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Development and Optimization of Niosomal In-Situ Gel Loaded With Fluconazole an Efficient Antifungal for Eye Infection

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ABSTRACT:
The aim of the present study is to formulate and evaluate niosomal insitu gel formulations as ocular drug delivery systems of Fluconazole to improve its therapeutic effect in a controlled manner. A 2x3 factorial design have been applied to develop niosome for optimization, by varying surfactant and soya lecithin concentration. Niosomal formulations were evaluated for entrapment efficiency, particle size, zeta potential and in vitro drug release. Particle size of the F2, F7 formulation was found to be 706.1nm and 915.8nm and zeta potential of the F2, F7 formulation was found to be -31.3 and -50.9mV respectively. The highest entrapment efficiency and drug content is observed in F7 niosomal formulation with 93.22 % and 91 % respectively. Since the formulation F7 showed maximum amount of %drug content, %drug entrapment efficiency. 2x3 factorial designs were applied by using QI MACROS 2022 to study effect and interaction on the response of % CDR and for the optimization process were performed by using DESIGN EXPERT-13 software.F7 niosomal formulation were selected as optimized and further used for niosomal in-situ gel by using the Carbopol 934 at 3 % w/v as a polymer (gelling agent). Further the prepared in-situ gel (GF7) was evaluated. Viscosity (759±3.051). Hence the results clearly showed that the in-situ gels have ability to retain the drug for prolonged periods. The % CDR ofniosomal in-situ gel formulation GF7 was found to be 89.92 % and which follows first order. The 'n' values for all the formulation were found to be more than 0.5. this indicates that the release approximates non-fickian diffusion mechanism.

Key words: Niosome, In-situ gel, Fluconazole, Carbopol 934, ocular drug delivery

I. INTRODUCTION

Despite eyes are among the most readily accessible organs in the body, good ocular bioavailability is still a challenging task. The bioavailability of ocular drugs in conventional system that is aqueous solution is usually low because of quick elimination from the eye reflex blinking and tear drainage. Corneal barrier also plays a significant role in low ocular bioavailability. Significant efforts have been made over decades to improve their ocular bioavailability of administered drug that is inserts collagen shield and colloidal system such as liposome's, niosomes, nanoparticles and nanocapsules.[1] Among all, niosomes come out to be the most promising application in ocular drug delivery. Treatment with niosomes system increases bioavailability reduces administration frequency and promotes drug targeting.

Infections by different fungal species is one of the most serious and challenging problems affecting the eye. The incidence of fungal infections increases in immune-compromised patients after organ transplantation, patients who receive chemotherapy and in intensive care units. Ocular fungal infections can be a result of eye traumas or surgeries complications.[2]

The most common sites for fungal infections of eyes are cornea, retina and vitreous. Also, they can involve other periocular tissues including lacrimal apparatus, conjunctiva, sclera, eyelids and bony orbit. If the infection was inadequately treated, corneal transplantation or vitrectomy might be required. Fungal infections can lead to irreversible sight damage or even blindness within days of the disease onset.[3]

However, due to the dramatic increase in the fungal resistance to drugs and the epidemiologic increase in the invasive fungal infections,there is a vital increasing need for exploiting new delivery systems that can increase the drug ocular availability and avoid the mechanisms of fungal resistance.[4]Fungal resistance is a consequence of the long term use of the antifungal drug for prophylaxis purposes or inadequate treatment caused by either low drug concentration or early discontinuation of medication due patient incompliance. This can lead

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to therapeutic failure which dictates more aggressive long term treatment. Thus, it mandates exploiting new effective alternative drug delivery systems. [5]

Fluconazole (FL), a synthetic fluorinated bis-triazole derivative, is one of the most important antimycotic agents with a broad spectrum and advantageous physicochemical properties. It is widely used for prophylaxis and treatment purposes for several candidal infections. It has intermediate molecular weight and short T1/2 in eye. Also, it has better safety profile compared to other antifungal analogues. These characteristics encourage ophthalmologists to prescribe it as a first line in Candidal infections treatment.[6]But ,slightly solubility of Fluconazole which is confronted with the problems of low bioavailability as the ophthalmic aqueous eye drops. The constraining barrier mechanisms of different eye layers, blinking, dynamic rapid tear turnover rate and the nasolarcrimal drainage into systemic circulation permit less than 5% of an applied topical drug dose of conventional aqueous eye drops to get access to intraocular tissues.[7]

Future trends in treating ocular diseases affecting anterior or posterior eye segments will be more focused on the use of non-invasive delivery routes and colloidal carriers. Recently, combining In-situ gel system within different colloidal has got extensive consideration in several studies and has revealed potentiality as an enabling tool in drug delivery system which improve retention of drug with improved bioavailabilty and better therapeutic effect.

II. MATERIALS AND METHODS:

Fluconazole pure drug was purchased from Karnatakaantibioticspharma.Ltd.., and span 40, Span 80 cholesterol and soya lecithin, methanol, Carbapol 934 was purchased from the SD fine chemicals, Mumbai. And the distilled water was produced in our research laboratory with a distillation unit.

Method of Preparation of niosomes:[8-10]

Niosome formulation containing Fluconazole was prepared by using thin film hydration method. Optimization of niosome formulation was done by preparing varying concentration of drug, surfactants (span40/span80), lecithin and cholesterol.

Accurately weighed amount of surfactant (Span40\ Span80), lecithin, cholesterol and drug (Fluconazole) were taken in a clean and dry wide mouthed glass vial and methanol (3ml) was added to it. After warming. All the ingredients were mixed well with a glass rod, open end of the glass bottle was covered with a lid to prevent the loss of solvents from it and warmed-over water bath at 60-70°C for about 5-10 min. until the surfactant mixture was dissolved completely. Then PBS (pH 7.4) was added and warmed on a water bath till clear solution was formed which was converted into niosomes on cooling. The obtained niosomes was preserved in the same glass bottle in dark condition.

Factorial design:

The niosomal were prepared by using 2^3 factorial designs.The effect of span 40 and span 80 have been evaluated by applying 2x3 factorial design.Vesicle forming capacity, % EE and % CDR were taken as parameter for optimizationby applying 2x3 factorial design and studied the effect and interaction.

Factorial design: 2×3
A: Cholesterol (low-50, high-150)
B: Span 40 (low-50, high-200)
C: Span 80 (low-50, high-200).

Table 1: 2^3 factorial designs of niosomal formulation

Formulation code	A	B	C
F1	-	-	-
F2	+	-	-
F3	-	+	-
F4	+	+	-
F5	-	-	+
F6	+	-	+
F7	-	+	+
F8	+	+	+

International Journal of Pharmaceutical Research and Applications
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CHARACTERIZATION OF PREPARED NIOSOME:

The prepared niosomal gel were evaluated for different parameters like FTIR, Drug Excipients compatibility, vesicle size analysis, viscosity, physical appearance, pH determination, Vesicle size analysis, Zeta potential analysis, drug content, entrapment efficiency, in-vitro diffusion study.the optimized niosomes was formulated by a niosomal in-situ gel and evaluated for Spreadability, viscosity, pH, drug content, in vitro release kinetics, in-vitro antifungal activity,(HET-CAM) Test, stability studies

In vitro release study:[11]

In vitro release study pattern of niosomal suspension was carried out in dialysis bag method. Fluconazole niosomal suspension equivalent to 10 mg was taken in dialysis bag and the bag was placed in a beaker containing 100 ml of pH: 7.4 Phosphate buffer. The beaker was placed over magnetic stirrer having stirring speed of 100 rpm and the temperature was maintained at 37+0.5°C. 1 ml sample was withdrawn periodically and were replaced by fresh buffer.

The samples were assayed by UV Spectrophotometer at 260 nm using phosphate buffer pH 7.4 as blank and cumulative % of drug released was calculated and plotted against time. The drug release was fitted to kinetic data analysis to understand the kinetic and mechanism of drug release.

