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DESIGN AND OPTIMIZATION OF MICROEMULSION FORMULATION FOR TOPICAL DELIVERY OF LORNOXICAM

Nandkishor Sudam Wani* Amol Pandurang Ilag and Niraj Nemichand Sanghai

Department of Pharmaceutics, STES's Sinhgad Institute of Pharmaceutical Sciences, Kusgaon (Bk.), Lonavala, Dist-Pune (Maharashtra), INDIA – 410 401.

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***Correspondence for Author**
Nandkishor Sudam Wani
Department of Pharmaceutics, STES's Sinhgad Institute of Pharmaceutical Sciences, Kusgaon (Bk.), Lonavala, Dist-Pune (Maharashtra), INDIA – 410 401.

ABSTRACT

The purpose of the present study was to assess and optimize the promising use of microemulsions as a topical drug delivery for poorly water soluble NSAID, lornoxicam. The pseudo-ternary phase diagrams were developed for various microemulsion formulations composed of Oleic acid (oil phase), Tween 80 (surfactant) and Propylene Glycol (co-surfactant) by using water titration method. Formulations of microemulsion with different proportions of oil, Surfactant (SAA), Co-surfactant (Co-SAA) was prepared and optimized using simplex lattice mixture design. The effect of independent variables (concentrations of surfactant: co-surfactant and water) were investigated on the particle size and cumulative amount of drug (lornoxicam) diffused per unit area (CADD) of excised mouse skin. The microemulsion was also evaluated for physicochemical properties like pH, viscosity, zeta potential and stability studies. The results showed that the optimized microemusion formulation was composed of oleic acid (5%, w/w), tween 80 (20% w/w), propylene glycol (30% w/w) and water (45% w/w). The mean particle diameter was 57.09 nm and the drug release was 29.53 %. Thus it was concluded that the permeating ability of lornoxicam was significantly increased from the microemulsion formulation.

KEY WORDS: Topical delivery, Lornoxicam, Microemulsion, Simplex lattice mixture design.

1. INTRODUCTION

Microemulsions (MEs) are clear, transparent (due to the size of droplets less than 100 nm, smaller than the wavelength of light), optically isotropic, biphasic, heterogeneous and

thermodynamically stable colloidal dispersions of oil and water, which are stabilised by an interfacial film of an SAA, in most cases in combination with a Co-SAA, typically of short to medium chain alcohols.[1] In recent years, they have emerged as promising vehicles for topical delivery of drugs as the result of their transparency, more solubilizing ability, ease of preparation, long-term stability, and may be sterilized by filtration, along with higher drug loading capacity and their thermodynamic stability.[2-5] They possess large solubilization capacity attributing to their immense interfacial area and the presence of various microdomains of different polarities.[6-7] They also significantly enhance penetration of hydrophilic, lipophilic and amphiphilic substances into and through biological membranes.[8-9] Besides, they are easy to formulate,[9-10] have relatively low viscosity[12] and have self preserving property.[13]

However, formulation of microemulsions might need high level of SAAs that might irritate the skin. Control of inflammatory disorders requires an organized approach to gain the advantage of medicinal agents. Alleviating of pain and lessening of inflammation are pressing objective to diminish the severity of indications.[14] Up on oral or topical administration, Non-steroidal anti-inflammatory drugs (NSAIDs) inhibits cyclooxygenase and consequently decreases the prostaglandin synthesis, reduces erythema and therefore are the most widely used for the treatment of pain, inflammation and sunburn.[15] Lornoxicam is a strong analgesic, anti-inflammatory and antipyretic NSAID of the oxicam class (chlortenoxicam) having an enhanced gastrointestinal safety profile compared to other NSAIDs.[16] It has been shown to be effective in the treatment of postoperative pain and rheumatoid arthritis (RA). It has accounted to have a restricted solubility and dissolution especially in an acidic environment.[17-18] It has short half life of 3-4 hrs, log P value 1.8 and molecular weight of 371.82 g/mol.[19] Lornoxicam is available in oral and parenteral formulations. Alkaline trometamol is utilized to suit the solubility constraint and to obtain a clear solution of 4.3 mg/ml for ready to use or reconstituted lornoxicam parenteral formulations.[20-21] Like other NSAIDs, the most common side effect of oral dosage of lornoxicam is gastrointestinal irritation. Thus, the possibility of delivering lornoxicam through the topical application at low doses is desirable. Literature survey also reveals that no topical application of lornoxicam has been reported till date. Hence in order to increase therapeutic efficacy of topical drug delivery, an attempt was made to design and optimize the microemulsion formulation for topical delivery of lornoxicam.

2. MATERIAL & METHODS

2.1 Materials

Lornoxicam was kindly supplied by Alkem Laboratory Ltd, Taloja, Navi Mumbai. Tween-80, Methanol was purchased from MERK Specialties, Mumbai. Oleic acid, Tween-60, Sesame Oil was procured from the Loba Chemicals, Mumbai. Propylene glycol, Glycerine, Span-80, Ethanol, Tween-20, Iso-Propyl Pyristate, Iso-Propyl Palmitate were procured from Ozone Chemicals, Mumbai. Capmul, Capmul C-8, was purchased from Abitec Corp, USA. Sodium Di-hydrogen Phosphate, Di-Sodium Hydrogen Phosphate, were purchased from Research Lab Fine Chemicals, Mumbai. Other chemicals are of HPLC or pharmaceutical grade. Double distilled water was used throughout the study.

