Research Article www.pharmaresearchlibrary.com/ajmps **ISSN: 2348-0165**

Asian Journal of Medical and Pharmaceutical Sciences

Aceclofenac Sodium Nanoemulsion: an Approach to Improve Anti-Inflammatory Therapy

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NCADDD, 25 July 2014, Organized by Department of Pharmaceutical Technology, Anna University, BIT Campus, Tiruchirappalli–620024, Tamil Nadu, India

Abstract

Nowadays drugs with low aqueous solubility and high permeability are present in high proportion. Poorly water-soluble compounds are frequently dropped in early stages in drug discovery process. Poorly-water soluble compounds are difficult to develop into drug products by using conventional formulation techniques. Nanotechnology is seen as a promising approach for the enhancement of drug solubility. In this study, we present an approach of nanosizing a drug/polymeric complex to increase both solubility and dissolution rate of poorly-water soluble drug aceclofenac, which is widely used as anti-inflammatory drug. Nanoemulsions were prepared by solvent evaporation technique with the aim to improve anti-inflammatory therapy. The prepared formulation was evaluated for drug excipients compatibility study, polydispersity index, particle size analysis, surface morphology, zeta potential, drug release features and stability. These study results indicate the suitability of formulation procedure for preparation of nanosized poorly-water soluble drug formulation with significantly improved in vitro dissolution rate and fast onset of therapeutic drug effect.

Keywords: Aceclofenac, Nanoemulsion, Span 80, Tween 80, Dissolution Anti-inflammatory.

Contents

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Manuscript ID: NCADDD2014-AJMPS2285

PAPER QR-CODE

1. Introduction

An ideal drug delivery system is that fulfills the objective of spatial placement and temporal delivery resulting maximized therapeutic effect and least toxicity. The oral route is one the most commonly used method for administration of drugs. The only disadvantages of this route are sluggish onset time, the possibility of erratic absorption, degradation of some specific drugs by digestive enzymes. The drug delivery industry scientists had used a wide range of methods to improve the dissolution rate of poorly water-soluble drugs. With the progress in time and growth of science and technology, the dosage forms have evolved from simple mixtures and pills into highly sophisticated technology intensive systems, which are known as novel drug delivery systems (NDDS). Different approaches have materialized into various forms of NDDS such as microemulsions, multiple emulsions, liposomes, niosomes, micospheres, pharmacosomes, virosomes, dendrimers, etc., Most often the problems associated with these

delivery systems are their stability and predictability in biological systems which reduce their clinical potential, although each one is associated with its own strong points [1,2].

Nowadays Nanotechnology is a rapidly expanding field. The pharmaceuticals developed on the basis of nanotechnology are termed as Nano-pharmaceuticals. Various nano-pharmaceticals currently being used in the process of development are nanoemulsions, nanospheres, nanotubes, nanoshells, nanocapsules, lipid nanoparticles and dendrimers[3]. In recent years, one of the most popular approaches is the incorporation of the active lipophilic component into the inert lipid vehicles[4]. Among the above carrier systems, Nanoemulsion emerged as a boon of nanotechnology in the form of a novel drug delivery system (NDDS).

Nanoemulsion is considered to be a thermodynamically or kinetically stable transparent liquid dispersion used in pharmaceuticals, biomedical aids, vehicles and also the most valuable options offered to improve the oral bioavailability of poorly water-soluble drugs and reduced inter subject bioavailability of drugs that shows great promise in the future pharmaceuticals [5-11]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs to reduce pain and inflammation. Aceclofenac, an NSAID, has been recommended orally for the treatment of rheumatoid arthritis and osteoarthritis. It also has anti-inflammatory, antipyretic, and analgesic activities. The oral administration of aceclofenac causes gastrointestinal ulcers and gastrointestinal bleeding with chronic use that causes anaemia[12-13]. On the basis of above literature information, our present aim was to develop oil-in-water nanoemulsion of aceclofenac to increase the solubility, consequently improve the oral bioavailability and to deliver the drug at treatment site for better therapeutic effect.

