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Novel Blend Microspheres of Poly(3-hydroxybutyrate) and Pluronic F68/127 for Controlled Release of 6-Mercaptopurine

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ABSTRACT: The objective of this article is to investigate the controlled release characteristics of 6-mercaptopurine (6-MP) loaded microspheres prepared from the blends of poly(3-hydroxybutyrate) (PHB) and Pluronic F68/127 by the oil-in-water emulsion-solvent evaporation technique. Formulations were prepared by taking different ratios of individual polymer components to achieve a maximum 79% encapsulation and extending the release time up to 24 h. Differential scanning calorimetry (DSC) suggested reduction in crystallanity of PHB after blending with Pluronic F127. The absence of chemical interactions between 6-MP and the blend matrix was confirmed by Fourier transform infrared (FTIR) spectroscopy, while the size of microspheres measured by optical microscopy ranged between 30 and 47 μm. X-ray diffraction (XRD) confirmed the crystalline nature of 6-MP even after encapsulation and surface morphology of the microspheres was investigated by scanning electron microscopy (SEM). *In vitro* release of 6-MP at 37°C in pH 7.4 phosphate buffer media indicated a dependence on the composition of Pluronic in the blend. The release data have been fitted to empirical equations to understand the release profile of 6-MP. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40196.

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INTRODUCTION

The use of biopolymers in controlled release (CR) applications has increased in recent years due to their efficient delivery and in extending the release time of short lived drugs over those of the conventional type dosage forms.[1] In systemic drug delivery,[2] biodegradable and biocompatible polymers like polycaprolactone (PCL), polylactic acid (PLA), poly(lactide-*co*-glycolide) (PLGA), chitosan, alginate, etc., have been used in desired sizes and shapes.[3–6] Development of CR matrices from such polymers is of value in pharmaceutical sciences to protect the drug from systemic degradation in order to improve its bioavailability by reducing toxic side effects.[7]

Among the many different polymers used in CR applications, polyhydroxyalkanoates (PHAs) are versatile materials synthesized using a wide range of microorganisms as intracellular energy and carbon storage materials and these exhibit interesting desired properties compared to some of the synthetically produced biodegradable polyesters[8,9] such as PLA and PCL. Nearly, more than 100 structurally different PHAs, having varying physico-mechanical properties and degradation kinetics are available,[10] of which poly(3-hydroxybutyrate) (PHB) is one of the widely studied polyesters due to its excellent biocompatibility, lack of toxicity as well as compatibility with tissues and blood. It undergoes hydrolytic degradation to produce D-(-)-β-hydroxy butyric acid as the monomer and is one of the constituents of blood produced by ketogenesis, which can be metabolized by extrahepatic tissues to supply energy.[11,12] Hence, degradation products of PHB are not toxic to the body and serve as ideal biomaterial in CR applications.

In the earlier literature, PHB microspheres have been studied by Zhao et al.[13] for the CR of bovine serum albumin (BSA). In that study, effect of polymer concentration in the oil phase, concentration of PVA in the continuous phase, volume ratio of inner water phase to oil phase and volume ratio of the primary emulsion to the external water phase were investigated. However, high crystalline and brittle nature of pure PHB and its relatively slow rate of *in vivo* hydrolytic degradation compared to other polyesters, limits its biomedical applications. These drawbacks can be overcome either by copolymerization or by blending with suitable polymers. In efforts to improve the CR properties of PHB, it was copolymerized with hydroxy valaric acid and hydroxyl hexanoic acid[14,15] as well as its blending with another polymer like chitosan, PEG, PCL, and PLA etc. to improve the CR properties of PHB.[16] Li et al.[17] prepared blend microspheres of PHB and PEG to investigate the effect of blend ratio as well as its crystallinity on the CR of BSA. Shih et al.[18] studied the effect of PHB/chitosan blend ratio on the morphology and crystallinity of drug-loaded microspheres. Kazuhiko

Table I. Formulation Details of 6-MP-loaded Microspheres Prepared from PHB and Pluronic F68/127% Along with Percentage Production Yield, % Encapsulation Efficiency (% EE) and Particle Size (μm)

Formulation codes	PHB (% w/w)	Pluronic F68 (% w/w)	Pluronic F127 (% w/w)	6-MP (mg)	Yield (%)	EE (%)	Particle size (μm)
CF	100	0	-	10	89.3 ± 2.2	77	47 ± 17
F1	90	10	-	10	87.2 ± 1.3	71	43 ± 7
F2	80	20	-	10	86.6 ± 1.7	69	39 ± 11
F3	70	30	-	10	84.8 ± 2.1	63	30 ± 8
F4	90	-	10	10	86.1 ± 2.4	67	44 ± 13
F5	80	-	20	10	84.6 ± 1.2	60	40 ± 11
F6	70	-	30	10	80.9 ± 1.9	54	33 ± 9
F7	90	10	-	20	88.3 ± 3.2	76	44 ± 12
F8	90	10	-	30	87.1 ± 2.1	79	44 ± 6

et al.[19] prepared the PHB microspheres by blending with a series of fatty acids and their alkyl esters to evaluate the CR properties of anticancer, antibiotic and aclarubicim drugs. In all these reports, much attention has been focused to reduce the crystallinity of PHB to improve its hydrophilicity.

In this study, 6-MP-loaded blend microspheres of PHB and Pluronic F68/127 were prepared by the oil-in-water emulsion-solvent evaporation technique to investigate their CR properties for 6-MP. Pluronics are well-known to be nontoxic and biocompatible amphiphilic triblock copolymers[20] of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO), i.e., PEO-*b*-PPO-*b*-PEO. Amphiphilic property of Pluronic copolymer has been used to enhance the biological stability and solubility of the poorly water-soluble drug by forming the core-shell micellar nanostructure.[21] Pluronics in aqueous solution at the concentration of 15–20% and higher exhibit unique property of reversible thermal gelation and is a liquid around 4–8°C, which can be changed to semi-solid gel at body temperature, making it attractive for investigating the CR properties.[22] Ma et al.[23] developed PCL/Pluronic blend microspheres, in which Pluronic was incorporated into the PCL matrix to enhance the release of the drug. Shelke et al.[24] developed PLGA/Pluronic nanoporous microspheres for the CR of repaglinide, wherein Pluronic was used as an amphiphilic filler to enhance the release of a hydrophobic drug.

The 6-MP is an antineoplastic agent used to treat various life-threatening diseases such as acute lymphoblastic leukemia, Non-Hodgkin-Lymphoma and Crohn's disease.[25,26] In pediatric population, acute lymphoblastic leukemia is the commonest malignancy (30% of cancers) with an incidence of 1 in 30,000 children.[27] The 6-MP with 37% bioavailability with its plasma half-life of 1–2 h has been used in almost all therapy protocols for acute lymphoblastic leukemia in childhood.[28] In the present study, Pluronic F68/127 is added to PHB to enhance the release rate of 6-MP extending up to the 24 h under physiological pH. The influence of Pluronic F68/127 composition of the blend on the morphology of blend microspheres and 6-MP release was investigated. The formulations were characterized by a variety of analytical techniques to understand their size, shape and morphology. *In vitro* release experiments were performed in pH 7.4 phosphate buffer at 37°C to understand the CR behavior and tested using several empirical relationships.

