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PREPARATION AND *INVITRO* CHARACTERISATION OF BOSENTAN MONOHYDRATE MUCOADHESIVE MICROSPHERES

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ABSTRACT

The present study involves the preparation and *in vitro* characterization of mucoadhesive microspheres of Bosentan Monohydrate a dual Endothelin receptor antagonist used in Pulmonary Arterial Hypertension with the aim to achieve controlled drug profiles in blood, to improve the therapeutic efficacy andthe patient compliance to use as an alternative therapy to conventional dosage form. The microspheres were prepared by emulsification solvent evaporation method using different polymer combinations in different ratios with HPMC K4M, HPMCK100M, Ethyl Cellulose andCarbopol 940P. Drug interaction studies by FTIR and DSC revealed that drug is compatible with the polymers in study. The prepared microspheres were charecterized for various parameters like particle size, practical yield, drug entrapment efficiency, swelling studies,*in vitro* drug release characteristics(in pH 1.2 buffer for first 2hrs, in pH 6.8 buffer up to 8 hrs and in pH 7.4 buffer up to 24 hrs.), *in vitro* mucoadhesion using goat intestine, muco adhesive strength and muco adhesive force. All the microspheres showed good swelling, mucoadhesive properties and good controlled release of drug. Among the different combinations of polymers in different ratios studied, the desired *in vitro* drug release(94.81% for 24 hrs) and highest *in vitro* mucoadhesion of 89% was found with the combination of Carbopol 940P and HPMC K100M with drug in the ratio of 1: 0.75:0.75(F3). The drug release from the F3 formulation followed Higuchi's matrix and Peppa's model.The *invitro* drug release of optimised formulation F3 was also compared with that of the marketed film coated tablets.The marketed formulation released 91% of drug with in one hour whereas the optimised formulation F3 showed a release of 94.81% of the drug over 24 hrs confirmed the prolonged release of the drug.

KEYWORDS: Bosentan Monohydrate, Pulmonary Arterial Hypertension, Solvent evaporation,drug entrapment efficiency, *in vitro* mucoadhesion ,controlled release.

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a chronic and progressive disease leading to right heart failure and ultimately death if untreated.[1] PAH results from chronic obstruction of small pulmonary arteries, which is due, at least in part, to endothelial and vascular smooth muscle cell dysfunction and proliferation.[2-3] Bosentan is an orally active, nonpeptidic, competitive dual endothelin (ET) receptor antagonist with high affinity for both ET_A and ET_B receptors and is used in the treatment of PAH.[4-6] Endothelin-receptor antagonism with oral bosentan is an effective approach to therapy for PAH. Bosentan significantly improved symptoms, exercise capacity, cardiopulmonary hemodynamics, quality of life and reduces clinical worsening in patients with idiopathic PAH or PAH associated with connective tissue diseases. Bosentan therapy was safe, well tolerated, confers therapeutic benefits in patients with PAH and has been extensively used as monotherapy.[7-10] The absolute bioavailability of Bosentan is about 50% and the half-life is 5.4 hours.[11]

Mucoadhesive drug delivery systems are commonly used to prolong the residence time of the dosage form at the site of application or absorption and to facilitate intimate contact of the dosage form with the underlying absorption surface to improve and enhance the bioavailability of drugs.[12-16] Microspheres, in general have the potential to be used for targeted and controlled release drug delivery but coupling of mucoadhesive properties to microspheres has additional advantages,like efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layers and specific targeting of drug to the absorption site achieved.[17] Hence, in the present study an attempt has been made to formulate and evaluate BMH loaded mucoadhesive microspheres using different polymers in combination to increase the Gatrointestinal residence time,there by

bioavailability and patient compliance with the prolonged release of the drug.

MATERIALS AND METHODS

Materials

Bosentan Monohydrate was a gift sample from MSN laboratories, Hyderabad. Bosentas tablets (Cipla PrivateLimited, Hyderabad, India), labelled to contain 62.5 mg Bosentan Monohydrate per tablet, were purchased from commercial sources inthe local pharmacy market. All polymers were obtained from Colorcon Asia Pvt Ltd, Goa, India. All other chemicals and solvents used were of analytical grade. The goat intestine for *in vitro* mucoadhesion was obtained from local slaughter house.

