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Research Article

Development and Evaluation of Novel Microemulsion Formulation for Oral Delivery of Aceclofenac.

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ABSTRACT

In this study, oil-in-water (O/W) system of microemulsion prepared to increase the solubility of poorly water soluble drug Aceclofenac which is a very efficient Non steroidal anti-inflammatory drug against serious diseases such as rheumatoid arthritis, as the poor solubility of Aceclofenac in water limits oral bioavailability. The solubility enhancers like different surfactants of Tween 80, Chremophor EL and Labrasol were used to solubilise the drug with medium chain triglycerides. The pseudoternary phase diagrams showed the suitability of selection of oils, surfactant and cosurfactant. Then Aceclofenac was mixed with the blend of Oil, surfactant, Cosurfactant to form homogeneous microemulsion followed by water titration. The microemulsion optimised by determination of mean droplet sizes, Zeta potential, viscosity, Invitro diffusion and In vivo studies. The droplet size of optimised formulation F2 was 87nm were successfully developed. They were able to improve the drug solubility up to 1000-fold. The zeta potential of -45mv showing the stability of microemulsion. The in-vitro drug diffusion of microemulsion through semi permeable membrane was compared with marketed formulation. The anti-inflammatory action of the formulation and the in-vivo absorption study investigation conducted in rat paw oedema experiment were significantly representing the efficacy of formulation.

KEYWORDS

Aceclofenac, Oil in water Microemulsions, Surfactants, Psedoternary Phase diagram.

1. INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) play significant role in treatment of inflammatory conditions like, osteoarthritis, rheumatoid arthritis and spondylitis[1]. Aceclofenac is one of the most effective NSAID molecule for the above treatments. It possesses derivative of diclofenac having less GIT disturbance[2]. The oral absorption of Aceclofenac is good with hepatic first pass metabolism[3]. Its elimination half life is about 4h, volume of distribution 25l, 99% of protein binding and 60-70 % of bioavailability after oral administration[4]. Now a days lipid based carriers such as microemulsions, nanoemulsions, solid dispersions, solid lipid nanoparticles are formulated for enhancement of the solubility and bioavailability of drugs[5].These above system of formulation where drug molecule is assumed to remain in solution throughout its residence time in the Gastro intestinal tract[6]. Microemulsion system improves the extent of drug absorption and also increases the overall bioavailability. Microemulsions considered as homogeneous transparent liquid, thermodynamically stable dispersions of water and oil, stabilized by a surfactant, usually in combination with a co surfactant (short chain alcohol). Moreover the absorption of the drug could be increased as lipids present from stimulation of biliary and pancreatic secretions by the gallbladder, having more gastric residence time[7].

Microemulsions are effective delivery systems for oral administration of lipophilic drugs as they are able to incorporate a wide range of drug molecules, increasing their solubilization and bioavailability, and reduce their toxicity[8]. Therefore, the objective of this work was to design and develop oil-in-water (O/W) microemulsion based on long- and medium-chain triglycerides in order to increase the solubility of Aceclofenac and enable its use by the oral route.

2. MATERIALS AND METHODS

2.1. Materials

Labrasol,Labrafac,Transcutol,Tween20,Tween80,PolyethyleneGlycol(PEG300),PropyleneGlycol(PG),Peceol,Myglyol were supplied by Gattefosse S.A. (Saint-Priest, France) were obtained as gift sample from Alkem Labs, Mumbai. Sodium hydroxide, Potassium hydrogen phosphate, Hydrochloride acid, Aceclofenac and HPLC grade methanol were purchased from Rankem (India).

2.2. Methods

2.2.1. Selection of oil, surfactant/Co-surfactant

Non-ionic surfactants (Tween 20,Tween 80) and the lipid(Miglyol) were weighed and put into number of screwcap test tubes having different ratios (w/w) of 0.1:0.9, 0.2:0.8, 0.3:0.7, 0.4:0.6, and 0.5:0.5 for 1 g per test tube, mixed together in vortex mixture (Spinix,Targons). After that 100 µL of demineral water was added to each test tube by help of micropipette. For each drop of water addition, the system was vortexed for 1min at room temperature. Visual observations were made, and the clarity or turbidity of each sample was inspected. The system was selected which was clear and less turbidity having the hydrophilic surfactant that best matched the tested lipid.The solubility of Aceclofenac determined in the above mediums were determined by dissolving an excess amount of aceclofenac in 2 mL of each of the selected Oils, Surfactants, and Co-surfactants in 5mL stoppered vials separately and vertexed using a vortex mixer. The mixture vials were then kept at room temperature for 48 hours under a shaker to get to equilibrium. The

equilibrated samples were kept out from the shaker and centrifuged at 5000 rpm for 10 minutes. After centrifugation the clear supernatant liquid collected and filtered using 0.45micron filter. The solubility of Aceclofenac was determined in oil, surfactant and cosurfactants by UV spectrophotometer at λmax of 275nm.

