WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 10, 1305-1328. Research Article ISSN 2277– 7105

FORMULATION AND EVALUATION OF ACECLOFENAC NANOEMULSION

Raj Kumar Mishra* Prof. Dr. S.K Prajapati Mr. G.C Soni

Institute of Pharmacy Bundelkhand University, Jhansi- 284128(UP) INDIA.

Article Received on
04 Oct 2014,

Revised on 30 Oct 2014,
Accepted on 25 Nov 2014

***Correspondence for Author**
Raj Kumar Mishra
Institute of pharmacy
Bundelkhand University ,
Jhansi- 284128(UP)
INDIA.

ABSTRACT

Present study is completely focused on the development of nanotechnology based drug delivery system, which not only provide desired action in predetermined testamonials but also show rapid and controlled release as per need. Nowdays we are very much aware of this thing that numerous of drugs are rejected from market every day the basic reason behind it is not this that these drugs are not potent to treat but sometimes they are not well absorved due to low strength of delivery system used ,so in the same concern present investigation is based on the development, evaluation of aceclofenac nanoemulsion. In which we have performed litrature review in which we found that delivery system like nanoparticles Nano gel niosomes proniosomes nanoemulsion nanosponges etc are very much fruitful for the delivery of poorly water soluble drugs which are very poorly absorved in conventional forms and hence low potency is seen First of all pre-formulation study is done in this course all the ingredients are tested for various phenomenon such as oil screening is done, safsol+oleic acid is selected as oil phase, calibration curve is plotted after UVabservation of drug to check out the purity of drug sample ACF after that IR of drug, surfactantand co-surfactant used i.e. ACF, PEG400, TWEEN 80 are done to check out the compatibility among them. As the formulation is based on the oral delivery so solubility profile is done for oils and drug partition coefficient of ACF was found i.e. Pseudo ternary phase diagram are plotted which make backbone for the formulation nanoemulsion using aqueous titration method from phase diagram different concentrations ofoil and surfectantand co-surfactant were selected based on the thermodynamic and dispensability test. Optimized formulation was selected forin vivo studyon the basis of higher drug release, optimum globule size minimum polydispersityvalue, lower viscosity and overall lower significant value of co-surfactant. The difference in t max

of nanoemulsion found to be (p<0.07)when compared to API drug suspension whereas difference was in significant when compared with tablet. The difference in Cmax of nanoemulsionwas very significant(p<0.01> when compared with conventional tablet and API drug suspension Bioavailability of nanoemulsion in comparison to the the conventional taletis better and has a relevant increase thus nanoemulsion can be used effectively to improve bioavailability of poorly water soluble drugs.

KEYWORDS: Poorly water soluble, bioavailability, pseudo ternary phase diagram, nanoemulsion.

INTRODUCTION [1,2]

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs to reduce pain and inflammation. [1] Aceclofenac, an NSAID, has been recommended orally for the treatment of rheumatoid arthritis and osteoarthritis. [2,3] It also has anti-inflammatory, antipyretic, and analgesic activities. [4] The oral administration of aceclofenac causes gastrointestinal ulcers and gastrointestinal bleeding with chronic use. [2] Because of gastrointestinal bleeding, it also causes anaemia. Using the transdermal route eliminates these side effects, increases patient compliance, avoids first-pass metabolism, and maintains the plasma drug level for a longer period of time. Therefore, an improved aceclofenac nanoemulsion formulation with a high degree of permeation could be useful in the treatment of locally inflamed skin and inflammatory and painful states of supporting structures of the body, such as bones, ligaments, joints, tendons, and muscles. There has been increased interest during recent years in the use of topical vehicle systems that could modify drug permeation through the skin. Many of the dermal vehicles contain chemical enhancers and solvents to achieve these goals. [5] But use of these chemical enhancers may be harmful, especially in chronic application, as many of them are irritants. Therefore, it is desirable to develop a topical vehicle system that does not require the use of chemical enhancers to facilitate drug permeation through the skin. One of the most promising techniques for enhancement of transdermal permeation of drugs is nanoemulsion or nanoemulsion. Nanoemulsions are thermodynamically stable transparent (translucent) dispersions of oil and water stabilized by an interfacial film of surfactant and cosurfactant molecules having a droplet size of less than 100 nm. [6,7] Many studies have shown that nanoemulsion formulations possess improved transdermal and dermal delivery properties in vitro, [8-16] as well as in vivo. [17-19] Nanoemulsions have improved transdermal permeation of many drugs

over the conventional topical formulations such as emulsions [20,21] and gels. [22,23] This article describes the potential of nanoemulsion systems in transdermal delivery of aceclofenac using nonirritating, pharmaceutically acceptable ingredients without using additional permeation enhancers, because excipients of nanoemulsions themselves act as permeation enhancers.

Experimental [2,3]

Materials

Aceclofenac was gifted from Parry Pharmaceutical Pvt. Ltd. (India). Castor oil, sunflower oil, Oleic acid, Triacetin, Span® 20, Span® 80, Tween® 20, Tween® 80, PEG 4000, Cremophor EL, Octanol, Ethanol, and PEG 6000 were purchased from S.D. Fine chemicals, Mumbai, India. All chemicals and solvents used in this study were of analytical reagent grade. Freshly distilled water was used throughout the work.

Solubility of Aceclofenac [3,4]

The solubility of aceclofenac in various oils (Castor oil, sunflower oil and Oleic acid), surfactants (Span® 20, Span® 80, Tween® 20 and Tween® 80, Cremophor EL) and cosurfactants (PEG 4000, Ethanol, and PEG 6000) was determined by dissolving an excess amount of aceclofenac in 2 ml of each of the selected oils, surfactants, and cosurfactants in 5-ml capacity stoppered vials separately. A combination of oils was also used for determination of solubility. An excess amount of aceclofenac was added to each 5-mL-capacity stoppered vial and mixed using a vortex mixer. The mixture vials were then kept at 37 ± 1.0 C in an isothermal shaker for 72 hours to get to equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 3000 rpm for 15 minutes. The supernatant was taken and filtered through a 0.45-µm membrane filter. The concentration of aceclofenac was determined in each oil, surfactant, cosurfactant, and combination of oils by UV spectrophotometer at their respective λ max

S.No	Oils	Solubility (mg/ml)
1	Oleic acid	52.01±1.77
2	Isoproylmyristate (IPM)	17.22±0.25
3	Olive oil	08.25±0.45
4	Triacetin	02.81±1.79
5	Jojoba oil	07.57±0.97
7	Castor oil	08.35±0.84
7	Groundnut oil	05.25±1.52
8	Liquid paraffin	04.19±1.78
9	Labrafac	11.30±0.58
10	Sefsol 218	55.42±3.24
11	Jojoba oil+ Oleic acid(1:1)	51.50±1.2
12	Triacetin + Oleic acid(1:1)	87.07±2.05
13	Labrafac + Oleic acid(1:1)	49.57±0.87
14	Sefsol + Oleic acid (1:1)	130.07±2.78
15	Tween-20	51.23±2.73
17	Tween-80	40.80±1.02
17	Cremophor EL	14.92±0.9
18	PEG-400	41.92±2.35
19	Carbitol	45.44±1.7

Pre-Formulation Studies [4]

Pre-formulation studies should focus on those physicochemical properties of compounds that could affect drug performance and development of an efficacious dosage form. prefomulation research relates to pharmaceutical analytical investigation that both proceed and support formulation development efforts for all dosage forms. Taking into account early pharmacological and biopharmaceutical data, pre-formulation studies yield key information necessary to guide the formulator and analyst toward development of an elegant,stable dosage from with good bioavailability.