Methods

Analytical method for construction of calibration curve

Pure BMH 100mg was dissolved in 100ml of 0.1N Hydrochloric acid. 10ml of this solution was further diluted to 100ml with same solvent to obtain 100µg/ml. From this solution (100µg/ml) suitable working solutions of different concentrations of 10, 20, 30, 40, 50 & 60µg/ml were prepared. The absorbance of these dilutions was measured at the determined lambda max of 272 nm. The standard graph of concentration versus absorbance was then plotted. Each point is an average of three determinations. Slope, y-axis intercept and regression coefficients were calculated. The same steps were repeated with pH 6.8 and 7.4 buffers.[18-20]

Method of preparation of BMH loaded mucoadhesive microspheres by emulsification solvent evaporation technique

Nine formulationsof Bosentan Monohydrate loaded microspheres were prepared by water in oil emulsification solvent evaporation technique, using three different polymers and changing drug: polymer ratios (1:0.5,1:1 and 1:1.5) as shown in Table 1. Carbopol has been widely used for the preparation of mucoadhesive drug delivery systems as it swells excessively upon being exposed to pH above 6.0.[21] As it causes irritation at the mucosal surface[22] it was, therefore, combined with HPMC and EC to optimize the mucoadhesion and swelling characteristics of microspheres and also to reduce its irritancy.[23-25]

Microspheres were prepared by dissolving a specific quantity of polymer in sufficient organic solvent(methanol: dichloromethane) (1:1) to produce polymeric solution then specific quantity of core material BMH was mixed with organic polymeric solution. This solution was added drop wise to 100 ml of light liquid paraffin containing 0.5% span 80 with constant stirring at 2000-2500 rpm using a three blade propeller for 5hours. After complete evaporation of organic phase the liquid paraffin was decanted and collected microspheres were washed three times with n-hexane to remove liquid paraffin and were dried.

Table 1: Composition of formulations of BMH loaded mucoadhesive microspheres.

Sr.No	Formulation code	Drug (g)	HPMC K100M(g)	HPMC K4M(g)	EC(g)	Carbopol 934p(g)	Drug:total polymer ratio
1	F1	0.5	0.125	-------	-------	0.125	1:0.5
2	F2	0.5	0.250	-------	-------	0.250	1:1
3	F3	0.5	0.375	-------	-------	0.375	1:1.5
4	F4	0.5	-------	0.125	-------	0.125	1:0.5
5	F5	0.5	-------	0.250	-------	0.250	1:1
6	F6	0.5	-------	0.375	-------	0.375	1:1.5
7	F7	0.5	-------	-------	0.125	0.125	1:0.5
8	F8	0.5	-------	-------	0.250	0.250	1:1
9	F9	0.5	-------	-------	0.375	0.375	1:1.5

Characterization of the microspheres

Micromeritic Properties

Angle of repose

Angle of repose of different formulations was measured according to fixed funnel method. The angle of repose was calculated using the Eq.[1] where θ is the angle of repose, h is the height of the pile, and r is the radius of the base of the pile.

$$\theta = \tan^{-1}(h/r), \ldots\ldots \text{Eq.[1]}$$

Bulk density and Tapped density

Bulk and tapped densities were measured by using 10 mL of graduated cylinder. The sample poured in cylinder, the volume occupied was measured initially and tapped mechanically for 100 times. Then tapped volume was noted. Bulk density and tapped density of the formulations were determined by using the Eqs[2] and[3]

Bulk density	=	$\frac{\mathbf{m}}{\mathbf{v_i}}$	=	$\frac{\textbf{Sample weight}}{\textbf{Sample Volume}}$	… Eq[2]

Tapped Density	=	$\frac{\mathbf{M}}{\mathbf{v_t}}$	=	$\frac{\textbf{Weight of microspheres}}{\textbf{Vol. of microspheres after 100 tappings}}$	……Eq[3]

where m is the mass of the drug (g), v_i is the initial volume (mL) and v_t is the tapped volume (mL).

Hausner's ratio

It is another parameter for measuring flowability of themicrospheres and is calculated using the Eq.[4]

Hausner's ratio=(bulk density/tapped density)×100…….Eq[4]

Compressibility Index

It is indirect measurement of bulk density, size and shape,surface area, moisture content, and cohesiveness of materialssince all of them can influence the compressibility index. It isalso called as carr's indexand is denoted by Ci. It wascalculated using theEq[5]. [27-30]

Carr's index=(bulk density−tapped density/bulk density)×100……..Eq.[5]

Particle size

The particle size of the microspheres was determined using an optical microscopy method.[31] The particle size of more than 300 microspheres were measured randomly.The average particle size was determined by using Edmondson's equation i.e. Eq[6]. [32]

$\mathbf{D_{mean}=\Sigma nd/\Sigma n}$.....Eq[6]

% Yield	=	$\frac{\textbf{Practical mass(Microspheres)}}{\textbf{Theoritical mass(Polymer+Drug)}}$	**x**	**100**	……Eq[7]

Wheren=number of microspheres measured, d=mean size range.

