International Journal of Drug Delivery 7 (2015) 01-12
http://www.arjournals.org/index.php/ijdd/index

Original Research Article

ISSN: 0975-0215

Miconazole Nitrate based cubosome hydrogels for topical application

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Abstract

Cubosomes are discrete, sub-micron, nano-structured particles of bicontinuous cubic liquid crystalline phase. Cubosomes consist of honeycombed (cavernous) structures separating two internal aqueous channels and a large interfacial area. They are the biocompatible novel approach for the drug delivery system. The controlled release application of these nanoparticles is of a great significance in cosmeceutical and pharmaceutical fields. The present study is concerned with the design and evaluation of a novel nano-particulate system; cubosomes, loaded with miconazole nitrate (MN); which is used as antifungal agent. Cubosome dispersions were formulated by emulsification technique using different concentrations of a lipid phase monoolein and the nonionic surfactant, Poloxamer 407. The prepared cubosomal dispersions were characterized regarding dimensional distribution, particle size, and in vitro drug release. The optimum formulae were incorporated in a CMC or HPMC based hydrogels, to form cubosomal hydrogels (cubogels). The cubogels were characterized regarding in vitro release of (MN), viscosity and pH. A comparison between the cubogels and a commercially available product, Miconaz® cream, was carried out to judge their efficacy. The drug release from the commercial preparation was lower than all the prepared nano-emulsion based gel formulations. G1 and G8 showed highest drug release percent (100%) after 8 hours, in contrast the marketed formulation released (44.8%) of the drug after 8 hrs. The in vitro Miconazole nitrate release data were fitted to Korsmeyerpeppa's release model. The formulation exhibited non-fickian transport with zero order kinetics. Formulae G1, G8 and G10, that showed both small droplet size and highest extent of drug release, were microbiologically evaluated against Candida albicans (C. albicans) using agar cup diffusion method. The selected formulae showed superior antimycotic activity compared to the commercially available formulation.

Keywords: Cubosome, Miconazole, Candida albicans, Cubogel, Topical delivery, Nanoemulsion.

Introduction

Miconazole nitrate is an imidazole derivative characterized by longer half-life and higher efficacy in the treatment of the protozoal and anaerobic bacterial infection of the vagina [1]. Miconazole nitrate (MN) is a broad-spectrum antifungal agent of the imidazole group. It acts inhibiting ergosterol biosynthesis, which causes lysis of fungal cell membranes because of the changes in both membrane integrity and fluidity and direct membrane damage of the fungal cells [2]. The drug is primarily used as a topical treatment for cutaneous mycoses; poor dissolution and lack of absorption make it a poor candidate for different route of administration. However, MN can be used as a systemic antifungal agent when amphotericin B or ketoconazole is either ineffective or contraindicated. MN's poor skin penetration capability presents a problem in the treatment of cutaneous diseases by topical application [3]. The stratum corneum is the target organ of anti-mycotic treatment, and the improvement of local bioavailability leads to enhanced efficacy of the applied formulation. For effective treatment, the drug must be delivered in sufficient concentration 1to the site of infection. Various approaches have been used to enhance the access of such poorly skin-partitioned drug molecules. Several reports have described the potential use of cubosomes in order to deliver drugs into the deep layers of the skin [3]. The aim of this study was to develop topical cubosomal dispersions loaded with Miconazole nitrate. The cubosomes were prepared by hot high-pressure homogenization method and were characterized in terms of particle size, morphology and in-vitro drug release followed by the incorporation of the optimal cubosomal dispersion in CMC or HPMC hydrogels (cubogels).The influence of the cubogels on ex-vivo drug skin permeation was evaluated and compared with a conventional gel. The emulsification of the cubic lipid phases in water results in the production of cubosomes that can be defined as nanoparticulate dispersal systems characterized by high biocompatibility and bioadhesivity [4]. Cubosomes are bicontinuous cubic phase liquid crystals have many properties that

make them appealing as a universal vehicle for drug delivery [5]. The surfactant assembles into bilayers that are twisted into a three dimension, periodic, minimal surface forming tightly packed structure, like "honeycombed" with bicontinuous domains of water and lipid. In the field of drug delivery, the bicontinuous cubic phases, particularly cubosomes, are promising vehicles since they are bioadhesive and have a property for controlled drug release [6]. They are capable of incorporating both hydrophobic and hydrophilic drugs and are found to protect drugs from degradation [7]. Cubosome could also enhance the drug deposition in the skin and showed excellent skin targeted characteristics [8].

Manufacturing Of Cubosomes

Cubosomes can be manufactured by two distinct methods [9 And10].

Top down Technique

Bulk cubic phase is first produced and by 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 differ in that they are a single thermodynamic phase and have periodic liquid crystalline structure. Cubic phases ruptures in a direction parallel to the shear direction, the energy required is proportional to the number of tubular network branches that rupture.

Bottom up Technique

In this cubosomes are allowed to form or crystallize from precursors. The bottom-up approach first forms the nanostructure 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 hydrotrope 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.

Materials and Methods

Materials

Miconazole nitrate was kindly donated as a gift from Medical union Pharmaceuticals company, Cithrrol ® HP-SO-(LK) RV06530 was used as the source of GMO and Synperonic (PE/F127) Flakes [Poloxamer 407 (P407)], were kindly supplied as a gift from croda. Polyvinyl alcohol, Polyethyleneglycol 400, propylene glycol, HPMC15000 were obtained from Sigma Chemical Co. (St. Louis, USA). Ethanol was purchased from ADWIC, (Egypt). Miconaz® skin cream (Miconazole nitrate 2% w/w, manufactured by Medical union Pharmaceuticals) was purchased from a local pharmacy store. All other materials were of analytical grade.

