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Original Article

EFFECT OF STRUCTURAL PARAMETERS OF BRIJ SURFACTANTS ON SELF-EMULSIFICATION OF POORLY SOLUBLE DRUG

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ABSTRACT

Objective: The objective of the present investigation was to optimize the excipient concentration, that is of oil, surfactant and co-surfactants to form a Self Emulsifying Drug Delivery Systems (SEDDS) using best possible combination of excipients. The present study aims to investigate the effect of homologous Brij surfactant on the self-emulsification of aceclofenac.

Methods: Three Brij surfactants Brij-35, Brij-58 and Brij-98 were selected for the study along with a common co-surfactant ethanol. The lipid carrier used was almond oil. The combinations of surfactants with ethanol were subjected to a pseudoternary diagram study.

Results: The best combination after the pseudoternary diagram study was found to be of Brij-58 and ethanol. The reason may be the difference in chains of Brij-35, Brij-58, Brij-98. The double bond of Brij-98 chain makes it rigid, whereas absence of unsaturation in Brij-58 imparts flexibility to its chain, leading to better shielding of the hydrophobic compartment when used along with ethanol. The Brij-35 chain consist of 12 carbons and Brij-58 chain consists of 16 carbons so latter offers larger core for drug solubilization. Simplex lattice design was used for optimization. Seven formulations were developed using almond oil, Brij-58, ethanol and evaluated. Formulation F2 was found to be best amongst all with globule size of 182 nm and zeta potential of-19.73 mV, indicating formation of stable microemulsion.

Conclusion: The surfactant possessing large and flexible chains along with less number of polyoxyethylene groups offers greater space for drug solubilization and better protection of the hydrophobic core and lead to finer microemulsification.

Keywords: Self emulsification, Microemulsion, Zeta potential, Brij, Globule size

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INTRODUCTION

Self-emulsifying drug Delivery Systems (SEDDS) are the pre-mixtures of a lipid carrier, surfactants and co-surfactants. SEDDS can be used to overcome poor solubility low permeability and to improve gastro-intestinal stability. These factors together make SEDDS an ideal lipid carrier for poorly soluble drugs [1]. SEDDS involves surfactants in their formulation, which further assist in improving the absorption of drug from gut, hence bioavailability [2]. The SEDDS spontaneously form small globules on aqueous dilution, which promotes their lymphatic transport, thus bypassing first-pass metabolism[3]. Surfactants are molecules which comprise of two chemical entities with different polarities. This amphiphilic property of surfactants make them an important excipient in the preparation of various drug delivery systems [4]. Surfactants at a concentration above Critical Micelle Concentration (CMC), forms a hydrophobic core which remains surrounded by a hydrophilic surface. The hydrophobic core enhance the drug solubilization by entrapping the poorly soluble drug in the core region [5]. Surfactant mixtures are of considerable interest in relation to lipid vesicular systems. The interaction between homologous surfactant to form mixed micelles remains a field of research [6]. Surfactant chain length in surfactant mixtures also effects various properties of lipid vesicles such as globule size, stability, entrapment, permeability [7]. This makes the surfactant mixtures a field of study in the development of SEDDS of drugs with poor aqueous solubility [8].

The Brij surfactants are a class of non-ionic surfactants which contains oxyethylene chain as hydrophilic part and hydrocarbon chain as the hydrophobic part. The Brij surfactants can serve as low toxicity stabilizers for microemulsion systems [9, 10]. Asfour MH *et al.* developed a SEDDS for the hydrophobic ion pair complex of cromolyn sodium using oleic acid, Brij 98, propylene glycol. The ex vivo intestinal permeability studies and *in vivo* evaluation indicated that the formulated self-emulsifying system has proven to be superior as compared to plain solution of cromolyn suspension for prophylaxis of asthma [11]. In this study, the effect of two Brij surfactants, Brij 35, Brij 58 and Brij 98 with varying chain length, 12 carbons and 23 polyoxyethylene units, 16 carbons and 20 polyoxyethylene units, 18 carbons and 20 polyoxyethylene units respectively; is investigated on self-emulsification of a poorly soluble drug aceclofenac [12]. This study aims to establish the selection of most preferable surfactant in the minimum possible concentration to form an optimized SEDDS. SEDDS can be employed for Biopharmaceutical Classification System (BCS) Class II and BCS Class IV drugs to enhance their bioavailability due to poor solubility [13]. The excipients used in formulating SEDDS plays a crucial role in improving absorption and, hence, bioavailability of poorly soluble drugs [14]. In the present investigation, the aim is to study the effect of chain length of Brij surfactants on efficiency of SEDDS of aceclofenac. The study focuses to optimize the surfactant concentration and to increase the bioavailability of poorly aqueous soluble drug aceclofenac using quality by design approach [15]. Aceclofenac is one of the BCS Class II drug. Aceclofenac is a non-steroidal anti-inflammatory drug which is widely used in treatment of acute and chronic pain associated with various maladies with minimum risk of adverse effect [16]. So aceclofenac is an ideal candidate to study the effect of surfactant mixtures on SEDDS.

MATERIALS AND METHODS

Materials

Brij-35 was purchased from loba Chemie; Brij-58 and Brij-98 was purchased from Sigma Aldrich Private limited. Ethanol was purchased from Merck life Sciences Pvt. limited. The model drug aceclofenac was gifted from Wilcure Remedies Pvt. limited, Indore. Distilled water was used for the construction of pseudoternary phase diagrams and dilutions. All other reagents and chemicals used were of analytical grade.

Methods

Screening of oil, surfactant and co-surfactant

Selected oils and, surfactants and co-surfactants were mixed in 1:1:1 ratio (w/w). The resultant mixture was then heated to 40°C and mixed till a homogeneity is achieved. 500 mg of mixture was

weighed accurately and dispersed into 10 ml of deionized water. The mixture was then evaluated for relative turbidity visually. The resultant mixture was then allowed to stand for 3 hours and transmittance was measured at 550 nm using Ultra Violet (UV)-Visible spectrophotometer [17].

Pseudoternary phase diagram study

The pseudoternary phase diagrams were constructed using almond oil, Brij-35, Brij-58 and Brij-98, along with ethanol as a co-surfactant. The diagrams were pseudoternary when Brij-35, Brij-58 and Brij-98 is used along with a definite ratio of ethanol. The different ratios of Brij-35, Brij-58 and Brij-98, which are used along with ethanol for the study, are presented in table 1. The almond oil is mixed with different surfactant mixtures (SMIX) in weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 [18]. The mixing is done with the aid of magnetic stirrer till a homogeneous mixing is accomplished. 5g of the mixture is withdrawn in a 500 ml beaker and water is added in 5% w/w increments of total mixture with continuous stirring at 30-40℃ till the final mixture contains 95% w/w of water. The transparent to translucent regions were then identified and used for formulation development [19, 20]. The experiment has been performed in triplicate and good correlation has been observed in all the sets of experiments.

