

Biodegradable Microspheres of Poly(3-hydroxybutyrate)/Poly(ϵ -caprolactone) loaded with Malathion Pesticide: Preparation, Characterization, and *In Vitro* Controlled Release Testing

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Received 12 March 2009; accepted 12 January 2010

DOI 10.1002/app.32082

Published online 12 May 2010 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: In this investigation, our aim was to evaluate the performance of a controlled release of malathion pesticide loaded in microspheres of pure polymers of poly(3-hydroxybutyrate) and poly(ϵ -caprolactone) (PCL) and of blends of these polymers with compositions of 70/30, 80/20, 90/10, 95/5, and 97/3 w/w. Microspheres were obtained by an emulsion–evaporation method to load a model pesticide. Scanning electron microscopy and differential scanning calorimetry were used to characterize the effect of the pesticide on the morphology and particles size of the biodegradable microspheres. The interaction between the matrix and

pesticide was determined by Fourier transform infrared spectroscopy. The test for controlled release was performed in an aqueous medium, and malathion was quantified by ultraviolet spectroscopy. The results show that the release of malathion was improved with the PCL content in the blends; this indicated the possibility of modulating the release of the pesticide in these biodegradable blends. © 2010 Wiley Periodicals, Inc. *J Appl Polym Sci* 117: 3419–3427, 2010

Key words: biodegradable; blends; microencapsulation; morphology; polyesters

INTRODUCTION

Biodegradable microsphere-based controlled release systems have been widely studied for pesticides and drug-delivery devices.¹ Polyhydroxyalkanoates are thermoplastic polyesters synthesized from bacteria after fermentation with renewable resources.² The most important polymer is poly(3-hydroxybutyrate) [P(3HB)], which has a melting point around 173–180°C and a glass-transition temperature around –5 to 5°C.³

On the other hand, poly(ϵ -caprolactone) (PCL) is a synthetic aliphatic polyester, which is also biodegradable. It is a semicrystalline polymer with a crystallinity degree that lies at approximately 50% and that has a glass-transition temperature of –70°C. In general, PCL acts as a polymeric plasticizer, enhancing the processability of the blend.⁴ Although homopolymers of P(3HB) and PCL are commonly used, their mechanical and degradable properties are diversified by blending with other biodegradable polymers;

this enhances the range of applications of the resulting polymer and controls its rate of biodegradation. Thus, different compositions display various degradation kinetics on the basis of the crystallinity degree of the polymer and the bulk surface area.⁵

The partial miscibility of P(3HB)/PCL blends have already been reported by several authors.^{4,6,7} The results show that it is possible to modulate the degradation of these blends through different compositions of the polymers.

Organophosphorus pesticides correspond to the most applied group of insecticides, and malathion (its molecular structure is shown in Fig. 1) is the most frequently used of these. It is slightly toxic via the oral route, with reported oral Lethal dose, 50% (LD₅₀) (rat, oral) values of 10,000–12,000 mg/kg.⁸ Recent research has revealed that alterations of acetylcholinesterase may play an important role in malathion resistance.⁹ Because of their nonspecific inhibition of acetylcholinesterase, which is present in insects and in humans; dissipation in the environment; and presence in spring waters,¹⁰ these represent a serious threat to wildlife and to public health, and the use of malathion is questionable because, in addition to its neurotoxic effects, technical products contain other cholinesterase inhibitors.¹¹

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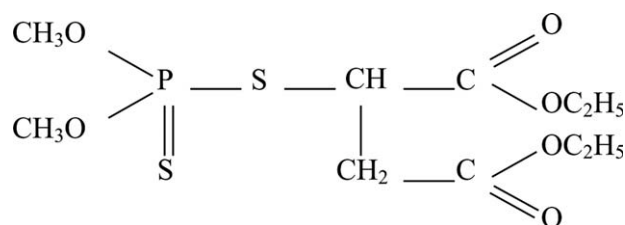


Figure 1 Structural formula of malathion.

Nonetheless, the use of pesticides is beneficial for decreasing crop loss both before and after harvest,¹² but on the other hand, there is a growing social desire to reduce the use of pesticides in agriculture and horticulture.^{13–15}

To reduce the harmful effects and the impact of these compounds on nontarget organisms, formulations for controlled release were developed with various methods, of which microencapsulation is one.¹⁶ The process can be carried out by different techniques for loading agrochemicals^{17–19} or other biological agents.^{20–22} Microencapsulated pesticides in polymeric devices have important advantages over conventional techniques: they reduce the exposure of workers to toxic materials, and less active agents often can be applied to achieve good biological efficacy. This procedure decrease the phytotoxicity, evaporative losses/or leaching through the soil and protects the active agent against environmental degradation.²³ This implies a reduction in the contamination of the environment because less active material is needed to maintain effective biological activity.^{24,25} Although there has been considerable research on various aspects of formulations for the controlled release of pesticides, commercial applications remain sporadic because these are generally

more difficult and expensive to engineer than the application of granules to the soil.¹⁹

Considering the characteristics of pure P(3HB) and PCL and previewing some results about the rate of the degradation of their blends,²⁶ in this study, we aimed to report the influence of different P(3HB)/PCL blends on the time of malathion release and to determine the interaction between the matrix and the loaded pesticide.