Preparation of niosomal in-situ gel[12]

The cold method described by Choi et al. (1998) will be followed with slight modification. Briefly, calculated quantities of the drug loaded niosomes and mucoadhesive polymer are to be stirred in de-ionized water maintained at 25 °C using a thermostatically controlled magnetic stirrer. This dispersion is then cooled to 4 °C in a refrigerator and kept overnight. In all the cases, pH of the mucoadhesive gels will be adjusted to 5.5-5.7 using suitable volume of 0.IN sodium hydroxide solution.

Biocompatibility test: Hen's Egg Test Chorioallantoic Membrane(HET-CAM)[13.14]

Development: Uponreceipt,the eggs will be placed incommercial incubators.On day10 of development,the eggs will be removed from the incubator and candled to determine the viability of the embryo. A rectangular window will be removed from the shel ldirectlyoverthe aircelland the eggmembrane willbe carefullymoistenedwith2- 3 mL 0.9% saline and returned to the incubator. The eggs are then dosed and observed continuously for 5minutes for the appearance oflys is,haemorr haging and/orcoagulation which is documented. In addition, the eggs are scored for severity at 1 and 5minutes.Theseverityofeach reactionafter 1and5 minutesis recorded.

Stability studies

The purpose of accelerated stability study is to provide evidence on the quality of a drug substance or drug product, which varies with time under the influence of a variety of environmental factors such as temperature, humidity and light. To confirm the stability of niosomal formulation, accelerated stability testing studies was performed for 6 months. The optimized formulation was kept at of 30ºC ± 2ºC and 65 ± 5%in stability chamber. Drug particle size, entrapment and drug release were fixed as physical parameters for stability testing.

IN-VITRO ANTIFUNGAL STUDIES

Procedure: Preparation and assay of bacterial inoculums

Fungal strain was streaked from stock solution to PBD Broth and incubated overnight at 27°C. Single colony from plate was transferred to the broth and incubated at 27°C, for 24 h and used as inoculums. The turbidity of the culture suspension was adjusted spectrophotometrically (range of 0.5–1.0) to the McFarland 0.5 turbidity standard (1.5×10^8 CFU/mL).

Antimicrobial activity by well diffusion method

Antimicrobial activity of given samples was investigated using well diffusion method. Test plates (diameter 10 cm) were prepared with 20 mL of respective potato dextrose agar media. After media get solidified, 100 µL of 24 h culture (1.5×10^8 CFU/mL) was added and uniformly spread over plates using L shaped rod. Well (about 6mm diameter) were made and the given 3 samples were added in given concentrations in respective wells. Plates were incubated at respective growth conditions for 24 h. Zones of inhibition of microbial growth around the well were measured and recorded after the incubation time. The inhibitory zone was considered theshortest distance (cm) from the outside margin of the samples to the initial point of the microbial growth.

International Journal of Pharmaceutical Research and Applications
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III. RESULTS:

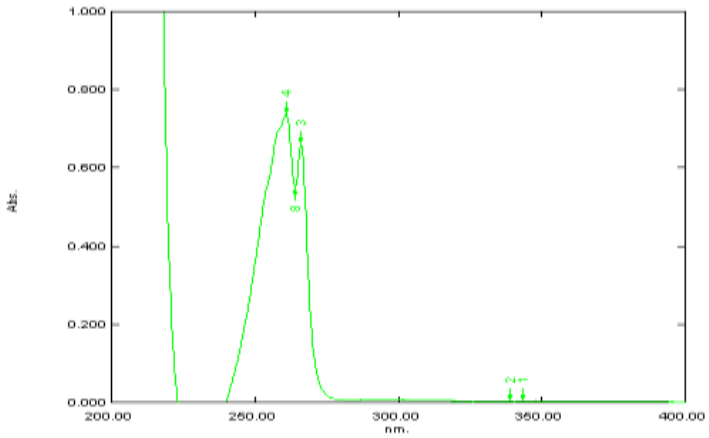

Figure 1- ƛ max of pure drug Fluconazole

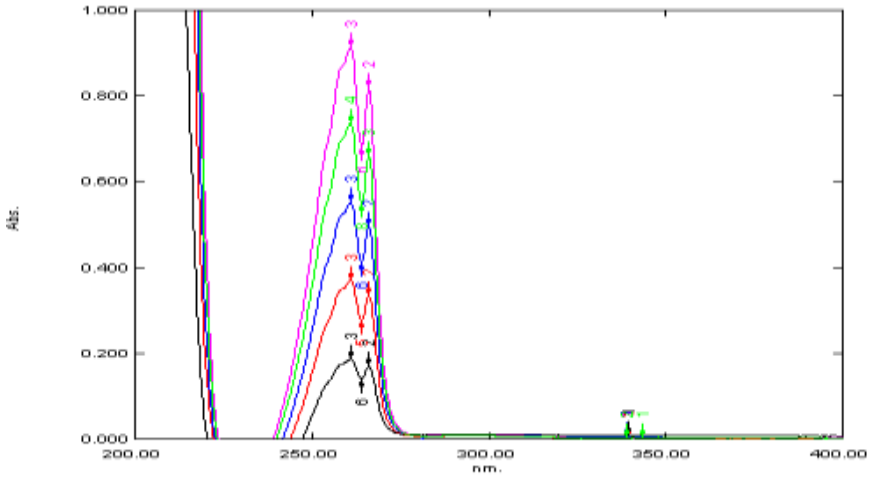

Figure 2: UV spectra of pure drug Fluconazole

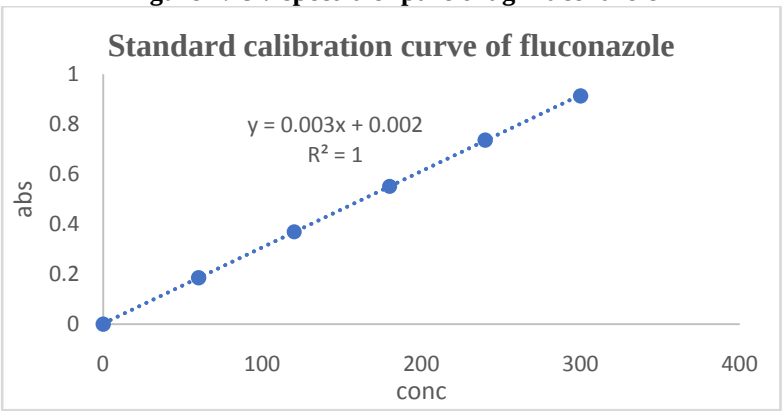

Figure 3: standard calibration curve of pure drug Fluconazole

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 1098-1115 www.ijprajournal.com ISSN: 2456-4494

