Research Article

Formulation and Evaluation of Itraconazole Oral Cubosomal Capsules

Vineetha Taadi*, Bala Tripura Sundari.I, Dr.Bhagawan Raju.M[2]
Department of pharmaceutics, Sri Venkateshwara College of Pharmacy, 86-Hitech city road Madhapur, Hyderabad, TN, India.
***Corresponding author's E-mail:** navineetha68@gmail.com

Received: 10-10-2019; **Revised:** 22-11-2019; **Accepted:** 30-11-2019.

ABSTRACT

Itraconazole is an anti-fungal drug with poor solubility and bioavailability. In order to improve its solubility cubosomal capsules loaded with Itraconazole were prepared. Cubosomes of Itraconazole were prepared by Top-down approach employing GMO (Glyceryl Monooleate) as lipid phase vehicle, poloxamer 407 as stabilizer and distilled water as aqueous phase. Formulations were characterized by optical microscopy, encapsulation efficiency, in-vitro drug release, particle size, zeta potential and SEM. Optimized formulation of cubosomal dispersion showed good entrapment efficiency of91.04% with maximum drug release of 86.21%, with efficient particle size of 479.2nm and zetapotential of -56.0mV. The optimized Itraconazole cubosomal dispersion was then made into granules by the addition of adsorbents such as starch and aerosil. The granules so prepared were then incorporated into hard gelatin capsules which are shown to have good flow properties and Suitability to be filled in capsules. Results suggest that the GMO cubosomes, as lipid nanocarrier could significantly enhance solubility of Itraconazole. In the present study the formulated oral cubosomal capsules of Itraconazole were found to have better solubility and good release rates when compared with marketed capsules of Itraconazole (SPORANOX). Thus, the study suggests that cubosomes can be used as nanocarrier in increasing the solubility and bioavailability of poorly soluble anti-fungal drugs.

Keywords: Cubosomes, Glyceryl monooleate, poloxamer, capsules, Itraconazole.

INTRODUCTION

Itraconazole is an azole derivative used as an antifungal drug used to treat number of fungal infections and belongs to BCS class II. Itraconazole is a highly selective inhibitor of fungal cytochrome P-450 sterol C-14 α-demethylation via the inhibition of the enzyme cytochrome P450 14α-demethylase.Cubosomes are a form of lyotropic liquid crystals. Lyotropic liquid crystal systems are formedby the addition of amphiphilic lipid with polar solvents like water or glycerine. Because oftheir properties, cubosomes are versatile systems, administrable by different ways such as orally, percutaneously and parenterally. Cubosomes are especially used to incorporate poor water soluble drugs. Itraconazole being a BCS class II drug it has poor water solubility. Independent of route of administration the effectiveness of drugs is challenged by its solubility. It also poses a major challenge for pharmaceutical companies developing new pharmaceutical products, since nearly half the active substances being identified through the new paradigm in high-throughput screening are either insoluble or poorly soluble in water.[1, 2]

Oral drug delivery system is the most preferred route for a drug to enter into blood stream especially when repeated and routine administration is necessary. However, for effective delivery through the oral route, a therapeutic agent must first dissolve in the gastrointestinal lumen[4]. The bioavailability of a BCS class II drug is rate-limited by its dissolution so that even a small increase in dissolution rate sometimes results in a large increase in bioavailability. Therefore, an enhancement of the dissolution rate of the drug is thought to be a key factor for improving the bioavailability of BCS Class II drugs. In this class of drugs, dissolution profile must be clearly defined and reproducible, since drug dissolution is rate controlling step in *In-vivo* drug absorption.[5,6]

Cubic phases are often founded sandwiched between lamellar and hexagonal liquid crystalline phases, especially in non ionic surfactant systems. The monoolein-water system uniquely posses a cubic phase region with broad compositional and temperature range. But surfactant packing concepts are more approaching. Normally monoolein has continuous hydrophilic head, hydrophobic tail end, producing reversed or inversed cubic phases, indicating the phases towards polar medium.[7-9]

MATERIALS AND METHODS

Itraconazole was a gift sample from Mylan laboratories pvt.ltd, Hyderabad, India. Glycerol Monooleate was a Sample from Mohini Organics P.Ltd. Mumbai. Poloxamer 407 (P-407) was obtained from Basf Chemicals Limited, Mumbai.

Preparation of Cubosomes

Top-down approach

The method employed in preparation of the Itraconazole cubosomes was Top-Down Approach. Different concentrations of Glyceryl Monooleate (GMO) and Poloxamer 407 as shown in the table below were accurately weighed and heated on the electric water bath

at a temperature of 40-50˚C until Poloxamer 407 completely dissolves in GMO. The Itraconazole drug was added to the above solution and mixed well. The obtained clear lipid solution was slowly added to distilled water and subjected to bath sonication for 30 minutes the resultant solution was white opaque dispersion without the presence of any aggregates. The prepared dispersions were stored in the closed glass vials at room temperature for 48 Hours, protected from light and later evaluations were carried out[10, 11]. Cubosomes using various concentrations of GMO were prepared as mentioned in the table no.1.

