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Formulation and evaluation of Lornoxicam loaded Lyotropic liquid crystalline gel

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ABSTRACT

GIT irritation is prominent limitation with the use of Non-steroidal anti-inflammatory drugs (NSAID's). There is rising interest in designing formulations which will deliver the drug at the site of action as topical gels, to avoid GIT irritation and other systemic side effects. Liquid Crystal phase has emerged as a novel material for the preparation of topical drug delivery system. In present study the attempt is made to prepare Lornoxicam loaded lyotropic liquid crystalline gel using glycerol monooleate. Glycerol monooleate is biocompatible, bioadhesive, penetration enhancer and sustain release agent. It also promotes ceramide extraction and enhancement of lipid fluidity in the stratum corneum region of the skin. Five formulation of lornoxicam were prepared and evaluated for parameters like drug content, viscosity, spreadability, Extrudability *In-vitro* drug release along with in vivo study. *In-Vitro* and *Ex-Vivo* drug release kinetics showed that there was 72.85% and 77.98% drug release within 48 hrs. Skin irritation test suggested that prepared formulation was safe for human use. *In-Vivo* evaluation of this formulation was done by carrageenan induced rat paw edema anti-inflammatory model.

Keywords: Lornoxicam, GMO, Lyotropic liquid crystal, Anti-Inflammatory, Topical drug delivery

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INTRODUCTION:

NSAIDs are the best choice to be administered through Topical drug delivery system in the management of diseases like, Osteoarthritis, rheumatoid Arthritis, and similar inflammatory disorders.[1]

Oral administration of NSAID shows side effects like Nausea, Vomiting, Heartburn, Gastric Ulceration, Epigastria. These drawbacks can be overcome by developing sustained release non-steroidal anti-inflammatory topical gel which is able to provide constant drug concentration at the site of administration. Hence attempt is to formulate a Liquid Crystalline gel (LCG) of Lornoxicam. Glycerol monooleate is used to formulate the LCG. (2) GMO is a mixture of the glycerides of oleic acid and other fatty acids, consisting mainly of the monooleate. GMO offer various Advantages like [3] Biocompatibility, Bioadhesiveness, Penetration Enhancer, Non Toxic, Non Irritant. Pluronic F 127 is used as a stabilizer. The prepared gel is having enhanced moisturizing ability. Moisture content of liquid crystalline system is retained for a long time. It promoted the ceramide extraction and enhancement of lipid fluidity in the stratum corneum region of the skin. [4] These gels are appropriate candidates for sustained release because the drug diffusion is reduced by a factor of 10 to 1000. [5] Clinical evidence indicates that topical gel is a safe and effective treatment option for use in the management of skin related disease. [6]

Topical Drug Delivery System :

Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. [7]

this avoids first pass effect, GIT irritation, with increased patient compliance and ease of application [8], [9]. The only major disadvantage associated is that it may cause allergic reaction to the skin, skin irritation, dermatitits[10]

MATERIALS & METHODS :

Materials:

Drug Lornoxicam was obtained as a gift sample from Macleods pharmaceuticals, Andheri, Campul Glyceryl

Monooleate (GMO) - 50, EP/NF was issued from Estelle chemicals ltd. Ahmednagar. Pluronic F – 127 (PF 127) was Sigma Aldrich. The solvents/chemicals used were obtained from the store of Pravara Rural college of Pharmacy, Loni. including the solvents/chemicals used were Hydrochloric acid, Potassium Chloride, Sodium hydroxide, Potassium dihydrogen phosphate, Ethanol etc. [11]

Method :

The lipid base (GMO) was melted at 37 ^{0}C and after complete melting of base the drug was added with constant stirring. At the same time the distilled water was heated in another beaker and surfactant (PF 127) was added to the aqueous base with constant stirring on a magnetic stirrer. Upon complete dissolution of the aqueous phase was added drop by drop to the lipid phase with constant stirring until a gel mass is formed.[12],[13]

Pre-formulation study : [14],[15],[16]

Confirmation of drug was carried out by using FT-Infrared Spectrophotometer, Differential Scanning Colorimeter (DSC) and Melting Point.

Preparation of Standard Calibration Curve :

Calibration curve of Lornoxicam in pH 6.8 buffer, pH 7.4 buffer and water was determined using UV visible spectrophotometer (UV Thermoscientific).

