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FORMULATION DEVELOPMENT AND EVALUATION OF DICLOFENAC SODIUM MICROEMULSION

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Abstract:
The aim of my present study is to Develop and Evaluate Microemulsion for topical application of Diclofenac sodium by using oleic acid at different ratios for the treatment of pain. Design of microemulsion formulation for topical use of drugs, having the potential to increase the solubility of poorly water soluble drugs. To avoid the first pass metabolism and there is a potential to deliver the drug in a controlled manner to minimize the adverse effect on the g.i.t like mild dyspepsia and heartburn to ulceration and hemorrhage. Reduction of dosing frequency due to longer duration of action. To improve patient compliance to provide sustained release drug for longer periods of time due to short half-life. To delivery of hydrophilic as well as lipophilic drug as drug carriers because of its improved drug solubilization capacity and long shelf –life. Microemulsion was prepared by water trituration method using oleic acid as oil phase, tween-80 as surfactant and polyethylen glycol-400 as co-surfactant. Different oils, surfactants and co-surfactants were screened to select ideal components of microemulsions with good solubility and excellent skin penetration of Diclofenac sodium. The solubility of diclofenac sodium was highest in oleic acid followed by olive oil, and isopropyl myristate, isopropyl palmitate. ME-3 was exhibited 98.54±0.26% higher drug content then other formulations. Among all formulations, the highest permeation flux of µg/cm^2/hour was observed in of formulation ME-3.

Keywords: *Microemulsion, Optical transparency, Particle size, Transmission Electron Microscopy, in –vitro skin permeation, FT-IR.*

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INTRODUCTION:

Microemulsions

In 1943, Hour and schulman visualized the existence of small emulsion like structures by electron microscopy and subsequently coined the term "Microemulsions". Microemulsions are isotropic, thermodynamically stable transparent (or translucent) systems of oil, water and surfactant, frequently in combination with a co-surfactant with

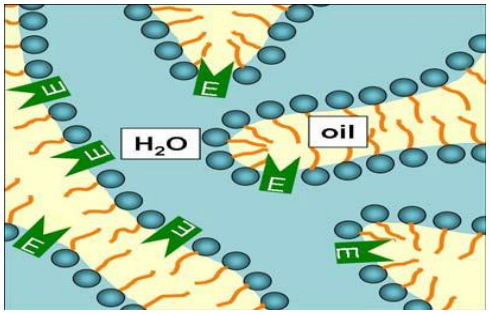


Fig 1: Microemulsion

a droplet size usually in the range of 10-100nm. Whereas the diameter of droplets in a kinetically stable emulsion is >500nm. Because the droplets are small , a microemulsion offers advantages as a carrier for drugs that are poorly soluble in water. These homogeneous systems , which can be prepared over a wide range of surfactant concentration and oil to water ratio, are all fluids of low viscosity [1-4].

Important Characteristics of Microemulsions [5,6]

- Thermodynamically stable (long Shelf – life)
- Optically Clear
- Particle size 10-100 nm
- High Surface area (high Solubilization Capacity)
- Small droplet size
- Enhanced drug Solubilization
- Ease formation (zero interfacial tension and almost spontaneous formatioin)
- Ability to be Sterilized by filtration
- Long - term Stability
- High Solubilization capacity for hydrophilic and lipophilic drugs
- Improved drug delivery

Table 1: Difference between Emulsion and Microemulsion

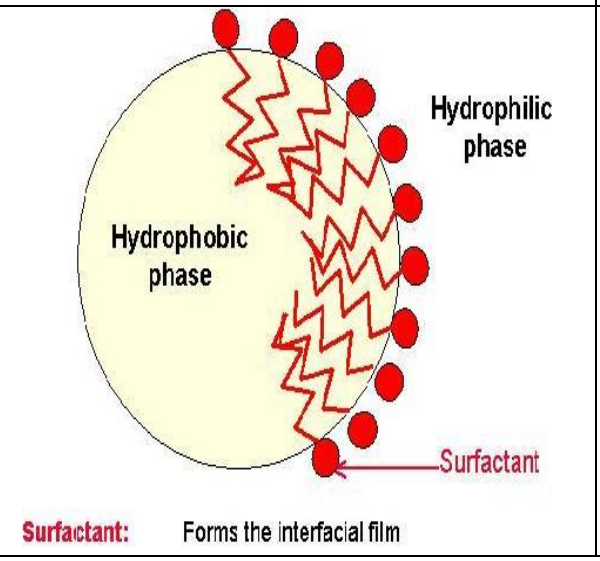
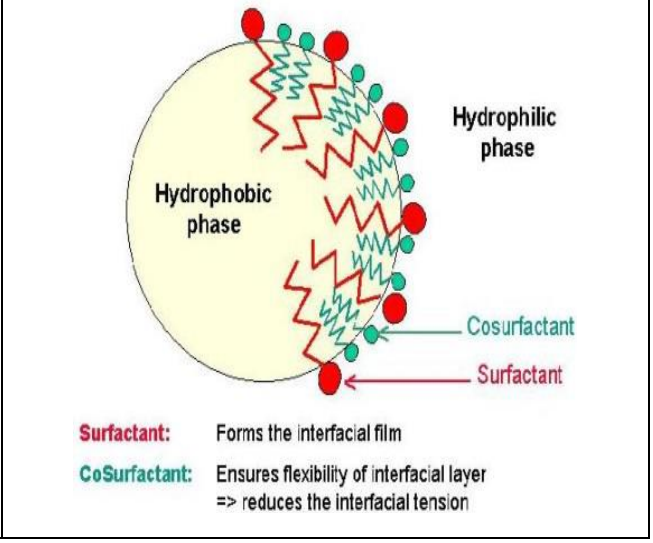
EMULSION	MICROEMULSION
	

Fig 1: Difference between Emulsion and Microemulsion

Table 1: Difference between Emulsion and Microemulsion

EMULSION	MICROEMULSION
Emulsion consist of roughly spherical droplets of one phase dispersed into the other.	They constantly evolve between various structures ranging from droplet like swollen micelles to bi- con tinuous structure
Thermodynamically unstable (Kinetically Stable)	Thermodynamically stable (Long shelf-life)
Inefficient molecular packing	Efficient molecular packing
Direct oil/water contact at the interface	No direct oil/water contact at the interface
High interfacial tension	Ultra low interfacial tension
High viscosity	Low viscosity with Newtonian behavior
Droplet diameter>500nm	10-100nm
Cloudy colloidal system	Optically transparent(Isotropic)
They are lyophobic	They are on the borderline between lyophobic and lyophilic colloids
Require intense agitation for their formation	Generally obtained by gentle mixing of ingredients.
Ordinary emulsion droplets, however small exist as individual entities until coalesance or ostwald ripening occurs	Microemulsion droplet may disapper within a fraction of a second while another droplet froms spontaneously elsewhere in the system

MATERIALS AND METHODS:

Materials Used

Diclofenac Sodium, Oleic acid, Olive oil, Castor oil, Isopropyl myristate, Isopropyl palmitate, Tween-80, Span-20, Polyethylen glycol-400, Isopropyl alcohol, N-butanol.

