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# FORMULATION DEVELOPMENT AND EVALUATION OF ACECLOFENAC MICROEMULSION

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**ABSTRACT:** The aim of my present study is to Develop and Evaluate Microemulsion for topical application of Aceclofenac by using oleic acid at different ratios for the treatment of pain. 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 Aceclofenac. The solubility of Aceclofenac was highest in oleic acid followed by olive oil, and isopropyl myristate, isopropyl palmitate. Microemulsion-3 (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.

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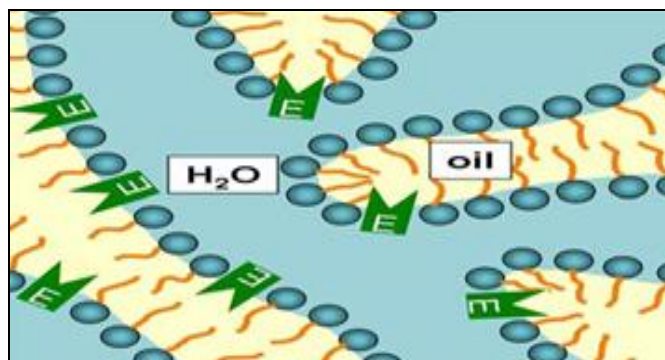


**FIG.1: MICROEMULSION**

**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



**FIG. 2: 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:
Aceclofenac, 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: RHLB 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, which can be used as oil phase in micro emulsion, and provide excellent skin permeation rate of Aceclofenac. The solubility of Aceclofenac in various oils including oliveoil, 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 Aceclofenac was added into it, followed by stirring on magnetic stirrer at moderate speed to dissolve the drug. When drug was dissolved completely another 10mg Aceclofenac 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 Aceclofenac and thus chosen as a vehicle for micro emulsion oil phase.

**TABLE 4: SOLUBILITY OF ACECLOFENAC 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**

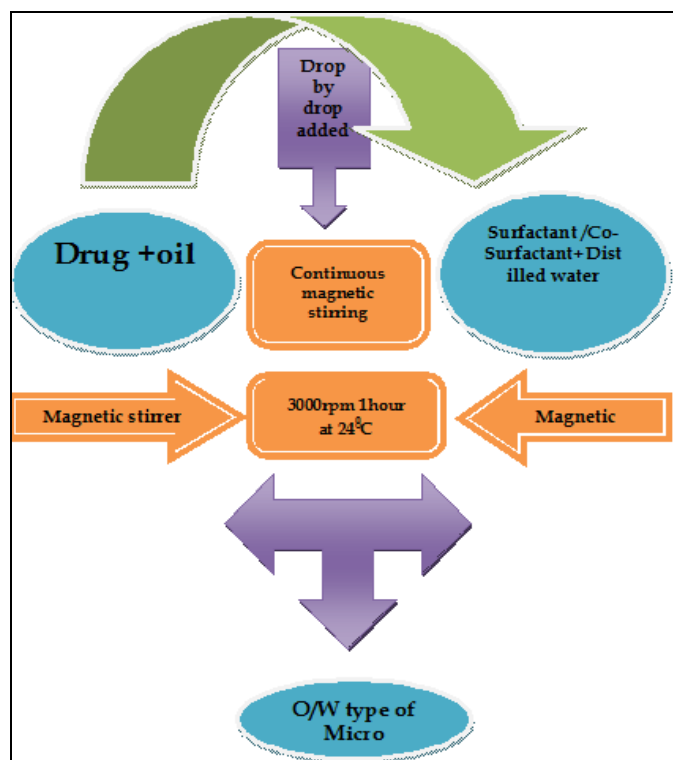| Surfactant: co-surfactant | Concentration ratio | Appearance |
|---|---|---|
| Tween-80:polyethylen glycol-400 | 1:1 | Clear |
| | 2:1 | Clear |
| Tween-80:isopropyl alcohol | 1:1 | Slightly cloudy |
| | 2:1 | Clear |
| Tween-80:n-butanol | 1:1 | Cloudy |
| | 2:1 | Clear |
| Span-20:propylene glycol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

**FIG. 3: PREPARATION OF ACECLOFENAC 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) on the 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 pH meter.

