Controlled release system for ametryn using polymer microspheres: Preparation, characterization and release kinetics in water

Renato Grillo [a,b], Anderson do Espirito Santo Pereira [b,c], Nathalie Ferreira Silva de Melo [a,b], Raquel Martins Porto [c], Leandro Oliveira Feitosa [c], Paulo Sergio Tonello [a], Newton L. Dias Filho [d], André Henrique Rosa [a], Renata Lima [c], Leonardo Fernandes Fraceto [a,b,*]

[a] Department of Environmental Engineering, UNESP – Univ Estadual Paulista, Avenida Três de Março, n° 511, CEP 18087-180 Sorocaba, SP, Brazil
[b] Department of Biochemistry, Institute of Biology, UNICAMP, Cidade Universitária Zeferino Vaz, s/n, Campinas, SP, Brazil
[c] Department of Biotechnology, University of Sorocaba, Sorocaba, SP, Brazil
[d] Department of Physics and Chemistry, UNESP – Univ Estadual Paulista, Ilha Solteira, SP, Brazil

ABSTRACT

The purpose of this work was to develop a modified release system for the herbicide ametryn by encapsulating the active substance in biodegradable polymer microparticles produced using the polymers poly(hydroxybutyrate) (PHB) or poly(hydroxybutyrate-valerate) (PHBV), in order to both improve the herbicidal action and reduce environmental toxicity. PHB or PHBV microparticles containing ametryn were prepared and the efficiencies of herbicide association and loading were evaluated, presenting similar values of approximately 40%. The microparticles were characterized by scanning electron microscopy (SEM), which showed that the average sizes of the PHB and PHBV microparticles were $5.92 \pm 0.74\,\mu m$ and $5.63 \pm 0.68\,\mu m$, respectively. The ametryn release profile was modified when it was encapsulated in the microparticles, with slower and more sustained release compared to the release profile of pure ametryn. When ametryn was associated with the PHB and PHBV microparticles, the amount of herbicide released in the same period of time was significantly reduced, declining to 75% and 87%, respectively. For both types of microparticle (PHB and PHBV) the release of ametryn was by diffusion processes due to anomalous transport (governed by diffusion and relaxation of the polymer chains), which did not follow Fick's laws of diffusion. The results presented in this paper are promising, in view of the successful encapsulation of ametryn in PHB or PHBV polymer microparticles, and indications that this system may help reduce the impacts caused by the herbicide, making it an environmentally safer alternative.

© 2010 Elsevier B.V. Open access under the Elsevier OA license.

1. Introduction

Herbicides are molecules used worldwide for weed control. However, these compounds and their degradation products show varying degrees of persistence and mobility in the environment, and can have toxic, carcinogenic, mutagenic and teratogenic potentials, as well as effects on the endocrine systems of non-target organisms, including humans [1]. To mitigate the toxicity of these compounds in the environment, new and improved controlled release systems are emerging that aim to increase the effectiveness of herbicides while minimizing their environmental impacts and aiding sustainable development.

Controlled release systems have also been applied extensively in the food and pharmaceutical industries for the release of active substances such as nutrients, drugs and aromas [2–7].

In this context, polymer micro- and nanoparticles have emerged among the new technologies under study as potential alternatives for the development of release systems in agribusiness. Polymer micro- and nanostructured systems can act as transport media for bioactive substances, and are able to alter the physicochemical properties of the substances they incorporate. In the case of herbicides, these systems can offer the following advantages: (a) reduction of the amount of chemical substance required for weed control; (b) diminished risk of environmental contamination; (c) reduction of energy consumption, since fewer applications are needed compared to conventional formulations; and (d) increased safety of the individuals who apply the product in the field.

The literature offers a wide range of release systems applicable to the bioactive compounds of interest in agriculture [3,7–11]. These include materials composed of silica [12], clays such as bentonites [13] and sepiolite [14], polymers such as alginate [15] and

* Corresponding author at: Department of Environmental Engineering, UNESP – Univ Estadual Paulista, Avenida Três de Março, n° 511, CEP 18087-180 Sorocaba, SP, Brazil. Tel.: +55 15 3238 3415; fax: +55 15 3228 2842.
E-mail address: leonardo@sorocaba.unesp.br (L.F. Fraceto).

lignin [16], and synthetic polymers such as polyhydroxyalkanoates (PHAs) [17], including polyhydroxybutyrate (PHB) and its copolymer hydroxyvalerate (PHBV), which are the most widely used PHA polymers [18]. The advantage of using polymers such as PHB and PHBV is that they are completely biodegradable, inexpensive, easily produced by the fermentation of a variety of bacteria and degrade during natural biological processes, making them important for the production of release systems for bioactive materials [19,20]. These polymers are isostatic and highly crystalline (55–80%), which decreases the speed of degradation in comparison to lactic (PLA) and glycolic (PGA) homo- and copolymers [21].

Ametryn is an herbicide of the s-triazine family, whose chemical structure presents an aromatic hexamer ring and which is widely used in the pre- and post-emergence control of broadleaf weeds and annual grasses [22]. This type of compound acts by inhibiting photosynthesis, particularly at the photosystem II level, leading to the blockage of electronic transport. Plants that are sensitive to these herbicides undergo chlorosis (leaf yellowing) followed by tissue necrosis [23]. Prolonged exposure of humans to ametryn can cause intoxication and contact dermatitis.

The aim of this work was to develop (involving preparation, characterization and evaluation of the release profile) a release system for the herbicide ametryn, using microparticles produced with two different polymers, PHB and PHBV, since these polymers are biodegradable and their degradation products are nontoxic to the environment [24]. The purpose of encapsulating ametryn in PHB or PHBV microparticles was to produce a modified release system that could enable the herbicide to be used more safely in agriculture, minimizing its environmental impact. The importance of the present work lies in the fact that the new formulations are not only safer in environmental terms, but also safer for the individuals who apply the product in the field, since smaller quantities of herbicide can be used. This is due to the modified release process, which minimizes possible environmental contamination while at the same time improving herbicidal activity.

