Functionalization of aliphatic polyesters by nitroxide radical coupling†

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Introduction

Biodegradable aliphatic polyesters, such as poly(lactic acid) (PLA), poly(butylene succinate) (PBS), and poly(butylene succinate-*co*-butylene adipate) (PBSA), are becoming increasingly important given that they have thermoplastic processability and thermo-mechanical properties.[1] However, their versatility and successful use as commodity plastics is limited, as is their exploitation in biomedical, electronic and optical sectors. The lack of reactive functionalities limits the use of biodegradable aliphatic polyesters in a series of demanding applications, where the precise control and placement of functionality is critical.

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There are two main approaches to obtain functionalized biodegradable aliphatic polyesters: chain-end and side-chain functionalization.[2] While a variety of examples of chain-end functionalized polyesters obtained by adding specific functional nucleophiles during the ring-opening polymerization (ROP) have been reported,[3] side-chain functionalization has been much less investigated. With side-chain functionalization greater quantities of functional pendant groups can be incorporated along the polymer backbone. However the preparation, which is typically made by the ROP of functionalized lactones or by the step-growth polymerization of functional monomers, is rather expensive, and is generally used only for materials aimed at special applications, such as biomedicine.

More attractive strategies based on post-polymerization modification methods include the polymerization of monomers with moieties that are inert towards the polymerization conditions, but which can be quantitatively converted into a broad range of functional groups. One example is the combination between ROP or step-growth polymerization with the click chemistry approach.[4,5]

Modification by radical grafting is an interesting and more convenient post-polymerization modification strategy for preparing side-chain functionalized polyesters, following a well-known approach used for preparing functionalized polyolefins.[6–8] In the case of biodegradable aliphatic polyesters, it

has been reported the peroxide modification, the radical grafting of maleic anhydride (MAH), and the preparation and use of polyester-*g*-MAH.[9–11] The reactivity of polyesters *versus* peroxide radicals was exploited in order to increase the polymer melt viscosity by branching and/or crosslinking.[12–15] Highly branched PLA and PBS were obtained by treating the molten polymers in an extruder or batch mixer with peroxides *via* a coupling reaction between macroradicals. The radical grafting of MAH on PBS, PLA, and copolyesters was also investigated. Carlson *et al.* and Mani *et al.*[16–18] firstly reported the preparation of MAH functionalized biodegradable polyesters by reactive extrusion and their use as interfacial adhesion promoters in blends and composites. This reaction was further investigated by modulating the MAH/peroxide ratio.[19] The grafting yield increased if the amount of peroxide was increased, but with a decrease in the extent of polymer crosslinking. In addition, Signori *et al.*[20] proposed the functionalization of PBSA with MAH in combination with cinnamate-like coagents to increase the grafting yield, following a method previously described for polyolefins.[8,21,22]

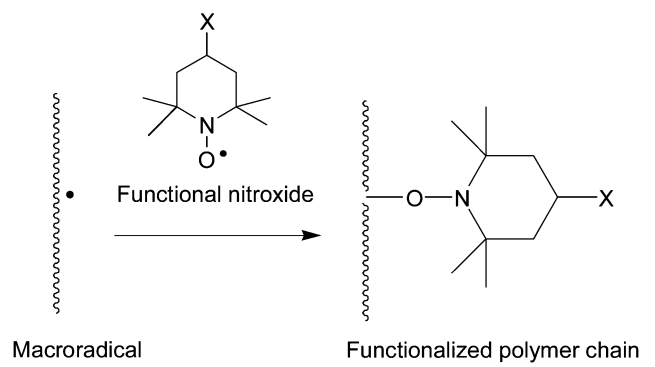
One of the main drawbacks of this approach is the difficulty in pinpointing the covalent grafting of MAH moieties both qualitatively and quantitatively. Indeed, infrared spectroscopy, which is generally used to show stretching vibrations associated with the functional groups, is not very suitable in this case because of the overlap between the C=O stretching signal of the covalent grafted moieties with the C=O stretching signal of the polyester chains. The functionalization degree is generally calculated by laborious acid-base titrations, and the low quantity of grafted groups that is generally achieved and the possible degradation of polymer chains by hydrolysis do not make this approach very reliable. Moreover, the radical functionalization method is affected by poor control and lack of selectivity. Consequently, a procedure providing excellent control of macroradical formation *versus* grafting of functional molecules, together with sensitive analytical techniques, is highly desirable. In this context the radical coupling reaction between a macroradical formed on the polymer backbone by H-abstraction and a functional nitroxide is a very interesting tool to graft specific functionalities onto polymer chains (Scheme 1).

All the attractive features of nitroxide radical coupling reactions, which generally refer to the coupling reaction between a nitroxide radical and a radical generated by Cu(I) or Cu(0) mediated activation, are summarized in Yang *et al.*[23] These kinds of reactions are generally robust, and highly tolerant *versus* functional groups. In addition, the rate of the coupling reaction between the carbon-centered radical and the stable nitroxide is close to the diffusion and is mainly limited by the formation rate of the carbon-centered radical. Importantly, this reaction is also reversible at high temperatures, and the reversibility can be exploited for further functionalization of the polymer through a competitive exchange with other functional nitroxides.

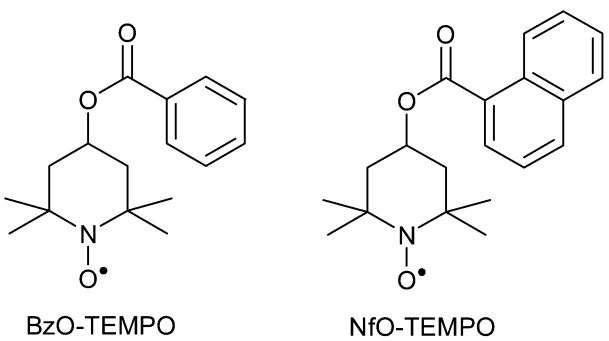
The use of a nitroxide radical coupling reaction to prepare polyethylene functionalized with 2,2,6,6-tetramethylpiperidine-oxly (TEMPO) derivatives was recently reported by our group.[24,25] 4-Hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (HO-TEMPO), 4-benzoyloxy-2,2,6,6-tetramethylpiperidine-1-oxyl (BzO-TEMPO), and 4-(1-naphthoate)-2,2,6,6-tetramethylpiperidine-1-oxyl (NfO-TEMPO) (Scheme 2), a pro-fluorescent nitroxide, were grafted by coupling reaction with polyethylene macroradicals. The method enabled polymer crosslinking to be limited and the number of grafted functional groups to be tuned.[26,27]

In the present paper, we extend our previous work to the radical modification of biodegradable aliphatic polyesters, such as PBS and PLA. Our aim was to (i) improve the grafting efficiency, (ii) limit side reactions, and, (iii) thanks to the use of the pro-fluorescent nitroxide, unequivocally demonstrate that it is really possible to functionalize aliphatic polyesters by a post-polymerization radical reaction.

BzO-TEMPO and NfO-TEMPO were successfully grafted on PBS at two different temperatures, whereas NfO-TEMPO was grafted on PLA in order to assess the versatility of the method. The grafting was confirmed by MALDI TOF MS analysis and fluorescence spectroscopy; the functionalization degree was estimated by UV-Vis and ^{1}H-NMR spectroscopy, whereas the preservation of molecular weight distribution was evidenced by SEC analysis. Finally, by combining theoretical calculations with experimental evidence collected by the EPR analysis of a functionalized PBS sample and by ^{1}H-NMR spectroscopy, a possible grafting site on the PBS chain was identified.



