Original Research Article

Formulation and Evaluation of Topical Gel Loaded with Fluconazole Niosomes.

.

ABSTRACT

Aim: The study aims to formulate and evaluate topical gel-loaded fluconazole niosomes. Fluconazole is a macrolide antibacterial used against various susceptible bacteria. Niosomes have a substantial role in the delivery of drugs as they can reduce toxicity and modify pharmacokinetics and bioavailability. Niosomes which are applied topically improve the deposition of drugs within stratum corneum and epidermis at the same time reducing systemic availability.
Methodology: In current investigation, fluconazole was entrapped into niosomes by thin-film hydration technique with the optimization of various process parameters like entrapment efficiency, vesicle size, shape and *in-vitro* drug release studies.
Results: Optimized formulations FNS5 and FNT4 prepared with Span-60 and Tween-60 exhibited vesicle sizes of 845.6 nm and 164.2 nm, zeta potential -10.2 mV and -46.4 mV indicates the formulation has good stability. The optimized niosomes were integrated into carbopol 934 and guar gum gels and then extensively characterized for zeta potential and vesicle size.
Conclusion: The study demonstrated that entrapment of drugs into niosomes led to prolonged drug release time, enhanced permeation and drug retention.

Keywords: Niosomes, Fluconazole, Carbopol 934, Guar gum.

1. INTRODUCTION

The pursuit of novel drug delivery system is to attain a steady-state concentration in blood or tissue that is the region of therapeutical effectiveness for extended span of time[1]. The method by which a drug is delivered influences the efficacy of the drug. Some drugs have an optimum concentration range that gives the maximum benefit and concentrations above or below this range can be toxic or produce no therapeutic benefit. Deliberate progression in efficacy of treatment is another strategy for severe diseases, involving multidisciplinary approach for delivering therapeutics to the target site[2].

To minimize loss/degradation of drugs, to avert harmful side-effects, to increase drug bioavailability and drug accumulation in the required zone, various drug delivery and drug targeting systems are currently under development. To acquire required drug delivery, carriers must hold the drug. Carriers are microparticles constituting either insoluble, soluble or biodegradable polymers, microcapsules, cell ghosts, liposomes, lipoproteins, micelles, etc. These carriers may be slow degrading, reactive to a stimulus (temperature, pH) or targeted (like conjugating with specific Ab)[2].

Targeting is controlling the movement of a drug-loaded system to the required site. Major mechanisms for managing release of drug to the required site are-

(i) Passive targeting: It implicates on comparison to healthy tissue, enhanced vascular permeability of tumour tissue causes antineoplastic agents' accumulation in solid tumours.

(ii) Active targeting: It implicates surface modulation of drug carriers with a ligand that is recognized by receptors of the inquisitive cells. Thus, precise targeting can be achieved by ligand-receptor interactions which are highly selective[2].

For the past 20 years, investigators have acknowledged the prospective aid of nanotechnology for yielding vast refinement in the delivery and targeting of drug. Enhancing delivery techniques that can decrease toxicity and show enhanced efficacy offers potential benefits to patients and offers new ventures concerning pharmaceutical and drug delivery companies. Another strategy for delivering drugs encompasses transversion through physical barriers like BBB, to target and enhance effectiveness or work on alternative and admissible routes for protein drug delivering, other than through the GIT where it gets degraded[2].

Colloidal systems for drug delivery like liposomes and niosomes have distinctive benefits over conventional dosage forms. These systems can serve as drug reservoirs and provide active substance release in control manner. In addition, modification of their composition/ surface can permit targeting.

Niosomes, are vesicles based on non-ionic surfactants designed as alternative controlled drug delivery systems to liposomes for overcoming difficulties associated with sterilization, large-scale production and stability. They are liposome-like vesicles built from hydrated mixtures comprising charge-inducing substances, non-ionic surfactants (like monoalkyl or dialkyl polyoxyethylene ether) and cholesterol. These vesicles do not form spontaneously. In the existence of an apt mixture of charge inducing agents and surfactants, thermodynamically stable vesicles are established.

Vesicle formation mechanism relying on the usage of non-ionic surfactant is not wholly apparent. The most familiar theory is closed bilayer formation in aqueous media by non-ionic surfactants based on its amphiphilic nature. Formation of this structure involves some energy input, for instance employing physical agitation (e.g., using the hand-shaking method) or heat (e.g., using the heating method)[3]. The hydrophobic parts of this closed bilayer orient in opposite direction to the aqueous solvent while the hydrophilic head faces the aqueous solvent as shown in **Fig.1**. It resembles phospholipid vesicles in liposomes and thus facilitates hydrophilic drug entrapment. Stability, low cost and consequent easier storing facility for non-ionic surfactants led to its exploitation as substitute to phospholipids.

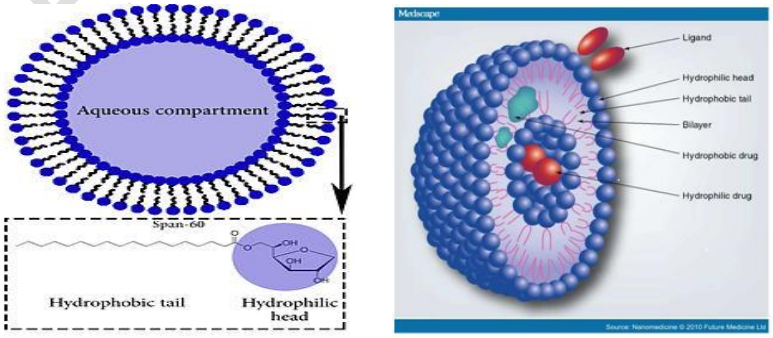


Fig. 1. Structure of Niosome.

2. MATERIALS

Fluconazole, Cholesterol, Chloroform, Methanol, Span, Tween, Sodium chloride, Disodium hydrogen orthophosphate, Potassium dihydrogen orthophosphate purchased from S.D Fine Chemicals Ltd.

3. METHODOLOGY

Study was divided into four sections:

A. Drug excipient compatability studies.
B. Preparation, evaluation followed by optimization of niosome with different combinations of non-ionic surfactants (Spans and Tweens).
C. Preparation, evaluation, optimization of gel.
D. Preparation, evaluation followed by optimization of niosomal gel prepared.

3.1 Preformulation Studies

3.1.1 Fourier Transform Infrared Spectroscopy Analysis of Fluconazole (pure drug)

The investigation was conducted to confirm that the sample is a pure drug. Fourier transform infrared (FTIR 8400s, Shimadzu Japan) spectra obtained for Fluconazole (pure drug) was checked. Potassium bromide disc method was used. The pellet was prepared with the dry sample by applying 10tons/inch^2 pressure for 10 min.[4,5].

3.2 Preparation, Evaluation and Optimization of Niosome with Various Non-Ionic Surfactants (Tween 60 & Span 60) Preliminary Trials

Preliminary trials were conducted with distinct non-ionic surfactants like span-60 and tween-60 with thin-film hydration technique by utilising cholesterol(CHO): surfactant(SUF): drug in ratios of 0.25:1:0.5, 0.5:1:0.5 and 1:1:0.5. Accurately weighed quantities of surfactant (span 60 and tween 60) and CHO were dissolved in a 100 ml round bottom flask(RBF) containing 10ml of chloroform and methanol. Weighed quantity of drug was added to solvent mixture. Then solvent mixture from the liquid phase was removed by flash evaporation at 60°C to obtain a thin film on the walls of the flask at 150 rpm. The vacuum was applied remove residual solvent completely. Hydration of dry lipid film was done with 10 ml PBS of pH 7.4 at room temperature.

The non-ionic surfactants chosen for the study was span 60 and tween 60 based on percent entrapment efficiency **(See Table 1)**. So, the preliminary work involved selecting suitable non-ionic surfactants in the optimized concentration. *In-vitro* dissolution profile of the formulations was evaluated. Non-ionic surfactant that showed better *in-vitro* dissolution profile with specifications was chosen for further studies[6–9].

Table 1. Preliminary trials of Fluconazole loaded Niosomes, cholesterol and non-ionic surfactant.

Tween 60			
Cholesterol (mg)	**Surfactant (mg)**	**Drug (mg)**	**CHO:SUF:DRUG ratio**
25	100	50	0.25:1:0.5
50	100	50	0.5:1:0.5

100	100	50	1:1:0.5
Span 60			
25	100	50	0.25:1:0.5
50	100	50	0.5:1:0.5
100	100	50	1:1:0.5

3.3 Characterization of the Prepared Niosomes

The niosomes prepared were assessed for their morphological characterization (size, shape), percent entrapment efficiency and *in-vitro* release.

3.3.1 Entrapment Efficiency Study

Niosomal dispersion within the Ephendroff tubes was taken and was centrifuged at 7300 rpm at 4ºC for 20-30 min in two cycles to isolate the drug-containing niosomes for unentrapped drug. Free drug determination was done using a clear fraction at 260nm spectrophotometrically. The vesicles pellet available in the precipitate was washed three times with saline phosphate buffer pH 7.4. After washing, to break the vesicles 5ml 0.9 % saline, 5ml of absolute alcohol: propylene glycol (1:1) were added. This was analysed spectrophotometrically at 260 nm [4,8,10,11].

Determination of the percent of drug entrapped was done by utilizing the formula:

$$\text{Entrapment efficiency (\%)} = \frac{(\text{The total amount of drug - amount of free drug}) \times 100}{\text{The total amount of drug}}$$

3.3.2 Size, Shape and Morphological Characterization

Optical microscopy at 45x resolution was utilised to confirm vesicle formation. The fixing of niosomal suspension over a glass slide was achieved by drying at ambient temperature, the dry thin film of niosomal suspension was observed for the establishment of vesicles. The microphotography of the niosomes was also acquired from microscope using a digital camera. The detailed surface characteristics of selected fluconazole niosomes formulation were observed using an electron microscope [5,9,12].

3.3.3 *In-vitro* Drug Release Study

Using membrane diffusion technique fluconazole release from niosomal formulations was specified. After removing the un-entrapped drug, a beaker containing 100 ml of pH 7.4 PBS comprising 10% v/v methanol (for maintaining sink condition) was utilized for dialysis of niosomes left. Magnetic stirrer was utilised for agitation and temperature of receptor medium was maintained at 37 ± 0.5°C. 5ml of aliquots were drawn periodically and then replaced with the same volume of medium. UV spectrophotometer was utilized for analysis of sample collected at 260nm. The tests were executed in triplicate [11,13].

3.4 Formulation of Gel

Different polymers in different concentrations were utilized to formulate gel, and carbopol 2% and guar gum 2% was established to be optimized **(See Table 2).**

Table 2. Formulation of gel with different polymers.

