Original Research Article

Design,Developmentand *In vitro* Assessment of Flurbiprofen Microemulsion Gel for Transdermal Drug Delivery

Abstract:

The aim of current research is to develop flurbiprofen microemulsion gel for transdermal delivery. To prepare a microemulsion oil, surfactant and co-surfactant selection is one of the important criteria. Selected to prepare different ratios of oil and surfactant mixture using ternary phase diagram.Based on results of physicochemical properties, we have optimized one formulation. The optimized microemulsion formulation incorporated into carbopol P 934 gel base. The *in vitro* diffusion of drug release shown maximum (98.6% in 12 hrs) for prepared microemulsion gel than marketed gel (60.5% in 16 hrs).

Keywords:Solubility;Microemulsion; Phase diagram; Gel; Permeation.

1. Introduction

Flurbiprofen, a potent nonsteroidal anti-inflammatory drug, is widely used for relief of pain in patients suffering from rheumatic diseases, migraine, sore throat and primary dysmenorrhoea. However, this drug has many gastrointestinal side effects produced by its oral administration, such as gastric bleeding and peptic ulcer. These effects are responsible for non-compliance among patients [1]. Flurbiprofen belongs to the biopharmaceutical class II of the biopharmaceutical classification system (BCS) of drugs; and the aqueous solubility of drugs of this class limits their permeation flux.The physicochemical properties of flurbiprofen, i.e., lipophilicity (log Koct= 4.24), low molecular weight and short elimination half-life make it a suitable candidate for transdermal drug delivery but its aqueous solubility is very low (0.03mg/ml) which hinders its skin penetration. The physicochemical properties of flurbiprofen make it a suitable candidate for transdermal drug delivery, which can overcome the drawbacks of oral administration. In this sense, microemulsions have been proved to increase the cutaneous absorption of lipophilic drugs when compared to conventional drug delivery systems [2].Microemulsions are a good example of pharmaceutical nano-technologic carrier systems which, during the recent decades, have increasingly attracted researchers as drug carriers for various therapeutics [3].Transdermal route of drug delivery has received increasing attention

Comment [BN1]: 1. Article is quite interesting and it is innovative. It has 18% of Plagiarism throughout the article. The source is Grammarly Plagiarism checker.
2. Other surfactants/co-surfactants could be included as in the form of comparison table which can provide advantage of this research study.
3. FTIR spectrum is not legible. Recommended to included high resolution image about 600 dpi.
4. Abbreviation need to be expanded in the first place of the word for better clarity. Please check the article thoroughly and incorporate.
5. Formatting of table/figures to be done as per the journal guidelines.
6. No table is included in the article. Recommended to include the obtained results in the form of table along with the advantage over the other surfactants.
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because it has the advantages of avoidance of hepatic first-pass metabolism, easier and convenient administration, possibility of immediate withdrawal of treatment, and potential of long-term controlled release with steady-state plasma drug levels and possibility for reduction of dose and for improving patient compliance [4]. Microemulsions form spontaneously with an average droplet diameter of 10 to 140nm. Microemulsions contain definite boundary between oil and water phases at which surfactant is located. Conventional surfactant molecules comprised polar head group region and non-polar tail region. Microemulsions may be asymmetric in shape, frequently adopting the shape of prolate ellipsoid [5-6].

2. Materials:

Flurbiprofenand oils were procured from Yarrow Chem products,Mumbai.Transcutol HP also obtained from Yarrow Chem products, Mumbai.

2.1.Methods:

Flurbiprofen UV calibration analysis was performed using phosphate buffer saline pH 7.4 (PBS). Linearity was found in the range of 2 to 12 µg/ml. Obtained samples were analysed at 247 nm.

2.2.Preformulation studies

The partition coefficient of Flurbiprofen was determined between the water and n-Octanol[7].