The objective of pre-formulation study is quantization of those physicochemical properties that will assist in developing a stable, safe and effective formulation with λmaximum bioavailability.

Physicochemical Charaterization Drug [5]

Physical Appearance

The drug sample was white powder. the drug substances color should be recorded by subjective description as well as by an means such as by comparison with standard color chips. The color of Aceclofenac was observed visually.

Odor

The odor of Aceclofenac was examined by cautiously smelling the head space of the drug container, which has been previously closed to allow volatiles to concentrate.

Melting Point

Melting Point is defined as the temperature at which the solid and liquid phases are equilibrium. The Melting Point of a drug can be measured by three techniques:

1. Capillary Method
2. Hot Stage Microscopy
3. Different Scanning Calorimeter Thermal Analysis.

A Melting Point determination is a good indication of purity of substance since the presence of relatively small amount of impurities can be detected by lowering as well as widening in the melting point range. Melting Point for drug Aceclofenac was determined using capillary method by finally powered the drug carefully dried in vaccum desiccators over silica gels over 24 hours. The capillary with the drug must be brought into contact with the heating medium at a temperature 5^0 below the expected lower limit of melting range the temperature is ramped about 1^0 per minute themelting range start when the substance begins to collapse and temperature noted when is completely molten it was found to bew 154^0.

Identification of Drug

Aceclofenac was indentified by ultraviolet spectroscopy and FTIR spectroscopy.

UV Special studies (λmax) of Aceclofenac

The UV spectrophotometry has been used for structural validation of drug in the identification studies. Molecules with structural unsaturation are able to absorb light within specific frequency range. The degree of unsaturation coupled with the presence of chromophores will influence the extent of absorption and whether UV(400-200 nm) or visible (800-400nm) light will absorb . The drug was dissolved in menthanol to produce 10 µg/ml solutions. this 10 µg/ml solution was scanned between 200-400 nm using the UV spectrophotometer (Shimadzu 1800 A,japan). the spectrum of the drug is given in fig. 6.1.

Fourier Transform Infrared (FTIR) Spectral Studies of Aceclofenac

The spectrum of Aceclofenac was obtained by means of a FTIR spectrophotometer. The KBr dispersion pellet of the given sample of Aceclofenac was prepared and spectrum was done by

FTIR spectrophotometer (Parkin Elmer, Singapore PTE Ltd). The spectrum is shown in fig. and characteristic peaks are given in tab.

Drug –Polymer Interaction Studies (Compatibility Studies)

The drug and polymer compatibility was characterized by means of FTIR spectroscopy. The compatibility was checked by making physical mixture of drug and polymer and then the FTIR analysis of the mixture was done. The peak should not be changed in FTIR spectra of mixtures and it should be similar to the pure drug and polymer FTIR spectra.

Partition Coefficient

The partition coefficient defined as the ratio of unionized drug distributed between the organic phase and aqueous phase in equilibrium for a drug delivery system. Partition coefficient provides a means of characterizing lipophillic and its ability to cross the lipoidal cell membrane in the oil/water system such as n-octanol/water.

partition coefficient of drug was determined in solvent system n-octanol /distilled water. Accurately weighed quantity of drug (10 mg) taken in stopper glass vials containing 5 ml of each solvent such as n-octanal and water. The glass vial final isolation of two phases were carried out by using the separating funnel were kept overnight for separation. The content of both phase were seprated. After appropriate dissolution, the aqueous phase was analyzed for cotrimazole against reagent blank solution using Shinmadzu 1700 UV spectrophotometer. The drug concentration in n-octanol phase is determined by subtracting the amount in aqueous phase from the total quantity of drug added to vial. The partition coefficient value P was calculated by following equation in below :

Po/w =

Where,

C organic = Concentration of drug in organic phase

C aqueous = Concentration of drug in aqueous phase

Po/w= Partition coefficient of drug in oil in water system.

Partition coefficient values of Aceclofenac

n-octanal/water	**7.3**

Solubility Studies of Drug

The solubility of Aceclofenac was determined in different solvents (e.g. distilled water, methanol, cholopharm ,DMSO phosphate buffer PH 7.4,6.8). A known amount of drug was transferred in a series of different solvents having 2 ml indifferent tissue culture tubes. Excess amount of drug was added to different solvent till the solution became saturated and these tubes were shaken by a mechanical shaker for 24 hours under constant vibration at constant temperature. After this period the solution were centrifuged. The supernatant was analyzed by UV spectrophotometer double beam were carried out before each sample to calculate the solubility of Aceclofenac in different solvent.

Spectrophotometer Estimation Aceclofenac

Standard curve of Aceclofenac in methanol

Preparation of Stock Solution

Accurately weighed Aceclofenac (10 mg) and transferred in a 100 ml volumetric flask and dissolved in a small amount of methanol by shaking gently and volume was made up to 100 ml with methanol. A working standard stock solution (10 µg/ml) was obtained by diluting 10 ml of this solution to 10 ml by methanol in a volumetric flask. 1 ml, 2 ml, 3 ml, 9 ml, of stock solution were transferred quantitevelly into series of 10 ml volumetric flask and volume was made up to 10 ml to produce solutions of concentration ranging 1 to 10 µg/ml.

Determination of λmax of Aceclofenac

the λmax of drug sample was determined by scanning 5 µg/ml standard stock solution in the range from 200-400 nm using Shimadzu -1700 UV spectrometer. The scan is shown in fig.

Preparation of Calibration curve

The calibration curve of Aceclofenac was prepared in methanol by preparing 1 to 10 µg/ml dilutions. Aliquot of 1,2,3,….10 ml of stock solution (10 µg/ml) were transferred quantitevelly into series of 10 ml volumetric flask and volume was made up to 10 ml to produce solutions of concentration ranging 1 to 10 µg/ml. The absorbance of solutions was determined at λmax (276 ml) against blank (menthnol). The absorption value is shown in table 3 and the linearity curve is shown in fig.

