Self-Emulsifying Systems of Aceclofenac by Extrusion/Spheronization: Formulation and Evaluation

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ABSTRACT
The objective of the present study was to increase the solubility and oral bioavailability of aceclofenac by formulating the solid self-emulsifying (SE) pellets. The pellets were prepared by extrusion/spheronization technique, using oleic acid, cremophor, lactose, polyvinyl pyrrolidone (PVP) K30 and microcrystalline cellulose. The solid self-emulsifying drug delivery systems (SEDDS) were characterized by scanning electron microscopy, infrared spectroscopy, differential scanning calorimetry, in vitro and in vivo pharmacokinetic studies. SE pellets exhibited uniform size (800 to 2000 µm) and were spherical in shape. Droplet size distribution of the optimized pellets (SF9) following self-emulsification in water was 234.9±16 nm. In vitro release of optimized pellets was higher (95±4.21%) compared with aceclofenac pure drug (40±4.55%) at the end of 15 min. In vivo pharmacokinetic studies in Wistar rats revealed that, AUC and C_{max} of SF9 were notably higher than aceclofenac pure drug. SEDDS showed feasible approach to improve the dissolution and bioavailability of poorly water-soluble drug aceclofenac.

Keywords: Extrusion, spheronization, pharmacokinetic, droplet size, oleic acid, cremophor, pharmacokinetics.

INTRODUCTION
Aceclofenac (2-[(2, 6-dichlorophenyl) amine] phenylacetoxyacetic acid), a non-steroidal anti-inflammatory drug (NSAID), is one of the promising molecule for arthritis treatment. It is indicated for pains of various etiologies, such as musculoskeletal pain, dental pain or post surgical pain [1]. Aceclofenac is an orally effective and well-tolerated drug among the NSAIDs, with a lower incidence of gastrointestinal adverse effects [2]. It has low aqueous solubility and, as a consequence, has low oral bioavailability [3]. Therefore, the improvement of dissolution
from its oral solid dosage forms is essential for enhancing its bioavailability and therapeutic efficacy. Poor oral bioavailability of a drug is often due to low solubility, degradation in gastrointestinal tract (GIT), low permeability and high first pass metabolism [4]. According to Biopharmaceutics Classification System, class II and class IV drugs have poor solubility leading to poor oral bioavailability, high intra and inter-subject variability and lack of dose proportionality [5]. For such drugs, rate determining step in absorption is mainly the dissolution rate in the GI tract [6].

To tackle the solubility challenges of poorly soluble drugs, strategies such as micronization, co-solubilisation, preparation of inclusion complexes, use of nanosuspensions, micellar solubilisation by surfactants, drug dispersion in carriers and lipid-based formulations are employed. Self-emulsifying drug delivery systems (SEDDS) or self-emulsifying oil formulations (SEOF) are physically stable, isotropic mixtures of oil, surfactant, co-surfactant and solubilised drug substance which are suitable for oral delivery in soft and hard gelatin (or hard hydroxypropylmethyl cellulose) capsules. Lipophilic drugs are incorporated into inert lipid carriers such as oils [7], surfactant dispersions [8], emulsions [9], self-emulsifying formulations [10], self-nanoemulsifying systems [11]; [12]; [13]; [14] and self-microemulsifying systems [15]. The improved drug absorption provided by self-emulsifying formulations is contingent upon the maintenance of the drug in the solubilised state until it can be absorbed from the GIT [16]. It spreads readily in the GI tract and the digestive motility of the stomach and the intestine provides the agitation necessary for self-emulsification when compared with emulsions, which are sensitive and metastable dispersed forms. SEDDS are physically stable formulations that are easy to manufacture. Thus, for lipophilic drug compounds that exhibit dissolution rate-limited absorption, these systems may offer an improvement in the rate and extent of absorption which result in more reproducible blood-time profiles to improve bioavailability.

Hence in the present study, an attempt has been made to formulate and evaluate self emulsifying drug delivery systems of aceclofenac in the form of pellets.