Characterization of Lornoxicam lyotropic liquid crystalline gel : [17],[18]

Appearance:

All the batches of Lornoxicam loaded liquid crystalline gel formulations were observed for appearance, color, and consistency.

pH :

2.0 gm. of gel was accurately weighed and dispersed in 20 ml of distilled water. The pH of dispersion was measured by using digital pH meter. This procedure was carried out in triplicate.

Viscosity : [19]

A Brookfield digital viscometer, cone and plate type of viscometer was used to determine viscosity of the formulations. The viscosity was measured 5 rpm after 30 seconds, by using spindle no.7

Drug content : [20]

0.50 gm. Lornoxicam, liquid crystalline gel was weighed accurately. It was added in 100 ml volumetric flask containing 100 ml of PBS 6.8. Resultant solution was kept for sonication for 30 min. for complete solubility of drug, and compared with pure absorbance at same wavelength and concentration. Thus % Assay was calculated this procedure was carried out in Triplicate.

Spreadability[13], [14] **:-**

A ground glass slide was fixed on the, block an excess of gel (about 2 gm) under study was placed on this ground slide. The gel was then sandwiched between the slide and another glass slide and in the dimensions of fixed ground slide and provide with the hook. A 1 kg weight was placed on the top of the two slides for 5min to expel air and provide a uniform film of gel of the gel between the slides. Excess of gel was scrapped of from the edges. The top plate was then subjected to pull off 80 gms. With the help of string attached to the hook and the time (in sec) require by top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better spreadability

Spreadability was then calculated using the following formula:

Where, $$S = M \times L/T$$

S = Spreadability of the gel

M = Weight in the pan (tied on the upper slide)

L = Length moved by the glass slide

T = the time taken to separate the slide completely from each other.

Extrudability [13]**:-**

The measurement of extrudability of each formulation was in triplicate and the average values are presented.

The Extrudability was than calculated by using the following formula,

Extrudability = Applied Weight to Extrude Gel from Tube (In gm) / Area (In Cm^2)

In-vitro **drug release:** [21], [22]

The in vitro drug release of the Lornoxicam loaded lyotropic liquid crystalline gel was performed to investigate the amount of drug released from a gel. Dialysis membrane (HIMEDEA) was used as diffusion membrane. Membrane was soaked in phosphate buffer 6.8 for 2 hour before subjecting to diffusion study. The membrane was positioned between the two cell halves of a glass chamber. The two compartments were held together with a clamp. The receiver/receptor compartment contained 25 ml of phosphate buffer. In the upper donor compartment 0.5 gm. of formulation was spread evenly on the membrane. The receptor phase (phosphate buffer) was continuously stirred with the help of magnetic stirrer at 300 rpm and maintained at 37^{o}C using a circulating water bath. At predetermined time intervals (1, 2, 4, 6, 8, 12, 25, 36, and 48h) 3 ml samples were collected from the receiver compartment and replace with fresh buffer solution. The samples collected from receiver compartment were analyzed for drug content using UV spectrometric method at 374 nm wavelength.

Ex-vivo **penetration study :** [23]

Male wistar rats (Animal ethical committee) weighing 200-250 g were used in this study. Rat abdominal skin was used as diffusion membrane. Three ml of the sample was withdrawn from the receiver compartment at time intervals 1, 2,3,4,5,6,12, up to 48 hours and the same amount of fresh buffer solution was added to maintain the sink condition in receiver compartment. The samples were analyzed spectrophotometrically at a wavelength of 374 nm. Percentage of Lornoxicam sample was determined by referring a previously prepared standard curve. This experiment was carried out for a period of 48 hours.

Skin irritation study :

Male wistar rats (Animal ethical committee) weighing 200-250 g; were used in this study. The animals were housed in propylene cages, with free access to standard laboratory diet and water. Animals were acclimatized for at least seven days before study then Lornoxicamlyotropic liquid crystalline gel was applied onto the dorsal skin of wistar rats area of 1" X 1" square. After a 24 hrs. exposure, the gel was removed; the animals were returned to the cages.

***In-vivo* study :**

To carry out in vivo anti-inflammatory studies approval was obtained from the Animal Ethical Committee, (PRCOP, Loni) and their guidelines were followed for the Studies. The sustained anti-inflammatory effect of the optimized formulation (F5) was evaluated by carrageenan induced in rat hind paw edema method. Young male wistar rats weighing (200-250 gm) were randomly divided into three groups.

