ISSN- O: 2458 - 868X, ISSN –P: 2458 - 8687

International Journal of Medical Science and Innovative Research (IJMSIR)

IJMSIR : A Medical Publication Hub

Available Online at: www.ijmsir.com

Volume – 2, Issue –5, September- October - 2017, Page No. : 318 - 329

Formulation and In vivo Evaluation of sustained release pellets of bosentan by pan coating process

G. Venkata Ramireddy[1*], K.V. Ramana Murthy[2]

[1]A.S.N. Pharmacy College, Tenali, Guntur- 522201

[2] Department of pharmaceutics, University College of pharmaceutical sciences, Andhra University, Visakapatanam, 530003

Correspondence Author: G. Venkata Ramireddy, A.S.N. Pharmacy College, Tenali, Guntur- 522201, India.

Conflicts of Interest: Nil

Abstract

The aim of the present study was to develop sustained release pellets of bosentan with eudragit RL 100 as rate retarding polymer by pan coating process. The prepared pellets were evaluated for drug content, particle size, subjected to Scanning Electron Microscopy (SEM), FT-IR and Differential Scanning Calorimetry (DSC) and evaluated for *in vitro* release. The drug content was in the range of 19.07% to 21.37%. The mean particle size of the drug loaded pellets was in the range 703-790 µm. It was found that among the various batches of formulations AF-9 and wasfound to release the drug over an extended period of time, i.e. up to 18 hrs. SEM photographs confirmed that the prepared formulations were spherical in nature with a smooth surface. The compatibility between drug and polymers in the drug-loaded pellets was confirmed by FT-IR and DSC studies. *In vivo* bioavailability studies indicated significance difference between bosentan coated pellets and pure drug. Therefore, the present bosentan coated pellets is considered to be potentially useful for the treatment where improved patient compliance and convenience is expected.

Keywords: Bosentan, pellets, Sustained release, eudragit RL 100, pan coating, in vivo studies.

Introduction

Coating pans have been used in pharmaceutical coating operations since the early 19th century when they were used extensively for sugar coating [1]. The first pelletization process for developing a sustained release dosage form in the coating pan can be traced to the 1956 patent by Blythe. This process involved layering a drug powder onto nonpareils using syrup as the adhesive solution. There have been 30 years of research and development experience in the powder layering technology since that patent, and a variety of products have been successfully developed and introduced into the market [2]. With time, the manufacture of pellets in conventional coating pans has developed from the art of earlier years into a much more sophisticated and controlled process. The basic components of conventional coating pan system are the rotating pan, air supply system, spray system, powder addition system, and air–exhaust system. In the powder layering technology, pellets are usually prepared by loading the micronized powders on the solid cores. Generally, this pelletization method involves the using of inert substrates, such as sugar spheres, and their enlargement by intermittently spraying a binder solution [3] and applying the active substance powder in a rotating coating pan or in a fluidized bed [4] Once the drug beads are prepared, they may be further coated with a protective coating to allow a sustained or prolonged release of the

drug [5]. Using a multiple-unit dosage form, pellets offer several advantages: Pellets disperse freely in the gastrointestinal tract and thus maximize drug absorption, reduce peak plasma fluctuations, and minimize side effects; high local concentrations of drug are avoided; there is flexibility in the development of oral dosage forms as pellets, so different drug substances (e.g. incompatible drugs) can be formulated and blended into a single dosage form; and immediate- and controlled-release pellets can be mixed to achieve the desired release pattern[6-10].

Materials and Methods

Materials: Bosentan was obtained as gift sample from Dr. Reddy's laboratories, Hyderabad, India. Eudragit RL 100 RS 100, Dibutyl phthalate, was obtained as gift samples from Dow Chemical's Asia pvt. Ltd., Mumbai. , Isopropyl alcohol was obtained from Loba chemi Pvt. Ltd., Mumbai.

Methods:

Nonpareil seeds were then transferred to the conventional coating pan having 20 cm diameter, baffled and pear shaped which was constructed with stainless steel. The varying concentration of coating solutions were formed by using eudragit RL100 in isopropyl alcohol with nonstop stirring for about 1hr and lastly dibutyl phthalate was incorporated to it. The speed of pan was set at 30 rpm. The coating solution was sprayed by using spray gun manually on drug loaded pellets. At the temperature of 40-45^{0}C the inlet air was locked and with intermittent spraying and drying the coating was done manually. Hot air oven was used for 3 hr at 40^{o}C for drying of coated pellets. While coating operation all the necessary parameters must be observed wisely to obtained the better coating. Negligence from any one of coating related parameter may have direct impact on the quality of the product.

Evaluation of sustained release pellets [12-14]

The pellets were evaluated for in process quality control tests. The tests were performed for sustained release pellets. They are angle of repose, bulk density, friability, drug content, *in vitro* dissolution and *in vivo* evaluation tests.

