FORMULATION AND EVALUATION OF PREDNISOLONE ACETATE MICRO EMULSIONS.

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KEYWORDS
Microemulsion, Prednisolone acetate, Solubility enhancement, Drug delivery, Controlled release, Stability studies.

ABSTRACT

Aim:
The present study aims to formulate and evaluate a microemulsion-based drug delivery system for Prednisolone acetate, a poorly water-soluble corticosteroid. The microemulsion system is designed to enhance the solubility, stability, and bioavailability of the drug for potential usage.

Methods:
Microemulsions were prepared using oil, surfactant and co-surfactant. The formulations were optimized using pseudo-ternary phase diagrams to identify the most stable composition. The prepared microemulsions were evaluated for physicochemical properties, viscosity, pH, and drug content. In vitro drug release was studied using a dialysis membrane, and kinetic modeling was applied to determine the release mechanism.

Results:
The optimized microemulsion formulation exhibited a transparent appearance, droplet size (295 nm), PDI (0.103), and zeta potential (-43mV), indicating stability. Drug content was found to be above 95%, with a controlled drug release profile suggesting a diffusion-based release mechanism.

Conclusion:
The formulated Prednisolone acetate microemulsion showed enhanced solubility, stability, and controlled drug release, making it a promising nanocarrier for improved drug delivery. This study highlights the potential of microemulsion technology in enhancing the therapeutic efficacy of poorly soluble drugs.

1.0 Introduction

Nowadays, pharmaceutical research is primarily focused on developing targeted drug delivery systems due to their inherent advantages, such as reduced drug dosage and an improved therapeutic benefit-risk ratio[1-3]. Targeted drug delivery involves precisely transporting the drug to the intended site at the right time and optimal dosage, regardless of the route or method of administration[4-6].

Fluid, optically clear, and thermodynamically stable, microemulsions are composed of four component mixtures: an oil phase, a water phase, a surfactant, and a co-surfactant[7–10]. A thermodynamically stable, optically transparent or translucent, low viscous, and isotropic microemulsion is created when a mixture of surfactant and co-surfactant is introduced to a biphasic system[11–14].

2.0 Materials and Methods

Prednisolone procured from Balaji Drugs, Gujarat, India. Tween 80 from S.R. Scientific and Surgicals, Hanamkonda, India. Tween 60 Chemisol Centre, Bangalore, India. Tween 20 Chemisol Centre, Bangalore, India. Cremophor RH40 Zeel

Pharmaceuticals, Mumbai, India. Ethanol Savvy Enterprise (Himedia), Secunderabad, India. Polyethylene glycol (PEG 400) S.R. Scientific and Surgicals, Hanamkonda,

2.1 METHODS

2.1.1 PREFORMULATION STUDIES[15]

This is an initial step to process the drug formulation. It includes the drug and excipient characterization which helps in better optimization of the formulation.

2.1.1.1 Detection of Wavelength

10 ml of ethanol was taken and into it 10 mg of drug was added results in producing 100 µg/ml. From this 10 µg/ml was made and scanned in the wave length range of 200-400 nm using ethanol as blank.

2.1.1.2 Solubility of drug in different solid lipids

1 g of solid lipid was taken and melted to its melting point. In to the melted lipid, drug was added until solubilized and checked for quantity dissolved.

2.1.1.3 Solubility of drug in different solvents and buffers

The excess quantity of drug was added to 10 ml of different solvents, pH 6, 6.4, 7, 7.4 phosphate buffers, ethanol and double distilled water in a 25 ml volumetric flask. Then flasks were properly capped and agitated at 37 ± 0.5°C in mechanical shaker for 48 hrs. Then samples were diluted using suitable diluents and again filtered using 0.45 µm membrane filters. Then samples were measured at 250 nm using UV-Vis spectroscopy.

2.1.1.4 Solubility of drug in various surfactant mixtures

Specified concentrations of Surfactant and co-surfactant ratios were taken inorder to solubilize the drug. Excess amount of PA was added to surfactant mixture. The mixtures were vortexed on isothermal shaker at 50 rpm for 2 days at 37°C to enhance solubilization. Quantity of PA was analysed at 250 nm.

2.1.1.5 Standard Calibration Curve of Prednisolone Acetate

- **Stock Solution Preparation:** 1000 µg/ml concentration of stock solution was prepared by making up with pH 6.4 buffer.
- **Preparation of Linearity Plot**

From the above stock solution 0.5-2.5 ml were pipetted out, to produce 5-25 µg/ml concentrations respectively. Standard plot was constructed by taking concentration on x-axis and absorbance on y-axis.