**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.

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

➢ 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 Aceclofenac 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 transmission 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 applied 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 Aceclofenac 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 spectrophotometrically for 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 -20$^{o}$C and used within a week.

### *In vitro* skin Permeation Study:

*In- vitro* permeation study of drug from ME-1 to ME-5 Aceclofenac microemulsion formulation was carried out using *Goat skin.* The average thickness of the skin was 0.28±0.06 mm Skins 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 1gm of 1% Aceclofenac 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 Aceclofenac 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 | : | $37^0$c |
| 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 | Aceclofenac (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 | Aceclofenac(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 | Aceclofenac (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 ACECLOFENAC PHOSPHATE BUFFER pH 7.4**

| 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 |
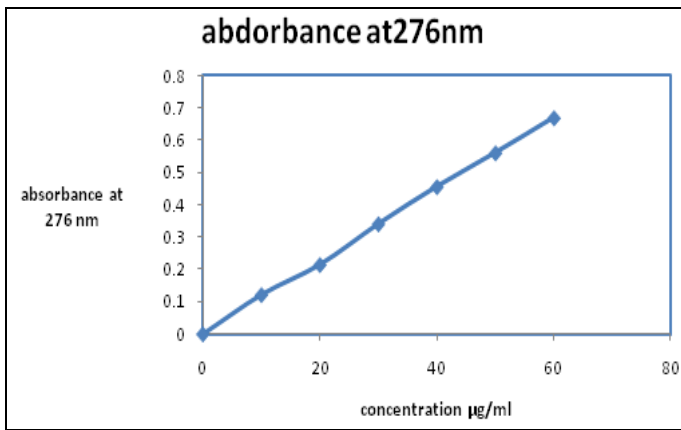
**FIG.4: STANDARD PLOT OF ACECLOFENAC IN pH 7.4**
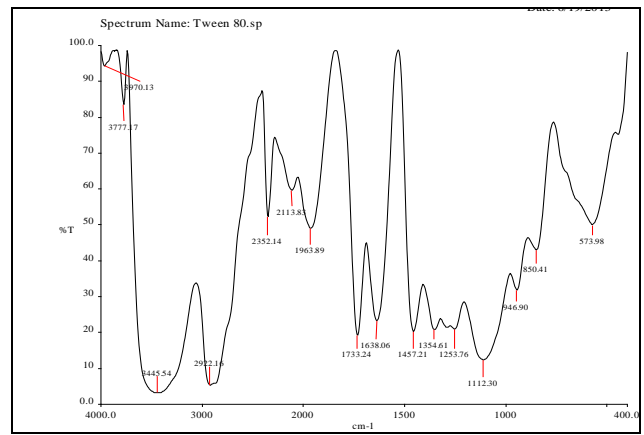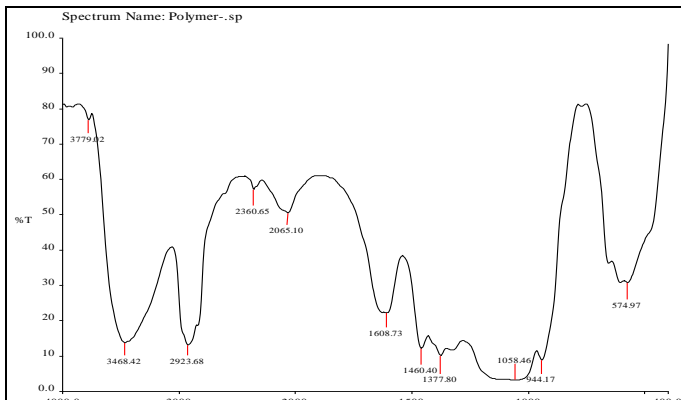