In vitro dissolution studies

Dissolution studies for each formulation were performed in a calibrated 8 station dissolution test apparatus (LABINDIA), equipped with paddles (USP apparatus II method) employing 900ml of distilled water containing 1% SLS as a medium. The paddles were operated at 50 rpm and the temperature was maintained at 37± 1^{o}C throughout the experiment. Samples were withdrawn at regular intervals up to 18 hrs and replaced with equal volume of dissolution medium to maintain the constant volume throughout the experiment. Samples withdrawn at various time intervals were suitably diluted with same dissolution medium and the amount of drug released was estimated by chromatographically at 272nm.

Drug Excipient Compatibility Studies:

The drug excipient compatibility studies were carried out by Fourier Transmission Infrared Spectroscopy (FTIR) method and Differential Scanning Colorimetry (DSC) method.

Differential Scanning Calorimetry (DSC) studies were carried out using DSC 60, having TA60 software, Shimadzu, Japan. Samples were accurately weighed and heated in sealed aluminium pans at a rate of 10°C/ min between 25 and 350°C temperature rang under nitrogen atmosphere. Empty aluminium pan was used as a reference.

FTIR spectra for pure drug, physical mixture and optimized formulations were recorded using a Fourier transform Infrared spectrophotometer. The analysis was carried out in Shimadzu-IR Affinity 1 Spectrophotometer.

The samples were dispersed in KBr and compressed into disc/pellet by application of pressure. The pellets were placed in the light path for recording the IR spectra. The scanning range was 400-4000 cm-1 and the resolution was 1 cm^{-1}.

The surface and shape characteristics of pellets were determined by scanning electron microscopy (SEM). Photographs were taken and recorded at suitable magnification.

In vivo studies

Twelve male Albino rabbits were selected for this study, all the animals were healthy during the period of the experiment. All efforts were made to maintain the animals under controlled environmental conditions (Temperature 25^{o}C, Relative Humidity 45% and 12 h alternate light and dark cycle) with 100 % fresh air exchange in animal rooms, uninterrupted power and water supply and rabbits were fed with standard diet and water ad libitum. The protocol of animal study was approved by the institutional animal ethics committee. The rats were fasted overnight before administration of the formulation (Bosentan coated pellets) and pure drug (Bosentan). The rabbits were randomly divided into two groups each group contains six animals. The Group A rabbits were received Bosentan coated pellets coated pellets and Group B received pure drugs Bosentan coated pellets administered orally (Dissolved in distilled water). Blood samples for pharmacokinetic analysis were obtained at different time intervals 0, 15, 30, 60, 120, 240, 360min and 24hrs after dosing. Blood samples were collected in heparinized tubes and were centrifuged for 10min at 3,000 rpm at room temperature. Rabbit plasma (0.5 ml) was prepared for chromatography by precipitating proteins with 2.5 ml of ice-cold absolute ethanol for each 0.5 ml of plasma. After centrifugation the ethanol was transferred into a clean tube. The precipitate was resuspended with 1 ml of acetonitrile by vortexing for 1 min. After centrifugation (5000 – 6000 rpm for 10 min), the acetonitrile was added to the ethanol and the organic mixture was taken to near dryness by a steam of nitrogen at room temperature. Internal standard losertan was added to the samples. The samples were analyzed by HPLC method.

Results and Discussion:

The formulated pellets were evaluated for various parameters. The results of the bulk density study concluded that the bulk density of all pellets was less than 1gm/ml. The value of angle of repose for all the batches of pellets was present from 9.440 to 11.210. As pellets of all the batches depicted angle of repose less than 20 hence excellent characteristic of flow was noted. After observing the table for friability study it was clearly seen that the friability for all the batches of pellets was less than 1%. Means the pellets were good. It was found after the study of SEM of pellets that surfaces of pellets was smooth and free from cavities and deformities. All the pellets were observed to be spherical in shapes. SEM also proved that uniform coating was done over pellets surfaces. DSC is an advanced technique by which the heat flows to or from a reference, which is monitored as a function of temperature or time, while the samples are subjected to a controlled temperature program. Thermal properties of pure drug were evaluated by Differential scanning caloriemetry using a diamond (DSC) (Mettler star 8.10). Accurately weighed 5-6 mg samples were hermetically sealed in aluminium pans and heated at a rate 50 ^{O}C/min from 50^{0}C to 250 ^{0}C temperature range under nitrogen flow of 25 ml/min. Results of DSC thermogram of pure Bosantan shows sharp endothermic peak at 107.49 ^{O}C confirms the crystalline nature of the Bosentan. The infrared spectra of bosentan pure drug alone and mixture of drug with excipients were recorded between 450 – 4000 cm^{-1} (Perkin Elmer FTIR). The FTIR spectra of the pure bosantan show

spectrum peak points at 752, 1020, 1083, 1112,1203, 1252, 1292, 1453, 1579, , 2962, 3064 and 3629 ± 1 cm^{-1}. The same peaks were appeared in the blend of drug with excipients. This indicated that there was no interaction between adrug and excipients.

