Modification of Poly(3-hydroxybutyrate)-*co*-Poly (3-hydroxyvalerate) with Natural Rubber

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ABSTRACT: Composites of poly(3-hydroxybutyrate)-*co*-poly(3-hydroxyvalerate) (PHBHV) with 6% of 3-hydroxyvalerate (HV) and natural rubber (NR) were prepared by a solvent-casting method. Different approaches were tested for the composite preparation. Both PHBHV and NR were dissolved in chloroform, followed by its evaporation, giving various layers. The mechanical properties and morphology of the obtained composites were evaluated by tensile tests and scanning electron microscopy (SEM), respectively. The obtained results demonstrated that the final composite has excellent mechanical properties when compared with PHBHV. SEM analysis unequivocally showed the excellent adhesion between the two polymeric layers. This new material was also tested as a drug delivering system using flurbiprofen as a model drug, and then the diffusion coefficients were determined. This article describes an easy method to produce a desirable composite from PHBHV and NR. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 116: 718–726, 2010

Key words: polyhydroxyalkanoates; natural rubber; renewable resources; composites; drug delivery systems

INTRODUCTION

The global market for biodegradable polymers exceeds 160 million dollars and it is expected to rise at an average annual growth rate of 12.6% to 300 million dollars by 2010.[1] The nondegradable plastics accumulate in the environment at a rate of 25 million tonnes per year, which indicates the extreme difficulty in treating this waste. The necessity to develop new degradable polymeric materials and/or composites is urgent as an answer to the problems concerning the global environment and solid waste management. Polyhydroxyalkanoates (PHAs) are polyesters of hydroalkanoates (HAs), synthesized by numerous bacteria as intracellular carbon and energy storage compounds and accumulate as granules in the cytoplasm of cells.[2] Recently, several of these polymers have been synthesized, being the subject of study of numerous scientists mainly due to the possibility of elimination after completion of the normal life cycle. Because of its biodegradability, wide industrial application could be expected.[3,4] However, characteristics such as the high melting points, the low impact strength, and the high price have prevented its widespread use. To obtain PHAs at a low price and with desirable mechanical properties, several approaches have been proposed by many authors. The use of cheap carbon sources has been suggested,[5] whereas other authors proposed the development of new composites by blending the PHAs with other materials.[3,4,6–8] Without this fundamental work it is not realistic to expect the total replacement of fuel-based polymers by the PHAs.[5] Even considering that biodegradability is a feature of all these polymers, without exception, new materials with the same properties as the oil-based polymers have to be developed. The pure poly(3-hydroxybutyrate) (HB), for example, is brittle and has a low extension to break.[9] These are limitations for potential applications.[5] Much research work has been published concerning the production of related copolymers,[10–12] because they can offer a potential solution for the improvement of the polymer properties. The HB and 3-hydroxyvalerate (3HV) copolymers system [poly(3-hydroxybutyrate)-*co*-poly(3-hydroxyvalerate) (PHBHV), with different HV ratios],[10,12,13] is popular because, when compared with P3HB, it presents a low melting point, and is less crystalline and ductile. It is easier to mold and is tougher.[14] Changes in the amount of 3HV can significantly change the thermomechanical properties of the copolymer.[5] Several authors have studied the preparation of this material using different conditions.[12,15–20]

For this work, a copolymer, PHBHV, with 6% HV was chosen. A new strategy for the preparation of composites made from biodegradable polymers (PHBHV and NR) is reported (Fig. 1).

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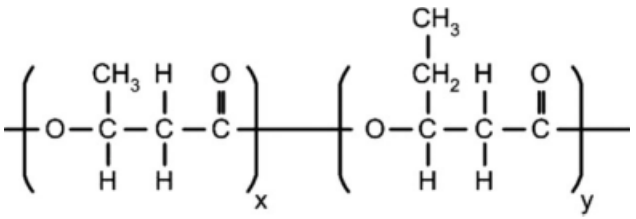

Figure 1 Structure of PHBHV.