2.2.1. Screening of oil, surfactants, Co surfactants

The oil, surfactant and co-surfactant for developing MEs of flurbiprofen was selected on the basis of solubility, surfactant efficiencyand water solubilization capacity. The solubility of flurbiprofen in various oils was determined employing shake flask method and drug content was analysed using UV-visible spectrophotometer at 247nm.The solubility of flurbiprofen in different oils, surfactants and Co surfactants were determined by taking 10 ml of oils or surfactant and Co surfactant in 50 ml conical flask and excess amount of drug was added. The conical flasks were closed with the help of Aluminium foil and were kept for stirring at 100 rpm at ambient temperature for 72 hrs. in orbital shaker. Samples were centrifuged for 15 min. The supernatant was separated, filtered and drug content was determined using UV-Visible Spectrophotometer [8].

3.2.2. Preparation of surfactant and Cosurfactant mass ratio for microemulsion

Surfactant was mixed with Co surfactant in ratios of 1:1, 2:1, 3:1 and 1:3 for study of phase diagrams. S_{mix}were chosen in decreasingconcentration of surfactant with respect to Co surfactant and *vice versa*. Different combinations in different weight ratios of oil and S_{mix}1:9, 2:8, 3:7, 4:6,

5:5, 6:4, 7:3, 8:2 were taken. Aqueous titration method was used for the construction of Pseudo ternary phase diagrams. It involves stepwise addition of water to each weight ratio of oil and S_{mix} and mixing of components by using magnetic stirrer.The mixtures were evaluated visually and microemulsion was identified as the region in the phase diagram where translucent, easily flowable and clear formulations are obtained [9].

Selected microemulsion formulations were chosen from themicroemulsion obtained domain

2.2.2. FTIR analysis of samples

FTIR studies were conducted by KBr pellet method. Required sampleof each excipient was tested for their spectrum analysis and the system equipped with opus software.The spectra were recorded in the wavelength range of400 to 4000 cm^{-1}.

3.3.Preparation of flurbiprofen microemulsions

For all the selected formulae, first, flurbiprofen (2.5%, w/v) wasdissolved gradually in the oil phase. Second, $S_{mix,}$ the resultant mixture was mixed under magnetic stirring. Third, the aqueous phase was added gradually to the oil/S_{mix}. Magnetic stirring was used to aid rapid emulsification [9].

3.4.Preparation of flurbiprofen microemulsion gel

Microemulsion gel was prepared by dispersing 1% (w/w) ofCarbopol 934 in sufficient quantity of distilled water. Thedispersion was kept in dark for 24 h and allowed to swell. The preparedcarbopol gel was neutralized with Triethanolamine (0.5%,w/w). 2.5% of flurbiprofen was dissolved in 8.33% (w/w) of clove oil.33.33% (w/w) mixture of S_{mix}ratioand this was added slowly tocarbopol gel. Remaining quantity of distilled water was added toit to get the final preparation 100% (w/w). It was added slowly tocarbopol gel and mixed it up well [10-11].

3.5. Preparation of flurbiprofen conventionalgel

Conventional flurbiprofen gel was prepared by dispersing 1%(w/w) of Carbopol 934 in sufficient quantity of distilled water. Thedispersion was kept in dark for 24h and allowed to swell. 2.5% of flurbiprofen was dispersed into gel. The prepared gel was neutralizedwith Triethanolamine (0.5%, w/w) and remaining quantity ofdistilled water was added to get the final preparation 100% (w/w) [10-11].

3.6. Evaluation of microemulsion gel [11-12]

3.6.1. Determination of drug content

I ml of prepared formulation was transferred into 10 ml of volumetric flask and volume was made up with methanol. The sample was filtered. From the above solution1 ml of solution was withdrawn and was transferred into 10ml of volumetric flask, the final volume was made upto the mark with pH 7.4 PBS. The solution the solution was filtered and the drug content was analysed by UV-Visible spectrophotometer.

3.6.2.Determination of pH

The pH values for microemulsion were determined at 25°C by ElicoL1614 pH meter.All measurements were carried out in triplicate.

