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Development and Evaluation of Nanoparticle Based Topical Gel for Antifungal Effect.

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Abstract:

Fluconazole is a synthetic antifungal agent belonging to the group of triazole. It is one of the commonly used antifungal agents for most kinds of fungal infections such as vulvovaginal candidiasis, oropharyngeal candidiasis, mucosal leishmaniasis, visceral leishmaniasis and dermatomycosis. The reason to select fluconazole is it overcomes all the side effects of the other fungal drugs like, Ketoconazole, Amphotericin B, Clotrimazole, and Miconazole. It is a BCS class III drug (high solubility and low permeability). Due to the retailoring properties they can over come physiological barriers and can help to guide their payload to specific cells or intercellular compartments by which side effects can be minimized and therapeutic benefits of a drug can be increased. By virtue of their small size and by functionalizing their surface with polymers and appropriate ligands, polymeric nanoparticles can also be targeted to specific cells and locations in the body.

Keywords: Nanoparticle**,** Biodegradable, Bioavailability, Dissolution, pharmacokinetic

Nanoparticles are one of the forms of novel drug delivery systems having the capability to release the drug at an optimum rate at the desired site of action. Nano particular formulations provide the liberty to use a wide range of polymers like synthetic, natural, biodegradable and non-biodegradable polymers.[1] The size range of the nanoparticles is 1 to 1000nm, but for the purpose of drug delivery, nanoparticles in the range of 50 – 500 nm are acceptable

depending on the route of administration.[2]

Nanoparticles have become one of the most active areas of research in the field of drug delivery due to their ability to deliver the drugs to the right place, at appropriate times, and in the right dosage. A wide variety of nanoparticles composed of a range of materials including lipids, polymers and inorganic materials have been developed resulting in delivery systems that vary in their physicochemical properties and their applications. The advantages of nano-encapsulation include the enhanced stability of labile drugs, controlled drug release and an enhanced drug bioavailability owing to the fact that particles in the nano-size range are efficient in crossing permeability barriers.

Typical problems associated with poorly soluble drugs are too low bioavailability and/or erratic absorption. The drugs with poor solubility and bioavailability can be improvised by the application of nanotechnology. The surface area of the drug particles increases after size reduction process which improves the dissolution rate. This promising approach rises to transform the drug particles to submicron size.[3]

Nanoparticles are efficient and versatile devices for drug delivery as they can improve crucial properties of a drug entity such as solubility, pharmacokinetic, bio-distribution and *in-vivo* stability. Due to their tailoring properties they can overcome physiological barriers and can help to guide their payload to specific cells or intercellular compartments by which side effects can be minimized and therapeutic benefits of a drug can be increased. By virtue of their small size and by functionalizing their surface with polymers and appropriate ligands, polymeric nanoparticles can also be targeted to specific cells and locations in the body. Depending on the polymer characteristics, polymeric nanocarriers can also be engineered in such a way that they

Methodology:

Preformulation studies:

Determination of λmax and Standard graph preparation:

Accurately weighed 50 mg of Fluconazole and was taken in a 100 ml volumetric flask and made up to 100 ml with methanol. Then the prepared solution was scanned in the ranged of 200 to 400 nm by using methanol as a blank in order to get standard stock solution containing

500µg/ml. The λmax was found to be 261nm.

Preparation of Fluconazole calibration curve:

50 mg of Fluconazole was weighed accurately and carefully transferred in 100 ml volumetric flask and dissolved in methanol and the volume is made up to the mark with methanol .(500µg/ml). From this solution 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5
ml was pipetted out and diluted to 10ml to get 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500µg/ml by using pH 7.4 phosphate buffer.

PH 7.4 phosphate buffer is used as a blank solution. Standard curve was prepared by plotting absorbance vs concentration at 261 nm using UV-Visible spectrophotometer.

Determination of melting point:

Determination of melting point gives an idea about purity of the drug. Melting point of Fluconazole was determined by capillary method. Fine powder of Fluconazole was filled in glass capillary tube (previously sealed on one end). The capillary tube is tied to thermometer and the thermometer was placed in tube containing liquid paraffin. The assembly was kept on heating and temperature was allowed to increase gradually. Temperature at which the powder melts was noticed.

