

Formulation, Development and Evaluation of Nano Ethosomal Gel of Tramadol Hydrochloride

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Abstract

Topical administration of drugs is better for local action and the efficiency of the topically administered drug is increased with Liposome, proliposomes and ethosomes. Tramadol HCl has used the local anaesthetic. Ethosomes were formulated using soya lecithin, cholesterol, ethanol and purified water using ultra shear homogenizer. Ethosomes were evaluated for vesicle size, shape, optical microscopy and *in-vitro* release study. FS05 and FC05 have better drug release profile than the other formulation. The ethosomes were entrapped in gel matrix of carbopol 980 in different concentration 0.75%, 1.00%, 1.25% and 1.50% w/w. The formulated gel formulation were evaluated with parameter drug content, pH, viscosity, spreadability, *in-vitro* release test, and *ex-vivo* study. The formulation FS05 C03 and FC05 C03 have better *in-vitro* and *ex-vivo* drug release profile which contains Carbopol 980 concentration 1.25%w/w. Both the formulation FS05 C03 and FC05 C03 were stable for 3 Months at room temperature and accelerated storage condition. The 3 M stability sample of FS05 C03 has maintained the drug release profile but the drug release profile of formulation FC05 C03 has dropped significantly.

Keywords: Tramadol hydrochloride; Ethosomes; Liposomes; Gel formulation

Introduction

Ethosomes are innovative nanovesicles containing the drug in a matrix of lipids, ethanol and water. The ethosomes are soft and a highly flexible vesicle efficiently penetrates through the skin and increases the drug delivery of drug molecules. Ethosomes are elastic vesicles made up of Phospholipids containing 20-45% ethanol. Ethanol also acts as a penetration enhancer by dissolving the skin lipids. The ethosomes overcomes the disadvantages of Liposomes and proliposomes such as less stability, scalability issues, leakage of drugs, fusion of vesicles and breaking of vesicles. Ethanol is a well-known permeation enhancer. Ethosomes are highly flexible which permits the elastic vesicles to squeeze themselves among the skin pores. Ethanol gives the net negative charge on the surface of ethosome vesicles due to which aggregation is avoided because of electrostatic repulsion. Ethosomes are much more stable than the Liposomes and proliposomes. Topically administered ethosomes increases the residence time of the drug molecule in the different layers of skin such as stratum corneum, epidermis and reduces the systemic absorption. Because of all these properties, ethosomes get easily permeated in the deeper layer of skin and circulation. Ethanol in deeper layers of skin leads to disruption of the skin which increases the lipid fluidity that allows enhanced permeation of drug molecule through the skin. Ethosomes fuses with the skin lipids to release the drug into the deeper layers of skin [1-4].

Tramadol HCl is an opioid analgesic which is used in the treatment of severe and chronic pain. Tramadol HCl is prescribed 3-4 times a day. The frequent dosing of Tramadol HCl leads to the increased incidence of side effects, non-compliance and development of tolerance especially in long-term used like osteoarthritis, arthritis, post-surgical pains etc. So, to mitigate the disadvantages associated with oral therapy, topical ethosomes to increase the better penetration Tramadol HCl [5,6].

Material and Methods

Material

Tramadol HCl (Glenmark), Soya Lecithin (Lipoids), Cholesterol (Qualigens), Ethanol (Rankem), Propylene glycol (BASF). Rests of the

reagents are used of Analytical reagent (AR) grade.

Method

Preparation of ethosomes:

Step 1: Solution A: Soya lecithin or Cholesterol were added in ethanol under stirring and continue stirring until it dissolves completely.

Step 2: Solution B: Tramadol HCl is added in of the remaining quantity of ethanol under stirring at room temperature and stirring is continued at room temperature until it forms the clear solution.

Step 3: Add Solution A to Solution B under ultra-shear homogenization and continue ultra-shear homogenization at speed 6000 rpm for 30 min.

Step 4: Add purified water under ultra-shear homogenization drop wise in the centre of the container. The dispersion is further homogenized for 15 min at room temperature with homogenization speed 6000 rpm.

Step 5: Formulated Ethosomes forms the cloudy homogenous liquid. The Ethosomes were stored at temperature $4^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for further evaluation (Table 1) [3,7-9].