3.6.3.Zeta potential determination

Zeta potential of samples were measured by Malvern Zeta sizer Samples were placed in clear disposable zeta cells and results were recorded. Before putting the fresh sample, cuvettes were washed with methanol and rinsed using the sample to be measured before each experiment.

3.6.4. Globule size measurements

The average droplet size of samples was measured at 25 °C by Malvern Zeta sizer

3.6.5.Conductivity measurements

The electric conductivity of ME was measured with a Malvern instrument and was done by using conductivity cell (with a cell constant of 1.0) consisting of two platinum plates separated by desired distance and having liquid between the platinum plate acting as a conductor.

3.6.6. *In vitro* drug release studies

Cellulose membrane was utilized to determine the release rate of flurbiprofen from different microemulsion formulations. The cellulose membrane was first hydrated in the distilled water solution at 25^{o}C for 24 hrs. The dialysis bag was prepared by tying one end of the membrane and the microemulsion formulation was transferred into membrane bag and then the other end of the bag was closed by tying. The bag was immersed into a beaker containing 50 ml phosphate buffer pH 7.4 buffer solution in such a way that the lower end of the bag remains in contact with solution of the beaker. The receptor fluid was constantly stirred by externally driven magnetic 100 600 rpm throughout the experiment. At 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 hrs time intervals, 3 ml sample was removed from receptor for spectrophotometric determination and replaced immediately with an equal volume of fresh receptor solution. Samples were analysed by UV visible spectrophotometer at247nm. The results were plotted as percent drug release versus time [9,13].

3.6.7. *In-vitro* skin permeation study

A Franz diffusion cell (area 12.57cm^2) with a goat skin was utilized to determine the Release rate of flurbiprofen from different microemulsion based gel formulations.Thegoat skin was first hydrated in the (pH 7.4) phosphate buffer solution at 25ºC for 1 hr. The skin was then clamped between the donor and receptor compartments of the cells Diffusion cell was filled with 25 ml of phosphate buffer (pH=7.4). The receptor fluid was constantly stirred by externally driven magnetic bars at 100rpm throughout the experiment. Flurbiprofen containing gel was accurately weighted and placed in donor compartment. At 0.5, 1, 2, 3, 4, 5, 6, 7, 8,9,10, 11, 12, 14,16 h time intervals, 2ml sample was removed from receptor for spectrophotometric determination and replaced immediately with an equal volume of fresh receptor solution. Samples were analysed by UV visible spectrophotometer at 247nm. The results were plotted as cumulative released drug percent versus time [9,13].

3.6.8. Release kinetics:

The analysis of drug release mechanism from a pharmaceutical dosage form is important but is a complicated process and is practically evident in the case of matrix systems. As a model-dependent approach, the diffusion was fitted to four popular release models such as zero-order, first order, Higuchi and korsemeyer-Peppas equations.The order of drug diffusion from the microemulsion based gel was described by using zero-order and first-order kinetics. The mechanism of drug release from the gel was studied by using Higuchi and Korsemeyer-Peppas equation.

3. Results and discussion:

Pre-formulation studies of drug were performed to characterized flurbiprofen. Partition coefficient of flurbiprofen was determined since to check whether the drug was suitable to topical application or not. All possible quality control tests were conducted and the results were presented as below