The dissolution revealed that batch AF1 released the highest quantity of medicament and batch AF15 removed the least amount of medicament in 18 hr. It was happened due to the fact that batch AF1 contains less Eudragit RL 100 and batch AF15contains more quantity of eudragit RL 100. The study was performed in triplicate style and batch AF9 released 91.33 % of medicament in 18 hr of study. As the released pattern of batch AF9 was best suited to the present designed research work and hence it was optimized. All the formulations were found to have following typical Zero order kinetics which was clearly indicated by their relatively higher r^2 values compared to that of First order regression co efficient values. All the formulations were found to be accepting Higuchian diffusion as release model, indicated by their relatively higher r^2values compared to that of Erosion model regression coefficient values. The dissolution data of all formulations were fitted to the Power law (Korsemeyer Peppas model) and the entire exponent 'n' values were found to be between 0.5-1, indicating that all the formulations were following Non-Fickian mode of drug release.

Based on the results obtained after performing physicochemical characteristics, *In vitro* drug release and stability studies on various technologies, among all formulations, AF9 capsules has shown best and satisfactory results. Hence, this formulation was considered as best and selected for further *In vivo* evaluations.

The mean bosentan concentrations time profiles for the pure bosentan and pellets of bosentan was measured and given in Table 4. The bioavailability parameters for the both formulations are summarized in Table 5. The prepared bosentan coated pellets shown more AUC indicated significant difference between oral solution and pellet formulation. Based on the data it was concluded that the two formulations exhibited significantly different plasma level-time profiles.

Conclusion

In this present work, core pellets were coated with the drug solution of bosentan by fluidized bed coating process. The core pellets were evaluated for physical properties and found to be within the limits. The drug layered pellets were also evaluated for drug content and in vitro drug release studies and found to be within Indian Pharmacopoeial limits. The optimized pellet formulation (AF9) of bosentan was characterized by SEM analysis to understand the pellet shape and surface morphology and found to be spherical in shape and smooth surface with minimal pores, indicating the uniform coating of the pellets. DSC and FTIR data revealed that no interactions takes place between the drug and polymers used in the optimized formulation. *In vivo* study indicated significance difference between bosentan coated pellets and pure drug, both exhibited significantly different drug plasma level - time profiles. Therefore, the present Bosentan coated pellets is considered to be potentially useful for the treatment where improved patient compliance and convenience is expected.

References

[1]. Walter GC. Conventional and specialized coating pans. Bristol-Mayers, Evansville, Indiana. 1989.

[2]. Blythe RH. Sympathomimetic preparation. U.S. Patent. 2, 738,303. 1956.

[3]. Armstrong NA. Tableting. In: Aulton ME, editor. Pharmaceutics: The science of dosage form design. New York: Churchill Livingstone; 1997.

[4]. Flament MP, Leterme P, Gayot A, Gendrot E, Bruna E, Cousin G. Development and industrial scale-up of tablets containing modifiedrelease pellets. Pharm Tech Eur 1994;2:19-25.

[5]. Laicher A, Lorck CA, Tobin J, Stanilaus F. Process optimization of pellet coating and drying using fluid bed production units. Pharm Tech Eur 1994;8:41-8.

[6]. Andrew BC, Shargel L. Modified-release drug products and targeted drug delivery system. In: Applied biopharmaceutics and pharmacokinetics. 3rd ed. USA: Appleton and Lange; 1941. p. 225-64.

[7]. Singh R, Poddar SS, Chivate A. Sintering of wax for controlling release from pellets. AAPS PharmSciTech 2007;8:E1-9.

[8]. Sellassie IG, Gordon RH, Fawzi MB. Evaluation of a high-speed pelletization process and equipment. Drug Dev Ind Pharm 1985;11:1523-41.

[9]. Ghebre-Shellasie I. Pellets: A general overview. In: Ghebre-Shellasie I, editor. Pharmaceutical pelletization technology. New York: Marcel Dekker Inc; 1989. p. 6-7. Vervaet C, Baert L, Remon J. Extrusion spheronisation: A literature review. Int J Pharm 1995;116:131-46.

[10]. Prabakaran L, Bajpai M. Novel micropelletization technique: Highly improved dissolution, wettability and micromeritic behavior of domperidone. Curr Drug Deliv 2006;3:307-13.

[11]. Holm P, Liska J, Clozel Ml. The endothelin antagonist bosentan: hemodynamic effects during normoxia and hypoxic pulmonary hypertension in pigs. J Thorac Cardiovasc Surg 1996; 112: 890–897.

[12].Provencher S, Sitbon O, Humbert M. Long-term outcome with first-line bosentan therapy in idiopathic pulmonary arterial hypertension. Eur Heart J 2006; 27: 589–595.

[13]. X. Wei et al. Sigmoidal release of Indomethacin from pectin matrix tablets: Effect of in situ cross linking by calcium cations. International Jour-nal of Pharmaceutics 2006; 318: 132–138.

[14]. Ishida et al. A novel approach to sustained pseudoephedrine release: Differentially coated mini-tablets in HPMC capsules. International Journal of Pharmaceutics 2008; 359: 46–52.

[15]. Chavan S, Anantwar S. Design and evaluation of once daily sustained release matrix tablets of Nicorandil. International Journal of Pharmacy and Pharmaceutical Sciences, 2011; 3(2): 13-18.