Methods Used

Preformulation Studies

Preformulation may be described as a stage of development process during which the researches characterize the physical, chemical, and mechanical properties of the drug substance to from effective, stable and safe dosage form hence, Preformulation studies are essential to characterize the drug for proper designing of the drug delivery system. The Preformulation studies which were performing in this include [7-9],

- ❖ Description
- ❖ Melting point
- ❖ Solubility
- ❖ Hygroscopic Nature
- ❖ Identification of drug sample
- ❖ Drug- excipient compatibility studies

Formulation Development

The pharmaceutical development studies have to be carried out with the purpose of selecting right dosage form and a stable formulation. These studies give detailed description of all the steps involved in the process of development of the finished procedure. Such details are intended towards identifying critical parameters involved in the process, which have to be controlled in order to give reliable and reproducible quality product [10-12].

Calculation of HLB value for o/w type of Microemulsions

The HLB of a non-o-ionic surfactant whose only hydrophilic portion is polyoxyethylene is calculated by using the formula

$$HLB = E/5$$

Where, E is the percentage by weight of ethylene oxide. A number of polyhydric alcohol fatty acid esters, such as glyceryl monostearate, can be estimated the formula

$$HLB = 20(1-S/A)$$

Where, S is the saponification number of the ester and A is the acid number of the fatty acid. The HLB of polyoxyethylene sorbitan monolaurate (tween-20)

For which S= 45.5 and A=276, is

$$HLB = 20(1-45.5/276)=15$$

Table 2: HLB Values of some Amphiphilic Agents

Substance	HLB Value
Oleic acid	1
Span -80	4.3
Span-20	8.6
Briji-30	9.5
Tween-80	15
Tween-20	16.7
Sodium oleate	18

Table 3: HLB for some oil phase ingredients for (O/W) and (W/O) emulsions

Oil phase ingredients	O/W emulsion	W/O emulsion
Cottonseed oil	6-7	-
Mineral oil	10-12	5-6
Castor oil	14	-
Lauric acid	16	-
Oleic acid	17	-

Selection of Oils

To find out the suitable oil, this can be used as oil phase in micro emulsion, and provide excellent skin permeation rate of Diclofenac Sodium. The solubility of Diclofenac Sodium in various oils including olive oil, castor oil, isopropyl myristate, isopropyl palmitate, oleic acid was measured at 25^0C. The solubility of olive oil, castor oil, isopropyl myristate, isopropyl palmitate, and oleic acid in oily mixtures was also measured [13-14].

Procedure:

About 10mg of oil was accurately weighed in 25 ml glass beaker and 100 mg of Diclofenac Sodium was added into it, followed by stirring on magnetic stirrer at moderate speed to dissolve the drug. When drug was dissolved completely another 10mg Diclofenac Sodium of was added and stirring was continued. Addition of drug was continued until the saturated solution is obtained. Finally, the total amount of drug consumed was determined by using UV-spectrophotometer at 276nm.

It was found that, oleic acid has consumed maximum amount of Diclofenac Sodium and thus chosen as a vehicle for micro emulsion oil phase.

Table 4: Solubility of Diclofenac Sodium in various oils at 25^0C

S.no	Drug solubility (In mg/10 g of oil)	Oils
1	120	Olive oil
2	140	Isopropyl myristate
3	150	Castor oil
4	120	Isopropyl palmitate
5	180	Oleic acid

Selection of surfactants and co-surfactants

The non-ionic surfactants do not ionize at any great extent in the solution, they are greatly compatible with both anionic and cationic substances; various nonionic surfactants like, span 20, Tween-20 and co-surfactants like, propylene glycol, isopropyl alcohol and b-butanol were subjected to titration. Finally, Tween -80 and propylene glycol were selected as an ideal surfactant and co-surfactant for the system.

Table 5: Selection of surfactant and co- surfactants for optimization of formulations

Surfactant:co-surfactant	Concentration ratio	Appearance
Tween-80:polyethylen glycol-400	1:1 2:1	Clear Clear
Tween-80:isopropyl alcohol	1:1 2:1	Slightly cloudy Clear
Tween-80:n- butanol	1:1 2:1	Cloudy Clear
Span-20:propylene glycol	1:1 2:1	Clear Cloudy
Span-20:isopropyl alcohol	1:1 2:1	Slight cloudy Cloudy
Span20:n-butanol	1:1 2:1	Cloudy Cloudy