2.1.2 FORMULATION STUDIES

2.1.2.1 Pseudoternary Phase Diagram Construction[16]

In order to construct phase diagrams hot water titration method was used. The surfactant and cosurfactant were blended in the ratios from 1:1 to 4:1. Solid lipids were utilized for this study. 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 (%w/w) ratios were used in order to form pseudo ternary diagram. Micro emulsions were named under these points.

2.1.2.2 Development of Drug Loaded Microemulsions[17-18]

From the results of the highest area, lipid-S_{mix} combination of phase diagram was selected. To the fixed quantity of hot S_{mix} and water 10 mg of PA was added under stirring. If the melt appears clear it was categorized as micro emulsion.

2.1.3 Characterization of PA MICRO EMULSION[19-20]

2.1.3.1 pH determination: By immersing the electrode directly into the micro emulsion using a digital pH meter at 25°C the apparent pH of all micro emulsions were determined.

2.1.3.2 Viscosity measurement: Brookfield viscometer at 25°C at 60 rpm by LV spindles no. 63 viscosity was identified.

2.1.3.3 Determination of Drug Content: The drug content of the ME formulation was determined by dissolving 1 ml consisting of 10 mg drug in 10ml of methanol. After suitable dilutions with methanol, absorbance was determined using the UV spectrophotometer at wavelength 250 nm.

2.1.3.4 *In-vitro* drug release: Franz diffusion cell was used to study diffusion. The receptor compartment consists of 20 ml of Phosphate buffer (pH 6.4). Prednisolone Acetate microemulsion formulation (equivalent to 5 mg of drug) is present in the donor compartment. At fixed time intervals samples were withdrawn from receptor compartment and analyzed for drug content.

2.1.3.5 Zeta potential and polydispersity index

Zeta potential, polydispersity index of optimized formulation was measured by dynamic light scattering by diluting l ml of ME with 10 ml distilled water.

3.0 Results and Discussion

3.1 PREFORMULATION STUDIES

Table 1: Physicochemical Properties of Drug

Drug	**Prednisolone Acetate**
Nature	Solid Crystalline Powder
Color	Colorless
Odor	Odorless
Melting point	240-248^{0}c
Water solubility	0.25mg/ml

3.2 Determination of λ_{max}:

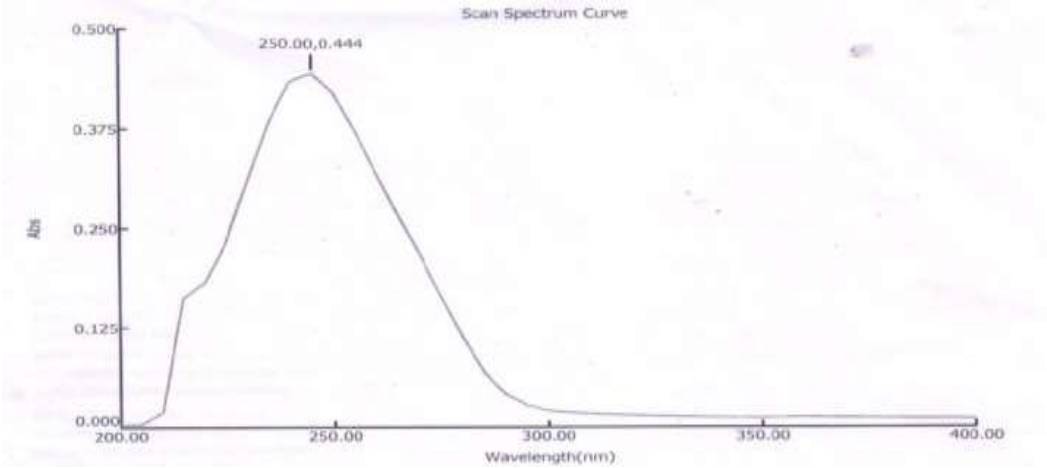

Fig 1: Wavelength of the drug at 250nm

The scanning studies revealed an absorption wavelength of 250 nm, which confirmed the presence of Prednisolone acetate. This wavelength is consistent with the known UV absorption characteristics of PA.

