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Preparation and Optimization of Topical Formulatons of Liquid Crystals of Clotrimazole for Increasing Permeability through Skin

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ABSTRACT

The aim of this work was to prepare liquid crystals of an antifungal drug clotrimazole to improve bioavailability and permeability of poorly water-soluble drug through the skin as generally shown by the liquid crystalline form of the drug. We have also prepared and optimized topical formulation of prepared liquid crystals of drug so as to produce a sustainable release from the formulation and thus effective antifungal therapy. 6 Topical formulations of prepared cubosomal liquid crystals of clotrimazole were formulated using different polymers (Carbopol 934, Carbopol 940 and HPMC) in different proportions. Optimized formulations were evaluated for various parameters like particle shape and size, drug entrapment efficiency and in vitro drug release profile etc. Comparative release rates of all six cubosomal gel formulations with that of marketed preparation (candid) reveal that cubosomal liquid crystalline dispersions of clotrimazole show better release rates in comparison to normal crystalline state of drug clotrimazole. Use of different polymers in gel formulation produces significant effects on release rates from the gel. Even different concentrations of a polymer have a considerable effect on release rates from a gel formulation which is reciprocal in terms of concentration i.e., when concentration of polymer is increased the release rate retards. Among all cubosomal gel formulations, F2 formulation containing 0.2 %w/v Carbopol 934 showed sustained release rate which was 66.513±2.225 % at the end of 24 hrs in comparison to 96.73±0.589 % for marketed preparation.

INTRODUCTION

Liquid crystals are a state of matter which appears to be fluid like when seen by naked eyes but appears as crystalline phase under optical polarized microscope [1]. In other words, liquid crystalline state, a new phase of matter also called as mesophase is an intermediate state having both the properties of solid crystals and ordinary liquids [2]. On experimental basis it is suggested that liquid crystals are thermodynamically stable phase [3]. Liquid crystals can be classified into two main categories i.e., ***Thermotropic*** liquid crystals and ***Lyotropic*** liquid crystals. **Thermotropic liquid crystalline** state are shown by large number of organic compounds with anisotropy of shape produced by either rise in temperature of solid and/or lowering the temperature of liquid [1]. **Lyotropic liquid crystals** are two component systems obtained when amphiphilic molecules are dissolved in a solvent to form a solution. Here the molecules come together to form "anisotropic aggregates" whereas the individual molecules come together to create anisotropy in case of thermotropic liquid crystals [4]. Recently lyotropic liquid crystal system has received importance due to their excellent potential as drug vehicle. The two important mesophases i.e., reversed cubic and hexagonal have been investigated for their ability to sustain the release of wide range of drugs. Though, the availability of only few materials which have properties to form liquid crystals in excess water limits their use, however, several preparation methods and characterization techniques make Liquid crystals as an effective tool of novel transdermal drug delivery system [5, 6].

Invasive fungal infections have increased in frequency worldwide in recent decades and have emerged as a major cause of illness and death [7]. Fungal infections in humans range from superficial and common to deeply invasive and disseminated [8]. However, the stratum corneum acts as a barrier to chemicals entering the human body and thus limited penetration of drugs through skin has produced a challenge before researchers to formulate an effective dermal delivery system. Among a large range of antifungal drugs, azole derivatives have proven their efficiency in curing dermal fungal infections among azoles; imidazole derivatives have come forth as promising candidates to cure fungal infections of skin [9]. Clotrimazole has future prospects both for its exploitation in new indications and further development of new formulations. Clotrimazole is an effective, safe and well tolerated drug having unusual chemistry that is widely used in the treatment of various skin, vulvovaginal and oropharyngeal fungal infections [9].

The aim of this work was to prepare liquid crystals of an antifungal drug through which we intended to improve bioavailability and permeability of poorly water-soluble drug through the skin as generally shown by the liquid crystalline forms of drugs. We also intended to prepare and optimize

topical formulation of prepared liquid crystals of drug so as to produce a sustainable release from the formulation and thus effective antifungal therapy. Topical formulations of prepared liquid crystals of clotrimazole have been formulated using different polymers (Carbopol 934, Carbopol 940 and HPMC) in different proportions. Optimized formulations have been evaluated for various parameters like particle shape and size, drug entrapment efficiency and in vitro drug release profile etc.

MATERIALS AND METHODS

MATERIALS: API clotrimazole and excipients- Glyceryl monooleate, Poloxamer 407, Carbopol 940was procured from Yarrow chem. Products, Mumbai. Carbopol 934 940was procured from Loba Chemie Pvt Ltd. All other chemicals- Hydroxyl propyl methyl cellulose 15 cps LR, Ethyl alcohol, Methyl alcohol, NaOH, Potassium di-hydrogen phosphate, Triethanolamine and Tween 80 was taken from labs and stores of department of Pharmacy, M.J.P. Rohilkhand University, Bareilly.

METHODS

Preformulation Studies

Prior to Preparation and evaluation of pharmaceutical dosage form containing active moiety, Preformulation Studies on various **physico-chemical properties** of procured drug was done along with **chemical authentication** by physical appearance, melting point determination and FT-IR spectra.

Physical Appearance

Drug sample was noted for its organoleptic properties simply done by visual inspection and compared with that of stated in references for standard drug [10, 11].

Melting point determination

Melting point of the drug sample was determined by capillary tube method using melting point apparatus (Macro Scientific Works). The drug sample was filled in 3 separate capillaries sealed at one end. The samples were heated slowly and observed continuously for most accurate results. The melting range was recorded which began when the sample first started to melt and ended when the sample was completely melted [11].

FT-IR spectrum of Clotrimazole drug

FT-IR spectra of pure drug Clotrimazole was carried out to check the authenticity of drug. Solid powder sample was dried in oven at around 300^0C, finely crushed, mixed with potassium bromide (1:10 ratio by weight) and pressed at 15000 psig to make a disc. The pellets were then scanned using FT-IR Spectrophotometer (Shimadzu IR Affinity 1, Tokyo, Japan). The wavelength ranged from 500 to 4500 cm^{-1} with a resolution of 4 cm^{-1}. The FT-IR spectra of pure drug were then compared with standard spectra given in analytical profiles for important peaks [11].

Development of standard analytical method

Literature review was silent on estimation of clotrimazole by UV spectrophotometric method. Therefore, UV spectrophotometric method for estimation of Clotrimazole was developed by plotting a standard curve for the same.

Determination of λ_{max} in methanol

As the drug has very poor solubility in water, a standard solution of Clotrimazole was prepared by dissolving 11.6 mg drug in 100 ml of methanol to prepare a stock solution having the concentration of 116µg/ml. From this stock solution 1ml was withdrawn in separate test tube and diluted up to 10 ml with methanol and was scanned between 200 nm to 400 nm [11].

Preparation of Standard Curve of Clotrimazole drug in methanol

Standard stock solution of Clotrimazole having concentration 116µg/ml was prepared by dissolving Clotrimazole in methanol. From this stock solution different concentrations of 11.6, 23.2, 34.8, 46.4, 58.0, 69.6, 81.2, 92.8, 104.4 and 116.0µg/ml were prepared by diluting with methanol. The absorbances of these solutions were measured at λ_{max}. A calibration curve of Concentration v/s Absorbance was plotted in Microsoft Excel.