2. Experimental

2.1. Materials

Ametryn (PESTANAL®), poly(3-hydroxybutyrate) (PHB, MW = 312,000 g/mol), poly(3-hydroxybutyrate-co-hydroxyvalerate) (PHBV, MW = 238,000 g/mol) and polyvinyl alcohol (PVA) were purchased from Sigma Chemical Co. The solvents employed for the chromatographic analysis were acetonitrile, HPLC grade methanol (JT Baker) and Milli-Q water. The solutions were filtered through 0.22 μm nylon membranes (Millipore®, Belford, USA).

2.2. Methodology

2.2.1. Preparation of polymer microparticles containing ametryn

Microparticles consisting of the PHB or PHBV polymers were prepared by forming an oil-in-water emulsion using the emulsion/solvent evaporation method [25,26]. 100 mg of polymer (PHB or PHBV) and 10 mg of herbicide were dissolved in 10 mL of chloroform at 50 °C to produce the organic phase. The aqueous phase (200 mL) was prepared at 50 °C using 0.5% (m/v) of polyvinyl alcohol. The organic phase was then transferred to the aqueous phase (at 50 °C), under magnetic stirring (1000 rpm, 15 min) [26]. After this step, the chloroform was evaporated from the emulsion under reduced pressure, at 50 °C, using a rotary evaporation system. The resulting microparticle suspension, containing a final herbicide concentration of 50 mg/L, was stored in an amber jar to prevent photodegradation of the herbicide. Microparticles were prepared without ametryn for use as controls, using the same method described above.

2.2.2. Evaluation of encapsulation efficiency and herbicide loading

PHB or PHBV microparticles containing herbicide (10 mg) were dissolved in 50 mL of methanol, and the percentage of ametryn encapsulated in the microparticles was determined by the HPLC ultrafiltration/centrifugation method [27,28].

The microparticle samples containing ametryn were centrifuged in ultrafiltration filters composed of regenerated cellulose with 30 kDa molecular size-exclusion pores (Microcon, Millipore®), and the filtrate was analyzed using a high-performance liquid chromatograph (HPLC, Varian® ProStar) coupled to a pump (PS 210), UV–vis detector (PS 325), Metatherm® furnace and autoinjector (PS 410). The chromatograms were processed using Galaxy Workstation software. The concentration of ametryn was calculated using a calibration curve. Specificity was tested in the presence of the components of the colloidal suspension, and it was found that these factors did not affect the quantification of ametryn.

The association rate of ametryn was determined from the difference between the concentration of the herbicide measured in the filtrate and its total concentration (100%) in the microparticle suspension.

The chromatographic conditions employed for the quantification were: mobile phase composed of acetonitrile/water (70:30, v/v), at a flow rate of 1.4 mL min^{-1}, and a Phenomenex Gemini chromatographic column (C$_{18}$ reversed phase, 5 μ 110 A, 150 mm × 4.6 mm). Ametryn was detected at a wavelength of 260 nm, using an ultraviolet (UV) detector. The injection volume was 100 μL, and all samples injected were previously filtered through a 0.22 μM polyethersulfone membrane (Millipore®). Total ametryn (100%) in the microparticle suspension was determined after diluting the suspension in acetonitrile. Methanol was used to dissolve the polymer, completely releasing the ametryn, which was quantified using the calibration curve. Measurements were performed in triplicate for each batch.

The encapsulation efficiency (EE, %) was expressed as the ratio between the amount of herbicide encapsulated by the microparticles and the total (100%) amount of herbicide, as described by Eq. (1) [9].

$$EE \ (\%) = \frac{W_s}{W_{total}} \times 100\% \tag{1}$$

where W_s is the weight of ametryn in the microspheres, and W_{total} is the weight of ametryn used in the formulation.

The herbicide loading (HL, %) was determined by mixing 4 mg of dry microparticles with 4 mL of methanol, and agitating the suspension for 4 h, until the particles had fully degraded and the ametryn had been released [7,9] HL was then calculated as the ratio between the amount of herbicide encapsulated by the microparticles and the final weight of the microparticles (Eq. (2)).

$$HL \ (\%) = \frac{W_s}{W_{P+S}} \times 100\% \tag{2}$$

where W_s is the weight of ametryn quantified in the microparticles, and W_{P+S} is the final weight of the microparticles (polymer + ametryn).

2.2.3. Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM, JSM-6700F, JEOL, Japan) was employed to evaluate the size distribution and surface morphology of the PHB and PHBV microparticles, with and without herbicide. The suspensions were filtered to collect the microparticles (with or without ametryn), which were then washed in distilled water (150 mL). The solids were dried overnight with Na$_2$SO$_4$ in a

desiccator. The solid samples of microparticles were then attached to metal holders (stubs) using double-sided tape, and coated with a layer of gold for 150 s, using a current of 25 mA. After coating, the stubs with the samples were placed in the scanning electron microscope for analysis and imaging (electromicrography).

2.2.3.1. Size distribution. The SEM micrographs were used to determine the diameters and size distribution profiles of the PHB and PHBV microparticles, with and without herbicide. Microparticle size was measured using the ImageJ 1.42 software program, and the size distributions employed OriginPro 7.0 software. At least 1000 spheres of each sample were used to determine the size distribution.

2.2.4. Study of ametryn release from the microparticles

The profile of ametryn release from the PHB (or PHBV) microparticles was determined based on *in vitro* release assays, using a dual-compartment system to observe the release profiles of the free herbicide alone and of the herbicide encapsulated in the microparticles. In this system, which was maintained under slight shaking at ambient temperature, a cellulose membrane (Spectrapore, with molecular exclusion pore size of 1000 Da) was employed to separate the donor compartment (containing 4 mL of herbicide solution or microparticle suspension) from the acceptor compartment (containing 50 mL of deionized water) [29]. The pore size of the membrane in this system did not permit the passage of microparticles, while the free herbicide could pass unimpeded through the membrane.