Percentage yield

The practical percentage yield was calculated from the weight of dried microspheres recovered from each batch in relation to the sum of the initial weight of starting materials. The percentage yield was calculated using the Eq.[7]. [33-34]

Drug Entrapment efficiency (DEE)

Fifty milligrams of weighed microspheres were crushed in a glass mortar and the powdered microspheres were suspended in 10 mL of pH 7.4 phosphate buffer solution. The solution was filtered after 24 h and the filtrate was analysed for drug content. The drug content was analyzed by measuring absorbance in a UV spectrophotometer at 272 nm using pH 7.4 phosphate buffer as blank.[35-36] The entrapment efficiency was calculated using the Eq[8]

Entrapment efficiency	=	$\frac{\textbf{Practical drug content}}{\textbf{Theoritical drug content}}$	**x**	**100**	....…….Eq[8]

Swelling Index (SI)

The equilibrium swelling studies were carried out to determine swelling index. A known weight (100 mg) of microspheres was placed in 500 ml of phosphate buffer solution (pH 7.4) and allowed to swell for the required period of time at 37 ± 0.5°C using the United State Pharmacopoeia (USP) dissolution test apparatus with the dissolution basket assembly at 50 rpm. To ensure complete equilibrium, samples were allowed to swell for 24 h. The excess surface adhered liquid drops were removed by blotting with soft tissue papers and the swollen microspheres were weighed to accuracy of 0.01 mg using an electronic microbalance. The microspheres were then dried in an oven at 60° C for 5 h until there was no change in the dried mass of the samples. Then SI was calculated from the Eq.[9]. [37-38]

Swelling index	=	$\frac{\mathbf{(W_2 - W_1)}}{\mathbf{W_2}}$	**x**	**100**	……..Eq[9]

Where, W_1 is the initial weight of the dry microparticles and W_2 is the weight of the swollen microparticles at equilibrium swelling in the media.

***In vitro* drug release studies**

The *in vitro* dissolution studies were performed at three different pH values: (i) 1.2 pH (simulated gastric fluid) (ii) 6.8 pH and (iii) 7.4 pH (simulated intestinal fluid). *In vitro* drug release studies were carried out using US Pharmacopoeia paddle type-II dissolution apparatus at 37 ± 0.5°C with constant stirring rate of 50 rpm. Microspheres equivalent to 50 mg of BMH were used for the studies. An accurately weighed sample was added into dissolution media consisting of 900 ml of 0.1 N (pH 1.2) HCl containing 1% sodium lauryl sulphate and dissolution was conducted for 2 h. The pH of dissolution medium was then adjusted topH 6.8with phosphate buffer and drug release study was carried out for further

3 h. Finally, the pH of dissolution medium was again changed with phosphate buffer to pH 7.4 and further dissolution was continued upto 24 h. A sample volume of 5 ml was withdrawn at regular intervals and replaced with equal volume of fresh dissolution medium. The sample was filtered and analyzed spectrophotometrically at 272 nm after suitable dilution. All dissolution studies were carried out in triplicate. The actual content in samples was read from a calibration curve prepared with standard BMH.[39-41]

Release kinetics

In order to understand the mechanism and kinetics of drug release, the result of the *in vitro* dissolution study of microspheres were fitted with various kinetic equations like Zero order, First order, Higuchi's and Hixson-Crowell cube root law. To find out the mechanism of drug release, first 60% drug release data were fitted in Korsmeyer-Peppas model.[42-51]

***Invitro* mucoadhesion studies**

a) *Invitro* wash off test

The mucoadhesive properties of the microspheres were evaluated by the *In vitro*Wash -off test.[52] A 4-cm piece of goat intestine mucosa was tied onto a glass slide using thread. Microspheres were spread (∽100) onto the wet, rinsed, tissue specimen and the prepared slide was hung on to one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in the beakers containing the simulated gastric fluid USP (pH 1.2) and the pH 7.0 Phosphatebuffer. At the end of 30 minutes, 1 hour and at hourly intervals up to 8 hours, the number of microspheres still adhering on to the tissue was counted.[53] The % mucoadhesion was calculated using Eq.[10]

$$\% \text{ mucoadhesion} = \frac{\text{No. of microspheres}}{\text{No. of microspheres applied}} \times 100 \quad \text{..Eq[10]}$$

b) Mucoadhesive strength and force

The mucoadhesive forces of microspheres were determined by means of the mucoadhesive force-measuring device according to the previously reported methods.[54-56]The pieces of stomach were stored frozen in phosphate buffer pH 7.4, thawed to room temperature before use. At the time of testing, a section of stomach was secured to the upper glass vial using cyanoacrylate adhesive.The diameter of each exposed mucosal membrane was1.5 cm.

