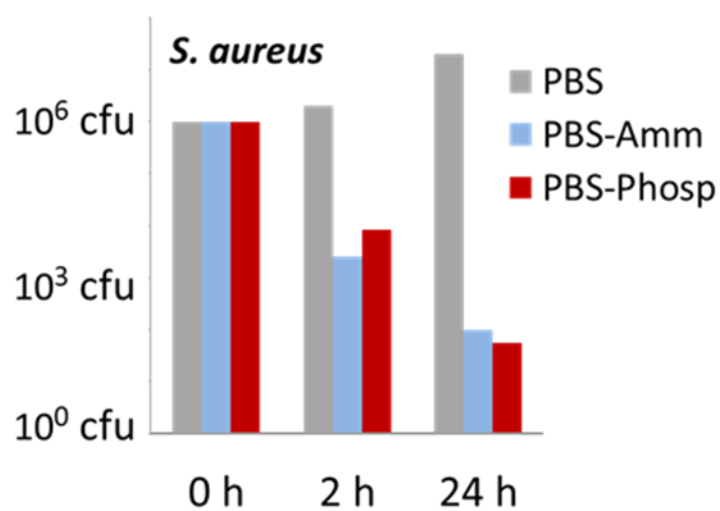
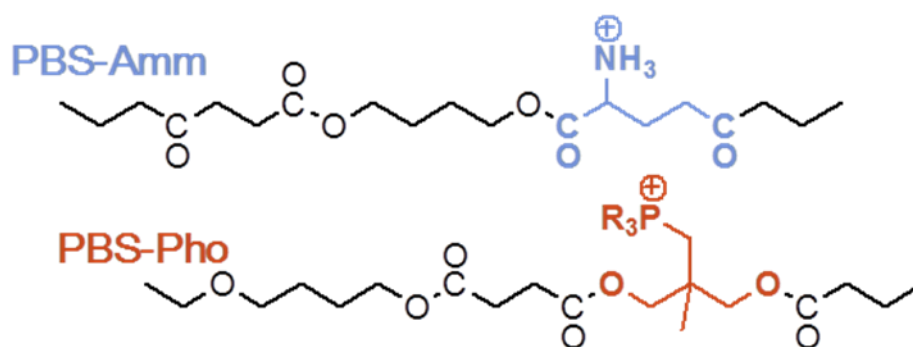


Graphical Abstract

Cationic poly(butylene succinate) copolyesters

Mayka Bautista, Antxon Martínez de Ilarduya, Abdelilah Alla,
Marc Vives, Jordi Morató^b and Sebastián Muñoz-Guerra



Highlights for Peer Review

Cationic poly(butylene succinate) copolyesters

Mayka Bautista, Antxon Martínez de Ilarduya, Abdelilah Alla,
Marc Vives, Jordi Morató and Sebastián Muñoz-Guerra
Universitat Politècnica de Catalunya

- A series of cationic copolyesters of PBS containing ammonium side groups was synthesized by enzymatic polycondensation.
- A series of cationic copolyesters of PBS containing phosphonium side groups was synthesized by organometallically catalyzed melt polycondensation.
- Both ammonium and phosphonium containing PBS copolyesters are thermally stable, semicrystalline and able to crystallize from the melt for moderate contents in ionic units.
- Both ammonium and phosphonium containing PBS copolyesters show high antimicrobial activity against both gram-positive and gram-negative bacteria.

Cationic poly(butylene succinate) copolyesters

Mayka Bautista^a, Antxon Martínez de Ilarduya^a, Abdelilah Alla^a,
Marc Vives^b, Jordi Morató^b and Sebastián Muñoz-Guerra^{a,*}

Universitat Politècnica de Catalunya

^aDepartament d'Enginyeria Química, ETSEIB, Diagonal 647, Barcelona 08028, Spain

^bHealth and Environmental Microbiology Lab & UNESCO Chair on Sustainability, EET, Edifici Gaia, Pg. Ernest Lluch/Rambla Sant Nebridi, Terrassa 08222, Spain

*Corresponding author: sebastian.munoz@upc.edu (S. Muñoz-Guerra Tel.: +34 93 4016680)

Abstract: The synthesis, characterization and comparative evaluation of properties of two series of cationic PBS copolyesters bearing respectively ammonium and tributylphosphonium side groups, are reported. The copolyesters with contents in ionic units up to 50 mole-% as well as the fully ionic homopolyesters were prepared by polycondensation in the melt catalyzed by CALB or TBT. Their M_n ranged between 20,000 and 5,000 g·mol⁻¹ depending of composition and the type of ionic group that is involved. All the copolyesters were non-water soluble and showed good thermal stability. They were semicrystalline with melting temperatures and enthalpies decreasing with the ionic contents. The interactions interplayed by the ionic groups restricted largely the molecular mobility and caused a significant increase in the melt viscosity and glass transition temperature of PBS and a decrease in crystallization rate. Both ammonium and phosphonium containing PBS copolyesters were able to be coupled with sulfonated PBS to generate ionic polymer blends with modified crystallizability. The presence of both ammoniums and phosphoniums provided PBS with remarkable antimicrobial activity against gram-positive and gram-negative bacteria.

Keywords: Poly(butylene succinate), polyelectrolytes, cationic polyesters, enzymatic polymerization, ionic polymeric complexes, biocidal polyesters.

1. Introduction

The interest in aliphatic polyesters has greatly increased along these last years due to the fact that they are among the most promising sustainable and biodegradable polymeric materials [1-3]. In addition to their applicability in packaging and coating, a good number of aliphatic polyesters are designed with adjusted biodegradation rate with the purpose of use them as temporal applications in the biomedical field [4-7]. Poly(butylene succinate) (PBS) is a typical biodegradable aliphatic polyester that has been traditionally produced using petroleum-based monomers. Recently PBS has emerged as a distinguished polymer mainly because its two monomers, succinic acid and 1,4-butanediol have become accessible from renewable resources [8]. Moreover PBS displays a satisfactory overall pattern of thermal and mechanical properties that makes it suitable for a wide range of end applications mainly in packaging and coating by using conventional melt processing techniques [9,10]. Nevertheless, the lack of functionality is a shortcoming of PBS that prevents expanding its use into new fields where polymer activity or polymer modifications are required.

The incorporation of ionic groups in a polymeric chain gives rise to important changes in both physical and chemical properties and provides it with certain chemical activity [11,12]. In fact, slightly charged polymers (ionomers) are known to be able to interact electrostatically with themselves or with other ionic polymers and small charged molecules to render unusual properties [13-15]. Aromatic copolyesters bearing sulfonated groups constitute an illustrative example of this approach [16-20]. In aliphatic polyester ionomers, property design may be combined with biodegradability to generate materials of exceptional interest as delivery systems for charged proteins and drugs, and in particular for transfection technology [21,22]. Anionic PBS ionomers and copolymers containing sulfonate groups have been recently studied by different authors [23-28], and their potential as biomaterial has been evaluated in some cases. On the contrary, polyester cationic ionomers are in a much less developed stage, in great part due to the difficulty found for introducing cationic groups into the polyester chain by melt polycondensation. The insertion of cationic units in polyesters is particularly attractive because it not only changes advantageously certain properties but in addition it is expected to provide antimicrobial activity. In this paper we wish to report on PBS copolyesters containing either ammonium or phosphonium side groups with a wide range of compositions. The work reported here embraces the synthesis and characterization of the two series of cationic PBS copolymers, the assessment of their basic properties and also a preliminary evaluation of their biocide activity.

To our knowledge, a recent paper published by us on PBS terpolyesters containing minor amounts of quaternized glutamic acid that were synthesized to be

used as nanocomposite compatibilizers, is the only reference on ammonium containing polyesters found in the accessible literature [29]. In such work, it was brought into evidence that the high temperatures required for melt polycondensation promoted the decomposition of the ammonium units with subsequent hindering of the polymer chain growth. These results have encouraged us to explore the enzymatic polycondensation for the synthesis of cationic PBS copolyesters. In fact, lipase-catalyzed polymerization in the melt is a recently developed method that allows producing aliphatic polyesters under mild conditions and avoiding residual metals [30,31]. Thus Azim *et al.* [32] reported the CAL-B mediated synthesis of PBS with a M_w close to 40,000. In this paper we describe the enzyme-catalyzed melt polycondensation of PBS copolyesters ($PBS_xG^1_y$) that incorporate different amounts of 2-aminoglutarate hydrochloride units (G^1) (x and y subscripts refer respectively to the mole-% in succinate and aminoglutarate units present in the copolyester).

Although the literature on phosphonium containing polymers is considerable, very short has been published on polyesters. Anderson *et al.* [33,34] synthesized successfully poly(ethylene terephthalate-co-ethylene isophthalate) (PET-co-PEI) ionomers end-capped with butyl *p*-carboxyphenyl diphenylphosphonium bromide, and Anthierens *et al.* [35] reported on poly(butylene adipate) functionalized with an alkyltriphenylphosphonium group that showed antimicrobial activity. In the present work the diol 2,2-(dihydroxymethyl)propyl-tributylphosphonium bromide (PPD) has been copolymerized with succinic acid and 1,4-butanediol (BD) to obtain poly(butylene-co-propylene succinate) copolyesters containing pendant quaternary phosphonium groups. These new cationic PBS ionomers are abbreviated as PB_xPP_yS where x and y subscripts refer respectively to the mole-% in 1,4-butylene and phosphonium propylene units that are present in the copolyester.

The influence of the cationic side groups on PBS properties has been examined in this work with particular attention paid to biocide activity. Given the enormous potential application of PBS in packaging and coating, the possibility of rendering PBS with biocide properties is a goal of primary technological interest. Antimicrobial agents based on natural or low-molecular-weight compounds may result in environmental contamination and toxicity to the human body due to uncontrolled diffusion [36]. Conversely, antimicrobial polymeric materials based on covalent linkages display high chemical stability and low volatility, and are able to exhibit therefore long-term activity [37,38]. Materials containing ammonium and/or phosphonium salts are probably the most widely studied and used as antimicrobial polymers. Accordingly, the biocide effect of the cationic PBS copolyesters synthesized in this work has been explored using *Escherichia coli* and *Staphylococcus aureus* as testing microorganisms.

2. Experimental

2.1. Materials

Dimethyl succinate (DMS) was purchased from Merck-Schuchardt. 1,4-butanediol (BD) (99%), L-glutamic acid dimethyl ester hydrochloride (2-ammonium dimethyl glutarate hydrochloride) (A-DMG·Cl), 1,1,1-tris(hydroxymethyl) ethane, tributylphosphine, diethyl carbonate and lipase from *Candida antarctica* immobilized on macroporous acrylic resin (CALB) were purchased from Sigma-Aldrich. Succinic acid (SA), hydrobromic acid and solvents used for purification and characterization were purchased from Panreac. All they were of either technical or high-purity grade and used as received without further purification. 2-(*N,N,N*-trimethylammonium) dimethyl glutarate iodide (TMA-DMG·I) was synthesized from A-DMG·Cl according to the general procedure described in the literature for amine quaternization [39]. The PBS ionomer (PBS₈₅SS₁₅) containing 15 mole-% of sulfonated succinate used for the complex formation study was synthesized as described in detail elsewhere [28].

2.2. Measurements

Intrinsic viscosities were measured with an Ubbelohde viscometer thermostated at 25 °C ± 0.1 °C. Size exclusion chromatography (SEC) was performed on a Waters system equipped with a refractive index detector using 1,1,1,3,3,3-hexafluoro-2-propanol as the mobile phase. Molecular weights and their distribution were calculated against poly(methyl methacrylate) standards using the Millenium 820 software. Absorbance measurements were performed with an UV-visible spectrophotometer UVmini-1240 using a 5 nm band-width. NMR spectra were recorded on a Bruker AMX-300 spectrometer operating at 300.1 MHz for ¹H and 75.5 MHz for ¹³C. About 10 mg for ¹H or 50 mg for ¹³C of polymer samples were dissolved in 1 mL of deuterated chloroform (CDCl₃). 64 and 5,000-10,000 scans were acquired with 32-K and 64-K data points and 1 and 2 s of relaxation delays for ¹H and ¹³C, respectively.

The thermal behavior of the polyesters was examined by differential scanning calorimetry (DSC) with a Perkin-Elmer DSC Pyris 1 instrument calibrated with indium and zinc for temperature and enthalpy. DSC data were obtained from 4 to 6 mg samples at heating and cooling rates of 10 °C·min⁻¹ under nitrogen circulation (20 mL·min⁻¹). Glass transition temperatures were taken as the inflection point of the heating DSC traces of melt-quenched samples recorded at 20 °C·min⁻¹. TGA measurements were performed on 10 to 15 mg of sample under a nitrogen flow of 20 mL·min⁻¹ at a heating rate of 10 °C·min⁻¹ and within a temperature range of 30-600 °C using a Mettler Toledo TGA/DSC 1 thermobalance. X-ray diffraction (XRD) patterns of copolyesters were recorded from powdered samples on the PANalytical X'Pert PRO

MPD θ/θ diffractometer using $\text{CuK}\alpha$ radiation of wavelength 0.1542 nm. For the analysis of the complexes requiring heating, XRD was carried out with the sample introduced in a Lindemann glass capillary of 1.0 mm of diameter. Tensile testing was performed using bone shape specimens ($2.7 \times 10 \text{ mm}^2$) that were cut from isotropic films obtained by hot pressing with a thickness of about 200 μm . Tensile tests were conducted at room temperature on a Zwick BZ2.5/TN1S universal tensile testing apparatus operating at a constant crosshead speed of $10 \text{ mm}\cdot\text{min}^{-1}$ with a 0.5 N preload and a grip-to-grip separation of 20 mm.

2.3. Synthesis of cationic PBS copolyesters containing ammoniums.

Candida antarctica lipase immobilized on macroporous acrylic resin (CALB) was dried under vacuum (0.1 mm-Hg) at 25 °C for 24 h. 2% (w/w) of CALB was transferred into a round-bottom flask containing a mixture of dimethyl succinate, L-glutamic acid dimethyl ester hydrochloride and 1,4-butanediol. A molar ratio 1.4:1 of BD to the total of diesters was used for the synthesis of all polyesters. The reaction mixture was mechanically stirred under heating at 80 °C for 72 h under a low nitrogen flow. Then a second 2% (w/w) CALB portion was added to the reaction mixture and the polycondensation reaction was initiated by raising the temperature to 90 °C and reducing the pressure to 40 mbar, and these conditions were maintained for 72 h. Finally a third 2% (w/w) portion of CALB was added, the temperature further increased to 115 °C and the pressure reduced to $1.6 \cdot 10^{-3}$ mbar, and the reaction left to proceed for 72 h more. The synthesis of $\text{PBS}_{50}\text{G}_{50}^1$ and PBG^1 required both longer reaction times (around 300 h) and higher amounts of enzyme (a total of 10% w/w) than in the other cases. The viscous mass finally formed was cooled down to room temperature and the atmospheric pressure was restored with a nitrogen flow to prevent degradation. The resulting solid mass was dissolved in chloroform and filtered using a PTFE membrane with 0.5 μm pore size. The product recovered after evaporation of the solvent was used for characterization and property evaluation without further treatment.

2.3. Synthesis of cationic PBS copolyesters containing phosphoniums.

2,2-(dihydroxymethyl)propyl-tributylphosphonium bromide (PPD-Br) was synthesized in two steps according to the procedures described in the literature [40,41]. 2-(Bromomethyl)-2-methyl-1,3-propanediol was firstly prepared from 1,1,1-tri(hydroxymethyl) ethane and then it was made to react with tributylphosphine to render PPD-Br. ^1H and ^{13}C NMR spectra of this compound and its precursor are available in the Electronic Supporting File (Figures ESI-1 and ESI-2).

The poly(butylene succinate) copolyesters containing tributylphosphonium groups (PB_xPP_yS) were obtained from succinic acid (SA) and mixtures of PPD-Br and BD containing 3, 5, 8, 10 and 15 mol-% of the former. The reactions were performed in a three necked, cylindrical-bottom flask equipped with a mechanical stirrer, a nitrogen inlet and a vacuum-distillation outlet. An excess 1.4:1 of BD to SA was used and titanium tetrabutoxide (TBT, 0.3 w/w % respect to monomers) was the catalyst of choice. Esterification reactions were carried out under a low nitrogen flow at 170 °C during 8 h. Polycondensation reactions were left to proceed at 190 °C under a 2.9·10⁻³ mbar vacuum for 8 h. Finally, the reaction mixture was cooled to room temperature and the atmospheric pressure was recovered with nitrogen to prevent degradation. The resulting solid mass without further treatment was used for the characterization and property evaluation unless otherwise stated.

