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Research Article

Formulation And Evaluation Of Luliconazole Loaded Cubosomal Gel For Topical Delivery

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ABSTRACT

Objective: The study aims to investigate novel formulation of the Luliconazole drug loaded cubosomal gel.

Methods: Top down technique was adopted to formulate cubosomal dispersion and then is converted to cubosomal gel. SEM test was performed to check the shape and average size of the cubosomes. FTIR test was performed to know if chemical interaction was taking place. Cubosomal gel was prepared using carbomer 934 and different evaluation of gel like viscosity test was performed using Brookfield viscometer, spreadability test was performed by slide method, Homogeneity, consistency also checked. Entrapment efficiency was calculated to know the drug entrapment percentage. Drug release study was also performed with the help of Franz diffusion cell. To see the activity of Luliconazole drug on *Candida Albicans*, in-vitro antifungal study was performed.

Results: SEM study shows that the particle size of cubosomes was found to be 80- 400 nm which is uniform and homogeneous throughout the sample and possess a cube shape. FTIR study shows no interaction between them. Entrapment efficiency of A1 formulation found to be 94 %. The cubosomal dispersion was incorporated into the gel by mixing. The viscosity, spreadability test, pH, consistency, homogeneity was performed which has given results within the limits. In vitro antifungal test was performed on *Candida albicans* and the zone of inhibition was found to be 9 mm, showing its activity. In vitro drug release study have shown that all three formulations are giving sustained release pattern of the drug which allow the drug to retain for longer periods of time. (A1) formulation was selected, giving promising results.

Conclusion: Formulation named A1 have given all the promising results which is needed for a cubosomal gel to be effective. However, clinical studies should be conducted to prove the effectiveness of this formula over the available marketed drug.

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INTRODUCTION

As fungal infections are one of the most often faced dermatological diseases across the world due to which millions and millions of peoples suffers from this, it can be serious or could be fatal. Though number of antifungal treatments are available but its prevalence rate had not reduced yet. Various researches have been done which has led to a discovery of new drug named Luliconazole which is R-enantiomer of Laniconazole. Various studies have showed though it's very useful but have limitation like very slow skin permeation and lesser skin retention on the effected site.^[11]

Luliconazole is a new candidate for anti-fungal infections to treat it topically. Its present therapy has showed limitations of very poor and slow skin permeation due to which the it is required to apply this repeatedly and for a long term for complete cure of disease.^[11] Cubosomes helps in achieving sustained release of the drug. Any drug which is either hydrophilic or lipophilic can be incorporated into cubosomes which help them in achieving a higher bioavailability of the drug. It also offers high drug pay load and incorporating the drug loaded cubosomes in emulgel helps in enhancing its penetration power and cure of infections.^[5]

They are sub-micron, discrete nanostructured particle made up of bicontinuous cubic liquid

crystalline phase. Their size range from 10nm to 1 μ m in diameter. They appear to be dots square shaped can be somewhat spherical in shape too. Dot is correlated with the presence of pore which have aqueous phase cubic phase in lipid water system, Luzzati and Husson are the one who identified this. Lutton results that Monoglyceride having C12 –c22 hydrocarbon chain especially Monoolein(Glycerylmonooleate) exhibit larger regions of cubic phase. The monoolein and water system have a region of cubic phase which helps to give a broad compositional and temperature range.^[2]

HISTORY

Cubosomes gets it early recognition in 1980 and the term was coined by the person named Larsson, that had reflected the cubic molecular crystallography and similarity to liposomes, few of anticancer drug have been successfully encapsulated and characterized as Cubosomes. They can be easily fractured and dispersed to shape a particulate dispersion which make it stable for a longer period of time. Determination of their structure that is honeycombed shaped was carried out by Luzzah and Husson.^[4]

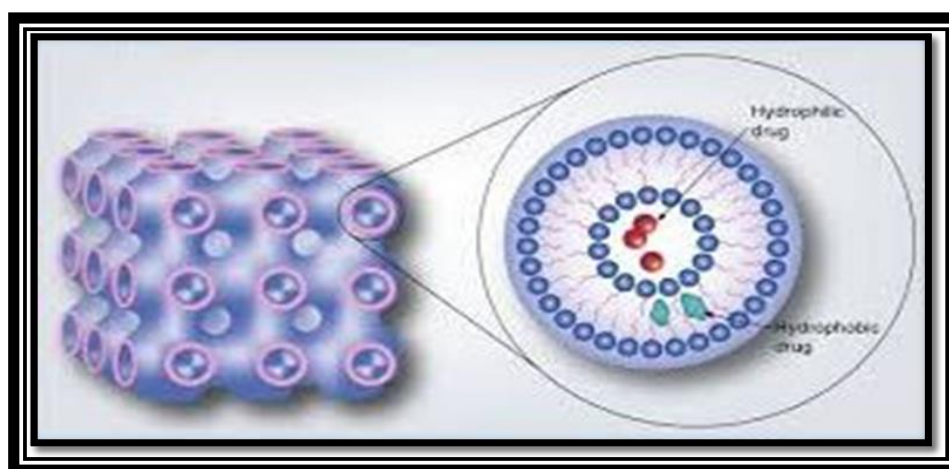


Figure. 1.1: *Cubosomes structure.*^[4]