2.2.2. Construction of pseudo ternary phase diagrams

The selection of Oil, Surfactant and Co surfactant was optimised by making suitable blends. Then the pseudoternary phase diagram constructed (Fig. 1) using Chemix software with respect to selected surfactant ,co surfactant mix (Table 2) with water consumed by titration followed by sonication. The systems were characterized by visual observation.

2.2.3. Development of microemulsion system

The pseudoternary phase diagrams facilitates to formulate the microemulsion, having ratios of oil, surfactant blend and water for the production of Oil/Water type were selected. The oil phase was mixed with the surfactant mix in the various w/w ratio respectively and water to produce 5ml. The mixture was vortexed and subjected to sonication at 140 V for 60 s (Sonilia,soltee,Italy).

2.2.4. Drug incorporation

Aceclofenac 100mg was added to the blank microemulsions (Table 3) and the systems were vortexed for 2 min. After stirring, the mixtures were left for 5, 15, 30, 45 and 60 min under magnetic stirring for the incorporation of Aceclofenac into the systems at 25 ± 0.1 °C. The microemulsions were centrifuged at 10,000 × *g* in a Hitachi Himac CP-80 Ultracentrifuge (USA) for 15 min to remove the undissolved drug. The supernatant liquid was kept and carefully filtered using a 0.22 μm membrane. The filtrate was diluted and dissolved in phosphate buffer pH 6.8 for the quantitative analysis.

2.2.5. Microemulsion characterization

2.2.5.1. Particle size

The particle size and distribution of formulations were evaluated by dynamic light scattering (DLS) using a Malvern-Zetasizer Nano ZS-90 (Malvern, Worcestershire, UK). To avoid multiple scatting effects formulations were first diluted 50 times with water and continuously before measurements to ensure the sample was homogeneous.

2.2.5.2. Quantitative analysis

The incorporation of Aceclofenac was determined by high performance liquid chromatography (HPLC) using (Shimadzu ULFC, DGU-205R) C18 column (25 cm ×5 mm, 5 μm). The mobile phase consisted of a solution containing methanol/water (80:20). An isocratic elution was performed with a flow rate of 1ml per minute. UV detection was performed at a wavelength of 275 nm and 20 μL of sample was injected for each analysis. To determine the linearity of the method different concentrations of Aceclofenac in the range 50 to 250 μg mL^{-1} were prepared and analyzed.

2.2.5.3. Zeta Potential

This is used to identify the charge of the droplets. In conventional emulsions, the charge on an oil droplet is negative due to presence of free fatty acids. Zeta potential is a measure of the magnitude of the electrostatic or charge repulsion/attraction between particles, and is one of the

fundamental parameters known to affect stability. Zeta potential of microemulsions were determined using Malvern Zetasizer Nano ZS-90.

2.2.5.4. Conductivity

Conductivity is a measure of the ability of water to pass an electrical current. The measurement of electrical conductivity gives the quantitative idea of the solubilisation of water phase in the selected mature containing oil phase, surfactant and co-surfactant. Conductivity measured by Meteller Tolledo conductive meter.

2.2.5.5. Rheological study

The Viscosities of microemulsion formulations were determined by using Brookfield Viscometer DV III Ultra Programmable Rheometer using spindle no. 64. Viscosity of a fluid is a measure of its resistance to gradual deformation by shear stress or tensile stress. For liquids, it corresponds to the informal concept of "thickness". Viscosity is a property arising from collisions between neighboring particles in a fluid.

2.2.5.6. Refractive index

The refractive index 'n' is a constant that describes travel of light from one medium to another. It is defined as n =c/ υ, where velocity of light (c) is the in the vacuum and the frequency light (υ) in the medium where light travels. It was measured using Abbe refractometer.

