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Original Article

FLUCONAZOLE NANOGEL: FABRICATION AND *IN VITRO* EVALUATION FOR TOPICAL APPLICATION

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ABSTRACT

Objective: The aim of this study is to develop and *in vitro* evaluation of prepared fluconazole nanogel for seborrheic dermatitis

Methods: Fluconazole nanogel was formulated to act against seborrheic dermatitis. The fluconazole nanoparticles were prepared by a simplified evaporation method and evaluated for particle size, entrapment efficiency, and percent *in vitro* drug release. The nanogel was also characterized based on parameters like particle size, percent entrapment efficiency, shape surface morphology, rheological properties, *in vitro* release $R^2 = 0.9046$, and release kinetics.

Results: The nanoparticle with a combination of Eudragit RS and Tween 80 showed the best result with particle size in the range of 119.0 nm to 149.5 nm, with a cumulative percent drug release of 95 % up to 18 h. The formulated nanogel with optimum concentration of HPMC authenticate with particle size 149.50±0.5 with maximum drug release (92.13±0.32) %.

Conclusion: Different percentages of polymers (ethyl-cellulose, eudragit, and tween 80) are used as variable components in the formulation of nanogel. The optimized batch showed good physical properties (flow index, spreadability, and viscosity) along with rapid drug release. Therefore, it can be concluded that nanogel containing fluconazole has potential application in topical delivery.

Keywords: Seborrheic dermatitis, Fluconazole, Nanogel, Topical application

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INTRODUCTION

Seborrheic dermatitis is a topical skin inflammatory disorder, mainly characterized by yellow-grey scales and poorly defined erythematous patches. It affects the sebum-rich areas such as skin, scalp, nasolabial folds, ears, eyebrows, and chest [1]. The frequency is more common in men than in women. The occurrence of the lesion also depends on the weather conditions [2, 3]. However, sunlight has some evidence to develop seborrheic dermatitis following psoralen+UVA therapy, also known as PUVA. Treatment of seborrheic dermatitis aims at reducing inflammatory processes and decreasing sebum production [4].

Besides, recent studies have revealed that tea tree oil (Melaleuca oil), honey, and cinnamic acid have antifungal activity against *Malassezia* species, which may be of benefit in the treatment of seborrheic dermatitis [5]. With the wide availability of preparations, including creams, shampoos, and oral formulations, antifungal agents are safe and effective in the treatment of seborrheic dermatitis [6]. This may, in part, be due to an abnormal or inflammatory immune response to these yeasts.

A variety of treatment modalities are available, including the eradication of the fungus, reducing, or treating the inflammatory process, and decreasing sebum production. These included some antifungal agents, corticosteroids, immunomodulators, and keratolytic. The characterization of these classes of drugs such as the anti-inflammatory properties inherent in many of the antifungal agents as well as the keratolytic properties of selenium, zinc, and tar preparations. The azoles including bifonazole, itraconazole, fluconazole, and ketoconazole represent the largest class of antifungals used in the treatment of fungal infection [7].

Antifungal agents are the mainstay of anti-seborrheic therapy, mostly in the azole form. These agents work by inhibiting ergosterol, an important component of the fungal cell wall, via interference with the fungal cytochrome P-450 (CYP 450) system. This causes an increase in the production of sterol precursors, a fungistatic process that does not allow the fungus to grow or reproduce. Fluconazole-first generation triazole is selected for the treatment of seborrheic dermatitis as it has fewer side effects but the weakest binding to human CYP 450 [4]. The nanogel of the drug Fluconazole shows better results as it has advantages over the simple topical products available in the market [5].

The present investigation aimed at formulating fluconazole nanogel, effective against seborrheic dermatitis. The research was mainly composed of two steps viz. loading of fluconazole to nanoparticle and second to incorporate these nanoparticles to a gel base forming nanogel. Nanogels are three-dimensional hydrogel materials crosslinked with swell polymers, increasing their efficiency to hold water. These are highly hydrophilic, increasing their biocompatibility to load drug molecules.

MATERIALS AND METHODS

Materials

Fluconazole was received as a gift sample from Unichem Laboratory, Ghaziabad. Eudragit RS was purchased from Sigma-Aldrich Pvt Ltd. Ethyl-cellulose and tween 80 were purchased from CDH Pvt. Ltd., New Delhi. All the solvents used were of analytical grade.