2.4. CALB activity test

The enzyme activity was tested according to the procedure described by Schwab *et al.* [42]. In this test, the *p*-nitrophenol (*p*NP) generated by methanolysis of *p*-nitrophenyl acetate (*p*NPAc) is followed by UV spectroscopy. 5 mL of a solution of *p*NPAc (7.25 mmol L⁻¹) in toluene and 20 μL of methanol were successively added to a stirred suspension of CALB (10 mg) in toluene (20 mL) at 40 °C. Aliquots of 0.5 mL were then withdrawn every 5 min and diluted with 9.0 mL of toluene. The *p*-nitrophenol concentration was determined by measuring the UV absorption at 304 nm (ϵ_{pNP} , 9344.5 M⁻¹ cm⁻¹ and ϵ_{pNPAc} , 2469.1 M⁻¹ cm⁻¹). The enzyme activity *a* (nmol of substrate converted by 1 mg of CALB per minute) was calculated using equations 1 and 2.

$$c = \frac{n \cdot Abs - \epsilon_{NPA} \cdot c_{0PNAC}}{\epsilon_{pNP} - \epsilon_{pNPAc}} \quad \text{Eq 1}$$

$$a = \frac{c \cdot V \cdot x}{M_{CALB} \cdot t} \quad \text{Eq 2}$$

2.5. Antimicrobial activity

The antimicrobial activity was performed based on the dynamic shake flask method described by Anthierens *et al.* with some modifications [35]. Lyophilized strains of the microorganisms from CECT (Spanish Collection of Culture Types) were resuspended in TSB (Tryptone Soy Broth) for *Escherichia coli* and *Staphylococcus aureus* following the CECT instructions and then incubated for 24 h at 37 °C, in TSA (Tryptone Soy Agar). The broth was then diluted in physiological peptone solution (PPS) containing 1 g of bacteriological peptone (Oxoid, Hampshire, United Kingdom) and 8.5 g of NaCl per liter to approximately 10⁶ to 10¹⁰ colony forming units (cfu)·mL⁻¹. 1 mL of this solution was used to inoculate the sterile 1.5 mL centrifugal tubes

(Eppendorf, Hamburg, Germany) containing 100 mg of each sample (PBS, PBS₈₄G^I₁₆, PBS₅₀G^I₅₀ and PBG^I for ammonium polymers and PBS, PB₉₇PP₃S, PB₉₀PP₁₀S, PB₈₅PP₁₅S for phosphonium polymers) to achieve finally an inoculation level of approximately 10⁵ to 10⁹ cfu·mL⁻¹ depending on the assay. The pH of the PPS solution was adjusted to 5.8 with 0.2% HCl. Assays were conducted per duplicate at 37 °C and under stirring (500 rpm). Samples were drawn at scheduled periods of time ranging from 30 min up to 24 h. 100 µL of each sample was then cultured in TSA petri plates per duplicate and incubated for 24 h at 37 °C, after which the colonies were counted. Petri Plate Count Assay was used to determine the microorganism concentration expressed as colony forming units. Counts out of the range of 30-300 cfu per plate were discarded.

3. Results and discussion

3.1. Synthesis of polyesters and copolyesters

The routes followed for the synthesis of PBS_xG^I_y and PB_xPP_yS polymers are depicted in Scheme 1. Initially the preparation of PBS copolyesters containing trimethylammoniums was pursued. Unfortunately, all attempts made to polymerize DMS with BD in the presence TMA-DMG-I were unsuccessful because the transesterification reaction was unable to proceed either in solution or in the bulk. Increasing the temperature or reducing the pressure to favor the release of methanol did not afford any positive result. Neither increasing amounts of CALB produced any perceivable effect. To elucidate if the enzyme activity was affected by the reaction components, activity tests for CALB were carried out using *p*-nitrophenyl acetate (*p*NPAC) as substrate in the presence and in the absence of TMA-DMG-I. The *p*-nitrophenol (*p*NP) released in these essays along a period of 60 min of incubation is plotted against time in Figure 1. Whereas in the absence of TMA-DMG-I the alcoholysis of *p*NPAC took place steadily, no reaction occurred in the presence of the quaternary ammonium salt. It was concluded therefore that the polycondensation reaction could not advance at a detectable rate because the enzyme was fully inhibited by TMA-DMG-I since the initial stage. Conversely, the same activity test carried out with A-DMG-Cl afforded more favorable results. CALB activity was also depressed in the presence of this compound but it was still remained over 50% after one hour of reaction. Apparently the inhibitory effect of the ammonium group on CALB activity is drastically enhanced by quaternization. It is worthy to point that this result contrasts with those reported by Lozano *et al.* [43], who found that ionic liquids made of

quaternary imidazolium salts were suitable for carrying out transesterification reactions mediated by CALB.

According to the enzyme activity results, $PBS_xG^l_y$ copolyesters were prepared by bulk polycondensation catalyzed by CALB since this method allows applying milder conditions than when organocatalysts are used. An excess of BD respect to the diesters was used to ensure the complete displacement of methanol, and temperature was increased gradually in order to minimize the decomposition of ammonium compounds and the denaturation of the enzyme. It is known that enzymes in general become inactive when they are exposed to temperatures above ~ 90 °C, especially in aqueous environments [42]. The advance of the transesterification reaction was followed by 1H NMR which was found to be complete in six days. The oligomeric mixture was polycondensated at 115 °C under a low pressure to favor the release of the excess of BD. As expected, the viscosity of the reaction mass increased with the content in G^l units due to the formation of ionic aggregates, which are known to act as thermoreversible crosslinks [18,44]. The preparation of $PBS_{50}G^l_{50}$ and PBG^l required increasing both the reaction time and the amount of added fresh enzyme in order to attain molecular weights within the range of those obtained for $PBS_xG^l_y$ containing lower amounts of ionic groups.

The influence of the tributylphosphonium compound PPD·Br on CALB activity was evaluated in a similar manner and results indicated that its effect was intermediate to those observed for the ammonium compounds. Nevertheless, at difference with $PBS_xG^l_y$, PB_xPP_yS polymers could be successfully synthesized by melt polycondensation using TBT catalyst. As usual, the reaction was made to proceed along two stages. The first stage was carried out at 170 °C under a nitrogen flow to facilitate the removal of the water generated by esterification. In the second stage, temperature was raised to 190 °C and high vacuum applied in order to promote the transesterification reaction between the oligomers formed in the first step and to favor the releasing of the excess of BD. A series of PB_xPP_yS copolyesters with contents in phosphonium units ranging from 3 to 15 mol-% in addition to the homopolyester PPPS made of PPD·Br as the only diol could be prepared by this procedure in high yields (>90%). It should be noted that the temperature used in these polycondensations was significantly lower than that usually applied in the synthesis of PBS because β -elimination occurs in the phosphonium containing units at high temperatures.

Synthesis results for the two series of ionic PBS copolyesters in addition to their respective parent homopolyesters are shown in Table 1. The PBS sample obtained by enzymatic polycondensation had an intrinsic viscosity of $0.9 \text{ dL}\cdot\text{g}^{-1}$ and a M_n of 25,000 $\text{g}\cdot\text{mol}^{-1}$ with a dispersity around 2. This is an excellent result for the enzymatic

synthesis of this polyester that proves the suitability of the applied procedure. In fact, the highest number-average molecular weights reported for enzymatically prepared PBS oscillate between 6,000 and 3,000 $\text{g}\cdot\text{mol}^{-1}$ [32]. The $\text{PBS}_x\text{G}_y^{\text{I}}$ copolyesters were obtained with M_n in the $\sim 5,000\text{-}13,000 \text{ g}\cdot\text{mol}^{-1}$ range and viscosities going from ~ 0.4 to $\sim 0.6 \text{ dL}\cdot\text{g}^{-1}$. $\text{PB}_x\text{PP}_y\text{S}$ copolyesters obtained by melt polycondensation had M_n in the $\sim 17,000\text{-}6,000$ range and viscosities between ~ 0.7 and $\sim 0.45 \text{ dL}\cdot\text{g}^{-1}$. It is apparent from these results that polymer molar mass tend to decrease with increasing contents in either G^{I} or PP units in the copolyesters. Accordingly minimum molecular weight were obtained for the ionic homopolyesters PBG^{I} and PPPS with M_n values of 4,500 and 6,000 $\text{g}\cdot\text{mol}^{-1}$, respectively. Such behavior is attributed to the difficulty for releasing 1,4-butanediol from the highly viscous molten reaction mixture at the relative low temperature at which polycondensations have to be carried out. This trend is more pronounced in the case of the $\text{PBS}_x\text{G}_y^{\text{I}}$ series likely due to the partially inhibitory effect of $\text{DMG}\cdot\text{Cl}$ on CALB.

The chemical structure and composition of the polyesters and copolyesters were ascertained by NMR spectroscopy. The ^1H and ^{13}C NMR spectra registered from representative members of the $\text{PBS}_x\text{G}_y^{\text{I}}$ and $\text{PB}_x\text{PP}_y\text{S}$ series with their corresponding peaks assignments are shown in Figures 2 and 3, respectively. An accurate assignment of the ^1H and ^{13}C spectra associated to the different protons and carbons signals present in the copolyesters could be achieved by 2D heteronuclear shift correlation (*hetcor*) analysis (Figures ESI-3 and ESI-4). The chemical composition was determined by integration of selected signals arising from G^{I} or PP units and signals of succinate or butylene units, respectively. As it can be observed in Table 1, the amount of ionic units incorporated into the PBS copolyester was slightly lower than that used in the feed, the differences being around 10 mole-% and practically constant along the whole series for both cases.

It should be noted that the ^1H NMR spectra of all $\text{PB}_x\text{PP}_y\text{S}$ copolyesters show a weak signal at 3.42 ppm due to the presence of dibutyleneoxide (DBO) units produced by the etherification side reaction between two hydroxybutyl units. The content in DBO of the copolyesters oscillated along the 2-4 mole-% range following roughly a trend parallel to their content in phosphonium units. The occurrence of this secondary reaction leading to the generation of ether groups in the polyester backbone was also reported for the synthesis of PET and PHT copolyesters containing sulfonated units [18,28]. In these cases much higher amounts of dialkylene glycol units were formed.

3.3. Thermal and mechanical properties

The thermal behavior of homopolyesters and copolyesters of the two series has been comparatively examined by TGA and DSC, and the thermal parameters estimated in this study are given in Table 2. The thermal stability was evaluated under inert atmosphere and the TGA traces recorded for the two sets of polyesters together with derivative curves for selected cases are shown in Figure 4. The comparative analysis of these traces indicates that the thermal stability of the ionic copolyesters steadily decreases with the incorporation of G^I or PPD units so both onset ($^{\circ}T_d$) and maximum rate decomposition ($^{\max}T_d$) temperatures range between the values displayed by their parent homopolyesters PBS and PBG^I or PBS and PPPS, respectively. Although the resistance to heat of the ionic copolyesters is significantly lower than that of PBS, the $^{\circ}T_d - T_m$ window is still wide enough as to allow their reversible melting and processing by thermal methods. In analogy with PBS, decomposition of PBS_xG^I_y copolyesters took place essentially in one stage whereas the PB_xPP_yS copolyesters degrade through a complex mechanism that involves two or three decomposition stages, depending on their composition (Figure 4b'). On the other hand, similar remaining weights were found for the two series after heating at 600 °C for similar copolyesters compositions but without apparent correlation with their ionic contents.

The heating DSC traces of the polyester samples that had been previously cooled by quenching from the melt showed the slope change characteristic of glass transition for all the studied compositions. The temperatures recorded for such changes revealed that the T_g of PBS steadily increased with the increasing content in ionic units. According to what should be reasonably expected, the incorporation of ionic groups caused a significant increase in the T_g of PBS. This enhancing T_g effect is clearly evidenced when the ionic homopolyesters are compared with PBS in this regard; both PBG^I and PPPS have T_g values much higher than PBS in spite that their molecular weights are several times lower. According to Shohamy *et al.* [45], these results are fully consistent with the effect on T_g that should be expected from the occurrence of ionic interactions.

The first heating DSC traces of the polyester coming directly from synthesis are depicted in Figure 5 for the two series. All copolyesters without exception produced first heating traces with endothermic peaks characteristic of melting which is demonstrative that all they were semicrystalline. Both T_m and ΔH_m were found to decrease steadily with the content in ionic units which is a sounded result since neither the ammonium nor the phosphonium side groups are expected to be able to accommodate in the PBS crystal lattice. Furthermore, crystallization from the melt was found to be only feasible for copolyesters with moderate ionic compositions, which is thought to be the consequence of the restricted chain mobility caused by ionic aggregation. Only

PBS_xG^l_y and PB_xPP_yS copolyesters containing up to 16 mole-% of G^l units and 10 mole-% of PP units, respectively, were able to crystallize from the melt and therefore to show melting at the second heating (second heating DSC traces are accessible in the . ESI file, Figure ESI-5). Lastly it is worthy to comment that PBGⁱ showed melting whereas no heat exchange was detected in the DSC traces recorded from the PPPS homopolymer. The absence of crystallinity in PPPS is a reasonably expected result given the great bulkiness of the pendant tributylphosphonium group. The general conclusion drawn from DSC results is that ionic groups depress the crystallinity of PBS and that such effect must be interpreted as a combination of two hindering factors: a) the incompatibility of the butylene succinate and the ionic units to cocrystallize within the same lattice, and b) the difficulty of the polymer chains to diffuse due to high melt viscosity of the ionic polyesters. The former is the simple consequence of the different constitution of the units whereas the latter is caused by the strong interactions that are characteristic for ionic charged polymers.

The tensile properties, such as Young's modulus (E), the maximum stress (σ_{\max}), and the elongation at break (ϵ_{\max}), were determined for amorphous samples of the PB_xPP_yS copolyesters with PP contents equal or below 8 mole-%. Copolyesters containing higher amounts of phosphonium units could not be evaluated because samples were too brittle and the same happened with copolyesters containing ammonium units. The stress-strain curves resulted from tensile essays are depicted in Figure 6 and the mechanical parameters measured therefrom are compared in Table 2. It was found that the E modulus increased and ϵ_{\max} decreased with the content of PP units whereas not clear trend was observed for tensile strength. It should be highlighted that the E modulus of PBS increased more than 100% when only 8 mole-% of PP units were incorporated in the chain which is a really amazing result that reveals the enormous stiff enhancing effect of the tributylphosphonium side group.

3.4. Crystal structure and crystallizability: coupling with anionic PBS copolyesters

Semicrystalline PBS_xG^l_y and PB_xPP_yS copolyesters were analyzed by powder XRD and the recorded profiles are compared in Figure 7 where PBS has been also included for reference. Essentially the same diffraction pattern comprising both peak spacing and intensity is shared by PBS and the ionic copolyesters. The pattern is distinguished by the presence of three sharp strong reflections at 4.5, 4.1 and 3.9 Å indicating that the monoclinic crystal structure of PBS [46] is retained in the copolyesters. As it has been reported for several random copolyesters containing unequal amounts of comonomers, the crystallinity attained in such systems is feasible because crystallites are formed exclusively from homogeneous polymer segments

made up of the major comonomer whereas the minor one is segregated to the amorphous phase [47,48]. Regarding the homopolyesters, no discrete scattering is detected in the profile recorded from PPPS according to the amorphous nature of this polymer. On the contrary, PBGⁱ produces a well-defined crystalline pattern that is radically dissimilar to that of PBS and indicates therefore the occurrence of a different crystal structure.

