Protecting Biodegradable Coatings Releasing Antimicrobial Agents

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ABSTRACT: This article describes the synthesis and *in vitro* analysis of poly(ester anhydride) antimicrobial protection coatings. Poly(ester anhydride)s composed of ricinoleic acid, sebacic acid, terephthalic acid, and isophthalic acid were used in this study. The polymers were compatible with various fillers commonly used in paint preparation. The *in vitro* experiments showed that the polymers are able to release diuron, an antimicrobial agent, for months. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 106: 3768–3777, 2007

Key words: biodegradable; coatings; dyes/pigments; films; polyaromatics

INTRODUCTION

The settlement and growth of living organisms on immersed surfaces lead to the deterioration of industrial structures such as ships' hulls, pipelines, and fish cages with severe financial consequences.[1,2] Among all the different solutions proposed, it is universally recognized that prevention can be achieved by the use of erodable coatings containing toxic molecules called biocides.[3,4] However, their widespread use has caused severe pollution in the ecosystem.[5,6] Environmental concerns have created considerable interest in producing a new generation of protective systems based on biodegradable polymers. Among these macromolecules, graft copolymers containing poly(lactic acid) side chains have revealed interesting properties. Nevertheless, their efficiency has been limited by their bulk erosion, which has reduced the mechanical characteristics of the coating.[7]

Polyanhydrides are degradable polymers that have been used in a number of applications, including biomaterials,[8,9] drug carriers,[10] and tissue engineering.[11] They have been investigated extensively for use in the controlled delivery of a number of drugs, including chemotherapeutics,[12,13] antibiotics,[13,14] anesthetics,[15] and polypeptides.[16] They have hydrophobic backbones with hydrolytically labile anhydride linkages. With different monomer building blocks, the erosion times can be varied from a few weeks to a few months.[17–21] Several studies have reported on the *in vitro* erosion of polyanhydrides and have underlined the possibility of obtaining surface erosion.[22–26] The insertion of some ester bonds into anhydride backbones reinforces the polymer and prolongs its degradation time.

This article describes the formulation of poly(ester anhydride)s releasing antimicrobial agents. The aims were (1) to study the effect of the formulation on the degradation and release of a biocide and the efficiency of the system and (2) to develop coatings to prevent the settlement and growth of undesired microorganisms. The use of a biodegradable coating for the extended release of antimicrobial agents provided the agent with long-term protection while being eliminated from the surface to allow new coating without accumulation of coatings or organisms.

Poly(ester anhydride)s were synthesized from ricinoleic acid (RA) and sebacic acid (SA). Both selected copolymers possessed the desired physicochemical properties for their use as coatings, such as a low melting point, hydrophilicity, flexibility, solubility, and biodegradability.[27]

EXPERIMENTAL

RA (85% pure; Fluka, Buchs, Switzerland); SA (99% pure; Sigma, Rehovot, Israel); terephthalic acid (TPA),

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isophthalic acid (IPA), and phthalic anhydride (PHA; 99% pure; Sigma); and diuron (98% pure; Sigma) were used without further purification; acetic anhydride (Biolab, Jerusalem, Israel) was also used in this study. All solvents that were used for analytical tests were high-performance liquid chromatography (HPLC) grade (Biolab) and were used without further purification. Other common solvents were analytical grade and were used without further purification.

IR spectroscopy (Vector 22 FTIR, Bruller, Germany) was conducted for prepolymer and polymer samples and for hydrolyzed samples cast onto NaCl plates from a dichloromethane solution. Thermal analyses were determined on a Metler TA 4000 differential scanning calorimeter calibrated with zinc and indium standards at a heating rate of 10°C/min (average sample weight = 10 mg) and on Stuart Scientific melting point SMP1 heater (Coatati, CA). Molecular weights of the polyanhydrides were estimated on a gel permeation chromotography (GPC) system consisting of a Waters 1515 isocratic HPLC pump with a Waters 2410 refractive-index detector (Waters, MA) and a Rheodyne (Coatati, CA) injection valve with a 20-μL loop (Waters Ma). Samples were eluted with $CHCl_3$ through a linear Styragel HR2 column (Waters; 7.8 × 300 mm) at a flow rate of 1 mL/min. The molecular weights were determined relative to polystyrene standards (Polyscience, Warrington, PA) with a molecular weight range of 4500–18,000 with a Breeze computer program. Concentration of diuron in buffer solutions was determined by an HPLC (Hewlett Packard, Waldbronn, Germany) system composed of an HP 1100 pump, an HP 1050 ultraviolet detector, and an HP ChemStation data analysis program with a C18 reverse-phase column (LichroCart 250-4, Lichrospher 100, 5 μm) with ultraviolet detection at 254 nm. A 50 : 50 (v/v) mixture of acetonitrile and distilled and deionized water at a flow rate of 1 was used as an eluent. Visual differences were monitored by a light microscope KL 1500 electronic (Ziess, Germany).

For HPLC/electrospray ionization mass spectrometry (ESIMS), an Agilent technologies Series 1100 vacuum degasser, an Esquire liquid chromatography pump, and an autosampler (Hewlett Packard, Waldbronn, Germany) were used. An aqueous solution (20 μL) was directly applied for electrospray ionization ion-trap mass spectrometry without a column. The quantification was based on peak area measurements. Specific $[M—H]^-$ ions of the different products resulting from degradation were isolated for tandem mass spectrometry fragmentation: the m/z values were 201 (SA), 297 (RA), 165 (PHA, TPA, and RA), and 231 (diuron). The solvent was a 10 : 40 : 50 H_2O/$(CH_3)_2CHOH$/ACN blend. The split ratio was 0.05%.

Scanning electron microscopy was carried out on a Jeol 6460LV microscope equipped with an Oxford INCA 300 X-ray microanalyzer. Samples were included in a low-viscosity epoxy resin blend (Epothin resin, Buehler, Germany). They were polished by a series of grindings (silicon carbide grinding paper P320 to P1200) with water as lubricant. Then, the polishing was performed with progressively finer abrasives with two grades of diamond polishing grit suspensions (9 μm and then 3 μm) and then alumina (0.05 μm). The polished specimens need to be sputter-coated with a thin film of conductive material (gold or carbon).

