	100	250	250	50
F5	100	250	200	50
F6	100	250	150	50

***F= Formulation**

Characterization of prepared cubosomal formulations

Entrapment efficiency

Entrapment efficiency of Isoconazole Cubosomes formulation was determined using centrifugation method. The entrapment efficiency of Isoconazole in Cubosomes vesicle was determined by ultracentrifugation, 10mL of Cubosomes formulation were collect in test tube. The amount of drug not entrapped in the Cubosomes was determined by centrifuging at 3,000 rpm and collect the supernatant, the supernatant layer was separated, diluted with water suitably and drug concentration was determined at 272nm using UV spectrophotometer (Esposito *et al.,* 2005).

$$\%\,Entrapment\,Efficiency = \frac{Therotical\,drug\,content - Practical\,drug\,content}{Therotical\,drug\,content} \times 100$$

Vesicle Size

Microscopic analysis was performed to determine the average size of prepared Cubosomes. Formulation was diluted with distilled water and one drop was taken on a glass slide and covered with cover slip. The prepared slide was examined under trinocular microscopic at 400 X. The diameters of more than 150 vehicles were randomly measured using calibrated ocular and stage micrometer. The average diameter was calculated using the following formula (Nithya *et al.,* 2018):

$$AverageDiameter = \frac{\Sigma n.d}{\Sigma\, n}$$

Where n = number of vesicles; d = diameter of the vesicles

Preparation of Cubosomal gel

The cubosomal gel was obtained by addition of weighted amount of carbomer (1 to 3% w/v) in distilled water and kept for half day

for getting to swell of carbomer and then add triethanolamine drop by drop up to pH 7. Propylene glycol is added to adjust the consistency. The obtained gel was then diluted with an appropriate amount of cubosomes dispersion (equivalent to 1% Isoconazole) in the gel then stir for 5 min, the cubosomal gel will be formed (Yasasvini *et al.,* 2017).

Table 2: Formulation ingredient for preparation of Cubosomal gel

F. Code	Carbomer (%)	TEA (ml)	Water (ml)
CG-1	1	0.2	100
CG-2	2	0.2	100
CG-3	3	0.2	100

*** CG = Cubosomal gel**

Evaluation of Cubosomes containing gel

Measurement of viscosity

Viscosity measurements of prepared topical Cubosomes based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm (Badie and Abbas, 2018).

pH measurements

pH of selected optimized formulations was determined with the help of digital pH meter. Before each measurement of pH, pH meter should be calibrated with the help of buffer solution of pH 4, pH 7 and pH 9.2. After calibration, the electrode was dipped into the vesicles as long as covered by the vesicles. Then pH of selected formulation was measured and readings shown on display were noted (Eldeeb *et al.,* 2019).

Drug content

Accurately weighed equivalent to 100 mg of topical Cubosomes gel was taken in beaker and added 20 ml of methanol. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 1.0 mL of filtered solution was taken in 10 mL capacity of volumetric flask and volume was made upto 10 mL with methanol. This solution was analyzed using UV-Spectroscope at λ_{max}272 nm (Bei *et al.,* 2009).

Extrudability study

Extrudability was based upon the quantity of the gel extruded from collapsible tube on application of certain load. More the quantity of gel extruded shows better extrudability. It was determine by applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube.

Spreadibility

Spreadibility of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response. It was determined by method reported by Multimer *et al.* (2006). An apparatus in which a slide fixed on wooded block and upper slide has movable and one end of movable slide tied with weight pan. To determine spreadibility, placing 2-5 g of gel between two slide and gradually weight was increased by adding it on the weight pan and time required by the top plate to cover a distance of 10 cm upon adding 80g of weight was noted. Good spreadibility show lesser time to spread.

$$Spreadability = \frac{Weight\ tide\ to\ Upper\ Slide\ x\ Length\ moved\ on\ the\ glass\ slide}{Time\ taken\ to\ slide}$$

***In-vitro* drug diffusion study**

The *in-vitro* diffusion study is carried by using franz diffusion cell. Egg membrane is taken as semi permeable membrane for diffusion (Nasr *et al.,* 2015). The franz diffusion cell has receptor compartment with an effective volume approximately 60 mL and

effective surface area of permeation 3.14sq.cms. The egg membrane is mounted between the donor and the receptor compartment. A two cm^2 size patch taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is surrounded by water jacket so as to maintain the temperature at 32±0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell. During each sampling interval, samples are withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling. The samples withdrawn and analyzed spectrophotometrically at 272nm.