EXPERIMENTAL

Materials

P(3HB) (weight-average molecular weight = 342,000 g/mol) was supplied from PHB-Industrial S. A. (Serana, S.P., Brazil) PCL (number-average molecular weight = 80,000 g/mol) was purchased from Aldrich, and technical malathion at a purity of 95–98% was obtained from Action Agro S. A. Gelatin and the surfactant Tween 80 were purchased from Vetec (Duque de Caxias, RJ, Brazil) and chloroform (analytical grade) was purchased from Synth (Diadema, SP, Brazil).

Microsphere preparation

P(3HB)/PCL microspheres were prepared by emulsion solvent evaporation. The experiments were performed with the following polymeric compositions: 0/100, 97/3, 95/5, 90/10, 80/20, 70/30, and 100/0 w/w.

Solutions of 5% (w/v) polymer in 5 mL of chloroform and 0.15 g of malathion were prepared (Fig. 2). The blends containing P(3HB) were heated up to 60°C with the solvent to dissolve the polymer. After cooling, the PCL and the pesticide were added. These blends were emulsified in a previously prepared solution (200 mL) of aqueous gelatin

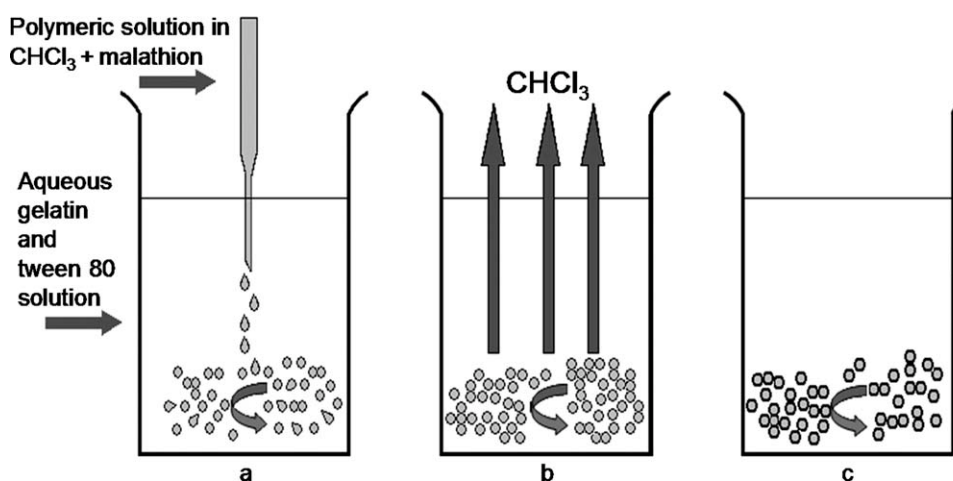


Figure 2 Microsphere preparation process by the emulsification-solvent evaporation method.

