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Rate-modulating PHBHV/PCL microparticles containing weak acid model drugs

Fernanda S. Poletto [a], Eliézer Jäger [c], Maria I. Ré [b], Sílvia S. Guterres [a], Adriana R. Pohlmann [a,c,*]

[a] *Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil*
[b] *Instituto de Pesquisas Tecnológicas do Estado de São Paulo (IPT), Divisão de Química, São Paulo, SP, Brazil*
[c] *Departamento de Química Orgânica, Instituto de Química, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil*

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Abstract

In this work, we aimed to evaluate the influence of the proportions of poly(*epsilon*-caprolactone) (PCL) in the poly(hydroxybutyrate-*co*-hydroxyvalerate) (PHBHV) blended microparticles on the drug release profiles of drug models and to determine the drug release mechanism. Diclofenac and indomethacin used as drug models showed encapsulation efficiencies close to 85%. The average diameters (122–273 μm) and the specific surface areas (26–120 $m^2\,g^{-1}$) of the microparticles were dependent on the PCL concentration in the blends. Differential scanning calorimetry (DSC) analyses showed that the microparticle preparation process influenced the thermal behavior of PHBHV, as well as the glass transition temperature of PHBHV increased with the presence of indomethacin. The release profiles, described by a biexponential equation, showed sustained phase half-lives varying from 131 to 912 min (diclofenac) and from 502 to 6300 min (indomethacin) depending on the decrease of the PCL concentration. The product between the diffusion coefficient and the drug solubility in the matrix ($DC_{s,m}$), which was proportional to the PCL concentration, was calculated by fitting the release data to the Baker–Lonsdale equation. The mechanism of release was mainly controlled by the drug diffusion and the drug release profiles were controlled by varying the PCL concentration systematically in the blended PHBHV/PCL microparticles.

Keywords: Drug release; Diffusion; Mathematical modeling; Microparticle; PHBHV; PCL

1. Introduction

Microparticles, which sizes range from 1 to 1000 μm, have been widely studied in the pharmaceutical field for drug release (Yang and Alexandridis, 2000) due to their advantages compared to the conventional pharmaceutical formulations. Those advantages include lower variability between patient responses (Kawashima et al., 1993), lower risk of dose dumping (Iwata et al., 1999) and higher patient comfort and compliance (Varde and Pack, 2004). In general, the microparticle matrices are composed of biodegradable and biocompatible polymers, such as poly(lactide) (PLA) and poly(lactide-*co*-glycolide) (PLGA) (Hombreiro-Pérez et al., 2000; Lin et al., 2005; Oh et al., 2006). An interesting alternative to these synthetic polymers are the poly(hydroxyalcanoates) (PHAs), which are polyesters produced by a great variety of microorganisms (Salehizadeh and van Loosdrecht, 2004). Poly(3-hydroxybutyrate) (PHB) and its copolymers with hydroxyvalerate (HV) are the most widely used PHAs (Amass and Tighe, 1998). They are atractive as biomedical devices due to their adequate biocompatibility, biodegradability and thermoprocessability (Chen and Wu, 2005). Although the PHB and the poly(hydroxybutyrate-*co*-hydroxyvalerate) (PHBHV) chemical structures are very similar to the highly popular synthetic biodegradable polymers, such as PLA and PLGA, they generally degrade at a much slower rate (Pouton and Akthar, 1996; Amass and Tighe, 1998). In this way,

* Corresponding author at: Departamento de Química Orgânica, Instituto de Química, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, CP 15003, Porto Alegre, CEP 91501-970, RS, Brazil.
Tel.: +55 51 33166274; fax: +55 51 33167304.
E-mail address: pohlmann@iq.ufrgs.br (A.R. Pohlmann).

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previous studies described the application of PHB and PHBHV microparticles as long-term drug delivery systems (Gangrade and Price, 1991; Wang et al., 2007). Nevertheless, more rapid drug release rates could be advantageous to enhance the applicability of the PHBHV microparticles, such as potential devices for oral administration.

In general, microparticle studies have showed that the rate of drug release is faster for higher porosity (Mandal et al., 2001; Le Ray et al., 2003; Ruan and Feng, 2003). Papaverine released from microparticles has been studied using poly(*epsilon*-caprolactone) (PCL) with different molecular weights (Jeong et al., 2003). The cristallinity has reduced as the PCL molecular weight has increased. So, the higher the molecular weight was, the greater the amorphous phase increased. The fast drug release rate has been observed using the highest PCL molecular weight. For those systems, the release was controlled by drug diffusion through the amorphous region of the polymer matrix.

Bovine serum albumin (BSA) has been faster released from PHBHV/PCL blended microparticles than BSA released from poly(ethylene adipate)/PCL blended microparticles (Atkins, 1997), because the former had pores on its surface and the latter showed smooth surface without pores. The release behavior of microparticles prepared with PHBHV containing different amounts of HV loading tetracycline in its acidic or neutral forms have been studied (Sendil et al., 1999). The drug has been completely released before the microparticle degradation, and the release data fitted to the Higuchi model. Microparticles prepared with PHBHV containing higher contents of HV have showed faster release rates probably due to the increase in the porosity of the matrix.

The control of the porosity in microparticles using PHBHV/PCL blends can be reached by modifying the PCL concentration in the blend (Embleton and Tighe, 1993, 2002). However, the correlation between the morphology and the drug release profile of those blended microparticles has not been established. So, the aim of this work was to determine the release behavior and the drug release mechanism of two drug models from PHBHV/PCL blended microparticles. Two series of microparticles were prepared with the proportions 100:0, 90:10, 70:30 and 50:50 (w/w), respectively, using diclofenac and indomethacin. Diclofenac (296 g mol^{-1}) and indomethacin (358 g mol^{-1}) previously employed as drug models (Pohlmann et al., 2002, 2004; Schaffazick et al., 2003; Fundueanu et al., 2005; Cruz et al., 2006; Şanlı et al., 2007) are phenyl or indol acetic acid derivatives. They were chosen as drug models in this study because both present pK_a values close to 4 (3.8 and 4.5, respectively) and similar water solubility (6×10^{-5} and 1×10^{-5} mol L^{-1}, respectively) (O'Connor and Corrigan, 2001; O'Brien et al., 1984).

