AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

An Approach to Enhance the Solubility and Bioavailability of Poorly Water Soluble Drug Aceclofenac by Self-Emulsifying Technique Using Natural Oil

Suyash Adhikari*[1], Senthil Kumar.K[1], Ankit Acharya[1], Mohammad Gulzar Ahmed[1], Gummadi Aswini[1] and Arjun Sapkota[2]
1.Department of Pharmaceutics, Sri Adichunchanagiri College of Pharmacy, B.G.Nagara, Karnataka -571448, India
2.Department of Pharmacology, Mallige College of Pharmacy, Banglore-90, Karnataka, India

ABSTRACT

Self-emulsifying drug delivery system (SEDDS) is an isotropic mixture of drug, oil, surfactant and co-surfactant which spontaneously forms emulsion in aqueous environment under gentle agitation. Many drugs are lipophilic in nature making them difficult for oral delivery as the GI environment is aqueous in nature. The objective of present work was to develop and evaluate the SEDDS of Aceclofenac using oleic acid as oil, tween 20 as surfactant and PEG 400 as co-surfactant. Compatibility studies showed no interaction between drug and excipients used. Ternary phase diagram was constructed to optimize the formulation. Formulated SEDDSs were evaluated for drug content, zeta-potential, robustness to dilution, globule size and *in-vitro* drug release. Globule size were between 151.1-182.3 nm and spherical in shape. *In-vitro* drug release study revealed that the drug release form the formulated SEDDSs were faster when compare to pure drug and marketed product. Formulation S6 showed the highest drug release i.e. 91.71% within 25 m. Formulations were stable during testing period of 3 months. From this work, it was cleared that the SEDDS of Aceclofenac was found to be significant in terms of releasing of the drug as compare to pure drug and marketed product.

Keywords: Self-emulsifying drug delivery system, Aceclofenac, Oleic Acid, Tween 20, PEG 400.

*Corresponding Author Email: adhisuyash@gmail.com
Received 5 May 2015, Accepted 06 June 2015

Please cite this article as: Adhikari S *et al*., An Approach to Enhance the Solubility and Bioavailability of Poorly Water Soluble Drug Aceclofenac by Self-Emulsifying Technique Using Natural Oil. American Journal of PharmTech Research 2016.

INTRODUCTION

Aceclofenac (2-[(2, 6- dichlorophenyl) amine] phenyllacetoxyacetic acid) is a non-steroidal anti-inflammatory drug used in the treatment of arthritis and for the pains of various etiologies, such as musculoskeletal pain, dental pain or post-surgical pain. Aceclofenac is orally effective NSAIDs with less gastrointestinal effects. Aceclofenac is poorly water soluble drugs as a result its absorption depends on dissolution rate. To increase the solubility of Aceclofenac, various approaches has been made, which includes surfactant addition, solid dispersion, micronization, permeation enhancers, salt formation, complexation and self-emulsifying techniques. As Aceclofenac is lipophilic drug, self-emulsification is considered as better technique for enhancing its the solubility as well as bioavailability, where dissolution step is not required as drug already is in solution[1].

Self-emulsifying drug delivery systems (SEDDS) are the mixtures of oils, surfactants, sometimes co-solvents, and ideally isotropic, which emulsify spontaneously to produce fine oil-in-water emulsions under gentle agitation when placed in aqueous phases[2]. After the administration, digestive motility of the stomach and the intestines will provide enough agitation for the spontaneous formation of emulsions. In the case of poorly water soluble drugs where absorption depends on dissolution, the SEDDS offer a way to improve the rate and extent of oral absorptions[3]. SEDDS have a unique property as they are able to form fine oil/water emulsions in gastro-intestinal fluids .These formed fine emulsion produce small droplets of oil dispersed in GIT that provide a large interfacial area increasing the activity of pancreatic lipase to hydrolyze the oil thereby promoting faster release of drug. Furthermore, surfactant used in the formulation increases the bioavailability of the drug by activations of different mechanisms, maintaining the drug in solution and thus, avoiding the dissolution step from the crystalline state and enhancing intestinal epithelial permeability at the same time[4]. Lipid exerts the effect on oral bioavailability of the drug by several mechanisms, including protection of the drug from enzymatic or chemical degradation in the oil droplets and activation of lipoproteins promoting the lymphatic transport of lipophilic drugs[5].

Natural oils such as oleic acid, sunflower oil, castor oil, soya bean oil, coconut oil and clove oil were used in formulation. Likewise surfactant and co-surfactant were also used. Oil dissolved the drug and surfactant helps in the spontaneous formation of emulsion in aqueous phase. Co-surfactant acts as co-solvent to increase the solubility of drug in oily phase or help in formation of micro-emulsion[4, 5].