3.1.FTIR Studies

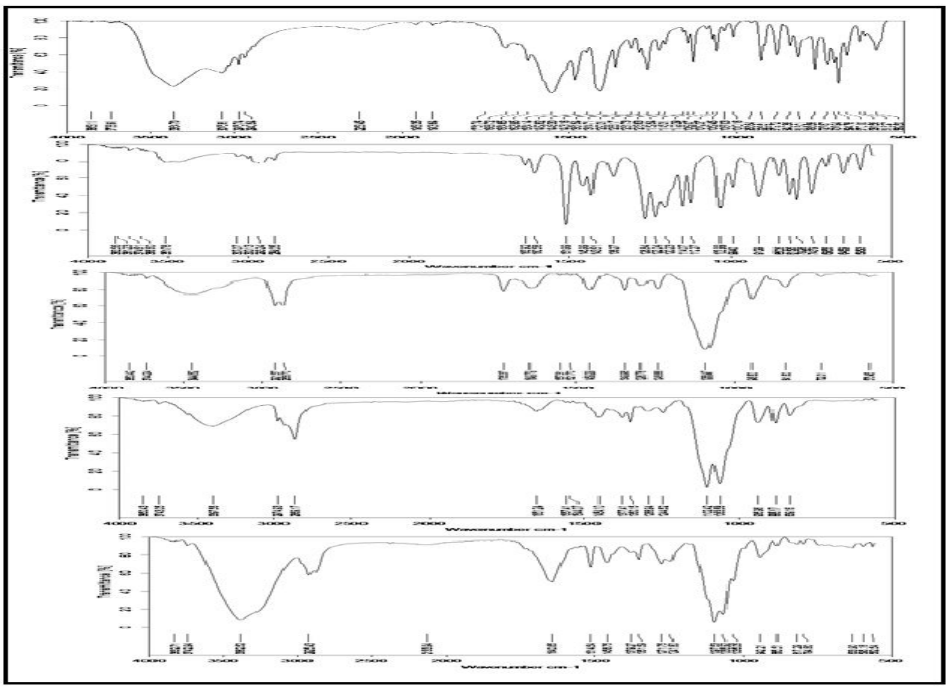


Fig. 1. FTIR Spectra of Flurbiprofen,Clove oil, Tween 80,Transcutol HP and Optimized Formulation

FTIR spectrum of physical mixture of excipients shows minor shifting of some peaks compared with FTIR spectrum of individual excipients and pure drug like O-H stretching of aliphatic acid from 3364.70 to 33362.52, C-H stretching of aliphatic methyl group from 22940.54 to 2925.43, C=O stretching of acid was completely disappear this is due to reaction of highly reactive C=O group with excipients, C-H bending of methyl group from 1482.82 to 1514.54, C-O stretching of acid from 1239.17 to 12.41.60. These minor shifts observed may be due to the formation of hydrogen bonds, attractive forces or dipole moment which are weak forces seen in the polar Vandewaalfunctional groups of drugs and excipients. The shifts seen due to the above-mentioned interaction may however support the formation of favourable vesicle shape, structure with good stability and sustained drug release.

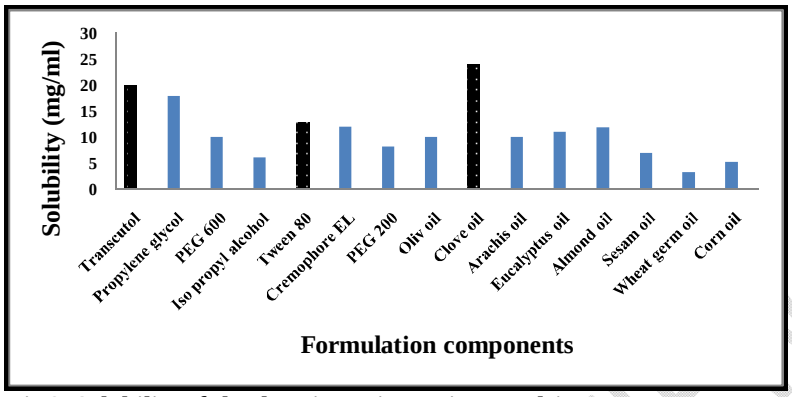
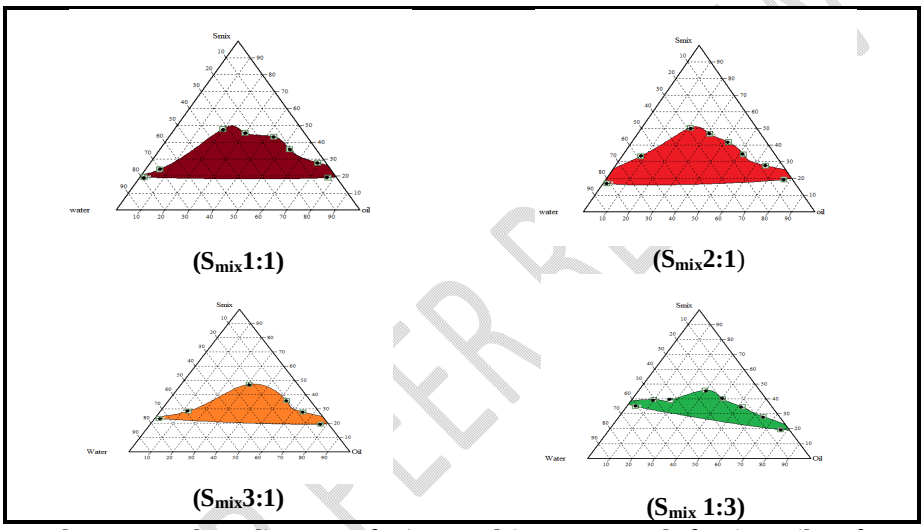


