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# FORMULATION AND DEVELOPMENT OF CUBOSOMES LOADED EMULGEL OF FLUCONAZOLE

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## ABSTRACT
The objective of this research work was to overcome the absorption problem of Emulgel and to develop a new topical formulation for chronic skin diseases, like fungal infection, acne, psoriasis etc. hence in this study the fluconazole loaded Cubosomal emulsion is incorporated in simple carbapol gel. As Cubosomes are the complex structural feature as like honeycombed maze and Cubosomes as very small particle size rages from 100-400 nm. Cubosomes can incorporate hydrophobic, hydrophilic and amphiphilic drug. This self-assembled nano particles formed by aqueous lipid and surfactant systems. Which forms tightly packed structure by assembling the lipid and surfactant into bilayers and twisted in three dimensional cubic structure. Cubosomes potential has greater drug loading abilities. Emulgel are the spontaneously favourite for the researchers as they shows the favourable properties such as thixotropic, greaseless, easily spredable, easily removable, non-staining. Fluconazole is BCS class II drug sparingly soluble in water. Fluconazole is antifungal drug used for treatment cutaneous candidiasis. The main objective behind this formulation is to deliver the drug which shows GIT irritation.

**KEYWORDS:** Emulgels, Cubosomes, nano particles, antifungal, Hydrophobic, Fluconazole.

## 1. INTRODUCTION

### 1.1 Emulgel

Topical drug delivery system can be define as the application of the drug containing formulation to the skin to directly treat cutaneous disorder. Topical drug delivery offers several advantages over conventional routes.

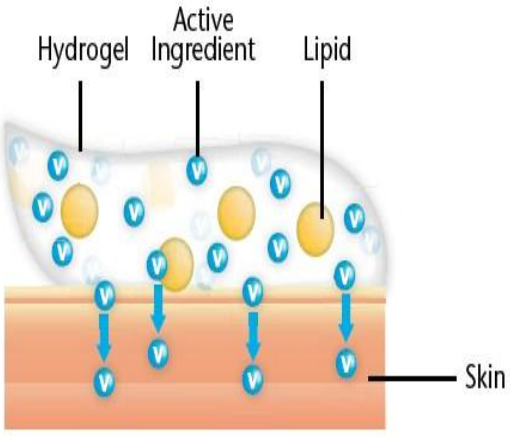



**Figure: Emulgel.**

When Emulsion and gel are used in combined form of dosage are reffered as Emulgel. In this the gel is actually colloid which is 99% of weight of the liquid. Emulsion may be oil in water or water in oil. Use of novel polymers with complex function as emulsifiers and thickness because the gelling capacity of this compound s allows the formulation of stable emulsion and cream by decreasing the viscosity of the aqueous phase. Presence of a gelling agent in the water phase converts a classical emulsion into Emulgel.

#### 1.1.1 Preparation of Emulgel


**Figure: Flow chart of Preparation of Emulgel.**

Preparation of Emulgel has reported by mohmmad et al (2004). In the following method gel sample is prepare by dispersing gelling agent into the water with pH of 6 to 6.5. Then after preparation of gel, The oil phase of the emulsion is prepare by dissolving emulsifying agent and stabilizing agent. Oil phase and water then mixed in proportion of 1:1 for good and stabilized Emulgel.

**1.1.2Advantages of Emulgel**

1. **Hydrotropic drugs can be easily incorporated into gels using d/o/w emulsion**

Most of the hydrophobic drugs cannot be incorporated directly into gel base because solubility act as barrier and problem arises during the release of the drug. Emulgel helps in the incorporation of hydrophobic drugs into the oil phase and then oily globules are dispersed in aqueous phase resulting in o/w emulsion.

2. **Better stability**

Other transdermal preparation are comparatively less stable than emulgels. Like powders are hygroscopic creams shows phase inversion or breaking and ointment shows rancidity to oil phase.

3. **Better loading capacity**

Other novel approaches like niosomes and liposomes are of nano size and due to vesicular structure may result in leakage and result in lesser entrapment efficiency but gels due to vast network have comparatively better loading capacity.

4. **Production feasibility and low preparation cost**

Preparation of Emulgel comprises of simpler and steps which increases the feasibility of the production. There are no specialized instruments needed for the production of Emulgel. Moreover materials used are easily available and cheaper. Hence, decreases the production cost of emulgels.

**1.2 Introduction of cubosomes**

Cubosomes are discrete, sub-micron, nanostructured particles of the bicontinuous cubic liquid crystalline phase. Such novel particles are utilized to encapsulate molecules which are poor water soluble druges. Fluconazole is sparingly water soluble drug so solubility is main constraint for bioavailability. The aim of this study is to investigate the potential of the cubosomal technique which is Nano carrier to improve the solubility of the fluconazole. Cubosomes are discrete submicron nanostructure formulation of bi-continuous cubic liquid crystalline phase whose size range is 10-500 nm in diameter they appear like dot square shaped each dotes corresponds to presence of pore size 510nm. Cubosomal gel have great potential in formulating nano size particulate system for topical delivery they show best advantages such as high drug pay load due to high internal surface area and cubic liquid structure. The purpose of present study to develop the fluconazole topical gel to increase the drug solubility by avoiding gastro intestinal irritation and excretion of the drug through urine.

1. **Liquid Cubosomes Precursors**

There is difficulty and expense of high shear dispersion of viscous bulk cubic phase to form cubosomes because it aggressive process of manufacturing· High energy process and expensive difficult to scale up and harmful to the fragile temperature sensitive active ingredient like protein. In cubosomes a strong driving force are exist for development of liquid phase to cubosomes to avoid the high energy processing and produced them in situ hence the hydrotropic dilution process are found to be consistently which produced smaller more stable cubosomes particles and growth are employed by nucleation crystallization and precipitation method. This is achieved by dissolving the monoolein in a hydro trope, such as ethanol, that prevents liquid crystalline formation. Subsequent dilution of this mixture spontaneously "crystallizes" or precipitates the cubosomes. Quid precursor process allows for easier scale up of cubosome preparations and avoids bulk solids handling and potentially damaging high energy Processes.

