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FORMULATION DEVELOPMENT AND CHARACTERIZATION OF MICROEMULSION SYSTEM FOR ANTI-CANCER DRUG TO ENHANCE THE SOLUBILITY AND BIOAVAILABILITY

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ABSTRACT

Background: Prostate cancer is cancer of the prostate gland. The prostate gland is located below the bladder and in front of the rectum. Finasteride (FNS) is one of the drugs of choice for the treatment of prostate cancer. But the conventional dosage form of this drug has low bioavailability so that, this research is designed to enhance the bioavailability of FSN using microemulsion. **Aim and Objectives:** Aim of the study is to formulation development and characterization of microemulsion system. The objectives of this research are formulation of the FNS microemulsion, characterization of FSN microemulsion and evaluation *in vitro* drug release study. **Methods:** FSN Microemulsion was prepared by water titration method using different concentrations of surfactant mix (1:1, 2:1, 3:1 and 4:1) and formulated Microemulsion was characterized for its drug content Droplet size analysis and Polydispersity Index Zeta potential Surface morphological analysis, pH measurements, Dye-solubility test, *In vitro* drug release and

Ex vivo drug release. **Results:** Particle size of the formulation was found to be between 722.5 to 3814 nm, Poly dispersity Index and Zeta potential shows between 0.692 to 1and -2.42 to -11.6 respectively. Based on the dye-solubility study 1:1 ratio formulation was selected as the best formulation. The TEM result shows spherical shape of FSN microemulsion. **Conclusion:** Results of this study concluded that, Surfactant (mix) ratio Tween 80: PEG 400 (F1) is the suitable surfactant ratio for the formulation of FNS microemulsion.

Keywords: Microemulsion, PEG 400, Tween 80, Zeta potential, *Ex vivo* release

1. INTRODUCTION

Solubility is an important physiochemical parameter that affects the absorption and effectiveness of desired drug. Failure in formulation development of the drug has been considered as the consequences of its poor aqueous solubility **[1]**. The reduced solubility and low dissolution rate in aqueous gastrointestinal tract most often lead to inadequate bioavailability of the drug **[2]**. Microemulsion is homogenous, transparent, thermodynamically stable dispersion of water and oil, stabilized by a surfactant, usually in combination with a co-surfactant. Microemulsions are macroscopically isotropic mixtures of at least a hydrophilic, a hydrophobic and an amphiphilic component. Microemulsion can enhance the solubility and bioavailability **[3]**. Finasteride (FNS) is non - steroidal anti-androgenic drug used to treat prostate cancer, it comes under BCS class II drug (low solubility and high permeability), FNS is a very poorly water soluble drug, FNS is lipophilic, and so it has low solubility **[4]**. By formulating micro-emulsion of FSN we can increase solubility of the drug.

2. MATERIALS AND METHODS

2.1. Materials

FSN, Tween-80, PEG-400 and Iso propyl myristate were purchased from Gatteffose, Merck science of pvt Ltd, Loba chemie, SDFCL ltd, Mumbai respectively and Dialysis membrane (pore cut off size 12000 - 14000 Dalton) was purchased from Himedia, Mumbai.

2.2. Methods

2.2.1. Preformulation studies

Preformulation tests were performed for the identification of the possible drug-excipients incompatibility. The compatibility studies were performed for the development of dosage form, preformulation studies is carried out to confirm that there was no interaction between the drug and excipients. Infrared spectrophotometer studies used to check the compatibility studies between

excipients and drug. Infrared Spectrum analysis of drug (FSN), oil (IPM), Surfactant (S) (Tween 80), Co-surfactant (PEG 400) and the physical mixtures of drug with the Excipients are obtained from FT-IR Spectrophotometer by KBr disk method. A known weight of samples are mixed with KBr powder and compressed to 10 mm discs by hydraulic press at pressure of 150 bars for 30s. The scanning range and resolution are 400 – 4000 cm^{-1} and 4 cm^{-1}. The spectra obtained were compared and interpreted for the shifting of functional peaks or the appearance and disappearance of new functional peaks **[5]**.