Fig.2. Solubility of the drug in various microemulsion components

Development of microemulsion formulation depends on physicochemical properties of drugs. Lipophilic drugs are encapsulated in oil phase of o/w microemulsions, whereas hydrophilic drugs are encapsulated in aqueous phase of w/o microemulsions. Solubility of the lipophilic drugs in the oil phase and hydrophilic drugs in aqueous phase is an important criterion for the selection of oils and water, respectively.Because flurbiprofen is hydrophobic drug, its solubility in oil is more important than water phase. The solubility of flurbiprofen in different oils was determined. The solubility of flurbiprofen was found to be highest in clove oil (23.9±1.24mg/ml) as compared to other oils. Therefore, clove oil was selected as the oil phase.

The most critical problem related in the development of microemulsion based drug delivery systems is the toxicity of the surfactants. Large amounts of surfactants may cause skin irritation when administered transdermally. It is therefore important to determine the surfactant concentration properly and use the minimum concentration in the development of microemulsion formulation. Another important criterion for surfactant selection is the hydrophilic lipophilic balance (HLB) value of surfactants. The right blend of surfactants with proper HLB value leads to the formation of a stable microemulsion formulation. After selection of oil (clove oil), surfactant was selected based on the highest solubilization capacity for the drug. In the present study, three surfactants (Tween 80, Cremophore EL, and PEG 200) were chosen for screening. As Tween 80 solubilized the maximum amount of flurbiprofen (12.9±1.52mg/ml), it was selected as the surfactant for the development of suitable o/w microemulsion. Addition of Co surfactants causes further reduction in the interfacial tension between oil phase and aqueous phase and increase the fluidity of the interface. Therefore, Transcutol HP, Propylene glycol, PEG

600, IPA were selectedas co surfactants. The solubility of flurbiprofen was found to be highest in Transcutol HP (19.9±1.06mg/ml) as compared to other Co surfactants. Therefore,Transcutol HP was selected as the Co surfactant.The zone of microemulsion formation can be explained with the help of the pseudo-ternary phase diagrams. Phase diagrams were constructed using clove oil as the oil phase and Tween 20 and Transcutol HP as the surfactant and cosurfactantrespectively. Effect of surfactant and co-surfactant mass ratio on microemulsion formation was assessed for the further optimization of the system.



(S_{mix}1:1) (S_{mix}2:1)

(S_{mix}3:1) (S_{mix} 1:3)

Fig.3.Pseudo-ternary phase diagrams of microemulsions composed ofvarious oil-surfactant S_{mix}ratios

It was observed that the surfactant alone was ineffective to reduce the o/w interfacial tension and failed to provide desirable microemulsion formulation. The amount of co-surfactant with respect to surfactant i.e.Smix ratio 1:1, the maximum amount of oil that could be solubilized was 30.30% (w/w) with45.45% (w/w) of Smix(1:1) at the maximum content of water area turn into easily flowable o/w microemulsion area in the presence of Transcutol HP as a co surfactant. This might be due to the fact that the incorporation of cosurfactant could have enhanced the penetration of the oil phase in the hydrophobic zone of the surfactant monomers, which in turn reduced the interfacial tension and increased the flexibility and fluidity of the interface, ultimately leading to increased entropy of the system.