It includes honeycomb like structure which separate the two aqueous channel internally with high interfacial area. They are nanoparticle

which is having a phase of liquid crystalline with cubic crystallographic symmetry which are made due to self-assembly made by

surfactant like molecules (amphiphilic). They form high internal surface area. these cubic phases viscosity like solid which makes a unique quality of them because of their bicontinuous type structures which are composed of two regions with water which was separated by a surfactant which is known to have controlled bilayer.

Amphiphilic molecules tend to form bicontinuous oil and water channel where bicontinuous term refers to two distinct regions which are hydrophilic separated by a bilayer. The interconnected structure results into a viscous gel which is clear and which is same in appearance and viscosity to cross linked polymer construct hydrogels.

Monoglyceride construct gels which are cubic in structure possess long range order when compared to hydrogels and due to the composition of lipid and water with excellent biocompatibility.^[4]

Manufacturing of Cubosomes:

- Top down technique
- Bottom up technique

Top down technique:

This technique is commonly used for Cubosomes preparation which involve two main steps. At first, mixing of lipid with a suitable stabilizer which form viscous cubic

aggregates. Secondly, dispersion of the produced in aqueous media with the help of high pressure or high energy homogenizer which then result in the formulation of Cubosomes.

The cubosomes which are prepared by this technique are found to be stable against aggregation for a very long time (up to a year). This procedure produces a problem when temperature sensitive bioactive agents are used.^[6]

Bottom up technique:

Commonly this approach is referring to as solvent dilution method, this process involves a step of dispersion of mixture which has Cubosomes forming lipid that is GMO, stabiliser and a hydro trope in water which is mixed by the application of minimal energy input. Hydrotrope plays an important role in this technique, it is added to dissolve lipids which is not soluble in water to form lipid precursors which prevent liquid crystals which forms at high concentration. It also solubilizes poorly soluble agent in aqueous media. Examples of hydro trope are sodium alginate, sodium benzoate. This method can be used with temperature sensitive agents. This also show long term stability because of the homogeneous dispersion of stabilizers on the surface of Nano vesicle produced.^[6]