Stability Studies

Stability study was carried out for drug loaded invasomal gel at two different temperatures i.e. refrigeration temperature (4.0±0.2°C) and at room temperature (25-28±2°C) for 3 weeks. The formulation subjected for stability study was stored in borosilicate container to avoid any interaction between the formulation and glass of container. The formulations were analyzed for any viscosity and % assay.

RESULTS AND DISCUSSION

The formulation and characterization of isoconazole-loaded cubosomes demonstrated promising results in terms of drug entrapment efficiency and vesicle size. Table 3 shows that the highest entrapment efficiency was observed in formulation F5, with a value of 75.65%, alongside an average vesicle size of 210.32nm. These characteristics suggest that F5 could effectively encapsulate isoconazole, potentially enhancing its bioavailability for topical application.

The average vesicle size is critical, as smaller vesicles tend to improve skin penetration. The optimized formulation F5, with its relatively small vesicle size, is expected to facilitate deeper penetration into the skin layers, which is essential for effective antifungal action. The zeta potential of -37.45 mV for F5 indicates good stability of the cubosomal formulation, as higher negative or positive values typically correlate with increased stability (Table 4).

Table 5 outlines the characterization of the cubosomal gel formulations. The viscosity of CG-2 was found to be 3569 cps with a drug content of 99.45%. These parameters are favorable for a gel intended for topical use, providing a good balance between spreadability and adherence to the skin. The extrudability of CG-2, at 165g, further supports its applicability for easy application.

In vitro drug release studies, as shown in Table 7, revealed that CG-2 exhibited a cumulative drug release of 98.85% over 12 hours, indicating a sustained release profile suitable for prolonged antifungal action. This sustained release is further supported by the regression analysis data in Table 8, where the formulation showed high correlation coefficients for both Higuchi and Korsmeyer-Peppas models, indicating a diffusion-controlled release mechanism.

Stability studies (Table 9) confirmed that the optimized formulation CG-2 maintained its characteristics over three months, with minimal changes in viscosity and drug content. The physical appearance remained largely normal, with some turbidity observed at specific time points, which suggests that the formulation's stability is robust under various conditions.

Table 3: Entrapment efficiency and average vesicle size of Isoconazole loaded Cubosomes

Formulation Code	% Entrapment efficiency	Average vesicle size (nm)
F1	68.89±0.32	295.65±0.25
F2	65.85±0.15	267.78±0.14
F3	70.36±0.26	248.96±0.36
F4	71.12±0.22	238.87±0.32
F5	75.65±0.18	210.32±0.44
F6	69.98±0.33	226.65±0.25