3.3 FTIR:

From the FTIR it was found that the drug is Predisolone Acetate

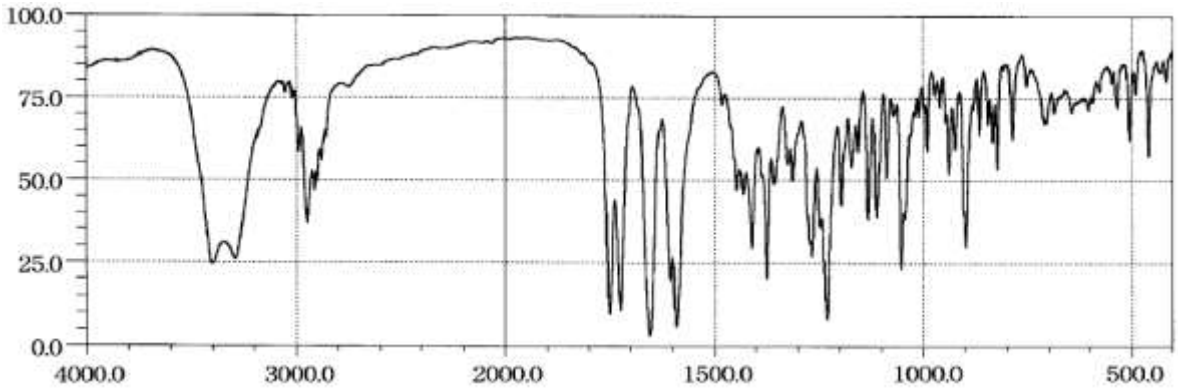

Fig 2: FTIR of PA

Table 2: FTIR data of Prednisolone Acetate

S. No.	Functional group	Functional group frequencies of drug as per IP standard (cm^{-1})	Functional group frequencies of procured drug (cm^{-1})
1	C=O stretching (Ketone, Ester)	1725 (s) 1700 (s)	1725.42 (s), 1702.92 (s).
2	C-O stretching (Alcohol)	1240 (s)	1243.64 (s).
3	C-O stretching (Alcohol)	1100 (s)	1102.15 (s)
4	C-O stretching (Ester)	1160 (s)	1153.38 (s)
5	C-O stretching (Ester)	1130 (s)	1128.53 (s)

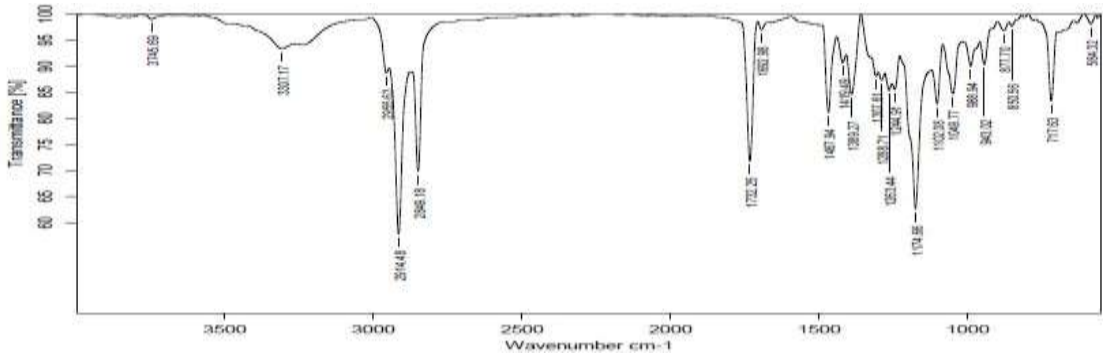

Fig: 3 FTIR of optimized Microemulsion

Table 3: Intrepretation of IR

S NO	FUNCTIONAL GROUP	OPTIMIZED FORMULATION
1	C=O	1736.2
2	C-O	1242.5
3	C-O(ALCHOL)	1104.2
4	C-O(ESTER)	1176.2
5	O-H(ACHOL)	1264.7