Table 1: Different ratios of surfactants and co-surfactants used

S. No.	SMIX	Surfactant (1% w/v)	Co-surfactant	Ratio of surfactant: Co-surfactant (SMIX)
1.	1	Brij-35	Ethanol	1:1
2.	2	Brij-58	Ethanol	1:1
3.	3	Brij-98	Ethanol	1:1

Optimization study

Optimization for the three formulation components for emulsification time, percentage transmission and drug release in 15 min was done using simplex lattice design. Seven runs were performed. Design points were represented on an equilateral triangle. The obtained data was fitted to most suitable model. Mathematical equations were generated which defines the relationship of one or a combination of components on selected parameters [21, 22]. The procedure for determination of emulsification time, percentage transmission and drug release are described under characterization section.

Development of SEDDS of aceclofenac

Formulations were developed using fixed ratios of oil, surfactant and co-surfactant selected on the basis of pseudoternary diagram and optimization studies; were taken and stirred using a magnetic stirrer with hot plate. 100 mg of drug was added to the mixture with continuous stirring on a magnetic stirrer till homogeneity is achieved [23, 24]. A final batch weight of 10 g was prepared, as shown in table 2.

Table 2: Composition of SEDDS formulations of aceclofenac

Formulation code	Drug (mg)	Almond oil proportion	Brij 58 proportion	Ethanol proportion
F1	100	0.20	0.50	0.30
F2	100	0.30	0.40	0.30
F3	100	0.30	0.37	0.33
F4	100	0.30	0.35	0.35
F5	100	0.20	0.56	0.24
F6	100	0.30	0.30	0.40
F7	100	0.25	0.50	0.50

Evaluation

Emulsification time

The emulsification ability of prepared formulations were determined using of United States Pharmacopoiea (USP) II dissolution apparatus (EDT-406Lx, Electrolab India Private limited, India). The formulations were filled in hard gelatin capsules and visually inspected for self-emulsification [25].

Percentage transmission

0.1 ml of the self-emulsifying pre-concentrate was added in 100 ml of distilled water and stirred for 5 min at 50 rpm at 37±0.5 °C. UV-visible spectrophotometer (UV1800, Shimadzu Corporation, Japan) was used to measure percentage transmission. The measurements were done at 650 nm. All samples were analyzed in triplicate [26].

pH

The pH of all the formulations was determined by using a digital pH meter (HI-98107, PHeP®, Hanna, USA). The readings were taken in triplicate and the mean was calculated [27].

Droplet size, polydispersity index and zeta potential analysis

0.1 ml of self-emulsifying preconcentrate was dispersed in 100 ml of distilled water. The mean globule size. Polydispersity index and zeta potential was determined using Malvern Zetasizer (Malvern, Nano Series ZS90, Malvern Instruments limited, UK) at 25°C. All the measurements were done in triplicate [28].

Rheology

The viscosities of the self-emulsifying pre-concentrates were determined using Ostwald viscometer (Fisher scientific) at 25 °±0.5 °C. the viscosity of given liquid is measured with respect to water using the relation described below [29]. The measurements were done in triplicate.

$$\eta1/\eta2 = t1d1/t2d2$$

where η1 and η2 are the viscosity coefficients of the liquids under study, d1, d2 are their densities and t1 and t2 are their times of flow of equal volume of liquids through the same capillary [30].

Refractive index

Refractive index of the developed SEDDS of aceclofenac at 25 °±1 °C was determined using Abbe's refractometer. Calibration of refractometer was achieved using distilled water. All readings were taken in triplicate [31, 32].

Tyndall effect

1 ml of optimized self-emulsifying pre-concentrate, F16 was diluted up to 100 ml with distilled water. A laser beam is passed from the dilution and is visually examined for scattering of light [33, 34].

Drug content

4-5 ml of the prepared formulations were dissolved in 100 ml methanol. The mixture is shaken well for 15-20 min and equilibriated for 24 hours. Filtration of the dispersed formulation

was done through 0.45 µm filter paper. The filtrate was assayed spectrophotometrically at 275 nm using a UV-Visible spectrophotometer [35].

***In vitro* dissolution study**

In vitro dissolution study of aceclofenac SEDDS was carried out at three different pH by using different dissolution media viz 0.1 M hydrochloric acid pH 1.5, acetate buffer pH 4.5 and phosphate buffer pH 6.8. The dissolution was performed by the aid of USP II dissolution apparatus (EDT-406Lx, Electrolab India Private limited, India) at 37±0.5 °C. 1 ml of the prepared formulations were dispersed in 900 ml of dissolution media rotated at 100 rpm for 240 min. Aliquots of 5 ml were withdrawn at interval of 5, 15, 30, 45, 60, 90, 120, and 240 min [36, 37]. Filtration of the withdrawn samples was done using 0.45 µm filter. Immediate replacement with the equivalent volume of fresh dissolution medium was done after withdrawal to maintain the sink conditions. Samples were analyzed using a UV-Visible spectrophotometer at 275 nm and percent dissolution efficiency at 5, 15, 30, 45, 60, 90, 120, 180 and 240 min was calculated [38, 39].

Statistical analysis

Significance of obtained data was determined using a one-way analysis of variance (ANOVA). p-values<0.05 were considered significant throughout the study. The measurements were done in triplicate in whole study. The data was analysed using Excel software (Excel 365, Microsoft, USA) [40, 41].

RESULTS

Selection of oil, surfactant and co-surfactant

The selected oil and surfactants are almond oil, Brij-35, Brij-58 and Brij-98. The solubility of aceclofenac in almond oil, Brij-35, Brij-58 and Brij-98 is shown in table 3.

Table 3: Solubility of aceclofenac in various surfactants and co-surfactants

S. No.	Surfactant	Solubility (mg/ml)*
1.	Almond oil	48.89±1.8
2.	Brij 35	52.32±2.3
3.	Brij 58	55.98±4.1
4.	Brij 98	44.26±5.3
5.	Ethanol	128.21±2.6

*Data indicate mean±SD, (n=3)

Pseudoternary phase diagram study

The phase diagrams were constructed using almond oil as lipid carrier, Brij-35, Brij 58 and Brij-98 as surfactants and ethanol as a co-surfactant combined together in 1:1 ratio. As per the study, the larger self-emulsifying region is observed in case of Brij-58 as compared to Brij-35 and Brij-98 as shown in fig. 1, fig. 2 and fig. 3.