The main objective of this work is to increase the solubility and bioavailability of Aceclofenac by self-emulsifying technique using natural oil, surfactant and co-surfactant.

MATERIALS AND METHOD

Drugs and chemicals:

Aceclofenac was obtained as gift sample from Vijayadeep Pharmaceutical Nepal. Oleic acid, PEG 400 and Tween 20 were procured from S.D. Fine Chem. Ltd, Mumbai, India. All other ingredients were analytical grade.

Compatibility study

IR spectroscopy is one of the important analytical techniques for chemical identification. The drug polymer interaction was studied by FT-IR spectroscopy. The spectra were recorded for pure drug using FT-IR.

Solubility study of drug in various excipients

Solubility of Aceclofenac was determined in different oils, surfactants and co-surfactants by dissolving an excess amount of Aceclofenac in 3ml of oil, and other components using a stirrer at $37^{o} \pm 0.5$ for 72 h. The equilibrated samples were then centrifuged at 1000 rpm for 30 m to remove the undissolved drug. The solubility of Aceclofenac was determined by analyzing the filtrate spectrophotometrically at 275 nm[6].

Oils used were oleic acid, olive oil, sunflower oil, castor oil, coriander oil.

Surfactants were span 80, tween 20, span 20, tween 80.

Co-surfactant was polyethylene glycol 200, 400.

Construction of Pseudo-ternary Phase Diagram

Pseudo-ternary phase diagrams of oil, surfactant/co-surfactant, and water were developed using the water titration method. The weight ratio of surfactant to co- surfactant (Km) was varied as 1:1, 2:1 and 3:1. For each pseudo ternary phase diagram at a specific surfactant/co-surfactant weight ratio, oil and surfactant/co-surfactant mixture were mixed thoroughly in different weight ratios (1:9, 2:8, 3:7, 4:6, 5:5, 132 6:4, 7:3, 8:2, and 9:1). Water was added drop by drop to the mixture of oil and surfactant/co-surfactant under magnetic stirring at 37 ° until the mixture became clear or persistent turbidity, and then the concentrations of the components were recorded[7].

Preparation of self-emulsifying drug delivery systems

The formulations were prepared by initially dissolving the accurately weighed amount of Aceclofenac in co-surfactant at 60^{o} in an isothermal water bath. Oil was then added and mixture was cooled to ambient temperature. Then surfactant and co-surfactant was added and the final

mixture was mixed by stirring until a clear solution was obtained. The formulation was equilibrated at ambient temperature for at least 48 h, and examined for signs of turbidity or phase separation prior to self-emulsification and particle size studies. Final formulation was filled in hard gelatin capsule and stored in well closed container[8] (table 1).

Table 1: Formulation development of Aceclofenac SEEDS

Formulation code	Drug in mg Aceclofenac	Surfactant: Co-surfactant (1:1) Tween 20:PEG 400	Oil Oleic acid
S1	50mg	40%	60%
S2	50mg	50%	50%
S3	50mg	60%	40%
S4	50mg	70%	30%
S5	50mg	80%	20%
S6	50mg	90%	10%

Evaluation of SEDDS of Aceclofenac

Formulated SEDDS of Aceclofenac was evaluated for various parameters such as compatibility, drug contents, globule size, drug release, release kinetic and stability studies.

Visual observation of self-emulsification efficiency

Self-emulsification property was performed by visual observations using USP XXII dissolution apparatus 2 where 1 ml of each formulation was added to 200 ml water maintained at temperature 37±0.5°. Gentle agitation was provided by dissolution apparatus paddle rotating at 50 rpm. The tendency to emulsify spontaneously was monitored visually and assessed using grading system[9] (table 2).

Table 2: Visual assessment of efficiency of self-emulsification

Grade	Dispersibility and appearance	Time
I	Rapid forming micro-emulsion which is clear or slightly bluish in appearance	< 1 min
II	Rapid forming, slightly less clear emulsion which has a bluish white appearance	< 2 min
III	Bright white emulsion (similar to milk in appearance)	< 3 min
IV	Dull, greyish white emulsion with a slightly oily appearance that is slow to emulsify	> 3 min
V	Exhibits poor or minimal emulsification with large oil droplets present on the surface	> 3 min

Droplet size analysis particle size measurements

A 10mg quantity of the SEDDS was placed inside the ring of the internally calibrated microscopic slide (Objective micrometer) and a drop of each non-solvent used above was added for a clearer view. The slide was covered with a cover slip and viewed under a binocular microscope at a magnification of 100. Different particles of the SEDDS from a particular batch were counted manually since they were sizeable enough to be distinguished (n = 100) and the mean value was taken[10].

