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ORIGINAL PAPER

Poly(hydroxybutyrate-co-hydroxyvalerate) microspheres loaded with atrazine herbicide: screening of conditions for preparation, physico-chemical characterization, and in vitro release studies

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Abstract Due to their widespread use in agriculture as well as in urban areas, agricultural chemicals are globally some of the most commonly encountered substances in waters. The objective of this study is to develop (including preparation and characterization) a new modified release system for the herbicide atrazine, employing poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) microspheres. The microspheres were prepared by the emulsification/solvent evaporation method, emulsifying an organic phase (atrazine and PHBV dissolved in chloroform) into an aqueous phase containing polyvinyl alcohol (PVA) as surfactant, under stirring, and then evaporating the solvent. A 2^{4-1} fractional factorial design, investigating the influence of four variables at two levels, was performed to obtain formulations with optimized association efficiencies. 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 size of the

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particles was assessed using scanning electron microscopy, which showed that the particles were rough-surfaced spheres. The results obtained are promising, since the formulations presented encapsulation efficiency near 25% and the release kinetics profile of atrazine was altered when it was encapsulated in the microparticles, indicating that these systems may be efficient in reducing the environmental impact caused by the herbicide, hence making it safer to use.

Keywords Microparticles · Atrazine · PHBV · Controlled release · Emulsification-Solvent evaporation · Microencapsulation

Introduction

Herbicides are chemical substances used in agriculture to control annual and perennial weeds, which compete for light, nutrients, and water, and therefore adversely affect the growth of crops and consequently productivity. Atrazine is a selective herbicide used to control pre- and post-emergence of weeds in various agricultural crops. It belongs to the triazine class of herbicides, whose action is based on inhibition of photosynthetic system II, with reduction of the flow of electrons that normally occurs from the water molecule to the $NADPH_2$ coenzyme during the photochemical step of photosynthesis. This causes an accumulation of electrons in the chlorophyll molecules, and therefore an increase of rates of oxidation reactions, above levels tolerable to the plant cells, resulting in death of the cells and therefore of the plant [1–3].

Many of the herbicides currently used, including atrazine, present problems related to their chemical stability, such as solubility, bioavailability, photodegradation, and soil absorption. Leaching of the chemicals can contaminate surface or subterranean waters, influencing water quality with consequences for society, biota and the wider environment [4, 5]. It is therefore important to develop systems that alter the physico-chemical properties of herbicides, modifying their release mechanisms, and consequently agricultural management practices, so that food production can meet current levels of demand while at the same time deleterious impacts are minimized.

Various herbicide and pesticide formulations are available on the market, e.g., granules, concentrates for preparing solutions, emulsions or suspension, wettable powders, microemulsions, water-dispersible granules, and seed treatments [6]. Currently, there is an increasing trend in agriculture toward use of modified release systems, which are intended to improve efficiency, reduce costs of application and minimize environmental impacts. The literature offers innumerable strategies for release systems of bioactive compounds of interest in agriculture [7–11]. These systems include those composed of silica [12], clays such as bentonites [13] and sepiolite [14], polymers such as alginate [15], lignin [16], and micro- and nanoparticles [17, 18]. Micro- and nanostructured systems can act as transport media for active substances, and offer advantages including improved physical, chemical, and biological stabilities, simple and reproducible preparation, and applicability to a wide range of chemicals [19–21].

Several materials are being investigated to promote the controlled release of bioactive chemicals. These include dendrimers [22], organic and inorganic microspheres [23], cyclodextrins [24], and natural biodegradable polymers [25, 26].

A class of polymers and copolymers that has received significant attention includes poly(lactic acid) (PLA) and its copolymer poly(lactic acid-*co*-glycolic acid) (PLGA), due to their excellent biocompatibility and biodegradability properties [27]. More recently, polyhydroxyalkanoates (PHAs), a class of biodegradable and biocompatible polyesters of biological origin, have attracted attention as biomaterials with considerable potential in biomedical and pharmaceutical applications [28]. The most common examples of these types of biomaterials are those containing monomeric hydroxyalkanoic acid units, such as poly(3-hydroxybutyric acid), PHB, and a related copolymer, poly(3-hydroxybutyrate-co-3-hydroxyvalerate), PHBV. These polymers are not produced by chemical synthesis; they are produced biosynthetically by bacteria from natural raw materials, and indeed can be readily broken down by microorganisms under different conditions, such as those present in sewage. A significant advantage of these compounds, therefore, is that they are both biocompatible and biodegradable [29, 30]. Preparation of micro/nanoparticles using these polymers can be achieved by various means, and in the case of water-insoluble substances the "salting-out" technique is useful. However, many factors can influence this type of system, including the concentration of the polymer, aqueous phase volume, active principle amount, agitation time and speed, agitation type, and organic phase volume. Statistical techniques, such as those employing factorial design, can be a valuable means of better understanding the influence of these variables on the system as a whole, as well as the interactions between the variables [31, 32]. The objective of this study is to develop a modified release system for the herbicide atrazine, using poly(hydroxybutyrate-*co*-hydroxyvalerate) (PHBV) microspheres.