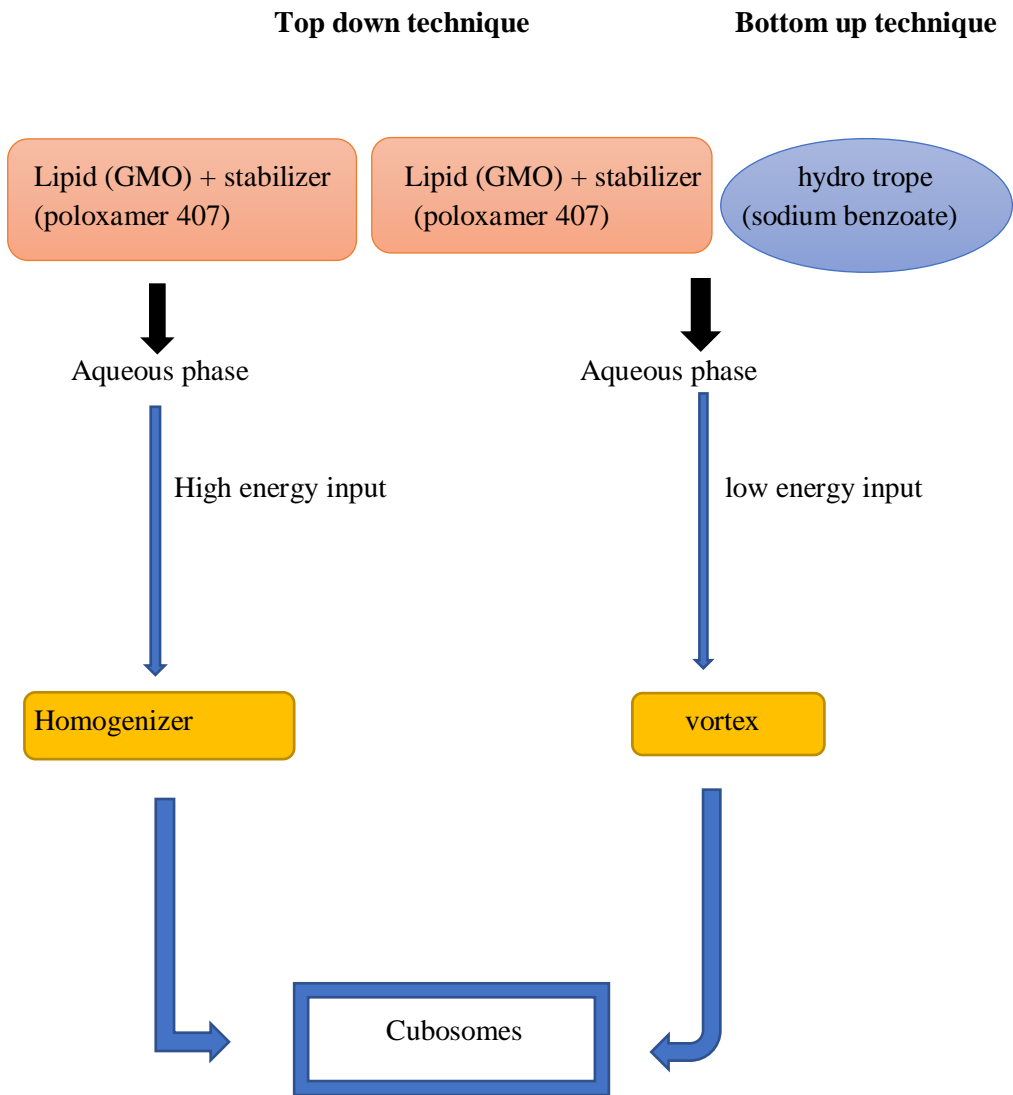


Figure 2: Diagrammatic illustration of the preparation approaches of Cubosomes.

Emulgel:

Topical delivery is failed for the administration of hydrophobic drug due to which more than one formulation can be combined to enhance drug delivery, emulgel is one of such

combinations which is a combination of emulsion and gel. It can be prepared as both, o/w and w/o type of emulsion which is then incorporated or placed with gel. [7]

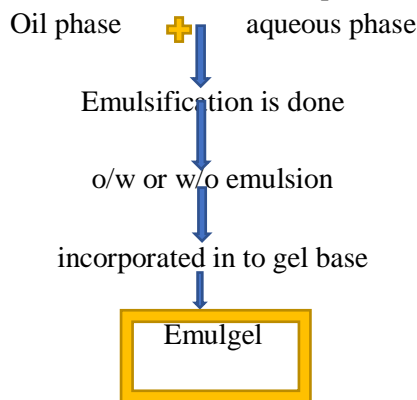


Fig.3: Process of emulgel preparation [7]

MATERIALS AND METHODS:

Luliconazole and Glyceryl Monooleate (GMO) was purchased from Yarrow Chem Product, pharmaceutical lab, Mumbai, Maharashtra, India. Poloxamer 407 and Carbopol 934 was obtained from Shambhunath Institute of Pharmacy, Prayagraj, India. All the reagents used in this study were of analytical grade. ^[9]

Preparation: Luliconazole loaded cubosomal vesicles

Luliconazole loaded Cubosomal vesicles was prepared using top-down method by adding stabilizer (poloxamer) with lipid (Glycerylmonooleate), lipid was firstly dissolved in methanol to get properly mixed. Luliconazole drug was then added by firstly getting dissolve with methanol and then mixed with the above solution, then this mixture was dispersed into aqueous solution by using homogenizer which is rotated at high speed helping in forming cubosomal vesicles. The

concentration of cubosomes vesicles was prepared as described in table 6.2. Glyceryl Monooleate and poloxamer 407 were mixed adding 50 ml water and melted in water bath at 60 degrees Celsius. To this, 200 mg of Luliconazole was mixed with methanol till it gets completely dissolve and then was added to this mixture and stirred. [9]

Then to this mixture 70-degree Celsius preheated distilled water 50 ml was added drop by drop while doing continuous stirring of the same. ^[9]

Then this was left aside for 24 - 28h to reach equilibrium. Two phase system was obtained after this which was then disturbed by stirring. The mixture was then homogenised at 8000-10000 rpm for 2 h at room temperature which help in the formation of liquid dispersion of cubosomes. Thus prepared cubosomes was kept at room temperature in glass bottles in dark place to avoid sunlight. ^[9]

Table 1: The different category of ingredient used in formulation with its function. ^[12]

Sr.No.	Ingredients	Function
1.	Luliconazole	Anti-fungal agent
2.	Poloxamer 407	Stabilizer
3.	Glyceryl Monooleate	Self-emulsifier
4.	Distilled water	vehicle
5.	Carbopol 934	Gelling agent
6.	Tri ethanolamine	pH adjustment
7.	Methyl paraben	preservative