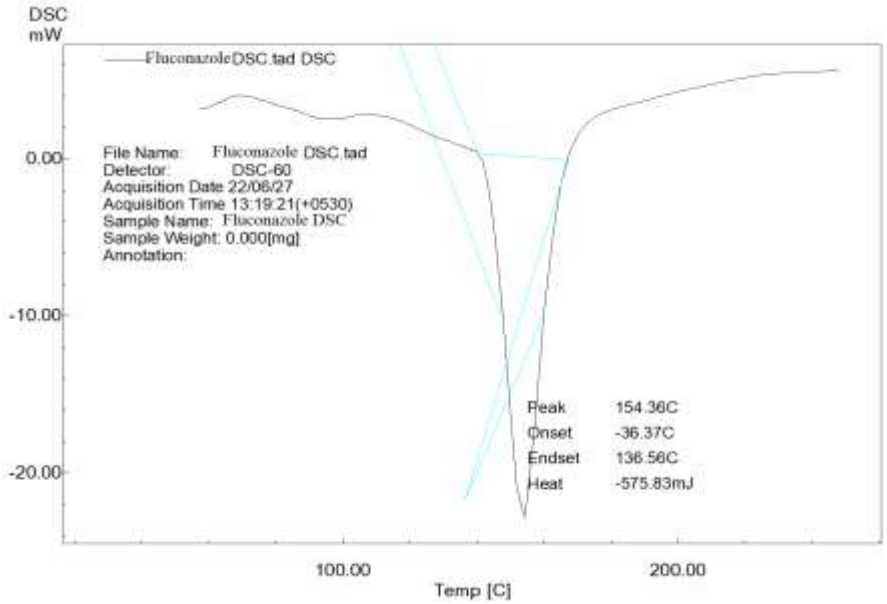

Figure 4: Thermal analysis results

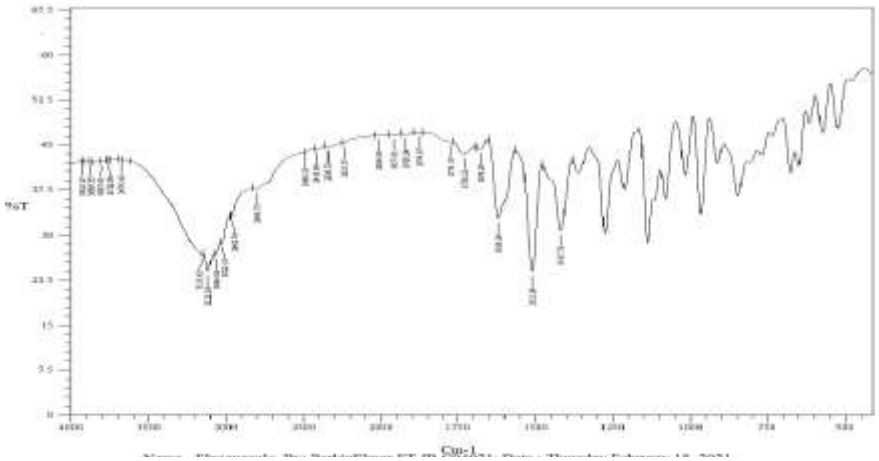

Figure 5: FT-IR spectra of pure drug Fluconazole

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 1098-1115 www.ijprajournal.com ISSN: 2456-4494

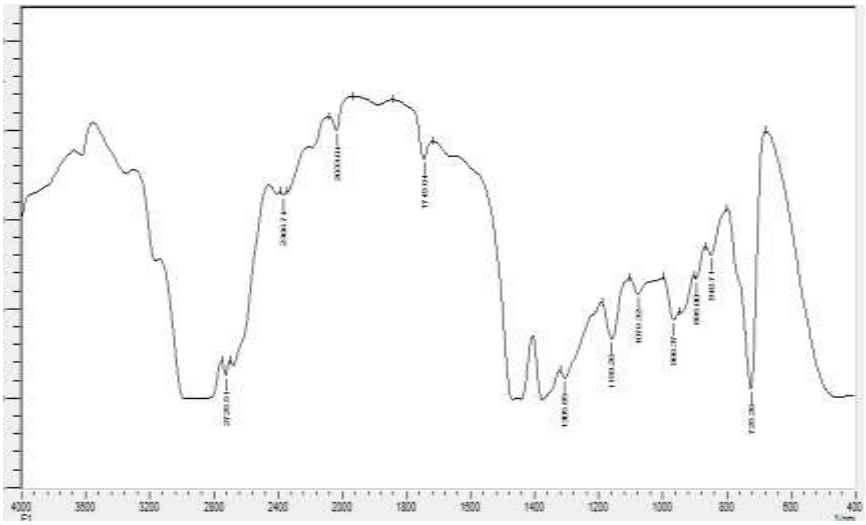

Figure 6: FT-IR spectra of pure drug with span40

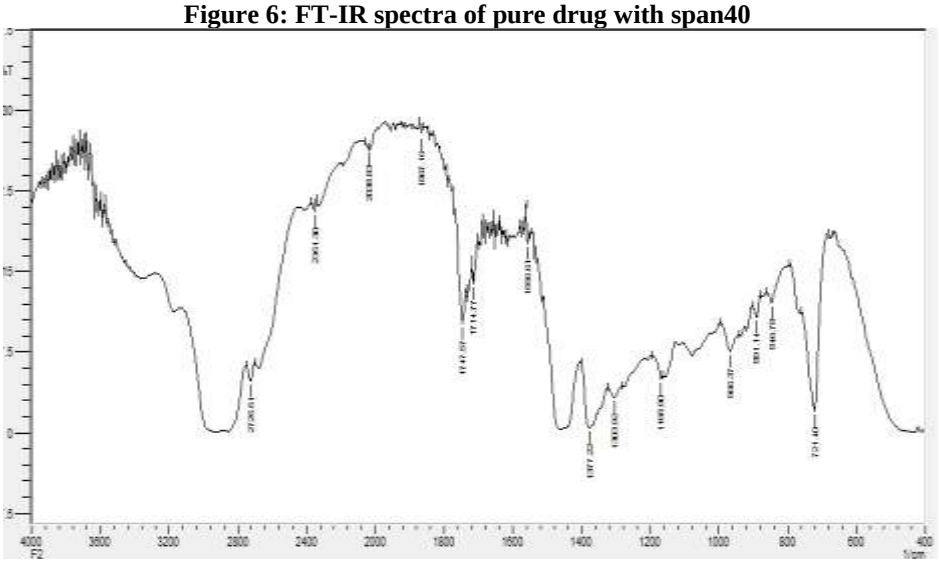

Figure 7: FT-IR spectra of pure drug with span80

DETERMINATION %DRUG CONTENT AND % ENTRAPMENT EFFICIENCY AND pH:

Table 2: Results % drug content and % entrapment and pH.

Formulation code	% Drug content	% Entrapment efficiency	pH
F1	72	85.161	6.4
F2	68	77.096	6.2
F3	80	82.58	6.4
F4	77	78.064	6.0
F5	87	89.322	7.0
F6	80	82.83	6.9
F7	91	93.225	7.3
F8	85	87.483	6.6

International Journal of Pharmaceutical Research and Applications
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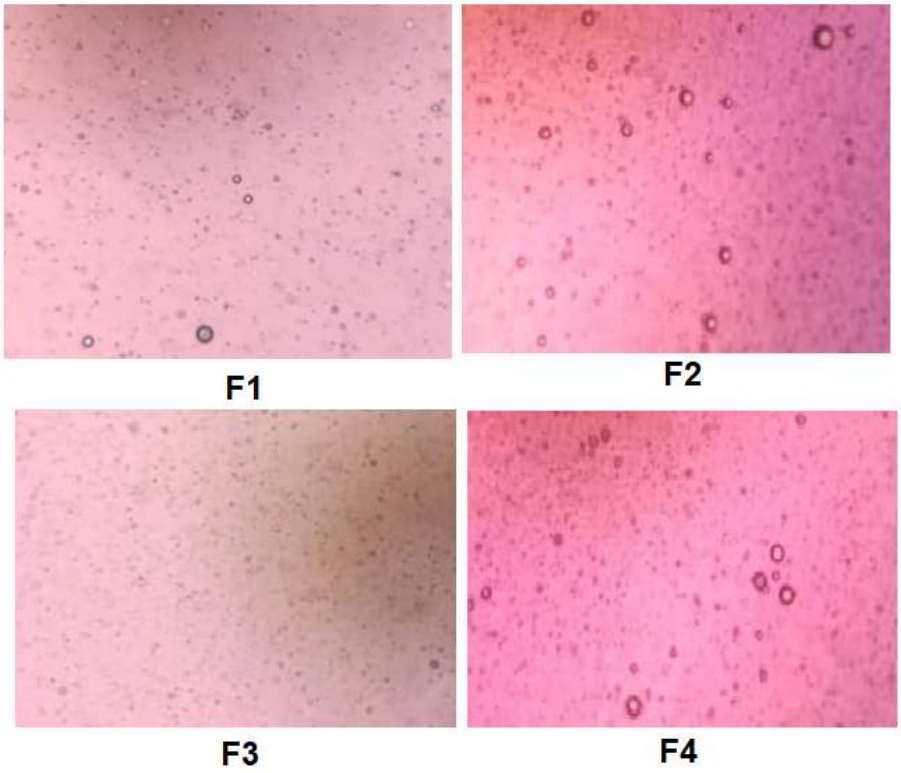

Figure 8: Microphotographs of niosomal gel formulation F1-F4.