2.2 Methods

2.2.1 Calibration curve of Lornoxicam in 0.1N methanolic NaOH & pH 7.4 Phosphate Buffer Saline

Lornoxicam stock solutions were prepared by dissolving 100 mg of drug in 100 ml of 0.1N methanolic NaOH & phosphate buffer saline pH 7.4 respectively. From this, 10 ml of sample was pipetted out and diluted up to 100 ml, making final concentration of 100µg/ml. Appropriate dilutions were made with alcoholic NaOH & PBS to give a concentration of 10 µg /ml; the resultant solution was scanned in UV range from 200-400 nm, which could be utilized for analysis and spectrum was recorded. A series of working solutions of concentration ranging from 4-24 µg/ml (Beers- Lambert range for lornoxicam) were prepared from a stock solution 1 & 2. The absorbance of these solutions was measured on UV double beam spectrophotometer (V-530, Jasco, Japan) at 381 nm. Calibration curve was prepared by plotting concentration on X-axis and absorbance on Y-axis.[22]

2.2.3 Differential Scanning Calorimetry (DSC)

Thermal analysis of the drug was performed on a Shimadzu DSC 60 which was calibrated for temperature and enthalpy using pure Indium. Drug (3-5 mg) was crimped in non-hermetic aluminium pans with lids and scanned from 50 to 300ºC at a heating rate of 10ºC/min under a constantly purged dry nitrogen atmosphere (flow rate 20 ml/min). The instrument was outfitted with a refrigerated cooling system. DSC study was conducted to confirm the purity of the drug sample.

2.2.3 Selection of Oils, Surfactants for Formulation Study

Various oils including Oleic acid, IPM, Eucalyptus oil and Capmul along with different surfactants including Tween-80, Tween-20 and Tween-60 and co-surfactants including Propylene glycol, Ethanol, Glycerine and Capmul C8 were screened for the formulation of microemulsion. The solubility of lornoxicam was determined by adding an excess amount of drug to the 10 ml of selected oil, surfactant, co-surfactant individually in 50 ml capacity stopper vials respectively. The mixtures were then stirred for 72 hours at room temperature on a rotary shaker. Further samples kept for 24 hours at room temperature to attain equilibrium. The equilibrated samples were centrifuged at 3000 rpm for 15 min. followed by filtration through a 0.45-µm membrane filter. The filtrates were diluted with methanolic NaOH and lornoxicam solubility was subsequently quantified by U.V. double beam spectrophotometer at λ_{max} 381 nm.[23-24] Excipients which offering outstanding solubility of lornoxicam were selected for further studies.

2.2.4 Construction of Pseudo-Ternary Phase Diagrams

The pseudo-ternary phase diagrams were constructed using water titration method to determine the microemulsion area and to detect the possibility of making microemulsions with different possible compositions of oil, surfactant/co-surfactant (Oleic acid, Tween80 and Propylene glycol) and water respectively. The ratios of surfactant to co-surfactants were selected to be 1:1, 2:1 and 3:1 with fixed 5 % oil amount. These mixtures (S/CoS) were mixed with the oil phase to give the weight ratios of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8 and 1:9. Water was added drop by drop and stirred using a magnetic stirrer at constant temperature until a homogeneous dispersion or solution was obtained. After each addition, the system was examined for the physical appearance. The end point of the titration was the point where the solution becomes transparent or translucent. The amount of the aqueous phase required to make the mixture turbid was noted.[25-26] The percentages of the various incorporated pseudo phases were estimated, and the same procedure was followed for the other S/CoS ratios. All the ratios of S/Co gives dotted area in pseudo ternary phase diagram. After construction of pseudo ternary diagrams for particular ratios, the diagram which shows the larger area of microemulsion existing zone that ratio was chosen for the further studies.

Table 2.1: Composition of ternary phase diagrams (Quantity in ml)

Oil: SAA/CoSAA ratio	Oil	1:1		2:1		3:1	
		SAA	Co-SAA	SAA	Co-SAA	SAA	Co-SAA
1:9	1.0	4.5	4.5	6.0	3.0	6.7	2.3
2:8	2.0	4.0	4.0	5.3	2.7	6.0	2.0
3:7	3.0	3.5	3.5	4.6	2.3	5.3	1.7
4:6	4.0	3.0	3.0	4.0	2.0	4.5	1.5
5:5	5.0	2.5	2.5	3.3	1.7	3.8	1.2
6:4	6.0	2.0	2.0	2.6	1.3	3.0	1.0
7:3	7.0	1.5	1.5	2.0	1.0	2.3	0.7
8:2	8.0	1.0	1.0	1.3	0.7	1.5	0.5
9:1	9.0	0.5	0.5	0.7	0.3	0.7	0.3

2.2.5 Design and Optimization for Lornoxicam Microemulsion

The formulation design for lornoxicam was done by using Simplex lattice by the help of Design Expert 8.0 software. The simplex lattice design for a three component system is represented by an equilateral triangle in two-dimensional space (Fig. 2.1). Seven batches were prepared as follows: one at each vertex (A, B and C), one at the halfway point between vertices (AB, BC and AC), and another one at the center point (ABC). In this design, three factors were assessed by altering their concentrations simultaneously and keeping their total concentration constant. The composition of oil phase (oleic acid) was kept constant throughout the experiment, i.e. 5% and the final compositions were adjusted with surfactant, co-surfactant and water. The different compositions of microemulsion were designed. The concentrations of surfactant, co-surfactant and water were selected as independent variables. The globule size and the cumulative amount of lornoxicam permeated through excised albino rat skins per unit area were taken as responses (Zhu et al., 2008).

