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DESIGN, FORMULATION AND CHARACTERIZATION OF TENOFOVIR MICROEMULSION AS ORAL DRUG DELIVERY

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ABSTRACT

The aim of the present research was to design, formulate and evaluate Tenofovir Microemulsion. Tenofovir, a Nucleotide Reverse transcriptase inhibitor belongs to the category of anti retro viral drug. The oral bioavailability of Tenofovir is 25% due to its poor water solubility. An attempt was made to enhance solubility by formulating oral Microemulsion of Tenofovir. The solubility of Tenofovir in various Oils, Surfactants and Cosurfactants were checked to optimize the components of Microemulsion. Pseudo ternary diagrams were constructed to identify the area of Microemulsion region. A Microemulsion system with oleic acid as Oil phase, Tween 20 as surfactant and Ethanol as Cosurfactant was developed for oral delivery of Tenofovir. To achieve the objective of present study, Microemulsion formulations were prepared by using different ratios of Oil, Surfactant: Cosurfactant and Water. The prepared formulations of Tenofovir were characterized for thermo dynamic stability studies, pH, transparency, viscosity, drug content and *in vitro* drug release. Particle size and Zeta potential of optimized formulation were found to be 54.30nm and -5.61mV. Among four formulations, TME4 shows highest drug release of 86.88%. The *invitro* release was found to follow Non-fickian diffusion mechanism. These results demonstrate the potential use of Microemulsion for improving the Bioavailability of poor water soluble compound Tenofovir.

Key words: Phase diagrams, Centrifugation, Kinetic models, Stability.

INTRODUCTION

Successful oral delivery of drugs has always remained challenge to the drug delivery field, since approximately 40% of the new drug candidates have poor water solubility associated with low bioavailability. Lipid-based formulations have attracted great deal of attention to improve the oral bioavailability of poorly water soluble drugs. In fact, the most novel approach is to incorporate lipophilic or hydrophilic drugs into inert lipid vehicles such as oils, surfactants through formulating in the form of microemulsions, self-emulsifying formulations. These lead to increased solubilization further increase in therapeutic efficacy. A microemulsion is a system of water, oil and an amphiphile which is a single optically isotropic and thermodynamically stable liquid solution. Microemulsions (μE) are usually in the range of 10-100 nm. 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 [1,2]. The main objective of the present work is to formulate Microemulsions, which is transparent unlike ordinary milky emulsion. This has provided impetus for active study of a design of suitable carrier, intelligent delivery system and approaches for the delivery.

Tenofovir disoproxil fumarate, the oral pro-drug of tenofovir, is a Nucleotide reverse transcriptase inhibitor. It inhibits viral polymerases by directly competing with the natural deoxyribonucleotide substrate and, after incorporation into deoxyribonucleic acid (DNA), by DNA chain termination.Tenofovir disoproxil fumarate is used to treat HIV and chronic Hepatitis. Due to its poor solubility in water and low bioavailability (25%) Tenofovir was selected as a candidate to formulate Microemulsion for oral delivery [3].

MATERIALS AND METHODS

Tenofovir was obtained as a gift sample from Cipla pharmaceuticals limited, Mumbai and other excipients and reagents purchased were oleic acid from CDH Laboratory reagent, New Delhi,India; Tween 20 from LOBA Chemicals Pvt Ltd, Mumbai,India; Ethanol from Changshu Yangyuon Chemicals, China. All other

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chemicals and reagents were of analytical grade and used without any further purification.

Screening of Oil for Microemulsion

The solubility of Tenofovir in various oils like Castor oil, olive oil, oleic acid, Eucalyptus oil, coconut oil, Isopropyl myristate was determined by dissolving an excess amount of Tenofovir in 5 ml of each of the selected oils in stoppered vials separately for the determination of solubility. The mixture vials were then kept in a shaker for 72 h to get to equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 3000 rpm for 30 min. The supernatant was taken and filtered through a 0.45 μm membrane filter. The concentration of drug was determined in each oils by UV spectrophotometer with suitable dilution with 0.1N HCL at their respective wavelength 260nm [4].

Screening of Surfactant and Co-Surfactant

Several surfactants including Tween-80, Tween-20 were screened. Co-surfactants were selected based on their capability to form stable microemulsion with relevant surfactants at a minimum concentration. Based on this, several co-surfactants including Polyethylene glycol 400 (PEG 400) and Ethanol were screened [4].