EXPERIMENTAL

Materials and Methods

The 6-Mercaptopurine (6-MP), poly(3-hydroxybutyrate) (PHB) and Pluronic F68/127 were all purchased from Sigma–Aldrich, St. Louis, MO. Analytical reagent grade chloroform and poly(vinyl alcohol) (PVA) of MW = 125,000 were purchased from s.d. Fine Chemicals, Mumbai, India. All other chemicals were used without further purification.

Preparation of Blend Microspheres

Blend microspheres of PHB and Pluronic F68/127 were prepared by oil-in-water (o/w) emulsion-solvent evaporation method.[24] Briefly, PHB and different concentrations of Pluronic F68/127 (0, 10, 20, and 30% w/w) were dissolved in chloroform (8 mL) to get 3% (w/v) polymer solution. Required amount of 6-MP (10, 20, or 30 mg) was dissolved in the above polymer solution and poured into 100 mL of 2 % (w/v) PVA solution under stirring at 1,000 rpm for 15 min using Eurostar stirrer (IKA Labortechnik, Germany). The resulting emulsion was further stirred for 6 h at 400 rpm to remove organic solvent, microspheres were isolated by tabletop centrifuge (Jouan, MR 23 I, France), washed two to three times with distilled water to remove the surface-adhered PVA and dried at 40°C for about 24 h. Different formulations were prepared by varying the amount of PHB and Pluronic F68/127 as stated above. Totally, nine formulations were prepared as per the codes given in Table I. The % PVA and speed of the overhead stirrer were used as the process parameters to prepare microspheres.

Microsphere Production Yield

The % yield of microsphere production was calculated by dividing weight of the collected microspheres by the initial weight of polymer plus amount of drug used to prepare the microspheres:

$$\%\,\text{Yield} = \left[\frac{W_m}{W_p}\right] \times 100 \qquad (1)$$

where W_m is the weight of dried microspheres and W_p is initial weight of the polymer plus the amount of drug used to prepare the microspheres:

Particle Size Measurements

Particle size analysis of the blend microspheres was performed by optical microscopy using a compound microscope (BESTO, Model 10A, Ambala, India). Small numbers of dry particles were spread onto a glass slide with a drop of paraffin oil and the glass slide was observed under compound microscope at the magnification of 100×. Around 500 particles were selected for each measurement using pre-calibrated ocular micrometer and the average value was considered.

Estimation of Drug Loading and Encapsulation Efficiency

The amount of encapsulated 6-MP in the microspheres was determined by UV spectrophotometer (Model Anthelic, Secomam, Ales, France) at the λ_{max} of 322 nm. About 10 mg of 6-MP loaded microspheres was dissolved in 5 mL of chloroform under vigorous shaking at ambient temperature for about 5 h. After dissolving the microspheres completely, 50 mL of phosphate buffer solution (PBS) of pH 7.4 was added to dissolve 6-MP in the microspheres by evaporating the chloroform. The resulting solution was filtered and analyzed by UV spectrophotometer to calculate encapsulation efficiency (EE) as:

$$\%\ \text{6-MP loading} = \left[\frac{\text{Weight of 6-MP in microspheres}}{\text{Weight of microspheres}}\right] \times 100 \quad (2)$$

$$\%\ \text{Encapsulation efficiency} = \left[\frac{\text{Theoretical 6-MP loading}}{\text{Actual 6-MP loading}}\right] \times 100 \quad (3)$$

Scanning Electron Microscopic (SEM) Study

The surface morphology of 6-MP-loaded blend microspheres was examined by SEM (Joel, JSM-840A scanning electron microscope, Tokyo, Japan). Microspheres were fixed on the supports with carbon-glue and coated with a gold layer using gold sputter coater (Joel, JFC-1100E sputter coater, Tokyo, Japan) in a high vacuum evaporator (coating thickness, 20 nm). Samples were observed by SEM under 20 kV energy.

Preparation of Films

Films of PHB with and without PLF127 were prepared by film casting method. PHB was blended with PLF127 in ratios of 100: 0, 90 : 10, 80 : 20, and 70 : 30% w/w. Polymers were dissolved in chloroform to prepare 3% (w/v) solution, stirred at 60°C for 5 min and poured onto glass plate to evaporate the solvent.

Differential Scanning Calorimetric (DSC) Studies

DSC (Rheometric Scientific, Surrey, UK) was performed on the blend films of PHB/PLF127 for all blend compositions 100 : 0, 90 : 10, 80 : 20, and 70 : 30 by heating the solid films from 10 to 200°C at the heating rate of 10°C min^{-1} in an inert nitrogen atmosphere by maintaining a flow rate of 20 mL min^{-1}. The melting enthalpy of PHB was obtained from the thermograms to calculate the crystallanity (X_c) of PHB in the blend films using:

$$X_c = \left[\frac{\Delta H_f \times W_{PHB}}{\Delta H_0}\right] \times 100 \quad (4)$$

where ΔH_f is melting enthalpy of the sample (J g^{-1}), ΔH_o is the melting enthalpy of 100% crystalline PHB, which is assumed[13] to be 146 J g^{-1} and W_{PHB} is the weight fraction of PHB in the sample.

Fourier Transform Infrared (FTIR) Spectral Study

FTIR spectra were taken on a Nicolet (Model Impact 410, Milwaukee, WI) spectrophotometer to explore the chemical stability of 6-MP in the microspheres by studying the interaction between 6-MP and the polymer. Samples were crushed with KBr and pellets were obtained under a pressure of 600 kg cm^{-2}. FTIR spectra of plain PHB, plain Pluronic F127 and placebo PHB/Pluronic F127 blend microspheres, 6-MP-loaded blend microspheres (F3) and pristine 6-MP were all scanned between 4000 and 500 cm^{-1}.

X-ray Diffraction (XRD) Study

The physical state of 6-MP in PHB/Pluronic F127 blend microspheres was evaluated by XRD using the X-ray diffractometer (Bruker Model D8 Advance, Germany) by recording each scan in the range of $2\theta = 3$ to 80° at the scan rate of 3° min^{-1}.

In Vitro Release Studies

In vitro release of 6-MP from the blend microspheres was investigated in simulated intestinal fluid (pH = 7.4) using USP apparatus-I dissolution tester (Dissotest, LabIndia, Mumbai, India). Blend microspheres equivalent to 10 mg of 6-MP were suspended in 3 mL of release media and placed in a dialysis membrane bag with molecular cutoff between 12,000 and 14,000 Da. The bag was tied and inserted inside the basket, immersed in 200 mL of dissolution medium and maintained at 37°C at 100-rpm stirring speed. The 5-mL aliquots were then withdrawn at different time intervals and filtered through a 0.45 mm filter. To maintain the sink condition, dissolution medium was replenished with 5 mL of fresh solution to measure the concentration of 6-MP by UV spectrophotometer at $\lambda_{max} = 322$ nm. The *in vitro* release data were taken in triplicate for each sample and average values were considered in data analysis and graphical display.