Scheme 1 Simplified mechanism of grafting of a functional nitroxide onto a polymer chain.



Scheme 2 Structure of 4-benzoyloxy-2,2,6,6-tetramethylpiperidine-1-oxyl (BzO-TEMPO) and 4-(1-naphthoate)-2,2,6,6-tetramethylpiperidine-1-oxyl (NfO-TEMPO).

Experimental

Materials

Poly(butylene succinate) (PBS) Bionolle 1001 supplied by Showa HighPolymer Co, Ltd., Japan, MFI (2.16 kg/190 °C) 1.5 g/10 min, and poly(lactic acid) (PLA) 2002D 96% L-lactide supplied by NatureWorks®, USA, MFI (2.16 kg/190 °C) 4–8 g/10 min, were used as polymer matrices. Before processing, PBS and PLA were dried in a vacuum oven for 18 h at 80 °C and 110 °C, respectively. Benzoyl peroxide (BPO, Aldrich), di(*tert*-butylperoxyisopropyl)benzene (mixture of isomers) (DTBPIB, Perkadox 14S-FL, Akzo Nobel), 4-benzoyloxy-2,2,6,6-tetramethylpiperidine-1-oxyl (BzO-TEMPO, Fluka), chloroform-d (99.98% d, Aldrich), 1,4-dinitrobenzene (Aldrich), 1,4-dimethoxybenzene (Aldrich), *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene] malononitrile (Aldrich) were used as received. 4-(1-Naphthoate)-2,2,6,6-tetramethylpiperidine-1-oxyl (NfO-TEMPO) was prepared as previously reported.[25]

Melt functionalization procedure

The melt reactions were carried out in an internal batch mixer (Brabender Plastograph OHG47055) with a chamber of 30 cc. Torque and temperature data were acquired by Brabender Mixing software Win-Mix ver.1.0. The PBS functionalization reactions using BPO as a peroxide were performed at 120 °C, 50 rpm and 6 min (6 min was selected as the time of mixing since the half-life of BPO at 120 °C is about 3 min) (Table 1). The functionalization of PBS using DTBPIB as a radical initiator was carried out at 150 °C, 50 rpm and 14 min (14 min was selected as the time of mixing since the half-life of DTBPIB at 150 °C is about 10.6 min) (Table 1). In a typical experiment, 25 g of PBS were introduced in the hot Brabender chamber, the functionalizing agent was added two minutes after the matrix had melted and the peroxide was introduced in the Brabender chamber one minute later. In order to remove unreacted reagents and byproducts of the peroxide decomposition, small pieces of PBS samples were weighed (around 1.5 g), placed into a cellulose extraction thimble, extracted with boiling methanol for 15 h, and finally vacuum dried to constant weight.

The functionalization reaction of PLA, using DTBPIB as a radical initiator, was carried out at 180 °C, 50 rpm and 14 min (Table 2). The half-life of DTBPIB at 180 °C is 0.6 min; thus, it can be reasonably hypothesized that at the end of the mixing all the peroxide was consumed. In a typical experiment, 25 g of PLA were introduced into the hot Brabender chamber, the functionalizing agent was added two minutes after the matrix had melted and the peroxide was introduced into the Brabender chamber one minute later. The functionalized PLA sample was purified by dissolution in chloroform at room temperature and collected by precipitation in diethyl ether. The procedure was repeated twice.

Table 1 PBS radical functionalization: feed composition, molecular weight, functionalization degree

	Feed composition		Functionalization degree[a]		Molecular weight and distribution		
Sample name	Peroxide (mol%)	f-TEMPO[b] (mol%)	FD_{UV}[c] (mol%)	FD_{NMR}[d] (mol%)	M_n (g mol^{-1})	M_w (g mol^{-1})	M_w/M_n
PBS[e]	—	—	—	—	17 700	42 180	2.38
PBS-120	—	—	—	—	16 900	42 700	2.53
PBS-BPO-120	1	—	—	—	n.d.[f]	n.d.[f]	n.d.[f]
PBS-BT1-BPO-120	1	0.5	0.15	n.d.	15 630	35 100	2.24
PBS-BT2-BPO-120	1	1	0.24	0.24	15 060	34 410	2.28
PBS-NfT1-BPO-120	1	0.5	0.26	n.d.	15 130	33 860	2.24
PBS-NfT2-BPO-120	1	1	0.30	0.29	16 980	39 910	2.35
PBS-150	—	—	—	—	17 420	39 470	2.26
PBS-DTBPIB-150	1	—	—	—	n.d.[f]	n.d[f]	n.d[f]
PBS-BT2-DTBPIB-150	1	1	0.14	n.d	15 970	39 150	2.45

[a] The functionalization degree represents the moles of the grafted functional groups per 100 moles of monomeric units. [b] f-TEMPO: functional TEMPO derivative. [c] Determined by the UV-Vis calibration curve. [d] Determined by ^{1}H-NMR analysis. [e] PBS pristine polymer extracted with boiling MeOH for 15 h. [f] Not determined because the sample is partially insoluble in $CHCl_3$.

Table 2 PLA radical functionalization: feed composition, molecular weight, functionalization degree

	Feed composition		Functionalization degree[a]		Molecular weight and distribution		
Sample name	Peroxide (mol%)	f-TEMPO[b] (mol%)	FD_{UV}[c] (mol%)	FD_{NMR}[d] (mol%)	M_n (g mol^{-1})	M_w (g mol^{-1})	M_w/M_n
PLA	—	—	—	—	111 460	199 340	1.79
PLA-180	—	—	—	—	108 750	184 730	1.70
PLA-DTBPIB-180	0.5	—	—	—	n.d.[e]	n.d.[e]	n.d.[e]
PLA-NfT2-DTBPIB-180	0.5	1	0.04	0.04	69 290	168 070	2.43

[a] The functionalization degree represents the moles of grafted functional groups per 100 moles of monomeric units. [b] f-TEMPO: functional TEMPO derivative. [c] Determined by the UV-Vis calibration curve. [d] Determined by ^{1}H-NMR analysis. [e] Not determined because the sample is partially insoluble in $CHCl_3$.

The abbreviation used for the samples is *polymer-f-TEMPO(1 or 2)-peroxide-temperature.* This indicates the type of polymer (PBS or PLA), the type of nitroxide (BT = BzO-TEMPO or NfT = NfO-TEMPO) in two different concentrations marked as 1 or 2, and the peroxide (BPO or DTBPIB), which is always added in the same concentration for each series of samples, and the reaction temperature. For example, the code PBS-BT1-BPO-120 identifies a sample obtained by reacting at 120 °C PBS, BzO-TEMPO in the lowest concentration (see Tables 1 and 2 for the exact value) and BPO.

Characterization

Number average molecular weight (M_n) and weight average molecular weight (M_w) as well as dispersity (M_w/M_n) of the samples were determined using size exclusion chromatography (SEC), Agilent Technologies 1200 Series. The instrument was equipped with an Agilent degasser, an isocratic HPLC pump, an Agilent refractive index (RI) detector, and two PLgel 5 μm MiniMIX-D columns conditioned at 35 °C. Chloroform ($CHCl_3$) was used as the mobile phase at a flow rate of 0.3 mL min^{-1}. The system was calibrated with polystyrene standards in a range from 500 to 3×10^5 g mol^{-1}. Samples were dissolved in $CHCl_3$ (2 mg mL^{-1}) and filtered through a 0.20 micron syringe filter before analysis. M_n and M_w were determined using Agilent ChemStation software.

