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PREPARATION AND CHARACTERIZATION OF NANOGEL DRUG DELIVERY SYSTEM CONTAINING CLOTRIMAZOLE AN ANTI-FUNGAL DRUG"

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ABSTRACT

The present work is to formulate, characterize and evaluate the Clotrimazole nanogel. The nanogel of clotrimazole is prepared by solvent diffusion method (high speed homogenization) using Carbopol 940 and Locust bean gum as polymers and triethanolamine as a gelling agent. And they are characterized for FTIR studies, Surface morphology, Particle size analysis, and evaluated for homogeneity, pH, spreadibility, extrudability, Drug content studies, viscosity, in-vitro diffusion and stabilities studies. FTIR studies revealed that the drug and polymer are compatible with each other during preparation. The average particle size ranges from 410 nm to 530 nm. The nanogel formed a moderately spherical and smooth in surface as observed in SEM. Homogeneity and extrudability studies reveal that the nanogel was homogenous and easily extrudable. The pH data shows all the formulation are in the range of 6.1 to 6.9 and they are in compatible to skin pH. Viscosity studies shows the results in the range of 3268-3528 cps, and having good viscous property. The drug content studies of formulations were from 82.16 to 90.15 %. *In vitro* diffusion studies of prepared nano gel follow Pappas's dissolution kinetics with controlled release mechanism. And by fitting in Korsemeyer-Peppa's equation it shows non Fickian kinetics. From the stability studies data, it was found that there was no such difference in drug content and *In-vitro* drug release. This indicates the prepared nano gel formulations are stable. Formulation F9 shows good results for the invitro diffusion studies for controlled release.

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INTRODUCATION

Transdermal delivery system of drugs is a novel drug delivery system. This system breaks many barriers in drug therapy like need of assistance and uncomfortable administration. Transdermal delivery has many advantages over conventional modes of drug administration. like it potentially decreases side effects, avoids hepatic first pass metabolism and improves patient compliance. Nano gel are semisolid systems in which a liquid phase is constrained within a three-dimensional polymeric matrix (consisting of natural or synthetic polymers) in which a high degree of physical (or sometimes chemical) cross-linking has been introduced.[1]

Nanogels are potential form of the delivery of large number of drugs to different organs of the body owing to their high biocompatibility, high drug loading capacity, high biodegradability (and hence low cytotoxicity), good permeation capabilities and tissue mimicking properties. Their high-water retention makes them ideal capable of incorporation of bulky drugs like proteins, peptides, oligonucleotides and other macromolecules.[2]

Nanogel have three-dimensional structure formed by chemically or physically cross-linked polymers with hydrophilic or amphiphilic macromolecular chains, able to swell, by holding a great amount of water, with no dissolving but maintaining the structure intact. The great water content correlates with the fluid-like transport properties for the biologically active molecules significantly smaller than the gel pore size.[3]

Fungal infection of skin is now-a-days one of the common dermatological problem. The physicians have a wide choice for treatment from solid dosage to semisolid dosage form and to liquid dosage formulation. Among the topical formulation clear transparent gels have widely accepted in both cosmetics and pharmaceuticals.[4]

The vagina is a region of administration with a high contact surface to obtain local or systemic effects. This anatomical area represents special interest for government health systems for different sexually transmitted infections. However, the chemical changes of the vagina, as well as its abundant mucus in continuous exchange, act as a barrier and a challenge for the development of new drugs. For these purposes, the development of new pharmaceutical forms based on Nanoparticles has been shown to offer various advantages, such as Bio adhesion, easy penetration of the mucosa, and controlled release, in addition to decreasing the adverse effects of conventional pharmaceutical forms.[5]

Clotrimazole is a broad-spectrum antimycotic drug mainly used for the treatment of *Candida albicans* and other fungal infections. A synthetic, azole antimycotic, clotrimazole is widely used as a topical treatment for tinea pedis (athlete's foot). Clotrimazole is a very well-tolerated product with side effects, although there is some drug resistance appearing among immunocompromised patients. Clotrimazole is the drug of choice for the topical treatment of tinea cruris and tinea corporis caused by isolates of Trichophyton rubrum, Trichophyton mentagrophytes, Epidermophyton floccosum, Microsporum canis and C. albicans. It is also widely used in the topical treatment of vulvovaginal and oropharyngeal candidiasis.[6]