The NR is found in the latex of the *Hevea braziliensis* tree, and its principal constituent is *cis*-poly(isoprene), formed from the isoprene monomer (Fig. 2). Besides being a natural polymer with all the advantages related, it has a range of unique physical properties that contribute for its wide industrial application. Its extremely high extensibility generated by low mechanical stress and the complete recovery after mechanical deformation are the key properties, justifying the belief that in 2020, the NR "consumption" will be around 28.1 million tonnes.[21]

The mechanical properties are one of the most important aspects of a material as they determine its final application. This work focuses on the modification of PHBHV with NR to provide a material with enhanced mechanical properties. NR has been used for the mechanical properties modifications of a wide range of materials.[22–27] An increase in toughness in thermoplastics can be generally achieved by adding rubber particles. However, a decrease in strength is observed.[27] In the literature, a study on the thermal behavior of PHBHV (with a hydroxyvalerate content of 12% mol) and epoxidized natural rubber (NR) blends is reported. These polymers are immiscible and any reaction between them is confined to the interfacial region.[28] To the best of our knowledge, there is no report in the literature that deals with composites prepared from the two components considered in this work.

The aim of this study is to develop a new composite prepared from degradable polymers that will be used in new applications of high value, at relatively low prices.

EXPERIMENTAL

Materials

The NR [*cis*-poly(isoprene)] was purchased from Sigma-Aldrich (St. Louis, MO). The PHBHV with 6% in PHV, $\overline{M_n}$ = 222,699 g/mol, and 99.77% purity, *N*,*N*′-dimethylacetamide (DMAc), *N*,*N*′-dimethylformamide (DMF), chloroform, and flurbiprofen (purity >98%) were supplied by Sigma-Aldrich. All of the chemicals were used as received.

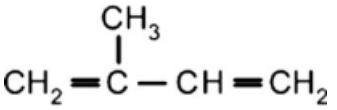

Figure 2 Isoprene monomer.

Solubilization tests

The work began with the solvent selection for the materials. From the literature, DMAc, DMF, and chloroform were identified as possible solvents for these biodegradable polymers.[29] All of these were tested, to establish one with the capacity to dissolve both PHBHV and NR, at room temperature. The tests showed that, regardless the amount of PHBHV and NR (w/w), the solubility in chloroform was excellent.

Composite preparation

Blend preparation

The first attempt to prepare the composite consisted of the dissolution of PHBHV and NR at the same time, followed by the evaporation of the chloroform in a Petri dish.

Chloroform-based solutions of PHBHV were prepared by dissolving 0.34 g of the polymer in 10 mL of chloroform, at 25°C, during 24 h. To each PHBHV solution, different volumes of a chloroform-based NR solution (3%, w/w; 4, 10, 15, and 20 mL) were added. After mixing for 4 h, the samples were placed in glass Petri dishes and kept at room temperature for 24 h to allow gradual solvent evaporation.

Evaporation Rate

The other parameter studied was the solvent evaporation rate. From the industrial standpoint this can be very important, as it determines the time necessary for the preparation. For this purpose, the films were dried in Petri dishes either covered with filter papers with different porosities or uncovered. This allowed the study of the influence of the solvent evaporation rate on the final morphology, keeping the temperature constant.

TABLE I
Thickness of PHBHV Films with Different Polymer Contents

PHBHV (g)	Thickness (mm)
0.1	<0.01
0.2	0.02
0.5	0.05
0.7	0.07

TABLE II
Composition of the Films Prepared

Film	PHBHV (g)/ chloroform (mL)	NR (g)/ chloroform (mL)	Drug (g)
1	0.4/15	–	–
2	–	0.8/15	–
3	0.4/15	–	0.02
4	–	0.8/15	0.04
5	0.4/15	0.8/15	–
6	0.4/15	0.8/15	0.06
7	0.8/15	0.8/15	0.08

Table I shows the variation of the film thickness with the amount of polymer used for the same solvent (10 mL) using the same Petri dish.

Natural Rubber films

In the preparation of the NR films, the same procedure as that described earlier for the PHBHV films was used.

Composite preparation

To prepare a rubber film, 1 g of NR was dissolved in 100 mL of chloroform. The NR was allowed to completely dissolve during 24 h at 25°C. Then 20 mL of this rubber solution was dropped in a Petri dish and the solvent was allowed to evaporate overnight at room temperature. A solution of 0.1 g of PHBHV in 10 mL of chloroform was prepared, and after the total dissolution, it was dropped on the surface of NR film, and the solvent was allowed to evaporate slowly over 24 h at room temperature. The final composite appearance met the required criteria, and preliminary tensile test showed that the required mechanical properties had been provided.

Drug load

The introduction of flurbiprofen was achieved by adding 5% in weight (PHBHV+NR) of this drug to the PHBHV solution before the film preparation process was undertaken.

The formulations used for the preparation of the films are presented in Table II.