The vials were equilibriated and maintained at 37°C for 10 min. Then, one vial with a section of tissue was connected to the balance and the other vial was fixed on a height-adjustable pan. A constant amount of microspheres were addedon this vial to expose the tissue. The height of the vial was adjusted so that the microspheres could adhere to the mucosal tissues of both vials. Immediately, a constant force of 0.5 N was applied for 2 minutes to ensure intimate contact between the tissues and the samples. The vial was then moved upwards at constant speed, it was connected to the balance. Weights were added at a constant rate to the pan on the other side of the modified balance of the used device until the two vials were separated. During measurement, 150 µL of simulated gastric solution (0.1 N HCl, pH 1.2) was evenly spread onto the surface of the test membrane. The bioadhesive force, expressed as the detachment stress in g/cm^2, was determined from the minimal weights that detached the tissues from the surface of each formulation using the Eq.[11]

$$\textbf{Mucoadhesive strength (gcm}^{-2}\textbf{)} = \textbf{mA} \quad \text{..... Eq[11]}$$

Where *m* is the weight added to the balance in grams and *A* is the area of tissue exposed. All the above experiments were conducted in triplicates.The gastric mucosa was changed for each measurement. The mucoadhesive force was calculated using the Eq.[12].[57-59]

$$\textbf{Mucoadhesive force(N)} = \frac{\text{Mucoadhesion Strength(g)}}{\textbf{1000}} \times \textbf{9.81} \quad \text{…Eq[12]}$$

Drug-Excipient Compatibility studies

Fourier transform infrared spectroscopy (FTIR)

The FT-IR spectrum of, pure drug, Pure drug in combination with each polymer and optimized formulation of microspheres were recorded using a FTIR spectrophotometer (Shimadzu, Kyoto, Japan). in the range of 4000-500 cm^{-1}.

Differential scanning calorimetry (DSC)

Differential scanning calorimeter (Shimadzu, Japan) was used to monitor thermal events during heating.[60] DSC measurements were carried out for pure drug and optimized formulation of microspheres on a modulated DSC apparatus (Shimadzu DSC 60, Calorimeter Tokyo, Japan) with thermal analyzer.

RESULTS AND DISCUSSION

Analytical Method for Construction of calibration curve

In order to conduct the *in vitro* drug dissolution studies calibration curve was plotted to determine R^2 and the equation of straight line is used to calculate drug release. Calibration curves of BMH in 0.1 N HCl (pH 1.2) and phosphate buffers (pH6.8 and7.4) were constructed against the respective buffers as blank at lambda max of 272 nm and are represented in "**Fig.1".**

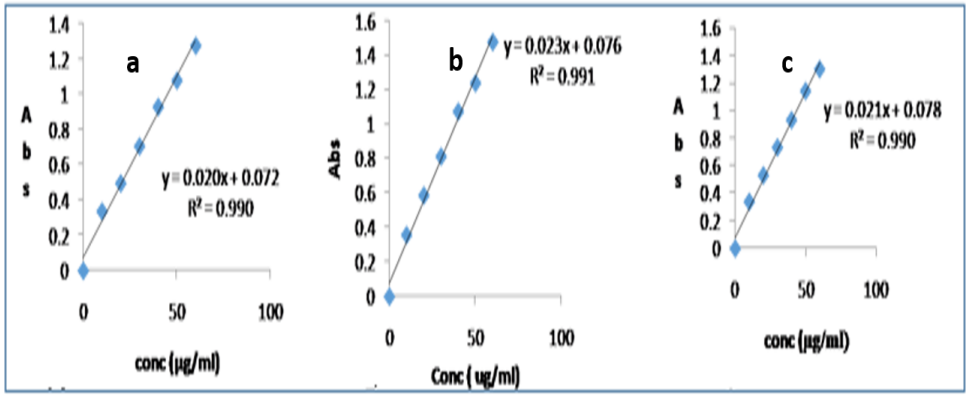

Fig. 1: Calibration curves of BMH in a) pH 1.2 buffer b) pH 6.8buffer c) pH 7.4buffer.

Micromeritic properties

The flow properties of BMH mucoadhesive microspheres were estimated by studying their bulk density, tapped density, Carr's index, Hausner's ratio and angle of repose. All the batches of microspheresshowed angle of repose value within the range of 30° to 35°, were found to be free flowing and confirmed good packing properties andcompressibilityas shown in the **Table 2**.

Table 2 : Micromeritic Properities of BMH loaded mucoadhesive microspheres.