2. **Powdered Cubosomes Precursors**

Powdered cubosome precursors are composed of dehydrated surfactant coated with polymer. Such powders offer advantages to liquid phase hydrotropic cubosome precursors. Hydration of the precursor powders forms cubosomes with a mean particle size of 600 nm as confirmed.

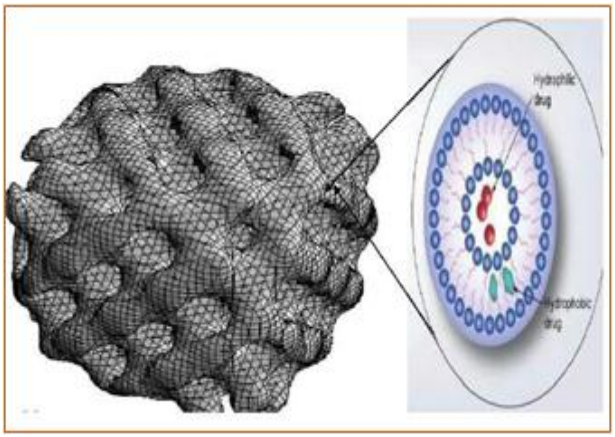


**Figure 1.1. Structure of Cubosomes.**

By light scattering and cryo-TEM[7]the lipids used to make cubosomes are waxy, sticky solids. Water-soluble non-cohesive starch coating on the waxy lipid prevents agglomeration and allows control of particle size. Spray drying is excellent processes for this purpose the encapsulation of particles are done. The process provide easy route to preload active drug in to cubosomes prior to driving. Spray-drying experiments are required a Pulvis Basic Unit. That is a cylindrical chamber having a cyclone collector and air existed. The nozzle having liquid orifice size of 0.1 cm is used to incorporate liquid

to spray-dried into the top of the spray-dryer body. The air pressure having the 300 Kpa is pump by which orifice size is 0.25-cm. Drying of liquid feed done by the heated, drying air at $200^0$C that flows down and passes to the nozzle. That prevents any oxidation ofthe monoolein at the elevated temperatures and liquid crystalline material are form that provide the high shear to disperse the high-viscosity.

### 1.2.1 Cubosomes Application

- High drug payloads due to high internal surface area and cubic crystalline structures.
- Relatively simple method of preparation.
- Control released of solubilized substance is the most popular application of cubosomes.
- It provides the drug in Nano size 10-500 nm. It mostly used in melanoma therapy. Enhances the solubility of the poor water soluble drug.
- Capability of encapsulating hydrophilic, hydrophobic and amphiphilic substances.
- Targeted release actions are shown.
- It produced high level of dilution.

### 1.2.2 Manufacture of Cubosomes

Cubosomes can be manufactured by two distinct methods:

- Top down technique.
- Bottom up technique.

**Top down technique**

The top-down method, the most common method to use for the preparation of Cubosomes. Bulk cubic phase is first produced by the application of high energy such as high pressure homogenization; it is processed into cubosomes nanoparticles. Bulk cubic phase resembles a clear rigid gel formed by water-swollen cross linked polymer chains. The cubic phases are differ in that they are a single thermodynamic phase and have periodic liquid crystalline structure. Cubic phase's ruptures in a direction parallel to the shear direction, the energy required is proportional to the number of tubular network[14]. The cubic phase's exhibits yield stress that increases with increasing amount of bilayer forming surfactant and oils. Warr & Chen gave the cubic phases may behave as lamellar phases during dispersion with increasing shear, dispersed liquid crystalline particles are forming at intermediate shear rates, whereas defect free bulk phase reforms at higher shear rate.

**Bottom up Technique**

In this cubosomes are allowed to form or crystallize from precursors. The bottom-up approach first forms the Nano-structure building blocks and then assembles them into the final material. It is more recently developed technique of cubosome formation, allowing cubosomes to form and crystallize from precursors on the molecular length scale. The key factor of this technique is hydro trope that can dissolve water insoluble lipids into liquid precursors. This is a dilution based approach that produces cubosomes with less energy input when compared top down approach. This method is more robust in large scale production of cubosomes. The cubosomes at room temperature is by diluting monoolein-ethanol solution with aqueous poloxamer 407 solution. The cubosomes are spontaneously formed by emulsification. Another process is also developed to produce the cubosomes from powdered precursors by spray drying technique. Spray dried powders comprising monoolein coated with starch or dextran form cubosomes on simple hydration. Colloidal stabilization of cubosomes is immediately provided by the polymers.

### 1.2.3 Preparation method of cubosomes

The cubosomes dispersion carried out by the Fabrication method and Emulsification method.

**i) Fabrication method**

GMO/P407 cubic gel GMO 5% and P407 1.0% were firstly melted at the 600 C in hot water bath the X amount of drug is kept in to the melted mass and stirred continuously to dissolve. Deionized water is added drop by drop and vortex mixers are set to the homogenization. It kept in to 48 hrsat the room temperature the optically isotropic cubic gel are form and it disturbed by mechanical stirring the crude dispersion was subsequently fragmented by sonicater probe having the energy 200W under the cool temperature at the 200C in water bath for the 20 min.

**ii) Emulsification method**

In this method the GMO and P407 are put in to the water and it followed the ultra-sonication the 5% GMO and 1% P407 and 5% ethanol in 89% water are taken GMO and P407 are melted at the 600C and mixed the ethanoic solution was added to the melting. The resultant mixture is added drop wise to deionized water preheated at the 700C. Ultra sonicated at maximum power 130kW for 50min at the same temperature the disperse solution are kept in ambient temperature and protected from light.