Preparation of Liquid crystal-based Gel of Clotrimazole

Topical gel of clotrimazole based on liquid crystals was prepared by following steps-

1. Preparation and optimization of clotrimazole loaded cubosome dispersion.

1.1. Preparation of clotrimazole loaded cubosome dispersion.

1.2. Optimization of Poloxamer 407 concentration through characterization.

1.3. Optimization of Glyceryl monooleate (GMO) concentration.

2. Preparation of cubosomal topical gel of clotrimazole.

1.1 Preparation of Clotrimazole loaded cubosomes dispersion

Cubosomal dispersion of clotrimazole was prepared by Top-down method using GMO as oil phase and poloxamer 407 as surfactant. First of all, accurately weighed quantities of Glyceryl monooleate (GMO) and Poloxamer 407 were mixed and melted on an electric water bath at a temperature of 60°C and then drug (Clotrimazole) was added to this. To the resultant mixture appropriate quantity of preheated distilled water was added and stirred continuously for 15 to 20 minutes at 500 rpm until completely dissolved. The preparation was then kept aside for 2 hours and after that it was sonicated for 20 minutes.

1.2 Optimization of Poloxamer 407 concentration through characterization

For optimization of proportion of poloxamer 407 first of all five cubosomal dispersions (P1, P2, P3, P4 and P5) were

prepared in which amount of GMO was kept constant at 10% w/v (10 gm for 100 ml cubosomal dispersion) and five different concentrations- 1%, 2%, 3%, 4% and 16% w/w poloxamer 407 to that of GMO were taken to prepare cubosomal dispersions. Compositions have been shown in the table 1. Then characterization of these five dispersions was done by following methods/techniques.

Table 1: Formulation of cubosomes for Optimization of Poloxamer 407 concentration

Dispersion code	Glyceryl Monooleate (%w/v)	Poloxamer 407 (%w/w of GMO)	Clotrimazole (mg)	Water (up to 100ml)
P1	10	1	500	100
P2	10	2	500	100
P3	10	3	500	100
P4	10	4	500	100
P5	10	16	500	100

Physical appearance and homogenicity:

The physical appearance was determined simply by shaking the formulations to check the physical stability. The homogenicity of the formulations was inspected using microscope.

Optical Microscopy: The size and shape of the cubosomes were identified using compound optical microscope. Samples were suitably diluted with purified distilled water prior to visualization.

Transmission Electron Microscopy (TEM): Cubosome morphology and size analysis was done by Transmission electron microscopy. TEM was performed at SAIF Lab AIIMS, New Delhi. The prepared samples were firstly negatively stained with phosphotungstic acid solution and transferred to a carbon coated grid. Excess sample was removed by filter paper and then dried at room temperature and then microphotographs were taken.

Entrapment efficiency: Effect of concentration of poloxamer 407 on cubosome formation was also studied by estimating drug entrapment efficiency of cubosomes. The free unentrapped drug was estimated by separating the cubosomes from dispersion. For the purpose of separating entrapped drug in cubosomes from unentrapped clotrimazole centrifugation method was used. Cubosomes were centrifuged in cooling microfuge at 10^{o} C at 6000 rpm for 30 minutes. After that supernatant was collected from the resulting solution and this supernatant was then suitably diluted with methanol and estimated for free drug in UV visible spectrophotometer at 260.8 nm using methanol as blank. The % entrapment efficiency was calculated, using formula.

$$\% \text{ EE} = [\text{Total drug} - \text{free drug}] / [\text{Total drug}] \times 100$$

1.3 Optimization of GMO concentration

For optimization of proportion of GMO six cubosomal dispersions (G1, G2, G3, G4, G5 & G6) were prepared using optimized concentration (16% w/w poloxamer 407 to that of GMO), as determined through previous step, and now varying the concentration of GMO as 2.5%, 5%, 7.5%, 10%, 25% and 50% w/v to that of dispersion. Compositions of these six dispersions are given in the table 2. Thus prepared six dispersions were analyzed through following characterization methods and techniques to select the optimized cubosomal dispersion.

Table 2: Formulation of cubosomes using optimized Poloxamer 407 concentration

Dispersion code	Glyceryl Monooleate (%w/v)	Poloxamer 407 (16 %w/w of GMO)	Clotrimazole (mg)	Water (up to 100 ml)
G1	2.5	0.4	500	100
G2	5	0.8	500	100
G3	7.5	1.2	500	100
G4	10	1.6	500	100
G5	25	4.0	500	100
G6	50	8.0	500	100

Optical Microscopy: The size and shape of the cubosomes were identified using compound optical microscope. Samples were suitably diluted with purified distilled water prior to visualization.

Entrapment efficiency: Effect of concentration of poloxamer GMO on cubosome formation was also studied by estimating drug entrapment efficiency of cubosomes. The free unentrapped drug was estimated by separating the cubosomes from dispersion. For the purpose of separating entrapped drug in cubosomes from unentrapped clotrimazole ultracentrifugation method was used. Cubosomes were centrifuged in cooling microfuge at 10^{o} C at 6000 rpm for 30 minutes. After that supernatant was collected from the

resulting solution and this supernatant was then suitably diluted with methanol and estimated for free drug in UV visible spectrophotometer at 260.8 nm using methanol as blank. The % entrapment efficiency was calculated by the following formula.

$$\% EE = \frac{[\text{Total drug} - \text{free drug}] \times 100}{[\text{Total drug}]}$$

2. Preparation of Clotrimazole cubosomal topical gel

In optimization of GMO proportions G4 cubosomal dispersion was found optimum. A sufficient quantity of 300 ml of G4 cubosomal dispersion containing 0.5% drug was prepared through previously stated Top-down method. This dispersion was then used for preparing cubosomal topical gel of clotrimazole. Gels were prepared by dispersing three polymers - **Carbopol 934, Carbopol 940 & HPMC** separately in sufficient quantity of water in two concentrations of **0.1%** and **0.2% w/v** to result into six formulations **F1, F2, F3, F4, F5 and F6.** To each formulation 0.5ml tween 80 was added as penetration enhancer and stirred well. The triethanolamine was added drop by drop to neutralize the gel and was kept overnight to remove the air bubbles. The obtained gel was then diluted with appropriate quantity of (5 ml) cubosomal dispersion G4 and stirred for 5 minutes to get final gel formulations. Compositions of these gel formulations are given in the table 3.

Table 3: Formulation of Clotrimazole cubosomal topical gels

Formulation code	Cubosomes dispersion (ml)	Carbopol 934 (gm)	Carbopol 940 (gm)	HPMC 15 (gm)	Tween 80 (ml)	Triethanol-amine (ml)	Water (ml) Up to 100%
F1	5	0.1	-	-	0.5	0.125	100
F2	5	0.2	-	-	0.5	0.125	100
F3	5	-	0.1	-	0.5	0.125	100
F4	5	-	0.2	-	0.5	0.125	100
F5	5	-	-	0.1	0.5	0.125	100
F6	5	-	-	0.2	0.5	0.125	100

Evaluation of cubosomal topical Gel formulations

Physical appearance: The prepared cubosomal gels were examined visually for their color, clarity, homogeneity and presence of foreign dust particles.

pH of gel: The pH of the cubosomal gel was determined using digital pH meter**.** 2.5 gm of gel dispersed in 25 ml of water before determination.