S.No	Formulation	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Carr's Index	Hausner Ratio	Angle of Repose (θ)
1	F1	0.52±0.36	0.65±0.44	20.02±0.32	1.21±0.33	34.2±0.31
2	F2	0.55±0.48	0.64±0.32	26.21±0.36	1.16±0.32	35.5±0.42
3	F3	0.49±0.22	0.57±0.18	14.04±0.16	1.18±0.12	33.2±0.20
4	F4	0.48±0.11	0.55±0.20	12.72±0.15	1.14±0.14	32.4±0.14
5	F5	0.50±0.18	0.58±0.16	13.79±0.17	1.16±0.15	33.0±0.12
6	F6	0.53±0.21	0.61±0.11	13.11±0.15	1.15±0.12	32.1±0.22
7	F7	0.56±0.36	0.63±0.44	11.09±0.32	1.12±0.33	34.8±0.31
8	F8	0.51±0.48	0.65±0.32	21.53±0.36	1.27±0.38	34.3±0.42
9	F9	0.47±0.11	0.58±0.20	14.63±0.15	1.23±0.14	33.9±0.14

(Values are given as Mean ± SD, where n = 3.).

Physicochemical Properties

The various physico chemical properties of the microspheres like mean particle size, percentage yield (PY), drug entrapment efficiency(DEE), swelling index, are reported in **Table 3.**

The mean particle size was increased with increase in polymer concentration which might be due to the fact that as polymer concentration increases it produces a significant increase in the viscosity, leading to an increase of the emulsion droplet size and finally a higher microsphere size.[61] It has been reported that higher molecular weight polymers show better precipitation of polymer at the boundaryphase of the droplets owing to the increase of hydrophobicity.[62] The microspheres were formulated using different grades ofHPMC polymers (F1-F6), which vary in molecular weight thusresultin different viscosities and sizes. Based on the results, it can be inferred that as thedrug polymer ratio increased, percentage yield was also increased.[63] The % entrapment efficiency for the different formulations significantly increased with increase in polymer concentration.The HPMCK100M and carbopol microspheres(F3)produced the highest % entrapment efficiency of 93.5%±1.99 because an increase in polymer concentration resulted in formation of larger microspheres entrapping greater amount of drug.[64] The results of swelling indexclearly indicated that as the concentration of the mucoadhesive polymer in the formulations increased, the swelling index was also increased, may be due to the hydrophilic property of the polymer.

***Invitro* Mucoadhesion studies**

The results of mucoadhesion studiesas reported in **Table 3** showed that all the formulations (F1 to F9) found to have satisfactory mucoadhesive strength and could adequately adhere to mucosa. The strength was dependent on the property of bioadhesive polymers, which on hydration, adheres to mucosal surface and on the concentration of the polymer used as well. The increase in mucoadhesive strength may be due to the swelling of the polymer aiding in the interpenetration of polymeric chains with the mucin present on the gastric mucosa. Swelling of the polymer also leads to the formation of matrix, thereby retarding the release of drug from the formulation.[47,65] High concentration of polymer imparts larger penetration with maximum

adhesion.[66] Anionic polymers are more mucoadhesive than cationic and non-ionic polymers. Carbomers (derived from poly acrylic acid polymers) have not only negatively charged but are also mucoadhesive. Nonionic polymers, including hydroxypropyl methylcellulose, ethylcellulose and methylcellulose, present a weaker mucoadhesive force compared to anionic polymers.[67] These polymers are often used as a rate-controlling membrane to modulate the drug release from dosage forms with organic or aqueous coating techniques.[68-70] The higher mucoadhesion of Carbopol microspheres may also be attributed to the higher molecular weight of Carbopol than HPMC.[71] As the polymer ratio (CP:EC) decreased in F7-F9, the percentage of mucoadhesion conversely increased; since the greater amount of polymer results in a higher amount of free –OH (hydroxyl) groups, which are responsible for reacting with the sialic acid groups within the mucous network.[65,72] Thus, sialic cid groups are not available for mucoadhesion.

Most of the hydrophilic polymers have the ability to absorb water and swell. This can increase the potential to adhere onto mucosal surfaces. HPMC is a nonionic polymer containing only hydroxyl groups, which can form weak hydrogen bonds with mucous layers. Furthermore, owing to its slow rate of hydration, it can form a strong surface gel that efficiently adheres onto the mucosal surface and remains in contact for a longer time.For this reason, it can be characterized as one of the most effective mucoadhesive polymers.[73-74] It was also observed that, as the concentration of HPMC increased in the microspheres(F1-F6)the mucoadhesive force also increased. Increase in the polymer amount may provide more adhesive sites and polymer chains for interpenetration with mucin, resulting consequently in the augmentation of bioadhesive strength. The effect of concentration of HPMC K100M (F1-F3)was found to be more significant than that of HPMC K4M(F4-F6). This could be attributed to the high viscosity of HPMC K100M resulting in extensive interpenetration into the mucous layer and forming a stronger surface gel.[75]

Table. 3: Results of various parameters of BMH loaded mucoadhesive microspheres.