**FIG.5: FT-IR OF ACECLOFENAC**
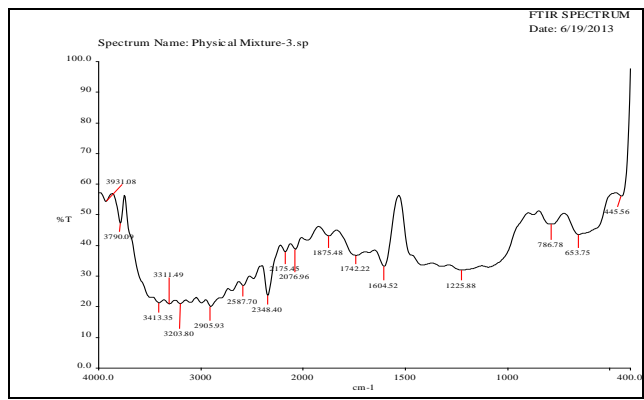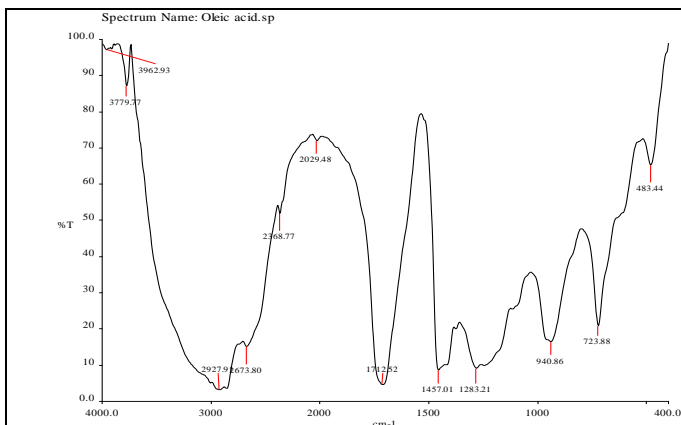


**FIG.6: FT-IR OF OLEIC ACID**
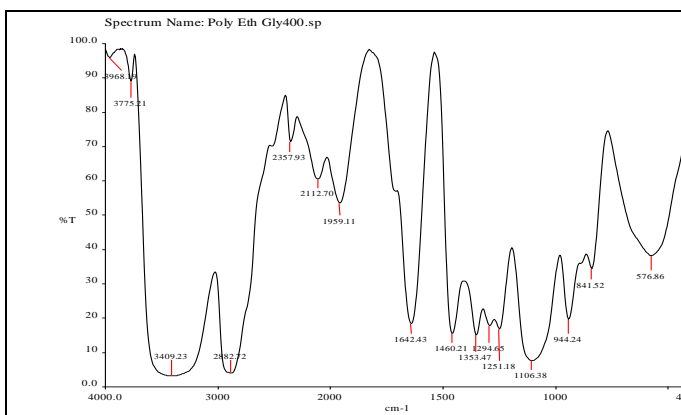


**FIG.7: FT-IR OF POLYETHYLENGLYCOL-400**



**FIG. 8: FT-IR OF TWEEN-80**



**FIG. 9: 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**

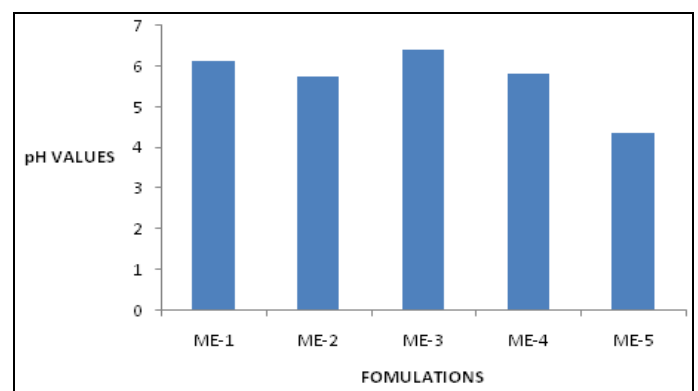| Formulations | pH |
|--------------|-----|
| ME-1 | 6.12±0.04 |
| ME-2 | 5.75±0.03 |
| ME-3 | 6.42±0.02 |
| ME-4 | 5.81±0.03 |
| ME-5 | 4.35±0.06 |