Clotrimazole is a lipophilic drug {log k o/w = 4.1} and slow dissolution in water, privies study has been carried out to improve solubility of clotrimazole by microcapsule, liposome, suspension with HPMC and nanosphere, cyclodextrins inclusion complex and solid dispersion technique using mannitol as carrier. Continuing that research our study is to gain insight into the effect of water-soluble polymers [natural and synthetic] by formulation it has topical preparation as nano gel.[7]

MATERIALS AND METHOD

Materials

Clotrimazole was gifted sample from Arathi pharmaceutical Mumbai. Carbopol 940, locust bean gum was purchased from yarrow chemicals Pvt. Ltd. And all other chemicals and reagent used were of analytical reagent grade.

Method

Emulsion – Solvent Diffusion Method[8]

The nanogel is prepared from modified Emulsion Solvent Diffusion method. It is having 4 steps.

Step I in the first step Accurately weighed quantity of drug is dissolved in ethanol and propylene glycol with stirring (organic phase).

Step II In the second step aqueous phase is prepared by using Carbopol -940 dissolved in water with continuous stirring and heat for a 20min in a magnetic stirring. And the drug phase is sonicated under ultrasonic bath Sonicator for 10min.

Step III In this step drug phase is added drop by drop into aqueous phase during high speed homogenization for 30 min at 6000rpm to from emulsion. The emulsion is converted into nanodroplet by homogenizer results in o/w emulsion formed.

Step IV In this step o/w emulsion is homogenized for 1 hour at 8000rpm and triethanolamine is added with continues stirring to from nanogel.

Formulation of Clotrimazole Nanogels

Table No: 1. Different concentrations of polymer and same concentration of drug.

Formulation Code	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
Clotrimazole (mg)	200	200	200	200	200	200	200	200	200
LBG (mg)	200	400	600	800	-	-	-	-	400
Carbopol -940 (mg)	-	-	-	-	200	400	600	800	400
Ethanol (ml)	10	10	10	10	10	10	10	10	10
Propylene Glycol (ml)	4	4	4	4	4	4	4	4	4
Triethanolamine (ml)	4	4	4	4	4	4	4	4	4
Water	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs

* LBG = locust bean gum

The Prepared nanogel were evaluated by following parameters[9-14]

FTIR study

Infrared absorption spectroscopy (IR) of Clotrimazole and Nanogel formulations (F1 to F9) were done using Bruker FTIR (ATR) spectroscopy to ascertain compatibility in all the formulations.

Scanning Electron Microscopy (SEM)

Shape and surface morphology of the Nanogel prepared with optimized parameters was observed by scanning electron microscopy.

Particle size

The particle size analysis revealed that, the Nanogel was in the nanometre range. The size of the nanoparticles was affected by the homogenization time and the concentration of Carbopol 940.

Zeta Potential

The stability of the formulated Nanogel was evaluated by Shivaji university, Kolhapur. measuring the zeta potential of the Nanogel (it shown between the desired range ±30 mV).

Homogeneity

The developed nanogels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

pH

The pH of various nanogel formulations was determined by using digital pH meter.

Spreadability

It was determined by wooden block and glass slide apparatus. Weights about 20g were added to the pan and the time were noted for upper slide (movable) to separate completely from the fixed slides.
Spreadability was then calculated by using the formula:

$$S = M.L / T$$

Where,
S = Spreadability, M = Weight tide to upper slide, L = Length of glass slide
T = Time taken to separate the slide completely from each other

Extrudability

The formulations were filled in to collapsible aluminium tubes. The tubes were pressed to extrude the 0.5 cm ribbon of the gel in 10 second and the extrudability of formulations was checked.

Drug content studies

The weight equivalent to 100 mg of gel was taken and transferred to a 100 ml standard flask. 25 ml of ethanol and 25 ml of 7.4 pH phosphate buffer were added and shaken for about half an hour and the volume was made up to 100 ml with 7.4 pH phosphate buffer. The above solution was filtered and 5 ml of filtrate was taken and diluted to 100 ml with 7.5 pH phosphate buffer. The absorbance of the resulting solution was measured at 245 nm and the content of clotrimazole was calculated.