Experimental

Materials

Polyvinyl alcohol (PVA), poly(hydroxybutyrate-*co*-hydroxyvalerate) and atrazine (purity $> 98\%$) were supplied by Sigma-Aldrich. Chloroform was obtained from Merck® (Brazil) and methanol from Tedia® (Brazil). All aqueous solutions were prepared using deionized water (Milli-Q system, Millipore, Belford, USA). All remaining analytical grade reagents were obtained from local suppliers. The solutions were filtered using Millipore 0.22-μm nylon membranes.

Experimental design

Since the preparation of microparticles involves consideration of the various factors that influence their ultimate characteristics, there has been an increasing use of statistical tools to better understand the response of the system, including screening of conditions by means of experimental design procedures [31]. Full or partial

Table 1 2^{4-1} fractional factorial design matrix applied to PHBV polymeric microspheres

Formulation	PHBV (mg)	[PVA] (% m/v)	AS (rpm)	OPV (mL)
1	+1	−1	+1	−1
2	+1	−1	−1	+1
3	−1	+1	−1	+1
4	+1	+1	+1	+1
5	−1	−1	+1	+1
6	−1	+1	+1	−1
7	+1	+1	−1	−1
8	−1	−1	−1	−1

Table 2 Levels of the factors used in the 2^{4-1} fractional factorial design matrix applied to PHBV polymeric microspheres

Factor	Level	
	Low (−1)	High (+1)
Polymer mass (mg)—PHBV	100	200
PVA concentration (% m/v)—[PVA]	0.25	0.5
Agitation speed (rpm)—AS	1000	2000
Organic phase volume (mL)—OPV	10	15

factorial designs are often used to define those variables that are most important in influencing the response of the system. Full factorial design is valuable for preliminary studies and the initial stages of optimization, while fractional models are used when the optimization involves a large number of factors, with a number of variables greater than or equal to 4. In this study, a 2^{4-1} fractional factorial design (Table 1) was used to assess the influences of four variables, at two levels. The independent variables tested were PHBV polymer mass, PVA concentration, agitation speed (AS), and volume of the organic phase (OPV) (Table 2), with the values used being based on a previous study [17]. The dependent variables assessed were encapsulation efficiency, the time required for 50% release of atrazine (t_{50}) and the microsphere size distribution. The formulations corresponding to each test were prepared in duplicate to estimate the experimental error.

Statistical analysis of the effects and interactions of the different parameters was performed using StatGraphics Plus 5.1 software, with significance levels of 95% ($p < 0.05$). From this analysis empirical linear mathematical models were obtained, containing terms for the experimental variables as well as interactions between the variables.

PHBV microsphere preparation

Atrazine-containing PHBV microspheres were prepared by a modified version of the method proposed by Coimbra et al. [33], using the traditional oil-in-water (O/W) emulsion solvent evaporation technique. Firstly, 10 mg of atrazine were dissolved in a solution of PHBV in chloroform, containing 100 or 200 mg of polymer, according to the experimental design. The organic dispersion was then dropped

slowly into a mechanically stirred PVA solution, to form the O/W emulsion, over 30 min with continuous stirring. After this period, the mixture was maintained agitated under a fume extraction hood, until the organic solvent had evaporated. The volume of the final dispersion (200 mL) was adjusted to achieve a final atrazine concentration of 0.05 mg/mL. The suspensions were centrifuged, and washed three times with deionized water to remove the surfactant and the unassociated herbicide. The microparticles were dried in a desiccator, and subsequently used in tests of association efficiency and for characterization using scanning electron microscopy. Another portion of each formulation was stored for future use in release tests. The proportions of ingredients used in each formulation were established according to the experimental design described in "Experimental design".