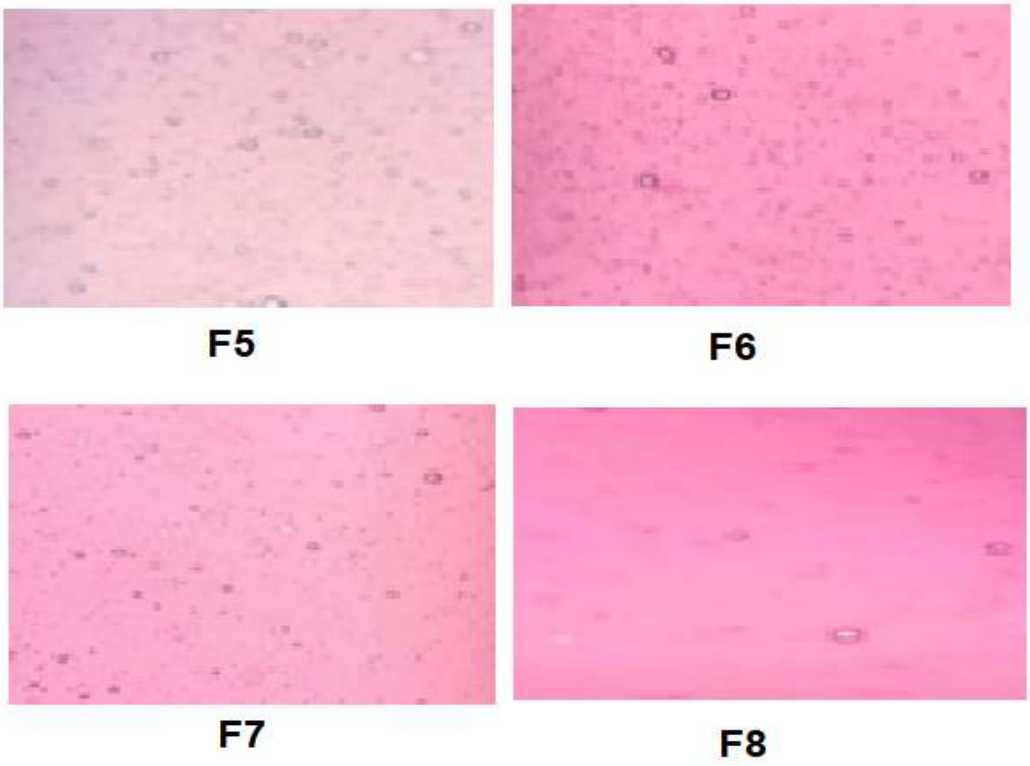

Figure9: Microphotographs of niosomal gel formulation F5-F8.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 1098-1115 www.ijprajournal.com ISSN: 2456-4494

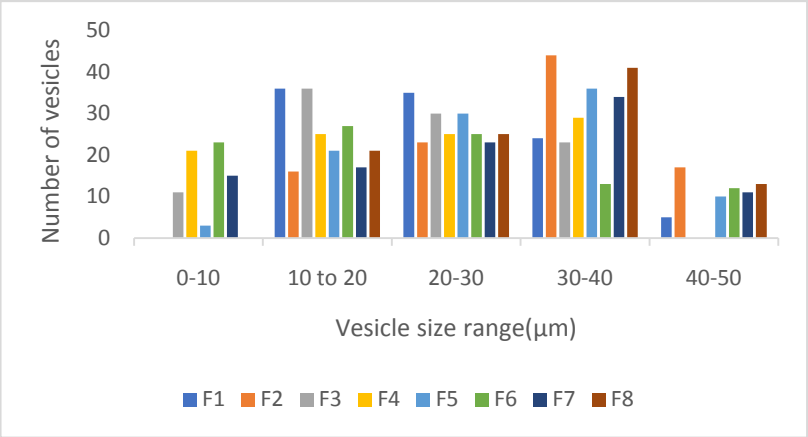

Figure .10: vesicle size distribution of niosomal gel formulation F1-F8.

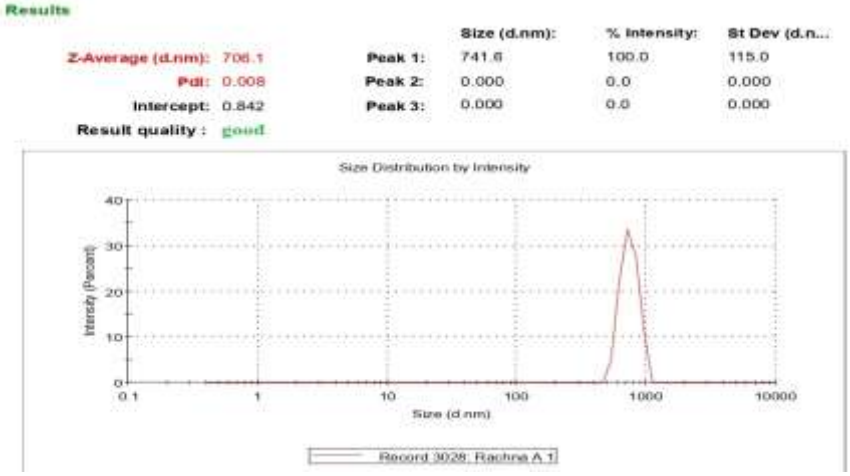

Figure 11: Particle size of niosomal formulation F2.

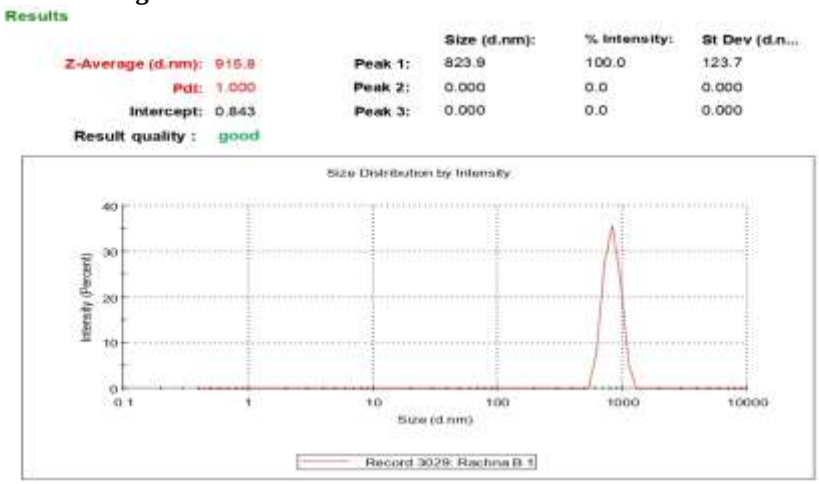

Figure 12: Particle size of niosomal formulation F7.

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Volume 7, Issue 5 Sep-Oct 2022, pp: 1098-1115 www.ijprajournal.com ISSN: 2456-4494

Table 3: Zeta potential and particle size analysis.

Formulation code	Zeta potential(mv)	Particle size(nm)
F2	-31.3	706.1
F7	-50.9	915.8

IN – VITRO DRUG RELEASE:

Table 4: Drug diffusion study of niosomal formulation F1-F8.

