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PREPARATION AND STUDY OF EFAVIRENZ MICROEMULSION DRUG DELIVERY SYSTEM FOR ENHANCEMENT OF BIOAVAILABILITY

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ABSTRACT

The purpose of study was to develop a microemulsion drug delivery system to enhance the bioavailability of Efavirenz(EFV). The microemulsion was prepared using efavirenz(drug), Capryol 90(oil), Tween 20 and Cremophor EL(surfactant) and Transcutol HP (co-surfactant) and water. The optimized formula for microemulsion was determined using phase diagrams which were constructed prior to the formulation. The physical evaluation like globule size, zeta potential, pH, conductance, drug content, stability etc. were performed along with *in vitro* and *ex vivo* studies. The pharmacokinetics studies were also performed on male albino rat. The optimized formulation of efavirenz microemulsion had globule size and zeta potential of was found to be 15.8±0.71nm and -12.6±0.23mv respectively. The optimized formulation showed higher drug release in *in vitro* and *ex vivo* studies as compared to pure drug suspension. Efavirenz microemusion shows 1.88 folds increase in peak plasma concentration in comparison to pure drug suspension and AUC was found to increase 2.15 fold in comparison to pure drug suspension. The size reduction by microemulsion would improve the bioavailability of the drug in vivo after oral administration.

KEYWORDS: Microemulsion, Efavirenz, Pharmacokinetic, Bioavailability.

INTRODUCTION

The oral route is the most acceptable and simple route for drug delivery, but administration of hydrophobic drugs through this route is nearly 50 % hampered due to its poor solubility.

Recent progresses in chemistry have led to the production of a number of new compounds. The developed compounds have limited solubility which affects the bioavailability of the drug. Thus it is necessary to improve the solubility of the compounds to enhance their bioavailability so that they can be therapeutically exploited. Bioavailability has important clinical implications as both pharmacologic and toxic effects are proportional to dose. The selection of an appropriate dosage form is also a critical because a dosage form with poor drug delivery can make a useful drug worthless.[1]

Microemulsion drug delivery systems have gained great importance as a promising technology to improve the solubility/dissolution and thus bioavailability of poorly water-soluble drugs. A considerable amount of individual reports about the credible microemulsion formulations has been published during the last two decade and the number is continuously increasing every year. Furthermore, successful commercialization of the products Sandimmune Neoral (Cyclosporin A), Fortovase (Saquinavir) and Norvir® (Ritonavir) is sufficient to establish the commercial viability of this delivery strategy.

The microemulsion concept was introduced as early as the 1940s by Hoar and Schulman who field generated a clear single-phase solution by titrating a milky emulsion with hexane.[4] Schulman and coworkers (1959) subsequently coined the term microemulsion.[5] Micro emulsions are thus defined as "A system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution". The spontaneous formation of an emulsion upon drug release in the GIT advantageously presents the drug in a dissolved form and the small droplet size provides a large interfacial surface area for drug absorption. Additionally, the specific components of microemulsion help the intestinal lymphatic transport of drugs which would be very useful in reducing the first pass effect of the drug. Main mechanisms include increasing membrane fluidity to facilitate transcellular absorption, opening tight junction to allow paracellular transport, inhibiting P-gp and/or Cytochrome P450 (CYP450) enzymes to increase intracellular concentration and residence time by surfactants, and stimulating lipoprotein/chylomicron production by lipid,[6-9] Oral absorption of numerous drugs has been enhanced by microemulsion.

In HAART non-nucleoside reverse transcriptase inhibitor is usually included as first choice of drug. EFV shown to be effective in the treatment of human immunodeficiency virus (HIV) type 1. However, the clinical efficacy of EFV is strongly limited by its low soluble nature. Apart from the insolubility, EFV is principally metabolized by the cytochrome P450 system

to hydroxylated metabolites with subsequent glucuronidation of these hydroxylated metabolites. These metabolites are essentially inactive against HIV-1. The current therapeutic scenario demands a strong need for a delivery strategy that can improve the therapeutic efficacy of EFV by means of increasing its solubility or by reducing its first pass metabolism. EFV belongs to BCS class II and problem with this potentially useful drug in HAART is that it is very low soluble (0.00855mg/ml) and bioavailability not available. EFV has Log P 4.6 which indicate lipophilic nature of drug.[2,3] Challenge in to development of formulation of low soluble drug is the solubilization. O/w microemulsions is a promising system for the incorporation of poorly water soluble drugs due to the high solubilization capacity and in addition, the potential for enhanced absorption. Additionally, the solution like feature of microemulsion could provide advantage of dose uniformity. Among the various drug delivery systems, microemulsion may be a better choice to solve the problems of solubility and at last bioavailability. In order to increase the solubility of EFV, capryol 90 (oil), tween 20 and cremophor EL (surfactant) and transcutol HP (co-surfactant) have been used to prepare microemulsion.

Among the various drug delivery systems, microemulsion may be a better choice to solve the problems of solubility and bioavailability.

The objectives of the present investigation were to formulate microemulsion of EFV and to evaluate its potential to improve solubility/dissolution and thus oral bioavailability of EFV by carrying out *in vivo* studies in rat. The stability of EFV in developed microemulsion has also been investigated in the present investigation.

