WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 13, Issue 2, 495-509. Research article ISSN 2277–7105

FORMULATION AND EVALUATION OF MICROEMULSION FOR CO-ADMINISTRATION OF HYDROPHILIC AND HYDROPHOBIC ANTI-TUBERCULAR DRUGS

Triveni N. (B.Pharm)* and Deepthi Swapna P. R.

Department of Industrial Pharmacy, Aditya Bangalore Institute for Pharmacy Education and Research, Bangalore-560064.

Article Received on
25 Nov. 2023,

Revised on 16 Dec. 2023,
Accepted on 06 Jan. 2024

DOI: 10.20959/wjpr20232-30359

***Corresponding Author**
Triveni N. (B.Pharm)
Department of Industrial Pharmacy, Aditya Bangalore Institute for Pharmacy Education and Research, Bangalore-560064.

ABSTRACT

A microemulsion has been formulated for co-administration of hydrophobic (Rifampin) and hydrophilic (Isoniazid) anti-tubercular drugs were prepared through Oil in water phase to enhance the solubility of combination drugs. The O/W microemulsion were prepared by using Oleic acid, Tween 80 and Ethanol and Evaluated for characteristic, pH, Viscometer, drug content, Zeta potential, drug release and stability study.

KEYWORDS: Microemulsion, Hydrophilic and Lipophilic, Tween 80, Smix.

INTRODUCTION

Microemulsions are optically clear, thermodynamically stable, isotropic liquids. It is the mixture of oil, water and surfactant, often used in conjunction with the use of co-surfactants, typically with droplet sizes in the range of 10-100 nm. The microemulsion serves as a drug carrier for parenteral, topical and oral administration.Drug-resistant tuberculosis (DR-TB) continues to pose a threat to public health, most importantly resistance to the most effective first-line drug, rifampicin. Resistance to rifampicin and isoniazid is defines multidrug-resistant tuberculosis (MDR-TB).

The present study to enhance the solubility of combination drugs by using Isoniazid (hydrophilic) and Rifampin (hydrophobic) anti-tubercular drugs.

MATERIALS AND METHODS

i) Materials

Rifampin, Isoniazid was purchased and performed identification test through FTIR. Oleic acid, Tween 80, Ethanol was purchased from Aone Scientific Solutions.

ii) Microemulsion Preparation & Phase Behaviour

The Microemulsion consisting of oil (Oleic acid), surfactant (Tween 80), cosurfactant (Ethanol) and distilled water. The elucidation of the microstructure was carried out along a dilution line of constant oil–surfactant mixing ratio of 1:1, 1:2, 1:3 and the concentration of oil and water is varied and phase behaviour mapping of microemulsions as reported.

iii) Drug Incorporation in Microemulsion

For the preparation of drug loaded microemulsion formulations of 10 are prepared using (1:1) Smix ratios were selected from the single-phase region (microemulsion) of phase diagram and the drugs were incorporated. INH were dissolved into the pre-weighed hydrophilic component, whereas RIF was dissolved into the pre-weighed hydrophobic component of the system at a concentration of 1% (w/w) under stirring followed by the addition of remaining components.

RESULTS AND DISCUSSION

A. PREFORMULATION STUDIES

1. Organoleptic characteristics

The Organoleptic characteristics like general description and colour was determined and confirmed that drug samples are Rifampin and Isoniazid.

2. Solubility

a) Solubility of Rifampin

It is determined by dissolving 1 mg of Rifampin, transfer to a 10 ml test tube and add 10 ml in methanol shake well. The Rifampin is soluble in methanol.

b) Solubility of Isoniazid

Weigh 1mg of Isoniazid sample and transfer into 10 ml test tube and add 10 ml of water and shake well.

3. Identification of pure drug

Identification of Rifampin and Isoniazid was performed using FT-IR Spectrophotometry.

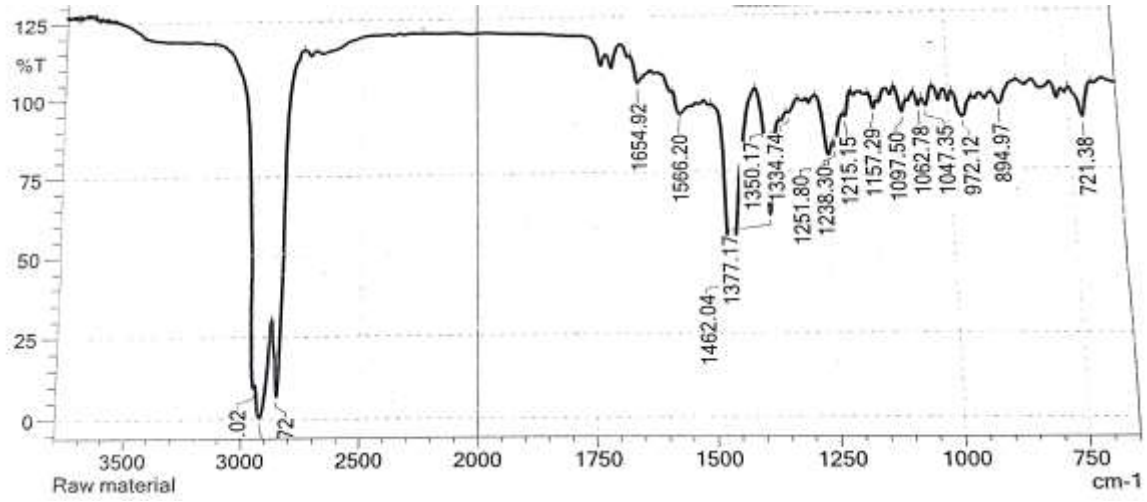

Figure 1: FTIR Spectra for Rifampin.