Standard curve of Aceclofenac in distilled water [6]

Preparation of Stock Solution

Accurately weighed Aceclofenac (10 mg) and transferred in a 100 ml volumetric flask and dissolved in a small amount of methanol by shaking gently and volume was made up to 100 ml with distilled water. A working standard stock solution (10µg/ml) was obtained by diluting 10 ml of this solution to 100 ml by distilled water in a volumetric flask.

Preparation of Calibration Curve

The calibration curve of Aceclofenac was prepared in distilled water by preparing 1 to 10 µg/ml dilutions. Aliquots of 1,2,3,…..10 ml of stock solution (10 µg/ml) were transferred quantitevelly into a eries of concentration ranging 1 to 10 µg/ml. The absorbance of solutions was determined at λmax (276 nm) against blank (distilled water).

Standard Curve of Aceclofenac in Phosphate Buffer (Ph 7.4)

Preparation of phosphate buffer saline PH 7.4 (PBS 7.4)

Accurately weighed 1.44 gm of disodium hydrogen phosphate ($Na_2HPO_4.2H_2O$), 0.24 gm of potassium dihydrogen phosphate (KH_2PO_4), 8.00 gm of Sodium Chloride (NaCl) and 0.20 gm of Potassium Chloride (KCl) and dissolved in sufficient distilled water to produce 1000 ml.

Preparation of Stock Solution

Aceclofenac (10mg) was accurately weighed and transferred in 100 ml volumetric flask and drug dissolved in a small quantity of methanol by gentle shaking and volume make up to 100 ml with phosphate buffer saline. A working standard stock solution (10 µg/ml) was obtained by diluting 10 ml of the solution to 100 ml by PBS 7.4 in a volumetric flask.

Determination of λmax of Aceclofenac [7]

The λmax of the drug sample was determined by scanning 10 µg/ml standard stock solution in the range from 200-400 nm using Shimadzu 1700 UV spectrometer.

Preparation of Calibration Curve

The calibration curve of Aceclofenac was prepared in Phosphate Buffer (Ph 7.4) by preparing 1 to 10 µg/ml dilutions. The aliquots of 1,2,3…..10 ml of stock solution (10 µg/ml) were transferred quantetivelly into a series of 10 ml volumetric flask and volume was made up to produce solutions of concentration ranging at λmax (276nm) against blank (Phosphate

buffer saline Ph 7.4). The absorption value is shown in table the linearity regressed curve is shown in fig.

Result of Preformulation

Physical State—Solid amourphous powder

Colour—White Powder

Odor—Characteristic

Taste—Bitter

Loss on Drying

An accurately weighed quantity of drug was taken in a clean, dried previously weighed bottle and dried in oven at 105^0C for 2 hours.

Weight of empty bottle = 10.9010 g (a)

Weight of empty bottle +Aceclofenac (before drying) = 11.9030 g (b)

Weight of empty bottle +Aceclofenac (after drying) = 11.890 g (c)

Weight of drug before drying d(b-a) = 1.002 g

Weight of drug after drying d(c-a) =.988 g

Loss on Drying = (d-e/d) x 100 =1.002-0.988/1.002x100 = 1.39%]

Table no1 Absorbance values with respect to different concentration of stock solution of ACF at 276 nm.

Concen-tration	Absor-bance
0	0.000
2	0.055
4	0.119
6	0.174
8	0.235
10	0.286
12	0.359
14	0.401
16	0.478
18	0.538
20	0.591

