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Research Article

DEVELOPMENT AND EVALUATION OF AN OCULAR ANTI-INFLAMMATORY MICROEMULSION

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Abstract

The main objective of this study is to formulate Bromfenac Microemulsion with prolonged duration of action which will help in increasing its topical bioavailability. Bromfenac belongs to the category of NSAID. A calibration curve of Bromfenac was taken in USP Phosphate buffer pH 7.4 at 268nm. Oleic acid, Poloxamer 188 and Propylene glycol were found to have maximum absorbance during solubility studies. Titration method was used to construct Pseudo-ternary phase diagram varying the ratio of Poloxamer 188 and Propylene glycol such as 1:1, 1:2, 1:3 and 2:1, diluting it appropriately with water. Oleic acid was then added drop-wise till turbidity was observed. Microemulsion was characterized for particle size, Osmolality, drug content and Viscosity. *In-Vitro* diffusion study was carried out on Keshary-chien membrane permeation cell to evaluate release pattern. Freeze thaw cycling and centrifugation were carried out to check stability of formulation. It was found from in-vitro diffusion studies of formulation that microemulsion of Bromfenac exhibited better penetration than marketed formulation. Particle size of formulation at optimized composition was found to be <100 nm (Malwern-zeta sizer).

A stable formulation requiring less frequency of administration could be prepared. The rate of penetration was found to be faster than the marketed formulation.

Keywords: Bromfenac, Diffusion, Microemulsion, Ocular.

Introduction

A Promising management of eye ailments take off effective concentration of drug at the eye for sufficient period of time. Ocular drug delivery is hampered by the barriers protecting the eye. The bioavailability of the active drug substance is often the major hurdle to overcome. Significant challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage. Development of newer, more sensitive diagnostic techniques and novel therapeutic agents continue to provide ocular delivery systems with high therapeutic efficacy. Poor bioavailability of drugs from ocular dosage forms is mainly due to the precorneal loss factors which include solution drainage, lacrimation, tear dynamics, tear dilution, tear turnover, conjuctival absorption, nonproductive absorption, transient residence time in the cul-de-sac, and the relative impermeability of the corneal epithelial membrane are the major challenges to anterior segment drug delivery following topical administration. Due to these physiological and anatomical constraints, only a small fraction of the drug, effectively 1% or even less of the instilled dose, is ocularly absorbed. To be clinically effective, topical formulation has to posses balance between lipophilicity and hydrophilicity with higher contact time.[1]Microemulsions have attracted large interest in the pharmaceutical industry as drug delivery systems due to their improved drug solubilisation properties, increased shelf life and ease of preparation. They normally consist of an aqueous phase, an oil phase, a surfactant and a co-surfactant, when the concentrations of these components are favourable, they spontaneously emulsify to form a monodisperse, thermodynamically stable, transparent microemulsion. Many drugs that are insoluble in aqueous media are prepared as preconcentrates consisting of oil, surfactant and co-surfactant. This is then diluted with water to form the microemulsion prior to administration to the patient.[2] Hoar and Schulman introduced the word microemulsion (ME), which they defined as a transparent solution obtained by titrating a normal coarse emulsion with medium-chain alcohols. The short to medium-chain alcohols are generally considered as co-surfactants in the ME system. Microemulsions are thus defined as ‘a system of water, oil and ampiphile which is a single optically isotropic and thermodynamically stable liquid solution.[3] Advantages of ME over coarse emulsion include its ease of preparation due to spontaneous formation, thermodynamic stability, transparent and elegant appearance, increased drug loading, enhanced penetration through the biological membranes, increased bioavailability and less inter- and intra-individual