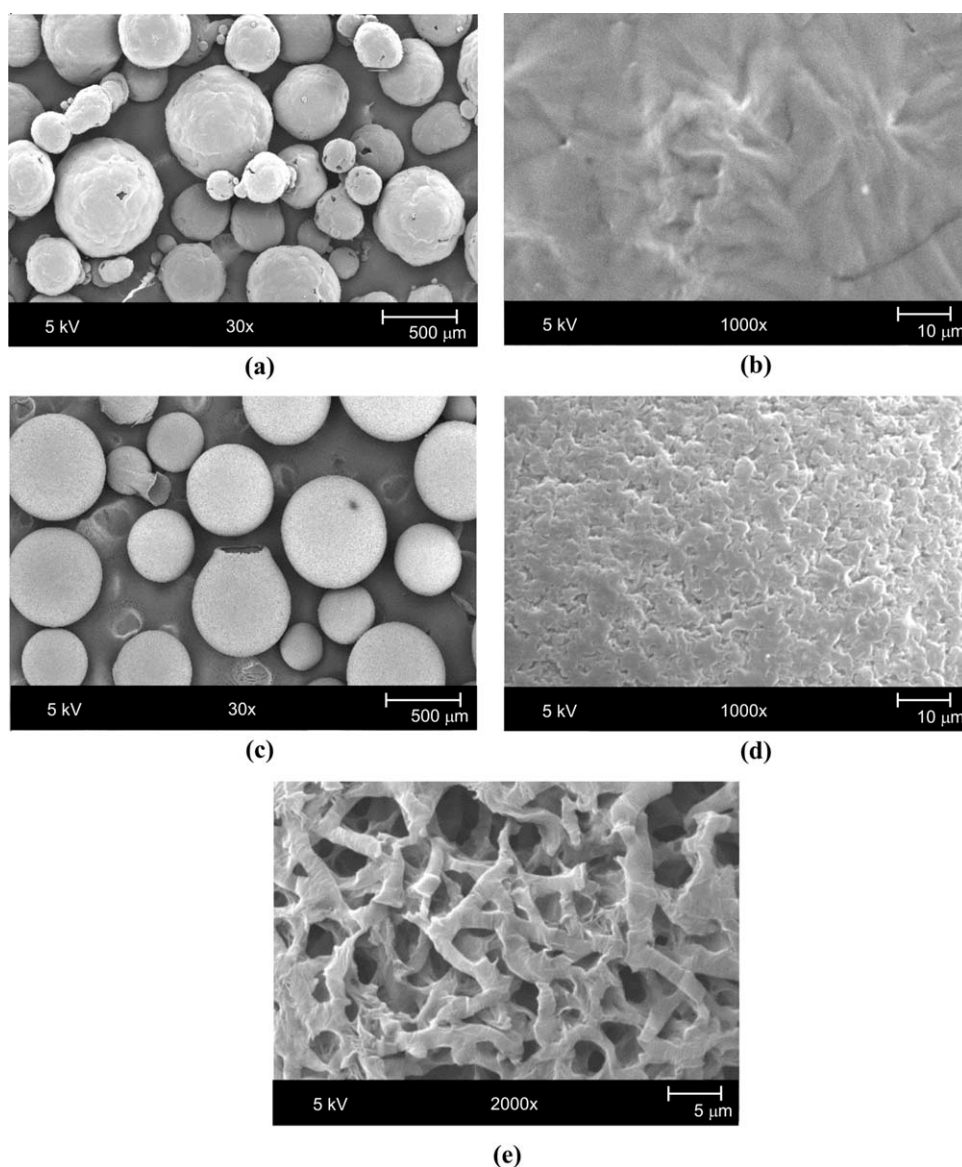


Figure 3 SEM micrographs of (a, b) neat PCL and (c, d) P(3HB) microspheres. The images in (b) and (d) present morphological details of the spheres surfaces and the image in (e) is the surface morphology of the P(3HB)/PCL 80/20 microsphere.

(1% w/v) and Tween 80 (0.05% w/v). The mixture was kept overnight under stirring at 700 rpm and 25°C. The microspheres were collected by vacuum filtration, washed three times in distilled water, dried at room temperature, and stored in a desiccator at room temperature before use. The same method was applied to obtain pure polymer microspheres. Each batch was prepared in triplicate.

Microsphere characterization

Scanning electron microscopy (SEM)

The surfaces and morphology of the microspheres were observed and documented by SEM (Zeiss DSM 940A) (Germany) under 5 kV of accelerated tension. The samples were coated with a thin layer of gold

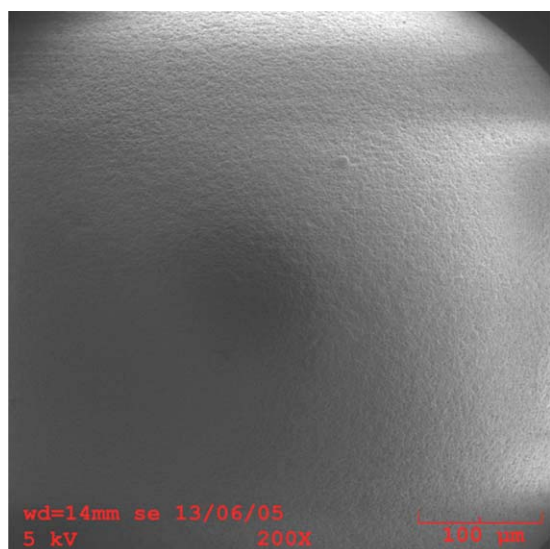
by vacuum deposition with a BALTEC SCD 050 sputter coater. The mean diameters and size distribution of the microspheres were estimated from the SEM micrographs.

Differential scanning calorimetry (DSC)

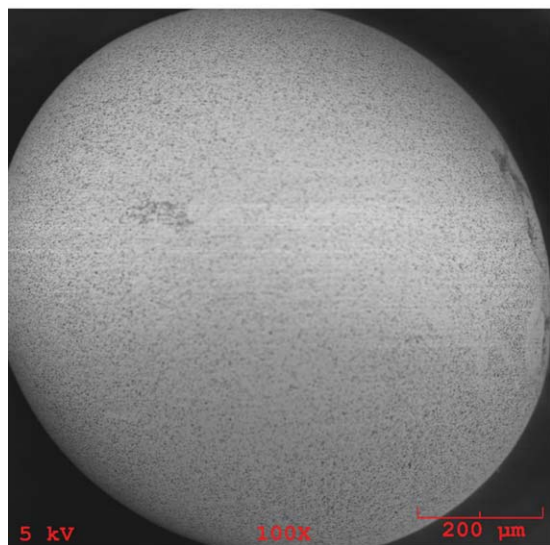
The microspheres were evaluated by DSC with a Shimadzu DSC-50 (USA) thermal analyzer under a nitrogen atmosphere. Samples sealed in aluminum pans were heated in a unique step from -50 to 110°C at heating rate of 10°C/min.

