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DEVELOPMENT AND CHARACTERIZATION OF FLURBIPROFEN LOADED NIOSOMAL IN-SITU GEL FOR OPHTHALMIC DRUG DELIVERY SYSTEM

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ABSTRACT:

Niosome are non-ionic surfactant-based liposomes, obtained in the hydration of artificial non-ionic surfactants, without or with addition of cholesterol or additional lipids. Theintention of the current study was to organize and evaluate the *in-situ* niosomal gel loaded with flurbeprofen for the ophthalmic drug delivery system. Flurbeprofen, a nonsteroidal anti- infammatory drug (NSAIDs) is used to relief pain and inflammation. It carries out anti- inflammatory, analgesic and antipyretic activities. Flurbeprofen loaded Niosomes investigate the connection among the non-ionic drug / surfactant ratio with the adding of cholesterol was successfully organized with the thin film hydration way and compare the result of different grade of span used (20, 40 and 60) with the different ratio of cholesterol. Niosomes have been identified by drug entrapment efficiency, drug content, particle size and in-vitro diffusion study. Niosomes prepared using span 60 and cholesterol in the ratio (1:1) f4 showed greater entrapment efficiency (79.2%).

Keywords: Niosomes, in-situ gel, flurbiprofen, TEM.

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INTRODUCTION

The drug delivery is one of the toughest among the all challenges faced by pharmaceutical industry. The original ophthalmic solution, suspension and ointment dosage forms are distinctly no longer adequate to fight against some present virulent diseases[1]. The conventional ophthalmic formulations marketed as solution, suspension and ointment have numerous limitations which results in a low bioavailability of the drug in the ocular cavity. The particular goal of scheming a therapeutic system is to accomplish a perfect measure of drug at the target site during the appropriate time period. The ocular disposition and the abolition of a therapeutic agent are based on the physico-chemical properties and on the adequate anatomy and physiology of the eye[2].
Numerous methods have been practiced to increase the bioavailability and time of the therapeutic properties of ocular drugs. The two main methods are the following:

✓ Depends on the use of sustained drug delivery systems, which transmit controlled andconstant delivery of ophthalmic drugs.

✓ Includes maximization of corneal drug absorption and minimization of precorneal drugloss[3,4].

Flurbiprofen is a non-steroidal, anti-inflammatory drug (NSAIDs). It is used as part of the treatment of mild to moderate pain and symptoms of chronic arthritis. Flurbiprofen has related to a low rate of serum enzyme augmentation during therapy and in exceptional gear of clinically clear sensitive liver injury[5-10].

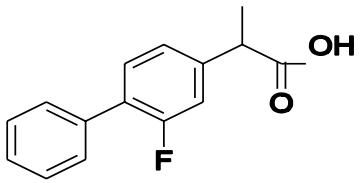

Fig 1. Chemical Structure of Flurbiprofen

Molecular Formula- $C_{15}H_{13}FO_2$
IUPAC name- 2-(3-fluoro-4-phenylphenyl) propanoic acid
Molecular weight- 244.265 gm/mol
Melting point- 110-111 °C
Water solubility- 8mg/l (at 22°C)
Mechanism of action
Comparable with other NSAIDs, the anti-inflammatory effect of flurbiprofen is produced by the reversible inhibition of cyclooxygenase (COX), the enzyme dependable for the transfer of arachidonic acid to prostaglandin G2 (PGG2) and PGG2 to prostaglandin H2 (PGH2) in the synthesis of prostaglandins. This appropriately reduces the concentration of occupational prostaglandins in inflammation, pain, swelling and fever. Flurbiprofen is a non-selective-COX

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inhibitor and also limits the progress of COX-1 and 2. Furthermore, it is one of the most attractive among the most effective NSAIDs in terms of inhibitory movement of prostaglandins[11].

MATERIALS AND METHODS

Materials

Table 1. List of chemicals

Chemical Name	Manufacture
Flurbiprofen	Gift Sample
Span 20	Yarrow Chem Products
Span 40	Yarrow Chem Products
Span 60	Yarrow Chem Products
Cholesterol	Yarrow Chem Products
Chloroform	Finar
Methanol	Finar
Distill Water	Lab

Table 2. List of Instrument and Equipment Used

Instruments	Manufacture
Electronic weighing machine	Citizen
UV spectrophotometer	SHIMATSZU-1800
Rotatory vaccum evaporator	Buchi type
Ultrasonic bath sonicator	Scientech
Probe sonicator	Scientech
pH meter	Scientech

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Cooling centrifuge	Remi
Stability chamber	Scientech
Humidity chamber	Scientech

A successful test was performed to formulate *in-situ* niosomal gel with Flurbiprofen containing different degrees of non-ionic surfactant (Span 20, Span 40 and Span 60). The effect of different types of non-ionic surfactants at different concentrations was studied. Total of 12 formulations have been established and evaluated.

Characterization of Drug

Solubility studies[12]

Preformulation solubility screening was performed to select an appropriate solvent system to dissolve the drug and also to test its solubility in the solution medium (pH 7.4) of flurbiprofen solubility in methanol, lipids and saline.

Table 3. Solubility studies of drug in different solvent

Solvent	Result
Water	Sparingly soluble
Alcohol	Soluble
Methanol	Freely soluble
Acetone	Freely soluble
Phosphate buffer pH 7.2	Soluble
Chloroform	Soluble

Appearance- White crystalline powder

Standard Calibration Curve for Flurbiprofen in Phosphate Buffer (pH 7.4)

The 244 nm UV absorption data and pure Flurbiprofen concentration estimates showedlinearity (R^2= 0.999) in the concentration range of 2-10µg/ml which passes through the source and follows the Lambert beer law. The values of slope and intersection of the obtained calibration curve are respectively 0.13 and 0.002[13].

Standard Calibration Curve of Flurbiprofen in methanol

The UV absorption data at 244nm and concentration estimates of pure Flurbiprofen showed linearity (R^2= 0.998) over the concentration range of 2-10µg/ml passing through origin and it

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follows the Lambert-beer's law. Slope and intercept values of calibration curve obtained are 0.082 and 0.020 respectively[14].

Table 4. Calibration curve of flurbiprofen in phosphate buffer (pH 7.4)

Concentration(µg/ml)	Absorbance
2	0.201±0.2015
4	0.392±0.3951
6	0.598±0.5981
8	0.776±0.775
10	0.973±0.975

Compatibility studies of Flurbiprofen and non-ionic surfactants

Infra-red spectrum of pure drug Flurbiprofen as well as drug with non-ionic surfactant shows characteristic absorption peaks shown in (fig.3.6-3.8). All the typical peaks of Flurbiprofen were present in spectra thus representative compatibility connecting drug and non-ionic surfactants. Shows that there was no major modify in the medication[15].