A 4800 MALDI TOF/TOF™ analyzer (Applied Biosystems, Framingham, MA, USA) mass spectrometer was used to acquire MALDI spectra. This instrument was equipped with an Nd:YAG laser with 355 nm wavelength of <500 ps pulse and 200 Hz repetition rate. MALDI-TOF mass spectra were recorded in reflectron and in positive ion mode. Polymer samples were dissolved in $CHCl_3$ at a concentration of 5–7 mg mL^{-1}. *trans*-2-[3-(4-*tert*-Butylphenyl)-2-methyl-2propenylidene]-malononitrile (0.1 M in $CHCl_3$/THF) was selected as the matrix. Appropriate volumes of polymer and matrix solutions were mixed in order to obtain 1 : 1 and 1 : 2 ratios (sample/matrix; v/v). An aliquot of 2 μL of each sample/matrix mixture was spotted onto the MALDI sample holder and slowly dried to allow matrix crystallization. MS data were processed using DataExplorer 4.4 (Applied Biosystems). The mass resolution was about 12 000 (full width at half maximum (FWMH)) and the mass accuracy was 1–2 ppm in the mass range m/z 400–1000 and 8–50 ppm in the mass range m/z 1000–2000.

UV-Vis absorption spectra of polymer solutions were recorded at room temperature with a Perkin-Elmer Lambda 25 UV-Vis Spectrometer. PBS solutions were prepared by dissolving 50 mg of each sample in 5 mL of $CHCl_3$. A quantitative analysis of the samples was obtained by referring to calibration curves. PBS/BzO-TEMPO and PBS/NfO-TEMPO $CHCl_3$ solutions at known compositions were prepared (as described in the ESI†). The UV-Vis absorption spectra of the calibration samples were recorded and linear calibration curves were obtained by plotting the absorbance of the chromophore at a specific wavelength *versus* the concentration of the chromophore.

A Bruker AV 400 (400 MHz) equipped with a 5 mm multinuclear probe with reverse detection was used to record the ^{1}H-NMR spectra. 2048 scans were recorded with an acquisition time of 5 seconds at 32 °C. About 40 mg of each polymer sample were dissolved in 1 mL of chloroform-d ($CDCl_3$) at room temperature and a known amount of a solution of 1,4-dinitrobenzene (reference compound, RC) or of 1,4-dimethoxybenzene in the case of PLA, in $CDCl_3$ (6.6×10^{-3} M) was added. The resulting solution was analyzed.

Steady-state fluorescence spectra of PBS-*g*-(NfO-TEMPO) and PLA-*g*-(NfO-TEMPO) films were acquired at room temperature under isotropic excitation using a Perkin-Elmer Fluorescence Spectrometer LS45 controlled by FL Winlab software and equipped with the Front Surface accessory.

X band EPR spectra were obtained by a Varian E112 spectrometer, controlling the temperature with a Varian E257 temperature control unit. The EPR spectrometer was interfaced to an IPC 610/P566C industrial grade Advantech computer by means of a data acquisition system. This consisted of an acquisition board capable of acquiring up to 500 000 12 bit samples per second,[28] and a software package specially designed for EPR experiments.[29] The spectra were run by placing a small amount of the PBS-BT2-BPO-120 sample into a quartz tube (internal diameter 3 mm) and by gradually increasing the temperature.

Full optimization of the molecular geometries and spin density surfaces of PBS macroradicals formed by H-abstraction *via* peroxide radicals were carried out using Spartan'10 (Wavefunction, Inc., Irvine, CA, USA) running on a Linux workstation. DFT data were obtained by adopting the B3LYP (Becke, three-parameter, Lee–Yang–Parr)[30,31] exchange-correlation functional formulated with the Becke 88 exchange functional[32] and the correlation functional of Lee, Yang and Parr,[33] adopting the 6-31G** base set of functions, which is appropriate for calculations of a split-valence plus-polarization quality.

Results and discussion

Preparation and structural characterization of functionalized aliphatic polyesters

A systematic study of PBS functionalization with BzO-TEMPO and NfO-TEMPO was carried out at 120 °C using BPO as a radical initiator. This temperature, which is above the melting temperature of PBS (114 °C from technical data sheet), was selected because it induces the thermal BPO decomposition in an interval of time that easily fits the duration of the mixing step.

Initially PBS was treated at 120 °C without adding any reagents (PBS-120, blank sample) and then its reactivity *versus* BPO radicals was investigated by treating the molten polymer with 1 mol% BPO (PBS-BPO-120) (Fig. 1). This amount of peroxide is enough to induce a concentration of macroradicals that promotes the formation of 60–80% gel content.[12–15]

After the peroxide had been added, an immediate increase in the torque was observed, which indicates an increase in the melt viscosity during the run. This effect is due to the coupling reaction between PBS macroradicals, which are formed by hydrogen abstraction from polymer chains. Indeed, PBS-BPO-120 is only partially soluble in $CHCl_3$, thus confirming that

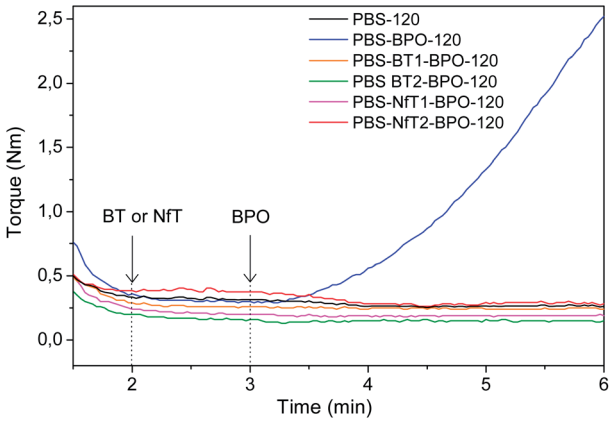


Fig. 1 Torque curves of PBS-120, PBS-BPO-120, PBS-*g*-(BzO-TEMPO), and PBS-*g*-(NfO-TEMPO) samples.

branching/crosslinking occurred during the treatment of PBS with the peroxide. Nonetheless, this undesired reaction needs to be controlled during the functionalization in order to obtain a functionalized material in which the initial structure of the polymer is preserved. Two different concentrations of both functional nitroxides were chosen: 1 mol%, corresponding to the maximum number of peroxide radicals that can be produced during the run (considering that each run was carried out for a time corresponding to the half-life time of the peroxide); 0.5 mol%, that is half of the peroxide concentration. The latter corresponds to the concentration of primary radicals considering that generally only 50% of primary radicals are active in the H-abstraction, the rest being lost by β-scission reaction.[7] While the PBS reaction with BPO demonstrated an appreciable increase in the torque, no increase in the torque was observed for the four-functionalization runs. Importantly, this effect is consistent with a suppression of the coupling reaction between PBS macroradicals. The SEC analysis of the purified samples (completely soluble in $CHCl_3$) indicated a slight decrease in both M_n and M_w if compared with PBS-120 and pristine PBS, probably due to some limited degradation (Table 1 and Fig. S1†). However, in the case of PBS-NfT2-BPO-120, prepared using the highest amount of NfO-TEMPO, the molecular weight values were very close to those of PBS-120.