Figure 2: Image of cubosomal dispersion:

Table 2: Composition of drug loaded cubosomal vesicles:^[10]

Formulation code	Glycerylmonooleate (%w/v)	Poloxamer407 (% w/w)	Luliconazole (mg)	Water (100%)
A1	15	1	200	100
A2	25	1	200	100
A3	35	1	200	100

Preparation of Luliconazole cubosomes emulgel

Emulgel was prepared by allowing cubosomes dispersions to swell using Carbopol 934 in distilled water for 30 min in magnetic stirrer. The carbopol 934 was sprinkled over distilled water and then was allowed it to get swell a bit then with the help of magnetic stirrer it was mixed for 30 min. To this Luliconazole loaded

cubosomes dispersion was added drop wise with continuous mixing.

To this 0.5% of triethanolamine was added to maintain the needed pH and is checked using pH meter.

Methyl paraben was added as preservative. the obtained formulation was then evaluated for pH, homogeneity, spreadibility, viscosity. [10]



Figure 3: Image of cubogel:

Characterization of Luliconazole loaded cubosomes vesicles

Visual inspection: The dispersions are examined visually for homogeneity, its colour, macroscopic aggregates, bubbles formation, disposition of material on the surface of glass vials.

Scanning electron microscopy (SEM) analysis

The SEM of the cubosomal sample was carried out by type of electron microscope which make the use of high energy electron beam. When the electron collides with the sample, signal is

produce which give the information about morphology of the sample, composition. The carbon coating of the material is done by using JEOI vacuum evaporator which makes the sample conducting [14]

SEM images were taken by using EPMA i.e. electron pro microanalyses JEOI -j*an 8100. the average size and structure was determined. [14]

Luliconazole entrapment efficiency (EE%) in cubosomes

Luliconazole entrapped in cubosomes was determined by ultrafiltration centrifugation.

Freshly prepared Luliconazole dispersion 5 gm was diluted with 10 ml of deionized water, 3 ml of the diluted sample was placed in centrifuge tube and centrifuge at 4 degrees Celsius at 4000 rpm for 15 min.

Free Luliconazole in filtrate was diluted with methanol and was measured using a validated HPLC assay.

The amount of entrapped Luliconazole was obtained by subtracting the amount of free drug from the total drug incorporated in 5 gm of cubosomes dispersion. ^[15]

$$\text{EE\%} = \frac{\text{total drug fed initially} - \text{amount of drug in filtrate}}{\text{Total drug fed initially}} \times 100$$

Evaluation of Luliconazole loaded cubosomes emulgel:

FTIR:

Spectra of drug and gel formulation were taken and analysed for the presence of any incompatibility by seeing stretch. This is tool which is needed for identification of functional group present in the sample, to identify organic compound, structural analysis of compounds like organic, polymer and natural compound. every functional group in the sample has specific vibration and can be observed via IR spectra as the fingerprints.

This analysis was done on Luliconazole drug, and Luliconazole loaded cubosomal gel. The FTIR Spectra was recorded at 4000-400cm⁻¹ using PerkinElmer Spectrum Version 10.4.00. ^[14]

pH:

pH was determined by using Digital pH meter (361, Systronics). In this measurement pH meter which was attached with electrode was cleaned with distilled water and then was dried later the electrode was immersed in the cubosomal gel and the results was obtained in the triplicate manner and the average is calculated. ^[14]

Viscosity:

It is a measurement of the fluid resistance. Unit of viscosity are Centipoise, Poise, Pascal second. It was determined by using Brook field viscometer. 63no. spindle was obtained to check and is rotated at 10 rpm to 100 rpm. ^[14]

Spreadability:

It is a process which possess an important role in the administration of a dose of a pre

It was determined taking wooden block and glass slide apparatus. Then the formulation was accurately weighed to 10g and added to the pan and then the time was noted for the upper slide to get completely detached from the fixed slides spreadability was the calculated using the given formula that is,

$$S = M.L/T$$

Where,

S is spreadability, M is weight applied to upper slide, L is length of glass slide, T is time required by the slide to get completely detached from the other slide. ^[10]

Homogeneity:

All developed gel was tested for homogeneity by inspecting it visually after the gel get set in the container. They were tested for the presence of any aggregates and for their appearance. ^[9]

Consistency:

This measurement was done by dropping a cone which is previously being attached to a holding rod from a fixed distance in a way that it should drop on a centre of the glass cup with the formulation. The measurement was taken of the penetrated cone from the surface of the gel to the tip of the cone penetrated the gel and after 10 secs the distance is measured which is travelled by the cone in this time. ^[9]

In vitro antifungal study:

Microbial strain: Candida Albicans (MCCB 0290) was obtained from Microbial Culture Collection bank, Microbiology department, SHIATS, Naini ,Prayagraj.