2. Materials and Method

Drugs and Chemicals

Aceclofenac was obtained as gift samples from Cipla Pharmaceuticals, Mumbai, India; ethanol, hydrochloric acid, tween 80, span 80 and Isopropyl myristate were obtained from Loba Chemie Pvt., Ltd., Mumbai, India; methanol, ethanol, potassium dihydrogen phosphate, sodium hydroxide and sodium chloride were obtained from SD fine **chemicals, India. Dialysis membrane was procured from Sigma Aldrich, USA.**

Methodology

Formulation of Nanoemulsion

Homogenous organic phase was prepared by dissolving drug in various composition of isopropyl myristate, water miscible solvent and lipophilic surfactant. The homogenous organic phase was injected in the aqueous phase containing various proportion of hydrophilic surfactant under magnetic stirring (4000 rpm). The o/w emulsion was formed instantaneously by diffusion of the organic solvent in the external aqueous phase leading to the formation of nanodroplets. The magnetic stirring was maintained for 10 min to let the system reach equilibrium. Water miscible solvent residues were left to evaporate under slow mechanical stirring of the nanoemulsion at room temperature for about 8 hours. Formulation composition is shown in Table 1.

Drug-Excipients Compatibility Studies

Excipients are integral components of almost all pharmaceutical dosage form. Compatibility studies are very important for the successful formulation of any dosage form. Commonly DSC, FT-IR, TLC and UV techniques are used for the determination of drug compatibility. Fourier Transform Infrared Spectroscopy (FTIR) and UV studies were used for the evaluation of physicochemical compatibility and interactions, which helps in the prediction of interaction of the drug with excipients used in the formulation. The earlier reports on drug-excipient interactions recommended that 1:1 ratio of drug and excipient maximizes the possibility of interaction and helps in easier detection of incompatibilities14. Therefore, in the present study 1:1 ratio was used for the preparation of physical mixtures and analyzed for compatibility studies.

Particle size distribution & Polydispersity Index

Particle size was determined by Photon Correlation Spectroscopy (PCS) using a Zetasizer 3000 (Malvern Instruments, UK). This analysis yields the mean diameter (z-average, measuring range between 20 and 1000 nm) at 25º C, and at an angle of 90 degree (n=10). The PCS analysis yields a mean diameter (z-average) as a light intensity-weighed size of bulk population and the polydispersity index as a measurement for the width of a particle size distribution. Polydispersity index (dimensionless measure for the broadness of a particle size distribution) of the prepared formulation was determined by instrument software.

Zeta Potential

The electrophoretic mobility was obtained by a Laser Doppler Anemometer connected with the Malvern zetasizer instrument. A suitable amount of sample (50-100µL) was diluted with 5ml of water (0.45µm) and injected in the electrophoretic cell of the instrument where a potential of±150mV was set. The zeta potential values were calculated by the instrument software using Smoluchosky equation.

Transmission Electron Microscopic (TEM) Analysis

TEM helps to visualize the inherent matrix of individual particles and its shape. A drop of the suitably diluted sample was placed on to a holey carbon coated copper grid and left for 10minutes. Then grid was kept inverted and a drop of phosphotungstic acid (PTA) was applied to the grid for 10s. Excess of PTA was removed by absorbing on a filter paper and the grid was analyzed using the TECNAI-10 (PHILIPS) operated at 70-80kV at 17500 x magnification.

Drug Content

The amount of drug present in the Aceclofenac nanoemulsion was determined using phosphate buffer solution at pH 7.4. Measurements of the absorbance were carried at 273nm in UV-Visible spectrophotometer (Shimadzu, Japan, Model: 1700), finally the percentage of drug content was calculated.

In-Vitro Release Studies

In-vitro release studies were carried out by using dialysis membrane bag method. The dialysis membrane was conditioned by soaking in phosphate buffer 7.4 for 24 hours. Aceclofenac nanoemulsion of about 1mL was taken in the dialysis membrane and immersed in 200 mL of phosphate buffer solution (pH 7.4). A sample of 5 mL was withdrawn from the dissolution setup at regular intervals for 24 hours and an equal volume of phosphate buffer (pH 7.4) was replaced to maintain a sink condition. Samples were analyzed by using UV spectrophotometer at 273 nm and the amount of drug release was calculated and compared with the marketed oral dosage form.