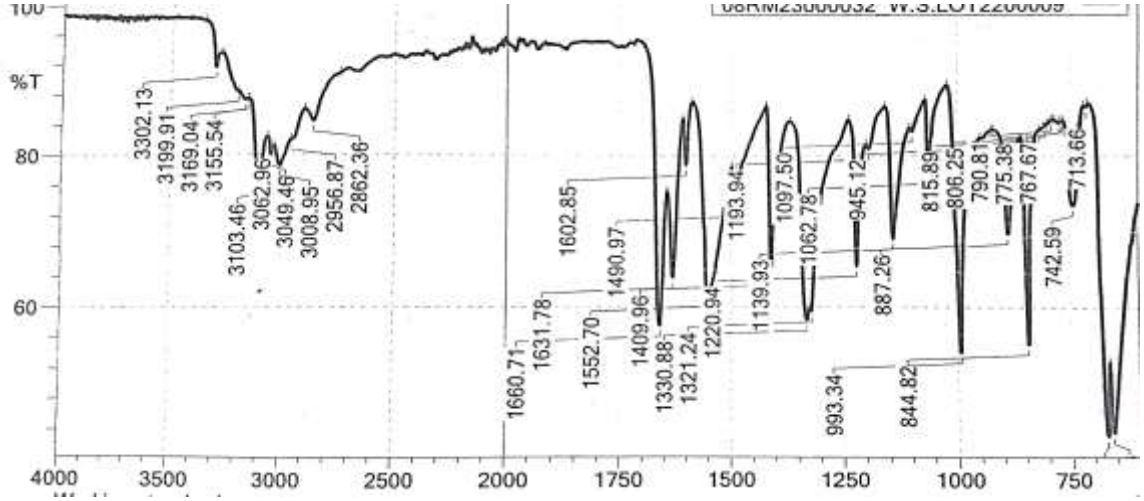

Figure 2: FTIR Spectra for Isoniazid.

4. Determination of melting point

The study was carried out and found that the drug melted at 145˚C for Rifampin and 172˚C for Isoniazid and indicated that the drug is pure.

5. Determination of absorbance maximum (λmax)

The λmax of Rifampin was determined in sodium hydroxide is scanned in the wavelength region of 200-400 nm and the λmax is found at 337 nm.

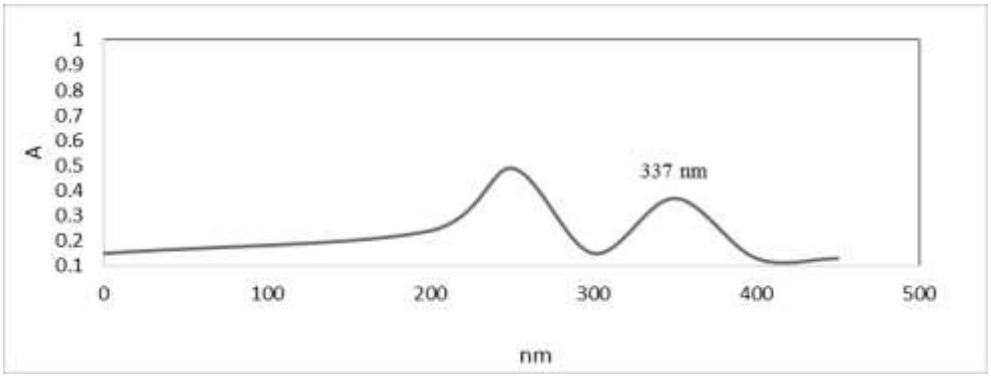

Figure 3: Absorption maxima of Rifampin.

The standard solution of each drug (25µgm/ml) in sodium hydroxide is scanned in the wavelength region of 200-400 nm and the λmax is found at 244 nm for Isoniazid which is significant for the pure drug.

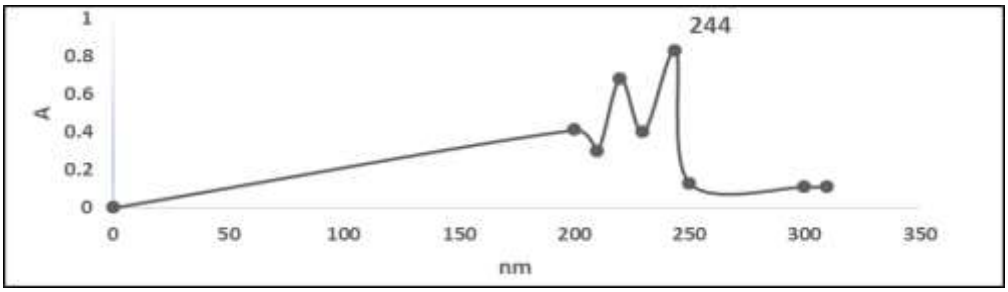

Figure 4: Absorption maxima of Isoniazid.