Fourier transformed infrared (FTIR) spectroscopy

P(3HB) and PCL films unloaded and loaded with malathion were prepared by casting with chloroform



(a)



(b)

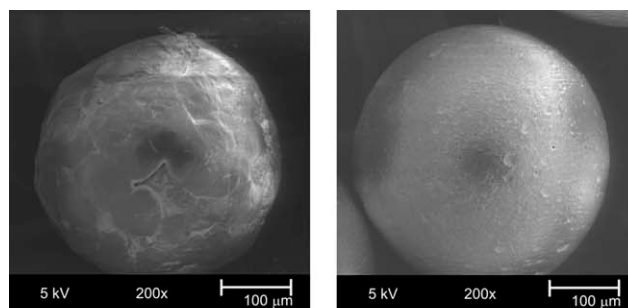
Figure 4 SEM micrographs of the unloaded P(3HB)/PCL microspheres with compositions (a) 95/5 and (b) 97/3. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

as a solvent. The samples had the same compositions used for the microspheres studies. Spectral scanning was done in the range between 4000 and 450 cm^{-1} with a PerkinElmer Fourier Spectrum One B (England).

Controlled released of malathion

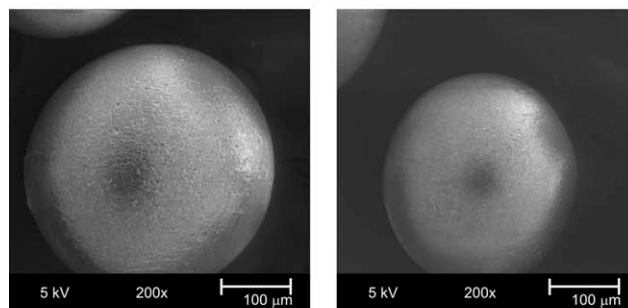
To determine pesticide release, the test was performed in duplicate. An Erlenmeyer flask containing precisely 120 mg of each microsphere and 100 mL of distilled water was closed and maintained under stirring at 100 rpm in a B. Braun Certomat S shaker

at room temperature. The samples were removed, filtered, and replaced in 100 mL of fresh distilled water at predetermined time intervals. The pesticide release was determined by ultraviolet spectroscopy (Hach DR/4000U spectrophotometer) at the maximum absorbance of malathion at $\lambda_{\text{max}} = 215 \text{ nm}$, where $\epsilon = 8.82 \text{ L g}^{-1} \text{ cm}^{-1}$.



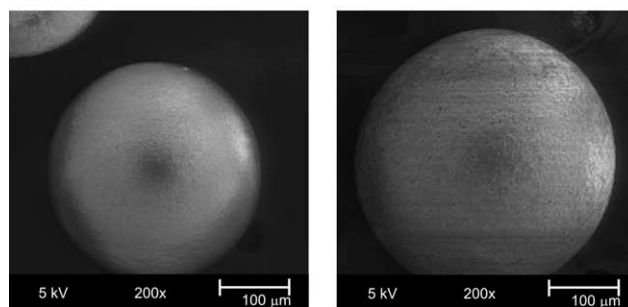
(a)

(b)



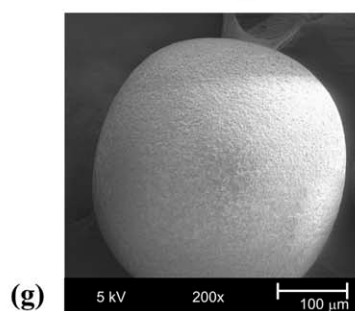
(c)

(d)



(e)

(f)



(g)

Figure 5 SEM micrographs of uniform malathion-loaded microspheres in different P(3HB)/PCL compositions: (a) 0/100, (b) 70/30, (c) 80/20, (d) 90/10, (e) 95/5, (f) 97/3, and (g) 100/0.

TABLE I
Values of the Range of Sizes of Unloaded and Malathion-Loaded P(3HB)/PCL Microspheres

	Range of diameter (μm)					
P(3HB)/PCL	100/0	97/3	95/5	80/20	70/30	0/100
Unloaded	250–1000	800–1200	600–900	75–550	75–550	250–1000
Malathion-loaded	200–500	200–500	200–500	200–500	200–500	200–500

RESULTS AND DISCUSSION

Microsphere characterization

The shape and surface morphology and the size estimation of the microparticles were evaluated by SEM. The SEM micrographs (Fig. 3) revealed that microspheres of neat PCL [Fig. 3(a,b)] and neat P(3HB) [Fig. 3(c,d)] exhibited a spherical and rough surface morphology. P(3HB)/PCL microspheres with 70/30, 80/20, and 90/10 compositions presented interwoven filaments on their surfaces, which were especially visible on the surface of the 80/20 composition [Fig. 3(e)]; this was attributed to phase separation of the polymers. This kind of morphology was associated with low miscibility of P(3HB) and PCL for this composition, which was in agreement with previously reported results.^{6,7} On the other hand, the morphologies observed on the surface of the P(3HB)/PCL microspheres with compositions of 95/5 and 97/3 were homogeneous and presented a roughness similar to the microspheres of neat P(3HB) (Fig. 4).

SEM micrographs of the malathion-loaded microspheres are shown in Figure 5. The morphologies of the loaded microspheres of neat PCL [Fig. 5(a)] and P(3HB) [Fig. 5(g)] were similar to that of the unloaded neat polymer microspheres (Fig. 4). The effect of malathion was perceptible in the morphology of the blends when compared to the unloaded blend microspheres. These particles presented porosity and roughness as the microspheres of neat P(3HB).