2.2.5.7. Transmittance

Transmittance (T) is measured by using UV Visible Spectrophotometer (Perkin Elmer, L-25). It is the fraction of radiant energy that having entered a layer of absorbing matter reaches its farther boundary. It is a measurement of how much light passes through a substance. Transmittance is defined as the ratio of the intensity of incident light: intensity of transmitted light i.e. if the intensity of incident light is I and the intensity of transmitted light is I_0 , then $T=I/I_0$ At times, this fraction may be represented as a percentage, where it is called the percentage transmittance **(**%T**)**.

2.3. In vitro diffusion study

In Vitro study is carried out by using Franz Diffusion Cell Apparatus. The drug release of all formulation, pure drug and marketed product through semi permeable membrane from donner chamber to receiver chamber was calculated for a period of 30 minutes. The study of *in vitro* drug release profile (Fig.2) showed the best available process that can quantitatively assure about the bioavailability of drug from its formulation that mimics the environment of biological.

2.4. In vivo study

The in-vivo anti inflammatory effect study was carried out by the approval from the Institutional Animal Ethics Committee Dadhichi college of pharmacy (regd. no: 1200/AC/08/CPCSEA) Odisha, India and the formal guidelines were adopted for the test. To find out anti-inflammatory action of the optimized formulation F2, the carrageenan-induced hind paw edema method used developed by Winter et al in albino Sprague-Dawley (SD) rats. Three groups of SD rats weighing 280 to 350g were taken i.e. Control group, Test (Formulation F2) group, and Reference group, each group containing six rats. The rats were placed with temperature of $25^0C \pm 1^0C$ and relative humidity of 55% ± 5% maintaining standard laboratory conditions. They were kept in polypropylene cages keeping 6 rats per cage, having standard laboratory diet (i.e. Lipton) and water ad libitum. According to the surface area ratio and the weight of the rats, the dose was

calculated. Both Reference and test group given drug orally 30 minutes before injecting carageenan 0.1ml 1% solution where only 0.9% sodium chloride injected to control group.

2.5. Stability study

The stability studies of formulation F2 carried out by packing it in borosil glass screw capped tubes and stored at specific condition of 25°C/60% relative humidity (RH) and accelerated condition 40°C/75% RH for a period of 3 month in a stability chamber (REMI, India). The drug content was calculated on 1st, 2nd, and 3rd months, and their physical nature observed for appearance, pH and viscosity.

3. RESULTS AND DISCUSSION

3.1. Characterization of Oil. Surfactant and Co-surfactant

Selection of Oil, Surfactant and co-surfactant was carried out by instant emulsification method. All the ingredients were pharmaceutically acceptable, and generally regarded as Safe category. It is important that the highest solubility of drug in oil because it help to maintain the drug in soluble form in dispersion medium. Moreover the selection of surfactant with respect to safety needs more attention. Non ionic surfactants are less toxic than the ionic one. The most important parameter is Hydrophilic Lipophilic Balance (HLB) value. For O/W microemulsion the HLB value must be more than 10.Hence the optimum HLB value depends the proportion of surfactant mixture resulting stability of microemulsion.

3.2. Construction of pseudoternary phase diagrams

As per the pseudoternary phase diagrams concerned the formulations developed by mixing Oil with the surfactant blends M1 to M6 followed by titration with water. For instance, large areas microemulsions are detected, as well as smaller areas of bicontinuous phase (Fig.1). Formation of microemulsion systems (the shaded area) was observed at room temperature. Phase behavior pattern of these formulations significantly determines the water phase, oil phase, surfactant concentration, and cosurfactant concentration with which the transparent, single phase low viscous microemulsion system was formed. The optimum formulation of microemulsion contained M2 in ternary plot which contains Miglyol and Chremophor, with Labrasol and PEG 300 at 5% and 39.2% respectively consuming maximum water i.e. 56%.

3.3. Physico chemical Evaluation of microemulsions

The various Physico chemical evaluation carried out to find out optimized formulation was given in Table 4, Table 5.The formulation F2 was satisfactory with optimum viscosity 49.5cP.The experimented microemulsion system exhibits electro conductivity 19.2 μΩ. Both the parameters concluded that the system was Oil/Water type .The refractive index (=1.41) of optimized formulation F2 was seemed to the value of water (=1.333). Also the transmittance of the subject formulation was 99.4%.From this it is conformed about the transparency of system. The particle size (43nm) and Zeta potential (-45.5mv) of optimized formulation indicates the stability of microemulsion system among formulations. The assay was found to 99.2% in formulation F2 which was highest among the formulations.