Table 5. Drug-excipient compatibility studies

Sample no.	Excipient	Ratio
1	Flurbiprofen (drug)	-
2	Drug+ span20	1:1
3	Drug + span40	1:1
4	Drug + span60	1:1
5	Drug + cholesterol	1:1

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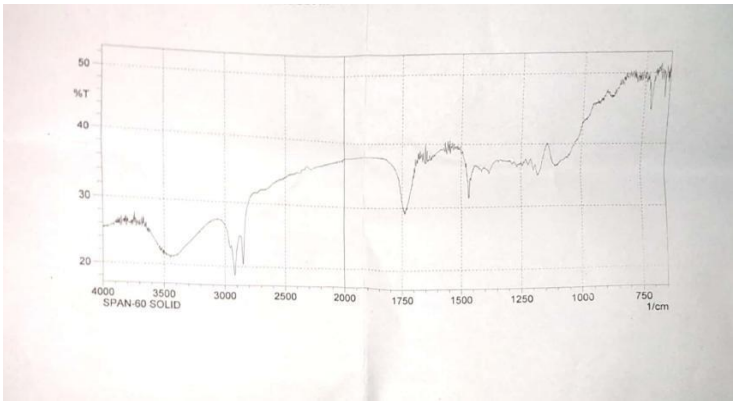

Fig 2. IR spectra of Flurbiprofen

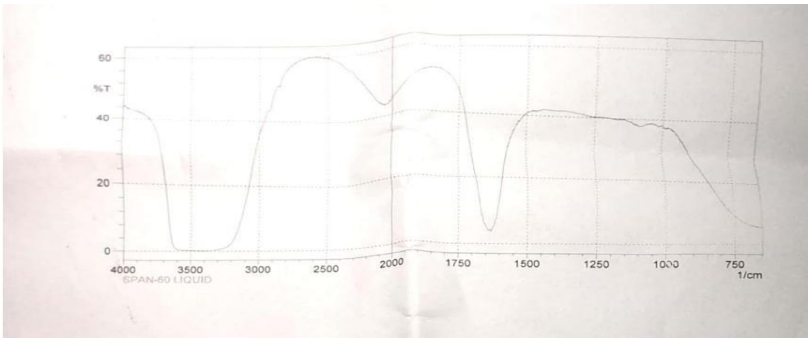

Fig 3. IR spectra of span-60 Solid

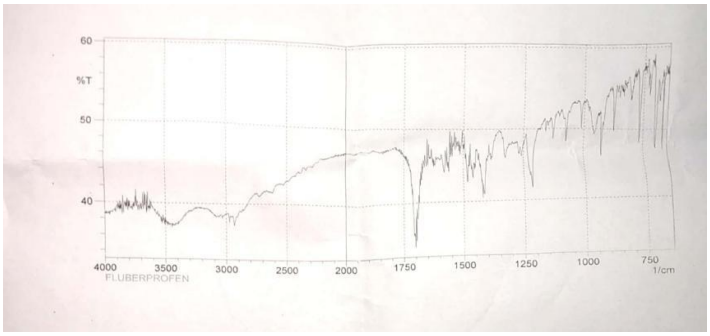

Fig. 4. IR spectra of span-60 liquid

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FORMULATION OF FLURBIPROFEN NIOSOMES

Composition of Niosomes

Table 6. Niosomes with varying Cholesterol: Surfactant Molar Ratio (Span 20)

Drug(mg)	Cholesterol(mg)	Span 20 (mg)	Solvent (Chloroform:Methanol) (2:1)
50	100	100	15
50	95	105	15
50	90	110	15
50	85	115	15

Table 7. Niosomes with varying Cholesterol: Surfactant Molar Ratio (span 40)

Drug(mg)	Cholesterol(mg)	Span 40 (mg)	Solvent (Chloroform:Methanol) (2:1)
50	100	100	15
50	95	105	15
50	90	110	15
50	85	115	15

Table 8. Niosomes with varying Cholesterol: Surfactant Molar Ratio (span 60)

Drug(mg)	Cholesterol(mg)	Span 60(mg)	Solvent(Chloroform:Methanol)(2:1)
50	100	100	15
50	95	105	15
50	90	110	15
50	85	115	15

Preparation of flurbiprofen niosomes[8]

Thin Film hydration method-

In this technique, cholesterol and span of different degree with different ratio were dissolved in chloroform and methanol, the drug was disintegrate in the solvent and the solvent was evaporated at a temperature of 60°C, using a rotary vacuum evaporator. The combination of a thin layer of cholesterol and surfactant was mixed in the round bottom flask.

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The aqueous phase of the phosphate buffer solution pH 7.4 (10 ml) was added to the round bottom flask at 60°C and stirred for about 15 minutes, which results in a fine dispersion of the mixture and theniosome is produced[16].

CHARACTERIZATIONS PARAMETERS[17-20]

Solubility

After Solubility analysis it was establish that Flurbiprofen drug was liberally soluble in acetone, methanol, phosphate buffer (pH 7.4).

Appearance

After the visual examination appearance of formulation is important parameter of ophthalmic drug delivery. All formulations were originate to be clear exposed in table.

pH

The pH values for all formulations have been established as acceptable in the 7.0-7.4 range as a printout. The pH was in a suitable range and therefore wouldn't cause any pain after the running of the formulations.

Particle size analysis

Particle size analysis was perform for the optimized formulation using the Malvern zeta sizer. The Zeta size of the F12 formulation was found 734nm.

Zeta potential

Zeta potential are performed for optimized formulation of Flurbiprofen loaded niosomes batch no. F12.

Drug Entrapment

The highest drug entrapment efficiency was 88.2% (F12) and lower drug entrapment efficiency. 75.71% was observed with the formulation (F3). When the concentration of the interval increases and the concentration of cholesterol decreases, the efficiency of trapping of the niosomal formulation increases. Section60 shows greater entrapment efficiency.

Drug content

Drug content formulations are revealed in table 3.1 among the niosomal formulations highest drug content was observed 89.34% in (F11) formulation and the lowest drug content was observed 65.02% in (F3) formulation. As there is increase in non-ionic surfactant concentration there is increase in drug content of niosomal formulations.

In-vitro drug release study

The release of the in-vitro formulation drug has been studied for all formulations. The medium consists of 900 ml of pH 7.4 buffered phosphate buffer at 50 rpm using paddle at 37° C ± 0.5° C. The drug liberate from a formulation is revealed in table 3.2-3.14.

In-vitro drug release kinetics

The drug release kinetics from that formulation was adapted in the Korsmeyer model and the R^2 value was fixed at 0.98.

Rheological studies

The viscosity of the *in-situ* embedded Niosomal gel is a vital factor in determining the abode time of the drug in the eye. Because of the viscosity of HPMC, the optimized in situ niosomal gel was fixed at 5.1 cps.

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Drug entrapment analysis

The entrapment of the drug from the optimized formulation was 88.2%.

Drug content analysis

The drug contented of the optimized formulation was 89.34%.

Accelerated stability studies for the optimized formulation

The optimized formulation (F11) was evaluated for refrigeration temperature stability studies. The result revealed that no changes in visual appearance, clarity, pH, viscosity, drug content and drug entrapment were determined at periodic intervals for each formulation found satisfactory.

