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Original Research Article

FORMULATION AND IN-VITRO EVALUATION OF BOSENTAN MONOHYDRATE MICROSPHERES

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ABSTRACT

The present study performed by Formulation and Evaluation of microspheres of Bosentan monohydrate which is an anti-hypertensive drug. Microspheres were formulated with various materials like HPMC K4M, HPMC K15M, HPMC K100M, Carbopol 934P as rate controlling polymers. The microspheres were prepared by Solvent evaporation method. The variant proportion of the polymers HPMC K100M, Carbopol showed significant difference in the release rates. The drug release rate decreased as the concentration of carbopol is increased.

Key words: Bosentan monohydrate, microspheres, antihypertensive, solvent evaporation method.

INTRODUCTION

Bosentan monohydrate ($C_{27}H_{29}N_5O_6S$) is a dual endothelin receptor antagonist important in the treatment of pulmonary artery hypertension (PAH) by blocking the action of endothelin molecules that would otherwise promote narrowing of the blood vessels and lead to high blood pressure.Endothelin-1 (ET-1) is a neurohormone, the effects of which are mediated by binding to ET_A and ET_B receptors in the endothelium and vascular smooth muscle. ET-1 concentrations are elevated in plasma and lung tissue of patients with pulmonary arterial hypertension, suggesting a pathogenic role for ET-1 in this disease. Bosentan is a specific and competitive antagonist at endothelin receptor types ET_A and ET_B. Bosentan has a slightly higher affinity for ET_A receptors than for ET_B receptors. It is freely soluble in acetone and dichloromethane. The bioavailability of Bosentan monohydrate is 50% with a half life of 4-5 hours. Therefore, it was chosen as a model drug for preparation of microspheres. The goal in designing sustained or controlled delivery system is to reduce the frequency of the dosing or to increase effectiveness of the drug by localization at the site of action, reducing the dose required or providing uniform drug delivery. So, the controlled release dosage form is a dosage form that release one or more drugs continuously in a predetermined pattern for a fixed period of time, either systemically or to a specified target organ. Controlled release dosage forms provide a better control of plasma drug levels, less dosage frequency, less side effects, increased efficacy and constant delivery. Microspheres can be defined as solid spherical and polymeric particles ranging in size from 1 to 1000µm. The mechanism of drug release is either dissolution or diffusion of drug. These are made up of polymeric (or) waxy (or) other protective materials. Microspheres have played a vital role in the development of controlled/sustained release drug delivery systems. Microspheres provide constant and prolonged therapeutic effect. Reduces the dosing frequency and thereby improve the patient compliance. They could be injected into the body due to the spherical shape and smaller size. Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effects. Microsphere morphology allows a controllable variability in degradation and drug release. The purpose of this work is to develop microspheres of Bosentan monohydrate by using different rate controlling polymers.

MATERIALS AND METHODS

Materials

Bosentan monohydrate was obtained as a gift sample from Alkem Pvt.Ltd. HPMC K4M, HPMC K15M, HPMC K100M, Carbopol 934p was obtained as a gift sample from Colorcon India Pvt.Ltd . All

other ingredients, reagents and solvents were of analytical grade.

Methods

Solvent evaporation method: The microspheres were prepared by solvent evaporation method.The drug and polymers were dissolved in dichloromethane and methanol. This solution was dispersed in 100 ml of liquid paraffin light containing 0.5% Span 80 in a 250 ml beaker. The dispersion was stirred at 500 rpm for 90 min. After the stirring time, microspheres were centrifuged, washed several times with n-hexane,ether and finally with acetone. The microspheres were dried at 50˚C and stored in desiccator. Total 17 batches were prepared with different drug : polymer ratios using combinations of HPMC/CARBOPOL . For all batches rpm was maintained at 500 and temperature was maintained at 15ºC. Total seventeen formulations were prepared with different drug polymer ratio. These seventeen formulations were included in the optimization study and evaluated.

EVALUATION PARAMETERS:

The prepared microspheres were evaluated for Particle size analysis, Percentage yield, Drug entrapment effiency, Drug content, SEM and In vitro drug release studies.

Particle size analysis: The particle size of microspheres was determined using optical microscopy method. Particle size of all the batches of the formulated beads in a sample was measured with an optical micrometer fitted with a calibrated eye piece. Calibration of the microscope was done prior to particle size measurement of the beads. Approximately 625 beads were counted for particle size using a calibrated optical microscope. All readings are average of three trials ± SD .

