INTRODUCTION

It is estimated that 40% or more of active substances being identified through combinatorial screening programs are poorly soluble in water [1]. Poor solubility is not only a problem for the formulation development and clinical testing; it is also an obstacle at the beginning when screening new compounds for pharmacological activity. There is a definite need for smart technological formulation approaches to make such poorly soluble drugs bioavailable [2].

Multi-particle dosage forms (MPDFs) are receiving immense attention as alternative drug delivery systems for oral drug delivery even though single-unit dosage forms have been widely used for decades [3]. MPDFs offer advantages over conventional dosage forms such as (1) predictable, reproducible and short gastric residence time, (2) less inter and intra-subject variability, and (3) improve bioavailability [4,5]. Pelletization is often called a size enlargement process that involves the production of agglomerates relatively with a narrow size range of 0.5 mm–2 mm [6,7] and they are called pellets. Pellets have free-flowing properties and low porosity of about 10%. Since their multi-particle nature offers many important pharmaceutical as well as technological advantages over conventional single unit solid dosage form, these formulations are preferred over other solid dosage forms [8]. In the past two decades, pellets have established their position for many reasons [9].

Pellets offer greater flexibility in pharmaceutical solid dosage form design and development. They flow freely and pack easily without significant difficulties, resulting in uniform and reproducible fill weight of capsules and tablets. Ethylcellulose, a hydrophobic polymer which has more controlled release properties and used in the preparation of many dosage forms. Therefore, in this study, it was used to extend the drug release [10,11].

Extendable drug delivery systems come under controlled drug delivery systems, which are used to extend the release rate of drugs over an extended rate of period [12,13].

Bosentan is an endothelin receptor antagonist (ERAs). Patients with PAH have elevated levels of endothelin, a potent blood vessel constrictor, in their plasma and lung tissue. Bosentan blocks the binding of its receptors, thereby negating endothelins deleterious effects. Its oral bioavailability is approximately 50%, and food does not affect its absorption. It has a terminal elimination half-life of 5 h [14].

From the above information on Bosentan, it is essential to formulate extended release (ER) dosage forms with least side effects as it has the oral bioavailability 50%, it is essential to increase bioavailability, and the half-life also need to be prolonged to improve the patient compliance by reducing the frequency of administration. This drug is indicated for the pulmonary artery hypertension; a chronic condition requires prolonged drug release.

MATERIALS AND METHODS

Materials

Bosentan was obtained as a free sample from Aurobindo Pharma Ltd, Hyderabad. MCC spheres were obtained from Aurobindo Pharma, HPMC E5 was obtained from Colorcon Asia, Ethyl Cellulose, Eudragit of different grades, and Magnesium stearate were obtained from Tini
Pharma Pvt., Ltd. All other chemicals and reagents used in the study were of analytical grade.

**Preparation methods**

**Method for estimation of Bosentan**

High-performance liquid chromatography (HPLC) method for the estimation of Bosentan was used for routine analysis of Bosentan in its formulations such as drug content and in vitro dissolution and drug content uniformity.

**Preparation of mobile phase**

The mobile phase was prepared using phosphate buffer of pH 7.4 and Acetonitrile at a ratio of 40:60 (Buffer:acetonitrile).

**Preparation of stock solution**

Accurately weighed 100 mg of Bosentan was transferred into 100 ml volumetric flask and dissolved in few ml of mobile phase and then further diluted and make up the volume up to 100 ml with same mobile phase.

**Preparation of standard solutions**

From the above stock solution, standard concentrations of 50, 100, and 150 µg/ml solutions and the samples were filtered through 0.45 µm Millipore filter. The samples were loaded into the Shimadzu HPLC System model No 1020 CHT with autosampler. A standard calibration curve was plotted using the standard peak areas versus concentration.

![Fig. 1: Calibration curve of Bosentan in 0.1 N hydrochloric acid](image)

Table 1: Peak areas of the Bosentan

<table>
<thead>
<tr>
<th>Concentration in µg/ml</th>
<th>Peak area</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>615768</td>
</tr>
<tr>
<td>100</td>
<td>1232177</td>
</tr>
<tr>
<td>150</td>
<td>1868566</td>
</tr>
</tbody>
</table>

**Drug loading on MCC spheres**

MCC spheres were loaded into the fluid bed processor [15-19], the process parameters were adjusted as per the machine setting. The MCC spheres then preheated to the required temperature as per the set parameters. The drug solution was then sprayed onto the spheres using the peristaltic pump. The process parameters were monitored and then adjusted as per the pellets fluidization and weight gain. The prepared pellets were then dried for 12 h in a hot air oven to remove the complete moisture.