Drug Release Kinetics

The mathematical models are used to evaluate the kinetics and mechanism of drug release from the nanoemulsion. The model that best fits the release data was selected based on the correlation coefficient (r2) value in various models. The model that gives high R value was considered as best fit of release data.

Stability Study of Aceclofenac nanoemulsion

The prepared nanoemulsion was tightly sealed in amber colored bottles and kept in a place at room temperature. Then at regular intervals the stability samples was tested for its physicochemical parameters and drug content. The stability study was carried out for a period of 3 months (0, 1, 2 & 3 months).

3. Results and Discussion

Low oral bioavailability of poorly water-soluble drug poses a major challenge during drug formulation development. In Nanoemulsion the drug is loaded into the inner phase of these systems and delivered by lymphatic route, bypassing the enzymes in the gastrointestinal tract (GIT) and reducing the pre systemic clearance and hepatic first pass metabolism. Nanoemulsion have a higher solubilization capacity, better thermodynamic stability, long self-life, rapid onset of action, the nanosized droplets leading to enormous interfacial areas of the drug associated with nanoemulsion would influence solubilization behavior, transport properties as well as absorption across the mucosa are an important promising factor to achieve sustained and optimum targeted drug delivery with reduced inter subject bioavailability of drugs more reproducible.

FT-IR and UV studies were performed to investigate chemical interactions between drug and the excipients. The FTIR of aceclofenac shows intense bands at 1769.55, 1716.32, 1503.91 and 1250.35cm-1 corresponding to the functional groups C=O, -COOH, -NH and –OH groups; these characteristic bands were present in the formulation composition. No new bands or shift in characteristic peaks were appeared. IR spectra are shown in Fig. 1a to 1e. In UV technique, the UV spectrum of drug is super impossible with the spectrum obtained with drug excipients mixtures and there is no change in the λmax 273 nm between the drug and drug excipients mixtures. FT-IR and UV results revealed that there is no interaction between the drug and the excipients used in the formulation. Ten different compositions of formulations were prepared with various ratios of drug and polymer. The best formulation was optimized based on the particle size. Since, reduced particle size helps in the improvement of solubility of poorly soluble drug thereby increase in the dissolution and bioavailability.

The polydispersity (PDI) of optimized formulation F8 was measured and the value was found to be 0.175; which indicates the broad distribution of particles. An increase or decrease in the particle size of the drug in a formulation can affect its in vitro release and subsequently its bioavailability. For emulsion based products, the particle size of droplets of the internal phase have an impact on the stability of emulsion itself. The mean particle size was found to be 17.51 nm. The particle size distribution is shown in figure 2.

TEM for the prepared formulations was examined using TECNAI-10 (PHILIPS) operated at 70-80kV at 17500 x magnification. The TEM results showed that the particles were of nanometer in size range with uniform, spherical with smooth surface. The TEM image is shown in figure 3. The zetapotential was determined by using Malvern zetasizer, it was found to be 0.179 mV; the zeta potential greater than +30 mV and smaller than −30 mV is normally considered stable. The formulation shows an accepted value for good stability, the zeta potential distribution is shown in figure 4. In vitro release studies are generally employed as a quality control tool for product release and in

predicting toxicity of the formulations. In order to assess the ability of a formulation to deliver a drug, it is important to determine the drug's release rate from its vehicle. In-vitro drug release study of aceclofenac nanoemulsion was performed in open tube method by using dialysis membrane. The cumulative percent release was found to be 99.1 % for 5 hrs. A comparative study was performed with the marketed tablet to know the pattern of drug release.

Based on the release study results formulation F8 shows maximum release in 5 hrs. Hence formulation F8 has been taken for the comparative in vitro release studies with the market formulation. A comparative release study was performed with the marketed tablet to know the pattern of drug release. Comparative release study results revealed that 99.0% w/w and 78.1% w/w of drug release was observed after 5 hours for Nanoemulsion formulation and marketed product respectively. The release results revealed that aceclofenac nanoemulsion showed faster onset of action. This also proves the advantage of nanoemulsion in delivering aceclofenac to improve anti-inflammatory therapy. The comparative in vitro release result is shown in Figure.5.