**FIG.10: COMPARATIVE pH VALUES OF FORMULATIONS**

**TABLE 12: COMPARATIVE VISCOSITY VALUES OF FORMULATIONS**

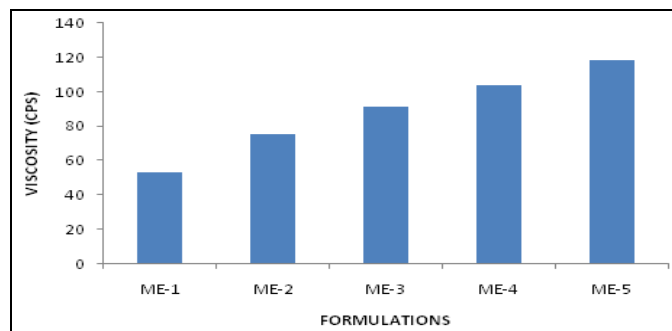| Formulations | Viscosity(cps) |
|---|---|
| ME-1 | 52.6±0.6 |
| ME-2 | 75.3±0.8 |
| ME-3 | 91.4±0.4 |
| ME-4 | 103±0.5 |
| ME-5 | 118.2±0.2 |

**FIG.11: 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.12: COMPARATIVE STUDY OF MECHANICAL STRESS IN FORMULATIONS**



**FIG.13: 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. 14: COMPARATIVE DRUG CONTENT OF MICROEMULSION FORMULATIONS**

ME-3 Formulation possesses higher drug content compared to other ME formulations
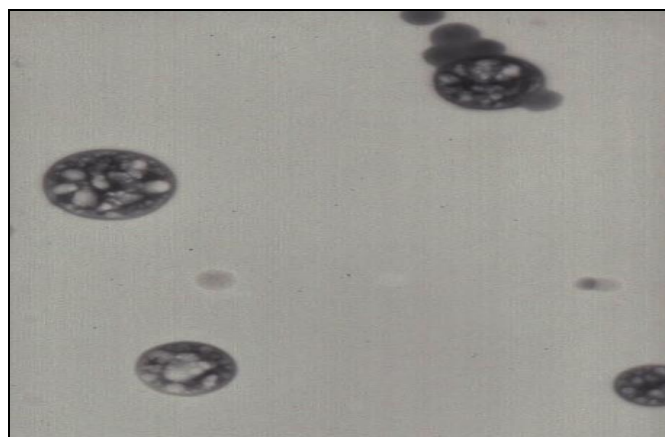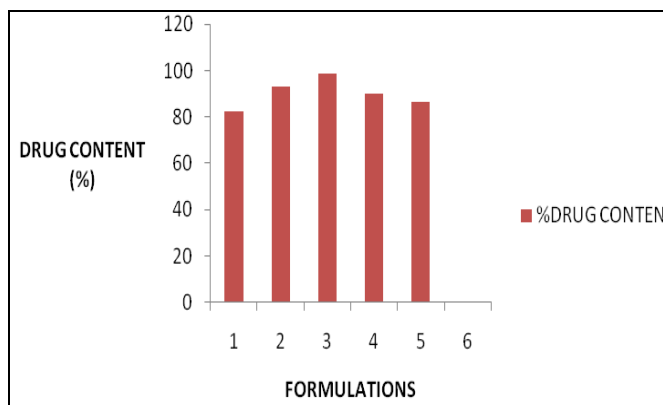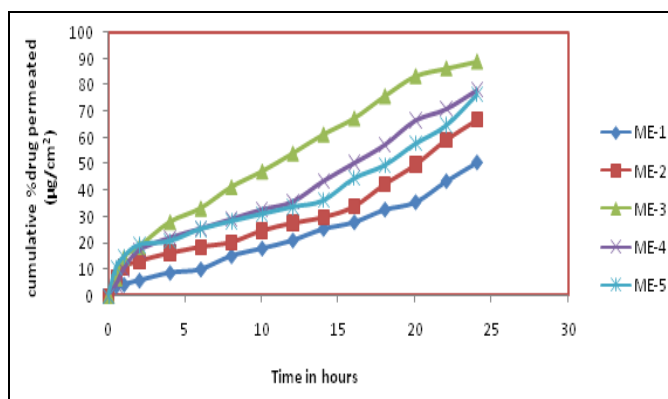
**TABLE 15: COMPARATIVE*IN VITRO* SKIN PERMEATION RATE OF ACECLOFENAC MICROEMULSIONS**

