Biodegradable conductive composites of poly(3-hydroxybutyrate) and polyaniline nanofibers: Preparation, characterization and radiolytic effects

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Abstract. Poly(3-hydroxybutyrate) is a biodegradable polyester produced by microorganisms under nutrient limitation conditions. We obtained a biodegradable poly(3-hydroxybutyrate) composite having 8 to 55% of chemically *in situ* polymerized hydrochloric acid-doped polyaniline nanofibers (70–100 nm in diameter). Fourier transform infrared spectroscopy and X-rays diffractometry data did not show evidence of significant interaction between the two components of the nanocomposite, and polyaniline semiconductivity was preserved in all studied compositions. Gamma-irradiation at 25 kGy absorbed dose on the semiconductive composite presenting 28% of doped polyaniline increased its conductivity from $4.6 \cdot 10^{-2}$ to 1.1 S/m, while slightly decreasing its biodegradability. PANI-HCl biodegradation is negligible when compared to PHB biodegradability in an 80 day timeframe. Thus, this unprecedented all-polymer nanocomposite presents, at the same time, semiconductivity and biodegradability and was proven to maintain these properties after gamma irradiation. This new material has many potential applications in biological science, engineering, and medicine.

Keywords: nanocomposites, gamma-irradiation, biodegradable polymers, polyaniline, poly(hydroxybutyrate)

1. Introduction

Biodegradable polymers are defined as macromolecules in which the primary degradation mechanism is through the action and metabolism of microorganisms [1]. In general, biodegradable polymer materials are degraded into biomass, carbon dioxide, and/or methane. Thus, the macromolecular backbone suffers breakdown and is used as a source of carbon and energy.

A major forefront for the application of biodegradable polymers is in medical science and technology. They are used as temporary substitutes for natural tissues and degrade *in vivo* over a predetermined period of time generating safe end products. Among

these materials, polyhydroxyalkanoates (PHAs) [2] along with poly(α -hydroxy acids) [3] are the most used biodegradable polymers.

PHAs are polyesters produced by microorganisms under nutrient limitation conditions [4]. Poly(3-hydroxybutyrate) (PHB) and copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (PHB-co-HV) can be considered as the most known PHAs. They present biodegradability and good biocompatibility and are frequently used, as neat substances or composites, for fabrication of medical supplies, including: sutures, screws, bone plates, orthopedic pins, guided tissue repair/regeneration devices, nerve guides, vein valves, bone marrow scaffolds,

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ocular cell implants, substitutes for skin and dura tissue, wound dressing, and hemostats [2]. PHB-co-HV can also be used as biodegradable drug carriers in implants loaded with therapeutic substances such as antibiotics [5] or anticancer drugs [6]. Among all PHAs, PHB appears to have a broader range of applications due to its good combination of mechanical, biological, and surface properties [7].

The previously-cited PHB characteristics turn this polymer into a very interesting matrix for the fabrication of multifunctional materials, namely polymer nanocomposites. In spite of their innumerable potential applications, scientific information on nanocomposites based on PHB and other PHAs are rather limited. Reports on the fabrication of PHB [8] or PHB-co-HV [8, 9] composites through melting intercalation showed that improved thermal tensile properties could be achieved in composites presenting nanodispersions of organoclay on PHB-co-HV matrix, although, thermal degradation of PHB after melting process might have counterbalanced the improving effect of this filler on the nanocomposite [9]. Nanocomposites of PHB-co-HV/multiwalled carbon nanotubes are reported to exhibit higher thermal stability than PHB-co-HV itself [10].