2. Materials and methods

2.1. Materials

Diclofenac (sodium salt) and indomethacin were obtained from Sigma (St. Louis, USA) and poly(*epsilon*-caprolactone) (PCL, MW = 65,000 g mol^{-1}) was supplied from Aldrich (Strasbourg, France). Poly(3-hydroxybutyrate-*co*-hydroxyvalerate) (PHBHV, MW = 300,000 g mol^{-1}) was kindly gifted by PHB Industrial (São Paulo, Brazil). Poly(vinyl alcohol) (PVA, MW = 200,000 g mol^{-1}, 88% hydrolyzed) was supplied by Delaware (Porto Alegre, Brazil). All other chemicals and solvents presented analytical grade and were used as received.

2.2. Preparation of free acid diclofenac

An aqueous solution (400 mL) of sodium diclofenac (3.0 g, 9.43 mmol) was acidified with 5 mol L^{-1} HCl. The precipitate (free acid form of diclofenac) was filtered and recrystallized from ethanol/water 1:1 (v/v). Colorless crystals were obtained with 90% of yield and characterized by infrared analysis.

IR ν_{max} (cm^{-1}): 3300 (NH), 2500–3200 (OH), 1710 (C=O), 1600 and 1580 (C=C), 1500 and 1450 (aromatic rings).

2.3. Preparation of microparticles by single emulsion/solvent evaporation method

Microparticles were prepared by an oil-in-water (o/w) emulsion/solvent evaporation method (Conti et al., 1995) with modifications. PHBHV (200–400 mg), PCL (0–200 mg) and 0.135 mmol of drug, diclofenac (acid form) or indomethacin, were dissolved in 10 mL of chloroform. In parallel, an aqueous phase (40 mL) containing 1% (w/v) PVA was prepared. The aqueous phase was added into the organic phase at 50 °C under magnetic stirring (1200 rpm, 10 min) (Lionzo et al., 2007). The chloroform was evaporated from the emulsion at 50 °C under reduced pressure (Büchi RE120, Switzerland). The suspension was filtered (25 μm) to collect the microparticles, which were then washed with distilled water (150 mL). The powders were maintained in a dessicator for 24 h. Formulations were prepared using different proportions of PHBHV and PCL: 100:0, 90:10, 70:30 and 50:50 (w/w), respectively. The microparticles containing diclofenac were called DIC-0 (0% PCL), DIC-10, DIC-30 and DIC-50 (50% PCL) and the formulations containing indomethacin were called IND-0, IND-10, IND-30 and IND-50. Placebo microparticles (without drug) were also prepared and they were called MP-0, MP-10, MP-30 and MP-50.

2.4. Drug loading and encapsulation efficiency

The drug loading was determined by dissolving accurately weighed amounts of microparticles (approximately 10 mg) in 50 mL of chloroform. Subsequent filtration (0.45 μm) was performed for UV measurements. Diclofenac was detected at 278 nm and indomethacin at 264 nm. The experiments were carried out in triplicates of three batches ($n = 9$). The analytical methods were validated according to the following characteristics: linearity, precision, accuracy and specificity (ICH, 1996). The encapsulation efficiency for each formulation was calculated by the correlation between the theoretical

and the experimental drug concentrations and expressed as percentages.

2.5. Drug release profiles

In vitro drug release profiles were determined as described by Beck et al. (2004) with modifications. The microparticles (weight equivalent to 8.44×10^{-3} mmol of drug) were suspended in 50 mL of phosphate buffer pH 7.4 ± 0.1. Total drug concentration in microparticle suspensions was less than 10% of its solubility in water. The suspensions were maintained at $37.0 \pm 0.5\,^{\circ}C$ under constant magnetic stirring (Velp Multistirrer, Germany) in closed glass flasks. Two milliliters of supernatant were collected at predetermined time intervals and filtered (0.45 μm Millipore® filters). The volume was replaced by adding 2 mL of phosphate buffer solution ($37.0 \pm 0.5\,^{\circ}C$). The filtered samples were analyzed by UV spectroscopy at 280 nm (diclofenac) or at 266 nm (indomethacin) and then the cumulative drug release was calculated. The analytical methods were validated according to linearity, precision, accuracy and specificity (ICH, 1996). The experiments were carried out in duplicates of three batches ($n = 6$). In parallel, the same procedure was performed to evaluate the dissolution of the free drugs in triplicates ($n = 3$).

2.6. Microparticle size determination

The mean diameter over the volume distribution ($d_{4.3}$) and particle size distribution (SPAN) were determined using a Malvern Mastersizer 2000 laser diffraction instrument (Malvern Instruments, UK). The determination of the microparticle sizes was carried out in water containing surfactant, as a non-dissolving dispersion medium, and the particles were suspended by ultrasound during the measurements. This experiment was carried out in triplicate. The size distribution was expressed by the SPAN value (Raffin et al., 2006), which was calculated using the following equation:

$$\text{SPAN} = \frac{d_{0.9} - d_{0.1}}{d_{0.5}} \quad (1)$$

where $d_{0.9}$, $d_{0.1}$ and $d_{0.5}$ are the diameters at 90%, 10% and 50% cumulative volumes, respectively.

2.7. Specific surface area

The specific surface areas were determined by the Brunauer–Emmett–Teller multipoint technique (BET) (Brunauer et al., 1938) on a volumetric apparatus using nitrogen (vapor) as probe. A homemade equipment with a vacuum line system employing a turbomolecular vacuum pump (Edwards *1.5 EXC 120, England) was used. The samples (100 mg) were previously degassed under vacuum at 25 °C for 3 h. Adsorption isotherms were determined from the volume of nitrogen (vapor) adsorbed onto the surface of samples immersed in liquid nitrogen as a function of relative pressure (50, 100, 150 and 200 mmHg) (Raffin et al., 2006). The pressure measurements were made using a capillary Hg barometer and also an active Pirani gauge. Alumina was used as standard reference.

2.8. Scanning electron microscopy

Microparticle surface morphologies were evaluated by scanning electron microscopy (SEM) (Jeol Scanning Microscope, JSM-5800, Tokyo, Japan) after they had been carbon coated and gold sputtered (Jeol Jee 4B SVG-IN, Tokyo, Japan) (Centro de Microscopia Eletrônica/UFRGS, Porto Alegre, Brazil).