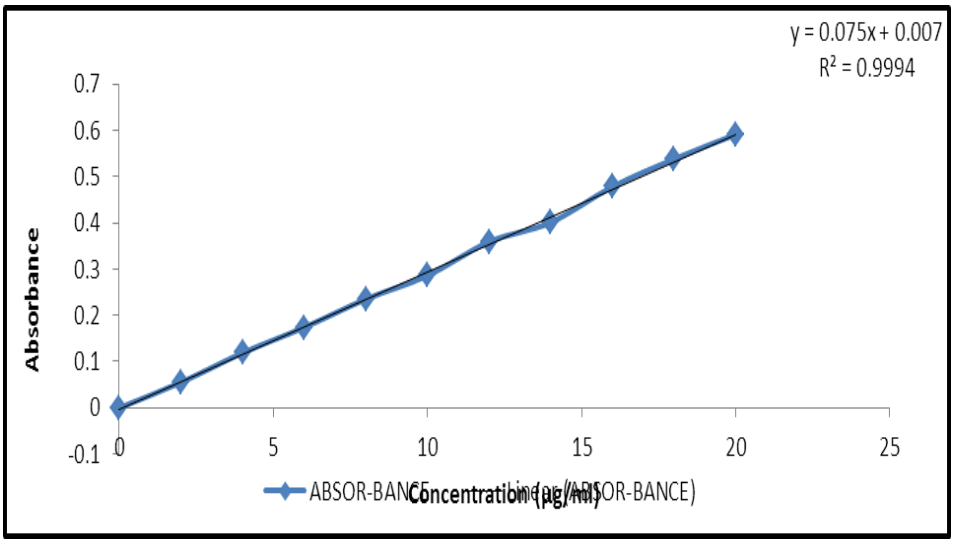


Fig no 1 . Calibration curve of aceclofenac

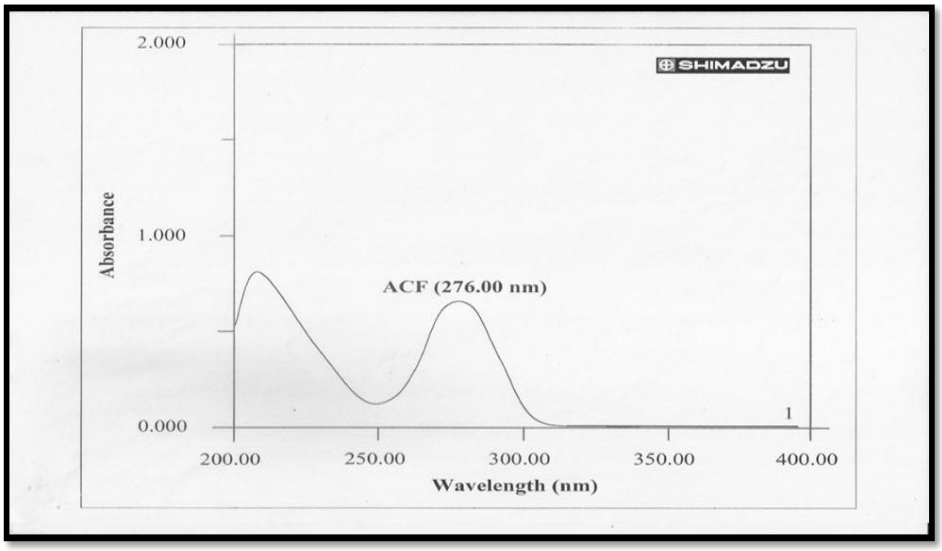
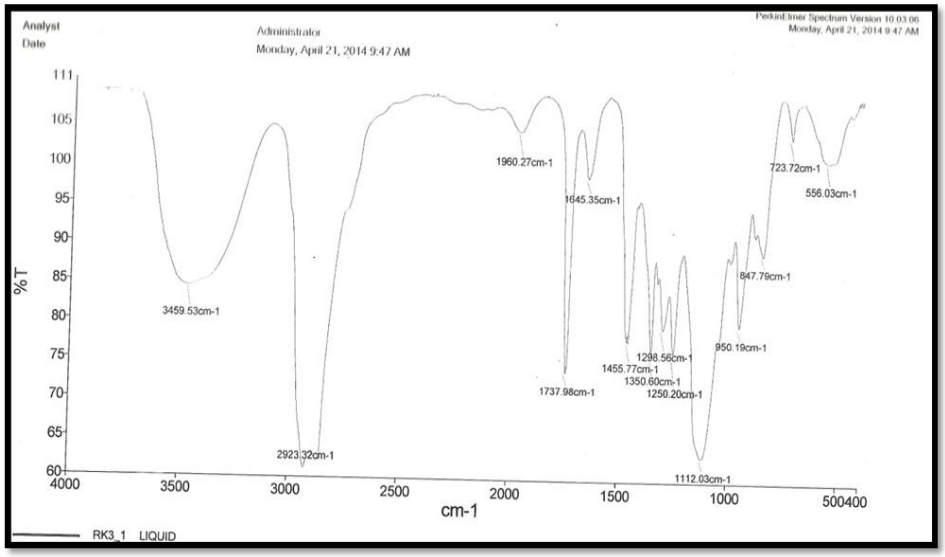
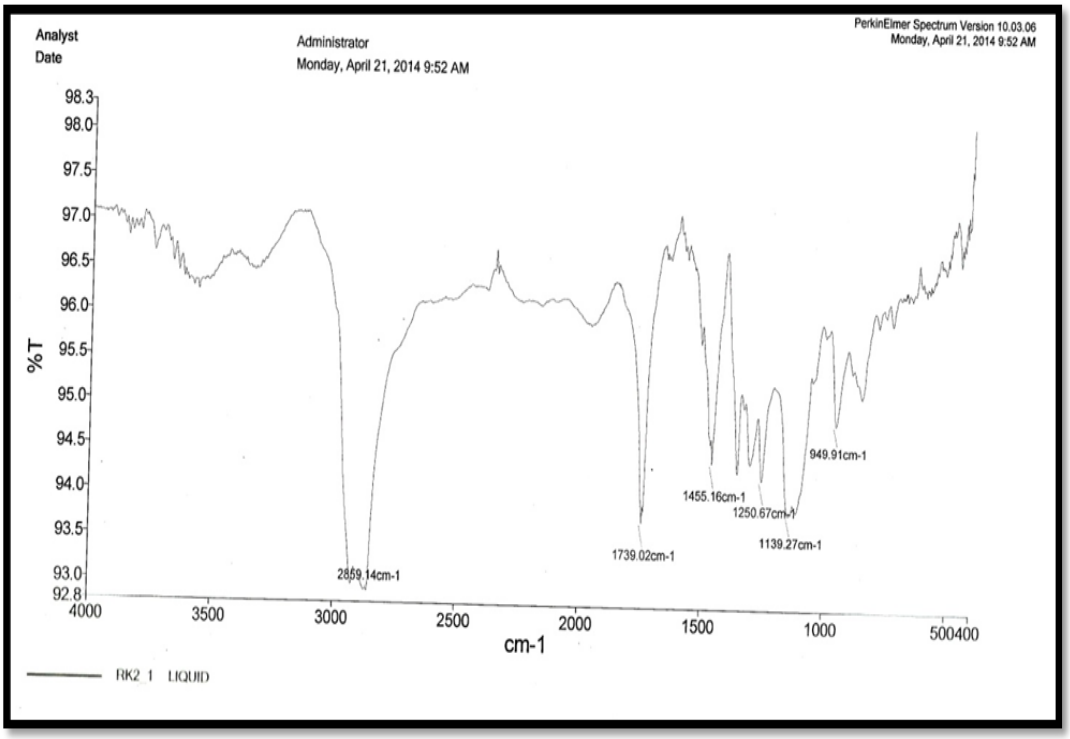


Fig no 2 Absorption spectra of ACF at 276 nm.

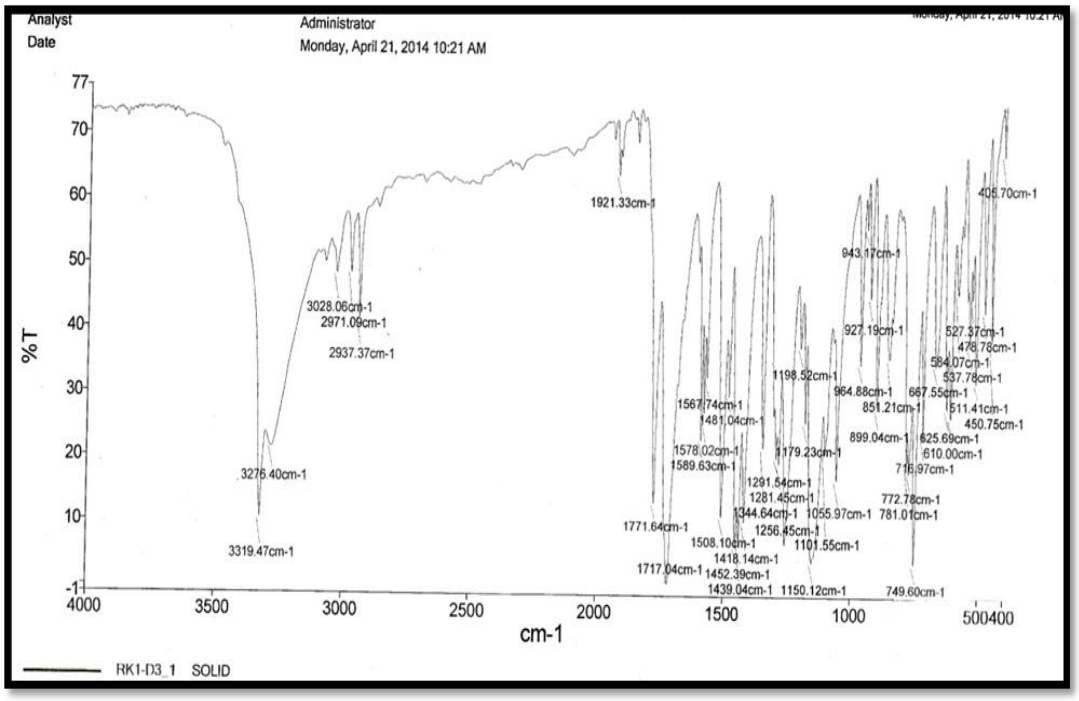


IR Spectra of ACF Drug

S.no	Peak(cm-1)	Groups
1	3318.58	N-H stretching
2	1717.14	C=O stretching
3	1506.70	C-H stretching
4	1147.85	CO-C stretching
5	749.46	C-CL streching