Methods

Preparation of fluconazole nanoparticle

Fluconazole nanoparticles were prepared by using simplified evaporation methods [8]. On the variable of different concentrations of polymers, six formulations were formulated (table 1). The polymers were mixed with a solvent (ethanol), forming an organic phase following the addition of fluconazole. The aqueous phase was prepared separately by dissolving tween 80 with water. Both phases were mixed by adding organic phase dropwise with continuous stirring [9]. The organic phase was later allowed to evaporate overnight. The mixture is then centrifuged to separate the nanoparticles [10].

Table 1: Formulation table for preparing fluconazole nanoparticles

S. No.	Formulation code	Ethylcellulose (mg)	Eudragit RS 100 (mg)	Tween 80 (%)
1	A1	450	-	0.5
2	A2	450	-	1.0
3	A3	450	-	1.5
4	A4	-	450	0.5
5	A5	-	450	1.0
6	A6	-	450	1.5

Characterization parameters for fluconazole nanoparticles

Particle size and polydispersity index (PDI)

The undiluted sample of nanoparticles were transferred in cuvette and particle size was measured by using dynamic light scattering (DLS) apparatus. The results were analyzed and performed in triplicate [11].

Percentage drug entrapment efficiency (%)

1 ml of nanoparticle sample is taken for centrifugation at 7168 RCF (relative centrifugal force) for 50 min. The supernatant was collected, washed, and filtered through membrane (0.45 micron) filter paper. The absorbance of the sample was noted, and the actual entrapped drug was calculated using the below-mentioned formula: The readings were taken in triplicate [12].

$$\text{Total drug concentration} = \frac{\text{Totalamountofdrug} - \text{Amountofdruginsupernatant}}{\text{Totalamountofdug}} \times 100$$

***In vitro* drug release study**

Keshary-Chien cell (K-C) was used for performing in vitro release study. These are thermo-regulated by using a water jacket at 37±0.5 °C. Phosphate buffer (pH 6.5) was used as a receptor medium. Nanoparticles (equivalent to 1 mg of fluconazole) were placed in the donor compartment. Dialysis membrane 70 (Hi-Media, Mumbai, India) having a pore size of 2.4 nm and a molecular weight cut-off between 12,000-14,000 Da was used as a donor compartment. At predetermined time intervals, 5 ml of the samples were withdrawn from the receiver compartment and replaced by the same volume of freshly prepared PBS (pH 6.5). The samples were analyzed at the wavelength of 254 nm using a UV spectrophotometer. The readings were taken in triplicate [13].

Preparation of fluconazole nanogel

The prepared and optimized nanoparticles were loaded to a hydrogel base to form a fluconazole nanogel. The hydrogel was formulated using ingredients and quantities as mentioned in table 2. Three polymers were used to optimize the hydrogel formulation, the three polymers used are carbopol 940, HPMC, and methylcellulose. Different concentrations of polymer aqueous solution (antisolvent) were prepared by dispersing the calculated amount of polymer in warm water with constant stirring. Then add the previous mixture containing the drug using a syringe to a polymer mixture followed by constant stirring at 7168 RCF by a homogenizer. Methyl and propylparaben as preservatives were added slowly with continuous stirring until gel formation [14].

Table 2: Formulation table for preparing fluconazole nanogel

S. No.	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
1	Fluconazole (mg)	1	1	1	1	1	1	1	1
2.	Carbopol 940	0.5	1	-	-	-	-	-	-
3.	Methyl cellulose	-	-	2	4	-	-	-	-
4.	HPMC	-	-	-	-	1.5	2	-	-
5.	Pectin	-	-	-	-	-	-	3	4
6.	Glycerine	10	10	10	10	10	10	10	10
7.	Propylene glycol	20	20	20	20	20	20	20	20
8.	Methylparaben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
9.	Propylparaben	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
10.	Purified water to	100	100	100	100	100	100	100	100

Characterization of fluconazole nanogel

Rheological parameters

Viscosity

Brookfield viscometer was used to analyze the viscosity of the formulated nanogel. The formulated nanogel was taken in beakers and analyzed by setting spindle number and rpm. The readings were taken in triplicate.