Table No. 1 : Carrageenan induced paw edema test

S.N.	Groups	Animal	Weight	N
1	Control	Male Wistar Rat	200-250	06
2	L-LLCG	Male Wistar Rat	200-250	06
3	Volini (Marketed)	Male Wistar Rat	200-250	06

*N = Number of rats

The animals were kept under standard laboratory conditions, at a temperature of 25±1°C. The animals were housed in poly propylene cages, with standard laboratory diet. Topical dose of the Lornoxicam loaded lyotropic liquid gel was calculated based in the weight of the rats according to the surface area ratio. The dorsal side of the rat skin was shaved 12h before.

L-LLCG and marketed formulation (Volini) were applied on the dorsal region of all animals except in control group half an hour before sub plantar injection of carrageenan in distilled water.

Stability study : [20]

The ICH Guidelines Q1 a (R2) have established that long term stability testing should be done at 25°C/60%RH; stress testing should be done at 40°C/75% RH for 6 months. If significant change occurs at these stress condition, then the formulation should be tested at an intermediate change condition i.e. 30°C/75%RH. Table No 2 shows different temperatures and period of stability testing.

Table No. 2. ICH Guidelines for stability study

Study	Storage condition	Time period
Long term*	25°C±2°C/60%RH±5%RHor 30°C±2°C/65%RH±5%RH	12 month
Intermediate**	30°C±2°C/65%RH±5%RH	6 month
Accelerated	40°C±2°C/75%RH±5%RH	6 month

*It is up to the applicant to decide whether long term stability study are performed at 25°C±2°C/60%RH±5%RH or 30°C±2°C/65%RH±5%RH

**If 30oC±2°C/65%RH±5%RH is a long term condition there is no intermediate condition.

Final formulation which was taken for stability. The prepared Lornoxicam loaded lyotropic crystalline gel was packed in aluminum collapsible tubes (10g) and subjected to stability studies at 30°C±2°C/65%RH±5%RH for a period of 3 months. Samples were withdrawn at 15-day time intervals and evaluated for pH and Drug content.

Stability Protocol :

Table No. 3: Stability protocol

Stability Study	(Intermediate condition)
Storage condition	**Duration**
30°C±2°C/65%RH±5%RH	3 month

RESULT& DISCUSSION :

Identification and Conformation of Lornoxicam :

***Infrared Spectroscopy* :**

Infrared spectroscopy of pure Lornoxicam was carried out for the conformation of the drug.

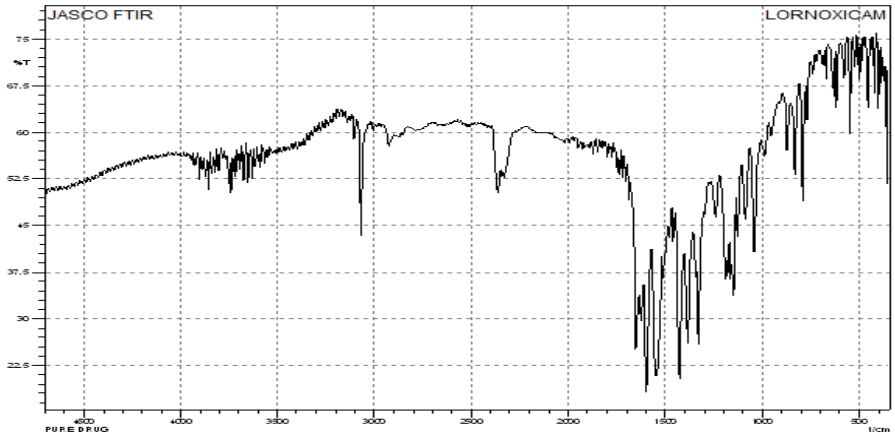

Fig. 1: FT-IR Spectrum of Lornoxicam

The principal peaks corresponds to the structural features of Lornoxicam are found due to presence of C-H stretching of primary carbon at 2889.37 cm^{-1} the peaks at 2850-2950 cm -1 and 1064.71 cm -1 indicated presence of free OH stretching of carboxylic acid. Peak of 1705 cm-1 confirms presence of C=O stretching of carboxylic acid.

***Differential scanning Calorimetry* (DSC) :**

The thermal properties of drug can be evaluated by means of DSC.

