

## Research Article



## Biodegradable PHBV/PEO Blend Microspheres for Controlled Release of Bosentan Monohydrate: Preparation, Characterization and *In Vitro* Release Studies

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### ABSTRACT

The aim of this study was to prepare Polymeric blend microspheres of poly (3-hydroxybutyrate-co- 3- hydroxyvalerate) (PHBV) and poly (ethylene oxide) by solvent evaporation method using poly (vinyl alcohol) (PVA) as a stabilizer. Bosentan monohydrate (BMH), an anti pulmonary arterial hypertensive drug was encapsulated successfully into these microspheres. Various formulations were prepared by varying the ratios of PHBV, PEO and drug. The prepared microspheres were characterized by various instrumental techniques like Fourier transform spectroscopy (FTIR), Differential scanning calorimetry (DSC), X-ray diffraction (X-RD) and Scanning electron microscopy (SEM). FTIR spectral data showed the blending of PHBV and PEO polymers. DSC and X-RD studies of BMH loaded blend microspheres confirmed the molecular level distribution of drug in the polymer matrix. SEM images of the microspheres indicated the formation of porous spherical particles with distinct size. Successful encapsulation of the drug up to 82.5 % was achieved. Controlled release behavior of the BMH drug loaded microspheres was studied in pH 7.4 buffer media. *In vitro* results indicated that the drug release rate was dependent on the amount of PEO and % of drug present in the polymer matrix. All these results indicated that the prepared microspheres are potentially useful in drug delivery systems and the prolonged release rate was observed up to 12 h.

**Keywords:** Bosentan Monohydrate, *In vitro* release, Microspheres, Poly (3-hydroxybutyrate-co-3-hydroxyvalerate), Poly (ethylene oxide).

### INTRODUCTION

Controlled drug delivery technology represents the more rapidly advancing area in recent years due to the involvement of multidisciplinary scientists, who are contributing to the human health care related problems. The drug delivery systems offer numerous advantages as compared to conventional dosage forms including improved efficiency, reduced toxicity, and improved patient compliance and convenience.<sup>1</sup> During the past three decades, numerous oral delivery systems have been developed to act as drug reservoirs from which the active substance can be released over a defined period of time at a predetermined and controlled rate.<sup>2</sup> The oral controlled release formulation have been developed for those drugs that are easily absorbed from the gastrointestinal tract (GIT) and have a short half-life and are eliminated quickly from the blood circulation.<sup>3</sup> As these will release the drug slowly into the GIT and maintain a constant drug concentration in the plasma for a longer period of time.<sup>4</sup> In the recent past, biodegradable synthetic and natural polymers have been extremely used to develop the controlled release (CR) formulations to decrease the release rates of drugs having short plasma life.<sup>5-6</sup>

Several biodegradable controlled drug delivery devices (e.g., microspheres, microcapsules, and nanoparticles) are available and can be used for various *in vitro* and *in vivo* applications. Among these micro and nanoparticles appears to be the most promising, as these systems

facilitate the diffusion through biological barriers.<sup>7</sup> These particles protect the liable compound (e.g., proteins and peptides) from degradation in the GIT.<sup>8</sup> In the search for systems that can lead to a controlled, sustained or verctorized release of active principles with the required properties of biocompatibility and biodegradability, the polyhydroxyalkanoate (PHA) polymers are particularly appropriated to this purpose.<sup>9-11</sup> Among these PHBV is the most widely used PHA, characterized by improved ductility and processability, due to the incorporation of 3-hydroxyvalerate (3HV) into the PHB main chains, which results in an enhanced chain flexibility, reduced crystallinity degree and consequent decreased glass transition and melting temperatures.<sup>12-13</sup> Currently PHAs are widely investigated for bioresorbable devices, drug delivery systems and tissue-engineered scaffolds.<sup>14-15</sup> In particular, PHB and PHBV have been extensively explored as biomaterials for *in vitro* and *in vivo* studies, and used for various biomedical devices such as sutures, prosthetic devices, drug delivery systems and surgical applications.<sup>16-20</sup> However it was demonstrated that the potential application of PHAs, such as PHB and PHBV, in biomedical devices is remarkably limited by their relatively high crystallinity, low flexibility and brittle nature.<sup>21</sup>