SEM analyses

Because of the nonconductive nature of our samples, 300 A gold layer was deposited by cathodic pulverization. The images presented correspond to secondary electrons and were observed in a JEOL JSM 5330 scanning electron microscope (SEM). The acceleration voltage of 5 kV was used.

Tensile tests

Before tensile testing, all of the samples were dried under vacuum at 30°C for at least 72 h to thoroughly remove traces of the chloroform. Tensile testing was carried out on a Chatillon TCD 1000 tensile testing unit at a crosshead speed of 10 mm/min.[17,26] An average of four measured values was taken for each sample.

Controlled drug release tests

Composite pieces were gently washed with water to remove possible drug that might be adsorbed at the surface. Dried composite pieces of about 40 mg were placed in a physiological saline solution (0.05*M* NaCl, pH = 6.64) at 37°C. At predetermined instants of time (1, 2, 4, 6, 12, 24, and 96 h), a small aliquot of release medium was withdrawn from the flask. The sample absorbance was determined (λ = 247 nm), and the amount of drug released (assuming equal dispersion of the drug within the film) was inferred by means of a calibration curve (Fig. 3) previously obtained, using the ultraviolet absorption spectroscopy technique (Spectrophotometer Jasco V-530).

RESULTS AND DISCUSSION

Composite preparation

The films obtained indicated that homogeneity was not achieved with the first preparation method. The NR was not uniformly distributed in the films. Nevertheless, in the film containing the residue from the addition of the 4 mL of NR solution, the regions with rubber segregation were almost undetectable.

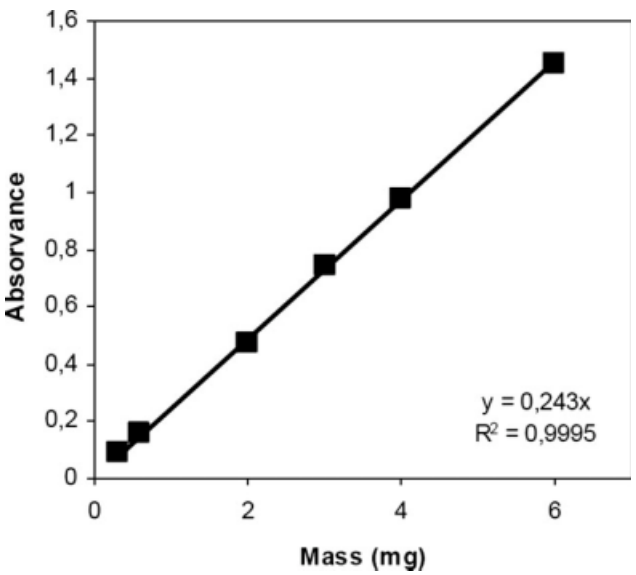

Figure 3 Calibration curve: absorbance versus drug mass.

TABLE III
Films Used in the Tensile Tests

Film	PHBHV (g)	NR (g)	Drug (g)
1	0.4	0	0
2	0	0.8	0
5	0.4	0.8	0
6	0.4	0.8	0.06
7	0.8	0.8	0.08

Several attempts were made to overcome the heterogeneity obtained in the films, namely variation of the agitation speed and dissolution time. However, with this procedure, it was not possible to prepare a homogeneous film. For the final intended applications of this composite, film homogeneity is extremely important to achieve the same drug delivery ratio in all parts of the film. Taking into account the results obtained, another approach, based on two polymeric layers, was used.

To get a PHBHV film that is free from imperfections, the solvent evaporation must be slow. Only by this means it is possible to guarantee that the PHBHV chains have enough time to form successive layers, producing a smooth film with a constant thickness. This result confirms the important role, described in the literature, of solvent evaporation rate, which is a key factor for the final blend's performance.[30] The regular and smooth surface is normally ascribed to a low degree of crystallization.[31]

The film preparation of PHBHV and NR showed different process characteristics. For the PHBHV film preparation, shrinking of the film was not observed as the solvent was evaporated. With NR it was observed that, regardless of the surface covered by the solution (NR + chloroform), during the evaporation process, the NR film shrank. This typical behavior is a consequence of the type of bonds that NR establish between its chains, and can explain why it is impossible to prepare these films by dissolving the two polymers at the same time. When the PHBHV and NR are dissolved at the same time, the PHBHV chains avoid the NR shrinking process, resulting in a heterogeneous film. Several NR films have been prepared using this procedure without any observed problems. Thus one can conclude that the quality of the NR films does not depend on the solvent evaporation speed. An opposite trend was found for the PHBHV films.