Methods

Preparation of MN loaded cubosomes Preparation of cubosome dispersions was based on the emulsification of monoglyceride/surfactant mixtures in water [11]. In particular, the monoglyceride based lipidic phase was glyceryl monooleate. Poloxamer 407 was used as surfactant in ratio to glyceryl monooleate of 1:9, 1:6, 1:3 and 1:15. The concentration of the glyceryl monooleate/surfactant mixture was 5% w/w with respect to the total weight of the dispersion. Composition of prepared MN loaded cubosomes is presented in table 1. PVA and PG were used in addition to poloxamer as a stabilizing agent for the dispersion, and it was added by solubilization at 80 C in the aqueous phase in concentration of 2.5% w/w with respect to the disperse phase. Briefly, GMO and Poloxamer 407 were melted on a hot plate. MN was dispersed in the molten mixture. The molten mixture was then added dropwise to the aqueous phase at 70 C under mechanical stirring at 1500 rpm [12]. Dispersions were maintained under stirring for two hours, afterwards the dispersions were subjected to homogenization at 15000 rpm at 60 C for one minute and then were cooled to room temperature up to the solidification of lipid droplets [13]. Dispersions were stored in glass bottles at room temperature for further investigations.

In vitro characterization of prepared MN loaded cubosomes

Determination of MN content

MN loaded cubosomes were mixed with methanol and sonicated for 10 min to obtain a clear solution [11]. Concentrations of MN were determined spectrophotometrically λmax 272 nm.

$$\textbf{Drug Content} = \frac{\textbf{Acutual Yeild}}{\textbf{Theoritical Yeild}} \textbf{X 100}$$

Table 1. Composition of prepared MN loaded cubosomes

Dispersions	Disperse phase (5% w/w with respect to the total weight of the dispersion)			Additive (gm)			MN	water (gm)
	Lipid monoolein (gm)	Surfactant (gm)	Poloxamer 407	PVA	Oliec acid	PG		
D1	2.25		0.25	-	-	-		46.5
D2	2.143		0.357	-	-	-		46.5
D3	2.25		0.25	-	0.55	-		45.95
D4	1.875		0.625	-	1.13	-		45.37
D5	2.25		0.25	2.5	-	-		44
D6	2.143		0.357	2.5	-	-		44
D7	1.875		0.625	-	-	-	2%	46.5
D8	2.343		0.157	-	-	-		46.5
D9	2.343		0.157	2.5	-	-		44
D10	2.25		0.25	-	-	2.5		44
D11	2.143		0.357	-	-	2.5		44
D12	2.343		0.157	-	-	2.5		44
D13	1.875		0.625	-	-	2.5		44
D14	1.875		0.625	2.5	-	-		44

Particle size analysis of cubosomes

Particle size analysis of the dispersions was performed using a Zetasizer 3000 PCS (Malvern Instr., England) equipped with a 5 mW helium neon laser with a wavelength output of 633 nm. Measurements were made at 25 C, angle 90 , run time at least 180 sec. Data interpreted by the method of cumulants.

High Resolution Transmission electron microscopy (HRTEM)

The samples were prepared by placing 5 µl droplet of the prepared dispersion onto a 300 mesh carbon coated copper grid, and allowing cubosomes to settle for 3–5 min. Excess fluid was then removed by wicking it off with an absorbent paper. The samples were then viewed on a JEOL Model- (JEM- 2100) 200KV transmission electron microscope with resolution of 0.1432 nm and magnification power of 1.5 M.

In vitro release of MN from prepared cubosomes and the kinetic analysis of the data

The in-vitro release of miconazole nitrate from different cubosomes was performed using the dialysis method [1]. The semipermeable cellophane membrane was obtained from Sigma which has a molecular wt cut off 12,000 Daltons was previously soaked in phosphate buffer of pH 6.4 and dried, and was stretched over the open end of a glass tube having a diameter of 3 cm. An amount of MN cubosomes equivalent to 50 mg drug was spread on the membrane to occupy all 3 cm diameter circle. The tubes were then immersed upside – down in a 250 ml beaker containing 100 ml phosphate buffer pH 6.4 which is preheated and maintained at 37 ± 1ºC in a constant temperature water bath. The tubes height was adjusted so that the membrane was just below the surface of the release medium. The whole assembly was shaken at 50 rpm during the entire time of diffusion. For each gel sample, 3ml was withdrawn at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8 and 24 hours´ time intervals and replaced by equal volumes of fresh release medium maintained at the same temperature. Samples were measured spectrophotometrically at λmax 272 nm against the same used phosphate buffer as a blank using Jenway spectrophotometer (Model 6105UV/Vis, Eng-land). The amounts of drug released were calculated on the basis of the standard curve previously constructed. Sink condition was maintained throughout the experiment. To study the drug release mechanism of each formulation, the release data were fitted to the general exponentional function: Mt / M0 = ktn; where Mt / M0 represents the fractional uptake of solvent (or release of a solute) normalized with respect to the equilibrium conditions; n is a diffusion exponent characteristics of the release mechanism, and k denotes properties of the polymer and the drug. This equation has been used frequently, due to its utility in describing the relative importance of Fickian (n = 0.5) and Case II (n 1.0) transport in anomalous diffusion. Kinetic studies were performed by adjusting the release profiles to Higuchi and zero order equations [14].

Preparation of hydrogels loaded with MN cubosomes (cubogels)

Cubogels were prepared by sprinkling HPMC (1.5% w/v) or CMC (2%w/v) over a specified amount of liquid cubosome dispersion and stirred with mechanical stirrer. Propyl paraben sodium salt (0.1% w/w) was added as a preservative to the preparation. Stirring was continued until a thin hazy dispersion, without lumps, was formed. For complete gel dispersion it was necessary to leave samples overnight in the refrigerator [14]. For cubogels containing 5% PEG or PG, they added to the dispersion followed by the addition of gelling agent. The compositions of the prepared cubogels are shown in table 2.