Table 1: Formulation of drug loaded pellets

S. No	Ingredients (gm)	AF 1	AF 2	AF 3	AF 4	AF 5	AF 6	AF 7	AF 8	AF 9	AF10	AF11	AF12	AF13	AF14	AF15
1	Bosentan	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
2	Eudragit RS 100	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
3	Nonpareil seeds	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
4	Isopropyl alcohol(ml)	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200
Composition of coating solution																
1	Eudragit RS100	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5
2	Isopropyl alcohol	200	200	200	200	200	200	200	200	200	200	200	200	200	200	200
3	Dibutyl phthalate	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

Table 2: Physical properties data of pellets

Batch	Bulk density (gm/ml)	Tapped density (gm/ml)	Angle of repose (^{0})	% friability	% drug content
AF1	0.78±0.015	0.80±0.025	10.18	0.04	19.07±0.25
AF2	0.71±0.009	0.86±0.091	10.38	0.05	19.10±0.34
AF3	0.79±0.004	0.86±0.004	11.22	0.03	19.16±0.11
AF4	0.81±0.023	0.81±0.011	9.44	0.03	20.57±0.24
AF5	0.79±0.004	0.88±0.012	9.54	0.04	21.34±0.14
AF6	0.73±0.022	0.84±0.022	10.18	0.05	19.89±0.14
AF7	0.82±0.008	0.82±0.008	10.32	0.04	19.36±0.63
AF8	0.76±0.025	0.87±0.025	11.48	0.05	19.86±0.52
AF9	0.76±0.025	0.82±0.011	9.88	0.06	19.78±0.21
AF10	0.80±0.003	0.80±0.009	9.52	0.03	20.13±0.13
AF11	0.72±0.007	0.81±0.008	10.65	0.06	19.07±0.21
AF12	0.72±0.007	0.88±0.007	11.29	0.05	20.21±0.32
AF13	0.75±0.004	0.88±0.08	9.71	0.04	19.13±0.57
AF13	0.74±0.012	0.84±0.011	11.38	0.03	20.21±0.57