TIME	F1	F2	F3	F4	F5	F6	F7	F8
0	0	0	0	0	0	0	0	0
1	12.451	6.451	9.677	6.451	5.903	6.451	9.677	3.225
2	28.903	19.354	22.58	12.903	19.354	16.129	29.032	9.677
4	39.258	32.258	38.709	29.032	31.129	28.258	38.709	29.032
6	59.032	41.935	58.064	38.709	49.096	41.612	51.612	41.612
8	68.258	54.838	70.032	54.516	64.967	57.741	74.193	53.87
10	85.419	66.774	82.935	63.87	72.87	67.419	86.774	66.129
12	94.483	72.258	90.387	80.645	84.838	72.096	89.354	73.322

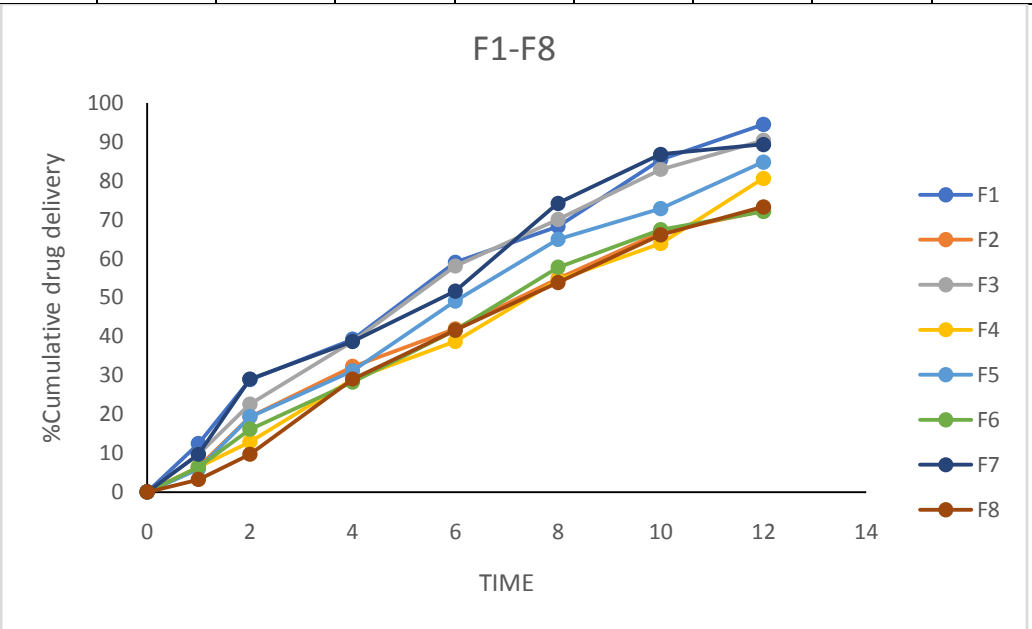

Figure 13: In–vitro diffusion profile of F1-F8.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 1098-1115 www.ijprajournal.com ISSN: 2456-4494

Table 5: Results of release kinetics.

Formulation code	% CDR	Zero order	First order	Higuchi plot	peppas plot	
					r^2	'n'
F1	94.483	0.9804	0.9321	0.9673	0.727	1.286
F2	72.258	0.9829	0.9924	0.9596	0.8147	1.326
F3	90.387	0.9807	0.9471	0.9629	0.7812	1.348
F4	80.645	0.9972	0.9885	0.9234	0.8567	1.369
F5	84.838	0.9886	0.9885	0.9449	0.9746	1.027
F6	72.096	0.9856	0.9885	0.9451	0.8376	1.348
F7	89.354	0.9688	0.9885	0.9547	0.7579	1.327
F8	73.322	0.9889	0.9885	0.9281	0.9264	1.521

EvaluationofFluconazole niosomal in-situ gel

The optimized niosomes F7was formulated into niosomal in-situ gel by the use ofCarbopol 934 gels containing 3% w/w gel.

Table6:Spreadability,Viscosity,pHand %drugcontentofniosomal in-situ gel

Formulationcode	Spreadability	Viscosity	pH	%Drugcontent
F7-G1	2.3 ± 0.03	759±3.051	6.0 ± 0.23	93.68 ± 0.16

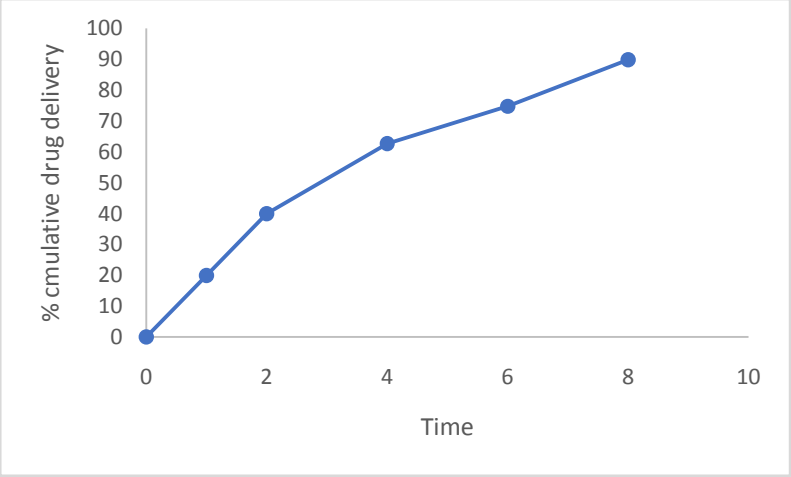

Figure 14: %Cumulative drug deliveryofF7-G1.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 1098-1115 www.ijprajournal.com ISSN: 2456-4494

Table7:Release kinetics profile of F7-G1

Formulationcode	Zeroorder	Firstorder	Higuchi	Peppas	n'value
LM3-G1	0.9481	0.9936	0.9832	0.605	1.464

Biocompatibility test: - Hen's Egg Test Chorioallantoic Membrane (HET-CAM)test

Figure 15 : Controlled before application **Figure 16 : Controlled after application**

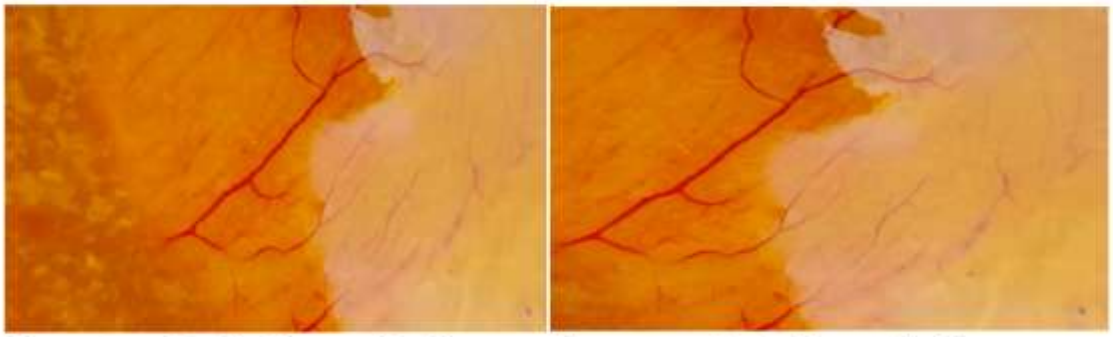

Figure 17: Test before application **Figure 18: Test after application**

Table :8Scoring forirritationtestingwiththe HET-CAMtest method

Effect	Time(minutes)		
	0.5	**2.0**	**5.0**
Hyperaemia	0.0	0.0	0.0
Haemorrhage	0.0	0.0	0.0
Coagulation	0.0	0.0	0.0

In-Vitro Antifungal Activity

In-vitro antifungal effect to evaluate the efficacy of optimized formulation and drug, marketed product against antifungal evaluation was carried out using fungal strain Candida albicans. The antifungal activity by well-diffusion method was performed atconcentration of drug 5µg/ml, and 5µg/ml gels, otoflu (marketed gel) as standard placed in welland measured zone of inhibition. Candida albicans was used as standard for fungi. The results are shown in Table 9 and Figure 19.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 1098-1115 www.ijprajournal.com ISSN: 2456-4494