Robustness to dilution

Robustness of dilution is carried out for SEDDS for ensuring that the emulsion formed have similar properties at different dilutions to achieve uniform drug release profile and to ensure that the drug will not get precipitated at higher dilutions in *in-vivo* which may significantly retard the absorption of the drug from the formulation. SEDDS were diluted to 100-fold and 1000-fold with pH 6.8 phosphate buffer solutions for studying robustness to dilution. The diluted emulsions were stored for 12 h at 37.0±0.5° and observed for any signs of phase separation and drug precipitation[10].

Drug content

SEDDS equivalent to 50mg Aceclofenac was extracted into 100 ml of Phosphate buffer pH 7.4: water (1:4). The extract was then analyzed after suitable dilutions spectrophotometrically at 275 nm[11].

Zeta Potential Measurement

Electrical charges play an important role in determining the interaction between the particles of the dispersed phase and the resultant physical stability of the system, particularly for those in the colloidal size range. The potential between the tightly bound surface liquid layer (shear plane) of the particle and the bulk phase of the solution is called as zeta potential. The measurement of the zeta potential tells about the stability. Emulsion with zeta-potential greater than ±30 mV is stable. Zeta potential was observed with the help of Malvern Zetasizer[10].

Transmission Electron Microscopy

Transmission electron microscope is use as a visualizing aid for the observation of morphology of droplets. SEDDS is diluted with water (1/100). A drop of the diluted emulsion is directly deposited on the holey film grid to observe the morphology of formulations. To improve the contrast, the samples were treated with a 1 wt % phosphotungstic acid solution for 2 h, deposited on copper grids, and allowed to dry for 48 h before TEM examination. The homogeneous and spherical droplets in emulsion were observed [10].

Dissolution studies

Dissolution profiles of the self-emulsified formulations were determined using USP I Basket method at 37 ° and a rotating speed of 100 rpm in a 900 ml of pH6.8 phosphate buffer. The membrane selected was pretreated by soaking it in the dissolution medium for 24 h prior to commencement of each experiment. A 750mg quantity of the formulated SEDDS from each batch was placed in a polycarbonate dialysis membrane containing 2ml of the dissolution medium, securely tied with a thermo-resistant thread and then placed in the appropriate chamber of the

release apparatus containing the dissolution medium. Samples (5 ml) withdrawn after 5 m were filtered using a whatman filter and subjected to spectrophotometric analysis. The sample volume was replaced each time with equal quantity of fresh medium [10].

Stability studies:

Stability study of prepared formulations was carried out in two different conditions i.e 25 ^{o}/60 % RH and 40 ^{o}/75 % RH for 3 months. Change in drug content, physical appearance and dissolution profile of the best formulation were studied. This gives idea about the stability of formulation during study[12].

RESULTSAND DISCUSSION

Compatibility studies

All the characteristic peaks of Aceclofenac were present in the spectrum of drug and polymer mixture, indicating compatibility between drug and polymer. The spectrum confirmed that there was no significant change in the chemical integrity of the drug. IR spectrums were shown in table 3 and figure 1, 2.