Preparation of gel:

Step 1: Ethosomes equivalent to 1% of Tramadol HCl is calculated.

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Step 2: Dissolve Disodium EDTA under stirring in purified water. Carbopol 980 is added under stirring and allows hydrating under stirring for 60 min.

Step 3: Ethosomes are dispersed in the hydrated Carbopol 980 slurry under stirring and stirring continued for 30 min.

Step 4: pH of the formulation is adjusted to 4.30 to 4.70, a thick gel is formed (Table 2) [10,11].

Evaluation of ethosomes:

Vesicles shape: Shape and Size of ethosomes were measured by the digital motic microscope. Ethosomes are dispersed in Mineral oil. Ethosomes dispersion is placed on the glass slide and focused under 100x object lens. The image was snapped by digital motic microscope using three different fields [12-14].

Vesicles size: Formulated ethosomes were analyzed by Malvern Sizer for vesicle size. Ethosomes were dispersed in purified water and the dispersion was placed in a clear disposable zeta cell for 90 seconds. The reading was taken in triplicate.

Zeta potential: Zeta potential of ethosomes was measured using laser dropper anemometry using Zetasizer. Ethosomes were dispersed in purified water. The dispersion of ethosomes was placed in the electrophoretic cell where the potential of 150 mV was established. The analysis is performed in triplicate.

Optical microscopy: The Ethosomes are diluted with Liquid paraffin oil and mounted on glass slides. The dispersion is fixed with cover slip on the glass slide. The Microscopic examination is conducted using Motic Digital Microscope under 40X object lens. The 500 particles were calculated from different field and mean and a median is calculated.

Drug entrapment efficacy: Entrapment efficacy of ethosomes

was performed by Ultra-centrifugation method. The indirect method is used to determine entrapment efficiency by measuring the untrapped drug. The ethosomes have subjected the dispersion for ultracentrifugation (Remi) at 24000 rpm for 60 min. The clear supernatant layer is removed. The supernatant layer and sediment were analyzed for Tramadol HCl content by UV Spectrophotometer at 271 nm. Each sample was prepared in duplicate [15,16].

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug in supernatant and sediment}} \times 100$$

In-vitro drug release for ethosomes: Ethosomes were spread on egg membrane equivalent amount 100 mg of drug uniformly. This egg membrane is mounted on the donor compartment of Franz diffusion cell. The receptor compartment is filled with 24 ml of pH 7.4 Phosphate buffer. 1 ml Samples are withdrawn at specific time points 15 min, 30 min, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr, 6 hr, 8 hr, 10 hr and 12 hr. To maintain the sink condition at each time point replaces the withdrawn sample amount with fresh buffer solution. The study is carried out for 12 h [17-19].

Evaluation of gel:

Drug content: Gel formulations were evaluated for drug content (assay). The drug is extracted from the gel in the solvent mixture of methanol and water under sonication. The sample was analyzed by UV-spectrophotometer [20,21].

pH: pH of the formulation is determined by using direct method. 10 g of the sample was taken in glass vial and electrode is dipped in sample bulk. The stable readings were noted [22,23].

Viscosity: Viscosity is determined using a Brookfield Cone and Plate Viscometer Model Cap 2000+. Sample approximately 100 mg was kept on the plate and set method of 100 rpm for 1 min is run [24,25].

Spreadability: The spreadability study was performed using a Brookfield Texture Analyzer. The sample is filled in the female cone and spread properly. Run the test at set parameters [25,26].

In-vitro drug release: Gel formulation was spread on egg membrane equivalent amount 100 mg drug uniformly. This egg membrane is mounted on the donor compartment of Franz diffusion cell. The receptor compartment is filled with 24 ml of pH 7.4 Phosphate buffer. 1 ml Samples are withdrawn at specific time points 15 min, 30 min, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr, 6 hr, 8 hr, 10 hr and 12 hr. To maintain the sink condition at each time point replaces the withdrawn sample amount with fresh buffer solution. The study is carried out for 12 hrs [27,28].