S.No.	Ingredients	1%	2%	3%	4%
1.	Carbopal 934	+	++	+++	-
2.	HPMC K15M	+	+	+	++
3.	Guar gum	+	++	+++	-
4.	Xanthan gum	+	++	+++	-
5.	HPMC K100M	++	-	-	-

***Note:** + Gel not formed, ++ Gel formed, +++ Hard gel.*

3.5 Niosomal Gel Formulation

By integrating optimized formulation into a suitable gel base appropriate gel was formulated. Gel base selected for incorporating niosomes was carbopol 2% w/w and guar gum 2% w/w in mixture of water and glycerol (7:3) the dispersion obtained was neutralized[10,14] and made viscous by addition of a sufficient amount of triethanolamine by the cold mechanical method **(See Table 3).**

Table 3. Formulation design for Niosomal gel.

S.No	Ingredients	Percentage
1.	Niosome formulation	10 %
2.	Carbopol	2 %
3.	Triethanolamine	QS
4.	Water	QS to 100 %

3.6 Niosomal Gel Evaluation

3.6.1 Physical appearance

Niosomal gel was inspected for colour, clarity, homogeneity and presence of foreign particles[15,16].

3.6.2 pH

25ml distilled water dispersed with 2.5g of gel was taken and then utilizing digital pH meter the pH was measured[10,16,17].

3.6.3 Rheological study

Brookfield programmable DV III ultra-viscometer was employed to determine viscosity. Spindle no. CP 52 at the optimum speed of 0.01 rpm was operated to measure viscosity[18–20].

3.6.4 Zeta potential, particle size and size distribution

Zeta sizer (HORIBA-SZ-100Z) was employed to ascertain particle size based on dynamic light scattering. The dilute suspension of nanoparticles was prepared in double-distilled water and sonicated for 30 seconds on an ice bath. Examination of sample was executed at a scattering angle of 1730, at 25^{o}C. Zeta potential was measured using a zeta sizer based on electrophoretic mobility at 25^{o}C. The Polydispersity index (PI) was also estimated to determine particle size distribution [10,20,21].

3.6.5 *In vitro* drug diffusion study

Here, study equipment comprises glass cylinder that is open at either end. Dialysis membrane (that was soaked in distilled water 24hrs before use) was affixed to one side of the cylinder with assistance of an adherent substance. Gel coequal to 10mg fluconazole is placed in cell (i.e., the donor compartment), further submerged in beaker constituting 100ml of pH 7.4 PBS comprising 10% v/v methanol, which serve as receptor compartment. The entire assembly was arranged such that lower end of the cell holding gel is just past the surface of diffusion medium (1-2mm deep) agitation of medium was achieved by employing magnetic stirrer at 37 ± 0.5°C. 5ml aliquots were drawn from the receptor compartment and replaced with similar volume of fresh buffer. Utilizing UV-visible spectrophotometer at 260 nm collected sample was analyzed [7,22,23].

3.7 Preparation and Comparison Study of Optimized Formulation with Niosomal Gel and Pure Drug.

The thin-film hydration technique was employed for preparing niosomes of fluconazole. Accurately weighed non-ionic surfactant and chloroform were taken in a clean RBF, and to this solution of methanol and chloroform (1:1) was added. The RBF was affixed to rotary evaporator at 60ºC, for 30 mins under vacuum at 150 rpm. Hydration of formed thin film was done with pH 7.4 phosphate buffer saline comprising drug at room temperature for 20 mins which resulted in milky white suspension [10,19,20]. (**See Table 4)**

Table 4. Formulation of Fluconazole loaded Niosomes.

S. No	Span/Tween (mg)	Cholesterol (mg)	Drug (mg)	Chloroform: Methanol (1:1)	PBS pH 7.4
1.	100	100	50	5 ml: 5 ml	5 ml
2.	150	100	50	5 ml: 5 ml	5 ml
3.	200	100	50	5 ml: 5 ml	5 ml

3.8 Calculation of Release Kinetics for FNG

Mathematical equations for calculation of release kinetics and interpretation of diffusion mechanisms are specified in **Table 5** and **6.**

Table 5. Applied mathematical models to the diffusion data of Fluconazole gel.

Model	Equation	Plot a graph	Parameters
Zero order	$Q_t = Q_o + K_o t$	% drug release versus time	K_o – release rate constant
First order	$\ln Q_t = \ln Q_o + K_1 t$	Log % drug release versus time	K_1- release rate constant
Higuchi release	$Q_t = K_H t^{1/2}$	% drug release versus time square root of time	K_H – Higuchi constant
Korsmeyer-peppas	$Q_t/Q_8 = K_k t_n$	Log % drug release versus time log time	n- release exponent

Release coefficient (r^2) was calculated for all formulations. Releases component “n” was calculated from Korsemeyer Peppas equation[10,17].

Table 6. Interpretation of diffusion release mechanisms from “n” value.

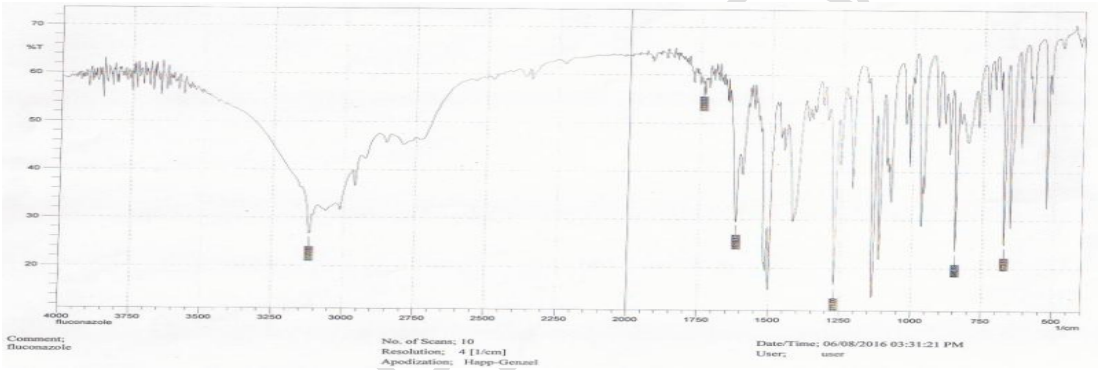
Release Exponent (n)	Drug transport mechanism	Rate as a function of time
< 0.5	Fickian diffusion	$t^{0.5}$
0.5 < n < 1.0	Anomalous transport	t^{n-1}
1.0	Case-II transport	Zero order release
> 1.0	Super case-II transport	t^{n-1}

4. RESULTS AND DISCUSSION

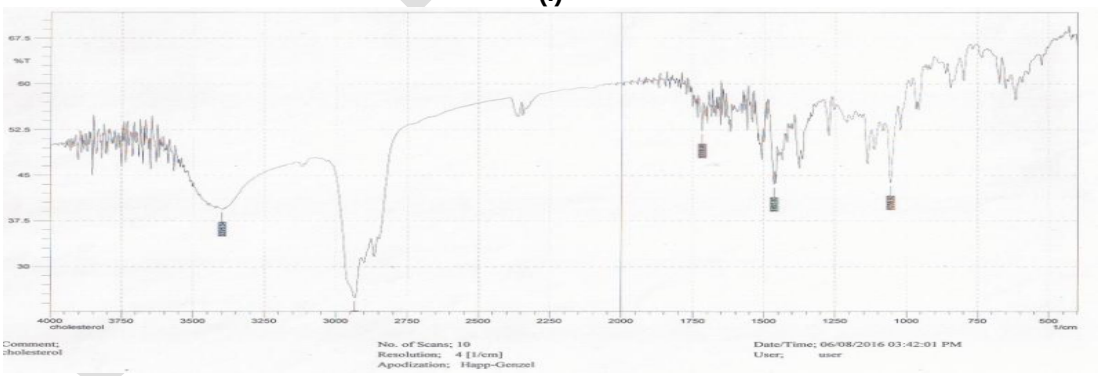
4.1 Drug- Excipient Compatibility Studies

4.1.1 Fourier Transforms Infrared Spectroscopy Analysis

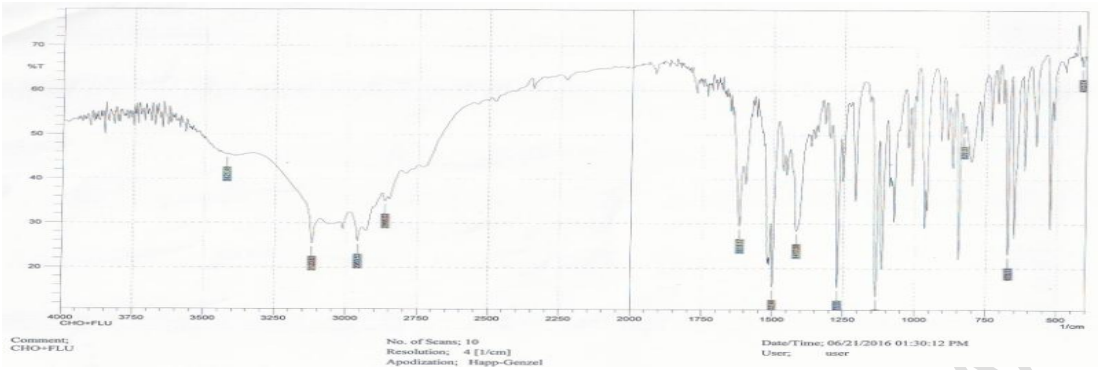
FTIR study was conducted to ascertain if any interactions exist between pure drug (Fluconazole) and the excipient employed. It was executed by the KBr pellet method and scanned[9,12]. The FTIR spectra of the pure drug and the polymer-physical mixture blends are displayed in **Fig.2** and **Fig.3.**



(i)

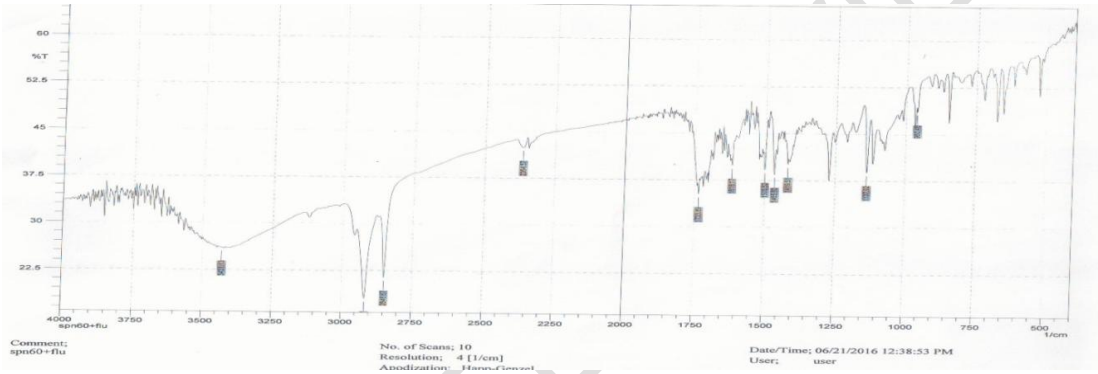


(ii)