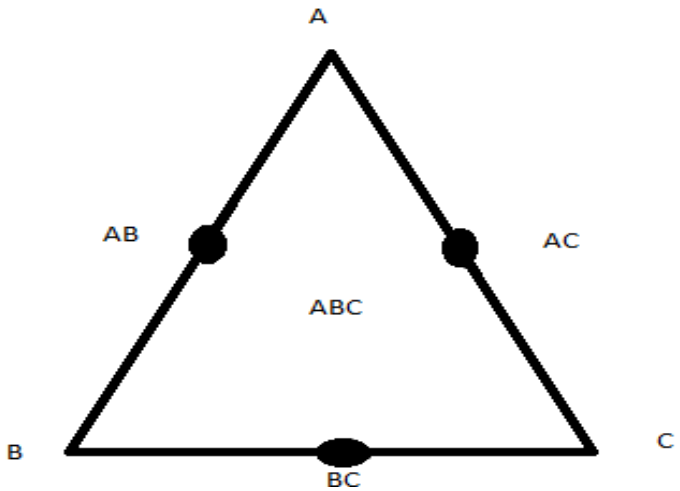


Fig. 2.1: Equilateral triangle representing simplex lattice design for three components.

2.2.6 Preparation of lornoxicam loaded microemulsion

The microemulsion system was prepared by mixing oil, surfactant, co-surfactant with an appropriate amount of drop by drop water. The microemulsion obtained by magnetic stirring for 30 mins at ambient temperature. Then a fixed quantity of lornoxicam was dissolved in mixture under ultrasonication. The final concentration of lornoxicam in microemulsion system was 0.075%w/w.

2.2.7 Characterization and Evaluation of Microemulsion

The prepared batches of microemulsion (F1-F7) were evaluated for optical transparency, globule size, phase separation, pH, viscosity, and zeta potential. Optical transparency of the formulations were determined by inspecting the sample in clear and transparent container under the presence of good light against reflection into the eyes, and viewed against black and white illuminated background.[25] Microemulsion system were subjected to centrifugations at 5,000 rpm for a period of 10 min. and examined for any change in phase separation.[27] The average globule size was measured using a NANOPHOX (NX0088) Cross corrélation. The measurement was performed at 25°C.[24] A 10% dispersion of formulation was prepared in distilled water and pH was determined by using Lab India pH meter standardized with standard buffers of pH 4 and pH 7.4.[3] The viscosities of microemulsion were measured using a Brookfield rotational viscometer equipped with the spindle no. 64. The measurement was performed at ambient temperature and in triplicate (Zhao X et al., 2006). Zeta potential is determined by using Zetasizer. Zeta potential is essentially useful for assessing flocculation since electrical charges on particles influence the rate of flocculation.

In vitro Diffusion study

The abdominal skin samples were obtained from male Wistar rats weighing 220±20 g. After hair was shaved, the skin was excised from the abdominal region of each sacrificed rat and the subcutaneous fat and other extraneous tissues were trimmed. The excised rat skins were washed, then stored at 4°C and used within 24 hours after the skin harvest.[24] The in vitro permeation rate of lornoxicam from various microemulsion formulations were determined to evaluate the effects of the formulation factors. The diffusion experiments were performed using Franz diffusion cells having an effective diffusion area of 2 cm^2 with an excised rat skin (Wistar Albino rat) at 37 ± 0.2°C. The receptor compartment was filled with 20 ml of phosphate buffer saline of pH 7.4. The receptor fluid was constantly stirred by magnetic

bead. Accurately weighed 2 ml of lornoxicam microemulsion was placed in the donor compartment. One ml of sample aliquots were withdrawn from the receptor fluid at predetermined time intervals up to 8 hours from the beginning of experiment. An equal volume of the fresh phosphate buffer was immediately replenished after each withdrawal sampling. The amount of drug diffused across the skin was quantified by analyzing the samples with UV spectrophotometer. The steady state flux (Jss) was calculated from the slope of graph of Cumulative Amount of Drug Diffused per unit area (CADD) vs. Time.[3,23,25]

Skin irritation studies

Optimized formulation and its blank microemulsion without any drug were selected as test formulations for the skin irritation studies. All samples were applied to the shaved skin on the back of six Wistar Albino rats, and then rats were secured. On one side of the back, a blank microemulsion and on another side optimized formulation was applied for each day. The animals were observed and evaluated for any sign of erythema or oedema for a period of 3 days.[25]

Stability studies

The optimized formulation was stable (particle size, phase separation, pH and drug content) at 37°C during 3 months storage. The centrifuge test was performed to assess the physical stability. Microemulsion was stored at 5, 15, 25 and 37°C to check the clarity and phase behavior stability. The drug content in the formulation was estimated by UV spectrophotometer.

3. RESULTS AND DISCUSSION

3.1 Calibration curve of Lornoxicam in 0.1N methanolic NaOH & pH 7.4 Phosphate Buffer Saline

The ultra violet scan of 10 µg/ml of lornoxicam was showed the absorbance peaks at (λ_{max}) 290 and 381 nm. The calibration curve of lornoxicam was prepared with 4-20 µg/ml concentration. The plot of different concentrations of lornoxicam versus absorbance was found to be linear in the concentration range 4-24 µg/ml at 381 nm. The absorbance values of lornoxicam were shown in Table 3.1.

Table 3.1: Absorbance data of Lornoxicam

Sr. No.	Concentration (µg/ml)	Absorbance in 0.1N Methanolic NaOH	Absorbance in pH 7.4 PBS
1	4	0.2127	0.2122
2	8	0.3585	0.4701
3	12	0.5071	0.5138
4	16	0.6711	0.6993
5	20	0.8278	0.9410

Slope, correlation coefficient and intercept on Y-axis values were found to be 0.052, 0.999 & 0.038 for 0.1N Methanolic NaOH whereas 0.061, 0.962 & 0.042 for pH 7.4 PBS, respectively. Studies were performed using solvents like 0.1N methanolic NaOH for evaluation of drug content and PBS (pH 7.4) for drug diffusion.