An energy dispersive X-ray spectroscopy (EDX) analysis of the elements was carried out with an Oxford INCA 300 system. To determine their distribution, Smart Map acquisition was used. Smart Map performed the simultaneous acquisition of X-ray data from each pixel on the image area. Diuron was directly quantified from chlorine.

Polymer synthesis

Poly(isophthalic-*co*-terephthalic-*co*-sebacic-*co*-ricinoleic ester anhydride) with a 13 : 51 : 19 : 17 weight ratio [P(IPA–TPA–SA–RA)] was prepared by anhydride condensation.

IPA (40 g) was refluxed in acetic anhydride (1 : 10 w/v) for 10 min and filtered to discard the unreacted acid, and the excess of acetic anhydride and was evaporated to dryness. TPA (10 g) was refluxed in acetic anhydride (1 : 10 w/v) similarly, and 15 g of SA was refluxed in acetic anhydride (1 : 5 w/v) for 30 min. The acetated acids were combined together (2 : 8 : 3 IPA–TPA–SA weight ratio) and stirred at 60°C to achieve a homogeneous mixture. The polymerization was performed at 170°C and 0.1 mmHg for 1.5 h. RA (13 g, 17% w/w) was incorporated into the polyanhydride backbone by a transesterification reaction that was performed in bulk at 120°C for 4 h. The hydroxyl group of RA reacted with the anhydride groups to form an ester bond and released IPA–TPA–SA oligomers with carboxylic terminals. The transesterification reaction was followed by GPC analysis and was terminated when the molecular weight reached a minimal constant value. The oligomers were repolymerized by anhydride polycondensation at 170°C and 0.1 mmHg for 3 h. The polymer had typical IR absorptions at 1732 and 1810 cm^{-1} (symmetrical and asymmetrical anhydride C=O stretching bands). ^{1}H-NMR confirmed the RA insertion and creation of the ester bond (t, 1H C**H**—O—CO). The polymer had typical aromatic peaks at 7.7 ppm (m, 1H, OC—CH—C**H**—CH—CO), 8.3–8.5 ppm (m, 2H, OC—C**H**—CH—C**H**—CO and 4H, OC—C**H**—C**H**—CO—C**H**—C**H**), and 9 ppm (s, 1H, OC—C**H**—CO). The polymer was highly soluble in acetone and dichloromethane.

Poly(ricinoleic-*co*-phthalic-*co*-isophthalic ester anhydride)s [P(RA–PHA–IPA)s] containing 0–40% IPA were prepared by anhydride condensation.

Segments with ester bonds were prepared by transesterification reaction between RA and PHA. RA (50 g) and PHA (37.3 g; 1.5 : 1 m/m ratio) were stirred together overnight at 120–125°C. The product was dissolved in dichloromethane, filtered to discard the unreacted PHA, and evaporated. The product was yellow oil.

We prepared the ricinoleyl phthalate prepolymer by refluxing the dicarboxylic acid in acetic anhydride (1 : 10 w/v) for 20 min, filtering, and evaporating to dryness. According to GPC analysis, 80% of the product was represented by the peak of about 500 Da (PHA + RA), and 20% of the product was represented by the peak of about 1000 Da (PHA + 3RA). We prepared the IPA prepolymer by refluxing in acetic anhydride (1 : 10 w/v) for 10 min, filtering the unreacted IPA, and evaporating to dryness. Both prepolymers were combined together [0–40% (w/w) IPA prepolymer] and were stirred for 30 min at 100°C. The polymerization was performed at 170°C and 0.1 mmHg for 1.5 h. All of the polymer had typical IR absorptions at 1732 and 1800 cm^{-1} (symmetrical and asymmetrical anhydride C=O stretching bands).

Formulation and immersion procedure

Paint was formulated with this polymer (Table I). All the ingredients were dispersed under vigorous agitation (2000 rpm) for 1 h. Then the paints were filtered through a sifter (100 μm).

Polycarbonate plates were coated with an automatic film applicator (Sheen 1137). The wet films were 200 μm thick. After drying, plates were immersed in water at 20°C. Water was analyzed at each sampling date and changed to prevent medium saturation.

In vitro hydrolytic degradation of the polymers

To determine the hydrolytic behavior of the polymers in buffer phosphate, samples of 200 mg of P(IPA–TPA–SA–RA) 13 : 51 : 19 : 17 w/w and P(RA–PHA–IPA)s were incubated in 50 mL of a 0.1*M* phosphate buffer solution (pH 7.4) at 37°C and 100 rpm. The degradation was monitored by molecular weight and weight loss, water absorption, and disappearance of the anhydride peak by IR. For the first 3 days, it was possible to separate the core and cortex of P(IPA–TPA–SA–RA) and analyze them separately by GPC and IR. After that period, the core content was insignificant and very problematic for separation. The physical appearance of P(RA–PHA–IPA)s did not allow this separation.

TABLE I
Compositions of the Varnishes and Paint for the *In Vitro* Studies

	Polymer (%)	Chloroform/ xylene (%)	Diuron (%)	Charge (%)
Varnish	50	50		
Loaded varnish	29	63	8	
Paint	18	37	6	39

To determine the hydrolytic behavior of the polymers in water, varnishes and paints were prepared. Their compositions are given in Table I. The degradation was monitored by molecular weight loss, water absorption, and the release of SA, RA, and IPA.

Diuron release from the polymer formulations

To study the release of diuron in buffer phosphate, samples of 100 mg of P(IPA–TPA–SA–RA) 13 : 51 : 19 : 17 w/w and P(RA–PHA–IPA) (40% w/w IPA) were loaded with 20% diuron and placed in 50 mL of buffer phosphate solution.

At each time point, a sample was withdrawn from the dissolution medium, and the medium was replaced with fresh buffer. The samples were analyzed by HPLC. All experiments were done in duplicate.