The data for the sizes of the unloaded and malathion-loaded P(3HB)/PCL microspheres are presented in Table I. The low miscibility of the two polymers resulted in a broad range of sizes for the microspheres, between 75 and 1200 μm for the unloaded blends and the neat polymers. The presence of malathion decreased the range of the sizes for the malathion-loaded microspheres to 200–500 μm , and more homogeneous microspheres formed, independent of the polymer composition.

Table II presents the values of the melting temperatures (T_m 's) of pure PCL and the P(3HB)/PCL blends from the unloaded and malathion-loaded microspheres, as obtained from DSC. P(3HB) is a highly crystalline thermoplastic with a relatively high T_m (170–180°C).⁶ However, the heating of all the samples was only done up to 110°C, to prevent the thermal degradation of malathion, which is very toxic.

Through this method, the influence of the P(3HB) percentage in the blends and the effect of the addition of malathion were monitored by the T_m of PCL.

In the absence of the pesticide, the addition of P(3HB) to the microspheres promoted the reduction of the T_m of the PCL from 64 to 58°C; this showed that the blend composition had an influence on the crystallization of PCL.

From the DSC data of the loaded P(3HB)/PCL microspheres, the influence of the pesticide on the thermal behavior of the blends was evaluated. However, the addition of 30% (w/w) malathion had an even greater effect in reducing the T_m of PCL from 64 to 55°C. The presence of malathion in the P(3HB)/PCL microspheres led to the reduction of the T_m 's of PCL for values in the range of 45°C. This result indicates that the combination of P(3HB) and malathion had a synergistic effect in reducing the T_m of PCL and suggests that there was some affinity between the malathion and the polymers studied. Figure 6 shows the DSC curves for the unloaded and malathion-loaded 80/20 P(3HB)/PCL, compared with the curve of the unloaded neat PCL.

The interaction between the pesticide and the neat PCL and P(3HB) polymers was also evident in the infrared spectra presented in Figures 7 and 8, respectively. In both figures, the characteristic absorption peaks of malathion appeared at 1016 cm^{-1} for the P–O asymmetrical vibration and at 656 cm^{-1} for the C–S axial vibration, and a further strong peak was revealed at 838 cm^{-1} , arising from the C–O–C stretching of the epoxide ring, and new peaks were not observed in the mixture. These observations suggest that some weak physical interactions between the pesticide and the polymers took place.

Controlled release of malathion

The calibration curve of the malathion absorbance at 215 nm is presented in Figure 9, and the release profiles of malathion from the microspheres are

TABLE II
 T_m of Pure PCL and the P(3HB)/PCL Blends for the Unloaded and Malathion-Loaded Microspheres

	T_m (°C)			
P(3HB)/PCL	0/100	70/30	80/20	95/05
Unloaded	64	62	57	58
Malathion-loaded	55	49	45	45

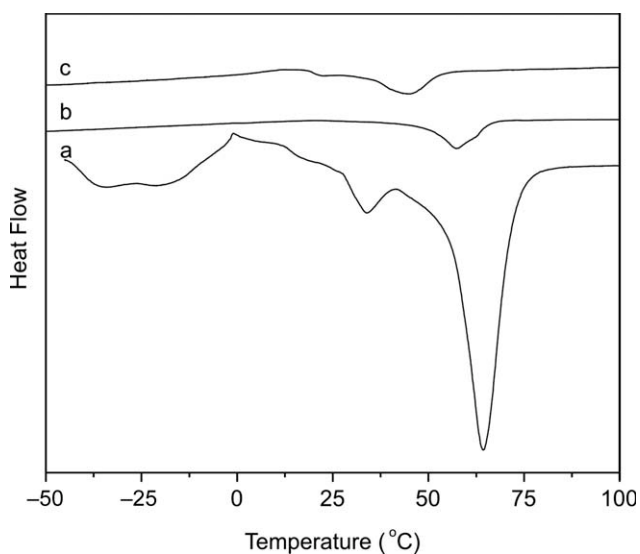


Figure 6 DSC curves of the microspheres: (a) unloaded neat PCL, (b) malathion-unloaded P(3HB)/PCL 80/20, and (c) malathion-loaded P(3HB)/PCL 80/20.

displayed in Figure 10. After 5 h of assay, 50% of the pesticide was released from all of the microspheres studied. At approximately 250 h, all of the curves reached the equilibrium.