Table 1: Formulations of Cubosomes Using Varying Concentrations of GMO

Formulation code	Glyceryl monooleate (GMO) (%W/V)	Poloxamer-407 (% W/W)	Itraconazole (mg)	Distilled water (%W/V upto 100%)
F1	1	1	60	100
F2	3	1	60	100
F3	3.5	1	60	100
F4	6	1	60	100
F5	6.5	1	60	100
F6	9.5	1	60	100
F7	12	1	60	100
F8	12.5	1	60	100
F9	15	1	60	100
F10	17.5	1	60	100
F11	20	1	60	100
F12	22.5	1	60	100

Evaluation of Cubosomes

Surface Morphology

The size and shape of the cubosomes was determined using the Optical Microscopy (Olympus CH20i) and the Scanning Electron Microscopy (SEM-Hitachi S 3700N). SEM provides access to the surface morphology of the cubosomes. The samples were examined at suitable accelerating voltage of 15-20 kV, at different magnification.

Particle size Analysis

The particle size and Zeta potential of cubosomes was determined by dynamic light scattering technique using the Horiba Particle Size Analyzer. Samples were diluted in particle-free purified water and measured at 25˚C.

Zeta Potential

The Zeta potential of the cubosomes was determined using the Zetasizer (Malvern Instruments). Zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion system. Zeta potential is the key indicator of the stability of the dispersions. The magnitude of the zeta potential indicates the degree of electrostatic repulsion between adjacent, similarly charged particles in dispersion.

Entrapment Efficiency (E.E)

Entrapment efficiency of the cubosomes is defined as the percentage amount of drug which is entrapped by the cubosomes. The Cubosomal dispersions were subjected to the centrifugation (Remi R24 Research Centrifuge) at 15000 RPM for 90 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 262 nm. The percentage of entrapment efficiency was determined from the equation:

$$E.E\% = \frac{(\text{Total Drug}) - (\text{Free Drug})}{(\text{Total Drug})} * 100$$

***In-vitro* Drug Release Studies**

In-vitro drug release studies were performed using the Franz Diffusion cell i.e. Bi-chambered donor receiver compartment model and this was placed on a magnetic stirrer and temperature was adjusted to 37±0.5˚C. One end of the compartment was covered with the Gelatin sheet, which was previously soaked in warm water. 0.1 N hydrochloric acid (0.1 N HCl) with 0.25% sodium lauryl sulphate (SLS) of pH 1.2 was placed in the receptor compartment. Cubosomal formulation was placed on the membrane, which was in contact with receptor medium. Samples were withdrawn from the receptor compartment at specified time intervals of 30, 60, 90, 120, 150, 180 minutes. The receptor medium was replaced with the equal amounts of fresh pH 1.2 solutions after each withdrawal. The samples were analyzed for drug content using a UV Spectrophotometer at 262 nm respectively.

Preparation of itraconazole loaded cubosomal oral capsules

Cubosomal formulation F9 (15% GMO and 1% Poloxamer 407) was optimized for preparation of capsules. To the optimized Cubosomal formulation, Starch (CF1-CF6) and Aerosil (CF7-CF10) was added separately to obtain a wet mass. Then the wet mass was passed through the sieve no. 16 and granules were obtained. The obtained Cubosomal granules were air dried at room temperature and were filled into the "0" sized capsules. The formulation of Itraconazole Cubosomal oral capsules were depicted in the table no.2

Table 2: Formulation of Cubosomal Granules Using Starch and Aerosil

Formulation code	Cubosomal dispersion (ml)	Starch powder (gms)	Formulation code	Cubosomal dispersion (ml)	Aerosil (gms)
CF1	10	1.4	CF6	10	0.2
CF2	10	1.6	CF7	10	0.4
CF3	10	1.8	CF8	10	0.6
CF4	10	2	CF9	10	0.8
CF5	10	2.2	CF10	10	1

Evaluation of Itraconazole Cubosomal Granules

Surface Morphology

The size and shape of the Cubosomal granules was determined using the Optical Microscopy (Olympus CH20i) and the Scanning Electron Microscopy (SEM-Hitachi S 3700N). SEM provides access to the surface morphology of the cubosomes. The samples were examined at suitable accelerating voltage of 15-20 kV, at different magnification.

Particle size Analysis

The particle size and Zeta potential of Cubosomal granules was determined by dynamic light scattering technique using the Horiba Particle Size Analyzer. Samples were diluted in particle-free purified water and measured at 25˚C.

Zeta Potential

The Zeta potential of the Cubosomal granules was determined using the Zetasizer (Malvern Instruments). Zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion system. Zeta potential is the key indicator of the stability of the dispersions. The magnitude of the zeta potential indicates the degree of electrostatic repulsion between adjacent, similarly charged particles in dispersion.

Flow Properties

The Flow properties of the Cubosomal granules were studied by measuring the quality parameters such as Tapped Density, Bulk Density, Hausner's Ratio, Angle of Repose, Compressibility Index or Carr's Index.

(a) Angle of repose

It is the parameter related to inter-particulate friction or resistance to movement between the particles. This is the maximum angle possible between surface of pile of powder or granules and the horizontal plane.