To confirm the grafting of the functional TEMPO molecules on PBS chains, the methanol residue fractions of PBS-BT2-BPO-120 and PBS-NfT2-BPO-120 were characterized by MALDI TOF. Several successful applications of MALDI-TOF MS for the determination of the structural architecture of biodegradable macromolecules, including their topology, composition, chemical structure of the end groups, have been reported.[34–37] Fig. 2 and 3 show two enlarged portions of the MALDI mass spectra of the methanol residues of PBS-BT2-BPO-120 and PBS-NfT2-BPO-120, respectively.

In both the samples, the most abundant ions were assigned to sodium-cationized cyclic PBS chains ($m/z = 1571 + n \times 172$) and to singly-charged sodium and potassium adducts of linear species with diverse end groups due to unfunctionalized PBS chains, in agreement with previous results[35,36] (see the ESI† for details). In addition, in the case of PBS-BT2-BPO-120, sodiated ions at $m/z = 1620 + n \times 172$ and $m/z = 1692 + n \times 172$ were

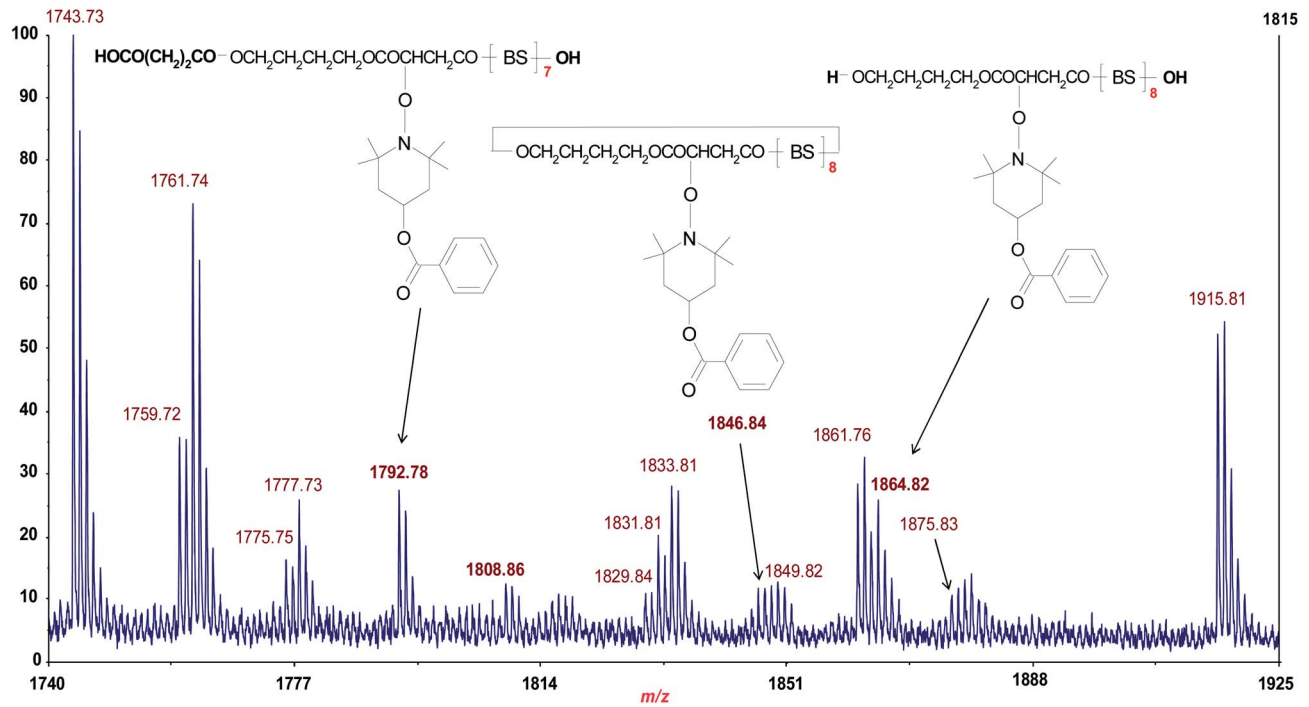


Fig. 2 Enlarged portions of MALDI mass spectrum of PBS-BT2-BPO-120 sample. The most abundant ions at m/z 1743 + $n \times 172$ are due to sodium-cationized cyclic chains. In bold the m/z values refer to sodiated (1792, 1846, and 1864) or potassiated (1808) linear and cyclic PBS chains functionalized with a BzO-TEMPO molecule (BS = $O(CH_2)_4OCO(CH_2)_2CO$).

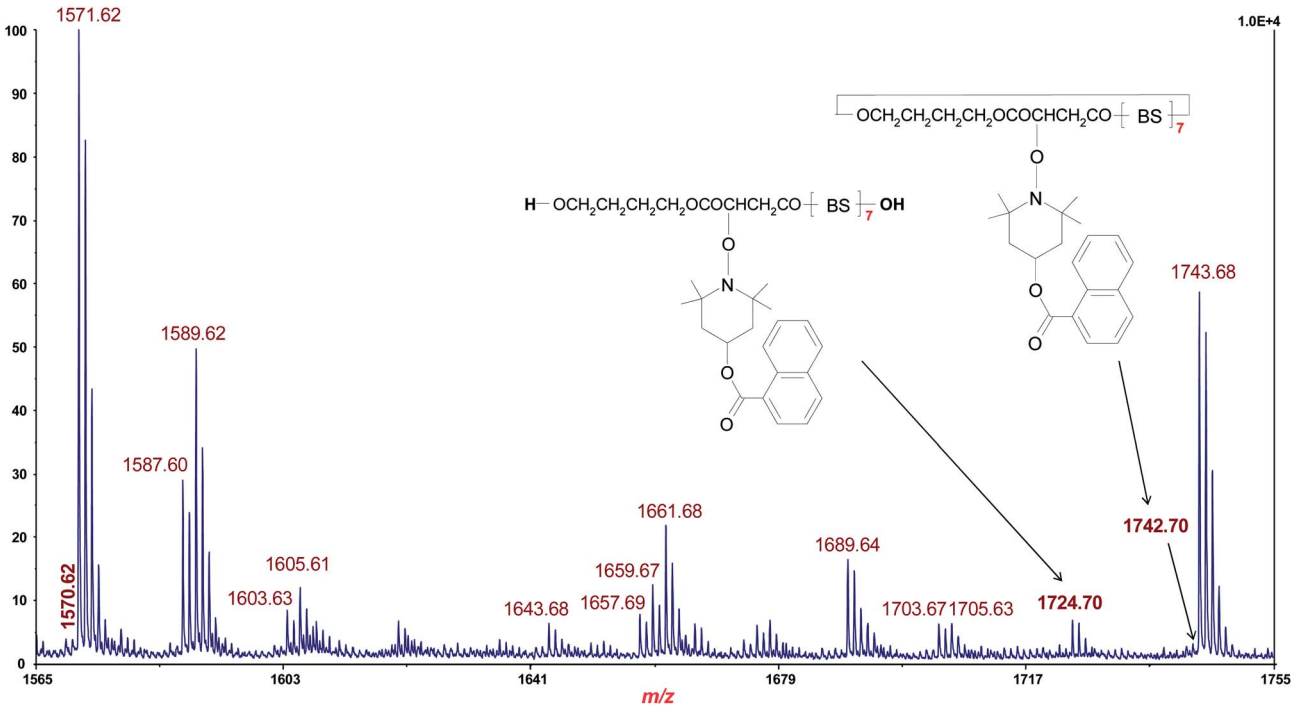


Fig. 3 Enlarged portions of MALDI mass spectrum of the PBS-NfT2-BPO-120 sample. The most abundant ions at m/z 1571 + $n \times 172$ are due to sodium-cationized cyclic chains. In bold the m/z values refer to sodiated (1570, 1724 and 1742) linear and cyclic PBS chains functionalized with an NfO-TEMPO molecule (BS = $O(CH_2)_4OCO(CH_2)_2CO$).