To draw a release model, we tested the approached analytical solution derived from Fick's law for diffusion in a sphere.¹⁷ The following assumptions were, thus, made to simplify the problem:

1. The microspheres were considered to be one spherical isotropic microparticle.
2. The initial malathion concentration in the microspheres was below solubility (malathion presents a solubility in water of 145 $\mu\text{m}/\text{mL}$).
3. Two matter transfers took place: the liquid entering the microspheres and the pesticide

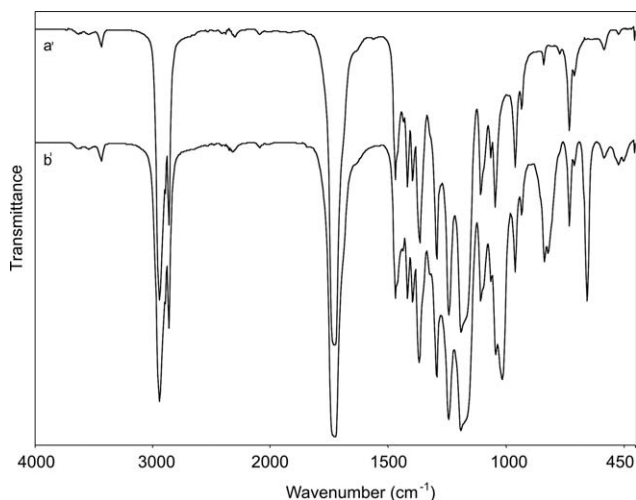


Figure 7 FTIR spectra of the (a) unloaded and (b) malathion-loaded PCL films.

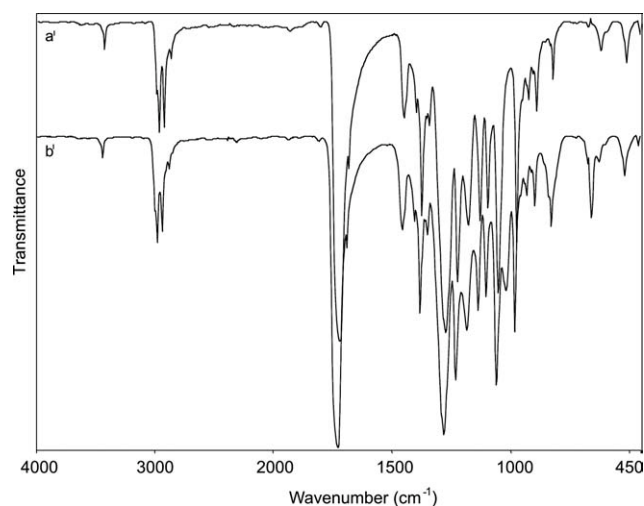


Figure 8 FTIR spectra of the (a) unloaded and (b) malathion-loaded P(3HB) films.

leaving the microspheres. We studied only the second transfer because it was difficult to measure the first one.

4. Both of these transfers were controlled by transient diffusion throughout the microspheres.

We supposed that the release of the pesticide model from this system involved four steps:

1. Penetration of water into the microspheres.
2. Dissolution of the active agent.
3. Diffusion of the active agent in to the matrix.
4. Transfer of the malathion into the solution.

By considering that the microparticles were spherical and isotropic and that the pesticide initial concentration was below the pesticide solubility, we approximated a short time release by Fick's second

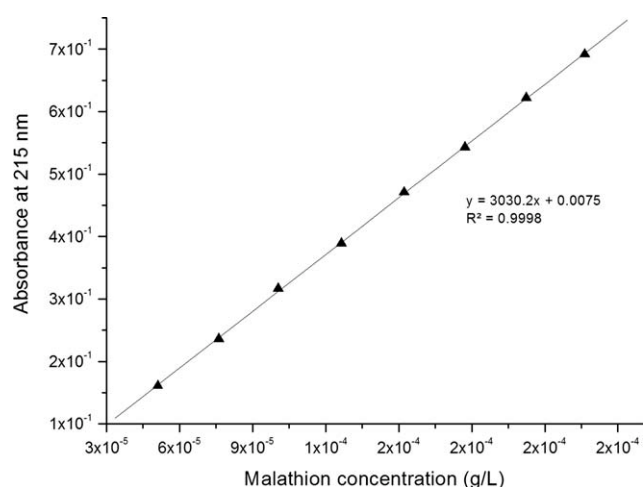


Figure 9 Calibration curve of the malathion absorbance at 215 nm.

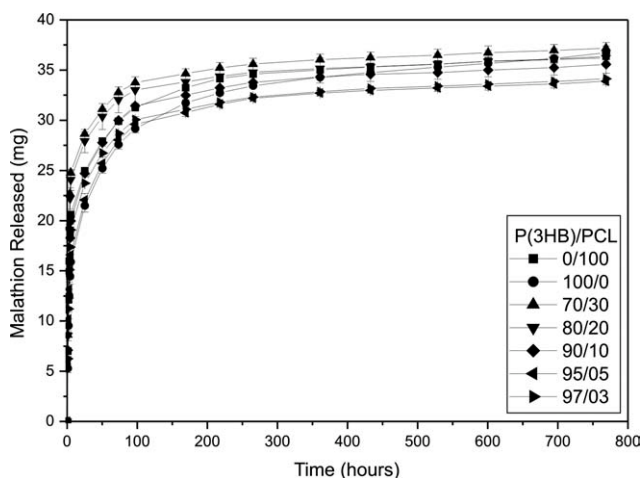


Figure 10 Release profiles of malathion as a function of time from the P(3HB)/PCL microspheres with several compositions.

law, as shown in Figure 11 which shows the linear dependence of the percentage of malathion released and the square root of time ($t^{1/2}$). The release results are given in Table III.