Ex-vivo drug release: Based on the viscosity, spreadability, consistency and stability data of all the formulation, formulation FS05 C03 and FC05 C03 were selected for the *ex-vivo* drug release studies. Freshly excised ear skin of Porcine was soaked in pH 7.4 Buffer for 12 hr. Gel formulation was spread on skin equivalent amount 100 mg drug uniformly. This skin is mounted on the donor compartment of

Formulation Code	Concentration of individual Ingredients				
	Soya lecithin (%)	Cholesterol (%)	Ethanol (%)	Drug (%)	Water (%)
FS 01	0.5	-	50	0.5	49
FS 02	0.6	-	50	0.5	48.9
FS 03	0.8	-	50	0.5	48.7
FS 04	1	-	50	0.5	48.5
FS 05	0.8	-	60	0.5	38.7
FS 06	1	-	60	0.5	38.5
FSC 01	0.6	0.2	60	0.5	38.75
FC 01	-	0.3	50	0.5	49.4
FC 02	-	0.4	50	0.5	49.35
FC 03	-	0.5	50	0.5	49.3
FC 04	-	0.6	50	0.5	49.25
FC 05	-	0.5	60	0.5	39.2
FC 06	-	0.6	60	0.5	39.1

Table 1: Formulation Composition for Ethosomes.

Ingredients	Formulation Code							
	FS05 C01	FS05 C02	FS05 C03	FS05 C04	FC05 C01	FC05 C02	FC05 C03	FC05 C04
Ethosomes Equivalent 1%	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Disodium EDTA	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Carbopol 980	0.75	1.00	1.25	1.50	0.75	1.00	1.25	1.50
Triethanolamine	QS to adjust pH 4.40 to 4.60							
Purified Water	QS to 100	QS to 100	QS to 100	QS to 100	QS to 100	QS to 100	QS to 100	QS to 100

Table 2: Formulation Composition for gel formulation.

Franz diffusion cell. The receptor compartment is filled with 24 ml of pH 7.4 Phosphate buffer. 1 ml Samples are withdrawn at specific time points 15 min, 30 min, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr, 6 hr, 8 hr, 10 hr, 12 hr, 16 hr and 24 hr. To maintain the sink condition at each time point replaces the withdrawn sample amount with fresh buffer solution. The study is carried out for 24 hrs. Each sample solutions were prepared in duplicate [29-33].

Stability study: The stability study was conducted in the storage condition $40^{\circ}\text{C} \pm 2^{\circ}\text{C}/75\% \pm 5\% \text{RH}$ and $25^{\circ}\text{C} \pm 2^{\circ}\text{C}/60\% \pm 5\% \text{RH}$ for 3 Months. The stability samples were evaluated for description, pH, Viscosity, drug content and *in-vitro* drug release [34-36].

Results and Discussion

Vesicles shape

All the formulation has shown the spherical shape of ethosomes. Results are tabulated in Table 3.

Vesicles size

Vesicles sizes of the formulated ethosomes are in the range of 939 nm to 1012 nm. Results are tabulated in Table 3. All the formulations show vesicles sizes in the nano range. Nano ethosomes can be formulated with this composition.

Zeta potential

Zeta Potential of the formulation was found in the range -40 to -53 mV. Results are tabulated in Table 3.

Optical microscopy

All the Ethosomes sizes are found in mean globule sized $0.94 \mu\text{m}$ to $0.99 \mu\text{m}$ through optical microscopy. Results are tabulated in Table 3. All ethosomes are in spherical in nature.

Drug entrapment efficiency

Drug Entrapment efficiency was measured by both the Direct and indirect method. Both the method has shown approximately similar results. T Results are tabulated in Table 4. The highest entrapment efficiency found with FS 04, FS 05, FS 06, FC 04, FC 05 and FC 06. The concentration of Soya lecithin in 0.8% to 1% and Cholesterol 0.5% to 0.6% has the highest drug entrapment efficiency.