Table 2. Compositions of the prepared cubogels

Gel	Dispersion [a]	Polymers
G1	D1	HPMC 1.5% + PEG 5%
G2	D1	HPMC 1.5% + PG 5%
G3	D1	CMC 2%
G4	D1	CMC 2% + PEG 5%
G5	D7	HPMC 1.5% + PEG 5%
G6	D7	HPMC 1.5% + PG 5%
G7	D7	CMC 2%
G8	D7	CMC 2% + PEG 5 %
G9	D8	HPMC 1.5% + PEG 5%
G10	D8	HPMC 1.5% + PG 5%
G11	D8	CMC 2%
G12	D8	CMC 2% + PEG 5%
G13	D10	HPMC 1.5% + PEG 5%
G14	D10	HPMC 1.5% + PG 5%
G15	D10	CMC 2%
G16	D10	CMC 2% + PEG %
C1	-	HPMC 1.5% + PEG 5%
C2	-	HPMC 1.5% + PG 5%
C3	-	CMC 2% + PEG %

A All formulae contained 2% MN.

In vitro characterization of prepared cubogels

Evaluation of the physical properties of the prepared cubogels

Determination of pH

The pH of cubogels was measured on digital pH meter standardized using pH of 4.0 and 7.0 standard buffers before use [14].

Drug content studies

The drug content studies of MN cubogels were investigated as prescribed previously.

Viscosity measurement

A Cole parmer viscometer was used to measure the viscosity of the prepared gel bases. The spindle was rotated at 10 rpm. Samples of the bases were allowed to settle over 30 min at room temperature before the measurements were taken [14].

In vitro release of MN from the prepared cubogels and kinetic analysis of the release results

The in vitro release studies of MN cubogel were investigated as prescribed previously. In vitro drug release from commercial product Miconaz® was carried out using the same procedure.

Ex vivo permeation of MN cubogels, through rabbit skin

Preparation of a rabbit skin

Dorsal full-thickness skin of male rabbit was obtained from white Newzealand rabbits weighing 3-4 kg. The skin was carefully removed from animals and after sacrificing them, the hair was clipped without damaging the skin. The fat was removed with the aid of scissor and skin was washed and the excised full thickness rabbit skin samples were stored at 20oC prior to use [15]. The excised full thickness rabbit skin samples were equilibrated by soaking in buffer solution of pH 6.4 for about one hour before beginning of each experiment.

Ex vivo permeation studies

The first three formulations that gave best Q (the cumulative amount of drug penetrates through the unit surface area of the membrane) after 24 hours were chosen for in vitro permeation studies of MN through dorsal rabbit skin. The same procedure for testing permeation through cellulose membrane was applied with the exception that the prepared skin samples were mounted on the donor compartment of the assembled permeation cell with the stratum conium facing upward and the dermal side facing downward with permeation area of 3.14cm2. The donor compartment was kept hanged on the receptor compartment and secured tightly with the help of clamps. The receptor compartment was then filled with 100 ml of phosphate buffer solution of pH 6.4. The temperature of media was maintained at 37±0.5oC. The amount of drug permeated through rabbit skin per unit area (μg/cm2) was plotted against time (hours) [15].

Calculation of permeation parameters across rabbit skin

Different permeation parameters of MN was calculated such as steady state fluxes (Jss) that were calculated from the slope of the portion of the amount permeated through unit area of the rabbit skin versus time plot (14). Permeability coefficient (Kp) through rabbit skin, Diffusion coefficient (D) within the rabbit skin and lag time (tL) were calculated from the penetration data.

Microbiological study of MN

The agar medium was prepared by dissolving 65 gm of sabouraud dextrose agar powder per liter of distilled water and was sterilized using autoclave at 121 C for 20 min. The indicator isolate of Candida albicans (ATCC 90028) was inoculated into sabouraud's dextrose agar plate and was incubated for 48 hr prior to testing. Once actively growing colonies of Candida albicans is obtained, a single well isolated colony was picked with a wire loop from sabouraud's dextrose agar plate and inoculated in sterilized normal saline and its turbidity is adjusted to match that of standard of 0.5M McFarland barium sulfate, so that it contains approximately 107 cells/ml. The isolate of Candida albicans was seeded from standardized suspension to a concentration of 3 µl per ml in agar medium, the seeded agar medium was poured into Petri-dishes (15cm diameter) to a depth of about 4mm and numbers of wells in each dish were cut using Wassermann tube of 6mm diameter. The antifungal efficacy of MN cubogels and commercial MN cream (Miconaz®) was determined by performing agar-cup diffusion assay. The effectiveness of the prepared MN cubogels against Candida albicans was studied, by applying 0.4 gm of the gel and an equivalent weight of commercial cream on the sabouraud dextrose agar which was previously seeded with Candida albicans. The Petri dishes were then incubated at 25OC.The effectiveness of the prepared gel was compared with plain gel contains 0% of MN and the commercial MN cream Miconaz®. The zones of growth inhibition were measured for all the tested samples. Each type of the samples was tested in triplicate [16]. The inhibition zone of growth of Candida albicans was measured in mm after 24 and the mean inhibition zone was then calculated.

Statistical analysis

Statistical analysis of the results was performed using one-way analysis of variances (ANOVA) to determine the significance of differences between groups; a P value less than 0.05 was considered statistically significant.

Results and discussion

Determination of MN content

Drug content was determined in order to make sure that the added amount of MN is present in the cubosome dispersion. Cubosome dispersions had drug content ranging from 86.6% to 94.56% of the added amount of MN as shown in table 3.

