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FORMULATION AND *IN VITRO* EVALUATIONS OF FLOATING MICROSPHERES OF BOSENTAN

Maliha Khanam*[1] and Shahid Mohammed[1]

[1]*Department of Pharmaceutics, Deccan School of Pharmacy, Darussalam, Aghapura, Hyderabad, India.

ABSTRACT

The study is to formulate and evaluate bosentan floating microspheres using different polymers i.e. Sodium alginate, Xanthum gum and Gum karaya in different ratios. Bosentan is a dual endothelin receptor antagonist important in the treatment of pulmonary artery hypertension (PAH), with an bioavailability of less than 50%. Bosentan monohydrate has maximum absorption in stomach region. Therefore, in the present study an attempt as been made to formulate Bosentan floating microsphers which can be expected to pro long the gastric residence time of active compounds and reduce the variability of transit. They are capable of increasing the bioavailability of drugs that are mainly absorbed in the upper gastro intestinal tract. For that purpose, drug release has to be controlled. For the formulation, three bio compatible polymers Sodium alginate, Guargum and Gum karaya were chosen in varying proportions with the drug. Iono tropic gelation method was used to prepare microspheres employing different solvent to dissolve the drug and the polymer. The prepared formulations were characterized for their percentage yield, micro meritic properties, morphology, buoyancy studies, drug entrapment, drug release studies. Percentage Drug entrapment efficiency of F1 to F3 ranges from 62 to 89% for microspheres containing Soldium alginate as polymer, formulations F4 to F6 ranges from 56 to 92% for microspheres containing Guar gum as polymer and formulations F7 to F9 ranges from 67 to 82% for microspheres containing gum karaya as polymer. Almost all the formulations showed fairly acceptable values for all the parameters evaluated.

KEYWORDS

Bosentan, Pulmonary arterial hypertension, Sodium Alginate, Gum karaya and Ionotropic gelation method.

Author for Correspondence:
Maliha Khanam,
Department of Pharmaceutics,
Deccan School of Pharmacy,
Darussalam, Aghapura, Hyderabad, India.

Email: malihakhanam25@gmail.com

INTRODUCTION

The oral controlled release system is defined as the system which provides the delivery of drugs at predictable and reproducible kinetics for predetermined period of time throughout the course of GIT and also the system that target the drug delivery to a specific region within the GI tract for either local or systemic action in the body.

All the pharmaceutical products which can be formulated for the systemic delivery of the drugs via the oral route of administration, irrespective of the mode of delivery either immediate release, sustained or controlled release, and the design of dosage form can be solid or liquid dispersion, must be developed within the intrinsic characteristics of GI physiology. Hence the formulation for the development of successful oral drug delivery systems consists of basic understanding of (i) Physicochemical, pharmacokinetic and pharmacodynamic characteristics of the drug (ii) the anatomic and physiologic characteristics of the gastrointestinal tract and (iii) physicochemical characteristics and the drug delivery mode of the dosage form to be designed[1,2].

The important areas of the potential challenge in the development of oral controlled drug delivery systems are[3,4]: -

1. For the development of a controlled drug delivery system: To develop a oral controlled release drug delivery system capable of delivering a drug at a therapeutically effective rate to a desirable site for duration required for optimal treatment.
2. Modulation of the gastrointestinal transit time: To modulate the time of GI transit so that the drug delivery system developed can be transported to a target site or to the nearby region of an absorption site and reside there for a prolonged period of time to maximize the delivery of a drug dose.
3. To minimize the hepatic first pass elimination: If the drug to be delivered is subjected to extensive hepatic first-pass elimination, preventive measures should be devised to either bypass or minimize the extent of hepatic metabolic effect.

SCOPE OF THE STUDY

The Oral controlled conventional dosage forms suffer from mainly two adversities. The short gastric retention time (GRT) and unpredictable gastric emptying time (GET). A relatively brief GI transit time of most drug products hinder the formulation of single daily dosage forms. Altering the gastric emptying can overwhelm these problems. Therefore it is necessary, to formulate a controlled release dosage form that gives an extended GI residence time.

Extended release controlled dosage form with prolonged residence time in stomach are highly desirable for the drugs having following criteria,

i. That are locally active in the stomach,
ii. That have an absorption window in the stomach or in the upper small intestine,
iii. That are unstable in the intestinal or colonic environment,

Have low solubility at high pH values. Oral drug delivery is the most widely utilized route of administration among all the routes that have been explored for the systemic delivery of drugs. Oral route is considered as most natural, convenient and safe due to its ease of administration, uncomplicated, patient compliance, and cost-effective manufacturing process[1].

The all pharmaceutical products which are formulated for the systemic delivery via the oral route of administration, irrespective of the mode of delivery can be (immediate, sustained or controlled release) and design of dosage form (solid or liquid dispersion), needs to be developed within the intrinsic characteristics of GI physiology[4].

EXPERIMENTAL WORK

Pre formulation studies

Pre formulation studies can be defined as a phase of the research and development process where the formulation needs to characterizes the physical, chemical and mechanical properties of the new drug substance, to check for its stability, safety and effective dosage form. Ideally, the Pre formulation phase begins early in the discovery process such as the appropriate physical and chemical data is available to aid the selection of new chemical entities that enters the development process during this evaluation possible interaction with various inert ingredients intended for use in final dosage form are also considered in the present study.

Organoleptic properties

The color, odor and taste of the drug were recorded using descriptive terminology.

Solubility

The solubility of the drug sample was carried out in different solvents (aqueous and organic) according to I.P. The results are then compared with those mentioned in the official books and Indian Pharmacopoeia

Melting point

The melting point of Bosentan was determined by capillary method using digital melting point apparatus.

ANALYTICAL METHODS

Standard curve

Preparation of standard solution

Stock solution-I

100mg of Bosentan was accurately weighed into 100ml volumetric flask and dissolved in small quantity of buffer. The volume was made with 0.1N HCL to get a concentration of 1000µg/ml (SS-I).