### 1.3 Mechanism action of antifungal drugs

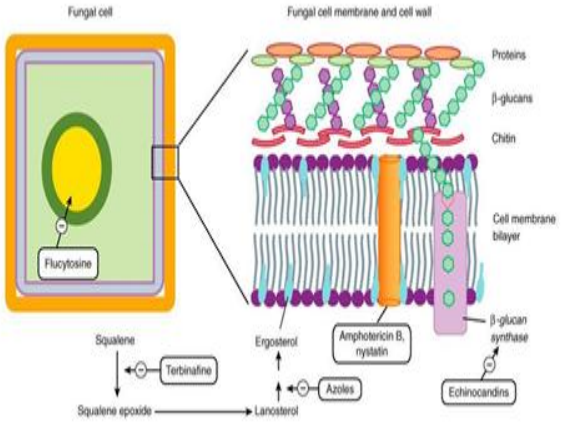



Figure: Targets of Antifungal Drugs

(Source: Katzung BG, Masters SB, Trevor AJ:Basic and Clincial Pharmacology, 11th Edition).

Amphotericin B has been the mainly used in antifungal therapy since its release in the 1950s. This agent emerged as the preferred polyene over the more toxic agent in this class, Nystatin. Nystatin has since been relegated to topical and localized therapy because of its unfavorable adverse effect profile. The polyene agents exert their antifungal activity via binding to ergo sterol in the fungal cell membrane. This disrupts cell permeability and results in rapid cell death. To date, Amphotericin B remains the broadest-spectrum antifungal agent available, with activity against many clinically relevant yeasts and molds. During the 1990s, newer lipid preparations of amphotericin B, including amphotericin B lipid complex (Abelcet, Enzon), liposomal amphotericin B (AmBisome, AstellasPharma US) and amphotericin B colloidal dispersion (Amphotec; Three Rivers Pharmaceuticals) were developed to alleviate drug toxicity. These agents possess the same spectrum of activity as amphotericin B deoxycholate. Each agent has been shown to decrease nephrotoxicity in comparison with the conventional preparation of amphotericin B. However, with the exception of findings for histoplasmosis, data supporting increased efficacy of the lipid products against common opportunistic fungal pathogens are lacking.

Amphotericin B and Griseofulvin remained the only systemic therapeutic options for invasive fungal disease until the early 1970s, when flucytosine (Ancobon; Valeant Pharmaceuticals) was released. Flucytosine is a pyrimidine analog that exerts antifungal activity via inhibition of both DNA synthesis and protein synthesis in the fungal cell. It also holds the distinction of being part of the only routinely recommended combination antifungal regimen for treatment of cryptococcal meningitis .Unfortunately, the toxicity of this agent and the rapid development of resistance when used as monotherapy have precluded its routine use for the treatment of other invasive infections.

In 1979, the first systemic azole antifungal agent, ketoconazole, was introduced. Azole agents exert their antifungal activity by blocking the demethylation of lanosterol, thereby inhibiting ergosterol synthesis. Ketoconazole was followed chronologically by Fluconazole (Diflucan; Pfizer), Itraconazole (Sporanox; Janssen Pharmaceuticals) and Voriconazole (Vfend; Pfizer). Four investigational agents remain in development: Posaconazole, which has been submitted for US Food and Drug Administration approval and Ravuconazole, BAL8557 and Albaconazole, which remain under study. Each agent offers a specific antifungal spectrum. Earlier agents in the class demonstrated potent activity against some, but not all, yeasts and Itraconazole had some activity against molds, including Aspergillus species. The newer, expanded-spectrum triazoles have been shown to have cidal activity against a wide spectrum of moulds, as well as enhanced activity against Candida species and other yeasts.

The echinocandins represent the newest class of antifungals. Caspofungin (Cancidas; Merck) was released in 2001. This was followed by Micafungin (Mycamine; Astellas Pharma US) in 2005 and Anidulafungin (Eraxis; Pfizer). The mechanism of activity of the echinocandins is inhibition of the production of (1→3) β-D-glucan, an essential component in the fungal cell wall. The spectrum of activity is therefore limited to pathogens that rely on these glucan polymers and is less broad than the spectrums of the polyene or azole agents. The echinocandins exhibitfungicidal activity against many Candida species, making this drug class a desirable alternative to the azole agents, which exhibit only static activity against yeasts. Because mammalian cells have no (1→3)-β-D-glucanin a cell wall, the echinocandins have very few toxic adverse effects in humans.

### 1.4 Challenges of topical drug delivery system

The challenge of developing a successful topical product stems from the several requirements that a formulation must meet

#### 1.4.1 Container Selection and Product Stability

Depending on the properties of the combinedingredients, a dispensing container will be chosen (i.e., tube, jar, can, etc.) to provide a stablephysicochemical environment that protects theactive compound(s) from chemical degradation. The formulation can be a liquid or semi-solid, monophasic or multiphasic (e.g., oil-inwater or water-in-oil); it is largely dependent on the characteristics of the active compound(s) and on the condition of the skin to be treated.

#### 1.4.2 Skin Penetration

Once the product is applied on the skin, a complex interaction occurs between the formulation, the active compounds, and the skin itself. The penetration of the active compound(s) into the skin follows Fick's first law of diffusion, which describes the transfer rate of solutes as a function of the concentration of the various ingredients, the size of the treatment surface area, and the permeability of the skin. However, the skin's permeability can be influenced by many factors, such as the drying, moisturizing, or occluding effects of the excipients in the formulation, which, in combination, can modulate the release of the product at the treatment site. In acne, the site of action is inside the pilosebaceous unit and, therefore, an efficacious anti acne formulation should facilitate the penetration of the active compound(s) into this extremely lipophilic environment.