Viscosity

The viscosity of the formulations (gel) was determined at 25°C by using Brookfield viscometer with spindle no. S-96 at 1 rpm and viscosity was measured in cps. The measurement of each formulation was done in triplicate and average values are calculated.

***In- vitro* diffusion study**

Cellophane membrane was used for this study in Frantz Diffusion Cell. 100mg of Nanogel is placed in donor compartment with which is filled phosphate buffer 7.4. The membrane was mounted between the compartments of the Frantz Diffusion Cell. Reservoir compartment was filled with phosphate buffer 7.4. The study was carried out at 37± 10^0 C and speed was adjusted to 100 to 120 rpm and it is carried out for 24 hours. 5 ml of sample was withdrawn from reservoir compartment by the help of hypodermic syringes at half an hour interval for 2 hours, then one-hour interval for 10 hours and finally 6 hrs to next 24 hrs. And absorbance was measured spectrophotometrically at 245nm. Each time the reservoir compartment was replenished with the 5 ml fresh volume of phosphate buffer 7.4 pH solution to maintain constant volume.

Stability studies

Optimised formulation was subjected for a stability testing for 45days per ICH norms at a temperature of 40º ± 2ºC and RH of 75%. optimised formulation was analysed for the change in drug content and *in-vitro* diffusion studies by procedure stated earlier.101-103

RESULTS

UV Spectrophotometric study:

Standard calibration curve of Clotrimazole in Phosphate buffer (7.4 pH)

Table No. 2 shows the absorbance of standard solution of Clotrimazole ranging from 2 to 10 µg/ml in phosphate buffer pH 7.4 (n=3). The curve was found to be linear in the range of 2 to 10 µg/ml at λ_{max} 262nm. The regression value was found to be 0.9996 as shown in Figure No. 1.

Table No: 2. Standard Calibration curve of Clotrimazole in Phosphate buffer 7.4.

Sl. No	Concentration (µg/ml)	Absorbance	
			RSD (%)
1	0	0.000	0.000
2	2	0.134	0.134±0.014
3	4	0.269	0.269±0.020
4	6	0.390	0.390±0.017
5	8	0.515	0.515±0.019
6	10	0.642	0.642±0.022

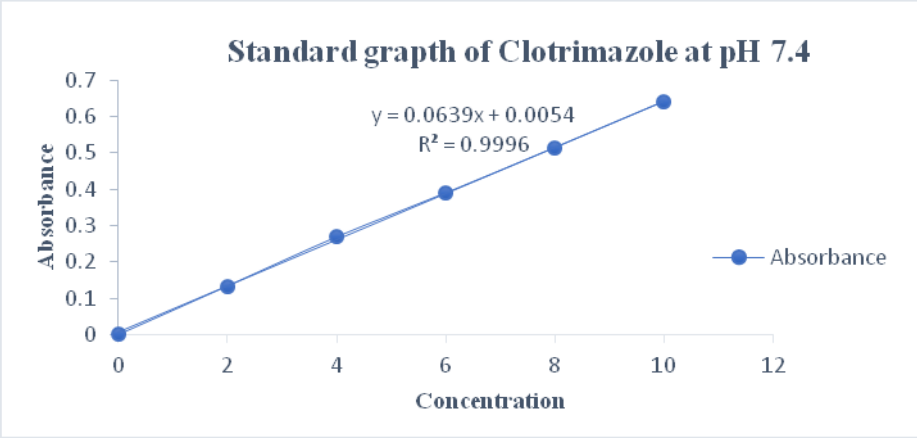


Figure No: 1. Standard Calibration curve of Clotrimazole in Phosphate buffer 7.4.

FTIR studies:

From the FTIR spectra it has been observed that there is no molecular shifting in functional group as if that of pure drug specified in pharmacopeia hence there is a compatibility b/w the drug & polymer. The individual FTIR spectra of pure drug clotrimazole and polymer Carbopol-940 and locust bean gum as well as a combination spectrum of the drug and polymer and Formulation F9 are shown in Figure No 2 and 3 It was found that the drug was compatible with polymer in physical mixture.