Construction of Phase Diagrams

On the basis of drug solubility in various microemulsions components, different combination of oil, water and surfactant/cosurfactant were selected. The pseudo-ternary phase diagrams of oil, surfactant: cosurfactant and water were developed using surfactant titration method. The mixtures of oil and water at certain weight ratios varying from 1:9 to 9:1 were titrated with surfactant/co-surfactant mix in a dropwise manner. Pseudo ternary phase diagram was achieved by titrating with four different ratios of Surfactant and Cosurfactant (1:1,1:2.2:1,4:1) until it turns from hazy to transparent. After the identification of microemulsion region in the phase diagrams, the microemulsion formulations were selected at desired component ratios in order to form the Microemulsion [5,6,7].

Formulation Design

On the basis of the solubility studies, oleic acid was selected as the oil phase. Tween 20 and Ethanol were selected as surfactant and cosurfactant respectively. Distilled water was used as an aqueous phase. Predetermined amounts of the drug were dissolved in the required quantity of oil, surfactant and cosurfactant with varying ratios as described in table 1. Distilled water was added to the above mixture as a fixed ratio. Surfactant and co-surfactant were added gradually with continuous stirring, which resulted in the formulation of a transparent and homogenous Microemulsion [4].

Characterization of Microemulsion

Thermodynamic Stability Studies

To overcome the problem of metastable formulation, thermodynamic stability tests were performed. Prepared formulations were centrifuged at 3000 rpm for 30 min and then examined for Phase separation. Those formulations that did not show any phase separation were taken for the heating and cooling cycle at temperature of 4°C and 45°C for 48 h were done. The formulations were then observed for phase separation. The formulations which were stable at these temperatures, those formulations that survived thermodynamic stability tests were selected for the further studies [4].

Type of Microemulsion

The type of microemulsion was identified by the staining method. The water-soluble dye methylene blue and the oil-soluble dye Sudan red were equally added onto the blank microemulsion and Tenofovir loaded Microemulsion to evaluate the diffusion speed of the two dyes. If the blue dye diffuses faster than the red dye, then the type of microemulsion is o/w, and vice versa for the w/o [8].

pH Determination

The pH of the each formulation was found to be measured by a digital pH meter standardized using pH 4.0 and 7.0 standard buffers before use [9,10].

Rheological Characterization

The rheological studies of samples were carried out with Brookfield Digital viscometer (LV DV-E model) using S-18 spindle number. The developed formulations were poured into the small sample adaptor of the Brookfield viscometer and the angular velocity increased gradually from 0.5 to 100rpm. The system was calibrated using Brookfield viscosity standard fluids [11].

Transmittance Test

Transparency of microemulsion was checked by measuring transmittance at 650 nm with 0.1N HCL as blank by using UV spectrophotometer [12].

Drug Content Estimation

Microemulsion containing 100mg drug was dissolved in 100ml 0.1N HCL taken in volumetric flask. Then the solvent was filtered,1ml was taken in 50ml volumetric solution and diluted up to the mark with 0.1N HCL and analyzed spectrometrically at 260nm. The concentration of Tenofovir in mg/ml was obtained by using standard calibration curve of the drug. Drug content studies were carried out in triplicate for each formulation batch [9].

***In Vitro* Drug Release Studies of Tenofovir**

Tenofovir ME 100mg equivalent weight was filled in hard gelatin capsule shell and drug release studies was carried out for each formulation by using Dissolution test apparatus (DS8000 Model) Type II. Under pH condition simulative gastro intestinal tract 900 ml dissolution media was taken. The basket rotation was adjusted to 100 rpm, the temperature being maintained at 37 ± 0.5^{o} c throughout the study. A buffer of pH1.2 was used as dissolution medium for a period of 2hrs as average

gastric emptying time is 2hrs. 5ml of sample of dissolution medium was withdrawn and replaced with fresh dissolution medium. The samples were analyzed for drug concentration by UV-Visible spectrophotometer at 260nm.The data obtained from the in-vitro dissolution studies was subjected for kinetic treatment to obtain the order of release and best fit model [4,13].

Particle Size and Zeta Potential Measurement

The particle size and zeta potential of the optimized microemulsion was determined by dynamic light scattering with Zetasizer Nano ZS ver.6.34 (Malvern Instruments Ltd., Malvern, Vadodara).

RESULTS AND DISSCUSSION

Preparation and optimization of microemulsion

The solubility of Tenofovir amongst various oils investigated was found to be highest in oleic acid (26.69±0.3mg/ml). Amongst surfactant, Tween 20 showed maximum solubility (33.31±1.5 mg/ml) followed by Tween 80. Ethanol showed highest solubility among the co-surfactants (41.57±0.6mg/ml), followed by PEG.