The properties of a polymeric material can be properly tailored and improved by several methods, including annealing, copolymerization, and blending.<sup>22-28</sup> In particular blending is a practical and economical approach



to modulate the properties of polymer, obtaining an easily processable material with preservation of the main properties of the moieties.<sup>24-28</sup> Thus in order to reducing crystallinity degree and increasing flexibility and hydrophilicity of PHBV, blending it with other suitable polymers is desirable. Among the employed synthetic polymers, poly (ethylene oxide) (PEO) is a biocompatible hydrophilic, nontoxic and water soluble polymer, widely used in chemical, cosmetic, and pharmaceutical industries. PEO gels produced in water can be dehydrated and the material produced is extremely hydrophilic and possesses a good bio adhesive property.<sup>29</sup> Due to its properties, PEO is used in various drug delivery systems. Christine et al. have reported PEO blend copolymer micelles as a delivery vehicle for dihydrotestosterone.<sup>30</sup> Zeng and Pitt have also reported PEO blend nanoparticles with cross linked cores as drug carrier. PEO is a good drug delivery vehicle in pharmaceutical industries.<sup>31-33</sup> Recently, our group is actively involved in the development of blend polymeric systems for the control release of various types of drugs.<sup>5-6</sup>

Several methods have been used to prepare microparticles, for both natural and synthetic polymers.<sup>34</sup> Some of the commonly reported processes of preparing microparticles from biodegradable polymers include polymer phase separation, emulsion-solvent evaporation/solvent extraction, supercritical fluid and spray drying techniques.<sup>35-36</sup> Among this emulsion-solvent evaporation/solvent extraction technique in particular single emulsion method is very useful to encapsulate hydrophobic drugs through oil-in-water (O/W) emulsification process. In this process polymer is dissolved in a water-immiscible, volatile organic solvent such as chloroform or dichloromethane, and the drug is dissolved or suspended into the polymer solution. The resulting mixture is emulsified in a large volume of water in the presence of an emulsifier.<sup>37-39</sup>

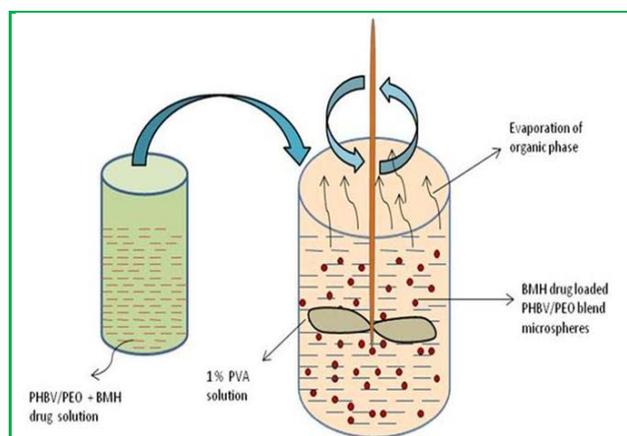
Bosentan monohydrate (4-tert-butyl-N-[6-(2-hydroxyethoxy)-5-(2-methoxyphenoxy)-2(pyrimidin-2-yl)pyrimidin-4-yl][benzene-1-sulfonamide monohydrate) often called as Tracleer, is a dual endothelin receptor antagonist, important in the treatment of pulmonary artery hypertension (PAH). Tracleer improves the exercise ability and decreases the rate of clinical worsening in patients with WHO Class III or IV symptoms of PAH, by blocking the binding of endothelin to its receptors, thereby negating endothelin's deleterious effects.<sup>40-41</sup> From literature survey, it was noticed that there were no reports on drug delivery involving BMH loaded microparticles obtained from PHBV and PEO as polymers. These polyesters are attractive materials for controlled release drug applications due to their biodegradability. Hence, in the present work the authors have selected PHBV/PEO blend microspheres by solvent evaporation method, for controlled release of bosentan monohydrate and the results are presented here.