Sr.No.	Formula-tion	Particle size (µm)	%DEE	%PY	%Swelling Index	% CDR	% Mucoadhesion	Mucoadhesive strength (gm)	Mucoadhesive force (N)
1	F1	296±1.69	71.1±1.58	73.3	68± 0.18	99.61±2.22 (14hrs)	81 ±2.65	8.45±0.85	0.86±0.72
2	F2	321±2.85	88.1±1.79	80.1	74±0.28	97.81±2.56 (22hrs)	86.33 ± 3.06	8.67 ±0.94	0.88 ± 0.63
3	F3	340±1.89	93.5±1.99	80.5	85±0.32	94.81±3.12 (24hrs)	89±2.29	8.9±0.77	0.92±0.455
4	F4	291±3.51	76.4±1.68	79.9	60±0.18	99.93±2.14 (12hrs)	79±2.87	7.8±0.36	0.80±0.54
5	F5	294±2.41	88.6±1.67	80.4	68±0.14	98.86±3.31 (14hrs)	81±1.96	8.00 ±0.43	0.82 ± 0.64
6	F6	300±2.69	89.4±2.01	81.4	72±0.24	97.82±1.56 (16hrs)	85.23±2.95	8.5±0.78	0.87±0.88
7	F7	355±2.64	79.5±1.35	80.5	56.4±0.97	97.44±1.85 (16hrs)	70±3.3	6.5±0.88	0.68±0.55
8	F8	369±2.57	88.3±1.87	85.2	60.67±0.72	93.40±1.63 (20hrs)	68 ± 2.65	6.33 ±0.76	0.65 ± 0.78
9	F9	372±2.78	89.5±2.11	87	65±0.88	90.5±2.12 (24hrs)	66±3.45	6.13±0.89	0.63±0.35

(*Values are given as Mean ± SD, where n = 3*.).

***Invitro* drug release studies**

The cumulative percent release of BMH from different formulations is shown in "Fig. 2" and Table 3. Drug release from these microspheres was slow, extended and dependent on the type of polymer and concentration of polymer used. Decrease in the rate and extent of drug release was observed with the increase in polymer concentration used in microspheres as shown inTable 3 & "Fig 2". This may be attributed to the greater degree of swelling upon hydration with greater mucoadhesive polymer content in the microspheres, which increase the density of the polymer matrix and the diffusional path length to traverse the drug molecules lead to slowdrug release.[76-77]

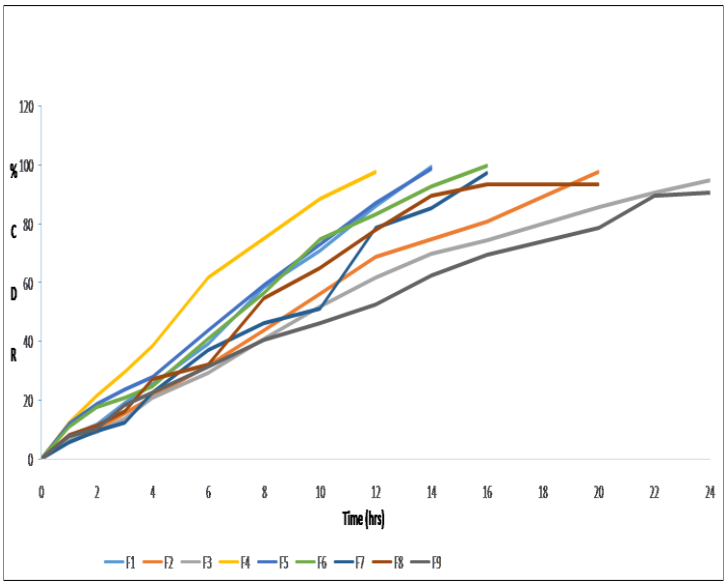

Fig. 2. *Invitro* dissolution profile of BMH from different mucoadhesive microspheres.

Drug Release kinetics

The data of drug release kinetics is compiled in Table 4. The models giving a correlation coefficient close to unity were taken as the order of release. The drug release mechanism from the microspheres was diffusion controlled as plots of the amount released versus square root of time was found to be linear. Therefore it was concluded that diffusion was the main mechanism of drug release from the microspheres. The correlation coefficient (r^2) was in the range of 0.942-0.992 for various formulations. Straight lines were obtained when percentage of drug released vs. time was plotted in accordance with zero order equation, indicated that drug release followed zero order kinetics as correlation coefficient of zero order drug release is close to unity than the correlation coefficient of first order drug release.