2.2.2. Optimization method of preparation of Microemulsion

The pseudoternary phase diagrams consisting of oil, water and surfactant/cosurfactant and cosurfactant mixture of different HLB values were constructed using water titration method. Tween 80 was combined with different types of cosurfactant such as PEG 400. The ratio of surfactant to cosurfactant was fixed at 1:1 on the weight basis. Each oil was mixed with surfactant or surfactant and cosurfactant mixture at ratios of 1:9, 1:8,1:7,1:6,1:5,1:4,1:3,1:2 and 1:1. Three types of surfactant (Tween 80 and span 80, Tween80, Span 80, Tween 80 and PEG 400) were used. Distilled water was added in increments of 100µL by micropipette at room temperature. The samples were vigorously mixed with a vortex mixer for 2 min and kept at room temperature for 24 h to reach equilibrium before the next addition of water **(Figure 1) [6, 7]**.

2.2.3. Formulation of FSN Microemulsion

FSN Microemulsion was prepared by water titration method using different concentrations of surfactant mix (1:1, 2:1, 3:1 and 4:1). In this method dispersion of required quantity drug (10 mg) in appropriate quantity of oil which is required for the solublisation of drug. The mixture was homogenized and accurately weighed quantity of Surfactant: Cosurfactant blends were added in small portion with stirring to it. The blends were mixed thoroughly using magnetic stirrer and drop wise double distilled water to it with continuous stirring around 1 hour **[8] (Table 1)**.