## MATERIALS AND METHODS

Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) granules (PHV content 12 mol %), Poly (ethylene oxide) (PEO) (MW ~ 125, 000) were purchased from Sigma-Aldrich, USA. Poly (vinyl alcohol) (PVA) (MW ~ 125, 000) with a degree of hydrolysis of 86-89%, Chloroform (CHCl<sub>3</sub>, 99%) were purchased from S. D. Fine Chemicals, Mumbai, India. A model drug Bosentan monohydrate (BMH) was obtained as a gift sample from R. L. Fine Chem., Bangalore. Double distilled water was used throughout the research work.

### Preparation of PHBV/PEO Blend Microspheres

Blend microspheres of PHBV/PEO were prepared by solvent evaporation technique. Briefly, 250 mg of PHBV and 250 mg of PEO were dissolved in 10 ml of chloroform. After obtaining homogenous solution of both the polymers, 50 mg of BMH drug solution was added and stirred until homogeneous solution was formed. The resulting blend solution was slowly added to the 500 ml beaker containing 100 ml of 1 % PVA solution (stabilizing agent) through a glass syringe. The emulsion was stirred at the continuous stirring speed of 600 rpm using Euro Star (IKA Labortechnik, Germany) high speed stirrer for 3 to 4 h. The chloroform was completely removed by evaporation under these conditions and the resulting product was washed twice with distilled water and the separated microspheres were vacuum-dried at 40 °C for 12 h. Similarly various formulations are used to prepare microspheres as listed in Table 1. These prepared microspheres were kept in a desiccator for further analysis.



**Scheme 1:** Fabrication of BMH drug loaded PHBV/PEO blend microspheres by solvent evaporation method.

### Characterization Techniques

#### Fourier Transform Infrared Spectroscopy

The FTIR spectra of plain drug, unloaded microspheres and drug-loaded microspheres were recorded using Perkin Elmer spectrophotometer (Model impact 410, Wisconsin, MI, USA). The samples were crushed finely with potassium bromide (KBr) to make pellets under hydraulic pressure of 700 dynes/m<sup>2</sup> and scanned between 4000 and 400 cm<sup>-1</sup>.

### Differential Scanning Calorimetry Studies

DSC curves of unloaded PHBV/PEO microspheres, loaded PHBV/PEO microspheres and pure drug were recorded using a DSC (Model – SDT Q600, USA) by heating from 40 to 600°C at a heating rate of 10 °C/min under nitrogen atmosphere (flow rate, 20 ml/min).

### Scanning Electron Microscopy Studies

A few microspheres were taken on a copper stub and sputtered with gold for 2 min. These gold coated microspheres were mounted on the SEM instrument (JEOL, JSM-6360, Kyoto, Japan) and photographs were

taken at a required magnification at room temperature. The acceleration voltage used was 10 kV with the secondary electron image as a detector.

### X-Ray Diffraction Studies

X-Ray diffraction (X-RD) patterns of unloaded microspheres, drug-loaded microspheres and pure drug were recorded using a Rigaku Giegerflex diffractometer (Tokyo, Japan) equipped with Ni-filtered CuK $\alpha$  radiation ( $\lambda = 1.5418 \text{ \AA}$ ) at 40 kV and 50 mA. The dried microspheres of uniform size were mounted on a sample holder, and the patterns were recorded in the 2 $\theta$  range of 0- 60°.

**Table 1:** Formulation Parameters, Encapsulation Efficiencies and release kinetic parameters of Microspheres prepared from various formulations

Formulation code	PHBV (% w/w)	PEO (%w/w)	BMH loading (%w/w)	% Encapsulation efficiency $\pm$ SD	n	k	Correlation coefficient, r
PP-1	70	30	10	58.1 $\pm$ 0.4	0.5912	0.0889	0.9390
PP-2	70	30	20	63.3 $\pm$ 0.7	0.4240	0.6917	0.9219
PP-3	70	30	30	67.9 $\pm$ 1.3	0.6262	0.1178	0.9825
PP-4	60	40	10	61.8 $\pm$ 0.6	0.6430	0.1482	0.9677
PP-5	60	40	20	69.7 $\pm$ 2.2	0.6699	0.1751	0.9561
PP-6	60	40	30	75.2 $\pm$ 1.6	0.4955	0.6430	0.9134
PP-7	50	50	10	71.4 $\pm$ 0.3	0.6589	0.4009	0.8886
PP-8	50	50	20	77.2 $\pm$ 0.9	0.6592	0.3132	0.9721
PP-9	50	50	30	82.5 $\pm$ 0.2	0.7521	0.2864	0.9917