3.2 DSC and FTIR study of Lornoxicam

The DSC scan with a sharp exothermic peak (Fig. 3.1) at 223.3°C corresponding to lornoxicams melting transition temperature was observed. The purity of drug was further supported by FTIR studies. The observed peaks with functional groups were 1646 cm^{-1} (C=O primary amide), 1595 cm^{-1} & 1553 cm^{-1} (N-H bending, secondary amide), 1378, 1326 and 1148 cm^{-1} (O=S=O Streching), 831 cm^{-1} (-CH aromatic ring, bending), 767 cm^{-1} (C-Cl bending) and 3067 cm^{-1} (=C-H aromatic stretch).

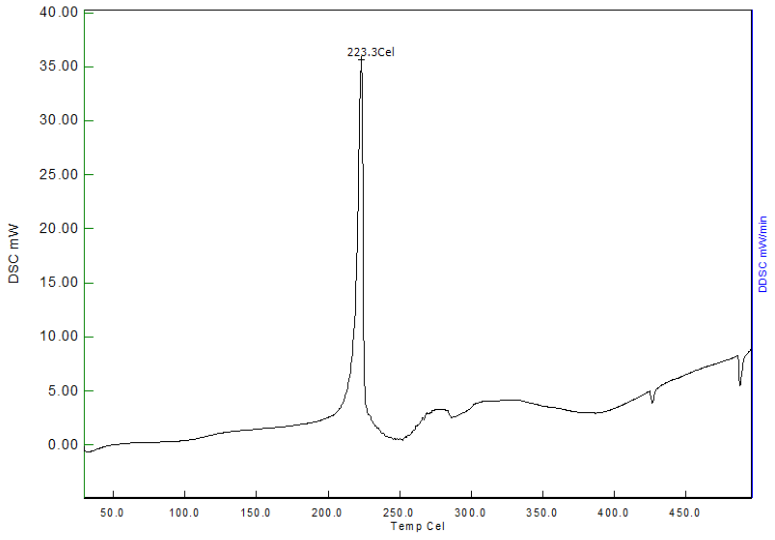


Fig. 3.1: DSC graph of Lornoxicam

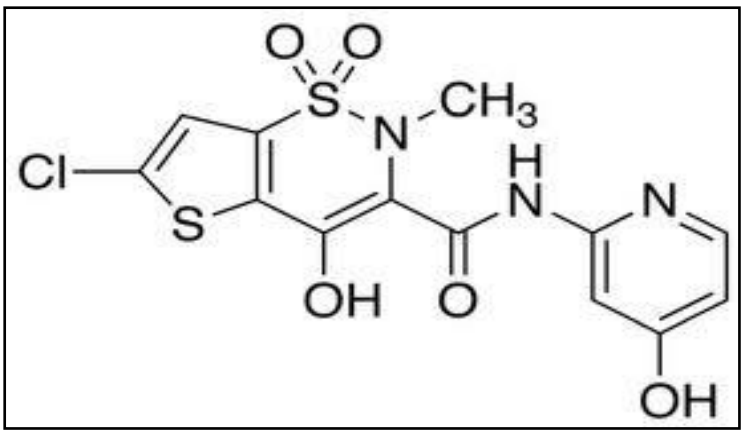


Fig. 3.2: Structure of Lornoxicam

3.3 Solubility study and Selection of Oil, Surfactant and co-surfactant for Formulation of microemulsion:

Table 3.2: Solubility data of Lornoxicam (µg /ml)

Oils	Solubility	Surfactants	Solubility	Co-surfactants	Solubility
Oleic acid	282.2	Ethanol	193.7	Tween-80	680.9
IPM	029.4	Capmul C8	240.3	Tween-60	608.3
Capmul	242.2	Glycerin	118.5	Tween-20	530.6
Eucalyptus Oil	183.0	Propylene glycol	294.8		

A choice of excipients which are reported as a well effective oil phases, were used to analyze the solubility of lornoxicam. In four oils, the oleic acid[25] showed highest solubility followed by capmul,[24] Eucalyptus Oil[28] and IPM.[23] It was also reported that oleic acid was a powerful enhancer for dermal delivery since it could increase fluidity of lipid portion of the stratum corneum which resulted in a permeation enhancing effect, so oleic acid was the choice of oil phase for the preparation of microemulsions containing lornoxicam. Thus the great solubilizing ability of the microemulsions results in the better dermal flux, and consequently to larger concentration gradient towards the skin. In surfactants and cosurfactants, tween 80 and propylene glycol showed a maximum solubility of lornoxicam compared to other surfactants and cosurfactants, respectively as shown in table 3.2. Microemulsion is an optically transparent system hence one of the important criteria for microemulsion preparation is that the selected oil and surfactant combination should show very high % transmittance (~ 99%). It was observed that the combination of oleic acid and Tween-80 showed 99.52 % transmittance and hence proved the choice of components for the preparation of microemulsion. Also Tween-80 and PG can be able to act as penetration enhancer. Thus in present study, the oleic acid, Tween-80 and PG were subsequently used as oil, surfactant and cosurfactant for the formulation of microemulsion containing lornoxicam.