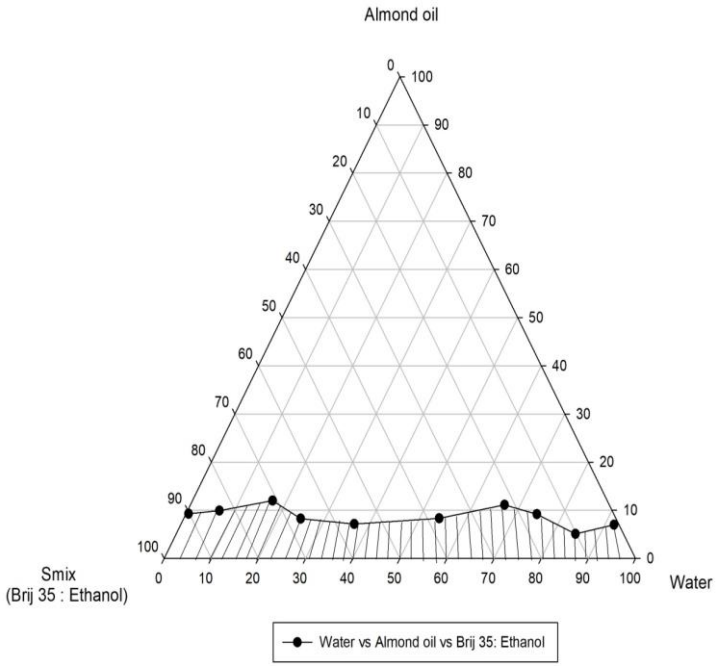

Fig. 1: Pseudoternary phase diagram of almond oil, SMIX1 (Brij 35: Ethanol in 1:1 ratio) and water-containing system (the shaded region represents the micro emulsifying region)

Simplex lattice experimental design

Emulsification time

The emulsification time for prepared batches is shown in table 4. The data is analyzed by regression for mixtures at 95% confidence interval. ANOVA was applied to establish the correlation between emulsification time and excipients concentration. Emulsification time depends on relative proportions of almond oil, Brij-58 and ethanol. The mixture contour plot and mixture surface plot for emulsification time is presented in fig. 4 (A and B). The model fit summary is presented in table 5.

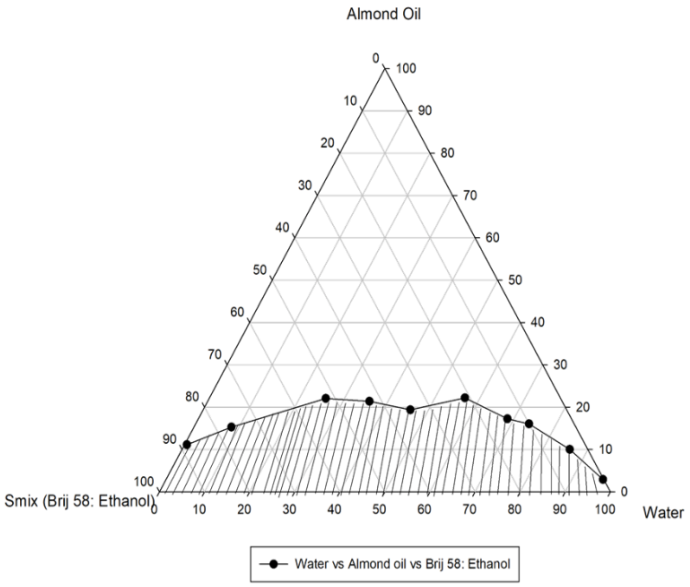

Fig. 2: Pseudoternary phase diagram of almond oil, SMIX2 (Brij 58: Ethanol in 1:1 ratio) and water-containing system (the shaded region represents the micro emulsifying region)

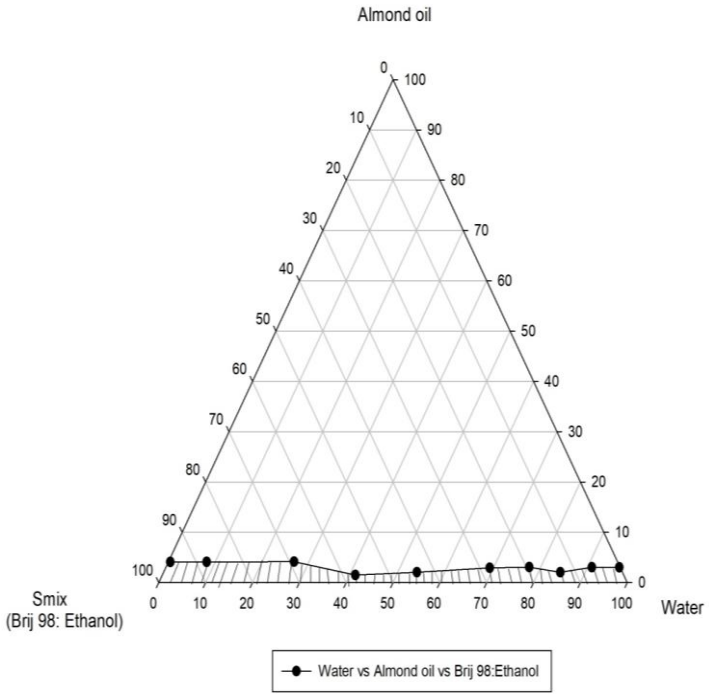

Fig. 3: Pseudo ternary phase diagram of almond oil, SMIX3 (Brij 98: Ethanol in 1:1 ratio) and water-containing system (the shaded region represents the micro emulsifying region)

Table 4: Emulsification time, percentage transmission and Drug release in 15 min of prepared design formulations

Formulation code	Emulsification time* (sec)	Percentage transmission* (%)	Drug release in 15 min (%)
FD1	40.27±1.00	82.42±0.31	18.11±0.61
FD2	17.16±1.21	85.49±0.28	23.42±0.37
FD3	31.18±1.31	88.36±0.47	32.76±0.29
FD4	15.31±2.16	89.26±0.61	28.72±0.41
FD5	19.44±1.37	92.11±0.29	33.71±0.44
FD6	40.28±1.21	95.51±0.31	39.34±0.42
FD7	34.47±1.27	98.25±0.28	44.49±0.27

*Data indicate mean±SD (n=3)

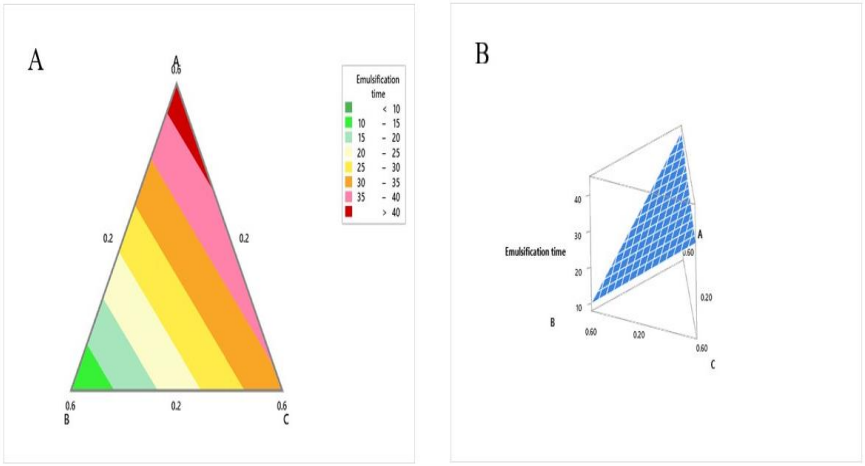

Fig. 4: A: Mixture contour plot of emulsification time, B: Mixture surface plot of emulsification time