**ER coating on drug-loaded pellets**

The drug-loaded pellets were prepared using ethyl cellulose and Eudragit. The solvent used in polymer solution was IPA and MDC. Tributyl citrate was used as plasticizer. Initially, ethylcellulose was added to the IPA and MDC 30:70 RATIO. The solution was then stirred for 30 min then plasticizer was added to it finally talcum and magnesium stearate was added and stirred to get the uniform dispersion. The prepared solution was sprayed on to the drug-loaded pellets.

**ER coating on drug-loaded pellets**

The drug-loaded pellets were loaded into fluid bed processor. The pellets were preheated up to 45°C. The inlet and exhaust temperatures were set as per the requirement. The polymer solution was then sprayed on to the drug-loaded pellets. The different ratio or percent of the polymer ethylcellulose alone and in combination with Eudragits were prepared and loaded on to the pellets. The pellets were then dried in a tray dryer overnight for complete evaporation of the solvent. The formula for ER coating solution of different polymers individually and in combination on drug-loaded pellets was shown in Table 2.

**Evaluation of ER pellets**

**In vitro dissolution studies**

Dissolution studies for each prepared formulation were performed in a calibrated dissolution test apparatus (LABINDIA), equipped with paddles (USP apparatus II method). 900 ml of 0.1 N hydrochloric acid solution was used as a dissolution medium. The paddles were operated at 50 rpm, and the temperature was maintained at 37±0.5°C throughout the experiment. Dissolution samples were withdrawn from the apparatus at regular intervals, i.e., 1, 2, 3…up to 24 h and replaced with equal volume of dissolution medium to maintain the sink condition throughout the experiment. Samples were withdrawn at various time intervals were suitably diluted with same dissolution medium, and the amount of drug released was estimated by chromatographically at 272 nm.

**In vivo study design**

**In vivo study of Bosentan**

In vivo study of Bosentan, ER pellets were performed in healthy rabbits (New Zealand, White) of either sex weighing (3.0–3.3 kg) were divided...
into two separate groups, each group consisting of 6 animals. The first group received conventional beads of Bosentan (80 mg) and the second group received the microbeads of Bosentan contain 80 mg of Bosentan. The beads were put behind the tongue to avoid biting by rabbit. Food was withdrawn from the rabbits before 12 h of drug administration and until 24 h post dosing. All rabbits have free access to water during the study period. The Institute Animal Ethical Committee approved the study protocol. PROTOCOL NO: 012/MRIPS/CPSEA-IAEC/HYD/2018.

**Standard calibration curve**

Preparation of standard solution 10 mg of Bosentan monohydrate was weighed accurately and transferred into 100 ml volumetric flask. Ensured to dissolve the un-dissolved portions of drug by sonicating and final volume was made with mobile phase. Aliquot of 1 ml was taken and further diluted to 10 ml to get a concentration of 1 µg/ml.

**Blood sampling**

Blood samples were collected at 0.25, 0.5, 1.0, 1.5, 2, 3, 4, 6, 8, 12, and 24 h from marginal ear vein. Blood collected was centrifuged at 2000–

---

**Table 3: Standard calibration curve of Bosentan in rabbit plasma**

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Peak Area ratios of Bosentan (S/IS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.1</td>
<td>0.052007</td>
</tr>
<tr>
<td>0.2</td>
<td>0.104633</td>
</tr>
<tr>
<td>0.3</td>
<td>0.160475</td>
</tr>
<tr>
<td>0.4</td>
<td>0.212688</td>
</tr>
<tr>
<td>0.5</td>
<td>0.254922</td>
</tr>
<tr>
<td>0.6</td>
<td>0.306102</td>
</tr>
<tr>
<td>0.7</td>
<td>0.349369</td>
</tr>
<tr>
<td>0.8</td>
<td>0.401659</td>
</tr>
<tr>
<td>0.9</td>
<td>0.466127</td>
</tr>
<tr>
<td>1</td>
<td>0.514828</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

In vitro dissolution studies were performed for all prepared pellets to find out the drug release, drug release mechanisms and also to know the effect of concentration and proportion of polymers used for extending the drug release from the core of the formulations. Among all the formulations, CB12 formulation was selected as best or optimized formulation based on the drug release and its physical properties. The formulation CB12 was extended the drug release up to 24 h, and it was prepared with Ethylcellulose N 50 (ECN50) and Eudragit polymer. Results were shown in Figs. 2-5.