3.4 DSC:

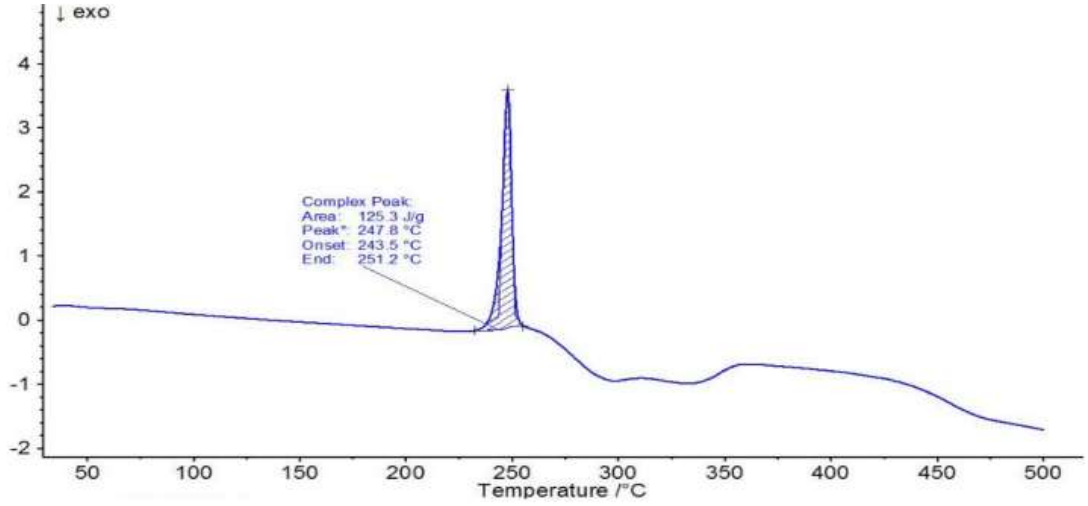

Fig 3: DSC of Prednisolone Acetate Pure drug

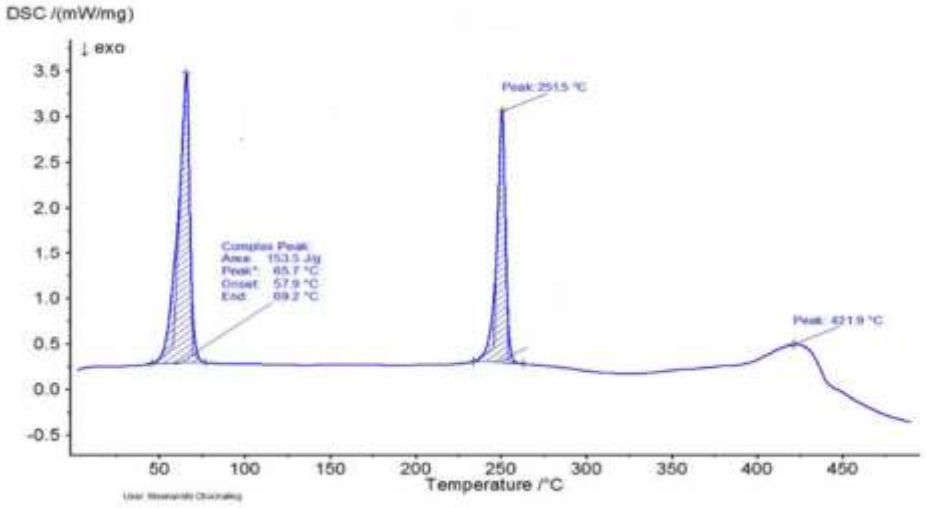

Fig 4: DSC of Optimized Formulation

The thermogram indicated that the drug was a crystalline compound, exhibiting a sharp endothermic peak at 247.8°C, which corresponds closely to its melting point of 242.2°C. Based on these thermal characteristics, the API was identified as Prednisolone acetate.

3.5 Solubility of drug in various solid lipids:

Table 4: Solubility of drug in different Lipids

Solid Lipid	**Amount (mg/gm)[a]**
Palmitic Acid	125.3±2.2
Stearic Acid	129.1±1.4
GMS	138.2±0.5
Cetyl Palmitate	136.1±1.7
Tripalmitin	142.3±0.9
Tristearin	147.5±0.6

A Mean±S.D., n=3

From the above studies, it was proved that PA has good solubility in Tristearin, when compared to other lipids selected for solubility studies.