IR Spectra's:

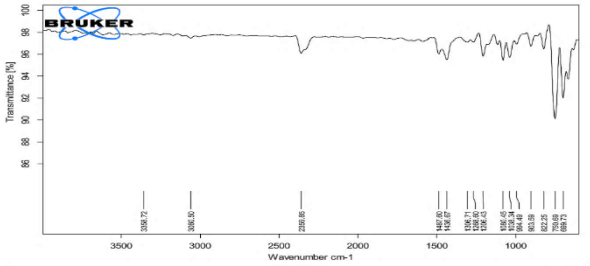


Figure No: 2. IR spectra of pure drug.

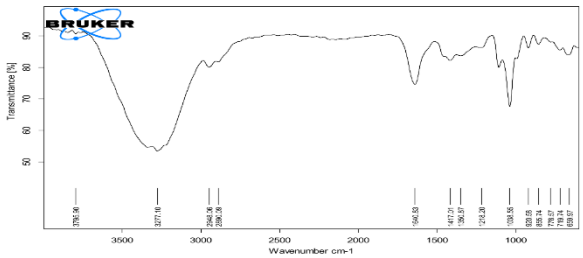


Figure No: 3. IR Spectra of Nanogel Formulation F_9.

Scanning Electron Microscopy (SEM)

Determination of surface morphology and Shape was done by ZEISS EVO. US scanning Electron Microscope.

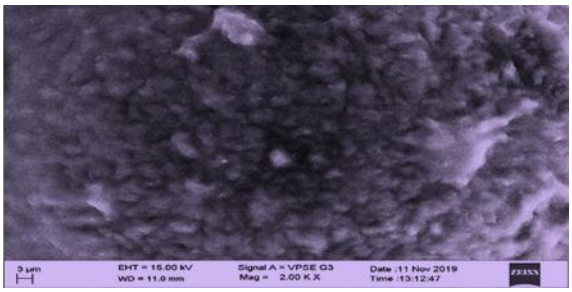


Figure No: 4. SEM Images of Formulation F_9(a).

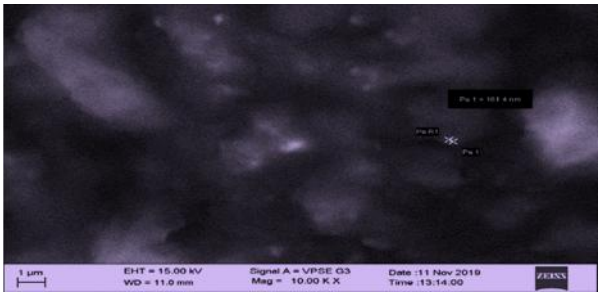


Figure No: 5. SEM Images of Formulation F_9 b.

Particle size analysis

The particle size determination of the Nanogel were carried out by using Particle size analyser (Zetasizer).

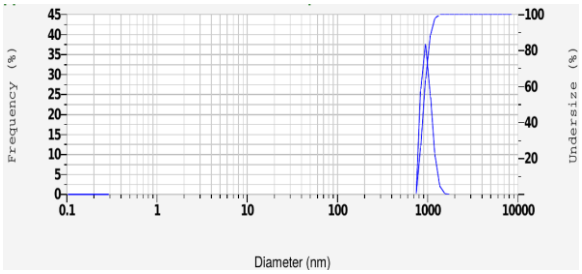


Figure No: 6. Particle Size distribution by intensity.

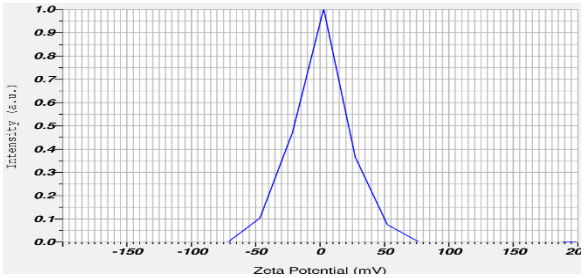


Figure No: 7. Zeta potential (mV).

Table No: 3. Particle size analysis of Nanogel containing Clotrimazole.

Formulation code	Particle size analysis (nm)
F_1	460
F_2	440
F_3	530
F_5	412
F_4	490
F_6	480
F_7	432
F_8	420
F_9	410

Homogeneity

All the gel formulations (F_1-F_9) showed good homogeneity with absence of lumps. Gels were found to be transparent and were free from presence of particles, uniformity of gel, aggregates, foreign matter and phase separation. Results are shown in Table No. 4

Determination of pH

The pH of different formulation from F_1 to F_9 were showed in **Table No. 4.** The pH varies from one formulation to another according to their polymer ratios with drug.