The regression coefficient (R2) for the drug release kinetic studies were found to be 0.987, 0.981, 0.966, 0.878 and 0.946, for Hixon crowell model, Korsmeyer peppas, Higuchi plot, Zero order and first order model respectively. The study shows that the Aceclofenac nanoemulsion follows First order and Hixon crowell release kinetics. Stability of the emulsion is dependent on the particle size. As the particle size reduces to the Nano size, the surface energy of the particles will be increased and they tend to agglomerate. So stabilizers are used which will decrease the chances of Ostwald ripening and improving the stability of the emulsion by providing a steric or ionic barrier. The physicochemical parameters like appearance, particle size and in vitro release were found to be satisfacotry, and there is no change with respected to the initial analysis results.

Table 1: Compositions of Nanoemulsion formulation

S.No	Ingredients	Formulation									
		F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
1.	Drug (mg)	100	100	100	100	100	100	100	100	100	100
2.	Isopropyl myristate (ml)	6	5	5	4	4	3	2	1	1	1
3.	Span80 (ml)	3	4	3	3	2	1	1	2	1	3
4.	Methanol (ml)	1	1	2	2	1	1	2	2	3	1
5.	Ethanol (ml) (co-surfactant)	10	9	9	8	8	8	8	8	8	8
6.	Tween80 (ml)	10	9	8	9	8	8	8	8	8	8
7.	Water (ml)	20	22	23	26	27	29	29	29	29	29