In vitro drug release studies: In-vitro drug release from prepared gel formulations and one market formulation (candid) was carried out in phosphate buffer pH 7.4 (skin simulator media) using self-fabricated modified diffusion cell as shown in figure 5.1. We fabricated diffusion cell by hanging a 50 ml bottomless beaker in a 250 ml beaker and binding cellophane membrane, which was previously boiled for two hrs., to the bottom end of inner bottomless beaker. The inner beaker acted as donor cell whereas outer beaker was used as receiver cell. Now 10 ml formulation was uniformly spread over the cellophane membrane of donor cell and 0.2 ml of phosphate buffer of pH 7.4 was added to prevent the formulation from becoming dry during experiment. Now receptor compartment (250 ml outer beaker) was filled with 100 ml dissolution media i.e., phosphate buffer pH 7.4 and donor cell (50 ml bottomless) was hanged inside it in such a way to remain in continuous contact with the surface of dissolution media. The assembly was placed on magnetic stirrer with hot plate. The magnetic bead was placed in receptor compartment and stirred at 50 rpm at 37±2°C temperature.5 ml of receptor fluid was removed after 1, 2, 3, 4, 5, 6 and 24 hrs. for estimation of released drug. Each removed sample was replaced with same volume of fresh receptor fluid. The concentration of drug was determined in U.V spectrophotometer at 260.8 nm using self-plotted standard curve.

RESULTS AND DISCUSSION

Preformulation studies

Physical appearance

The physical appearance of the procured drug sample was found to be same as that of the official monographs. Results are shown in table 4.

Melting Point

The average melting point of procured drug Clotrimazole was found to be 143.83 °C which is within the range (141-145 °C) given in official monograph. Results have been shown in table 5.

FTIR Spectrum

For authentication of procured drug its FTIR spectrum (Fig. 1) was taken and compared with standard FTIR spectrum of clotrimazole. FTIR spectrum of procured drug showed a weak peak for aromatic C-H stretching at 3063.09 cm^{-1}, a weak peak at 2922.28 cm^{-1} , a weak and a medium peak for aromatic C=C and C=N stretching at 1649.21 cm^{-1} and 1491.04 cm^{-1} , a strong peak at 1210.38 cm^{-1} , two medium

peaks at 906.58 cm^{-1} and 823.64 cm^{-1} , a very strong peak at 761.91 cm^{-1} for aromatic C-H out of plane bending and two very strong and strong peaks at 704.05 cm^{-1} and 669.33 cm^{-1} for aromatic C-H out of plane bending. Thus, presence of all the characteristic peaks with same intensity as that of drug clotrimazole infers that procured drug is clotrimazole. Attempts have been made to clarify this comparative analysis of FTIR spectra through tabulating the data regarding peaks in table 6.

Development of standard analytical method

For quantitative estimation of Clotrimazole U.V. spectrophotometric method was developed according to method described in methodology.

The average **λ max of Clotrimazole in methanol** was found to be 260.8 nm. Results have been shown in table 7.

The **standard curve of Clotrimazole** was prepared by the different concentrations of Clotrimazole in methanol as already described in methodology. The absorbances are reported in the table 8. The data was regressed to obtain a straight line. The **R^2 value** was found to be **0.9983** indicating linearity in calibration curve (Fig. 2). The developed calibration curve follows Beer Lambert`s law good enough in the concentration range used in the experimentation. This standard curve has been used to calculate the release rate of drug from various formulations in the experiment.

Regressed Equation for standard curve: **y = 0.0021x + 0.0048**

R^2 = 0.9983

Cubosomal Topical Gel of clotrimazole

1.1 Preparation of Clotrimazole loaded cubosomal dispersion

Clotrimazole loaded cubosomal dispersion was prepared by Top-down method as described in methodology

1.2 Optimization of Poloxamer 407 concentration

Physical appearance: The prepared cubosomes dispersion was opaque, milky in color, viscous & homogeneous

Optical Microscopy (Vesicle shape and size): Microscopy was done using Olympus CX21 at 10X and 40X magnifications to visualize the shape and size of cubosomal vesicles. It was observed that by increasing the Poloxamer 407 concentration the size of vesicles was decreased but number of vesicles formation was increased that reached to the range of nanometers. Results have been shown through images shown in figure 3.

TEM: Transmission electron Microscopy done at 200 KV and at various magnifications of X15000, X19500, X29000 and X43000 revealed the morphology of cubosomes present in dispersion. The particles size of cubosomes was found to be between the ranges of **13.76 – 103.72 nm** as shown in figures 4 to 8.

Entrapment Efficiency: Entrapment efficiency done for various dispersions with different poloxamer 407 concentrations, showed that 33.632±0.669, 38.140±0.542, 42.776±0.240, 53.601±0.252 and 78.426±0.385% drug was entrapped in dispersion P1, P2, P3, P4 and P5 respectively which contains 1%, 2%, 3%, 4% and 16% poloxamer 407 w/w to that of GMO. The data shows that on increasing the concentration of poloxamer 407, entrapment efficiency also increased. Cubosomal dispersion P5, containing 16% w/w poloxamer to that of GMO showed the highest entrapment efficiency of 78.427±0.385% which is above the desirable level of 75% entrapment efficiency. Results are shown in table 9 and figure 9.

1.3 Optimization of GMO concentration

Optical microscopy revealed that cubosomes formation started when concentration of GMO reached above 10%. Results have been shown in figure 10.

Entrapment efficiency: Entrapment efficiency done for various cubosomal dispersions with different concentration of GMO showed that 64.616±0.291%, 67.728±0.557%, 73.601±0.4337%, 79.31±0.579%, 83.25±2.997% & 89.598±2.325% drug was entrapped in dispersion G1, G2, G3, G4, G5 & G6 containing 2.5%, 5%, 7.5%, 10%, 25% & 50% of GMO respectively. Results show that on increasing the concentration of GMO entrapment efficiency also increased. The highest entrapment efficiency was found to be 89.598±2.325% at 50% concentration of GMO. However, the desired entrapment efficiency of 75% was achieved at 10% concentration of GMO. Data have been shown in table 10. When the effect of concentration of GMO on entrapment efficiency was studied and plotted on a graph, it was also revealed that though after increasing the concentration of GMO beyond 10% entrapment efficiency increased, however, the increase was not considerable and proportionate to the increment in GMO concentration as shown by figure 11.

Evaluation of Cubosomal gel formulations

Physical appearance: The formulated gels were found to be homogeneous and viscous in nature.

pH: The average pH of samples was found to be in the range of 6.367-6.8. The pH of formulations F1, F2, F3, F4, F5, and F6 were found to be 6.8±0.300, 6.6±0.200, 6.5±0.361, 6.567±0.451, 6.367±0.451 and 6.767±0.306 respectively. The pH of all the formulations is close to the pH of skin. This implies that all prepared gel formulation would not produce any irritation to the skin. The pH of gel was highest for formulation F1 (6.8±0.300) and least for formulation F5 (6.367±0.451). Results are shown in table 11.

In vitro release study: In vitro drug release studies of all the total six cubosomal gel formulations and comparison with release rate of marketed gel (candid 15gm) formulation suggested that in the first hour release rates of all the six formulations were quite higher in comparison with the marketed gel preparation. The release rates were 20.06±1.342, 16.273±1.516, 25.816±0.106, 25.106±0.656, 30.813±1.206 and 26.806±1.148 % for F1, F2, F3, F4, F5, and F6 respectively whereas release rate was only 8.713±0.595 % for marketed preparation at the end of first hour. Data have been shown in table 12.

All the six cubosomal gel formulations showed much better release rates in comparison to the marketed preparation up to sixth hour of release except F2 which showed release rate of 23.35±1.815 % as compared to 24.94±0.589 % of marketed preparation at the end of fifth hour and 27.36±1.417 % as compared to 31.063±1.452 % of marketed preparation at the end of sixth hour. The higher release rate of all the six cubosomal gel formulations in comparison to marketed preparation can be attributed to the use of cubosomal dispersion for gel formulation which showed faster release of drug, as was expected from the liquid crystalline state. However, slowdown in release for F2 gel formulation, in comparison to marketed preparation, could be due to higher cross linking and thus higher viscosity and rigidity of the polymer Carbopol 934 used in the formulation F2.