Release Kinetics Analysis

To investigate the kinetics of 6-MP release from blend microspheres, *in vitro* release data have been fitted to Zero-order, First-order, Higuchi rate equation and Hixson–Crowell cube root equation, respectively[29,30]:

$$Q_t = Q_0 - K_0 t \quad (5)$$

$$\ln Q_t = \ln Q_0 - K_1 t \quad (6)$$

$$Q_t = K_h t^{1/2} \quad (7)$$

$$Q_t^{1/3} = Q_0^{1/3} - K_0 t \quad (8)$$

In the above equations, Q_0 is initial amount of drug in the blend microspheres, Q_t is amount of drug in the microspheres after t in hours of dissolution; K_0, K_1, and K_h are the respective rate constants. To confirm the release mechanism, *in vitro* release data have also been fitted to Korsmeyer et al equation[30,31]:

$$M_t/M_\infty = Kt^n \quad (9)$$

Here, M_t/M_∞ is the fraction of drug released at time t, K is the kinetics rate constant and n refers to release exponent whose, value can be used to assess the nature of release mechanism.

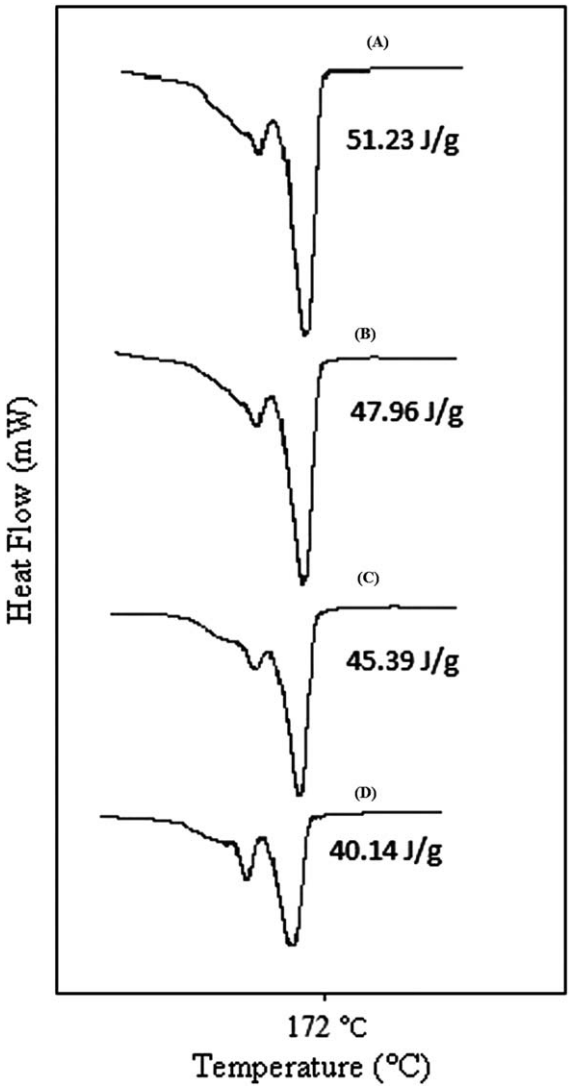

Figure 1. DSC thermograms of (A) plain PHB and its blend with (B) 10%, (C) 20%, and (D) 30% (w/w) of Pluronic F127.

RESULTS AND DISCUSSIONS

In the present study, PHB and Pluronic F68/127 blend microspheres were prepared by o/w emulsion-solvent evaporation method and used for investigating *in vitro* release kinetics of 6-MP. Solutions of the blends and 6-MP in chloroform were poured into aqueous solution of PVA used as a stabilizer with stirring and by evaporating chloroform, the solid microspheres were formed to compute % yields of blend microspheres using eq. (1). These data are presented in Table I. By using the solvent evaporation technique, 92% of placebo PHB microspheres were also obtained, but for 6-MP loaded plain PHB microspheres, the yield was 89% due to the low encapsulation of 6-MP compared to actual drug loading. However, the microspheres prepared with higher concentration of Pluronic F68/127 showed lower % yield, because hydrophilic Pluronic might have leached out of the blend into the continuous aqueous phase at the organic-aqueous interface as seen from the SEM images of the blend microspheres i.e., high Pluronic content showed more wrinkled surfaces (Figure 5). Pluronic F68 containing blend microspheres showed high % yield than Pluronic F127 containing blend microspheres due to more hydrophilic nature of Pluronic F127 than Pluronic F68. However, the initial 6-MP-loading did not affect the production yield of the blend microspheres. Formulation codes along with % yield, particle size and % EE are given in Table I.

Particle Size Measurements

Particle size data are presented in Table I show a dependence on Pluronic content of the blend. For example, formulations prepared with higher amount of Pluronic showed smaller size and vice versa. As can be seen, F3 (with 30 wt% Pluronic F68) has smaller particle size (30 ± 8 μm) than F2 (with 20 wt% Pluronic F68), the latter has a size of 39 ± 11 μm, which is still smaller than F1 (with 10 wt% Pluronic F68) for which the size is 43 ± 7 μm. Microspheres prepared from PHB (without Pluronic) are bigger in size 47 ± 17 μm compared to blend microspheres. Blending of PHB with Pluronic in different weight ratios might reduce the viscosity of polymer solution than that of pure PHB, since viscosity of the polymer solution has a significant effect on the size of microspheres. For high viscous polymer solution, it is difficult to breakdown into smaller droplets, thus leading to bigger size microspheres, but not much difference can be observed in particle size data even after changing Pluronic from F68 to F127 as well as by increasing the initial 6-MP loading.

Encapsulation Efficiency (EE)

Encapsulation efficiency of all the formulations calculated from eqs. (2) and (3) presented in Table I, vary from 54 to 79%, thus showing a dependence on Pluronic content of the blend, type of pluronic as well as initial drug loading. For plain PHB microspheres, EE of 77% decreased with increasing Pluronic composition the blend. For instance, with increasing Pluronic F68 from 10% (w/w) to 30% (w/w), EE values decrease from 71 to 63% and similar trend is observed for Pluronic F127 containing microspheres.