variability in drug pharmacokinetics. Microemulsions have the ability to deliver large amounts of water and topically applied agents in to the skin than water alone or other traditional vehicles because they act as a better reservoir for a poorly soluble drug through their capacity for enhanced solubilization.[4,5] Microemulsions have been widely studied to enhance the bioavailability of the poorly soluble drugs. They offer a cost effective approach in such cases. Microemulsions have very low surface tension and small droplet size which results in high absorption and permeation. Interest in these versatile carriers is increasing and their applications have been diversified to various administration routes in addition to the conventional oral route. This can be attributed to their unique solubilization properties and thermodynamic stability which has drawn attention for their use as novel vehicles for drug delivery. The results obtained have been indeed very promising. In recent past, microemulsion formulation of a poorly soluble immunosuppressant was marketed as a soft capsule which contains a mixture of drug dissolved in oil and surfactant. [6,7] Microemulsion formulation made the bioavailability and plasma concentration profiles of the drug more reproducible which is clinically important in the case of drugs showing serious adverse effects. This is a significant step forward in the delivery of poorly soluble drugs. Microemulsion systems are also now being increasingly investigated for transdermal [8-12], ocular [13] , nasal [14, 15], pulmonary [16], vaginal [17, 18] , rectal [19,20] and intravenous drug delivery [21,22] .These systems may also be used for sustained release of drugs by formulating ocular preparations recently; more attention has been focused on microemulsions for ocular delivery of drugs. The present research work describes the formulation of Bromfenac Microemulsion. Bromfenac belongs to the category of NSAID which acts by inhibiting COX -2. In this study, we tried to develop a new formulation of Bromfenac in microemulsion base for ocular application which will improve the patient compliance. Microemulsions containing 0.09%w/w Bromfenac were formulated and examined the diffusion and in vitro skin penetration of Bromfenac from them.[23]

Materials and Method

Bromfenac was obtained as a gift sample from Enaltec laboratories, Ambernath, India and other excipients and reagents purchased were Oleic acid From CDH Laboratory Reagent, Ambernath, India; Poloxamer 188 from BASF, India; propylene glycol was supplied generously by Merck Pvt Ltd worli, India; and all other chemicals and reagents were of analytical grade.

Experimental Work

Determination of solubility of Bromfenac

Solubility of drug in various oils, surfactants and co-surfactants were carried out in order to select the components to be used for formulation development. List of the components is given in Table no 1.

Table No-1: List of components used in solubility study.

Oil	Oleic Acid, Triacetin, Labrafac, Isopropyl myristate, Ethyl oleate and Olive oil.
Surfactant	Poloxamer 108, Poloxamer 188, Cremophor RH 40, Tween 80, Tween 20, PEG 200
Co-surfactant	Propylene Glycol, Butanol.

Saturated solution of drug in individual 5 ml of oil, surfactant, and co-surfactant were prepared and kept in orbital shaker at 100 RPM for 48 hrs at 37±2^{0}C. Suspension was centrifuged at 5000 RPM, supernatant was collected. The required dilutions were carried out with methanol was done. According to absorbance value; the solubility of Bromfenac was estimated by UV method at 268nm. [24]

Compatibility Study

Compatibility study was carried out using FT-IR (shimadzu 1800).

Drug was evaluated by IR for confirmation of functional groups. The components selected by solubility study was checked for compatibility with drug. Individual component with drug was evaluated for any incompatibility and finally drug along with formulation was checked and compatibility was obtained, result was shown in figure 3.

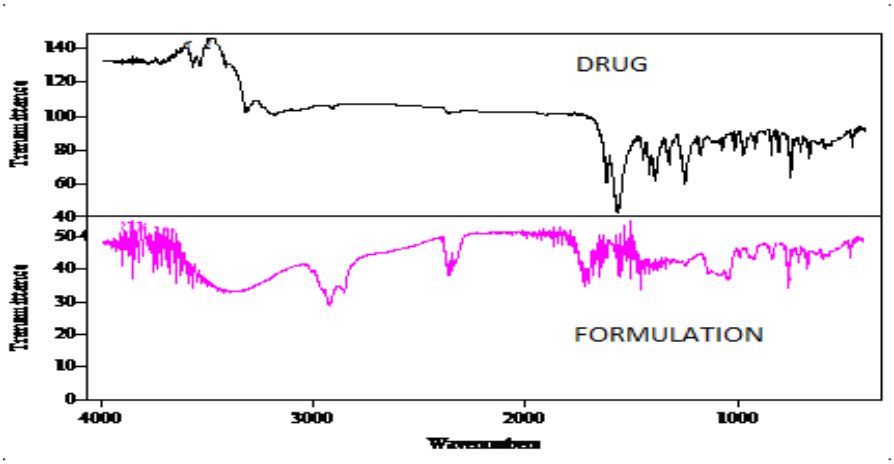

Figure 3: compatibility study between drug and formulation.

Construction of Pseudoternary Phase Diagram

The boundaries of microemulsion in the phase diagrams were determined by using titration method using PCP disso v3 to determine the microemulsion existence region with the component mixtures. Four components selected according to the solubility were oleic acid, Poloxamer 188, propylene glycol and phosphate buffer USP (7.4).