The equation for Angle of Repose (θ) is:

$\theta = \text{Tan}^{-1}\, h/r$ Where, h= height and r = radius

(b) Bulk Density

Bulk density of granules was determined by pouring gently 1.5 g of granules through a glass funnel into 25 ml of measuring cylinder. The volumes occupied by the samples were recorded.

Bulk Density = Weight of the sample in grams/ Volume occupied by sample

(c) Tapped Density

The granules were taken in a measuring cylinder and were tapped 100 times or until there is no change in the volume occupied. It is given by:

Tapped Density = Weight of the sample in grams/ Volume occupied by sample

(d) Compressibility Index and Hausner's Ratio

Compressibility Index = (Tapped Density – Bulk Density) / Tapped density * 100

Hausner's Ratio = Tapped Density/ Bulk Density

Drug Content

Granules equivalent of 5mg of the drug were accurately weighed and transferred to 100 ml volumetric flask. The solution was made up to the volume with pH 6.8 Phosphate Buffer. The resultant solution was filtered and suitably diluted and analyzed using UV Visible spectrophotometer at 262 nm using pH 1.2 (0.1N HCl with 0.25% SLS) as a blank.

In-vitro Dissolution Studies

In-vitro dissolution test was carried out using USP Type I Dissolution Apparatus. 0.1N HCl with 0.25% SLS (pH-1.2) was used as dissolution media. 900ml volume of dissolution medium was used along with a basket speed of 50 rpm was selected. The temperature of the medium was maintained at 37±0.5˚C. Aliquots of 5ml were collected at 30, 60, 90, 120, 150, 180 minutes time

intervals and the same amount of fresh dissolution medium was replaced into the dissolution vessels. The collected samples were filtered and suitably diluted to analyze using UV Visible spectrophotometer at 262 nm.

Release Kinetics

The optimized Cubosomal oral capsule (CF4) was studied for release kinetics. Coefficient of correlation values were calculated for the linear curves obtained by regression analysis of the plots.

Zero order release rate kinetics

To study the zero order release kinetics the release rate data are fitted to the following equation.

$$F=K_0.t$$

Where F is the drug release, 'K' is the release rate constant and t' is the release time. The plot of % drug release versus time is linear.

First order release rate kinetics

The release rate date are fitted to the following equation

$$Log\ (100-F) = kt$$

A plot of log % drug release versus time is linear.

Higuchi release model:

To study the Higuchi release kinetics, the release rate data were fitted to the following equation,

$$F=kt^{1/2}$$

where 'k' is the Higuchi constant In higuchi model, a plot of model, a plot of % drug release versus square root of time is linear.

Korsemeyer's and peppas release model:

The release rate data were fitted to the following equation

$$M_t/M_\infty = k.t^n$$

Where, M/M is the fraction of drug released,

K is the release constant

T is the time taken to release

n is diffusion exponent, if n is equal to 0.89, the release is zero order. If n is equal to 4.45 the release is best explained by Fickian diffusion, and if 0.45 < n <0.89 then the release is through anomalous diffusion or nonfickian diffusion (Swellable & Cylindrical Matrix)

Stability Studies

Accelerated stability studies were conducted as per the ICH guidelines at 25˚C±2˚C at 60% ± 5% Relative Humidity at sampling intervals of 30, 60 and 90 days respectively. The drug content was determined periodically.

RESULTS AND DISCUSSION

Surface Morphology

From figure.no.1 it was observed that the cubosomes have smooth surfaces and were cubic shaped. This states that the cubosomes are capable of incorporating the large amounts of the Itraconazole which in turn helps in increasing the drug release.

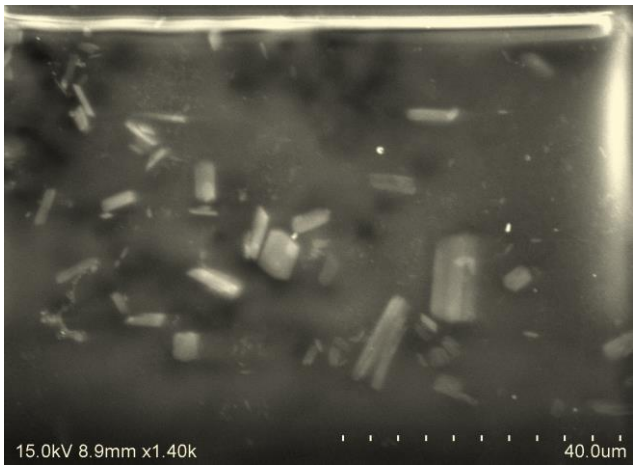

Figure 1: Scanning Electron Microscopic Images of Itraconazole Cubosomes.

Particle size Analysis and Zeta Potential of cubosomes

From figure.2 it was found that the average particle size of the Itraconazole Cubosomes was 479.2nm which states that the obtained cubosomes were with the good structural integrity to deliver the Itraconazole for longer time.

The Zeta Potential of the Itraconazole Cubosomes was found to be -56.0mV as shown in figure.2, which in turn states that the formulated Itraconazole Cubosomes were stable in the dispersion and thereby withstand the conditions of varying pH while delivering the drug within the body.