With regard to particle size analysis data, with increasing content of pluronic, the size of microspheres decrease due to a reduction in the size of microemulsion during the preparation of microspheres. As the size of microemulsions decrease, total surface area in contact with continuous phase becomes large and 6-MP molecules on the surface of microemulsion may get dissolved in the water phase, giving the low values for EE. However, solidification of microemulsion is quite rapid at higher concentration of PHB due to the formation of a polymer layer

Table II. DSC Results of the Various PHB-Pluronic F127 Blend Films

PHB (% w/w)	PLF127 (% w/w)	T_m (°C)	ΔH_f (J g^{-1})	X_c (%)
100	0	172.1	51.21	35.08
90	10	170.6	47.96	29.56
80	20	168.3	45.39	24.87
70	30	163.8	40.14	19.25

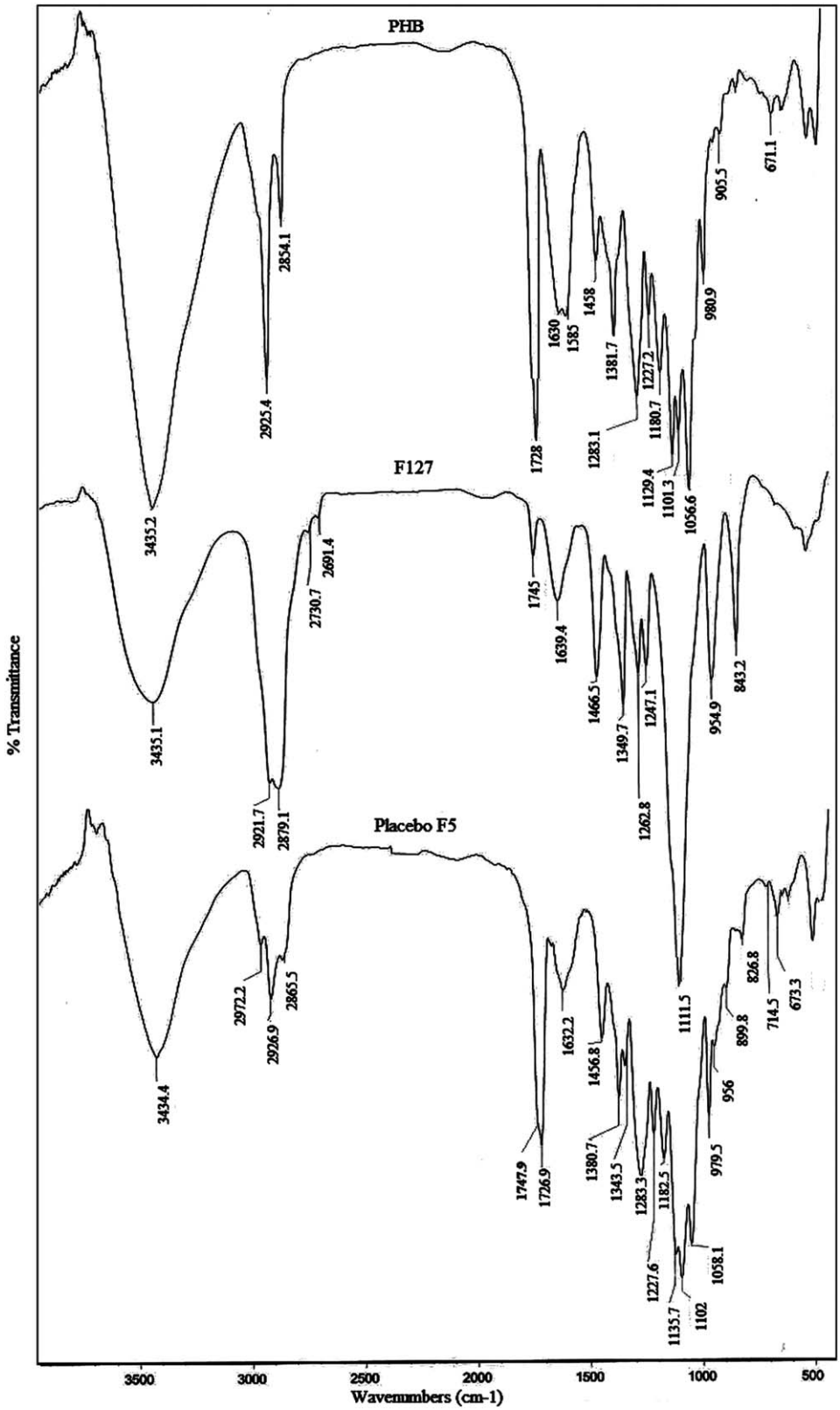

Figure 2. FTIR spectra of PHB, Pluronic F127 and Placebo blend microspheres.

between oil-water interfaces that becomes more aggressive for PHB. However, by increasing the Pluronic content, time taken to form the interfacial polymer layer also increases, thereby allowing drug particles to diffuse into water phase, thus lowering the EE. By changing the Pluronic type from F68 to F127, a decrease in EE is observed. For instance, formulation F2 with

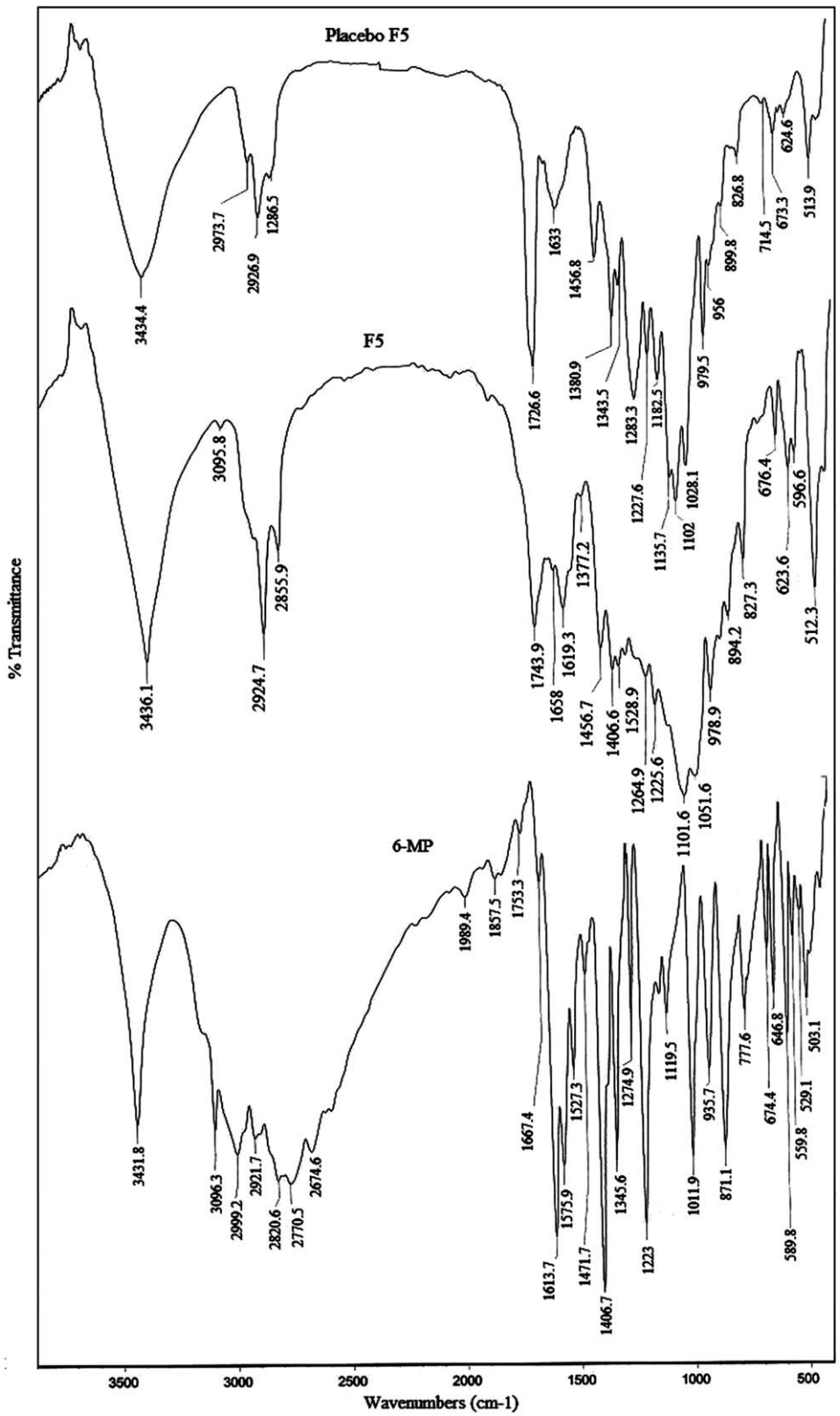

Figure 3. FTIR spectra of placebo blend microspheres, 6-MP-loaded blend microspheres (F5) and plain 6-MP.