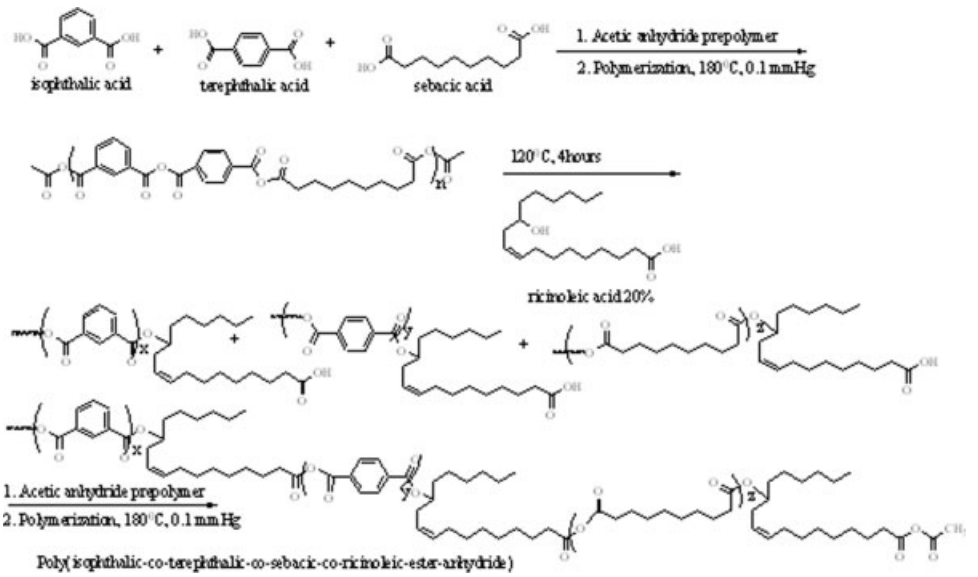
The diuron release from varnish and paint formulations (Table I) was studied by the immersion of these samples in 250 mL of water at room temperature. At each time point, a sample was withdrawn from the dissolution medium and the medium was replaced by fresh buffer. The samples were analyzed by HPLC/ESIMS. All experiments were done in duplicate.

RESULTS AND DISCUSSION

Polymer synthesis

P(IPA–TPA–SA–RA) 13 : 51 : 19 : 17 w/w was prepared by anhydride condensation. The synthesis is presented in Scheme 1. The molecular weight and melting point are given in Table II.

P(RA–PHA–IPA) containing 0–40% IPA were prepared by anhydride condensation. The synthesis is given in Scheme 2. The transesterification reaction between RA and PHA (1.5 : 1 m/m) was performed overnight at 125°C in a sealed glass flask to avoid evaporation of the reagents. RA–PHA ester bond and RA–RA ester bond can be distinguished by in ^{1}H-NMR analysis. The first has a typical peak at 5.1 ppm and the second at 4.8 ppm. According to the ^{1}H-NMR analysis, the RA–PHA always contained 20% RA–RA ester bonds. Together with the GPC data, we concluded that the polymer repeating units were 80% RA–PHA and 20% RA–RA–RA–PHA. The



Scheme 1 Synthesis of P(IPA–TPA–SA–RA) from IPA, TPA, SA, and RA: activation by acetic anhydride of IPA, TPA, and SA; anhydride condensation for 4 h; transesterification with RA esterification for 3 h at 120°C until a constant low molecular weight was reached; activation by acetic anhydride; and polycondensation for 4 h until a constant maximum molecular weight was reached.

aromatic area showed five typical peaks at 7.5 ppm (m, 2H, C**H**—CO—O—CH) for RA–PHA–RA molecules, 7.7 ppm (d, 1H, C**H**—CO—O—CH) for RA–PHA molecules, 7.8 (q, 1H, C**H**—CH—C—CO-O—CH), 7.9 ppm (q, 1H, C**H**—CH—CH—C—CO-O—CH), and 8 ppm (d, 1H, C**H**—CO—OH). All the polymers had typical IR absorptions at 1732 and 1800 cm^{-1} (symmetrical and asymmetrical anhydride C=O stretching bands). The molecular weights and melting points are given in Table II. There was no effect of the different percentages of IPA on the molecular weight.

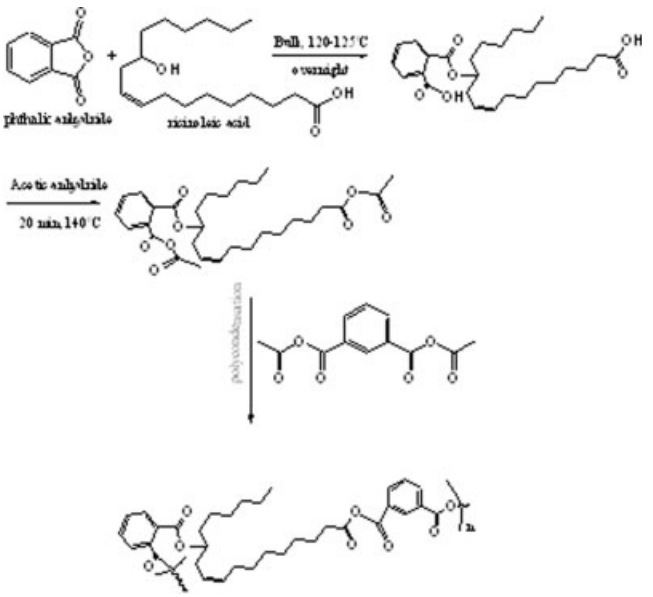
In vitro hydrolytic degradation of the polymers and erosion

The objectives of this experiment were to study (1) the effect of the surrounding aging medium on hydrolysis kinetics, (2) the effect of the formulation (the presence of a bioactive and additives), and (3) the effect of the specimen geometry. Consequently, complementary analytical methods were used to study the hydrolysis and the erosion of these polymers. For clarity, it is important to distinguish between the terms *degradation* and *erosion*. The term *degradation* refers to the chain scission process by which polymer chains are cleaved into oligomer or monomer units. The term *erosion* refers to mass loss from the bulk polymer. In other words, erosion could be considered as the sum of several elementary processes that result in the elimination of the polymer mass over time, one of which is degradation. The aim of the degradation protocol was to obtain chemical and physicochemical information about the polymer degradation during their immersion in various media: the disappearance of anhydride bonds by IR, monitoring of the weight loss of the specimen, changes in the polymer molecular