Standard calibration curve of Bosentan in rabbit plasma

Linearity was observed within the range of 0.1–1 µg/ml with correlation coefficient of 0.999 showed in Fig. 6 and the values were shown in Table 3. The retention time of etodolac (I/S) was found at 4.132 min (Fig. 7).

Pharmacokinetic assessment

All the pharmacokinetic results were shown in Table 4. The plasma kinetic data were assessed with KINETIKA 5.0 software. Fig. 8 shows the mean comparative data plot of the mean plasma concentration of the Bosentan in both test (ER formulation) and reference (conventional formulation). The mean peak plasma concentration of test (T) formulation C \(_{\text{max}}\) 3.412 µg/ml was gradually reached in 3.0 h. In case of conventional reference formulation (R), the C \(_{\text{max}}\) was 5.074 µg/ml which was reached in 1.5 h. The concentration maximum of the test formulation (T) was lower when compared with reference (R) formulation. The increase T \(_{\text{max}}\) was clearly indicates the drugs availability for the longer duration. Tables show the kinetic data of Bosentan conventional formulation (R) and ER formulations (T), respectively.

The reference (R) formulation was absorbed rapidly, the T \(_{\text{max}}\) reached in about 1.5 h. After reaching the T \(_{\text{max}}\), the drug starts rapid elimination and the concentration gradually reduced. In case of test (T) formulation, the T \(_{\text{max}}\) achieved gradually, and the drug availability was long time.

The AUC \(_{0-t}\) of the reference (R) was found to be 7.312 µg min/ml, the increase in AUC \(_{0-t}\), of about 10.42 ± 1.52 µg min/ml was observed in the test (T) formulation, and this clearly indicates the drug availability for long period of time.

Decrease in elimination rate constant (K \(_{\text{el}}\)) from 0.181 h \(^{-1}\) (Reference) (R) to 0.093 h \(^{-1}\) (Test) indicates the lower release rate of drug in the body.

The half-life (T \(_{\text{1/2}}\) ) of the reference (R) and test (T) formulations were 3.75 h and 7.45 h, respectively, which were significantly different. Thus, the prolonged T \(_{\text{1/2}}\) is another indication on the in vivo performance of the Bosentan ER beads.

The overall C \(_{\text{max}}\), T \(_{\text{max}}\), AUC \(_{0-t}\), K \(_{\text{el}}\) and T \(_{1/2}\) were completely different between both test and reference formulation. Therefore, the prepared formulation was releasing the drug for a prolonged period of time.

CONCLUSION

A fluid bed coating method was successfully applied to make Bosentan prolonged-release pellets. Drug solution was loaded into the MCC spheres, and then the drug-loaded spheres were coated with ECN50, and Eudragit NE30D in different proportions of polymers were used.
among those formulation CB12 with 1:1 proportion of ECN50 and Eudragit was shown better drug release. The in vitro release profiles indicated that the release of Bosentan from the pellets exhibited a controlled release behavior. In vivo studies were conducted in New Zealand rabbits. Based on the results of in vivo study, the overall Cmax, T1/2, AUC0-24, Kmax, and T1/2 were completely different between both test and reference formulation. Therefore, the ready formulation was releasing the drug for a prolonged period. Finally, the present work demonstrates the feasibility of controlled delivery of Bosentan utilizing MCC-based pellets.

CONFLICTS OF INTEREST
No conflicts of interest.

AUTHOR’S CONTRIBUTION
We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

Mr. Narender Karra collected the data, analyzed the data, all the laboratory work performed, and wrote the introduction, discussion, and the material and method part.

Dr.P. Narayana Raju, supervisor of this study helped in selection of the topic, procuring chemicals, drugs from different laboratories, in the preparation of manuscript, designing and conducting of this study. DrR siva kumar, cosupervisor of this study, helped me a lot in drafting the manuscript and proofreading.

ACKNOWLEDGMENT
The authors are thankful to our guide P. Narayana Raju and coguide R. Siva Kumar for their support to complete our research work.

The authors are thankful to the management and principle of Malla Reddy Institute of Pharmaceutical Sciences for providing all facilities for this research work.

REFERENCES