For study the effect of concentration of polymer on release rate, the release rates from formulations containing the same polymer but different concentration of polymer were compared. Cubosomal gel formulation F1, containing 0.1 %w/v Carbopol 934 showed releases of 20.06±1.342, 24.993±1.100, 30.313±2.330, 38.480±2.226, 42.890±3.102, 50.41±1.606 and 70.387±1.078 % whereas cubosomal gel formulation F2, containing 0.2% w/v Carbopol 934 showed 16.273±1.516, 17.776±1.615, 19.176±1.562, 21.53±1.810, 23.35±1.815, 27.36±1.417 and 66.513±2.225 % at the end of 1, 2, 3, 4, 5, 6 and 24 hrs. respectively. Cubosomal gel formulation F3, containing 0.1 %w/v of Carbopol 940, showed 25.816±0.106, 33.656±1.669, 40.383±1.312, 45.05±2.325, 51.636±3.165, 56.46±1.496 and 79.017±1.184 % whereas cubosomal gel formulation F4, containing 0.2% w/v Carbopol 940 showed 25.106±0.656, 33.66±1.398, 37.956±2.117, 41.056±2.110, 45.963±1.858, 52.62±1.790 and 74.757±0.675 % at the end of 1, 2, 3, 4, 5, 6 and 24 hrs. respectively. Cubosomal gel formulation F5, containing 0.1 %w/v of HPMC 15, showed 30.813±1.206, 37.53±1.343, 44.043±1.991, 50.896±0.784, 60.7±1.700, 64.716±2.237 and 88.563±0.598% whereas cubosomal gel formulation F6, containing 0.2% w/v HPMC 15 showed 26.806±1.148, 35.75±1.568, 41.68±0.757, 49.44±1.708, 56.933±2.015, 59.206±1.090 and 81.907±1.484 % at the end of 1, 2, 3, 4, 5, 6 and 24 hrs. respectively. This comparative analysis shows that on increasing the concentration of polymers used in gel formulations, the release rate is retarded.

On comparing the above stated release data for analysis of release rates for different polymers used in cubosomal gel formulations, was found that release rate was higher from formulations containing polymer HPMC 15 in comparison to formulations containing Carbopol and formulations containing Carbopol 940 showed higher release rates than formulations containing Carbopol 934. The comparative release rates from all the six formulations were as following order -

F5 >F3 >F1 and F6 >F4 >F2

The explanation of the above pattern of release rates can be assigned to the hydrophilic nature and the level of cross linking in the polymers used in gel formulation. Further, the higher release rates from formulations F3 and F4 containing polymer Carbopol 940 in comparison to formulation F1 and F2 containing Carbopol 934 is in quite accordance with the established fact for carbomer polymers that higher the grade values, higher is the M_C [12] value (parameter of elasticity to swollen gel) and lower the viscosity and rigidity.

Comparison of release rates of all six cubosomal gel formulations with that of marketed preparation (Fig. 12) revealed that all the cubosomal gel formulations after a certain period of time showed lower release rate than marketed preparation. Among all cubosomal gel formulations, F2 formulation containing 0.2 %w/v Carbopol 934 showed sustained release rate which was 66.513±2.225 % at the end of 24 hrs. in comparison to 96.73±0.589 % for marketed preparation.

Table 4: Organoleptic characteristics of Clotrimazole

Parameters	For Standard Drug Clotrimazole [10, 11]	For Procured drug sample
Physical appearance	Crystalline powder	Crystalline powder
Color	White or pale yellow	White
Odor	Odorless	Odorless
Taste	Tasteless	Tasteless

Table 5: Average melting point of Clotrimazole

Sample number	Melting point (°C)
1	145.6
2	142.8
3	141.5
Average melting point	143.3

Table 6: FTIR spectrum peaks for drug Clotrimazole

Wave number (cm^{-1}) [11] Standard peaks of drug Clotrimazole	Assignment [11]	Similar peaks in FTIR spectra of procured drug (cm^{-1})
3170, 3115, 3085, 3075 (w)	Aromatic C-H stretching	3063.09 (w), 2922.28 (w)
1585, 1570, (w)	Aromatic C=C, C=N stretching	1649.21 (w)
1510, 1500, 1450 (m)	Aromatic C=C, C=N stretching	1491.04 (m), 1437.99 (m)
770, 760, 750 (vs)	Aromatic C-H out of plane bending	761.91 (vs)
720 ,700, 680 (s-vs)	Aromatic C-H out of plane bending	704.05 (vs), 669.33 (s)

(w = weak, m = medium, s = strong, vs = very strong)

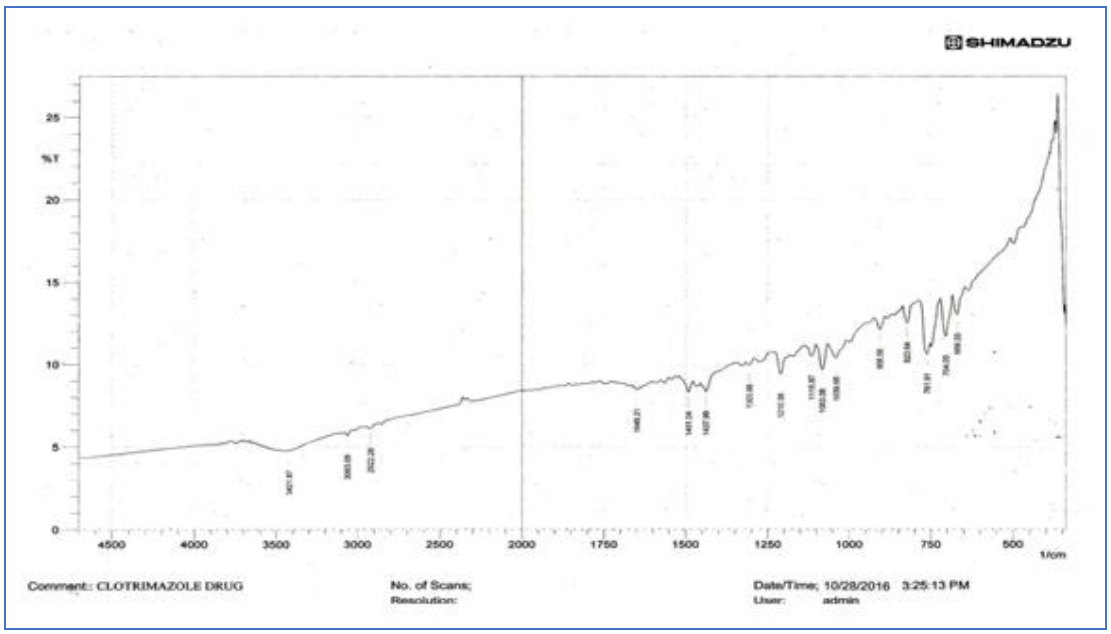


Figure: 1 FT-IR spectrum of Clotrimazole.