MATERIALS AND METHODS

MATERIALS

EFV was obtained as a gift sample from Aurobindo Pharma, Hyderabad, India. Acconon CC_6 and Capmul MCM were obtained as a gift sample from Abitec corporation. Mumbai, India. Cremohpor EL purchased from Sigma Aldrich. Labrafac PG, Transcutol HP, Capryol 90, Labrasol, Miglyol 812, Kolliphor MCT 70 and Lauraglycol 90 were obtained as gift sample from Gattefosse, Mumbai. Tween 80 and Tween 20 were purchased from S.D fine chemical Mumbai, India. All other reagents used were of analytical grade.

Solubility study

The solubility of EFV in various oil, surfactant and co-surfactant were determined by dissolving excess amount drug in 1ml of each of oils, surfactants and co-surfactants. The mixer was kept on cyclomixer for 10 mins and then were kept on a mechanical shaker at 25^{o}C for 48 h and kept for equilibrium. The equilibrated samples were centrifuged at 5000 rpm for 15 min. The supernatant was withdraw and filtered through 0.45 µm membrane filter.[10] The concentration of drug was determined in each of oils, surfactants, and co-surfactants by UV spectrophotometer at the 247 nm wavelength.

Pseudo Ternary Phase Diagram Study

The pseudoternary phase diagram of surfactant co-surfactant mixture (Smix), oil and doubled distilled water was plotted by water titration method. The ratio of surfactant(S) to co-surfactant (CoS) was fixed at different ratios of 1:1,2:1 and 3:1 on the weight basis for each phase diagram. The oil phase mixed with the mixture composed of surfactant and co-surfactant in the ratio of(volume basis) 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9(w/w). A water titration technique was employed for the preparation of the pseudoternary phase diagrams. Based on the technique distilled water was added drop wise to the mixture of oil/surfactant/co-surfactant at room temperature. After each water addition, the mixture was stirred in a beaker until homogenous solution was obtained and was examined for appearance and flow property. The end point of the titration was the point in which the system becomes turbid or cloudy. The quantity of water necessary to make the mixture turbid was noted. The pseudo-ternary phase diagram composed of specific two regions: biphasic emulsion region and monophasic microemulsion region. In each titration runs, a number of end points were noted down as critical points between this two regions. This point was a specific where a significant change in the appearance (turbidity) of the system occurred. The boundaries between these two regions were drawn on the phase diagram by joining together these critical points. Three such phase diagrams were constructed taking ratio of surfactant/co-surfactant as 1:1, 2:1, and 3:1. Based on these diagrams appropriate quantity of selected oils, S & CoS were taken for the preparation of EFV microemulsions.[3]

Preparation of Drug Loaded Microemulsion

Microemulsion was prepared by using the water titration method by keeping constant ratio (3:1) of S/CoS (selected from phase diagram). Capryol 90 as a oil, a mixture of surfactants Tween 80, Cremophor EL and co-surfactant Transcutol HP added and mixed properly, to this

EFV was added and mixed till dissolved and finally distilled water as external phase was added dropwise, after addition of each drop, the mixture was stirred and observed until clear and transparent microemulsion was obtained. Various formulations from the monophasic area of the phase diagram were prepared using different concentration of oil, S/CoS and water.

Optimization of Microemulsion

Various batches of microemulsions were prepared by water titration method and the formulation was optimized in terms of particle size, clarity, percentage transmittance, percentage drug content and zeta potential and in-vitro drug release.

Characterization of EFV loaded Microemulsion

Percentage drug content

The percentage drug content of the formulation was analysed by dissolving 1 ml of the formulation in 10 ml methanol. After suitable dilutions with methanol, absorbance was determined using the UV spectrophotometer (UV 1700, Shimadzu, Japan) keeping blank microemulsion as control at wavelength 247 nm.

pH measurement

The apparent pH of the prepared formulations was measured (in triplicate) by using calibrated digital pH meter (Pico$^+$ Labindia, Mumbai, India) at ambient temperature with glass electrode at 25 ± 1˚C.

Dilution test

The microemulsions were further taken for the dilution test for visual assessment and were assessed either clear, less clear or milky appearance. Dilution tests are based on the fact that the emulsion is miscible with the liquid that forms its continuous phase. The microemulsions resulting from dilution with continuous phase should not show any separation atleast after 24 hours of storage. [6]

Dye solubility test

It is also known as the stain test in which a dye is sprinkled onto the surface of the emulsion indicates the nature of continuous phase. With an o/w emulsion there is rapid incorporation of a water soluble dye into the system where as with w/o emulsion the dye forms microscopically visible clumps. The reverse happens on addition of an oil soluble dye. These tests essentially identify the continuous phase.

Percentage transmittance

Transparency of the microemulsion was determined by measuring the percentage transmittance at 650nm against distilled water as blank using UV-Visible spectrophotometer (UV, 1700, Shimadzu, Japan).

Viscosity measurement

Rheological measurements were performed at 25± 1˚C using a Brookfield digital viscometer DV-1, prime, equipped with spindle 64 and rotation at 100 rpm in triplicate.