Table 3. Drug content, Z-average and PDI of measured cubosomes (n = 3) Particle size analysis of cubosomesParticle

Dispersions	Drug content (%) ± SD	Z-average (d.nm) ± SD	PdI ± SD
D1	92.45	137.0±1.40	0.215±0.02
D2	87.23	172.1±1.68	0.301±0.004
D3	86.6	149±0.658	0.273±0.0007
D4	87.45	133.8±0.39	0.219±0.23
D5	90.34	501.4±1.15	0.544±0.4
D6	91.42	197.8±0.30	0.219±0.03
D7	89.65	148.5±2.98	0.233±0.004
D8	88.32	163.3±0.97	0.226±0.05
D9	87.49	225.8±1.82	0.421±0.01
D10	94.56	128.9±0.77	0.215±0.03
D11	93.55	152.8±1.07	0.301±0.03
D12	89.97	148.6±1.66	0.417±0.02
D13	86.78	131.7±0.26	0.239±0.005
D14	90.12	169.9±0.28	0.205±0.01

size measurement was done to confirm that particles of the dispersion are all of nanometer range. Z-average of all the measured dispersions is shown in table 3. Z-average of all dispersions was in the nanometer range (average particle size

values ranged from 128.9 nm to 501.4 nm), with a polydispersity index of < 1. The cubosomes of diameter 100 nm are considered small cubosomes while those of diameter > 200 nm are larger cubosomes [12]. The addition of PVA as a stabilizer at ratio of 2.5% resulted in a significant increase in particle size at $p < 0.05$(> 200 nm for D5 and D9) as shown in table 3, although the increase in surfactant concentration with PVA (2.5 %) results in a significant decrease at $p < 0.05$ in the particle size again. This result is in agreement with the results obtained by Esposito et al [13].The addition of PG at ratio of 2.5% resulted in a significant increase in particle size (D 10, D11, D12 and D13).Results of the one-way ANOVA showed that the total concentration of surfactant and PVA had significant effect on the average particle size (Z-average (d.nm)) of cubosomes ($p < 0.05$).

High Resolution Transmission electron microscopy (HRTEM)

The high resolution transmission electron micrographs showed that the investigated cubosome (D1) is in the nano-size, which confirms the results of particles size measurement (figure 1). Micrographs reveal the coexistence of the particles and vesicles characterized by cubic organization and well separated from each other.

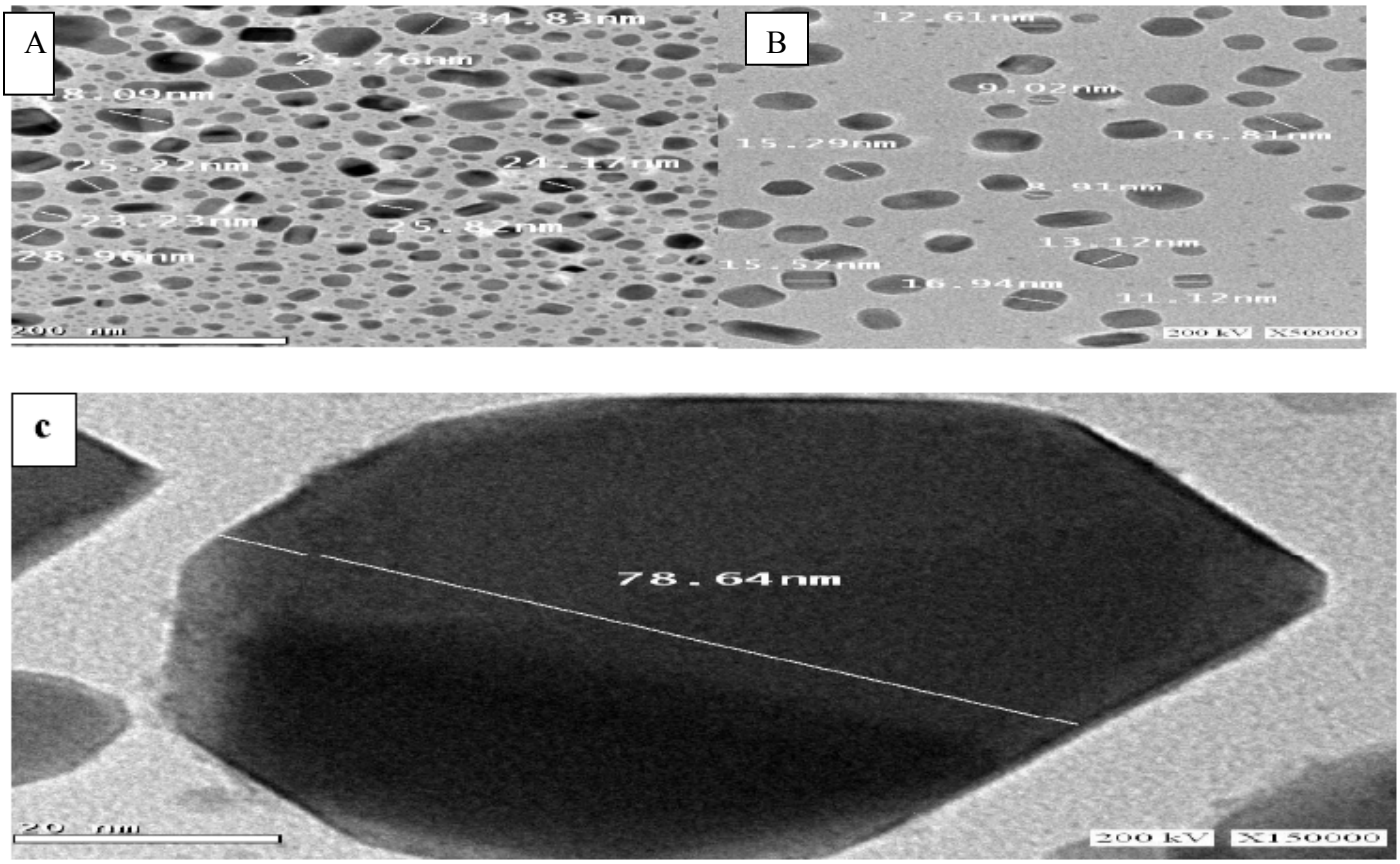

Figure 1. High resolution transmission electron micrographs of cubosome dispersions (D1); (a) 50 kx, (b) 20 kx and (c) 1500 kx, the cubosomes are cubic in shape, nano-sized and well separated from each other.