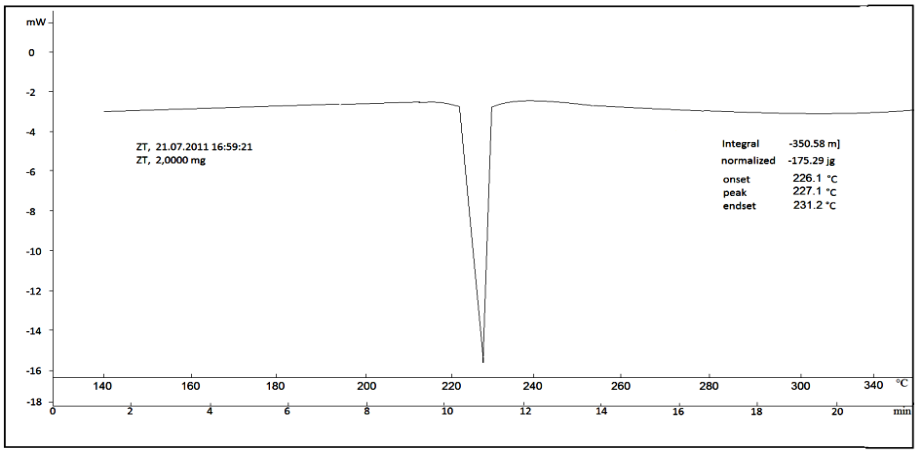

Fig. 2 : DSC Thermogram of Lornoxicam

The DSC thermogram of Lornoxicam was typical of amorphous substance, exhibiting a sharp endothermic peak at 227.1°C corresponding to its melting and decomposition reported peak temperature was 226-231°C

***Melting point* :**

Melting point of Pure drug (Lornoxicam) was determined by capillary method.

Table No. 4 : Melting point of drug by capillary method

Sample	Melting point (Practical)	Melting point (Theoretical)
Drug	225-230°C	226 °C

Drug-Excipient Interaction Study :

Fourier Transform Infrared (FT-IR) Spectroscopy:

Fig.3: FT-IR spectrum of drug with physical mixture (PF 127).

From the FTIR spectra it is observed that, peak at 1739 cm^{-1} confirms presence of C=O stretching and peak at 1204 cm^{-1} further conform the ester group, and peak at 3735 cm^{-1} confirms free OH group of monooleate. The principal peaks correspond to the structure features of Lornoxicam are found due to presence of C-H stretching of primary carbon at 2889.37 cm^{-1}. The peaks at 2850-2950 cm^{-1} and 1064.71 cm^{-1} indicate presence of free OH stretching of carboxylic acid.

From the FT-IR spectra it is observed that, all the characteristic peaks of drugs appear in the spectra of physical mixture at the same wave number indicating no interaction between drug, lipid and polymer.

Stanard Calibration Curves :

Standard calibration curves of lornoxicam were studied at pH 6.8,7.4, and in water (Table no. 5)

Table No.5: Calibration curve of Lornoxicam in Water, pH 6.8, & pH 7.4:

Sr. No	Conc (µg/m)	Abs. (nm)		
		pH 6.8	*pH 7.4*	*Water*
1	0	0	0	0
2	2	0.081	0.121	0.067
3	4	0.148	0.258	0.143
4	6	0.221	0.409	0.198
5	8	0.309	0.556	0.258
6	10	0.399	0.668	0.338

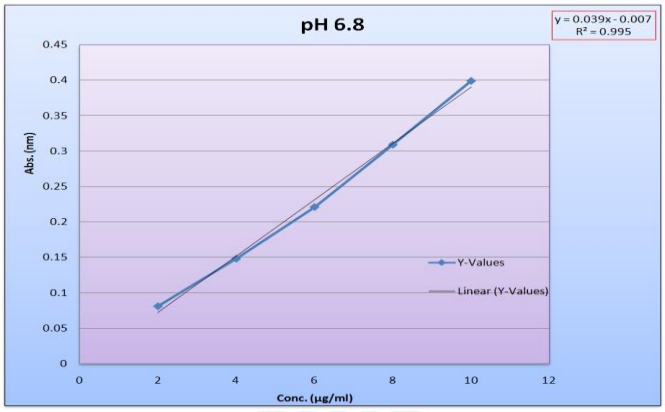

Fig. 4: Calibration of Lornoxicam in pH 6.8.