The ratios of surfactant to co-surfactants were chosen to be 1:2, 1:1, 2:1, and 3:1, and such mixtures were prepared. These mixtures of surfactant/co-surfactants were mixed with the water phase to give the weight ratios of 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90. Oil was added drop by drop and stirred using a magnetic stirrer until a homogeneous dispersion or solution was obtained. After each addition, the system was examined for the appearance and flow properties. The end point of the titration was the point where the solution turn to gel or turbid. The quantity of the oil phase required to make the mixture turbid was noted. The percent composition of the different incorporated phases was then calculated and the same method was followed for the other S/CoS ratios.

Method of Preparation

Bromfenac containing microemulsions were formulated by mixing oil, surfactant, and co-surfactant with varying component ratio as described in Table 2. 0.09 % w/w of Bromfenac was dissolved in this mixture and then an appropriate amount of water was added to the mixture drop by drop with constant stirring on magnetic stirrer. Bromfenac containing microemulsion was obtained spontaneously on stirring the mixtures at ambient temperature. All microemulsions were stored at ambient temperature.

Table-2: Formulation of Bromfenac Microemulsion.

INGREDIENTS (%W/W)	ME-1	ME-2	ME-3	ME-4	ME-5	ME-6
Bromfenac	0.09	0.09	0.09	0.09	0.09	0.09
Oleic acid	3	3	3	3	3	3
Poloxamer 188 + propylene glycol mixture	10	12	15	18	20	25
Phenyl mercuric nitrate	0.01	0.01	0.01	0.01	0.01	0.01
Phosphate buffer (7.4)	87	85	82	79	77	72

Physicochemical Evaluation

Particle size measurement:

Particle size of microemulsion was determined by using particle size analyser. (malwern zeta sizer).

pH measurement:

pH was measured by using PH meter (EQ-610,Equip-tronics Digital pH meter) at 25 ± 2^0C. pH meter was previously calibrated with buffers of pH 4, pH 7, and pH 9.2.

Viscosity: The viscosities of microemulsions were measured with a Brookfield rotational viscometer (RV, Brookfield Inc., USA) equipped with spindle no. 4. The measurement was done at 30°C at 5 rpm.

Percentage Transmittance:

The transmittances of each batch were checked by UV (shimadzu 1800) at 650 nm, taking pH 7.4 USP phosphate buffer as blank.

Analysis of Drug content:

The 10 ppm of each batch in phosphate buffer USP 7.4 was prepared. The absorbance of each batch at 268nm was observed and the drug content was calculated.

Percent drug efficiency= Observed absorbance/Actual absorbance * 100.

Results and Discussion

(A) **Solubility Studies**

To develop microemulsion formulations for ocular delivery of poorly water-soluble drug, proper selection of components are needed. The optimizations of the components to be used in formulating microemulsion were decided based on the solubility of Bromfenac in the various oils, surfactants and co-surfactants.

The solubility of Bromfenac from various oils found to be highest in oleic acid 22.2066 mg/ml. Various surfactant were used, Poloxamer 188 showed the highest solubility 123.0062 mg/ml. Propylene glycol as co-surfactant showed the highest solubility 16.1041 mg/ml. Based on the solubility studies of Bromfenac in oil, surfactant and co-surfactant, Oleic acid, Poloxamer 188, Propylene glycol could be the most appropriate combination for development of microemulsion. The solubility data is shown in Table 2 and the respective Graph is shown in Figure1 and 2.