6. Compatibility study

The physical stability of the excipient with drug product needs to be determined. The drug is mixed with the components used in the formulation in 1:1 ratio. The sample mixture is then placed in two closed containers each at room temperature 25±1°C and accelerated temperatures 40±1°C for 4 weeks. The mixtures were then observed for any specific changes by visual observations.

Preparation of Microemulsion

Table no. 1: Preparation of Microemulsion.

Sl. No.	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
1.	Rifampin (mg)	10	10	10	10	10	10	10	10	10	10
2.	Isoniazid (mg)	10	10	10	10	10	10	10	10	10	10
3.	Oleic acid (%w/v)	7	9	5	8	4	7	5	6	7	11
4.	Smix (1:1) (Tween 80: Ethanol)	9	8	9	6	7	9	10	9	8	5
5.	Distilled water (%w/v)	4	3	6	6	9	4	5	5	5	4
Final Volume (%w/v)		20	20	20	20	20	20	20	20	20	20

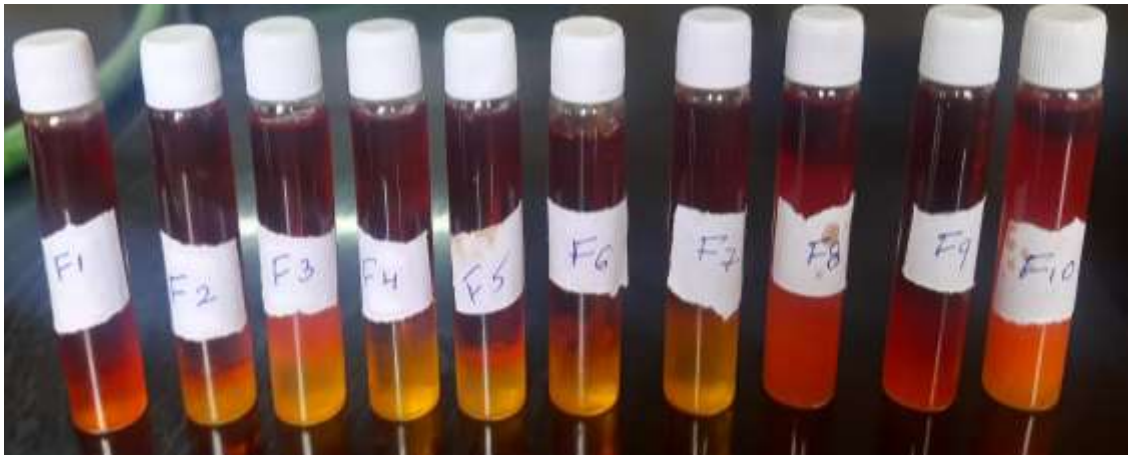

Figure 5: Preparation of Drug containing Microemulsion.

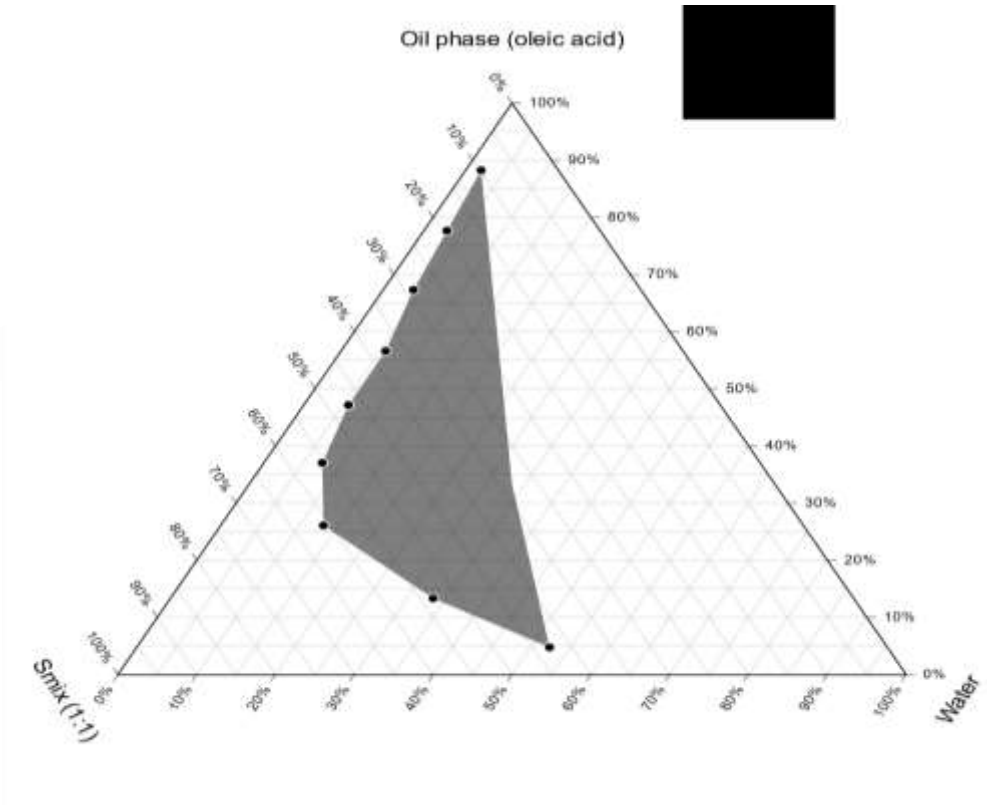

Figure 6: Pseudo Ternary Phase Diagram of Smix (1:1).