This study was carried out to see the biological activity against microorganism which was determined with the help of agar diffusion

medium using cup plate technique. Cubogel loaded with Luliconazole drug was taken in cup which is already being seeded throughout with *Candida Albicans*. this plate was incubated for 24 h and temp. 37⁰ C and the result was obtained in triplicate manner.^[10]

In –vitro drug Release study

The in vitro release of Luliconazole from the cubosomes dispersion was performed. It was taken out using Franz diffusion cell also known as chambered donor receiver compartment model. This cell was placed on a magnetic stirrer and its temperature was maintained to 37 degrees Celsius^[10]

Upper side of the chamber was put with Himedia dialysis membrane (cut of molecular weight 12000-14000), which was soaked into phosphate buffer pH6.8 for almost about 12 h. phosphate buffer 6.8 was made by using potassium dihydrogen phosphate and disodium hydrogen phosphate in 1000 ml water of HPLC grade and was vacuum filtered using 0.2-micron filter and was then kept in the receptor cell. Then 1 gram of the formulation was

poured on the dialysis membrane, which was in the contact with the receptor medium (phosphate buffer) Samples were taken out at every 2 h time interval and the medium was again added with the same buffer that is phosphate buffer pH 6.8.^[10]

Then the samples were analysed for the Luliconazole drug using HPLC at wavelength 276nm.^[10]

Results and discussion:

Visual examination:

Luliconazole loaded cubosomal dispersions was visually inspected for colour, homogeneity, aggregates and found to be a homogeneous dispersion without any settlement of aggregates and the colour is white throughout the solution.

Shape examination from projection microscope:

While making the formulation, the samples were also observed in the microscope with 10X resolution to observe the shape of the sample and a cubical shape was observed with a little honey come like structure within.

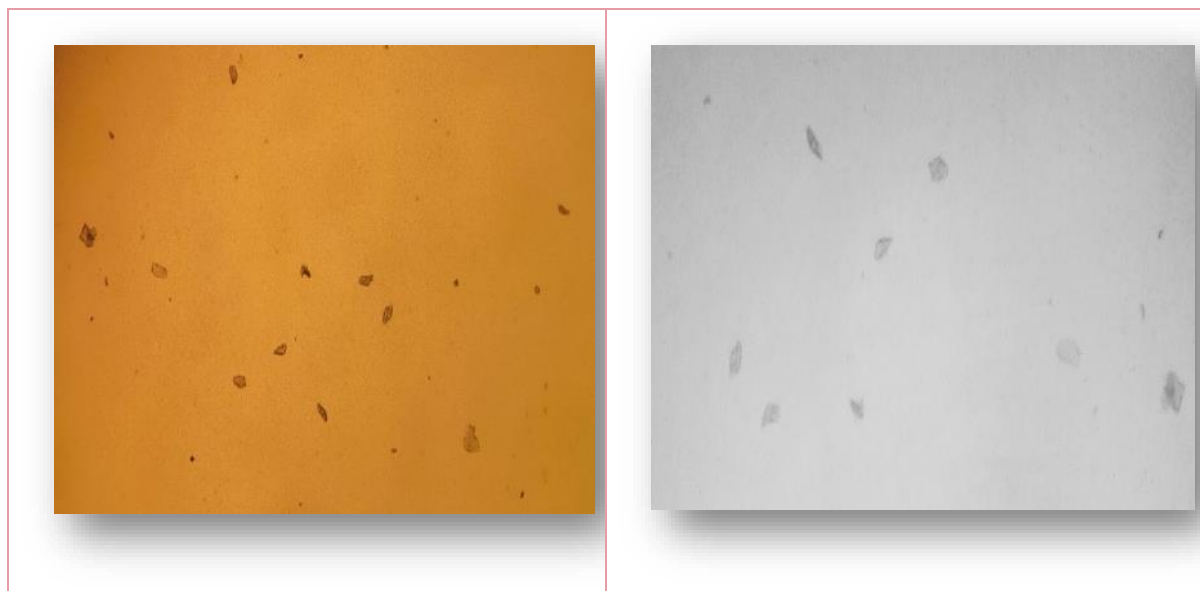


Figure 4: images of cubosomes from projection microscope.

Scanning electron microscopy (SEM) analysis

SEM is a technique in which incident beam scan the sample surface and generate signals. Shape and size of the sample can be obtained.

The average size of the particle was found to be 80-400 nm. in sample and the composition was uniform and homogenous in all over the sample. The shape of the particle was found to be cubosomal in nature.