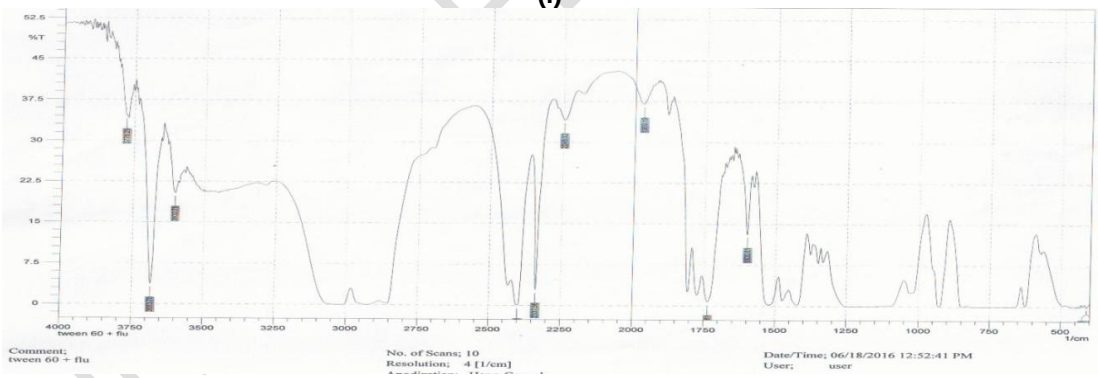


(iii)

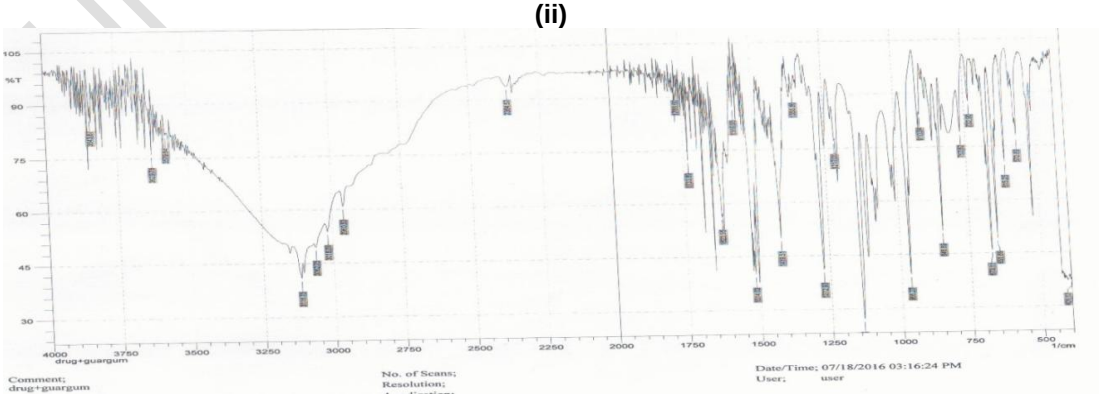
Fig. 2. FTIR spectra of (i) Fluconazole (ii) Cholesterol (iii)Fluconazole + Cholesterol.



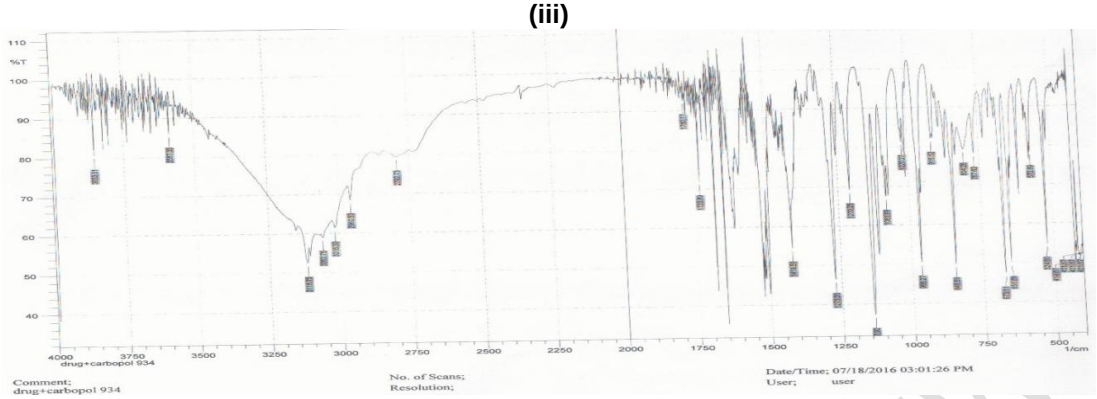
(i)



(ii)



(iii)



(iv)

Fig. 3. FTIR spectra of (i) Fluconazole + Span 60 (ii) Fluconazole + Tween 60 (iii) Fluconazole + Guar gum and (iv) Fluconazole+ Carbopal 934.

Table 7. Interpretation of fluconazole IR spectra

Region in cm^{-1}	Types of vibrations	Functional group
3120.61	O-H stretching	Alcohols group
2960.53	C-H stretching	Alkane group
1618.17	C=C	Alkynylyne group
1271.0	C-H stretching	Alkane group
1413.72	C-H bending	Alkane group
1519.80	C=N stretching	Alkane group
1116.71	C-O stretching	Acid group

From the above IR spectra **Fig.2(i)** and **Table 7,** the peaks illustrating fluconazole were similar in pure drug and the blend of fluconazole with other excipients used, it indicates that there exist no interactions; it was noted that the functional peaks retained after addition of excipients to the drug. Hence, it denotes that drug was stable and compatible throughout the process with excipients used.

4.2 Preparation and Optimization of Niosomes by Thin-Film Hydration Method

Vesicles were initially prepared with varying concentrations of cholesterol[6–8,11]. The optimized formulations were picked for further studies depending on vesicle formation with excellent clarity by photomicrographical examination as enlisted in **Table 8.**

Table 8. Preliminary trials of Fluconazole loaded Niosomes, cholesterol and non-ionic surfactant.

Tween 60				
Cholesterol (mg)	**Surfactant (mg)**	**Drug (mg)**	**CHO:SUF:DRUG ratio**	**Observation (Vesicles formed)**
25	100	50	0.25:1:0.5	+
50	100	50	0.5:1:0.5	++
100	100	50	1:1:0.5	+++

Span 60				
25	100	50	0.25:1:0.5	+
50	100	50	0.5:1:0.5	++
100	100	50	1:1:0.5	+++

***Note:** + Good, ++ Very good, +++ Excellent.*

Among all formulations incorporated with the drug, 1:1:0.5 ratio of cholesterol: surfactant: fluconazole was excellent as observed in **Table 8**.

4.3 Entrapment Efficiency of Niosomes

Evaluating the delivery potential of system requires the dominant criterion to be defined i.e., the entrapment of drug within a vesicular carrier. All the formulations were assessed for their entrapment efficiency in an attempt to examine impact of niosome composition, i.e., the quantity and kind of non-ionic surfactants (Spans and Tweens) and the approach of preparation on drug loading capacity; as illustrated in **Table 9 and Fig.4**, non-ionic surfactants, type and quantity used for niosomes preparation and the approach of preparation affected the entrapment efficiency[5,9,11].

Table 9. Entrapment efficiency of Niosomes.

Formulation code	Surfactant	SUF:CHO:DRUG Ratio	% Entrapment efficiency
FS1	Span 60	1: 1: 0.5	77.62±0.26
FS2	Span 60	1.5: 1: 0.5	93.92±0.35
FS3	Span 60	2: 1: 0.5	84.95±0.14
FS4	Span 60	2.5: 1: 0.5	92.51±0.61
FS5	**Span 60**	**3: 1: 0.5**	**95.12±0.49**
FT1	Tween 60	1: 1: 0.5	82.31±0.38
FT2	Tween 60	1.5: 1: 0.5	91.2±0.28
FT3	Tween 60	2: 1: 0.5	87.64±0.67
FT4	**Tween 60**	**2.5: 1: 0.5**	**94.91±0.72**
FT5	Tween 60	3: 1: 0.5	94.31±0.57

** **Note:** Values are represented as Mean ± SD, n=3.*

All formulation has shown results in a range of 77.62±0.26 to 95.12±0.49. Formulation FS5 comprising Span 60 has demonstrated maximum entrapment efficiency i.e., 95.12±0.49 and FT4 containing Tween 60 has demonstrated maximum entrapment efficiency i.e., 94.91±0.72.

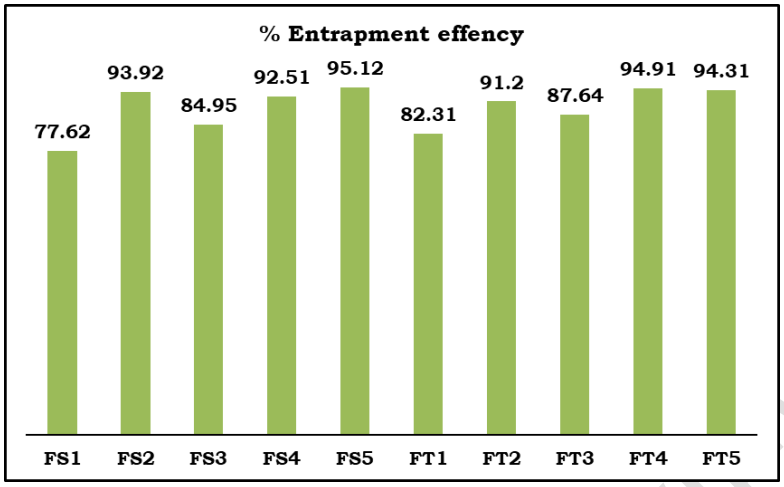
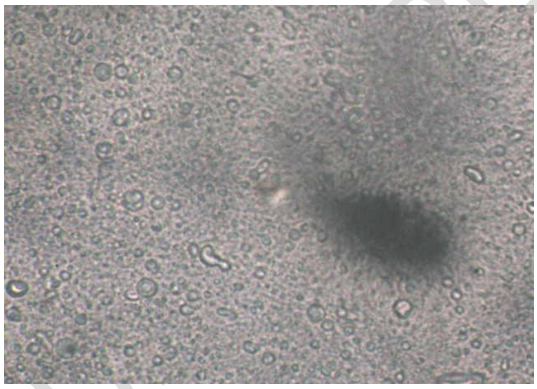


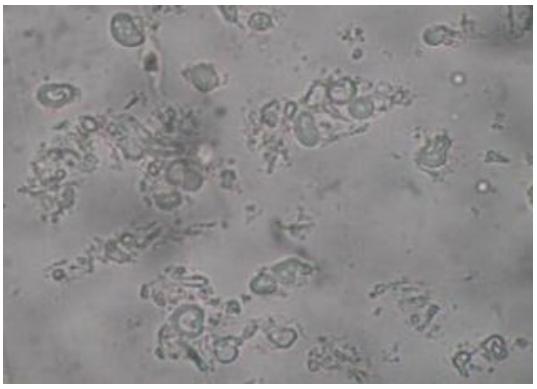
Fig. 4. Bar diagram showing the entrapment efficiency of prepared Niosomes.