2.9. Infrared spectroscopy

Infrared spectra were recorded on a spectrophotometer (Shimadzu FT-IR 8300, Japan). The microparticles and the drugs, diclofenac and indomethacin, were analyzed using KBr tablets. Thin films of PHBHV and PCL were also analyzed.

2.10. Differential scanning calorimetry

Differential scanning calorimetry (DSC) curves were obtained using a Perkin-Elmer DSC-4 (USA). All the experiments were performed from −20 to 250 °C at a scanning rate of $10\,^{\circ}C\,min^{-1}$. The instrument was calibrated using indium, as standard, and all the samples (microparticles, physical mixtures and raw materials) weighed less than 10 mg. Thermograms were obtained by the first heating cycle in order to evaluate the influence of the process of microparticle preparation on the thermal properties of the samples.

2.11. Drug release profiles analyses

2.11.1. Model independent method

The dissolution efficiency (DE) (Eq. (2)) was used to compare the drug release profiles (Costa and Lobo, 2001).

$$\text{DE} = \frac{\int y\mathrm{d}t}{y_{100} \times t} \times 100 \quad (2)$$

where $\int y\mathrm{d}t$ is the area under the dissolution curve up to a certain time t and $y_{100} \times t$ is the area of the rectangle described by 100% of drug dissolution in the same time. Each replicate ($n = 3$) was used to calculate the DE values of formulations, which are expressed as average values and their standard deviation. Data were evaluated by one-way analysis of variance (ANOVA) as statistical method (SigmaStat for Windows version 3.5).

2.11.2. Monoexponential and biexponential equations

The fit of the mathematical semi-empirical monoexponential (Eq. (3)) and biexponential (Eq. (4)) models to the experimental dissolution data was carried out using the Scientist® 2.0 software (MicroMath®, USA). The mathematical models were selected considering the best plot adjustment, the correlation between experimental points and theoretical profiles and the higher

value of model selection criteria (MSC), furnished by the software.

$$C = 1 - [C_0\, e^{-kt}] \quad (3)$$

$$C = 1 - [A\, e^{-\alpha t} + B\, e^{-\beta t}] \quad (4)$$

where C is the concentration of drug released at time t, C_0 the drug loading, k, α and β are the observed kinetic rate constants, A and B are the parameters which reflect the portion of the initial concentrations of drug that contributed to the burst and sustained phases, respectively.

2.11.3. Baker–Lonsdale equation

The drug release profiles were analyzed using the Baker–Lonsdale model (Eq. (5)).

$$\frac{3}{2}\left[1 - \left(1 - \frac{M_t}{M_{\inf}}\right)^{2/3}\right] - \frac{M_t}{M_{\inf}} = \frac{3DC_{s,m}}{r_0^2 C_0} t \quad (5)$$

where M_t is the amount of drug released at time t and M_{inf} the total amount of drug-loaded in the microparticles; D, C_0 and $C_{s,m}$ are the diffusion coefficient of drug, the drug loading, and the drug solubility in the matrix, respectively; r_0 is the radius of the sphere, and t is the elapsed time for release. The parameter C_0 was experimentally obtained by UV quantification (Section 2.4), and the particle radius, r_0, was determined by laser diffraction (Section 2.6). The fitting of drug release data to the Baker–Lonsdale equation was performed by the least squares method assuming that the diffusion coefficient was constant for all time intervals for all formulations. Therefore, the $DC_{s,m}$ product was obtained from the angular coefficient of each curve.

3. Results and discussion

3.1. Microparticle drug loading, encapsulation efficiency and particle size

Diclofenac and indomethacin microparticles presented drug loading between 83.9 and 101.4 mg g^{-1} and high encapsulation efficiencies ranging from 81% to 87% (Table 1). The mean diameters ($d_{4.3}$) ranged from 122 to 274 μm for all formulations (Table 1). The particle size slightly decreased according to the increase of the PCL concentrations for the diclofenac-loaded and placebo formulations. On the other hand, the indomethacin loading increased the sizes of microparticles with higher contents of PHBHV (IND-0 and IND-10). Furthermore, the SPAN values calculated for all the samples in this study were lower than 2, indicating that the particle size distribution is adequately narrow.

3.2. Morphological analysis

SEM images of microparticles (Fig. 1) showed that the particles were spherically shaped and the increase in the PCL concentration from 0% to 50% resulted in the augmentation of the number and/or the size of the pores in the microparticles. In addition, microparticles prepared with 50% of PCL showed disrupted walls and a hollow core. The SEM micrographs also revealed crystals on the surface of the microparticles for both series of drug-loaded formulations. The specific surface area determined by nitrogen isotherms showed that the increase in the PCL concentration produced a significant increase in the surface area independently of the presence of the drugs (Table 1).

3.3. DSC analysis

Endothermic peaks were visualized on the thermograms of the isolated polymers (PHBHV and PCL) (Fig. 2). The melting temperatures (T_m) obtained for the raw materials were 171.6 °C

Table 1
Morphological and drug entrapment parameters corresponding to the PHBHV and the PHBHV/PCL microparticles

Sample	Drug loading (mg/g)	EE (%)[a]	MVD[b]	Particle size distribution				Specific surface area (m^2/g)
			$d_{4.3}$ (μm)	$d_{0.9}$ (μm)	$d_{0.1}$ (μm)	$d_{0.5}$ (μm)	SPAN	
MP-0	–	–	214.9 ± 1.8	360.0	99.3	195.0	1.3	33
MP-10	–	–	187.8 ± 0.8	317.8	87.8	169.4	1.3	82
MP-30	–	–	176.2 ± 0.2	296.8	82.0	159.0	1.3	109
MP-50	–	–	122.0 ± 2.4	190.9	65.8	111.1	1.1	120
DIC-0	87.8 ± 4.1	87 ± 2	187.0 ± 1.4	324.1	79.8	168.8	1.4	26
DIC-10	83.9 ± 3.7	83 ± 2	199.4 ± 1.5	334.1	91.5	178.4	1.4	91
DIC-30	85.4 ± 7.8	84 ± 6	160.9 ± 1.0	289.5	67.8	137.2	1.6	111
DIC-50	84.2 ± 5.0	84 ± 4	153.9 ± 0.1	239.2	84.9	142.5	1.1	120
IND-0	96.8 ± 7.0	83 ± 3	271.6 ± 1.5	466.8	112.0	248.1	1.4	26
IND-10	96.4 ± 6.1	81 ± 3	273.8 ± 0.1	461.5	117.4	254.4	1.3	80
IND-30	97.0 ± 6.6	82 ± 3	174.0 ± 1.0	293.1	64.3	127.0	1.8	103
IND-50	101.4 ± 2.2	82 ± 2	177.9 ± 2.2	299.3	86.2	161.8	1.3	110

[a] Encapsulation efficiency.
[b] Mean volume diameter.