Spreadability

The gel was evaluated by using a glass slide method. The formulated gel was kept between the two sides within a pre-marked circle (1 cm). The pre-weighted plate was kept above the gel for 5 min [12]. The increase in the diameter due to gel spreading was noted and calculated using the formula mentioned below:

$$\text{Spreadability} = \text{mass} \times \text{length/time}$$

pH determination

pH of the nanogel formulation was measured using a pH meter. For this determination, 1 g of nanogel was weighed and dispersed in 10 ml of distilled water. This was kept for 4–5 min for taking the actual pH value. The reading was taken in triplicate.

Drug content

Nanogel was dissolved in 10 ml of ethanol and thereby drug content was measured. The mixture was centrifuged at 448 RCF for 1 h. The supernatant liquid was withdrawn, and samples were analyzed using a UV spectrophotometer at 260 nm.

Particle shape and surface morphology

The particle shape and surface morphology of prepared nanogel was visualized by scanning electron microscopes (Cart Zeiss EV018). One drop of the sample was placed on a slide, and excess water was left to dry at room temperature. The testing slides of samples were prepared by lightly sprinkling samples on double-sided adhesive tape on an aluminum stub. The samples were coated with gold to a thickness of 200 to 500 °A under an argon atmosphere using a gold sputter module in a high vacuum evaporator. The samples were then randomly scanned, and photomicrographs were taken at different magnifications with SEM [15].

Transmission electron microscopy (TEM)

TEM was used to visualize (morphology and structure) of nanogel. The samples were dried on a copper grid and adsorbed with filter paper. After drying, the sample was viewed under the microscope at different magnifications at an accelerating voltage of 100 kV.

Drug release kinetics studies

From the characterization parameters one optimized formulation was selected and evaluated for release kinetics to understand the release mechanism of the formulated nanogel. For this, data obtained from *in vitro* drug release was plotted for zero-order

equation, first-order equation, Korsmeyer's Peppas equation, and Higuchi's equation.

Drug-excipients compatibility studies

While formulating fluconazole nanogel, interaction studies were performed. No interaction was found while performing interaction studies using Fourier transform Infrared Spectroscopy technique. The results have been illustrated in fig. 1.

***In vitro* drug release studies**

The various nanogel batches were evaluated using the Keshary-Chien cell (K-C) cell. The procedure used for *in vitro* release study was the same as employed for fluconazole nanoparticles.

RESULTS AND DISCUSSION

Preparation of fluconazole nanoparticle

Fluconazole nanoparticles were prepared by the solvent evaporation method in six different batches.

Characterization for fluconazole nanoparticles

Nanoparticles of fluconazole were successfully prepared and optimized to find out the best formulation which is to be loaded to a hydrogel base. The optimization was based on particle size, percent entrapment efficiency, and *in vitro* drug release.

Drug-excipients compatibility studies

Particle size and polydispersity index and percentage drug entrapment efficiency (%)

The result obtained from particle size analysis and %EE have been mentioned in table 3. From the results obtained it can be concluded that Formulation A6 (particle size of 149.50±0.5 nm) shows better % EE and has optimum percent drug release. The smaller size of nanoparticles will help to increase the permeation of the drug through the biological membrane at the target site indicating its efficiency to deliver the drug through a topical route [16].