UV Absorption Maxima (λ_{max}) of Bosentan sample in 0.1NHcl

Stock II: 10ml of above solution was then further diluted to 100ml with 0.1N HCL to get a stock solution of 100µg/ml. UVscanning was done for 100µg/ml drug solution from 200-400 nm using 0.1 as a blank in Shimadzu, UV 2450 spectrophotometer. The wavelength maximum was found to be at 273 nm.

Preparation of working standard solutions:

Further, from SS-II aliquots of 0.2ml, 0.4ml, 0.6ml, 0.8ml and 1ml were pipetted out into 10ml volumetric flasks. The volume was made up with 0.1N HCL to get the final concentrations of 10, 15, 20, 25, and 30µg/ml respectively. The absorbance of each concentration was measured at 273nm.

Calibration curve for the estimation of Bosentan

Calibration curve of Bosentan was estimated in 0.1N HCL (Table No.1).

Preparation of Floating Microspheres of Bosentan

Floating microspheres were prepared by the ionotropic gelation method. Various concentration of polymer in suitable solvents were mixed well with the Bosentan with different ratios of polymer as shown in Table No.2 and this pasty, mass was introduced into 50ml of aqueous saline phase containing 2 % (2 g) Calcium chloride ($CaCl_2$). The system is stirred using propeller at 300 rpm at room temperature for an hour. The drug loaded floating microspheres formed were filtered, washed and dried in a hot air oven at 60˚C.

PREFORMULATION STUDIES

Spectroscopic studies

Determination of λ max

A solution of 10µg/ml of Bosentan was scanned in the range of 200 to 400nm. The Drug Exhibited a λ max at 280nm in simulated gastric fluid pH 1.2 and had good reproducibility. Correlation between the concentration and absorban ce was found to be near to 0.9995, with a slope of 0.0349 and intercept of 0.0097.

Standard calibration curve

In the pre-formulation study, it was found that the λ max of bosentan monohydrate by spectrophoto metric method in 0.1N HCL was found to be 273nm.

PREFORMULATION PARAMETERS

All the formulations were evaluated for bulk density, tapped density, % compressibility, hausner's ratio and angle of repose. The results of % compressibility, hausner's ratio and angle of repose were found to be <16, <1.25 and <30 respectively. These results show that the formulations have very good flow properties (Table No.3).

Compatibility studies

Drug polymer compatibility studies were carried out using Fourier Transform Infra Red spectroscopy to establish any possible interaction of Bosentan with the polymers used in the formulation. The FT-IR spectra of the formulations were compared

with the FTIR spectra of the pure drug. The results indicated that the characteristic absorption peaks due to pure Bosentan have appeared in the formulated microspheres.

Drug-Excipient Compatibility study (FTIR)

Mean Particle Size

Mean particle size was determined by optical microscopy and the average particle size was calculated. The results were shown in Table No.5.

***IN-VITRO* DRUG RELEASE KINETICS**

For understanding the mechanism of drug release (Table No.6) and release rate kinetics of the drug from dosage form, the *In-Vitro* drug dissolution data obtained was fitted various mathematical models such as zero order, First order, Higuchi matrix, and Krosmeyer-Peppas model. The values are compiled in Table No.7. The coefficient of determination (R^2) was used as an indicator of the best fitting for each of the models considered. The kinetic data analysis of all the formulations reached higher coefficient of determination with the Zero order (R^2 = 0.985). From the coefficient of determination and release exponent values, it can be suggested that the mechanism of drug release follows Korsmeyer-Peppas model along with non-Fickian diffusion mechanism which leading to the conclusion that a release mechanism of drug followed combination of diffusion and spheres erosion.

STABILITY STUDIES OF BOSENTAN OPTIMIZED FORMULATION

The optimized formulation of Bosentan (F5) were subjected to short-term stability testing by storing the microspheres at room temperature 25°C/ 60% RH.

Results from stability studies indicate that the formulated microspheres are stable for a period of 3 months under room temperature i.e., 30˚C temp and 65±5% RH. There were no remarkable changes were observed during the period of storage.

The optimized formulation of Bosentan (F5) were subjected to accelerated stability testing by storing the microspheres at accelerated temperature 40°C/ 70% RH (Table No.8 and 9).

Table No.1: Calibration curve for the estimation of Bosentan

S.No	Concentration (µg /ml)	Absorbance
1	0	0
2	10	0.171
3	15	0.279
4	20	0.404
5	25	0.504
6	30	0.585

Table No.2: Formulation of Bosentan Floating Microspheres

S.No	Ingredients(mg)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	Bosentan	500	500	500	500	500	500	500	500	500
2	Sodium Alginate	500	750	1000	-	-	-	-	-	-
3	Xanthum Gum	-	-	-	500	750	1000	-	-	-
4	Karaya gum	-	-	-	-	-	-	500	750	1000
5	$NaHCO_3$	150	150	150	150	150	150	150	150	150
6	Water (ml)	100	100	100	100	100	100	100	100	100

Table No.3: Micro particulate Analysis

S.No	Formulation code	Bulk density (g/cc)	Tapped density (g/cc)	Carr's Index	Hausner Ratio	Angle of repose(θ)
1	F1	0.45±0.045	0.52 ± 0.09	15.60±0.2	1.15±0.02	28.06± 0.31
2	F2	0.45±0.045	0.50 ± 0.07	12.23±0.6	1.11±0.04	27.58± 0.15
3	F3	0.44±0.044	0.50 ± 0.09	12.58±0.8	1.13±0.08	28.44± 0.11
4	F4	0.45±0.045	0.52 ± 0.04	15.19±0.1	1.15±0.06	28.36± 0.13
5	F5	0.44±0.044	0.52± 0.01	15.48±0.6	1.18±0.08	28.52± 0.19
6	F6	0.45±0.045	0.51 ± 0.04	13.48±0.8	1.13±0.09	29.32± 0.19
7	F7	0.51±0.045	0.59 ± 0.04	14.48±0.8	1.15±0.09	29.69± 0.19
8	F8	0.45±0.041	0.52 ± 0.10	15.60±0.21	1.15±0.04	28.06± 0.41