Ionic coupling between polyelectrolytes of opposite sign is known to proceed with generation of a stable heterocomplex that distinguishes in displaying properties different to its individual components [49,50]. In order to investigate this issue, a sample of anionic PBS copolyester containing sulfonate units was used as counterpart for the cationic copolyesters studied in this work. Thus nearly isocompositional copolyester pairs PB₈₄Gⁱ₁₆S/PB₈₅SS₁₅ and PB₈₅PP₁₅S/ PB₈₅SS₁₅ were made to interact in 1,1,1.3,3,3-hexafluoro-2-propanol solution, and the structure and thermal behavior of the product resulting in each case upon evaporation to dryness was examined by TGA, DSC and XRD. Data collected in this study for the mixed compounds (PBS-Gⁱ/SS and PBS-PP/SS) are compared with those of the three neat ionic copolyesters in Table 3. The thermal stability of the complexes slightly decreased with respect to the cationic components probably due to the presence of the less stable anionic copolyester. Regarding T_m , no noteworthy differences were observed between the complex and the isolated components probably because the density of ionic interactions is too low as to disturb crystallite size. On the contrary the T_g was found to increase significantly in the complexes which makes sense since the ionic interactions are expected to take place exclusively in the amorphous phase. Nevertheless, the most noticeable differences between complexes and individual copolyesters were those concerning crystallization at cooling from the melt (Figure 8). PB₈₄Gⁱ₁₆S crystallized homogeneously in one single peak whereas crystallization of PBS-Gⁱ/SS showed a second peak at lower temperature (Figure 8a). Differences in crystallization became even clearer for the PB₈₅PP₁₅S/ PB₈₅SS₁₅ system. As it is seen in Figure 8a', no crystallization could be detected for none of the two isolated components whereas a strong exothermic peak was observed for the PBS-PP/SS complex. The distinctive crystallization features observed in the blends are due to the nucleating effect exerted by the microcrystallites that are generated by specific interactions between the opposite charged ionic groups. Such effect was ascertained by a comparative isothermal crystallization study carried out on the PB₈₅PP₁₅S/ PB₈₅SS₁₅ system. Details of this study are given in the ESI file (Figure ESI-6). Kinetics parameters resulting from the application of the Avrami approach (Table 3) revealed that crystallization of PB₈₅SS₁₅ took place with a $t_{(1/2)}$

approximately double than the complex with no perceivable changes in the crystallization mechanism.

PBS-Gⁱ/SS and PBS-PP/SS complexes were examined by XRD in order to get insight the complex structure and corroborate the occurrence of ionic microcrystallites. The XRD profiles registered from the two isolated ionic copolyesters and their corresponding complex are shown in Figures 8b and 8b' for the two systems. In both cases the three compared profiles display the pattern characteristic of PBS with strong peaks at 3.9, 4.1 and 4.5 Å indicating that the crystal structure of the cationic copolyesters is retained in the complexes. What is really noteworthy is the presence in the trace recorded from PBS-PP/SS of two sharp peaks of low intensity at about 2.9 and 3.4 Å. These peaks do not disappear after heating the sample at temperatures above the T_m and they are made to correspond to small crystallites made of ionic motives. After cooling the diffraction pattern characteristic of PBS was recovered with the two small peaks unchanged. It can be concluded therefore that the ionic crystallites preserved in the molten state act as effective nuclei in the crystallization of PBS.

3.5. Antimicrobial activity

As the last part of this research the antimicrobial activity of the cationic copolyesters of PBS was explored against *E. coli* and *S. aureus*. The ammonium containing PBS copolyesters tested were PBS₅₀Gⁱ₅₀ and PBS₈₄Gⁱ₁₆ in addition to the homopolyester PBGⁱ. The selected phosphonium containing PBS copolyesters were PB₉₇PP₃S, PB₉₀PP₁₀S and PB₈₅PP₁₅S. A PBS sample was also tested for reference. The results obtained from these essays are represented in Figure 9.

In all cases the response of PBS was similar to the blank indicating that this polyester does not exert perceivable biocide activity. On the contrary, the results observed for the PBS copolyesters containing ionic groups clearly brought into evidence their microbial effect. Thus a complete inactivation of the colony was observed in a few minutes for *E. coli* cultured in the presence of PBGⁱ and also a drastic decreasing in viability was observed after a few hours in the presence of the PBS₅₀Gⁱ₅₀. It is worthy to compare these results with those reported by Riva *et al.* [51] for poly(ϵ -caprolactone) grafted with ammonium salts; they observed a fast and complete inactivation of the *E. coli* by PCL containing 30 mol-% of ammonium groups after 1 h when a concentration of $\sim 10 \text{ g}\cdot\text{L}^{-1}$ of active groups were used in the dynamic shake flask method. The inactivation for *S. aureus* was even more noticeable in spite that this bacterium is known to be much more resistant to adverse factors. PBGⁱ was able to completely inactivate cell proliferation in a period of a few hours and PBS₅₀Gⁱ₅₀ made to fall the CFU concentration in about five orders after less than one day of

incubation. The results obtained for $PBS_{84}G_{16}^I$ were uneven and no clear conclusions can be derived from them.

The trend observed for the PB_xPP_yS series was highly depending on composition and a close comparison with the $PBS_xG_y^I$ series is not feasible because copolyesters with high ionic compositions are not available for the former. An inspection of the data shown in Figure 9 for both *E. coli* and *S. aureus* reveals that no clear effect is displayed by copolyesters containing 3% and 10% of phosphonium groups whereas the biocide activity of $PB_{85}PP_{15}S$ becomes unmistakably manifested. In fact, the presence of this copolyester in both cultures promoted a steady decay of CFU of about five orders after only one day of incubation. It is also remarkable that the antibacterial effect of this copolyester is clearly perceived since the beginning of the assay.

The above reported observations lead to conclude the following: a) The biocide effect of both ammonium and phosphonium containing PBS copolyesters is highly depending on composition so it is only clearly manifested above certain contents in ionic groups. b) The phosphonium containing PBS copolyesters are particularly active and they display a sustained biocide effect with similar intensity for the two tested bacteria.

4. Conclusions

Two series of cationic PBS copolyesters containing ammonium and tributylphosphonium side groups in contents up to 50 %-mole in addition to the entirely ionic homopolyesters have been synthesized. The high sensitivity to heat of cationic compounds made necessary to apply polymerization conditions less severe than usual. CALB enzymatic mediated polycondensation at temperatures close to 100 °C has been proved to be a suitable method to produce ammonium containing PBS copolyesters. PBS copolyesters bearing phosphonium groups could be successfully prepared by melt polycondensation assisted by titanium catalyst by applying relative lower temperatures.

The cationic PBS copolyesters display a panel of properties that reflects the presence of the ionic units in the polyester chain. Their thermal stability decreases respect to PBS for the two series but all the copolyesters continue to be heat resistant enough as to be processed by melting. They are semicrystalline and they adopt the same crystal structure as PBS although their melting temperature and crystallinity decrease with the content in ionic groups. On the contrary, the T_g of PBS regularly increases with the insertion of either ammonium or phosphonium units due the decreasing in chain mobility caused by strong ionic interactions. Such interactions are also responsible for the high melt viscosity displayed by these cationic PBS

copolyesters so that only those with moderate ionic contents are able to crystallize from the melt under non-isothermal conditions. Blending with anionic PBS copolyesters containing sulfonate groups entails formation of ionically compensated microcrystallites that are resistant to temperature and are able to act as effective nucleating agent in the crystallization from the melt.

Ammonium and phosphonium containing PBS copolyesters show a noticeable biocide activity against both *gram*-positive and *gram*-negative bacteria that is strongly depending on composition. The copolyesters containing 50 mole-% of ammonium groups or 15% of phosphonium groups display strong biocide in periods of time of hours, which is a very interesting result given the potential of PBS as packaging and coating material. Not only copolymerization but also blending of PBS with cationic containing copolyester master-batches will be promising approaches to attain PBS formulations with antimicrobial activity.

Acknowledgements

Authors are grateful to Prof. Katja Loos (Gröningen University, NL) for valuable comments and suggestions. They also acknowledge the financial support received from MINECO with grant MAT2012-38044-CO3-03 and the UPC-Research grant awarded to Mayka Bautista Betancur.

References

- [1] Miller, SA. Sustainable Polymers: Opportunities for the next decade. *ACS Macro Letters* 2013; 2:550-554.
- [2] Rydz J, Wanda S, Kyulavska M, Christova, D. Polyester-based (bio)degradable polymers as environmentally friendly materials for sustainable development. *Intern J Molec Sci* 2015; 16:564-596.
- [3] Hillmyer MA, Tolman, WB. Aliphatic polyester block polymers: renewable, degradable, and sustainable. *Acc Chem Res* 2014; 47:2390-2396.
- [4] Williams CK. Synthesis of functionalized biodegradable polyesters. *Chem Soc Rev* 2007; 36:1573-1580.
- [5] Barrett DG, Yousa MN. Design and applications of biodegradable polyester tissue scaffolds based on endogenous monomers found in human metabolism. *Molecules* 2009; 14:4022-4050.
- [6] Mohamed F, van der Walle CF. Engineering biodegradable polyester particles with specific drug targeting and drug release properties. *J Pharm Sci* 2008; 97:71-87.
- [7] Ikada Y, Tsuji H. Biodegradable polyesters for medical and ecological applications. *Macromol Rapid Comm* 2000; 21:117-132.
- [8] Zeikus JG, Jain MK, Elankovan P. Biotechnology of succinic acid production and markets for derived industrial products, *Appl. Microbiol Biotech* 1999; 51:545-552.
- [9] Xu, J and Guo, B-H. Poly(butylene succinate) and its copolymers: Research, development and industrialization *Biotech J* 2010; 5:1149-1163.
- [10] Ichikawa Y, Mizukoshi T. Bionolle (Polybutylenesuccinate). *Adv Polym Sci* 2012; 245:285-313.
- [11] Eisenberg A. Glass transitions in ionic polymers. *Macromolecules* 1971; 4:125-128.
- [12] Hoover MF, Butler GB. Recent advances in ion-containing polymers. *J Polym Sci, Part C-Polym Symp* 1974; 45:1-37.
- [13] Utracki LA, Walsh DJ, Weiss RA. Polymer alloys, blends, and ionomers: An overview. 1989; *ACS Symp Series* 1989; 395:1-35.
- [14] Lundberg RD, Duvdevani I. Shear-thickening behavior of ionomers and their complexes. *ACS Symp Series* 1991; 462:155-175.
- [15] Hara M, Sauer JA. Mechanical-properties of ionomers. *J Macrom Sci, Rev in Macrom Chem Phys* 1994. C34:325-373.
- [16] Ali I, Dolui SK. Synthesis of polyester containing ionic comonomer through interfacial polymerization. *J Polym Mat* 2004; 21:61-66.
- [17] Gaona O, Kint D, Martínez de Ilarduya A, Alla A, Bou J, Muñoz-Guerra S. Preparation and hydrolytic degradation of sulfonated poly(ethylene terephthalate) copolymers. *Polymer* 2003; 44:7281-7289.

- [18] Bautista M, Martínez de Ilarduya A, Alla A, Muñoz-Guerra S. Sulfonated poly(hexamethylene terephthalate) copolyesters: enhanced thermal and mechanical properties. *J Appl Polym Sci* 2013; 129:3527-3535.
- [19] Bougarech A, Abid M, Gouanve E, Espuche E, Abid S, El Gharbi R, Fleury, E. Synthesis, characterization and water sorption study of new biobased (furanic-sulfonated) copolyesters. *Polymer* 2013; 54:5482-5489.
- [20] Bougarech A, Abid M, DaCruz-Boisson F, Abid S, El Gharbi R, Fleury E. Modulation of furanic-sulfonated isophthalic copolyesters properties through diols units control. *Eur Polym J* 2014; 58: 207-217.
- [21] Storey RF, Deng ZD, Glancy TP, Peterson DR, US Patent 5668288, 1997.
- [22] Han SI, Yoo Y, Kim DK, Im SS. Biodegradable aliphatic polyester ionomer. *Macromol Biosci* 2004; 4:199-207.
- [23] Han SI, Im SS, Kim DK. Dynamic mechanical and melt rheological properties of sulfonated poly(butylene succinate) ionomers. *Polymer* 2003; 44:7165-7173.
- [24] Li HY, Chang J, Cao AM Wang JY. In vitro evaluation of biodegradable poly(butylene succinate) as a novel biomaterial. *Macromol Biosci* 2005; 5:433-440.
- [25] Han SI, Kang SW, Kim BS, Im SS. A novel polymeric ionomer as a potential biomaterial: crystallization behavior, degradation and in-vitro cellular interactions. *Adv. Funct. Mater* 2005; 15:367-374.
- [26] Lim JS, Kim JH. New application of poly(butylene succinate) (PBS) based ionomer as biopolymer: a role of ion group for hydroxyapatite (HAp) crystal formation. *J Mater Sci* 2009; 44:6398-6403.
- [27] Park SB, Hwang SY, Moon CW, Im SS, Yoo ES. Plasticizer effect of novel PBS ionomer in PLA/PBS ionomer blends. *Macromol Res* 2010; 18:463-471.
- [28] Bautista M, Martínez de Ilarduya A, Alla A, Muñoz-Guerra S. Poly(butylene succinate) ionomers with enhanced hydrodegradability. *Polymers* 2015; 7:1232-1247.
- [29] Bautista M, Martínez de Ilarduya A, Alla A, Muñoz-Guerra S. Poly(butylene succinate) ionomers and their use as compatibilizers in nanocomposites. *Polym Compos* 2016; DOI: 10.1002/pc.23454
- [30] Kobayashi S, Uyama H, Kimura S, Enzymatic polymerization. *Chem Rev* 2001; 101:3793-3818.
- [31] Xu J, Guo B-H. Microbial succinic acid, its polymer poly(butylene succinate), and applications, in: G.G.-Q. Chen (Ed.), *Plastics from Bacteria*, Springer Berlin, Heidelberg, 2010, pp. 347-388.
- [32] a) Jiang, Y, Alberda van Ekenstein, GOR., Woortman, AJJ, Loos, K. Fully biobased unsaturated aliphatic polyesters from renewable resources: Enzymatic synthesis, characterization, and properties. *Macromol. Chem. Phys.* 2014; 215:2185-2197. b) Azim H, Dekhterman A, Jiang ZZ, Gross RA. *Candida antarctica* lipase B-catalyzed synthesis of poly(butylene succinate): shorter chain building blocks also work. *Biomacromolecules* 2006; 7:3093-3097.
- [33] Anderson EB, Unal S, Long TE. Synthesis and association of phosphonium cation-containing polyesters for food packaging applications. *Polym Prepr* 2007; 48:731-732.
- [34] Anderson EB, Unal S, Long TE. Phosphonium cations as a new avenue for electrostatic interactions in polymer design. *Polym Prepr* 2008; 49:262-263.
- [35] Anthierens T, Billiet L, Devlieghere F, Du Prez F. Poly(butylene adipate) functionalized with quaternary phosphonium groups as potential antimicrobial packaging material. *Innov Food Sci Emerg Technol* 2012; 15:81-85.

- [36] Fuchs AD, Tiller JC, Contact-active antimicrobial coatings derived from aqueous suspensions. *Angew Chem Int Ed* 2006; 45:6759-6762.
- [37] Majumdar P, Lee E, Gubbins N, Stafslieen SJ, Daniels J, Thorson CJ, Chisholm BJ. Synthesis and antimicrobial activity of quaternary ammonium-functionalized POSS (Q-POSS) and polysiloxane coatings containing Q-POSS. *Polymer* 2009; 50:1124-1133.
- [38] Zheng A, Xue Y, Wei D, Li S, Xiao H, Guan Y. Synthesis and characterization of antimicrobial polyvinyl pyrrolidone hydrogel as wound dressing. *Soft Matter* 2014; 12: 179-187.
- [39] Chen FCM, Benoiton NL. New method of quaternizing amines and its use in amino-acid and peptide chemistry. *Can J Chem* 1976; 54:3310-3311.
- [40] Mindemark J; Bowden, T. Synthesis and polymerization of alkyl halide-functional cyclic carbonates. *Polymer* 2011; 52:5716-5722.
- [41] Zhang M, Hemp ST, Allen MH, Carmean RN, Moore RB, Long TE. Water-dispersible cationic polyurethanes containing pendant trialkylphosphoniums. *Polym. Chem* 2014; 5:3795-3803.
- [42] Schwab LW, Polyamide synthesis by hydrolases, PhD Thesis, University of Groningen, The Netherlands, 2010.
- [43] Lozano P, De Diego T, Carrie D, Vaultier M, Iborra JL, Enzymatic ester synthesis in ionic liquids. *J Mol Catal, B Enzym* 2003; 21:9-13.
- [44] Greener J, Gillmor JR, Daly RC. Melt rheology of a class of polyester ionomers, *Macromolecules* 1993; 26:6416-6424.
- [45] Shohamy E, Eisenberg A. Effect of ion aggregation on some melt properties of styrene ionomers. *J Polym Sci, Polym Phys* 1976; 14:1211-1220.
- [46] Ihn K, Yoo E, Im S. Structure and morphology of poly(tetramethylene succinate) crystals. *Macromolecules* 1995; 28:2460-2464.
- [47] Li B, Yu J, Lee S, Ree M. Poly(ethylene terephthalate co ethylene isophthalate) - relationship between physical properties and chemical structures. *Eur Polym J* 1999; 35:1607-1610.
- [48] Quintana R, Martínez de Ilarduya A, Rudé E, Kint DPR, Alla A, Galbis JA, Muñoz-Guerra S. Poly(ethylene isophthalate)s: effect of the tert-butyl substituent on structure and properties. *Polymer* 2004; 45:5005-5012.
- [49] Chelushkin PS, Lysenko EA, Bronich TK, Eisenberg A, Kabanov VA, Kabanov A V. Polyion complex nanomaterials from block polyelectrolyte micelles and linear polyelectrolytes of opposite charge: 1. Solution behavior. *J Phys Chem B* 2007; 111:8419-8425.
- [50] Chelushkin PS, Lysenko EA, Bronich TK, Eisenberg A, Kabanov VA, Kabanov A V. Polyion complex nanomaterials from block polyelectrolyte micelles and linear polyelectrolytes of opposite charge. 2. Dynamic properties. *J Phys Chem B*. 2008; 112:7732-77388.
- [51] Riva R, Lussis P, Lenoir S, Jérôme C, Jérôme R, Lecomte P. Contribution of "click chemistry" to the synthesis of antimicrobial aliphatic copolyesters. *Polymer* 2008; 49:2023-2028.