Table 5: Model summary

Parameter	S	R-sq	R-sq(adj)	Press	R-sq(pred)
Emulsification time	5.94258	83.96%	75.95%	516.714	41.34%
Percentage transmission	3.06315	79.56%	69.34%	67.8622	63.04%
Drug release in 15 min	4.64705	82.33%	73.50%	135.778	72.23%

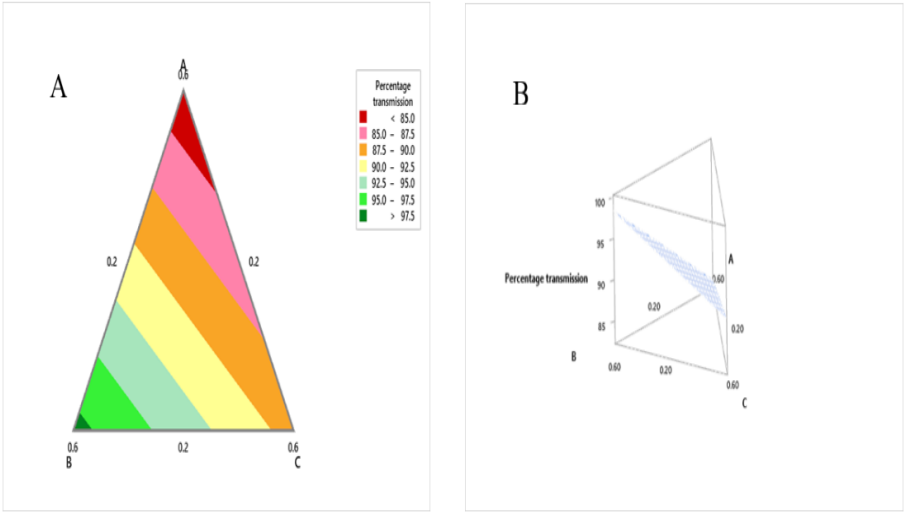

Fig. 5: A: Mixture contour plot of percentage transmission, B: Mixture surface plot of percentage transmission

Percentage transmission

The percentage transmission for prepared batches is shown in table 4. The data is analysed by regression for mixtures at 95% confidence interval. ANOVA was applied to establish correlation between percentage transmission and excipients concentration. The mixture contour plot and mixture surface plot for emulsification time is presented in fig. 5 (A and B). The model fit summary is presented in table 5.

Drug release in 15 min

The drug release in 15 min for prepared batches is shown in table 4. The data is analysed by regression for mixtures at 95% confidence interval. ANOVA was applied to establish correlation between drug release in 15 min and excipients concentration. The mixture contour plot and mixture surface plot for emulsification time is presented in fig. 6 (A and B). The model fit summary is presented in table 5.

The overlay plot is plotted by the overlapping of contour plots of emulsification time, percentage transmission and drug release in 15 min. The white region in the fig. 7 below indicates the feasible region for emulsification time within upper and lower limits of 50 seconds and 1 second, respectively, for percentage transmission for upper and lower limits of 100% and 90%, respectively, drug release in 15 min for upper and lower limits of 50% and 25% respectively.

Characterization of SEDDS of aceclofenac

Emulsification time

It was observed that all the formulations containing Brij-58 got spontaneously dispersed as soon as they got released from the hard gelatin capsule. The time taken for complete emulsification is minimum for formulation F4 and maximum for formulation F1 as shown in table 6.

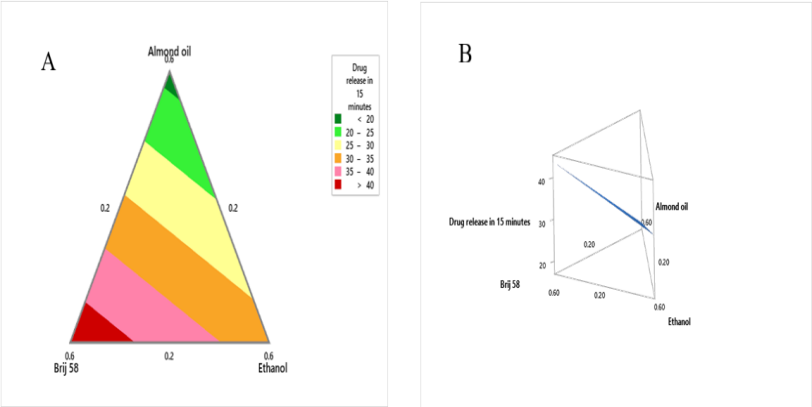

Fig. 6: A: Mixture contour plot of percentage transmission, B: Mixture surface plot of percentage transmission

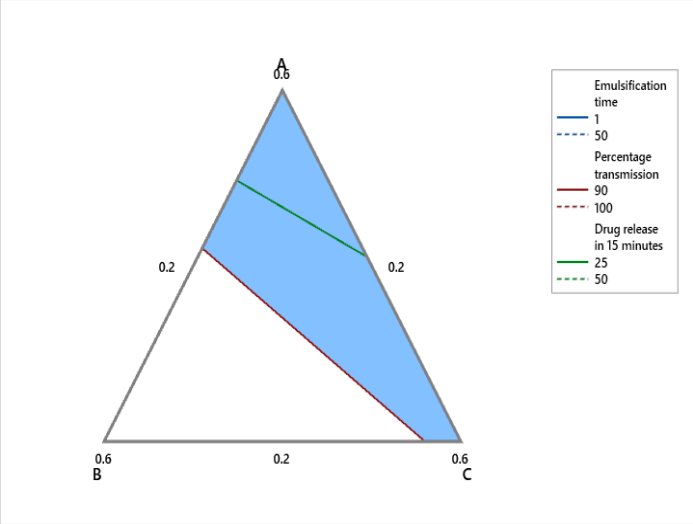

Fig. 7: Overlay plot of emulsification time, percentage transmission and drug release in 15 min

Table 6: Emulsification time, percentage transmission, pH, refractive index of prepared SEDDS formulations

Formulation code	Emulsification time* (sec)	Percentage transmission* (%)	pH*	Refractive index*
F1	38.12±1.41	90.31±0.23	7.3±0.47	1.49±0.5
F2	15.43±2.24	96.43±0.39	7.3±0.36	1.43±0.4
F3	33.31±1.15	91.24±0.41	7.3±0.62	1.42±0.5
F4	13.29±2.11	93.33±0.38	7.3±0.33	1.48±0.3
F5	17.37±1.31	91.62±0.52	7.3±0.65	1.44±0.4
F6	37.24±2.31	95.41±0.33	7.3±0.39	1.47±0.4
F7	31.52±1.46	90.72±0.47	7.3±0.40	1.42±0.5

*Data indicate mean±SD (n=3)

Percentage transmission

The percentage transmission of all the prepared formulations is shown in table 6. It has been observed that the percentage transmission is highest for formulation F2 and lowest for formulation F1.

pH

The pH values of all the formulations were found to be in the range of 7.3±0.33 to 7.3±0.65 and do not change upon dilution. The pH values of the prepared formulations are shown in table 6.