Entrapment Efficiency (E.E) of cubosomes

It was found that among all the Itraconazole Cubosomal formulations with varied concentrations of GMO, the Cubosomal formulation (F9) with 15% GMO and 1% P407 has the highest entrapment efficiency of 91.04% when compared with the remaining formulations as shown in table no.3.

***In-vitro* Drug release Studies of cubosomes**

The drug release studies were performed on the formulations with entrapment efficiency above 85% i.e. F6, F9 and F12 were selected (see table no.4)and the formulation F9 shows higher drug release of 86.21% when compared with rest of the formulations (see figure.3). Hence the formulation F9 was selected for further studies and to formulate into the Itraconazole Cubosomal Oral Capsules.

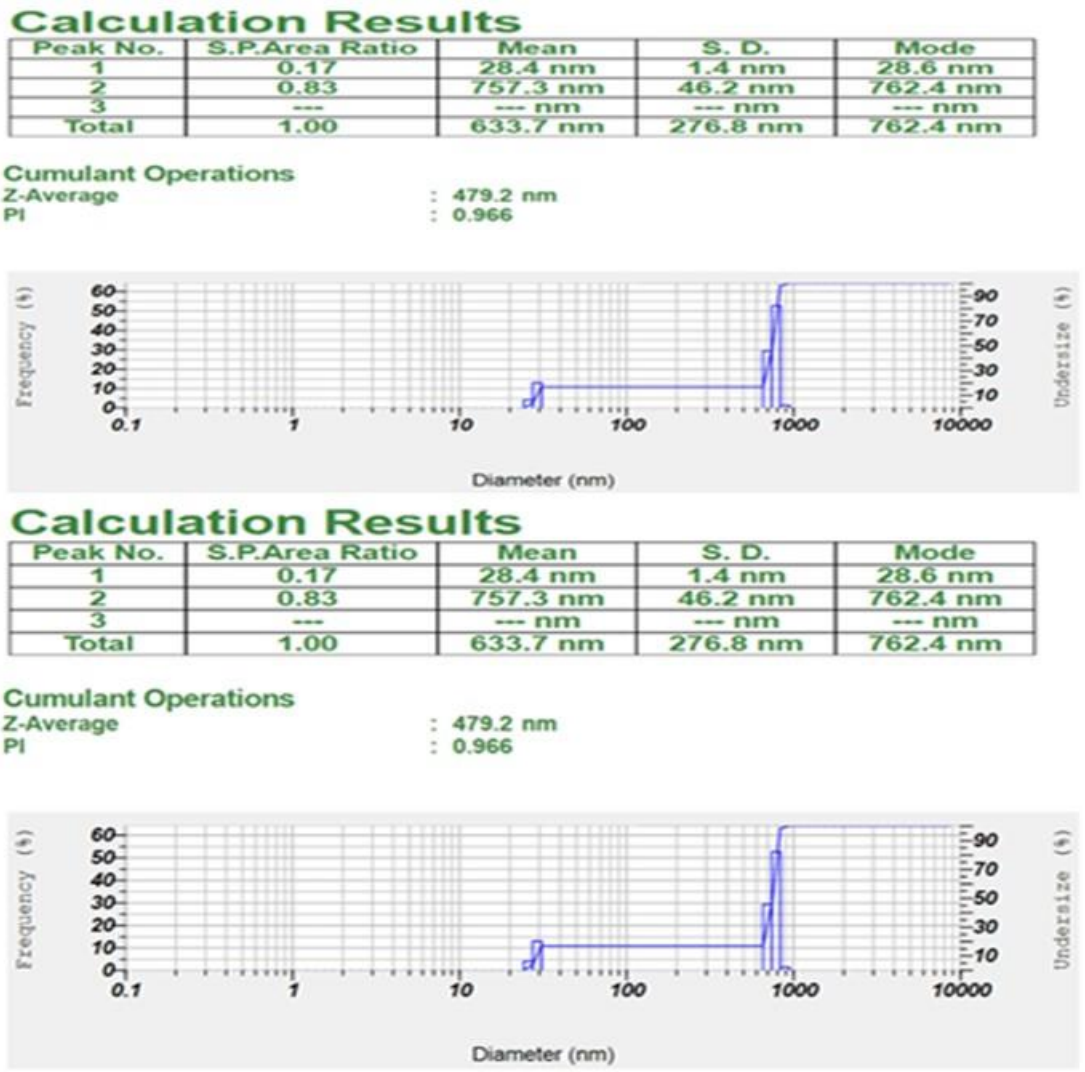

Figure 2: Particle size and Zeta Potential of Itraconazole Cubosomes

Table 3: Drug Entrapment Efficiency of Itraconazole Cubosomes (F1-F12)

Formulation Code	%EE ± SD	Formulation Code	%EE ± SD	Formulation Code	%EE ± SD
F1	78.39±1.02	F5	82.36±1.22	F9	91.04±0.33
F2	79.01±1.11	F6	88.17±0.89	F10	80.59±0.30
F3	69.59±0.95	F7	84.72±1.06	F11	76.73±0.99
F4	80.42±1.68	F8	79.98±0.79	F12	85.12±0.44