The samples were collected in the acceptor compartment as a function of time, and analyzed by HPLC at a wavelength of 260 nm (at 15 min intervals during the first hour, 30 min intervals during the second hour, and at hourly intervals thereafter until the peak area stabilized). The area values were converted into the amount (%) of herbicide released as a function of time [30]. All the measurements were made in triplicate, and followed the dissolution sink condition [31].

2.2.4.1. Release efficiency (RE). The release efficiency (RE, %), first suggested by Khan and Rhodes [32], is a useful parameter that can provide information on the release kinetics. This term can be defined as the area under the release curve in a given time interval, and allows different formulations containing the same active substance to be compared. The release efficiency was calculated based on the values obtained from the area under the curve (AUC) of the herbicide release profile (for the free herbicide or the herbicide in the microparticle suspensions) at time intervals (t) ranging from zero to 4.3 days. This calculated value was then divided by the area above the theoretical curve of a rectangle, assuming a release of 100% between times zero and 4.3 days (AUC 100%) [32]. The RE was expressed in percentage terms, and can be defined by Equation 3.

$$\text{RE} \;\; (\%) = \frac{\text{AUC(zero} - 4.3)}{\text{AUC(100\%)}} \times 100\% \tag{3}$$

2.2.4.2. Mathematical modeling of ametryn release. The release profiles of bioactive compounds in microparticle polymer systems can be modeled mathematically in order to obtain information concerning the release mechanism. The model described by Peppas is able to predict release mechanisms based on processes governed by Fick's laws of diffusion, non-Fickian processes and Case II diffusion [33,34]. Using this model, it is possible to calculate the release constant (k) of the polymer-active compound system, and the diffusion exponent (n) characteristic of the release mechanism. A value of $n = 0.5$ is expected for Fickian diffusion, while values of $n = 1.0$ and $0.5 < n < 1.0$ are expected for Case II diffusion and non-Fickian diffusion, respectively [33–39]. The model proposed by Korsmeyer

and Peppas [40] was applied to the release curves in order to characterize the mechanism of release of the herbicide encapsulated in the PHB and PHBV microparticles. These curves can explain how the molecules are released from polymer matrix systems, enabling determination of the values of k, n and the linear correlation coefficient [35–39].

3. Results and discussion

3.1. Characterization of the microparticles containing ametryn herbicide

In earlier work, we used a 2^{4-1} fractional factorial design to identify the optimum conditions for encapsulation of ametryn (data not shown). The influence of four variables, at two levels, was examined in order to obtain formulations with optimized association efficiencies. It was observed that there was a greater dependence of association efficiency on PVA concentration (negative) and the mass of polymer (positive), with lesser influence of both stirring speed and organic phase volume. The present work was performed using the microparticle formulations containing ametryn that had been previously optimized using the experimental design procedure.

The PHB and PHBV polymer microparticles containing ametryn were prepared, and the efficiency of association of the herbicide was evaluated by the method proposed by Schaffazick [27]. The encapsulation efficiencies (EE, %) of the PHB:AMT and PHBV:AMT microparticles were 34.3% and 38.2%, respectively. The herbicide loadings were 13.2% and 11.1% for the PHB and PHBV microparticles, respectively. These values are similar to those reported in the literature for formulations involving the association of other compounds with microparticles prepared using the same polymers [7,9,41–43].

Bazzo et al. [41] studied the use of PHB microparticles containing chitosan with two bioactive compounds, and found similar encapsulation efficiency values. Sendil et al. [42] found association efficiency values of 30% for PHBV microparticles containing tetracycline, while Grillo et al. [43] showed that the encapsulation efficiency of the herbicide atrazine in PHBV microparticles was higher than 30%. The low efficiency of encapsulation of bioactive substances in some microparticles could be related to the use of high concentrations of emulsifiers, as is the case for the PHB and PHBV microparticles, where poly-vinyl alcohol (PVA) is used in the preparation procedure. The emulsifier increases the solubility of the herbicide in the aqueous phase, and therefore reduces the association of the compound [44–47]. This could be useful in agricultural practices, since a controlled release system in which only 30% of the herbicide is incorporated within a carrier enables the fraction of the active compound that is not associated with the microparticles to be immediately released at the application site, where it acts rapidly, while the remaining fraction is progressively released with time.

Scanning electron microscopy (SEM) was used to examine the influence of the encapsulation of ametryn on physical and morphological characteristics of the microparticles. Fig. 1 presents micrographs obtained for the PHB and PHBV microparticles containing ametryn.

Both PHB and PHBV microparticles were spherical, although their surface aspects differed. The PHB microparticles presented a smooth surface with a few surface pores, while the PHBV microparticles were rough with numerous surface pores. Morphological analysis of the surface of the microparticles is very important, since knowledge of the surface characteristics can help shed light on the mechanism of release of the herbicide associated with them. In other words, the larger the number of pores, the greater the probability of the solvent coming into contact with the interior
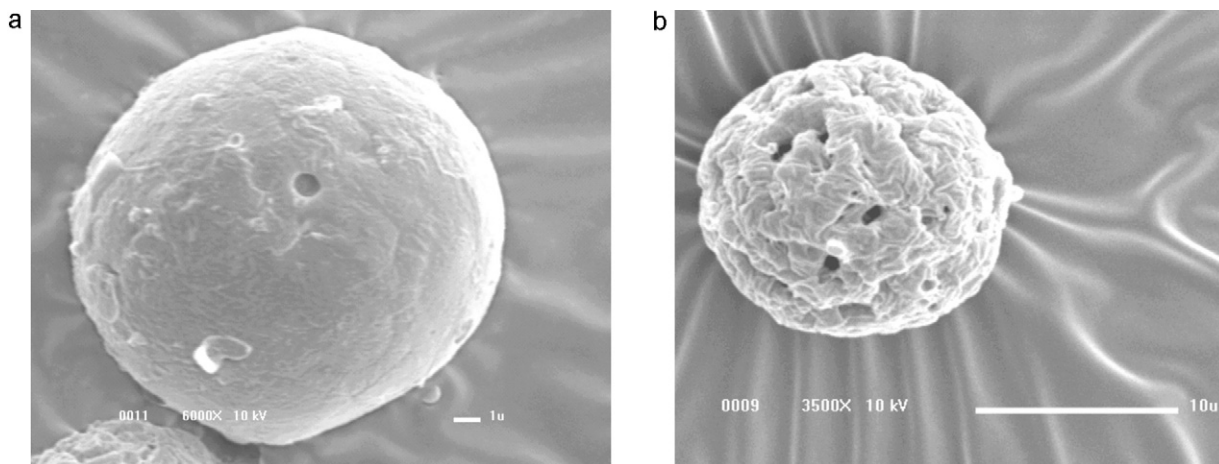
Fig. 1. Images of the polymer nanoparticles: (a) PHB, and (b) PHBV.