Formulation Code	Vesicle Shape	Vesicle Size (nm)	Zeta Potential (mV)	Optical Microscopy
FS 01	Spherical	975	-47.62	0.95
FS 02	Spherical	963	-45.12	0.97
FS 03	Spherical	971	-52.64	0.95
FS 04	Spherical	981	-42.17	0.97
FS 05	Spherical	991	-40.49	0.99
FS 06	Spherical	952	-46.45	0.96
FSC 01	Spherical	1012	-43.16	0.98
FC 01	Spherical	974	-45.91	0.95
FC 02	Spherical	962	-50.45	0.95
FC 03	Spherical	943	-51.98	0.96
FC 04	Spherical	939	-53.01	0.94
FC 05	Spherical	981	-49.81	0.98
FC 06	Spherical	974	-49.34	0.97

n: all the readings are executed in triplicate and average of three reading were recorded

Table 3: Vesicles Shape, size, Zetapotential and Optical Microscopy data.

Formulation Code	Entrapment Efficiency	
	Direct method	Indirect Method
FS 01	65.34	67.45
FS 02	68.93	70.13
FS 03	75.12	76.98
FS 04	78.23	79.34
FS 05	79.9	80.45
FS 06	80.57	81.67
FSC 01	73.94	75.39
FC 01	60.12	62.17
FC 02	61.89	64.01
FC 03	69.26	72.28
FC 04	78.51	80.48
FC 05	79.72	80.91
FC 06	80.69	82.51

Table 4: Drug Entrapment Efficiency in Ethosomes.

In-vitro drug release for ethosomes

The highest drug release found with FS 05 (85.75%) and FC 05 (86.17%). Results are tabulated in Table 5 and graphically represented in Figures 1 and 2. The lower concentration of soya lecithin and cholesterol has initial faster drug release but after 4 hours, they have shown slower drug release. Higher levels of alcohols in the formulation have better drug release if the concentration of either soya lecithin or cholesterol is constant.

Initial drug release for batches FC 04 and FS 03 is higher up to 5 hr and then drug release is lower compared to other formulation. But drug release for batch FC 05 and FS 05 is initially low till 5 hr and after that the drug release is constant.

Evaluation of gel

Drug content: Assay (% drug content) is found in the range 96.2% to 99.3%. Results are tabulated in Table 6. Assay (% drug content) of all the formulations is in an acceptable range.

pH: pH of all the formulation was found in the range 4.49 to 4.58. Results are tabulated in Table 6. All the pH range is acceptable for formulation.

Viscosity: The viscosity of all formulation is in the range of 1.76 to 2.83 cPs. Results are tabulated in Table 6. The increase in the concentration of the polymer, the viscosity of the formulation increases. All the formulations have shown polymer concentration-dependent viscosity. The viscosity of the formulation may affect the drug release from the formulation.

Spreadability: Spreadability of the formulation is found in the range 10.78 to 14.94. Results are tabulated in Table 6. Spreadability of the formulation is inversely proportional to the viscosity of the formulation. The increase in the concentration of Polymer the spreadability decreases.

***In-vitro* drug release:** Amount of drug release from all the gel formulation was found in the range of 70-83%. Maximum drug release found with formulation 83.45% with soya lecithin formulation and 84.45% with Cholesterol formulation. Results are tabulated in Table 7 and graphically represented in Figures 3 and 4. The concentration of Polymer in the formulation has a significant impact on the *in-vitro* drug release from the formulation. The increase in the polymer concentration in the formulation, drug release from formulation

decreases. The drug release from the formulation decreases with an increase in polymer concentration, this may be due to increase in viscosity of the formulation with the increase in polymer concentration.

FC05 C01 has higher drug release when compared with other formulation this may be due to lower concentration of polymer in formulation. Formulation with same polymer concentration but with different composition of Ethosomes has different drug release pattern. Higher concentration of polymer retards the drug release due to high viscosity; May high viscosity of formulation retards the drug diffusion.

Ex-vivo drug release: Ex-vivo release and in-vitro drug for both the formulation is almost similar. The results are tabulated in Table 7 and graphically represented in Figures 5 and 6. In 24 h the ex-vivo drug release from formulation FS05 C03 is 85.45% and FC05 C03 is 81.98%. Both the formulation has shown similar drug release pattern. The formulation FS05 C03 has better drug release than the formulation FC05 C03.