4.4 Microscopic Characterization of SPAN 60 and TWEEN 60

Optical microscopy at 45x resolution was employed to confirm vesicle formation. The fixing of niosomal suspension over glass slide was achieved by drying at ambient temperature, the dry thin film of niosomal suspension was observed for the establishment of vesicles. Digital camera was utilized for microphotography of niosomes **Fig.5**. The detailed surface characteristics of selected fluconazole niosomes formulation were observed using an electron microscope[5,9,12]. A triangular research microscope with a Fuji film digital camera was utilised to examine the surface morphology of niosomes and the vesicles appeared to be spherical in shape as illustrated in **Fig.5.**



(a)



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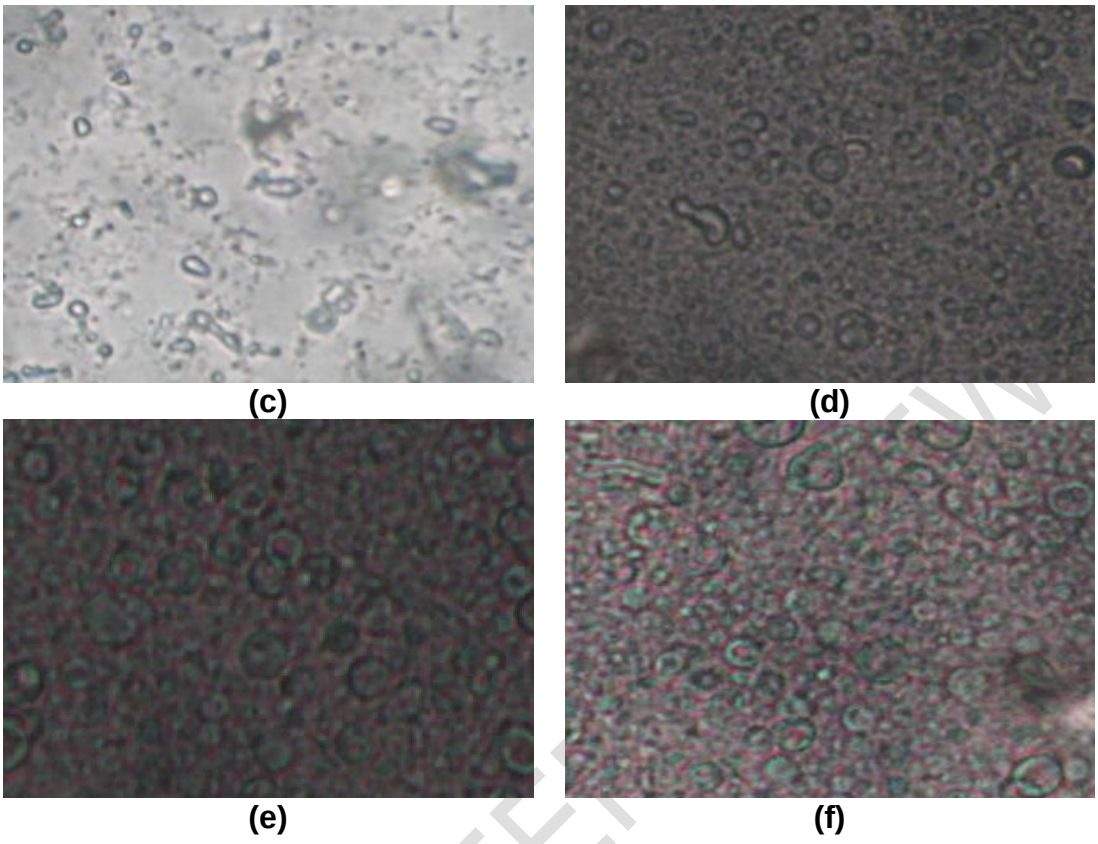


(c) **(d)**

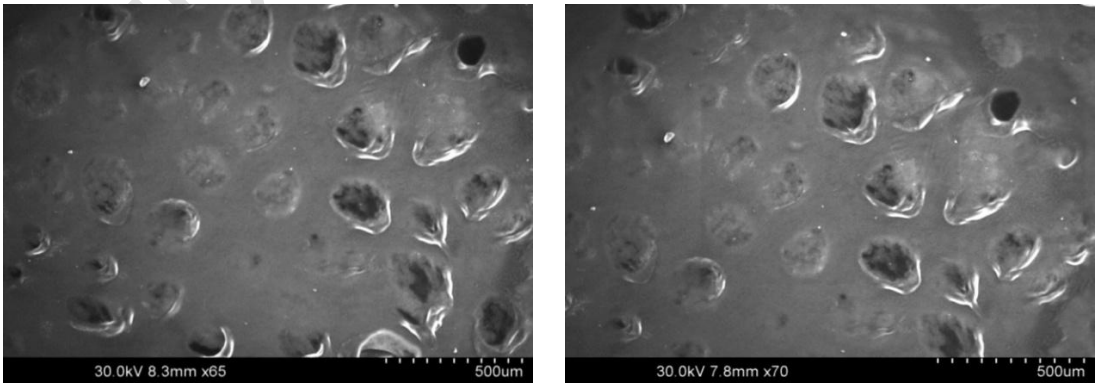
(e) **(f)**

Fig. 5. Microscopic characterization of (a) Span 60 (25mg), (b) Span 60 (50mg), (c) Tween 60 (25mg), (d) Tween 60 (50mg), (e) Span 60 (100mg) and (f) Tween 60 (100mg).

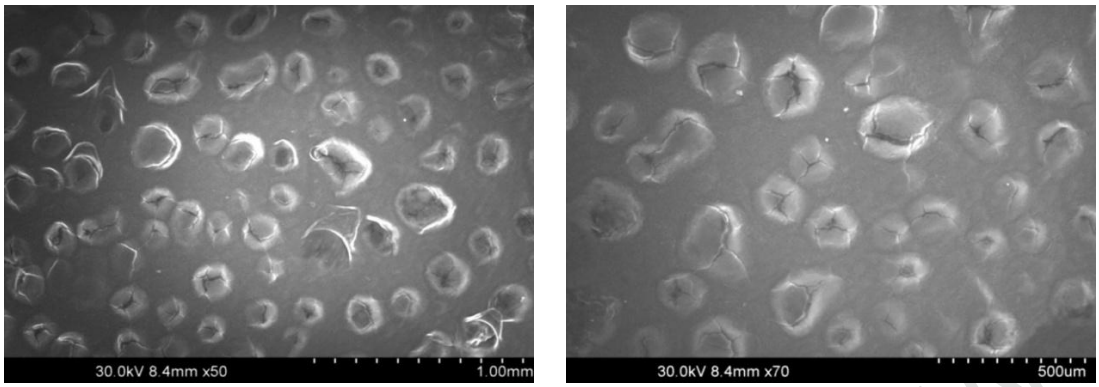
4.5 Evaluation of Fluconazole Niosomes and Niosomal Gel

4.5.1 Particle Size and Morphology

For evaluating prepared niosomes and niosomal gel, scanning electron microscopy (SEM), vesicle size and zeta potential were analysed by HORIBA-SZ-100Z instrument[10,14–16].



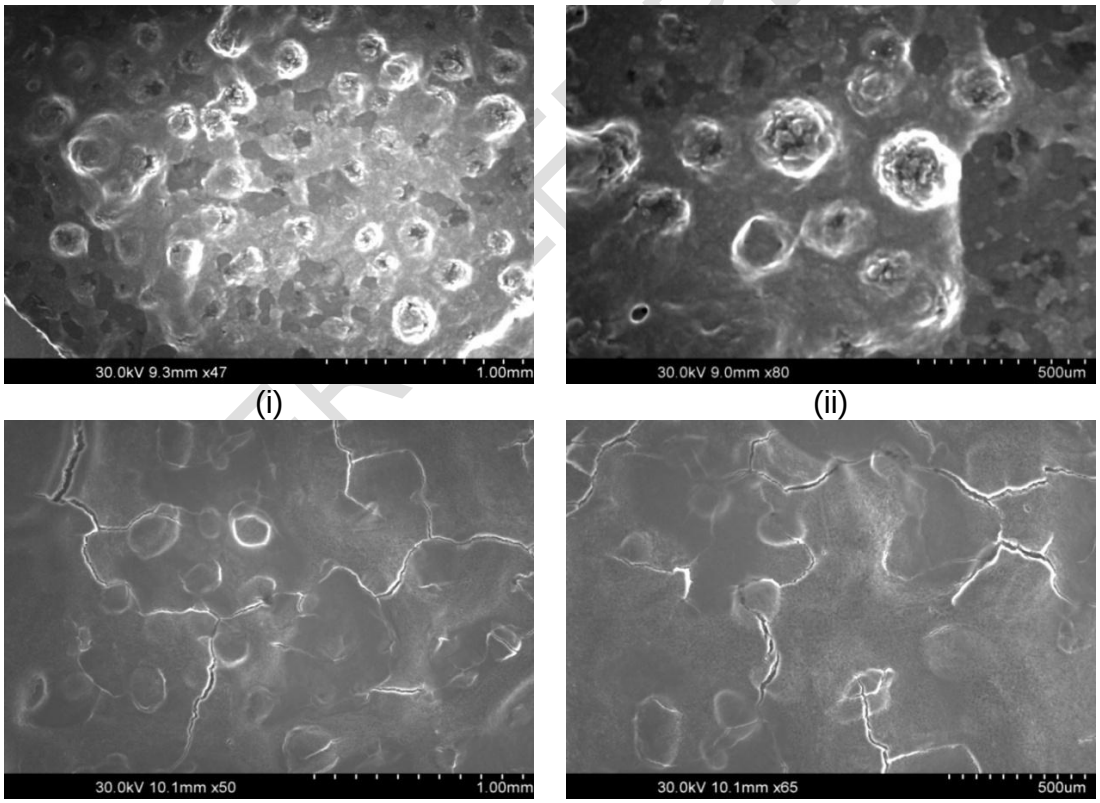
(i) **(ii)**



(iii) **(iv)**

Fig. 6. Surface morphology of Fluconazole Niosomes (i) and (ii) FNS5 Fluconazole Niosome using Span 60, (iii) and (iv) FNT4 Fluconazole Niosome using Tween 60.