Preparation of Diclofenac sodium Microemulsions by Water Trituration method

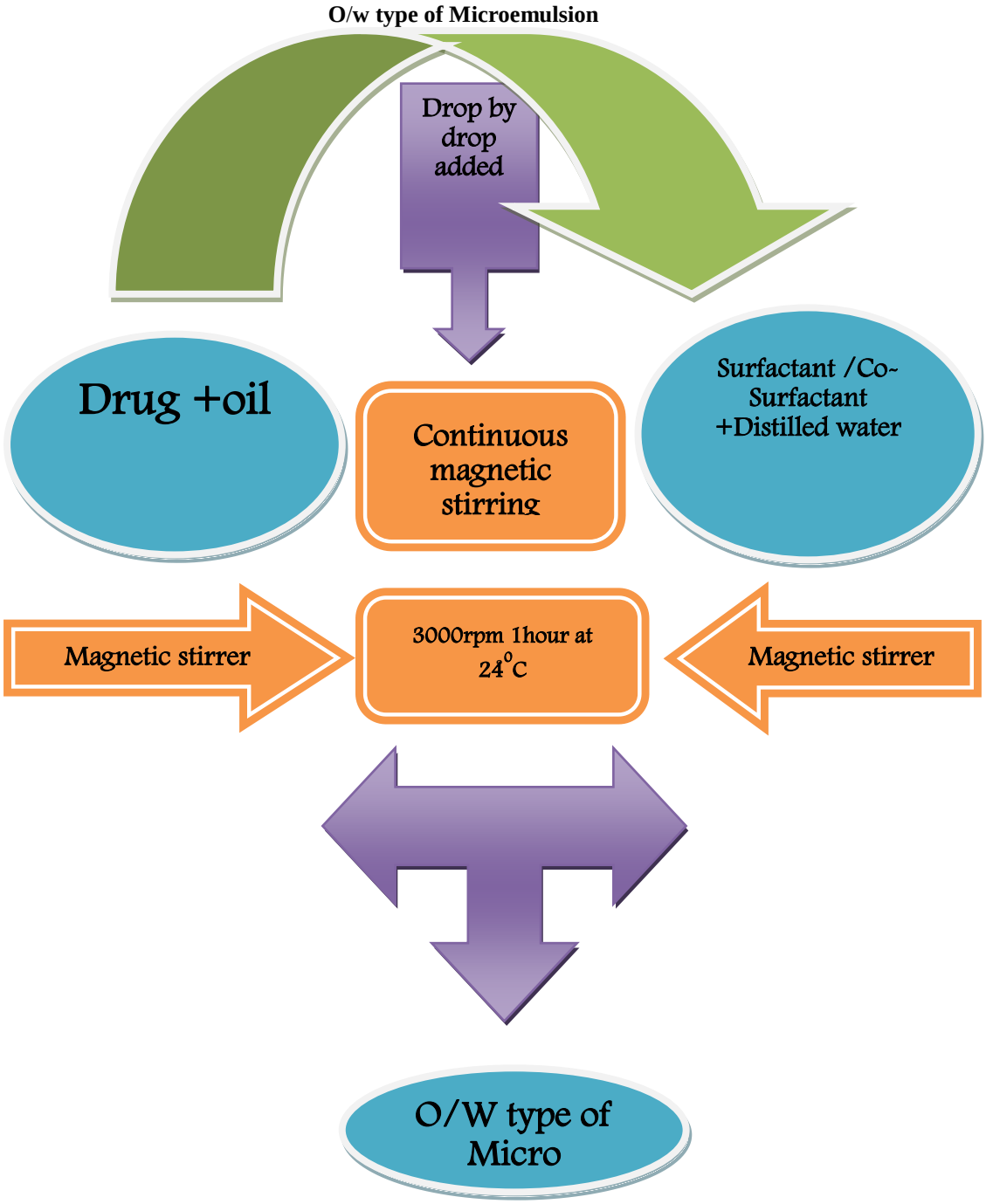


Fig 2: Preparation of Diclofenac Microemulsion

Characterization of Microemulsions [15-16]

Optical Transparency

Optical transparency of the formulation was 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.

Determination of pH

pH is measured using a pH meter of a glass electrode. pH fundamentally represents the value of hydrogen ion activity in solutions. It is defined by the equation given below . This value well accords with the logarithm of the reciprocal of hydrogen ion concentration in dilute solutions.

$$pH = pHS + \frac{E - ES}{2.3026\,RT/F}$$

Where pHs = pH value of a pH standard,
E= electromotive force (volt) onthe combination of

glass and reference Electrode in a sample solution; the constitution of the cell

Es = electromotive force (volt) on the combination of glass and reference Electrode in a pH standard, the constitution of the cell

R = gas constant,

T = absolute temperature,

F = Faraday constant.

The values of 2.3026 RT/F (volt) at various temperature of solutions.

The pH was measured in microemulsion formulations using a ELICOLI120 pH meter that was calibrated before formulation use with buffered solutions at pH 4 and Ph 9.2

A defined amount of formulation was taken and diluted with calibrated distilled water and mixed well. The electrode of the pH meter was immersed in the prepared formulation for pH determination.

About 2gm of formulation was dispersed into 20ml of distilled water and pH was determined by pHmeter.

Viscosity Measurements

This procedure determines the viscosity of a fluid by the use of a Brookfield viscometer. Viscosity is the measure of fluid friction which can be considered as the internal friction resulting when a layer of fluid is made to move in relationship to another layer. Viscosity is a measure of the ratio of shearing stress to rate of shear.

$$\frac{\text{Shear Stress (dynes)}}{\text{Rate of Shear (cm/sec}} = \text{Poise}$$

- Check to confirm that the viscometer has been calibrated. If not, calibrate using software.
- The sample container and quantity should be approximately the same as for the Calibration standard. Equilibrate the temperature of the sample to the temperature designated in the specification (±1°C).
- Confirm that the viscometer is level using the bubble level on the back of the instrument. For the Brookfield LV-II, the instrument with spindle attached and the speed set as designated in the product specification. The main display will flash 00.0 after 10 seconds.
- Immerse the spindle designated in the product specification into the sample to the groove on the spindle shaft. Do not allow air bubbles to be formed. Attach the spindle to the viscometer.[

The spindle should not touch the bottom or sides of the container and should be centered. Reconfirm that the viscometer in level.

Mechanical Stress Study

The chemical and physical stability of micro emulsion with Diclofenac Sodium were evaluated *via* phase separation by mechanical stress study.

The different microemulsion formulation (ME-1 to ME-5) were centrifuged (Remi centrifuge) at 2000 rpm for different time interval (10min, 30min, and 60min) and noted down the volume of phase separation of formulation.

Particle Shape and Surface Morphology

Transmission Electron Microscopy (TEM)

Morphology and structure of the microemulsion were studied using trensmission electron microscopy with Topcon 002B operating at 200kv (Topcon, paramus, NJ) and capable of point –to – point resolution. In order to perform transmission electron microscopy observations, a drop of the microemulsion was suitably diluted with water and appiled on a carbon- coated grid, then treated with a drop of 2% phosphotungstic acid and left for 30sec. The coated grid was dried under vacuum and then taken on a grid holder and observed under the transmission electron microscope.

Atomic Force Microscopy (AFM)

An atomic force microscope is an excellent for visualising particles with sizes ranging from 1 nm to 10 µm. Another advantage of the AFM is its simplicity of operation and that the AFM requires minimal sample preparation.Additionally, the AFM can operate in air, liquid or a vacuum. In comparison to traditional techniques for single particle analysis of sub-µm particles, the AFM gives three- dimensional profiles.

It is possible to make quantitative measurements of particle size with an AFM. It can easily measure particle sizing parameters as long as the particle is > 100nm. If the particle size is less than 100nm special considerations must be taken into account.

Particle Size Measurement

Determination of particle size distribution by particle size analyzer:

The selected best Diclofenac Sodium microemulsion formulations were subjected to laser particle counting method. Here the sample was injected into the sample delivery and controlling chamber. Then, suitable solvent was pumped through the chamber. Now a beam of laser light was allowed to fall on the sample cell. After required number of runs, they were directed towards the detector. From this the particle size range and the average mean particle size of the formulation can be studied. The average particle size of Microemulsion formulations can be determined using particle size analyzer.

Drug content Analysis

1ml of microemulsion formulation was transferred into a beaker containing 10ml methanol. The content of the beaker were stirred for 30 minutes and then kept for 24hr. After 24hr the content of beaker were transferred into centrifuge tube and centrifuged at the 3000 rpm for 10 min. Supernatant was separated and filtered. Then 0.1 ml of the supernatant was diluted appropriately

with phosphate buffer saline (PBS) pH 7.4 and assayed spectrophotometricallyfor drug content.

***In Vitro* Skin Permeation Study**

Preparation of Goat skin:

Selected formulations were further studied for skin permeation using goat ear skin, obtained from the slaughter house after sacrificing the animal within 1 hour. The average thickness of the goat skin was0.28±0.06 mm and then the hair was removed from the upper portion of skin surface using an animal hair clipper, and, subsequently, full thickness of the skin was harvested. The fatty layer, adhering to the dermis side, was removed by surgical scalpel. Finally, these excised skins were thoroughly rinsed with distilled water and packed in aluminum foils. The skin sample were stored at -20ºC and used within a week.