Table 7: λ_{max} of Clotrimazole in methanol

S. No.	Average λ max (nm)	Average absorbance
1	260.8	0.243
2	254.8	0.218
3	247.1	0.221
4	226.9	0.145

Table 8: Absorbance for standard curve in methanol

S. No.	Concentration of drug (µg/ml)	Absorbance at λ_{max} (260.8 nm)
1.	11.6	0.031
2.	23.2	0.056
3.	34.8	0.074
4.	46.4	0.103
5.	58.0	0.123
6.	69.6	0.151
7.	81.2	0.175
8.	92.8	0.192
9.	104.4	0.216
10.	116.0	0.246

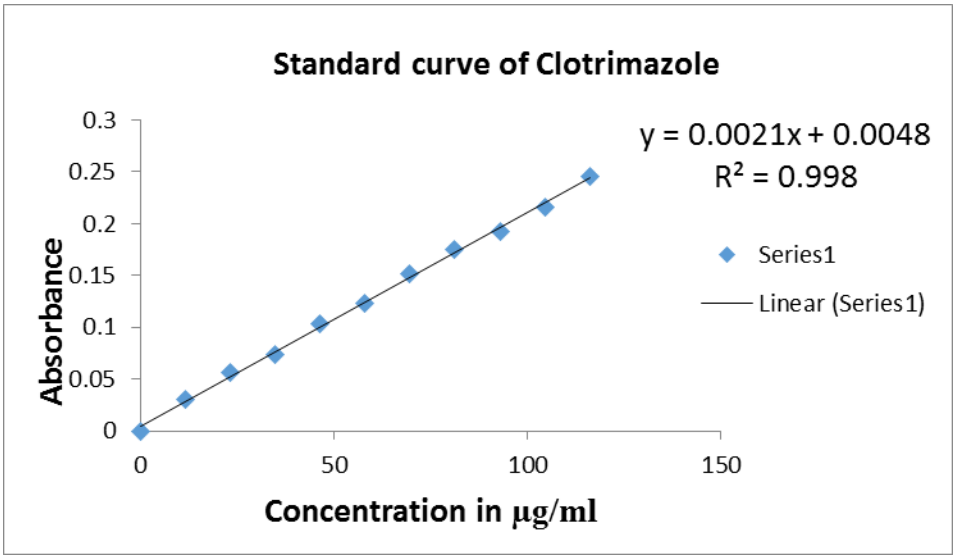
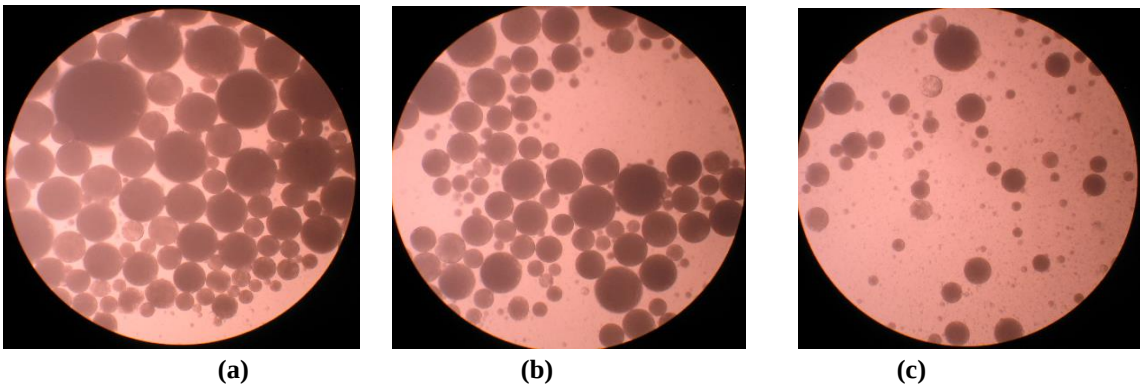
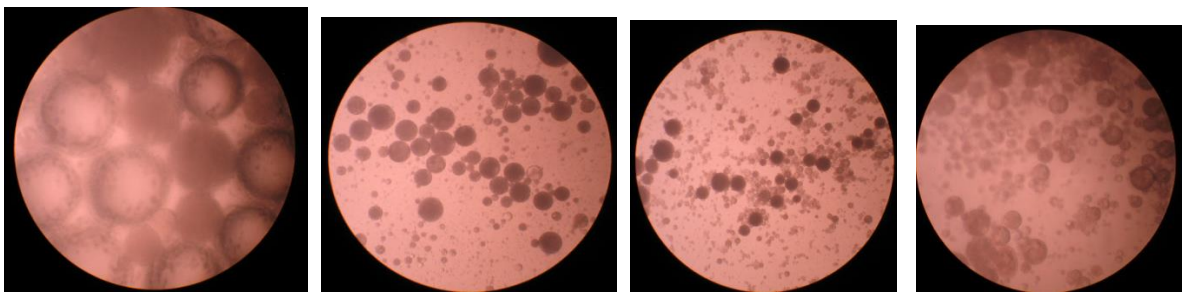


Figure 2: Calibration curve of Clotrimazole in methanol.



(a) **(b)** **(c)**



(d) **(e)** **(f)** **(g)**

(a) cubosomes with 1% poloxamer (10X) **(b)** cubosomes with 2% poloxamer (10X) **(c)** cubosomes with 3% poloxamer(10X) **(d)** cubosomes with 3% poloxamer (40X) **(e)** cubosomes with 4% poloxamer (10X) **(f)** cubosomes with 16% poloxamer (10X) **(g)** cubosomes with 16% poloxamer (40X)

Figure 3: Optical microscopy of cubosomes.