of the particle, aiding the release of the herbicide molecules [39]. Grillo et al. [43] showed that PHBV microparticles, prepared using the same methodology as that used in the present study, exhibited roughness and high porosity, and that these features were important for the release mechanism of the herbicide atrazine.

The SEM micrographs were analyzed to determine and compare the size distribution profiles of the different polymer microparticles. Fig. 2 shows representative images used for determining the size distribution, and graphs of the size distributions of the PHB and PHBV microparticles without herbicide.
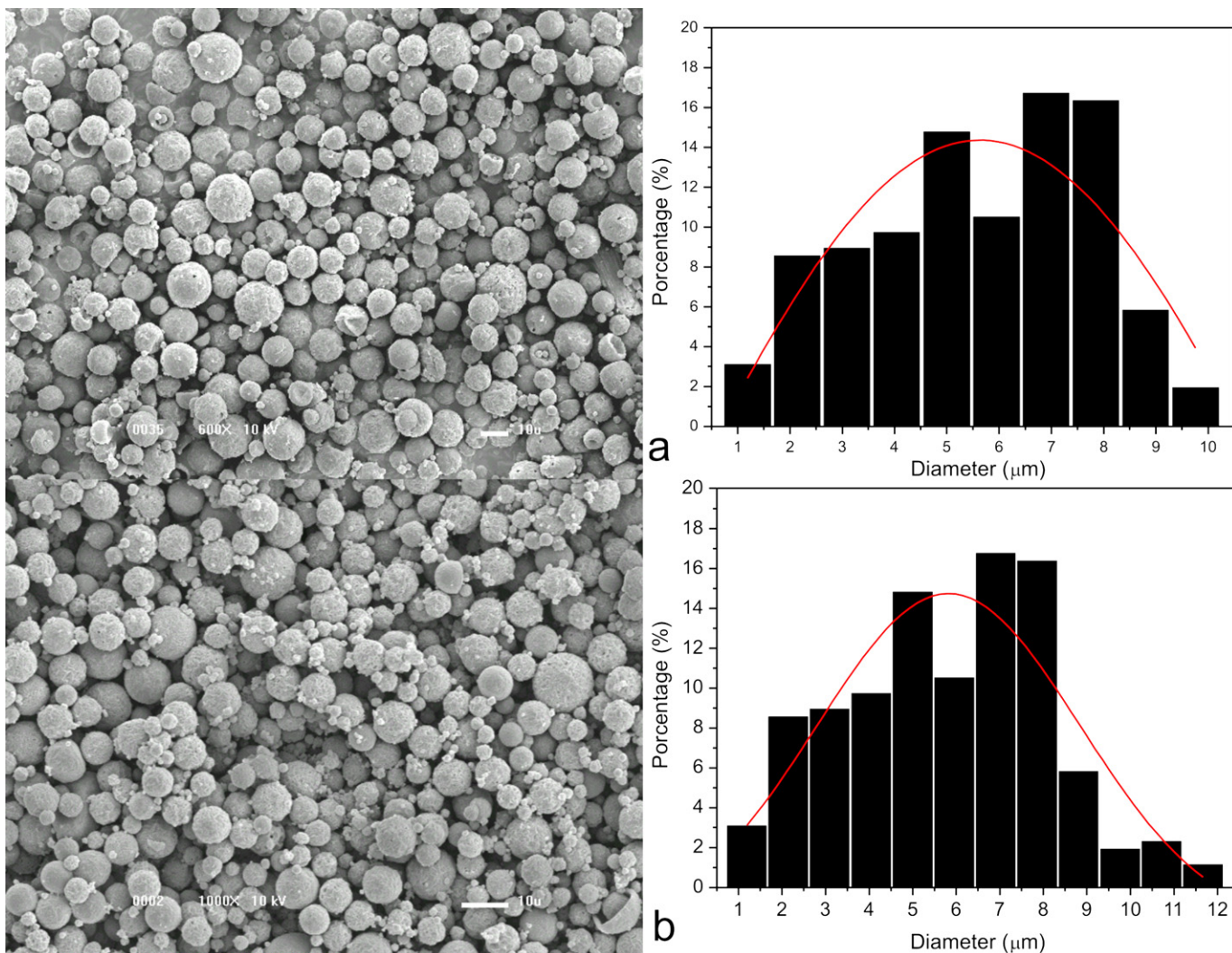