In the present work, we report the development of a simple, straightforward method to fabricate PHB/polyaniline (PANI) nanocomposites. PANI is an intrinsically conductive polymer (ICP) which has been extensively studied over the last twenty years, in both theoretical [11, 12] and practical points-of-view [13, 14]. This polymer combines striking properties such as metal-like characteristics [15], reversible doping level [16], and good biocompatibility [17]. PANI presents four oxidation states, which can arranged in crescent order of oxidation level as leucoemeraldine, emeraldine, nigraniline and pernigraniline. Each one of these substances presents a base and a salt form [18]. The electrically conductive form of PANI is the emeraldine salt, shown in Figure 1. Such versatile polymer attracts researchers from a number of areas, leading to fre-

quent reports on PANI practical uses. In recent years, our research group reported a new application of PANI as a radiostabilizing agent in nanocomposites of poly(methyl methacrylate) (PMMA) and PANI nanofibers submitted to radiosterilization [19, 20].

The composites presented in this work have, at the same time, biodegradability and semiconductivity and are made of *in situ* chemically polymerized HCl-doped polyaniline (PANI) nanofibers as fillers and the bacterially-produced Brazilian commercial PHB as the matrix.

Our methodology of choice for the preparation of nanofibers embedded into PHB matrix is based on the rapid mixing of the oxidant agent with an initial short period of stirring, an approach firstly presented by Huang and Kaner, in 2006 [21], to produce bulky quantities of PANI nanofibers in water and other solvents. Following the addition of the agent, fast consumption of the oxidant occurs, and polymer chain overgrowth is prevented. In such conditions, PANI nanofibers are allowed to form, as nanostructures of this particular morphology appear to be intrinsic to PANI and other ICPs [21, 22]. Previous publications on *in situ* polymerization of PANI in polymer matrix emulsion were based on traditional slow, dropwise addition of reagents and continuous stirring [23, 24], which frequently results in irregular PANI agglomerates. [21, 25]. In order to produce PHB/PANI nanofiber composites, we performed aniline (Ani) polymerization reaction in the presence of emulsion of PHB in water/chloroform/SDS. In 2007, Ali and co-workers [26] prepared semiconducting PANI/polyvinyl alcohol (PVA) composites containing fibrous nanoclusters and aggregates of PANI, by irradiating a solution of HCl, Ani and PVA with gamma rays up to 50 kGy. Recently, some nanofiber composites of PHB and PANI were fabricated by electrospinning method [27]. However, to our best knowledge, *in situ* chemical polymerization of PANI resulting in nanofibers embedded into polymer matrix was not reported, so far. In this work, we obtained good quality HCl-doped PANI nanofibers/PHB composites presenting semiconductivity and biodegradability. Thus, a new methodology is suggested for the manufacturing of a polymer matrix nanocomposite.

PHB/PANI nanocomposites were gamma-irradiated at 25 kGy dose, the standard sterilization absorbed

Figure 1. Polyaniline emeraldine salt, an electrically conducting form of polyaniline. A^- is a counter-ion.

dose for medical supplies, in order to assess the effects of such procedure on its electrical conductivity and biodegradability. Our results demonstrate that gamma-irradiation improved electrical conductivity while presenting little influence on the biodegradability of the nanocomposite.

Many uses of this new material can be possible, e.g., as supports for electrically stimulated/guided cell growth, similarly to what is proposed for other PANI/biodegradable polymer nanocomposites [28, 29].

2. Experimental

Aniline monomer (Ani), ammonium peroxydisulfate (APS) and ammonium hydroxide (NH_4OH) (Vetec, Rio de Janeiro, Brazil), methanol, chloroform and hydrochloric acid (HCl) (Dinâmica, Londrina, Brazil), and sodium dodecylsulfate 99% (SDS) (Sigma-Aldrich, Saint Louis, USA) were of analytical grade. Ani was treated with stannous chloride for 24 h and vacuum distilled prior to use. Chloroform was dried and distilled. Commercial PHB (BIOCYCLE®, M_w 530 kg/mol, PHB Industrial S. A., Usina da Pedra, Brazil) was purified by extraction with methanol in a Soxhlet apparatus for 48 h and air dried before use. Other chemicals were used as received.