Percentage yield: The yields of production of microbeads of various batches were calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microbeads and percent production yields were calculated as per the Formula mentioned below.

Percentage yield =

(practical yield/theoretical yield) ×100

Drug entrapment efficiency : Drug entrapment efficiency of Bosentan microspheres was performed by accurately weighing 80mg of drug equivalent microspheres and suspended in 100 ml of 7.4 pH phosphate buffer and it was kept on a side for 24 hours. Then, it was stirred for 15 mins and filtered. After suitable dilution, Bosentan content in the filtrate was analyzed Spectrophotometrically at 262 nm using U.V.Spectrophotometer.

Estimation of Drug content: Equivalent weight of microspheres was weighed and dissolved in 5ml of water and methanol mixture in a standard flask Shake for 30min and then make up with 7.4 pH phosphate buffer and then centrifuge it.

from that take 5ml of solution in 50 ml standard flask make up with 7.4 PH phosphate buffer . Generally, the drug content in any formulation should fall within the limit of 90 – 110%. About 25mg of microspheres were weighed and added to 50 ml of 7.4 Ph phosphate buffer. The resulting mixture was agitated on mechanical shaker for 24 hrs, then solution was filtered and the drug content was estimated at 262 nm spectrophotometrically after suitable dilution.

Scanning electron microscopy analysis (SEM):

The shape and surface characteristics were determined by scanning electron microscopy (model-JSM, 35CF, jeol, Japan) using gold sputter technique. The particles were Vacuum dried, coated to 200 Aº thicknesses with gold palladium using prior to microscopy. A working distance of 20nm, a tilt of zero-degree and accelerating voltage of 15kv were the operating parameters. Photographs were taken within a range of 50-500 magnifications.

In vitro drug release studies:

In vitro drug release from Bosentan microspheres was performed using USP Apparatus 1 in 900 mL of 0.05 M potassium phosphate buffer pH 7.4 stirred at 37 °C and 100 rpm maintaining sink conditions. The accurately weighed Bosentan microspheres were enclosed in a sieve, placed in the basket, and processed for dissolution testing. All the Bosentan microspheres stayed in the basket during 24-h dissolution testing (i.e., no particles diffused out of the sieve). Dissolution samples (5 mL) were withdrawn at regular intervals (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22 and 24h) using an auto sampler with replacement of equal volumes of fresh medium. The samples were filtered through a 0.45-µm filter and analyzed spectrophotometrically at 262 nm in triplicate. Drug concentration was calculated using a calibration curve.

Dissolution media	pH 7.4 Phosphate buffers
Volume	900 ml
Apparatus	Paddle
Speed	50 rpm
Time	0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 18, 20, 22 and 24 hrs
Aliquot withdrawn	5ml
Aliquot replaced	5ml
Wavelength	262nm

DRUG RELEASE KINETICS:

Zero order release rate kinetics:

To study the zero order release kinetics the release rate data are fitted to the following equation

$$F=K_0t$$

Here, F is the fraction of drug release

K_0 is the rate constant

T is the release time

First order model:

This model has also been used to describe absorption and/elimination of drug, the release of the drug which followed first order kinetic can be expressed by the equation

$$\text{Log } C=\log c_0-kt/2.303$$

Where, C_o is the initial concentration of drug

K is the first order rate constant

t = is the time

Higuchi release model:

To study the higuchi release kinetics, the release rate data was fitted to the following eqution

$$F=K_H.t^{1/2}$$

Where, F is the amount of the drug release

Kh is the release time

t is the release time

Korsmeyer and peppas model:

The release rate date were fitted to the following eqution,

$$Mt/M8=K_M.t^n$$

Where, Mt/M8 is the fraction of drug release

K_M is the release constant

t is the release time

RESULTS

Table.1.Composition of the Formulations

Formulation	Drug (mg)	HPMC K4M (mg)	HPMC K15M (mg)	HPMC K100M (mg)	carbopol 934 (mg)	Ratio	Methanol, Dichloro-methane (ml)
F1	500	500	-	-	-	1:1	20
F2		750	-	-	-	1:1.5	20
F3		1000	-	-	-	1:2	20
F4		-	500	-	-	1:1	20
F5		-	750	-	-	1:1.5	20
F6		-	1000	-	-	1:2	20
F7		-	-	500	-	1:1	20
F8		-	-	750	-	1:1.5	20
F9		-	-	1000	-	1:2	20
F10		250	250	-	-	1:1	20
F11		375	375	-	-	1:1.5	20
F12		500	500	-	-	1:2	20
F13		-	-	250	250	1:1	25
F14		-	-	375	375	1:1.5	25
F15		-	-	500	500	1:2	25
F16		-	-	250	500	1:1.5	25
F17		-	-	250	750	1:2	25
		Liquid paraffin (ml) – 100 ml Span 80% - 0.5					

Table.2. Micromeritic properties of BMH

Formulation	Angle of repose	Bulk density	Tapped density	Car's index	Hausner's ratio
F1	25.43±0.1	1.041±0.3	1.16±0.1	11.4±0.320	1.114±0.015
F2	26.46±0.2	1.02±0.4	1.12±0.2	9±0.208	1.09±0.015
F3	23.31±0.1	1.01±0.2	1.11±0.1	9±0.320	1.09±0.013
F4	26.89±0.17	1.02±0.28	1.11±0.21	8±0.342	1.08±0.016
F5	29.14±0.1	0.96±0.24	1.03±0.27	7±0.401	1.07±0.019
F6	28.14±0.2	0.95±0.24	1.03±0.27	9.5±0.210	1.095±0.012
F7	29.1±0.1	0.94±0.2	1.03±0.2	9±0.237	1.095±0.015
F8	28.2±0.1	0.96±0.2	1.04±0.2	8±0.238	1.08±0.018
F9	27.1±0.4	1.041±0.3	1.16±0.1	11.4±0.342	1.114±0.016
F10	25.1±0.4	1.02±0.4	1.12±0.2	9±0.282	1.08±0.018
F11	29.14±0.1	0.96±0.24	1.03±0.27	7±0.313	1.07±0.015
F12	28.14±0.2	0.95±0.24	1.03±0.27	9.5±0.196	1.095±0.011
F13	29.1±0.1	0.94±0.2	1.03±0.2	9±0.254	1.095±0.016
F14	28.2±0.1	0.96±0.2	1.04±0.2	8±0.195	1.08±0.010
F15	25.1±0.4	1.041±0.3	1.16±0.1	11.4±0.156	1.114±0.019
F16	25.1±0.4	1.02±0.4	1.12±0.2	9±0.164	1.09±0.016
F17	24.1±0.4	1.02±0.4	1.12±0.2	9±0.188	1.09±0.013

n=3; ±=standard deviation

Table.3. Particle size, %yield, Drug Entrapment Efficiency, Drug Content

Formulation code	Particle size (µm)	% yield	Entrapment efficiency	Drug content
F1	106.5±2.3	93.70±1.28	87.04±1.92	98.56±0.63
F2	110±2.21	87.82±2.01	78.68±2.1	98.48±0.91
F3	103.4±1.42	92.70±1.19	85.04±1.87	97.59±1.97
F4	102.5±1.3	85.95±1.98	76.87±1.91	98.64±2.01
F5	103.2±0.9	94.82±2.16	88.35±2.67	98.46±3.22
F6	103±2.8	86.90±3.05	75.69±1.91	98.78±1.4
F7	108.6±1.7	93.25±1.37	86.98±2.08	99.11±2.1
F8	106±2.35	85.82±2.01	76.68±2.1	97.46±2.4
F9	103.8±1.8	93.70±1.28	87.04±1.92	98.95±1.8
F10	102.1±1.3	87.82±2.01	78.68±2.1	97.65±1.6
F11	102.9±1.4	85.95±1.98	76.87±1.91	96.89±2.1
F12	101.9±1.7	94.82±2.16	88.35±2.67	98.28±1.7
F13	104.2±1.2	86.90±2.45	75.72±1.94	98.73±1.9
F14	105.1±1.5	93.55±1.37	86.68±2.08	97.89±1.92
F15	106.2±1.3	85.35±1.98	76.84±1.98	98.48±2.08
F16	101.8±1.1	86.27±2.05	76.68±2.12	99.24±1.91
F17	100.8±1.6	98.70±1.87	92.02±1.07	99.92±2.67