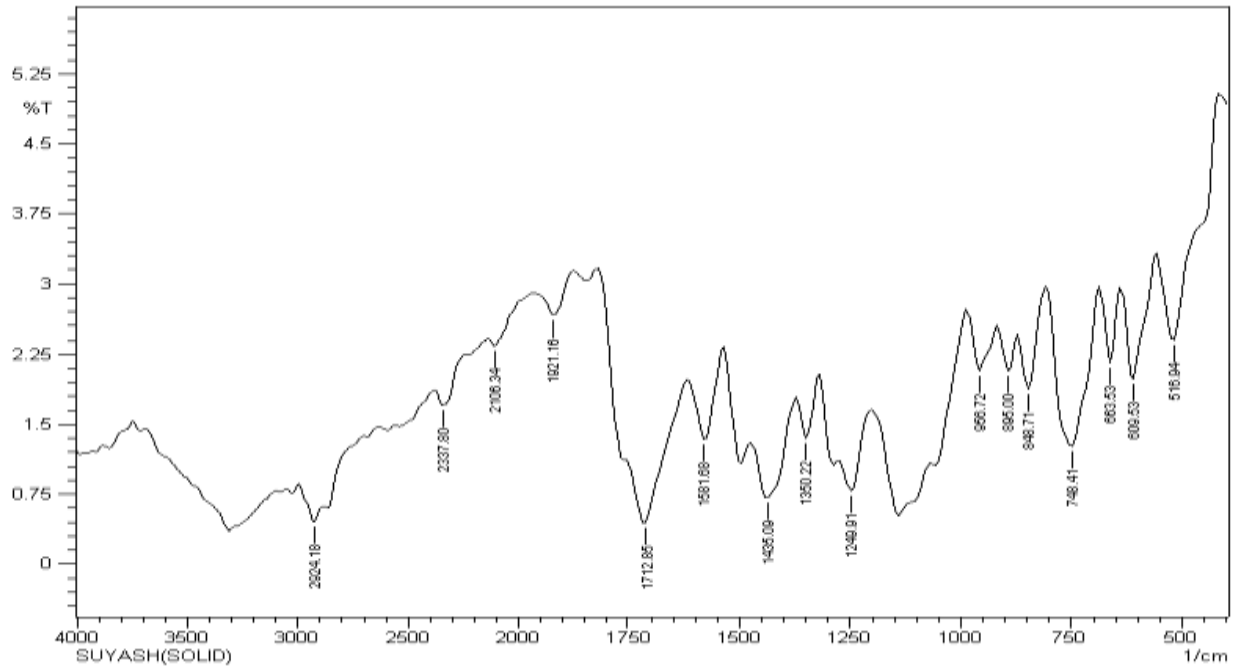

Figure 1: FT-IR Spectrum of pure drug, Aceclofenac

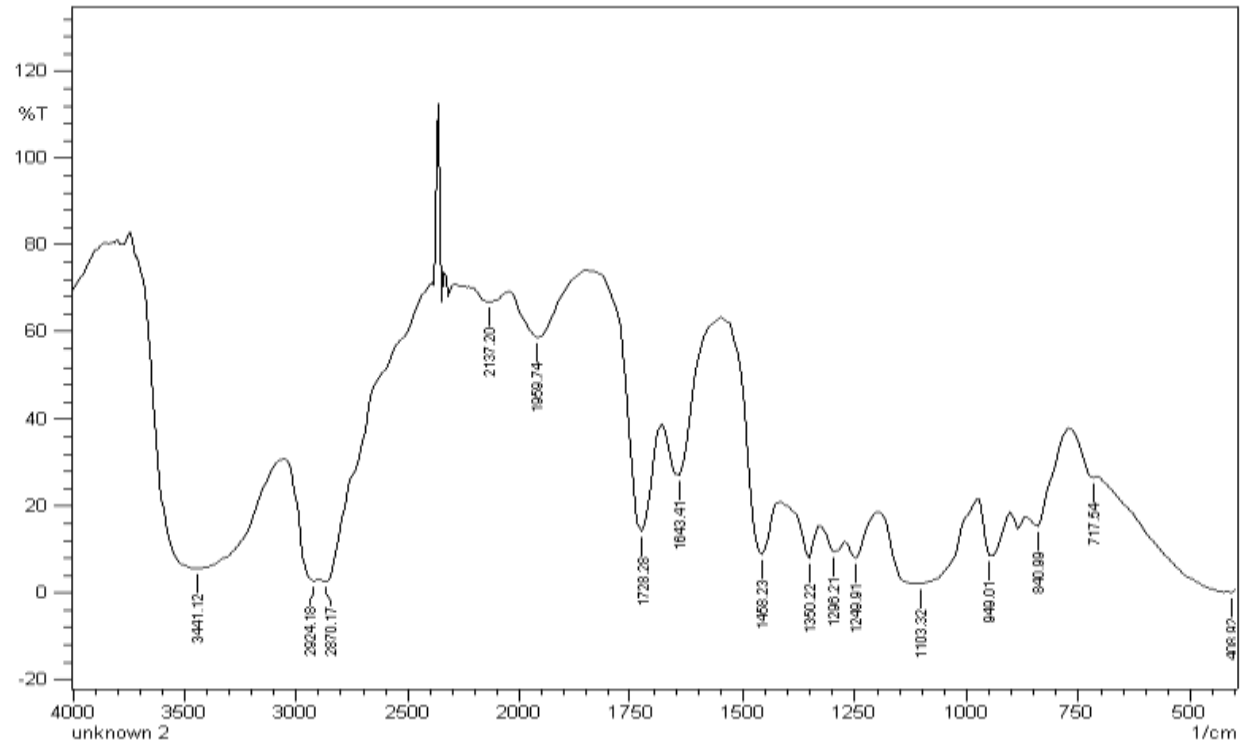

Figure 2: FT-IR Spectrum of drug and its physical mixtures

Table 3: Interpretation of FTIR- spectrum

Ingredients	Functional groups with wave number (cm^{-1})			
	C-O(s)	C-N (s)	O-H (b)	C-H (B)
Pure drug	1712.85	1249.91	1436.09	748.41
Drug + Physical mixtures	1728.28	1240.89	1458.32	725.26