Encapsulation efficiency

The herbicide content of the microspheres was determined by UV spectrophotometry. A weighed amount (10 mg) of atrazine-loaded PHBV microspheres (dried in a desiccator) was dissolved in 1 mL of chloroform, followed by the addition of 9 mL of methanol to precipitate the polymer. The resulting suspension was centrifuged, and the supernatant filtered and analyzed using a UV spectrophotometer (Varian, model V-550), at a wavelength of 262 nm, to evaluate the amount of atrazine incorporated in the microparticles. A calibration curve was generated, at the same wavelength, using standard solutions of atrazine in chloroform/methanol (1:9) mixtures. Measurements were performed in triplicate for each batch. The encapsulation efficiency (EE, %) was expressed as the ratio between the experimentally measured herbicide and the theoretical herbicide loading, as described by Eq. 1.

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

where W_s is the amount of atrazine quantified and W_{total} is the theoretical amount of atrazine (weight/amount of atrazine and polymer).

Physicochemical characterization

Scanning electron microscopy

The shape and surface morphology of the microspheres were analyzed by scanning electron microscopy (SEM). Previously dried microspheres were placed on double-sided tape attached to a metal surface, and coated with copper on a sputter coater for 150 s using a current of 25 mA. Observations were performed at 10 kV, using a JEOL (Japan) model JSM-6700F microscope.

Size distribution

The size distribution of the particles was determined from the SEM images, using 100 individual size values (assessed using a circle equivalent diameter) to calculate

the mean particle size in each formulation. The microparticle sizes were measured using the ImageJ 1.42 software program, and size distributions were determined using OriginPro 7.0 software.

Release of atrazine from the microspheres

The in vitro atrazine release tests were performed using the formulations that showed the best association efficiencies. The profile of atrazine release from the PHBV microparticles was determined based on assays using a dual-compartment system to observe the release profile of the free herbicide alone and of the herbicide encapsulated in the microparticles. In this system, a cellulose membrane (Spectrapore, with molecular pore-exclusion size of 1000 Da.) was employed, which separated the donor compartment (containing 2 mL of solution of herbicide in water or in suspensions with PHBV microparticles) from the acceptor compartment containing 80 mL of deionized water (at pH 7.4, adjusted with NaOH or HCl) [34]. The system was maintained under slight agitation, at ambient temperature. The pore size of the membrane in this system does not permit the passage of microparticles, only the free herbicide. Aliquots were withdrawn from the receptor compartment at regular intervals (0, 15, 30, 45, 60, 90, 120, 150, 180, 300, and 360 min), and then filtered and analyzed by UV spectrophotometry. Deionized water at pH 7.4 (adjusted with NaOH or HCl) was then supplied to maintain the acceptor compartment at a constant volume (80 mL). The experiments were carried out in triplicate of three batches ($n = 9$). All the measurements were made in triplicate, and followed the dissolution sink condition [35]. The sink condition was maintained throughout the experiment, since 20% of the acceptor volume was sufficient to solubilize all the herbicide during the entire period.

Mathematical modeling

Mathematical modeling of the release profiles of bioactive compounds in polymeric systems was employed to obtain information on the mechanism of release of atrazine from the microparticles. Only the microparticles that showed good EE values were used to study the mechanism of release. Zero order, first order, Higuchi, Korsemeyer-Peppas, and Weibull theoretical models were applied to the release curves to obtain information on the nature of the release mechanism [36–39].

Results and discussion

Characterization and optimization of the PHBV microspheres

Factorial design is increasingly used in research planning, because it provides the maximum amount of information and requires the least number of experiments. In the present study, eight formulations were used, for which the dependent variable values are provided in Table 3.

Table 3 2^{4-1} factorial design matrix results: encapsulation efficiency (%) and particle size distribution of the PHBV polymeric microspheres

Formulation	Encapsulation efficiency (%)[a]	Mean size (μm)[b]
1	5.3 ± 5.8	12.4 ± 0.9
2	3.3 ± 1.4	13.3 ± 1.1
3	14.5 ± 10.8	6.6 ± 0.3
4	5.8 ± 2.6	5.1 ± 0.4
5	3.3 ± 2.8	4.5 ± 0.3
6	9.0 ± 12.1	6.5 ± 0.6
7	24.7 ± 2.1	24.0 ± 0.1
8	12.5 ± 7.8	5.1 ± 0.9

[a] $n = 6$ (two batches in triplicate)

[b] $n = 3$ (mean size of 100 particles per micrograph)

Statgraphics 5.1 software was the tool employed to design and analyze the response variables, and to generate the polynomial models. Model simplification was carried out by eliminating insignificant parameters in the polynomial equations resulting from multiple regression analysis. The models obtained to describe the dependent variables were selected taking into account their statistical significance ($p < 0.05$). In addition, graphical representations such as Pareto and principal effect charts are often used to aid in data sorting, and were employed here to assess the results obtained for the dependent variables analyzed [40].