Conductance measurement

Electrical conductivity has been usually used as a standard technique to study the phase behavior. The principle for phase determination by conductivity is the ability of microemulsion to conduct an electric current, which is measured in Scm^{-1} or μScm^{-1}.The conductivity of sample microemulsion was measured by dipping electrode at temperature 25°C until equilibrium and reading become stable. Reproducibility was checked for certain samples and no significant differences were observed.[11]

Thermodynamic stability study

Microemulsions are thermodynamically stable system and are formed at specific concentration of oil, surfactant/co-surfactant and water, with no phase separation and/or creaming and cracking. It is the thermostability that differentiates microemulsions from emulsions which has kinetic stability and eventually phase separate. Thus, the selected formulations of EFV loaded microemulsions were subjected to different thermodynamic stability test by using heating cooling cycle, centrifugation and freeze thaw cycle stress tests. Physical stability was continuously monitored throughout the experiment. Various aspects like phase separation, turbidity etc. at room temperature were observed.

a) Heating cooling cycle

Three cycles between refrigerator temperature 4˚C and 45˚C with storage at each temperature, not less than 48h was studied. Those formulations which were stable at these temperatures were subjected to centrifugation test.

b) Centrifugation

Passed formulations from heating cooling cycle were centrifuged at 3500 rpm for 30min. All formulations that did not show any phase separation were taken for the freeze thaw stress test.

c) Freeze thaw cycle

Three freeze thaw cycles between -21˚C and +25 ˚C with storage at each temperature for not less than 48h was done for the formulations. Those formulations, which passed these thermodynamic stress tests, were further taken for the characterization.[12]

Droplet size and Zeta potential

The mean droplet size of emulsion globules was determined by using photon correlation spectroscopy (which analyse the fluctuations in light scattering due to Brownian motion of the particles) using Malvern Zetasizer Nano zs able to measure sizes between 10-3000nm.[13] To determine globule size and PDI, microemulsion system was diluted with distilled water and then measured with Malvern zeta sizer (nano sizer) using clear disposable zeta cell.

Refractive Index

Refractive index of microemulsions was determined using an Abbey type refractometer in triplicate (n=3).

Transmission electron microscopy

Optimized formulation was examined morphologically by transmission electron microscopy. After 10 fold dilution with water, a drop of microemulsion was placed on a piece of parafilm; a carbon-coated pioloform grid was placed on the top of the drop and left for one minute. The excess fluid was removed with the filter paper. Negative staining was then performed by placing the grid on a drop of 2% phosphotungsten acid (PTA) for one minute and excess of PTA was removed with filter paper. The grids were examined under a transmission electron microscope.

Stability Study

The formulation was stored in two different condition 2-8^{o}C and 25±2^{o}C, 60%RH±5%RH over six months. After one month of interval time, drug content, percentage transmittance and particle size were analyzed.[14]

***In-vitro* release study**

In vitro release study was performed by dialysis membrane method.[15] A dialysis membrane, with a molecular weight cut-off between 12,000 and 14,000, was used. EFV microemulsion and pure drug suspension (each equivalent to 50 mg) were placed in donor compartment. The receptor compartment was filled with 1 % SLS (Sodium lauryl Sulphate) solution. At

predetermined interval, sample (5ml) was collected from receptor compartment and replaced the same volume of fresh media in order to keep system under sink condition. The absorbance was measured using a UV-visible spectroscopic method at a wavelength of 247 nm, using 1% SLS solution as a blank. Same procedure followed while performing drug release of pure drug suspensions. The % release of drug was calculated and graph was plotted against time. All tests were performed in triplicate and results were expressed as mean ± S.D.

***Ex-vivo* release study**

Male albino rats (250-300 g) were sacrificed and the tissues duodenum was isolated carefully. These tissues were thoroughly washed with cold Ringer's solution to remove the mucous and lumen contents. Then each of formulations (Pure drug suspension and EFV-Microemulsion) was filled in piece of rat duodenum. The further study by keeping the 50 ml of 1% SLS solution kept as receptor phase. Whole study was carried out with continuous carbogen supply & gentle stirring condition at 37 ± 2˚C.[16] Samples were collected at different time intervals, replace the same volume with diffusion media and analyze the samples at 247 nm by UV-visible spectroscopic method for the estimation of drug release.

***In-vivo* study**

The animal experiment was approved by institutional animal ethics committee of Pharmacy department, MSU,Baroda, India working under the guidance of CPCSEA (protocol number MSU/PHARMA/IAEC/2013/37). Male albino rats were obtained from biochemistry department from The M.S University of Baroda.

Pharmacokinetic study was performed in 8-10 week older albino rats of weight (250-300gm). The experimental procedure was reviewed and approved by institutional animal ethical committee. Twelve albino rats were divided in two groups and kept under standard laboratory condition 25±2 ˚C, relative humidity 55±5%, in animal cage with free access to standard laboratory diet and water ad libitum. Each group of animals was received dose of EFV formulation equivalent to 10mg/kg EFV. Prior to administration drug to animals were starved overnight.[17]

Group 1= Pure drug suspension

Group 2= microemulsion

Calibration plot of EFV in plasma was constructed over a range of (50-5000 ng/ml) in plasma (r^2=0.9936) and validated for inter and intraday differences and the differences were within

acceptable range. The blank plasma samples were spiked with stock solution. The protein precipitation was carried out by addition of acetonitrile. For 0.2 ml of plasma sample, 200 µl of acetonitrile was used. The separation of precipitate from organic phase was achieved by centrifugation at 4000 rpm for 10min. The supernatant was evaporated in vacuum evaporator. The residue was reconstituted with mobile phase. The mobile phase consisted of 97 volumes of buffer (13.8 gm of monobasic sodium phosphate in 1000 ml distilled water, pH adjusted to 2.4-2.6 with orthophsphoric acid) and 3 volumes of methanol. The resulting solution was analysed with HPLC. Calibration curves were drawn by plotting peak area of curve vs drug concentration. Program parameters where: Flow rate-1.2 ml/min, Detection wavelength-247 nm, Run time- 10 min. The column was equilibrated by passing at least 150-200 ml of mobile phase. 20µl of sample was loaded using syringe through rheodyne injector.