Based on the n values, the drug release from the formulations exhibited non-fickian diffusion (demonstrated super case-II transport(n > 0.89) mechanism controlled by swelling of the polymeric matrix.

Table 4. The data of release kinetics of BMH loaded mucoadhesive microspheres.

						Correlationcoefficient						
Sr.No	**Formulation**	**Zero Order**		**First Order**		**Hixson Crowell**		**Higuchi**		**Koresmayer**		
		K_0	R^2	K_1	R^2	K_{HC}	R^2	K_{HC}	R^2	N	R^2	Kkp
1	F1	7.249	0.997	-0.066	0.923	-0.239	0.88	6.199	0.991	0.98	0.98	0.46
2	F2	5.07	0.991	-0.066	0.831	-0.15	0.948	5.141	0.989	0.934	0.983	0.41
3	F3	4.109	0.978	-0.049	0.955	-0.119	0.992	5.002	0.986	0.945	0.983	0.37
4	F4	8.319	0.986	-0.122	0.889	-0.266	0.972	9.47	0.992	0.918	0.991	0.62
5	F5	7.058	0.997	-0.108	0.774	-0.224	0.917	7.005	0.964	0.916	0.985	0.51
6	F6	6.588	0.989	-0.076	0.924	-0.232	0.918	5.978	0.985	0.897	0.976	0.50
7	F7	6.223	0.987	-0.079	0.808	-0.182	0.913	5.571	0.992	1.07	0.982	0.32
8	F8	5.374	0.949	-0.068	0.944	-0.161	0.963	6.667	0.984	0.952	0.966	0.42
9	F9	3.789	0.986	-0.04	0.947	-0.103	0.983	5.621	0.997	0.901	0.991	0.36

Among the 9 formulations prepared it was evident that the carbopol940p -EC (F7-F9) microspheres showed lower mucoadhesion and more controlled release than cabopol940P HPMC combination (F1-F6). Between Carbopol 940P- HPMCK100 and HPMC K4M grades, the combination withHPMCK100 (F1-F3) has released the drug in a more controlled fashion and exhibited better mucoadhesion than with HPMCK4 M (F4-F6). Among HPMCK100(F1-F3) formulations, F3 had exhibited more sustained drug release i.e, 94.81% in 24 hours and maximum mucoadhesive properties (89% Mucoadhesion**).** Hence formulation F3 was selected as optimised formulation which was used for comparision with marketed tablet.

Drug-Excipient Compatibility studies

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of pure BMH, BMH with polymers and optimized microsphere formulation F3 are reported in "**Fig.3".** Positions of peaks in FTIR spectra of pure BMH were compared with the spectrum of BMH containing microspheres. Characteristic IR absorption peaks of pure BMH of the aromatic N-H stretch (1597 cm^{-1}), O-H stretch (3316 cm^{-1}) and C-H bending

(2919cm^{-1}) were present in the spectrum of the BM H loaded microspheres.

FTIR analysis revealed that there was no interaction between the drug and the polymers. The FTIR spectra of the pure drug and formulation indicated that the characteristic peaks due to pure BMH have appeared in microspheres and the positions of characteristic peaks of BMH were not altered after their successful entrapment in the microspheres, suggesting the absence of interactions between the drug and other components of the formulation indicated the stability of drug during microencapsulation process.

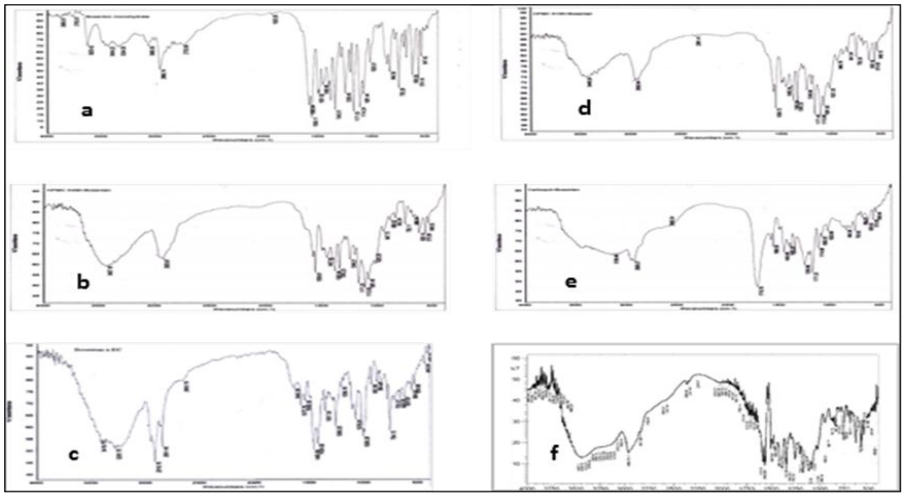

Fig 3: FTIR Spectra of a) Pure BMH b) BMH and HPMCK100M c) BMH and HPMCK4M d) BMH and Carbopol 940P e) BMH and EC f) Optimized formulation.