TABLE II
Characteristics of the Synthesized Polymers

	Solubility in dichloromethane and acetone	Appearance	M_n[a]	M_w[b]	Melting point (°C)[c]
P(RA–PHA)	High	Brown, gluelike liquid	4,700	5,100	−58.5
P(RA–PHA) + 10% IPA	High	Brown, gluelike liquid	4,800	5,600	−38.4
P(RA–PHA) + 20% IPA	High	Brown, gluelike liquid	4,500	5,100	−24.2
P(RA–PHA) + 30% IPA	High	Off-white paste	4,300	5,200	−10
P(RA–PHA) + 40% IPA	Very low	Off-white solid	3,900	4,800	69.5
P(IPA–TPA–SA–RA)	High	Brown, sticky solid	8,000	12,000	112

[a] Number-average molecular weight.
[b] Weight-average molecular weight.
[c] Determined with differential scanning calorimetry.



Scheme 2 Synthesis of P(RA–PHA–IPA)s from RA, PHA, and IPA: transeterification of PHA by RA at 120°C in bulk overnight, activation by acetic anhydride, and polycondensation with different amounts of IPA for 4 h until a constant maximum molecular weight was reached.

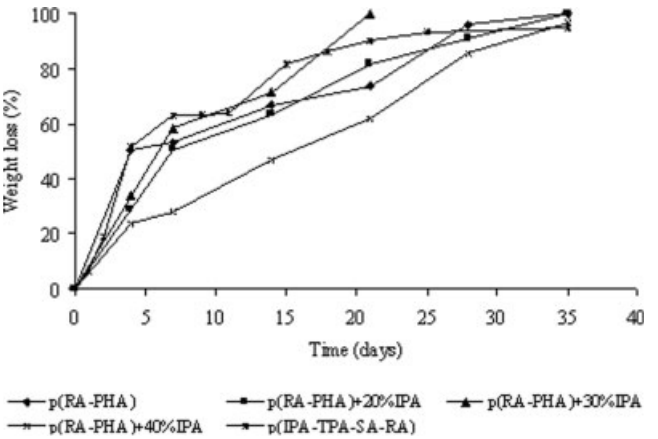


Figure 1 Hydrolysis of P(IPA–TPA–SA–RA) and P(RA–PHA–IPA)s monitored by weight loss. Hydrolysis was conducted in 0.1*M* phosphate buffer (pH 7.4) at 37°C. The standard deviation was not more than ±5%.

weight by GPC, and the monitoring of the products resulting from the polymer hydrolysis by HPLC coupled with ESIMS.

To monitor water uptake, two different protocols were used: weighting wet and lyophilized samples at each sampling date and Karl–Fisher titration for water content.

To evaluate the effect of the surrounding medium on the degradation, the polymers were tested under reference conditions: in the phosphate buffer (a 0.1*M* phosphate buffer solution at pH 7.4 and 37°) and in distilled water (at 20°C).

The influence of the formulation and sample preparation were studied by comparison of the blank polymer (as films or as cylinders) and the polymers in paint (obtained by the mixture of the polymer with additives).

Effect of the surrounding degradation medium and sample preparation

Cylinders (5 × 10 mm^2) made of the P(IPA–TPA–SA–RA) were fully eroded for 6.5 weeks in buffer phosphate (Fig. 1). The color differences between the core and the cortex are shown in Figure 2(a–c). The core had the brownish color of the nonhydrolyzed polymer [Fig. 2(a)], whereas the cortex had a white color like the monomers [Fig. 2(b,c)]. According to the IR analysis, the cortex contained anhydride bonds only for the first 24 h from the first immersion in water. After this period, the cortex included only acid and ester bonds. Both core and cortex were analyzed by GPC. The results are summarized in Figure 3. The changes in the number-average molecular weight as a function of the immersion time indicated a fast degradation of the cortex to its acid components. The water absorption, established by the weight of wet and lyophilized samples, was terminated after 18 days because the sample developed a jellylike cortex. It was easily crumbled once the sample was pulled out the medium. The polymer reached its maximum water absorption of 16.5% w/w after 4 days, and a constant content (8–9% w/w) was achieved after 11 days (Fig. 4).

The water uptake of P(IPA–TPA–SA–RA) thin film of 200 μm (obtained after dissolution in aromatic solvents and application on polycarbonate plates) is presented in Figure 4. After a fast absorption for the first 3 days of immersion, the water uptake steadily increased to reach 8% w/w, which was similar to the value obtained in buffer phosphate. The decrease in the number-average molecular weight was similar to the cortex one: the degradation of anhydride bonds was complete after 3 days of immersion, which could be explained by the thin film (200 μm).

The composition of the products resulting from the polymer hydrolysis was determined by ESIMS.

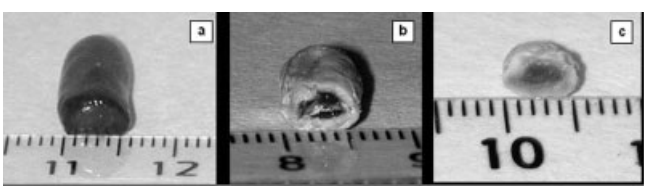


Figure 2 Hydrolysis of P(IPA–TPA–SA–RA) monitored by the core–cortex visual difference by light microscope: (a) before hydrolysis, (b) after 24 h of hydrolysis, and (c) after 96 h of hydrolysis. Hydrolysis was conducted in 0.1*M* phosphate buffer (pH 7.4) at 37°C.