Droplet size, polydispersity index and zeta potential analysis

Finer droplet size results in better performance of self-emulsifying systems and can lead to achieve good bioavailability. The globule size of the optimized formulation, F2 was found to be 182.63±3.700 nm (mean±SD, n=3) and the polydispersity index was found to be 0.351±0.012 (mean±SD, n=3). The size distribution by intensity of the optimized formulation is shown in fig. 8A. The apparent zeta potential of the optimized formulation F2 was found to be-19.73±0.321 mV as shown in fig. 8B.

Rheology

The graphical representation of the viscosity values of different formulations is shown in fig. 9. The viscosity values follow the order F6<F7<F3<F4<F2<F1<F5.

Refractive index

All the self-emulsifying formulations were found to have a refractive index in the range of 1.42 to 1.49. The refractive index of different self-emulsifying formulations is shown in table 6.

Tyndall effect

The laser light scattering through all the formulations was observed. It was observed that the laser light illuminated most clearly through formulation F2, which indicates the formation of oil in water microemulsion, as shown in fig. 10.

Drug content

The drug content of all the prepared formulations were found to be above 90%. The highest drug content was found in case of formulation F2 i. e. 96.31%. The drug content of prepared self-emulsifying drug delivery systems is shown in table 7.

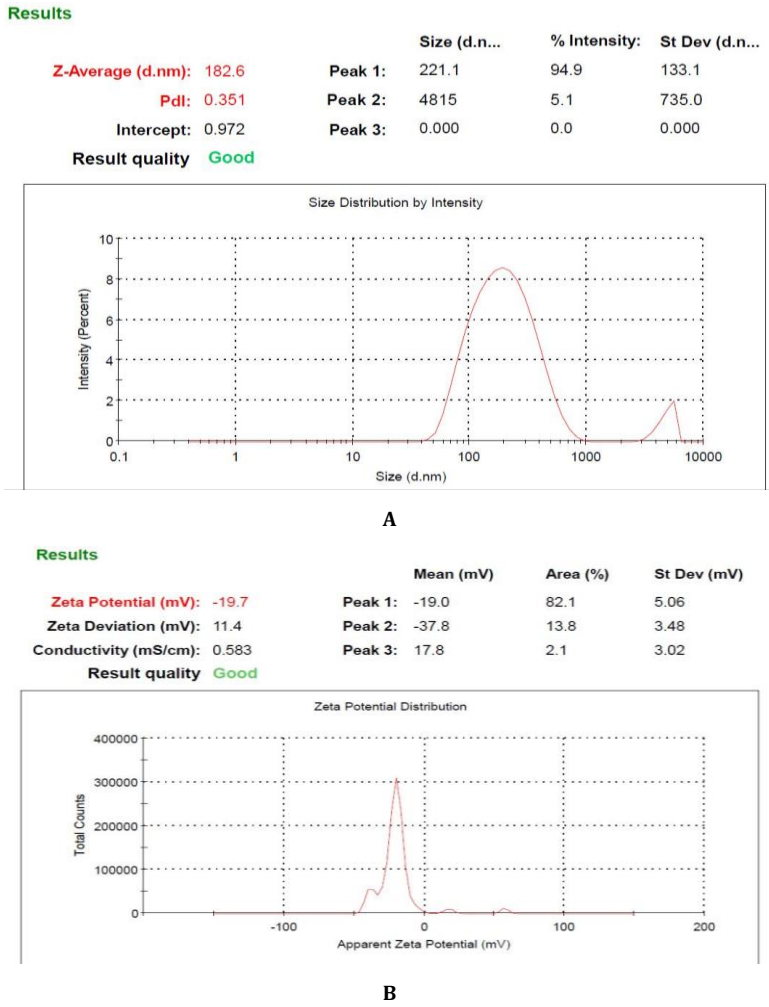

Fig. 8: (A) Size distribution by intensity of optimized self-emulsifying system of aceclofenac, (B) Apparent zeta potential (average) of optimized self-emulsifying system of aceclofenac

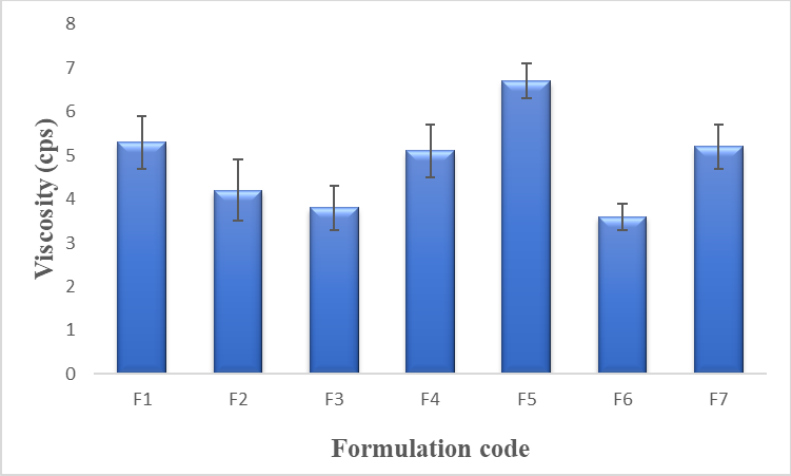

Fig. 9: Viscosity of prepared SEDDS preconcentrates (Data indicate mean±SD, n=3)

Fig. 10: laser light scattering through formulation F2

Table 7: Drug content of the prepared SEDDS formulations

S. No.	Formulation code	Drug content*= Practical content/Theoretical × 100 (%)
1.	F1	94.84±0.25
2.	F2	96.31±0.36
3.	F3	94.49±0.28
4.	F4	93.44±0.37
5.	F5	94.54±0.48
6.	F6	95.77±0.62
7.	F7	94.48±0.53

*Data indicate mean±SD (n=3)

***In vitro* dissolution study**

The dissolution profile of aceclofenac from various self-emulsifying formulations was determined and compared with the dissolution of plain aceclofenac in phosphate buffer pH 6.8, acetate buffer solution pH 4.5, 0.1 M hydrochloric acid solution pH 1.5. In 60 min the drug released from the developed aceclofenac formulations F1, F2, F3, F4, F5, F6, F7 was found to be highest in phosphate buffer pH 6.8. The dissolution data from phosphate buffer pH 6.8, acetate buffer solution pH 4.5, 0.1 M hydrochloric acid solution pH 1.5 is presented in table 8, table 9 and table 10, respectively. The graphical representation of the data shown in fig. 11A, fig. 11B and fig. 11C respectively.