Fig. 2. SEM micrographs (10 kV, bar = 10 μm) and microparticle size distribution: (a) PHB, and (b) PHBV.
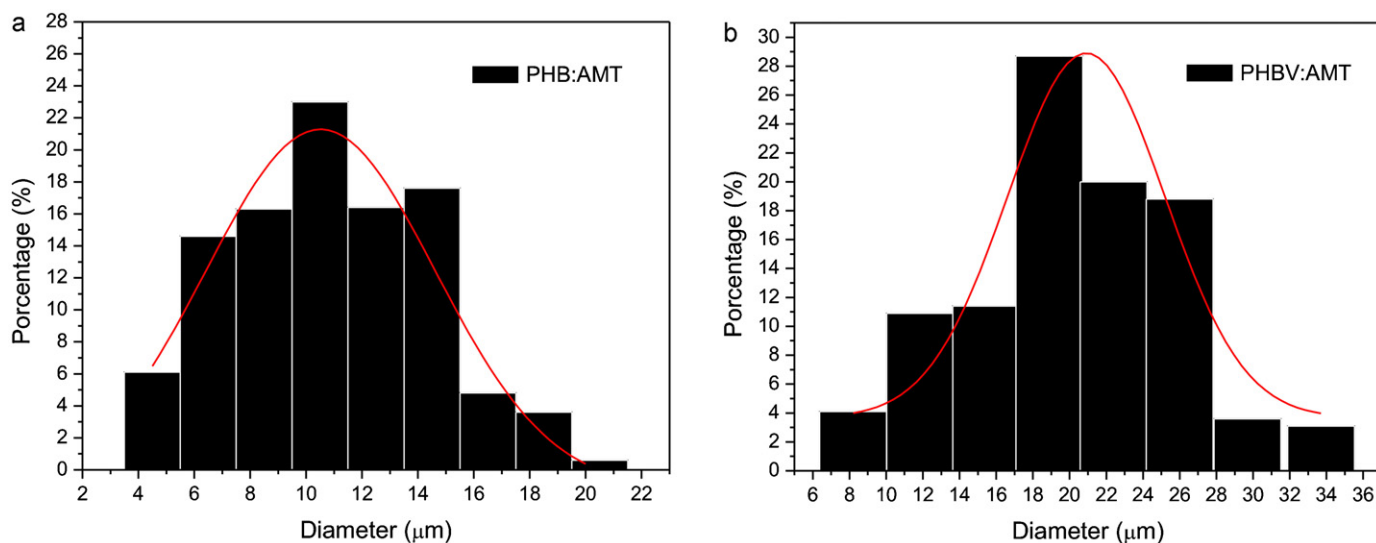
Fig. 3. Size distribution of PHB and PHBV microparticles containing ametryn: (a) PHB/AMT, (b) PHBV/AMT.

From Fig. 2, the PHB and PHBV microparticles (without herbicide) presented size distributions in the ranges 1–10 μm and 1–12 μm, respectively. The average sizes of the PHB and PHBV microparticles were 5.92 ± 0.74 μm and 5.63 ± 0.68 μm, respectively, indicating that there were no significant differences in size (using the unpaired t-test, $p < 0.05$). These data demonstrate that although the surfaces of the particles displayed distinct characteristics, they possessed similar size distributions.

The same size distribution analysis was performed for the PHB and PHBV microparticles containing ametryn (Fig. 3).

As can be seen in Fig. 3, both types of microparticle (PHB and PHBV) showed an increase in size, with distributions in the ranges 3–21 μm and 6–35 μm for the PHB:AMT and PHBV:AMT microparticles, respectively. Similar size ranges have been reported in the literature for microparticles produced with the same polymers but containing other bioactive substances [41,43]. The mean sizes of the PHB and PHBV microparticles containing ametryn were 10.6 ± 0.43 μm and 20.5 ± 0.93 μm, respectively. These differences in microparticle size were due to the encapsulation of the herbicide in the microparticles, as described in the literature for other molecules [41,43]. Suave et al. [48] investigated the size and morphology of microparticles of poly(3-hydroxybutyrate)/poly(e-caprolactone) containing malathion, and also observed alteration of the size of the microparticles when they were associated with the pesticide.

The surface morphology of the particles was not affected by the presence of ametryn, with the PHB microparticles remaining smooth and the PHBV microparticles remaining rough (data not shown).

3.2. Release kinetics of ametryn from microparticles

The release assays provided the release profiles of ametryn, either free or encapsulated in the PHB and PHBV microparticles, as a function of time (Fig. 4). In this assay, the herbicide passes through the pores of the membrane while the microparticles do not, hence allowing observation of the effect of the association of the herbicide on its speed of release from the polymer matrix of the microspheres. During the assay, aliquots were collected at preestablished times and the herbicide was quantified by HPLC. The results were expressed in terms of the percentage release.

Fig. 4 illustrates the release profiles of ametryn encapsulated in PHB and PHBV microparticles as a function of time (up to approx-

imately 4.5 days), at ambient temperature. It should be noted that in the tests using microparticles containing herbicide, a fraction of non-encapsulated herbicide was present in the donor compartment (only about 40% of the herbicide was incorporated into the particles). This means that the effect of the encapsulation of this herbicide in the microspheres was not as marked as that described by several previous authors [11,49,50], whose studies involved release tests using microparticles resuspended in an aqueous medium (possibly also containing some co-solubilizer). In our release tests, we aimed to evaluate the release profile of the colloidal suspension prepared without processing by filtration and drying of the spheres, which would undoubtedly have resulted in much longer release times comparable with the previous reports [11,49,50].

Analysis of the release kinetics curves indicated that free ametryn was released much more rapidly than when it was encapsulated in the microparticles, with nearly 100% release after 1.2 days. In contrast, when associated with the PHB and PHBV microparticles, the amount of herbicide released in the same period of time declined significantly, to 75% and 87%, respectively. This modification of the release behavior of pesticides after association with microstructured polymer systems has been widely reported for other bioactive compounds [11,42,43,48–51].
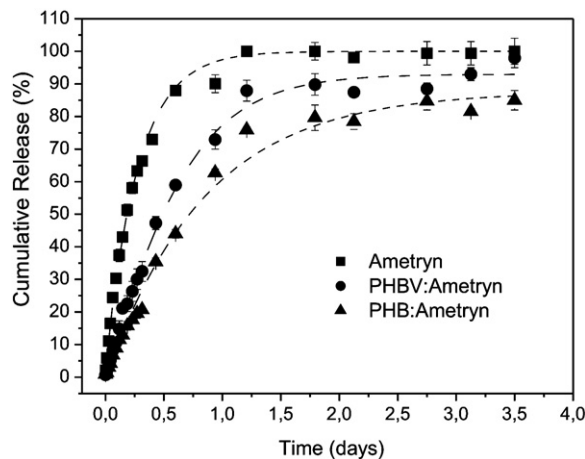

Fig. 4. Results of *in vitro* release assays, comparing the kinetic profiles of pure AMT and AMT encapsulated in PHB and PHBV microparticles at ambient temperature ($n = 3$).