Solubility studies:

The self-emulsifying formulation consisted of oil, surfactants, co-surfactants and drug. SEDDS should be a clear and monophasic liquid at ambient temperature when introduced to aqueous phase; hence it should have good solvent properties to allow presentation of drug in solution. The solubility of Aceclofenac in different vehicles is shown in figure 3. Based on this study, oil (oleic acid), surfactant (Tween 80), co-surfactant (PEG-400) was selected for the formulation of SEDDS of Aceclofenac.

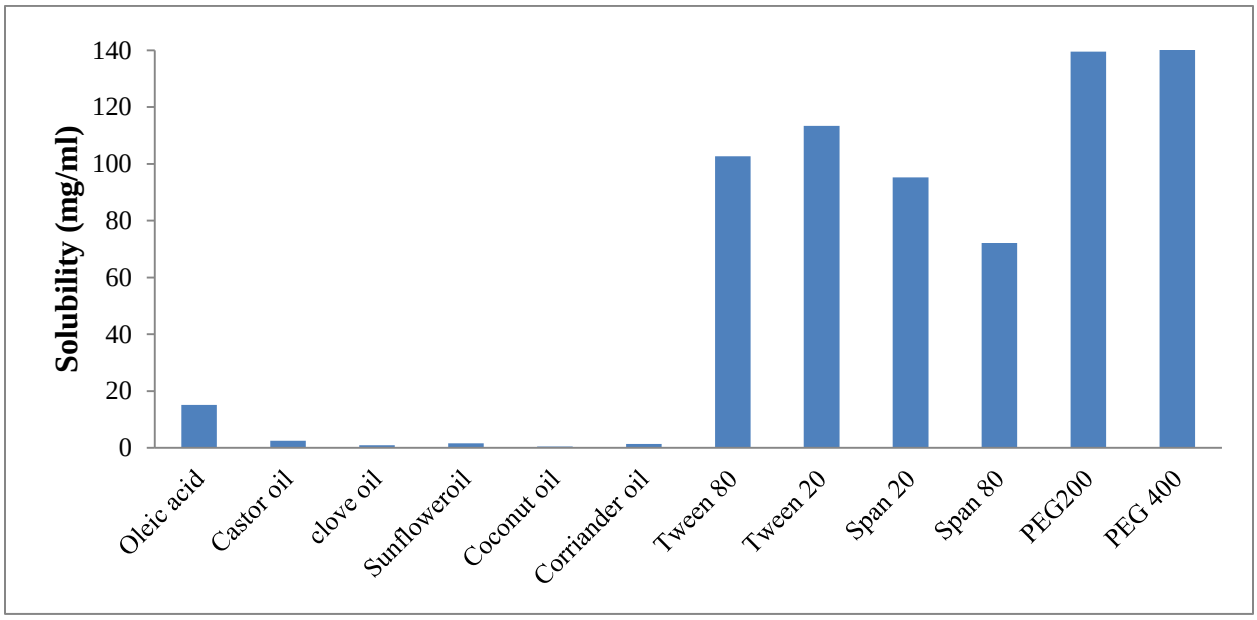

Figure 3: Solubility of Aceclofenac in different oils, surfactants and co-surfactants

Phase diagram study

SEDDS form fine oil-water emulsion with only gentle agitation, upon its introduction into aqueous media. Since the free energy required to form an emulsion is very low, the formation of emulsion spontaneous. Surfactants form a layer around the emulsion droplets and reduce the interfacial energy as well as providing a mechanical barrier to coalescence. The visual test measures the apparent spontaneity of emulsion formation. The series of SEDDS were prepared and their self-emulsifying properties were observed visually. Pseudo ternary phase diagrams were constructed to identify the self-emulsify regions and optimized concentration of oil, surfactant, and co-surfactant was used for formulation (figure 4, 5 and 6). From this study surfactant: co-surfactant (Km) ratio 1:1 has been selected as the optimized concentration.