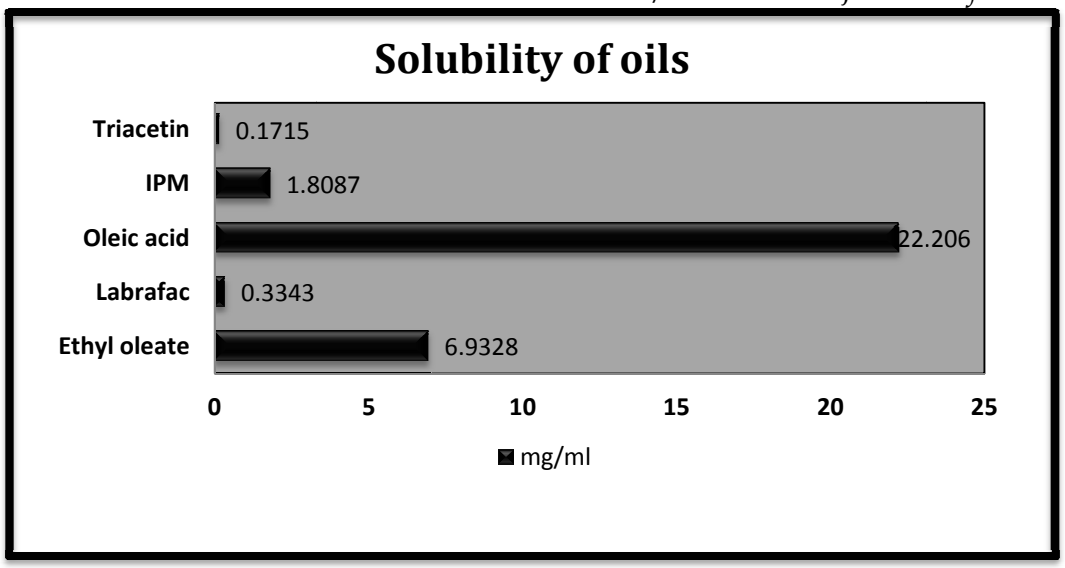

Figure -1: Solubility of Bromfenac in different oils.

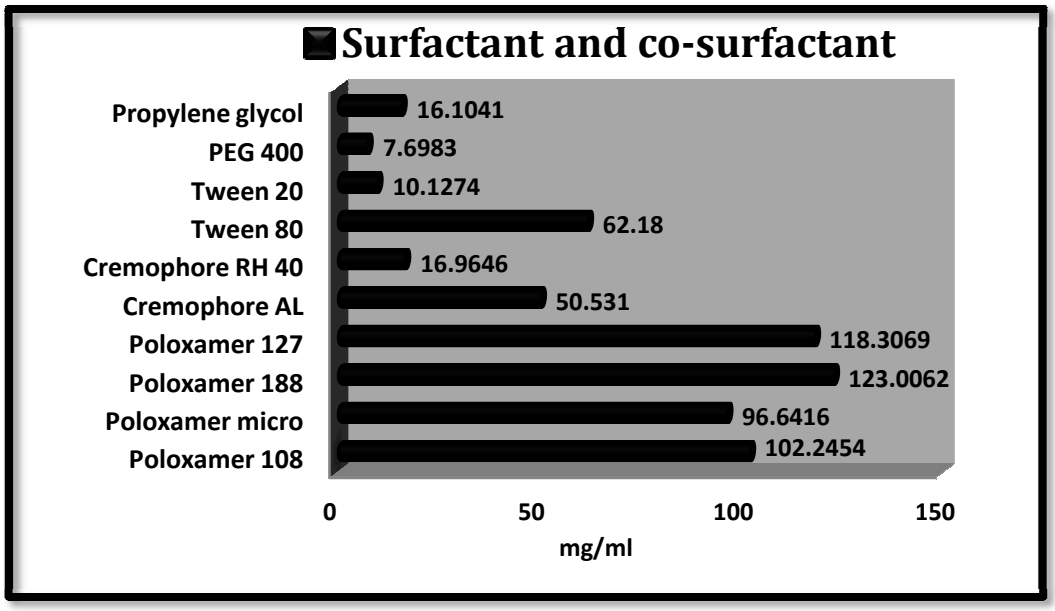

Figure -2 solubility of Bromfenac in different surfactant and co-surfactant.

(B) Pseudo-ternary phase diagram study

The microemulsion existence region was determined by constructing phase diagrams. Oleic acid, Poloxamer 188 and Propylene glycol were found to have maximum solubility based on the absorbance values. The translucent region presented in phase diagram reveals the microemulsion existence region. No distinct conversion from water-in oil (w/o) to oil-in-water (o/w) microemulsion was observed. The rest of region on the phase diagram represents the turbidity on visual inspection. Pseudo-ternary phase diagram were constructed by varying the ratio of Poloxamer 188 and

Propylene glycol in different ratios of 1:1, 1:2, 1:3 and 2:1 and diluting it appropriately with phosphate buffer USP 7.4. Oleic acid was then added drop-wise till turbidity or gelling was observed. The ratio of 1:2 was found to show an increase in Microemulsion existence region as per the results obtained from phase diagram studies. The phase study reveals that with an increase in the ratio of surfactant, the microemulsion region is also expanded. The maximum proportion of oil was incorporated in increasing ratio of oil: surfactant/co-surfactant. .Pseudo ternary phase diagram were shown in Figure no 4.