AF14	0.77±0.018	0.86±0.012	11.29	0.05	19.97±0.13
AF15	0.74±0.005	0.83±0.004	9.71	0.06	19.74±0.24

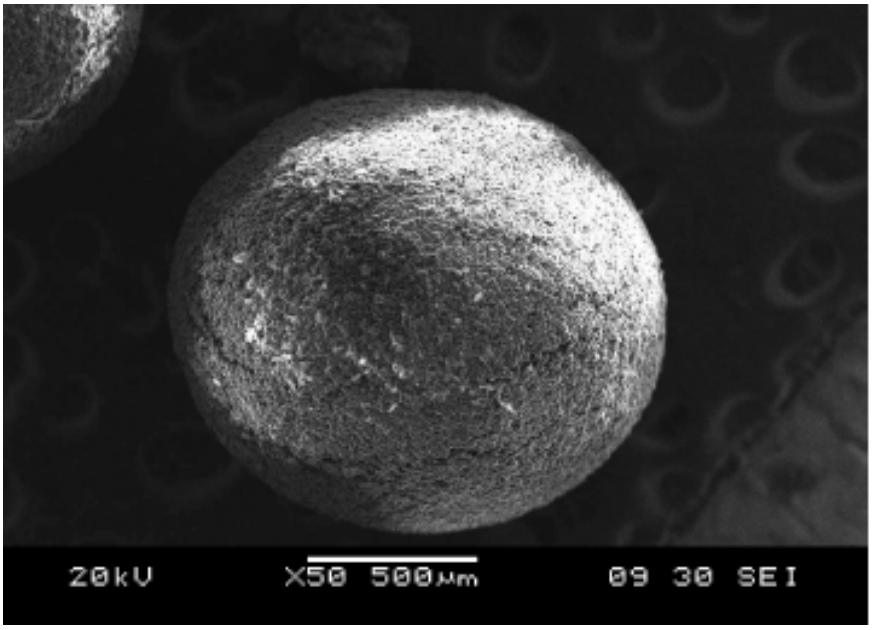


Figure 1: SEM of bosentan pellet

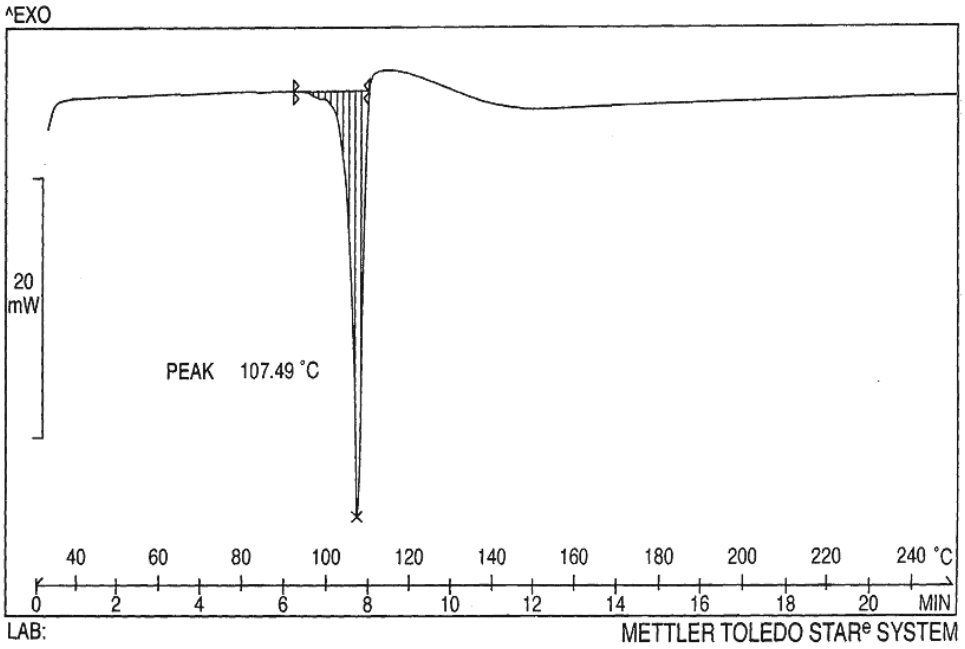


Figure 2: DSC thermogram of bosentan

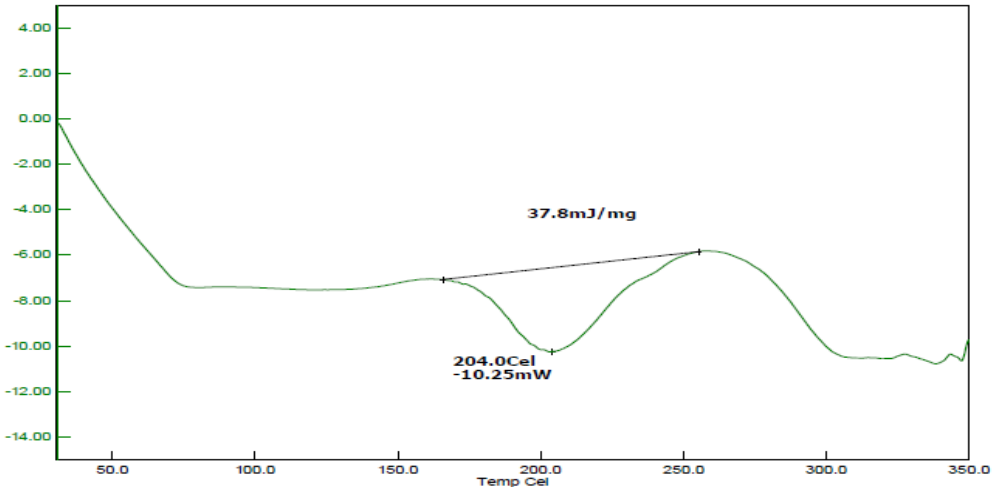


Figure 3: DSC thermogram of Eudragit RL100