Differential scanning calorimetry

The thermograms of pure BMH and F3 formulation are presented in "**Fig.4"**. Pure BMH has shown a sharp endothermic peak at116.73°C due to the melting of BMH, but, in the case of BMH -loaded microspheres, no sharp peak was observed at 116.7°C, suggested that BMH may be molecularly encapsulated in the microspheres.

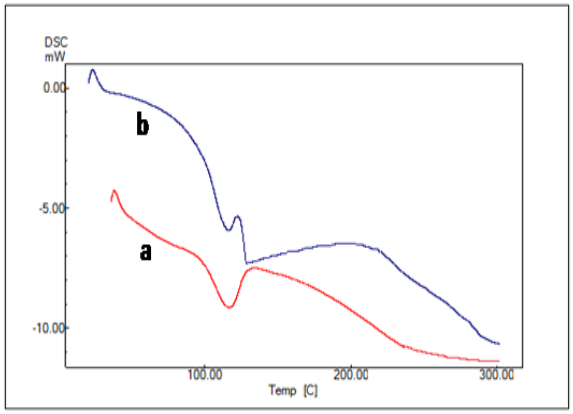

Fig. 4: Differential scanning calorimetry thermograms of (a) Pure BMH (b) Optimised Formulation (F3).

Comparision of the optimised formulation (F3) with marketed formulation

The *invitro* drug release of optimised formulation F3 was compared with that of the marketed film coatedtablets (BOSENTAS) labeled to contain 62.5mg of Bosentan Monohydrateper tablet. The comparative *invitro* release profilesis shown in "**Fig.5".** The marketed formulation released 91% of the drug within one hour where as the 94.81% drug was released for a period of 24 hrs in case of the optimised formulation F3. This shows that the drug release was controlled from the optimized formulation for 24 hrs which might be because of slow release and increased gastrointestinal retention of the prepared mucoadhesive microparticulate system.

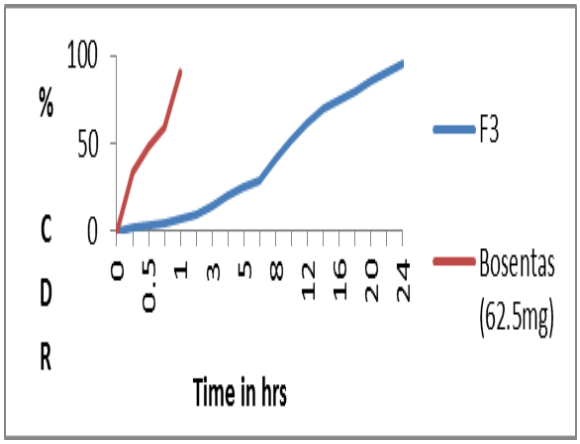

Fig. 5 Comparison of dissolution profiles of marketed product and optimized formulation F3.

CONCLUSION

Bosentan Monohydrate loaded mucoadhesive microspheres were successfully prepared by emulsification solvent evaporation method. From the present study, it was concluded that, carbopol 940P, HPMC K4M, HPMC K100M were compatible with Bosentan Monohydrate(BMH) based on the results

obtained from compatibility studies and hence are suitable for formulationof mucoadhesive microspheres.The prepared microspheres exhibited well-controlled and delayed release pattern. The carbopol 940P, HPMC Microspheres(F1-F6) exhibited good mucoadhesiveproperties as observed in *in vitro* wash-offtest when compared to a carbopol940P-EC microspheres(F7-F9). Varying degrees of sustained drugrelease were obtained for microspheresformulated with EC(F7-F9) and HPMC, (F1-F6) in combination with carbopol940pand amongst which carbopol 940P – EC combination(F7-F9) has shown the most sustaining of drug release than all. The drug release mechanism was non-fickian type controlled by swelling and relaxation of polymer chain. This study proved that, the addition of carbopol 940P increases the viscosity and swelling of microspheres there by controls the release of drug and improves the mucoadhesive properties. Hence, it can be concluded that the mucoadhesive microspheres can be successfully formulated by using Carbopol940P, HPMC K4M, HPMC K100M (F1-F6) and amongthe developed formulations, F3 is promising for the controlled oral delivery of Bosentan Monohydrate to treat PAH.

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