Cationic poly(butylene succinate) copolyesters

Mayka Bautista^a, Antxon Martínez de Ilarduya^a, Abdelilah Alla^a,
Marc Vives^b, Jordi Morató^b and Sebastián Muñoz-Guerra^{a,*}

Universitat Politècnica de Catalunya

^aDepartament d'Enginyeria Química, ETSEIB, Diagonal 647, Barcelona 08028, Spain

^bHealth and Environmental Microbiology Lab & UNESCO Chair on Sustainability, EET, Edifici Gaia, Pg. Ernest Lluch/Rambla Sant Nebridi, Terrassa 08222, Spain

*Corresponding author: sebastian.munoz@upc.edu (S. Muñoz-Guerra Tel.: +34 93 4016680)

Abstract: The synthesis, characterization and comparative evaluation of properties of two series of cationic PBS copolyesters bearing respectively ammonium and tributylphosphonium side groups, are reported. The copolyesters with contents in ionic units up to 50 mole-% as well as the fully ionic homopolyesters were prepared by polycondensation in the melt catalyzed by CALB or TBT. Their M_n ranged between 20,000 and 5,000 g·mol⁻¹ depending of composition and the type of ionic group that is involved. All the copolyesters were non-water soluble and showed good thermal stability. They were semicrystalline with melting temperatures and enthalpies decreasing with the ionic contents. The interactions interplayed by the ionic groups restricted largely the molecular mobility and caused a significant increase in the melt viscosity and glass transition temperature of PBS and a decrease in crystallization rate. Both ammonium and phosphonium containing PBS copolyesters were able to be coupled with sulfonated PBS to generate ionic polymer blends with modified crystallizability. The presence of both ammoniums and phosphoniums provided PBS with remarkable antimicrobial activity against gram-positive and gram-negative bacteria.

Keywords: Poly(butylene succinate), polyelectrolytes, cationic polyesters, enzymatic polymerization, ionic polymeric complexes, biocidal polyesters.

1. Introduction

The interest in aliphatic polyesters has greatly increased along these last years due to the fact that they are among the most promising sustainable and biodegradable polymeric materials [1-3]. In addition to their applicability in packaging and coating, a good number of aliphatic polyesters are designed with adjusted biodegradation rate with the purpose of use them as temporal applications in the biomedical field [4-7]. Poly(butylene succinate) (PBS) is a typical biodegradable aliphatic polyester that has been traditionally produced using petroleum-based monomers. Recently PBS has emerged as a distinguished polymer mainly because its two monomers, succinic acid and 1,4-butanediol have become accessible from renewable resources [8]. Moreover PBS displays a satisfactory overall pattern of thermal and mechanical properties that makes it suitable for a wide range of end applications mainly in packaging and coating by using conventional melt processing techniques [9,10]. Nevertheless, the lack of functionality is a shortcoming of PBS that prevents expanding its use into new fields where polymer activity or polymer modifications are required.

The incorporation of ionic groups in a polymeric chain gives rise to important changes in both physical and chemical properties and provides it with certain chemical activity [11,12]. In fact, slightly charged polymers (ionomers) are known to be able to interact electrostatically with themselves or with other ionic polymers and small charged molecules to render unusual properties [13-15]. Aromatic copolyesters bearing sulfonated groups constitute an illustrative example of this approach [16-20]. In aliphatic polyester ionomers, property design may be combined with biodegradability to generate materials of exceptional interest as delivery systems for charged proteins and drugs, and in particular for transfection technology [21,22]. Anionic PBS ionomers and copolymers containing sulfonate groups have been recently studied by different authors [23-28], and their potential as biomaterial has been evaluated in some cases. On the contrary, polyester cationic ionomers are in a much less developed stage, in great part due to the difficulty found for introducing cationic groups into the polyester chain by melt polycondensation. The insertion of cationic units in polyesters is particularly attractive because it not only changes advantageously certain properties but in addition it is expected to provide antimicrobial activity. In this paper we wish to report on PBS copolyesters containing either ammonium or phosphonium side groups with a wide range of compositions. The work reported here embraces the synthesis and characterization of the two series of cationic PBS copolymers, the assessment of their basic properties and also a preliminary evaluation of their biocide activity.

To our knowledge, a recent paper published by us on PBS terpolyesters containing minor amounts of quaternized glutamic acid that were synthesized to be

used as nanocomposite compatibilizers, is the only reference on ammonium containing polyesters found in the accessible literature [29]. In such work, it was brought into evidence that the high temperatures required for melt polycondensation promoted the decomposition of the ammonium units with subsequent hindering of the polymer chain growth. These results have encouraged us to explore the enzymatic polycondensation for the synthesis of cationic PBS copolyesters. In fact, lipase-catalyzed polymerization in the melt is a recently developed method that allows producing aliphatic polyesters under mild conditions and avoiding residual metals [30,31]. Thus Azim *et al.* [32] reported the CAL-B mediated synthesis of PBS with a M_w close to 40,000. In this paper we describe the enzyme-catalyzed melt polycondensation of PBS copolyesters ($PBS_xG^1_y$) that incorporate different amounts of 2-aminoglutarate hydrochloride units (G^1) (x and y subscripts refer respectively to the mole-% in succinate and aminoglutarate units present in the copolyester).

Although the literature on phosphonium containing polymers is considerable, very short has been published on polyesters. Anderson *et al.* [33,34] synthesized successfully poly(ethylene terephthalate-co-ethylene isophthalate) (PET-co-PEI) ionomers end-capped with butyl *p*-carboxyphenyl diphenylphosphonium bromide, and Anthierens *et al.* [35] reported on poly(butylene adipate) functionalized with an alkyltriphenylphosphonium group that showed antimicrobial activity. In the present work the diol 2,2-(dihydroxymethyl)propyl-tributylphosphonium bromide (PPD) has been copolymerized with succinic acid and 1,4-butanediol (BD) to obtain poly(butylene-co-propylene succinate) copolyesters containing pendant quaternary phosphonium groups. These new cationic PBS ionomers are abbreviated as PB_xPP_yS where x and y subscripts refer respectively to the mole-% in 1,4-butylene and phosphonium propylene units that are present in the copolyester.

The influence of the cationic side groups on PBS properties has been examined in this work with particular attention paid to biocide activity. Given the enormous potential application of PBS in packaging and coating, the possibility of rendering PBS with biocide properties is a goal of primary technological interest. Antimicrobial agents based on natural or low-molecular-weight compounds may result in environmental contamination and toxicity to the human body due to uncontrolled diffusion [36]. Conversely, antimicrobial polymeric materials based on covalent linkages display high chemical stability and low volatility, and are able to exhibit therefore long-term activity [37,38]. Materials containing ammonium and/or phosphonium salts are probably the most widely studied and used as antimicrobial polymers. Accordingly, the biocide effect of the cationic PBS copolyesters synthesized in this work has been explored using *Escherichia coli* and *Staphylococcus aureus* as testing microorganisms.

2. Experimental

2.1. Materials

Dimethyl succinate (DMS) was purchased from Merck-Schuchardt. 1,4-butanediol (BD) (99%), L-glutamic acid dimethyl ester hydrochloride (2-ammonium dimethyl glutarate hydrochloride) (A-DMG·Cl), 1,1,1-tris(hydroxymethyl) ethane, tributylphosphine, diethyl carbonate and lipase from *Candida antarctica* immobilized on macroporous acrylic resin (CALB) were purchased from Sigma-Aldrich. Succinic acid (SA), hydrobromic acid and solvents used for purification and characterization were purchased from Panreac. All they were of either technical or high-purity grade and used as received without further purification. 2-(*N,N,N*-trimethylammonium) dimethyl glutarate iodide (TMA-DMG·I) was synthesized from A-DMG·Cl according to the general procedure described in the literature for amine quaternization [39]. The PBS ionomer (PBS₈₅SS₁₅) containing 15 mole-% of sulfonated succinate used for the complex formation study was synthesized as described in detail elsewhere [28].

2.2. Measurements

Intrinsic viscosities were measured with an Ubbelohde viscometer thermostated at 25 °C ± 0.1 °C. Size exclusion chromatography (SEC) was performed on a Waters system equipped with a refractive index detector using 1,1,1,3,3,3-hexafluoro-2-propanol as the mobile phase. Molecular weights and their distribution were calculated against poly(methyl methacrylate) standards using the Millenium 820 software. Absorbance measurements were performed with an UV-visible spectrophotometer UVmini-1240 using a 5 nm band-width. NMR spectra were recorded on a Bruker AMX-300 spectrometer operating at 300.1 MHz for ¹H and 75.5 MHz for ¹³C. About 10 mg for ¹H or 50 mg for ¹³C of polymer samples were dissolved in 1 mL of deuterated chloroform (CDCl₃). 64 and 5,000-10,000 scans were acquired with 32-K and 64-K data points and 1 and 2 s of relaxation delays for ¹H and ¹³C, respectively.

The thermal behavior of the polyesters was examined by differential scanning calorimetry (DSC) with a Perkin-Elmer DSC Pyris 1 instrument calibrated with indium and zinc for temperature and enthalpy. DSC data were obtained from 4 to 6 mg samples at heating and cooling rates of 10 °C·min⁻¹ under nitrogen circulation (20 mL·min⁻¹). Glass transition temperatures were taken as the inflection point of the heating DSC traces of melt-quenched samples recorded at 20 °C·min⁻¹. TGA measurements were performed on 10 to 15 mg of sample under a nitrogen flow of 20 mL·min⁻¹ at a heating rate of 10 °C·min⁻¹ and within a temperature range of 30-600 °C using a Mettler Toledo TGA/DSC 1 thermobalance. X-ray diffraction (XRD) patterns of copolyesters were recorded from powdered samples on the PANalytical X'Pert PRO

MPD θ/θ diffractometer using $\text{CuK}\alpha$ radiation of wavelength 0.1542 nm. For the analysis of the complexes requiring heating, XRD was carried out with the sample introduced in a Lindemann glass capillary of 1.0 mm of diameter. Tensile testing was performed using bone shape specimens ($2.7 \times 10 \text{ mm}^2$) that were cut from isotropic films obtained by hot pressing with a thickness of about 200 μm . Tensile tests were conducted at room temperature on a Zwick BZ2.5/TN1S universal tensile testing apparatus operating at a constant crosshead speed of $10 \text{ mm}\cdot\text{min}^{-1}$ with a 0.5 N preload and a grip-to-grip separation of 20 mm.

2.3. Synthesis of cationic PBS copolyesters containing ammoniums.

Candida antarctica lipase immobilized on macroporous acrylic resin (CALB) was dried under vacuum (0.1 mm-Hg) at 25 °C for 24 h. 2% (w/w) of CALB was transferred into a round-bottom flask containing a mixture of dimethyl succinate, L-glutamic acid dimethyl ester hydrochloride and 1,4-butanediol. A molar ratio 1.4:1 of BD to the total of diesters was used for the synthesis of all polyesters. The reaction mixture was mechanically stirred under heating at 80 °C for 72 h under a low nitrogen flow. Then a second 2% (w/w) CALB portion was added to the reaction mixture and the polycondensation reaction was initiated by raising the temperature to 90 °C and reducing the pressure to 40 mbar, and these conditions were maintained for 72 h. Finally a third 2% (w/w) portion of CALB was added, the temperature further increased to 115 °C and the pressure reduced to $1.6 \cdot 10^{-3}$ mbar, and the reaction left to proceed for 72 h more. The synthesis of $\text{PBS}_{50}\text{G}_{50}^1$ and PBG^1 required both longer reaction times (around 300 h) and higher amounts of enzyme (a total of 10% w/w) than in the other cases. The viscous mass finally formed was cooled down to room temperature and the atmospheric pressure was restored with a nitrogen flow to prevent degradation. The resulting solid mass was dissolved in chloroform and filtered using a PTFE membrane with 0.5 μm pore size. The product recovered after evaporation of the solvent was used for characterization and property evaluation without further treatment.

2.3. Synthesis of cationic PBS copolyesters containing phosphoniums.

2,2-(dihydroxymethyl)propyl-tributylphosphonium bromide (PPD-Br) was synthesized in two steps according to the procedures described in the literature [40,41]. 2-(Bromomethyl)-2-methyl-1,3-propanediol was firstly prepared from 1,1,1-tri(hydroxymethyl) ethane and then it was made to react with tributylphosphine to render PPD-Br. ^1H and ^{13}C NMR spectra of this compound and its precursor are available in the Electronic Supporting File (Figures ESI-1 and ESI-2).

The poly(butylene succinate) copolyesters containing tributylphosphonium groups (PB_xPP_yS) were obtained from succinic acid (SA) and mixtures of PPD-Br and BD containing 3, 5, 8, 10 and 15 mol-% of the former. The reactions were performed in a three necked, cylindrical-bottom flask equipped with a mechanical stirrer, a nitrogen inlet and a vacuum-distillation outlet. An excess 1.4:1 of BD to SA was used and titanium tetrabutoxide (TBT, 0.3 w/w % respect to monomers) was the catalyst of choice. Esterification reactions were carried out under a low nitrogen flow at 170 °C during 8 h. Polycondensation reactions were left to proceed at 190 °C under a 2.9·10⁻³ mbar vacuum for 8 h. Finally, the reaction mixture was cooled to room temperature and the atmospheric pressure was recovered with nitrogen to prevent degradation. The resulting solid mass without further treatment was used for the characterization and property evaluation unless otherwise stated.