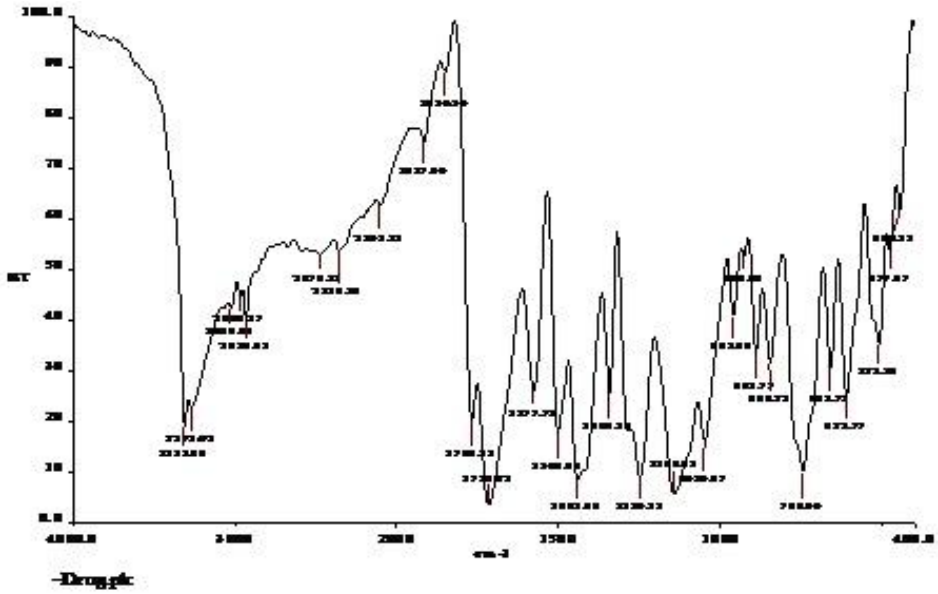

Figure 1a: FTIR spectrum of Aceclofenac

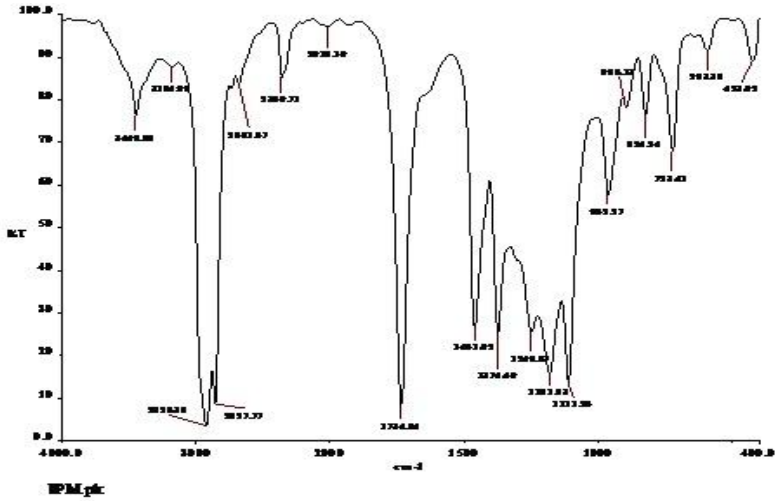

Figure 1b: FTIR spectrum of Isopropyl myristrate

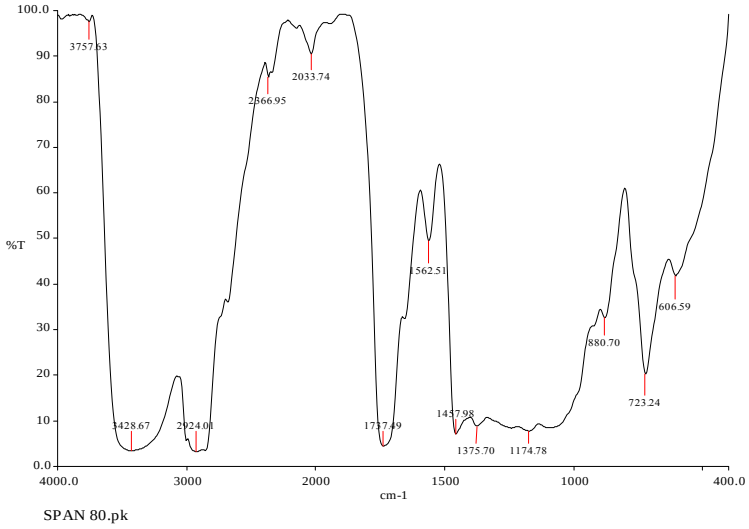

Figure 1c: FTIR spectrum of Span80

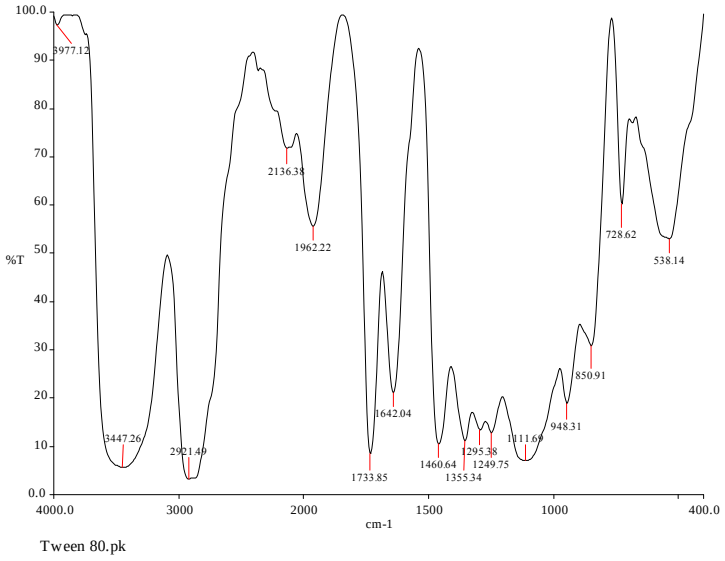

Figure 1d: FTIR spectrum of Tween80

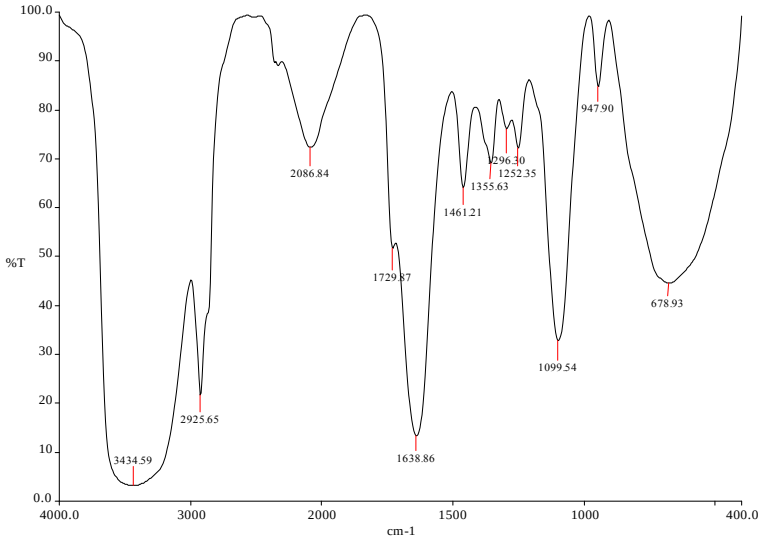

Figure 1e: FTIR spectrum of Aceclofenac nanoemulsion

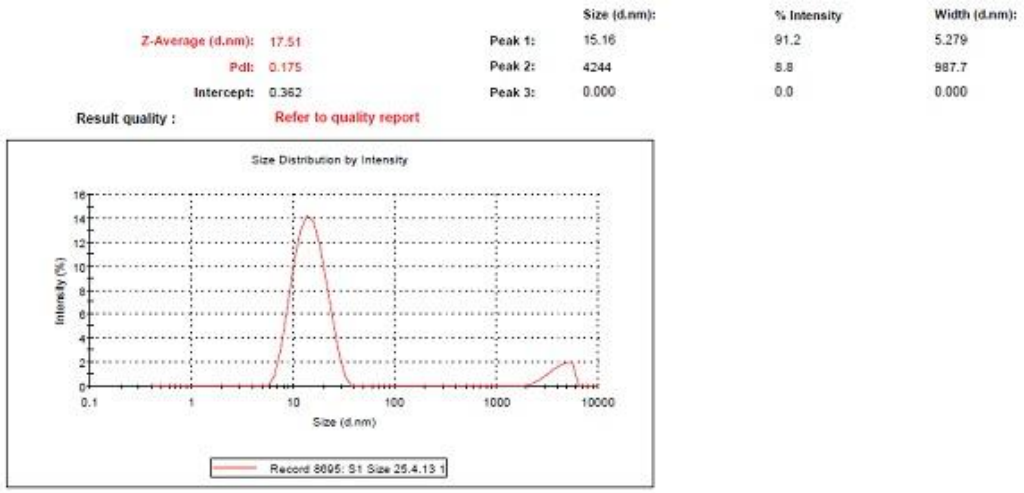

Figure 2: Particle size distribution of optimized formulation

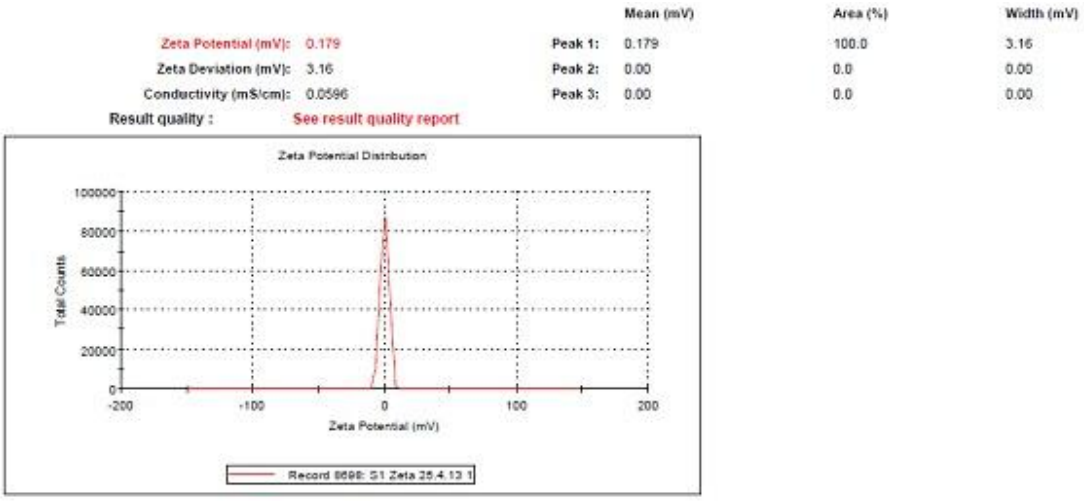

Figure 3: Zeta Potentialof optimized formulation

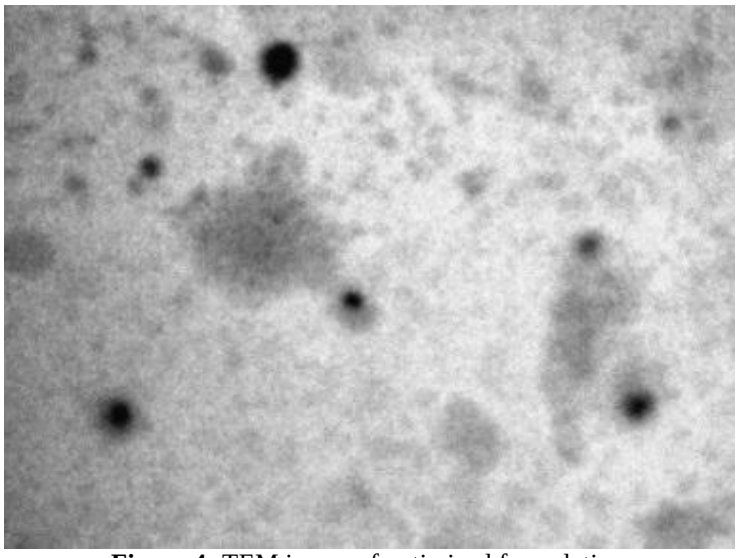