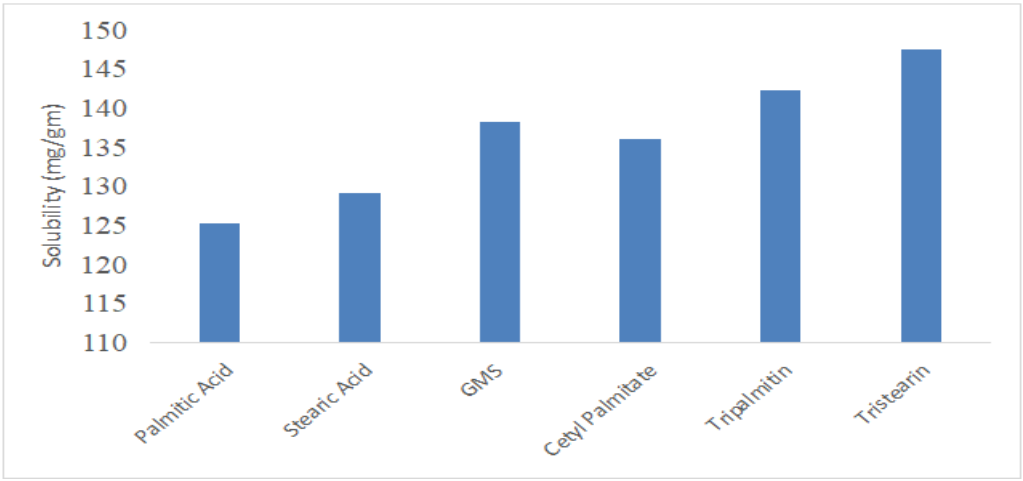

Fig 5: Comparison of solubility of drug in various solid lipids

3.6 Solubility of drug in various solvents and buffers

Table 5: Solubility of drug in various solvents and buffers

Name of the Solvent	Solubility(mg/ml)[a]
Ethanol	23.3±0.12
Water	0.19±0.11
SNES(pH)	5.99±0.26
pH 6.0	3.06±0.11
pH 6.4	3.33±0.14
pH 7.0	2.14±0.07
pH 7.4	1.87±0.09

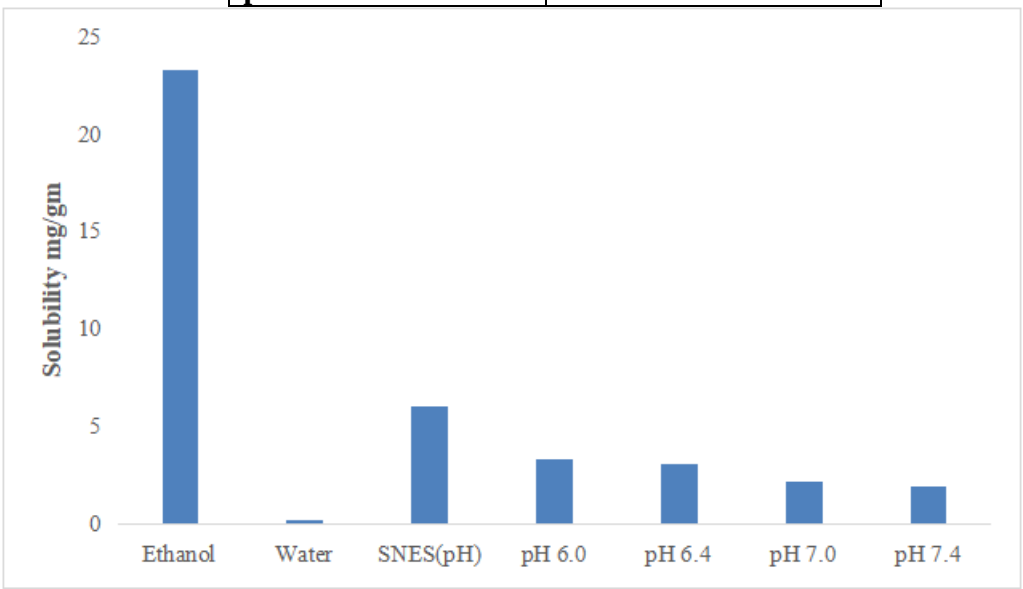

Fig 6: Solubility of PA in various solvents and Buffers

The results showed that the drug exhibited low solubility in water but high solubility in ethanol. Additionally, in terms of pH-dependent solubility, as the pH increased, the solubility of the drug decreased.