B. EVALUATION TEST FOR MICROEMULSIONS CONTAINING RIFAMPIN AND ISONIAZID

1) Optical Transparency

The Optical transparency of the formulation was determined by examining the sample in clear and transparent container under the presence of good light suitable for eye reflection and observing against a black and white illuminated background.

Table No. 2: Optical Transparency.

Sl. No.	Formulation	Appearance
1.	F1	Clear
2.	F2	Clear
3.	F3	Red Opalescent
4.	F4	Clear
5.	F5	Clear
6.	F6	Clear
7.	F7	Clear
8.	F8	Red Opalescent
9.	F9	Clear
10.	F10	Red Opalescent

2) pH

The pH values are measured for microemulsion F1-F10 using a pH meter of a glass electrode. pH essentially indicates the level of hydrogen ion activity in a solution.

A certain amount of the formulation was taken and diluted with calibrated distilled water and mixed well. To measure the pH value, the electrode of the pH meter was immersed in the prepared formulation.

Table No. 3: pH values.

Sl. No.	Formulation	pH
1.	F1	7.0
2.	F2	7.2
3.	F3	7.1
4.	F4	6.8
5.	F5	6.6
6.	F6	6.8
7.	F7	6.9
8.	F8	7.0
9.	F9	6.7
10.	F10	7.0

3) Viscosity

The viscosity measurements of drug-loaded microemulsions were determined by using Brookfield viscometer. Accurately weighed 10 g of microemulsion was transferred to 50 ml glass beaker. Spindle no 6 was selected and it is immersed into the emulsion. The viscometer was operated at 10 rpm until the reading gets stabilized and reading was recorded in centipoise. According to the literature, the viscosity of the formulation after gelation should be between 50 – 50,000 cps.

Table No. 4: Viscosity values.

Sl. No.	Formulation	Viscosity (cps)
1.	F1	75.3±0.8
2.	F2	80.2±0.4
3.	F3	103.5±05
4.	F4	85.2±0.7
5.	F5	88.2±0.3
6.	F6	98.2±0.4
7.	F7	95.2±0.7
8.	F8	78.2±0.6
9.	F9	91.4±04
10.	F10	80.2±0.8

All samples exhibited Newtonian flow behaviour, as expected from microemulsions. It could be noted that the viscosity values tended to increase slightly when the water concentrations increased or when the system turned into oil/water type because oil/water microemulsions have higher viscosities than those of water/oil systems.

4) Centrifugation

Table No. 5: Mechanical stress.

Sl. No.	Centrifugation Time (min)	% Phase Separation									
		F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
1.	10	-	-	-	-	-	1	1	2	-	2
2.	20	-	6	2	5	4	4	5	5	3	3
3.	40	-	10	3	7	8	8	8	10	4	10

The chemical and physical stability of microemulsion containing Rifampin and Isoniazid was evaluated using phase separation with mechanical stress study.

The 10 microemulsion formulations (F1-F10) were centrifuged (Remi centrifuge) at 2000 rpm for different time intervals (10 min, 20 min, and 40 min) and the amount of phase separation of the formulations were reported.

The F1 Microemulsions are not having any phase separation and F3, F9 are having less phase separation when compared to the other microemulsions.

5) Analysis of Drug content

1ml of microemulsion formulations were transferred to a beaker containing 10 ml methanol. The content of the beaker was stirred for 30 minutes and then held for 24hr. After 24hr the contents of the beaker were transferred to a centrifuge tube and centrifuged at the 2000 rpm

for 10 minutes. The Supernatant was separated and filtered. Then, 0.1 ml of the supernatant was appropriately diluted with Phosphate Buffer Saline (PBS) pH 7.4 and active ingredient content was determined Spectrophotometrically.

Table No. 6: Drug Content in Microemulsion.

Sl. No.	Formulation	Drug Content of RIF (%)	Drug Content of INH (%)
1.	F1	91.25±0.08	92.75±0.12
2.	F3	84.10±0.15	80.10±0.08
3.	F9	86.78±0.25	88.13±0.35

The drug content analysis for microemulsion were performed by using phosphate buffer (7.4) and the good results are obtained for F1 microemulsion for Rifampin is 91.25% and Isoniazid is 92.75%

6) Zeta Potential Determination

The microemulsion 1ml was dispersed in a disposable zeta cell. Palladium electrode was inserted in distilled water in the cuvette and the analysis was performed at 25°C.

The measurements were done in triplicate and average value was reported, which measures the distribution of the electrophoretic mobility of particles using the laser Doppler velocity technique. The analyser calculated the zeta potential from the measured velocity using the Smoluchowski equation.

Table No. 7: Evaluation of Microemulsion.