Scanning electron microscopy was utilized to characterize the surface morphology, vesicle structure[7,10,22] and shape using Span 60 and Tween 60 was spherical, confirming the vesicular characteristics as displayed in **Fig.6** and **Fig.7.**



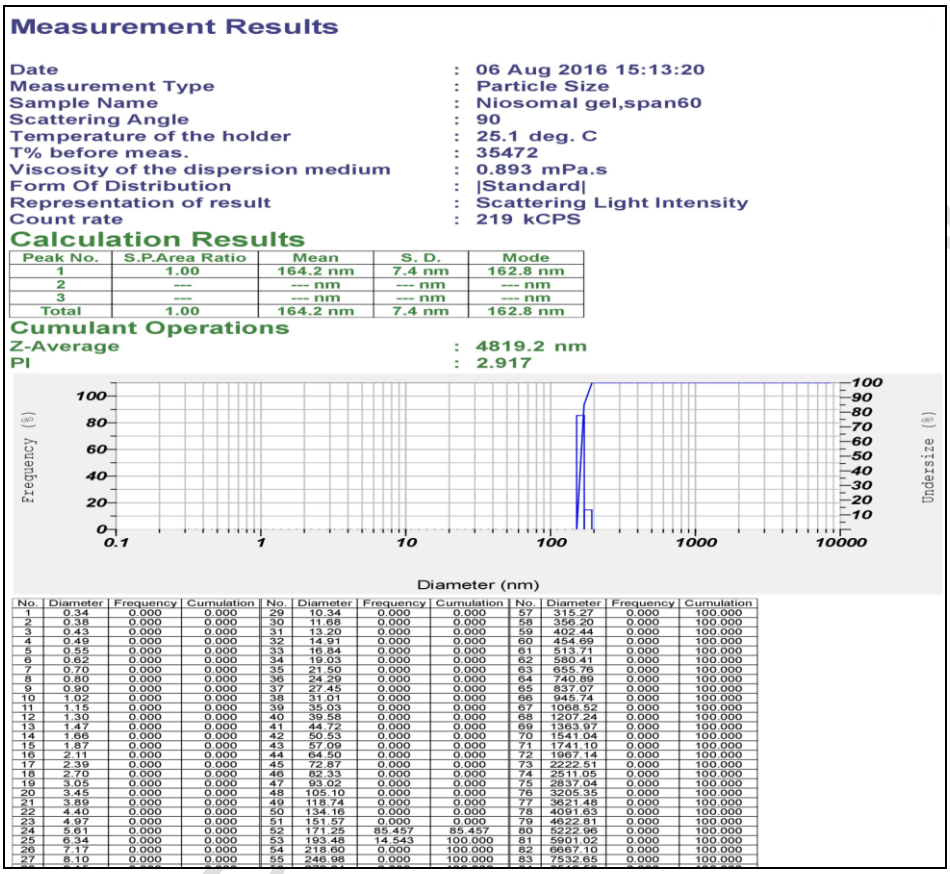
(i) (ii)

(iii) (iv)

Fig. 7. Surface morphology of Fluconazole Niosomes (i) and (ii) FCS niosomal gel using Span 60, (iii) and (iv) FCT niosomal gel using Tween 60.

4.5.2 Vesicle Size Analysis

Optimized niosomes vesicle size was examined by HORIBA-SZ-100Z zeta-sizer. Formulation FCS and FCT showed mean particle size 164.2±7.4 and 845.6±298.8 nm respectively as depicted in **Fig. 8** and **Fig. 9**.



Measurement Results

Date	:	06 Aug 2016 15:13:20
Measurement Type	:	Particle Size
Sample Name	:	Niosomal gel,span60
Scattering Angle	:	90
Temperature of the holder	:	25.1 deg. C
T% before meas.	:	35472
Viscosity of the dispersion medium	:	0.893 mPa.s
Form Of Distribution	:	|Standard|
Representation of result	:	Scattering Light Intensity
Count rate	:	219 kCPS

Calculation Results

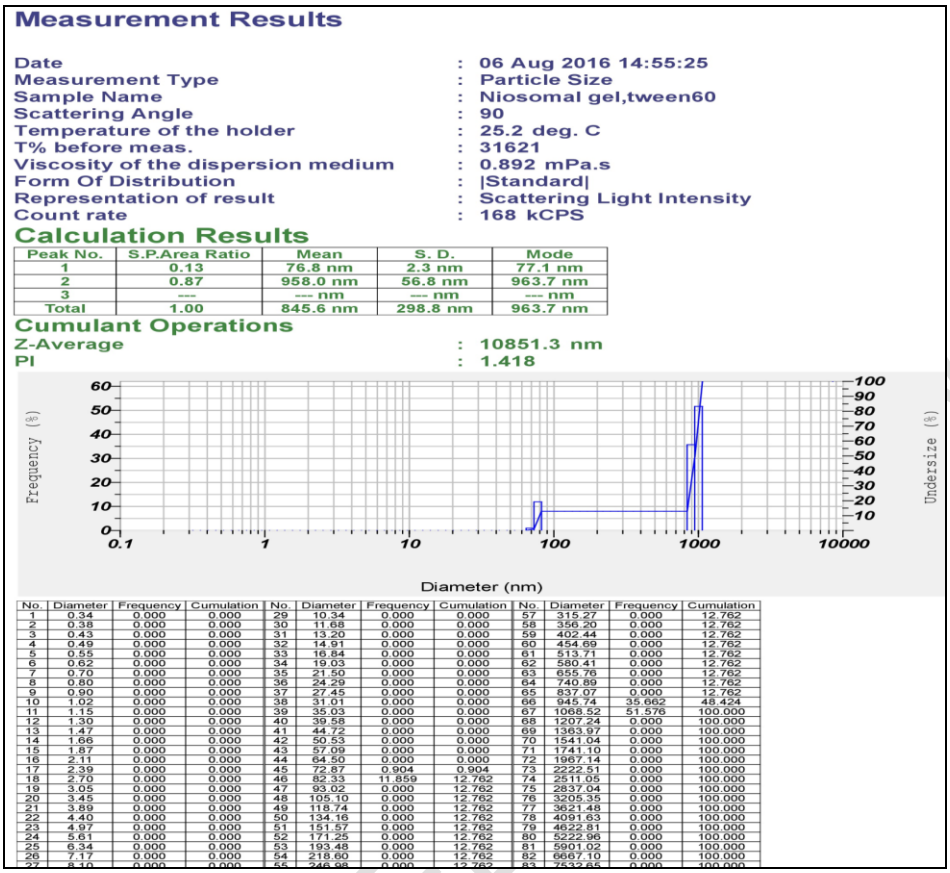
Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	164.2 nm	7.4 nm	162.8 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	164.2 nm	7.4 nm	162.8 nm

Cumulant Operations

Z-Average	:	4819.2 nm
PI	:	2.917

No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation
1	0.34	0.000	0.000	29	10.34	0.000	0.000	57	315.27	0.000	100.000
2	0.38	0.000	0.000	30	11.68	0.000	0.000	58	356.20	0.000	100.000
3	0.43	0.000	0.000	31	13.20	0.000	0.000	59	402.44	0.000	100.000
4	0.49	0.000	0.000	32	14.91	0.000	0.000	60	454.69	0.000	100.000
5	0.55	0.000	0.000	33	16.84	0.000	0.000	61	513.71	0.000	100.000
6	0.62	0.000	0.000	34	19.03	0.000	0.000	62	580.41	0.000	100.000
7	0.70	0.000	0.000	35	21.50	0.000	0.000	63	655.76	0.000	100.000
8	0.80	0.000	0.000	36	24.29	0.000	0.000	64	740.89	0.000	100.000
9	0.90	0.000	0.000	37	27.45	0.000	0.000	65	837.07	0.000	100.000
10	1.02	0.000	0.000	38	31.01	0.000	0.000	66	945.74	0.000	100.000
11	1.15	0.000	0.000	39	35.03	0.000	0.000	67	1068.52	0.000	100.000
12	1.30	0.000	0.000	40	39.58	0.000	0.000	68	1207.24	0.000	100.000
13	1.47	0.000	0.000	41	44.72	0.000	0.000	69	1363.97	0.000	100.000
14	1.66	0.000	0.000	42	50.53	0.000	0.000	70	1541.04	0.000	100.000
15	1.87	0.000	0.000	43	57.09	0.000	0.000	71	1741.10	0.000	100.000
16	2.11	0.000	0.000	44	64.50	0.000	0.000	72	1967.14	0.000	100.000
17	2.39	0.000	0.000	45	72.87	0.000	0.000	73	2222.51	0.000	100.000
18	2.70	0.000	0.000	46	82.33	0.000	0.000	74	2511.05	0.000	100.000
19	3.05	0.000	0.000	47	93.02	0.000	0.000	75	2837.04	0.000	100.000
20	3.45	0.000	0.000	48	105.10	0.000	0.000	76	3205.35	0.000	100.000
21	3.89	0.000	0.000	49	118.74	0.000	0.000	77	3621.48	0.000	100.000
22	4.40	0.000	0.000	50	134.16	0.000	0.000	78	4091.63	0.000	100.000
23	4.97	0.000	0.000	51	151.57	0.000	0.000	79	4622.81	0.000	100.000
24	5.61	0.000	0.000	52	171.25	85.457	85.457	80	5222.96	0.000	100.000
25	6.34	0.000	0.000	53	193.48	14.543	100.000	81	5901.02	0.000	100.000
26	7.17	0.000	0.000	54	218.60	0.000	100.000	82	6667.10	0.000	100.000
27	8.10	0.000	0.000	55	246.98	0.000	100.000	83	7532.65	0.000	100.000

Fig. 8. Vesicle size of Fluconazole carbopal Span 60 Niosomal gel.