Table No.4: Percentage yield and Percentage drug entrapment efficiency of the prepared microspheres

S.No	Formulation code	% Yield	% Buoyancy after 12 hrs	% Drug entrapment efficiency	% Swelling Index
1	F1	80	63	62.66	33.32
2	F2	83.33	67	72	35.66
3	F3	85	75	89	30.91
4	F4	86	79	56	32.33
5	F5	87.22	89	92	38.11
6	F6	80	85	72	38.18
7	F7	88	70	80	36.55
8	F8	82	76	82	37.32
9	F9	80	84	67	35.66

Table No.5: Average particle size of Bosentan microspheres

S.No	Batches	Mean Particle Size(µm)
1	F_1	540 µm
2	F_2	602 µm
3	F_3	644 µm
4	F4	612 µm
5	F5	528 µm
6	F6	624 µm
7	F7	588 µm
8	F8	598 µm
9	F9	626 µm

Table No.6: Percentage cummulative drug release for all formulations

TIME(hrs)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	23	18	16	28.4	16.25	14	25.3	23	11.30
2	32	27.2	24	40.3	21.3	20	37.2	38	19.6
3	41.5	36	31	49.7	28.6	26	44.3	45	25.4
4	57.6	45	42	55.3	30.4	28	52.4	50	28.2
5	68.2	53	49	62.4	38.2	38	57.8	54	36.3
6	79.7	67	54	68.3	44.3	42	65.2	63	40.4
7	99.3	72	58.7	76.9	51.6	48	70.8	69	46.8
8	-	99.1	100.4	83.2	57.2	54	89.2	88	79.3
10	-	-	-	96.9	78.3	63	100.1	99.8	101.2
12	-	-	-	-	99.2	88.1	-	-	-

Table No.7: *In-vitro* drug release kinetics data for Formulation F5

Zero order		First order		Higuchi's data		Korsmeyer-Peppas data	
Time (h)	% CDR	Time (h)	Log % CD Remaining	SQR Time	% CDR	Log Time	Log % CDR
1	16.25	1	1.922	1	16.25	0	1.21
2	21.3	2	1.895	1.414	21.3	0.301	1.328
3	28.6	3	1.853	1.732	28.6	0.477	1.456
4	30.4	4	1.842	2	30.4	0.602	1.482
5	38.2	5	1.790	2.236	38.2	0.698	1.582
6	44.3	6	1.745	2.449	44.3	0.778	1.646
7	51.6	7	1.684	2.645	51.6	0.845	1.712
8	57.2	8	1.631	2.828	57.2	0.903	1.752
10	78.3	10	1.336	3.162	78.3	1	1.893
12	86.2	12	1.139	3.464	86.2	1.079	1.935

Table No.8: Stability studies of optimized formulation at room temperature

Time	Colour	Drug entrapment efficiency ± St.D. at Room Temperature	Cumulative % drug release ± St.D.
First day	White	92.00± 0.91	86.20±0.55
30days	White	91.84 ± 0.23	86.01±0.72
60 days	White	91. 06 ± 0.62	85.62±0.65
90 days	White	90. 92 ± 0.31	85.20±0.98

Table No.9: Stability studies of optimized formulation at Accelerated temperature

Time	Colour	Drug entrapment efficiency ± St.D. at accelerated Temperature	Cumulative % drug release ± St.D.
First day	White	92.00± 0.91	86.20±0.55
30days	White	91.72 ± 0.21	85.71±0.10
60 days	White	91. 01 ± 0.90	85.12±0.88
90 days	White	90. 66 ± 0.01	85.00±0.12