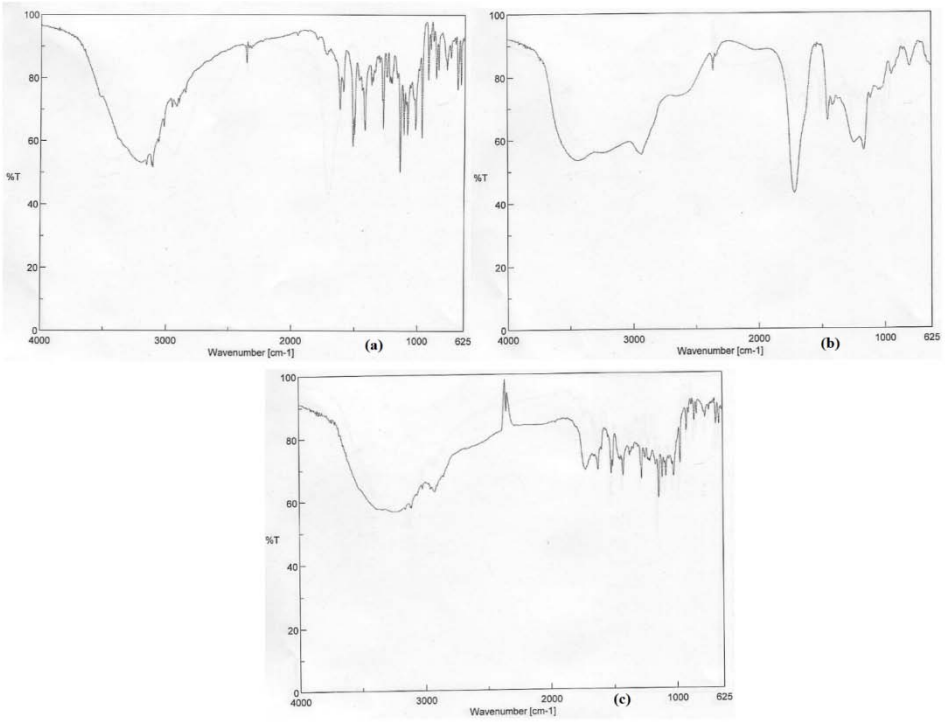

Fig. 1: Fourier transformation infra-red radiation (FTIR) images showing drug-excipient interaction of sample: (a) Drug and excipients, (b) drug (fluconazole), and (c) excipients. No new peak in the sample indicates drug excipient compatibility

Table 3: Particle size and percent entrapment efficiency

Formulation code	Particle size (nm)	EE%
A1	119.46±0.5	83.66±0.58
A2	110.83±0.76	91.36±1.1
A3	140.83±1.04	79.50±0.88
A4	150.36±0.47	94.20±0.88
A5	135.93±0.95	95.80±0.55
A6	149.50±0.5	96.03±0.96

***In vitro* drug release of fluconazole nanoparticles**

The *in vitro* drug release obtained from fluconazole nanoparticles has been depicted in fig. 2. The optimized formulation (A6) was selected and further loaded to a nanogel base.

Characterization of fluconazole nanogel

An optimized formulation of fluconazole nanoparticles was successfully loaded to form nanogel.

Results obtained from physical and rheological parameters have been depicted in table 4, which concluded that the formulation exhibited good viscosity, spreadability, and an optimum pH. The formulation was having a good viscosity, spreadability, homogeneity, pH, and visual inspection. The result obtained showed that the formulation is having an optimum viscosity and spreadability. Rheological properties such as flow index, flow behavior, and Coefficient of determination have been illustrated in table 4.

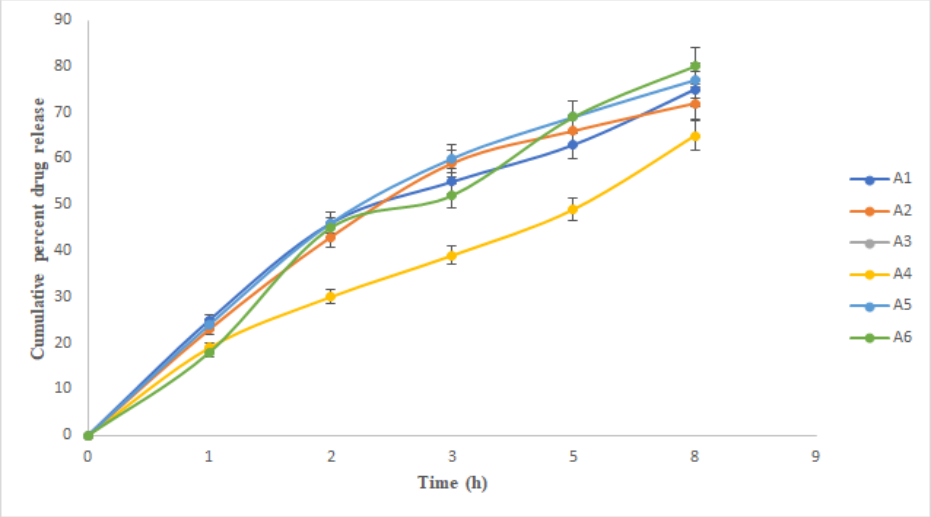

Fig. 2: Cumulative percent *in vitro* drug release of fluconazole nanoparticles showing that half of the nanoparticles are released in the first 2 hr and 90% release occurs in 8 hr. Among all batches, the A6 batch showed maximum cumulative release with 149.50±0.5 particle size and 96.03±0.96 entrapment efficiency and n=3, mean±SD

Table 4: Evaluation of physical and rheological properties of fluconazole nanogel batches