(C) Physicochemical Evaluation

Microemulsions were evaluated by dilution test to confirm the stable formulation.

Centrifugation and freeze thaw cycle was done to check the stability of formulation. Freeze thaw cycle was carried out at -20^0C for 24 hr and for $+25^0$C for 24 hr. 6 cycles were done. Centrifugation was carried out at 5000 rpm for 1hr. The result of droplet size was found to be <100nm, pH of the formulation was 7-7.4, viscosity, drug content, osmolality and stability is given in below Table no 3.

Table-3: Solubility data of Bromfenac in different oil, surfactant and co-surfactant.

	INGREDIENTS	**Absorbance (mg/ml)**
OIL	Triacetin	0.1715
	Oleic acid	22.2066
	IPM	1.8087
	Labrafac	0.3343
	Ethyl oleate	6.9328
SURFACTANT & CO – SURFACTANT	poloxamer 108	102.2454
	polo micro	96.6416
	poloxamer 188	123.006
	polo 127	118.3069
	Cremophor AL	50.5310
	cremo RH 40	16.9646
	Propylene glycol	62.1800
	PEG 200	10.1274
	Tween 20	7.6983
	Tween 80	16.1041

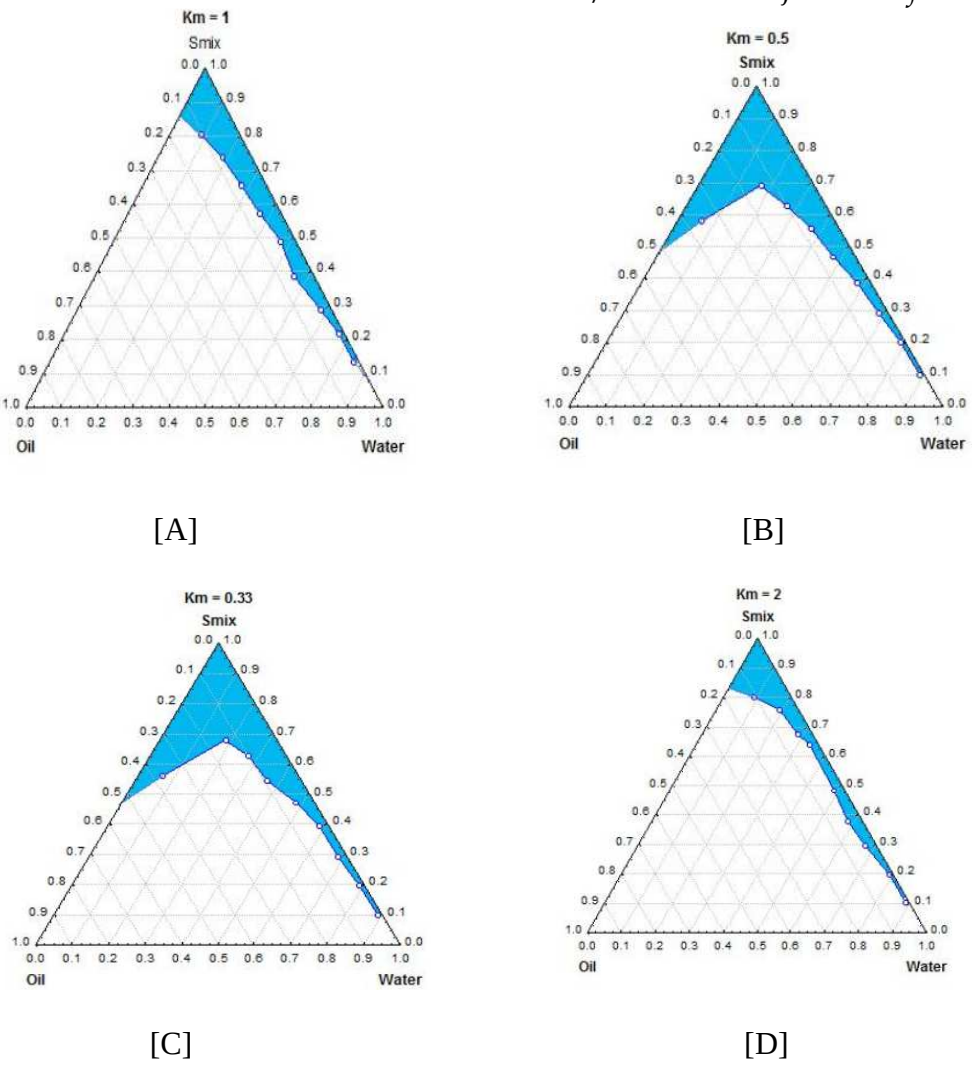

Figure 4: Pseudo ternary phase diagram with various ratios of surfactant-co-surfactant A] 1:1 B] 1:2 and C] 1:3 D] 2:1