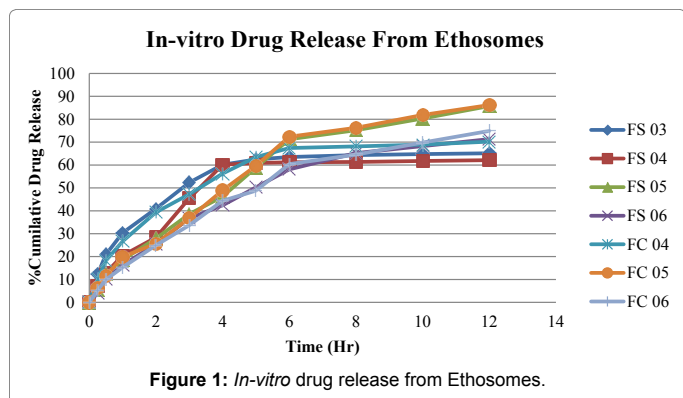
Formulation

Stability study: Formulations FC05 C03 and FS05 C03 have shown good stability for 3 M stability study at temperature condition 40°C ± 2°C/75% ± 5%RH and 25°C ± 2°C/60% ± 5% RH. All the Physicochemical stability results were tabulated in Table 8. The in-vitro drug release of 3 M sample is tabulated in Table 9. Both the formulations are physically and chemically stable for 3 Months at Room temperature and accelerated storage conditions.

Time	Formulation Code						
	FS 03	FS 04	FS 05	FS 06	FC 04	FC 05	FC 06
% Cumulative amount of Drug Diffused							
0	0	0	0	0	0	0	0
15 min	12.29	7.12	5.23	3.99	10.23	6.45	5.34
30 min	20.93	12.94	12.34	10.34	18.23	11.45	9.98
1 Hr	30.34	20.45	18.52	16.23	26.67	19.93	15.28
2 Hr	40.91	28.34	27.91	25.49	39.34	25.67	24.95
3 Hr	52.34	45.42	38.56	36.71	47.23	36.78	33.67
4 Hr	60.12	60.12	46.23	42.38	56.24	48.92	44.38
5 Hr	62.45	60.89	58.91	50.23	63.45	59.67	48.79
6 Hr	63.51	61.19	71.25	58.12	67.41	72.37	60.38
8 Hr	64.32	61.32	75.18	65.23	68.12	76.23	64.56
10 Hr	64.78	61.78	80.31	68.27	68.92	81.87	69.81
12 Hr	65.13	62.13	85.75	71.3	70.19	86.17	74.89

n: Diffusion study is carried out in duplicate and average reading were recorded.

Table 5: In-vitro drug release study data for ethosomes.

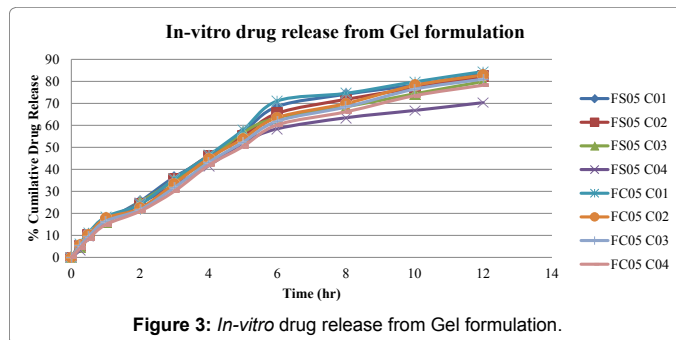
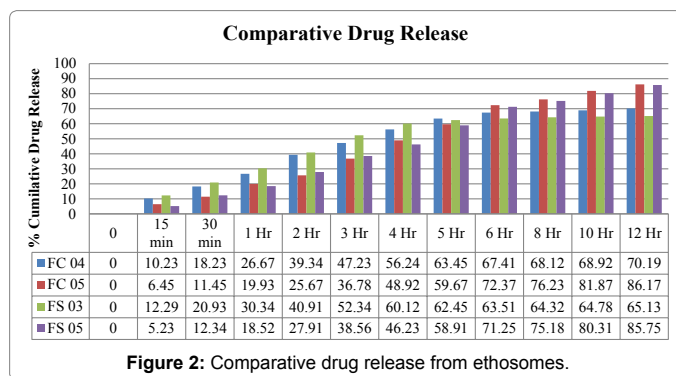


Formulation Code	% Drug Content	pH	Viscosity cPs	Spread-ability
FS05 C01	99.1	4.55	1.76	10.78
FS05 C02	98.0	4.54	2.12	11.24
FS05 C03	99.2	4.51	2.45	12.56
FS05 C04	98.8	4.56	2.78	14.35
FC05 C01	98.3	4.58	1.80	11.11
FC05 C02	99.3	4.57	2.23	12.23
FC05 C03	97.1	4.51	2.36	13.08
FC05 C04	96.2	4.49	2.83	14.94

Table 6: Drug content, pH, viscosity and Spreadability data.