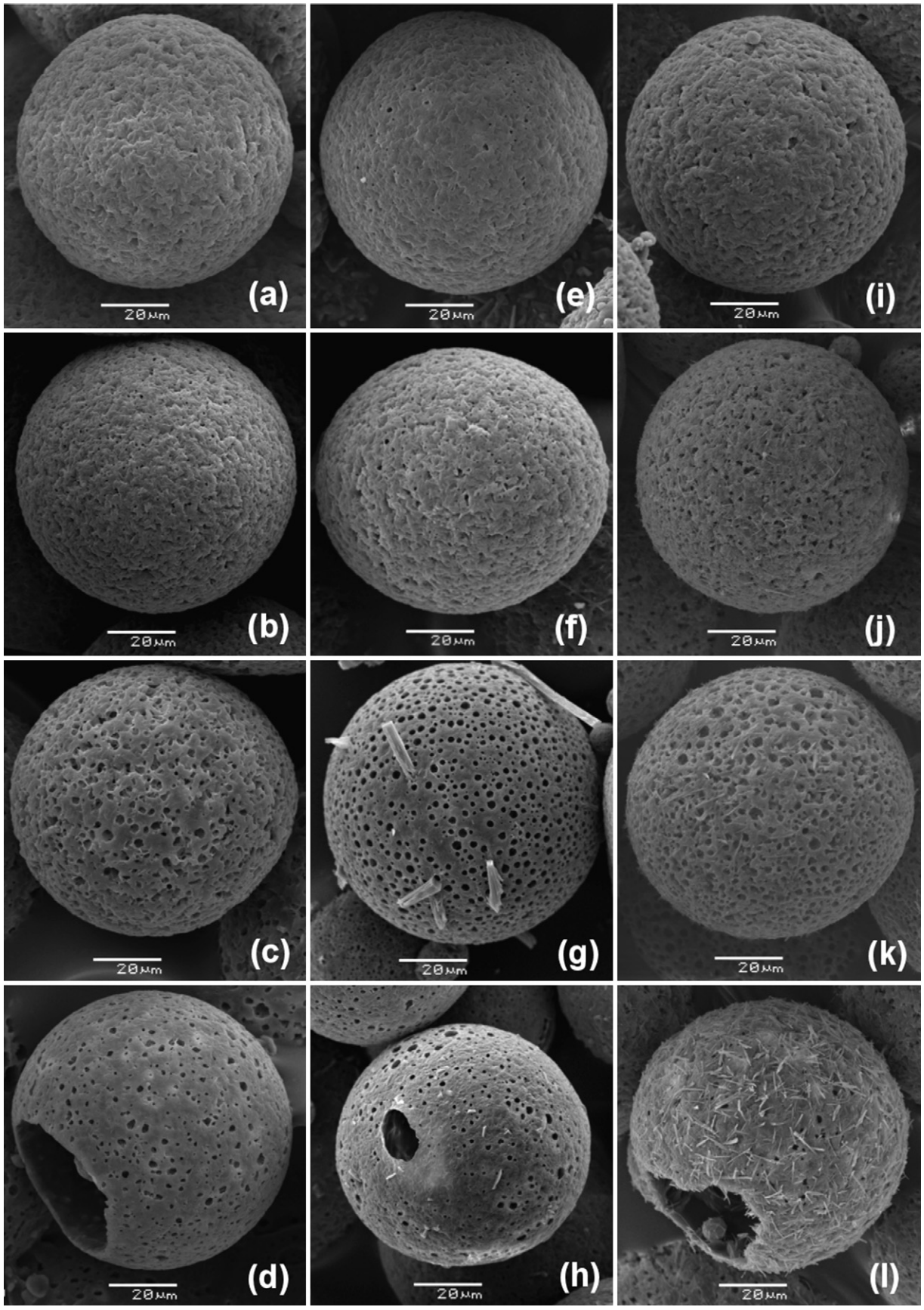

Fig. 1. SEM images of microparticles: (a) MP-0, (b) MP-10, (c) MP-30, (d) MP-50, (e) DIC-0, (f) DIC-10, (g) DIC-30, (h) DIC-50, (i) IND-0, (j) IND-10, (k) IND-30 and (l) IND-50 (15 kV, bar = 20 μm).

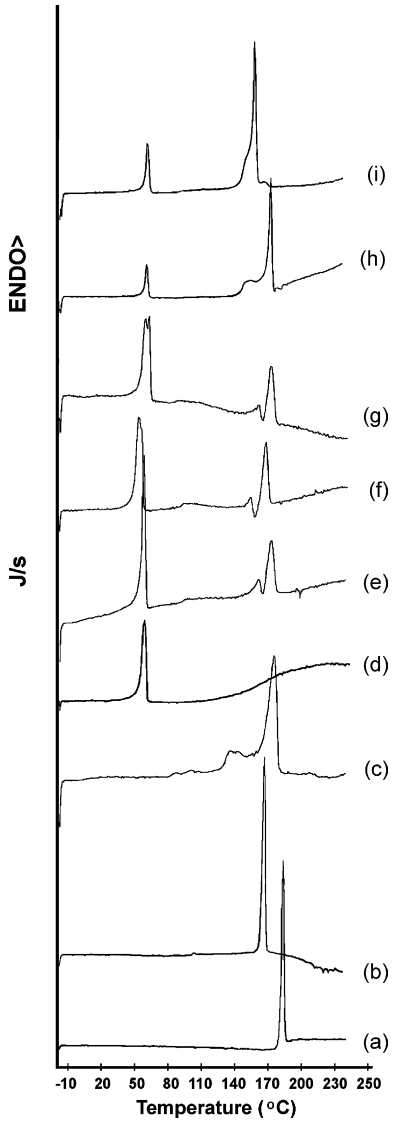

Fig. 2. DSC thermograms of: (a) diclofenac, (b) indomethacin, (c) PHBHV (raw material), (d) PCL (raw material), (e) MP-50, (f) DIC-50, (g) IND-50, (h) diclofenac/MP-50 physical mixture (1:1, w/w) and (i) indomethacin/MP-50 physical mixture (1:1, w/w).