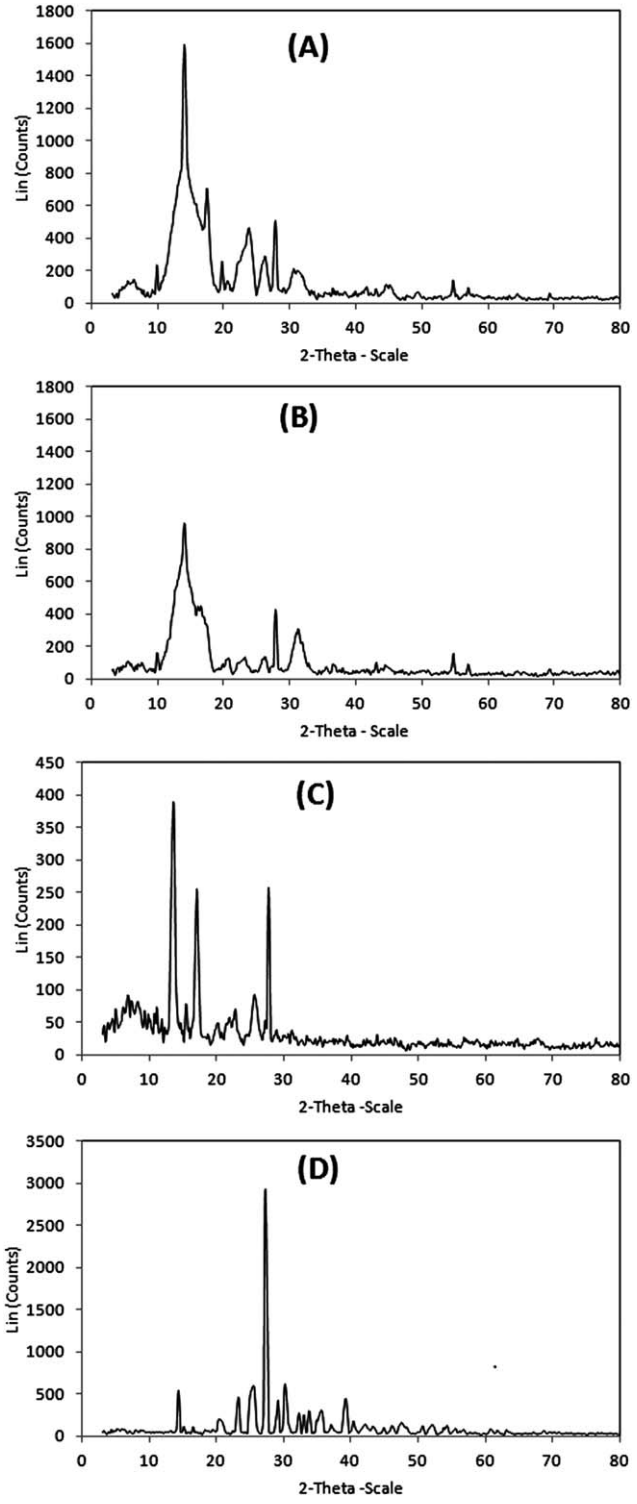

Figure 4. XRD patterns of (A) plain PHB, (B) placebo blend microspheres, (C) 6-MP-loaded blend microspheres (F5) and (D) plain 6-MP.

20% (w/w) of Pluronic F68 showed 69% of EE, whereas formulation F5 with 20% (w/w) of Pluronic F127 showed 60% EE. Similarly, F1 and F3 exhibit lower values of EE than those of F4 and F6, due to more hydrophilic nature of Pluronic F127 than F68. While preparing the microspheres, hydrophilic wall component (Pluronic) of the blend might have leached out along with the hydrophobic 6-MP by forming the micelles. Because 6-MP is hydrophobic, hydrophobic part of pluronic (PPO) might form the core with 6-MP, but hydrophilic part of pluronic (PEO) projects towards the continuous phase, leading to high dissolution of 6-MP into aqueous continuous medium, thus giving low EE values. The % EE also shows a dependence on the initial drug loading i.e., formulation loaded with higher amount of drug shows higher EE value (i.e., F8 containing 30 mg of 6-MP) of initial drug-loading showed 79 % EE, whereas F1 loaded with 10 mg of 6-MP exhibited 71% EE. On the other hand, F7 with 20 mg initial loading of 6-MP showed an intermediate value of 76% for EE, probably due to accumulation of more 6-MP particles at high drug loading.

Crystallanity of PHB

DSC results (Figure 1) are used to evaluate the effect of PLF127 on the crystallanity of PHB, which shows decrease of melting temperature of the blend with increasing Pluronic F127 content compared to the plain PHB. These results are shown in Table II. Pluronic F127 acts as a plasticizer, which might have weakened the intermolecular forces between adjacent polymer chains. However, plasticizer Pluronic F127 would retard the PHB chains from lamellae formation by reducing its chain mobility, thus resulting into thinner lamellae and such thinner lamellae exhibit lower melting temperature. The crystallinity of PHB in the sample was determined according to eq. (4) and the results shown in Table II suggest that crystallinity of PHB decreases with increasing Pluronic F127 content of the blend, because the plasticizer Pluronic F127 hinders the nucleation of PHB to promote the formation of smaller spherulites, thus increasing the flexibility of blends compared to plain PHB chains.[13,32]

Fourier Transform Infrared (FTIR) Spectral Study

FTIR spectra of plain PHB, Pluronic F127 and placebo blend microspheres i.e., F2 without 6-MP are shown in Figure 2. Plain PHB has a peak at 3435 cm^{-1} that corresponds to the stretching vibrations of end O—H group. The peak at 1728 cm^{-1} is due to C=O stretching, whereas those at 2925 and 2854 cm^{-1} correspond, respectively to asymmetric and symmetric C—H stretchings of methylene groups of PHB; its bending vibrations are observed at 1458 and 1381 cm^{-1}. Peaks in the range of 1283–1056 cm^{-1} are assigned to C—O—C group stretching vibrations.[17] In case of Pluronic F127, a broad band at 2879 cm^{-1} corresponds to CH_2 stretching of propylene oxide unit. Pluronic F127 is characterized by its O—H, C—O—C, and C—H stretching frequencies, which are also present in the PHB moiety. Thus, PHB/Pluronic F127 blend shows almost similar peaks as that of PHB.