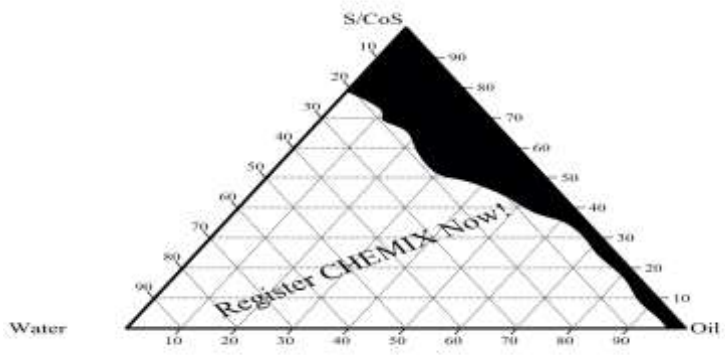

Figure 4: Ternary plot of Tween 20 and PEG 400 (1:1)

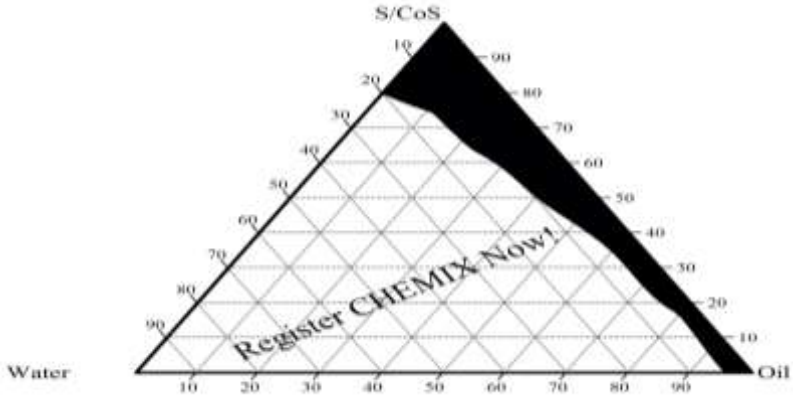

Figure 5: Ternary plot of Tween 20 and PEG 400 (2:1)

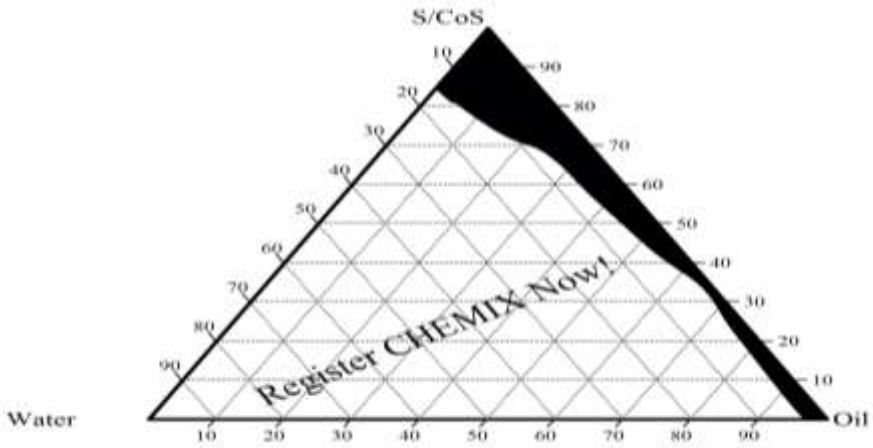

Figure 6: Ternary plot of Tween 20 and PEG 400 (3:1)

Drug content estimation

Drug content of the all Formulations was done using UV spectrophotometer (Shimadzu UV-1800). Drug content of all formulations were found in the range of 97.23%-99.45%, obtained results were in B.P limits.

Globule size

Globule sizes of all the formulations were found in the range of 151.1nm-182.3 nm. Globule size analysis showed, as the concentration of surfactant and co-surfactant increases the globule size of emulsion decreases thus forming micro- emulsion. Formulation S6 has smallest globule size of 151.1nm and S1 has largest globule size of 182.3 nm.

Visual observation of self-emulsification efficiency

Rate of emulsification of different formulations are shown in the table 4. Formulation containing higher concentration of surfactant showed less emulsification times as they get easily dispersed in water. Formulation S6 has less emulsification time.

Table 4: Self-emulsification time of SEDDS formulations

Formulations	Self-emulsification time	Visual appearance
S1	134 sec	Milky appearance
S2	112 sec	Less clear emulsion with rapid formation
S3	108 sec	Less clear emulsion with rapid formation
S4	93 sec	Less clear emulsion with rapid formation
S5	57 sec	Clear or Slightly bluish with rapid formation
S6	42 sec	Clear or Slightly bluish with rapid formation

In-vitro drug release profiles of Formulations

In-vitro drug release of different SEDDS formulations were compared with marketed product and pure drug and results were shown in figure 7. Dissolution studies were performed for the SEDDS formulations in 6.8 pH phosphate buffer. As compare to drug release of pure drug (38.4%) and marketed product (70.1%), drug release of SEDDS was more than 83% within 25 m of dissolution studies. Drug release from the SEDDS is droplet size dependent. This means emulsion with smaller droplet size has larger interfacial area, thus promoting the faster release of drug from formulation. Since the droplet size of formulation S6 is less compare to other formulations, drug release from S6 formulation is more i.e. 91.7% within 25 m.