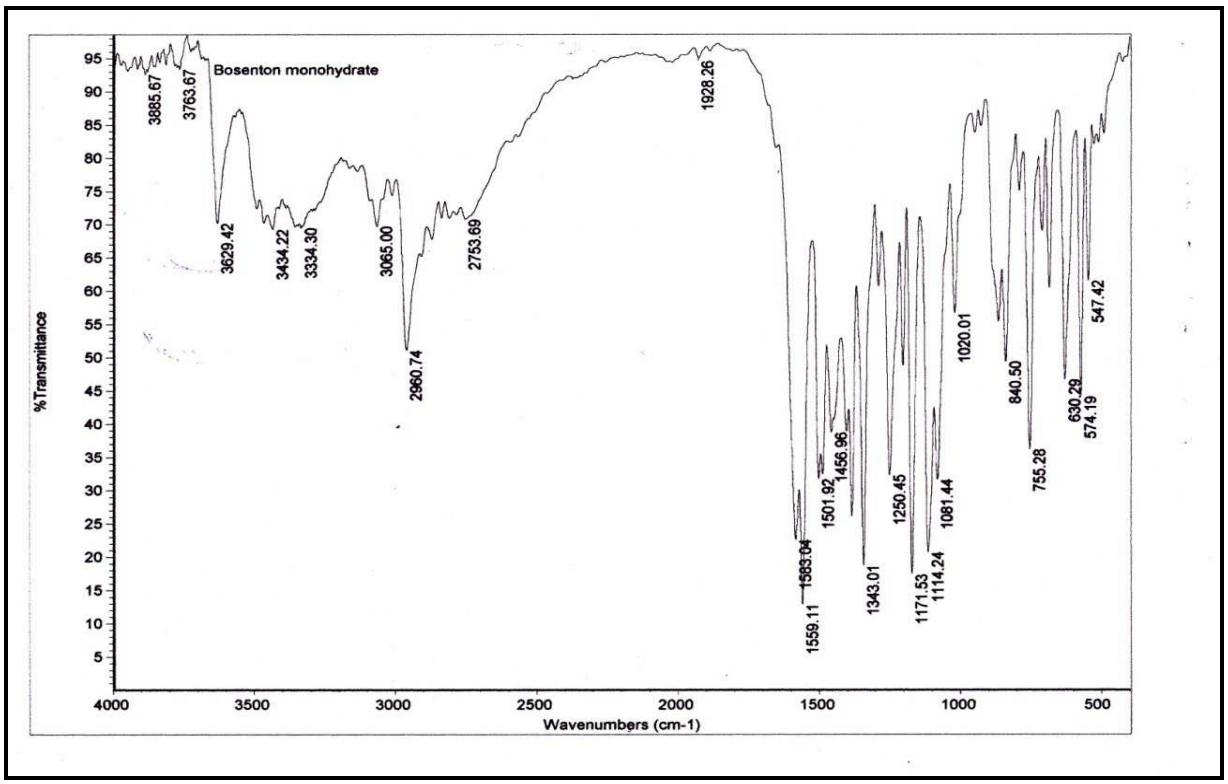

Figure No.1: FTIR SPECTRUM OF BOSENTAN PURE DRUG

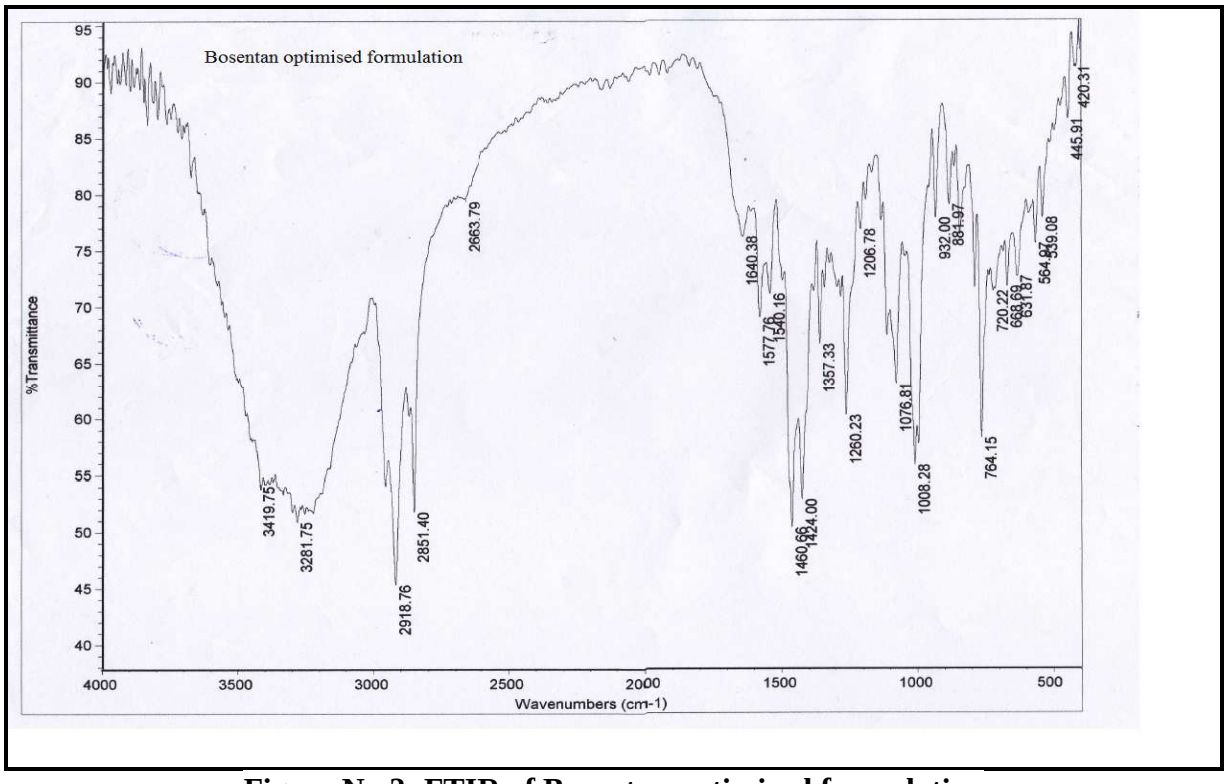

Figure No.2: FTIR of Bosentan optimized formulation

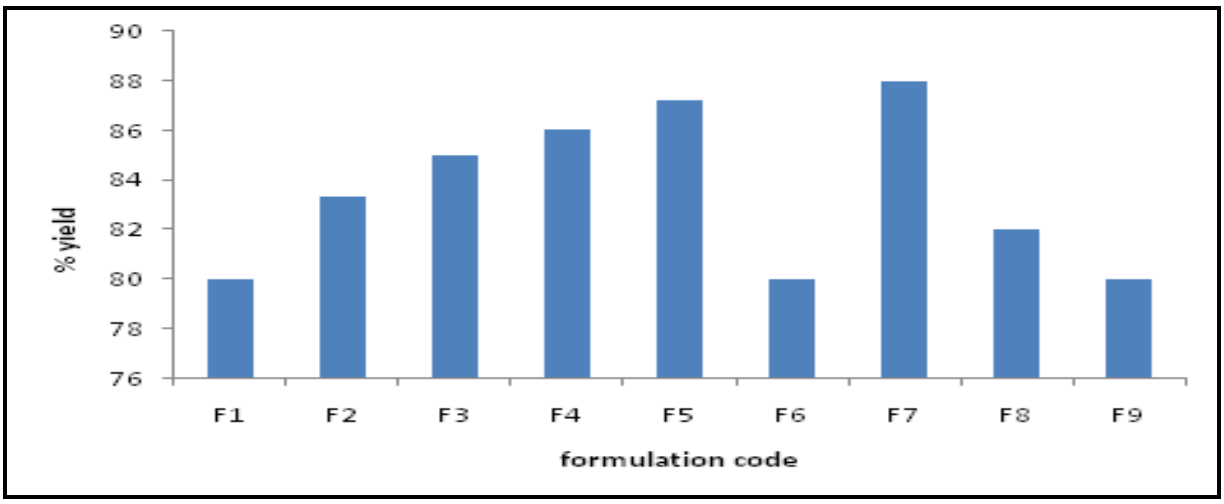

Figure No.3: Graph for % yield vs Formulation code

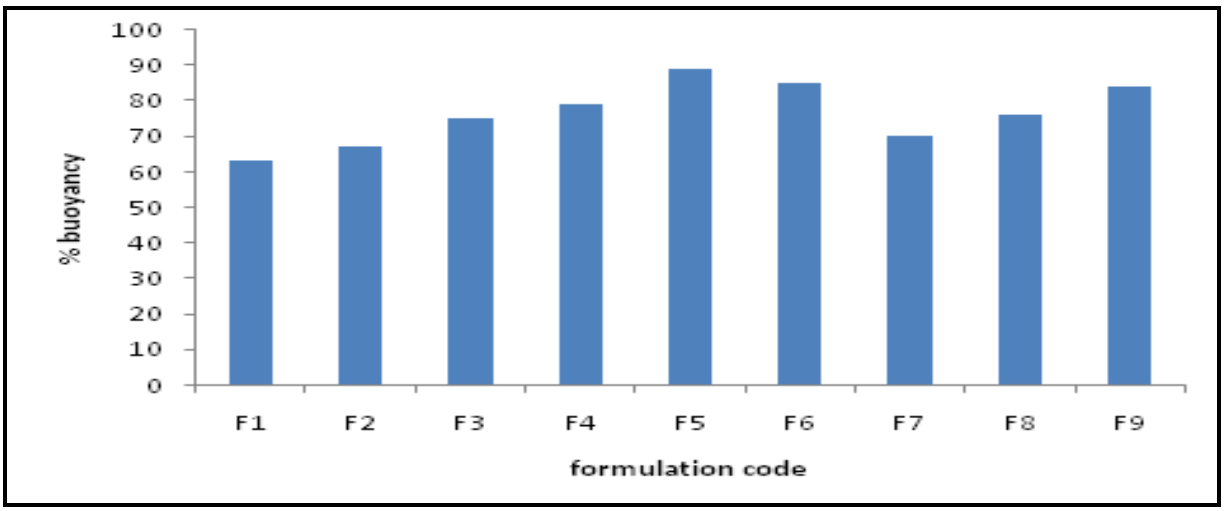

Figure No.4: Graph for % Buoyancy vs Formulation code

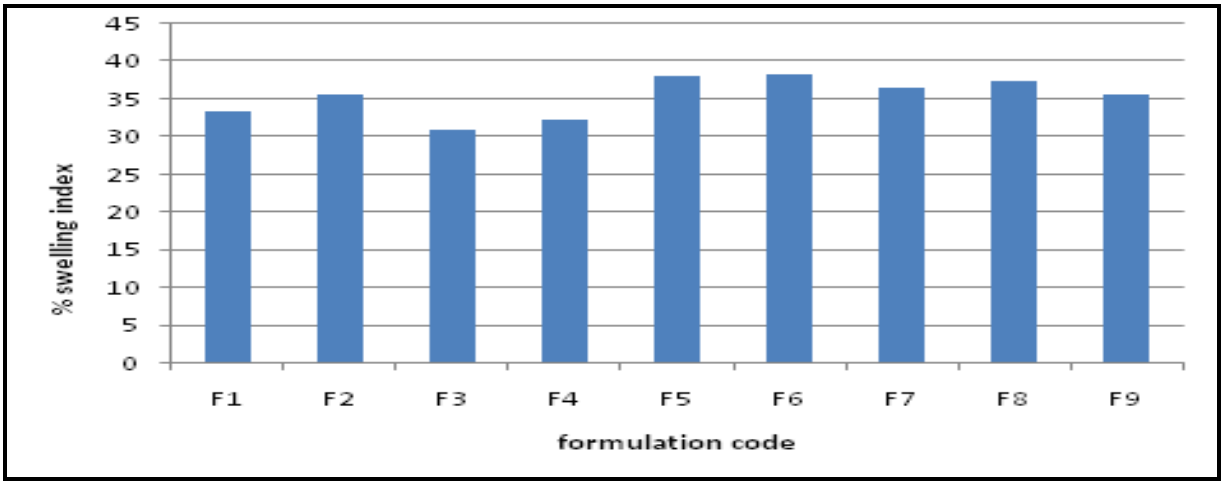

Figure No.5: Graph for % swelling index vs Formulation code

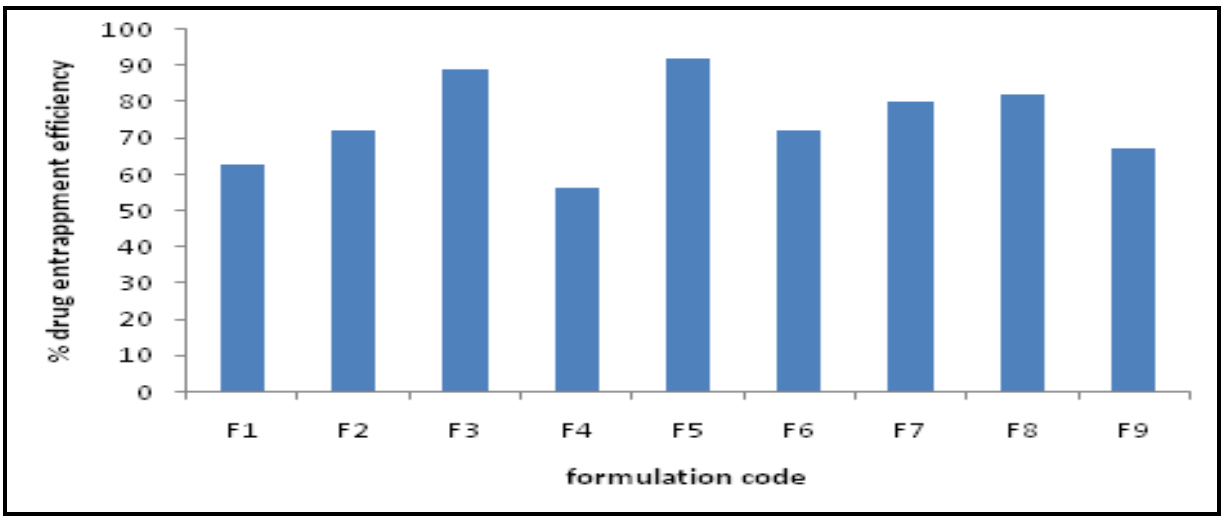

Figure No.6: Graph for % drug entrapment efficiency vs Formulation code

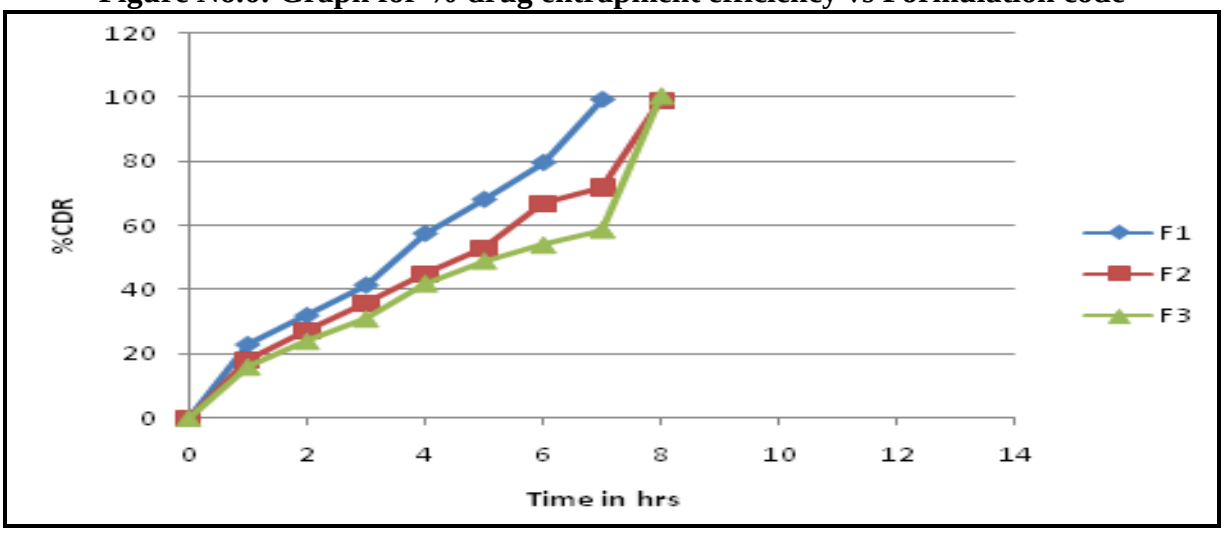