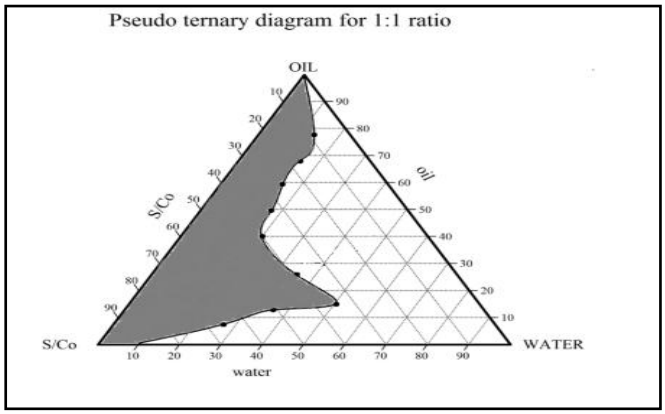
3.4 Preparation of Microemulsion

3.4.1 Construction of pseudo-ternary phase diagrams

The construction of pseudo-ternary phase diagrams was used to get suitable concentration range of components in the area of forming microemulsion. The pseudo-ternary phase diagrams of microemulsions composed of oleic acid; tween-80, PG and distilled water with various surfactants to cosurfactant ratios were shown in Fig. 3.3 (a, b and c). The area of microemulsion became enlarged as surfactant to cosurfactant ratio increased, reaching the maximum at surfactant to cosurfactant ratio of 2:1. The data for constructing pseudo ternary diagrams were given in table 3.3. Unshaded part in each phase diagram indicates the region of two immiscible phases, whereas all plotted points indicates the instantaneous formation of microemulsions for respective oil to water ratios with specific amount of surfactant/cosurfactant ratio. From diagrams it was concluded that microemulsion existing zone was more with the surfactant: cosurfactant ratio of 2:1 as compared to the other ratios (1:1) and (3:1). Hence 2:1 ratio of surfactant:co-surfactant was promising for preparation of microemulsion.

Table 3.3: Data for Phase Diagram of S/Co (Tween 80: PG)

Oil: S/Co ratio	1:1		2:1		3:1	
	% Oil	**% S/Co**	**% Oil**	**% S/Co**	**% Oil**	**% S/Co**
1:9	7.30	65.69	5.21	46.88	5.13	46.15
2:8	12.74	50.96	10.93	43.72	11.63	46.51
3:7	14.85	34.65	18.07	41.57	17.96	41.92
4:6	25.81	38.71	22.73	34.09	25.00	37.50
5:5	40.00	40.00	26.32	26.32	27.32	27.32
6:4	49.59	33.06	35.50	23.08	37.27	24.84
7:3	59.32	25.42	50.00	21.43	45.75	19.61
8:2	67.80	16.95	54.79	13.70	59.70	14.93
93:1	77.59	8.62	70.87	7.87	68.70	7.63



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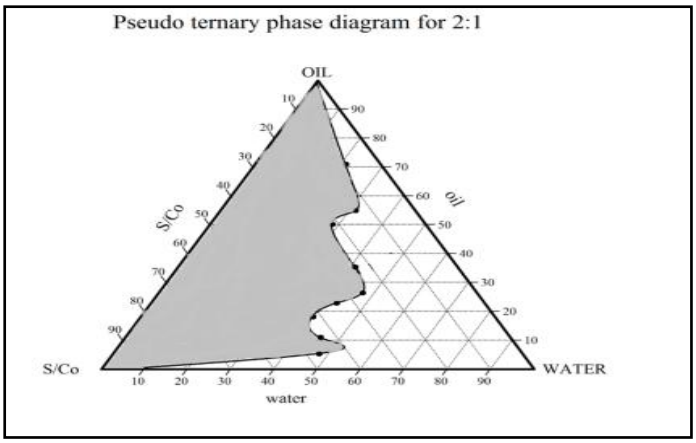

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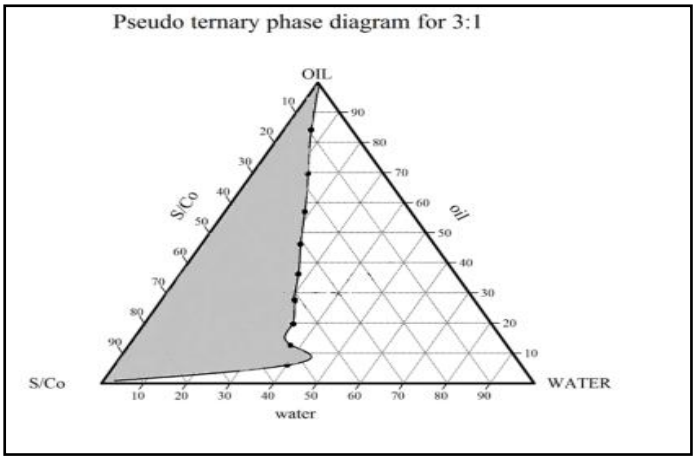

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Fig. 3.3 Microemulsion region of ternary phase diagrm having of S/Co (Tween 80: PG) ratio a) 1:1, b) 2:1 and c) 3:1.

The increasing concentration of surfactant in S/Co ratio leads to rise in the microemulsion region because of enhanced hydrolipophilicity, where as further rise in the surfactant concentration leads to too much hydrophilicity (Tween-80, HLB-15) which fells to emulsification with oil phase. The surfactant: co-surfactant quantity was required up to 40-50% to form a microemulsion. Therefore the quantity of surfactant and cosurfactant was selected in the range of 15-40% while designing prototype formulation.

3.4.2 Design and Optimization of Microemulsion

A simplex lattice experiment design was adopted to optimize the composition of microemulsion.[5] In this design three factors were evaluated by changing their concentration simultaneously and keeping their total concentration constant. Because of high content, oleic acid could cause skin irritation, the 5% was chosen as oil phase in this study. Also it was well

reported the relationship between hydration effect of stratum corneum and dermal permeation, 40-65% water content was chosen as water phase. Both the surfactant and cosurfactant were chosen in 15-40%. To simplify the actual concentrations of surfactant, cosurfactant and water, simplex lattice method was used. The minimum concentration corresponds to zero and maximum concentration corresponds to one. The different microemulsion formulations were prepared by water titration method as per detailed compositions of table 3.4. The drug was dissolved with the aid of ultrasonication.