Time	Formulation Code							
	FS05 C01	FS05 C02	FS05 C03	FS05 C04	FC05 C01	FC05 C02	FC05 C03	FC05 C04
% Cumulative Amount of Drug Diffused								
0	0	0	0	0	0	0	0	0
15min	5.23	5.12	4.56	3.45	6.25	5.89	5.34	4.18
30min	10.44	10.14	10.45	9.78	11.23	10.14	9.56	8.38
1 hr	16.61	15.98	16.01	16.45	18.45	17.78	16.49	14.89
2 hr	25.48	24.36	25.02	24.59	24.12	22.31	21.65	20.98
3 hr	36.56	35.64	34.87	32.84	35.78	33.45	31.73	30.06
4 hr	45.02	46.23	44.23	41.67	46.38	44.98	43.16	41.84
5 hr	56.78	55.19	56.76	51.98	57.88	53.91	52.38	50.37
6 hr	68.56	65.49	63.47	58.45	71.09	63.56	61.92	60.25
8 hr	74.12	71.82	68.45	63.46	74.56	69.92	68.35	66.15
10 hr	78.34	76.98	74.34	66.78	79.78	78.16	76.45	73.49
12 hr	83.45	82.34	79.91	70.33	84.45	82.89	81.02	78.26

Table 7: In-vitro drug release study data for ethosomes.



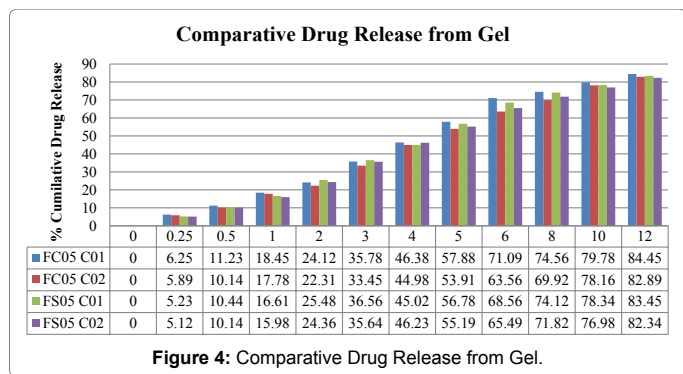


Figure 4: Comparative Drug Release from Gel.

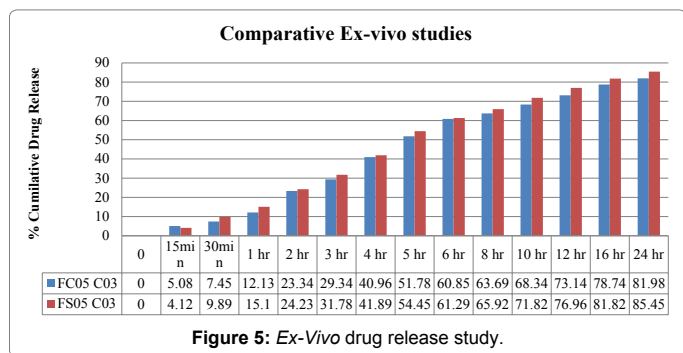


Figure 5: Ex-Vivo drug release study.

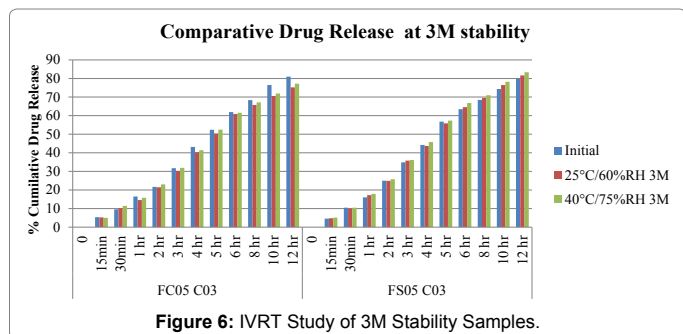


Figure 6: IVRT Study of 3M Stability Samples.