IRSpectrum of tween 80



IR Spectrum of polymer+ACF

Procedure [7,8]

For each phase diagram, oil and specific Smix ratio was mixed thoroughly in different volume ratios from 1:9 to 9:1 in different small glass test tubes. Sixteen different combinations of oil and each Smix, 1:9, 1:8, 1:7, 1:7, 1:5, 2:8 (1:4), 1:3.5, 1:3, 3:7 (1:2.3), 1:2, 4:7 (1:1.5), 5:5 (1:1), 7:4 (1:0.7), 7:3 (1:0.43), 8:2(1:0.25), 9:1 (1:0.1) were made so that maximum ratios were covered for the study to delineate the boundaries of phases precisely formed in the phase diagrams.For the determination of existence zone of microemulsion, pseudoternary phase diagrams were constructed using water titration method (Shafiq et al., 2007). To construct pseudoternary phase diagrams, the oil phase (oleic acid: Sefsol, 1:1) was mixed with different ratio of surfactant and cosurfactant (Tween 20 and Carbitol® respectively) and mixture was titrated with distilled water until it turned turbid. Examine each and every point I detailed and note it down. Pseudo ternary phase diagrams were drawn by using data obtained in aqueous titration method as shown in Figure. (A-G). The amount of water added to give water concentration in the range of 5-95% of total volume at 5% intervals. After every 5% addition of the water to the oil and Smix mixture, visual observation were made as shown in . The ratio of surfactant and co surfactant (Tween 20 and Carbitol®) were used for the titration are, 1:0,1:1,1:2,1:3,2:1,3:1 and 4:1 respectively.

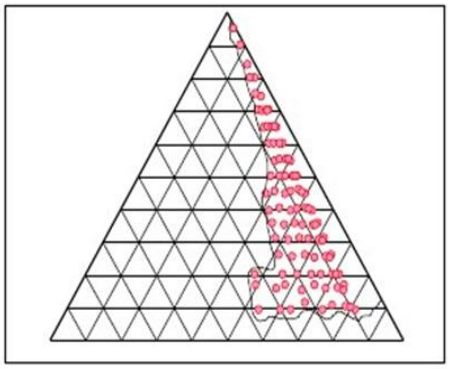


Figure D Phase diagram of Smix 1:

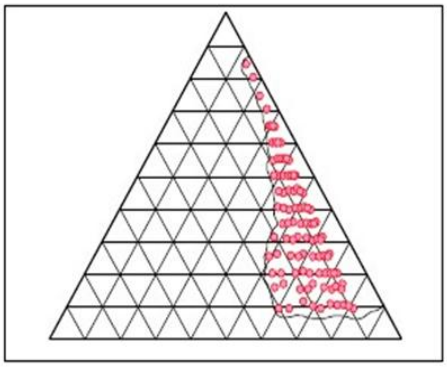


Figure C Phase diagram of Smix 1:2

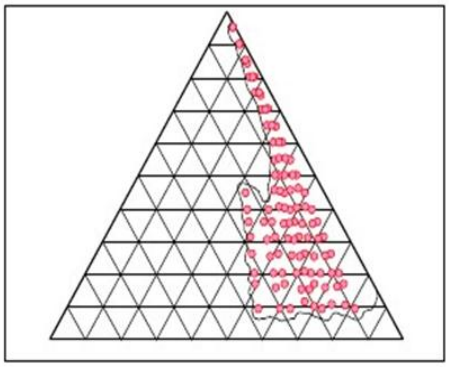


Figure E Phase diagram of Smix

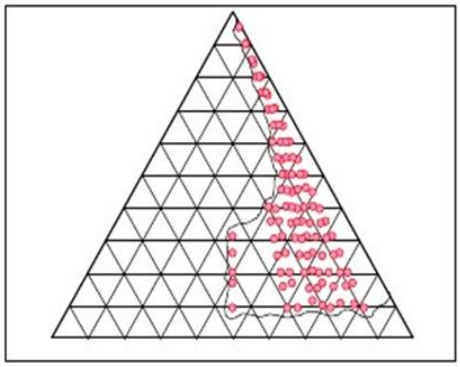


Figure F Phase diagram of Smix 3:1

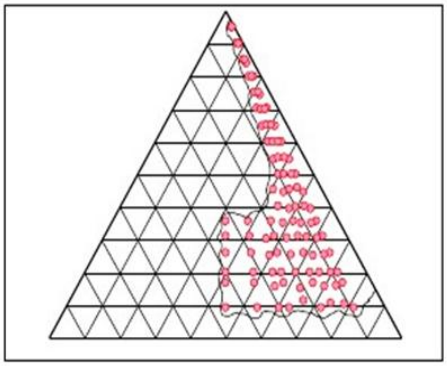


Figure G Phase diagram of Smix 4:

Preparation of Nanoemulsion [9,10]

The formulations were prepared by mixing appropriate amount of surfactant and cosurfactant and then oily part added, mix the formulation until completely dispersion occurs at room temperature. Then appropriate amount of drug was added and the final mixture was mixed by vortexing until a transparent solution was obtained12. Composition of selected nanoemulsion formulations are given in table 1.

Selection of the Formulations from Phase Diagram

From each phase diagram constructed, different formulations were selected from nanoemulsion region so that drug could be incorporated into the oil phase; therefore, following criteria were made for the selection of different formulation from phase diagrams:

The dose of Aceclofenac varies between 5 mg to 20 mg. But most frequently used dose is 10 mg.

1. The oil concentration should be such that it dissolves the drug (10 mg) easily.
2. From each phase diagram different concentration of oil, which solubilized 10 mg of Aceclofenac , was selected at a difference of 5% (10, 15 and 20%).
3. For each percentage of oil selected, that formula was taken from the phase diagram, which used minimum concentration of Smix for its nanoemulsion formation.
4. The emphasis for the selection of formulations was given on the minimum concentration of the Smix, from the phase diagram.

As per saturation solubility studies of Aceclofenac in oily mixture (Sefsol 218 : Oleic acid ::1:1), around 130 2.78 mg of drug was solubilized per ml of mixtures. The concentration, 10% (100 µL) of oil in 1 mL of formulation is just able to solubilize 10 mg of Aceclofenac . Therefore 10% was selected as the least oil concentration to be taken for one mL formulation from the phase diagram. The drug stock solutions in oil mixture were prepared in such a way that 10 mg dose is present in each formulation complying the oil percentage for each formulas as shown in the Table 7.5.