Table 9. Accelerated stability studies for the optimized formulation

Solvent	λ_{max}	Absorbance	Concentration (µg/ml)	Amount(mg)
Methanol	244	0.345	28.02	28.02
sphate buffer7.4	245	0.201	15.02	15.02
Dichloromethane	246	0.205	04.05	04.05
Distilled water	250	0.121	11.04	11.04

RESULTS AND DISCUSSION

Pre-formulation study

Table 10. Pre-formulation Studies

S. N.	Parameter	Results
1	λ_{max}	
	Methanol	244
	Phosphate buffer pH7.4	243
2	R^2	
	Methanol	0.998
	Phosphate buffer pH7.4	0.999
3	**Solubility**	
	Methanol	Freely soluble
	Ethanol	Freely soluble
	Phosphate buffer pH7.4	Freely soluble

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Table 11. Determination of clarity and pH

Formulation	Visual appearance	pH (at 25^0C)			
		1st	2nd	3rd	pH (Mean±S.D)
F1	Clear	7.3	7.2	7.2	7.233
F2	Clear	7.2	7.1	7.3	7.200
F3	Clear	7.2	7.0	7.0	7.066
F4	Clear	7.1	7.2	7.2	7.166
F5	Clear	7.2	7.2	7.4	7.266
F6	Clear	7.3	7.3	7.1	7.233
F7	Clear	7.2	7.0	7.2	7.133
F8	Clear	7.1	7.2	7.2	7.166
F9	Clear	7.3	7.2	7.2	7.233
F10	Clear	7.4	7.4	7.3	7.366
F11	Clear	7.2	7.2	7.2	7.200
F12	Clear	7.0	7.0	7.0	7.033

Table 12. Determine of drug entrapment and drug content

Formulation	Percentage of Drug Entrapment	Percentage of Drug content
F1 (span20)	80.00	75.20
F2 (span20)	78.20	69.45
F3 (span20)	75.71	65.02
F4 (span20)	78.05	70.51
F5 (span40)	79.51	79.20
F6 (span40)	75.20	80.74
F7 (span40)	76.52	85.12
F8 (span40)	75.42	80.11
F9 (span60)	80.10	85.21
F10 (span60)	82.52	80.33
F11 (span60)	85.21	89.34
F12 (span60)	88.20	85.21

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Table 13. In-vitro Drug release study (F1-F12)

Time (hrs.)	Abs.	Conc.	1 ml	5 ml	900 ml	CDR	%CDR
0.5	0.085	0.791667	0.00079	0.0039583	0.712500	00.712500	01.096154
1.0	0.125	1.208333	0.00120	0.0060416	1.087500	01.800000	02.769231
2.0	0.211	2.104167	0.00210	0.0105208	1.893750	03.693750	05.682692
3.0	0.317	3.208333	0.00320	0.0160416	2.887500	06.581250	10.125000
4.0	0.437	4.458333	0.00445	0.0222916	4.012500	10.593750	16.298080
5.0	0.654	6.718750	0.00671	0.0335937	6.046875	16.640630	25.600960
6.0	0.132	1.281250	0.00128	0.0064062	1.153125	17.793750	27.375000
7.0	0.153	1.500000	0.00150	0.0075000	1.350000	19.143750	29.451920
8.0	0.187	1.854167	0.00185	0.0092708	1.668750	20.81250	32.01923
9.0	0.197	1.958333	0.00195	0.0097916	1.762500	22.57500	34.73077
10.0	0.210	2.093750	0.00209	0.0104687	1.884375	24.45938	37.62981
11.0	0.229	2.291667	0.00229	0.0114583	2.062500	26.52188	40.80288
12.0	0.267	2.687500	0.00268	0.0134375	2.418750	28.94063	44.52404

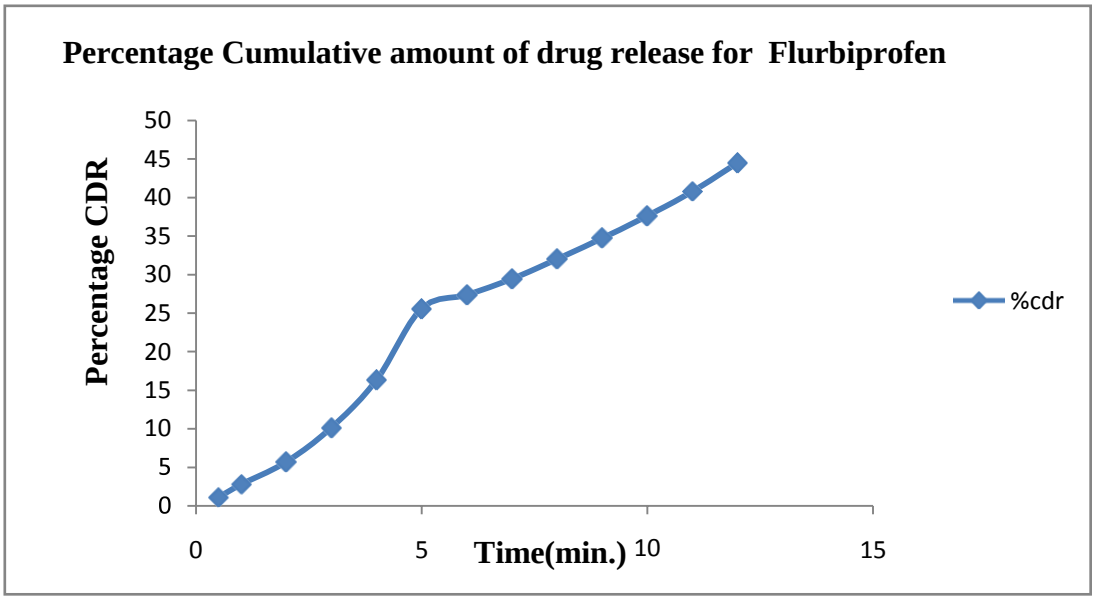

Fig. 5. Percentage Cumulative amount of drug release for Flurbiprofen

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Table 14. In-vitro release study of drug (F1) Formulation (span 20)

Time (hrs.)	Abs.	Conc.	1 ml	5 ml	900 ml	CDR	%CDR
0.5	0.111	1.062500	0.001065	0.0053125	0.95625	00.95625	01.471154
1.0	0.185	1.833333	0.001833	0.0091666	1.65000	02.60625	04.009615
2.0	0.345	3.500000	0.003500	0.0175000	3.15000	05.75625	08.855769
3.0	0.567	5.812500	0.005812	0.0290625	5.23125	10.98750	16.903850
4.0	0.921	9.500000	0.009500	0.0475000	8.55000	19.53750	30.057690
5.0	0.137	1.333333	0.001333	0.0066666	1.20000	20.73750	31.903850
6.0	0.152	1.489583	0.001489	0.0074479	1.34062	22.07810	33.966350
7.0	0.185	1.83333	0.00183	0.0091666	1.65000	23.72813	36.504810
8.0	0.201	2.00000	0.00200	0.0100000	1.80000	25.52813	39.274040
9.0	0.237	2.37500	0.00237	0.0118750	2.13750	27.66563	42.562500
10.0	0.268	2.69791	0.00269	0.0134895	2.42812	30.09375	46.298080
11.0	0.290	2.92708	0.00292	0.0146354	2.63437	32.72813	50.350960
12.0	0.300	3.03125	0.00303	0.0151562	2.72812	35.45625	54.548080

Table 15. In-vitro release study of drug (F2) Formulation (span 20)