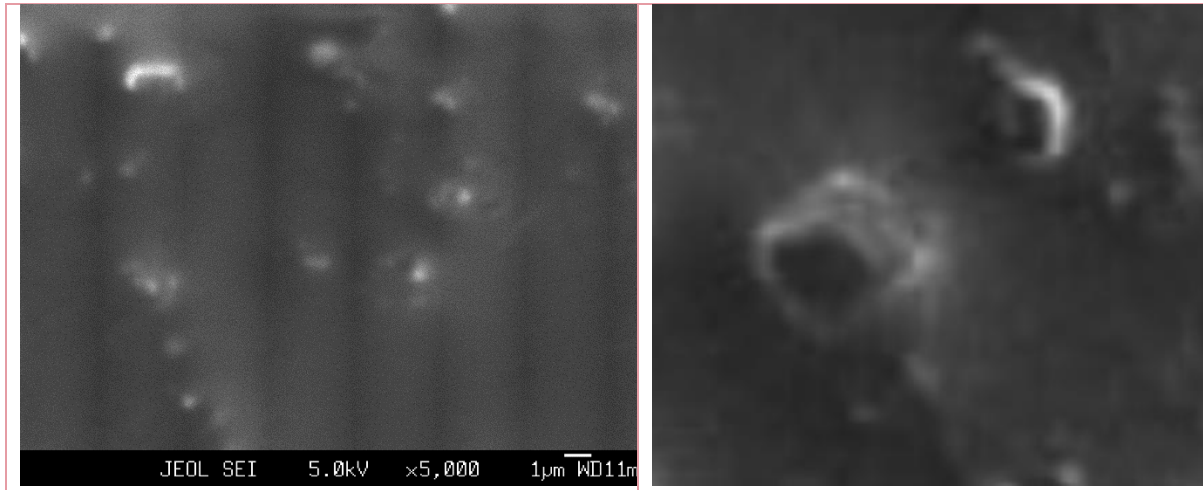


Figure 5: Sample 1 A1 formulation: (Images)

Entrapment efficiency EE%

The EE% for the prepared Luliconazole loaded cubosomes was found to be $92.00 \pm 0.5\%$ to $94.32 \pm 0.8\%$ and the drug content of optimised formulation A1 was found to be $94.32 \pm 0.8\%$

as shown below which shows that the Luliconazole was successfully entrapped into the cubosomes to be a promising delivery system for lipid soluble drugs.

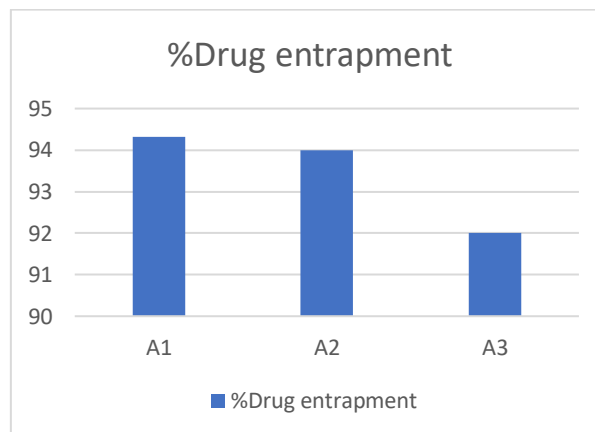
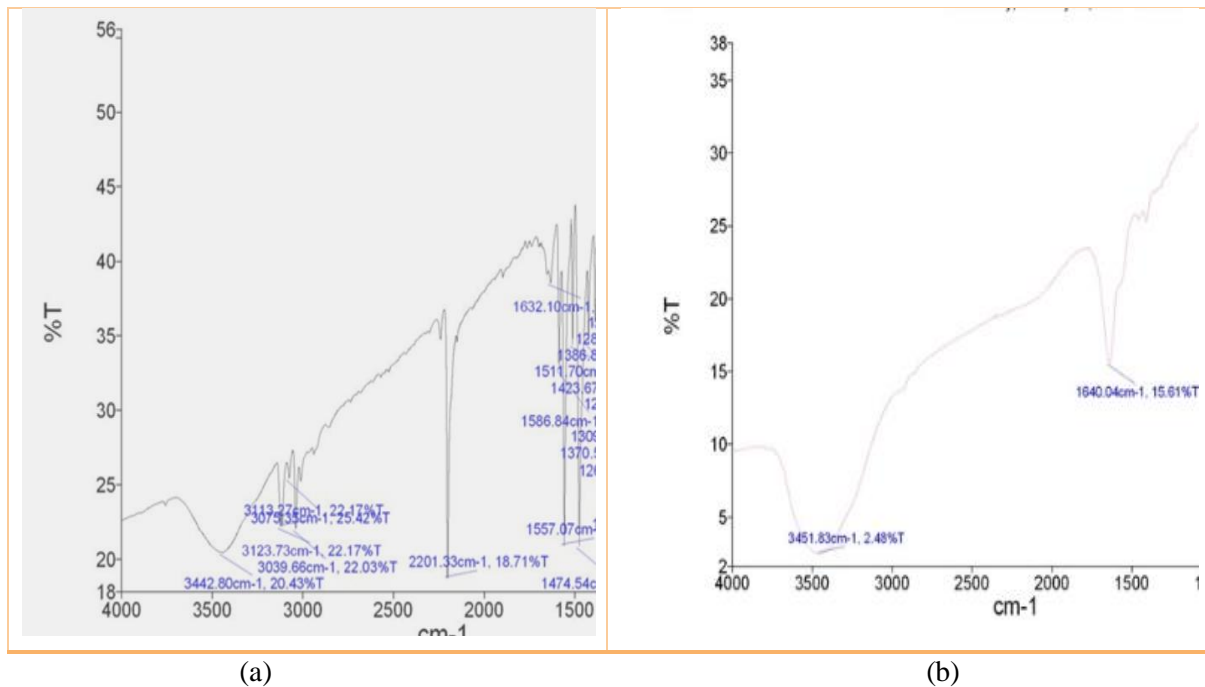