With these boundary conditions, the pesticide-release mechanisms in the earlier stages of the process could be given by the following equation:

$$\frac{M_t}{M_\infty} = kt^n$$

where M_t is the cumulative amount of the active agent released at time t and M_∞ is the total amount released at equilibrium. This model, also called the *Peppas equation*,²⁷ is very frequently used, and the result is that M_t is proportional to t^n when we suppose that k is a constant incorporating the structural and geometric characteristics of the system. For spherical devices, the exponent derived is 0.43 by the release mechanism governed by diffusion.²⁸

By plotting M_t/M_∞ as function of $t^{0.43}$ (Fig. 12), we were able to verify that the Peppas model was obeyed and that the kinetic profile of malathion

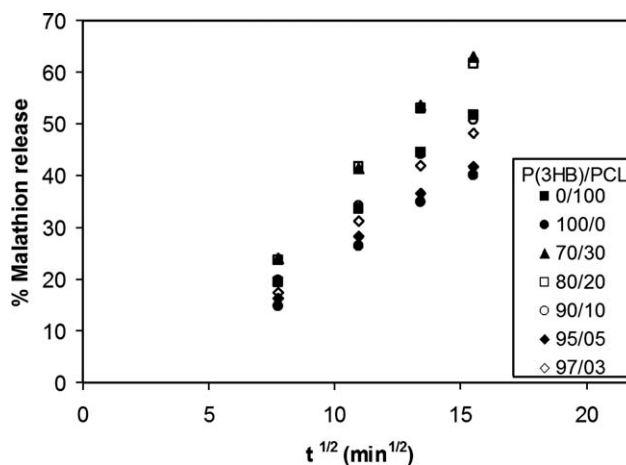


Figure 11 Fickian behavior of malathion release from the microspheres.

release (Table IV) at the earlier stages could be assumed as a Fickian diffusion mechanism.

Analyzing the releasing curves of the P(3HB)/PCL microspheres, we found that the 70/30 composition presented the fastest kinetics of release, whereas the 95/5 and 97/3 compositions had a slower release profile. The 80/20 composition showed similar kinetics to the initial release of the 70/30 blend, but after 100 h of assay, the release became slower and ended with the same profile as that of the neat polymers. The analysis of the average deviation showed that the 80/20 composition could take the behavior of both the 90/10 and 70/30 blends. The rate of release for the 90/10 blend was similar to that of the neat PCL; however, it had a slower release when compared to the pure polymer. When only the releasing profile from the microspheres of the neat polymers was evaluated, there was a faster release for the PCL in the first 500 h of assay.

The release of an active agent can occur through fracture mechanics, by the action of temperature and pH, by the solubility in the medium, and also by diffusion through the degradation of the material.^{29,30} The faster release of the 70/30 blend may have been due to the greater degradation of the 70/30

TABLE III
Release of Malathion from the Microspheres

P(3HB)/PCL matrix	Kinetic equation	R^2	% E_{valid}^a	% E_∞^b
0/100	$4.2029t - 12.727$	0.9977	51.68	101.03
100/0	$3.3161t - 10.431$	0.9947	40.12	102.18
70/30	$5.0534t - 14.649$	0.9984	63.00	103.84
80/20	$4.9095t - 13.385$	0.9951	61.68	100.61
90/10	$4.0434t - 10.947$	0.9952	50.77	98.83
95/5	$3.3117t - 8.6941$	0.994	41.74	94.12
97/3	$4.0511t - 13.511$	0.9954	48.30	94.86

^a The the percentage of malathion released where the kinetic equation is valid.

^b The percentage of malathion released at equilibrium.

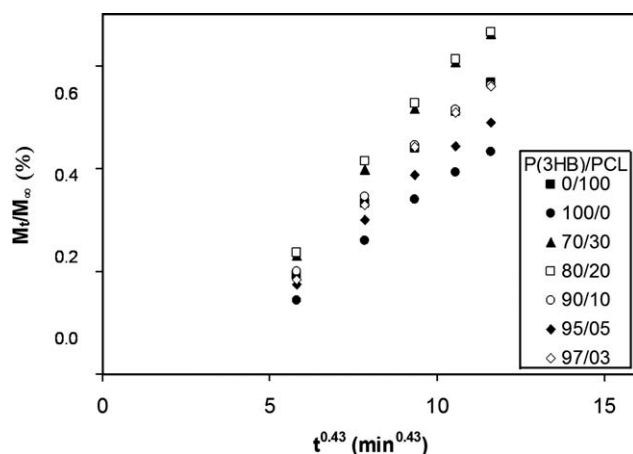


Figure 12 Percentage of malathion released from the microspheres as determined from the Peppas model.

P(3HB)/PCL blend because of the immiscibility of the polymers. Vogelsanger et al.²⁶ studied the biodegradation of P(3HB)/PCL blends and observed that the degradation was mainly facilitated by the amorphous region; this confirmed that the immiscibility of the binary blend increased the kinetics of degradation. It was also observed that 90/10, 95/5, and 97/3 P(3HB)/PCL microspheres had kinetics of total release that were slower than those of neat PCL. It was supposed that there was a greater affinity between the polymers and the pesticide in these blend compositions.