Table 9: Well Diffusion assay against C. albicansusing given Samples

Sample Name	(ZOI in cm)
Gel	3.0 cm
Fluconazole (Drug)	2.6 cm
Market product (Otoflu)	1.4 cm

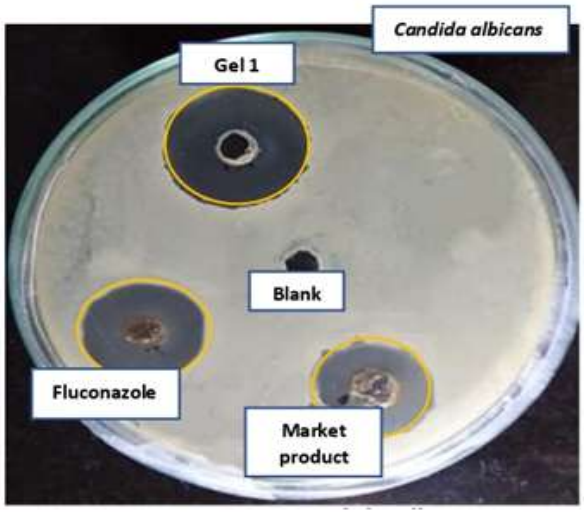

Figure 19: The Antifungal activity against C. albicans

Stability Studies

Table 10: comparison of % drug content of niosomal in-situ gel formulation (GF7) after 3 months of storage at 5°C±3°C, 30°C±2°C/65%RH

Temperature in °C	Drug content (%)
5°±3°C	93.14
30°±2°C/65%RH	91.46

International Journal of Pharmaceutical Research and Applications
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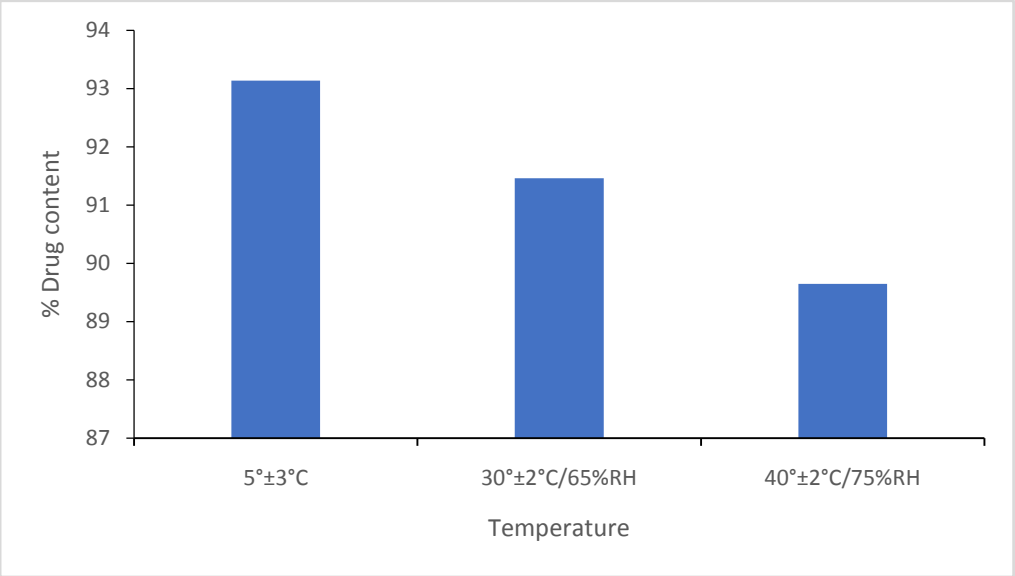

Figure 20: comparison of % drug content of niosomal in-situ gel at 5°C±3°C, 30°C±2°C/65%RH and 40°C±2°C/75%RH.

Table 11: In-vitro release study of a GF7 niosomal in-situ gel after 3 months storage at at 5°C±3°C, 30°C±2°C/65%RH

Time(hrs)	% Cumulative drug release	
	5°±3°C	30°±2°C/65%RH
0	0	0
1	16.88	15.47
2	25.62	22.36
4	42.14	40.35
6	78.48	77.13
8	89.01	88.94

Effect of parameters on % Cumulative drug release(%CDR):

Table 5.12: ANOVA of Effect of parameter on response of % Cumulative drug release(%CDR):

Anova	Factor	df	SS	MS	F	Effect	Contrast	p
Source	Cholesterol	1	461.2	461.2	14.81616	-15.19	-60.74	0.16
	span 40	1	12.6	12.6	0.404812	2.51	10.04	0.64
	Cholesterol X span 40	1	10.6	10.6	0.339908	2.3	9.20	0.66
	span 80	1	41.2	41.2	1.324395	-4.54	-18.16	0.46
	Cholesterol X span 80	1	1.3	1.3	0.041123	0.8	3.20	0.87
	span 40 X span 80	1	0.3	0.3	0.00856	0.365	1.46	0.94
	Cholesterol X span 40 X span 80	1	31.1	31.1	1	-3.945	-15.78	0.50

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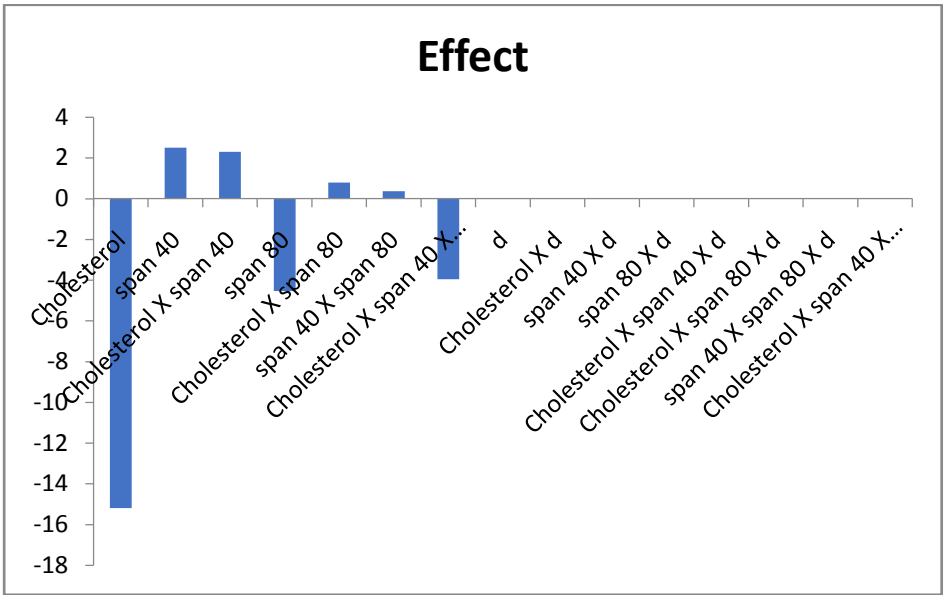