Table 3.4 Design of Microemulsion Formulations

Formulation	Lornoxicam	Oleic acid	Tween-80	PG	Water
F1	75 mg	5 %	15 % (0)	15 % (0)	65 % (1)
F2	75 mg	5 %	40 % (1)	15 % (0)	40 % (0)
F3	75 mg	5 %	15 % (0)	40 % (1)	40 % (0)
F4	75 mg	5 %	27.5 % (0.5)	15 % (0)	52.5 % (0.5)
F5	75 mg	5 %	15 % (0)	27.5 % (0.5)	52.5 % (0.5)
F6	75 mg	5 %	27.5 % (0.5)	27.5 % (0.5)	40 % (0)
F7	75 mg	5 %	23.33% (0.33)	23.33 % (0.33)	48.33 % (0.33)

3.5 Evaluation of Prepared Microemulsion

All the formulation batches were analyzed for the optical transparency, globule size, viscosity, pH and zeta potential. All the formulation batches were found to be transparent in nature. The globule size of all prepared formulations observed in between 0-500 nm range which was acceptable range for microemulsions. Formulation F2 shown the least globule size as compared to the all other microemulsions, this might be due to presence of appropriate surfactant, cosurfactant and oil concentration. The surfactant and co-surfactant reduces the interfacial tension formed between oil and water phase and helps to reduce the globule size. Droplet size distribution of formulation F2 was given in figure no: 3.6 which were measured by NANOPHOX cross correlation.

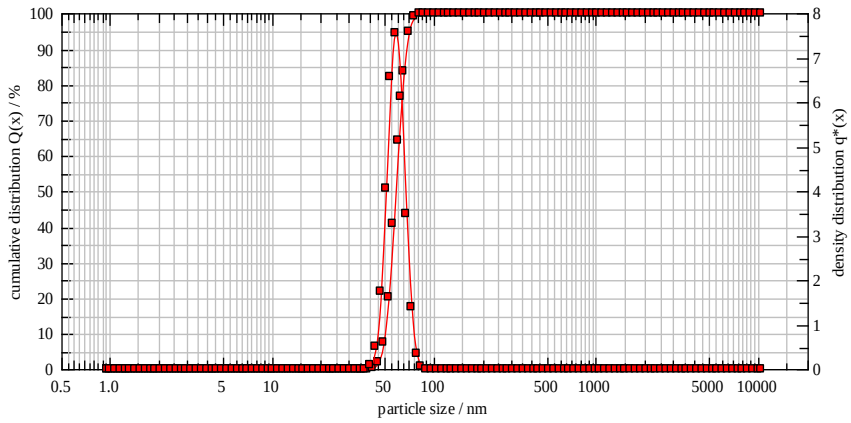

Fig. 3.4 Globule size distribution of microemulsion

The microemulsion being the combination of oil, surfactant, cosurfactant and water, these could affect the viscosity of the formulation. Formulation F2 which contains least amounts of water, with proper amount of surfactant, and it may be due to which it shows highest viscosity as compared to all formulations. Formulation F1 shows least viscosity this could be due to presence of high concentration of water in formulation and very low concentration of the surfactant. Also as the concentration of surfactant co-surfactant mixture increases the viscosity of formulation get increased.[29] None of the microemulsion systems showed signs of phase separation on centrifugation at 1000 rpm for 30 minutes. This result provided a rapid and full proof identification of the system as microemulsion, and which was the sign of stability of microemulsion.

Table 3.5: Characterization Mcroemulsion Formulations

Formulation	Optical activity	Globule Size (nm)	Viscosity (cp)	Phase Separation	pH
F1	Transparent	85.72	60	No	6.20
F2	Transparent	57.09	78	No	6.25
F3	Transparent	195.44	69	No	6.21
F4	Transparent	342.96	65	No	6.30
F5	Transparent	142.72	73	No	6.12
F6	Transparent	141.9	67	No	6.35
F7	Transparent	165.58	70	No	6.25

The pH of all formulations was found in between 6-6.5 which was acceptable for pH of skin.[3] This is an important parameter as the skin pH ranges between pH 5.5-6.5. The results of optical transparency, globule size, viscosity and pH were showed in table 3.5. The zeta potential of optimized formulation F2 was determined by using Zetasizer. The microemulsion having -3.15 charge on each globule, and that could responsible for the repulsion of globule from each other and that not allows the globule to settle down for longer period of time, indirectly causing the long stability of the F2 formulation.

Table 3.6: Zeta Potential of F2 Formulation

Results			Mean (mV)	Area (%)	Width (mV)
Zeta Potential(mV)	-3.15	Peak 1	-2.59	93.9	5.09
Zeta Deviation (mV)	6.25	Peak 2	-19.6	5.5	2.72
Conductivity(mS/cm)	0.0995	Peak 3	17.2	0.6	3.00

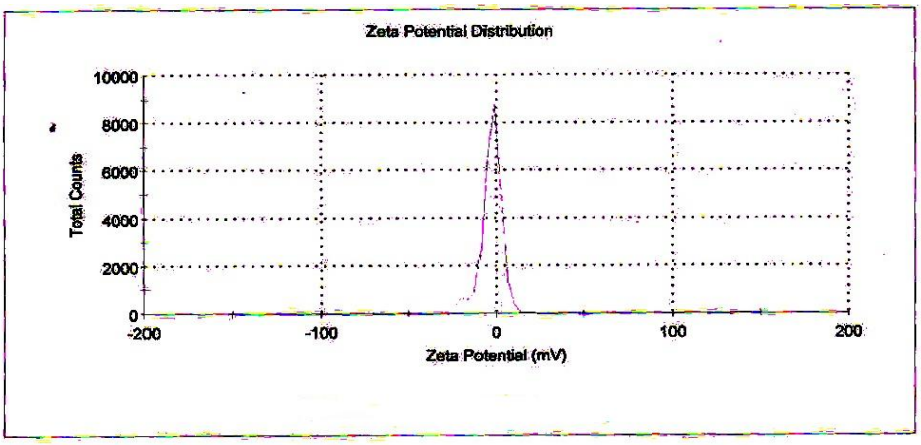

Fig. 3.5: Zeta potential of F2 microemulsion

3.5.1 Skin Irritation Studies

The skin irritation studies did not showed any visible irritation after application of F1-F7 formulations for 3 days on the skin of Wistar Albino rats. Neither erythma nor oedema was observed on the skin of Wistar Albino rats. But rubefaction appeared on some rat's skin on first day which was subsequently disappeared on the second and third day. The microemulsion might reduce the skin irritation which was likely induced by the active ingredient in many cases. This leads to make the microemulsion system viable for the topical drug delivery (Chen et al., 2006;).