Table 4: *In-vitro* Drug release profile of Itraconazole Cubosomes

Time (mins)	%DR of F6	%DR of F9	%DR of F12
0	0	0	0
30	6.88±1.12	20.16±1.06	14.42±1.89
60	18.26±1.01	39.84±1.16	23.09±0.38
90	26.22±1.00	52.37±1.28	32.16±1.78
120	33.41±1.02	67.81±0.98	46.82±1.48
150	48.76±1.09	76.34±1.98	56.91±0.14
180	61.23±1.00	86.21±1.32	66.88±0.78

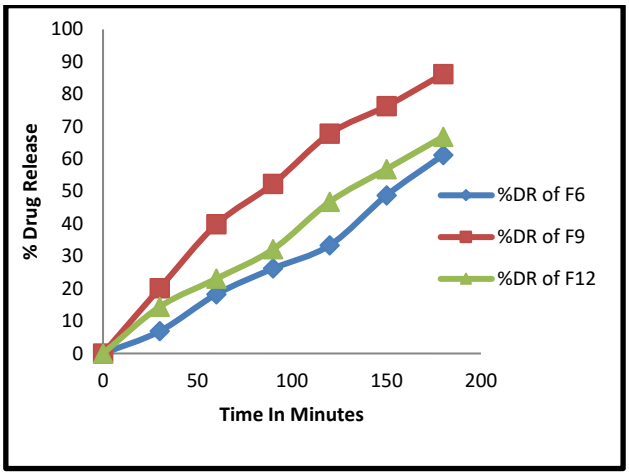

Figure 3: *In-vitro* Drug release profile of Itraconazole cubosomes in 0.1N HCl

Evaluation of Itraconazole Cubosomal Granules

Surface Morphology

From figure.4 it was found that the surface morphology of the Itraconazole Cubosomal Granules was ranging from 4-15 micrometers. This helps in determining the flow properties of the granules such as Angle of Repose, Bulk Density, Tapped Density, Compressibility Index and Hausner's Ratio.

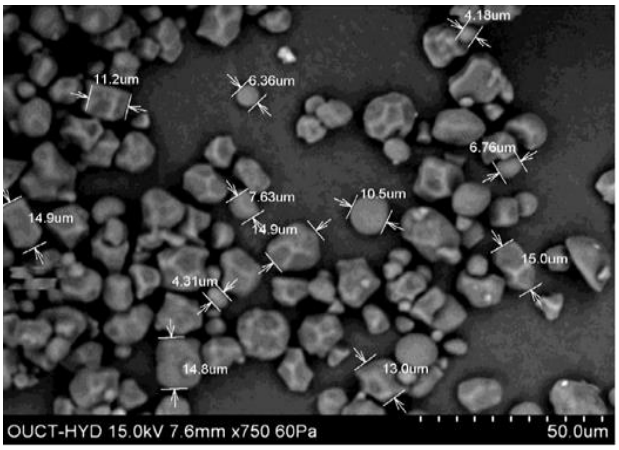

Figure 4: Scanning Electron Microscopic images of Itraconazole Cubosomal Granules

Particle size Analysis

From figure.5 it was found that the particle size of the Itraconazole Cubosomal Granules was 2034.9 nm.