Measurement Results

Date	:	06 Aug 2016 14:55:25
Measurement Type	:	Particle Size
Sample Name	:	Niosomal gel,tween60
Scattering Angle	:	90
Temperature of the holder	:	25.2 deg. C
T% before meas.	:	31621
Viscosity of the dispersion medium	:	0.892 mPa.s
Form Of Distribution	:	|Standard|
Representation of result	:	Scattering Light Intensity
Count rate	:	168 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	0.13	76.8 nm	2.3 nm	77.1 nm
2	0.87	958.0 nm	56.8 nm	963.7 nm
3	---	--- nm	--- nm	--- nm
Total	1.00	845.6 nm	298.8 nm	963.7 nm

Cumulant Operations

Z-Average	:	10851.3 nm
PI	:	1.418

No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation	No.	Diameter	Frequency	Cumulation
1	0.34	0.000	0.000	29	10.34	0.000	0.000	57	315.27	0.000	12.762
2	0.38	0.000	0.000	30	11.68	0.000	0.000	58	356.20	0.000	12.762
3	0.43	0.000	0.000	31	13.20	0.000	0.000	59	402.44	0.000	12.762
4	0.49	0.000	0.000	32	14.91	0.000	0.000	60	454.69	0.000	12.762
5	0.55	0.000	0.000	33	16.84	0.000	0.000	61	513.71	0.000	12.762
6	0.62	0.000	0.000	34	19.03	0.000	0.000	62	580.41	0.000	12.762
7	0.70	0.000	0.000	35	21.50	0.000	0.000	63	655.76	0.000	12.762
8	0.80	0.000	0.000	36	24.29	0.000	0.000	64	740.89	0.000	12.762
9	0.90	0.000	0.000	37	27.45	0.000	0.000	65	837.07	0.000	12.762
10	1.02	0.000	0.000	38	31.01	0.000	0.000	66	945.74	35.662	48.424
11	1.15	0.000	0.000	39	35.03	0.000	0.000	67	1068.52	51.576	100.000
12	1.30	0.000	0.000	40	39.58	0.000	0.000	68	1207.24	0.000	100.000
13	1.47	0.000	0.000	41	44.72	0.000	0.000	69	1363.97	0.000	100.000
14	1.66	0.000	0.000	42	50.53	0.000	0.000	70	1541.04	0.000	100.000
15	1.87	0.000	0.000	43	57.09	0.000	0.000	71	1741.10	0.000	100.000
16	2.11	0.000	0.000	44	64.50	0.000	0.000	72	1967.14	0.000	100.000
17	2.39	0.000	0.000	45	72.87	0.904	0.904	73	2222.51	0.000	100.000
18	2.70	0.000	0.000	46	82.33	11.859	12.762	74	2511.05	0.000	100.000
19	3.05	0.000	0.000	47	93.02	0.000	12.762	75	2837.04	0.000	100.000
20	3.45	0.000	0.000	48	105.10	0.000	12.762	76	3205.35	0.000	100.000
21	3.89	0.000	0.000	49	118.74	0.000	12.762	77	3621.48	0.000	100.000
22	4.40	0.000	0.000	50	134.16	0.000	12.762	78	4091.63	0.000	100.000
23	4.97	0.000	0.000	51	151.57	0.000	12.762	79	4622.81	0.000	100.000
24	5.61	0.000	0.000	52	171.25	0.000	12.762	80	5222.96	0.000	100.000
25	6.34	0.000	0.000	53	193.48	0.000	12.762	81	5901.02	0.000	100.000
26	7.17	0.000	0.000	54	218.60	0.000	12.762	82	6667.10	0.000	100.000
27	8.10	0.000	0.000	55	246.98	0.000	12.762	83	7532.65	0.000	100.000

Fig. 9. Vesicle size of Fluconazole carbopal Tween 60 Niosomal gel.

These results might be attributed to relationship noticed between niosome size. A drop in surface energy with rising hydrophobicity results in smaller vesicles. Previous studies have indicated a condensing ability of methanol for lipid vesicles. It was documented that with higher methanol concentration, owing to formation of a phase with interpenetrating hydrocarbon chains membrane thickness of vesicles recedes.

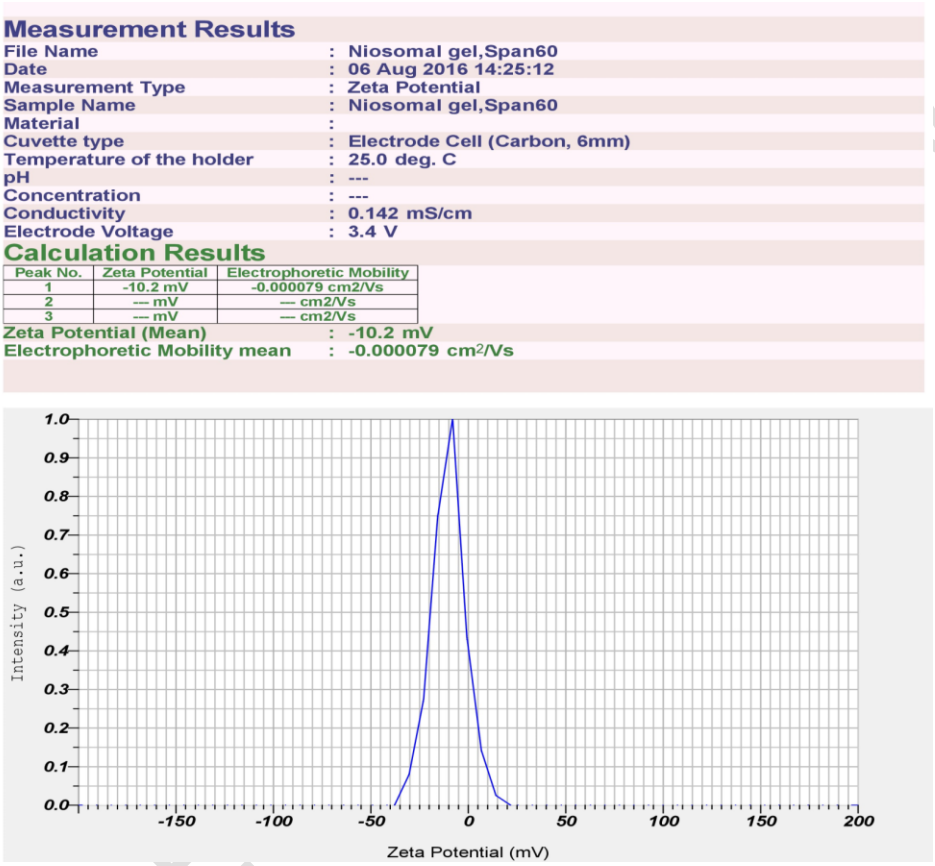
Also, methanol may induce modification of the net charge of the system that results in decreased mean particle size due to some degree of steric stabilization. Furthermore, the achievement of nano vesicles helps in passage across the anatomical constraints of skin.

The polydispersibility index (PDI) was recorded to be 2.917 and 1.418 for formulations FCS and FCT respectively. Polydispersity index is ratio of standard deviation to mean particle size signifying uniformity of particle size within a formulation. The formulation was in nanosize range with PDI less than 0.3 indicating a homogeneous distribution of particles within the formulation while a value greater than 0.3 indicates heterogeneous nature and low PDI ascertains narrow variation in size distribution and consistency of particle size within the formulation[18,19,22].

4.5.3 Zeta potential of niosomal gel

The parameter that infers the thermodynamic stability of nanosize vesicular system is zeta potential. Optimized niosomal gel FCS and FCT showed zeta potential -10.2 mV and -46.4

mV as displayed in **Fig.10** and **Fig.11**. The zeta potential of -50 mV to +50 mV from the literature indicates a fairly stable formulation. The potential stability of a colloidal system is indicated by magnitude of zeta potential. The existence of larger negative or positive zeta potential in all particles of gel results in repulsion and the tendency of particles to come close is lost. The formation of cationic vesicles and higher zeta potential is denoted by the existence of positive charge. Strong electrostatic repulsions due to high zeta potential, either negative or positive, results in a more stable system[10,17,19,20].



Measurement Results

File Name	: Niosomal gel,Span60
Date	: 06 Aug 2016 14:25:12
Measurement Type	: Zeta Potential
Sample Name	: Niosomal gel,Span60
Material	:
Cuvette type	: Electrode Cell (Carbon, 6mm)
Temperature of the holder	: 25.0 deg. C
pH	: ---
Concentration	: ---
Conductivity	: 0.142 mS/cm
Electrode Voltage	: 3.4 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-10.2 mV	-0.000079 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	: -10.2 mV
Electrophoretic Mobility mean	: -0.000079 cm^2/Vs

Fig. 10. Zeta potential of Niosomal gel Span 60.

Measurement Results

File Name	:	Niosomal gel,tween60
Date	:	06 Aug 2016 14:14:11
Measurement Type	:	Zeta Potential
Sample Name	:	Niosomal gel,tween60
Material :		
Cuvette type	:	Electrode Cell (Carbon, 6mm)
Temperature of the holder	:	25.1 deg. C
pH	:	---
Concentration	:	---
Conductivity	:	0.092 mS/cm
Electrode Voltage	:	3.9 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-46.4 mV	-0.000360 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	:	-46.4 mV
Electrophoretic Mobility mean	:	-0.000360 cm^2/Vs

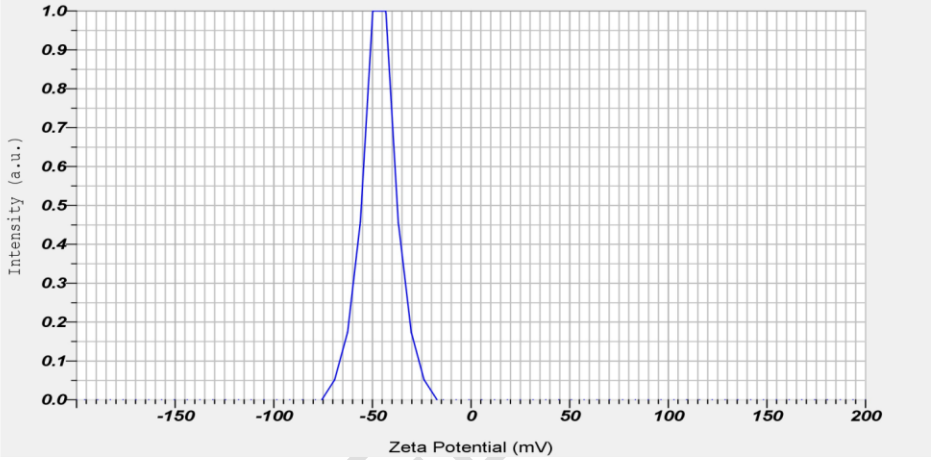


Fig. 11. Zeta potential of Niosomal gel Tween 60.