Drug-excipients and Compatibility study:

A successful formulation of a stable and effective solid dosage form depends on careful selection of the excipients that are added to facilitate administration, promote the consistent release and bioavailability of the drug and protect it from degradation. If the excipients are new and have not been used in formulations containing the active substance, the compatibility studies are of more importance.

FTIR:

The compatibility of the drugs with the excipients was determined by subjecting the physical mixture of the drug and the polymers of the formulation to infrared absorption spectral analysis (FTIR). Any change in the chemical composition of the drugs after combining it with the polymer was investigated with I.R spectral analysis.

Formulation of fluconazole nanoparticles: [54, 55]

The fluconazole nanoparticles were prepared by a nanoprecipitation method. The formulation plan is shown in Table 4.3.7. Fluconazole 10mg and Eudragit RL 100 10mg, 20mg and 30mg were dissolved in 3ml Ethanol. The internal organic phase solutions were slowly injected at the rate of (1ml/minute) into 20ml of the external aqueous solution containing stabilizing agent (Poloxamer 188) at various concentrations such as (0.5, 0.75 and 1% w/v) in double distilled water, and the mixtures were then stirred at 500 rpm for 4 hours at room temperature. Internal organic phase solutions are always composed of solvents, making the drug and Eudragit RL 100 soluble completely, and the external aqueous phase comprises aqueous solution, sometimes with or without surfactant in it. The surfactant can penetrate into the fluconazole nanoparticles during the nanoprecipitation process to form a stable nanoparticle delivery system. The aqueous phase immediately turned into milky bluish opalescence due to the formation of the nanoparticle suspension. Ethanol was completely removed by rotary vacuum evaporation using a water bath maintaining at 32°C. The Fluconazole nanoparticles formed were isolated, washed three times with distilled water, and freeze-dried.

Table 4.3.7: Formulation chart of fluconazole nanoparticles (F1- F9)

Formulation Code	Drug: Eudragit RL100	Ethanol (ml)	Poloxamer 188 (%)	Distilled water (ml)
F1	1:1	3	0.5	20
F2	1:2	3	0.5	20
F3	1:3	3	0.5	20
F4	1:1	3	0.75	20
F5	1:2	3	0.75	20
F6	1:3	3	0.75	20
F7	1:1	3	1	20
F8	1:2	3	1	20
F9	1:3	3	1	20

Particle size distribution and polydispersity index:

The average particle size and size distribution are important parameters because they influence the physicochemical properties and biological fate of the NP after in vivo administration. The mean size of the fluconazole nanoparticles was determined using Nano particle Analyzer SZ-100, HORIBA scientific.

***In- vitro* diffusion studies:**[2]

The *in-vitro* drug release of fluconazole nanoparticles was studied by using Franz diffusion apparatus. Freshly prepared pH 7.4 phosphate buffer was used as the diffusion medium. Cellophane membrane previously soaked overnight in the distilled water was tied to one end of a specially designed glass cylinder (open at both ends). Stability Studies:[2]

Stability studies were carried out on optimized formulation (F5) at 40°C ± 2°C /75% ± 5%RH in stability chamber (Thermo lab) and 4°C in refrigerator for 30 days. The optimized formulation stored in the sealed in aluminium foil. After 30 days, evaluation studies were carried out.

4.4. Preparation of nanoparticle based gel: [4]

Six formulations of fluconazole gel were prepared using carbopol 934 and carbopol 940 as a gelling agent with different ratios of 0.3%, 0.5% and 0.7 %. Specified quantity of carbopol 934 and carbopol 940 were soaked overnight as mentioned in the formulation chart.

Table 4.5: Formulation chart of fluconazole nanoparticles gel (F5).

