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Evaluation of antibacterial activity of branched quaternary ammonium grafted green polymers

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ABSTRACT

This study describes the antibacterial activity evaluation of film-shaped polymers based on biodegradable chains of polylactic acid (PLA), polycaprolactone (PCL) and polyhydroxybutyrate (PHB) in branched structures with quaternary ammonium pendant groups. The materials were tested against both Gram-negative (*Escherichia coli* and *Salmonella* Typhimurium) and Gram-positive (*Listeria monocytogenes*) bacteria. Minimal inhibitory and maximal tolerated concentrations of the quaternary ammonium monomers were first determined. Films of pure quaternary ammonium functionalized polymers (QAFPs) and of blends of QAFPs with commercial matrices were studied to determine their inhibition effects and their bactericidal activity. A high antibacterial activity was found, up to 5.6 log, 4.4 log, and 4.2 log CFU.mL-1 reduction for respectively *Escherichia coli (Salmonella* Typhimurium and *Listeria monocytogenes* after only one hour of bactericidal tests, 6.7 log, 5.2 log and 4.9 log CFU.mL^{-1} reduction was observed for the respectively cited bacteria after 24 h, making these materials potentially applicable in many fields.

1. Introduction

Antimicrobial polymers are in growing interest especially in food industry; many works have been carried out on these materials and their applications in food packaging or as active coatings in various fields. Few polymers are intrinsically antibacterial such as chitosan and polycations, so the antibacterial activity is generally incorporated into the polymer and many methods can be followed to reach this purpose, the best known being the dispersion of antimicrobial agents in the polymeric matrix as additives (Benavides, Villalobos-Carvajal, & Reyes, 2012; Colak et al., 2015; Severino, Ferrari, Vu, Donsi, & Salmieri, 2015; Stroescu, Stoica-Guzun, & Jipa, 2013). Antimicrobial polymers can also be obtained by polymerization of active monomers (Colonna et al., 2012; Coneski, Fulmer, Giles, & Wynne, 2014; Ganewatta & Tang, 2015; Zhou et al., 2014), modification and functionalization of polymers (Criado, Fraschini, Salmieri, & Lacroix, 2014) or by grafting monomers including active groups in their structure onto polymer chains (Cerkez, Worley, Broughton, & Huang, 2013; Riva et al., 2008). The antimicrobial polymeric materials act in different ways according to both their structure and the kind of antimicrobial active groups: some materials work by releasing antimicrobial agents in a contaminated medium (Buonocore et al., 2004; Imran et al., 2012; Raval, Naik, Amin, & Patel, 2014; Muriel-Galet, Cran, Bigger, Hernández-Muñoz, & Gavara, 2015) while others act by

Abbreviations: ADCl, [2-(Acryloyoxy)ethyl]trimethyl ammonium chloride; MADCl, [2-(Methacryloyloxy)ethyl]trimethyl ammonium chloride; QAFP, Quaternary ammonium functionalized polymer; PLAgAD, Polylactic acid based QAFP grafted by ADCl; PLAgMAD, Polylactic acid based QAFP grafted by MADCl; PCLgAD, Polycaprolactone based QAFP grafted by ADCl; PCLgMAD, Polycaprolactone based QAFP grafted by MADCl; PHBgAD, Polyhydroxybutyrate based QAFP grafted by ADCl; PHBgMAD, Polyhydroxybutyrate based QAFP grafted by MADCl; PLA, commercial polymer matrix of polylactic acide; PCL, commercial polymer matrix of polycaprolactone; O.D., Optical density; MIC, Minimal inhibitory concentration (wt.%); MTC, Maximal tolerated concentration (wt.%); CFU, Colony forming units.

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contact with the targeted microorganisms (Abreu et al., 2015; Eknoian, Worley, Bickert, & Williams, 1999).

Different antibacterial active chemical groups are involved in the elaboration of antibacterial polymers (Jain et al., 2014; Park et al., 2001; Nonaka, Hua, Ogata, & Kurihara, 2003; de Moura, Mattoso, & Zucolotto, 2012; Paillot, Jegat, Becquart, & Taha, 2016; Zhang et al., 2006) and among them the quaternary ammonium group has a high antibacterial efficiency against both Gram-negative and Gram-positive bacteria (Lee, Roh, Lee, Song, & Jang, 2016; Majumdar et al., 2009). The quaternary ammonium-containing monomers are commercially available under various structures with large possibilities of chemical reactivity. In previous works (Belkhir, Shen, Chen, Jegat, & Taha, 2015; Belkhir, Jegat, & Taha, 2016), new quaternary ammonium-bearing polymers based on polylactic acid (PLA), polycaprolactone (PCL) or polyhydroxybutyrate (PHB) segments were synthesized with well-defined design and low average molecular weight.

Some antibacterial molecules may lose their activity after chemical treatments or modification, or after thermal treatments during melt processing. In practice, antibacterial materials prepared by mixing active entities with polymer matrices present an important disadvantage: the active species are released faster than needed so the antibacterial activity does not last long, note that in some cases the release of antibacterial entities is prohibited. The use of quaternary ammonium grafted polymers as antibacterial entities mixed with polymer matrices can be a solution for all these disadvantages. Several polyester matrices such as PLA and PCL are interesting for this aim and are more and more used in various applications such as food packaging or cosmetic industry and many works are in progress to enlarge their application fields.