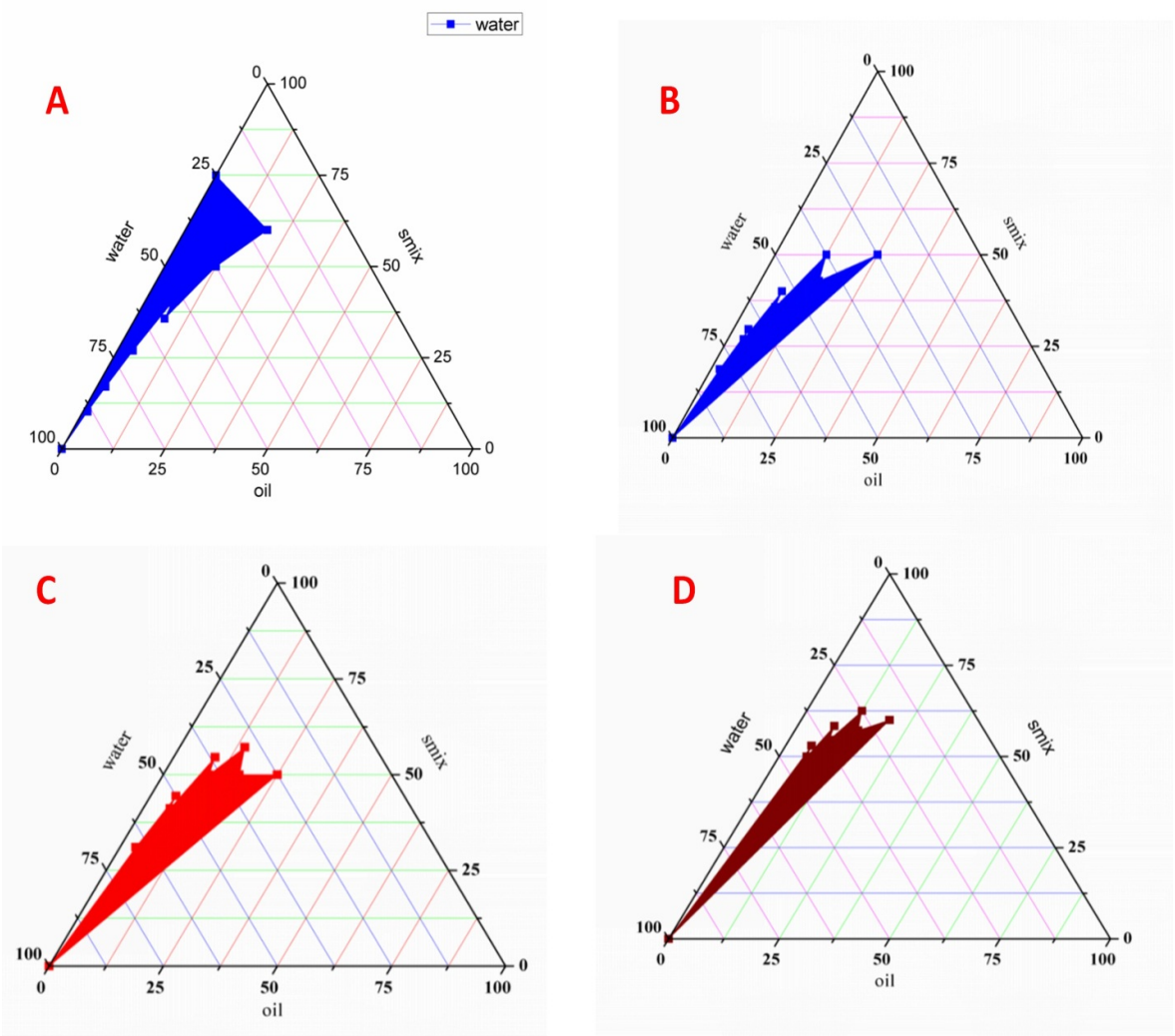

Figure 1: Optimization method of preparation of Microemulsion (Ternary phase diagrams 1:1, 2:1, 3:1 and 4:1)

Table 1: Formulation development of Microemulsion

Formulation code	S (mix) ratio Tween 80: PEG 400	Oil: S (mix) Ratio	FSN mg	S (mix) ml	Oil mL (IPM)	Water ml	Final Volume ml
F-1	1:1	1:9	10	4	0.4	0.6	5
F-2	2:1	1:9	10	3.8	0.4	0.8	5
F-3	3:1	1:9	10	3.6	0.4	1.0	5
F-4	4:1	1:9	10	3.4	0.4	1.2	5

2.2.4. Determination of drug content

Sample containing 10 mg equivalent FSN microemulsions were dissolved and the volume is made up to 10ml with 0.1 N Hcl buffer. From the above solution 1ml was pipetted out and made up to10ml with 0.1 N Hcl buffer. The absorbance of resulting solution is determined at λ_{max} (213nm) using UV Spectrophotometer (UV-1700 agilent

UV technologies, Japan) and the drug content is estimated **[9]**.

2.2.5. Droplet size analysis and Polydispersity Index

The droplet size of the emulsion was determined by Zeta sizer 1000 HS. The formulation (0.1 mL) was dispersed in 50 mL of water in a volumetric flask and gently mixed by inverting the flask. Measurement was done using a Zeta sizer 1000 HS. Light scattering was monitored at 25^0C at a 90^0 angle **[10]**.

2.2.6. Zeta potential

The magnitude of the zeta potential values gives an indication about the stability of the formulation **[11]**. The limit for the zeta potential value of the particle in the formulated microemulsion was above± 30.

2.2.7. Surface morphological analysis, Dye-solubility test and pH measurements

The surface morphology of the prepared drug loaded Microemulsion were examined under Transmission electron microscope **[12]**. The aqueous dispersion (one drop) was placed over a copper grid of 400 meshes coated with carbon film. Finally, the sample was air dried prior to placing it in the TEM instrument for analysis. Water soluble dye, methylene blue solution 10 µL was added to the emulsion, if the continuous phase is water (o/w emulsion), the dye will dissolve uniformly throughout the system **[13]**. The apparent pH of the formulation was measured by a pH meter in triplicate at 25^0C, the pH not only affect the stability the emulsion but also alter the solubility and bioavailability **[14]**.