for PHBHV and 56.4 °C for PCL. The T_m values of each polymer in the physical mixtures, prepared at the proportions of 90:10, 70:30 and 50:50 (w/w), were 171.4, 170.8 and 171.3 °C for PHBHV and 56.5, 57.2 and 57.2 °C for PCL. The T_m values of the mixtures were similar to those of the raw materials. The T_m values for the PCL in the placebo microparticles (MP-0, MP-10, MP-30 and MP-50) (Table 2) were similar to the value determined for the PCL raw material, indicating that this polymer maintained its characteristics after the microparticle preparation process. On the other hand, PHBHV in the placebo microparticles showed two melting peaks indicating that the thermal characteristic of PHBHV was modifyied by the preparation process. As previously reported, the thermal behavior of PHB was altered after microparticle preparation by emulsification/solvent evaporation process (Martin et al., 2000).

The T_m values of PHBHV and PCL in the microparticles were shifted to lower values after loading diclofenac or indomethacin (Table 2). The melting peak for diclofenac (raw material) was observed at 181.4 °C and for indomethacin (raw material) at 162.6 °C. However, for the drug-loaded microparticles no peak of the drugs was observed on the thermograms (Fig. 2). In order to investigate the sensitivity of this technique to detect the drugs in the concentration that they were used in the formulations, two thermograms for each drug were recorded using the mixtures of drug and MP-50 microparticles at the proportions of 1:9 and 1:1 (w/w). The curves showed that the melting peaks of PHBHV were overlapped with the peaks of the drugs. In this way, the melting peaks of diclofenac and indomethacin could not be detected in the microparticle curves at the concentrations used in the formulations. Fig. 2 shows the thermograms of the raw materials, microparticles containing 50% of PCL and physical mixtures of drugs with MP-50 microparticles.

The PHBHV (raw material) showed a glass transition temperature (T_g) at −4.29 °C. In contrast, PHBHV in the placebo microparticles showed higher T_g values (−1.1 to 1.0 °C), demonstrating that the mobility of this polymer chains was reduced in the microparticles. Similar T_g values of PHBHV for placebo microparticles containing or not PCL were observed. These results indicated that PHBHV and PCL are immiscible blends, in agreement with previous reports (Chun and Kim, 2000; Qiu et al., 2005). The presence of diclofenac in the formulations slightly affected the T_g of PHBHV in the microparticles. However, the T_g values of PHBHV for indomethacin-loaded microparticles were higher than the values observed for the placebo microparticles (Table 2), suggesting an antiplasticizing effect caused by indomethacin. In general an antiplasticizing agent is a solute molecule that presents high affinity with the polymer (Slark, 1997). So, the results suggest that indomethacin is at least in part molecularly interacting with PHBHV amorphous region, while diclofenac would be dispersed as aggregates in the microparticle polymer matrix.

3.4. Infrared characterization

PHBHV infrared spectrum presented peaks at 1735 and 3440 cm^{-1}, corresponding to the C=O (stretching) and to the OH groups. The peaks at 2940 and 2960 cm^{-1} are related to the C–H bond of saturated carbons (Fig. 3a). PCL is also an aliphatic polyester and its spectrum is similar to that of PHBHV (Fig. 3b). Diclofenac showed peaks at 3300 cm^{-1} (NH), 1710 cm^{-1} (CO), 1600 and 1580 cm^{-1} (C=C), 1500 and 1450 cm^{-1} (aromatic rings), as well as a large band at 2500–3200 cm^{-1} (OH) (Fig. 3c). Indomethacin showed peaks at 3000 and 2800 cm^{-1} (C–H), 1710 cm^{-1} (carboxylic acid C=O), 1640 cm^{-1} (amide C=O), 1600 and 1580 cm^{-1} (C=C) and a large band at 2500–3200 cm^{-1} (OH) (Fig. 3d). The analysis of drug-loaded microparticles showed peaks of diclofenac corresponding to the amine stretching (3300 cm^{-1}) and C=C stretching (1600 cm^{-1}), as well as the peak of indomethacin corresponding to C=C stretching (1600 cm^{-1}) (Fig. 3f and g, respectively). The peaks observed for PHBHV and PCL in

Table 2
Thermal properties of PHBHV and PHBHV/PCL microparticles

Sample	PHBHV			PCL	
	T_m (°C)[a]	T_g (°C)[b]	ΔH_m (J/g)[c]	T_m (°C)	ΔH_m (J/g)
MP-0	159.6; 172.2	0.4	10.30; 33.10	–	–
MP-10	158.6; 171.1	−1.1	11.03; 28.76	55.8	4.97
MP-30	159.0; 171.4	1.0	14.09; 27.17	61.2	23.41
MP-50	159.6; 171.0	−1.0	3.97; 15.51	56.5	29.51
DIC-0	152.9; 167.2	0.4	9.15; 36.66	–	–
DIC-10	153.3; 167.2	−1.6	4.10; 32.10	49.1	4.72
DIC-30	153.0; 167.4	1.3	6.52; 26.71	54.0	17.97
DIC-50	153.6; 167.1	−0.6	3.05; 17.56	53.1	30.14
IND-0	154.0; 167.4	3.8	3.43; 28.80	–	–
IND-10	155.3; 169.0	3.5	13.96; 30.64	49.4	3.47
IND-30	154.5; 168.1	3.6	3.26; 19.81	54.2	15.38
IND-50	156.7; 168.4	4.2	4.18; 15.67	54.5	28.51

[a] Melting temperature.
[b] Glass transition temperature.
[c] Melting enthalpy.

the drug-loaded microparticles were similar to those determined for the placebo microparticles (Fig. 3e) and for the raw materials.