Table 8: *In vitro* release of aceclofenac from prepared self-emulsifying pre-concentrates in 0.1 M hydrochloric acid solution (pH 1.5)

Time (min)	% Cumulative drug release*								
Formulation code	**5**	**15**	**30**	**45**	**60**	**90**	**120**	**180**	**240**
F1	15.21±0.5	20.42±0.4	54.80±0.4	62.90±0.6	81.91±0.3	81.62±0.3	82.21±0.5	81.71±0.4	81.71±0.5
F2	15.87±0.4	22.22±0.4	54.61±0.3	70.60±0.4	82.41±0.3	82.41±0.5	83.62±0.4	83.66±0.5	83.53±0.3
F3	16.81±0.3	31.21±0.5	55.90±0.3	71.40±0.5	90.31±0.4	92.53±0.4	92.51±0.5	92.62±0.3	92.51±0.3
F4	16.36±0.5	20.51±0.6	45.43±0.4	60.70±0.3	73.80±0.5	73.60±0.5	74.11±0.4	73.52±0.4	73.52±0.5
F5	15.80±0.3	20.31±0.4	50.44±0.5	57.90±0.5	78.91±0.4	78.34±0.5	78.64±0.3	78.91±0.4	78.88±0.3
F6	16.78±0.4	20.81±0.5	50.98±0.6	60.80±0.6	79.90±0.5	79.91±0.4	79.88±0.5	79.90±0.4	79.89±0.4
F7	14.99±0.4	21.47±0.4	54.88±0.5	62.88±0.4	80.87±0.3	80.62±0.4	81.76±0.5	81.66±0.4	81.52±0.5
Plain Aceclofenac	11.14±0.4	20.90±0.3	47.41±0.3	53.52±0.5	53.60±0.4	53.43±0.4	53.32±0.5	53.52±0.5	53.52±0.5

*Data indicate mean±SD (n=3)

Table 9: *In vitro* release of aceclofenac from prepared self-emulsifying pre-concentrates in acetate buffer solution (pH 4.5)

Time (min)	% Cumulative drug release*								
Formulation code	**5**	**15**	**30**	**45**	**60**	**90**	**120**	**180**	**240**
F1	15.20±0.4	21.30±0.2	56.80±0.5	68.40±0.4	84.43±0.4	84.33±0.4	84.71±0.3	84.68±0.4	84.41±0.3
F2	16.32±0.2	21.50±0.4	62.70±0.4	71.30±0.3	85.70±0.5	85.74±0.2	86.47±0.5	85.73±0.4	85.70±0.3
F3	19.50±0.6	34.50±0.3	60.70±0.6	75.22±0.6	90.54±0.4	90.54±0.2	91.65±0.4	90.73±0.5	90.71±0.4
F4	17.19±0.4	22.16±0.4	49.90±0.3	62.32±0.5	75.21±0.3	75.64±0.3	75.48±0.3	75.71±0.6	75.71±0.3
F5	18.38±0.4	22.30±0.6	53.40±0.5	60.23±0.4	81.24±0.6	82.54±0.3	82.43±0.3	82.24±0.3	82.24±0.5
F6	18.41±0.3	23.32±0.3	54.42±0.5	64.36±0.2	80.89±0.3	80.89±0.3	81.46±0.2	81.32±0.4	81.46±0.4
F7	15.55±0.4	20.85±0.5	57.21±0.4	66.71±0.4	84.79±0.5	84.79±0.3	84.79±0.3	84.79±0.4	84.79±0.5
Plain Aceclofenac	11.28±0.6	41.24±0.5	58.19±0.4	58.18±0.4	58.24±0.5	58.37±0.3	58.71±0.3	58.57±0.3	58.57±0.3

*Data indicate mean±SD (n=3)

Table 10: *In vitro* release of aceclofenac from prepared self-emulsifying pre-concentrates in phosphate buffer solution (pH 6.8)

Time (min)	% Cumulative drug release*								
Formulation code	**5**	**15**	**30**	**45**	**60**	**90**	**120**	**180**	**240**
F1	15.31±0.3	19.21±0.4	58.80±0.4	72.90±0.5	88.91±0.6	88.32±0.4	88.63±0.3	88.91±0.4	88.91±0.3
F2	21.94±0.3	40.34±0.5	59.70±0.3	74.50±0.5	93.54±0.5	94.27±0.2	94.43±0.5	94.27±0.4	94.27±0.3
F3	16.80±0.3	22.76±0.4	61.30±0.3	77.63±0.5	90.32±0.4	90.77±0.2	90.62±0.4	90.62±0.5	90.62±0.4
F4	18.22±0.4	24.31±0.3	51.73±0.4	65.21±0.4	79.35±0.3	79.35±0.3	78.62±0.3	79.35±0.6	79.35±0.3
F5	15.60±0.3	22.20±0.3	55.80±0.3	66.35±0.3	85.22±0.4	86.38±0.3	86.14±0.3	86.62±0.3	86.62±0.5
F6	19.88±0.4	32.26±0.5	58.93±0.5	68.88±0.3	86.73±0.4	86.79±0.3	86.79±0.2	86.75±0.4	86.75±0.4
F7	15.24±0.5	20.98±0.4	58.21±0.4	71.52±0.4	87.44±0.5	87.44±0.3	87.44±0.3	87.44±0.4	87.44±0.5
Plain Aceclofenac	12.22±0.4	20.10±0.3	55.20±0.4	58.40±0.3	58.74±0.3	58.58±0.3	58.68±0.3	58.69±0.3	58.55±0.3

*Data indicate mean±SD (n=3)