## 2. Formulation development of Fluconazole Cubosomes.

### 2.1 Preparation of cubosomes.

**Table 1: The different functional categories of ingredients used in formulation.**

| Sr.No. | Ingredients | Functional category |
|---|---|---|
| 1. | Fluconazole | Anti-fungal Agent. |
| 2. | Poloxomer 407 | Stabilizer |
| 3. | Glycerylmonooleate | Self-emulsifier |
| 4. | Distilled water | Vehicle |
| 5. | Carbopol 940 | Gelling agent |
| 6. | Sodium hydroxide | pH adjustment |

Accurately weighted quantity of 4.5% Glyceryl monooleate (GMO) and 0.5% poloxamer 407polymer mixed and melted in a water bath at $60^0$C to this mixture add diclofenac sodium drug and stir until completely dissolved, then to this solution add drop by drop preheated (upto $70^0$C) distilled water of suitable quantity (95%) by continuous stirring, after complete addition of water kept aside for one day to attained equilibration, there is a formation two phase system and it is disturbed by stirring.

This whole system is taken into subjected for homogenization at 8000-10000 rpm for 2hr. under at room temperature. Thus formed liquid dispersion of cubosomes was kept at a room temperature, avoids direct sunlight and which will used for further study.

### 2.1.1 Optimization of formula.

A $3^2$ full factorial design was used in order to investigate the joint influence of the 2 formulation variables are mention but in trial batches the middle values of factorial shows good result hence the set formula not obeys the rule of factorial design.

**Table 2: Full factorial design.**

| Coded level | Glyceryl Monooleate% | Poloxomer 407% |
|---|---|---|
| | X1 | X11 |
| -1 | 3.2 | 0.2 |
| 0 | 4.0 | 1.0 |
| +1 | 4.8 | 1.8 |

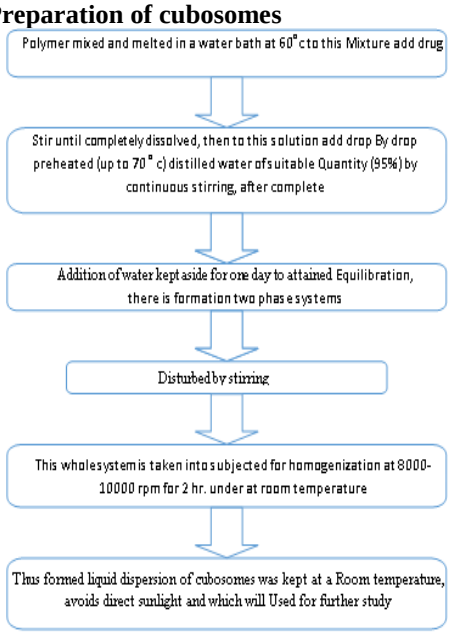
In this design, two factors are evaluated, each at three levels and experimental trials are perform at all 9 possible combination. The amounts of poloxomer and glyceryl monooleate were selected as independent variables.

### 2.1.2 Optimization of cubosomesformulation.[30,32]

**Table 3: Optimization table**

| Formulation | Glyceryl Monooleate | Poloxomer 407 | Fluconazole (gm) | Water % |
|---|---|---|---|---|
| F1 | 4.8% | 0.2% | 1 | 100 |
| F2 | 4.6% | 0.4% | 1 | 100 |
| F3 | 4.4% | 0.6% | 1 | 100 |
| F4 | 4.2% | 0.8% | 1 | 100 |
| F5 | 4.0% | 1.0% | 1 | 100 |
| F6 | 3.8% | 1.2% | 1 | 100 |
| F7 | 3.6% | 1.4% | 1 | 100 |
| F8 | 3.4% | 1.6% | 1 | 100 |
| F9 | 3.2% | 1.8% | 1 | 100 |

### 2.1.3 Preparation of cubosomes




### 2.1.4 Cubosomes biphasic solution

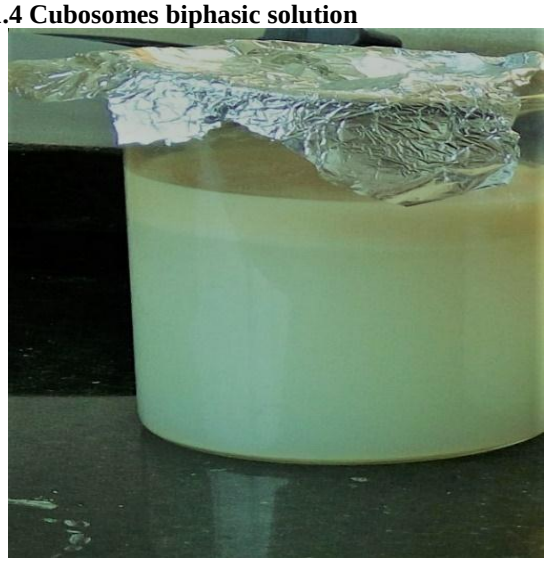


**Figure 1: Cubosomes biphasic solution.**

On the basis of the entrapment efficiency and particle size of cubosomes the batch are selected for gel formulation. Fluconazole is water insoluble drug having high permeability but low solubility in biological fluid hence the solubility is main concern in this study. Investigation and analysis of solubility study of cubosomes show increase the solubility in three folds. The particle size considers for the solubility results which batch show the efficient result. Reduction the particle size solubility is enhances. Micro ionization is a one of the most useful technique which reduced the particle size in micrometer but in cubosomes it reduced in the nanometer size by the high pressure homogenization technique.