Sr. No.	Formulation	Globule Size (nm)	Poly Dispersity Index (PDI)	Zeta Potential (mV)
1.	F1	175.1	0.191	-3.62 mV
2.	F3	91.26	0.169	-3.51 mV
3.	F9	152	0.431	-3.75 mV

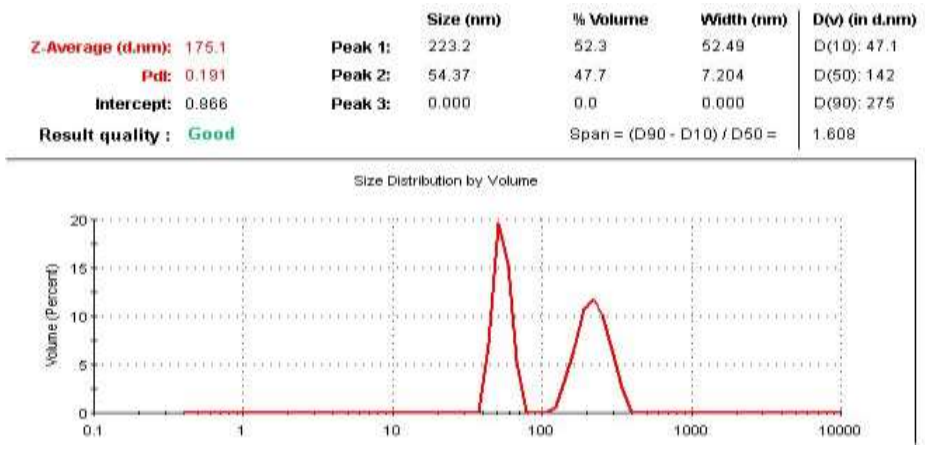

Figure 7: Globule size of F1 Microemulsion by Malvern Zeta Sizer.

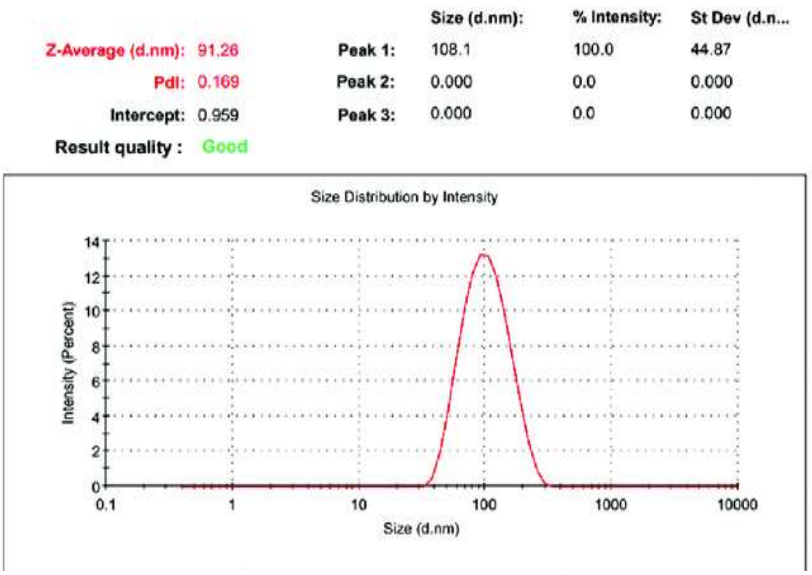

Figure 8: Globule size of F3 Microemulsion by Malvern Zeta Sizer.

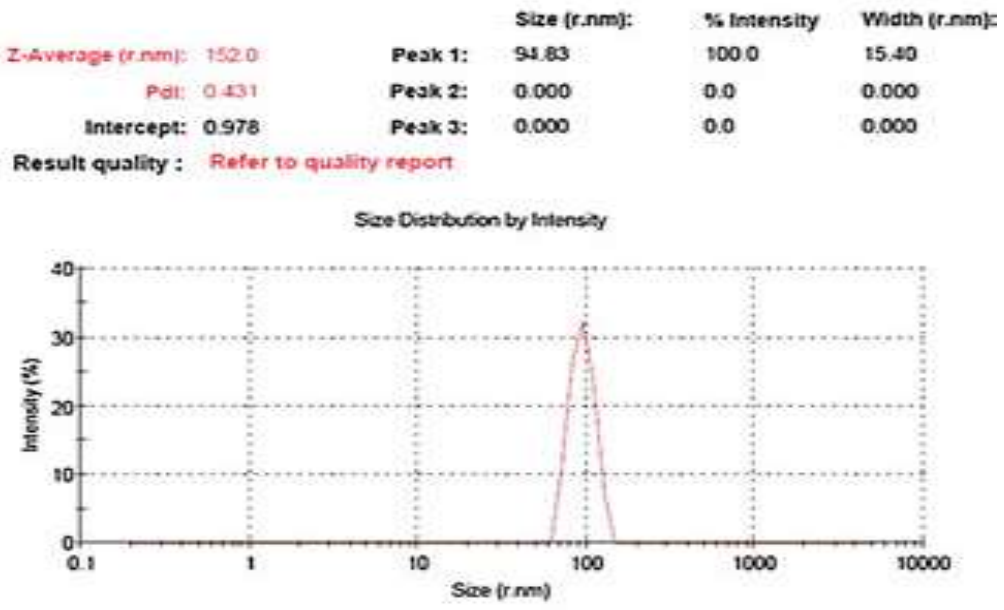

Figure 9: Globule size of F9 Microemulsion by Malvern Zeta Sizer.