A typical procedure for the preparation of PHB/PANI nanocomposites was based on Ruckenstein and Yang [23]: a solution of 0.2 g of SDS in 2 ml of distilled deionized water was placed in a 50 ml Becker flask. To a 5 ml chloroform solution containing 0.4 g of PHB, previously dissolved under reflux for 24 hours, was added 0.12 g of Ani, thus Ani to PHB ratio in mass (Ani:PHB) equaled 0.3. The resulting mixture was added to the aqueous solution under intense magnetic stirring, until forming an emulsion. A solution of 0.19 g of the oxidant agent, APS, in 10 ml of HCl $1 \text{ mol} \cdot \text{dm}^{-3}$ was then placed at once in the emulsion and the stirring was kept working just long enough to allow an homogeneous mixing of the solutions and until an initial changing in the color of the system was perceptible (approximately 40 s). The reaction was left to proceed for 3 hours at room temperature, then quenched with methanol. The precipitate formed was filtered in an Hirsch funnel, washed with methanol followed by water and HCl $1 \text{ mol} \cdot \text{dm}^{-3}$. The green coarse powder obtained was dried at room tempera-

ture in a desiccator until constant weight and named as composite I. Similar procedure was followed to produce composites having initial Ani:PHB of 0.75, 1.0 and 1.5, keeping all components but Ani in the same proportion (composites I, III and IV, respectively). For comparison, PANI was synthesized in the absence of PHB in the above-described conditions. For the same reason, the PHB chloroform solution was submitted to the emulsification process in the presence of APS and SDS and left to stand for 3 hours before precipitation of the polymer with methanol. Yields were calculated considering 100% as the sum of Ani, PHB and SDS weights in the reactional media [23, 24]. Samples were prepared in duplicate. PANI content in each polymer was determined gravimetrically by extraction of the soluble PHB matrix in a Soxhlet extractor with chloroform. Residual insoluble doped PANI was dried in desiccator until constant weight. Gamma irradiation of powder samples were done in air, at room temperature, in a Gammacell irradiator (220 SN 65R Source Model Number: C-198 MDS Nordion Inc, Kanata, Canada) at 25 kGy dose and $2.61 \text{ Gy} \cdot \text{s}^{-1}$ dose rate.

Room temperature electrical conductivity measurements were performed in an electrometer Keithley (model 617 Keithley Instruments Inc. Cleveland, USA) by the two-probe method on pellets of power samples pressed at 3 MPa for 30 s. Samples were left to rest for 72 h before the readings. The morphologies of the composites were investigated by scanning electron microscopy (SEM) (JEOL JSM-5900, Tokyo, Japan) on gold-coated samples. Fourier Transform Infrared spectrometry (FTIR) experiments were performed on KBr pellets (Bruker IFS66, Ettlinger, Germany). The X-ray diffraction patterns (Rigaku D/max-2200, Texas, USA) were taken with $\text{CuK}\alpha$ radiation, $1.54 \text{ \AA}$, 40 kV, 20 mA, in the range of diffraction angle $2\theta = 5\text{--}35^\circ$ in a continuous scanning type at 1.2° per min. The background and the amorphous halo were subtracted according to the methodology established by Ruland [30]. Biodegradability determination followed modified Sturm's test conditions [31], according to ASTM D5338-98 [32]. 0.8 g of the samples and 100 g of inoculant medium (worm humus, Gnumus, Vitória de Santo Antão, Brazil, 50% of dry solids) mixed in 200 ml of distilled water were used in each experiment. Measurements were interrupted

when CO₂ production remained approximately the same as the control sample for at least ten days. The accumulation of CO₂ (P) was calculated according to the Equation (1):

$$P = \sum_{n=1}^i \frac{m_{ai} - m_{ci}}{m_{ci}} \quad (1)$$

where n – number of days, m_{ai} – mass of CO₂ produced by a sample in the i day, m_{ci} – mass of CO₂ produced by the control sample in the i day.