The optimized batch is selected by the analyzing the solution under the Malvern instrument which show result of P.D.I ratio and particle size of cubosomes in nanometer as well as zeta potential analysis report is analyzed. The entrapment efficiency of cubosomes is find out by the centrifugation method the solution is centrifuged for 3 hrs at 10000 to 15000 rpm. On that basis F3 batch is selected for the formation of emulgel of cubosome formulation.

### 2.1.5 Preparation of cubosomes emulgel formulation[7,8]

Cubosomes emulgel obtain by the addition of the weighted amount of the carbopol 2% in distilled water and kept it for half day for gelling and swelling of carbopol and then addition of 1% sodium hydroxide which maintain the pH and obtain gelling consistency. The obtain gel is diluted with the cubosomes solution containing only entrap consistency of cubosomes it stir continuously and make its homogeneous emulgel formulation.

## 3. RESULT AND DISCUSSION

### 3.1 Evaluation of Cubosomal liquid

1. **pH measurement.**
2. **Particle size analysis.**
3. **Zeta potential.**
4. **Entrapment efficiency.**

### 3.1.1 pH measurement

pH of formulation is determine by using digital pH meter by immersing the electrode in gel formulation and pH is measure.

**Table 4: Observation table of pH.**

| Sr.No. | Formulation code | Observe pH (S.D) |
|---|---|---|
| 1 | **F1** | **5.9 ± 0.01** |
| 2 | **F2** | **5.78 ±0.01** |
| 3 | **F3** | **6.35 ±0.02** |
| 4 | **F4** | **6.28 ±0.03** |
| 5 | **F5** | **5.95 ±0.02** |
| 6 | **F6** | **6.05 ±0.02** |
| 7 | **F7** | **5.75 ±0.02** |
| 8 | **F8** | **6.52 ±0.01** |
| 9 | **F9** | **6.31 ±0.01** |

### 3.1.2 Particle size, polydispersity index

Particle size analysis determine by the (Nano ZS, Malvern, Worcestershire, UK) instrument at 25ºC, Which is based on the Brownian motion. Samplewere diluted in particle free purified water to scattering intensity approximately 150300keps. The mean z-average diameter and polydispersity indices were obtain by cumulative analysis using MALVERN software.

**Table 5: Particle size and PDI ratio of optimized batch.**

| | | Size (d.nm): | % Intensity | Width (d.nm): |
|---|---|---|---|---|
| **Z-Average (d.nm): 147.5** | **Peak 1** | **145.7** | **100.0** | **45.20** |
| **PdI: 0.351** | **Peak 2** | **0.000** | **0.0** | **0.000** |
| **Intercept: 0.949** | **Peak 3** | **0.000** | **0.0** | **0.000** |

- **Graph of size distribution and PDI ratio of cubosomes particles**

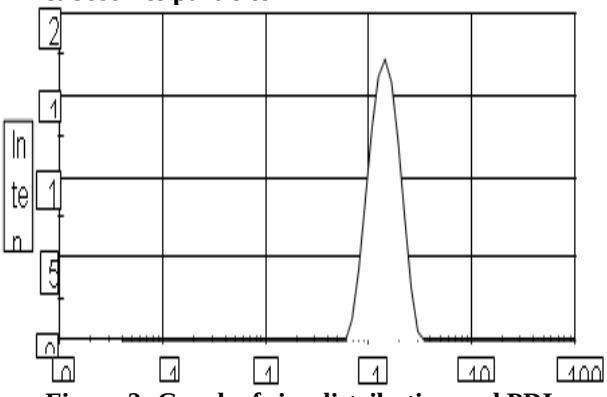


**Figure 2: Graph of size distribution and PDI.**

### 3.1.3 Zeta potential

Zeta potential is key indicator of the stability of formulation. The magnitude of zeta potential indicates the degree of electronic repulsion between adjusts, similarly charge particle in dispersion.

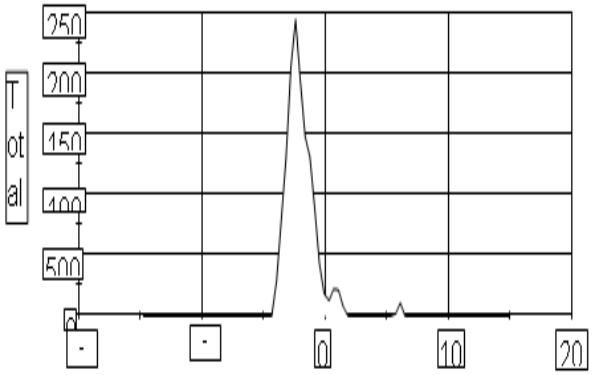



**Figure 3: Graph of Zeta Potential Distribution.**

**Table 6: Zeta Potential.**

| | | Mean (mV) | Area (%) | Width (mV) |
|---|---|---|---|---|
| **Zeta Potential (mV): -19.8** | **Peak 1** | **-21.7** | **94.5** | **8.95** |
| **Zeta Deviation (mV): 13.1** | **Peak 2** | **8.20** | **4.6** | **3.60** |
| **Intercept: 0.949** | **Peak 3** | **60.1** | **0.9** | **1.40** |

### 3.1.4 Entrapment efficiency

Entrapment efficiency is defined as the percentage amount of drug which is entrapped by the cubosomes. For the determination of entrapment efficiency, the unentrapped drug was first separated by centrifugation at 15000 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 261 nm.

$$\% \text{ Drug Entrapment} = \frac{\text{Total amount of drug} - \text{unentraped drug}}{\text{total amount of drug}} \times 100$$