### Estimation of Drug Loading and Encapsulation Efficiency

Specific amount of drug loaded dry microspheres were vigorously stirred in a beaker containing 10 ml of 7.4 pH buffer solution to extract the drug from microspheres. The solution was then filtered through 0.2  $\mu\text{m}$  membrane filter and assayed by UV spectrophotometer (Lab India-UV 3000<sup>+</sup>) at the  $\lambda_{\text{max}}$  of 220 nm. The results of % BMH loading and encapsulation efficiency were calculated using Equations (1) and (2). Each determination was made in triplicate. The averages of triplicate measurements of these results are compiled in Table 1.

$$\% \text{ Drug loading} = \left[ \frac{\text{Amount of drug in microspheres}}{\text{Amount of microspheres}} \right] \times 100 \quad \text{..... (1)}$$

$$\% \text{ Encapsulation efficiency} = \left[ \frac{\text{Actual loading}}{\text{Theoretical loading}} \right] \times 100 \quad \text{..... (2)}$$

### In Vitro Release

*In vitro* drug release studies have been carried out by *dissolution* experiments using the tablet dissolution tester (Labindia, DS 8000, Mumbai, India) equipped with eight baskets, each containing 600 ml of phosphate buffer medium (pH 7.4) at  $37 \pm 0.5^\circ\text{C}$  under 100 rpm. A weighed quantity of each sample was placed in these baskets and at regular time intervals, aliquot samples were withdrawn, and analyzed with an UV spectrophotometer at fixed  $\lambda_{\text{max}}$  value of 220 nm.

## RESULTS AND DISCUSSION

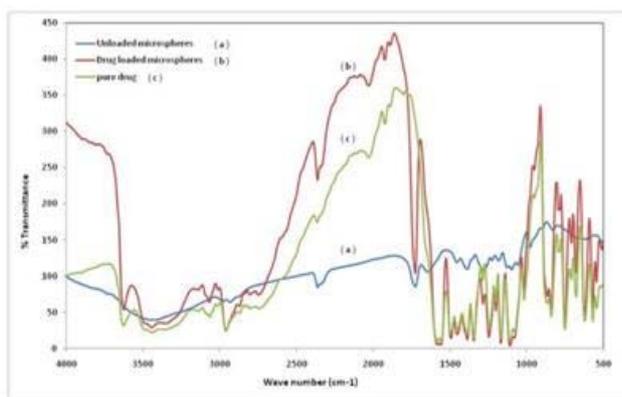
### FTIR Spectral Studies

Figure 1, shows the FTIR spectra of (a) Unloaded microspheres, (b) drug loaded microspheres and (c) Pure drug. The FTIR spectrum of unloaded microspheres and drug loaded microspheres shows characteristic absorption band at 1748 and 1739  $\text{cm}^{-1}$  respectively corresponding to  $-\text{C}=\text{O}$  stretching frequency of ester. The shift in frequency from 1748 to 1739  $\text{cm}^{-1}$  is due to the formation of hydrogen bonding interactions between drug molecules and polymer chains ( $-\text{NH}$  group of drug molecules and  $-\text{C}=\text{O}$  group of ester). The FTIR spectrum of drug loaded and unloaded microspheres are giving all the characteristic functional bands for the functional groups present in PHBV and PEO indicating the physical blending of two polymers. From the spectra we may notice that Figure 1b, is having all the drug bands, indicating that no chemical bond between drug and polymers was formed during microencapsulation, so we may conclude that drug has maintained its identity in the prepared polymer microspheres.