n=3; ±=standard deviation

Table.4. Invitro drug release of F1-F6 Formulations

Time	F1	F2	F3	F4	F5	F6
1	58.6±0.6	48.7±0.5	30.6±0.7	35.7±0.7	26.81±0.6	19.7±0.6
2	69.7±0.9	60.6±0.3	38.9±0.3	49.6±0.2	35.4±1.3	31.5±0.3
3	79.3±0.4	68.7±0.2	43.8±0.5	50.7±0.1	47.8±0.5	42.6±0.6
4	87.6±1.1	75.8±1.9	50.6±0.2	61.3±0.8	59.4±0.2	53.6±1.5
5	99.8±0.6	84.9±0.5	64.9±0.3	75.4±0.2	67.8±0.3	61.8±0.2
6	-	98.9±0.2	68.6±0.4	85.6±0.5	83.6±0.9	80.7±0.5
7	-	-	70.3±0.6	98.5±0.9	99.8±0.7	91.7±0.5
8	-	-	99.8±1.5	-	-	99.5±0.6
9	-	-	-	-	-	-
10	-	-	-	-	-	-
12	-	-	-	-	-	-
14	-	-	-	-	-	-
16	-	-	-	-	-	-
18	-	-	-	-	-	-
22	-	-	-	-	-	-
24	-	-	-	-	-	-

n=3; ±=standard deviation

Table.5. Invitro drug release of formulations F7-F12

Time	F7	F8	F9	F10	F11	F12
1	40.9±0.4	32.6±1.97	20.9±1.5	21.7±1.5	10.8±0.6	10.94±1.25
2	52.3±1.3	46.8±1.92	28.9±2.1	31.3±2.24	15.30±0.89	21.6±2.23
3	59.4±0.6	50.6±2.67	40.7±2.8	36.8±2.89	21.6±1.23	30.1±1.09
4	67.8±1.5	61.2±1.5	48.3±1.29	43.4±1.25	31.45±1.28	42.6±1.56
5	76.8±1.8	70.6±2.1	55.6±1.86	58.9±1.9	34.8±2.25	49.8±2.04
6	85.9±1.9	79.6±2.08	68.7±1.32	71.8±2.68	51.3±2.28	59.3±2.87
7	99.8±2.1	84.6±0.6	74.3±2.65	79.4±1.92	58.9±1.25	68.7±3.25
8	-	91.9±1.9	81.56±3.25	83.6±2.05	67.4±1.56	87.6±3.65
9	-	99.29±1.5	85.45±1.89	87.25±1.09	73.25±1.98	93.45±1.56
10	-	-	92.1±1.25	98.25±3.01	82.45±2.36	98.99±1.28
12	-	-	98.9±2.08	-	90.99±2.67	-
14	-	-	-	-	97.92±1.56	-
16	-	-	-	-	-	-
18	-	-	-	-	-	
22	-	-	-	-	-	-
24	-	-	-	-	-	-

n=3; ±=standard deviation

Table.6. Invitro drug release of formulations F13-F17

Time	F13	F14	F15	F16	F17
1	63.5±1.98	58.95±1.02	49.89±1.63	29.5±2.06	8.96±1.56
2	72.5±2.25	64.95±1.56	58.45±1.95	38.59±2.65	12.25±1.26
3	87.5±1.65	70.25±2.56	64.54±2.65	42.53±2.28	17.85±2.25
4	92.5±0.89	79.23±2.86	71.23±1.23	54.55±2.23	22.58±1.8
5	99.89±1.089	83.45±3.24	77.32±1.56	62.23±2.45	29.89±3.25
6	-	87.85±2.56	85.25±2.65	69.45±3.25	36.53±1.96
7	-	92.45±2.87	89.95±3.25	72.55±1.56	40.23±2.89
8	-	97.29±2.92	94.95±3.56	79.88±1.98	42.25±2.25
9	-	-	98.29±3.85	85.23±1.56	47.25±1.68
10	-	-	-	89.99±2.56	54.55±2.36
12	-	-	-	94.88±2.87	62.89±3.25
14	-	-	-	99.83±2.96	70.12±2.89
16	-	-	-	-	79.89±1.5
18	-	-	-	-	88.93±1.65
22	-	-		-	94.35±1.95
24	-	-	-	-	97.43±2.65

n=3; ±=standard deviation

Table.7.Stability studies of optimized formulation physico-chemical parameters.