When cosurfactant concentration was tripled i.e.Smixratio 1:3 the total area of microemulsion decreased as compared to Smix ratio 1:1and 2:1. The maximum amount of oil i.e. 20.07% (w/w)

could be solubilized by using 47.29% (w/w) and 46.87% (w/w) of Smixat the ratio of 1:1 and 2:1 respectively at the maximum content of water. High concentration of co surfactant appeared to have a destabilizing effect on the formation of microemulsion resulting into substantial reduction of microemulsion area. Similarly, when the surfactant concentration of Smix was increased from 1:1 to 2:1and 3, depletion in microemulsion region wasobserved in comparison to 1:1. It might be due to insufficient co surfactant concentration, required to reduce the interfacial tension and provide the flexibility of the interfaces.

However, microemulsionregion of Smix ratio 2:1 was higher than Smixratio, 3:1 and 1:3. Smix 1:1and 2:1 showed the maximum area as compared to the other ratios indicating that surfactant and cosurfactant mass ratio (Smix) have pronounced effect on phase properties i.e. size and position of microemulsion zone. Such effect is attributed to differences in the packing of surfactant and cosurfactant at the o/w interface.

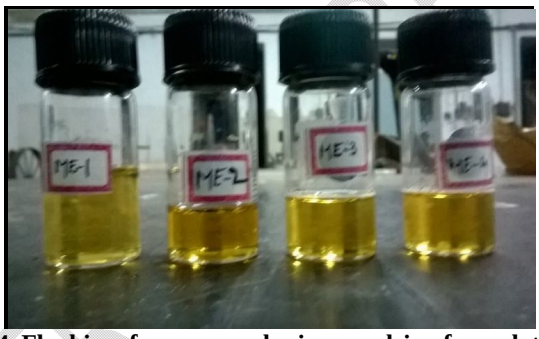


Fig.4. Flurbiprofen prepared microemulsion formulations

Microemulsion containing flurbiprofen 2.5% were preparedusing clove oil as the oil phase, Tween 80 as the surfactant, and Transcutol HP as the cosurfactant using phase titration (spontaneous emulsification)method. S_{mix}(Tween 80 and Transcutol HP) was used at differentratio.No change was observedin the phase behaviour of the pseudo ternary phase diagram whenflurbiprofen was incorporated in the formulations, showing desirablestability of microemulsions.

Results of current research work indicated that development of successful microemulsion formulation of flurbiprofen with optimum characteristics. The drug content of different formulations was found to be in the range of 96.49±0.70 to 98.17±0.49 represents almost uniform distribution of drug throughout the formulation. The pH values of all formulations were found to be of 5.64±0.02 to 5.78±0.04, indicates near to neutral pH. The conductivity values

were shown that formulation is o/w type. The formulations were clear and transparent.The conductivity values of prepared formulations were found to be 142.24 to 155.57µS/cm respectively. The mean diameter was found to be 16.17 to 94.73. It indicated that all the formulations were found to be within the required range of nm (10-100nm). The zeta potential of prepared microemulsion was found to be -0.054 to -16.30mV. It represents that the formulations were found to be stable.

***In-vitro* drug release studies**

The *in-vitro* drug release profiles of ME1, ME2, ME3 and ME4 were shown in Fig.6. The maximum % amount of drug release was observed from ME4 (98.4%) than compare with other formulations ME1, ME2 AND ME3(72.8, 79.2 and 79.6) so that the formulation components were influence effectively on release profiles of microemulsion formulations.Considering the globule size, zeta potential and other characteristics, the flurbiprofen microemulsion formulation (ME4) was selected as optimized formulation and subjected to further study.