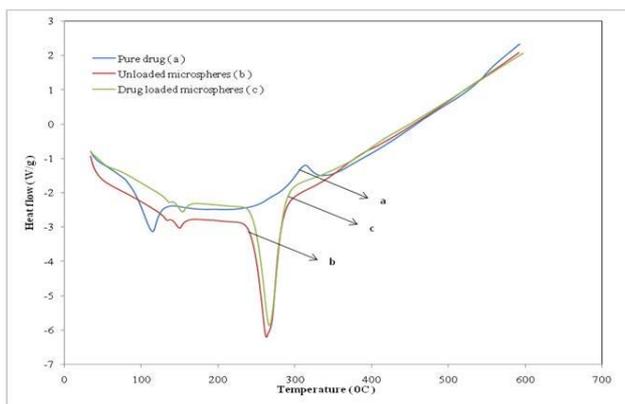
### DSC Studies

DSC thermograms of (a) Pure BMH drug, (b) Unloaded microspheres and (c) Drug loaded microspheres are showed in Figure 2. The unloaded microspheres showed two peaks at 150°C and 270°C due to endothermic

transition of the blend polymers. In case of drug-loaded microspheres, two peaks were observed at 154°C and 276 °C respectively. Thermogram of the pure BMH drug showed an onset melting peak at 105 °C. From Figure 2, we may conclude that no peak corresponding to BMH drug was observed in the drug-loaded microspheres, suggesting that most of the drug was uniformly dispersed in polymer matrices at molecular level. This result is reinforced by the XRD patterns of micro particles in which no characteristic crystalline peaks of the drug were observed.



**Figure 1:** FTIR spectra of (a) Unloaded microspheres, (b) Drug-loaded microspheres and (c) Pure drug.



**Figure 2:** DSC thermograms of (a) Pure drug, (b) Unloaded microspheres, and (c) Drug-loaded microspheres.

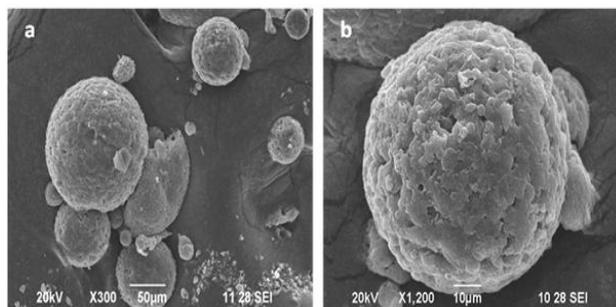
**SEM Studies**

The scanning electron micrographs of BMH drug loaded PHBV/PEO microspheres at low and high magnifications are shown in Figure 3 a, b. The microspheres are spherical shaped with a rough and porous surface. According to the literature the appearance of pores can be attributed to the speed of the solvent evaporation.<sup>42</sup> In the SEM micrographs polymeric debris seen around some particles could be due to the method of particle production (i.e., simultaneous particle production and formulation of the blend matrix).

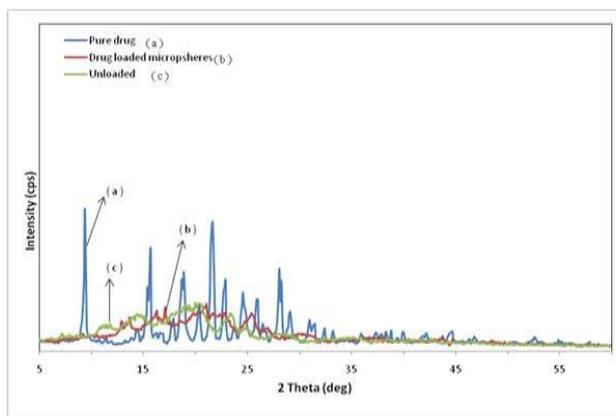
**X-RD Studies**

X-ray diffractogram of (a) pure drug, (b) drug loaded microspheres and (c) unloaded microspheres are presented in Figure 4. BMH drug has shown the

characteristic intense peaks at 2θ of 9.5 0, 16.1, 18.5, 22.2, 23.7 and 27.6 due to its crystalline nature. These peaks are not present in the drug loaded microspheres and in unloaded microspheres. This indicates that drug is dispersed at a molecular level in the polymer matrix and hence, no crystals were found in the drug loaded matrix.