In vitro release of MN from prepared cubosomes and the kinetic analysis of the data

In vitro release profile of MN from cubosomes was performed in Phosphate buffer at pH of 6.4 using the dialysis method as shown in figure2. The release of MN from its solution was very slow; it releases about 6.8% after 7 h. The dispersion containing PVA (D5, D6, D9 and D14), about 21, 44.8, 9.8 and 15.9 % respectively of MN was released during the first 7hrs, The addition of PVA resulted in significant decrease in the percent of drug released after 2, 4 and 7 hours at $p < 0.05$, as demonstrated in figure 2b. This result is in agreement with the results of Morsi et al [11]. PVA is a polyol which is known to be an excellent size controlling agent and stabilizer [11]. For the dispersion (D1, D7, D8 and D10) which

contain no PVA, about 100.52, 100.8, 98.28 and 70.28 % respectively of MN was released during the first 7hrs.

The in vitro release characteristics of percutaneous cubosomes showed that the drug release is directly proportional to the concentration of GMO and inversely proportional to the concentration of P-407 i.e. the cubosomes showed decrease in percent drug release when using of lower concentration of GMO and higher concentration of P-407 polymer. It was found that in the presence of less poloxamer 407 concentration, cubic particles exists in Pn3 m (CD) patterns, it has been observed that at low concentration of Poloxamer 407, most of the polymer adheres to the surface of cubosome particles and a few participate in the formation of the internal cubic structure [17]. At high poloxamer 407 concentrations, the polymer is incorporated into the bulk of the cubic-phase matrix and Im3 m (CP) patterns appear in the dispersions. It is suggested that the cubic particles with cP structure have lower drug release efficiency, and the drug release is more restricted than that of the particles with cD structure [18]. Dispersion containing oleic acid (D3andD4) showed a significant reduction in drug release percent (13.3 and 25.2% respectively) at $p< 0.05$ after 7 hours. This is may be due to an increase in lipophilicity in the formulation which attracts the hydrophobic drug towards itself leading to slow release from the nanoparticles [8].

Kinetic studies for drug release from different dispersions

The kinetic analysis of the release data is shown in table 4, and it can be observed that the release of most formulae (D1, D3, D4, D6, D7, D9, D10, D12 and D13) exhibited non- Fickian transport according to Korsmeyerpeppa's release model. And most of formulae followed zero –order kinetics (D1, D3, D6, D7, D9, D10, D12, D13 and D14) as indicated from the highest coefficient of determination (r).