unambiguously assigned to linear PBS chains functionalized with a BzO-TEMPO molecule and terminated, respectively, by succinic acid at both chain ends or with carboxyl and hydroxyl end groups. Sodium adducts of cyclic PBS species functionalized with a BzO-TEMPO molecule ($m/z = 1674 + n \times 172$) were also detected. Similarly, signals at $m/z = 1724 + n \times 172$ were observed in the MALDI mass spectrum of the methanol residual fraction of the PBS-NfT2-BPO-120 sample and were assigned to sodiated cyclic PBS chains functionalized with an NfO-TEMPO molecule. Less abundant ions related to linear PBS chains terminated with carboxyl and hydroxyl end groups and functionalized with an NfO-TEMPO molecule ($m/z = 1742 + n \times 172$) were also detected.

While MALDI-TOF MS revealed the grafting, further proof of the PBS functionalization was obtained by UV-Vis analysis of purified samples (residues to methanol extraction, to avoid non reacting functionalizing agents and byproducts). Besides confirming the successful functionalization, UV-Vis spectroscopy led to the quantitative determination of the moles of the grafted functionalities. The characteristic absorptions of BzO-TEMPO centered at 275 and 282 nm (enlargement in Fig. 4a) and due to the π–π^* transition of the aromatic ring were observed in the spectra of the functionalized samples (Fig. 4a). Similarly, in the case of PBS-*g*-(NfO-TEMPO) samples, the UV-Vis spectra (Fig. 4b) showed an absorption band centered at 298 nm, which is characteristic of the π–π^* transition of the aromatic moiety of NfO-TEMPO (enlargement in Fig. 4b).[25]

We quantified the functional grafted groups by determining the functionalization degree (FD = moles of grafted functional groups per 100 moles of monomeric units) using two calibration curves based on standard solutions at known concentrations of PBS and BzO-TEMPO or NfO-TEMPO (ESI Fig. S2 and S3†). The resulting linear correlations were used to quantify the chromophore concentration in the functionalized PBS samples from solutions of a known polymer concentration (assuming similar extinction coefficients of grafted and free chromophores), and the FD was determined accordingly (Table 1). Although rather low, the FDs are of the same order of magnitude as the values reported in the literature with regard to the functionalization of polyesters with MAH and peroxide, which range between 0.4 and 1.5 wt%.[10,18] Indeed, the highest FD obtained with NfO-TEMPO corresponds to 0.6 wt%, but the amount of functionalizing agent used is notably lower (*i.e.* 0.8–1.9 wt% NfO-TEMPO *versus* 3–8 wt% MAH) and the conversion ranges between 33–50% rather than 15–30% for MAH grafting. This suggests an enhanced yield for the nitroxide radical coupling functionalization methodology. In addition, in the case of PBS the moles of grafted moieties per 100 moles of monomeric units are comparable to those obtained with polyethylene using the same functionalization method.[24,25] Although a direct comparison is not fully appropriate because the reaction temperature and the peroxide adopted were different, in the case of PBS the amount of nitroxide used to obtain the functionalization by controlling the final molecular weight of the polymer was three times lower than that used with polyethylene. This may be due to a better control of macroradical side reactions in polyesters compared to polyolefins, which suggests that the nitroxide radical coupling reaction is a well-performing functionalization method for this kind of polymer too.

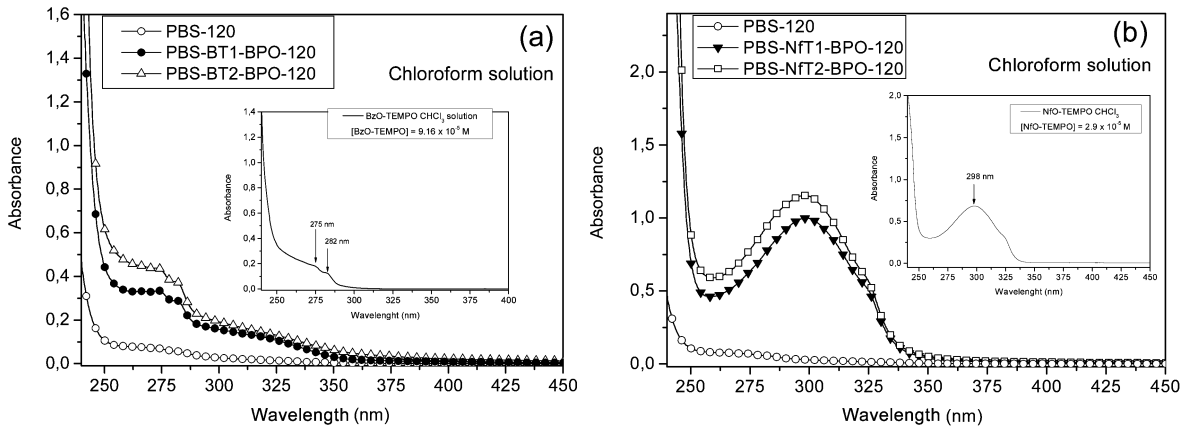


Fig. 4 UV-Vis absorption spectra of PBS-*g*-(BzO-TEMPO) (a) and PBS-*g*-(NfO-TEMPO) samples (b). The insets show the spectra of BzO-TEMPO and NfO-TEMPO.

The FD of the two PBS-*g*-(BzO-TEMPO) samples increased with the BzO-TEMPO concentration keeping the peroxide moles constant. In contrast, in the PBS-*g*-(NfO-TEMPO) samples, the FD values were similar (around 0.3 mol%) irrespectively of the NfO-TEMPO concentration in the feed. The data seem to indicate that the NfO-TEMPO is more likely than BzO-TEMPO to graft to PBS:FD values were higher, and for the highest FD, the molecular weight of the polymer was closer to the pure PBS. This different behavior may be due to a different reactivity of the functional TEMPO molecules, depending mainly on their solubility/dispersibility in the molten polymer.

To corroborate the outcomes of the UV-Vis procedure, the FD values of PBS-BT2-BPO-120 and PBS-NfT2-BPO-120 were determined by ^{1}H-NMR spectroscopy (Table 1). In order to get a complete attribution of polymer signals, the ^{1}H-NMR spectrum of the purified PBS-120 sample was acquired first. The signals observed in this spectrum are in agreement with the previously reported analysis of Bionolle 1001,[36] which contains low fractions of 1,6-hexamethylene diisocyanate and aromatic diisocyanate[38] used as chain extenders (Fig. 5a and S4 in the ESI†). Besides the typical PBS signals, the spectra of the functionalized samples (Fig. 5b and c) highlighted signals due to the grafting of the functional TEMPO derivatives in the spectral region between 7 and 9 ppm and a signal at about 5.3 ppm, which can be attributed to the methine proton on the TEMPO ring in the alpha position compared to the ester functional group (H_h, Fig. 5b and c).[25]