2.4. CALB activity test

The enzyme activity was tested according to the procedure described by Schwab *et al.* [42]. In this test, the *p*-nitrophenol (*p*NP) generated by methanolysis of *p*-nitrophenyl acetate (*p*NPAc) is followed by UV spectroscopy. 5 mL of a solution of *p*NPAc (7.25 mmol L⁻¹) in toluene and 20 μL of methanol were successively added to a stirred suspension of CALB (10 mg) in toluene (20 mL) at 40 °C. Aliquots of 0.5 mL were then withdrawn every 5 min and diluted with 9.0 mL of toluene. The *p*-nitrophenol concentration was determined by measuring the UV absorption at 304 nm (ϵ_{pNP} , 9344.5 M⁻¹ cm⁻¹ and ϵ_{pNPAc} , 2469.1 M⁻¹ cm⁻¹). The enzyme activity *a* (nmol of substrate converted by 1 mg of CALB per minute) was calculated using equations 1 and 2.

$$c = \frac{n \cdot Abs - \epsilon_{NPA} \cdot c_{0PNAC}}{\epsilon_{pNP} - \epsilon_{pNPAc}} \quad \text{Eq 1}$$

$$a = \frac{c \cdot V \cdot x}{M_{CALB} \cdot t} \quad \text{Eq 2}$$

2.5. Antimicrobial activity

The antimicrobial activity was performed based on the dynamic shake flask method described by Anthierens *et al.* with some modifications [35]. Lyophilized strains of the microorganisms from CECT (Spanish Collection of Culture Types) were resuspended in TSB (Tryptone Soy Broth) for *Escherichia coli* and *Staphylococcus aureus* following the CECT instructions and then incubated for 24 h at 37 °C, in TSA (Tryptone Soy Agar). The broth was then diluted in physiological peptone solution (PPS) containing 1 g of bacteriological peptone (Oxoid, Hampshire, United Kingdom) and 8.5 g of NaCl per liter to approximately 10⁶ to 10¹⁰ colony forming units (cfu)·mL⁻¹. 1 mL of this solution was used to inoculate the sterile 1.5 mL centrifugal tubes

(Eppendorf, Hamburg, Germany) containing 100 mg of each sample (PBS, PBS₈₄G^I₁₆, PBS₅₀G^I₅₀ and PBG^I for ammonium polymers and PBS, PB₉₇PP₃S, PB₉₀PP₁₀S, PB₈₅PP₁₅S for phosphonium polymers) to achieve finally an inoculation level of approximately 10⁵ to 10⁹ cfu·mL⁻¹ depending on the assay. The pH of the PPS solution was adjusted to 5.8 with 0.2% HCl. Assays were conducted per duplicate at 37 °C and under stirring (500 rpm). Samples were drawn at scheduled periods of time ranging from 30 min up to 24 h. 100 µL of each sample was then cultured in TSA petri plates per duplicate and incubated for 24 h at 37 °C, after which the colonies were counted. Petri Plate Count Assay was used to determine the microorganism concentration expressed as colony forming units. Counts out of the range of 30-300 cfu per plate were discarded.

3. Results and discussion

3.1. Synthesis of polyesters and copolyesters

The routes followed for the synthesis of PBS_xG^I_y and PB_xPP_yS polymers are depicted in Scheme 1. Initially the preparation of PBS copolyesters containing trimethylammoniums was pursued. Unfortunately, all attempts made to polymerize DMS with BD in the presence TMA-DMG-I were unsuccessful because the transesterification reaction was unable to proceed either in solution or in the bulk. Increasing the temperature or reducing the pressure to favor the release of methanol did not afford any positive result. Neither increasing amounts of CALB produced any perceivable effect. To elucidate if the enzyme activity was affected by the reaction components, activity tests for CALB were carried out using *p*-nitrophenyl acetate (*p*NPAC) as substrate in the presence and in the absence of TMA-DMG-I. The *p*-nitrophenol (*p*NP) released in these essays along a period of 60 min of incubation is plotted against time in Figure 1. Whereas in the absence of TMA-DMG-I the alcoholysis of *p*NPAC took place steadily, no reaction occurred in the presence of the quaternary ammonium salt. It was concluded therefore that the polycondensation reaction could not advance at a detectable rate because the enzyme was fully inhibited by TMA-DMG-I since the initial stage. Conversely, the same activity test carried out with A-DMG-Cl afforded more favorable results. CALB activity was also depressed in the presence of this compound but it was still remained over 50% after one hour of reaction. Apparently the inhibitory effect of the ammonium group on CALB activity is drastically enhanced by quaternization. It is worthy to point that this result contrasts with those reported by Lozano *et al.* [43], who found that ionic liquids made of

quaternary imidazolium salts were suitable for carrying out transesterification reactions mediated by CALB.

According to the enzyme activity results, $PBS_xG^l_y$ copolyesters were prepared by bulk polycondensation catalyzed by CALB since this method allows applying milder conditions than when organocatalysts are used. An excess of BD respect to the diesters was used to ensure the complete displacement of methanol, and temperature was increased gradually in order to minimize the decomposition of ammonium compounds and the denaturation of the enzyme. It is known that enzymes in general become inactive when they are exposed to temperatures above ~ 90 °C, especially in aqueous environments [42]. The advance of the transesterification reaction was followed by 1H NMR which was found to be complete in six days. The oligomeric mixture was polycondensated at 115 °C under a low pressure to favor the release of the excess of BD. As expected, the viscosity of the reaction mass increased with the content in G^l units due to the formation of ionic aggregates, which are known to act as thermoreversible crosslinks [18,44]. The preparation of $PBS_{50}G^l_{50}$ and PBG^l required increasing both the reaction time and the amount of added fresh enzyme in order to attain molecular weights within the range of those obtained for $PBS_xG^l_y$ containing lower amounts of ionic groups.

The influence of the tributylphosphonium compound PPD·Br on CALB activity was evaluated in a similar manner and results indicated that its effect was intermediate to those observed for the ammonium compounds. Nevertheless, at difference with $PBS_xG^l_y$, PB_xPP_yS polymers could be successfully synthesized by melt polycondensation using TBT catalyst. As usual, the reaction was made to proceed along two stages. The first stage was carried out at 170 °C under a nitrogen flow to facilitate the removal of the water generated by esterification. In the second stage, temperature was raised to 190 °C and high vacuum applied in order to promote the transesterification reaction between the oligomers formed in the first step and to favor the releasing of the excess of BD. A series of PB_xPP_yS copolyesters with contents in phosphonium units ranging from 3 to 15 mol-% in addition to the homopolyester PPPS made of PPD·Br as the only diol could be prepared by this procedure in high yields (>90%). It should be noted that the temperature used in these polycondensations was significantly lower than that usually applied in the synthesis of PBS because β -elimination occurs in the phosphonium containing units at high temperatures.

Synthesis results for the two series of ionic PBS copolyesters in addition to their respective parent homopolyesters are shown in Table 1. The PBS sample obtained by enzymatic polycondensation had an intrinsic viscosity of $0.9 \text{ dL}\cdot\text{g}^{-1}$ and a M_n of 25,000 $\text{g}\cdot\text{mol}^{-1}$ with a dispersity around 2. This is an excellent result for the enzymatic

synthesis of this polyester that proves the suitability of the applied procedure. In fact, the highest molecular weight reported for enzymatically prepared PBS is about 3,000 $\text{g}\cdot\text{mol}^{-1}$ [32]. The $\text{PBS}_x\text{G}_y^{\text{I}}$ copolyesters were obtained with M_n in the $\sim 13,000$ - $5,000$ range and viscosities ranging from ~ 0.4 to ~ 0.6 $\text{dL}\cdot\text{g}^{-1}$. $\text{PB}_x\text{PP}_y\text{S}$ copolyesters obtained by melt polycondensation had M_n in the $\sim 17,000$ - $6,000$ range and viscosities between ~ 0.7 and ~ 0.45 $\text{dL}\cdot\text{g}^{-1}$. It is apparent from these results that polymer molar mass tend to decrease with increasing contents in either G^{I} or PP units in the copolyesters. Accordingly minimum molecular weight were obtained for the ionic homopolyesters PBG^{I} and PPPS with M_n values of 4,500 and 6,000 $\text{g}\cdot\text{mol}^{-1}$, respectively. Such behavior is attributed to the difficulty for releasing 1,4-butanediol from the highly viscous molten reaction mixture at the relative low temperature at which polycondensations have to be carried out. This trend is more pronounced in the case of the $\text{PBS}_x\text{G}_y^{\text{I}}$ series likely due to the partially inhibitory effect of DMG-Cl on CALB.

The chemical structure and composition of the polyesters and copolyesters were ascertained by NMR spectroscopy. The ^1H and ^{13}C NMR spectra registered from representative members of the $\text{PBS}_x\text{G}_y^{\text{I}}$ and $\text{PB}_x\text{PP}_y\text{S}$ series with their corresponding peaks assignments are shown in Figures 2 and 3, respectively. An accurate assignment of the ^1H and ^{13}C spectra associated to the different protons and carbons signals present in the copolyesters could be achieved by 2D heteronuclear shift correlation (*hetcor*) analysis (Figures ESI-3 and ESI-4). The chemical composition was determined by integration of selected signals arising from G^{I} or PP units and signals of succinate or butylene units, respectively. As it can be observed in Table 1, the amount of ionic units incorporated into the PBS copolyester was slightly lower than that used in the feed, the differences being around 10 mole-% and practically constant along the whole series for both cases.

It should be noted that the ^1H NMR spectra of all $\text{PB}_x\text{PP}_y\text{S}$ copolyesters show a weak signal at 3.42 ppm due to the presence of dibutyleneoxide (DBO) units produced by the etherification side reaction between two hydroxybutyl units. The content in DBO of the copolyesters oscillated along the 2-4 mole-% range following roughly a trend parallel to their content in phosphonium units. The occurrence of this secondary reaction leading to the generation of ether groups in the polyester backbone was also reported for the synthesis of PET and PHT copolyesters containing sulfonated units [18,28]. In these cases much higher amounts of dialkylene glycol units were formed.

3.3. Thermal and mechanical properties

The thermal behavior of homopolyesters and copolyesters of the two series has been comparatively examined by TGA and DSC, and the thermal parameters

estimated in this study are given in Table 2. The thermal stability was evaluated under inert atmosphere and the TGA traces recorded for the two sets of polyesters together with derivative curves for selected cases are shown in Figure 4. The comparative analysis of these traces indicates that the thermal stability of the ionic copolyesters steadily decreases with the incorporation of G^l or PPD units so both onset ($^{\circ}T_d$) and maximum rate decomposition ($^{max}T_d$) temperatures range between the values displayed by their parent homopolyesters PBS and PBG^l or PBS and PPPS, respectively. Although the resistance to heat of the ionic copolyesters is significantly lower than that of PBS, the $^{\circ}T_d - T_m$ window is still wide enough as to allow their reversible melting and processing by thermal methods. In analogy with PBS, decomposition of PBS_xG^l_y copolyesters took place essentially in one stage whereas the PB_xPP_yS copolyesters degrade through a complex mechanism that involves two or three decomposition stages, depending on their composition (Figure 4b'). On the other hand, similar remaining weights were found for the two series after heating at 600 °C for similar copolyesters compositions but without apparent correlation with their ionic contents.

The heating DSC traces of the polyester samples that had been previously cooled by quenching from the melt showed the slope change characteristic of glass transition for all the studied compositions. The temperatures recorded for such changes revealed that the T_g of PBS steadily increased with the increasing content in ionic units. According to what should be reasonably expected, the incorporation of ionic groups caused a significant increase in the T_g of PBS. This enhancing T_g effect is clearly evidenced when the ionic homopolyesters are compared with PBS in this regard; both PBGⁱ and PPPS have T_g values much higher than PBS in spite that their molecular weights are several times lower. According to Shohamy *et al.* [45], these results are fully consistent with the effect on T_g that should be expected from the occurrence of ionic interactions.

The first heating DSC traces of the polyester coming directly from synthesis are depicted in Figure 5 for the two series. All copolyesters without exception produced first heating traces with endothermic peaks characteristic of melting which is demonstrative that all they were semicrystalline. Both T_m and ΔH_m were found to decrease steadily with the content in ionic units which is a sounded result since neither the ammonium nor the phosphonium side groups are expected to be able to accommodate in the PBS crystal lattice. Furthermore, crystallization from the melt was found to be only feasible for copolyesters with moderate ionic compositions, which is thought to be the consequence of the restricted chain mobility caused by ionic aggregation. Only PBS_xG^l_y and PB_xPP_yS copolyesters containing up to 16 mole-% of G^l units and 10 mole-% of PP units, respectively, were able to crystallize from the melt and therefore to

show melting at the second heating (second heating DSC traces are accessible in the . ESI file, Figure ESI-5). Lastly it is worthy to comment that PBGⁱ showed melting whereas no heat exchange was detected in the DSC traces recorded from the PPPS homopolymer. The absence of crystallinity in PPPS is a reasonably expected result given the great bulkiness of the pendant tributylphosphonium group. The general conclusion drawn from DSC results is that ionic groups depress the crystallinity of PBS and that such effect must be interpreted as a combination of two hindering factors: a) the incompatibility of the butylene succinate and the ionic units to cocrystallize within the same lattice, and b) the difficulty of the polymer chains to diffuse due to high melt viscosity of the ionic polyesters. The former is the simple consequence of the different constitution of the units whereas the latter is caused by the strong interactions that are characteristic for ionic charged polymers.

The tensile properties, such as Young's modulus (E), the maximum stress (σ_{\max}), and the elongation at break (ϵ_{\max}), were determined for amorphous samples of the PB_xPP_yS copolyesters with PP contents equal or below 8 mole-%. Copolyesters containing higher amounts of phosphonium units could not be evaluated because samples were too brittle and the same happened with copolyesters containing ammonium units. The stress-strain curves resulted from tensile essays are depicted in Figure 6 and the mechanical parameters measured therefrom are compared in Table 2. It was found that the E modulus increased and ϵ_{\max} decreased with the content of PP units whereas not clear trend was observed for tensile strength. It should be highlighted that the E modulus of PBS increased more than 100% when only 8 mole-% of PP units were incorporated in the chain which is a really amazing result that reveals the enormous stiff enhancing effect of the tributylphosphonium side group.

3.4. Crystal structure and crystallizability: coupling with anionic PBS copolyesters

Semicrystalline PBS_xG_y^l and PB_xPP_yS copolyesters were analyzed by powder XRD and the recorded profiles are compared in Figure 7 where PBS has been also included for reference. Essentially the same diffraction pattern comprising both peak spacing and intensity is shared by PBS and the ionic copolyesters. The pattern is distinguished by the presence of three sharp strong reflections at 4.5, 4.1 and 3.9 Å indicating that the monoclinic crystal structure of PBS [46] is retained in the copolyesters. As it has been reported for several random copolyesters containing unequal amounts of comonomers, the crystallinity attained in such systems is feasible because crystallites are formed exclusively from homogeneous polymer segments made up of the major comonomer whereas the minor one is segregated to the amorphous phase [47,48]. Regarding the homopolyesters, no discrete scattering is

detected in the profile recorded from PPPS according to the amorphous nature of this polymer. On the contrary, PBGⁱ produces a well-defined crystalline pattern that is radically dissimilar to that of PBS and indicates therefore the occurrence of a different crystal structure.

Ionic coupling between polyelectrolytes of opposite sign is known to proceed with generation of a stable heterocomplex that distinguishes in displaying properties different to its individual components [49,50]. In order to investigate this issue, a sample of anionic PBS copolyester containing sulfonate units was used as counterpart for the cationic copolyesters studied in this work. Thus nearly isocompositional copolyester pairs PB₈₄Gⁱ₁₆S/PB₈₅SS₁₅ and PB₈₅PP₁₅S/ PB₈₅SS₁₅ were made to interact in 1,1,1.3,3,3-hexafluoro-2-propanol solution, and the structure and thermal behavior of the product resulting in each case upon evaporation to dryness was examined by TGA, DSC and XRD. Data collected in this study for the mixed compounds (PBS-Gⁱ/SS and PBS-PP/SS) are compared with those of the three neat ionic copolyesters in Table 3. The thermal stability of the complexes slightly decreased with respect to the cationic components probably due to the presence of the less stable anionic copolyester. Regarding T_m , no noteworthy differences were observed between the complex and the isolated components probably because the density of ionic interactions is too low as to disturb crystallite size. On the contrary the T_g was found to increase significantly in the complexes which makes sense since the ionic interactions are expected to take place exclusively in the amorphous phase. Nevertheless, the most noticeable differences between complexes and individual copolyesters were those concerning crystallization at cooling from the melt (Figure 8). PB₈₄Gⁱ₁₆S crystallized homogeneously in one single peak whereas crystallization of PBS-Gⁱ/SS showed a second peak at lower temperature (Figure 8a). Differences in crystallization became even clearer for the PB₈₅PP₁₅S/ PB₈₅SS₁₅ system. As it is seen in Figure 8a', no crystallization could be detected for none of the two isolated components whereas a strong exothermic peak was observed for the PBS-PP/SS complex. The distinctive crystallization features observed in the blends are due to the nucleating effect exerted by the microcrystallites that are generated by specific interactions between the opposite charged ionic groups. Such effect was ascertained by a comparative isothermal crystallization study carried out on the PB₈₅PP₁₅S/ PB₈₅SS₁₅ system. Details of this study are given in the ESI file (Figure ESI-6). Kinetics parameters resulting from the application of the Avrami approach (Table 3) revealed that crystallization of PB₈₅SS₁₅ took place with a $t_{(1/2)}$ approximately double than the complex with no perceivable changes in the crystallization mechanism.