The microemulsion existence region was determined by constructing phase diagrams. From the pseudo phase diagrams it was concluded that highest Microemulsion zone was obtained for the Microemulsions having Tween 20 and Ethanol in the ratio of 4:1.

Evaluation of Physical Stability of Microemulsion Formulations

Thermal stability and Centrifugation Studies on formulations

Microemulsions are thermodynamically stable system composed of fixed proportion of oil, surfactant/cosurfactant and water which does not tends to show any phase separation after the centrifugation.

Type of Microemulsion,Viscosity and pH of the formulation

All Microemulsion formulations were W/O that it contains water in internal phase and oil in the external phase. All formulations had clear transparent yellowish to pale yellow colour. The viscosity of the selected formulation was determined on Brookfield viscometer by using CP-18 Spindle 10 rotations per minute (RPM) at constant temperature. The viscosity of formulation TME-4 (56.7cps) was lower than that of any other formulation. The percentage drug content was evaluated in all the formulations of w/o Tenofovir microemulsions. It was observed that percentage drug content of TME 4 was more than other formulations.

***In vitro* drug release studies**

The overall cumulative drug release for formulations TME1, TME2, TME3 and TME4 were found to be 65.72%, 62.66%, 76.21 and 86.88% respectively. Among all formulations TME 4 shows highest drug release compared to other formulations. The values of diffusion coefficient (n) for formulations TME1 to TME4 are 0.8793, 0.8594, 0.8846 and 0.9056 respectively which indicates Anomalous mechanism. Peppa's was found to be best fit model for all formulations.

Measurement of particle size and zeta potential

Globule size of Microemulsion was given in figure 4. Optimized Microemulsion showed particle size ie., 54.3 nm. Zeta potential result of optimized Microemulsion was found to be -5.61mV.

Table 1. Final formulation with different oil concentration

Formulation code	Oil %	S:CoS %	Water%
TME 1	25.5	70	4.5
TME 2	26.1	70	3.9
TME 3	26.7	70	3.3
TME 4	27	70	3.0

Table 2. Thermal and Centrifugation stability of Tenofovir Microemulsions

Microemulsion formulations	Thermal stability			Centrifugation stability at 3000rpm
	Storage at 4°c	Storage at room temp.	Storage at 45°c	
TME 1	√	√	√	√
TME 2	√	√	√	√
TME 3	√	√	√	√
TME 4	√	√	√	√

√ indicates microemulsions at particular conditions are stable, no phase separation.

Table 3. Physicochemical Parameters of developed Microemulsion

Formulation code	pH	Viscosity	%Transmittance	Drug content
TME 1	4.70±0.02	69.2±1.3 cps	89.08±0.4%	92.5±1.4%
TME 2	4.80±0.02	65.9±2.8cps	91.13±0.8%	93.5±0.9%
TME 3	4.85±0.02	58.8±1.2cps	93.52±0.6%	94.8±0.2%
TME 4	4.87±0.02	56.7±1.1 cps	95.74±0.5%	95.3±0.8%

Table 4. Effect of temperature on stability of the optimized Microemulsion formulation (n=3,mean±SD)

Temperature (°c)	Phase separation		%transmittance		% of Assay	
	After 4mnths	After 6mnths	After 4mnths	After 6mnths	After 4mnths	After 6mnths
2-8 °c	No	No	95.08±0.8	94.87±1.3	95.23±0.2	94.30±0.1
Room temperature	No	No	95.72±1.2	94.48±1.5	94.23±0.4	93.60±1.1
Elevated temperature	No	No	95.22±1.2	93.80±0.6	94.00±0.7	93.28±0.3

Figure 1. % Solubility of Tenofovir in different oils, Surfactants and Cosurfactants