The ^{1}H-NMR spectrum of PBS-BT2-BPO-120 shows three signals (H_a, H_b, H_c) in the 7–8 ppm range due to the aromatic protons (see molecule structure in Fig. 5b for attributions). For the quantitative determination of the grafted groups, a known amount of a reference compound (1,4-dinitrobenzene, RC) was dissolved in a deuterated chloroform solution of the functionalized polymer at a known concentration.[24,25] A comparison between the area of the peak of the RC protons at 8.39 ppm with those of the aromatic protons of the grafted BzO-TEMPO unit enabled the FD_{NMR} to be evaluated (Table 1). Similarly, in the case of PBS-NfT2-BPO, the ^{1}H-NMR spectrum (Fig. 5c) showed five multiplet signals (H_a–H_g) in the 8.9–7.4 ppm range, which are all due to protons of the naphthalene ring. Note that the FD values collected by UV calibration and NMR determination (Table 1) are in a very good agreement, thus underlining that the UV-Vis methodology, through the use of appropriate calibration curves, can be successfully used to quantitatively evaluate the FD of PBS samples functionalized with BzO-TEMPO and NfO-TEMPO. We attributed the signal at 9.76 ppm in the spectra of the functionalized samples to the formation of aldehyde groups[35,36] probably deriving from a partial thermo-oxidative degradation of the polymer which occurs during the functionalization process.

Interestingly, by comparing the spectrum of PBS-120 (Fig. 5a) and the spectra of functionalized samples (Fig. 5b and c), a triplet at about 4.3 ppm emerges in the spectra of functionalized samples. On the basis of the chemical shift and multiplicity, this signal can be attributed to a methine proton in the alpha position compared to the carbonyl group of the succinic acid unit of PBS substituted by the functional TEMPO moiety (H_i, Fig. 5b and c). The presence of this signal supports the mechanism of grafting previously reported, which proposes the insertion of the functional moieties onto the dicarboxylic acid units of the polyester.[10,18]

This experimental evidence was tentatively confirmed with quantum mechanical calculations made to predict the site of H-abstraction from the PBS backbone *via* peroxide radicals and, therefore, the type of macroradical formed. The theoretical enthalpy determined at the B3LYP/6-311+G** level was lower for the PBS macroradical formed by H-abstraction from the CH_2 in the alpha position compared to the carbonyl group of the succinic acid unit (Fig. S5–S7 in the ESI†). In order to provide direct evidence of the grafting site, an EPR study was carried out on a functionalized PBS sample to tentatively intercept the EPR signals of PBS macroradicals formed by the homolytic cleavage of the C–ON bond induced by the temperature increase. Purified PBS-BT2-BPO-120 was heated in the EPR cavity and spectra were registered at different temperatures. At the beginning of the experiment (at 40 °C), the spectrum exhibited a weak asymmetric three-line signal due to free BzO-TEMPO entrapped in the rigid polymer phase, whose presence is due to the

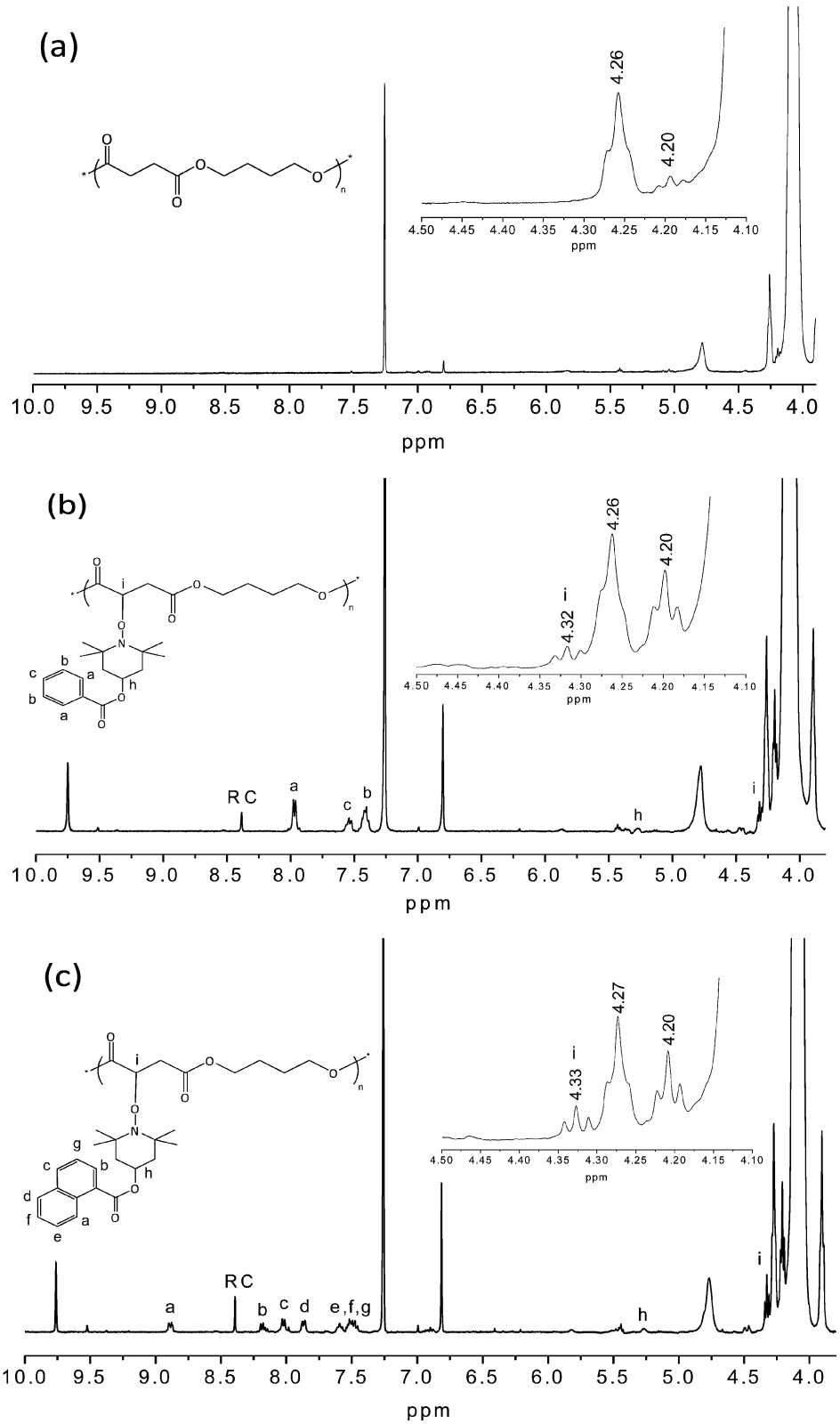


Fig. 5 ^{1}H-NMR spectra of PBS-120 (a), PBS-BT2-BPO-120 (b), and PBS-NfT2-BPO-120 (c).

equilibrium between grafted and ungrafted species.[25] By gradually increasing the temperature, the nitroxide signal became similar to the signal of a nitroxide radical that is free to rotate (Fig. 6), the integrated area at 100 °C corresponding to a 3.67×10^{15} spin. A further increase in the temperature up to 155 °C produced an increase in the EPR signal due to the TEMPO

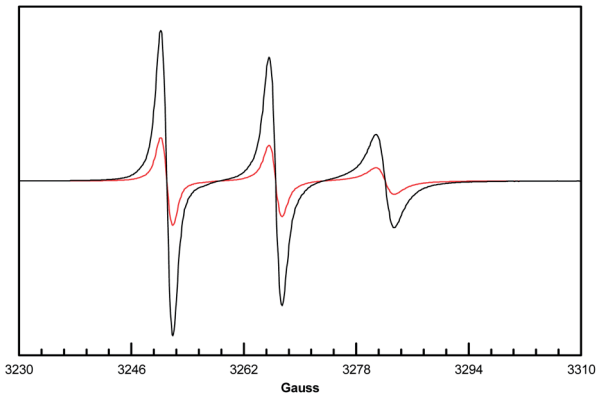


Fig. 6 EPR spectrum of PBS-BT2-BPO-120 registered at 100 °C (red line) and at 155 °C after 5 min at this temperature (black line).

radicals, thus suggesting that the homolytic cleavage of the nitroxide–PBS bond had occurred. The integrated area of this signal after 5 minutes at this temperature corresponds to a 1.28×10^{16} spin, which is about 10% of the FD of this sample.