***In Vitro* skin Permeation Study**

In- vitro permeation study of drug from ME-1 to ME-5 Diclofenac Sodium microemulsion formulation was carried out using *Goat skin.* The average thickness of the skin was 0.28±0.06 mmSkins were allowed to hydrate for 1 hour before being mounted on the open ended diffusion with the stratum corneum facing the donor compartment and the dermal side faced the receiver compartment.

The receptor compartment was consist of 400ml of phosphate buffer (pH 7.4) in 500ml beaker and its temperature was maintained at 37 ±0.5°C and stirred at 300rpm throughout the experiment. About 1 gm of 1% Diclofenac Sodium microemulsion was placed in *Goat skin* tied to the one end of open – ended glass cylinder that was then dipped into freshly prepared phosphate buffer on magnetic stirrer. Sample were taken from receptor medium at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, and 8, 9, 10, 11, 12, 23, 24hrs and replaced immediately with an equal volume of fresh phosphate buffer equilibrated at 37 ±0.5°C. All the samples were analyzed for Diclofenac Sodium content at 276nm by UV-spectrophotometer. Cumulative amount of drug permeation was calculated from standard calibration curve.

Permeation Study:

Apparatus : Open ended diffusion cylinder
Speed : 300rpm
pH : 7.4
Time : 1-24hrs
Temperature: 37^0c
Λmax : 276nm

Table 6: Formulation of trial batch I (F1-F5) Surfactant: co-surfactant (1:1)

S.no	Ingredients	Formulations				
		F1	F2	F3	F4	F5
1	Diclofenac Sodium (mg)	10	10	10	10	10
2	Oleic acid(%w/v)	2	2	2	2	2
3	Tween-80(%w/v)	1	2	3	4	5
4	Polyethylen glycol - 400(%w/v)	1	2	3	4	5
5	Distilled water(%w/v)	26	24	22	20	18
6	Final volume(%w/v)	30	30	30	30	30

Table 7: Formulation of trial batch II (F6-F10) Surfactant: co-surfactant (2:1)

S.no	Ingredients	Formulations				
		F6	F7	F8	F9	F10
1	Diclofenac Sodium(mg)	10	10	10	10	10
2	Oleic acid (%w/v)	2	2	2	2	2
3	Tween-80 (%w/v)	2	4	6	8	10
4	Polyethylen glycol-400 (%w/v)	1	2	3	4	5
5	Distilled water(%w/v)	25	22	19	16	13
6	Final volume (%w/v)	30	30	30	30	30

Table 8: Compositions of the selected Microemulsion Formulation

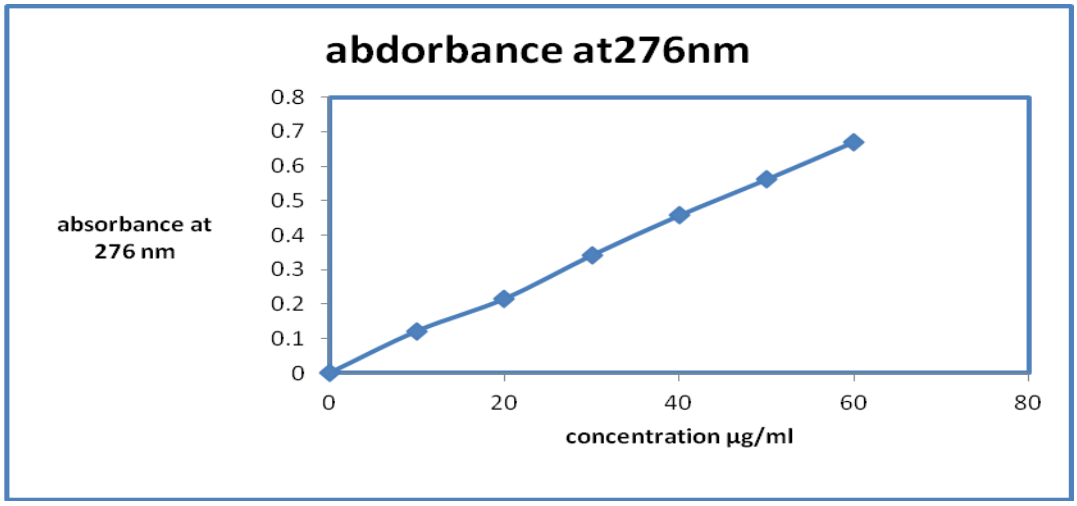
S.no	Formulation	Diclofenac Sodium (mg)	Oleic acid(%w/v)	Tween-80(%w/v)	PEG-400(%w/v)	Distilled water (%w/v)	Final volume(%w/v)
1	ME-1	10	2	6	3	19	30
2	ME-2	10	4	6	3	17	30
3	ME-3	10	6	6	3	15	30
4	ME-4	10	8	6	3	13	30
5	ME-5	10	10	6	3	11	30

RESULTS:

Table 9: Standard Plot of Diclofenac Sodium phosphate buffer pH7.4

S.no	Concentration (µg/ml)	Absorbance at 276nm
1	10	0.1219
2	20	0.2151
3	30	0.3413
4	40	0.4568
5	50	0.5615
6	60	0.6702