Figure 22: Effect of Parameter on response of % Cumulative drug release

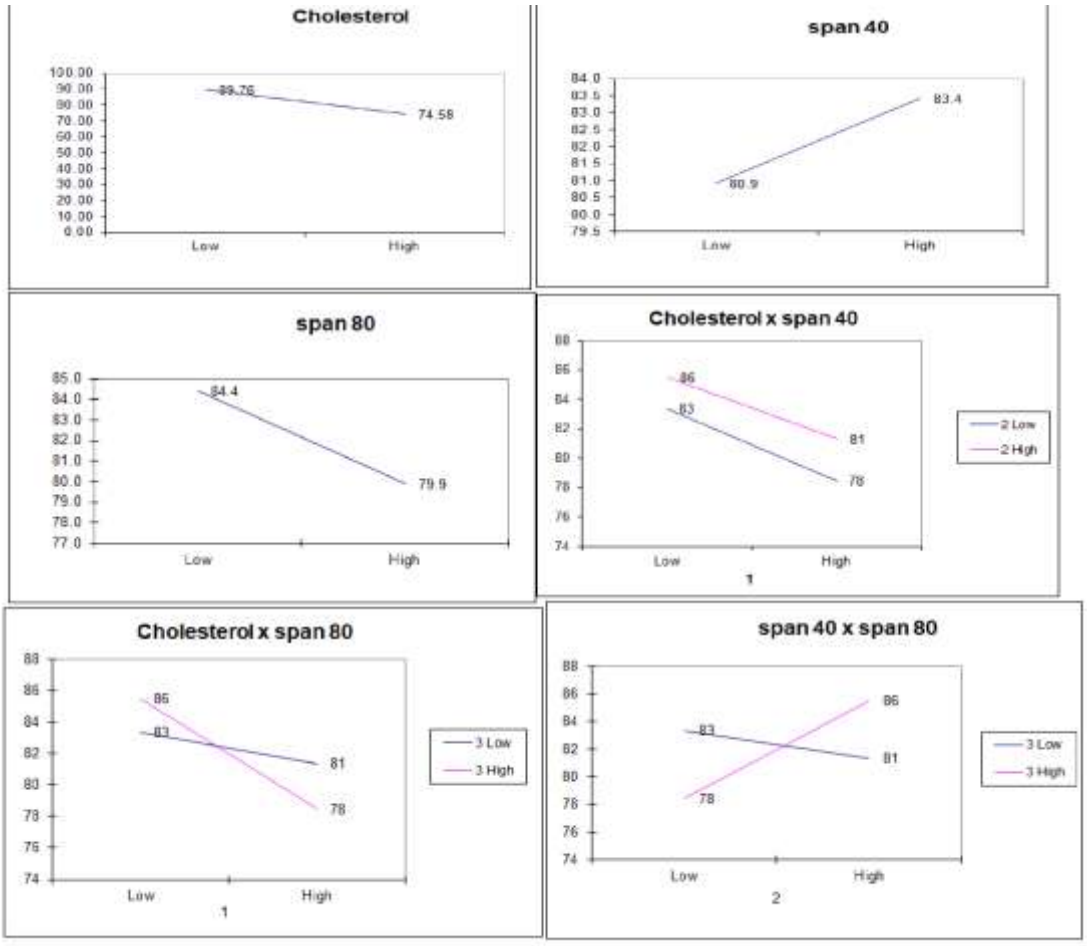

Figure 5.23: Interaction effect of parameter on response of cumulative drug delivery

International Journal of Pharmaceutical Research and Applications
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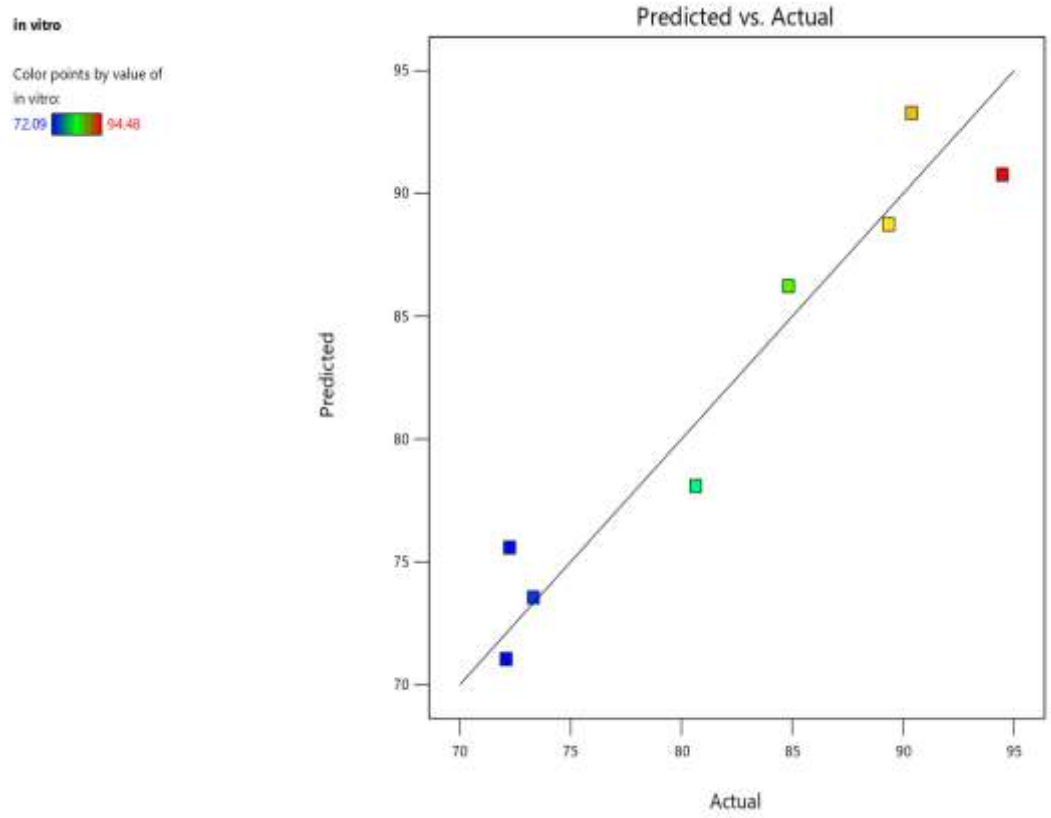

Figure 24: Predicted v/s actual relation of % cumulative drug delivery

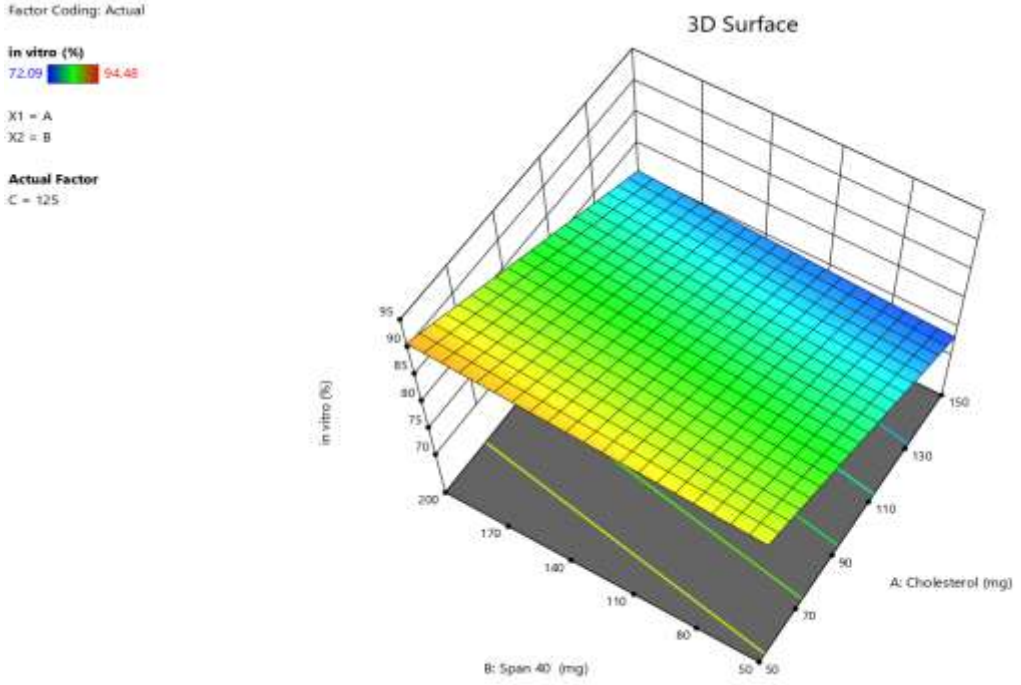

Figure25: 3D Surface response plot for the response % cumulative drug delivery

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IV. DISCUSSION:

The purity of the drug was determined by Differential scanning calorimetry (DSC) we determined melting point of pure drug was found to be 154.36^0 C as shown in Figure 4, observed that the value within the standard Indian pharmacopoeia (IP) limits confirming the purity of the drug. The ʎ max of Fluconazole in phosphate buffer pH:7.4 was found to be 260nm and UV spectrum was shown in Figure 1 and 2. Standard curve of Fluconazole obeys the Beer's law in concentration range 0-10µg/ml (Figure 3) in phosphate buffer pH 7.4 with regression of coefficient of r^2= 1 and slope of 0.003. The calibration spectra of different concentration was shown inFigure3.