3.5.2 *In vitro* diffusion study from microemulsion

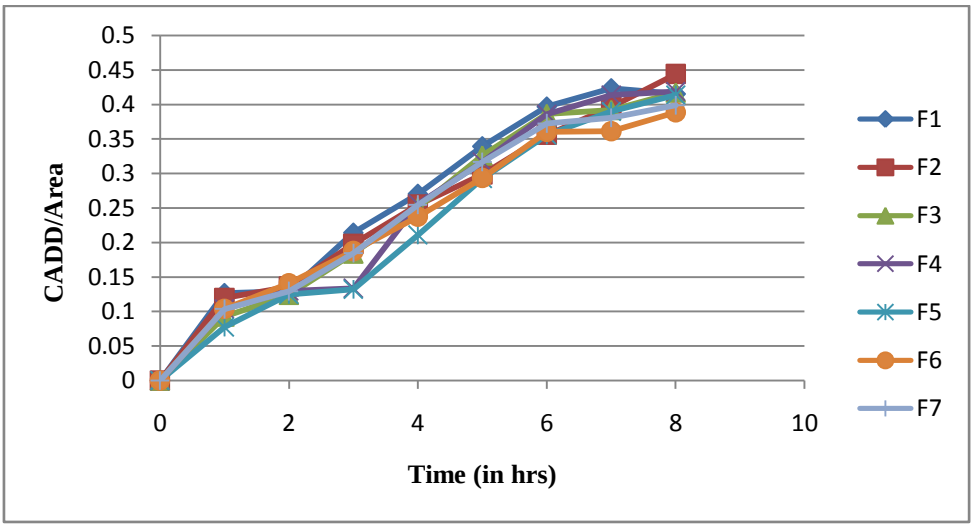

Fig. 3.6 Cumulative amount of Lornoxicam diffused/ unit area from microemulsion formulations through excised rat skin using Franz diffusion cell.

The in vitro drug release profile of lornoxicam microemulsions through excised rat skin were represented in above figure 3.6 and table 3.7. All the formulation shown the drug release about 25-30% through excised albino rat skin within the 8 hours time period, which was acceptable for the topical formulations and were meant for the localized effect, not the systemic effect.

Table 3.7: Percutaneous Permeation Parameters of Lornoxicam through Excised Rat Skin from Different Microemulsion Formulations

Formulation	% Drug Release	CADD/unit area	Flux(Jss) ($mg/Cm^2/hr$)	Permeability Coefficient (Kp)
F1	25.92	0.1238	0.0162	0.0108
F2	29.59	0.1413	0.0261	0.0101
F3	27.90	0.1333	0.0207	0.0138
F4	27.56	0.1316	0.0209	0.0159
F5	27.82	0.1329	0.0194	0.0129
F6	27.67	0.1321	0.0201	0.0134
F7	27.72	0.1324	0.0204	0.0135

Formulation F2 had smallest droplet size with greater % drug release, highest cumulative drug release (CADD/unit area), higher flux value (Jss) and better permeability coefficient (Kp) than the other microemulsion formulations, hence F2 was considered as optimized formula for microemulsion preparation. Though topical formulation ideally should releases minimum drug concentration in the systemic circulation, yet one has to consider other parameters like the particle size which also affects on the permeation. Formulation 2 also had the better viscosity as compare to other formulations as far as topical application is concern. When microemulsion was applied on skin, the oil of microemulsion was expected to penetrate the stratum corneum and to stay, intact in the whole horny layer altering both the lipid and the polar pathways. The drug dissolved in oil phase of microemulsion can intercalate between the lipid chains of the stratum corneum, thereby destabilizing its bilayer structure. Subsequent interactions will lead to enhanced permeability of the drug. Also the surfactant Tween-80 assists in the penetration of drug which was in high concentration in the formulation.

3.5.3 Stability Studies

Stability studies of microemulsion subjected to accelerated stability study at 40°C and 75% relative humidity for 1, 2 and 3 months, respectively; and there was no change in physical parameters and pH of microemulsion.

4. CONCLUSION

The microemulsion of lornoxicam was designed by simplex lattice mixture design and the formulation F2 containing a 5% oleic acid, 40% Tween and 15% PEG was the optimized batch which showed smaller particle size of 57.09 nm, viscosity of 78 CP and good stability along with drug delivery on topical application of poorly water soluble drug. Finally, results revealed that topical formulation of Lornoxicam microemulsion was becoming promising system to overcome shortcomings associated with conventional delivery.