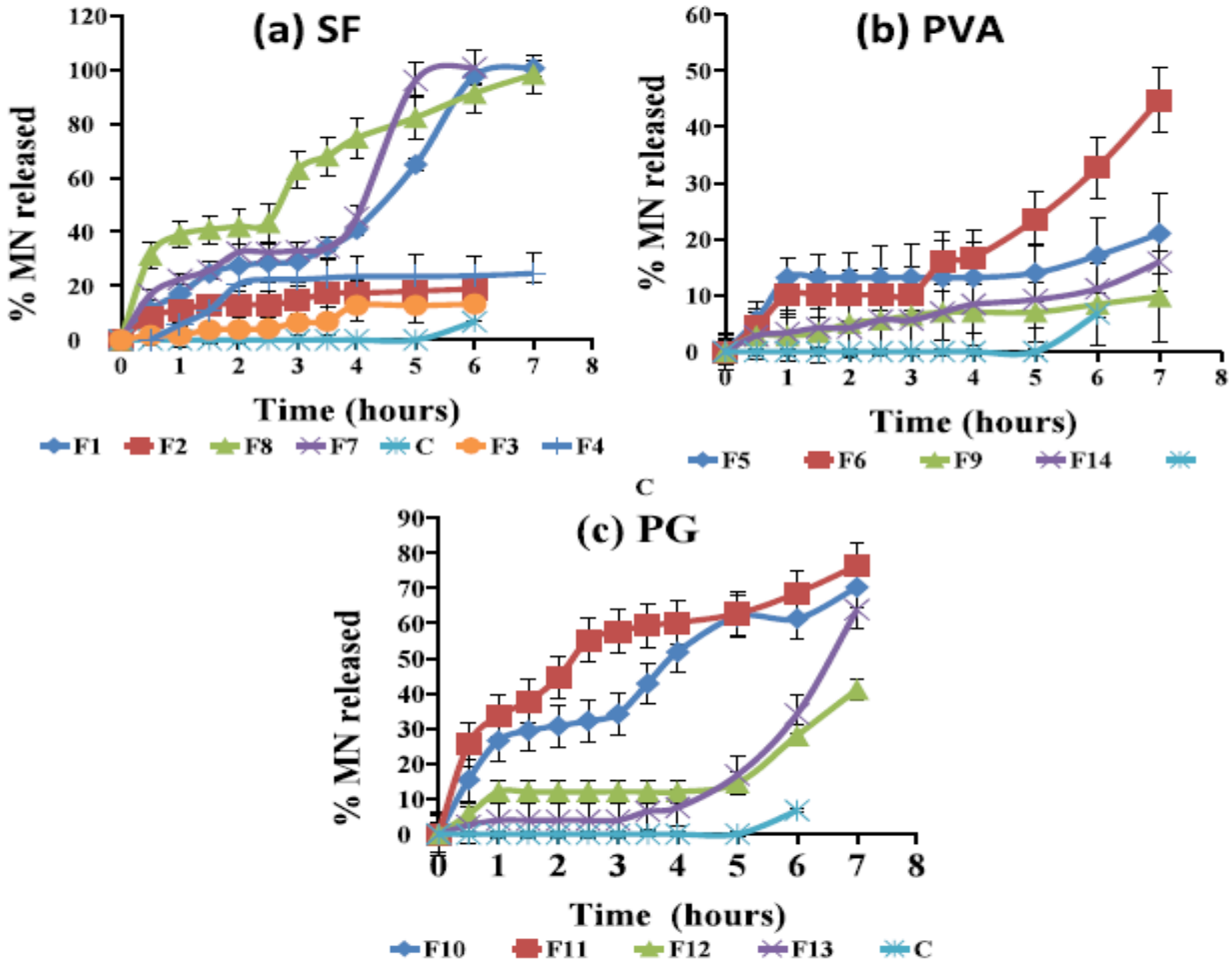

Figure 2. In vitro release profiles of (a) stabilizer free cubosomes, (b) PVP 2.5% cubosomes and (c)PG 2.5% cubosomes in comparison with control (Miconazole nitrate solution 2%)

Table 4. Kinetic studies for drug release from different dispersions

Dispersions	(n) value	r	Zero order kinetics		Diffusion		Mechanism of drug release
			R	K	R	K	
D1	0.807	0.948	0.9549	0.0124	0.906	5.95	Non- Fickian, Zero order
D2	0.235	0.977	0.949	0.03	0.972	0.317	Fickian, Diffusion
D3	0.958	0.951	0.949	0.041	0.928	1.003	Non- Fickian, Zero order
D4	0.558	0.888	0.731	0.036	0.886	1.100	Non-Fickian, Diffusion
D5	0.332	0.833	0.824	0.024	0.828	0.643	Fickian, Diffusion
D6	0.740	0.911	0.9405	0.091	0.881	2.275	Non- Fickian, Zero order
D7	0.664	0.884	0.905	0.253	0.847	5.89	Non- Fickian, Zero order
D8	0.465	0.946	0.980	0.183	0.968	4.823	Fickian, Diffusion
D9	0.520	0.003	0.964	0.0176	0.974	0.473	Non-Fickian, Zero order
D10	0.548	0.97	0.977	0.136	0.968	3.61	Non-Fickian, Zero order
D11	0.414	0.988	0.9501	0.119	0.981	3.27	Fickian, Diffusion
D12	0.518	0.808	0.8401	0.067	0.772	1.64	Non-Fickian, Zero order
D13	1.054	0.809	0.845	0.129	0.759	3.089	Non-Fickian, Zero order
D14	0.32	0.792	0.878	0.045	0.83	0.145	Fickian, Zero order

Preparation of hydrogels loaded with MN cubosomes (cubogels)

Determination of pH

The pH of cubogels was found to be in the range of 5.2 to 6.2 (table 5) which was within the acceptable limits for topical application.

Drug content studies

Cubogels had drug content ranging from 85.9% to 93.12% of the added amount of MN

Viscosity measurement

The viscosity of semisolid preparations plays an important role in drug release from the vehicles and greatly affects drug bioavailability (19). The viscosity of the cubogels could be observed from table (5). Viscosity was ranged from 348.3 to 633.5 Ps.

Table5. Viscosity and pH of the prepared cubogels

Gel	Viscosity (Ps) ± SD	pH	Gel	Viscosity (Ps) ± SD	pH	Gel	Viscosity (Ps)± SD	PH
G1	355.2 ± 2.3	6.2	G7	443.3 ± 6.4	5.6	G13	387.9 ± 13.1	5.2
G2	348.3 ± 7.8	5.6	G8	421.2 ± 7.8	5.8	G14	410.3 ± 12.3	5.5
G3	436.8 ± 10.2	5.6	G9	512.7 ± 12.3	5.2	G15	455.2 ± 10.2	5.5
G4	450.2 ± 12.7	5.9	G10	544.6 ± 11.9	5.3	G16	437.5 ± 8.4	5.5
G5	377.9 ± 5.8	5.7	G11	613.4 ± 10.3	5.2			
G6	367.3 ± 9.8	5.8	G12	633.5 ± 9.2	6.2			

In vitro release of MN from prepared cubogels and the kinetic analysis of the data

The in vitro release results of MN from different cubogels in comparison with commercial MN cream (Miconaz®) are represented in figure 2. Percent MN released after 2 h can be arranged in a descending order: G8 (48.44)> Miconaz ® (25.2) > G1 (22.4) = G10 (22.4) > G9 (17.64) > G15 (15.4). Percent MN released after 4 h can be arranged in a descending order: G8 (81.2) > G1 (50.4) > G10 (33.6) > Miconaz ® (28.84) > G4 (25.2). While Percent MN released after 8 h can be arranged in a descending order: G8 (100) = G1 (100) > G5 (74.76) > G6 (70.56)

> G10 (51.24) >Miconaz ® (44.8). It was found that G1 and G8 showed a significant high percent MN released in comparison with other cubogels and commercial MN cream Miconaz® at $p < 0.05$.The kinetic analysis of the release data is shown in table 5, and it can be observed that the release of most formulae (G1, G3, G4, G5, G6, G11, G12, G15and G16) exhibited non- Fickian transport according to Korsmeyerpeppa's release model. And most of formulae followed zero –order kinetics (G2, G3, G4, G6, G7, G9, G10, G11, G12, G13, G14 and G15) as indicated from the highest coefficient of determination (r).