Table 1
Release constants (k), correlation coefficient (r) and diffusion exponent (n) obtained by adjusting the curves of the release kinetics of the herbicides encapsulated in the polymers.

Parameters	PHB:AMT	PHBV:AMT
Release constant (k)	0.64 min^{-1}	1.00 min^{-1}
Diffusion exponent	$n = 0.83$	$n = 0.90$
Correlation coefficient	$r = 0.996$	$r = 0.997$

To better quantify the differences in the release profiles, the release efficiencies (RE %) were calculated from the areas under the herbicide release profile curves, for a given time interval (t), using the method described in the literature [32]. The RE values were 73.9% and 84.6% for the PHB and PHBV microparticles, respectively, indicating that ametryn was released faster from the PHBV microparticles.

The difference observed between the release profiles of encapsulated ametryn was unlikely to have been due to the encapsulation rates, which were similar for both polymers, but could be explained by the structural characteristics of the microparticles. The association of PHB with hydroxyvalerate (HV) causes a plastification effect in the microparticles, increasing the polymer's free volume and reducing its crystallinity. The advantage of polymer plastification is that it increases the speed of release of the associated compound, due to lower resistance to diffusion inside the microparticle. Similar studies conducted by Gangrade and Price [52] on progesterone encapsulation in PHB and PHBV (9–24% of HV) also concluded that the higher the HV content the lower the polymer's crystallinity, due to the break in interchain regularity, rendering the particle more porous and increasing the speed of release of the active principle. Moreover, PHB has a lower speed of degradation by *in vivo* hydrolysis than PLA and PHBV, which degrade more easily [19].

Another factor that could influence the release profile is the presence of crystals in solution, which might occur if the herbicide was not fully dissolved during the preparation of the microparticles, and which could influence the release kinetics. However, the presence of herbicide crystals was not detected in the micrographs.

The herbicide release profile curves were analyzed to obtain information on the possible mechanisms governing the release process [53]. The release of a compound associated with microparticles involves several mechanisms, including desorption from the surface of the polymer matrix, diffusion of the active principle through the pores of the polymer matrix or the polymer wall, disintegration of the microparticles and subsequent release of the active principle, and dissolution and erosion of the polymer matrix or wall [27,53].
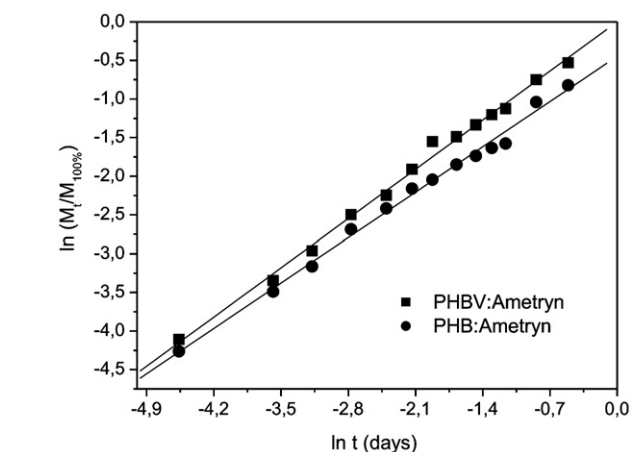
Several mathematical models are used extensively to analyze the characteristics of release of active substances from polymer systems [39]. The results obtained from the *in vitro* release assay (Fig. 4) were analyzed by the model described by Peppas [33–39]. Table 1 and Fig. 5 present the values obtained for k, n and the correlation coefficient, for ametryn release from the PHB and PHBV microparticles.

The Peppas model, which is based on a semi-empirical equation [40], is frequently employed in the absence of information about a system's release mechanism. In the present study, the values found for the release constant k were 0.64 min^{-1} and 1.00 min^{-1}, respectively, for the PHB:AMT and PHBV:AMT systems, indicating that ametryn was released more rapidly from the PHBV microparticles. Values of the release coefficient (n) were 0.83 and 0.90 for the PHB:AMT and PHBV:AMT systems, respectively. These data indicate that for both types of microparticle the ametryn release mechanism involved diffusion processes due to anomalous transport, not governed by Fick's laws of diffusion, but by diffusion and relaxation of the polymer chains.

4. Conclusions

In this study, the herbicide ametryn was incorporated into microparticles composed of two polymers, PHB and PHBV, with encapsulation efficiencies of ~40%. SEM analysis revealed that the microparticles were spherical but had different surface properties (smooth or rough with pores). The formulations of PHB and PHBV microparticles without herbicide showed similar size distributions. However, encapsulation of the herbicide in PHB and PHBV microparticles increased the size distributions of the microparticles of both polymers. The release profile of ametryn was modified by encapsulation in the microparticles, which provided a slower and more sustained release compared to the release kinetics of the free herbicide. This is a desirable feature in the use of herbicides, since it diminishes their impacts on ecosystems, human health and the environment. Mathematical modeling revealed that the ametryn release mechanism was the same for both types of polymer microparticle. The new carrier system has the potential to reduce harmful effects of the herbicide, as well as provide a safer handling system.