Values were corrected to take in account only PHB percentage in the total sample mass.

Figure 2. SEM images of neat PHB (a) $\times 1000$, (b) $\times 30000$; PHB/PANI-HCl (c) nanocomposite II, nanocomposite IV. Residual PANI-HCl salt after extraction of PHB fraction with chloroform (e) $\times 1700$ and (f) $\times 20000$.

3. Results and discussion

3.1. Morphology of PHB and PHB/PANI composites

Scanning electron micrographs of treated PHB samples exhibited spherical granules ranging from 10 μm (Figure 2a) to 260 nm (Figure 2b) in diameter. Granules of PHB found in the cytoplasm of the bacteria *Alcaligenes eutrophus*, the microorganism used in the production of Brazilian PHB, presented diameters from 200 to 500 nm [33]. Larger granular structures may be formed during extraction process. Composites of PHB and HCl-doped PANI (PHB/PANI-HCl) presented distinct morphologies from the neat PHB. Figures 2c and 2d show electron micrograph images from composites II and IV where fibers or rods with diameters around 70–100 nm are visualized. After extraction of PHB with chloroform, residual PANI-HCl has a sponge-like appearance (Figure 2e). A network of fibrils can be identified from a higher magnification image (Figure 2f). Images of composite I did not allow a clear visualization of PANI nanostructures. These results characterize formation and good dispersion of PANI-HCl nanofibers into the PHB matrix when polymerized *in situ* with rapid mixing of APS.

Previous works on PANI composites fabricated by *in situ* emulsion polymerization with polystyrene [23] and PMMA [24] used traditional slow addition of reagents during PANI synthesis. Information on the morphology of PANI structures and the methodology for calculating its content in the composite were not given at that time. At the present it is difficult to infer about the role of each emulsion component on the final morphology of PANI when rapid mixing is performed. In one hand, intrinsic fibrillar morphology is reported to be prevalent in both aqueous and organic media [21]. Moreover, nanofibrillar morphology is also observed when PANI synthesis occurs at the interface of water/immiscible organic solvent biphasic systems [21, 34]. Hence, it is arguable the formation of such structures regardless the existence of two solutions of immiscible solvents in an emulsified media, which is

the environment found in our experiment. On the other hand, we used SDS, a surfactant agent to promote emulsification. Since surfactant agents are known to act as soft templates for PANI nanofibers fabrication [35, 36], the SDS influence on nanofiber formation cannot be ruled out.

Our present approach appears to be very promising for the fabrication of fibrillar PANI nanocomposites, because it precludes the use of hard templates, electrospinning apparatus, or ionizing radiation sources in order to obtain nanofibers, and, at the same time, makes possible to embed inexpensive, insoluble, self-assembled HCl-doped PANI nanofibers directly into host polymer matrix.

3.2. Yields, PANI content and electrical conductivity of the PHB/PANI-HCl nanocomposites

Composites obtained as described in the previous section were analyzed for yields, PANI content, and conductivity. Results are shown in Table 1. Nanocomposite I presented the highest yield among the studied materials, followed by nanocomposite IV. Neat PHB conductivity was in the range of 10^{-12} S/m, while all nanocomposite materials obtained were above the electrical percolation threshold and presented semiconductivity. Since PHB is an electrical insulator, conductivity presented by the composites is due to PANI-HCl. For nanocomposites I–III, conductivities were situated in the same order of magnitude, around $1.0 \cdot 10^{-2}$ S/m. PANI pristine polymerized in the emulsion water/chloroform had a conductivity of 1.2 S/m which is close to the conductivity reached by composite IV, $7.0 \cdot 10^{-1}$ S/m. Fluctuations in conductivity of PANI and its composites prepared under controlled conditions may be attributed to a large number of factors, including the presence of humidity [16], morphology and the dispersion of the filler in the composite [37]. Thus, all the samples presented comparable conductivities and are suitable candidates for applications as semiconducting biomaterials.