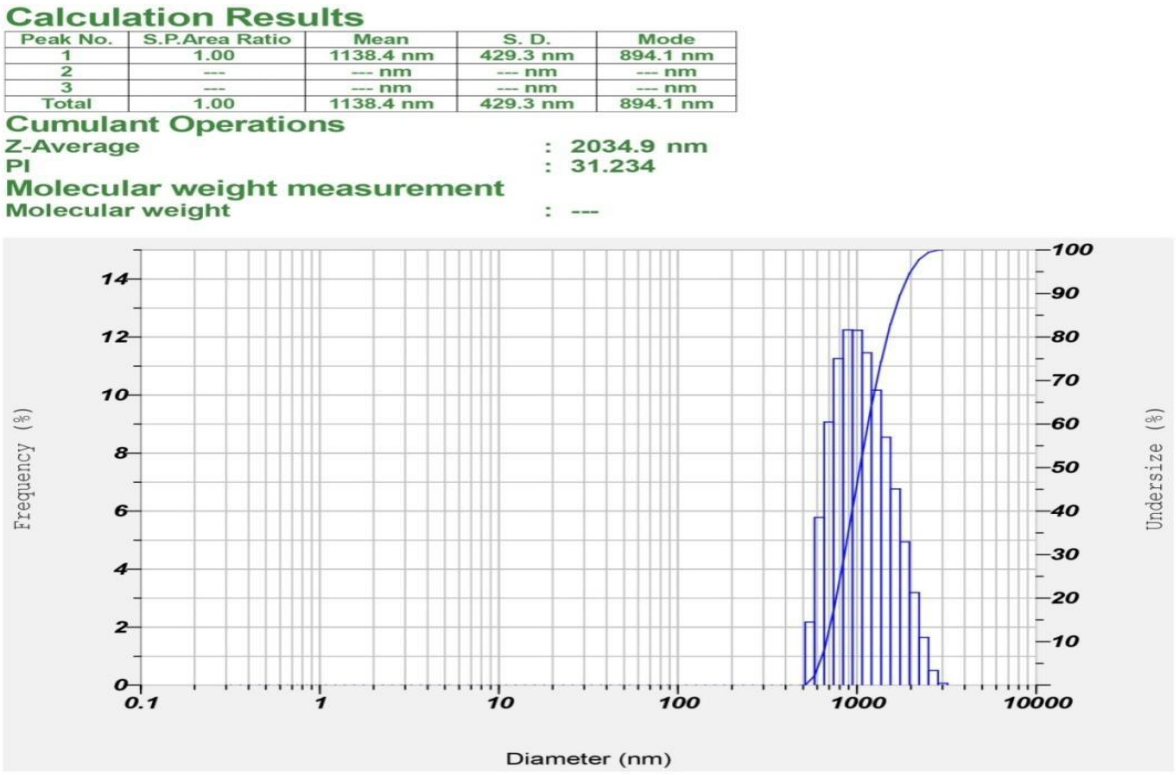

Figure 5: Particle Size of Itraconazole Cubosomal Granules

Flow Properties and Drug content

The flow properties of cubosomal granules were depicted below in the table no.5. The optimized Itraconazole Cubosomal granule formulation (CF4) is shown to have bulk density was 0.39gm/ml, tapped density was 0.49 gm/ml, Carr's Index was 12.24, Hausner's ratio was 1.18 and the values showed low intra particulate friction between the granules. The angle of repose was found to be 26.79 indicating the good flow properties of the granules. The granules were found to be free flowing with no intra and inter particulate friction and can be efficiently suitable to be filled into the capsules.

Drug content

From the below table no.5 it was found that the drug content of the Itraconazole Cubosomal granules was decreasing with the increasing concentrations of the

Starch and Aerosil as well. The drug content of the optimized Itraconazole Cubosomal Oral Capsules formulation CF4 was found to be 88.94%.

Table 5: Flow properties of the Itraconazole Cubosomal Granules.

Formulation Code	Angle of Repose	Bulk Density	Tapped Density	Hausner's Ratio	Carr's Compressibility Index	%Drug Content
CF1	25.48	0.34	0.25	1.26	12.76	76.59
CF2	30.27	0.29	0.27	1.20	16.58	82.27
CF3	36.52	0.31	0.38	1.11	18.79	85.43
CF4	26.79	0.39	0.49	1.18	12.24	88.94
CF5	34.76	0.42	0.40	1.25	18.30	79.12
CF6	34.89	0.37	0.57	1.09	10.43	83.76
CF7	31.47	0.3	0.19	1.12	18.72	77.45
CF8	33.29	0.44	0.46	1.27	12.46	77.49
CF9	27.38	0.43	0.45	1.25	17.29	79.14
CF10	37.73	0.35	0.52	1.32	19.32	84.71

In-vitro Dissolution Studies

The *in-vitro* drug release studies were performed on the formulations with drug content more than 80% i.e. CF2, CF3, CF4, CF6 and CF10 (table no.6). The optimized formulation CF4 was showing a release of 73.54% at the end of 3 Hours when compared with remaining formulations (figure no.6).

Table 6: *In-vitro* Dissolution profile of the Itraconazole Cubosomal Oral Capsules

Time (Mins)	%DR of CF2	%DR of CF3	%DR of CF4	%DR of CF6	%DR of CF10
0	0	0	0	0	0
30	4.99±0.18	5.48±1.49	14.39±1.48	4.58±1.43	7.37±1.19
60	16.48±0.49	12.34±1.34	28.78±0.48	10.06±1.08	12.96±0.98
90	25.35±0.34	19.12±1.79	40.66±0.89	17.88±1.19	19.88±0.76
120	34.26±1.92	24.96±1.48	52.63±0.46	21.46±1.96	27.11±1.36
150	42.9±1.18	31.45±0.47	64.88±1.37	28.84±1.51	33.46±1.79
180	46.79±0.44	42.76±1.23	73.54±1.38	34.48±1.03	41.59±1.53