Particle size analysis of microemulsion globules was determined using Malvern zeta sizer instrument. The results show in the Table No. 7 and Figure 7, 8 & 9 shows that the globule size in microemulsion formulation F1, F3, F9 that is 175.1nm, 91.26nm and 152nm, from which F2 was lesser than other formulation (Figure 8). It was found that the average particle size of F3 was approximately 91.26nm.

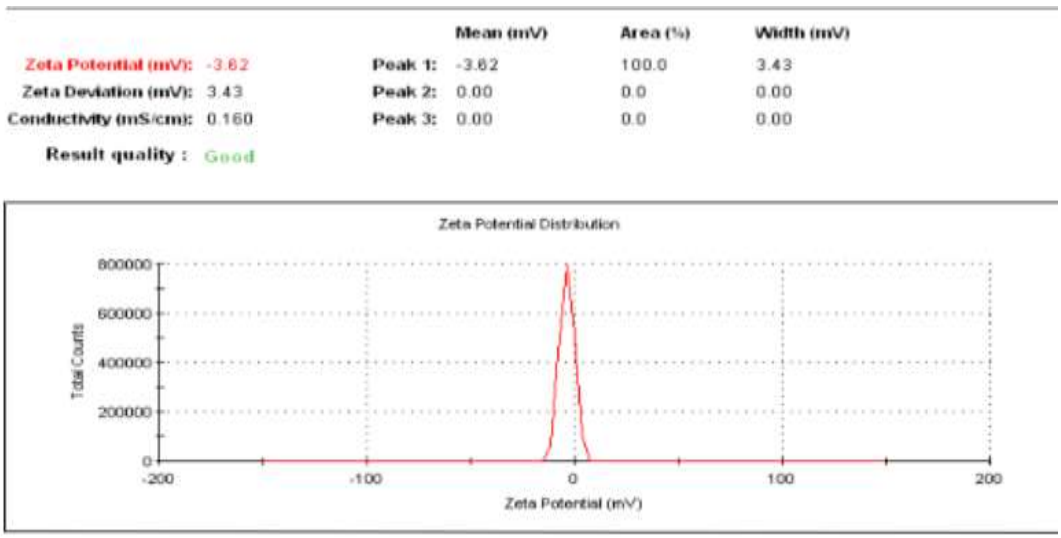

Figure 10: Zeta Potential of Microemulsion by Malvern Zeta Sizer.

The zeta potential represents an index for globule size of microemulsion stability.

The results show in the table No. 7 and figure 10. This stability is important in preventing aggregation of particles. The zeta potential of the optimized formulation was found to be -3.62 milli volt (mV). Higher the zeta potential maximum is the stability of microemulsion.

7) Dissolution Studies

The microemulsion is adsorbed on to the solid particle adsorbent by using colloidal silica and appeared as slightly damp and filled in to the hard gelatin capsule. The dissolution medium was phosphate buffer (pH 7.4) was choosen and the basket dissolution apparatus USP-I is selected. For a medium volume of 900ml, a basket speed of 50 rpm was chosen. The medium was maintained at 37 ± 0.5°C. The dissolution vessels were covered to minimize evaporation. At regular intervals, 5 ml aliquots were collected and the same amount of fresh dissolution medium was replaced into dissolution vessel to maintain the sink condition throughout the experiment. The collected aliquots were filtered using Whatman filter No. 1, and further diluted suitably to analyse using UV method at λmax.

Table No. 8: *in- vitro* drug dissolution data of microemulsion (F1, F3, F9).

Time (mins)	% cumulative drug release					
	F1		F3		F9	
	Rifampin	Isoniazid	Rifampin	Isoniazid	Rifampin	Isoniazid
0	0	0	0	0	0	0
15	7.89	26.70	5.45	20.12	6.21	18.57
30	15.45	33.56	10.75	28.33	12.12	26.70
45	20.46	63.12	15.21	57.25	16.17	57.12
60	26.75	80.25	22.15	65.68	21.85	65.25

90	32.45	85.52	30.12	77.54	28.92	75.52
120	46.21	90.12	38.62	85.14	40.12	86.12
150	54.51	90.21	45.12	85.25	50.12	86.67
180	65.65	90.15	55.45	85.66	66.12	86.71
210	76.65	90.12	65.12	85.28	75.15	86.78
240	88.12	91.02	80.14	85.12	86.12	86.81

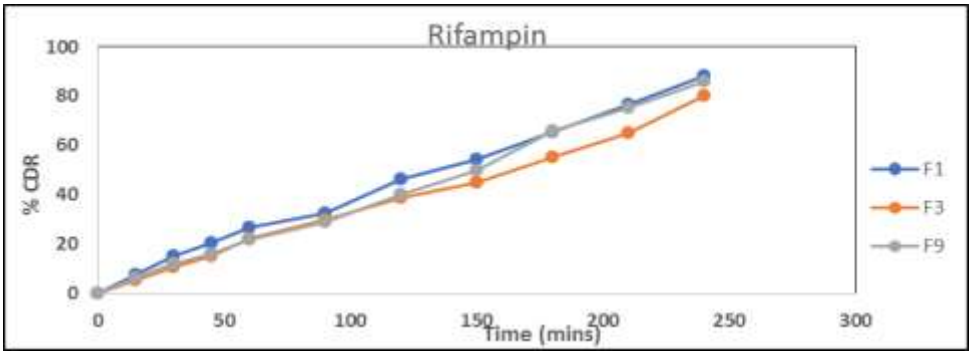

Figure 11: In-vitro drug release profile of Rifampin (F1, F3, F9).