Time (hrs)	Abs.	Conc.	1ml	5ml	900ml	CDR	%CDR
0.5	0.043	0.354167	0.000354	0.00177083	0.318750	00.31875	00.49038
1.0	0.162	1.593750	0.001593	0.00796875	1.434375	01.75312	02.69711
2.0	0.301	3.041667	0.003041	0.01520833	2.737500	04.49062	06.90865
3.0	0.598	6.135417	0.006135	0.03067708	5.521875	10.01250	15.40385
4.0	0.975	10.06250	0.010062	0.05031250	9.056250	19.06875	29.33654
5.0	0.125	1.208333	0.001208	0.00604166	1.087500	20.15625	31.00962
6.0	0.15	1.468750	0.001467	0.00734375	1.321875	21.47813	33.04327
7.0	0.175	1.729167	0.001791	0.00864583	1.556250	23.03438	35.43750
8.0	0.191	1.895833	0.001895	0.00947916	1.706250	24.74063	38.06250
9.0	0.201	2.001001	0.002124	0.01123400	1.812302	26.54063	40.83173
10.0	0.221	2.208333	0.002208	0.01104166	1.987500	28.52813	43.88942
11.0	0.261	2.625000	0.002625	0.01312500	2.362500	30.89063	47.52404
12.0	0.281	2.833333	0.002833	0.01416666	2.550000	33.44063	51.44712

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Table 16. In-vitro release study of drug (F3) Formulation (span 20)

Time (hrs)	Abs.	Conc.	1ml	5ml	900ml	CDR	%CDR
0.5	0.070	0.635417	0.00063541	0.003177083	0.571875	00.57187	00.879808
1.0	0.180	1.781250	0.00178125	0.008906250	1.603125	02.17510	03.346154
2.0	0.221	2.208333	0.00220833	0.011041667	1.987512	04.16251	06.403846
3.0	0.305	3.083333	0.00308333	0.015416667	2.775121	06.93751	10.673081
4.0	0.451	4.604167	0.00460416	0.023020833	4.143751	11.08125	17.048082
5.0	0.499	5.104167	0.00510416	0.025520833	4.593750	15.67510	24.115381
6.0	0.605	6.208333	0.00620833	0.031041667	5.587501	21.26251	32.711542
7.0	0.801	8.250012	0.00825022	0.041250021	7.425012	28.68751	44.134623
8.0	0.902	9.302083	0.00930083	0.046510417	8.371875	37.05938	57.014420
9.0	0.132	1.281250	0.00121250	0.006406250	1.153125	38.21251	58.788461
10.0	0.202	2.010417	0.00210417	0.010052083	1.809375	40.02188	61.572120
11.0	0.231	2.312501	0.00231250	0.011562501	2.081250	42.10313	64.774041
12.0	0.245	2.458333	0.00245833	0.012291667	2.212501	44.31563	68.177880

Table 17. In-vitro release study of drug (F4) Formulation (span 20)

Time (hrs)	Abs.	Conc.	1ml	5ml	900ml	CDR	%CDR
0.5	0.051	0.427083	0.000427083	0.002135417	0.384375	00.38437	00.59134
1.0	0.110	1.052083	0.001052083	0.005260417	0.946875	01.33125	02.04807
2.0	0.201	2.012120	0.002120123	0.011212002	1.812021	03.13125	04.81730
3.0	0.299	3.020833	0.003020833	0.015104167	2.718751	05.85120	09.12021
4.0	0.421	4.291667	0.004291667	0.021458333	3.862512	09.71250	14.94231
5.0	0.548	5.614583	0.005614583	0.028072917	5.053125	14.76563	22.71635
6.0	0.609	6.251200	0.006251251	0.031251205	5.625212	20.39063	31.37019
7.0	0.780	8.031250	0.008031250	0.040156250	7.228125	27.61875	42.49038
8.0	0.925	9.541667	0.009541667	0.047708333	8.587510	36.20625	55.70192
9.0	0.121	1.166667	0.001166667	0.005833333	1.051201	37.25625	57.31731
10.0	0.230	2.302083	0.002302083	0.011510417	2.071875	39.32813	60.50481
11.0	0.245	2.458333	0.002458333	0.012291667	2.212512	41.54063	63.90865
12.0	0.253	2.541667	0.002541667	0.012708333	2.287512	43.82813	67.42788

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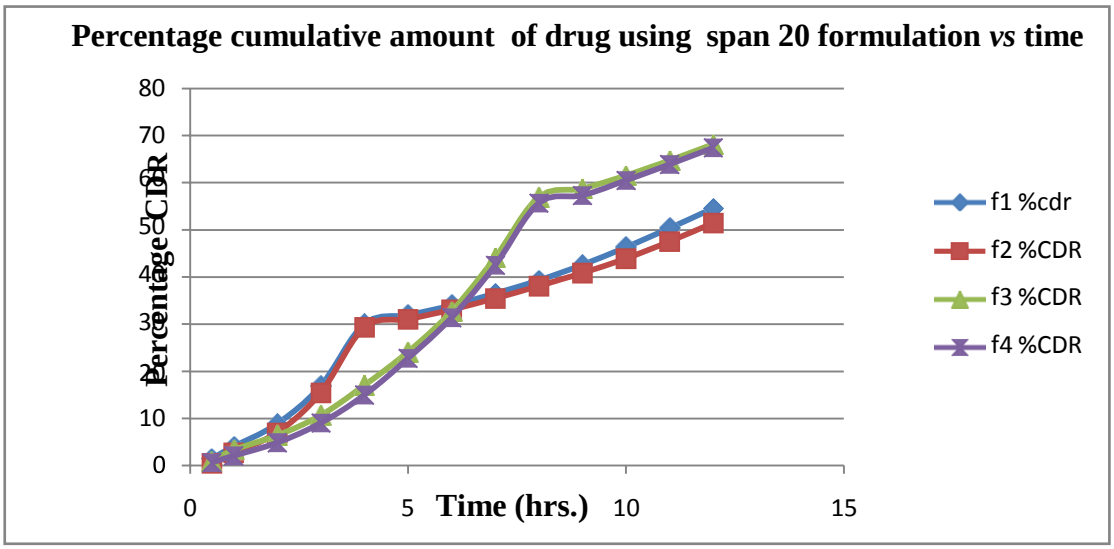

Fig. 6. Percentage cumulative amount of drug using span 20 formulation *vs* time

Table 18. In-vitro release study of drug (F5) Formulation (span 40)

Time (hrs)	Abs.	Conc.	1ml	5ml	900ml	CDR	%CDR
0.5	0.119	1.145833	0.001145833	0.005729167	1.03125	01.03125	01.586538
1.0	0.163	1.604167	0.001604167	0.008020833	1.44375	02.47501	03.807692
2.0	0.315	3.187501	0.003187510	0.015937510	2.86875	05.34375	08.221154
3.0	0.457	4.666667	0.004666667	0.023333333	4.21021	09.54375	14.682690
4.0	0.495	5.062501	0.005062511	0.025312501	4.55625	14.1012	21.692310
5.0	0.698	7.177083	0.007177083	0.035885417	6.45937	20.5593	31.629810
6.0	0.770	7.927083	0.007927083	0.039635417	7.13437	27.6937	42.605771
7.0	0.119	1.145833	0.001145833	0.005729167	1.03125	28.7250	44.192311
8.0	0.149	1.458333	0.001458333	0.007291667	1.31250	30.0375	46.211541
9.0	0.258	2.593750	0.002593750	0.012968751	2.33437	32.37188	49.802881
10.0	0.278	2.802083	0.002802083	0.014010417	2.52187	34.89375	53.682692
11.0	0.301	3.041667	0.003041667	0.015208333	2.73751	37.63125	57.894230
12.0	0.324	3.281250	0.003281250	0.016406250	2.95312	40.58438	62.437512

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Table 19. In-vitro release study of drug (F6) Formulation (span 40)