4.5.4 *In-vitro* diffusion of niosomes

In-vitro diffusion studies for fluconazole pure drug, along with prepared niosomes in pH 7.4 PBS solution, was performed for 11hrs and the optimized formulations were evaluated for 24hrs[11,13].

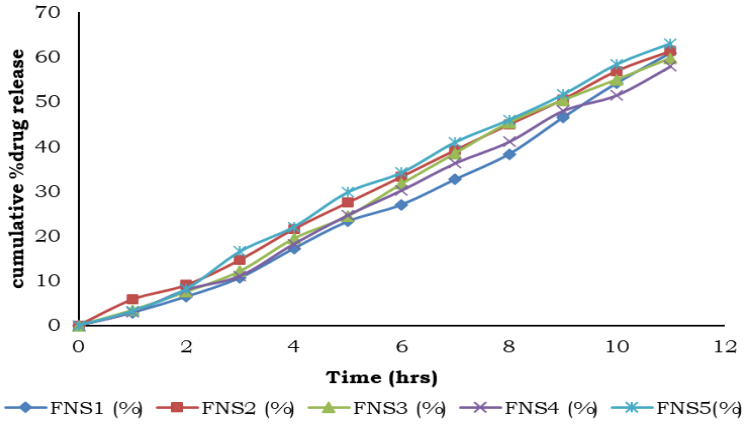


Fig. 12. Diffusion profile of Span 60 formulations.

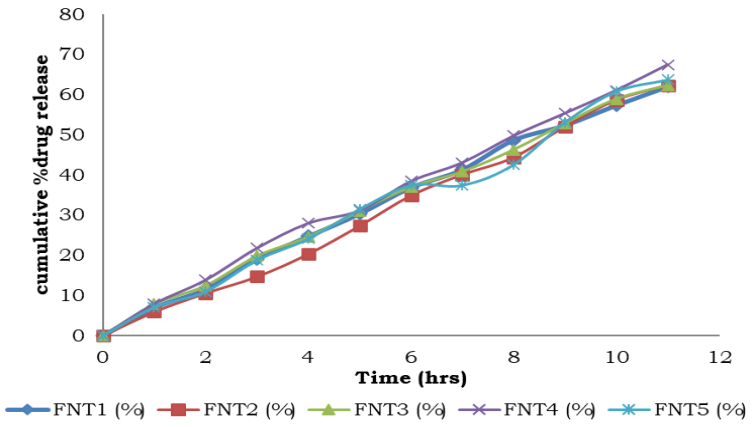


Fig. 13. Diffusion profile of Tween 60 formulations.

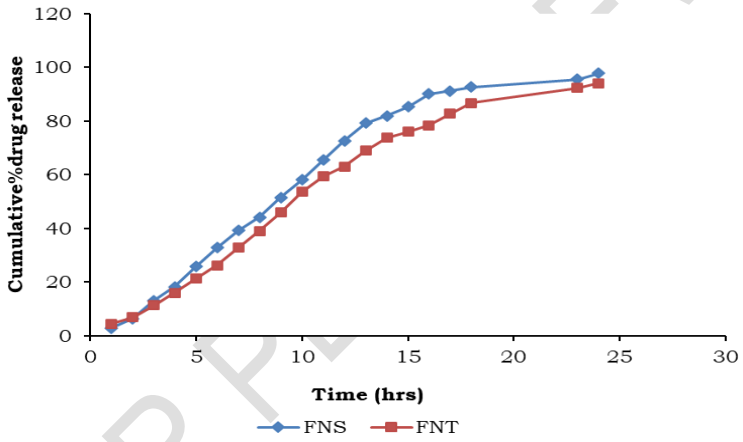


Fig. 14. Diffusion profile of optimized formulations.

The *in-vitro* diffusion studies were conducted on dialysis membrane using Franz diffusion cells for all the formulation[11,13]. Samples were collected periodically every 1hr and analyzed using a UV-Visible double beam spectrophotometer at 260 nm.

Formulation FNS5 and FNT4 were ascertained to be optimized because at 11hrs they exhibited drug release 63.03±0.24 and 67.42±0.64 respectively (**Fig.12** and **Fig.13**). For the extended-release of drug, formulations were additionally studied up to 24hrs and the outcomes were in the scale of 96.01±0.2 and 94.03±0.27 (**Fig.14**).

4.5.5 Model-dependent kinetics

Formulations prepared were fit into the model-dependent kinetics such as zero, first-order, Higuchi and Peppas models.

Table 10. Model dependent kinetics.

Formulations	Zero order	First order	Higuchi	Korsmeyer peppas		Release Mechanism
	r	r	r	r	n	
FNS5	0.944	0.733	0.974	0.977	0.042	Fickian diffusion
FNT4	0.954	0.786	0.986	0.990	0.815	Anomalous transport

Release kinetics for each formulation was plotted against time to fit zero order, first order, Higuchi kinetic model and Korsmeyer Peppas equations. The regression value and 'n' values were acquired from the plots. The mechanism by which drug release took place was examined for all formulations using the 'n' value. Amongst all the formulations, FNS5 and FNT4 were optimized. Formulation, FNS5 displayed zero-order kinetics and Korsmeyer Peppas following fickian diffusion[10,17] Formulation, FNT4 displayed zero-order kinetics and Korsmeyer Peppas following Anomalous transport (**Table 10**).

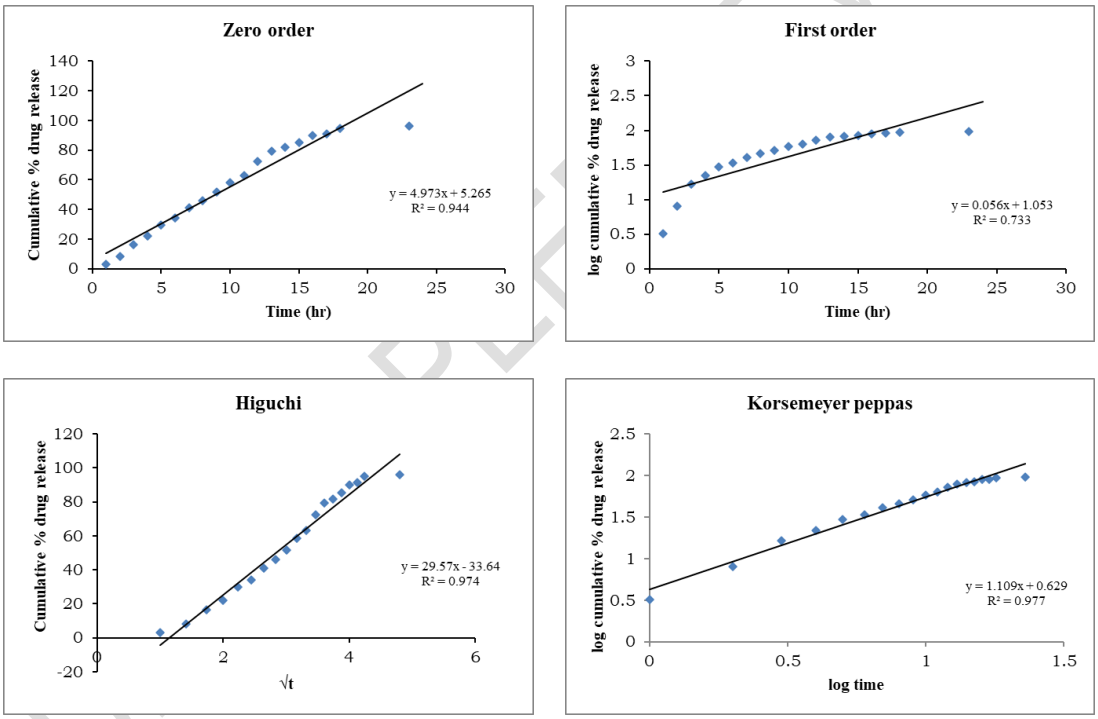


Fig. 15. Model dependent kinetics of formulation FNS5.

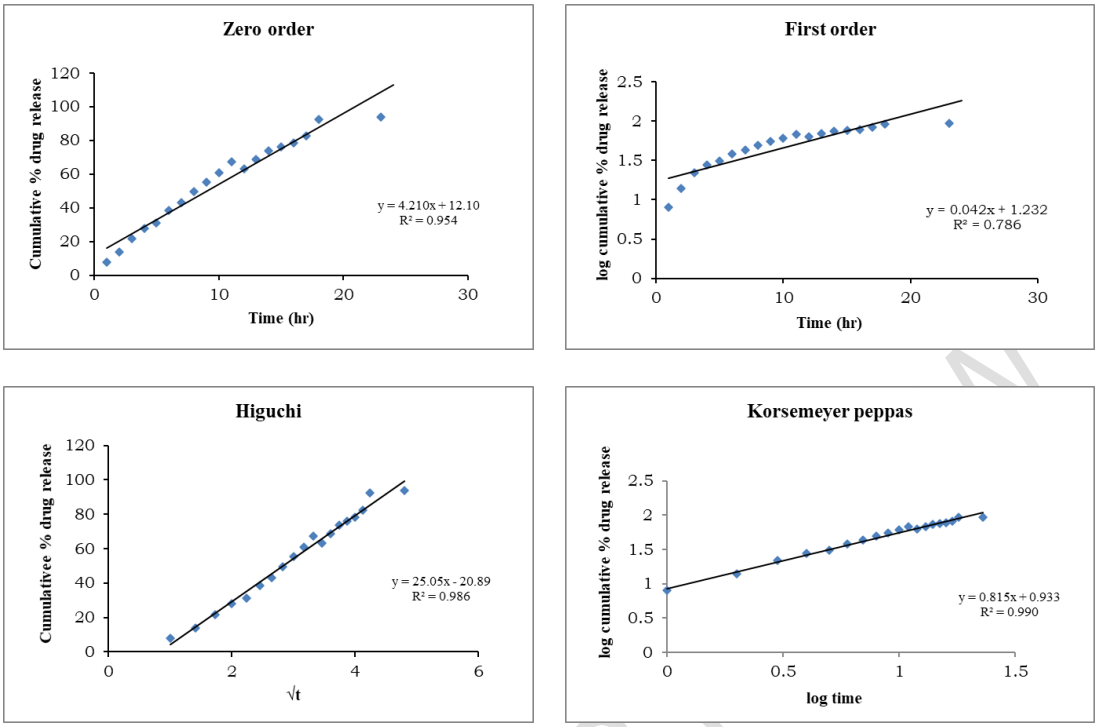


Fig. 16. Model dependent kinetics of formulation FNT4.

4.5.6 Formulation and evaluation of gel

Formulation of gel was done utilizing various polymers in different concentrations followed by its evaluation. Results specified that carbopal 2% and guar gum 2% to be optimized[10,14] (**See Table 10)**.