Table : Selected formulations from pseudoternary phase diagram of Smix ratio 1:1, 1:2, 1:3

Smix Ratio (S:CoS)	Code	Percentage v/v of different components in formulation		
		Oil	Smix	Aque
1:1 (Figure 7.2 B)	1	10	30	70
	2	10	38	55
	3	15	38	47
	4	15	45	45
	5	20	40	45
	7	20	40	35
	7	25	45	35
	8	25	31	30

	9	10	40	59
1:2 (Figure 7.2 C)	10	10	40	50
	11	15	45	45
	12	15	38	40
	13	20	40	47
	14	20	37	45
	15	25	40	49
	17	25	37	35
	17	10	40	53
	18	10	44	50
	19	15	50	41
1:3 (Figure 7.2 D)	20	15	48	35
	21	20	48	32
	22	20	50	30

Table : Selected formulations from pseudoternary phase diagram of Smix ratio 2:1, 3:1 & 4:1.

Smix Ratio (S:CoS)	Code	Percentage v/v of different components in Formulation		
		Oil	Smix	Aque
2:1 (Figure 7.2 E)	23.	25	35	55
	24.	25	40	50
	25.	10	42	43
	26.	10	45	40
	27.	15	40	40
	28.	15	45	35
	29.	20	38	37
	30.	20	40	50
3:1 (Figure 7.2 F)	31.	25	40	45
	32.	25	42	42
	33.	10	43	40
	34.	10	45	40
	35.	15	40	35
	36.	15	45	35
	37.	20	40	38
	38.	20	38	35
4:1 (Figure 7.2 G)	39.	25	40	50
	40.	25	45	45
	41.	10	45	40
	42.	10	50	35
	43.	15	45	35

	44.	15	50	30
	45.	20	40	35
	46.	20	45	30

Development of Drug Containing Nanoemulsion Formulation

The drug stock solutions in oil mixture were prepared in such a way that 10 mg dose is present in each formulation complying the oil percentage for each formulae as shown selected from the phase diagram. This was prepared by dissolving the 1000 mg of drug individually in the 10, 15, 20 and 25 mL of oily mixture, which complies the 10%, 15%, 20% and 25% oil compositions respectively in the formulae. The drug stock table is shown in the Table 7.4. Table 7.5: Preparation of drug stock for each formulae selected in phase diagram.

S.NO	Oil percentage in formulations	Amount of drug (mg)	Volume of oil (mL)	Final concentration (mg/µL)
1	10%	1000	10	10 mg/100 µL
2	15%	1000	15	10 mg/150 µL
3	20%	1000	20	10 mg/200 µL
4	25%	1000	25	10 mg/250 µL

Screening of Formulations on the Basis of Thermodynamic Stability Studies [10]

Microemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant:co-surfactant mixture and water, with no phase separation, creaming or cracking. It is the thermostability which differentiates micro emulsion from emulsions that have kinetic stability and will eventually phase separate (Lawrence and Rees., 2000). The thermodynamic stability studies was performed on the basis of following tests.

i. Centrifugation Study

The selected formulations were centrifuged (REMI, India) at the 5000 rpm for 30 mins and observed for phase separation, creaming or cracking. The formulations which showed maximum stability (no creaming, cracking, phase seperation) were selected and studied for heating-cooling cycle, freeze-thaw cycles and Dispersibility tests (Tables 7.7 and 7.7).

Heating Cooling Cycles

It is used to see the stressed effect of heating and cooling on the nanoemulsion`s stability. In this study the formulations were kept at 450 c and at 0 ^{0}C temperature for not less then 48 hrs for each temperature cycle (Tables 7.7 and 7.7)

Freeeze –thaw cycles (Accelerated ageing)

This test was performed for accelerated stability testing of nanoemulsion formulations. In this study the formulations were exposed at two different temperatures i.e -210C and 210C for each temperature cycles not than 24 hrs. For the better estimation of accelerated stability studies three such cycle should be run for each batch of formulation (Tables 7.7 and 7.7). The formulations which showed the maximum stability were selected for further study. Table 7.7: Thermodynamic stability tests of different selected formulations from Smix ratio 1:1, 1:2, 1:3

Oil used: Sefsol 218+Oleic acid (1:1), Carbitol, Surfactant used: Tween 20, Cosurfactant used: External phase: Distilled water								
Smix Ratio (S:CoS)	**S.No.**	**Percentage v/v of different components in formulations**			**Observations based on the thermodynamic stability studies**			**Inference**
		Oil	**Smix**	**Water**	**H/C**	**Cent**	**Freez**	
1:1 (Figure 2.1 B)	1	10	10	15	ˣ	ˣ	ˣ	Failed
	2	15	20	20	√	√	√	Passed
	3	25	25	10	√	√	√	Passed
	4	10	15	15	√	√	√	Passed
	5	20	20	25	√	√	√	Passed
	7	25	10	10	√	√	√	Passed
	7	15	15	20	√	√	√	Passed
	8	20	30	38	√	ˣ	√	Passed
1:2 (Figure 2.1 C)	9	38	45	40	ˣ	√	√	Failed
	10	40	45	31	√	√	√	Passed
	11	40	40	45	√	√	√	Passed
	12	38	40	37	√	√	√	Passed
	13	40	37	40	√	√	√	Passed
	14	44	50	48	√	√	√	Passed
	15	48	50	70	-	√	√	Failed
	17	55	47	45	-	√	√	Failed
1:3 (Figure 2.1 D)	17	45	35	35	√	√	√	Passed
	18	30	59	50	√	√	√	Passed
	19	45	40	47	√	√	√	Passed
	20	45	49	35	√	√	√	Passed
	21	53	50	41	√	√	√	Passed
	22	35	32	30	ˣ	ˣ	√	Failed

H/C = Heating-cooling cycle, Cent = Centrifugation, Freeze = Freeze-thaw cycle

Table 7.7: Thermodynamic stability tests of different selected formulations from Smix ratio 2:1,

Oil used: Sefsol 218+Oleic acid (1:1) , Cosurfactant used: Carbitol,					Surfactant used: Tween 20, External phase: Distilled water			
Smix Ratio (S:CoS)	**S.No.**	**Percentage v/v of different components in formulations**			**Observations based on the thermodynamic stability studies**			**Inference**
		Oil	**Smix**	**Water**	**H/C**	**Cent**	**Freez**	
2:1 (Figure 2.1 E)	23	25	35	55	√	√	√	Passed
	24	25	40	50	√	√	√	Passed
	25	10	42	43	√	ˣ	-	Failed
	27	10	45	40	-	√	√	Passed
	27	15	40	40	-	√	√	Passed
	28	15	45	35	√	√	√	Passed
	29	20	38	37	ˣ	√	√	Passed
	30	20	40	50	√	√	√	Passed
3:1 (Figure 2.1F)	31	25	40	45	√	ˣ	-	Failed
	32	25	42	42	-	ˣ	-	Failed
	33	10	43	40	-	ˣ	-	Failed
	34	10	45	40	-	√	√	Passed
	35	15	40	35	√	√	ˣ	Passed
	37	15	45	35	-	√	√	Passed
	37	20	40	38	-	-	-	Failed
	38	20	38	35	-	√	√	Passed
4:1 (Figure 2.1G)	39	25	40	50	√	√	√	Passed
	40	25	45	45	ˣ	-	-	Failed
	41	10	45	40	√	√	√	Passed
	42	10	50	35	√	ˣ	-	Failed
	43	15	45	35	√	ˣ	-	Failed
	44	15	50	30	√	ˣ	-	Failed
	45	20	40	35	-	√	√	Passed
	47	20	40	30	√	√	√	Passed

H/C = Heating-cooling cycle, Cent = Centrifugation, Freez = Fre

Characterization of Nanoemulsion Droplet Size Measurements [11,12]

Size analysis of nanoemulsion was carried out by dynamic light scattering with zetasizer hsa 3000 (Malvern instruments ltd., Malvern, U.K). Samples were placed in square glass cuvettes and droplet size analysis was carried out at Temperature 250 C, for 80 second duration.