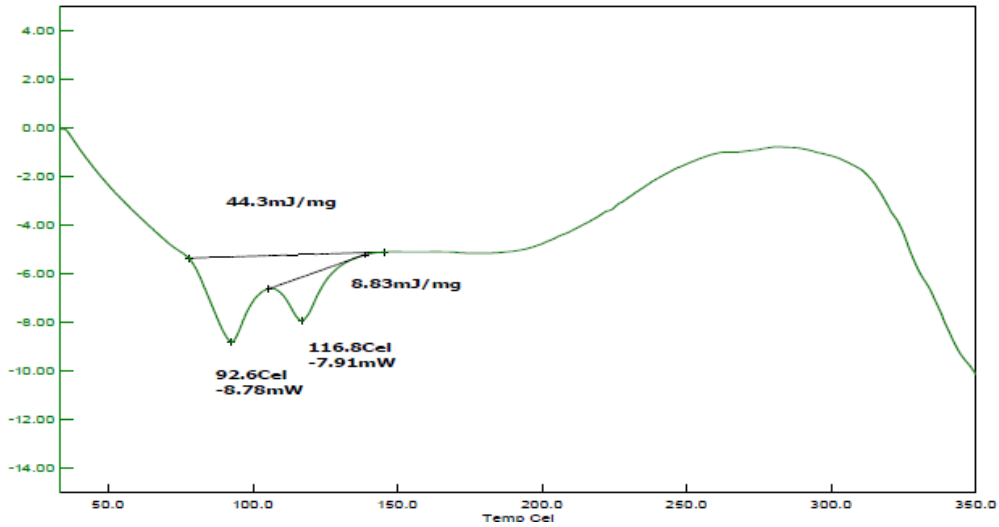
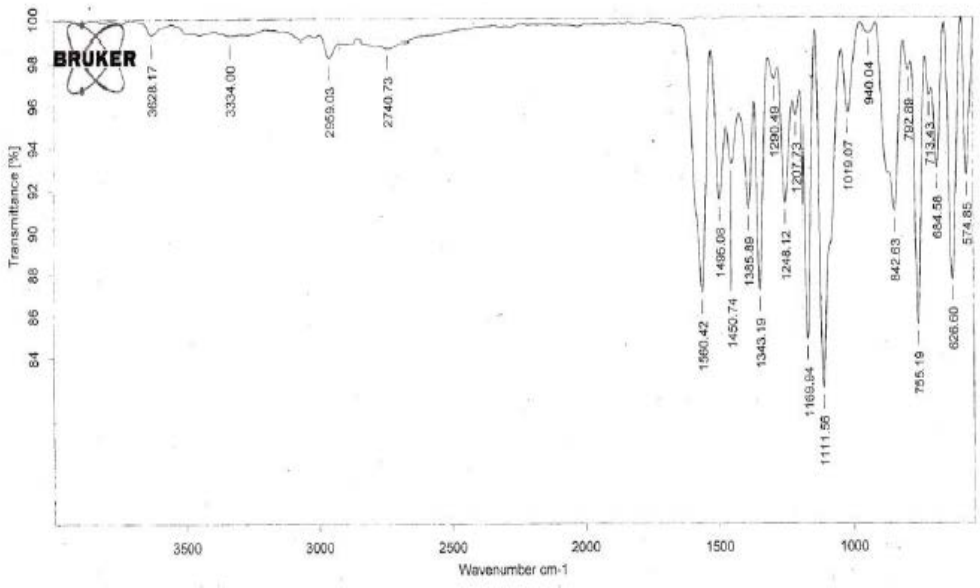
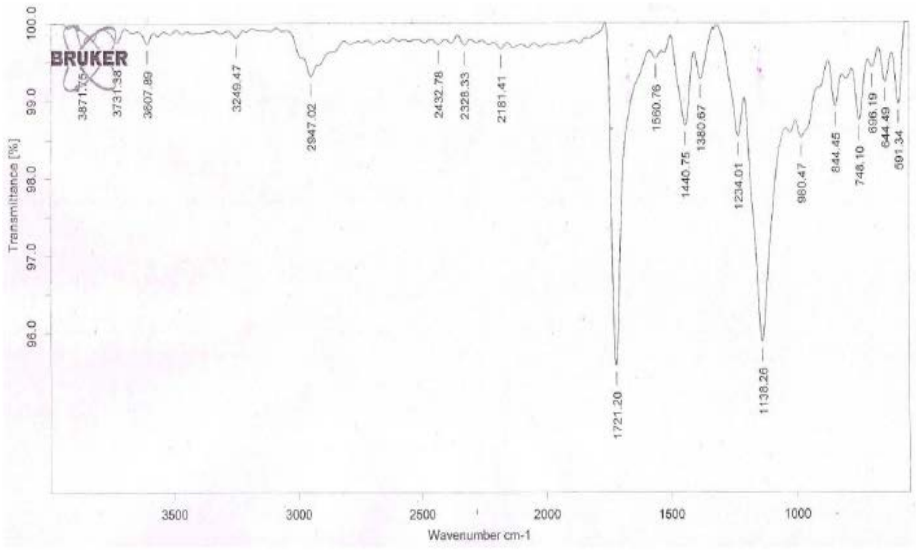


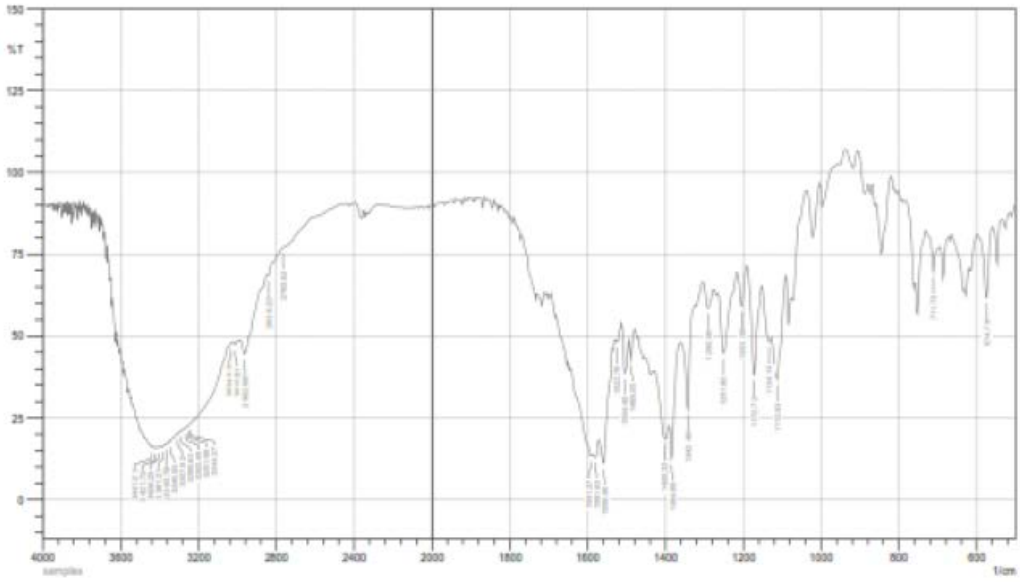
Figure 4: DSC thermogram of Drug+Eudragit RL100



a)



b)



c)

Figure 5: FT-IR spectrum of a)bosentan b) Eudragit RL100 c)physical mixture bosentan+ Eudragit RL100