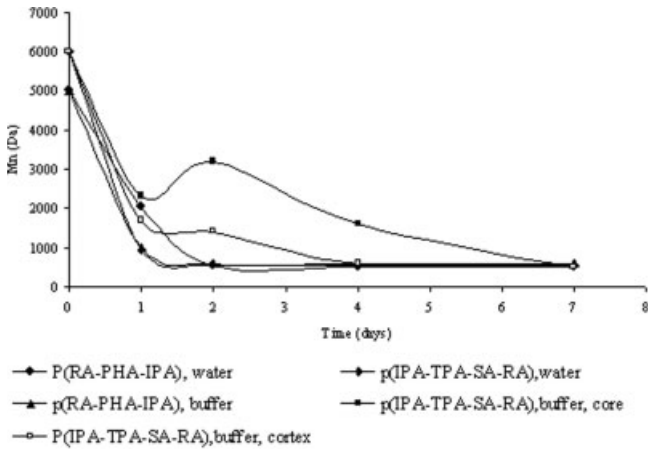


Figure 3 Core–cortex hydrolysis of P(IPA–TPA–SA–RA) 13 : 51 : 19 : 17 w/w and hydrolysis of P(RA–PHA–IPA) (40% IPA) monitored by number-average molecular weight loss as determined by GPC. Hydrolysis was conducted in 0.1*M* phosphate buffer (pH 7.4) at 37°C and in water at 20°C.

At each sampling date, the water was collected, analyzed, and replaced to prevent the risk of medium saturation. IPA and TPA, which had close chemical structures and identical molecular masses, were detected at the same retention time and were not quantified separately. The results are summarized in Figure 5. The RA was not released (only 0.35% after 3 months of immersion), and only the release of SA, IPA, and TPA remained weak (ca. 15% after 3 months of immersion).

The same analytical protocol was used to determine the degradation of P(RA–PHA–IPA)s, which contained 0–40% IPA monomer units. As shown in Figure 1, in buffer phosphate, the four polymers underwent rapid degradation: 100% of weight loss

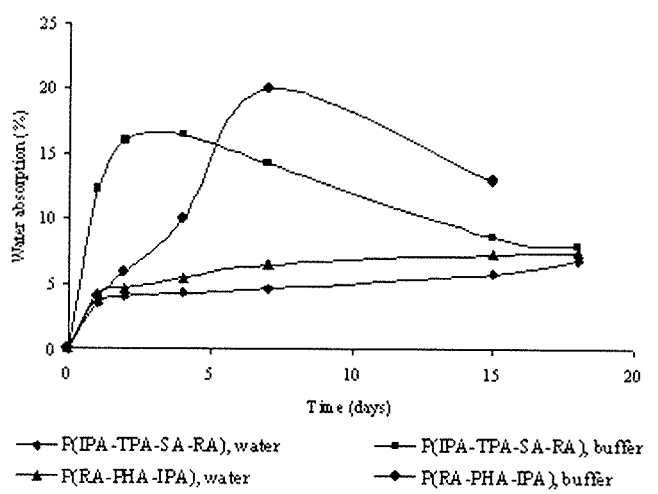


Figure 4 Water absorption of P(IPA–TPA–SA–RA) 13 : 51 : 19 : 17 w/w and P(RA–PHA–IPA) (40% IPA) determined by Karl–Fisher titration. The water absorption was conducted in 0.1*M* phosphate buffer (pH 7.4) at 37°C and in water at 20°C. The standard deviation was not more than ±5%.

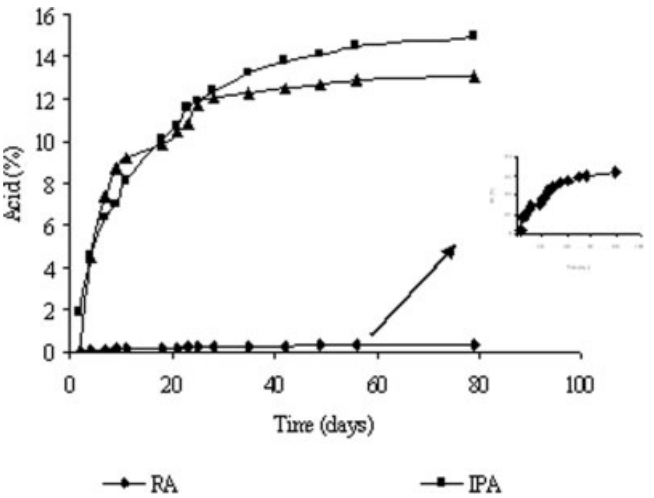


Figure 5 Hydrolysis of P(IPA–TPA–SA–RA) 13 : 51 : 19 : 17 w/w monitored by the release of RA, TPA, SA, and IPA to the aqueous medium as determined by ESIMS. The standard deviation was not more than ±5%.

was observed after 1 month of immersion. No direct correlation could be made between the IPA amount and the degradation rate: the polymer containing 30% IPA units degraded faster than the 0 and 20% ones, which degraded faster than the 40% IPA containing polymer. The water absorption determined by the gravimetric method reached its maximum after 1 week for all polymers. The most stable polymer with 40% IPA had the highest water absorption of 58% w/w that decreased with degradation until complete elimination.

In water, the polymer with 40% IPA was studied (designed as film). The water uptake was fast during the first 3 days of immersion and increased regularly to reach the value of about 7% w/w after 3 weeks. In this case, the medium or the sample preparation seemed to have a great effect on water penetration in the polymer. In buffer phosphate, as cylinders, the P(RA–PHA–IPA)s exhibited a large water absorption (fivefold the value obtained in seawater for the films). The difference could be explained by the two analytical methods used: in the first case, gravimetry, which requires a time-consuming preparation of the samples (lyophilization, weighting), and in the second case, the Karl–Fisher titration, which is a direct technique. The monitoring of the molecular weight decrease showed that the experimental conditions that we used did not modify the degradation by a significant mean: the hydrolysis of the anhydride bonds seemed to be completed after 3 days of immersion. No effect of the IPA content was observed.