3.7 Solubility of drug in various surfactant mixtures

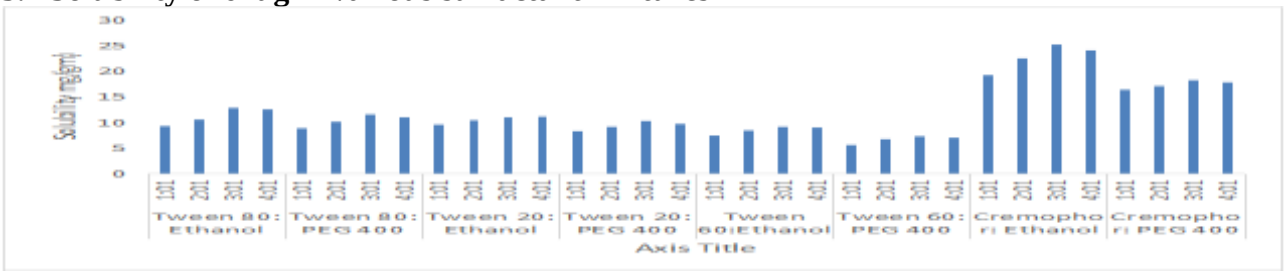

Fig 7: Solubility of drug in various surfactant mixture

Table 6: Solubility of drug in Surfactant Mixture

Name of the surfactant Mixture	Ratio of surfactant Mixture	Solubility(mg/gm)
Tween 80: Ethanol	1:1	9.33±0.25
	2:1	10.6±0.39
	3:1	12.8±0.12
	4:1	12.6±0.44
Tween 80: PEG 400	1:1	8.9±0.1
	2:1	10.2±0.05
	3:1	11.56±0.02
	4:1	11.03±0.36
Tween 20: Ethanol	1:1	9.57±0.1
	2:1	10.4±0.15
	3:1	11.01±0.11
	4:1	11.1±0.06
Tween 20: PEG 400	1:1	8.3±0.1
	2:1	9.1±0.5
	3:1	10.3±0.2
	4:1	9.7±0.04
Tween 60:Ethanol	1:1	7.5±0.07
	2:1	8.5±0.36
	3:1	9.1±0.12
	4:1	9.0±0.4
Tween 60: PEG 400	1:1	5.6±0.33
	2:1	6.8±0.15
	3:1	7.3±0.6
	4:1	7.1±0.12
Cremophor: Ethanol	1:1	19.2±0.1
	2:1	22.5±0.40
	3:1	25.2±0.33
	4:1	24.1±0.15
Cremophor: PEG 400	1:1	16.4±0.17
	2:1	17.1±0.6
	3:1	18.2±0.1
	4:1	17.9±0.04

The solubility of the drug in various surfactant mixture ratios was studied and presented in Table 5 and Figure 6. The results indicated that as the surfactant mixture

ratio increased from 1:1 to 3:1, the drug's solubility also increased. Hence, the range from 1:1 to 3:1 was selected.

3.8 Construction of Calibration Curve

Table 7: Linearity curve values of PA in pH6.4

S.No	Concentration (µg/ml)	Absorbance (±SD)
1	0	0
2	5	0.199±0.021
3	10	0.399±0.35
4	15	0.799±0.15
5	20	0.887±0.07
6	25	0.995±0.47

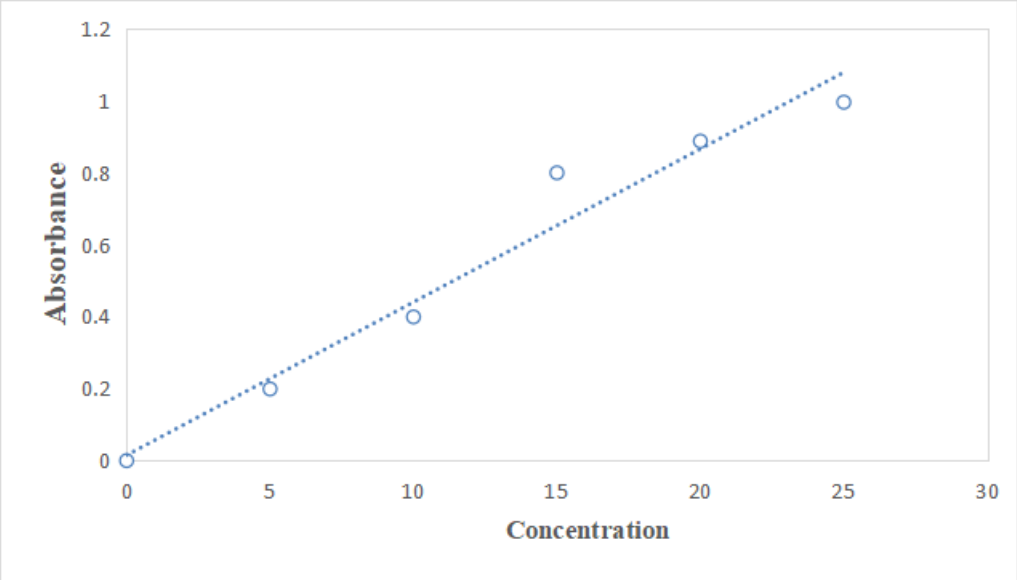

Fig 8: Calibration curve

Table 6 and Figure 7 display the calibration curve along with its corresponding values. An R^2 value of 0.9997 demonstrates a highly linear relationship, confirming the curve's accuracy within the 5-25 µg/ml concentration range.