PBS-Gⁱ/SS and PBS-PP/SS complexes were examined by XRD in order to get insight the complex structure and corroborate the occurrence of ionic microcrystallites. The XRD profiles registered from the two isolated ionic copolyesters and their corresponding complex are shown in Figures 8b and 8b' for the two systems. In both cases the three compared profiles display the pattern characteristic of PBS with strong peaks at 3.9, 4.1 and 4.5 Å indicating that the crystal structure of the cationic copolyesters is retained in the complexes. What is really noteworthy is the presence in the trace recorded from PBS-PP/SS of two sharp peaks of low intensity at about 2.9 and 3.4 Å. These peaks do not disappear after heating the sample at temperatures above the T_m and they are made to correspond to small crystallites made of ionic motives. After cooling the diffraction pattern characteristic of PBS was recovered with the two small peaks unchanged. It can be concluded therefore that the ionic crystallites preserved in the molten state act as effective nuclei in the crystallization of PBS.

3.5. Antimicrobial activity

As the last part of this research the antimicrobial activity of the cationic copolyesters of PBS was explored against *E. coli* and *S. aureus*. The ammonium containing PBS copolyesters tested were PBS₅₀Gⁱ₅₀ and PBS₈₄Gⁱ₁₆ in addition to the homopolyester PBGⁱ. The selected phosphonium containing PBS copolyesters were PB₉₇PP₃S, PB₉₀PP₁₀S and PB₈₅PP₁₅S. A PBS sample was also tested for reference. The results obtained from these essays are represented in Figure 9.

In all cases the response of PBS was similar to the blank indicating that this polyester does not exert perceivable biocide activity. On the contrary, the results observed for the PBS copolyesters containing ionic groups clearly brought into evidence their microbial effect. Thus a complete inactivation of the colony was observed in a few minutes for *E. coli* cultured in the presence of PBGⁱ and also a drastic decreasing in viability was observed after a few hours in the presence of the PBS₅₀Gⁱ₅₀. It is worthy to compare these results with those reported by Riva *et al.* [51] for poly(ϵ -caprolactone) grafted with ammonium salts; they observed a fast and complete inactivation of the *E. coli* by PCL containing 30 mol-% of ammonium groups after 1 h when a concentration of $\sim 10 \text{ g}\cdot\text{L}^{-1}$ of active groups were used in the dynamic shake flask method. The inactivation for *S. aureus* was even more noticeable in spite that this bacterium is known to be much more resistant to adverse factors. PBGⁱ was able to completely inactivate cell proliferation in a period of a few hours and PBS₅₀Gⁱ₅₀ made to fall the CFU concentration in about five orders after less than one day of incubation. The results obtained for PBS₈₄Gⁱ₁₆ were uneven and no clear conclusions can be derived from them.

The trend observed for the PB_xPP_yS series was highly depending on composition and a close comparison with the $PBS_xG^I_y$ series is not feasible because copolyesters with high ionic compositions are not available for the former. An inspection of the data shown in Figure 9 for both *E. coli* and *S. aureus* reveals that no clear effect is displayed by copolyesters containing 3% and 10% of phosphonium groups whereas the biocide activity of $PB_{85}PP_{15}S$ becomes unmistakably manifested. In fact, the presence of this copolyester in both cultures promoted a steady decay of CFU of about five orders after only one day of incubation. It is also remarkable that the antibacterial effect of this copolyester is clearly perceived since the beginning of the assay.

The above reported observations lead to conclude the following: a) The biocide effect of both ammonium and phosphonium containing PBS copolyesters is highly depending on composition so it is only clearly manifested above certain contents in ionic groups. b) The phosphonium containing PBS copolyesters are particularly active and they display a sustained biocide effect with similar intensity for the two tested bacteria.

4. Conclusions

Two series of cationic PBS copolyesters containing ammonium and tributylphosphonium side groups in contents up to 50 %-mole in addition to the entirely ionic homopolyesters have been synthesized. The high sensitivity to heat of cationic compounds made necessary to apply polymerization conditions less severe than usual. CALB enzymatic mediated polycondensation at temperatures close to 100 °C has been proved to be a suitable method to produce ammonium containing PBS copolyesters. PBS copolyesters bearing phosphonium groups could be successfully prepared by melt polycondensation assisted by titanium catalyst by applying relative lower temperatures.

The cationic PBS copolyesters display a panel of properties that reflects the presence of the ionic units in the polyester chain. Their thermal stability decreases respect to PBS for the two series but all the copolyesters continue to be heat resistant enough as to be processed by melting. They are semicrystalline and they adopt the same crystal structure as PBS although their melting temperature and crystallinity decrease with the content in ionic groups. On the contrary, the T_g of PBS regularly increases with the insertion of either ammonium or phosphonium units due the decreasing in chain mobility caused by strong ionic interactions. Such interactions are also responsible for the high melt viscosity displayed by these cationic PBS copolyesters so that only those with moderate ionic contents are able to crystallize from the melt under non-isothermal conditions. Blending with anionic PBS copolyesters

containing sulfonate groups entails formation of ionically compensated microcrystallites that are resistant to temperature and are able to act as effective nucleating agent in the crystallization from the melt.

Ammonium and phosphonium containing PBS copolyesters show a noticeable biocide activity against both *gram*-positive and *gram*-negative bacteria that is strongly depending on composition. The copolyesters containing 50 mole-% of ammonium groups or 15% of phosphonium groups display strong biocide in periods of time of hours, which is a very interesting result given the potential of PBS as packaging and coating material. Not only copolymerization but also blending of PBS with cationic containing copolyester master-batches will be promising approaches to attain PBS formulations with antimicrobial activity.

Acknowledgements

Authors are grateful to Prof. Katja Loos (Gröningen University, NL) for valuable comments and suggestions. They also acknowledge the financial support received from MINECO with grant MAT2012-38044-CO3-03 and the UPC-Research grant awarded to Mayka Bautista Betancur.

References

- [1] Miller, SA. Sustainable Polymers: Opportunities for the next decade. *ACS Macro Letters* 2013; 2:550-554.
- [2] Rydz J, Wanda S, Kyulavska M, Christova, D. Polyester-based (bio)degradable polymers as environmentally friendly materials for sustainable development. *Intern J Molec Sci* 2015; 16:564-596.
- [3] Hillmyer MA, Tolman, WB. Aliphatic polyester block polymers: renewable, degradable, and sustainable. *Acc Chem Res* 2014; 47:2390-2396.
- [4] Williams CK. Synthesis of functionalized biodegradable polyesters. *Chem Soc Rev* 2007; 36:1573-1580.
- [5] Barrett DG, Yousa MN. Design and applications of biodegradable polyester tissue scaffolds based on endogenous monomers found in human metabolism. *Molecules* 2009; 14:4022-4050.
- [6] Mohamed F, van der Walle CF. Engineering biodegradable polyester particles with specific drug targeting and drug release properties. *J Pharm Sci* 2008; 97:71-87.
- [7] Ikada Y, Tsuji H. Biodegradable polyesters for medical and ecological applications. *Macromol Rapid Comm* 2000; 21:117-132.
- [8] Zeikus JG, Jain MK, Elankovan P. Biotechnology of succinic acid production and markets for derived industrial products, *Appl. Microbiol Biotech* 1999; 51:545-552.
- [9] Xu, J and Guo, B-H. Poly(butylene succinate) and its copolymers: Research, development and industrialization *Biotech J* 2010; 5:1149-1163.
- [10] Ichikawa Y, Mizukoshi T. Bionolle (Polybutylenesuccinate). *Adv Polym Sci* 2012; 245:285-313.
- [11] Eisenberg A. Glass transitions in ionic polymers. *Macromolecules* 1971; 4:125-128.
- [12] Hoover MF, Butler GB. Recent advances in ion-containing polymers. *J Polym Sci, Part C-Polym Symp* 1974; 45:1-37.
- [13] Utracki LA, Walsh DJ, Weiss RA. Polymer alloys, blends, and ionomers: An overview. 1989; *ACS Symp Series* 1989; 395:1-35.
- [14] Lundberg RD, Duvdevani I. Shear-thickening behavior of ionomers and their complexes. *ACS Symp Series* 1991; 462:155-175.
- [15] Hara M, Sauer JA. Mechanical-properties of ionomers. *J Macrom Sci, Rev in Macrom Chem Phys* 1994. C34:325-373.
- [16] Ali I, Dolui SK. Synthesis of polyester containing ionic comonomer through interfacial polymerization. *J Polym Mat* 2004; 21:61-66.
- [17] Gaona O, Kint D, Martínez de Ilarduya A, Alla A, Bou J, Muñoz-Guerra S. Preparation and hydrolytic degradation of sulfonated poly(ethylene terephthalate) copolymers. *Polymer* 2003; 44:7281-7289.
- [18] Bautista M, Martínez de Ilarduya A, Alla A, Muñoz-Guerra S. Sulfonated poly(hexamethylene terephthalate) copolyesters: enhanced thermal and mechanical properties. *J Appl Polym Sci* 2013; 129:3527-3535.

- [19] Bougarech A, Abid M, Gouanve E, Espuche E, Abid S, El Gharbi R, Fleury, E. Synthesis, characterization and water sorption study of new biobased (furanic-sulfonated) copolyesters. *Polymer* 2013; 54:5482-5489.
- [20] Bougarech A, Abid M, DaCruz-Boisson F, Abid S, El Gharbi R, Fleury E. Modulation of furanic-sulfonated isophthalic copolyesters properties through diols units control. *Eur Polym J* 2014; 58: 207-217.
- [21] Storey RF, Deng ZD, Glancy TP, Peterson DR, US Patent 5668288, 1997.
- [22] Han SI, Yoo Y, Kim DK, Im SS. Biodegradable aliphatic polyester ionomer. *Macromol Biosci* 2004; 4:199-207.
- [23] Han SI, Im SS, Kim DK. Dynamic mechanical and melt rheological properties of sulfonated poly(butylene succinate) ionomers. *Polymer* 2003; 44:7165-7173.
- [24] Li HY, Chang J, Cao AM Wang JY. In vitro evaluation of biodegradable poly(butylene succinate) as a novel biomaterial. *Macromol Biosci* 2005; 5:433-440.
- [25] Han SI, Kang SW, Kim BS, Im SS. A novel polymeric ionomer as a potential biomaterial: crystallization behavior, degradation and in-vitro cellular interactions. *Adv. Funct. Mater* 2005; 15:367-374.
- [26] Lim JS, Kim JH. New application of poly(butylene succinate) (PBS) based ionomer as biopolymer: a role of ion group for hydroxyapatite (HAp) crystal formation. *J Mater Sci* 2009; 44:6398-6403.
- [27] Park SB, Hwang SY, Moon CW, Im SS, Yoo ES. Plasticizer effect of novel PBS ionomer in PLA/PBS ionomer blends. *Macromol Res* 2010; 18:463-471.
- [28] Bautista M, Martínez de Ilarduya A, Alla A, Muñoz-Guerra S. Poly(butylene succinate) ionomers with enhanced hydrodegradability. *Polymers* 2015; 7:1232-1247.
- [29] Bautista M, Martínez de Ilarduya A, Alla A, Muñoz-Guerra S. Poly(butylene succinate) ionomers and their use as compatibilizers in nanocomposites. *Polym Compos* 2016; DOI: 10.1002/pc.23454
- [30] Kobayashi S, Uyama H, Kimura S, Enzymatic polymerization. *Chem Rev* 2001; 101:3793-3818.
- [31] Xu J, Guo B-H. Microbial succinic acid, its polymer poly(butylene succinate), and applications, in: G.G.-Q. Chen (Ed.), *Plastics from Bacteria*, Springer Berlin, Heidelberg, 2010, pp. 347-388.
- [32] Azim H, Dekhterman A, Jiang ZZ, Gross RA. *Candida antarctica* lipase B-catalyzed synthesis of poly(butylene succinate): shorter chain building blocks also work. *Biomacromolecules* 2006; 7:3093-3097.
- [33] Anderson EB, Unal S, Long TE. Synthesis and association of phosphonium cation-containing polyesters for food packaging applications. *Polym Prepr* 2007; 48:731-732.
- [34] Anderson EB, Unal S, Long TE. Phosphonium cations as a new avenue for electrostatic interactions in polymer design. *Polym Prepr* 2008; 49:262-263.
- [35] Anthierens T, Billiet L, Devlieghere F, Du Prez F. Poly(butylene adipate) functionalized with quaternary phosphonium groups as potential antimicrobial packaging material. *Innov Food Sci Emerg Technol* 2012; 15:81-85.
- [36] Fuchs AD, Tiller JC, Contact-active antimicrobial coatings derived from aqueous suspensions. *Angew Chem Int Ed* 2006; 45:6759-6762.
- [37] Majumdar P, Lee E, Gubbins N, Stafslie SJ, Daniels J, Thorson CJ, Chisholm BJ. Synthesis and antimicrobial activity of quaternary ammonium-functionalized POSS (Q-POSS) and polysiloxane coatings containing Q-POSS. *Polymer* 2009; 50:1124-1133.

- [38] Zheng A, Xue Y, Wei D, Li S, Xiao H, Guan Y. Synthesis and characterization of antimicrobial polyvinyl pyrrolidone hydrogel as wound dressing. *Soft Matter* 2014; 12: 179-187.
- [39] Chen FCM, Benoit NL. New method of quaternizing amines and its use in amino-acid and peptide chemistry. *Can J Chem* 1976; 54:3310-3311.
- [40] Mindemark J; Bowden, T. Synthesis and polymerization of alkyl halide-functional cyclic carbonates. *Polymer* 2011; 52:5716-5722.
- [41] Zhang M, Hemp ST, Allen MH, Carmean RN, Moore RB, Long TE. Water-dispersible cationic polyurethanes containing pendant trialkylphosphoniums. *Polym. Chem* 2014; 5:3795-3803.
- [42] Schwab LW, Polyamide synthesis by hydrolases, PhD Thesis, University of Groningen, The Netherlands, 2010.
- [43] Lozano P, De Diego T, Carrie D, Vaultier M, Iborra JL, Enzymatic ester synthesis in ionic liquids. *J Mol Catal, B Enzym* 2003; 21:9-13.
- [44] Greener J, Gillmor JR, Daly RC. Melt rheology of a class of polyester ionomers, *Macromolecules* 1993; 26:6416-6424.
- [45] Shohamy E, Eisenberg A. Effect of ion aggregation on some melt properties of styrene ionomers. *J Polym Sci, Polym Phys* 1976; 14:1211-1220.
- [46] Ihn K, Yoo E, Im S. Structure and morphology of poly(tetramethylene succinate) crystals. *Macromolecules* 1995; 28:2460-2464.
- [47] Li B, Yu J, Lee S, Ree M. Poly(ethylene terephthalate co ethylene isophthalate) - relationship between physical properties and chemical structures. *Eur Polym J* 1999; 35:1607-1610.
- [48] Quintana R, Martínez de Ilarduya A, Rudé E, Kint DPR, Alla A, Galbis JA, Muñoz-Guerra S. Poly(ethylene isophthalate)s: effect of the tert-butyl substituent on structure and properties. *Polymer* 2004; 45:5005-5012.
- [49] Chelushkin PS, Lysenko EA, Bronich TK, Eisenberg A, Kabanov VA, Kabanov A V. Polyion complex nanomaterials from block polyelectrolyte micelles and linear polyelectrolytes of opposite charge: 1. Solution behavior. *J Phys Chem B* 2007; 111:8419-8425.
- [50] Chelushkin PS, Lysenko EA, Bronich TK, Eisenberg A, Kabanov VA, Kabanov A V. Polyion complex nanomaterials from block polyelectrolyte micelles and linear polyelectrolytes of opposite charge. 2. Dynamic properties. *J Phys Chem B*. 2008; 112:7732-7738.
- [51] Riva R, Lussis P, Lenoir S, Jérôme C, Jérôme R, Lecomte P. Contribution of “click chemistry” to the synthesis of antimicrobial aliphatic copolyesters. *Polymer* 2008; 49:2023-2028.

Table 1. Composition and molecular weights of $\text{PBS}_x\text{G}_y^{\text{I}}$ and $\text{PB}_x\text{PP}_y\text{S}$ copolyesters.