FTIR spectra of nascent 6-MP, 6-MP-loaded blend microspheres and placebo blend microspheres are shown in Figure 3. For 6-MP, a sharp peak at 3432 cm^{-1} corresponds to the secondary amine N—H stretching, while its bending vibrations appear at 1527 cm^{-1}. Vinylic C-H stretching vibrations are observed at 3094 cm^{-1}, while the imine group C=N stretching is seen at 1667 cm^{-1}. A sharp band at 1223 cm^{-1} is assigned to C—N stretchings. In case of drug-loaded microspheres, the

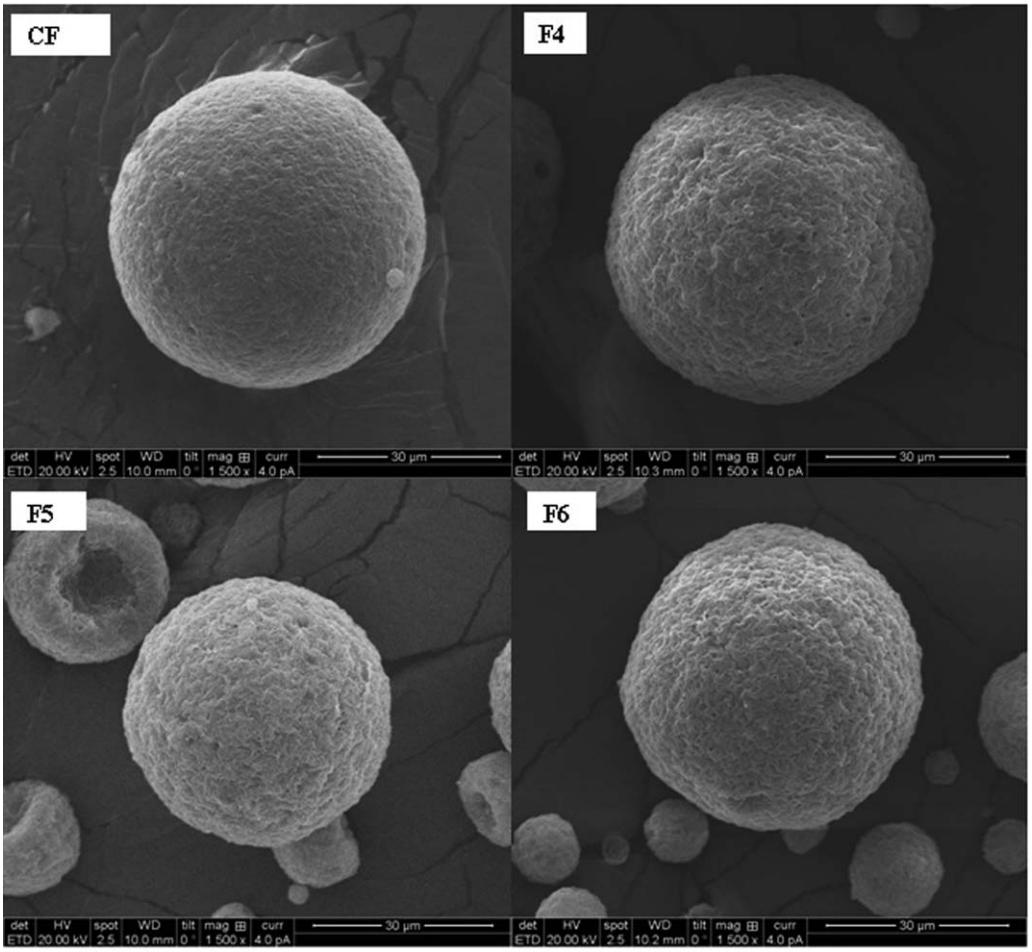

Figure 5. SEM micrographs of plain PHB (CF) microspheres and blend microspheres containing (F4) 10% (w/w), (F5) 20% (w/w), and (F6) 30% (w/w) of Pluronic F127 at ×1500 magnifications.

intensity of peak at 3435 cm^{-1} increased due to drug's secondary amine N—H stretching vibrations and a new peak appearing at 3094 cm^{-1} is due to vinylic C—H stretching frequency of 6-MP. The main functional groups of 6-MP are also observed in drug-loaded microspheres, even though some peaks of 6-MP have merged in broad peaks of the blend polymer, suggesting that the reactive sites of 6-MP i.e., functional groups have not undergone any reaction with the polymer, confirming the chemical stability of 6-MP in PHB/Pluronic F127 blend matrix.

X-ray Diffraction (XRD) Study

The X-ray diffractograms of plain PHB and its blend with Pluronic F127 were used to evaluate the influence of Pluronic F127 on the crystallinity of PHB. XRD pattern of pure PHB presented in Figure 4(A) shows the characteristic peaks at 2θ of 14°, 17.5°, 19.7°, 23.8°, 26.5°, and 27.8° due to orthorhombic PHB lattice.[12] Compared to XRD patterns of plain PHB, the locations of peaks in XRD patterns of PHB/Pluronic F127 blend remains the same, but intensity of the peaks have diminished as shown in Figure 4(B), suggesting that the presence of Pluronic F127 as a plasticizer in the blend might have decreased the crystallanity of PHB. The XRD analysis was also carried out to investigate the physical state of 6-MP after encapsulation. XRD patterns of 6-MP-loaded PHB/Pluronic F127 blend microsphere and pristine 6-MP are presented in Figure 4(C,D), respectively. Pristine 6-MP has the characteristic XRD peaks at 2θ of 14.4°, 23.3°, 25.6°, 27.3°, 29.2°, and 30.2°, but for 6-MP-loaded blend microspheres, intense peaks are observed at 2θ of 13.8°, 17.1°, 25.7°, and 27.7°, indicating the presence of characteristic peaks of 6-MP along with the peaks corresponding to the blend matrix. The existence of 6-MP peaks in the XRD pattern of 6-MP-loaded blend microspheres suggests that encapsulated 6-MP is in crystalline nature.

Scanning Electron Microscopic (SEM) Study

Surface morphology of the drug-loaded microspheres was analyzed by SEM to investigate the influence of Pluronic F127. Typical SEM images taken at 1500× magnification on CF, F4, F5, and F6 formulations shown in Figure 5 confirm spherical nature for the microspheres, but images were taken at 4000× (Figure 6) magnification on the same formulations to study the effect of Pluronic F127 on the surface morphology of blend microspheres. Microspheres prepared using plain PHB showed rough surfaces due to crystallization during the transformation of microdroplets into microspheres, thus introducing microstructural phase differences. In case of Pluronic F127 blend microspheres, the observed wrinkles on the surface as well as