Encapsulation efficiency

Here, we used the Statgraphics 5.1 software package to evaluate the effects of independent variables ([PHBV] [PVA], AS, OPV) and the influence of the combination of two or more independent variables (e.g. [PHBV] × AS). An empirical linear model was constructed from the results of the statistical analysis and the data obtained from the experimental design (statistically significant values). Simplification of the model was achieved by eliminating insignificant parameters (those with higher p values) from the polynomial equations resulting from multiple regression analysis.

Eight formulations were prepared, as described in Table 1, and the encapsulation efficiency results were analyzed using the Statgraphics 5.1 software. The values of the effects and interactions, together with standard deviations and statistical significances, are presented in Table 4.

The EE values for atrazine in the PHBV microspheres (Table 3) ranged between 3.3% (formulations 2 and 5) and 24.7% (formulation 7). Previous studies involving other bioactive compounds have found EE values close to those obtained in this study [41–43]. In this study, PHBV was dissolved in chloroform (a solvent compatible with the polymer), together with atrazine (which has a low water solubility of 70 ppm), with subsequent emulsification using an aqueous phase containing polyvinyl alcohol surfactant. Grillo et al. [17] showed that the interaction between atrazine and PHBV is governed by van der Waals type interactions (hydrophobic contacts), or even hydrogen bonding between the amino groups of the

Table 4 Standardized effects of factors on the encapsulation efficiency of atrazine in PHBV microspheres

	Effect	Standard deviation (±)	*p* value
Average	15.705	2.08	
A: [PHBV]	15.675	4.17	0.0071*
B: [PVA]	8.315	4.17	0.0864
C: AS	4.475	4.17	0.3189
D: OPV	−18.480	4.17	0.0030*
AC	14.505	4.17	0.0103*
AD	−19.365	4.17	0.0024*
CD	8.145	4.17	0.0918

* Significant at 95% confidence level ($p < 0.05$)

atrazine molecule and carbonyls of the PHBV, and the encapsulation efficiency was around 30%. Encapsulation efficiencies lower than 40% have been obtained in other studies reported in the literature [44, 45], which employed different active compounds but the same microencapsulation methodology.

An empirical linear model (Eq. 2) was constructed from the results of the statistical analysis and the data obtained from the experimental design (Table 4). The statistically significant values obtained were fitted by a linear model using the least squares method ($r = 0.9161$).

$$\begin{aligned} \mathrm{EE} = {} & 15.70 - 18.4\ \mathrm{OPV} + 15.67[\mathrm{PHBV}] + 14.50\,[\mathrm{PHBV}] \times \mathrm{AS} \\ & - 19.36[\mathrm{PHBV}] \times \mathrm{OPV} \end{aligned} \tag{2}$$

The regression coefficients obtained showed that EE was significantly and differently influenced by the three factors investigated. From Eq. 2, it can be seen that the organic phase volume (OPV) exerted the greatest (negative) influence on EE, with smaller volumes increasing EE. The water solubility of atrazine is low (70 ppm at 25 °C) compared to its solubility in organic solvents such as chloroform (52000 ppm at 25 °C) and methanol (18000 ppm at 25 °C) [45]. It has previously been shown that increasing the organic phase volume increases the solubility of the compound in this medium, hence reducing its affinity for the polymer chains [41]. In this case, increase of the organic phase volume caused greater dissolution of atrazine in this phase, with a reduction of the amount in the polymeric matrix, so that this variable interfered negatively with the association rate.