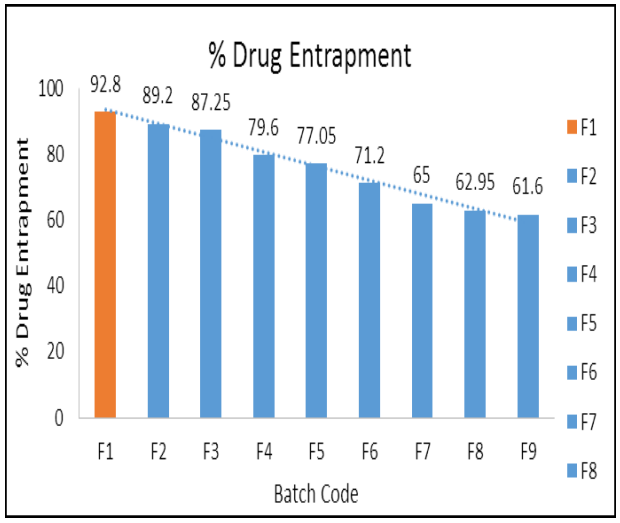



**Figure 4: Entrapment efficiency of Cubosomes.**

### 3.1 Evaluation of the Fluconazole Cubosomal Emulgel formulation[29,30,31]

1. pH measurement.
2. Viscosity measurement.
3. Drug content.
4. Clarity.
5. Diffusion study.
6. Drug kinetic release.
7. Antimicrobial activity.
8. Accelerated stability study.
9. Statical analysis.

### 3.1.1 pH measurement

The obtain Cubosomal emulgel were analyzed by digital pH meter by immersing the electrode in gel formulation and pH is measure which is previously calibrated by the pH 7.

### 3.1.2 Viscosity measurement

Viscosity of the different formulation was determine at room temperature using a Brook field viscometer. A Cole parmer viscometer was used to measure the viscosity of the prepared gel bases. The spindle was rotated at 10 rpm to 100 rpm and viscosity were observe.

**Table 7: Viscosity of Cubosomal Emulgel.**

| Sr.No. | Rpm | Viscosity (cps) |
|---|---|---|
| 1 | **10** | **6908** |
| 2 | **20** | **6249** |
| 3 | **30** | **5389** |
| 4 | **50** | **4528** |
| 5 | **100** | **3656** |

### 3.1.3 Drug content

Drug loaded cubosomes were mixed with methanol and sonicated for 10 min to obtain a clear solution. Concentrations of drug were determined spectrophotometrically at max 242 nm.

$$\text{Drug Content} = \frac{\text{Actual yield}}{\text{therotical}} \times 100$$

**Table 8: Evaluation of Fluconazole Cubosomes.**

| Sr.No. | Evaluation test | Observation |
|---|---|---|
| 1 | pH measurement | 6.8-7.4 |
| 2 | Clarity | Opaque |
| 3 | Homogeneity | Homogeneous |
| 4 | Drug content | 97.30% |

### 3.2.4 Diffusion study

In vitro skin permeation studies were performed by using a Franze diffusion cell with a receptor compartment capacity of 50ml. The synthetic cellophane membrane was mounted between the donor and receptor compartment of the diffusion cell. The formulated cubosomes gel of 1gm was placed over the drug release membrane (In the donor compartment) and the receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4. The whole assembly was fixed on a magnetic stirrer and the solution in the receptor. And the solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 50 rpm; the temperature was maintained at 37 ± 0.50°c by surrounding water in jacket. The samples of 1ml were withdrawn at time interval of 1, 2, 3, 4, 6, 8 and 12 hours and analyzed for drug content UV. Spectro-photometrically at 287nm against blank. The

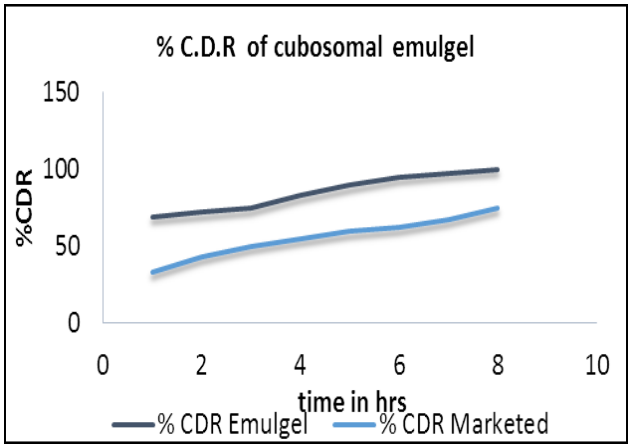



**Figure 8: Diffusion Study.**

receptor phase was replaced with an equal volume of phosphate buffer at each time of sample withdrawal. The cumulative amounts of drug from cubosomes permeated through synthetic membrane plotted.

### 3.2.5 Drug kinetic analysis

Dug kinetic analysis done for the Fluconazole emulegel were considered for various kinetic model for the optimized batch F3.