3.5. In vitro drug release kinetics

3.5.1. Drug release profiles

Diclofenac (pure drug) was totally dissolved after 60 min in phosphate buffer pH 7.4 ± 0.1 (37.0 ± 0.5 °C) (Fig. 4).

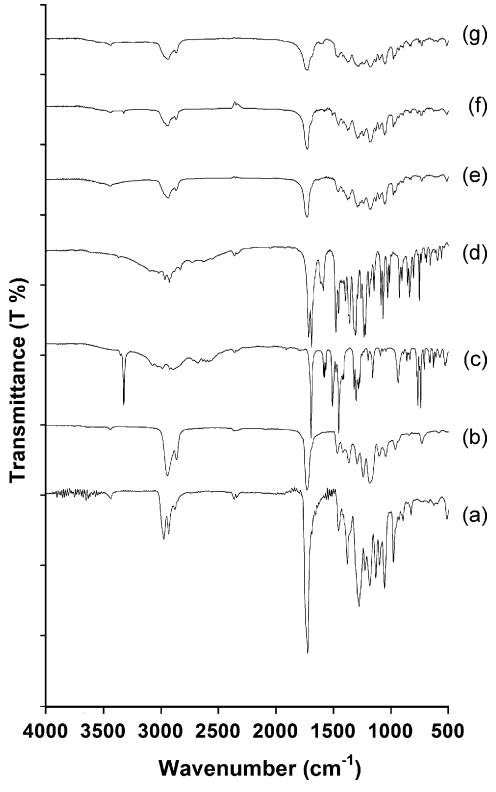

Fig. 3. FT-IR spectra of (a) PHBHV, (b) PCL, (c) diclofenac, (d) indomethacin, (e) MP-50, (f) DIC-50 and (g) IND-50.

In contrast, diclofenac-loaded microparticles released different amounts of drug after 60 min, from 23.6 ± 3.0% (DIC-0) to 36.6 ± 4.3% (DIC-50). Diclofenac was totally released from the microparticles after 8 h (DIC-50), 13 h (DIC-30), 45 h (DIC-10) and 47 h (DIC-0). Indomethacin (pure drug) was totally dissolved after 120 min in phosphate buffer pH 7.4 ± 0.11 (37.0 ± 0.5 °C). On the other hand, the indomethacin released from the microparticles at the same time period ranged from 20.4 ± 1.1% (IND-0) to 37.0 ± 1.8% (IND-50). Indomethacin was totally released from the microparticles after 24 h (IND-50) and 42 h (IND-30). After 48 h, 48.7 ± 2.7% and 50.5 ± 4.1% of indomethacin were released from the microparticles IND-10 and IND-0, respectively.

The dissolution efficiencies (DE) were calculated to compare the drug release profiles (Table 3). ANOVA showed significant difference ($P < 0.05$) for the DE values of the formulations containing diclofenac (DIC-0, DIC-10, DIC-30 and DIC-50). This statistical analysis indicated that the drug dissolution efficiencies were not similar among the formulations. On the other hand, the Tukey test showed that the DE of DIC-0 and DIC-10 were similar ($P > 0.05$), while the DE of DIC-30 and DIC-50 were significant different ($P < 0.05$) from

Table 3
Comparison of drug release profiles of diclofenac and indomethacin-loaded PHBHV/PCL microparticles through the dissolution efficiency (DE)

Formulation	DE
DIC-0	99.838 ± 7.034
DIC-10	92.859 ± 4.239
DIC-30	77.349 ± 7.155
DIC-50	75.324 ± 7.654
IND-0	91.411 ± 3.265
IND-10	89.684 ± 6.265
IND-30	78.553 ± 1.803
IND-50	72.344 ± 2.324

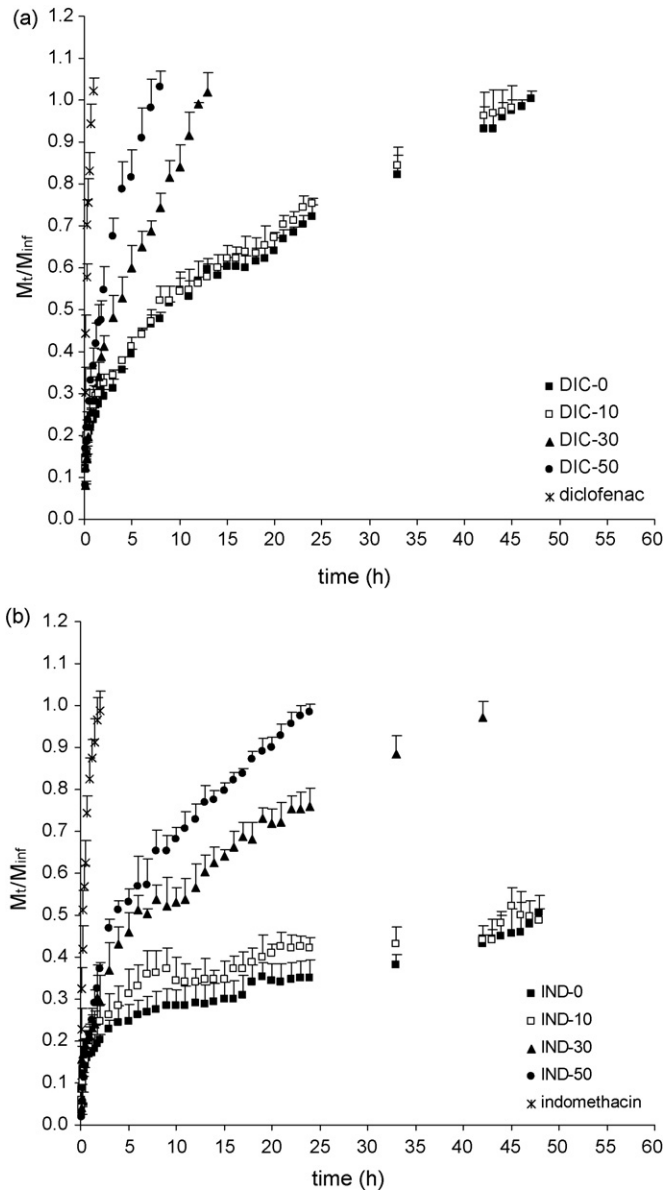

Fig. 4. Drug release profiles of diclofenac and indomethacin from PHBHV and PHBHV/PCL microparticles and dissolution profiles of the free drugs in buffer pH 7.4 ± 0.1 (37 °C ± 0.5).