Spreadability

Spreadability diameter for different formulations F_1-F_9 showed good spreadability i.e. gel is easily spreadable. The results are shown in **Table No. 4.**

Extrudability

For a good gel formulation, it should extrude easily from the container. The extrudability of all formulations was found to be good. The result shown **Table No.4**

Drug content

The drug content of all the formulations from F_1 to F_9 are shown in **Table No. 4.** There is no much difference in the drug content of each formulations. So, the effect of polymers ratios is less considerable here.

Viscosity

All the formulations of Nanogel were subjected to Brookfield viscometer used to measure the viscosity (in cps) by dropping a cone attached to a holding rod from distance of 10 cm in such a way that, it should fall on centre of the glass cup filled with Nanogel. The results are shown in **Table No. 4.**

Table No: 4. Evaluation of formulated batches of Nanogel.

Formulation code	Homogeneity	pH	Spreadibility (cm)	Extrudability	Drug Content	Viscosity (cps)
F_1	Homogenous	6.1	2.5	+	82.16%	3459
F_2	Homogenous	6.3	3.2	+	86.72%	3356
F_3	Homogenous	6.2	2.9	++	84.75%	3268
F_4	Homogenous	6.5	3.4	++	83.45%	3498
F_5	Homogenous	6.2	2.6	+	80.24%	3295
F_6	Homogenous	6.3	2.9	++	85.67%	3501
F_7	Homogenous	5.9	3.2	+	82.15%	3340
F_8	Homogenous	6.4	2.8	++	88.65%	3351
F_9	Homogenous	6.9	3.5	++	90.15%	3528

+Satisfactory, ++Good

***In-vitro* diffusion studies**

The drug release form the Nanogel was studied by Franz diffusion cell method. The in vitro release profiles of clotrimazole from Nanogel are shown in **Table No.5.** The cumulative percentage release of Clotrimazole Nanogel were varied depends on the drug polymer ratio for 24 hrs.

***In-vitro* Diffusion Studies: -**

Table No: 5. *In-vitro* drug release kinetics of Nanogel formulation F1 to F9.

Time (hr)	% Drug Release $\bar{X}$ ± SD n=3								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
0.5	7.3269	13.0123	16.2356	10.625	18.0326	6.2316	9.562	15.632	11.866
1	12.0365	18.321	24.5746	16.0238	26.135	13.5623	15.652	22.561	17.762
1.5	16.2365	23.562	28.6234	26.6861	36.5629	19.2356	20.565	25.599	21.632
2	23.6031	27.6215	33.653	32.5623	48.2689	26.1576	28.623	30.562	24.152
3	27.9658	39.5201	39.6892	37.0653	53.156	28.2364	35.496	33.125	27.31
4	31.6892	47.2653	48.9608	45.3685	65.2305	32.659	43.213	39.154	33.423
5	45.6042	55.8052	56.6391	50.256	71.9689	36.589	50.289	43.125	39.438
6	54.6512	62.6529	67.0653	62.2943	82.136	41.0653	57.562	48.268	42.643
7	65.356	77.6531	79.3506	69.3291	83.2653	52.68	63.163	52.691	48.621
8	72.623	78.903	82.6204	75.683	84.631	56.1203	69.136	64.523	57.961
9	80.361	84.7568	85.6086	78.263	86.2369	62.1269	75.139	70.562	63.682
10	83.653	76.2751	86.361	84.264	87.135	68.6329	81.276	79.263	69.432
11	85.263	83.2613	87.623	86.621	89.445	73.263	86.896	82.652	72.891
12	85.9632	85.263	88.2306	88.652	90.563	84.632	89.123	85.785	77.138
18	86.2631	89.2646	88.263	90.123	92.426	89.532	90.458	88.253	85.631
24	89.6503	90.6382	90.638	93.456	93.185	93.862	92.695	90.350	94.82