Formulation code	G1	G2	G3	G4	G5	G6
Fluconazole nanoparticles equivalent to fluconazole =	250mg	250mg	250mg	250mg	250mg	250mg

Carbopol 934 (gm)	0.3	0.5	0.7	0.3	0.5	0.7
Carbopol 940 (gm)	0.3	0.5	0.7	0.3	0.5	0.7
Ethanol (ml)	2	2	2	2	2	2
Propylene glycol (%)	20	20	20	20	20	20
Glycerine (%)	10	10	10	10	10	10
Methyl paraben (%)	0.03	0.03	0.03	0.03	0.03	0.03
Propyl paraben (%)	0.01	0.01	0.01	0.01	0.01	0.01
Triethanolamine (ml)	0.2	0.2	0.2	0.2	0.2	0.2
Distilled water	q.s to make 50gm	q.s to make 50gm	q.s to make 50gm	q.s to make 50gm	q.s to make 50gm	q.s to make 50gm

4.4.1. Measurement of pH:

The pH of gel formulations are determined by digital pH meter.1 g of gel is dissolved in 100 ml distilled water and stored for two hours. The measurement of pH of each formulation is done in triplicate and average values are calculated.

4.4.2. Drug content studies:

Accurately weighed 1 g of gel was transferred into 10 ml volumetric flask containing 5 ml of saline phosphate buffer (pH 7.4) and stirred for 30 min followed by sonication. The volume was made up to 10 ml with saline phosphate buffer (pH 7.4). 5 ml of the above solution was further diluted to 10 ml with saline phosphate buffer (PH 7.4). The absorbance was measured using Shimadzu 1800 UV Visible spectrophotometer at 261 nm.

4.4.3. Spreadability:

The Spreadability of all formulations was determined by using horizontal plate method. 1 g of gel was placed between two horizontal glass plates and standard weight (125 g) was tied

on the upper glass plate. The whole set was held in the vertical position. The time was noted for the plate to slide off from the other plate. The spreadability was calculated from the following formula,

S = (m x l) / t

Where 'S' is the spreadability coefficient, 'm' is the weight tied to the upper slide, 'l' is the length of glass slide and't' is the time taken.

4.4.4. Viscosity measurement:

Viscosity of the gel was determined by using Brookfield viscometer. Accurately weighed 25 gm of fluconazole gel was transferred to 50 ml glass beaker. Spindle no 6 was selected and it is immersed into the gel. The viscometer was operated at 10 rpm

until the reading gets stabilized and reading was noted in centipoises. It was noted from the literature that the formulations after gelling should have a viscosity of 50 – 50,000 cps.

4.4.5. *In-vitro* diffusion studies: [2]

In-vitro diffusion study was carried out in a Franz diffusion cell using cellophane membrane which is soaked overnight in distilled water. The membrane was tied to the donor compartment and mounted on the reservoir compartment of Franz diffusion cell containing 150 ml of pH 7.4 phosphate buffer. 1 gm of fluconazole gel was placed over the cellophane membrane of donor compartment. Whole set was placed on the magnetic stirrer. The study was carried out at 37±0.5 ºC and 100 rpm for 12 h. Samples were withdrawn from the sampling port of reservoir compartment at regular intervals and absorbance was measured using Shimadzu 1800 UV visible spectrophotometer at 261 nm.

4.4.6. Mathematical modeling of drug release profile: [56]

The % Drug release from the Fluconazole nanoparticle gel at different time intervals were fitted to zero order kinetics and first order kinetics model, Higuchi model and Korsemeyer-Pappas model to characterize mechanism of drug release.

Table 4.6.7: Diffusional Exponent and Mechanism of Diffusional Release from swellable Controlled release System of Different Geometries.

Slab	Cylinder	Sphere	Drug Release Mechanism
0.5	0.45	0.43	Fickian diffusion
0.5 < - < 1.0	>0.45-<0.89	>0.43-<0.85	Anomalous transport(NonFickian)
1.0	0.89	0.85	Zero-order
>1.0	>0.89	>0.85	Case II Transport
>>1.0	>>1.0	>1.0	Super Case II Transport

4.4.7. Stability: [3]

Stability testing of drug product being as a part of drug discovery and ends with the commercial product, to assess the drug and formulation stability, stability studies were done. The stability study was carried out for the optimised formulation (G5), subjecting to a temperature of 40 ± 2°C and 75 ± 5% RH and 4°C in refrigerator for 1 month. After 1 month the samples were analysed for the physical characteristics, drug content and *in-vitro* diffusion study.