The second most important factor was the amount of PHBV, with greater quantities of PHBV increasing EE, in agreement with the literature [37, 46–48]. This can be explained by increased viscosity of the organic phase at higher polymer concentrations, which causes a decrease in diffusion of molecules from the organic phase to the aqueous phase, thus allowing greater association with the microspheres. Several studies have shown that increasing the amount of polymer in formulations increases the EE of the active compounds [29, 46–48], while others have found no significant influence [49]. In addition, no influences of the amount of PVA or agitation speed were observed, so that these parameters were therefore deemed to be

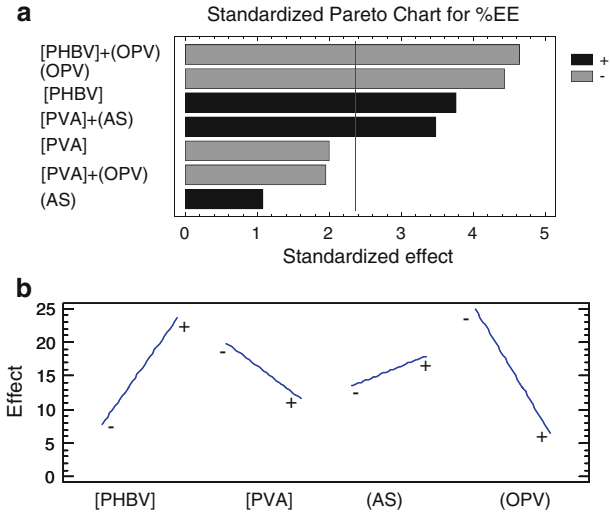

Fig. 1 Influence of **a** factors and **b** principal effects on the encapsulation efficiency of PHBV microspheres containing atrazine. Results obtained with a 95% level of confidence

unimportant for encapsulation efficiency (Table 4, $p > 0.05$). In this study, Eq. 2 was modelled using the factors that caused important effects on the system ($p < 0.05$).

Pareto (Fig. 1a) and principal effects (Fig. 1b) graphs were plotted to better illustrate the EE results obtained using the 2^{4-1} factorial design. Figure 1a shows that the interactions between [PHBV] and OPV, and the variable OPV, were significant positive influences on the system, while the interactions between [PHBV] and AS, and the variable [PHBV], were significant negative influences.

In principal effects graphs, a greater slope of the line indicates a larger influence of the parameter on the system, with positive or negative slopes reflecting positive or negative influences, respectively. Figure 1b shows that the variables [PHBV] and AS exerted positive influences, while the influences of [PVA] and OPV were negative. The most influential variables were [PHBV] and OPV.

Size and morphology

Figure 2 illustrates the surface morphology of the PHBV microspheres containing atrazine, for the formulations analyzed by SEM. Figure 2a (formulation 3) and b (formulation 7) shows the presence of spherical, rough surfaced, and highly porous particles, characteristic of the typical morphology of PHBV and attributed to the highly crystalline nature of the polymer [17, 33, 50]. Although we did not measure the porosity, it is possible to observe that the microspheres exhibited large surface cavities, which probably derived from the emulsion/evaporation process and collision between the particles during hardening. However, spherical particles were not obtained in all the formulations employed. Formulation 1 (Fig. 2c) presented