DIC-0. Comparing the DE values of formulations containing indomethacin (IND-0, IND-10, IND-30 and IND-50) all values were significant different ($P < 0.05$) using ANOVA, whereas the Tukey test showed no significant difference between IND-0 and IND-10, but those DE values were significant different ($P < 0.05$) from IND-30 and IND-50. The statistical analyses demonstrated that the release profiles were dependent of the PCL concentration among formulations prepared with the same drug.

3.5.2. Semi-empirical modeling of drug release profiles

In order to determine the drug release half-lives, semi-empirical mathematical modeling of profiles was carried out. The best model to describe the pure drug dissolution profiles was the monoexponential equation, which gave the kinetic constants k of $0.0602 \pm 0.0009\ \text{min}^{-1}$ (diclofenac) and $0.0365 \pm 0.0009\ \text{min}^{-1}$ (indomethacin). On the other hand, the biexponential model better described the experimental data for diclofenac and indomethacin released from microparticles (Table 4). The parameters which reflected the portion of the initial concentrations which contributed to the burst phases ranged from 0.13 to 0.17 for diclofenac and from 0.18 to 0.32 for indomethacin. In addition, the parameters which reflect the portion of the initial concentrations that contributed to the sustained phases ranged from 0.76 to 0.86 for diclofenac and from 0.69 to 0.75 for indomethacin. Based on the kinetic constants α and β from the biexponential equation, the half-lives ($t_{1/2}$) of the burst and the sustained phases were calculated. They ranged from 5 to 15 min (burst) and from 2 h 11 min to 15 h 12 min (sustained) for diclofenac-loaded microparticles, as well as from 41 to 55 min (burst) and from 8 h 22 min to 105 h (sustained) for indomethacin-loaded microparticles. The half-lives of the sustained phases increased according to the decrease of the PCL concentration in the formulations independently of the drug, diclofenac or indomethacin. The lower release rate observed for indomethacin compared to the rate for diclofenac from microparticles, containing the same PCL concentration, was probably a consequence of the type of the encapsulated drug, as well as the kind of drug interaction with the polymer matrix. As previously discussed, DSC data suggested that indomethacin is at least in part molecularly interacting with PHBHV, while aggregates of diclofenac are dispersed in the matrix of microparticles.

3.5.3. Baker–Lonsdale equation

Various mechanisms can be used to control the drug release, such as fickian diffusion, swelling and degradation/erosion (Kim, 2000). Mathematical modeling of drug release profiles can be used to identify the drug transport mechanism and to simulate the effect of the device design parameters on the resulting drug release kinetics (Siepmann and Peppas, 2001; Siepmann et al., 2004). The choice of the model is related to the characteristics of the drug and the matrix (Kim, 2000). PHBHV and PCL are hydrophobic polymers and, as a consequence, their swelling in aqueous medium are negligible (Terada and Marchessault, 1999; Avella et al., 2000). Also, their erosion can be considered irrelevant until several months (Maia et al., 2004; Sinha et al., 2004; Dhanaraju et al., 2006). In this way, considering the conditions used for the release experiments in this work (48 h in phosphate buffer pH 7.4) the diffusion would be the main mechanism of the drug release. Mathematical modeling of the release profiles using the Baker–Lonsdale equation was performed to verify this hypothesis.

According to this model, the drug must be uniformly dispersed in the spherically shaped matrix, the release mechanism should be primarily diffusion and the total drug loading must be greater than the drug solubility in the matrix (Costa and Lobo, 2001). Our results showed that the drugs (diclofenac and indomethacin) are in excess in the microparticles. SEM analyses demonstrated the presence of drug crystals on the microparticle

Table 4
Observed rate constants (a and b, adimensional; α and β, min^{-1}), correlation coefficients and MSC obtained by fitting diclofenac and indomethacin release profiles from PHBHV and PHBHV/PCL microparticles to the monoexponential and to the biexponential equations

Parameters	DIC-0	DIC-10	DIC-30	DIC-50
Monoexponential				
r	0.8907	0.8794	0.9589	0.9810
MSC	1.58	1.44	2.58	3.19
k	$0.00123 \pm 7 \times 10^{-5}$	0.00116 ± 0.00018	0.00367 ± 0.00032	0.00749 ± 0.00142
Biexponential				
r	0.9872	0.9920	0.9846	0.9969
MSC	3.52	3.99	3.29	4.53
a	0.17 ± 0.11	0.17 ± 0.03	0.13 ± 0.06	0.17 ± 0.01
b	0.77 ± 0.02	0.76 ± 0.02	0.86 ± 0.04	0.85 ± 0.01
α	0.07080 ± 0.05185	0.04695 ± 0.01595	0.11469 ± 0.01974	0.13764 ± 0.02752
β	$0.00076 \pm 8 \times 10^{-5}$	$0.00076 \pm 9 \times 10^{-5}$	0.00292 ± 0.00045	0.00529 ± 0.00088
Parameters	IND-0	IND-10	IND-30	IND-50
Monoexponential				
r	0.2800	0.5741	0.8979	0.9625
MSC	0.02	0.59	1.66	2.54
k	$0.00033 \pm 8 \times 10^{-6}$	$0.00038 \pm 3 \times 10^{-5}$	0.00134 ± 0.00019	0.00224 ± 0.00028
Biexponential				
r	0.9868	0.9878	0.9921	0.9977
MSC	3.57	3.40	3.95	5.18
a	0.19 ± 0.02	0.25 ± 0.04	0.19 ± 0.06	0.32 ± 0.03
b	0.75 ± 0.03	0.69 ± 0.0045	0.71 ± 0.06	0.70 ± 0.05
α	0.01443 ± 0.00948	0.01249 ± 0.00228	0.01431 ± 0.00044	0.01671 ± 0.00317
β	$0.00013 \pm 2 \times 10^{-5}$	$0.00011 \pm 2 \times 10^{-5}$	$0.00080 \pm 2 \times 10^{-6}$	$0.00138 \pm 4 \times 10^{-5}$