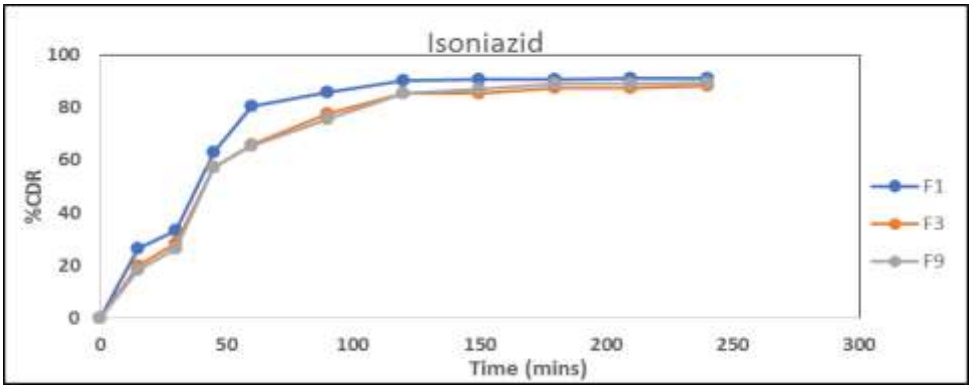

Figure 12: In-vitro drug release profile of Isoniazid (F1, F3, F9).

In-vitro drug dissolution profiles obtained for all formulations (F1, F3, F9) were shown in Table No.8 and Figure 11 and 12. The cumulative percent drug release after 4 hours was found formulation F1, F3 and F9 respectively.

8) Kinetic modelling of drug release profile

The % Drug release from the Rifampin and Isoniazid microemulsions at different time intervals were fitted to zero order kinetics and first order kinetics model, Higuchi model and Korsemeyer-Pappas model to characterize mechanism of drug release. Coefficient of correlation values were calculated for the linear curves obtained by regression analysis of the plots.

The In vitro drug release data was subjected to goodness of fit test by linear regression analysis according to zero order and first order kinetic models in order to determine the mechanism of drug release.

When the regression coefficient values of zero order and first order plots were compared, it was observed that 'R2' values for Rifampin of first order plots were in range of 0.8848 to 0.9157 and zero order plots were in range of 0.9933 to 0.9972 indicating drug release from most of the formulations was found to follows zero order kinetics and for Isoniazid of Korsmeyer peppas were in range of 0.8277 to 0.9033 drug release were shows the zero order and first order plots.

9) Stability studies

Table No. 9: Stability Study of microemulsion.

Sr. No.	Evaluation parameter	Time (Days) accelerated condition at 30±2˚C & 65±5%RH for 90 days									
		Before storage		0th day		30th day		60th day		90th Day	
		RIF	INH	RIF	INH	RIF	INH	RIF	INH	RIF	INH
1.	Drug Content	91.25	92.75	91.25	92.75	90.81	91.12	90.02	90.81	89.12	89.69
2.	Appearance	Clear		Clear		Clear		Clear		Clear	
3.	pH	7.0		6.8		6.9		6.6		6.4	
4.	Viscosity	75.3±0.8		72.2±0.4		73.8±0.7		74.8±0.6		72.4±0.6	

The results of the stability studies indicated that the microemulsions did not show any changes in the appearance, drug content, pH, viscosity during the stability study period of 3 month.

CONCLUSION

The study demonstrates that microemulsion formulations can be used to improve the solubility of the poorly water-soluble drugs. Oleic acid was chosen as the vehicle for the aqueous phase of the microemulsion because it consumes the greatest amount of Rifampin (hydrophobic) and Isoniazid (hydrophilic). Tween80 and ethanol in appropriate ratios were selected as an ideal surfactants and co-surfactants.

A Preformulation study was conducted on the properties and solubility of microemulsion containing Rifampin and Isoniazid. Compliance with melting point and limit values specified in the literature. FTIR spectra showed no interaction between Rifampin and Isoniazid. Based on the pseudo-ternary phase diagram of Smix (1:1) and surface presence of microemulsions and characterized by optical transparency, pH, Viscosity and Centrifugation for F1-F10

formulation containing Smix (1:1). The evaluation studied for 3 formulations based on the centrifugation result F1, F3, F9 are selected based on low phase separation of microemulsions, Drug content, zeta potential, dissolution study and stability study for 90 days. Among the selected microemulsion F1 shows optimum incorporation of rifampin and isoniazid in the preparation and remained stable, clear and no phase separation in the microemulsion. Their stability was demonstrated after being exposed centrifugation stress tests. Their viscosity and droplet size indicate that they are suitability for oral delivery systems and their pH values are within the physiological range. The active ingredients content of the formulation revealed that the rifampin content was 91.25±0.08 and Isoniazid content was 92.75±0.12. This study highlighted that the efficacy of Rifampin follows zero order kinetics and Isoniazid formulations follows the korsemeyer-peppas model and is non-fickian (anomalous). In-vitro drug release studies showed that the amount of Rifampin and Isoniazid from the formulation F1 exhibited favourable release rates compared to other formulations. The desired drug release for rifampin was about 88.12 % after 4 hours and the desired drug release for isoniazid about 90.12% after 2 hours. Stability studies were conducted for the best formulation F1. The results of studies showed no significant changes, suggesting that the formulation is stable.