Fig 3: Standard plot of Diclofenac Sodium in pH 7.4



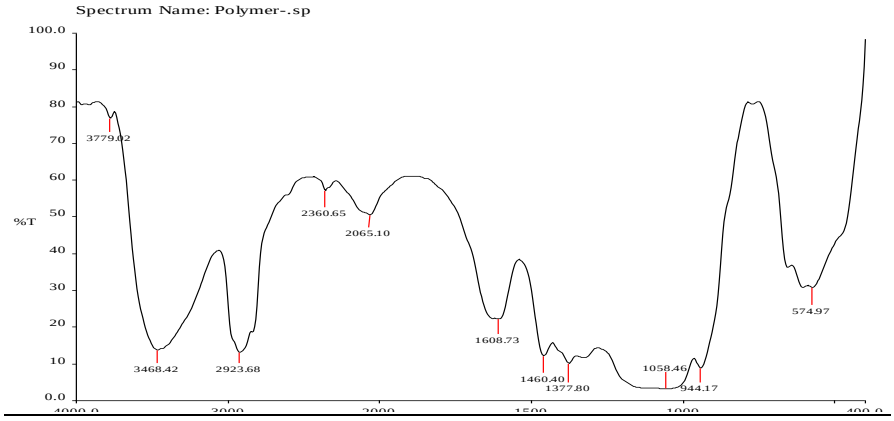


Fig 4: FT-IR OF Diclofenac sodium

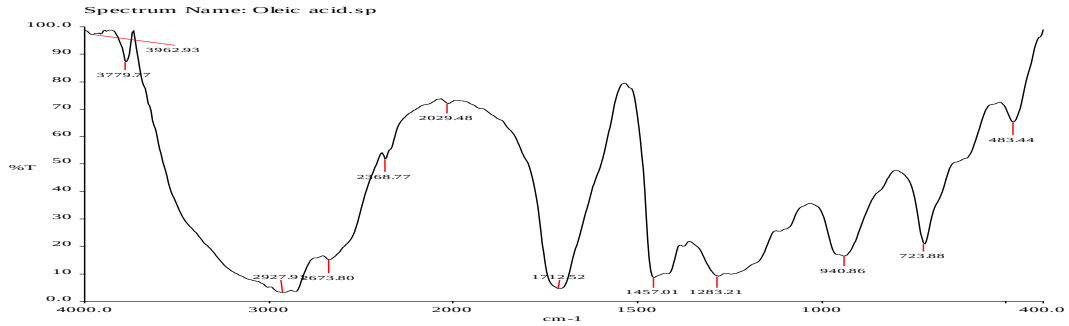


Fig 5: FT-IR of oleic acid

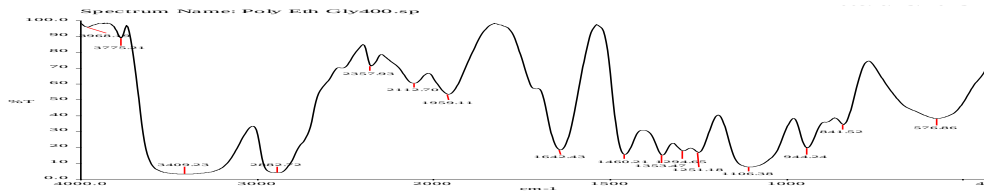


Fig 6: FT-IR OF Polyethylenglycol-400

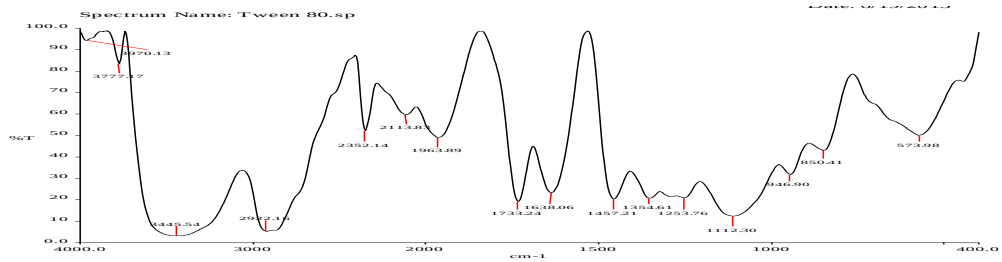


Fig 7: FT-IR of Tween-80

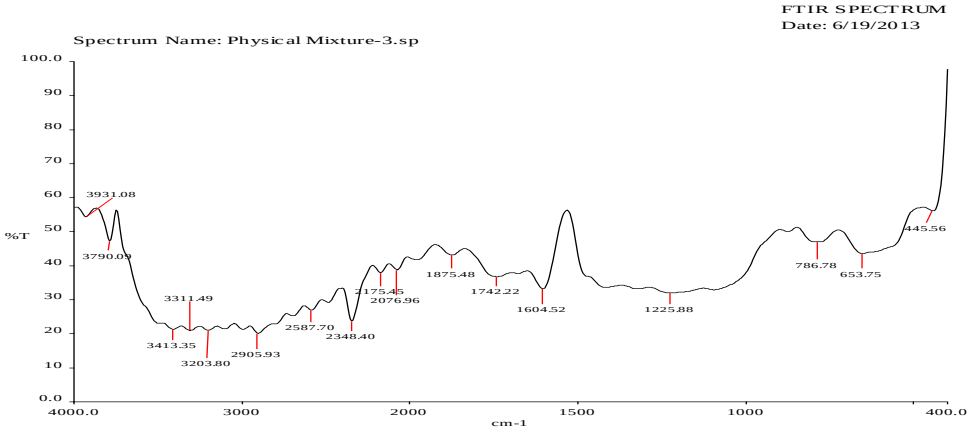


Fig 8: FT-IR of Mixture

Table 10: **Appearance of Formulations**

Formulation	Appearance
ME-1	Milky
ME-2	Opalescent
ME-3	Clear
ME-4	Milky
ME-5	Milky

Table 11: Comparative pH values of Formulations

Formulations	pH
ME-1	6.12±0.04
ME-2	5.75±0.03
ME-3	6.42±0.02
ME-4	5.81±0.03
ME-5	4.35±0.06

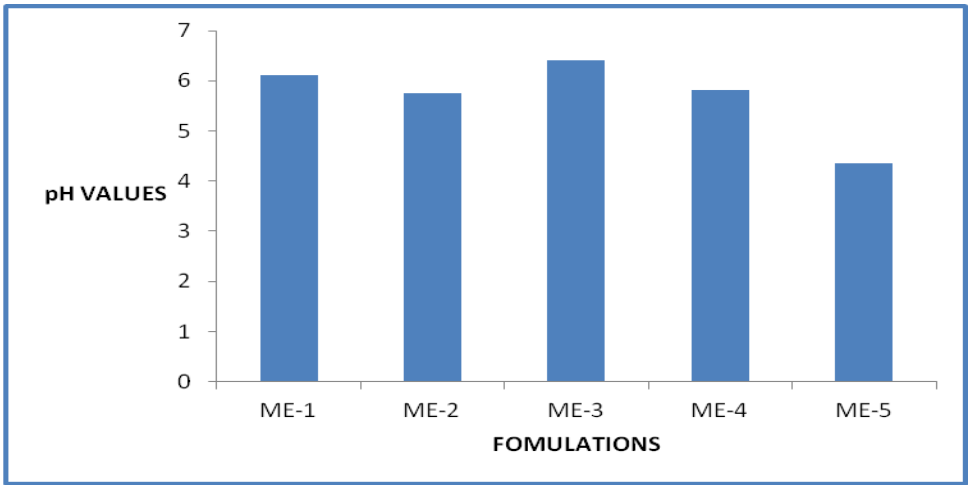


Fig: 9- Comparative pH values of Formulations

Table 12: **Comparative Viscosity values of Formulations**

Formulations	Viscosity(cps)
ME-1	52.6±0.6
ME-2	75.3±0.8
ME-3	91.4±0.4
ME-4	103±0.5
ME-5	118.2±0.2