2.2.8. *In vitro* drug release

Known quantity of microemulsion about 1 ml of formulation was placed in dialysis membrane bag with molecular cut off 12kda Dalton, then it was tied and placed in 250 ml of 0.1 N HCl. The entire system was kept at 37^0C with continuous constant magnetic stirring at 100 rpm **[15, 16]**. 2 ml of sample was withdrawn and simultaneously 2 ml of 0.1 N Hcl was replaced into the system to maintain the sink condition **[17, 18]**. The study was carried out up to 3 h time period. The amount of FSN in the release medium was evaluated using UV-spectrometer at 213 nm **[19]**.

2.2.9. *Ex vivo* drug release

5 ml of formulation was placed in a goat intestine then it was tied and placed in 500 ml of 0.1 N HCl. The entire system was kept at 37^0C with continuous constant magnetic stirring at 100 rpm **[20, 21]**. 2 ml of sample was withdrawn and simultaneously 2 ml of 0.1 N HCl was replaced into the system to maintain the sink condition. The study was carried out up to 3 hour time period. The

amount of FSN in the release medium was evaluated using UV-spectrometer at 213 nm **[22]**.

3. RESULTS AND DISCUSSION

3.1. Preformulation studies

The I.R spectrum of IPM Shows its Characteristics 3627cm-1 Showed (O-H stretch), and 1734cm-1 (C-O stretch), and 963cm-1 (C-H bend).The FTIR spectrum of Tween 80 shows absorption band as follows (cm-1): 1735, 1248 and 1108(C-O stretch), 948 (C-H bend), 1298 (C-N stretch) and 1640 (C=C stretch). The FTIR Spectrum of PEG 400 Shows its Characteristics band for 3371cm^{-1} (O-H stretch), 1455cm^{-1} (C-H bend) and 1103cm^{-1} (C-O stretch).FSN was identified by its absorption band C-H bend at 949-722 cm^{-1}, C-H stretch at 2923 cm^{-1}. At 1733 C=O bend absorption band was appeared. Absorption band for C-O stretch was found at 1249 cm^{-1} and 1106cm^{-1}.

3.2. Drug content

The drug content of the F1-F4 formulations was found to be 90.32, 80.87, 83.04 and 87.3 respectively. The results indicate that F1 formulation having higher drug content compared with other formulations.

3.3. Particle size and Polydispersity index

For characterization of FSN microemulsion particle size was determined using dynamic light scattering method, an apparatus Nano ZS (Malvern Instrument) operating in back-scattering mode at an angle of 173º. The particle size and polydispersity index values are shown in **Table 2**. The results indicate that F1 has lesser particle size (722.5nm) among all other formulations.

3.4. Zeta potential and pH

The magnitude of the zeta potential values gives an indication about the stability of the formulation. The zeta potential of F1-F2 formulations was found to be -27.8, -16.3, -20.6 and -16.3 respectively. The results indicate that F1 formulation has good result among other formulations. The pH of F1-F2 formulations was found to be 5.48±0.03, 5.57±0.04, 5.62±0.05 and 5.54±0.05 respectively. The pH of microemulsion acceptable limit is 4-6. Microemulsion formulation may also have the accepting limit.

3.5. Surface morphological analysis

The Surface morphology of the FSN microemulsions was characterized using Transmission Electron Microscope (TEM). The **Figure 2** shows the surface morphology of FSN microemulsion (F1-F4). The Particle was in the size range 500nm. The surface morphology was nearly uniform in all particles and also appeared in most spherical shape. Methylene blue is water soluble so the dye is added to the microemulsion. Oil

droplets and water droplets can be viewed by optical microscope. Dye with F1 and F2 is shown in **Figure 3**. Dye-Solubility study of these formulations 1:1, 2:1, 3:1 and 4:1 the dye uniformly distributed throughout the microemulsion. Based on the dye-solubility study 1:1 ratio formulation was selected as the best formulation.