Based on the physico-chemical and spectroscopic analysis, concluded that the microemulsion containing Rifampin and Isoniazid can be used for the oral drug delivery for the treatment of Tuberculosis.

ACKNOWLEDGEMENT

I am immensely thankful to chairman, Dr. B.A. Vishwanath sir for providing the facilities to carry on the academic research in the institution and guidance of Mrs. Deepthi Swapna P.R M.Pharm (Ph.D.) Dep. of Industrial Pharmacy, Aditya Bangalore Institute for Pharmacy Education and Research, Bangalore, for their valuable guidance, inspiration and constructive criticism throughout my research work.

REFERENCES

1. Gurpreet Kaur, SK Mehta, Sandeep Kumar, Gaurav Bhanjana, Neeraj Hibachi. Coencapsulation of hydrophobic and hydrophilic antituberculosis drugs in synergistic Brij 96 microemulsions: a biophysical characterization. journal of Pharmaceutical Sciences, 2015; 104(7): 2203-2212.
2. Malathi, S., and S. Balasubramanian. “PLGA Nanoparticles for Anti Tuberculosis Drug

Delivery." Advanced Materials Research Oct., 2012; 584: 465–469.

3. Malathi, S., and S. Balasubramanian. "PLGA Nanoparticles for Anti Tuberculosis Drug Delivery." Advanced Materials Research Oct., 2012; 584: 465–469.
4. Mehta S K, Kaur G, Bhasin KK. Tween-embedded microemulsions--physicochemical and spectroscopic analysis for antitubercular drugs. AAPS Pharm SciTech, Mar., 2010; 11(1): 143-53. doi: 10.1208/s12249-009-9356-5
5. Buya, A.B.; Witika, B.A.; Bapolisi, A.M.; Mwila, C.; Mukubwa, G.K.; Memvanga, P.B.; Makoni, P.A.; Nkanga, C.I. Application of Lipid-Based Nanocarriers for Antitubercular Drug Delivery: A Review. Pharmaceutics, 2021; 13: 2041.
6. Raouf Bhat, Farooq Ahmad Wani, Kamalakanta Behera, Abbul Bashar Khan, Rajan Patel, Formulation of biocompatible microemulsions for encapsulation of anti-TB drug rifampicin: A physicochemical and spectroscopic study, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2022; 645: 128846. ISSN 0927-7757, https://doi.org/10.1016/j.colsurfa.2022.128846.
7. Kaur Gurpreet, Assessment of Brij 96 embedded microemulsions as carrier for anti-tuberculosis drug rifampicin. American Scientific Publishers, 2014; 3: 18-22(5).
8. Kaur G, Chiappisi L, Prevost S, Schweins R, Gradzielski M, Mehta, Surinder K Mehta, Probing the microstructure of nonionic microemulsions with ethyl oleate by viscosity, ROESY, DLS, SANS, and cyclic voltammetry. ACS publication, 2012; 28: 10640–10652.
9. Ryo Maeda, Tatsuya Ito, Tatsuaki Tagami, Takemasa Takii, Tetsuya Ozeki. Development of dried emulsion/mannitol composite microparticles through a unique spray nozzle for efficient delivery of hydrophilic anti-tuberculosis drug against alveolar macrophages. Biological and Pharmaceutical Bulletin, 2019; 42(11): 1846-1853.
10. Mehanna, M.M, Mohyeldin, S.M, Elgindy, N.A. Respirable nanocarriers as a promising strategy for antitubercular drug delivery. J. Control. Release, 2014; 187: 183–197.
11. Kumar, V., Abbas, A.K., Fausto, N., Mitchell, R.N. Robbins Basic Pathology. 8th ed.; Saunders Elsevier: Philadelphia, PA, USA, 2007; 516–522.
12. Kumar, N., Kumar, P.; Kumar, M.; Kumar, R. Nanotechnology: A focus on treatment of Tuberculosis. Int. J. Drug Deliv, 2011; 3: 25–42.
13. Eleraky N.E, Allam A. Hassan, Omar S.B., M.M. Nanomedicine Fight against Antibacterial Resistance: An Overview of the Recent Pharmaceutical Innovations. Pharmaceutics, 2020; 12(2): 142.
14. Bysu Sujatha, Himabindu E., Sowjanya Bttu, Abbulu Konde. Microemulsions-A review. Journal of Pharmaceutical Sciences and research., 2020; 12(6): 750-753.

15. Schulman, J.H, Stoeckenius, W Prince, L.M. Mechanism of Formation and Structure of Microemulsions by Electron Microscopy. ACS publication, 1959; 63(10): 1677-1680.