Formulation Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-17	25^{0}C/60%RH % Release	97.43	97.40	97.38	97.35	Not less than 85 %
F-17	30^{0}C/75% RH % Release	97.47	97.41	97.39	97.37	Not less than 85 %
F-17	40^{0}C/75% RH % Release	97.45	97.42	97.36	97.32	Not less than 85 %
F-17	25^{0}C/60% RH Assay Value	99.92	99.89	99.86	99.85	Not less than 90 % Not more than 110 %
F-17	30^{0}C/75% RH Assay Value	99.93	99.89	99.87	99.86	Not less than 90 % Not more than 110 %
F-17	40^{0}C/75% RH Assay Value	99.91	99.88	99.86	99.84	Not less than 90 % Not more than 110 %

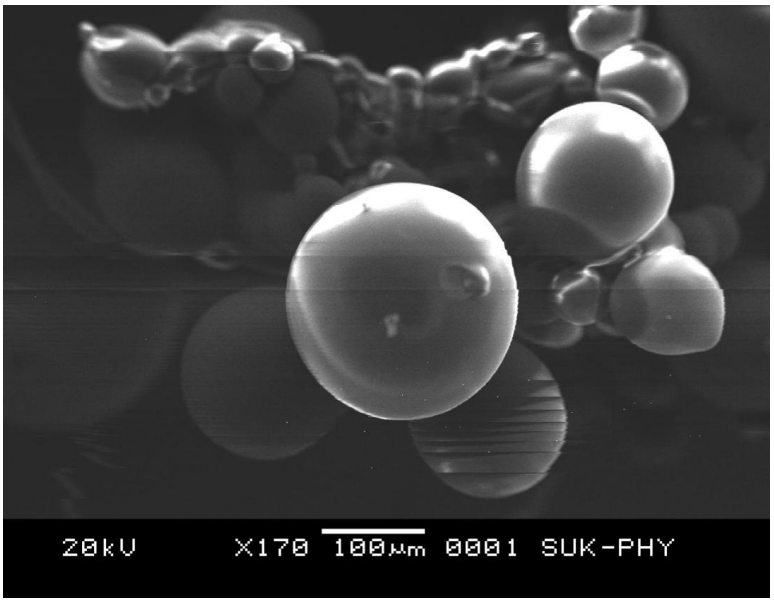

Fig.1. SEM image of microspheres

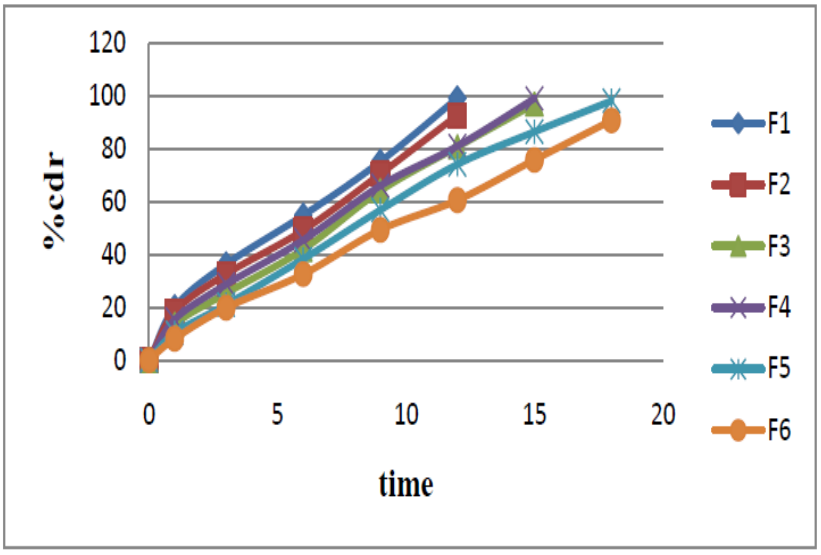

Fig.2. *In Vitro* Dissolution Profile For Batches F1 – F6

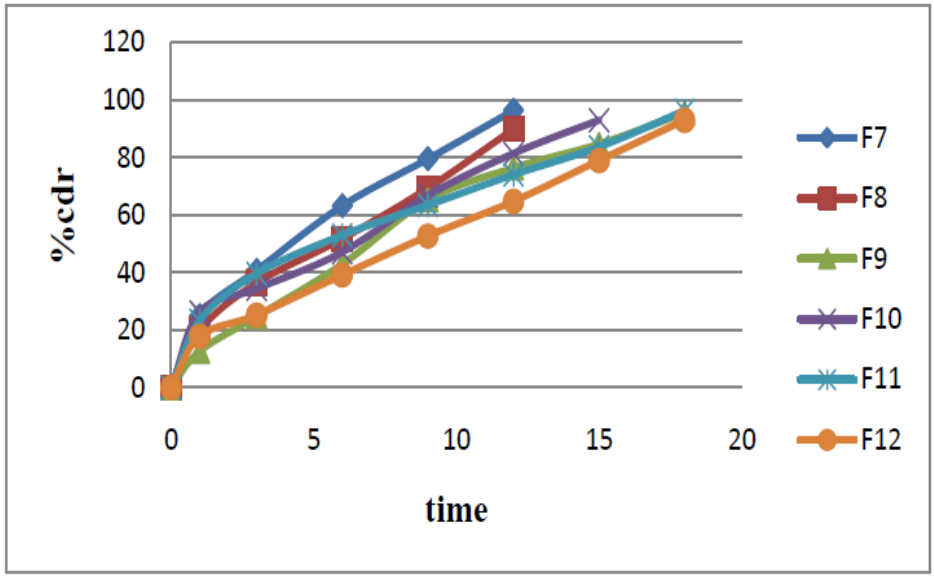

Fig.3. *In Vitro* Dissolution Profile For Batches F7 – F12

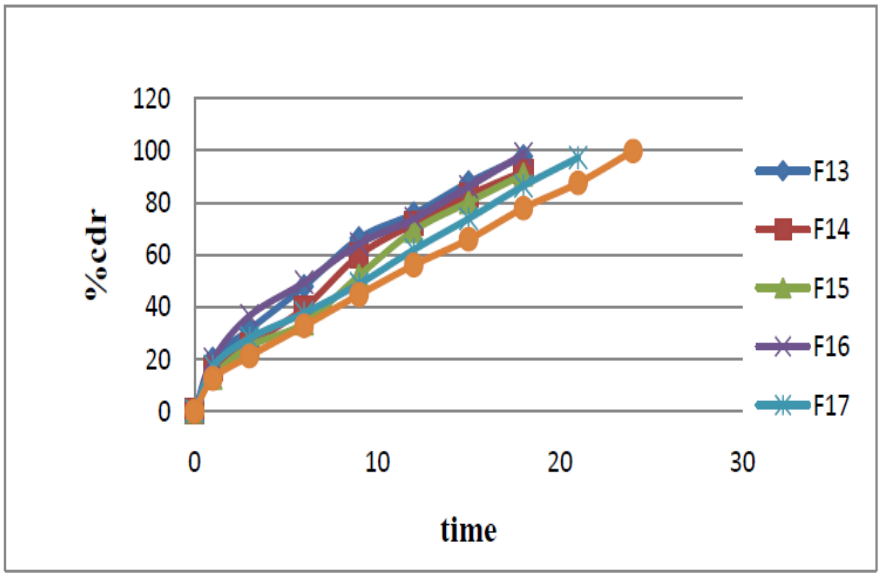

Fig.4.*In Vitro* Dissolution Profile For Batches F13– F17

Kinetic studies for F-17 formulation:

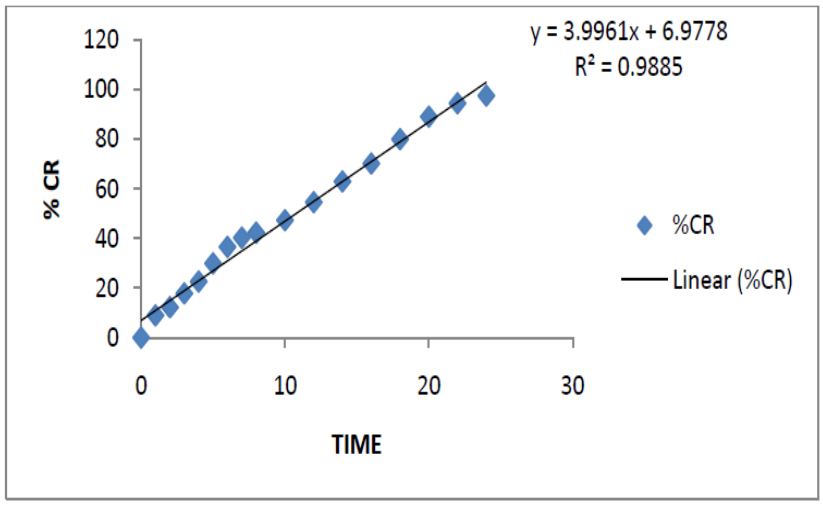

Fig.5.Graphical representation of Zero order kinetic studies

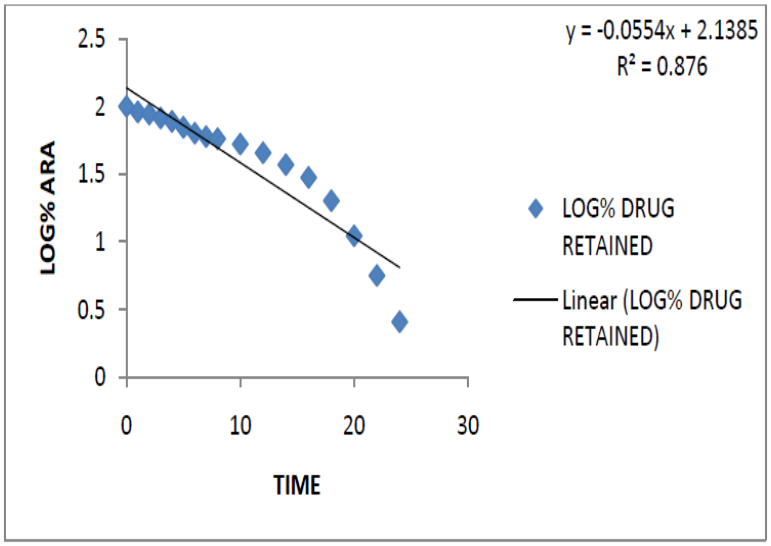

Fig.6. Graphical representation of First order kinetic studies

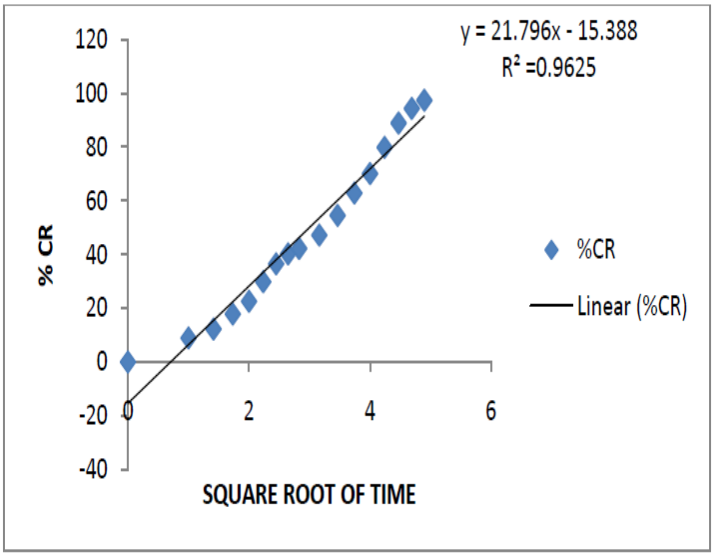

Fig.7.Graphical representation of Higuchi mechanism of release

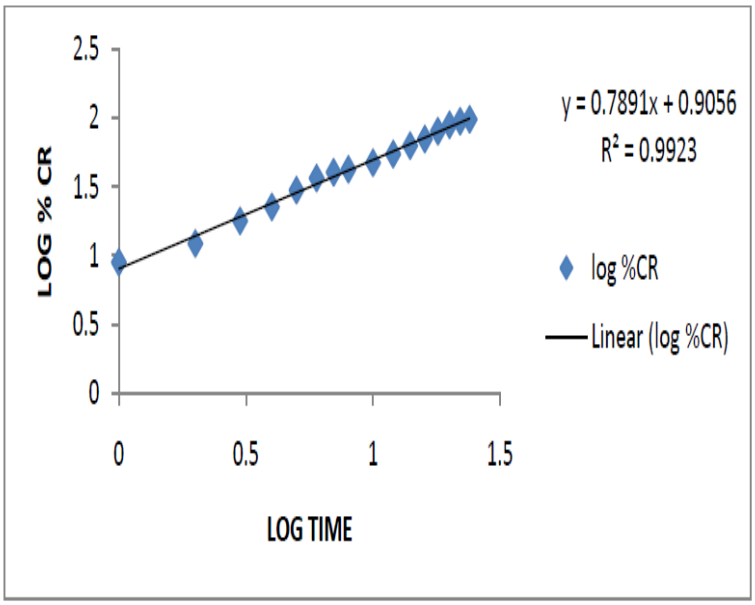

Fig.8.Graphical representation of Peppas mechanism of release

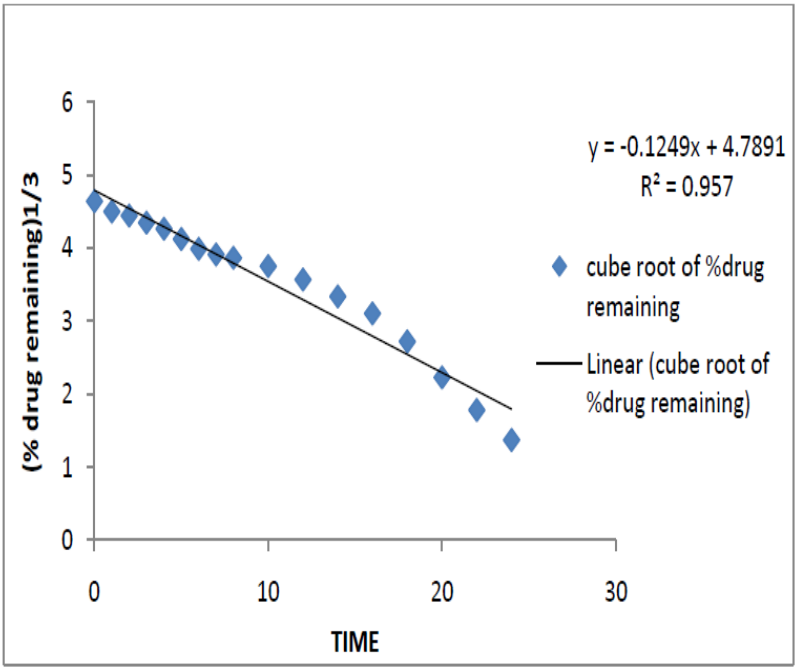

Fig.9.Graphical representation of Hixson & Crowell mechanism of release

DISCUSSION

Evaluation of physical parameters of microspheres:

The important parameters in the formulation of microsphers were evaluated and reported in Table 2 . The bulk density and tapped density varied from 0.94 to 1.041 and 1.03 to 1.16 respectively. Hausner`s s ratio and carr`s index varied from 1.07 to 1.114 and 7 to 11.4 respectively. The results showed that microspheres had good flow property and compressibility.

Evaluation of microspheres:

The particle size was found to be in the range of 101.9 to 110. The percentage yield, entrapment efficiency and drug content was found to be in the range of 85.82 to 94.8, 75.72 to 88.35 and 96.89 to 98.56.

The stability studies were conducted for the formulation F17. Initially the drug release was observed and then stored for three months in respective conditions. After 3 months the drug release was observed.

Dissolution Studies:

Based on the objectives of the present investigation, the microspheres were evaluated for release of Bosentan monohydrate. Dissolution studies were attempted. The results are shown in Table 6.The dissolution data reveals that the rate of dissolution was decreasing linearly with increasing concentration of polymer. By using the polymers like HPMC K100M and Carbopol in combination satisfactory results were found. In kinetic data formulation F-17 (97.43%) follows Zero order and Koresmeyer-peppas mechanism of drug release.

CONCLUSION

The present investigation carried out to develop microspheres of Bosentan monohydrate. Microspheres were prepared by using Solvent evaporation method. The release of Bosentan monohydrate from the formulations is proportional to the concentration of polymers. As the concentration of polymers increases, the drug release rate decreases. Result of the study based on in vitro performance clearly suggests that extended release can be achieved by incorporating hydrophobic and hydrophilic polymers.

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