Mechanical properties

The films were cut approximately with 120 mm of length and 10 mm of width. All of the specimens were evaluated individually to exactly determine the cross area used in tension expression (Tension = Force/Area). The thickness of the films was precisely measured. This was possible due to its smooth surface. The composition of the films used in the tensile measurements is presented in Table III.

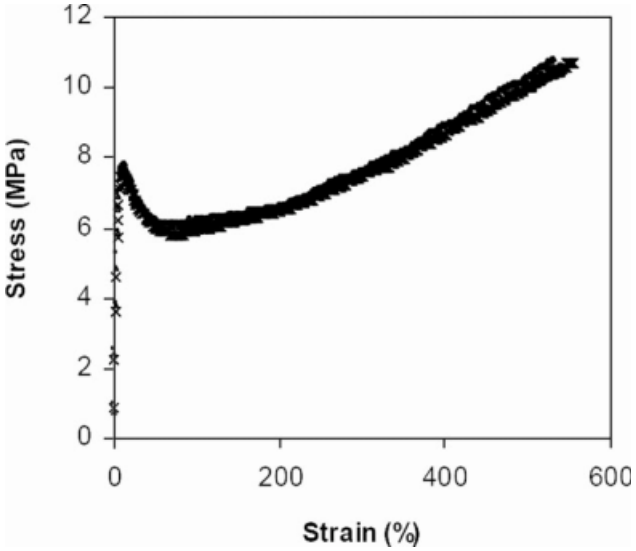

Figure 4 Variation in stress (MPa) with strain (%) for three samples prepared from different regions of Film 7.

The films developed homogeneous properties during preparation, an observation that is supported by the results obtained for the different specimens prepared from the same film. Figure 4, for example, shows the results obtained for Film 7.

Figure 4 shows the high quality match between the three specimens obtained from different parts of the film, once more confirming the homogeneity of the film that was obtained using the proposed procedure.

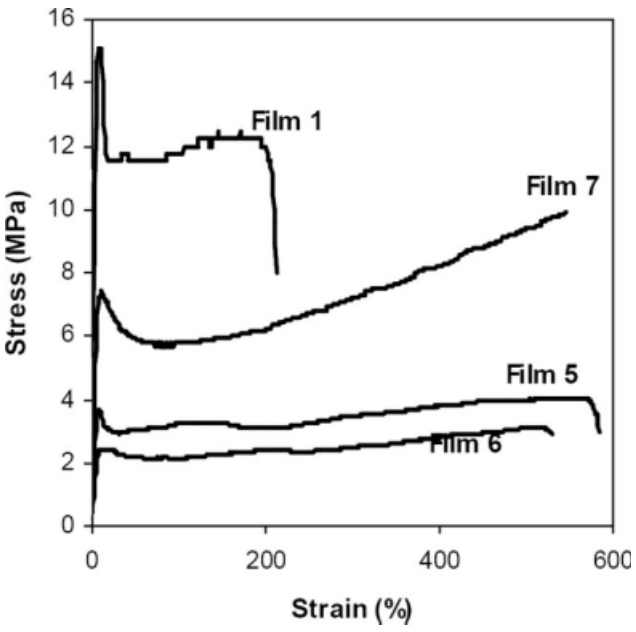

Figure 5 Variation in stress (MPa) with strain (%) for Films 1, 5, 6, and 7.

TABLE IV
Comparison Between the Mechanical Properties of the Films Tested

Film	Young's modulus (MPa)	Tensile strength (MPa)	Strain at break (%)	Stress at break (MPa)	Toughness (MPa)
1	1344.4	15.1	212.1	8.0	2443.2
5	309.9	3.7	583.8	3.0	1959.1
6	220.4	2.4	529.5	2.9	1398.3
7	733.2	7.3	547.7	9.9	4179.1

Figure 5 shows the mechanical properties that were obtained for the films tested.

The strain–stress curves vary greatly for the different polymers and can even vary for the same polymer. To avoid unwanted changes and possible errors, some parameters such as the crosshead speed and the temperature (around 23°C) were kept constant. Before tests, the samples were placed in a excicator to keep the moisture constant.