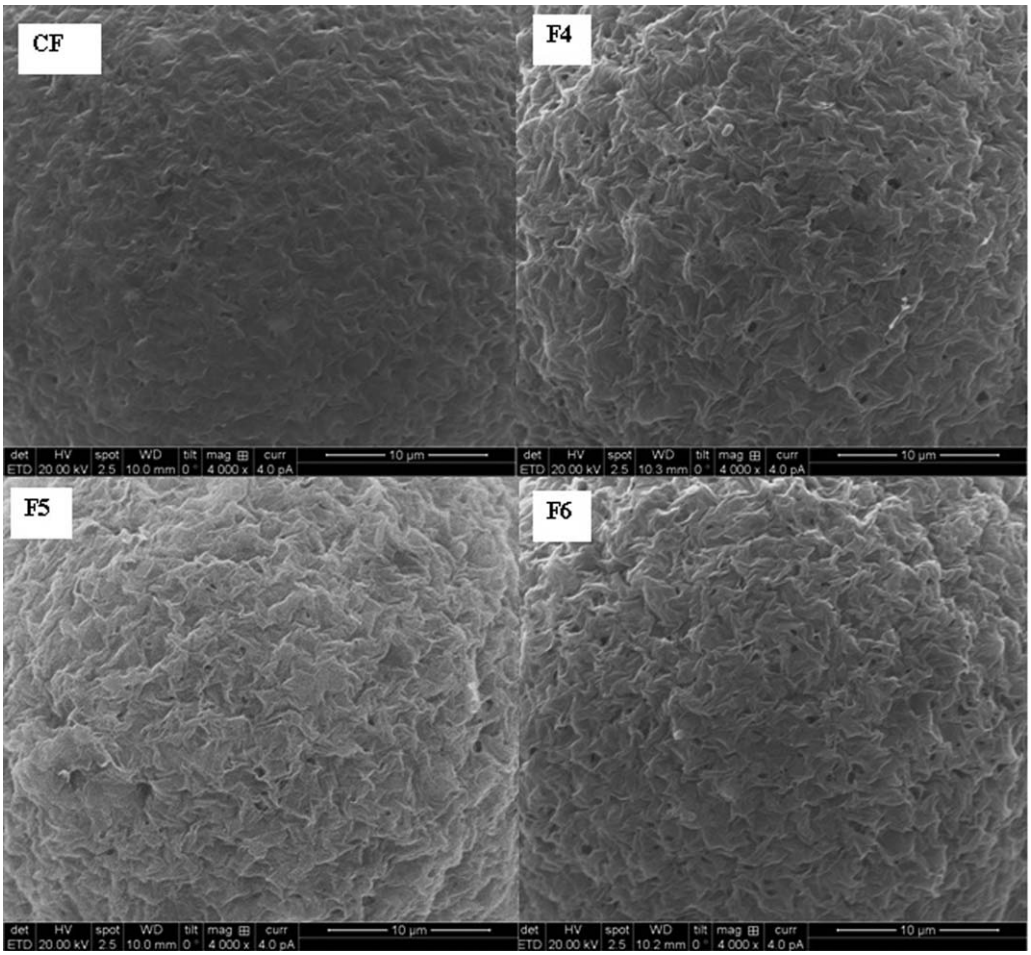

Figure 6. Surface SEM images of 6-MP-loaded plain PHB (CF) and PHB/Pluronic F127 blend microspheres formed with 10% (w/w) (F4), 20% (w/w) (F5), and 30% (w/w) (F6) of Pluronic F127 at ×4000 magnifications.

folding increased with increasing Pluronic F127 content of the blend, due to hydrophilic nature of the Pluronic F127, which might have leached out into water phase during the emulsification and solvent evaporation steps.

In Vitro Release Studies

To understand the *in vitro* release of 6-MP from the blend microspheres, experiments were done in pH 7.4 phosphate buffer media to simulate the physiological condition. The results of % cumulative release vs. time for CF, F1, F2, and F3 are compared in Figure 7(A) that show the effect of pluronic F68 on the *in vitro* release of the blend microspheres. The % cumulative release is higher for F3 than F2, which in turn, shows higher release rate than F1. On the other hand, control formulation (CF) exhibits a lesser release rate than F1, F2, and F3. The plain PHB microspheres released only 58% of 6-MP at the 16th hour, whereas formulations F1, F2 and F3 prepared, respectively by taking 10, 20, and 30% (w/w) Pluronic F68 released 71, 85, and 100% of 6-MP, respectively at 16th hour. The effect of Pluronic F127 content for formulations F4, F5, and F6 on the *in vitro* release profile is compared in Figure 7(B). Microspheres containing Pluronic F127 show similar trend of cumulative release as that of blend microspheres containing Pluronic F68 i.e., at the 16th hour of the release, but formulation F4 prepared with 10 % (w/w) of Pluronic F127 released 81% of MP, whereas formulation F5 prepared with 20% (w/w) of Pluronic F127 showed 95% release of 6-MP. Formulation F6 prepared with 30% (w/w) of Pluronic F127 shows 100% cumulative release, but with increasing Pluronic F68/F127 content of the blend, the % cumulative release of 6-MP also increase due to the hydrophilic nature of Pluronic.

PHB is a hydrophobic polyester and with increasing composition of hydrophilic Pluronic (F68/F127), hydrophillicity of the blend matrix hase increased, resulting in easy transport of release media into blend microspheres, thereby giving a fast release of 6-MP. When pluronic containing blend microspheres are in contact with the release media, pluronic molecules that are present on the surface of the blend microspheres diffuse into the release media, creating more pores on the surface such that the release media easily penetrates inside the microspheres, thus leading to higher drug diffusion into the release media, giving higher release of 6-MP. The particle size analysis showed that blend microspheres prepared with higher pluronic content showed smaller particle size compared to plain PHB microspheres. However, surface area of the microspheres in contact with the release media is more in case of smaller microspheres compared to bigger ones. Such a surface/volume ratio may offer

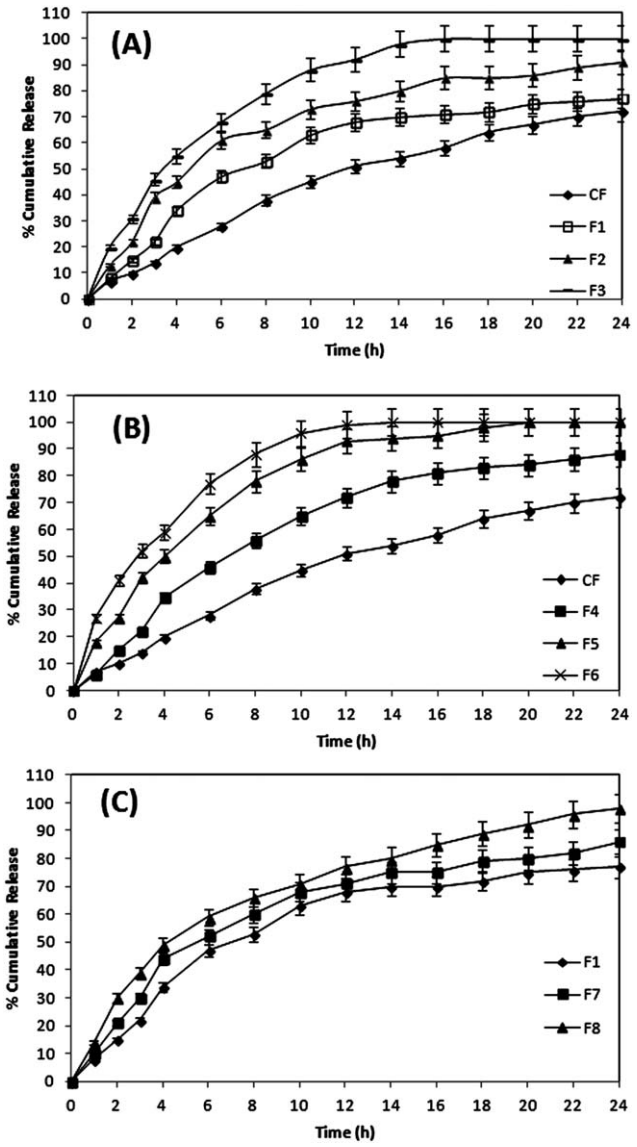

Figure 7. (A) Effect of Pluronic F68 content on i*n vitro* release profiles of PHB/Pluronic F68 blend microspheres, (B) effect of Pluronic F127 content on i*n vitro* release profiles of PHB/Pluronic F127 blend microspheres and (C) effect of % 6-MP loading on *in vitro* release profiles of PHB/Pluronic F68 blend microspheres.