**EXPERIMENTAL SECTION**

**Materials**
Aceclofenac was obtained as gift sample from Lupin Research Centre Pune, India. Oleic acid was purchased from Chemical Solvent Centre, Mumbai, India. Lemon oil was purchased from Genuine Chemical, Mumbai, India. Microcrystalline cellulose (MCC PH101) was purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Lactose was purchased from Labort fine chem. Pvt. Ltd., Surat, India. Polyvinylpyrrolidone (PVP K30) was purchased from Sisco Research Laboratories Pvt. Ltd, Mumbai, India. Cremophor, Lutrol 600 and Lutrol 400 were purchased from Signet Chemical Corporation, Mumbai, India. Tween-80 and Propylene glycol (PG) were purchased from Universal Laboratories, Mumbai. All the chemicals and reagents used were of analytical grade.

**Preparation of self emulsified pellets**
The molten blend was prepared by mixing surfactants (cremophor or tween 80) in cosolvents (propylene glycol or Lutrol 600 or Lutrol 400) with oil (oleic acid or lemon oil) in different proportions (Table:1) in a china dish at 70°C, followed by mixing the prepared blend with drug until a creamy dispersion was produced. Blending of excipients (MCC PH101, lactose and PVP K30) was carried out by physical mixing in different proportions. Creamy dispersion was then mixed thoroughly until a mass suitable for extrusion was obtained. Pellets were prepared by using single screw extruder (Umang Pharmatech Pvt Ltd., Mumbai, India) screen with a 0.5mm
aperture at 60 rpm, and put in a spheronizer having a plate diameter 2.0 mm for 5 min at 1,000 rpm to obtain spherical pellets [17]. The pellets were then dried in a vacuum oven and were sieved.

Table 1: Composition of aceclofenac self emulsifying pellets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>SF1</th>
<th>SF2</th>
<th>SF3</th>
<th>SF4</th>
<th>SF5</th>
<th>SF6</th>
<th>SF7</th>
<th>SF8</th>
<th>SF9</th>
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<td>Cremophor</td>
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</table>

Coating of capsules by enteric polymer
Capsules were coated with pH-sensitive poly (meth) acrylate copolymer (Eudragit L100-55) coating solution (Table: 2) and filled with optimized SF9 self-emulsifying pellets, to achieve site-specific drug release. Dip coating method was used to obtain uniform coating on the capsule. Different weight gain of coating levels on the capsules was studied. Capsules were ruptured in HCl itself when they were coated at the weight gain of 10% w/w and 15% w/w. Further at 25% w/w, coating capsules were intact in HCl. Hence weight gain of 25% w/w was found to be suitable for enteric coated capsules.

Table 2: Composition of coating solution

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eudragit L100</td>
<td>5%</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>97%</td>
</tr>
<tr>
<td>Water</td>
<td>3%</td>
</tr>
<tr>
<td>Triethyl citrate</td>
<td>0.9%</td>
</tr>
</tbody>
</table>

Solubility and Drug content
For the determination of solubility, excess quantity of pure aceclofenac and pellets were taken in 10 ml of different media (distilled water, pH 1.2, phosphate buffer pH 6.8 and pH 7.4) separately, kept on a shaking water bath (100 agitations/min) for 24h at room temperature. The solutions were then filtered through a 0.45µm membrane filters and the amount of the drug dissolved was analyzed spectrophotometrically at 273nm. Drug content of prepared pellets was also determined in suitable media.

Drug-excipient compatibility studies

Differential scanning calorimetry (DSC)
DSC was performed using DSC-60, (Shimadzu, Japan) to study the thermal behaviour of drug alone, mixture of drug and polymer and prepared optimized formulation (SF9). The samples were heated in sealed aluminium pans, under nitrogen flow (30 ml/min) at a scanning rate (5°C/min) from 25°C to 250°C.
Fourier transform infrared spectroscopy (FTIR)

Infrared spectroscopy was conducted using a Shimadzu FTIR 8300 spectrophotometer and the spectrum was recorded in the region of 4000 to 400 cm$^{-1}$. Samples (drug alone and formulation SF9 separately) were mixed with Potassium bromide (200-400 mg) and compressed into discs by applying a pressure of 5 tons for 5 min in a hydraulic press. The compressed disc was placed in the light path and the spectrum was obtained.

Particle size analysis

Prepared samples of pellets (20g) were passed through a set of sieves number 10, 20 and 40 with aperture sizes of 0.42, 0.84, and 2.0 mm respectively. The samples were shaken for 10 min on a mechanical shaker (Geologists Syndicate Pvt. Ltd, India). The samples retained on sieve number 20 were used for further studies.