Figure 4: TEM image of optimized formulation

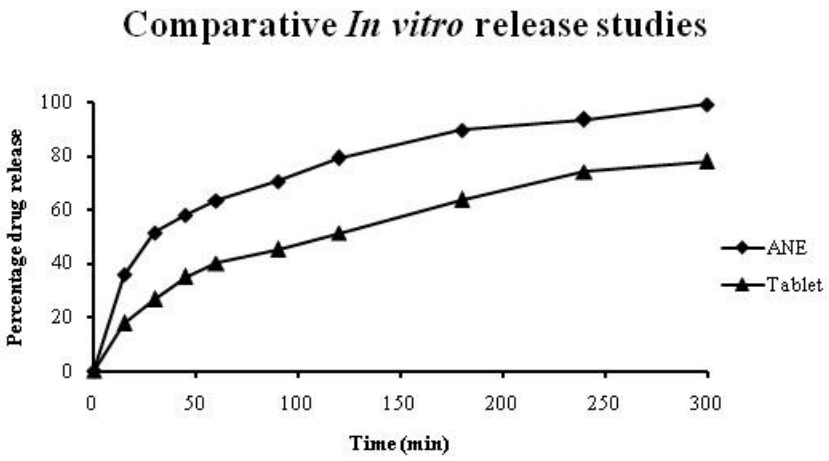

Figure 5: Comparative *in-vitro* release profile of optimized formulation and marketed tablet

4. Conclusion

Solubilization and drug release rate are two of important factors that which affects the bioavailability of poorly water-soluble compounds. Recently nanosizing techniques are used for low oral bioavailability compounds to improve and increase drug release rate. In this study an approach to prepare a suitable procedure for preparation of aceclofenac Nanoemulsion by solvent evaporation was designed. The optimized formulation shows increased drug release rate leads to improve the bioavailability. The nanosized droplets leading to enormous interfacial areas of the drug associated with nanoemulsion would influence solubilization behavior, transport properties as well as absorption across the mucosa to provide site-specific targeting. The results clearly indicated the suitability of formulation procedure for preparation of nano-sized poorly water soluble drug with significant improvement of the in vitro dissolution rate, and thus possibly improve oral bioavailability and to produce better therapeutic effect than the existing conventional tablet.

5. References

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