3.9 Development of Drug Loaded Microemulsions

The phase diagram showed that as the S_{mix} concentration increased, the ME region expanded from a 1:1 to a 3:1 ratio. However, a concentration of 4:1 resulted in unfavorable outcomes. As a result, a S_{mix} ratio of 3:1 (Cremophor and ethanol) was selected, and the lipid composition was adjusted during the formulation development.

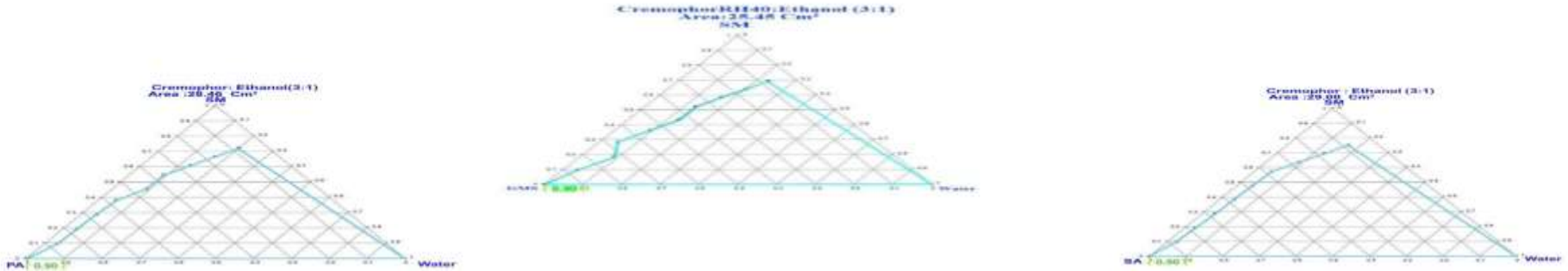

Fig 9: The ternary phase diagrams of ME consisted of PT, GMS, SA / cremophor RH40/Ethanol/ Water at various S/CoS ratio 3:1 at 60°C. Area under the curve represented as emulsification region.

Fig 10: The ternary phase diagrams of ME consisted of CP, TP, TS / cremophor RH40/Ethanol/ Water at various S/CoS ratio 3:1 at 60°C. Area under the curve represented as emulsification region.

Six ternary phase diagrams were selected based on the largest isotropic ME region for each lipid component. These selected phase diagrams are presented in Fig 9 and 10.

3.10 Characterization of the Micro Emulsions prepared:

Drug Content: 98.2 ± 0.1% to 99.3 ± 0.1% is the range of drug content present in the ME formulations.

Viscosity: Viscosity analysis of the microemulsion formulations revealed values within the range of 220-240 cps. An upward trend in viscosity was observed with increasing surfactant concentration, indicating its direct influence on the formulation's rheological properties.

pH determination: The pH of the microemulsion formulations was recorded within the range of 6.4 ± 0.33 to 6.6 ± 0.22, ensuring compatibility with physiological conditions.

Drug release studies: Fig 11 illustrates the in vitro drug release profile of six formulations. Among them, the formulation containing Tristearin (ME6) exhibited the highest drug release, whereas the other formulations demonstrated comparatively lower release rates.

Zeta potential, Droplet size and PDI: The zeta potential of ME6 was recorded as -43 mV, indicating a negative surface charge. The formulation exhibited a particle size of 295 nm with a polydispersity index (PDI) of 0.103. (Fig. 12).