Figure No.7: Dissolution graph for formulation F1-F3

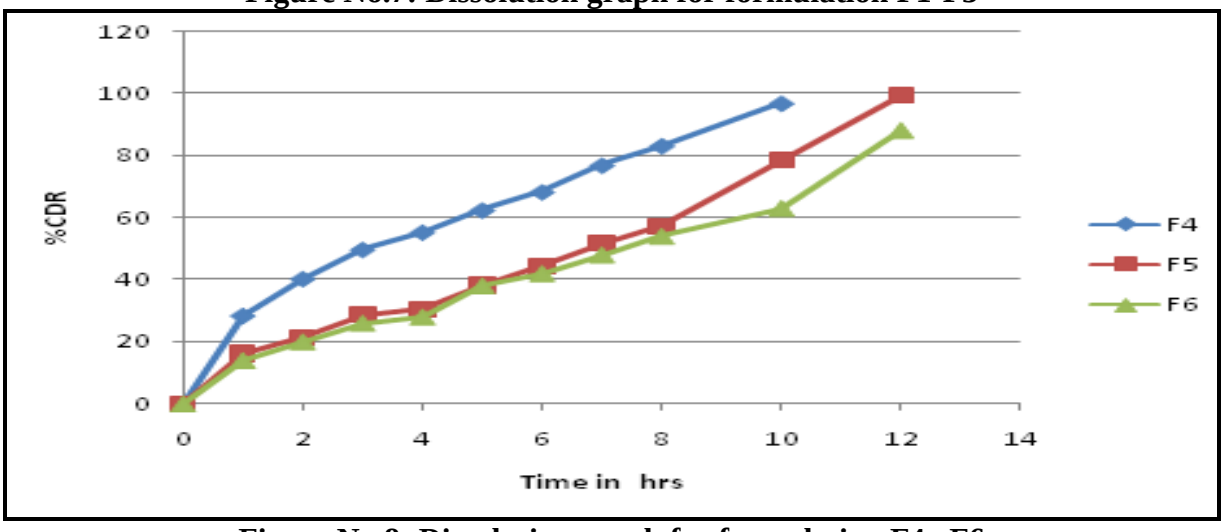

Figure No.8: Dissolution graph for formulation F4 –F6

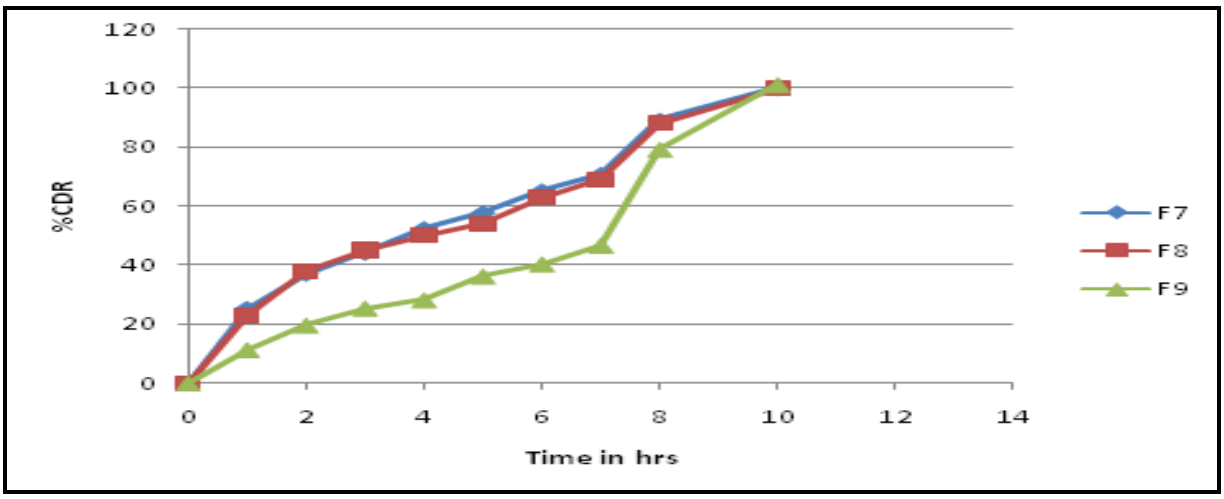

Figure No.9: Dissolution graph for formulation F7-F9

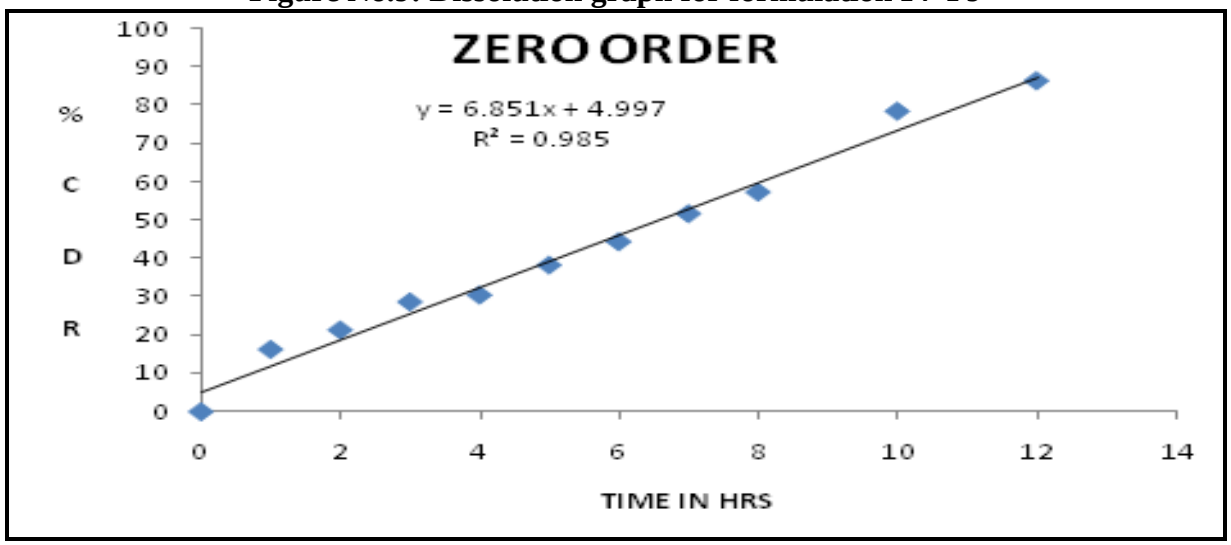

Figure No.10: Zero order kinetic graph for F5 batch

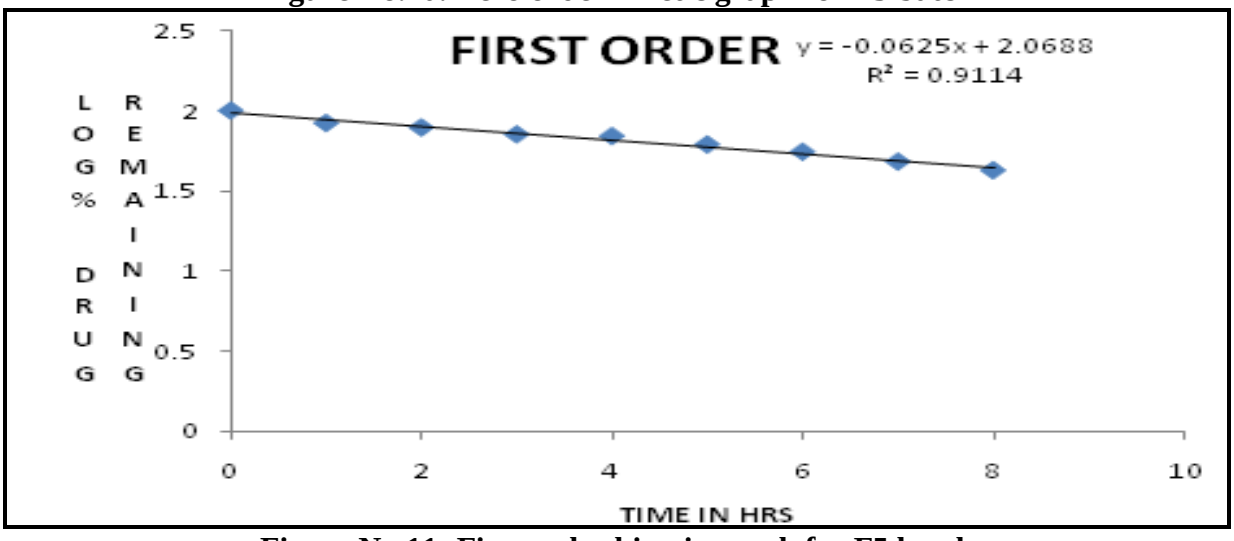

Figure No.11: First order kinetic graph for F5 batch

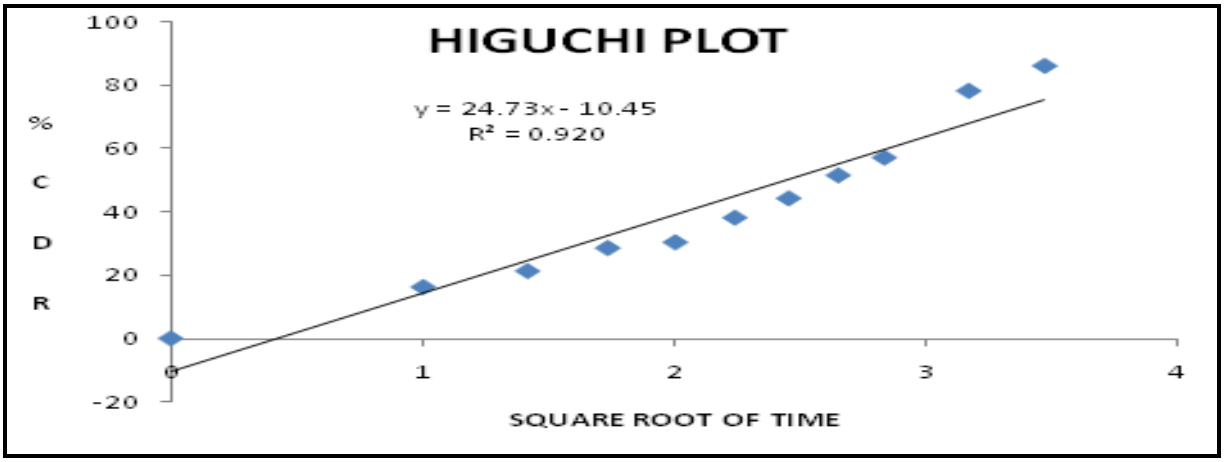

Figure No.12: Higuchis model kinetic graph for F5 batch

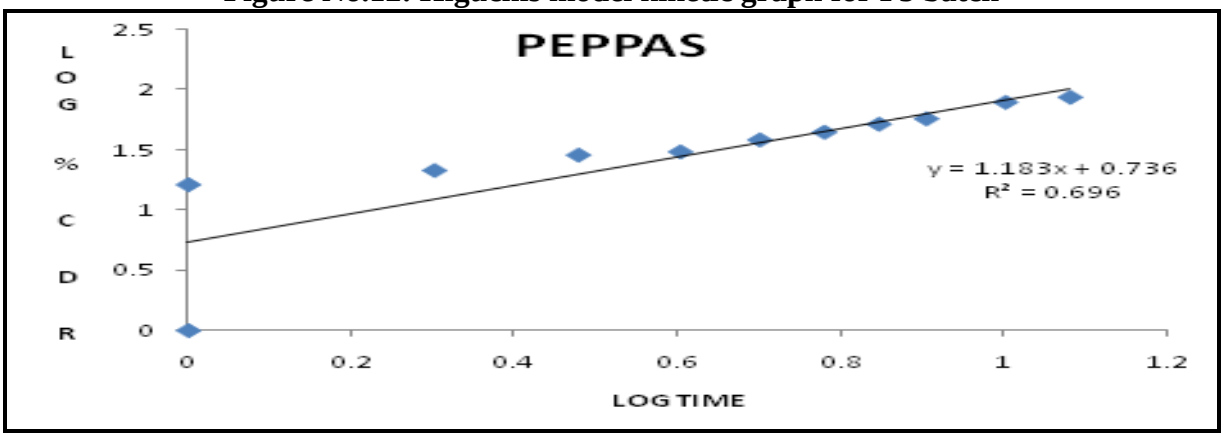

Figure No.13: Peppas model kinetic graph for F5 batch

CONCLUSION