PLA is a biosourced and biodegradable polymer, easily processable with interesting mechanical and thermal properties. PCL is highly biodegradable and abundant, with high thermal stability and excellent characteristics even in the melt or the solid state.

The aim of this study was to determine the antibacterial activity of films made of polymers including PLA, PCL or PHB segments in branched structures having pendant quaternary ammonium groups, synthesized in previous works (Belkhir et al., 2015; Belkhir et al., 2016), against Gram-negative bacteria (*Escherichia coli* and *Salmonella Typhimurium*) as well as a Gram-positive bacterium (*Listeria monocytogenes*), before and after their melt processing. The unintended release of the quaternary ammonium polymer outside the matrix was also verified.

2. Materials and methods

Three quaternary ammonium functionalized polymers (QAFPs) were used as antibacterial products. The structure of these polymers was branched and based on biodegradable polyester chains of PLA, PCL or PHB. Fig. 1 shows a representation of these polymers highlighting the branched structure with three arms and the active sites formula on the chain ends. Their synthesis and physicochemical characterizations were described in previous publications (Belkhir et al., 2015; Belkhir et al., 2016); three-step synthesis protocol was followed: The first step consists of the synthesis of branched polymers with isocyanate functions at the chain ends by mixing dihydroxy telechelic polymer (PLA-diol, PCL-diol or PHB-diol) with glycerol and 4,4′-Methylenebis(cyclohexyl isocyanate) in DMF at 80 °C, the second one allowed the

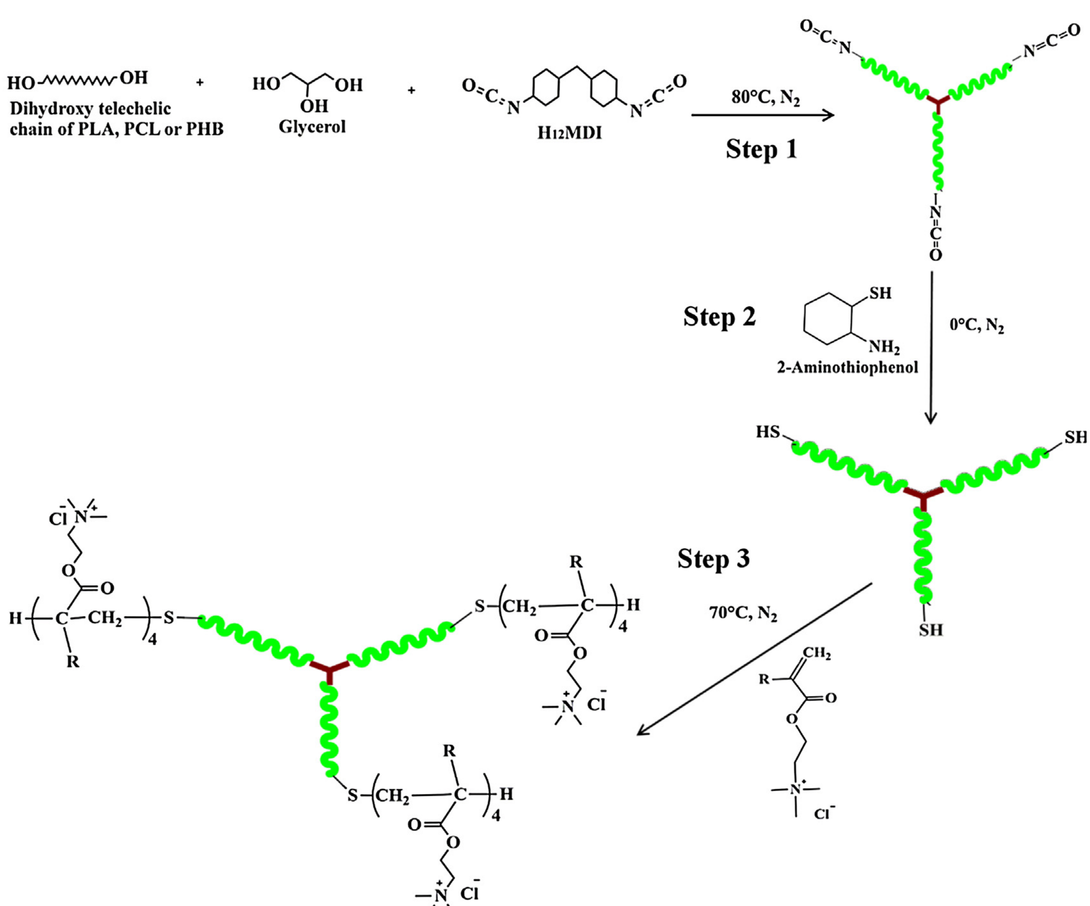

Fig. 1. The three-step synthesis of the quaternary ammonium functionalized polymers (QAFPs).