Scanning electron microscopy (SEM)

The surface morphology of the pellets was studied by scanning electron microscopy (JEOL, JSM 50A, Tokyo, Japan). An appropriate sample of pellets was mounted on metal (aluminium) stubs. The samples were mounted using double-sided adhesive tape and fractured with a razor blade. The samples were sputter-coated with gold/palladium for 120 seconds at 14 mA under argon atmosphere for secondary electron emissive SEM and observed for morphology at acceleration voltage of 15 KV.

Droplet shape and size analysis [18]

The size of the droplets released from the pellets was determined in water and phosphate buffer pH 6.8 (T=37.0$^\circ$C) to check possible diameter variations during the in vitro dissolution test. Samples of 5ml were collected from the dissolution media and filtered through polycarbonate membrane syringe filter. The size of the submicron dispersed liquid phase was determined using a light scattering technique (Nano ZS – Zetasizer, Malvern, UK). The shape of the droplets was captured by microscope.

Dissolution studies

The in vitro dissolution studies were carried out using USP type II dissolution apparatus in phosphate buffer pH 6.8 [17]. The dissolution study of enteric coated capsules filled with optimised formulation SF9 was carried out in 900 ml of 0.1N HCl for 2 h, then the capsule was transferred to phosphate buffer pH 6.8 which was already kept in a thermostatically controlled water bath, maintained at 37±0.5$^\circ$C for 1 h. The paddle rotation speed was 75 rpm. The samples were withdrawn at predetermined time intervals subsequently replaced with fresh media. The sample solutions were then filtered through 0.45µm membrane filters and the drug concentration was analyzed spectrophotometrically at 273nm.

Stability Studies

The stability study of the optimized formulation (SF9) was done as per ICH guidelines (40±2 $^\circ$C/75±5%RH). The samples were withdrawn at predetermined time intervals i.e. 1, 3 and 6 months and evaluated for physical characteristics and drug content.

Preclinical studies

The preclinical study (pharmacokinetic studies) was carried out in Wistar rats and was approved by the Institutional Animal Ethical Committee, Kasturba Medical College, Manipal University, Manipal (Approval No: IAEC/KMC/31/2009-2010).
Pharmacokinetic study
Pharmacokinetic studies were carried out in Wistar rats (200–250 g). The overnight fasted animals were divided into two groups of six rats each and treated orally as shown below

**Group I**: Pure aceclofenac powder in 0.5 % sodium carboxymethylcellulose (Na CMC)

**Group II**: Self emulsifying pellets (SF9) equivalent quantity of aceclofenac in 0.5 % sodium carboxymethylcellulose (Na CMC)

The blood samples were collected at predetermined intervals of 0, 0.5, 1, 2, 4, 6 and 8 h of post dose into heparinised tubes from the retro orbital sinus. The plasma was separated immediately by using cool centrifugation at 4000 rpm for 10 minutes and the separated plasma was stored at -72°C until analysis.

Analysis of drug in plasma
Aceclofenac in rat plasma was analyzed by RP-HPLC method [19]. The HPLC system (Shimadzu Class VP series having class VP 6.12 version software) consisted of two pumps (LC-10AT VP), a variable wavelength programmable UV/Vis detector (SPD-10A VP), a system controller (SCL-10A VP) and an RP C-18 column (Grace Vydac C18; 250 cm x 4.6 mm; 5µ), mobile phase was methanol + 0.3% TEA pH 7.0 (65:35 v/v) and flow rate was 1.0 ml/min, injection volume was 20 µl; temperature was 25°C; run time was 15 min and the detection wavelength was 273 nm.

In the preparation of sample solutions, 25 µl of internal standard solution (500µg/ml) and 200 µl of acetonitrile were added to 100 µl of rat plasma and mixed for a min. To this, diluent was added (675 µl) up to 1 ml. The resulting solution was vortexed for a min and centrifuged at 10000 rpm for 10 min. The supernatant layer was separated and analyzed using RP-HPLC system. The response factor (peak area ratio of drug peak area to the internal standard peak area) of the standard solution and the sample were calculated and the concentration of the aceclofenac present in the plasma samples was calculated from the calibration curve. When blank rat plasma was analysed, no interference was observed in the analysis of drug. The peaks were well resolved (retention time: 10.006 min for aceclofenac and 11.833 min for venlafaxine). The data was analyzed using PK Solutions 2.0TM Non compartmental pharmacokinetic data analysis software to calculate the pharmacokinetic parameters ($C_{max}$, $T_{max}$, and area under the curve (AUC), mean residential time (MRT) and elimination half-life ($t_{1/2}$)).