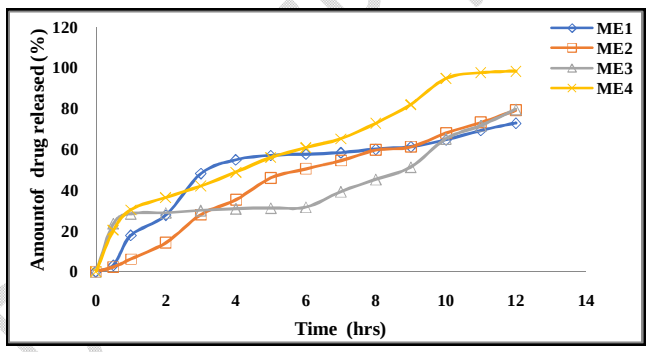


Fig.5. Comparative drug release profiles of microemulsion formulations

***In vitro* permeation studies**

Goat skin was used to study the skin permeation of flurbiprofen loaded microemulsion gel and flurbiprofen conventional gel preparations. To ensure stable collection conditions of phosphate buffer saline pH 7.4 was used as receptor fluid.Thecumulative amount of flurbiprofen obtained from the microemulsion based gel i.e., 98.6 µg/cm^2, which was 6 times than that of flurbiprofen conventional gel at 16 hrs. (33.68 µg/cm^2).The skin permeation rate ($J_{ss,}$ µg/(cm^2.h)) of microemulsion based gel was higher than the flurbiprofen conventional gel which showed the addition of carbopol P934 to microemulsion could decreased the permeability of flurbiprofen

markedly. Therefore, it can be concluded that the addition of carbopol P 934 to microemulsion into the microemulsion should delay drug release. Based on drug release kinetics, drug transport was found to be diffusion(Zero order) hence we concluded that the release pattern of drug from the microemulsion based gel was found to be zero order drug release and non fickian type diffusion from the network structure of carbopol P934 polymer.

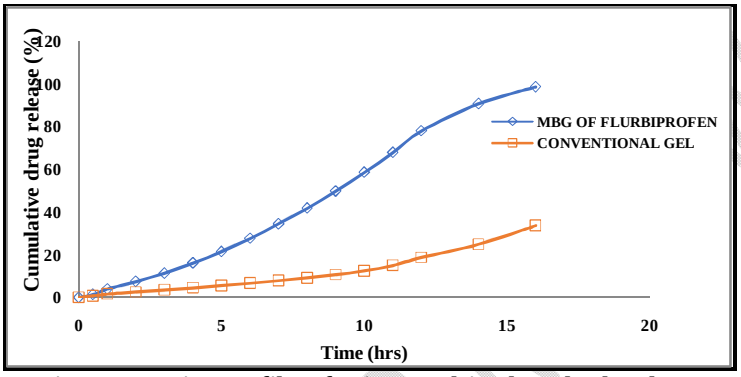


Fig.6: Comparative permeation profiles of Microemulsion based gel and conventional gel

Conclusion

Successfully developed flurbiprofen microemulsion gel. Our current research aims to overcome the first-pass metabolism and other associated drawbacks from the oral route.Based on the solubility of oils and surfactants we have selected clove oil, Tween 80 and Transcutol HP were selected for the preparation of flurbiprofen microemulsion system. The ratios such as 1:1 and 2:1 was produced more stable microemulsion regions than other ratios. Among the four developed microemulsion formulations, ME4 shown highest *in vitro* percentage drug release (98.4 ± 0.22%) in 12 hrs. The skin permeability study of flurbiprofen microemulsion based gel revealed that drug permeation at faster rate from the microemulsion based gel than the conventional gel. Hence, the microemulsion offers a promising approach to increase the solubility and sustained permeability of poorly water-soluble drug of flurbiprofen.

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