Batches	Physical properties			Rheological properties			
	Colour	Spreadability (cm)	pH	Coefficient of determination (R^2)	Flow index	Viscosity* (cp) (η)	Flow behavior
F1	Shiny transparent	4.5±0.21	6.1±0.2	0.916±0.04	0.238±0.00	1709±92.1	Shear-thinning
F2	Shiny transparent	4±0.95	5.99±0.29	0.9291±0.04	0.235±0.01	1918±47.9	Shear-thinning
F3	Transparent	4.5±0.2	6.1±0.12	0.9976±0.04	0.225±0.01	1449±71.8	Shear-thinning
F4	Transparent	3.6±0.15	6.13±0.8	0.9823±0.04	0.210±0.01	2083±98.1	Shear-thinning
F5	Pale, translucent	5±0.23	5.60±0.15	0.908±0.04	0.230±0.00	1012±48.2	Shear-thinning
F6	Pale, translucent	5±0.2	5.67±0.14	0.908±0.04	0.230±0.01	1036±50.6	Shear-thinning
F7	Opaque, buff	5±0.22	3.6±0.9	0.9304±0.04	0.121±0.00	1247±61.4	Shear-thinning
F8	Opaque, buff	3.5±0.12	3.7±1.2	0.9819±0.04	0.143±0.00	2289±110.3	Shear-thinning

Drug content

The formulated fluconazole nanogel was evaluated for drug content parameters. The drug content was found to be in the range of 75.30±0.43 % to 95.33±0.49%. The results have been shown in table 5.

Table 5: Drug content of fluconazole nanogel batches

Formulations	Drug content (%)
F1	85.43±0.45
F2	77.33±0.49
F3	80.23±0.58
F4	94.13±0.32
F5	92.13±0.32
F6	93.46±0.45
F7	95.33±0.49
F8	75.30±0.43

SEM

The nanogel was visualized by scanning electron microscopy (SEM). They were found to be spherical, having a smooth surface (fig. 3). The average range of particle size was found to be 101.9 nm to 127.5 nm, having the particle size in the optimal range, which is having excellent morphological properties with minimum toxicity [17].

TEM

The sample was viewed under the microscope at different magnifications at an accelerating voltage of 100 kV. The prepared nanoparticles, showing the spherical shape and particle size of 100-130 nm.

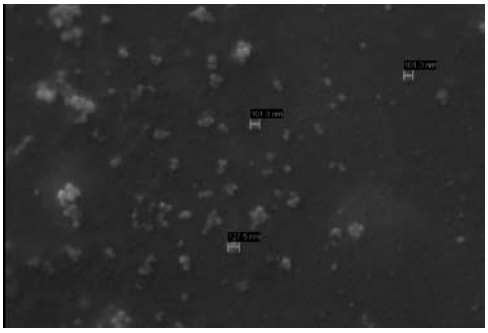

Fig. 3: SEM (scanning electron microscopy) of optimized fluconazole nanogel batch showing the range±113.6 nm, n=3, mean±SEM

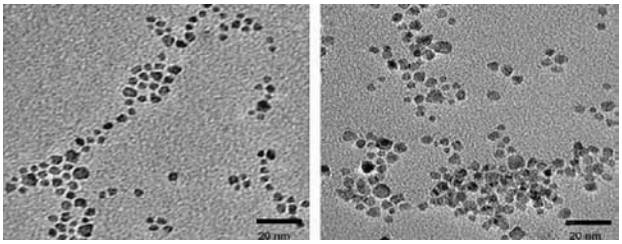

Fig. 4: TEM (transition electron microscopy) of fluconazole optimized nanogel formulation showing the spherical shape and size of average±112 nm, n=3, mean±TEM

In vitro drug release

The *in vitro* release study showed the release of above 90 % up to 18 h. (fig. 5). The model or equation that best fits the release data was evaluated by correlation coefficient (r) and the value of n, particularly for Korsmeyer Peppas's Equation. The release study was best explained by Higuchi's equation, shown in fig. 6. From the above, it is indicated that the drug release will be diffusion controlled, depending on the swelling criteria of cross-linked polymers present in the hydrogel