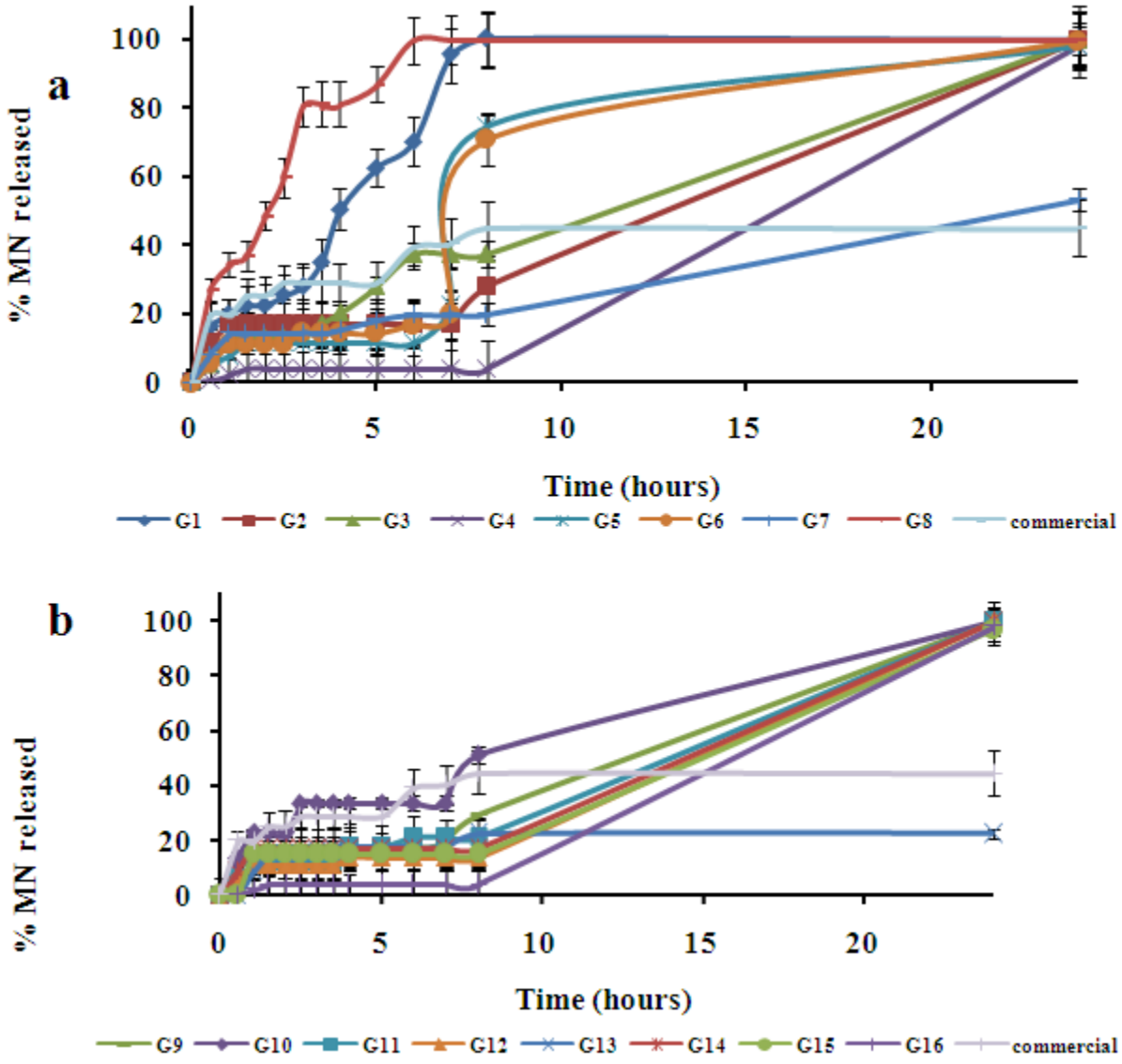

Figure 3 (a and b). In vitro release profiles cubogels in comparison with commercial MN cream (Miconaz®)

Table 6. The kinetic analysis of MN release from the prepared cubogels

Cubogels	(n) value	r	Zero order kinetics		Diffusion		Mechanism of drug release
			R	K	R	K	
G1	0.624	0.921	0.759	0.067	0.866	3.39	Non- Fickian, Diffusion
G2	0.410	0.752	0.953	0.06	0.854	2.4	Fickian, Zero order
G3	0.725	0.964	0.992	0.066	0.969	2.89	Non- Fickian, Zero order
G4	1	0.862	0.948	0.06	0.941	3.01	Non-Fickian, zero order
G5	0.694	0.826	0.886	0.069	0.856	2.988	Non-Fickian, Diffusion
G6	0.688	0.865	0.909	0.069	0.876	2.97	Non- Fickian, Zero order
G7	0.378	0.899	0.985	0.029	0.928	1.23	Fickian, Zero order
G8	0.425	0.910	0.617	0.046	0.791	2.61	Fickian, Diffusion
G9	0.416	0.77	0.958	0.06	0.863	0.934	Fickian, Zero order
G10	0.454	0.934	0.975	0.056	0.948	2.44	Fickian, Zero order
G11	0.588	0.910	0.976	0.063	0.898	2.6	Non-Fickian, Zero order
G12	0.514	0.804	0.949	0.063	0.847	2.5	Non-Fickian, Zero order
G13	0.411	0.732	0.962	0.061	0.872	2.45	Fickian, Zero order
G14	0.464	0.844	0.936	0.0594	0.831	2.33	Fickian, Zero order
G15	0.77	0.777	0.938	0.06	0.846	2.4	Non-Fickian, Zero order
G16	1	0.834	0.933	0.067	0.82	2.6	Non-Fickian, Zero order
Control	0.259	0.932	0.764	0.017	0.878	0.914	Fickian, Diffusion

Ex vivo Permeation of MN cubogels, through Rabbit Skin

Permeation results of MN from different gel bases and commercial MN cream (Miconaz®) using dorsal rabbit skin are shown in figure 4. It was found that the permeation of MN through dorsal rabbit skin from all gel bases was significantly higher than that obtained from the commercial MN cream. Archana et al. found that the hydrogels with cubosomes showed better permeability when compared to the hydrogels with pure drug. This shows that cubosomes enhance the permeability of the drug [20].