Fig: 10-Comparative Viscosity values of Formulations

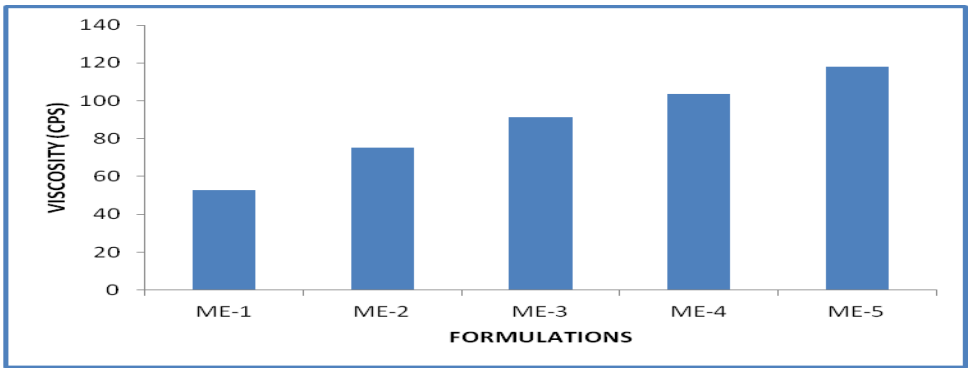
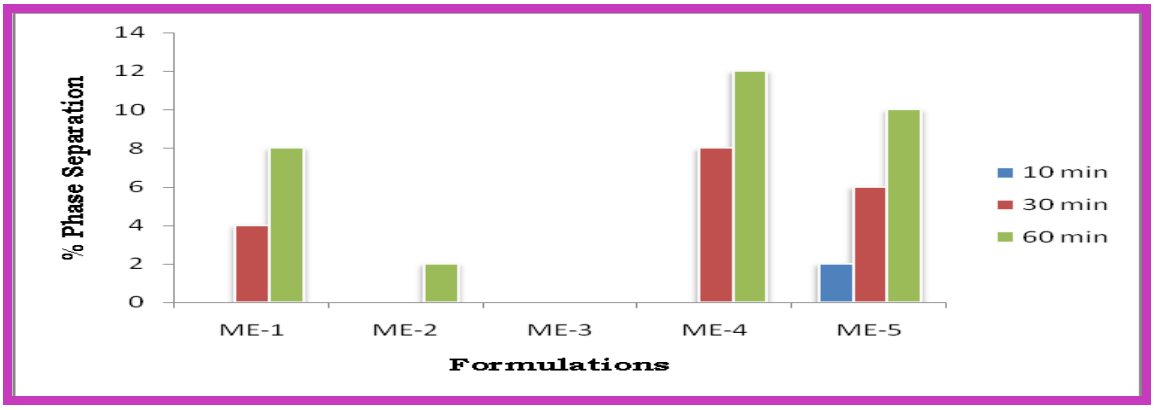


Table 13: Comparative study of mechanical stress in Formulations

S.NO	Centrifugation time(min)	%Phase separation				
		ME-1	ME-2	ME-3	ME-4	ME-5
1	10	-	-	-	-	2
2	30	4	-	-	8	6
3	60	8	2	-	12	10

Fig 11: Comparative study of mechanical stress in Formulations



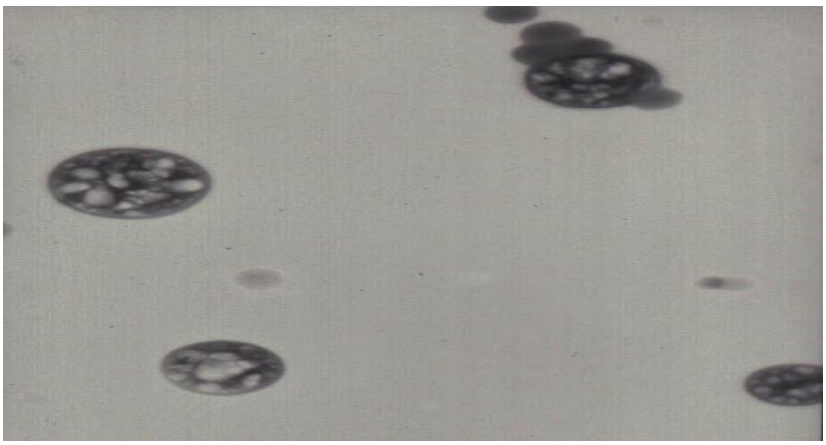
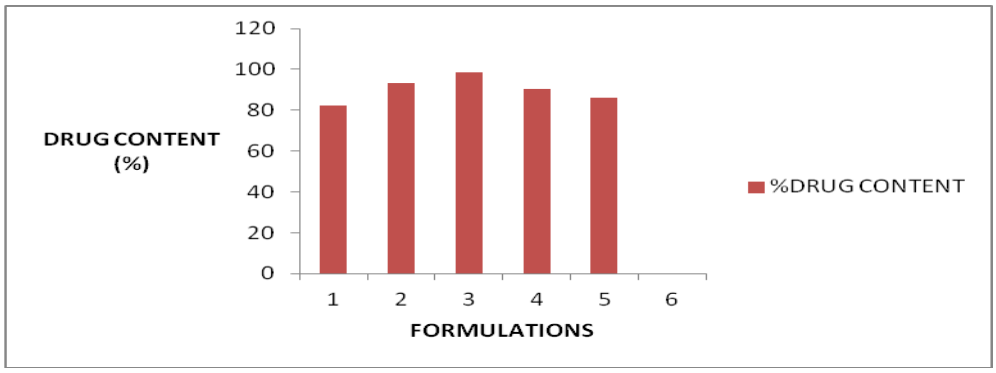


Table 14 Fig: 12-TEM Photo graph of Formulation (ME-3)

Table 14: Comparative drug content of Formulations

Formulations	Drug content (%)
ME-1	82.42±0.32
ME-2	93.12±0.54
ME-3	98.54±0.26
ME-4	90.21±0.42
ME-5	86.34±0.28

Fig 13: comparative drug content of Microemulsion formulations



ME-3 Formulation possesses higher drug content compared to other ME formulations

Table 15: Comparative *in vitro* Skin permeation rate of Diclofenac Sodium Microemulsions

Time in hours	Cumulative % drug permeated (µg/cm^2)				
	ME-1	**ME-2**	**ME-3**	**ME-4**	**ME-5**
0	0	0	0	0	0
0.5	3.71±0.12	7.06±0.032	6.59±0.42	4.22±0.12	10.70±1.02
1	4.32±0.02	11.03±0.42	14.56±0.31	10.02±0.02	14.70±0.34
2	5.84±2.11	13.15±2.12	18.59±2.10	17.23±0.31	19.39±0.15
4	8.76±0.21	16.22±1.25	27.96±0.24	21.98±1.02	20.79±0.52
6	10.09±1.13	18.52±0.02	33.12±0.15	25.44±1.32	25.41±0.12
8	15.14±0.02	20.25±1.01	41.24±1.02	29.05±2.01	28.36±0.32
10	18.02±0.21	24.65±0.21	47.14±0.21	32.72±0.02	31.14±2.53
12	21.80±1.04	27.53±1.31	53.98±2.25	35.68±0.12	33.80±1.28
14	25.44±0.01	29.73±0.20	61.19±0.20	43.53±0.21	36.22±0.28
16	27.96±0.02	33.77±0.35	67.24±1.01	50.34±1.20	44.66±0.2
18	32.76±2.24	42.23±2.25	75.68±0.13	57.30±2.03	49.66±1.26
20	35.57±0.31	49.62±0.14	83.24±0.20	66.56±0.41	57.80±0.02
22	43.53±0.24	59.03±0.21	86.12±2.02	70.92±0.25	64.76±1.25
24	50.63±0.02	66.56±2.32	88.79±0.15	78.13±1.02	76.40±0.15