1. Preformulation studies:

1.1. Determination of Melting Point:

The melting point of Fluconazole was found to be (138-140) ˚C.

1.2. Determination of wavelength maxima of fluconazole:

The solutions was scanned in the range of 200- 400 nm to fix the maximum wavelength, and maximum absorption of Fluconazole. The λ max was found to be 261nm in both methanol and pH 7.4 phosphate buffer.

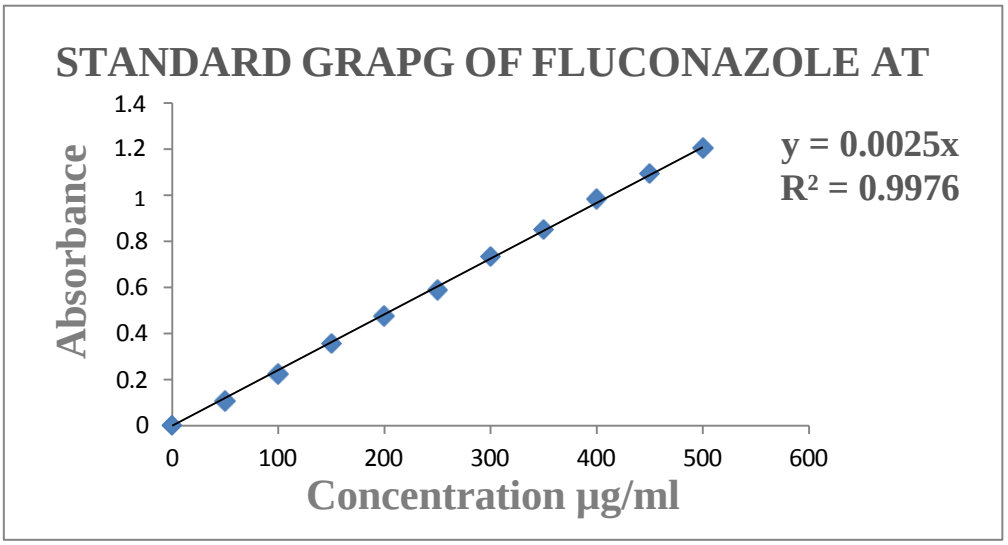
Standard calibration Curve of Fluconazole at λ max 261 nm in phosphate buffer (pH 7.4):

Fluconazole obeyed Beer's law in the range from 50-500 µg/ml. The absorbance is shown in the table 5.2 and standard graph in figure 5.2.

Table 5.2: Concentration and absorbance of the prepared solutions:

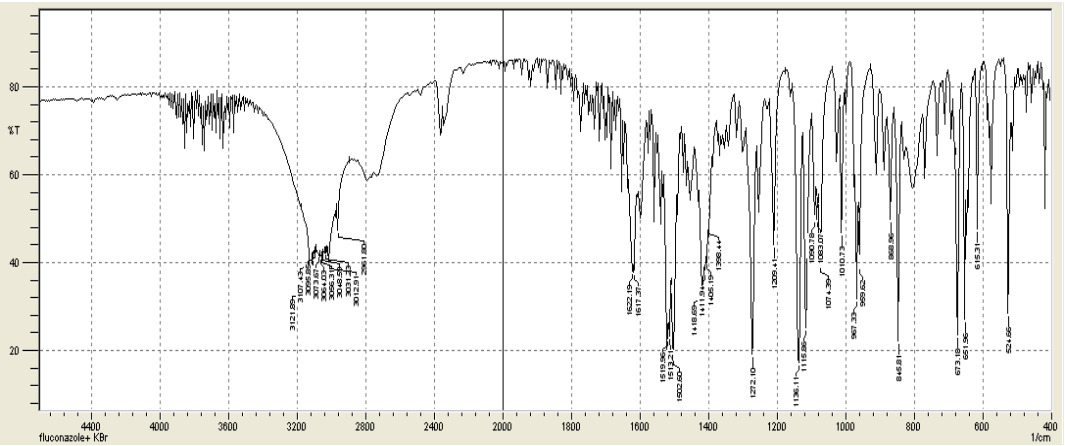
Sl.No.	Concentration (µg/ml)	Absorbance
1.	0	0.0
2.	50	0.105
3.	100	0.222
4.	150	0.355
5.	200	0.474
6.	250	0.587
7.	300	0.733
8.	350	0.849
9.	400	0.982
10.	450	1.094
11.	500	1.205