**Figure 3:** SEM micrographs of blend microspheres loaded with BMH drug: (a) group of microspheres at low magnification and (b) single microsphere at high magnification.



**Figure 4:** X-RD patterns of (a) pure drug, (b) Drug loaded microspheres, (c) Unloaded microspheres.

**In Vitro Release Study**

**Drug-Release Kinetic Parameters of Different Formulations**

Drug-release kinetics were estimated by plotting the cumulative release data vs time by fitting the data to a simple exponential equation.<sup>43</sup>

$$\left[ \frac{M_t}{M_\infty} \right] = kt^n \dots\dots\dots (3)$$

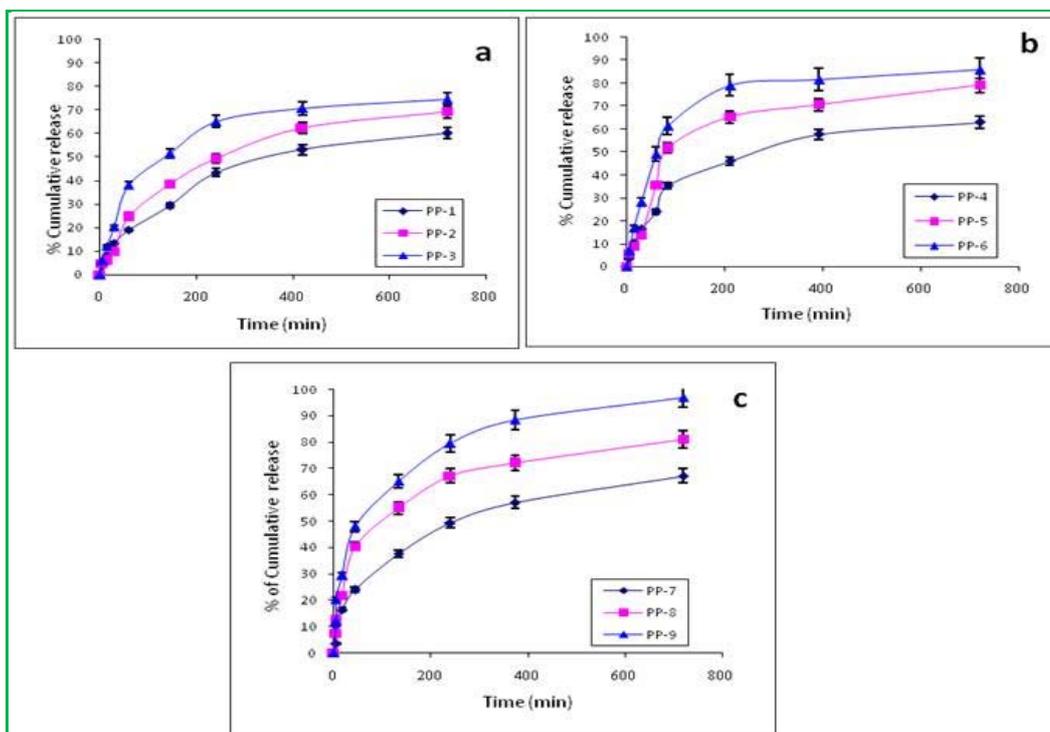
Where  $\frac{M_t}{M_\infty}$  represent the fractional drug release at time t, k is a constant characteristic of the drug-polymer system, and n is an empirical parameter characterizing the release mechanism. Using the least square procedure, we have the values of n and k for all the formulations and these values are given in Table 1. If n = 0.5, then drug diffuses and releases from the polymer matrix following a Fickian diffusion. For n > 0.5, anomalous or non-Fickian type drug diffusion occurs. If n = 1, a completely non-Fickian type or case-II release kinetics is operative. The intermediary

values ranging between 0.5 and 1.0 are attributed to the anomalous type transport.<sup>43</sup>

In the present study, the  $k$  and  $n$  values showed a dependence on the extent of drug loading and PEO content in the PHBV/PEO blend microspheres. Values of  $n$  for microspheres prepared using varying amounts of PEO (30, 40, and 50 wt %) keeping BMH constant (20 wt %) have ranged from 0.4240 to 0.6592. The drug loaded microspheres have shown  $n$  values ranging from 0.4240 to