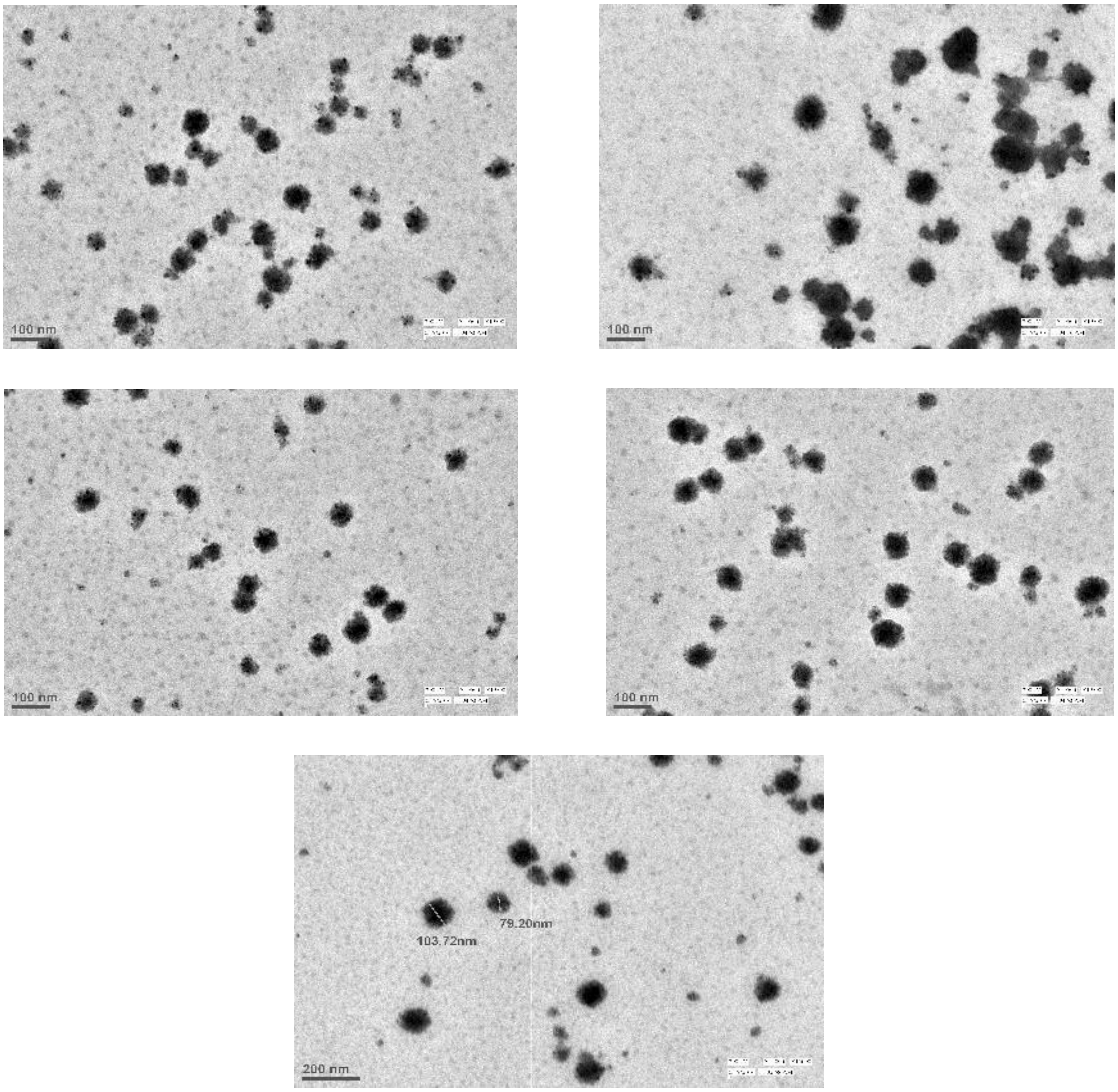


Figure 4: TEM micrographs of P_1 dispersion (1% of poloxamer 407 w/w to that of GMO).

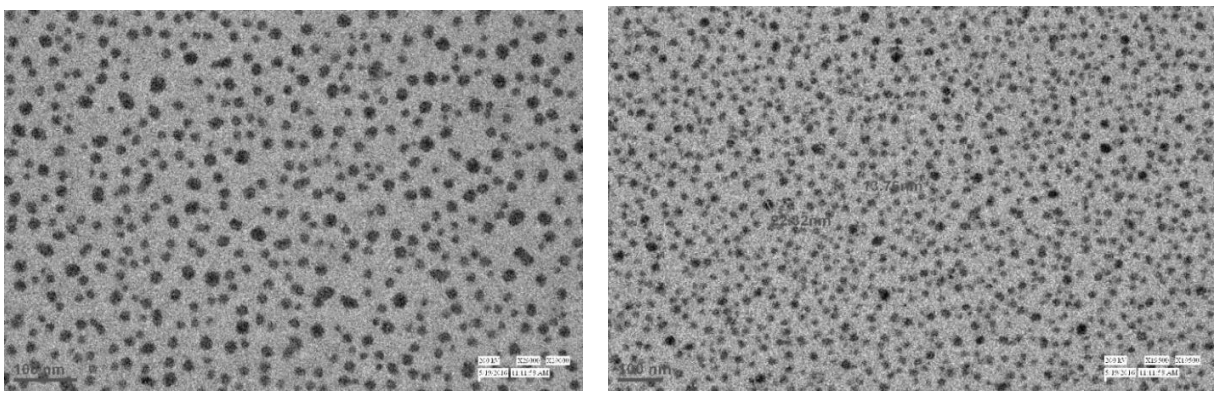


Figure 5: TEM micrographs of P_2 dispersion (2% of poloxamer 407 w/w to that of GMO).

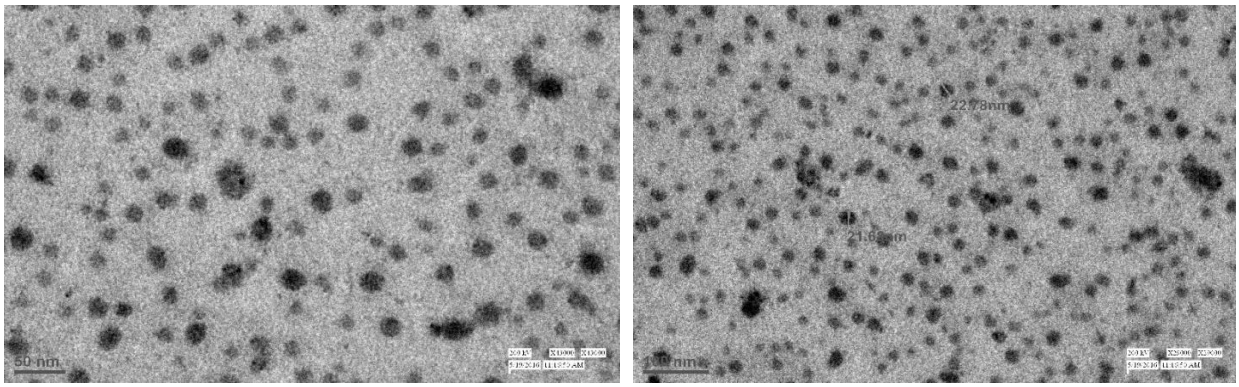


Figure 6: TEM micrographs of P_3 dispersion (3% of poloxamer 407 w/w to that of GMO).

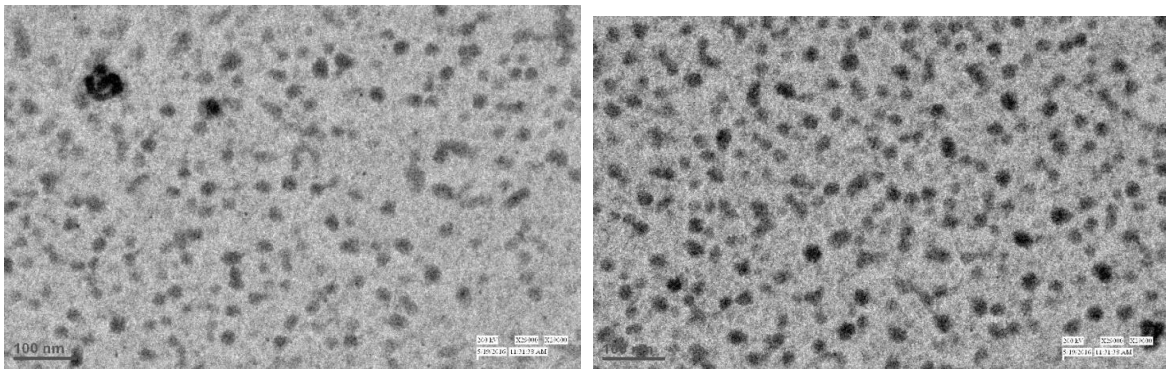


Figure 7: TEM micrographs of P_4 dispersion (4% of poloxamer 407 w/w to that of GMO).