Blood samples (0.5 ml) were collected through retro-orbital vein into heparinized tubes at 0, 1, 2, 4, 6, 8 and 24 h after administration. Blood samples were centrifuged at 4000 rpm, 4 ^{o}C for 10 min using a high speed centrifuge machine and plasma samples were withdrawn, after that 0.5 ml of Acetonitrile was add to precipitate out the plasma, vortex for 2 min and kept in centrifugation at 4000 rpm for 10 min and samples were stored at -20 ^{o}C till analysed. The drug plasma concentration values were determined from the calibration curve. Plasma concentrations (ng/ml) versus time (h) profiles were prepared and peak plasma concentration (Cmax) and time of its occurrence (Tmax) were read directly from pharmacokinetic plot [18,19]. One compartmental pharmacokinetic analysis was performed. Mean residence time (MRT) was calculated as area under the first moment curve (AUMC) divided by AUC. All the pharmacokinetic parameters were calculated by using kinetica® 5.1 software.

RESULTS AND DISCUSSION

Solubility study

After performing solubility study in different vehicle, it was found that EFV exhibited maximum solubility in the Capryol 90 as oil, Cremophor EL and Tween 20 as surfactant and Transcutol HP as co surfactant showed in Figure 1.

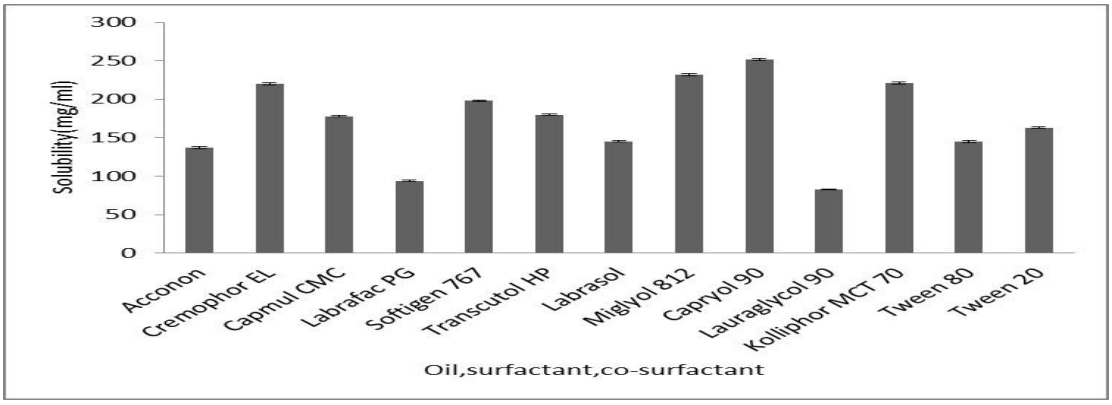

Figure 1: Graphical representation of solubility studies of different potential vehicles.

Pseudoternary phase diagram study

In microemulsion system, the decrease in the free energy required for the emulsion formation consequently improves the thermodynamic stability of the microemulsion formulation. Therefore, the selection of oil and surfactant, and the mixing ratio of oil to S/CoS, play an important role in the formation of the microemulsion. This can be certain by pseudo ternary phase diagram as it differentiates the microemulsion region. One can select the microemulsion region from pseudoternary phase diagram (Figure 2) for Capryol 90: Tween 20/cremophor EL: Transcutol HP system. In ratio1:1 microemulsion is not stable because phase separation is seen after centrifugation of microemulsion.In ratio 2:1 precipitation of microemulsion was observed after some time. In ratio 3:1 microemulsion was found most stable and 50mg drug loading was found hence 3:1 is selected for further preparation.