Table 8: Evaluation parameters of PA ME

Formulation Code	S_{mix} Ratio	Lipid	pH	Viscosity (cps)	% Drug content
ME1	3:1	Palmitic Acid	6.4±0.12	220±1.2	83.2
ME2	3:1	Stearic Acid	6.4±0.46	223±1.5	77.2
ME3	3:1	GMS	6.5±0.19	226±2.3	76.1
ME4	3:1	Cetyl Palmitate	6.5±0.33	231±1.7	69.3
ME5	3:1	Tripalmitin	6.6±0.47	232±2.2	88.4
ME6	3:1	Tristearin	6.6±0.66	2332.5	99.2

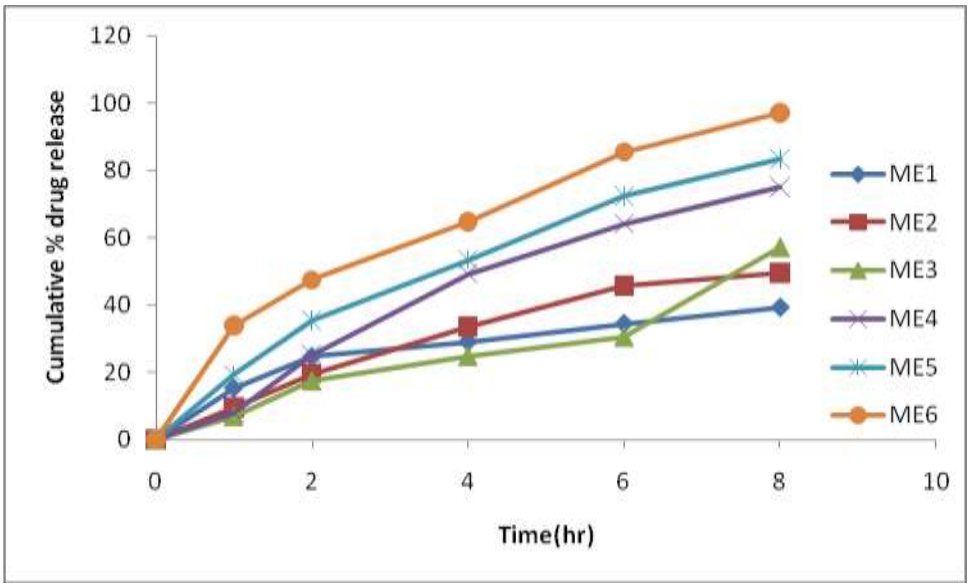

Fig 11: Cumulative % drug release

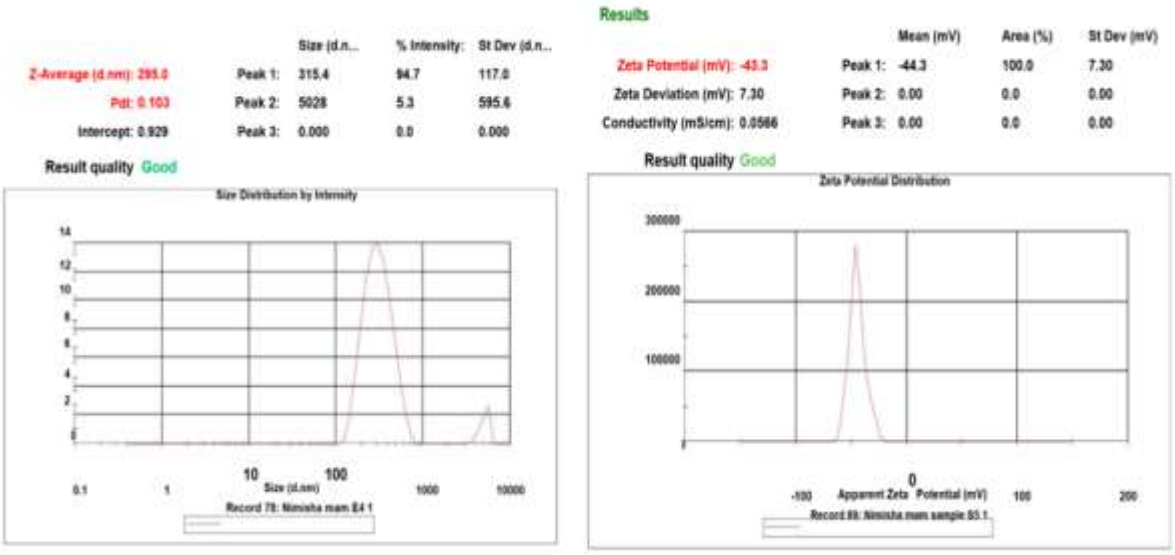

Fig 12: Zeta potential and Particle size of Prednisolone Acetate

4.0 Conclusion

In the present study, various formulations of Prednisolone acetate were developed as microemulsions to enhance solubility. Based on the evaluation of different physicochemical properties, the ME6 formulation was identified as the optimal batch. The findings suggest that microemulsion-based formulations can effectively improve the solubility of Prednisolone acetate.

5.0 References

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