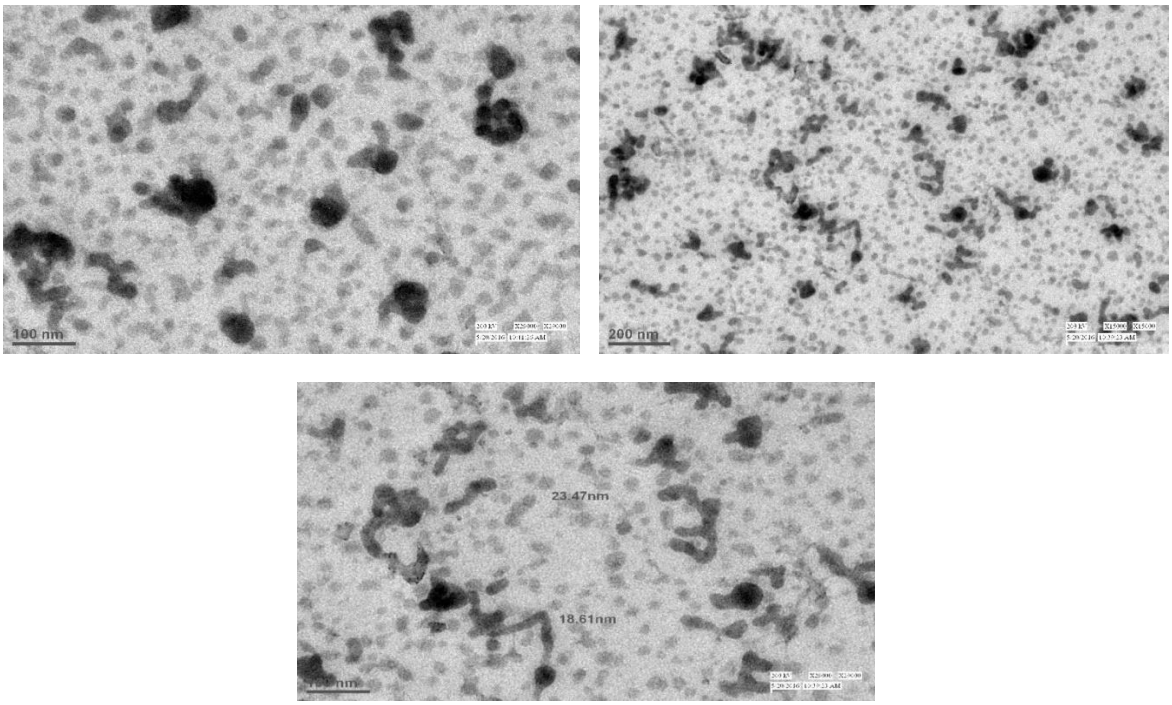


Figure 8: TEM micrographs of P_5 dispersion (16% of poloxamer 407 w/w to that of GMO).

Table 9: Effect of Poloxamer 407 concentrations on entrapment efficiency of cubosomes

S.NO	CONCENTRATION OF POLOXAMER 407	% ENTRAPMENT EFFICIENCY*
1	0	0
2	1	33.632±0.669
3	2	38.140±0.542
4	3	42.776±0.240
5	4	53.601±0.252
6	16	78.426±0.385

*** Each value represents mean ± S.D. of three observations**

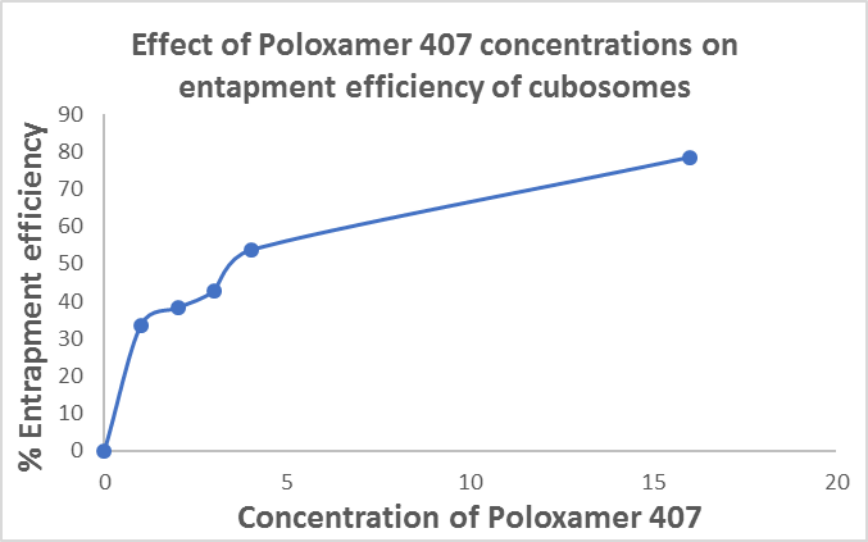


Figure 9: Effect of Poloxamer 407 concentrations on entrapment efficiency of cubosomes.

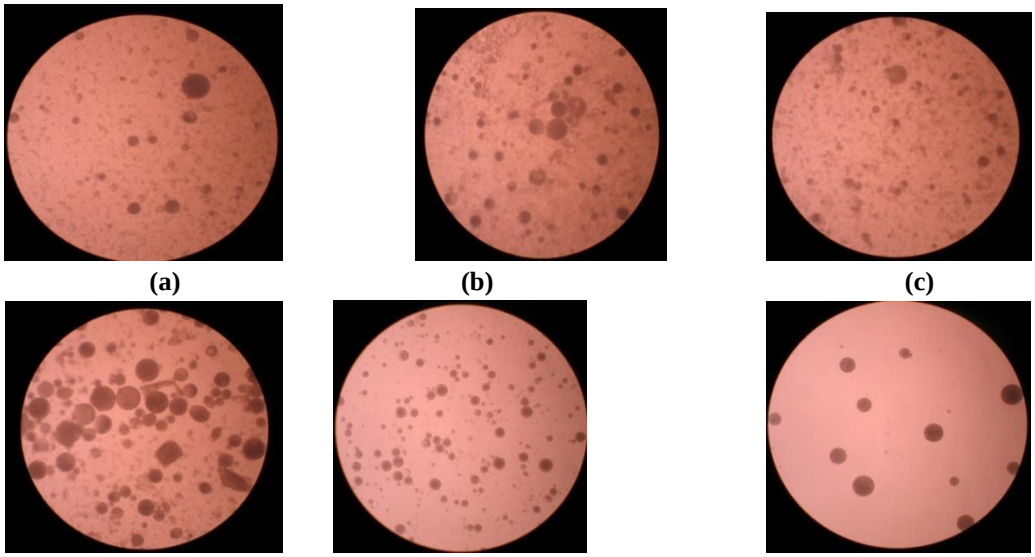


Figure 10: Structures of cubosomes **(a)** 2.5% GMO concentration at 10X. **(b)** 5% GMO concentration at 10X**. (c)** 7.5% GMO concentration at 10X. **(d)** 10% GMO concentration at 10X. **(e)** 25% GMO concentration at 10X. **(f)** 50% GMO concentration at 10X.