Fig 5.2: Standard calibration curve of Fluconazole.



1.3. Drug-Excipient Compatibility Studies:

Fig.no. – 5.3.1: FTIR Characteristics Peaks of Pure Fluconazole Drug



Evaluation of nanoparticles:

Table: 5.4 Evaluation of nanoparticles (F1 to F9)

Formulation code	Particle size (nm)	Polydispersity index	Zeta potential (mV)	Entrapment efficiency (%)	% Yield	Drug content
F1	47.7	0.558	-25.9	28.41%	72.32%	59%
F2	34.3	0.338	-21.7	90.8%	78.45%	68%
F3	42.0	0.345	-16.4	89.55%	79.13%	70%
F4	48.9	0.229	-26.4	86.48%	67.56%	88%
F5	20.9	0.377	-26.6	95.78%	94.25%	97.38%
F6	40.5	0.461	-25.3	92.68%	92.01%	87%
F7	16.8	0.342	-16.9	26.78%	81.87%	70.34%
F8	43.6	0.406	-11.6	65.97%	83.16%	79%

F9	28.3	0.555	-21.0	86.77%	86.78%	89%

Fig.no. – 5.4.1: Particle Size Distribution and Zeta potential of Formulation F1.

Table 5.6: *In-vitro* diffusion release of fluconazole nanoparticle (F5)

% Cumulative Drug Release of F1 to F5					
Time (h)	**F1**	**F2**	**F3**	**F4**	**F5**
0	0	0	0	0	0
1	21.5%	34.9 %	5.8%	23.8%	36.02%
2	30.3%	41.5%	15.8%	36.3%	42.54%
4	38.9%	55.4%	26.9%	41.6%	59.76%
6	45.4%	67.8%	34.6%	50.4%	63.34%
8	52.6%	76.9%	38.1%	58.7%	70.03%
10	59.8%	88.9%	41.8%	69.1%	85.72%
12	62.92%	93.5%	46.3%	75.4%	95.5%

% Cumulative Drug Release of F6 to F9				
Time (h)	**F6**	**F7**	**F8**	**F9**
0	0	0	0	0
1	12.3%	7.3%	15.5%	10.12%
2	27.8%	15.2%	24.33%	19.3%
4	36.9%	22.5%	39.1%	32.5%
6	44.2%	27.6%	48%	48.6%
8	51.3%	33.8%	55.6%	59.2%
10	57.9%	36.1%	61.3%	65.3%
12	61.8%	40.2%	63.8%	74.8%

Fig no. 5.6.1: *In-vitro* diffusion release of fluconazole nanoparticle (F1 to F5)