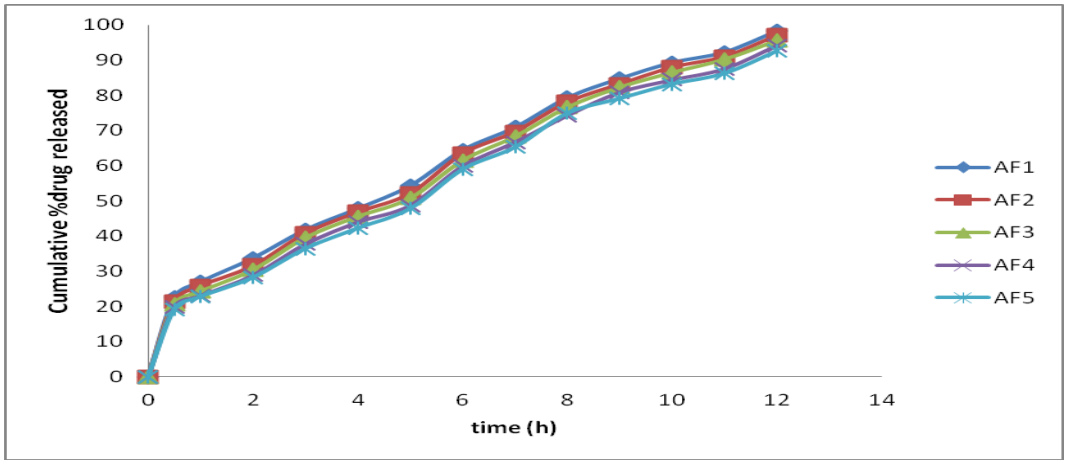


Figure 6: Drug release profile of AF1 TO AF5 formulations

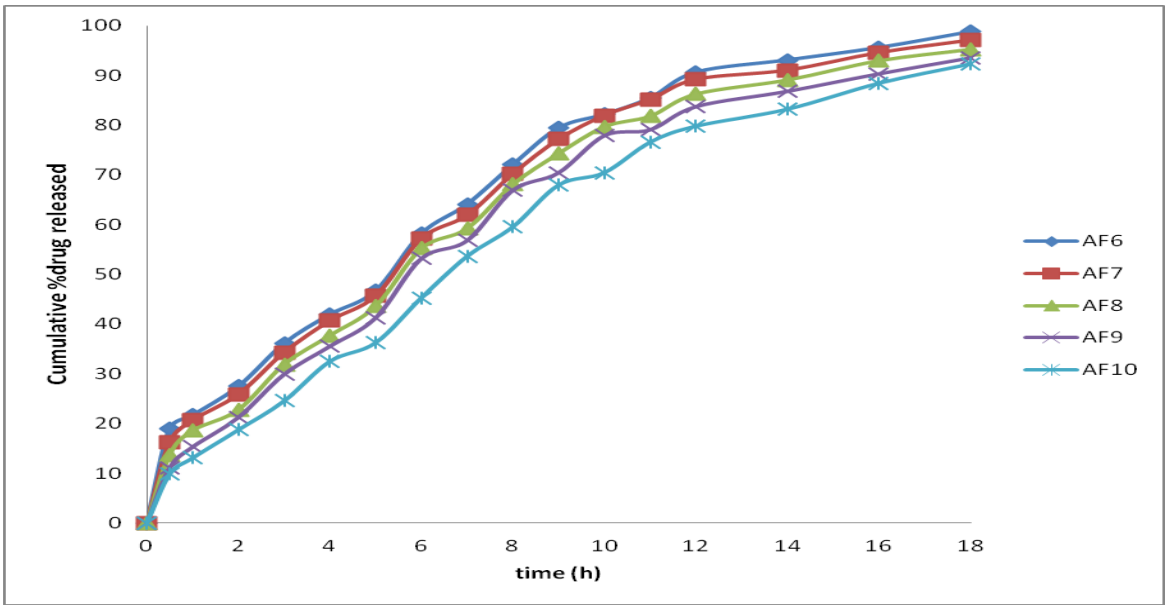


Figure 7: Drug release profile of AF6 TO AF10 formulations

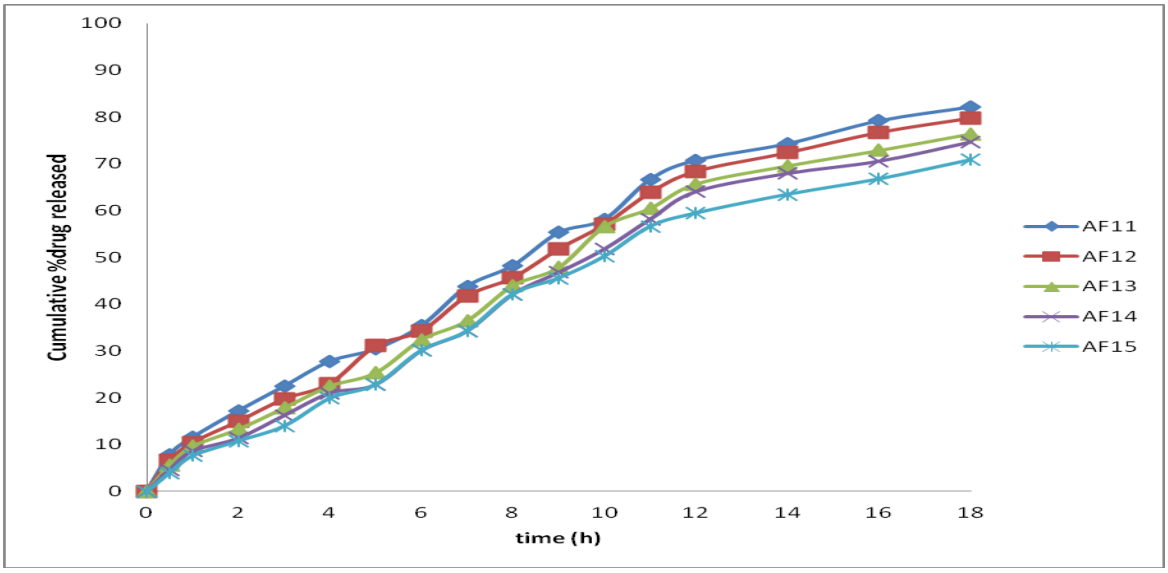


Figure 8: Drug release profile of AF11 TO AF15 formulations

Table 3: kinetic drug release data of bosentan from AF1 TO AF15 formulations

Formulation code	Zero order	First order	Higuchi	Korsemeyer pepas	'n' value
AF1	0.9913	0.8588	0.9799	0.9693	0.49
AF2	0.9906	0.8799	0.9782	0.9671	0.51
AF3	0.9916	0.9237	0.9775		0.52
AF4	0.9915	0.9317	0.9746	0.9628	0.53
AF5	0.9906	0.9416	0.9726	0.9619	0.53
AF6	0.9900	0.9593	0.9726	0.9608	0.54
AF7	0.9916	0.9636	0.9749	0.9719	0.57

AF8	0.9896	0.9730	0.9730	0.9739	0.61
AF9	0.9892	0.9782	0.9768	0.9845	0.67
AF10	0.9941	0.9736	0.9668	0.9776	0.7