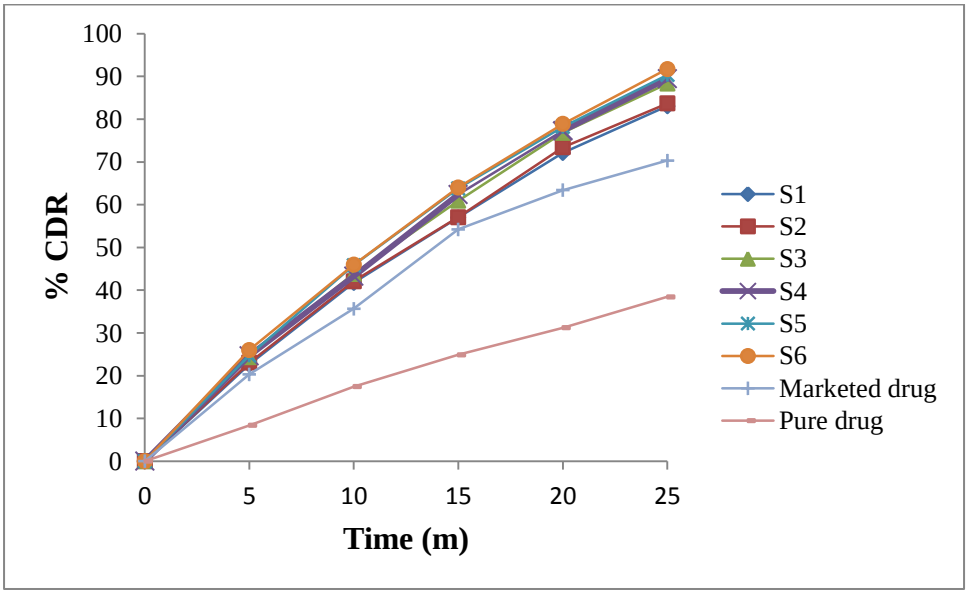

Figure 7: *In-vitro* drug release profile formulation S1-S6, Marketed drug and pure drug

Release Kinetics studies

Drug release data of all formulations were subjected to various kinetics models. From kinetic studies it was found that value of 'r' for first order ranged from 0.9602- 0.9887 and zero order ranged from 0.9814-0.9869, which is nearer to 1 compared to Higuchi's square root 0.8722-0.971.

So the formulation followed mix order pattern (zero order and first order) (table 5). The values for 'n' were above 0.89 which indicates that all the formulation followed supper class-II mechanism.

Table 5: Release exponent values and release rate constant values for formulations

Formulation code	Zero order R^2	First order R^2	Higuchi's plots R^2	Korsmeyer-Peppas plots R^2	N
S1	0.9861	0.9815	0.9016	0.9539	1.364
S2	0.9858	0.9799	0.8844	0.9531	1.366
S3	0.9814	0.9859	0.917	0.9517	1.382
S4	0.9869	0.9602	0.8726	0.9519	1.385
S5	0.9818	0.9887	0.9781	0.9509	1.3913
S6	0.9845	0.9674	0.9322	0.947	1.394

Zeta-potential of formulations

Greater the zeta potential (negative) means greater the stability of emulsions. It prevents droplet coalescence upon random collisions of particles leading to repulsive forces which can stabilize the formulation. The zeta potential of formulation S6 (-54.8 ± 1.3 mV) was high when compared to other formulations. So the formulation S6 was the most stable formulation among all formulations. Results of zeta potential of all formulations were shown in table 6.

Table 6: Zeta-potential of Aceclofenac SEEDS formulations

Formulation	Zeta-potential
S1	-25.2±1.3
S2	-29.3±1.5
S3	-35.7±2.5
S4	-44.6±1.7
S5	-46.2±1.0
S6	-49.8±1.3

Robustness to dilution

Robustness to dilution was carried out in 6.8 pH phosphate buffer to see any phase separation or precipitation in the formulation after diluting. Formulation S1 and S2 showed the sign of phase separation while remaining formulation didn't show any sigh of phase separation. Since the oil used is natural the one (oleic acid), more amount of surfactant is required for the stable emulsion. Formulation S3-S6 contains more concentration of surfactant, thus forming stable emulsion in all dilutions. Results of robustness to dilution of SEDDS formulations were shown in table 7.