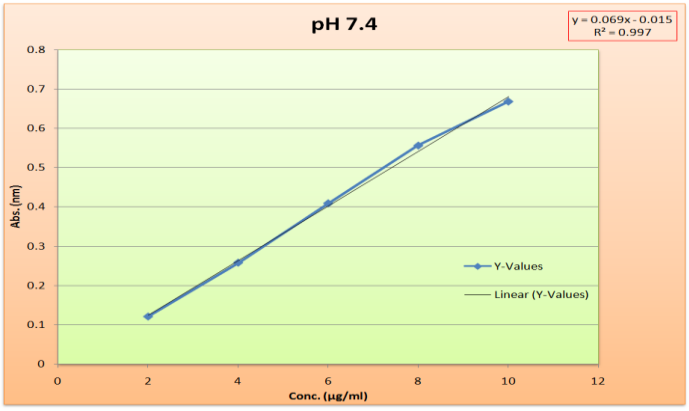

Fig. 5: Calibration curve of Lornoxicam in pH 7.4.

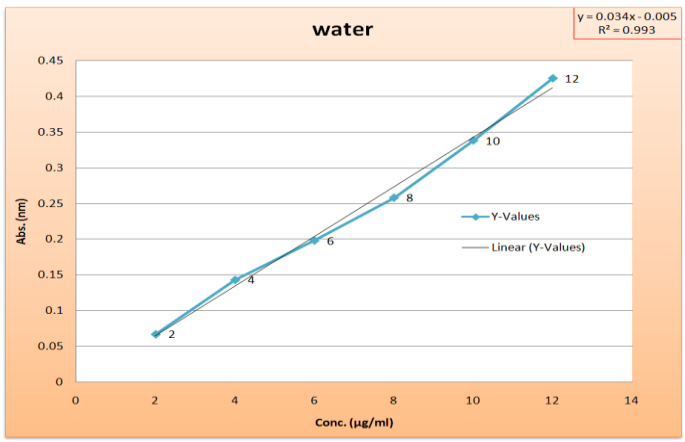

Fig. 6: Calibration of Lornoxicam in Water.

5.Charactarization of Lornoxicam Lyotropic Liquid Crystalline Gel :

Physical Appearance :

The Lornoxicam loaded lyotropic liquid crystalline gel (L-LLCG) formulations were observed under dark background. The observations for various formulations are reported in table no. 10 showed yellow viscous preparation with smooth, homogenous and consistent appearance.

Table No. 6 : Appearance of L-LLCG formulation batches

S.N.	Formulations	Appearance
1	F1	Transparent, less viscous
2	F2	Slight phase separation
3	F3	Translucent, smooth, viscous
4	F4	Translucent, smooth, viscous
5	F5	Translucent, smooth, viscous

pH :

The pH of formulations was determined using digital pH meter and were found to be in the pH values required for topical application.

Table No. 7 : pH values of L-LLCG formulation batches

S.N.	Formulations	pH values
1	F1	6.6
2	F2	6.8
3	F3	7.0
4	F4	6.9
5	F5	7.2

Viscosity :

The viscosity for various formulation batches tabulated in Table No. 8.

Table No. 8: Viscosity study of L-LLCG formulation batches

S.N.	Formulations	Viscosity (cps)
1	F1	1130
2	F2	1850
3	F3	7520
4	F4	7730
5	F5	9200

From the result it was observed that viscosity is increased with increase in proportion of GMO.

Spreadability & Extrudability:

Table1 No. 9: Spreadability study & Extrudability study of L-LLCG

Formulation batch	Spreadability (gm.cm/sec.)	Extrudability (gm./cm2)
F1	11.96	12.97
F2	12.69	13.43
F3	13.78	14.88
F4	14.11	15.09
F5	14.79	15.61

Spreadability was measured on the basis of 'slip' and 'drag' characteristics of gel by using ground glass slide and results were found in the range of acceptance. (Table No. 9)

Drug Content :

Lornoxicam content in each formulation was determined and reported in table no. 10

Table No. 10: Drug content of L-LLCG formulation batches.

S.N.	Formulation	Drug content %
1	F1	65.58
2	F2	70.21
3	F3	76.50
4	F4	83.32
5	F5	88.78

The drug content of optimized formulations (F5) was carried out in triplicate and average drug content was found to be 88.78 %.