The curve shape of Film 1 is indicative of semicrystalline polymers that are above T_g, materials with a necking region, typical of almost all polymers that are not crosslinked. This corresponds to a region where the same stress causes considerable elongation. Just before the onset of necking, this test shows a region of maximum stress, usually seen as the hump, the intensity of which deeply depends on the testing rate. It is possible to eliminate the hump

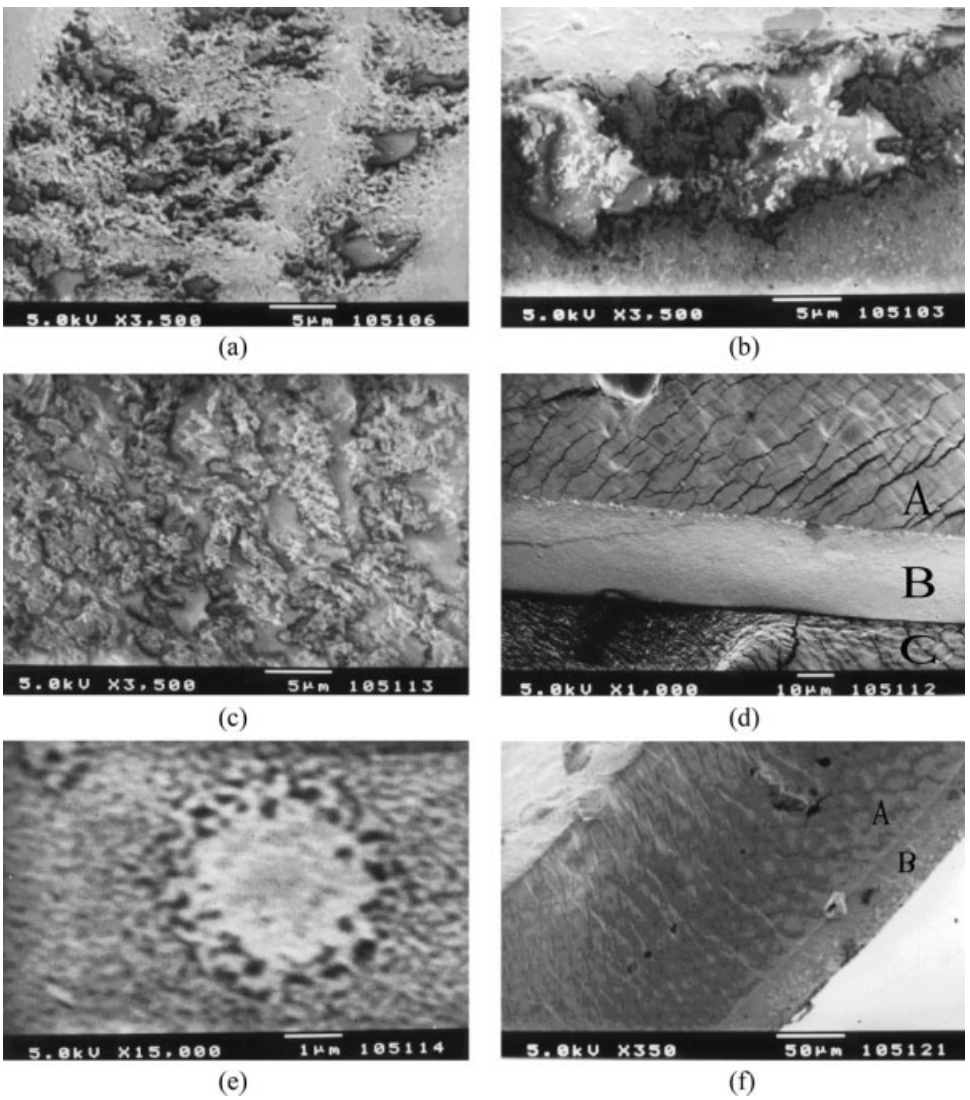

Figure 6 Scanning electron micrograph of surface morphologies of the films. (a) Film 1, PHBHV surface. (b) Film 1, side view. (c) Film 5, NR surface. (d) Film 5, side view: A, NR; B, PHBHV; C, adhesive used. (e) Film 6, surface. (f) Film 6, side view.

TABLE V
Composition of the Films Used in Drug Delivering Purposes

Film	PHBHV (g)	NR (g)	Drug (g)
3	0.4	–	0.02
5	0.4	0.8	–
6	0.4	0.8	0.06

when a very slow speed is used. The hump appearance results from the difficulty in dissipating the heat to the specimen caused by the stress applied. This excess of heat causes softening of the polymer that easily results in a strain increase rather than that maintain at the same level throughout necking.[32]

The addition of NR to the composite, as expected, approaches the composite curve to an elastomeric behavior. The mechanical properties taken from the plots showed in Figure 5 are presented in Table IV.