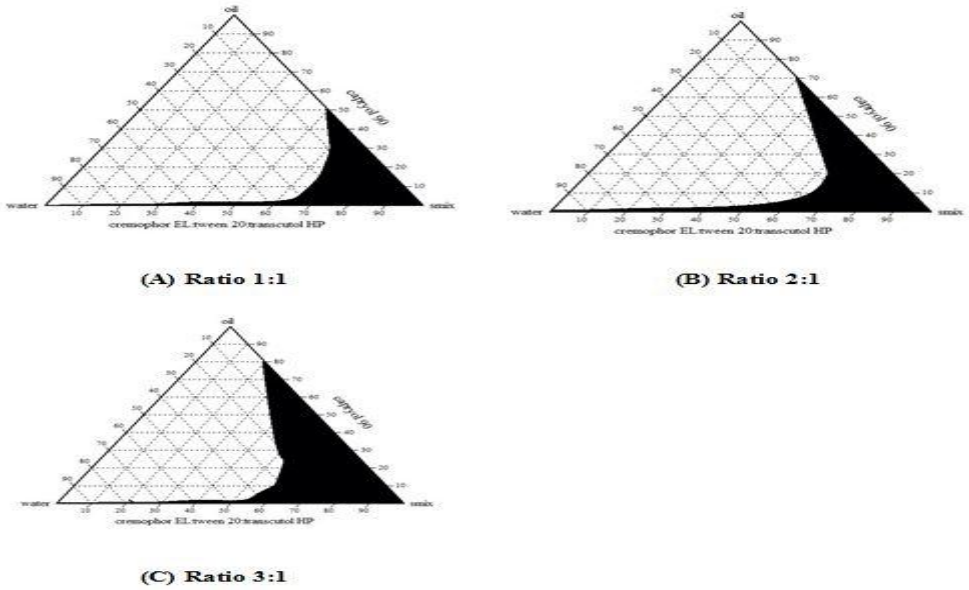

Figure 2: Pseudoternary phase diagrams of Capryol 90(oil), Tween 20 and Cremophor EL (1:1) (surfactant) and Transcutol HP (co-surfactant) and water system.

Composition of microemulsion

From above phase diagram various microemulsion were formulated in different concentration and their particle size and %transparency were determined. F4, F5 and F6 have been selected as shown in Table 1**.** The selected formulations were used for further evaluation and studies.

Table 1: Composition of Microemulsion

Formulations Code	Oil (%)	Surfactant /co-surfactant (%)	Water (%)	Particlesize (nm)	%Transparency
F1	6	45	49	38.95	98.66
F2	6	50	44	33.13	94.64
F3	6	55	39	30.32	97.66
F4	8	45	47	19.08	99.43
F5	8	50	42	15.80	98.13
F6	8	55	37	17.38	98.66
F7	10	45	45	55.74	95.43
F8	10	50	40	62.15	96.64
F9	10	55	35	67.19	95.20

Characterization of EFV loaded Microemulsion

Drug content

Drug content of optimized formulation was found to be 96.19±3.6%W/W.

pH measurement

The excipient used in formulation decides the pH of the final preparation. Change in pH may change zeta potential of formulation which in turn can affect the stability of preparation. Optimized microemulsion formulation has pH 5.8.

Dilution test

In dilution test, water was added to microemulsion, it was readily miscible with the system indicating that the prepared microemulsion is of o/w type.

Dye solubility test

The dye methyl orange used is water soluble and it is found to be distributed uniformly through-out the microemulsion.It can thus be concluded that the type of microemulsion is o/w type.

Percentage transmittance

In the present study, % transmittance was found to be 98.13 ± 0.066. The high value of % transmittance indicates that the system is optically clear which a pre-requisite for microemulsion.

Viscosity measurement

It has been observed that the viscosity of the microemulsion differed, generally, with the surfactant used, microemulsions containing Cremophor EL had higher viscosity values relative to those containing Tween 80. The viscosity of the optimized formulation was found to be in the range of 178.8 ± 2.14 cps.

Conductivity measurement

Conductivity measurements provide a means of determining whether a microemulsion is oil-continuous or water-continuous.Conductivity value of optimized microemulsion formulation was found to be 98.34±02µS.

The results of the characterization tests of optimized formulation are shown in Table 2.

Thermodynamic stability of microemulsion

Microemulsions are thermodynamically stable systems and are formed at a particular concentration of oil, surfactant and water, with no phase separation, creaming or cracking. Thus, the selected formulations were subjected to different thermodynamic stability by using heating cooling cycle, centrifugation and freeze thaw cycle stress tests. Those formulations, which survived thermodynamic stability tests, were taken for characterization with different physiochemical attributes. Result of thermodynamic stability study of drug loaded microemulsions shown in Table 3.

Table 2: Different test result of optimized formulation

Test	Result
Assay (%w/w)	96.19±3.6
Transmittance (%)	98.13 ± 0.06%
pH	5.8± 0.03
Conductivity	98.34±02 µS
Refractive Index	1.432
Viscosity	178.8± 2.14 cps

Table 3: Thermodynamic stability study

Formulation code	Particle Size (nm)			Inference
	Initial	After heating cooling cycle	After freeze thaw cycle	
F4	19.08	34.12	28.37	Fail
F5	15.80	15.45	15.97	Pass
F6	17.38	67.35	90.89	Fail

Droplet size and Zeta potential

Droplet size is an important parameter of microemulsion system for evaluating its stability and bioavailability of drug. Particle size is charatertics for ensuring that microemulsion is efficient dosage form and having good stability. Particle size of optimized formulation was found to be 15.80 nm and PDI was 0.145. The obtained result is shown in Figure 3. The small size of formulated emulsion confirms the high stability of formulation and indicates the possibility of enhanced permeation through the mucus membrane. The zeta potential of optimed formulation was found to be -12.6mv which is shown in Figure 4.