Time (hrs)	Abs.	Conc.	1ml	5ml	900ml	CDR	%CDR
0.5	0.610	6.156250	0.00615625	0.030781251	5.540625	05.54062	08.524038
1.0	0.120	0.947917	0.00094791	0.004739583	0.853125	06.39375	09.836538
2.0	0.257	2.583333	0.00258333	0.012916667	2.325102	08.71875	13.413460
3.0	0.384	3.906251	0.00390625	0.019531251	3.515625	12.23438	18.822121
4.0	0.577	5.916667	0.00591666	0.029583333	5.325120	17.55938	27.014421
5.0	0.748	7.697917	0.00769791	0.038489583	6.928125	24.48750	37.673081
6.0	0.911	9.395833	0.00939583	0.046979167	8.456251	32.94375	50.682691
7.0	0.107	1.020833	0.00102083	0.005104167	0.918750	33.86251	52.096152
8.0	0.137	1.333333	0.00133333	0.006666667	1.212012	35.06250	53.942312
9.0	0.196	1.947917	0.00194791	0.009739583	1.753125	36.81563	56.639420
10.0	0.140	1.364583	0.00136458	0.006822917	1.228125	38.04375	58.528851
11.0	0.280	2.822917	0.00282291	0.014114583	2.540625	40.58438	62.437501
12.0	0.321	3.251201	0.00325102	0.016251200	2.925120	43.50938	66.937511

Table 20. In-vitro release study of drug (F7) Formulation (span 40)

Time (hrs)	Abs.	Conc.	1ml	5ml	900ml	CDR	%CDR
0.5	0.166	1.635417	0.001635417	0.00817708	1.471875	01.47187	2.264423
1.0	0.327	3.312510	0.003312512	0.01656251	2.981250	04.45312	6.850962
2.0	0.385	3.916667	0.003916667	0.01958333	3.525102	07.97812	12.27404
3.0	0.627	6.437511	0.006437512	0.03218750	5.793751	13.77188	21.18751
4.0	0.410	4.072917	0.004072917	0.02036458	3.665625	17.43751	26.82692
5.0	0.107	1.020833	0.001020833	0.00510416	0.918750	18.35625	28.24038
6.0	0.138	1.343751	0.001343750	0.00671875	1.209375	19.56563	30.10096
7.0	0.160	1.572917	0.001572917	0.00786458	1.415625	20.98125	32.27885
8.0	0.110	1.052083	0.001052083	0.00526041	0.946875	21.92813	33.73558
9.0	0.217	2.166667	0.002166667	0.01083333	1.951201	23.87813	36.73558
10.0	0.259	2.604167	0.002604167	0.01302083	2.343751	26.22188	40.34135
11.0	0.190	1.885417	0.001885417	0.00942708	1.696875	27.91875	42.95192
12.0	0.318	3.218750	0.003218750	0.01609375	2.896875	30.81563	47.40865

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Table 21. In-vitro release study of drug (F8) Formulation (span 40)

Time (hrs)	Abs.	Conc.	1ml	5ml	900ml	CDR	%CDR
0.5	0.110	0.947917	0.00094791	0.00473958	0.853125	00.85312	01.31250
1.0	0.180	1.781251	0.00178125	0.00890625	1.603125	02.45625	03.77884
2.0	0.665	6.833333	0.00683333	0.03416666	6.151201	08.60625	13.24038
3.0	0.837	8.625012	0.00862501	0.04312512	7.762512	16.36875	25.18269
4.0	0.961	9.906251	0.00990625	0.04953125	8.915625	25.28438	38.89904
5.0	0.105	1.120121	0.00112012	0.00501210	0.912012	26.18438	40.28365
6.0	0.145	1.416667	0.00141666	0.00708333	1.275145	27.45938	42.24519
7.0	0.194	1.927083	0.00192708	0.00963541	1.734375	29.19375	44.91346
8.0	0.234	2.343751	0.00234375	0.01171875	2.109375	31.30313	48.15865
9.0	0.201	1.989583	0.00198958	0.00994791	1.790625	33.09375	50.91346
10.0	0.279	2.812512	0.00281251	0.01406251	2.531250	35.62512	54.80769
11.0	0.316	3.197917	0.00319791	0.01598958	2.878125	38.50313	59.23558
12.0	0.321	3.250122	0.00325120	0.01625121	2.925124	41.42813	63.73558

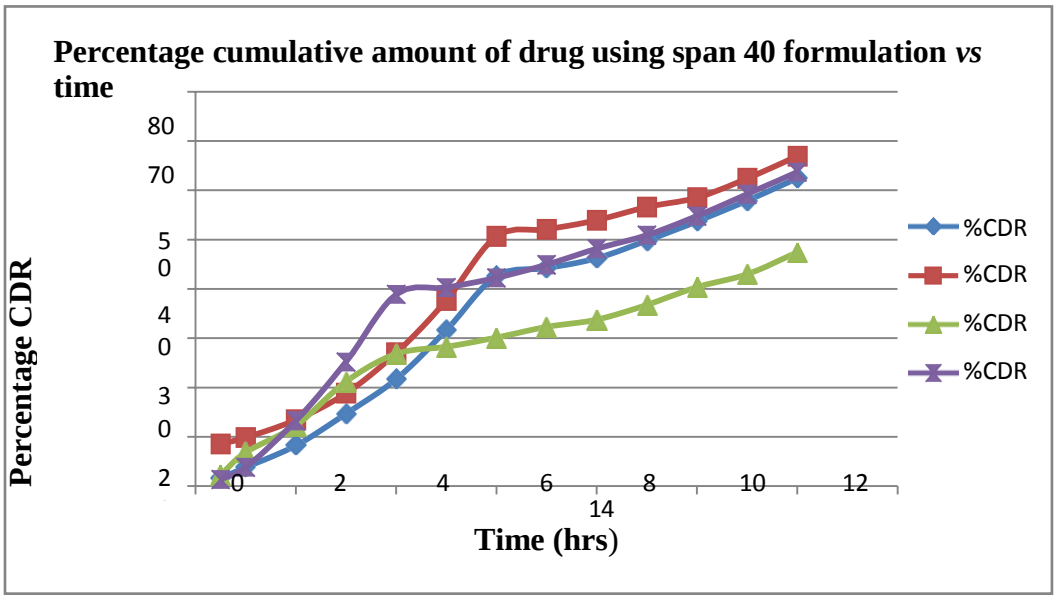

Fig. 7. Percentage cumulative amount of drug using span 40 formulation *vs* time

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Table 22. In-vitro release study of drug (F9) Formulation (span 60)

Time (hrs)	Abs.	Conc.	1ml	5ml	900ml	CDR	%CDR
0.5	0.029	0.208333	0.00208333	0.001041667	0.187502	00.18750	0.288462
1.0	0.105	1.120121	0.01012121	0.005012011	0.912110	01.08750	1.673077
2.0	0.180	1.781252	0.01781251	0.008906251	1.603125	02.69062	4.139423
3.0	0.296	2.989583	0.02989583	0.014947917	2.690625	05.38125	8.278846
4.0	0.436	4.447917	0.04447917	0.022239583	4.003125	09.38437	14.43751
5.0	0.658	6.760417	0.06760417	0.033802083	6.084375	15.46875	23.79808
6.0	0.989	10.20833	0.01020833	0.051041667	9.187512	24.65625	37.93269
7.0	0.121	1.166667	0.01166667	0.005833333	1.050211	25.70625	39.54808
8.0	0.180	1.781251	0.01781251	0.008906250	1.603125	27.30938	42.01442
9.0	0.228	2.281252	0.02281251	0.011406250	2.053125	29.36250	45.17308
10.0	0.201	1.989583	0.01989583	0.009947917	1.790625	31.15313	47.92788
11.0	0.317	3.208333	0.03208333	0.016041667	2.887522	34.04063	52.37019
12.0	0.339	3.437512	0.03437501	0.017187500	3.093751	37.13438	57.12981