Table 1. Yields, PANI-HCl contents, and conductivity of PHB/PANI-HCl composites

Composite PHB/PANI	Ani/PHB ratio	Yield of PHB/PANI [%]	PANI-HCl [%]	PANI-HCl/Ani [%]	Conductivity [S/m]
I	0.30	93	8	43	$2.1 \cdot 10^{-2}$
II	0.75	51	28	45	$4.6 \cdot 10^{-2}$
III	1.00	46	55	64	$1.8 \cdot 10^{-2}$
IV	1.50	71	48	68	$7.0 \cdot 10^{-1}$

As composite II presents good conductivity and the lower PANI-HCl content in which nanofibers could be visualized, it was chosen for experiments of gamma irradiation and biodegradation. After exposed to a 25 kGy absorbed dose, conductivity of nanocomposite II increased in two orders of magnitude and reached 1.1 S/m. Hence, nanocomposite semiconductivity was maintained after irradiation procedure. This result opens up an opportunity for the fabrication of gamma rays-sterilizable, biodegradable, electroactive supports for a number of biotechnology applications.

Very little information is available on conductivity behavior of chemically synthesized PANI exposed to gamma-rays. A direct comparison with previous published results is rather difficult, since most reports are based on conductivity onset observed when gamma radiation induces doping process in insulator PANI emeraldine base, resulting in semiconducting PANI forms, either in composites [38–41] or pristine PANI films [42]. Thus, conductivity increases observed were of several orders of magnitude.

Our material presented semiconducting filler content above the electrical percolation limit, hence radiation-induced conductivity enhancements were smaller than those evidenced in the previously-cited works. Conversely, in a report on conducting PANI pellets subjected to up to 400 kGy dose, no increase in conductivity was detected in the 0–200 kGy dose range, with a less than 25% increase in the 300–400 kGy range [43]. The increase in conductivity was consistent with the increase in spin or radical concentration detected by Electron Paramagnetic Resonance (EPR) measurements. These and other defects are capable of acting as charge carriers throughout ICPs polymer chains [44–46], thus, explaining the higher conductivity observed.

3.3. FTIR analysis

FTIR characterization of PHB and PANI-HCl, nanocomposites II (before and after irradiation at 25 kGy) and nanocomposite IV are presented in Figure 3. PHB spectrum (Figure 3a) exhibits C=O stretching at 1724 cm^{-1} and C–O stretching at 1283 cm^{-1} . These results are in good agreement with previous reports on FTIR data of PHB [47]. PANI-HCl spectrum (Figure 3b) exhibits band at $\sim 1573\text{ cm}^{-1}$ attributed to C=N stretching of the

Figure 3. FTIR spectra of (a) PHB, (b) PANI HCl, (c) nanocomposite IV, (d) non-irradiated nanocomposite II and (e) nanocomposite II irradiated at 25 kGy

quinoid diimine unit ($\text{N}=\text{Q}=\text{N}$). C–C aromatic ring stretching of the benzenoid diamine unit ($\text{N}-\text{B}-\text{N}$) appears at 1481 cm^{-1} [48]. Based on previous reports, the very close intensities presented by these two bands identify the emeraldine oxidation state of PANI [49]. The $\sim 1140\text{ cm}^{-1}$ band is a vibrational mode of $\text{B}-\text{NH}^+=\text{Q}$ or $\text{B}-\text{NH}^+=\text{B}$. Both bands are related to the doping level of PANI-HCl and can be used as a comparison of PANI doping process when formed *in situ* in the PHB matrix. Nanocomposites II and IV spectra (Figures 3c–3e) exhibit the same major bands present in PHB and PANI-HCl spectrum. Only marginal shifts were detected in these nanocomposites, thus intermolecular interactions between the two components are negligible, e.g., $n-\pi$ interactions of the unshared pair of electron of the carbonyl group in PHB and the π -electrons of the aromatic ring of PANI-HCl are not as perceptible in these composites, as they are in blends of other carbonyl/aromatic polymers [50, 51]. Comparison between the changing in intensity of ~ 1303 and $\sim 1140\text{ cm}^{-1}$ bands shows that doping level of PANI HCl in these nanocomposites are similar to the doping level of the product obtained in the absence of PHB, even in the gamma-irradiated nanocomposite II (Figure 3e).