***In-vitro* drug release :**

The in vitro drug release from Lornoxicam loaded lyotropic liquid crystalline gel was evaluated by in vitro diffusion study using Franz diffusion cell, dialysis membrane was used in phosphate buffer pH 6.8

Table No. 11 : In Vitro drug release study of formulation batches :

Time (hr.)	F1	F2	F3	F4	F5
0	0	0	0	0	0
1	1.097	2. 921	1.291	3.978	7.683
2	1.892	3.944	2.567	5.1467	8.834
3	2.635	5.852	3.727	6.616	9.018
4	3.659	9.179	4.837	7.245	10.824
5	3.790	9.880	5.958	8.211	11.634
6	4.770	10.797	6. 50	9.951	14.968
7	5.470	12.128	7.723	10.447	17.627
8	6.702	13.922	8.965	11.116	22.557
9	7.359	15.786	11.414	13.532	27.861
10	8.970	17.932	14.102	17.485	33.885
11	10.254	19.182	17.217	27.563	39.282
12	12.054	21.016	22.163	36.127	49.892
24	19.243	30.771	36.515	43.129	58.113
36	24.482	38.149	39.536	49.668	68.792
48	26.724	45.338	48.850	58.525	72.853

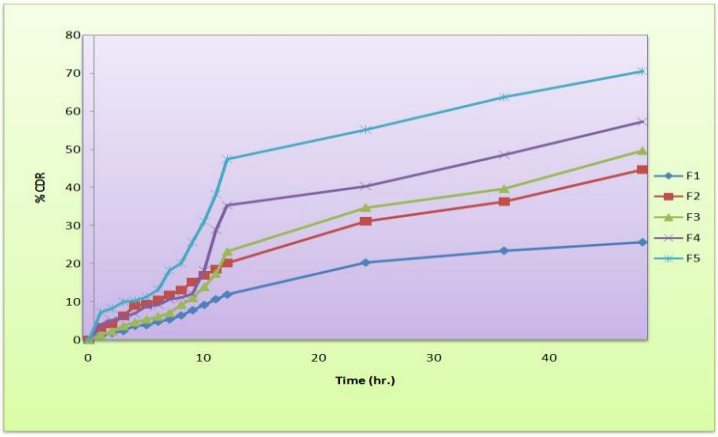

Fig. 8: In vitro drug release study from formulation Batches by using Franz diffusion cell.

Cumulative drug release from batch F1, F2, F3, F4, and F5 in 48 hours was found to be 26.724, 45.338, 48.850, 58.525, 72.853 % respectively. Result indicated that, batch F5 showed high permeation and hence drug release.

***Ex-vivo* study :**

The Ex vivo drug release study of optimize (F5) formulation, Rat abdominal skin (0.22µ) was used as diffusion membrane. Diffusion study was performed for 48hrs

Table No. 12: % ex vivo drug release study optimizes batch (F5) formulation.

Time (Hrs.)	% Drug Release
0	0
1	7.387
2	12.642
3	14.463
4	16.352
5	21.672
6	25.534
7	31.138
8	31.166
9	43.260
10	47.155
11	52.354
12	62.855
24	70.958
36	74.689
48	77.986

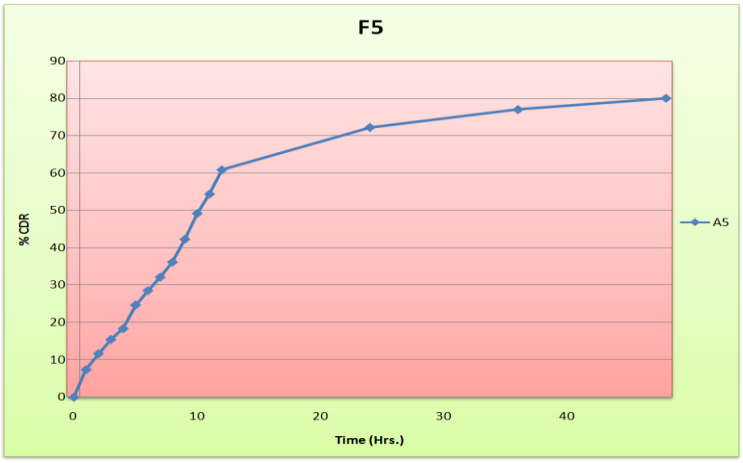

Fig. 7: Ex-vivo drug release study from optimize batch (F5) Formulation using franz diffusion cell.

Cumulative drug release from Lornoxicam lyotropic liquid crystalline gel in 48 hrs was found to be 77.98.

Skin irritation study :

The result of skin irritation study based on visual observation, the observation revealed that, L-LLCG (F5) formulation was non-sensitizing and safe for use, as there is no irritation produced after application of L-LLCG formulation to the rat skin.