Table 23. In-vitro release study of drug (F10) Formulation (span 60)

Time (hrs)	Abs.	Conc.	1ml	5ml	900ml	CDR	%CDR
0.5	0.057	0.51021	0.00051201	0.00251021	0.452121	00.45122	00.69230
1.0	0.910	9.28125	0.00928125	0.04640625	8.353125	08.80312	13.54327
2.0	0.325	3.29166	0.00329166	0.01645833	2.962512	11.76563	18.10096
3.0	0.609	6.25102	0.00625102	0.03125121	5.625121	17.39063	26.75481
4.0	0.878	9.05208	0.00905208	0.04526041	8.146875	25.53751	39.28846
5.0	0.106	1.01041	0.00101041	0.00505208	0.909375	26.44688	40.68751
6.0	0.155	1.52083	0.00152083	0.00760416	1.368751	27.81563	42.79327
7.0	0.194	1.92708	0.00192708	0.00963541	1.734375	29.55021	45.46154
8.0	0.246	2.46875	0.00246875	0.01234375	2.221875	31.77188	48.87981
9.0	0.253	2.54166	0.00254166	0.01270833	2.287512	34.05938	52.39904
10.0	0.262	2.63541	0.00263541	0.01317708	2.371875	36.43125	56.04808
11.0	0.283	2.85416	0.00285416	0.01427083	2.568751	39.01212	60.12012
12.0	0.336	3.40625	0.00340625	0.01703125	3.065625	42.06563	64.71635

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Table 24. In-vitro release study of drug (F11) Formulation (span 60)

Time (hrs)	Abs.	Conc.	1ml	5ml	900ml	CDR	%CDR
0.5	0.047	0.395833	0.03958331	0.01979167	0.356251	00.35625	00.54807
1.0	0.124	1.197917	0.01197917	0.05989583	1.078125	01.43437	02.20673
2.0	0.221	2.208333	0.02208333	0.01104166	1.987512	03.42187	05.26442
3.0	0.458	4.677083	0.04677083	0.02338541	4.209375	07.63125	11.74038
4.0	0.849	8.751201	0.08751221	0.04375121	7.875102	15.50625	23.85577
5.0	0.127	1.229167	0.01229167	0.06145833	1.106251	16.61251	25.55769
6.0	0.140	1.364583	0.01364583	0.06822917	1.228125	17.84063	27.44712
7.0	0.179	1.770833	0.01770833	0.08854167	1.593751	19.43438	29.89904
8.0	0.207	2.062511	0.02062512	0.01031250	1.856252	21.29063	32.75481
9.0	0.268	2.697917	0.02697917	0.01348958	2.428125	23.71875	36.49038
10.0	0.274	2.760417	0.02760417	0.01380208	2.484375	26.20313	40.31251
11.0	0.305	3.083333	0.03083333	0.01541666	2.775105	28.97813	44.58173
12.0	0.337	3.416667	0.03416667	0.01708333	3.075012	32.05313	49.31250

Table 25. In-vitro release study of drug (F12) Formulation (span 60)

Time (hrs)	Abs.	Conc.	1ml	5ml	900ml	CDR	%CDR
0.5	0.107	1.020833	0.01020833	0.05104167	0.918751	00.91875	01.41346
1.0	0.239	2.395833	0.02395833	0.01197916	2.156250	03.07512	04.73076
2.0	0.360	3.656250	0.03656251	0.01828125	3.290625	06.36562	09.79326
3.0	0.536	5.489583	0.05489583	0.02744791	4.940625	11.30625	17.39423
4.0	0.630	6.468750	0.06468751	0.03234375	5.821875	17.12813	26.35096
5.0	0.936	9.656250	0.09656250	0.04828125	8.690625	25.81875	39.72115
6.0	0.127	1.229167	0.01229167	0.06145833	1.106251	26.92512	41.42308
7.0	0.136	1.322917	0.01322917	0.06614583	1.190625	28.11563	43.25481
8.0	0.177	1.751021	0.01751022	0.08752102	1.575212	29.69063	45.67788
9.0	0.222	2.218750	0.02218751	0.01109375	1.996875	31.68750	48.75120
10.0	0.256	2.572917	0.02572917	0.01286458	2.315625	34.00313	52.31251
11.0	0.288	2.906251	0.02906251	0.01453125	2.615625	36.61875	56.33654
12.0	0.318	3.218751	0.03218750	0.01609375	2.896875	39.51563	60.79327

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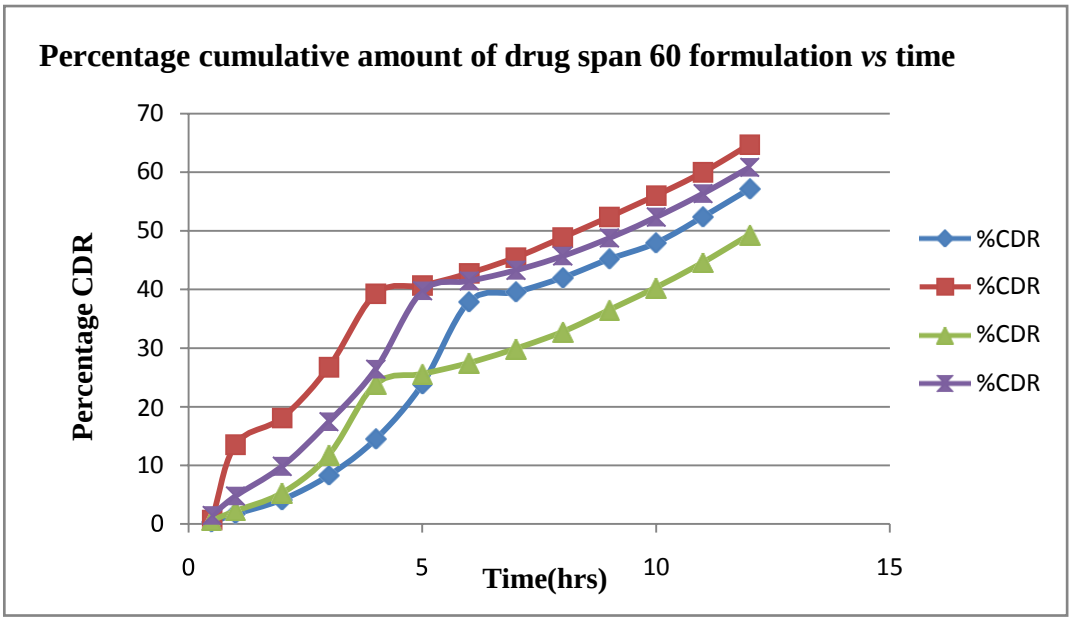