In general, when the release profile of the P(3HB)/PCL blends were analyzed, the time

TABLE IV
Kinetic Equation to the Malathion Release in the Neat Polymers and the Blends

P(3HB)/PCL matrix	Kinetic equation (%)	R^2
0/100	$0.0652t - 0.1799$	0.9964
100/0	$0.0501t - 0.1386$	0.9925
70/30	$0.0754t - 0.1983$	0.9952
80/20	$0.063t - 0.1558$	0.991
90/10	$0.063t - 0.1558$	0.9923
95/5	$0.0547t - 0.1345$	0.9932
97/3	$0.0658t - 0.1893$	0.9923

required to release all of the malathion was found to be proportional to the concentration of PCL in the blend. The presence of the PCL in the blend accelerated the release of the pesticide. Thus, variations of the proportion between the components of the blend could be achieved at different times of release.

Through the release curves, it was also possible to determine the efficiency of the encapsulation of malathion in the microspheres of P(3HB), PCL, and their blends with the emulsification–evaporation of the solvent method. Considering that 120 mg of microspheres before the release assay should have theoretically contained 36 mg of malathion, we confirmed a high efficiency of microencapsulation for all of the compositions.

The microspheres submitted to malathion release were also analyzed by SEM, and Figure 11 shows the effect of the malathion release from PCL, 90/10 P(3HB)/PCL, and P(3HB) microspheres. The PCL

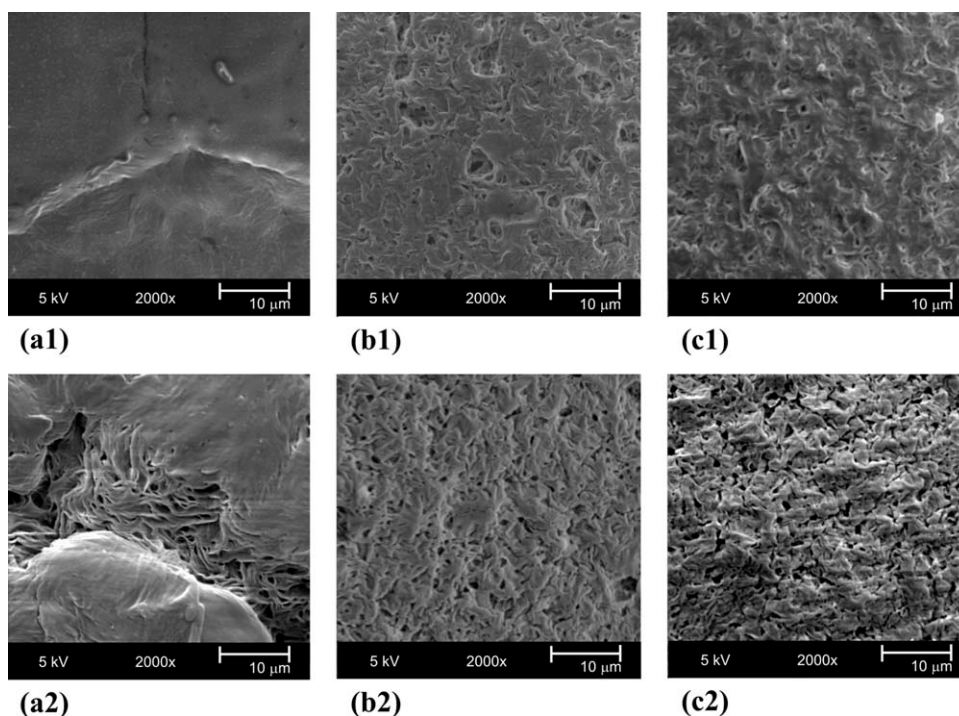


Figure 13 Surface micrographs of malathion-loaded microspheres (1) before and (2) after the release assay: (a) PCL, (b) P(3HB)/PCL 90/10, and (c) P(3HB).

microspheres that were initially compacted became damaged. The porosity shown by these microspheres was attributed to an initial aqueous degradation in the release media.

CONCLUSIONS

SEM analysis showed the influence of the insecticide malathion on the morphology and particle size of P(3HB), PCL, and P(3HB)/PCL microspheres in different compositions. The introduction of 30% malathion to the microspheres generated a uniform particle size distribution in the range 200–500 μm , and the morphology of all of the microspheres in several compositions of the blends became similar to the microspheres of P(3HB).

The DSC results show that the combination of P(3HB) and malathion presented a synergistic effect in reducing the T_m of PCL; this indicated some affinity between the polymers and malathion.

The profile of the release of malathion could be modulated by changes in the compositions of the blends, and the kinetics of release were proportional to the content of PCL in the blend. It was observed that p(3HB)/PCL blends with 20 and 30% of PCL released the malathion in a shorter time than the other blends and neat polymers. The results from the release assay also indicate the viability of obtaining homogeneous microspheres of PCL, P(3HB), and its blends with a simple method with a high efficiency of encapsulation.

This work was carried out with Financial support from Conselho Nacional de Desenvolvimento Científico e Tecnológico (Brazilian Research Council) and Fundo de Apoio à Pesquisa (FAP-UNIVILLE). The authors thank PHB Industrial S. A. for the donation of the samples. The authors also thank Rainer Jonas for his fruitful discussion and valuable suggestions for this work.

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