Interestingly, in addition to the main three-line signal due to the nitroxide radical, with a nitrogen isotropic hyperfine splitting constant (AN) of 15.3 G and a carbon (C13 isotope) isotropic hyperfine splitting constant (AC) of 6.8 G, the EPR spectrum collected at 155 °C also revealed a very weak signal (labeled as "b" in Fig. 7) with a proton splitting constant of 21 G.

This signal, which is partially covered by the strong nitroxide signal, indicates the formation of a carbon-centered radical coupled with one alpha proton, which can be attributed to the PBS macroradical likely generated by the detachment of the nitroxide. The intensity of this signal could be very low because of the very high reactivity of carbon-centred radicals, which at high temperatures can give rise to recombination reactions even with the free nitroxide.

In order to demonstrate the versatility of our method, the PBS functionalization was also investigated at a higher temperature by testing a different type of peroxide. PBS was functionalized with BzO-TEMPO at 150 °C using DTBPIB as a peroxide. The addition of the DTBPIB to the molten polymer led to a gradual increase in the torque thus highlighting the coupling reaction between macroradicals. Similarly to the previous set of reactions carried out at 120 °C, the addition of the functional TEMPO molecule suppressed the formation of branched/crosslinked fractions: the torque–time curve was flat for the PBS-BT2-DTBPIB-150 run (Fig. S8 in the ESI†), the functionalized polymer was completely soluble in $CHCl_3$ and SEC results showed only a slight decrease in both M_n and M_w compared with PBS-150 and pristine PBS (Table 1). An analysis of the functionalized sample by UV-Vis spectroscopy confirmed the success of the reaction (Fig. S9 in the ESI†), although the FD was lower than the value obtained using the same quantity of reagents and working at 120 °C (Table 1). The reason for this difference may the loss of part of the BzO-TEMPO by coupling reactions with methyl radicals formed by the thermal degradation of DTBPIB, as previously observed.[24] Although this run did not show clear advantages in terms of FD as a consequence of the change in the peroxide, it represents a useful scale-up test in terms of the extrusion process.

The PLA functionalization was tested using NfO-TEMPO as a functional molecule in order to easily prove the grafting thanks to the emission from the functionalized product. The peroxide addition again caused a marked increase in the torque values (Fig. 8) plus the formation of a $CHCl_3$ insoluble material due to coupling reactions between PLA macroradicals, as reported in the literature.[17]

The functionalization run PLA-NfT2-DTBPIB-180 did not show the same torque increment, which is probably due to the coupling between PLA macroradicals and NfO-TEMPO giving the desired functionalized product. However, the SEC analysis carried out after purification of the sample showed a partial decrease in both M_n and M_w and an increase in the dispersity (Table 2). Accordingly, although the coupling reaction between PLA macroradicals was suppressed, a degradation effect was found. Given that coupling is a bimolecular reaction, the macroradicals concentration was probably reduced, thus inhibiting coupling between macroradicals, though some of them were likely involved in degradation probably by a β-scission

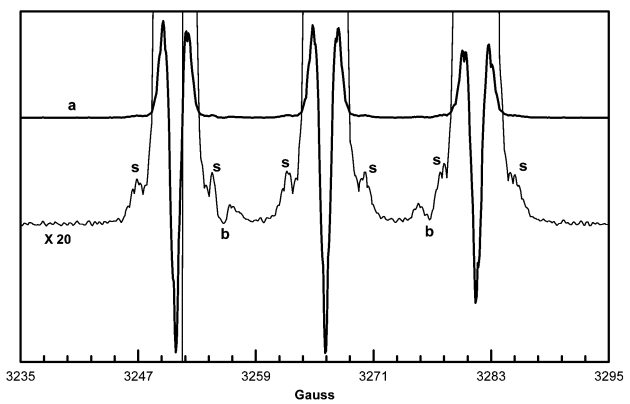


Fig. 7 EPR spectrum (second derivative) of PBS-BT2-BPO-120 registered at 155 °C: "a" is the signal of detached BzO-TEMPO radicals; "s" signals are due to C^{13} satellites, $g_{iso} = 2.00472$; "b" is the signal of PBS macroradicals formed by detachment at high temperature of the grafted f-TEMPO, $g_{iso} = 2.00492$.

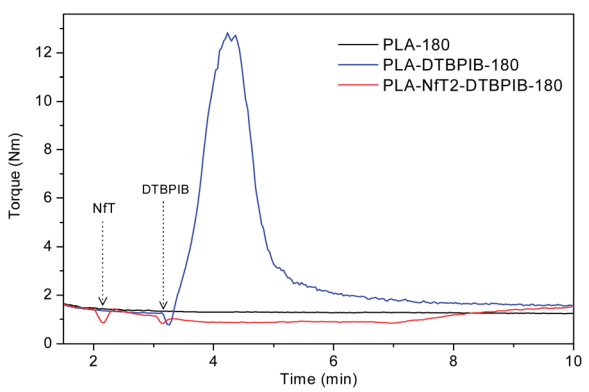


Fig. 8 Torque curves of PLA-180, PLA-DTBPIP-180, and PLA-NfT2-DTBPIB-180.

reaction.[17,42] The UV-Vis analysis of the purified functionalized sample revealed an absorption band centered at 299 nm (Fig. S10 in the ESI†) which confirms the presence of the grafted chromophore (naphthoic group). To roughly estimate the FD, the absorbance of the band at 298 nm was plotted *versus* the PBS/NfO-TEMPO calibration curve previously reported. A very low FD = 0.04 mol% was found, which was confirmed by ^{1}H-NMR analysis (Fig. S11 in the ESI†). As reported in the literature, the PLA macroradical is generated by H-abstraction at the tertiary carbon atom, which is thus likely to be the grafting site of the functional nitroxide.[16,9,43]

Fluorescent properties of aliphatic polyesters grafted with NfO-TEMPO

In our previous work on polyethylene radical functionalization with NfO-TEMPO,[25] we showed that the fluorescence emission spectrum of NfO-TEMPO was characterized by a very low emission intensity, whereas in the case of the metoxy-derivative of NfO-TEMPO (which can be considered as a model compound of the grafted TEMPO), the maximum emission position and its intensity was very close to that of 1-naphthoic acid. In agreement with the literature, molecules containing a nitroxide tethered to a fluorophore show dramatically reduced fluorescence due to intramolecular quenching of the fluorophore excited singlet state.[39–41] In contrast, the free radical trapping of the nitroxide moiety yields a diamagnetic alkoxyamine and restores the emission from the fluorophore. Because of this behavior, if the pro-fluorescent nitroxide is grafted on the backbone of PBS, a fluorescence emission for the functionalized polymer is expected. Fluorescent emission spectra of PBS, PBS-NfT1-BPO and PBS-NfT2-BPO were thus collected from polymer films (Fig. 9). The spectra of the two functionalized samples showed a large emission band centered at 365 nm, as similarly observed for polyethylene-*g*-(NfO-TEMPO),[25] thus confirming once again that grafting had been successful. The difference observed in the emission intensity of the samples reflects the FD of PBS-NfT1-BPO-120 and PBS-NfT2-BPO-120.