References

[1] E.D. Armas, R.T.R. Monteiro, P.M.M.A.P. Antunes, F. Santos, P.B. Camargo, Uso de agrotóxicos em cana-de açúcar na bacia do rio Corumbataí e o risco de poluição hídrica, Quim. Nova 30 (2007) 1119–1127.
[2] N.F. Melo, R. Grillo, A.H. Rosa, L.F. Fraceto, Study of the interaction between Hydroxymethylnitrofurazone and 2-Hydroxypropyl-beta-Cyclodextrin, J. Pharm. Biomed. Anal. 47 (2008) 865–869.
[3] R. Grillo, N.F.S. De Melo, D.R. De Araújo, E. De Paula, A.H. Rosa, L.F. Fraceto, Polymeric alginate nanoparticles containing the local anesthetic bupivacaine, J. Drug Target. 18 (2010) 688–699.
[4] R. Grillo, N.F.S. Melo, C.M. Moraes, R. Lima, C.M. Menezes, E.I. Ferreira, A.H. Rosa, L.F. Fraceto, Study of the interaction between hydroxymethylnitrofurazone and 2-hydroxypropyl-beta-cyclodextrin, J. Pharm. Biomed. Anal. 47 (2008) 295–302.
[5] C.M. Moraes, E. De Paula, A.H. Rosa, L.F. Fraceto, Physicochemical stability of poly(lactide-co-glycolide) nanocapsules containing the local anesthetic Bupivacaine, J. Braz. Chem. Soc. 21 (2010) 995–1000.
[6] R. Duncan, L.W. Seymour, Controlled Release Technologies: A Survey of Research and Commercial Applications, Elsevier Advanced Technology, Oxford, 1989.

Fig. 5. Results of the analyses of PHB and PHBV microparticles containing ametryn, using the mathematical model of Peppas.

[7] Z. El Bahri, J.L. Taverdet, Elaboration and characterisation of microparticles by pesticide model, Powder Technol. 172 (2007) 30–40.

[8] K. Hirech, S. Payan, G. Carnelle, L. Brujes, J. Legrand, Microencapsulation of an insecticide by interfacial polymerization, Powder Technol. 130 (2003) 324–330.

[9] Z. El Bahri, J.L. Taverdet, Optimization of an herbicide release from ethylcellulose microspheres, Polym. Bull. 54 (2005) 353–363.

[10] B. Singh, D.K. Sharma, A. Gupta, In vitro release dynamics of thiram fungicide from starch and poly(methacrylic acid)-based hydrogels, J. Hazard. Mater. 154 (2008) 278–286.

[11] B. Singh, D.K. Sharma, R. Kumar, A. Gupta, Development of a new controlled pesticide delivery system based on neem leaf powder, J. Hazard. Mater. 177 (2010) 290–299.

[12] T.K. Barik, B. Sahu, V. Swain, Nanosilica—from medicine to pest control, Parasitol. Res. 103 (2008) 253–258.

[13] M. Fernández-Pérez, F. Flores-Céspedes, E. González-Pradas, M. Villafranca-Sánchez, S. Pérez-García, F.J. Garrido-Herrera, Use of activated bentonites in controlled-release formulations of atrazine, J. Agric. Food Chem. 52 (2004) 3888–3893.

[14] C. Maqueda, J. Villaverde, F. Sopena, T. Undabeytia, E. Morillo, Novel system for reducing leaching of the herbicide metribuzin using clay-gel-based formulations, J. Agric. Food Chem. 56 (2008) 11941–11946.

[15] M. Fernádez-Pérez, Controlled release systems to prevent the agro-environmental pollution derived from pesticide use, J. Environ. Sci. Health B 42 (2007) 857–862.

[16] F.J. Garrido-Herrera, I. Daza-Fernández, E. González-Pradas, M. Fernández-Pérez, Lignin-based formulations to prevent pesticides pollution, J. Hazard. Mater. 168 (2009) 220–225.

[17] H. Salehizadeh, M.C.M. Van Loosdrecht, Production of polyhydroxyalkanoates by mixed culture: recent trends and biotechnological importance, Biotechnol. Adv. 22 (2004) 261–279.

[18] W. Amass, B. Tighe, A review of biodegradable polymers: use, current developments in the synthesis and characterization of biodegradable polyesters, blends of biodegradable polymers and recent advances in biodegradable studies, Polym. Int. 47 (1998) 89–144.

[19] C.W. Pouton, S. Akhtarb, Biosynthetic polyhydroxyalkanoates and their potential in drug delivery, Adv. Drug Delivery Rev. 18 (1996) 133–162.

[20] K.C. Reis, J. Pereira, A.C. Smith, C.W.P. Carvalho, N. Wellner, I. Yakimets, Characterization of polyhydroxybutyrate-hydroxyvalerate (PHB-HV)/maize starch blend films, J. Food Eng. 89 (2008) 361–369.

[21] K. Sudesh, T. Fukui, T. Iwata, Y. Doi, Factors affecting the freeze-fracture morphology of in vivo polyhydroxyalkanoate granules, Can. J. Microbiol. 46 (2000) 304–311.

[22] A.H. Tennant, P. Peng, A.D. Kligerman, Genotoxicity studies of three triazine herbicides: in vivo studies using the alkaline single cell gel (SCG) assay, Mutat. Res. 493 (2001) 1–10.

[23] L. Prade, R. Huber, B. Bieseler, Structures of herbicides in complex with their detoxifying enzyme glutathione S-transferase—explanations for the selectivity of the enzyme in plants, Structure 11 (1998) 1445–1452.

[24] O.N. Voïnova, G.S. Kalacheva, I.D. Grodnitskaia, T.G. Volova, Microbial polymers as a degradable carrier for pesticide delivery, Prikl. Biokhim. Mikrobiol. 45 (2009) 427–431.

[25] B. Conti, I. Genta, T. Modena, F. Pavanetto, Investigation on process parameters involved in polylactide-co-glycolide microspheres preparation, Drug Dev. Ind. Pharm. 21 (1995) 615–622.

[26] M.I.Z. Lionzo, M.I. Ré, S.S. Guterres, A.R. Pohlmann, Microparticles prepared with poly(hydroxybutyrate-co-hydroxyvalerate) and poly((-caprolactone) blends to control the release of a drug model, J. Microencapsul. 24 (2007) 175–186.

[27] S.R. Schaffazick, S.S. Guterres, L.L. Freitas, A.R. Pohlmann, Physicochemical characterization and stability of the polymeric nanoparticle systems for drug administration, Quim. Nova 5 (2003) 726–737.

[28] A.C. Kilic, Y. Capan, I. Vural, R.N. Gursoy, T. Dalkara, A. Cuine, A.A. Hincal, Preparation and characterization of PLGA nanospheres for the targeted delivery of NR2B-specific antisense oligonucleotides to the NMDA receptors in the brain, J. Microencapsul. 22 (2005) 633–641.