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Conflict of interest : Nil

6. REFERENCES

1. Djekic L, Primorac M. (The influence of cosurfactants and oils on the formation of pharmaceutical microemulsions based on PEG-8 caprylic/capric glycerides). Int. J. Pharm. 2008; 352: 231–239.
2. Liu CH, Chang FY, Hung DK. (Terpene microemulsions for transdermal curcumin delivery: effects of terpenes and cosurfactants). Colloids Surf. B: Biointerfaces. 2011; 82: 63–70.
3. Hathout RM, Woodman TJ, Mansour S, Mortada ND, Geneidi AS, Guy RH. (Microemulsion formulations for the transdermal delivery of testosterone). Eur. J. Pharm. Sci. 2010; 40: 188–196.
4. Yuan JS, Ansari M, Samaan M, Acosta EJ. (Linker-based lecithin microemulsions for transdermal delivery of lidocaine). Int. J. Pharm. 2008; 349: 130–143.
5. Zhu W, Yu A, Wang W, Dong R, Wu J, Zhai G. (Formulation design of microemulsion for dermal delivery of penciclovir). Int. J. Pharm. 2008; 360:184–190.
6. Pakpayat N, Nielloud F, Fortune R, Tourne-Peteilh C, Villareal A, Grillo I, Bataille B. (Formulation of ascorbic acid microemulsions with alkyl polyglycosides). Eur. J. Pharm. Biopharm. 2009; 72: 444–452.
7. Chaiyana W, Saeio K, Hennink WE, Okonogi S. (Characterization of potent anti-cholinesterase plant oil based microemulsion). Int. J. Pharm. 2010; 401:32–40.

8. Yuan JS, Yip A, Nguyen N, Chu J, Wen XY, Acosta EJ. (Effect of surfactant concentration on transdermal lidocaine delivery with linker microemulsions). Int. J. Pharm. 2010; 392: 274–284.
9. Araujo LM, Thomazine JA, Lopez RF. (Development of microemulsions to topically deliver 5-aminolevulinic acid in photodynamic therapy). Eur. J. Pharm. Biopharm. 2010; 75: 48–55.
10. Kogan A, Rozner S, Mehta S, Somasundaran P, Aserin A, Garti N, Ottaviani. (Characterization of the nonionic microemulsions by EPR. I. Effect of solubilized drug on nanostructure) J. Phys. Chem. B. 2009; 113: 691–699.
11. Lawrence MJ, Rees GD. (Microemulsion-based media as novel drug delivery systems). Adv. Drug Deliv. Rev. 2000; 45: 89–121.
12. Zhao XY, Cao Q, Zheng LQ, Zhang GY. (Rheological properties and microstructures of gelatin-containing microemulsion-based organogels) Colloids Surfaces A: Physicochem. Eng. Aspects. 2006; 281: 67–73.
13. Date AA, Nagarsenker MS. (Parenteral microemulsions: an overview). Int. J. Pharm. 2008; 355: 19–30.
14. Foye WO, Thomas LL, David AW. Principles of Medicinal Chemistry. 4th ed^{n}; 1995; Williams and Wilkins, USA: 335
15. Bissett DL, Chatterjee R, Hannon DP. (Photoprotective effect of topical anti-inflammatory agents against ultraviolet radiation- induced chronic skin damage in the hairless mouse). Photodermatol. Photoimmunol. Photomed. 1990; 7:153–158.
16. Radhofer-Welte S, Rabasseda X. (Lornoxicam, a new potent NSAID with an improved tolerability profile). Drugs Today. 2000; 36: 55–76.
17. Hitzenberger G et al. (Pharmacokinetics of lornoxicam in man). Postgrad Med J. 1990; 66: 22–26.
18. Radhofer-Welte S et al. (Comparative bioavailability of lornoxicam as single doses of quick-release tablet, standard tablet and intramuscular injection: a randomized, open-label, crossover phase-I study in healthy volunteers). Clin Drug Investig. 2008; 28: 345–351.
19. Rosenow D. (A comparison of patient-controlled analgesia with lornoxicam versus morphine in patients undergoing lumbar disk surgery). Anesthesia & Analgesia (International Anesthesia Research Society). 1998; 86: 1045-1050.

20. Møller PL, Nørholt SE. (Analgesic efficacy of quick-release versus standard lornoxicam for pain after third molar surgery: a randomized, double-blind, placebo-controlled, single-dose trial). Clin Drug Investig. 2008; 28: 757–766.
21. Saricaoglu F et al. (Effect of intra articular injection of lornoxicam on the articular cartilage & synovium in rat). Indian J Med Res. 2008; 127: 362–365.
22. Prajapati P, Vaghela V, Rawtani D. (Azeotropic mixture used for development and validation of Lornoxicam in bulk and its tablet dosage form by spectrophotometric method). Journal of pharmaceutical analysis. 2012; 2 (4): 306-309.
23. Baroli B, Arturo Lopez-Quintela M *et al.* (Microemulsion for topical delivery of 8-methoxsalen). Journal of Controlled Release. 2000; 69: 209-218.
24. Yuan Y, Li S, *et al.* (Investigation of microemulsion system for transdermal delivery of Meloxicam). International Journal of Pharmaceutics. 2006; *321:* 117–123.
25. Chen H, Chang X, Du D, *et.al*. (Microemulsion-based hydrogel formulation of Ibuprofen for topical delivery). International Journal of Pharmaceutics. 2006; 315: 52–58.
26. Panapisal V, Charoensri S, Tantituvanont A. (Formulation of Microemulsion Systems for Dermal Delivery of Silymarin). AAPS PharmSciTech. 2012; 1-11.
27. Rhee Y, Choi J *et al.* (Transdermal delivery of Ketoprofen using microemulsions). International Journal of Pharmaceutics. 2001; *228*: 161–170.
28. Biruss B, Kahlig H, Valenta C. (Evaluation of an eucalyptus oil containing topical drug delivery system for selected steroid hormones). International Journal of Pharmaceutics, 2007; 328 (2), 142-151.
29. Zhao X, Liu J *et al.* (Enhancement of transdermal delivery of Theophylline using microemulsion vehicle). International Journal of Pharmaceutics. 2006; 1-7.