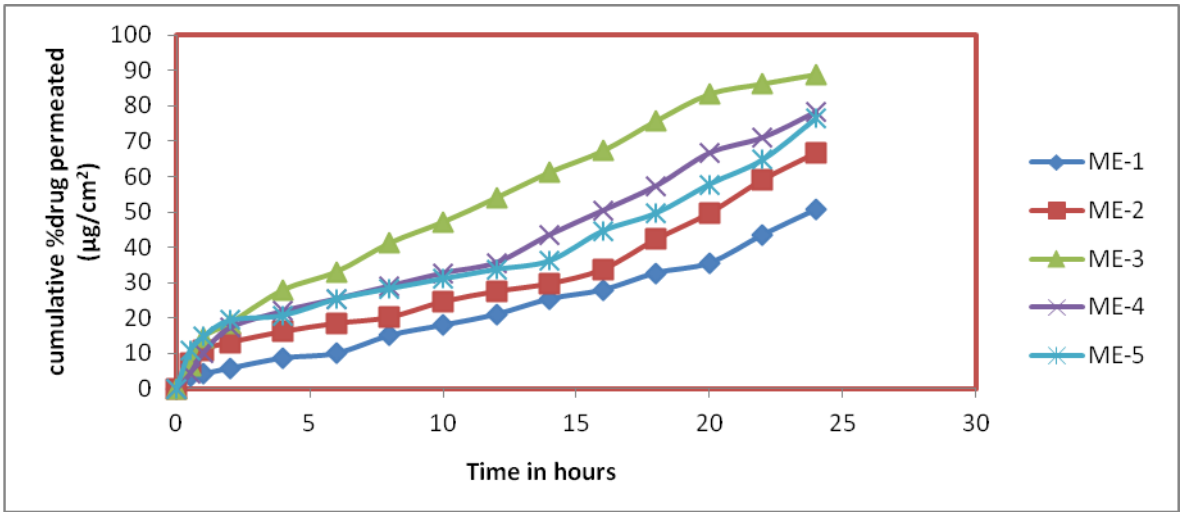


Fig 14: Comparative *in vitro* Skin permeation rate of Diclofenac Sodium Microemulsions

The Comparative graph indicates ME-3 had higher skin Permeation rate compared other microemulsion formulations.

Table 16: Model fitting of the in vitro Permeation data of various Diclofenac Sodium Microemulsions

Formulations	r^2 Value				
	Zero order	First order	Higuchi	Korsmeyer-peppas	Diffusional exponent (n)
ME-1	0.978	0.817	0.899	0.999	0.7
ME-2	0.941	0.817	0.845	0.563	1.9
ME-3	0.989	0.958	0.981	0.988	1.0
ME-4	0.982	0.817	0.933	0.644	2.0
ME-5	0.955	0.817	0.873	0.913	1.1

Table 17: Release kinetics of ME-3

Time in hrs	Log Time	√Time	% Cumulative drug release	Log Cumu. %drub release	Log % Cumu.of drug remaind
0.50	- 0.3010	0.71	6.59	0.8189	1.9704
1.00	0.0000	1.00	14.55	1.1629	1.9317
2.00	0.3010	1.41	18.59	1.2693	1.9107
4.00	0.6021	2.00	27.96	1.4465	1.8576
6.00	0.7782	2.45	33.11	1.5200	1.8254
8.00	0.9031	2.83	41.24	1.6153	1.7691
10.00	1.0000	3.16	47.24	1.6733	1.7232
12.00	1.0792	3.46	53.98	1.7322	1.6629
14.00	1.1461	3.74	61.18	1.7866	1.5891
16.00	1.2041	4.00	67.24	1.8276	1.5153
18.00	1.2553	4.24	75.67	1.8789	1.3861
20.00	1.3010	4.47	83.24	1.9203	1.2243
22.00	1.3424	4.69	86.12	1.9351	1.1424
24.00	1.3802	4.90	88.79	1.9484	1.0496