The quantification of the products resulting from polymer hydrolysis in surrounding water confirmed the previous results obtained for P(IPA–TPA–SA–RA) (Fig. 6). The RA was not leached out: only 1%

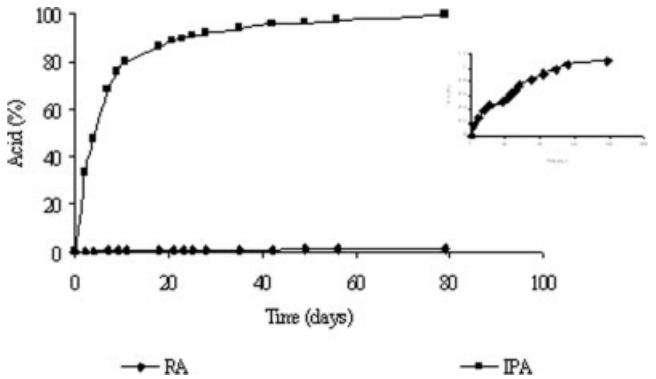


Figure 6 Hydrolysis of P(RA–PHA–IPA) (40% IPA) monitored by the release of RA, TPA, SA, and IPA to the aqueous medium as determined by ESIMS. Hydrolysis was conducted in water at 2°C. The standard deviation was not more than ±5%.

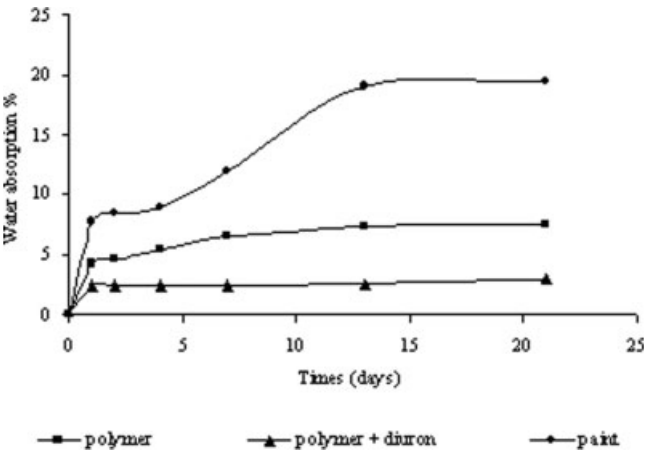


Figure 7 Effect of the formulation on water absorption for P(RA–PHA–IPA) (40% IPA) loaded with paint, diuron, and unloaded polymer. The water absorption was conducted in water at 20°C. The standard deviation was not more than ±5%.

remained after 80 days of immersion. This was in agreement with a study made on the degradation of another RA-based polymer: poly(sebacic-*co*-ricinoleic ester anhydride).[28] The HPLC analyses of the degradation medium showed that RA left the polymer matrix not as a single molecule but in the form of dimers, trimers, and tetramers. To the contrarily, in the case of IPA and PHA units, the release was very significant: about 90% after 80 days. As shown, the chemical structures of the monomer units had a great effect on the leaching out of degradation byproducts. This result may be essential for the conception of polymer films, which have to keep their film properties during immersion.

Effect of the formulation

The mixing of fillers, additives, and active molecules with a polymer may modify its degradation properties. In this study, to evaluate the ability of a potential application in antimicrobial paints, the poly (ester anhydride)s were blended with an herbicide (diuron) as summarized in Table I. The P(IPA–TPA–SA–RA) had poor solubility in xylene, and it was not sufficient to allow for formulation in paint.

The following curves present the water uptake (Figs. 7 and 8) and the release of degradation byproducts (Figs. 9 and 10) for the two polymers. The experimental data showed that no significant difference was observed between the polymer and the blend, the polymer and diuron, for P(IPA–TPA–SA–RA). In the case of P(RA–PHA–IPA), the paint and the blend clearly underwent a different degradation patterns. The water uptake was considerably slower: after 1 week of immersion, the water absorption reached a steady state (2 and 6% w/w, respectively for the blend and the paint), whereas the polymer continued to absorb water until the 15th day of immersion (20% w/w).

This formulation modified the permeability of the film and probably reinforced its hydrophobicity because of the added molecules. We reached a similar conclusion by considering the kinetics of the byproduct leachout. Figure 9 presents the experimental results obtained by the titration of PHA and IPA units in the releasing medium. The presence of diuron prevented the release of degradation byproducts: the percentage release in the case of loaded polymer or paint was six times as inferior as that of the polymer (15% w/w and 90% w/w, respectively). Nevertheless, the degradation seemed to be effective in all the cases: the molecular weight decreased rapidly as soon as the immersion began (data not shown).

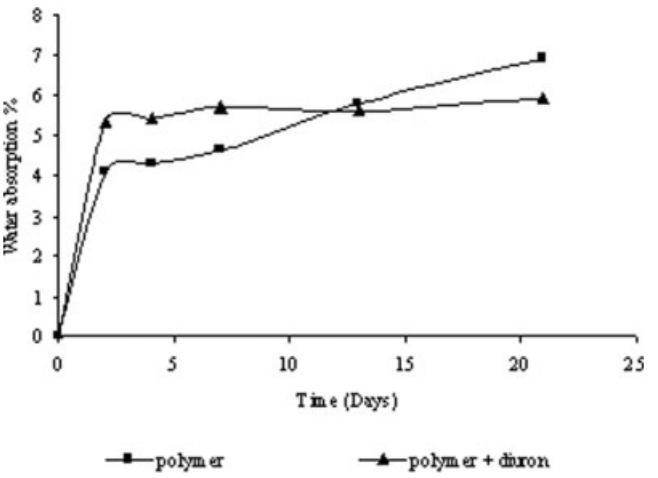


Figure 8 Effect of the formulation on water absorption for P(IPA–TPA–SA–RA) 13 : 51 : 19 : 17 w/w loaded with diuron and unloaded polymer. The water absorption was conducted in water at 20°C. The standard deviation was not more than ±5%.

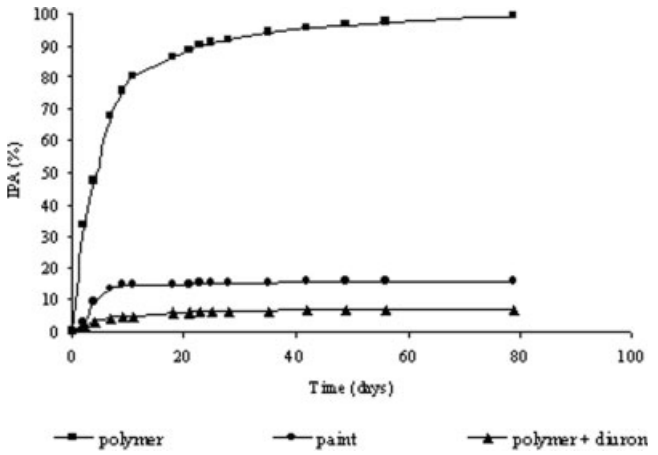


Figure 9 Effect of the formulation on the hydrolysis of P(RA–PHA–IPA) (40% IPA) loaded with paint, diuron, and unloaded polymer monitored by the release of RA, TPA, SA, and IPA and as determined by ESIMS. Hydrolysis was conducted in water at 20°C. The standard deviation was not more than ±5%.