***In vivo* study :**

Carrageenan induced Rat paw edema test :

The Carrageenan induced paw edema test carried out by using digital plethyesmometer as shown in image.

Fig.8: *In-Vivo* study of carrageenan induced paw edema

The Anti-inflammatory effect of developed Lornoxicam lyotropic crystalline gel formulation (F5) was compared with marketed volini gel. (table 13). The result showed that % edema inhibition for L-LLG was 32.16 %, 53.71 and 68.83 % and for marketed gel was 41.19 %, 59.57 % and 56.10 % after 3, 6 and 12 hr respectively. Initially the percent (%) inhibition value was low for Lornoxicam loaded lyotropic liquid crystalline gel formulation (F5) but after 3 hr. there was significant enhancement in the value which indicated the sustained release of Lornoxicam from the formulation F5($p<0.05$).

Table No. 13: Observation table for carrageenan induced paw edema

Group	Formulation	N	Mean wt. (g)	Time (h)	Mean % Edema	% inhibition
1	Control	6	250	1	28.7	-
				3	66.9	-
				6	51.2	-
				12	40.1	-
2	L-LLCG	6	240	1	25.1	8.75 %
				3	45.4	32.16 %
				6	23.7	53.71
				12	12.5	• 68.83 %
3	Marketed gel	6	245	1	28.2	11.78 %
				3	39.4	41.19 %
				6	20.7	59.57 %
				12	17.6	56.10 %

N : Number of rats in each group

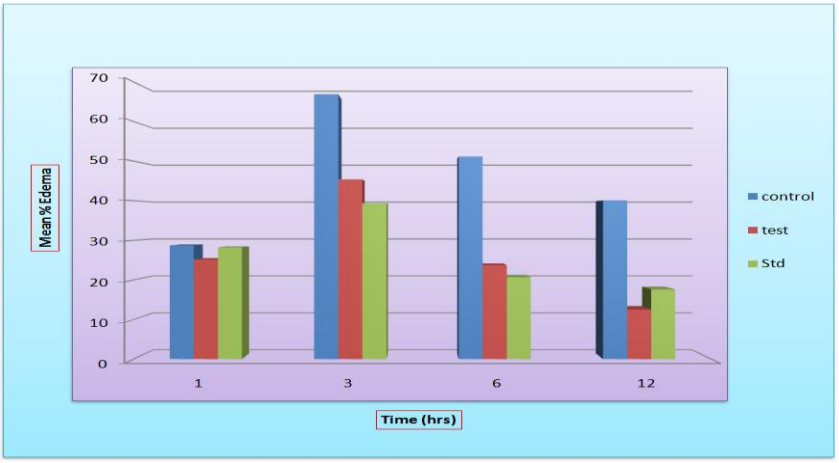

Fig. 9 : Percent inhibitions of rat paw edema volume

Accelerated stability study :

The accelerated stability studies were carried for developed optimized formulation (F5) was analyzed for pH and drug content. Samples were withdrawn at 15 days interval. After 6 weeks studies, formulation showed slight changes in pH, but it were in acceptable limits (±5). no definite change observed for drug degradation as show table.

Table No. 14: Stability study of L-LLCG formulation :

FORMULATION (F5)	Weeks	PH	Drug content (%)
Lornoxicam Loaded Lyotropic Liquid Crystalline Gel	0	6.6	90.15
	3	6.6	89.85
	6	6.7	88.75
	9	6.7	88.68

CONCLUSION :

From the summary we can be concluded that the lyotropic liquid system provides a novel material for the preparation of topical drug delivery system for the poorly water soluble drug (Lornoxicam). To achieve this aim, we have selected glycerol monooleate for the preparation of lyotropic liquid crystal because it is biocompatible, bioadhesive penetration enhancer and sustained release agent. According to Ex-vivo study drug release was found to be 77.98% in 48 hrs. Shows sustained release action. It means the formulated lyotropic liquid crystal exhibit good penetration into the skin and the systemic circulation. Also this formulation was evaluated for spreadability and extrudability.

The anti-inflammatory activity of this gel formulation in rat hind paw edema revealed that Lornoxicam was delivered to the inflammation site at a sustained rate over a period of 12 hr. These results suggest the feasibility of the topical gel formulation of Lornoxicam loaded lyotropic liquid crystalline gel.

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