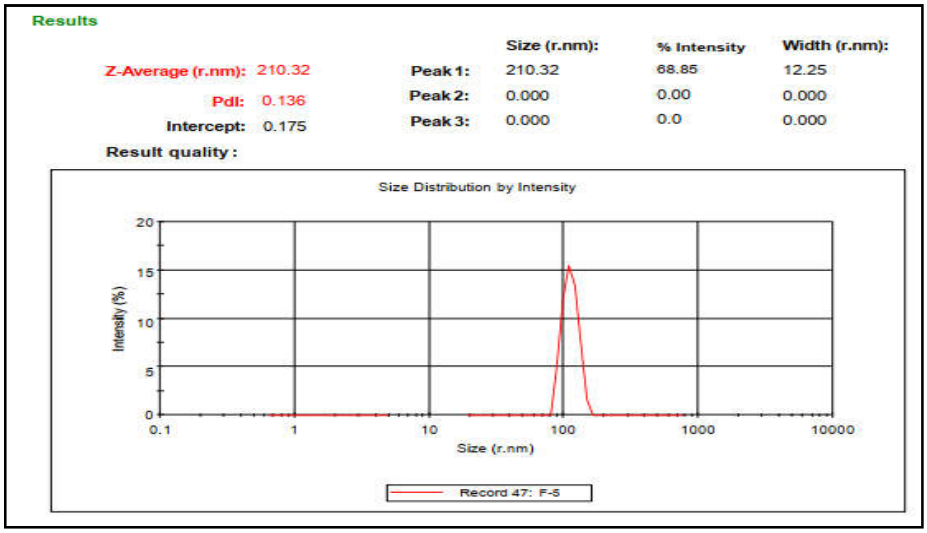

Figure 1: Graph of average vesicle size (nm) of optimized formulation F5

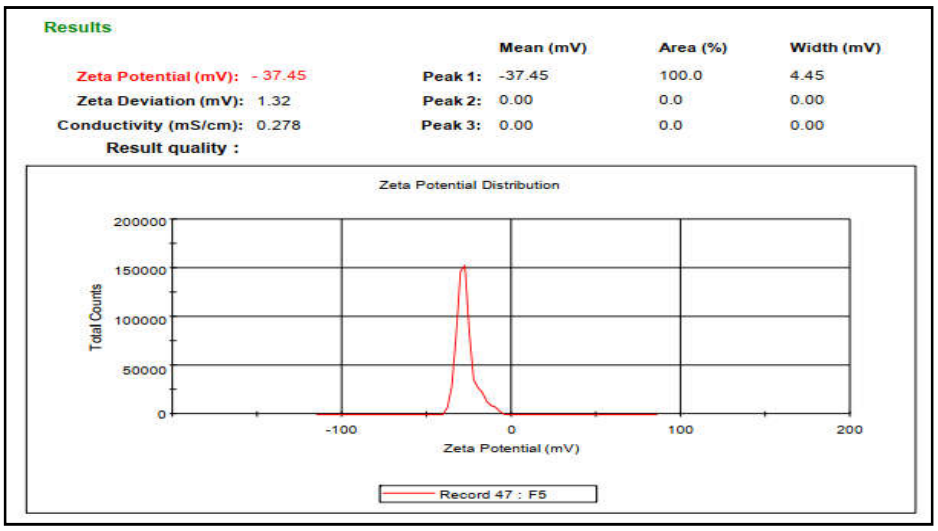

Figure 2: Graph of zeta Potential (mV) optimized formulation F5

Table 4: Characterization of optimized formulation of Cubosomes

Formulation	Average vesicle size (nm)	% Entrapment efficiency	Zeta Potential (mV)
F5	75.65±0.18	210.32±0.44	-37.45

Table 5: Characterization of Cubosomes gel

Gel formulation	Viscosity (cps)	pH	Drug Content (%)	Extrudability (g)	Spreadibility (g.cm/sec)
CG-1	3645±15	6.78±0.02	96.65±0.12	185±5	12.32±0.32
CG-2	3569±12	6.82±0.05	99.45±0.25	165±7	11.45±0.25
CG-3	3475±19	6.53±0.03	95.74±0.32	149±4	10.32±0.14

Table 6: *In vitro* drug release study of gel formulation

S. No.	Time (hr)	% Cumulative Drug Release*		
		CG-1	CG-2	CG-3
1	0.5	26.65	23.32	20.23
2	1	38.85	34.45	33.12
3	2	52.23	46.65	41.12
4	4	69.98	53.32	51.36
5	6	92.23	64.45	61.32
6	8	98.85	74.54	69.98
7	10	99.12	86.65	76.65
8	12	99.45	98.85	85.45

Table 7: *In vitro* drug release study of optimized gel formulation CG-2

S. No.	Time (hr)	% Cumulative Drug Release*
1	0.5	23.32
2	1	34.45
3	2	46.65
4	4	53.32
5	6	64.45
6	8	74.54
7	10	86.65
8	12	98.85

Table 8: Regression analysis data of optimized gel formulation CG-2

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R^2	R^2	R^2	R^2
CG-2	0.9766	0.7718	0.9803	0.9825

Table 9: Stability of optimized formulation of Cubosomes gel CG2

Characteristic	Time (Month)					
	1 Month		2 Month		3 Month	
Temp.	4.0±0. 2°C	25-28±2°C	4.0 ±0. 2°C	25-28±2°C	4.0 ±0. 2°C	25-28±2°C
Viscosity (cps)	3569	3540	3550	3425	3545	3345
Drug Content (%)	99.45	99.10	99.25	98.25	99.10	97.58
Physical Appearance	Normal	Turbid	Normal	High turbid	Normal	High turbid

CONCLUSION

The formulation of isoconazole-loaded cubosomes demonstrates a promising strategy for enhancing topical delivery, offering effective encapsulation, favorable release profiles, and stability. Future studies could focus on clinical evaluations to validate the efficacy and safety of this formulation for treating skin infections.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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