These results underline the potential effect of formulation on polymer aging and its intricacy: two polymers with close chemical structures did not lead to the same conclusions. For P(RA–PHA–IPA), in the presence of diuron, the permeability of the film was shortened, which led to reduced water uptake and byproduct release.

In vitro bioactive molecule release

Diuron, a common hydrophobic and stable herbicide used in agriculture and in antifouling paints, was used as a reference molecule.

The release of diuron was monitored by titration following the two aging protocols (buffer phosphate versus water) and for polymer and paint. We carried out the titration by determining the released amount in the surrounding medium by HPLC or ESIMS and by quantifying the concentration of molecules remaining in the film by EDX.

Figure 11 describes the diuron release data obtained for the two copolymers immersed in buffer or water. In the case of P(IPA–TPA–SA–RA), only the blend of the polymer with diuron was studied. Similar release patterns were found for the release in buffer phosphate and water: about 32% of the molecules were released after 25 days of immersion. In the case of P(RA–PHA–IPA), both the aging conditions and formulation had a great effect on the release. When the polymer was formulated as paint, the release was enhanced: 60% of the molecules were released after 25 days, and then a constant release was reached. In the case of the polymer, the release was restricted to 18%. This result could be explained by the larger permeability of the film, which was already observed in the water uptake. Film porosity and texture were dependent on the pigment loading and also the wide variation in particle size and shape. The scanning electron micrographs of paint surfaces (data not shown) showed that there were enough paint vehicles to fill the interstices between the particles: the critical pigment volume concentration was not reached. Consequently, the variation of permeability could be explained by changes in the interactions between macromolecular chains of the polymers due to the presence of fillers.

In buffer at 37°C, 30% of the diuron was released to the surrounding medium after 25 days. The increase in temperature affected the diffusion rate.

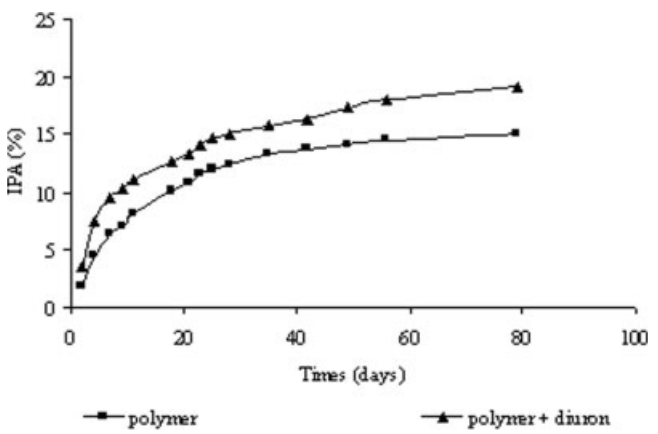


Figure 10 Effect of formulation on the hydrolysis of P(IPA–TPA–SA–RA) 13 : 51 : 19 : 17 w/w loaded with diuron and unloaded monitored by the release of RA, TPA, SA, and IPA and as determined by ESIMS. Hydrolysis was conducted in water at 20°C. The standard deviation was not more than ±5%.

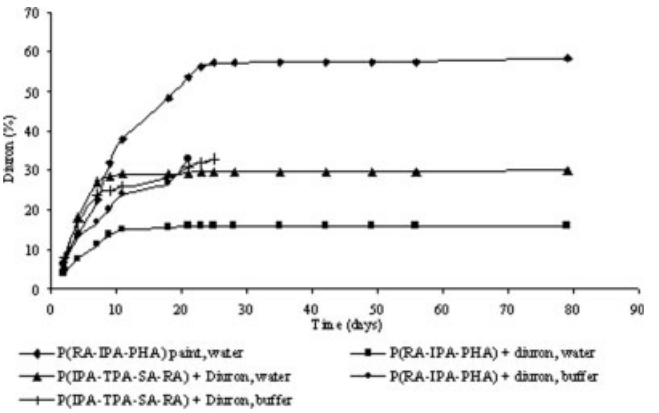


Figure 11 *In vitro* release of diuron from P(IPA–TPA–SA–RA) 13 : 51 : 19 : 17 w/w and P(RA–PHA–IPA) (40% IPA) matrices. Diuron release was conducted in 0.1*M* phosphate buffer (pH 7.4) at 37°C and in water at 20°C. The drug content in the releasing medium was determined by HPLC for the buffer solutions and by ESIMS for the water solutions. The standard deviation was not more than ±5%.

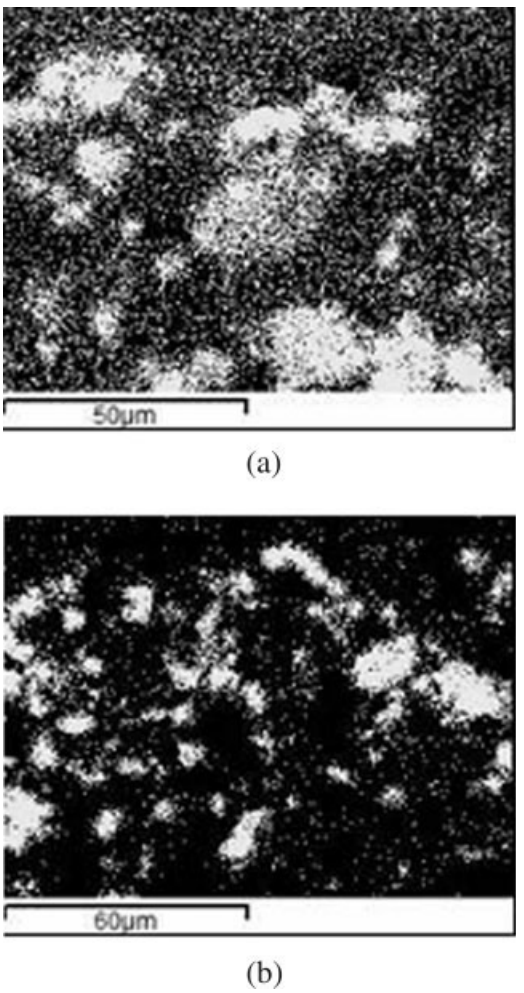


Figure 12 X-ray maps of distribution of diuron in the cross section of films (a) before immersion and (b) after 2 months of immersion in water at 20°C.