Zeta Potential Measurements [13]

Zeta potential for nanoemulsion was determined using zetasizer hsa 3000 (Malvern instrument ltd., UK). Samples were placed in clear disposable zeta cells and results were recorded. Before putting the fresh sample, cuvettes were washed with the methanol and rinsed using the sample to be measured before each experiment14.

Transmission Electronic Microscopy (TEM) [14]

Morphology and structure of the nanoemulsion were studied using Transmission Electron Microscopy (TEM) LEO 912AB EFTEM. To perform the TEM observations, samples were placed on a formvar carbon-coated copper grid (200 mesh in-1) and then stained with 1% phosphotungstic acid. The excess phosphotungstic acid on the sample was gently wiped off using filter paper and examined after drying for about half an hour at room temperature.

Stability *Temperature Stabilit[y*[15]

Shelf life as a function of time and storage temperature was evaluated by visual inspection of the nanoemulsion system at different time period. aceclofenac nanoemulsion was diluted with purified distilled water to determine the temperature stability of samples. Samples were kept at three different temperature ranges (4°C, room temperature) and observed for any evidences of phase separation, flocculation or precipitation.

Centrifugation [16]

In order to estimate metastable systems, the optimized nanoemulsion formulation was diluted with purified distilled water. Then nanoemulsion was centrifuged (Remi laboratories, Mumbai, India) at 10,000 rpm for 30 minute at room temperature and observed for any change in homogenecity of nanoemulsions.

Formulation of Aceclofenac Nanoemulsion based Gel [17,26]

Nanoemulsion base gel was prepared by dispersing the 1 g of the Carbopol 934 in a sufficient quantity of distilled water. After complete dispersion, the Carbopol 934 solution was kept in the dark for 24 hours for complete swelling. Then the aceclofenac loaded nanoemulsion was slowly added to the viscous solution of Carbomer 934 under magnetic stirring13. The pH values were subsequently regulated to 6-9. Then other ingredients like isopropyl alcohol, PEG-400, PG and triethanolamine were added to obtain a homogeneous dispersion of gel.

RESULT AND DISCUSSION [18,19]

Excipient Selection

The excipients selected needed to be pharmaceutically acceptable, nonirritating, and nonsensitizing to the skin and to fall into the GRAS (generally regarded as safe) category. Higher solubility of the drug in the oil phase was another important criterion, as it would help the nanoemulsion to maintain the drug in solubilized form. Safety is a major determining factor in choosing a surfactant, as a large amount of surfactants may cause skin irritation. Non-ionic surfactants are less toxic than ionic surfactants. An important criterion for selection of the surfactants is that the required hydrophilic lipophilic balance (HLB) value to form the o/w nanoemulsion be greater than 10. The right blend of low and high HLB surfactants leads to the formation of a stable nanoemulsion formulation.30 In this study, we selected Cremophor EL as a surfactant with an HLB value of 15. Transient negative interfacial tension and fluid interfacial film are rarely achieved by the use of single surfactant; usually, addition of a cosurfactant is necessary. The presence of cosurfactant decreases the bending stress of interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form nanoemulsions over a wide range of composition.31,32 Thus, the cosurfactant selected for the study was Ethanol, which has an HLB value of 4.2. Aceclofenac is a highly lipophilic drug, and its physicochemical properties suggest that it has good potential for transdermal drug delivery. Therefore, in the present study different nanoemulsions were prepared for transdermal delivery of aceclofenac.

Solubility of Aceclofenac [20,27]

The maximum solubility of aceclofenac was found in Oleic acid (30.76 ± 1.27 mg/gm) as compared to other oils and combinations of oils (Table 2). High drug solubility was found in Tween® 80 (70 ± 2.18) and Ethanol (32 ± 1.10). Therefore, Tween® 80 and Ethanol were selected as surfactant and cosurfactant, respectively, for the phase study.

Pseudo-Ternary Phase Diagram [21]

A pseudo ternary phase diagram of the investigated quaternary system water/Oleic acid/ Tween® 80/Ethanol is presented in Figure 1. Formation of nanoemulsion system (the shaded area) was observed at room temperature. Phase behavior investigation of this system demonstrated the suitable approach to determining the water phase, oil phase, surfactant concentration, and cosurfactant concentration with which the transparent, 1-phase low-viscous nanoemulsion system was formed. The phase study revealed that the maximum

proportion of oil was incorporated in nanoemulsion systems when the surfactant-to-cosurfactant ratio (km) was 4:1. Moreover, when the composition (% wt/wt) of surfactant mixture (Smix) in a nanoemulsion preparation was <40%, the formulation was less viscous. From pseudoternary phase diagrams.

Characterization of Nanoemulsion Base Gel Droplet Size Measurements [22]

The mean droplet size and polydispersity index were calculated from intensity, volume and bimodal distribution assuming spherical particles. All the nanoemulsion had small average droplet diameter between 10 to 100 nm. A small droplet sizes are very much prerequisite for drug delivery as the oil droplets tend to fuse with the skin thus providing a channel for drug delivery. Polydispersity index (PI) is a measure of particle homogenicity and it varies from 0.0 to 1.0. The closer to zero the polydispersity value the more homogenous are the particles. Formulations showed their PI in between 0.134 to 0.394 that indicates acceptable homogenicity. Zeta potential of all Nanoemulsion formulation was found between -7.02 to -0.044 mV in the 100 times diluted Nanoemulsion formulation consists of non-ionic components which show relatively neutral charge, it means it will not affected by body membrane charge during absorption.