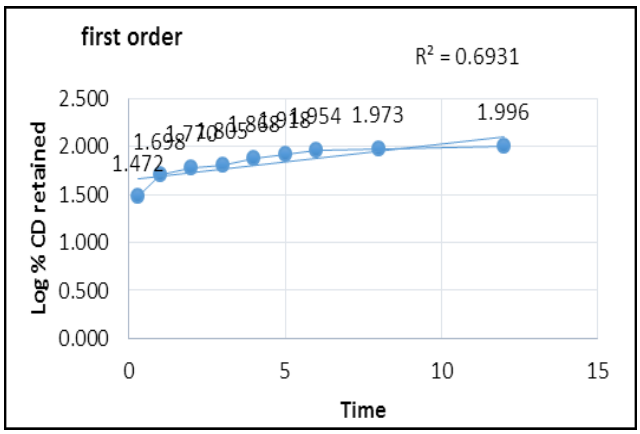



**Figure 8: Zero order.**

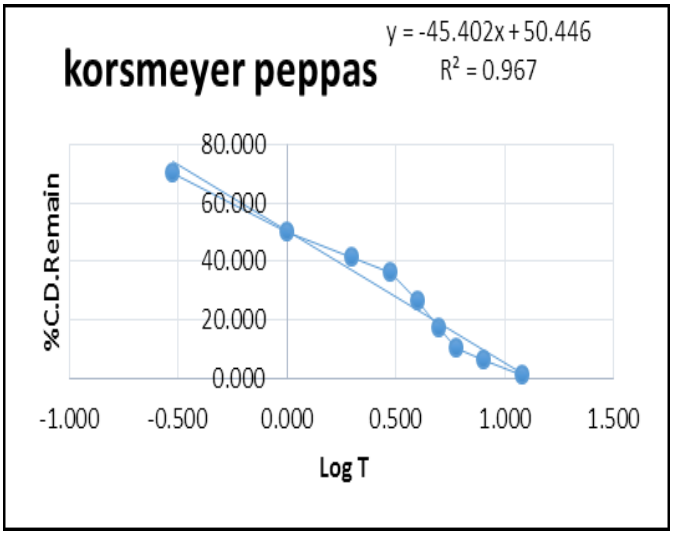



**Figure 9: First order.**

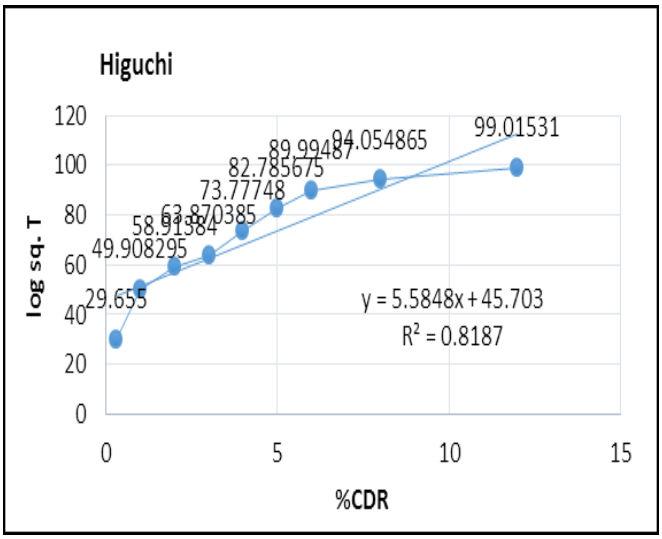



**Figure 10: Korsmeyer peppas.**

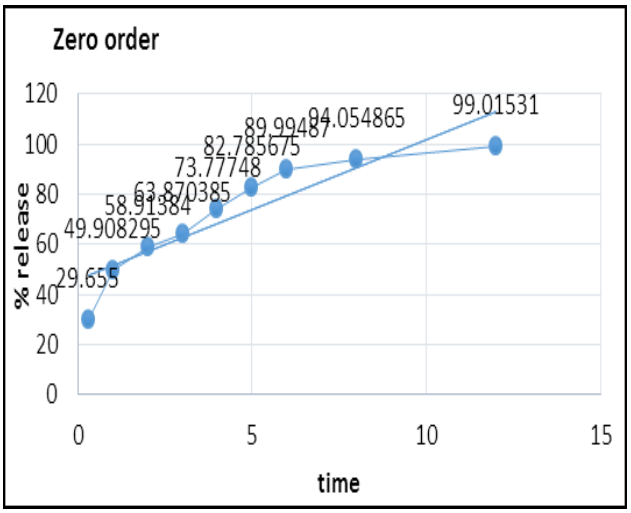



**Figure 11: Higuchi.**

### 3.2.6 Antimicrobial study

An agar diffusion method used for determination the antifungal activity of formulation. Standard petri dish 9

cm containing medium to depth of 0.5cm were used. The sterility of the lots was controlled before used. Inocula were prepared by suspending 1-2 colonies of candida albicans (NCIM NO.3102) FROM 24 hr. Cultures in Sabouraud's medium in to tube contain 10 ml of sterile saline. The tubes were diluted with saline. The inoculum spread over the surface of agar medium. The plate was dried at 35ºC for 15 min prior to placing the formulation. The boars of 0.5 cm diameter were prepaid and 25µl sample of formulation (1%w/v) were added in the bores. After incubation at 35ºC for 24 hr. the zone of inhibition around the boars are measure.

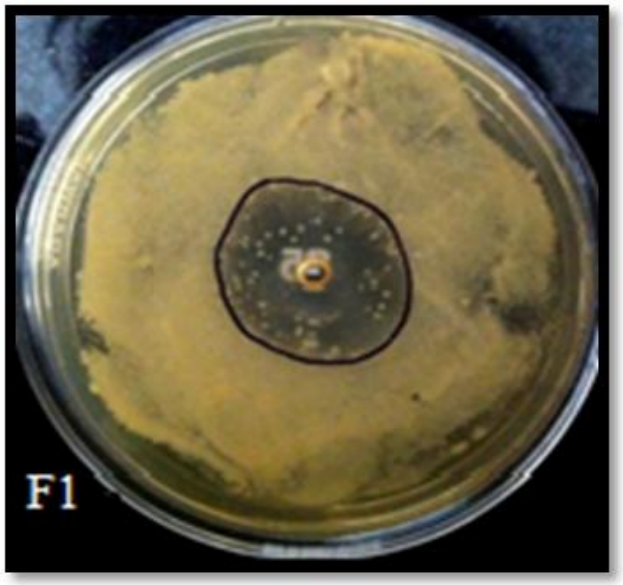



**Figure 11: Antimicrobial study.**

### 3.2.7 Accelerated stability study

Stability study were taken after 3 month according to ICH guideline through accelerated stability study for optimized batch formulation show compatible result pH of formulation and zeta potential that two factor are consider.