Parallel to the chromatographic analysis of the releasing medium, EDX analysis of the polymer remnants was performed. It enabled us to quantify the content of active molecules retained in the film and led to their spatial distribution. It presented many other advantages, including no limit on the aging conditions (e.g., salt amounts) and the visualization of the biocides. Figure 12 displays the X-ray maps obtained for diuron before and after 2 months of immersion. The percentage of released molecules obtained by EDX analysis is also indicated. Before immersion, the distribution was inhomogeneous; many clusters 20 µm in length were observed.

The two X-ray maps indicate a significant decrease in diuron concentration in the thickness of paint film during the immersion. The percentage of released molecules reached 50% after 2 months.

These data were confirmed by the titration of released molecules in the surrounding water. The calculated percentages determined by the two methods were similar: for example, after 2 months of immersion, the ratio of released molecules was 0.50 and 0.55 by EDX analysis and ESIMS, respectively.

These results showed that poly(ester anhydride)s could be used as matrix for the controlled release of biocides from paint. The kinetics of release may be modulate by choosing the nature of fillers used and make it possible to maintain protection for many months.

CONCLUSIONS

This article presents the synthesis of copolymers composed of RA, SA, IPA, TPA, and PHA and the study of their hydrolysis and controlled release in buffer phosphate and in water with complementary analytical methods. Preliminary trials of formulation by the mixture of the polymer with various fillers proved that poly(ester anhydride)s were compatible with paint preparation. The results underline that both aging conditions and formulation modify the polymers during immersion. In the case of P(RA–PHA–IPA), the permeability of the film was increased and promoted water uptake, degradation of the polymer, and release of byproducts and diuron. The results will enable us to enlarge the use of these copolymers from the medical field to potential environmental applications.

References

1. Champ, M. A. Sci Total Environ 2000, 258, 21.
2. Abbott, A.; Abel, P. D.; Arnold, D. W.; Milne, A. Sci Total Environ 2000, 258, 5.
3. Evans, S. M.; Birchenough, A. C.; Brancato, M. S. Mar Pollut Bull 2000, 40, 204.
4. Boxall, A. B. A.; Comber, S. D.; Conrad, A. U.; Howcroft, J.; Zaman, N. Mar Pollut Bull 2000, 40, 898.
5. Alzieu, C.; Sanjuan, J.; Michel, P. Mar Pollut Bull 1986, 17, 494.
6. Evans, S. M.; Leksono, T.; McKinnel, P. D. Mar Pollut Bull 1995, 30, 14.
7. Langlois, V.; Vallée-Réhel, K.; Péron, J. J.; Le Borgne, A.; Walls, M.; Guérin, P. Polym Degrad Stab 2002, 76, 411.
8. Uhrich, K. E.; Gupta, A.; Thomos, T. T. Macromolecules 1995, 19, 2184.
9. Muggli, D. S.; Burkoth, A. K.; Keyser, S. A. Macromolecules 1998, 31, 4120.
10. Domb, A. J.; Langer, R. Macromolecules 1989, 22, 2117.
11. Uhrich, K. E.; Cannizzaro, S.; Langer, R.; Shakesheff, K. Chem Rev 1999, 99, 3181.
12. Sampath, P.; Brem, H. Cancer Control 1998, 5, 130.
13. Park, E. S.; Maniar, M.; Shah, J. C. J Controlled Release 1998, 52, 179.
14. Stephens, D.; Li, L.; Robinson, D. J Controlled Release 2000, 63, 305.
15. Masters, D. B.; Berde, C. B.; Dutta, S. Pharm Res 1993, 10, 1527.
16. Carino, G. P.; Jacob, J. S.; Mathiowitz, E. J Controlled Release 2000, 65, 261.
17. Domb, A. J.; Maniar, M. J Polym Sci Part A: Polym Chem 1993, 31, 1275.

18. Domb, A. J.; Nudelman, R. J Polym Sci Part A: Polym Chem 1995, 33, 717.
19. Guo, W. X.; Huang, K. X.; Tang, R.; Chi, Q. Polymer 2004, 45, 5743.
20. Leong, K.; Broot, B.; Langer, R. J Biomed Mater Res 1985, 19, 941.
21. Uhrich, K. E.; Whitaker, K.; Schmeltzer, R. Polym Mater Sci Eng 2001, 84, 215.
22. Leong, K.; Domb, A. J.; Ron, E.; Langer, R. Encyclopedia of Polymer Science and Technology; Wiley: New York, 1989; Vol. 10, p 648.
23. Leong, K.; D'Amore, P.; Maletta, M.; Langer, R. J Biomed Mater Res 1986, 20, 51.
24. Brem, H.; Mahaley, S.; Vick, N.; Black, K.; Schold, S.; Burger, P.; Friedman, A.; Kenealy, J. J Neurosurg 1991, 74, 441.
25. Brem, H.; Piantadosi, S.; Burger, P.; Walker, M.; Schold, S. Lancet 1995, 345, 1008.
26. Goepferich, A. Biomaterials 1996, 17, 103.
27. Krasko, M. Y.; Shukanov, A.; Ezra, A.; Domb, A. J. J Polym Sci Part A: Polym Chem 2003, 41, 1059.
28. Krasko, M. Y.; Domb, A. J. Biomacromolecules 2005, 6, 1877.