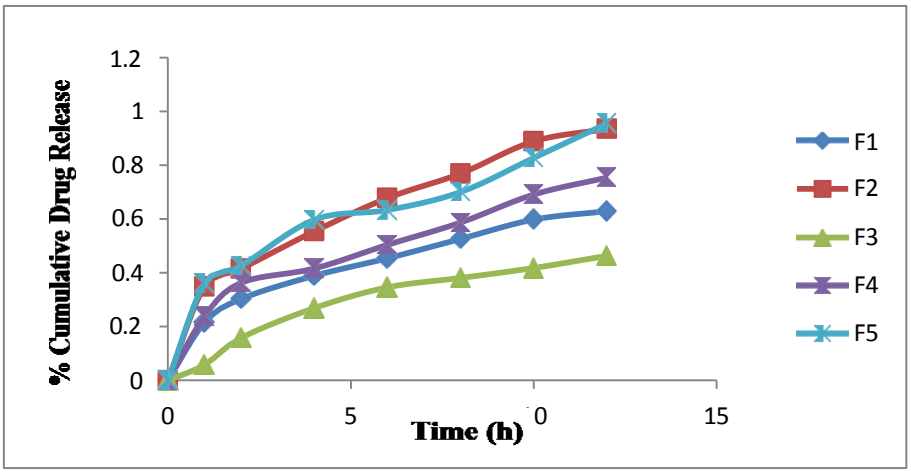
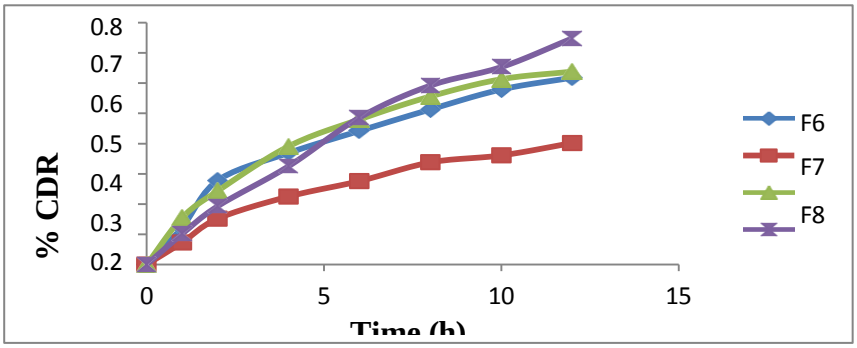


Fig no. 5.6.2: *In-vitro* diffusion release of fluconazole nanoparticle (F6 to F9)



5.4: Evaluation of Fluconazole nanoparticle gel:

Table 5.8: Evaluation of Fluconazole nanoparticle gel

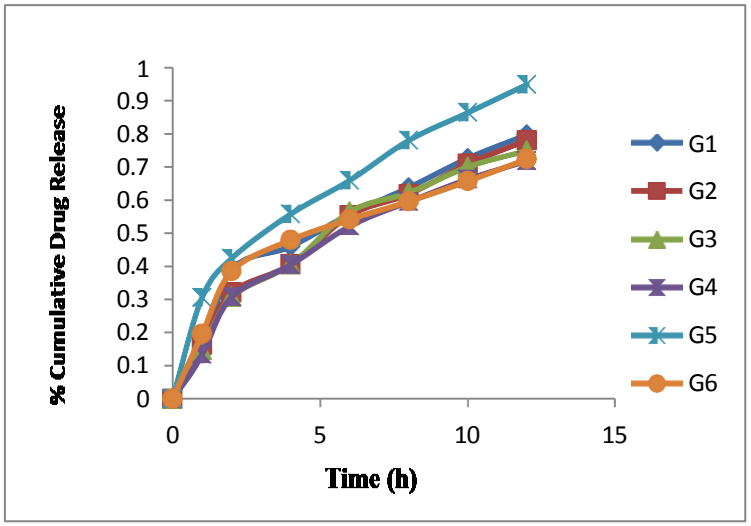
Formulation code	Percentage yield (%)	Drug content (%)	pH	Spreadability (gm.cm/sec)	Viscosity (cps)
G1	91.5%	89.9%	6.8	11.0	6,900
G2	93.1%	90.31%	7.1	11.1	8,300
G3	96.6%	93.0%	6.9	10.5	7,115
G4	92.8%	91.11%	6.85	10.7	9,200

G5	98.7%	97.5%	7.0	11.2	15,200
G6	98.0%	95.0%	7.21	10.9	12,100

5.5: *In-vitro* diffusion release of Fluconazole nanoparticle gel (G5):