The results (Young's modulus,[2,33] tensile strength,[33] and strain at break[18]) obtained for the Film 1, only PHBHV, are very similar to those presented in the literature.

In the literature,[27] it is frequently pointed out that the desirable increase in toughness is generally achieved by modification (or blending) with NR. However, other properties may be a compromise between strength and/or stiffness. The results show this trend. The increment of toughness and of strain to break achieved with the NR addition is remarkable, considering the results obtained for Film 7. Analyses of results obtained for the Films 5 and 6, notwithstanding the fact that toughness decreased, show that the ratio of PHBHV/NR used in these two films was too low, leading to a lower value of tensile strength. Taking into account the result obtained for Film 5 and Film 6, the presence of the drug seems to have little influence on the mechanical properties. Considering the information that is available, it is impossible to establish the exact cause of the effect of the drug that is described. One reason could be that the drug has a tendency to migrate toward interface of PHBHV/NR. The decrease in the strength at break is normally assumed to be inevitable when elastomers are used to modify the mechanical properties. This decrease is not pronounced.

The Young's modulus was calculated from the initial slope of the stress–strain plot. The results presented in Table IV show a decrease of Young's modulus when NR was used, indicating that the PHBHV/NR composite is much softer and more flexible than PHBHV. The film that was prepared with a higher percentage of PHBHV showed an increase in the Young's modulus that can be ascribed to the increment of stiffness due to PHBHV.

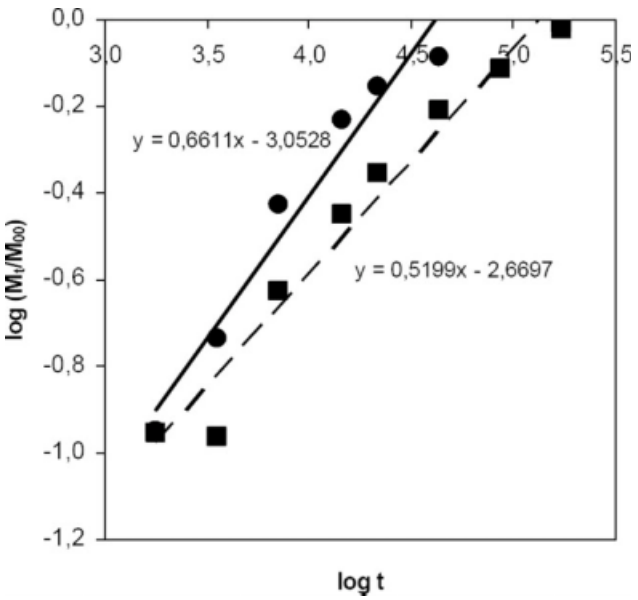

Figure 7 Relationship between $\log(M_t/M_\infty)$ for Film 3 (PHBHV) (•) and Film 6 (PHBHV+NR) (■).

These results show that an optimum ratio PHBHV/NR has to be found to obtain a composite with enhanced mechanical properties, as can be observed by the results between Films 6 and 7.

The capacity to resist before break is tremendously increased when the NR is used. As expected, the rubber particles not only act as stress concentrators initiating multiple crazing at low applied stresses but also extend and deform with the crazed matrix, providing stability against premature fracture. From the results obtained, with this mechanical test, one

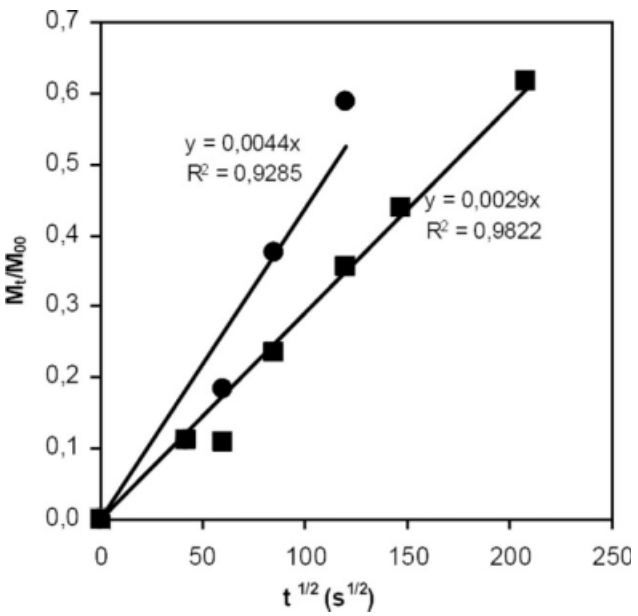

Figure 8 Relationship between M_t/M_∞ for Film 3 (PHBHV) (•) and Film 6 (PHBHV+NR) (■).