surfaces (Fig. 1) indicating that the microparticles were saturated by the drugs. Although the SEM images showed that the microparticles presented a hollow core, they were considered as solid spheres in the modeling, since the drugs were enclosed in the polymeric wall of microparticles forming a matrix-like structure. Since the microparticles have macropores (Fig. 1), D is an apparent diffusion coefficient. The apparent diffusion coefficient (D) of a molecule through a porous matrix can be defined as the ratio between the diffusion coefficient through a non-porous matrix ($D_{n\text{-}p}$) and a factor which comprises the porosity (ε) and the tortuosity (τ) of the matrix (Kim, 2000). Considering that the determination of $D_{n\text{-}p}$ is not experimentally feasible, the $DC_{s,m}$ values were used to compare the formulations. The $DC_{s,m}$ values were calculated from the angular coefficients of the curves using the Baker–Lonsdale equation (Fig. 5) varying from 5.65×10^{-16} to 2.23×10^{-15} $m^2\ s^{-1}$ (diclofenac) and from 1.52×10^{-16} to 1.05×10^{-15} $m^2\ s^{-1}$ (indomethacin) (Table 5). The $DC_{s,m}$ increased according to the augmentation of the PCL concentration for each series. The correlation coefficients after fitting data to the Baker–Lonsdale equation presented values higher than 0.9177 (Table 5) indicating that the modeling satisfactorily described the data. So, the main drug transport from microparticles was controlled by diffusion validating the hypothesis raised above.

Table 5
Parameters (a, b, r) obtained by fitting diclofenac and indomethacin release profiles from PHBHV and PHBHV/PCL microparticles to the Baker–Lonsdale equation

Sample	Baker–Lonsdale model parameters			
	a ($\times 10^{-4}$)	b ($\times 10^{-3}$)	r	$DC_{s,m}$ ($\times 10^{-16}$ $m^2\ s^{-1}$)
DIC-0	79.2 ± 11.5	13.8 ± 8.8	0.9656 ± 0.0072	5.63 ± 0.82
DIC-10	60.1 ± 6.2	4.8 ± 1.5	0.9890 ± 0.0062	4.64 ± 0.48
DIC-30	275.9 ± 32.0	19.3 ± 3.4	0.9374 ± 0.0171	14.1 ± 1.64
DIC-50	483.1 ± 143.8	14.9 ± 8.1	0.9817 ± 0.0136	22.3 ± 6.64
IND-0	9.2 ± 2.2	4.7 ± 1.1	0.9696 ± 0.0079	1.52 ± 0.36
IND-10	9.9 ± 2.0	9.9 ± 3.7	0.9177 ± 0.0610	1.66 ± 0.33
IND-30	79.5 ± 12.3	4.7 ± 1.9	0.9730 ± 0.0137	5.40 ± 0.83
IND-50	141.9 ± 17.0	7.6 ± 6.1	0.9863 ± 0.0037	10.50 ± 1.27

a: angular coefficient, b: linear coeficient, r: correlation coefficient.

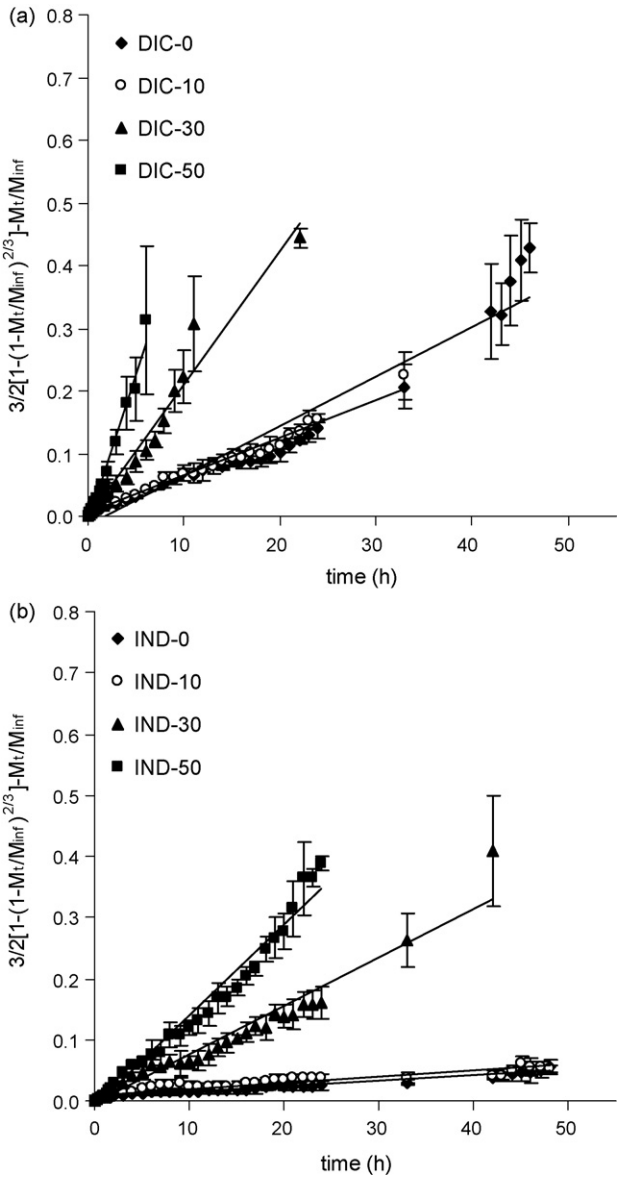

Fig. 5. Modeling of (a) diclofenac release profiles and (b) indomethacin release profiles to the Baker–Lonsdale equation.

4. Conclusions

The DSC analyses showed that the microparticle preparation process influenced the thermal behavior of PHBHV, as well as that indomethacin acted as an antiplasticizing agent for PHBHV. The release rates of diclofenac and indomethacin from microparticles increased according to the increase in the PCL concentration in the blends. Drug release profiles were adequately fitted to the Baker–Lonsdale equation, indicating a diffusion release mechanism. Finally, the drug release profiles were controlled by varying the PCL concentration in the blended PHBHV/PCL microparticles.

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