Figure 6: Percentage entrapment efficiency

FTIR: -

Luliconazole showed the peaks at: 3442.80 cm^{-1} O-H stretch, Phenols group, 1632.10 cm^{-1} C-C stretch, 3123.73 cm^{-1} C-H stretch, 3039.66 cm^{-1} C-H stretch. After the

formulation of cubosomal gel, its shows peaks at 3451.83 cm^{-1} O-H stretch, 1640.04 cm^{-1} C=C stretch, which depicts there is no chemical interaction happen between the drug and gel.



(a) (b)
 Figure 7: FTIR spectra of Luliconazole drug (a), FTIR spectra of Luliconazole loaded cubosomal gel(b).

pH:

The pH of all the formulation were observed in the range of 6.8 to 7.2, which indicated that the

prepared formulation is compatible with skin pH.



Figure 8: Image of cubogel

Viscosity:

The result of viscosity showed that when the amount of carbopol 934 get increased from 0.5 to 1%, viscosity of the gel also gets increased.

So by testing 4 formulation having different amount of gelling agent carbopol 934(1%) was selected showing optimum result as shown below:

Table: Viscosities in cps of cubosomal gel at different rpm.

Rpm	Viscosity(cps)
10	8198
20	7259
30	5383
50	4529
100	3656

Spreadability:

It was observed that increasing the carbopol 940 concentration is indirectly proportional to spreadability as the carbopol concentration increased the gel strength also increased with decrease in spreadability. A good gel takes less time to spread with good spreadability. The spreadability was found to be 13.9,14.01,14.2(g.cm/sec) respectively of three formulations.

Homogeneity:

The developed gel when prepared using 1% carbopol have given good results otherwise with every increase in 0.5 % the gel showed lumps and of more viscous nature.

Consistency:

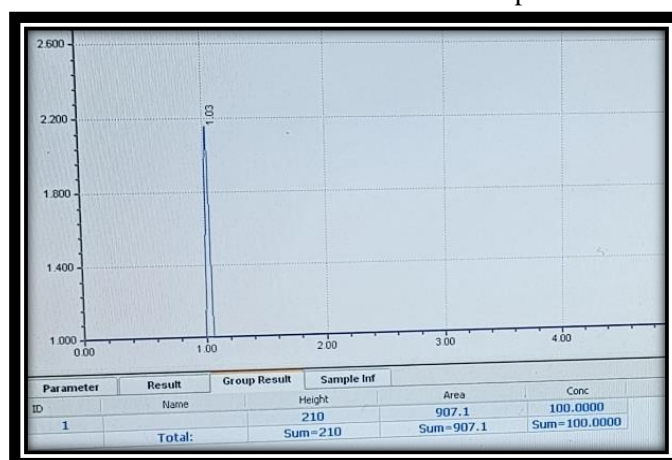
It reflects the capacity to gel when squeezed should get ejected in uniform and desired quantity. Distance travelled by the cone was 10 mm for all the formulations.

In- vitro antifungal activity:

The in-vitro ant- fungal property of Luliconazole loaded cubosomal gel was determined by using cup and plate method. In present study Salbouraud dextrose agar is used as a culture media for culturing candida albicans. Luliconazole loaded cubogel was placed in the plate already having fungal strain after this the plate is incubated for 48 hours and zone of inhibition of the cubogel were observed which was found 9 mm which shows that the Luliconazole is capable of inhibiting fungal growth.