% Cumulative Drug Release of G1 to G6						
Time (h)	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**
0	0	0	0	0	0	0
1	13.65%	16.42%	14.66%	13.42%	30.54%	19.56%
2	38.96%	32.07%	30.69%	30.71%	42.32%	38.46%
4	45.89%	40.54%	40.5%	40.37%	55.70%	47.89%
6	55.71%	55.3%	56.4%	52.04%	65.85%	54.1%
8	63.7%	61.7%	62.10%	59.4%	77.92%	59.5%
10	72.53%	70.8%	69.9%	66.21%	86.26%	65.6%
12	79.61%	77.9%	74.81%	71.71%	94.75%	72.3%

Fig no. 5.9: *In-vitro* diffusion release of fluconazole nanoparticle gel (G1 to G6)



5.6 : Drug release kinetics of formulation G5:

Table 5.10: Kinetics of drug release of G5 Formulation

Formulation code	Zero order kinetics	First order kinetics	Higuchi model	Korsemeyer-peppas model		Mechanism of Drug Release
	R^2	R^2	R^2	R^2	N	
F5	0.9731	-20.14	0.94	0.9879	0.6569	Non-Fickian

Fig. 5.10.1: Zero order plot for drug release kinetics of G5 formulation.

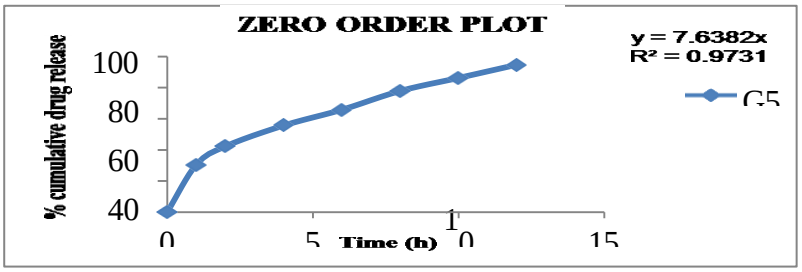


Fig. 5.10.2: First order plot for drug release kinetics of G5 formulation.

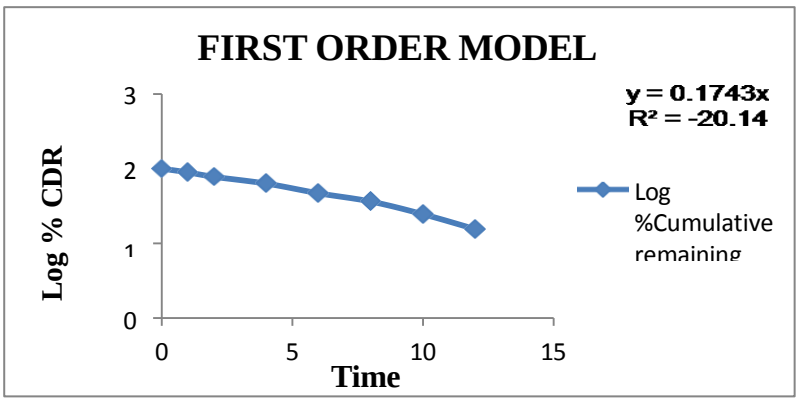


Fig. 5.10.3: Higuchi plot for drug release kinetics of G5 formulation.