RESULTS AND DISCUSSION

The SE pellets were prepared by extrusion/spheronization technique. Effect of different formulation variables on solubility, drug content, particle size, micromeritic properties, surface morphology and *in vitro* release were determined.

**Solubility and Drug content**

The drug content was found to be uniform among the different batches of prepared pellets and ranged from 95.13 to 98.92 %. Solubility of drug was found to be increased for all the prepared batches of pellets and the optimised formulation SF9 showed the maximum solubility.

**Drug-excipient compatibility studies**

The possible interactions between drug and the excipients were investigated by FTIR spectroscopy and DSC analysis. The DSC thermograms (Figure 1) of pure aceclofenac showed a sharp endotherm at 151.78 °C and optimized formulation SF9 showed at 152.40°C which
indicated the absence of any interaction between drug and excipients. This observation was further supported by FTIR analysis, where pure aceclofenac showed major peaks at 3315.74, 3271.38, 2929.97, 2359.02, 1768.78, 1579.75, 1504.53, 1444.73, 1342.50, 1249.91, and 663.53 cm$^{-1}$ and SF9 IR spectra revealed no considerable changes when compared with that of pure drug thereby indicating the absence of any interaction.

**Figure 1: DSC of pure aceclofenac and optimise formulation (SF9)**

<table>
<thead>
<tr>
<th>DSC mW</th>
<th>SF9</th>
<th>Pure drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>-30.00</td>
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<tr>
<td>-20.00</td>
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<td>-10.00</td>
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<td>0.00</td>
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<td>10.00</td>
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<td>20.00</td>
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<td>30.00</td>
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</tbody>
</table>

**Micromeritic Properties**
The self emulsifying pellets were evaluated for bulk density (BD), tapped density (TD), compressibility (Carr’s) index and angle of repose. The bulk density of different formulations SF1, SF2, SF3, SF4, SF5, SF7 and SF9 were found to be in the range of 0.21 to 0.57 and tapped density from 0.37 to 0.71 respectively. BD and TD of formulations SF6 and SF8 were not performed due to formation of a sticky mass. The compressibility index (29.99%) indicated poor flowability of aceclofenac. Angle of repose was determined for the measurement of flowability. The formulations SF1, SF2 and SF9 showed improved flowability in the form of pellets. This was further supported by the value of Hausner’s ratio (<1.25). The improved flowability of self emulsifying pellets may be due to good sphericity and small size of granules.

**Particle size analysis**
Sieve analysis was carried out for the formulations SF1 to SF9, except SF6 and SF8. Results showed that a majority of the particles were retained on the sieve no. 20 and were in the size range of 800 to 2000 µm.

**Scanning electron microscopy, droplet shape and size analysis**
The surface morphology of self emulsifying pellets (SF9) was studied using SEM (figure 2a). All formulated pellets were spherical in shape with uniform size shown at low magnification, details about the surface characteristics of pellets were obtained with increasing the magnification. Figure: 2b showed typical surface features of the pellets with an apparently smooth surface.
Surface morphology of optimized formulation (SF9) using SEM

Figure: 3a Photomicrographs of emulsion droplets of optimise formulation (SF9)

Figure: 3b Emulsion droplet size distribution by intensity of formulation SF9

The droplet size of the emulsion was determined by Malvern particle size analyzer and shape was studied by photomicrograph. Droplet size distribution of SF9 pellets following self-emulsification in water was 234.9 ± 16 nm. Emulsion droplet shape and mean emulsion droplet size of formulation SF9 are presented in figure 3a and 3b respectively. When dissolution of the formulation SF9 was carried out, SF9 dispersed in to fine emulsion droplets (Figure 3b). The
probable explanation to the enhanced absorption could be that the smaller the droplet size, the larger the interfacial surface area, which facilitate and improve drug absorption [20].