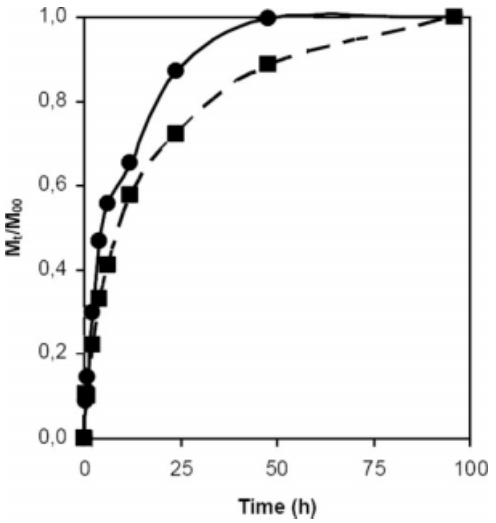

Figure 9 Accumulation release weight for Film 3 (PHBHV) (●) and Film 6 (PHBHV+NR) (■).

$$\frac{M_t}{M_\infty} = Kt^n \quad (1)$$

where, $\frac{M_t}{M_\infty}$ is the fractional drug release after time t, K is the kinetic constant, and n is the diffusion exponent. Graphical representation of log $\frac{M_t}{M_\infty}$ versus log t (Fig. 7), which results from the linearization of eq. (1), allowed the establishment of the drug release mechanism for this composite.

The results obtained show a diffusional coefficient of 0.66 and 0.52 for Films 3 and 6, respectively, indicating a Ficknian diffusion mechanism for both films. From a practical standpoint, this means that flurbiprofen is released from the interior of the film to the surrounding medium by permeation,[31] and that drug diffusion is the rate-limiting step rather than polymer swelling.

can conclude that PHBHV and NR have a strong interaction and that there is a possibility of morphology changes that arise from the mixture of these two polymers.

Morphology

Figure 6(a) shows an SEM of the Film 1, 100% PHBHV. The film has a coralloid surface with many pores and protrusions in different sizes that range approximately from 1 to 6 μm. The surface is clearly heterogeneous due to the different contents of PHB and PHV. The cross-sectional view [Fig. 6(b)] shows the structural heterogeneity of the film. The contrast between the PHBHV and NR can be observed in Figure 6(c). Figure 6(c) shows the adhesion between the two polymer surfaces, showing that both surfaces are compatible and supporting the interpretation of the results obtained in the tensile tests.

Figure 6(f) shows that the adhesion between the PHBHV layer and NR is maintained even when the drug has been added to the system. One can conclude that the introduction of the drug in the film does not induce any significant structural change. However, the possibility of drug migration to the interface of the two materials that cannot be observed by SEM must be considered.

Controlled drug release tests

The release kinetics of the drug was studied with the films indicated in Table V.

Several authors have proposed a simple, semiempirical equation that can be used to evaluate drug release based on a water-soluble drug from polymeric materials[34]:

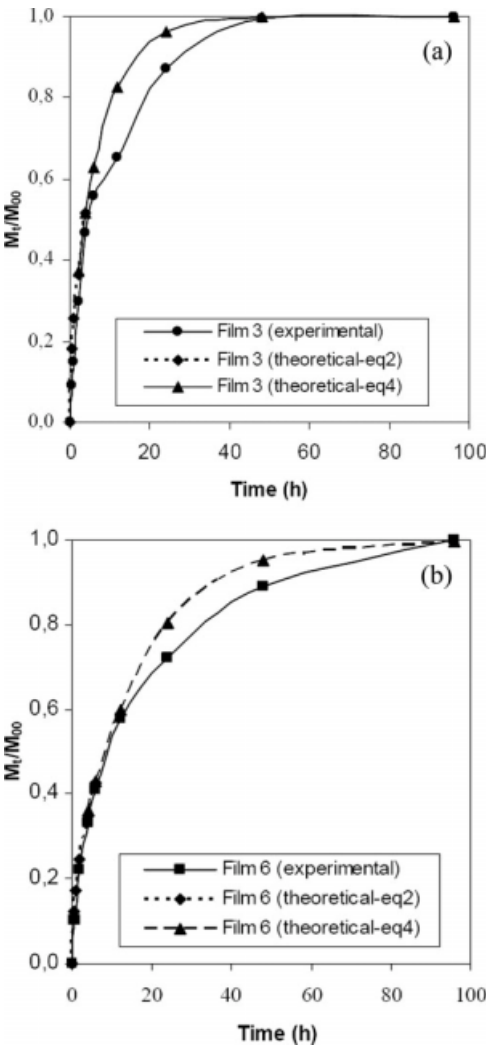

Figure 10 Comparison between theoretical and experimental drug release curves: (a) Film 3 (PHBHV) and (b) Film 6 (PHBHV+NR).

The diffusion coefficient was obtained from eq. (2), which is valid if $0 \leq \frac{M_t}{M_\infty} \leq 0.6$:

$$\frac{M_t}{M_\infty} = 4\left[\frac{Dt}{l^2\pi}\right]^{1/2} \quad (2)$$

where, D is the diffusion coefficient and l is the thickness of the film. It should be noted that eq. (2) is only valid because of the thinness of the films in both cases, and the diffusion from the film can be considered to be one-dimensional.[35]

For $\frac{M_t}{M_\infty} \leq 0.6$, the drug release behavior is linear, and D can be obtained from the slope, m [eq. (3)], of the graphical representation $\frac{M_t}{M_\infty}$ versus $t^{1/2}$.

$$m = \frac{4D^{1/2}}{l\pi^{1/2}} \quad (3)$$

Figure 8 shows the representation of $\frac{M_t}{M_\infty}$ versus $t^{1/2}$ for the first release points.

The diffusion coefficients determined for Films 3 and 6 were $\times 10^{-11}$ 6.6 $\times 10^{-11}$ cm^2/s and $\times 10^{-10}$ 5.4 $\times 10^{-10}$ cm^2/s, respectively. The difference in the thickness of each film does not allow any accurate comparison between the absolute values to be made. Many factors may influence the final value, namely, change in the polymer chains mobility, the drug mobility, and the average pore size. Figure 9 shows the behavior of drug release from Films 3 and 6.

From Figure 9 it is possible to observe that the rate of the release of the drug is faster from the PHBHV film. This result could mean that NR has an important role in the rate of drug release. Thus, rate of release of the drug can be controlled by changing the NR content. For both films, the entire dose was released and matched the amount used, which shows that there was no undesirable interaction between the materials that were used in the composite preparation and the flurbiprofen.

Figure 10 gives a comparison between the theoretical drug release curve and the experimental one. The theoretical values were obtained from eq. (2) for $0 < \frac{M_t}{M_\infty} < 0.6$. For release ratios that were above 0.6, eq. (4) was used, as usual.[36] D was assumed to be constant due to the low solubility of the drug in the polymers.

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\exp\left[-\frac{\pi^2 Dt}{l^2}\right] \quad (4)$$

Film 5 was used as a control to check whether the compounds used in the preparation could release any chemical compounds that might interfere with the absorbance detected in UV spectroscopy. No impurities were detected.

CONCLUSIONS

The results obtained in this work show that NR is a suitable material for use in blends with PHBHV. The SEM images clearly show that the adhesion between the layers of the two polymers is good, which is in accordance with the results obtained in the tension tests. Several attempts to produce the composite showed that it is impossible to obtain truly homogeneous blends from these two materials. The biodegradable polymer used goes from a behavior that is typically associated with a semicrystalline polymer, above T_g, to a behavior that resembles more an elastomer material.

This composite has suitable properties for use in drug delivery, confirming the PHAs potential for controlled drug release, as described in the literature.[20] The theoretical curves fit the experimental data and the mechanism that describes the drug release behavior is the Ficknian, indicating that drug diffusion is the rate-limiting step. The results presented unequivocally showed that NR has a strong influence over the composite's behavior. In the drug delivering system studied, the amount of NR seems to effectively control the drug release rate. The mechanical tests reveal some changes in the tensile curves that comes from drug addition to the films. A complete understanding of such general trend is essential if one is to furthermore improve the mechanical properties of films presented in this work. The results presented in this work open a real possibility of using NR to enhance the mechanical properties of PHBHV.

It is generally believed that interfacial adhesion between the dispersed rubber particles and the matrix plays an important role in the toughening of polymers.[25] The mechanical test data and the information taken from the SEM analysis shows that PHBHV and NR have some compatibility.

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