Copolyester	Copolyester composition ^a	Copolyester composition ^b	DBO content ^c	Molecular weight			
	S/G ^I or B/PP ^a	S/G ^I or B/PP	mol-%	$[\eta]^{\text{d}}$	M_n^{e}	M_n^{f}	\bar{D}^{f}
Ammonium containing polymers							
PBS	100/0	100/0	-	0.90	25,000	31,000	1.9
$\text{PBS}_{97}\text{G}_3^{\text{I}}$	97/3	97.6/2.6	-	0.60	13,000	11,000	2.1
$\text{PBS}_{90}\text{G}_{10}^{\text{I}}$	90/10	90.7/9.3	-	0.45	5,400	5,000	2.5
$\text{PBS}_{84}\text{G}_{16}^{\text{I}}$	84/16	85.7/14.3	-	0.40	5,500	4,500	2.6
$\text{PBS}_{50}\text{G}_{50}^{\text{I}}$	50/50	56/44	-	0.36	4,900	-	-
PBG^{I}	0/100	0/100	-	0.35	4,500	-	-
Phosphonium containing polymers							
PBS	100/0	100/0	-	1.00	-	18,200	2.5
$\text{PB}_{97}\text{PP}_3\text{S}$	97/3	97.1/2.9	1.8	0.68	-	16,700	2.1
$\text{PB}_{95}\text{PP}_5\text{S}$	95/5	95.2/4.8	2.7	0.64	-	11,800	2.2
$\text{PB}_{92}\text{PP}_8\text{S}$	92/8	92.7/7.3	3.9	0.54	-	22,000	2.2
$\text{PB}_{90}\text{PP}_{10}\text{S}$	90/10	90.4/9.6	3.9	0.49	-	10,000	2.3
$\text{PB}_{85}\text{PP}_{15}\text{S}$	85/15	86.1/13.9	2.9	0.44	-	6,300	2.4
PPPS	0/100	0/100	-	0.31	-	6,000	3

^aMolar ratio of comonomers in the feed.^bComposition of the copolyester (mol/mol) determined by ¹H NMR.^cComposition in dibutyleneoxide units in mol-% determined by ¹H NMR^dIntrinsic viscosity (dL·g⁻¹) measured in dichloroacetic acid at 25 °C.^eNumber-average molecular weight (M_n) (g·mol⁻¹) determined by NMR end-group analysis.^fNumber-average molecular weight (M_n) (g·mol⁻¹) and dispersity (\bar{D}) determined by GPC.

Table 2. Thermal and mechanical properties of PBS homopolymer and PB_xPP_yS copolyesters.

Copolyester	TGA			DSC					Stress-strain parameters ^g		
	T_d^a (°C)	T_d^{max} ^b (°C)	RW^c (%)	T_g^d (°C)	T_m^e (°C)	ΔH_m^e (Jg ⁻¹)	T_c^f (°C)	ΔH_c^f (Jg ⁻¹)	E (Mpa)	σ_{max} (Mpa)	ϵ_{max} (%)
Ammonium containing polymers											
PBS ^h	376	408	0.9	-39	114 (113)	101 (68)	71	-69	-	-	-
PBS ₉₇ G ₃ ^l	374	407	1.1	-37	114 (113)	96 (74)	70	-72	-	-	-
PBS ₉₀ G ₁₀ ^l	369	406	3.2	-35	107 (106)	58 (43)	53	-43	-	-	-
PBS ₈₄ G ₁₆ ^l	367	406	1.8	-34	108 (108)	90 (72)	56	-71	-	-	-
PBS ₅₀ G ₅₀ ^l	343	391	10	-27	77 (81)	63 (37)	-	-	-	-	-
PBG ^l	292	342	16	-10	85	52 (-)	-	-	-	-	-
Phosphonium containing polymers											
PBS ^h	356	405	3	-37	113 (114)	77 (67)	78	-62	403±3	26±1	10±0.5
PB ₉₇ PP ₃ S	301	337/391	4	-37	111 (111)	71 (67)	70	-60	583±10	28±1	8±0.3
PB ₉₅ PP ₅ S	294	328/387	5	-37	111 (109)	68 (66)	68	-59	736±30	29±1	5±0.5
PB ₉₂ PP ₈ S	288	322/373	5	-36	105 (104)	48 (56)	58	-53	893±40	19±2	1.4±0.3
PB ₉₀ PP ₁₀ S	288	318/356/383	5	-34	104 (102)	42 (35)	58	-35	-	-	-
PB ₈₅ PP ₁₅ S	283	308/352/401	4	-31	94 (89)	41 (32)	-	-	-	-	-
PPPS	310	325/353/423	3	-29	-	-	-	-	-	-	-

^aDegradation temperature at which a 10% weight loss was observed in TGA traces at 10 °C·min⁻¹.

^bTemperature of maximum degradation rate.

^cRemaining weight at 600 °C.

^dGlass transition temperature taken as the inflection point of the heating DSC traces of melt-quenched samples recorded at 20 °C·min⁻¹.

^eMelting temperatures and enthalpies were registered at a heating rate of 10 °C·min⁻¹. In parenthesis, values recorded at the second heating.

^fCrystallization temperatures and enthalpies were registered at cooling from 200 °C at 10 °C·min⁻¹.

^gYoung's modulus, maximum tensile stress and elongation at break measured at room temperature on a Zwick BZ2.5/TN1S.

^hPBS samples used for reference and prepared by the method respectively applied for each series.

Table 3. Compared properties of sulfonated and phosphonium/ammonium copolyesters of PBS and their equimolar mixtures.

	TGA-DSC							Isothermal crystallization			WAXS
	Ionic (mol-%)	$[\eta]^a$ (dL·g ⁻¹)	M_w^b (g·mol ⁻¹)	T_d^c (°C)	T_g^d (°C)	T_m^e (°C)	T_c^f (°C)	$t_{(1/2)}$ (min)	n	$-\log K$	d (Å)
PBS ₈₅ SS ₁₅	13.8	0.74	33,000	315	-32	106	-	3.8	2.1	1.4	3.9, 4.1, 4.5
PBS ₈₄ G ^I ₁₆	14.3	0.40	12,000	367	-34	108	56	-	-	-	3.9, 4.1, 4.5
PBS ₈₅ PP ₁₅ S	13.9	0.44	15,000	283	-31	89	-	-	-	-	3.9, 4.1, 4.5
PBS(G ^I -SS)	-	0.59	-	327	-34	105,109	63,56	-	-	-	3.9, 4.1, 4.5
PBS(PP-SS)	-	0.68	-	289	-29	104	48	1.9	1.9	0.5	3.9, 4.1, 4.5 (2.9, 3.4)

^aIntrinsic viscosity (dL·g⁻¹) measured in dichloroacetic acid at 25 °C.

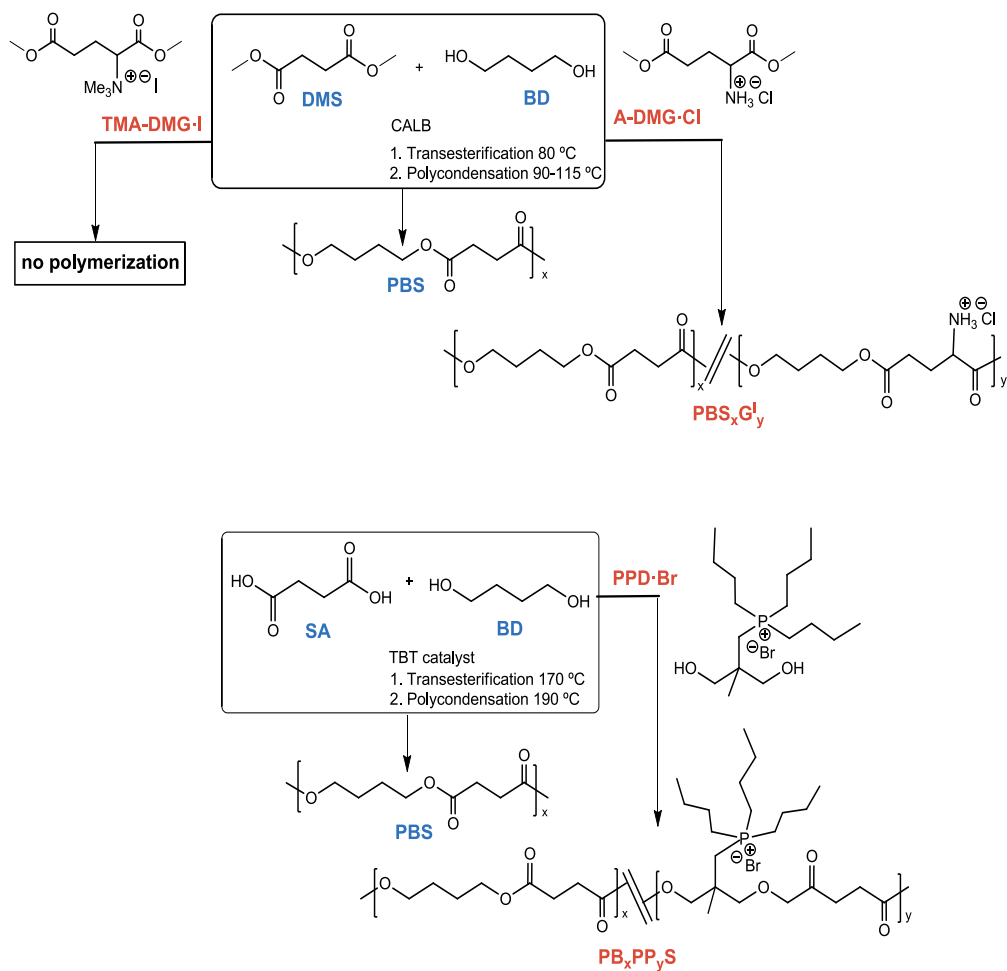
^bWeight-average molecular weight (M_w) (g·mol⁻¹) determined by GPC.

^cDegradation temperature at which a 10% weight loss was observed in TGA traces at 10 °C·min⁻¹.

^dGlass transition temperature taken as the inflection point of the heating DSC traces of melt-quenched samples recorded at 20 °C·min⁻¹.

^eMelting temperatures were registered at a heating rate of 10 °C·min⁻¹.

^fCrystallization temperatures and enthalpies were registered at cooling at 10 °C·min⁻¹.



Scheme 1. Synthesis routes to cationic PBS copolyesters. Top: Ammonium containing copolyesters prepared by enzymatic polycondensation. Bottom: Phosphonium containing copolyesters prepared by melt polycondensation catalyzed by TBT.

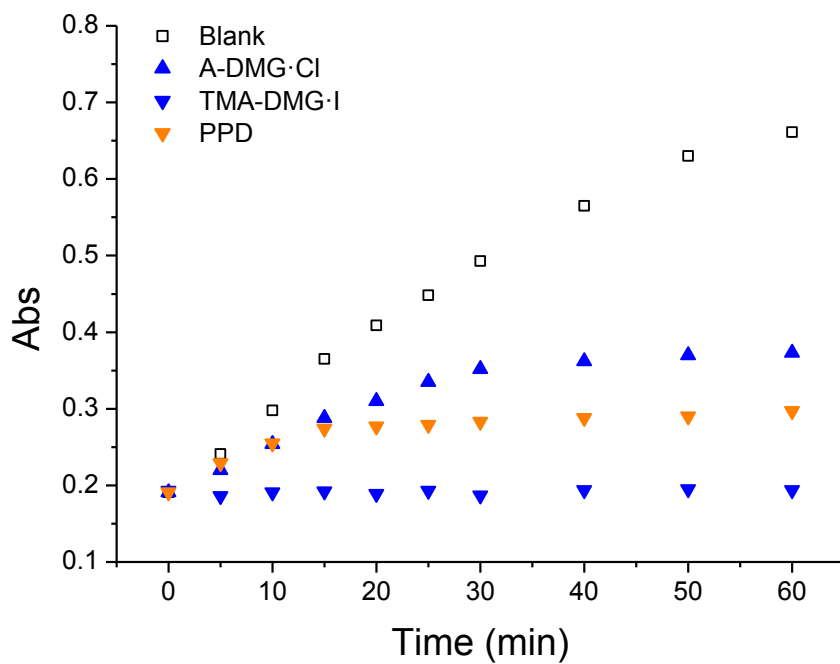


Figure 1. Loss of activity of CALB in the presence of ammonium and phosphonium compounds.

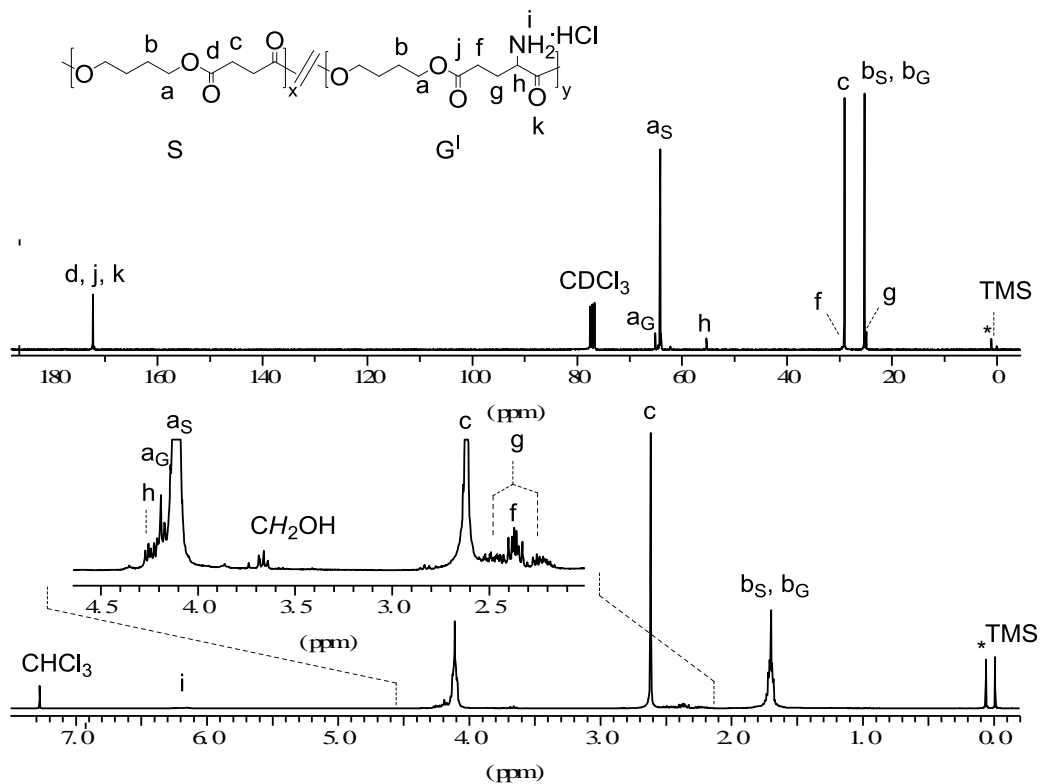


Figure 2. 1H NMR (bottom) and ^{13}C NMR (top) spectra of $PBS_{84}G'_{16}$. The asterisked peak arises from methyl silicone impurity.

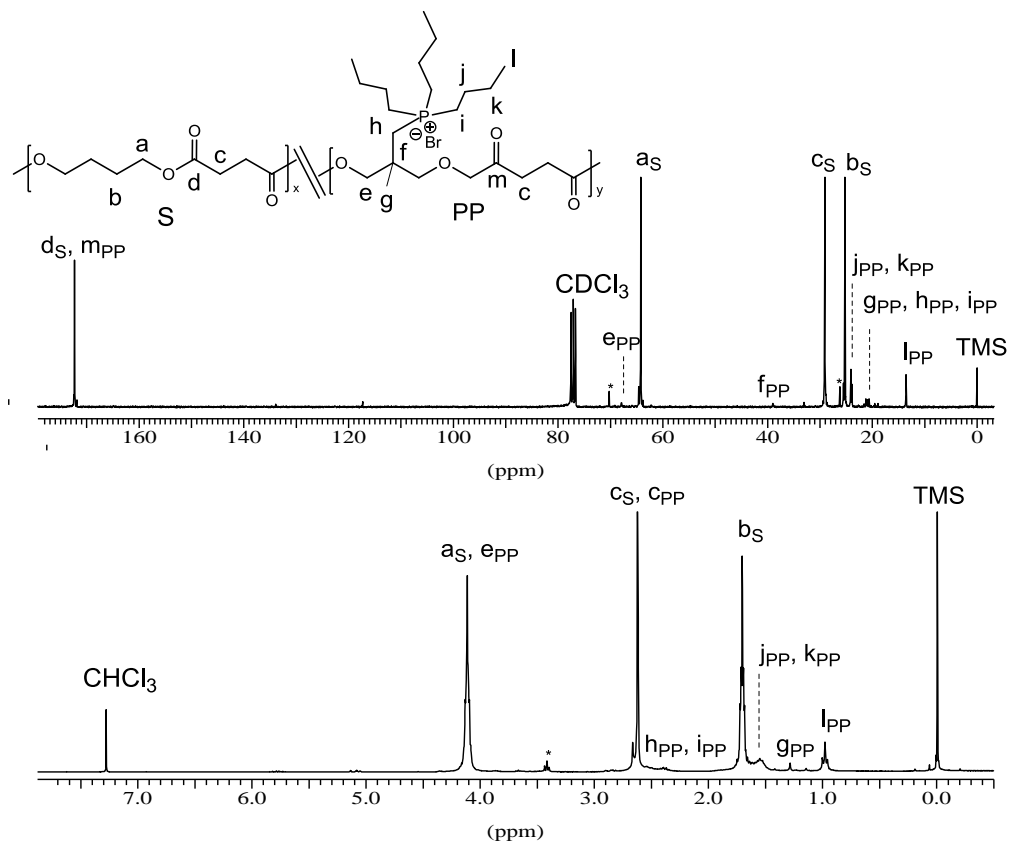


Figure 3. ¹H NMR (bottom) and ¹³C NMR (top) spectra of PB₉₅PP₅S. Peaks arising from DBO units are labelled with asterisks.