Table 7: Robustness to dilution of SEDDS formulation

Formulation	Phase separation	Precipitation
S1	Phase separation	No precipitation
S2	Phase separation	No precipitation
S3	No phase separation	No precipitation

S4	No phase separation	No precipitation
S5	No phase separation	No precipitation
S6	No phase separation	No precipitation

Transmission Electron Microscopy (TEM)

TEM of the formulation S6 is shown in the figure 8. Shapes of the globules were spherical as seen in the TEM analysis. Size of particle were in the range between 150-180nm.

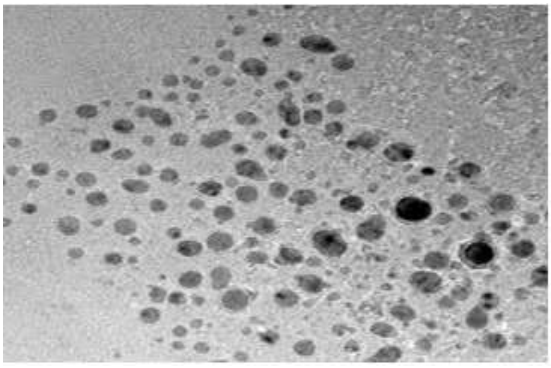

Figure 8: TEM analysis of SEDDS

Stability study

Stability studies of an optimized formulation S6 was done at two storage conditions i.e. 25^{o}/60 % RH and 40^{o}/75% RH for the period of 3 months and evaluated for drug content, release profile and physical appearance. Results indicated that there was no significant loss in drug content, release profile and physical appearance. Thus the prepared formulation was physico-chemically stable throughout the study period.

CONCLUSION

Self-emulsifying drug delivery systems are a promising approach for improving the oral bioavailability of hydrophobic drugs. The present work is an attempt to formulate SEDDSs of Aceclofenac using different concentration of oil, surfactant and co-surfactant. A SEED is a novel application in drug delivery and will solve the problems associated with the delivery of poorly aqueous soluble drugs.

REFRENCE

1. Uppugalla SR, Rathnanand M, Srinivas P, Deepak K, Kumar A, Priya S. Self-emulsifying systems of aceclofenac by extrusion/spheronization: formulation and evaluation. J Chem Pharm Res 2011; 3 (2) : 280-289.
2. Patel PA, Chaulang GM, Akolkotar A, Mutha SS, Hardikar SR, Bhosale AV. Self-emulsifying drug delivery system: a review. Res J Pharm Tech 2008; 1(4): 313-22.

3. R. Neslihan Gursoy, Simon Benita. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. Biomed Pharmacother 2004; 58(3): 173-82.

4. Suman K, Chandrasekhar VS, Balaji. Approaches for the development of solid self-emulsifying drug delivery systems and dosage forms. Asian J Pharma Sci 2009; 4(4): 240-253.
5. C. W. Pouton. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and self-micro emulsifying drug delivery systems. Eur J Pharm Sci 2000; 11(2): 93-08.
6. Eskandar M, Anayatollah S, Fatemeh L. Preparation and evaluation of Tretinoin micro emulsion based on pseudo-ternary phase diagram. Adv Pharma Bulletin 2012; 2(2): 141-47**.**
7. Wenli L, Rui T, Wenjing H, Yuntao J, Liangke Z. Preparation and evaluation of self-micro emulsifying drug delivery system of baicalein Q13. Sci Verse Science Direct 2012; 1-8.
8. Maulik JP, Natvarlal MP, Ritesh BP, Rakesh PP. Formulation and evaluation of self-microemulsifying drug delivery system of Lovastatin. Asian J Pharma Sci 2010; 5(6): 266-75.
9. Saritha D, Penjuri SCB, Nagaraju R. Formulation and evaluation of self-emulsifying drug delivery system (SEDDS) of Indomethacin. Int J Res Pharm Sci 2014; 4(2): 17-23.
10. Maulik JP, Natvarlal MP, Ritesh BP, Rakesh PP. Formulation and evaluation of self-microemulsifying drug delivery system of Lovastatin. Asian J Pharm Sci 2010; 5(6): 266-75.
11. Patel PA, Chaulang GM, Akolkotar A, Mutha SS, Hardikar SR, Bhosale AV. Self-emulsifying drug delivery system: a review. Res J Pharm Tech 2008; 1(4): 313-22.

s