3.6. *In vitro* and *Ex vivo* drug release

The *in vitro* and *Ex vivo* study results of F1-F4 formulations are shown in **Figure 4**. In overall formulations, F1 formulation exerts maximum drug release 3 h. The initial burst release followed by the sustained release was observed in all formulation. The initial burst release may be probably caused by the drug absorbed on the surface of FSN microemulsion. Selected formulations were further studied for intestine permeation using goat intestine. *Ex vivo* drug permeation was examined through goat intestine over a period of 3 h in 0.1 N HCl solutions at 37^0C for the optimized microemulsions and the corresponding pure drug. There was an increase in intestinal permeability for optimized formulation compared to pure drug due to presence of surfactants and cosurfactants combination, generally used as permeation enhancers. Formulation F-1 (94.19%) shows highest permeation and F-4(88.85%) shows lowest permeation in intestine. The maximum permeation could be due to having the lowest droplet size and lowest viscosity of all the formulations. Thus the drug diffused at a faster rate from the microemulsion system. Hence we postulate that the developed FNS microemulsion shown desirable *in vitro* and *Ex vivo* characteristics for drug delivery.

Table 2: Average particle size and Polydispersity index values

S. No	Formulations	Average Particle Size (nm)	Polydispersity Index
1	F-1	722.5	0.692
2	F-2	2362	1
3	F-3	2019	1
4	F-4	3814	1