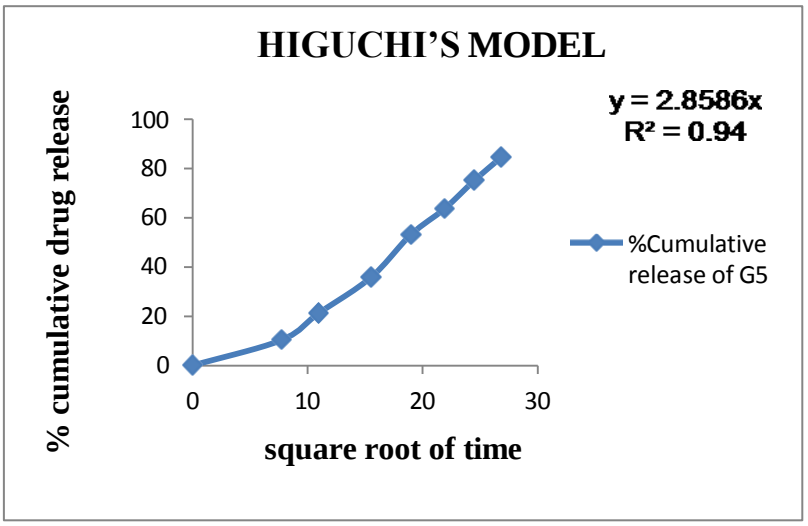
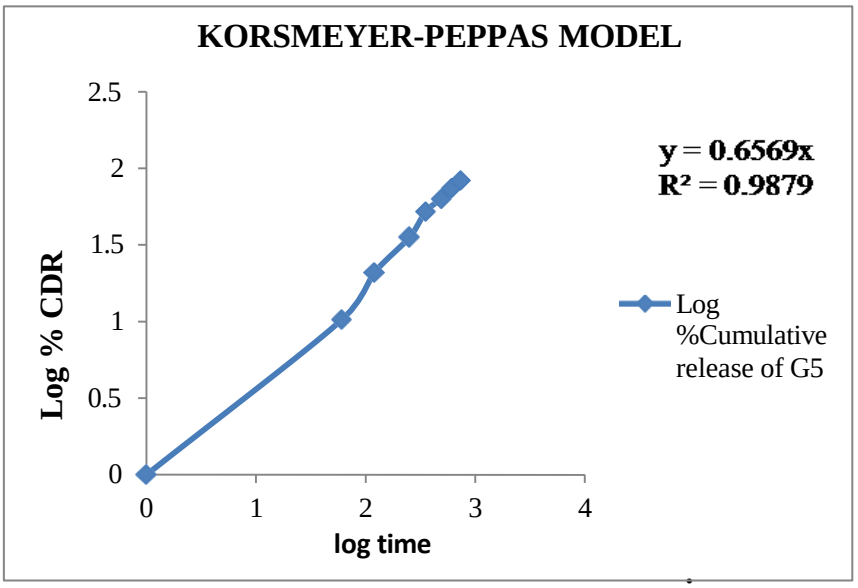


Fig.5.10.4: Peppas plot for drug release kinetics of G5 formulation



DISCUSSION

In the present study, an attempt was made to formulate nanoparticle based fluconazole gel for efficient delivery of drug to the skin. Topical and transdermal drug delivery systems offer several advantages over oral delivery systems. These delivery systems include gel, patch, cream, ointment and lotion. However it has been found so many side-effects were proved by the oral delivery system of fluconazole and here to overcome the side-effects of oral dosage

form, the dosage form has been changed by development and evaluation of nanoparticles based topical gel containing antifungal drug fluconazole.

Fluconazole is a synthetic antifungal agent belonging to the group of triazole. It is one of the commonly used antifungal agents for most kinds of fungal infections such as vulvovaginal candidiasis, oropharyngeal candidiasis, mucosal leishmaniasis, visceral leishmaniasis and dermatomycosis. The reason to select fluconazole is it overcomes all the side effects of the other fungal drugs like, Ketoconazole, Amphotericin B, Clotrimazole, and Miconazole. It is a BCS class III drug (high solubility and low permeability).

Preformulation studies:

1.3.1 Determination of melting point:

The melting point of the procured drug sample was found to be 140ºC which was lying within the reported range of 138-140ºC. It complies with the pharmacopeia standards, thus indicating the purity of the drug sample.

6.1.2. Determination of λ max (wavelength of maximum absorption):

Drug solution was scanned in the UV region (200-400nm) to find out the wavelength of maximum absorption (λmax). The λmax was found to be 261 nm. So the standard

calibration curve of Fluconazole was developed at this wave length. This was in accordance with the literature.

6.1.3 Standard graph of Fluconazole in methanol and phosphate buffer (pH7.4):

The standard graph was found to be linear in the range of 50-500 µg/ml. The R^2 and the slope were found to be 0.9976 and 0.0025 respectively. (Fig no.5.2)

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