Drug excipient compatibility studies were carried out using FT-IR. The characteristic peak obtained of pure drug (Fluconazole), and their mixture (drug, cholesterol, span40, soya lecithin) and (drug, cholesterol, span 80, soya lecithin) was shown in Figure 5 and 6,7. The characteristic peak of pure drug also found in physical mixture indicating there was no significant interaction between the drug and excipients. Different formulations of Fluconazole niosomes were prepared by thin films hydration method using surfactants (span-40, span80), cholesterol, methanol and chloroform. Span 40 were used as surfactants which is used to entrap wide range of drugs in niosomes. Span 80 were used to blend alcohol with gasoline. The cholesterol improves the stability of bilayer membrane of vesicles and methanol and chloroform was used as nasal penetration enhancer and for providing softness to the vesicles. The particle size analysis also done by using Malvern particle size analyser for the optimized formulation of F2 and F7 The average particle size was found to be F2 -706.1nm, F7-915.8nm. The data was shown in Figure 11 to 12. The percentage entrapment efficiency of Fluconazole in different niosomal formulations were shown in and table3. Highest Entrapment efficiency was observed in F7 with 93.22%. The high drug entrapment may be observed due to increase in the surfactant ratio. Drug content for all formulation was shown in Table3. Zeta potential of optimized formulation F2 and F7 formulations were measured by zeta analyzer and found that -31.3, -50.9 for the respective formulation, which are shown in Table 4.

In vitro release study of Fluconazole from various niosomal formulations was conducted for 12 hrs by using dialysis membrane. Cumulative % drug release was plotted against time (t). The % drug release from F1-F8 was observed as follows F1-94.48%, F2-72.25%, F3-90.38%, F4-80.64%, F5-84.83%, F6-72.09%, F7-89.35%, F8-73.22%. The increase in surfactant (span 40, span80) ratio from F1 to F8 causes decrease in the drug release, the release was more controlled by increasing the surfactant ratio. All the formulation released the drug in a controlled manner. The in vitro release data were shown in Figure 13. In vitro release profiles of all the formulation were fitted to various kinetic model and from the results Table 5. and release profile represented table , and it was found that all the formulation follows first order. The 'n' values for all the formulation were found to be more than 0.5. This indicates that the release approximates non-fickian diffusion mechanism.

The formulation F7 showed maximum amount of %drug content, %drug entrapment efficiency, and drug will release in controlled manner for prolonged time. and hence F7 niosomal formulation were selected for as optimized and further used for ocular niosomal in-situ gel. The viscosity of in-situ gels of various formulations was determined and formulation GF7 showed 759±3.051 cps. The result of in vitro release of Fluconazole from the in-situ gel formulation is given in Figure 21. However, the results clearly showed that the in-situ gels have ability to retain the drug for prolonged perisods. The % CDR of niosomal in-situ gel formulation GF7 was found to be 89.92% and which follows first order. The 'n' values for all the formulation were found to be more than 0.5. this indicates that the release approximates non-fickian diffusion mechanism.Hen s Egg. Test Chorioallantoic Membrane (HET-CAM) Test Figure 15 to 16 illustrates the observations given by the HET-CAM test in the case of application of the controlled, test formulation. When applied to the CAM to the controlled substance over a period of 5 minutes, the blood vessels in the CAM go initially from the time zero Figure 15 to the appearance shown in Figure 16 after 5 minutes there was no sign of lysis, haemorrhage and coagulation was observed. Then applied to the CAM to the test substance over a period of 5 minutes, the blood vessels in the CAM go initially from the time zero Figure 17 to the appearance shown in Figure 18 safter 5 minutes there is no irritant effect on the embryo and there was no sign of lysis, haemorrhage and coagulation was observed and score shown in Table 8.

Antifungalactivitybythewell-diffusionmethodwasperformedatdifferentconcentrations of 5µg/ml drug, 5µg/ml in-situ gel. 5 µg/ml of Otoflu (marketed gel) was used as a standard. (Table 9). The in-vitroantifungal effect of

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Volume 7, Issue 5 Sep-Oct 2022, pp: 1098-1115 www.ijprajournal.com ISSN: 2456-4494

optimized niosomal in-situ gel formulations, and the drug has done against using fungal strain Candida albicans. The zone ofinhibitionwasmeasuredintermsofcentimeters.Itwa sobservedthatthezone of inhibition increased in a dose-dependent manner in the tested concentration range.The results of the well-diffusion method showed that the in-situ gel, marketed product and drug shows antifungal effect against Candida albicans individually and when the drugand in-situ gel arecombinedintheformof niosomes showedgoodantifungaleffectas shown in Figure 19. The zone of inhibitions was measure 3.0 cm for 5µg/ml of in-situ gel and 2.6 cm for 5µg/ml of drug, 1.4 cm for 5µg/ml of marketed product the zone of inhibition was observed These niosomal in-situ gel formulationshave more effective in inhibiting the growth of Candida albicans fungal strain used inthe study. Optimized niosomal in-situ gel GF7 have a better antifungal effect than marketed product this proved that thesynergisticeffectcouldbeachievedby niosomal in-situ gel and Fluconazole drug.

Stability studies the accelerated stability study for optimized niosomal in-situ gel formulation F7 was performed for 3 months according to ICH guidelines. Drug content, pH: % CDR were fixed as physical parameters for stability studies of selected formulation F7 shows that negligible changes in drug content, pH: % This revealed that the formulation stable on storage at and 30°C±2°C the results were given in the table 10 and figure 20.

2x3 factorial designs were applied by using QI MACROS 2022 and for the optimization process were performed by using DESIGN EXPERT-13 software, and the results are obtained as follows.In- vitro diffusion studies Effect of cholesterol on %CDR: Increasing the concentration of cholesterol from low to high level, the %CDR have decreased for the formulations 89.76-74.58 with the difference of 15.18%. The results revealed that %EE have been decreased when we change the cholesterol concentration from low to high level.Effect of span 40 on entrapment efficiency: Increasing the concentration of span 40 from low to high level, the %CDR have increased for the formulations 80.9-83.4 with the difference of 2.5%. The results revealed that %CDR have been increased when we change the span 40 concentration from low to high level.Effect of span 80 on entrapment efficiency: Increasing the concentration of span 80 from low to high level, the %CDR have increased for the formulations 84.4-79.9 with the difference of 4.5%. The results revealed that %CDR have been decreased when we change the span 80 concentration from low to high level.Interaction effect: We observed interaction on %CDR the cholesterol combined with the span 40, it doesn't show any interaction effect, but interaction occurs between cholesterol and span 80 and span 40 and span 80.

V. CONCLUSION:

A successful attempt was made to develop niosomal in-situ gel for ocular drug delivery of Fluconazole by utilizing 2 X 3 factorial design by (Span 40 and span 80): cholesterol, soya-lecithin using thin film- hydration technique. Fluconazole was successfully entrapped within the non-ionic surfactant vesicles. The in-vitro permeation studies suggest that niosomal in-situ gel formulations enhance the rate of permeation of the drug across eye. This penetration enhancement effect may be attributed to both the presence of non-ionic surfactants and cholesterol. Niosomal in-situ gel system prepared with Span 40 and Span 80 exhibited optimum entrapment efficiency and has shown potential for delivery Fluconazole as antifungal drug.

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