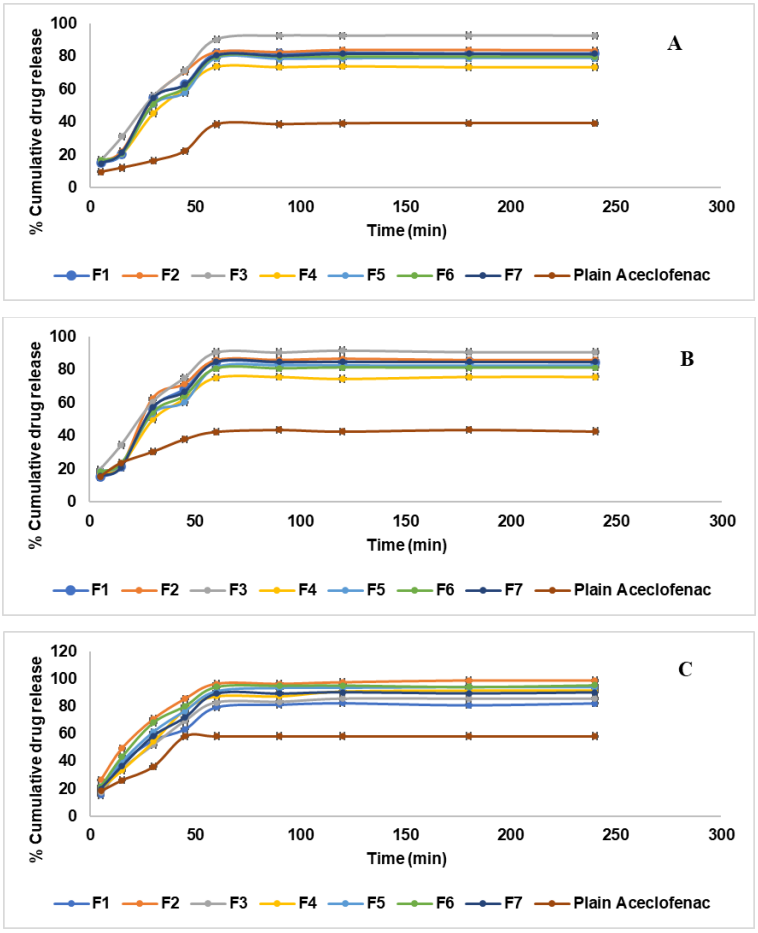

Fig. 11: (A) Percentage cumulative drug release of aceclofenac from the prepared formulations and plain drug in 0.1 M hydrochloric acid solution (pH 1.5); (B) Percentage cumulative drug release of aceclofenac from the prepared formulations and plain drug in acetate buffer solution (pH 4.5); (C) Percentage cumulative drug release of aceclofenac from the prepared formulations and plain drug in phosphate buffer solution (pH 6.8), (Data indicated as mean, n=3)

DISCUSSION

Aceclofenac is a BCS Class-II drug and hence it exhibits poor aqueous solubility. To deliver the aceclofenac successfully *in vivo* it must exhibits good solubility in the carrier system. Hence on the basis of good solubility of aceclofenac in the almond oil and Brij-35, Brij-58 and Brij-98, these are selected as vehicles for development of self-emulsifying drug delivery system of aceclofenac.

The microemulsions formed with Brij-35 and Brij-58 along with ethanol have found to have greater stability in case of water contents as high as>95%, as compared to the microemulsions formed with Brij-98 and ethanol. The optical clarity was also better in case of Brij-35 and Brij-58 containing systems. It has been observed that in case of Brij-98 containing systems, the high surfactant concentration is required to form a microemulsion. Also it has been observed that Brij 58+ethanol-containing systems are superior in case of optical clarity among all the three surfactants. The reason which can be attributed for the same is the difference in structure of the surfactants. Brij-35 contains hydrophobic chain of 12 carbons and Brij-58 contains hydrophobic chain of 16 carbons, so the hydrophobic core radius is greater in case of Brij-58; hence it acts as better solubilizer for the drug. However, in case of Brij-98 the hydrophobic chain length consists of 18 carbons which means large core for drug solubilisation but still the microemulsifying-region as well as optical clarity is less in case of Brij 98+ethanol containing systems. The reason which can be attributed for this observation is the double bond present in the structure of Brij-98 as depicted in fig. 12A leads to decreased flexibility of chain of the surfactant due to which the hydrophobic core in which the drug is intended to get dissolved get exposed to water surface due to, which reduced solubilization of drug in the core results, which leads to poor micro–emulsification of the system [42]. However, in case of Brij-35 and Brij-58 containing systems, the chain of surfactant possess higher flexibility due to which the folding of chain of Brij-35 and Brij-58 is likely to occur and thus it provides better shielding along with the shielding effect of ethanol of the hydrophobic core from the outside environment as shown in fig. 12B [43]. However, Brij-58 has greater microemulsifying region and optical clarity as compared to Brij-35 containing systems is large drug solubilisation core as well as a smaller number of polyoxyethylene groups in Brij-58. The number of polyoxyethylene groups in Brij-58 is 20 and in Brij-35 is 23. As the number of polyoxyethylene group is more in Brij-35 this means more hydration of Brij-35+ethanol containing systems, which may lead to less protection of hydrophobic core of Brij-35 containing systems from outside environment [44].

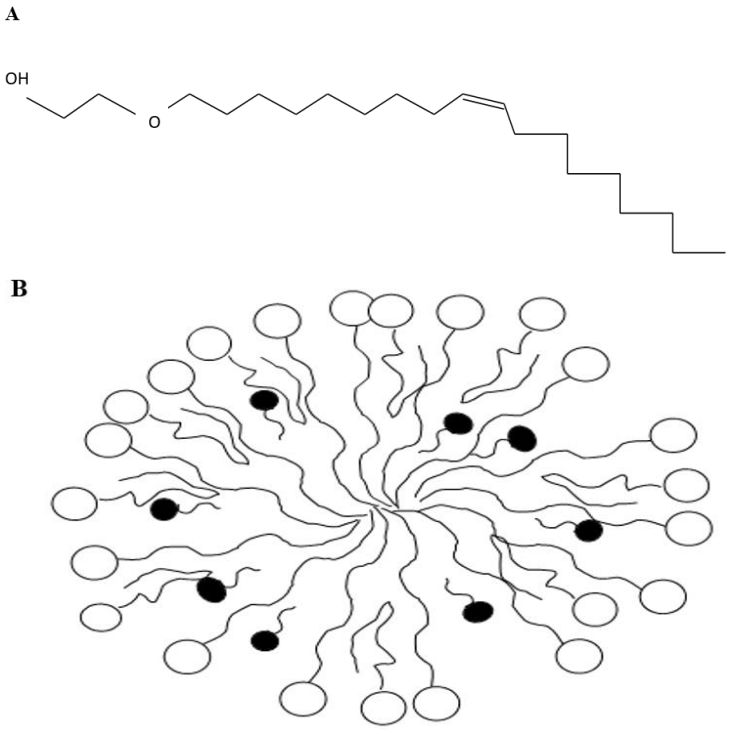

Fig. 12: (A) Structure of Brij 98; (B) Folding of Brij 58 and Brij 35 chains which provide better shielding to hydrophobic core (the filled black circles represents the ethanol molecules)

On applying simplex lattice design, it is evident from mixture contour plot and mixture surface plot that as the proportion of Brij-58 and increases the emulsification time decreases; with increase in proportion of oil it increases. The model fit summary indicates good fit with R square value of 83.96% and adjusted R square value of 75.95%. ANOVA indicates good correlation between excipients concentration and emulsification time (p value<0.05). Multiple regression analysis is used to generate equation 1, the high coefficient of oil (64.1) indicates that an increase in concentration of oil will lead to an increase in emulsification time, negative coefficient of Brij 58 (-18.9) indicates that increase in the concentration of Brij-58 leads to decrease in emulsification time, low coefficient of ethanol (42.1) indicates that concentration of ethanol has very little effect on emulsification time.