(D) *In Vitro* Permeation study

All the *in-vitro* permeation studies were carried out in Keshary-chien diffusion cells. It closely simulates the *in-vivo* situation since the membrane was exposed to ambient conditions while the receiver temperature is 37 ± 1^0C. Dialysis Membrane was used as a membrane for the experiments. The receptor compartment was filled with 10 ml of USP pH 7.4 phosphate buffer system. The solution in the receptor compartment was constantly stirred by means of Teflon coated magnetic bead on a magnetic stirrer, so that the hydrodynamic conditions of the system were maintained. Bromfenac equivalent to 1 mg was applied uniformly on the membrane from both marketed and laboratory formulations. The opening of the donor compartment was covered by foil, in order to prevent loss due to evaporation. An aliquot of 1 ml was removed from the receptor medium at intervals of 1, 2, 3, 4, 5, 6, 7, 8 hours and replaced

immediately with the same volume of the medium. Two optimized batches were found to show prolonged release of drug as compared to the marketed formulation.[25] Percent release of the drug is given in Table No.4.

Table no-4: **Result of pH, Droplet size, viscosity, Osmolality, Drug content and stability.**

Parameter	ME-1	ME-2	ME-3	ME-4	ME-5	ME-6
pH	7.0	7.4	7.2	7.1	7.0	7.1
Droplet size	45.91	83.35	74.32	76.45	61.34	65.83
Viscosity	23	20	21	14	18	22
Osmolality	0.2	0.209	0.216	0.301	0.312	0.317
Drug content	94	92	91	90	95	86
Stability	+	-	+	-	-	-

A (+) = passes the stability study, B (-) = Does not pass the stability

Table no-5: Percent release of the drug from the formulation.

Time(min)	% release of drug	
	ME-1	ME-2
0	0	0
30	26.98	27.76
60	34.01	33.23
120	40.06	45.62
180	56.84	58.64
240	65.40	70.30
300	71.99	75.13
360	77.25	80.35

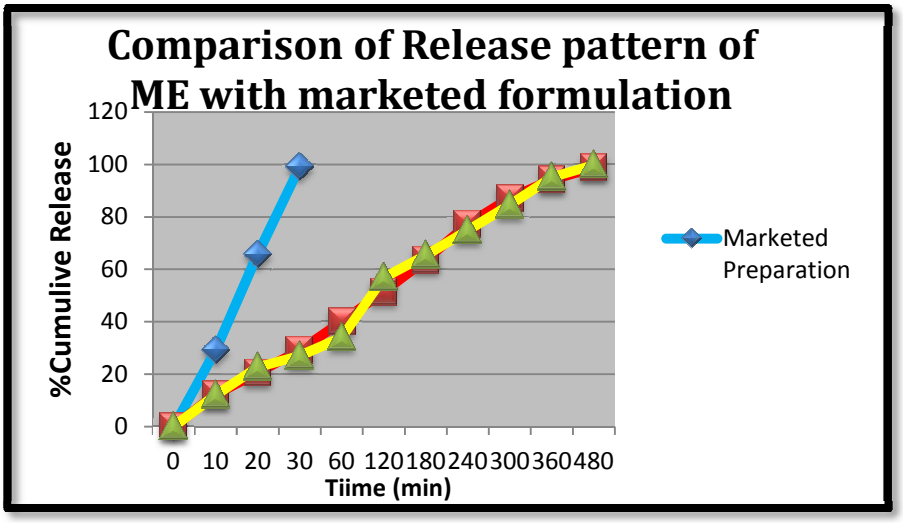

Fig-5 : Graphical Representation of percent release of the drug.

Conclusion

A Microemulsion was prepared which showed a longer duration of action as compared to marketed formulation. Out of 6 formulations two were found to be stable. According to release pattern a formulation requiring less frequency of administration could be prepared. The rate of penetration was found to be faster and the problems of drainage could be successfully avoided with this formulation.

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