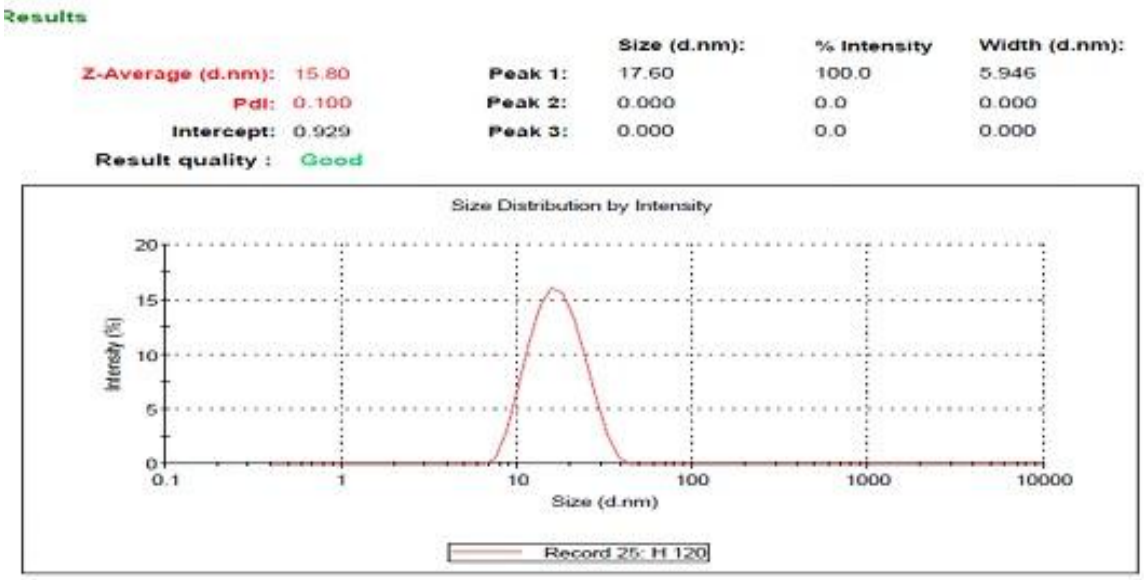

Figure 3: Particle size distribution

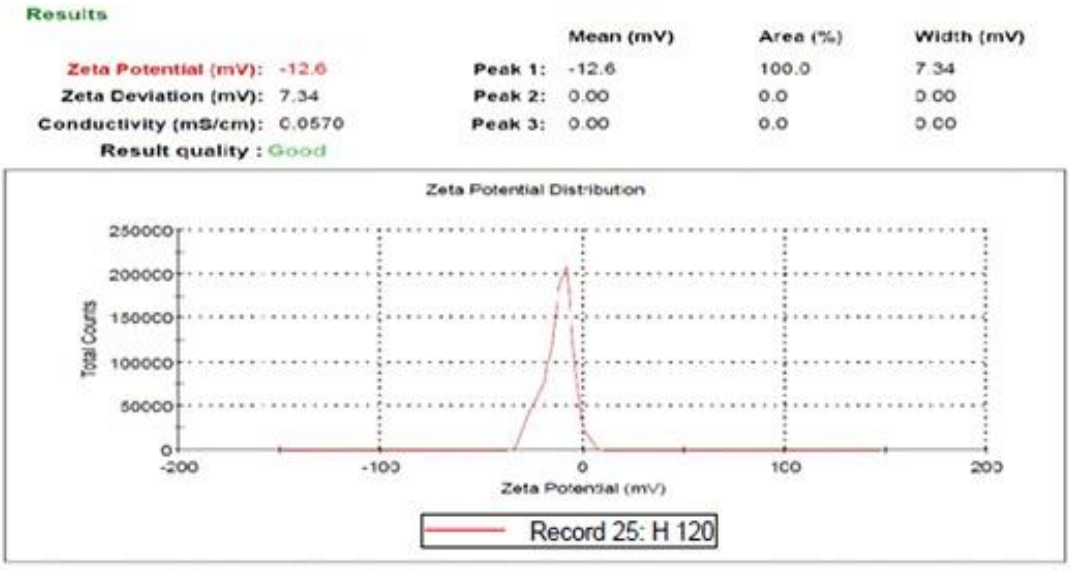

Figure 4: Zeta Potential

Refractive Index

Refractive index of microemulsions by using an Abbey type refractometer was found to be 1.432.

TEM analysis

The morphology of optimized formulation F5 microemulsion was studied using TEM. The TEM image is shown in Figure 5. The microemulsion seems to have uniformly distributed spherical drug loaded globules. The globule size seemed to be in agreement with the result obtained from globule size analysis using Zetasizer.