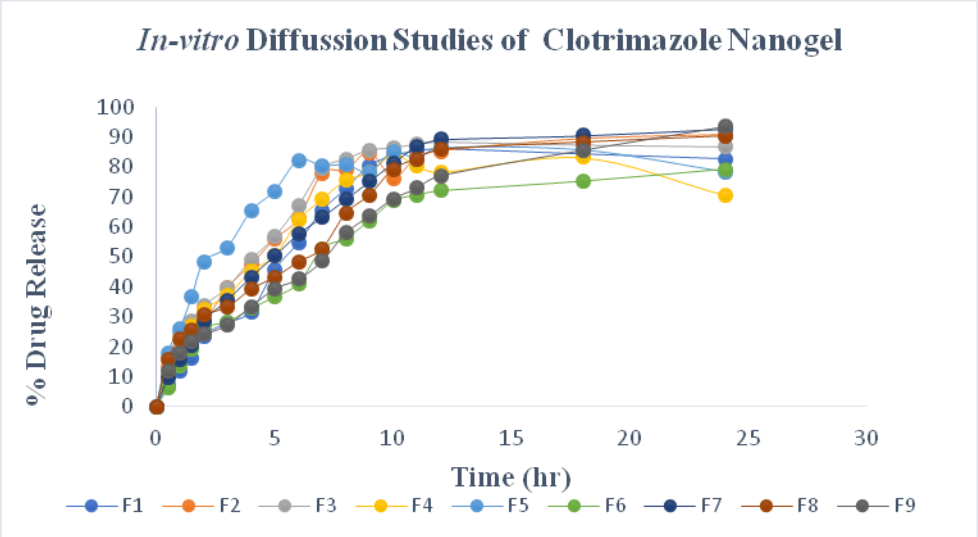
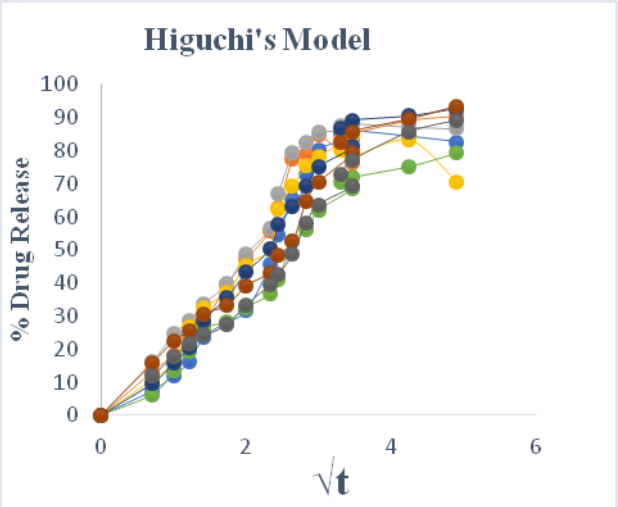


Figure No:8. Graph of *In-vitro* drug release of Nanogel formulations F1 to F9.



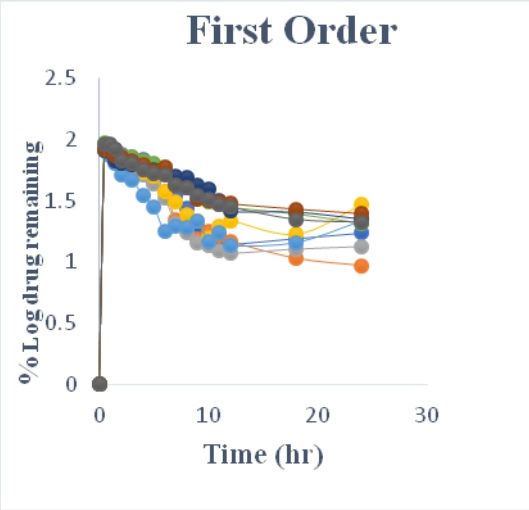


Figure No:9. Graph of *In-vitro* drug release of Nanogel formulations F1 to F9.

Figure No:10. Graph of higuchi model F1 to F9 formulation.