higher release of 6-MP from the blends containing higher Pluronic content. Formulations containing Pluronic F127 (F4, F5, and F6) exhibit higher release rates than those containing Pluronic F68 (F1, F2, and F3) due to high molecular weight and more hydrophilic nature[33,34] of Pluronic F127 compared to Pluronic F68.

Figure 7(C) displays the release profiles of blend microspheres with different 6-MP-loadings that showed that highest 6-MP-loaded formulation displayed higher release rates than those formulations containing lower 6-MP loading. Blend microspheres loaded with 30 mg of 6-MP (F8) released ~98% of 6-MP in 24 h, whereas the blend microspheres loaded with 20 mg of 6-MP (F7) released only ~86% of 6-MP. Formulation F1 with 10 mg of initial 6-MP loaded formulation released ~77% 6-MP, but the release was quite slower at lower concentration of 6-MP. During the dissolution process, release media in the immediate vicinity of the microsphere surface might have dissolved the surface adhered drug particles due to high loading of 6-MP leading to the formation of voids on the particle surface, which are then occupied by the release media. Hence, if more of drug is present in the microspheres, more numbers of voids are created to form large free void space for the release media to enter into the microspheres, which will enhance the release rate. However, the availability of free void space is small for microspheres loaded with lesser amount of drug to induce sustained release of 6-MP[35] at lower loading of 6-MP.

Release Kinetics

To study the drug release kinetics from blend microspheres, the release data were fitted to Zero order, First order, Higuchi square root and Hixson–Crowell cube root equations [eqs. (5–8)] to compute correlation coefficients (r), whose values are presented in Table III. Release data of plain PHB (CF) as well as formulations F1, F7, and F8 loaded with different amounts of 6-MP are best fitted with the First order rate equation as indicated by the values of correlation coefficients (see Table III). Thus, drug release rate is dependent on the initial amount of drug in the microspheres. On the other hand, 6-MP release from F2, F3, F4, and F5 formulations are best fitted with the Hixson–Crowell equation, which indicates the drug release to be dependent on the surface area as well as size of the microspheres. However, the release data of F6 is best fitted with Higuchi square root equation, indicating the dependence of 6-MP release on square root of release time.[29,30]

As per Korsmeyer et al.[31] equation [eq. (9)], estimated n values along with correlation coefficients presented in Table III show the values below 0.43, which indicates that the drug release follows Fickian diffusion, while the values of n between 0.43 and 0.85 are indicative of both diffusion controlled as well as swelling-controlled release, but the values >0.85 indicate swelling-controlled release that is related to polymer relaxation phenomenon during swelling.[36] If n value is >1, then drug release follows Super Case II transport mechanism. The n values for F1, F2, and F3 are 0.95, 0.94, and 0.75, respectively, whereas for F5 and F6, the respective n values are 1.09, 0.76, and 0.57. This suggests that blend microspheres prepared taking a small amount (10% w/w) of Pluronic F68/127 released the 6-MP because of the relaxation of polymer chains that followed swelling controlled release. On the other hand, for blend microspheres prepared with higher (20 and 30% w/w) amount of Pluronic F68/127, the 6-MP release is occurs as result of diffusion and relaxation of polymeric chains due to swelling, which followed the anomalous release. In any case, drug release due to swelling is the result of distanglement of polymer chains, whereas drug release due to diffusion is due to leaching of hydrophilic pluronic in the blend along with drug particles. The n value for F1, F7, and F8 are 0.95, 0.88, and 0.81, respectively that suggests that increasing the drug loading from 10 to 30 mg, the release of 6-MP shifts from swelling controlled to anomalous type mechanism; In case of plain PHB microspheres, n value is 1.19, indicating that 6-MP release follows

Table III. Correlation Coefficient (r) Values of all the Formulations Estimated for Different Empirical Equations

Formulation codes	Zero order [eq. (4)]	First order [eq. (5)]	Higuchi [eq. (6)]	Hixson–Crowell [eq. (7)]	Korsemeyer et al. [eq. (8)]	
					n	r
CF	0.964	0.994	0.921	0.989	1.19	0.991
F1	0.973	0.986	0.887	0.985	0.95	0.989
F2	0.984	0.984	0.903	0.985	0.94	0.979
F3	0.974	0.994	0.960	0.996	0.76	0.992
F4	0.983	0.987	0.869	0.990	1.09	0.984
F5	0.976	0.991	0.951	0.993	0.76	0.983
F6	0.896	0.974	0.998	0.954	0.57	0.997
F7	0.931	0.982	0.929	0.971	0.88	0.974
F8	0.926	0.987	0.957	0.973	0.81	0.973

Super Case II transport,[12] due to distanglement of PHB chains from swelling.

CONCLUSIONS

The present study reports on the development of novel blend microspheres of PHB and pluronic F68/127 prepared by emulsion-solvent evaporation technique used in the CR of 6-MP and EE of 79 and 87% of production yield were achieved by this method. The sizes of the microspheres are in the range 30 to 47 μm. Spherical shape of the blend microspheres as well as wrinkles on the surface are due to the presence of Pluronic as indicated by SEM images. The composition of PHB to pluronic F68/127 in the blend showed a direct effect on the size distribution of microspheres, surface morphology, production yield and EE. DSC confirmed the decrease in crystallanity of PHB after blending with Pluronic F127. FTIR confirmed the presence of 6-MP in the microspheres, suggesting no chemical interactions between 6-MP and polymers. XRD confirmed the crystalline nature of 6-MP in the blend and drug release was dependent mainly on the amount of Pluronic F68/127 in the blend i.e., release increased with increasing pluronic content. Hydrophilicity of the blend microspheres and their release of 6-MP increased with increasing composition of Pluronic F68/127 of the blend. The *in vitro* cumulative release data when analyzed by the empirical equations indicated the dependence on the Pluronic F68/127 content, drug concentration, size, surface area of the microspheres and the time of release. The mechanism of drug release as analyzed by n values calculated by fitting the cumulative release data to Kormayer et al. equation showed that Pluronic is mainly responsible for 6-MP release by the diffusion mechanism, whereas chain relaxation of PHB and Pluronic are responsible for its swelling related release.

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