3.4. In-Vitro Diffusion study

In vitro diffusion studies were performed to compare the release of drug from different microemulsion formulations, Pure drug and Marketed formulation (i.e. Aceclofenac Tablet

100mg) given in fig.2 .In vitro diffusion was highest i.e. 92.59% in formulation F2 with compared to other formulations and marketed product i.e. 69.8%. The significant difference in Aceclofenac permeation among microemulsion formulations was observed. It may due to the presence of mean droplet size of internal phase, which were significantly smaller in formulation F2.

3.5. In-Vivo study

This study based on higher drug permeation, lowest droplet size and lowest viscosity of formulation F2 was selected to carry out in vivo anti-inflammatory effect. The anti-inflammatory and sustaining action of the optimized formulation was evaluated by the carrageenan induced hind paw edema method carried out in Wistar rats. The percent inhibition value in Test group for formulation F2 after 3 hours of oral administration was found to be high i.e., 82.2% ($P<0.05$) as compared with Reference group. The enhanced anti-inflammatory effects of formulation F2 could be due to the enhanced absorption of Aceclofenac through the gastro intestinal membrane.

4. CONCLUSION

On the basis of highest drug permeation, lowest droplet size, lowest viscosity, and optimum surfactant and cosurfactant concentration, we selected formulation F2 which contained Labrasol (10.64% w/w), Chremophor (16.12% w/w), Miglyol (4% w/w), PEG (5% w/w), and distilled water (64.5% w/w) for use in vivo studies. The in vivo studies revealed a significant increase in the anti-inflammatory effects with formulation F2. From in vitro and in vivo data it can be concluded that the developed formulation F2 was the best formulation. Moreover the 3 month accelerated stability study revealed the formulation was stable and no significant deviation found in assay, physical appearance, pH, Viscosity.

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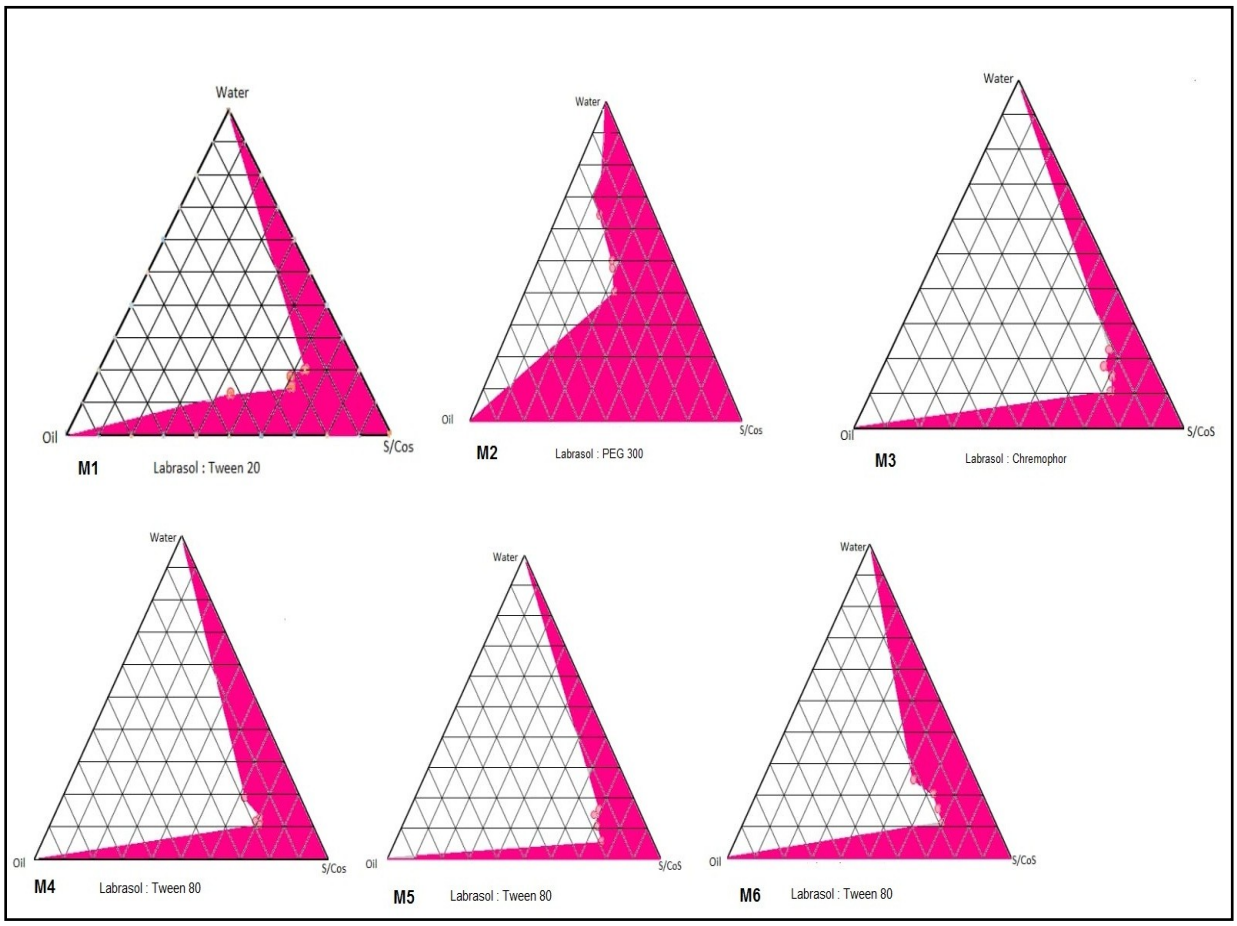