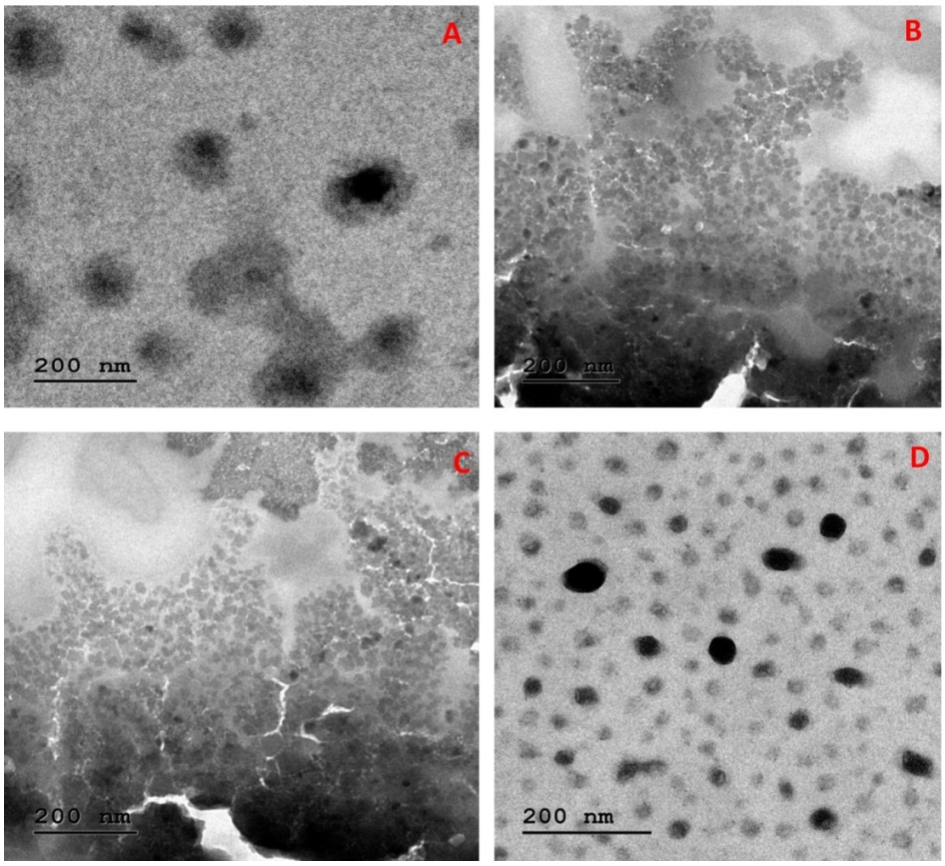

Figure 2: TEM images of F-1, F-2, F-3 and F-4 formulations

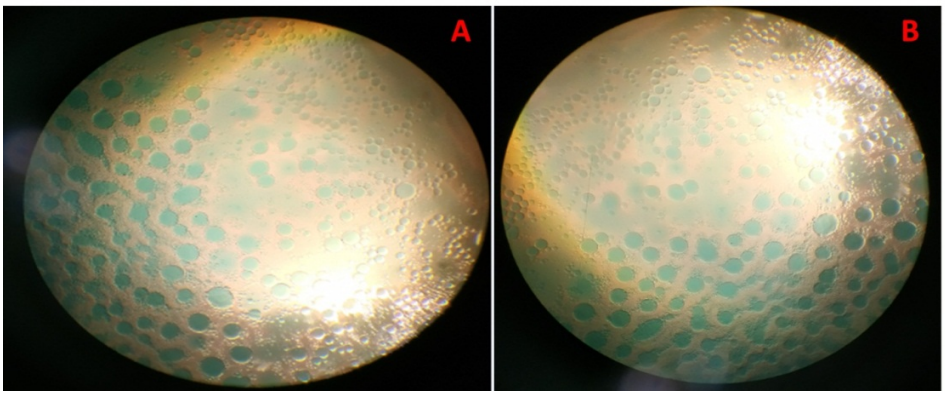

Figure 3: Dye with F-1 and F-2

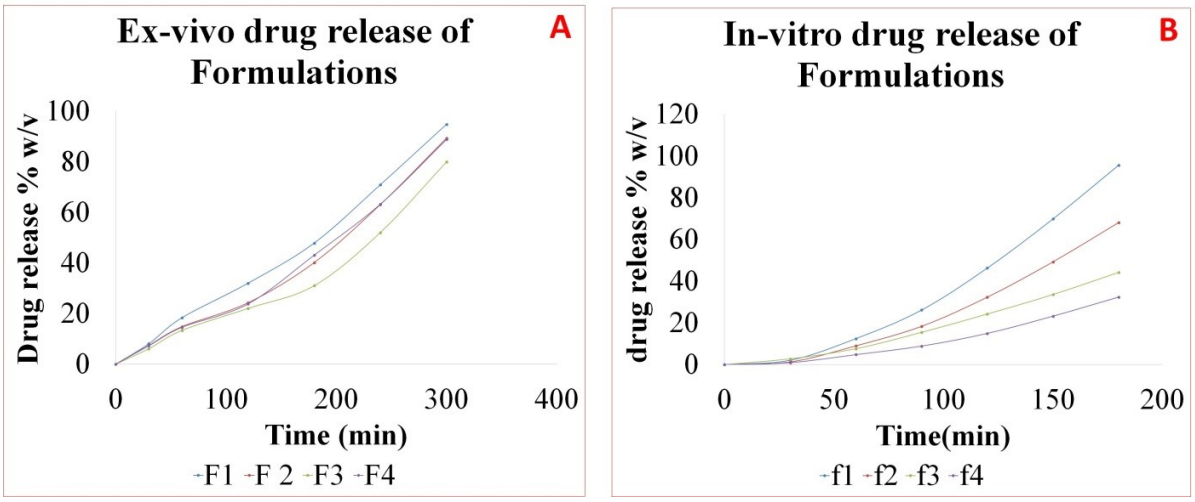

Figure 4: ***In vitro*** **and** ***Ex vivo*** **drug release of FSN Microemulsion**

4. CONCLUSION

Results of this study concluded that, S (mix) ratio Tween 80: PEG 400 (F1) is the suitable surfactant ratio for the formulation of FNS microemulsion. Future direction of this research is to perform clinical trials and commercialization of the prepared formulation.

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