Calculation of Permeation Parameters for MN formulations using rabbit skin

The permeation data were treated according to zero order or membrane diffusion controlled model and the permeation parameters calculated according to Fick's law, like flux (Jss), Diffusion Coefficient (D), Permeation Coefficient (P) and Lag time (tL) were obtained. Permeation parameters obtained, according to Fick's law are shown in table 7. The flux coefficient values of G1, G8 and G10 were significantly higher than that of commercial MN formulation at $p < 0.05$.

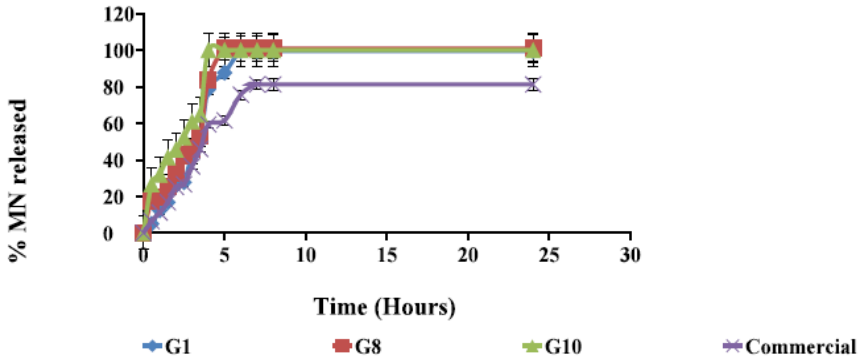

Figure4. Ex vivo permeation of MN cubogels in comparison with commercial MN cream (Miconaz®)

Table 7. The permeation parameters of MN cubogels in comparison with commercial MN cream (Miconaz®)

Gel	Steady-state flux Jss (µg.cm^{-2}hr^{-1})	Lag time(tL) (hr)	Diffusion coefficient (D) (cm^2.hr^{-1})	Permeability coefficient (cm hr^{-1})
G1	3119.72	1.284	1.028E-07	0.3119
G8	3201.08	1.050	1.257E-07	0.3201
G10	2676.08	0.066	1.997E-06	0.2676
Commercial cream	1834.18	0.466	2.829E-07	0.1834

Microbiological study of MN

The cubogel formulations (G1, G8 and G10) were further evaluated for antifungal activity against Candida albicans by agar cup method. The results of zone of inhibition of MN cubogels were compared to the commercial (Miconaz® cream). The zones of inhibition of various formulations are represented graphically in figure 5. These results demonstrated that, the antifungal activity of the cubogel formulations were significantly higher than that of the commercial product although there is no significant differences between the prepared cubogel formulations ($p<0.05$). No significant zone of inhibition was observed in case of plain gel which contain 0% of MN which indicates that the components of micro emulsion, except miconazole nitrate, have no antifungal activity. cubosomes acts as carriers for drug delivery to the particular site of action, the antifungal activity is created by the drug incorporated into the cubosomes. This enhanced antifungal activity is due to enhanced penetration of cubosomes containing drug through the fungal cell wall and inhibiting the ergosterol synthesis [21]. The greater in vitro antifungal activity of cubogels than marketed cream may be due to the smaller globule size with its larger surface area as compare to the normal emulsion present in the cream. This may be the reason for greater drug release from cubogels preparation [22].

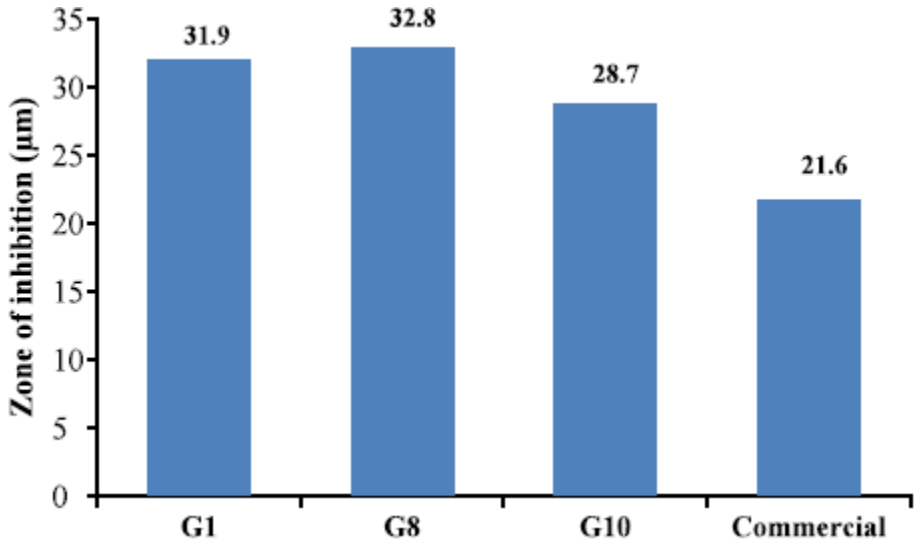

Figure 5. Histogram showing the mean values of inhibition zones of MN cubogels and its commercial cream at 24 hrs.

Conclusion

In this work, cubogels were formulateded to deliver Miconazole Nitrate for topical administration. From in-vitro release, ex vivo permeation and microbiological study data it can be concluded that the developed cubogels have a great potential for topical drug deliver

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