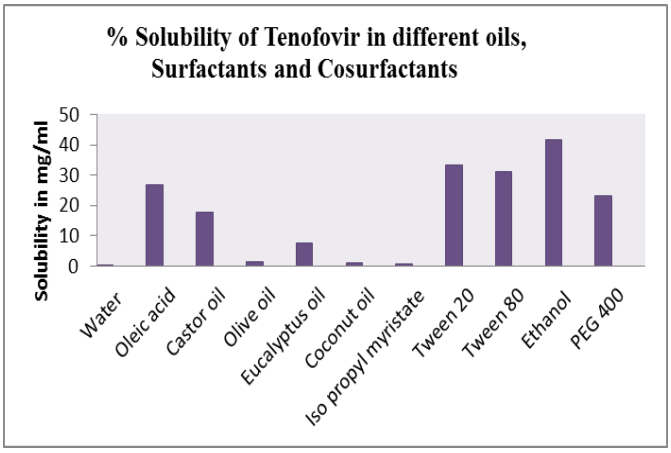


Figure 2. Pseudo phase diagram containing surfactant and cosurfactant ratio 4:1

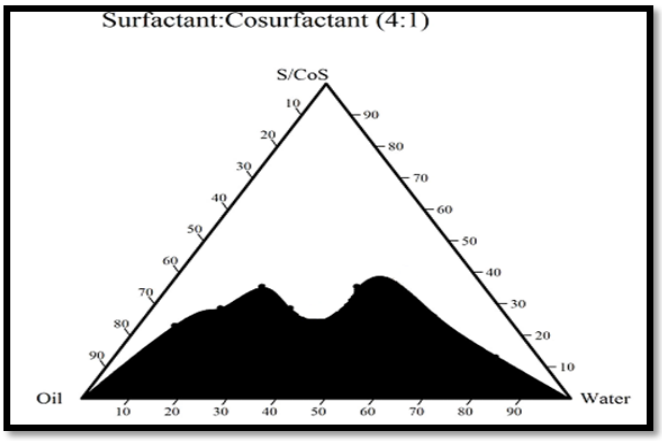


Figure 3. Comparative cumulative percent drug release versus time plots for formulations TME 1-4

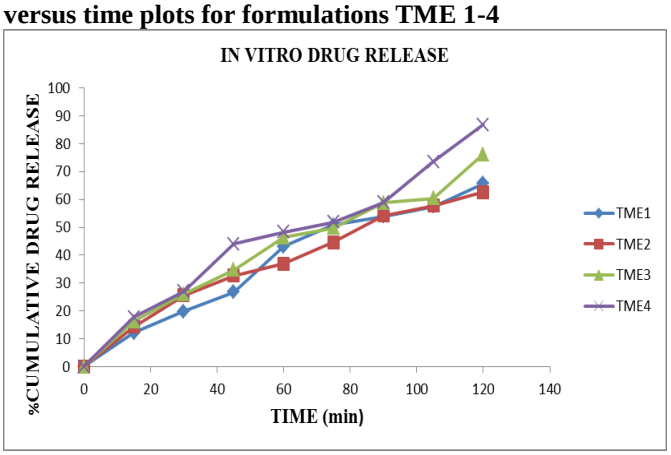
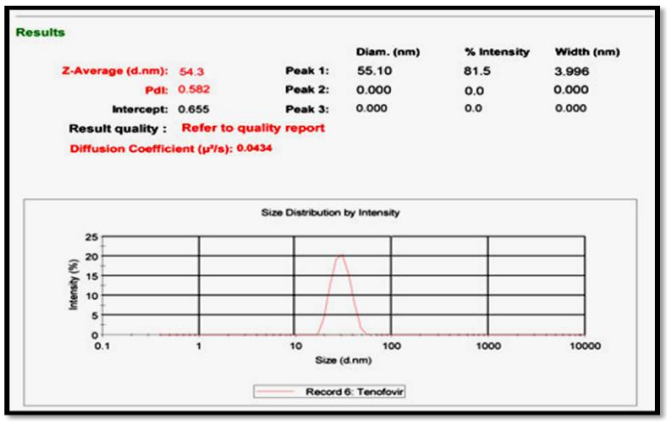


Figure 4. Average globule size of optimized microemulsion



Stability studies

The studies revealed that there is slight reduction in the drug content and % transmittance after storage for 6months. Results were recorded in table 4. From the data it was indicated that the optimized Tenofovir Microemulsion can stable up to 6months.

CONCLUSION

The study demonstrates that the developed Microemulsion TME 4 formulation containing oleic acid (27%), Tween 20 (28%), Ethanol (7%) and water (3%) is a transparent, less viscous system and stable system. Results from Invitro studies revealed that developed TME 4 possessed higher rate of drug release compared to the other formulations. This increased dissolution rate and increased solubility can ultimately lead to increase bioavailability of Tenofovir. Phase diagrams indicated more width Microemulsion region with increase in surfactant ratio. It was confirmed that drug content of TME 4 was more than the other formulations this may be because of increased solubility of drug in the oil. Particle size and Zeta potential of optimized formulation were found to be 54.30nm and -5.61mV.The stability studies confirmed that the optimized formulation can stable up to 6months. However, further studies on animals and human beings are needed to be performed before this formulation can be commercially exploited.

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