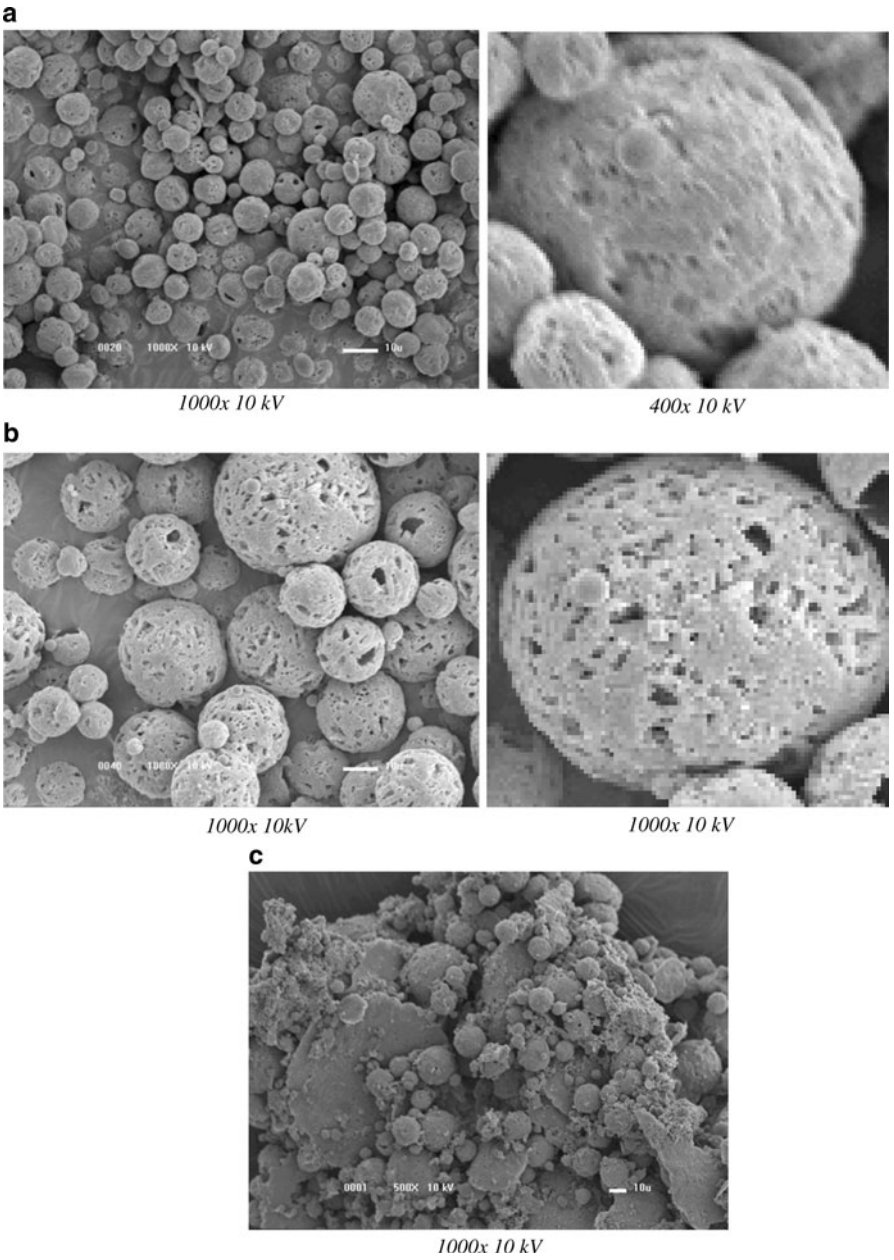

Fig. 2 Scanning electron micrographs of PHBV microparticles containing atrazine: **a** formulation 3, **b** formulation 7, and **c** formulation 1

plate-like aggregates, probably due to the formulation composition and the methodology used, indicating that the combination of conditions was not able to promote quantitative microsphere formation.

Particle size results and statistics are provided in Tables 3 and 5. Mean particle sizes ranged between 4.5 μm (formulation 5) and 24 μm (formulation 7). As previously, a linear model ($r = 0.9934$) described the relationship between the various parameters (Eq. 3).

$$\begin{aligned}\text{Size} = {} & 9.945 + 7.606[\text{PHBV}] - 5.096\ \text{AS} - 4.418\ \text{OPV} + 1.433[\text{PVA}] \\ & - 4.836[\text{PHBV}] \times \text{AS} \ - 4.548[\text{PHBV}] \times \text{OPV}\end{aligned} \quad (3)$$

From the model, it can be seen that the factor most influential on particle size was the amount of polymer, which showed a positive effect. This increase of particle size with the increase of polymer concentration is well documented in the literature [33], and can be explained by the increasing viscosity of the organic phase, which is therefore less likely to disperse into small droplets. Another factor that influences the particle size distribution is AS, which exerts a negative effect. This is interesting, since the greater the agitation, the smaller the particle size, indicating that more homogeneous mixing of the formulation components results in smaller particles. The organic phase volume also negatively influenced particle size, because a modification in the volume of solvent can alter the dissolution of atrazine in chloroform. PVA showed a positive influence on size, due to increased solubilization of the formulation components, increasing aggregation and therefore size. The Pareto and principal effects graphs shown in Fig. 3a, b illustrate these effects. The quantities of PHBV and PVA influenced the system positively, while AS, OPV, and the interactions [PHBV] × AS and [PHBV] × VFO showed negative influences. According to Fig. 3b, [PHBV], AS, and OPV influenced the system most strongly, while [PVA] had little effect.