Table 10: Effect of GMO concentrations on entrapment efficiency of cubosomes

S. NO.	CONCENTRATION OF GMO	% ENTARMENT EFFICIENCY*
1	0	0
2	2.5	64.616±0.291
3	5	67.728±0.557
4	7.5	73.601±0.437
5	10	79.314±0.579
6	25	83.25±2.997
7	50	89.598±2.325

*** Each value represents mean ± S.D. of three observations**

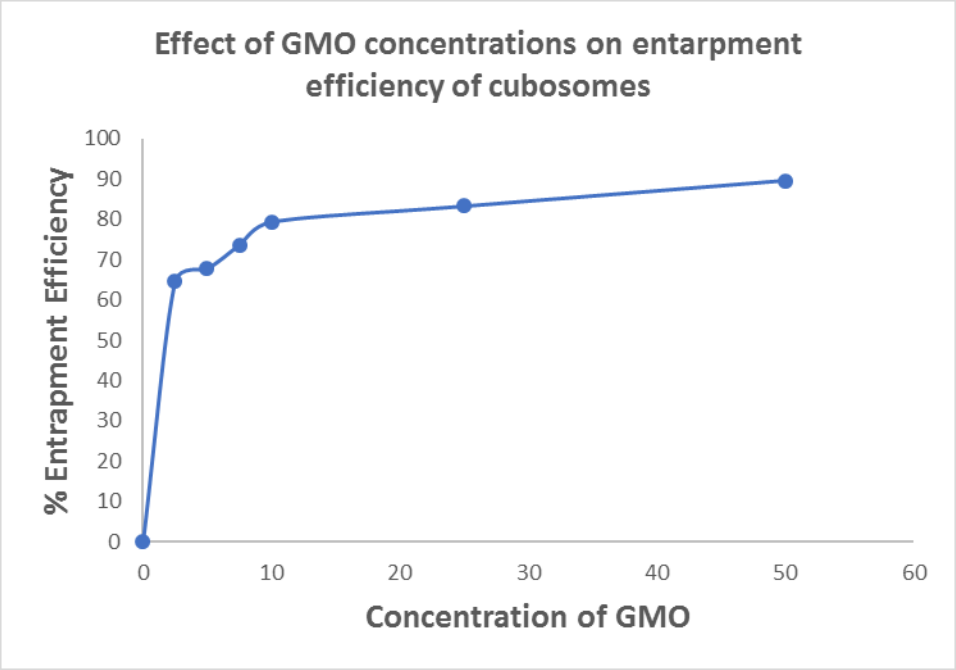


Figure: 11 Effect of GMO concentrations on entrapment efficiency of cubosomes.

Table 11: pH of Clotrimazole cubosomal topical gel formulations

S.NO	FORMULATION CODE	AVERAGE pH*
1	F1	6.8±0.300
2	F2	6.6±0.200
3	F3	6.5±0.361
4	F4	6.567±0.451
5	F5	6.367±0.451
6	F6	6.767±0.306

*** Each value represents mean ± S.D. of three observations**

Table: 12 In-vitro drug release of Clotrimazole cubosomal topical gel formulations

Cumulative % Drug Release*							
Time (h)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**Marketed preparation**
1	20.06±1.342	16.273±1.516	25.816±0.106	25.106±0.656	30.813±1.206	26.806±1.148	8.713±0.595
2	24.993±1.100	17.776±1.615	33.656±1.669	33.66±1.398	37.53±1.343	35.75±1.568	12.55±1.030
3	30.313±2.330	19.176±1.562	40.383±1.312	37.956±2.117	44.043±1.991	41.68±0.757	15.256±0.512
4	38.480±2.226	21.53±1.810	45.05±2.325	41.056±2.110	50.896±0.784	49.44±1.708	19.293±0.586
5	42.890±3.102	23.35±1.815	51.636±3.165	45.963±1.858	60.7±1.700	56.933±2.015	24.94±0.589
6	50.41±1.606	27.36±1.417	56.46±1.496	52.62±1.790	64.716±2.237	59.206±1.090	31.063±1.452
24	70.387±1.078	66.513±2.225	79.017±1.184	74.757±0.675	88.563±0.598	81.907±1.484	96.73±0.589

*** Each value represents mean ± S.D. of three observations**

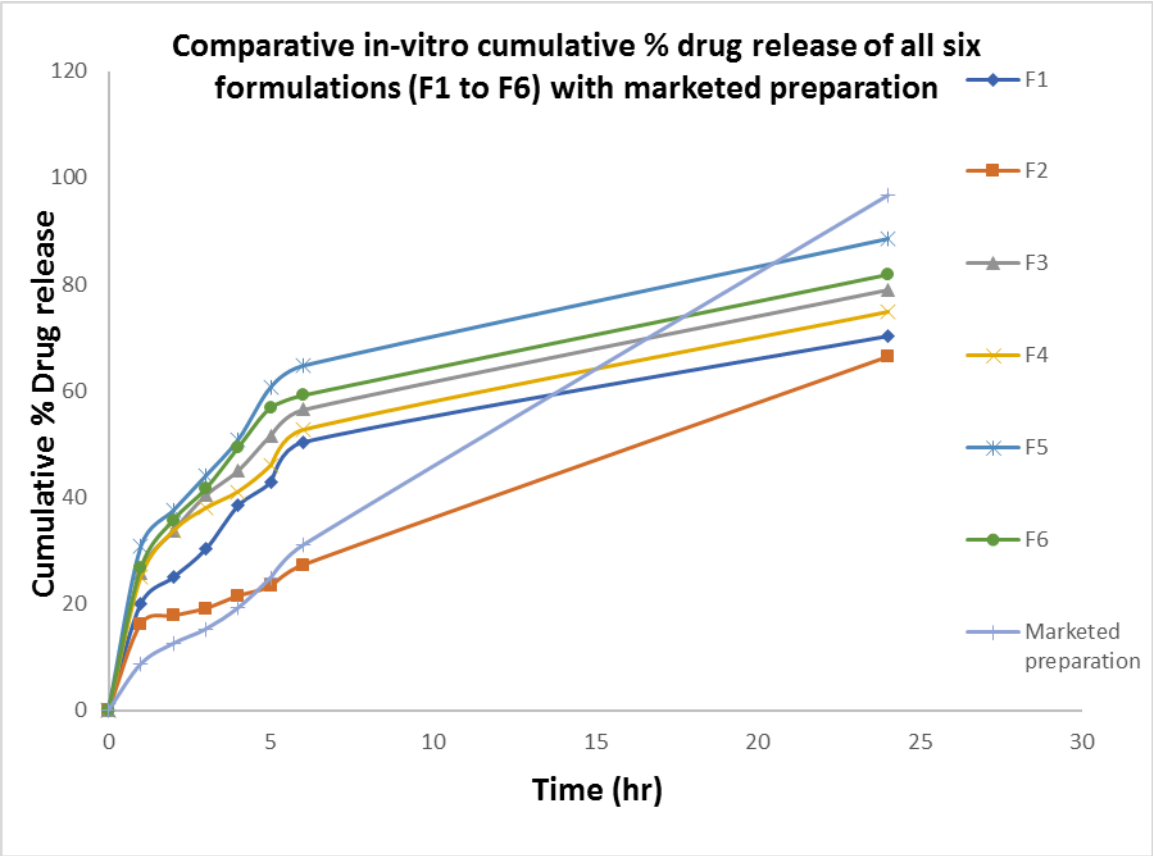


Figure 12: Comparative in-vitro cumulative % drug releases of all six formulations (F1 to F6) with marketed preparation

CONCLUSION

From present study following inferences can be withdrawn –

- Comparative release rates of all six cubosomal gel formulations with that of marketed preparation (candid) reveal that cubosomal liquid crystalline dispersions of clotrimazole show better release rates in comparison to normal crystalline state of drug clotrimazole.
- Use of different polymers in gel formulation produces significant effects on release rates from the gel.
- Even different concentrations of a polymer have a considerable effect on release rates from a gel formulation which is reciprocal in terms of concentration i.e., when concentration of polymer is increased the release rate retards.

From above stated inferences withdrawn from present study, it can be concluded that for topical preparation a better bioavailability can be achieved by use of liquid crystalline dispersions of drug which can result in an early and effective therapy of topical ailments. At the same time, if one intends to sustain the release of drug from the topical gel formulation use of suitable polymer in a suitable concentration can lead to desirable pattern of sustained release. However further investigation is suggested for optimization of cubosomal gel formulations according to desirable pattern of one's requirements.

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