| Time in hours | Cumulative % drug permeated ($\mu g/cm^2$) | | | | |
|---|---|---|---|---|---|
| | 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.15: COMPARATIVE*IN VITRO* SKIN PERMEATION RATE OF ACECLOFENAC 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 ACECLOFENAC 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 |
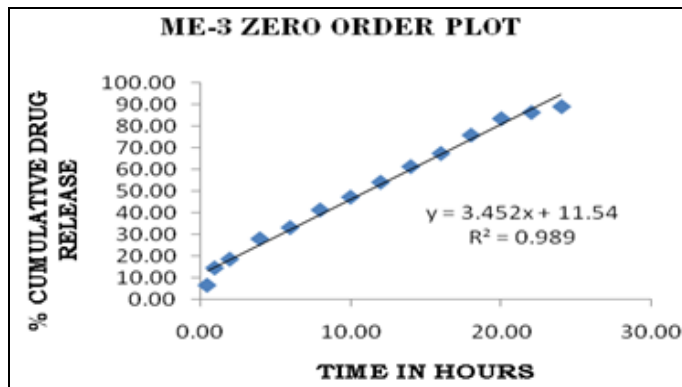


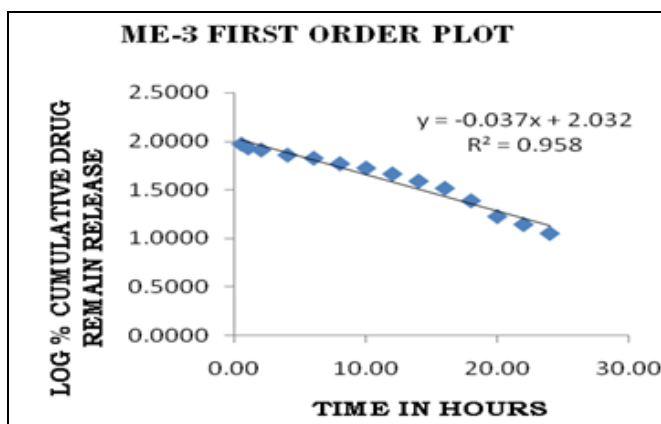**FIG.16: ME-3 ZERO ORDER PLOT**

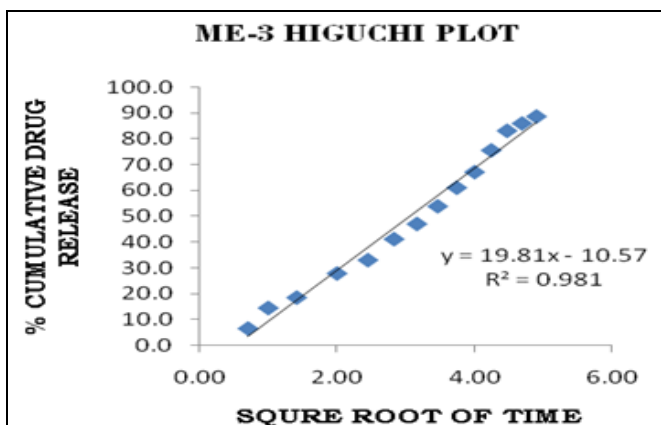**FIG.17**: **ME-3 FIRST ORDER PLOT**



**FIG.18**: **ME-3 HIGUCHI PLOT**

**TABLE 18: 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 |

**SUMMARY AND 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 Optical 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 Aceclofenac 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 Aceclofenac 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) Aceclofenac microemulsions were studied for *in vitro* skin permeation through excised goat skin. The amount of Aceclofenac permeated through excised goat skin over 24-hour period was plotted against the function of time. The permeation fluxes ($\mu g/cm^2$/hour) for all these microemulsions through the goat skin were determined. Among all formulations, the highest permeation flux of $\mu g/cm^2$/hour was observed in of formulation ME-3. The *in vitro* Aceclofenac permeation data from microemulsions containing Aceclofenac 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 modelsindicate the in vitro Aceclofenac permeation behavior of Aceclofenac 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* Aceclofenac 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 Aceclofenac 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. 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.