Fig. 8. Percentage cumulative amount of drug using span 60 formulation *vs* time

Table 26. Drug content of different formulation of gel

Formulation	F9	F10	F11	F12
Drug content	79.83	83.12	90.31	83.22

Table 27. Evaluation of optimized Niosomal *in-situ* gel

Formulation	Clarity	pH	Viscosity(cps)	%Drugcontent	%Drug Entrapped
F11 *in-situ* gel	Turbid	7.2	5.2	90.30	75.05

Table 28. In-vitro study of optimized formulation gel (F9)

Time (hrs)	Abs.	Conc.	1ml	5ml	900ml	CDR	%CDR
1	0.061	0.531251	0.000531251	0.002656251	0.478125	00.47812	00.73557
2	0.981	10.11458	0.010114583	0.050572917	9.103125	09.58125	14.74031

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3	0.152	1.489583	0.001489583	0.007447917	1.340625	10.92188	16.80288
4	0.207	2.062510	0.002062510	0.010312510	1.856251	12.77813	19.65865
5	0.171	1.677083	0.001677083	0.008385417	1.509375	14.28751	21.98077
6	0.254	2.552083	0.002552083	0.012760417	2.296875	16.58438	25.51442
7	0.272	2.739583	0.002739583	0.013697917	2.465625	19.05120	29.30769
8	0.329	3.333333	0.003333333	0.016666667	3.102120	22.05120	33.92308
9	0.388	3.947917	0.003947917	0.019739583	3.553125	25.60313	39.38942
10	0.391	3.968751	0.003968751	0.019843751	3.571875	29.17502	44.88462
11	0.461	4.708333	0.004708333	0.023541667	4.237512	33.41250	51.40385
12	0.517	5.291667	0.005291667	0.026458333	4.762512	38.17501	58.73077

Table 29. In-vitro study of optimized formulation gel (F10)

Time (hrs)	Abs.	Conc.	1ml	5ml	900ml	CDR	%CDR
1	0.068	0.614583	0.0614583	0.03072917	0.553125	00.55312	00.85096
2	0.105	1.012100	0.0101212	0.05120121	0.900212	01.45312	02.23557
3	0.137	1.333333	0.0133333	0.06666667	1.200111	02.65312	04.08173
4	0.178	1.760417	0.0176041	0.08802083	1.584375	04.23750	06.51923
5	0.206	2.052083	0.0205208	0.01020417	1.846875	06.08437	09.36057
6	0.229	2.291667	0.0229166	0.01148333	2.062500	08.14687	12.53365
7	0.268	2.697917	0.0269791	0.01349583	2.428125	10.57500	16.26923
8	0.304	3.072917	0.0307291	0.01534583	2.765625	13.34063	20.52404
9	0.318	3.218750	0.0321875	0.01603750	2.896875	16.23750	24.98077
10	0.366	3.718750	0.0371875	0.01853750	3.346875	19.58438	30.12981
11	0.390	3.968750	0.0396875	0.01983750	3.571875	23.15625	35.62500
12	0.465	4.750210	0.0475002	0.02370012	4.275012	27.43125	42.20192

Table 30. In-vitro study of optimized formulation gel (F11)

Time (hrs)	Abs.	Conc.	1ml	5ml	900ml	CDR	%CDR
1	0.040	00.32291	0.0322917	0.01614583	0.290625	00.29062	00.44711
2	0.980	10.11458	0.0101458	0.05052917	9.103125	09.39375	14.45191
3	0.136	1.322917	0.0132291	0.06614583	1.190625	10.58438	16.28365
4	0.200	1.989583	0.0198958	0.09947917	1.790625	12.37500	19.03846
5	0.231	2.312500	0.0231250	0.01562500	2.081250	14.45625	22.24038
6	0.260	2.614583	0.0261458	0.01072917	2.353125	16.80938	25.86058
7	0.285	2.875000	0.0287500	0.01475000	2.587500	19.39688	29.84135

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8	0.313	3.166667	0.0316666	0.01533333	2.850110	22.24688	34.22596
9	0.340	3.447917	0.0344791	0.01739583	3.103125	25.35010	39.01200
10	0.360	3.656250	0.0365625	0.01821250	3.290625	28.64063	44.06250
11	0.404	4.114583	0.0411458	0.02052917	3.703125	32.34375	49.75962
12	0.439	4.479167	0.0447916	0.02395833	4.031250	36.37500	55.96154

Table 31. In-vitro study of optimized formulation gel (F12)

Time (hrs)	Abs.	Conc.	1ml	5ml	900ml	CDR	%CDR
1	0.043	0.354167	0.0354167	0.01770833	0.318750	00.31875	00.49038
2	0.067	0.604167	0.0604167	0.03020833	0.543750	00.86250	01.32692
3	0.980	10.11458	0.0114583	0.05052917	9.103125	09.96562	15.33173
4	0.134	1.302083	0.0102083	0.06510417	1.171875	11.13750	17.13462
5	0.183	1.812500	0.0182500	0.09062500	1.631250	12.76875	19.64423
6	0.265	2.666667	0.0266667	0.01333333	2.400000	15.16875	23.33654
7	0.283	2.854167	0.0284167	0.01470833	2.568750	17.73750	27.28846
8	0.100	0.947917	0.0947917	0.04739583	0.853125	18.59063	28.60096
9	0.307	3.104167	0.0314167	0.01520833	2.793750	21.38438	32.89904
10	0.333	3.375000	0.0335000	0.01675000	3.037500	24.42188	37.57212
11	0.396	4.031250	0.0401250	0.02156250	3.628125	28.05000	43.15385
12	0.431	4.395833	0.0439583	0.02179167	3.956250	32.00625	49.24038

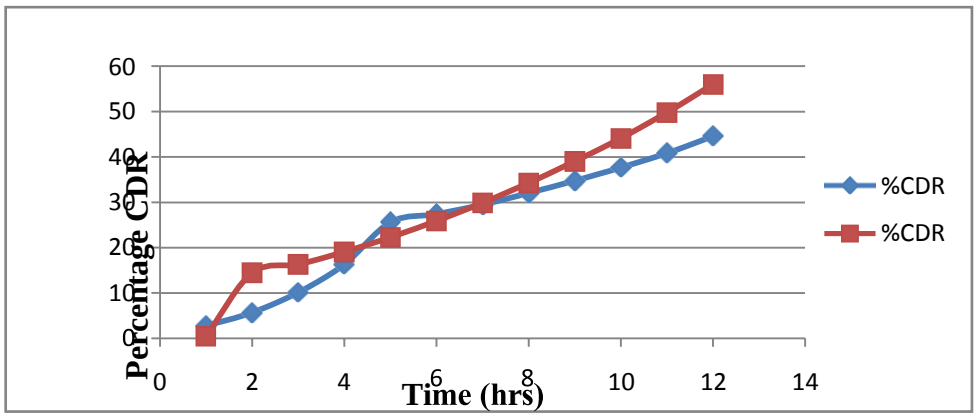

Fig. 9. In-vitro drug release of gel pure drug and optimized formulation F12

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Table 32. Accelerated stability study of optimized formulation F12

Parameter	15 days	30 days	45 days
pH	07.20	07.20	07.30
Drug entrapment	85.00	83.38	83.20
Viscosity	04.34	04.28	04.11
Drug content	93.21	93.03	92.98