3.4. XRD analysis

X-ray diffractogram of PHB is shown in Figure 4a. Diffraction peaks in $2\theta = 13.4, 16.8, 20, 22.2$ and 25.5° have a similar pattern when compared with previous crystallographic data for this material [52]. The degree of crystallinity calculated through Ruland's method considers the total area of the diffracted peaks subtracted of the amorphous halo

Figure 4. X-ray diffractograms of (a) PHB, (b) nanocomposite II (28% of PANI), (c) nanocomposite IV (45% of PANI) and (d) PANI-HCl

[30]. Our results indicated a degree of crystallinity of 53%. This method was previously used to assess the crystalline fraction of as-supplied Brazilian PHB with similar results [53]. PHB/PANI-HCl nanocomposites II and IV diffraction patterns are very close to those exhibited by neat PHB (Figures 4b and 4c). Nevertheless, it is possible to notice a slight shift to higher angles with the increase of PANI-HCl content, indicating a decrease in the interplanar distance of PHB crystals. In neat PANI-HCl diffractogram, two broad, relatively intense peaks at 20.3 and 25.1° can be seen, along with two less intense 8.5 and 15.0° (Figure 4d). This pattern is related to amorphous polyanilines synthesized in the presence of surfactant agents such as SDS and dodecylbenzenesulfonate (SDBS) [54]. When prepared in the absence of such agents or secondary dopants, PANI diffractograms tend to exhibit a single broad weak peak at $2\theta = 24.7^\circ$ [54, 55]. This evidence may indicate a direct inclusion of SDS surfactant used in the emulsion polymerization. Nevertheless, the absence of new crystalline orders corroborates the assumption of poor interaction between PHB and PANI. Since some characteristic diffraction peaks of PANI-HCl partially overlapped those of PHB, crystalline fraction calculations for the nanocomposites did not present consistent results.

3.5. Biodegradability of PHB/PANI-HCl nanocomposite

Sturm's test was applied to powders of PHB, PANI-HCl and nanocomposite II. Attempts to promote biodegradation of neat non-irradiated and irradiated PANI-HCl under the tested conditions did not result

in any appreciable CO_2 production. Changes in PHB biodegradation could be perceived after 10 days. Non-irradiated PHB began to biodegrade releasing lower amounts of CO_2 than the other samples and remained this way until the 50th day. Similarly, irradiated PHB started to evolve lower quantities of CO_2 than non-irradiated and irradiated nanocomposite II around the 15th day. Nanocomposite II presented similar behavior in non-irradiated and irradiated samples until the 22th day, when the irradiated samples started to exhibit a small decrease in CO_2 production. Non-irradiated nanocomposite II maintained higher rates of CO_2 release until the 35th, when biodegradation of both nanocomposites subsided. Neat PHB samples, in turn, showed rampant increase in CO_2 production after the 45th to approximately the 70th day, when both sample media started to show signs of exhaustion. Figure 5a shows P versus time (in days). Figure 5b shows the first derivate of P as a function of time (dP/dT) and reveals the differences in biodegradation behavior of the studied samples. Nanocomposite samples have a markedly higher biodegradation around the first month of testing (region I) while neat PHB samples showed increased activity starting in the second month of observations (region II), when practically no activity is detected for nanocomposites biodegradation. CO_2 accumulated values in region II are higher for irradiated neat PHB as evidenced by higher peaks heights for this sample.