Transmission Electronic Microscopy (TEM) [23,24]

TEM determination is one of the studies conducted in order to confirm the particle size obtained by the laser scattering spectroscopy. Because recently people started to have doubts about measurements made by using laser scattering spectroscopy method which usually need significant dilution of samples. In the TEM image the nanoemulsion appeared dark and the surroundings were bright. The micrograph exhibits, the droplets size of the sample were in the range of nanoemulsion. Nanoemulsion and nanoemulsion base gel were stable at 4 °C and at room temperature in the presence of aceclofenac. There was no significant change of particle size, phase separation and degradation of aceclofenac observed up to 3 months. The centrifuged tests revealed that nanoemulsion and nanoemulsion base gel were remained homogenous without any phase separation throughout the test indicates good physical stability of both preparations.

Transmission Electron Microscopic Image of Aceclofenac Nanoemulsion Showing Size of Some Oil Droplets. Stability.

CONCLUSION [25,28]

In this work, nanoemulsion base gel with suitable viscosity was constructed to deliver aceclofenac for topical administration. The nanoemulsion base gel formulation of aceclofenac containing 10% of oil phase (Oleic acid), 45% of surfactant mixture (Cremophor EL and Ethanol) and 43 % of distilled water has been optimized. From *in vitro* data it can be concluded that the developed nanoemulsion- based gel have great potential for topical drug delivery.

ACKNOWLEDGEMENT

Authors wish to acknowledge all those who are involved directly or indirectly for compilation of this article.

REFERENCES

1. Alvarez-Figueroa MJ, Blanco-Mendez J. Transdermal delivery of methotrexate: iontophoretic delivery from hydrogels and passive delivery from microemulsions. Int J Pharm, 2001; 215: 57Y65.
2. Attwood D, Mallon C, Ktistis G, Taylor CJ. A study on factors influencing the droplet size in non-ionicoil-in-water microemulsions. Int J Pharm, 1992; 88: 417Y422.
3. Baboota S, Al-Azaki A, Kohli K, Ali J, Dixit N, Shakeel F. Development and evaluation of a nanoemulsion formulation for transdermal delivery of terbinafine PDA. J. Pharm. Sci. Technol, 2007; 61: 276–285.
4. Baboota S, Shakeel F, Kohli K. Formulation and evaluation of once a day transdermal gels of diclofenac diethylamine. Methods Find Exp Clin Pharmacol, 2006; 28: 109-114.
5. Changez M, Varshney M. Aerosol-OT nanoemulsions as transdermal carriers of tetracaine hydrochloride. Drug Dev. Ind. Pharm, 2000; 26: 507–512.
6. Chen H, Chang X, Weng T, Du D, Li J, Xu H, Yang X. Nanoemulsion-based hydrogel.
7. Cohen BA, Prose N, Schachner LA. Acne. In: L. A. Schachner, R. C. Hansen (eds.), Pediatric Dermatology. Vol. I, Churchill Livingstone, New York, 1988, 663-694.
8. Craig DQM, Barker SA, Banning D, Booth SW. An investigation into the mechanisms of self-emulsification using particle size analysis and low frequency dielectric spectroscopy. Int J Pharm, 1995; 114: 103-110.
9. Delgado-Charro MB, Iglesias-Vilas G, Blanco-Mendez J, Lopez-Quintela MJ, Marty MA, Guy JP. Delivery of a hydrophilic solute through the skin from novel microemulsion systems. Eur J Pharm Biopharm, 1997; 43: 37-42.

10. Dreher F, Walde P, Walter P, Wehrli E. Interaction of a lecithin microemulsion gel with human stratum corneum and its effect on transdermal transport. J Control Rel, 1997; 45:131-140.
11. Eccleston J. Microemulsions. In: Swarbrick J, Boylan JC, eds. Encyclopedia of Pharmaceutical Technology. vol. 9. New York, NY: Marcel Dekker, 1995: 375-421.
12. Escribano E, Calpena AC, Queralt J, Obach R, Domenech J. Assessment of diclofenac permeation with different formulations: anti-inflammatory study of a selected formula. Eur J Pharm Sci, 2003; 19:203-210.
13. Faiyaz S, Shafiq S, Sushma T, Farhan J, Khar R, Ali M. Development and bioavailability assessment of ramipril nanoemulsion formulation, Eur. J. Pharm. Biopharm, 2007; 66: 227– 243.
14. Formulation of ibuprofen for topical delivery. Int. J. Pharm, 2006; 315: 52– 58.
15. Gasco MR, Gallarate M, Pattarino F. In vitro permeation of azelaic acid from viscosized microemulsions. Int J Pharm, 1991; 69: 193Y196.
16. Gonzalez E, Cruz C, Nicolas R, Egido J, Herrero-Beaumont G. Long-term effects of nonsteroidal anti-inflammatory drugs on the production of cytokines and other inflammatory mediators by blood cells of patients with osteoarthritis. Agents Actions, 1994; 41: 171-178.
17. Gosh MN. Fundamentals of Experimental Pharmacology. Kolkata, India: Hilton and Company, 2005: 192.
18. Kawakami K, Yoshikawa T, Moroto Y, et al. Microemulsion formulation for enhanced absorption of poorly soluble drugs, I: prescription design. J Control Rel, 2002; 81: 65-74.
19. Kazimiera A, Katarzyna Z, Agnieszka H, Adam J. Biocompatible nanoemulsions of dicephalic aldonamide-type surfactants: Formulation, structure and temperature influence. Journal of Colloid and Interface Science, 2009; 334: 87–95.
20. Kemken J, Ziegler A, Muller BW. Influence of supersaturation on the pharmacodynamic effect of bupranolol after dermal administration using microemulsions as vehicle. Pharm Res, 1992; 9: 554Y558.
21. Kreilgaard M, Kemme MJB, Burggraaf J, Schoemaker RC, Cohen AF. Influence of a microemulsion vehicle on cutaneous bioequivalence of a lipophilic model drug assessed by microdialysis and pharmacodynamics. Pharm Res, 2001; 18: 593Y599.
22. Kreilgaard M, Pedersen EJ, Jaroszewski JW. NMR characterization and transdermal drug delivery potentials of microemulsion systems. J Control Rel, 2000; 69: 421-433.

23. Kreilgaard M. Dermal pharmacokinetics of microemulsion formulations determined by in-vitro microdialysis. Pharm Res, 2001; 18: 367-373.
24. Kreilgaard M. Influence of nanoemulsions on cutaneous drug delivery. Adv. Drug Deliv. Rev, 2002; 54: 77–98.
25. Kriwet K, Muller-Goymann CC. Diclofenac release fromphospholipid drug systems and permeation through excised human stratum corneum. Int J Pharm, 1995; 125: 231-242.
26. Ktistis G, Niopas I. A study on the in-vitro percutaneous absorption of propranolol from disperse systems. J Pharm Pharmacol, 1998; 50: 413-419.
27. Lapasin R, Grassi M, Coceani N. Effects of polymer addition on the rheology of o/w icroemulsions. Rheol. Acta, 2001; 40: 185–192.
28. Lapasin R, Grassi M, Coceani N. Effects of polymer addition on the rheology of o/w nanoemulsions. Rheol. Acta, 2001; 40: 185–192.