Table 33. In-vitro drug release of formulation F12

Time(min.)	Log time	Time ½	% cdr	Log% cdr	% cumulative drug remaining	Log % cumulative drug remaining
0	0	0	0	0	100	2
030	1.477121	05.47722	01.41346	0.150284	98.586538	1.993817
060	1.778151	07.74596	04.73076	0.674931	95.269231	1.978952
120	2.079181	10.95445	09.79326	0.990927	90.206731	1.955238
180	2.255273	13.41641	17.39423	1.240405	82.605770	1.917010
240	2.380211	15.49193	26.35096	1.420796	73.649040	1.867167
300	2.477121	17.32051	39.72115	1.599021	60.278850	1.780164
360	2.556303	18.97367	41.42308	1.617242	58.576920	1.737009
420	2.623249	20.49390	43.25481	1.636034	56.745190	1.753929
480	2.681241	21.90890	45.67788	1.659705	54.322120	1.734976
540	2.732394	23.23790	48.75000	1.687974	51.250000	1.709693
600	2.778151	24.49490	52.31250	1.718605	47.687500	1.678404
660	2.819544	25.69047	56.33654	1.750790	43.663460	1.640118
720	2.857332	26.83282	60.79327	1.783855	39.206730	1.593360

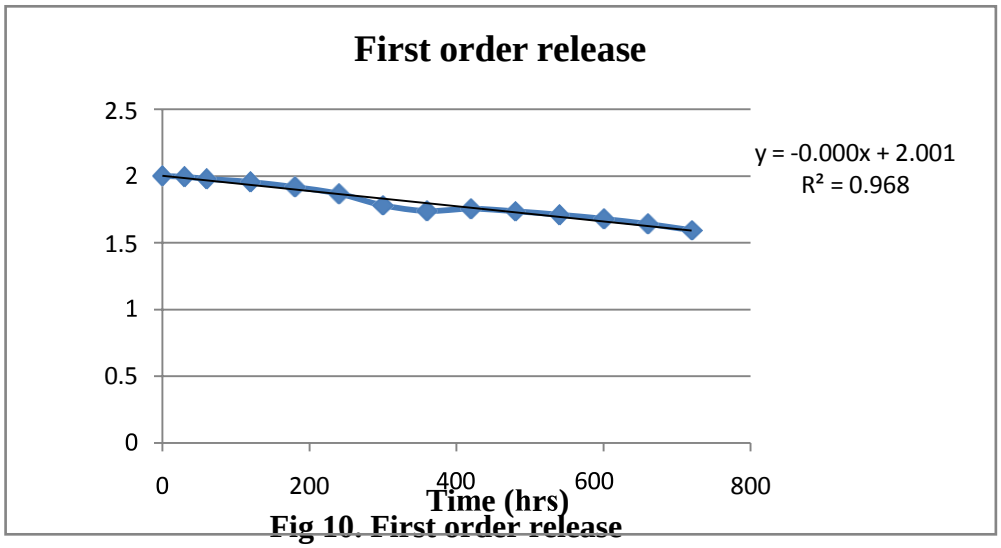

Fig 10. First order release

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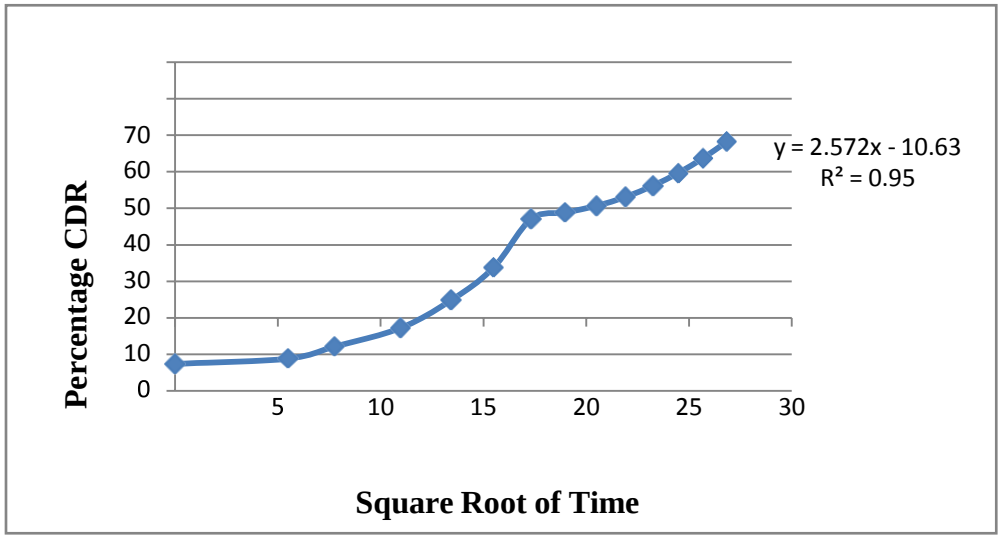

Fig 11. Higuchi model

Table 34. Release pattern of Flurbiprofen niosomes

Zero orderkinetics	First order kinetics	Higuchi model	Korsmeyer-peppasmodel	
R^2	R^2	R^2	R^2	N
0.952	0.968	0.951	0.870	0.935

Ocular efficacy is directly related to ocular bioavailability, which could be improved by increasing corneal drug penetration and prolonging the cessation time of the precorneal drug. A major increase in the precorneal abode time of the drug can be achieved and, consequently, better bioavailability through the use of administration systems based upon theconcepts of *in-situ* Niosomal gel. Therefore, the development of Niosomal *in-situ* eye gel has an advantage over other forms of ophthalmic dosage (eye drops, suspensions, ointments) to increase penetration and bioavailability of the cornea.

- In *in-situ* ophthalmological gel was subjected to preliminary assessment, such as the graphic appearance, clarity, pH and drug content. The whole formulation was clear.
- Using a methanol and phosphate buffer (pH 7.4), a simple spectrophotometric process was developed for the opinion of Flurbiprofen. Absorption maxima by UV- spectrophotometer were obtained at 244 nm, 244 nm, 243 nm, respectively, which follow the beer's-Lambert law.
- The infra-red spectra of the uncontaminated Flurbiprofen drug and the drug with non- ionic surfactant show characteristic absorption peaks. All typical Flurbiprofen peaks were present in the spectra, which is a compatibility with the drug and non-ionic surfactant. It does not show an important possibility in the drug.
- The melting point of Flurbiprofen was established at 117^0c as reported in the literature, indicating the clarity of the drug sample.

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- Flurbiprofen is originally freely soluble in methanol, ethanol, chloroform. It was also soluble in the phosphate buffer (pH 7.4).
- For all batches, the pH value is acceptable in the series of 7.0 to 7.4. Therefore, they wouldn't cause any pain after the administration of the formulations.
- Among the Niosomes formulations show lowest drug content (75.2%) was observed with formulation F7 (span 20), whereas formulation F12 (span 60) showed highest drug content (89.34%).
- Among the formulations Niosome efficiency was observed lower entrapment (78.05%) with formulation F4 (span20) and F12 showed a greater efficiency of entrapment (88.2%) span 60 included.
- They were corneal permeability *ex-vivo* Niosomes loaded with Flurbiprofen was evaluated comparatively with pure drug (Flurbiprofen) using isolated goat cornea, shows formulation increased corneal permeability of Flurbiprofen when supplemented with puredrug.

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CONFLICT OF INTEREST
Authors declared for none conflict of interest.

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