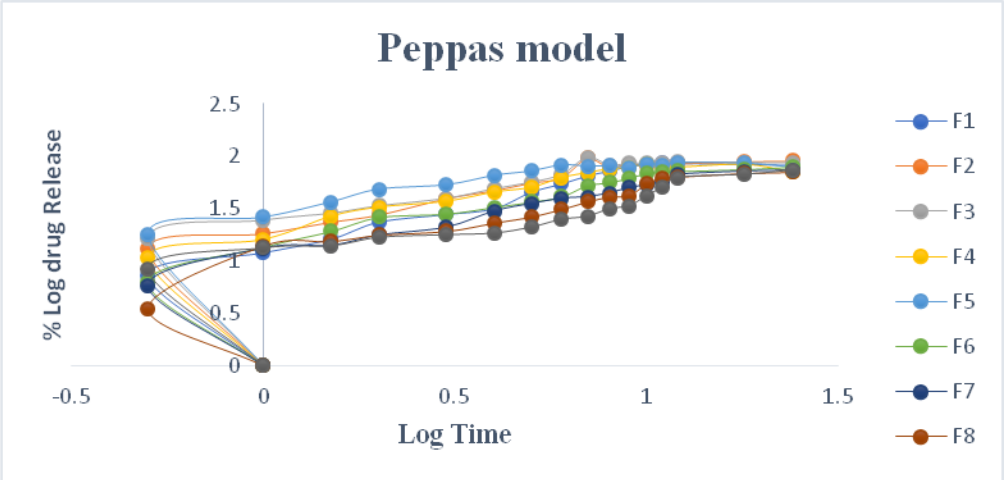


Figure No:11. Graph of Peppas model drug release of Nanogel formulations F1 to F9.

Table No: 6. Regression co-efficient value and 'n' value of different Nanogel.

Formulation Code	Zero order		First order		Higuchi	Peppas	
	n	R^2	n	R^2	R^2	n	R^2
F1	4.545	0.674	0.055	0.7608	0.836	0.732	0.911
F2	3.229	0.691	0.035	0.9341	0.875	0.677	0.929
F3	3.186	0.764	0.033	0.8791	0.962	0.683	0.955
F4	3.358	0.747	0.029	0.7931	0.992	0.723	0.970
F5	3.514	0.844	0.053	0.9293	0.936	0.625	0.971
F6	3.645	0.747	0.027	0.8367	0.927	0.832	0.960
F7	3.343	0.941	0.068	0.9230	0.979	0.797	0.982
F8	3.379	0.805	0.024	0.9340	0.956	0.826	0.969
F9	3.996	0.957	0.030	0.8865	0.961	0.850	0.975

Stability studies

The stability study was performed as per ICH guidelines. The Optimized formulations of gel were filled in the collapsible tubes and stored at different temperatures and humidity conditions, viz.250 C ± 20C/ 60% ± 5% RH, 30 ^{0}C ± 2 ^{0}C/ 65% ± 5%RH, 40 ^{0}C ± 2 ^{0}C/ 75% ± 5% RH for a period of 45 days and samples were analysed drug content and drug release studies.

Table No :7. Drug content studies.

Formulation Code	Drug content	
	Before stability test	After stability test
F9	88.15%	87.20%

Table No: 8. *In- vitro* diffusion studies.

Formulation Code	Percentage of drug diffusion	
	Before stability test	After stability test
F9	89.16±0.025	88.26±0.015

The diffusion study of optimized nanogel was studied according to earlier procedure and determine the drug diffusion rate.

CONCLUSION

It can be concluded that the experimental study carried out that the formulation of a Nanogel containing antifungal drug yields a formulation with spherical and smooth surface, nano in size range. The prepared nanogel was opaque, without any lumps, particle and aggregates. So, all the formulations are homogenous. Based on all the factors the nanogel drug delivery system F_9 shows good drug content compare to other. The particle size of the nanogel formulation is optimum and it is less than 1000 nm. So, it concluded that the particles are in tiny and nano in size range. All nanogel formulations shows pH in the range of 6.1 to 6.9. Formulation F_9 shows highest pH of 6.9. Because the pH range of nanogel were 1 to 7 pH. Based on the spreadibility diameter study it shown the nanogel is having good spreadibility. Nanogel formulations shown viscosity range from 3268-3528 cps. It concluded that they are stable in nature. Formulation F_9 shows highest percentage of drug release compare to other formulations. *In-vitro* diffusion studies show F_9 formulation shows controlled release pattern of drug from the formulation. The formulation was found to be stable in short term stability studies. Here we have selected F9 has an optimized formulation which shown good morphological features, drug content efficiency and controlled drug release.

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