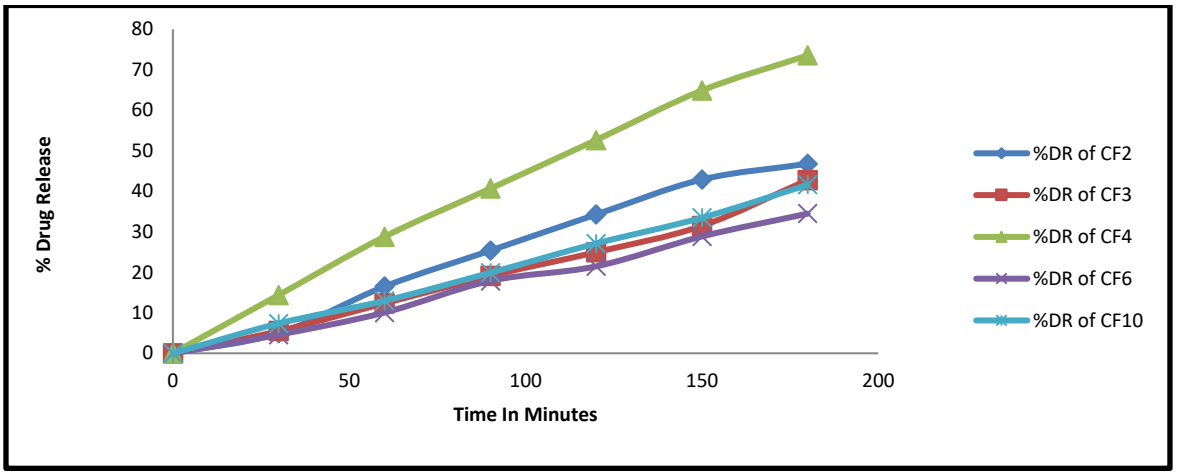

Figure 6: *In-vitro* Dissolution profile of Itraconazole Cubosomal Oral Capsules

Release Kinetics

The mechanism of Itraconazole Cubosomal release from capsules was studied by fitting the data obtained from *In-vitro* release studies into zero-order, first-order, Higuchi's, Korsmayer-peppas kinetic models. From the obtained values of correlation coefficient it was found that optimized formulation CF4 showed Zero order drug release as shown in figure.7.

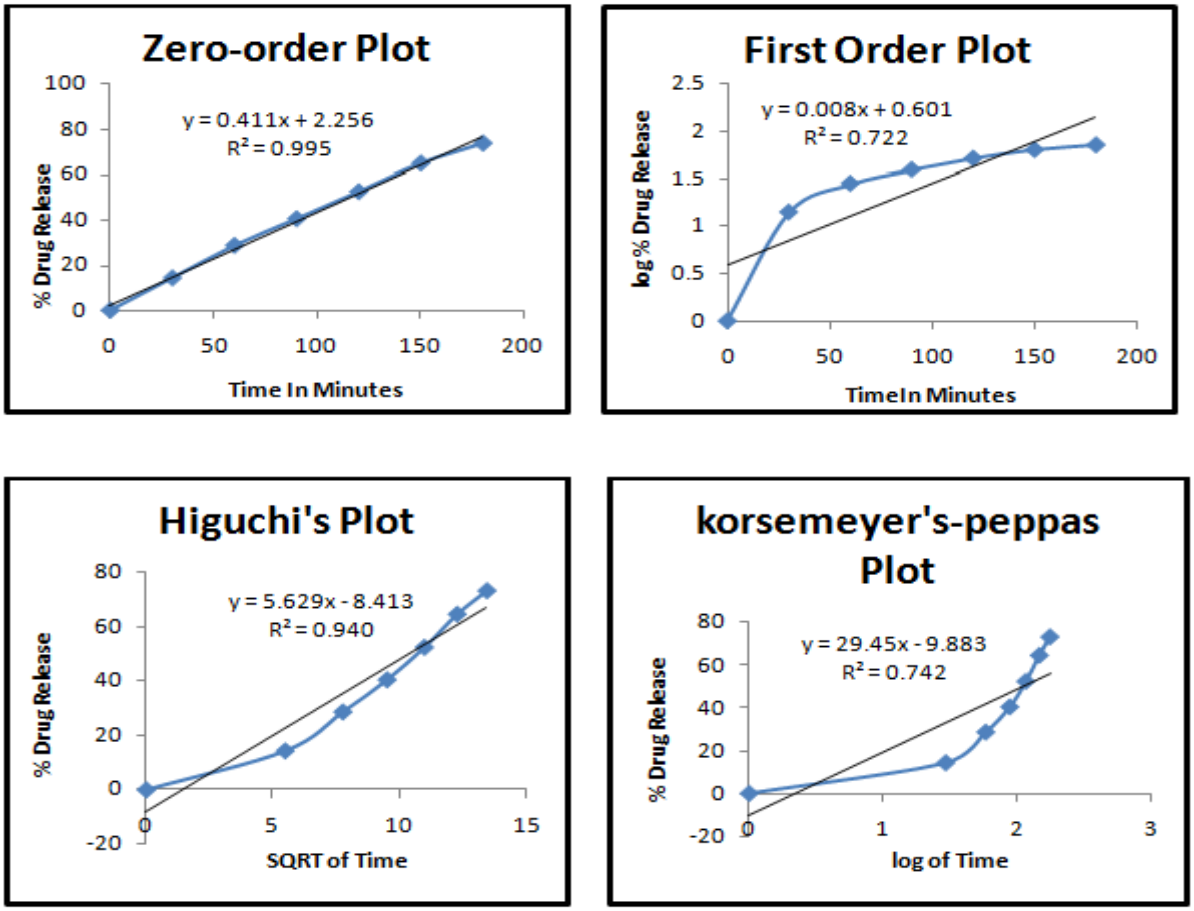

Figure 7: Zero, First, Higuchi's and Korsemeyer'speppas plots for *In-vitro* Dissolution Studies of Itraconazole Cubosomal oral capsules.

Stability Studies

% Drug content determination of the optimized formulations was depicted in table no.7 and found that there was no significant change. At the first month of storage the drug content was found to be 87.48% and at the end of the third month it was found to be 86.21% at 25˚C±2˚C and 60% RH. Thus we may conclude that the drug does not undergo degradation on storage.