**Dissolution studies**

*In vitro* release of aceclofenac from optimized formulation (SF9) and pure drug was carried out in 6.8 pH phosphate buffer (Figure 4a). The enteric coated capsule filled with SF9 was carried out in 0.1 N HCl for the first 2 h and followed by pH 6.8 phosphate buffer for 1 h (Figure 4b). The *in vitro* drug release from Eudragit L100-55 coated capsules filled with optimized formulation was 0.45% in the pH 1.2 at the end of 2 h and in pH 6.8, maximum release (95% release in 30 min) was observed. The result demonstrates better gastric resistance of the formulated pellets using Eudragit L100-55. The enteric polymer, Eudragit L100-55, is an anionic copolymer based on methacrylic acid and ethyl acrylate, with free carboxyl groups in a ratio of 1:1 with the ester groups. The carboxylic groups begin to ionize in aqueous media at pH 5.5 and above, rendering the polymer resistant to the acidic environment of the stomach but soluble in intestinal fluid [17]. Thus, the study concluded that the formulated SEDDS can be used as an alternate drug delivery system for the aceclofenac.

*Figure: 4a In vitro release of aceclofenac from optimized formulation (SF9) and pure drug in 6.8 pH phosphate buffer*

![](image1)

*Figure: 4b In vitro release of aceclofenac from enteric coated capsule filled with optimized formulation (SF9) in 0.1 N HCl followed by 6.8 pH phosphate buffer*

![](image2)

**Stability studies**

The results of accelerated stability studies carried out according to ICH guidelines. Self emulsifying pellets (SF9) did not show any physical changes during the study period. Drug content was found to be more than 95% at the end of 6 months in accelerated conditions. The
values for drug content (n =3; mean ± S.D.) for SF9 pellets were, Initial (99.92 ± 0.23), 1 month (98.15 ± 0.54), 3 months (97.63 ± 0.75), 6 months (94.95 ± 0.45). This indicates that the prepared self emulsifying pellets (SF9) are stable at accelerated storage conditions and are suitable for in vivo studies in animal models.

Preclinical studies:
Aceclofenac absorption after oral administration was rapid with all two groups as indicated by low T_max value of 0.25 h. However, the C_max value was high with formulation SF9 compared with aceclofenac pure drug, indicating maximum absorption of drug. The elimination half-life (t_{1/2}) of aceclofenac pure drug (1.3±0.09 h) with formulation SF9 (1.6±0.07 h) is indicative of slow elimination of drug from body in the form of pellets (Table 3). It was further supported by low elimination rate constant value (0.46±0.01 h^{-1}) of self emulsifying pellets in comparison with pure drug (0.55±0.01 h^{-1}). Self emulsifying pellets showed high area under the curve value (1.4±0.05 µg/ml) in comparison with pure drug (1.1±0.05 µg/ml) indicating the greater bioavailability of drug from formulation SF9. Hence the pharmacokinetic study indicates rapid absorption and higher bioavailability of drug from formulation SF9 in comparison with pure drug. This could be due to improved solubility and dissolution rate of drug from prepared self emulsifying pellets.

Table 3: Pharmacokinetic parameters from the plasma concentration-time curves

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pure drug</th>
<th>Formulation SF9</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_max (µg/ml)</td>
<td>1.09±0.09</td>
<td>1.41±0.11</td>
</tr>
<tr>
<td>T_max (h)</td>
<td>0.25±0.49</td>
<td>0.25±0.55</td>
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<tr>
<td>t_{1/2} (h)</td>
<td>1.3±0.09</td>
<td>1.6±0.07</td>
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<tr>
<td>AUC_{0-8} (µg/ml)</td>
<td>1.1±0.05</td>
<td>1.4±0.05</td>
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<tr>
<td>MRT (h)</td>
<td>1.1±0.46</td>
<td>1.5±0.06</td>
</tr>
<tr>
<td>K_e (h^{-1})</td>
<td>0.55±0.01</td>
<td>0.46±0.01</td>
</tr>
<tr>
<td>CL (L/hr)</td>
<td>9.4±0.01</td>
<td>6.8±0.03</td>
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<tr>
<td>Vd (L)</td>
<td>17.15±0.03</td>
<td>19.66±0.04</td>
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</tbody>
</table>

Figure 5: Plasma drug concentration-time curve
CONCLUSION

Bioavailability is major concern of the most therapy involving with hydrophobic drugs. The current study demonstrated a successful and simple method to prepare self-emulsifying aceclofenac pellets, where pellets produced emulsion with uniform droplet size to enhance its aqueous solubility and dissolution rate. The prepared SE pellets showed better in vitro and in vivo performance in comparison with pure drug. This potential technique has ability to develop stable dosage forms which can be scaled up.

Acknowledgement

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REFERENCES