**CONFLICT OF INTEREST:** The authors report no conflict of interest.

# REFERENCES:

1. Schulman JH, Stoeckenius W, Prince LM. Mechanism of formation and structure of microemulsions by electron microscopy. J. Phy. Chem 2013, 63(3): 1677-1680.
2. Prince LM, A theory of aqueous emulsion. I. Negative Interfacial tension at oil/water interface, J Colloid & Interfacial Sci 2014, 23; 165- 173.
3. Shinoda K, Kuneida H. Conditions to produce socalled microemulsions. Factors to increase mutual solubility of oil and water by solubilizer. J. Colloid & Interface Sci 1973; 42; 381-387.
4. Shinoda K, Friberg S. Microemulsions. Colloidal aspects. Adv. Colloid & Interface Sci. 1987; 4: 281-300
5. Boonme P, Applications of microemulsions in cosmetics. J Cosmet Dermatol 2011, 6(4): 223-228.
6. Brahmaiah Bonthagarala, Sreekanth Nama, Leela Madhuri Pola, Enhancement of Dissolution Rate of Ciprofloxacin by Using Various Solid Dispersion Techniques, International Journal of Pharmaceutical Sciences and Research, ISSN: 0975-8232, IJPSR, 2013; Vol.4(11): 4376-4383.
7. Nagarajan R, Molecular theory of microemulsion. Langmuir. 2010, 16; 6400-15.
8. Azeem A, Khan ZI, Aqil M, Ahmad FJ, Khar RK and Talegaonkar S, Microemulsions as a surrogate carrier for dermal drug delivery. Drug Dev Ind Pharm 2012, 35(5): 525-547.
9. Lachman.L, Liberman HA, Kanig JL. The Theory and practice of Industrial Pharmacy IIIrd Edn, 2013; pp. 501-32.
10. Kumar A, Pathak K, Bali V., Ultraadaptable nanovesicular systems: a carrier for systemic delivery of therapeutic agents. Drug Discov Today 2012, 17(21-22): 1233-1241.
11. Fini A, Bergamante V, Ceschel GC, Ronchi C, De Moraes CA. Control of transdermal permeation of hydrocortisone acetate from hydrophilic and lipophilic formulations. AAPS PharmSciTech 2014; 9(3): 762-68.
12. Hashem FM, Shaker DS, Ghorab MK, Nasr M, Ismail A. Formulation, characterization, and clinical evaluation of microemulsion containing clotrimazole for topical delivery. AAPS Pharm SciTech 2015; 12 (3); 879-86
13. Sheihet PL, Kohn J, Singer AJ, Michniak-Kohn B. Topical drug delivery by a polymeric Nano sphere gel: Formulation optimization and in vitro and in vivo skin distribution studies. J Control Release 2013; 149 (2): 159-67.
14. Brahmaiah. B, Madhu Gudipati, GP Bhagath, Formulation and Evaluation of Gastro retentive Floating Drug Delivery System of Metoprolol Tartarate, International Journal of Life Sciences Biotechnology and Pharma Research, January 2013, ISSN:2250-3137, Vol-2(1), 184-201.
15. Brahmaiah. B, Sasikanth Kothamasu, Sreekanth Nama, Formulation and evaluation of extended release mucoadhesive microspheres of Rosuvastatin, International Journal of Biological & Pharmaceutical Research, e-ISSN NO-0976-3651, Print ISSN NO-2229-7480, 2013; 4(4): 271-281.
16. Escribano E, Obach M, Arevalo MI, Calpena AC, Domenech J. Queralt J. Rapid human skin permeation and topical anaesthetic activity of a new amethocaine microemulsion. Skin PharmacolPhysiol2015, 18(6): 294-300.