[29] A. Paavola, J. Yliruusi, Y. Kajimoto, E. Kalso, T. Wahlström, P. Rosenberg, Controlled release of lidocaine from injectable gels and efficacy in rat sciatic nerve block, Pharm. Res. 12 (1995) 1997–2002.

[30] D.R. De Araújo, C.M.S. Cereda, G.B. Brunetto, L.M.A. Pinto, M.H.A. Santana, E. De Paula, Encapsulation of mepivacaine prolongs the analgesia provided by sciatic nerve blockade in mice, Can. J. Anesth. 51 (2004) 566–572.

[31] M. Aulton, Dissolution and Solubility, Pharmaceutics: The Science of Dosage from Design, 2nd ed., Churchill Livingstone, Edinburgh, 2002, 15–32 (Chapter 2).

[32] K.A. Khan, C.T. Rhodes, The concept of dissolution efficiency, J. Pharm. Pharmacol. 27 (1975) 48–49.

[33] P.L. Ritger, N.A. Peppas, A simple equation for description of solute release. II. Fickian and anomalous release from swellable devices, J. Control. Release 5 (1987) 37–42.

[34] P.L. Ritger, N.A. Peppas, A simple equation for description of solute release I. Fickian and non-fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs, J. Control. Release 5 (1987) 23–36.

[35] D. Hariharam, N.A. Peppas, R. Bettini, P. Colombo, Mathematical analysis of drug delivery from swellable systems with partial physical restrictions or impermeable coatings, Int. J. Pharm. 112 (1994) 47–54.

[36] P. Colombo, R. Bettini, G. Massimo, P.L. Catellani, P. Santi, N.A. Peppas, Drug diffusion front movement is important in drug release control from swellable matrix tablets, J. Pharm. Sci. 84 (1995) 991–997.

[37] G. Colombo, R. Padera, R. Langer, D.S. Kohane, Prolonged duration anesthesia with lipid-protein-sugar particles containing bupivacaine and dexamethasone, J. Biomed. Mater. Res. A 75 (2005) 458–464.

[38] C. Ferrero, A. Muñoz-Ruiz, M.R. Jiménez Castellanos, Fronts movement as a useful tool for hydrophilic matrix release mechanism elucidation, Int. J. Pharm. 202 (2000) 21–28.

[39] C. Costa, J.M.S. Lobo, Modeling and comparison of dissolution profiles, Eur. J. Pharm. Sci. 13 (2001) 123–133.

[40] R.W. Korsmeyer, N.A. Peppas, Macromolecular and modeling aspects of swelling-controlled systems, in: T.J. Roseman, S.Z. Mansdorf (Eds.), Controlled Release Delivery Systems, Marcel Dekker Inc., New York, USA, 1991.

[41] G.C. Bazzo, E. Lemos-Senna, A.T.N. Pires, Poly(3-hydroxybutyrate)/chitosan/ketoprofen or piroxicam composite microparticles: preparation and controlled drug release evaluation, Carbohydr. Polym. 77 (2009) 839–844.

[42] D. Sendil, I. Gursel, D.L. Wise, V. Hasircia, Antibiotic release from biodegradable PHBV microparticles, J. Control. Release 59 (1999) 207–217.

[43] R. Grillo, N.F. Melo, R. Lima, R. Lourenço, A.H. Rosa, L.F. Fraceto, Characterization of atrazine-loaded biodegradable poly(hydroxybutyrate-co-hydroxyvalerate) microspheres, J. Polym. Environ. 18 (2010) 26–32.

[44] M. Cea, P. Cartes, G. Palma, M.L. Mora, Atrazine efficiency in an andisol as affected by clays and nanoclays in ethylcellulose controlled release formulations, R. C. Suelo Nutr. Veg. 10 (2010) 62–77.

[45] F. Sopeña, A. Cabrera, C. Maqueda, E. Morillo, Ethylcellulose formulations for controlled release of the herbicide alachlor in a sandy soil, J. Agric. Food Chem. 55 (2007) 8200–8205.

[46] F. Sopeña, C. Maqueda, E. Morillo, Norflurazon mobility, dissipation, activity, and persistence in a sandy soil as influenced by formulation, J. Agric. Food Chem. 55 (2007) 3561–3567.

[47] R. Fernandez-Urrusuño, J.M. Gines, E. Morillo, Development of controlled release formulations of alachlor in ethylcellulose, J. Microencapsul. 17 (2000) 331–342.

[48] J. Suave, E.C. Dall'Agnol, A.P.T. Pezzin, M.M. Meier, D.A.K. Silva, Biodegradable microspheres of poly(3-hydroxybutyrate)/poly(e-caprolactone) loaded with Malathion pesticide: preparation, characterization, and in vitro controlled release testing, J. Appl. Polym. Sci. 117 (2010) 3419–3427.

[49] C. Maqueda, J. Villaverde, F. Sopeña, T. Undabevtia, E. Morillo, Effects of soil characteristics on metribuzin dissipation using clay-gel-based formulations, J. Agric. Food Chem. 57 (2009) 3273–3278.

[50] X. Liu, W.S. Heng, L.Q. Paul, L.W. Chan, Novel polymeric microspheres containing norcantharidin for chemoembolization, J Control. Release 116 (2006) 35–41.

[51] Y. Wang, X. Wang, K. Wei, N. Zhao, S. Zhang, J. Chen, Fabrication, characterization and long-term in vitro release of hydrophilic drug using PHBV/HA composite microspheres, J. Mater. Lett. 61 (2007) 1071–1076.

[52] N. Gangrade, J.C. Price, Poly(hydroxybutyrate-hydroxyvalerate) microspheres containing progesterone: preparation, morphology and release properties, J. Microencapsul. 8 (1991) 185–202.

[53] M. Polakovic, T. Gorner, R. Gref, E. Dellacherie, Lidocaine loaded biodegradable nanospheres II. Modelling of drug release, J. Control. Release 60 (1999) 169–177.