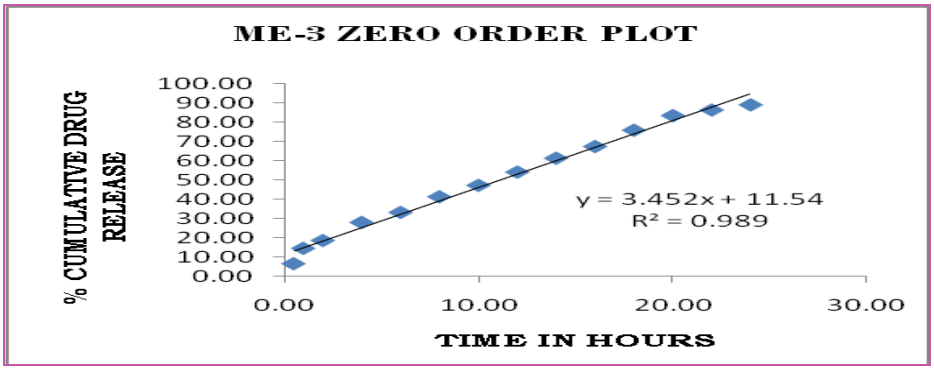


Fig 15: **ME-3 Zero order plot**

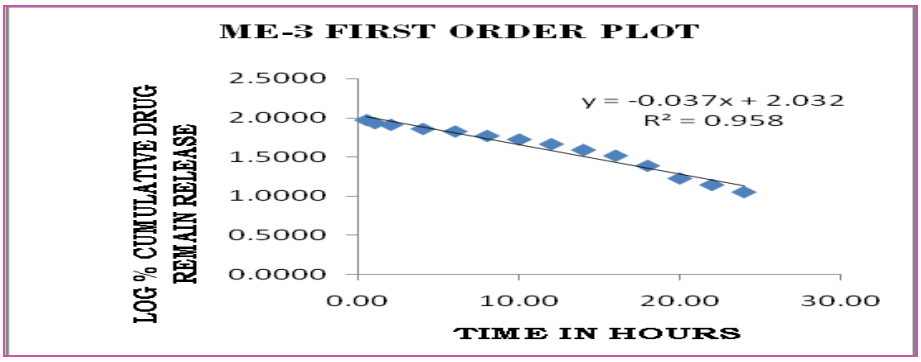


Fig 16: **ME-3 First order plot**

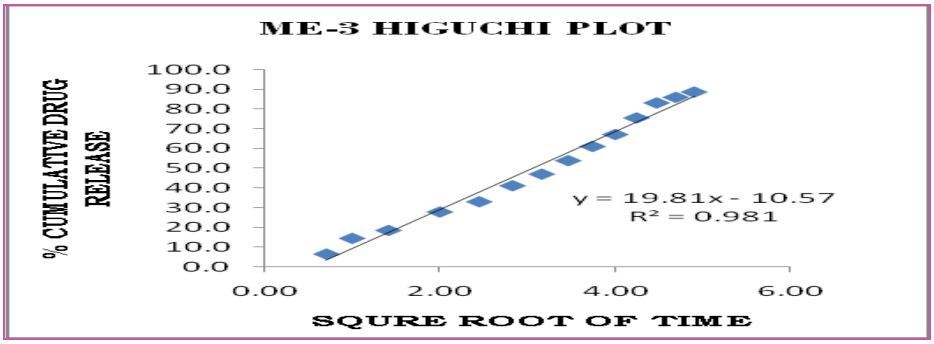


Fig 17: **ME-3 Higuchi plot**

Table18: Stability Study of Micro Emulsion (ME-3)

S.No	Formulation ME-3	Before storage	Stored at $40^0C \pm 2^0C$ and 75%±5%RH		
			1st month	2nd month	3 rd month
1	Drug content (%)	98.54	98.23	98.01	97.36
2	pH	6.42	6.31	6.01	5.65
3	Viscosity(cps)	91.4	90.2	90	90

CONCLUSION:

In order to identify the optimum ME formulation containing oleic acid, Tween-80, polyethylenglycol-400(PEG-400) were selected from the trial formulations ratios (1:1,2:1). (F1-F10) trial formulation in two ratios (1:1,2:1) of microemulsion formulations were prepared by varying the amount of surfactant/co-surfactant.

In 1:1 and 2:1 ratios of trial formulations stable microemulsions were not formed. Turbid and conventional emulsions based visual observation. From ten trial formulations one **(F8) formulation** were selected on 2:1 ratio for optimizing the formulations. Finally drug, surfactant and co-surfactant is kept constant and oil amount was changed.

The microemulsion (ME-1to ME-5 were subjected to the study of **Opticle transparency**. ME-3formulation were optically clear, transparent and elegant in appearance when compared to other microemulsions

The **pH** values of ME-1 TO ME-5 (6.12±0.04, 5.75±0.03, 6.42±0.02, 5.81±0.03, 4.35±0.06) units only. In all cases pH showed the smallest changes. The pH value of optimized Diclofenacsodium microemulsion formulation (ME-3) was 6.42±0.02 suitable for topical application because the pH of skin in the range 5.5 to 7.0.

It was clear from the **viscosity of** microemulsion systems (ME-1 to ME-5) that the developed formulations have gradually increased (52.6±0.6cps, 75.3±0.8cps, 91.4±0.4cps, 103.5±0.5cps, 118.2±0.2cps).All samples exhibited Newtonian flow behaviour, as expected from microemulsions.

The Centrifugation stress test of formulations ME-3 shows good physical stability and no phase separation when were centrifuged at 2000 rpm for 60mins.

The morphology of Microemulsion was characterized using **Transmission Electron Microscopy**. The TEM image of optimized best formulation showed that globules were spherical in shape and had smooth surface. The results TEM further indicated the existence of an isotropic dispersion of spherical droplets, leading to the assumption of inverse micelles because of the proportion of the constituents. The particle size distribution of the best microemulsion formulation (ME-3) ranged from 0.276µm to 1.231µm with the average droplet size of (ME-3) 0.78µm. The result shows that the droplet diameter decreases with increasing ratio of oil and surfactant: co-surfactant. Due to the small droplet size of (ME-3) microemulsion, its surface areas are assumed to be high. Therefore, droplets of microemulsion settled down to close contact with the skin providing high concentration gradient and improved Diclofenac sodium permeation from formulation (ME-3). The average droplet size of the best microemulsion formulation (ME-3) was determined by **Atomic Force Microscopy.** The average droplet sizes of ME-3 0.88µm and 0.45µm.

The mean percent **drug content** in microemulsion formulations (ME-1 to ME-5) was found to be respectively. (82.42±0.32%, 93.12±0.54%, 98.54±0.26%, 90.21±0.46%, 86.34±0.28%). **ME-3 was exhibited 98.54±0.26% higher drug content then other formulations.**

The permeation capability of the microemulsion formulations were evaluated by conducting the *in – vitro* **skin permeation** experiments. ME-(1-5) Diclofenac sodium microemulsions were studied for *in vitro* skin permeation through excised goat skin. The amount of Diclofenac sodium permeated through excised goat skin over 24-hour period was plotted against the function of time. The permeation fluxes (µg/cm^2/hour) for all these microemulsions through the goat skin were determined. **Among all formulations, the highest permeation flux of µg/cm^2/hour was observed in of formulation ME-3.**

The *in vitro* Diclofenac Sodium permeation data from microemulsions containing Diclofenac Sodium through excised goat skin were evaluated kinetically by various mathematical models like zero-order, first –order, and Korsmeyer-Peppas model. The results of the curve fitting into these above-mentioned **Mathematical models** indicate the in vitro Diclofenac Sodium permeation behavior of Diclofenac Sodium microemulsions (ME-1 to ME-5). When respective correlation coefficients were compared, ME-1, ME-2, ME-3, ME-4 and ME-5. ME-3 followed the zero-order release (r^2=0.982) over a period of 24 hours. Again, the Korsmeyer-Peppas model was employed in the *in vitro* Diclofenac Sodium permeation behavior analysis of these formulations to find out permeation mechanism: Fickian (nonsteady) diffusional release when n≤ 0.5, case –II transport (zero-order) when n≥1, and non-Fickian, "anomalous" release when the value of n is in between 0.5 and 1.

The determined values of diffusion exponent (n=0.1) in ME-3 indicating that the drug permeation from Diclofenac Sodium microemulsion followed the non-Fickian, "anomalous" mechanism.

Stability of the prepared microemulsion formulations was assessed using accelerated temperature study. The drug content, pH, viscosity of the best formulation ME-3 were subjected to stability studies at 40^{0}C/75% RH up to 3 months. The results are summarized in 38. ME-3 Sample showed excellent results in these studies. Drug degradation was found to be in the range (98.23, 98.01%, and 97.36%) after three months. Viscosity values after three months compared to the initial viscosity were in the range 0-1cps, while pH changed 6-5 units only. In all cases, ME-3 showed the smallest changes in these parameters. Overall results from the stability studies indicated that the microemulsions were chemically stable for three months.

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