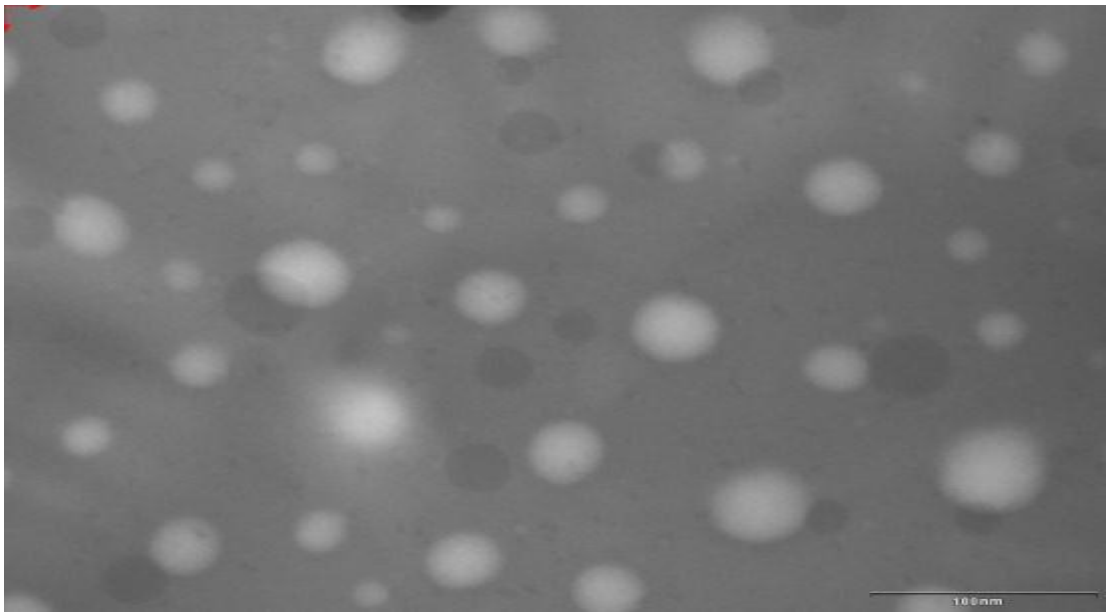

Figure 5: TEM image of microemulsion

***In-Vitro* release study**

To understand the release study *In vitro* release was performed. *In vitro* drug release studies for EFV loaded microemulsion and pure drug suspension are shown in Figure 6. Pure drug suspension released only 44.65 % drug in 4 h whereas drug release from microemulsion was around 94.78 %. The figure 6 shows significant change in drug release of microemulsion formulation compared to pure drug suspension. It may due to reduction in size of globules and solubility enhancer used in formulation.

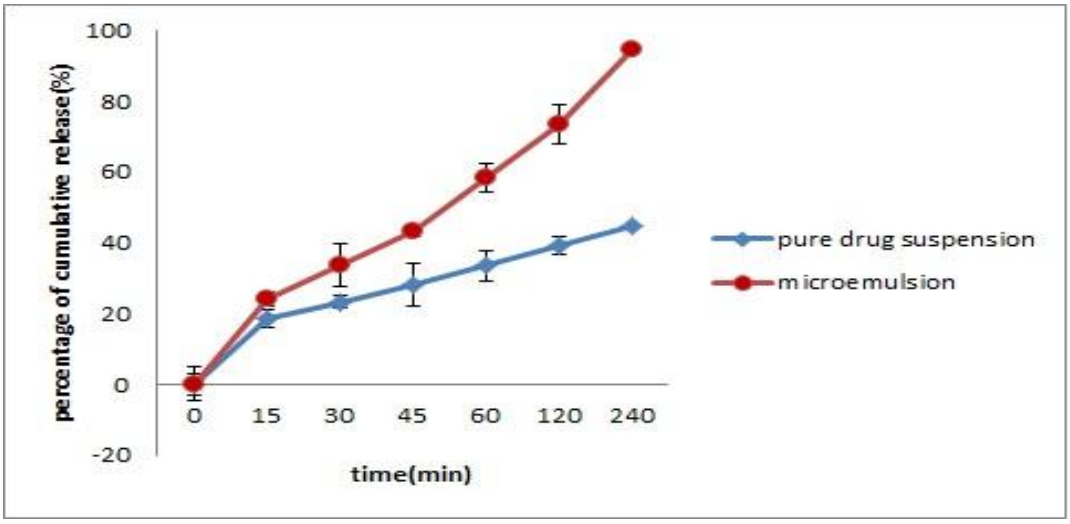

Figure 6: In vitro release of pure drug suspension and microemulsion

***Ex-vivo* release study**

From *Ex-vivo study*, it was found that release of drug from microemulsion is 96.37% in 6h as compared to pure drug suspension which is only 47.85% as in shown Figure 7 .The results in this study are similar to *in vitro* release studies.