Formulation Code	Condition	Time Point	pH	Viscosity	% Drug Content
FC05 C03	40°C/75%RH	1M	4.53	2.53	99.1
		2M	4.48	2.42	98.6
		3M	4.45	2.32	97.9
	25°C/60%RH	1M	4.55	2.52	99.8
		2M	4.49	2.43	99.4
		3M	4.45	2.35	98.9
FS05 C03	40°C/75%RH	1M	4.57	2.51	99.7
		2M	4.52	2.45	99.2
		3M	4.46	2.34	98.4
	25°C/60%RH	1M	4.52	2.47	99.8
		2M	4.55	2.52	99.5
		3M	4.52	2.46	98.7

Table 8: Results for the Physicochemical test for 3M Stability Study Sample.

Drug release from the formulation FS05 C03 has comparable with the initial sample while in case of formulation FC05 C03 the drug has been dropped significantly. The drug release profile at accelerated storage condition is slightly higher when compared to room temperature sample. This may be due to slight drop in viscosity at

Time	Formulation Code					
	FS05 C03			FC05 C03		
	Initial	3M 25°C/60%RH	3M 40°C/75%RH	Initial	3M 25°C/60%RH	3M 40°C/75%RH
	% Amount of Drug Released					
0	0	0	0	0	0	0
15 min	4.56	4.78	5.12	5.34	5.23	4.98
30 min	10.45	9.98	10.45	9.56	10.23	11.45
1 hr	16.01	17.23	17.89	16.49	14.56	15.78
2 hr	25.02	24.89	25.78	21.65	21.42	23.02
3 hr	34.87	35.78	36.13	31.73	30.23	31.89
4 hr	44.23	43.67	45.78	43.16	40.35	41.41
5 hr	56.76	55.83	57.34	52.38	50.34	52.45
6 hr	63.47	64.56	66.76	61.92	60.85	61.56
8 hr	68.45	69.54	70.98	68.35	65.71	67.13
10 hr	74.34	76.45	78.24	76.45	70.56	71.94
12 hr	79.91	81.67	83.34	81.02	75.23	77.18

Table 9: In-vitro drug release Results of 3M Stability Study Sample.

accelerated storage condition which is affecting the drug release profile. Viscosity of the formulation affects the drug release profiles may be due change in diffusion rate due to change mobility of drug molecules.

Conclusion

Ethosomes has more stability and penetration than the liposomes. The stable ethosomes can be formulated with using the composition soya lecithin, cholesterol, ethanol and water. The concentration of ethanol increases in the formulation of ethosomes, the stability and drug release from the formulation increases. Drug loaded Ethosomes are suitable to entrap in the gel matrix for suitable drug application. Stable Gel formulation is formulated with different concentration (0.75%, 1.0%, 1.25%, 1.5%w/w) of Carbopol 980. Ethosomes were evaluated for vehicle shape, size, zeta potential, and drug release and entrapment efficiency. The ethosome formulation FS05 (0.8% Soya lecithin 60% ethanol) and FC05 (0.3% Cholesterol and 60% ethanol) have drug release profile amongst all the formulations. The gel formulation FS05 C03 (1.25% Carbopol) and FC05 C03 (1.25% Carbopol) has better *in-vitro* drug release profile amongst all the formulation. The increase in the concentration of Carbopol 980 has the direct impact on the viscosity and drug release profile. The increase in Carbopol 980 concentration viscosity of the formulation increases and decreases the drug release from the formulation. The *ex-vivo* study has shown a similar release pattern to the *in-vitro* release study from both the formulation FS05 and FC05. Both the formulations were stable for 3 M at room temperature and accelerated temperature storage condition. Formulation FS05 C03 has shown similar drug release pattern to the initial sample while formulation FC05 C03 has shown a significant drop in drug release from the formulation. Samples at accelerated storage condition have shown slightly higher drug release than room temperature.

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