**Table 9: Stability study.**

| Sr.No | Consider parameter | Observation |
|---|---|---|
| 1 | **Zeta potential** | -14.1 |
| 2 | **pH** | 6.4 |

**Table 10: Zeta potential.**

| | Mean (mV) | Area (%) | Width (mV) |
|---|---|---|---|
| Zeta potential (mV): | Peak 1: -14.1 | 100.0 | 5.83 |
| Zeta deviation (mV): | Peak 2: 0.00 | 0.0 | 0.00 |
| Condutivity (mS/cm): | Peak 3: 0.00 | 0.0 | 0.00 |

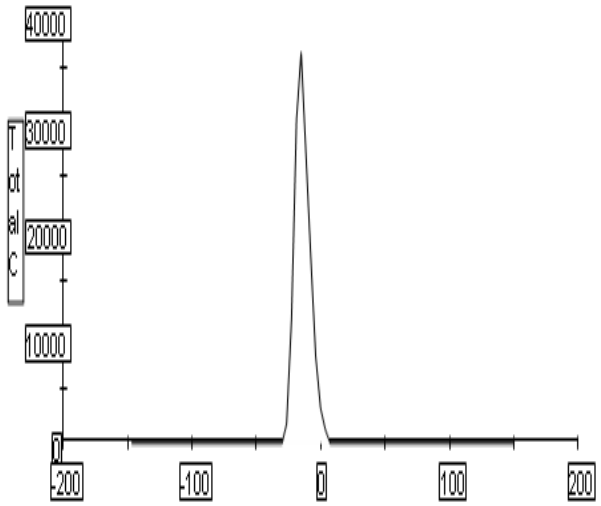



**Figure 12: Graph of Zeta potential Distribution.**

### 3.2.8 Statistical analysis[45]

In order to compare the results ANOVA (Design expert dx 7) was used. Stability data were compared using ANOVA test. Data reported A statistically significant difference was considered at is not less than 0.05.

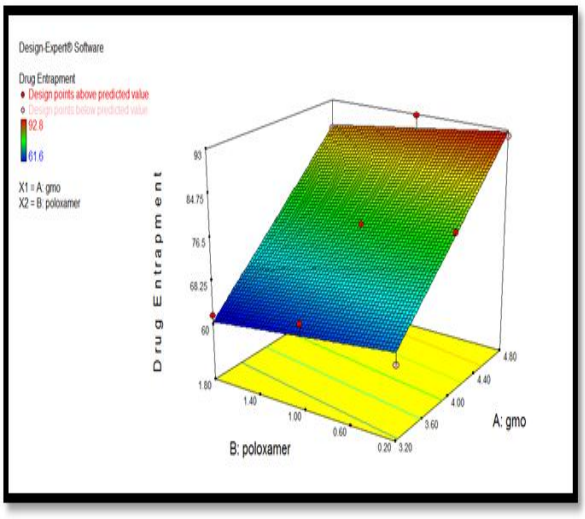



**Figure 13: 3D plot of % Drug Entrapment.**

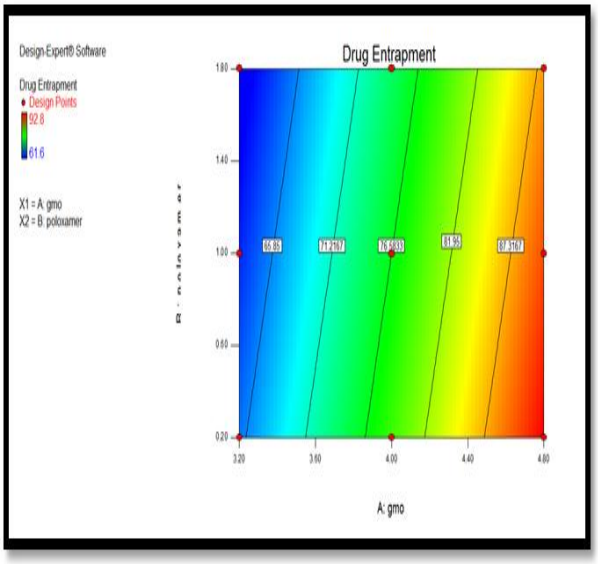



**Figure 14: Counter plot of Drug Entrapment.**

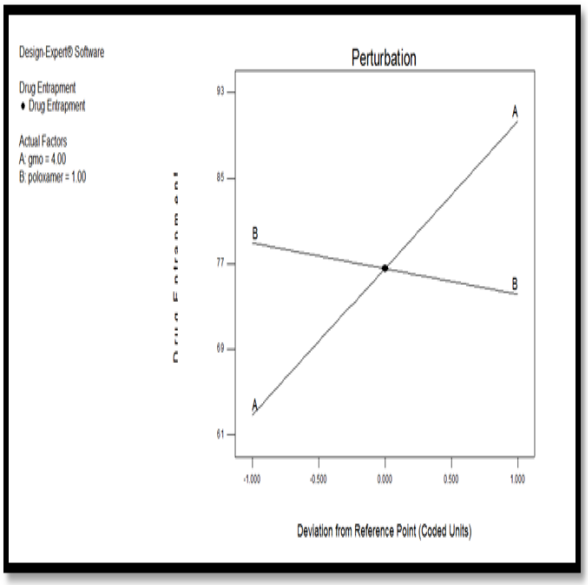



**Figure 15: Cross over design.**

## 4. SUMMARY AND CONCLUSION

Cubosomes are nano sized structures formed by dispersion of bicontinous cubic liquid crystalline phases. Fluconazole is successfully loaded in cuboseomes with 98%. Drug loading ability of Cubosomes was enhance. Emulgel was formulated and evaluated with frequently good absorption as compare to the marketed formulation. Hence, conclusion was drawn from above research is by Cubosomal emulsification method the Cubosomal Emulgel loaded with Fluconazole is can be shows good activity than other topical preparation of same drug with better stability.

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