YET = 64.1A-18.9B+42.1C-180AB-307AC-157 BC…….. (1)

In above equation A, B, C are almond oil, Tween 80 and PEG 400, respectively. The terms AB, AC and BC shows the interaction between main effects A, B and C.

Percentage transmission depends on relative proportions of almond oil, Brij-58 and ethanol. It is evident from the mixture contour plot and mixture surface plot that as the proportion of Brij-58 increases the percentage transmission increases. The model fit summary indicates good fit with R square value of 79.56% and adjusted R square value of 69.34%. ANOVA indicates good correlation between excipients concentration and emulsification time (p value<0.05). Multiple regression analysis is used to generate equation 2, the high coefficient of Brij-58 (110.56) and ethanol (87.38) indicates that increase in concentration of Brij-58 and ethanol will lead to an increase in percentage transmission, relatively low coefficient of almond oil (72.65)

indicates that concentration of almond oil has comparatively low effect on percentage transmission.

YPT= 72.65A+110.56B+87.38C-36AB+80AC-45BC…… (2)

In above equation A, B, C are almond oil, Brij-58 and ethanol, respectively. The terms AB, AC and BC shows interaction between main effects A, B and C.

Drug release depends on relative proportions of almond oil, Brij-58 and ethanol. It is evident from mixture contour plot and mixture surface plot that as the proportion of Brij-58 increases the drug release increases; with an increase in proportion of oil it decreases. The model fit summary indicates good fit with R square value of 82.33% and adjusted R square value of 73.50%. ANOVA indicates good correlation between excipients concentration and emulsification time (p value<0.05). Multiple regression analysis is used to generate equation 3, the high coefficient of Brij-58 (62.33) and ethanol (32.34) indicates that an increase in concentration of Brij-58 and ethanol will lead to increase in drug release.

YDR = 1.05A+62.33B+32.34C+10AB+28704AC-165BC…… (3)

In above equation A, B, C are almond oil, Brij-58 and ethanol, respectively. The terms AB, AC and BC shows the interaction between main effects A, B and C.

Characterization of SEDDS of aceclofenac indicated that, formulation F4 consists of a total of 70% of surfactant concentration and formulation F1 consists of a total of 80% of surfactant concentration, this indicates 35:35 proportion of Brij58: ethanol leads to swift emulsification. The emulsification time for different formulations follows the order F4<F2<F5<F7<F3<F6<F1. This indicates that high surfactant concentration leads to quick dispersion. Also, it was observed that all the formulations got dispersed within one minute, which indicates good dispersibility of the formulations.

The percentage transmission for all the formulations follows the order F1<F7<F3<F5<F4<F6<F2. The results show that maximum transparency is found when surfactant concentration is 70% and oil proportion is 30%. This may be due to the fact that given quantity of drug gets solubilized completely in 30% oil and 70% (when Brij-58 is 40% and ethanol is 30%) surfactant mixture (SMIX) and the same mixture is able to form very fine dispersion. Also it has been observed that when Brij 58 concentration is relatively high as compared to oil then the transparency decreases as in case of formulation F8 and F12. However, it is observed that the transparency of all the formulations was above 90% which is an indicative of good micro emulsification [45].

The results indicates that pH of all the formulations is near to the physiological pH of 7.4 [46].

The particle size indicates that the self-emulsifying preconcentrate is capable of forming a fine microemulsion upon dilution [47]. The polydispersity index indicates that the formed microemulsion is homogeneous [48].

The apparent zeta potential indicates that the formed microemulsion possess high stability [49].

The viscosity values in general, indicate that the formulations which contain high oil proportion and very high concentration of Brij-58 have high viscosity values [50]. Formulation F12 has the highest viscosity values at 20 rpm and it contains 20% of almond oil, 56% of Brij-58 and 24% of ethanol, the reason which can be attributed to this may be the high concentration of Brij-58 which may have led to the formation of highly concentrated micellar solution.

The result indicates that the refractive index of prepared formulations is close to refractive index of water 1.33, so it can be inferred that all the formulations were isotropic in nature [51].

The observed tyndall effect confirms that the given solution contains particles of colloidal dimensions; hence it can be inferred that the microemulsion has been formed [52].

The highest drug content was found in case of formulation F2 i. e. 96.31%, which indicates that when Brij-58 and ethanol are used in 40:30 ratio, respectively that lead to development of optimized globule size and the hydrophobic area, which is sufficient to hold the drug in its highest possible concentration with respect to present study [53].

The dissolution rate of aceclofenac from the plain drug was found to be quite low as compared to developed formulations. Also it has been observed that the drug release increases slightly with increase in pH, which may be due to high solubility of acidic drugs in basic media. The drug release was found to be highest from formulation F2 and follows the order F4<F5<F6<F7<F1<F3<F2 in phosphate buffer pH 6.8. The drug release indicates that the formulation with highest drug content showed highest drug release. Also the, the formulation F2 contains 40:30 ratio of Brij-58 and ethanol, respectively, which indicates this combination produce lowest interfacial tension and spontaneously converts into microemulsion [54].

CONCLUSION

The effect of the difference in chain length of different non-ionic surfactants of Brij series has been investigated on the self-emulsifying efficiency of SEDDS of aceclofenac. largest microemulsifying region was found to be in case of Brij 58 and ethanol-containing systems as inferred from the pseudoternary diagram study. The reason which can be accounted for this observation is lesser number of polyoxyethylene groups in Brij-58 and flexible linear chains due to absence of double bond, which leads to lesser hydration and more protection of hydrophobic core.

It has been observed that when Brij surfactants are used the surfactant having longer hydrophobic chain length, absence of unsaturation and lesser number of polyoxyethylene groups (as in case of Brij-58) leads to increased self-emulsifying efficiency and results in finer microemulsion formation. The stability of the microemulsion so formed is found to be good with a zeta potential value of-19.73 mV.

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AUTHORS CONTRIBUTIONS

1. Shailendra Chouhan: Study conception, design and data analysis, manuscript preparation.

2. lalit Singh Chauhan: Overall guidance and data revision and checking.

3. Hemant Khambete: Guidance, data analysis and data interpretation, manuscript preparation.

All authors reviewed the results and approved the final version of the manuscript.

CONFLICTS OF INTERESTS

Declared none

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