Table 11. Formulation of gel with different polymers.

S.No.	Ingredients	1%	2%	3%	4%	Gel formed ratio
1.	**Carbopal 934**	**+**	**++**	**+++**	**-**	**2%**
2.	HPMC K15M	+	+	+	++	4%
3.	**Guar gum**	**+**	**++**	**+++**	**-**	**2%**
4.	Xanthan gum	+	++	+++	-	2%
5.	HPMC K100M	++	-	-	-	1%

***Note:** + Gel not formed, ++ Gel formed, +++ Hard gel.*

I*n-vitro* diffusion studies were done for drug-loaded gel containing carbopol (2%) and guar gum (2%), and results displayed release to be 94.67±0.76 at 5 hrs and 97.63±0.85 at 6 hrs for formulations[14–16] FCG (2%) and FGG (2%).

Table 12. Evaluation of gel.

FORMULATIONS	pH	Viscosity (cps) (2%)	Spreadability (gm/cm^2)
FNCT	6.8	67,865.58	17.20

FNCS	6.4	71,462.12	21.73
FNGT	6.2	63,240.03	23.96
FNGS	6.7	73,173.14	35.21

***Note:** Values are expressed as Mean ± SD, n=3*

All formulations displayed results in following range for evaluated parameters i.e., pH 6.2 to 6.8, spreadability 17.20 to 35.21 gm/cm^2, viscosity 63,240.03 to 73,173.14 cps (**See Table 12**).

4.5.7 *In-vitro* drug release studies

Franz diffusion cell in pH 7.4 PBS was employed for *in-vitro* evaluation studies of fluconazole niosomal gel.

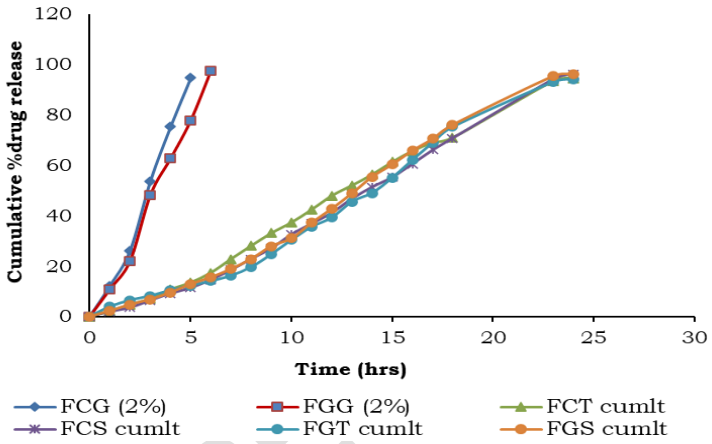


Fig. 17. Diffusion profile of Niosomal gel.

The *in-vitro* diffusion studies were carried on dialysis membrane using Franz diffusion cells for each formulation. Samples were collected periodically every 1hr and examined utilizing UV-Visible double beam spectrophotometer at 260 nm[10,17,18,22,23].

Amongst all formulations, FCT and FCS were ascertained to be optimized as at 24 hrs, the release was 94.51±0.68 and 96.21±0.26 **(See Fig.17).**

4.5.8 Model dependent kinetics for niosomal gel

Table 13. Model dependent kinetics.

Formulations	Zero order	First order	Higuchi	Korsmeyer peppas		Release Mechanism
	r	r	r	R	n	
FNCT	0.974	0.826	0.957	0.887	0.057	Fickian diffusion
FNCS	0.974	0.86	0.926	0.995	1.303	Super case II transport
FNGT	0.978	0.931	0.915	0.61	1.104	Super case II transport
FNGS	0.987	0.872	0.926	0.992	1.251	Super case II transport

The release kinetics for all formulations was plotted against time to fit zero-order, first-order, Higuchi kinetic model and Korsmeyer Peppas equations. The regression value and ‘n’ values were acquired from the plots. The mechanism by which drug release took place was examined for all formulation using the 'n' value. Amongst all the formulations, FCT and FCS were optimized. Formulation, FCT displayed zero-order kinetics and Higuchi model following fickian diffusion[10,17]. Formulations, FCS displayed zero-order kinetics and Korsmeyer Peppas following super case II transport (**Table 13**).

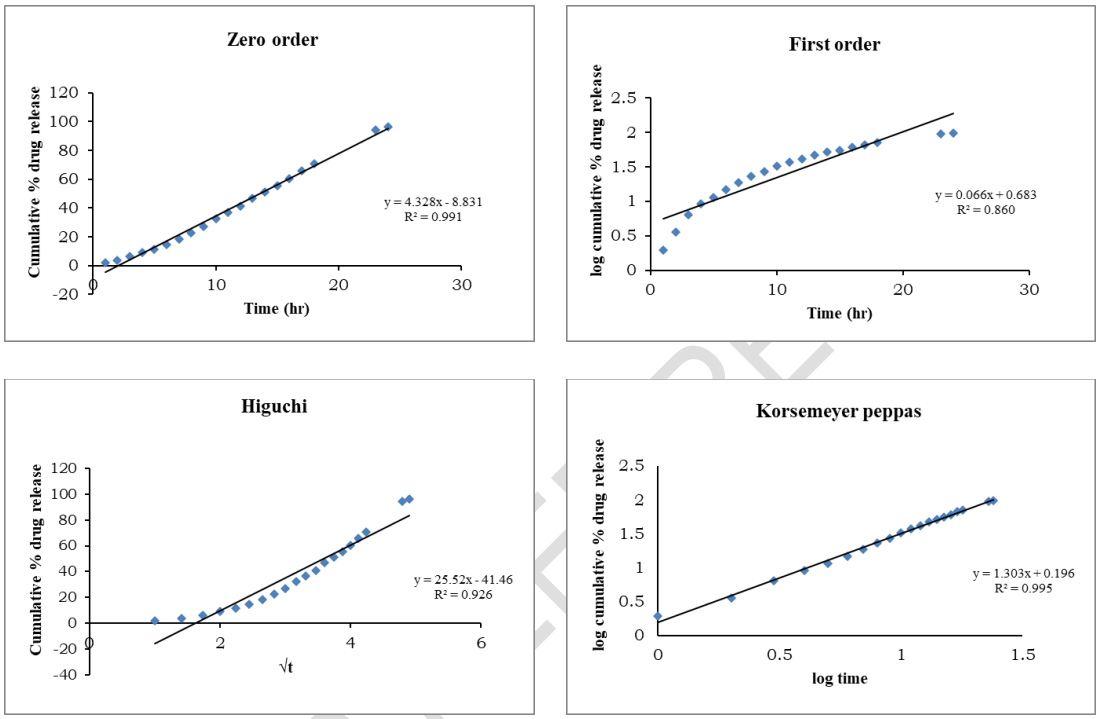
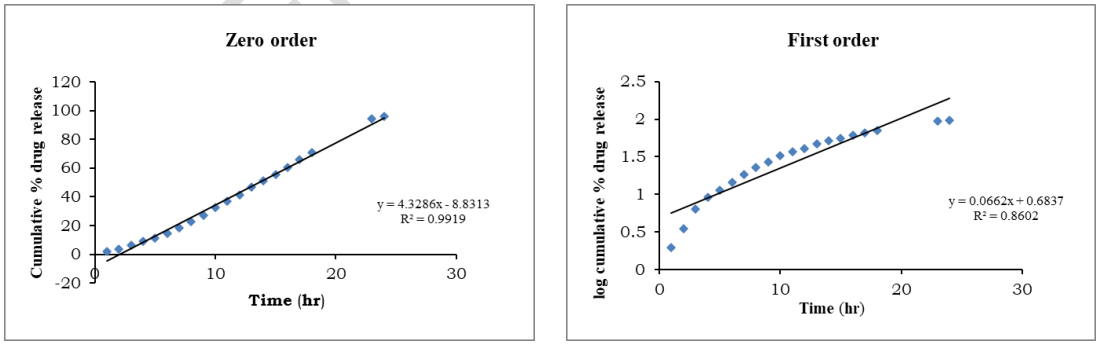


Fig. 18. Model dependent kinetics of formulation FCT.



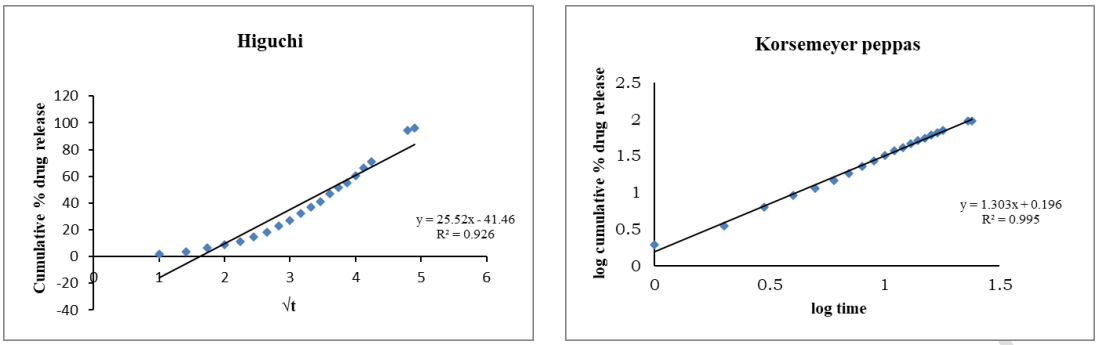


Fig. 19. Model dependent kinetics of formulation FCS.

5. CONCLUSION

An endeavour has been made to formulate, evaluate fluconazole niosomal gel and optimize the concentrations of non-ionic surfactants by thin-film hydration method.

The entrapment efficiency of 95.12±0.49 and 94.91±0.72 was exhibited by FS5 comprising Span 60 and FT4 comprising Tween 60. The *in-vitro* diffusion studies for various formulations were conducted. The optimized formulation FNCS and FNCT consisting of Span 60 and Tween 60 developed by thin-film hydration method exhibited vesicle size 845.6 and 164.2 nm respectively, zeta potential of -10.2 mV and -46.4 mV implies that the formulation has good stability.

Accordingly, niosomal gel could be a recommendable carrier to deliver fluconazole in contrast to conventional topical gel loaded with pure drug.

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO NOT INTEND TO USE THESE PRODUCTS AS AN AVENUE FOR ANY LITIGATION BUT FOR THE ADVANCEMENT OF KNOWLEDGE. ALSO, THE RESEARCH WAS NOT FUNDED BY THE PRODUCING COMPANY RATHER IT WAS FUNDED BY PERSONAL EFFORTS OF THE AUTHORS.

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