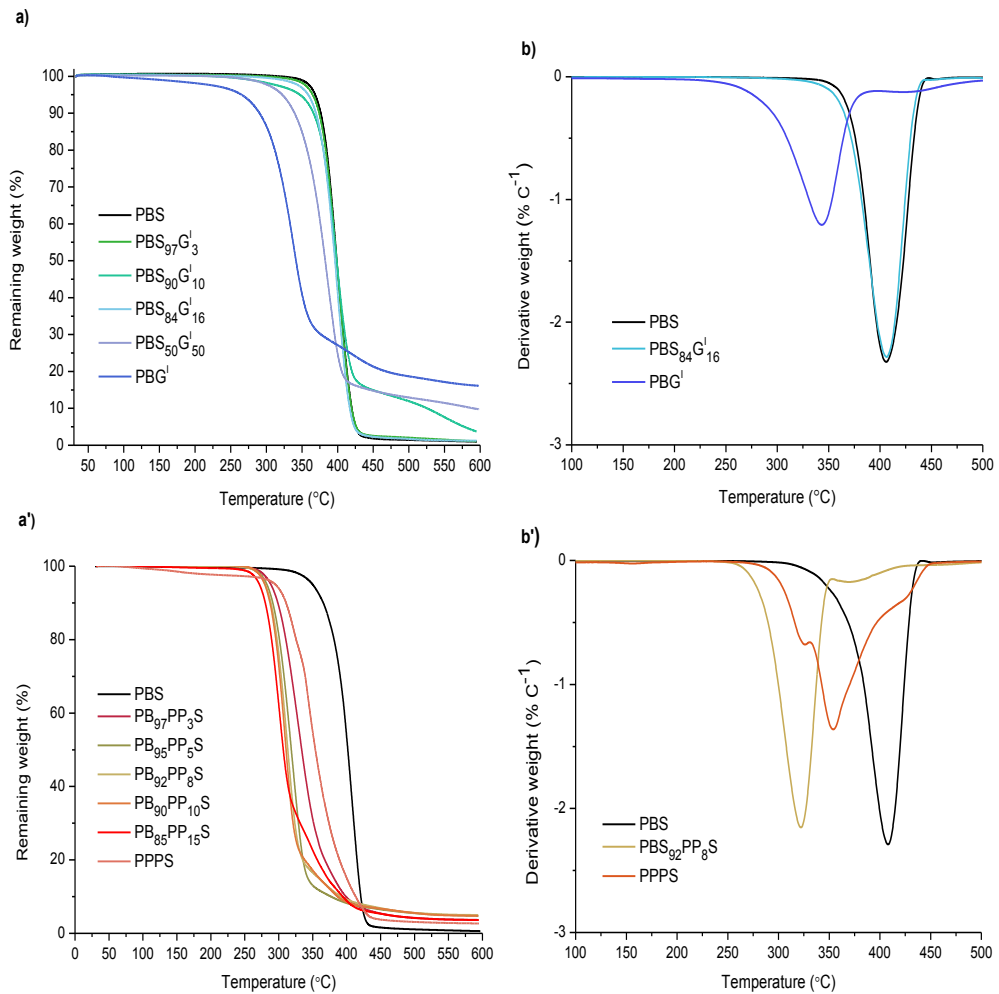


Figure 4. TGA traces (a, a') and derivative curves (b,b') of PBS_xG_y^I and PB_xPP_yS copolyesters.

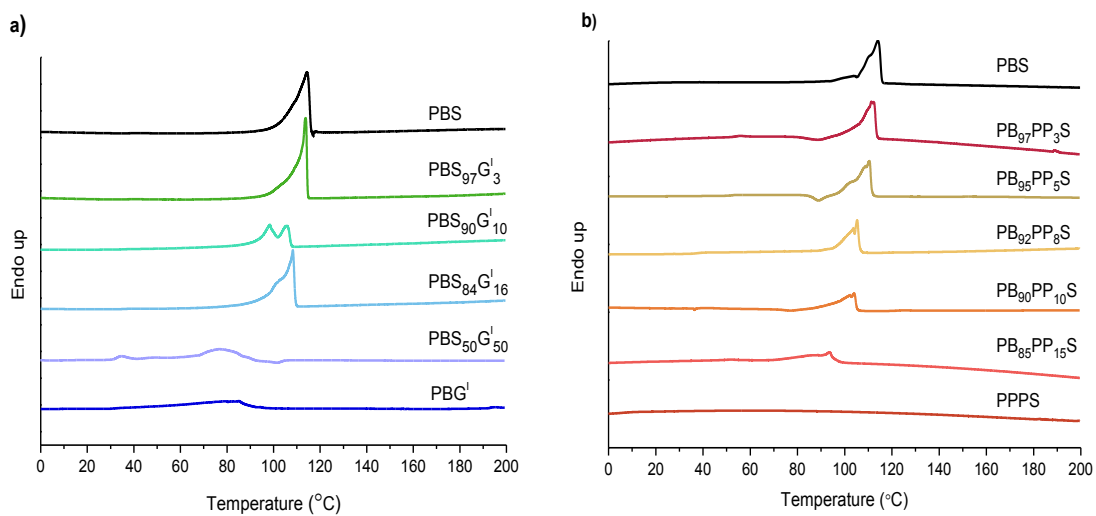


Figure 5. DSC traces at first heating of $PBS_xG_y^I$ (a) and PB_xPP_yS (b) copolyesters.

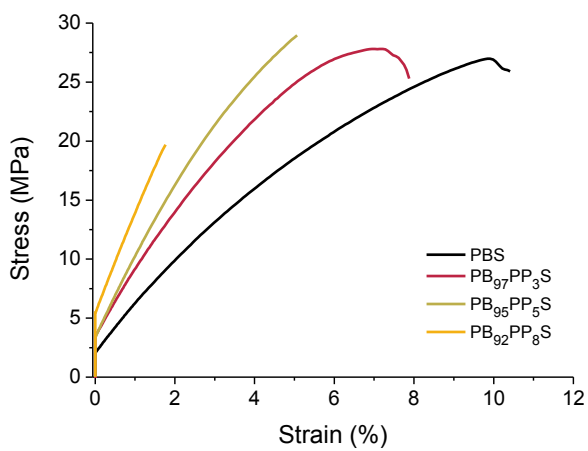


Figure 6. Stress-strain curves of PB_xPP_yS copolyesters.

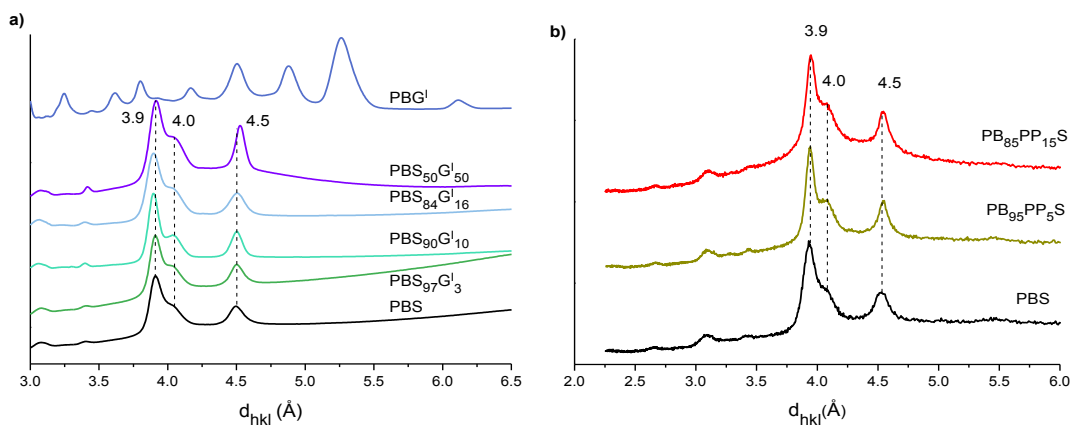


Figure 7. Powder XRD profiles of $PBS_xG_y^I$ (a) and PB_xPP_yS (b) copolyesters.

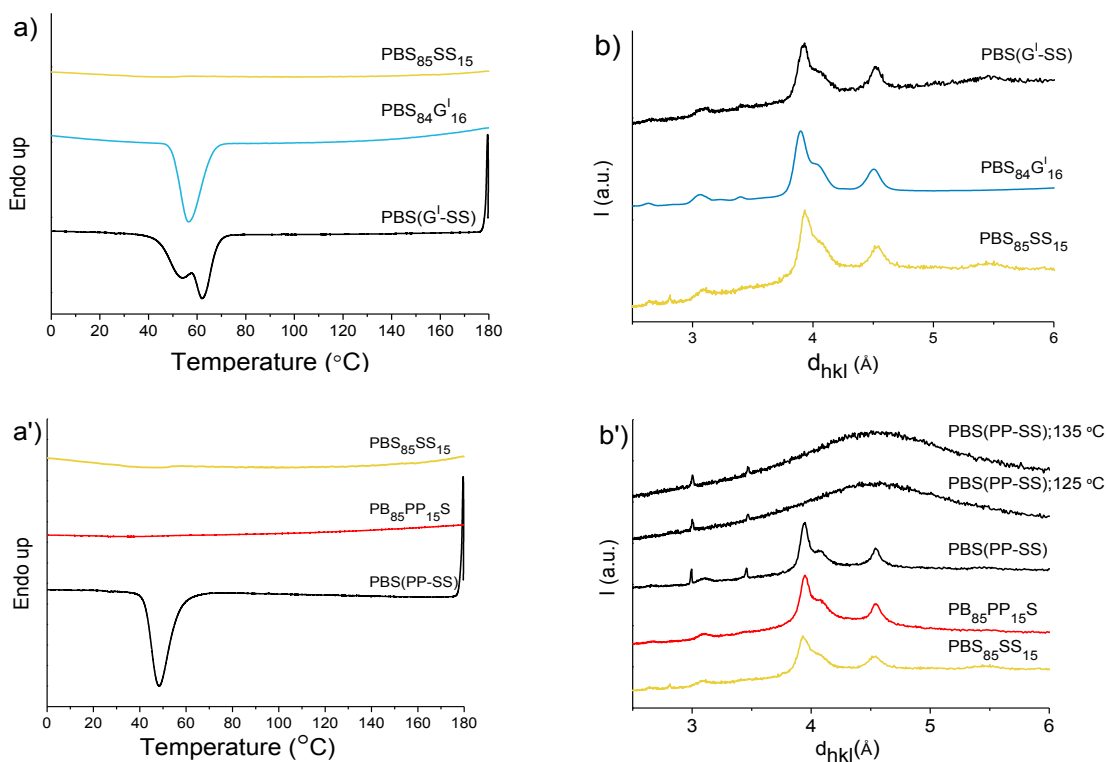


Figure 8. Non-isothermal crystallization and crystalline structure of heterocomplexes made of ionic copolymers of PBS compared to their respective components. a, a') DSC traces registered at cooling from the melt. b, b') Powder XRD profiles. In b') the profiles registered at temperatures above T_m are also included.

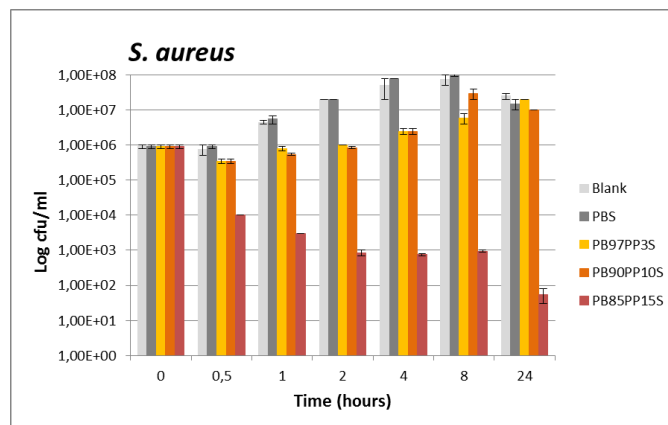
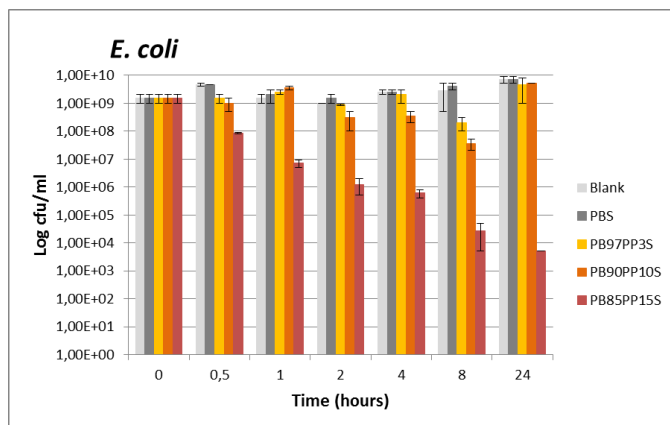
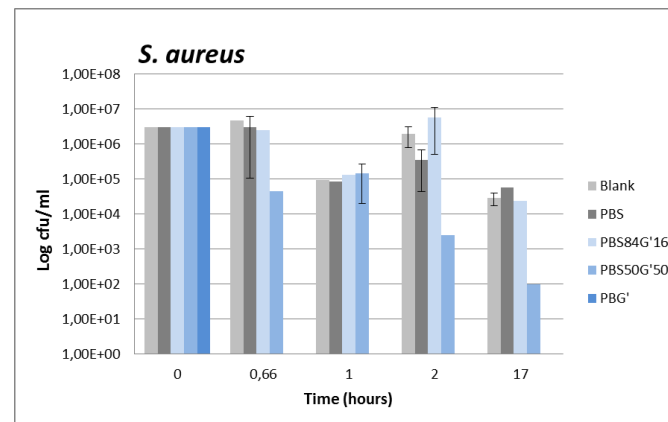
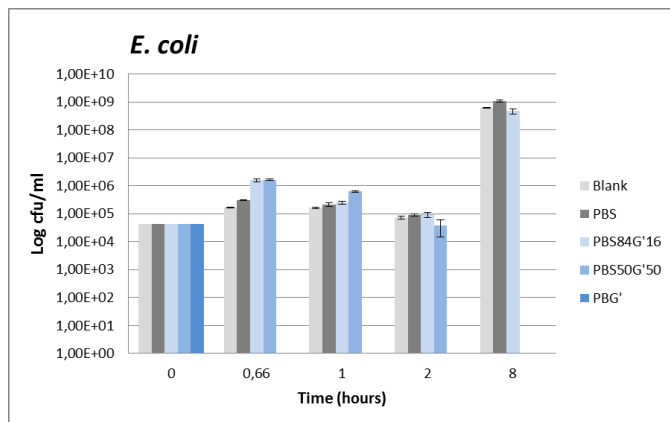


Figure 9. Microbial colony counts along the dynamic shake flask experiment for *Escherichia coli* and *Staphylococcus aureus* in contact with PBS and PBS copolyesters containing ammonium (top) and phosphonium units (bottom).

Supplementary Material

[Click here to download Supplementary Material: MBB-5 EPJ-2015-ESI.doc](#)