Fig. 1- Pseudo ternary phase diagrams of Microemulsion Composition.

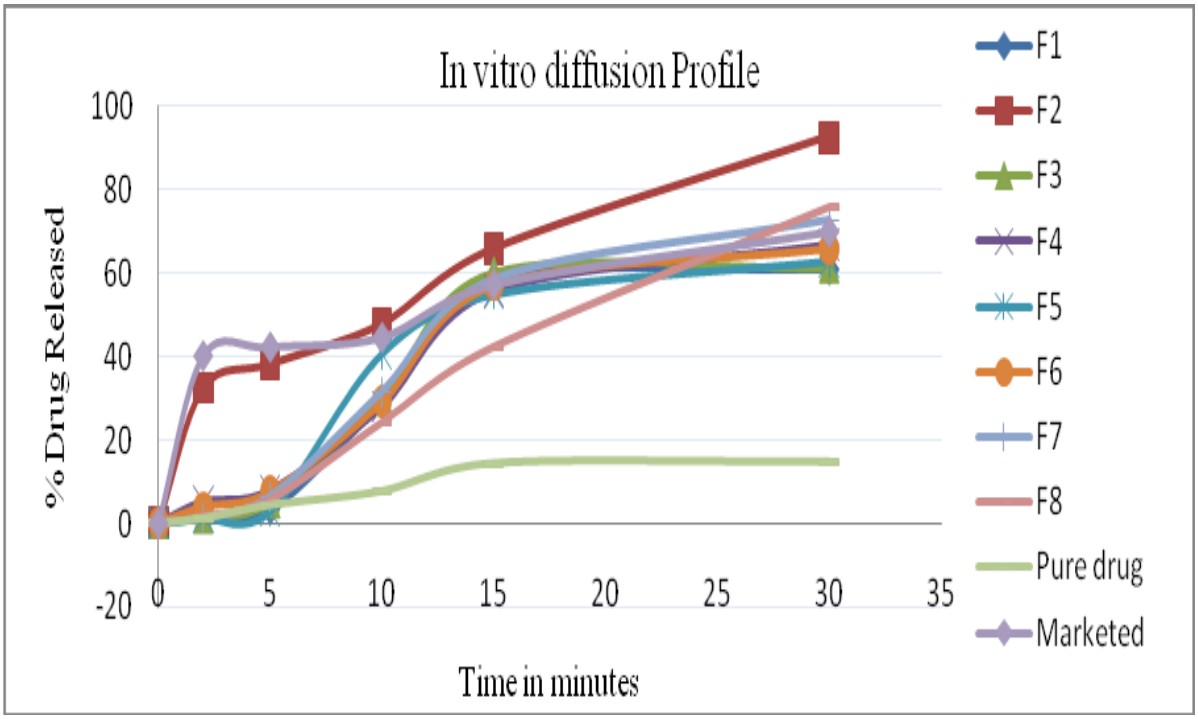

Fig. 2- Diffusion study of microemulsions.

Table 1: Solubility of Surfactant and Co-surfactant.