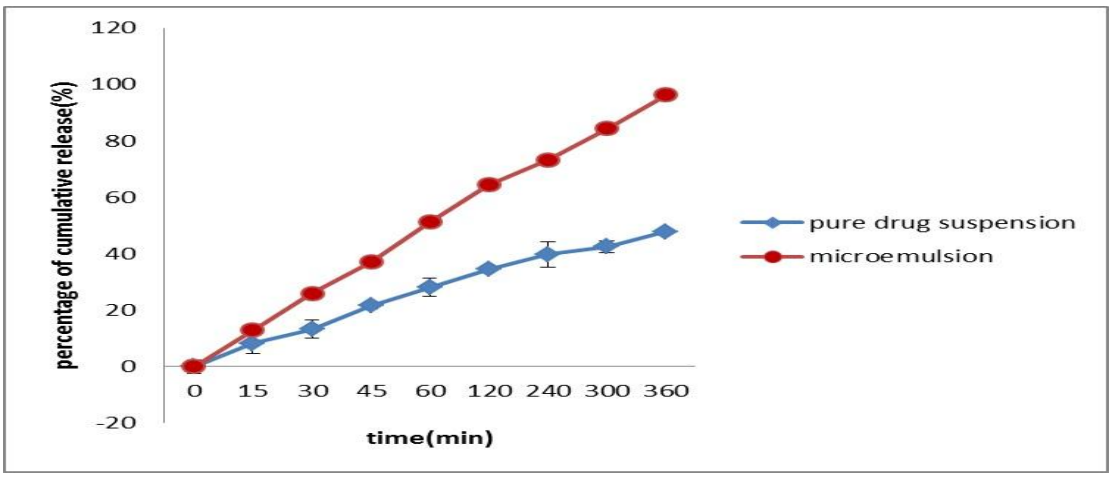

Figure 7: Ex-vivo release study of pure drug suspension and microemulsion

***In-vivo* study**

All the pharmacokinetic parameters for the developed formulation were evaluated in male albino rats after a single oral administration of pure drug suspension and EFV-Microemulsion (EFV-M) by respective plasma concentration model analysis. Pure drug suspension has shown peak plasma concentration (Cmax) at 1650.5±456.2 (ng/ml) and AUC_t 20220.0±345.2 (ng.h/ml), where as in EFV-M Cmax at 3102.9±189.42 (ng/ml) and AUC_t 43530±120.1 (ng.h/ml). EFV-M show 1.88 fold increases in peak plasma concentration and 2.15 fold

increase in AUC_t in comparison to pure drug suspension shown in Figure 8 and Table 4. The significant change as observed during the pharmacokinetic study while comparing microemulsion and pure drug suspension. EFV has low solubility in water and it belongs to BCS II drug, EFV has low dissolution rate due to this charactertics it has low oral bioavailability. Size reduction and solubility are key factors for improving oral bioavailability of this drug. Globule size in EFV microemulsion was achieved 15.80nm. Due to decrease in size, its saturation solubility and surface area has been increased. Surfactant and co-surfactant are permeation enhancers which may help to increase permeability of intestinal membrane. It could significantly increase the oral bioavailability of EFV by the combined effects of enhanced solubility, P-gp inhibition in the gastro intestinal walls and permeability enhancing effects.

Table 4: Pharmacokinetic study (Mean± SD, n=3)

Pharmacokinetic Parameter	Pure drug suspension	EFV-Microemulsion
Cmax (ng/ml)	1650.5±456.2	3102.9±189.42
Tmax (h)	2.00±0.32	4±0.49
$K_{el}(h^{-1})$	0.084±0.012	0.061±0.024
AUC_t(ng.h/ml)	20220.0±345.21	43530±120.1
AUC(ng.h^2/ml)	198200±412	101159±621
MRT (h)	12.39±0.21	30.62±0.45
$t_{1/2}$	8.23±0.24	23.34±0.25

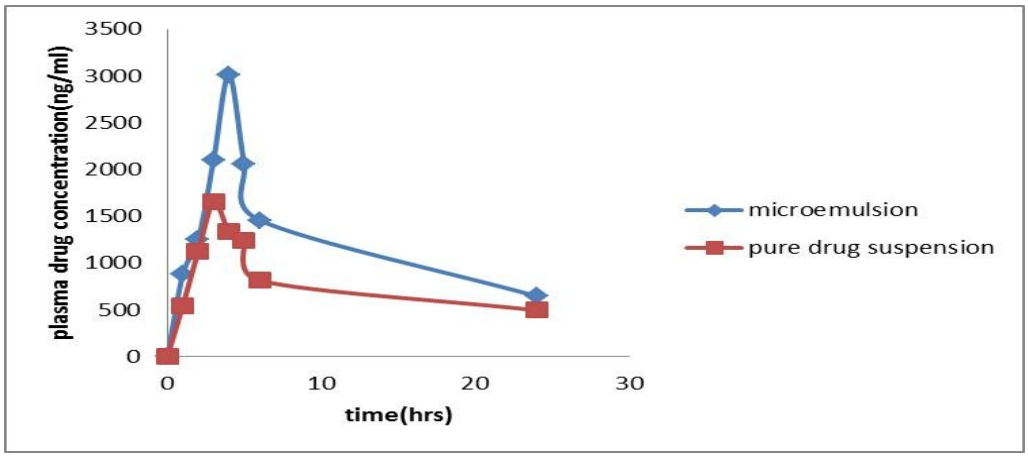

Figure 8: Pharmacokinetic study

Stability study

It was found that there was no significant change in the particle size and assay value when microemulsion was stored at 2-8°C and 25°C ± 2°C/60±5%RH over 6 months as shown in Table 5 . The formulation maintained their clarity throughout the stability period.

Table 5: Stability study of selected formulation (Mean± SD, n=3)

Temp.	2-8°C		25°C ± 2°C/60±5%RH	
Month	Particle size nm	Assay (%W/W)	Particle size nm	Assay (%W/W)
0	15.80±0.71	98.64 ±0.44	15.80±0.71	98.64 ±0.44
1	15.35±0.34	97.91 ±0.25	14.89±0.23	98.02 ±0.48
2	16.15±0.21	98.14 ±0.54	15.45±0.45	98.23 ±0.36
3	15.34±0.03	98.74 ±0.54	16.73±0.25	98.15 ±0.62
6	15.76±0.24	98.27 ±0.41	15.76±0.56	98.59 ±0.53

CONCLUSION

The microemulsion formulation was successfully prepared by water titration method. The prepared microemulsion drug content was 50mg/ml. Particles size was found to 15.80±0.71 nm. Microemulsion found to thermodynamically stable and physically stable for 6 months at 2-8^{o}C and 25^{o}± 2^{0}C. The oral bioavailability of microemulsion was enhanced to 2.15 fold compared to pure drug suspension. However further more studies in higher animals and human is need to perform before this formulation can used commercially.

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