Gamma irradiations affected the studied materials in opposite ways: while irradiated neat PHB reached higher values of accumulated CO_2 production than non-irradiated neat PHB samples, irradiated nanocomposite materials showed lower CO_2 accumulation values. Irradiation with gamma rays provokes main chain scissions in PHB structure. In the range of 0–50 kGy, the G value (number of scissions per 100 eV of absorbed energy) for BIOCYCLE® is 15.7 [53]. This number of scissions is large enough to reduce the initial molar mass in one order of magnitude after a 25 kGy absorbed dose. Such damage might ease the biodegradation process in neat samples and explain higher CO_2 production of these materials. In nanocomposites, radiolysis may not be as remarkable because of a putative radiostabilization of PANI-HCl on PHB matrix, in the same fashion reported for PMMA [19, 20]. Moreover,

Figure 5. a) Accumulation of CO₂ (P) of powder samples of PHB: non-irradiated (—) and irradiated at 25 kGy (—□—); PHB/PANI-HCl (28% of PANI-HCl) non-irradiated (—●—) and irradiated 25 kGy (—○—). Results are normalized by the P value of the control (inoculant media). Results for the nanocomposite II were also corrected by the percentage of PHB present (72%). b) First derivative of P in time. Region I shows roughly the first month of observations, when intense peaks associated with nanocomposite biodegradation are present, while neat PHB samples show relatively low CO₂ accumulation. Region II delimits approximately the final 40 days of observation, when most of the neat PHB biodegradation occurs.

differences in biodegradation of irradiated neat PHB matrix and PANI nanocomposite may be governed by other factors, as minor alterations caused by irradiation on the surface of the nanocomposite. PANI presents good biocompatibility, either in its emeraldine, nigraniline or leucoemeraldine oxidation states [17]. Moreover, the presence of electrically conducting PANI in polymer nanocomposites with biodegradable polymers allows the fabrication of suitable substrates for tissue engineering, in which electrical stimulation is important to promote cell proliferation and tissue regeneration [28, 29].

PHB/PANI nanocomposites presented in this work have the basic requirements to become one of these multifunctional nanotechnology materials, as they are composed of biocompatible polymers and possess electrical semiconductivity.

Many factors, besides materials biocompatibility may also interfere in substrate performance, e.g. minor synthesis residues such as oligoanilines and toxic unreacted aniline monomers. Thus, further investigations are needed in order to assess the viability of cell attachment and proliferation on the surface of the nanocomposites presented in this work, as well as the influence of electrical stimulation on these and other cytological processes.

4. Conclusions

PHB/PANI-HCl nanocomposites were obtained by *in situ* emulsion polymerization of HCl-doped PANI, with rapid mixing of oxidant, in the presence of dissolved PHB. The nanofiller presented fibrillar or rod-like shape and 70–100 nm in diameter. Conductivity of the nanocomposites were in the range of 10^{-4} to 10^{-2} S/cm. FTIR analysis revealed similar doping levels in PANI-HCl when embedded into the PHB matrix or in the pristine form. XRD patterns of the semicrystalline PHB were present in nanocomposites and PHB diffractograms. PANI-HCl peaks suggested some degree of association with a surfactant agent, SDS, used to promote emulsification. Nevertheless, FTIR and XRD data did not show evidence of significant interactions between PHB and PANI-HCl nanofibers in the composite. Biodegradation experiments showed that PHB biodegradability is enhanced after irradiation at 25 kGy. Biodegradation of non-irradiated and irradiated PHB occur more intensely after 30 days, but with a larger accumulated production of CO₂ detected for the irradiated samples. PHB/PANI-HCl nanocomposites present a faster biodegradation than PHB in the first month of measurements. After this period, nanocomposite biodegradation subsided. Irradiated samples exhibited a decrease in the biodegradation process of the nanocomposite after the 22th day of testing. Therefore, accumulated CO₂ production is higher in the non-irradiated samples. Neat PANI-HCl biodegradation is negligible when compared to PHB biodegradability in an 80 day timeframe.

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