S/Cos	Mean Solubility (mg/ml) (n=3) ±SD
Labrasol	42.65 ± 0.93
Chremophor	29.37± 0.74
Tween 20	17.6 ± 0.46
PEG 300	0.133 ± 0.15
Tween 80	19.8 ± 0.53
Ethanol	4.33 ± 0.79
PG	6.42 ± 0.15
PEG	2.68 ± 0.38

Table 2: Oil and surfactant blend with water.

Composition	Oil (w/w)	Surfactant/Co surfactant (w/w)	Water(w/ w)
M1	Miglyol+Cremophor (10%)	Labrasol+Tween20 (40%)	50%
M2	Miglyol+Cremophor (5%)	Labrasol+PEG300 (39.2%)	56%
M3	Miglyol+Cremophor (10.14%)	Labrasol+Tween20 (59.52%)	20.3%
M4	Miglyol+Cremophor (22.62%)	Labrasol+PG (56.56%)	20.81%
M5	Miglyol+Transcutol (14.7%)	Labrasol+PEG-300 (69.11%)	16.17%
M6	Labrafac (22.12%)	Labrasol+Tween 80 (53.09%)	24.77%

Table 3: Composition of the Aceclofenac microemulsions.

Ingredients	Formulations							
	F1	F2	F3	F4	F5	F6	F7	F8
Aceclofenac	100 mg							
Labrasol	1.29g	0.532g	0.738 g	0.7g	1.2g	0.7g	0.8g	0.9g
Chremophor	0.487g	0.806g	0.277 g	0.5g		___	0.5g	0.487g
Miglyol	0.405g	0.201g	0.5g	0.5g	0.5g	-----	0.5g	0.64g
Tween 20	2.11g	-------	1.20g	------	0.3g	------	------	2.11g
PEG 300	--------	0.251g	0.46g	------	0.5g	-------	------	0.57g
Propylene	--------	-------	-------	------	0.25g	0.85g	0.6g	-------

Glycol								
Glycerin	---------	------	0.992 g	2.05g	0.2g	0.65g	-----	0.35g
Transcutol	--------	-------	-------	------	0.55g	------	-----	--------
Labrafac	---------	------	-------	------	------	0.6g	------	---------
Tween 80	---------	--------	--------	0.5g	0.8g	1.15g	------	---------
Water	1.087g	3.225g	1.015 g	1.01g	1.23g	1.05g	2.71g	1.382g

Table 4: Physico chemical evaluation of Microemulsions.

Parameters	**Formulations**			
	F1	**F2**	**F3**	**F4**
Particle size (nm)	47 ±0.34	43±0.12	72±0.64	64 ±0.21
Zeta potential (mv)	-33.7±0.74	-45.1±0.93	-24.9±0.85	-39.7±1.12
Viscosity (cP)	53.1±1.41	49.5±0.97	58.5±1.17	67.2±1.87
Electro conductivity (µΩ)	13.3±0.28	19.2±1.11	16.2±0.89	13.9±0.72
pH	4.52±1.16	5.01±0.49	4.54±1.97	4.3±0.89
Refractive Index	1.44±1.14	1.41±1.82	1.41±1.26	1.43±1.98
Transmittance at 650nm (%T)	93.1±1.57	99.4±1.09	92.9±2.51	91.3±2.63
%Assay (HPLC)	97.8 ±1.1	99.2 ±0.97	96.8 ±1.24	97.4 ±0.83

Table 5: Physico chemical evaluation of Microemulsions.

Parameters	**Formulations**			
	F5	**F6**	**F7**	**F8**
Particle size (nm)	59 ±0.22	54 ±0.31	59±0.49	82 ±0.37
Zeta potential (mv)	-36.8±2.51	-30.4±1.54	-7.3±0.54	-12.6±1.75
Viscosity (cP)	61.2±1.35	62.3±1.82	55.9±2.13	68.2±1.14
Electro conductivity (µΩ)	15.2±1.13	14.1±1.64	13.5±1.19	11.9±1.64
pH	4.65±1.21	4.76±0.69	4.66±0.47	4.89±0.98
Refractive Index	1.42±2.01	1.43±1.98	1.40±2.48	1.44±2.04
Transmittance at 650nm (%T)	99.2±1.08	94.2±1.75	91.7±2.61	92.6±1.63
%Assay	95.2 ±1.64	98.3 ±1.08	97.1 ±1.04	98.6 ±1.77