Atrazine release profile

The release profiles of atrazine are illustrated in Fig. 4, from which it can be seen that the herbicide was completely released after 360 min. The same tests, using either free or associated atrazine, were performed for atrazine from formulation 3, as previously described for atrazine in formulation 7 (data not shown). Small differences can be observed in the release profiles, as a function of encapsulation efficiency and particle size. The times required for 50% release ($t_{50\%}$) were 20 min

Table 5 Standardized effects of factors on the size of PHBV microspheres containing atrazine

	Effect	Standard deviation (±)	*p* value
Average	9.945	0.15	
A: [PHBV]	7.606	0.31	0.0000*
B: [PVA]	1.433	0.31	0.0020*
C: AS	−5.096	0.31	0.0000*
D: OPV	−4.418	0.31	0.0000*
AB	0.248	0.31	0.4562
AC	−4.836	0.31	0.0000*
AD	−4.548	0.31	0.0000*

* Significant at 95% confidence level ($p < 0.05$)

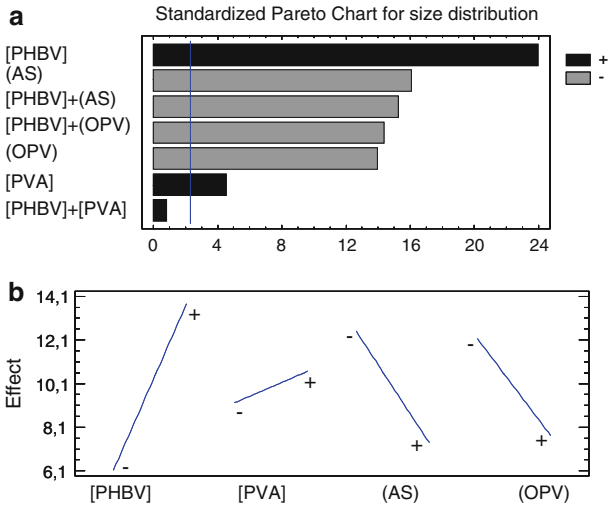

Fig. 3 Influence of **a** factors and **b** principal effects on the size distribution of PHBV microspheres containing atrazine. Results obtained with a 95% level of confidence

for free atrazine [17] and 70 min for atrazine in formulations 3 and 7, indicating modulation of the release profiles following encapsulation in the PHBV microspheres. The same $t_{50\%}$ value was obtained for formulations 3 and 7, which had average particle sizes of 6.6 and 24.0 μm, respectively, suggesting little influence of total surface area on measured release rates. Grillo et al. [17] described three steps that are important for herbicide release from microparticles in an aqueous medium: (1) inhibition of entry of the release medium into the microparticles, (2) dissolution of the herbicide in the microparticle matrix, and (3) herbicide release into the aqueous medium by a diffusion process.

The use of mathematical models can provide important information on the mechanisms of release of molecules from structured systems [43]. These can include desorption from the surface, diffusion through the pores of the matrix or polymeric wall, disintegration or erosion [17, 31, 43]. The mathematical models previously described [17, 41, 42] were applied to obtain information on the possible mechanisms involved in the release of atrazine from the PHBV microspheres. Table 6 provides the parameters obtained using the five different models (zero order, first order, Higuchi, Korsemeyer-Peppas, and Weibull).

From Table 6, the model which best fitted the atrazine release profiles was the Weibull model, which after adjustment provided the best correlation coefficients. The Weibull model is described by Eq. 4.

$$\log[-\mathrm{In}(1-(m))] = b\log(t-T_i) - \log a \tag{4}$$

where m is the accumulated fraction of the compound m in solution at time t; a is a scale parameter which defines the time scale of the process; b is a shape parameter that characterizes the curve as exponential, sigmoid or parabolic; T_i is a location parameter representing the lag time before onset of the release (usually zero).