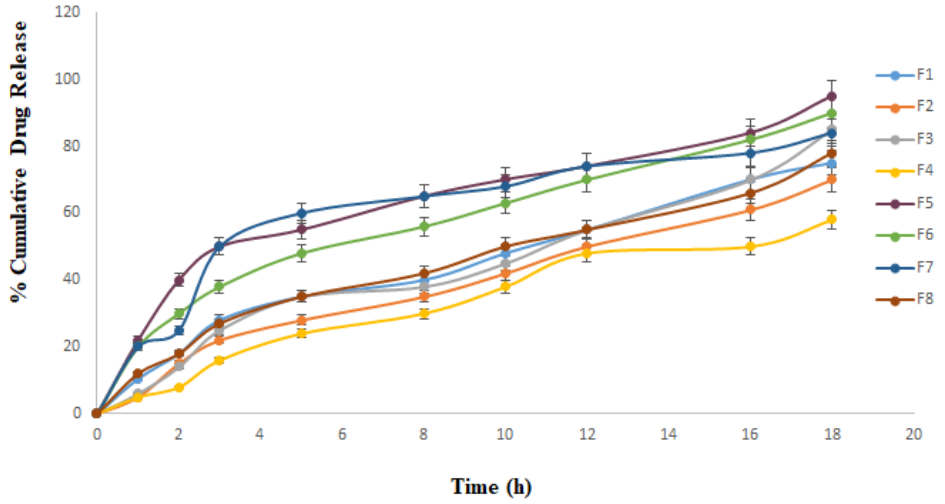

Fig. 5: Cumulative percent *in vitro* drug release of F1-F8 batches of fluconazole nanogel shows around 50% of drugs were released in the starting first 10 h of total release and 90% drug releases in 18 h, with maximum release of F5, n=3, mean±SD

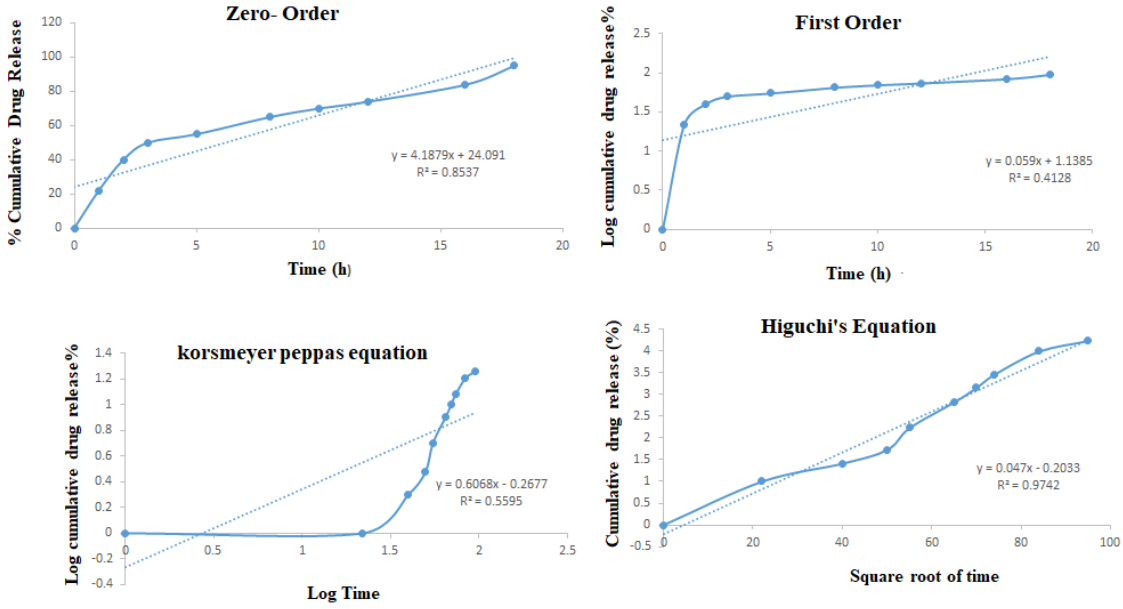

Fig. 6: Drug release kinetics of optimized fluconazole nanogel showed the highest linearity was R^2 = 0.971, followed by zero-order (R^2= 0.853), Korsmeyer-Peppas model (R^2= 0.559), and first-order equation (R^2 = 0.412). All values are expressed as, n = 6, mean±SD

CONCLUSION

Fluconazole was successfully incorporated into a gel base. The nanogel shows a good flow index, spreadability, and viscosity. The release profile shows drug release for a prolonged period. The nanogel attains a good percentage of drug content in the formulation. Therefore, it can be concluded that nanogel containing fluconazole has potential application in topical delivery.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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