Similarly to PBS-*g*-(NfTO-TEMPO) samples, a fluorescent PLA sample was obtained, in which the optical properties were transferred from the chromophore to the polymer. The fluorescence emission spectrum of the PLA-NfT2-DTBPIB-180 film (Fig. 10) showed a weak but visible band centered at 360 nm, similar to the one observed in the analogous functionalized PBS samples. The low emission intensity is in agreement with the low FD of this sample.

This thus demonstrates the possibility to simply transfer the optical properties of the chromophore to the biodegradable polymer using a post-polymerization method. From these results it appears that low functionalization degrees are sufficient to give fluorescence, which is a property not observed in the pure material. This is indeed the main objective of a post-polymerization functionalization reaction: to impart new properties without changing the original characteristics of the polymer. Such a fluorescent feature could be exploited to develop sensors or anti-counterfeit packaging. The polyesters carrying integral fluorescent moieties could also be exploited to

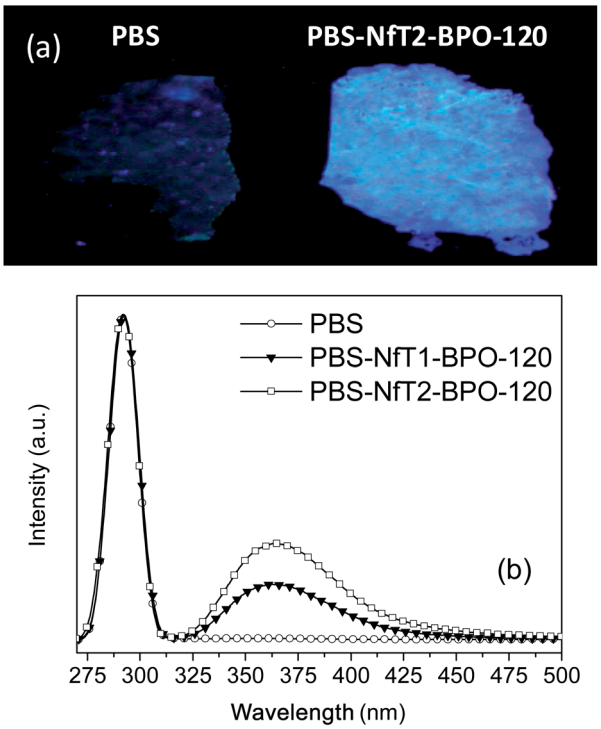


Fig. 9 Digital images of PBS and PBS-NfT2-BPO-120 under excitation with a long-range UV lamp ($\lambda = 254$ nm) (a), and fluorescence emission spectra ($\lambda_{exc} = 290$ nm) of PBS, PBS-NfT1-BPO-120, and PBS-NfT2-BPO-120 film (b). The spectra are normalized with respect to the scattering intensity.

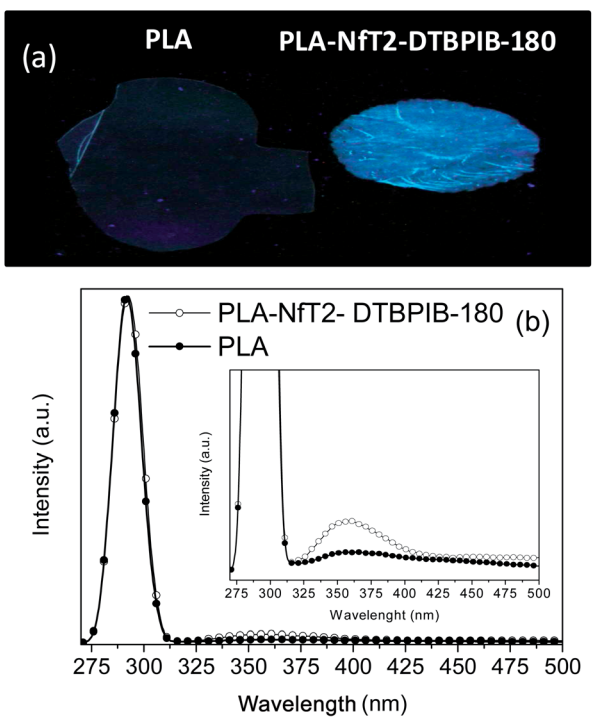


Fig. 10 Digital images of PLA and PLA-NfT2-DTBPIB-180 under excitation with a long-range UV lamp ($\lambda = 254$ nm) (a) and fluorescence emission spectra ($\lambda_{exc} = 290$ nm) of PLA, PLA-NfT2-DTBPIB-180 film (b). The spectra are normalized with respect to the scattering intensity.

design fluorescent biodegradable and biocompatible polymers for biomedical applications.[44]

Conclusions

The preparation of functionalized PBS and PLA samples by a post-polymerization method based on nitroxide radical coupling between polyester macroradicals and TEMPO derivatives carrying different functionalities was investigated for what we believe was the first time. This approach was successful in promoting grafting by limiting the radical-induced cross-linking/branching of the polymer substrate. The grafting occurred by the coupling between polyester macroradicals, formed by H-abstraction, and functional nitroxides and, consequently, the concentration of macroradicals was limited, thus enabling the molecular weight to be well controlled.

Two different series of functionalized PBS samples were obtained at 120 °C by grafting BzO-TEMPO and pro-fluorescent NfO-TEMPO. The grafting was clearly shown by MALDI-TOF MS, which enabled the sodium adducts of the linear PBS chains functionalized with BzO-TEMPO and NfO-TEMPO to be unambiguously detected. Besides confirming the success of the functionalization reaction, UV-Vis and ^{1}H-NMR spectroscopy also led to the evaluation of the FD, which ranged between 0.15 and 0.30 mol%. In addition, combining EPR analysis, theoretical calculations and ^{1}H-NMR spectroscopy, demonstrated that the grafting site of PBS derives from the hydrogen abstraction from one of the two CH_2 groups of the succinic acid unit. Our data indicate that the NfO-TEMPO is more likely than BzO-TEMPO to graft to PBS. The FD values were higher, and the molecular weight of the polymer was closer to that of the pure PBS for the highest FD.

The feasibility of this new methodology was also tested by changing the experimental conditions and by performing the reaction on PLA. Also in this case, although with a lower FD value, the occurrence of grafting was proved with a good control of the molecular weight. For both PBS and PLA, the fluorescence emission spectra not only provided clear evidence of the grafting of NfO-TEMPO, but also revealed a fully restored fluorophore emission, thus obtaining a fluorescent polymer.

We believe that our method provides a convenient and versatile route for introducing different functional groups on biodegradable aliphatic polyesters using a range of functional TEMPO derivatives. It is particularly attractive considering the ever-increasing demand for specific functionalized polyesters to replace commodity plastics in several applications such as films for packaging. It also provides a tool in polymer tailoring as demonstrated by the fluorescent feature exhibited by the functionalized polyesters, which might prove useful in applications such as fluorescent tracks and labels, or sensors.

Acknowledgements

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