In Vitro release study:

It was performed for all formulation from which A1 was selected. The drug release profile of all formulations are given in figure no 11 and it was found that increasing GMO concentration from 15 to 35 % does not affect the release pattern of the drug as much. It was observed that after 8 hrs cubosomes formulation was sustained over a period of time when compared to Luliconazole drug powder.



*Figure 9: crude drug peak at wavelength 276.
Retention time of the drug was found to be at 1.03*

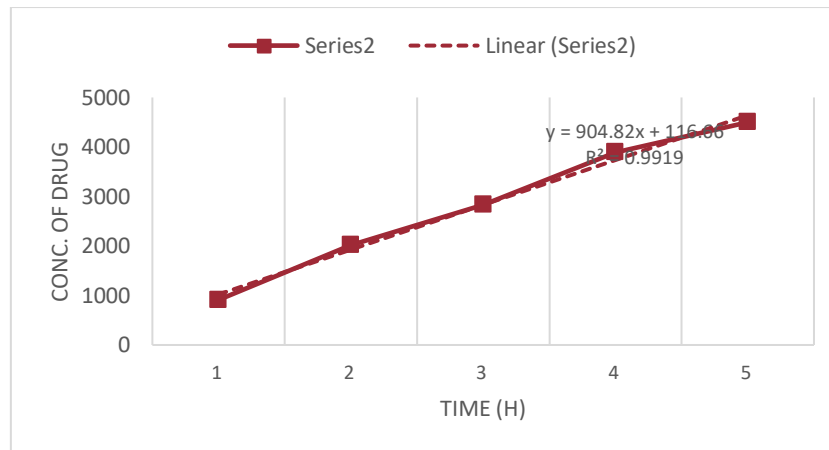


Figure 10: Drug release of Luliconazole drug powder

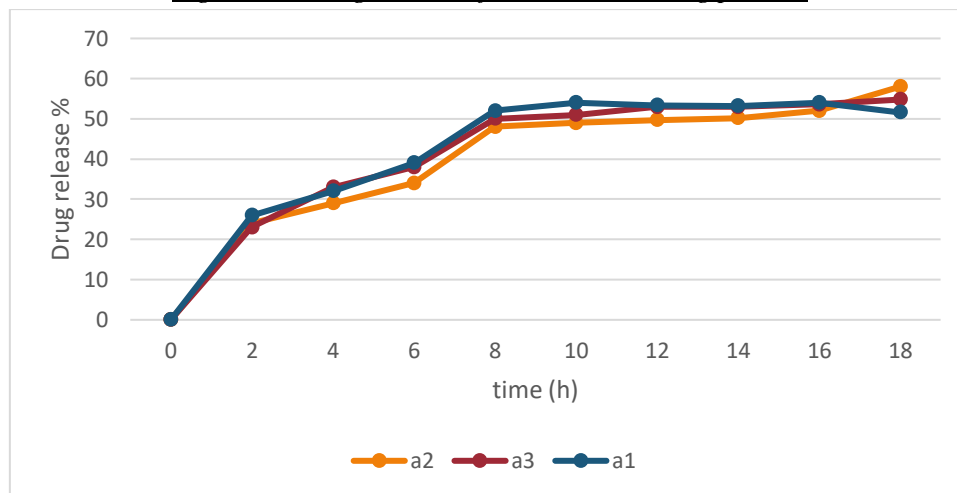


Figure 11: Drug release % of cubogel all three formulation

DISCUSSION:

Topical delivery of the drug allows local delivery of the drug to the site with least side effect but have some limitations which is overcome by making cubosomal formulation loaded with Luliconazole drug which provide effective treatment of the same overcoming the limitation. It is also showing its efficacy towards *Candida Albicans*. Cubosomes allow high pay load of the drug, lipophilic and hydrophilic drug, both can be incorporated into cubosomes. It also gives sustained release of the drug which help in providing better treatment for the infection. Overall A1 formulation have shown the best result out of three in every aspect.

CONCLUSION:

Luliconazole loaded cubosomal gel was prepared successfully and three formulations

having different GMO % was made out of which A1 formulation have shown the best result out of three. Cube like structure was found in SEM study with 80 – 400 nm size which is spread throughout the sample homogeneously. FTIR study showed no incompatibility between them. In vitro Antifungal study was performed which shows the inhibition of 9 mm which means that the Luliconazole drug was showing its activity against *Candida albicans*. Various evaluations of gel were also carried out like pH, viscosity, spreadability, consistency, drug release study, entrapment efficiency which had shown the promising results giving effective results. In Vitro drug release study have shown the sustained release pattern of the drug after 8 hrs which help the drug to get retained on the site for longer duration of time. Entrapment efficiency have shown 94.32 % drug (A1)

which is entrapped in. However, clinical studies should be conducted to prove the effectiveness of this formula over the available marketed products.

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