Table 7: Stability study data for optimized Itraconazole Cubosomal Oral Capsule (CF4)

Stability Conditions	% Drug Content CF4		
	Number of Months		
25˚C±2˚C/60% RH	1	2	3
	87.48	86.84	86.21

Comparison of *In-vitro* Dissolution studies of Optimized Itraconazole Cubosomal Oral Capsules with the *In-vitro* Dissolution studies of the Sporanox capsules

The drug release of the optimized Itraconazole Cubosomal oral capsules are compared with the drug release of the Sporanox capsules (100mg). From Figure no.8 it was found that the drug release of the optimized Itraconazole Cubosomal oral capsules drug release was found to be 73.54% in 03 hours and the Sporanox capsules was found to be 65.12% in 3 hours.

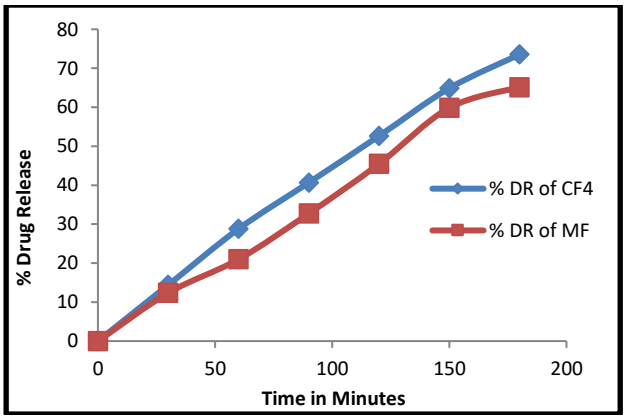

Figure 8: *In-vitro* Dissolution profile of Optimized Itraconazole Cubosomal Oral Capsules and the Marketed Itraconazole Capsules.

CONCLUSION

An attempt was made to develop Itraconazole Cubosomal Oral Capsules to investigate the potential of Cubosomes as lipid nanocarrier to improve the solubility and bioavailability of Itraconazole. Formulation F9 containing 1% Poloxamer 407 and 15% GMO concentration was optimized based on the drug release, good entrapment efficiency and greater stability. Formulation CF4 containing starch showed cubic structure with higher drug release of 73.54% at the end of the 03 Hours in 0.1N HCl when compared with marketed formulation (Sporanox-65.12%). It can be stated that the objective of the study was met. Cubosomal system is a viable alternative to conventional dosage forms by virtue of its ability to increase the solubility, bioavailability and drug release resulting in better patience compliance.

Acknowledgment: The author is thankful to I. Bala Tripura Sundari M.Pharm, (Ph.D.) for her support and valuable suggestions in completing this article.

REFERENCES

1. Hiendrawan ST, Hartanti AW, Veriansyah BA, Widjojokusumo ED, Tjandrawinata RR. Solubility enhancement of ketoconazole via salt and cocrystal formation. Int J Pharm Pharm Sci., 7(7), 2015, 160-4.
2. Shelat P, Mandowara VK, Gupta DG, Patel SH. Formulation of curcuminoid loaded solid lipid nanoparticles in order to improve oral bioavailability. Int. J. Pharm. Pharm. Sci., 7(6), 2015, 278-82.
3. Nanam PK, Thadkala K, Sailu C, Aukunuru J. Investigation of various practical techniques to enhance dissolution of ezetimibe from oral tablets: A comparative study. Journal of Young Pharmacists., 6(1), 2014, 8.
4. Raju A, Reddy AJ, Satheesh J, Jithan AV. Preparation and characterisation of nevirapine oral nanosuspensions. Indian journal of pharmaceutical sciences. 76(1), 2014 Jan, 62.
5. Devara RA, Habibuddin MO, Aukunuru JI. Enhancement of dissolution rate of poorly soluble drug itraconazole by nanosuspension technology: its preparation and evaluation studies. Asian J Pharm Clin Res. 11(4), 2018, 414-21.
6. Kawabata Y, Wada K, Nakatani M, Yamada S, Onoue S. Formulation design for poorly water-soluble drugs based on biopharmaceutics classification system: basic approaches and practical applications. International journal of pharmaceutics. 420(1), 2011 Nov 25, 1-0.
7. Thandanki M, Kumari PS, Prabha KS. Overview of Cubosomes: A Nano Particle. Int. J. Res. Pharm. Chem. 11, 2011, 2231-781.
8. Luzzati V, Husson F. The structure of the liquid-crystalline phases of lipid-water systems. The Journal of cell biology. 12(2), 1962 Feb 1, 207-19.
9. Luzzati V, Tardieu A, Gulik-Krzywicki T, Rivas E, Reiss-Husson F. Structure of the cubic phases of lipid–water systems. Nature. 220(5166), 1968 Nov, 485.
10. Esposito E, Eblovi N, Rasi S, Drechsler M, Di Gregorio GM, Menegatti E, Cortesi R. Lipid-based supramolecular systems for topical application: a preformulatory study. AAPS PharmSci. 5(4), 2003 Dec 1, 62.
11. Rosen M. Delivery system handbook for personal care and cosmetic products: technology, applications and formulations. William Andrew; 2005 Sep 23.

Source of Support: Nil, **Conflict of Interest:** None.