AF11	0.9961	0.9647	0.9581	0.9855	0.69
AF12	0.9976	0.9695	0.9596	0.9846	0.74
AF13	0.9939	0.9624	0.9432	0.9816	0.77
AF14	0.9926	0.9601	0.9378	0.9808	0.81
AF15	0.9951	0.9770	0.9052	0.9498	0.85

Table 4: Plasma concentration of bosentan oral formulation and pellets

Time (hrs)	Oral solution	AF9
0	0	0
0.5	283.9	28.75
1	398.5	59.63
2	175.63	114.57
4	81.32	225.36
8	39.56	148.56
12	5.36	112.65
16	0	79.63
24	0	34.53

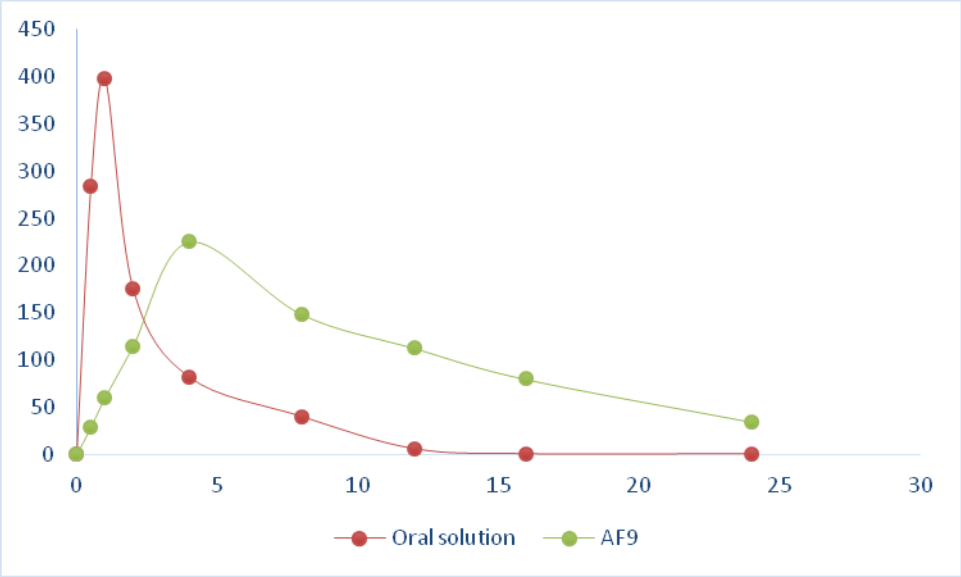


Figure 9: Plasma concentration of bosentan oral formulation and pellets

Table 5: *In vivo* pharmacokinetic parameters of pellet formulation

Parameter	Oral solution	Formulation AF9
C_{max} (ng/ml)	398.5	225.36
T_{max} (hr)	1	4
$t_{1/2}$ (hr)	2.98	5.34
Kel (/hr)	0.234	0.138
$AUC_{(0-t)}$ (ng-hr/ml)	1245	2978
$AUMC_{(0-t)}$ (ng-hr*hr/ml)	5234	29245
MRT (hr)	4.5	13