The present study has been a satisfactory attempt to formulate a floating Microspheres of Bosentan with a view to control the release of the drug. From the experimental results it can be concluded that,

Biocompatible polymers like can be Sodium alginate, guargum and gum karaya used to formulate a floating Microspheres. Good percentage drug entrapment and practical yields were obtained with the polymers.

The formulations showed good flow properties, suggesting that, in future they could be easily and successfully packed and developed into a capsule dosage form. Among all formulations F5 formulation with drug: polymer (1:2) was found to be satisfactory in terms of excellent micro meritic properties, percent yield (87.22%), drug entrapment efficiency (92%), percent buoyancy (89%), and highest *in vitro* drug release of 96.2% in sustained manner over a extended period of time for 12 hrs. Thus the prepared microspheres proved to be a potential candidate as a micro particulate controlled release drug delivery device in this era of patenting novel and controlled release formulations.

The overall curve fitting into various mathematical models was found to be on average. The formulations F5best fitted into zero order and shows non fickian diffusion mechanism. Formulated microspheres were stable and compatible at the room and accelerated temperature and humidity in storage for 90days.

Thus, the formulated floating microspheres seem to be a potential candidate as an oral gastro retentive controlled drug delivery system in prolonging the drug retention stomach and increasing the bioavailability of drug.

FUTURE SCOPE

Further detailed stability studies and *in vivo* bioavailability studies are to be done to establish the efficacy of these formulations. *In vitro - in vivo* or relation study are to be done to establish the guarantee of efficacy and bioavailability of the formulation.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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