0.7521 (see Table 1), indicating a shift from erosion type release to swelling controlled non-Fickian transport. Correlation coefficients,  $r$  obtained while fitting the release data are in the range of 0.8886 to 0.9917, but non-Fickian trends are due to a reduction in the regions of low micro viscosity and closure of micro cavities in the swollen state of the IPN matrix. Similar findings were reported elsewhere.<sup>43</sup>

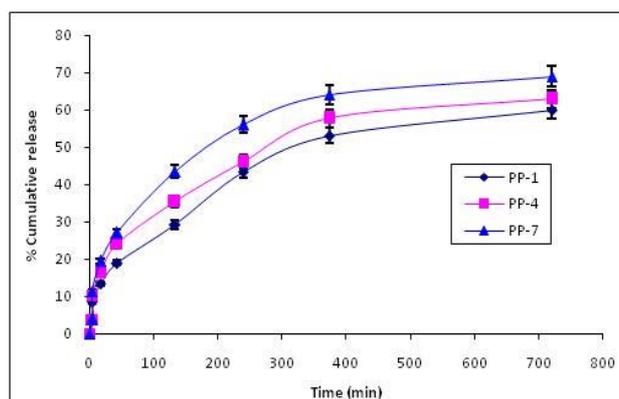
### Effect of Drug Content



**Figure 5:** % Cumulative release of BMH drug through microspheres prepared from various formulations (according to the Table 1) at pH 7.4.

To understand the effect of drug loading on *in vitro* release profiles for formulations PP-1 to PP-9 are compared in Figure 5 a, b and c. The formulations exhibited EE in the range of 58.1 to 82.5 %, which is due to lesser solubility of BMH drug in the polymer solution and due to the loss of drug in the PVA solution. These release data showed that formulations containing highest amount of BMH (30 wt %) displayed higher release rates than those containing lower amount of BMH drug. Formulation containing highest amount of drug released 82.5 % of the total encapsulated drug. On the other hand, formulations containing lower amount of drug have released only 58.1 % of BMH drug. Thus, sustained release was observed for the formulation containing lower amount of drug. Thus, the release rates are slower for lower amounts of BMH drug in the matrix, probably due to the availability of more free void spaces through which a lesser number of drug molecules will transport. For all the drug loaded formulations, a prolonged release of BMH drug occurred for about 720 min.

### Effect of PEO Content



**Figure 6:** % Cumulative release of BMH drug through PHBV/PEO blend microspheres containing 10 wt% of drug with different amounts of PEO (PP-1) 30 wt%, (PP-4) 40 wt%, and (PP-7) 50 wt% at pH 7.4.

The effect of PEO content on encapsulation efficiency and *in vitro* release rates in formulations PP-1, PP-4 and PP-7 are presented in Figure 6. In these formulations *in vitro*

release profiles of 10 wt% drug prepared by taking different amounts of PEO are shown. The % cumulative release is higher for PP-7 as compared to PP-4 and PP-1, i.e. faster release rates were observed from formulations prepared with higher amount of PEO than those formulations prepared from lower amount of PEO. Therefore, as the PEO content of the polymer matrix increases, the release rate also increases due to the hydrophilic nature of PEO. As the hydrophilic nature of the blend system increases the swelling of microspheres increases which in result to a fast release of drug.

## CONCLUSION

The present study reports on the development, characterization and *in vitro* evaluation of bosentan monohydrate drug incorporated into the blend microspheres of PHBV and PEO, prepared by solvent evaporation method using PVA as a stabilizer. Molecular level dispersion of drug was confirmed by the DSC and X-RD analysis of drug loaded microspheres. SEM pictures have shown the formation of porous and spherical surface morphology of prepared microspheres. *In vitro* release results showed that the amount of BMH drug release, increased with an increase in PEO and increase in drug percentage. Controlled release of BMH drug for more than 12 h was noticed from PHBV/PEO blend microspheres. The encapsulation efficiency was found to vary between 58.1 to 82.5 % depending upon the blend composition and the amount of drug loading.

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