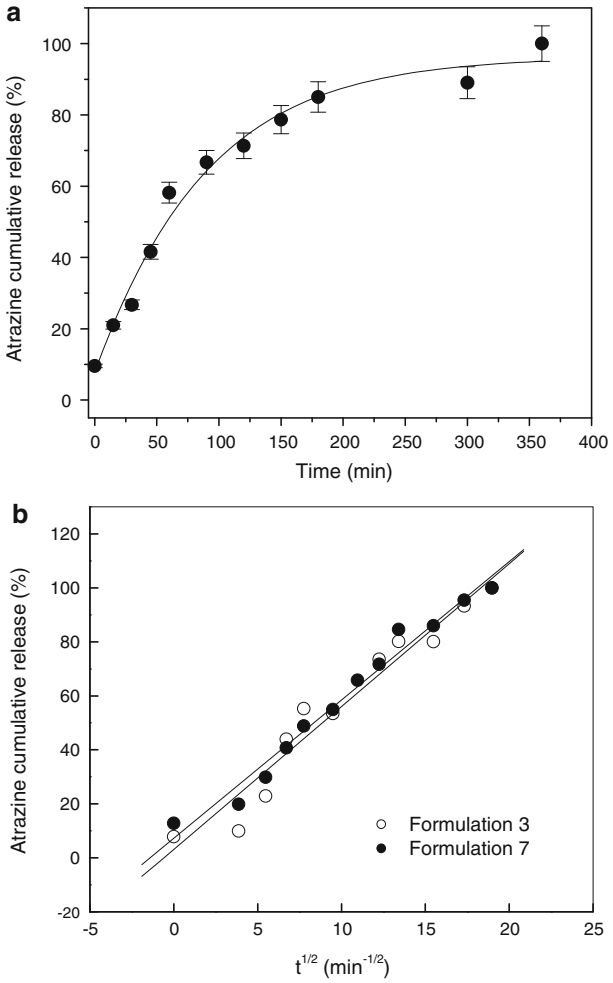

Fig. 4 **a** Release profile of atrazine-loaded PHBV microspheres and **b** Results obtained using the Higuchi mathematical model for formulations 3 and 7

According to Segale et al. [51], a value of *b* close to 1 indicates that the shape of the release curve is exponential, and that the release follows a diffusive mechanism. The value of the *a* parameter, which is related to the rate of the release process, decreases with increasing particle size.

Here, adjustment of the release curves to the Weibull model gave values of parameter *b* close to 1, indicating that the release followed an exponential profile. The values of parameter *a* obtained for formulation 3 (average size of 4.5 μm) and formulation 7 (average size of 24 μm) were 0.020 and 0.010, respectively, indicating that parameter *a* decreased with increasing particle size. The parameter values obtained in this study are very similar to those described by Segale et al. [51] for the release of propafenone from different sized microparticles composed of

Table 6 Values of the parameters obtained after application of mathematical models to the release curves of atrazine associated with PHBV microparticles

	Zero order	First order	Higuchi	Korsemeyer-Peppas	Weibull
Formulation 3					
Release constant (k)	0.238	0.0047 min^{-1}	5.125 $min^{-1/2}$	0.387 min^{-n}	
Parameter a					0.020
Parameter b					0.980
Correlation coefficient (r)	0.843	0.945	0.988	0.970	0.997
Formulation 7					
Release constant (k)	0.243 min^{-1}	0.0057 min^{-1}	5.290 $min^{-1/2}$	0.492 min^{-n}	
Parameter a					0.010
Parameter b					0.977
Correlation coefficient (r)	0.787	0.923	0.973	0.941	0.979

cetearyl alcohol and Pluronic® F68, where values of a were in the range 0.007–0.040 for microparticle sizes between 9 and 63 μm.

The use of the Weibull model for description of release profiles is often criticized because of the lack of a kinetics basis and the non-physical nature of its parameters [51–53]. For this reason, and to confirm the indications obtained from the Weibull model, the kinetics model described by Higuchi was also employed. The Higuchi model showed a good fit for both formulations (Table 6; Fig. 4b), proving that a diffusive mechanism was mostly responsible for release of atrazine from the microparticles. The Higuchi model describes the release of a solute from the particles according to a diffusion mechanism described by Fick's law. Analysis of the data gave values of the kinetic constant, k, of 5.125 $min^{-1/2}$ and 5.290 $min^{-1/2}$ for formulations 3 and 7, respectively, indicating that the formulations had very similar release profiles, in agreement with the results discussed above, and that the process of atrazine release from the polymeric matrix, based on Fickian diffusion, was a function of the square root of time. The observed mechanism of release of atrazine from the microparticles was the same as that reported by Grillo and co-workers [18] for bioactive compounds loaded onto alginate/chitosan nanoparticles (where kinetic constants were $\sim$6 $min^{-1/2}$).

Conclusions

This study demonstrates the importance of evaluating various parameters during the preparation of microparticles, since the concentrations of the formulation components used, and the way in which they are manipulated, can dramatically alter the characteristics of the resultant systems. The use of experimental design was shown to be extremely helpful in selection of the optimum preparation conditions for

atrazine release systems employing PHBV microspheres. Further study is in progress to determine the toxicity and mode of action of these new formulations.

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